FORMULATION AND EVALUATION OF DAPOXETINE HCL NANOPARTICLE CAPSULES

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Submitted By

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CERTIFICATE

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF NANOPARTICLE DRUG DELIVERY SYSTEM OF DAPOXETINE HCL"** is a bonafide work done by **Mr. M.SELVAKUMAR (Reg.No:261611306), Department of Pharmaceutics, College of Pharmacy, Madurai Medical College** in partial fulfillment of The TamilNadu Dr.M.G.R Medical University rules regulations for award of **MASTER OF PHARMACY IN PHARMACEUTICS** in under my guidance and supervision during the academic year 2017–2018.

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CONTENTS

LIST OF ABBREVIATIONS

%	:	Percentage
^{0}C	:	Celsius
cm	:	Centimeter
EE	:	Entrapment Efficiency
FT-IR	:	Fourier transform infrared
gm	:	Gram
Hcl	:	Hydrochloric Acid
Hrs	:	Hours
IP	:	Indian Pharmacopoeia
KBr	:	Potassium Bromide
β-CD	:	Beta cyclodextrins
NPs	:	Nanoparticles
Log	:	Logarithm
mg	:	Milligram
ml	:	milliliter
mm	:	Millimeter
nm	:	Nanometer
NDDS	:	Novel Drug Delivery System
SDDS	:	Smart Drug Delivery System
µg	:	Microgram
pH	:	Potential of Hydrogen
RH	:	Relative Humidity

Rpm	:	Revolution per Minute
SEM	:	Scanning Electron Microscopy
UV	:	Ultra Violet
IR	:	Infra red
λmax	:	Maximum Absorbance
BCS	:	Biopharmaceutical Classification System
Conc.	:	Concentration
CDR	:	Cumulative Drug Release
e.g.	:	Example
Etc.	:	Excetra
FDA	:	Food and Drug Administration
mts	:	Minutes
ppm	:	Parts Per Million
SD	:	Standard Deviation

CHAPTER I

INTRODUCTION

INTRODUCTION

NANOTECHNOLOGY

Nanoscience has been variously defined at different fora, books, journals and the web, yet one thing is common; it involves the study of the control of matter on an atomic and molecular scale. This molecular level investigation is at a range usually below 100 nm. In simple terms, a nanometer is one billionth of a meter and the properties of materials at this atomic or subatomic level differ significantly from properties of the same materials at larger sizes. Although, the initial properties of nanomaterials studied were for its physical, mechanical, electrical, magnetic, chemical and biological applications, recently, attention has been geared towards its pharmaceutical application, especially in the area of drug delivery.

This is because of the challenges with use of large size materials in drug delivery, some of which include poor bioavailability, in vivo stability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, generalized side effects, and plasma fluctuations of drugs. Of recent, several researches in nanodrug delivery have been designed to overcome these challenges through the development and fabrication of nanostructures. It has been reported that, nanostructures have the ability to protect drugs from the degradation in the gastrointestinal tract; the technology can allow target delivery of drugs to various areas of the body. **(Supriya A et al., 2018)**

The technology enables the delivery of drugs that are poorly water soluble and can provide means of bypassing the liver, thereby preventing the first pass metabolism Nanotechnology increases oral bioavailability of drugs due to their specialized uptake mechanisms such as absorptive endocytosis and are able to remain in the blood circulation for a long time, releasing the incorporated drug in a controlled fashion, leading

to less plasma fluctuations and minimized side-effects. Nanoscale size nanostructures are able to penetrate tissues and are easily taken up by cells, allowing for efficient delivery of drugs to target sites of action. Uptake of nanostructures has been reported to be 15–250 times greater than that of microparticles in the 1–10 um range. Nanotechnology improves performance and acceptability of dosage forms by increasing their effectiveness, safety, patient adherence, as well as ultimately reducing health care costs. It may also enhance the performance of drugs that are unable to pass clinical trial phases. Nanotechnology definitely promises to serve as drug delivery carrier of choice for the more challenging conventional drugs used for the treatment and management of chronic diseases such as cancer, asthma, hypertension, HIV and diabetes.

Nanotechnology is derived from the Latin word “Nano”, which means dwarf. One nanometer (nm) is equal to one-billionth of a meter, or else about the width of 6 carbon atoms or 10 water molecules. A human hair is approximately 80,000 nm wide, and a red blood cell is approximately 7000 nm wide. Atoms are smaller than 1 nm, however many molecules including some proteins range between 1 nm and larger.The theoretical foundations of nanotechnologies were first laid out in 1959 by the physicist Richard Feynman in his lecture, There’s plenty of room at the bottom. Feynman explored the possibility of manipulating material at the scale of individual atoms and molecules, imagining the whole of the Encyclopedia Britannica written on the head of a pin and foreseeing the increasing ability to examine and control matter at the nanoscale. The term nanotechnology was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to engineer materials precisely at the nanometer level. The primary driving force for miniaturization at that time came from the electronics industry, which aimed to develop tools to create smaller (and therefore faster and more complex) electronic devices on silicon chips. In medicine and pharmaceutics, nanotechnology is used to improve human health at a molecular level. The novel and

potential applications of nanotechnology in pharmaceutics are; development of diagnostic tools, formulation of drug carrier systems and gene therapy. The advantages of nanotech drugs compared to conventional counterparts lie on the basis of particle size. Drugs/drug products with nano dimension can be used at a lower concentration and can lead to early onset of bioactivity. Nano drug delivery systems (nanopharmaceutics) are, but not limited to, nanocapsules, nanospheres, nanosponges, nanoemulsions, solid lipid nanoparticles, nanovesicular systems (liposomes, niosomes), molecular systems (inclusion complexes) and nanocrystals. **(Ahmed et al., 2016)**

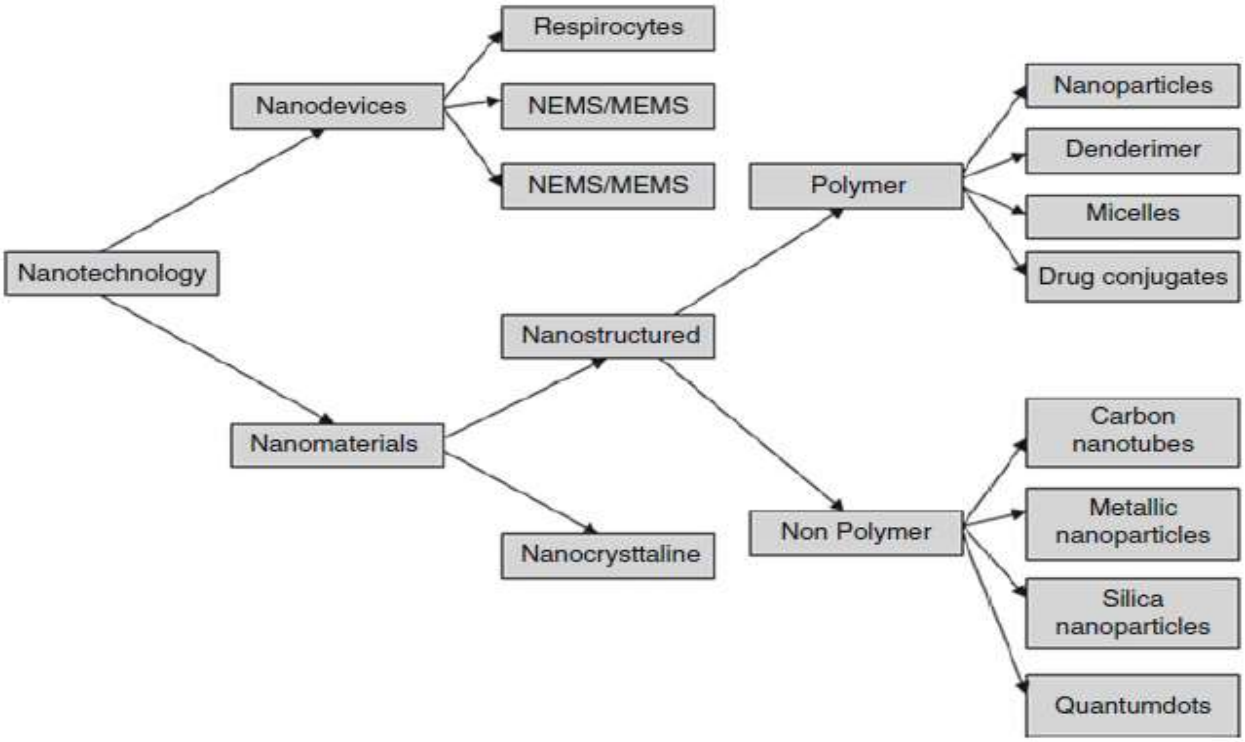


FIG: 1 Various systems of Nanotechnology

NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS

1. SMART DRUG DELIVERY SYSTEMS

One of the most outstanding achievements in the drug delivery field was the development of smart drug delivery systems (SDDSs), also called stimuli-sensitive delivery systems. The concept is based on rapid transitions of a physicochemical property of polymer systems upon a stimulus. This stimulus includes physical (temperature, mechanical stress, ultrasound, electricity, light), chemical (pH, ionic strength), or biological (enzymes, biomolecules) signals and such stimuli can either be internal, resulting from changes in the physiological condition of a living subject, or ‘‘external’’ signals, artificially induced to provoke desired events. SDDS provides a programmable and predictable drug release profile in response to various stimulation sources **(Masilamanb K et al., 2015).**

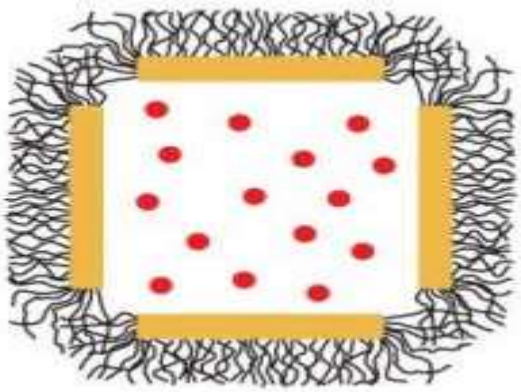


FIGURE 2: Smart drug delivery system-Gold nanocage covered with polymer

Depending on the desired applications, one may design different drug delivery systems for enhanced therapeutic efficiency with low systemic toxicity and side effects. SDDS has several advantages compared to conventional drug delivery systems. The conventional **(Hamed Hamishehkar et al., 2015)** controlled release systems are based

on the predetermined drug release rate irrespective of the environmental condition at the time of application. On the other hand, SDDS is based on the release-on-demand strategy, allowing a drug carrier to liberate a therapeutic drug only when it is required in response to a specific stimulation. The best example of SDDS has been self-regulated insulin delivery systems that can respond to changes in the environmental glucose level. One of the most widely used SDDSs has been polymeric micelles. Many polymeric micelles consisting of hydrophobic and hydrophilic polymer blocks have been developed. They have been found to dissolve water-insoluble drugs, such as doxorubicin or paclitaxel, at high concentrations. When administered to the body, drug release from polymeric micelles usually depends on simple diffusion, degradation of the micelle blocks, or disruption of the micelles by body components.

The release kinetics of the loaded drug can be modulated by varying the degradation rate of hydrophobic polymer blocks, but because the degradation rate is usually very slow, the loaded drug is released by diffusion from polymeric micelles. This slow release by passive diffusion may not be desirable, as the polymeric micelles reaching the target site need to release their contents fast. To solve this problem, smart polymeric micelles have been designed to liberate the loaded therapeutic agent at the targeted site fast. For example, Poly (ethylene glycol)-b-polyhistidine (PEG-b-PHis) forms micelles only over the pKb of the polyhistidine block (pH 6.5–7.0). It is interesting to know that, the pKb can be adjusted by varying the molecular weight of polyhistidine. Since solid tumors have a slightly acidic environment, a small reduction in pH to less than 7 at the tumor site triggers dissociation of the polymeric micelle to release its contents. In a separate study, PEG-b-polyhistidine micelles containing doxorubicin effectively killed multi-drug resistant MCF-7 cells at pH 6.8. Similarly, Hruby et al, (33) reported that, SDDS can achieve a highly localized drug accumulation at target sites even though it is administered parenterally. It is therefore postulated that, SDDS with enhanced targeting property is highly promising in

increasing the efficiency and efficacy of therapy while at the same time minimizing side effects

2. POLYMER–DRUG CONJUGATES

Polymer–drug conjugates are a class of polymer therapeutics that consists of a water-soluble polymer that is chemically conjugated to a drug through a biodegradable linker. The idea started in 1975 when Ringsdorf proposed the use of polymer–drug conjugates to deliver hydrophobic small molecules. The reasoning was that, small molecule drugs, especially hydrophobic compounds, have a low aqueous solubility and a broad tissue distribution profile such that, administration of the free drug may result in serious side effects. Therefore, conjugation of these compounds to hydrophilic, biocompatible polymers would significantly increase their aqueous solubility, modify their tissue distribution profile and enhance their plasma circulation half-life.

An important attribute of colloidal systems is their hydrodynamic diameter, which are typically about 3–20 nm for polymer–drug conjugates and between 10 and 200 nm for colloidal particles such as micelles or liposomes. The colloidal nature or size of these vehicles can facilitate their retention within the circulation for prolonged periods, in comparison to low molecular weight small molecules. One major difference between polymer–drug conjugates and delivery systems that contain physically entrapped drug (e.g., micelles and liposomes) is that the drug is chemically conjugated to the polymer and therefore these systems qualify as new chemical entities (NCE). Classification as an NCE is often accompanied by additional development and regulatory hurdles that must be met in order to receive approval. Over the last decade, polymer conjugate technology has proven to be a viable formulation strategy. There have been reports (36 – 38) of bioconjugation of protein and peptide to PEG been able to significantly improve the efficacy of these macro- molecular drugs by increasing their stability in the presence of proteases and decreasing their immunogenicity. Studies have also shown that by using PEG in a

specific molecular weight range, the fast renal clearance and mononuclear phagocytic system uptake of the drugs can be prevented or delayed leading to a prolonged plasma half-life for the conjugated molecules. Successful applications have led to several FDA-approved products e.g. Neulasta®. The first practical use of polymer therapeutics that resulted in an FDA-approved anti-cancer treatment was the introduction of PEG-Lasparaginase (Oncaspar1) in 1994. This conjugate is composed of PEG polymer (MW ~ 5 kD) attached to the enzyme, L asparaginase, and is used for the treatment of acute lymphoblastic leukemia (39). In fact, polymer–drug conjugate itself can be considered as a nanovehicle. Various conjugates have been developed and clinically tested.

One of the major advantages of polymer–drug conjugates is prolonged circulation in the blood stream by retarding degradation/metabolism/excretion rates of the conjugated drugs. Many peptide and protein drugs cannot be delivered by oral administration because of their large molecular weights. Even when administered directly into the blood stream, they do not remain in the blood for a long time due to fast degradation and metabolism, limiting the clinical applications. The circulation times of these drugs have increased substantially by conjugation with polymers, such as PEG. A good example is the glucagon-like peptide-1, which regulates food uptake and insulin release. The peptide is a very useful therapeutic agent for diabetic patients, but it is liable to degradation by a plasma enzyme, dipeptidyl dipeptidase IV, but by introducing one PEG chain, Lee et al (40) showed that its half-life could be increased up to 40 folds over the natural form. Very often, low molecular weight drugs with high hydrophobicity are used for conjugation with attendant reduction in the degradation/ clearance rate as well as the toxicity of the conjugated drug. The therapeutic effect is achieved upon hydrolysis inside the target cells to release the original drug. The polymers used in conjugation usually have stimuli-responsiveness, imparting unique properties into the conjugated drug such that, its activities can be turned on or off by external signals. For example, the catalytic activity

endoglucanase 12A upon conjugation could be turned on by application of UV light or high temperature because, it was conjugated with either photosensitive or thermo-sensitive polymers. The active site of the enzyme was exposed by collapsing the conjugated long polymer chain by external stimuli. Once visible light was turned on or the temperature was lowered, the enzyme activity vanished due to the blocking of the active site by the extended polymer chain **(Bhatt Neha et al., 2013).**

3. MULTIFUNCTIONAL DRUG CARRIERS

A multifunctional drug delivery system (MDDS) refers to drug carrier that has multiple properties of prolonged blood circulation, passive or active localization at specific disease site, stimuli-sensitivity, ability to deliver drug into intracellular target organelles, and/or imaging ability (45). Technically therefore, it has two or more functions, infact, SDDS and polymer–drug conjugates discussed above can be considered MDDS. In addition to 74 Recent Advances in Novel Drug Carrier Systems delivering drugs, MDDS can carry out the second function, such as stimuli-responsiveness or hydrolysis inside cells. Some reported MDDS include the biotin-tagged pH-sensitive polymeric micelles based on a mixture of PLA-b-PEG-b-PHis-biotin (PLA=poly (L-lactic acid)) and PEG-b-PHis block copolymers by Lee et al (46) in which the targeting moiety, biotin, was masked until the carrier was exposed to an expected environment of pH 7.0. Once the nanocarrier was internalized to cancer cells by ligand– receptor interactions, lowered pH (< 6.5) destabilized the carrier resulting in a burst release of the loaded drug and that of Lukyanov et al (47), where a pH-degradable PEG-b-phosphatidylethanolamine (PE) liposome had anti-myosin monoclonal antibody as well as TAT or biotin attached on its surface.

4. ORGANIC/INORGANIC COMPOSITES

An inorganic-organic composite usually comprises an inorganic phase and a film forming organic phase. A typical green approach to developing an inorganic-organic composite involves the selection of film forming organic phase from starches having a

degree of polymerization; degree of substitution and viscosity such that the substituted starches are insoluble in water during mixing but dissolve at a higher processing temperature during forming, setting or drying of the composite. Thus, excessive migration of the starch is prevented and the composite is substantially strengthened. There has also been reports on the lab-on-a-chip approach, which embodies micron- or nano-sized machines composed of sophisticated circuits. Small devices have many advantages including portability/disposability, low cost, high reproducibility, high-throughput screening, and multiple functionalities in a single device.

Recently, combined with other technologies such as optics, single molecular imaging, or cell/protein-based assay systems, biomedical lab-ona- chip devices have become an important part of drug discovery and diagnosis, but its application in drug delivery systems based on are just beginning to appear. As rightly noted by several authors, to release a drug from a nanodevice is more complicated than to perform assay or screening drug candidates, this is because, successful drug delivery requires at least four components namely; drug reservoir, pump, valve, and sensor (58). Drugs can be placed either in a fabricated reservoir or in conventional micro /nanoparticles. Other important organic/inorganic composites are metal nanoparticles, such as silver, iron oxide, or gold nanoparticles, coated with hydrophilic polymers. Their major application has been as theranostics. Only recently, Hirsch et al, (59) developed gold nanoshell, which provided tunable emission light for bioimaging. Importantly, is the fact that, gold nanoparticles can be detected by X-ray and emit thermal energy by excitation making it very useful for medical imaging and thermal therapy (theranostics). In a related report, Corot et al, (60) developed super paramagnetic iron oxide nanoparticles for magnetic resonance imaging (MRI) of the whole body. Mechanistically, these nanoparticles are primarily engulfed by monocyte or macrophage after intravenous administration. However, uptake of super

paramagnetic iron oxide by macrophage does not induce activation of nearby cells making it suitable for diagnosis of inflammatory or degenerative diseases.

Approaches to improve the solubility or to increase the available Surface area for dissolution classified as

- ❖ Physical modification are
- ❖ Chemical modifications

The Physical modifications are

- ➢ Decreasing particle size (micronization, Nano suspensions)
- ➢ Formation of polymorphs/pseudo polymorphs (including solvates)

The chemical modifications are (Bhatt Neha et al., 2013)

- ➢ Synthesis of soluble prodrugs and salts
- ➢ Preparation of drug dispersions in carriers (eutectic mixtures, nonmolecular solid dispersions, solid solutions).
- ➢ Approaches such as adding a surfactant/co-solvents
- ➢ Complexation with cyclodextrine or/and preparing oil–in-water emulsions for intravenous applications have been developed for poorly water-soluble drugs
- ➢ Thus, bioavailability of poorly water-soluble drugs will be affected positively when their dissolution rate is increased. These drugs show serious adverse clinical effects like non- steady absorption due to variability among patients and individual patient dosing. Drugs which have high permeability but low solubility (Class II according to Biopharmaceutics Classification System) are not easily dissolved so they may not be absorbed from the GI tract sufficiently. Moreover, such drugs incorporated into conventional dosage forms are usually affected by the fasted or fed state of the patient. This situation eventually causes inappropriate dosing and low bioavailability.

PHARMACEUTICAL APPROACHES

For improving solubility of drugs, in order to enhance oral bioavailability. Solubilization poorly water-soluble drugs increases dissolution rate and absorption leading to a significant improvement of drug bioavailability. Approaches to improve the solubility or to increase the available surface area for dissolution are classified as physical and chemical modifications. Physical modifications are decreasing particle size (micronization, nanosuspensions), formation of polymorphs/pseudopolymorphs (including solvates), complexation/solubilization (use of surfactants or cyclodextrines, addition of cosolvents) and preparation of drug dispersions in carriers (eutectic mixtures, non-molecular solid dispersions, solid solutions).

Approaches such as adding a surfactant/cosolvent, complexation with cyclodextrins or/and preparing oil–in-water emulsions for intravenous applications have been developed for poorly water-soluble drugs, but these approaches have limited application since the active drug substance must have specific physicochemical properties (for example, cyclodextrins must have suitable molecular weight for optimal conjugation of the conical structure with the drug for such applications to be successful). Solid dispersions are theoretically one of the appropriate methods for increasing dissolution rate, but molecules in the amorphous state are not thermodynamically stable; they can convert to the crystal form during storage. The use of surfactants or co-solvents sometimes leads to increased side effects and toxic reactions in the body **(Abdul Hasan Sathali et al.,2015).**

Potential disadvantages of salt forms include, high reactivity with atmospheric carbon dioxide and water resulting in precipitation of the poorly water-soluble drug. Polymorphs are different crystalline forms of a drug that may have different physico chemical properties and biological activities with respect to morphology, density, melting point, hardness, compression, solubility and bioavailability.

Therefore, the preparation of actual drug polymorphs is crucial during

preformulation studies. An alternative drug delivery approach called micronization, has been developed to overcome poor solubility in water. Micronization of poorly soluble drugs, increases the dissolution rate of the drug due to the increase in surface area, but does not change the saturation solubility. In order to increase solubility and oral bioavailability, going down to the micron level may sometimes not be sufficient, so the next step, going down to the nano level, may be necessary.

INFLUENCE OF PARTICLE SIZE DISSOLUTION RATE Vs PARTICLE SIZE

Intrinsic solubility can be explained as the number of moles of a substance per liter which dissolves in a particular solvent. Thus, dissolution is the process by which a solid substance dissolves. There are many factors that affect solubility; such as pH, co-solvent, surfactants, temperature and particle size. By changing these factors, solubility of active drug substances can be modified. Dissolution rate has been first described by Noyes and Whitney in 1897. In 1904, Nernst and Brunner explored the dissolution rate constant and diffusion coefficient of solutes. According to the Nernst Brunner equation, dissolution rate is proportional to the surface area of the active drug substance in contact with the dissolution medium.

$$Dw/dt = D/h \times S\ (Cs - Ct)$$

Where,

dw/dt-: Dissolution rate (mg/s)

S- Effective surface area of the solid drug (cm2)

Cs-Ct- Concentration gradient (mg/mL)

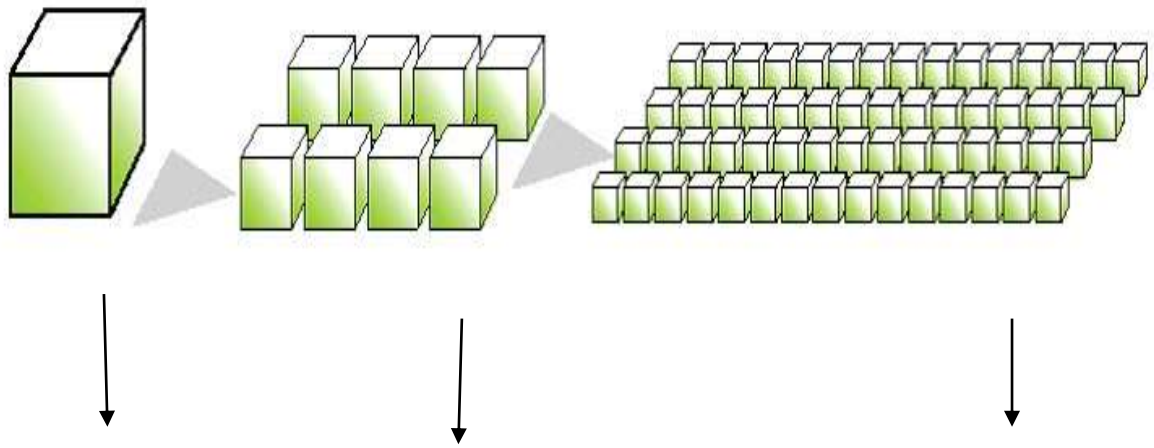
Cs- Saturated concentration (mg/mL)

Ct-Concentration of the solute at time t (mg/mL)

h- Effective diffusion layer thickness (cm)

D-Diffusion coefficient (cm/s)

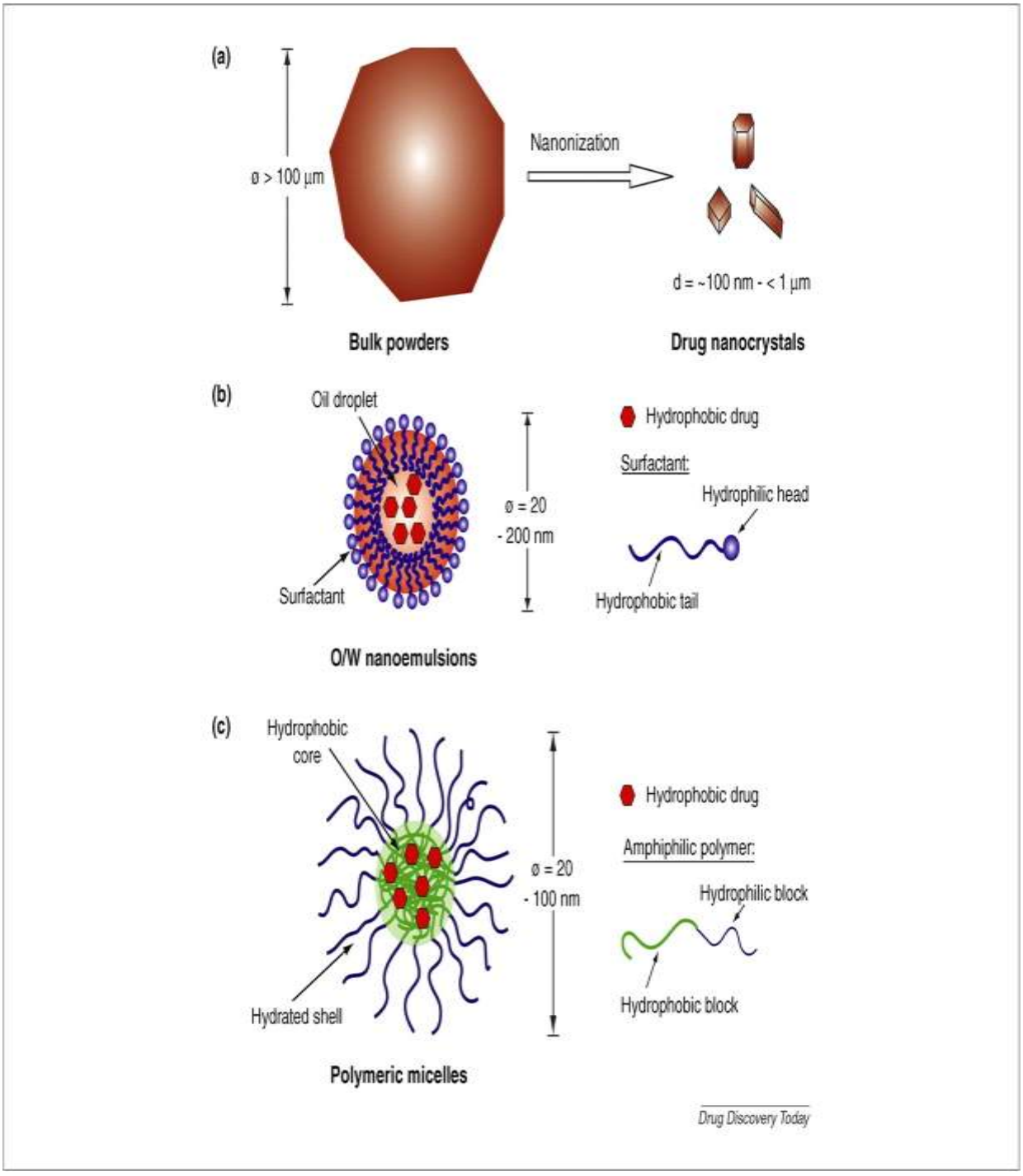
It can be deduced from the formula that changing particle size of the active drug substance, it is possible to change the specific surface area and the dissolution rate of the active drug substance in body fluids . With this basic information in hand, it is clear that the dissolution rate and bioavailability of poorly water soluble drugs can be increased by decreasing the particle size of active drug substances. Additionally, particle size reduction results in a decrease in the diffusion layer thickness surrounding the drug particles and an increased concentration gradient between the surface of the drug particles **(Tugba gulsun *et al*., 2009).**



(Total surface area 6cm^2) (Total surface area 12cm^2) (Total surface area 24cm^2)

FIGURE 3: Surface Area Enlargement by Particle Size Reduction Nanonization strategies for Poorly Water Soluble Drugs (Wang ZH et al.,2015).

SCHEMATIC OF DIFFERENT NANONIZATION STRATEGIES TO INCREASE DRUG SOLUBILITY AND BIOAVAILABILITY:



CHAPTER II

NANOPARTICLE- REVIEW

CHAPTER II

NANOPARTICLES - A REVIEW

Nanotechnology employs knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering. It has immense applications in almost all the fields of science and human life. Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Depending upon to the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carrier of DNA in gene therapy, and their ability to deliver proteins, peptides and genes. The input of today's nanotechnology is that it allows real progress to achieve temporal and spatial site-specific delivery. The market of nanotechnology and drug delivery systems based on this technology will be widely felt by the pharmaceutical industry. In recent years, the number of patents and products in this field is increasing significantly. . Several terminologies have been used to describe nanoparticulate drug delivery systems.

In most cases, either polymers or lipids are used as carriers for the drug, and the delivery systems have particle size distribution from few nanometers to few hundred nanometers. Nanomedicines is a large subject area and includes nanoparticles that act as biological mimetic (e.g. functionalized carbon nanotubes),"nanomachics"(e.g. those made from interchangeable DNA parts and DNA scaffolds such as octahedron and stick cube) ,nanofibers and polymeric nanoconstructs as biomaterials (e.g. molecular self-assembly and nano-fibers of peptides and peptideamphiphiles for tissue engineering) , shape memory polymers as molecular switches, nanoporous membranes) , and nanoscale microfabrication based devices (e.g. silicon microchips for drug release and micro machined hollow needles and two dimensional needles assay from single crystal silicon), sensors and laboratory diagnostics. Recent developments in nanotechnology have shown that nanoparticles (structures smaller than 100 nm in at least one dimension) have a great potential as drug carriers. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favorable material for biomedical applications **(Yuminoki K et al 2014).**

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.

OBJECTIVES

- Decrease drug resistance
- Decrease toxicity
- Enhance oral bioavailability
- Enhance rate of dissolution
- Enhance solubility
- Increase the stability of drug and formulation
- Increase drug targeting ability and patient compliance
- Increase surface area
- Reduce the dose needed

ADVANTAGES

1. This technology provides entrapment of active contents and side effects are less.
2. It provides improved stability, elegance and formulation flexibility.
3. It is non-mutagenic.
4. Non-irritating, non-toxic.
5. It provide extended release condition which is continuous action up to 12hr.
6. Drug is protected from degradation.
7. Therapeutic provide onset of action, formulations are cost effective.
8. It can be used to mask unpleasant flavours and to convert liquid substances to solids. Less harmful side effects (since smaller quantities of the drug have contact with healthy tissue).
9. Nanoparticle particles are soluble in water, so encapsulation can be done within the nanoparticle, by the addition of chemical called an adjuvant reagent.
10. Particles can be made smaller or larger by varying the proportion of cross-linker to polymer.
11. Easy scale-up for commercial production.

12. The drug profiles can be vary from fast, medium to slow release in case of dosing therapy.

13. Predictable release.

14. Biodegradable.

Disadvantages

1) nanoparticles include only small molecules.

2) Depend only upon loading capacities

The selection criteria of matrix materials depend on many factors such as:

(a) Size of nanoparticles required;

(b) Inherent properties of the drug, e.g., aqueous solubility and stability;

(c) Surface characteristics such as Charge and Permeability;

(d) Degree of biodegradability, biocompatibility and toxicity;

e) Drug release profile desired; and

(f) Antigenicity of the final product.

Limitations of Nanoparticles (An TT *et al.,* 2014):

a) Small size and large surface area can lead to particleparticle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

b) In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

Preparation of Nanoparticles

Nanoparticles preparation is most frequently by three methods:

(1) Dispersion of preformed polymers;

(2)Polymerization of monomers; and

(3) Ionic gelation or coacervation of hydrophilic polymers.

However, other methods such as supercritical fluid technology and particle replication in non-wetting templates have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry.

DISPERSION OF PREFORMED POLYMERS

Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticle from poly (lactic acid) (PLA); poly (D,L-glycolide),PLG; poly (D,Llactide- coglycolide) (PLGA) and poly(cyanoacrylate) (PCA), This technique can be used in various ways as described further:

SOLVENT EVAPORATION METHOD

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed **(Bin Du *et al*.,2013).**

SPONTANEOUS EMULSIFICATION OR SOLVENT DIFFUSION METHOD

This is a modified version of solvent evaporation method. I this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents a interfacial turbulence is created between the two phases leading to the formation

As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

POLYMERIZATION METHOD

In this method, monomers are polymerized to form nanoparticle in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles.

COACERVATION OR IONIC GELATION METHOD

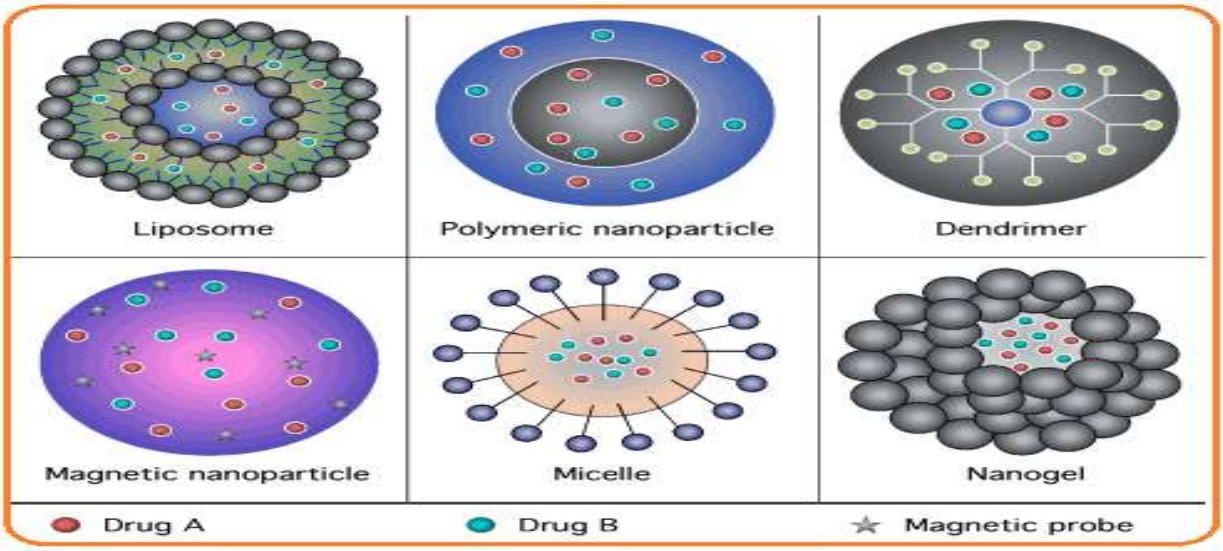
The nanoparticles preparation is carried by using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Developing a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. In this method, positively charged amino-group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer.

Supercritical fluid technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe.

A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), nontoxicity, nonflammability nonflammability, and low price. The most common processing techniques involving supercritical fluids are supercritical antisolvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure, Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive **(Abdul Hasan Sathali. A., and Gopinath.M., *et al*., 2013).**

TYPES OF NANOPARTICLES:



SOLID LIPID NANOPARTICLES

Solid lipid nanoparticles (SLN) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles as a colloidal carrier system for controlled drug delivery. Main reason for their development is the combination of advantages from different carriers systems like liposomes and polymeric nanoparticles. SLN have been developed and investigated for parenteral, pulmonal and dermal application routes. Solid Lipid Nanoparticles consist of a solid lipid matrix, where the drug is normally incorporated, with an average diameter below 1 µm. To avoid aggregation and to stabilize the dispersion, different surfactants are used that have an accepted GRAS (Generally Recognized as Safe) status. SLN have been considered as new transfection agents using cationic lipids for the matrix lipid composition. Cationic solid lipid nanoparticles (SLN) for gene transfer can be formulated using the same cationic lipids as for liposomal transfection agents.

POLYMERIC NANOPARTICLES

In comparison to SLN or nanosuspensions polymeric nanoparticles (PNPs) consists of a biodegradable polymer. The advantages of using PNPs in drug delivery are many, being the most important that they generally increase the stability of any

volatile pharmaceutical agents and that they are easily and cheaply fabricated in large quantities by a multitude of methods. Also, polymeric nanoparticles may have engineered specificity, allowing them to deliver a higher concentration of pharmaceutical agent to a desired location. Polymeric nanoparticles are a broad class comprised of both vesicular systems (nanocapsules) and matrix systems (nanospheres).

DENDRIMERS

Dendrimers, a unique class of polymers, are highly branched macromolecules whose size and shape can be precisely controlled. Dendrimers are fabricated from monomers using either convergent or divergent step growth polymerization. The well-defined structure, mono dispersity of size, surface functionalization capability, and stability are properties of dendrimers that make them attractive drug carrier candidates. Drug molecules can be incorporated into dendrimers via either complexation or encapsulation. Dendrimers are being investigated for both drug and gene delivery, as carriers for penicillin, and for use in anticancer therapy.

NANOTUBE

Carbon nanotubes (CNTs; also known as buckytubes) are allotropes of carbon with a cylindrical nanostructure. Nanotubes have been constructed with length-to-diameter ratio of up to 132,000,000:1, which is significantly larger than any other material. These cylindrical carbon molecules have novel properties which make them potentially useful in many applications in nanotechnology, electronics, optics, and other fields of materials science, as well as potential uses in architectural fields. They may also have applications in the construction of body armor. They exhibit extraordinary strength and unique electrical properties, and are efficient thermal conductors. Nanotubes are members of the fullerene structural family, which also includes the spherical bucky balls. The ends of a nanotube may be capped with a hemisphere of the bucky ball structure. Their name is derived from their size, since the diameter of a nanotube is on the

order of a few nanometers (approximately 1/50,000th of the width of a human hair), while they can be up to 18 centimeters in length (as of 2010). Nanotubes are categorized as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs).Chemical bonding in nanotubes is described by applied quantum chemistry, specifically, orbital hybridization. The chemical bonding of nanotubes is composed entirely of sp2 bonds, similar to those of graphite. These bonds, which are stronger than the sp3 bonds found in diamonds, provide nanotubules with their unique strength. Moreover, nanotubes naturally align themselves into "ropes" held together by Van der Waals forces **(Afifa Bathool et al., 2012).**

NANOWIRE

A nanowire is a nanostructure, with the diameter of the order of a nanometer (10−9 meters). Alternatively, nanowires can be defined as structures that have a thickness or diameter constrained to tens of nanometers or less and an unconstrained length. At these scales, quantum mechanical effects are important — which coined the term "quantum wires". Many different types of nanowires exist, including metallic (e.g., Ni, Pt, Au), semiconducting (e.g., Si, InP, GaN, etc.), and insulating (e.g., SiO2, TiO2). pharmacokinetics monitoring of diabetes, and health care. In such plans, future medical nanotechnology is expected to employ Nano robots injected into the patient to perform work at a cellular level. Such Nano robots intended for use in medicine should be non-replicating, as replication would needlessly increase device complexity, reduce reliability, and interfere with the medical mission. Instead, medical nanorobots are posited to be manufactured in hypothetical, carefully controlled nanofactories in which nanoscale machines would be solidly integrated into a supposed desktop-scale machine that would build macroscopic products. The most detailed theoretical discussion of nanorobotics, including specific design issues such as sensing, power communication, navigation, manipulation, locomotion, and onboard computation,

has been presented in the medical context of nanomedicine by Robert Freitas. Some of these discussions remain at the level of unbuildable generality and do not approach the level of detailed engineering.

APPLICATIONS OF NANOPARTICLES:

Tumor targeting using Nanoparticulate delivery system

The rational of using nanoparticles for tumor targeting is based on (1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active nanoparticles. (2) Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.An experiment demonstrated in mice treated with doxorubicin incorporated into poly (isohexylcynoacrylate) nanospheres that higher concentration of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin **(Ansari KA *et al.,* 2011).**

Nanoparticles for oral delivery of peptide and proteins:

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. The surface area of human mucosa extends to 200 times that of skin 62. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and

chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug deliverysystem and the epithelia cells in the GI tract.

Nanoparticles for Gene delivery

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cellmediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system.

Targeting of nanoparticles to epithelial cells in the GI tract using ligands

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract can be classified into those the surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors.

