PREPARATION AND EVALUATION OF NANOPARTICLES CONTAINING IMATINIB MESYLATE AND THE COMPLEX OF IMATINIB MESYLATE COBALT (II) CHLORIDE

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
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Submitted by
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APRIL 2017

CERTIFICATE

This is to certify that the M.Pharm dissertation entitled **"Preparation and Evaluation of Nanoparticles Containing Imatinib Mesylate and the Complex of Imatinib Mesylate Cobalt (II) Chloride"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai was carried out by **M.Raja (Reg. 261510155)** in the Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under my direct supervision, guidance and to my fullest satisfaction.

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This is to certify that **Mr.M.Raja (Reg. 261510155),** Department of Pharmaceutics, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, worked on the *In vitro* and *Invivo* anticancer activity of nanoparticles containing Imatinib Mesylate in Post Graduate Pharmacology Laboratory which is a part of his dissertation work entitled **"Preparation and Evaluation of Nanoparticles Containing Imatinib Mesylate and the Complex of Imatinib Mesylate Cobalt (II) Chloride"** being submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai under my supervision to fullest satisfaction.

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CONTENTS

ABBREVIATIONS

nm	:	nanometer
ml	:	milliliter
min	:	minutes
hrs	:	hours
mm	:	millimeter
µm	:	micrometer
PBS	:	phosphate buffer saline
IPA	:	Iso propyl alcohol
FT-IR	:	fourier transform-infra red spectroscopy
WHO	:	World Health Organization
MDX	:	Metadoxine
SA	:	Sodium Alginate
Pdi	:	Poly dispersive index
mg	:	milligram
kg	:	kilogram
L	:	liter
gm	:	gram
u.v.	:	Ultra-violet
mV	:	millivolt
log	:	logarithm
rpm	:	Rotation per minute
cm	:	centimeter
KBR	:	Potassium bromide
%EE	:	Percentage entrapment efficiency
K_0	:	Zero order rate constant
C_0	:	Concentration at zero time

R^2	:	regression value
kV	:	kilo volt
^0C	:	Degree Celsius
BBB	:	Blood Brain Barrier
NPs	:	Nanoparticles
PVA	:	Poly Vinyl Alcohol
HPMC	:	Hydroxy Propyl Methyl Cellulose
CML	:	Chronic Myeloid Leukemia
CHR	:	Complete Monitoring Committee
SMC	:	Study Monitoring Committee
$CoCl_2$ NPs	:	Cobalt chloride nanoparticles
XRD	:	X-Ray Diffraction
DLA	:	Dalton's Lymphoma Ascities
I.P	:	IntraPeritonieal
MST	:	Mean Survival Time
ILS	:	Increase Life Span

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INTRODUCTION

Nanotechnology, the term invented by Norio taniguchi in 1974 at the University of Tokyo, provides opportunities to assimilate science and technology in life and physical sciences at nanolevel. Nanotechnology is the integration of science and engineering disciplines to produce products either with or containing nanoscale particles. Thus, it is applied in various areas of biomedical sciences. Indeed, this technology helps to improve the innovation of biomarkers, helps in molecular diagnosis, drug delivery, regenerative medicine, cellular trafficking, bio imaging and gene delivery.

The US FDA specifies that nanotechnology involves: research and technology development at atomic, molecular or macromolecular levels, in the length scale of approximately 1-100 nm ; creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size; and the ability to control or manipulate on atomic scale. Nanoparticles have range of potential application in short term in new cosmetics, textiles and paints. These technologies can increase the potency of traditional small molecules of drugs in addition to potentially providing a mechanism for treating previously incurable diseases (Shinde et al., 2012).

Nanoparticles are sub-nanosized colloidal structures composed of natural or synthetic or semi synthetic polymer. The first reported nanoparticles were based on non-biodegradable polymeric systems (polyacrylamide, polymethyl-methacrylate and polystyrene etc.) (Birrenbach and Speiser, 1976; Kreuter and Speiser, 1976) The polymeric nanoparticles can carry drug(s) or proteinaceous substances, i.e. antigen(s). These bioactive are entrapped in the polymer matrix as particulates enmesh or solid solution or may be bound to the particle surface by physical adsorption or chemical.

Nanomedicine is a subset of nanotechnology, they use tiny particles which are more than 10 million times smaller when compared to human body. In nanomedicine, particles are very smaller than the living cell and due to this, nanomedicine has many revolutionary opportunities in fighting against all types of cancer, neurodegenerative disorders and even other diseases (Shinde et al.,2012).

The emergence of nanotechnology has made a significant impact on clinical therapeutics in the last two decades. Advances in bio compatible nanoscale drug carriers such as liposomes and polymeric nanparticles have enabled more efficient and safer delivery of a myriad of drugs. Advantages in nanoparticle drug delivery, particularly at the systemic level, include longer circulation half-lives, improved pharmaco kinetics and reduced side effects. In cancer treatments, nanoparticles can further rely on the enhanced permeability and retention effect caused by leaky tumor vasculatures for better drug accumulation at the tumor sites. These benefits have made therapeutic nanoparticles a promising candidate to replace traditional chemotherapy, where intravenous injection of toxic agents poses a serious threat to healthy tissues and results in dose-limiting side effects. Currently, several nanoparticle-based chemotherapeutics have emerged on the market, while many are undergoing various stages of clinical or preclinical development.

Mainly there are two types of nanoparticles, They are

1) Nanospheres - A matrix type like structure in which drug is dispersed in polymer matrix.

2) Nanocapsule - In this drug is encapsulated in central volume surrounded by continuous polymeric sheath (Nagavarma et al., 2012).

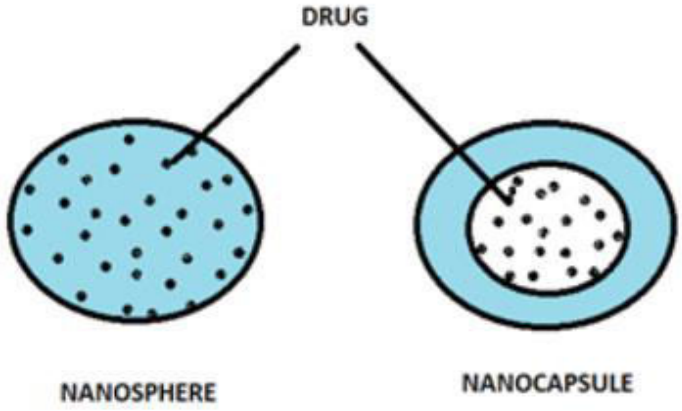

Figure: 1 Various type of nanoparticles

Advantages of Nanoparticles

- Easy preparation.
- They are targeted drug delivery.
- Due to their small size, they penetrate small capillary and taken by the cells which allows drug accumulation at target sites in the body.
- They have a good control over size and size distribution.
- They have good protection of the encapsulated drug.
- They have a longer clearance time.
- They have a high therapeutic efficacy.
- They have high bioavailability.
- Stable dosage forms of drug which are either unstable or have unacceptably low bioavailability in non nano particulate dosages forms.
- Increased surface area results in faster dissolution of active agents in an aqueous environment.
- They have faster dissolution that equates to greater bioavailability.
- Smaller drug doses are enough.
- There is a reduction in fed/fasted variability.
- Less toxic in nature (Shinde et al., 2012).

Disadvantages of Nanoparticles

- ❖ Extensive use of polyvinyl alcohol as a detergent.
- ❖ There are Issues with toxicity.
- ❖ There are limited targeting abilities.
- ❖ Discontinuation of therapy is impossible.
- ❖ They produce cytotoxicity.
- ❖ They can cause pulmonary inflammation and pulmonary carcinogenicity.
- ❖ They can even cause Alveloarar inflammation.
- ❖ The disturbance of autonomic balance caused by nanoparticles have direct effect on heart and vascular function (Shinde et al.,2012).

