DEVELOPMENT AND OPTIMIZATION OF OSMOTICALLY CONTROLLED ORAL DRUG DELIVERY SYSTEMS FOR HIGHLY AND POORLY WATER SOLUBLE DRUGS

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The Tamil Nadu Dr. M.G.R. Medical University, Chennai

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DOCTOR OF PHILOSOPHY

In

PHARMACY

By

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"DEVELOPMENT AND OPTIMIZATION OF OSMOTICALLY CONTROLLED ORAL DRUG DELIVERY SYSTEMS FOR HIGHLY AND POORLY WATER SOLUBLE DRUGS"** *being submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for the award of degree of* **DOCTOR OF PHILOSOPHY** *in* **PHARMACY** *was carried out by* **Mrs. SONA P.S,** *at* **RVS COLLEGE OF PHARMACEUTICAL SCIENCES**, Sulur, Coimbatore, *under my direct supervision and guidance to my fullest satisfaction. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.*

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DECLARATION

I hereby certify that I am the sole author of this thesis entitled **"DEVELOPMENT AND OPTIMIZATION OF OSMOTICALLY CONTROLLED ORAL DRUG DELIVERY SYSTEMS FOR HIGHLY AND POORLY WATER SOLUBLE DRUGS"** *and that neither any part of this thesis nor the whole of the thesis has been submitted for a degree to any other University or Institution. I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or, are fully acknowledged in accordance with the standard referencing practices. I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis review committee.*

Mrs. Sona.P.S

Place: Coimbatore

Date:

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This thesis is fruit of infinite encouragement, guidance and cooperation received from multidirectional aiming for a unidirectional goal. Today, at the acme of my dissertation, with heartiness, I gratefully remember my parents, teachers, friends, relatives and well wishers; as one flower makes no garland. This presentation would not have taken shape without their whole hearted encouragement and live involvement.

*On this occasion of successful completion of my work, I offer my salutation to the **Almighty**, with whose showering of blessing, this task was, ventured without any hindrance.*

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Sona.P.S

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

CRDDS	Controlled release drug delivery systems
CR	Controlled release
AUC	Area under the curve
MSC	Maximum safe concentration
OCODDS	Osmotically controlled oral drug delivery systems
OT/OTs	Osmotic tablet/Osmotic tablets
OODS	Oral osmotic delivery systems
BCS	Biopharmaceutical classification system
OROS	Osmotically regulated oral system
PPOP	Push pull osmotic pump
DoE	Design of experiments
UV	Ultra violet
λ_{max}	Lamda max
DSC	Differential scanning colorimetry
OCOT	Osmotically controlled oral tablet
PEO	Poly ethylene oxide
NaCl	Sodium chloride
SLS	Sodium lauryl sulphate
PG	Propylene Glycol
CA	Cellulose acetate
DCP	Di calcium phosphate
IPA	Iso propyl alcohol
BHT	Butylated hydroxy toluene
DL	Drug layer
PL	Push layer
Factor A	Poly ethylene oxide in drug layer
Factor B	Sodium chloride in drug layer
Factor C	Sodium lauryl sulphate in drug layer
Factor D	Poly ethylene oxide in push layer

Factor E	Sodium chloride in push layer
Factor F	Sodium lauryl sulphate in push layer
Factor G	Propylene Glycol
Factor H	Weight gain
PCUR	Percentage cumulative release
R^2	Release rate constant
Wt gain	Weight gain
ANOVA	Analysis of variance
SL	Significant level
p-value	Probability value
FDS curve	Fraction of design space curve
RS plot	Response surface plot
CI	Confidence interval
TI	Tolerance interval
RH	Relative humidity
HPLC	High performance liquid chromatography
C_{max}	Maximum plasma concentration
t_{max}	Time to reach maximum plasma concentration
T ½	Half life
K_{el}	Elimination constant
mg	Milligram
ml	Milliliter
µg	Microgram
mm	Millimeter
cm	Centimeter
nm	Nanometer
hrs	Hours
^0C	Degree Celsius
Abs	Absorbance
con	Concentration

Chapter -1
INTRODUCTION

1. INTRODUCTION

1.1. CONTROLLED DRUG DELIVERY SYSTEMS [1, 2-5]

Controlled release drug delivery systems (CRDDS) offer many advantages over conventional dosage forms like improved patient compliance and convenience and reduced adverse effects. A constant therapeutic plasma concentration of the drug within the therapeutic index of the drug over extended periods was maintained in CRDDS. Figure No. 1.1.1.

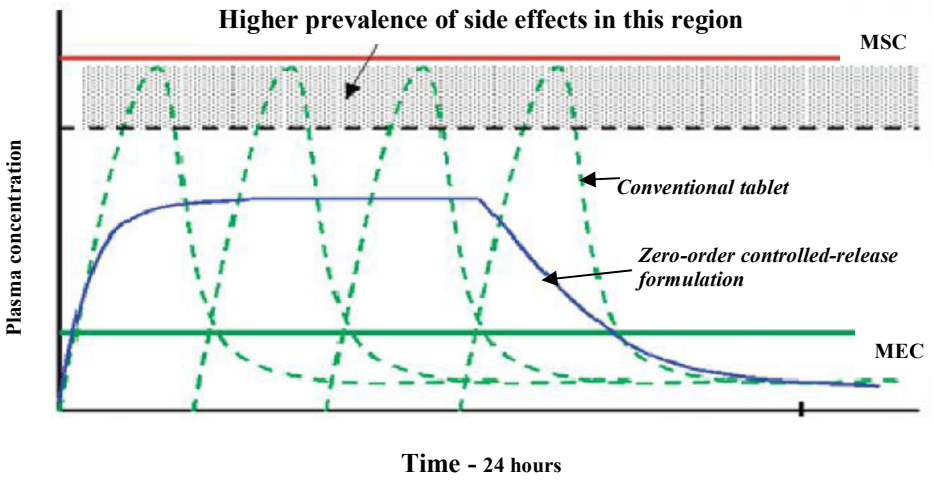


Figure No.1.1.1: Plasma drug concentration profiles of controlled release and conventional formulations

In conventional oral dosage forms, the resulting pattern of concentration of drug in plasma widely varies and this may cause unpredictable and undesired clinical effects. Variations of the blood concentration above the MSC may result in adverse effects. With CR products drug entry with a precise extent, rate, or timing into the blood can be programmed or achieved. Release of the drugs from all other conventional dosage forms except intravenous dosage forms follows first-order kinetics. This results in irregular high and low concentrations and only a brief optimal therapeutic level. But the controlled release systems release the drug at a constant rate (zero order) for a definite time period. This results in consistent concentration of drug in tissue and plasma. In order to maintain blood

concentrations within the therapeutic index, frequent dosing will be done for drugs with short half life. Frequency of dosing and patient compliance is inversely related. CR products have the potential to improve patient compliance by reducing the number of daily doses.

1.2. ADVANTAGES OF OSMOTIC DRUG DELIVERY SYSTEM [5]

- Zero order delivery rate can be achievable.
- Pulsed or delayed drug delivery is obtainable.
- *In vitro* delivery rate can be accurately predicted using mathematical equations.
- High level of *in vivo* correlation.
- Rate of delivery is independent of pH variations in the gastrointestinal tract environment.
- Rate of delivery is independent of agitations like GI motility.
- Rate of release from osmotic system is well predictable and programmable.
- Drugs are delivered from the system in the solution form which is ready for absorption.
- Delivery rate is nearly independent of delivery orifice size within limits.
- Device is reasonably simple to produce.
- Drugs with extensively altering solubility's can be included.

1.3. DRUG CANDIDATE SELECTION FOR OCODDS [4-5]

The selection of the OCODDS technology should be done only after studying the pharmacokinetic profile of the drug under consideration. In order to formulate a successful extended-release dosage form, drugs which have higher permeability, less pre systemic metabolism, no absorption window can be chosen. Ideal BCS classes are I, II and V. For BCS class I, the solubilisation step is usually quick and not rate-limiting. So permeability characteristics determine the drug release and absorption. For BCS class II and V, solubility is rate limiting and drug should be delivered in an oversaturated solution. Consistent absorption of the over saturated solution is a mandatory throughout the GI tract for class II drugs. If

not, the use of OCODDS may not be beneficial because of the impaired bioavailability. Drug solubility and dosage strength are the main two criteria that should be taken care of during the device selection for OCODDS. In order to deliver highly soluble drugs elementary osmotic pumps, controlled porosity osmotic pumps and swellable core osmotic pumps are considered to be better than other devices. Osmotic devices using self emulsifying technologies can be used for poorly soluble drugs. But push pull and push stick osmotic pumps can indiscriminately be used for both highly and poorly soluble drugs.

1.4. PUSH PULL OSMOTIC TABLETS [1- 37]

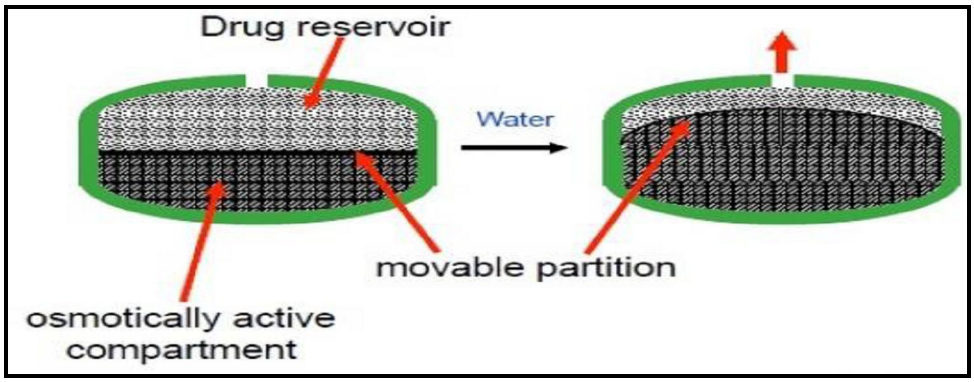


Figure No.1.1.2: Osmotic push pull pump

Push pull osmotic tablet is a tailored made elementary osmotic pump intended to release the drug at zero order rate. This device resembles a bilayer coated tablet. The upper layer contains drug, polymeric osmotic agent and other tablet ingredients. This polymeric osmogen can form a suspension of drug in situ when this tablet later imbibes water. The other layer contains osmotic agent, polymer, colouring agent and other tablet excipients. The layers are made and punched together to form a bilayer single core. A layer of semipermiable membrane coat of reasonable thickness is applied over the bilayer tablet. A small opening can be drilled on the membrane usually on the drug layer with the help of mechanical or laser drill. The balance between the osmotic pressure created by the osmotic agent present both in drug and push layer was responsible for the perfect zero order release. This device is equally suitable for both highly and poorly soluble drugs.

1.5. DESIGN OF EXPERIMENTS [38-51]

The QbD (Quality by design) is a systemic approach to pharmaceutical development. ICH Q8 guidance, states that "quality cannot be tested into products; it should be built in by design". This new advance to development of products could increase efficiencies, provide flexibility, regulatory support and offer significant business benefits. The FDA publicized a new initiative (cGMP for the 21st Century: A Risk based Approach) in 2002. This initiative proposed to modernize the FDAs regulation of pharmaceutical quality, and establish a new regulatory agenda focused on risk management, QbD, and quality system. The initiative of the FDA challenged industry to look beyond quality by testing (QbT) for ensuring product quality and performance. An important part of QbD is to understand how process and formulation parameters affect the product characteristics and subsequent optimization of these parameters. This is done with the help of design of experiments.

1.5.1. Importance of Design of experiments.

Design of experiments (D_OE) extensively helps the designers to figure out simultaneously the main as well as the interaction effects among the vast number of factors which are affecting the actual outcome. D_OE helps to pin point the responsive parts and sensitive areas in your process that cause problems in the outcome of the process. The findings can be used to furnish a fruitful process. The major advantages of DoE can be summarised as follows,

- A one factor at a time approach (OFAT) adopted by most of the industries burden the manufacturer with large number of experimental trials, which is time consuming and costly. Well designed experimental trials reduce both the problems.
- Systematic study of the interaction of factors can be done, which are not possible with OFAT experiments.
- Factor space and design space can be identified. The forecast of the response in the factor space can be done as per the requirements. The optimization of the product as well as process can be efficiently performed.

1.5.2. Flow chart of the proceedings using DoE

The systematic steps of optimization of products using DoE is shown in the flow chart given below,

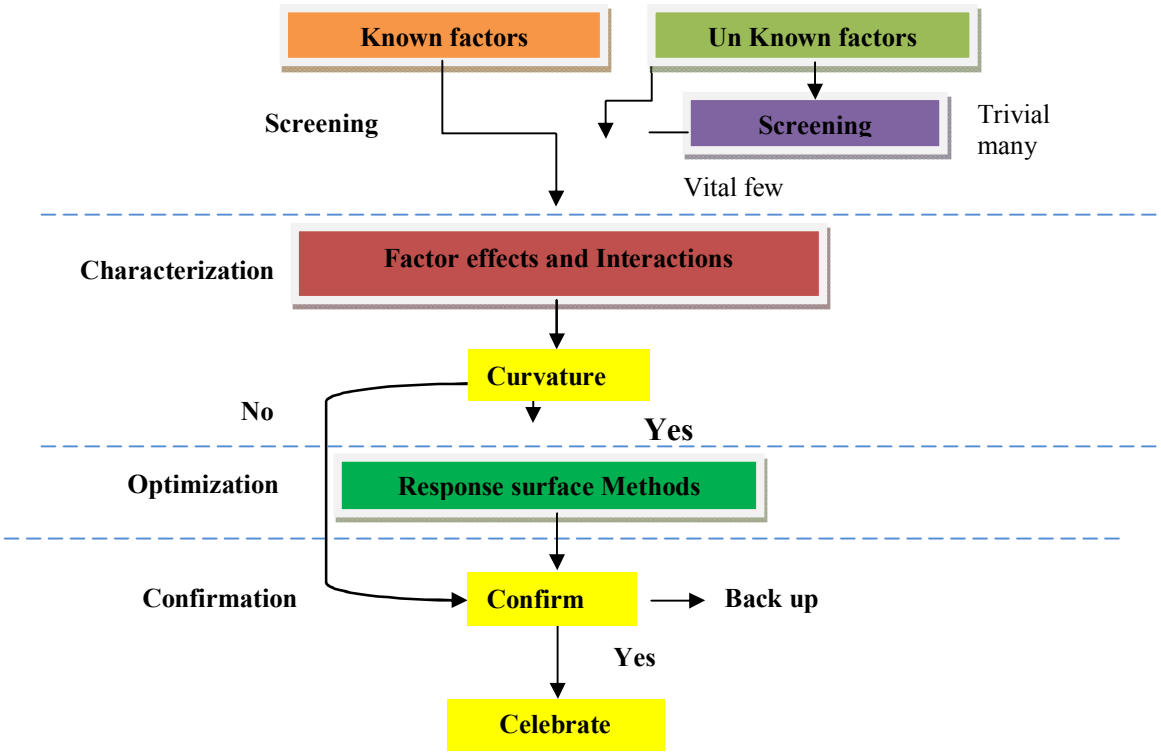


Figure No 1.1.3: Systematic steps of design of experiments [43]

1.5.3. Screening studies

When large number of factors were affecting a process/ product outcome, it is essential to identify the few vital factors. Screening designs are used for reducing the number of factors down to the few that have vital effects. These important factors are then examined more closely using other design models. In a screening design, each continuous factor is usually set at two levels to economize on the number of runs required. The design consists of a fraction of the possible combinations of factor levels.

1.5.4. Factor influence study

When vital factors are identified, a factor influence study will be done to find out the magnitude of the factor influences. When sufficient literatures are

available, researcher can skip the screening study and start with factor influence study. Full factorial designs and fractional factorial design are used for factor influence study.

1.5.5. Full factorial designs

A full factorial D_OE is a planned set of tests on the response variable(s) with one or more inputs (factors) with all combinations of levels. Commonly used full factorial designs are 2 level full factorial designs and 3 level full factorial designs.

1.5.6. Fractional factorial designs

Fractional factorial design in the following way: "*A factorial experiment in which only an adequately chosen fraction of the treatment combinations required for the complete factorial experiment is selected to be run.*" [49] Full factorial designs are the first choice for any problems. But the main disadvantages of these designs are the existence of large number of runs. For a 2^6 full factorial design 64 runs should be executed. A good number of center point runs also to be added to this design to predict the linearity/non linearty of the model.

Fractional factorial designs or partial design offers the flexibility of performing the runs in fractions such as ½, ¼, 1/8, 1/16 etc. Various approaches that ensure a fitting, choice of runs can be used. The basic purpose of a fractional factorial design is to economically investigate cause-and-effect relationships of significance in a given experimental setting.

Lower resolution fractional factorial designs are only used to study the main effects as the main effects are aliased with two way or 3 way interactions. So these deigns are called as screening designs.

Designs with resolution five or higher are used estimate main effects as well as interaction effects. The main effects are aliased with 4 way/5 way interaction which can be negligible. The results obtained are considered to be accurate than the lower resolution fractional factorial designs. Theses designs can easily augmented to complete a second-order design if non linearity present.

Chapter- 2
AIMS AND OBJECTIVES

2. AIMS AND OBJECTIVES

2.1. AIMS OF THE STUDY

For a successful drug therapy the concentration of the drug in the plasma should be maintained constant within the therapeutic index (TI) throughout the treatment period. It avoids the fluctuations in plasma concentration of drug and improves the patient compliance. Diseases which need a longer duration of treatment (sometimes a life time) require more careful medications and drug delivery systems for the better patient compliance and comfort. Controlled release drug delivery systems, release the drug at a controlled and constant manner within the therapeutic index throughout the treatment period. Osmotic pumps are such a device which can strictly maintain a controlled release of the drug in the blood plasma within the therapeutic index up to the desired time period.

The drug candidates were selected after extensively studying the drug properties for the suitability of osmotic drug delivery systems. Ropinirole HCl is a potent antiparkinsons agent having a half life of 6 hrs. It is highly soluble in water (BCS class I) and absorbed fastly achieving the peak plasma concentration within 1-2 hours. Ivabradine HCl which is an anti ischemic drug having half life 2 hrs, highly soluble (BCS class I) and rapidly absorbed after oral administration. Therefore, both these drugs are potential candidates for controlled drug delivery formulations; however, controlling its release is a challenging task due to its high water solubility.

Carvedilol phosphate which is an Alpha/beta-adrenergic blocking agent having half life 7-10 hrs and Nisoldipine, a calcium channel blocker having a half life of 7 -12 hrs. Both these drugs are poorly water soluble (BCS class II) making the candidates extremely problematic for any type of extended/controlled drug delivery systems.

So our work aims to develop a suitable dosage form for successfully delivering both the categories of drugs which can release the selected drugs at a zero order rate throughout the treatment period. As tablets are considered one of the best acceptable dosage forms, an OCODDS in the form of tablets was chosen as the drug delivery system for the selected drugs. The selected drugs show extreme solubility characteristics. So development of push pull type osmotic tablets was planned for the successful delivery of the drugs as this is the only device which is equally suitablefor the complete as well as constant delivery of both highly and poorly soluble drugs.

So aim of our study is to develop and optimize push pull osmotic tablets of highly water soluble drugs (Ropinirole HCl, Ivabradine HCl) and highly water insoluble drugs (Carvedilol phosphate, Nisldipine) that deliver the drug at zero order rate up to 24 hours.

The major challenges during the development of push pull osmotic tablets are the optimization of core as well as coating parameters to achieve the desired release profile. So a factor influence study of core variables and coating membrane variables on the release profile of the selected drugs from the device were planned using design of experiments. As the selected drugs show extreme solubility characteristics, the study also focus on the extensive comparison of different core and membrane variables affecting the release profile of the highly and poorly water soluble drugs. The study also aims on the optimization of the significant variables/factors of both core and membrane using numerical optimization and desirability approach.

2.2. OBJECTIVES OF THE STUDY

- To develop and evaluate an OCODDS (Push pull osmotic tablets) of highly and poorly water soluble drugs.
- To study and optimize core as well as membrane parameters affecting the release profile using design of experiments (DoE)
- To compare and conclude the effect of different parameters of the formulation on the release profile/pattern of the selected drugs from the device using different statistical tools.
- To optimize the push pull osmotic tablet formulation of all the four selected drugs using numerical optimization and desirability techniques.
- To study the factor influence on the desirability function of highly and poorly soluble drugs.
- To conduct the stability studies of the selected optimized formulations as per ICH guidelines.
- To conduct an *in vivo* animal studies for the selected optimized formulation of all the four drugs.

Chapter -3
LITERATURE REVIEW

3. LITERATURE REVIEW

Wakode R et al, 2010 [52] developed push pull osmotic tablets of Pramipexole. *Invivo* efficiency of the once a day formulation was evaluated. The formulation contained bilayered tablets of drug layer and polymer layer coated with cellulose acetate membrane with water soluble pore forming agents. Different pharmacokinetic parameters were estimated. The developed formulation maintained plasma levels of pramipexole with in the TI for time duration of 24 hrs.

Wakode R et al, 2012 [53] developed and characterized an extended release push-pull osmotic oral system which can deliver Pramipexole at a constant rate. A bilayer osmotic drug delivery device with an orifice at the drug layer was developed. They studied the effect of the concentration of a pore-forming agent such as PEG 400, pH of dissolution media, dibutyl phthalate, the effect of agitation and osmotic agents on drug release. The release of the drug was found to follow zero order kinetics. Release of the drug was increased with an increase in osmotic pressure.

Mane SS et al, 2012 [54] focused on various components of osmotic systems, their role in controlling drug release, different types of ODDS in research phase and some formulations available in market.

Zhang ZH et al, 2011 [55] for the formulation of push –pull osmotic pump tablets an expert system was built. A vast number of poorly soluble drugs were studied. Rules regarding the PPOP was created and recorded in the database for preparations containing poorly soluble drugs and pharmaceutical excipients. Large number of articles available was also studied. A back propagation (BP) neural network was used for the prediction of release behaviour of the drug from the systems.

Chaudhary A et al, 2011 [56] developed a micro porous bilayer oral drug delivery system for colon targeting. Dicyclomine hydrochloride and Diclofenac potassium were the drugs of choice. Various formulation variables were studied for their effect on the drug release. Osmogen, hydroxy propyl methyl cellulose, sodium carboxy methyl cellulose and pore former were studied. *In vitro* dissolution results confirmed that the system showed acid-resistant, timed release. A zero order release up to 24 hrs was reported.

Waterman KC et al, 2011 [57] described an osmotic, oral, controlled-release capsule. Constant delivery rates ($t_{80\%}$=6 or 14 h) obtained was reported which was independent of drug properties (e.g., solubility or drug loading). The findings will be useful for rapid development of formulations of drugs. This concept type formulation offer greater flexibility for the clinical trials.

Sankar C et al, 2011 [58] developed osmotic tablets as well as capsules of Amoxicillin and combination with Tinidazole. Further preparation of osmogen coated tablets was done for evaluating coat and core osmogen effects. Prepared osmotic pump tablets as well as capsules would be a better alternative in complete eradication of H. Pylori infection with improved patient compliance and reducing toxicity, bacterial resistance.

Thakor RS et al, 2010 [59] reviewed different types of oral osmotic devices, various features and different factors affecting drug release from these devices, and its critical formulation factors. The release of drug(s) from osmotic systems was governed by different formulation factors such as solubility and osmotic pressure of the core component(s), nature of the rate-controlling membrane and size of the delivery orifice. With the help of optimization of process and formulation factors, development of osmotic systems to deliver drugs of varied nature at a pre-programmed rate was done.

Malaterre V et al, 2009 [60] studied different factors and its effect on the drug release. Polynomial equations and mathematical assumptions and statistical predictions were used to optimize the push pull osmotic system. Isradipine (ISR)

and Chlorpheniramine (CPA) were selected as the model drugs. Different significant factors and its effects on the release rate and extend were studied. To predict the drug delivery kinetics of the formulated push pull osmotic tablets the suggested mathematical models were used and found to be useful and efficient for optimization.

Rathore GS et al, 2009 [61] described various oral osmotically controlled devices. Formulation aspects, clinical applications and different evaluation methods were explained.

Liu L et al, 2008 [62] developed a bilayer-core osmotic pump device (OPD) which does not require mechanical formation of the orifice during manufacturing. Optimization of the formulations variables was done with the help of orthogonal design. Similarity factor (f2) was used to evaluate various formulations. The preparated bilayer-core tablets were coated with highly soluble pore forming agent which will produce drug delivery pores *insitu.* Both release media and agitation rates were not affecting the rate and extend of the drug release. A 24 hr release with zero order was achievable which was proven highly predictable and reproducible.

Pramod Kumar et al, **2009** [63] developed a unitary core osmotic pump system of Tramadol hydrochloride (TRH). Different formulation factors affecting drug delivery kinetics were identified as expandable polymer and plasticizer, thickness of the coating membrane. The effect of the above said factors were closely monitored and reported. A positive relationship was found between level of plasticizer and osmotic pressure with release rate. But the change in the expandable polymer and core thickness had a negative effect on the release rate.

Patel VK et al, 2012 [64] developed a push-pull osmotic pump for zero order delivery of Lithium carbonate for a period of 24 hr. The effect of various formulation variables on bilayer core tablet and its semi permeable coating along with orifice diameter have been investigated and optimized for desired drug release profile. An inverse relationship was found between membrane thickness

and drug release. But a direct effect was reported for the amount of pore formers.

Tang X et al, 2013 [65] designed and evaluated Gliclazide push-pull osmotic pump (PPOP) coated with aqueous colloidal polymer dispersions-Eudragit(®) RL 30D and Eudragit(®) RS 30D. The influence of diacetin, diethyl phthalate, dibutyl sebacate and triethyl citrate on the free Eudragit (®) RL 30D and Eudragit (®) RS 30D films as plasticizers on drug release were studied. Among these four plasticizers, diacetin offered the smoothest surface of the cast films, and it displayed greatest water vapor transmission coefficient. Free RL and RS films with diacetin also exhibited greatest erosion compared with the other three plasticizers.

Derakhshandeh K et al, 2014 [66] designed a permeable osmotically driven drug delivery system for controlling the release of Buspirone from the delivery system. The core of the tablets was prepared by direct compression technique and coated using dip-coating. The effect of various processing variables such as the amount of osmotic agent, the amount of swellable polymer, concentration of the core former, concentration of the plasticizer, membrane thickness and quantum of orifice on drug release from osmotic pump were evaluated.

Sharkheliya DB et al, 2013 [67]developed push pull osmotic tablets of Glipizide. Cellulose acetate NF (CA-398-10 NF) in a concentration of 8 % w/w for 10.0 mg tablet was optimized as coating polymer and Polyethylene Glycol 3350 NF in a concentration of 0.284% as pore former for Glipizide tablets.

Veronica C et al, 1999 [68] demonstrated efficiently the advantages of designed experiments over one factor at a time (OFAT) experiments. Real life examples were demonstrated to justify the major significances of the designed experiments in various research areas.

Liu L et al, 2000 [69] prepared sandwiched osmotic tablet system of Nifedipine consists of a trilayer osmotic tablet surrounded by a SPM with two

micro openings on both the sides. The push layer was sandwiched between two drug layers. By this formulation maximum drug loading can be achievable. Different formulation variables were studied and its effects on the release rate were reported. The study reported a similar release rate and profile up to 24 hrs as that of the marketed push pull osmotic tablet of Nifedipine.

Ketjinda W et al, 2011[70] prepared oral push–pull osmotic device of Felodipine. A complex of chitosan (CS) and poly(acrylic acid) (PAA) was used as osmogen. The effects of different variables like compression forces, type of plasticizers and polymer concentrations on release profile of the drug were studied. The study revealed that a 12 hrs or 24 releases with a zero order was programmable by changing the plasticizer. A prolonged lag time and slower release of the drug was obtainable with dibutyl sebacate as plasticizer. But by using polyethylene glycol 400 a shorter lag time and faster release was achieved.

Jinghua Y et al, 2011 [71] determined the effects of various concentrations of cellulose acetate to PEG, solvent systems and molecular weights of PEG on the permeability of CA-free films and thermo mechanical properties. Statistical approaches were used for analyzing the effect of the above mentioned factors on the responses. These researchers reported graphical and mathematic representations of the effect of factors on the responses.

Mutyaba MR al, 2011[72] developed and optimized an osmotically controlled drug delivery system of Diclofenac sodium. A three-level three-factorial Box–Behnken experimental design was used to characterize and optimize three formulation parameters, i.e. level of osmotic agent, pore former and plasticizer. Initial level of pore former had a positive effect on the release rate of drug, but membrane weight and osmotic pressure had a negative effect.

Patel KN et al, 2013 [73] optimized and evaluated push pull osmotic pump (PPOP) tablets of Nicardipine hydrochloride (NH). A 3^2 full factorial design was employed to optimize the amount of osmotic agent (X_1) and osmopolymer (X_2) as

independent variables that influence the drug release. Solubility of the NH was improved by preparing inclusion complex using β-Cyclodextrin. Optimization of amount of osmotic agent, and osmopolymer were done to obtain the predicted drug release. From the *in vitro* drug release study, it was reported that the release rate is increased with the amount of osmotic agent and osmopolymer.

Saini S et al, 2012 [74] formulated and evaluated colon targeted drug delivery system using microbially triggered osmotically controlled approach. central composite design - face centred was used to study the effect on independent factors (concentration of sodium chloride, polyethylene glycol, and chitosan) on percentage cumulative release and disintegration time. The research revealed that solubilising agent chitosan had a major significant effect on the drug release than the other factors.

Malaterre V et al, **2009** [75] developed a push–pull osmotic system to transport poorly soluble drugs. Different core tablet factors were studied for its effect on the drug release kinetics and loadability. The study revealed that either core factors or the membrane characteristics can be modulated for obtaining the desired release profile. Changes in the concentrations of swellable polymer in the drug layer and osmotic agent in the drug layer were a better option than changing the membrane characteristics to obtain the desired release. Effect of the drug loading on the release rate was also investigated. An undisturbed 24 hrs zero order release was reported up to 20% of the drug loading. This could be achieved by carefully tailoring concentration of osmotic agent proportions and by selecting viscous-grade polymers.

Malaterre V et al, 2009 [76] investigated the use of magnetic resonance imaging (MRI) for determining the mechanism of release from push–pull osmotic Device. A new benchtop apparatus was demonstrated in this research paper. A Non invasive study was carried out to characterize the hydration and swelling kinetics by monitoring the signal intensity profiles of both PPOS layers. High degree of correlation was observed between release of drug and kinetics of

hydration. The work showed that the tablet core composition, high osmotic pressure developed by the push layer, the hydration of both the drug and the push layers were the significant factors controlling the hydration and swelling. A proper balance between the factors will leads to an effective drug release.

Muthulingam C et al, 2013 [77] developed and optimized osmotic drug delivery system of Lamotrigine, an anticonvulsant drug using design of experiments. Design expert was used to study the impact of formulation variables of core tablets and the functional coating variables in two different stages. The formulation development reveals that the polyethylene oxide of drug layer and the push layer, sodium chloride of push layer and polyethylene glycol of the functional coating impacted the release profile at 24 hours.

Anschütz M et al, 2010 [78] compared the bioavailability of two osmotically controlled extended release tablets of Nifedipine. Dosage forms were administered in both fasted and fed conditions using human volunteers. The study reported that both products compared were not bioequivalent with each other.

Sharma AR et al, 2012 [79] prepared push pull osmotic drug delivery system for a highly insoluble drug, an antipsychotic category. The main aim was to improve the site specification and to provide the controlled release of drug for once-a-day drug delivery system with zero order drug release profile with applying drug release kinetic modeling. This study revealed that the osmotic agent proportion, drug layer polymer grade and plasticizer proportion in the membrane has to be optimized for the better release profiles.

Kumudhavalli MV et al, 2011 [80] developed and validated spectrophotometric methods for the determination of Ropinirole in pharmaceutical formulation. Quantitative determination of Ropinirole in pharmaceutical formulation was carried out by UVspectrophotometric method using λ_{max} at 249 nm. The method showed high specificity and linearity in the concentration range of 10-30 μg/ml.

Nashatizadeh MM et al, 2009 [81] described Ropinirole prolonged release once-daily, 24-hour formulation of Ropinirole. Mechanism of actions, ADME, side effects and drug interactions were reported in detail.

Sreekanth N et al, 2009 [82] developed a simple and accurate RP-HPLC for the estimation of Ropinirole hydrochloride. The proposed method had permitted the quantification of Ropinirole hydrochloride over linearity in the range of 5-50µg/ml.

Bhuvana K et al, 2011 [83] described the mechanism of action, pharmacokinetics, dose, clinical studies, drug interactions, uses and adverse effect of Ivabradine in this review article**.**

Maheshwari S et al, 2010 [84] developed a highly sensitive, selective, reproducible, and rapid and stability indicating RP-HPLC and spectrophotometric method has been developed and validated successfully for analysis of a new anti angina agent Ivabradine HCl in solid dosage form. Linearity of both the methods was achieved in the range 4.2 to 31.6 µg ml^{-1} with a correlation coefficient (r^2) ≥ 0.999.

Theivarasu C et al, 2010 [85] developed a new and rapid method indicating ultraviolet spectroscopic methods for the estimation of Carvedilol in pure form and in their respective formulations. The absorbance of Carvedilol was measured at 241nm in the wavelength range of 200 - 350 nm. The linear calibration range was found to be 50% - 150%.

Ketema G et al, 2012 [86] developed a simple, rapid and specific RP-HPLC method has been developed and validated for determination of Carvedilol in bulk and tablet formulations. Linearity was obtained in a concentration range of 30 to130 µg/ml with a correlation coefficient (r^2) of 0.999.

Nirupa Rani Y et al, 2013 [87] developed a simple, sensitive and specific spectrophotometric method for the determination of Carvedilol, an alpha adrenergic receptor blocker, anti hypertensive drug in pure form and in pharmaceutical formulations by UV visible spectroscopic methods. The adequate drug solubility and maximum sensitivity was found in chloroform. The λ_{max} or the absorption maxima of the drug was found to be 286 nm. The calibration range was studied from 50% -150% and correlation was found to be $R^2 = 0.998$ which was within the limits of ICH guidelines.

Zhang HF et al, 2002 [88] established a method of reverse phase high performance liquid chromatography (HPLC) for the determination of Nisoldipine in human plasma. RP HPLC was carried out on ODS C 18 column and moracizine was used as internal standard to determine Nisoldipine human plasma concentrations. The calibration was linear over the range of $0.7 \sim 64.32 ng \cdot ml^{-1}$, lowest plasma limitation of determination was $0.7 ng \cdot ml^{-1}$ and 0.4ng was the lowest amount of determination.

Gupta A et al, **2010** [89] developed a discriminatory dissolution method for Nisoldipine. The media selection was done by solubility study of drug in different pH as well as in different surfactant solution. Volume of media was found by calculating sink condition. Further method selection at different rotation speed and volume of media and their discriminating power was evaluated using simple model independent approach. Sodium lauryl suphate, 1.0% was found to be most suitable surfactant.Discriminating dissolution method for Nisoldipine is paddle at 60 rpm, 500 mL of 1.0% sodiul lauryl sulphate solution.

Safhi MM et al, 2011 [90] developed a spectrophotometric method in ultraviolet region for the determination of Nisoldipine in bulk and in pharmaceutical formulations. Absorption maxima for Nisoldipine was reported as 237 nm The range of concentrations studied was 4 -40µl.

Bertera F et al, 2012 [91] assessed cardiovascular effects and pharmacokinetics of Carvedilol in fructose-fed rats using pharmacokinetic–pharmacodynamic (PK–PD) modeling. Carvedilol showed enantioselective pharmacokinetic properties with increased distribution in fractose rats compared with normotensive animals. An enhanced hypotensive activity of Carvedilol was found in fructose rats compared with Carvedilol fed rats, which is not related to enhance sympatholytic activity.

Klippert P et al, 1998 [92] developed and validated a high-performance liquid chromatographic method with fluorescence detection for the quantification of Ivabradine and its N-demethylated metabolite in plasma (rat, dog, human) and human urine. Concentration ranges from 2.0 to 500 ng/ml in urine and 0.5 to 100 ng/ml in plasma were used for plotting the calibration curves.

Guan J et al, 2010 [93] developed a gastric-resident osmotic pump tablet of Famotidine. Incorporation of iron powder as a gas-formation and density-increasing agent was done to increase the gastric residence. Influence of different factors were done and optimized with the help of Central composite design-response surface methodology. The drug release profile was dependant on NaCl content, iron powder content, polyethylene oxide (Mw 1,000,000) content, and weight gain. *Invivo* animal study was performed using beagle dogs.

Xu H et al, **2013** [94] developed an ascending release push-pull osmotic pump (APOP) system with a novel mechanism and an easy manufacture process. In order to slow down the drug layer hydration rate an expanding polymer (Polyox WSR N-12K) was introduced in the form of suspension agent. Different core as well as coating parameters were studied and their influence in the release rate was reported. An *in vivo* study was performed using beagle dogs. *invivo* study revealed that paliperidone plasma concentration was increased gradually up to 19 h.

Gaylen ZM et al, 1985 [95] prepared and evaluated controlled porosity osmotic system. Study revealed that a zero order release was obtainable by modulating the wall thickness, osmotic pressure difference across the wall,

permeability of the polymer component in the semi permeable membrane, level of soluble additives, drug loading and total solubility of the core tablet. pH and agitation speed has no effect on the rate of release.

VenhoVMK et al, 1996 [96] developed a method for comparing bioavailability of Carbamazepine in rabbits. Three different brands of Carbamazepine tablets were used for the analysis. The Carbamazipine tablets were administered to the pharynx of the rabbit by plastic catheter-rubber balloon device. The 24 hrs bioavailability profile was created for both Carbamazepine and Carbamazepine-10,11-epoxide in serum. No rabbit to rabbit variation was reported.

Huang Y et al, 1990 [97] developed a RP-HPLC for the determination of m-Nisoldipine in plasma. A mobile phase of methanol- KH_2PO_4 with flow rate of 1ml/min was used. A two compartment model featured the pharmacokinetic process of m-Nisoldipine after its IV injection to rats and rabbits.

Ramji JV et al, 1999 [98] conducted a study in human volunteers in an open two-way crossover design. Four healthy non patient male subjects aged 40± 49 years. The studies showed that drug-related material was virtually all absorbed from the GI tract following peroral administration of Ropinirole hydrochloride to the animal species used for the toxicological evaluation of the compound.

Soltani SI et al, 2012 [99] developed, precise, sensitive and simple HPLC method for simultaneous determination of Losartan and Carvedilol in human plasma and urine. The liquid-liquid micro extraction methods were used. For the separation a Waters® ODS column (250 × 4.6 mm) was used. Mobile phase of 15 mM sodium dihydrogen phosphate buffer (pH 4.0)/acetonitrile/2-propanol (70/27.5/2.5, v/v/v), and detected by a UV detector were used.

Parasuraman S et al, 2010 [100] explained the approved blood collection techniques for laboratory animals like rodents, lagomorphs and non rodents. The methods were explained with the help of original photographs of the procedures.

Chapter -4
SCOPE AND PLAN OF WORK

4. SCOPE AND PLAN OF WORK

4.1 SCOPE OF WORK

The research work focus on the development and optimization of once daily push pull osmotically controlled oral tablets of two highly water soluble drugs – Ropinirole HCl (treatment of Parkinson's disease) Ivabradine HCl (Anti ischemic drug) and two highly water insoluble drugs- Nisoldipine (anti hypertensive drug) , Carvedilol phosphate (anti hypertensive drug).

Extensive factor influence study planned on the different formulation factors affecting the release of the drug from the push pull osmotic oral tablets with the help of design of experiments would allow an easy determination of design space to achieve the optimum release pattern.

Comparison of the different significant factors and its effects on the release pattern of push pull osmotic oral tablets of highly and poorly water soluble drugs will be giving the manufacturer an easy reference for the different critical factors taken care while formulating the dosage form.

Identification and submission of design space with the help of desirability approach and point predictions to the FDA would allow the manufacture to make changes in the formulations without obtaining further regulatory approvals.

The applied new approach to drug development using DoE could increase efficiencies, provide regulatory relief and flexibility, and offer important business benefits throughout the product's life cycle.

4.2. PLAN OF WORK

- Selection of area of interest
- Selection of suitable drug delivery systems
- Study of the need of work
- Study of feasibility of work
- Identification of drugs
- Identification of excipients and polymers
- Literature review
- Procurement of drugs and excipients
- Pre formulation studies
- Analytical methods development
- Identification of process as well as product parameters
- Screening of factors
- Product development of push pull osmotic tablets of the selected drugs with the help of DoE
- Evaluation of OCODDS
- Factor influence study
- Optimization of formulations
- Stability Studies
- *In vivo* studies
- Analysing the results of all the formulations
- Final conclusion
- Scope of future work

Chapter -5
MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1. LIST OF MATERIALS

Table No. 5.1.1: List of materials used in the project

SLNo	Raw Materials	Manufacturer / Supplier
1	Ropinirole HCl	Alembic Ltd., Vadodara
2	Ivabradine HCl	Alembic Ltd., Vadodara
3	Carvedilol phosphate	Alembic Ltd., Vadodara
4	Nisoldipine	Alembic Ltd., Vadodara
5	Butylated Hydroxy Toluene	Merck Millipore.,Mumbai
6	Polyethylene oxide 400 K(WSR N)	Signet Chemical Pvt. Ltd., Mumbai
7	Polyethylene oxide 7000K	Signet Chemical Pvt. Ltd., Mumbai
8	Sodium Lauryl Sulphate	Merck Millipore, Mumbai
9	Sodium chloride	Merck Millipore, Mumbai
10	Cellulose acetate(CA-398-10NF)	SignetChemical Pvt. Ltd., Mumbai
11	Dibasic calcium phosphate	Innophos., India
12	Magnesium stearate	Ferro India Pvt. Ltd., Pune
13	Iron oxide red	Chemdyes Corporation, Vadodara
14	Propylene Glycol	S.D Fine., Mumbai, India
15	Acetone	S.D Fine., Mumbai, India
16	Disodium hydrogen phosphate	S.D Fine., Mumbai, India
17	Potassium di hydrogen phosphate	S.D Fine., Mumbai, India
18	Formic acid	S.D Fine., Mumbai, India
19	Sodium hydrogen phosphate	S.D Fine., Mumbai, India
20	Acetonitrile	S.D Fine., Mumbai, India
21	Sodium octyl sulfate	S.D Fine., Mumbai, India
22	EDTA	S.D Fine., Mumbai, India
23	Ammonium formate	S.D Fine., Mumbai, India
24	Tri fluroacetic acid.	S.D Fine., Mumbai, India
25	Methanol	S.D Fine., Mumbai, India
26	2-Propanol	S.D Fine., Mumbai, India

5.2 LIST OF INSTRUMENTS

Table No. 5.2.1: List of Instruments used in the project

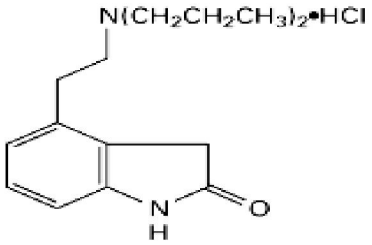
SL. No.	Instruments	Manufactures/ Suppliers
1	Electronic Balance	Mettler Toledo.,India
2	Pfizer Hardness Tester	Pfizer Pvt., Ltd.
3	Friability Test Apparatus	Electro Lab., India
4	Tap Density Tester	Electro Lab., India
5	Tablet Disintegration Test Apparatus	Electro Lab., India
6	Sieve Shaker	Endecotts., UK
7	Tablet Dissolution Tester	Electro Lab., India
8	U.V Spectroscopy	Shimadzu., Japan
9	H.P.L.C	Schimazu., Japan
10	Sieve / Sifter	Microteknik., Ambala
11	Tablet Coater	Glatt (India)., Mumbai
12	Vernier Caliper	Tresna Pvt. Ltd., USA
13	Tray Drier	Nutronics., India
14	Compression Machine	Cadmach Pvt Ltd., India
15	Micro Drill Press	Cameron.,Canada
16	Vortex Mixer	Scientific industries Inc., USA
17	Refrigerated Micro Centrifuge	USA scientific., USA

5.3. DRUG PROFILE

5.3.1 Ropinirole hydrochloride [101, 102]

Description

It is an orally administered non-ergoline dopamine agonist used for the treatment of idiopathic Parkinson's disease. The structural formula is:



4-*[2-(dipropylamino) ethyl]-1, 3-dihydro-2H-indol-2-one monohydrochloride*

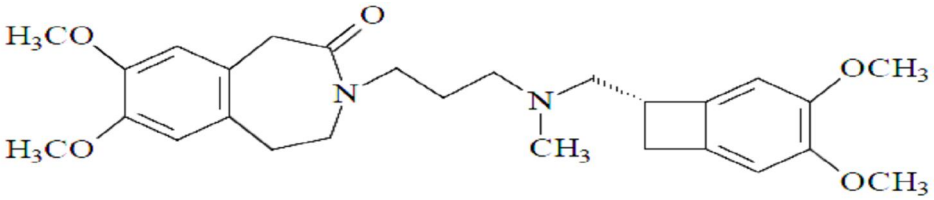
Table No.5.3.1: Properties of Ropinirole HCl

Properties	Description
Appearance	White to yellow solid
Molecular formula	**$C_{16}H_{24}N_2O$•HCl**
Molecular Mass	296.84 (260.38 as the free base)
Category	non-ergoline dopamine agonist
Use	For the treatment of idiopathic Parkinson's disease
Solubility	133 mg/ml in water
Melting Point	243° to 250°C
BCS Class	Class I
Log P	3.16
Pk_a	15.55
Protein Binding	40%
Bio availability	55% (First pass metabolism)
Metabolism	Extensively metabolized by the liver
V_d	7.5 l/kg
T ½	6 hours
T_{max}	1-2 hours
Dosing	1 to 8 mg 3 times daily
Route of elimination	Urine
Strength	0.25 mg, 0.5 mg, 1 mg, 2 mg, 3 mg, 4 mg, or 5 mg(IR). 2 mg, 4 mg, 6 mg, 8 mg, and 12 mg(XR)
Available Marketed products	Requip Tiltab(Glaxo) Tablets ,REQUIP XL tablets

5.3.2. Ivabradine HCl[83,102,103]

Description

Ivabradine HCl is a pure heart rate lowering agent having selective and specific inhibition of the cardiac pacemaker $I_f$current that controls the spontaneous diastolic depolarization in the sinus node. It is used for the symptomatic treatment of chronic stable angina pectoris patients



3-(3-{[((7S)-3,4-Dimethoxybicyclo[4.2.0]octa-1,3,5-trien-7-yl)methyl] methyl amino} propyl)-1,3,4,5tetrahydro-7,8-dimethoxy-2H-3-benzazepin-2-one,hydrochloride.

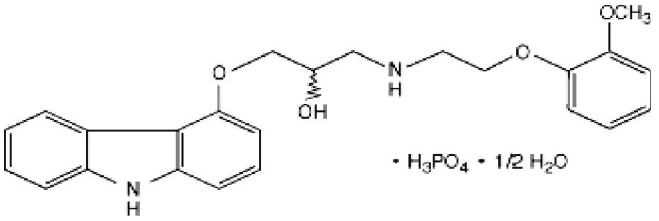
Table No. 5.3.2: Properties of Ivabradine HCl

Properties	Description
Appearance	White to slightly yellow powder
Molecular formula	**$C_{27}H_{35}N_2O_5$**
Molecular Mass	468.585 g/mol
Category	Anti ischemic drug
Use	Chronic stable angina pectoris
solubility	Highly soluble (> 10 mg / ml)
Melting Point	193 – 196 ^{o}C
BCS Class	Class I
Log P	2.71
Protein Binding	70 -75 %
Bio availability	40 %
Metabolism	Hepatic (Cytochrome CYP 3 A4)
V_d	100 l
$T_{½}$	2 hrs
T_{max}	1 hr
Dosing	Bid (5 mg Twice daily, increased up to 7.5 mg twice daily after 3-4 weeks)
Route of elimination	Faceus and urine
Strength	5, 7.5 mg tablets
Pka	8.6
Available products	Ivabrad (Lupin) Ivabrid (Piramal), Procoralan (Servier) Coralan (Servier)

5.3.3. Carvedilol Phosphate[104]

Description

Carvedilol phosphate is a nonselective β-adrenergic blocking agent with α1-blocking activity. It is a recemic mixture of the following structure,



(2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol phosphate salt (1:1) hemihydrates

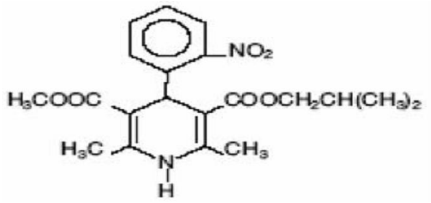
Table No. 5.3.3: Properties of Carvedilol phosphate

Parameter	Description
Appearance	White to off-white powder
Molecular Formula	$C_{24}H_{26}N_2O_4$
Molecular Mass	406.5
Category	Alpha/beta-adrenergic blocking agent
Use	Treatment of mild-to-severe chronic heart failure, and essential hypertension
Solubility	Poorly soluble in water (0.583 mg/L)
Melting Point	114-115 C
BCS class	BCS II
LogP	3.8
pKa	15.00
Protein binding	98%
Bioavailability	25 – 35%
Metabolism	Hepatic
V_d	115 l
$T_{½}$	7 – 10 hrs
T_{max}	30 min
Route of elimination	via the bile into the faeces
Strength	3.125, 6.25, 12.5, 25 mg (IR) 10, 20, 40 80 mg (ER)
Dosing	bid (IR) Once daily (ER)
Food effect	Rate of absorption is slowed
Contraindication	Bronchial asthma or related bronchospastic conditions
Available marketed brands	Carvil (Zydus Cadila), Coreg (GSK), Dilatrend (Roche), Eucardic (Roche), and Carloc (Cipla) , Coreg CR (GSK)

5.3.4. Nisoldipine [105]

Description

It is a calcium channel blocker used for the treatment of hypertension.



Nisoldipine is 3, 5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methyl-propyl ester

Table No. 5.3.4: Properties of Nisoldipine

Parameter	Description
Appearance	Yellowish crystalline Powder
Molecular Formula	$C_{20}H_{24}N_2O_6$
Molecular Mass	388.4
Category	Anti Hypertensive (calcium channel blocker)
Use	Treatment of hypertension
solubility	Poorly soluble (5.77mg/L)
Melting Point	150 - 155 C
BCS class	BCS II
LogP	3.63
pKa	< 3.0
Protein binding	99%
Bioavailability	5%
Metabolism	Pre-systemic metabolism in the gut wall, Cytochrome P450 3A4
Vd	350 l
$T_{½}$	7-12 hours
T_{max}	6-12 hrs
Route of elimination	Urine
Strength	8.5,17,20,25.5,30,34,40mg(All extended release tablets)
Dosing	Once a day
Side effects	Peripheral Edema, Headache ,Dizziness,
Contraindication	Pregnancy, Lactation ,Hepatic function impairment
Available marketed brands	Sular (Shionogi Pharma, Inc, atlanta)

METHOD DEVELOPMENT[80-90,107,108]

5.4.1. UV method development for the evaluation of formulations

I. Determination of λ_{max} of the selected drugs

Accurately weighed drug was dissolved in 100ml volumetric flask containing freshly prepared dissolution medium. After proper dilution a 20 µg/ml was used for the spectrum scanning within the range of 200-400 nm using UV spectrophotometer. The λ_{max} was scanned using test solution prepared in 6.8 pH phosphate buffer. The λ_{max} was identified where the drug shows maximum absorbance.

A double beam UV-visible Spectrophotometer (Shimadzu, UV-1800, Japan), attached to a computer software UV probe 2.34, with a spectral width of 2 nm, wavelength accuracy of 0.2 nm and pair of 1 cm matched quartz cells was used for the analysis.

II. Preparation of standard stock solution

Accurately weighed drug was dissolved in 100ml/250 ml volumetric flask containing freshly prepared medium /solvents. 10 ml ethanol/ acetone or 1% SLS solution can be used for the solubilisation of poorly soluble drugs. The obtained solution of the drug was used as standard stock solution.

III. Preparation of calibration curve

From the stock solution, suitable dilutions were prepared in the corresponding solvent to produce standard curve of the drugs. Absorbance of each solution was measured against diluted media at the corresponding λ_{max} of the drug using UV/Visible Spectrophotometer. Samples were analyzed in triplicate, and the average values were used for plotting the graph of absorbance versus concentration (µg/ml). Linearity range, regression equation, slope and R^2 were determined.

5.5. PRE FORMULATION STUDY [109-115]

5.5.1. Organoleptic characteristics

The color, odor and taste of the drug were characterized and recorded using descriptive terminology.

5.5.2. Solubility studies

Solubility is defined as the amount of substance that passes into solution to achieve a saturated solution at constant temperature and pressure.

The solubility of the drugs was determined by the shake flask method[113]. Solubility study of drugs were done in four different medium 0.1N HCl, acetate buffer pH 4.5,Phosphate buffer pH 6.8, Phosphate buffer pH7.4 and distilled water. According to this method the compound is added in surplus to medium and shaken on an orbital shaker upto24 hr. The saturation is confirmed by the observation of the presence of un-dissolved material. After filtration of the slurry a sample analysis can be done. Both filtration and analysis should be performed under the same temperature as the solubility determination to minimize loss of volatile components. The amount of solute contained in the sample is determined by UV spectroscopic method. Solubility of the drug substance is expressed in mg/ml. USP suggests according to the solubility study the drugs can be categorized as,

Table No.5.5.1:Categorizing the API according to the solubility study[110]

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1-10
Soluble	10-30
Sparingly soluble	30-100
Slightly soluble	100-1000
Very slightly soluble	1000-10000
Practically insoluble or insoluble	Greater than or equal to 10000

5.5.3. Particle size and distribution[110]

Theparticle size of the drugs was determined by Malvern particle sizer. The basic theory of particle size distribution is laser diffraction. Equipment specifications and the parameters kept constant during the study is given below,

Equipment	:	Malvern Master Sizer 2000 equipped with Vacuum unit and Air compressor
Mode	:	Dry Powder
Dry Powder Feeder	:	SCIROCCO 2000
Lens	:	Auto lens
Size range	:	0.02 – 2000 µm
Beam Length	:	10 mm
Software	:	Malvern Master Sizer 2000

Instrument Parameters:

Vibration Feed Rate	:	50% (or adjust if necessary)
Dispersion air pressure	:	1.5 bar
Particle RI	:	1.5
Dispersant RI	:	1.0
Measurement Time	:	6 Seconds
Measurement Snaps	:	6000
Background Time	:	6 Seconds
Background Snaps	:	6000
Measurement	:	Default

Procedure:

Take about 10 gm of the test sample into dry powder feeder. Bulk samples of the material were scoop sampled and placed into the vibratory hopper of the scirocco dry dispersion unit and consecutive repeat measurements undertaken in order to assess the reproducibility of measurement which is a function of the homogeneity of the material (or otherwise). The mass flow was adjusted until a stable and correct particle concentration was achieved at 4-bar and then left

constant for the remainder of the experiments. Enter the above parameters. Measure the particle size of the sample and report the result. Determine the particle size as an average of 3 replicate measurements and report the average result. Report the average of the measurement of each volume distribution in μm.

5.5.4. Density [110-115]

I. Bulk Density

An accurately weighed quantity of powder, which was previously passed through mesh size 40 carefully poured into graduated cylinder. The powder bed was made uniform without disturbing. The volume was measured directly from the graduation marks on the cylinder. The volume measure was called as the bulk volume and the bulk density is calculated by following formula;

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Bulk volume}}$$

II. Tapped Density

After measuring the bulk volume the same measuring cylinder was set into tap density apparatus. The tap density apparatus was set to 250 taps per minute and operated for 500 taps. Volume was noted as (V_a) and again operated for 750 taps and volume was noted as (V_b). If the difference between V_a and V_b not greater than 2% then V_b is consider as final tapped volume. The tapped density is calculated by the following formula;

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume}}$$

5.5.5. Carr's index [compressibility index]

It is one of the most important parameter to characterize the nature of powders and granules. It can be calculated from the following equation;

$$\text{Carr's index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Table No. 5.5.2: Flow property scale

Flow property	C.I (%)	Hausner ratio
Excellent	≤10	1.00 – 1.11
Good	11 – 15	1.12 – 1.18
Fair	16 – 20	1.19 – 1.25
Passable	21 – 25	1.26 – 1.34
Poor	26 – 31	1.35 – 1.45
Very poor	32 – 37	1.46 – 1.59
Very, very poor	>38	>1.60

5.5.6. Hausner's ratio

Hausner's ratio is an important character to determine the flow property of powder and granules. This can be calculated by the following formula;

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Value < 1.25 indicate good flow (=20% Carr)

While > 1.50 indicate poor flow (=35% Carr)

5.5.7. Angle of repose

It is defined as the angle between the free surfaces of a pile of powder to the horizontal plane. It was measured using static angle response method (fixed height cone). The relationship between angle of repose and type of flow is shown in Table No.5.5.3.

Table No. 5.5.3: Flow property scale in terms of angle of repose

Flow property	Angle of repose (degrees)
Excellent	25 – 30
Good	31 – 35
Fair-aid not needed	36 – 40
Passable – may hang up	41 – 45
Poor – must agitate, vibrate	46 – 55
Very poor	56 – 65
Very, very poor	>66

5.5.8. Drug excipients interaction study

Study of drug–excipient compatibility is an important process in the development of a stable dosage form, as incompatibility between drug and excipients can alter the stability and bioavailability thereby, affecting its safety and/or efficacy.

Procedure

5 mg of drug (1: 1) with excipient, to maximize the like hood of observing an interaction is taken. Mixture should be examined under N_2 to eliminate oxidative and pyrrolytic effects at heating.Around 1-1.5 mg of each of these samples was weighed in a sample pan and subjected to programmed heating. Differential scanning calorimetric analysis was performed on Metler Toledo. The temperature calibration was performed using indium as the standard. Samples were crimped in a standard aluminum pan and heated from 50 to 270 °C at a heating rate of 5 °C/min under constant purging of dry nitrogen at 50 ml/min. An empty pan, sealed in the same manner as the samples, was used as reference.

Table No.5.5.4: Instrumental conditions

Instrument	DSC 8000
Temperature program	Heat from 30oC to 180^{o}C at rate of 10^{o}C/min
Sample weight	~1.5 mg
Purge gas	30ml/min
Sample pan	Standard aluminium crucible

5.6. PRODUCT DEVELOPMENT AND OPTIMIZATION OF PUSH PULL OSMOTIC TABLETS OF THE SELECTED DRUGS

5.6.1. Dose calculation[116 -119]

Amount of drug to be incorporated in the push pull OTs was done with the help of the Robinson- Erickson equation if no marketed extended release formulation of the selected drugs was available. If XR marketed formulations of the selected drugs were available it was calculated after studying the labeled claim and the molecular weight of the active moiety. The Robinson – Eriksenequation for amount to be incorporated in a SR/CR product is given in Table No. 5.6.1.

Table No. 5.6.1: Robinson - Eriksen equation for the dose calculation

Sl No	Parameter	Equation	Terms used	units
1	Elimination rate constant(K_{el})	0.693/t ½	t ½ = biological half life	h^{-1}
2	Zero-order release rate (K_0)	$C_p.V_d.K_{el}$	C_p = Peak plasma concentration or steady state plasma concentration C_{ss} V_d = Volume of distribution K_{el} = Elimination rate constant	mg/h
3	Initial dose (D_b)	$C_p.V_d.1/F$	F = Absolute Bio availability	mg
4	Corrected initial dose (D_i)	$D_b - (T_p.K_0)$	D_b = Initial dose T_p = Time to peach peak plasma concentration (C_{max}) K_0 = Zero-order release rate	mg
5	Maintenance dose (D_m)	$K_0.T$	T = the number of hours up to which the release is desired	mg
6	Total dose (W)	$D_i + D_m$	D_i = Corrected initial dose D_m = Maintenance dose	mg

5.6.2. Screening of the factors affecting release profile of the drug from push pull osmotic tablets

While formulating any pharmaceutical preparation it is essential to study the different parameters/ factors (process or product) affecting response/ effect of the dosage form. This will give the formulator a better chance to make improvement in the preparation at the early stages itself.Fractional factorials are widely used for screening experiments, where we try to identify which factors have a major effect and which factors are not relevant. They are often used in the early stages of a project when the major features of the project are little understood.

5.6.3. Soft ware used

Design-Expert 9.0.0.7 Trial version from stat ease Inc, Minneapolis was used for the study.

5.6.4. Product development, factor influence study and optimization of push–pull osmotic tablets[120-124,41-79]

A fractional factorial design with 8 selected factors **2^{8-4} (1/32 fraction)** with **Resolution IV** was selected for the study. 16 trials with 4 center points were planned for the factor influence study. The selected factors with levels chosen are given in the Table No.5.6.2 and 5.6.3. The responses selected for the study and the weightage given to each response were given in the Table No.5.6.4.The design matrix for the factor influence study in the coded terms is given in the Table No.5.6.5 .

Table No.5.6.2: Ingredients used for the formulation of push pull OT of the selected drugs

Ingredients	Range selected	Function of the excipients
PULL LAYER		
API	Ropinirole HCl /Ivabradine HCl/ Nisoldipine/Carvedilol phosphate	
PEO 400 K(WSR N)	10 – 100% of API (%w/w)	Suspending agent
Sodium chloride (NaCl)	1-10% of total core weight (w/w)	Osmotic agent
SLS	1 – 5% of the drug layer (w/w)	Wicking/ Solubilizing agent
BHT (butylated Hydroxy toluene)	0.1% of the PEO drug later(w/w)	Anti oxidant
Dicalcium phosphate	q s	Diluents
Magnesium stearate	1% of the drug layer (w/w)	lubricant
PUSH LAYER		
PEO 7000K(WSR 303)	5-50% of the drug layer(w/w)	Extending polymer
Sodium chloride(NaCl)	5- 50 % of the extender (w/w)	Osmotic agent
SLS	1 – 5% of the push layer	Wicking /Solubilising agent
BHT	0.1% of PEO Push layer (w/w)	
Dicalcium phosphate	qs	Diluents
Magnesium stearate	1% of the push layer	Lubricant
Ferric oxide red	0.1% of the push layer	Colouring agent
COATING		
Cellulose acetate(CA-398-10NF)	According to the weight gain	Semi Permeable Membrane
Weight gain	10 -20 % of the tablet core weight	
Propylene Glycol	1 -10% of polymer	Flux regulator/plasticizer

Table No.5.6.3: Selected factors with levels for the factor influence study

Factors		Levels		
		Min (-1)	Max (+1)	Central points(0)
1	PEO in the drug layer (% w/w of the API)	10	100	50
2	NaCl in drug layer(% w/w of the total core weight)	1	10	5.5
3	SLS in the drug layer (%w/w of the drug layer)	1	5	3
4	PEO in the push layer (% w/w of the drug layer)	5	50	27.5
5	Sodium chloride in the push layer (% w/w of the extender)	5	50	27.5
6	SLS in the push layer(%w/w of the push layer)	1	5	3
7	Propylene Glycol (% w/w the polymer)	1	10	5.5
8	Weight gain (%)	10	20	15

Table No. 5.6.4: Response selected for the factor influence study

Response	Unit	Weightage
Cumulative release at 24 Hrs	%	+++++
R^2	----	++++
Lag time	Hrs	+++

Table No.5.6.5: Design matrix of the factor influence study(coded terms)

Std order	PEO DL	NaCl DL	SLS DL	PEO PL	NaCl PL	SLS PL	PG	Wt Gain
1	-1	-1	-1	-1	-1	-1	-1	-1
2	1	-1	-1	-1	1	-1	1	1
3	-1	1	-1	-1	1	1	-1	1
4	1	1	-1	-1	-1	1	1	-1
5	-1	-1	1	-1	1	1	1	-1
6	1	-1	1	-1	-1	1	-1	1
7	-1	1	1	-1	-1	-1	1	1
8	1	1	1	-1	1	-1	-1	-1
9	-1	-1	-1	1	-1	1	1	1
10	1	-1	-1	1	1	1	-1	-1
11	-1	1	-1	1	1	-1	1	-1
12	1	1	-1	1	-1	-1	-1	1
13	-1	-1	1	1	1	-1	-1	1
14	1	-1	1	1	-1	-1	1	-1
15	-1	1	1	1	-1	1	-1	-1
16	1	1	1	1	1	1	1	1
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0

I. Formulation of push pull osmotic tablets

The common processes for the formulation of push pull osmotic tablets were show in the flow chart given below,

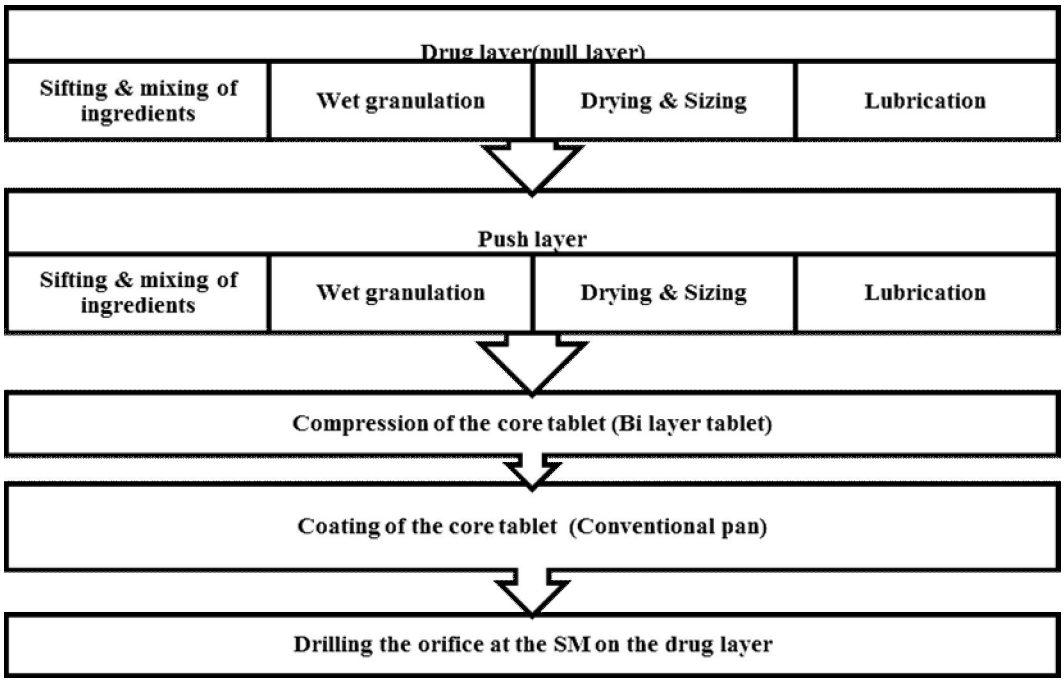


Figure No.5.6.1: Schematic flow chart for the formulation of push pull osmotic tablets

A. Preparation of granules of push pull osmotic tablets [55, 56, 58, 62, 64-67, 72-79, 112-115]

i) Preparation of drug layer

All the ingredients of the drug layer were weighed accurately and individually passed through mesh number 40 sieve. The ingredients except magnesium stearate were mixed in geometrical manner in a poly bag. Dough was prepared by adding sufficient quantity of isopropyl alcohol. The wet mass was passed through mesh number 10 sieve to obtain the granules. Granules were then dried in tray drier at 40˚c for 1 hour and passed through 20 mesh sieve. 10%fines were taken and lubricated with magnesium stearate. This mixture was then added to the granules.

ii) Preparation of push layer

All the ingredients of the drug layer were weighed accurately and individually passed through 40 mesh sieve. The ingredients except magnesium stearate were mixed in geometrical manner in a poly bag. Dough was prepared by adding sufficient quantity of isopropyl alcohol. The wet mass was passed through 10 mesh sieve to obtain the granules. Granules were then dried in tray drier at 40˚c for 1 hour and passed through 20 mesh sieve. 10%fines were taken and lubricated with magnesium stearate in a poly bag. Ferric oxide (Dye) was also added in the mixture.

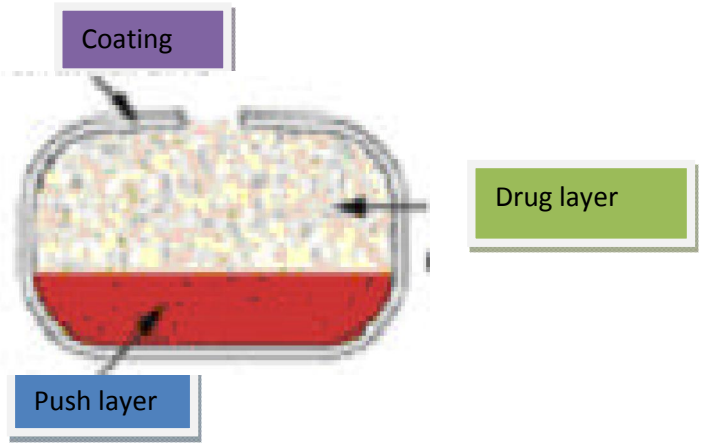


Figure No. 5.6.2: Components of push pull osmotic tablets

B. Compression of blend for making push pull osmotic tablets

The prepared granules of both the pull and push layer were weighed separately in sachets. Push layer was compressed first using rotary tablet compression machine and a thin tablet was made. Then drug layer was added by setting the dye cavity. A final sharp compression was carried out. By this bilayer tablet was made. Hardness was adjusted while compressing the granules (3.5-5 kg/cm^2). 5 mm round normal biconcave punch was used for the compression of the core bilayer OTs of the selected drugs.

C. Coating of the core tablets

i) Method of preparation of polymeric coating solution

Accurately weighed quantity of Propylene Glycol was added to 10 ml of water. 90 ml acetone was added slowly with stirring. Cellulose acetate was added with stirring and completely dissolved it. The compositions of used coating solution along with quantity are listed in Table No.5.6.6.

Table No. 5.6.6: Composition of coating solution

Ingredients	Quantity
Cellulose acetate	According to the formula
Propylene Glycol	According to the formula
Water : Acetone	10 : 90

ii) Coating of core tablet

The prepared bi layer tablets were then coated with prepared coating solution. Coating of core tablet was done by conventional coating method in coating pan by maintaining the parameters given in the Table No. 5.6.7 constant. The manual coating procedure was used based on intermittent spraying and drying techniques. 10 tablets were removed at an interval of 30min and increase of weight was noted down until it was observed sufficient %wt gain. Coated tablets were allowed to dry completely in a hot air oven at 60^0C to remove the residual solvent and finished by standard polishing procedure.

Table No. 5.6.7: Parameters maintained during coating process

Parameters	Value
Batch size	100 tablets
Pan diameter	18 cm
Pan rotating speed	32 rpm
Inlet air temperature	60˚c
Spray pressure	50 -60 mm hg
Spray rate	1 ml/min
Nozzle diameter	1 mm
Distance between tablet bed and spray gun	12 -14 cm

D. Drilling of coated tablets

The drug delivery orifice having diameter of 0.6 mm was made on the surface of one side of the tablets using Micro drill (Cameron, Canada). High speed stainless steel drill bits were used for drilling.

II. Evaluation of formulations

A. Blend evaluation

The tapped density, bulk density, carr's index and hausner's ratio was determined for granules prepared for both drug and push layer. The procedure was given the pre formulation study section 5.5.4 to 5.5.7.

B. Tablet evaluation

i) Diameter

The diameter of the tablets was determined using a digital vernier calliper. Five tablets from each of the formulation were used and average values were calculated.

ii) Thickness

The thickness of the tablets was determined using a digital vernier calliper. Five tablets from each type of formulation were used and average values were calculated.

iii) Weight variation

To find out weight variation, 20 tablets of each of the formulation were weighed individually using an electronic balance, average weight was calculated and individual tablet weight was then compared with average value to find the deviation in weight from average weight. The specifications for tablets to pass the weight variation test as per pharmacopoeia of India are mentioned in Table No.5.6.8.

Table No. 5.6.8: Limits of weight variation test

IP/BP	Limit	USP
80 mg or less	10%	130mg or less
More than 80mg or Less than 250mg	7.5%	130mg to 324mg
250mg or more	5%	More than 324mg

iv) Friability

20 tablets were weighed accurately and placed in roche's friabilator. Friability was evaluated as the percentage weight loss of 20 tablets tumbled in a friabilator for 4 min at 25 rpm. The tablets were then de-dusted and the loss in weight caused by fracture or abrasion was recorded as the percentage friability. Friability range as per IP is not more that 2% of average weight of tablet.

v) Hardness

It was measured by Pfizer hardness tester. The tablet was held along its oblong axis in between the two jaws of the tester. At this point, reading should be zero kg/cm^2. Then constant force was applied by pressing the arms until the tablet fractured. The value at this point was noted in kg/cm^2

vi) Orifice diameter[125]

The orifice diameter was determined by optical microscopy under 40 X. A calibrated eye piece micro meter was used for the study. Five tablets were placed individually on the glass slide under the eye piece of the microscope and the number of divisions covering the orifice was noted. The number of division was multiplied with the standard value of the eye piece micrometer and the diameter was recorded.

vii)* Drug *content

One core osmotic tablet (without coating) was crushed in mortar and pestle and added in 100ml volumetric flask. For water insoluble drugs 10-15 ml of ethanol was added to dissolve the drug. Volumetric flask was made up to 100 ml with distilled water. It was shaken for 15 minutes. Filter the solution if necessary. From that 1 ml was taken out and diluted up to 10 ml with distilled water in 10 ml

volumetric flask and its absorbance was measured using UV spectrophotometer at the corresponding λ_{max} of the drugs. Further calculate % of label claim present using following formula. The test was performed in triplicate and reported the results.

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/tablet)} \times 100}{\text{Label claim (mg/tablet)}}$$

viii) Percentage weight gain

Percentage weight gain was determined to find out the weight gain during coating**.** Randomly selected 10 sample core tablets were weighed before coating and the weight was denoted as W_o. The tablets were subjected to coating as per the method specified in the section 5.6.4(IC). At specified intervals the weights of coated tablets were recorded which is denoted as W_t. The % wt gain of tablet coat was calculated using following formula.

$$\% \text{ weight gain} = (W_t - W_o)/W_o * 100$$

ix) In vitro dissolution study

Dissolution test was performed using an USP II paddle apparatus at 37˚C± 0.5˚C in 900 ml of pH 6.8phosphate buffer. Paddle speed was kept at 50 rpm.1-10% of SLS was used in the dissolution media while analysing Carvedilol phosphate and Nisoldipine formulations. Samples were withdrawn after predetermined time intervals of 1, 2,3,4,6,8,12,16,20,24 hrs. The drug content was measured using an UV spectrophotometer at the corresponding λmax. Samples were suitably diluted and absorbance was measured. Six tablets were tested and the average absorbance was reported. Dissolution profile of each trial was constructed by taking time (time in hrs in X axis and percentage cumulative release at Y axis. The regression analysis was performed to find out the best fit of the curves and R^2 values were recorded.

C. Coating film evaluation[126-130]

The measurement of mechanical properties gives an indication of the

strength and elasticity of the film. Here the film of cellulose acetate with plasticizer was evaluated. The free films were prepared by film casting method (8.5 ml in a petri dish of diameter 7.3 cm). The cellulose acetate films were prepared using solvent evaporation method. The composition is shown in Table No.5.6.9. Propylene glycol and water were mixed together. Acetone was added to the mixture with stirring. Cellulose acetate was then added gradually under stirring. Stirring was continued for another 2 hrs to dissolve the cellulose acetate completely. Add the remaining acetone and stir for 30min - 1hrs. Degas the solution for 3 hrs. Polymeric coating solution was poured into plastic petri dishes. Petri dishes with polymeric coating solution were then left overnight (18 hr) for air drying. The film properties like folding endurance, appearance were evaluated on the next day. The effect of plasticizer on the elastic property of the films was reported.

TableNo.5.6.9: Composition for making film

SL No	Ingredients	Quantity (mg)					
		T1	T2	T3	T4	T5	T6
	Cellulose acetate	9.4	8.6	18.8	17.3	13.5	19
	Acetone: Water	90:10	90:10	90:10	90:10	90:10	90:10
	Propylene Glycol	0.09	0.86	0.19	1.73	0.74	0

i) Appearance

It was observed by visual inspection.

ii) Folding endurance

A film strip of 2 cm X 2 cm was repeatedly folded and unfolded at the same place till it breaks. The number of times, the film could be folded at the same place, without breaking was recorded as the value of folding endurance.

III. Factor influence study of the formulations

After completion of the evaluation of each trial the selected responses

were analyzed with the help of design expert software. The statistical data obtained were studied thoroughly. The ANOVA data was studied to check the model validity and significant factors. Model suitability was checked by regression analysis. A suitable model will be having significant model terms and non significant lack of fit and curvature. If the curvature and lack of fit in the model were not significant, the 2 level designs can be used for optimization.

A. FDS curve and standard error graph evaluation

Before starting the experiment the FDS curve and the standard error distribution of the proposed design should be studied. The FDS (Fraction of design space) graph is a line graph showing the relationship between the "volume" of the design space (area of interest) and amount of prediction error. The curve indicates what fraction (percentage) of the design space has a given prediction error or lower. In general, a lower and flatter FDS curve is better. Lower is more important than flatter. A lower curve translates to a higher Fraction of Design space - more of the design has useful precision.

B. StdErr of Design (Standard Error) Graph

It is the contour plots showing the standard error of prediction for areas in the design space. These values are reflective of the design only, not of the response data. Generally, these graphs should have relatively low (less than 1) standard error across the region of interest.

C. Analysis of responses

PCUR at 24 hrs, $R^2$and lag time values determined for the trials were analysed with the help of design expert software.

i) Half Normal plot

The half-normal plot is used to select the factors producing significant effects. Larger effects (absolute values) will appear in the upper-right section of the plot. When the selection of statistically significant terms is complete, the p-

value should above 0.10 to indicate there is no significant deviation from the assumption of normality for the non-selected factors.This can be done with the help of Shapiro-Wilk Normality Test.

ii) Normal plot

For 2-level factorial designs, this plot can be used to choose significant effects. A plot of the ordered values of a sample versus the expected ordered values from the true population will be approximately a straight line.

iii) Pareto chart

Pareto chart is a bar graph for the clear identification of the significant factors. Two different colors are used for the identification of significant as well as non significant effects. The blue color indicates the negative effect and the orange color indicates the positive effect of the factors on the selected responses. t value and the bonferroni limit were used for the identification of the significant factors.

iv) ANOVA and regression analysis

The results obtained for the study design is analysised with the help of design expert software and significance of factors were found out by ANOVA analysis. The hypothesis were tested with a level of significance 5 % ($p < 0.05$). From the ANOVA analysis significant factors were identified. Other statistical parameters like lack of fit, R^2, R^2_{adj},$R^2_{Predicted,}$ Adequate precision, PRESS were also estimated.

v) Polynomial equation

From the regression analysis of the responses, the mathematical equation can be constructed which can be used for the prediction of the responses at any selected levels of the factors. If the suggested model for the optimization is linear, the following linear model would be used,

$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 .....+ \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 +$ error

vi) Test for the assumptions of ANOVA

The normal probability plot, residuals*vs.* predicted, residuals *vs*. run and

predicted *vs.* actual were studied for testing the assumptions of ANOVA.

❖ **Normal probability test**

The normal probability plot indicates whether the residuals follow a normal distribution, in which case the points will follow a straight line. Expect some moderate scatter even with normal data. Look only for definite patterns like an "S-shaped" curve, which indicates that a transformation of the response may provide a better analysis.

❖ **Residuals vs. Predicted**

This is a plot of the residuals versus the ascending predicted response values. It tests the assumption of constant variance. The plot should be a random scatter (constant range of residuals across the graph).

❖ **Residuals vs Run**

This is a plot of the residuals versus the experimental run order. It allows checking for lurking variables that may have influenced the response during the experiment. The plot should show a random scatter. Trends indicate a time-related variable lurking in the background.

❖ **Predicted vs Actual**

A graph of the observed (actual) response values versus the predicted response values. It helps to detect a value, or group of values, that are not easily predicted by the model. The data points should be split evenly by the 45 degree line. If they are not, a transformation to improve the fit should be tried.

❖ **Box cox plot**

This plot provides a guideline for selecting the correct power law transformation. A recommended transformation is listed, based on the best lambda value, which is found at the minimum point of the curve generated by the natural log of the sum of squares of the residuals.

vii) The perturbation graph

This graph shows the effect of all the factors in a single display. The magnitude and the sign of the effect can well understand from the graph.

viii) Interaction Graph

An interaction occurs when the response is different depending on the settings of two factors. Plots make it easy to interpret two factor interactions. If they appears with two non-parallel lines, indicating that the effect of one factor depends on the level of the other. The "I beam" range symbols on the interaction plots are the result of least significant difference (LSD) calculations. If the plotted points fall outside the range, the differences are unlikely to be caused by error alone and can be attributed to the factor effects. If the "I beams" overlap there is not a significant difference (95% confidence is default) between the two points.

ix) Contour plots and response surfaces plots

Contour plot is a 2D graphical representation of the effect of less than 3 factors on a single response. Response surface plots are the 3D version of the contour plot. A better understanding of influence of factors on the responses will be possible with the response surface plots.

x) Cube plot

This plot will represent the effect of 3 factors at a time on a selected response. A better understanding of the effect of factors on the responses would be possible with this graph.

IV. Numerical optimization with the help of desirability

When there are only 2-3 process variables a relatively straight forward approach can be adopted to optimize several responses by overlaying the contour plots for each response. When there are more than 3 variables overlaying contour plots become awkward, because the contour plot is two dimensional and k-2 of the design variables must be held constant to construct the graph. Therefore there is practical interest in more formal optimization methods for multiple responses.

A. Optimization of the push pull osmotic tablets of the selected drugs

When more than two factors were significant, overlay plot does not give a complete idea about the optimization. But it can be done with the help of desirability function. It is a simple mathematical method to find the optimum

formulation. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function.

B. Point prediction

Point Prediction allows to enter levels for each factor or component into the current model. The L% CI (confidence interval) is the range in which one can expect the process average to fall into L% of the time. The L% PI (prediction interval) is the range in which one can expect the next outcome at the current setting to fall into L% of the time. The proportion L% TI (tolerance interval) is the range in which one can expect P% of all population outcomes to occur given L% confidence estimating the true mean and standard deviation of the population.

C. Check point batch

In order to check the model validity, any three optimum solution batch suggested by the software were practically prepared and evaluated for the selected responses**.** The confidence interval/prediction interval was used to assess the outcomes. If the outcome is within the confidence / prediction interval the model validity is assured.

D. Optimized batch

The optimized batch from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. It is not essential that the desirability should be 1.

E. Desirability contour plot and RS plot

They are the graphical representation of change in factors on the desirability function. It will be giving a better visualization of achieving the optimum condition by changing two factors at a time. Desirability plots shows

how all the targeted optimum conditions are met by changing two factors at a time.

5.6.5. Stability study[131-134]

The stability study was carried out for selected optimized formulations as per ICH guidelines. ICH storage conditions used are $25^0C \pm 2^0C$ (60% ± 5%RH), $30^0C \pm 2^0C$ (65% ± 5%RH) and $40^0C \pm 2^0C$ (75% ± 5%RH). The tablets of the best formulation were placed in screw capped, high density polyethylene bottles and stored at various ICH storage conditions for a period of 6 months. The samples were analyzed for physical appearance, In-vitro dissolution, and assay at regular interval. The storage conditions and the time period of the stability study is given below,

- 25^0C / 60%RH – 1M , 2 M & 3 M
- 30^0C / 65%RH – 3 M & 6 M
- 40^0C / 75%RH - 3 M & 6 M

5.7 *INVIVO* ANIMAL STUDIES OF THE OPTIMIZED FORMULATIONS

5.7.1. Standard calibration curves[135-139]

Simple, accurate, precise and sensitive high-performance liquid chromatographic (HPLC) method was used for quantification of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in rabbit plasma samples from individual bioavailability study.

The working standard solutions were prepared by diluting the stock solution with the corresponding mobile phase. Well stored (poly propylene tubes at -20 ^{0}C) drug free plasma samples (Rabbit Plasma) were spiked after precipitating the plasma proteins with desired concentration of drug and the internal standard (IS) prepared from the stock solution. Vortex the spiked samples for 20 sec and equilibrated for 10 min before analysis. The conditions and system parameters selected for the plasma drug analysis using RP-HPLC was given in the Table No.5.8.1. Calibration curves were constructed by plotting the ratio of S/IS (peak area of the drug Vs peak area of the internal standard) area versus concentrations. Retention time (min) and validation parameters like, linearity, range, correlation coefficient, slope, intercept, LOD and LOQ were determined.

5.7.2. Bio availability studies of the optimized formulations[141-154]

6 healthy Rabbits (New Zealand, White) of either sex weighing 3.0 - 3.5 kg divided in to two groups were selected for each study. A total 24 animals were used.The institutional animal ethical committee of RVS college of pharmaceutical sciences, Sulur approved the study protocol.

I. Administration of the prepared optimized formulation to the animals [96,148,149.153]

For each drugs, the animals were divided into 2 groups each consisting of 3 animals. First group received reference product

- Ropinirole HCl - ROPARK-XL:SUN Pharmaceuticals;India,

- Ivabradine HCl - Ivabrad: Lupin Pharmaceuticals;India,
- Carvedilol phosphate - Cardivas-CR: Sun Pharmaceuticals;India
- Nisoldipine – Sular; Shionogi Inc;USA
- and second group received the optimized formulation of the selected drugs.

II. Collection of blood samples[148-154]

The samples are collected from the marginal ear vein of the rabbit at an interval of 0,1,2,3,4,5,6,7,8,9,10,12,16,24,36 and 48hrs.Ear was cleaned with 95% v/v alcohol and local anesthetic cream was applied on the collection site10 min prior to sampling. (If required, the o-xylene a topical vasodilator may be applied topically on the collection site to dilate blood vessels). A 26G needle was used for collecting blood from animal marginal vein. Clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding.Each sample was separately collected in to purple top EDTA tubes.

III. Separation of plasma from the blood samples[142, 150-154]

The samples in the tubes were centrifuged in a refrigerated centrifuge (Lab bench top centrifuge micro centrifuge 5407 +Rotor) with an rpm 5000 at 4 ^{0}C for 20 minutes. The supernatant liquid separated (plasma) was immediately transferred in to poly propylene tube with the help of a Pasteur pipette.

IV. Extraction of drug from the plasma

A. Step -1: Protein precipitation from the plasma

1ml of plasma was mixed with 2 ml acetone in micro poly propylene tube. Centrifuge the plasma using micro centrifuge (5000 rpm for 7min). Supernatant was transferred in to a conical tube and diluter with aqueous buffer/water.

B. Step -2: Extraction of drugs from the plasma

i) Extraction of Ropinirole HCl

Condition a low displacement C_{18} SPE cartridge 3ml (Baker) using 3 column volume of methyl alcohol (MeOH) and 3 column volume of water. 1ml

plasma was injected to the solid phase extraction cartridge. Wash the column with 10 ml of water and 10 ml of acetonitrile (MeCN). After the washing process the drug fraction was eluted with 3.5 ml MeCN: water: Ammonia (100:2:0.5).The elute obtained was evaporated to dryness under stream of nitrogen at 35^{o}C. Reconstitute the residue with 300 µL mobile Phase (MeCN: 70 mM pH 3.8 ammonium formate buffer 25: 75). The mobile phase also contains 0.3 % ethylene diamine tetra acetic acid (EDTA) and 0.005% sodium octyl sulphate.

ii) Extraction of Ivbradine HCl

The plasma samples (1ml) were alkalinized by adding 250 µl of buffer solution at pH 13 (0.2 M NaOH - 0.2 M KCl– H_2O, 66:25:9, v/v/v).After vortexing, the sampleswereextracted using solid-phase extraction on anASPEC system (Gilson, Villiers-Le-Bel, France). The 100 mg/ ml cyano cartridges (Baker, Noisy-le-Sec waters) were conditioned with 2 ml ofacetonitrile followed by 3 ml of purified water. Thesample was then applied to thepreconditioned cartridge, washed with 3 ml of purified water and eluted with 3 ml of acetonitrile. The eluent was evaporated to dryness under a nitrogen stream at 37^{o}C. The residue was dissolved in 300 µl of 0.01 *M* HCl, vortexed for 1 min, transferred into a vial and at least 30 µl were injected into the chromatographic system.

iii) Extraction of Carvedilol phosphate

To a 15 ml conical tube add 1ml of 250 mM sodium dihydrogen phosphate buffer. The mixture of disperse (acetone containing the supernatant obtained from the step 1)/ extraction solvent ($CHCl_3$) in the ratio of 500/100 µL was injected very quickly and vigorously. A cloudy emulsion was formed in the tube. The emulsion was centrifuged in micro centrifuge at 2500 rpm for 5 min.

The extraction solvent collected at the bottom of the tube was transferred in to a micro tube and the supernatant portion was discarded. The extraction solvent was evaporated at 60 ^{0}C in an oven. The residue was dissolved in the 50 µL of mobile phase.

iv) Extraction of Nisoldipine

200 µL of NH_4OH was added gradually to 1 ml plasma with gentle shaking. 5ml of ternary butyl methyl ether was added to it. The mixture was centrifuged at 1600 x g for 10 min. The supernatant layer was evaporated to dryness with vacuum aspirator at 50 ^{0}C. The residue was dissolved in the mobile phase (60 µL). 20 µL was then injected to the HPLC.

Table No.5.7.1:HPLC variable kept constant during the calibration curve

Parameters	**Ropinirole HCl** [98,140]	**Ivabradine HCl** [92,141]	**Carvedilol phosphate** [99,146]	**Nisoldipine** [97]
HPLC variables				
Column	Phenomenex C18 column (250 mm × 4.6 mm id, 5 µm particle size)	Nova-Pak C8 (150 x 4.6 mm i.d, 4 µm particle size, Waters).	C 18 ODS -3(250 X 4.6 mm, 5 µm particle size)	C 18 column (30cm x3.9 mm i.d(Bondapak, Waters)
Mobile Phase	MeCN : 70 mM pH 3.8 ammonium formate buffer (25: 75)	Acetonitrile: 0.025 M Potassium dihydrogen Phosphate (containing 0.3% v/v 1 M HCl) 22:78	15 mM Sodium hydrogen phosphate buffer pH 4.0/ Acetonitrile/2-Propanol (70/27.5/2.5,v/v/v)	Methanol-KH_2PO_4 of 15 mM/l (v/v)
Flow rate	Iml/min	1 ml/min	2ml/min	1ml/min
Injection Volume	10 -100 µL	20µL	20µL	20µL
Column tem	25 ^{0}C	25 ^{0}C	25 ^{0}C	22 ^{0}C
Detector used	UV	Fluorescence	UV	UV
Detection λ max/ λ em	250nm	λ_{em} 328 nm after excitation of the analytes at λ_{exc} 283 nm.	254nm	254nm
Internal Standard	4-(2-di-N,N-propylaminoethyl) 7-methoxy-2-(3H)-indoline HCl	S16070	Amitriptyline	Diazepam

V. Analysis of the plasma samples

Each processed plasma samples taken at an interval of 0,1,2,3,4,5,6,7,8,9,10,12,16,24,36 and 48 hrs were analyzed with the help of HPLC maintaining the HPLC conditions given in the Table No.5.7.1. The concentration present in each samples were interpreted from the standard calibration curve.

VI. AUC Curve and determination of pharmacokinetic parameters[90-100,154]

AUC plot was drawn by taking the time on X axis and concentration on Y axis. Pharmacokinetic parameters like t_{max}, C_{max}, AUC_{0-t},$AUC_{0-\infty}$,K_{el}, $t_{1/2}$ were determined. The AUC curve and the pharmacokinetic parameters of the test were compared with the reference product. Student's t -test was used to determine the significant difference between the values. The significant level used was 5% (P=0.05).

VII. Bioavailability study protocol No: 1

PROTOCOL NO: IAE 1012/C/10/CPCSEA – 2011-1

Study objective

To study the rate and extent of absorption of optimized push pull osmotic tablets of Ropinirole HCl (12mg)

Study protocol

Study Title	Oral bioavailability study of push pull osmotic tablets of Ropinirole HCl (12mg), in 6 healthy rabbits (New Zealand, White) of either sex weighing 3.0 - 3.5 kg in fasting conditions.
Study Objectives	To study the rate and extent of absorption of optimized push pull osmotic tablets of Ropinirole HCl (12mg).
Study Design	Parallel design.
Sample size	6 healthy rabbits (New Zealand, White) of either sex weighing 3.0 -3.5 kg).
Study treatments	Reference (R) – Marketed XR formulation (12mg) Test (T) - Optimized formulation of push pull osmotic tablets of Ropinirole HCl (12mg).
Introduction	Ropinirole HCl an orally administered non-ergoline dopamine agonist used for the treatment of Idiopathic Parkinson's disease
Dose	A single oral dose of either (R) – Marketed XR formulation (12mg)or test treatment (T) Ropinirole HCl push pull OT (12mg), along with water.
Dietary Plan	Food was withdrawn from the rats 12 hr before drug administration. Until 24 hr post dosing food was not given to the animals. All rats have access to water during the study period.
Sampling Schedules	At defined time intervals blood samples will be collected from marginal ear vein.
Bio analytical Method	Ropinirole HCl will be estimated in plasma using a validated analytical method.
Pharmacokinetic Parameters	t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, k_{el} and $t_{1/2}$ will be determined from the plasma concentration data of Ropinirole HCl.
Ethical Considerations	The study will be carried out as per the ICH- Guidelines.