NANOTECHNOLOGY IN MEDICINE APPLICATION:

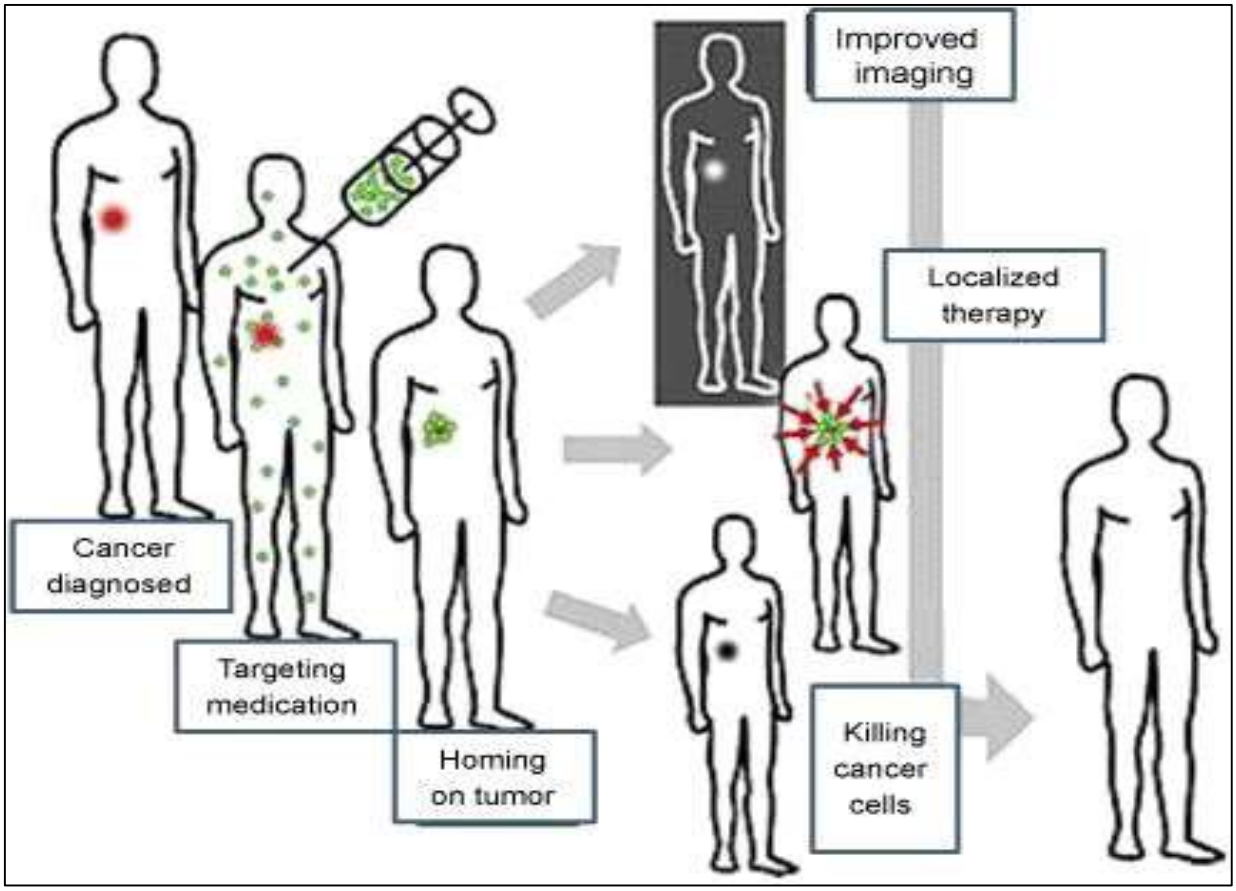
Anti-Microbial Techniques (Maiti S *et al*., 2012):

One of the earliest nanomedicine applications was the use of nanocrystalline silver, which is as an antimicrobial agent for the treatment of wounds, A nanoparticle cream has been shown to fight staph infections. The nanoparticles contain nitric oxide gas, which is known to kill bacteria. Studies on mice have shown that using the nanoparticle cream to releasenitric oxide gas at the site of staph abscesses significantly reduced the infection. Burn dressing that is coated with nanocapsules containing antibotics. If a infection starts the harmful bacteria in the wound causes the nanocapsules to break open, releasing the antibotics. This allows much quicker treatment of an infection and reduces the number of times a dressing has to be changed. A welcome idea in the early study stages is the elimination of bacterial infections in a patient within minutes,instead of delivering treatment with antibiotics over a period of weeks.

Absorption enhancement using non-specific interactions

In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticlesutilises less than 1% of mucosal surface area Using polymers such as chitosan, starch or poly (acrylate) can increase the paracellular per-meability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic,

it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated.



Applications Of Nanocrystals By Various Routes Of Administration

Nanoparticles for drug delivery into the brain:

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. Relatively impermeable endothelial cells characterize the BBB with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor mediated transport systems in the BBB.

For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis.

It has been reported poly(butylcyanoacrylate) nanoparticles was able to deliver hexapeptidedalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80. OX26MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of lipsosomes. However, recently, Jiet al. demonstrated that brain uptake of lactoferrin, an ironbinding glycoprotein belonging to the transferrin (Tf) family, is twice that of OX26 and transferrrinin vivo. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain **(Singling Tang *et al*., 2011).**

CHAPTER III

LITERATURE REVIEW

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LITERATURE REVIEW

Supriya A et al., 2018 developed Formulation and Evaluation of Capsules of Asenapine Maleate Loaded Chitosan Nanoparticles. Asenapine Maleate is an antipsychotic drug which has poor bioavailability due to its insolubility in water and belongs to biopharmaceutics Classification-IV. The aim of this study is to enhance the bioavailability of Asenapine Maleate by the preparation of Nanoparticles using ionic gelation method. In present work different formulations were prepared by using different ratios of polymer, Tween 80 (stabilizer) and sodium tri polyphosphate (cross linking agent). Prepared Nanoparticle was evaluated for its Particle Size, zeta potential, scanning electron microscopy, Percentage practical yield, Drug Entrapment Efficiency, and in-vitro drug releas studies. NPs formulation was subsequently loaded into hard gelatin capsules that were evaluated for preformulation studies, in-vitro dissolution and pharmacokinetic behavior. The optimized CSNPs was found with particle size of 41.1 nm, zeta potential -9.9 Mv, percentage practical yield was 97.5%. Entrapment efficiency (%EE) of 65.7%, scanning electron microscopy irregular shape. The in-vitro release profile was found to be 92.5% sustained up to 510 minutes. Thus, incorporation of Asenapine maleate into CSNPs results in enhanced bioavailability when compared to pure drug.

Ahmed et al., 2016 developed the optimized freeze-dried finasteride nanoparticles (NPs) were prepared from drug nanosuspension formulation that was developed sing the bottom–up technique. The effects of four formulation and processing variables that affect the particle size and solubility enhancement of the NPs were explored using the response surface optimization design. The optimized formulation was morphologically characterized using transmission electron microscopy (TEM).

Physicochemical interaction among the studied components was investigated. Crystalline change was investigated using X-ray powder diffraction (XRPD). Crystal growth of the freeze-dried NPs was compared to the corresponding aqueous drug nanosuspension. Freeze-dried NPs formulation was subsequently loaded into hard gelatin capsules that were examined for in vitro dissolution and pharmacokinetic behavior. Results revealed that in most of the studied variables, some of the quadratic and interaction effects had a significant effect on the studied responses. TEM image illustrated homogeneity and shape of the prepared NPs. No interaction among components was noticed. XRPD confirmed crystalline state change in the optimized NPs. An enhancement in the dissolution rate of more than 2.5 times from capsules filled with optimum drug NPs, when compared to capsules filled with pure drug, was obtained. Crystal growth, due to Ostwald ripening phenomenon and positive Gibbs free energy, was reduced following lyophilization of the nanosuspension formulation. Pharmacokinetic parameters from drug NPs were superior to that of pure drug and drug microparticles. In conclusion, freeze-dried NPs based on drug nanosuspension formulation is a successful technique in enhancing stability, solubility, and in vitro dissolution of poorly water-soluble drugs with possible impact on the drug bioavailability responses. TEM image illustrated homogeneity and shape of the prepared NPs. No interaction among components was noticed. XRPD confirmed crystalline state change in the optimized NPs. An enhancement in the dissolution rate of more than 2.5 times from capsules filled with optimum drug NPs, when compared to capsules filled with pure drug, was obtained. Crystal growth, due to Ostwald ripening phenomenon and positive Gibbs free energy, was reduced following lyophilization of the nanosuspension formulation. Pharmacokinetic parameters from drug NPs were superior to that of pure drug and drug microparticles. In conclusion, freeze-dried NPs based on drug nanosuspension

formulation is a successful technique in enhancing stability, solubility, and in vitro dissolution of poorly water-soluble drugs with possible impact on the drug bioavailability.

Masilamanb K et al., 2015 developed a new delivery systems for the controlled release of drugs is one of the most innovative fields of research in pharmaceutical sciences. Nanoparticles specially designed to release the drug in the vicinity of target sites. The aim of this study was to formulate and evaluate the Lansoprazole nanoparticles to improve the therapeutic efficacy of Lansoprazole by loading in nanoparticle drug delivery system. Lansoprazole loaded chitosan nanoparticles (CNP1 to CNP10) were prepared by ionotropic gelation method. The formulated nanoparticles were evaluated for external morphological characters, determination of particle size analysis, zeta potential, drug content, entrapment efficiency and *in-vitro* release studies. The drug content for the Lansoprazole loaded chitosan nanoparticles varied from 69.5±7.2% to 87.9±1.2%. The entrapment efficiencies were found to be minimum and maximum of 39.3±2.6% and 85.6±1.2%, the optimum entrapment efficiency was found to be 85.3±0.8%, particle size varied from 360±12nm to 814±62nm, zeta potential values were in positive and increased from 11.2±1.2mV to 18.7±0.4mV. *In-vitro* release of drug follows zero order and showed sustained release behavior for a period of 24 hr. The study demonstrated the successful preparation of sustained release polymeric nanoparticles of Lansoprazole.

Hamed Hamishehkar et al., 2015 developed the Evaluation of Different Methods for Preparing Nanoparticle Containing Gammaoryzanol for Potential Use in Food FortificationGammaoryzanol is a natural antioxidant and could provide beneficial as used in the food products due to the antioxidant activity and potential health benefits. Nanotechnology has been introduced into several aspects of the food science, including encapsulation of materials and used as delivery systems. The field of nanoparticle delivery systems for nutrients and nutraceuticals has been expanding over the last decades. The aim of this work was evaluation of different methods for preparation of polymeric nanoparticle containing gammaoryzanol. Nanoprecipitation technique, where polymer and gammaoryzanol were dissolved in acetone, nano-emulsion template method, by stepwise addition of water in to the oil phase consisting of ethyl acetate, gammaoryzanol and surfactant mixtures, as well as emulsification solvent evaporation technique, where gammaoryzanol and polymer were dissolved in ethyl acetate and chloroform, were used for preparation of nanoparticles. Two ratio of gammaoryzanol-polymer (Ethyl cellulose) (1:2 and 1:4) as well as different solvents and surfactants were used in these methods to produce gammaoryzanol nanoparticles. Among these methods, solvent evaporation technique has been successfully employed to produce gammaoryzanol loaded nanoparticles with desired characteristics.

Natarajan Tamilselvan et al.,2015 developed the Formulation and characterization of anti-alzheimer's drug loaded chitosan nanoparticles and its In vitro biological evaluation. The main objective of the study is to formulate hydrophilic drug loaded sustained release nanoparticles with the size of 200 nm and to increase the encapsulation efficiency of the drug. The nanoparticles were prepared by simple ionic gelation method using various concentrations of chitosan and TPP. The prepared nanoparticles were evaluated for particle size, shape, charge,

encapsulation efficiency, *in vitro* drug release and *in vitro* cytotoxicity. The optimized drug loaded nanoparticles showed the size of 125 ± 4mn with PDI 0.25 ± 0.05, potential of +40 ± 2mV, encapsulation efficiency of 65.5 ± 1.2% and the drug release of 68.4 ± 1.6% with an initial burst effect up to one hour followed by sustained release up to 24 hrs. Further the optimized formulation was subjected to investigate the cytotoxicity of CS-NP in SH-SY-5Y cell lines it revealed that the cell viability was above 90% without any toxicity. These preliminary results demonstrate that the possibility of delivering hydrophilic drugs to brain with enhanced encapsulation efficiency.

Bhatt Neha et al., 2013 worked in drug delivery to the brain using polymeric nanoparticles**.** Nanoparticle drug carriers consist of solid biodegradable particles in size ranging from 10 to 1000 nm (50–300 nm generally). The use of minute particles as drug carriers for targeted treatment has been studied over a long period of time. A selective accumulation of active substances in target tissues has been demonstrated for certain so-called nanocarrier systems that are administered bound to pharmaceutical drugs. Great expectations are placed on nanocarrier systems that can overcome natural barriers such as the blood-brain barrier (BBB) and transport the medication directly to the desired tissue and thus heal neurological diseases that were formerly incurable. Polymeric Nanoparticle have been shown to be promising carriers for CNS drug delivery due to their potential both in encapsulating drugs, hence protecting them from excretion and metabolism, and in delivering active agent across the blood – brain barrier without inflicting any damage to the barrier Different polymers have been used and different strategies like surface modification have been done to increase the retention time of nanoparticles.

Graves et.al., 2015 developed and evaluation of biodegradable nanoparticles for the oral delivery of fenretinide. Fenretinide is an anticancer drug with low water solubility and poor bioavailability. The goal of this study was to develop biodegradable polymeric nanoparticles of fenretinide with the intent of increasing its apparent aqueous solubility and intestinal permeability. Three biodegradable polymers were investigated for this purpose: two different poly lactide-co- studies. The release kinetics was analyzed using zero-order model, first-order model and higuchi's square root equation. FT-IR was performed to know the compatibility of the drug with various excipients and SEM analysis was performed to know the morphology of the pellet. All the 10 formulations are kept for Stability studies and carried out for 3 months at 40°C/75 %RH and 25°C/60 % RH according to ICH guidelines. The optimized formulation F10 was shown desirable *in vitro* acid and buffer drug release during the stability period and comparable to the innovator.

Amighi K *et al*., 2005, prepared nifedipine nanoparticles using high pressure homogenization. Nanoparticals were characterized in terms of size, morphology and redispersion characteristics following water removal. Saturation solubility and dissolution characteristics were investigated and compared to the un- milled commercial NIF to verify the theoretical hypothesis on the benefit of increased surface area. Crystalline state evaluation before and following particle size reduction was also conducted through differential scanning calorimeter (DSC) and Powder X-Ray diffraction (PXRD). Through this study it has been shown that initial crystalline state is maintained following particle size reduction and that the dissolution characteristics of Nifedipine nanoparticles were significantly increased in regard to the commercial product. This approach should have a general applicability to many poorly water soluble drug entities.

Abdul Hasan Sathali et al.,2015 developed and evaluation of Amisulpride Nanocrystal tablets. Amisulpride is an antipsychotic drug which belongs to BCS type II clacification.The present study aims to develop nanocrystal of Amisulpride in order to enhance solubility and dissolution rate by decreasing particle size of drug. The amisulpride nanocrystal with small and uniform particle size were successfully prepared by emulsion solvent diffusion method is based on the high pressure homogenization technique using β-cyclodextrin, sodium lauryl sulphate, hydroxy propyl methyl cellulose E15 and polyvinyl alcohol as stabilizers at different concentrations. The compatibility studies was done by infrared spectroscopy and differential scanning calorimetry showed that no interaction between the drug and stabilizers. The amisulpride nanocrystals were evaluated for drug content, invitro dissolution study, SEM, X-ray powder diffraction, particle size distribution, zeta potential and solubility studies. The X-ray powder diffraction (XRPD) confirmed that there was no change in the crystalline state by this size reduction process. The presence of stabilizers made the nanocrystal formulations more stable. The solubility and in vitro dissolution studies suggested that the dissolution rate when compared to pure drug. It was showed that BCD 1.8% concentration gives better drug release and enhances the solubility. The amisulpride nanocrystal tablets were successfully prepared from the best formulation by direct compression method. Recompression and post compression evaluation studies are also performed. Amisulpride nanocrystal tablet showed better drug release profile when compared to market and amisulpride tablet.

Wang ZH et al.,2015, developed and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. The aim of the present study was to prepare and evaluate a paclitaxel nanocrystal-based formulation stabilized by serum protein transferrin in a non-covalent manner. The pure paclitaxel nanocrystals were first prepared using an antisolvent precipitation method augmented by sonication. The serum protein transferrin was selected for use after evaluating the stabilizing effect of several serum proteins including albumin and immunoglobulin G. The formulation contained approximately 55-60% drug and was stable for at least 3months at 4°C. In vivo antitumor efficacy studies using mice inoculated with KB cells demonstrate significantly higher tumor inhibition rate of 45.1% for paclitaxel-transferrin formulation compared to 28.8% for paclitaxel nanosuspension treatment alone. Interestingly, the Taxol(®) formulation showed higher antitumor activity than the paclitaxel-transferrin formulation, achieving a 93.3% tumor inhibition rate 12days post initial dosing However, the paclitaxel-transferrin formulation showed a lower level of toxicity, which is indicated by a steady increase in body weight of mice over the treatment period. In comparison, treatment with Taxol(®) resulted in toxicity issues as body weight decreased. These results suggest the potential benefit of using a serum protein in a non-covalent manner in conjunction with paclitaxel nanocrystals as a promising drug delivery model for anticancer therapy.

Lockhart *et al.,*2015 developed Polyester nanoparticles (NPs) co- loaded with tamoxifen (TAM) and quercetin (QT) to investigate the loading, release and in vitro metabolism of a dual drug formulation. The NPs are made in 2 variations, 4% and 8% crosslinking densities, to evaluate the effects on metabolism and release kinetics. The NP-4% formulation with a particle size of 89.3 ± 14.8 nm was found to have loading percentages of 6.91 ± 0.13% TAM and 7.72 ± 0.15% QT after targeting 10% (w/w) each. The NP-8% formulation with a particle size of 91.5 ± 9.8

nm was found to have loading percentages of 7.26 ± 0.10% TAM & 7.80 ± 0.12% QT. The stability of the formulation was established in simulated gastrointestinal fluids, and the metabolism of TAM was shown to be reduced 2-fold and 3-fold for NP-4%s and NP-8%s, respectively, while QT metabolism was reduced 3 and 4-fold. The implications for improved bioavailability of the NP formulations were supported by cytotoxicity results that showed a similar efficacy to free dual drug formulations and even enhanced anti-cancer effects in the recovery condition. This work demonstrates the suitability of the nanosponges not only as a dual release drug delivery system but also enabling a regulated metabolism through the capacity of a nanonetwork. The variation in crosslinking enables a dual release with tailored release kinetics and suggests improved bioavailability aided by a reduced metabolism.

Umar PS *et al.,* 2015 developed clotrimazole loaded – cyclodextrin nanoparticles containing vaginal gels using polymers like hydroxyl propyl methyl cellulose (HPMC), Sodium Carboxy Methyl Cellulose (NaCMC), Methyl cellulose and Carbopol. The gel formulations were prepared with a view to improve permeability of drug. The prepared gels were evaluated for pH, viscosity, spreadability, extrudability, and mucoadhesive time and invitro diffusion study. The correlation coefficient values revealed that the diffusion profiles follows zero order kinetics and the mechanism of drug release was governed by peppas model. The diffusion exponent of release profiles (slope) has a value of (n> 0.5) which indicates non fickian diffusion. It was found that the clotrimazole loaded nanoparticles containing gels prepared with HPMC showed good extrudability, homogeneity, spreadability and required diffusion rate in comparison with other. Formulations and was selected as suitable candidate to be delivered through vaginal route at controlled rate.

Gouri sankar *et al.,* 2015 developed simvastatin loaded - cyclodextrin nanoparticles with carbonyldiimidazole as a crosslinker and chitosan as ucoadhesive agent for oral drug delivery for enhancing the solubility of simvastatin. Solid dispersion technique has been used for drug incorporation. The particle size loaded nanosponge was found to be between 350-600nm with minimum PI (0.374-0.417).The loaded nan osponges have shown more solublisation efficiency in comparison with plain simvastatin and – cyclodextrin complex. It was found that solubility of simvastatin was enhanced more than 20- folds with a nanosponge system. The zeta potential was sufficiently high (- 26.8 to -29.5Mv) which indicates formation of a stable colloidal nanosuspension. The simvastatin loaded nanosponges were characterized by FT-IR, DSC and powder x-ray diffraction (XRD) studies and it confirmed the interactions of simvastatin with nanosponges. The *invitro* drug release of simvastatin was controlled over a prolonged period of time and was influenced by the crosslinker ratio. The formulations were stable under the conditions of storage.

Singling Tang *et al*., 2011, improved the oral bioavailabilty of genistein, by nanoparticles. Nanoparticles were prepared by the nanoprecipitation technique using Eudragit E100 as carrier. The drug loaded nanoparticles were spherical on observation by transmission electric microscopy(TEM). Release of drug from the genistein nanoparticles was two times greater than that from the conventional capsules. These esults suggested that a nanoparticle system was a potentially promising formulation for the efficient delivery of poorly water soluble drugs by oral administration.

Sureshkumar P *et al.,* 2015 developed Miconazole loaded cyclodextrin nanoparticle were prepared by cross linking - cyclodextrin with carbonate bonds of diphenyl

carbonate in different proportions, which are porous as well as nanosized.Drug was incorporated by solvent evaporation method by dissolving the drug in various solvents like ethanol, methanol, acetone and chloroform. The formulated nanosponges are influenced by the solvent used for drug loading by solvent evaporation technique. Based on the drug encapsulation efficiency, drug content and extent of sustained nature, the gel prepared with polymer and crosslinking agent in 1:1 ratio, chloroform as a solvent and carbopol as a felling agent(F12) formulation was concluded to be the best formulation zero order release kinetics and mechanism of drug release was governed by peppas model. The diffusion exponential coefficient values were found to be in between 0.9402 to 1.1864 indicating non fickian diffusion mechanism. The optimized formulation has good spreadability extrudability and mucoadhesive nature. The pH and viscosity of the formulation were appropriate for the vaginal drug delivery and nanosponge technique was a better choice for the burst effect.

Shende PK *et al.,*2015 developed Meloxicam loaded β-cyclodextrin-based nanoparticles to enhance their solubility and stability and to prolong release using different methods that included physical mixing, kneading and sonication. Particle size, zeta potential, encapsulation efficiency, stability study results, in vitro and in vivo drug release study results, FTIR, DSC and XRPD were used as characterization parameters. SEM studies revealed that the particle sizes of the nclusion complexes of meloxicam were within the range of 350 ± 5.69-765 ± 13.29 nm. The zeta potentials were sufficiently high to obtain stable formulations. In vitro and in vivo release studies revealed the controlled release of meloxicam from the nanosponges for 24h. The interaction of the meloxicam with the nanoparticles was confirmed by FTIR and DSC. A XRPD study revealed that the crystalline nature of

meloxicam was changed to an amorphous form due to the complexation with the nanoparticles. A stability study revealed that the meloxicam nanosponges were stable. Therefore, β-cyclodextrin-based nanosponges represent a novel approach for the controlled release of meloxicam for anti-inflammatory and analgesic effects.parameters. SEM studies revealed that the particle sizes of the inclusion complexes of meloxicam were within the range of 350 ± 5.69-765 ± 13.29 nm. The zeta potentials were sufficiently high to obtain stable formulations. *In vitro* and *in vivo* release studies revealed the controlled release of meloxicam from the nanosponges for 24h. The interaction of the meloxicam with the nanosponges was confirmed by FTIR and DSC. A XRPD study revealed that the crystalline nature of meloxicam was changed to an amorphous form due to the complexation with the nanosponges. A stability study revealed that the meloxicam nanosponges were stable. Therefore, β-cd- based NS represent a novel approach for the controlled release of meloxicam for anti-inflammatory and analgesic effects.

Wang ZH et al., 2015 developed and evaluation of transferrin-stabilized paclitaxel nanocrystal formulation. The aim of the present study was to prepare and evaluate a paclitaxel nanocrystal-based formulation stabilized by serum protein Tansferrin in a non-covalent manner. The pure paclitaxel nanocrystals were first prepared using an antisolvent precipitation method augmented by sonication. The serum protein transferrin was selected for use after evaluating the stabilizing effect of several serum proteins including albumin and immunoglobulin G. The formulation contained approximately 55-60% drug and was stable for at least 3months at 4°C. In vivo antitumor efficacy studies using mice inoculated with KB cells demonstrate significantly higher tumor inhibition rate of 45.1% for paclitaxel-transferrin formulation compared to 28.8% for paclitaxel nanosuspension treatment alone.

Interestingly, the Taxol(®) formulation showed higher antitumor activity than the paclitaxel-transferrin formulation, achieving a 93.3% tumor inhibition rate 12days post initial dosing. However, the paclitaxel-transferrin formulation showed a lower level of toxicity, which is indicated by a steady increase in body weight of mice over the treatment period. In comparison, treatment with Taxol resulted in toxicity issues as body weight increased. These results suggest the potential benefit of using a serum protein in a non-covalent manner in conjunction with paclitaxel nanocrystals as a promising drug delivery model for anticancer therapy.

Yuminoki K et al 2014, developed preparation and evaluation of high dispersion stable nanocrystal formulation of poorly water-soluble compounds by using povacoat**.** In this study, we reported the application of Povacoat®, a hydrophilic polyvinyl alcohol copolymer, as a dispersion stabilizer of nanoparticles of poorly water-soluble compounds. In addition, the influence of aggregation of the nanoparticles on their solubility and oral absorption was studied. Griseofulvin (GF) was used as a model compound with poor water solubility and was milled to nanoparticles by wet bead milling. The dispersion stability of GF milled with Povacoat® or the generally used polymers (polyvinyl alcohol, hydroxypropylcellulose SSL, and polyvinylpyrrolidone K30) was compared. Milled GF suspended in ovacoat® aqueous solution with mannitol, added to improve the disintegration rate of freeze-dried GF, exhibited high dispersion stability without aggregation (D90 = ca. 0.220 μm), whereas milled GF suspended in aqueous solutions of the other polymers aggregated (D90 > 5 μm). Milled GF with Povacoat® showed improved aqueous solubility and bioavailability compared with the other polymers. The aggregation of nanoparticles had significant impact on the solubility and bioavailability of GF. Povacoat® also prevented the

aggregation of the various milled poorly water-soluble compounds (hydrochlorothiazide and tolbutamide, etc.) more effectively than the other polymers. These results showed that Povacoat® could have wide applicability to the development of nano formulations of poorly water- soluble compounds.

Sezione di Scienze de et al 2014 Developed formulation strategy and evaluation of nanocrystal piroxicam orally disintegrating tablets manufacturing by freeze-drying. Piroxicam (PRX) is a non-steroidal anti-inflammatory drug characterized by a poor water solubility and consequently by a low oral bioavailability. In this work, different nanocrystal orally disintegrating tablets (ODT) were prepared to enhance piroxicam dissolution rate and saturation solubility. PRX nanocrystals were prepared by means of high pressure homogenization technique using poloxamer -188 as stabilizer. Three different ODTs were prepared with the same nanosuspension using different excipients in order to study their effect on the PRX dissolution properties. PRX nanocrystal size and zeta potential were determined by photon correlation spectroscopy. Additional characterization of PRX nanocrystal ODT was carried out by infrared spectroscopy, X-ray powder diffractometry, differential scanning calorimetry. Dissolution study was performed in stilled water (pH 5.5) and compared with PRX coarse suspension ODT, PRX/poloxamer 188 physical mixture, Bulk PRX samples and a PRX commercial ODT. All PRX nanocrystal ODT formulations showed a higher drug dissolution rate than coarse PRX ODT. PRX nanocrystal ODT prepared using gelatin or croscarmellose as excipient showed a higher PRX dissolution rate compared with the commercial formulation and ODT prepared using xanthan gum. Overall results confirmed that improved PRX dissolution rate is due to the increased surface-to-volume ratio due to the nanosized drug particle but also revealed the important role of different

excipients used.

Vidya viswanad *et al.,* 2014 developed cephalexin loaded Nanoparticles were prepared by solvent evaporation method by using hydroxy ethyl cellulose and poly vinyl alcohol polymers.The particle size and entrapment efficiency was around the range of 200- 400 nm and 88.5%- 95.6% respectively.Five different formulations of hydrogel prepared by using carbopol 934 with varying concentration of penetration enhancer (Propylene glycol). The invitro release studies revealed that the formulation with higher concentration of penetration enhancer (15% Propylene glycol) showed greater release from the kinetic study, the best linearity was found with first order and Higuchi's equation .The permeation studies showed that the formulation having high concentration of permeation enhancer showed good skin permeation.

An TT *et al.,* 2014 developed isradipine nanosuspension by using the son precipitation method for stable nanosuspensions. Nanosuspension systems were prepared upon various factors including amplitude and the time length of ultrasonication. The dissolution test was performed according to the USP paddle method in intestinal fluid (pH 6.8). The crystalline structure of drug, the molecular interaction, morphology and size of nanosuspension were also investigated to determine the mechanism of dissolution enhancement. The sonoprecipitation method with use of HPMC showed its potential in enhancement of the drug release rate. Stable nanosuspension was significantly depended on amplitude and time of ultrasonication since these factors affected on the size of nanoparticles. The synergistic effects of reduction of drug crystallinity and particle size could increase the dissolution rate of isradipine by providing a stable nanosuspension. This work may contribute to a new strategy for improvement dissolution rate of isradipine.

Bhatt Neha et al., 2013 worked in drug delivery to the brain using polymeric nanoparticles**.** Nanoparticle drug carriers consist of solid biodegradable particles in size ranging from 10 to 1000 nm (50-300 nm generally. The use of minute particles as drug carriers for targeted treatment has been studied over a long period of time. A selective accumulation of active substances in target tissues has been demonstrated for certain so-called nanocarrier systems that are administered bound to pharmaceutical drugs. Great expectations are placed on nanocarrier systems that can overcome natural barriers such as the blood-brain barrier (BBB) and transport the medication directly to the desired tissue and thus heal neurological diseases that were formerly incurable. Polymeric Nanoparticle have been shown to be promising carriers for CNS drug delivery due to their potential both in encapsulating drugs, hence protecting them from excretion and metabolism, and in delivering active agents across the blood – brain barrier without inflicting any damage to the barrier. Different polymers have been used and different strategies like surface modification have been done to increase the retention time of nanoparticles.

Zuki Abu Bakar Zakaria *et al*., 2013, synthesized biobased calcium carbonate nanocrytals had demonstrated to be an effective carrier for delivery of anticancer drug Doxorubicin (DOX). These nanocrystls displayed high levels of selectivity and specificity in achieving effective cancer cell death without non- pecific toxicity. The $CaCo_3$/DOX nanocrystals were relatively stable at neutral pH (7.4), resulting in slow release of drug, but, progressively dissociated in acidic pH (4.8) and faster release of DOX. They indicated that, $CaCo_3$/DOX nanocrystals were more sensitive and gave a greater reduction in breast cancer cell growth by controlled and targeted cancer therapy than free DOX.

Bin Du *et al.*,2013 developed oral nanocrystal capsules were produced using nanocrystal formulations in order to optimize dissolution properties of poorly soluble drug glimepiride and improve its bioavailability. The mean particle size and polydispersity index were investigated systematically, and the optimal values were 0.2% glimepiride (w/v), 1.2% Lipoid S100, 0.6% PEG 6000 (w/v), 0.6% PVPK 30 w/v), 500W and 2 min, respectively. Characterization of glimepiride nanocrystal was carried out by X-PRD, DSC and SEM *Invitro* dissolution test, the nanocrystal-loaded capsules of glimepiride showed an evident increase in dissolution rate compared to micronized and market capsules. The in vivo studies demonstrated that a marked enhancement of bioavailability of nanocrystal-loaded capsules was superior compared to the marketed formulation and microcrystal-loaded capsules, which may reduce the risk of side effects by allowing a reduction in either the dose or its frequency of administration.

Mbo D *et al.*,2013 developed Cyclodextrin-based nanosponges (NS) are solid nanoparticles, obtained from the cross-linking of yclodextrins .In this work, new carboxylated cyclodextrin-based nanosponges (Carb-NS) carrying carboxylic roups within their structure were purposely designed as novel Acyclovir carriers. TEM measurements revealed their spherical shape and size of about 400 nm.The behaviour of Carb-NS, with respect to the incorporation and delivery of Acyclovir, was compared to that of NS, previously investigated as a drug carrier. DSC, XRPD and FTIR analyses were used to investigate the two NS formulations. The results confirm the incorporation of the drug into the NS structure and NS-Acyclovir interactions. The Acyclovir loading into Carb- NS was higher than that obtained using NS, reaching about 70% (w/w). *In vitro* release studies showed the release kinetics of Acyclovir from Carb-NS to be prolonged in comparison with those observed with NS, with no initial burst effect. The NS uptake into cells was evaluated

using fluorescent Carb-NS and revealed the nanoparticle internalization. Enhanced antiviral activity against a clinical isolate of HSV-1 was obtained using Acyclovir loaded in Carb-NS.

Abdul Hasan Sathali. A., and Gopinath.M., *et al*., 2013, developed Paliperidone nanocrystal in order to enhance the solubility and dissolution rate by decreasing the particle size of the drug and also sustained the drug release profile by using EudragitL100 as polymer at different ratio. The Paliperidone nanocrystals were successfully prepared by nanoprecipitation method using different stabilizers (PVP K30, Poloxamer 1888, Poloxamer 407, combination of PVP K30 and Poloxamer 407, combination of PVP K30 and Poloxamer 188). The formulations were evaluated for entrapment efficiency, morphology, size distribution, zeta potential, solubility studies and stability studies. The presence of stabilizers made the nanocrystal formulation more stable. The solubility studies and Invitro dissolution studies suggested that the nanocrystal formulations can improve the bioavailability of Paliperidone when compared to pure drug. The X-ray powder diffraction (XRPD) confirmed that, there was no change in the crystalline state by this size reduction process. Stability studies were favorable for the development of this nanocrystal formulation.

Bhay S. Sapre et al., 2012 developed the Design of a buccal mucoadhesive, nanoparticles based delivery system of fluoxetine**.** The study was attempted to develop an alternative oral mucosal delivery of nanoparticles based system for antidepressant drug. The aim was to formulate a novel, transmucosal (buccal), polymeric nanoparticles based mucoadhesive system (diskettes) that could deliver fluoxetine hydrochloride with bypass first pass effect, with relative rapid onset, higher absorption and sustain release effect to increase bioavailability compared to oral

absorption. In this study, the drug was encapsulated into poly (methyl vinyl ether / maleic anhydride) (Gantrez MS-955) mucoadhesive polymer nanoparticles. The drug bearing nanoparticles were prepared by emulsion solvent evaporation method. The effect of critical formulation variables like, polymer concentration, emulsifier concentration and process variable like rate of homogenization were studied for the particle size distribution; drug entrapment efficiency and mucoadhesion. The dependent variables of the formulations were optimized using 32 full factorial designs and defined in mathematical equations. The desired values of response variables were found by contour plots generated using the design-expert® 8 version 8.0.6.1 software. The drug encapsulated polymeric nanoparticles were gently compacted along ethyl cellulose layer into small round shaped diskettes for facilitating buccal application in such a way that polymer layer adhere with buccal mucosa. The gently compacted diskettes gave dose precision through uniform drug distribution, high surface areas for better drug releases with sustain effect and without disrupter of nanoparticles. The in vitro studies of the diskettes included mucoadhesion and drug release profile were characterized. The in vivo studies were performed on rats. A significant improvement in the pharmacokinetic parameters of bioavailability like Cmax, Tmax and AUC was observed when compared with oral solution. The stability study was conducted on the optimized formulation at accelerated conditions.

Afifa Bathool et al., 2012 dveloping the formulate and characterize atorvastatin loaded chitosan loaded nanoparticles prepared by solvent evaporation method for sustained release. Low oral bioavailability of Atorvastatin calcium (14%) due to an extensive high first-pass effect makes it as prime target for oral sustained drug delivery. Weighed amount of drug and polymer were dissolved in suitable organic solvent DMSO and 2% acetic acid as an organic phase. This solution is added drop

wise to aqueous solution of Lutrol F68 and homogenized at 25000rpm followed by magnetic stirring for 4hrs. Nanoparticles were evaluated for its particle size, scanning electron microscopy (SEM), Fourier-Transform infrared spectroscopy (FTIR), percentage yield, drug entrapment and for in vitro release kinetics. Among the four different ratios, 1:4 ratio showed high drug loading and encapsulation efficiency. SEM studies shows that prepared nanoparticles were spherical in shape with a smooth surface. Particle size of prepared nanoparticles was found to be in range between 142 nm to 221 nm. FTIR and DSC shows drug to polymer compatibility ruling out any interactions. *In vitro* release study showed that the drug release was sustained up to 7 days. Hence, prepared nanoparticles proved to be promising dosage form for sustained drug delivery of atorvastatin reducing dosing frequency, thus increasing the patiesnt compliance.

Maiti S *et al*., 2012, studied liquid-crystal and nanocrystal technology for solubilization of poorly water soluble drugs. Any material with a dimension of less than 1 micrometer i.e. 1000 nm, should be referred to as nanoparticles, not a nanocrystal. Crystalline nanoparticles are often provide single domain crystalline system that can be studied to provide information, that can be help to explain the behavior of macroscopic samples of similar material. Nanocrystal is also a registered trademark of Elan Pharma International Ltd (Ireland). That improves the bioavailability of drugs by nanoscale particles that can be suspended in liquid, made into powder, pressed into tablets or encapsulated. As decreased size of the drug particles, may increase oral bioavailability of sparingly water soluble drugs. Drugs nanocrystals can be used for chemical stabilization of chemically labile drugs. The increased stability can be explained by a shield effect of the surfactant.

Ansari KA *et al.,* 2011 developed resveratrol loaded with cyclodextrin-based nanoparticles (NP).They have been used to increase the solubility and stability of poorly soluble actives. This study aimed at formulating complexes of resveratrol with β-cyclodextrin nanopartices in different weight ratios. DSC, FTIR and X-ray powder diffraction (XRPD) studies confirmed the interaction of resveratrol with NP. XRPD showed that the crystallinity of resveratrol decrease after encapsulation. The particle sizes of resveratrol-loaded NP are in between 400 to 500 nm with low polydispersity indices. Zeta potential is sufficiently high to obtain a stable colloidal nanosuspension. TEM measurement also revealed a particle size around 400 nm for NP complexes. The *in vitro release* and stability of resveratrol complex were increased compared with plain drug. Cytotoxic studies on HCPC-I cell showed that resveratrol formulations were more cytotoxic than plain resveratrol. The permeation study indicates that the resveratrol NP formulation howed good permeation in pigskin. The accumulation study in rabbit mucosa showed better accumulation of resveratrol NP formulation than plain drug. These results signify that resveratrol NP formulation can be used for buccal delivery and topical application.

Singling Tang *et al*., 2011, improved the oral bioavailabilty of genistein, by nanoparticles. Nanoparticles were prepared by the nanoprecipitation technique using Eudragit E100 as carrier. The drug loaded nanoparticles were spherical on observation by transmission electric microscopy(TEM). Release of drug from the genistein nanoparticles was two times greater than that from the conventional capsules. These esults suggested that a nanoparticle system was a potentially promising formulation for the efficient delivery of poorly water soluble drugs by oral administration.

Peng Quan *et al*., 2011, developed solid formulation containing Nitrendipine nanoparticle for oral delivery. Nitrendipine nanoparticles were prepared by using a tandem precipitation homogenization process. The optimal process was follows firstly nitrendipine/acetone solution(100mg/ml)was added to a polyvinyl alcohol solution (1mg/ml) at 10^{o}c, then the presuspension was homogenized for 20 cycles at 1000bar. The invitro dissolution rate of the nanocrystal was significantly increased and compared with the physical mixture and commercial tablets. The Cmax of the nanoparticle was approximately 15 folds and 10 folds greater than that of physical mixture and commercial tablet, respectively. And the $AUC_{0\rightarrow24}$ of the nanocrystals was approximately 41 folds and 10 folds greater than that of physical mixture and commercial tablet, respectively. The spray drying method was found to be suitable for conversion of the nanoparticle into solid form.

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Ansari KA *et.al.,* 2011 developed resveratrol loaded with cyclodextrin-based nanoparticles (NS).They have been used to increase the solubility and stability of poorly soluble actives. This study aimed at formulating complexes of resveratrol with β-cyclodextrin nanoparticles in different weight ratios. DSC, FTIR and X-ray powder diffraction (XRPD) studies confirmed the interaction of resveratrol with NS. XRPD showed that the crystallinity of resveratrol decrease after encapsulation. The particle sizes of resveratrol-loaded NP are in between 400 to 500 nm with low polydispersity indices. Zeta potential is sufficiently high to obtain a stable colloidal nanosuspension. TEM measurement also revealed a particle size around 400 nm for NP complexes. The *in vitro* release and stability of resveratrol complex were increased compared with plain drug. Cytotoxic studies on HCPC-I cell showed that resveratrol formulations were more cytotoxic than plain resveratrol. The permeation study indicates that the resveratrol NP formulation showed good permeation in pigskin. The accumulation study in rabbit mucosa showed better accumulation of resveratrol NP formulation than plain drug. These results signify that resveratrol NP formulation can be used for buccal delivery and topical application.

Muhammed Rafeeq P E et al., 2010 developed isoniazid in chitosan Nanoparticles inorder to enhance bioavailability and to reduce dose frequency.Chitosan was dissolved in acetic acid aqueous solution at various concentrations; Drug was dispersed in above Chitosan solution kept over magnetic stirrer at room temperature. It shows good encapsulation efficiency. And good release profile follows first order release kinetics. From all these results it concludes that formulation No 2 I the best formulation and which is recommended for future studies like Nano dry powder preparation.

Adlin Jino Nesalin. J *et al*., 2009, formulated Flutamide nanoparticles using chitosan polymer by ionic gelation technique. Nanoparticles of different core : coat ratio were formulated and analysed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. The drug loading capacity of nanoparticles containing drug : polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 63.3 ± 0.43, 66.3 ± 0.58, 68.1 ± 0.38, 75.2 ± 0.52, 71.0 ± 0.46. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation F4 registered highest entrapment of 75.2%. The particle size distribution was found to be 400nm by scanning electron microscopy (SEM) analysis. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core : coat ratio 1:4 was showed better sustained release for about 12hours as compared to other formulations.

Levent Oner *et al*., 2009, studied the major application areas of nanotechnology in pharmacy is nanoparticular drug delivery systems. Preparation of drug nanocrystals to improve the solubility of poorly water soluble drugs for oral delivery is also one of the important applications. Nanocrystal dispersion comprises water, active drug substance and a stabilizer. Different techniques can be used to prepare nanocrystal formulation of a drug powder such as homogenization, co-precipitation, spray drying and milling. Nanocrystals are physically stable due to the presence of stabilizers. The advantage of Nanocrystals formulations are enhanced oral bioavailability, improved dose proportionality, reduced food effects, suitability for administration by all routes and possibility of sterile filtration due to decreased particle size range. This technology will be substantially useful for the manufacture of poorly water soluble drug products for oral delivery.

Dlin Jino Nesalin. J *et al*., 2009, formulated Flutamide nanoparticles using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analysed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. The drug loading capacity of nanoparticles containing drug : polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 were found to be 63.3 ± 0.43, 66.3 ± 0.58, 68.1 ± 0.38, 75.2 ± 0.52, 71.0 ± 0.46. Thus there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The formulation F4 registered highest ntrapment of 75.2%. The particle size distribution was found to be 400nm by scanning electron microscopy (SEM) analysis. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core : coat ratio 1:4 was showed better sustained release for about 12hours as compared to other formulations.

ADLIN JINO NESALIN J et al., 2009 dveloped th formulation and evaluation of nanoparticles containing flutamide. Flutamide, a substituted anilide, is a potent antiandrogenic that has been used in the treatment of prostate carcinoma having short biological half-life of 5-6 hrs; thus it is a good candidate for the formulation of sustained release dosage form. In the present work nanoparticles of Flutamide were formulated using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

Muller H et *al*., 2007, investigated the feasibility of nanosuspension technology by high pressure homogenization to enhance the chemical stability of ascorbyl

palmitate (AP), followed by lyophilization. Sodium dodecyl sulfate (SDS) and Tween 80 were chosen as emulsifying agents to stabilize the develop AP nanosuspension. After 3 months of storage at 3 different temperature (4^oC, 25^oC and 40^oC), the photon correlation spectroscopy (PCS) analysis of AP nanosuspension revealed that the mean particle size of these stabilized with SDS significantly increased compared to those stabilize with Tween 80. The percentage of AP remaining in nanosuspension stabilized with Tween 80 was higher than 90% after 3months storage at 4^oC, 25^oC and 40^oC. To increase the chemical stability of AP nanosuspension. A drug powder was prepared by lyophilization. The effect of the presence of cryoprotectant terhalose on the physical stability was evaluated at different concentrations. After redispersing the lyophilized product the mean size of AP nanosuspensions without terhalose was significantly higher compared with system with terhalose. It was found that the mean size of AP nanosuspensions stabilized with Tween 80 remained in the nanometer range and the amount of the active determined by HPLC, was more than 90% when stored at 3 different temperatures during 3 months. From the X-Ray diffracto grams, it was shown that AP remained in a crystalline state which is physiochemically and thermodynamically more stable than AP in an amorphous state.