Properties of Nanoparticles

- Due to their smaller size, the nanoparticles readily disperse in water forming a clear colloidal dispersion and is suitable for injection via fine gauge needles.
- The preparation is very sterile and a pyrogenic.
- No gross change can occurs when gelatin nanoparticles are autoclaved at 121°C for 15 mins.
- By experimental data, it is found that the nanoparticles are non-antigenic.

Physiochemical and biological consideration in preparation of nanoparticles

The large number of molecule and manufacturing methods and possibility to stabilize these particles by freeze drying has offered more advantage over the other colloidal carrier systems eg, liposomes. However, during preparation of the nanoparticles, the following factors should be taken into consideration.

Choice of materials and methods

- It depends on the property of the drug which is associated with the nanoparticles carrier. Thus for hydrophobic drugs the continuous aqueous phase should be used for only for hydrophilic drug appear hydrophobic continuous phase should be used.
- Natural macromolecules like proteins and cellulose are degradable base for the formulation of nanoparticles just like human serum albumin, bovine serum albumin, ethyl cellulose carrier and gelatin.
- The opposite continuous phase increases absorption of drug to the nanoparticles remain in the body and it depends up on the material of which the carrier is made i.e., different degradation & elimination rates are obtained by using different materials & methods.

ex: Gelatin - very short periods of degradation and elimination

Albumin- medium fast degeneration and elimination

Long chain polycyanoacrylate–Long period for degradation and elimination.

Poly methacrylate – Very long period of degradation and elimination.

- Short persistent time carriers is intended when rapid elimination rate is necessary to avoid accumulation.
- Long persistence is necessary incase of vaccination.

Release consideration

There are two mechanisms commonly considered in explaining solute transport through a polymer matrix, they are

a) Pore mechanisms

b) Partition mechanisms.

Stability

The drug released from nanoparticles in plain solvents may be rapid compared to some of the material used like polycyanoacrylate depolymorises in aqueous media. So nanoparticles should not be stored in aqueous media and in addition, all the types of nanoparticles can be freeze dried.

Toxicity

Till now, there are very less results available on the toxicity of nanoparticles in blood and body distribution studies. No adverse reactions are reported.

Structure

According to Birren back nanoparticles are spherical in shape, they are non-toxic polymeric material with entrapped bioactive material. Particles diameter lies between 20-35µm.

Polymers used in preparation of nanoparticles

The polymers should be compatible with the body, they should be (non-toxicity) and (non- antigenicity) and they should be biodegradable and biocompatible.

Natural polymers

The most commonly employed natural polymers in preparation of polymeric nanoparticles are

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

- **Synthetic polymers**
- Polylactides (PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters
- Polycyanoacrylates
- Polycaprolactone
- Poly glutamic acid
- Poly malic acid
- Poly(N-vinyl pyrrolidone)
- Poly(methyl methacrylate)
- Poly(vinyl alcohol)
- Poly(acrylic acid)
- Poly acrylamide
- Poly(ethylene glycol)
- Poly(methacrylic acid (Nagavarma et al., 2012).

Mechanisms of drug release

The polymeric nanoparticales delivers the drug at the tissue site by any one of the 3 general physic chemical mechanisms. mainly by

1. Swelling of the polymeric nanoparticles first by hydration followed by release of drug through diffusion.
2 An enzymatic reaction which results in either rupture or cleavage or degradation of the polymer at the delivery site, there by release of the drug from the entrapped inner core.
3 Dissociation of the drug from the polymer or its de-adsorption/release from the swelled nanoparticles (Nagavarma et al., 2012).

Classification of nanoparticles

There are many approaches for classification of nano materials. Nano particles can be classified based on

- One dimensions
- two dimensions
- three dimensions

One dimension nanoparticles

One dimensional system of classification includes thin film or manufactured surfaces and has been used for decades in electronics, chemistry and engineering. Production of thin films (sizes1-100 nm) or monolayer is now common in the field of solar cells or catalysis. This thin films are used in technological applications, like information storage systems, chemical and also biological sensors, fibre-optic systems, magneto-optic and optical device (Sovan et al., 2011).

Two dimension nanoparticles

Carbon nanotubes (CNTs)

Carbon nanotubes are hexagonal network of carbon atoms, they are 1 nm in diameter and 100 nm in length, in the form of layer of graphite rolled into cylinder. CNTs are of two types, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs).The small dimensions of carbon nanotubes, combined with remarkable physical, mechanical and electrical properties, make them a unique materials. They show metallic or semi conductive properties, depending on how the carbon leaf is wound on itself. The density that nanotubes can be able to carry is extremely high and can reach even one billion amperes per square meter making it a superconductor. The mechanical strength is sixty times greater than the best steels. Carbon nanotubes have a great capacity for molecular absorption and offer a three dimensional configuration and they are chemically very stable (Sovan et al., 2011).

Three dimension nanoparticles

Fullerenes (Carbon 60):

Fullerenes are spherical cages. They contain 28 to more than 100 carbon atoms. a hollow ball which is composed of interconnected carbon pentagons and hexagons, resembles a soccer ball. Fullerenes are class of materials having unique physical properties. They can be exposed to extreme pressure and it regain their original shape when the pressure is released. They do not combine with each other and used as lubricants. They have good electrical properties and it has been used them in the electronic field, like application ranging from data storage to production of solar cells. Fullerenes offer potential application in the area of nano electronics. therefore fullerenes are empty structures with dimensions which are similar to several biological active molecules (Sovan et al., 2011).

Nano particles preparation

Nanoparticles are prepared from a variety of materials like proteins polysaccharides and synthetic polymers. The selection of matrix materials depends on many factors such as:

- Size of nanoparticles needed
- Inherent properties of the drug, like aqueous solubility and stability
- Surface characteristics like charge and permeability
- Degree of biodegradability, biocompatibility and toxicity
- Drug release profile desired
- Antigenic property of the final product

Nanoparticles preparation is most frequently by three Methods

1. From dispersion of preformed polymers
2. By polymerization of monomers
3. Ionic gelation or co-acervation of hydrophilic polymers (Nagavarma et al., 2012).

4. PREPARATION TECHNIQUES OF NANOPARTICLES

The selection of the appropriate method for the preparation of nanoparticles depends on the physicochemical characteristics of the polymer and the drug to be loaded. On the contrary, the preparation techniques largely determine the inner structure, in vitro release profile and the biological fate of these polymeric delivery systems (Kreuter et al., 1991). Two types of systems with different inner structures are apparently possible including:

- A matrix type system consisting of an entanglement of oligomer or polymer units (nanoparticles/nanospheres).
- A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (nanocapsules).

The drug can either be entrapped within the reservoir or the matrix or otherwise be adsorbed on the surface of these particulate system. The polymers are strictly structured to a nanometric size range particle(s) using appropriate methodologies.