VIII. Bioavailability study protocol No:2

PROTOCOL NO: IAE 1012/C/10/CPCSEA – 2011-2

Study objective

To study the rate and extent of absorption of optimized push pull osmotic tablets of Ivabradine HCl (10.123 mg)

Study protocol

Study Title	Oral bioavailability study of push pull osmotic tablets of Ivabradine HCl (10.1233mg), in 6 healthy rabbits (New Zealand, White) of either sex weighing 3.0 -3.5 kg under fasting conditions.
Study Objectives	To study the rate and extent of absorption of optimized push pull osmotic tablets of Ivabradine HCl (10.123mg).
Study Design	Parallel design.
Sample size	6 healthy rabbits (New Zealand, White) of either sex weighing 3.0-3.5 kg).
Study treatments	Reference (R) – Marketed conventional formulation (10 mg) Test (T) - Optimized formulation of push pull osmotic tablets of Ivabradine HCl (10.123mg).
Introduction	Ivabradine HCl is a pure heart rate lowering agent used for the symptomatic treatment of chronic stable angina pectoris patients with normal sinus rhythm, commonly used when contraindication or intolerance to beta blockers.
Dose	A single oral dose of either test treatment (R) – Marketed conventional formulation (10 mg) or Test (T) Ivabradine HCl push pull OT(10.123mg), along with water.
Dietary Plan	Food was withdrawn from the rats 10.123 hr before drug administration. Until 24 hr post dosing food was not given to the animals. All rats have access to water during the study period.
Sampling Schedules	At defined time intervals blood samples will be collected from marginal ear vein.
Bio analytical Method	Ivabradine HCl will be estimated in plasma using a validated analytical method.
Pharmacokinetic Parameters	t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, k_{el} and $t_{1/2}$ will be determined from the plasma concentration data of Ivabradine HCl.
Ethical Considerations	The study will be carried out as per the ICH- Guidelines.

IX. Bioavailability study protocol No: 3

PROTOCOL NO: IAE 1012/C/10/CPCSEA – 2011-3

Study objective

To study the rate and extent of absorption of optimized push pull osmotic tablets of Carvedilol phosphate (10mg)

Study Title	Oral bioavailability study of push pull osmotic tablets of Carvedilol phosphate (10mg), in 6 healthy rabbits (New Zealand, White) of either sex weighing 3.0 -3.5 kg under fasting conditions.
Study Objectives	To study the rate and extent of absorption of optimized push pull osmotic tablets of Carvedilol phosphate (10mg).
Study Design	Parallel design.
Sample size	6 healthy rabbits (New Zealand, White) of either sex weighing 3.0- 3.5 kg).
Study treatments	Reference (R) – Marketed XR formulation (10mg) Test (T) - Optimized formulation of push pull osmotic tablets of Carvedilol phosphate (10mg).
Introduction	Carvedilol phosphate is a nonselective β-adrenergic blocking agent with α1-blocking activity.
Dose	A single oral dose of either (R) – Marketed XR formulation (10mg) or test treatment (T) Carvedilol phosphate push pull OT (10mg), along with water.
Dietary Plan	Food was withdrawn from the rats 12 hr before drug administration. Until 24 hr post dosing food was not given to the animals. All rats have access to water during the study period.
Sampling Schedules	At defined time intervals blood samples will be collected from marginal ear vein.
Bio analytical Method	Carvedilol phosphate will be estimated in plasma using a validated analytical method.
Pharmacokinetic Parameters	t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, k_{el} and $t_{1/2}$ will be determined from the plasma concentration data of Carvedilol phosphate
Ethical Considerations	The study will be carried out as per the ICH- Guidelines.

X. Bioavailability study protocol No: 4

PROTOCOL NO: IAE 1012/C/10/CPCSEA – 2011-4

Study objective

To study the rate and extent of absorption of optimized push pull osmotic tablets of Nisoldipine (8.5mg)

Study protocol

Study Title	Oral bioavailability study of push pull osmotic tablets of Nisoldipine (8.5mg), in 6 healthy rabbits (New Zealand, White) of either sex weighing 3.0- 3.5 kg under fasting conditions.
Study Objectives	To study the rate and extent of absorption of optimized push pull osmotic tablets of Nisoldipine(8.5mg)
Study Design	Parallel design
Sample size	6 healthy rabbits (New Zealand, White) of either sex weighing 3.0 -3.5 kg)
Study treatments	Reference (R) – Marketed XR formulation (8.5 mg) Test (T) - Optimized formulation of push pull osmotic tablets of Nisoldipine (8.5mg)
Introduction	Nisoldipine is a calcium channel blocker used for the treatment of Hypertension.
Dose	A single oral dose of either Reference (R) – Marketed XR formulation of 8.5 mg or test treatment (T) push pull OT Nisoldipine (8.5mg), along with water.
Dietary Plan	Food was withdrawn from the rats 12 hr before drug administration. Until 24 hr post dosing food was not given to the animals. All rats have access to water during the study period.
Sampling Schedules	At defined time intervals blood samples will be collected from marginal ear vein.
Bio analytical Method	Nisoldipine will be estimated in plasma using a validated analytical method.
Pharmacokinetic Parameters	t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, k_{el} and $t_{1/2}$ will be determined from the plasma concentration data of Nisoldipine
Ethical Considerations	The study will be carried out as per the ICH- Guidelines.

Chapter -6
RESULT AND ANALYSIS

6. RESULTS AND ANALYSIS

6.1. METHOD DEVELOPMENT FOR THE ANALYSIS OF THE SELECTED DRUGS

6.1.1. Determination of λ_{max} of the selected drugs

λ_{max} of the drugs was determined by the method suggested in the chapter section 5.4.1(I). The solutions (20ppm) were scanned in the UV range 200 – 400 for the determination of the λ_{max}.

Table No.6.1.1: λ_{max} of drugs

Drug	Amount of drug taken(mg)	Solvent	Standard stock solution (µg/ ml)	UV scanning range(nm)	λ_{max} (nm)
Ropinirole HCl	10	6.8 PH	100	200-400	250
Ivabradine HCl	10	6.8 PH	100	200-400	286
Carvedilol phosphate	10	6.8 PH	100	200-400	285.5
Nisoldipine	10	6.8 PH	100	200-400	235.5

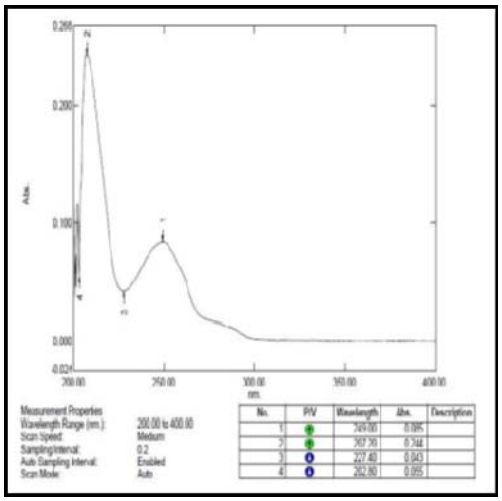
The results of the study were given in the Table No.6.1.1.The UV spectrum of the Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in pH 6.8 was given in the Figure No.6.1.1, 6.1.2, 6.1.3 and 6.1.4 respectively.

6.1.2. Calibration curves of the selected drugs

The standard curves were prepared and plotted as described in methodology chapter 5.4.1(II-III). The λ_{max} of the drugs were shown in the table No.6.1.1. The concentrations prepared and the corresponding absorbance measured was tabulated in Table No.6.1.2. The R^2, Linear regression equation and slope of the calibration curves were shown in the table No.6.1.2. Standard plot of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in phosphate buffer pH 6.8 were shown in Figure No.6.1.5, 6.1.6, 6.1.7 and 6.1.8 respectively.

Table No.6.1.2: Results of the spectrophotometric analysis of selected drugs

Drugs	Dilutions (mcg /ml)	Absorbance (nm)
		PH 6.8
Ropinirole HCl	4	0.129
	8	0.253
	12	0.399
	16	0.532
	20	0.686
	24	0.7935
	28	0.9467
R^2		**0.999**
Y		**0.0341x - 0.0117**
Ivabradine HCl	5	0.082
	10	0.1495
	15	0.219
	20	0.2924
	25	0.359
	30	0.4223
R^2		**0.999**
Y		**= 0.0137x + 0.0137**
Nisoldipine	4	0.1254
	8	0.3183
	12	0.4828
	16	0.666
	20	0.8274
	24	0.9864
R^2		**0.999**
Y		**0.043x - 0.0338**
Carvedilol phosphate	2	0.1548
	4	0.2849
	6	0.4212
	8	0.5418
	10	0.657
	12	0.7956
	14	0.9219
R^2		**0.999**
Y		**0.0635x + 0.0312**



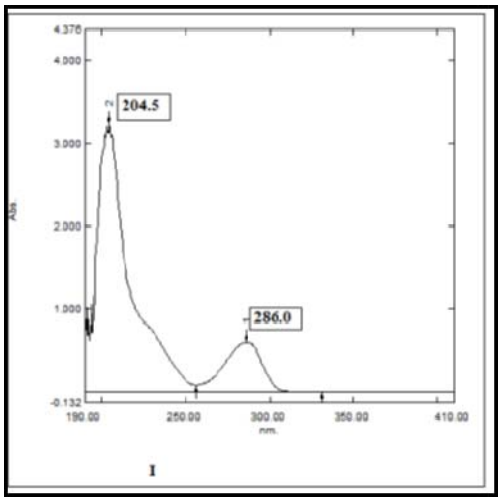
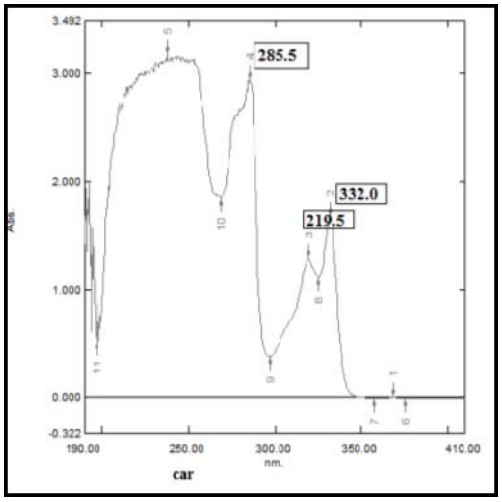


Figure No. 6.1.1: Ropinirole HCl Spectra in pH 6.8 Phosphate buffer
Figure No.6.1.2: Ivabradine HCl Spectra in pH 6.8 Phosphate buffer



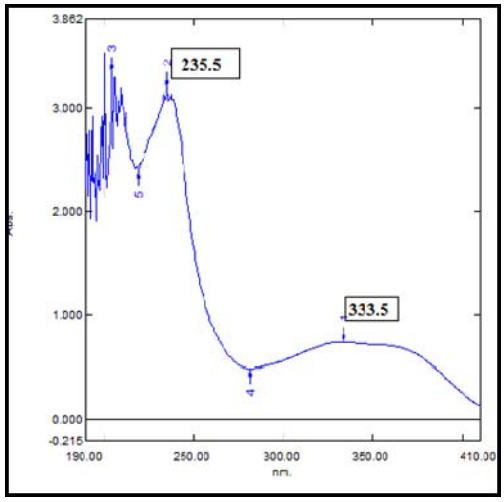


Figure No .6.1.3: Carvedilol Phosphate Spectra in pH 6.8 Phosphate buffer
Figure No.6.1.4: Nisoldipine Spectra in pH 6.8 Phosphate buffer

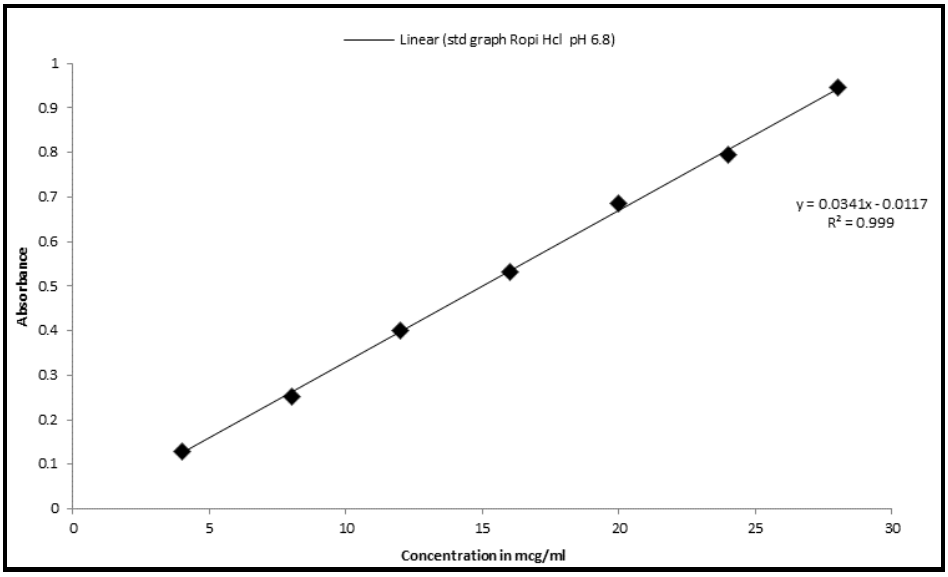


Figure No .6.1.5: Standard graph of Ropinirole HCl in pH 6.8 phoshate buffer solution

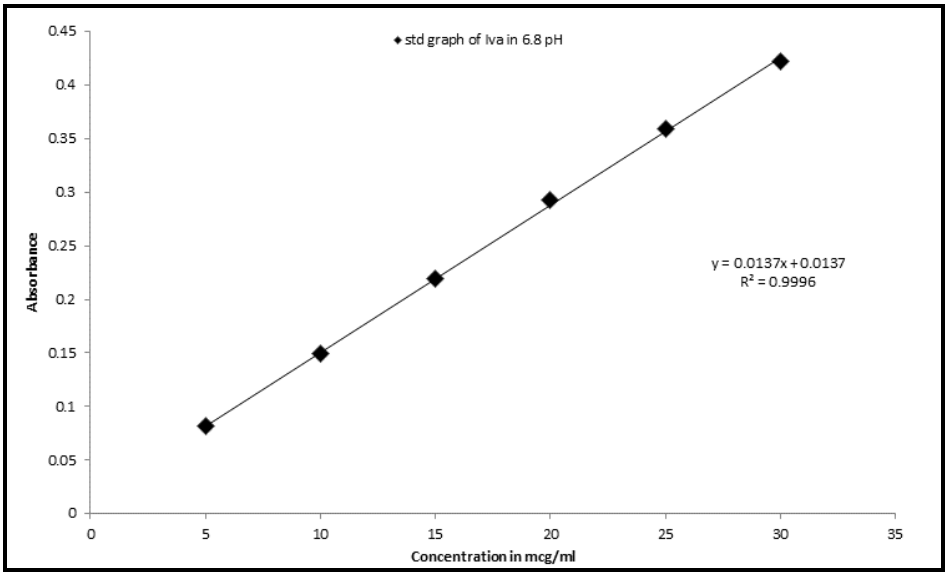


Figure No .6.1.6: Standard graph of Ivabradine HCl in pH 6.8 phosphate buffer solution

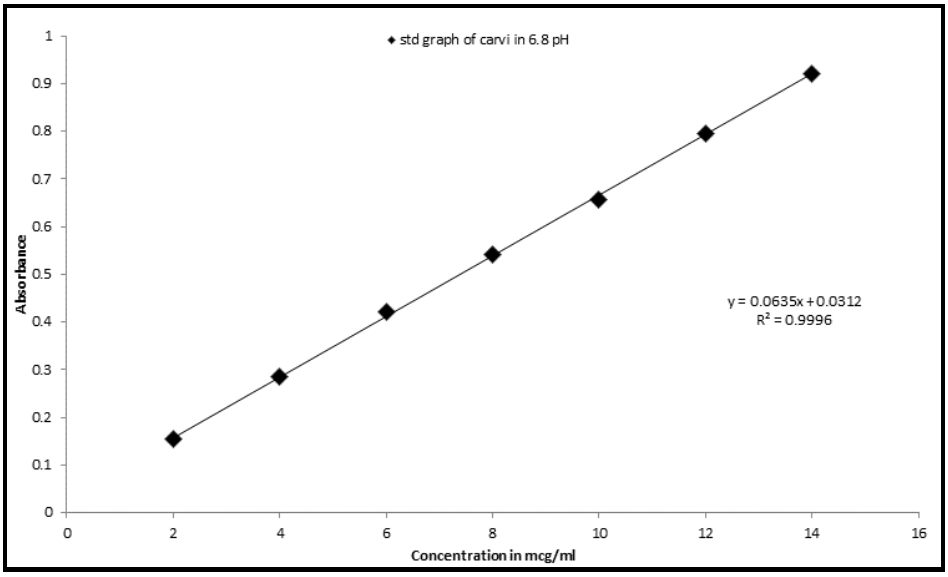


Figure No .6.1.7: Standard graph of Carvedilol Phosphate in pH 6.8 phosphate buffer solution

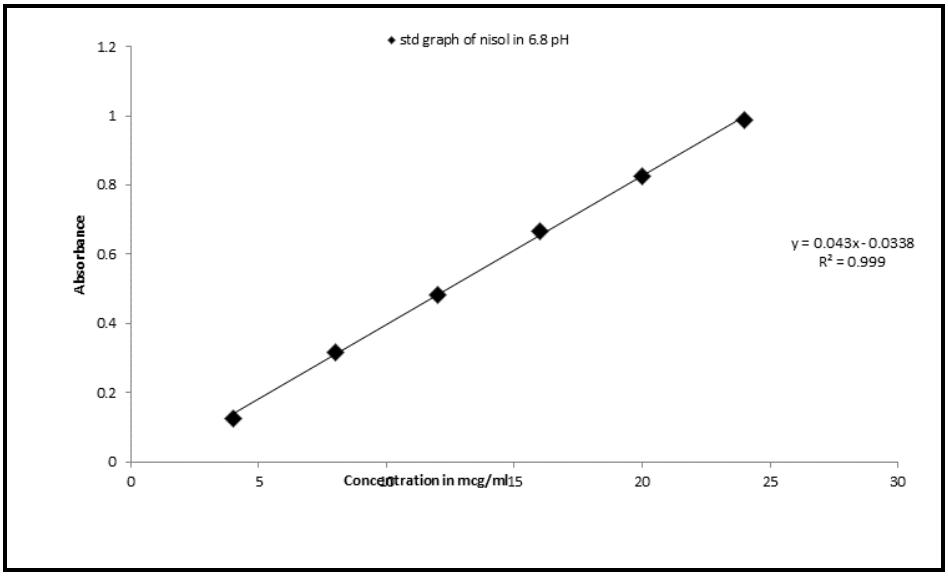


Figure No .6.1.8: Standard graph of Nisoldipine in pH 6.8 phosphate buffer solution

6.2. PREFORMULATION STUDIES

6.2.1 Organoleptic properties

The organoleptic properties of the selected drugs were given in the Table No.6.2.1.

Table No.6.2.1: Organoleptic properties of the selected drugs

Drugs	Colour	State	Odour
Ropinirole HCl	Pale yellow	Amorphous solid	Odourless
Ivabradine HCl	White or whitish	Crystalline powder	Odourless
Carvedilol Phosphate	White to off white	Crystalline powder	Odourless
Nisoldipine	Pale yellow to yellow	Crystalline powder	Odourless

6.2.2. Solubility study

Solubility of the drugs was found out in various solvents. The test was performed according to the methodology given in section 5.5.2. The data was tabulated in Table No.6.2.2.

Table No.6.2.2: Solubility of selected drugs in different pH solutions

Drugs	Solubility in solvents (mg/ml)				
	Water	0.1 N HCl	PH 4.5	PH 6.8	PH 7.4
Ropinirole HCl	130.58	128.93	127.56	125.31	129.82
Ivabradine HCl	52.6	22.4	54.3	64	52.6
Carvedilol phosphate	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
Nisoldipine	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble

6.2.3. Particle size and distribution

The particle size of the selected drugs was determined by malvern master sizer. The procedure of the particle size distribution is given in the section 5.5.3. The average particle size of the selected drugs was given in the Table No.6.2.3. The particle size distribution of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine was given in the figure No. 6.2.1, 6.2.2, 6.2.3 and 6.2.4 respectively.

Table No .6.2.3: Average particle size of the selected drugs

SL No	Drugs	Average Particle size (µm)	
1	Ropinirole HCl	D (0.1)	0.537
		D (0.5)	2.282
		D (0.9)	8.633
2	Ivabradine HCl	D (0.1)	5.007
		D (0.5)	18.513
		D (0.9)	47.155
3	Carvedilol phosphate	D (0.1)	8.11
		D (0.5)	27.35
		D (0.9)	73.36
4	Nisoldipine	D (0.1)	1.439
		D (0.5)	6.670
		D (0.9)	18.768

6.2.4. Density

The Bulk density, tapped density, carr's index, hauser ratio and angle of repose of the selected drugs were determined as per the methods suggested in the section 5.5.4-5.5.7. The results of the study were recorded in the Table No.6.2.4.

Table No. 6.2.4: Density and flow property of the selected drugs

Sl No	Drugs	Tapped density(g/cc)	Bulk density (g/cc)	Hausner's ratio	Carr's index	Angle of repose
1	Ropinirole HCl	0.435	0.310	1.4032	28.73	39.6
2	Ivabradine HCl	0.4618	0.4013	1.15	13.10	35
3	Carvedilolphosphate	0.415	0.294	1.4115	29.15	38
4	Nisoldipine	0.521	0.353	1.4759	32.24	42

6.2.5. Drug excipients interaction study

DSC study was carried out in order to identify of the interaction of the drug with the selected formulation ingredients. The procedure for the study was given in the chapter section 5.5.8. The melting point of the drugs as well as the ingredients was given in the Table No.6.2.5 for the better understanding of the thermogram. Thermograms for each drug were taken to study the interactions of the drug with the excipients used in the formulation. Two thermograms of each drug were taken.

1) Drug alone

2) Drug with all the ingredients (Final formulation)

The Figure No.6.2.5 and 6.2.6 showed the thermograms of Ropinirole HCl alone and Ropinirole HCl push pull osmotic tablets respectively. The Figure No.6.2.7 and 6.2.8 showed the thermogram of Ivabradine HCl alone and Ivabradine HCl push pull osmotic tablets respectively. The Figure No.6.2.9 and 6.2.10 showed the thermogram of Carvedilol phosphate alone and Carvedilol phosphate push pull osmotic tablets respectively. The Figure No.6.2.11 and 6.2.12 showed the thermogram of Nisoldipine alone and Nisoldipine push pull osmotic tablets respectively.

Table No. 6.2.5: Melting points of the drugs as well as the formulation ingredients

SL No	Description	Melting point
1	Ropinirole HCl	243 - 250°C
2	Ivabradine HCl	190-198°C
3	Carvedilol phosphate	114.9°C
4	Nisoldipine	152 ^{0}C
5	Butylated hydroxy toluene	70°C
6	Polyethylene oxide	65–70°C
7	Sodium lauryl sulphate	204–207°C
8	Sodium chloride	804°C
9	Cellulose acetate	230–300°C
10	Dibasic calcium phosphate	Does not melt
11	Magnesium stearate	117–150°C
12	Iron oxide red	NA
13	Propylene Glycol	−59°C

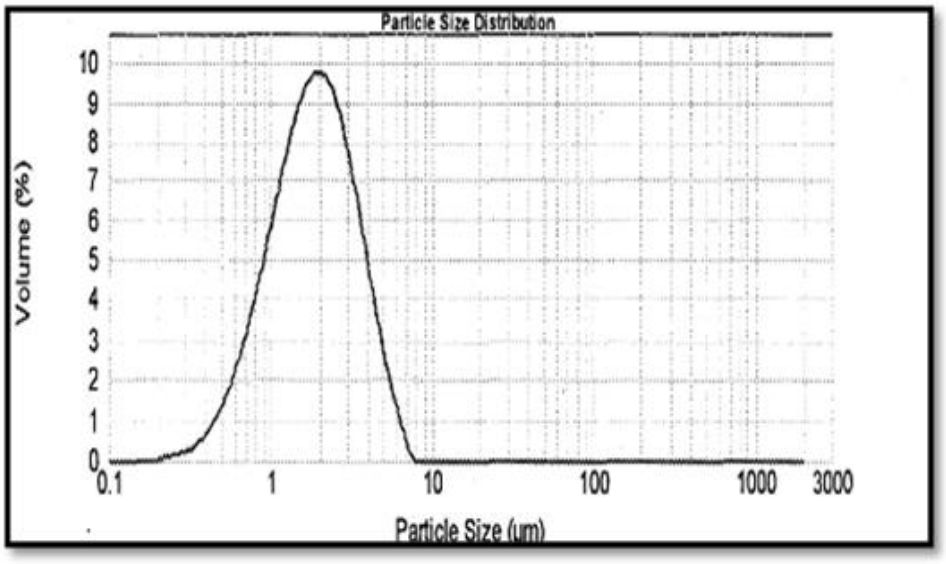


Figure No.6.2.1: Particle size distribution of Ropinirole HCl

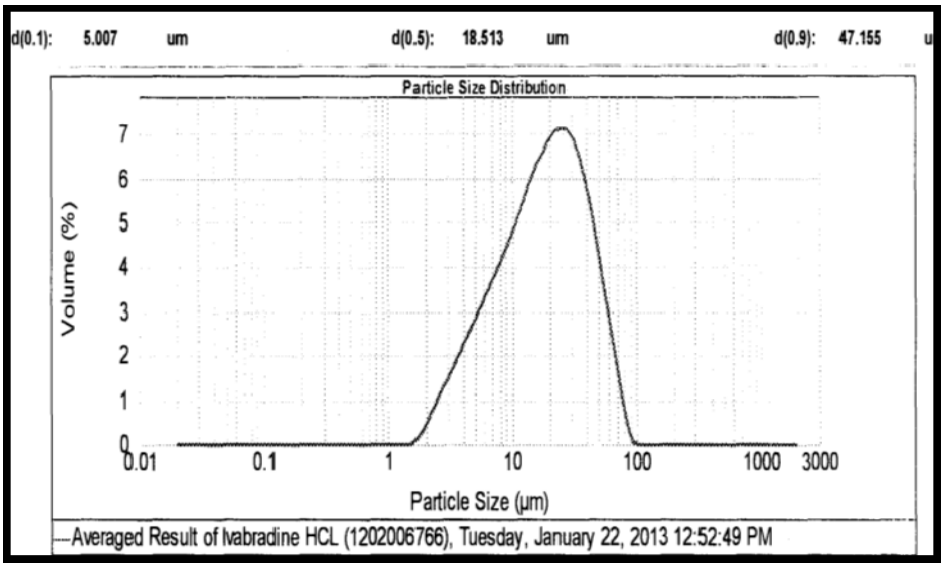


Figure No. 6.2.2: Particle size distribution of Ivabradine HCl

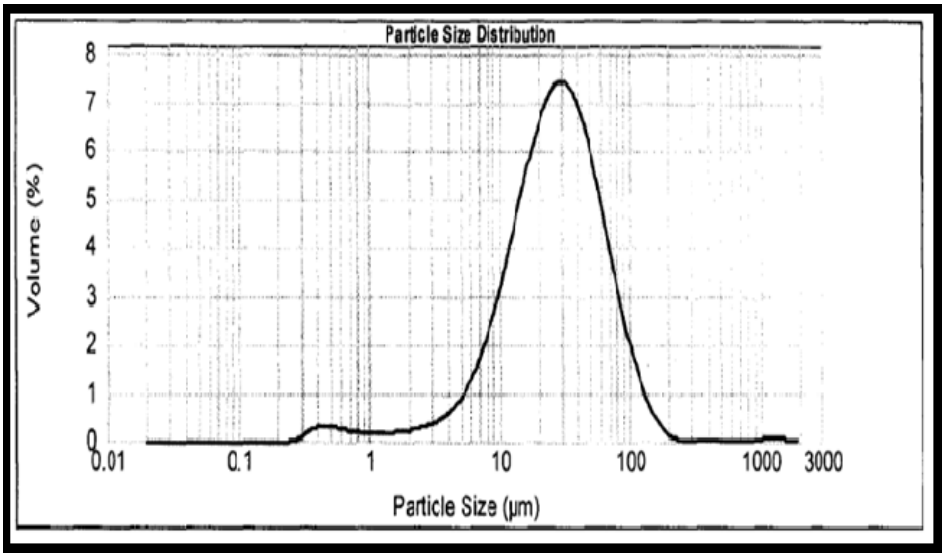


Figure No.6.2.3: Particle size distribution of Carvedilol phosphate

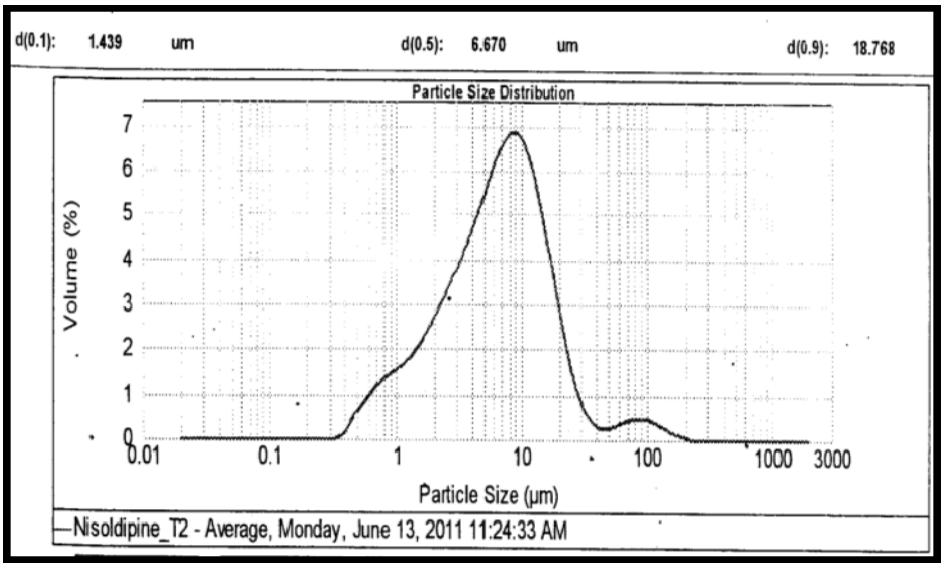


Figure No.6.2.4: Particle size distribution of Nisoldipine

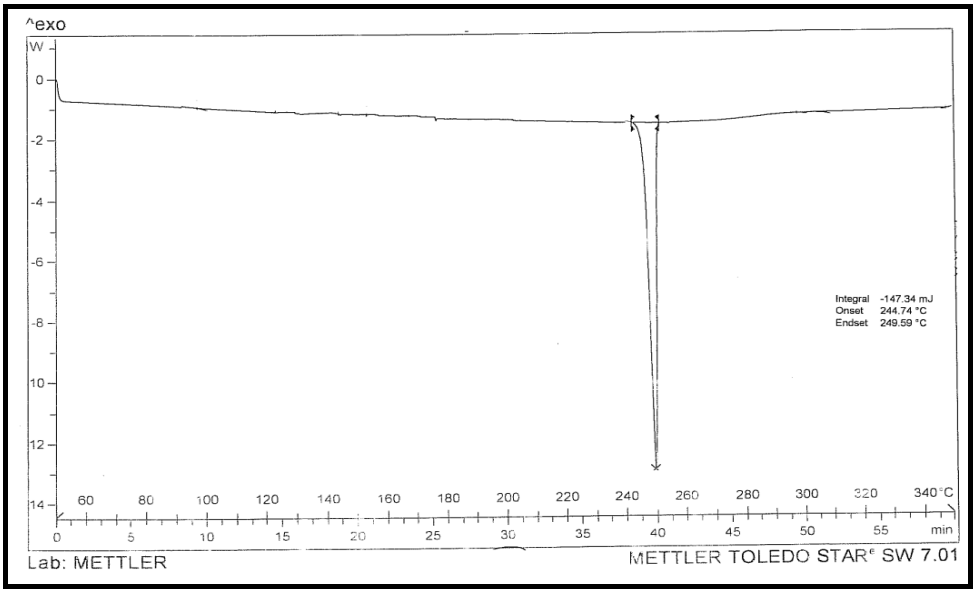


Figure No.6.2.5: DSC of Ropinirole HCl

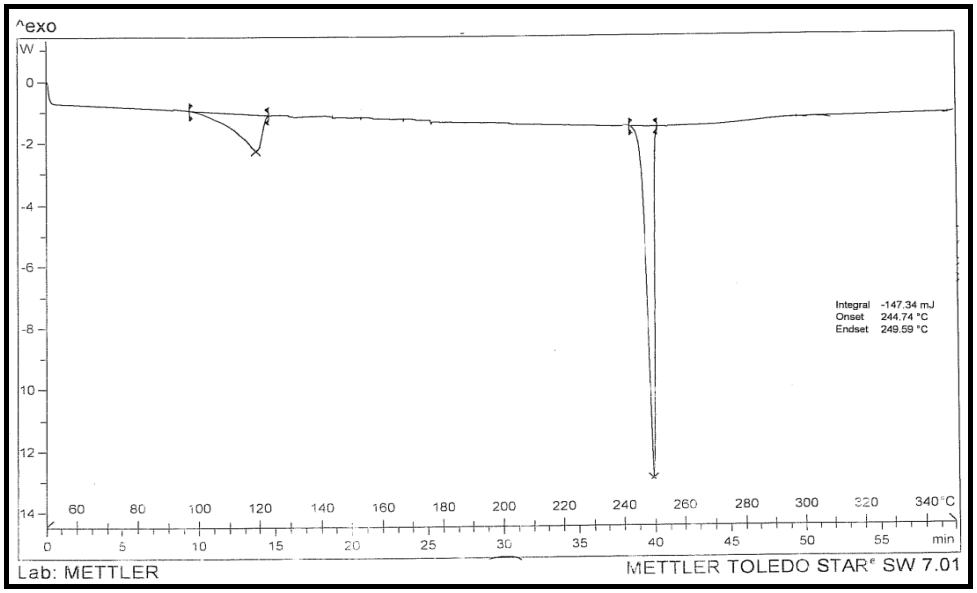


Figure No. 6.2.6: DSC of Ropinirole HCl push pull osmotic tablets

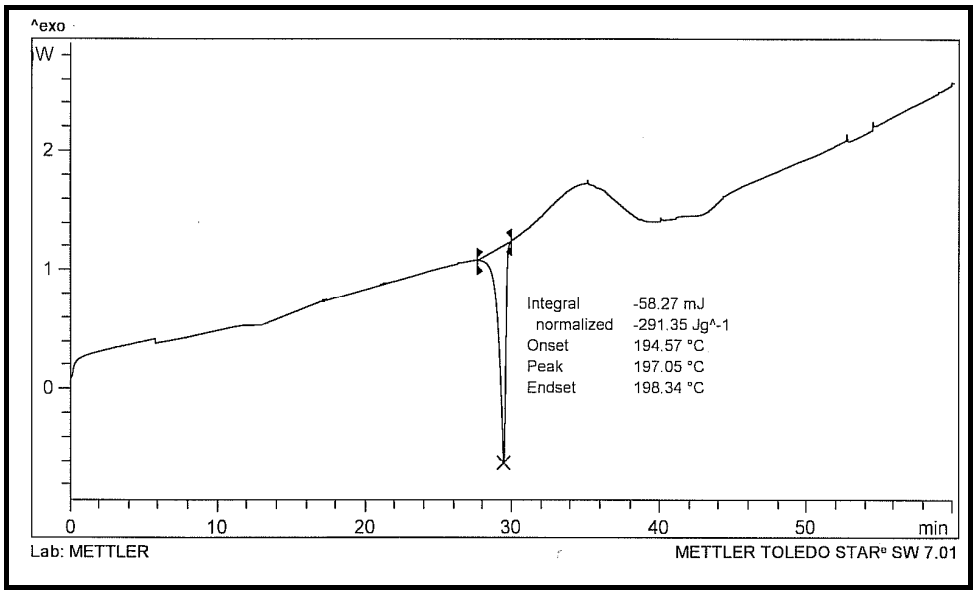


Figure No.6.2.7: DSC of Ivabradine HCl

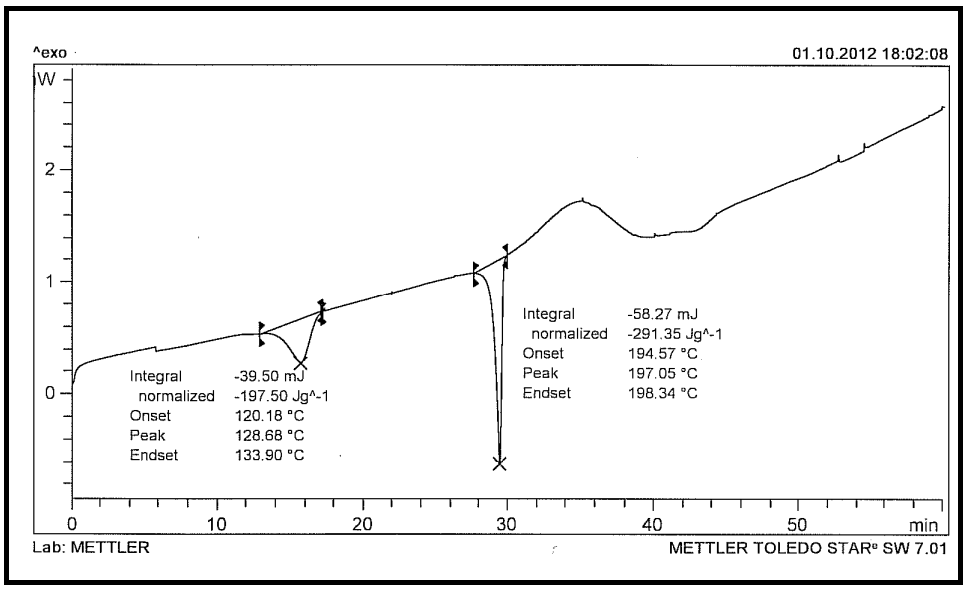


Figure No.6.2.8: DSC of Ivabradine HCl push pull osmotic tablets

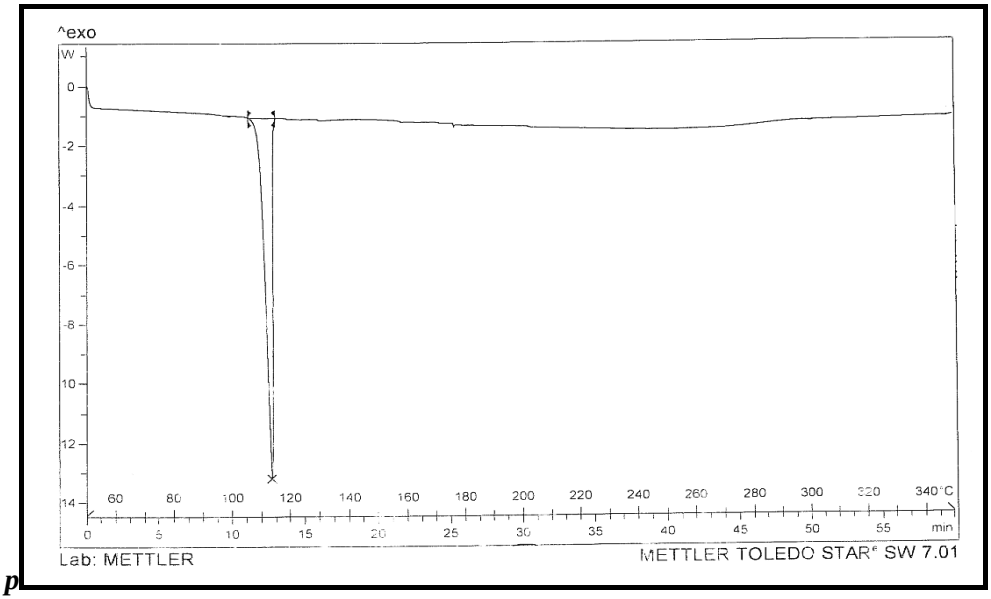


Figure No. 6.2.9: DSC of Carvedilol Phosphate

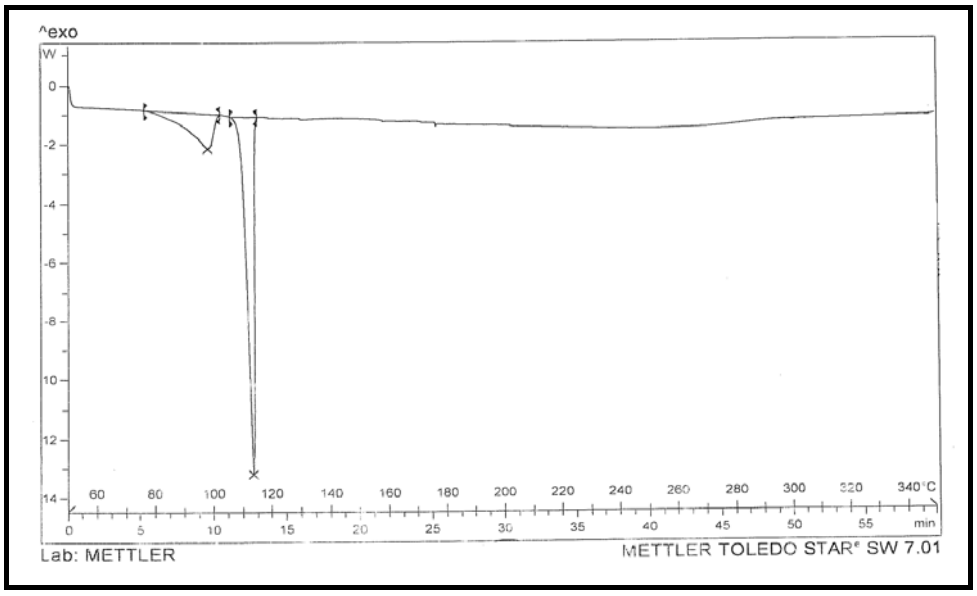


Figure No. 6.2.10: DSC of Carvedilol Phosphate push pull osmotic tablets

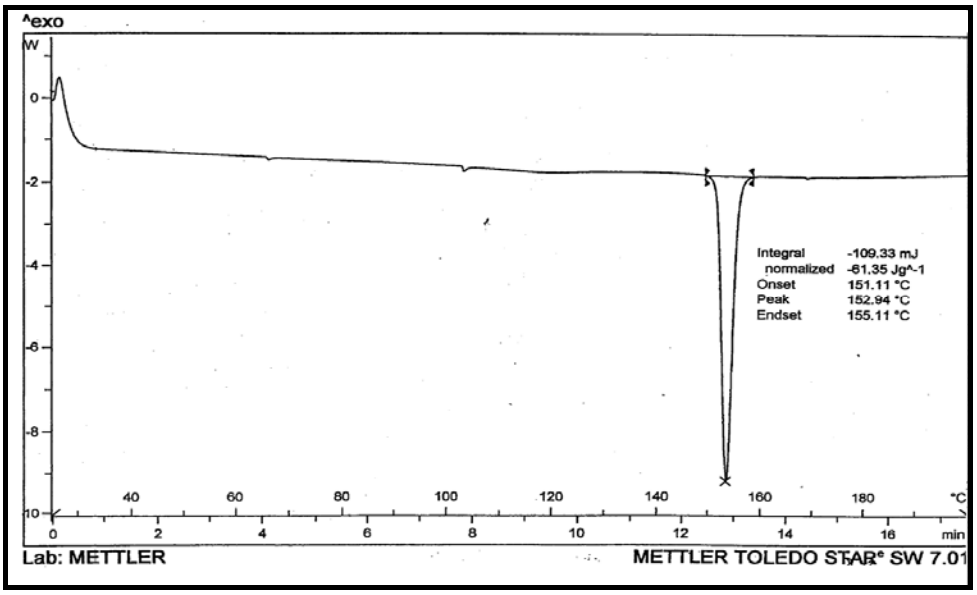


Figure No. 6.2.11: DSC of Nisldipine

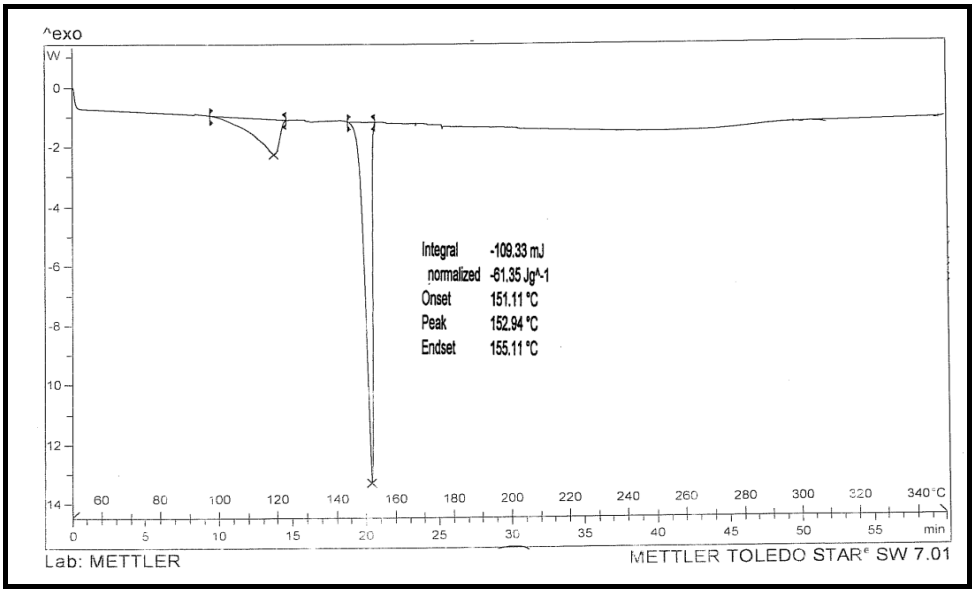


Figure No. 6.2.12: DSC of Nisoldipine push pull osmotic tablets

6.3. PRODUCT DEVELOPMENT AND OPTIMIZATION OF PUSH PULL OSMOTIC TABLETS OF THE SELECTED DRUGS

6.3.1. Calculation of Dose

As the present study concentrated on the formulation of once daily osmotic tablets of the selected drugs, the dose incorporated in the device should be fixed before the formulation development. Extended release once daily tablets of Ropinirole HCl, Carvedilol phosphate and Nisoldipine were available in the market. Available marketed products and its strength were given in the Table No.5.3.1, 5.3.3 and 5.3.4 respectively. For Ivabradine HCl, only immediate release formulations were available (5 and 7.5 mg). No extended release tablets were available in the market. So it was essential to calculate the dose to be incorporated in the osmotic tablets. The procedure for the calculation of dose was given in the section 5.6.1. The total dose incorporated was determined with the help of the Robinson – Eriksen equation [1,2,3,4].

The total dose of Ivabradine HCl was determined according to the Robinson - Ericksen equation and the dose of the drug chosen was given in the Table No. 6.3.1.

Table No.6.3.1: Dose incorporated in the push pull osmotic tablets

SL.No	Parameters	Ropinirole HCl	Ivabradine HCl	Carvedilol phosphate	Nisoldipine
1	Elimination rate constant(K_{el})	12 mg	0.3465 h^{-1}	10 mg	8.5mg
2	Zero-order release rate(K_0)		0.3465 mg/hr		
3	Initial dose (D_b)		2.5 mg		
4	Corrected initial dose(D_i)		2.1535 mg		
5	Maintenance dose (D_m)		7.9695 mg		
6	Total dose (W)		10.123 mg		

6.3.2. Screening of the factors affecting release profile of the drug from push - pull osmotic tablets

Vast number of the factors affects the release of the drug from the push pull osmotic tablet system. In this study, all the process parameters were kept constant and studied the effect of product parameters on the responses. An extensive literature survey was done and the different factors affecting the release of the drug from the system was summarized in the Table No.6.3.2.

Table No.6.3.2: Factor affecting the push pull osmotic drug delivery systems

Factors	Effect
Hardness	No effect
Pore size	No effect
Drug loading	Effect
Solubility of the drug	Effect
Surface area of the tablets	Effect
Osmotic agent in the DL(Types and concentration)	Effect
Osmotic agent in the PL(Types and concentration)	Effect
Suspending agent DL(Types and concentration)	Effect
Extender in the PL(Types and concentration)	Effect
Solubilizing agent DL(Types and concentration)	Effect
Solubilizing agent PL(Types and concentration)	Effect
Other functional ingredients	No effect
Coating polymer(Types and concentration)	Effect
Coating thickness	Effect
Weight gain	Effect
Plasticizer(Types and concentration)	Effect
Pore former(Types and concentration)	Effect
Dissolution media	No effect
Agitation speed	No effect
Osmotic agent in the dissolution media	No effect
pH of the dissolution media	No effect

6.3.3. Product development and optimization of push –pull osmotic tablets of Ropinirole HCl

I. Formulation of push pull osmotic tablets of Ropinirole HCl

The Factor influence study batches of Ropinirole HCl R1- R20 were formulated according to the methodology given in the section 5.6.4 (I A-D), which explains preparation of granules, compression of core bilayer tablets, coating of core tablets and drilling of coated tablets. Quantity of the ingredients was set according to the range specified in the literatures. The compositions taken for preparation of factor influence study batches were shown in Table No.5.6.2. The levels and responses fixed for the study was given in Table No. 5.6.3 and 5.6.4.

The design table in coded values for the formulation development of Ropinirole HCl osmotic tablets was given in the Table No.5.6.5. The final formula for the factor influence study and optimization of Ropinirole HCl push pull OT was shown in the Table No. 6.3.3 and 6.3.4.

II. Evaluation of the formulations

The batches R1- R20 were evaluated simultaneously while preparing. They were subjected to blend as well as whole tablet evaluation. The procedures for the evaluation were given in the chapter section 5.5.4 - 5.5.7 and 5.6.4(II A&B).

A. Blend evaluation

The prepared granules of both the layers i.e. drug layer and push layer were evaluated by means of various tests. The tests were carried out according to the methodology given in the chapter section 5.5.4 to 5.5.7. The results of the various blend evaluation were mentioned in the Table No.6.3.5.

B. Tablet evaluation

The prepared tablets were evaluated for weight variation, hardness, friability assay, weight gain, pore size, diameter and thickness. The tests were performed as per the methodology given in chapter section 5.6.4(IIB). The results of various tests were shown in Table No.6.3.6.

Table No. 6.3.3: Formula for the trial R1- R10

S. No.	Ingredients	R1 mg/tab	R2 mg/tab	R3 mg/tab	R4 mg/tab	R5 mg/tab	R6 mg/tab	R7 mg/tab	R8 mg/tab	R9 mg/tab	R10 mg/tab
Drug Layer (DL)											
1	Ropinirole HCl	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68
2	DCP	33.00	20.68	24.45	12.13	31.00	18.67	22.45	10.12	33.00	20.68
3	PEO 400 K	1.368	13.68	1.368	13.68	1.368	13.68	1.368	13.68	1.368	13.68
4	NaCl	0.950	0.950	9.500	9.500	0.950	0.950	9.500	9.500	0.950	0.950
5	BHT	0.001	0.014	0.001	0.014	0.001	0.014	0.001	0.014	0.001	0.014
6	SLS	0.50	0.50	0.50	0.50	2.50	2.50	2.50	2.50	0.50	0.50
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg .stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total weight of DL		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer (PL)											
9	PEO 7000 K	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	25.00	25.00
10	NaCl	0.13	1.25	1.25	0.13	1.25	0.13	0.13	1.25	1.25	12.50
11	DCP	39.62	38.50	36.70	37.82	36.70	37.82	39.62	38.50	14.18	2.93
12	BHT	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.03	0.03
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg.stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of PL		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	9.4	17.3	18.8	8.6	8.6	18.8	17.3	9.4	17.3	9.4
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.09	1.73	0.19	0.86	0.86	0.19	1.73	0.09	1.73	0.09
Total weight of coating		9.5	19.0	19.0	9.5	9.5	19.0	19.0	9.5	19.0	9.5
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**114.0**	**104.5**	**114.0**	**104.5**

Table No. 6.3.4: Formula for the trial R11- R20

S. No.	Ingredients	R11 mg/tab	R12 mg/tab	R13 mg/tab	R14 mg/tab	R15 mg/tab	R16 mg/tab	R17 mg/tab	18 mg/tab	R19 mg/tab	R20 mg/tab
Drug Layer (DL)											
1	Ropinirole HCl	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68
2	DCP	24.45	12.13	31.00	18.68	22.45	10.13	21.56	21.56	21.56	21.56
3	PEO 400 K	1.368	13.68	1.368	13.68	1.368	13.68	7.524	7.524	7.524	7.524
4	NaCl	9.500	9.500	0.950	0.950	9.500	9.500	5.225	5.225	5.225	5.225
5	BHT	0.001	0.014	0.001	0.014	0.001	0.014	0.008	0.008	0.008	0.008
6	SLS	0.500	0.500	2.500	2.500	2.500	2.500	1.500	1.500	1.500	1.500
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg stearate	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total weight of DL		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer (PL)											
9	PEO 7000 K	25.00	25.00	25.00	25.00	25.00	25.00	13.75	13.75	13.75	13.75
10	NaCl	12.50	1.25	12.50	1.25	1.25	12.50	3.78	3.78	3.78	3.78
11	DCP	4.73	15.98	4.73	15.98	14.18	2.93	23.81	23.81	23.81	23.81
12	BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
13	SLS	0.45	0.45	0.45	0.45	2.25	2.25	1.35	1.35	1.35	1.35
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg.stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of PL		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	8.6	18.8	18.8	8.6	9.4	17.3	13.5	13.5	13.5	13.5
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.86	0.19	0.19	0.86	0.09	1.73	0.74	0.74	0.74	0.74
	Total Weight of Coating	9.5	19.0	19.0	9.5	9.5	19.0	14.3	14.3	14.3	14.3
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**109.3**	**109.3**	**109.3**	**109.3**

Table No. 6.3.5: Blend evaluation of the DL and PL of Push pull OT of Ropinirole HCl

Trials	Angle of repose		Bulk density(g/ml)		Tapped density(g/ml)		Hausner's ratio		Carr's index (%)	
	DL	PL	DL	PL	DL	PL	DL	PL	DL	PL
R1	28.33	26	0.812	0.617	0.911	0.692	1.12	1.12	10.87	10.83815
R2	25.43	27.14	0.734	0.618	0.834	0.694	1.14	1.12	11.99	10.95101
R3	28.55	27.75	0.834	0.751	0.953	0.841	1.14	1.12	12.49	10.70155
R4	29.65	26.56	0.789	0.622	0.893	0.73	1.13	1.17	11.65	14.79452
R5	27.48	28.39	0.761	0.627	0.853	0.698	1.12	1.11	10.79	10.17192
R6	26.87	27.14	0.645	0.715	0.743	0.83	1.15	1.16	13.19	13.85542
R7	28.9	29.65	0.721	0.597	0.846	0.699	1.17	1.17	14.78	14.59227
R8	29.86	27.14	0.654	0.752	0.734	0.85	1.12	1.13	10.90	11.52941
R9	27.65	26	0.823	0.793	0.953	0.891	1.16	1.12	13.64	10.99888
R10	26.89	28.39	0.721	0.648	0.803	0.727	1.11	1.12	10.21	10.86657
R11	27.89	28.39	0.679	0.616	0.774	0.686	1.14	1.11	12.27	10.20408
R12	28.75	27.14	0.856	0.632	0.953	0.723	1.11	1.14	10.18	12.58645
R13	27.33	25.88	0.745	0.672	0.845	0.745	1.13	1.11	11.83	9.798658
R14	28.12	26.22	0.734	0.61	0.835	0.696	1.14	1.14	12.10	12.35632
R15	27.56	27.12	0.823	0.623	0.932	0.7	1.13	1.12	11.70	11
R16	29.6	25.99	0.699	0.712	0.795	0.795	1.14	1.12	12.08	10.44025
R17	27.68	27.43	0.865	0.654	0.991	0.743	1.15	1.14	12.71	11.97847
R18	29.44	29.88	0.789	0.61	0.894	0.693	1.13	1.14	11.74	11.97691
R19	28.11	28.56	0.814	0.689	0.939	0.783	1.15	1.14	13.31	12.00511
R20	29.18	27.9	0.777	0.643	0.867	0.715	1.12	1.11	10.38	10.06993

Table No.6.3.6: Whole tablet evaluation of Push pull OT of Ropinirole HCl

Trial	Wt variation (n =20)	Diameter mm (n=5)	Thickness mm (n=5)	Hardness (n=6) Kg/cm^2	Friability (%)	Assay (%)	Weight gain (%)	Pore size (mm)
R1	103.1±0.005	5.08±0.14	3.5±0.14	3.5±0.2	0.36	100±1.56	10.02±0.23	0.60
R2	115.5±0.04	5.12±0.16	3.6±0.11	3.8±1	0.746	99.9±1.82	20.09±0.02	0.60
R3	116 ±.006	5.0±0.18	3.5±0.13	3.7±0.5	0.626	102.54±1.7	20.04±0.78	0.60
R4	102.8 ± 0.08	5.14±0.11	3.6±0.11	4±0.2	0.344	100.1±1.03	10.08±0.76	0.60
R5	105.3 ± 0.01	5.14±0.15	3.4±0.14	4.2±0.1	0.22	100.3±0.87	10.16±0.80	0.60
R6	114 ±0.09	5.13±0.18	3.5±0.10	4.1±0.2	0.571	99.99±0.99	20.08±0.97	0.60
R7	114 ± 0.04	5.12±0.14	3.6±0.11	3.8±0.5	0.735	100±2.78	20.17±0.62	0.60
R8	103.89 ±0.07	5.13±0.15	3.6±0.13	3.8±0.8	0.447	99.78± 1.56	10.15±0.59	0.60
R9	114 .35± 0.05	5.1±0.18	3.6±0.10	3.6±0.6	0.809	99.34±2.67	20.04±2.98	0.60
R10	104.56 ± 0.01	5.1±0.08	3.6±0.24	3.5±0.5	0.681	99.56±1.2	10.28±0.13	0.60
R11	104.78 ± 0.08	5.16±0.06	3.6±0.11	3.4±0.4	0.453	101.33±1.78	10.07±0.03	0.60
R12	113.78 ± 0.07	5.2±0.12	3.5±0.13	3.6±0.45	0.838	100±1.6	21.08±0.23	0.60
R13	114 .59± 0.13	5.1±0.06	3.6±0.14	3.7±0.34	0.72	99.45±1.12	20.09±0.55	0.60
R14	103.87±0.034	5.04±0.1	3.4±0.21	3.6±0.22	0.35	99.78±2.6	10.04±0.73	0.60
R15	104.5 ±0.14	5.12±0.08	3.4±0.23	3.4±0.62	0.83	101±0.98	10.09±0.92	0.60
R16	114 ± 0.23	5.2±0.08	3.5±0.24	3.7±0.44	0.12	100±1.52	20.06±0.82	0.60
R17	109.25 ± 0.3	5.1±0.05	3.6±0.21	3.5±0.38	0.22	101±2.82	15.18±0.76	0.60
R18	109.25 ± 0.2	5.14±0.06	3.5±0.14	3.6±0.48	0.53	100±1.76	15.07±0.84	0.60
R19	109.25 ± 0.1	5.1±0.04	3.5±0.12	4.2±0.03	0.35	98±2.890	15.06±0.79	0.60
R20	109.67±0.12	5.16±0.04	3.6±0.13	4.1±0.07	0.47	99.98±1.12	15.13±1.4	0.60

C. *In-vitro* dissolution tests

In vitro dissolution study was carried out for all the batches and the test was carried out as per methodology given in the section 5.6.4(IIBix). The release profile of R1 to R20 batches were shown Table No.6.3.7, 6.3.8 and in Figures 6.3.1 and 6.3.2.

Table No.6.3.7*: *Dissolution profile of R1 to R10 batches

Time (Hrs)	Cumulative drug release (%)									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	
2	2.2±0.3	0	0	8±0.5	5.3±0.6	0	2.5±0.7	4.26±1.1	0	2.2±0.7
3	9.5±1.8	9.9±0.5	2.6±0.5	14.3±1.1	11.5±0.9	5±0.8	12±1.2	8.88±0.9	8.5±0.8	8.5±0.9
4	17.1±3.1	15.3±1.3	9.1±1.1	23.5±2.1	28.5±0.8	10±0.2	20.7±2.3	16.22±2.3	15.3±0.4	14.6±1.4
6	26.3±1.2	19.7±2.4	15.4±0.6	32.2±3.2	43±1.1	16±0.4	28.6±1.3	29.56±1.2	23.5±0.9	28.1±2.3
8	36.6±2.6	22.5±3.2	24.8±2.1	40±1.4	54.4±1.5	20±1.2	35.8±2.6	39.4±1.8	31.2±1.2	38.2±1.5
12	57.9±2.2	50±1.4	45.7±0.9	57.5±3.1	65.4±2.1	27±2.1	48±3.1	56.2±3.4	48.8±3.6	66.6±1.3
16	65.8±1.5	55.2±2.1	56.7±1.2	78.9±3.7	78±3.2	33±1.1	62±1.1	76.6±3.7	65.2±2.3	70.3±2.2
20	70.9±2.8	57.6±2.4	67±2.4	100±1.1	80±1.4	36±1.7	74±1.8	89.3±1.2	79.1±3.2	75.2±1.7
24	75.2±3.2	58.9±3.1	70±1.2	100±2.7	85±1.7	38±1.5	85±3.2	100±1.7	90.9±1.1	77±3.8

Table No.6.3.8: Dissolution profile of R11 to R20 batches

Time (Hrs)	Cumulative drug release (%)									
	R11	**R12**	**R13**	**R14**	**R15**	**R16**	**R17**	**R18**	**R19**	**R20**
0	0	0	0	0	0	0	0	0	0	0
1	5±0.9	0	0	0	0	0	0	2.1±1.1	2.3±1.6	1.9±1.1
2	8±0.7	0	0	4±1.1	5±1.1	7±2.1	7.9±1.1	6.4±2.5	5.7±0.7	4.8±3.2
3	15.2±1.1	8.1±0.7	2±0.8	12.3±2.8	10±2.1	13±1.5	8.7±2.6	9.1±1.7	8.1±0.6	8.3±2.5
4	27.4±2.1	13.3±0.6	8.1±1.2	21.2±2.1	17.5±3.2	20.2±1.8	16.3±1.4	14.5±1.3	13.3±1.2	14.3±1.2
6	40±3.7	16.5±1.4	16.4±1.2	36.4±2.1	24.3±1.7	29.5±1.7	24.1±2.7	26.9±1.6	24.6±3.6	23.7±2.3
8	55.4±3.8	20.3±1.6	24.3±2.1	49.24±3.7	30.9±3.1	45.3±1.8	38.6±2.2	39.8±3.7	36.3±2.9	33.9±2.9
12	72.6±2.1	30.7±3.1	35.6±3.6	80±3.2	44.1±3.4	59.88±2.6	66.3±3.5	65±4.3	60±2.2	61.3±2.6
16	89±1.2	38.5±1.2	40.2±3.2	88±1.9	57.7±2.1	80.4±2.8	84.5±2.8	82.2±4.8	77.1±1.3	72.5±1.7
20	100±3.2	47.3±3.2	41.9±1.1	90.2±1.9	70±4.1	97±3.1	88.4±2.4	87.8±3.2	80.7±2.5	78.2±1.5
24	100±4.2	55.8±1.4	45.3±3.8	93±1.1	80.8±2.3	100±1.5	90±1.2	92.2±3.1	84.9±1.8	85.1±2.6

D. Coating Evaluation

The coating evaluation was done by formulation of mechanical film in a Petri dish as it was described in methodology section 5.6.4(IIC). The results were shown in Table No.6.3.9. As the plasticizer concentration increases the folding endurance increases.

Table No.6.3.9: Coating film evaluation

SL No	parameters	Quantity (mg)					
		T1	T2	T3	T4	T5	T6
1	Physical appearance	Smooth opaque film	Smooth opaque film	Smooth Opaque film	Smooth opaque film	Smooth opaque film	Smooth opaque film
2	Folding endurance	276±45	400±37	290±60	425±53	366±40	70 ±49

III. Factor influence study

The *in vitro* evaluation of all the 20 trials was performed and the necessary values for the factor influence study were recorded. The results of the factor influence study were given in the Table No.6.3.10.

A. FDS curve

The FDS graph for the selected design with the selected factors and responses showed a flatter curve. The curve indicates a high FDS so the design space predicted by the selected model had useful precision. The graph was given in the Figure No.6.3.3.

***B.* Standard error graph**

Standard error graph is a contour plot showing the standard error of prediction for areas in the design space. The standard error of prediction for areas in the design space for the different factors were found to be between 0.52 – 0.60. So it was proven that the standard error throughout the design space was relatively very low. The entire design space will be having a very less prediction error for the selected design. Figure No.6.3.4.

Table No.6.3.10: Result of the factor influence study

SL No	Responses		
	PCUR at 24 hrs	R^2	Lag time (t10%)
R1	75	0.922	4.3
R2	59	0.889	3.7
R3	70	0.954	4.2
R4	100	0.997	2.8
R5	85	0.868	3.2
R6	38	0.946	4.2
R7	85	0.997	3.4
R8	100	0.988	3.2
R9	90	0.997	4.3
R10	77	0.886	3.9
R11	100	0.979	2.8
R12	54	0.998	4.5
R13	45	0.871	4.7
R14	93	0.855	3.7
R15	81	0.998	3.3
R16	100	0.980	3.2
R17	90	0.928	3.7
R18	92	0.943	3.5
R19	85	0.939	3.9
R20	81	0.9242	3.7

C. Analysis of the responses

1. Cumulative release at 24 hrs

The cumulative release of the different formulations R1-R20 were studied and analyzed. The different factors affecting the PCUR at 24 hrs were identified and studied with the help of different evaluation graphs and data explained below,

i) Half normal plot

The half-normal plot shown in the Figure No. 6.3.5 was used to identify the significant factors affecting PCUR at 24 hrs. From the graph it was evident that the factors which were affecting the cumulative release up to 24 hrs were B (NaCl DL), G (propylene glycol) and H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.634. This indicated the non significance of the non selected factors**.**

ii) Normal plot

From the normal plot shown in the Figure No. 6.3.6 it was evident that the factor B, G and H were significantly away from the normal straight line. Shapiro-wilk normality test displayed the p value as 0.499. This indicated that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

From the pareto chart shown in the Figure No. 6.3.7, it was clearly evident that the factors B (NaCl DL), G (propylene glycol), H (weight gain) were significantly affecting the PCUR at 24 hrs. Factor B and G had apositve effect and H had a negative effect on the response.The magnitude of the effect can be written as G> H> B. Non significant term effects and interaction effects were present below the t limit.

iv) ANOVA and Regression analysis

ANOVA and regression analysis for the PCUR at 24 hrs is given in the Table No.6.3.11. In this case B (p *=0.0097)*, G(p *=0.0012)*, and H(p *=0.0013)* are significant model terms. The Model F-value of 13.37 implied that the model was significant. The "Lack of Fit F-value" of 10.2 implied that the lack of fit was not significant relative to the pure error. Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit was observed . No interactions were significant. The "Pred R-Squared" of 0.5574 is in reasonable agreement with the "Adj R-Squared" of 0.6734 indicating the linearity of the model. Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Our ratio was12.559 indicates an adequate signal. So this model can be used to navigate the design space.

v) Polynomial equation

From the regression analysis the polynomial equation which can represents the cumulative percentage release at 24 hrs can be formed. The positive sign of the coefficients in the equation indicates the positive effect and the negative sign indicates the negative effect on the response.

PCUR at 24 hrs = 80.47+8.00* B +10.75 * G-10.63* H*(coded values)*

PCUR at 24 hrs = 88.9583 +1.77778* NaCl DL +2.38889 *Propylene Glycol -2.12500 * Weight gain (*Actual values)*

Table No. 6.3.11: ANOVA and Regression analysis for the PCUR at 24 hrs

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	4679.25	3	1559.75	13.37	0.0002	*significant*
B-NaCl DL	*1024*	*1*	*1024*	*8.78*	*0.0097*	
G-PG	*1849*	*1*	*1849*	*15.85*	*0.0012*	
H-Weight Gain	*1806.25*	*1*	*1806.25*	*15.48*	*0.0013*	
Residual	1749.7	15	116.65			
Lack of Fit	*1723.7*	*13*	*132.59*	*10.2*	*0.0995*	*not significant*
Pure Error	*26*	*2*	*13*			
Cor Total	6430	19				
Regression analysis						
Std. Dev.	10.8		R-Squared	0.7278		
Mean	80		Adj R-Squared	0.6734		
C.V. %	13.5		Pred R-Squared	0.5574		
PRESS	2646.8		Adeq Precision	12.559		

vi) Tests for the assumptions of the ANOVA

The ANOVA assumtions were tested and studied with the help of various graphs shown in the Figure No. 6.3.8**.**

- *The normal probability plot:* The plot indicated that residuals follow a normal distribution, as the points followed a straight line. The curve does not follow any pattern like S curve.
- *Residuals Vs Predicted***:** It tests the assumption of constant variance. The plot was a random scatter (constant range of residuals across the graph.) This confirmed the constant variance in the experiments performed.
- *Residuals vs Run***:** This is a plot of the residuals versus the experimental run order. It checks for lurking variables that may have influenced the response during the experiment. The plot shown a random scatter. Absence of any trends in the graph indicated that no time-related variable lurking in the background.

- *Predicted vs. Actual*: This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The lamda value was 1 and no transformation was needed.

vii) The perturbation graph

By changing the concentration of factor B (NaCl DL) and G (propylene glycol) from minimum to maximum, PCUR at 24 hr was increased from 75 to 85 and 72 to 93 respectively. But a decrease in the response (from 92 to72) was observed with an increase in the factor H (weight gain).

viii) Contour plot and RS plot

Figure No.6.3.9 shows the contour plot and response surface plot of the simultaneous effect of NaCl DL and propylene glycol on the PCUR at 24 hrs at a time. At lower concentration of propylene glycol, NaCl DL had lesser effect on the response. High PCUR at 24 hrs would be expected at high levels of both the factors. From the RS plot it was evident that the propylene glycol had a greater effect than NaCl DL on PCUR.

Figure 6.3.10 shows the change in PCUR at 24 hr with the change in weight gain and NaCl DL at a time. NaCl DL had a positive effect and weight gain had an opposite effect on the response. At low levels of weight gain, the NaCl DL had a prominent effect. At high weight gain even high concentrations of NaCl DL would not produce PCUR greater than 80 %. From the surface plot it was evident that the effect of weight gain had a greater effect on the PCUR than NaCl DL.

Figure No.6.3.11 shows the simultaneous effect of weight gain and propylene glycol on the PCUR at 24hrs. At 10 % of the weight gain the response was more prone to slight changes in propylene glycol. But at a higher weight gain even a 10% of propylene glycol was not sufficient to achieve 80% release at 24hrs. From the response surface plot, it was evident that with increase in the

concentration of propylene glycol the PCUR at 24 hrs was increased. The weight gain had a reciprocating effect on the PCUR at 24 hrs. Figures show that at both the levels of propylene glycol change in weight gain had a negative effect on the response.

ix) Cube plots

Cube plots are useful for representing the effects of three factors at a time. They shows the predicted values from the coded model for the combinations of the –1 and +1 levels of any three factors that we select. The combined effect of B (NaCl DL), G (propylene glycol), and H (weight gain) were shown in Figure No.6.3.12. When all the three factors were at minimum the PCUR at 24 hrs was about 72.34, and at maximum it was around 88.59. But a similar response can be achieved by keeping propylene glycol at its minimum, NaCl DL at its maximum and weight gain at its minimum.

2. Analysis of responses –Release rate constant (R^2)

i) Half normal plot

From the graph shown in the Figure No.6.3.13, it was evident that the factor affecting the release rate constant (R^2) were B (NaCl DL) and E (NaCl PL). The Shapiro-Wilk normality test displayed the p value as 0.450, indicates the non significance of the non selected factors**.** So no other factors except B and E were affecting the zero order release rate constant.

ii) Normal plot

Form the normal plot shown in the figure it was apparent that the factors B (NaCl DL) and E (NaCl PL) were significantly away from the normal straight line. Shapiro-Wilk Normality test displayed the p value as 0.450 indicating that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

The Pareto chart shown in Figure No.6.3.15 represents the significant effect of B (NaCl DL) and E (NaCl PL) on the zero order release rate constant.

Both the factors crossed the t limit, confirmed the obvious effect of these factors on the zero order rate constant. The magnitude of the effect can be written as B > E. With an Increase in the concentration of factor B, the R^2 approached unity, but increase in the concentration of factor E had an opposite effect. No other terms were significant as they all were below the t limit.

iv) ANOVA and Regression analysis

The significance level selected for the study was 5 % and the p value was 0.05. The Model F-value of 16.10912 implied that the model was significant. Factors B (p =<*0.0003)*, E (*0.0035)* were significant model terms. Value of 0.0846 implied that the lack of fit was not significant relative to the pure error. This means that the polynomial model was fitting all of the design points well. Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit was observed. No interactions were significant.

Table No. 6.3.12: ANOVA and Regression analysis for the effect of factors on R^2

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.0321	2	1.6057E-02	16.109	0.0001	*significant*
B-NaCl DL	0.0204	1	2.0449E-02	20.516	0.0003	
E-Sodium chloride PL	0.0116	1	1.1664E-02	11.702	0.0035	
Residual	0.0159	16	9.9673E-04			
Lack of Fit	0.0157	14	1.1248E-03	11.248	0.0846	*not significant*
Pure Error	0.0002	2	1.0000E-04			
Cor Total	0.0481	19				
Regression analysis						
Std. Dev.	0.0315		R-Squared	0.6681		
Mean	0.9281		Adj R-Squared	0.6266		
C.V. %	3.401		Pred R-Squared	0.6178		
PRESS	N/A		Adeq Precision	8.888		

v) Polynomial equation

The linear model polynomial equation representing R^2 is as follows,

R^2 = 0.93592 +0.036 * B - 0.027 * E *(coded terms)*

R^2 = 0.925523 + 7.94444E-003 * NaCl in drug layer - 1.20000E-003 * Sodium chloride in the push layer *(actual terms)*

vi) Test for the assumption of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No. 6.3.16.

- *The normal probability plot:* Residuals follow a normal distribution, as the points in the plot followed a straight line.
- *Residuals vs Predicted:* The plot showed a random scatter (constant range of residuals across the graph.) This confirmed the constant variance in the experiments performed.
- *Residuals vs Run:* The plot showed a random scatter. Absence of any trends in the graph indicated that no time-related variable lurking in the background.
- *Predicted vs. Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot:* The lamda value was 1 and no transformation was needed.

vii) The perturbation graph

The perturbation graph shown in the Figure No.6.3.16 explained the effect and sign of the significant factors on the zero order release rate constant. With the change in the concentration of NaCl DL from minimum to maximum, an increase in zero order rate constant from 0.89 to 0.96 was produced. But an increase in the NaCl PL produced a decrease in the response from 0.95 – 0.90.

viii) Contour plots and RS plots

Figure No.6.3.17 shows the contour plot and response surface plot for the simultaneous effect of factor B (NaCl DL) and E (NaCl PL) at a time. From the plot it was obvious that the factor B had a positive effect and E had a negative effect. High levels of NaCl DL and low levels of NaCl PL yield a better R^2 value. The change in concentration of NaCl DL was more evident at low level of NaCl PL. At high levels of NaCl PL even a high level of NaCl DL failed to produce an R^2 value more than 0.94. From the surface plot, the larger effect of NaCl DL than the NaCl PL was clearly understood.

3. Analysis of responses – lag time

i) Half normal plot

Figure No. 6.3.18 shows the half normal plot of the effect of factors on the R^2. The significant factor affecting the lag time was identified as B (NaCl DL) G (propylene glycol), H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.122. This indicated the non significance of the non selected factors**.** So no other factors except B, G, and H were affecting the lag time**.**

ii) Normal plot

Figure No. 6.3.19 shows the normal plot of the effect of factors on lag time. The factor B, G, and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.122**.** This indicated that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

Figure No. 6.3.20 shows the pareto chart of effect of factors on the lag time in terms of t value. The factors significantly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect. The magnitude of the effect of significant factors on the lag time can be written as G > H> B. No other factors or interaction terms were significant as they have not crossed the t limit.

iv) ANOVA and regression analysis

The significance level selected for the study was 5 % and the p value was 0.05. The Model F-value of 23.8514 implied that the model selected was significant. Factors B (3.292E-04*)*, G (9.855E-05*)* and *H (*1.467E-04*)* were the significant model terms affecting the lag time. Value of 2.7202, implied that the lack of fit was not significant relative to the pure error. This means that the polynomial model was fitting all of the design points well. Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit was observed. No interaction terms were significant. The "Pred R-Squared" of 0.6945 was in reasonable agreement with the "Adj R-Squared" of 0.7830, indicating the linearity of the model. "Adeq Precision was 16.3598 indicates an adequate signal. So this model can be used to navigate the design space.

Table No.6.3.13: ANOVA and regression analysis of the effect of factors on the lag time

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4.575	3	1.525	23.8514	3.800E-06	*significant*
B-NaCl DL	1.3225	1	1.3225	20.6843	3.292E-04	
G-PG	1.69	1	1.69	26.4321	9.855E-05	
H-Weight gain	1.5625	1	1.5625	24.4379	1.467E-04	
Residual	1.023	16	0.0639			
Lack of Fit	0.943	13	0.0725	2.7202	2.224E-01	*not significant*
Pure Error	0.08	3	0.0267			
Cor Total	5.598	19				
Regression analysis						
Std. Dev.	0.2529		R-Squared	0.8173		
Mean	3.71		Adj R-Squared	0.7830		
C.V. %	6.8156		Pred R-Squared	0.6945		
PRESS	1.7103		Adeq Precision	16.3598		

v) Polynomial equation

The polynomial equation representing the lag time can be written as,

lag time = 3.91 -0.2875 * B -0.325 * G +0.3125 * H *(Coded terms)*

lag time = 3.81711- 0.063888889* NaCl DL- 0.07222 * Propylene Glycol + 0.0625 * weight gain *(Actual terms)*

vi) Test for Assumptions of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No.6.3.21.

- *Normal probability plot*: The plot indicates that residuals followed a normal distribution, as the points followed a straight line.
- *Residuals vs Predicted:* The plot was a random scatter (constant range of residuals across the graph). This confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: The plot showed a random scatter. The graphs didn't follow any trends' indicates that no time-related variable lurking in the background.
- *Predicted vs. Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The lamda value was 1 and no transformation was needed.

vii) The perturbation graph

The graph explains the effect and sign of the significant factors on lag time. It showed that the change in the concentration of factors B and G from minimum to maximum, lag time was decreased. The factor H had an opposite effect. The factor G had a major effect on the lag time.

viii) Contour plots and RS plots

Figure No. 6.3.22 shows the contour plot and RS plot of the simultaneous effect of factor B (NaCl DL) and the G (propylene glycol) on lag time. At low level of propylene glycol, change in NaCl DL from minimum to maximum had produced a decrease in lag time from 4 to 3.3 hrs. But at high levels of propylene glycol this was 3.3 to 2.8 hrs. NaCl DL had lesser effect at the high level of propylene glycol. Response surface clearly represented the chief effect of propylene glycol.

The Figure No.6.3.23 shows the contour plot and RS plot of the simultaneous effect of factors B (NaCl DL) and H (weight gain) on lag time. NaCl DL had a negative effect on the lag time. Weight gain had an opposite effect. The desired effect was produced at low levels of weight gain and high levels of NaCl DL. NaCl DL had a prominent effect at low levels of weight gain.

Figure No.6.3.24 shows the contour plot and response plot of the combined effect of factors G (propylene glycol) and H (weight gain) on lag time at a time. Weight gain had a positive effect on the lag time and propylene glycol had a reverse effect. Effect of propylene glycol was more pronounced at low weight gain. From the RS plot the greater effect of the propylene glycol was well understood.

ix) Cube plots

The cube plot shown in Figure No. 6.3.25 explains the combined effect of B (NaCl DL), G (propylene glycol), and H (weight gain). When all the three factors were at minimum, the lag time was 4.01 hr, and at maximum it was around 3.41hrs.The lowest lag time was observed when NaCl DL and propylene glycol were at maximum and weight gain at its minimum. This cube plot also well represented the major effect of propylene glycol in all the levels of the other factors.

IV. Numerical optimization with the help of desirability

From the factor influence study it was found that the model suggested was linear and no significant lack of fit and curvature effect were found for any of the responses. So no quadratic model was chosen for optimization. The same 2 level design was used for further optimization.

A. Optimization of the push pull osmotic tablets of Ropinirole HCl

When more than two factors were significant, overlay plot does not give a complete idea about the optimization. In our study three factors were significantly affecting the lag time as well as PCUR at 24 hrs. So for a better understanding the numerical optimization was chosen. Desirability function was selected as the tool for optimization. The constraint fixed for the optimization was given in the Table No.6.3.14 and the solutions of the numerical optimization were given in the Table No.6.3.15.

Table No.6.3.14: Constraints of optimization of Ropinirole HCl push pull OTs

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
B:NaCl DL	is in range	1	10	1	1	3
E:NaCl PL	is in range	10	50	1	1	3
G:PG	is in range	1	10	1	1	3
H:weight gain	is in range	10	15	1	1	3
PCUR	is in range	95	100	1	1	5
R^2	Maximize	0.855	0.998	1	1	4
lag time	Minimize	2.8	4.7	1	1	3

B. Point prediction

The point prediction for the solution 2, 6 and 18 were given in the Table No.6.3.16. The same batches were selected as the check point batches. The confidence interval, prediction interval and the tolerance interval were given in the Table No.6.3.16.

C. Check point batch

To confirm the validity of the model, three formulations (solutions 2, 6 and 18) from the solutions were selected and formulated. The dissolutions were performed as per the method specified in section 5.6.4(IIBix). Table No.6.3.17 showed the values obtained from the dissolution study. All the responses were within the confidence interval and tolerance limits of the point predicted by the software. Hence it can be concluded that the model suggested for the design was a success and can be used for further predictions.

D. Optimized batch and evaluations

The optimized batch (ROB) from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. The composition of the optimized batch was given in the Table No.6.3.18. The optimized batch was prepared as per the procedure mentioned in the materials and method section 5.6.4(I). The blend as well as the whole tablet evaluation was performed as per the methods specified in section 5.6.4(II). The result of the study was given in the Table No.6.3.19. *In vitro* study was performed as per the methods mentioned in the section 5.6.4(IIBix) and the findings were given in the Table No. 6.3.19 and the Figure No.6.3.26.

Table No.6.3.15: Numerical solutions for the optimization of Ropinirole HCl push pull OTs

No	PEO DL*	NaCl DL	SLS DL*	PEO PL*	NaCl PL	SLS PL*	PG	Weight gain	CUR	R^2	lag time	Desirability
1	11.38	10.00	4.94	12.51	5.00	1.23	9.98	14.39	100.00	0.9989	3.00	0.9622
2	**27.97**	**10.00**	**4.94**	**26.69**	5.00	**1.59**	**9.68**	**14.00**	**100.00**	**0.9989**	**3.00**	**0.9622**
3	78.93	10.00	2.70	27.35	5.00	1.43	9.60	13.97	100.00	0.9989	3.00	0.9622
4	48.37	10.00	3.12	28.40	5.00	3.08	9.59	13.95	100.00	0.9989	3.00	0.9622
5	14.35	10.00	3.63	31.55	5.00	2.29	9.58	13.94	100.00	0.9989	3.00	0.9622
6	**10.10**	**9.87**	**1.55**	**11.17**	5.00	**1.03**	**6.38**	**10.03**	**100.33**	**0.9989**	**3.00**	**0.9622**
7	99.79	10.00	4.62	16.81	5.00	3.89	8.54	12.77	100.00	0.9989	3.00	0.9622
8	99.83	10.00	4.69	43.10	5.00	4.32	8.49	12.72	100.00	0.9989	3.00	0.9622
9	45.40	10.00	3.63	49.90	5.00	1.68	8.51	12.74	100.00	0.9989	3.00	0.9622
10	70.42	10.00	3.08	11.72	5.00	1.49	6.77	10.08	100.00	0.9989	3.00	0.9622
11	76.02	10.00	1.39	14.88	5.00	2.37	6.58	10.57	100.00	0.9989	3.00	0.9622
12	61.17	10.00	3.03	29.10	5.00	3.01	6.55	10.53	100.00	0.9989	3.00	0.9622
13	67.71	10.00	3.18	37.42	5.00	2.31	6.49	10.47	100.00	0.9989	3.00	0.9622
14	83.39	10.00	2.24	32.78	5.00	3.99	6.43	10.40	100.00	0.9989	3.00	0.9622
15	38.20	10.00	4.83	49.17	5.00	1.31	6.36	10.31	100.00	0.9989	3.00	0.9622
16	59.28	10.00	1.00	24.28	5.21	3.17	8.60	12.83	100.00	0.9989	3.00	0.9622
17	85.41	10.00	3.61	10.41	5.00	1.55	6.23	10.17	100.00	0.9989	3.00	0.9622
18	**14.35**	**10.00**	**4.97**	**49.29**	**5.00**	**4.90**	**6.10**	**10.02**	**100.00**	**0.9989**	**3.00**	**0.9622**
19	70.29	9.71	3.64	49.24	5.00	2.22	9.69	13.82	100.00	0.9965	3.00	0.9621
20	100.00	9.72	1.01	30.68	5.50	4.49	10.0	14.19	99.97	0.9965	3.00	0.9616
21	58.66	9.75	3.05	38.25	5.50	3.55	6.70	10.49	100.00	0.9965	3.00	0.9614
22	49.62	10.00	4.44	46.34	6.00	1.00	10.0	15.11	98.51	0.997	3.10	0.9610
23	64.37	10.00	2.84	31.08	6.00	2.62	10.0	14.41	100.00	0.997	3.00	0.9604
24	10.25	10.00	2.61	33.35	5.00	4.47	10.0	15.67	97.33	0.9963	3.14	0.9598

No	PEO DL*	NaCl DL	SLS DL*	PEO PL*	NaCl PL	SLS PL*	PG	Weight gain	CUR	R^2	lag time	Desirability
25	82.71	10.00	1.17	44.98	13.97	1.00	9.98	14.39	100.00	0.9859	3.06	0.9582
26	88.29	10.00	2.52	10.38	5.00	5.00	5.48	10.52	97.47	0.9955	3.14	0.9568
27	15.10	10.00	1.92	43.86	13.95	5.00	9.61	14.05	99.83	0.9855	3.07	0.9567
28	71.62	9.56	5.00	13.07	10.00	1.02	5.62	10.00	98.13	0.9868	3.13	0.9559
29	100.00	9.93	1.00	29.45	15.25	1.00	8.45	12.61	100.00	0.9863	3.06	0.9507
30	97.90	10.00	4.65	47.14	15.50	3.00	6.22	10.16	100.00	0.9826	3.07	0.9464
31	93.20	10.00	3.56	27.99	25.00	2.73	7.74	11.87	100.00	0.977	3.07	0.9407
32	55.48	9.96	4.97	49.72	25.10	4.99	8.51	12.71	100.00	0.9799	3.06	0.9323
33	30.06	10.00	3.48	30.01	25.24	4.18	6.08	10.00	100.00	0.9739	3.07	0.9295
34	45.68	10.00	4.71	14.75	25.30	2.96	6.06	10.00	99.97	0.9735	3.07	0.9277
35	85.33	10.00	1.58	14.62	27.53	2.66	6.07	10.00	99.98	0.9698	3.07	0.8997
36	43.86	9.98	1.57	10.32	26.00	4.37	8.24	12.50	99.83	0.9652	3.07	0.8962
37	80.01	10.00	4.60	35.65	30.00	1.69	8.62	12.86	100.00	0.9681	3.07	0.8782
38	99.98	9.85	1.01	47.88	35.5	5.00	6.83	10.72	100.00	0.9612	3.07	0.8724
39	10.27	9.88	5.00	50.00	35.01	4.41	8.93	13.28	99.64	0.9617	3.07	0.8688
40	80.74	9.83	3.51	10.65	34.10	3.25	6.46	10.29	100.00	0.96	3.07	0.8621
41	100.00	9.78	2.47	17.39	5.	1.27	10.0	14.23	99.99	0.9544	3.06	0.8401
42	95.05	10.00	1.28	12.83	35.14	4.72	6.08	10.00	100.00	0.9644	3.07	0.8347
43	54.32	10.00	4.49	12.20	45.54	5.00	6.18	10.44	99.33	0.9525	3.09	0.8323
44	99.88	10.00	4.83	27.92	47.00	1.06	6.62	10.62	100.00	0.9489	3.07	0.7750
45	10.04	10.00	1.55	34.94	48.00	5.00	6.08	10.00	100.00	0.9486	3.07	0.7736

*Non significant factors

Table No.6.3.16: Prediction of the responses

Solution 2							
/Response	**Pred Mean**	**Std Dev**	**SE Mean**	**CI for mean**		**99% ofPopulation**	
				95% CI low	**95% CI high**	**95% TI low**	**95% TI high**
PCUR	100.0000	10.4605	4.1884	91.1210	108.8789	54.4907	145.5092
R^2	0.9989	0.0307	0.0128	0.9838	1.0179	0.8578	1.1239
lag time	3.00	0.2529	0.1012	2.8477	3.2769	1.9622	4.1624
Solution 6							
PCUR	100	10.4604	4.381	90.711	109.288	54.15	145.84
R^2	0.9989	0.0306	0.0128	0.9837	1.0179	0.8546	1.1238
lag time	3.00	0.252	0.105	2.83	3.28	1.95	4.12
Solution 18							
PCUR	100	10.4604	4.1702	91.15	108.84	54.52	145.47
R^2	0.9989	0.0306	0.0128	0.9837	1.0179	0.8578	1.1238
lag time	3.00	0.2528	0.1008	2.84	3.27	1.96	4.16

Table No.6.3.17: Check point batches for the model validation of the Ropinirole HCl push pull OT

Batches	PCUR at 24 hrs	R^2	Lag time (hrs)
Solution 2	98.88 ±3.38	0.998±0.004	3.05±0.04
Solution 6	100.03 ±2.56	0.9988±0.008	2.99±0.34
Solution 18	101.2±2.50	0.998±0.015	3.08±0.007

Table No.6.3.18: Composition of optimized batch of Ropinirole HCl

SL.No	Ingredients Drug Layer	Optimized batch(ROB)	
		Mg/tab	(%w/w)
1	Ropinirole Hydrochloride	13.68	
2	Dibasic calcium phosphate	24.285	
3	PEO 400 K	1.382	10.1
4	Sodium chloride	9.377	9.87
5	BHT	0.001382	
6	SLS	0.775	1.55
7	IPA	qs	
8	Magnesium stearate	0.500	
	Total weight of drug layer	50	
	Push layer		
9	PEO 7000 K	5.59	11.17
10	Sodium chloride	0.28	5
11	Dibasic calcium phosphate	36.37	
12	BHT	0.005585	
13	SLS	0.46	1.03
14	Iron oxide Red	0.8	
15	IPA	qs	
16	Magnesium stearate	1.5	
	Total weight of Push layer	45	
	Total weight of un coated tablet	95	
	Functional coating		
15	Cellulose acetate	8.9	
16	Acetone	q.s	
18	Water	q.s	
19	Propylene Glycol	0.57	6.4
	Total Weight of Coating	9.53	10.03
	Total tablet weight	104.5	
Responses	**CUR at 24 hrs (%)**	**R^2**	**Lag time**
Predicted	100	0.9989	3.00
Observed	100.03±2.56	0.9988±0.008	2.99±0.34

Table No.6.3.19: Optimized batch evaluation

Trial	Wt variation (n =20)	Diameter (n=10)	Thickness (n=10)	Hardness (n=6)	Friability (%)	Assay (%)	Wtgain (%)	Pore size (mm)
ROB	104.5±0.01	5.12±0.11	3.62±0.13	4.5±0.4	0.63	99.98 ±0.65	10.17± .58	0.6

Dissolution Profile											
Time(hrs)	0	1	2	3	4	6	8	12	16	20	24
PCUR	0	0	5.0± 0.03	10.1 ±0.8	15.4 ±0.9	25.5 ±1.8	34.7 ±1.5	52.1 ±3.3	69.2 ±4.0	85.5 ±1.5	100.03± 1.5

E. Desirability contour plot and RS plot

They are the graphical representation of change in factors on the desirability function. It will be giving a better visualization of achieving the optimum condition by changing two factors at a time. Desirability plots showed how all the targeted optimum conditions are met by changing two factors at a time. The Figure No.6.3.27 shows how factor G (propylene glycol) and B (NaCl DL) affects the desirability. Higher desirability was achieved at maximum level of NaCl DL (more than 9%) and a lower concentrations propylene glycol (<5%). Lower concentrations of both factors yield desirability less than 0.6. High levels of both the factors also showed low desirability.

Figure No.6.3.28 shows the effect of factors B (NaCl DL) and H (weight gain) on desirability. The desirability was highest at high concentration of NaCl DL (9-10%) and low concentration of weight gain (10-12%). Higher weight gain (>15%) had desirability zero even at higher concentrations of propylene glycol.

Figure No.6.3.29 shows the desirability contour plot of factors H (weight gain) and G (propylene glycol). A larger portion of the contour plot showed the desirability close to one, indicates that these two factors were the major factors for achieving the desired optimum conditions

The Figure No. 6.3.30 shows the desirability contour plot and RS plot of effect of factors E (NaCl PL) and H (weight gain). From the plot it was evident that a wide range of NaCl PL can be used to get a better desirability. Weight gain was again proven as one of the stringent factors, as slight changes showed a drop in the desirability from 1 to 0.2. Weight gain more than 12.5 was having zero desirability at all the levels of NaCl PL.

The Figure No. 6.3.31 shows the desirability contour plot and the RS plot of simultaneous effect of factors B (NaCl DL) and E (NaCl PL) on the desirability. Optimum conditions were reached while keeping the NaCl DL at high level and the NaCl PL at low level. Below 3% of NaCl DL change in concentration of NaCl PL had little effect on the desirability. Change in concentration of NaCl PL from low to high desirability decreases.

Figure No. 6.3.32 shows the contour plot and RS plot of the effect of change in concentration of factors E (NaCl PL) and G (propylene glycol) on the desirability. Combinations of propylene glycol greater than 6 and lesser than 4 had a desirability zero at all levels of NaCl PL. Desirability increased with decreasing concentration of NaCl PL by keeping propylene glycol concentration between 3.5- 5.75%.

