DEVELOPMENT AND CHARACTERIZATION OF AN ANTIMICROTUBULAR TAXANE LOADED MPEG-b-PCL NANOPARTICLES

Dissertation Submitted in partial fulfillment of the requirement for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI.

DEPARTMENT OF PHARMACEUTICS

K.M.COLLEGE OF PHARMACY

UTHANGUDI, MADURAI – 625107

APRIL - 2012

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND CHARACTERIZATION OF AN ANTIMICROTUBULAR TAXANE LOADED MPEG-b-PCL NANOPARTICLES"** submitted by **Mr. RAJASEKHARREDDY V** to The Tamilnadu Dr.M.G.R.Medical University, Chennai, in the partial fulfilment for the award of Master of Pharmacy in Pharmaceutics at K.M. College of Pharmacy, Madurai, is a bonafide work carried out by him under my guidance and supervision during the academic year 2011-2012.

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ACKNOWLEDGEMENT

It affords me immense pleasure to acknowledge with gratitude the help and guidance rendered to me by a host of people, to whom I owe a substantial measure for the successful complements of this project.

I owe my great debt of gratitude and heartful thanks to ***Prof. M.Nagarajan, M. Pharm, M.B.A, DMS (IM) DMS (BM)*** *Corrospondent, K. M. College of Pharmacy, Madurai for providing me with all the facilities and encourangement for the successful completion of my thesis work.*

I express my deep sense thanks to ***Dr.S.Jayaprakash, Ph.D,*** *Principal and H.O.D., Dept. of pharmaceutics for their invaluable advice, suggestion and encourangement extended throughout the work.*

I express my wholehearted gratitude to my esteemed teacher and guide, ***Mr. S.Mohamed Halith, M.Pharm, (Ph.D)*** *Asst.Professor Dept. of Pharmaceutics, for his zealous guidance, indefatigable support and constant encourangement for the entire period of m thesis work.*

I prevail my sense of gradtitude to, ***Mr. K.K. Pillai, Mrs. A. Abirami, Mrs. S. Chitra karthikeyini,*** *Asst.Professors in pharmaceutics, for their invaluable advice, suggestion and encourangement extended throughout the work.*

My sincere thanks goes to ***Prof.M.S.Prakash,*** *M.Pharm, HOD of pharmaceutical Analysis and* ***Mr.M.Boopathi,*** *Asst.Professor of Pharmaceutical Analysis, for their valuable help and support during the analytical part of my work.*

A special word of thanks to ***all the professors and Assistant Professors*** *of all departments for their kind holly hortatory constant encouragement and expertise during this course.*

I extend my thanks to ***M.Shanthi,B.A, M.Li.Sc,*** *Librarian,* ***Mrs.Ayyamal*** *Lab Assistant and all other non teaching staff members of our college for their co-operation.*

I cannot forget to express my gratitude to all my classmates with grateful thanks my friends and all my juniors who helped me directly and indirectly for the successful completion of my project work.

I also take a chance to thank my ***Father*** *for the support rendered throughout my project work.*

My parents and brothers deserve special mention for their inseparable support and prayers. I am greatly indebted to them for their love, encouragement and inspiration which have been inexhaustible source of support and strength.

Rajasekharreddy V

Dedicated to My Dad

CONTENTS

ABBREVATIONS

AIDS	Acquired Immune deficiency syndrome
AUC	Area under curve
CDR	Cumulative drug release
^{0}C	Degree Celsius
%EE	Percentage Encapsulation Efficiency
FTIR	Fourier Transform Infrared
gm	Gram
hr	Hour
IR	Infrared Spectroscopy
mg	Milligram
µg/ml	Microgram per Milliliter
MPEG	Methoxy Poly (Ethylene glycol)
MPS	Mononuclear phagocytic system
NDDS	Novel Drug Delivery Systems
PBS	Phosphate Buffer Saline
PCL	Poly (ε-caprolactone)
PDI	Poly dispersive index
PTX	Paclitaxel
RPM	Rotation per minute
RES	Reticulo Endothelial system
WHO	World Health Organisation

1. INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site of action in the body, to achieve promptly and then maintain the desired drug concentration.

Conventional drug delivery system achieves as well as maintains the drug concentration within the therapeutically effective range needed for treatment only when taken several times a day. These results in a significant fluctuation in drug level, dose related side effects and poor patient compliance [1]. These problems were totally alleviated due to the advent of Novel drug delivery systems (NDDS) in the domain of drug delivery.

Novel drug delivery system has two important prerequisites one was controlled drug delivery and another was the targeted drug delivery, it means that delivering the drug at a rate directed by the needs of the body, over the period of treatment and exactly nearer to the site of action.

Controlled & Novel delivery envisages optimized drug in the sense that the therapeutic efficacy of a drug is optimized, which also implies nil or minimum side effects. It is expected that the 21st century would witness great changes in the area of drug delivery. The products may be more potent as well as safer.

Target specific dosage delivery is likely to overcome much of the criticism of conventional dosage forms. The cumulative outcome could be summarized as optimized drug delivery that encompasses greater potency & greater effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance [2].

1.1 Targeted drug delivery system [3, 4]

Drug targeting can be defined as the ability to target a therapeutic agent specifically to desired site of action with little or no interaction with non target tissue thus minimizes the side effects and increases the therapeutic index of drugs.

Paul Ehrlich described the targeting of the drugs to the desired site of action in the body as “magic bullet”.

The efforts to improve drug effectiveness in therapeutics have been assisted by parallel developments in molecular and cell biology. Such developments supported the successful generation of target oriented drug delivery systems.

Targeted drug delivery systems were acceptable under following conditions,

i. Drugs with low specificity and low therapeutic index.
ii. Stability of the drug is poor.
iii. Drugs which were having short biological half life and also large volume of distribution.

1.1.1 Levels of drug targeting:

The various levels of targeting the drug to the site of action were as follows:

1. Passive targeting
2. Inverse targeting
3. Active targeting
4. Dual targeting
5. Double targeting and
6. Combination targeting.

Passive targeting

Drug delivery Systems that were targeting the systemic circulation were generally known as “passive” delivery systems. In this type of targeting, drug delivery system were taken up by Reticuloendothelial cells occurs in the physiological fluids and then accumulate in the spleen and liver and they become the targeting agents for the delivery to those compartments.

Inverse targeting

Inverse targeting was based on principle to avoid the passive uptake of particulate carriers by RES. This was the reversion of passive targeting and hence the process was known as inverse targeting. The strategies that were used to overcome the defence mechanism of RES or to achieve inverse targeting modification of the size, surface charge, composition and hydrophilicity of carriers were modified. And also to suppress the function of RES macromolecules like Dextran sulphate were used for RES blockade and leads to the impairment of host defence system.

Active targeting

The targeting of the drugs to the specific sites in the body by forming the drug-carrier complexes by the use of ligands to increase receptor mediated, specific delivery of drug (s) was known as active targeting.

Dual targeting

The dual targeting has offers the increased therapeutic efficacy by targeting drug carrier molecules, which have same beneficial therapeutic effects. These types of drug conjugates have synergistic effect in drug delivery.

Double targeting

Double targeting was a type of targeting for drug delivery systems with the combination made between spatial control and temporal control of drug delivery.

Combination targeting

Combination targeting was used for the site-specific delivery of peptide based drugs. These systems were equipped with carriers and polymers for a specific approach of targeting the drug to the site of actions.

1.1.2 Limitations of Targeted drug delivery:

The problems associated with drug targeting strategies were as follows

- Clearance of targeting systems was rapid for especially carriers of antibody targeting.
- Immune reactions against carrier systems.
- Tissue heterogenecity of targeting system was reported.
- Localizations of drug targeted systems into tumor cells were less.
- Diffusion and distribution of released drug leads to no-specific accumulation of drug in other than target site.

1.1.3 Carriers used in targeted drug delivery system:

Carriers were required for the transportation of the loaded active moieties to the desired site of action or nearer to the target.

An ideal carrier engineered as targetable device should have following features:

- They should be able to cross membrane barriers in the body.
- They must be recognized and selectively uptake by the target cells.
- The drug and carrier should be stable in physiological fluids.
- They should be non-antigenic and biodegradable after uptake and release of the active moiety.

Several carriers were used for targeted delivery were classified based on the nature of their origin as follows

- Endogenous (low & high density lipoproteins)
- Exogenous (Biodegradable polymeric drug carriers)

Table 1: Carrier systems used for Targeted Drug Delivery

S.No	Type of carrier	Example
1	Colloidal Carriers	a) Vesicular systems Niosomes, Liposomes. b) Microparticulate systems Microparticles, Nanoparticles, Magnetic Microspheres, Nanocapsules.
2	Cellular Carriers	Resealed erythrocytes, antibodies
3	Micellar delivery systems	Micelles, mixed micelles and polymeric micelles
5	Macro molecular carriers	a) Proteins, glycoproteins, artificial viral envelopes. b) Glycosylated polymers. c) Mabs, antibody enzyme complex. d) Toxins, conjugates. e) Lectins and polysaccharides.

1.2 NANOPARTICLE BASED TARGETED DRUG DELIVERY [5]

Nanoparticles are one of the novel colloidal drug delivery systems have the properties of controlled drug delivery as well as site specific delivery. Nanoparticles are the sub-nanosized colloidal carriers whose particle size ranges from 10-1000nm. These are made of non-biodegradable and biodegradable polymers.

The polymeric nanoparticles were able to carry the active moieties by entrapping in their polymer matrices as particulates or adsorbed on to the surface of particles by physical or chemically adsorption. The drugs may be added during preparation of nanoparticles or to the previously prepared nanoparticles.

Advantages of polymeric nanoparticles

- They release the drug at a controlled rate and targeted drug release.
- They have the ability in drug utilization by decreasing the initial drug loading.
- They will provide better convenience and improved patient compliance.
- They decrease the toxicity and occurrence of adverse reactions associated with conventional drug delivery systems.
- The polymeric nanoparticles can be freeze-dried and obtained as dry powder form.
- They were nontoxic, biodegradable, biocompatible and relatively stable.

Types of nanoparticles [6]

There were two types of nanoparticles

- Nanospheres
- Nanocapsules and
- Nanomicelles

Nanospheres : These were solid core Spherical particulates which are nanometre in size range in which drug is embedded within the matrix or adsorbed on to the surface.

Nanocapsules : These were the vesicular systems in which drug is essentially encapsulated within the central volume surrounded by an embryonic continuous polymeric stealth.

Nanomicelles: These were the micellar delivery systems consists of inner hydrophobic core with entrapped drug and outer hydrophilic shell made from amphiphilic block copolymers with size range of 10 to 200 nm.

Types of polymers used for Preparation of Polymeric Nanoparticles[7]

Polymers used for preparation of nanoparticles were of two types

1. **Natural hydrophilic polymers**

These were amphiphilic macromolecules obtained from natural sources like Proteins (Albumin, Gelatin) and polysaccharides (Alginates or Agarose).

Disadvantage with natural polymers were antigenicity and conditional biodegradability

2. Synthetic hydrophobic polymers

These hydrophobic polymers were synthesized chemically. These polymers were investigated for their biomedical applications and consequently their safety and biodegradation patterns.

These polymers were either pre-polymerized or synthesized during nanoparticle preparation.

1. Pre-polymerized polymers were Poly (ε-caprolactone) , Poly (lactic acid) and Poly (lactide-co-glycolide)
2. Polymers that were prepared during preparation of nanoparticles were Poly (butylcyanoacrylates), Poly hexylcyanoacrylate andPoly methyl (methacrylate)

1.3 PREPARATION OF NANOPARTICLES [8]**:**

Nanoparticles were prepared by many methods. The choice of the method of preparation depends on the solubility characteristics of the incorporating drugs and type of the raw materials that were going to be used in the Nanoparticle formulations.

Nanoparticles were mainly prepared by the two types of processes.

They were

1. Dispersion of the preformed polymers along with active moiety.
2. Polymerization of monomer.

For dispersion of the preformed polymers, several methods have been used to prepare polymeric nanoparticles such as

- Emulsion Solvent evaporation method.
- Nanoprecipitation method
- Dialysis method and
- Supercritical fluid spraying method

Emulsion Solvent evaporation method:

Single Emulsion evaporation method was the most commonly used method to load the hydrophobic drugs into polymeric nanoparticles. In this method, an organic mixture of polymer and drug was emulsified in an aqueous solution with surfactant or stabilizer to make an oil-in-water emulsion. The nanosized emulsion droplets were usually induced by homogenization. After that, organic solvent used in the preparation was evaporated by rotary vaccum evaporator and nanoparticles were collected by centrifugation and filtration methods.

Double emulsion solvent evaporation method was a multiple emulsion method or modified method of single emulsion method which encapsulates the water soluble drugs.

In this method an aqueous solution of the water soluble compound was first emulsified into an organic solution of polymer to form primary water-in-oil (w/o) emulsion. And this primary w/o emulsion was then poured into a large volume of water with stabilizer to form multiple emulsions.

Nanoprecipitation method:

Nanoprecipitation was also known as interfacial deposition method or emulsification diffusion method.

In this method drug and polymer were dissolved into water-miscible solvent such as acetone. This organ solution was added drop wise to the aqueous phase having a stabilizer or surfactant which sterically stabilised the formed nanoparticles. In the nanoprecipitation method, an interfacial turbulence will be created from the spontaneous diffusion of water-soluble solvent to water, leading to the formation of nanoparticles.

Dialysis method:

Dialysis method was used for preparing stabilizer free nanoparticulate system. A stabilizer free nanoparticulate system was not only has the advantages of ease of preparation but also preventing the possible side effects of emulsifier.

In this method drug and polymer were first dissolved in water-miscible solvent such as DMF or THF. Then solution was transferred into a dialysis membrane with certain molecular weight cut off and dialysis was performed against large volume of distilled water for about two days to remove free drug and organic solvent. The formed nanoparticles were separated by centrifugation or filtration methods.

Supercritical fluid spraying method:

Supercritical fluid spraying method was a technique used for preparation of the nanoparticles without the use of any toxic organic solvent and stabilizer.

In this method the drug and polymer were solubilised in a supercritical fluid and then the solution was expanded through a nozzle. The supercritical fluid will undergo evaporation during spraying process and the drug solute will eventually precipitate into smaller nanoparticles.

Polymerization method

In this method the nanoparticles were prepared from monomers by poly-condensation reactions. Polymerization includes emulsion polymerization and interfacial polymerization.

Emulsion polymerization was developed into a chain of polymers from single monomers which was initiated by radical formation. The residual monomers were removed by filtration. The drug was incorporated on to the surface of nanoparticles by adsorption.

For interfacial polymerization, monomer contained aqueous phase and organic phase were brought together by mechanical force. Drug was dissolved in the polymerization medium either before the addition of monomer or at the end of the polymerization reaction and finally, nanoparticles was recovered by ultracentrifugation method.

1.4 EVALUATION OF NANOPARTICLES:

The nanoparticles were evaluated for various parameters they were:

1. Percentage yield
2. Drug loading
3. Entrapment efficiency
4. Size and morphology
5. In vitro drug release studies
6. Stability testing

Percentage Yield:

Percentage yield is of great importance in industrial perspective, when costly raw materials chosen for preparation of nanoparticles. It plays a crucial role in determining whether the preparation procedure chosen for incorporating a active compound into the polymeric particles was efficient. The overall yield of a procedure is simply expressed as

$$\% \text{ Yield} = \frac{\text{The total amount of raw materials added}}{\text{Amount of nanoparticles}} \times 100$$

The initial amount of raw materials corresponds to the amount of active compound, polymer and certain amounts of stabilizing agents or surfactants being adsorbed during the preparation.

Drug loading:

The drug loading or loading content was expressed as

$$\text{Drug loading} = \frac{\text{Amount of the drug in the nanoparticles}}{\text{Amount of the drug added to the formulation}}$$

% Entrapment efficiency:

Drug entrapment efficiency represents the amount of the drug which has been incorporated into the polymer matrix or absorbed onto the particle surface.

$$\%\text{Entrapment Efficiency} = \frac{\text{Amount of the drug entrapped}}{\text{Amount of the drug added}} \times 100$$

Drug loading was done after separation of free drug from bounded drug in nanoparticles. drug loading can be determined after ultracentrifugation of a nanoparticulate suspension by dissolving the formed sediment with an appropriate solvent to extract the entrapped drug from polymer matrices and further subsequent analysis was carried out by using methods such as spectrophotometry, spectrofluorophotometry, high performance liquid chromatography to determine the amount of drug loaded in the nanoparticles.

Size and Morphology:

Scanning Electron Microscopy and Photon Correlation Spectroscopy and were used to study the particle size and surface characteristics of nanoparticles.

Scanning Electron Microscopy was widely used in the field of nanotechnology to study the surface properties such as type of structure and smoothness of prepared nanoparticles. It has high resolution and relatively easy sample preparation.

Photon Correlation Spectroscopy (PCS) was a laser light scattering method useful to the measure the particles ranging from 5nm to 5μm. The average mean sizes of particles were determined by this method.

Invitro release studies:

In vitro release studies should be in principle was useful for the prediction of in vivo kinetics. Unfortunately, due to the very small size of the particles, the release rate observed in vivo can differ greatly from the release obtained in a buffer solution.

Depending on the type of polymer, drug release from nanoparticles can take place through following two processes:

- The drug may diffuse out of the polymer through solid matrix: to allow complete release from the polymer.
- The solvent may penetrate into the interstices of nanoparticles and dissolve the drug, which then diffuses into the surrounding medium.

In vitro release study of entrapped drug in nanoparticles can be studied by several experimental methods. Such as

1. Dialysis bag diffusion
2. In situ method
3. Sample and separation techniques
4. Ultra filtration at low pressure.

Stability of drug nanoparticles [9]:

Stability is one of the important concerns in ensuring efficacy and safety of the parentrally administered nanosuspensions.

The stability problems would arise during preparation, shipping and storage. For example because of the high pressure and temperature during preparation can cause Crystallinity change in drug particles and during the storage and shipping of the products stability problems like crystal growth, agglomeration and sedimentation were seen.

Therefore, stability problems with drug loaded nanoparticles have drawn a significant attention during the nanosuspension preparation.

The stability issues related to the nanosuspensions were divided into physical and chemical stability.

Physical stability:

The common physical stability problems related to nanoparticles were

- Sedimentation/ creaming
- Agglomeration and
- Crystal growth

Sedimentation or creaming issues were generally reduced by decreasing the particles size during the suspension preparation.

Agglomeration was seen in the thermodynamically unstable suspension because of large surface area creates larger surface energy; according to that particles tend to agglomeration to minimize the surface energy. The most common strategy to overcome the agglomeration was adding stabilisers to the suspensions. The selection of stabilizers was based on their ability to provide the wetting properties to the surfaces of particles to prevent particles agglomeration.

Crystal growths in nanosuspensions were raised due to the changes in the particle size and size distribution. A narrow size distribution with uniform particle size and also added stabilisers can alleviate effects of crystal growth in colloidal suspensions.

Chemical stability

Chemical stability in nanosuspensions was mainly due to the molecular interactions between the interactions between the drugs, polymer and other excipients in the formulation. Because of their incompatibility degradation impurities were liberated and were effectively analysed by HPLC. These incompatibilities were eliminated by performing preformulation studies during the formulation development.

1.5 PHARMACEUTICAL ASPECTS OF NANOPARTICLES:

Nanoparticles that were prepared from different methods were should be free from potential toxic impurities, easier to handle, storage and administration and also they should be sterilised if intravenous administration was advocated.

The following were three important process parameters that were performed after the fabrication of nanoparticles by different processing methods.

They were
a. Purification.
b. Freeze drying.
c. Sterilisation.

1.5.1 PURIFICATION OF NANOPARTICLES:

Nanoparticles prepared by different methods, might have yield toxic impurities in the Nanoparticle formulations which were traces of organic solvents, residual monomers, polymerisation catalysts, stabilizers, electrolytes and polymer aggregates.

The most commonly used methods to purify the nanoparticulate preparation were

a. Ultra-centrifugation method.
b. Dialysis membrane method and
c. Gel filtration method.

Ultra-centrifugation method:

In this method the prepared nanoparticle suspension was taken into a centrifuge tube and subjected to high centrifugation to sediment the particles at the bottom of tube. By this method the residual monomers and excess amounts of stabilizers and electrolytes were removed by separating out the supernatant. This method suffers with the problems of more time consuming and particle aggregation.

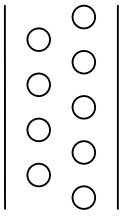
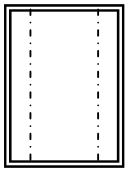
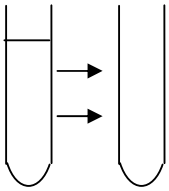
Dialysis membrane method:

By this method low molecular weight impurities and stabilizers were removed by dialysing with dialysis fluids. This method cannot separate high molecular weight impurities.

Gel filtration method:

Gel filtration method was an efficient method in removing the low molecular weight impurities, residual monomers and polymerisation catalysts but suffers the separation of the large polymer aggregates from Nanoparticle suspensions.

Table.2 Various techniques used for purification of Nanoparticles:

S. No	Purification methods	Schematic principle	Remarks
1	Gel filtration		High molecular weight substances and impurities are difficult to remove.
2	Dialysis		High molecular weight impurities are difficult to remove; time consuming process; scaling up is difficult.
3	Ultra-centrifugation		Aggregation of particles; Time consuming process; Scaling up difficult

1.5.2 LYOPHILISATION OF NANOPARTICLES [10,11]:

Lyophilisation or freeze drying is a dehydration technique, in which dehydration takes place while the product is in a frozen state and under a controlled vaccum condition.

Lyophilisation involves the freezing of the Nanoparticle solution and subsequent sublimation of its bound and unbound water content under controlled vaccum to get a free flowing powder material.

The following were the advantages of lyophilisation of Nanoparticles

- Freeze drying of nanoparticles prevents the solubilisation and degradation of polymer in the solvents used for formulation.
- It prevents the drug leakage, desorption and degradation by improving the drug association with nanoparticles and also by removing the unbound and bound water in the Nanoparticle suspensions.
- Lyophilised nanoparticles were easy to handle and store and helps in the long term preservation of nanoparticles.
- It preserves the physicochemical properties of prepared nanoparticles like elegant freeze dried cake and shorter reconstitution time and unchanged encapsulation efficiency.
- Freeze drying has an accepted method for processing and stabilising heat sensitive products like niosomes, nanomicelles and nanoemulsions throughout the storage conditions.