1. Amphiphilic macromolecule cross-linking
    a. Heat cross-linking
    b. Chemical cross-linking
2. Polymerization based methods
    a. Polymerization of monomers in situ
    b. Emulsion (micellar) polymerization
    c. Dispersion polymerization
    d. Interfacial condensation polymerization
    e. Interfacial complexation

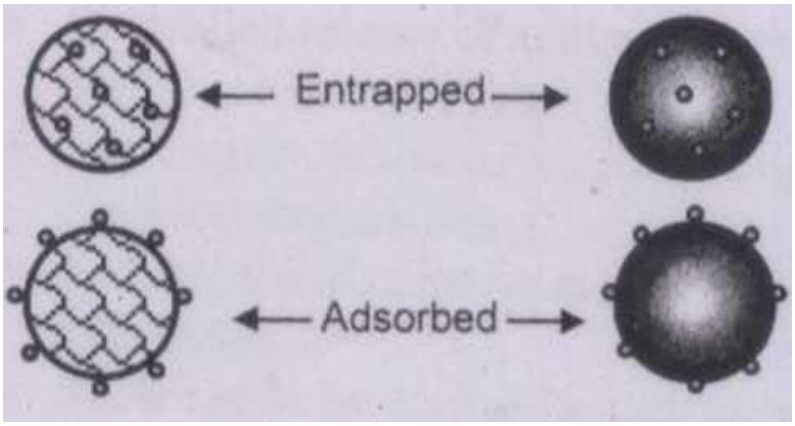

Figure: 2 Nanoparticles/Nanospheres and Nanocapsules with the mode of drug entrapment

3. Polymer precipitation methods

   a. Solvent extraction/evaporation

   b. Solvent displacement (nanoprecipitation)

   c. Salting out (Nagavarma et *al.*, 2012)

Polymers used for the Preparation of Nanoparticles and Nanocapsules

Polymer use	Technique	Candidate drug
Hydrophilic Albumin, gelatin	Heat denaturation and cross-linking in W/O emulsion Desolvation and cross-linking in aqueous medium	Hydrophilic
Alginate, chitosan Dextran	Cross-linking in aqueous medium Polymer precipitation in an organic solvent	Hydrophilic and protein affinity Hydrophobic
Hydrophobic Poly(alky Icy anoacrylates)	Emulsion polymerization Interfacial O/W polymerization	Hydrophilic hydrophobic
Polyesters Poly(lactic acid), Poly(lactide-co-glycolide), poly (e-caprolactone)	Solvent extraction-evaporation Solvent displacement Salting out	Hydrophilic & hydrophobic Soluble in polar solvent Soluble in polar solvent

Targeted and controlled drug delivery by(Khar et al., 2006)

Solvent evaporation method

In this method, the polymer is dissolved in a organic solvent like dichloromethane, chloroform or ethyl acetate, and also used as the solvent in dissolving the hydrophobic drug. The mixture of polymer and drug solution is emulsified in an aqueous solution that contain a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After stable emulsion is formed, the organic solvent is evaporated by reducing the pressure or by continuous stirring. Particle size is influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. To produce small particle size, often a high speed homogenization or ultra sonication is used (Nagavarma et al., 2012).

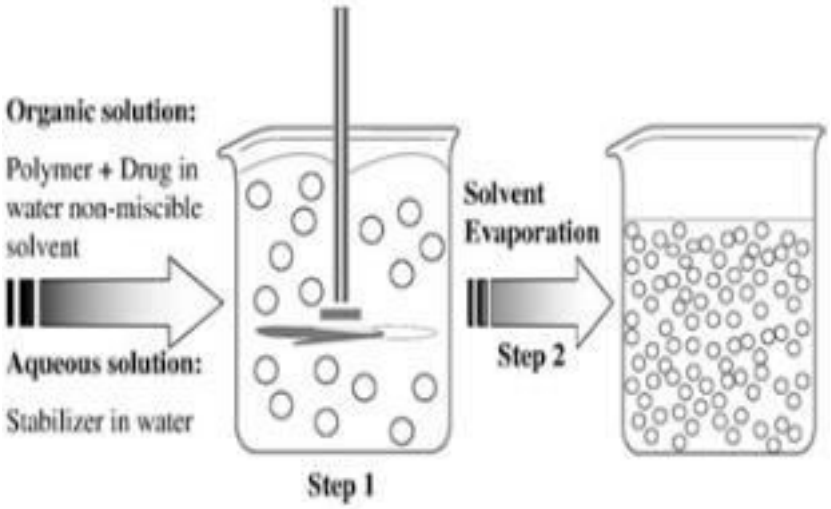

Figure : 3 Solvent evaporation method

Nano precipitation method

Nano precipitation also called solvent displacement method involves the precipitation of a preformed polymer from an organic solution and also the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant. The polymer generally PLA, is dissolved in water-miscible solvent having intermediate polarity, which leads to the precipitation of nanospheres. This

phase is then injected into a stirred aqueous solution which contains a stabilizer as a surfactant. Polymer deposited on the interface between the water and the organic solvent, caused due to fast diffusion of the solvent, leads to the formation of a colloidal suspension.

To facilitate formation of colloidal polymer particles in the first step of the procedure, phase separation is done with a totally miscible solvent which is also a non solvent of the polymer (Nagavarma et al., 2012).

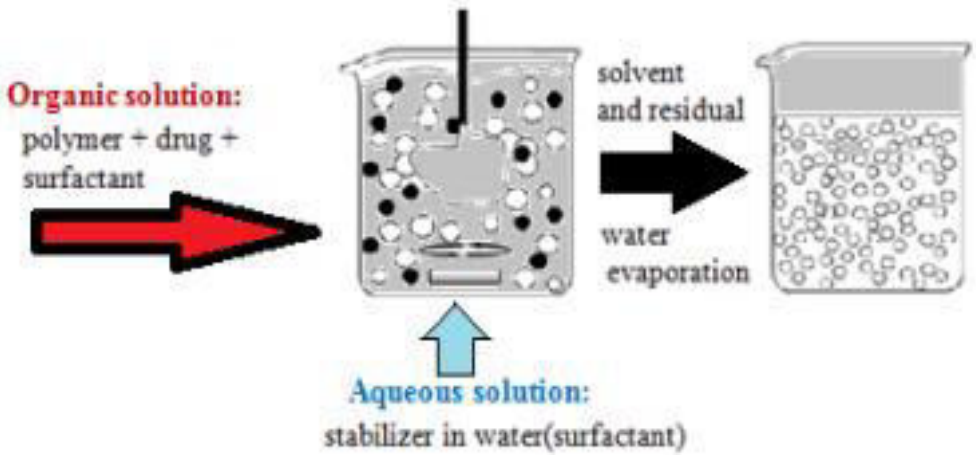

Figure : 4 Nano precipitation method

Solvent Displacement / Precipitation method

Solvent displacement method involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium either in the presence or absence of surfactant. Polymers, drug, and or lipophilic surfactant are dissolved in a semi- polar water miscible solvent such as acetone or ethanol. Then the solution is poured or injected into an aqueous solution containing stabilizer with magnetic stirring. Nano particles are formed instantaneously by the rapid solvent diffusion.

Then the solvent is removed from the suspensions under reduced pressure. The addition rate of the organic phase into the aqueous phase affect the size of particle, it was observed that a decrease in both particles size and drug entrapment occurs when the rate of mixing of the two phase increases. This method is well suited mostly for poorly soluble drugs. Nanosphere size, drug release and yield is effectively controlled by adjusting preparation parameters. Adjusting polymer concentration in the organic phase is found to be useful in the preparation of smaller size nanospheres which is restricted to a limited range of the polymer to drug ratio (Sovan et al., 2011).

Emulsification/solvent diffusion method

It is Modified version of solvent evaporation method in which The encapsulating polymer is made to dissolve in a partially water soluble solvent like propylene carbonate and is saturate with water to confirm the initial thermodynamic equilibrium of both the liquids. To produce the precipitation of the polymer and the formation of nanoparticles, it is important to promote the diffusion of the solvent of dispersed phase by diluting with an excess of water when the organic solvent is partly miscible with water or either with another organic solvent in the opposite case. Subsequently, the polymer-water saturated solvent phase is made emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion of the external phase and leads to the formation of nanospheres or nanocapsules, based on the oil-to-polymer ratio. At last, the solvent is eliminated by evaporation or filtration, based on its boiling point (Nagavarma et al., 2012).