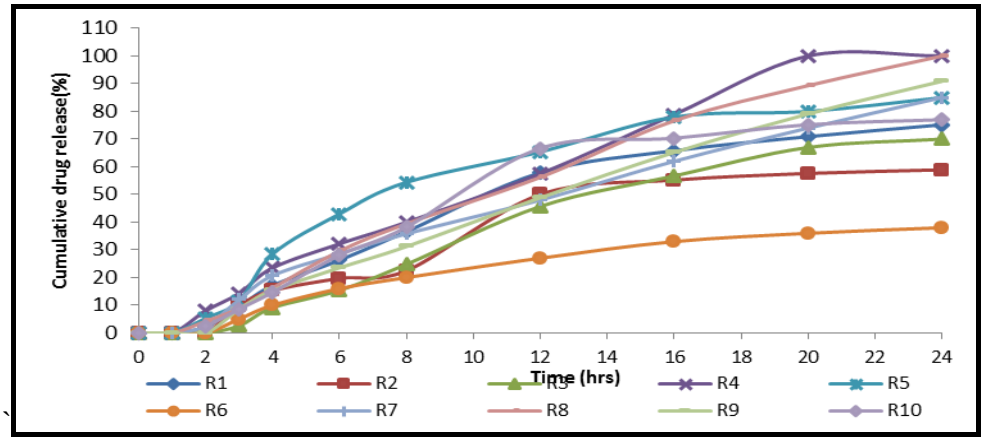


Figure No.6.3.1: Dissolution profile of R1 to R10 batches

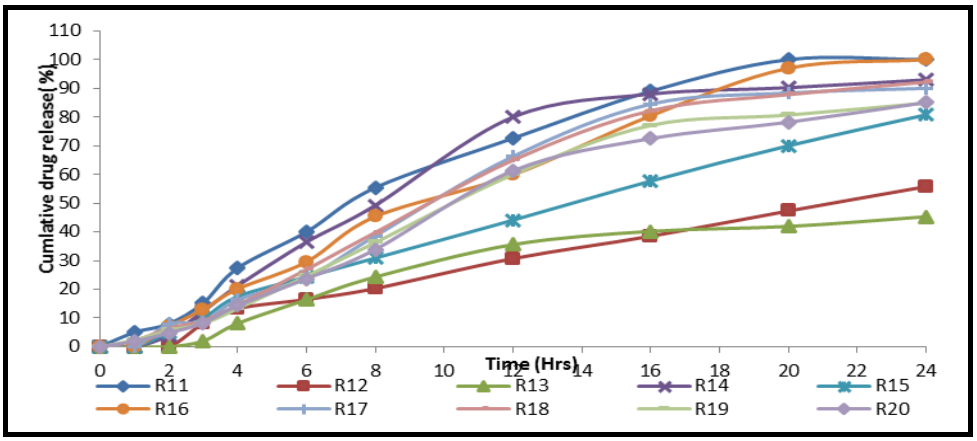


Figure No.6.3.2: Dissolution profile of R11 to R20 batches

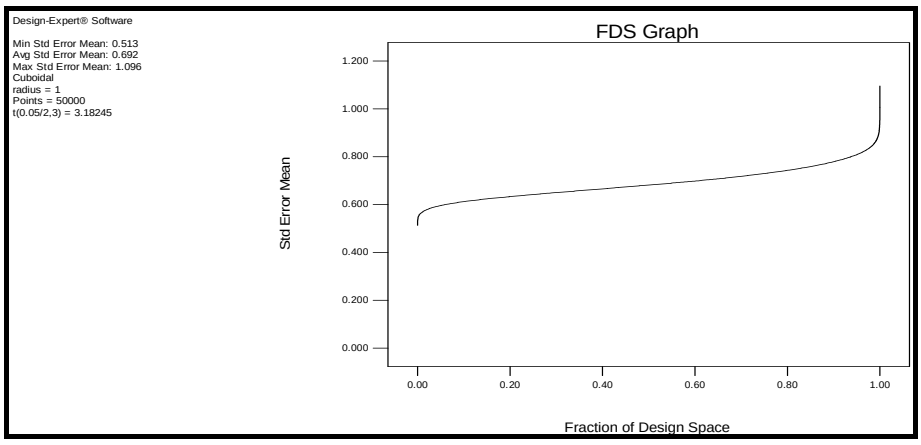


Figure No.6.3.3: FDS graph of the design selected for the FI study & Optimization

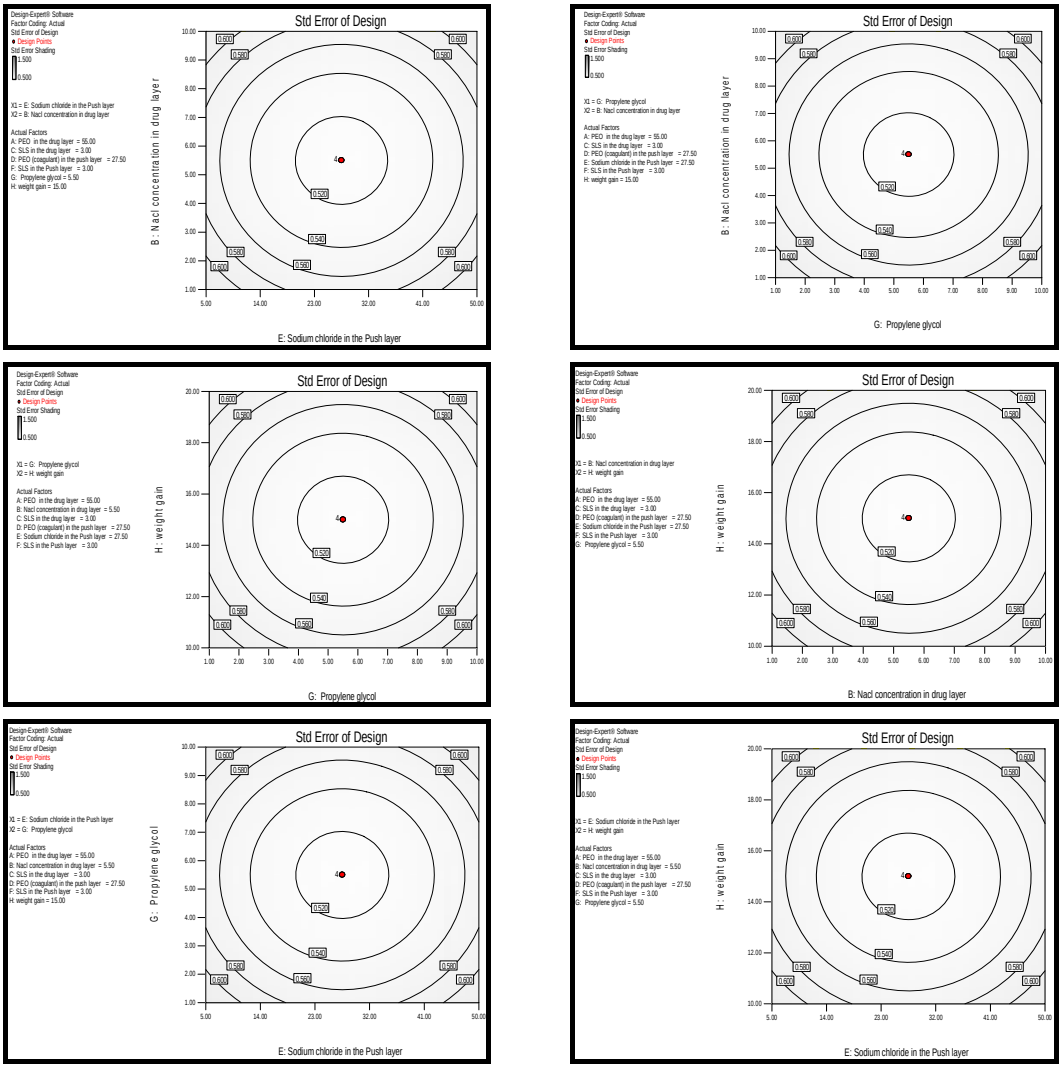


Figure No.6.3.4: Standard error Contour plots of the FI study & optimization

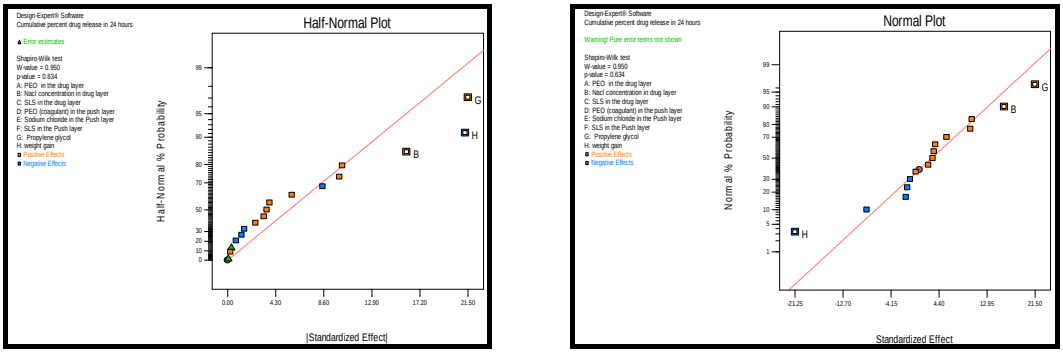


Figure No.6.3.5: Half normal plot for the effect of the factors on the PCUR at 24 hrs, Figure No.6.3.6: Normal plot for the effect of the factors on the PCUR at 24 hrs

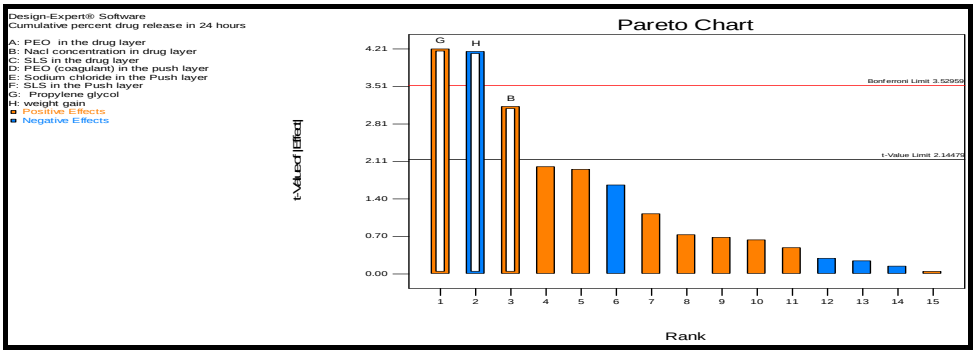


Figure No. 6.3.7: Pareto chart for the effect of the factors on the PCUR at 24 hrs

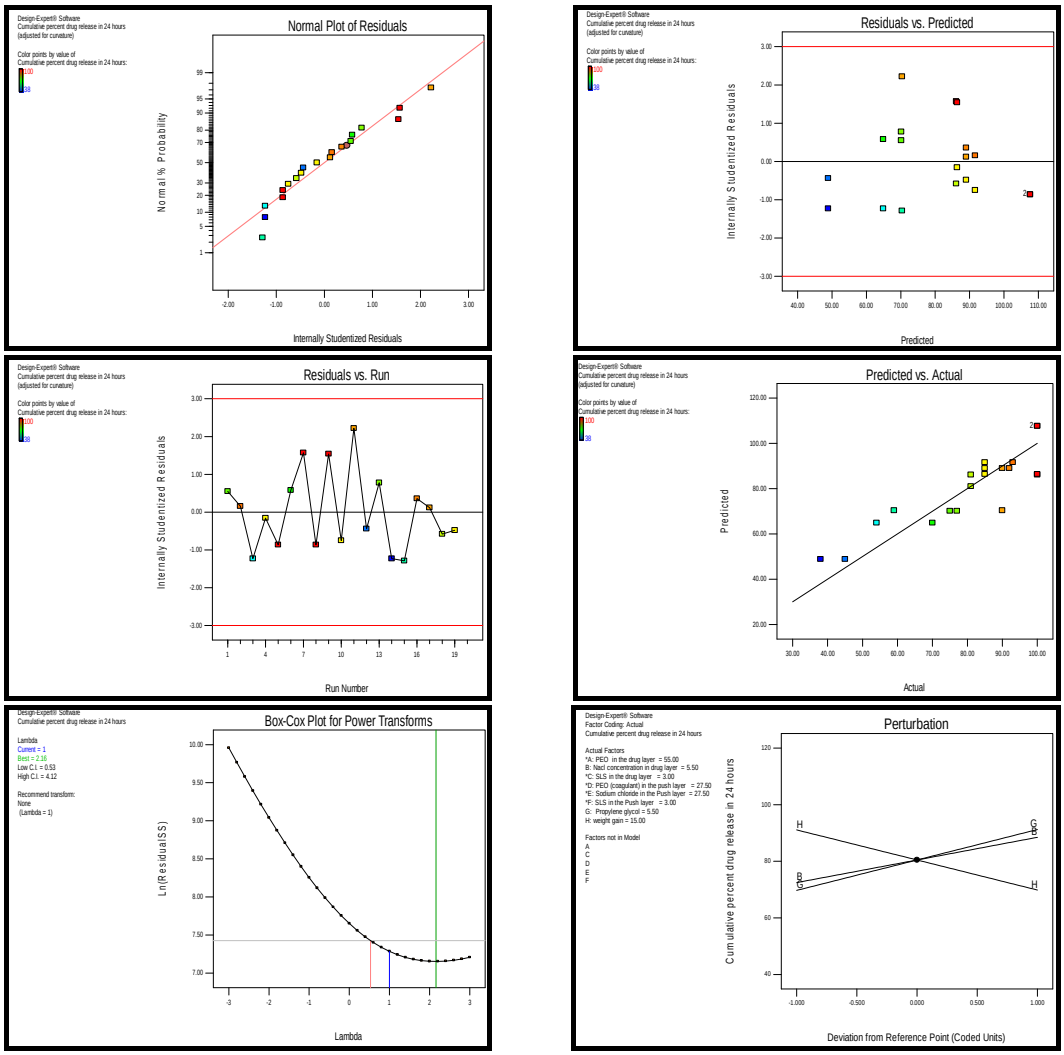


Figure No.6.3.8: Plots for the testing the assumptions of ANOVA and perturbation curve-PCUR at 24 hrs

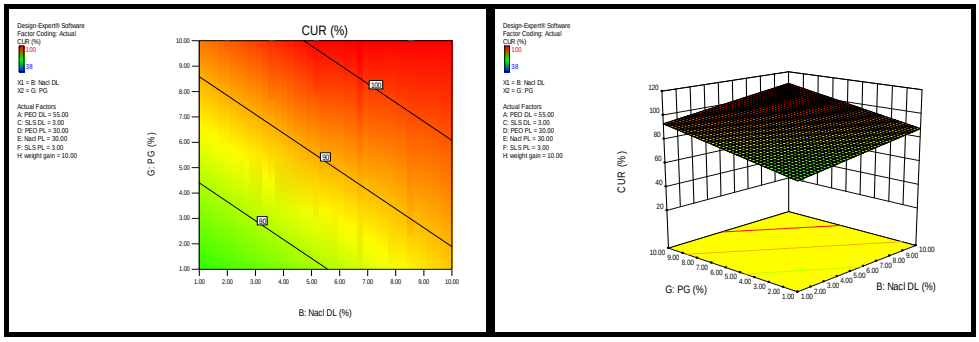


Figure No.6.3.9: Contour plot and RS Plot -
Effect of NaCl DL and Propylene Glycol on PCUR at 24 hrs

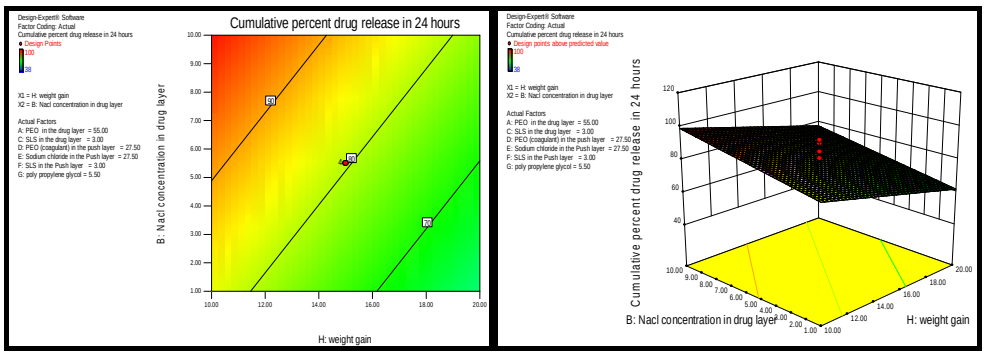


Figure No.6.3.10: Contour plot and RS Plot -
Effect of NaCl in DL and Weight gain on PCUR at 24 hrs

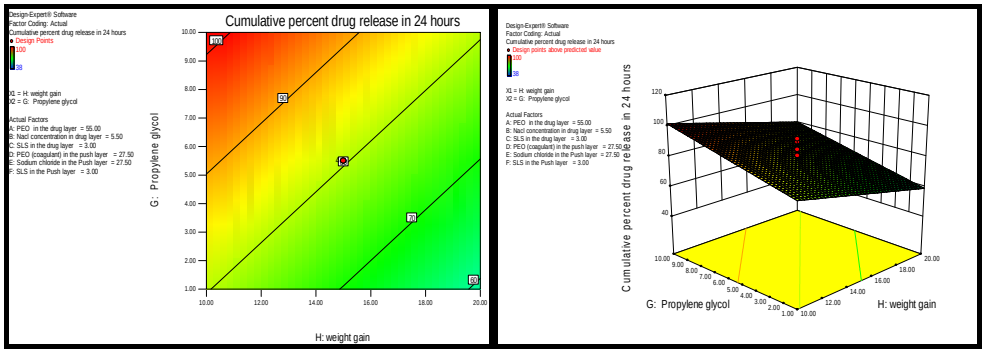


Figure No.6.3.11: RS Plot and Contour plot -
Effect of Weight gain and Propylene Glycol on PCUR at 24 hrs

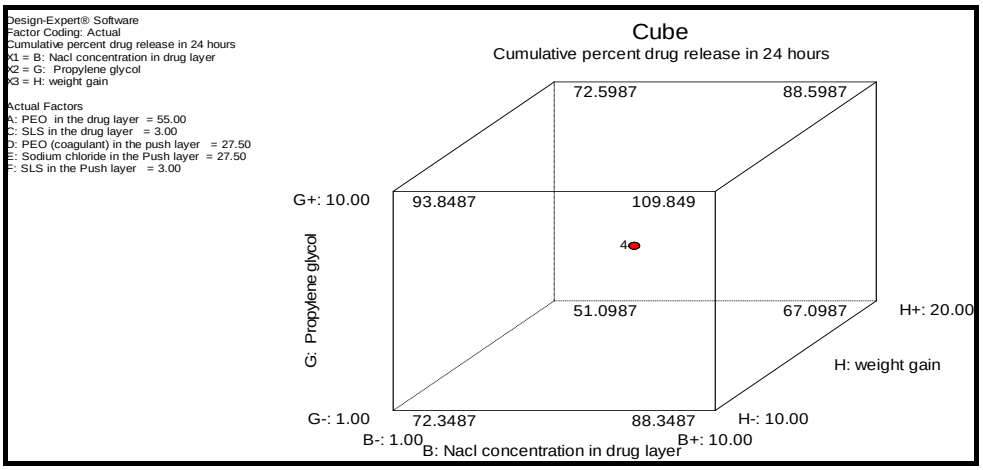
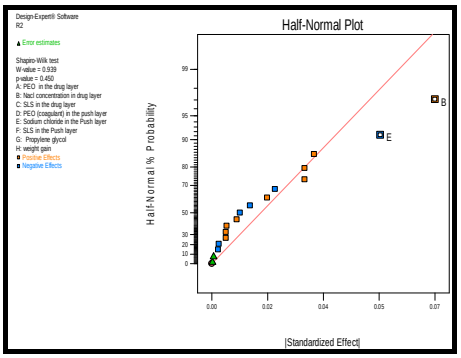


Figure No.6.3.12: Cube plot of the effect of NaCl DL, Propylene Glycol and Weight gain on PCUR at 24 hrs



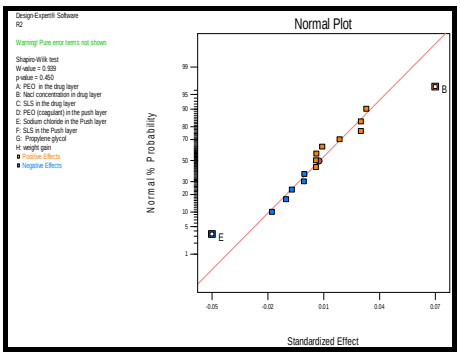


Figure No.6.3.13: Half Normal plot of the effect of the factors onR^2
Figure No.6.3.14: Normal plot of the effect of the factors onR^2

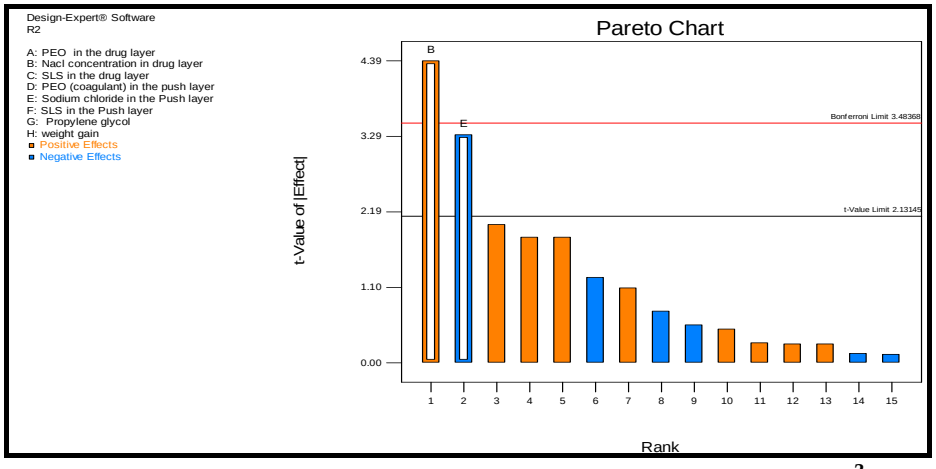


Figure No.6.3.15: Pareto chart of the effect of the factors onR^2

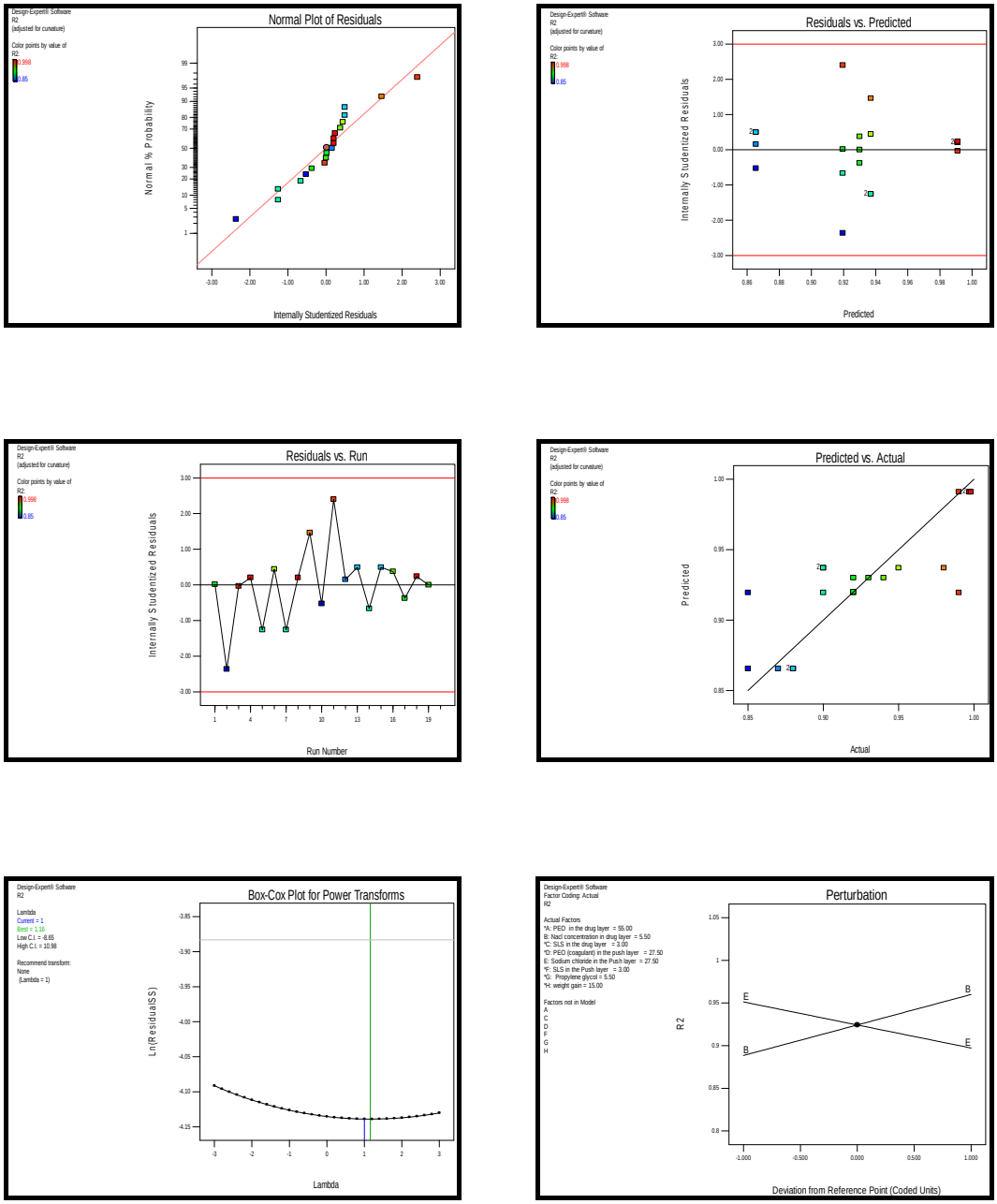
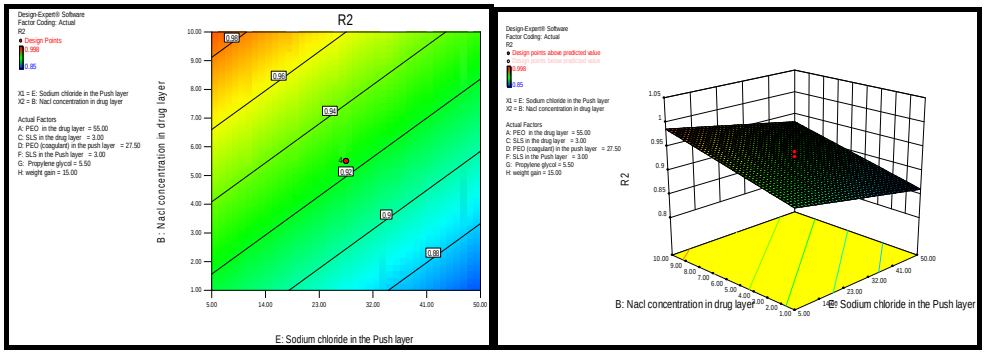


Figure No. 6.3.16: Plots for Testing the assumptions of the ANOVA and pertubation Curve



Figureo-6.3.17: Contour plot and RS Plot – Effect of NaCl DL and NaCl PL on R^2

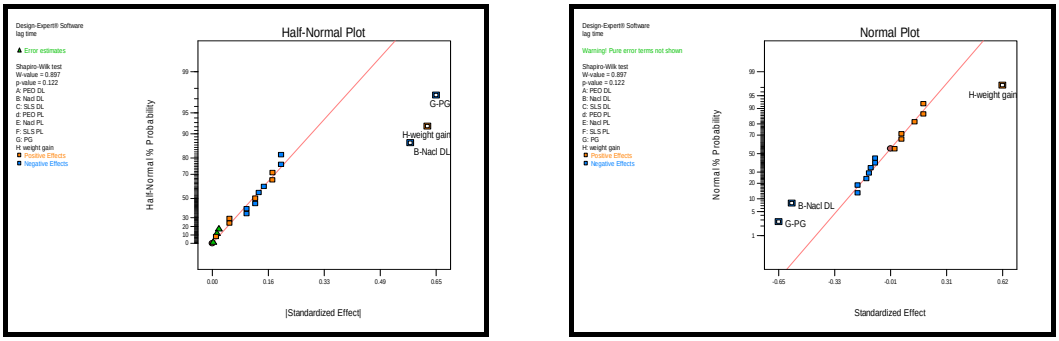
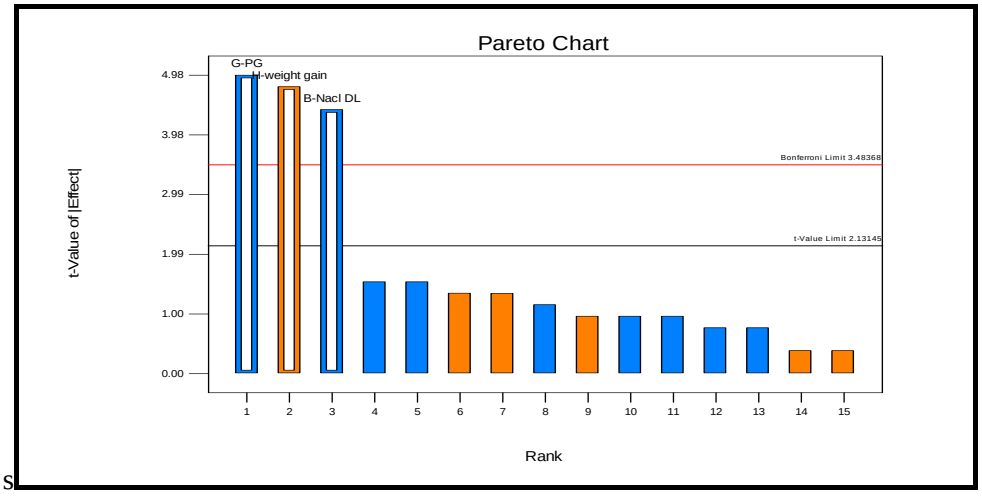


Figure No. 6.3.18: Half Normal plot of the effect of the factors on lag time
Figure No.6.3.19: Normal plot of the effect of the factors on lag time



S

Figure No. 6.3.20: Pareto chart of the effect of the factors on lag time

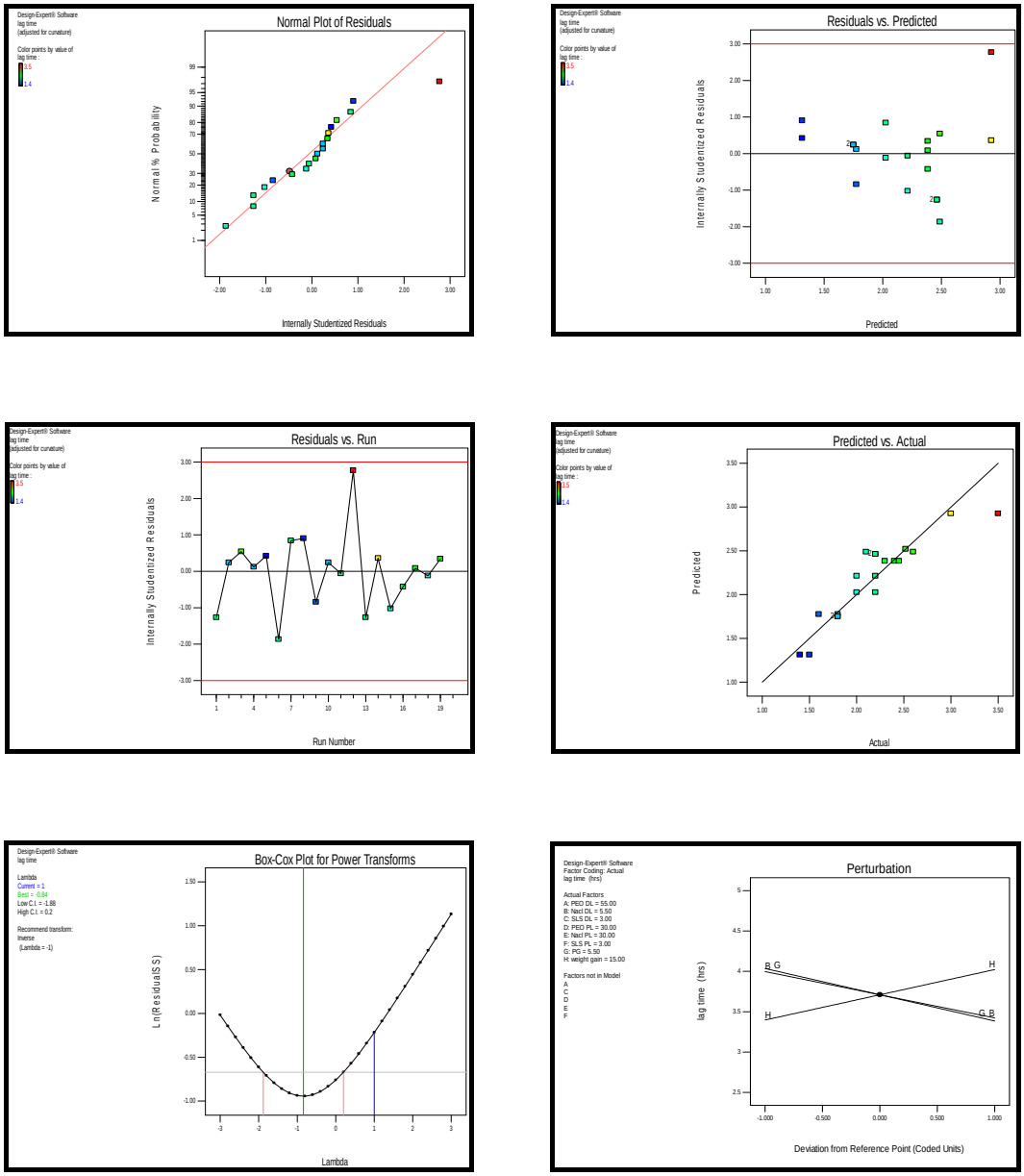


Figure No. 6.3.21: Plots for Testing the assumptions of the ANOVA and pertubation Curve

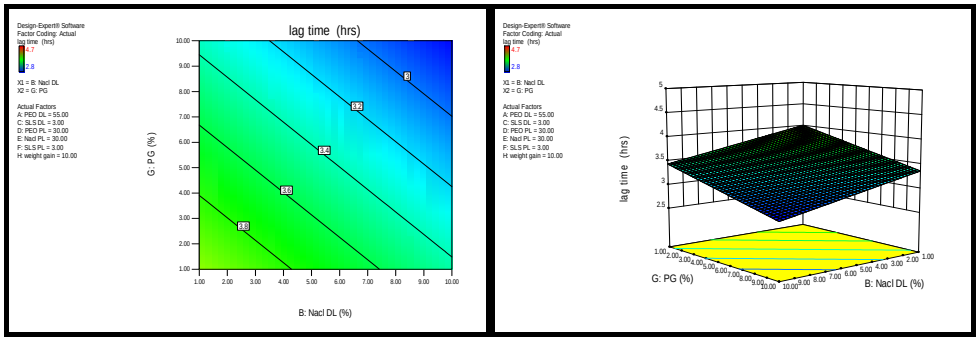


Figure No.6.3.22: Contour plot and RS Plot – Effect of NaCl DL and Propylene Glycol on lag time

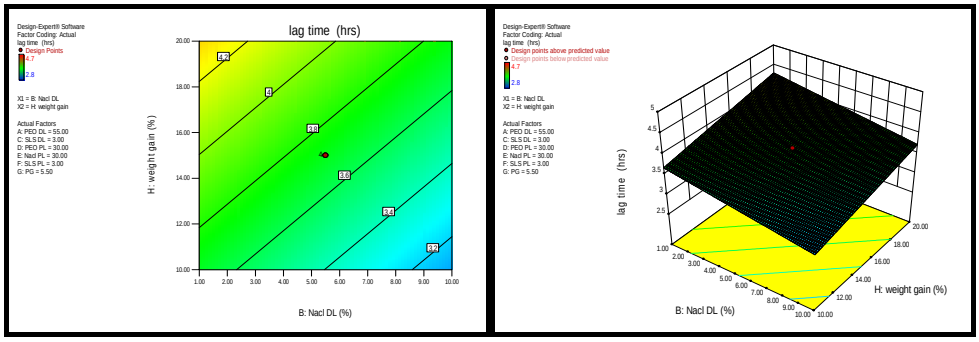


Figure No.6.3.23: Contour plot and RS Plot – Effect of NaCl DL and Weight gain on lag time

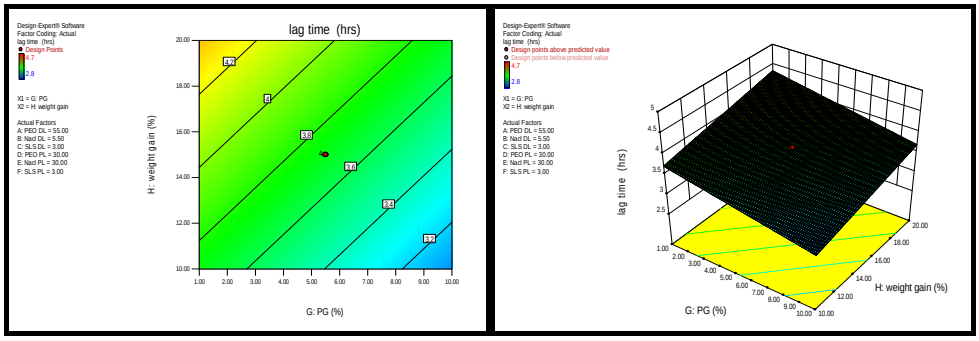


Figure No.6.3.24: Contour plot and RS Plot – Effect of Weight gain and Propylene Glycol on lag time

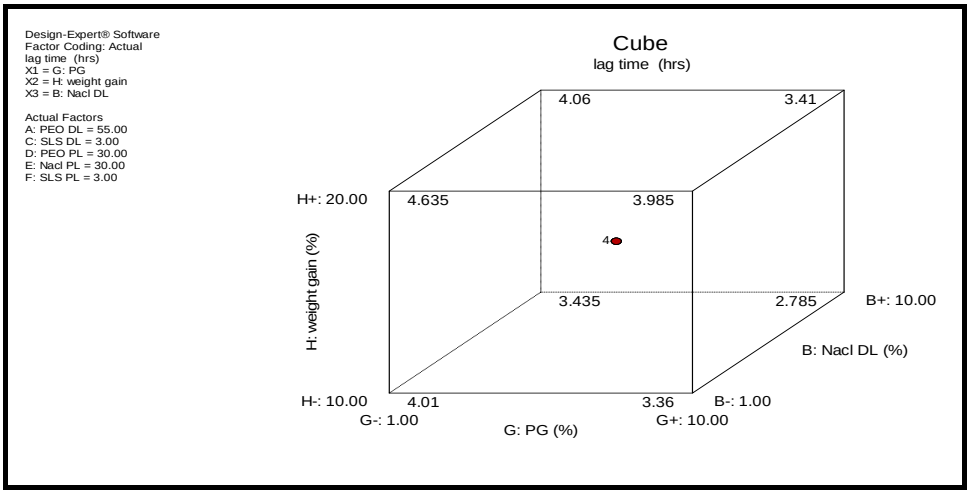


Figure No.6.3.25: Cube plot of effect of NaCl DL, Propylene glycol and Weight gain on lag time

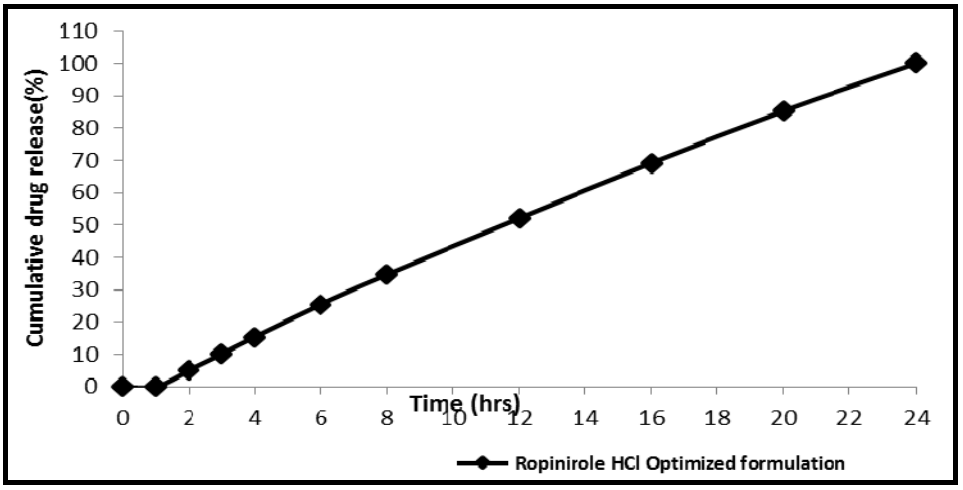


Figure No.6.3.26: In-vitro dissolution study of the optimized batch

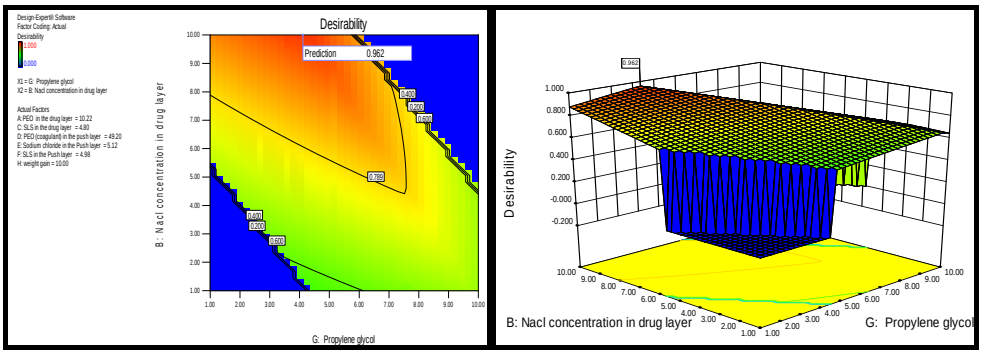


Figure No.6.3.27: Desirability contour plot and RS plot – Effect of NaCl DL and propylene Glycol

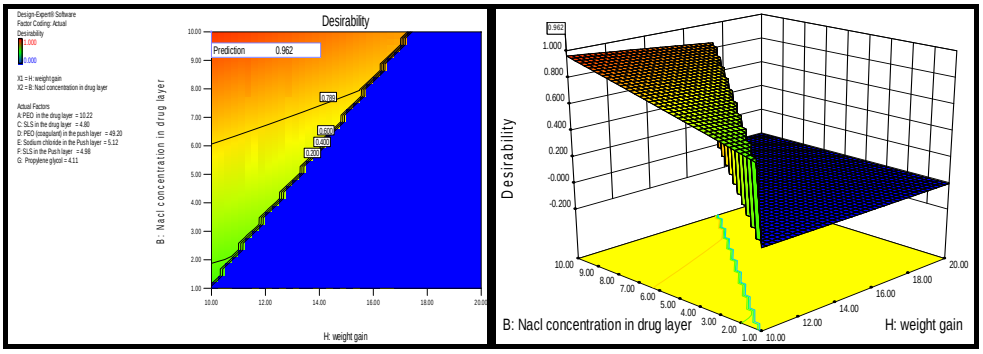


Figure No.6.3.28: Desirability contour plot and RS plot – Effect of NaCl DL and weight gain

.

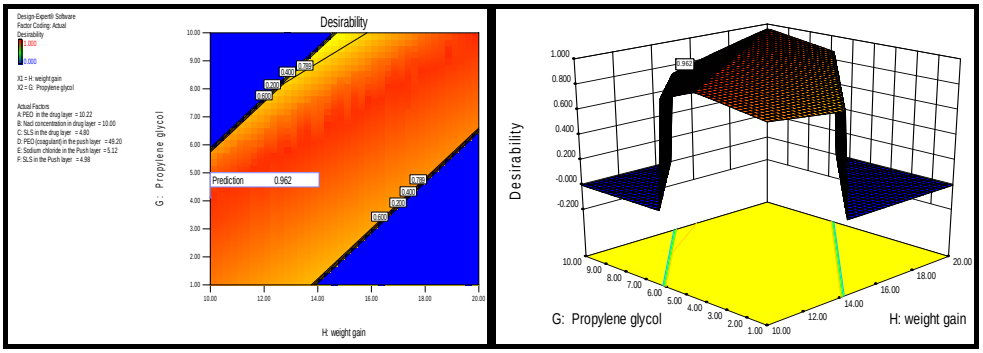


Figure No.6.3.29: Desirability contour plot and RS plot – Effect of Weight gain and Propylene glycol

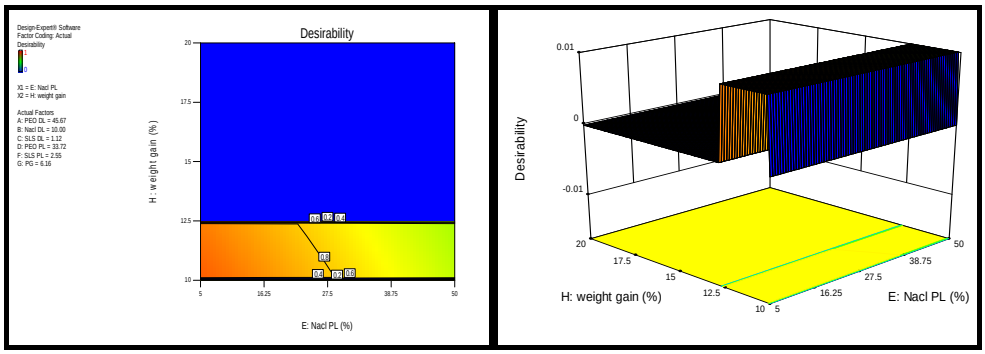


Figure No.6.3.30: Desirability contour plot and RS plot – Effect of Weight gain and NaCl PL

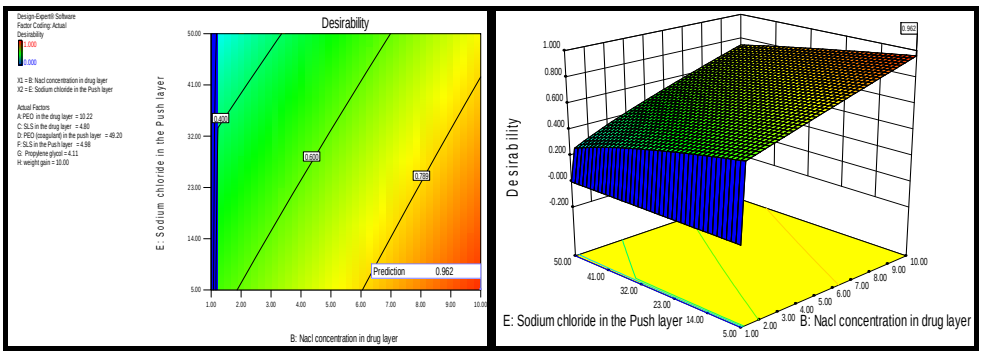


Figure No.6.3.31: Desirability contour plot and RS plot – Effect of NaCl DL and NaCl PL

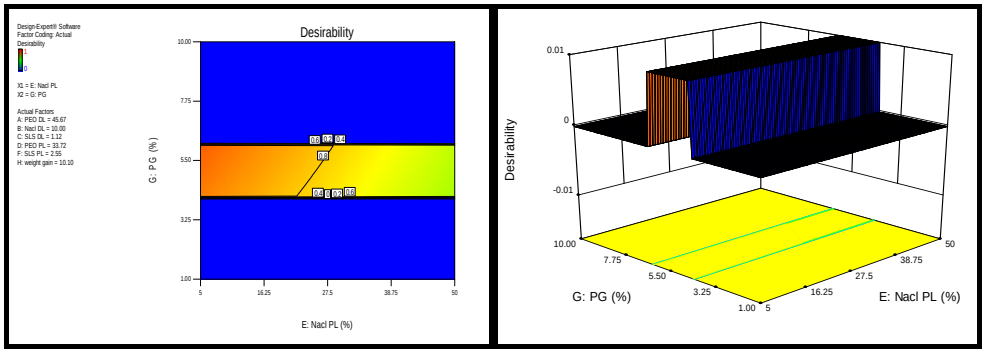


Figure No.6.3.32: Desirability contour plot and RS plot – Effect of NaCl PL and Propylene Glycol

6.3.4. Product development and optimizationof push –pull osmotic tablets of Ivabradine HCl

I. Formulation of push pull osmotic tablets of Ivabradine HCl

The Factor influence study batches of Ivabradine HCl (IB1 to IB20) were formulated according to the methodology given in the section 5.6.4(I A-D) which explains preparation of granules, compression of core bilayer tablets, coating of core tablets and drilling of coated tablets. Quantities of the ingredients were set according to the range specified in the literatures. The compositions taken for preparation of factor influence study batches were shown in Table No.5.6.2. The levels and responses fixed for the study was given in Table No.5.6.3 and 5.6.4.

The design table in coded values for the formulation development of Ivabradine HCl osmotic tablets was given in the Table No.5.6.5. The final formula for the factor influence study and optimization of Ivabradine HCl push pull OT was shown in the Table No. 6.3.20 and 6.3.21.

II. Evaluation of the formulations

The batches IB1 to IB20 were evaluatedsimultaneously while preparing. They were subjected to blend as well as whole tablet evaluation. The procedures for the evaluation were given in the chapter section 5.6.4(IIA&B).

A. Blend evaluation

The prepared granules of both the layers i.e. drug layer and push layer were evaluated by means of various tests. The tests were carried out according to the methodology given in the section 5.5.4 to 5.5.7.The results of the various blend evaluation were mentioned in the table No.6.3.22.

B. Tablet evaluation

The prepared tablets were evaluated for weight variation, hardness, friability, assay, weight gain, pore size diameter and thickness. The tests were performed as per the methodology given in chapter section 5.6.4(IIB). The results of various tests were shown in Table No.6.3.23.

Table No. 6.3.20:Formula for the trials IB1- IB10

S. No.	Ingredients	IB1 mg/tab	IB2 mg/tab	IB3 mg/tab	IB4 mg/tab	IB5 mg/tab	IB6 mg/tab	IB7 mg/tab	IB8 mg/tab	IB9 mg/tab	IB10 mg/tab
Drug Layer											
1	Ivabradine HCl	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913
2	DCP	36.06	26.21	27.50	17.66	34.04	24.21	25.50	15.66	36.05	26.21
3	PEO400 K	1.091	10.913	1.091	10.913	1.091	10.913	1.091	10.913	1.091	10.913
4	NaCl	0.950	0.950	9.500	9.500	0.950	0.950	9.500	9.500	0.950	0.950
5	BHT	0.001	0.011	0.001	0.011	0.001	0.011	0.001	0.011	0.001	0.011
6	SLS	0.50	0.500	0.500	0.500	2.500	2.500	2.500	2.500	0.500	0.500
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg.stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total weight of drug layer		50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Push layer											
9	PEO 7000 K	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	25.00	25.00
10	Sodium chloride	0.13	1.25	1.25	0.13	1.25	0.13	0.13	1.25	1.25	12.50
11	DCP	39.62	38.50	36.70	37.82	36.70	37.82	39.62	38.50	14.18	2.93
12	BHT	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.03	0.03
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg. stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of Push layer		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	9.4	17.3	18.8	8.6	8.6	18.8	17.3	9.4	17.3	9.4
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.09	1.73	0.19	0.86	0.86	0.19	1.73	0.09	1.73	0.09
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	19.0	9.5	19.0	9.5
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**114.0**	**104.5**	**114.0**	**104.5**

Table No. 6.3.21:Formula for the trials IB11- IB20

S. No.	Ingredients	IB11 mg/tab	IB12 mg/tab	IB13 mg/tab	IB14 mg/tab	IB15 mg/tab	IB16 mg/tab	IB17 mg/tab	IB18 mg/tab	IB19 mg/tab	IB20 mg/tab
Drug Layer											
1	Ivabradine HCl	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913	10.913
2	DCP	27.495	17.664	34.045	24.214	25.495	15.664	25.854	25.854	25.854	25.854
3	PEO 400 K	1.091	10.913	1.091	10.913	1.091	10.913	6.002	6.002	6.002	6.002
4	NaCl	9.50	9.500	0.950	0.950	9.500	9.500	5.225	5.225	5.225	5.225
5	BHT	0.001	0.011	0.001	0.011	0.001	0.011	0.006	0.006	0.006	0.006
6	SLS	0.50	0.50	2.50	2.50	2.50	2.50	1.50	1.50	1.50	1.50
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	
8	Mg. stearate	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total weight of drug layer		50.000	50.000	50.000	50.000	50.000	50.000	50.000	50.000	50.000	50.000
Push layer											
9	PEO 7000 K	25.00	25.00	25.00	25.00	25.00	25.00	13.75	13.75	13.75	13.75
10	NaCl	12.50	1.25	12.50	1.25	1.25	12.50	3.78	3.78	3.78	3.78
11	DCP	4.73	15.98	4.73	15.98	14.18	2.93	23.81	23.81	23.81	23.81
12	BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
13	SLS	0.45	0.45	0.45	0.45	2.25	2.25	1.35	1.35	1.35	1.35
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	
16	Mg.sterate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of Push layer		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	8.6	18.8	18.8	8.6	9.4	17.3	13.5	13.5	13.5	13.5
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.86	0.19	0.19	0.86	0.09	1.73	0.74	0.74	0.74	0.74
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	14.3	14.3	14.3	14.3
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**109.3**	**109.3**	**109.3**	**109.3**

Table No.6.3.22: Blend evaluation of the DL and PL of push –pull OTs of Ivabradine HCl

Trials	Angle of repose		Bulk density(g/ml)		Tapped density(g/ml)		Hausner's ratio		Carr's index(%)	
	DL	PL	DL	PL	DL	PL	DL	PL	DL	PL
IB 1	25.44	23.12	1.12	0.98	1.32	1.12	1.17	1.14	15.15	12.5
IB 2	26.33	22.87	1.05	0.92	1.3	1.09	1.23	1.18	19.23	15.59
IB3	24.88	25.08	1	0.89	1.2	1.02	1.2	1.14	16.66	12.74
IB4	25.87	23.44	1.11	0.95	1.32	1.08	1.18	1.13	15.90	12.03
IB5	25.69	22.55	0.97	0.87	1.15	0.99	1.18	1.13	15.65	12.12
IB6	24.36	25.03	1.14	0.93	1.4	1.12	1.22	1.20	18.57	16.96
IB7	25.08	24.8	1.11	0.88	1.36	1.04	1.22	1.18	18.38	15.38
IB8	26.56	23.17	1.2	0.84	1.46	0.97	1.21	1.15	17.80	13.40
IB9	27.09	24.33	1.09	0.9	1.35	1.06	1.23	1.17	19.25	15.09
IB10	25.87	24.56	1.12	0.86	1.38	0.99	1.23	1.15	18.84	13.13
IB11	26.15	24.97	1.15	0.92	1.4	1.1	1.21	1.19	17.85	16.36
IB12	25.33	22.34	1.18	0.94	1.43	1.1	1.21	1.17	17.48	14.54
IB13	26.55	25.11	1.12	0.87	1.38	1.05	1.23	1.20	18.84	17.14
IB14	24.33	25.32	1.1	0.79	1.34	0.88	1.21	1.11	17.91	10.22
IB15	28.56	24.42	1.06	0.95	1.25	1.1	1.17	1.15	15.23	13.63
IB16	24.88	23.33	1.09	0.86	1.3	1.02	1.19	1.18	16.15	15.68
IB17	26.67	24.14	1.12	0.78	1.37	0.95	1.223214	1.21	18.24	17.89
IB18	27.98	24.38	1.22	0.86	1.46	0.99	1.196721	1.15	16.43	13.13
IB19	24.65	24.54	1.22	0.88	1.5	1.05	1.229508	1.19	18.66	16.19
IB20	25.77	25.63	1.08	0.96	1.34	1.12	1.240741	1.16	19.40	14.28

Table No.6.3.23:Whole tablet evaluation push pull OTs of Ivabradine HCl

Trials	Wt variation (n =20)	Diameter mm(n=5)	Thickness mm(n=5)	Hardness Kg/cm^2 (n=6)	Friability (%)	Assay (%)	Weight gain(%)	Pore size (mm)
IB1	103.8±1.31	5.2±0.10	3.62±0.02	4.6±0.08	0.56	102±2.3	10.2±1.2	0.60
IB2	113.5±1.53	5.14±0.11	3.64±0.05	4.6±1.2	0.68	98±1.4	20.05±1.2	0.60
IB3	114.56 ±1.8	5.10±0.16	3.57±0.12	4.8±0.88	0.78	101.2±2.1	20.5±0.88	0.60
IB4	104.5 ± 1.05	5.02±0.12	3.61±0.01	5±0.68	0.86	99.2±1.8	10.06±0.17	0.60
IB5	103.6 ± 2.32	5.04±0.15	3.5±0.06	4.4±1.6	0.48	98.3±2.1	10.12±0.04	0.60
IB6	114.6 ±3.61	5.13±0.10	3.61±0.06	5.1±0.82	0.66	99.8±2.9	20.06±0.05	0.60
IB7	113.5 ± 2.0	5.02±0.14	3.7±0.06	3.8±2.01	0.88	101.3±2.1	20.08±0.12	0.60
IB8	105.62 ±1.7	5.04±0.15	3.8±0.03	4.4±1.2	0.75	99.6±1.8	10.08±0.02	0.60
IB9	114 ± 2.52	5.11±0.12	3.64±0.08	4.4±0.62	0.54	101.2±2.5	20.06±0.03	0.60
IB10	103.8 ± 1.01	5.01±0.08	3.63±0.04	4.8±0.56	0.59	99.3±1.7	10.04±0.01	0.60
IB11	104.9 ± 1.1	5.06±0.06	3.61±0.11	4.8±0.4	0.89	101±1.7	10.06±0.07	0.60
IB12	115.34 ± 2.40	5.12±0.02	3.62±0.03	4.6±0.45	0.92	100.8±2.1	20.02±0.08	0.60
IB13	113.67 ± 1.49	5.1±0.06	3.64±0.21	4.4±0.68	0.78	100.6±1.9	20.06±0.04	0.60
IB14	103.8±1.38	5.13±0.08	3.58±0.01	4.2±.0.44	0.82	99.7±2.1	10.14±0.01	0.60
IB15	103.92 ±1.93	5.12±0.08	3.72±0.10	4.8±0.68	0.69	98.2±2.1	10.06±1.2	0.60
IB16	114.5 ± 1.35	5.12±0.08	3.64±0.06	4.7±0.22	0.78	99.62±2.12	20.06±0.05	0.60
IB17	109.2 ±3.63	5.14±0.05	3.68±0.09	4.5±0.56	0.69	97.9±2.1	15.1±.0.05	0.60
IB18	109.2± 2.52	5.12±0.06	3.74±0.04	4.98±0.09	0.52	99.28±1.2	15.08±0.12	0.60
IB19	108.5 ± 1.45	5.1±0.04	3.8±0.02	4.96±0.03	0.91	100.2±2.5	15.02±0.01	0.60
IB20	109.45 ±0.8	5.13±0.04	3.78±0.02	4.58±0.17	0.76	99.8±1.6	15.05±1.1	0.60

C. *Invitro* dissolution tests

In vitro dissolution study was carried out for all the batches as per methodology given in the chapter section 5.6.4(IIBix).The release profile of IB1 to IB20 batches were shown Table No.6.3.24 and 6.3.25, Figure No. 6.3.33 and 6.3.34.

Table 6.3.24: Dissolution profile of formulation IB1 – IB10

Time (hrs)	Cumulative drug release (%)									
	IB1	IB2	IB3	IB4	IB5	IB6	IB7	IB8	IB9	IB10
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	7.3±0.5	4.4±0.1	0	5.5±0.2	5.2±0.8	1±0.08	0
3	7.8±0.33	8±0.1	10±0.2	10.1±0.5	7.5±2.1	1.2±0.1	8.3±0.7	10±0.6	3.8±0.4	3±0.3
4	9.3±2.1	12.1±1.2	10.1±1.2	20.3±1.9	12.3±1.2	8.9±0.6	15.3±1.9	19.1±0.4	9.3±1.5	9.6±1.9
6	22.1±1.8	25.4±1.9	25.1±0.7	30.3±1.5	32.5±0.6	12.1±0.7	22.4±1.8	26±0.4	20.1±3.1	25±2.8
8	30.5±3.2	34.2±0.7	33.3±2.1	45.6±4.3	47.2±0.6	19.3±1.1	30.2±1.1	45. ±1.83	33.1±1.6	35±2.8
12	48.5±2.2	42.6±1.1	51.4±2.7	61.1±2.3	65.6±1.2	23.2±1.8	44.1±4.3	70.3±2.6	53.2±1.9	65±2.8
16	57.4±1.1	47.1±2.3	60.2±2.6	80.3±4.2	71.1±.18	28.1±2.7	58.6±3.6	82.1±3.2	68.3±2.3	70.3±3.5
20	65.2±2.1	49.3±2.6	65.4±1.8	100±3.2	78.4±2.1	33.4±3.9	71.2±3.8	92.3±4.1	75.6±3.1	75.2±4.7
24	70.1±1.8	52.3 ±2.8	70.4±1.5	100±4.5	82.2±2.9	35.3±3.8	84.2±2.5	100±3.4	82.5±2.9	76±2.6

Table No.6.3.25: Dissolution profile of formulation IB11 – IB20

Time(hrs)	Cumulative drug release (%)									
	IB11	IB12	IB13	IB14	IB15	IB16	IB17	IB18	IB19	IB20
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	7.4±0.2	0	0	0	5.2±0.5	3.8±0.3	0	1±0.06	0	2.5±0.1
3	17.3±1.1	3.3±0.1	0	3.3±0.2	9.1±0.4	9.8±0.1	7.6±0.6	8.6±0.1	6.6±0.3	8.8±0.1
4	25.5±3.2	7.2±0.1	5±0.3	8.9±0.4	13.5±1.7	23.8±0.8	10±0.06	15.8±0.16	10±0.6	18.8±0.6
6	45.1±4.1	14.3±0.6	16±0.8	28.1±1.6	20.5±2.3	35.4±2.6	20.2±1.1	24.6±1.7	20.5±1.8	27.4±1.3
8	54.3±2.6	19.3±0.7	27.6±2.7	40.1±1.3	28.2±3.2	46.9±0.5	34.8±3.2	33.9±1.9	34±1.5	39.7±4.1
12	76.2±1.8	28.4±1.9	39.7±2.6	72.3±1.7	42.4±1.2	70.4±4.2	59.1±1.4	54.7±2.9	53±0.4	58.1±1.5
16	90.5±2.5	38.2±2.3	45±2.9	86.1±3.3	55.3±2.9	89±4.8	73±1.4	70.3±3.6	70.4±1.1	72.5±1.9
20	100±2.8	46.4±2.8	47.1±1.1	88.2±3.8	67.5±3.1	99.1±1.3	76.7±3.2	74.4±4.2	75.2±2.3	76.8±3.2
24	100±1.8	55.1±3.3	50±2.8	90.2±1.2	78.5±0.6	100±2.3	80.5±1.7	79.2±1.5	80.2±3.3	79±1.2

D. Coating Evaluation

The coating evaluation was done as per the methodology described in chapter section 5.6.4(IIC). The results were shown in Table No.6.3.9.From the study it was proven that the elastictity of the film increased as the plastisizer concentration increases.

III. Factor influence study

The *invitro* evaluation of all the 20 trials were performed and the nessessary values for the factor influence study was recorded. The design matrix and the responses for the factor influence study was given in the Table No.6.3 26.

Table No.6.3.26: Result of the factor influence study

Trials	CUR at 24 hr	R^2	Lag time
IB1	72	0.952	4.1
IB 2	52	0.849	3.5
IB 3	70	0.932	4
IB 4	100	0.997	3
IB 5	82	0.871	3.5
IB 6	35	0.953	4.5
IB 7	84	0.999	3.6
IB 8	100	0.955	3
IB 9	83	0.960	4.2
IB 10	76	0.858	4.1
IB 11	100	0.969	2.9
IB 12	55	0.998	4.7
IB 13	50	0.857	5
IB 14	90	0.861	3.5
IB 15	78	0.998	3.5
IB 16	100	0.978	3.1
IB 17	80	0.926	4
IB 18	79	0.946	3.4
IB 19	80	0.933	4
IB 20	79	0.923	3.6

A. FDS curve

The FDS graph for the selected design with the selected factors and responses was shown as flatter curve.The curve indicated a high FDS. so the design space predicted by the selected model had useful precision. The graph is given in the Figure No.6.3.35.

B. Standard error graph

The standard error prediction for areas in the design space for the different factor were shown in Figure No.6.3.36.The values were found to be between 0.25–0.4. So it was proven that the standard error through out the design space was relatively very low. The entire design space will be having a very less prediction error for the selected design.

C. Analysis of the responses

1. Cumulative release at 24 hrs

The cumulative release of the different formulations were analysed and different factors affecting the PCUR at 24 hrs were identified with the help of different evaluation tools explained below,

i) Half normal plot

The half-normal plot shown in the Figure No.6.3.37 was used to identify the significant factors affecting the PCUR at 24 hrs. From the graph it was evident that the factor which were affecting the cumulative release up to 24 hrs were B (NaCl DL), G (propylene glycol) H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.499. This indicated the non significance of the non selected factors**.**

ii) Normal plot

From normal plot shown in the Figure No.6.3.38, it was evident that the factor B,G and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.499. Thisindicated that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

From the pareto chart shown in Figure No.6.3.39, it was clearly evident that the factors B (NaCl DL), G (propylene glycol) and H (weight gain) were significantly affecting the PCUR at 24 hrs. All the significant factors crossed the t limit. Factors G & H crossed the Bonferroni limit. The magnitude of the

effect can be written as G > H > B. With an Increase in the concentration of NaCl DL and propylene glycol, PCUR at 24 hr was found to be increased. But with an increase in the weight gain, PCUR at 24 hr was decreased. Non significant term effects and interaction effects were present below the t limit.

iv) ANOVA and regression analysis

In this case B (p *=0.0062)*, G (p *=0.0018)*, and H(p *=0.0024)*were the significant model terms. The Model F-value of 12.29 implied that the model was significant. The "Lack of Fit F-value" of 5.00 implieed the lack of fit was not significant relative to the pure error. No lack of fit, curvature effect and interactions were significant. The "Pred R-Squared" of 0.4937 was in reasonable agreement with the "Adj R-Squared" of 0.6407. This indicated the linearity of the model. Adeq Precision" was 11.729 indicated an adequate signal. So this model can be used to navigate the design space.

Table No.6.3.27: ANOVA and Regression analysis for the effect of factors on the PCUR at 24 hrs

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob >F	
Model	4489.19	3	1496.4	12.29	*0.0002*	*significant*
B-NaCl drug layer	1207.56	1	1207.56	9.92	*0.0062*	
G- Propylene Glycol	1701.56	1	1701.56	13.98	*0.0018*	
H-Weight gain	1580.06	1	1580.06	12.98	*0.0024*	
Residual	1947.76	16	121.74			
Lack of Fit	1861.76	13	143.21	5	0.1056	*not significant*
Pure Error	86	3	28.67			
Cor Total	6436.95	19				
Regression analysis						
Std. Dev.	11.03		R-Squared	0.6974		
Mean	78.05		Adj R-Squared	0.6407		
C.V. %	14.14		Pred R-Squared	0.4937		
PRESS	3259.27		Adeq Precision	11.729		

v) Polynomial equation

From the regression analysis the polynomial equation which can represents the PCUR at 24 hrs can be constructed. The positive sign of the coefficients in the equation indicates the positive and the negative sign indicates the negative effect on the response. Larger the coefficients larger will be the

effects.

Cumulative percent drug release in24 hours = +78.05+8.69 * *B*+10.31* *G* - 9.94* H*(coded units)*

Cumulative percent drug release in 24 hours= +84.64028+1.93056 * NaClin drug layer+2.29167 *Propylene Glycol -1.98750 * weight gain *(actual units)*

vi) Tests for the assumptions of the ANOVA

The ANOVA assumtions were tested and studied with the help of various graphs shown in the Figure No.6.3.40.

- *The normal probability plot:* The plot indicated that residuals follow a normal distribution, as the points follows a straight line.
- *Residuals vs Predicted*: The plot showed a random scatter (constant range of residuals across the graph).This confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: The plot showed a random scatter. Absence of any Trends in the graph indicated that no time-related variable lurking in the background.
- *Predicted vs.Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms:*The lamda value was1 and no transformation was needed.

vii) The perturbation graph

The pertubation curve shown in the Figure No.6.3.40 explained the effect and sign of the significant factors on the PCUR at 24 hrs. It showed that a change in the concentration of factor B from minimum to maximum have produced an increase in PCUR 24 hr from 70 to 85. For factor G the increase in response was still higher (65 to 90). But increase in factor H showed a decrease in the response from 88 to 67.

viii) Contour plot and RS plot

Figure No.6.3.41 shows the contour plot and response surface plot of the

simultaneous effect of factors B(NaCl DL) and G(propylene glycol) on the PCUR at 24 hrs. At lower concentration of propylene glycol, NaCl DL had little effect on the response.High PCUR was obtained at high levels of both the factors. From the RS plot it was evident that the propylene glycol had a greater effect than NaCl DL.

Figure No.6.3.42,the contour plot and RS plot showed the change in PCUR at 24hr with the change in factors H (weight gain) and B (NaCl DL). NaCl DL had a positive effect and weight gain had an opposite effect on the response. At the low levels of weight gain, NaCl DL had a prominent effect. At higher weight gain even a high concentration of NaCl DL was not able to produce 80 % cumulative release of the drug. Form the surface plot it was evident that weight gain had a greater effect on the the response than NaCl DL.

Contour plot and RS plot shown in Figure No.6.3.43 explained the simultaneous effect of factors H(weight gain) and G(propylene glycol) on the PCUR at 24hrs. At 10 % of the weight gain the response was more prone to slight changes of proplylene glycol. But at a higher weight gain even a 10% of propylene glycol was not sufficient to achieve 80% CUR at 24hrs. From the response surface plot, it was evident that, as the concentration of propylene glycol increases, the PCUR will be increasing. The weight gain had a reciprocating effect on the PCUR. RS plot showed that at both the levels of propylene glycol, change in weight gain had a negative effect on the response.

ix) Cube plot

This cube plot shown in Figure No. 6.3.44 explained the combined effect of B, G, and H. When all the three factors were at minimum the PCUR at 24 hrs was about 68.9875, and at maximum it was around 87.1125. But a similar response can be achieved by keeping propylene glycol at its minimum, NaCl DL at its maximum and weight gain at its minimum.

2. Analysis of responses - Rate constant(R^2)

i) Half normal plot

From the graph shown in the Figure No.6.3.45 it was evident that the factor which were affecting the release rate constant (R^2) were B (NaCl DL) and E (NaCl PL). The Shapiro-Wilk normality test displayed the p value as 0.741.This indicated the non significance of the non selected factors**.** So no other factors except B and E were affecting the zero order release rate constant.

ii) Normal plot

From the normal plot shown in the Figure No.6.3.46 it was understood that the factor B and E were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.741 indicated that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

The pareto chart shown in Figure No.6.3.47 represents the significant effect of B and E on the zero order rate constant. Both the factors crossed the t and Bonferroni limit. This confirmed the obvious effect of these factors on the zero order rate constant. The magnitude of the effect can be written as B > E. R^2 approched unity with an increase in the concentration of factor B. But increase in the concentration of factor E had an opposite effect. No other terms were significant, as they all were below the t limit.

iv) ANOVA and regression analysis

The Model F-value of 29.11 implied the model was significant. Factors B (p *<0.0001)*, E(*0.0002)*were the significant model terms.The "Lack of Fit F-value" of 0.3706 implied the lack of fit was not significant. This means that the polynomial model was fitting all of the design points well. The model selected was significant and no lack of fit was observed. No interactions were significant. The "Pred R-Squared" of 0.6794 was in reasonable agreement with the "Adj R-

Squared" of 0.7474 indicated the linearity of the model. Adeq Precision 13.807indicated an adequate signal. So this model can be used to navigate the design

Table No.6.3.28:ANOVA and Regression analysis of the effect of factors on the R^2

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F	
Model	*0.042*	*2*	*0.021*	*29.11*	< 0.0001	*Significant*
B-NaCl DL	*0.027*	*1*	*0.027*	*36.83*	*<0.0001*	
E-NaCl PL	*0.016*	*1*	*0.016*	*21.39*	*0.0002*	
Residual	0.012	17	7.30E-04			
Lack of Fit	0.011	14	7.86E-04	1.68	0.3706	*not significant*
Pure Error	1.40E-03	3	4.67E-04			
Cor Total	0.055	19				
Regression analysis						
Std. Dev.	0.027		R-Squared	0.774		
Mean	0.93		Adj R-Squared	0.7474		
C.V. %	2.91		Pred R-Squared	0.6794		
PRESS	0.018		Adeq Precision	13.81		

v) Polynomial equation

The polynomial equation representing the R^2 can be witten as follows,

R^2 = 0.93 + 0.041* *B* - 0.031 * *E(Coded values)*

R^2 = 0.91577 +9.10833E-003* NaCl concentration in drug layer - 1.38833E-003* Sodium chloride in the push layer *(Actual values)*

vi) Test for the assumption of ANOVA

- *The normal probability plot:* The plot indicated that residuals follow a normal distribution.
- *Residuals vs Predicted*: The plot was a random scatter (constant range of residuals across the graph.) confirmed the constant variance in the experiments performed .
- *Residuals vs Run*: The plot showed a random scatter. The graphs did not follow any treands indicates that no time-related variable lurking in the background.

- *Predicted vs. Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms:* The graph showed that the lamda value was 1 and no transformation was needed.

vii) The perturbation graph

The plot shown in Figure 6.3.48 explained the effect and sign of the significant factors on the zero order rate constant. It showed that the change in the concentration of NaCl DL from minimum to maximum produced an increase in zero order rate constant from 0.89 to 0.98. But an increase in the NaCl PL produced a decrease in the response from 0.96 to 0.90.

viii) Contour plots and RS plots

Figure No.6.3.49 shows the contour plot and response surface plot for the simultaneous effect of factor B(NaCl DL) and E (NaCl PL) on R^2. From the plot it was obvious that the factor B had a positive effect and E had a negative effect. High levels of NaCl DL and low levels of NaCl PL yields a better R^2 value. The change in concentration of NaCl DL was more evident at low level of NaCl PL. At high levels of NaCl PL even a high level of NaCl DL fails to to produce a R^2 value more than 0.95. From the surface plot the larger effect of NaCl DL than the NaCl PLwas clearly understood.

3. *Analysis of responses – lag time*

i) Half normal plot

Figure No.6.3.50 shows the half normal plot of the effect of factors on the lag time. The significant factor affecting the lag time was identified as B (NaCl in the DL) G (propylene Glycol) and H(weight gain). The Shapiro-Wilk normality test displayed the p value as 0.415. This indicateed the non significance of the non selected factors**.** So no other factors except B, G and H were affecting the lag time**.**

ii) Normal plot

Figure 6.3.51 shows the normal plot of the effect of factors on the lag time. The factor B, G and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.415. This indicated that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

FigureNo.6.3.52 shows the pareto chart of the effect of factors on lag time in terms of t value.The factors signficanly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect. Propylene Glycol had greater effect on the lag time. The magnitude of the effect of significant factors on the lag time can be written as G > H> B. No other factors or interaction terms were significant as they did not cross the t limit .

iv) ANOVA and regression analysis

The Model F-value of 20.35 implied that the model selected was significant. Factors B (*0.0017)*, G(*<0.0003)and H (0.0009)* were the significant model terms affecting the lag time. The "Lack of Fit F-value" of *17.19*,implied that the lack of fit was not significant relative to the pure error. This means that the polynomial model was fitting all of the design points well. Hence from the ANOVA analysis it was proved that the model selected was significant and no lack of fit was observed. No interaction terms were found to be significant. The "Pred R-Squared" of 0.6188 was in reasonable agreement with the "Adj R-Squared" of 0.7188, indicated the linearity of the model. "Adeq Precision was 13.862 indicated an adequate signal. So this model can be used to navigate the design space.

v) Polynomial equation

The polynomial equation for the lag time can be written as,

Lag time = 3.76 - 0.2875 * B - 0.35 * G +0.3125 * H *(Coded values)*

Lag time = 3.5211- 0.0639* NaCl DL-0.0722 * Propylene Glycol + 0.0625 * Weight gain *(Actual terms)*

Table No.6.3.29: ANOVA and regression analysis of the effect of factors on the lag time

Source	Sum ofSquares	df	Mean Square	F Value	p-value Prob > F	
Model	4.84	3	1.62	17.19	*< 0.0001*	*significant*
B-NaCl DL	1.32	1	1.32	14.08	*0.0017*	
G- PG	1.96	1	1.96	20.86	*0.0003*	
H-weight Gain	1.56	1	1.56	16.63	*0.0009*	
Residual	5.0E-004	15	0.094	1.05	0.5544	
Lack of Fit	1.50	*12*	*0.095*	17.19	< 0.0001	*not significant*
Pure Error	1.23	*3*	*0.090*			
Cor Total	0.27	19				
Regression analysis						
Std. Dev.	0.31		R-Squared	0.7632		
Mean	3.76		Adj R-Squared	0.7188		
C.V. %	8.15		Pred RSquared	0.6188		
PRESS	2.42		Adeq Precision	13.862		

vi) Test for assumptions of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No.6.3.53.

- *The normal probability plot:*The plot indicates that residuals follow a normal distribution.
- *Residuals vs Predicted*: The plot was a random scatter (constant range of residuals across the graph) confirmed the constant variance in the experiments performed.
- *Residuals vs Run:* The graph did not follow any treands indicated that no time-related variable lurking in the background.
- *Predicted vs. Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms:* The lamda value was 1 and no transformation was needed.

vii) Perturbation graph

This plot explained the effect and sign of the significant factors on lag

time. It showed that the change in the concentration of B and G from minimum to maximum produced a decrease in the lag time. The factor H had an opposite effect. The factor G had a major effect on the lag time.

viii) Contour plots and RS plots

Figure No.6.3.54 shows the contour plot and RS plot of the simultaneous effect of factor B(NaCl DL) and the G(propylene glycol) on lag time. At low levels of propylene glycol, change in NaCl DL produced a change in lag time from 4.5 to 3.8 hrs. Bt at high levels of propylene glycol it was from 3.7 to 3.2. NaCl DL had lesser effect at the high levels of propylene glycol.Response surface plot clearly represented the chief effect of factor G.

The Figure No.6.3.55 shows the contour plot and RS plot of the simultaneous effect of factor B(NaCl DL) and H(weight gain) on lag time. NaCl DL had a negative effect on the lag time ie, increase in the concentration from low to high had produced a decrese in lag time. But the weight gain had an opposite effect. The desired effect was produced while keeping low weight gain and high concentration of NaCl DL. NaCl DL had a prominent effect at low weight gain.

Figure No.6.3.56 shows the contour plot and response plot of the combained effect of factors G(propylene glycol) and H(weight gain) on lag time. Weight gain had a positive effect on the lag time and propylene glycol had a reverse effect.Effect of propylene glycol was more pronounced at low weight gain. From the RS plot the greater effect of the propylene glycol was well understood.

ix) Cube plots

This cube plot shown in Figure No.6.3.57 explains the combined effect of B, G and H. When all the three factors were at minimum the lag time 4.08 hr, and at maximum it was around 3.435. A better response would be observed when NaCl DL and propylene glycol at maximum and weight gain at its minimum.

IV. Numerical optimization with the help of desirability

From the factor influence study it was found that the model suggested was linear and no significant lack of fit and curvature were found for any of the responses. So no quadratic model was chosen for optimization. The same 2 level design was used for further optimization.

A. Optimization of the push pull OTs of Ivabradine HCl

When more than two factors were significant, overlay plot does not give a complete idea about the optimization. In our study three factor were significantly affecting the lag time as well as PCUR at 24 hrs. So the numerical method with desirability function was selected as the tool for optimization. The constaint fixed for the optimization was given in the Table No.6.3.30.The solutions of the numerical optimization was given in the Table No.6.3.31.

B. Point prediction

The point prediction for the solution 1, 2 and 15 were given in the Table No.6.3.32. The same batches were selected as the check point batches. The confidence interval and the tolerance interval for the solutions were given in the Table No.6.3.32.

Table No.6.3.30: Constraints fixed for the optimization of Ivabradine HCl push pull OTs

Constraints	Goal	Lower Limit	Upper Limit	Importance
Name				
B:NaCl concentration DL	is in range	1	10	3
E:Sodium chloride PL	is in range	5	50	3
G: Propylene glycol	is in range	1	10	3
H:Weight gain	is in range	10	20	3
PCUR drug release 24 hrs	is in range	95	100	5
R^2	maximize	0.84	0.999	4
lag time	minimize	2.9	5	3

C. Check point

To confirm the validity of the model, three formulations (1, 2 and 15) from the solutions were selected and formulated. The dissolutions were performed as per the method specified in section 5.6.4(IIBix). The values obtained from the dissolution study were given in the Table No.6.3.33. All the responses were within the confidence Interval and tolerance limits of the point predicted by the software. Hence it can be concluded that the model suggested for the design was a success and can be used for further predictions.

D. Optimized batch and evaluations

The optimized batch (IBOB), solution 1 from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. The composition of the optimized batch was given in the Table No.6.3.34. The optimized batch was prepared as per the procedure mentioned in the materials and method section 5.6.4(I). The blend as well as the whole tablet evaluation was performed as per the methods specified in section 5.6.4(II). The result of the study was given in the Table No.6.3.35. The invitro study was performed as per the methods mentioned in the section 5.6.4(IIBix) and the findings were given in the Table No.6.3.35 and in the Figure No.6.3.58.

Table No.6.3.31:Numerical solutions for the optimization of the Ivabradine HCl Push pull OTs

No	PEO DL*	NaCl DL	SLS DL*	PEO PL*	NaCl PL	SLS PL*	PG	weight gain	PCUR	R^2	lag time	Desirability
1	**11.18**	**10.00**	**2.06**	**36.80**	**5.00**	**3.88**	**7.43**	**10.01**	**101.0**	**0.9999**	**2.97**	**0.9649**
2	**57.79**	**10.00**	**2.72**	**14.28**	**5.00**	**2.14**	**9.99**	**13.51**	**99.98**	**0.9999**	**3.00**	**0.9649**
3	66.57	10.00	4.51	10.96	5.00	3.35	10.00	13.52	100.0	0.9999	3.00	0.9649
4	19.22	10.00	2.24	31.07	5.00	2.88	10.00	13.51	100.0	0.9999	3.00	0.9649
5	100.00	9.98	4.90	47.70	5.00	3.01	10.00	13.50	100.0	0.9999	3.00	0.9649
6	11.16	10.00	1.95	36.79	5.00	4.87	9.50	12.94	100.0	0.9999	3.00	0.9649
7	98.46	10.00	4.51	19.11	5.00	2.53	9.43	12.86	100.0	0.9999	3.00	0.9649
8	98.99	10.00	1.00	6.55	5.00	3.76	9.40	12.82	100.0	0.9999	3.00	0.9649
9	13.28	10.00	1.04	20.60	5.00	4.45	9.39	12.81	100.0	0.9999	3.00	0.9649
10	96.21	10.00	1.30	7.42	5.25	4.53	9.53	12.98	100.0	0.9999	3.00	0.9649
11	94.55	10.00	4.87	31.46	5.11	2.92	9.84	13.42	99.82	0.9999	3.00	0.9649
12	87.23	10.00	4.66	47.84	5.00	3.22	8.37	11.64	100.0	0.9999	3.00	0.9649
13	96.81	10.00	2.23	8.45	5.00	4.83	8.13	11.36	100.0	0.9999	3.00	0.9649
14	52.47	10.00	2.20	32.60	5.00	2.19	8.05	11.27	100.0	0.9999	3.00	0.9649
15	**41.06**	**10.00**	**3.72**	**47.92**	**5.00**	**4.20**	**6.99**	**10.05**	**99.99**	**0.9999**	**3.00**	**0.9649**
16	86.73	10.00	1.00	41.83	5.00	1.86	7.46	10.01	100.0	0.9999	3.00	0.9649
17	65.09	10.00	3.23	34.96	5.00	1.77	7.31	10.41	100.0	0.9999	3.05	0.9645
18	83.90	10.00	4.22	13.44	5.00	3.88	7.10	10.18	100.0	0.9999	3.05	0.9645
19	10.62	10.00	1.55	34.60	5.00	1.00	7.03	10.09	100.0	0.9999	3.05	0.9645
20	10.60	10.00	3.15	22.31	5.00	1.97	6.97	10.02	100.0	0.9999	3.05	0.9645
21	15.31	10.00	4.97	48.76	5.00	4.78	6.98	10.03	100.0	0.9999	3.05	0.9645
22	50.99	10.00	4.63	50.00	5.00	3.38	6.94	10.00	99.98	0.9999	3.05	0.9645
23	85.97	10.00	5.00	30.01	5.01	2.45	6.98	10.07	99.92	0.9999	3.05	0.9645
24	86.73	10.00	1.00	41.83	5.00	1.86	7.46	10.58	100.0	0.9999	3.04	0.9619

No	PEO DL*	NaCl DL	SLS DL*	PEO PL*	NaCl PL	SLS PL*	PG	weight gain	PCUR	R^2	lag time	Desirability
25	11.18	10.00	2.06	36.80	5.00	3.88	7.43	10.55	100.0	0.9999	3.04	0.9619
26	65.09	10.00	3.23	34.96	5.00	1.77	7.31	10.41	100.0	0.9999	3.05	0.9617
27	83.90	10.00	4.22	13.44	5.00	3.88	7.10	10.18	100.0	0.9999	3.05	0.9615
28	10.62	10.00	1.55	34.60	5.00	1.00	7.03	10.09	100.0	0.9999	3.05	0.9613
29	10.60	10.00	3.15	22.31	5.00	1.97	6.97	10.02	100.0	0.9999	3.05	0.9613
30	41.06	10.00	3.72	47.92	5.00	4.20	6.99	10.05	99.99	0.9999	3.05	0.9613
31	15.31	10.00	4.97	48.76	5.00	4.78	6.98	10.03	100.0	0.9999	3.05	0.9612
32	50.99	10.00	4.63	50.00	5.00	3.38	6.94	10.00	99.98	0.9999	3.05	0.9611
33	85.97	10.00	5.00	30.01	5.01	2.45	6.98	10.07	99.92	0.9999	3.05	0.9607
34	44.71	10.00	2.96	38.15	19.44	5.00	9.62	15.57	95.03	0.981	3.19	0.8634
35	100.00	10.00	3.37	15.23	25.88	2.35	9.52	12.96	100.0	0.9724	3.03	0.8619
36	99.13	10.00	4.21	23.57	25.63	1.10	7.27	10.70	99.33	0.9735	3.07	0.8567
37	100.00	9.94	4.58	33.60	21.01	1.00	9.87	15.79	95.08	0.9791	3.19	0.8548
38	10.00	7.55	5.00	31.44	13.20	1.00	8.99	10.00	99.95	0.9900	3.04	0.8530
39	10.08	10.00	3.90	49.99	29.15	1.97	6.76	10.02	99.52	0.9680	3.06	0.8393
40	35.23	9.62	4.98	50.00	22.88	1.52	8.43	13.85	95.00	0.9764	3.20	0.8342
41	18.71	10.00	3.77	44.44	33.05	1.00	6.95	10.00	100.0	0.9625	3.05	0.8219
42	100.00	9.86	1.26	16.17	32.80	4.98	7.07	10.00	100.0	0.9628	3.05	0.8189

*Non significant factors

Table No.6.3.32: Prediction of the responses

Response	Predicted Mean	Std Dev	SE Mean	CI for mean		99% ofPopulation	
				95% CI low	95% CI high	95% TI low	95% TI high
Solution 1							
PCUR at 24 hrs	101	11.033	4.6876	90.062	109.936	51.526	148.472
R^2	0.9999	0.0330	0.0138	0.9863	1.0245	0.8525	1.1383
Lag time	2.97	0.3065	0.1302	2.7537	3.3058	1.6832	4.3763
Solution 2							
PCUR at 24 hrs	99.98	11.03	4.6790	90.053	109.891	51.514	148.430
R^2	0.9999	0.0330	0.0138	0.9863	1.0245	0.8525	1.1383
Lag time	3.00	0.3065	0.1300	2.7552	3.3063	1.6847	4.3769
Solution 15							
PCUR at 24 hrs	99.99	11.033	4.5133	90.4322	109.567	51.8315	148.168
R^2	0.9999	0.0330	0.0138	0.9863	1.0245	0.8525	1.1383
lag time	3.00	0.3065	0.1254	2.7700	3.3016	1.6977	4.3739

Table No.6.3.33: Check point batches for the model validation of the Ivabradine HCl OTs

Batches	PCUR at 24 hrs	R^2	Lag time
Solution 1	100.02 ± 1.5	0.9985 ± 0.023	3.03±0.13
Solution 2	98.79 ± 3.9	0.9988±0.003	3.01±0.3
Solution 15	100.5 ±3.9	0.9993±0.0010	3.0±0.05

Table No.6.3.34: Composition of optimized batch

SL.No	Ingredients	Optimized batch(IBOB) mg/tab	(%w/w)
Drug Layer			
1	Ivabradine HCl	10.91	
2	Dibasic calcium phosphate	26.839	
3	PEO 400 K	1.220	11.18
4	Sodium chloride	9.500	10
5	BHT	0.00122	
6	SLS	1.030	2.06
7	IPA		
8	Magnesium stearate	0.500	
	Total weight of drug layer	**50**	
Push layer			
9	PEO 7000 K	18.40	36.8
10	Sodium chloride	0.92	5
11	Dibasic calcium phosphate	21.62	
12	BHT	0.0184	
13	SLS	1.75	3.88
14	Iron oxide Red	0.8	
15	IPA		
16	Magnesium stearate	1.5	
	Total weight of Push layer	45	
	Total weight of un coated tablet	95	
Functional coating			
15	Cellulose acetate	8.8	8.8
16	Acetone	q.s	
18	Water	q.s	
19	Propylene Glycol	0.66	7.4
	Total Weight of Coating	9.5	10.01
	Total tablet weight	104.5	
Responses	**PCUR at 24 hrs**	**R^2**	**Lag time(hrs)**
Predicted	101	0.9999	2.97
Observed	100.02 ± 1.5	0.9985 ± 0.023	3.03±0.13

Table No.6.3.35: Optimized batch evaluation

Trial	Wt variation (n =20)	Diameter (n=10)	Thickness (n=10)	Hardness (n=6)	Friability (%)	Assay (%)	Weight gain(%)	Pore size (mm)
IBOB	104.5 ±0.14	5.13 ±0.5	3.6 ±0.13	4.3 ±0.8	0.75	98.78 ±2.3	10.09 ±0.10	0.6

Dissolution Profile											
Time	0	1	2	3	4	6	8	12	16	20	24
PCUR	0	0	4.8 ± 0.7	9.8 ±0.4	14.7 ±1.8	22.9 ±1.7	31.9 ±2.1	46.9 ±2.2	65.7 ±3.0	85.4 ±1.1	100.02 ± 1.5

E. Desirability contour plot and RS plot

Desirability plots shows how all the targetted optimum conditions are met by changing two factors at a time. The Figure No.6.3.59 shows how factor NaCl DL and propylene glycol affect the desirability. Higher desirability will be acheived at maximum level of NaCl DL (more than 9%) and a lower concentarions propylene glycol (<5%). Lower concentrations of both factors yield a desirability less than 0. 6.

Figure 6.3.60 shows effect of weight gain and NaCl DL on desirability. The desirability was higest at high concentration (>9) of NaCl DL and the low concentration (less than 14) of weight gain.

Figure 6.3.61 shows the desirability contour plot of weight gain and propylene glycol. A larger portion of the contour plot shows the desirability close to one, indicated that these two were the major factors for acheiving the desired optimum conditions.

The Figure No.6.3.62 shows the desirability contuor plot of NaCl PL and the weight gain.Form the plot it was evident that a wide range of NaCl PL can be used to get desirability more than one. Weight gain was again proved as one of the inflexible factors as a a slight change in factor showed a greater leap in the desirability from 1 to 0.2 .

The Figure No.6.3.63 shows the desirability contour plot and the RS plot of simultaneous effect of NaCl DL and NaCl PL on the desirability. Optimum conditions reached while keeping the NaCl DL at high level and the NaCl PL at low level. Below 3 % of NaCl DL,change in concentration of NaCl PL had no effect on the desirability. Change in the concentration of NaCl PL from low to

high produced a decrease in the desirability.

Figure No.6.3.64 shows the desirability contour plot and the RS plot for simultaneous effect of propylene glycol and NaCl PL.Desirability increased with decresing concentration of propylene glycol and NaCl PL.High levels of propylene glycol had a desirability zero at low level of NaCl PL.