Lyoprotectants or Cryopreservatives :

Lyoprotectants were used to protect the formulation from stress that was generated during freezing and dehydration stages of Lyophilisation.

Some of the lyoprotectants that were used for lyophilisation were as follows

- Mannitol
- Sucrose
- Poly(vinyl alcohol)
- Poly(vinyl pyrrolidone)

Lyophilisation cycle can be divided into three steps

- Freezing.
- Primary drying and
- Secondary drying

1. **Freezing step:**

Freezing step was a solidification stage in the lyophilisation of the liquid suspension; during this stage the water which was in unbound form was freezes into ice crystals. This results in the increased concentration of suspension. The concentrated suspension gets solidified on further increasing the freezing duration and yields a crystalline, amorphous or combined amorphous-crystalline form. The small percentage of water that remained in the liquid state and did not freeze was called bound water.

2. Primary Drying:

During this stage, ice crystals that were formed in freezing step were sublimated under controlled vaccum and temperature conditions. At the end of sublimation step Porous plugs were formed on the surface of lyophilised cake it represents the spaces that were occupied by ice crystals.

3. **Secondary Drying:**

Secondary drying removes the bound water from the product which was not sublimated during primary drying.

1.5.3 STERILISATION OF NANOPARTICLES:

Nanoparticles which were intended for IV administration should be sterilised thoroughly to remove pyrogens which reported to cause the increased levels of endotoxins.

The different methods that were used to sterilize the Nanoparticle formulations were

a. Membrane filters.
b. Irradiation technique and
c. Autoclave methods

Membrane filtration:

The nanoparticle suspensions which were having size less than 0.2µm were effectively sterilised through the 0.22µm membrane filters than the particles with a size range of 0.25-1.0µm were did not filtered through the membrane.

Irradiation technique:

In this method the Nanoparticle formulations were subjected to Gamma radiations. Heat sensitive formulations were effectively sterilised by this method.

Autoclave method:

It is a type of aseptic method which completely eliminates the effects of endotoxins but was prone to show an impact on the physicochemical properties of nanoparticles like particle size, stability and release characteristics.

1.6 AN OVERVIEW OF CANCER CHEMOTHERPHY [12]

Cancer was a group of neoplastic diseases that occur in human of all age groups and races as well as in all animal species. The specific types of cancer are related to multiple factors include race, age, sex, genetic predisposition and exposure to the carcinogen.

Cancer is a disease of uncontrolled cell division and metastatic. It was generally considered to be due to the clonal expansion of a singe neoplastic cell.

Cancer tumors were of two types

1. Benign tumors, these were non metastatic tumors with limited clonal expansion.
2. Malignant tumors, these were metastatic in nature with uncontrolled cell division and harmful effects on physiological processes.

Mode of action of chemotherapeutic agents [13]

Most of antineoplastic agents affect cell cycle process during the mitosis by acting specifically on processes such as function of mitotic spindle and DNA synthesis.

Antitumor drugs were better in killing the cancers cells during DNA synthesis and active division stage itself because at that stage a tumor was young and also most of its cells were making DNA.

The major problems in cancer chemotheraphy were the toxic drugs effects on normal cells and the rapid clearance of the drug from the tumor cells [14]. Rapidly dividing normal cell like bone marrow cells involved in the immune defence system were also destroyed by the present day chemotheraphy. Hair loss, increased susceptibility to infections and Nausea were adverse effects associated with anticancer drugs [15].

1.7 NANOTECHNOLOGY IN ANTICANCER DRUG DELIVERY [16]:

Nanotechnology based therapeutics in the cancer chemotherapy have the advantages of the increased therapeutic drug efficacy, reduced adverse effects or toxicity to normal healthy cells and tissues, and improved patient compliance.

It plays an important role in satisfying the delivery of anticancer drugs particularly to the site of action with a minimum effective concentration over a period of time.

Nanosize based drug carriers like polymeric nanomicelles , polymer-drug conjugates, solid lipid nanoparticles and polymeric nanoparticles have numerous benefits over the conventional dosage forms like injections and oral solids [17].

A successful formulation for the delivery of chemotherapeutic agents should have the following features

- It should act as a better therapeutic drug carrier for targeted delivery.
- They should have longer circulation time.
- Smaller size to escape from the scavenging effect of phagocytes.
- They should avoid the opsonisation.
- They should enhance tumor uptake of drug.
- And promote endocytosis of drug loaded carrier.

The above features were hold good by delivering the anticancer drugs through the polymeric micellar nanotechnology, because they were longer time circulating nanoparticles with stealth properties [18].

The primary function of micellar drug delivery was to improve the solubility and stability of hydrophobic drugs. This was achieved by entrapping the drug in the hydrophobic core of polymeric micelle. And the long circulation time, reduced plasma protein binding, uptake by phagocytes and RES cells were removed because of the presence of hydrophilic shell on the outer surface of nanomicelles [19].

The most commonly used nanomicelles in the cancer drug delivery were derived from the amphiphilic block copolymers like MPEG-b-PCL copolymer, MPEG-b-PLA copolymer, MPEG-b-PLGA copolymer and MPEG-b-PEGMA copolymer. These polymeric micelles have capacity to encapsulate the various anticancer drugs such as Paclitaxel , Docetaxel and Doxorubicin.

So, the polymeric micellar nanotechnology has a very good capability of loading and targeting the anticancer drugs in the treatment of various cancer conditions in the human body.

2. LITERATURE REVIEW

Kevin Letchford *et al.,* (2009) formulated the Paclitaxel loaded nanomicelles with two types of MPEG-b-PCL diblock copolymers, differs in the PCL block lengths of either 19 or 104 and evaluated the encapsulation efficiency and in vitro release behaviour of these two types of Paclitaxel loaded polymeric micelles. The results were showed that the polymeric micelles having PCL block length of 104 units have superior encapsulation and release behaviour than the PCL block with 19 units. Hence, they concluded that amphiphilic diblock copolymers with PCL block length of 104 units were suitable for the preparation of Paclitaxel nanomicelles [20].

T. Musumeci *et al.,* (2006) developed and characterised Paclitaxel loaded poly(lactide) PLA and poly (lactide-co-glycolide) PLGA nanospheres by solvent displacement method and lyophilised by freeze drying method to increase the stability of nanoparticle formulation for longer periods of time to ensure the good physico-chemical properties upon reconstitutions and also reported the effects of various lyoprotectants (mono- and disaccharides and poly alcohols) at various concentrations on nanoparticles particle size and surface charge characteristics [21].

Hongliang Xin *et al.,* (2010) prepared Paclitaxel nanomicelles by o/w emulsion and evaporation technique and evaluated drug loading coefficient (DL%) and encapsulation efficiency (ER%) with the different amounts of Paclitaxel, which were co-dissolved with polymeric micelle in the processes of polymeric micellar nanoparticles and reported that with increase in PTX feeding, the encapsulation efficiency decreased, but the drug-loading coefficient increased firstly and then decreased sharply because of the increased hydrophobic interactions between PTX molecules and copolymers as the amount of PTX increased [22].

Xiaowei Dong *et al.,* (2009) developed a Cremophore free lipid based Paclitaxel nanoparticles by completely eliminating the mixture of Cremophore EL (polyethoxylated castor oil) and dehydrated alcohol which were in the conventional Taxol formulation, reported to cause serious side effects such as hypersensitivity reactions after IV in fusion and studied in vitro cytotoxicity in human breast cancer MDA-MB-231 cells in comparison with Taxol. The lipid based nanoparticles have similar anticancer activity to Taxol [23].

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Xintao Shuai *et al.*, (2004) developed the polymeric micellar nanoparticles for Doxorubicin delivery with different compositions of diblock copolymers of MPEG-b-PCL and evaluated the effects of increasing PCL block length in the copolymer composition on micellar size and drug loading content (DL %). The results revealed that increase in the PCL block length increases micellar size on increasing PCL length and DL% was significantly less than anticipated. They concluded that compromised molecular interactions and PCL crystallinity would be the reason for decreased drug loading content [50].

Weihui Xie *et al.*, (2007) synthesized the amphiphilic MPEG-PCL diblock copolymers by using catalyst yttrium oxide Y $(DBMP)_3$ in the presence of PCL , MPEG has a macro initiator by ring opening polymerisation method and controlled the molecular weights of copolymers by adjusting MPEG and PCL molar ratios. They characterised the diblock structure of the copolymers by ^1H NMR spectroscopy and also investigated the thermal and crystallisation properties by DSC and POM (polarised optical microscopy). The results were indicated that diblock copolymers were linear in structure and crystallization properties were depended on length of PCL block lengths as per DSC and POM reports [51].

Peng Wei Dong *et al.*, (2010) encapsulated the Honokoil, an anticancer drug into the star shaped PCL-b-PEG copolymeric nanomicelles by sef assembly without any organic solvent by using ultrasonication energy and characterised the obtained nanomicelles for % drug loading, EE and in vitro drug release in comparison with free Honokoil. The results were found that the % drug loading and EE were higher because of the increased solubility of lipophilic drug in hydrophobic core of PCL copolymer. The drug release profiles showed that release was in a biphasic manner with initial burst in 8hrs and then slow and controlled release for 168 hrs when compared to free Honokoil which was released completely in initial burst phase for 8 hrs, it was clearly observed that the release rate of drug from micelles was slower than free Honokoil [52].

Sosnik A *et al.*, (2003) synthesised the MPEG-PCL oligomers by reacting the blends of MPEG and PCL in different ratios by ring opening polymerisation method and investigated the influence of PCL block length on crystallisable properties of oligomers by DSC analysis, because the crystallinity effects the rate of degradation of oligomers. The thermograms of DSC showed that oligomers comprising longer PCL blocks (>5) were only crystallized at the body temperatures and the oligimers with PCL blocks <5 were able to crystallized at low temperatures (4 and 25^0C) [53].

Donghui Zheng *et al.*, (2009) developed Docetaxel-loaded MPEG-b-PCL nanoparticles by nanoprecipitation method. The obtained nanoparticles were evaluated for encapsulation efficiency (%), in vitro drug release and its antitumor efficacy against malignant melanoma was evaluated both in vitro and in vivo. The results were indicated that the EE% was high because of Docetaxel lipophilicity and also drug was released in a continuous and sustained

manner throughout the release study. The in vitro and in vivo studies proved that cytotoxicity was achieved in a dose and time dependent manner with good in vitro in vivo correlation [54].

May P Xiong *et al.,* (2008) encapsulated a lipophilic Geldanamycin prodrug in MPEG-b-PCL micelles by emulsion evaporation method and evaluated the pharmacokinetic parameters such as release, AUC, volume of distribution and total clearance in comparison with the conventional free Geldanamycin prodrug formulation by in vivo anti tumor activity. They results showed that there was a 72 fold enhancement in the AUC, a 21fold decrease in volume of distribution, and a 11fold increase in clearance and also drug was released in a sustained manner. They suggested that formulating the Geldanamycin prodrug in micellar form is a effective way to overcome the problems associated with conventional delivery [55].

Sa Won Lee *et al.,* (2011) formulated the Docetaxel loaded MPEG-PDLLA nanomicelles and evaluated the morphological characteristics such as mean diameter, PDI and pharmacokinetic characteristics like in vivo cytotoxicity and hypersensitivity reactions in comparison with Docetaxel injection. The prepared micelles were in size range of 10-50nm with narrow size distribution. Pharmacokinetic study in mice and Beagle dogs have shown the reduced side effects of hypersensitivity reactions and retained the antitumoral activity in comparison with Docetaxel injection [56].

Maria Teresa Peracchia *et al.,* (1997) formulated the PEG-coated amphiphilic diblock and multiblock copolymeric nanoparticles by emulsification diffusion method and investigated the encapsulation efficacy and in vitro drug release characteristics. The results were indicated that %EE and drug release were dependent on chemical composition of hydrophobic core as well as the surface density and molecular weight of hydrophilic PEG shell of copolymers [57].

Lusianna Ostacolo *et al.,* (2010) formulated the Docetaxel loaded PEO-PCL nano micelles derived from copolymers of linear triblock and 4-arm star diblock copolymers and evaluated the encapsulation efficiency and drug release between two types of copolymers. The results were showed that the loading ability and release rate in buffered saline were similar for two types of copolymers [58].

Junko Matsumoto *et al.*, (1999) developed Progesterone loaded poly (L-lactide)-poly (ethylene glycol)-poly (L-lactide) nanoparticles by solvent evaporation method and evaluated drug entrapment and amount of drug release of nanoparticles. The drug entrapment efficiencies were around 70% and amount of drug released increased as the PEG content and molecular weight of copolymers increased. They concluded that change in the PEG content, and PEG Mw could able to control the drug release from the nanoparticles [59].

Luis A. Guzman *et al.*, (1996) formulated the nanoparticles using polylactic-polyglycolic acid (PLGA) copolymer by emulsification solvent evaporation technique. Fluorescently labeled nanoparticles were administered by intraluminal infusion, amount of the nanoparticle that reached that reached the target site and biocompatibility were evaluated. Dexamethasone was entrapped into the particles to evaluate the therapeutic potential in the prevention of neointimal formation. The results were indicated that the nanoparticles successfully penetrated into the vessel wall and stayed for up to 14 days after a single intraluminal infusion and also significantly decreased neointimal formation [60].

Rubianna M. Mainardes *et al.*, (2005) designed the Praziquantel loaded PLGA nanoparticles by emulsion solvent evaporation method and studied the effects of formulation variables on the particle size distribution of nanoparticles. The results showed that sonication time, homogenization speed and time, drug and polymer ratio, PVA % and ratio between organic and aqueous phases have the significant effects on the size distribution of nanoparticles[61].

Chang Gu Keum *et al.*, (2011) formulated the Docetaxel-loaded PLGA nanoparticles by Emulsion evaporation technique. They studied the effects of various process parameters such as surfactant concentration, sonication time and power, type of evaporation method and time, centrifugation speed and initial drug loading that influences the particle size, encapsulation and release kinetics of drug loaded nanoparticles and also specified the limits for that parameters to achieve better formulation characteristics [62].

Ganesh. G. N. K *et al.*, (2011) formulated the Docetaxel loaded liposomes by dried thin film method and evaluated the in vitro drug release kinetics of the encapsulated drug by fitting the dissolution data to the various kinetic models like zero, first order and Korsmeyer- Peppas equation. The results were indicated that drug release follows first order kinetics and case II transport mechanism of drug release when applied to Korsmeyer-Peppas model [63].

2. LITERATURE REVIEW

Kevin Letchford *et al.,* (2009) formulated the Paclitaxel loaded nanomicelles with two types of MPEG-b-PCL diblock copolymers, differs in the PCL block lengths of either 19 or 104 and evaluated the encapsulation efficiency and in vitro release behaviour of these two types of Paclitaxel loaded polymeric micelles. The results were showed that the polymeric micelles having PCL block length of 104 units have superior encapsulation and release behaviour than the PCL block with 19 units. Hence, they concluded that amphiphilic diblock copolymers with PCL block length of 104 units were suitable for the preparation of Paclitaxel nanomicelles [20].

T. Musumeci *et al.,* (2006) developed and characterised Paclitaxel loaded poly(lactide) PLA and poly (lactide-co-glycolide) PLGA nanospheres by solvent displacement method and lyophilised by freeze drying method to increase the stability of nanoparticle formulation for longer periods of time to ensure the good physico-chemical properties upon reconstitutions and also reported the effects of various lyoprotectants (mono- and disaccharides and poly alcohols) at various concentrations on nanoparticles particle size and surface charge characteristics [21].

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Mohammad Reza Nabid *et al.*, (2011) synthesized biodegradable amphiphilic star shaped MPEG-PCL copolymer by ring opening polymerisation method and evaluated the properties such as hydrodynamic radius, viscosity and micellar stability in comparison with linear amphiphilic copolymers. They star shaped copolymers have smaller hydrodynamic radius, lower viscosity and improved micellar stability when compared to linear copolymers of same molecular weight and composition. They concluded that the developing newer types of star shaped copolymers based on PCL and PEG with unique feed ratios may provide novel insights in fabricating the superior drug carriers in clinical applications in drug delivery systems [48].

Yodthong Baimark *et al*., (2009) formulated Ibuprofen loaded MPEG-b-PCL nanospheres by emulsification solvent diffusion method and evaluated the influences of PCL block length and drug loading content on characteristics of nanospheres and drug release pattern. The results where showed that nanospheres with and without drug loading where found of spherical shape and smooth surface with a size less than 150nm. Encapsulation efficiency was decreased with increase in the PCL length and also drug was released in a sustained manner and concluded that drug release rate was depended on the PCL block length and the initial drug loading content [49].

Xintao Shuai *et al.*, (2004) developed the polymeric micellar nanoparticles for Doxorubicin delivery with different compositions of diblock copolymers of MPEG-b-PCL and evaluated the effects of increasing PCL block length in the copolymer composition on micellar size and drug loading content (DL %). The results revealed that increase in the PCL block length increases micellar size on increasing PCL length and DL% was significantly less than anticipated. They concluded that compromised molecular interactions and PCL crystallinity would be the reason for decreased drug loading content [50].

Weihui Xie *et al.*, (2007) synthesized the amphiphilic MPEG-PCL diblock copolymers by using catalyst yttrium oxide Y $(DBMP)_3$ in the presence of PCL , MPEG has a macro initiator by ring opening polymerisation method and controlled the molecular weights of copolymers by adjusting MPEG and PCL molar ratios. They characterised the diblock structure of the copolymers by ^1H NMR spectroscopy and also investigated the thermal and crystallisation properties by DSC and POM (polarised optical microscopy). The results were indicated that diblock copolymers were linear in structure and crystallization properties were depended on length of PCL block lengths as per DSC and POM reports [51].

Peng Wei Dong *et al.*, (2010) encapsulated the Honokoil, an anticancer drug into the star shaped PCL-b-PEG copolymeric nanomicelles by sef assembly without any organic solvent by using ultrasonication energy and characterised the obtained nanomicelles for % drug loading, EE and in vitro drug release in comparison with free Honokoil. The results were found that the % drug loading and EE were higher because of the increased solubility of lipophilic drug in hydrophobic core of PCL copolymer. The drug release profiles showed that release was in a biphasic manner with initial burst in 8hrs and then slow and controlled release for 168 hrs when compared to free Honokoil which was released completely in initial burst phase for 8 hrs, it was clearly observed that the release rate of drug from micelles was slower than free Honokoil [52].

Sosnik A *et al.*, (2003) synthesised the MPEG-PCL oligomers by reacting the blends of MPEG and PCL in different ratios by ring opening polymerisation method and investigated the influence of PCL block length on crystallisable properties of oligomers by DSC analysis, because the crystallinity effects the rate of degradation of oligomers. The thermograms of DSC showed that oligomers comprising longer PCL blocks (>5) were only crystallized at the body temperatures and the oligimers with PCL blocks <5 were able to crystallized at low temperatures (4 and 25^0C) [53].

Donghui Zheng *et al.*, (2009) developed Docetaxel-loaded MPEG-b-PCL nanoparticles by nanoprecipitation method. The obtained nanoparticles were evaluated for encapsulation efficiency (%), in vitro drug release and its antitumor efficacy against malignant melanoma was evaluated both in vitro and in vivo. The results were indicated that the EE% was high because of Docetaxel lipophilicity and also drug was released in a continuous and sustained

manner throughout the release study. The in vitro and in vivo studies proved that cytotoxicity was achieved in a dose and time dependent manner with good in vitro in vivo correlation [54].

May P Xiong *et al.,* (2008) encapsulated a lipophilic Geldanamycin prodrug in MPEG-b-PCL micelles by emulsion evaporation method and evaluated the pharmacokinetic parameters such as release, AUC, volume of distribution and total clearance in comparison with the conventional free Geldanamycin prodrug formulation by in vivo anti tumor activity. They results showed that there was a 72 fold enhancement in the AUC, a 21fold decrease in volume of distribution, and a 11fold increase in clearance and also drug was released in a sustained manner. They suggested that formulating the Geldanamycin prodrug in micellar form is a effective way to overcome the problems associated with conventional delivery [55].

Sa Won Lee *et al.,* (2011) formulated the Docetaxel loaded MPEG-PDLLA nanomicelles and evaluated the morphological characteristics such as mean diameter, PDI and pharmacokinetic characteristics like in vivo cytotoxicity and hypersensitivity reactions in comparison with Docetaxel injection. The prepared micelles were in size range of 10-50nm with narrow size distribution. Pharmacokinetic study in mice and Beagle dogs have shown the reduced side effects of hypersensitivity reactions and retained the antitumoral activity in comparison with Docetaxel injection [56].

Maria Teresa Peracchia *et al.,* (1997) formulated the PEG-coated amphiphilic diblock and multiblock copolymeric nanoparticles by emulsification diffusion method and investigated the encapsulation efficacy and in vitro drug release characteristics. The results were indicated that %EE and drug release were dependent on chemical composition of hydrophobic core as well as the surface density and molecular weight of hydrophilic PEG shell of copolymers [57].

Lusianna Ostacolo *et al.,* (2010) formulated the Docetaxel loaded PEO-PCL nano micelles derived from copolymers of linear triblock and 4-arm star diblock copolymers and evaluated the encapsulation efficiency and drug release between two types of copolymers. The results were showed that the loading ability and release rate in buffered saline were similar for two types of copolymers [58].

Junko Matsumoto *et al.*, (1999) developed Progesterone loaded poly (L-lactide)-poly (ethylene glycol)-poly (L-lactide) nanoparticles by solvent evaporation method and evaluated drug entrapment and amount of drug release of nanoparticles. The drug entrapment efficiencies were around 70% and amount of drug released increased as the PEG content and molecular weight of copolymers increased. They concluded that change in the PEG content, and PEG Mw could able to control the drug release from the nanoparticles [59].

Luis A. Guzman *et al.*, (1996) formulated the nanoparticles using polylactic-polyglycolic acid (PLGA) copolymer by emulsification solvent evaporation technique. Fluorescently labeled nanoparticles were administered by intraluminal infusion, amount of the nanoparticle that reached that reached the target site and biocompatibility were evaluated. Dexamethasone was entrapped into the particles to evaluate the therapeutic potential in the prevention of neointimal formation. The results were indicated that the nanoparticles successfully penetrated into the vessel wall and stayed for up to 14 days after a single intraluminal infusion and also significantly decreased neointimal formation [60].