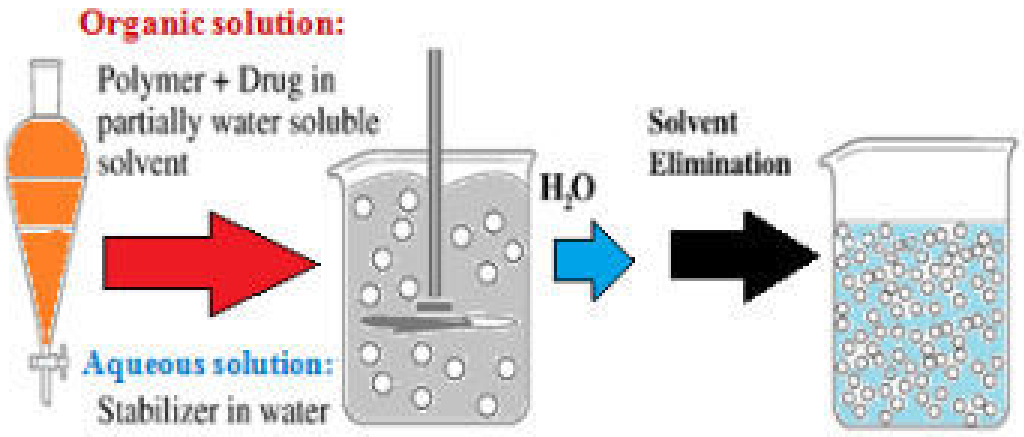

Figure: 5 Emulsification / solvent diffusion method

Salting out method

Salting out is the separation of a water miscible solvent from aqueous solution through a salting out effect. This procedure is considered as a modification of the emulsification/solvent diffusion. Polymer and drug are first dissolved in a solvent such as acetone, which is then emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non- electrolytes such as sucrose) and also a colloidal stabilizer such as poly vinyl pyrolidone or hydroxyl ethyl cellulose. This oil/water emulsion is then diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus induces the formation of nanospheres (Nagavarma et al., 2012).

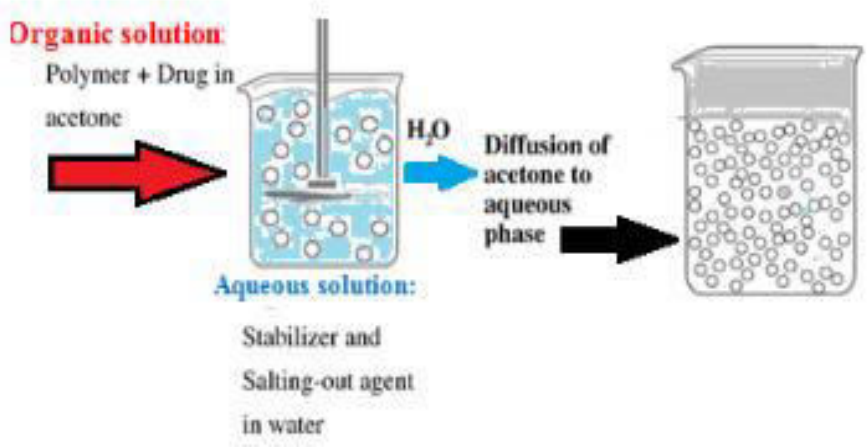

Figure:6 Salting out method

Dialysis

Dialysis is a simple, effective method for the preparation of small, narrow-distributed PN. Polymer is first dissolved in an organic solvent and then placed inside a dialysis tube with molecular weight cut off. Dialysis is performed against a non-solvent miscible with former miscible. The displacement of the solvent inside is followed by the aggregation of polymer due to a loss of solubility and homogeneous suspensions of nanoparticles formed (Nagavarma et al., 2012).

Polymerization method

In this, monomers are polymerized to give nanoparticle in aqueous solution. Drug is incorporated by dissolving in the polymerization medium or either by adsorption on to the nanoparticles after polymerization is completed. The nanoparticle suspension is later purified to remove stabilizers and surfactants employed in polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique is for making polybutylcyanoacrylate or poly (alkyl cyanoacrylate) nanoparticles (Nagavarma et al., 2012).

Emulsion polymerization

Emulsion polymerization and development of many polymer materials have recently increased. A typical formulation used in mini-emulsion polymerization comprises of water, monomer mixture, co-stabilizer, surfactant, and a initiator. The emulsion polymerization differs from mini-emulsion polymerization in the use of a low molecular mass compound as co-stabilizer and also in the use of a high-shearing device (ultrasound etc). Mini-emulsions are stabilized, and requires high-shear to get a steady state, they have an interfacial tension much greater than zero 31. The polymer nanoparticles are prepared by using Mini-emulsion method only (Nagavarma et al., 2012).

Micro-emulsion polymerization

Micro-emulsion polymerization is a new approach for making nano sized polymer particles and has gained significant attention. Emulsion and micro-emulsion polymerization appear similar because both methods produces colloidal polymer particles of high molar mass, but they are entirely different kinetics. Particle size and the average number of chains per particle is small in micro-emulsion polymerization. In micro-emulsion polymerization, an initiator, which is water-soluble, is being added to the aqueous phase of a thermodynamically stable micro-emulsion that contains swollen micelles. The polymerization starts from this thermodynamically stable, spontaneously formed state and depends on high quantities of surfactant systems, which has an interfacial tension at the oil/water interface close to zero (Nagavarma et al., 2012).

Interfacial polymerization

It is one of the well-established methods for preparation of polymer nanoparticles. It involves step polymerization of two reactive monomers or agents, which is dissolved in two phases respectively (i.e., continuous-and dispersed-phase), the reaction takes place at the interface of the two liquids. Nanometer-size

hollow polymer particles are synthesized by interfacial cross-linking reactions like poly addition or poly condensation or radical polymerization. Oil-containing nanocapsules are obtained by polymerization of monomers at oil/water interface of fine oil-in water micro-emulsion. The organic solvent, which s completely miscible with water is serving as a monomer vehicle and interfacial polymerization of the monomer may occur at the surface of the oil droplets that is formed during emulsification. To enhance nanocapsule formation, aprotic solvents, such as acetone and acetonitrile is used. Protic solvents, like ethanol, n-butanol and isopropanol, is found to enhance the formation of nanospheres with nanocapsules (Nagavarma et al., 2012).

Control living/radical polymerization

Radical polymerization include the lack of control in the molar mass, the molar mass distribution, the end functionalities and the macromolecular architecture. The limitations are due to the unavoidable fast radical–radical termination reactions. The recent emergence of many Controlled or 'Living' Radical Polymerization (C/LRP) processes has opened a new area by involvement of an old polymerization technique. The factors that contributes to this trend of the C/LRP process is increased environmental concern and also a sharp growth of pharmaceutical and medical applications for hydrophilic polymers (Nagavarma et al., 2012).

Co-acervation or ionic gelation method

The nanoparticles is prepared by using biodegradable hydrophilic polymers like chitosan, gelatin and sodium alginate. A method for preparing hydrophilic chitosan nanoparticles by ionic gelation is developed. In this method, positively charged amino-group of chitosan interact with negative charged tripolyphosphate to form co acervates with a size which is in the range of nanometer (Nagavarma et al., 2012).