Akas M *et al.*, 2006 developed Long-alkyl chain functionalized poly (propyleneimine) dendrimer, poly(ethyleneimine) hyperbranched polymer, and beta-cyclodextrin derivatives, which are completely insoluble in water, have the property of encapsulating organic pollutants from water. Ceramic porous filters can be impregnated with these compounds resulting in hybrid organic/ inorganic filter modules. These hybrid filter modules were tested for the effective purification of water, by continuous filtration experiments, employing a variety of water pollutants. It has been established that polycyclic aromatic hydrocarbons (PAHs) can be removed very efficiently (more than 95%), and final concentrations of several ppb (microg/ L) are easily obtained. Representatives of the pollutant group of trihalogen methanes (THMs), monoaromatic hydrocarbons (BTX), and pesticides (simazine) can also

be removed (>80%), although the filters are saturated considerably faster in these cases.

Amighi K *et al*., 2005, prepared nifedipine nanoparticles using high pressure homogenization. Nanoparticals were characterized in terms of size, morphology and redispersion characteristics following water removal. Saturation solubility and dissolution characteristics were investigated and compared to the un-milled commercial NIF to verify the theoretical hypothesis on the benefit of increased surface area. Crystalline state evaluation before and following particle size reduction was also conducted through differential scanning calorimeter (DSC) and Powder X-Ray diffraction (PXRD). Through this study it has been shown that initial crystalline state is maintained following particle size reduction and that the dissolution characteristics of Nifedipine nanoparticles were significantly increased in regard to the commercial product. This approach should have a general applicability to many poorly water soluble drug entities.

CHAPTER-IV

AIM OF WORK

CHAPTER-IV

AIM OF THE WORK

A major hurdle in pharmaceutical formulation is water insolubility of most of the drugs affecting stability and bioavailability of the drug, if the drug is also insoluble in organic medium, it is difficult to deliver the drug in a sufficiently bioavailable form and hence it is a great challenge to formulation researchers to overcome such difficulty. Although some approaches are available for enhancing the dissolution of poorly soluble drugs but has certain draw backs like low drug loading and large doses. However, a new solution to poorly water soluble drug candidates is now available i.e. nanonisation and it leads to much more soluble, more biologically available and safer dosage form of poorly soluble and poorly bioavailable drugs. During the last two decades, many modern technologies such as high throughput screening, combinatorial chemistry, and computer-aided drug design in the pharmaceutical research and development area is leading to a vast number of drug candidates possessing a very good efficacy.

Many of these drug candidates are exhibiting poor aqueous solubility. Although some approaches are available for enhancing the dissolution of poorly soluble drugs, but has certain drawbacks like low drug loading and large dose. Dapoxetine Hcl as a highly potent serotonin-transporter inhibitor and increase the synaptic serotonin level. In vitro studies suggest that dapoxetine is cleared by multiple enzyme systems in the liver and kidneys, primarily CYP2D6, CYP3A4, and flavin monooxygenase (FMO1). Following oral dosing in a clinical study designed to explore the metabolism of 14C-dapoxetine, dapoxetine was extensively metabolised to multiple metabolites primarily through the following biotransformational pathways: N-oxidation, N-demethylation, naphthyl hydroxylation, glucuronidation and sulfation. There was

evidence of presystemic first-pass metabolism after oral administration. The absolute oral bioavailability of Dapoxetine Hcl following oral administration is mean 42% and half-life 1-1.6hour. Dapoxetine hydrochloride is a white to slightly yellow powder. It is freely soluble in methanol, propylene glycol, some organic solvents (eg. N,N-dimethylformamide) and slightly soluble in ethanol. The solubility of dapoxetine hydrochloride in aqueous media is a function of the pH (soluble at pH 3.9, sparingly soluble at pH 2.1 and insoluble at pH >7.0).

Drug nanoparticles are pure solid drug particles with crystalline character and a particle size in the nanometer range. In recent years, drug nanoparticles have been the subject of much interest due to their novel physical properties, which mainly depend on particle size. It has been reported that the dissolution rate and saturation solubility of some water insoluble drugs can be increased by reduction of the particle size; hence, the bioavailability is improved. However, it is difficult to modify the *in vitro* release profiles of drug nanoparticles in the carrier free drug delivery system.

The present study is carried out to develop nanoparticles of Dapoxetine Hcl in order to enhance the solubility and bioavailability by decreasing the particle size of the drug. Drugs nanoparticles are prepared by solvent evaporation method. Solubility and dissolution profile of obtained nanoparticles are compared with pure drug. The best formulation is selected based on the drug content, *invitro* dissolution studies, particle size and solubility studies. Dapoxetine Hcl nanoparticle capsules are formulated from the best formulation and invitro dissolution profile is compared with the Dapoxetine Hcl capsules and Dapoxetine Hcl capsules.

CHAPTER V

PLAN OF WORKS

CHAPTER V

PLAN OF WORK

Step-I

Preformulation Studies Standard Curves for Dapoxetine

a) Determination of λmax of Dapoxetine Hcl in 0.01N Hydrochloric acid.
b) Calibration curve for the Dapoxetine Hcl at λmax in 0.01N Hydrochloric acid.
c) Determination of λmax of Dapoxetine Hcl in Phosphate buffer pH 6.8.
d) Calibration curve for the Dapoxetine Hcl at λmax in Phosphate buffer pH 6.8

Step-II

Drug –Polymer Interaction Studies

- Fourier Transform Infrared spectroscopic (FT-IR) studies.

Step-III

Preparation of Dapoxetine Hcl Loaded Controlled Release Nanoparticle by Inclusion Complex Methods

a) Solvent Evaporation Method

Nanoparticles prepared by using different polymers like Ethyl cellulose, Chitosan, HPMC K100, β-cyclodextrin are using dissolved in 20 ml of dichloromethane. Add this mixture into 100 ml aqueous solution of Polyvinyl Alcohol (PVA) in dist. water . Stir the mixture at 1000 rpm for 2 hours in a magnetic stirrer. Then filter the product and dry it an oven at 40^0C for 24 hours.

b) Hyper Cross-linked (Betacyclodextrin Nanoparticles)

Nanoparticles obtained by reacting Cyclodextrin with a Cross-linker (Diphenyl Carbonate). It Synthesize by in neutral and Acid forms. The average diameter of a Nanoparticle is below 1 µm but fractions below 500 nm can be selected

Step-IV

Characterization of *Dapoxetine Hcl* Loaded Controlled Release Nanoparticles Using Inclusion and Non-Inclusion Complex

- Determination of Production Yield
- Determination of drug content.
- Determination of drug entrapment efficiency
- Solubilization efficiency
- Determination of particle size, polydispersity index & zeta potential.
- *Invitro* release studies of Dapoxetine Hcl loaded nanoparticles by using inclusion and Non-inclusion complex
- Kinetics of drug release
- Stability studies
- *Invivo* studies of nanosponge formulation

Step-V

Selection and Evaluation of Best Formulation

The best formulation is selected depending on the results obtained from

- ✓ Production yield

- ✓ Particle size, Polydispersity Index & Zeta potential
- ✓ Entrapment Efficiency
- ✓ Solublization efficiency
- ✓ *In-vitro* drug release studies
- ✓ Release Kinetics
- ✓ Stability studies

Step-VI

Evaluation of Best Formulation

- ✓ Particle Size, Poly dispersity Index & Zeta potential
- ✓ Scanning Electron Microscopy (SEM)
- ✓ X-ray Powder Diffraction (XRPD) analysis.
- ✓ Release Kinetics
- ✓ *Invivo* studies of the nanoparticles formulation.

Step - VII

Formulation of Dapoxetine Hcl Nanoparticles Loaded Capsules

Dapoxetine Hcl Nanoparticles capsules are formulated by Hand filling method. microcrystalline cellulose is used as a diluents, magnesium stearate is used as a Lubricant. All the ingredients are passed through #60mesh separately. Then the ingredients are weighed and mixed in geometrical order after sufficient mixing of drug as well as other components and formulated into capsules. Dapoxetine Hcl Nanoparticles capsules are prepared by hand filling method without using any solubility enhancement method for comparison of *invitro* drug release studies

with Dapoxetine Hcl Nanoparticles capsules and Dapoxetine Hcl conventional capsules.

Step - VIII

Evaluation of Dapoxetine Hcl Nanoparticles Capsules

a) Compatibility Studies

- Fourier transform Infrared spectroscopic (FTIR) studies. .

b) Pre compressional evaluation of powder blend:

- Angle of repose
- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Drug content

c) Post compressional evaluation of Nanoparticles loaded Dapoxetine Hcl Capsules

- General appearance
- Length of capsules
- Weight variation
- Drug content
- *Invitro* release studies

d) Comparison of *Invitro release* of Dapoxetine Hcl Nanoparticles capsules with Dapoxetine Hcl capsules.

CHAPTER-VI

MATERIALS AND EQUIPMENTS

CHAPTER-VI

MATERIALS AND EQUIPMENTS

S.No.	Materials	Manufacturer
1	Dapoxetine Hcl	Gift sample from *Drug* Testing Laboratory, Chennai, India.
2	Ethyl cellulose	Gift sample from Shasun Pharmaceuticals, Chennai, India.
3	Chitosan	Gift sample from Madras Pharma, Chennai, India.
4	HPMC K100 M	Gift sample from Shasun Pharmaceuticals, Chennai, India.
5	Poly vinyl alcohol	Pharmafabrikon, Madurai, India.
6	Dichloromethane	Central Drug House (P) Ltd, New Delhi, India.
7	Ethanol	Central Drug House (P) Ltd, New Delhi, India.
8	Betacyclodextrin	Central Drug House (P) Ltd, New Delhi, India.
9	Diphenyl Carbonate	Central Drug House (P) Ltd, New Delhi, India.
10	Empty Hard gelatin capsules	Gift sample from Strides Shasun , Pudhucherry, India.
11	Microcrystalline Cellulose	Paris Dakner , Madurai, India.
12	Magnesium Stearate	Universal scientific appliances, Madurai, India.

MATERIALS AND EQUIPMENTS

S.No.	Equipments	Company
1	Electronic Weighing Balance	A&D Company HR 200,Japan.
2	Mechanical Strirrer	Remi Motors,India.
3	Magnetic Stirrer	MC Dalal & Co India
4	UV Visible Spetrophotometer	Shimadzu,Japan.
5	Digital Tablet Dissolution Test	Disso 2000,Lab India.
6	X-ray Machime	Stallion 20,Elpro Internationak Ltd.
7	Digital Scanning Calorimeter	DSC 60 Shimadzu
8	SEM Analyzer	Hitachi,Japan
9	Malvern Zetasizer and Particle size analyser	Malvern instruments,UK
10	Ultracentrifuge	Eppendorf centrifuge 5417 R,Germany
11	Ultrasonic Processor	MODROBS - AICTE
12	Stability Chamber	Inlab Equipments Madras PVT (LTD)

CHAPTER VII

DRUG PROFILE

CHAPTER VII

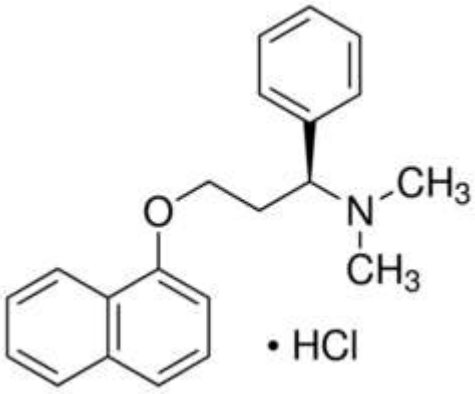
DRUG PROFILE

DAPOXETINE HCL

Synonym

- Dapoxetina
- Dapoxetinum

Structural Formula



Empirical Formula

$C_{21}H_{23}NO$

Chemical Name

(S)-N,N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine hydrochloride

methyl-1-phenyl-ethyl ester)

Molecular Weight

305.4134

Chemical Data

Melting Point : 181.75

Boiling Point : 160-170

Solubility Profile : Soluble in Soluble in DMSO (68MG/ML AT 25^0C

Soluble in chloroform, methanol, ethanol

Description	**:**	White powder
Bioavailability	**:**	Mean 42%
Protein Binding	**:**	>99%
Half-Life	**:**	1-1.6 hours
pH range	**:**	5.87
Log P	**:**	(Octanol/Water Partition Coefficient) 6.8
Dose Range	**:**	30mg, 60 mg

Identification

λmax at 292 nm in UV spectrophotometer

Pharmacodynamic Properties

Pharmacological studies show Dapoxetine Hcl to be a highly potent serotonin-transporter inhibitor. Although this pharmacological activity is similar to that of clomipramine and conventional selective serotonin reuptake inhibitors (SSRIs), chemical features of the structure of dapoxetine and its pharmacokinetic profile differentiate it from other SSRIs. For instance, and unique among SSRIs, it is not a halogenated compound), while all other SSRIs contain one or more halogen atoms. The molecular structure of dapoxetine also includes a naphthyl moiety and it is possible that these features underpin the physicochemical and pharmacokinetic properties of the molecule. In recent Phase III trials, the efficacy of dapoxetine was tested in patients with moderate to severe PE who, at baseline, had a measured mean IELT of <1 min (an IELT inclusion criteria of <2 min was used throughout trials with Dapoxetine Hcl).

In the light of the rapid peak drug concentration, patients were asked to take Dapoxetine (30 or 60 mg) or placebo, on-demand, at 1–3 h before anticipated sexual intercourse. This contrasts with other PE studies in which

patients were obliged to take their medication daily for several weeks, to detect any benefit. Even those studies that purport to examine 'on-demand' drug dosing, use long pre-intercourse dosing intervals such as 3–8 h or pre-load patients with the trial drug for several weeks.

Pharmacokinetic Properties

Absorption

Dapoxetine is a white powder substance and water- insoluble. Taken 1–3 hours before sexual activity, it is rapidly absorbed in the body. Its maximum plasma concentration (C_{max}) is reached 1–2 hours after oral administration. The C_{max} and AUC (Area Under the plasma vs. time Curve) are dose dependent. The C_{max} and Tm (time needed to obtain the maximum plasma concentration) after single doses of dapoxetine 30 mg and 60 mg are 297 and 498 µg/mL at 1.01 and 1.27 hours respectively. A high fat meal does reduce the C_{max} slightly, but it is insignificant. In fact, food doesn't alter dapoxetine pharmacokinetics. Dapoxetine can be taken with or without food.

Distribution

Dapoxetine is absorbed and distributed rapidly in the body. Greater than 99% of dapoxetine is bound to the plasma protein. The mean steady state volume is 162 L. Its initial half-life is 1.31 hours (30 mg dose) and 1.42 hours (60 mg dose,) and its terminal half life is 18.7 hours (30 mg dose) ansd 21.9 hours (60 mg dose).

Biotransformation

In vitro studies suggest that dapoxetine is cleared by multiple enzyme systems in the liver and kidneys, primarily CYP2D6, CYP3A4, and flavin monooxygenase (FMO1). Following oral dosing in a clinical study designed to explore the metabolism of 14C-dapoxetine, dapoxetine was extensively

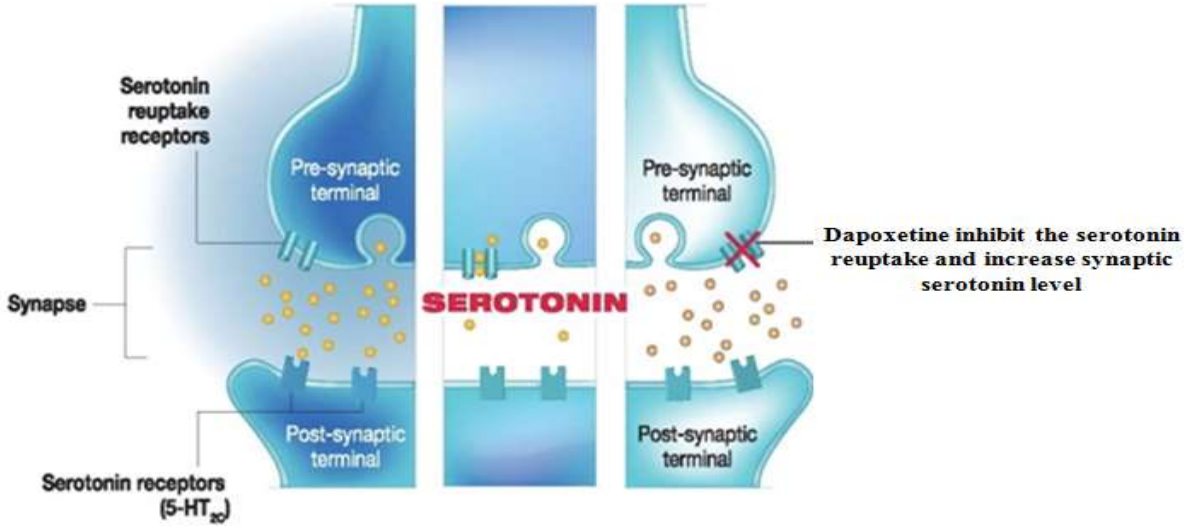
metabolised to multiple metabolites primarily through the following biotransformational pathways: N-oxidation, N-demethylation, naphthyl hydroxylation, glucuronidation and sulfation. There was evidence of presystemic first-pass metabolism after oral administration. Intact dapoxetine and dapoxetine-N-oxide were the major circulating species

in the plasma. In vitro studies show that dapoxetine-N-oxide was inactive in a battery of in vitro binding and transporter studies. Additional metabolites include desmethyldapoxetine (DED) and didesmethydapoxetine, which account for less than 3% of the circulating medicinal product related material. In vitro binding studies indicate that DED is equipotent to dapoxetine and didesmethydapoxetine has approximately 50% of the potency of dapoxetine. The unbound exposure of DED is approximately 1/2 of the free exposure of dapoxetine. The unbound Cmax of DED is estimated to be 20-25% of dapoxetine Cmax in the absence of intrinsic or extrinsic factors that may change exposure levels.

Excretion

The metabolites of dapoxetine were primarily eliminated in the urine conjugates. Unchanged active substance was not detected in the urine. Dapoxetine has a rapid elimination, as evidenced by a low concentration (less than 5% of peak) 24 hours after dosing. There was minimal accumulation of dapoxetine following daily dosing. The terminal half-life is approximately 19 hours following oral administration. The half-life of DED is similar to that of Dapoxetine Hcl

Mechanism of Action



Onset and Duration of Efficacy

The on-demand efficacy of dapoxetine in PE is the key feature that sets the drug apart from other PE treatments. Although various authors have claimed 'on-demand' efficacy for other treatments (e.g. the conventional SSRIs), close inspection of the studies often reveals methodological failings. Often hybrid treatment regimens have been used, comprising periods of chronic drug administration followed by on-demand treatment. Moreover, double-blind, placebo-controlled trials are the exception rather than the rule. These idiosyncratic protocols make the results difficult to assess, and true on-demand efficacy has not been convincingly shown for existing agents.

The efficacy of on-demand dapoxetine suggests that the drug, despite its biochemical similarities to other SSRIs, may have a different mechanism of action in PE, possibly due to its pharmacokinetic profile or physicochemical properties.

Tolerability/Safety

The most common and a dose-dependent adverse effect with both the conventional SSRIs and dapoxetine is nausea. Even so, nausea after dapoxetine is

mainly mild, transient and related to the presence of dapoxetine in the body. Other adverse effects occurred less frequently and were not consistently dose-related.

One of the largest issues when treating PE with conventional daily dosing of SSRIs is the high incidence of sexual side-effects: chronic treatment with these SSRIs reduces libido and compromises erectile function, possibly due to the adaptive receptor and messenger changes inherent in their actions. Of patients on chronic SSRIs, 30–50% experience sexual side-effects. By contrast, sexual side-effects are infrequent with dapoxetine. On-demand dapoxetine has no significant adverse effects on libido (<1%) or erection (<4%), presumably because it does not persist in the body and thus there are no adaptive changes.

Side effects of Dapoxetine hydrochloride.

- Nausea
- Dizziness
- Diarrhea
- Anorgasmia,
- Erectile dysfunction,
- Diminished libido

Contraindications

- contraindicated when used in combination with monoamine oxidase inhibitors (MAOIs)
- Dapoxetine should also not be used in combination with thioridazine or other drugs that inhibit the CYP450 2D6 hepatic enzyme may raise plasma thioridazine levels. Administration of thioridazine alone may lead to prolongation of the QTc interval associated with severe ventricular arrhythmias such as torsades de pointes and sudden cardiac death.
- Dapoxetine should also not be used in combination with the drug pimozide

- Dapoxetine should not be used in men with moderate to severe hepatic impairment and in those receiving CYP3A4 inhibitors such as ketoconazole, ritonavir, and telithromycine.
- Dapoxetine can also not be used in patients with heart failure, permanent pacemaker, or other significant ischemic heart disease.
- Caution is advised in men receiving thioridazine, monoamine oxidase inhibitors, SSRIs, serotonin-norepinephrine reuptake inhibitors, or tricyclic antidepressant. If a patient stops taking one of these drugs, he should wait for 14 days before taking dapoxetine..

Interactions

- With *phosphodiesterase inhibitors* (PDE5 inhibitors)
- With *ethanol*

Brand Names

- **Dasutra**
- **Kutub**
- **SD-Pill**
- **Sustinex**
- **TD-Pill**

(Drug bank, Clark's analysis, Martindale Wikipedia, drugs.com)

CHAPTER VIII

EXICIPIENTS PROFILE

CHAPTER VIII

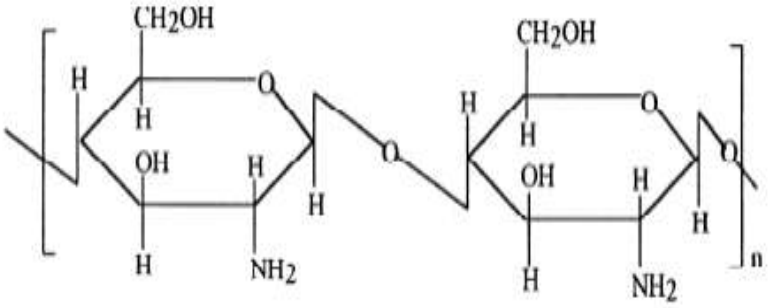
EXICIPIENTS PROFILE

CHITOSAN

Synonym

- Poliglusam
- Deacetylchitin
- Chicol
- Chitosan hydro chloridum

Structure



Emprical Formula

$C_{56}H_{103}N_9O_{39}$

Molecular Weight

1526.464 g/mol

Description

Appearance	:	Powder
Color	:	White or creamy white powder
Odor	:	Odourless
Taste	:	Tasteless

Solubility

- 97% in 1% Acetic Acid

- Sparingly soluble in water
- Practically insoluble in ethanol (95%)
- Neutral or alkali solutions at pH above approximately 6.5

pH

4.0 - .6.0 (1% w/v aqueous solution)

Functional Category

- Coating agent
- Controlled release agent
- Viscosity increasing agent
- Mucoadhesive material
- Film former

Storage Conditions

- Chitosan should be stored in a tightly closed container in a cool and dry place.
- It should b stored at a room temperature of 2 - 8^0C.

Incompatibility

Chitosan is incompatible with strong oxidizing agent

Handling Precaution

- It is compatible
- Open flames should be avoided
- It is temperature sensitive and should not be heated above 200^0C.
- It should be handled in a well-ventilated environment.

Regulatory Status

Chitosan is registered as a food supplement in some countries.

Application

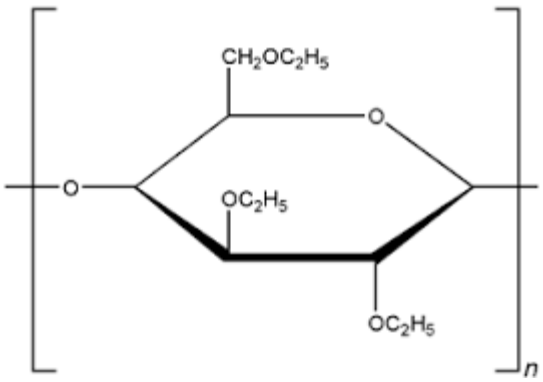
- ❖ Controlled drug delivery
- ❖ Peptide delivery
- ❖ Colonic drug delivery
- ❖ Gene delivery
- ❖ Pharmaceutical formulation including gel, films, beads microsphere, tablet and liposomes (**Singling Tang *et al*., 2011).**

ETHYL CELLULOSE

Synonym

- Aquacoat ECD
- Aqualon
- Ethocel
- Sucrelease

Structure



Emprical Formula

C12H23O6 (C12H22O5) n$C_{12}H_{23}O_5$

Molecular Weight

40, 000

Description

Color : White to light tan-coloured powder

Odor : Odourless

Taste : Tasteless

Melting Point

$1650^0 - 1850^0$ C

Solubility

Practically insoluble in propylene glycol, glycerine and water freely soluble in chloroform, ethanol, ethyl acetate, methanol and toluene.

Functional Category

- Coating agent
- Flavouring fixative
- Tablet binder
- Tablet filler
- Viscosity - increasing agent.

Storage Conditions

It should be stored at a temperature not exceeding 3280^0 C (900F) in a dry area away from all sources of heat.

Handling Precautions (Muhammed Rafeeq P E et al., 2010):

- To prevent fine dust clouds of ethyl cellulose from reaching potentially explosive levels in the air.

- ❖ It may be an irritant to the eyes and eye protection should be worn.

Regulatory Status

Included in the FDA inactive ingredients

BETA CYCLODEXTRIN

Synonyms

- ❖ b-Cyclodextrin beta-cycloamylose
- ❖ Beta-dextrin
- ❖ Betadexum

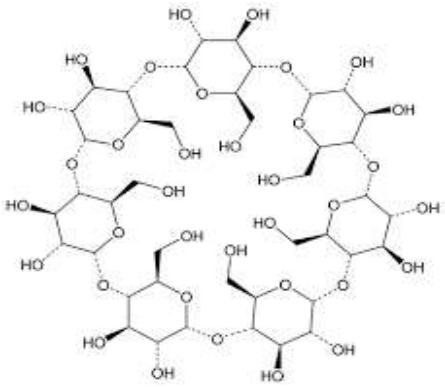
Empirical Formula

$C_{42}H_{70}O_{35}$

Molecular Weight

1135g/Mol

Structural Formula



Functional Category

- ❖ Solubilizing agent
- ❖ stabilizing agent

Description

Cyclodextrin occur as white, practically odorless, fine crystalline powders, having a slightly sweet taste. Some cyclodextrin derivatives occur as amorphous powders.

Pharmacopeial Specifications

Solubility

Soluble 1 in 200 parts of propylene glycol, 1in50 of water at 208C, 1 in 20 at 508C; practically insoluble in acetone, ethanol (95%), and methylene chloride.

Specific Rotation

D25= þ162.08;

Surface Tension (at 258c)

1 mN/m (71 dynes/cm)

Stability and Storage Conditions

- β-Cyclodextrin is stable in the solid state if protected from high humidity.
- β-Cyclodextrins should be stored in a tightly sealed container, in a cool, dry place.

Method of Manufacture

Betacyclodextrin is produced by the action of the enzyme cyclodextrin glucosyl transferase upon starch or a starch hydrolysate. An organic solvent is used to direct the reaction that produces betacyclodextrin, and to prevent the growth of microorganisms during the enzymatic reaction. The insoluble complex of betacyclodextrin and organic solvent is separated from the non cyclic starch, and the organic solvent is removed in vacuum so that less than 1 ppm of solvent remains in the betacyclodextrin. The betacyclodextrin is then carbon treated and crystallized from water, dried, andcollected **(Peng Quan *et al*., 2011).**

Safety

 nontoxic and nonirritant and cyclodextrin are approved for use in food products and orally administered pharmaceuticals in a number of countries.

 Cyclodextrins are not irritant to the skin and eyes, or upon inhalation.

 There is also no evidence to suggest that cyclodextrins are mutagenic or teratogenic.

Handling Precautions

It should be handled in a well-ventilated environment

Applications

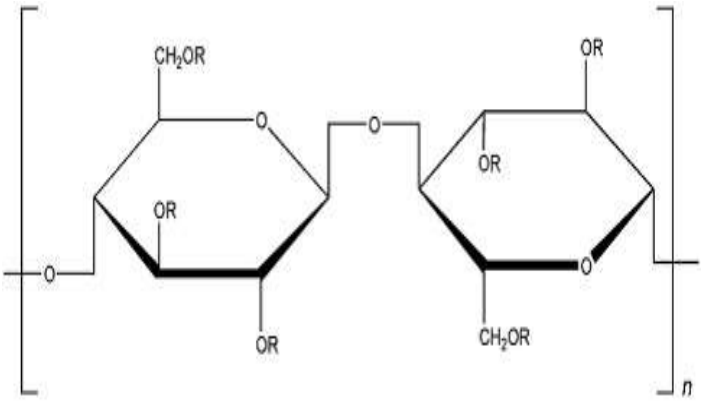
- Cyclodextrin may be used to form inclusion complexes with a variety of drug molecules, resulting primarily in improvements to dissolution and bioavailability
- Primarily used in tablet and capsule formulation
- Bioavailability enhancement
- Cholestrol free products
- Multifunctional dietary fiber
- Other food applications
- Aerosols
- Clinical applications

HYDROXY PROPYL METHYL CELLULOSE

SYNONYM:

- Hypromellose.
- Methocel

STRUCTURE:



where R is H, CH3, or CH3CH(OH)CH2

EMPIRICAL FORMULA:

It is a partly O-methylated and O-(2-hydroxypropylated) cellulose. It is available in several grades that vary in viscosity and extent of substitution.

MOLECULAR WEIGHT:

10,000–15, 00,000 Dalton

DESCRIPTION:

- **Colour:** white or creamy-white fibrous or granular powder
- **Odour:** odourless

TASTE:

Tasteless

Solubility:

Soluble in cold water, forming a viscous colloidal solution; practically insoluble in hot water, chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents. Some grades are swellable in ethanol.

Melting point:

Browns at 190–2008C; chars at 225–2308C

METHOD OF MANUFACTURE:

A purified form of cellulose, obtained from cotton linters or wood pulp, is reacted with sodium hydroxide solution to produce a swollen alkali cellulose that is chemically more reactive thanuntreated cellulose. The alkali cellulose is then treated with chloromethane and propylene oxide to produce methyl hydroxypropyl ethers of cellulose. The fibrous reaction product is then purified and ground to a fine, uniform powder or granules. Hypromellose can then be exposed to anhydrous hydrogen chloride to induce depolymerization, thus producing low viscosity grades.

Typical viscosity values for 2 % (w/v) aqueous solutions of different viscosity grades of hpmc at 20°c:

Methocel K100 Premium LVEP100

Methocel K4M Premium : 4000

Methocel K15M Premium : 15000

Methocel K100M Premium : 100 000

Methocel E4M Premium : 4000

Methocel F50 Premium 50

Methocel E6 Premium LV 6

Methocel E15 Premium LV 15

Methocel E50 Premium LV 50

Metolose 60SH : 50, 4000, 10 000

Metolose 65SH : 50, 400, 1500, 4000

Metolose 90SH : 100, 400, 4000, 15 000

STORAGE CONDITION:

It should be stored in a well-closed container, in a cool, dry place.

HANDLING PRECAUTION:

- Hypromellose dust may be irritant to the eyes and eye protection is recommended.
- Excessive dust generation should be avoided to minimize the risks of explosion.
- Hypromellose is combustible.

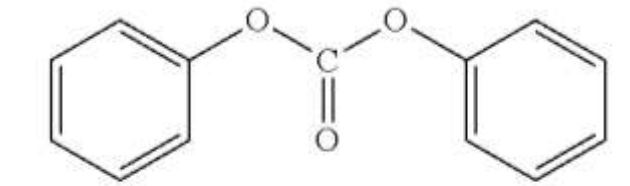
(Hand book of Pharmaceutical Excipients by Raymond C. Row)

DIPHENYL CARBONATE

Synonym

Phenyl Carbonate

Structure



DPC

Emprical Formula

C13 H10 O3

Molecular Weight

214.216 g/mol.

Melting Point

83^0C

Boiling Point

$306\ ^0\ C$

Solubility

Soluble in Ethanol, diethyl ether, Carbon tetrachloride ,acetic acid insoluble in water

Description

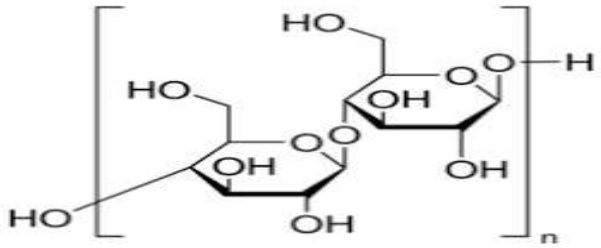
White powder

Applications

Polycarbonates can be prepared by transesterifying diphenyl carbonate with Bisphenol A, and phenol is a co-product. Phosgene is avoided as a result. These polycarbonates may be recycled by reversing the process transesterifying the polycarbonate with phenol to yield diphenyl carbonate and bisphenol A.

MICROCRYSTALLINE CELLULOSE

Structural Formula



Non-proprietary Name

- BP: Microcrystalline Cellulose
- JP: Microcrystalline Cellulose
- PhEur: Cellulose, Microcrystalline
- USP-NF: Microcrystalline Cellulose

Synonyms

- Avicel PH; Cellets; Celex; cellulose gel; hellulosum microcristallinum;
- Celphere; Ceolus KG; crystalline cellulose; E460; Emcocel;
- Ethispheres; Fibrocel; MCC Sanaq; Pharmacel; Tabulose; Vivapur.

Empirical Formula

$(C_6H_{10}O_5)_n$

Molecular weight

36000g/mol

Functional Category

- Adsorbent and suspending agent
- Tablet and capsule diluents
- Tablet disintegrant

.**Description**

. Microcrystalline cellulose is a purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles.

Solubility

Slightly soluble in 5% w/v sodium hydroxide solution; practically insoluble in water, dilute acids, and most organic solvents

pH

pH 5.0-7.5

Melting point

260 – 270^{0}C.

Density

0.337 g/cm3

Incompatibilities

Microcrystalline cellulose is incompatible with strong oxidizing agents.

Method of manufacture

Microcrystalline cellulose is manufactured by controlled hydrolysis with dilute mineral acid solutions of a-cellulose, obtained as a pulp from fibrous plant materials. Following hydrolysis, the hydrocellulose is purified by filtration and the aqueous slurry is spray dried to form dry, porous particles of a broad size distribution.

Stability & Storage condition

Microcrystalline cellulose is a stable though hygroscopic material. The bulk material should be stored in a well-closed container in a cool, dry place.

Applications

1. Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluents in oral tablet and capsule formulations where it is used in both wet-granulation and direct-compression Processes.

2. In addition to its use as a binder/diluent, microcrystalline cellulose also has some lubricant and disintegrant properties that make it useful in tableting.

3. Microcrystalline cellulose is also used in cosmetics and food products;

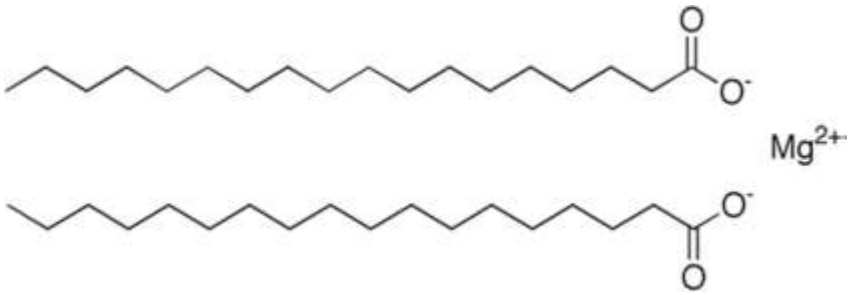
Handling precaution

Microcrystalline cellulose may be irritant to the eyes. Gloves, eye protection, and a dust mask are recommended.

(Hand book of Pharmaceutical Excipients by Raymond C. Rowe et.al. 2009)

MAGNESIUM STEARATE

Structure



Structural Formula

[CH3 (CH2)16COO] 2Mgs

Nonproprietary Names

- BP: Magnesium Stearate
- JP: Magnesium Stearate
- PhEur: Magnesium Stearate
- USP-NF: Magnesium Stearate

Synonyms

- Dibasic magnesium stearate
- Magnesium distearate
- Magnesiistearas
- Magnesium octadecanoate;
- Octadecanoic acid
- Magnesium salt
- Synpro90.

Empirical formula

C36H70MgO4

Molecular weight

591.24 g/mol

Description

Magnesium stearate is a very fine, light white, precipitated or milled, impalpable.

Powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. The powder is greasy to the touch and readily adheres to the skin.

Functional categories

Tablet and capsule lubricant.

Solubility

Practically insoluble in ethanol, ethanol (95%), ether and water; slightly soluble in warm benzene and warm ethanol (95%).

Melting point

117–150˚C

Density

1.092 g/cm3

Loss on drying

46.0%

Stability and storage conditions

Magnesium stearate is stable and should be stored in a well-closed container in a cool, dry place

Incompatibilities

Incompatible with strong acids, alkalis, and iron salts. Avoid mixing with strong oxidizing materials.

Applications

It is primarily used as a lubricant in capsule and tablet manufacture.

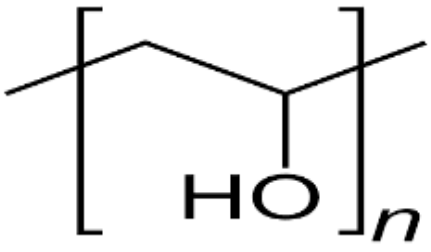
(Hand book of Pharmaceutical Excipients. Pharmaceutical Press, London. 5thedtition)

POLYVINYL ALCOHOL

Synonyms

- Airvol
- Elvanol
- Gohsenol

Structural formula



Emprical formula

(C2H4O)n

Functional category

- Coating agentLubricant
- Stabilizing agent
- Viscosity-increasing agent

Description

Polyvinyl alcohol occurs as an odorless, white to cream-colored granular powder.

Solubility

Soluble in water, slightly soluble in ethanol, insoluble in organic solvents.

Applications

- Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations

- It is used as a stabilizing agent for emulsions

Safety

- PVA is generally considered a non-toxic material.
- It is non-irritant to the skin and eyes at concentrations up to 10%; concentrations up to 7% are used in cosmetics.

Handling precaution (Ansari KA *et.al.,* 2011)

- protection and gloves recommended
- PVA dust may be an irritant on inhalation
- Handle in a well-ventilated environment

CHAPTER IX

EXPERIMENTAL PROTOCOL

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EXPERIMENTAL PROTOCOL

1. CALIBRATION OF DAPOXETINE HCL

a) Preparation of Dissolution Medium

0.01 N Hydrochloric acid

0.85 ml of Hydrochloric acid diluted in 1000 ml distilled water.

Phosphate buffer pH 6.8

50ml of 0.2M potassium dihydrogen phosphate is placed in 200 ml volumetric flask. 22.4 ml of 0.2M sodium hydroxide is added and makeup to the volume with distilled water.

0.2 M Potassium dihydrogen phosphate

27.218 gm of potassium dihydrogen phosphate is dissolved and diluted to 1000 ml with distilled water.

0.2M Sodium hydroxide

8 gm of sodium hydroxide is dissolved and makeup to 1000 ml with distilled water.

b) Determination of absorption maximum (λmax) (Supriya A et al., 2018)

A known weight (100 mg) of drug (Dapoxetine Hcl) is dissolved in 100ml of methanol to make a primary stock solution (1000µg/ml). The stock solution is further diluted using a 0.01N hydrochloric acid solution to 10µg/ ml concentration. The resultant solution is scanned in the range of (200- 400nm) by UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) to get absorption maximum (λ max). And, also a absorption maximum is estimated similarly using phosphate buffer pH (6.8).

c) Preparation of calibration curve

From the above prepared stock solution, (5 to 50µg/ml) concentration solutions are prepared using the 0.01N hydrochloric acid solution. The absorbance of these solutions is measured at λ max by UV- spectrophotometer (UV-1700 Shimadzu corporation, Japan). A standard curve is plotted using concentration on X-axis and the absorbance obtained on Y-axis. And also a standard curve is prepared similarly using phosphate buffer pH (6.8).

2. PREFORMULATION (COMPATABILITY) STUDIES

Compatibility Studies for Drug and Excipients

The compatibility studies were performed for the development of dosage form, preformulation studies is carried out to confirm that there was no interaction between the drug and excipients. Infra spectrophotometer studies used to check the compatibility studies between excipients.

***Fourier Transform-Infra Red (FT-IR) Studies (*ADLIN JINO NESALIN J et al., 2009)**

Infrared Spectrum analysis of drug (Dapoxetine Hcl), polymers (chitosan, ethyl cellulose, hydroxyl propyl methyl cellulose, Betacyclodextrin, Diphenyl Carbonate) and the physical mixtures of drug with the polymers are obtained from FT-IR Spectrophotometer (Shimadzu, Japan) by KBr disk method. It is an important tool plays a major role in determining the compatibility between the drug and polymer. A known weight of samples are mixed with KBr powder and compressed to 10-mm discs by hydraulic press at pressure of 150 bar for 30 s. The scanning range and resolution are 400 – 4000cm^{-1} and 4cm^{1}. The spectra obtained are compared and interpreted for the shifting of functional peaks or the appearance and disappearance of new functional peaks **(Afifa Bathool et al., 2012).**

3. FORMULATION OF DAPOXETINE HCL NANOPARTICLES

Solvent Evaporation Method (Ansari KA *et al.,* 2011):

Nanoparticles prepared by polymers like chitosan, ethyl cellulose, hydroxyl propyl methyl cellulose; Betacyclodextrin, Diphenyl Carbonate and polyvinyl alcohol were prepared by solvent evaporation method. Disperse phase consisting of Dapoxetine (100 mg) and requisite quantity of polymers dissolved in 20 ml solvent (dichloromethane) was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for two-three hours on a magnetic stirrer. The nanoparticles formed were collected by filtration through whatman filter paper and dried in oven at 50 ^{0} C for 2 hours. The dried nanoparticles were stored in vaccumdesicater to ensure the removal of residual solvent **(Ahmed et al., 2016).**

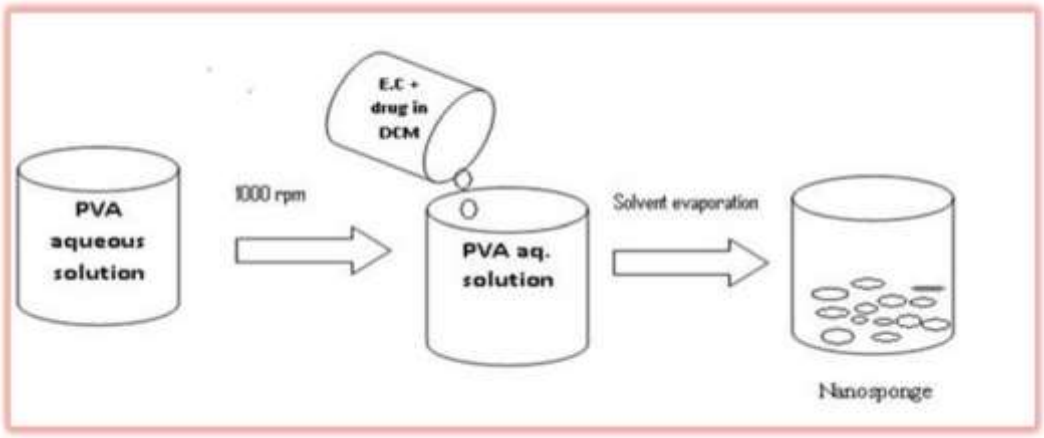

Figure: 1

Hyper-Cross Linked (Betacyclodextrin) Nanoparticles

β-cyclodextrin Nanoparticles was prepared by using diphenylcarbonate for cross linking process Briefly, an amount of anhydrous cyclodextrin(1:1,1:2,1:3,1:4) was put to react in melted diphenylcarbonate at 90^{0} C for atleast 5 hours. Then,

the solid was ground in mortar and Soxhlet extracted with ethanol to remove either impurities or unreacted diphenylcarbonate. The reaction was carried out using a cross-linked excess, at four different molar ratios, e.g. 1:1,1:2,1:3,1:4 (β-CD:cross linker). After purification, NP was stored 25^0C until further use. This reaction was also carried out in the presence of ultrasound, and two different types of NP, namely, crystalline (1:1 ,1:2, 1:3,1:4 NPs), and Paracrystalline were formed based on the process conditions of the synthesis **(Sureshkumar P *et al.,* 2015).**

Preparation of Dapoxetine Hcl Loaded Nanoparticles

Dapoxetine Hcl was dispersed in aqueous suspensions of the various types of nanoparticles in a ratio of 1:1, 1:2, 1:3, 1:4 (drug to NP by weight) and was stirred for 24 hours. After 24h, the suspension were centrifuged at 2000rpm for 10 min to separate the uncomplexed drug as a residue below colloidal supernatants were freeze dried to obtain drug loaded NP formulations, named as F1:1 F1:2 F1:3 F1:4 and Fpara depending upon the ratio of β-CD: cross-linker. The drug- loaded NS formulations were stored in a covered vaccum desicator at room temperature.