Rubianna M. Mainardes *et al.*, (2005) designed the Praziquantel loaded PLGA nanoparticles by emulsion solvent evaporation method and studied the effects of formulation variables on the particle size distribution of nanoparticles. The results showed that sonication time, homogenization speed and time, drug and polymer ratio, PVA % and ratio between organic and aqueous phases have the significant effects on the size distribution of nanoparticles[61].

Chang Gu Keum *et al.*, (2011) formulated the Docetaxel-loaded PLGA nanoparticles by Emulsion evaporation technique. They studied the effects of various process parameters such as surfactant concentration, sonication time and power, type of evaporation method and time, centrifugation speed and initial drug loading that influences the particle size, encapsulation and release kinetics of drug loaded nanoparticles and also specified the limits for that parameters to achieve better formulation characteristics [62].

Ganesh. G. N. K *et al.*, (2011) formulated the Docetaxel loaded liposomes by dried thin film method and evaluated the in vitro drug release kinetics of the encapsulated drug by fitting the dissolution data to the various kinetic models like zero, first order and Korsmeyer- Peppas equation. The results were indicated that drug release follows first order kinetics and case II transport mechanism of drug release when applied to Korsmeyer-Peppas model [63].

3.1 AIM OF THE WORK

- The main objective of the work was to develop a controlled release dosage form of Paclitaxel nanoparticles using MPEG-b-PCL amphiphilic block copolymer for enhancing solubility of the drug and reducing severe side effects related with the solvents in conventional delivery system.
- The Amphiphilic block copolymers have the property of forming the self stabilised nanomicelles at their CMC in the aqueous solutions with inner hydrophobic polyester core and outer hydrophilic stealth with MPEG. The inner hydrophobic core in the nanomicelles has high capability to entrap lipophilic drugs and contributes for increased solubility of hydrophobic drug in aqueous solutions.
- Paclitaxel is one of the most commonly used cytotoxic antimicrotubular taxane. It is highly lipophilic and insoluble in water, due to the low solubility in water taxanes were administered by dissolving in Cremophore EL (polyethoxylated castor oil) and ethanol which causes serious side effects like sever hypersensitivity reactions and neurotoxicity, when administered intravenously. These severe side effects limit the use of conventional dosage form.
- So, the inclusion of this Taxanes in polymeric nanomicelles has proved to be a good approach to eliminate side effects of Cremophore EL and improved antitumor activity.
- Design of Nanoparticulate drug delivery of Paclitaxel will improve the patient compliance, because of the reduced hypersensivity reactions of solvent Cremophore EL and non target side effects associated with conventional delivery system.
- The prepared Paclitaxel nanoparticles were further lyophilized by freeze drying process to improve the stability of nanoparticles during the storage condition.

3.2 PLAN OF THE WORK

IT WAS PLANNED TO CARRY OUT THE PRESENT STUDY AS FOLLOWS:

STAGE 1:

Preformulation studies involve the observation of physical and chemical data available. The identification of raw materials and preliminary compatibility studies between drug and copolymer to be done using infrared spectroscopy.

STAGE 2:

Formulation of MPEG-b-PCL nanoparticles with Paclitaxel in various Proportions of drug and copolymer by Emulsion solvent evaporation technique.

STAGE 3:

In vitro characterization

a. Entrapment efficiency
b. Study on in vitro drug release
c. Release kinetics

STAGE 4:

Physical characterization of nanoparticles

a. Particle size analysis
b. Zeta potential
c. Scanning Electron Microscopy
d. Polydispersity index

STAGE 5:

Short term stability studies

4. MATERIALS AND INSTRUMENTS

4.1 MATERIALS USED

S.No	Ingredients	Manufactures
1.	Paclitaxel	Celon Laboratories Ltd, Hyderabad.
2.	MPEG-b-PCL copolymer	Celon Laboratories Ltd, Hyderabad.
3.	Polyvinyl Alcohol	Merck Chemicals, Mumbai.
4.	Dichloromethane	Merck chemicals, Mumbai.
5.	Acetonitrile	Merck chemicals, Mumbai.
6.	Isopropyl alcohol	Merck chemicals, Mumbai.
7.	PBS buffer Capsule	Sigma Aldrich, USA
8.	Methanol	Merck chemicals, Mumbai.
9.	Glycerine	Fisher scientific, Mumbai. .
10.	Potassium Bromide	Merck chemicals, Mumbai.
11.	Water for injection	Celon Laboratories Ltd, Hyderabad.
12.	Cellulose acetate membrane filters 0.22 µm	Raylab, New Zealand.
13.	Whatman filter papers	Madhu chemicals Ltd, Hyderabad.

4.2 INSTRUMENTS USED

S.No	Instruments	Manufactures
1.	UV spectrophotometer 1601	Shimadzu corporation, Kyoto, Japan.
2.	Infra Red spectroscopy	Bruker, Germany.
3.	Shear homogenizer	Ika Ultra turrax, Germany.
4.	Rotary vacuum evaporator	Buchi, Germany.
5.	Lyophilizer	Lyo Lab, LSI, India.
6.	Electronic Balance	Sartorius, Germany.
7.	Mechanical stirrer	Remi instruments, India.
8.	Ultra Centrifuge (Beckman)	Centrifuge instruments Ltd, China.
9.	Bath Sonicator	Intech Labs, Hyderabad, India.
10.	Scanning Electron Microscopy	Field instruments, Japan.
11.	Particle size analyser	Malvern, UK.
12.	Zeta potential analyser	Malvern, UK.
13.	Stability chamber	Osworld, Mumbai, India.

5.1 DRUG PROFILE

Paclitaxel[64]

Paclitaxel is a drug used in cancer chemotherapy. It is isolated from the bark of the Yew tree, Taxous brevifolia. It polymerizes the microtubules and leading to the cell death.

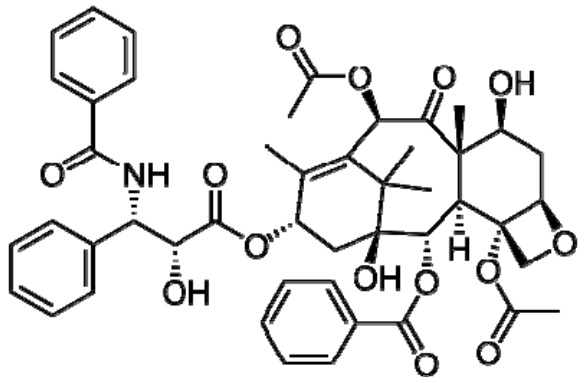
Paclitaxel is used in the treatment of ovarian and breast cancers [67].

Synonyms : 7-epi-Paclitaxel, 7-epi-taxol

Molecular Formula : $C_{47}H_{51}NO_{14}$

Molecular weight : 853.9061 g/mol

Molecular Structure [65]**:**



Systematic IUPAC name [66]**:**

(1S, 2S, 7R, 10S, 12R)-4, 12-bis(acetyloxy)-1,9-dihydroxy-1-(3S-2-hydroxy-3-phenyl-3-propoxy-17-methyl-12-oxo-7-heptacyclo[11.3.1] dec-13-en-2-yl benzoate.

Paclitaxel Identifier:

CAS Number : 33069-62-4

PHYSICO-CHEMICAL DATA [67, 68]

Physical description : White crystalline solid

Melting point : 216-217^{0}C

Solubility : Freely soluble in ethanol, soluble in dichloromethane, sparingly soluble in isoproponal and insoluble in water

Hydrophobicity : Log p 3.96

Dissociation constant : 11.99

PHARMACOKINETIC DATA [69]

Absorption : IV infusion

Bioavailability : Oral (< 10%)

Route : Intravenous administration

Distribution : 227 to 688 Lt/m^2

Half-life : It shows a distribution half-life of 0.54 hours and elimination half-life of 5 to 6 hours.

Protein binding : > 90% of the drug is extensively bind to the plasma proteins.

Metabolism : Hepatic metabolism**.**

Paclitaxel was metabolized by the cytochrome P450 isozyme.

Excretion : Less than 10% of the drug is eliminated in unchanged form in urine and also the smaller amount of drug is eliminated through the faeces.

Storage Condition : 2-8^{0}C and protected from light.

Mechanism of Action[70]:

Paclitaxel promotes the assembly of microtubules by binding to tubulin dimers and then stabilizes microtubules by preventing their depolymerisation. This results in the inhibition of dynamic reorganisation of network of microtubules that is essential during the interphase stage of mitotic cellular division.

Pharmacokinetics [71]:

Absorption:

Paclitaxel is administered as a 3-hour infusion of 135 to 175 mg/m^2 every 3 weeks, or as a weekly 1-hour infusion of 80 to 100 mg/m^2. Longer infusions (96 hours) have yielded significant response rates in breast cancer patients. Pharmacokinetics was assessed by non compartment and model independent methods.

Distribution:

The average distribution half life of Paclitaxel was 0.34 hours in plasma. About 98% of drug is bound to plasma proteins and the presence of antiulcer drugs did not affect protein binding of Paclitaxel.

Metabolism:

Paclitaxel was metabolized in the liver by Cyt P450 isozyme into 6α-hydroxypaclitaxel.

Excretion:

Paclitaxel was mainly excreted by urine. Renal excretion is nonlinear and decreases with increased dose. The excretion through fecal route was very less when compared to renal excretion.

Side effects:

The most common side effects observed with the Paclitaxel are

- Hair loss.
- Low red blood cell count (Anaemia).
- Tired or short of breath.
- Numbness or tingling in the hands or feet.

- Nausea.
- Abnormal heart beat.
- Infections.
- Diarrhoea

Adverse reactions:

The most common adverse effects observed with Paclitaxel are

- Neutropenia.
- Hypersensitivity reactions.
- Hypotension.
- Bradycardia.
- Peripheral neuropathy.
- Myalgia/Arthalgia.

Formulations [72, 73]:

- Ambraxane 100 mg; Nanoparticle albumin-bound paclitaxel.
- Taxol 5mg/ml; Paclitaxel injection.

Combinations with other drugs:

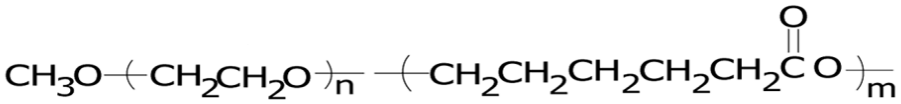
Paclitaxel is sometimes prescribed for lung and breast cancers[74]. AIDS releated kaposis sarcoma in combination with the Cisplatin for the treatment of Breast cancer in combination with Doxorubicin.

5.2 Methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) diblock copolymer[75, 76]:

Synonym:

MPEG-b-PCL polymer micelle

Structure :



Description : It is an odourless and tasteless, white (or) off white semi crystalline powder.

MPEG content : M_n is 5000 g/mol (or) 5 kilo Daltons (or) 114 MPEG units

PCL content : M_n is 24,700 g/mol (or) 24.7 kilo Daltons (or) 104 PCL units

CMC : 0.9 Mg/L

Solubility : It is freely soluble in Dicholoromethane and insoluble in water

Melting point : T_m of MPEG is 41.9^0C

T_m of PCL is 57^0C

viscosity : 13 – 20 cp

Temperature (Tg) : -45^0C (glass transition temperature)

Temperature (Tc) : 58^0C (collapse temperature)

Functional Category: Entrapping agent, entraps the drug in the polymeric micelle and also act as a solubilising agent for hydrophobic drugs [78].

Recent developments in drug delivery systems provide promising ways to maximize the localization of the drug toward the tumor while minimizing systemic toxicity. Latest progress in polymer science has greatly improved the efficiency of drug carriers in encapsulating and delivering chemotherapeutics, among which the amphiphilic block copolymers have attracted the most interest.

Amphiphilic block copolymers tend to form polymeric micelles at lower critical micellar concentrations with inner hydrophobic core and outer hydrophilic shell[77]. These micelles are the promising candidates for hydrophobic anticancer drugs because of the easier incorporation into the hydrophobic core without destroying outer shell which is able to stabilize the system [78, 79].

In most of diblock copolymers the hydrophilic part is MPEG because of its hydrophilicity, biocompatibility, low toxicity, negligible antigenicity and immunogenicity[81]. It enables nanoparticles to escape from scavenging of the RES after the systemic administration[80].

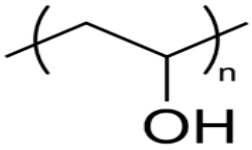
The hydrophobic blocks, forming the core are composed of biocompatible, biodegradable polyesters such as poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), poly(caprolactone) (PCL) and have proven to be very effective in solubilising hydrophobic drugs. Among them PCL was chosen to be a good hydrophobic core because of its good encapsulation ability and slow biodegradability[81, 82].

MPEG-b-PCL is an effective amphiphlic diblock copolymer in carrying hydrophobic cancer drugs such as Paclitaxel and Docetaxel for controlled and targeted delivery to the tumor sites.

5.3 Polyvinyl Alcohol[83, 84]

Synonyms: Polyviol, vinol, covol, PVA.

Structure:



Molecular form : $(C_4 H_6 O_2)_n$

Molecular weight : 30,000 -2, 00,000

IUPAC name : Ethenol

Appearance : It is an odourless, Flake White and granular or semi crystalline powder.

Solubility : Soluble in water and insoluble in Methanol, Acetone

Functional category : Emulsifying agent or Stabilizing agent.

Melting point : 230^0C

Boiling point : 228^0C

Viscosity : 20-28 Mpa.s

pH value : 5-7 for a 1% w/w aqueous solution.

Functional category : Non-ionic surfactant and Viscosity builder.

Applications : Used in the topical formulations, particularly in ophthalmic products as a viscosity builder and stabilizer in emulsions.

Storage **:** PVA undergoes slow degradation at 190^0C and rapid degradation at 200^0C. So, it was stored at room temperature.

Importance of Stabilizer [41, 85]:

Stabilizer was used to stabilize the dispersed phase droplets formed during emulsification and inhibit coalescence. Surfactants or emulsifiers were act as stabilizers.

Surfactants were amphipathic in nature and hence align themselves at the droplet surface to promote stability by lowering the free energy at the interface between two phases, moreover the creation of a charge or steric barrier at the droplet surface confers resistance to coalescence and flocculation.

The amount and the type of emulsifier used play a significant role in determine the morphology, size, encapsulation efficiency and release profile.

The higher amounts of stabilizers in Nanoparticle formulations causes the changes such as

- Bio-degradability.
- Bio-distribution.
- Particle cellular uptake.
- Drug release behaviour.

6. EXPERIMENTAL INVESTIGATIONS

6.1 STANDARD CALIBRATION CURVE OF PACLITAXEL[41]:

By UV spectroscopic method:

Paclitaxel was estimated spectroscopically at 227 nm and it obeys Beer-Lamberts law in the range of 5-40 µg/ml.

Solvent media:

The solvent media used was a mixture of 50:50 v/v of Acetonitrile: pH 7.4 Phosphate buffer saline.

a) Preparation of pH 7.4 Phosphate buffer Saline solution:

One PBS capsule (Sigma Aldrich) was dissolved in 200 ml of deionized water and yields a pH of 7.4 at 25^0C.

b) Preparation of stock solution:

Accurately weighed 100 mg of Paclitaxel was taken into the 100 ml standard flask and dissolved with 100ml of solvent to get a solution concentration of 1000 µg/ml.

c) Preparation of sample solution:

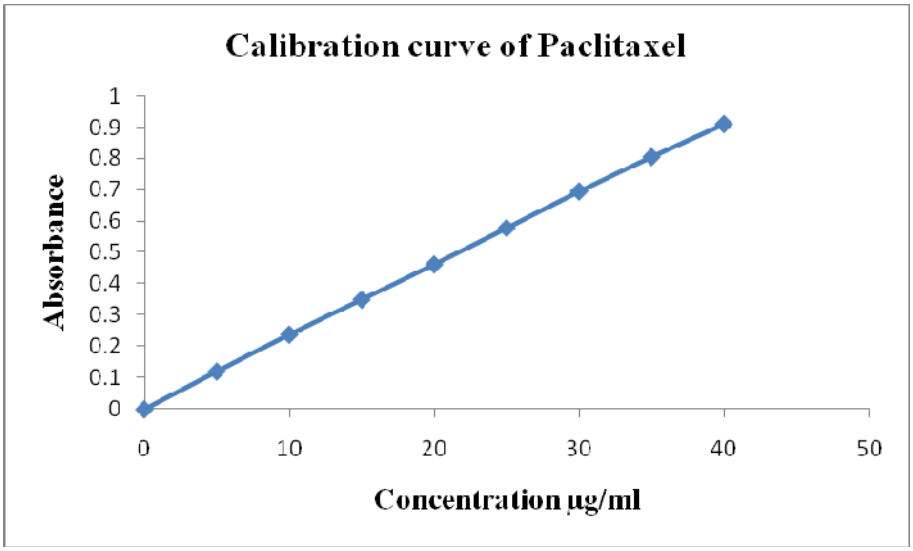
From the stock solution aliquots ranging from 0.5 to 4 ml were pipetted out and diluted 100ml with PBS pH 7.4 to get the concentrations of 5, 10, 15, 20, 25, 30, 35 and 40µg/ml respectively. Then, the absorbance of each solution was measured at 227 nm UV spectrphotometrically against drug free solvent media as blank. Absorbance values were plotted against respective concentrations to establish a standard calibration curve.

STANDARD CALIBRATION CURVE OF PACLITAXEL:

Table No: 5 Standard readings of Paclitaxel by UV spectroscopy

S. No.	Concentration (µg/ml)	Absorbance at 227nm
1.	5	0.121
2	10	0.239
3.	15	0.351
4.	20	0.463
5.	25	0.579
6.	30	0.696
7.	35	0.806
8.	40	0.911

Graph No: 1 Standard graph of Paclitaxel in 50:50 v/v Acetonitrile : pH 7.4 PBS



The linear equation was obtained as

$$R^2 = 0.999 \text{ and } Y = 0.022x+0.006$$

Correlation coefficient value (R^2) indicated that there was a linear correlation between concentration and absorbance.

From the standard calibration curve of drug it was concluded that, the paclitaxel obeys Beer-Lambert's law in the concentration range of 5-40 μg/ml. The absorbance values and standard curve were shown in Table No: 5 and Graph No: 1.

6.2 PREFORMULATION STUDIES:

Compatibility studies by IR Spectroscopy [86, 87, 28]:

Infrared spectroscopic analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample.

IR spectroscopy was used to identify and predict any interactions or incompatibilities between the components in the formulation. Therefore, in the present work, a study was carried out using IR spectrophotometer to confirm the absence of any possible chemical interactions between the drug, polymer and other excipients in the formulation.

In the present study, the following IR spectra were recorded

a) Pure sample of Paclitaxel.

b) MPEG-b-PCL copolymer.

c) Polyvinyl alcohol.

d) A mixture of Paclitaxel and MPEG-b-PCL copolymer.

e) A mixture of Paclitaxel and MPEG-b-PCL copolymer and PVA.

One part of sample and three parts of potassium bromide of IR grade were triturated in mortar. A small amount of triturated sample was taken into a pellet maker and compressed at 15 tons pressure in hydraulic press to form a semi transparent pellet. Then the pellet was kept in sample holder and was scanned from 4000 to 400 cm^{-1} in IR spectrometer (Bruker)

and the peaks were identified. IR spectra was compared and checked for any shifting in functional peaks and non involvement of functional groups.

The compatibility between the drug, copolymer and other excipients were evaluated by using IR peak matching method.