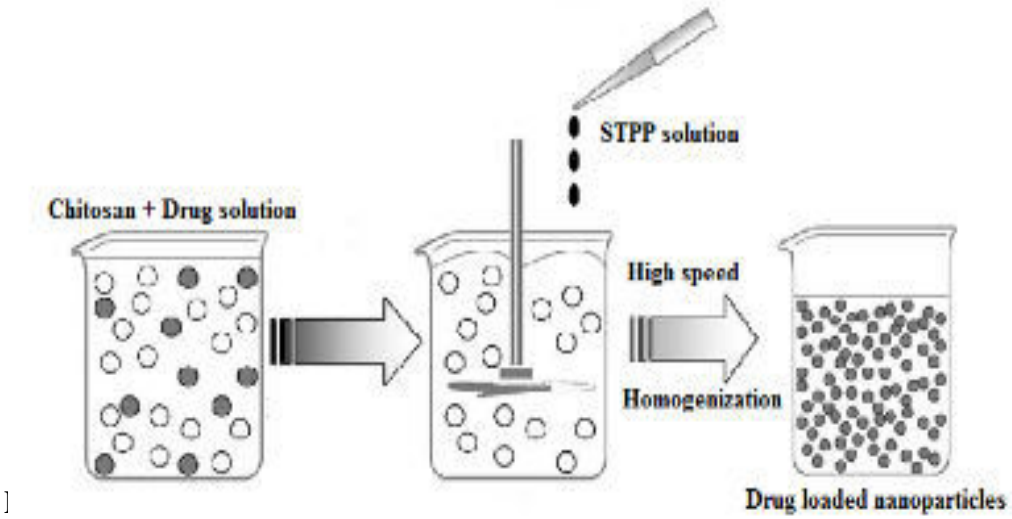

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Figure 7 : Co-acervation or ionic gelation method

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods needs the use of organic solvents which is hazardous to the environment and to physiological systems. Hence, the supercritical fluid technology is an alternative to produce biodegradable micro- and nanoparticles just because supercritical fluids are found to be environmentally safe. A supercritical fluid is defined as a solvent at a temperature above its critical temperature, in which the fluid remains as a single phase regardless of pressure. Supercritical CO_2 (SC CO_2) is widely used supercritical fluid because of the mild critical conditions (Tc = 31.1°C, Pc = 73.8 bars), nontoxicity, non flammability and low price. The common processing techniques for supercritical fluids are Supercritical Anti-Solvent (SAS) and Rapid Expansion of Critical Solution (RECS).

The process of SAS involves liquid solvent, eg methanol, that is completely miscible with the supercritical fluid (SC CO_2), mainly to dissolve the solute which is to be micronized; at the process conditions, because the solute is

not soluble in supercritical fluid, the extract of solvent by supercritical fluid leads to formation of precipitation of the solute, which results in the formation of nanoparticles. RECS differs from that of the SAS process. In that, solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is made to rapidly expand through a small nozzle in a region lower pressure, As a result the solvent power of supercritical fluids decreases and the solute eventually precipitates out (Nagavarma et al., 2012).

Drug may be incorporated into nanoparticles by any one of the following ways

- By preparing a solution of drug in the polymer.
- By solid dispersion of the drug in the polymer.
- By the surface adsorption of the drug.
- By chemically binding of the drug to the polymer

Modification of nanoparticles which alter the drug release rate

- Nanoparticles coated with plasma proteins creates a significant additional diffusion barrier which retards the drug release.
- Direct contact of nanoparticles with biological or artificial membrane enhances drug delivery to these membranes in comparison to simple solutions.

NANOPARTICLES CHARACTERIZATION

Measurement of particle size and zeta Potential

Photon Correlation Spectroscopy (PCS) and Laser Diffraction (LD) are the most powerful techniques for measurements of particle size. PCS also known as dynamic light scattering measures the fluctuation of the intensity of the scattered light which is caused by particle movement. This method covers a size range from a few nanometers to about 3 microns. PCS is a good tool for characterize nanoparticles, but it is not able to detect larger micro particles. The physical stability of optimized SLN dispersed is generally more than 12 months. ZP measurements allow predictions about the storage stability of colloidal dispersion (Kumar et al., 2014).

Particle size

Particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by electron microscopy. The major application of nanoparticles is in drug release and drug targeting. It has been found that particle size affects the drug release. Smaller particles offer larger surface area. As a result, most of the drug loaded onto them will be exposed to the particle surface leading to fast drug release. On the contrary, drugs slowly diffuse inside larger particles. As a drawback, smaller particles tend to aggregate during storage and transportation of nanoparticles dispersion. Hence, there is a compromise between a small size and maximum stability of nanoparticles. Polymer degradation can also be affected by the particle size. For instance, the degradation rate of poly (lactic-co-glycolic acid) was found to increase with increasing particle size *in vitro* (Kumar et al., 2014).

Scanning Electron microscopy

Scanning Electron Microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum, and the electron beam can damage the polymer. The mean size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution (Kumar et al., 2014).

Determination of Entrapment Efficiency (EE)

The EE was determined by analyzing the free drug content in the supernatant obtained after centrifuging the Nanoparticles suspension in high speed centrifuge at 15000 rpm for 30 min using Remi centrifuge (Mumbai, India). The EE was calculated as follows: (Havanoor et al., 2014).

$$Entrapment\ Efficiency = \frac{\text{Practical Drug Content}}{Theoritical\ Drug\ Content} X100$$

Drug release kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs time), first order (log cumulative percentage of drug remaining vs time), Higuchi model (cumulative percentage of drug released vs. square root of

time), Korsmeyer-Peppas model were applied to interpret the drug release kinetics from the formulations. The best-fit model was decided based on the highest regression values (r^2) of obtained release data of formulations (Balaiah et al., 2012).

Infrared spectroscopy (IR)

Shimadzu IR-470 spectrometer was used to record the IR spectrum of nanoparticles from 400 to 4000 cm^{-1}. The sample was grounded with Potassium Bromide (KBr) and pressed to a suitable size disk for measurement.

***In vitro* drug release**

The *In vitro* drug release profile of the prepared Imatinib Mesylate nanoparticles were investigated by USP Type I dissolution apparatus in using distilled water as dissolution medium. At a temperature of 37°C±2°C and the stirrer was rotated at 50 rpm speed. Nanoparticles containing Imatinib Mesylate equivalent 250 mg is introduced into the basket and involved in the dissolution medium transferred into the vessel containing 900 ml of purified water.

At specific intervals, 1 ml aliquot of the dissolution medium was sampled (the pooled sample was replaced with a fresh purified water), filtered by whatmann filter paper (pore size: 0.45 pm) and determine the amount of drug from UV absorbance of the sample solution, in comparison with a standard solution having a known concentration of raw drug. (Mansouri et al., 2011)

LITERATURE REVIEW

- **Kassem et al., (2017)** This research purposed to formulate an optimized imatinib mesylate (IM) loaded nanoparticles to improve its chemotherapeutic efficacy. The influence of 3 formulation factors on size (Y1), zeta potential (Y2), entrapment capacity percentage (Y3), the percentage of initial drug release after 2 h (Y4) and the percentage of cumulative drug release after 24 h (Y5) were studied and optimized using Box-Behnken design. Optimum desirability was specified and the optimized formula was prepared, stability tested, morphologically examined, checked for vesicular bilayer formation and evaluated for its in vitro cytotoxicity on 3 different cancer cell lines namely MCF-7, HCT-116, and HepG-2 in addition to1 normal cell line to ensure its selectivity against cancer cells. The actual responses of the optimized IM formulation were 425.36 nm, _62.4 mV, 82.96%, 18.93%, and 89.45% for Y1, Y2, Y3, Y4, and Y5, respectively.