4. CHARACTERIZATION OF DAPOXETINE HCL NANOPARTICLES

All the formulations are evaluated for its production yield, particle size, polydispersity index, zeta potential, drug content, entrapment efficiency, solubilization efficiency and *In vitro* drug release studies and Kinetics of drug release studies **(Akas M *et al.*, 2006).**

a) Production yield

The production yield of the Nanoparticles is calculated for each batch by dividing the total weight of product (M) by the total expected weight of drug and polymer. Weight of nanoparticles (M).

$$\%\ Yield = \frac{Actual\ Yield}{Theoretical\ Yield} \times 100\%$$

b) Theoretical drug loading

Theoretical drug loading in nanoparticles is estimated by using the following formula,

$$\text{Drug Loading Content (\%)} = \frac{\text{weight of drug in nanoparticles}}{\text{weight of nanoparticles}} \times 100$$

c) Determination of drug content

Sample containing 100 mg equivalent Dapoxetine Hcl Nanoparticles are dissolved and the volume is made upto 100ml with pH 6.8 phosphate buffer. From the above solution 10 ml is pipette out and made upto 100 ml with phosphate buffer. The absorbance of resulting solution is determined at λmax (292nm) using UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan) and the drug content is estimated.

d) Entrapment Efficiency

For the drug entrapment efficiency tests, the nanoparticles of F1- F16 were performed. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The 10mg of the nanoparticle was analyzed by dissolving sample in 10ml of distilled water. After the drug was dissolved, 10 ml of clear layer of dissolved drug is taken **(Abdul Hasan Sathali et al.,2015).**

Thereafter, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 292nm (U.V Spectrophotometer, systronics). The test was repeated with another sample. The amount of the drug in the suspension was analyzed by centrifugation at 500rpm for 5 minutes and by measuring the concentration of the drug in the clear supernatant layer by the UV-spectrophotometric

method. The test was again repeated with another sample. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticles suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ Entrapment Efficiency} = \frac{\text{Total amount of drug} - \text{Concentration of drug}}{\text{Total amount of drug}} \times 100$$

e) Determination of Particle size and Zeta potential

The mean particle size (z-average), polydispersity index (PI) and zeta potential of dapoxetine nanoparticles formulations are determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd.,UK). The freeze dried powders are redispersed with water to obtain a proper scattering intensity before measurement **(Natarajan Tamilselvan et al.,2015).s**

f) *In vitro* dissolution studies

I) Non- inclusion complex method (Solvent evaporation method)

USP dissolution apparatus Type I (Basket method) at rotation speed of 100 rpm is used for *invitro* testing of drug dissolution of solvent evaporation method formulation. For each batch, sample of 10 mg equivalent Dapoxetine Hcl nanoparticles containing in enteric capsules are taken and subjected to dissolution studies with 900 ml 0.01N hydrochloric acid (2 hours) and phosphate buffer pH 6.8 (10 hours) as dissolution medium. Bath temperature is maintained at 37±0.5°C throughout study.

A sample (1.4 ml) of the solution is withdrawn from the dissolution apparatus. Samples were taken at appropriate time intervals for 1 hour, 2 hour,3 hour, upto 12 hourls sampling for up to 6 hours and finally for twelth hour. Fresh dissolution

medium was replenished each time when sample is withdrawn to compensate the volume.

Absorbance values of sample solutions are measured against respective buffer solutions at λmax (292nm) in UV Spectrophotometer (UV-1700 Shimadzu corporation, Japan). The cumulative percentage drug release is calculated.

ii) Inclusion complex method (Hyper cross-linker nanoparticles)

The *invitro* release was carried out using multi compartment rotating cells with a dialysis membrane (Sartorious, cut off 12,000Da). The donor phase consisted of formulations containing fixed amount of Dapoxetine Hcl nanoparticles in 0.01N hydrochloric acid &Phosphate buffer at pH6.8 (1.4ml), The receiving phase consisted of phosphate buffer, pH 6.8 added with 0.5%w/v sodium lauryl sulphate (1ml) to maintain proper sink conditions .The receiving phase was completely withdrawn and replaced with fresh medium after fixed time intervals. Suitably diluted and analyzed using UV spectrophotometer at 292 nm. The experiment was carried out in triplicate.

g) *Invitro* Kinetic analysis

In order to investigate the drug release mechanism from Controlled release nanoparticles formulations, the percentage cumulative drug release data is analyzed with following mathematical model **(Amighi K *et al*., 2005).**

- Zero-order
- First order
- Higuchi
- Hixson-Crowell cube root law
- Korsmeyer-peppas model.

The zero order rate Equation describes the systems where the drug release rate is independent of its concentration.

$$Q_t = Q_0 + K_0 t$$

Where, Qt is the amount of drug dissolved in time t, Q0 is the initial amount of drug in the solution (most times, Q0= 0) and K0 is the zero order release constant expressed in units of concentration/time .To study the release kinetics, data obtained from in vitro drug release studies are plotted as cumulative amount of drug released versus time.

The first order Equation describes the release from a system where the release rate is concentration dependent.

$$\text{Log } C = \log C_0 - kt / 2.303$$

Where C is the concentration of the drug at time (t), C0 is the initial concentration of the drug and k is the first-order release rate constant. The data obtained are plotted as log cumulative percentage of drug remaining vs. time. **Higuchi** described the release of drugs from porous, insoluble matrix as a square root of time dependent process based on Fickian diffusion as shown in following Equation.

$$F\ t = Q = KH \times t^{1/2}$$

Where Q is the amount of drug released in time t.

This model is based on the hypotheses that

- initial drug concentration in the matrix is much higher than drug solubility
- drug diffusion takes place only in one dimension (edge effect must be negligible)
- drug particles are much smaller than system thickness
- matrix swelling and dissolution are negligible
- drug diffusivity is constant and
- perfect sink conditions are always attained in the release environment.

The data obtained are plotted as cumulative percentage drug release versus square root of time. **Hixson and Crowell** (1931) recognized that the Particles' regular area is proportional to the cube root of its volume. They derived the equation

$$W_0 1/3 - Wt 1/3 = \kappa t$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form, Wt is the remaining amount of drug in the pharmaceutical dosage form at time t and κ (kappa) is a constant incorporating the surface volume relation. The equation describes the release from systems where there is a change in surface area and diameter of particles. To study the release kinetics, data obtained from in vitro drug release studies are plotted as cube root of drug % remaining in matrix vs. time.

Korsmeyer – Peppas model describes the fraction of drug release relates exponentially with respect to time **(Sureshkumar P *et al.,* 2015).**

$$Mt / M\infty = K\ t\ n$$

Where Mt / M∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. In this model, the value of n characterizes the release mechanism of drug. To study the release kinetics, data obtained from Invitro drug release studies are plotted as log cumulative percentage drug release versus log time **(Bhatt Neha et al., 2013).**

Release exponent (n)	**Drug transport**	**Rate as a function of time**
0.5	Fickian diffusion	$t^{-0.5}$
0.45< n-0.89	Non-fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super case II	t^{n-1}

Table: 1

5. SELECTION AND EVALUATION OF BEST FORMULATION

Selection of best formulation

The best formulation selection is based on the results obtained from

- Production yield
- Drug content
- particle size, poly dispersity Index and Zeta potential
- entrapment efficiency
- solubilization efficiency
- In vitro drug release studies
- kinetics of drug release.

6. Evaluation of Best Formulation

a) Infrared (IR) spectroscopic studies (Ansari KA *et al.,* 2011)

Infrared spectrum (IR) analysis is carried out for the selected best formulations (F6) to find out the interactions between the drug and excipients used as per the procedure mentioned on drug polymer interaction studies.

b) Determination of Particle Size

The particle size distribution was determined by using dynamic light scattering technique (DLS) technique used for the particle size distribution in is a Malvern particle size analyzer (Nano ZS 90, Malvern Instruments Ltd., UK). In this technique the particle sizes of batch of the nanoparticles were observed and from the standard deviation and mean particle size of the nanoparticles, the polydispersity index (PDI) was calculated. The poly dispersity index is the indication for the nature of dispersity. The freeze dried powders are redispersed with water to obtain a proper scattering intensity before measurement.

c) Morphological studies of nanoparticles by using Scanning Electron Microscopy (SEM)

Morphological evaluation of the selected Nanoparticles formulation is carried out in scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan).All samples are examined on a brass stub using carbon double-sided tape. Powder samples are glued and mounted on metal sample plates. The samples are gold coated (thickness ≈15–20 nm) with a sputter coater (Fison Instruments, UK) using an electrical potential of 2.0kV at 25 mA for 10 min. An excitation voltage of 20 kV was used in the experiments **(Natarajan Tamilselvan et al.,2015).**

d) X-ray Powder Diffraction (XRPD) analysis

The crystalline state of the samples, including the drug and freeze-dried powders are studied in X-ray diffractometer (XRD-462, Digaku, Japan). XRPD is carried out in symmetrical reflection mode using Copper line as the source of radiation and the wavelength is set at 1.5405A˚. Standard runs using a 40 kV and 30mA in this process. Samples are performed with a scanning rate of 0.1000^0/min and the scanning range of the 2 from the initial angle 4˚to the final angle 90.˚

e) Stability Studies

The nanoparticle formulation F1-F16 was subjected to stability studies according to ICH guidelines by storing at 25^0C/60% RH for 30 days and 40^0C /75% RH for 30 days. These samples were analyzed and checked for changes in physical appearance, drug content and Entrapment efficiency*, invitro* drug release studies at regular intervals. The observations of short term storage conditions and accelerated conditions indicate no significant changes in the parameter even when it was subjected to stress testing for a period of 2 months. When the formulation was studied for short – term storage conditions, the drug content in the formulation within the 95% confidence interval and hence the slight

decrease in the drug content and entrapment efficiency was statistically not significant.

7. PREPARATION AND EVALUATION DAPOXTINE HCL NANOPARTICLES LOADED CAPSULES

Dapoxetine Hcl nanoparticles capsules were formulated by Hand filling method. Micro crystalline cellulose is used as a binder and diluents; magnesium stearate is used as a Lubricant. All the ingredients are passed through #60mesh separately. Then the ingredients were weighed and mixed in geometrical order after sufficient mixing of drug as well as other components and formulated into capsules. Dapoxetine Hcl nanoparticle capsules were prepared by Hand filling method without using any solubility enhancement method for comparison of *invitro* drug release studies with Dapoxetine Hcl nanoparticles capsules and Dapoxetine Hcl capsules.

(a) Pre compressional evaluation of powder blend

i). Angle of repose

The flow characteristics are evaluated by determining angle of repose. Improper Flow of powder is due to frictional forces between the particles. These frictional forces are quantified by angle of repose. Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane. Angle of repose is calculated using the equation.

$$\tan \theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where,

h = height of pile

r = radius of the base of the pile

θ = angle of repose

Angle of repose	Type of flow
<20˚	Excellent
20˚-30˚	Good
30˚-35˚	Moderate
35˚-40˚	Poor
>40˚	Very poor

Table: 2

ii) Bulk Density

Apparent bulk density is determined by pouring pre sieved drug excipients blend into a graduated cylinder and measuring the volume and weight “as it is”. It is expressed in g/mL and is given by,

$$D_b = M / V_O$$

Where, Db is the bulk density, M is the mass of powder and VO is the Bulk volume of the powder.

iii). Tapped density (Yuminoki K et al 2014):

It is determined by placing a graduated cylinder, containing a known mass of drug excipients blend, on mechanical tapping apparatus. The tapped volume is measured by tapping the powder to constant volume. It is expressed in g/mL.

$$D_t = M / V_t$$

Where, **Dt** is the tapped density, **M** is the mass of powder and **Vt** is the tapped volume of the powder.

iv) Compressibility index (or) Carr’s Index (I)

Compressibility index is an important measure that can be obtained from the bulk and tapped densities. A material having values less than 20 to 30% is defined as the free flowing material, based on the apparent bulk density and tapped density.

The percentage compressibility of the bulk drug is determined by using the following formula.

$$I = Dt – Db / Dt \times 100$$

Where, I is the Compressibility index, Dt is the tapped density of the powder and Db is he bulk density of the powder **(Natarajan Tamilselvan et al.,2015).**

Compressibility index (%)	Type of flow
10	Excellent
11-15	Good
15-20	Fair
21-25	Passable
26-31	Poor
32-37	very poor

Table:3

v). Hausner's ratio

It indicates the flow properties of the powder. The ratio of Tapped density to bulk density of the powder or granules is called Hausner's ratio. **H = Dt / Db**

Where, H is the Hausner's ratio, Dt is the tapped density of the powder and Db is the bulk density of the powder (**Wang ZH et al., 2015).**

Hausner's	Type of flow
1-1.11	Excellent
1.12-1.18	Good
1.19s-1.25	Fair
1.26-1.34	Passable
1.35-1.45	Poor
1.46-1.54	very poor
>1.60	very very poor

Table : 4

vi) Drug content

Weight of the powder material equivalent to 10 mg of Dapoxetine Hcl nanoparticles capsule is taken and transferred into 100 ml volumetric flask. Then 30 ml of Phosphate buffer pH 6.8 is added slowly, mixed properly and the volume is made up to 100 ml with Phosphate buffer pH 6.8. The above solution is filtered and 10 ml of filtrate is taken into 100 ml volumetric flask and made up to final volume with Phosphate buffer and the drug content is estimated by measuring the absorbance at λmax 292 nm using a UV- spectrophotometer. The drug content is determined by using the formula, **(Ansari KA *et al.,* 2011)**

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

ii) Post Compression Evaluation of Capsules

a) General Appearance

Size of Capsule : 4(100mg)

Shape of Capsule : Tube

Color of Capsule : Dark brown

e) Weight Variation Test

Twenty capsules are selected at random, individually weighed. Open the capsules without losing any part of the shell and remove the content as completely as possible. Weigh the shell. The difference between weight and weight of the content. The uniformity of weight is determined according to I.P. specification. As per IP not more than two of individual weights should deviate from average weight by more than 10% and none deviate more than twice that percentage**.** The following percentage deviation in weight.

Average Net weight of	**Percentage deviation**	**Number of tablets**
Less than 300mg	**±**10%	Minimum 18
300mg or more	**±**7.5%	Minimum 18

Table: 5

vi) Drug content uniformity

Ten capsules are weighed and taken contents from capsules without losing the drug. A quantity of powder weighing equivalent to 100 mg of drug is taken in a 100ml volumetric flask and Phosphate buffer pH 6.8 is added. The solution is filtered using membrane filter (0.45µm) and 10 ml of filtrate is taken into 100 ml volumetric flask and made up to final volume with Phosphate buffer pH 6.8. Then its absorbance is measured at 292nm using UV Visible spectrometer. The amount of drug present in one capsule is calculated using standard graph. The capsules comply with the test if

not more than one of the individual values thus obtained is outside the limits 85 to 115 % of the average value and none is outside the limits of 75 to 125%.

$$\text{Drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

vii) *In vitro* drug release studies

Dissolution characteristics of the formulated dapoxetine hcl nanoparticles loaded capsules were carried out using USP Type I (Basket) dissolution test apparatus for 12hrs (**Wang ZH et al., 2015).**

Method

900 ml of 0.01N Hydrochloric acid for 2 hours and 10 hours in Phosphate buffer pH 6.8 was filled in dissolution vessel and temperature of the medium is set at 37°C ± 0.5°C. One capsule of best formulation is placed in each dissolution vessel and the rotational speed of basket was set at 75rpm. 1.4ml of sample is withdrawn at pre determined time interval of every one hour for up to 12 hours and same volume of fresh medium is replaced immediately. The withdrawn sample is diluted to 10ml in volumetric flask and filtered through 0.45µ membrane filter. The resultant samples are analyzed for drug content at 292nm using UV-Visible spectrophotometer.

Parameter	Specifications
Dissolution Medium	0.01N Hydrochloric acid Phosphate Buffer pH 6.8
Temperature	37.0 ± 0.5 °C
Initial Volume	900ml
Rotation Speed	75rpm
Drawn Volume	1.4 ml
Running Time	2 hrs in 0.01N Hydrochloric acid 10 hrs in Phosphate Buffer pH 6.8

Table: **6**

8. Comparison of *Invitro* drug release studies of Dapoxetine Hcl nanoparticles capsules with Dapoxetine Hcl capsules (Sureshkumar P *et al.,* 2015):

Comparison of *invitro* drug release studies of Dapoxetine Hcl nanoparticles capsule with Dapoxetine Hcl capsules performed using dissolution tester (Basket type, LABINDIA 2000, India). Capsules were added to the 900 ml of 0.1N Hydrochloric Acid at 37°C ± 0.5°C, which is stirred with a rotating basket at 75 rpm. 1.4ml samples are withdrawn from the dissolution apparatus at the time in travel of 1,2,3 and upto 12 hours. Equal volume of fresh medium is replaced in to the dissolution medium after each sampling to maintain its constant volume throughout the test. Assay carried out using UV spectrophotometer (Shimdzu 1700UV/Visible Spectrophotometer, Japan) at 292 nm **(ADLIN JINO NESALIN J et al., 2009).**

9. Preclinical evaluation:

The present investigation is undertaken to study the effect of anti depressant activity of Dapoxetine Hcl nanoparticles in experimental rats.

Experimental animals:

Albino Wister rats weighing 180-220 gm were used for the study. The rats were in the animal house of the Dept. of pharmacology K.M. College of Pharmacy, Madurai. Under suitable conditions of housing, temperature, ventilation and nutrition were used for anti-depressant activity. The animal were fed with standard pelleted diet and distilled water and libitum was maintained at 21-23^{0}C under a constant 12 hours light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA/IAEC.

Standard drug:

Imipramine (15 mg/kg) is used for antidepressant activity in a standard drug.

Experimental design:

Experimental rats are randomly divided in to 4 groups and each group contains six animals.

- ***Group I*** - Received 10ml/kg of normal saline-Intra peritoneally.
- ***Group II*** - Received 15mg/kg Imipramine-Intra peritoneally
- ***Group III*** - Received 2.7mg/kg pure drug-Intra peritoneally
- ***Group IV*** - Received 2.7mg/kg formulated drug-Intra peritoneally.

Statistical analysis:

- Results are expressed as mean ± SEM.
- Data was analyzed by one way ANOVA was used for multiple comparison followed Newman Keul's multiple range test.
- $P<0.05$ was considered a statistical significance.

Forced swimming test:

- The most frequently used behavioral model for screening anti-depressant like activity in rats. Rats were individually forced to swim in open-glass chamber (25 * 15*25 cm) containing fresh water to a height of 15 cm and maintained at 23-25^{0}C. At this height of water, animals were not able to support themselves by toughing the bottom or the side walls of the chamber with their hand paws or tail.
- Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period.
- Rats were considered to be immobile when they ceased struggling and remained floating motionless in water. Making only these movements necessary to keep their head above water.
- Values were mean ± S.E.M for (n=6). Expressed as the time (in sec) of 6 animals in each group and a $P<0.01$ values are significantly different from normal control.

CHAPTER XI

RESULTS AND DISCUSSION

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RESULTS AND DISCUSSION

I. Standard curves for Dapoxetine Hcl:

a) Determination of (maximum absorption) λmax:

The maximum absorbance (λmax) of the Dapoxetine Hcl was estimated by scanning the drug solution (10µ/ml) between 200 nm-400 nm regions in the UV spectrophotometer. The obtained spectrum that the absorption maximum (λmax) at 292 nm in 0.01N Hydrochloric Acid and 292 nm in Phosphate Buffer pH 6.8.The result was shown in **Figure: 1A & 1B.**

0.1N Hydrochloric Acid:

The λmax of Dapoxetine Hcl was determined by adding 10 ml of 10µ/ml 0.01N Hydrochloric acid and scanning the solution of drug using UV spectrophotometer and was measured at 292 nm .The absorbance of the solutions (5-50 µ/ml) was measured in UV spectrophotometer at λmax of 292nm. The correlation coefficient was found to be γ=0.9994. The Dapoxetine Hcl obeys the Beers law within the concentration of 5 to 50 µg/ml. The results were given in **Table: 1A & FIG: 2A.**

Phosphate Buffer pH 6.8:

The λmax of Dapoxetine Hcl was determined by adding 0.2M 10 ml of 10µ/ml Phosphate Buffer pH 6.8 solution and scanning the solution of drug using UV spectrophotometer and was measured at 292 nm .The absorbance of the solutions (5-50 µ/ml) was measured in UV spectrophotometer at λmax of 292nm. The correlation coefficient was found to be γ=0.9996. The Dapoxetine Hcl obeys the Beers law within the concentration of 5 to 50 µg/ml. **(Wang ZH et al.,2015).** The results were given in **Table: 1B & FIG: 2B**

II. PREFORMULATIN (COMPATABILITY) STUDIES:

Fourier Transform- Infra Red (FT-IR) Studies:

FT-IR spectrum of the drug and polymers were shown in the **figure: 3A-3L** IR spectroscopy was used to investigate the interactions between and drug. The FT-IR spectral analysis of Dapoxetine Hcl alone showed that the principal peaks were observed at wave numbers confirming the drug **(Singling Tang *et al*., 2011).**

S.No	Functional	Range	Wave	Intensity
1	N-H Stretching	3300-3500cm^{-1}	3469 cm^{-1}	Medium
2	O-H Stretching	2500-3300 cm-1	2544 cm^{-1}	Strong
3	C-H Stretching	2700-3300 cm^{-1}	2627 cm^{-1}	Medium
4	C=O Stretching	1780-1650 cm^{-1}	1629 cm^{-1}	Strong
5	C-N Stretching	1230-1020 cm^{-1}	1062 cm^{-1}	Medium

Table: **1**

In the FT-IR spectra of physical mixture of Dapoxetine Hcl and ethyl cellulose, Dapoxetine Hcl and Chitosan, Dapoxetine Hcl and H P M C K 1 0 0 M , Dapoxetine Hcl and Betacyclodextrin a n d Diphenyl carbonate the major peaks of Dapoxetine Hcl were observed at wave numbers 3469 cm^{-1}, 2544 cm^{-1}, 2627 cm^{-1}, 1629 cm^{-1}, 1062 cm^{-1}, It was confirmed that there are no major shifting as well as any loss of functional peaks between the spectra of drug and the physical mixture. The drug and individual polymer (physical mixture) confirmed that are no interaction between the drug and polymer **(Masilamanb K et al., 2015).**

III. PREPARATION OF DAPOXETINE HCL NANOPARTICLES:

1. Solvent Evaporation Method:

Controlled release nanoparticles of Dapoxetine Hcl were prepared by using Solvent evaporation method Using different ratio of polymers **Table 2A & 2B** shows the composition of various prepared controlled release nanoparticles .The Drug (Dapoxetine Hcl) and polymer (Ethyl cellulose) was in the ratio of 1:1, 1:2, 1:3 and 1:4 for F1,F2, F3 and F4 respectively, Dapoxetine Hcl and polymer (Chitosan) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F5, F6, F7 and F8 respectively, Dapoxetine Hcl and polymer (HPMC K100 M) in the ratio of 1:1, 1:2, 1:3 and 1:4 for F9, F10, F11, and F12 respectively. Disperse phase consisting of Dapoxetine Hcl (100 mg) dissolved in methanol and requisite quantity of polymers dissolved in 20 ml solvent (dichloromethane) was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for two to three hours on a magnetic stirrer. The nanoparticles formed were collected by filtration through whatman filter paper and dried in oven at 50 ^{0} C for 2 hours. The dried nanoparticles were stored in vaccum desicator to ensure the removal of residual solvent **(Umar PS *et al.,* 2015).**

2. Hyper Cross-Linked β-Cyclodextrin Method:

Dapoxetine Hcl and polymer Betacyclodextrin and Diphenyl carbonate in the ratio of 1:1, 1:2, 1:3 and 1:4 for F13, F14, F15 and F16 respectively Shown in **Table.2B.** A series of four types of β-cyclodextrin Nanoparticles was prepared by using diphenylcarbonate for cross linking as previously prepared. Briefly, an amount of anhydrous cyclodextrin was put to react in melted diphenylcarbonate at 90^{0}C for atleast 5 hours. Then, the solid was ground in mortar and Soxhlet extracted with methanol to remove either impurities or unreacted diphenylcarbonate. The

reaction was carried out using a cross linked excess, at four different molar ratios, e.g. 1:1, 1:2, 1:3, 1:4 (β- CD:cross linker). After purification, NP was stored 25^0C until further use. This reaction was also carried out in the presence of ultrasound, and two different types of NP, namely, crystalline (1:1, 1:2, 1:3, 1:4 NP), and paracrystalline (1:1, 1:2, 1:3, 1:4 NP Para), were formed based on the process conditions of the synthesis **(Supriya A et al., 2018).**

2.1 Preparation of Dapoxetine Hcl Loaded Nanoparticles:

Dapoxetine Hcl was dispersed in aqueous suspensions of the various types of nanoparticles in a ratio of 1:1, 1:2, 1:3 & 1:4 (drug to NP by weight) and was stirred for 24 hours. After 24h, the suspension were centrifuged at 2000rpm for 10 min to separate the uncomplexed drug as a residue below colloidal supernatants were freeze dried to obtain drug loaded NP formulations, named as F1:1 F1:2 F1:3 F 1:4 and Fpara depending upon the ratio of β-CD: cross-linker. The drug- loaded NP formulations were stored in a covered vaccum desicator at room temperature (**Singling Tang *et al*., 2011).**

IV. CHARACTERIZATION OF CONTROLLED RELEASE NANOPARTICLES:

A) Production Yield:

The Production yield of prepared controlled release nanoparticles (F1-F16) was shown in **Table: 4** Increasing polymer ratio in the formulation led to increase the product yield**).**The low percent yield in some formulations may also due to nanoparticles lost during successive decantation during drying process. The percentage yield of produced nanoparticles F1 to F16 is shown in **figure: 4**

B) Determination of drug content:

Sample containing 100 mg equivalent Dapoxetine Hcl Nanoparticles are dissolved and the volume is made upto 100ml with pH6.8 phosphate buffer. From the above solution 10 ml is pipette out and made upto 100 ml with phosphate buffer. The Absorbance of resulting solution is determining at λmax (292 nm) using UV spectrophotometer (UV-1700 Shimadzu corporation, Japan) and the drug content is estimated shown in **Table: 4 & FIG: 6. (Ansari KA *et.al.,* 2011)**

C) Theoretical drug loading:

Theoretical drug loading for controlled release nanoparticles formulation F1- F16 shown in **Table: 4 & FIG: 5.**

D) Solubilization efficiency:

Solubilization efficiency of pure drug (Dapoxetine Hcl) and selected best formulation F2, F6. F10, F14 were shown in **Table-5** and **Figure.8.** Nanoparticle formulation F6 showed highest solubility in distilled water (9.66mg/10ml) as compared with pure drug (1.56 mg/10ml). Thus the solubility of nanoparticle formulation was increased when compared to pure drug. The solubility of formulation and pure drug in phosphate buffer pH (6.8) were 8.08 mg/10ml and 9 . 9 1 0 g/10ml respectively. Thus the solubility of Dapoxetine Hcl in nanoparticle formulation was

increased when compared to pure drug. Hence, the noticeable increased saturation solubility of Dapoxetine Hcl in the formulation of nanoparticle was mainly attributed to the decreased particle size and increased surface area. The results can be explained by the Ostwald–Freundlich equation which demonstrates that the saturation solubility of the drug increases with reduction of particle size.

E) Entrapment efficiency:

Drug entrapment efficiency for prepared controlled release nanoparticles was observed in the range from 54.53 % to 90.94 %. The Dapoxetine Hcl loaded Ethyl cellulose controlled release nanoparticles was found to be 63.43% to 74.85% for formulations F1 to F4 respectively. Dapoxetine Hcl loaded Chitosan controlled release nanoparticles was found to be 58.44 % to 90.94 % for formulations F5 to F8 respectively. The Dapoxetine Hcl loaded HPMC K100 M controlled release nanoparticles was found to be 58.64 % to 76.57 % for formulations F9 to F12 respectively. The Dapoxetine Hcl loaded Betacyclodextrin controlled release nanoparticles was found to be 54.53% to 69.92 % for formulations F13 to F16 respectively. Among the different drug polymer ratios investigated Formulation F6 (Chitosan 1:4 ratio), showed the maximum capacity for drug entrapment efficiency as shown in **figure: 7.** (**Wang ZH et al.,2015).** Drug entrapment efficiency was increased with increasing polymer concentration in Nanoparticle shown in **Table 4 & Figure: 7.**

F) Particle Size Analysis:

The particle size, polydispersity index and zeta potential for nanoparticle formulation are shown in **Table 7.** The particle size analysis revealed that the particle size measured by laser light scattering method is around 200-400 nm with low poly dispersity index values (0.812), as known , the polydispersity index is a parameter used to define the particle size distribution of nanoparticles. It is a dimension less number and it values ranges from 0.5-0.7 for mono dispersed particles, values greater than 0.7 are characteristic of samples with a broad size distribution. Therefore, it can be stated that the particle size distribution is unimodel, having a narrow range and a homogenous size distribution. Particle size of nanoparticles shown in **figure: 11.** When the polymer to drug ratio was increased, the proportion of larger particles was high, because the viscosity of the polymer and drug dispersion was increased with increase of polymer to drug ratio**.**

G) Zeta Potential:

The zeta potentials of Dapoxetine Hcl nanoparticle F6 formulations shown in **Table: 7 & Figure: 11**, were sufficiently high (+38.1 mV) probably due to presence of chitin groups structure which ensure physical stability between nanoparticle through electrostatic repulsion, and thereby avoiding aggregations. Increase in the charge on the particles is directly proportional to the polymer ratio used in the formulation. This is mainly because of more cation on the surface of the particles; higher is the stability of the nanoparticles **(Masilamanb K et al., 2015).**

H) *In vitro* Release studies:

The dissolution study was carried out in 0.1N Hydrochloric acid for 2 hours and phosphate buffer of pH 6.8 for the 10 hours. The results of *in-vitro* drug release studies from the controlled release Nanoparticles of Dapoxetine Hcl are

shown in the **Table-6A-6D.** The cumulative percentage drug release after 12 hours was found to be 85.44% 82.69%, 79.77%, and 73.13% for the formulations of F1 to F4, 94.25% , 96.93%, 92.87% and 90.22% for the formulations of F5 to F8, 90.93%, 8 5.49%, 8 0.52% and 77.47% for the formulations of F9 to F12, 91.09 %, 8 6 . 1 9 % , 81.05% and 7 2 . 2 9 % for the formulation of F13 to F16 . It was found that the drug release was prolonged up to 12 hrs. Dapoxetine Hcl loaded chitosan nanoparticles F6 formulation exhibited good controlled release characteristics. The release rate was related to drug: polymer ratio. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. The initial burst effect of some formulations may be due to presence of drug particle on the surface of the nanoparticles, this initial drug release may also attribute as a desired effect to ensure Minimum effective concentration of drug to produce pharmacological action **(Umar PS *et al.,* 2015).**

Initial burst release of nanoparticle formulation was probably due to the Dapoxetine Hcl which was present in the formulations as the inclusion complex; also it was adsorbed or encapsulated as non- inclusion complex on the nanoparticle surface the complexation of Dapoxetine Hcl in nanoparticle formulations. Thus, it was expected that the increased concentration of polymer, which retarded the release of dapoxetine hcl from nanoparticle formulations might be useful for controlling its release. The F6 formulation showed the controlled release due to increase concentration of polymer but F5 formulation showed the intermediate release .This may be happened because of nanopores on the surface of nanoparticles which depend on the concentration of polymer. Another biorelevant consideration is that transit time of a drug through the absorptive area of the gastrointestinal tract is between 9 to 12 hours this includes 2-3 hours of gastric residence time. The release of Dapoxetine Hcl from prepared Ethyl cellulose nanoparticles showed cumulative

percentage drug release in the range of 73.13% to 85.44% and Chitosan nanoparticles showed drug release in the range of 90.22% to 96.93% and HPMC K100 M nanoparticles showed in the range of 77.47% to 90.93%.and Betacyclodextrin nanoparticles showed in the range of 72.29% to 91.19% **(Singling Tang *et al*., 2011).** The cumulative percentage drug release for formulations F1 to F16 as shown in **figure: 12B-12E.**

I) Kinetic analysis:

The release data was modeled for Zero order, First order, Higuchi model, Hixson- Crowell model, Korsmeyer-Peppas model. The correlation coefficient of F1 to F16 formulations for Zero order, Higuchi, Hixson-Crowell and first order equations was shown in **Table 8.** Formulation F6 (Chitosan NP 1:2 ratios) was found high correlation to zero order kinetics 0.990 respectively **Figure: 13A &13 D** as well as Higuchi plot 0.879 **Figure: 15A &15D** rather than Hixson-Crowell models.

Higuchi model (Bhay S. Sapre et al., 2012):

The release kinetics of all the formulations is best fitted the Higuchi model. Higuchi model with R2 values ranges from 0.816 to 0.926 for F6. From these higuchi's model values, the release kinetics showed purely diffusion controlled. The release kinetics is shown in **figure: 15A-15D**.

Korsmeyer-peppas model (Masilamanb K et al., 2015)

The drug release was proportional to square root of time, indicating that the drug release from polymeric (Chitosan) microcapsules was diffusion controlled. The data obtained was also put in Korsemeyer-peppas equation in order to find out release exponent (n value ranges is 0.410 to 0.691**),** which describes the drug release mechanism by Non Fickian diffusion. The release kinetics is shown in **Figure: 16A-16D**.

J) Stability studies (Bhay S. Sapre et al., 2012):

Stability studies of the prepared nanoparticles were carried out, by storing formulation F6 at 25°C ± 2°C/ 60% ± 5% RH in humidity control oven for sixty days. Drug content, entrapment efficiency studies & invitro release studies were determined after 60 days. The results of drug content after 60 days of stability testing at different storage conditions are shown in **(Table 9)**. Formulation (F6) showed slight decrease in drug content (97.95%)respectively and entrapment efficiency (91.00%) at 25 ^{0}C $\pm$ 2^{0} C /60% RH after 60 days of storage whereas at 40^{0}±2°C/75% RH the formulation F6 showed significant decrease in the drug content (98.00%) and entrapment efficiency (89.91%) after 60 days of storage. The observations of the storage conditions are shown in **Table 9,** results indicated no significant changes in the parameter even when it was subjected to testing for 2 months when F6 was studied for short term storage conditions, the drug content in the formulation within the 95% confidence interval and hence slight decrease in the drug content was statistically not significant. From the stability studies it was confirmed that nanoparticle formulations of Dapoxetine Hcl remained more stable at storage conditions **(Abdul Hasan Sathali. A., and Gopinath.M., *et al*., 2013).**

K) I*nvivo studies* of nanoparticle formulation Experimental Animals:

IV.SELECTION OF BEST FORMULATION:

The best formulations were selected by Production yield, Entrapment Efficiency, Particle size, Zeta potential , *In vitro* drug release and kinetic drug release. From the above results of characterization **F6** was selected as the best formulation shown **Table-4 & 7**

Because,

- Production yield

- Entrapment efficiency
- Solubilization efficiency
- Particle size, Zeta potential & Polydispersity Index
- *Invitro* dissolution studies
- Release kinetics: closest linearity to zero order kinetics(0.990),
- Non-Fickian diffusion mechanism.

S.No	Formulation Code	Production Yield	Entrapment Efficiency	Particle Size (nm)	Zeta Potential	Solubilization Efficiency
1	F6	82.88	90.94	628	+38.1	9.910

Table-2

V. EVALUATION OF BEST FORMULATION:

The selected best formulation **F6** was subjected to,

- FT-IR
- Particle size, Zeta potential & Polydispersity Index
- Scanning Electron Microscopy
- X-ray powder diffraction study
- *Invivo* studies of nanoparticle formulation (F6).

1) FTIR Spectroscopic study:

The results obtained from FTIR study of best formulation was shown in **Figure: 18.** The peaks obtained in the pure Dapoxetine Hcl were also found in final formulation, which indicates that there is no interaction between the drug and excipients.

2) SEM Analysis of Best Formulation (Ansari KA *et al.,* 2011)

The morphology of the nanoparticles by solvent evaporation method were investigated by Scanning electron microscopy (SEM) It was observed by SEM analysis that the nanoparticles were uniformly spherical in shape. The particle and porous nature of the nanoparticles with rough surface and presence of holes /hollow cavity due to the collapse of the wall of the nanoparticles during in situ drying process. Thus the rate of solvent removal from the embryonic nanoparticles exerts an influence on the morphology of the end product. Porous structure was observed on the surface due to the rapid diffusion of the solvent, there is a possibility of rupture of the capsule wall. SEM photographs were shown in **Figure-20A-20E.**

3) X-Ray Powder Diffraction Study:

The XRPD patterns of pure drug Dapoxetine Hcl with Chitosan NP formulation (F6) was presented in **(Figure 21)**. The XRPD patterns of pure drug showed us sharp peaks (at 2θ 18.96°, 22.74 ° and 25.36°) which are the characteristic of a crystalline compound. Drug crystallinity peaks were also detectable in formulation. This result confirmed that the characteristic peaks were still preserved indicating the crystalline state was not changed. As we know, the amorphous form can generally enhance the dissolution rate and bioavailability of drugs due to its high-energy. According to that principle and with the XRPD

analysis considered, the enhancement of dissolution rate of Dapoxetine Hcl may be due to the reduction of particle size or the influence of polymers rather than the appearance of amorphous form. Moreover, compared with the amorphous form, the maintenance of crystalline state was beneficial to a long-term stability.

4) Invivo studies of nanoparticle formulations Experimental Animals:

Dapoxetine Hcl loaded nanoparticles could be able to maintain the serotonin level in depression over the period of observation (12 h) by selecting the suitable polymers. The results can be attributed to the efficacy of formulated Dapoxetine Hcl nanoparticles in treating depression as well as its role in maintaining the therapeutic levels until extended period of time. These observations could be also explained as the nanoparticle drug delivery system containing drug Dapoxetine Hcl, enhance its solubility, absorption as well as remain longer period in systemic circulation *invivo* studies.

5) Result of invivo studies of anti depressant activity of Dapoxetine Hcl nanoparticles in experimental rats:

- ***Forced swim test*** **(Ansari KA *et al.,* 2011) :**

Table 11 - values shown that animals treated with pure drug and formulated drug (2.7 mg/kg) showed decrease in their immobility times, which was significant (68.0 ± 1.20) and 72.0 ± 1.28) when compared with control (152.0v ±4.35). Similarly animals treated with Imipramine (15mg/kg) as expected showed a significant decrease in the immobility time (54.0 ± 1.05), ($p<0.01$).

VI. PREPARATION OF DAPOXETINE HCL LOADED NANOPARTICLE CAPSULES:

The Dapoxetine Hcl nanoparticle capsules of best formulation **(F6)** were prepared by using hand filling method. The individually weighed powder blends of Dapoxetine Hcl nanoparticle along with other excipients mentioned in **Table 12** were formulated into capsules. Each Dapoxetine Hcl nanoparticle capsules contains 30mg equivalent of Dapoxetine Hcl, microcrystalline cellulose is used as a diluent, magnesium stearate is used as a lubricant. The prepared Dapoxetine Hcl nanoparticle capsules were dark brown in color and tube in shape.

a) Compatibility Studies:

FT-IR Spectroscopy (Ansari KA *et.al.*, 2011)

FT-IR spectrum of the drug and polymers were shown in the **figure:21** IR spectroscopy was used to investigate the interactions between and drug. The FT-IR spectral analysis of Dapoxetine Hcl alone showed that the principal peaks were observed at wave numbers confirming the drug.