The spectra and their interpretations with pure drug are as follows

Table: 06 IR spectra of pure Paclitaxel:

Frequency (cm^{-1})	**Groups assigned**
3440.65	O- H stretching
2944.36	C-H Aromatic stretching
1646.87	C = O stretching
1370.32	C - N stretching
1096.62	C-O stretching
984.12	C- H in plane bending
632.40	C- H out plane bending

Table: 07 IR spectra of MPEG-b-PCL copolymer:

Frequency (cm^{-1})	Groups assigned
3449.38	O - H stretching
2959.20	C-H Aromatic stretching
1640.23	C = O stretching
1374.79	C - N stretching
1103.13	C-O stretching
982.91	C- H in plane bending
644.98	C- H out plane bending

Table: 08 IR spectra of Poly vinyl alcohol:

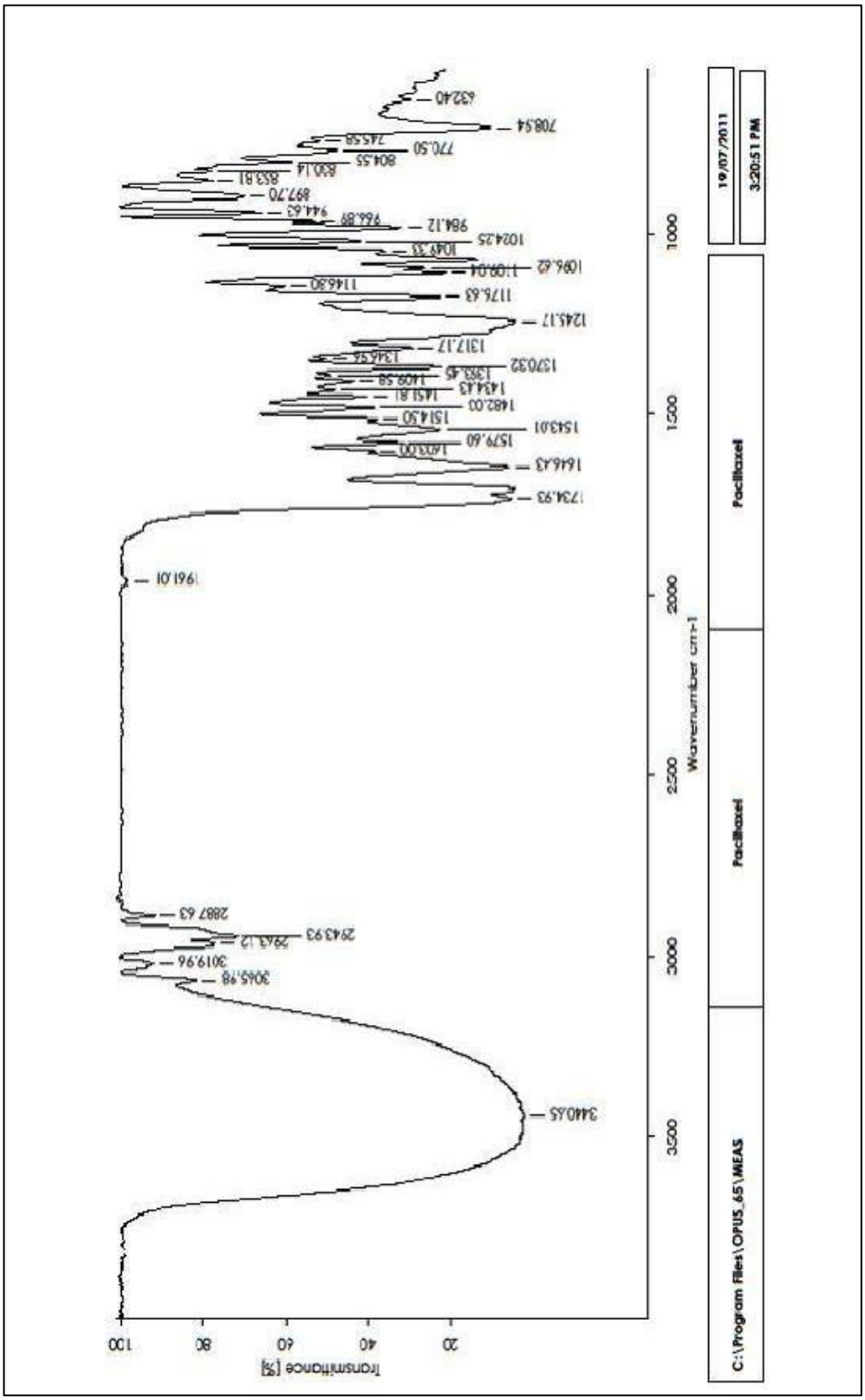
Frequency (cm^{-1})	Groups assigned
3406.10	O- H stretching
1649.61	C = O stretching
1378.69	C - N stretching
1096.28	C-O stretching
946.08	C- H in plane bending
652.27	C- H out plane bending

Table: 09 IR spectra of Paclitaxel and MPEG-b-PCL copolymer:

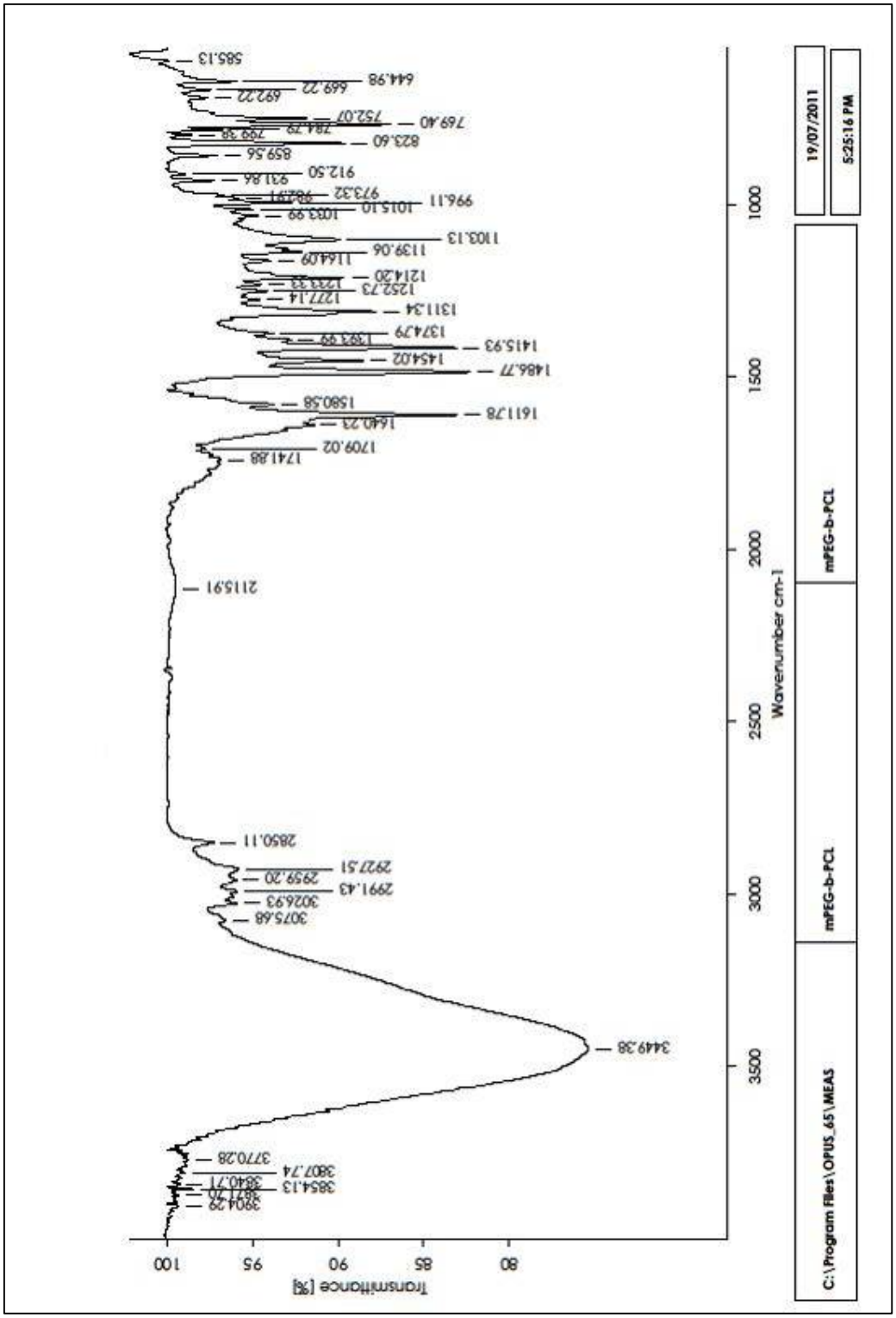
Frequency (cm^{-1})	Groups assigned
3440.28	O- H stretching
2944.49	C-H Aromatic stretching
1646.70	C = O stretching
1370.50	C - N stretching
1095.64	C-O stretching
983.17	C- H in plane bending
632.53	C- H out plane bending

Table: 10 IR spectra of Paclitaxel and MPEG-b-PCL copolymer and Poly vinyl alcohol:

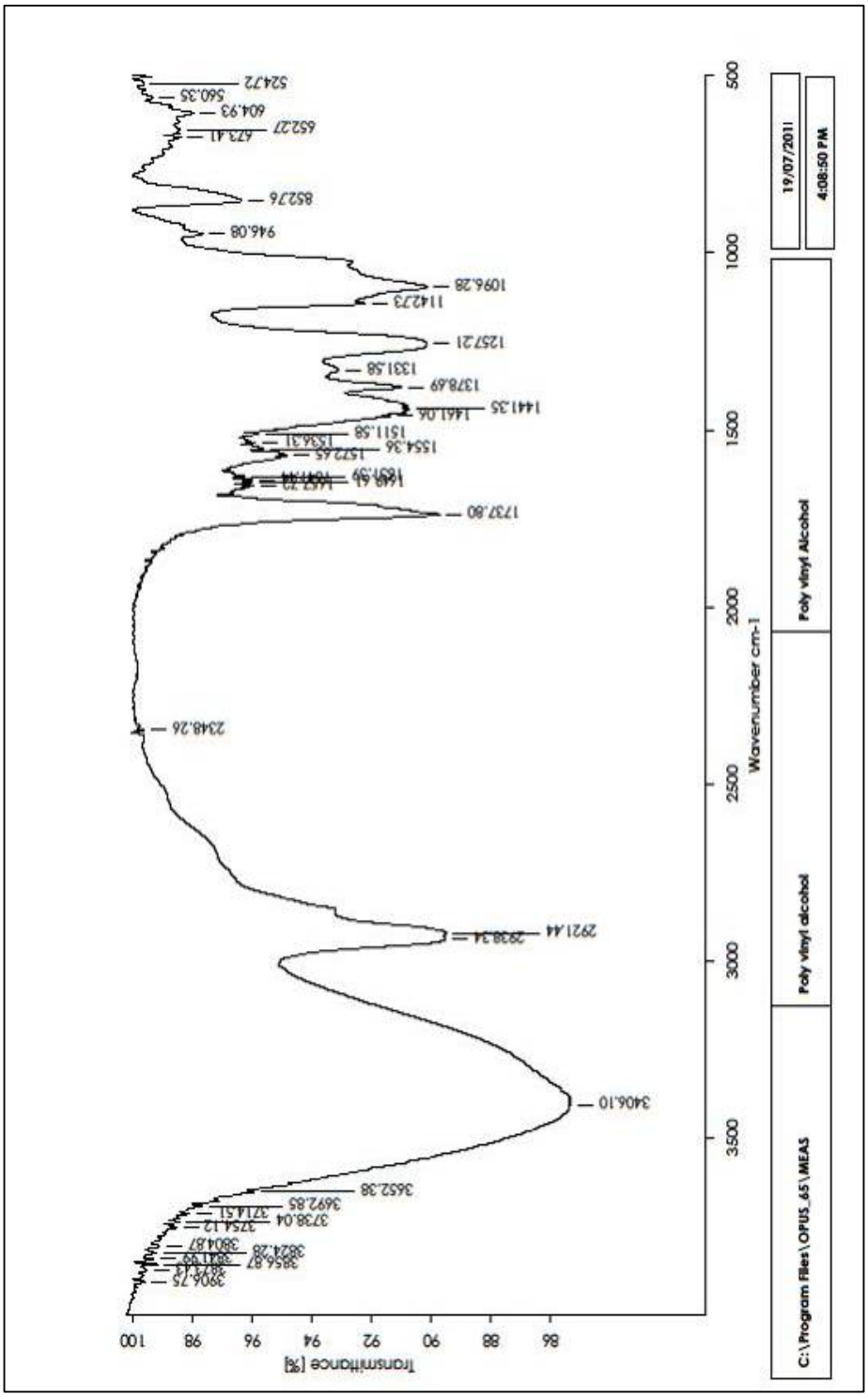
Frequency (cm^{-1})	Groups assigned
3440.53	O- H stretching
2944.36	C-H Aromatic stretching
1646.87	C = O stretching
1370.75	C - N stretching
1095.92	C-O stretching
983.99	C- H in plane bending
632.93	C- H out plane bending



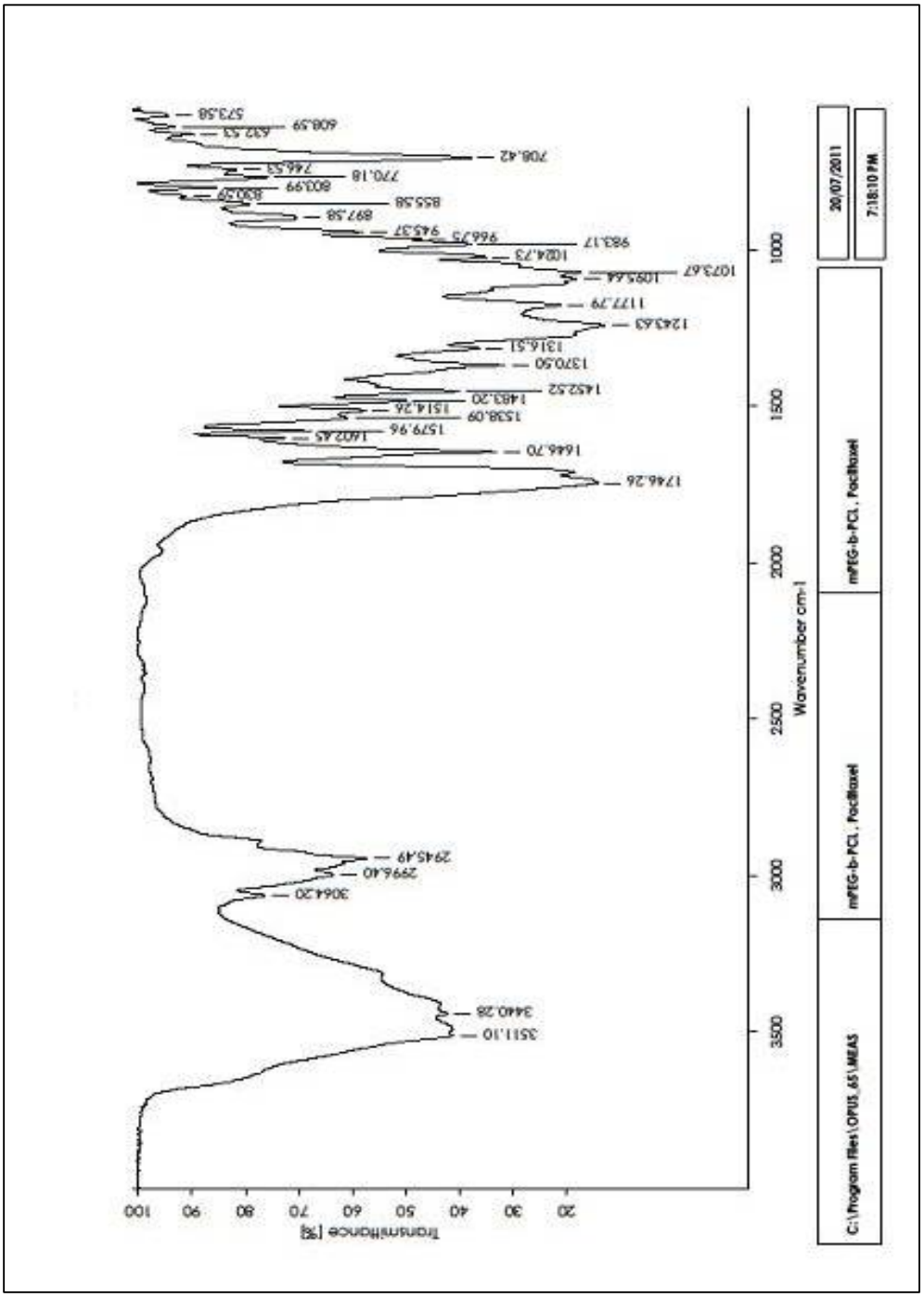
SPECTRA NO: 1 FTIR OF PACLITAXEL



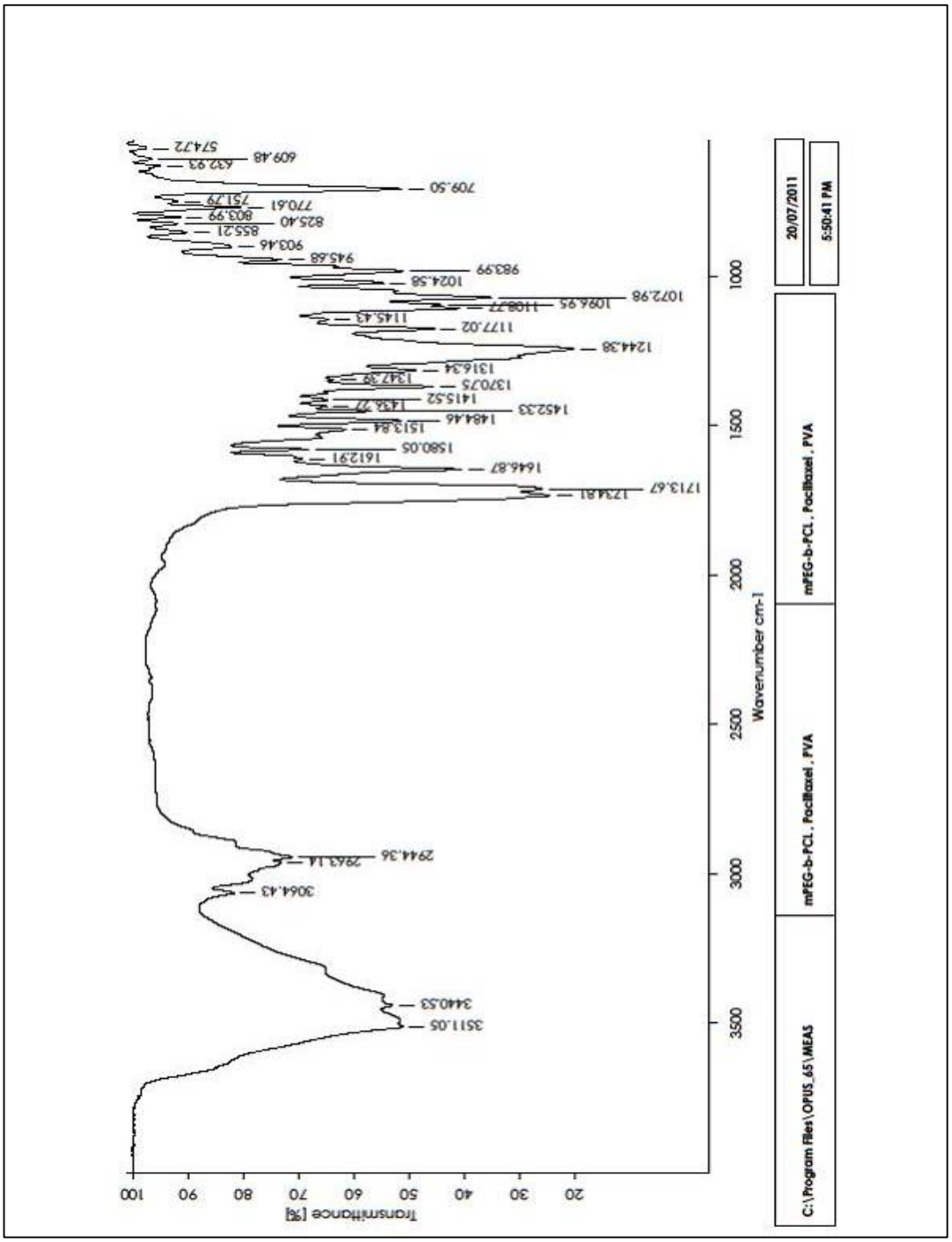
SPECTRA NO: 2 FTIR OF MPEG-b-PCL COPOLYMER



SPECTRA NO: 3 FTIR OF POLYVINYL ALCOHOL



SPECTRA NO 4: FTIR OF MPEG-b-PCL & PACLITAXEL



SPECTRA NO: 5 FTIR OF PACLITAXEL, MPEG-b-PCL AND PVA

Table No: 11 Interpretations of FTIR Spectra for pure Paclitaxel drug.

Functional Groups	Assessment peaks of pure drug(cm^{-1})	Range of Groups (cm^{-1})
O- H stretching	3440.65	3450-3250
C-H Aromatic stretching	2944.36	2973-2547
C = O stretching	1646.87	1850-1550
C - N stretching	1370.32	1390-1300
C-O stretching	1096.62	1260-1000
C- H in plane bending	984.12	941-803
C- H out plane bending	632.40	< 700

Table No: 12 Interpretations of FTIR Spectra for pure drug and drug copolymer mixture

Functional Groups	Assessment peaks of pure drug (cm^{-1})	Assessment peaks of drug, copolymer (cm^{-1})
O- H stretching	3440.65	3440.28
C-H Aromatic stretching	2944.36	2945.49
C = O stretching	1646.87	1646.70
C - N stretching	1370.32	1370.50
C-O stretching	1096.62	1095.64
C- H in plane bending	984.12	983.17
C- H out plane bending	632.40	632.53

Table No: 13 Interpretations of FTIR Spectra for pure drug and drug, copolymer & PVA mixture

Functional Groups	Assessment peaks of pure drug (cm^{-1})	Assessment peaks of Drug, copolymer & PVA (cm^{-1})
O- H stretching	3440.65	3440.53
C-H Aromatic stretching	2944.36	2944.36
C = O stretching	1646.87	1646.87
C - N stretching	1370.32	1370.75
C-O stretching	1096.62	1096.92
C- H in plane bending	984.12	983.99
C- H out plane bending	632.40	632.93

It was observed that the assessment peaks which were seen in the IR spectra of pure Paclitaxel were also appeared in the Paclitaxel, copolymer and PVA mixture, which confirmed the absence of any chemical interactions between the drug, copolymer and other excipients. The results were shown in Table No: 11, 12 and 13 and Spectra No: 1, 2, 3, 4 and 5.

6.3 PREPARATION OF PACLITAXEL NANOPARTICLES[41]

6.3.1 Procedure for the preparation of Paclitaxel nanoparticles

The preparation of nanoparticles with MPEG-b-PCL copolymer was carried out by Emulsion solvent evaporation technique.

All the batches of nanoparticles were prepared by emulsion solvent evaporation technique. For the preparation of nanoparticles, organic and aqueous solutions were required.

Preparation of organic solution:

Composition: Dichloromethane - 2 ml

Paclitaxel - 40 mg

MPEG-b-PCL copolymer- Quantities were specified in table no: 3

Accurately weighed 40 mg of the Paclitaxel was dissolved into1.5 ml of dichloromethane solvent, then added an accurately weighed quantity of MPEG-b-PCL copolymer as specified in the table no: (14) to the previously dissolved 1.5 ml organic solution then add remaining 0.5 ml DCM solvent and sonicated for 10mins in the bath sonicator for the complete solubility of drug and copolymer.

Preparation of Aqueous solution:

Composition: Poly vinyl alcohol: 200 mg

Water for Injection: quantity sufficient

Accurately weighed 200 mg of PVA was dissolved into the 100 ml of WFI at 65^0C with frequent stirring for preparing 0.2% PVA solution and then filtered through the 0.2 micron whatmann membrane filter under vaccum. From this, 20 ml of solution was taken for emulsion preparation. The PVA solution was used as a stabilizer to stabilize the formed emulsion droplets.

For the stable emulsion preparation, the organic and aqueous solutions were should be in the ratio of 1:10.

EMULSION SOLVENT EVAPORATION METHOD:

In this method organic solvent was evaporated during the process of Nanoparticle formulation.

The organic solution containing the drug and polymer was added drop wise into aqueous solution of PVA (0.2%) for 1minute through the micropipette during the homogenization under high shear homogenizer at 12,000 rpm. The homogenization was carried out for 10-15 min under ice bath for the better entrapment of drug into the copolymer matrix.