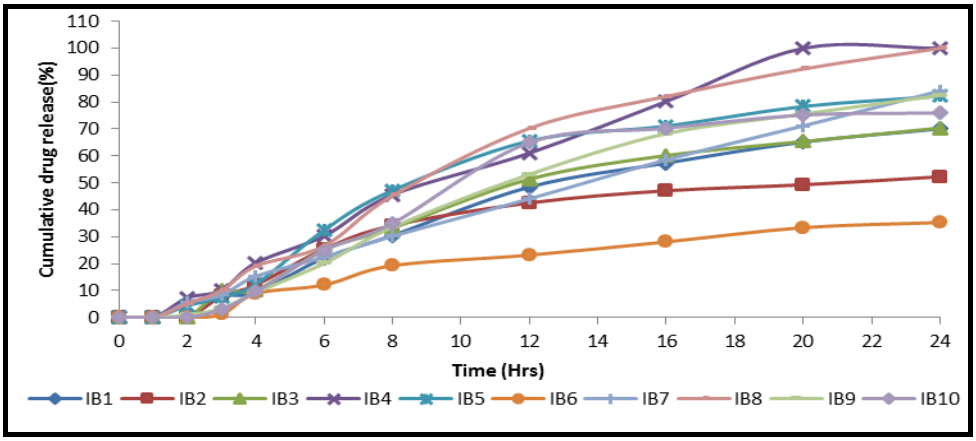


Figure 6.3.33: Dissolution profile of IB 1- IB 10

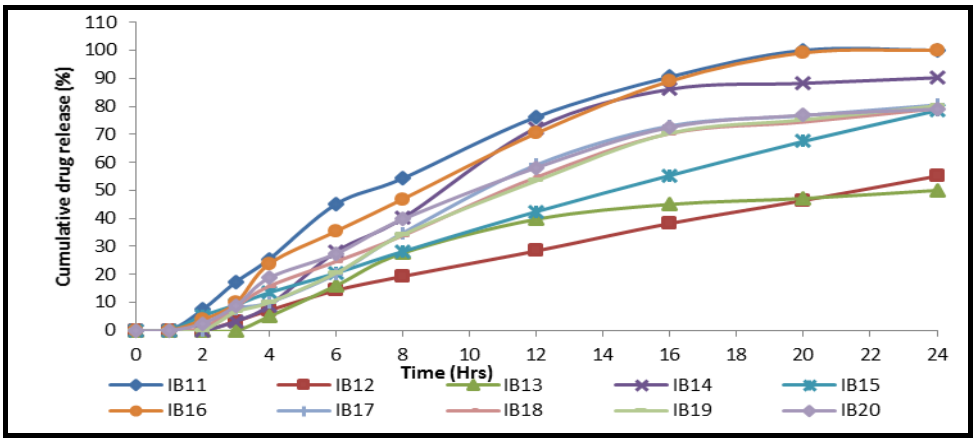


Figure No.6.3.34: Dissolution profile of IB11- IB 20

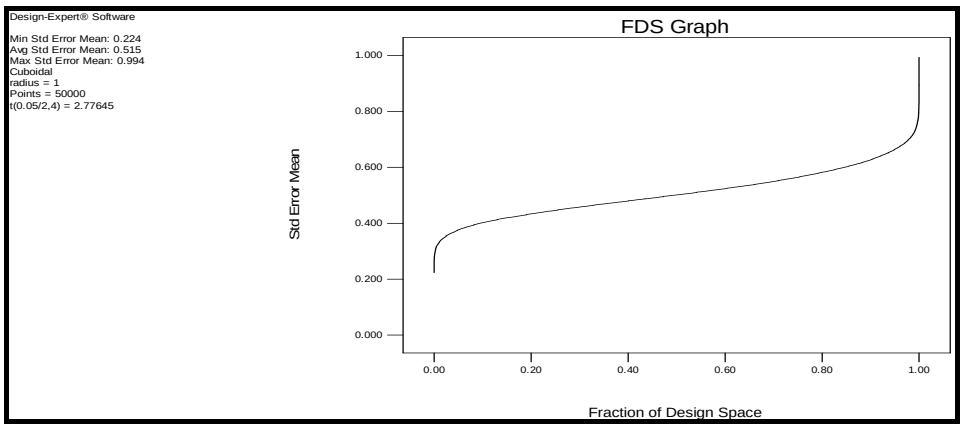


Figure No.6.3.35: FDS graph of the design selected for the factor influence study and optimization

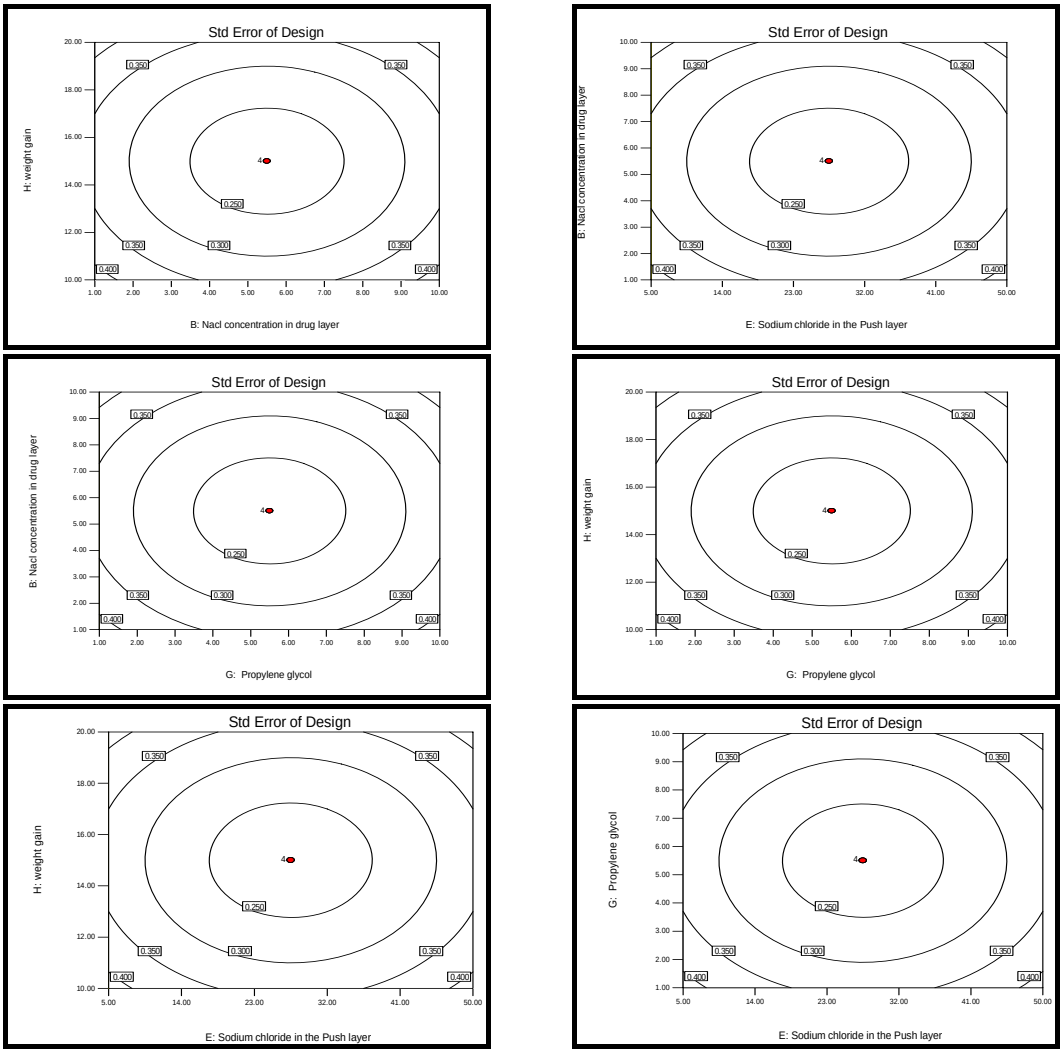


Figure No.6.3.36: Standard error contour plots of FI study & optimization

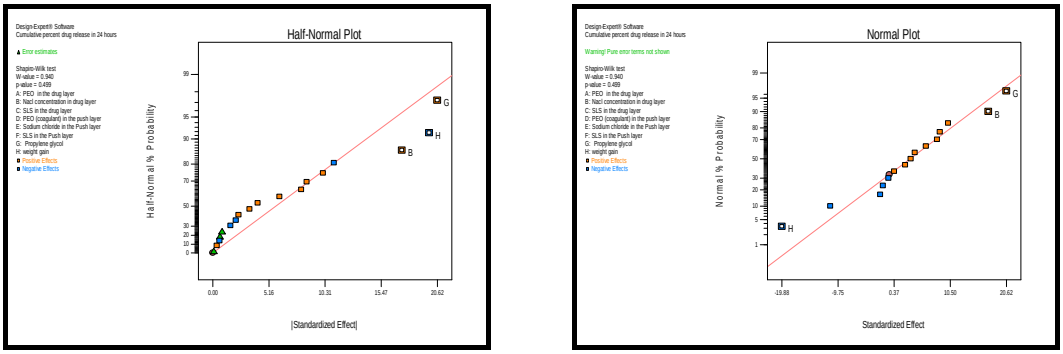


Figure No.6.3.37: Half normal plot for the effect of the factors on the PCUR at 24 hrsFigure No.6.3.38: Normal plot for the effect of the factors on the PCUR at 24 hrs

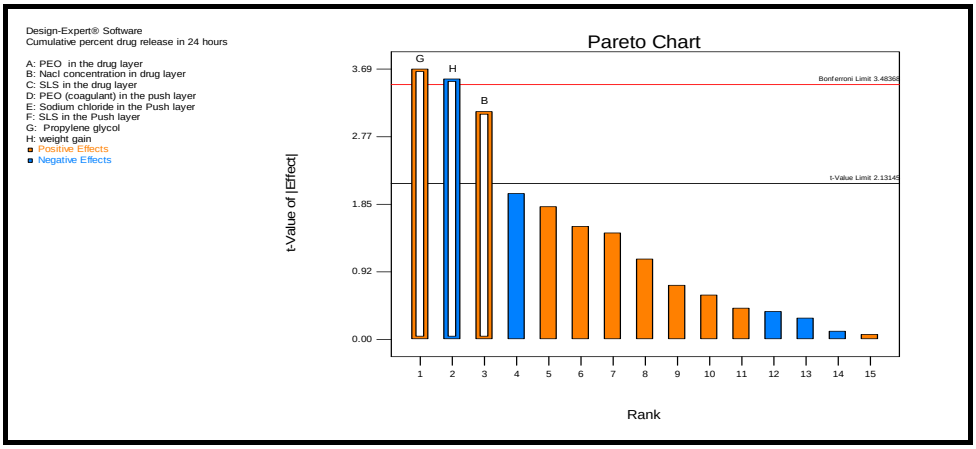


Figure No.6.3.39: The pareto chart for the effect of the factors on the PCUR at 24 hrs

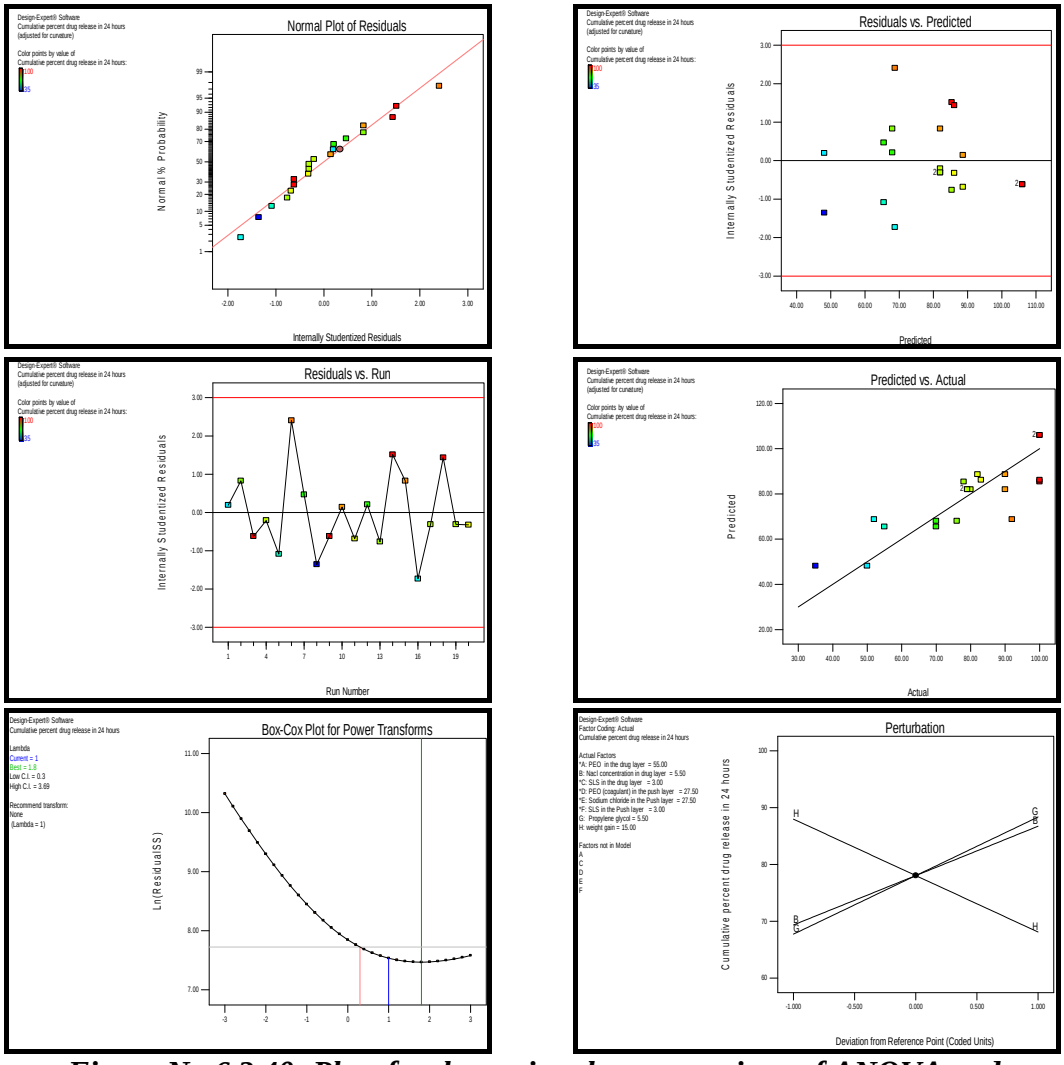


Figure No.6.3.40: Plots for the testing the assumptions of ANOVA and perturbation curve

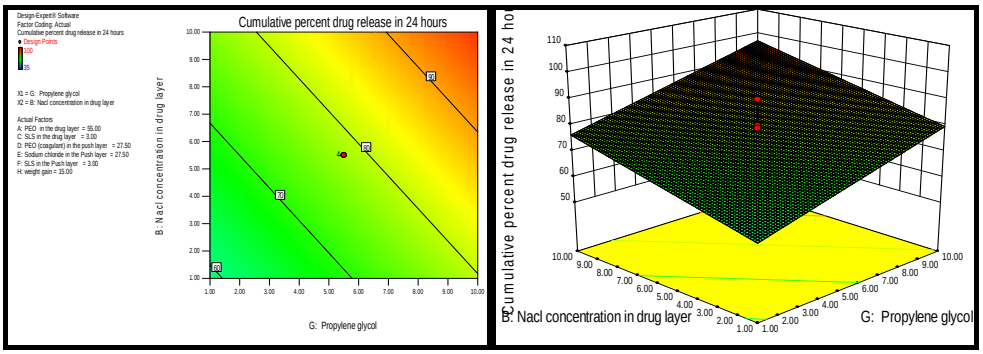


Figure No 6.3.41:Contour plot and RS Plot - Effect of NaCl DL and Propylene Glycol on PCUR at 24 hrs

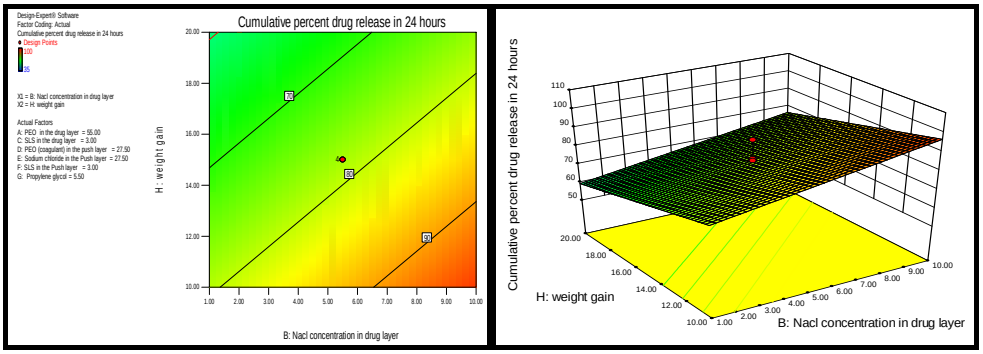


Figure No.6.3.42: Contour plot and RS Plot - Effect of NaCl in DL and weight gain on PCUR at 24 hrs

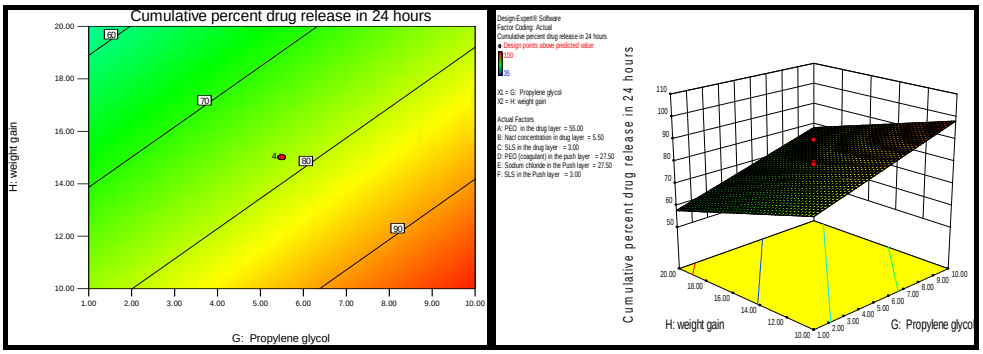


Figure No.6.3.43:Contour plot and RS Plot - Effect of Propylene Glycol and weight gain on PCUR at 24 hrs

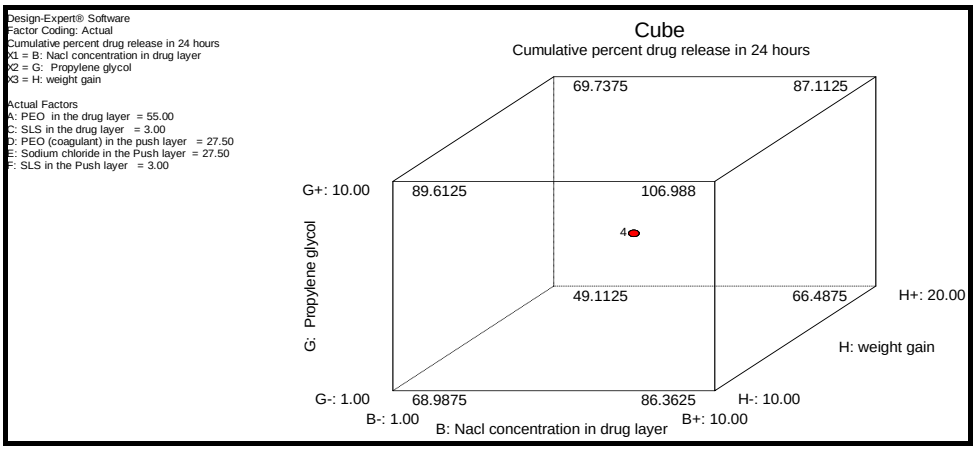
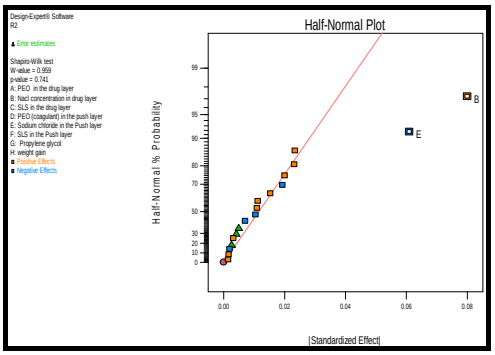


Figure6.3.44:Cube plot of the Effect of NaCl DL, Propylene Glycol and Weight gain on PCUR at 24 hrs7



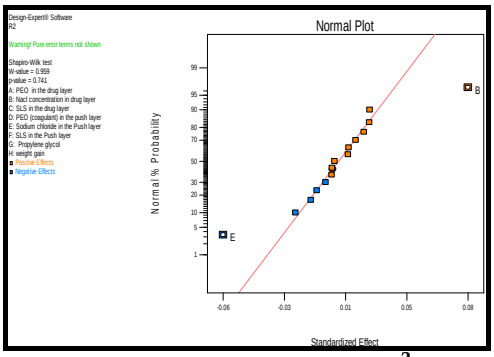


Figure No.6.3.45: Half Normal plot of the effect of the factors on R^2
Figure No.6.3.46: Normal plot of the effect of the factors on R^2

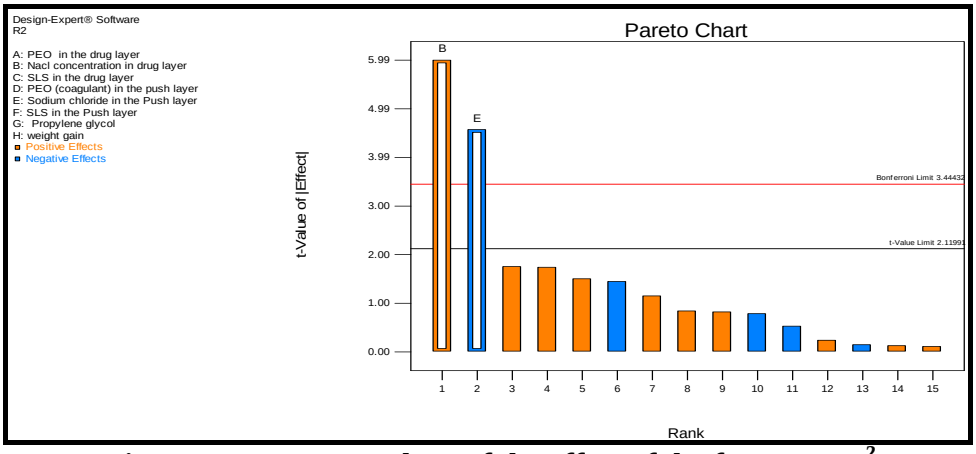
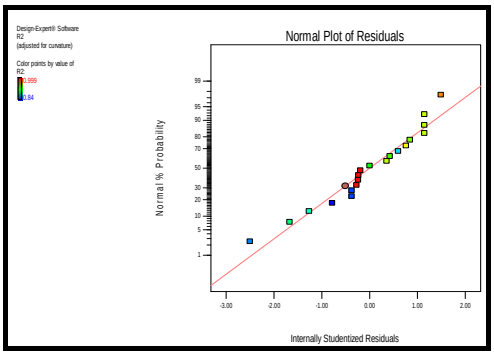
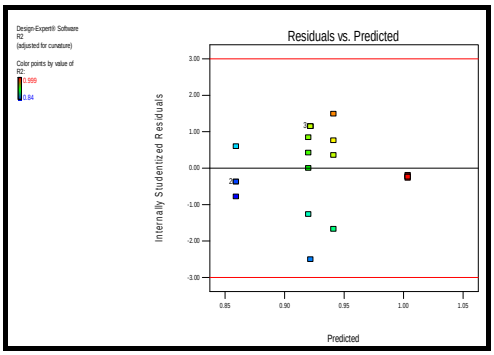
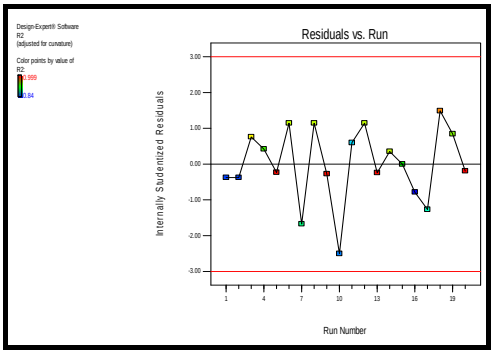
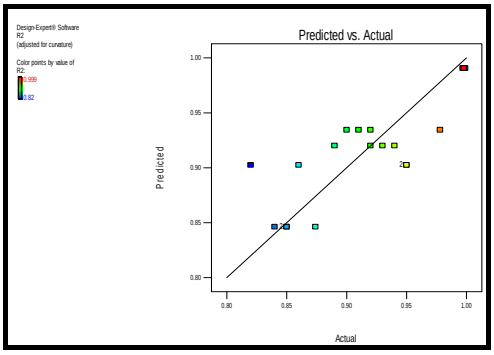


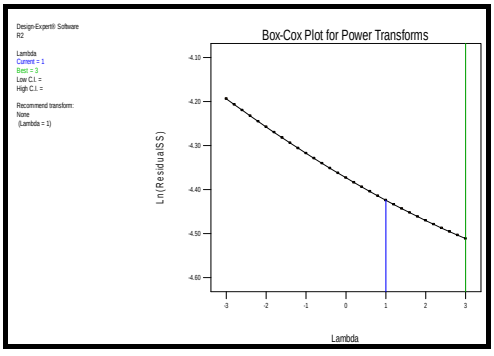
Figure6.3.47: Pareto chart of the effect of the factors on R^2











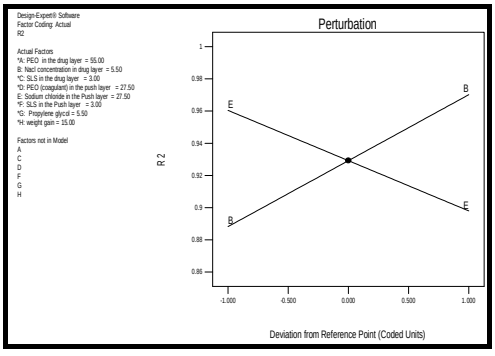


Figure No.6.3.48: Plots for Testing the assumptions of the ANOVA and pertubation Curve

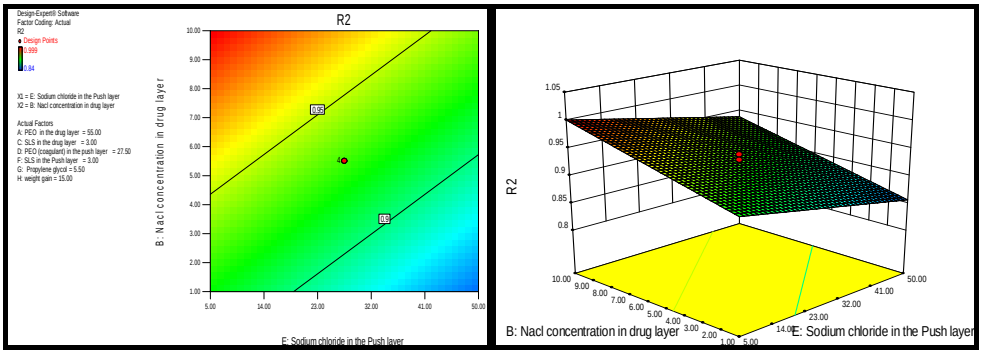
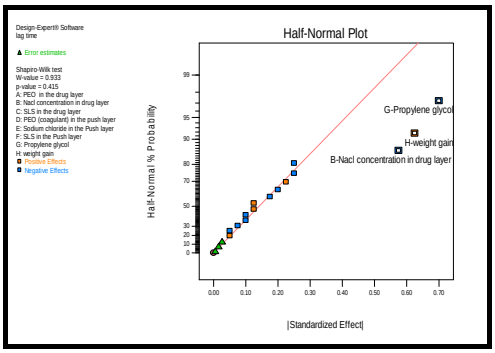


Figure No.6.3.49:Contour plot and RS Plot – Effect of NaCl DL and NaCl PL on R^2



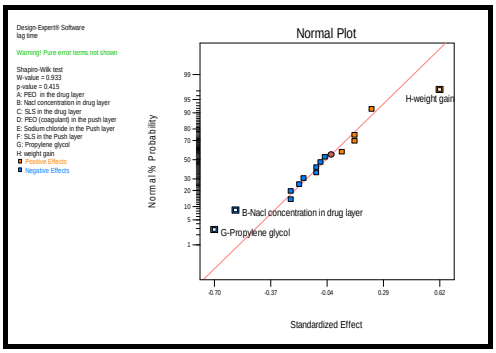


Figure No.6.3.50:Half Normal plot of the effect of the factors on lag time
Figure No.6.3.51:Normal plot of the effect of the factors on lag time

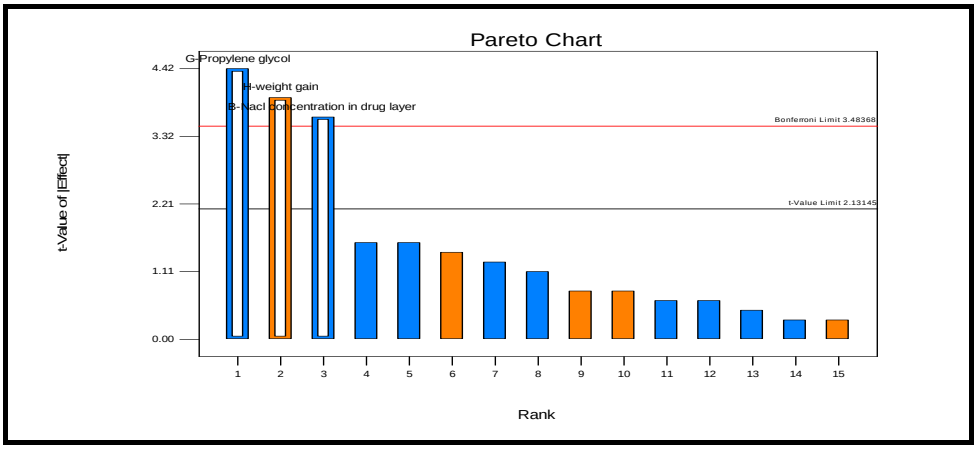


Figure No.6.3.52:Pareto chart of the effect of the factors on lag time

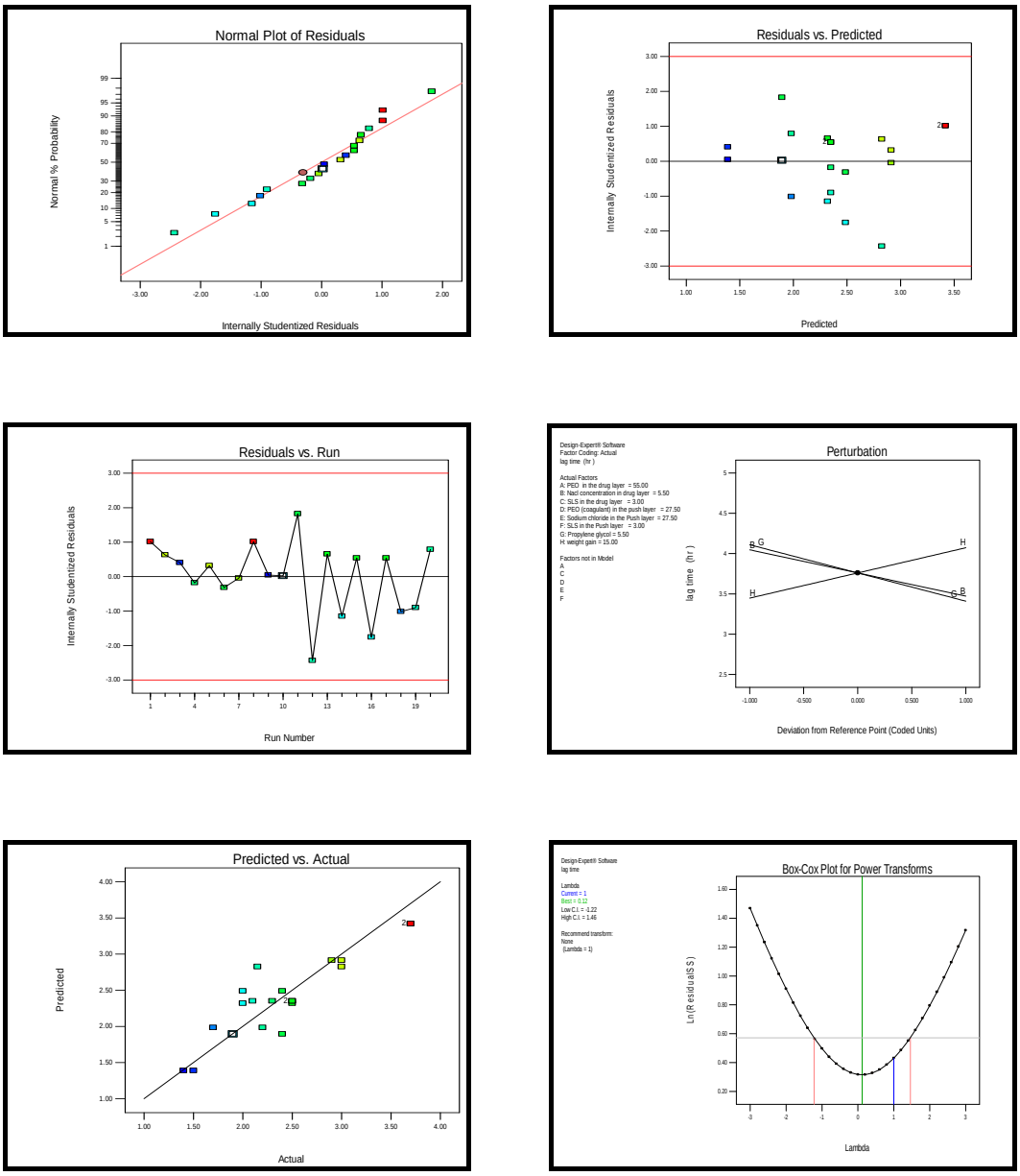


Figure No.6.3.53: Plots for testing the assumptions of the ANOVA and pertubation Curve

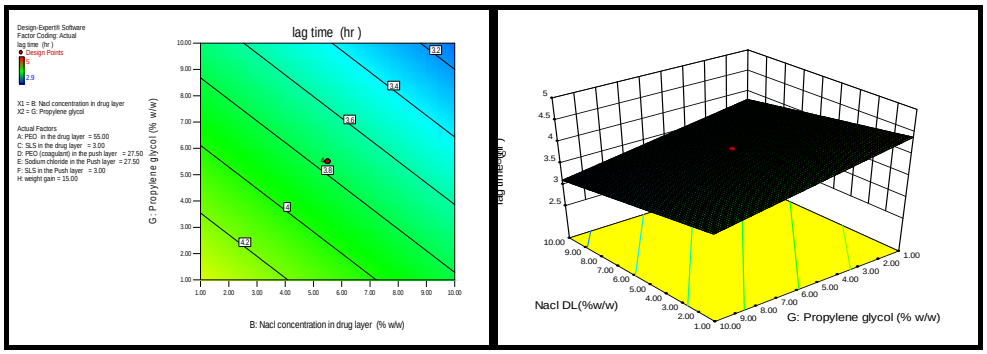


Figure No.6.3.54: Contour plot and RS Plot –
Effect of NaCl DL and Propylene Glycol on lag time

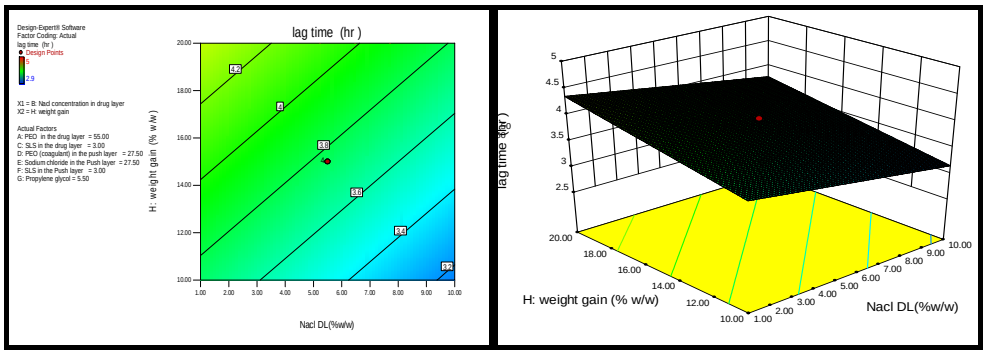


Figure 6.3.55: Contour plot and RS Plot –
Effect of NaCl DL and weight gain on lag time

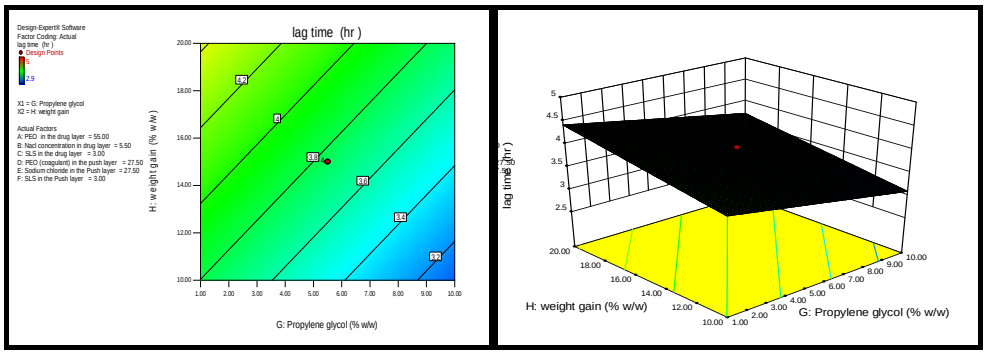


Figure No.6.3.56:Contour plot and RS Plot –
Effect of Weight gain and Propylene Glycol on lag time

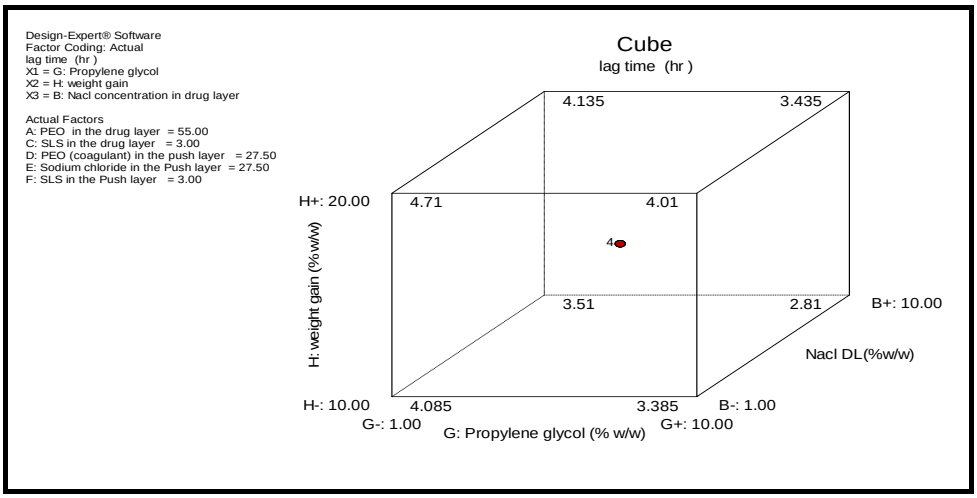


Figure No.6.3.57:Cube plot of effect of NaCl DL, Propylene glycol and Weight gain on lag time

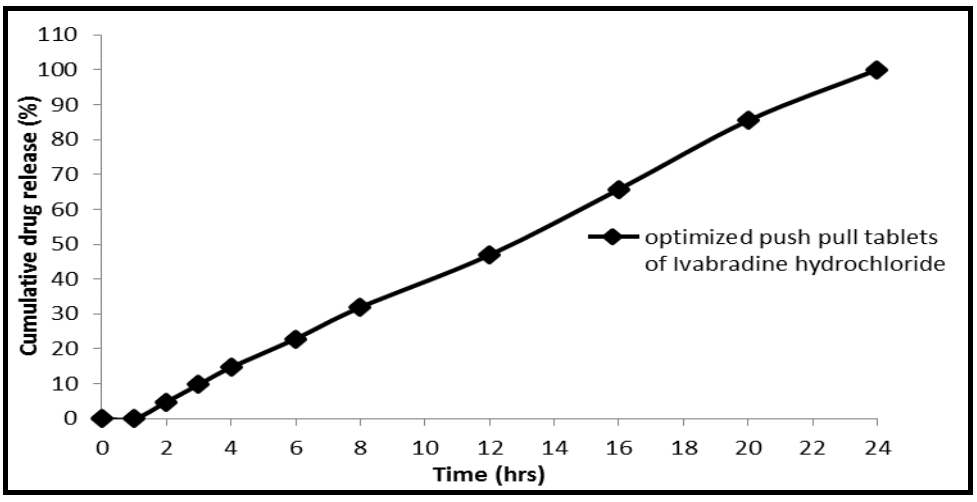


Figure No.6.3.58: Invitro dissolution study of the optimized batch

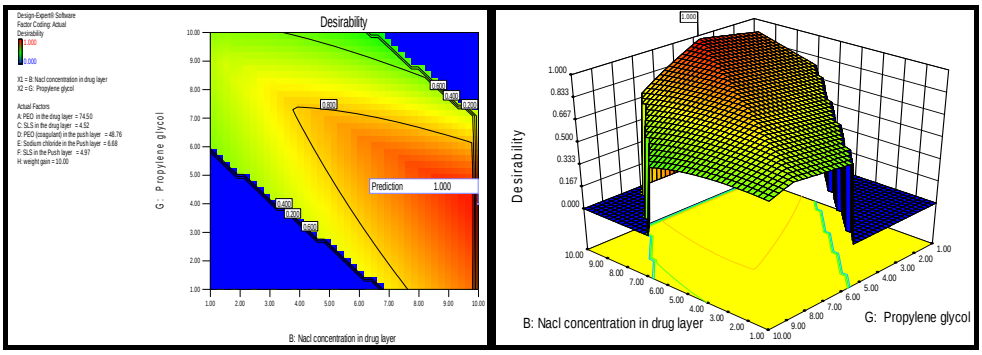


Figure No.6.3.59: Desirability contour plot and RS plot – Effect of NaCl DL and propylene Glycol

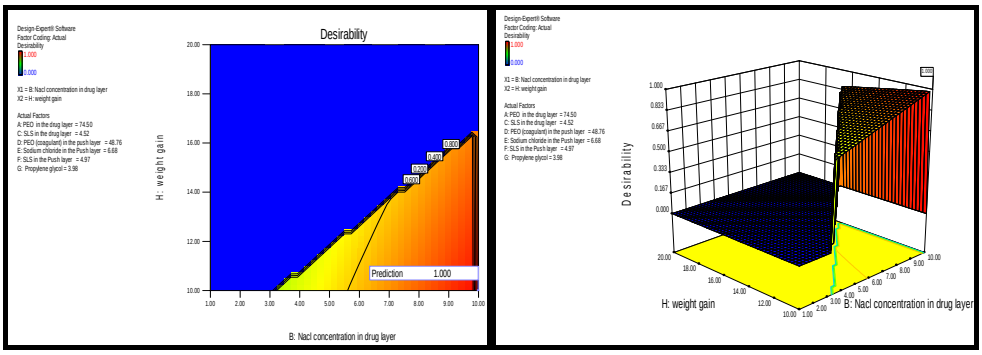


Figure No.6.3.60: Desirability contour plot and RS plot – Effect of NaCl DL and weight gain

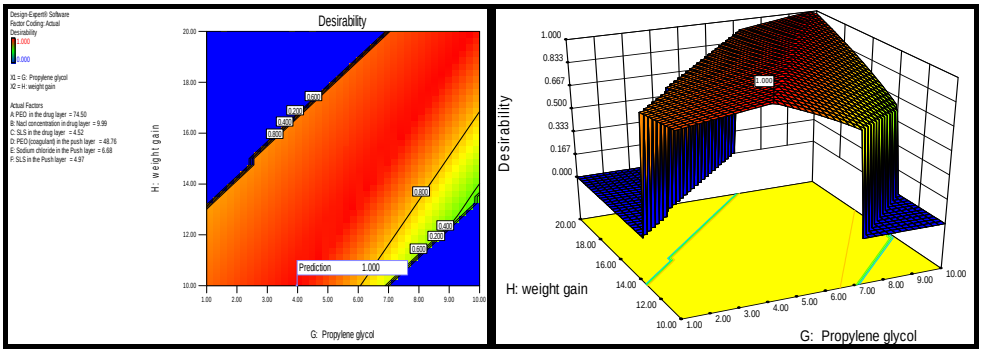


Figure No.6.3.61: Desirability contour plot and RS plot – Effect of Weight gain and Propylene Glycol

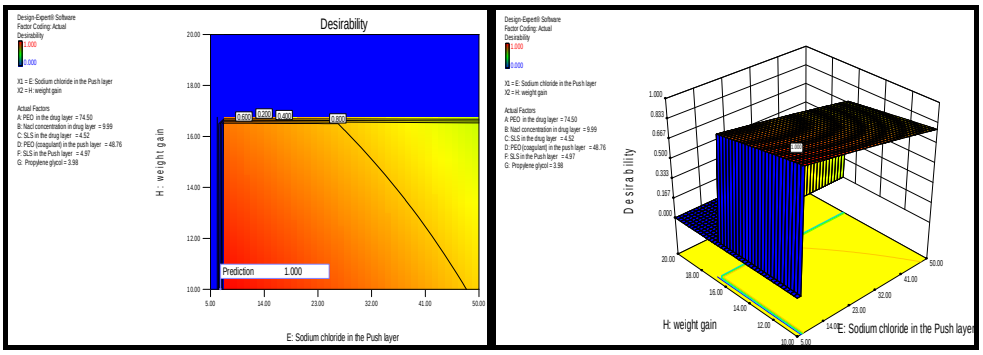


Figure No.6.3.62: Desirability contour plot and RS plot – Effect of Weight gain and NaCl PL

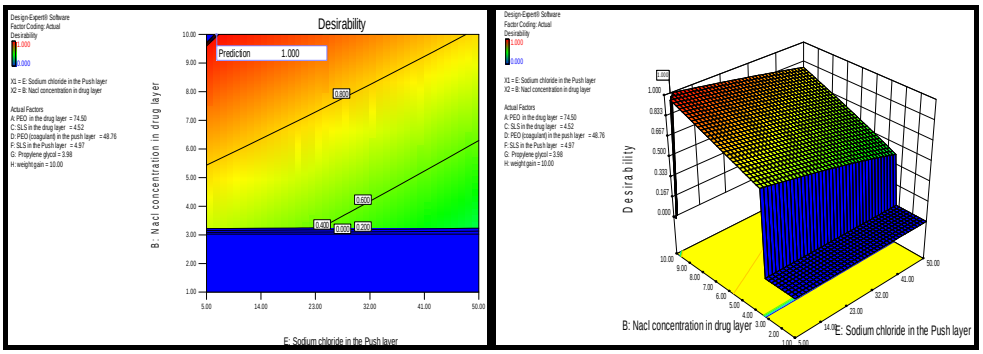


Figure No.6.3.63: Desirability contour plot and RS plot – Effect of NaCl DL and NaCl PL

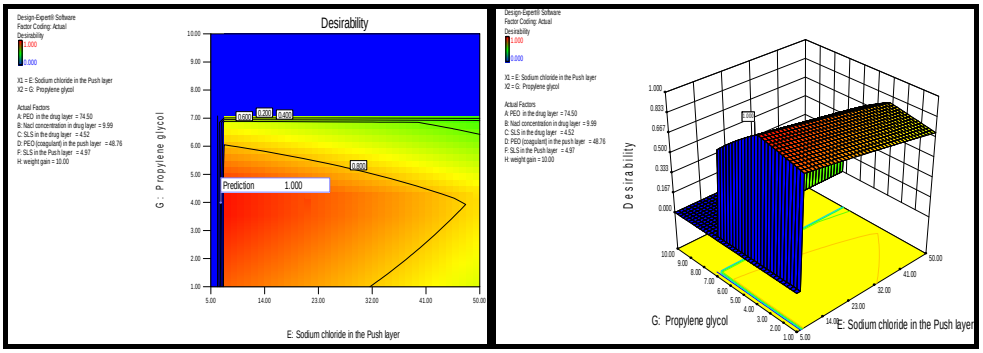


Figure No.6.3.64: Desirability contour plot and RS plot – Effect of NaCl PL and Propylene Glycol

6.3.5. Product development and optimization of push – pull osmotic tablets of Carvedilol phosphate

I. Formulation of push pull osmotic tablets of Cavedilol phosphate

The factor influence study batches of Carvedilol phosphate C1 to C20 were formulated according to the methodology given in the chapter 5.6.4(IA-D), which explains preparation of granules, compression of core bilayer tablets, coating of core tablets and drilling of coated tablets. Quantities were set according to the range specified in the literatures. The compositions taken for preparation of Factor influence study batches are shown in Table No.5.6.2. The levels and responses fixed for the study was given in Table No 5.6.3 and 5.6.4.

The design table in the coded values for the formulation development of Carvedilol phosphate osmotic tablets was given in the Table No.5.6.5. The final formula for the factor influence study and optimization of Carvedilol phosphate push pull OT are shown in the Table No.6.3.36 and 6.3.37.

II. Evaluation of the formulations

The batches C1 to C20 were evaluated simultaneously while preparing. They were subjected to blend as well as whole tablet evaluation. The procedures for the evaluation were given in chapter 5.6.4(IIA&B).

A. Blend evaluation

The prepared granules of the layers i.e, drug layer and push layer were evaluated by means of various tests. The tests were carried out according to the methodology given in the section 5.5.4 to 5.5.7. The results of the various blend evaluation are mentioned in the Table No.6.3.38.

B. Tablet evaluation

The prepared tablets were evaluated for weight variation, hardness, friability, assay, weight gain, pore size, diameter and thickness. The tests were performed as per the methodology given chapter section 5.6.4(IIB). The results of various tests are shown in Table No.6.3.39.

Table No: 6.3.36: Formula for the trial C1- C10

SL. No.	Ingredients	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
		mg/tab	mg/tab	Mg/tab	mg/tab	mg/tab	mg/tab	mg/tab	mg/tab	mg/tab	mg/tab
Drug Layer (DL)											
1	Carvedilol phosphate	10	10	10	10	10	10	10	10	10	10
2	DCP	37.05	28.04	28.50	19.50	35.05	26.04	26.50	17.50	37.05	28.04
3	PEO 400 K	1.000	10.00	1.000	10.00	1.000	10.00	1.000	10.00	1.000	10.00
4	Sodium chloride	0.950	0.950	9.500	9.500	0.950	0.950	9.500	9.500	0.950	0.950
5	BHT	0.001	0.010	0.001	0.010	0.001	0.010	0.001	0.010	0.001	0.010
6	SLS	0.500	0.500	0.500	0.500	2.500	2.500	2.500	2.500	0.500	0.500
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg. stearate	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total weight of DL		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer (PL)											
9	PEO 7000 K (WSR 302)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	25.00	25.00
10	Sodium chloride	0.13	1.25	1.25	0.13	1.25	0.13	0.13	1.25	1.25	12.50
11	DCP	39.62	38.50	36.70	37.82	36.70	37.82	39.62	38.50	14.18	2.93
12	BHT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg.sterate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of PL		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	9.4	17.3	18.8	8.6	8.6	18.8	17.3	9.4	17.3	9.4
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.09	1.73	0.19	0.86	0.86	0.19	1.73	0.09	1.73	0.09
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	19.0	9.5	19.0	9.5
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**114.0**	**104.5**	**114.0**	**104.5**

Table No: 6.3.37: Formula for the trial C11- C20

[	Ingredients	C11 mg/tab	C12 mg/tab	C13 mg/tab	C14 mg/tab	C15 mg/tab	C16 mg/tab	C17 mg/tab	C18 mg/tab	C19 mg/ tab	C20 mg/tab
Drug Layer (DL)											
1	Carvedilol phosphate	10	10	10	10	10	10	10	10	10	10
2	DCP	28.50	19.50	35.05	26.05	26.50	17.50	27.27	27.27	27.27	28.04
3	PEO 400 K	1.000	10.00	1.000	10.00	1.000	10.00	5.500	5.500	5.500	10.00
4	Sodium chloride	9.500	9.500	0.950	0.950	9.500	9.500	5.225	5.225	5.225	0.950
5	BHT	0.001	0.010	0.001	0.010	0.001	0.010	0.006	0.006	0.006	0.010
6	SLS	0.500	0.500	2.500	2.500	2.500	2.500	1.500	1.500	1.500	0.500
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Magnsium stearate	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Total weight of DL		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer (PL)											
9	PEO 7000 K	25.00	25.00	25.00	25.00	25.00	25.00	13.75	13.75	13.75	25.00
10	Sodium chloride	12.50	1.25	12.50	1.25	1.25	12.50	3.78	3.78	3.78	12.50
11	DCP	4.73	15.98	4.73	15.98	14.18	2.93	23.81	23.81	23.81	2.93
12	BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.03
13	SLS	0.45	0.45	0.45	0.45	2.25	2.25	1.35	1.35	1.35	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Magnsium stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of PL		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	8.6	18.8	18.8	8.6	9.4	17.3	13.5	13.5	13.5	13.5
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.86	0.19	0.19	0.86	0.09	1.73	0.74	0.74	0.74	0.14
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	14.3	14.3	14.3	14.3
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**109.3**	**109.3**	**109.3**	**109.3**

Table.6.3.38: Blend evaluation of the DL and PL of push –pull OT of Carvedilol phosphate

Trials	Angle of repose		Bulk density(g/ml)		Tapped density(g/ml)		Hausner's ratio		Carr's index (%)	
	DL	PL	DL	PL	DL	PL	DL	PL	DL	PL
C1	31.2	28.5	0.854	0.685	0.998	0.775	1.1686	1.1314	14.4289	11.6129
C2	32.5	28.2	0.836	0.679	0.976	0.77	1.1675	1.1340	14.3443	11.8182
C3	30.8	27.9	0.848	0.682	0.982	0.772	1.1580	1.1320	13.6456	11.6580
C4	31.7	28.5	0.85	0.659	0.99	0.748	1.1647	1.1351	14.1414	11.8984
C5	31.4	29.1	0.855	0.6761	0.995	0.764	1.1637	1.1300	14.0704	11.5052
C6	32.2	27.9	0.849	0.664	0.989	0.739	1.1649	1.1130	14.1557	10.1488
C7	32.2	28.5	0.839	0.671	0.999	0.759	1.1907	1.1311	16.0160	11.5942
C8	31.4	28.8	0.856	0.685	0.998	0.769	1.1659	1.1226	14.2285	10.9233
C9	29.9	28.4	0.845	0.655	0.988	0.736	1.1692	1.1237	14.4737	11.0054
C10	31.5	28.9	0.845	0.67	0.988	0.755	1.1692	1.1269	14.4737	11.2583
C11	31.6	29.2	0.838	0.679	0.979	0.764	1.1683	1.1252	14.4025	11.1257
C12	32	27.9	0.858	0.657	1	0.739	1.1655	1.1248	14.2000	11.0961
C13	31.8	27.8	0.845	0.685	0.987	0.777	1.1680	1.1343	14.3870	11.8404
C14	30.6	29.5	0.849	0.676	0.996	0.762	1.1731	1.1272	14.7590	11.2861
C15	31.3	29.7	0.837	0.68	0.996	0.759	1.1900	1.1162	15.9639	10.4084
C16	31.5	29.8	0.853	0.699	0.989	0.785	1.1594	1.1230	13.7513	10.9554
C17	32.4	27.8	0.839	0.677	0.982	0.761	1.1704	1.1241	14.5621	11.0381
C18	29.9	28.7	0.847	0.675	0.989	0.759	1.1677	1.1244	14.3579	11.0672
C19	30.7	28.6	0.838	0.67	0.986	0.755	1.1766	1.1269	15.0101	11.2583
C20	30.6	29.2	0.844	0.688	0.987	0.779	1.1694	1.1323	14.4883	11.6816

Table No.6.3.39: Whole tablet evaluation of push pull OT of Carvedilol phosphate

Trial	Wt variation (n =20)	Diameter (mm) (n=5)	Thickness (mm) (n=5)	Hardness Kg/cm^2) (n=6)	Friability (%)	Assay (%)	Weight gain %)	Pore size (mm)
C1	104.3±1.67	5.12± 0.2	3.5±0.03	3.6±0.5	0.63	100±1.2	10.11±1.3	0.60
C2	114±1.21	5.15±0.1	3.5±0.01	3.7±0.5	0.51	99± 2.3	20.05±1.5	0.60
C3	113 ±2.33	5.08±0.12	3.5±0.03	3.8±0.3	0.45	101±3.4	20.11±0.8	0.60
C4	104.8 ± 2.25	5.14±0.08	3.6±0.01	4±0.7	0.53	95±1.8	10.2±0.1	0.60
C5	104.2 ± 1.13	5.20±0.05	3.5±0.03	4.1± 0.6	0.55	97±1.4	10.13±0.04	0.60
C6	113 ±1.54	5.0±0.10	3.6±0.02	4.2±0.3	0.64	99±2.2	20.13±0.25	0.60
C7	115 ± 2.23	5.18±0.04	3.5±0.01	3.9±0.5	0.67	100±1.45	20.03±0.12	0.60
C8	103.8 ±1.18	5.13±0.08	3.6±0.03	3.8±0.8	0.48	98±2.5	10.12±0.3	0.60
C9	113.2 ± 1.84	5.12±0.01	3.6±0.02	4.1±0.1	0.67	100±2.2	20.2±0.5	0.60
C10	103.9 ± 2.04	5.15±0.06	3.5±0.04	3.8±0.9	0.66	99±2.4	10.1±0.05	0.60
C11	104 ± 2.25	5.08±0.05	3.5±0.01	3.7±0.5	0.45	102±1.1	10.05±0.15	0.60
C12	114 ± 1.97	5.09±0.07	3.7±0.03	4.2±0.45	0.56	99±2.1	20.08±0.18	0.60
C13	115 ± 1.54	5.07±0.02	3.6±0.04	4.1±0.04	0.66	98±1.7	20.07±0.23	0.60
C14	104±1.43	5.08±0.06	3.5±0.01	4.0±0.24	0.64	100± 2.3	10.06±0.23	0.60
C15	104.8 ±1.89	5.11 ±0.03	3.4±0.13	3.7±0.12	0.55	100±1.3	10.1±0.11	0.60
C16	113.9 ± 1.99	5.1±0.05	3.5±0.05	3.8±0.46	0.52	99±2.3	20.2±0.5	0.60
C17	110.2 ± 1.58	5.15±0.01	3.5±0.04	3.7±0.15	0.55	101±1.3	15.04±0.14	0.60
C18	108.56 ± 1.34	5.11±0.03	3.7±0.04	3.9±0.15	0.61	100±1.6	15.1±0.08	0.60
C19	109.2 ± 1.62	5.13±0.04	3.7±0.12	3.9±0.2	0.59	99±2.3	15.03±0.15	0.60
C20	109.67±0.12	5.12±0.02	3.8±0.03	4.1±0.05	0.55	98±2.5	15.2±0.3	0.60

C. *In vitro* dissolution tests

In vitro dissolution study was carried out for all the batches and the test was carried out as per methodology given in the chapter section 5.6.4(IIBix). The release profile of C1 to C20 batches are shown Table No.6.3.40, 6.3.41 and Figure No.6.3.65 &6.3.66.

Table No.6.3.40: Dissolution profile of C1- C10 batches

Time (hrs)	Cumulative drug release (%)									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	3.3±0.15	0	0	0	0	0	0
3	2±1.8	5.1±0.6	0	10.1±.6	8.5±0.6	3.3±0.25	0	9±0.2	1±0.1	2±0.01
4	6.2±1.2	10±3.4	8.2±0.7	17.9±0.3	12±1.3	8.6±0.6	11.6±1.2	13±2.1	8.5±1.3	9.2±1.1
6	16.5±2.3	17.3±1.2	13.1±0.7	28.7±1.2	25.6±1.1	14.3±0.3	16.3±1.6	17.6±2	14.3±1.1	13.3±1.3
8	22.3±1.5	25.3±1.3	17.5±1.1	37.9±2.5	32.5±1.3	20.3±0.8	21.8±1.5	22.4±2.7	24.4±0.8	25.6±2.5
12	27.4±3.1	47.4±1.9	24.3±1.2	47.3±2.3	43.7±1.2	24.4±0.9	35.8±0.8	29.6±0.9	32.4±1.9	59.6±2.1
16	32.6±1.5	65.6±1.8	32.7±0.9	64.7±3.8	57.9±1.8	26.6±0.6	41.7±1.3	37.7±1.3	36.2±1.8	73.3±2.2
20	33.5±1.4	80±2.8	41±0.3	73.4±1.8	64.7±1.3	28.3±2.3	48.9±2.1	48.9±2.3	40.1±2.2	85±2.5
24	34.1±0.6	85.3±2.2	47.3±1.2	81.9±1.9	75.3±2.4	30±0.5	54.4±1.4	59.8±4.5	42±0.3.8	100±2.3

Table No.6.3.41: Dissolution profile of C 11- C 20 batches

Time (hrs)	Cumulative drug release (%)									
	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	5	0	0	0	0	4.1±0.7	0	0	0	0
3	10.2±0.1	0	0	3±0.4	8.5±0.7	8.3±0.6	5.2±0.2	6.5±1.2	4.5±0.1	5±0.2
4	13.5±0.2	6.0±0.5	6.6±0.3	10.0±0.7	15.3±.1.1	15.1±1.2	10.1±0.3	11.8±1.1	9.20±0.5	10.0±0.9
6	21.1±1.2	18.5±1.1	16.4±1.0	25.2±1.6	25.1±1.4	26.5±2.2	20.3±0.4	19.5±1.3	16.4±1.2	19.5±0.3
8	28.5±1.5	25.6±1.1	23.1±1.2	46.3±1.8	30.2±1.8	35.8±2.5	28.4±0.7	27.6±1.2	25.2±1.3	29.2±1.2
12	41.5±2.4	32.3±2.5	30.5±3.1	72.3±5.1	40.7±4.1	52.4±1.4	48.4±0.9	49.4±2.8	44.1±2.7	45.3±2.5
16	54.8±0.8	47.2±2.3	42.6±1.2	80.5±3.6	48.5±1.2	69.5±4.2	55.7±2.1	61.1±2.1	50.3±3.9	56.7±3.3
20	67.9±3.2	57.6±4.5	48.3±2.1	85.9±3.7	55.3±2.8	85.3±1.4	64.2±4.2	65.3±4.8	60.2±3.5	65.2±4.9
24	79.5±4.4	64.3±2.9	50.2±2.5	89.8±3.3	62.1±1.2	100.0±1.8	70.0±1.4	72.4±4.4	65.0±4.3	70.0±4.9

D. Coating Evaluation

The coating evaluation was done by formulation of mechanical film in a Petri dish as described in methodology chapter section 5.6.4(IIC). And the results are shown in Table No.6.3.9.

III. Factor influence study

The *in vitro* evaluations of all the 20 trials were performed and the necessary values for the factor influence study were recorded. The result of the factor influence study was given in the Table No.6.3.42.

Table No. 6.3.42: Result of Factor influence study

Trials	PCUR at 24 hrs	R^2	Lag time
C1	34	0.866	4.7
C 2	85	0.978	4
C3	47	0.998	4.5
C4	82	0.975	3
C5	75	0.976	3.5
C6	30	0.877	4.5
C7	54	0.975	3.7
C8	60	0.995	3.5
C9	42	0.880	4.5
C10	100	0.968	4.2
C11	79	0.999	3
C12	64	0.989	4.8
C13	50	0.954	5
C14	90	0.863	4
C15	62	0.976	3.4
C16	100	0.997	3.5
C17	70	0.950	4
C18	72	0.945	3.8
C19	65	0.955	4.2
C20	70	0.959	4

A. FDS Curve

Figure No.6.3.67 shows the FDS graph for the selected design with the selected factors and responses. It showed a flatter curve. This indicated a high FDS. So the design space predicted by the selected model had useful precision.

B. Standard error graph

The standard error of prediction for areas in the design space for the different factor were found to be between 0.25 – 0.45. So it was proven that the standard error throughout the design space was relatively very low. The entire design space will be having a very less prediction error for the selected design. The graphs are shown in Figure No.6.3.68.

C. **Analysis of responses**

1. Cumulative release at 24 hrs

The cumulative release of the different formulations were studied and analyzed for the different factors affecting the same. The different evaluation graphs and data are explained below,

i) Half normal plot

The half-normal plot was used to select effects to be included in the model. From the graph shown in Figure No.6.3.69, it was evident that the factor which were affecting the PCUR up to 24 hrs were A (PEO DL), G (propylene glycol), E (NaCl PL), D (PEO PL) and H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.746. This indicated the non significance of the non selected factors**.** Two interaction AC (PEO DL-SLS DL) and AD (PEO DL-PEO PL) terms were also found significant.

ii) Normal plot

Figure No.6.3.70 shows the normal plot for the effect of factors on PCUR at 24 hrs. The factor A, D, E, G and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.746. This indicated that the remaining (unselected) terms were normally distributed. Two interaction AC (PEO DL-SLS DL) and AD (PEO DL-PEO PL) terms were also found significant.

iii) Pareto chart

Figure No.6.3.71 shows the pareto chart for the effect of factors on PCUR at 24 hrs. From the pareto chart also it was clearly evident that the factors A,D,E,G and H were significantly affecting the PCUR at 24 hrs. All the factors crossed the t limit and Bonferroni limit. The magnitude of the effect can be written as PEO DL >Propylene Glycol >NaCl PL >PEO PL> Weight gain. The orange color indicates the positive effect and the blue color indicates the negative effect. So with an increase in the concentration of PEO DL, propylene glycol, NaCl PL and PEO PL, PCUR at 24 hr was increased. But increase in the weight gain decreased the PCUR at 24 hr. AC and AD are probabliy significant as these two terms were with in the t and B limit. Other non significant term effects and interaction effects were present below the t limit.

iv) ANOVA and Regression analysis

From the ANOVA analysis the significant model terms were identified as (A =2.2E-05*)*, G (p =3.5E-05*)*, (1.4E-04) D (4.5E-04) and H (p =8.9E-04*)*. From the ANOVA analysis it was proven that the model selected was significant and no lack of fit was observed. AC and AD interactions were also significant. But no curvature effect was identified. This was an indication that the same model can be used for optimization. The "Pred R-Squared" of 0.781 was in reasonable agreement with the "Adj R-Squared" of 0.901 indicating the linearity of the model. Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Our ratio was 16.76 indicates an adequate signal. So this model can be used to navigate the design space.

v) Polynomial equations

From the regression analysis the polynomial equation which can represents the cumulative percentage release at 24 hrs can be formed. The positive sign of the coefficients in the equation indicates the positive and the negative sign indicates the negative effect on the response. Larger the coefficients larger will be the effects.

The linear model polynomial equation representing the response can be written as,

CUR at 24 hrs = 66.55+ 10.5 * A +7.5* D+ 8.625* E +10* G -6.875* H-5.625* AC +4.625* AD *(coded terms)*

CUR at 24 hrs = 49.3201 +0.1952 * PEO in the drug layer +0.0821 * PEO in the push layer +0.3833 * Sodium chloride in the Push layer +2.2222 * Propylene Glycol -1.375* Weight gain -0.0291 * PEO in the drug layer * SLS in the drug layer +0.0046 * PEO in the drug layer * PEO in the push layer *(actual terms)*

Table No.6.3.43: ANOVA analysis for the effect of factors on the PCUR at 24 hr

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	7059	7	1008.429	25.64	2.6E-06	*significant*
A-PEO DL	1764	1	1764	44.85	*2.2E-05*	
D-PEO PL	900	1	900	22.88	*4.5E-04*	
E-NaCl PL	1190.25	1	1190.25	30.26	*1.4E-04*	
G-Propylene Glycol	1600	1	1600	40.68	*3.5E-05*	
H- Weight gain	756.25	1	756.25	19.22	*8.9E-04*	
AC	506.25	1	506.25	12.87	*3.7E-03*	
AD	342.25	1	342.25	8.70	*1.2E-02*	
Residual	471.95	12	39.32917			
Lack of Fit	445.2	9	49.46667	5.54	0.092875	*not significant*
Pure Error	26.75	3	8.916667			
Cor Total	7530.95	19				
Regression analysis						
Std. Dev.	6.27		R-Squared	0.93		
Mean	66.55		Adj R-Squared	0.901		
C.V. %	9.42		Pred R-Squared	0.781		
PRESS	1645.92		Adeq Precision	16.76		

vi) Tests for the assumptions of the ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No.6.3.72.

- *The normal probability plot:* The plot indicated that residuals follow a normal distribution, as the point follows a straight line.

- *Residuals vs Predicted*: The plot showed random scatter (constant range of residuals across the graph). This confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: Absence of any trends in the graph indicated that no time-related variable lurking in the background.
- *Predicted vs. Actual*: This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The lamda value was 1 and no transformation was needed.

vii) Perturbation graph

The graph shows the change in PCUR at 24 hrs with the an increase in the concentration of factor A, G, E and D from minimum to maximum were from 55 to 75, 57 to 73, 58 to 70 and 59 to 69 respectively. But an increase in the factor H showed decrease in the response (73 to 60).

viii) Interaction graphs

Figure No. 6.3.73 shows interactions AC and AD. Both the plots showed non parrellel lines. The AC interaction showed that when SLS DL concentration was high, increase in concentraion of PEO DL had a lesser effect than SLS DL at lower concentration. SLS which is a solubilizing agent, has an impact on the solubilization of the drug and intern the release of the drug. PEO DL effect was more prominent if the concentration of SLS was less.

The LSD bars at high level and low level of PEO PL overlapped when the concentration of PEO DL kept at minimum. This showed that when PEO DL at minimum, no significant change in the PCUR at 24hr even if the concentration of PEO PL was at maximum.

ix) Contour plots and RS plots

Figure No.6.3.74 shows the contour plot and RS plot of the simultaneous effect of factors A (PEO DL) and D (PEO PL) on the PCUR at 24 hrs. At lower

concentration of PEO DL even a higher concentration of PEO PL could not produce the desired effect. High PCUR would be expected at high levels of both the factors. From the RS plot it was evident that PEO DL had a greater effect on the PCUR at 24 hrs.

Figure No 6.3.75 shows the contour plot and RS plot of the simultaneous effect of PEO DL and NaCl PL on the PCUR at 24 hrs. Lower concentrations of both the factors helped to release 60-70% of the drug from the dosage form. A higher concentration of both the factors produced greater than 80 % release at 24 hrs. Both the factors had a positive effect on the release of the drug form the OTs. At higher level of PEO DL, change in concentration of NaCl PL had a greater effect.

Figure No.6.3.76 shows the contour plot and RS plot for the change in PCUR at 24hr with change in PEO DL and propylene glycol. Both the factors had a positive effect on the response. At the high levels of PEO DL, the propylene glycol had a prominent effect. At low levels of PEO DL even a high concentration of propylene glycol would not produce more than 75 % of drug release. From the surface plot it was evident that both the factor had almost similar effect on the PCUR at 24 hrs at its low and high concentrations.

Figure No.6.3.77 shows the contour plot and RS plot of change in PCUR at 24hr with change in PEO DL and weight gain. PEO DL had a positive effect and weight gain had an opposite effect on the response. At low levels of weight gain PEO DL showed prominent effect. At higher weight gain even a high concentration of PEO DL would not produce more than 70 % PCUR at 24 hrs. From the surface plot it was evident that the effect of PEO DL had a greater effect on the PCUR on both high and lower concentrations of weight gain.

Figure No.6.3.78 shows the contour plot and RS plot which explains the concurrent effect of PEO PL and NaCl PL on PCUR at 24 hrs. Form the plots it

was evident that both the factors had a positive effect on the response. Both the factors showed almost similar effects on the lower and higher concentrations of the other.

Figure No.6.3.79 shows the contour plot and RS plot of contemporaneous effect of PEO PL and propylene glycol on PCUR at 24 hrs. Both the factors had a positive effect on the response. RS plot clearly shows the prominent effect of propylene glycol at both the levels of PEO PL. Higher levels of both the factors executed a better release from the dosage form.

Figure No.6.3.80 shows the contour plot and RS plot representing the simultaneous effect of PEO PL and weight gain on PCUR at 24 hrs. PEO PL had a positive effect and weight gain had an opposite effect on the PCUR at 24 hrs. A more pronounced effect of PEO PL was visible at lower weight gain. At higher weight gain a maximum concentration of PEO PL failed to produce a CUR more than 70%.

Figure No.6.3.81 shows the contour plot and RS plot representing the simultaneous effect of NaCl PL and weight gain on PCUR at 24 hrs. NaCl PL had a positive effect and weight gain had a negative effect on the response. Effect of NaCl PL was more prominent at low weight gain. At higher weight gain even a maximum level of NaCl PL does not produce a PCUR more than 70 %.

Figure No.6.3.82 shows the contour plot and RS plot representing the concomitant effect of NaCl PL and propylene glycol at a time on PCUR at 24 hrs. Both the factors had positive effect on the response. From the RS plot, the prominent effect of propylene glycol was clearly understood. Effect of NaCl PL was more pronounced at higher level of propylene glycol and vice versa. A better release was observed at higher levels of both the factors.

Figure No.6.3.83 shows the contour plot and RS plot representing the simultaneous effect of weight gain and propylene glycol on PCUR at 24 hrs. From the plot it was evident that weight gain had a negative effect and propylene glycol

had a positive effect on the response. From the RS plot, the prominent effect of propylene glycol was clearly understood. Effect of propylene glycol was more pronounced at low level of weight gain. A better release was observed at low weight gain and higher levels of propylene glycol.

x) Cube plots

Figure No 6.3.84 shows the combined effect of A, G, and H. When all the three factors were at minimum the PCUR at 24 hrs was about 52.925, and at maximum it was around 80. 175. But a higher release can be achieved by keeping propylene glycol at its maximum, PEO drug layer at its maximum and weight gain at its minimum.

Figure No.6.3.85 and shows the combined effect of PEO PL, NaCl PL and weight gain. At low levels of all the factors the PCUR at 24 hrs was 57.3. When they were at high levels the PCUR was 75.8. A better release was observed when the PEO PL, NaCl PL were at its maximum and weight gain was at its minimum (89.55%).

Figure 6.3.86 shows the combined effect of PEO DL, NaCl PL and PEO PL. At low levels of all the factors the PCUR at 24 hrs was 44.55. When they are at high levels the release was 97.8.

Figure.6.3.87 shows the combined effect of propylene glycol, weight gain and PEO PL. At low levels of all the factors the PCUR at 24 hrs was 55.925. When they were at high levels the release was 77.175. A 90.925 % release was observed when the PEO PL and propylene glycol were at maximum and weight gain at its minimum.

2. Analysis of responses - Rate constant (R^2)

i) Half normal plot

From half normal plot shown in Figure No.6.3.88, it was evident that the factor which have affected the release rate constant (R^2), were B (NaCl DL) and

E(NaCl PL). An AC interaction was also found significant. The Shapiro-Wilk normality test displayed the p value as 0.451. This indicated the non significance of the non selected factors**.** So no other factors except B and E were affecting the zero order release rate constant.

ii) Normal plot

From the normal plot shown in the Figure No. 6.3.89 it was evident that the factors B and E are significantly away from the normal straight line. An interaction AC was also found significant. Shapiro-Wilk normality test displayed the p value as 0.451 indicating that the remaining (unselected) terms are normally distributed.

iii) Pareto chart

The pareto chart shown in Figure No.6.3.90 represent the significant effect of B and E on the zero order release rate constant. Both the factors crosses the t and Bonferroni limit confirmed the obvious effect of these factors on the zero order release rate constant. The magnitude of the effect can be written as, NaCl in the drug leyer > NaCl in the push layer. Both the factors had a positive effect on the R^2. AC(PEO DL- SLS DL) interaction was also found significant. No other terms are significant as they all were below the t limit.

iv) ANOVA and Regression Analysis

The Model F-value of 50.2001 implied the model was highly significant. Factors B ($p = 2.95E^{-08}$), E ($1.65E^{-05}$) are significant model terms. AC interaction was also found significant ($p = 1.40E^{-03}$). The "Lack of Fit F-value" of 3.7867 implied that the lack fit was not significant relative to the pure error. No curvature effect was reported. This means that the polynomial model was fitting all of the design points well. The "Pred R-Squared" of 0.8384 was in reasonable agreement with the "Adj R-Squared" of 0.8860, indicated the linearity of the model. Adeq Precision 17.9000 indicated an adequate signal. So this model can be used to navigate the design space.

Table No.6.3.44: ANOVA and Regression analysis for the effect of factors on R^2

Source	Sum of Squares	df	Mean Square	F Value	p-value	Prob > F
Model	0.0358	3	0.01195	50.2001	2.31E-08	*significant*
B-NaCl DL	0.0236	1	0.02356	98.9959	2.95E-08	
E-NaCl PL	0.0087	1	0.00874	36.7302	1.65E-05	
AC	0.0035	1	0.00354	14.8742	1.40E-03	
Residual	0.0038	16	0.00024			
Lack of Fit	0.0036	13	0.00028	3.7867	1.50E-01	*not significant*
Pure Error	0.0002	3	0.00007			
Cor Total	0.0397	19				
Regression analysis						
Std. Dev.	0.0154		R-Squared	0.9040		
Mean	0.9496		Adj R-Squared	0.8860		
C.V. %	1.6247		Pred R-Squared	0.8384		
PRESS	0.0064		Adeq Precision	17.9000		

v) Polynomial equations

The polynomial equation representing the R^2 can be written as follows,

R^2 = 0.94955 +0.038375 * B +0.02337* E - 0.014875 * AC *(coded values*)

R^2 = 0.8814 +0.0085 * NaCl concentration in drug layer +0.0010 *Sodium chloride in the Push layer -4.4355e^{-005} * PEO in the drug layer * SLS in the drug layer *(actual values)*

vi) Test for the assumption of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs show in the Figure No.6.3.91.

- *The normal probability plot*: The plot indicated that residuals followed a normal distribution. The curve did not follow any pattern like S curve.
- *Residuals vs Predicted*: The plot was a random scatter (constant range of residuals across the graph.) confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: The plot showed a random scatter. The plot did not follow any trends indicates that no time-related variable lurking in the background.

- ❖ *Predicted vs. Actual*: This graph represented a good relationship between actual and predicted responses.
- ❖ *Box-Cox Plot for Power Transforms*: The lamda value was 1 and no transformation was needed.

vii) The perturbation graph

This graph showed the effects and signs of the significant factors on the zero order rate constant. It showed that as the concentration of NaCl DL changed from minimum to maximum an increase in zero order rate constant from 0.92 to 0.98 was produced. An increase in the NaCl PL has changed the response from 0.925 to 0.97.

viii) Interaction graphs

The Figure No. 6.3.92 shows the interaction plot of PEO DL and SLS DL(AC).The plot showed the effect of change in the concentration of PEO DL at low and high level of SLS DL. Both the lines were not parrellel to each other , indicating that the effect of one factor depends on the level of the other. At high level of SLS, change in concentration of PEO DL had a negative impact on R^2. At medium level of PEO DL, high and low concentration of SLS produced no significant difference on R^2 value. No overlapping of the I beams of both high and low levels indcated that there was a significant difference (95% confidence was default) between the two points.

ix) Contour plots and RS plots

Figure No. 6.3.93 shows the contour plot and RS plot for the simultaneous effect of factor B and E at a time. From the plot it was obvious that the factor B and E had a positive effect on zero order release rate constant. High levels of NaCl DL and PL yields a better R^2 value. The effect of change in concentration of NaCl DL was more evident at high level of NaCl PL and vice versa. From the surface plot the larger effect of NaCl DL than the NaCl PL was clearly understood.

Figure. 6.3.94 shows the contour plot and RS plot for the concurrent effect

of factor A (PEO DL) and C (SLS DL) at a time on zero order rate constant. The curved lines indicate the nonlinearity in the response with change in levels of factors.

3. Analysis of responses – Lag time

i) Half normal plot

Figure 6.3.95 shows the half normal plot of the effect of factors on lag time. The significant factor affecting the lag time was identified as B (NaCl DL) G (propylene glycol) and H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.233. This indicated the non significance of the non selected factors**.** So no other factors except B, G and H were affecting the lag time**.**

ii) Normal plot

Figure No.6.3.96 shows the normal plot of the effect of factors on lag time. The factor B, G and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.233 indicating that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

Figure No.6.3.97 shows the pareto chart of effect of factors on the lag time in terms of t value. The factors significantly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect on the response. The magnitude of the effect of significant factors on the lag time can be written as G > H> B. Propylene Glycol had comparatively greater effect on the lag time. There was not much variation in the t value for all the 3 factors. So it can be considered that all the 3 factors are equally affecting the lag time. No other factors or interaction terms were significant as they had not crossed the t limit.

iv) ANOVA and regression analysis

The Model F-value of 22.6058 implied that the model selected was

significant. Factors B (*0.0004)*, G (*< 0.0002)* and H *(0.0003)* were the significant model terms affecting the lag time. No interaction terms were significant. The "Lack of Fit F-value" of 3.2578, implied that the lack of fit was not significant relative to the pure error. This means that the polynomial model was fitting all of the design points well. Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit and curvature effect were observed.

Table No.6.3.45: ANOVA and Regression analysis for the effect of factors on the lag time

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.0750	3	1.6917	22.6058	*8.09E-06*	*significant*
B-NaCl DL	1.5625	1	1.5625	20.8797	*0.0004*	
G-Propylene Glycol	1.8225	1	1.8225	24.3541	*0.0002*	
H- Weight gain	1.6900	1	1.6900	22.5835	*0.0003*	
Curvature	0.0005	1	0.0005	0.0067	0.9359	
Residual	1.1225	15	0.0748			
Lack of Fit	1.0425	12	0.0869	3.2578	0.1801	*not significant*
Pure Error	0.0800	3	0.0267			
Cor Total	6.1980	19				
Regression analysis						
Std. Dev.	0.2649		R-Squared	0.8188		
Mean	3.9900		Adj R-Squared	0.7848		
C.V. %	6.6398		Pred R-Squared	0.6963		
PRESS	1.8823		Adeq Precision	16.4585		

v) Polynomial equation

The polynomial equation for the lag time can be written as,

Lag time = 3.99 - 0.3125 * B - 0.3375 * G + 0.325 * H (*coded terms)*

Lag time = 3.8094 - 0.0694 * NaCl concentration in drug layer -0.075 * Propylene Glycol + 0.065 * Weight gain *(actual terms)*

vi) Test for assumptions of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs show in the Figure No.6.3.98.

- *Normal probability plot*: The residuals followed a normal distribution, as the points followed a straight line.
- *Residuals vs Predicted*: Random scatter (constant range of residuals across the graph) plot confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: The plot showed a random scatter. The graphs did not follow any trends indicated that no time-related variable lurking in the background.
- *Predicted vs. Actual*: This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The lamda value was 1 and no transformation was needed.

vii) Perturbation graph

This graph explained the effects and signs of the significant factors on lag time. From the graph it was evident that the change in the concentration of B and G from minimum to maximum had produced a decrease in the lag time. The factor H had an opposite effect. The factor G had comparatively larger effect on the lag time.

viii) Contour plots and RS plots

Figure No.6.3.99 shows the contour plot and RS plot of the simultaneous effect of factor B (NaCl DL) and the G (propylene glycol) at a time on lag time. Both the factors had a negative effect on the response. Effect of propylene glycol was more prominent at high levels of NaCl DL. Change in concentration of NaCl DL had little effect at high levels of propylene glycol. Response surface plot clearly represented the chief effect of propylene glycol.

Figure No.6.3.100 shows the contour plot and RS plot, of the simultaneous effect of factor B (NaCl DL) and H (weight gain). NaCl DL had a negative effect on the lag time. i.e., lag time had decreased as the concentration changed from low to high. But the weight gain had an opposite effect. Low weight gain and high concentration of NaCl DL produced the desired effect. NaCl had prominent effect at low weight gain.

Figure No 6.3.101 shows the contour plot and response plot of the combined effect of G (propylene glycol) and H (weight gain) at a time. Weight gain had a positive effect on the lag time and propylene glycol had a reverse effect. Effect of propylene glycol was more pronounced at low weight gain. From the RS plot the greater effect of the propylene glycol was well understood.

ix) Cube plots

Figure No.6.3.102 showed the combined effect of B, G and H. When all the three factors were at minimum the lag time was 4.315 hr, and at maximum it was around 3.66 hr. Lowest lag time was observed while keeping NaCl DL and propylene glycol at maximum and weight gain at its minimum. This cube plot also well represented the major effect of propylene glycol in all the levels of the other factors.

IV. Numerical optimization with the help of desirability

From the factor influence study it was found that the model suggested was linear and no significant lack of fit and curvature were found for any of the responses. So No quadratic model was chosen for optimization. The same 2 level design was used for further optimization.

***A.* Optimization of the push pull osmotic tablets of Carvedilol phosphate**

When more than two factors were significant, overlay plot does not give a complete idea about the optimization. In our study three and four factors were significantly affecting the lag time and PCUR at 24 hrs respectively. So for a

better understanding the numerical optimization was chosen. Desirability function was selected as the tool for optimization. The constraint fixed for the optimization was given in the Table No.6.3.46. The solutions of the numerical optimization were given in the Table No.6.3.47.

Table No.6.3.46: Constraints fixed for the optimization of Carvedilol phosphate push pull OTs

Constraints Name	Goal	Lower Limit	Upper Limit	Importance
A:PEO DL	*is in range*	10	100	3
B:NaCl DL	*is in range*	1	10	3
C:SLS DL	*is in range*	1	5	3
D:PEO PL	*is in range*	5	50	3
E:NaCl PL	*is in range*	5	50	3
G:Propylene Glycol	*is in range*	1	10	3
H: Weight gain	*is in range*	10	20	3
CUR at 24 hrs	*is in range*	95	100	5
R^2	*maximize*	0.98	0.999	4
lag time	*minimize*	3	5	3

B. The point prediction

The point prediction for the solution 1, 2 and 3 were given in the Table No.6.3.48. The same batches were selected as the check point batches. The confidence intervals and the tolerance intervals were given in the Table No.6.3.48.

C. Check point

To confirm the validity of the model three formulations from the solutions (1, 2 and 3) were selected and formulated as discussed in the chapter 5.6.4(I). The dissolutions were performed as per the method specified in section 5.6.4(IIBix). The values obtained from the dissolution study were given in the Table No.6.3.49. All the responses were within the Intervals and tolerance limits of the point predicted by the software. Hence it can be concluded that the model suggested for the design was a success and can be used for further predictions.

D. Optimized batch and evaluations

The optimized batch (COB) from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. Solution

2 was selected as the optimized formulation. The composition of the optimized batch was given in the Table No. 6.3.50. The optimized batch was prepared as per the procedure mentioned in the chapter section 5.6.4(I). The blend as well as the whole tablet evaluation was performed as per the methods specified in section 5.6.4(II). The result of the study was given in the Table No.6.3.51. The *in vitro* study was performed as per the methods mentioned in the section 5.6.4(IIBix) and the findings were given in the Table No.6.3.51 and in the Figure No.6.3.95.

Table No.6.3.47: Numerical solutions for the optimization of the Carvedilol phosphate push pull OTs

Solutions												
Number	**PEO DL**	**NaCl DL**	**SLS DL***	**PEO PL**	**NaCl PL**	**SLS PL***	**PG**	**Wt gain**	**CUR at 24 hrs**	**R^2**	**lag time**	**Desirability**
1	**89.74**	**9.99**	**2.91**	**37.55**	**36.71**	**4.53**	**10.00**	**10.15**	**100.00**	**0.999**	**3.01**	**0.996**
2	**77.09**	**9.97**	**3.24**	**45.81**	**38.29**	**2.97**	**9.84**	**10.00**	**99.99**	**0.999**	**3.01**	**0.9966**
3	**99.17**	**9.88**	**4.08**	**31.51**	**46.17**	**2.01**	**10.0**	**10.09**	**99.93**	**0.999**	**3.03**	**0.9945**
4	93.28	9.99	4.06	33.76	44.64	*3.21*	10.0	10.00	99.47	0.999	3.015	0.9939
5	69.84	10.00	2.80	38.69	36.71	3.80	9.99	10.00	95.06	0.999	3.02	0.9938
6	83.22	9.99	4.27	37.05	43.45	1.77	9.79	10.00	97.83	0.999	3.03	0.9933
7	94.36	9.51	2.87	31.43	39.36	3.23	10.00	10.00	99.50	0.999	3.05	0.9894
8	89.59	9.49	2.37	24.98	36.61	1.45	10.00	10.08	95.00	0.999	3.06	0.9881
9	75.71	9.19	2.03	30.74	38.73	1.13	9.93	10.03	95.00	0.999	3.08	0.9830
10	64.74	9.06	4.14	46.24	44.93	4.11	10.00	10.00	98.76	0.999	3.08	0.9826
11	77.43	8.86	3.24	28.72	45.17	1.15	10.00	10.00	95.63	0.999	3.09	0.9795
12	75.44	10.00	1.20	49.25	30.85	1.00	8.95	10.08	98.62	0.999	3.10	0.9785
13	70.51	8.58	1.04	37.80	40.70	1.03	10.00	10.00	98.16	0.999	3.11	0.9753
14	58.93	8.66	2.61	49.09	45.18	4.98	10.00	10.05	98.72	0.999	3.11	0.9750
15	61.69	9.20	4.41	43.94	43.82	1.48	9.27	10.00	95.00	0.999	3.13	0.9725
16	69.21	8.31	2.28	29.12	45.89	1.36	10.00	10.02	95.04	0.999	3.13	0.9707
17	53.85	8.86	3.04	48.91	44.24	4.70	9.96	10.58	95.71	0.999	3.13	0.9705
18	100.00	8.29	2.78	15.27	46.08	3.33	10.00	10.03	95.04	0.999	3.14	0.9703
19	54.43	7.97	4.12	47.89	49.64	5.00	10.00	10.07	98.46	0.999	3.16	0.9647
20	82.17	10.00	1.10	49.36	28.26	3.86	10.00	13.08	99.05	0.999	3.22	0.9524
21	54.51	7.95	1.41	43.98	49.99	2.51	9.11	10.01	95.34	0.999	3.22	0.9502
22	91.72	6.89	1.19	18.24	44.90	4.88	10.00	10.00	98.18	0.999	3.23	0.9487
23	68.86	7.86	2.66	32.56	49.62	1.00	8.90	10.00	95.00	0.999	3.25	0.9453
24	72.18	9.25	5.00	47.58	47.81	1.91	10.00	12.81	97.48	0.999	3.25	0.9444

Solutions												
Number	**PEO DL**	**NaCl DL**	**SLS DL***	**PEO PL**	**NaCl PL**	**SLS PL***	**PG**	**Wt gain**	**CUR at 24 hrs**	**R^2**	**lag time**	**Desirability**
25	95.46	10.00	4.70	46.88	49.15	1.78	7.18	10.46	100.00	0.999	3.26	0.9429
26	62.92	8.28	4.20	40.77	49.48	1.03	9.92	12.06	95.00	0.999	3.27	0.9387
27	90.51	8.03	2.81	23.46	48.22	5.00	9.74	11.60	95.31	0.999	3.28	0.9385
28	87.33	9.61	1.36	49.87	30.19	3.61	7.21	10.01	99.87	0.999	3.25	0.9352
29	73.53	7.90	2.49	44.90	48.52	1.00	8.56	10.34	100.00	0.999	3.29	0.9348
30	72.92	7.31	1.02	39.55	47.62	5.00	8.86	10.00	100.00	0.999	3.29	0.9343
31	96.55	6.21	1.36	17.96	48.85	2.18	9.75	10.00	100.00	0.999	3.30	0.9334
32	77.47	8.19	2.63	47.14	46.96	1.00	7.68	10.04	100.00	0.999	3.32	0.9286
33	95.47	10.00	3.56	48.06	41.12	2.67	8.63	13.12	100.00	0.999	3.32	0.9279
34	88.98	6.05	1.00	18.57	49.82	3.38	10.00	10.57	99.01	0.999	3.33	0.9265
35	77.99	10.00	5.00	49.58	44.45	4.99	7.30	10.00	96.10	0.998	3.22	0.9262
36	98.53	6.00	1.30	8.48	49.12	4.09	9.39	10.00	95.00	0.999	3.34	0.9236
37	97.65	9.54	3.27	39.53	41.99	1.00	10.00	14.66	98.17	0.999	3.35	0.9210
38	94.95	10.00	3.84	49.46	43.04	1.33	9.95	15.68	100.00	0.999	3.39	0.9114
39	98.50	10.00	1.00	46.95	22.16	3.44	4.94	10.05	96.01	0.999	3.40	0.9094
40	100.00	8.01	2.74	36.88	47.50	1.40	6.68	10.00	100.00	0.999	3.40	0.9082
41	100.00	10.00	1.17	41.53	22.85	1.47	10.00	15.78	96.91	0.999	3.39	0.9078
42	89.98	8.78	1.30	50.00	34.49	1.85	5.90	10.00	99.97	0.999	3.41	0.9070
43	89.94	9.60	4.67	47.71	49.76	1.00	4.85	10.00	95.00	0.999	3.43	0.9018
44	77.83	9.29	4.74	47.95	48.44	5.00	10.00	16.00	95.00	0.999	3.45	0.8955
45	96.72	9.99	4.63	47.67	49.02	4.79	10.00	16.95	98.17	0.999	3.47	0.8921
46	100.00	9.42	4.18	47.55	49.99	5.00	3.70	10.00	96.03	0.999	3.53	0.8770
47	99.03	8.33	3.32	39.76	49.97	3.39	9.99	16.10	99.57	0.999	3.53	0.8769
48	99.99	9.71	4.39	49.91	49.84	5.00	10.00	17.74	100.00	0.999	3.54	0.8740
49	71.15	8.29	1.00	49.93	42.16	3.19	9.83	16.11	95.19	0.999	3.54	0.8730
50	99.99	8.96	3.74	46.27	49.43	1.22	10.00	17.53	100.00	0.999	3.58	0.8642

Table No.6.3.48: Prediction of the responses

Solution 1							
	Predicted			CI for Mean		99% of Population	
Response	**Mean**	**Std Dev**	**SE Mean**	**95%CI low**	**95%CI high**	**95%TI low**	**95%TI high**
PCUR at 24 hrs	100	6.271	3.066	93.317	106.676	70.055	129.93
R^2	0.999	0.007	0.003	0.993	1.005	0.967	1.031
lag time	3.01	0.265	0.128	2.754	3.297	1.835	4.216
Solution 2							
Response		Predicted		CI for Mean		99% of Population	
	Mean	**Std Dev**	**SE Mean**	**95%CI low**	**95%CI high**	**95%TI low**	**95%TI high**
PCUR at 24 hrs	99.99	6.271	3.147	93.133	106.845	69.902	130.07
R^2	0.999	0.007	0.003	0.993	1.005	0.967	1.031
lag time	3.01	0.265	0.128	2.758	3.299	1.838	4.219
Solution 3							
	Predicted			CI for Mean		99% of Population	
Response	**Mean**	**Std Dev**	**SE Mean**	**95%CI low**	**95%CI high**	**95%TI low**	**95%TI high**
PCUR at 24 hrs	99.93	6.271	3.420	92.483	107.385	69.361	130.50
R^2	0.999	0.007	0.003	0.993	1.005	0.967	1.031
lag time	3.029	0.265	0.128	2.758	3.300	1.839	4.219

Table No .6.3.49: Check point batches for the model validation of the Carvedilol phosphate push pull OTs

Batches	PCUR at 24 hrs	R^2	Lag time
Solution 1	99.08 ±2.5	0.998±0.004	3.07±0.1
Solution 2	99.08 ±1.8	0.998±0.003	3.05± 0.03
Solution 3	100±2.1	0.999±0.012	3.03±0.05

Table No.6.3.50: Composition of the optimized batch

SLNo	Ingredients	Mg/tab	(%w/w)
	Drug Layer		
1	Carvedilol Phosphate	10	
2	Dibasic calcium phosphate	20.692	
3	PEO 400 K	7.709	77.09
4	Sodium chloride	9.472	9.97
5	BHT	0.007709	
6	SLS	1.620	3.24
7	IPA		
8	Magnesium stearate	0.500	
	Total weight of drug layer	50	
	Push layer		
9	PEO 7000 K	22.91	45.81
10	Sodium chloride	8.77	38.29
11	Dibasic calcium phosphate	9.67	
12	BHT	0.022905	
13	SLS	1.34	2.97
14	Iron oxide Red	0.8	
15	IPA		
16	Magnesium stearate	1.5	
	Total weight of Push layer	45	
	Total weight of un coated tablet	95	
	Functional coating		
15	Cellulose acetate	8.6	
16	Acetone	q.s	
18	Water	q.s	
19	Propylene Glycol	0.85	9.8
	Total Weight of Coating	9.5	10.00
	Total tablet weight	104.5	
Responses	**CUR at 24 hrs (%)**	**R^2**	**Lag time**
Predicted	99.99	0.999	3.01
Observed	99.08± 1.8	0.998±0.003	3.05±0.03

Table No.6.3.51: Optimized batch evaluation

Trial	Wt variation (n =20)	Diameter (n=10)	Thickness (n=10)	Hardness (n=6)	Friability (%)	Assay (%)	Weight gain(%)	Pore size (mm)
COB	103.5 ±2.4	5.13 ±0.04	3.52 ±0.08	4.5 ±0.4	0.74	101.3 ±2.7	10.12 ±0.07	0.60

Dissolution Profile of Carvedilol phosphate optimized formulation											
Time	0	1	2	3	4	6	8	12	16	20	24
PCUR	0	0	4.5± 0.02	9.9± 0.1	15.3 ±0.6	23.3 ±1.2	32.3 ±2.4	47.6 ±2.8	63.4 ±4.5	79.3 ±5.1	99.0 8 ±1.8

E. Desirability contour plot and RS plot

Desirability plots show how all the targeted optimum conditions are met by changing two factors at a time. The Figure No.6.3.104 shows how PEO DL and NaCl DL affect the desirability. Higher desirability was achieved at maximum levels of NaCl DL (more than 9%) and PEO DL. Change in concentration of PEO DL 65- 85 % and NaCl DL 9 -10 % showed desirability more than 0.8. Desirability of 0.2-0.8 was observed while keeping PEO DL 65-85% and NaCl DL greater than 7.75.