S.No	Functional Groups	Range	Wave Number	Intensity
1	N-H Stretching	3300-3500cm^{-1}	3469 cm^{-1}	Medium
2	O-H Stretching	2500-3300 cm-1	2544 cm^{-1}	Strong
3	C-H Stretching	2700-3300 cm^{-1}	2627 cm^{-1}	Medium
4	C=O Stretching	1780-1650 cm^{-1}	1629 cm^{-1}	Strong
5	C-N Stretching	1230-1020 cm^{-1}	1062 cm^{-1}	Medium

In the FT-IR spectra of physical mixture of Dapoxetine Hcl nanoparticle (best formulation), Dapoxetine Hcl and Chitosan, with other excipients like microcrystalline cellulose and magnesium stearate the major peaks of Dapoxetine Hcl were observed at wave numbers 3469 cm^{-1}, 2544 cm^{-1}, 2627 cm^{-1}, 1629 cm^{-1}, 1062 cm^{-1}. It was confirmed that there are no major shifting as well as any loss of functional peaks between the spectra of drug and the physical mixture. The drug and individual polymer (physical mixture) confirmed that are no interaction between the drug and polymer **(Clark's analysis of drugs and poisons, 3rd edition).**

i) Angle of repose (θ):

The Angle of repose for formulated powder blend in the range of **$32^0$62'** which indicates **good flow** properties of powder blend. The results were given in the **Table 10 & Figure: 23.**

ii) Bulk density (gm/ml):

The bulk density of powder blends was in the range of **0.4629 gm/ml,** which indicates, that the powder blends were not bulky. **(Singling Tang *et al*., 2011).** The results were shown in the **Table 10** & **Figure: 24.**

iii) Tapped density (gm/ml):

The tapped density of powder blends was in the range of **0.5618 gm/ml,** the results were shown in the table, which indicates smaller particles to occupy the voids between larger particles. The results were shown in the **Table 10** & **Figure: 24**

iv) Compressibity index (%):

Compressibility index were found to be in between **17.59 %** which indicates that the powder blend have **good flow** property **(Abdul Hasan Sathali et al.,2015).** The results were shown in **Table 10 & Figure: 23.**

v) Hausner's ratio (Abdul Hasan Sathali et al.,2015):

The Hausner's ratio of powder blend was found to be in the range of **1.21 %** which indicates **good flow** properties of powder blend. The results were shown in the **Table 10 & Figure:24.**

vi) Drug content (%) (Bhay S. Sapre et al., 2012) :

The percentage drug content of formulation (F6) was found to be in between **93.20%** ensured the uniformity of drug content within the limits of IP 2014 The results were shown in **table 13 & Figure: 25.**

Post Compression Evaluation of Dapoxetine Hcl Nanoparticles:

- **General Appearance**
- Size of Empty Hard gelatin capsules : 4 (100mg)
- Color of Capsules : Dark brown
- Shape of capsules : Tube

➢ Weight Variation (Ansari KA *et al.,* 2011):

In the formulations, the weight variation of Dapoxetine Hcl loaded capsule was ranges for Dapoxetine Hcl NP & F6 (88.47 – 108.47mg). Formulated capsules were passed the weight variation test as the % weight variation was within the pharmacopoeial limits of ± 10% of the average weight, which proved good uniformity. The results were shown in **Table 13.**

➢ Estimation of drug content (Peng Quan *et al*., 2011):

The percentage of drug content for best formulation (F6) was found to be in the ranges from 93.20% which is within acceptable limits, showed that the drug was uniformly distributed in the formulation. Hence the percentage of drug content of the formulation complies with official specifications as per IP (Limits: not less than 85% and not more than 115%). The results were shown in **Figigure:25 & Table 13.**

➢ Comparison of Invitro Release Studies of Dapoxetine Hcl Nanoparticle Capsules with Dapoxetine Hcl Capsules:

The *Invitro* dissolution studies of the formulations of Dapoxetine Hcl nanoparticle loaded capsules and Dapoxetine Hcl capsule were carried out in Hcl (0.01 N Hcl) for 2 hour and Phosphate Buffer pH 6.8 for 10 hours .The study was performed for 12 hrs, and cumulative drug release was calculated at different time intervals. The *invitro* drug release profiles for the formulation Dapoxetine **(Shende PK *et al.,*2015).** Hcl Capsule & F6 was tabulated in **Table14.** The plot of cumulative drug release (vs) time (hr) was plotted F6 and depicted as shown in **Fig.19 (Singling Tang *et al*., 2011).**

TABLE 2C: FORMULATION TABLE FOR CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL BY SOLVNT EVAPORATION METHOD

S. No	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16
1	Dapoxetine Hcl (mg)	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300	300
2	Ethyl cellulose (mg)	300	600	900	1200												
3	Chitosan (mg)					300	600	900	1200								
4	HPMC K100 (mg)									300	600	900	1200				
5	B-CD (mg)													300	600	900	1200
6	Polyvinyl alcohol(%w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
7	Dichlro Methane (ml)	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
8	Distilled water(ml)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

TABLE : 8 KINETICS OF INVITRO RELEASE FROM CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL

Formulation Code	Zero order Kinetics		First order Kinetics		Higuchi Model		Korsemeyer-peppas model		Hixon Crowell	
	R^2 value	$K_0(mg/h^{-1})$	R^2 value	$K_1(/h^{-1})$	R^2 value	$K_H(mg/h^{-1})$	R^2 value	n value	R^2 value	$K_H(h^{-1/3})$
F1	0.925	6.527	0.895	0.062	0.918	26.33	0.9663	0.576	0.967	0.172
F2	0.981	7.165	0.965	0.057	0.870	25.73	0.9774	0.433	0.939	0.161
F3	0.985	7.458	0.976	0.050	0.907	27.12	0.983	0.538	0.988	0.170
F4	0.983	7.110	0.892	0.060	0.868	24.83	0.9643	0.416	0.976	0.148
F5	0.995	8.812	0.728	0.0095	0.899	31.26	0.9625	0.523	0.980	0.207
F6	0.990	7.903	0.900	0.091	0.879	28.78	0.9876	0.597	0.967	0.173
F7	0.995	8.322	0.932	0.089	0.904	30.64	0.9608	0.486	0.961	0.155
F8	0.994	8.050	0.938	0.079	0.911	29.77	0.9608	0.460	0.902	0.136
F9	0.986	7.978	0.927	0.078	0.923	29.83	0.9844	0.480	0.980	0.207
F10	0.995	7.181	0.927	0.062	0.915	26.60	0.9651	0.490	0.967	0.173
F11	0.995	6.806	0.26	0.054	0.894	24.92	0.9585	0.572	0.961	0.155
F12	0.964	6.277	0.858	0.046	0.816	22.30	0.9702	0.574	0.902	0.136
F13	0.974	8.023	0.966	0.078	0.926	30.02	0.9717	0.502	0.990	0.206
F14	0.996	8.322	0.903	0.080	0.891	29.61	0.9762	0.552	0.956	0.210
F15	0.982	7.793	0.971	0.064	0.895	28.01	0.9897	0.513	0.983	0.179
F16	0.971	6.317	0.889	0.043	0.831	2168	0.9832	0.520	0.921	0.130

TABLE 1 A: CALIBRATION OF DAPOXETINE IN 0.01N HYDROCHLORIC ACID

S.No.	Concentration in µg/ml	Absorbance ± SD
1	5	0.0821 ± 0.0006
2	10	0.1469 ± 0.0060
3	15	0.2164 ± 0.0070
4	20	0.2792 ± 0.0070
5	25	0.3534 ± 0.0080
6	30	0.4247 ± 0.0090
7	35	0.5025 ± 0.0040
8	40	0.5701 ± 0.0090
9	45	0.6399 ± 0.0080
10	50	0.6953 ± 0.0020
Regression Value: 0.9994 ± 0.000081649		

TABLE 1 B: CALIBRATION OF DAPOXETINE IN PHOPHATE BUFFER pH 6.8

S.No.:	Concentration in µg/ml	Absorbance ± SD
1	5	0.0726 **±** 0.0015
2	10	0.1390 ± 0.0005
3	15	0.2085 ± 0.0004
4	20	0.2720 ± 0.0005
5	25	0.3399 ± 0.0019
6	30	0.4076 ± 0.0019
7	35	0.4732 ± 0.0012
8	40	0.5373 ± 0.0006
9	45	0.6152 ± 0.0049
10	50	0.6763 ± 0.0023
Regression Value:0.9996 ± 0.00008144		

TABLE: 2A FORMULATION TABLE FOR CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL

S.NO:	FORMULATION CODE	DRUG : POLYMER RATIO	DRUG (DAPOXETINE HCL)	CHITOSAN	ETHYL CELLULOSE
1	F1	1 : 1	300	300	300
2	F2	1 : 2	300	600	600
3	F3	1 : 3	300	900	900
4	F4	1 : 4	300	1200	1200
5	F5	1 : 1	300	300	300
6	F6	1 : 2	300	600	600
7	F7	1 : 3	300	900	900
8	F8	1 : 4	300	1200	1200
9	F9	1 : 1	300	300	300
10	F10	1 : 2	300	600	600
11	F11	1 : 3	300	900	900
12	F12	1 : 4	300	1200	1200
13	F13	1 : 1	300	300	300
14	F14	1 : 2	300	600	600
15	F15	1 : 3	300	900	900
16	F16	1 : 4	300	1200	1200

TABLE: 2B FORMULATION TABLE FOR CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL

S.NO	FORMULATION CODE	DRUG : POLYMER RATIO	DRUG (DAPOXETINE)	HPMC KM100	B-CD	DPC
1	F1	1 : 1	300	300	300	300
2	F2	1 : 2	300	600	600	600
3	F3	1 : 3	300	900	900	900
4	F4	1 : 4	300	1200	1200	1200
5	F5	1 : 1	300	300	300	300
6	F6	1 : 2	300	600	600	600
7	F7	1 : 3	300	900	900	900
8	F8	1 : 4	300	1200	1200	1200
9	F9	1 : 1	300	300	300	300
10	F10	1 : 2	300	600	600	600
11	F11	1 : 3	300	900	900	900
12	F12	1 : 4	300	1200	1200	1200
13	F13	1 : 1	300	300	300	300
14	F14	1 : 2	300	600	600	600
15	F15	1 : 3	300	900	900	900
16	F16	1 : 4	300	1200	1200	1200

TABLE: 3 FORMULATION & RPM FOR THE CONTROLLED RELEASE NANOPARTICLES

Formulation Code	Drug: Polymer Ratio	Drug (mg)	Polymer (mg)	RPM
F1	1 : 1	300	300	1000
F2	1 : 2	300	600	1000
F3	1 : 3	300	900	1000
F4	1 : 4	300	1200	1000
F5	1 : 1	300	300	1000
F6	1 : 2	300	600	1000
F7	1 : 3	300	900	1000
F8	1 : 4	300	1200	1000
F9	1 : 1	300	300	1000
F10	1 : 2	300	600	1000
F11	1 : 3	300	900	1000
F12	1 : 4	300	1200	1000
F13	1 : 1	300	300	1000
F14	1 : 2	300	600	1000
F15	1 : 3	300	900	1000
F16	1 : 4	300	1200	1000

TABLE: 4 PRODUCTION YIELD, THEORETICAL YIELD, ACTUAL LOADING, DRUG CONTENT &ENTRAPMENT EFFICIENCY FOR CONTROLLED RELEASE NANOPARTICLES

S. No.:	Formulation Code	Production yield (%)	Theoretical Loading (%)	Experimental Drug Content (%)	Entrapment Efficiency (%)
1	F1	81.8	61.09	90.22 ± 0.71	67.71
2	F2	78.22	42.61	95.01 ± 0.74	74.85
3	F3	83.58	29.91	89.57 ± 0.92	63.43
4	F4	81.57	24.43	86.88 ± 0.91	68.12
5	F5	78.00	64.10	82.56 ± 1.01	69.25
6	F6	82.88	40.21	98.20 ± 0.57	90.94
7	F7	80.25	31.45	90.17 ± 0.56	77.94
8	F8	79.80	25.06	91.34 ± 0.40	58.44
9	F9	80.50	62.11	91.03 ± 0.40	67.99
10	F10	76.11	43.79	94.03 ± 0.25	76.57
11	F11	83.17	30.06	91.46 ± 0.86	62.28
12	F12	80.40	24.87	86.33 ± 1.47	58.64
13	F13	77.83	64.24	91.88 ± 0.57	69.92
14	F14	79.00	42.19	93.21 ± 0.67	65.26
15	F15	81.08	30.83	89.28 ± 0.31	54.53
16	F16	80.73	24.77	86.11 ± 0.78	68.76

TABLE : 5 COMPARISON OF SOLUBILITY OF SELECTED FORMULATION WITH PURE DRUG (DAPOXTINE HCL)

SL NO.	SOLVENT USED	SOLUBILITY IN EACH SOLVENT(mg/10ml)				
		PURE DRUG	F2	F6	F10	F14
1	Distilled Water	1.56±0.010	8.79±0.014	9.66±0.018	9.04±0.013	8.430±0.015
2	0.01N Hydrochloric Acid	2.146±0.021	2.586±0.018	3.172±0.025	2.277±0.010	2.577±0.017
3	Phosphate Buffer pH6.8	8.073±0.015	9.235±0.011	9.910±0.048	9.132±0.069	9.101±0.076

TABLE: 6A IN-VITRO RELEASE PROFILE OF ETHYL CELLULOSE CONTROLLED RELEASE NANOPARTICLES OF DAPOXTINE HCL

Time in hours	Cumulative % Release (Ethyl Cellulose)							
	F1 (1:1)		F2 (1:2)		F3 (1:3)		F4 (1:4)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
1	1.29	0.33	2.70	0.33	1.75	0.06	1.15	0.17
2	7.93	0.43	11.70	1.95	8.85	0.35	5.22	0.75
3	22.93	0.73	20.71	0.22	16.14	0.82	4.52	1.91
4	30.43	0.45	25.59	0.25	24.48	0.97	21.02	0.34
5	35.24	0.87	31.30	1.41	33.16	0.70	25.81	0.54
6	41.26	0.89	35.99	1.47	43.46	1.55	37.49	0.77
7	47.70	0.82	42.61	0.57	52.50	1.18	43.43	0.71
8	55.52	1.05	49.77	0.88	61.00	1.15	51.27	0.76
9	62.67	0.81	55.35	0.65	66.02	0.74	57.16	1.31
10	68.90	1.12	61.54	1.33	70.23	1.46	63.16	0.60
11	75.10	0.86	76.44	1.75	75.06	0.72	68.36	0.67
12	85.44	1.21	82.69	1.02	79.77	0.93	73.13	0.57

TABLE: 6B IN-VITRO RELEASE PROFILE OF CHITOSAN CONTROLLED RELEASE NANOPARTICLES OF DAPOXTINE HCL

Time in hours	Cumulative % Release (Chitosan)							
	F5 (1:1)		F6 (1:2)		F7 (1:3)		F8 (1:4)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
1	1.92	0.21	2.63	0.24	2.13	0.21	2.13	0.24
2	9.98	0.32	19.04	0.67	8.48	0.66	8.63	0.66
3	19.96	0.53	19.86	0.65	20.05	0.45	20.05	0.44
4	28.47	0.73	28.08	0.31	28.92	0.25	29.37	0.69
5	37.19	0.43	34.14	0.86	38.55	1.19	38.16	1.12
6	45.07	1.07	40.80	1.08	46.31	0.788	46.22	0.78
7	55.54	0.87	47.23	0.77	55.93	1.46	55.43	0.27
8	66.21	1.61	54.69	1.10	63.21	0.94	63.46	0.43
9	73.05	1.26	64.11	0.98	71.09	0.49	70.77	1.43
10	83.45	1.19	73.88	0.45	80.11	1.04	77.44	2.13
11	89.27	0.23	83.92	1.72	88.52	1.29	84.34	2.57
12	94.25	0.55	96.93	0.69	92.87	1.28	90.22	0.31

TABLE: 6C IN-VITRO RELEASE PROFILE OF HPMC K100 CONTROLLED RELEASE NANOPARTICLES OF DAPOXTINE HCL

Time in hours	Cumulative % Release (HPMC K100)							
	F9 (1:1)		F10 (1:2)		F11 (1:3)		F12 (1:4)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
1	2.10	0.22	1.98	0.06	1.61	0.25	1.98	0.25
2	9.90	0.16	10.11	0.14	8.91	0.65	7.12	1.04
3	20.23	1.31	21.34	1.92	17.56	0.66	11.61	1.41
4	31.54	0.79	30.42	0.44	23.15	0.87	15.87	0.34
5	44.47	1.41	35.22	0.87	31.17	0.52	20.68	0.79
6	51.21	0.37	41.36	0.89	37.29	0.09	26.02	1.20
7	58.69	0.63	47.69	0.81	42.26	1.07	31.63	0.68
8	65.02	0.71	55.52	1.05	48.91	0.12	38.76	1.20
9	71.94	0.41	62.67	0.81	55.91	0.55	45.72	1.08
10	76.14	1.36	68.90	1.04	64.35	0.61	56.72	1.61
11	83.15	1.69	76.43	0.96	72.87	1.32	66.01	1.50
12	90.93	0.85	85.49	0.73	80.52	0.78	77.47	1.66

TABLE: 6D IN-VITRO RELEASE PROFILE OF BETACYCLODEXTTRIN CONTROLLED RELEASE NANOPARTICLES OF DAPOXTINE HCL

Time in hours	Cumulative % Release (Betacyclodextrin)							
	F13 (1:1)		F14 (1:)		F5 (1:3)		F16 (1:4)	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
1	1.84	0.01	1.84	0.55	1.59	0.39	1.29	0.02
2	10.01	0.07	9.45	0.88	7.94	1.05	8.90	0.76
3	19.49	0.48	18.99	1.26	13.82	1.81	13.12	0.41
4	34.52	1.75	24.59	2.36	21.05	1.23	16.97	0.62
5	44.52	1.32	31.18	0.76	35.55	1.06	21.58	0.55
6	54.10	1.44	44.48	1.59	44.65	1.09	26.45	0.53
7	59.22	1.03	53.27	2.06	52.43	0.25	31.54	0.09
8	66.82	1.91	61.98	2.10	60.62	1.07	37.45	0.87
9	73.09	1.02	69.03	0.07	68.41	1.21	45.17	0.54
10	77.77	1.09	75.77	1.12	72.36	1.18	56.86	1.83
11	83.59	0.06	84.63	0.79	77.41	0.79	67.33	1.55
12	89.09	1.35	91.19	1.34	81.05	1.34	72.29	1.91

TABLE: 7 PARTICLE SIZE, PDI, ZETA POTENTIAL & DRUG RELEASE FOR BEST FORMULATION (F6)

MEAN DIAMETER(nm)	628
PDI	0.812
ZETA POTENTIAL(mV)	38.1
% OF DRUG RELEASE	96.93

TABLE: 9 STABILITY STUDY OF BEST FORMULATION (F6) STORED AT 25°C±2^{0}C/65%RH AND 40°C/ 70%RH

EVALUATION PARAMETER	STORING TEMPERATURE	Initial	30 Days	60 Days
% Drug content	25^{0}C± 2^{0}C/65%RH	98.20 ±0.16%	98.10 ±0.27%	97.95±0.17%
	40°C ± 2^{0}C /70%RH	98.20 ±0.16%	98.08 ±0.28%	98.00± 0.17%
% Entrapment efficiency	25^{0}C± 2^{0}C/65%RH	90.94 ±0.28%	90.12 ±0.28%	90.00±0.15%
	40°C ±2^{0}C /70%RH	90.94±0.28%	90.07 ±0.28%	89.91±0.15%
Cumulative % drug Release	25^{0}C±2^{0}C/65%RH	96.93±0.47%	96.56±0.31%	96.12±0.218%
	40°C ±2^{0}C /70%RH	96.93±0.47%	96.56±0.31%	96.02±0.218%

TABLE:10 PRECOMPRESSION STUDIES FOR ISRADIPINE NANOPARTICLE CAPSULES (F6)

Formulation code	F6
Angle of Repose (Ɵ) ±SD*	$32^0\,62'$
Bulk Density (G/Ml) ±SD*	0.4629 ± 0.06
Tapped Density (G/Ml) ±SD*	0.5618 ± 0.10
Hausner Ratio ±SD	1.21 ± 0.51
Compressibility index (%) ±SD*	17.59 ± 0.83

TABLE: 11 *INVIVO* STUDIES OF ANTI DEPRESSANT ACTIVITY OF DAPOXETINE HCL NANOPARTICLES IN EXPERIMENTAL RATS:

Groups	Treatment	Drug mg/kg	Immobility period Mean ± S.E.M.
G-I	Control	10 ml/kg	152. ± 4.35
G-II	Std. control Imipramine	15 mg/kg	54.0 ± 1.05
G-III	Pure Drug	2.7 mg/kg	68.0 ± 1.20
G-IV	Formulated Drug	2.7 mg/ kg	72.0 ± 1.2

TABLE: 12 COMPOSITION OF DAPOXETINE HCL – CHITOSAN NANOPARTICLES

SL NO.	INGREDIENTS	FOR 1 CAPSULES (mg)	FOR 50 CAPSULES (g)
1	Dapoxetine Hcl – Chitosan nanoparticles (equivalent to 30 mg of Dapoxetine Hcl)	75.5mg	3.775gm
2	Microcrystalline cellulose	23.5mg	1.175gm
3	Magnesium stearate	1mg	0.050gm

TABLE: 13 POST COMPRESSION FOR DAPOXETINE HCL NANOPARTICLE & BEST FORMULATION OF F6 CAPSULES

FORMULATION CODE	LENGTH OF CAPSULE (mm)	WEIGHT VARIATION ±10% (±15mg)	DRUG CONTENT % ±SD*
DAPOXETINE HCL CAPSULES	17	144.15	88.15 ± 0.010
F6	17	146.23	93.20 ± 0.012

TABLE: 14 *IN-VITRO* RELEASE PROFILE OF DAPOXETINE HCL AND F6 CAPSULES OF CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL

Cumulative % Drug Release (CHITOSAN)				
Time in Hours	**Dapoxetine Hcl Capsules**		**F6(1:4) Chitosan NP Capsules**	
	Mean	**±SD**	**Mean**	**±SD**
1	1.98	0.25	2.63	0.24
2	8.12	1.04	11.04	0.67
3	15.61	1.41	19.86	0.65
4	21.87	0.34	28.08	0.31
5	30.68	0.79	34.14	0.86
6	39.02	1.20	40.80	1.08
7	46.63	0.68	47.23	0.77
8	52.76	1.20	54.69	1.10
9	60.72	1.08	64.11	0.98
10	67.72	1.61	73.88	0.45
11	74.01	1.50	83.92	1.72
12	82.47	1.66	96.93	0.69