After that, the homogenized solution was stirred at 8000 rpm with mechanical stirrer for 1hour at room temperature. During this step the formed nanoparticles were hardened and organic solution is slowly evaporated. For removing the traces of Dichloromethane in the aqueous solution, the solution was evaporated by using Rotary evaporator (Buchi) at 25^0C with 40 rpm for 30 min by applying a vaccum of about 25 mm Hg.

Filter the Nanoparticle solution through cellulose acetate membrane filter, under vaccum to get the sterile formulation. Then, the collected nanoparticles were redispersed in 20 ml of Water for injection for lyophilisation. Here, the PVA over the particles was dissolved in to the solution and act as a lyoprotectant for protecting the formulation against the freezing and drying stress during freeze drying [10].

Finally, the Nanoparticle suspension was transferred into a 50 ml glass vial and sealed with a rubber stopper followed by an over seal (aluminium) and then wrapped in aluminium foil to protect from light. Then, the glass vials were stored in refrigerated condition.

TABLE: 14 COMPOSITIONS OF PACLITAXEL NANOPARTICLES

S.No	Formulation code	Amount of drug(mg)	Amount of copolymer (mg)	Drug: carrier ratio
1.	F1	40	140	1:3.5
2.	F2	40	160	1: 4
3.	F3	40	180	1:4.5
4.	F4	40	200	1: 5
5.	F5	40	220	1:5.5
6.	F6	40	240	1: 6
7.	F7	40	260	1:6.5
8.	F8	40	280	1: 7
9.	F9	40	300	1:7.5
10.	F10	40	320	1: 8

From the above formulations, the best formulation was selected based on the amount of the drug loaded or percentage entrapment efficiency and in vitro drug release behaviour. Then, the selected or optimised formulation was further lyophilised by freeze drying method to increase the stability of nanoparticle formulation during storage.

6.3.2 Freeze drying method to lyophilise the Nanoparticle formulation:

Lyophilisation or freeze drying has become an accepted method of processing heat sensitive products that require long term storage at temperatures above freezing. During the freeze drying dehydration of aqueous solution (unbound and bound water) was carried, while the product suspension was in a frozen state, under a controlled vaccum condition.

As per the Entrapment efficiency (%) and in vitro drug release results, formulation F7 was found to be desirable than other formulations. Hence, it was chosen for freeze drying or lyophilisation.

Lyophilisation of Nanoparticle formulation was carried out in three stages[11]

a) Freezing (solidification).

b) Primary drying. (ice sublimation)

c) Secondary drying. (desorption of unfrozen water)

Freezing:

During this step the Nanoparticle suspension was freezed at -45^{0}C for 490 min, then the liquid suspension is cooled and ice crystals are formed and the suspension becomes concentrated. Total duration of freezing was 565 minutes. At the end of freezing step, the concentrated suspension was in an amorphous form or combined amorphous-crystalline form.

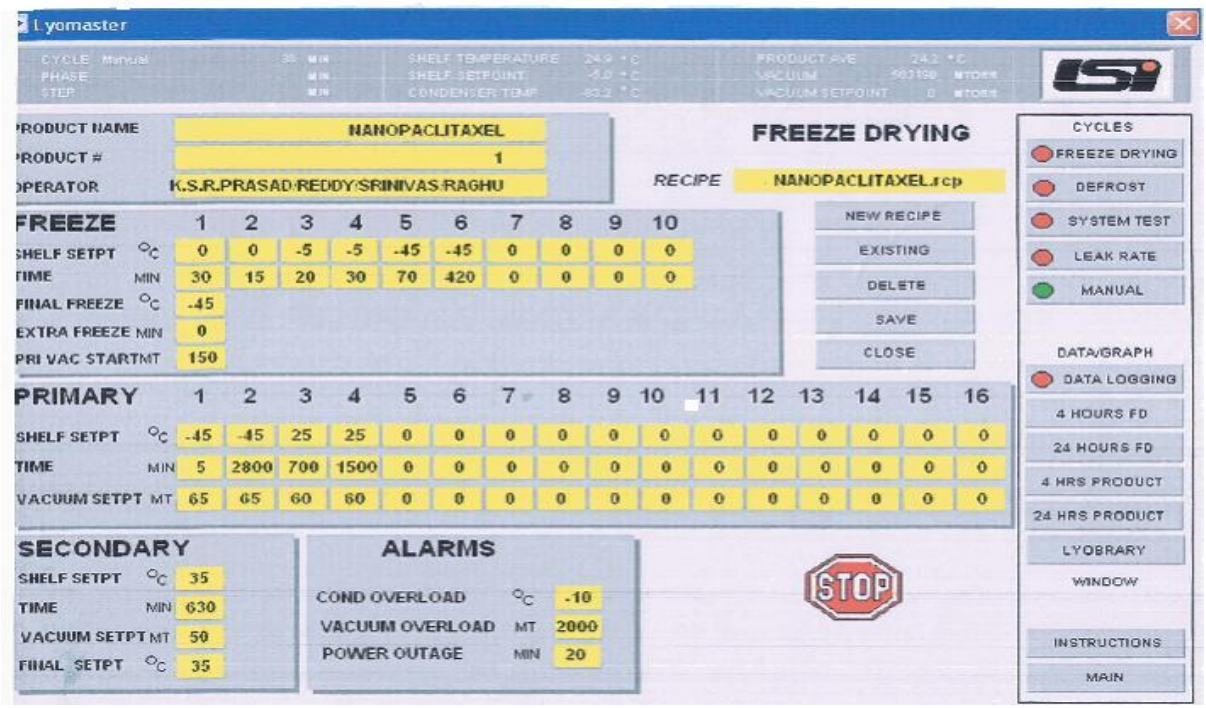
Primary Drying:

In the primary drying, the ice crystals that were formed in the frozen product were sublimated at 25^{0}C under controlled vaccum of 60-65 MT for 2200 min. The total duration of PD was 83 hour. At the end of drying, porous plugs were formed those represents the spaces occupied by ice crystals.

Secondary drying:

In the secondary drying, bound water present in the product was removed at 35^{0}C under controlled vaccum of 65 MT for 630 minutes, which was not separated out as ice during the freezing was removed.

The three stages were detailed in the following freeze dried nanoparticle recipie [92].



6.3.3 Reconstitution of lyophilised Nanoparticle formulation:

Finally, the formed lyophilised cake was reconstituted slowly with 20 ml of pH 7.4 phosphate buffer saline for 1min to prevent foaming and allowed to settle for 5 minutes for proper wetting of the lyophilized cake and then gently swirl or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs.

6.4 EVALUATION OF PACLITAXEL NANOPARTICLES:

6.4.1 PHYSICAL CHARACTERISATION OF NANOPARTICLES:

The optimised Nanoparticle formulation before and after the freeze drying were evaluated by studying their physical properties like

- Particle size analysis
- Polydispersity index
- Zeta potential analysis
- SEM analysis

6.4.1.1 Particle size Analysis:

The paclitaxel loaded nanoparticles before and after lyophilisation technique were subjected to Laser diffraction particle size analysis for determining the Nanoparticles size and their distribution. It was carried out by MALAVERN INSTRUMENTS in Indian institute of chemical technology. Hyderabad.

6.4.1.2 Zeta potential analysis:

Zeta potential is an important physical characteristic that evaluates the potential stability of colloidal systems. It is an aid in predicting long-term stability of colloidal dispersions.

The significance of zeta potential was that if the particles in the suspension have a large negative or positive zeta potential then they will tend to repel each other and they were electrically stabilized. However, if the particles have low zeta potential values then there will be no force to prevent particles coagulation or flocculation.

The prepared nanoparticles were subjected to zeta potential analysis before and after lyophilisation by MALVERN ZETASIZER V 6.01 in Indian institute of chemical technology. Hyderabad.

6.4.1.3 Polydispersity index [88]:

Polydispersity index is an index or variation or spread with in the particle size distribution of a given dispersion sample. It was carried out along with particle size analysis by Malvern instruments in IICT, Hyderabad.

6.4.1.4 Scanning electron microscopy:

Determination of surface morphology (spherical structure, smoothness and formation of aggregates) of nanoparticles before and after the lyophilisation were carried out by scanning electron microscopy. Samples for SEM were mounted on metal studs and were magnified to X 5000.

6.4.2 INVITRO CHARACTERIZATION OF NANOPARTICLES:

6.4.2.1 Entrapment Efficiency:

The Entrapment efficiency of Nanoparticle preparation was determined by UV absorbance of drug at 227 nm by UV spectrophotometry [41].

Nanoparticle suspension was subjected to the ultracentrifugation at 14,000 rpm for 30 mins to remove the supernatant containing excess of Paclitaxel, the sediment was formed at the bottom of the centrifuge tube. It was vortexed with 2 ml Dichloromethane for 15 min to extract the entrapped drug from the core of the nanoparticles. And add the solvent media (Acetonitrile : pH 7.4 PBS 50: 50 v/v) to the solution to solubilise the released drug then purged with nitrogen gas to evaporate the dichloromethane[85], until the clear solution is obtained. After that, the solution is filtered through the 0.2 micron syringe filter to remove the polymer aggregates.

The 1ml of filtered solution was taken and suitably diluted with solvent media. Then, the absorbance of diluted solution was measured spectrophotometrically and the drug concentration in the filtered solution was obtained by comparison with the standard calibration curve which previously constructed.

The following equation was used to find out entrapment efficiency of paclitaxel in Nanoparticle formulation

$$\text{Entrapment efficiency}(\%) = \frac{\text{Amount of Drug entrapped}}{\text{Amount of drug used for preparation}} \times 100$$

For all batches, EE (%) was performed in triplicate and the average result was calculated.
The entrapment efficiency was also performed for the optimised lyophilised formulation F7.

The best formulation is the one which has good entrapment efficiency and desired drug release profile. So, it was decided to do the in vitro drug release studies for all the formulations.

6.4.2.2 In-vitro drug release studies:

By UV spectrophotometric method[41]:

In vitro release of Paclitaxel from Nanoparticle formulation was carried out by using dialysis bag diffusion technique[22].

The prepared Nanoparticle formulation was placed in a dialysis bag membrane having molecular weight cut off 14,000 daltons, which was previously soaked for overnight, both the ends were tied tightly and dropped into a beaker containing 200 ml of diffusion solvent medium pH 7.4 PBS, maintained at 37^0C and stirred with the help of a magnetic stirrer. Aliquots (1ml) of release medium were withdrawn at different time intervals (8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 and 96 hr) and the sample was replaced with same amount of fresh solvent medium to maintain the constant volume.

Then, the samples were diluted with (Acetonitrile : pH 7.4 PBS 50: 50 v/v) and analysed spectrophotometrically at 227 nm. The percentages of drug released at different time intervals were calculated from standard calibration curve and the results were tabulated.

The release study was also performed for the lyophilised formulation F7 after reconstitution with 20 ml pH 7.4 PBS.

6.4.2.3 Release kinetics[89,88]

To analyze the in vitro release data of Nano formulations, various kinetic models were used to describe the release kinetics. The zero order release states that drug release rate was independent of its concentration. The first order release describes, the release rate from the system was concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process was based on Fickian diffusion. The results of *in vitro* release profile obtained for an optimised freeze dried F7 formulation were plotted in modes of data treatment as follows:

1. Zero - order kinetic model – Cumulative % drug released versus time.
2. First - order kinetic model – Log cumulative percent drug remaining versus time.
3. Higuchi's model – Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log time.

a. Zero order kinetics:

Zero order release would be predicted by the following equation:

$$A_t = A_0 - K_0 t$$

Where

A_t = Drug release at time't'

A_0 = Initial drug concentration.

K_0 = Zero- order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero – order kinetics and its slope is equal to Zero order release constant K_0**.**

b. First order kinetics:

First - order release could be predicted by the following equation:

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

Where,

C = Amount of drug remained at time‘t’

C_0 = Initial amount of drug.

K = First - order rate constant (hr^{-1}).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follows first order kinetics. The constant ‘K_t’ can be obtained by multiplying 2.303 with the slope value.

c. Higuchi’s model:

Drug release from the matrix devices by diffusion has been described below

Higuchi’s classical diffusion equation:

$$Q = [D_{Є} / \tau(2A - ЄCs\ )\ Cs\ t]^{1/2}$$

Where,

Q = Amount of drug release at time ‘t’

D = Diffusion coefficient of the drug in the matrix.

A = Total amount of drug in unit volume of matrix.

Cs = Solubility of drug in the matrix.

Є = Porosity of the matrix.

τ = Tortuosity.

t = Time (hrs at which q amount of drug is released.

Above equation can be simplified assumed that 'D', 'Cs' and 'A' are constants. Then the equation becomes

$$Q = Kt^{1/2}$$

According to the equation, if cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism.

d. Korsmeyer equation / Peppa's model[91]:

Korsmeyer described a simple relationship to find the mechanism of drug release from polymeric system. To study the mechanism of drug release from the Nanoparticle formulation, the release data was fitted to the Korsmeyer-Peppa's law equation, which was used to describe the drug release behaviour from polymeric systems.

$$M_t / M_\alpha = Kt^n$$

Where,

M_t / M_α = Fraction of drug released at time't'.

K = Constant

n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides,

$$\text{Log } M_t / M_\alpha = \text{Log } K + n \text{ Log } t$$

In the above equation, "n" value was used to characterize the various release mechanisms as mentioned in the table below

Table 15: Diffusion exponent and solute release mechanism for cylindrical shape

S.No	Diffusion	Exponent (n) Overall solute diffusion mechanism
1.	0.45	Fickian diffusion
2.	0.45<n<0.89	Anomalous (non-Fickian) Diffusion
3.	0.89	Case-II transport
4.	n>0.89	Super case-II transport

6.5 STABILITY STUDIES OF PACLITAXEL NANOPARTICLES [92]:

The formulation F7 was chosen for stability studies because the entrapment efficiency and percentage drug release was high when compared to other formulations and also it was lyophilised. The lyophilized Nanoparticle formulations of F7 were kept for three months at 5^0C and 25^0C/65% RH in a stability chamber (Osworld). At the time interval of one month samples were withdrawn from the stability chamber and evaluated the following characteristics for 3 months.

A) Entrapment efficiency (%)

B) In vitro drug release

7. RESULTS AND DISCUSSION

7.1 In vitro characterisation

7.1.1 Entrapment efficiency of Paclitaxel nanoparticles

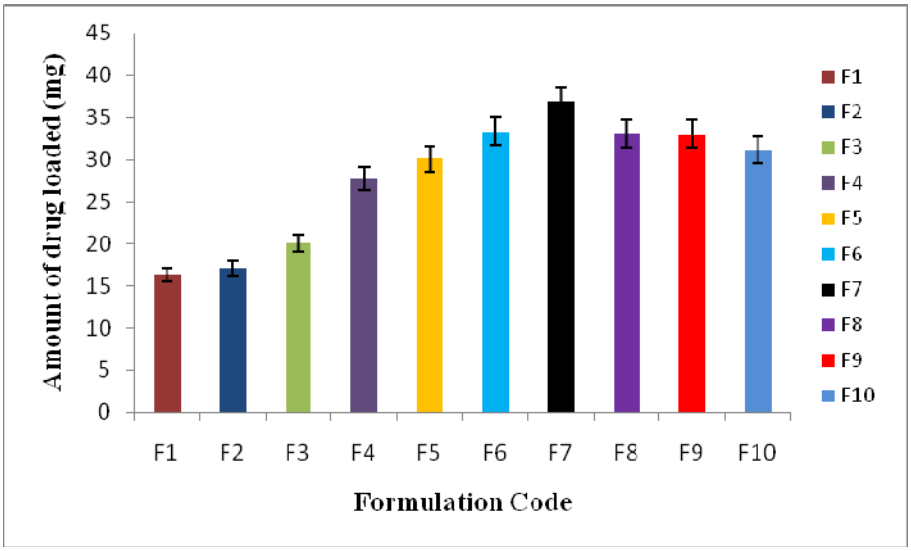
The entrapment efficiency (%) of the Paclitaxel loaded Nanoparticle formulations (F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10) and an optimised freeze dried formulation F7 was determined and tabulated.

Table No:16 Entrapment efficiency of formulations with drug and copolymer Before lyophilisation (n=3), values were the mean of 3 experiments ± S.D

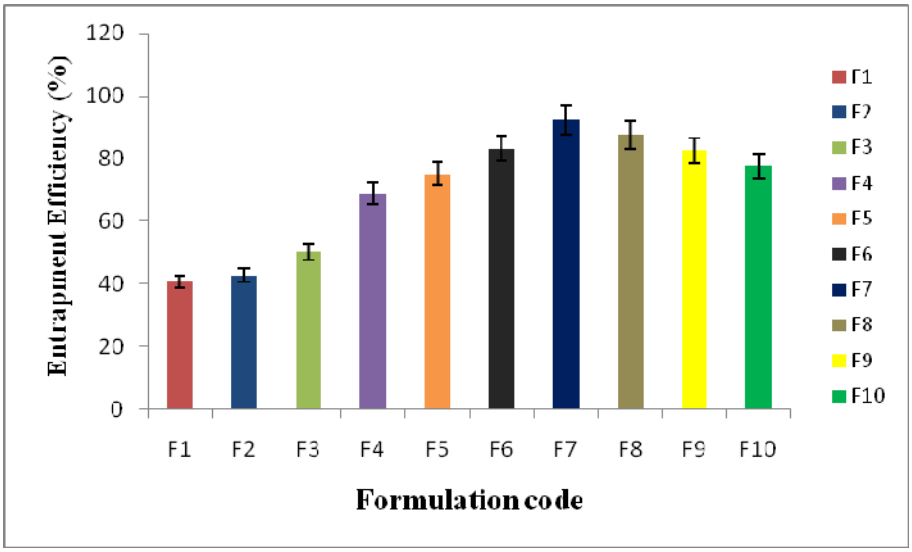
S.N0	Formulation code	Amount of drug entrapped(mg)	Percentage Entrapment efficiency
1.	F1	16.31±0.17	40.76±0.28
2.	F2	17.12±0.13	42.81±0.32
3.	F3	20.13±0.42	50.34±0.80
4.	F4	27.73±0.45	68.72±0.73
5.	F5	30.07±0.24	75.19±0.60
6.	F6	33.30±0.19	83.26±0.56
7.	F7	36.79±0.17	92.0±0.36
8.	F8	35.07±0.32	87.6±1.08
9.	F9	33.02±0.36	82.57±0.78
10	F10	31.09±0.33	77.78±1.08

Table No: 17 Entrapment efficiency of formulation F7 with drug and copolymer After lyophilisation (n=3). Values were Mean ± S.D

S.N0	Formulation code	Amount of drug entrapped(mg)	Percentage Entrapment efficiency
1.	F7	36.76±0.14	91.96±0.18



Graph No: 2 Amount drug loaded plot for F1, F2, F3, F4, F5, F5, F6, F7, F8, F9, F10 Formulations. All the values were plotted by using Mean± S.D (n=3).



Graph No: 3 Percent Entrapment efficiency plot for F1, F2, F3, F4, F5, F5, F6, F7, F8, F9, F10 Formulations, All the values were plotted by using Mean± S.D (n=3).

The % entrapment efficiency was determined by ultracentrifugation method and it was found that the formulations F1, F2 and F3 has showed entrapment efficiencies of 40.76±0.28%, 42.81±0.32%, 50.34±0.80% with drug loading contents of 16.31±0.17mg, 17.12±0.13mg and 20.13±0.42mg respectively. The low % entrapment efficiencies were due to lower drug loading contents because of lesser availability of PCL carboxyl groups of copolymer to entrap the drug.

The formulations F4, F5 and F6 has showed the entrapment efficiencies of 68.72±0.73%, 75.19±0.60%, 83.26±0.56% with drug loading contents of 27.73±0.45mg, 30.07±0.24mg and 33.30±0.19mg respectively. The % entrapment efficiencies were increased with increase in loading contents because of increased copolymer concentrations with respect to the drug and also due to availability of PCL carboxyl groups of copolymer to entrap the drug.

The F7 formulation has showed entrapment efficiency of 92.0±0.36% with a higher drug loading content of 36.79±0.17mg. This was because of maximum availability of PCL carboxyl groups to entrap the drug. Then, the formulations F8, F9 and F10 has showed % entrapment efficiencies of 87.6±1.08%, 82.57±0.78% and 77.78±1.08% with drug loading contents of 35.07±0.32mg, 33.02±0.36mg and 31.09±0.33mg respectively. The gradual decrease in the % entrapment efficiency then compared to formulation F7 was due to increased copolymer concentration because of higher hydrophobic or steric interactions between the carboxyl groups of PCL copolymer and drug.

Hence the F7 formulation has shown highest % entrapment efficiency with higher drug loading content when compared with other formulations. So, it was selected as an optimized formulation and then it was freeze dried by lyophiliser.

The entrapment efficiency of lyophilised F7 formulation was found to be 91.96±0.18% with drug content of 36.76±0.14mg. This signifies that there was no change in drug loading content and entrapment efficiency before and after lyophilisation of formulation F7.

The results of amount of drug entrapped and percentage entrapment efficiencies of Nanoparticle formulations were shown in table no: 16 and17 and graph no: 2 and 3.

7.1.2 In vitro Drug release profile of Paclitaxel Nanoparticles

The in vitro drug release studies were performed for the prepared Nanoparticle formulations (F1, F2, F3, F4, F5, F6, F7, F8, F9 and F10) and also for optimised freeze dried formulation F7. Results were tabulated and graphs were made by plotting cumulative % drug release against time in hours on the y axis and x axis respectively.

The in vitro drug release characteristics for prepared Nanoparticle formulations were evaluated with the help of release profiles in graphical plots.

The formulations F1, F2 and F3 showed a drug release of 35.6%, 42.87% and 50.46% at 96 hr respectively. The drug release was increased from F1 to F3, because of the increased % entrapment efficiencies due to the availability of increased carboxyl groups of copolymer.