- **Snehalatha et al., (2016) investigated** Etoposide-loaded were prepared using nanoprecipitation and emulsion solvent evaporation techniques using -co-glycolic acid and poly(ε-caprolactone) in presenceof Pluronic F68, respectively. Effect of formulation variables like stabilizer concentration, amount of polymer, and drug was studied. These parameters were found to affect particle size, zeta potential, drug content, and entrapment efficiency of nanoparticles. The methods produced nanoparticles with good entrapment efficiency of around 80%. Recovery of nanoparticles was as high as 95% and drug content was around 1.5%. Increase in lactide content decreased the release of etoposide *in vitro* and poly(ε- caprolactone) nanoparticles retarded etoposide release for 48 hr. The results show the suitability of polylactide-co-glycolic acid and poly(ε-caprolactone) nanoparticles as potential carriers for controlled delivery of etoposide.

- **Alexis et al., (2016)** investigated nanoparticles as drug delivery systems enable unique approaches for cancer treatment. Over the last two decades, a large number of nanoparticle delivery systems have been developed for cancer therapy, including organic and inorganic materials. Many liposomal, polymer–drug conjugates, and micellar formulations are part of the state of the art in the clinics, and an even greater number of nanoparticle platforms are currently in the preclinical stages of development. More recently developed nanoparticles are demonstrating the potential sophistication of these delivery systems by incorporating multifunctional capabilities and targeting strategies in an effort to increase the efficacy of these systems against the most difficult cancer challenges, including drug resistance and metastatic disease. Here, we will review the available preclinical and clinical nanoparticle technology platforms and their impact for cancer therapy.

- **Kalepua et al., (2015)** investigated The emerging trends in the combinatorial chemistry and drug design have led to the development of drug candidates with greater lipophilicity, high molecular weight and poor water solubility.One of largest-selling anticancer drugs Imatinib is marketed as a salt form, Imatinib mesylate .The drug exhibits poor solubility and hence mesylate salt was used for its development, which is soluble in wate at pH<5.5. Among the two polymorphic forms (α and β), generated by Imatinib salt, the β forms more stable with acceptable pharmaceutical properties. However, additional marketing rights were assigned to the innovator due to their patent protection of the β form.

Over the past two decades, nanoparticle technology has become a well-established and proven formulation approach for poorly-soluble drugs. Reducing a drug's particle size to sub-micron range is referred to as ‘nanonization’. In the field of pharmaceuticals, the term ‘nanoparticle’ is applied to structures less than 1 μm in size.

- **Jeena et al., (2015)** carried out the antitumor and cytotoxic activity of ginger essential oil (zingiber officinale roscoe). GEO showed potent in vitro cytotoxic activity against DLA and EAC cell lines. IC50 value for DLA cell line was 11 µg/ml and for EAC cell lines 18 µg/ml. The IC50 of GEO was found to be 41 µg/ml against the L929 cell mg/kg and 1000 mg/kg body weight) significantly reduced the volume of solid tumor development by 54.4% and 62.4% respectively. The life span was increased up to 50% in 1000 mg/kg b. wt GEO treated ascites tumor induced animals

- **Fitzgibbon et al., (2015)** investigated clinical outcome of surgical revascularization using autologous vein graft is limited by vein graft failure attributable to neointima formation. Platelet-derived growth factor (PDGF) plays a central role in the pathogenesis of vein graft failure. Therefore, we hypothesized that nanoparticle (NP)-mediated drug delivery system of PDGF-receptor (PDGF-R) tyrosine kinase inhibitor (imatinib mesylate: STI571) could be an innovative therapeutic strategy. Uptake of STI571-NP normalized PDGF-induced cell proliferation and migration. Excised rabbit jugular vein was treated ex vivo with PBS, STI571 only, FITC-NP, or STI571-NP, then interposed back into the carotid artery position. NP was detected in many cells in the neointima and media at 7 and 28 days after grafting. Significant neointima was formed 28 days after grafting in the PBS group; this neointima formation was suppressed in the STI571-NP group. STI571-NP treatment inhibited cell proliferation and phosphorylation of the PDGF-R-β but did not affect inflammation and endothelial regeneration. STI571-NP-induced suppression of vein graft neointima formation holds promise as a strategy for preventing vein graft failure.

- **Mehrotra et al., (2015)** developed the incorporation of lomustine, a hydrophobic anticancer drug into PLGA nanoparticles by interfacial deposition method was optimized. Based on the optimal parameters, it was found that lomustine-PLGA nanoparticles with acceptable properties could be obtained. Optimization of formulation variables to control the size and drug entrapment

efficiency of the prepared nanoparticles seems to be based on the same scientific principles as drug-loaded nanoparticles prepared by nanoprecipitation, solvent evaporation method. The process was the most important factor to control the particle size, while both the drug-polymer interaction and the partition of drug in organic and aqueous phases were the crucial factors to govern the drug entrapment efficiency and biodistribution profiles of prepared nanoparticles in albino mice showed higher plasma drug concentration for longer period of time, elevated drug concentration in lungs and slow elimination from kidney. No toxic effects of prepared nanoparticles were observed in histopathological examination of lungs and kidney. The systematic investigation reported here promises the development of PLGA nanoparticles loaded with lomustine when tested in Lung Cancer cell line L132 and toxicological/ histopathological studies in albino mice.

- **Jana et al., (2014)** Prepared Felodipine nanoparticles using poly (D, L-lactic-co-glycolic acid) were prepared by single emulsion solvent evaporation technique and the physico-chemical characterization of prepared nanoparticles confirmed the particles were nanosize range with smooth and spherical morphology. Further, the compatibility of drug-polymer combination was analyzed by FTIR and DSC study. The in vitro drug release study of PLGA nanoparticles showed longer duration of drug release with reduced burst release compared with pure felodipine. The in vitro drug release data were fitted with various mathematical models to establish the drug release mechanism from the nanoparticles and found to follow mixed order kinetics.

- **Akagi et.al (2014**) Platelet–derived growth factor (PDGF) is implicated in the pathogenesis of pulmonary arterial hypertension (PAH). Imatinib, a PDGF-receptor tyrosine kinase inhibitor, improved hemodynamics, but serious side effects and drug discontinuation are common when treating PAH. A drug delivery system using nanoparticles (NPs) enables the reduction of side effects while maintaining the effects of the drug. We examined the efficacy of imatinib-incorporated NPs (Ima-NPs) in a rat model and in human PAH-pulmonary arterial

smooth muscle cells (PASMCs). Ima-NPs significantly inhibited proliferation after 24 hours of treatment. Ima-NPs significantly inhibited proliferation Delivery of Ima-NPs into lungs suppressed the development of MCT-induced PAH by sustained antiproliferative effects on PASMCs.

- **Bingwang et al (2014)** developed the combination of chemotherapeutic agents with different pharmacological action has emerged as promising therapeutic strategy in the treatment of cancer. The antitumor activity of paclitaxel and etoposide loaded PLGA nanoparticles for the treatment of osteosarcoma. The resulting drug loaded PLGA NP exhibited a nanosized dimension with uniform spherical morphology. The NP exhibited a sustained release profile for both PTX and ETP throughout the study period without any sign of initial burst release. The combinational drug loaded PLGA NP enhanced cytotoxic effect in MG63 and saos-2 osteosarcoma cell lines. The greater inhibitory effect of nanoparticle combination would be of greater advantage during systemic cancer therapy.

- **Ruma Maji et.al (2014)** Four formulations o Tamoxifen citrate loaded plylactide-co-glycolide(PLGA)based nanoparticles(TNPs)were developed and chracterised.Their internalizatin by Michigan Cancer Foundation-7(MCF-7)breast cancer cells was also investigated.Nanoparticles were prepared by a multiple emulsion solvent evapouration method.Then the following studies were carried out:drug- excipients interaction studies using Fourier transform infrared spectroscopy(FTIR),surface morphology by filed emission scanning electron microscopy(FESEM),zeta potential and size ditribution using Zetasizer NanoZS90 and particle size analyzer and in vitro drug release.Invitro cellulr uptake of nanoparticles were assessed by confocal microscopy and their cell viability was studied and drug loaded nanoprticle were found to be moe cytotoxic than the free drug.