FIG: 1A- DETERMINATION OF λmax OF DAPOXETINE HCL IN 0.01N HCL

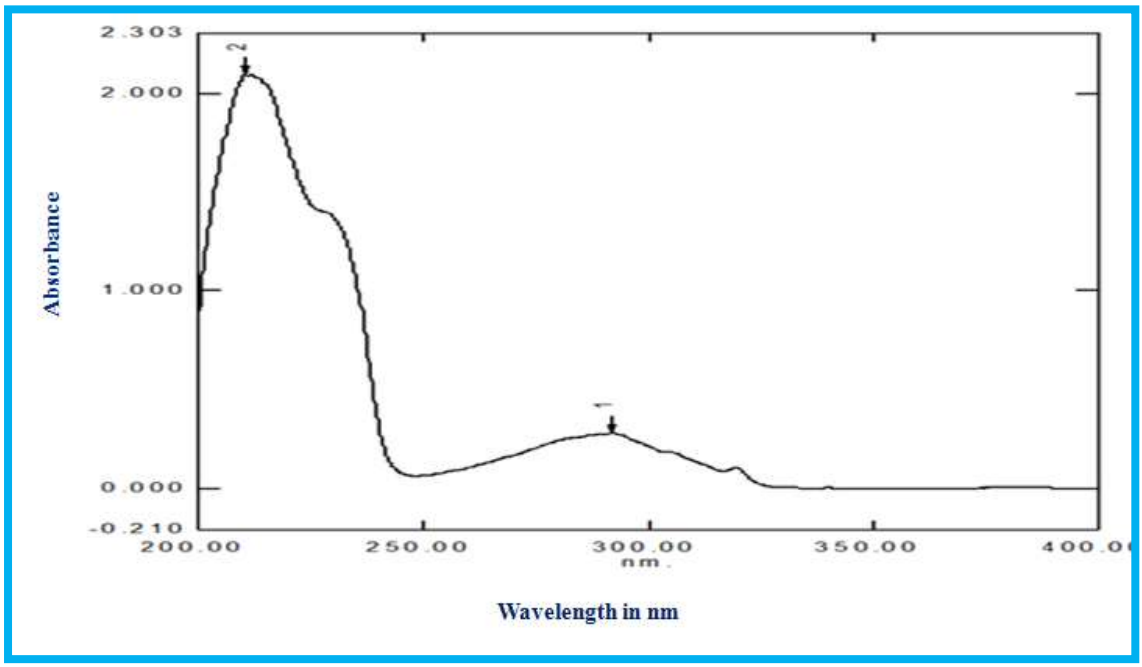


FIG: 1B- DETERMINATION OF λmax OF DAPOXETINE HCL IN PHOSPHATE BUFFER pH6.8

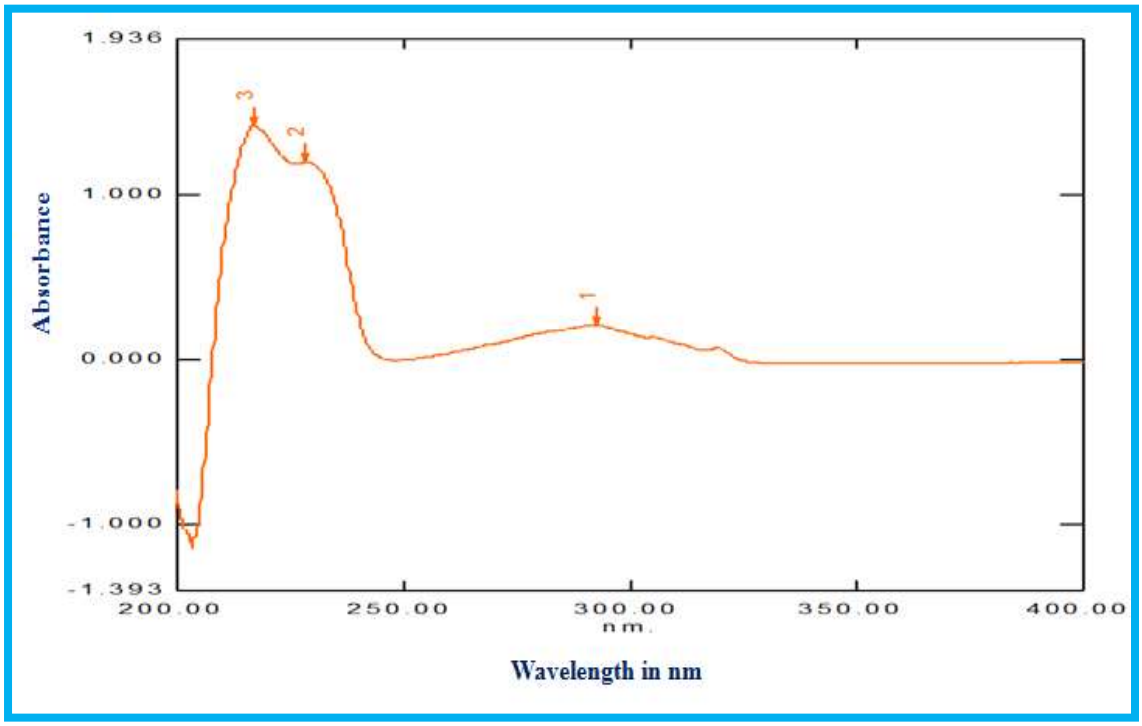


FIG: 2A-CALIBRATION OF DAPOXETINE HCL IN 0.01N HCL

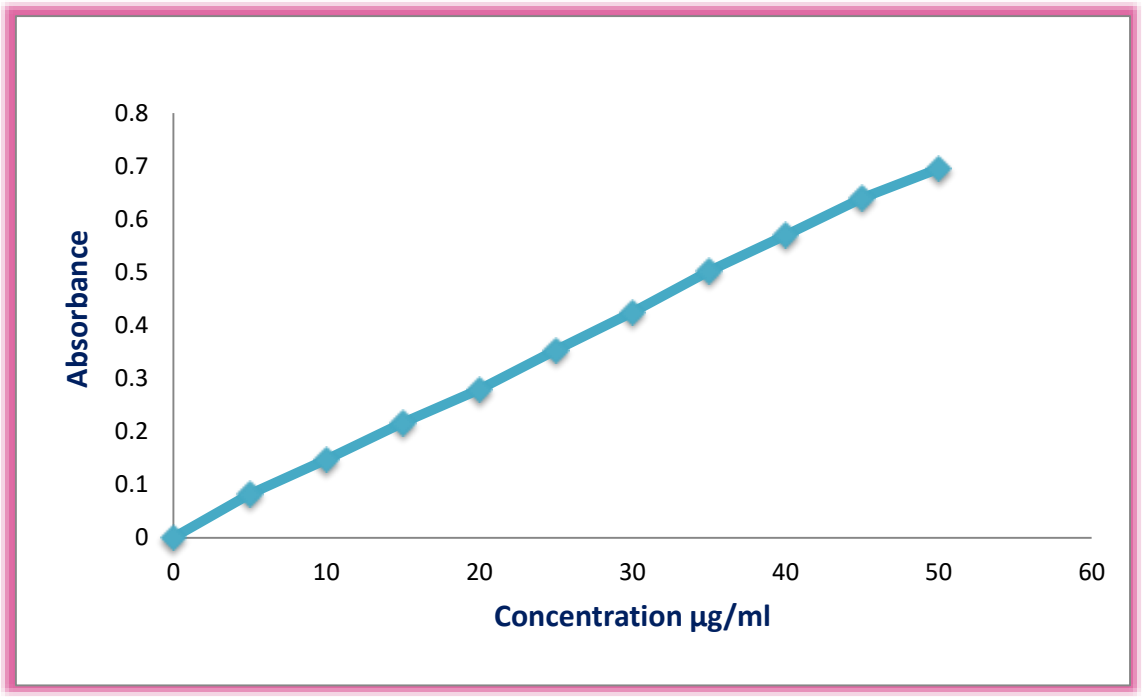


FIG: 2B-CALIBRATION OF DAPOXETINE HCL IN PHOSPHATE BUFFER pH 6.8

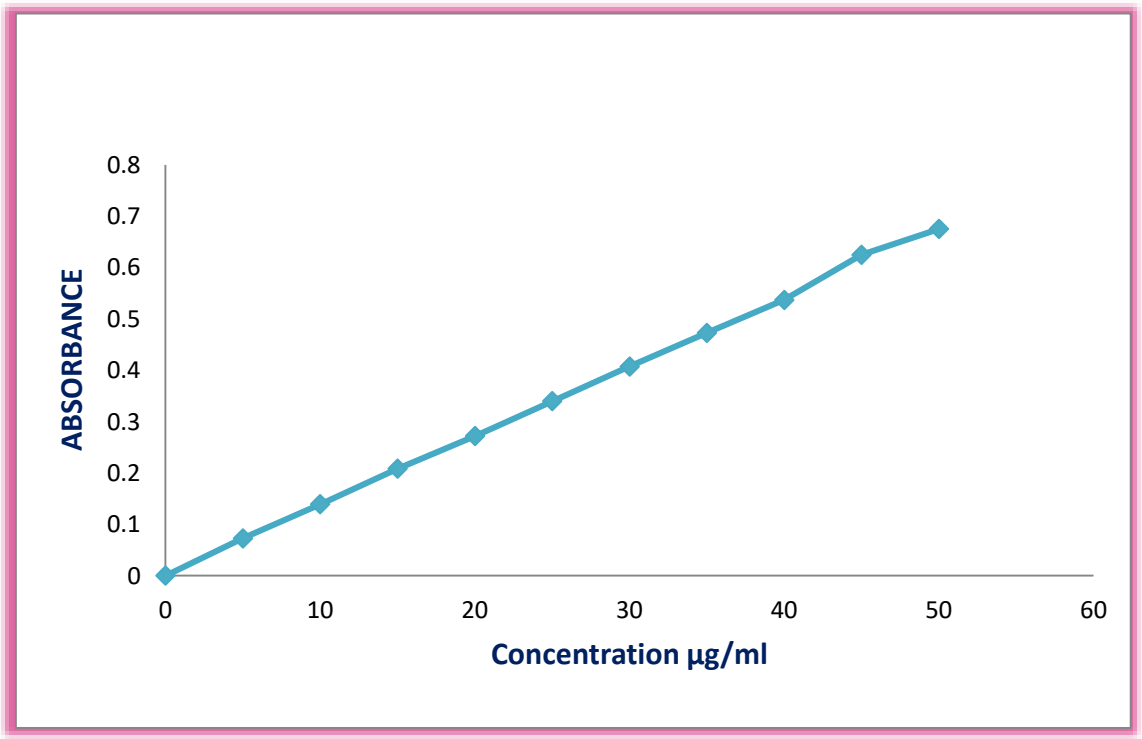


FIGURE: 3A-FT-IR SPECTRUM OF DAPOXETINE HCL

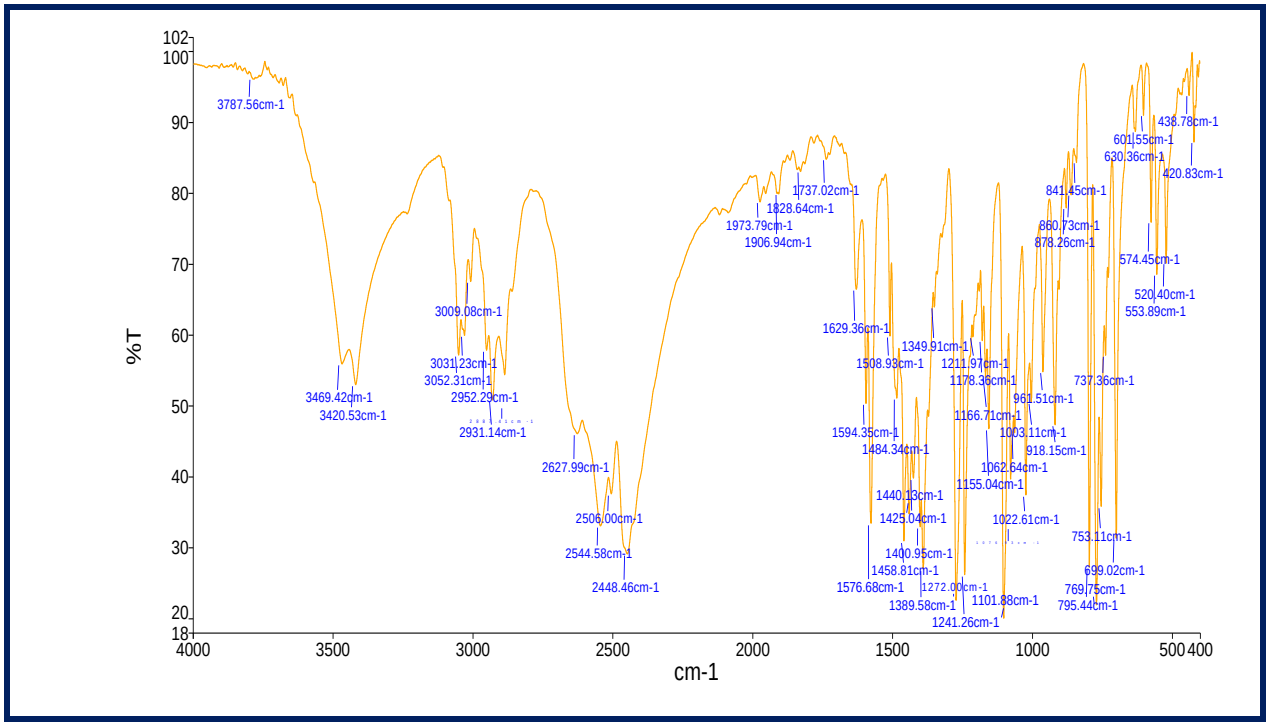


FIGURE: 3B-FT-IR SPECTRUM OF ETHYL CELLULOS

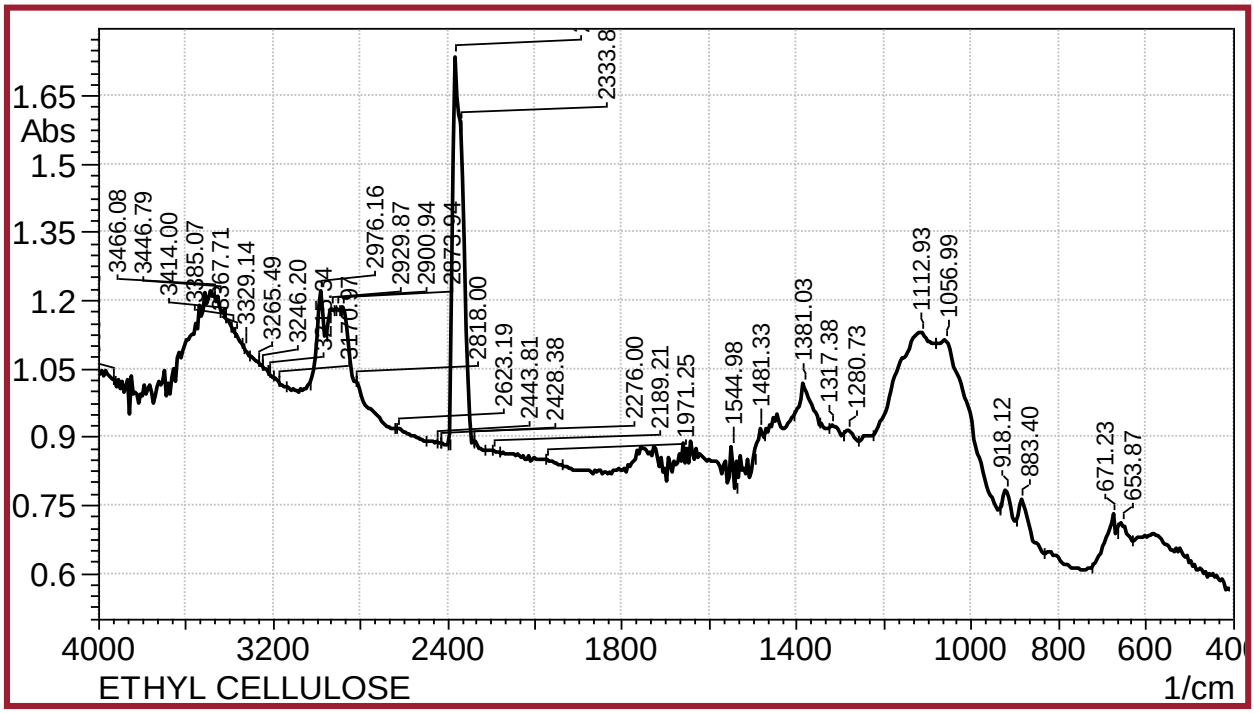


FIGURE: 3C-FT-IR SPECTRUM OF CHITOSAN

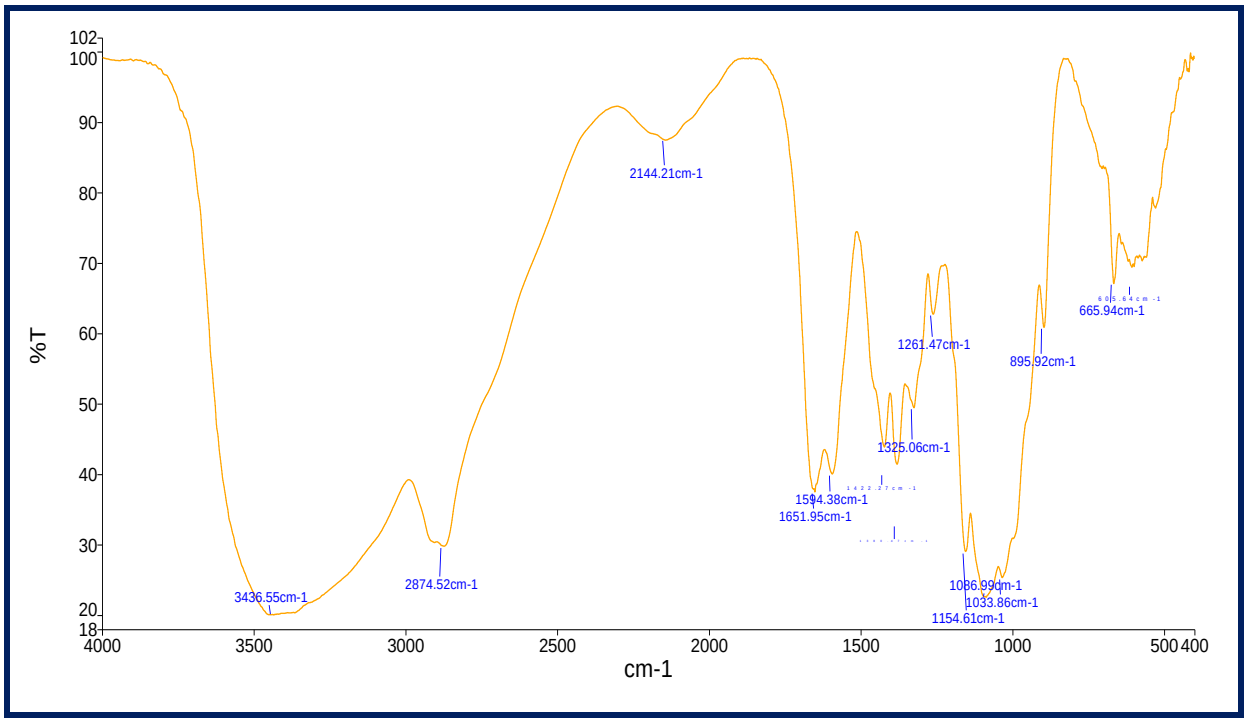


FIGURE: 3D-FT-IR SPECTRUM OF HPMC K100

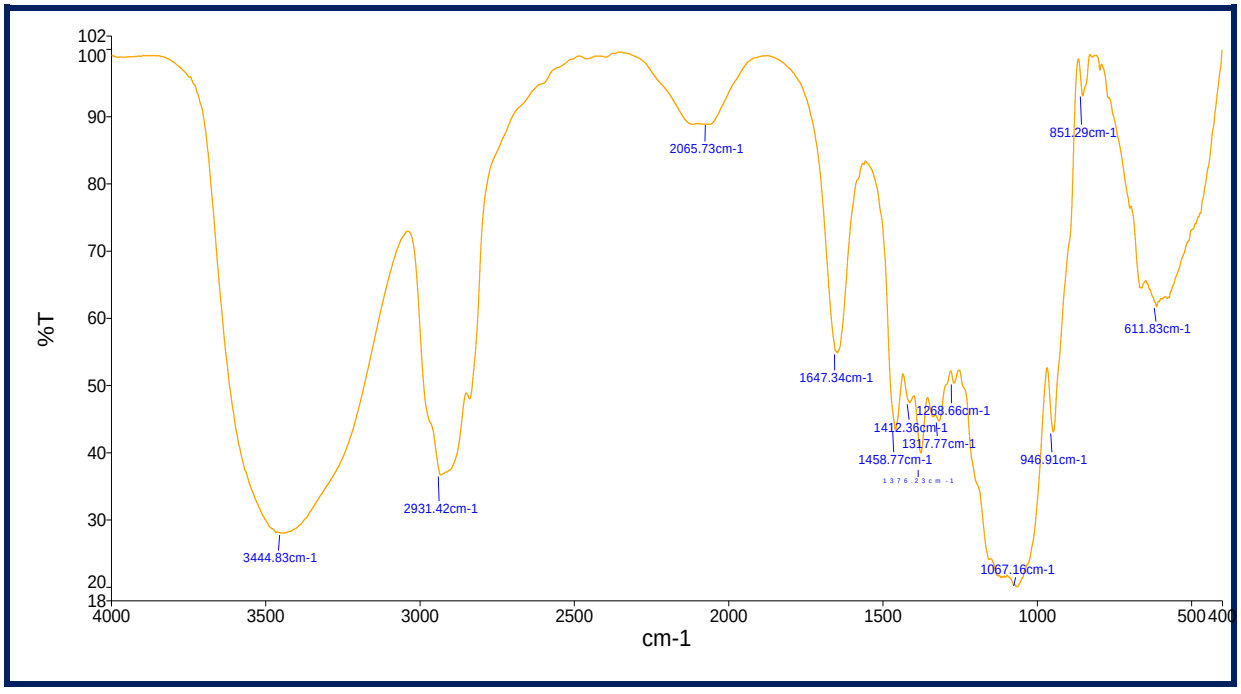


FIGURE: 3E-FT-IR SPECTRUM OF BETA-CYCLODEXTRIN

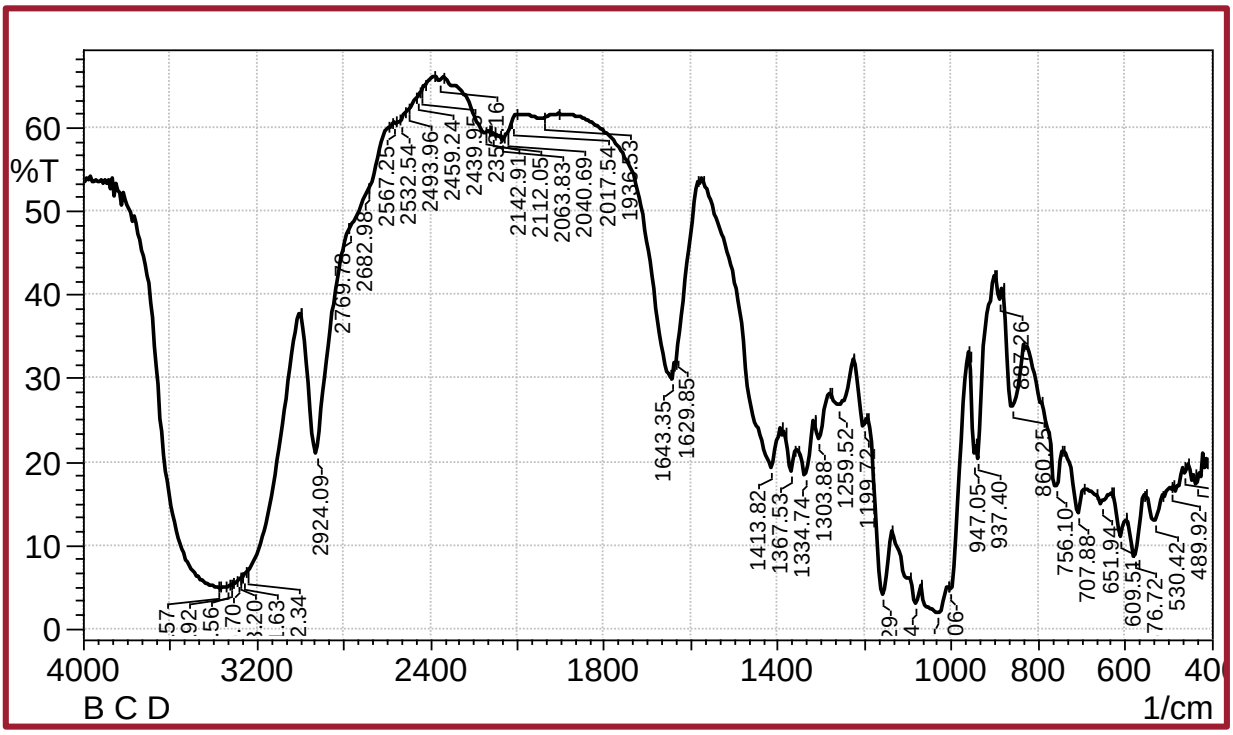


FIGURE: 3F-FT-IR SPECTRUM OF DIPHENYL CARBONATE

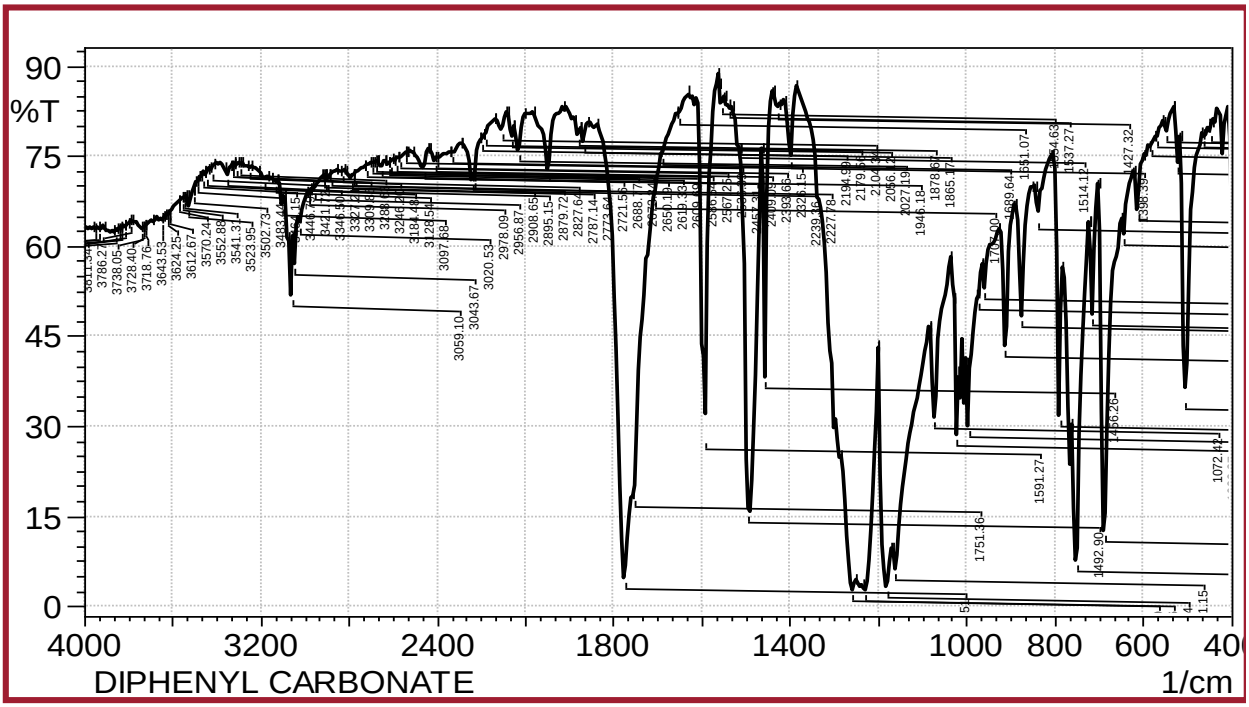


FIGURE: 3G-FT-IR SPECTRUM OF POLYVINYL ALCOHOL

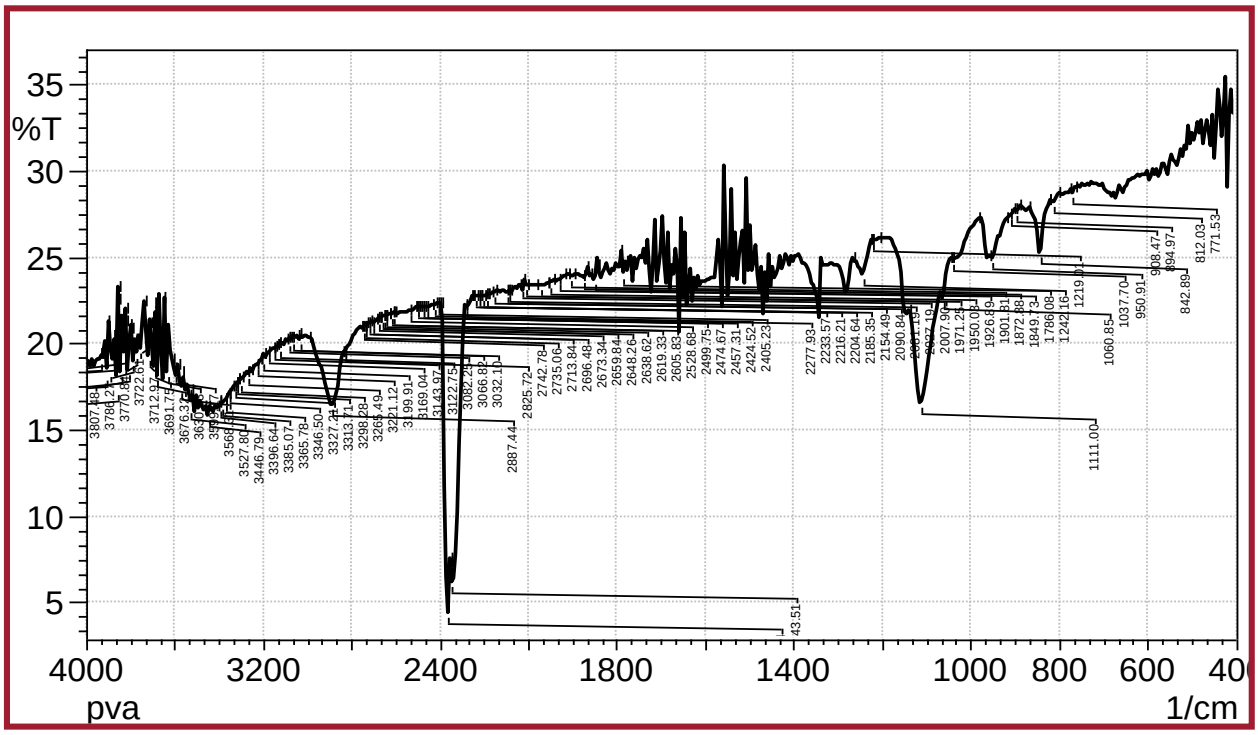


FIGURE: 3H- FT-IR SPECTRUM OF DAPOXETINE HCL + ETHYL CELLULOSE

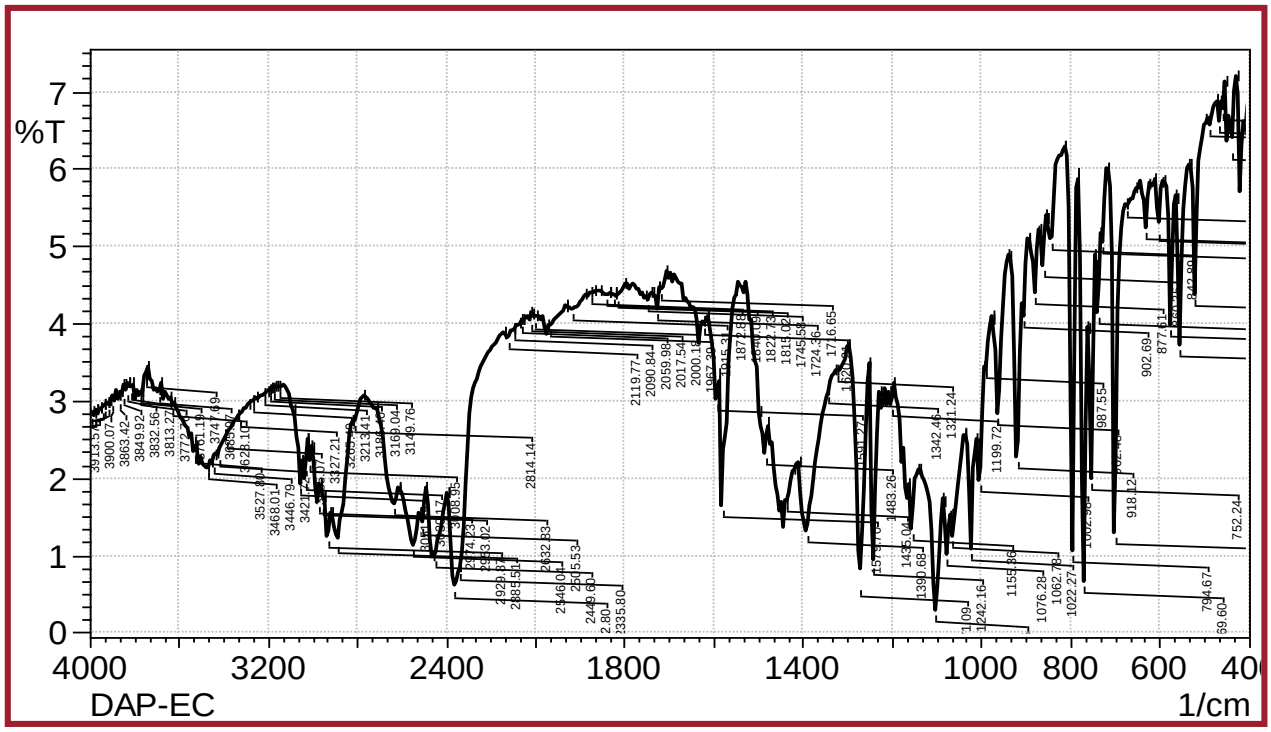


FIGURE: 3 I -FT-IR SPECTRUMS OF DAPOXETINE HCL + CHITOSAN

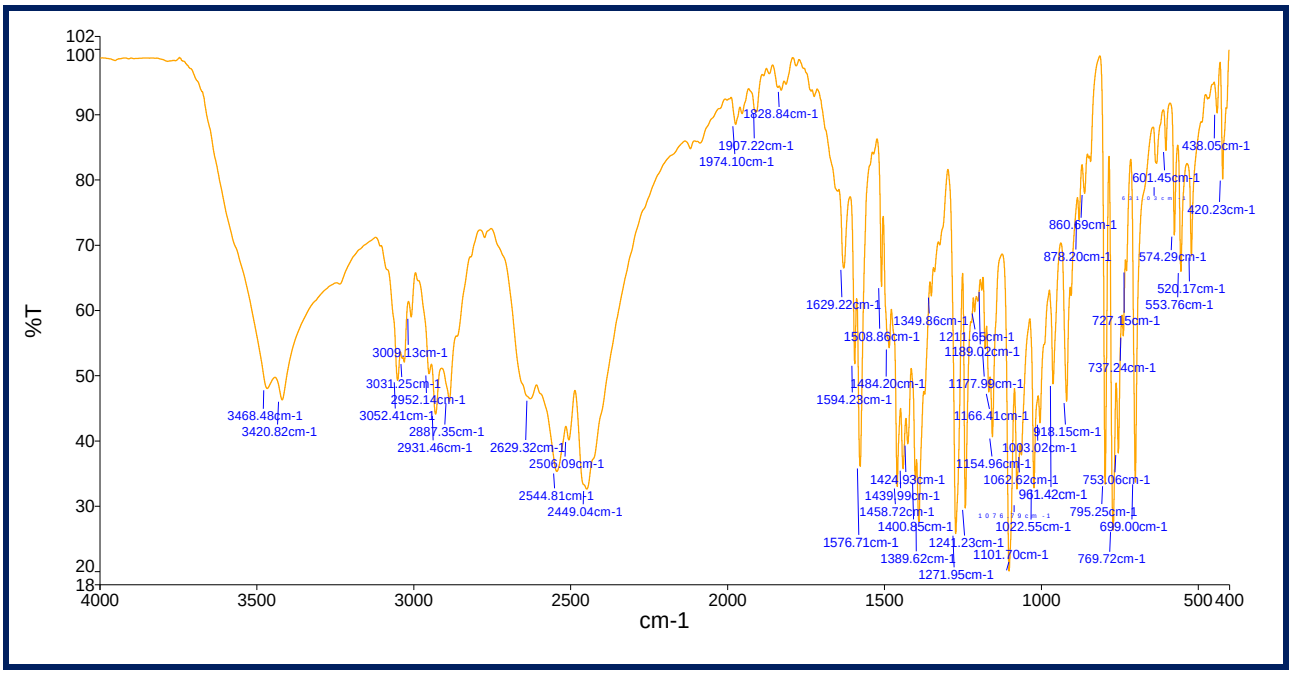


FIGURE: 3 J -FT-IR SPECTRUM OF DAPOXETINE HCL + HPMC K100

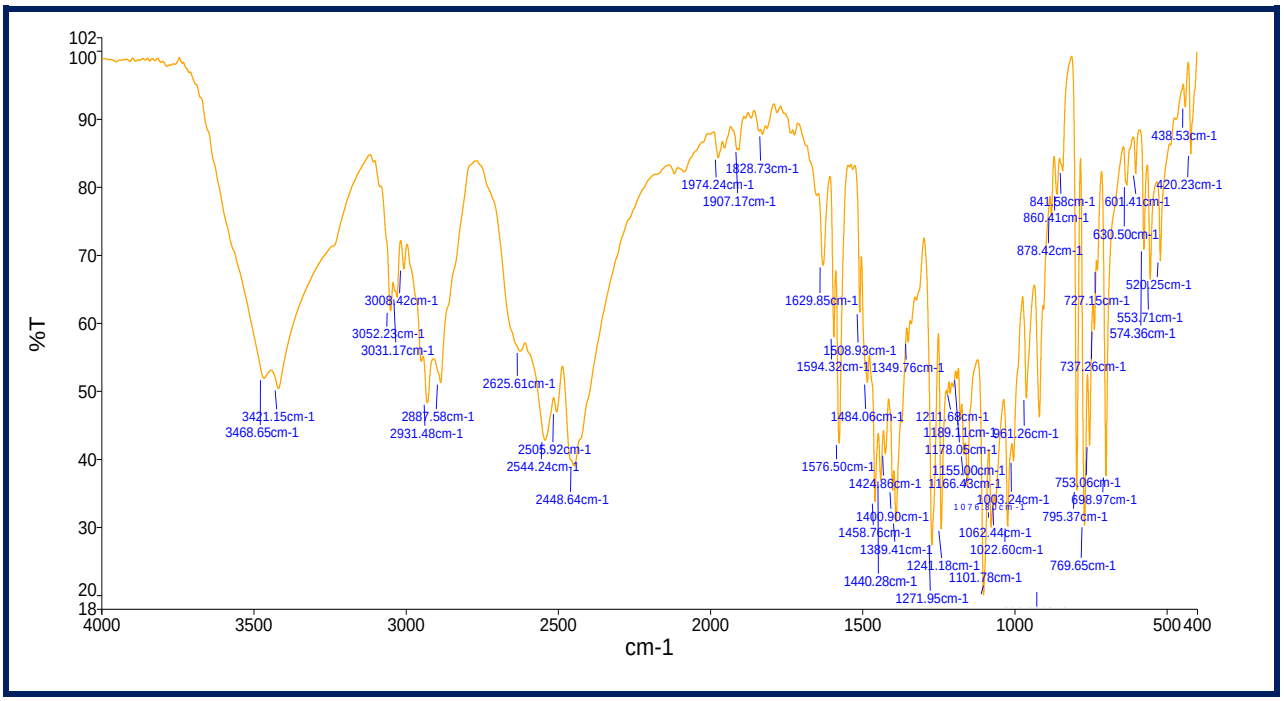


FIGURE: 3K-FT-IR SPECTRUM OF DAPOXETINE HCL + BETA-CYCDEXTRIN

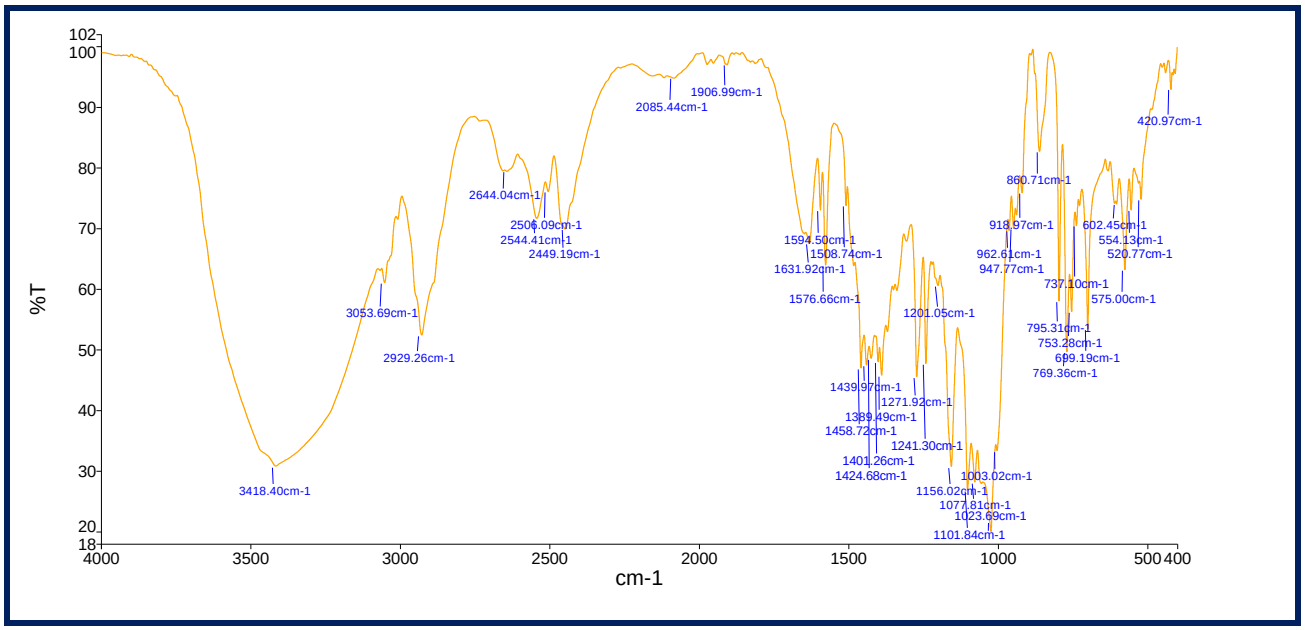


FIGURE: 3L -FT-IR SPECTRUM OF CHITOSAN+BETA-CYCLODEXTRIN + HPMC K100+ ETHYL CELLULOSE

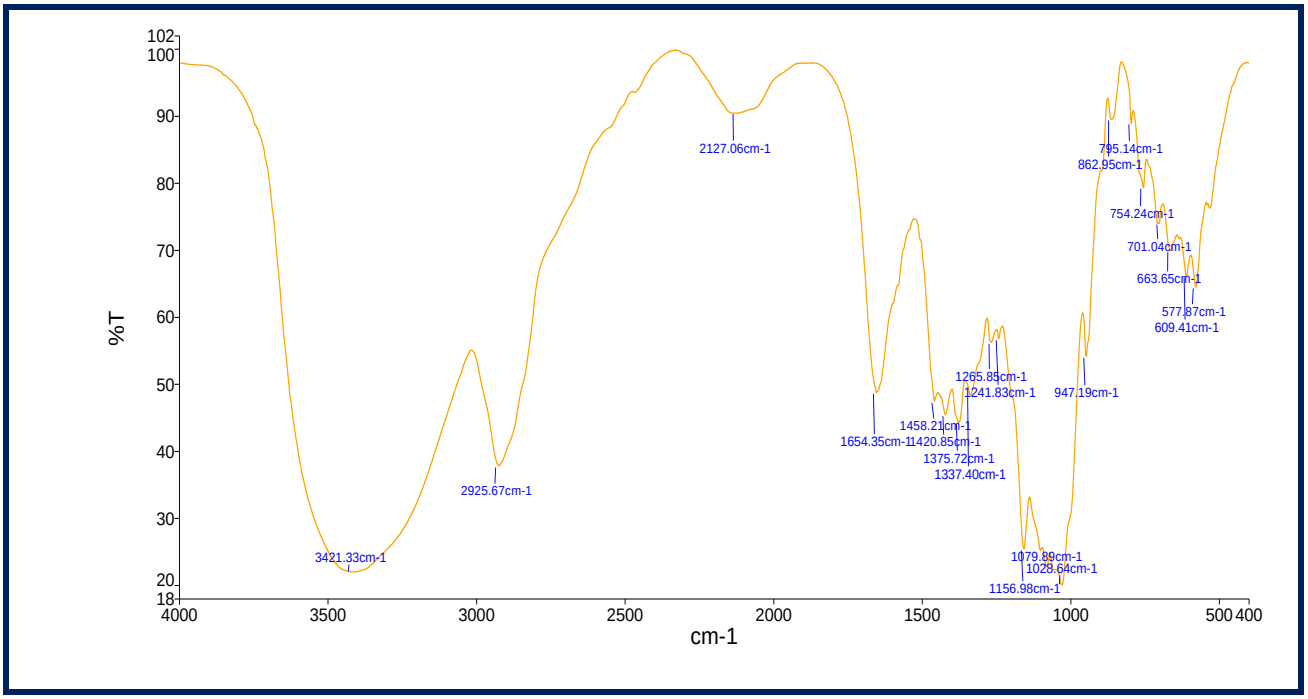


FIG :4 PRODUCTION YIELD OF CONTROLLED RELEASE NANOPARTICLES OF DAPOXETINE HCL

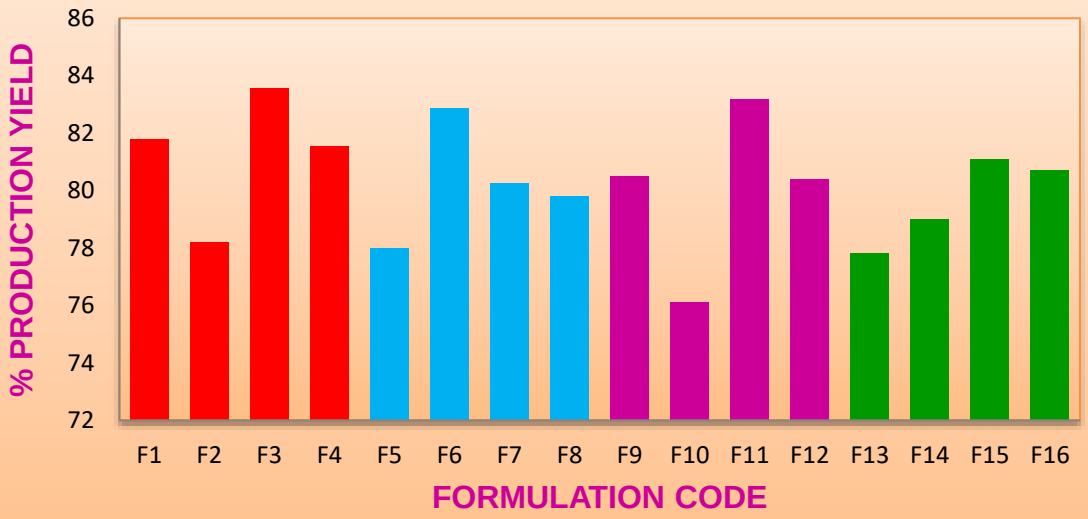


FIG: 5 THEORETICAL LOADING OF CONTROLLED RELEASE NANOPARTICLES OF DAPOXTINE HCL

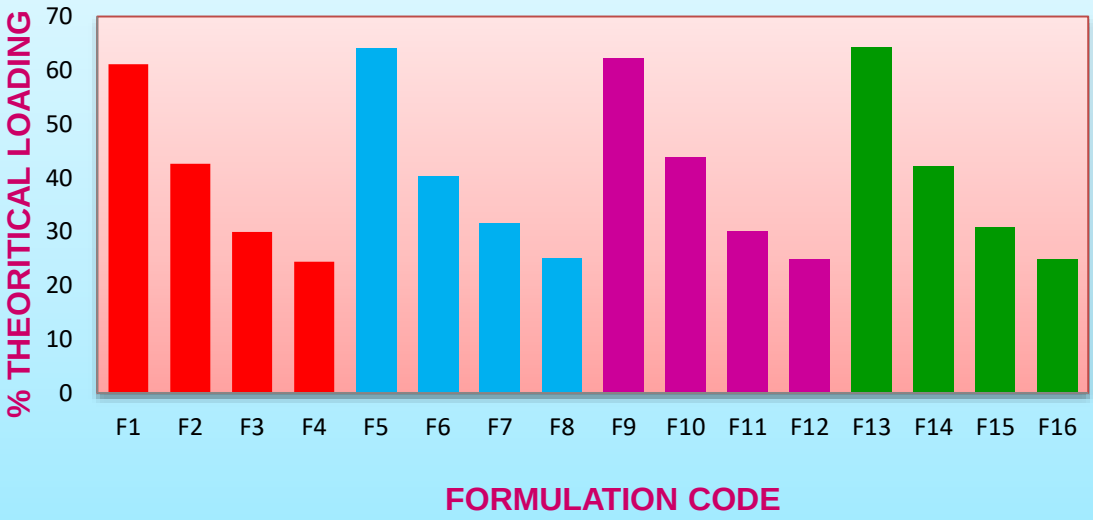


FIG: 6 DRUG CONTENT FOR CONTROLLED RELEASE OF DAPOXETINE HCL NANOPARTICLES

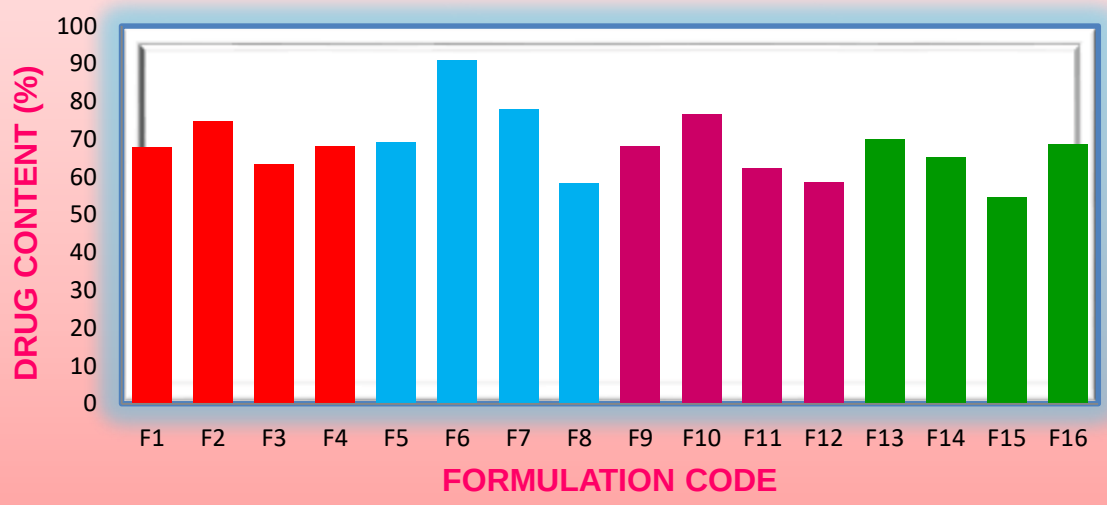


FIG. 7 ENTRAPMENT EFFICIENCY FOR DAPOXETINE HCL NANOPARTICLES

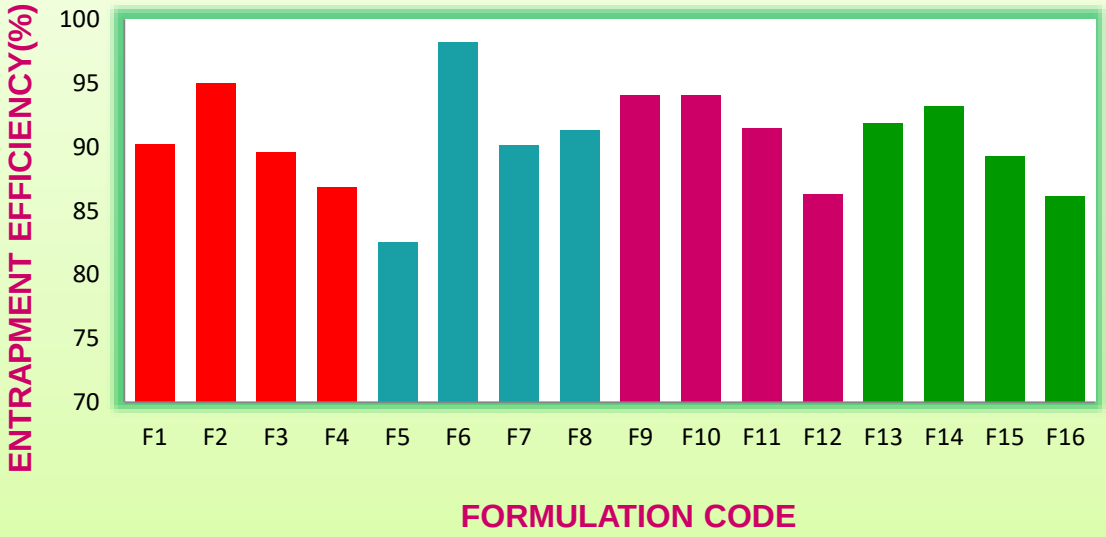


FIGURE : 8 SOLUBILITY EFFICACY OF FORMULATION IN DIFFERENT FORMULATION

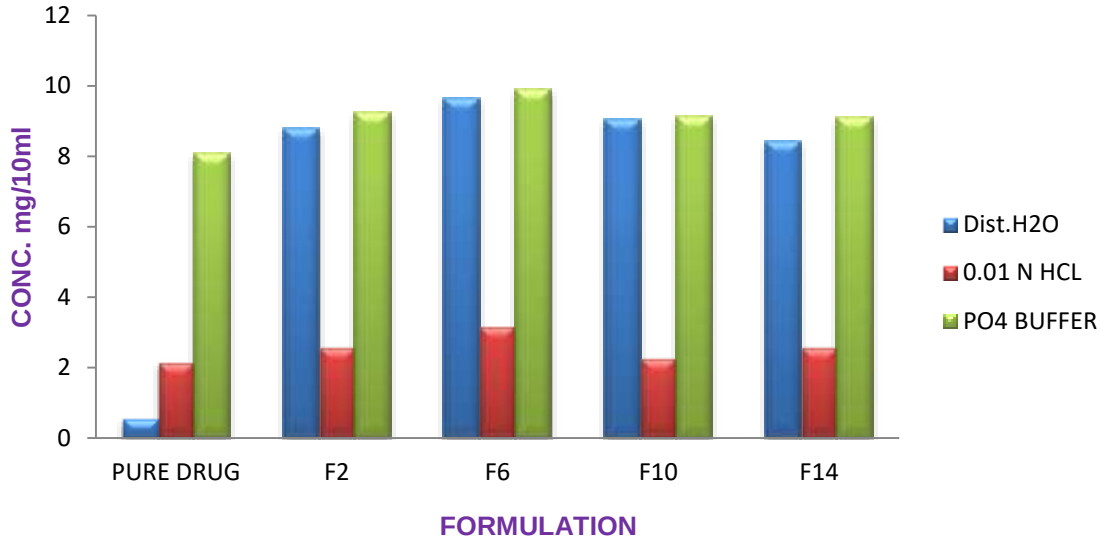


FIGURE :9 PARTICLS SIZE FOR CHITOSAN NPs (F6)

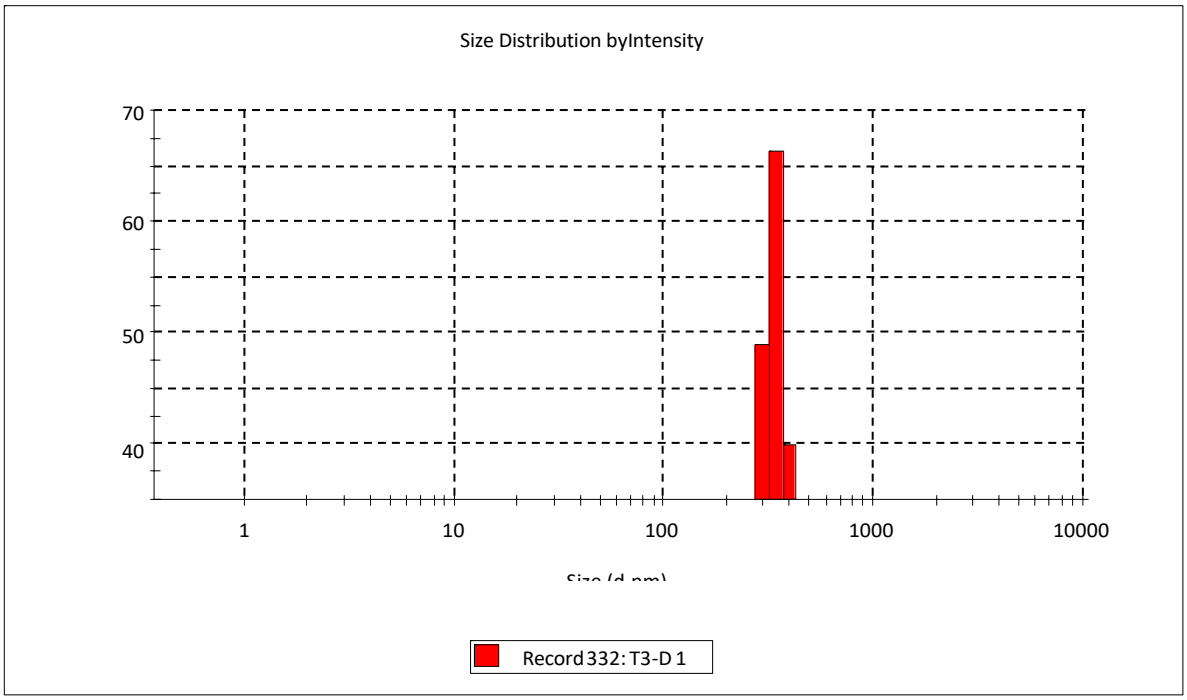


FIGURE: 10 ZETA POTENTIAL FOR CHITOSAN NPs (F6)

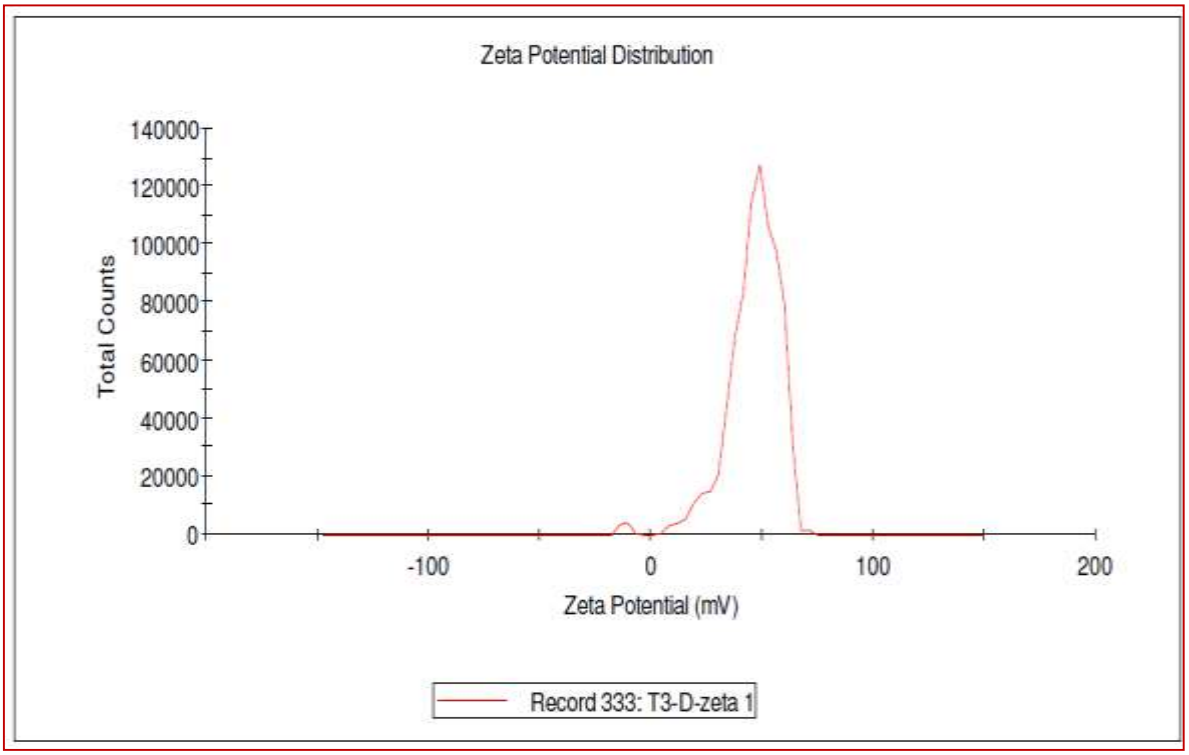


FIGURE: 11 PARTICLE SIZE & ZETAPOTENTIAL FOR BEST FORMULATION F6 (1:2) RATIO

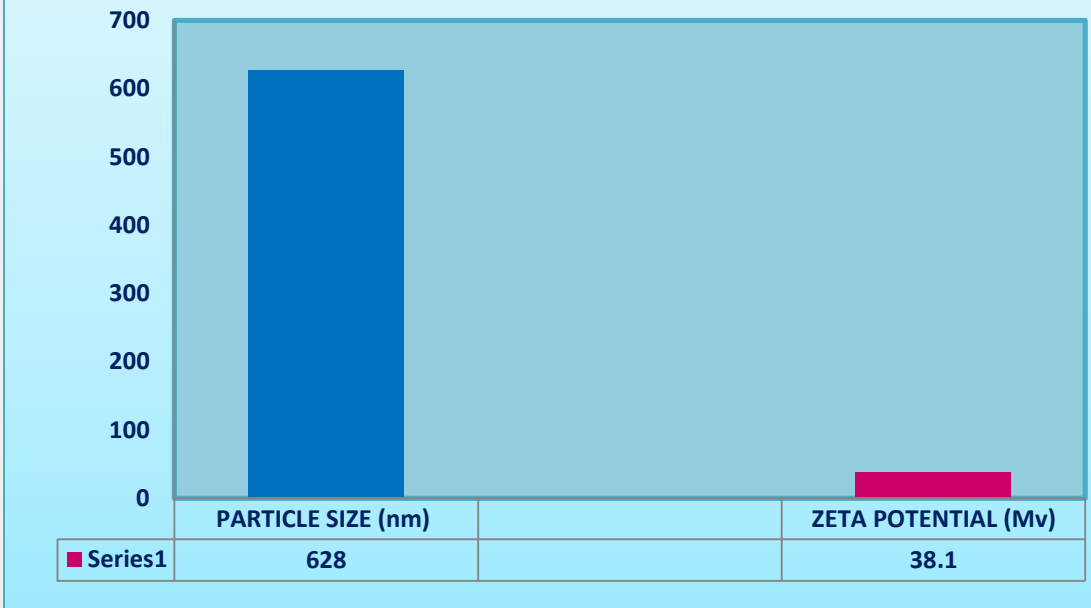


FIGURE : 12A INVITRO RELEASE PROFILE OF DAPOXETINE HCL PURE DRUG

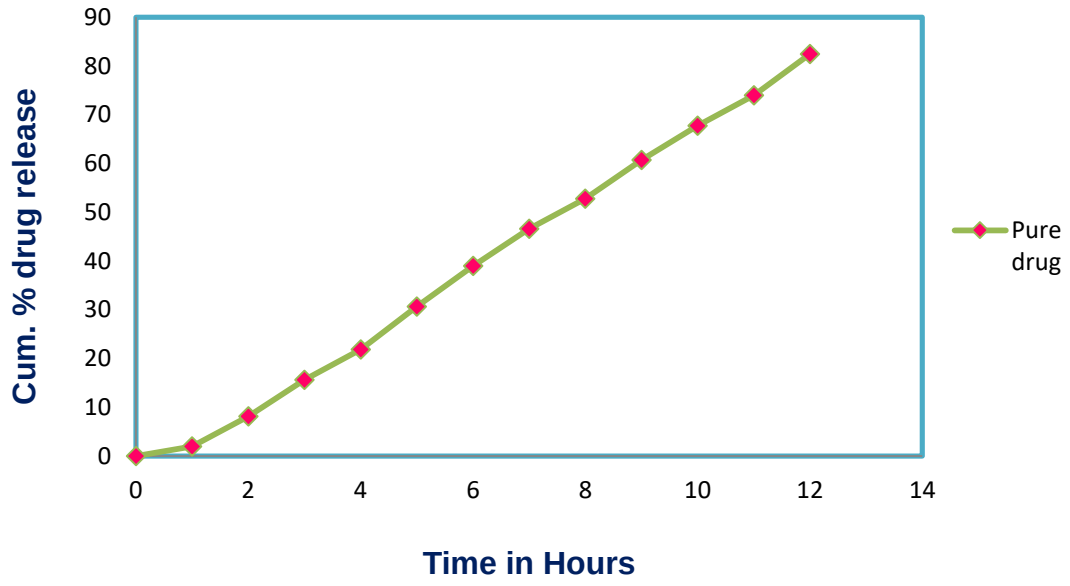


FIG: 12B COMPARISION OF INVITRO RELEASE PROFILEOF DAPOXETINE NANOPARTICLES CONTAINING ETHYL CELLULOSE AT DIFFERENT RATIOS