The Figure No. 6.3.105 shows how PEO DL and NaCl PL affects the desirability function. Combinations of 75 - 85 % of PEO DL and 30 -35% NaCl PL had a desirability >0.8.

The Figure No. 6.3.106 shows the effect of PEO DL and PEO PL on desirability function. Combinations of 60 -85 % of PEO DL and 30-42% of PEO PL had a desirability 1. All other combinations had desirability zero.

Figure No. 6.3.107 shows the desirability contour plot and RS plot of the effect of PEO DL and propylene glycol. Combinations of PEO DL 65 to 80% and propylene glycol 9-10% had desirability 1. Lower concentrations of both the factors had desirability zero.

Figure No. 6.3.108 shows the desirability contour plot and RS plot of the effect of PEO DL and weight gain. Combinations of PEO DL 60 to 80% and weight gain 10-13 % had desirability >0.8. Combinations of weight gain more than 13 % and PEO DL less than 60 % had desirability zero.

Figure No.6.3.109 shows the desirability contour plot and RS plot of the effect of PEO PL and NaCl DL. Combinations of PEO PL 27- 35 % and NaCl DL 9 -10% had desirability 1. Combinations of NaCl DL less than 7.5 % and PEO PL less than 27% & greater than 40% had desirability zero.

Figure No.6.3.110 shows the desirability contour plot and RS plot of the effect of NaCl PL and NaCl DL. Combinations of NaCl PL 27- 35 % and NaCl DL 9 -10% had desirability 1. Combinations of NaCl DL less than 7.5 % and NaCl PL less than 27% & greater than 40% had desirability zero.

Figure No 6.3.111 shows the desirability contour plot and RS plot of the effect of propylene glycol and NaCl DL. Combinations of NaCl DL 9-10% and propylene glycol 7.75 -10% had desirability 1. Combinations of NaCl DL less than 7.5 % and propylene glycol less than 8%, had desirability zero.

Figure No.6.3.112 shows the desirability contour plot and RS plot of the effect of weight gain and NaCl DL. Combinations of NaCl DL 9-10% and weight gain 10 -14% had desirability 1. Combinations of NaCl DL less than 9 % and weight gain greater than 14% had desirability zero.

Figure No. 6.3.113 shows the desirability contour plot and RS plot of the effect of PEO PL and NaCl PL. Combinations of NaCl PL 30-38% and PEO PL 28-40% had desirability greater than 0.80. A higher level (>35%) of NaCl PL and lower level (< 25%) had desirability zero.

Figure No.6.3.114 shows the desirability contour plot and RS plot of

the effect of PEO PL and propylene glycol. Combinations of propylene glycol 7.5-10% and PEO PL 30-40% had desirability greater than 0.80. All other combinations had desirability zero.

Figure No. 6.3.115 shows the desirability contour plot and RS plot of the effect of PEO PL and weight gain. Combinations of weight gain 10 -12.5 % and PEO PL 28 -40% had desirability 1. Other combinations having concentrations of PEO PL less than 27 % and weight gain more than 12.5% had desirability zero.

Figure No.6.3.116 shows the desirability contour plot and RS plot of the effect of NaCl PL and propylene glycol. Combinations of propylene glycol 7.5-10% and NaCl PL 25-38 % had desirability >0.4. All other combinations had desirability zero.

Figure No. 6.3.117 shows the desirability contour plot and RS plot of the effect of NaCl PL and weight gain. Combinations of weight gain 10-12.5% and NaCl PL 30-35 % had desirability >0.6. Higher weight gain and NaCl PL acquired the desirability zero.

Figure No.6.3.118 shows the desirability contour plot and RS plot of the effect of propylene glycol and weight gain. Combinations of weight gain 10 -12.5% and propylene glycol 7.75 -10 % had desirability greater than 0.6-0.8. Higher weight gain and lower propylene glycol concentrations acquired the desirability zero.

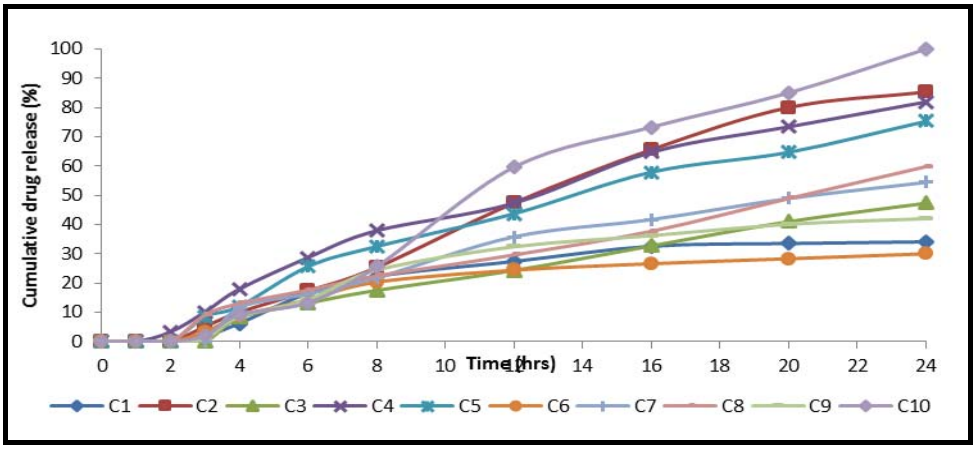


Figure No .6.3.65: Dissolution profile of C1- C10

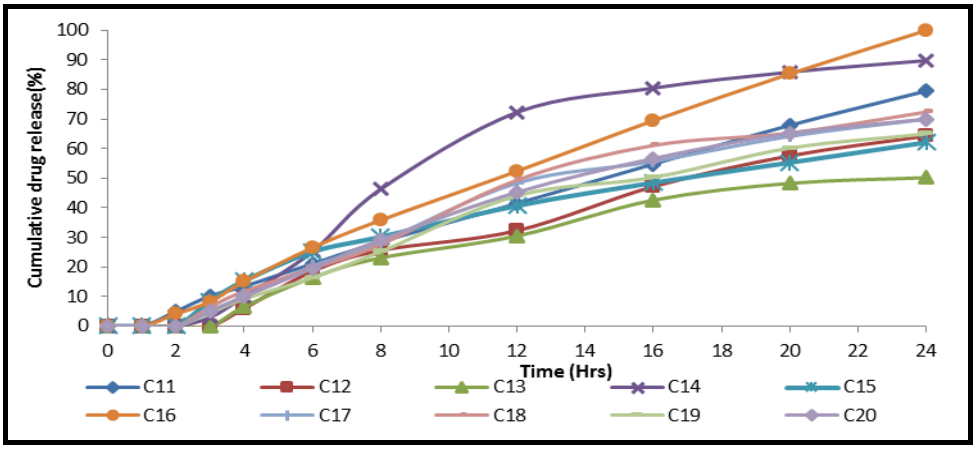


Figure No .6.3.66: Dissolution profile of C11- C20

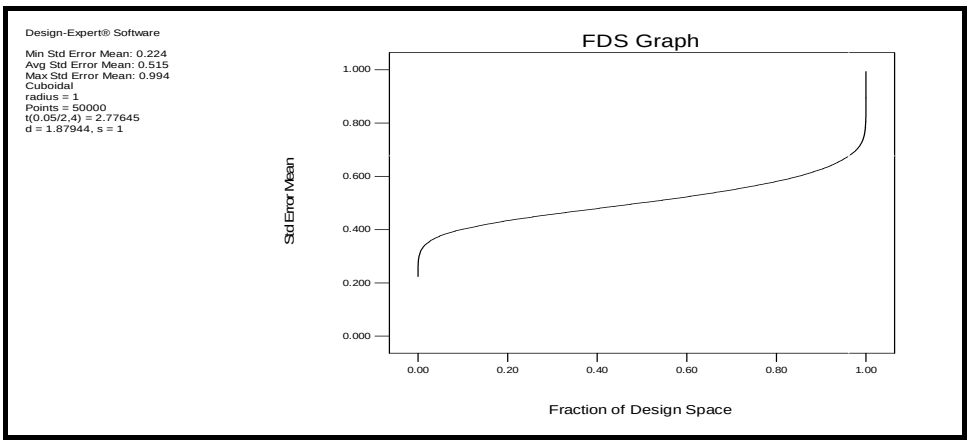
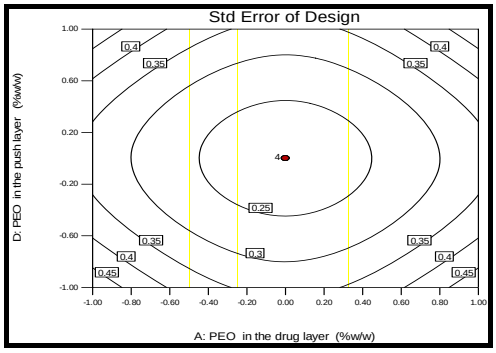
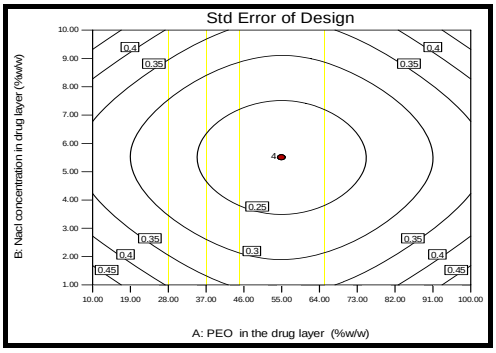
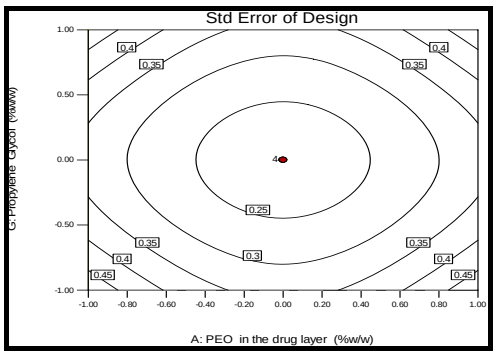
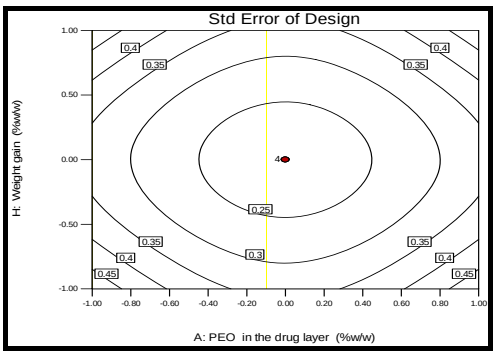


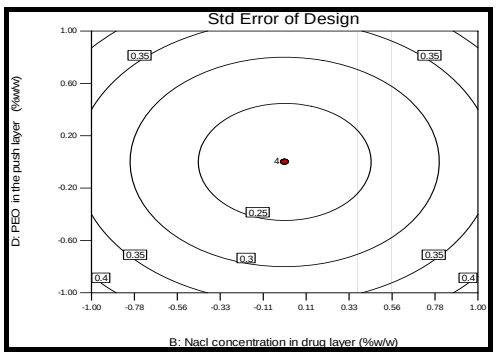
Figure No.6.3.67: FDS graph of the design selected for the FI study & optimization

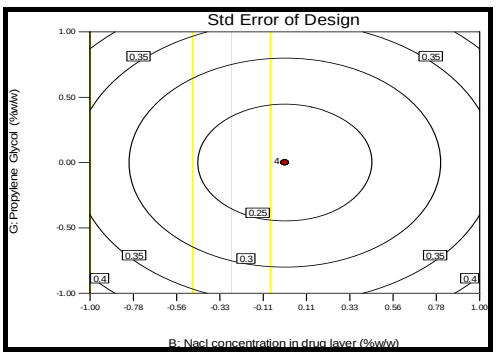

Std Error of Design
D: PEO in the push layer (%w/w)
A: PEO in the drug layer (%w/w)

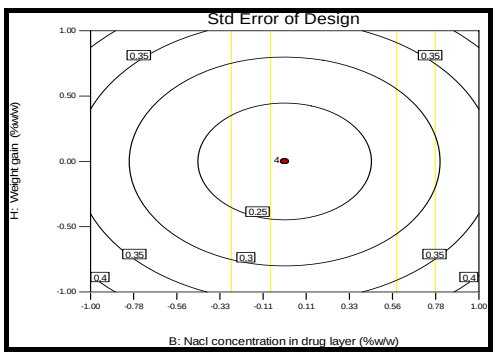

Std Error of Design
B: NaCl concentration in drug layer (%w/w)
A: PEO in the drug layer (%w/w)

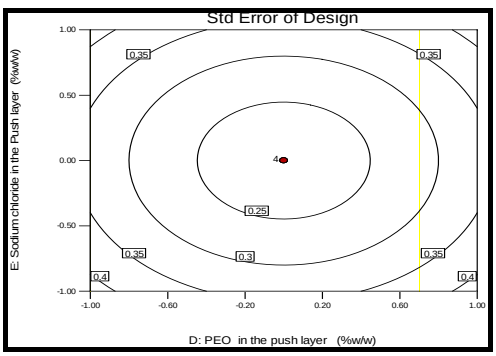

Std Error of Design
G: Propylene Glycol (%w/w)
A: PEO in the drug layer (%w/w)


Std Error of Design
H: Weight gain (%w/w)
A: PEO in the drug layer (%w/w)


Std Error of Design
D: PEO in the push layer (%w/w)
B: NaCl concentration in drug layer (%w/w)


Std Error of Design
G: Propylene Glycol (%w/w)
B: NaCl concentration in drug layer (%w/w)


Std Error of Design
H: Weight gain (%w/w)
B: NaCl concentration in drug layer (%w/w)


Std Error of Design
E: Sodium chloride in the Push layer (%w/w)
D: PEO in the push layer (%w/w)

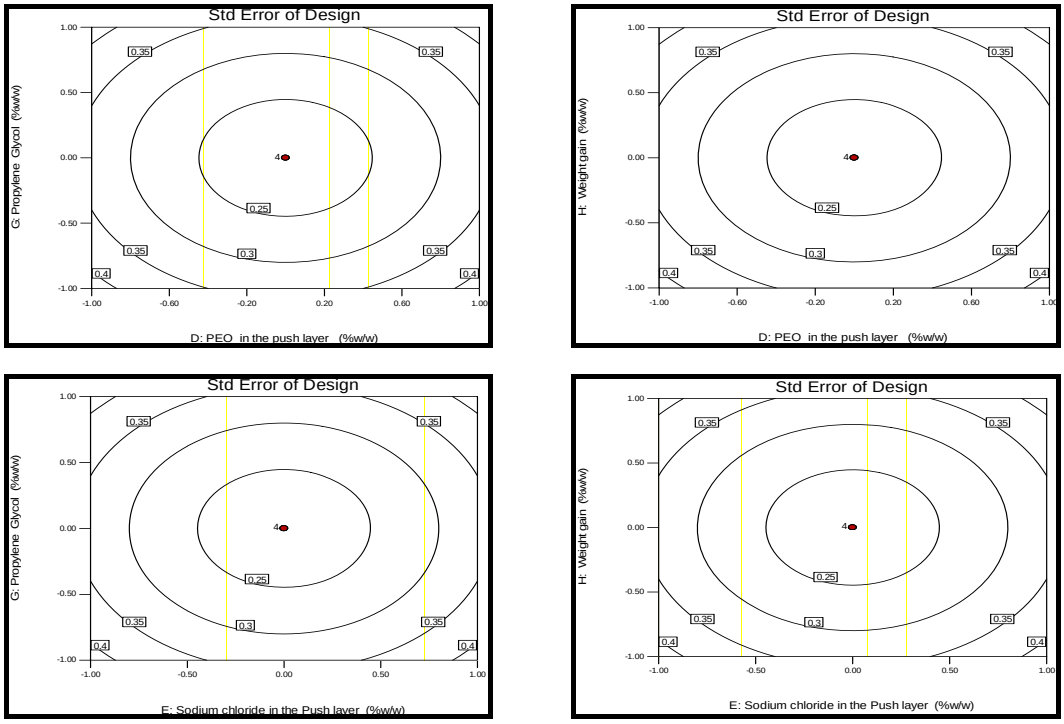


Figure No.6.3.68: Standard error contour plots of the FI study & optimization

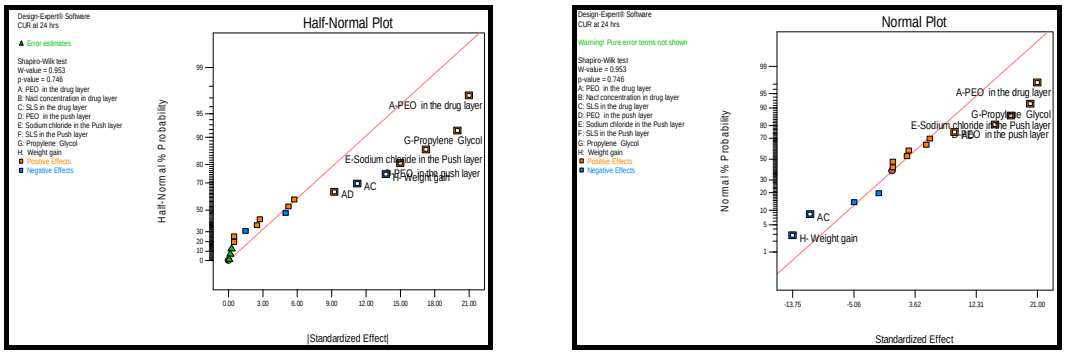


Figure.6.3.69: Half normal plot for the effect of the factors selected on the PCUR at 24 hrs, Figure.6.3.70: Normal plot for the effect of the factors selected on the PCUR at 24 hrs

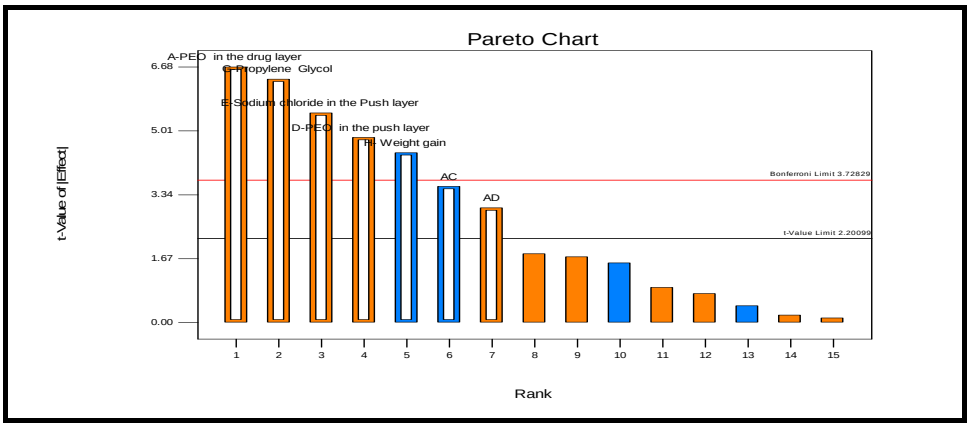


Figure No.6.3.71 : The pareto chart for the effect of the factors selected on the PCUR at 24 hrs

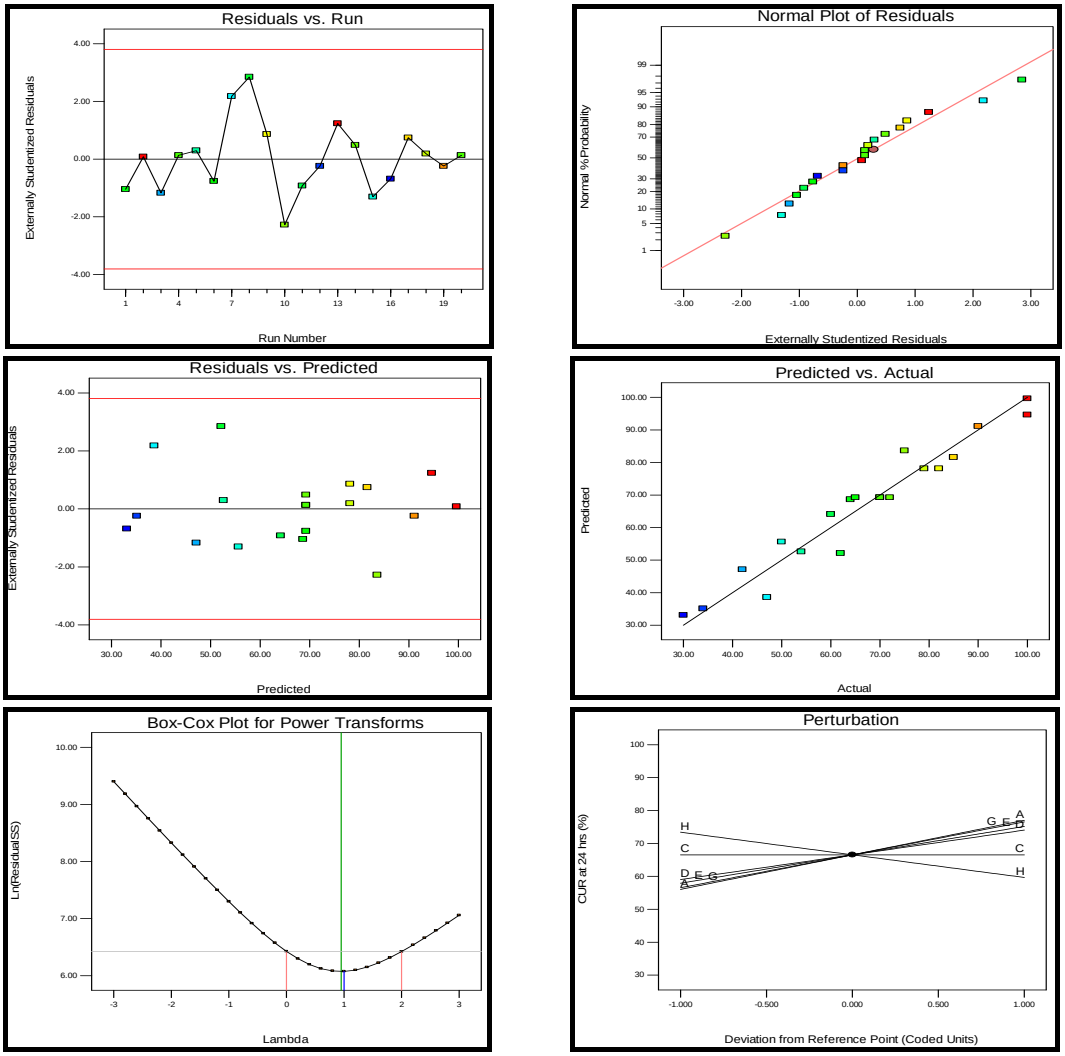


Figure No. 6.3.72: Plots for the testing the assumptions of ANOVA and perturbation curve for factors selected on the PCUR at 24 hrs

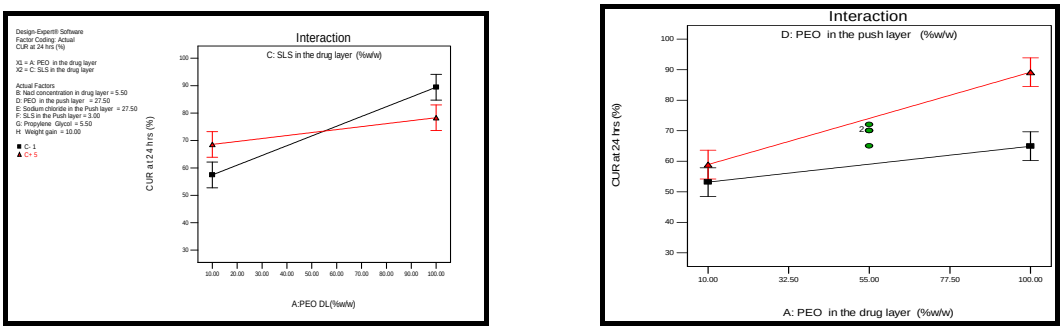


Figure No. 6.3.73: AD and AC interaction plots on PCR at 24 hrs

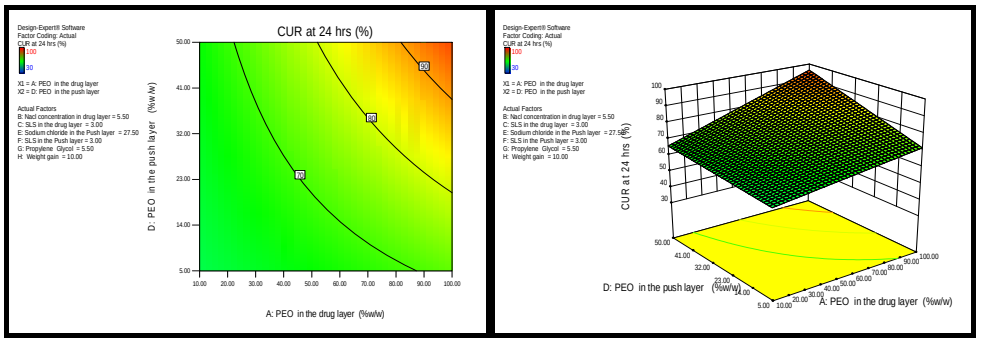


Figure No.6.3.74: Contour plot and RS Plot - Effect of PEO DL and PEO PL on PCUR at 24 hrs

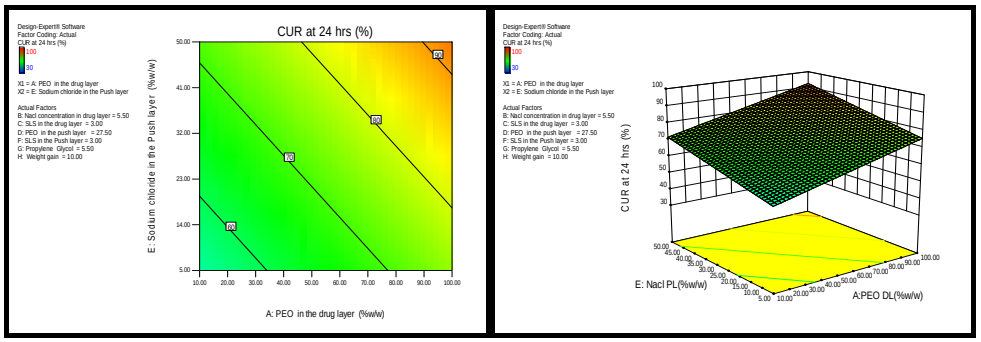


Figure.6.3.75: Contour plot and RS Plot -Effect of PEO DL and NaCl PL on PCUR at 24 hrs

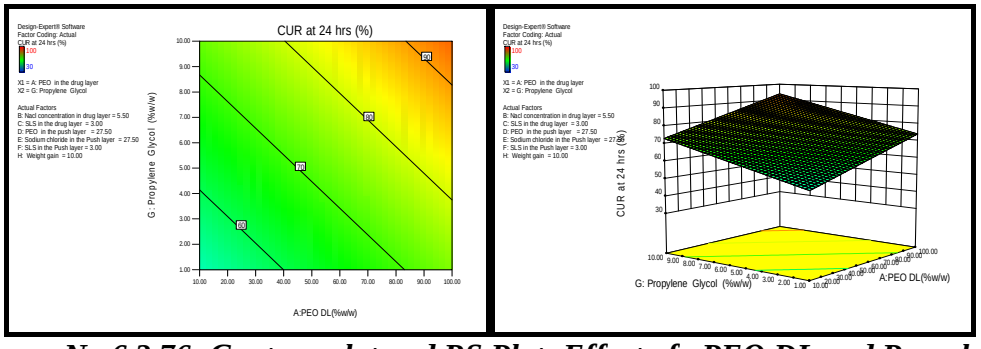


Figure No.6.3.76: Contour plot and RS Plot -Effect of PEO DL and Propylene Glycol on PCUR at 24 hrs

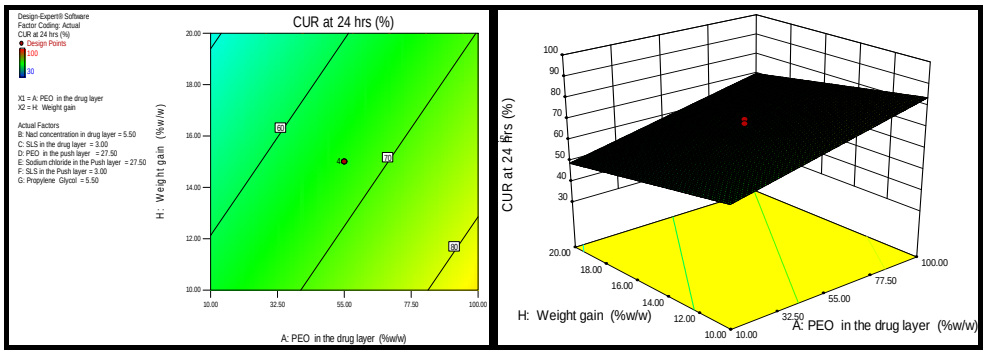


Figure No.6.3.77: Contour plot and RS Plot -Effect of PEO DL and weight gain on PCUR at 24 hrs

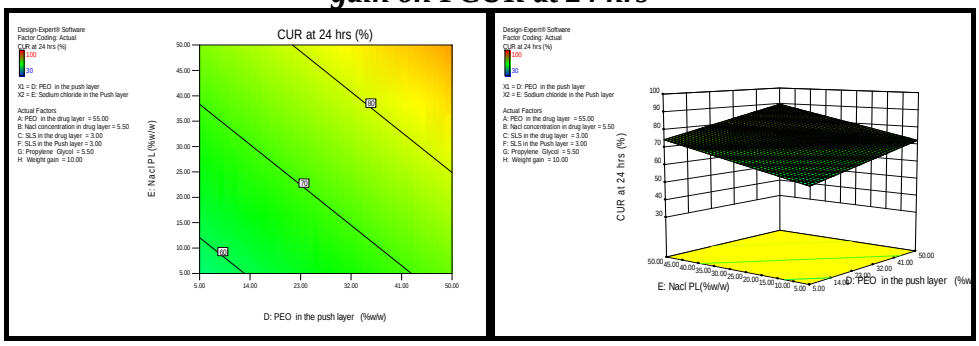


Figure No.6.3.78: Contour plot and RS Plot -Effect of PEO PL and NaCl PL on PCUR at 24 hrs

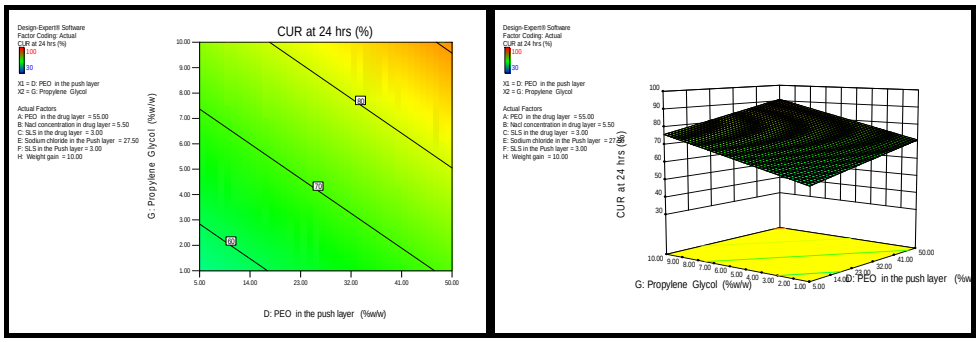


Figure No.6.3.79: Contour plot and RS Plot -Effect of PEO PL and Propylene Glycol on PCUR at 24 hrs

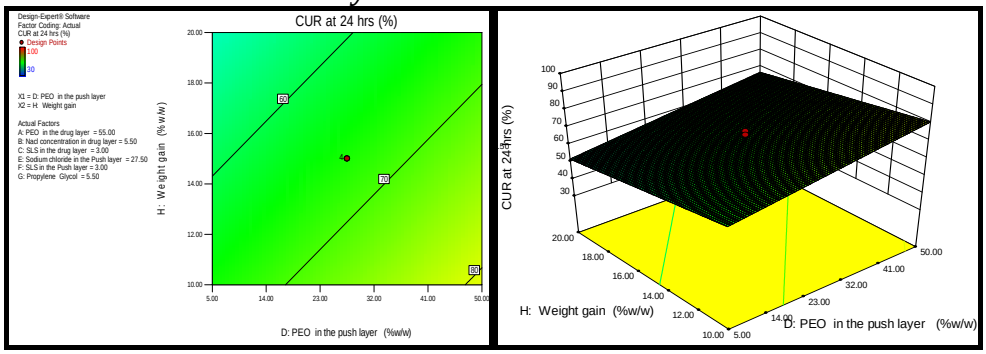


Figure No.6.3.80: Contour plot and RS Plot -Effect of PEO PL and weight /gain on PCUR at 24 hrs

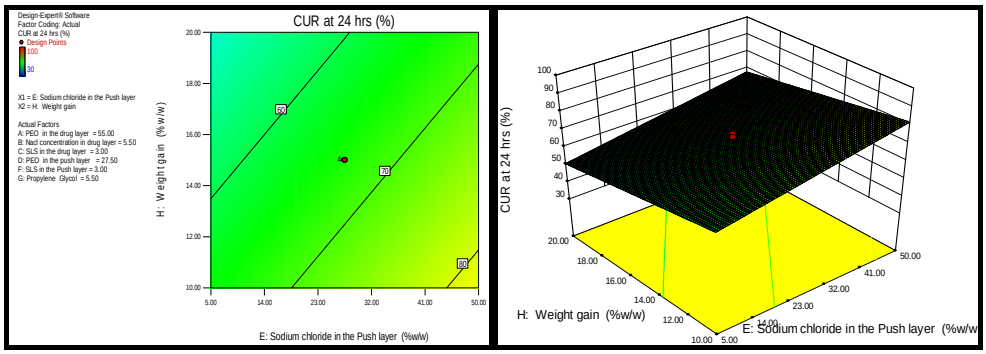


Figure No.6.3.81: Contour plot and RS Plot - Effect of NaCl PL and weight gain on PCUR at 24 hrs

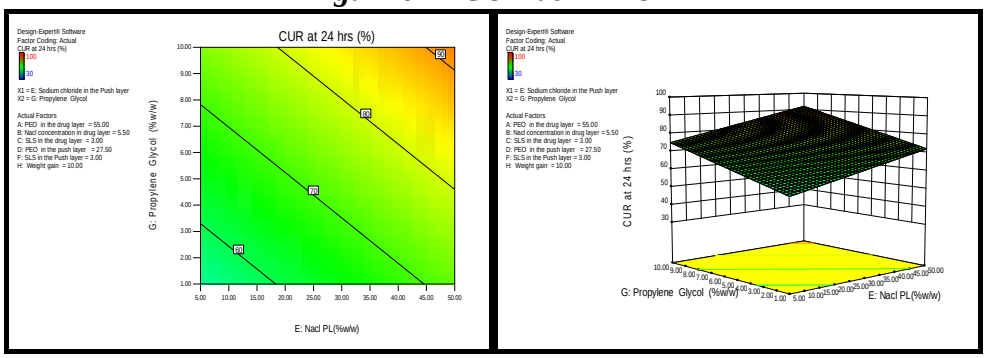


Figure No.6.3.82: Contour plot and RS Plot -Effect of NaCl PL and propylene glycol on PCUR at 24 hrs

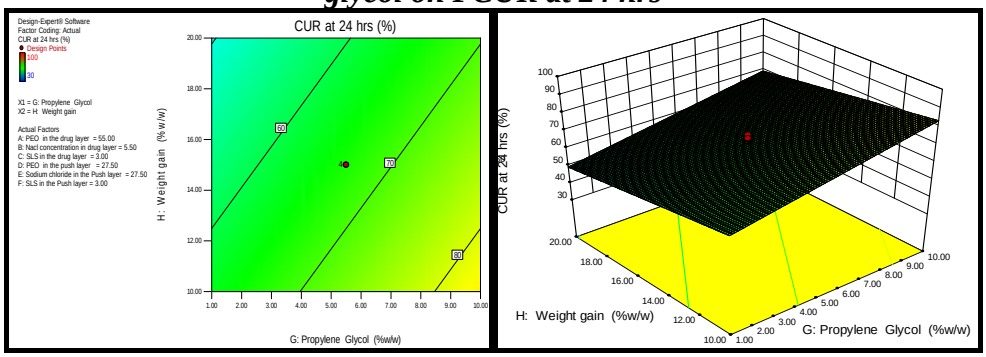
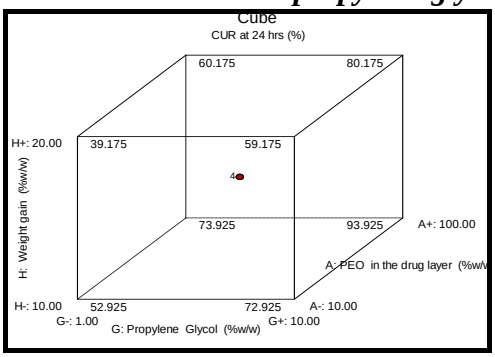


Figure No.6.3.83: Contour plot and RS Plot -Effect of weight gain and propylene glycol on PCUR at 24 hrs



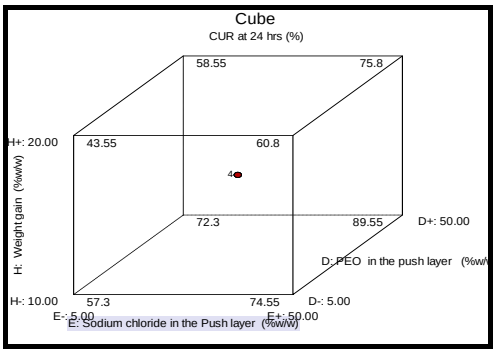
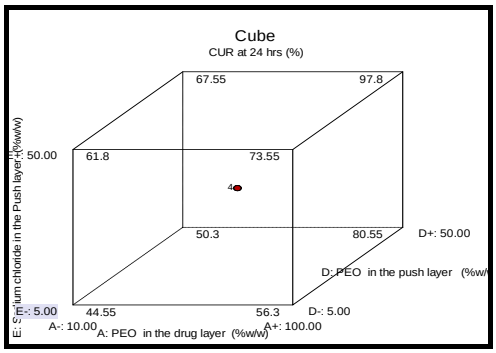


Figure No.6.3.84: Cube plot of the effect of PEO DL, Propylene Glycol and weight gain on PCUR at 24 hrs. Figure No.6.3.85: Cube plot of the effect of NaCl PL, PEO PL and Weight gain on PCUR at 24 hrs



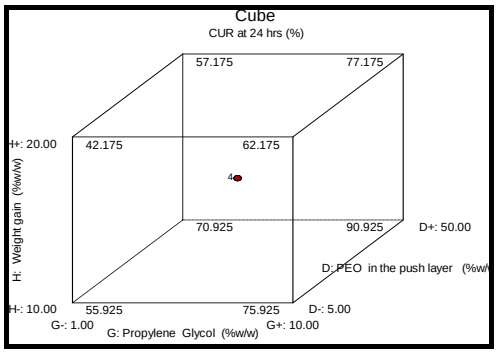
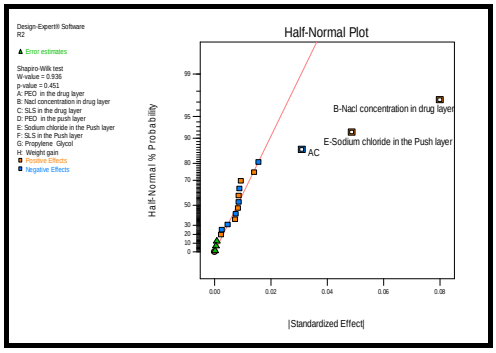


Figure No.6.3.86: Cube plot of the effect of PEO PL, NaCl PL and PEO DL on PCUR at 24 hrs. Figure No.6.3.87: Cube plot of the effect of PEO PL, Propylene Glycol and Weight gain on PCUR at 24 hrs



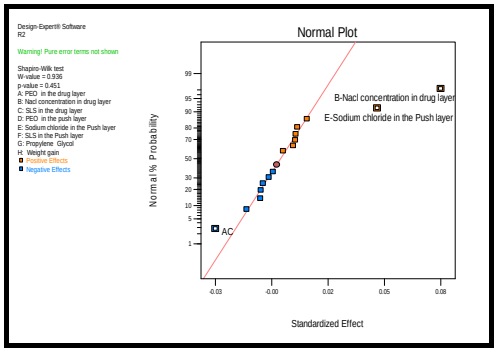


Figure No.6.3.88: Half Normal plot of the effect of the factors selected on R^2
Figure No.6.3.89: Normal plot of the effect of the factors selected onR^2

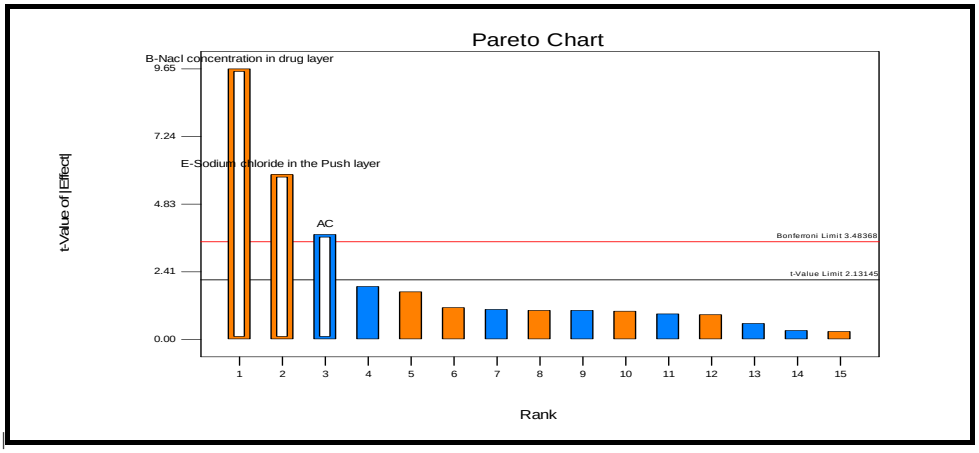


Figure No.6.3.90: Pareto chart of the effect of the factors selected onR^2

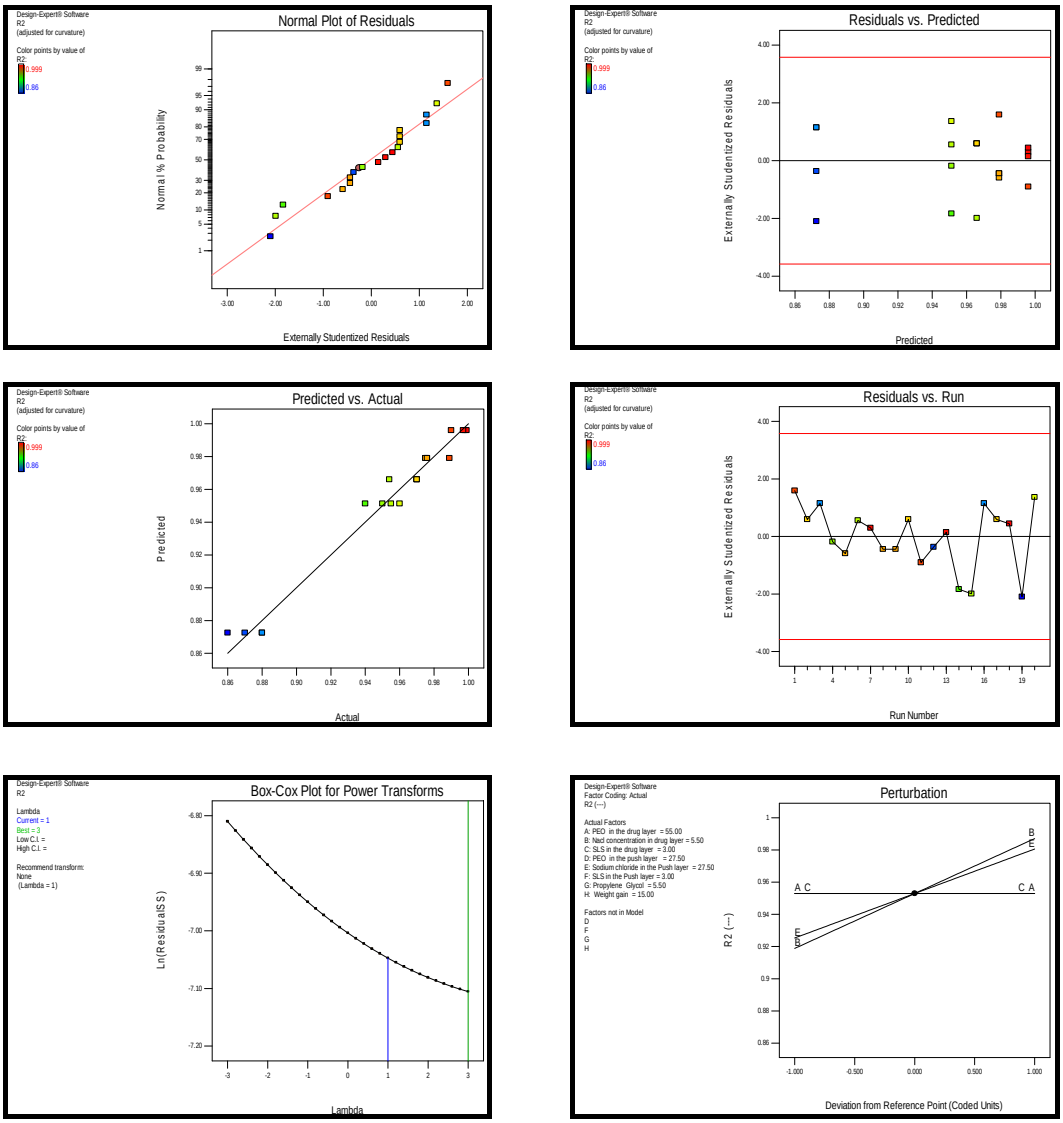


Figure No.6.3.91: Plots for testing the assumptions of the ANOVA and pertubation curve on R^2

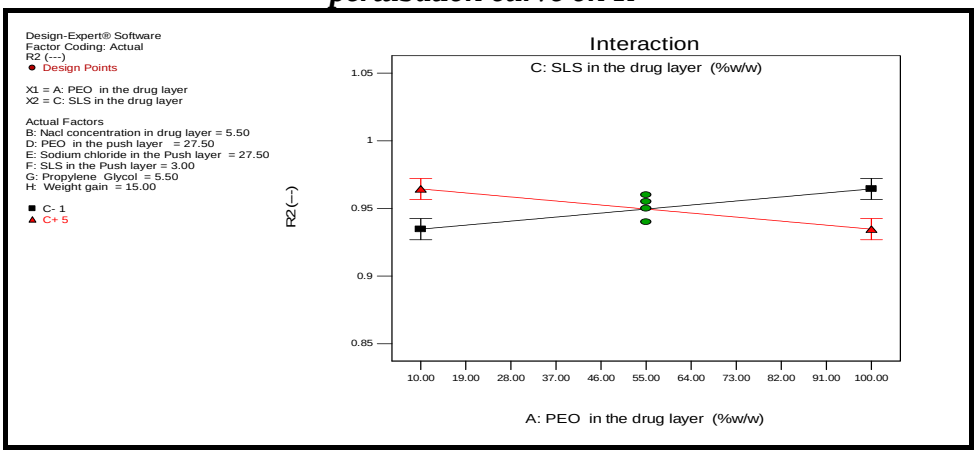


Figure No.6.3.92: AC interaction Plot on R^2

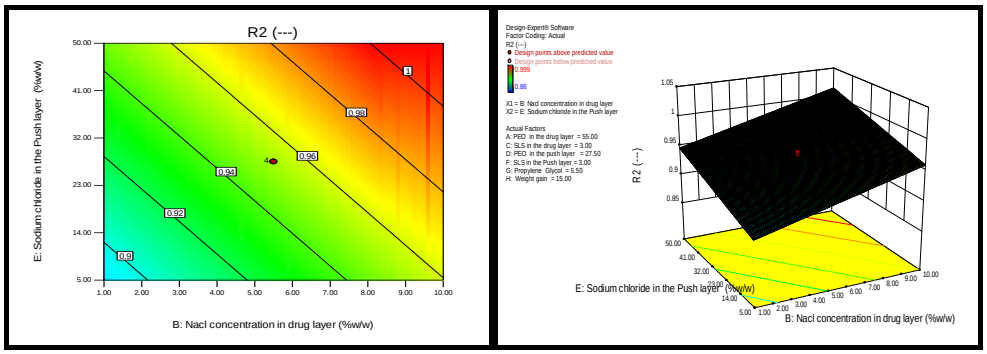


Figure No.6.3.93: Contour plot and RS Plot – Effect of NaCl DL and NaCl PL on R^2

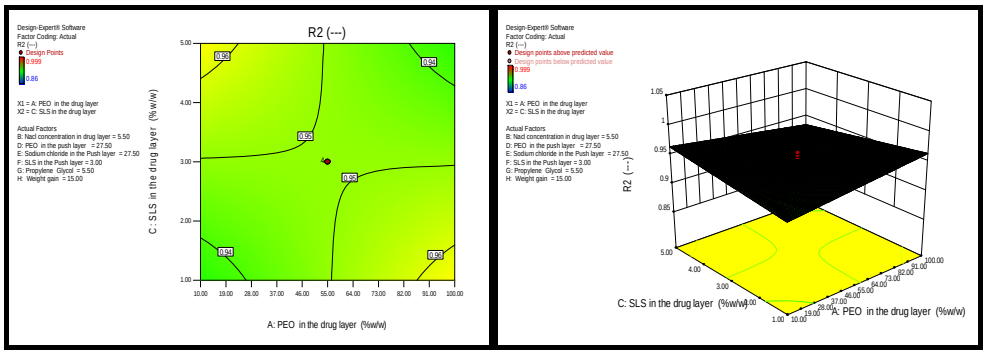


Figure No.6.3.94: Contour plot and RS Plot – Effect of PEO DL and SLS DL on R^2

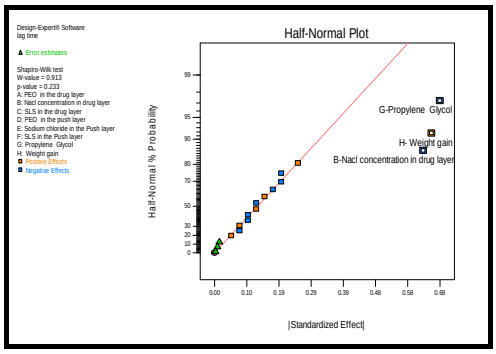


Figure No.6.3.95: Half Normal plot of the effect of the factors selected on lag time.

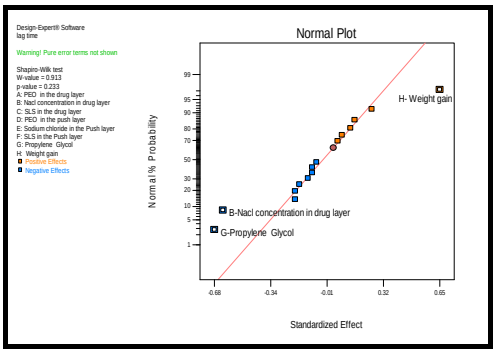


Figure No.6.3.96: Normal plot of the effect of the factors selected for on lag time.

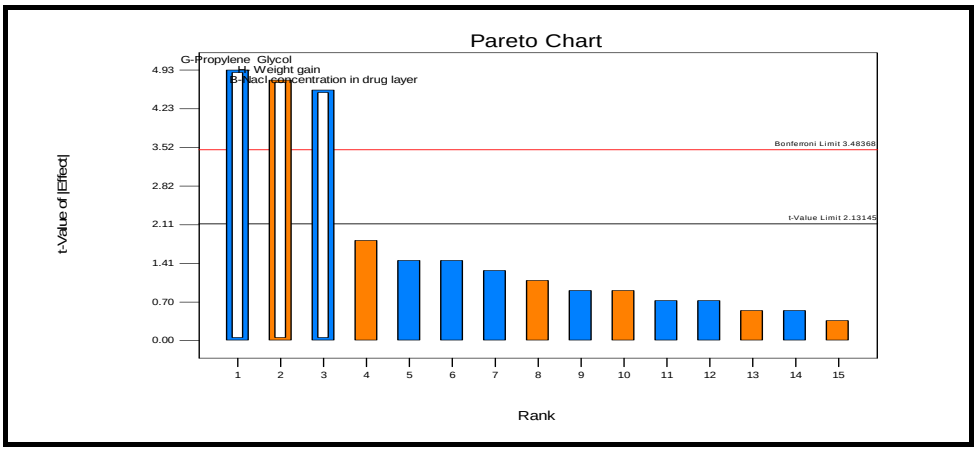


Figure No.6.3.97: Pareto chart of the effect of the factors selected on lag time

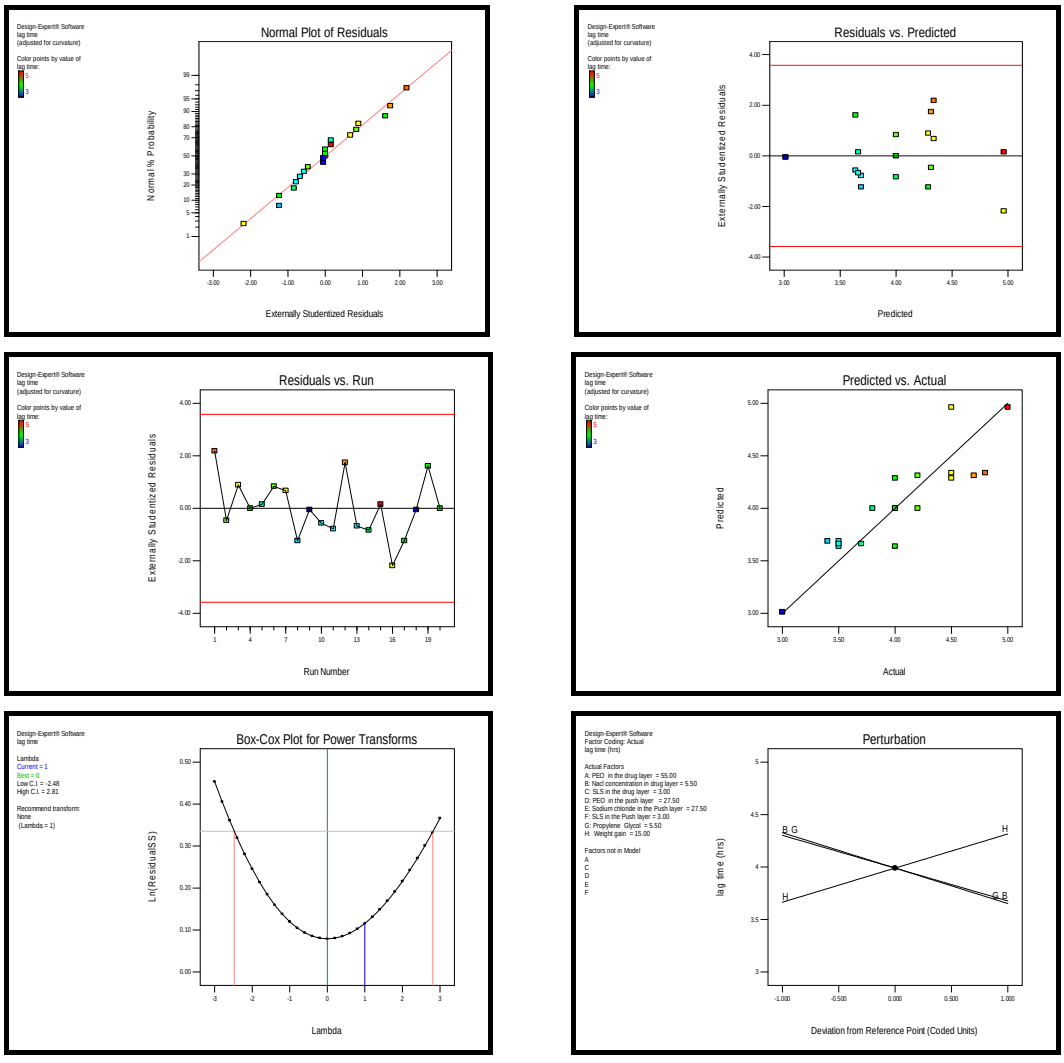


Figure .6.3.98: Plots for testing the assumptions of the ANOVA and pertubation curve

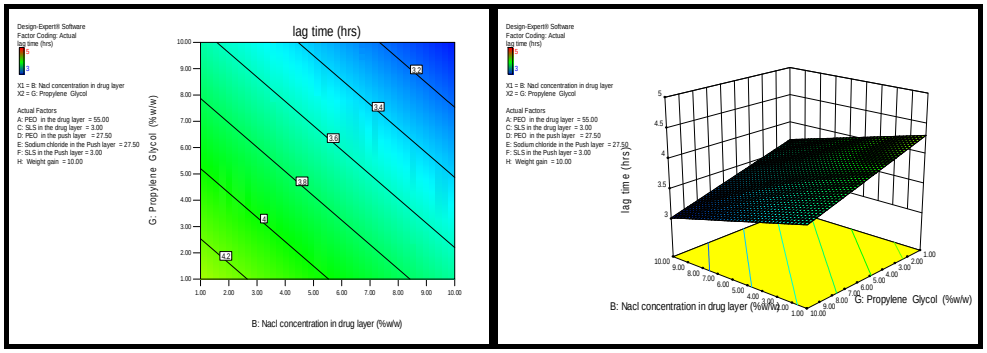


Figure No. 6.3.99 : Contour plot and RS Plot – Effect of NaCl DL and Propylene Glycol on lag time

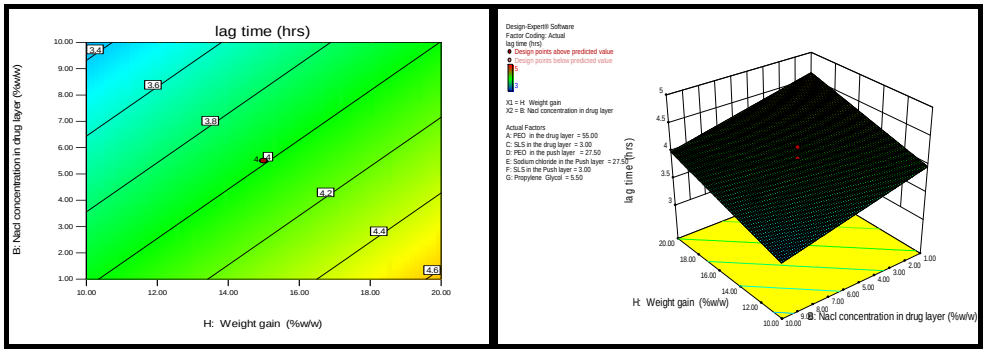


Figure No.6.3.100: Contour plot and RS Plot – Effect of NaCl DL and weight gain on lag time

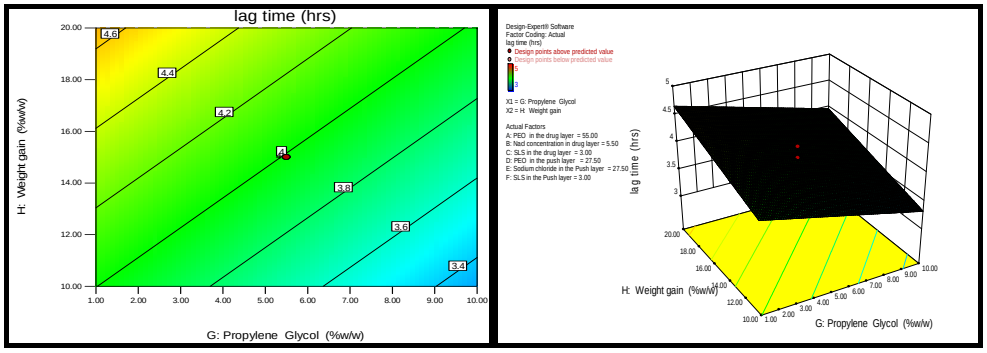


Figure No.6.3.101: Contour plot and RS Plot – Effect of weight gain and Propylene Glycol on lag time

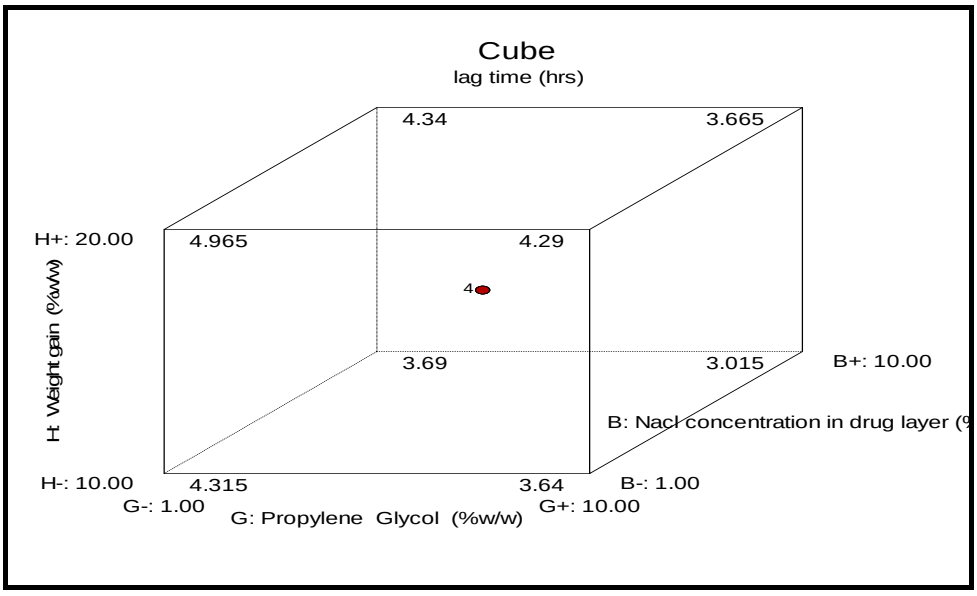


Figure No.6.3.102: Cube plot of effect of NaCl DL, Propylene Glycol and weight gain on lag time

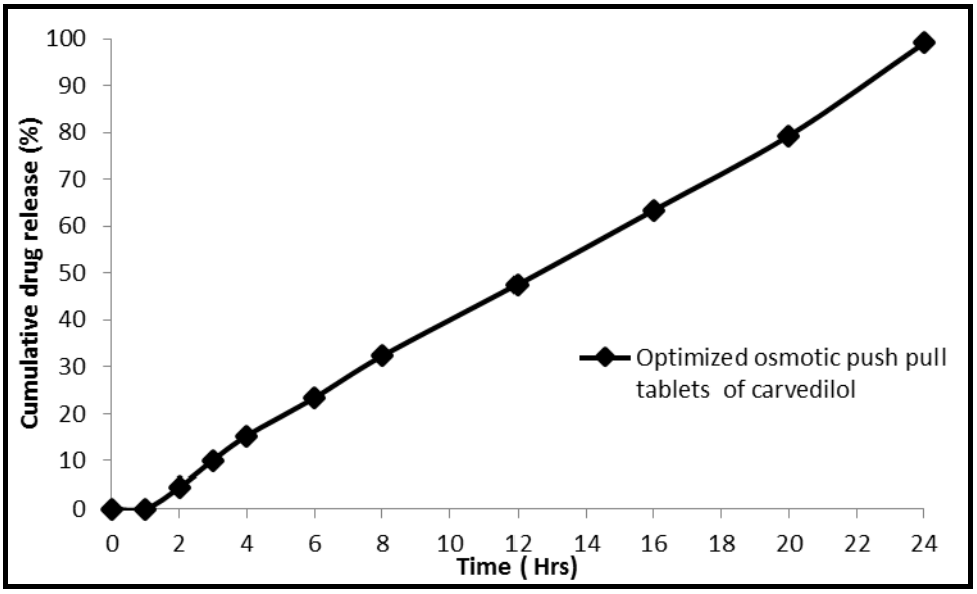


Figure No. 6.3.103: Invitro dissolution study of the optimized batch of Carvedilol phosphate push pull OT

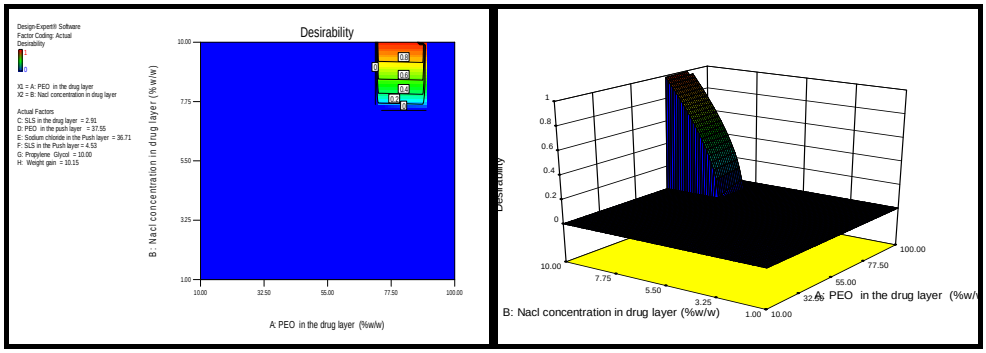


Figure No.6.3.104: Desirability contour plot and RS plot - Effect of PEO DL and NaCl DL

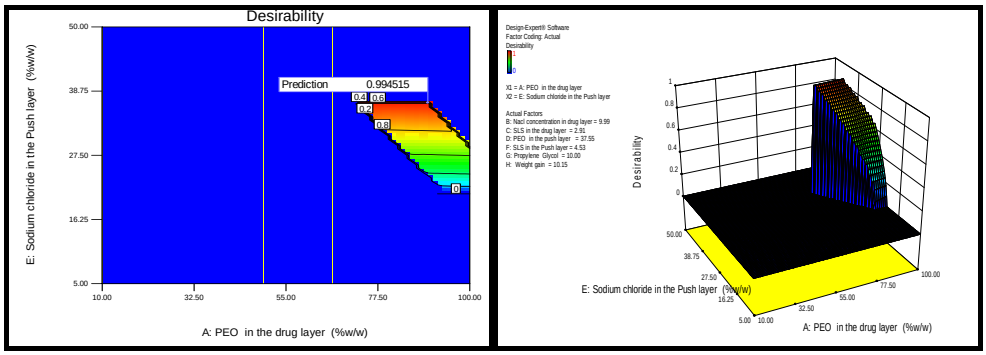


Figure No.6.3.105: Desirability contour plot & RS plot - Effect of PEO DL and NaCl PL

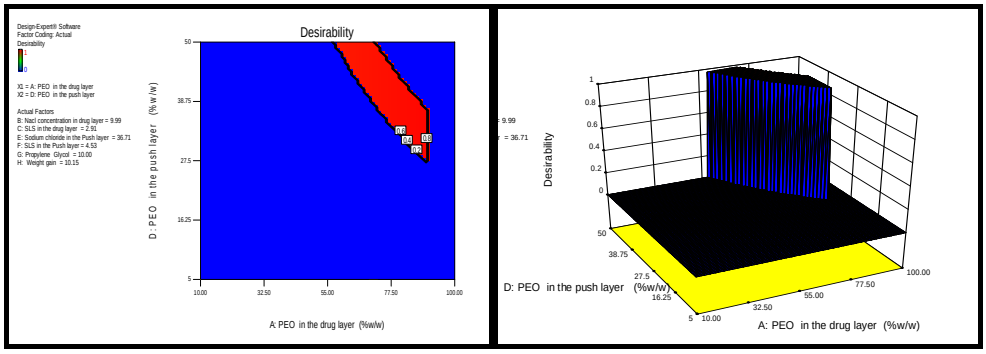


Figure .6.3.106: Desirability contour plot and RS plot – Effect of PEO DL and PEO PL

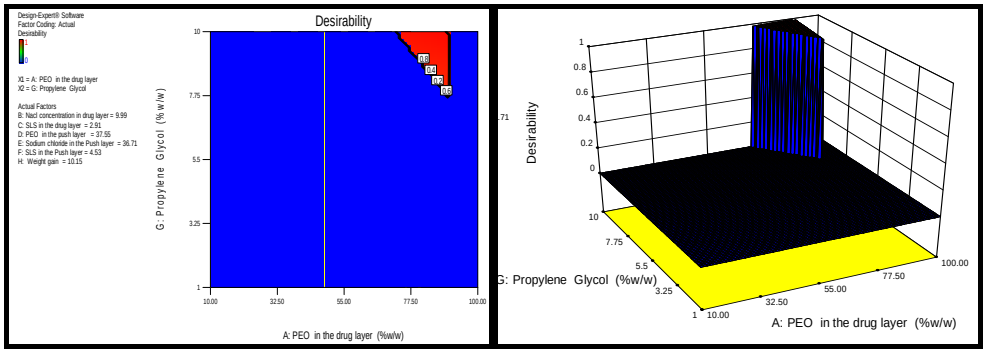


Figure .6.3.107: Desirability contour plot and RS plot - Effect of PEO DL and propylene glycol

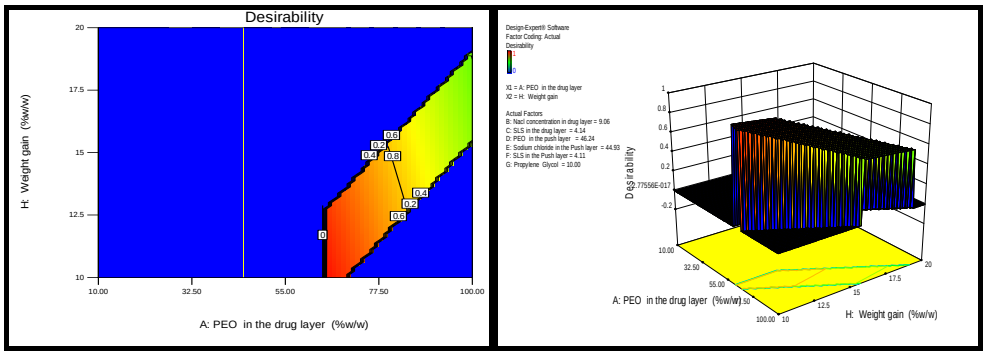


Figure.6.3.108: Desirability contour plot and RS plot – Effect of PEO DL and weight gain

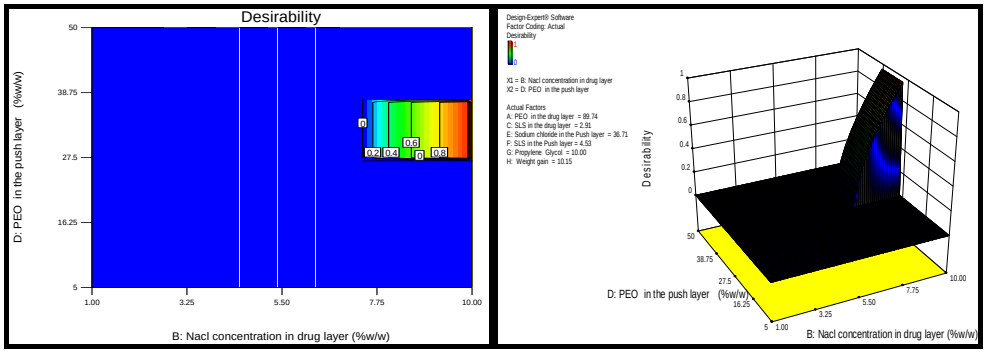


Figure 6.3.109: Desirability contour plot and RS plot –Effect of NaCl DL and PEO PL

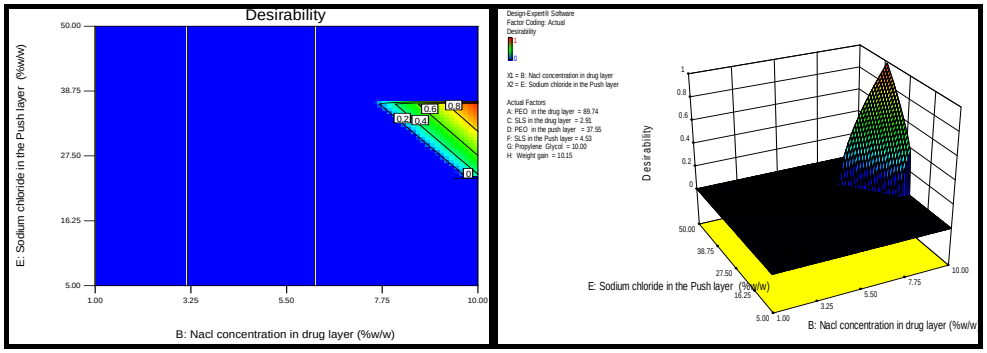


Figure .6.3.110: Desirability contour plot and RS plot – Effect of NaCl DL and NaCl PL

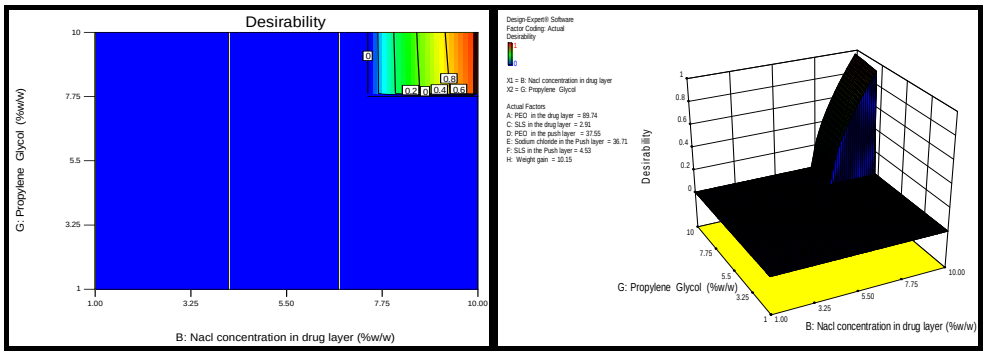


Figure.6.3.111: Desirability contour plot and RS plot – Effect of NaCl DL and Propylene Glycol

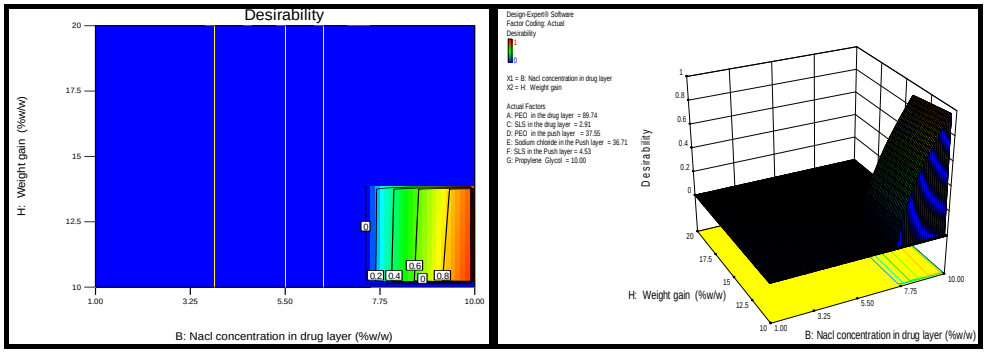


Figure.6.3.112: Desirability contour plot and RS plot – Effect of NaCl DL and weight gain

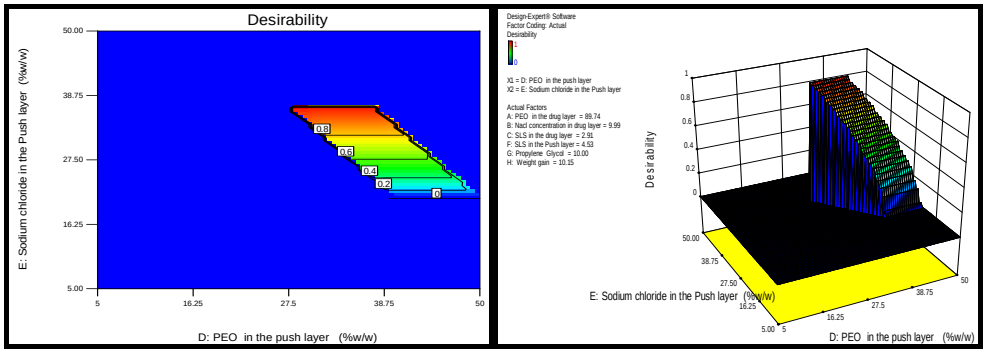


Figure No.6.3.113: Desirability contour plot and RS plot – Effect of PEO PL and NaCl PL

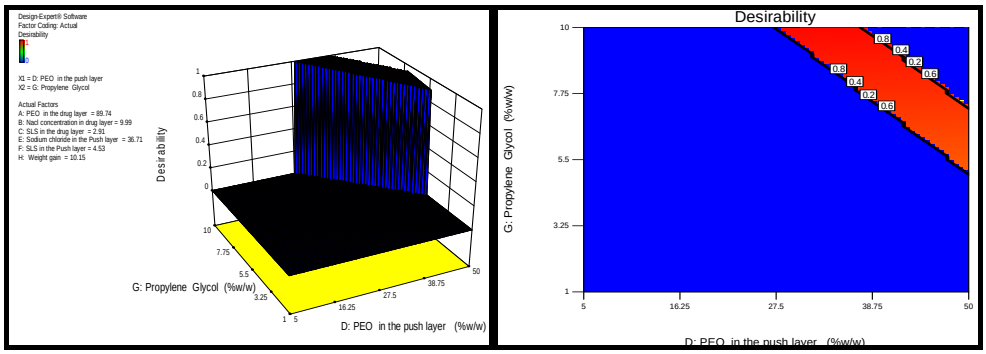


Figure No .6.3.114: Desirability contour plot and RS plot – Effect of PEO PL and propylene Glycol

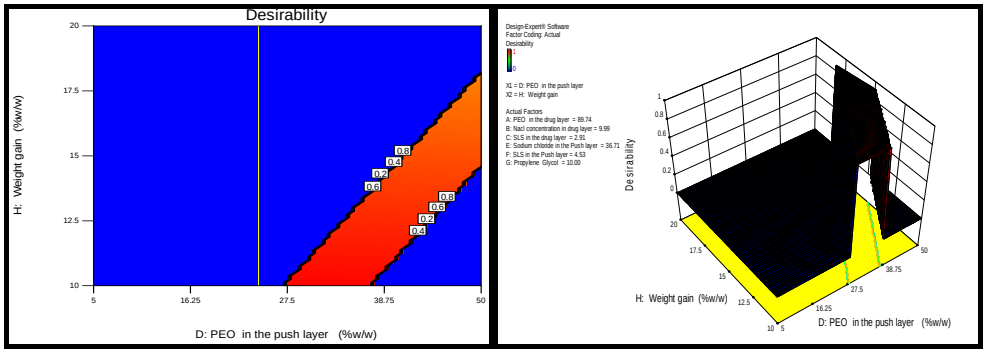


Figure No.6.3.115: Desirability contour plot and RS plot – Effect of PEO PL and weight gain

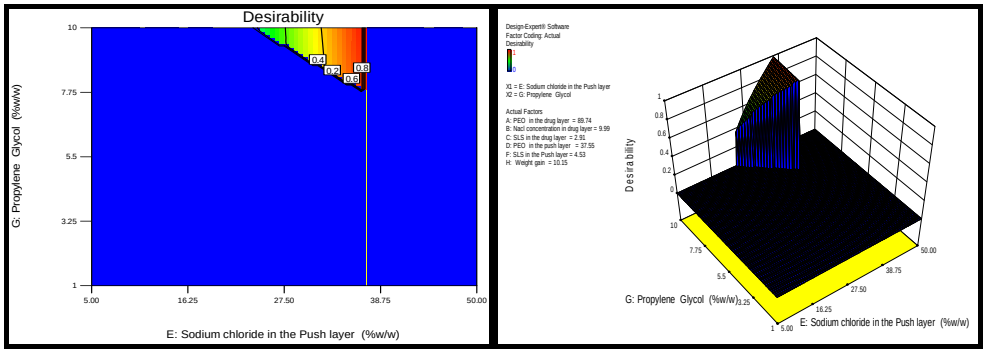


Figure No.6.3.116: Desirability contour plot and RS plot – Effect of NaCl PL and Propylene Glycol

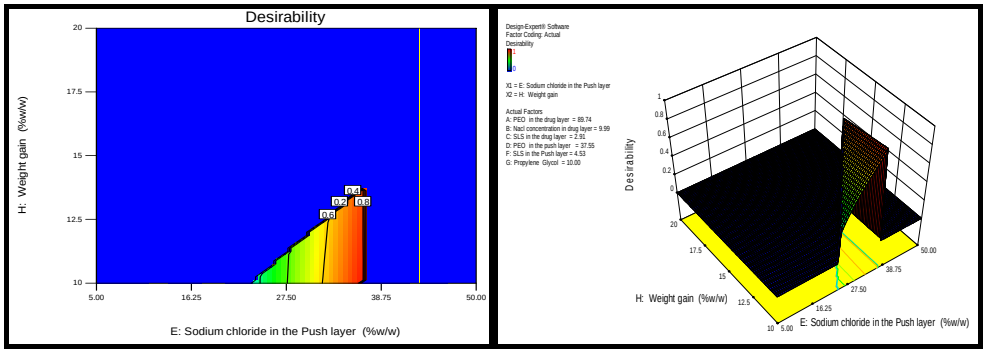


Figure No .6.3.117: Desirability contour plot and RS plot –Effect of NaCl PL and weight gain

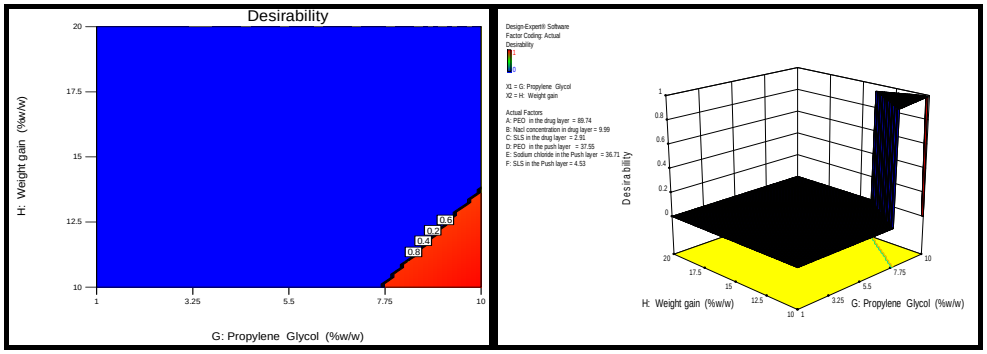


Figure No.6.3.118: Desirability contour plot and RS plot – Effect of Weight gain and Propylene Glycol

6.3.6. Product development and optimizationof push –pull osmotic tablets of Nisoldipine

I. Formulation of push pull osmotic tablets of Nisoldipine

The Factor influence study batches of Nisoldipine N1 to N20 were formulated according to the methodology given in the chapter section 5.6.4(IA-D), which explains preparation of granules, compression of core bilayer tablets, coating of core tablets and drilling of coated tablets. The compositions taken for preparation of factor influence study batches were shown in Table No. 5.6.2. The levels and responses fixed for the study was given in Table No 5.6.3 and 5.6.4.

The design table in coded values for the formulation development of Nisoldipine push pull osmotic tablets was given in the Table No.5.6.5.The final formula for the factor influence study and optimization of Nisoldipine push pull OT was shown in the Table No. 6.3.52 and 6.3.53.

II. Evaluation of the formulations

The batches N1 to N20 were evaluatedsimultaneously while preparing. They were subjected to blend as well as whole tablet evaluation. The procedures for the evaluation were given in the chapter 5.6.4(IIA&B).

A. Blend evaluation

The prepared granules of both the layers i.e. drug layer and push layer were evaluated by means of various tests. The tests were carried out according to the methodology given in the section 5.6.4 to 5.6.7. The results of the various blend evaluation are mentioned in the Table No.6.3.54.

B. Tablet evaluation

To monitor the product quality and for quantitative evaluation of tablet properties evaluation of tablets are necessary. The prepared tablets were evaluated for weight variation, hardness, friability, assay, weight gain, pore size and physical tests like diameter and thickness. The tests were performed as per the methodology givenin the section 5.6.4(IIB). The results of various tests are shown in Table No.6.3.55.

Table No.6.3.52: Formula for the trials N1- N10

S. No.	Ingredients	N1 mg/tab	N2 mg/tab	N3 mg/tab	N4 mg/tab	N5 mg/tab	N6 mg/tab	N7 mg/tab	N8 mg/tab	N9 mg/tab	N10 mg/tab
Drug Layer(DL)											
1	Nisoldipine	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50
2	DCP	38.70	31.04	30.15	22.49	36.70	29.04	28.15	20.49	38.70	31.04
3	PEO 400 K	0.85	8.50	0.85	8.50	0.85	8.50	0.85	8.50	0.85	8.50
4	Sodium chloride	0.95	0.95	9.50	9.50	0.95	0.95	9.50	9.50	0.95	0.95
5	BHT	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
6	SLS	0.50	0.50	0.50	0.50	2.50	2.50	2.50	2.50	0.50	0.50
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg. stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total weight of drug layer		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer(PL)											
9	PEO 7000 K	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	25.00	25.00
10	Sodium chloride	0.13	1.25	1.25	0.13	1.25	0.13	0.13	1.25	1.25	12.50
11	DCP	39.62	38.50	36.70	37.82	36.70	37.82	39.62	38.50	14.18	2.93
12	BHT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg. stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of Push layer		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	9.4	17.3	18.8	8.6	7.6	18.8	17.3	9.4	17.3	9.4
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.09	1.73	0.19	0.86	0.76	0.19	1.73	0.09	1.73	0.09
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	19.0	9.5	19.0	9.5
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**114.0**	**104.5**	**114.0**	**104.5**

Table No.6.3.53:Formula for the trials N11- N20

S. No.	Ingredients	N11 mg/tab	N12 mg/tab	N13 mg/tab	N14 mg/tab	N15 mg/tab	N16 mg/tab	N17 mg/tab	N18 mg/tab	N19 mg/tab	N20 mg/tab
Drug Layer(DL)											
1	Nisoldipine	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50	8.50
2	DCP	30.15	22.49	36.70	29.04	28.15	20.49	29.60	29.60	29.60	31.04
3	PEO 400 K	0.85	8.50	0.85	8.50	0.85	8.50	4.68	4.68	4.68	8.50
4	Sodium chloride	9.50	9.50	0.95	0.95	9.50	9.50	5.23	5.23	5.23	0.95
5	BHT	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01
6	SLS	0.50	0.50	2.50	2.50	2.50	2.50	1.50	1.50	1.50	0.50
7	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
8	Mg. stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total weight of drug layer		50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Push layer(PL)											
9	PEO 7000 K	25.00	25.00	25.00	25.00	25.00	25.00	13.75	13.75	13.75	25.00
10	Sodium chloride	12.50	1.25	12.50	1.25	1.25	12.50	3.78	3.78	3.78	12.50
11	DCP	4.73	15.98	4.73	15.98	14.18	2.93	23.81	23.81	23.81	2.93
12	BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.03
13	SLS	0.45	0.45	0.45	0.45	2.25	2.25	1.35	1.35	1.35	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
16	Mg. stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Total weight of Push layer		45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Total weight of un coated tablet		95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
Functional coating											
17	Cellulose acetate	8.6	18.8	18.8	8.6	9.4	17.3	13.5	13.5	13.5	14.2
18	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
20	Propylene Glycol	0.86	0.19	0.19	0.86	0.09	1.73	0.74	0.74	0.74	0.09
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	14.3	14.3	14.3	14.3
Total tablet weight		**104.5**	**114.0**	**114.0**	**104.5**	**104.5**	**114.0**	**109.3**	**109.3**	**109.3**	**109.3**

Table No.6.3.54: Blend evaluation of the DL and PL of push pull osmotic tablets of Nisoldipine

Trials	Angle of repose		Bulk density		Tapped density		Hausner's ratio		Carr's index	
	DL	PL	DL	PL	DL	PL	DL	PL	DL	PL
N1	28.3	26.9	0.756	0.612	0.852	0.692	1.1270	1.1307	11.2676	11.5607
N2	30.1	27.8	0.699	0.608	0.784	0.684	1.1216	1.1250	10.8418	11.1111
N3	29.7	26.9	0.734	0.618	0.836	0.691	1.1390	1.1181	12.2010	10.5644
N4	28.8	29.5	0.776	0.623	0.884	0.702	1.1392	1.1268	12.2172	11.2536
N5	30.2	27.5	0.696	0.617	0.783	0.712	1.1250	1.1540	11.1111	13.3427
N6	29.3	28.2	0.773	0.614	0.886	0.705	1.1462	1.1482	12.7540	12.9078
N7	27.5	27.2	0.779	0.628	0.883	0.713	1.1335	1.1354	11.7780	11.9215
N8	30.1	28.7	0.679	0.614	0.769	0.705	1.1325	1.1482	11.7035	12.9078
N9	29.2	27.3	0.784	0.608	0.892	0.717	1.1378	1.1793	12.1076	15.2022
N10	27.4	29.3	0.793	0.617	0.886	0.704	1.1173	1.1410	10.4966	12.3580
N11	31.2	26.5	0.755	0.629	0.894	0.717	1.1841	1.1399	15.5481	12.2734
N12	29.8	27.4	0.668	0.612	0.759	0.703	1.1362	1.1487	11.9895	12.9445
N13	28.6	28.3	0.749	0.603	0.866	0.688	1.1562	1.1410	13.5104	12.3547
N14	30.4	26.6	0.645	0.616	0.735	0.689	1.1395	1.1185	12.2449	10.5951
N15	28.9	27.9	0.776	0.68	0.882	0.759	1.1366	1.1162	12.0181	10.4084
N16	30.4	26.5	0.783	0.617	0.877	0.707	1.1201	1.1459	10.7184	12.7298
N17	29.3	27.9	0.675	0.611	0.764	0.701	1.1319	1.1473	11.6492	12.8388
N18	30.1	27.7	0.655	0.612	0.751	0.709	1.1466	1.1585	12.7830	13.6812
N19	20.4	26.8	0.678	0.617	0.783	0.712	1.1549	1.1540	13.4100	13.3427
N20	28.8	29.1	0.748	0.625	0.852	0.731	1.1390	1.1696	12.2066	14.5007

Table No.6.3.55: Whole tablet evaluation push pull OT of Nisoldipine

Trials	Wt variation (n =20)	Diameter(mm) (n=5)	Thickness(mm) (n=5)	Hardness (Kg/cm^2)(n=6)	Friability (%)	Assay (%)	Weight gain (%)	Pore size (mm)
N1	103.8±1.32	5.20± 0.1	3.40±0.02	4.6±0.2	0.99	98.2±1.2	10.1±0.2	0.60
N2	114.4±2.12	5.18±0.12	3.42±0.01	4.4±0.5	0.82	101.8±2.1	20.1±.18	0.60
N3	114.2 ±3.42	5.02±0.18	3.50±0.03	3.6±0.2	0.45	100.6±2.7	20.03±0.05	0.60
N4	104.5 ± 1.2	5.08±0.11	3.42±0.01	4.2±0.7	0.76	98.1±1.6	10.1±0.04	0.60
N5	104.8 ± 2.1	5.10±0.21	3.54±0.03	4.6± 0.1	0.75	99.1±1.1	10.1±0.02	0.60
N6	114 ±2.1	5.18±0.12	3.40±0.02	4.9±0.1	0.65	100.5±1.7	10.01±.0.05	0.60
N7	114.5 ± 1.29	5.12±0.02	3.52±0.01	3.8±0.4	0.78	101.6±0.8	20.06±0.01	0.60
N8	104.2 ±2.15	5.02±0.11	3.52±0.13	4.8±0.2	0.56	100.2±1.3	10.02±0.02	0.60
N9	114.2 ± 2.12	5.06±0.14	3.45±0.12	4.5±0.5	0.77	98.2±0.8	20.04±0.02	0.60
N10	104.3 ± 1.8	5.08±0.16	3.52±0.04	3.4±0.4	0.82	99.1±1.3	10.08±0.01	0.60
N11	104.3 ± 1.76	5.18±0.02	3.50±0.01	3.6±0.5	0.59	100.8±2.1	10.01±0.02	0.60
N12	114.86 ± 2.8	5.12±0.07	3.50±0.03	4.7±0.1	0.63	100.1±2.4	20.02±0.06	0.60
N13	114.3 ± 2.1	5.02±0.14	3.52±0.14	4.5±0.64	0.92	101.7±1.7	20.02±0.05	0.60
N14	104.8±1.5	5.16±0.10	3.54±0.11	4.8±0.24	0.71	102.1±1.0	10.1±0.001	0.60
N15	103.8 ±0.08	5.03 ±0.06	3.50±0.11	3.9±0.2	0.77	99.2±1.8	10.05±0.03	0.60
N16	114.9 ± 1.42	5.01±0.22	3.44±0.05	4.1±0.22	0.55	100.6±2.1	20.12±0.21	0.60
N17	108.4 ± 1.4	5.12±0.01	3.56±0.04	3.9±0.26	0.64	98.9±1.0	15.2±0.31	0.60
N18	108.74 ± 0.6	5.18±0.05	3.60±0.01	3.7±0.18	0.63	100.7±1.9	15.1±0.11	0.60
N19	109.6 ± 0.12	5.15±0.02	3.50±0.08	4.9±0.1	0.78	100.1±2.5	15.05±0.03	0.60
N20	108.9±0.18	5.14±0.06	3.42±0.03	4.5±0.02	0.61	101.6±2.1	15.01±0.12	0.60

C. Invitro dissolution tests

In vitro dissolution study was carried out for all the batches and the test was carried out as per methodology given in the 5.6.4(IIBix). The release profiles of N1 to N20 batches were shown in Table No.6.3.56, 6.3.57 and in Figure No.6.3.119, 6.3.120.