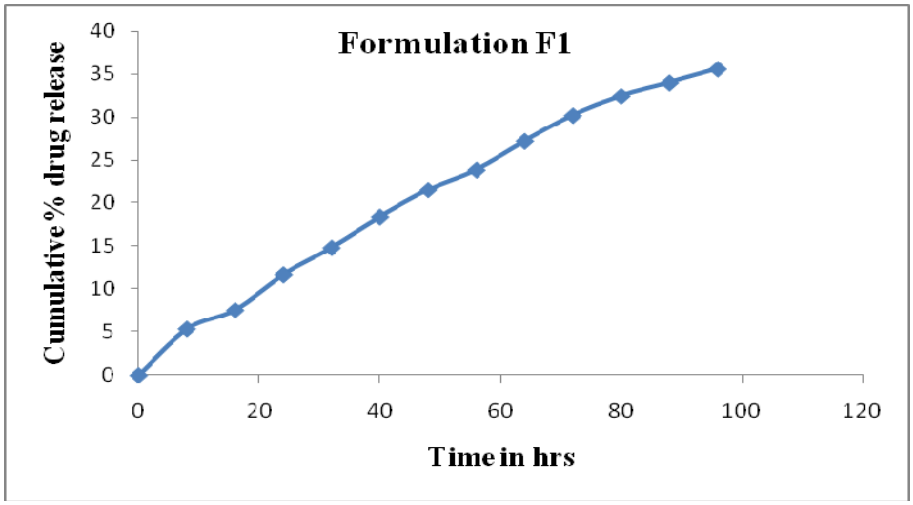
The formulations F4, F5 and F6 showed a drug release of 66.15%, 73.23% and 84.77%, at 96 hrs respectively. The percentage drug releases of formulations were increased due to increased entrapment efficiencies because of the increased copolymer concentration with respect to the drug or due to increase in carboxyl groups of copolymer to entrap the drug.

The formulation F7 has showed a drug release of 89.84% in 96 hours. The release of the drug was suddenly increased with a drug loading content of 36.79 mg due to the availability of maximum carboxyl groups of copolymer to entrap the drug. Then, the F8, F9 and F10 have showed a drug release of 83.06%, 79.54% and 78.34% respectively. The gradual decrease in drug release when compared with the F7 formulation was found to be due increased hydrophobic or steric interactions between drug and carboxyl groups of copolymer and also in between the carboxyl groups of copolymers. Hence, the F7 formulation was selected as the best formulation with highest percent drug release and also having the higher % encapsulation efficiency then compared to the other formulations.

So, it was selected for the freeze drying to increase the stability during storage. This freeze stabilized formulation F7 has showed drug release of 89.82% with a drug loading content of 36.76 mg. It was found that there was no significant change in percentage drug release for selected formulation F7 before and after lyophilisation. So, it was selected further to study the release kinetics.

Table No: 18 In vitro drug release data of F1 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	0.87	5.34	5.34
2.	16	1.23	7.56	7.58
3.	24	1.89	11.64	11.67
4.	32	2.41	14.78	14.83
5.	40	2.99	18.36	18.40
6.	48	3.50	21.49	21.55
7.	56	3.88	23.81	23.89
8.	64	4.42	27.13	27.21
9.	72	4.91	30.11	30.23
10.	80	5.27	32.36	32.46
11.	88	5.52	33.90	34.0
12.	96	5.78	35.47	35.60

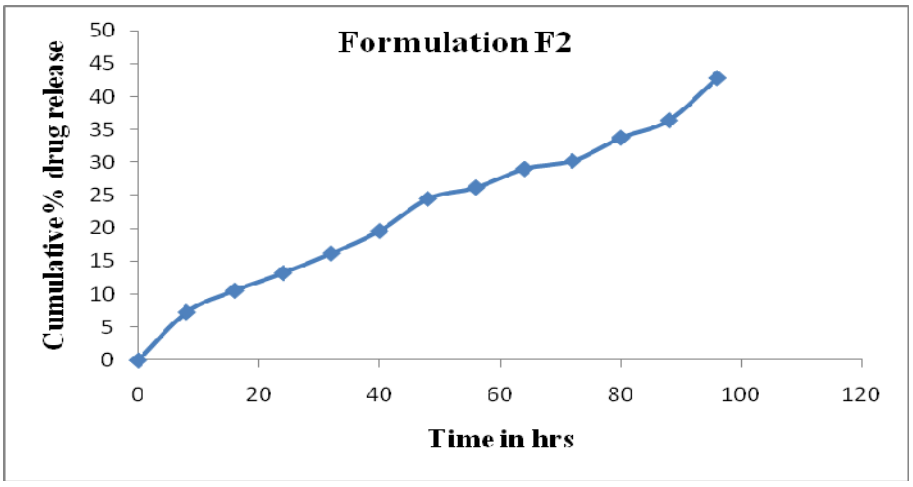


Graph No: 4 In vitro drug release profile of F1 formulation

Slope = 0.372 **Regression** = 0.987

Table No: 19 In vitro drug release data of F2 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	1.25	7.31	7.31
2.	16	1.81	10.57	10.60
3.	24	2.26	13.24	13.25
4.	32	2.77	16.19	16.24
5.	40	3.35	19.57	19.64
6.	48	4.19	24.48	24.57
7.	56	4.47	26.16	26.23
8.	64	4.95	28.93	29.04
9.	72	5.16	30.18	30.28
10.	80	5.76	33.67	33.79
11.	88	6.21	36.28	36.44
12.	96	7.31	42.75	42.87

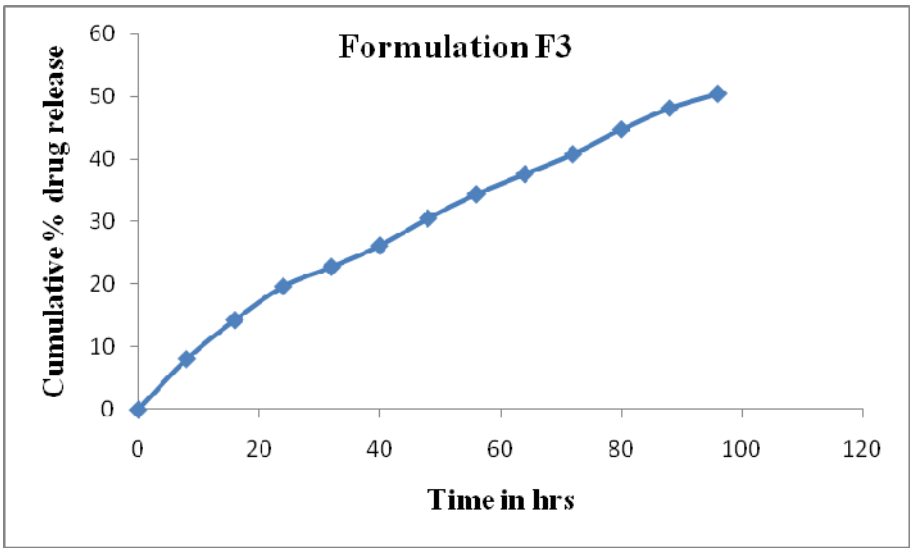


Graph No: 5 In vitro drug release profile of F2 formulation

Slope = 0.397 **Regression** = 0.985

Table No: 20 In vitro drug release data of F3 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	1.63	8.12	8.12
2.	16	2.88	14.34	14.34
3.	24	3.95	19.67	19.69
4.	32	4.57	22.72	22.80
5.	40	5.25	26.12	26.19
6.	48	6.13	30.48	30.58
7.	56	6.90	34.29	34.42
8.	64	7.54	37.47	37.62
9.	72	8.18	40.64	40.82
10.	80	8.97	44.57	44.76
11.	88	9.65	47.98	48.16
12.	96	10.11	50.24	50.46

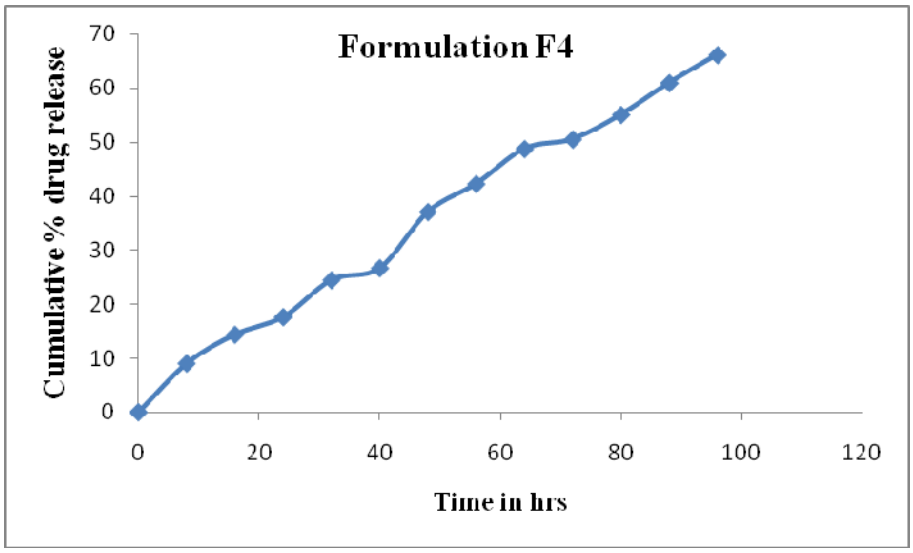


Graph No: 6 In vitro drug release profile of F3 formulation

Slope = 0.498 **Regression** = 0.982

Table No: 21 In vitro drug release data of F4 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	2.50	9.03	9.03
2.	16	3.97	14.35	14.36
3.	24	4.87	17.51	17.63
4.	32	6.76	24.38	24.46
5.	40	7.89	28.47	29.67
6.	48	10.15	36.93	37.08
7.	56	11.67	42.12	42.26
8.	64	13.46	48.54	48.74
9.	72	13.94	50.28	50.51
10.	80	14.87	54.93	55.07
11.	88	16.83	60.72	60.96
12.	96	18.26	65.88	66.15

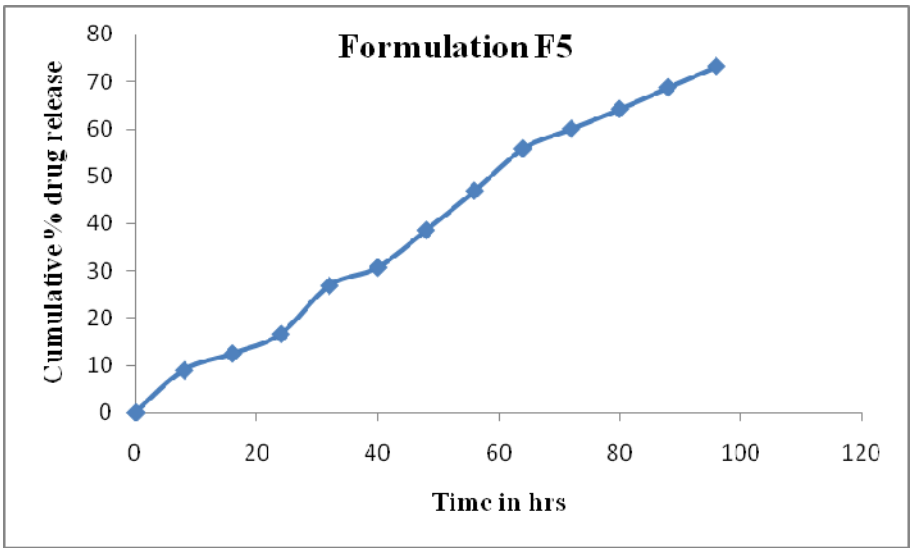


Graph No: 7 In vitro drug release profile of F4 formulation

Slope = 0.674 **Regression** = 0.992

Table No: 22 In vitro drug release data of F5 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	2.69	8.95	8.95
2.	16	3.73	12.43	12.45
3.	24	4.98	16.58	16.62
4.	32	7.86	26.17	26.34
5.	40	9.19	30.58	30.69
6.	48	11.55	38.42	38.56
7.	56	14.04	46.71	46.88
8.	64	16.72	55.63	55.83
9.	72	17.96	59.75	60.0
10.	80	19.22	63.94	64.21
11.	88	20.58	68.46	68.75
12.	96	21.92	72.91	73.23

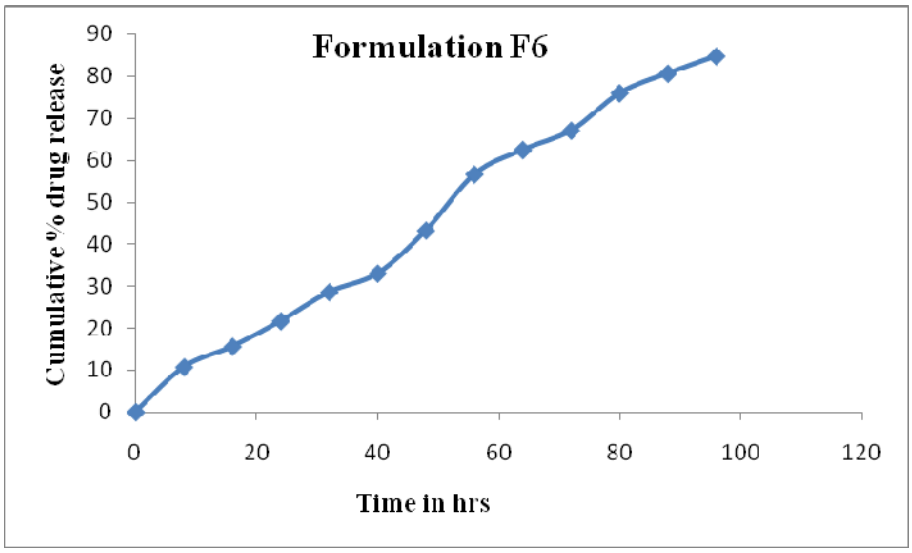


Graph No: 8 In vitro drug release profile of F5 formulation

Slope = 0.789 **Regression** = 0.991

Table No: 23 In vitro drug release data of F6 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	3.56	10.71	10.71
2.	16	7.20	15.58	15.60
3.	24	9.48	21.64	21.69
4.	32	10.95	28.47	28.57
5.	40	14.36	32.91	33.02
6.	48	18.79	43.14	43.28
7.	56	20.70	56.43	56.64
8.	64	22.24	62.18	62.44
9.	72	25.19	66.79	67.06
10.	80	26.74	75.65	75.97
11.	88	28.01	80.32	80.67
12.	96	31.09	84.12	84.77

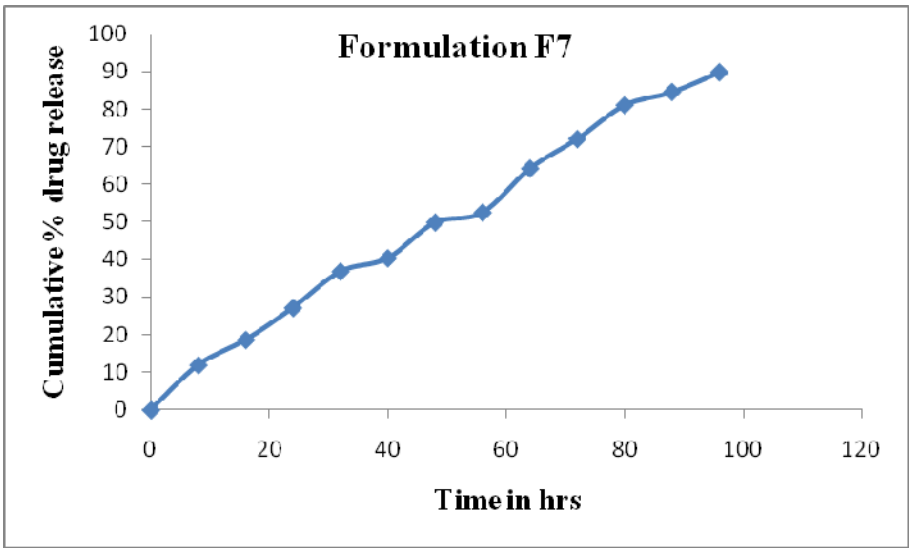


Graph No: 9 In vitro drug release profile of F6 formulation

Slope = 0.911 **Regression** = 0.991

Table No: 24 In vitro drug release data of F7 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	4.39	11.95	11.95
2.	16	6.84	18.61	18.65
3.	24	9.97	27.12	27.19
4.	32	13.50	36.71	36.83
5.	40	14.79	40.21	40.38
6.	48	17.99	48.91	49.91
7.	56	19.23	52.29	52.51
8.	64	23.54	64.01	64.24
9.	72	26.41	71.79	72.10
10.	80	29.71	80.78	81.11
11.	88	30.98	84.21	84.61
12.	96	32.90	89.43	89.84

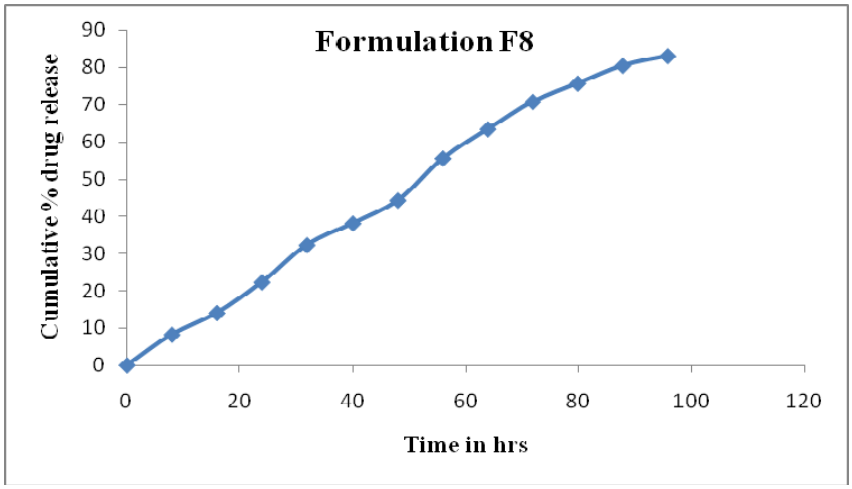


Graph No: 10 In vitro drug release profile of F7 formulation

Slope = 0.929 **Regression** = 0.993

Table No: 25 In vitro drug release data of F8 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	2.87	8.19	8.19
2.	16	4.91	14.01	14.04
3.	24	7.79	22.24	22.28
4.	32	11.27	32.15	32.24
5.	40	13.31	37.98	38.11
6.	48	15.47	44.12	44.30
7.	56	19.41	55.36	55.56
8.	64	22.15	63.17	63.43
9.	72	24.71	70.48	70.77
10.	80	26.43	75.38	75.71
11.	88	28.09	80.12	80.47
12.	96	28.99	82.67	83.06

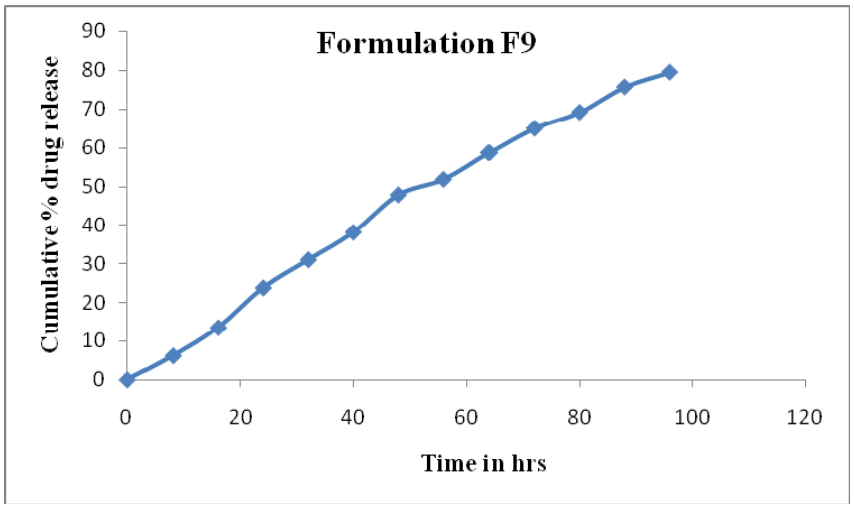


Graph No: 11 In vitro drug release profile of F8 formulation

Slope = 0.914 **Regression** = 0.991

Table No: 26 In vitro drug release data of F9 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	2.03	6.17	6.17
2.	16	4.45	13.48	13.50
3.	24	7.83	23.72	23.78
4.	32	10.20	30.91	31.01
5.	40	12.54	37.98	38.13
6.	48	15.65	47.42	47.58
7.	56	17.04	51.63	51.84
8.	64	19.30	58.46	58.70
9.	72	21.37	64.72	65.01
10.	80	22.75	68.91	69.22
11.	88	24.90	75.42	75.75
12.	96	26.14	79.17	79.54

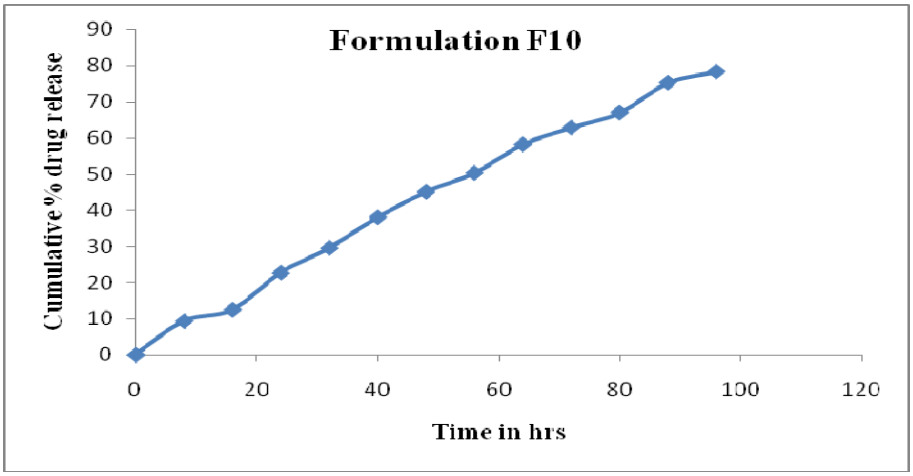


Graph No: 12 In vitro drug release profile of F9 formulation

Slope = 0.852 **Regression** = 0.990

Table No: 27 In vitro drug release data of F10 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	2.91	9.37	9.37
2.	16	3.87	12.46	12.49
3.	24	7.06	22.74	22.77
4.	32	9.16	29.48	29.57
5.	40	11.79	37.93	38.06
6.	48	13.96	44.92	45.09
7.	56	15.58	50.13	50.33
8.	64	18.02	57.98	58.21
9.	72	19.45	62.57	62.85
10.	80	20.74	66.73	67.02
11.	88	23.31	74.32	75.30
12.	96	24.24	77.98	78.34