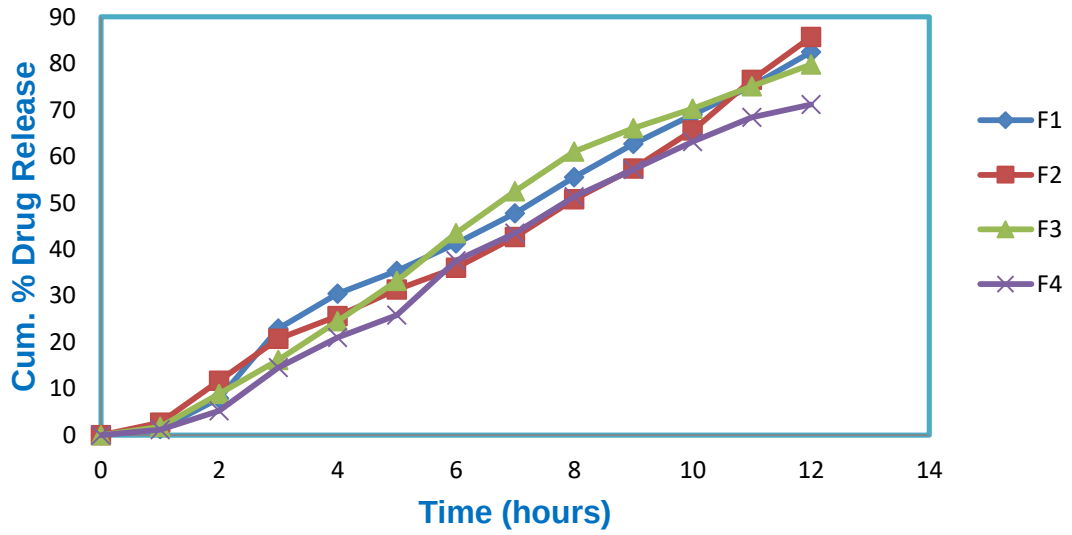


FIG:12C COMPARISION OF INVITRO RELEASE PROFILEOF DAPOXETINE NANOPARTICLES CONTAINING CHITOSAN AT DIFFERENT RATIOS

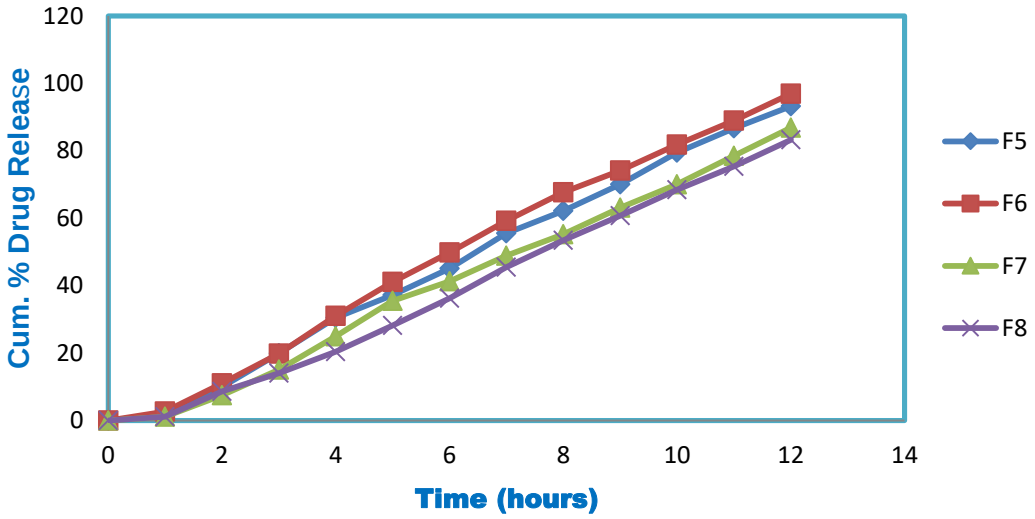


FIGURE: 12D COMPARISION OF INVITRO RELEASE PROFILEOF DAPOXETINE NANOPARTICLES CONTAINING HPMC K100 AT DIFFERENT RATIOS

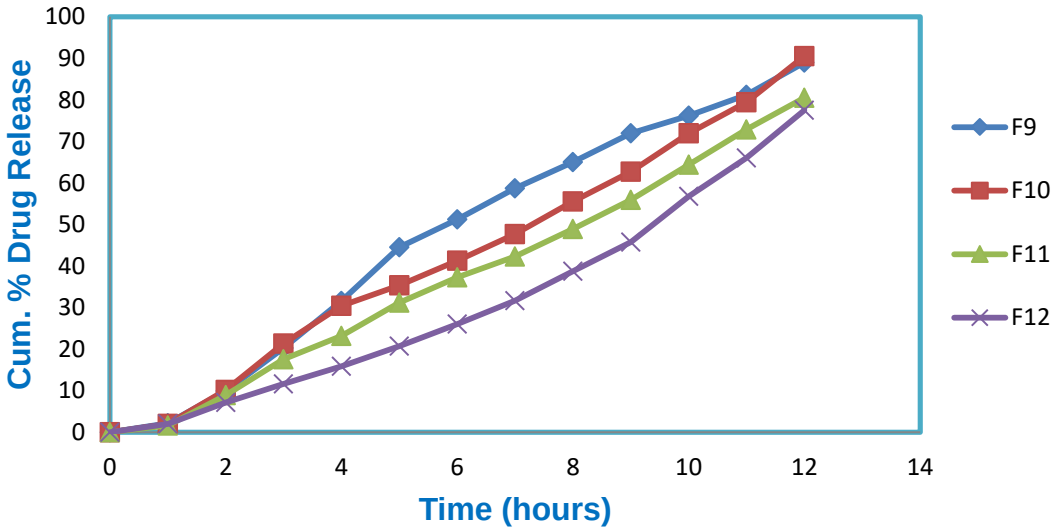


FIGURE: 12E COMPARISION OF INVITRO RELEASE PROFILEOF DAPOXETINE NANOPARTICLES CONTAINING BETACYCLODEXTTRIN AT DIFFERENT RATIOS

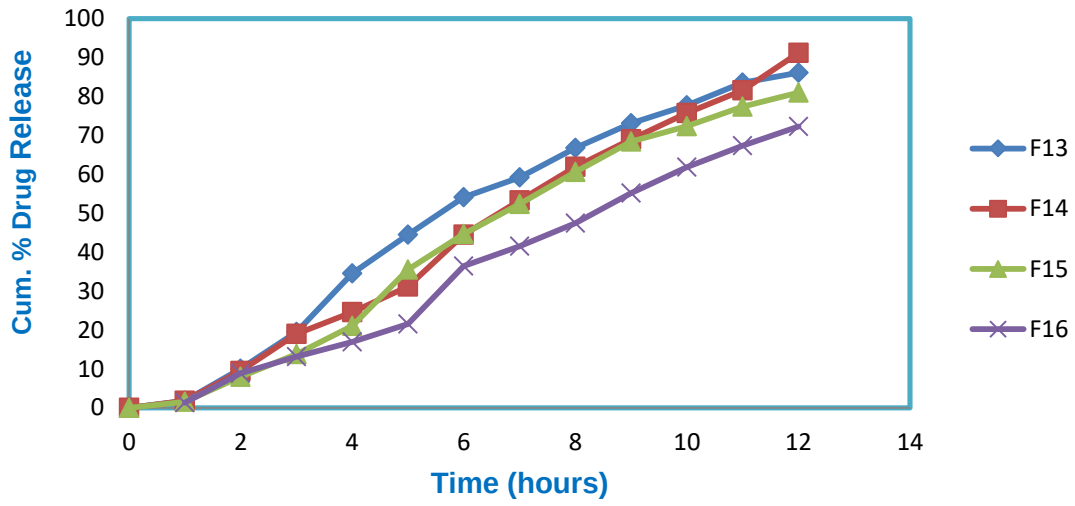


FIGURE:13A COMPARISION OF *INVITRO* ZERO ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

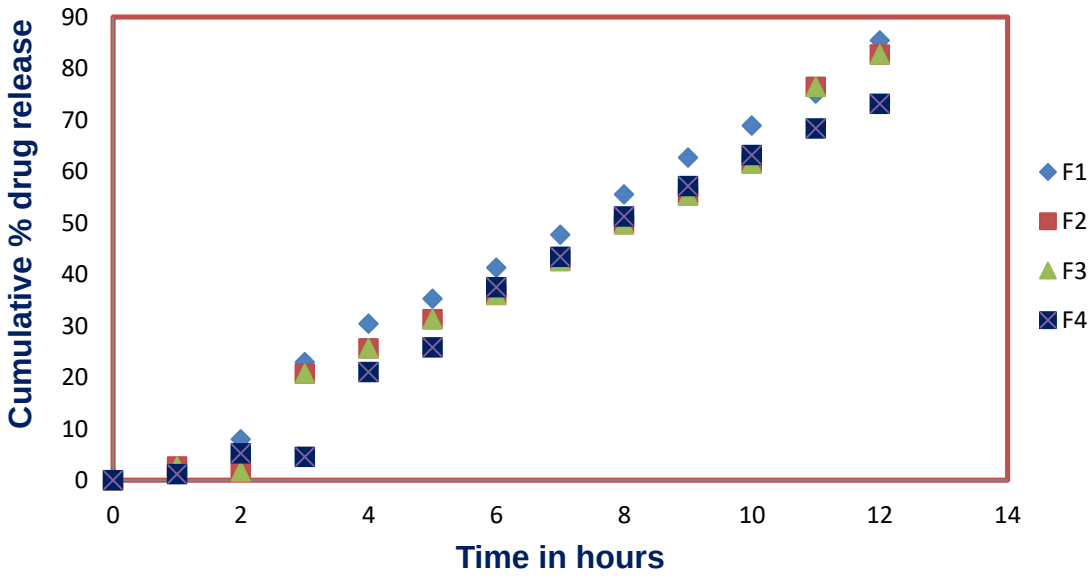


FIGURE:13B COMPARISION OF *INVITRO* ZERO ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED CHITOSAN NANOPARTICLES

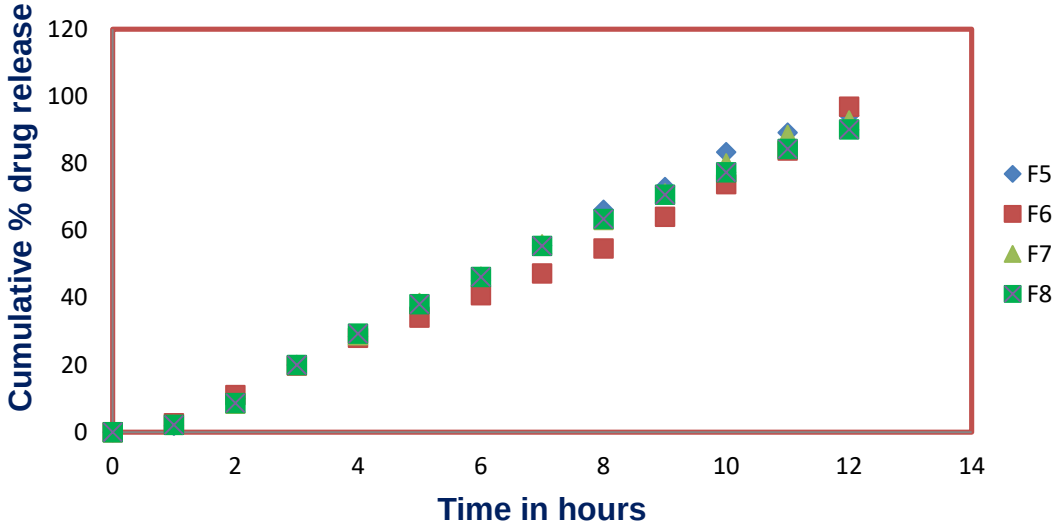


FIGURE:13C COMPARISION OF *INVITRO* ZERO ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED HPMC K100 M NANOPARTICLES

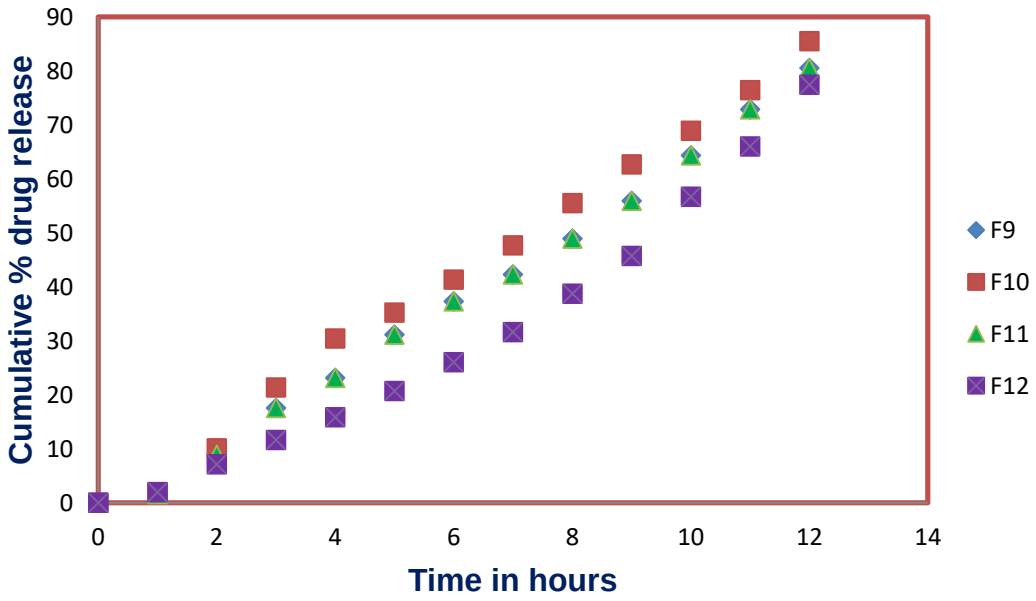


FIGURE:13D COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED β-CD NANOPARTICLES

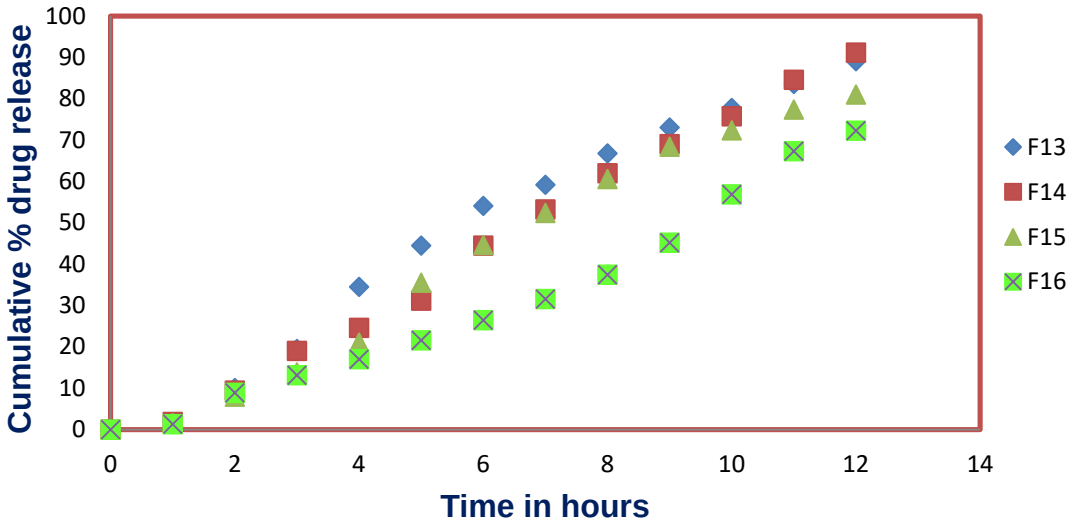


FIGURE:14A COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

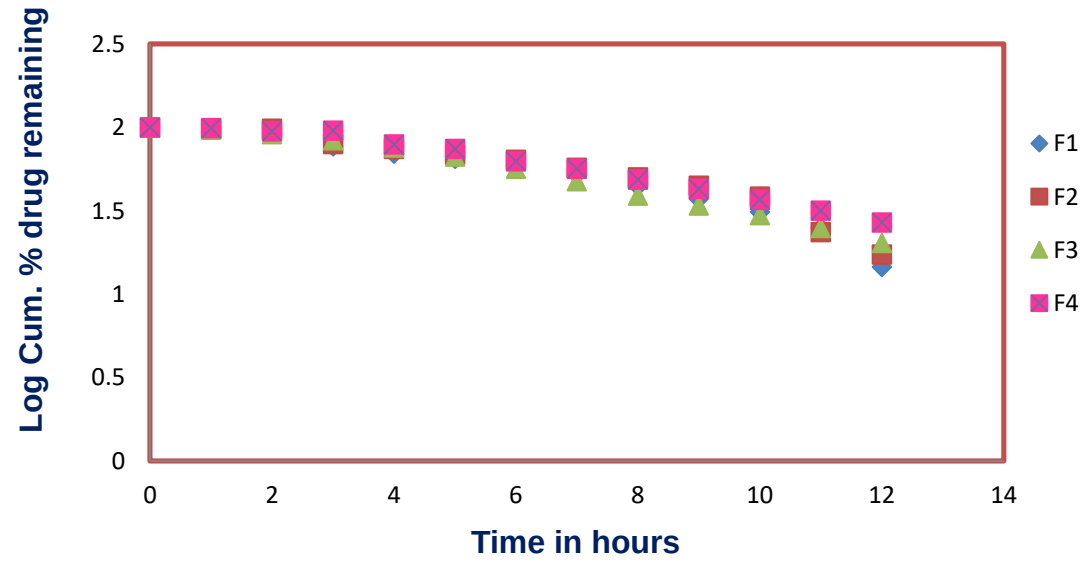


FIGURE:14B COMPARISION OF *INVITRO* FIRST ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

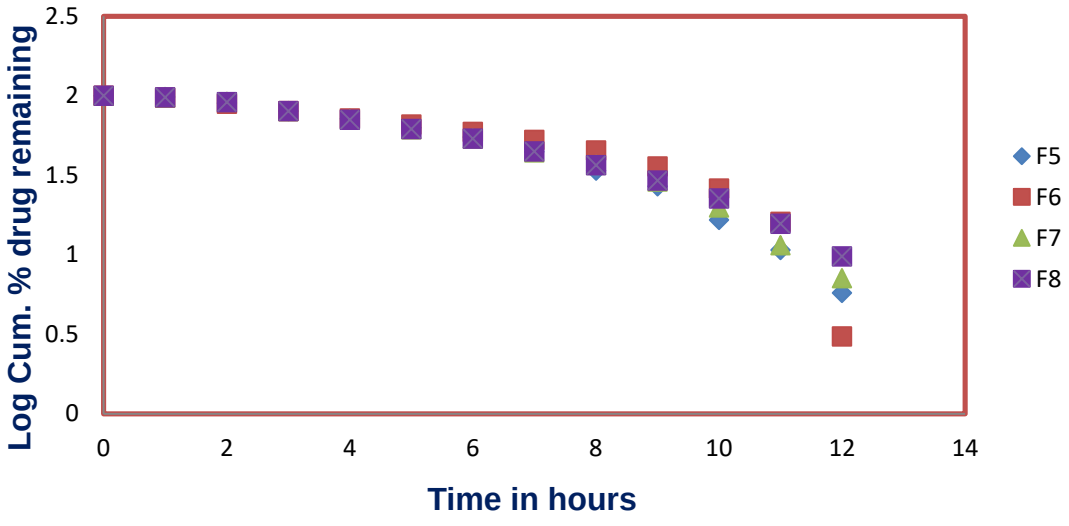


FIGURE:14C COMPARISION OF *INVITRO* FIRST ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

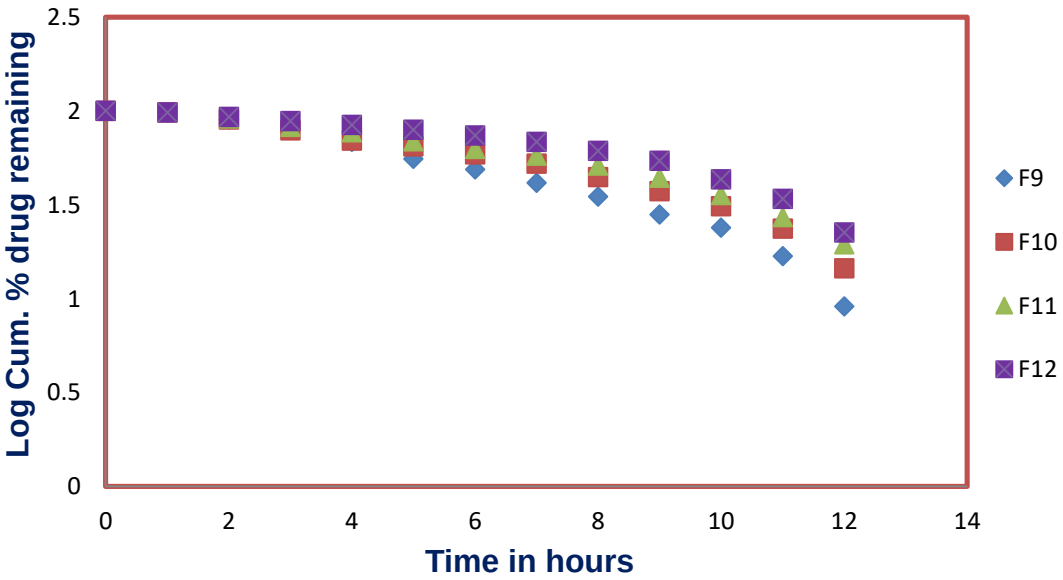


FIGURE:14D COMPARISION OF *INVITRO* FIRST ORDER RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

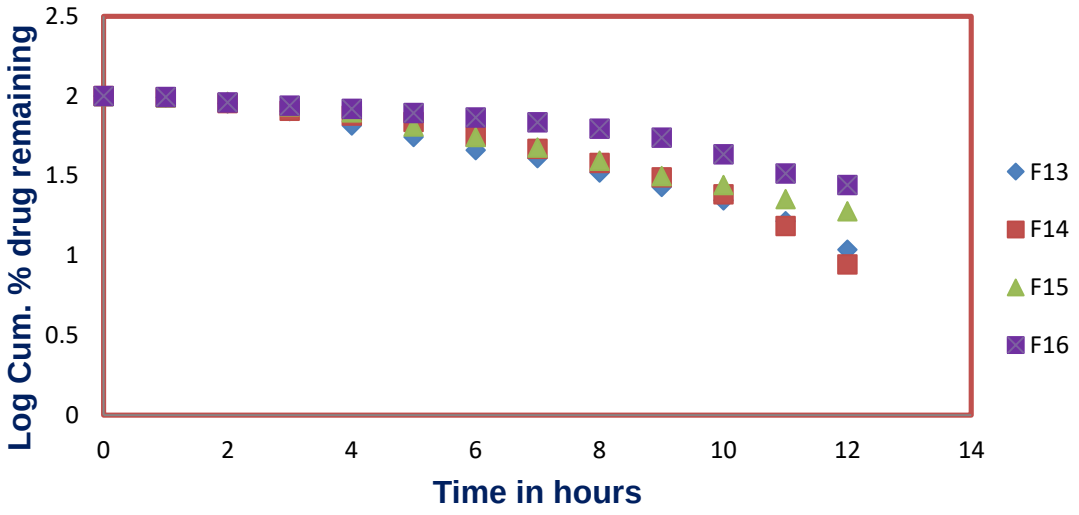


FIGURE:15A COMPARISION OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

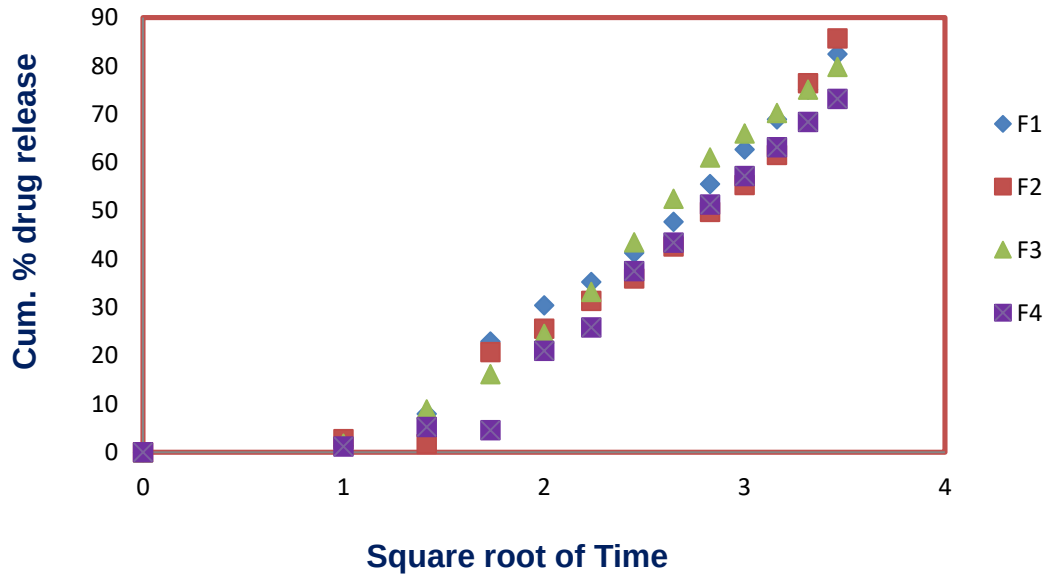


FIGURE:15B COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF DAPOXETINE HCL LOADED CHITOSAN NANOPARTICLES

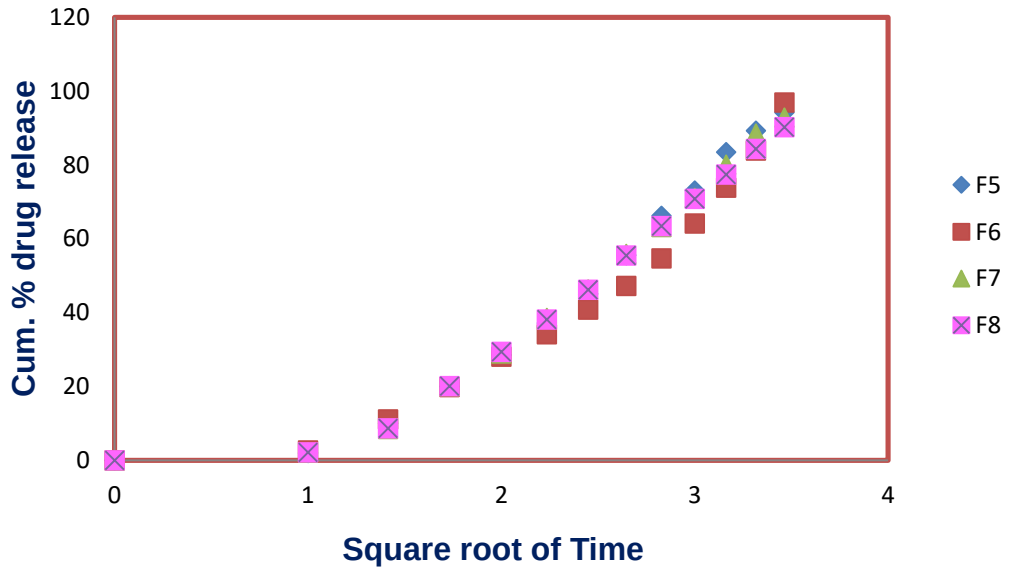


FIGURE:15C COMPARISION OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF DAPOXETINE HCL LOADED HPMC K100 M NANOPARTICLES

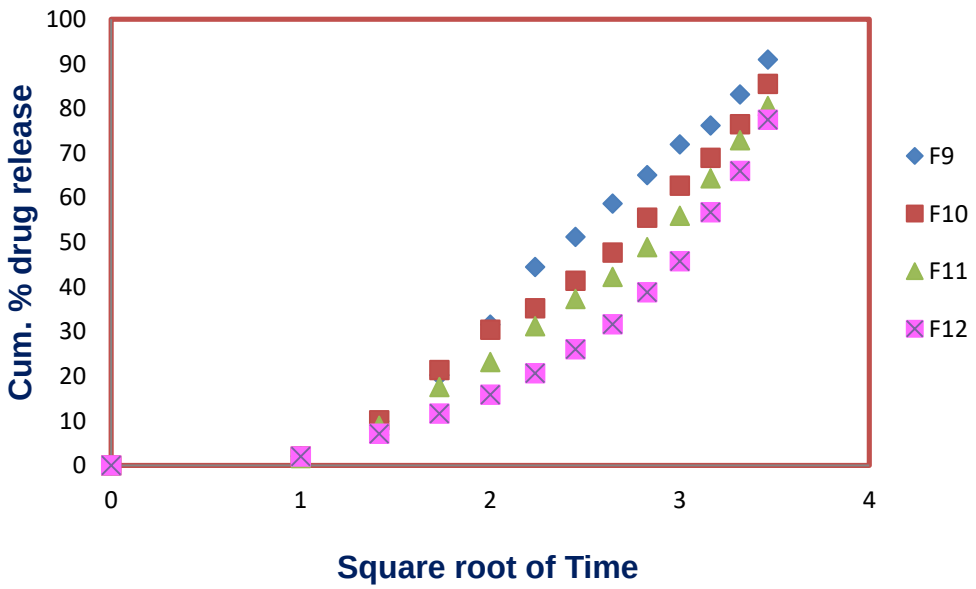


FIGURE:15D COMPARISION OF *INVITRO* HIGUCHI MODEL RELEASE KINETICS OF DAPOXETINE HCL LOADED HPMC K100 M NANOPARTICLES

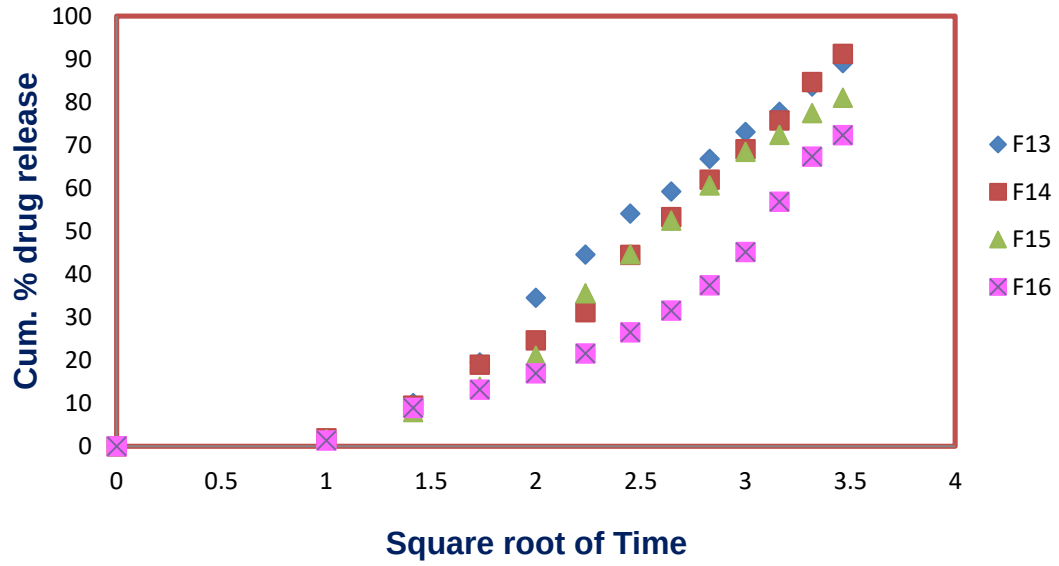


FIGURE:16A COMPARISION OF *INVITRO* KORSEMEYER-PEPPAS RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

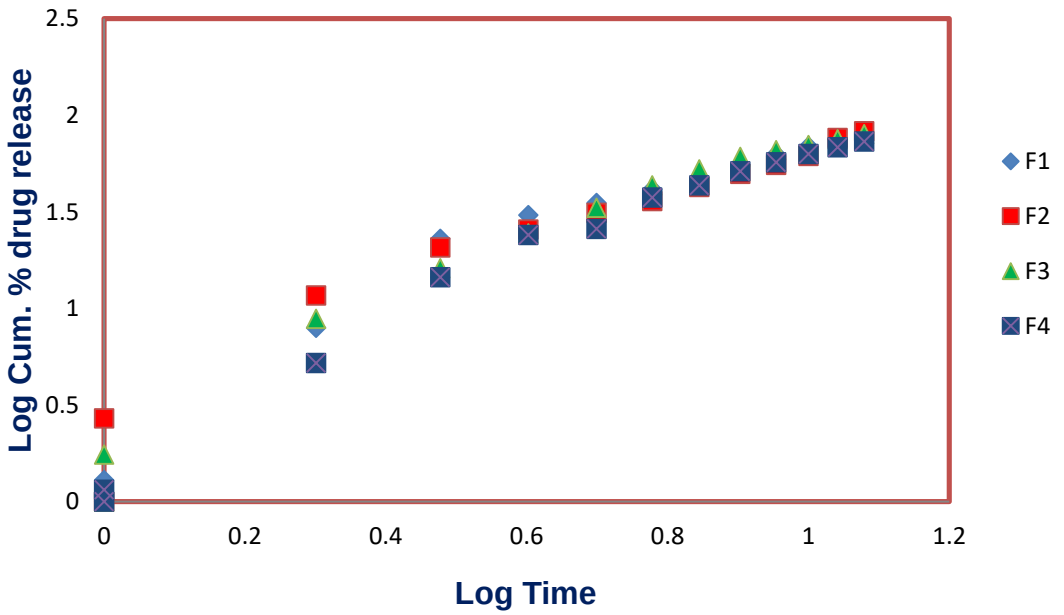


FIGURE:16B COMPARISION OF *INVITRO* KORSEMEYER-PEPPAS RELEASE KINETICS OF DAPOXETINE HCL LOADED CHITOSAN NANOPARTICLES

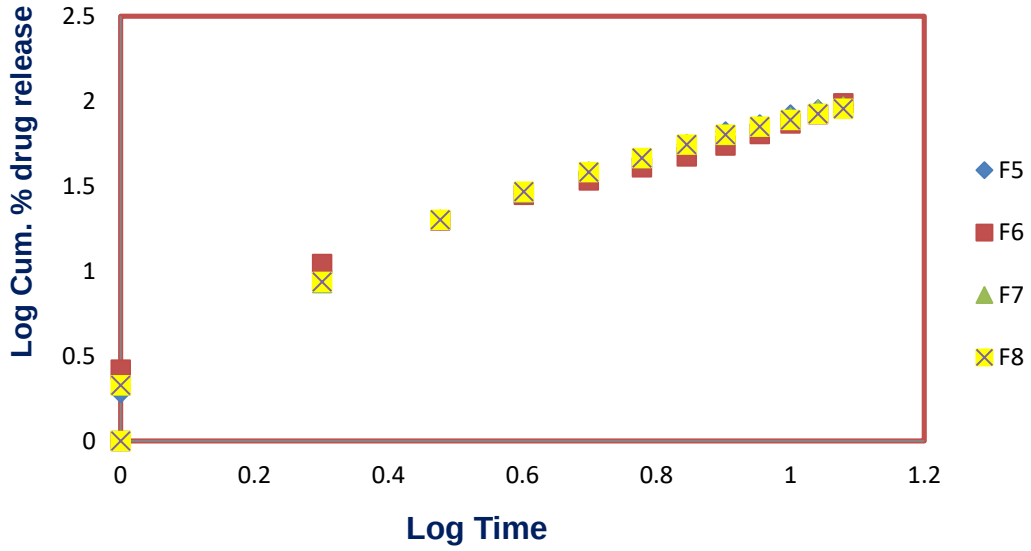


FIGURE:16C COMPARISION OF *INVITRO* KORSEMEYER-PEPPAS RELEASE KINETICS OF DAPOXETINE HCL LOADED HPMC K100 M NANOPARTICLES

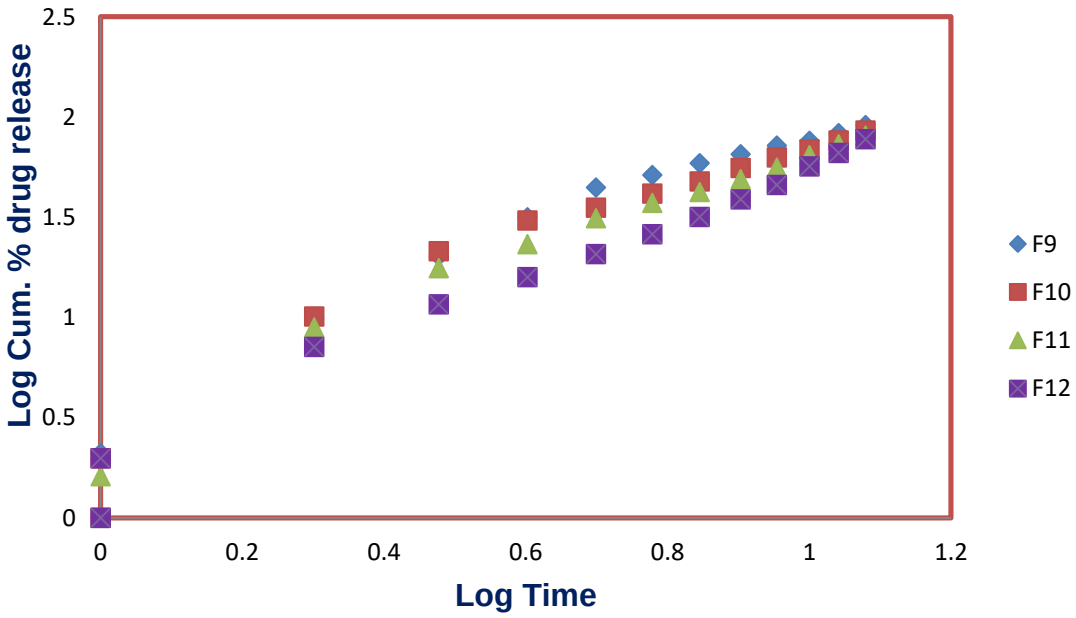


FIGURE:16D COMPARISION OF *INVITRO* KORSEMEYER-PEPPAS RELEASE KINETICS OF DAPOXETINE HCL LOADED β-CYCLODEXTRIN NANOPARTICLES

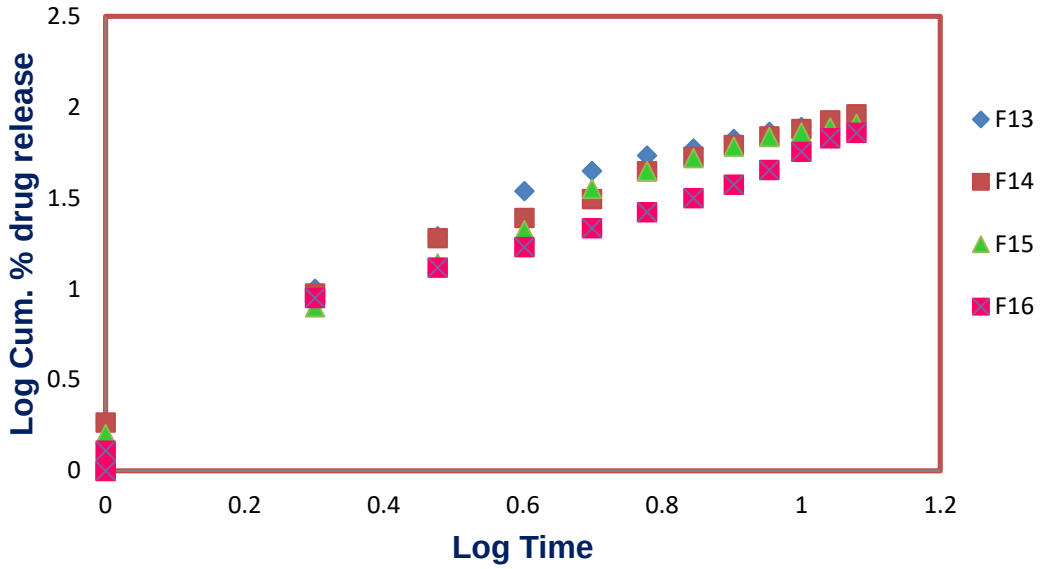


FIGURE:17A COMPARISION OF *INVITRO* HIXON-CROWELL RELEASE KINETICS OF DAPOXETINE HCL LOADED ETHYL CELLULOSE NANOPARTICLES

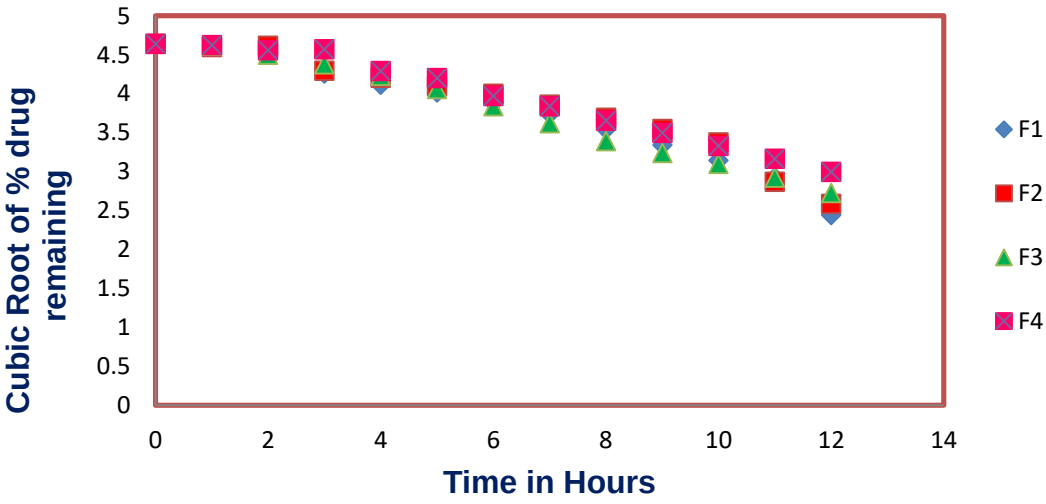


FIGURE:17B COMPARISION OF *INVITRO* HIXON-CROWELL RELEASE KINETICS OF DAPOXETINE HCL LOADED CHITOSAN NANOPARTICLES

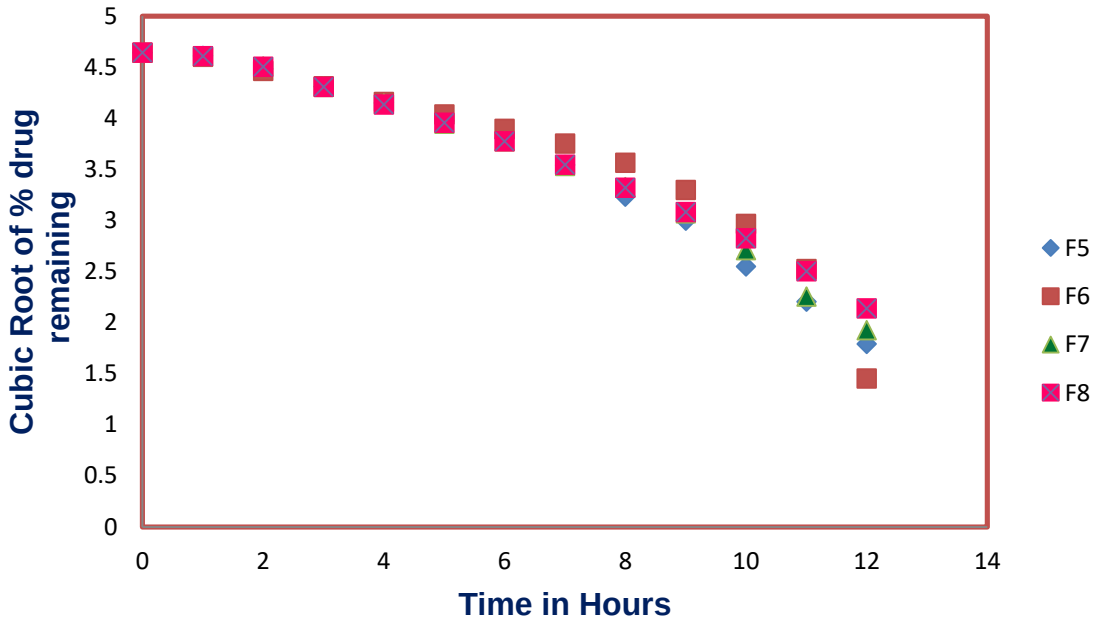


FIGURE:17C COMPARISION OF INVITRO HIXON-CROWELL RELASE KINETICS OF DAPOXETINE HCL LOADED HPMC K100 M NANOPARTICLES

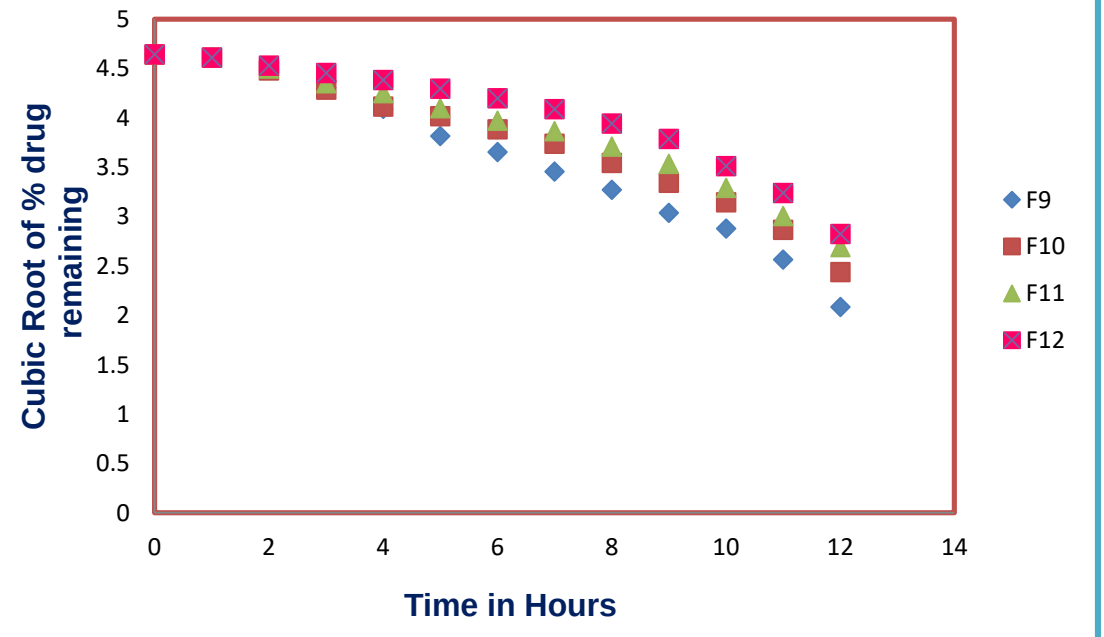


FIGURE:17D COMPARISION OF *INVITRO* HIXON-CROWELL RELEASE KINETICS OF DAPOXETINE HCL LOADED CHITOSAN NANOPARTICLES

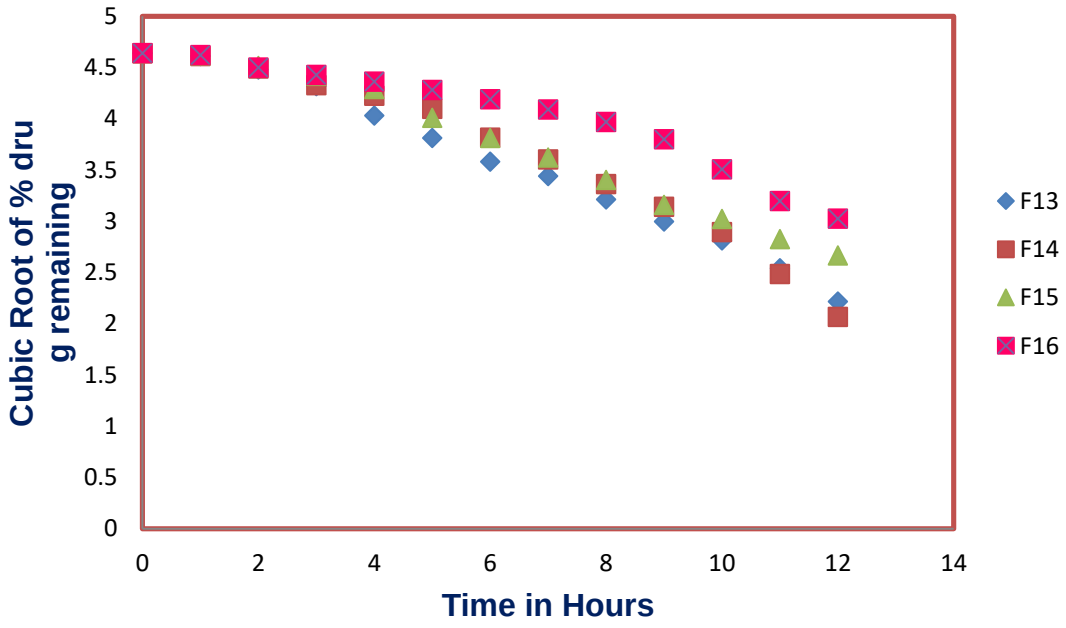


FIGURE: 18 FT-IR SPECTURM OF BEST FORMULATION F6 (1:2 DAPOXETIN HCL: CHITOSAN NANOPARTICLES)