- **Yass et al., (2014)** Prepared Sildenafil Citrate (SFC) nanoparticles using melt method was employed for preparing SFC – solid lipid microparticles dispersions (SFC – SLMDs), a non – solvent technique aid in the production of

drug – matrix dispersions with sustained release properties. Glyceryl behenate (GB) (Compritol® 888 ATO) was used as the retarding matrix and the results shown that as its ratio increase there was a decrease in the fine particle fraction, an increase in the drug content and a prolong drug release pattern. The best model fit the release data was Higuchi – Matrix model which indicates drug diffusion – controlled releasing mechanism. Thus, inhaled SFC – SLMDs dry powder will improve PAH treatment via drug localization at low doses and reducing the administration frequency.

- **Kumar et al., (2014)** developed Formulation and evaluation of nanoparticles containing artemisinin HCL is poorly soluble in water and a fast-acting blood schizonticide effective in treating the acute attack of malaria (including chloroquine – resistant and celebral malaria). Artemisinin are effective against multi-resistant strains of P. falciparum. The purpose of the present work is to minimize the dosing frequency, taste masking and toxicity and to improve the therapeutic efficacy by formulating Artemisinin HCl nanoparticles. Artemisinin HCl nanoparticles were formulated by solvent evaporation method using polymer poly(ε-caprolactone) with five different formulations. Nanoparticles were characterized by determining its particle size, polydispersity index, drug entrapment efficiency, particle morphological character and drug release. The particle size ranged between 100nm to 240nm. Drug entrapment efficacy was > 99%. The in-vitro release of nanoparticles were carried out which exhibited a sustained release of Artemisinin HCl from nanoparticles up to 24hrs. The results showed that nanoparticles can be a promising drug delivery system for sustained release of Artemisinin HCl.

- **Kotikalapudi et al., (2014)** investigated Prepared and Evaluated Domperidone loaded solid lipid nanoparticles (DOM-SLN). DOM loaded SLN were prepared by hot homogenization followed by ultrasonication technique. DOM- SLN were characterized for particle size, polydispersity index (PDI), zeta potential and entrapment efficiency and in vitro drug release behaviour were

investigated. P-XRD and DSC analysis were performed to characterize the state of drug and lipid modification. Shape and surface morphology were determined by transmission electron microscopy (TEM). SLN formulations were subjected to stability study over a period of 30 days. The mean particle size, PDI, Zeta potential and entrapment efficiency of optimized SLN were found to be 56 nm, 0.154, 34 mV,98.5 %. P-XRD and DSC studies revealed that DOM was in an amorphous state. Shape and surface morphology was determined by TEM revealed fairly spherical shape of nanoparticles.

- **Bharti et al., (2014)** Formulation and evaluation of gelatin nanoparticles for pulmonary drug delivery entrapment efficiency of all gelatin nanoparticles formulations were found to be in the range of 46.16 % - 58.60 %. The particle size of gelatin nanoparticle formulations(GNps1, GNps2, GNps3, GNps4) were found to be 179.9 nm, 198.4 nm, 252.4 nm and 1545 nm, respectively. The gelatin nanoparticle formulation GNps3 was selected best formulation depending upon the particle size less then 500nm and higher entrapment efficiency as compared to GNps1 and GNps2. The zeta potential of gelatin nanoparticles found to exhibit stability due to positive charge on the surface. The transmission electron microscopy indicated the spherical surface of the gelatin nanoparticles. The in vitro release was found to follow higuchi plot as compared to zero order plot, first order plot and krosmeyer peppas plot. Stability studies showed that gelatin nanoparticle formulation GNps3 was stable when tested for particle size, entrapment efficiency and in vitro drug release at refrigerated condition (5º±3ºC), at room temperature (25º±2ºC/65%±5% RH) and at accelerated condition (40º±2ºC/75%±5% RH) according to ICH guidelines for 6 months(180 days).

- **Parvin et al., (2014)** Formulated Dexamethasone solid lipid nano particles with stearic acid as solid lipid, lutrol F-68 as surfactant and tween-80 as stabilizer. SLNPs are prepared by hot homogenzation method at different ratio of drug, lipid, surfactant and stabilizer and designated as DNP1 to DNP6. In vitro dissolution study was performed using the USP type II apparatus (paddle method) at 50 rpm

to a temperature of 37°±0.5°C in distilled water containing 0.75% w/vSLS(sodium lauryl sulfate). The absorbance of sample was measured spectrophotometrically at λmax 239nm on a UV-Visible spectrophotometer. Release pattern of drug was found to follow zero order, first order and Korsmeyer-Peppasequations. Pure drug showed only 27.25% release in 50 min whereas the dexamethasone SLNPs showed faster (66.19%) *in vitro* drug release.

- **Thavamani et al., (2014)** investigated the Anticancer activity of *cissampelos pareira* against dalton's lymphoma ascites bearing mice. Methanol Extract of Cissampelos pariera (MECP) showed a potent cytotoxic activity, with an IC 50 value of 95.5 μg/ml and a significant ($p < 0.001$) decrease in packed cell volume, viable cell count, and an increased lifespan (54 and 72%). The hematological and serum biochemical profiles were restored to normal levels in MECP-treated mice. The MECP-treated group significantly ($p < 0.001$) decreased SOD, lipid peroxidation, and CAT to normal.

- **Firdhouse et al., (2013)** evaluated the biosynthesis of silver nanoparticles using the extract of *Alternanthera sessilis* - antiproliferative effect against prostate cancer cells. The biosynthesis of silver nanoparticles using the extract of *Alternanthera sessilis* was economical, non-toxic, and environmentally benign. The synthesized silver nanoparticles were stable due to the reducing and capping nature of phytoconstituents present in the aqueous extract of Alternanthera sessilis analyzed by FTIR spectra. The particle size of the synthesized silver nanoparticles is less than 50 nm which was confirmed by XRD and SEM analysis. Nanosilver shows good cytotoxic activity against prostate cancer cells and may serve as a potential anticancer drug for cancer therapy.

- **Karal-Yilmaz et al., (2012)** Poly(lactic-co-glycolic acid) nanoparticles loaded with imatinib mesylate has been developed as a new therapeutic strategy to prevent craniopharyngioma recurrence. Nanoparticles composed of different lactic/glycolic acid ratios, molecular weights and drug compositions were

synthesized and loaded with imatinib mesylate by modified double-emulsion/solvent evaporation technique and subsequently characterized by particle-size distribution, scanning electron microscopy, encapsulation efficiency and in vitro drug release. Inhibitory potential of imatinib containing nanaoparticles on tumor neovascularization was investigated on craniopharyngioma tumor samples by rat cornea angiogenesis assay. Results showed that nanoparticles in different LA:GA ratios [LA:GA 50:50 (G50), 75:25 (G25), 85:15 (G15)] considerably reduced neovascularization induced by recurrent tumor samples in an *in vivo* angiogenesis assay.

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- **Yin et al., (2012)** Percutaneous coronary intervention (PCI) has become the most common revascularization procedure for coronary artery disease. The use of stents has reduced the rate of restenosis by preventing elastic recoil and negative remodeling. However, in-stent restenosis remains one of the major drawbacks of this procedure. Drug-eluting stents (DESs) have proven to be effective in reducing the risk of late restenosis, but the use of currently marketed DESs presents safety concerns, including the non-specificity of therapeutics, incomplete endothelialization leading to late thrombosis, the need for long-term anti-platelet agents, the use of DESs, and progress in nanoparticle drug- or gene-eluting stents for the prevention and treatment of coronary restenosis.