Table No.6.3.56: Dissolution profile of N1 – N10 batches

Time (hrs)	Cumulative Drug release (%)									
	N1	**N2**	**N3**	**N4**	**N5**	**N6**	**N7**	**N8**	**N9**	**N10**
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	4	0	0	0	0	0	0
3	1±0.03	4.1±0.1	0	11.4±0.03	7.9±0.3	4.1±0.1	1±0.02	6±0.3	1±0.04	2±0.08
4	4.8±0.4	9.1±0.7	7±0.6	15.3±1.2	13.4±1.1	9±0.4	9.3±0.6	13.4±0.91	6.5±0.8	8.9±0.5
6	12.7±1.4	16.4±1.1	14±0.9	23.4±1.5	20.8±1.8	13.9±1.1	13.7±0.6	21.6±1.8	15.1±0.6	14.5±0.8
8	16.9±0.4	21.4±1.4	16.1±0.4	30.4±1.9	30.7±1.2	17.3±1.6	17.9±1.6	27.6±2.1	23.4±1.1	26.4±1.2
12	23.4±0.9	56.4±3.5	20.5±1.1	44.8±1.1	41.4±1.4	25.1±1.4	28.9±3.7	38.87±3.2	35.4±2.6	55.1±2.1
16	25.8±1.1	69.7±4.1	25.6±1.3	60.2±3.1	57.8±1.7	26.6±1.1	35.4±3.1	48.6±3.3	38.7±2.9	74.3±2.3
20	27.5±1.2	79.5±5.3	30.2±1.9	72.1±4.6	70.1±3.4	28.3±0.9	40.4±2.2	58.99±1.2	42.7±1.7	86.4±3.1
24	30.1±1.1	89±1.7	35±1.2	75.4±2.1	72.8±0.4	30±0.7	45.3±1.3	70±1.4	45±2.4	100±1.3

Table No .6.3.57: Dissolution profile of N11 – N20 batches

Time (hrs)	Cumulative Drug release (%)									
	N11	N12	N13	N14	N15	N16	N17	N18	N19	N20
0	0	0	0	0	0	0	0	0	0	0
1	1±0.05	0	0	0	0	0	0	0	0	0
2	5.6±0.4	0	0	0	0	2±0.03	0	1±0.03	2±0.01	0
3	11.5±1.2	0	0	5±1.1	6.6±0.5	7.4±1.1	5.2±1.1	5±0.5	4±2.3	5±2.3
4	14.5±1.6	7±1.1	5.2±0.4	9.8±0.6	12±0.8	16.2±1.1	9.1±0.3	10.1±0.2	8.1±0.5	8.5±0.4
6	19.6±1.1	17.2±1.5	14.4±1.3	27.5±1.4	23.5±1.1	26.5±2.4	23.5±1.7	22.7±1.5	22.7±1.2	16.2±1.1
8	26.6±1.9	22.56±2.1	20.7±0.7	49.9±2.5	28.5±1.1	35.8±2.6	30.4±3.2	29.9±2.8	34.2±1.6	25.3±1.1
12	38.5±1.3	30.1±2.1	28.9±1.1	76.4±5.4	38.1±2.5	51.7±3.1	40.6±2.8	43.6±2.7	44.6±2.6	35.3±0.2
16	51.9±4.2	48.2±2.4	38.8±3.1	84.1±1.6	47.9±3.8	68.3±2.5	49.4±1.1	53.4±3.2	53.2±2.8	45.3±2.6
20	65.1±3.3	55.7±2.8	42.7±1.5	87.9±1.9	54.8±1.2	84.3±0.7	59.7±3.6	59.9±1.1	60.5±1.1	54.3±1.1
24	75.5±0.6	62.2±1.4	45.3±1.8	95.8±0.6	61.7±2.1	100±0.6	65.3±3.9	64.8±0.5	66±1.8	58±1.7

D. Coating Evaluation

The coating evaluation was done by formulation of mechanical film in a Petri dish and it was described in methodology section 5.6.4(IIC). And the results were shown in Table No.6.3.9.

III. Factor influence study

The *invitro* evaluation of all the 20 trials was performed and the necessary values for the factor influence study were recorded. The design matrix and the responses for the factor influence study were given in the Table No.6.3.58.

Table No.6.3.58: Result of the factor influence study

Trials	PCUR at 24 hrs	R^2	Lag time
N1	30	0.920	4.7
N2	89	0.955	4.2
N3	35	0.999	4.7
N4	75	0.981	2.8
N5	72	0.975	3.6
N6	30	0.879	4.4
N7	45	0.978	3.8
N8	70	0.995	3.7
N9	45	0.883	4.7
N10	100	0.974	4.4
N11	75	0.999	2.8
N12	62	0.977	5
N13	45	0.949	5.1
N14	96	0.859	4.1
N15	62	0.972	3.6
N16	100	0.998	3.7
N17	65	0.955	4.2
N18	65	0.943	4
N19	66	0.959	4.4
N20	58	0.969	4.2

A. FDS graph

The FDS graph for the selected design with the selected factors and responses showed a flatter curve. This indicated a high FDS. So the design space predicted by the selected model had useful precision. The graph was given in the Figure No.6.3.121.

B. Standard error graph

The standard error of prediction for areas in the design space for the different factors were found to be between 0.25 – 0.45. So it was proven that the standard error throughout the design space was relatively very low. The entire design space will be having a very less prediction error for the selected design. Figure No.6.3.122.

C. Analysis of the responses

1. Cumulative release at 24 hrs

The cumulative release of the different formulations were studied and analyzed for the different factors affecting the same. The different evaluation graphs and data are explained below,

i) Half normal plot

The half-normal plot shown in the Figure No.6.3.123 was used to select significant effects to be included in the model. From the graph it was evident that the factor which were affecting the PCUR up to 24 hrs were A (PEO DL), G (propylene glycol), E (NaCl in PL), D (PEO PL) and H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.309. This indicated the non significance of the non selected factors**.** Interaction AC (PEO DL-SLS DL) was also found significant.

ii) Normal plot

From the normal plot shown in the Figure No.6.3.124 it was evident that the factor A, G, E, D and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.309. Thisindicated that the remaining (unselected) terms were normally distributed. Interaction AC (PEO DL-SLS in the DL) was also found significant.

iii) Pareto chart

From the pareto chart shown in Figure No.6.3.125 it was clearly evident that the factors A,G,E,D and H were significantly affecting the PCUR at 24 hrs. All the significant factors crossed the t limit and Bonferroni limit. The magnitude of the effect can be written as PEO DL >Propylene Glycol >NaCl PL >PEO PL> Weight gain.Increase in the concentration of PEO DL, Propylene Glycol, NaCl PL and PEO PL had increased PCUR at 24 hrs. But an increase in the weight gain had reduced the PCUR at 24 hrs. AC was probably significant as this was with in the t and B limit. Other non significant term effects and interaction effects were present below the t limit.

iv) ANOVA and regression analysis

In this case A (p =2.4E-06*)*, G (p =3.9E-05*)*, E (1.5E-04) D (1.8E-04) and H (p =3.4E-04*)* were significant model terms. The "Lack of Fit F-value" of 3.97 implied that the lack of fit was not significant relative to the pure error. Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit was observed. An AC interaction was significant. But no curvature effects were identified. The "Pred R-Squared" of 0.8102 is in reasonable agreement with the "Adj R-Squared" of 0.9038 indicating the linearity of the model. Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 was desirable. Our ratio was 17.71 indicates an adequate signal. So this model can be used to navigate the design space.

v) Polynomial equation

From the regression analysis the polynomial equation which can represent the PCUR at 24 hrs can be formed. The positive sign of the coefficients in the equation indicates the positive and the negative sign indicates the negative effect on the response. Larger the coefficients larger will be the effects. The magnitude of the effect of the factors on the response can be written as A (NaCl DL) >G (PG) >E (NaCl PL) >D (PEO PL) >H (Weight gain). Thelinear model polynomial equation can be written as,

CUR at 24 hrs = 64.25+13.3125 * A +8.6875 * D +8.8125 * E +10.1875 * G-8.0625 * H -4.3125 * AC *(codedterms)*

CUR at 24 hrs = 38.3264 +0.3443 * PEO DL+0.3861 * PEO PL +0.3917 * Sodium chloride PL +2.2639* Propylene Glycol -1.6125* Weight gain -0.0162 * PEO DL * SLS DL (*Actual terms)*

Table No.6.3.59: ANOVA and Regression analysis for the effect of factors selected on the PCUR at 24hrs

	Sum of Squares	df	Mean Square	F Value	p-value	Prob > F
Model	8283.87	6	1380.64	30.74	*5.9E-07*	*significant*
A-PEO DL	2835.56	1	2835.56	63.13	*2.4E-06*	
D-PEO PL	1207.56	1	1207.56	26.88	*1.8E-04*	
E-NaCl PL	1242.56	1	1242.56	27.66	*1.5E-04*	
G-PG	1660.56	1	1660.56	36.97	*3.9E-05*	
H- Weight gain	1040.06	1	1040.06	23.15	*3.4E-04*	
AC	297.56	1	297.56	6.62	*2.3E-02*	
Residual	583.875	13	44.913462			
Lack of Fit	542.87	10	54.28	3.9722	0.1416	*not significant*
Pure Error	41	3	13.66			
Cor Total	8867.75	19				
Regression analysis						
Std. Dev.	6.70		R-Squared		0.9342	
Mean	64.25		Adj R-Squared		0.9038	
C.V. %	10.43		Pred R-Squared		0.8102	
PRESS	1683.08		Adeq Precision		17.7184	

vi) Tests for the assumptions of the ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No .6.3.126.

- *The normal probability plot*: The plot indicated that the residuals followed a normal distribution, as the points followed a straight line.
- *Residuals vs Predicted*: The plot showed a random scatter (constant range of residuals across the graph). This confirmed the constant variance in the experiments performed.
- *Residuals vs Run*:This plot showed a random scatter indicated that no time-related variable lurking in the background.

- *Predicted vs. Actual*: This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The graph shows that the lamda value is 1 and no transformation is needed.

vii) Perturbation graph

This graph explained the effect and sign of the significant factors on the PCUR at 24 hrs. It showed that the change in the concentration of factor A, G, E and D from minimum to maximum produced an increase in the PCUR at 24 hr from 55 to 75, 57 to 73, 58 to 70 and 59 to 69% respectively. But an increase in the factor H showed a decrease in the response (from 73 – 60%).

viii) Interaction graphs

AC interaction graph shown in the Figure No.6.3.127 represented non parrellel lines. This indicated that, higher concentration of SLS DL would be producing a lesser effect while changing the concentartion of PEO DL form low to high than expected. Effectof PEO DL was more prominent at lower concentraions of SLS DL.

ix) Contour plots and RS plots

Figure No.6.3.128 shows the contour plot and response surface plot of the simultaneous effect of PEO DL and PEO PL on the PCUR at 24 hrs. At lower concentration of PEO DL, even a higher concentration of PEO PL produced only 50-65% CUR at 24 hrs. At higher concentration of PEO DL, change in PEO PL produced PCUR from 76 -93%. At lower concentration of PEO PL, change in PEO DL made a difference in PCUR form 50 -75 %. But at high concentration of PEO PL, it was 67- 92%. High PCUR would be expected at high levels of both the factors. From the RS plot it was evident that PEO DL had a greater effect on the PCUR at 24 hrs.

Figure 6.3.129 shows the contour plot and RS plot of the simultaneous effect of PEO DL and NaCl PL on the PCUR at 24 hrs. Lower concentrations of both the factors only helped to release 60-65 % of the drug from the dosage form. A higher concentration of both the factors had produced greater than 80 % release

at 24 hrs. Both the factors had a positive effect on the release of the drug from the OTs. At higher level of PEO DL, change in concentration of NaCl PL had a greater effect.

Figure No.6.3.130 shows the contour plot and RS plot of the concurrent effect of PEO DL and propylene glycol on PCUR at 24 hrs.Both the factors had a positive effect on the response. At high levels of PEO DL the PG had a prominent effect. At low levels of PEO DL even a high concentration of propylene glycol would not produce more than 65 % of drug release. From the surface plot it was evident that both the factors had almost similar effect on the PCUR at 24 hrs at its low and high concentrations.

Figure No.6.3.131 shows the contour plot and RS plot of the simultaneous effect of PEO DL and weight gain on PCUR at 24 hrs. PEO DL had a positive effect and weight gain had an opposite effect on the response. At low levels of weight gain, the selected levels of PEO DL showed a prominent effect. At high weight gain, even a high concentration of PEO DL would not produce more than 70 % PCUR at 24 hrs.

Figure No. 6.3.132 shows the contour plot and RS plot of the concurrent effect of PEO PL and NaCl PL on PCUR at 24 hrs. Form the plot it was evident that both the factors had a positive effect on the response. Both the factors showed almost similar effects on the lower and higher concentrations of the other.

Figure No.6.3.133 shows the contour plot and RS plot of the contemporaneous effect of PEO PL and propylene glycol on PCUR at 24 hrs. Both the factors had a positive effect on the response. RS plot clearly showed the prominent effect of propylene glycol at both the levels of PEO PL.

The contour plot and RS plot representing the simultaneous effect of PEO PL and weight on PCUR at 24 hrs was shown in the Figure No.6.3.134. PEO PL had a positive effect and weight gain had an opposite effect on PCUR at 24 hrs. A more pronounced effect of PEO PL was visible at lower weight gain. At low level

of PEO PL, change in weight gain has produced a change in PCUR from 63 to 48%. But at higher levels of PEO PL, the change was from 80 to 64%. At low level weight gain the change in concentration of PEO PL produced a shift of PCUR at 24 hrs from 63- 80 %. But at high weight gain this was 64 – 48 %.

The contour plot and RS plot representing the simultaneous effect of NaCl PL and propylene glycol on PCUR at 24 hrs was shown in the Figure No.6.3.135. At lower propylene glycol concentrations the change in concentration of NaCl PL had produced a shift of PCUR at 24 hrs from 53-72%. At higher propylene glycol concentration this was around 72–89%. A change in concentration of propylene glycol at lower NaCl PL had produced change in PCUR from 53- 73%. At higher NaCl PL concentration, the change was around 70 -89%.

Figure No.6.3.136 shows the contour plot and RS plot representing the concomitant effect of NaCl PL and weight gain on PCUR at 24 hrs. Both the factors had positive effect on the response. The magnitude of the effect of change in factors on both the levels of the other factor was approximately equal. So both the factors had an equal effect on the response.

Figure No.6.3.137 shows the contour plot and RS plot representing the simultaneous effect of weight gain and propylene glycol on PCUR at 24 hrs. From the plot it was evident that weight gain had a negative effect and propylene glycol had a positive effect on the response. Propylene Glycol had a prominent effect and it was more pronounced at low level of weight gain. At higher weight gain, even a high concentration of propylene glycol would be producing a PCUR < 65%.

x) Cube plots

Figure No.6.3.138 shows the combined effect of A, G, and H. When all the three factors were at minimum, the PCUR at 24 hrs was about 48.8%, and at maximum it was around 79.6%. But a higher release of 96% can be achieved by keeping propylene glycol at its maximum, PEO DL at its maximum and weight gain at its minimum.

Figure No.6.3.139 shows the combined effect of D, E and H. At low levels of all the factors the PCUR at 24 hrs was 55.36. When they were at high levels the release was 74.23%. A better release was observed when D and E were at its maximum & weight gain was at its minimum.

Figure No .6.3.140 shows the combined effect of factors A, D and E on PCR at 24 hrs. At low levels of all the factors, the PCUR at 24 hrs was 33.98%.When they were at high levels the release was 95.61%.

Figure No .6.3.141 shows the combined effect of factors D, G and H. At low levels of all the factors the PCUR at 24 hrs was 57.75%.When they were at high levels the release was 79.38%. A 95.5 % release was observed when factors D and G were at maximum and H at its minimum.

2. Analysis of responses - Rate constant (R^2)

i) Half normal plot

From the half normal plot shown in Figure No.6.3.142 it was evident that the factors which were affecting the release rate constant (R^2) were B (NaCl DL) and E (NaCl PL). The AC interaction was also found significant. The Shapiro-Wilk normality test displayed the p value as 0.18, indicated the non significance of the non selected factors**.** So no other factors except B and E were affecting the zero order release rate constant.

ii) Normal plot

The normal plot shown in Figure No.6.3.143 it was evident that the factors B and E were significantly away from the normal straight line. An interaction AC was also found significant. Shapiro-Wilk normality test displayed the p value as 0.18 indicating that the remaining (unselected) terms were normally distributed.

iii) Pareto chart

The pareto chart shown in Figure No 6.3.144 represent the significant effect of B and E on the zero order rate constant. Both the factors crossed the t and Bonferroni limit conirmed the obvious effect of these factors on the zero order

rate constant. The magnitude of the effect can be written as, B> E. The factors had a positive effect on the R^2. AC(PEO DL- SLS DL) interaction was also found significant. No other terms were significant as they all were below the t limit.

iv) ANOVA and Regression analysis

The Model F-value of 51.89implied thatthe model was highly significant. Factors B (p =6.852E-08*)*, E (2.28E-06*)* were the significant model terms. AC interaction was also found to be significant (p =0.0008184). The "Lack of Fit F-value" of 1.7442031 implied the lack of fit was not significant relative to the pure error. No curvature effect was reported. Hence from the ANOVA analysis it was proven that the model selected was significant with no lack of fit and curvature effect.

v) Polynomial equation

The polynomial equation representing the R^2 can be written as follows,

R^2 = 0.9557 +0.031875 * B +0.024375 * E - 0.014 * AC *(coded terms)*

R^2 = 0.8969+0.0070 * NaCl DL +0.0010 * NaCl PL-6.03611E-05 * PEO DL * SLS DL *(Actual terms)*

Table No.6.3.60: ANOVA and regression analysis for the effect of factors selected on the R^2

Source	Sum of Squares	df	Mean Square	F Value	p-valueProb > F	
Model	0.0289	3	0.0096	51.8993	*1.818E-08*	*significant*
B-NaCl DL	0.0163	1	0.0163	87.5846	*6.852E-08*	
E-NaCl PL	0.0095	1	0.0095	51.2173	*2.28E-06*	
AC	0.0031	1	0.0031	16.8960	*0.0008*	
Residual	0.0030	16	0.0002			
Lack of Fit	0.0026	13	0.0002	1.74420	0.3574	*not significant*
Pure Error	0.0003	3	0.0001			
Cor Total	0.0319	19				
Regression analysis						
Std. Dev.	0.0136		R-Squared	0.9068		
Mean	0.9557		Adj R-Squared	0.8893		
C.V. %	1.4255		Pred R-Squared	0.8464		
PRESS	0.0049		Adeq Precision	18.4647		

vi) Test for the assumption of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No.6.3.145.

- *The normal probability plot*: The plot indicates that the residuals follow a normal distribution, as the points follow a straight line. The curve does not follow any pattern like S curve.
- *Residuals vs Predicted*: The plot was a random scatter (constant range of residuals across the graph) confirmed the constant variance in the experiments performed.
- *Residuals vs Run:* The plot showed a random scatter. The graphs had not followed any trends indicated that no time-related variable lurking in the background.
- *Predicted vs. Actual:* This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms:* The lamda value was 1 and no transformation was needed.

vii) The perturbation graph

This graph explained the effect and sign of the significant factors on the zero order rate constant. It showed that the change in the concentration of NaCl DL from minimum to maximum produced an increase in zero order rate constant from 0.925 to 0.975. An increase in the NaCl PL has changed the response from 0.94 to 0.96.

viii) Interaction graphs

The Figure No.6.3.146 shows the interaction plot of PEO DL and SLS DL (AC interaction). The plot showed the effect of change in the concentration of PEO DL at low and high level of SLS DL. Both the lines were not parrellel to each other, indicated that the effect of one factor depends on the level of the other.

ix) Contour plots and RS plots

Figure No.6.3.147 shows the contour plot and RS plot for the simultaneous effect of factor B(NaCl DL) and E (NaCl PL) on R^2. From the plot it was obvious that the factor B and E had a positive effect on zero order release rates constant. High levels of NaCl DL and PL yields a better R^2 value. The effect of change in concentration of NaCl DL was more evident at high level of NaCl PL and vice versa. From the surface plot the larger effect of NaCl DL than the NaCl PL was clearly understood.Figure No.6.3.148 shows the contour plot and RS plot for the concurrent effect of factor A and C at a time on R^2. Non linearity was expected because of the curved lines.

3. Analysis of responses - lag time

i) Half normal plot

Figure No 6.3.149 shows the half normal plot of the effect of factors on lag time. The significant factors affecting the lag time were identified as B (NaCl DL) G (propylene glycol) and H (weight gain). The Shapiro-Wilk normality test displayed the p value as 0.289, indicated the non significance of the non selected factors**.** So no other factors except B, G, and H were affecting the lag time**.**

ii) Normal plot

Figure No.6.3.150 shows the normal plot of the effect of factors on lag time.The factor B, G and H were significantly away from the normal straight line. Shapiro-Wilk normality test displayed the p value as 0.289indicating that the remaining (unselected) terms were normally distributed.

***iii)* Pareto chart**

Figure No.6.3.151 shows the pareto chart of effect of factors on the lag time in terms of t value. The factors significantly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect on the response. The magnitude of the effect of significant factors on the lag time can

be written as G > H> B. Propylene Glycol had comparatively greater effect on the lag time. There was not much variation in the t value for all the 3 factors. So it can be considered that all the 3 factors were equally affecting the lag time. No other factors or interaction terms were significant as they all were present below the t limit.

iv) ANOVA and regression analysis

The Model F-value of 21.07 implied that the model selected is significant. Factors B (7.59E-04*)*, G (1.97E-04*)* and H *(*1.97E-04.*)* were the significant model terms affecting the lag time. The "Lack of Fit F-value" of 1.34E-01, implied that the lack of fit was not significant relative to the pure error. This means that the polynomial model was fitting all of the design points well.Hence from the ANOVA analysis it was proven that the model selected was significant and no lack of fit and curvature effect were observed. No interaction terms were significant.

Table No .6.3.61: ANOVA and Regression analysis of the effect of factors selected on the lag time

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5.9769	3	1.9923	21.0737	*8.37E-06*	*significant*
B-NaCl DL	1.6256	1	1.6256	17.1953	*7.59E-04*	
G-Propylene Glycol	2.1756	1	2.1756	23.0130	*1.97E-04*	
H- Weight gain	2.1756	1	2.1756	23.0130	*1.97E-04*	
Residual	1.5126	16	0.0945			
Lack of Fit	1.4326	13	0.1102	4.1326	1.34E-01	*not significant*
Pure Error	0.08	3	0.0267			
Cor Total	7.4895	19				
Regression analysis						
Std. Dev.	0.3075		R-Squared		0.79803	
Mean	4.1050		Adj R-Squared		0.76017	
C.V. %	7.4902		Pred R-Squared		0.66211	
PRESS	2.5306		Adeq Precision		15.36300	

v) Polynomial equation

Polynomial equations for the lag time can be written as,

Lag time = 4.105 - 0.31875 * B -0.36875 * G +0.36875 * H *(coded terms)*

Lag time = 3.8390 -0.0708 * NaCl DL -0.0819 * Propylene Glycol +0.07375 * Weight gain *(Actual terms)*

vi) Test for assumptions of ANOVA

The ANOVA assumptions were tested and studied with the help of various graphs shown in the Figure No.6.3.152.

- *The normal probability plot*: The plot indicated that the residuals followed a normal distribution, as the points followed a straight line. The curve does not follow any pattern like S curve.
- *Residuals vs Predicted*: The plot was a random scatter (constant range of residuals across the graph.) confirmed the constant variance in the experiments performed.
- *Residuals vs Run*: This plot showed a random scatter. So no time-related variable lurking in the background.
- *Predicted vs. Actual*: A graph of the predicted response values versus the actual response values. This graph represented a good relationship between actual and predicted responses.
- *Box-Cox Plot for Power Transforms*: The graph shows that the lamda value is 1 and no transformation is needed

vii) The perturbation graph

The perturbation graph shown in Figure No.6.3.152 explained the effect and sign of the significant factors on lag time. NaCl DL and propylene glycol had a negative effect and weight gain had a positive effect on the lag time.

viii) Contour plots and RS plots

Figure No.6.3.153 shows the contour plot of the simultaneous effect of factor B and the G on lag time at a time. Both the factors had a negative effect on the response. Propylene Glycol had similar effect on both the levels of NaCl DL

(change in lag time of 0.8). NaCl DL had a prominent effect when propylene glycol concentration was high and vice versa.

The Figure No.6.3.154 shows the contour plot and RS plot, of the simultaneous effect of factor B and the H at a time. NaCl DL had a negative effect on the lag time. ie, as the concentration had changed from low to high, the lag time decreased. But the weight gain had an opposite effect. The desired lag time was produced at low weight gain and high concentration of NaCl DL. NaCl DL had a prominent effect at low weight gain.

Figure No. 6.3.155 shows the contour plot and RS plot of the simultaneous effect of G (propylene glycol) and H (weight gain). Weight gain had a positive effect on the lag time and propylene glycol had a reverse effect. Both the factors had significant effect on both the levels of the other.

ix) Cube plot

This cube plot showed in Figure No.6.3.156 explained the combined effect of B, G and H. When all the three factors were at minimum the lag time was 4.425 hr and at minimum it was 4.52hrs. Lowest lag time was observed while keeping NaCl DL and propylene glycol at maximum and weight gain at its minimum (lag time was 3.05). This cube plot also well represents the major effects of propylene glycol and weight gain.

IV. Numerical optimization with the help of desirability

From the factor influence study it was found that the model suggested was linear and no significant lack of fit and curvature were found for any of the responses. So no quadratic model was chosen for optimization. The same 2 level design was used for further optimization.

A. Optimization of the push pull osmotic tablets of Nisoldipine

The constraint fixed for the optimization was given in the Table No.6.3.62. The solutions of the numerical optimization were given in the Table No.6.3.63.

Table No.6.3.62: Constraints fixed for the optimization of Nisoldipine push pull OTs

Constraints	Goal	Lower Limit	Upper Limit	Importance
A:PEO in the drug layer	*is in range*	10	100	3
B:NaCl concentration in drug layer	*is in range*	1	10	3
C:SLS in the drug layer	*is in range*	1	5	3
D:PEO in the push layer	*is in range*	5	50	3
E:Sodium chloride in the Push layer	*is in range*	5	50	3
G:Propylene Glycol	*is in range*	1	10	3
H: Weight gain	*is in range*	10	20	3
CUR at 24 hrs	*is in range*	95	100	5
R^2	*maximize*	0.859	0.999	4
lag time	*minimize*	3.8	5.1	3

B. Point prediction

The point prediction for the solution 1, 2 and 3 were given in the Table No.6.3.64. The same batches were selected as the check point batches. The confidence intervals and the tolerance intervals were given in the Table No.6.3.64.

Table No.6.3.63: Numerical solutions for the optimization of the Nisoldipine push pull OTs

Number	PEO DL	NaCl DL	SLS DL	PEO PL	NaCl PL	SLS PL*	Propylene Glycol	Weight gain	CUR at 24 hrs	R^2	lag time	Desirability
1	**89.90**	**10.00**	**3.59**	**30.66**	**41.07**	**3.27**	**10.00**	**10.00**	**98.37**	**0.9991**	**3.05**	**0.9504**
2	**86.39**	**10.00**	**4.13**	**30.45**	**49.31**	**4.01**	**10.00**	**10.00**	**99.77**	**1.0057**	**3.05**	**0.9504**
3	**92.63**	**10.00**	**1.33**	**31.98**	**30.63**	**1.14**	**10.00**	**10.00**	**99.59**	**1.0007**	**3.05**	**0.9504**
4	94.42	10.00	2.25	22.78	40.20	2.82	10.00	10.00	98.72	1.0059	3.05	0.9504
5	95.78	10.00	2.93	27.51	39.26	2.32	10.00	10.00	99.31	1.0008	3.05	0.9504
6	73.07	10.00	2.68	38.20	46.10	2.60	10.00	10.00	99.54	1.0086	3.05	0.9504
7	95.15	10.00	3.66	29.93	42.55	1.22	10.00	10.00	99.94	0.9998	3.05	0.9504
8	88.32	10.00	2.48	25.38	35.98	2.85	10.00	10.00	95.69	0.9994	3.05	0.9504
9	84.83	10.00	3.26	36.38	39.79	4.34	10.00	10.00	99.20	0.9997	3.05	0.9503
10	79.95	10.00	3.57	41.87	40.11	3.34	10.00	10.00	99.68	0.9990	3.05	0.9503
11	45.82	10.00	2.18	49.81	45.32	1.48	10.00	10.01	95.00	1.0057	3.05	0.9502
12	73.06	10.00	2.42	44.46	40.71	3.66	10.00	10.04	100.00	1.0035	3.05	0.9498
13	43.21	10.00	1.00	50.00	49.42	1.05	9.94	10.01	95.01	1.0077	3.05	0.9493
14	76.71	10.00	4.02	45.09	41.23	1.00	9.93	10.00	99.87	0.9990	3.05	0.9492
15	91.58	10.00	2.58	34.82	35.82	1.99	10.00	10.09	100.00	0.9990	3.06	0.9490
16	99.50	10.00	1.22	10.95	33.50	5.00	9.93	10.02	95.22	1.0064	3.06	0.9489
17	40.38	10.00	3.00	50.00	48.83	1.13	9.98	10.09	95.03	1.0107	3.06	0.9488
18	54.81	10.00	1.70	45.63	45.90	4.93	10.00	10.13	96.43	1.0075	3.06	0.9485
19	52.49	9.99	3.52	50.00	48.22	3.27	9.84	10.00	98.27	1.0102	3.06	0.9477
20	97.94	10.00	1.00	8.37	49.24	2.45	9.80	10.00	100.00	1.0245	3.06	0.9471
21	87.04	9.94	2.79	31.10	45.28	2.24	9.71	10.00	100.00	1.0075	3.08	0.9446
22	97.16	10.00	5.00	20.97	49.08	2.65	9.87	10.01	96.56	0.9978	3.06	0.9438
23	91.95	9.49	2.93	30.35	41.00	4.31	10.00	10.00	99.94	0.9990	3.09	0.9428
24	82.48	10.00	1.78	21.18	50.00	3.23	9.98	10.61	97.60	1.0172	3.09	0.9409
25	79.61	10.00	3.03	21.53	50.00	2.94	10.00	10.78	95.00	1.0118	3.11	0.9385

Number	PEO DL	NaCl DL	SLS DL	PEO PL	NaCl PL	SLS PL*	Propylene Glycol	Weight gain	CUR at 24 hrs	R^2	lag time	Desirability
26	100.00	10.00	1.00	20.66	22.99	4.00	10.00	10.27	95.29	0.9967	3.07	0.9376
27	61.68	9.02	3.51	37.63	45.16	1.28	10.00	10.00	95.13	0.9992	3.12	0.9359
28	83.09	10.00	1.48	46.96	31.93	3.21	9.03	10.01	99.89	0.9990	3.13	0.9336
29	62.92	9.26	2.46	50.00	46.37	5.00	9.50	10.00	100.00	1.0034	3.14	0.9310
30	100.00	10.00	1.00	22.11	20.82	3.27	10.00	10.17	95.14	0.9943	3.06	0.9301
31	67.47	10.00	4.44	48.28	50.00	4.72	9.97	11.29	100.00	1.0092	3.15	0.9299
32	38.31	8.41	4.01	48.26	50.00	4.85	9.91	10.00	95.00	1.0033	3.17	0.9253
33	58.60	8.91	1.01	49.28	50.00	4.37	9.40	10.00	99.77	1.0054	3.17	0.9240
34	68.96	10.00	3.56	49.99	50.00	3.97	8.14	10.00	99.54	1.0107	3.20	0.9184
35	89.04	10.00	1.00	22.59	50.00	4.19	7.95	10.00	98.11	1.0225	3.22	0.9150
36	95.28	7.77	2.52	5.00	49.07	1.00	10.00	10.00	95.10	0.9981	3.21	0.9140
37	75.44	10.00	4.57	38.56	50.00	1.00	7.83	10.02	95.14	1.0069	3.23	0.9125
38	84.87	7.38	1.00	9.62	47.34	1.00	10.00	10.04	95.00	0.9998	3.24	0.9106
39	68.69	8.68	1.03	35.92	42.84	3.38	10.00	11.30	95.00	0.9991	3.24	0.9104
40	85.63	10.00	3.22	48.33	29.32	4.99	10.00	10.00	100.00	0.9885	3.05	0.9101
41	97.11	6.90	1.01	7.93	49.34	3.06	10.00	10.00	99.98	1.0023	3.27	0.9038
42	94.99	10.00	4.53	43.85	46.86	4.99	9.08	12.27	99.56	0.9990	3.29	0.8987
43	83.38	9.99	1.00	49.72	29.92	5.00	7.04	10.00	96.44	0.9990	3.29	0.8983
44	100.00	8.01	1.42	5.00	46.43	2.79	8.73	10.00	95.06	1.0051	3.29	0.8981
45	85.75	9.81	4.36	50.00	45.29	2.11	9.91	13.19	99.89	0.9990	3.31	0.8956

Table No.6.3.64: Prediction of the responses

Solution 1							
Response	**Predicted**			**CI for Mean**		**99% of Population**	
	Mean	**Std Dev**	**SE Mean**	**95%CI low**	**95%CI high**	**95%TI low**	**95%TI high**
PCUR at 24 hrs	98.37	6.702	3.614	92.193	102.807	62.958	127.042
R^2	0.9991	0.014	0.005	0.988	1.010	0.940	1.058
lag time	3.05	0.265	0.132	2.705	3.266	1.772	4.199
Solution 2							
Response	Predicted			CI for Mean		99% of Population	
	Mean	**Std Dev**	**SE Mean**	**95%CI low**	**95%CI high**	**95%TI low**	**95%TI high**
PCUR at 24 hrs	99.77	6.702	3.377	91.714	102.306	63.387	126.633
R^2	1.0057	0.014	0.005	0.989	1.009	0.941	1.057
lag time	3.05	0.265	0.128	2.776	3.324	1.842	4.258
Solution 3							
Response	**Predicted**			**CI for Mean**		**99% of Population**	
	Mean	**Std Dev**	**SE Mean**	**95%nCI low**	**95% CI high**	**95% TI low**	**95%TI high**
PCUR at 24 hrs	99.59	6.702	3.333	88.821	103.221	64.477	127.565
R^2	1.0007	0.014	0.005	0.989	1.009	0.941	1.057
lag time	3.05	0.265	0.129	2.792	3.342	1.858	4.276

C. Check point

To confirm the validity of the model three formulations from the solutions were selected and formulated as discussed in section 5.6.4.I. The dissolutions were performed as per the method specified in section 5.6.4(IIBix). The Table No. 6.3.65 shows the value obtained from the dissolution study. All the responses were within the confidence Intervals and tolerance limits of the point predicted by the software. Hence it can be concluded that the model suggested for the design was a success and can be used for further predictions.

Table.6.3.65: Check point batches for the model validation of the Nisoldipine push pull OTs

Batches	PCUR at 24 hrs	R^2	Lag time
Solution 1	100 ±1.5	0.998	3.02±0.05
Solution 2	101.5 ± 3.1	0.999	2.9± 0.06
Solution 3	97.5±1.7	0.998	3.0±0.07

D. Optimized batch and evaluations

The Nisoldipine optimized batch (NOB) from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. Solution 1 was selected as the optimized batch. The composition of the optimized batch was given in the Table No.6.3.66. The optimized batch was prepared as per the procedure mentioned in the chapter section 5.6.4.I. The blend as well as the whole tablet evaluation was performed as per the methods specified in section 5.6.4.II. The result of the study was given in the Table No.6.3.67. The *in vitro* study was performed as per the methods mentioned in the section 5.6.4(IIBix) and the findings were given in the Table No.6.3.67 and in the Figure No. 6.3.157.

Table No. 6.3.66: Composition of the optimized batch

SL.No	Ingredients		
		Mg/tab	(%w/w)
	Drug Layer		
1	Nisoldipine	8.5	
2	DCP	22.056	
3	PEO 400 K	7.642	89.9
4	Sodium chloride	9.500	10
5	BHT	0.0076415	
6	SLS	1.795	3.59
7	IPA		
8	Magnesium stearate	0.500	
	Total weight of drug layer	50	
	Push layer		
9	PEO 7000 K	15.33	30.66
10	Sodium chloride	6.30	41.07
11	DCP	19.59	
12	BHT	0.01533	
13	SLS	1.47	3.27
14	Iron oxide Red	0.8	
15	IPA		
16	Magnesium stearate	1.5	
	Total weight of Push layer	45	
	Total weight of un coated tablet	95	
	Functional coating		
15	Cellulose acetate	8.6	
16	Acetone	q.s	
18	Water	q.s	
19	Propylene Glycol	0.95	10.0
	Total Weight of Coating	9.5	10.00
	Total tablet weight	104.5	
Responses	**CUR at 24 hrs (%)**	**R^2**	**Lag time**
Predicted	98.37	0.9991	3.05
Observed	100±1.5	0.998	3.02±0.05

Table No .6.3.67: Optimized batch evaluation

Trial	Wt variation (n =20)	Diameter (n=10)	Thickness (n=10)	Hardness (n=6)	Friability (%)	Assay (%)	Weight gain (%)	Pore size (mm)
NOB	105 ±0.07	5.18 ±0.11	3.6 ±0.13	4.6 ±0.5	0.63	98.7 ±2.1	10.1 ±0.04	0.60

Dissolution Profile											
Time	0	1	2	3	4	6	8	12	16	20	24
PCUR	0	0	3.5± 0.1	9.9± 0.3	14.8± 1.1	25.1± 2.2	34.5± 2.3	51.7± 3.6	68.7± 3.2	84.5± 1.7	100 ±1.5

E. Desirability contour plot and RS plot

Desirability plots shows how all the targeted optimum conditions are met by changing two factors at a time. The Figure No.6.3.158 shows the effect of PEO DL and NaCl DL on the desirability. Higher desirability will be achieved at maximum level of NaCl DL (more than 9%) and PEO DL (75 -95).

The Figure No.6.3.159 shows the effect of factor A (PEO DL) and E (NaCl PL) on the desirability function. Combinations of 74 -91 % of PEO DL and 30 -40% NaCl PL had desirability more than 0.8.

The Figure No.6.3.152 shows the effect of factors A (PEO DL) and D (PEO PL) on desirability function. Combinations of 70 -90 % of PEO DL and 30 - 45 PEO PL had desirability more than 0.8.All other combinations had desirability zero.

Figure No. 6.3 161 shows the desirability contour plot and RS plot of the effect of PEO DL and propylene glycol. Combinations of PEO DL 76 to 100% and propylene glycol 8-10% had desirability >0.8. Lower concentrations of both the factors had desirability zero.

Figure No. 6.3.162 shows the desirability contour plot and RS plot of the effect of PEO DL and weight gain. Combinations of PEO DL 70 to 80% and weight gain 10-12.5 % had desirability more than 0.8. Combinations of weight gain more than 13 % and PEO in DL less than 75 % had desirability zero.

Figure No.6.3.163 shows the desirability contour plot and RS plot of the effect of NaCl PL and NaCl DL. Combinations of NaCl PL 28 - 40 % and NaCl DL 9 -10% had desirability 1. Combinations containing NaCl PL less than 28 % and greater than 40% had desirability zero.

Figure No 6.3.164 shows the desirability contour plot and RS plot of the effect of PEO PL and NaCl DL. Combinations of PEO PL 28 - 40 % and NaCl DL 9 -10% had desirability 1.

Figure No.6.3.165 shows the desirability contour plot and RS plot of the effect of propylene glycol and NaCl DL. Combinations of NaCl DL 9-10% and propylene glycol 9 -10% had desirability 1.

Figure No.6.3.166 shows the desirability contour plot and RS plot of the

effect of weight gain and NaCl DL. Combinations of NaCl DL 9-10% and weight gain 10 -11% had desirability 1. Any combination having weight gain more than 11 had desirability zero.

Figure No.6.3.167 shows the desirability contour plot and RS plot of the effect of PEO PL and NaCl PL. Combinations of NaCl PL 40-45% and PEO PL 28 -30% had desirability greater than 0.80.

Figure No.6.3.168 shows the desirability contour plot and RS plot of the effect of PEO PL and propylene glycol. Combinations of propylene glycol 8 -10% and PEO PL 30 -40% had desirability greater than 0.80. All other combinations had desirability zero.

Figure No. 6.3.169 shows the desirability contour plot and RS plot of the effect of PEO PL and weight gain. Combinations of weight gain 10 -12.5 % and PEO PL 30 -40% had desirability 0.8. Other combinations having concentrations of PEO PL less than 30 % and weight gain more than 12.5% had desirability zero.

Figure No. 6.3.170 shows the desirability contour plot and RS plot of the effect of NaCl PL and propylene glycol. Combinations of propylene glycol 7.75-10% and NaCl PL 31-43 % had desirability greater than 0.6. All other combinations had desirability zero.

Figure No.6.3.171 shows the desirability contour plot and RS plot of the effect of NaCl PL and weight gain. Combinations of weight gain 10-12.5% and NaCl PL 28-40 % had desirability greater than 0.8. Higher weight gain and NaCl PL acquired the desirability zero.

Figure No.6.3.172 shows the desirability contour plot and RS plot of the effect of propylene glycol and weight gain. Combinations of weight gain 10-12.5% and propylene glycol 8 -10 % had desirability greater than 0.8. Higher weight gain and lower propylene glycol concentrations acquired the desirability zero.

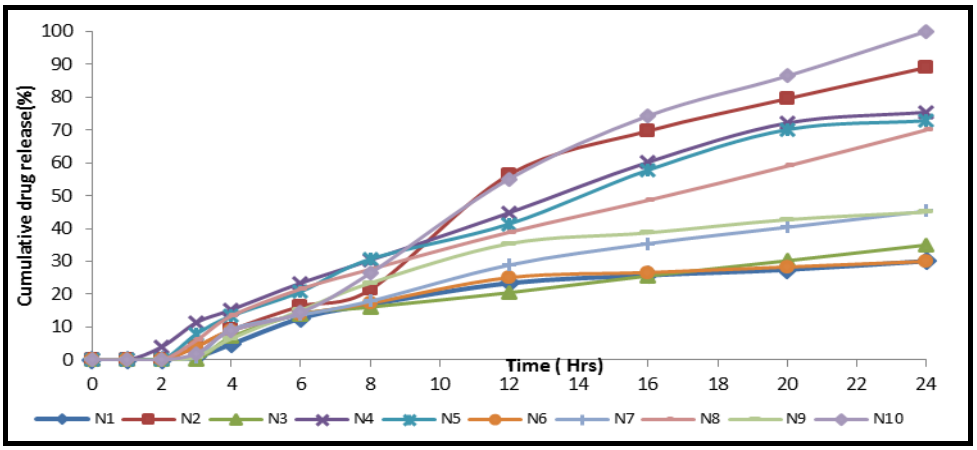


Figure No. 6.3 .119: Dissolution profile of N 1- N 10

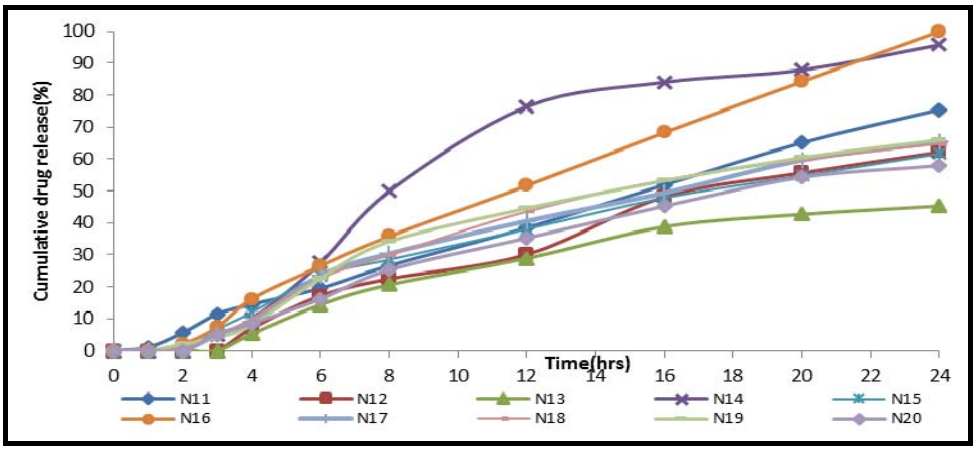


Figure No. 6.3.120: Dissolution profile of N11- N20

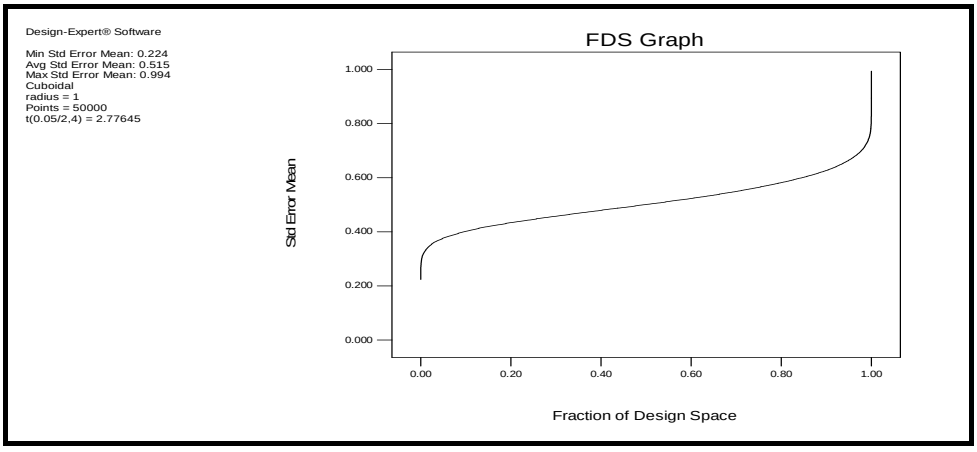
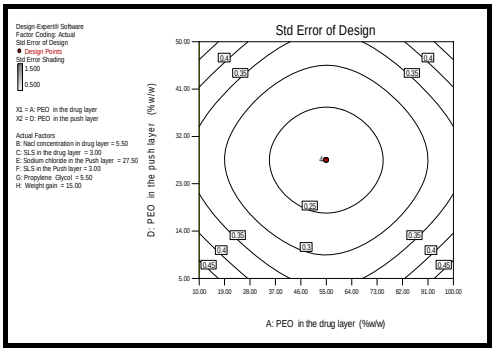
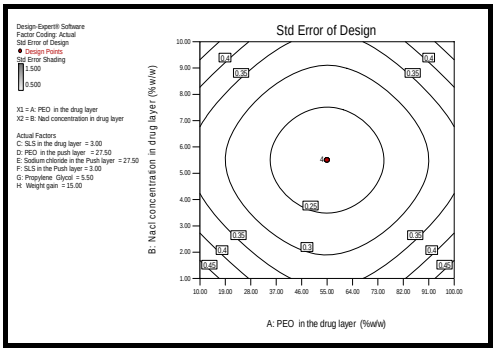


Figure No .6.3.121: FDS graph of the design selected for the FI study & optimization


Design-Expert® Software
Factor Coding: Actual
Std Error of Design
Design Points
Std Error Shading
1.500
0.500
X1 = A: PEO in the drug layer
X2 = D: PEO in the push layer
Actual Factors
B: NaCl concentration in drug layer = 5.50
C: SLS in the drug layer = 3.00
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
G: Propylene Glycol = 5.50
H: Weight gain = 15.00
Std Error of Design
D: PEO in the push layer (%w/w)
A: PEO in the drug layer (%w/w)


Design-Expert® Software
Factor Coding: Actual
Std Error of Design
Design Points
Std Error Shading
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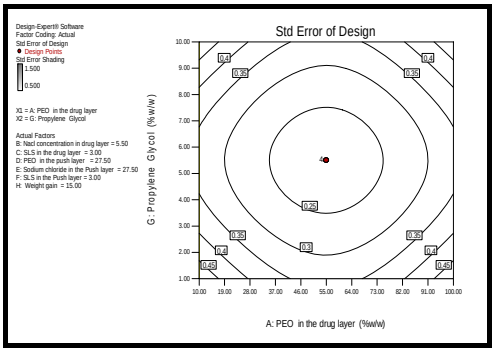
0.500
X1 = A: PEO in the drug layer
X2 = B: NaCl concentration in drug layer
Actual Factors
C: SLS in the drug layer = 3.00
D: PEO in the push layer = 27.50
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
G: Propylene Glycol = 5.50
H: Weight gain = 15.00
Std Error of Design
B: NaCl concentration in drug layer (%w/w)
A: PEO in the drug layer (%w/w)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Factor Coding: Actual
Std Error of Design
Design Points
Std Error Shading
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0.500
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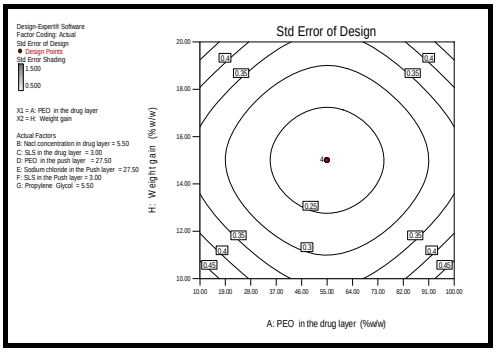
X2 = G: Propylene Glycol
Actual Factors
B: NaCl concentration in drug layer = 5.50
C: SLS in the drug layer = 3.00
D: PEO in the push layer = 27.50
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
H: Weight gain = 15.00
Std Error of Design
G: Propylene Glycol (%w/w)
A: PEO in the drug layer (%w/w)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Factor Coding: Actual
Std Error of Design
Design Points
Std Error Shading
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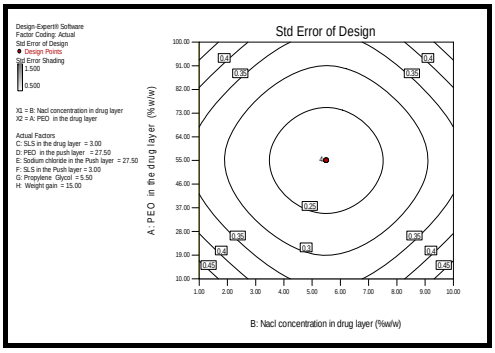
0.500
X1 = A: PEO in the drug layer
X2 = H: Weight gain
Actual Factors
B: NaCl concentration in drug layer = 5.50
C: SLS in the drug layer = 3.00
D: PEO in the push layer = 27.50
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
G: Propylene Glycol = 5.50
Std Error of Design
H: Weight gain (%w/w)
A: PEO in the drug layer (%w/w)


Design-Expert® Software
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Std Error of Design
Design Points
Std Error Shading
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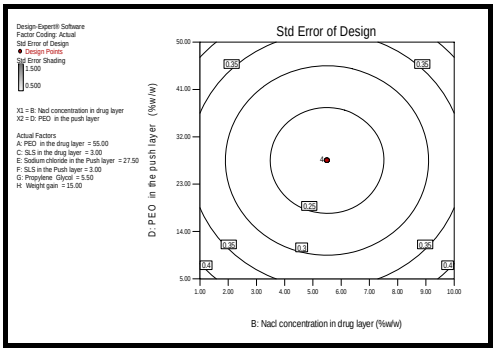
0.500
X1 = B: NaCl concentration in drug layer
X2 = A: PEO in the drug layer
Actual Factors
C: SLS in the drug layer = 3.00
D: PEO in the push layer = 27.50
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
G: Propylene Glycol = 5.50
H: Weight gain = 15.00
Std Error of Design
A: PEO in the drug layer (%w/w)
B: NaCl concentration in drug layer (%w/w)


Design-Expert® Software
Factor Coding: Actual
Std Error of Design
Design Points
Std Error Shading
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0.500
X1 = B: NaCl concentration in drug layer
X2 = D: PEO in the push layer
Actual Factors
A: PEO in the drug layer = 55.00
C: SLS in the drug layer = 3.00
E: Sodium chloride in the Push layer = 27.50
F: SLS in the Push layer = 3.00
G: Propylene Glycol = 5.50
H: Weight gain = 15.00
Std Error of Design
D: PEO in the push layer (%w/w)
B: NaCl concentration in drug layer (%w/w)

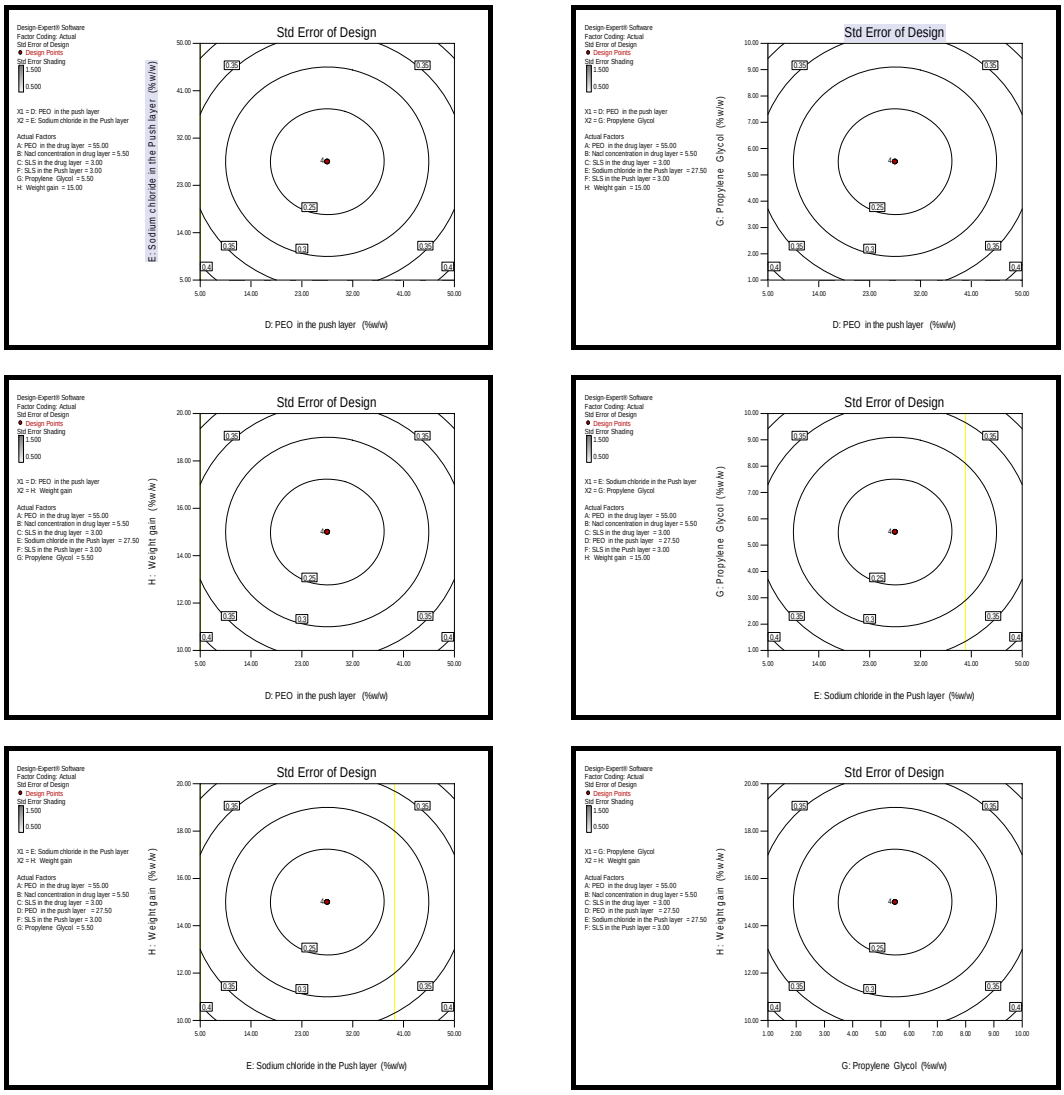
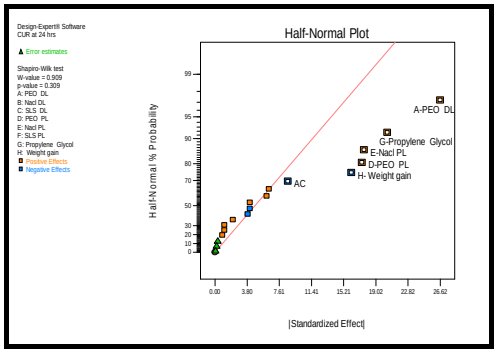


Figure No.6.3.122: Standard error contour plots of the design selected for the FI study & optimization



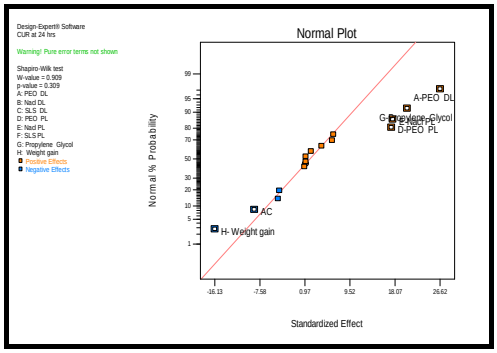


Figure.6.3.123: Half normal plot of the effect of the factors selected on the PCUR at 24 hrs. Figure .6.3.124: Normal plot for the effect of the factors selected on the PCUR at 24 hrs

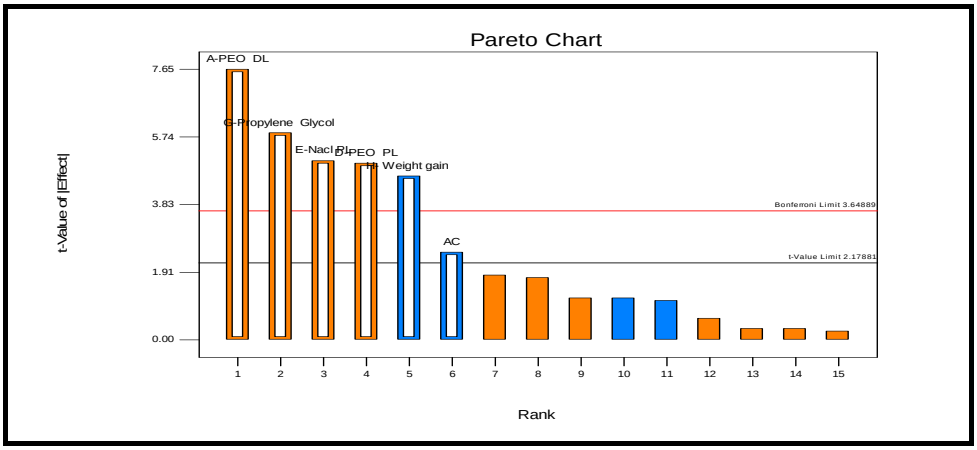


Figure .6.3.125: The pareto chart for the effect of the factors selected on the PCUR at 24 hrs

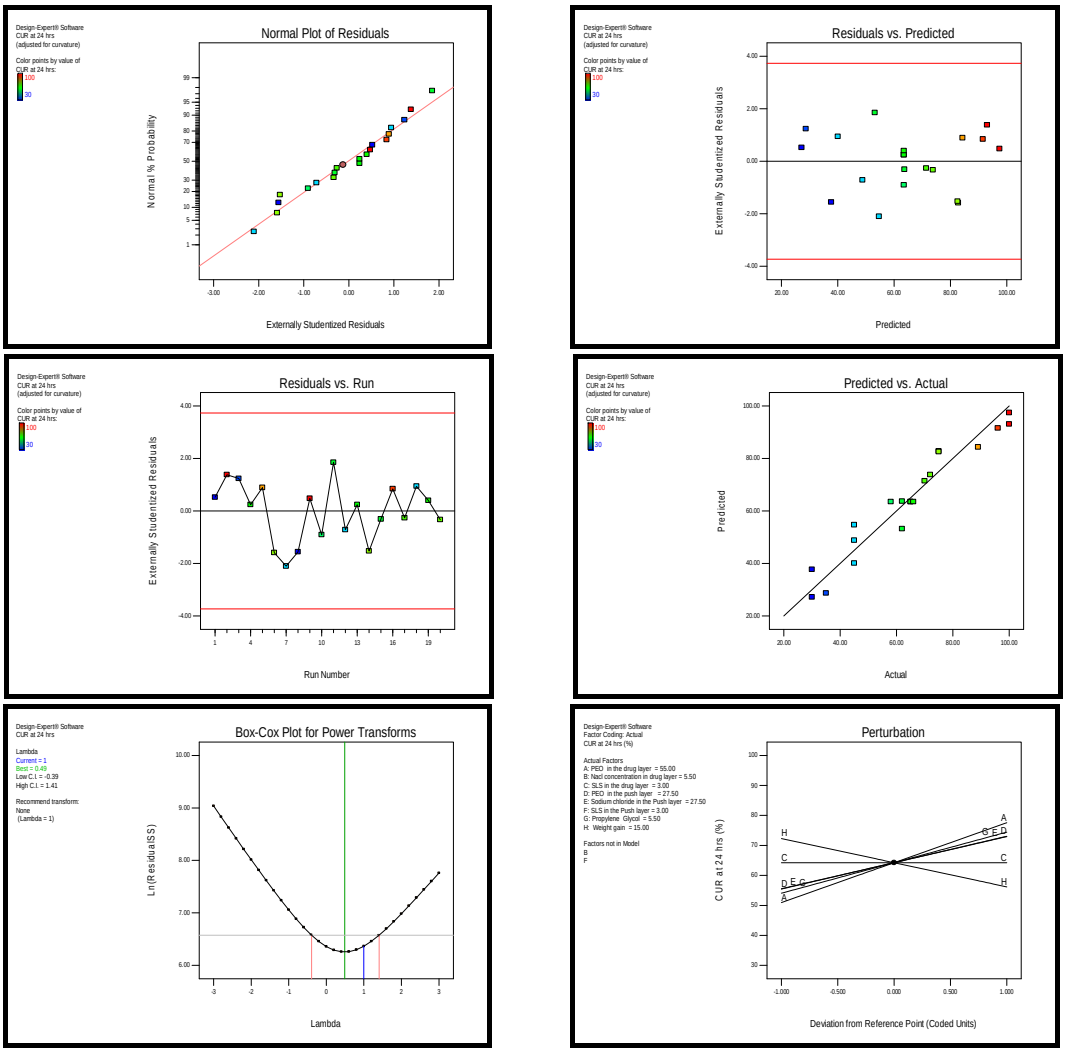


Figure No.6.3.126: Plots for the testing the assumptions of ANOVA and perturbation curve

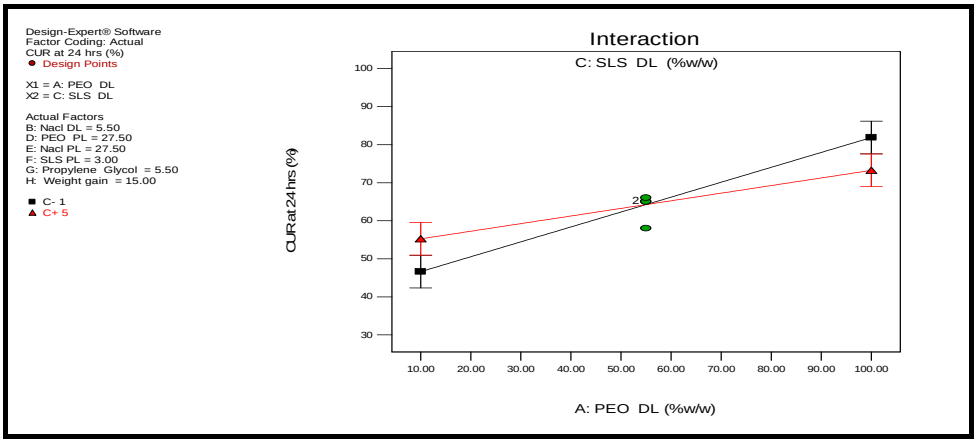


Figure .6.3.127: AC interaction on PCR at 24 hrs

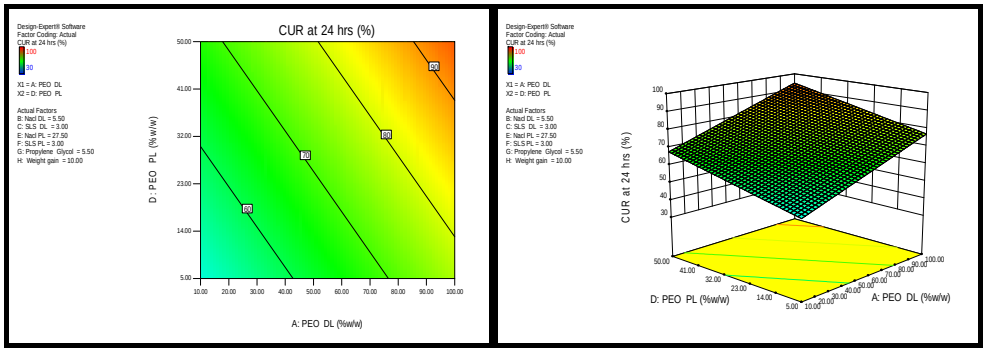


Figure .6.3.128: Contour plot and RS Plot - Effect of PEO DL and PEO PL on PCUR at 24 hrs

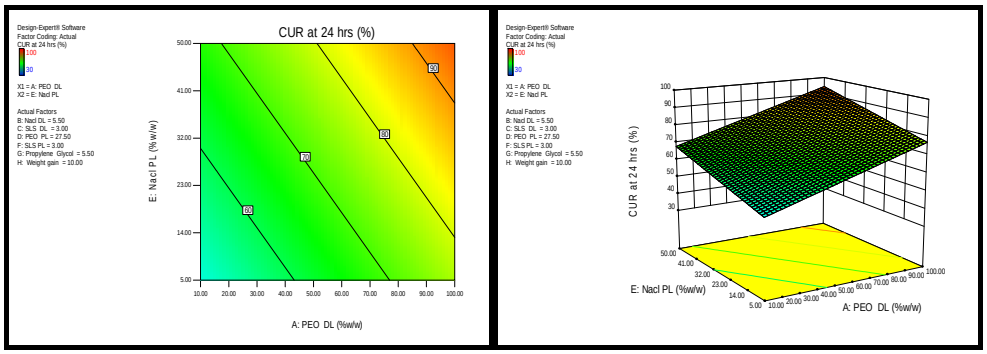


Figure .6.3 .129: Contour plot and RS Plot - Effect of PEO DL and NaCl PL on PCUR at 24 hrs

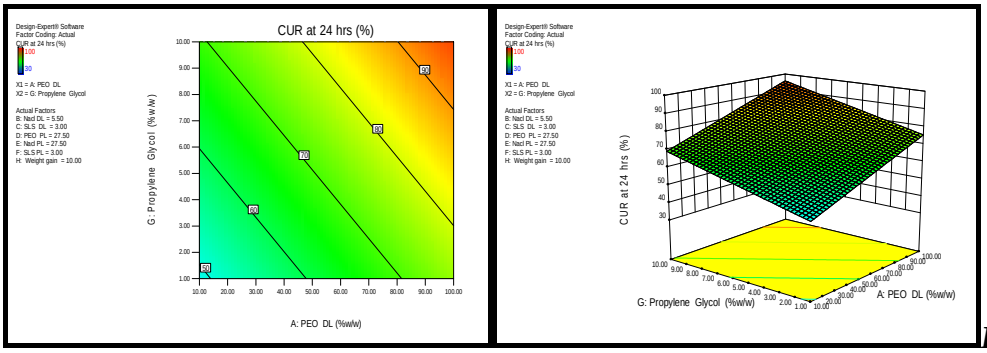


Figure .6.3.130: Contour plot and RS Plot - Effect of PEO DL and Propylene Glycol on PCUR at 24 hrs

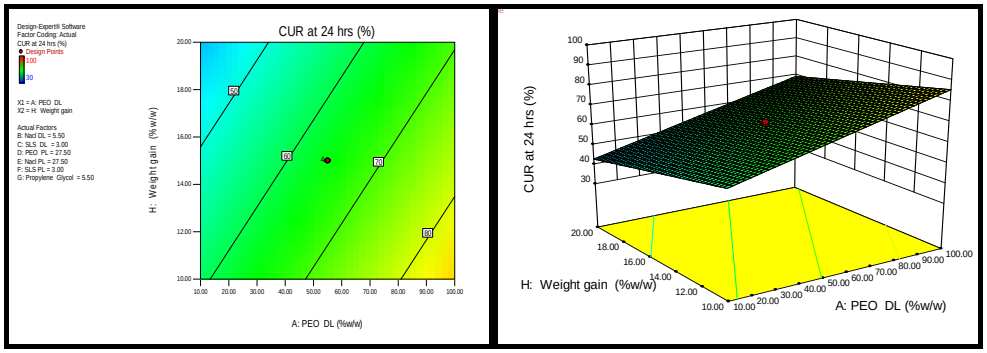


Figure No.6.3.131: Contour plot and RS Plot - Effect of PEO DL and weight gain on PCUR at 24 hrs

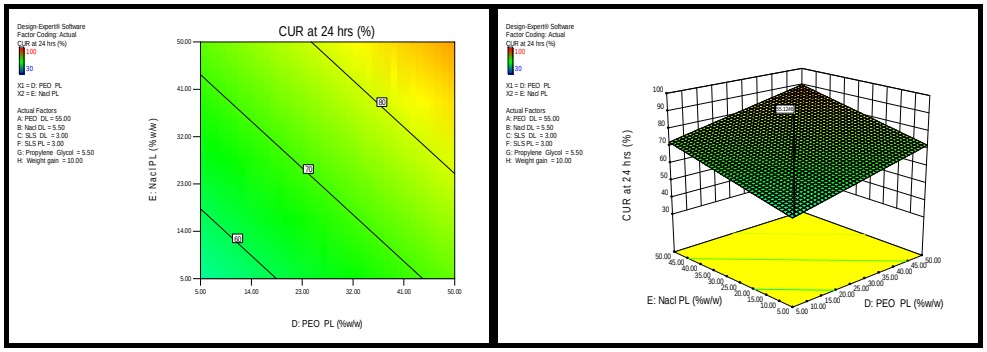


Figure No.6.3.132: Contour plot and RS Plot - Effect of PEO PL and NaCl PL on PCUR at 24 hrs

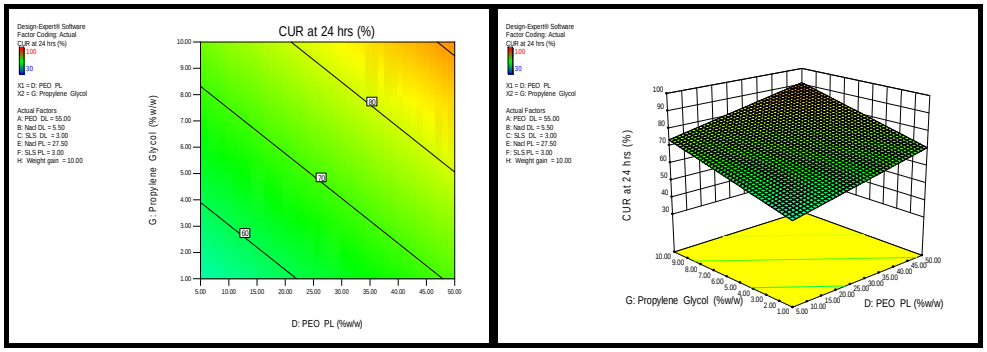


Figure No.6.3.133: Contour plot and RS Plot - Effect of PEO PL and Propylene Glycol on PCUR at 24 hrs

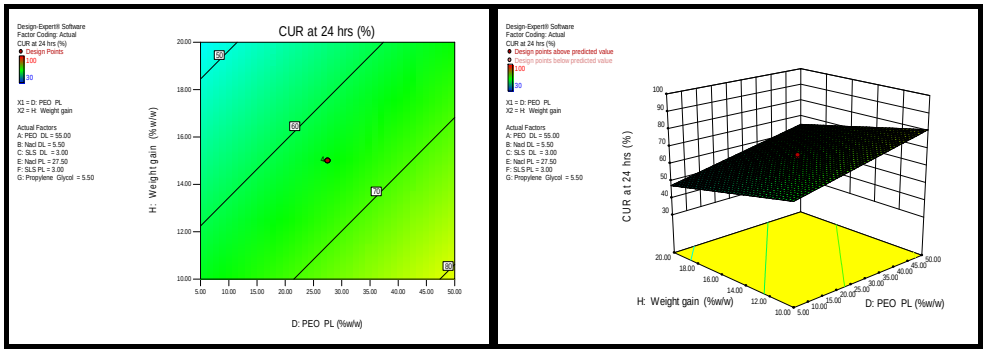


Figure No.6.3.134: Contour plot and RS Plot - Effect of PEO PL and weight gain on PCUR at 24 hrs

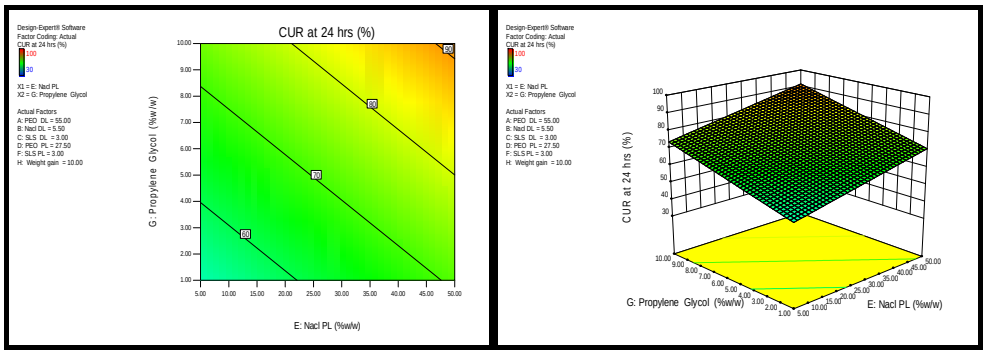


Figure No.6.3.135: Contour plot and RS Plot - Effect of NaCl PL and Propylene Glycol on PCUR at 24 hrs

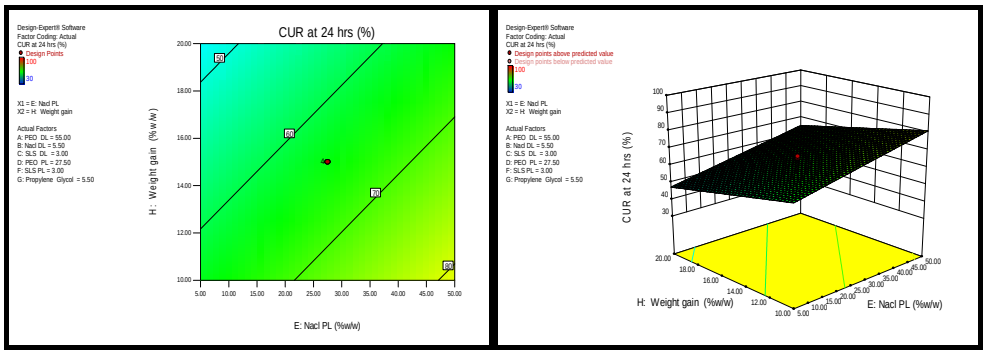


Figure No 6.3.136: Contour plot and RS Plot - Effect of NaCl PL and weight gain on PCUR at 24 hrs

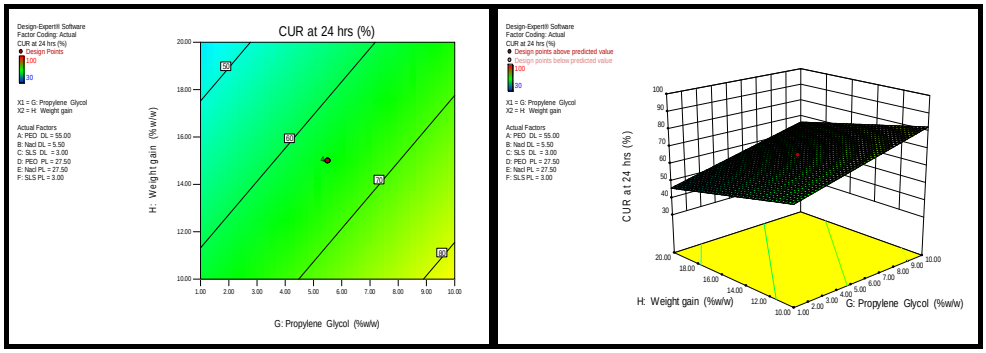


Figure No.6.3.137: Contour plot and RS Plot - Effect of weight gain and propylene glycol on PCUR at 24 hrs

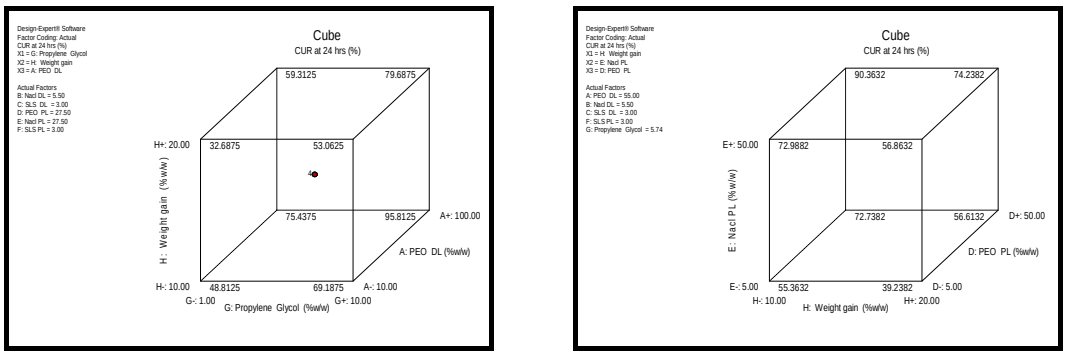


Figure No.6.3.138: Cube plot of the effect of PEO DL, Propylene Glycol and weight gain on PCUR at 24 hrs. Figure No.6.3.139: Cube plot of the Effect of NaCl PL, PEO PL and Weight gain on PCUR at 24 hrs

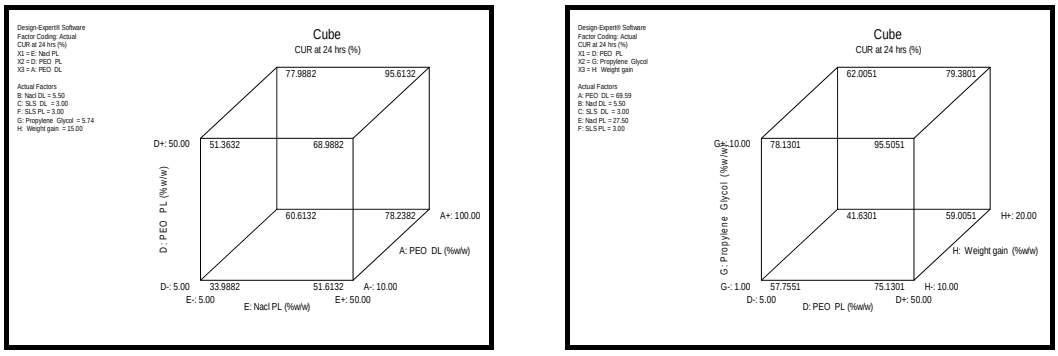
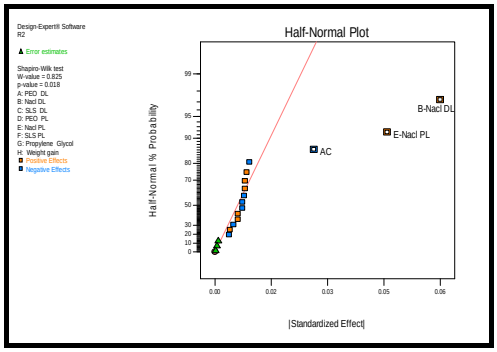


Figure No.6.3.140: Cube plot of the Effect of PEO PL, NaCl PL and PEO DL on PCUR at 24 hrs. Figure No.6.3.141: Cube plot of the Effect of PEO PL, Propylene Glycol and Weight gain on PCUR at 24 hrs



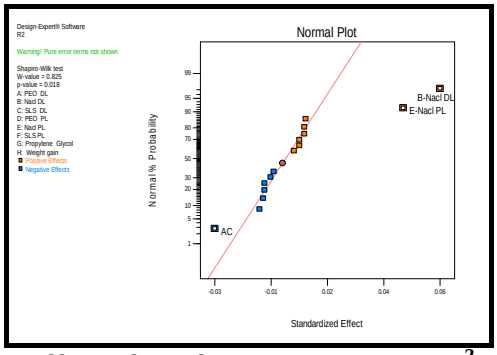


Figure No.6.3.142: Half Normal plot of the effect of the factors selected on R^2.
Figure No.6.3.143: Normal plot of the effect of the factors selected onR^2

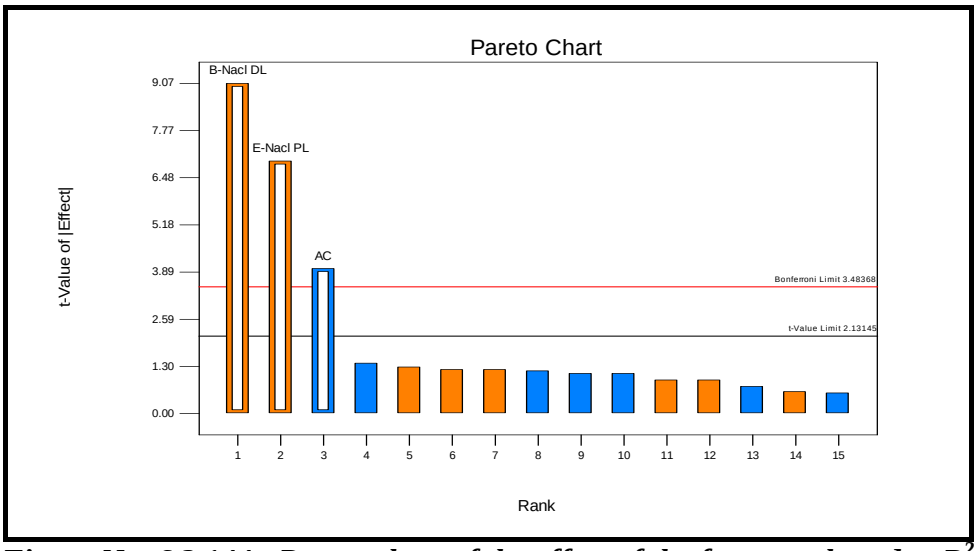


Figure No .6.3.144 : Pareto chart of the effect of the factors selected onR^2

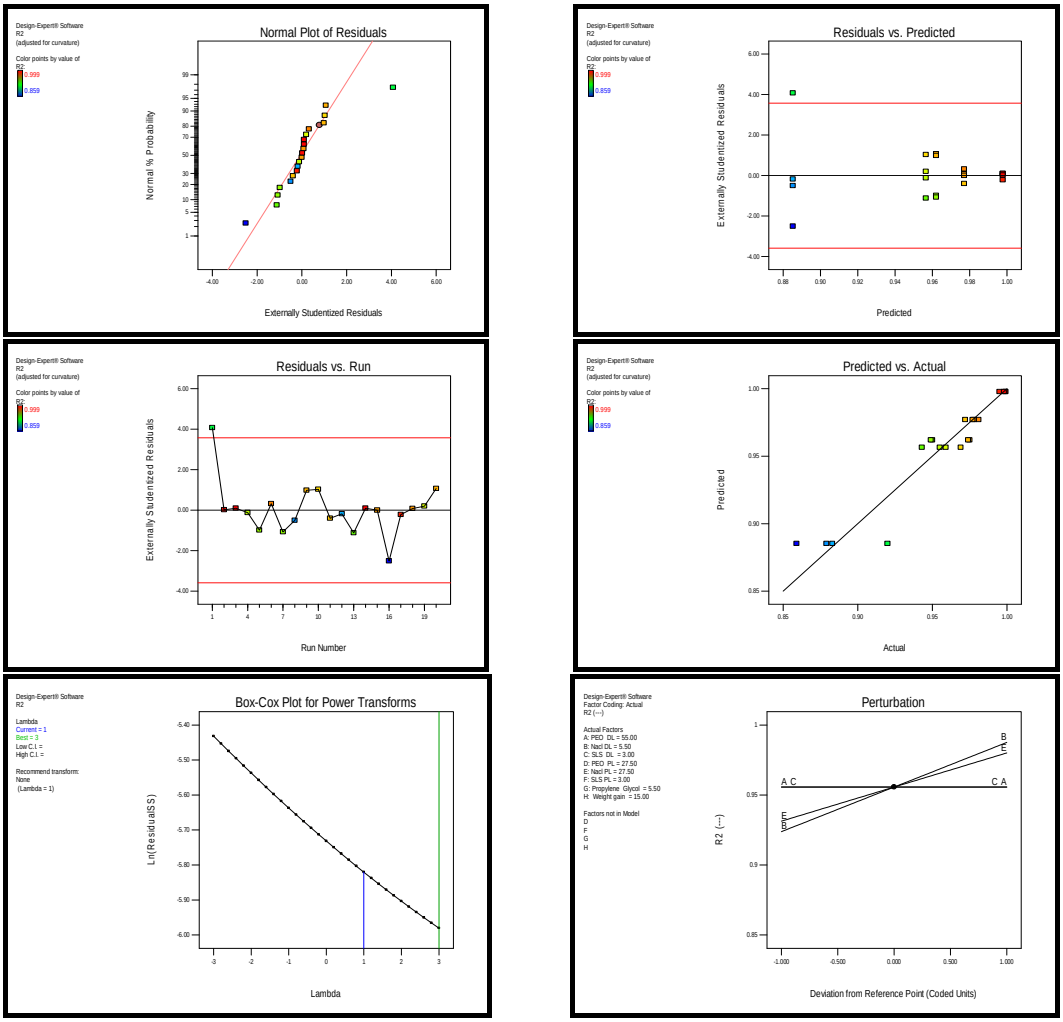


Figure No.6.3.145: Plots for Testing the assumptions of the ANOVA and pertubation Curve

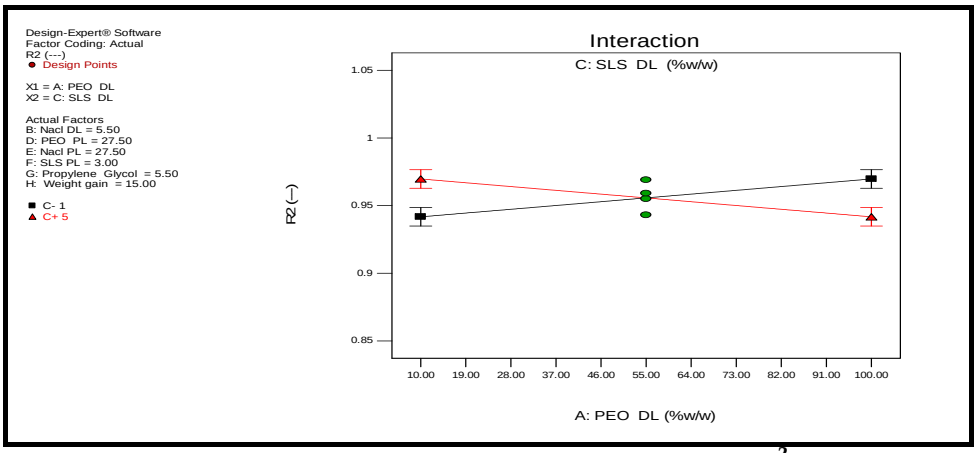


Figure No.6.3.146: AC interaction Plot on R^2

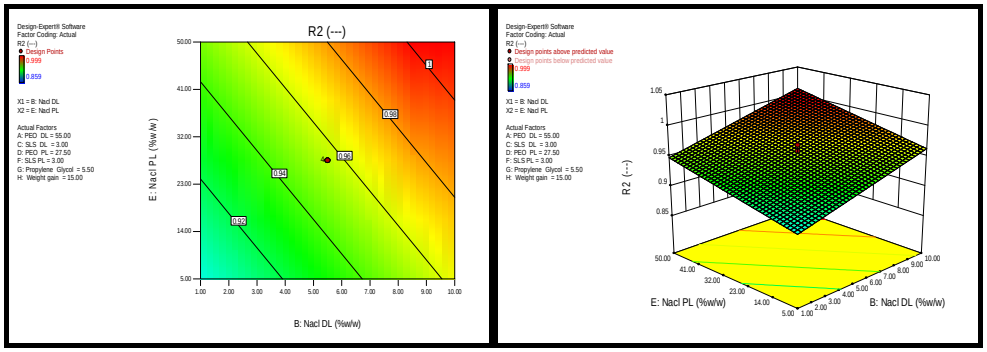


Figure No.6.3.147: Contour plot and RS Plot – Effect of NaCl DL and NaCl PL on R^2

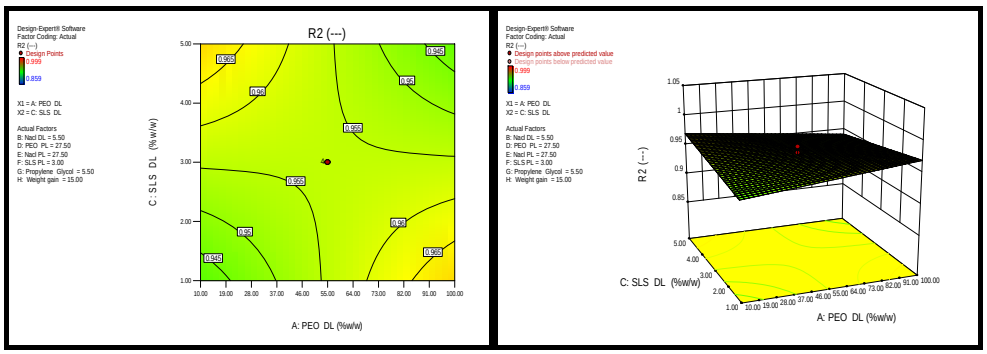


Figure No.6.3.148: Contour plot and RS Plot – Effect of PEO DL and SLS DL on R^2

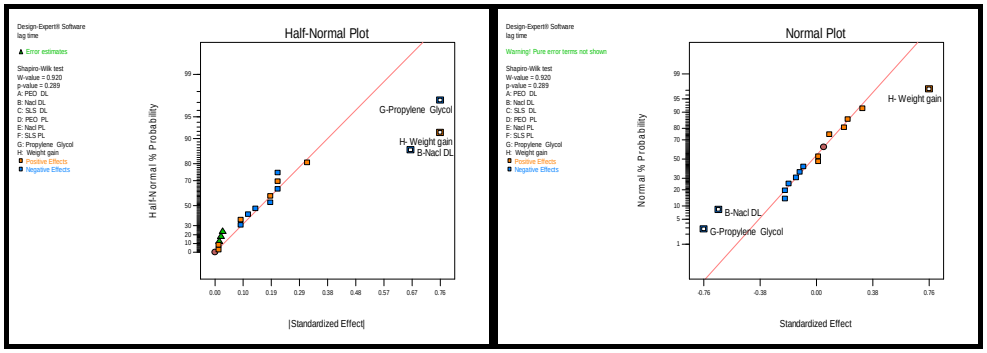


Figure No.6.3.149: Half Normal plot of the effect of the factors selected on lag time.