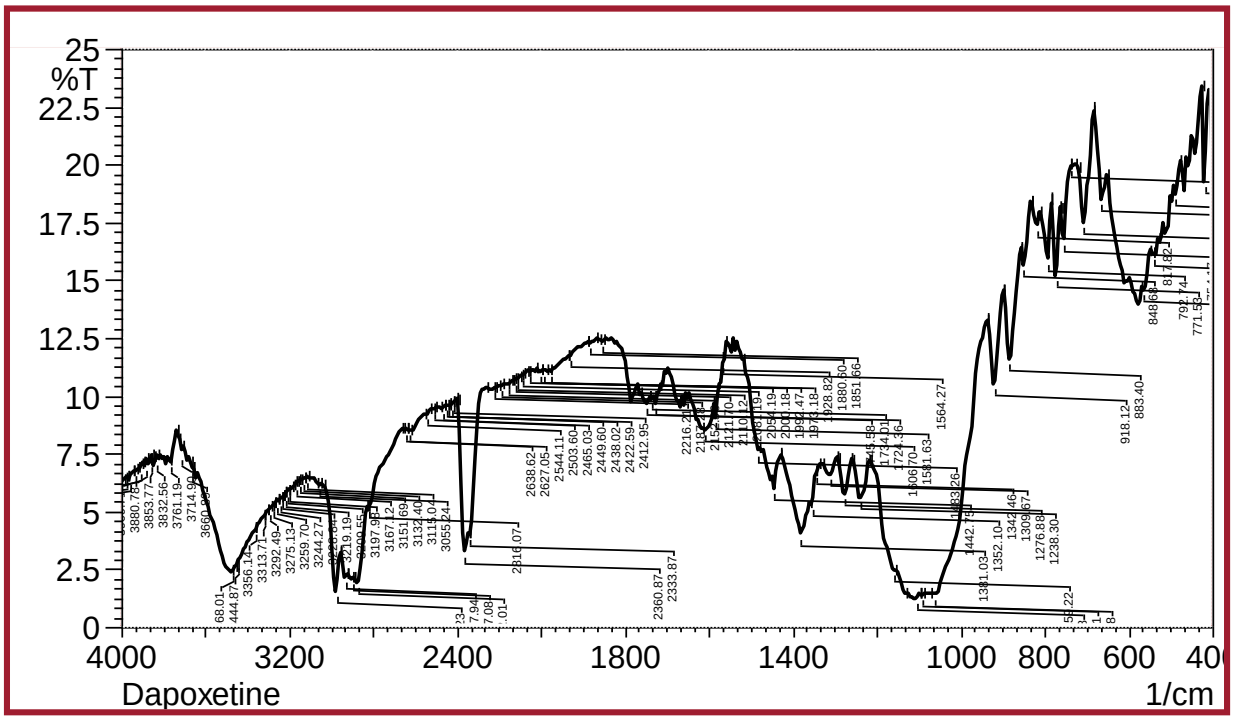


FIGURE:19 INVITRO RELEASE PROFILE OF DAPOXETINE HCL PURE DRUG AND BEST FORMULATION F6 (1:2)

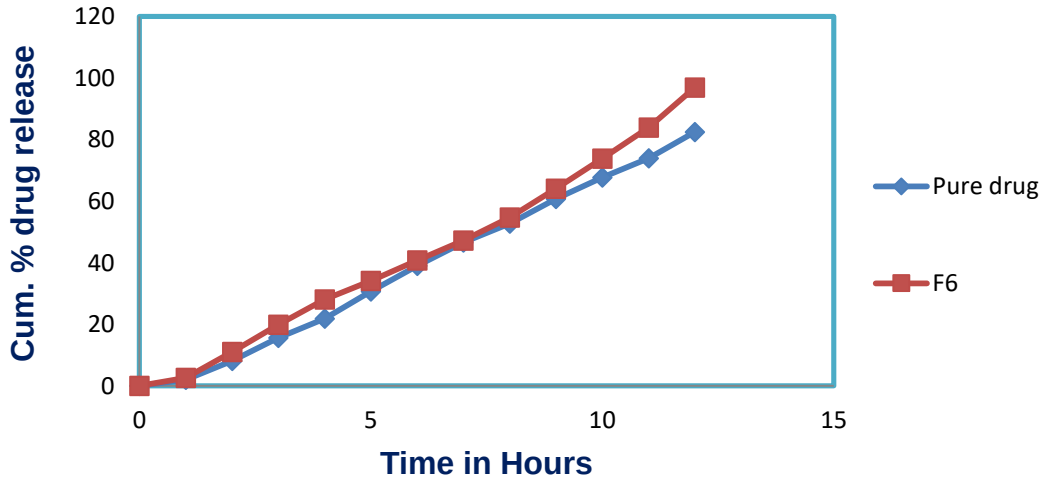


FIGURE: 20A SEM IMAGES OF DAPOXETIN HCL CHITOSAN NANOPARTICLES (F6):

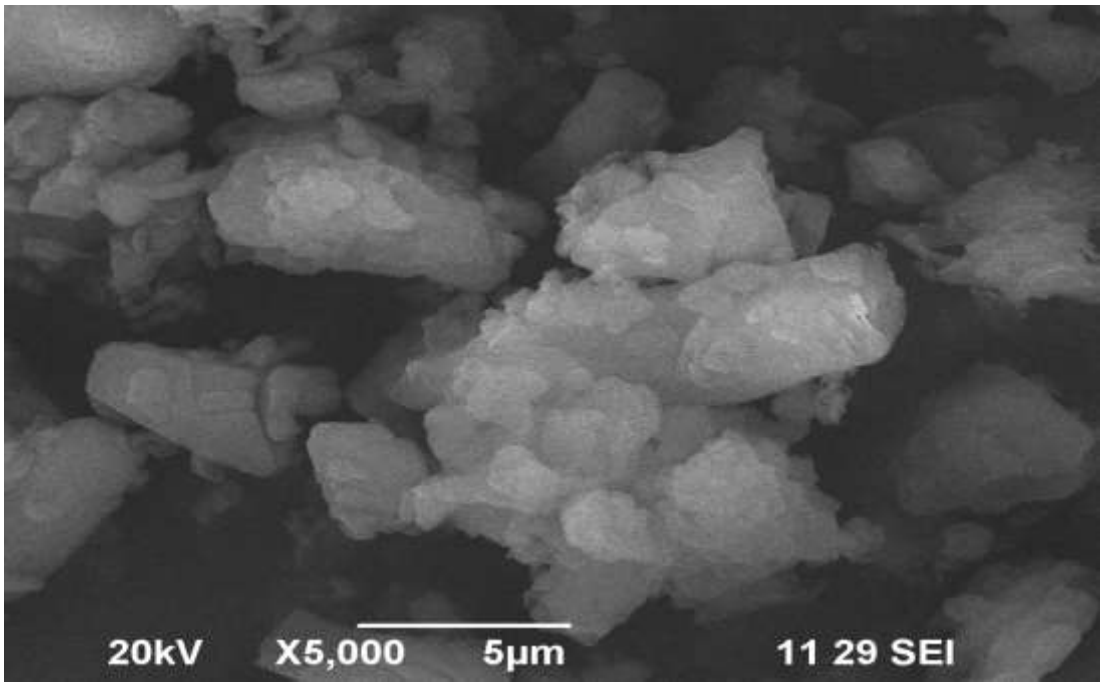


FIGURE: 20B SEM IMAGES OF DAPOXETIN HCL CHITOSAN NANOPARTICLES (F6):

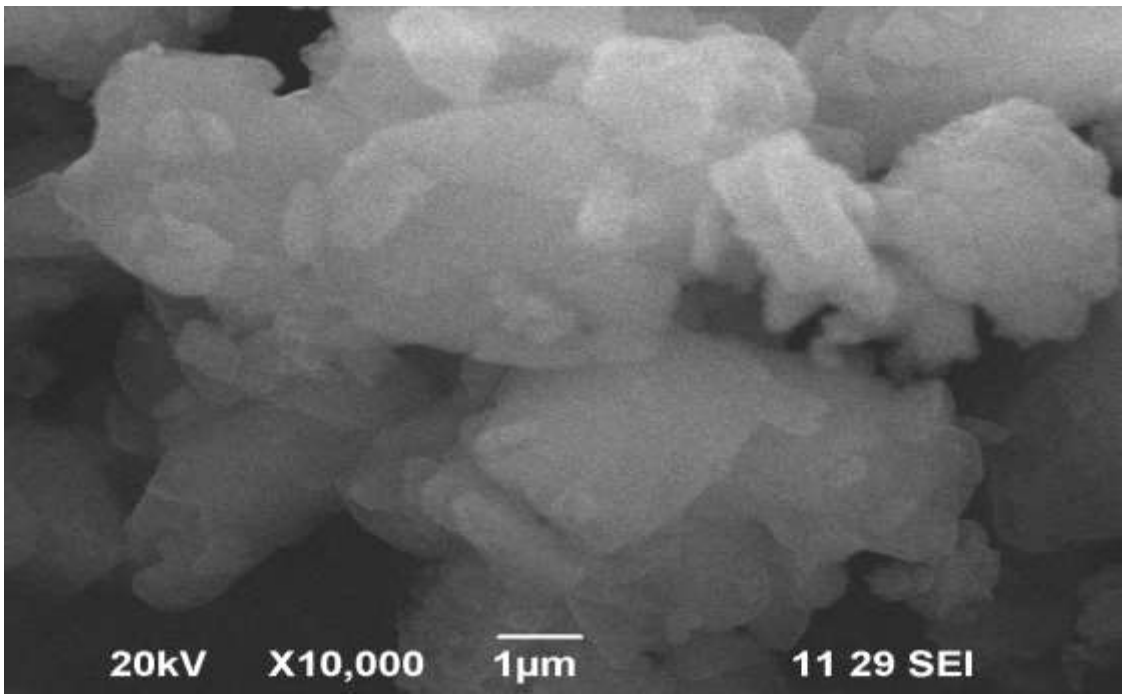


FIGURE: 20C SEM IMAGES OF DAPOXETIN HCL CHITOSAN NANOPARTICLES (F6):

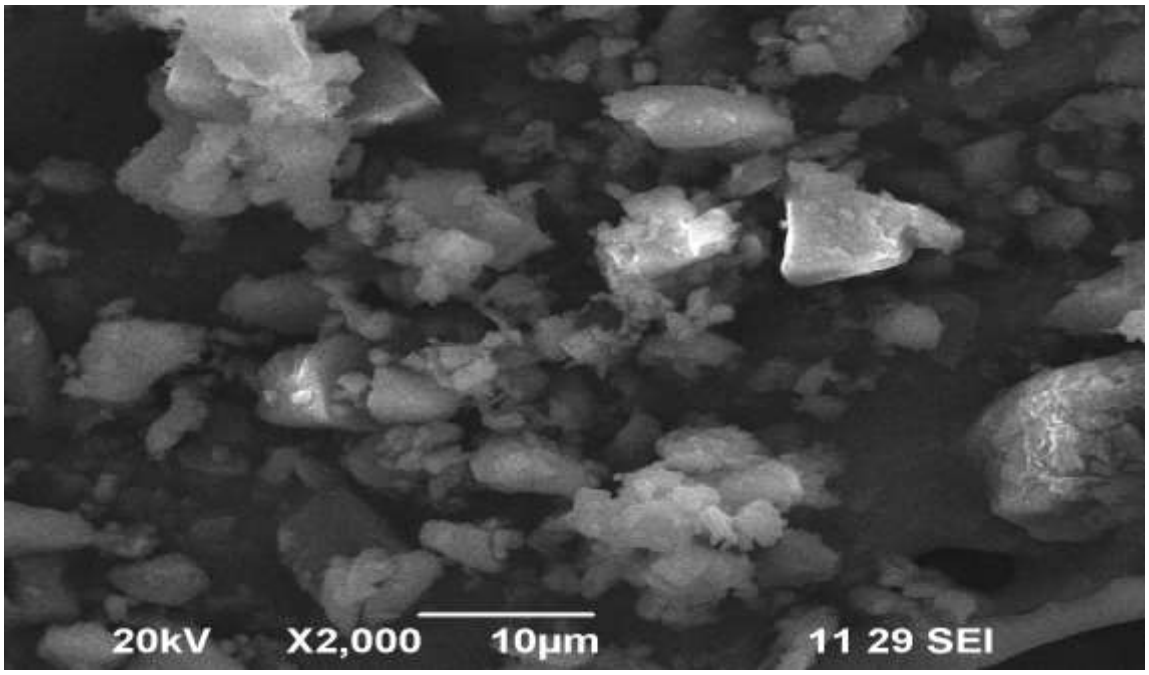


FIGURE: 20D SEM IMAGES OF DAPOXETIN HCL CHITOSAN NANOPARTICLES (F6):

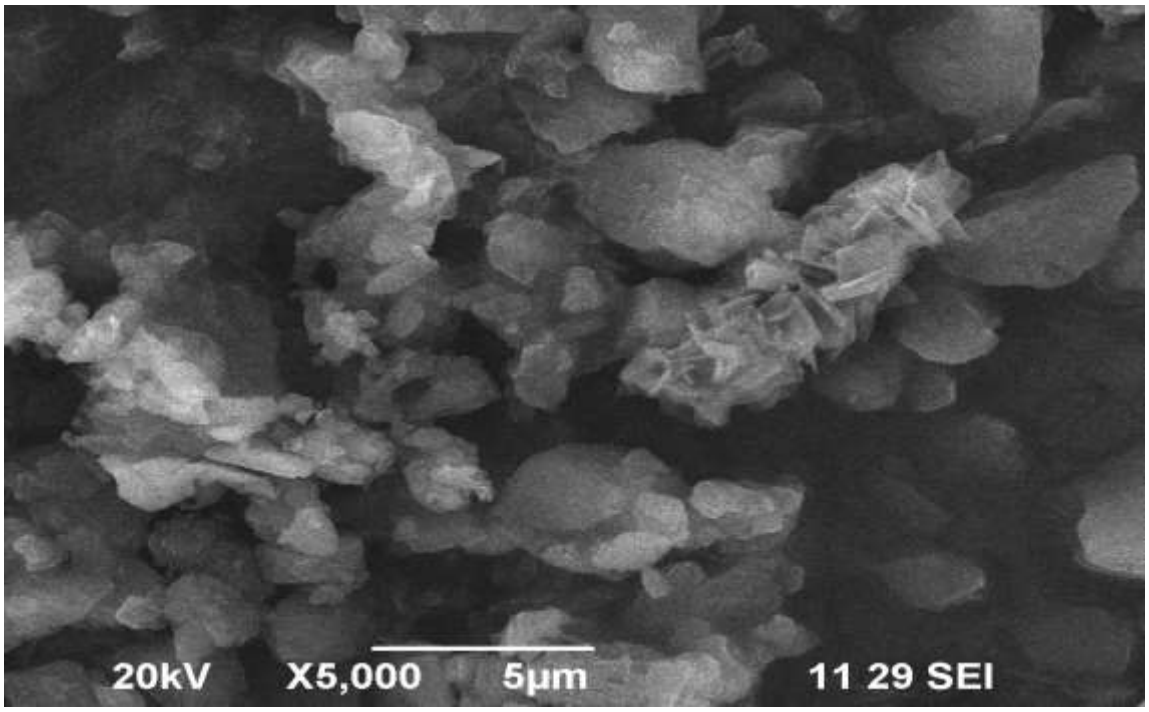


FIGURE: 20E SEM IMAGES OF DAPOXETIN HCL CHITOSAN NANOPARTICLES (F6):

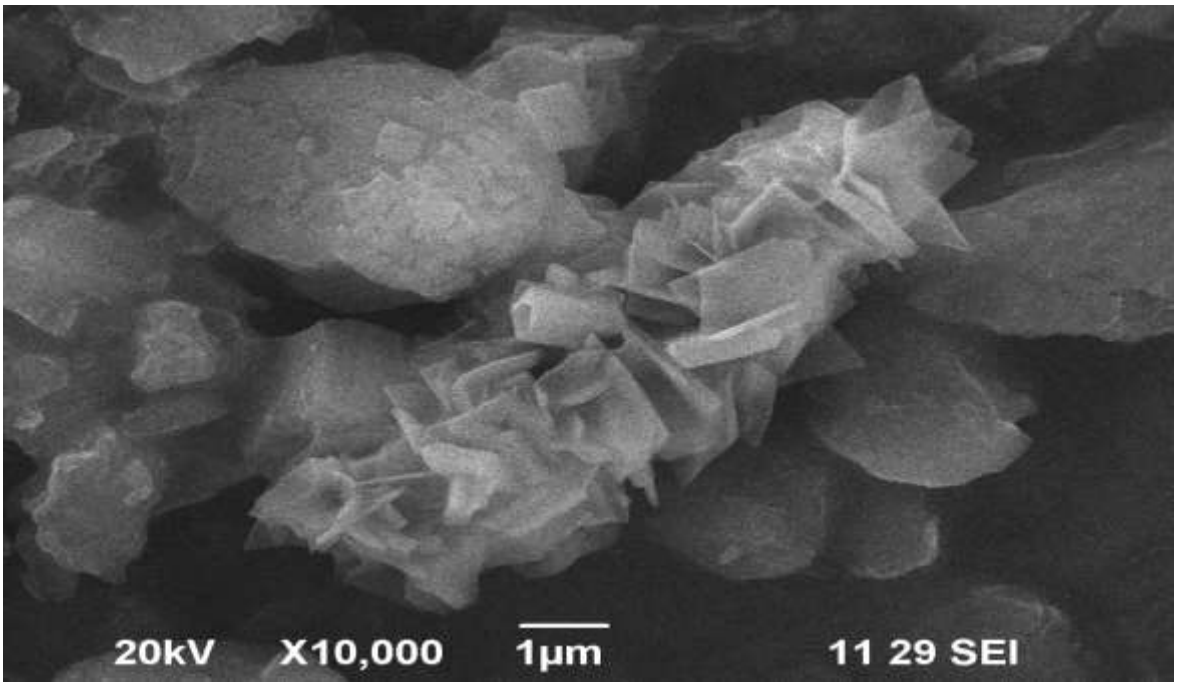


FIGURE: 21 POWDER DIFFRACTION OF CHITOSAN NPs (F6)

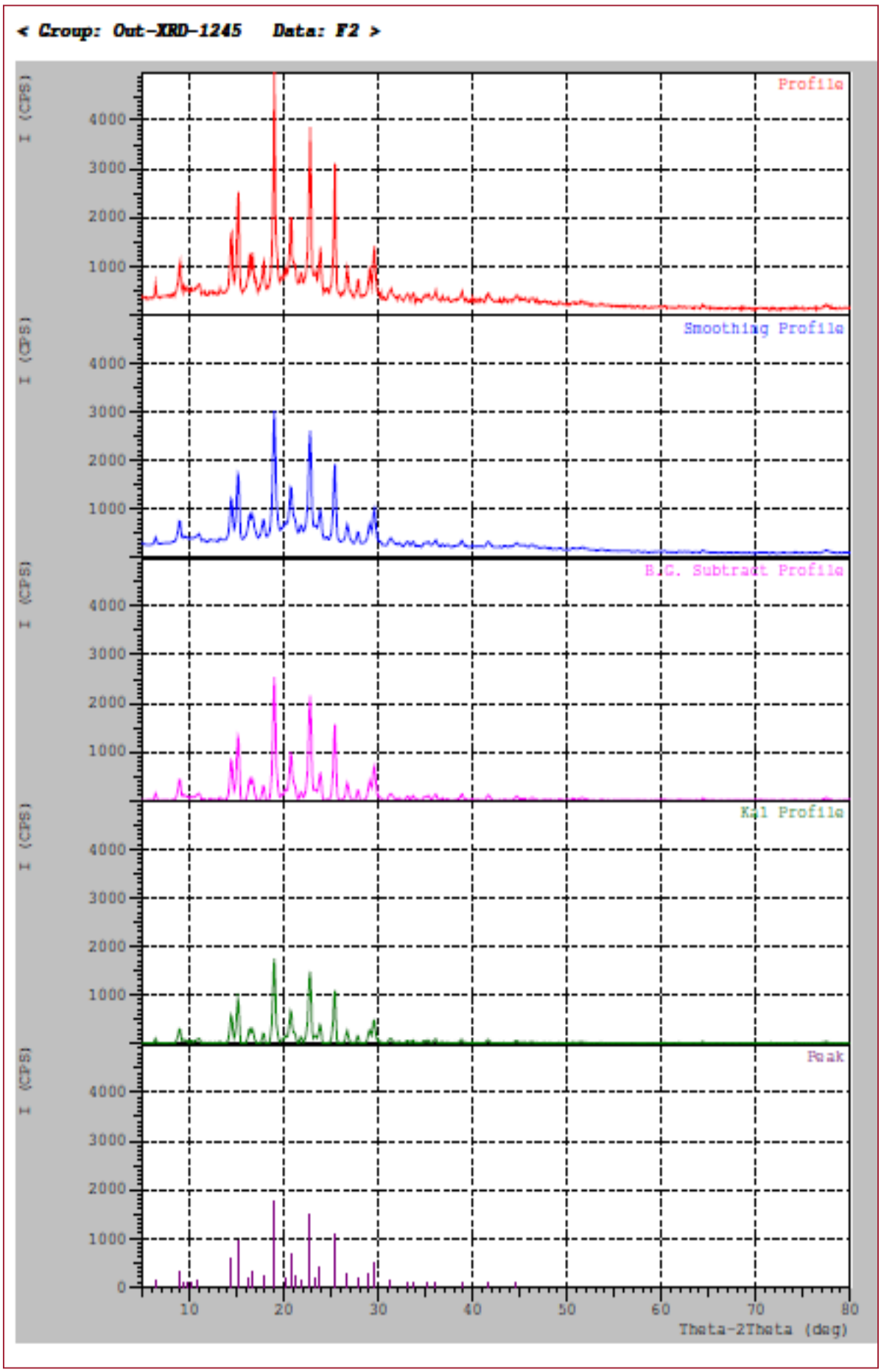


FIGURE: 22 FT-IR SPECTURM OF BEST FORMULATION F6 (1:2) DAPOXETINE HCL CHITOSAN NANOPARTICLES LOADED CAPSULES

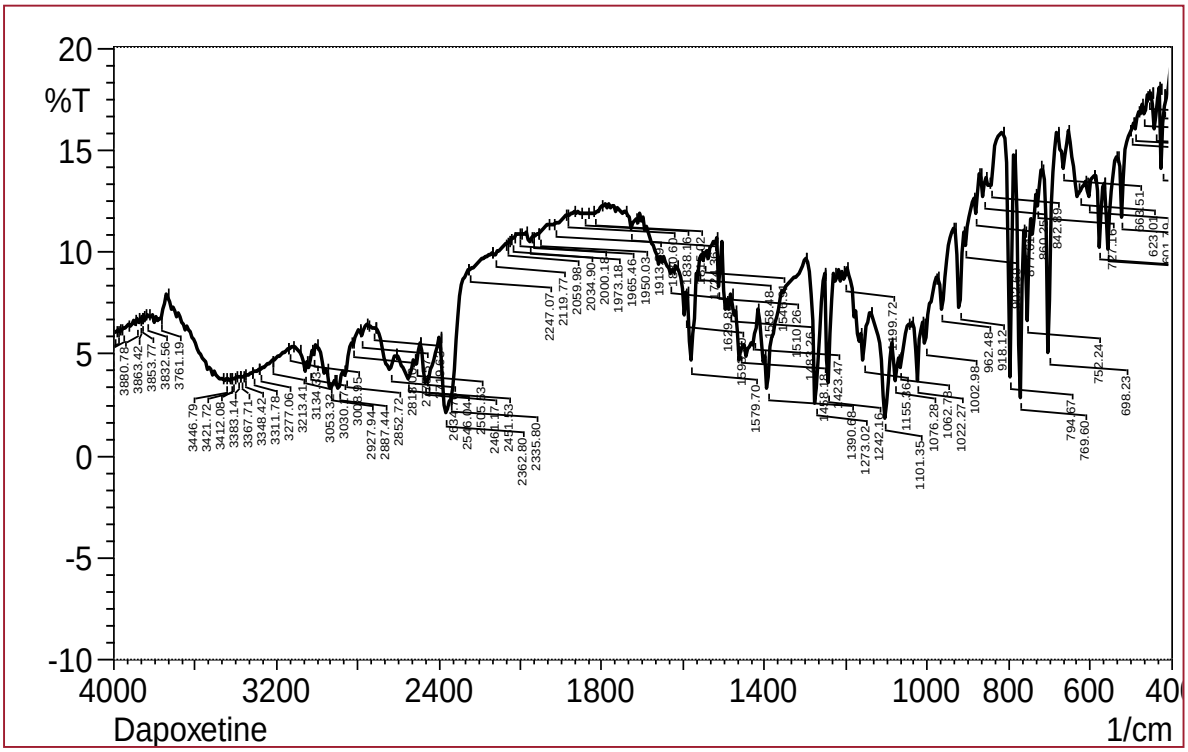


FIGURE: 23 ANGLE OF REPOSE & COMPRESSIBILITY INDEX FOR BEST (F6) FORMULATION

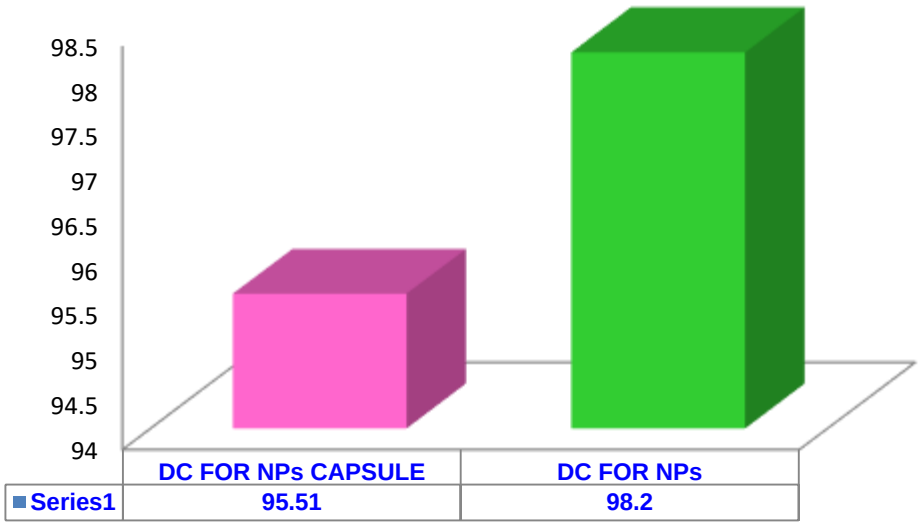
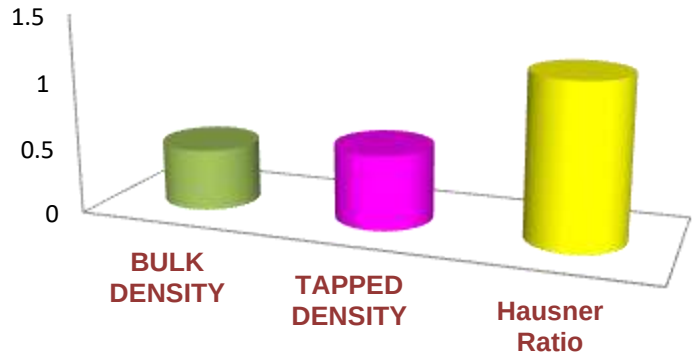


FIGURE:24 BULK DENSITY & TAPPED DENSITY FOR BEST (F6) FORMULATION



	BULK DENSITY	TAPPED DENSITY	Hausner Ratio
Series1	0.4629	0.5618	1.21

FIGURE:25 DRUG CONTENT FOR BEST (F6) FORMULATION

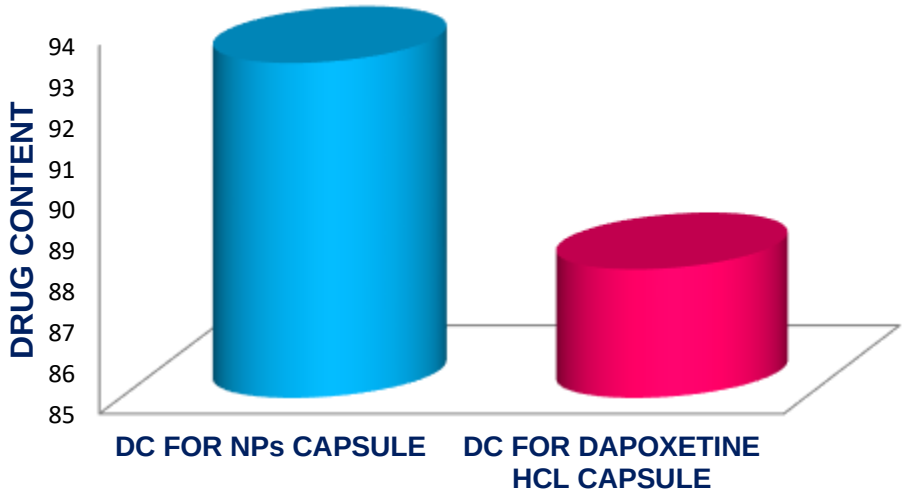


FIGURE:26 DRUG CONTENT FOR CHITOSAN NP (F6) AFTER STORED AT 25^0C+20C/RH 60% & 40^0C+20C/RH 75%

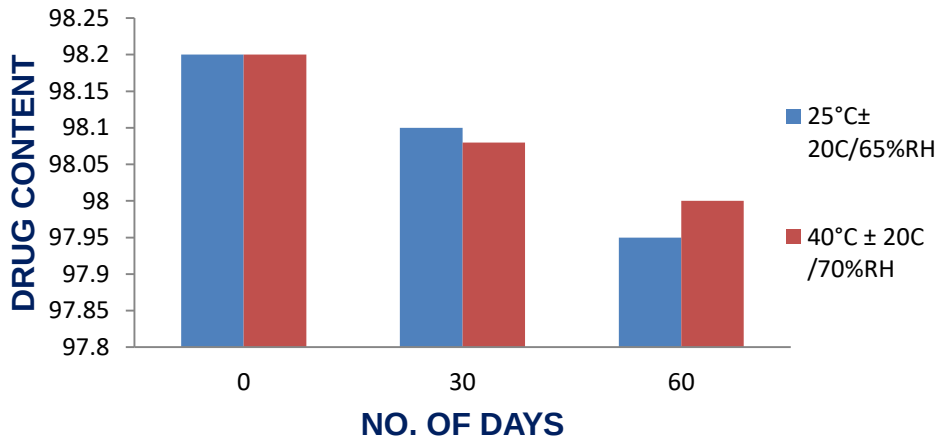


FIGURE :27 ENTRAPMENT EFFICIENCY FOR CHITOSAN N6 (F6) AFTER STORED AT 25^0C±20C/RH 60% & 40^0C±20C/RH 75%

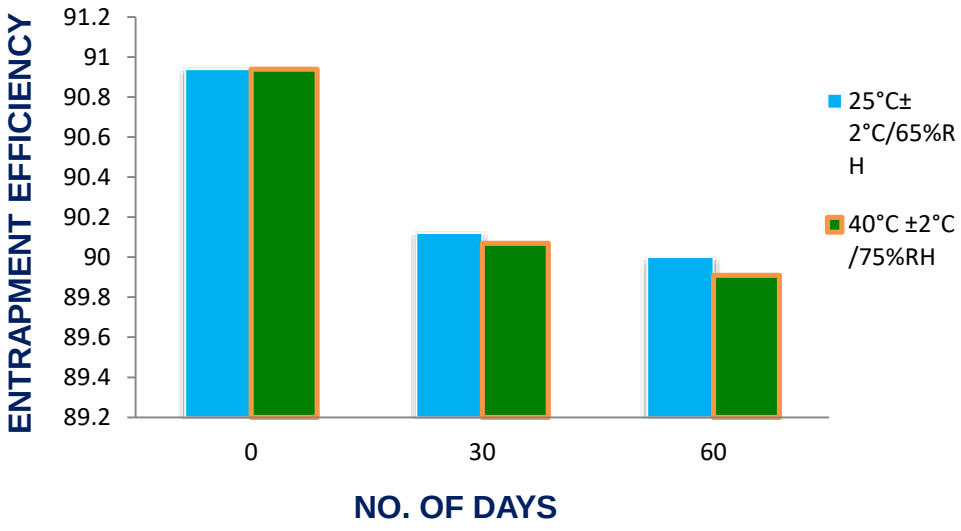
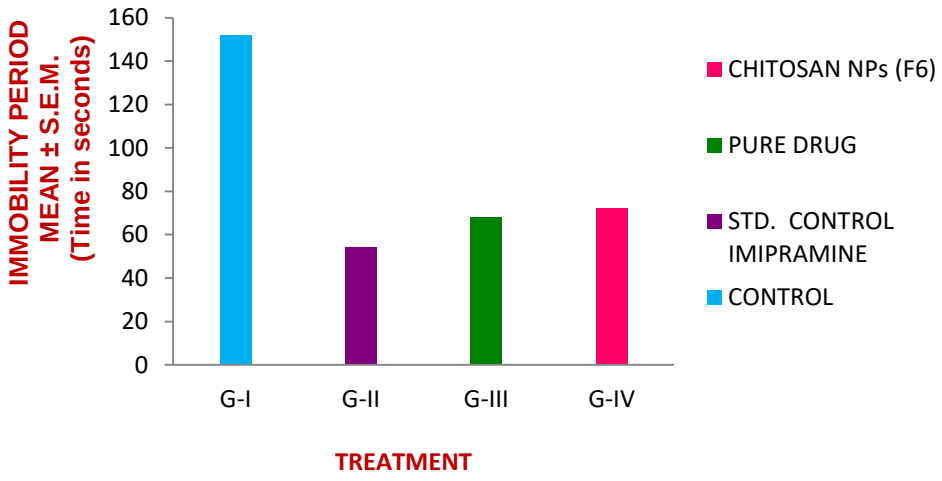


FIGURE: 28 INVIVO STUDIES OF ANTI-DEPRESSANT ACTIVITY ACTIVITY OF NANOPARTICLE FORMULATION



CHAPTER XI

SUMMARY AND CONCLUSION

CHAPTER-XI

SUMMARY AND CONCLUSION

- The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also to achieve and maintain the desired drug concentration.
- The λ max of Dapoxetine Hcl as found to be 292 nm in 0.01N Hydrochloric acid and 292nm in Phosphate buffer pH6.8 in 10µg/ml solution.
- Dapoxetine Hcl is a highly potent SSRI was used as a model drug to develop a controlled release formulation. Dapoxetine Hcl designed to prolong the release increase the drug bioavailability, diminish the side effects of irritating drugs and also reducing the frequency of administration, thereby improving the patient compliance and therapeutic efficacy.
- The Dapoxetine Hcl obeys the Beers law within the concentration of 5 to 50 µg/ml.
- Dapoxetine Hcl is having low solubility and short duration elimination half life (1-1.6 hour), the drug displays extensive first pass metabolism.
- The purpose of this study was to prepare Dapoxetine Hcl nanoparticles for control release of Dapoxetine Hcl to improve the oral bioavailability, enhance the solubility and dissolution rate by decreasing particle size of drug.
- Infrared spectroscopic studies confirmed that there was no interaction between drug and polymers.
- The controlled release Dapoxetine Hcl nanoparticles were prepared by Solvent evaporation by using Ethyl cellulose, Chitosan & HPMC K100 M and hyper-cross linked method using Betacyclodextrin and Diphenyl carbonate polymers of 1:1, 1:2, 1:3, 1:4 ratios.

- The production yield of the formulated controlled release nanoparticles (F1 to F16) in the range of 76.11% to 83.58 %.
- The drug content of the formulated controlled release nanoparticles (F1 to F16) in the range of 82.56 % to 98.20 %.
- The Theoretical loading of the formulated controlled release nanoparticles (F1- F16) in the range of 24.43 % to 64.24%.
- The entrapment efficiency increased with increasing the concentration of polymers and the formulations containing chitosan nanoparticles F6 (1:2) showed better entrapment (90.94%) among all formulation.
- The solubility of selected formulation (F6) in 0.2M Phosphate buffer pH6.8 increased when compared to pure drug.
- Particle size distribution was determined by Malvern zeta size, the size range for produced nanoparticles in the range of 200nm to 400 nm.
- The polydispersity index of selected nanoparticle formulation (F6) was 0.812 which indicated a narrow range and a homogeneous size distribution of particles.
- Zeta potential value of Dapoxetine Hcl nanoparticles showed a positive surface charge (+38.1 mV) this is because of more anion on the surface of the particles higher the charge higher is the stability of the nanoparticle.
- The *invitro* dissolution study was carried out in 0.01N Hydrochloric acid for 2 hours and phosphate buffer pH 6.8 for 10 hours. The formulations shows controlled release of drug upto 12 hrs and all formulations showed more than75% of drug release.
- The release kinetics showed that the formulations were complies with Zero order kinetics followed by diffusion controlled mechanism and Korsemeyar peppas n values were more than 0.4 indicating Nonfickian diffusion.

- The best formulation F6 was selected based on production yield, entrapment efficiency, solubilization efficiency, particle size, polydispersity index & zeta potential and *in-vitro* drug release and release kinetics.

- The best formulation F6 was evaluated by infrared spectroscopy, particle size, polydispersity index & zeta potential, Scanning Electron microscopy, X-ray powder diffraction method (X-PRD) and DSC.

- Best formulation of nanoparticles shown the extent of drug release was found to be F6 (96.93 %) in 12hrs.

- SEM studies confirmed the morphology of the nanoparticle formulation.

- The crystalline state of the nanoparticle formulation was not altered according to the XRPD analysis.

- The stability studies confirmed that the developed Dapoxetine Hcl nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various temperature and humidity conditions over a period of 2 month. One formulation were selected for *invivo* studies on mice. The administration of Dapoxetine Hcl loaded nanoparticle significantly inhibit the serotonin reuptake in the synaptic cleft similar trend but the extent of decrease is greater compared to standard drug. The sudden decrease of blood pressure after administration of Dapoxetine Hcl (standard) was due to burst release of drug from nanoparticle which was observed during invitro drug release studies. The anti-depressive activity of Dapoxetine Hcl loaded nanoparticles is lesser than control. Hence, Chitosan NP could be able to maintain the serotonin level over the period of observation(12h).The results

can be attributed to the efficacy of formulated Dapoxetine HCL nanoparticles in treating hypertension as well as its role in maintaintaing the therapeutic levels until extended period of time.

- The Dapoxetine Hcl nanoparticle & selected best formulation (F6) was formulated into capsules using suitable diluents (MCC) by hand filling method.
- The formulated capsules were analyzed for pre compression and post compression parameter drug content, *invitro* drug release studies.
- The pre compression parameters of all the formulations were within the required limit was indicated good flow property, suitable for formulation of the capsules.
- The post compression parameters such as general appearance, weight variation, uniformity of content and *invitro* studies of all formulations were within the acceptable limits.
- FTIR studies of selected best formulation shows that no interaction between the drug and excipients.
- Dapoxetine Hcl chitosan nanoparticle capsules showed increased drug release profile when compared with Dapoxetine Hcl capsules.

CONCLUSION

Hence, it was concluded that nanoparticle a good approach to release the drug in a controlled manner to the targeted site and enhance the solubility and dissolution property of Dapoxetine Hcl by solvent evaporation method for the successful incorporation of Dapoxetine Hcl with high entrapment efficiency. The solubility studies suggested that the nanoparticle formulations enhanced the bioavailability of Dapoxetine Hcl by improving its solubility and dissolution rate when compared to pure drug. Furthermore, it could be presumed that if the nanometer range of particles were obtained, the bioavailability might be increased. Thus the nanoparticles as controlled release formulations can be useful for delivery of short elimination half life, low bioavailability through orally. Thus nanoparticle drug delivery system provides site specific drug delivery and prolongs dosage interval and thus improving patient compliance. The *invivo* release studies results indicated the efficacy of formulated Dapoxetine Hcl nanoparticles in treating depression as well as its role in maintaining the therapeutic levels until extended period of time.

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