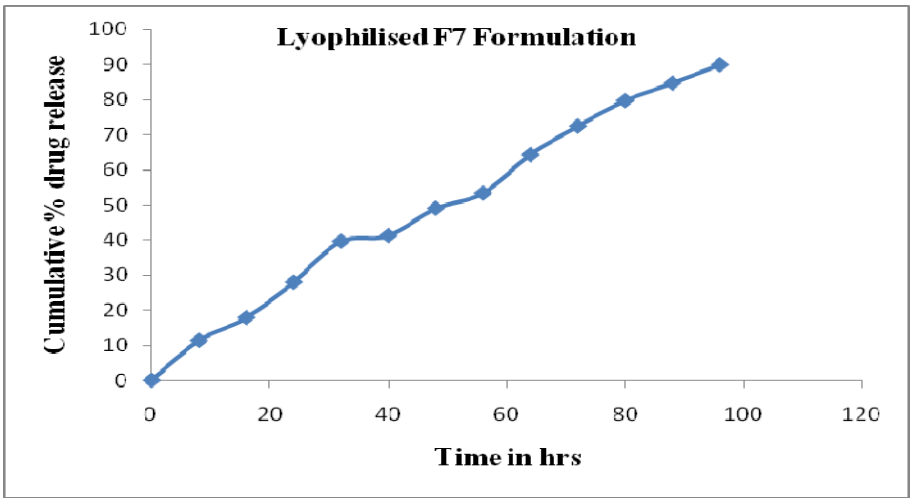


Graph No: 13 In vitro drug release profile of F10 formulation

Slope = 0.829 **Regression** = 0.992

Table No: 28 In vitro drug release data of Lyophilised F7 formulation

S.No	Time (hrs)	Amount of drug release (mg)	Percentage drug release	Cumulative % drug release
1.	8	4.18	11.35	11.35
2.	16	6.55	17.79	17.82
3.	24	10.26	27.86	27.92
4.	32	13.24	35.94	35.96
5.	40	15.11	41.0	41.17
6.	48	17.97	48.76	48.95
7.	56	19.54	53.02	53.25
8.	64	23.57	63.97	64.20
9.	72	26.54	72.01	72.32
10.	80	29.21	79.25	79.60
11.	88	31.77	84.19	84.57
12.	96	32.86	89.40	89.82

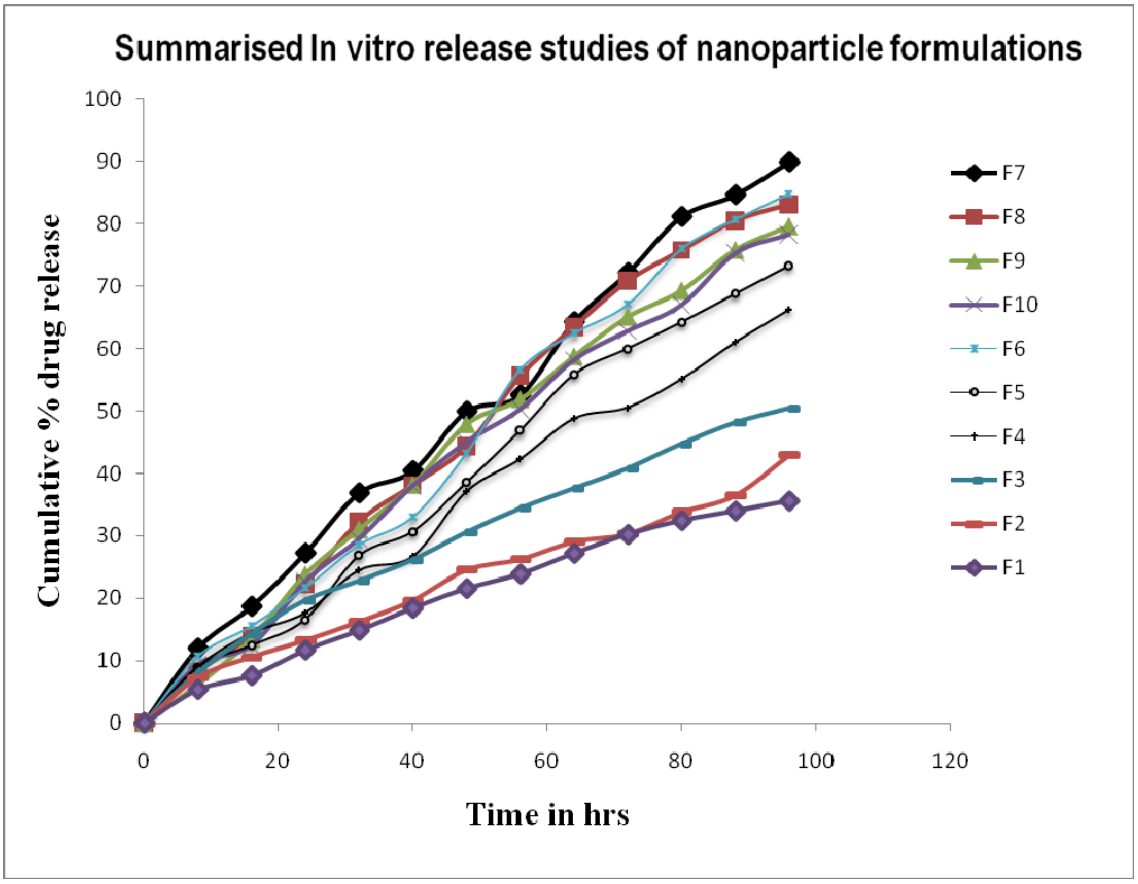


Graph No: 14 In vitro drug release profile of F10 formulation

Slope = 0.924 **Regression** = 0.993

Table No: 29 Summary of In vitro drug release of Paclitaxel nanoparticles

S.No	Formulation code	Cumulative % drug release at 96 hours
1.	F1	35.60
2.	F2	42.87
3.	F3	50.46
4.	F4	66.15
5.	F5	73.23
6.	F6	84.77
7.	F7	89.84
8.	F8	83.06
9.	F9	79.54
10.	F10	78.34



Graph No:15 Summarised Invitro drug release profiles of all Paclitaxel nanoparticle formulations

7.1.3 RELEASE KINETICS

The release kinetics was studied for an optimised freeze dried F7 formulation by plotting the graphs for different kinetic models by using the in vitro drug release data.

a) Zero Order Plot:

Table No: 30 Zero-Order Kinetics plot data for an optimized freeze dried F7 formulation

Time (hrs)	Cumulative % drug release
8	11.35
16	17.82
24	27.92
32	35.96
40	41.17
48	48.95
56	53.25
64	64.20
72	72.32
80	79.60
88	84.57
96	89.82

Graph No: 16 Zero-Order release profile for an optimized freeze dried F7 formulation

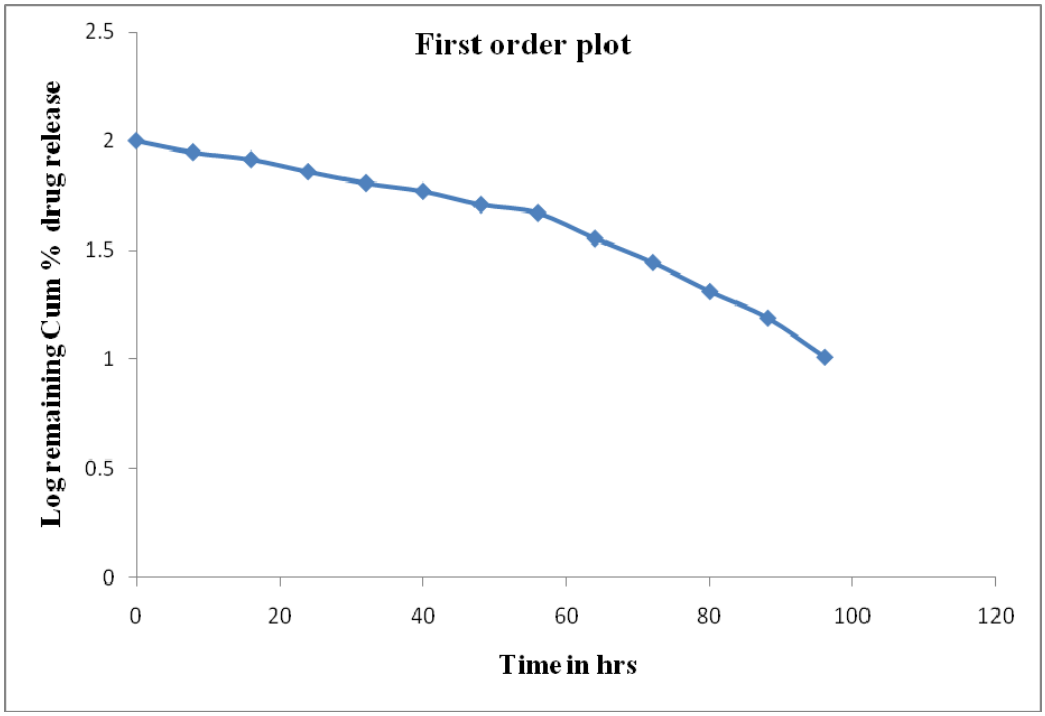
Zero order release kinetics	
Rate constant k_0	0.929 hr^{-1}
R^2	0.995

b) First Order Plot:

Table No: 31 First-Order Kinetics plot data for an optimized freeze dried F7 formulation

Time (hrs)	Log remaining Cumulative % drug release
8	1.9476
16	1.9147
24	1.8578
32	1.8064
40	1.7695
48	1.7079
56	1.6697
64	1.5530
72	1.4421
80	1.3096
88	1.1883
96	1.0081

Graph No: 17 First-Order release profile for an optimized freeze dried F7 formulation



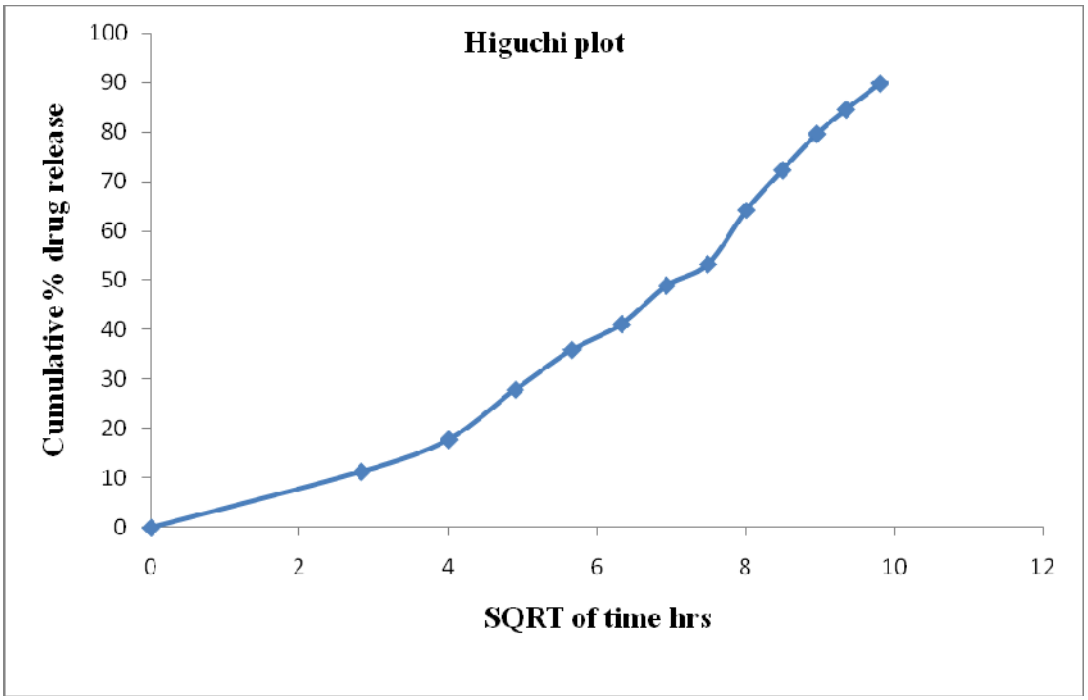
First order release kinetics	
Rate constant k_t	$0.02hr^{-1}$
R^2	0.935

c) Higuchi Plot:

Table No: 32 Higuchi Kinetics plot data for an optimized freeze dried F7 formulation

SQRT of time (hrs)	Cumulative % drug release
2.828	11.35
4.0	17.82
4.898	27.92
5.656	35.96
6.324	41.17
6.928	48.95
7.483	53.25
8.0	64.20
8.485	72.32
8.944	79.60
9.380	84.57
9,797	89.82

Graph No: 18 Higuchi release profile for an optimized freeze dried F7 formulation



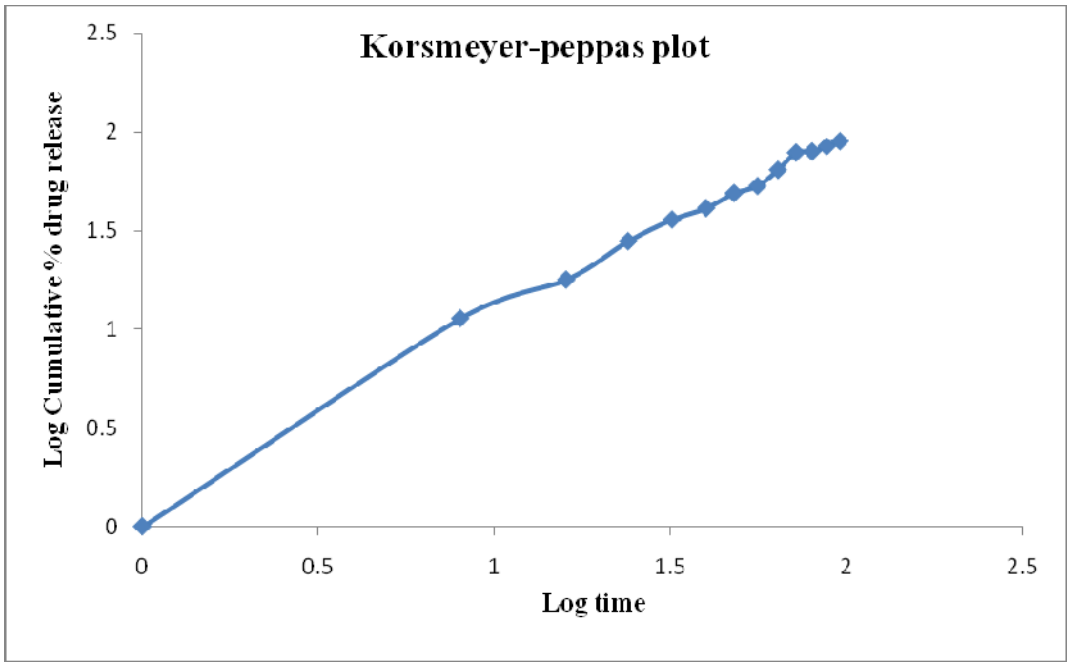
Higuchi release kinetics	
R^2	0.987

d) Korsmeyer – Peppas model kinetics plot:

Table No: 33 Korsmeyer- Peppas kinetics plot data for an optimized freeze dried F7 formulation

Log time (hrs)	Log cumulative % drug release
0.9030	1.0549
1.2041	1.2509
1.3802	1.5549
1.5051	1.5558
1.6020	1.6145
1.6812	1.6897
1.7481	1.7263
1.8061	1.8075
1.8573	1.8952
1.9030	1.9009
1.9444	1.9272
1.9822	1.9535

Graph No: 19 Korsmeyer-Peppas release profile for an optimized freeze dried F7 formulation



Korsmeyer-peppas model release kinetics	
R^2	n value
0.992	0.408

Zero order plot:

Zero order plot of a freeze dried formulation F7 was found to be linear with a regression value of 0.995, which signifies that the drug was released in a controlled manner from the nanoparticles during the release study.

First order plot:

The first order plot was made by plotting log remaining cumulative % drug release against time and the regression value was found to be 0.935 which indicates that drug release was not followed the first order rate kinetics.

Higuchi plot:

Higuchi plot was found to be of linear with a regression value of 0.987, which indicates that diffusion was one of the mechanisms of the drug release from Nanoparticle matrices.

Korsmeyer-Peppas plot:

The type of invitro mechanism of drug release was best explained by Korsmeyer- Peppas plot. The plot was found to be linear with R^2 value 0.992 and diffusion exponent 'n' value was 0.408, According to Korsmeyer-pepps equation, mechanism of drug release based on 'n' (given in the table no: 4), which indicates that mechanism of drug release from copolymer matrices was followed Fickian diffusion.

So, the kinetic studies of an optimized freeze dried F7 formulation with different kinetic models has showed that the Paclitaxel release profile was best fitted with zero order plot and release mechanism was found to be Fickian diffusion based on 'n' value from Korsmeyer-Peppas plot.

7.2 Physical characterization:

The Paclitaxel loaded MPEG-b-PCL Nanoparticle formulation before and after the lyophilisation was subjected to study particle size analysis, Zeta potential and poly dispersive index (PDI) by using Malvern instruments, Zetasizer ver. 6.01and surface morphology by Scanning electron microscopy (field instruments).

The results were shown in the Table No: 34, Reports 1, 2, 3, 4, 5, 6 and figures 2, 3.

Table No: 34 Physicochemical characteristics of an optimised Paclitaxel Nanoparticle formulation before and after Lyophilisation

S.No.	Formulation F7	Average particle Size (nm)	Zeta potential (mV)	Poly dispersive Index
1.	Before Lyophilization	169.7	- 19.6	0.195
2.	After Lyophilization	187.0	- 15.9	0.170

Report No: 1 Particle size distribution of formulation F7 before lyophilisation.

Size Distribution Report by Intensity

v2.0

Malvern

Sample Details

Sample Name: NP F7 BEFORE LYO
SOP Name: NP O1
General Notes:

File Name: NP F7
Record Number: 237
Material RI: 1.60
Material Absorbtion: 0.00
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Thursday, September 29, 20...

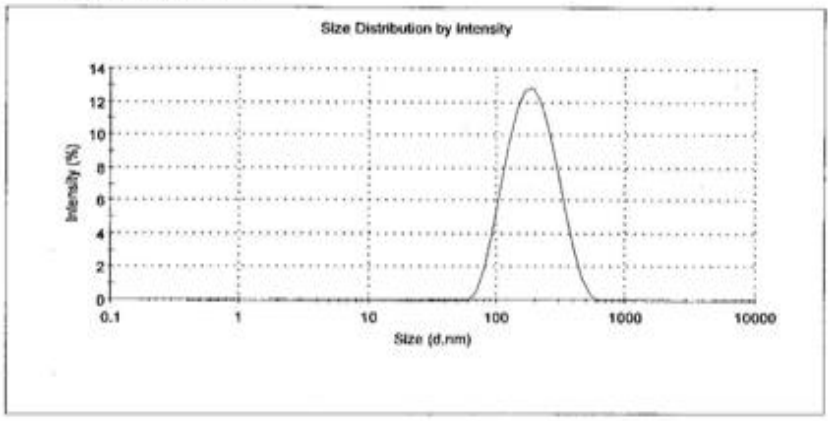
System

Temperature (°C): 25.0
Count Rate (kcps): 331.8
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 4.65
Attenuator: 5

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	169.7	**Peak 1:**	199.5	100.0	84.82
PdI:	0.195	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.950	**Peak 3:**	0.000	0.0	0.000

Result quality : Good



Report No: 2 Particle size distribution of formulation F7 before lyophilisation.

Size Distribution Report by Intensity

v2.0

Malvern

Sample Details

Sample Name: NP F7 BEFORR LYO
SOP Name: NP O1
General Notes:

File Name:	NP F7	**Dispersant Name:**	Water
Record Number:	237	**Dispersant RI:**	1.330
Material RI:	1.60	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.00	**Measurement Date and Time:**	Thursday, September 29, 20...

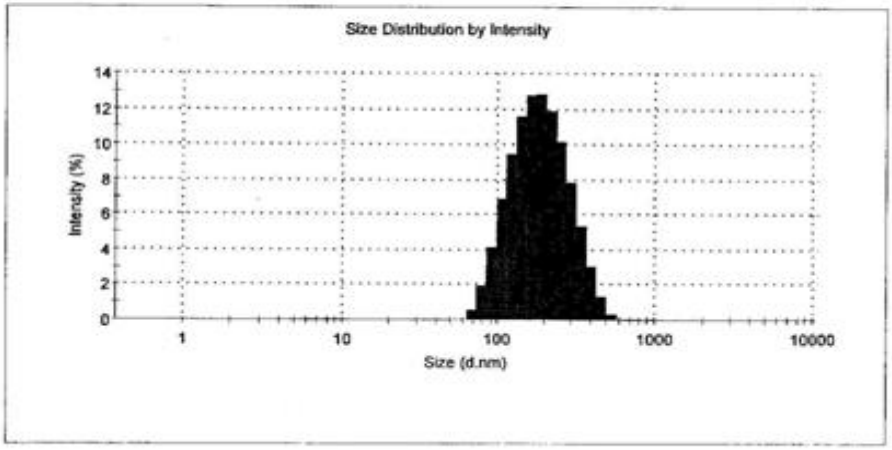
System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	331.8	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	5

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	169.7	**Peak 1:**	199.5	100.0	84.82
PdI:	0.195	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.950	**Peak 3:**	0.000	0.0	0.000

Result quality : Good



Report No: 3 Zeta potential of an optimised formulation F7 before lyophilisation.