Figure No.6.3.150: Normal plot of the effect of the factors selected on lag time

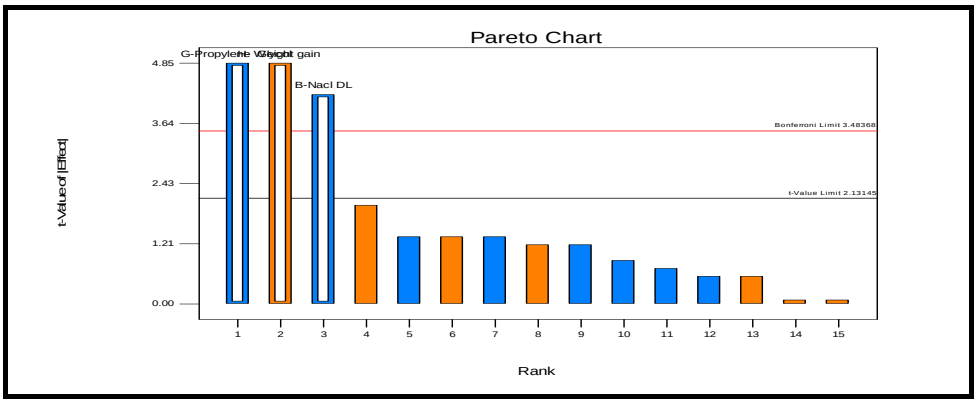


Figure No.6.3.151: Pareto chart of the effect of the factors selected on lag time

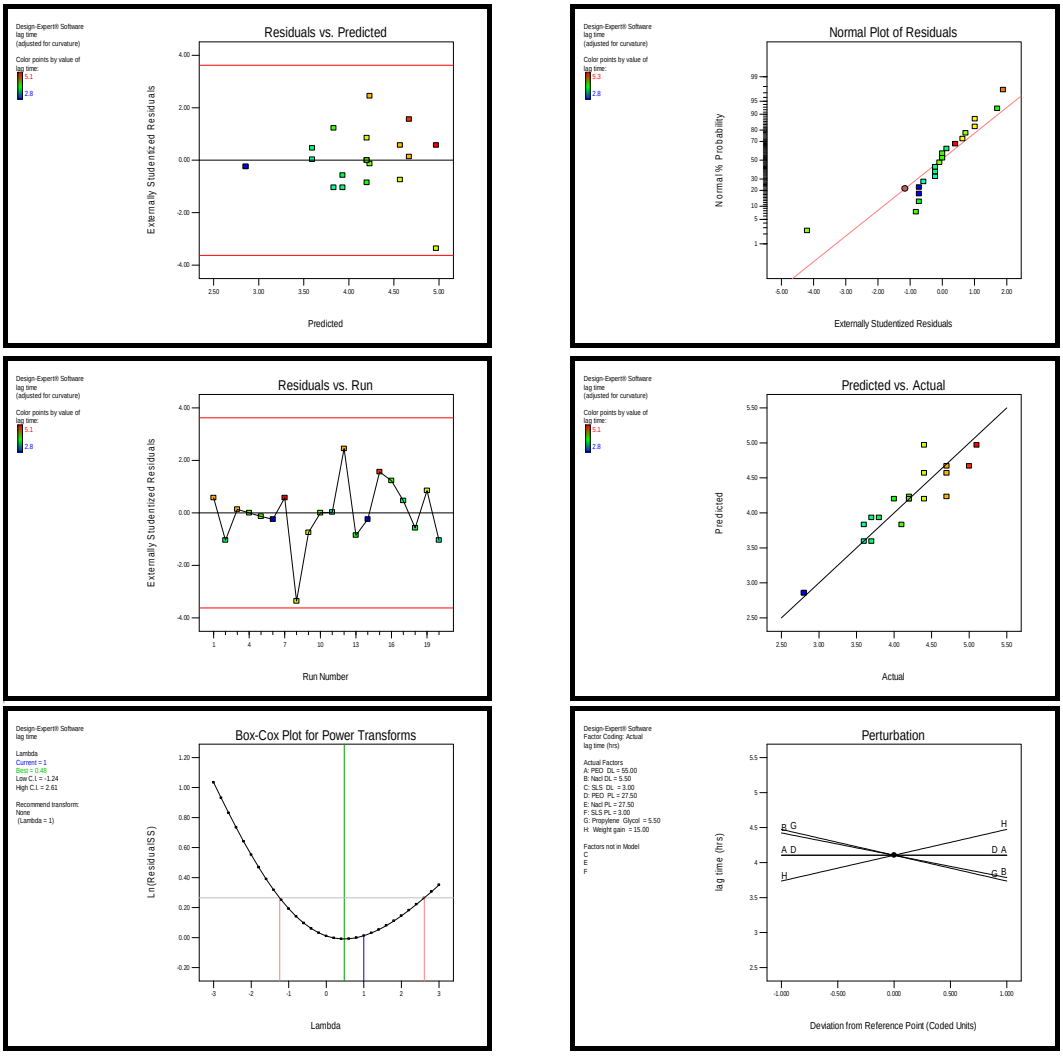


Figure No .6.3.152: Plots for Testing the assumptions of the ANOVA and pertubation Curve

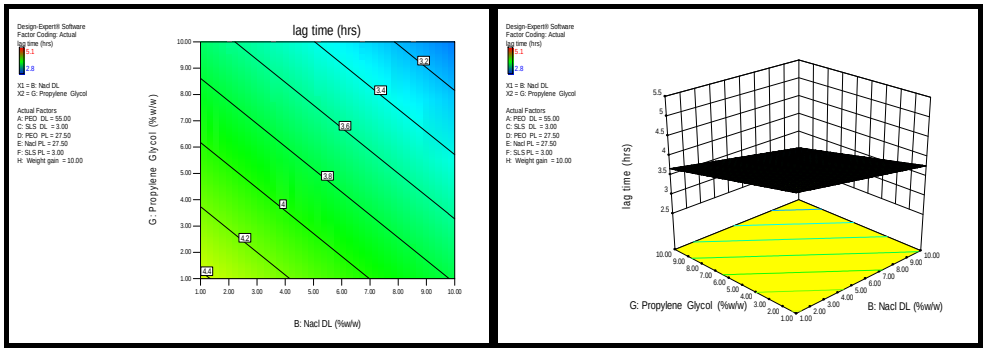


Figure No.6.3.153: Contour plot and RS Plot – Effect of NaCl DL and Propylene Glycol on lag time

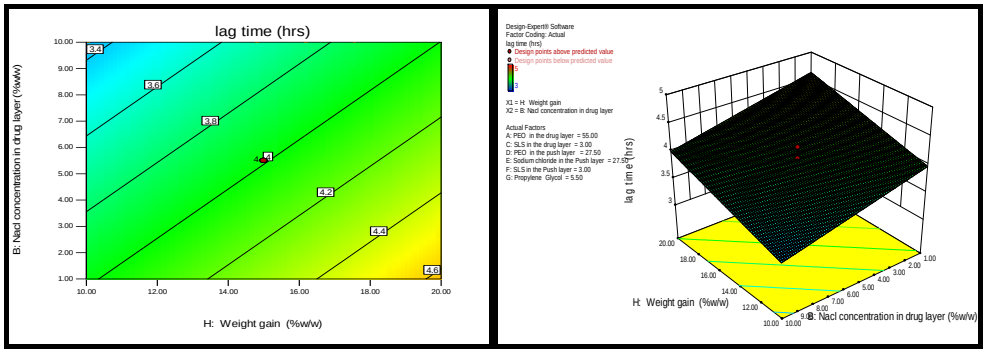


Figure No.6.3.154: Contour plot and RS Plot – Effect of NaCl DL and Weight gain on lag time

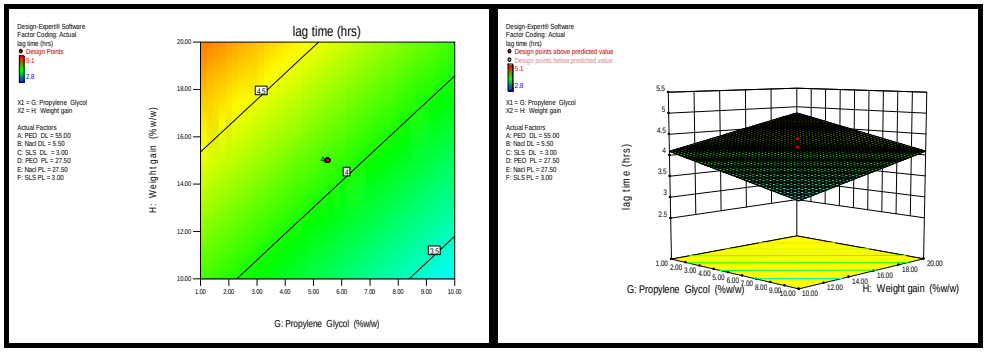


Figure No.6.3.155: Contour plot and RS Plot – Effect of Weight gain and Propylene Glycol on lag time

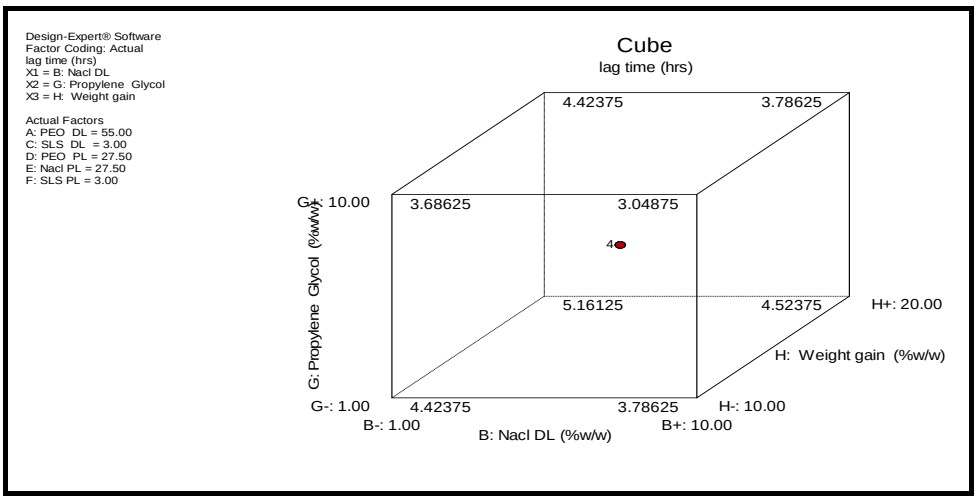


Figure No.6.3.156: Cube plot of effect of NaCl DL, Propylene Glycol and Weight gain on lag time

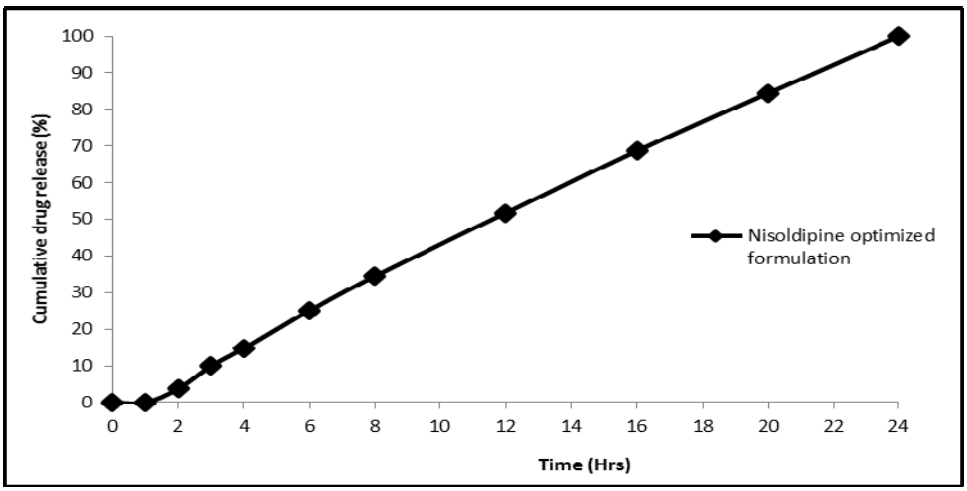


Figure No.6.3.157: In vitro dissolution study of the optimized batch of Nisoldipine push pull OT

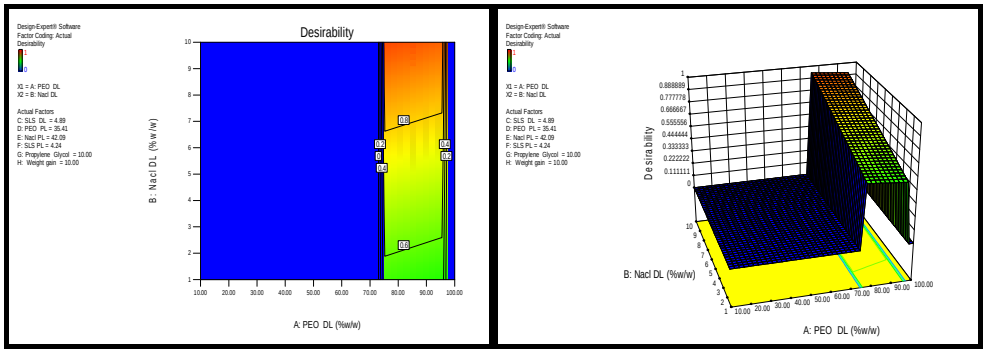


Figure No.6.3.158: Desirability contour plot and RS plot - Effect of PEO DL and NaCl DL

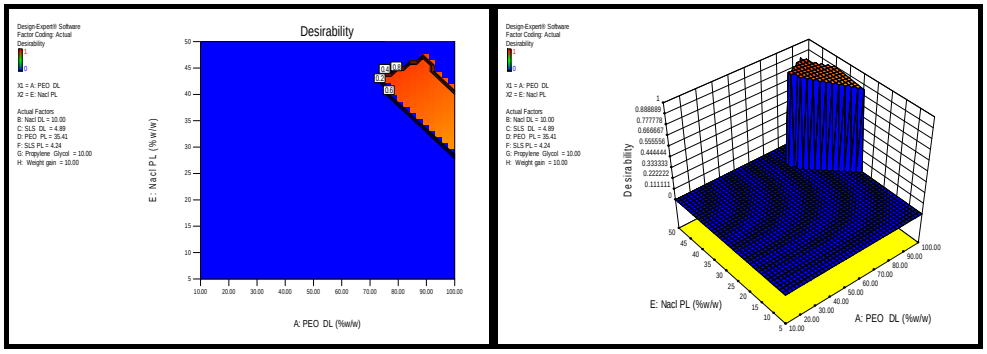


Figure No.6.3.159: Desirability contour plot & RS plot - Effect of PEO DL and NaCl PL

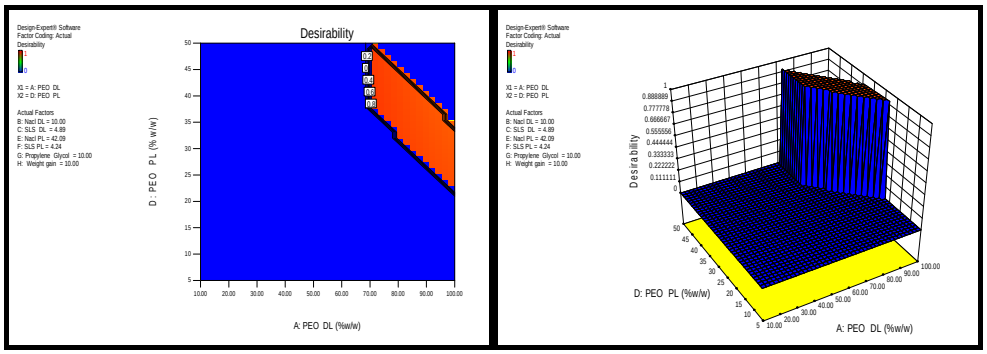


Figure No.6.3.160: Desirability contour plot and RS plot – Effect of PEO DL and PEO PL

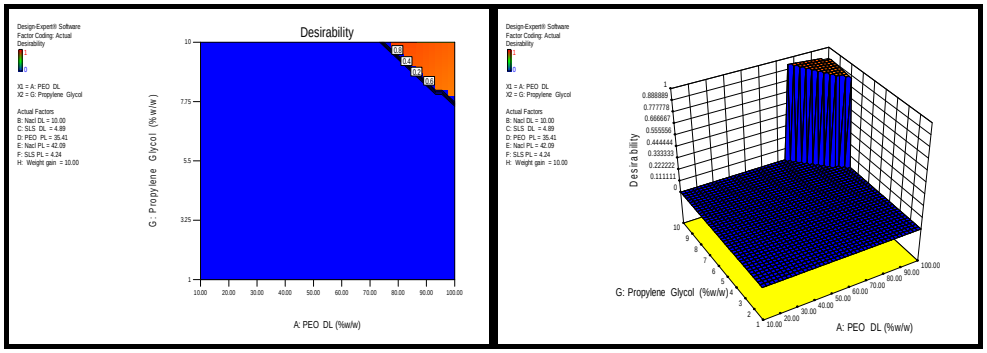


Figure No.6.3.161: Desirability contour plot and RS plot – Effect of PEO DL and Propylene Glycol

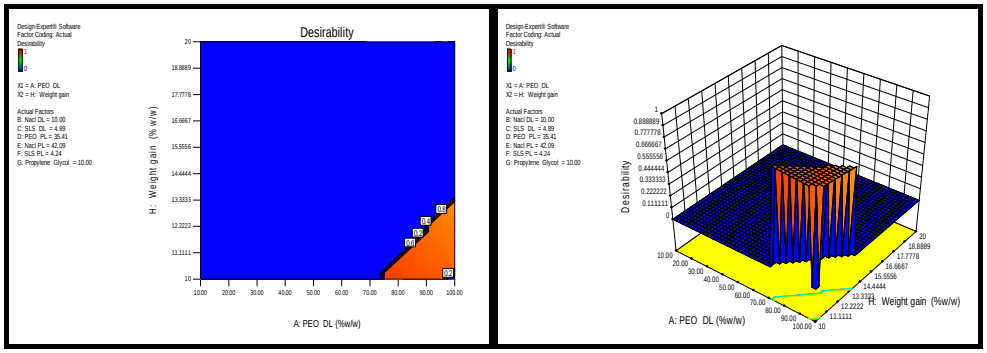


Figure No .6.3.162: Desirability contour plot and RS plot – Effect of PEO DL and Weight gain

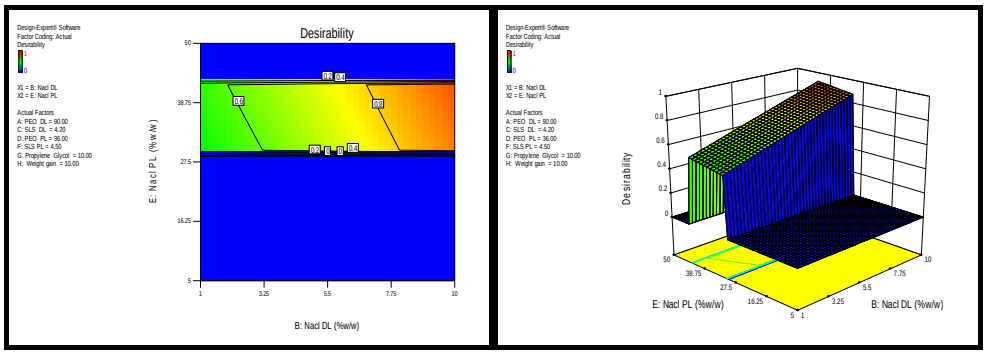


Figure No.6.3.163: Desirability contour plot and RS plot – Effect of NaCl DL and NaCl PL

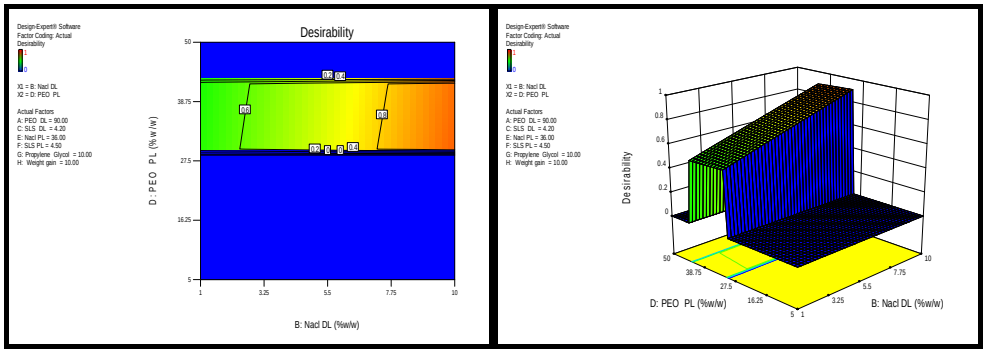


Figure No.6.3.164: Desirability contour plot and RS plot – Effect of NaCl DL and PEO PL

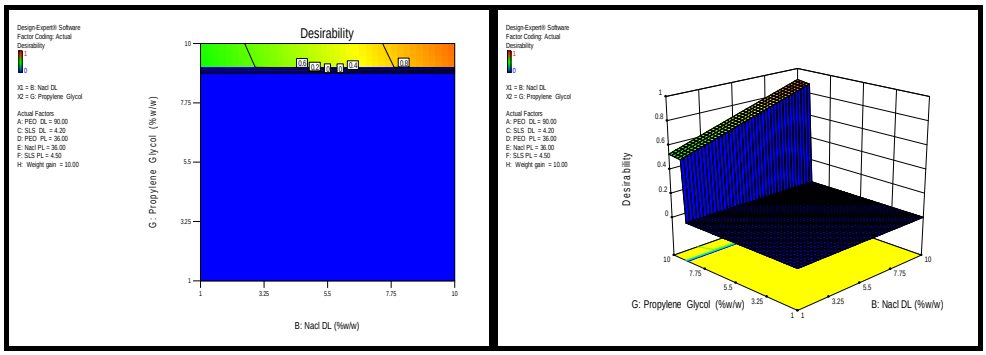


Figure No.6.3.165: Desirability contour plot and RS plot – Effect of NaCl DL and Propylene Glycol

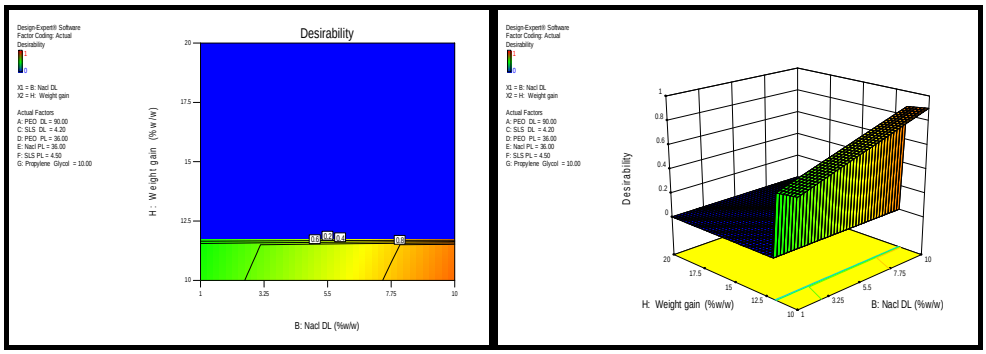


Figure No.6.3.166: Desirability contour plot and RS plot – Effect of NaCl DL and weight gain

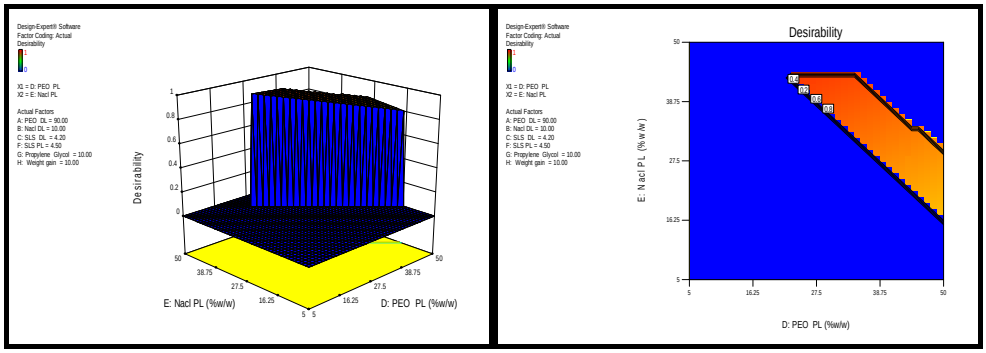


Figure No.6.3.167: Desirability contour plot and RS plot – Effect of PEO PL and NaCl PL

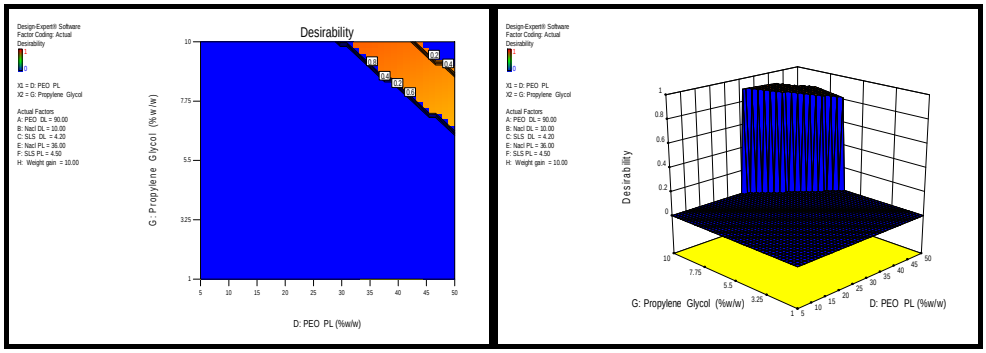


Figure No.6.3.168: Desirability contour plot and RS plot – Effect of PEO PL and Propylene Glycol

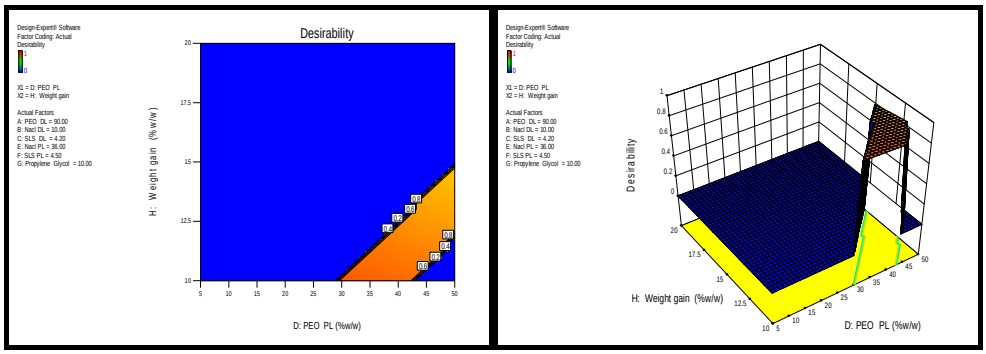


Figure No.6.3.169: Desirability contour plot and RS plot – Effect of PEO PL and weight gain

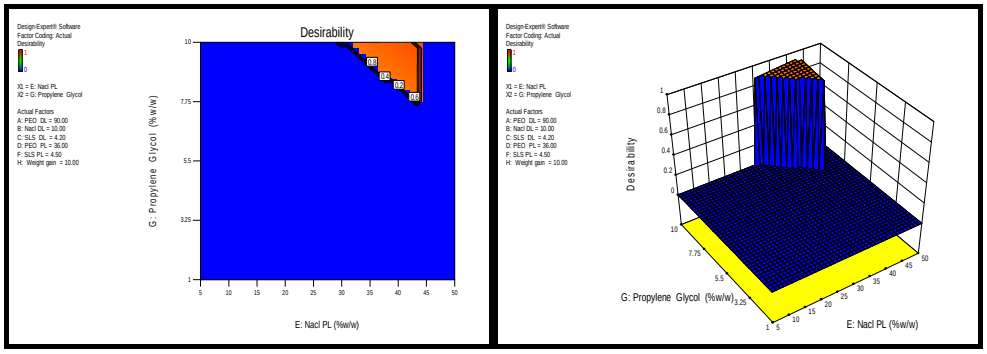


Figure No.6.3.170: Desirability contour plot and RS plot – Effect of NaCl PL and Propylene Glycol

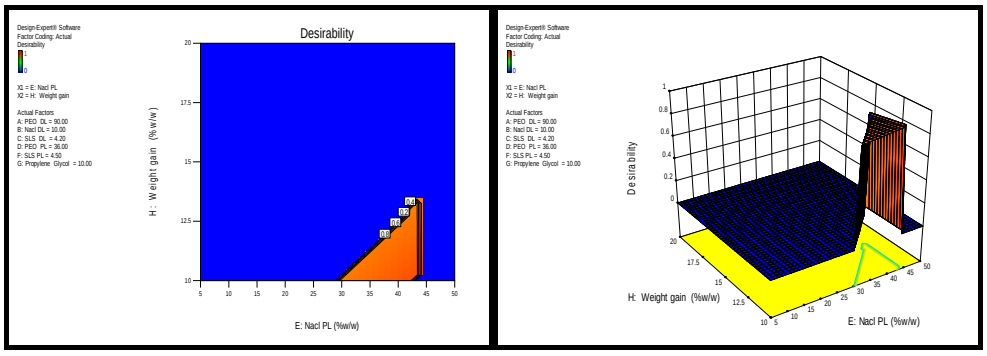


Figure No.6.3.171: Desirability contour plot and RS plot – Effect of NaCl PL and weight gain

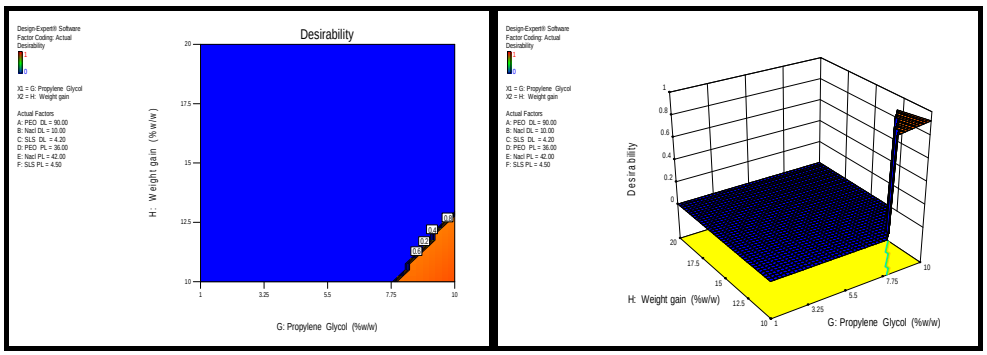


Figure No.6.3.172: Desirability contour plot and RS plot – Effect of weight gain and Propylene Glycol

6.3.7. Stability study of the optimized batches

I. Stability study of the optimized push pull osmotic tablets of Ropinirole HCl

Stability study was carried out on the optimized batches of the formulations of Ropinirole HCl, as per described in section 5.6.5 and the results were shown in the Table No: 6.3.68.

II. Stability study of the optimized push pull osmotic tablets of Ivabdadine HCl

Stability study was carried out on the optimized batch as per described in section 5.6.5 and the results are shown in the table No 6.3.69.

III. Stability study of the optimized push pull osmotic tablets of Carvedilol phosphate

Stability study was carried out on the optimized batch as per described in section 5.6.5 and the results are shown in the Table No.6.6.70.

IV. Stability study of the optimized push pull osmotic tablets of Nisoldipine

Stability study was carried out on the optimized batch as per described in section5.6.5 and the results are shown in the Table No.6.3.71.

Table No.6.3.68: Stability study of the optimized batch - Ropinirole HCl

Condition	Initial	40°C / 75%RH			30°C / 65%RH		25°C / 60%RH	
		1 M	2M	3M	3 M	6 M	3 M	6 M
Physical Change (color)	-	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Assay (%)	99.98±0.65	100.6±0.5	100.5±0.7	100.1±0.4	99.5±1.2	100.2±1.2	99.2±0.67	98.2±0.62
Weight variation(mg)	104.5±0.1	104.5± 0.2	104.5 ±0.1	104.5 ±0.2	103.8±2.3	104.1±1.8	103.2± 2.6	104±1.8
Hardness(kg/cm^2)	4.5±0.4	3.8±0.8	3.6±1.2	3.5±0.9	4.5±0.5	4.2±0.6	3.8±1.1	5±.0.1
Dissolution Profile of the optimized batch								
Time (in hours)	**Cumulative drug release (%)**							
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	5.2	4.8	5	4.8	5.4	4.7	0	0
3	10.1	11	10	11	10.5	10	10.2	11
4	15.4	16.3	16	18	17.5	15.1	15.1	15.5
6	25.5	25.5	24	26	25.3	24.1	25.3	25.3
8	34.5	33.5	34	35.1	35.2	34.7	32.1	34.2
12	52.1	53	50	49	53.4	52.1	53.1	50.4
16	69.2	69.5	68	68.5	68.6	67.5	68.2	68.1
20	85.5	88	88	89	85.3	85.3	84	85.3
24	100	101	99	99.3	98.3	100.7	98.5	100.2
R^2	**0.9988**	**0.997**	**0.999**	**0.9978**	**0.998**	**0.9979**	**0.9985**	**0.9988**
Lag time	**2.99**	**2.85**	**3.0**	**2.89**	**3.05**	**3.00**	**2.9**	**2.87**

Table No. 6.3 69: Stability study of the optimized batch - Ivabradine HCl

Condition	Initial	40°C / 75%RH			30°C / 65%RH		25°C / 60%RH	
		1 M	2M	3M	3 M	6 M	3 M	6 M
Physical Change (colour)	-	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Assay (%)	98.78±2.3	99.1±2.3	101±2.5	99.2±1.7	98.7±1.8	99.6±2.5	98.6±2.4	100.4±2.8
Weight variation(mg)	104.5±0.14	103.7±1.7	105.1±2.1	103.6±1.3	104.8±1.8	104.9±0.8	103.6±1.4	105.2±2.2
Hardness(kg/cm^2)	4.3±0.8	5.1±0.1	3.7±1.2	3.5±1.5	4.1±1.7	4.2±1.3	4.7±0.3	4.5±0.5
Dissolution Profile of the optimized batch								
Time (in hours)	**Cumulative drug release (%)**							
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	4.8	5.5	5.1	4.5	4.7	5.2	4.5	5
3	9.8	9.6	10.3	11	10.8	10.3	10	11
4	14.7	15.7	16	13.5	13.7	15	14.5	15.5
6	22.9	23.5	21.5	23.9	21.3	22.5	21.2	21.4
8	31.9	30.4	32.8	32.1	30.5	31.7	30.2	32.3
12	46.9	45.5	47.6	45.3	46.4	45.8	46.2	45.9
16	65.7	66.4	64.3	65.7	66.1	66.9	65.1	66.2
20	85.4	86	85.2	84.2	86.5	84.5	83.9	86.1
24	100.02	99.8	98.9	101.4	99.7	101.3	99.7	98.9
R^2	**0.9985**	**0.9979**	**0.9978**	**0.999**	**0.998**	**0.9979**	**0.9988**	**0.9975**
Lag time	**3.03**	**3.15**	**2.9**	**2.87**	**2.8**	**3.03**	**3.00**	**2.87**

Table No.6.3.70: Stability study of the optimized batch- Carvedilol phosphate

Condition	Initial	40°C / 75%RH			30°C / 65%RH		25°C / 60%RH	
		1 M	2M	3M	3 M	6 M	3 M	6 M
Physical Change (color)	-	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Assay (%)	101.3±27	102.1±1.9	99.3±2.7	101.2±2.3	98.6±1.3	99.2±0.8	100.3±1.1	99.6±2.4
Weight variation(mg)	103.5±24	104.7±1.4	103.7±1.6	103.2±2.1	104.5±0.6	103.8±0.5	104.7±1.1	103.8±1.1
Hardness(kg/cm^2)	4.5±0.4	3.8±0.8	3.5±0.7	4.5±0.4	3.7±1.1	3.4±0.4	5±0.2	4.6±0.3
Dissolution Profile of the optimized batch								
Time (in hours)	**Cumulative drug release (%)**							
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	4.5	3.8	4.2	5	5.2	4.1	4	3.6
3	9.9	10	11	9.8	9.5	10	10.5	11
4	15.3	15.1	16	16.7	14.7	14.3	15	15.8
6	23.3	24.2	23.8	22.7	22.1	24.7	25.1	23.7
8	32.3	32.3	30.7	31.6	30.5	32.9	33.4	32.4
12	47.6	48.1	48	47.5	47.1	48.9	47	48.2
16	63.4	65	64.8	65,2	62.1	62.9	63.7	65.1
20	79.3	78.1	79.6	77.5	78.7	79.2	79.8	77.4
24	99.08	100.4	110.8	101.5	98.3	99.6	99.1	100.3
R^2	**0.998**	**0.9975**	**0.987**	**0.997**	**0.999**	**0.999**	**0.999**	**0.998**
Lag time	**3.03**	**3.00**	**2.85**	**3.05**	**3.12**	**3.00**	**2.9**	**2.85**

Table No.6.3.71: Stability study of the optimized batch - Nisoldipine

Condition	Initial	40°C / 75%RH			30°C / 65%RH		25°C / 60%RH	
		1 M	2M	3M	3 M	6 M	3 M	6 M
Physical Change (color)	-	No Change	No Change	No Change	No Change	No Change	No Change	No Change
Assay (%)	98.7±2.1	98.2±3.2	100.3±1.6	98.7±2.1	100.2±3.6	99.2±1.7	98.7±2.7	101±2.5
Weight variation(mg)	105±0.07	103.8±2.1	103.8±0.6	105.2±1.3	104.8±0.3	103.9±1.2	104.2±2.1	103±0.7
Hardness(kg/cm^2)	4.6±0.5	4.8±0.5	4.5±0.7	3.9±0.7	4.7±0.1	4.4±0.7	4.7±0.5	4.7±0.8
Dissolution Profile of the optimized batch								
Time (in hours)	**Cumulative drug release (%)**							
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	3.5	3.5	4	4.5	3.8	3	4	4.2
3	9.9	9.2	10	10.1	11	9.5	9.5	10
4	14.8	14	15.2	15	14.1	13.8	15.5	14.3
6	25.1	24.8	25.7	23.9	25.1	24.4	23.2	24.8
8	34.5	35.9	36.2	34.3	35.1	34.7	33.5	36.1
12	51.7	50.3	52.5	51.7	52.7	50.4	51.8	52.8
16	68.7	69.3	67.6	68.1	67.5	66.5	69.7	68.1
20	84.5	85.9	83.1	84.8	83.5	86.1	85.2	84.8
24	100	99.2	98.4	100.1	102.3	98.4	99.3	99.8
R^2	**0.998**	**0.995**	**0.998**	**0.9975**	**0.997**	**0.9975**	**0.998**	**0.9988**
Lag time	**3.02**	**3.3**	**3**	**2.9**	**2.8**	**3.15**	**3.05**	**3**

6.4. *IN VIVO* ANIMAL STUDIES

6.4.1. Standard calibration Curve of the selected drugs in Rabbit plasma using RP- HPLC

Simple, accurate, precise and sensitive high-performance liquid chromatographic (HPLC) method was used for quantification of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in rabbit plasma samples. The study was performed as per the method suggested in the section 5.7.1 and 5.7.2. The HPLC conditions of the analysis were shown in the Table No. 5.7.1.

The calibration curve data of the drugs were shown in Table No 6.4.1 and 6.4.2.The calibration curve of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine was given in the Figure No.6.4.1, 6.4.2, 6.4.3, 6.4.4 respectively.

6.4.2. *In vivo* animal study and analysis of blood samples

The *invivo* animal study of the optimized push pull osmotic tablets of the selected drugs for the determination of pharmacokinetic parameters were performed according to the methods specified in the section 5.7.2(I-X).The result of the study was given in the Table No.6.4.3.

The data were analyzed Phoenix® WinNonlin® software. The pharmacokinetic parameters were determined for each formulation under study and reported in the Table No.6.4.4. The comparative plasma profiles of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine with their corresponding reference products were shown in Figure No.6.4.5, 6.4.6, 6.4.7 and 6.4.8 respectively.

Table No.6.4.1: Calibration curve data

Parameters	Ropinirole HCl	Ivabradine HCl	Carvedilol phosphate	Nisoldipine
Retention time Drugs(min)	8.5	8.6	8.2	5.6
Retention time IS (min)	10.1	12.1	6.5	7.9
Linearity range	20-100	50-200	50-500	20-120
Correlation Coefficient	0.999	0.999	0.999	0.999
Number of data points	5	4	6	6
Slope	0.006	0.001	0.001	0.004
Intercept	0.025	0.079	0.012	0.042
LOD(ng/ml)	5	0.25	5	2.5
LOQ(ng/ml)	10	0.5	10	5

TableNo.6.4.2: Standard calibration curve of the selected drugs in rabbit plasma by RP-HPLC

Drugs	Concentrations (ng/ml)	Peak Area		Ratio(S/IS)
		Drug	IS	
Ropinirole HCl (IS - 4-(2-di-N,N-propylaminoethyl)7-methoxy-2-(3H)-indoline HCl)	20	10686	68950	0.15
	40	21878	75826	0.29
	60	28656	65987	0.43
	80	39987	71565	0.56
	100	52642	76984	0.68
Ivabradine HCl (IS -S 1670)	50	14820	95687	0.15
	100	22890	102500	0.22
	150	32870	90890	0.36
	200	56856	110250	0.52
Carvedilol Phosphate (IS – Amitriptyline)	50	5466	66250	0.08
	100	9846	65870	0.15
	200	17960	64270	0.28
	300	25640	60850	0.42
	400	31540	56800	0.56
	500	47560	68540	0.69
Nisoldipine (IS – Diazepam)	20	5640	39600	0.14
	40	10260	45222	0.23
	60	13987	42656	0.33
	80	17860	41989	0.43
	100	20252	39265	0.52
	120	28954	46878	0.62

Table No.6.4.3: Plasma concentrations of the test and references products obtained from in vivo animal study

Time (hours)	Plasma concentrations (ng/ml)															
	Ropinirole HCl				Ivabradine HCl				Carvedilol phosphate				Nisoldipine			
	Push-Pull OTs	% CV	Marketed XR tablets	% CV	Push-Pull OTs	% CV	Marketed XR tablets	% CV	Push-Pull OTs	% CV	Marketed XR tablets	% CV	Push-Pull OTs	% CV	Marketed XR tablets	% CV
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	22.35	78.98	0	0	163.16	34.87	0	0	81.69	46.35	0	0	25.76	51.89
2	0	0	35.26	82.95	0	0	137.46	41.55	0	0	145.66	48.95	0	0	46.41	65.78
3	17.58	56.98	42.34	67.84	53.82	42.89	100.19	46.56	49.86	48.63	194.9	42.64	14.89	45.87	62.74	62.66
4	29.34	62.58	45.79	56.77	83.42	38.67	71.26	38.91	94.46	51.65	231.92	41.69	28.32	48.99	75.43	57.68
5	37.42	59.86	46.92	42.68	98.36	36.78	50.45	36.45	134.29	46.32	258.85	39.61	40.43	52.55	85.04	42.66
6	43.1	48.56	46.59	59.44	104.7	35.12	35.68	41.25	169.83	41.56	277.49	52.65	59.4	40.58	92.08	40.99
7	47.17	42.97	45.31	48.79	81.27	30.12	25.23	39.88	201.48	47.68	289.35	47.23	71.83	39.88	96.98	38.42
8	50.12	38.98	43.44	57.46	62.58	45.51	17.84	21.56	229.63	39.55	295.7	39.68	83.42	42.35	100.09	39.12
9	52.25	44.68	41.21	62.4	47.87	40.56	12.62	30.25	254.63	46.87	297.62	37.63	94.23	36.88	101.73	30.66
10	53.8	31.54	38.79	65.31	40.89	47.86	8.92	36.55	276.78	36.42	295.99	42.96	104.3	39.78	102.16	35.66
12	38.18	39.87	33.75	60.23	25.56	38.97	4.46	32.54	313.66	25.87	284.97	52.93	110.45	32.45	100.26	45.89
16	29.51	42.11	24.39	68.77	8.95	41.88	1.12	45.110	295.87	32.61	245.97	48.56	102.93	30.89	89.83	42.35
24	15.19	50.69	11.56	54.56	0.88	51.22	0	0	188.96	41.32	158.01	42.63	74.27	36.87	61.97	38.79
36	0	0	0	0	0	0	0	0	83.51	37.98	69.79	39.54	36.18	38.77	30.23	52.41
48	0	0	0	0	0	0	0	0	0	0	0	0	16.94	41.22	14	50.36

Table No.6.4.4: Pharmacokinetic parameters of the test and references products obtained from in vivo animal study

Formulation		t_{lag} (hrs)	t_{max} (hrs)	% CV	C_{max} (ng/ml)	% CV	AUC $_{last}$ (ng.hr/ml)	%CV	AUC $_{INF\_obs}$ (ng.hr/ml)	%CV
Ropinirole HCl	Push-Pull OTs	2	10	30.33	53.8	31.54	867.18	28.96	881.37	30.21
	Marketed XR tablets	0	5	42.68	46.92	42.68	837.38	34.56	846.76	30.98
Ivabradine HCl	Push-Pull OT	2	6	25.89	104.7	35.12	732.79	25.14	732.8	24.55
	Marketed IR tablets	0	1	20.26	163.16	34.87	648.06	28.68	648.07	26.47
Carvedilol phosphate	Push-Pull OTs	2	12	39.56	313.66	25.87	7367.17	39.57	7864.66	41.25
	Marketed XR tablets	0	9	36.87	297.62	37.63	7438.34	35.46	7844.38	39.65
Nisoldipine	Push-Pull OTs	2	12	31.88	110.45	32.45	2776.42	34.56	3051.56	36.12
	Marketed XR tablets	0	10	28.99	102.16	35.66	2745.75	35.87	2971.56	34.68

Table No.6.4.5: Result of the Student's t test at 5% significant level

Formulation		P value				
		t_{lag} (hrs)	**t_{max} (hrs)**	**C_{max} (ng/ml)**	**AUC_{last} (ng.hr/ml)**	**AUC_{INF_obs} (ng.hr/ml)**
Ropinirole HCl	Push-Pull OTs	0.015	0.008	0.13	0.158	0.223
	Marketed XR tablets					
Ivabradine HCl	Push-Pull OT	0.019	0.002	0.00995	0.073	0.08
	Marketed IR tablets					
Carvedilol phosphate	Push-Pull OTs	0.019	0.005	0.163	0.16	0.144
	Marketed XR tablets					
Nisoldipine	Push-Pull OTs	0.016	0.011	0.284	0.187	0.123
	Marketed XR tablets					

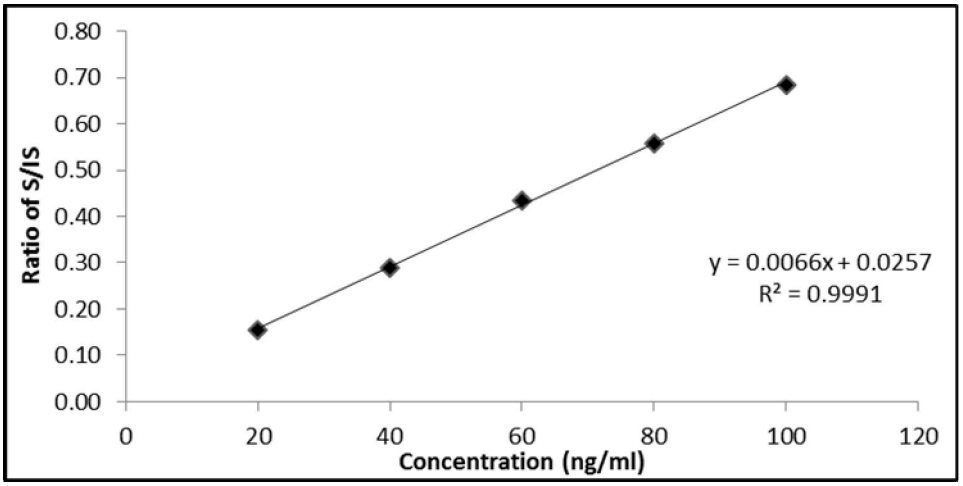


Figure No.6.4.1: Calibration curve of Ropinirole HCl

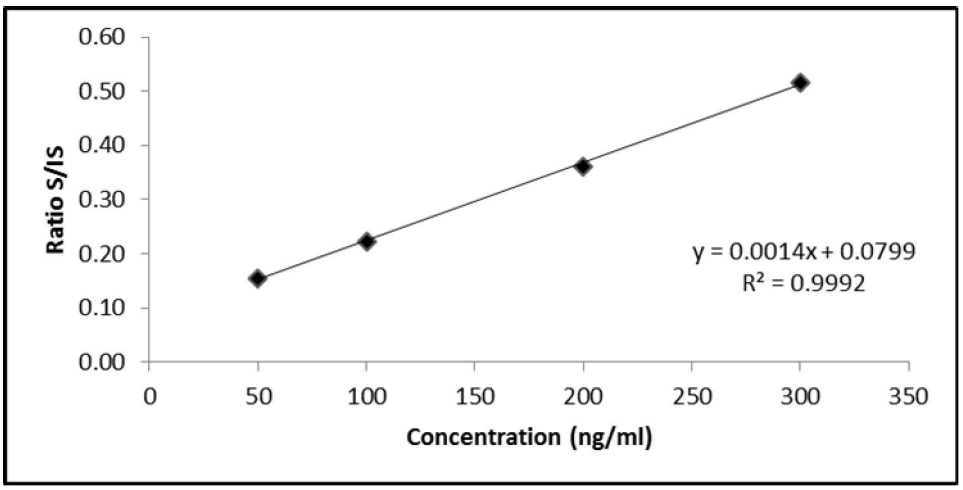


Figure No.6.4.2: Calibration curve of Ivabradine HCl

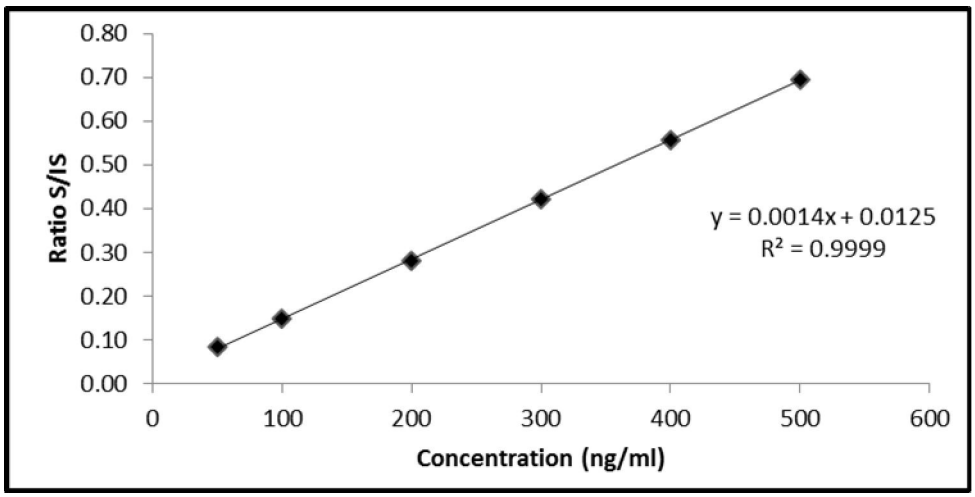


Figure No.6.4.3: Calibration curve of Carvedilol phosphate

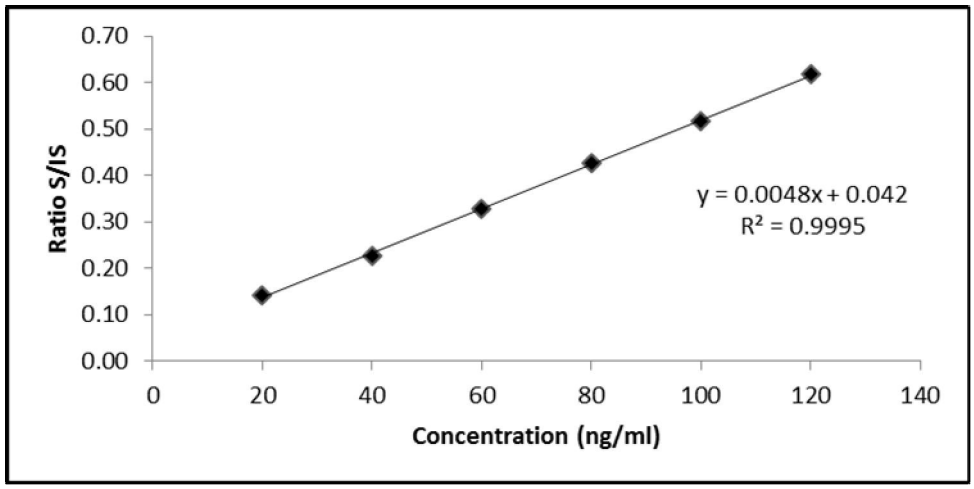


Figure No.6.4.4: Calibration curve of Nisoldipine

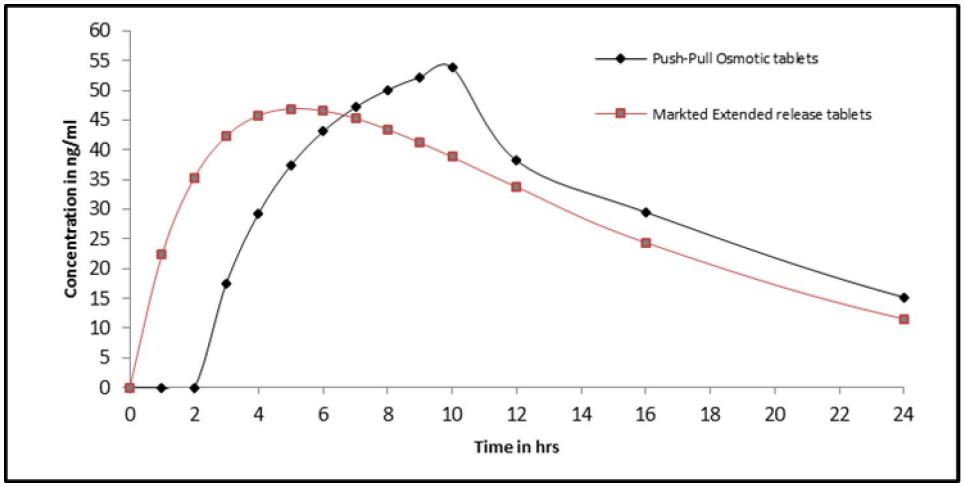
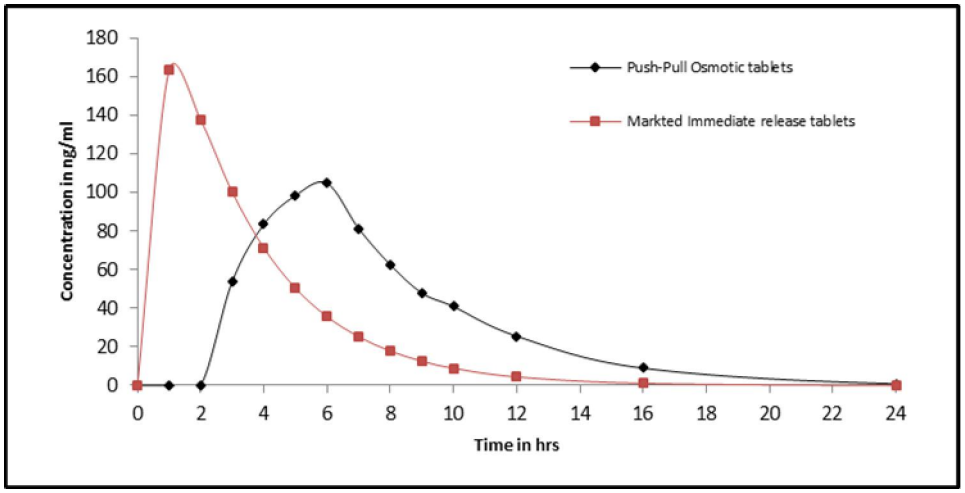


Figure No 6.4.5: Comparative plasma profiles of Ropinirole HCl



FigureNo.6.4.6: Comparative plasma profiles of Ivabradine HCl

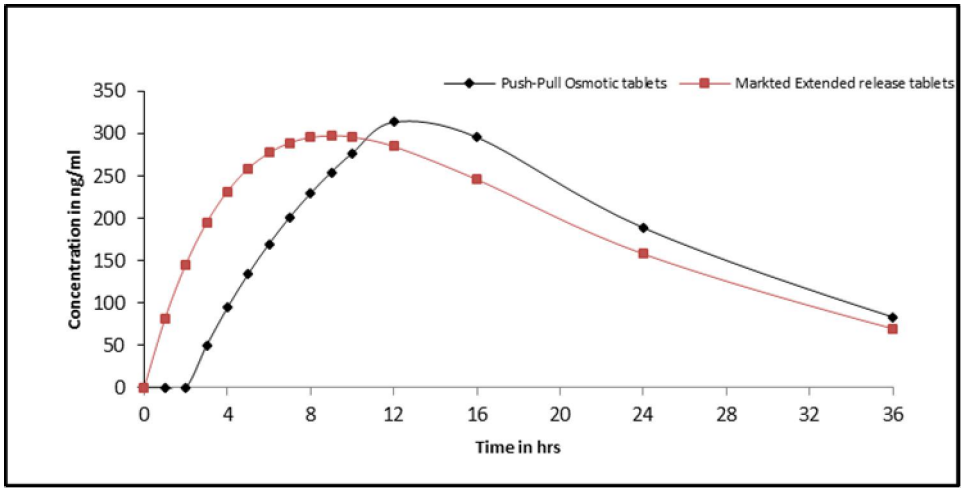


Figure No.6.4.7: Comparative plasma profiles of Carvedilol phosphate

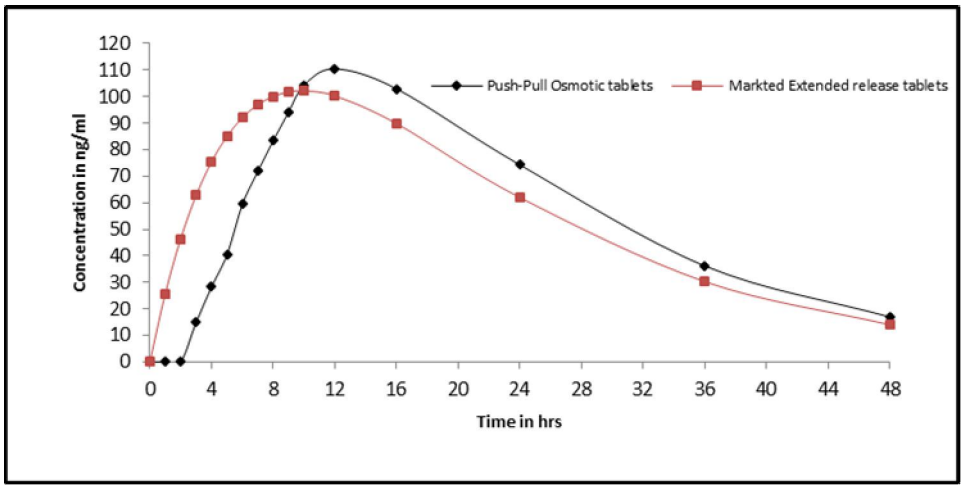


Figure No.6.4.8: Comparative plasma profiles of Nisoldipine

Chapter -7
DISCUSSION

7. DISCUSSION

7.1. ANALYTICAL DEVELOPMENT

$\lambda_{max}$of the selected drugs were identified by scanning the 20ppm solution of the corresponding drugsin the UV range 200- 400 nm using UV spectrophotometer.

The λ_{max} of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in pH 6.8 phosphate buffer solution was 250, 286, 285.5 and 235.5nm respectively. Thecalibration curves of the drugs were plotted at the corresponding $\lambda_{max}$and studied. The calibration curves were plotted for all the four selected drugs**.** The linearity, R^2 and the regression equations were recorded and studied.All the graphs showed a greater linearitywith R^2 value ranging from 0.999 – 0.9998. The regression equations were used for further calculations.

7.2. PRE FORMULATION STUDY

The pre formulation studies like organoleptic properties, solubility, flow property, particle size determination and drug – excipient interaction study were performed on the selected drugs. All the API available was found to be odorless. The color of the API ranges from white to yellow powder. Ropinirole HCl and Ivabradine HCl were available as amorphous powder, butCarvedilol phosphate and Nisoldipine were available as crystalline powder. Solubility study of the drugs on various media like water, 0.1 HCl, pH 4.5 acetate buffer, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer was performed for all the four drugs. Solubility of Ropinirole HCl was found to be 130.58 mg/ml in water. Not much variation was found in the solubility of Ropinirole HCl in other media. The solubility of Ivabradine HCl was found to be 52.6 mg/ml in water.In 0.1 N HCl, solubility of Ivabradine HCl was little lower compared to the solubility in other media. Carvedilol phosphate andNisoldipine were found to be insoluble in water.

Particle size of the API of the selected drugs was determined using Malvern zeta sizer. 90 % of the drug particles of all the four drugs were having particle size less than 75µm.

The density and the flow property of the selected drugs were determined. The flow property of the Ivabradine HCl was good. But other drugs were found to have poor flow property.

Drug interaction with excipients was done with the help of differential scanning calorimetry (DSC). The peaks appeared in the pure drug DSC plot and drug with tablet as well as coating excipients were almost same and were within the specified range. No addition or deletion of any peaks was observed in the spectra. So the excipients used in the study were compatible with the drugs.

7.3. FORMULATION DEVELOPMENT OF PUSH PULL OSMOTIC TABLETS OF HIGHLY AND POORLY SOLUBLE DRUGS

7.3.1. Dose calculation

For Ropinirole HCl, Carvedilol phosphate and Nisoldipine the amount to be incorporated in to the push pull osmotic tablets was selected according to the available marketed XR Product strengths. But for Ivabradine HCl it was determined with the help of Robinson- Eriksen equation**.**

7.3.2. Screening study

An extensive literature survey was done to identify the vital factors affecting the release profile of the drugs from the push pull osmotic tablets. The vital factors selected for the study were solubilizing agent in the DL, suspending agent in the DL, osmotic agent in the DL, Extender in the PL, solubilizing agent PL, weight gain, plasticizer in the coating.

7.3.3. Product development and optimization of push –pull osmotic tablets of highly soluble drugs

Push pull osmotic tablets of highly water soluble drugs Ropinirole HCl and Ivabradine HCl were designed and optimized with the help of design of experiments.

The design expert software was used for this purpose.A fractional factorial design (**2** $^{8-4}$ with **Resolution IV**) with 4 centre points was selected for the factor influence study after identifying the vital factors.

As the intention of the work was to identify and characterize the various formulation factors affecting the release pattern of the selected drugs from the OTs, the major responses selected for the study were cumulative percentage release at 24 hrs (target was >95%) , zero order rate of release (R^2 = 1) and lag time (3hrs).

The model suitability was checked with the help of FDS plot.The distribution of standard error was checked before starting the trials and confirmed the equal distribution of prediction error throughout the design space.

Trials R1 to R20 (Ropinirole HCl) and IB1- IB20 (Ivabradine HCl) were designed and formulated. Blend evaluation like tapped density, bulk density, angle of repose, carr's index, hausner's ratio of drug layer and push layer were performed for the formulations of both the drugs. Good flow property was observed for granules of both the layers. Whole tablet evaluations like weight variation, assay, friability, were also performed for all the 20 trials of each drug. None of the results deviates from the limits specified in the pharmacopoeias.

Diameter and thickness of the tablets of OT of highly soluble drugs were within the range of 5 - 5.2 mm and 3.4 - 3.6mm respectively. Pore size of all the trials was found to be 0.60 mm. Weight gain deviated not more than 2.5-3% from the actual weight gain expected.

The film evaluation was done to find out the plasticizer effect on the elasticity of the film. All the films formulated were smooth and opaque with folding endurance ranges from 231-478. The study showed that, as the plasticizer concentration increases the folding endurance increases. This means that increase in concentration of plasticizer increases the elasticity of the film.

In vitro dissolution was carried out and three responses were selected mainly PCUR at 24 hrs, R^2 and lag time value for the factor influence study and optimization of push pull OT formulations of both the drugs. All these values were analyzed with the help of design expert software and the result of the study was interpreted from ANOVA analysis and other statistical tests. The level of significance selected was 5% ($p<0.05$).

For all the selected responses the ANOVA assumptions were tested and studied with the help of various plots like normal plot of residuals, Residual Vs predicted, residual Vs run, actual Vs predicted. The effect of the factors on the responses was identified from the graphs such as half normal plot, normal plot, pareto chart and ANOVA analysis.

For highly water soluble drugs, the significant factors affecting the PCUR at 24 hrs were identified as propylene glycol (G), weight gain (H) and NaCl DL respectively. The factors affecting the zero order rate constant were NaCl DL (B) and NaCl PL (E) and for lag time it was propylene glycol (G), weight gain (H) and NaCl DL (B).

For both the drugs studied, the model was found to be significant and no lack of fit and interactions were reported for any of the responses. After eliminating the non significant terms, the linear polynomial equation representing responses were constructed and studied for both the drugs.Thesimultaneous effect of two significant factors on the selected responses was studied with the help of contour plots and RS plots.

From the plots it was evident that the propylene glycol was a major factor affecting the responses.A 100 % release up to 24 hrs would be suggestive when the weight gain was at its minimum and the sodium chloride at its maximum level and the propylene glycol at its higher levels.

The major factors affecting R^2 were found to be B (NaCl DL)> E (NaCl

PL).As the concentration of NaCl DL increases the R^2 increases. A greater linearity in the release profile was observed when NaCl DL was kept more than 9 %. But R^2 decreases with the increase in the concentration of the NaCl PL. A lower concentration of NaCl PL and NaCl DL more than 9% was preferred for achieving better linearity. R^2 drastically changed away from unity by decreasing the NaCl DL and increasing the NaCl PL.

Propylene glycol and NaCl DL had a negative effect on the lag time. But weight gain had an opposite effect.The lowest lag time was observed when NaCl DL and propylene glycol were at maximum and weight gain at its minimum. Simultaneous effect of all the significant factors on each response was also studied with the help of cube plots.

The model exhibits linearity without any curvature and lack of fit for all the three responses. So theoptimization was done using the same design points by numerical optimization.The achievement of the optimum conditions was demonstrated by desirability function.

The desirability contour plots and RS plots were studied for the optimum combinations of factors. Higher desirability will be achieved at maximum level of NaCl DL (more than 9%), lower concentrations propylene glycol and low concentration (<14%) of weight gain.NaCl PL had comparatively lesser significance for achieving the optimum.A wide range of weight gain- propylene glycol combinations can be used for achieving the optimum combinations. High level of propylene glycol had a desirability zero.

Point prediction on the 3 selected solutions was done with the help of the software. Confidence intervals and tolerance intervals for the responses were tabulated.The same three solution batches for each drug were prepared and evaluated as check point batches for confirming the model validity. The predicted responses were compared with the experimental values. The experimental values were within the CI of the predicted responses.Thus the model validity of the2^{8-4}fractional factorial design for the optimization of the osmotically controlled oral tablets of Ropinirole HCl and Ivabradine HCl was proved.so this model can be

used for the further predictions.

Hence the design space provided by the software can be very well used for the formulation modifications and better optimization of the highly soluble drugs according to the need of the manufacturer.

The optimized batch from the numerical optimization solutions was selected by considering the better feasibility of the trials and desirability. The optimized batch for both the drugs was formulated and evaluated.The blend as well as the tablet evaluations was performed and the results were within the limits specified in the pharmacopoeias. *In vitro* dissolution was performed and PCUR at 24 hr, R^2 lag time were recorded. The values were within the confidence limits predicted by the software. The Push pull OT optimized formulation of both the drugs was found to be releasing the drug in a zero order rate up to 24 hrs with a lag time of 3 hrs.

A stability study on the optimized batches of both the drugs was performed as per the ICH guidelines. No significant changes in any of the parameters were observed after 6 months.

So it can be concluded that during the study, stable optimized push pull OTs of highly soluble drugs (Ropinirole HCl and Ivabradine HCl) was successfully formulated and extensively studied the significant factors affecting the release pattern of the drug from the system with the help of design of experiments.

7.3.4. Product development and optimization of push –pull osmotic tablets of poorly soluble drugs

Push pull osmotic tablets of poorly soluble drugs (Carvedilol phosphate and Nisoldipine) were designed and optimized with the help of design of experiments. A fractional factorial design (**2^{8-4}** with **Resolution IV**) with 4 centre points were selected for the factor influence study after identifying the vital factors.

The major responses selected for the factor influence study and optimization, were cumulative percentage release at 24 hrs (target was > 95%), zero order rate of release ($R^2 = 1$) and lag time (3hrs).

The model suitability was checked with the help of FDS plot.The distribution of standard error was checked before starting the trials and confirmed the equal distribution of prediction error throughout the design space.

Total 20 trials were planned and formulated for each drug (Carvedilol phosphate and Nisoldipine). Blend evaluation like tapped density, bulk density, angle of repose, carr's index, hausner's ratio of both drug layer and push layer of OTs of poorly soluble drugs were performed. The flow property of blend was found to be good for both the drugs selected. Whole tablet evaluations like weight variation, assay, friability, were also performed for all the 20 trials of each drug. None of the results deviated from the limits specified in the pharmacopoeias.

Diameter and thickness of the OTsof poorly soluble drugs were within the range of 5- 5.2 mm and 3.4 - 3.6 mm respectively. Pore size of all the trials was found to be 0.60 mm. Weight gain deviated not more than 2.5-3% from the actual weight gain expected.

The coating film evaluation was done to study the plasticizer effect on the elasticity of the film and found that an increase in concentration of plasticizer produced an increases the elasticity of the film.

In vitro dissolution was carried out and three responses selected were analyzed with the help of design expert software and the result of the study was interpreted from ANOVA analysis and other statistical tests. The level of significance selected was 5% ($p<0.05$).

For all the selected responses the ANOVA assumptions were tested and studied with the help of various plots. The effect of the factors on the responses were confirmed from the graphs such as half normal plot, normal plot, pareto chart and from ANOVA analysis.

From the analysis the significant factors affecting the cumulative drug release at 24 hrs from the push pull OTs of poorly soluble drugs were identified as PEO DL (A), Propylene Glycol (G), NaCl PL (E) PEO PL (D) and Weight gain

(H) respectively. The magnitude of the effect on the PCUR at 24 hr was A > G> E >D > H.

ForCarvedilol phosphate OTs, AC (PEO DL- SLS DL) and AD (PEO DL-PEO PL) interactions were found significant (for PCUR at 24hrs). AC interaction plot shows that at higher concentrations of SLS DL, PEO DL had lesser effect compared to the lower level. But for Nisoldipine OTs only the AC (PEO DL- SLS DL) interaction was found significant.

The factors which were affecting the zero order release rate constant (R^2) was B (NaCl DL) > E (NaCl PL). A significant AC (PEO DL- SLS DL) interaction was also present for both the drugs. At high level of SLS DL change in concentration of PEO DL had a negative impact on the R^2.

The significant factors affecting lag time was in the order of G (Propylene glycol)> H > B. It can be considered that all the 3 factors were equally affecting the lag time. By Increasing the concentration of G and B a drastic decrease in the lag time was observed. No interaction terms were significant for factors affecting lag time for both the drugs.

The polynomial equations representingresponses were constucted for each response after eliminating the non significant terms for both the drugs.

Simultaneous effect of two factors on the PCUR at 24 hrs was studied with the help of contour plots and RS plots.

For PCUR at 24 hrs, factors PEO PL and NaCl PL had almost similar effect on the response. Higher levels of PEO DL, PEO PL, Propylene Glycol, NaCl PL and a lower weight gain had a better effect on the response. Unlike the highly water soluble drugs, no two factors can alone contribute more than 80 % release at 24 hrs. Combined effect of all the significant factors will leads to the desired effect. From the plots it was evident that the propylene glycol and PEO DL were the major factors affecting the responses. A 100 % release up to 24 hrs would be suggestive when the weight gain is at its minimum and the PEO DL, NaCl PL, PEO PL and propylene glycol were at its maximum level.

It was proven from the plots that R^2 close to unity was observedonly at NaCl DL more than 9% and NaCl PL 30 -35%. R^2 drastically changed by decreasing the NaCl DL andNaCl PL. Propylene glycol, NaCl DL and weight gain had almost equal effect on the lag time.

For lag time, it was evident that effect of propylene glycol was more prominent at high levels of NaCl DL. Change in NaCl DL had little effect at the high level of propylene glycol. NaCl DL had a prominent effect on the response at low weight gain.

Cube plots for the simultaneous effect of all the significant factors on the responses were also studied.

The model exhibited linearity without any curvature and lack of fit for all the responses. So theoptimization was done using the same design points by numerical optimization. The target kept for the optimization was PCUR at 24 hr more than 95%, lag time minimum as possible and R^2 to maximize to 1.

The desirability contour plots and RS plots were studied for the optimum combinations of factors. For Carvedilol phosphate higher desirability will be achievedat maximum level of NaCl DL(more than 9%)and a higher concentrations propylene glycol(7.75 -10%),higher levels of PEO DL (60-85%), PEO PL(30-35%), NaCl PL(30- 42%) and low weight gain(10-12.5%).

For Nisoldipine higher desirability will be achieved at NaCl DL (more than 9%), propylene glycol(8-10%), PEO DL (75-95%), PEO PL(25-40%), NaCl PL(30- 40%) and low weight gain(10-12.5%)

A point prediction on the 3 selected solutions was done with the help of the software for both the drugs. Confidence intervals and tolerance intervals for the responses were tabulated.

The same 3 solution batches for each drug were prepared and evaluated as check point batches for confirming the model validity. Thepredicted responses were compared with the experimental values.The experimental values were with in the CI of the predited responses.Thus the model selected i.e.,the 2^{8-4}fractional factorial design for the optimization of the osmotically controlled oral tablets of

poorly water soluble drugs was a validated one and can be used for the further predictions.

Optimized formulation was selected from the numerical solutions considering the desirability, and manufacturing condition preferences. The optimized formulation of each drug was evaluated. It was found that the optimized formulation of push pull OT of highly water insoluble drugs released the drug in a zero order rate up to 24 hrs with a lag time of 3 hrs.

The optimized formulations of both the selected drugs were kept for stability studies according to the ICH guideline. No changes in any of the parameters evaluated were found.

So it can be concluded that during the study, stable optimized push pull OTs of highly water insoluble drugs., Carvedilol phosphate and Nisoldipine were successfully formulated and the significant factors affecting the release pattern of the drug from the system were extensively studied with the help of design of experiments.

7.3.5. Comparison of the results of the factor influence study

I . Effect of PEO DL

For highly soluble drugs, Ropinirole HCl and Ivabradine HCl the suspending agent (PEO) in the DL has no significant effect on the PCUR, R^2 and lag time.

But for poorly soluble drugs suspending agent found to the highly significant factor affecting PCUR. PEO DL had a positive effect on PCUR at 24 hrs. Highly water insoluble drugs need high solubilization effect by any mode inside the system for the complete release of the drug. If the solubilization was impaired /not sufficient, the drug will remain inside as solid particle even if greater osmotic pressure was created inside the system. PEO 400 selected for the study offers an excellent solubilization helping the complete release of the drug. Higher concentrations (65-80%) of PEO DL had higher desirability.No effect of PEO DL was found on R^2 and lag time.

II. Effect of NaCl DL

For selected highly soluble drugs, NaCl DL is one of the significant factors affecting all the responses under investigation. An increase in concentration of NaCl DL had improved the PCUR, $R^2$and lag time. A greater than 9% concentration of NaCl DL had a higher desirability.

But for the selected poorly soluble drugs, NaCl DL had significant effect only on the R^2 and lag time. Surprisingly PCUR at 24 hrs is not affected by the change in concentration of NaCl DL.

III. Effect of Sodium lauryl sulphate DL

For the selected highly and poorly soluble drugs, SLS DL did not have a significant effect on any of the responses studied at 5 % SL.

But for poorly soluble drugs an AC interaction was reported on PCUR at 24 hrs. When SLS concentration was high increase in concentration of PEO DL had a lesser effect than when SLS is at lower concentration. SLS which is a solubilizing agent had an impact on the solubilization of the drug and intern the release of the drug.PEO effect was more prominent if the concentration of SLS is less.

IV. Effect of PEO PL

For the selected highly soluble drugs, any of the responses under investigation was not significantly affected by PEO PL. But for poorly soluble drugs PEO PL had a significant effect on the PCUR at 24 hrs. An increase in the PEO PL produced an increase in PCUR. A 30 - 40 % PEO PL had higher desirability.

V. Effect of NaCl PL

For the selected highly soluble drugs, NaCl PL had no significant effect on the responses except the release rate constant**.** R^2decreases with increase in concentration of NaCl PL. NaCl PL concentration below 15% had a higher desirability.

But for the selected poorly soluble drugs, NaCl PL was one of the significant factors affecting PCUR at 24 hrs and R^2. As the concentration of NaCl

PL increased, the PCUR at 24 hrs and R^2were found to have increased. NaCl PL had no effect on lag time. A higher concentration of NaCl PL (27-35%) had a higher desirability.

VI. Effect of Sodium laurylSulphate PL

For the selected highly and poorly soluble drugs, SLS PL was not significantly affected any of the responses studied at 5 % SL.

VII. Effect of Propylene Glycol in the coating

For the selected highly soluble drugs, propylene glycol was the most significant factor affecting the PCUR at 24 hrs and lag time. Propylene Glycol had no effect on R^2. An increase in concentration of propylene glycol had increased the PCUR at 24 hrs and decreased the lag time. But lower concentration (<5%) of propylene glycol had higher desirability in achieving the optimum conditions.

Forthe selected poorly soluble drugs also propylene glycol was found to be the most significant factor affecting the PCUR at 24 hrs and lag time. Propylene Glycol was not found to have significant effect on R^2. An increase in concentration of propylene glycol produced an increase in the PCUR at 24 hrs and decrease in the lag time. But higher concentrations (>7.5%) had higher desirability in achieving the optimum conditions.

VIII. Effect of weight gain

For the selected highly and poorly soluble drugs, weight gain was one of the significant factors affecting the PCUR at 24 hrs and lag time. This factor had no effect on the linearity of the release. For both the types of the drugs selected a lower weight gain showed highest desirability. As the tablet weight was low, a low weight coating would be sufficient to produce a semi permiable covering which can with stand the osmotic pressure inside the system.

7.3.6. Mechanism of release of the highly soluble drugs from push pull osmotic tablets

The release of the drug is mainly depends upon the amount of the water entered in to the system and the osmotic pressure created inside the systems. The water entry in to the system was controlled by the weight gain and the amount of

the plasticizer present in the coating. The osmotic pressure created would be directly proportional to the water entered in to the system and the concentration of the osmotic agent present in the system.So at lower weight gain if the plasticizer increases more amounts of the water influx produces, decreases the lag time and increase the PCUR.As the tablet weight was low, a low weight coating would be sufficient to produce a semi permeable covering which can with stand the osmotic pressure inside the system.

As the solubility of the drug was high, a lesser concentration of the propylene glycol would be sufficient to produces extra water influx apart from the water influx by osmosis. As the drugs were highly water soluble higher concentrations of propylene glycol would not be desirable, as this will create more influx of the water, and will facilitate the faster release from the system. The complete release of the drug would be possible by a lower weight gain(10-12%) , lower concentration of the plasticizer(<5%) and higher concentration of NaCl DL (>9%).As the drugs were soluble, no extra pressure in the form of osmotic pressure or the extender action by an expanding polymer is needed for the complete release from the system.

But Zero order release was a function of combined osmotic pressure created by the push layer and the PL. The pressure balance in side system is very much essential to release the drug in a zero order fashion. From the present work it was evident that a 10% concentration of the NaCl DL and lower concentrations of NaCl PL would be a better choice for the maximum linearity.

Lag time is the time at which 10% of the release is achieved. It can also be called as the t 10%. The delay in the drug release depends upon the time required for the water influx in to the device, mixing with the ingredients and its solubilization. Minimum 1- 4 hrs lag time would be acceptable for the osmotic drug delivery systems. During the release predictions done at the initial stages we have decided to get10% release by 3 hrs. The lag time can be altered by changing the concentrations of the plasticizer, weight gain and the osmotic pressure created inside the DL. No ingredient in the PL contributed to the lag time modifications.

From the study it can be concluded that for design and development of

push pull osmotic tablets, researchers can concentrate more on the coating and the ingredients in the drug layer compartment especially NaCl DL for a better release profile and linearity.

7.3.7. Mechanism of the release of a poorly soluble drug from the push pull osmotic tablets

The release of the drug is mainly depends upon the amount of the water entered in to the system and the osmotic pressure created inside the systems. The water entry in to the system was controlled by the weight gain and the amount of the plasticizer present in the coating. The osmotic pressure created would be directly proportional to the water entered in to the system and the concentration of the osmotic agent present in the system.So at lower weight gain if the plasticizer increases more amounts of the water influx will be produced. This will cause a decrease in the lag time and an increase the PCUR of the drug. NaCl DL would be producing the pressure for the initial release of the drug. But the complete release of the poorly soluble drugs was dependant on the high solubilization offered by the PEO DL, the osmotic pressure created in the PL and the extender action of the PEO PL. From the study it can be concluded that for design and development of osmotically controlled oral systems for poorly soluble drugs, both the core and coating parameters were equally important and carefully controlled for the better release profile.

As the tablet weight was low, a low weight coating would be sufficient to produce a semi- permeable covering which can with stand the osmotic pressure inside the system. As the solubility of the drug was very less, a higher concentration of the propylene glycol would be needed to produces extra water influx apart from the water influx by osmosis. As the drug was highly water insoluble, higher concentrations of propylene glycol would be desirable, as this will create more influx of the water needed, hence facilitate the faster release from the system. Extra pressure from the push compartment in the form of osmotic pressure and expanding polymers was necessary for the complete out flux of the

drug from the device. Apart from this complete solubilization was necessary for the 100% release of the drug form the system within the specified time.

Lag time was controlled by changing the concentrations of propylene glycol, weight gain and NaCl DL. During the release predictions done at the initial stages we have decided to getthe 10% release by 3 hrs. The lag time can be altered by changing the concentrations of the plasticizer, weight gain and the osmotic pressure created inside the DL. No ingredient in the PL contributed to the lag time modifications.

As zero order release is a function of combined osmotic pressure created by the drug layer and the push layer. From the present work it was evident that a higher concentration of the NaCl DL and higher concentrations of NaCl PL would be a better choice for the maximum linearity.

Considering all these observations, while formulating a push pull osmotic tablets of a poorly soluble drugs, manufactures have to concentrate on both core and coating parameters for the desired release profile.

7.4. STABILITY STUDY OF THE OPTIMIZED FORMULATIONS

Theoptimized formulations of all the selected drugs were subjected to stability study according to ICH guideline**.** Osmotic push pull optimized batch tablets were subjected to various stability evaluation tests such as physical evaluation for color change, weigh variation, hardness, assay and in vitro drug release. None of the parameters were changed significantly during the stability study. The in vitro dissolution study showed no significant difference in its pattern and amount. Hence it can be concluded that the formulations were stable.

7.5. *IN VIVO* ANIMAL STUDIES

7.5.1. Standard calibration curve of the selected drugs

Simple, accurate, precise and sensitive high-performance liquid chromatographic (HPLC) method was used for the quantification of Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine in rabbit plasma samples. Sharp peak with good separation of drugs was obtained during the study.

Good linearity of 0.999 was obtained for Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate, Nisoldipine within the selected range of 20 – 100 µg/ml, 50 – 300 µg/ml, 50-500 µg/ml and 20-120 µg/ml concentration respectively.

7.5.2. *In- vivo* animal study and pharmacokinetics of the optimized push pull OTs of the selected drugs and marketed drug products

The plasma kinetic data was assessed with Phoenix® WinNonlin® software. The marketed drug products of Ropinirole HCl, Carvedilol phosphate and Nisoldipine were available in the extended release dosage form. Hence the same strength of the osmotic tablets was used for the comparative *in-vivo* pharmacokinetic evaluation of the drug products. However Ivabradine HCl was not available in any of the extended or modified release dosage formulations. Hence the available immediate release dosage formulation was used.

The pharmacokinetic profile of the Ropinirole HCl, Ivabradine HCl, Carvedilol phosphate and Nisoldipine osmotic tablets showed a lag phase (zero drug concentration) of approximately 2.0 hours compared to the marketed extended/ immediate release dosage formulation. This could be due to the lag time observed in the dissolution profile of the osmotic tablets.

The time to reach maximum concentration (t_{max}) of the Ropinirole HCl, Carvedilol phosphate and Nisoldipine osmotic tablets were 10, 12 and 12hrs respectively. But for the marketed extended release products it was 5, 9 and 10 hrs respectively. The P value obtained from the "t" test was 0.008, 0.005, 0.01 respectively. This clearly showed that the $t_{max}$of the osmotic tablets were significantly different from the marketed products. So the null hypothesis was rejected. So it can be said that t_{max} of push pull OTs of the above said drugs were considered to be prolonged compared to the marketed release drug products. However the t_{max} of Ivabradine HCl osmotic tablet was 6 hours against 1 hours of the marketed immediate release drug product ($p = 0.002$). This could be due to the lag phase and due to the controlled release of the osmotic tablets compared to that of the marketed drug product.

The maximum drug concentration (C_{max}) in plasma observed for Ropinirole HCl, Carvedilol phosphate and Nisoldipine osmotic tablets were 53.8, 313.66 and 110.45 ng /ml respectively compared to that of 46.92, 297.62 and 102.16 ng/ml of the marketed drug products. The P value observed for the "t" test was 0.13, 0.163, and 0.284 respectively. From the t test it can be said that there were no significant difference observer for the C_{max} of the optimized OTs and the marketed products of the above mentioned selected drugs (at 5% significant level). This clearly shows that the C_{max} observed was well within the range of C_{max} of the marketed products. But for Ivabradine HCl a significant lowering of the C max was observed for the optimized formulation ($p = 0.009$). No dose dumping was observed in any of the optimized OTs studied. All the optimized formulations maintained the therapeutic drug concentration in the plasma to provide the desired effects. The extent of the drug in plasma (AUC) for the osmotic tablets and the marketed drug products were comparable.

Overall it could be concluded that Ropinirole hydrochloride, Carvedilol phosphate and Nisoldipine osmotic tablets provided a controlled release and maintained the drug concentration within the therapeutic level similar to that of the marketed drug products for 24 hours providing a once daily dosage regimen.

Since the Ivabradine HCl was not available in extended release dosage formulation, the osmotic tablets would provide a suitable alternative for the immediate release formulation which could lower the C_{max} however maintain the drug within the therapeutic window and reduce the side effects of the drug product. From the kinetic profile of the Ivabradine OTs, it was clear that a once daily formulation for the Ivabradine HCl osmotic tablets would not be feasible and can stick to a twice daily dosage regimen.

Chapter -8
SUMMARY AND CONCLUSION

8. SUMMARY AND CONCLUSION

In this research work, once daily push pull osmotic tablets of two highly soluble drugs (Ropinirole HCl and Ivabradine Hcl) and two poorly soluble drugs (Nisoldipine and Carvedilol phosphate) were developed,optimized and different factors affecting the release profile were extensively studied. The concept of QbD was applied and the design space was successfully obtained with the help of design expert soft ware. Different statistical tools like ANOVA, regression analysis were used for the study.

Analytical method was developed in pH 6.8 phosphate buffer for all the selected drugs. The drugs exhibited greater linearity at the selected ranges. Regression equations and R^2 were created and studied for all the drugs in both the solvents.

Pre formulation studies of the selected drugs like, organoleptic properties, solubility, flow property, particle size determination and drug–excipients interaction study were performed and reported.

The amount of the drug to incorporated in to the push pull osmotic tablets of the selected drugs were calculated with the help of the available labeled claim of the XR products and Robinson – eriksen equation.

An extensive literature survey was performed and the various vital factors affecting the drug release profile from the push pull OT were identified. Formulation development, factor influence study and optimization of the formulations were done with the help of design expert software. A fractional factorial design (**2^{8-4}** with **Resolution IV**) with 4 centre points were selected for the study. Push pull OTs of the selected drugs were formulated and both blend as well as whole tablet evaluations were performed. All the tests were within specified limits of the pharmacopoeia. In vitro dissolution study of all the trials of the selected drugs were carried out in triplicate. The selected responses like PCUR at 24 hrs, lag time and R^2 were reported for each trial. The responses were

analyzed with the help of design expert software and different significant factors affecting the selected responses were identified.

The factor influences were extensively studied and reported with the help of different plots like half normal plot, normal plots, Pareto charts, contour plots, RS plots and cube plots. ANOVA analysis was also performed for the identification significant factors. From the regression analysis the coefficients of significant factors were determined. Polynomial equations representing the responses were framed after eliminating the non significant factors.

Optimization of the push pull OTs of the selected drugs was done with the help of numerical optimization and desirability function. A better identification of design space was done with the help of desirability contour plots and RS plots. Check point batches of all the four selected drugs were formulated and evaluated for the design model validity.

Stability studies on the optimized push pull OT formulations of all the selected drugs were performed to assess their stabilityover time. The ICH guidelines were strictly followed during the stability study. None of the formulations showed any significant changes in any of the parameters evaluated.

An *In vivo* animal study of the optimized formulations of all the selected drugs was performed to assess the in vivo performance of the dosage form. Pharmacokinetic parameters like t_{max},C_{max}, AUC_{0-t},$AUC_{0-\infty}$,K_{el}, $t_{½}$ were determined and compared with the available marketed products of the selected drugs.

So it can be concluded that in this research work, stable optimized formulations of push pull OTs of highly and poorly soluble drugs were successfully formulated and extensively studied the significant factors affecting the release pattern of the drug from the system with the help of design of experiments.

Chapter -9
RECOMMENDATIONS

9. RECOMMENDATIONS

Further extension of this study can be concentrated on,

1) **Effect of process parameters on the release pattern of the drugs and identification of the design space:** Process parameters also have an influence on the release profile of the drug from the push pull OTs. Process as well as the product parameters cannot be varied at time to find out their effect on the release profile. In this study, all the process parameters were kept constant and studied the effect of product variables. A further study can be possible to optimize the process parameters while keeping the product variables constant.

2) ***Invivo* study using human volunteers:**A better understanding of the *in vivo* behavior of the optimized push pull osmotic tablets of the selected drugs is possible with human volunteers. So bioavailability studies on the optimized formulation of the push pull OTs of the selected drugs can be performed as an extension of this work.

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APPENDIX

RVS COLLEGE OF PHARMACEUTICAL SCIENCES

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COMMITTEE FOR THE PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS

Ref No: IAE1012/c/10/CPCSEA –Corres – 2011 10.06.2011

Sub: Approval of animal studies – project submitted for clearance – Resolutions Passed by Animal Ethical Committee meeting held on 8th June 2011.

Sir,

With reference to the subject citied above, **please find the enclosed list of projects cleared for the animal studies by the Institutional Animal Ethical Committee** of RVS College of Pharmaceutical Sciences, in its meeting held on 8th June 2011 at the college premises.

10/6/11

Dr. D. Benito Johnson
Secretary –Member
IAEC

Dr. D. BENITO JOHNSON. M.Pharm.Ph.D.
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No. of Resolutions passed –01.

Resolution No : 18

It is resolved to pass the clearances for the animal study of the Research "**DEVELOPMENT AND OPTIMIZATION OF OSMOTICALLY CONTROLLED ORAL DRUG DELIVERY SYSTEMS FOR HIGHLY AND POORLY WATER SOLUBLE DRUGS**" submitted by **Mrs. P.S.Sona,** Department of Pharmaceutics , RVS College of Pharmaceutical Sciences, 242 - B Trichy Main Road, Sulur, Coimbatore 641 402, Tamil Nadu.

/ True Copy Extract/

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Statistical optimization of osmotically controlled oral tablets of ropinirole hydrochloride using desirability approach

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ABSTRACT

Quality by design concept is nowadays widely used in the pharmaceutical product development and optimization. Regulatory authority FDA made QbD compulsory for its ANADAs applications. In the present study a fractional factorial design 2_{IV}^{8-4} was selected for the development of Push pull osmotic tablets of Ropinirole Hcl and optimization was done with the help of desirability function. The responses selected for the optimization were PCUR at 24 hrs, R^2, Lag time. FDS curve was used for the precision power of the design. A lower curve obtained indicates that more of the design has useful precision. Statistical tools like ANOVA and regression analysis were used for the identification of significant factors. The Significant level selected for the study was 5%. The study identified and quantified the effect of different variables on the responses. The magnitude of the effect of factors on the PCUR at 24 hr was G > H> B, R^2 was B > E and for lag time it was in the order of G> H> B. Numerical optimization was done by fixing the target PCUR at 24 hrs 85 -100 %, R^2 - 0.998 and lag time 3 hrs. The effect of various factors on the desirability function was represented with the help of desirability contour plots and RS plots. Higher desirability will be achieved at maximum level of Nacl DL and a lower concentrations propylene glycol. The desirability was highest at High concentration (>9) of Nacl and the low concentration(less than 14) of weight gain . A wide range of weight gain- propylene glycol combinations can be used for the optimization.

KEY WORDS: Ropinirole hydrochloride, Design of experiments, ANOVA, contour plot, RS plot, Desirability.

INTRODUCTION

Design of experiments (DoE) and quality by design (QbD) are comparatively newer approach in the field of Pharmacy. DoE was developed originally for agricultural purposes, but during World War II and thereafter it become a tool for quality improvement, along with statistical process control (SPC). Until 1980, DoE was mainly used in the process industries, may be because engineers are well versed with the mathematical and statistical concepts. Much attention to this field was not paid by the pharma researchers until, the FDA Announced a new initiative (cGMP for the 21st Century: A Risk based Approach) on 2002. FDA in its cGMP initiative, two important guidance documents was published as part of International Conference on Harmonization (ICH) guidelines: Q8 Pharmaceutical Development and Q9 Quality Risk Management. [1, 2, 3]

DoE is a planned approach for determining cause and effect relationship [4]. It also provides a statistical means for analyzing how numerous variables interact. Understanding the effect of variables on the response provides a better chance of successful product development and optimization. Optimization and factor influence study would be rather complicated when number of factors and responses were involved. In that case, the classical graphical optimization would be meaningless and awkward and the Numerical optimization with the help of desirability function would be a better option.

Ropinirole hydrochloride is an orally administered non-ergoline dopamine agonist used for the treatment of Parkinson's diseases. A once daily controlled release drug device would be beneficial for the successful management of the Parkinson's disease. Osmotically controlled systems strictly provides a zero order release maintains the plasma concentration constant, hence the most desirable dosage form.In this present study we adopt the applications of statistics optimization for the formulation and optimization Ropinirole Hcl osmotically controlled oral tablets .

A relatively straight forward approach to optimize several responses that works well when there are less than three variables and is to

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overlay the contour plots for each response. When there are more than three variables overlaying contour plots become awkward, because the contour plot is two dimensional and k-2 of the design variables must be held constant to construct the graph. When more than two factors were significant, overlay plot does not give a complete idea about the optimization. Therefore there is practical interest in more formal optimization methods for multiple responses called as desirability.Desirability function is a simple mathematical method to find the optimum. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals get combined into one desirability function. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals get combined into one desirability function [4]. In our study more than two variables were significant for each responses, hence adopted numerical optimization with desirability for optimization.

MATERIALS AND METHODS

MATERIALS

The Ropinirole Hcl was received as gift sample from Alembic Pharmaceuticals Limited; Butylated hydroxyl toluene, Sodium lauryl sulfate, Sodium chloride was received from Merck; Polyethylene Oxide and cellulose acetate was received from signet; Dibasic calcium phosphate from Innophos; Magnesium stearate from Ferro; iron oxide and Propylene glycol from Alembic Limited. Other regents were of analytical grade.

METHODS

I. Formulation and evaluations of Push pull osmotic tablets of Ropinirole Hcl [4, 5, 6]

A fractional factorial design with 8 selected factors **2^{8-4}** (ie 1/32 fraction) with **Resolution IV** was selected for the study. 16 trials with 4 centre points were planned for the study. The selected factors with levels chosen are given in the Table 1. The responses selected for the study is given in the Table: 2. The formula table showing all the ingredients taken in each trial is explained in the Table: 3.

Table 1: selected Factors with levels affecting osmotic push pull delivery system for the factor influence study

	Factors	Levels min	Levels max
1	PEO in the drug layer (% w/w of the API)	10	100
2	Nacl concentration in drug layer(% w/w of drug layer)	1	10
3	SLS in the drug layer (%w/w of the drug layer)	1	5
4	PEO (coagulant) in the push layer (% w/w of the drug layer)	5	50
5	Sodium chloride in the Push layer (% w/w % of the extender)	5	50
6	SLS in the Push layer(%w/w of the push layer)	1	5
7	Propylene Glycol (% w/w of the coating weight)	1	10
8	Weight gain (%)	10	20

Table 2: the response selected for the factor influence study

Response	Unit	Weightage
Cumulative release at 24 Hrs	%	+++++
R^2	-	++++
Lag time	Hrs	+++

Table 3: Formula table (trial 1 to 13)

S. No.	Ingredients	Trial-1 mg/tab	Trial-2 mg/t	Trial-3 mg/t	Trial-4 mg/t	Trial -5 mg/t	Trial-6 mg/t	Trial -7 mg/t	Trial -8 mg/t	Trial-9 mg/t	Trial-10 mg/t	Trial-11 mg/t	Trial-12 mg/t	Trial-13 mg/t
Drug Layer														
1	RH	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68	13.68
2	DCP	33.44	21.13	28.94	16.63	31.44	19.13	26.94	14.63	33.42	21.11	28.92	16.61	31.42
3	PEO 400 K	1.368	13.68	1.368	13.68	1.368	13.68	1.368	13.68	1.3	13.6	1.3	13.68	1.368
4	NaCl	0.5	0.5	5	5	0.5	0.5	5	5	0.5	0.5	5	5	0.5
5	BHT	0.0025	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.025	0.025	0.025	0.025	0.025
6	SLS	0.5	0.5	0.5	0.5	2.5	2.5	2.5	2.5	0.5	0.5	0.5	0.5	2.5
7	IPA													
8	Mg Sterate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Push layer														
9	PEO 7000 K	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	25	25	25	25	25
10	Nacl	0.125	1.25	1.25	0.125	1.25	0.125	0.125	1.25	1.25	12.5	12.5	1.25	12.5
11	DCP	39.62	38.49	36.69	37.82	36.69	37.82	39.62	38.49	14.17	2.92	4.725	15.97	4.72
12	BHT	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.02	0.025	0.025	0.025	0.025
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25	0.45	0.45	0.45
14	IOR	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
15	IPA													
16	Mg Sterate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
W UCT		95	95	95	95	95	95	95	95	95	95	95	95	95
Functional coating														
15	CA	9.31	18.05	18.90	7.6	8.55	18.81	17.1	9.405	18.05	9.31	8.55	18.90	18.81
16	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
18	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	PG	0.19	0.95	0.095	1.9	0.95	0.19	1.9	0.095	0.95	0.19	0.95	0.095	0.19
T TW		**104.5**	**114**	**114**	**104.5**	**104.5**	**114**	**114**	**104.5**	**114**	**104.5**	**104.5**	**114**	**114**

Table 3: Formula table (trial 14 to 20)

S. No.	Ingredients	Trial-14 mg/t	Trial-15 mg/t	Trial-16 mg/t	Trial-17 mg/t	Trial-18 mg/t	Trial-19 mg/t	Trial-20 mg/t
Drug Layer								
1	RH	13.68	13.68	13.68	13.68	13.68	13.68	13.68
2	DCP	19.11	26.92	14.61	24.03	24.03	24.03	24.05
3	PEO 400 K	13.68	1.368	13.68	7.524	7.524	7.524	7.524
4	NaCl	0.5	5	5	2.75	2.75	2.75	2.75
5	BHT	0.025	0.025	0.025	0.013	0.013	0.013	0.013
6	SLS	2.5	2.5	2.5	1.5	1.5	1.5	1.5
7	IPA							
8	Mg Sterate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Push layer								
9	PEO 7000 K	25	25	25	13.75	13.75	13.75	13.75
10	Nacl	1.25	1.25	12.5	3.781	3.781	3.781	3.781
11	DCP	15.97	14.17	2.92	23.80	23.80	23.80	23.80
12	BHT	0.025	0.025	0.025	0.013	0.013	0.013	0.013
13	SLS	0.45	2.25	2.25	1.35	1.35	1.35	1.35
14	IOR	0.8	0.8	0.8	0.8	0.8	0.8	0.8
15	IPA							
16	Mg Sterate	1.5	1.5	1.5	1.5	1.5	1.5	1.5
W UCT		95	95	95	95	95	95	95
Functional coating								
15	CA	8.55	9.405	17.1	13.46	13.46	13.46	13.46
16	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s
18	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	PG	0.95	0.095	1.9	0.783	0.783	0.783	0.783
T TW		**104.5**	**104.5**	**114**	**109.2**	**109.2**	**109.2**	**109.2**

RH – Ropinirole Hcl, DCP – Dicalcium Phosphate, PEO – Polyethylene oxide, Nacl – sodium Chloride, BHT –Butylated hydroxyl toluene, SLS – Sodium lauryl sulphate, IPA – Iso propyl alcohol,Mg state - Magnesium Sterate, IOR – Iron oxide red, CA – cellulose acetate, PG – Propylene Glycol, WUCT – Weight of uncoated tablets,TTW- Total Tablet weight.

II.Preparation of osmotic push pull tablets

The common processes for the formulation of push pull osmotic tablets were show in the flow chart given below.

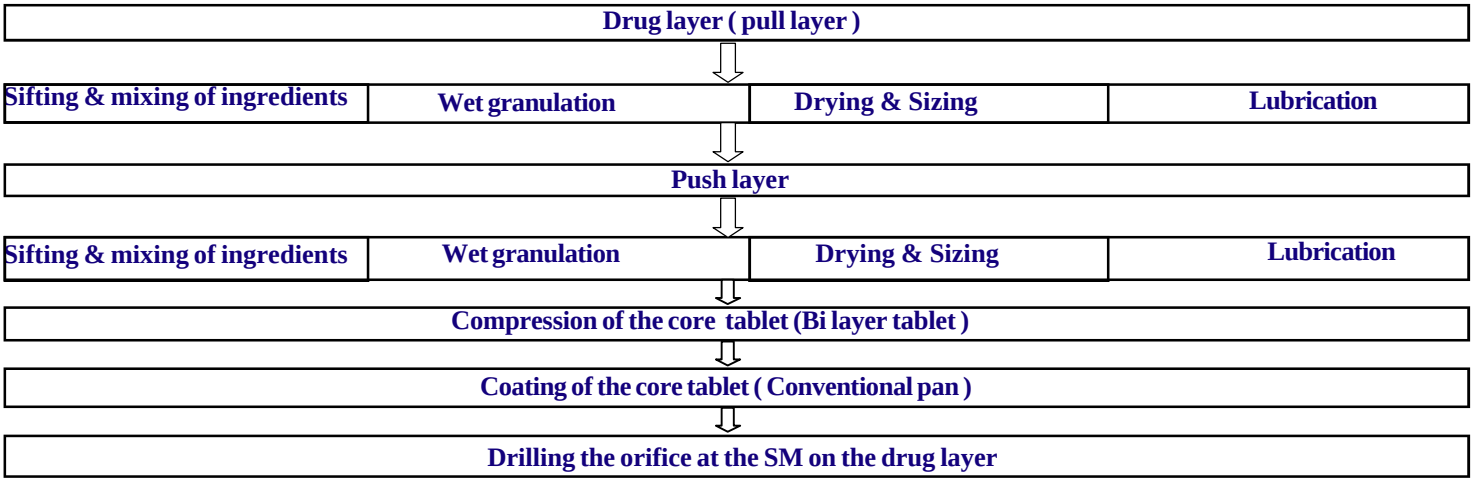


Fig 1: Schematic flow chart for the formulation of Push pull osmotic systems

III.Evaluation of the formulations [7,8,9]

The batches were evaluated simultaneously while preparing. They were subjected to blend as well as whole tablet evaluation.

a) .Blend evaluation

The prepared granules of both the layers i.e. drug layer and push layer were evaluated by means of various tests. The tapped density, Bulk density, Carr's index and Hauser's ratio was determined for granules prepared for both drug and push layer.

b).Tablet evaluation

To monitor the product quality and for quantitative evaluation of tablet properties evaluation of tablets are necessary. The prepared tablets were evaluated for weight variation, hardness, friability, Assay, weight gain and pore size and physical tests like diameter and thickness.

c).In vitro dissolution study

Dissolution test was performed using an USP II paddle apparatus (DS-8000, Lab India, Analytical instrument pvt ltd, Navi Mumbai, India.) at 37°C± 0.5°C in 900 ml of phosphate buffer 6.8. Paddle speed was kept at 50 rpm. Samples were withdrawn after predetermined time intervals of 1 ,2,4,6,8,12,16,20,24 hrs. The drug content was measured using an UV spectrophotometer at 250 nm. Samples were suitably diluted and absorbance was measured. Cumulative percentage drug released was calculated for each batch. The study was performed in triplicate and the average was reported. The data of % cumulative release from each trial batch were subjected to kinetic release studies to assess the fit into the zero-order release kinetics. The r2 value was found out to determine the best fit zero order release kinetics.

III.Statistical optimization of the formulation

After completion of the evaluation of the responses the statistical data were studied thoroughly. The ANOVA analysis would suggest the model validity. Model suitability was checked by regression analysis.if the curvature and lack of fit in the model were not significant the 2 level design can be used for optimization. The R^2 predicted, R2 adjusted, and adequate precision values for the regression analysis would suggest the model suitability.

a.FDS curve

Before staring the experiment the FDS curve of the proposed design would be studied .The FDS graph of the two level design with the selected factor and run was generated . The **FDS (Fraction of Design Space) Graph** is a line graph showing the relationship between the "volume" of the design space (area of interest) and amount of prediction error. The curve indicates what fraction (percentage) of the design space has a given prediction error or lower. In general, a lower and flatter FDS curve is better. Lower is more important than flatter. A lower curve translates to a higher Fraction of Design space - more of the design has useful precision.

B.Analysis of responses [10, 11]

For all batches granules were prepared, bilayer tablets were prepared, coated and drilled by micro drilling. All the batches were subjected to in vitro dissolution using USP II (paddle) apparatus up to 24 hour. The samples were withdrawn at an interval of 1,2,4,6,8,12,16,18,20,24 and analyzed using UV Spectrophotometer (Shimadzu1800) at λmax 250nm.. From graph of %cumulative drug release verses time, T90 and R^2 value and lag time were determined.

c. ANOVA and regression analysis

The results obtained for the study design was analyzed with the help of design expert software and significance of factors were found out by ANOVA analysis. The hypothesis were tested with a level of significance 5 % ($p < 0.05$) From the ANOVA analysis significant factors are identified..

D.Pareto chart

Pareto chart is bar graph for the clear identification of the significant factors. Two different colors are used for the identification of significant as well as non significant effects. The blue color indicates the negative effect and the orange color indicates the positive effect of the factors on the selected responses. T value and the Bonferroni limit is used for the identification of the significant factors.

IV.Numerical optimization with the help of desirability

The constraints fixed for the numerical optimization was given in the table:4. The weightage given for the responses was in the order of PCUR drug release at 24 hrs > R^2 > lag time.

Desirability contour plot and RS plot

They are the graphical representation of change in factors on the desirability function. It will be giving a better visualization of achieving the optimum condition by changing two factors at a time. Desirability plots shows how all the targeted optimum conditions are met by changing two factors at a time.

Table 4: The constraints of optimization of Ropinirole Hcl Push pull OCOTs

Constraints Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
B:Nacl concentration DL	is in range	1	10	1	1	3
E:Sodium chloride PL	is in range	5	50	1	1	3
G: Propylene glycol	is in range	1	10	1	1	3
H:weight gain	is in range	10	20	1	1	3
PCUR drug release in 24 hrs	is in range	95	100	1	1	5
R2	is target = 0.998	0.85	0.998	1	1	4
lag time	is Maximize	2.8	4.7	1	1	3

Table 5: Blend evaluation of the DL and PL of push –pull osmotic tabletsof Ropinirole Hcl

Trials	Angle of repose		Bulk density (g/ml)		Tapped density (g/ml)		Hausner's ratio		Carr's index(%)	
	DL	PL	DL	PL	DL	PL	DL	PL	DL	PL
1	28.33	26	0.812	0.617	0.869	0.682	1.003	1.043	6.5593	9.5308
2	25.43	27.14	0.734	0.618	0.8	0.674	1.113	1.091	8.2500	8.3086
3	28.55	27.75	0.834	0.751	0.901	0.811	1.079	1.081	7.4362	7.3983
4	29.65	26.56	0.789	0.622	0.853	0.71	1.056	1.044	7.5029	12.3944
5	27.48	28.39	0.761	0.627	0.823	0.668	1.114	1.066	7.5334	6.1377
6	26.87	27.14	0.645	0.715	0.723	0.77	1.113	1.077	10.7884	7.1429
7	28.9	29.65	0.721	0.597	0.826	0.679	1.135	1.137	12.7119	12.0766
8	29.86	27.14	0.654	0.752	0.734	0.81	1.178	1.052	10.8992	7.1605
9	27.65	26	0.823	0.793	0.923	0.861	1.034	1.086	10.8342	7.8978
10	26.89	28.39	0.721	0.648	0.803	0.727	1.056	1.122	10.2117	10.8666
11	27.89	28.39	0.679	0.616	0.734	0.686	1.087	1.113	7.4932	10.2041
12	28.75	27.14	0.856	0.632	0.923	0.723	1.198	1.044	7.2589	12.5864
13	27.33	25.88	0.745	0.672	0.815	0.745	1.174	1.082	8.5890	9.7987
14	28.12	26.22	0.734	0.61	0.805	0.666	1.112	1.113	8.8199	8.4084
15	27.56	27.12	0.823	0.623	0.902	0.7	1.002	1.096	8.7583	11.0000
16	29.6	25.99	0.699	0.712	0.795	0.795	1.116	1.11	12.0755	10.4403
17	27.68	27.43	0.865	0.654	0.931	0.723	1.183	1.113	7.0892	9.5436
18	29.44	29.88	0.789	0.61	0.854	0.673	1.2	1.065	7.6112	9.3611
19	28.11	28.56	0.814	0.689	0.899	0.753	1.22	1.126	9.4549	8.4993
20	29.18	27.9	0.777	0.643	0.837	0.698	1.055	1.034	7.1685	7.8797

RESULT AND DISCUSSION

I. II.Preparation of osmotic push pull tablets

Formulation development of Ropinirole Hcl push pull osmotic tablets were designed and optimized with the help of design of experiments. The design expert software was used for this purpose. A fractional factorial design (**2^{8-4}** with **Resolution IV**) with 4 centre points were selected for the study after identifying the vital factors. The responses selected for the factor influence as well as optimization were PCUR at 24 hrs, Zero order rate constant, and the lag time.

II.Evaluation of Formulation of Ropinirole Hcl push pull osmotic tablets

Blend evaluation

Blend evaluation of both drug layer and push layer were performed. The result of the blend evaluation was given in the Table: 5. The values fall within the range for angle of repose, Hauser's ratio and Carr's index confirming the good flow property of blend.

Tablet evaluations

The result of the whole tablet evaluation was given in the **Table: 6.** According to the results not a single tablet have deviation more than 5% of its weight so all the formulations were passed the weight variation test. . Diameter of the formulations was within the range of 5.2 ±0.5 -0.18 mmand thickness was 3.3 ±0.20-0.24mm the hardness was found to be within the range of 3.5 -4.2kg/cm ^{2}.Friability values were not more than 1%. The drug content was found to be within 99% to 102% range. The pore size was found to be 0.60 mm.. Weight gain of the tablets were found to be 10. 04- 10.28, 20. 04 – 21.08 and 15. 06 – 15.18.

Invitro dissolution study

The invitro evaluations of all the 20 trials were performed and the necessary values for the study were recorded. The design matrix and the responses for the study were given in the **table.7**. The invitro dissolution was carried out and three responses were selected mainly lag time, PCUR at 24 hrs and R^2. All these values were analyzed with the help of design expert software and the result of the study was interpreted from ANOVA analysis and other statistical tests. The level of significant selected was 5% ($p<0.05$).

Table No 6 : Push pull OCOT of Ropinirole (whole tablet) evaluation

Trial	Wt variation (n =20)	Diameter (n=5)	Thickness (n=5)	Hardness (n=6)	Friability (%)	Assay (%)	Weight gain(%)	Pore size(mm)
1	104.5±0.005	5.2±0.15	3.3±0.24	3.5±1.2	0.36	100±1.56	10.12±1.23	0.60
2	114±0.04	5.2±0.15	3.3±0.21	3.8±1	0.746	99.9±1.82	20.09±0.02	0.60
3	114 ±.006	5.2±0.18	3.3±0.23	3.7±0.5	0.626	102.54±1.7	20.04±0.78	0.60
4	104.5 ± 0.08	5.2±0.11	3.3±0.21	4±0.8	0.344	100.1±1.03	10.08±1.76	0.60
5	104.5 ± 001	5.2±0.15	3.3±0.24	4.2±0.6	0.22	100.3±0.87	10.16±0.80	0.60
6	114 ±0.09	5.2±0.18	3.3±0.20	4.1±0.2	0.571	99.99±0.99	20.18±0.97	0.60
7	114 ± 0.04	5.2±0.14	3.3±0.21	3.8±0.5	0.735	100±2.78	20.17±0.62	0.60
8	104.5 ±0.07	5.2±0.15	3.3±0.23	3.8±0.8	0.447	99.78± 1.56	10.15±1.59	0.60
9	114 ± 0.005	5.2±0.18	3.3±0.20	3.6±0.6	0.809	99.34±2.67	20.04±2.98	0.60
10	104.5 ± 0.01	5.2±0.08	3.3±0.24	3.5±0.5	0.681	99.56±1.2	10.28±0.13	0.60
11	104.5 ± 0.08	5.2±0.05	3.3±0.21	3.4±0.4	0.453	101.33±1.78	10.07±1.03	0.60
12	114 ± 0.07	5.2±0.12	3.3±0.23	3.6±0.45	0.838	100±1.6	21.08±1.23	0.60
13	114 ± 0.13	5.2±0.05	3.3±0..24	3.7±0.34	0.72	99.45±1.12	20.09±1.55	0.60
14	104.5±0.034	5.2±0.08	3.3±0.21	3.6±0.22	0.35	99.78±2.6	10.04±0.73	0.60
15	104.5 ±0.14	5.2±0.07	3.3±0.23	3.4±0.62	0.83	101±0.98	10.19±0.92	0.60
16	114 ± 0.23	5.2±0.08	3.3±0.24	3.7±0.44	0.12	100±1.52	20.26±0.82	0.60
17	109.25 ± 0.3	5.2±0.05	3.3±0.21	3.5±0.38	0.22	101±2.82	15.18±0.76	0.60
18	109.25 ± 0.2	5.2±0.06	3.3±0.24	3.6±0.48	0.53	100±1.76	15.07±1.84	0.60
19	109.25 ± 0.1	5.2±0.07	3.3±0.22	4.2±0.03	0.35	98±2.890	15.06±0.79	0.60
20	109.67±0.12	5.2±0.05	3.3±0.23	4.1±0.07	0.47	99.98±1.12	15.13±1.4	0.60

Table 7 : Design matrix in coded terms with responses

Sl No	Design matrix								Responses		
	PEO DL	Nacl DL	SLS DL	PEO PL	Nacl PL	SlS PL	PEG	Wt Gain	PCUR at 24 hrs	R^2	Lag time (t 10%)
1	-1	-1	-1	-1	-1	-1	-1	-1	75	0.922	4.3
2	1	-1	-1	-1	1	-1	1	1	59	0.889	3.7
3	-1	1	-1	-1	1	1	-1	1	70	0.954	4.2
4	1	1	-1	-1	-1	1	1	-1	100	0.997	2.8
5	-1	-1	1	-1	1	1	1	-1	85	0.868	3.2
6	1	-1	1	-1	-1	1	-1	1	38	0.946	4.2
7	-1	1	1	-1	-1	-1	1	1	85	0.997	3.4
8	1	1	1	-1	1	-1	-1	-1	100	0.988	3.2
9	-1	-1	-1	1	-1	1	1	1	90	0.997	4.3
10	1	-1	-1	1	1	1	-1	-1	77	0.886	3.9
11	-1	1	-1	1	1	-1	1	-1	100	0.979	2.8
12	1	1	-1	1	-1	-1	-1	1	54	0.998	4.5
13	-1	-1	1	1	1	-1	-1	1	45	0.871	4.7
14	1	-1	1	1	-1	-1	1	-1	93	0.855	3.7
15	-1	1	1	1	-1	1	-1	-1	81	0.998	3.3
16	1	1	1	1	1	1	1	1	100	0.980	3.2
17	0	0	0	0	0	0	0	0	90	0.928	3.7
18	0	0	0	0	0	0	0	0	92	0.943	3.5
19	0	0	0	0	0	0	0	0	85	0.939	3.9
20	0	0	0	0	0	0	0	0	85	0.9242	3.7

III.Statistical optimization of the formulation

The FDS graph for the selected design with the selected factors and responses showed a flatter curve. The curve indicates that a high FDS so the design space predicted by the selected model had useful precision. The graph is given in the **figure No: 2**

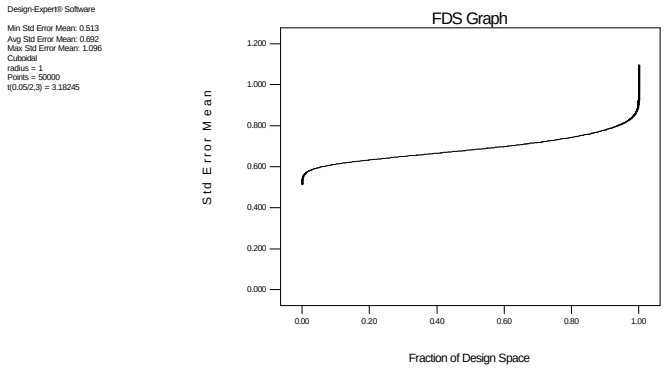


Fig 2: The FDS Curve

From the Pareto chart also it was clearly evident that the factors B, G, H are the significantly affecting the cumulative response at 24 hrs. All the factors cross the t limit and G and H crosses the Bonferroni limit. The magnitude of the effect can be written as Propylene glycol > weight gain > sodium chloride in the drug layer.

The Pareto chart represents the significant effect of B and E on the zero order rate constant. Both the factors crosses the t limit confirms the obvious effect of these factors on the zero order rate constant. The magnitude of the effect can be written as Nacl in the drug layer > Nacl in the push layer.

Figure shows the Pareto chart of effect of factors on the lag time in terms of T value. The factors significantly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect.

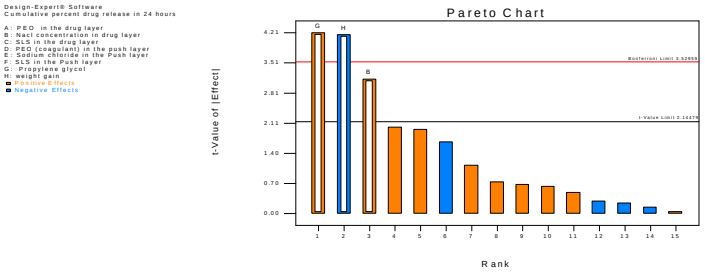


Figure 3: The pareto chart for the effect of the factors on the PCUR at 24 Hrs

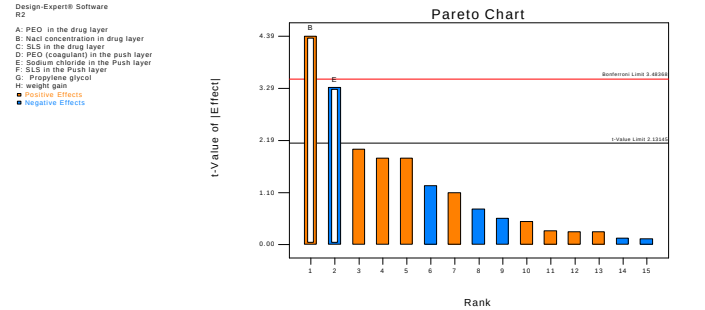


Figure 4: Pareto chart of the effect of the factors on$R^{2,}$

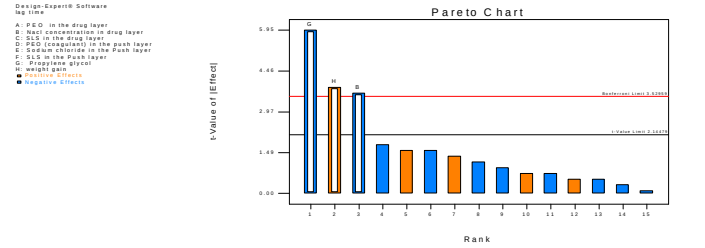


Figure 5: Pareto chart of the effect of the factors on lag time

ANOVA analysis

The result of the ANOVA analysis for the responses were given in the table : 8.

Table: 8 ANOVA Analysis of the responses

PCUR at 24 hrs Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Block	1.05	1	1.05			
Model	4679.25	3	1559.75	13.37	0.0002	significant
B-NaclDL	*1024*	*1*	*1024*	*8.78*	*0.0097*	
G-propylene glycol	*1849*	*1*	*1849*	*15.85*	*0.0012*	
H-weight gain	*1806.25*	*1*	*1806.25*	*15.48*	*0.0013*	
Residual	1749.7	15	116.65			
Lack of Fit	*1723.7*	*13*	*132.59*	*10.2*	*0.0927*	not significant
R2						
Block	*6.91E-05*	*1*	*6.9063E-05*			
Model	*0.032113*	*2*	*1.6057E-02*	*16.10912*	*0.0001*	significant
B-Nacl DL	*0.020449*	*1*	*2.0449E-02*	*20.51601*	*0.0003*	
E-Sodium chloride PL	*0.011664*	*1*	*1.1664E-02*	*11.70222*	*0.0035*	
Residual	*0.015948*	*16*	*9.9673E-04*			
Lack of Fit	*0.015748*	*14*	*1.1248E-03*	*11.24838*	*0.0846*	not significant
Lag time						
Block	0.12	1	0.12			
Model	3.65	3	1.22	18.61	< 0.0001	significant
B-Nacl DL	*0.77*	*1*	*0.77*	*11.71*	*0.0038*	
G-PEG	*2.03*	*1*	*2.03*	*31.05*	*< 0.0001*	
H-weight gain	*0.86*	*1*	*0.86*	*13.08*	*0.0025*	
Residual	0.98	15	0.065			
Lack of Fit	*0.97*	*13*	*0.075*	*12.78*	*0.0748*	*not significant*

All the three responses were analyzed with help of various statistical tools like ANOVA and regression analysis to find out the significant factors affecting the release of the drug from the system and the model suitability. From the analysis the significant factors affecting the Cumulative drug release were identified as propylene glycol (G). Weight gain (H) and sodium chloride in the drug layer respectively. The factors affecting the zero order rate constant were Nacl in the DL (B) and Nacl in the PL (E) and lag time was Propylene glycol (G) weight gain (H) and Nacl DL (B). The model was also significant and no lack of fit and interactions were reported for any of the responses. No interactions were reported in any of the responses. The effect of the factors on the responses was confirmed from pareoto chart. The magnitude of the effect on the PCUR at 24 hr was G > H> B, r^2 was B > E and for lag time it was in the order of G> H> B. Highly water soluble drugs does not need any suspending agent hence the PEO and SLS in the DL does not have any significant effect on the PCUR at 24hrs at 5% significance level.R^2 and lag time were also unaffected by the change in concentration of these factors. No factors in PL would be significantly affecting the lag time and CUR at 24hs. But R^2 had a negative relationship with increase in con of Nacl PL.

III.Numerical optimization with the help of desirability

Numerical optimization was done by keeping the target profile as shown in the methods. The solutions obtained for the optimization by the software was given in the table.

Desirability contour plot and RS plot

They are the graphical representation of change in factors on the desirability function. It will be giving a better visualization of achieving the optimum condition by changing two factors at a time. Desirability plots shows how all the targeted optimum conditions are met by changing two factors at a time. The figure: 6 shows how factor G and B affects the desirability. Higher desirability will be achieved at maximum level of Nacl (more than 9%) and a lower concentrations propylene glycol. Lower concentrations of both factors yield desirability less than 6.

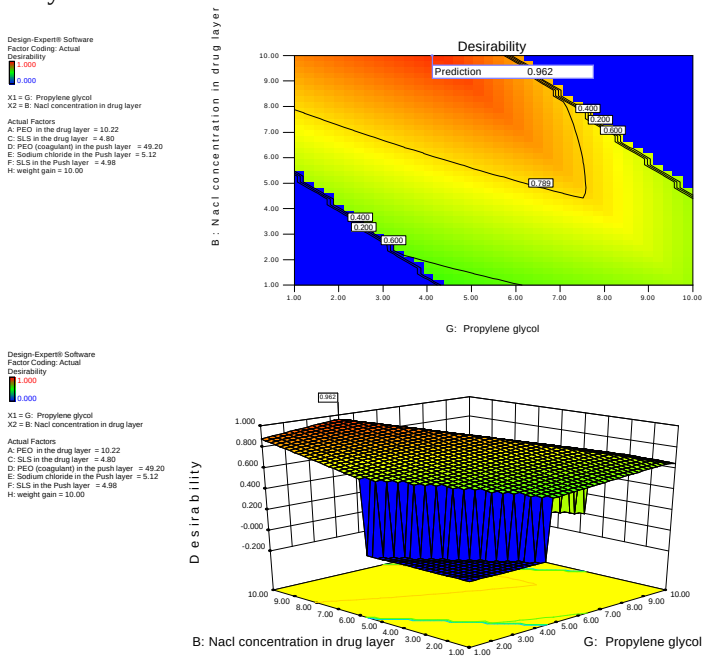


Table: 9 .The numerical optimization of Ropinirole Hcl Push pull OCOTs

Number	PEO DL*	Nacl DL	SLS DL*	PEO PL*	Nacl PL	SLS PL*	PG	Weight gain	CUR	R^2	lag time	Desirability
1	11.38	10.00	4.94	12.51	10.00	1.23	9.98	14.39	100.00	0.998	3.00	0.9622
2	27.97	10.00	4.94	26.69	10.00	1.59	9.68	14.00	100.00	0.998	3.00	0.9622
3	78.93	10.00	2.70	27.35	10.00	1.43	9.60	13.97	100.00	0.998	3.00	0.9622
4	48.37	10.00	3.12	28.40	10.00	3.08	9.59	13.95	100.00	0.998	3.00	0.9622
5	14.35	10.00	3.63	31.55	10.00	2.29	9.58	13.94	100.00	0.998	3.00	0.9622
6	10.10	9.87	1.55	11.17	10.00	1.03	6.38	10.03	100.00	0.998	3.00	0.9622
7	99.79	10.00	4.62	16.81	10.00	3.89	8.54	12.77	100.00	0.998	3.00	0.9622
8	99.83	10.00	4.69	43.10	10.00	4.32	8.49	12.72	100.00	0.998	3.00	0.9622
9	45.40	10.00	3.63	49.90	10.00	1.68	8.51	12.74	100.00	0.998	3.00	0.9622
10	70.42	10.00	3.08	11.72	10.00	1.49	6.77	10.08	100.00	0.998	3.00	0.9622
11	76.02	10.00	1.39	14.88	10.00	2.37	6.58	10.57	100.00	0.998	3.00	0.9622
12	61.17	10.00	3.03	29.10	10.00	3.01	6.55	10.53	100.00	0.998	3.00	0.9622
13	67.71	10.00	3.18	37.42	10.00	2.31	6.49	10.47	100.00	0.998	3.00	0.9622
14	83.39	10.00	2.24	32.78	10.00	3.99	6.43	10.40	100.00	0.998	3.00	0.9622
15	38.20	10.00	4.83	49.17	10.00	1.31	6.36	10.31	100.00	0.998	3.00	0.9622
16	59.28	10.00	1.00	24.28	10.21	3.17	8.60	12.83	100.00	0.998	3.00	0.9622
17	85.41	10.00	3.61	10.41	10.00	1.55	6.23	10.17	100.00	0.998	3.00	0.9622
18	14.35	10.00	4.97	49.29	10.00	4.90	6.10	10.02	100.00	0.998	3.00	0.9622
19	70.29	9.71	3.64	49.24	10.00	2.22	9.69	13.82	100.00	0.998	3.00	0.9621
20	100.00	9.72	1.01	30.68	10.02	4.49	10.0	14.19	99.97	0.998	3.00	0.9616
21	58.66	9.75	3.05	38.25	10.00	3.55	6.70	10.49	100.00	0.998	3.00	0.9614
22	49.62	10.00	4.44	46.34	10.01	1.00	10.0	15.11	98.51	0.997	3.10	0.9610
23	64.37	10.00	2.84	31.08	12.98	2.62	10.0	14.41	100.00	0.997	3.00	0.9604
24	10.25	10.00	2.61	33.35	10.00	4.47	10.0	15.67	97.33	0.9963	3.14	0.9598
25	82.71	10.00	1.17	44.98	13.97	1.00	9.98	14.39	100.00	0.9959	3.06	0.9582

Figure 6: The desirability contour plot and RS plot – Effect of Nacl DL and propylene Glycol

Figure :7 shows effect of weight gain and Nacl in the DL on desirability. The desirability was highest at High concentration of Nacl and the low concentration of weight gain.

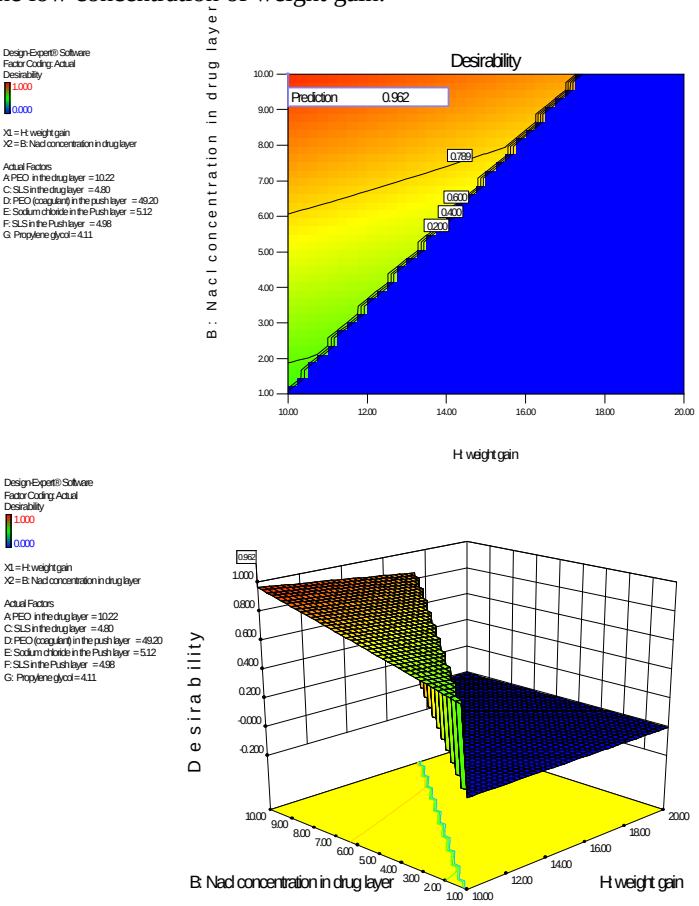
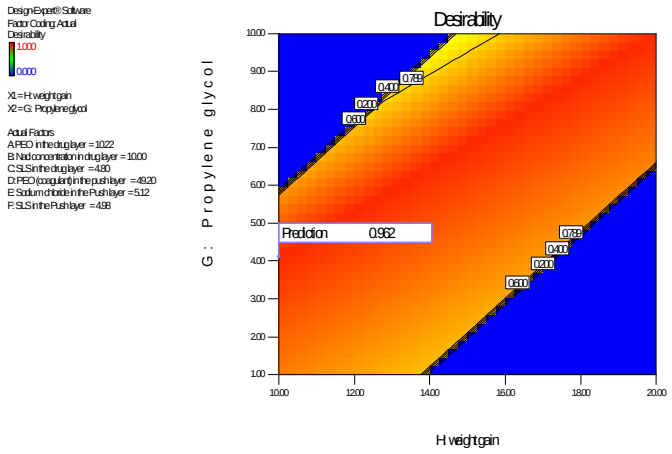


Figure 7 : The desirability contour plot and RS plot – Effect of Nacl DL and weight gain

Figure :8 shows the desirability contour plot of weight gain and propylene glycol. A larger portion of the contour plot shows the desirability close to one, indicates that these two factors were the major factors for achieving the desired optimum conditions.



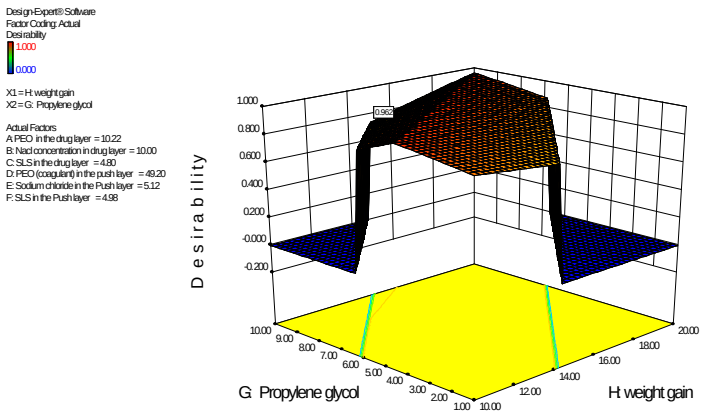
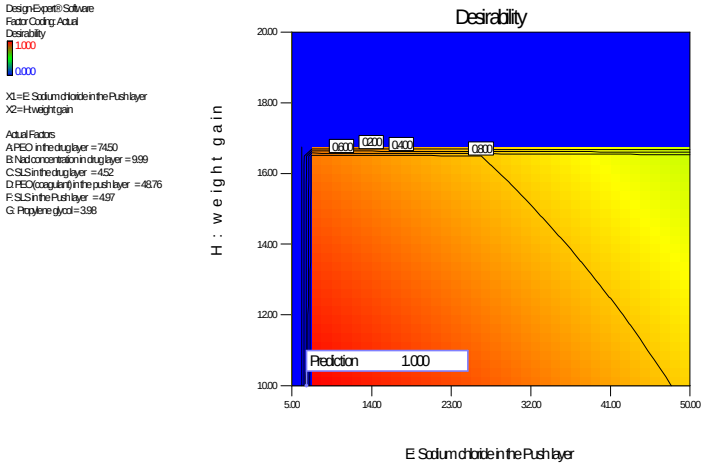


Figure 8 : The desirability contour plot and RS plot – Effect of Weight gain and Propylene glycol

The figure: 9 shows the desirability contour plot of Nacl in the Push layer and the weight gain .from the plot it was evident that a wide range of Nacl in PL can be used to get desirability more than one. Weight gain is again proved as one of the stringent factors as a slight change in factor shows a greater leap in the desirability from 1 to .2



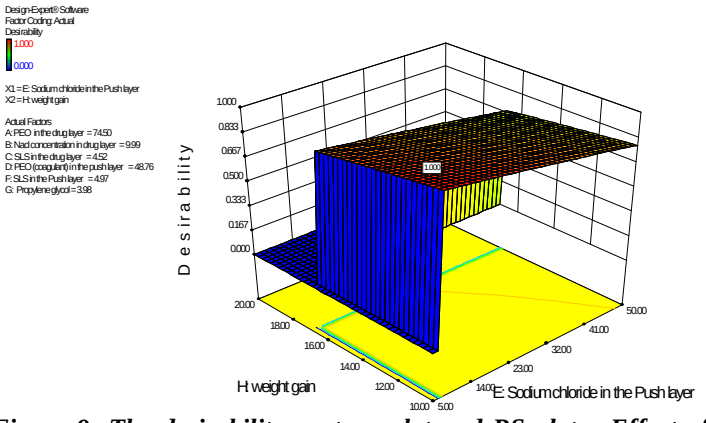


Figure 9: The desirability contour plot and RS plot – Effect of Weight gain and Nacl in the PL

The figure: 10 shows the desirability contour plot and the RS plot of simultaneous effect of Nacl in the DL and Nacl PL on the desirability. Optimum conditions reached while keeping the Nacl DL at high level and the Nacl in PL at low level. Below 3 % of Nacl DL change in concentration of Nacl PL had little effect on the desirability. Change in concentration of Nacl PL from low to high desirability decrease.

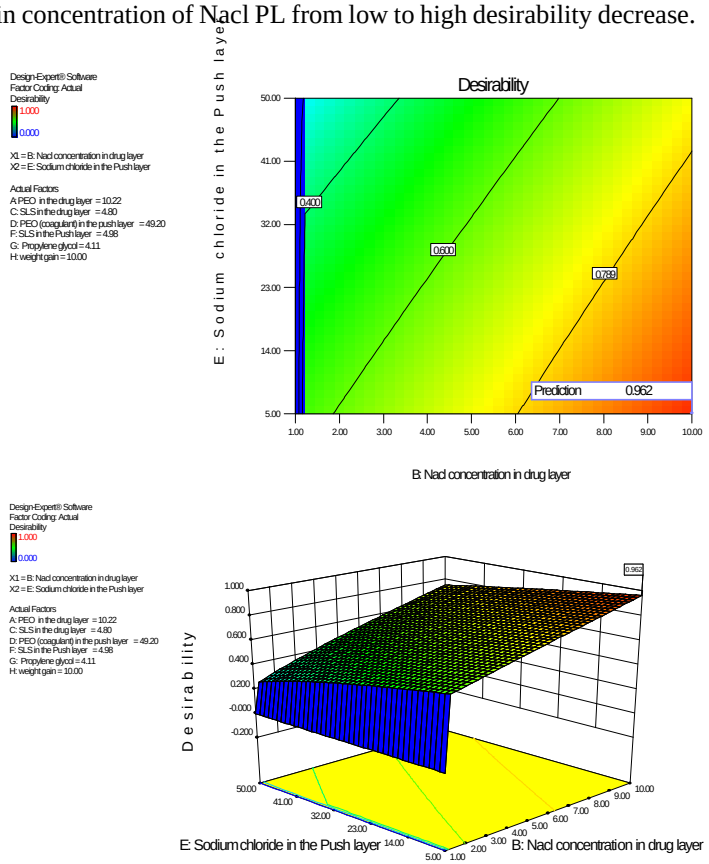


Figure: 10 .The desirability contour plot and RS plot – Effect of Nacl DL and propylene Glycol

The model exhibits linearity without any curvature and lack of fit for all the responses. So the optimization was done using the same design points by numerical optimization. The target kept for the optimization was PCUR at 24 hr more than 80% , lag time 3 hrs and R^2 0.999. The achievement of the optimum conditions was demonstrated by desirability function. A close to 1 desirability indicates all the targets were achieved. The desirability contour plots and RS plots were studied for the optimum combinations of factors. Higher desirability will be achieved at maximum level of Nacl (more than 9%) and a lower concentrations propylene glycol. The desirability was highest at High concentration (>9) of Nacl and the low concentration(less than 14) of weight gain .Nacl in the PL had comparatively less significant factor for achieving the optimum. A wide range of weight gain-propylene glycol combinations can be used for the optimization. High level of Propylene glycol had a desirability zero.

The release of the drug is mainly depends upon the amount of the water entered in to the system and the osmotic pressure created inside the systems. The water entry in to the system was controlled by the weight gain and the amount of the plasticizer present in the coating. The osmotic pressure created would be directly proportional to the water entered in to the system and the concentration of the osmotic agent present in the system. So at lower weight gain if the plasticizer increases more amounts of the water influx produces, decrease the lag time and increase the PCUR. From the study it can be concluded that for design and development of Push pull osmotically controlled oral system of ropinirole Hcl , researchers can concentrate more on the coating and the DL Nacl for the optimization .

Prediction of responses

The point prediction for the solution 2, 6 and 18 were given in the table: 10. The same batches were selected as the check point batches. The confidence interval, prediction interval and the tolerance interval were given in the table: 10. All the values of the responses were within the prediction interval and within the confidence interval. Thus the model selected i.e., the 2_{IV}^{8-4} fractional factorial design for the optimization of the osmotically controlled oral tablets of Ropinirole Hcl was a validated one and can be used for the further predictions.

Table: 10. The prediction of the responses

Solution 2								
Response	Predicted Mean	Observed	Std Dev	SE Mean	CI for 95% CI low	Mean 95% CI high	99% of 95% TI low	Population 95% TI high
CUR	100.0000	-	10.4605	4.1884	91.1210	108.8789	54.4907	145.5092
R2	0.9908	-	0.0307	0.0128	0.9638	1.0179	0.8578	1.1239
lag time	3.0623	-	0.2529	0.1012	2.8477	3.2769	1.9622	4.1624
Solution 6								
Response	Predicted Mean	Observed	Std Dev	SE Mean	CI for 95% CI low	Mean 95% CI high	99% of 95% TI low	Population 95% TI high
CUR	99.9999918	-	10.46049115	4.38163664	90.7113371	109.288647	54.1532983	145.846685
R2	0.99084622	-	0.030694702	0.01284003	0.96375612	1.01793631	0.85780867	1.12388376
lag time	3.06077298	-	0.252858656	0.10591613	2.83624081	3.28530515	1.95253311	4.16901284
Solution 18								
Response	Predicted Mean	Observed	Std Dev	SE Mean	CI for 95% CI low	Mean 95% CI high	99% of 95% TI low	Population 95% TI high
CUR	99.9999315	-	10.46049115	4.17056403	91.1587307	108.841132	54.5217462	145.478117
R2	0.99084989	-	0.030694702	0.0128405	0.9637588	1.01794097	0.85781152	1.12388825
lag time	3.06249332	-	0.252858656	0.10081393	2.84877734	3.2762093	1.9631613	4.16182534

Check point batch

To confirm the validity of the model three formulations from the solutions were selected and formulated as discussed in the methods. The dissolutions were performed as per the method specified in section in triplicate. The value obtained from the dissolution study was given in the table 11. All the responses were within the CI, Prediction interval and tolerance limits of the point predicted by the software. Hence it can be concluded that the model suggested for the study was a success and can be used for further predictions.

Table No 11: check point batches for the model validation of the Ropinirole Hcl osmotic tablets

Batches	PCUR at 24 hrs	R2	Lag time
Solution 2	100.18	0.998	3.05
Solution 6	99.8	0.9988	3.00
Solution 18	101.2	0.998	3.08

Hence it can be concluded that the design space provided by the software can be very well used for the formulation modifications and better optimization according to the need of the manufacturer.

CONCLUSION

Formulation and Optimization of the Push pull osmotic tablets of Ropinirole Hcl was successfully done with the help design of experiments. A better understanding on the achievement of target profile was well demonstrated and studied with the help of desirability function.

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Research Article

FACTOR INFLUENCE STUDY OF IVABRADINE HCL OSMOTIC PUSH PULL TABLETS USING FRACTIONAL FACTORIAL DESIGN

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ABSTRACT

A push pull osmotic tablets of Ivabdradine was formulated and different factors affecting the release profile were studied with the help of design of experiments. A fractional factorial design was used for the factor influence study. Various core and coating factors were selected for the study. The responses selected were lag time, Zero order rate constant, PCUR at 24 hrs. ANOVA and regression analysis were used for the identification of significant factors and constructing the polynomial equation representing the responses. A 5 % SL ($p< 0.05$) was chosen for the study. Various plots like Half normal plot, Normal plot, Pareto chart were also studied. The factors which were affecting the PCUR at 24 hrs were identified as Propylene glycol > weight gain> Nacl in the DL. The significant factors which were affecting the R^2 were Nacl DL> Nacl PL. The lag time for the drug release was greatly affected by PEG > Weight gain > Nacl in the DL. The simultaneous effect of two factors were represented and studied with the help of contour plots and response surface plots.

Keyword: Design of experiments, PCUR, Contour plot, Response surface plot, Factor influence study.

INTRODUCTION

Design of experiments is nowadays widely used for the pharmaceutical product development and optimization. One has to be very conscious while choosing the right design for any study. As the statistical designs are based on assumptions, a wise selection of design is mandatory for the success of the research. Plenty of designs are available for designing the experiments during the product development. The following flow chart will be showing the basic steps for the systematic approach followed while applying the DoE in product development. With the help of the designed experiments, the effects of multiple variables on the responses can be studied. When sufficient literatures are available about the different factors affecting the product as well as process, the first step, ie, the screening study can be omitted. Out of many trivial factors the vital factors were identified and can proceed with the factor influence study. Factor influence study will be helpful for identifying and quantifying the significant factors. So final optimization can only include the significant factors identified after factor influence study. This step will minimize the number of factors included in the optimization study thus drastically reducing the experimental trials.

Fractional factorial designs are reduced factorial designs which can be used when many vital factors are to be included in a factor influence study. In the present study, push pull osmotic tablets of ivabradine Hcl were developed with the help of fractional factorial design. Numbers of factors were identified as vital after screening study. So before optimization a factor influence study was performed to quantify the effect of the vital factors. This would be helpful for the optimization of the formulation where we can only concentrate on the highly significant factors obtained after factor influence study.

Thus the number of experimental trials can be further minimized and better design can be selected for the optimization. Ivabradine Hcl, A heart rate lowering agent used for the treatment of Symptomatic treatment of chronic stable angina pectoris in coronary artery disease adults with normal sinus rhythm. The formulation is available in the market as immediate release dosage form to be taken twice daily. A once daily Osmotic drug delivery system of Ivabradine Hcl was developed with an intention of more patient compliance. A zero order release, which reduces the fluctuations in the plasma concentration, is only expected in the case of osmotically controlled systems.

MATERIALS AND METHODS

Materials

The Ivabradine Hcl was received as gift sample from Alembic Pharmaceuticals Limited; Butylated hydroxyl toluene, Sodium lauryl sulfate, Sodium chloride was received from Merck; Polyethylene Oxide and cellulose acetate was received from signet; Dibasic calcium phosphate from Innophos; Magnesium stearate from Ferro; iron oxide and Propylene glycol from Alembic Limited. Other regents were of analytical grade.

Methods

I. Formulation and evaluations of Push pull osmotic tablets of Ropinirole Hcl [2]

A fractional factorial design with 8 selected factors **2 [8-4]**(ie 1/32 fraction) with **Resolution IV** was selected for the study. 16 trials with 4 centre points were planned for the study. The selected factors with levels chosen are given in the Table 1. The responses selected for the study were given in the Table: 2. The formula table showing all the ingredients taken in each trial is explained in the Table: 3 and 4.

The prepared granules of both the pull layer and push layer of trial 1- 20 were weighed separately in sachets. First drug layer was compressed using rotary tablet compression machine and made thin tablet and then push layer was added by setting the dye cavity and in the upper pull layer tablet was put as a plug and final sharp compression was carried out. By this bilayer tablets were made. Hardness was adjusted while compressing the granules. 5.0 mm biconcave punch was used in preparation of bi layer tablets.

Coating and drilling of core tablet.[3,4]

The prepared bi layer tablets were then coated with coating solution (Acetone :water 90:10) Coating of core tablet was done by conventional coating method in coating pan. 10 tablets were removed at an interval of 30min and increase of weight was noted down until it was observed sufficient %wt gain. Coated tablets were allowed to dry completely in a hot air oven at 60^0C and finished by standard polishing procedure. The drug delivery orifice having diameter of 0.6 mm was made on the surface of one side of the tablets(above the drug layer) by using Micro drill. High speed stainless steel drill bits were used for drilling.

Flow chart of the proceedings using DoE [1]

The systematic steps of the product optimization using DoE is shown in the flow chart given in figure.1

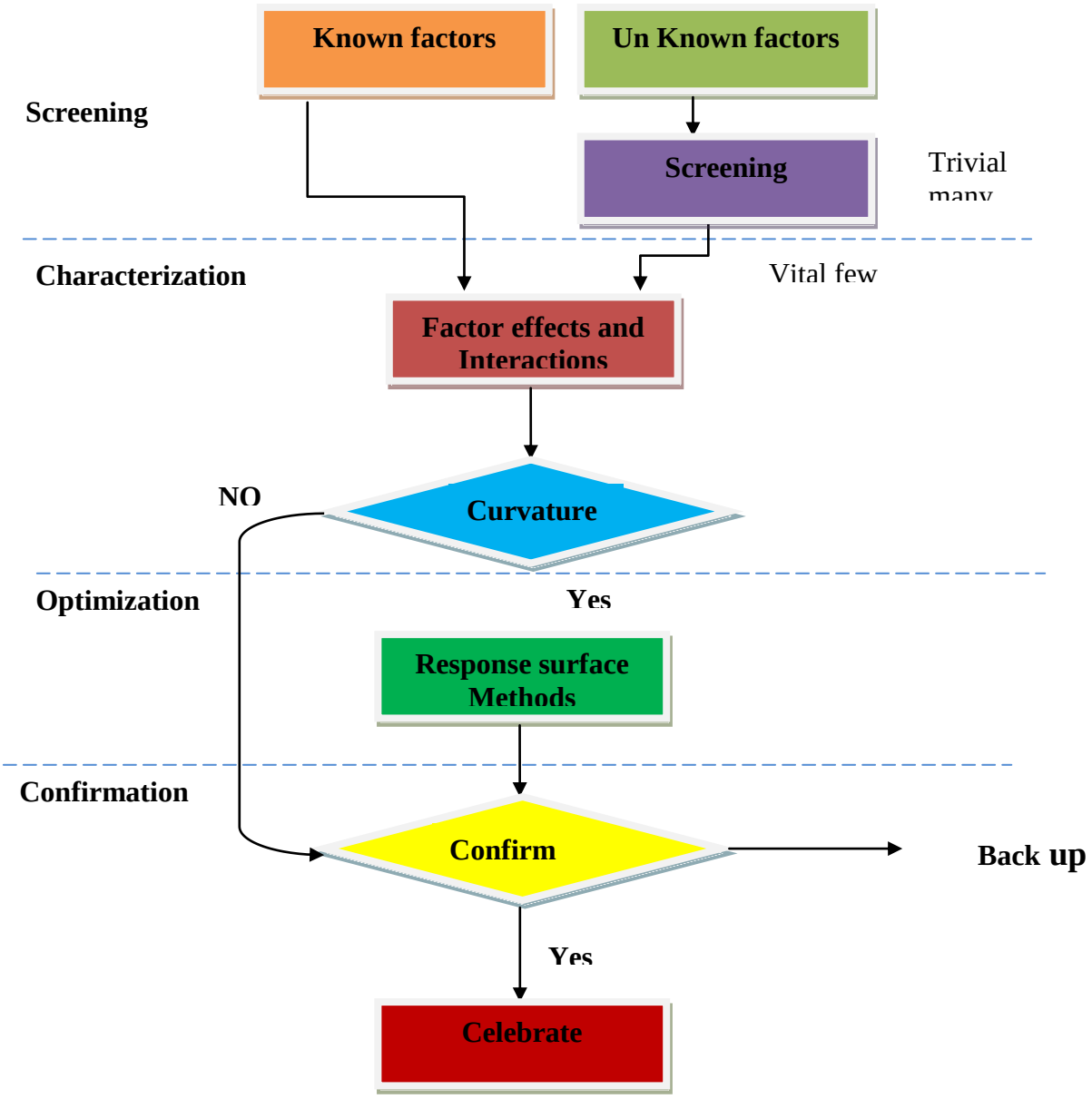


Fig. 1: It shows systematic steps of the product optimization using DoE

Table 1: It shows the selected Factors with levels affecting osmotic push pull delivery system for the factor influence study

Factors		Levels	
		Min(-)	Max(+)
1	PEO in the drug layer (% w/w of the API)	10	100
2	Nacl concentration in drug layer(% w/w core tablet)	1	10
3	SLS in the drug layer (%w/w of the drug layer)	1	5
4	PEO (coagulant) in the push layer (% w/w of the drug layer)	5	50
5	Sodium chloride in the Push layer (% w/w % of the extender)	5	50
6	SLS in the Push layer(%w/w of the push layer)	1	5
7	Propylene Glycol (% w/w of the coating weight)	1	10
8	Weight gain (%)	10	20

Table 2: It shows the selected response for the factor influence study

Response	Unit	Weightage
Cumulative release at 24 Hrs	%	+++++
R^2	----	++++
Lag time	Hrs	+++

Table 3: It shows the contents of the formulation IB 1-10

	Ingredients	IB 1	IB2	IB3	IB4	IB5	IB6	IB7	IB8	IB9	IB 10
1	ivabradine hydrochloride	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91
2	Dibasic calcium phosphate	36.49	26.67	31.99	22.17	34.49	24.67	29.99	20.17	36.47	26.65
3	PEO 400 K	1.09	10.91	1.09	10.91	1.09	10.91	1.09	10.91	1.09	10.91
4	Sodium chloride	0.50	0.50	5.00	5.00	0.50	0.50	5.00	5.00	0.50	0.50
5	BHT	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.025	0.025
6	SLS	0.50	0.50	0.50	0.50	2.50	2.50	2.50	2.50	0.50	0.50
7	IPA										
8	Magnsium stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
9	PEO 7000 K (WSR 302)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	25.00	25.00
10	Sodium chloride	0.13	1.25	1.25	0.13	1.25	0.13	0.13	1.25	1.25	12.50
11	Dibasic calcium phosphate	39.62	38.50	36.70	37.82	36.70	37.82	39.62	38.50	14.18	2.93
12	BHT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03
13	SLS	0.45	0.45	2.25	2.25	2.25	2.25	0.45	0.45	2.25	2.25
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA										
16	Magnsium stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
15	Cellulose acetate	9.3	18.1	18.9	7.6	8.6	18.8	17.1	9.4	18.1	9.3
16	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
18	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Propylene Glycol	0.19	0.95	0.10	1.90	0.95	0.19	1.90	0.10	0.95	0.19
Total Weight of Coating		9.5	19.0	19.0	9.5	9.5	19.0	19.0	9.5	19.0	9.5
Total tablet weight		104.5	114.0	114.0	104.5	104.5	114.0	114.0	104.5	114.0	104.5

Table 4: it shows the contents of the formulation IB 11-20

	ingredients	IB11	IB 12	IB 13	IB 14	IB15	IB16	IB17	IB18	IB19	IB20
1	ivabradine hydrochloride	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91	10.91
2	Dibasic calcium phosphate	31.97	22.15	34.47	24.65	29.97	20.15	28.32	28.32	28.32	28.32
3	PEO 400 K	1.09	10.91	1.09	10.91	1.09	10.91	6.00	6.00	6.00	6.00
4	Sodium chloride	5.00	5.00	0.50	0.50	5.00	5.00	2.75	2.75	2.75	2.75
5	BHT	0.025	0.025	0.025	0.025	0.025	0.025	0.014	0.014	0.014	0.014
6	SLS	0.50	0.50	2.50	2.50	2.50	2.50	1.50	1.50	1.50	1.500
7	IPA										
8	Magnsium stearate	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
9	PEO 7000 K (WSR 302)	25.00	25.00	25.00	25.00	25.00	25.00	13.75	13.75	13.75	13.75
10	Sodium chloride	12.50	1.25	12.50	1.25	1.25	12.50	3.78	3.78	3.78	3.78
11	Dibasic calcium phosphate	4.73	15.98	4.73	15.98	14.18	2.93	23.81	23.81	23.81	23.81
12	BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.01	0.01	0.01	0.01
13	SLS	0.45	0.45	0.45	0.45	2.25	2.25	1.35	1.35	1.35	1.35
14	Iron oxide Red	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
15	IPA										
16	Magnsium stearate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
15	Cellulose acetate	8.6	18.9	18.8	8.6	9.4	17.1	13.5	13.5	13.5	13.5
16	Acetone	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
18	Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
19	Propylene Glycol	0.95	0.10	0.19	0.95	0.10	1.90	0.78	0.78	0.78	0.78
Total weight of coating		9.5	19.0	19.0	9.5	9.5	19.0	14.3	14.3	14.3	14.3
Total tablet weight		104.5	114.0	114.0	104.5	104.5	114.0	109.3	109.3	109.3	109.3

In vitro dissolution study [5]

Dissolution test was performed using USP II paddle apparatus (DS-8000, Lab India, Analytical instrument pvt ltd, Navi Mumbai, India.) at 37˚C± 0.5˚C in 900 ml of phosphate buffer 6.8. Paddle speed was kept at 50 rpm. Samples were withdrawn after predetermined. time intervals of 1,2,3,4,6,8,12,16,20,24 hrs and the drug content was measured using an UV spectrophotometer at the 286 nm. Samples were suitably diluted and absorbance was measured. Cumulative percentage drug released was calculated for each batch. The study was performed in triplicate and the average was reported. The data of % cumulative release from each trial batch were subjected to kinetic release studies to assess the fit into the zero-order release kinetics. The R^2value was found out to determine the best fit zero order release kinetics.

Analysis of responses [6,7]

For all the batches, the Push pull osmotic tablets were formulated as per the procedure explained in the methods. All the batches were subjected to in vitro dissolution using USP II (paddle) Apparatus up to 24 hour. The samples were withdrawn at an interval of 1hr and analyzed using UV Spectrophotometer (Shimadzu1800) at 286 nm. From graph of %cumulative drug release verses time, lag time ($t_{10\%}$) T90 and R^2 values were determined.

ANOVA and regression analysis

The results obtained for the study design was analysed with the help of design expert software and significance of factors were found out by ANOVA analysis. The hypothesis were tested with a level of significance 5 % ($p < 0.05$)

Polynomial equation

From the regression analysis of the responses the mathematical equation can be constructed which can be used for the prediction of the responses at any selected levels of the factors. If the suggested model for the optimization is linear, the following linear model would be used,

$$Y= \beta_0 + \beta^1 X_1 + \beta_2 X_2 + \beta_3 X_3 \ldots\ldots\ldots\ldots + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 + \text{error}$$

Half Normal plot and normal plot

For 2-level factorial designs, this plot can be used to choose significant effects.

Normal plot

For 2-level factorial designs, this plot can be used to choose significant effects. They show up as outliers on the normal probability plot.

Pareto chart

Pareto chart is bar graph for the clear identification of the significant factors.

Contour plots and response surfaces plots

Contour plot is a 2D graphical representation of the effect of less than 3 factors on a single response. Response surface plots are the 3D version of the contour plot. A better understanding will be possible with the help of response surface plots.

RESULT AND DISCUSSION

Factor influence study of the Ivabdadine Hcl push pull osmotic tablets was done with the help of 2_{IV} [8-4] fractional factorial designs. Twenty trials were formulated as per the procedure given in the materials and methods.

The invitro dissolution of each trial was performed as per the procedure given in the materials and methods. The PCUR at 24 hrs, R^2, and the lag time was recorded and analysed with the help of design expert software 8.0.7.1 version. The result of the invitro dissolution profile was given in the Table.5.

Analysis of responses – PCUR at 24 hrs

With the help of the half normal plot, normal plot and pareto chart the significant factors affecting the PCUR was determined. The plots are given in the figures 2, 3 and 4. From the graph it was evident that the factor which are affecting the cumulative release up to 24 hrs are B (Nacl in the DL), G (Propylene glycol) H (the weight gain). The Shapiro-Wilk Normality Test indicates the non significance of the non selected factors. From the pareto chart also it was clearly evident that the factors B, G, H are the significantly affecting the cumulative response at 24 hrs. All the factors cross the t limit and G and H crosses the Bonferroni limit. The magnitude of the effect can be written as Propylene glycol > weight gain > sodium chloride in the drug layer.

Table 5: It shows the result of the dissolution study

Trial NO	CUR at 24 hr	R^2	Lag time
IB1	70.1	0.952	4.1
IB2	52.3	0.8489	3.5
IB3	70.4	0.9315	4
IB4	100	0.997	3
IB5	82.2	0.8713	3.5
IB6	35.3	0.9531	4.5
IB7	84.2	0.9993	3.6
IB8	100	0.9549	3
IB9	82.5	0.9598	4.2
IB10	76	0.8582	4.1
IB11	100	0.9689	2.9
IB12	55.1	0.9982	4.7
IB13	50	0.8573	5
IB14	90.2	0.8614	3.5
IB15	78.5	0.9978	3.5
IB16	100	0.9781	3.1
IB17	80.5	0.9263	4
IB18	79.2	0.9455	3.4
IB19	80.2	0.9331	4
IB20	79	0.9229	3.6

The significance level selected for the study was 5 % and the p value was 0.05. Values of "Prob > F" less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. In this case **B (p =***0.0062)***, G (p =***0.0018)***, and H (p =***0.0024)** are significant model terms. The Model F-value of 12.29 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise. The "Lack of Fit F-value" of 5.00 implies the Lack of Fit is not significant relative to the pure error.

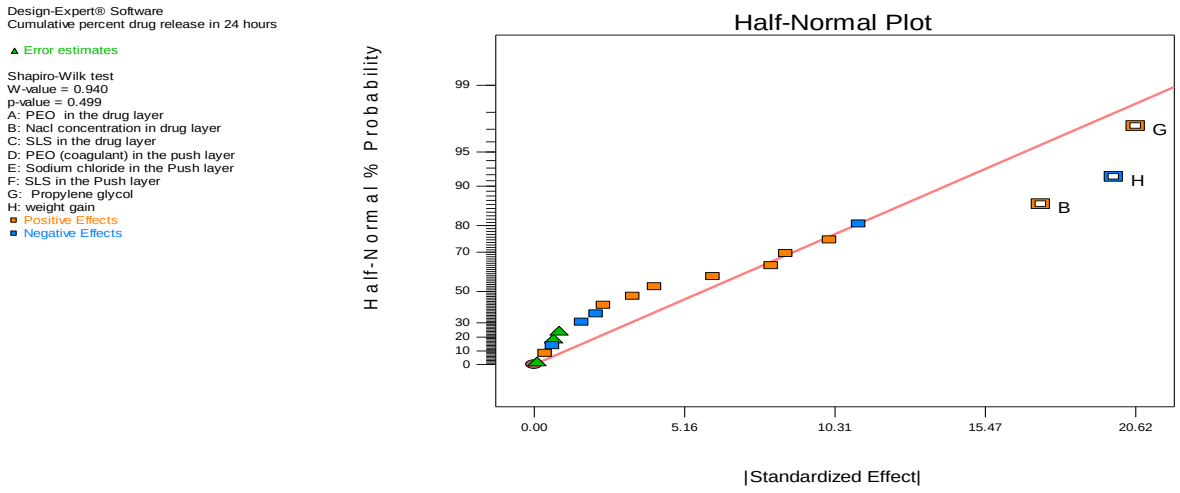


Fig. 2: It shows the Half Normal plot of the effect of the factors on PCUR at 24 hrs

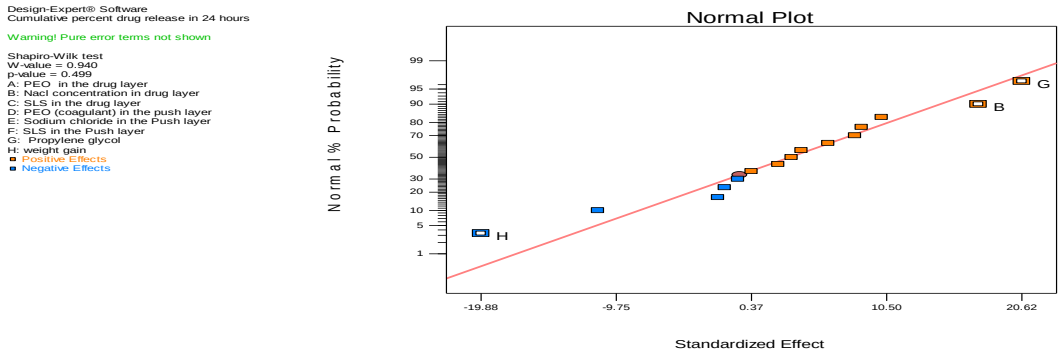


Fig. 3: It shows the Normal plot of the effect of the factors on PCUR at 24 hrs

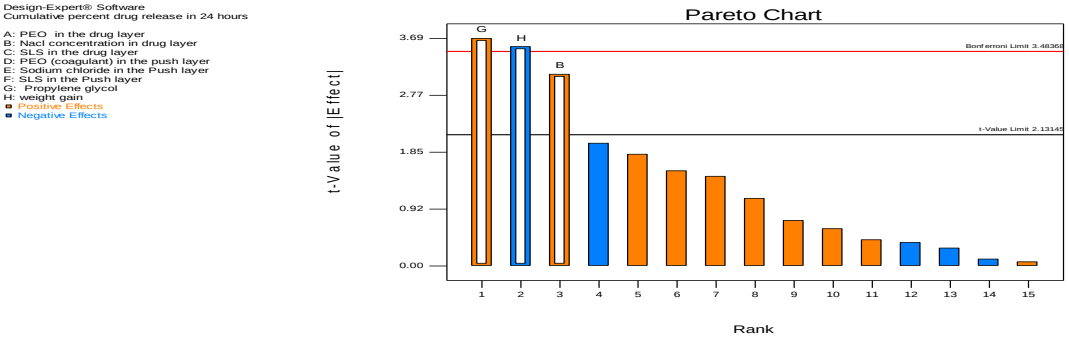


Fig. 4: It shows the Pareto chart of effect of the factors on PCUR at 24 hrs

The polynomial equation representing the PCUR at 24 hrs was given as,

Cumulative percent drug release in 24 hours = +84.64028+1.93056 * Nacl in drug layer +2.29167 * Propylene glycol -1.98750 * weight gain

Figure 5 shows the contour plot and response surface plot of the simultaneous effect of Nacl DL and Propylene glycol on the PCUR at 24 hrs at a time. At lower concentration of propylene glycol Nacl DL had little effect. High PCUR at 24 hrs would be expected at high levels of both the factors.

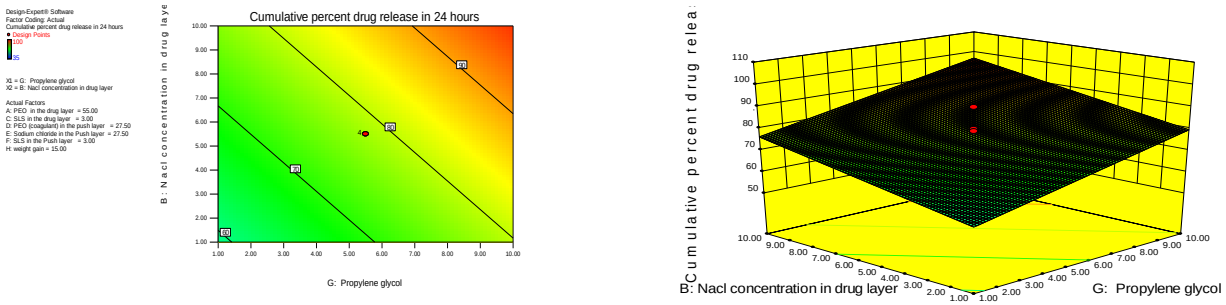


Fig. 5: It shows the Contour plot and RS Plot – Effect of Nacl DL and Propylene Glycol on PCUR at 24 hrs

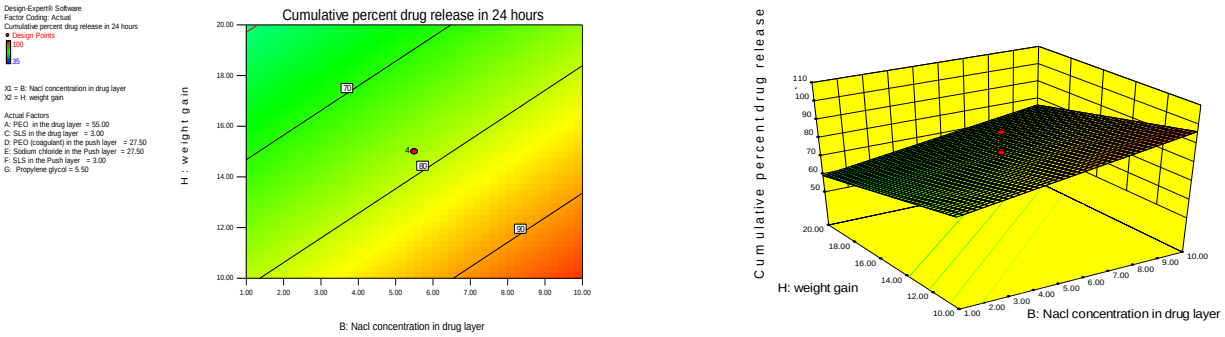


Fig. 6: It shows the Contour plot and RS Plot – Effect of Nacl DL and weight gain on PCUR at 24 hrs

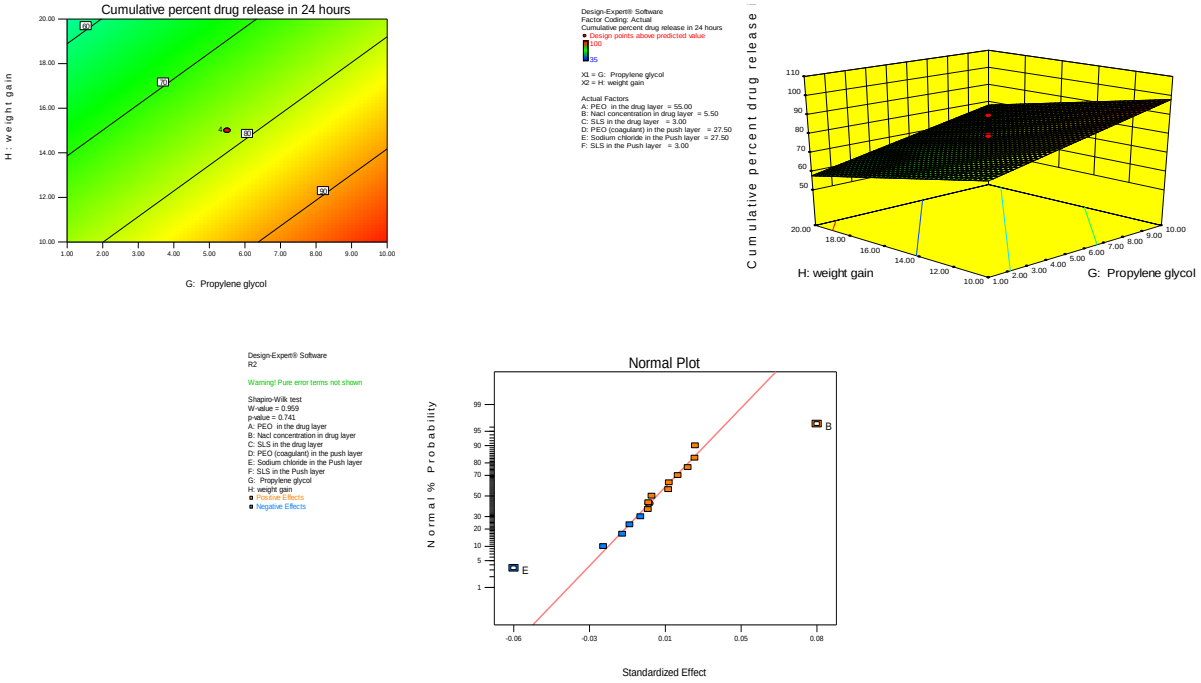


Fig. 7(1): It shows the Half Normal plot of the effect of the factors on R^2

Fig. 7: It shows the Contour plot and RS Plot – Effect of Nacl DL and weight gain on PCUR at 24 hrs

Figure.6 shows the Contour plot and Rs plot, showing the change in PCUR at24hr with the change in weight gain and Nacl.Nacl DL had a positive effect and Weight gain had an opposite effect on the response. At the low levels of weight gain the Nacl had a prominent effect.

Figure.7 shows the contour plot and RS plot showing the simultaneous effect of weight gain and propylene glycol on the PCUR at 24hrs. At 10 % of the weight gain the response is more prone to slight changes propylene glycol. But at a higher weight gain even a 10% of propylene glycol is not sufficient to achieve 80% release at 24hrs.

Analysis of responses - Rate constant(R^2)

With the help of the half normal plot and normal plot shown in figure 7 and 8, the significant factors affecting the $R^2$was determined. The plots are given in the figures and. From the graph it was evident that the factor which are affecting the release rate constant (R^2)were B (Nacl in the DL) and E(Nacl in the push layer). The Shapiro-Wilk Normality Test displayed the non significance of the non selected factors.

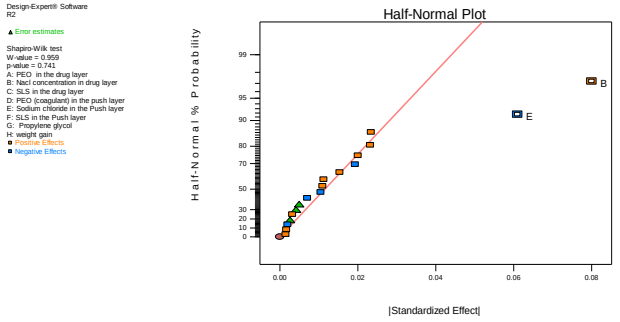
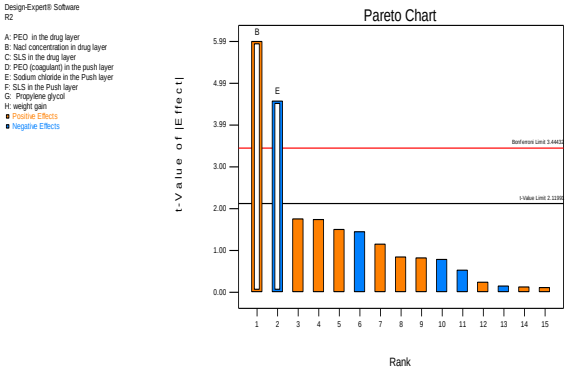


Fig. 8: It shows the half Normal plot of the effect of the factors



onR2

Fig.9: It shows the Pareto chart of effect of the factors on R^2

The Pareto chart shown in figure.9 represents the significant effect of B and E on the zero order rate constant. Both the factors crosses the t and Bonferroni limit confirm the obvious effect of these factors on the zero order rate constant. The magnitude of the effect can be written as Nacl in the drug layer > Nacl in the push layer. The F-value from the ANOVA analysis 29.11 implies the model is significant. Factors **B (p =<** *0.0001)*, **E(***0.0002)* are significant model terms.. The "Lack of Fit F-value" of 0.3706 implies the Lack of Fit is not significant relative to the pure error. The polynomial equation representing R^2 can be written,

R^2 =+0.91727+9.10833E-003* Nacl concentration in drug layer - 1.38833E-003* Sodium chloride in the Push layer. The Figure.10 shows the Contour plot and response surface plot for the simultaneous effect of factor B and E at a time. From the plot it is obvious that the factor B had a positive effect and E had a negative effect. High levels of Nacl in the DL and low levels of Nacl in the PL yields a better R^2 value. The change in concentration of Nacl in the DL is more evident at low level of Nacl in the PL.

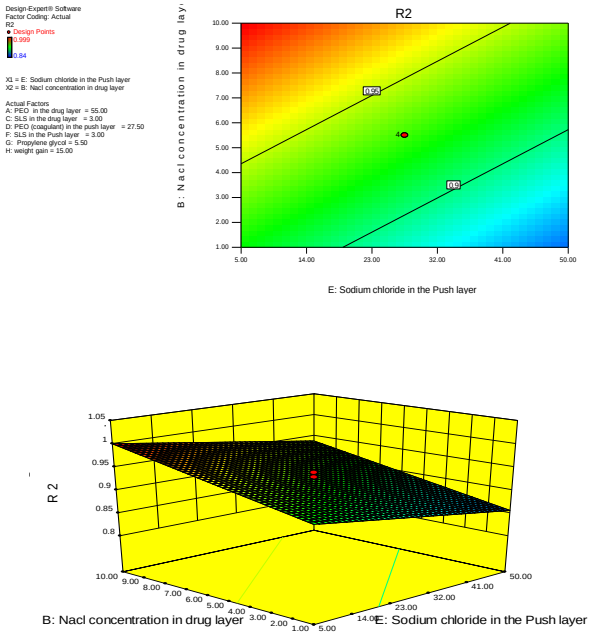


Fig. 10: It shows the Contour plot and RS Plot – Effect of Nacl DL and Nacl in PL on R^2

Analysis of responses – lag time

Figures 11, 12 show the half normal plot and normal plot of the effect of factors on the R^2. The significant factor affecting the lag time was identified as B (Nacl in the DL) G (propylene Glycol), H (the weight gain) The Shapiro-Wilk Normality Test displayed the non significance of the non selected factors**.** So no other factors except B, G, and H are affecting the lag time**.**

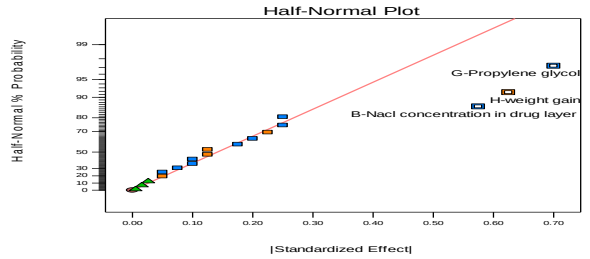


Fig. 11 : It shows the Half Normal plot of the effect of the factors on lag time

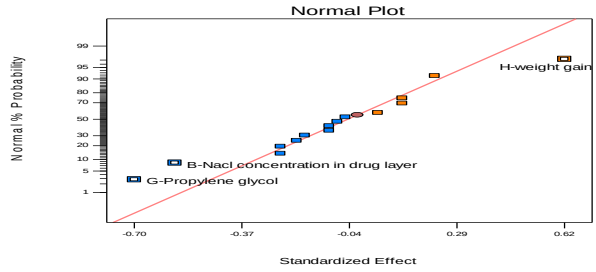


Fig. 12: It shows the Normal plot of the effect of the factors on lag time

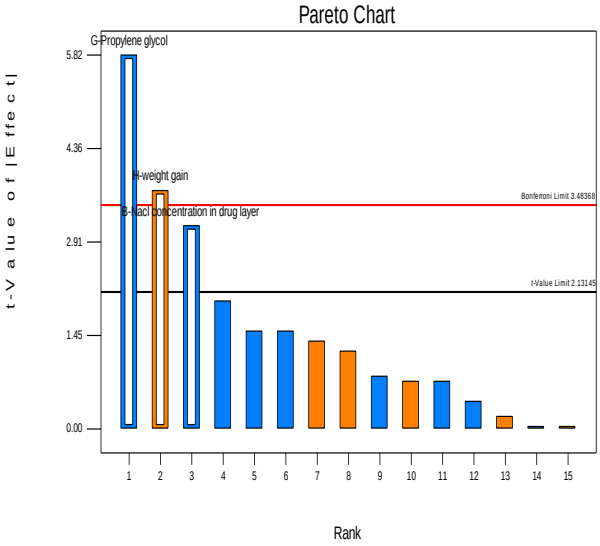


Fig. 13: It shows the Pareto chart of the effect of the factors on lag time

Figure 13 shows the Pareto chart of effect of factors on the lag time in terms of T value. The factors significantly affecting the lag time were G, H and B accordingly. G and B had a negative effect and H had a positive effect. Propylene glycol had greater effect on the lag time. The magnitude of the effect of significant factors on the lag time can be written as G > H> B. No other factors or interaction terms were significant as they does not crosses the t limit.

The significance level selected for the study was 5 % and the p value was 0.05. The Model F-value of 23.8514 implies the model selected is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Factors **B (**3.292E-[04]**), G(**9.855E-[05]*)and H (*1.467E-[04]*)*were the significant model terms affecting the lag time. The "Lack of Fit F-value" of 2.7202,implies that the Lack of Fit is not significant relative to the pure error. this means that the polynomial model is fitting all of the design points well. The polynomial equation representing the lag time was written as,

lag time =3.5211- 0.0639* Nacl DL- 0.0722 * PG + 0.0625 * weight gain (Actual terms)

Figure 14 shows the contour plot of the simultaneous effect of factor B and the G at a time. At low levels of Propylene glycol even 10% of Nacl in the level was not sufficient to produce the desired lag time. But at high level of Propylene glycol 1% or less Nacl can sufficient for the desired effect. Nacl had seldom effect at the high level of propylene glycol. Response surface clearly represents the chief effect of factor G.

The Figure 15 shows the Contour plot and Rs plot, of the simultaneous effect of factor B and the H at a time. Nacl had a negative effect on the lag time ie, as the concentration changes from low to high the lag time decreases. But the weight gain had an opposite effect. At low weight gain and high concentration of Nacl in the DL produces the desired effect. Nacl had a prominent effect at low weight gain. Figure 16 shows the contour plot and response plot of the combined effect of propylene glycol and weight gain at a time. Weight gain had a positive effect on the lag time and propylene glycol had a reverse effect. Effect of propylene glycol was more pronounced at low weight gain. From the RS plot the greater effect of the propylene glycol is well understood.

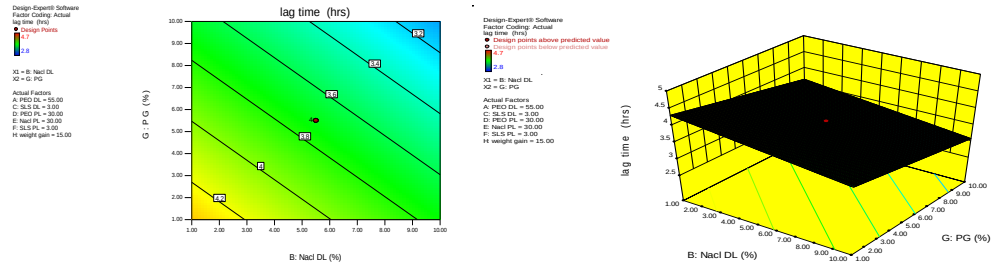


Fig. 14: It shows the Contour plot and RS Plot – Effect of Nacl DL and Propylene Glycol on lag time

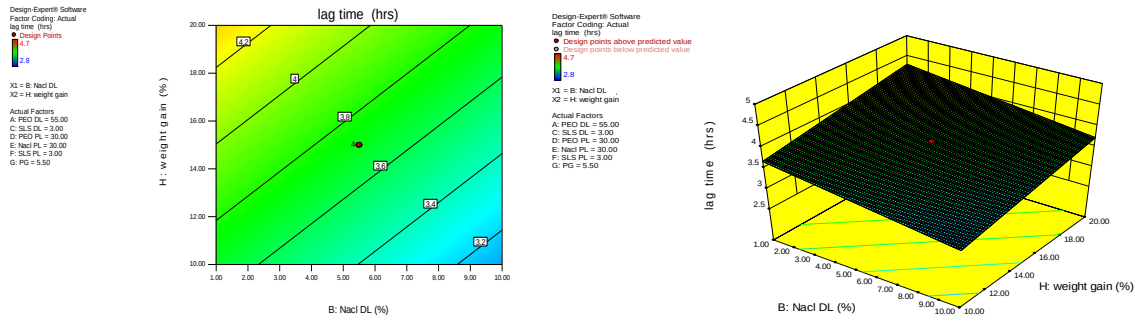


Fig. 15: It shows the Contour plot and RS Plot – Effect of Nacl DL and weight gain on lag time

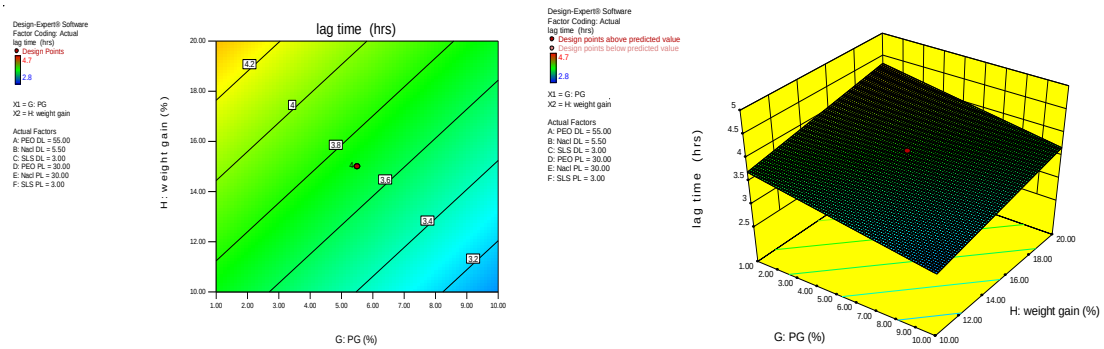


Fig. 16: It shows the Contour plot and RS Plot – Effect of Weight gain and Propylene Glycol on lag time

CONCLUSION

The factor influence study of the ivabradine Hcl push pull osmotic tablets was done with the help of 2 $_{IV}$ [8-4] fractional factorial design. Core factors and coating factors are combainly selected for the factor influence study. The effect of the factors on the responses like PCUR at 24 hrs, Zero order rate constant and lag time were studied. It was found from the study that the most significant factors which affecting the responses were Propylene glycol (plasticizer), weight gain and the Nacl in the DL. Researchers can concentrate more on the coating parameters and Nacl DL for the optimization of Ivabradine Hcl Push pull osmotic tablets. A better chance of variation with in the design space without affecting the desired profile can be possible with change in coating parameters for the formulation of a push pull osmotic tablets ivabradine Hcl.

Conflict of Interest: None

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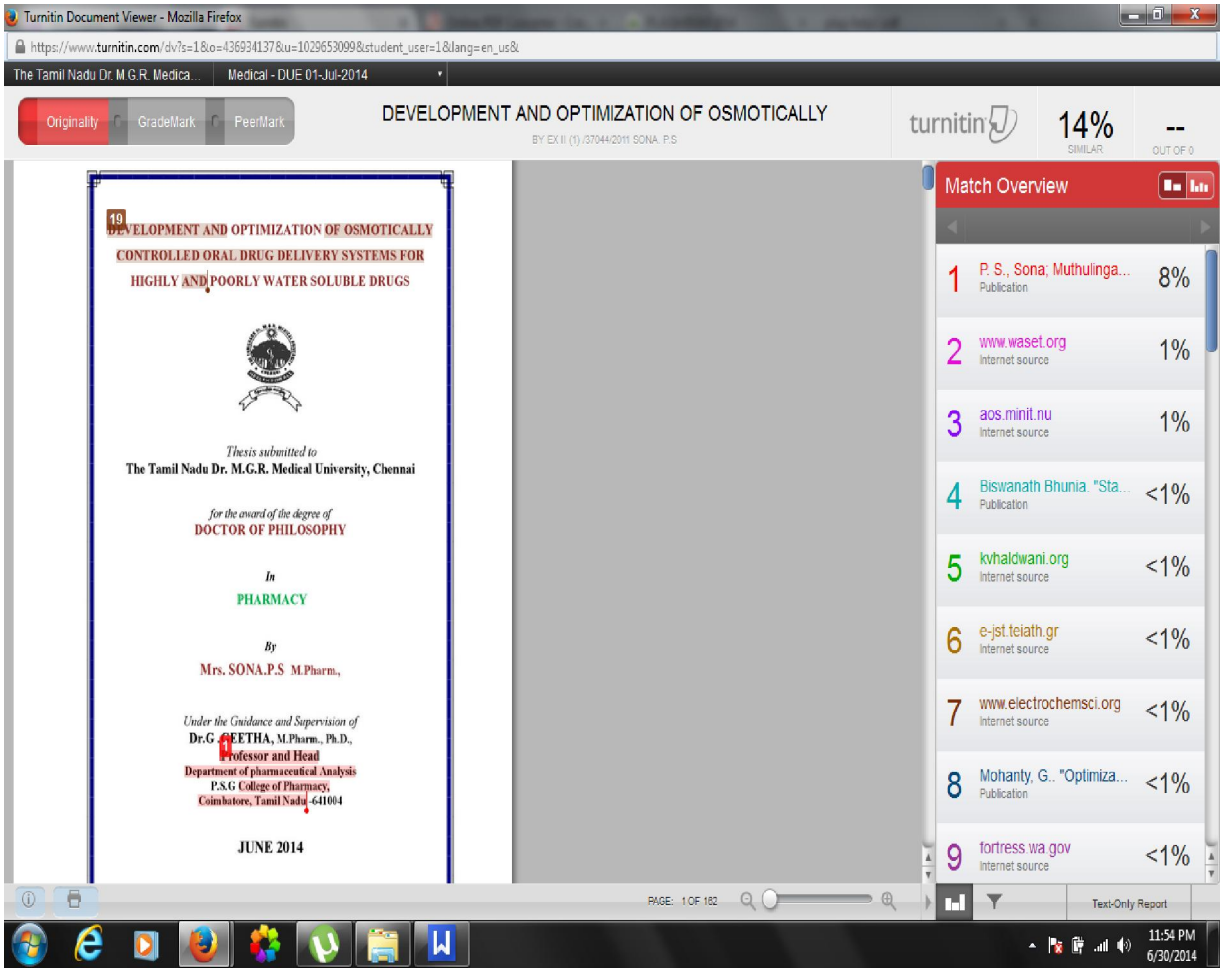
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