Zeta Potential Report

v2.2

Malvern

Sample Details

Sample Name: NP F7 BEFORE LYO
SOP Name: NP 02
General Notes:

File Name: NP F7
Record Number: 238
Date and Time: Thursday, September 29, 2011 1:...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

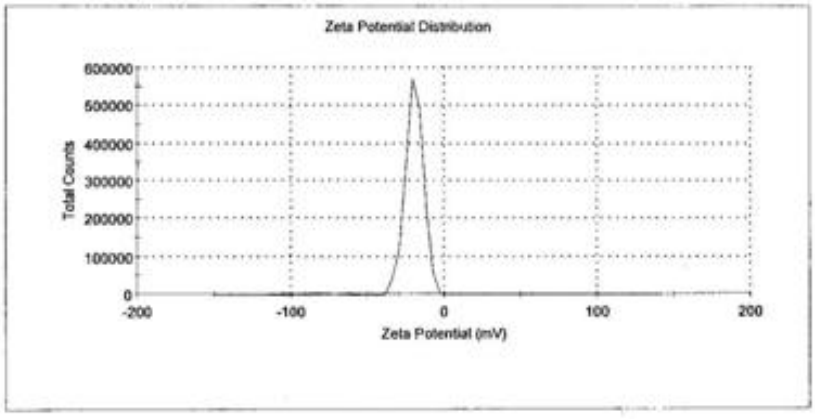
System

Temperature (°C): 25.0
Count Rate (kcps): 330.8
Cell Description: Zeta dip cell
Zeta Runs: 12
Measurement Position (mm): 4.50
Attenuator: 7

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -19.6	**Peak 1:**	-19.6	100.0	5.68
Zeta Deviation (mV): 5.68	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.852	**Peak 3:**	0.00	0.0	0.00

Result quality : Good



Report No: 4 Particle size distribution of formulation F7 after lyophilisation.

Size Distribution Report by Intensity

r2.0

Malvern

Sample Details

Sample Name: NP F7 AFTER LYO
SOP Name: NP 03
General Notes:

File Name: NP F7
Record Number: 7
Material RI: 1.60
Material Absorbtion: 0.00
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Thursday, October 20, 2011 ...

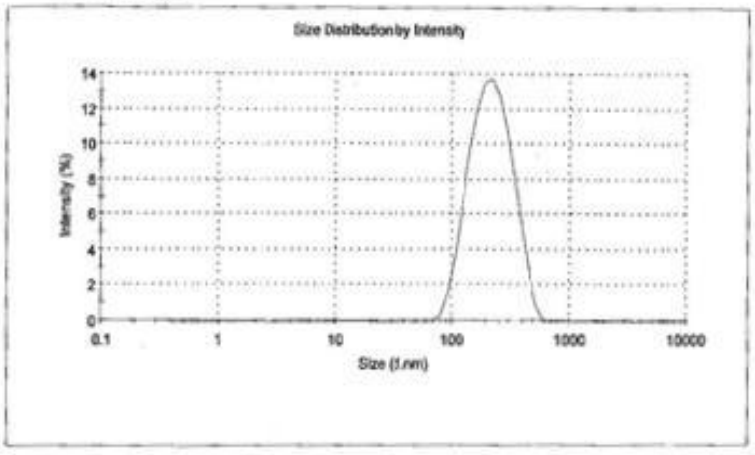
System

Temperature (°C): 25.0
Count Rate (kcps): 293.9
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 1.05
Attenuator: 4

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	187.0	Peak 1:	227.0	100.0	89.23
PdI:	0.170	Peak 2:	0.000	0.0	0.000
Intercept:	0.951	Peak 3:	0.000	0.0	0.000

Result quality : Good



Report No: 5 Particle size distribution of formulation F7 after lyophilisation.

Size Distribution Report by Intensity

v2.0

Malvern

Sample Details

Sample Name: NP F7 AFTER LYO
SOP Name: NP 03
General Notes:

File Name: NP F7
Record Number: 7
Material RI: 1.60
Material Absorbtion: 0.00
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Thursday, October 20, 2011 ...

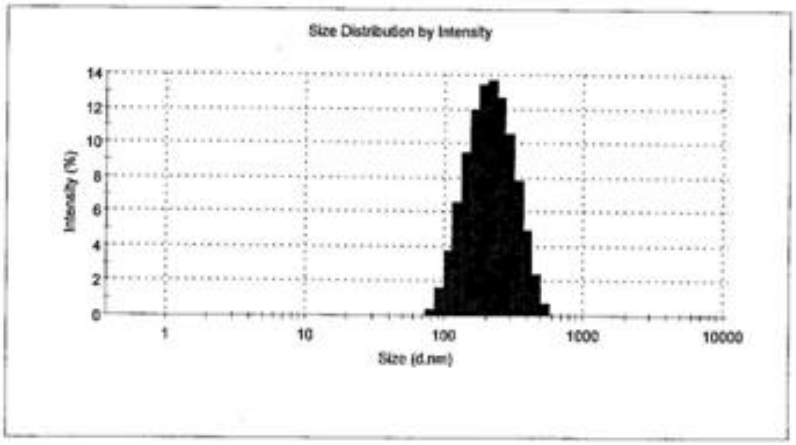
System

Temperature (°C): 25.0
Count Rate (kcps): 293.9
Cell Description: Disposable sizing cuvette
Duration Used (s): 60
Measurement Position (mm): 1.05
Attenuator: 4

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 187.0	**Peak 1:**	227.0	100.0	89.23
PdI: 0.170	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.951	**Peak 3:**	0.000	0.0	0.000

Result quality : Good



Report No: 6 Zeta potential of an optimised formulation F7 after lyophilisation.

Zeta Potential Report

v2.2

Malvern

Malvern Instruments Ltd. © Copyright 2008

Sample Details

Sample Name: NP F7 AFTER LYO
SOP Name: NP 04
General Notes:

File Name: NP F7
Record Number: 8
Date and Time: Thursday, October 20, 2011 4:16:...

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

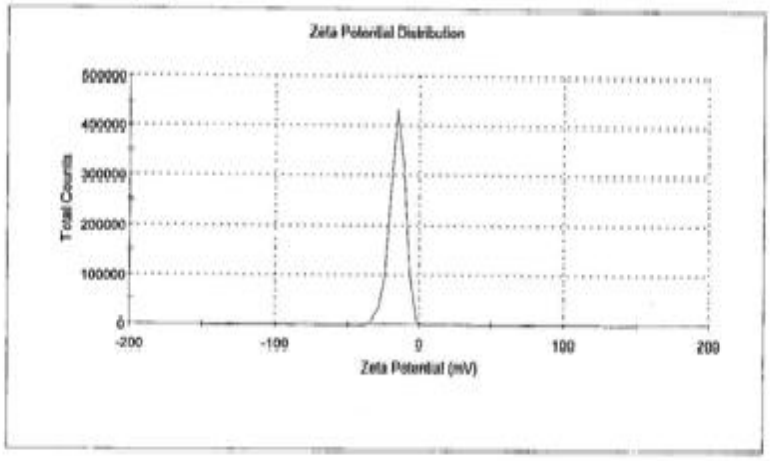
Temperature (°C): 25.0
Count Rate (kcps): 174.3
Cell Description: Zeta dip cell

Zeta Runs: 12
Measurement Position (mm): 4.50
Attenuator: 6

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -15.9	Peak 1:	-15.9	100.0	5.48
Zeta Deviation (mV): 5.48	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 1.03	Peak 3:	0.00	0.0	0.00

Result quality : Good



SCANNING ELECTRON MICROSCOPY

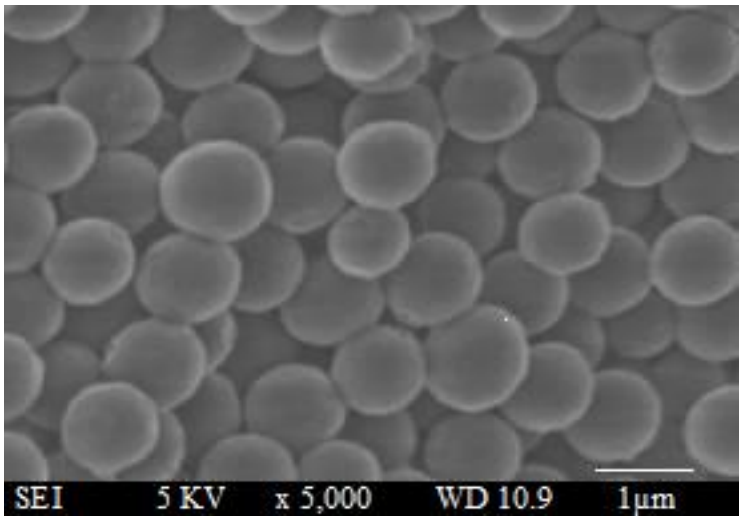


Fig No: 2 SEM photograph of Nanoparticle formulation F7 before Lyophilisation

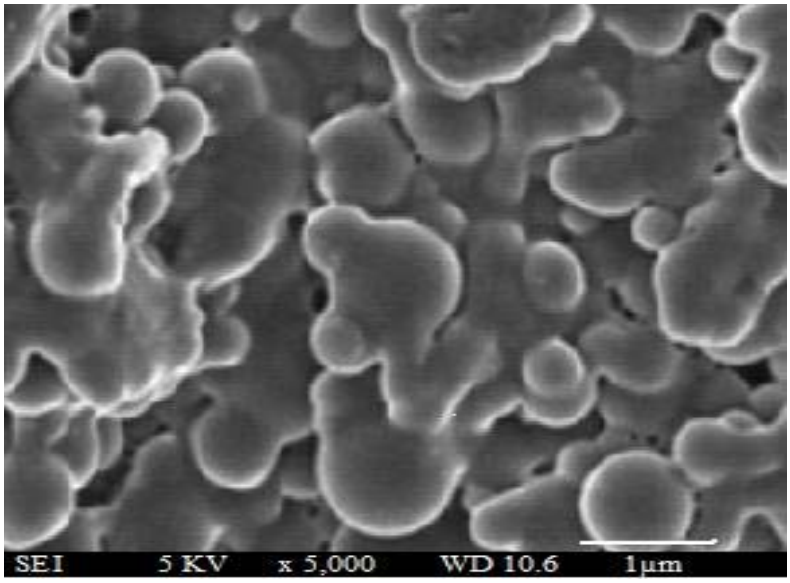


Fig No: 2 SEM photograph of Nanoparticle formulation F7 after Lyophilisation

7.2.1 Particle size:

The average particle size of the nanoparticle formulation F7, before and after lyophilisation was found to 169.7 nm and 187.0 nm respectively. The observed particle sizes were fall in the range of nano micellar level (10 nm-200 nm) and also the calculated ratio value of nanoparticle size before and after lyophilisation was nearer to the value of one which was an important indication of conservation of the nanoparticle size after the freeze drying. The results were shown in table no: 34 and reports: 1, 3.

7.2.2 Zeta potential:

The zeta potential values of the formulation F7, before and after freeze drying were -19.6 mV and -15.9 mV respectively. The decrease in the zeta potential was due to the effect of PEG in the outer shell of nanoparticles and also probably due to the adsorption of Paclitaxel on the nanoparticle surfaces during preparation process. The lower negative zeta potential results in a reduction of cell repulsion to the nanoparticles up to a certain degree and also contributes to the stability of nanosuspension. The results were shown in reports: 2, 4.

7.2.3 Scanning Electron Microscopy:

The SEM photographs of F7 formulation before and after freeze drying showed that, the colloidal nanoparticles before the freeze drying have a spherical shape with nanometer size range and smooth surfaces without any noticeable cracks or pin holes. The SEM image of after lyophilisation has shown matted like appearance or adhesion between the nanoparticles, this was because of lyoprotectant PVA around the nanoparticles.

7.2.4 Poly Dispersity index:

Poly dispersity index (PDI) values for the formulation F7, before and after lyophilisation were found to be 0.195 and 0.17. This lower PDI values indicates that the Nanoparticle formulation before and after freeze drying has mono dispersive characteristics and narrow particle size distribution. The results were shown in reports: 3, 5.

7.3 STABILITY STUDIES OF PACLITAXEL NANOPARTICLES:

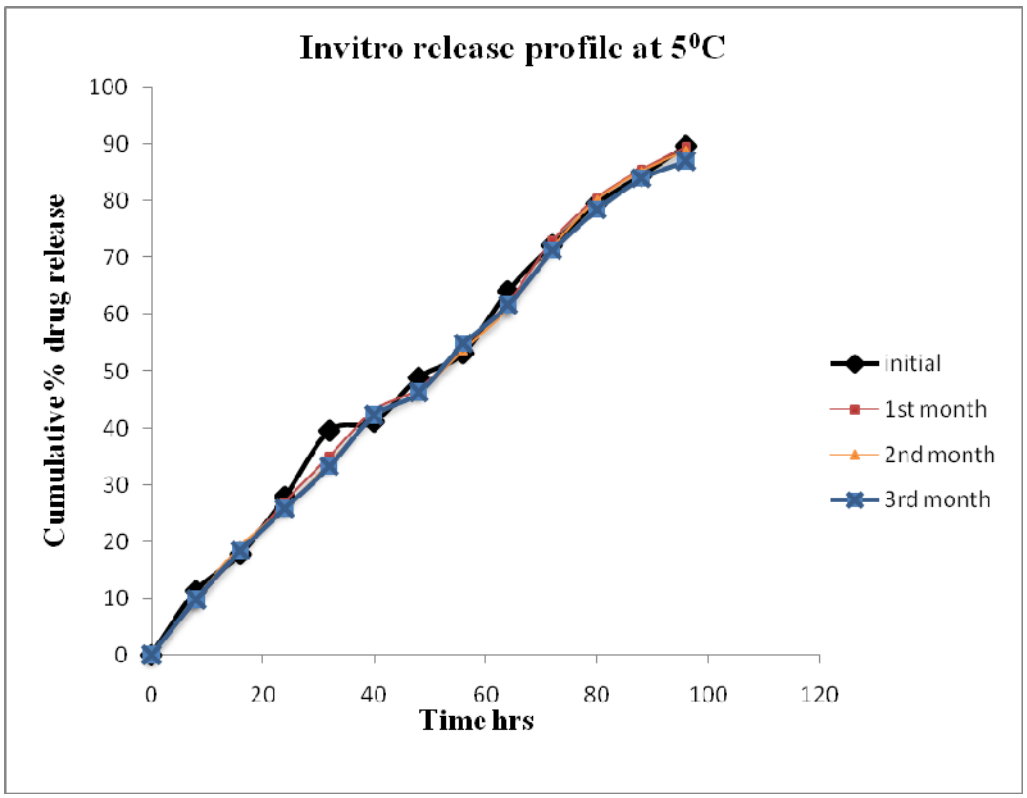
The stability studies for an optimised freeze dried formulation F7 was carried for 3 months. The evaluation of nanoparticles was carried out by testing Entrapment Efiiciency (%) and in vitro drug release in a interval of one month duration. The results of the tests were tabulated in the below table

Table No 35: Summarised stability studies for Freeze dried F7 formulation

S.No	Storage condition	Test parameters	1st month	2nd month	3rd month
1.	5^0C (Refrigerator)	Entrapment efficiency (%)	91.70%	91.60%	89.97%
		In vitro drug release (%)	89.52%	88.98%	87.89%
2.	25^0C/65% RH (Room temperature)	Entrapment Efficiency (%)	91.32%	89.67%	86.55%
		In vitro drug release (%)	89.68%	87.42%	86.38%

Table No: 36 Summarised stability study data of optimized freeze dried F7 formulation for 3 months at 5^0C

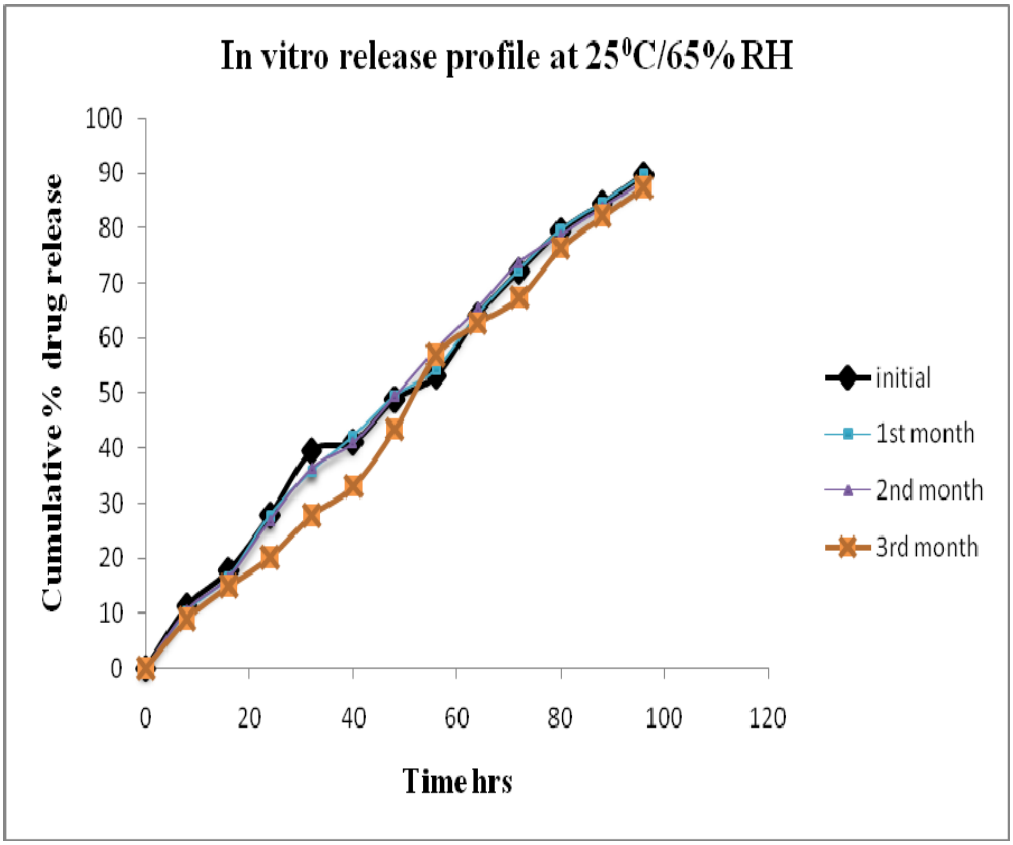
S.No	Time (hrs)	Cumulative % drug release			
		Initial	1st Month	2nd Month	3rd Month
1.	8	11.35	9.47	10.01	9.82
2.	16	17.82	18.15	19.31	18.39
3.	24	27.92	27.10	25.96	25.85
4.	32	35.96	34.99	33.83	33.28
5.	40	41.17	43.08	42.03	42.24
6.	48	48.95	46.95	46.30	46.29
7.	56	53.25	54.30	53.80	54.89
8.	64	64.20	62.13	61.10	61.69
9.	72	72.32	72.94	71.86	71.23
10.	80	79.60	80.52	80.24	78.50
11.	88	84.57	85.45	85.30	84.02
12.	96	89.82	89.52	88.98	87.89



Graph No: 20 Comparison of in vitro release profiles at 5^0C for 3 months

Table No: 37 Summarised stability study data of optimized freeze dried F7 formulation for 3 months at 25^0C/65% RH

S.No	Time (hrs)	Cumulative % drug release			
		Initial	1st Month	2nd Month	3rd Month
1.	8	11.35	10.23	10.58	9.02
2.	16	17.82	16.62	16.58	14.94
3.	24	27.92	27.81	26.92	20.24
4.	32	35.96	35.83	36.34	27.81
5.	40	41.17	42.08	40.93	33.12
6.	48	48.95	49.40	49.35	43.43
7.	56	53.25	54.28	57.95	56.91
8.	64	64.20	64.24	65.44	62.70
9.	72	72.32	72.34	73.75	67.35
10.	80	79.60	79.61	78.67	76.30
11.	88	84.57	84.57	83.77	82.09
12.	96	89.82	89.68	87.42	86.38



Graph No: 21 Comparison of in vitro release profiles at 25^0C/ 65% RH for 3 months

The results of the stability studies of the prepared Paclitaxel nanoparticles, reveals that the freeze dried formulation was stable in the refrigerated condition for a period of 3 months. The entrapment efficiency and drug release of the formulation at 5^0C did not showed any significant change during storage condition when compared with the same formulations stored at 25^0C/ 65% RH which has shown significant changes.

8. CONCLUSION

The present work entitled, “Development and characterisation of an Antimicrotubular Taxane loaded MPEG-b-PCL Nanoparticles” was aimed to develop a nanoparticulate drug delivery system of an anticancer drug Paclitaxel using a biodegradable amphiphilic diblock MPEG-b-PCL copolymer.

The main objective of the work was to develop a controlled release dosage form of Paclitaxel nanoparticles for reducing severe side effects associated with conventional delivery system like paclitaxel injection(Taxol), because of the low solubility of paclitaxel in water, it was first dissolved in Cremophore EL (polyethoxylated castor oil) and Ethanol and then administered, use of this solvent mixture cause serious side effects like sever hypersensitivity reactions, neurotoxicity, nephrotoxicity and hypotensive reactions on iv administration. So, the inclusion of Paclitaxel in nanoparticle formulations has proved to be a good approach to eliminate effects of Cremophore EL and Ethanol.

The preformulation studies were performed by using infrared spectroscopy, the obtained spectra revealed the absence of molecular interactions between the Paclitaxel, MPEG-b-PCL copolymer and PVA used in the formulation.

The Paclitaxel loaded nanoparticles were prepared by Emulsion solvent evaporation technique. The formulations containing drug: polymer in various proportions like 1: 3.5, 1: 4, 1:4.5, 1:5, 1:5.5, 1: 6, 1:6.5, 1:7, 1:7.5 and 1:8 were made and subjected to entrapment efficiency and in vitro dissolution studies. The results were indicated that formulation F7 containing drug: polymer in the ratio of 1:6.5 possessed an entrapment efficiency of 92% and a drug release of 89.84% and thereby considered to be the best formulation.

The optimized formulation F7 was lyophilized by freeze dryer to increase the stability of nanoparticle formulation during storage. It was further evaluated for % EE and drug release; the results showed that there was no significant change in EE% and in vitro drug release profile when compared with unlyophilised F7 formulation.

The release kinetics of an optimised lyophilised formulation showed zero order drug release and followed Fickian diffusion when applied to the Korsmeyer-peppas model for mechanism of drug release from copolymer matrices.

The particle sizes of the optimised Nanoparticle formulation F7 before and after lyophilisation were found to be in the size range of nanomicelles (10nm-200 nm) and the lower negative zeta potential values contributes to reduction of cell repulsion to the nanoparticles up to a certain degree and also stabilizes nanosuspension.

Stability testing of optimised freeze dried formulation F7 at 5^0 C and 25^0C/65 % RH for 3 months showed that formulation was remained stable only at 4^0 C throughout the study period. So, the storage condition proposed for the prepared paclitaxel Nanoparticle formulation was refrigerated (5^0C) condition.

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