- **Naik J P et al.,(2012)** Formulated and optimized Repaglinide (Rg) loaded Eudragit RL-100 nanoparticles using Eudragit RL-100 nanoparticles have been developed by High Pressure Homogenizer Emulsification (HPHE) -Solvent Evaporation method indifferent ratios. The method was optimized using design of experiments by employing a 3-factor, 3-level Design Expert (version 8.0.7.1) Statistical Design Software and was subjected to various studies for characterization including Transmission electron microscopy (TEM), X-ray diffraction (XRD), Encapsulation efficiency (%EE), Particle Size Analysis (PSA)

etc. These studies are favorably revealed that the mean particle diameter of optimized formulation was 50 nm with crystalline nature. Moreover, formulated nanoparticles were also subjected to Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC) for interaction between drug and polymer. The results were positive and showed that, there were no interaction between drug and polymer.

- **Kulathuran Pillai et al., (2012)** discussed the antitumor activity of ethanolic extract of *CNIDOSCOLUS CHAYAMANSA MCVAUGH* against Dalton's ascetic Lymphoma in mice. Both doses of EECC decreased average increase in body weight, reduced the packed cell volume (PCV) viable tumor cell count and increased the life span of DAL treated mice and brought back the hematological parameters, serum enzyme and lipid profile near to normal values. All the values were found to be statistically significant with control group at $p<0.001$.These observations are suggestive of the protective effect of extracts in the Dalton's Ascitic Lymphoma (DLA).

- **Masuda et.al (2011)** The use of currently marketed drug eluting stents(DES) present safety concerns, including an increased risk of late thrombosis in the range of 0.6% per year in patients, including acute coronary syndrome, which is thought to result from delayed endothelial healing effects. A new DES system targeting vascular smooth muscle cells without adverse effects on endothelial cells is therefore needed. Platelet derived growth factors (PDGF) plays a central role in the pathogenes of restenosis; therefore we hypothesised that Imatinib mesylate (PDGF receptor tyrosine kinase inhibitor) encapsulated bioabsorble polymeric nanoparticles(NP)-eluting stents attenuates in-stent neointima formation.

Imatinib NP attenuated the proliferation of vascular smooth muscle associated with inhibition of target molecule (phosphorylation of PDGF receptor-β), but showed no effect on endothelial proliferation.

- **Junjie Zou et al., (2011)** investigated Poly (lactic-co-glycolic acid) (PLGA) NPs containing rapamycin was prepared using an oil/water solvent evaporation technique. Nanoparticle size and morphology were determined by dynamic light scattering methodology and electron microscopy. In vitro cytotoxicity of blank, rapamycin-loaded PLGA (RPLGA) NPs was studied using MTT Assay. Rapamycin was efficiently loaded in PLGA nanoparticles with an encapsulation efficiency was 87.6. Thus concluded that sustained-release rapamycin from rapaymycin loaded NPs inhibits vein graft thickening without affecting the reendothelialization in rat carotid vein-to-artery interposition grafts

- **Sriram et al., (2010)** discussed the Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. Transmission Electron Microscope (TEM) showed that the purified nanoparticles were spherical with a mean diameter of 50 nm and the endotoxin assay revealed that the purified AgNPs were endotoxin-free. The AgNPs were found to be cytotoxic to tumor cells at concentrations of 500 nm and higher AgNPs at 500 nm decreased the viability of DLA cells to 50% of the initial level, and this was chosen as the IC50. The present study explores the potential antitumor activity of biologically synthesized AgNP in a DLA tumor system in vitro by activation of the caspase 3 enzyme. Histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs. These findings confirm the antitumor properties of AgNPs, and suggest that they may be a cost-effective alternative in the treatment of cancer and angiogenesis-related disorders.

- **Nesalin *et al*., (2009)** formulated and evaluated nanoparticles containing flutamide. Nanoparticles of Flutamide were formulated using chitosan polymer by ionic gelation technique. Nanoparticles of different core: coat ratio were formulated and analyzed for total drug content, loading efficiency, particle size and *in vitro* drug release studies. From the drug release studies it was observed that nanoparticles prepared with chitosan in the core: coat ratio 1:4 gives better sustained release for about 12 hrs as compared to other formulations.

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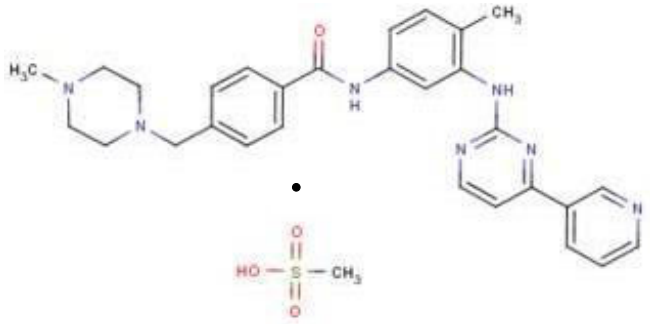
DRUG PROFILE

DESCRIPTION:

Imatinib mesylate is designated chemically as 4-[(4Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino] phenyl] benzamide methanesulfonate .

STRUCTURE:



DESCRIPTION:

Imatinib mesylate is a white to off-white to brownish or yellowish tinged crystalline powder.

MOLECULAR FORMULA:

$C_{29}H_{31}N_7O \cdot CH_4SO_3$

MOLECULAR WEIGHT:

589.7

SOLUBILITY:

Soluble in aqueous buffers ≤(pH 5.5) but is very slightly soluble to insoluble in neutral/alkaline aqueous buffers. In non-aqueous solvents, the drug substance is freely soluble to very slightly soluble in dimethyl sulfoxide, methanol and ethanol, but is insoluble in n-octanol, acetone and acetonitrile.

EXCIPIENTS USED:

Colloidal silicon dioxide (NF); crospovidone (NF); hydroxypropyl methylcellulose (USP); magnesium stearate (NF); and microcrystalline cellulose (NF). Tablet coating: ferric oxide, red (NF); ferric oxide, yellow (NF); hydroxypropyl methylcellulose (USP); polyethylene glycol (NF) and talc (USP).

CLINICAL PHARMACOLOGY

Mechanism of Action:

Imatinib Mesylate is a protein-tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in Chronic Myeloid Leukemia (CML). It inhibits proliferation and induces apoptosis in bcr-abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. In colony formation assays using *ex vivo* peripheral blood and bone marrow samples, imatinib shows inhibition of bcr-abl positive colonies from CML patients. *In vivo*, it inhibits tumor growth of bcr-abl transfected murine myeloid cells as well as bcr-abl positive leukemia lines derived from CML patients in blast crisis. Imatinib is also an inhibitor of the receptor tyrosine kinases for Platelet-Derived Growth Factor (PDGF) and Stem Cell Factor (SCF), c-kit, and inhibits PDGF- and SCF-mediated cellular events. *In vitro*, Imatinib inhibits proliferation and induces apoptosis in Gastrointestinal Stromal Tumor (GIST) cells, which express an activating c-kit mutation.

Pharmacokinetics:

Imatinib is well absorbed after oral administration with C_{max} achieved within 2-4 hours post-dose. Mean absolute bioavailability is 98%. Following oral administration in healthy volunteers, and its major active metabolite, the (N-desmethyl derivative), are approximately 18 and 40 hours, respectively. Mean imatinib AUC increases proportionally with increasing doses ranging from 25 mg-1,000 mg. There is no significant change in the pharmacokinetics of imatinib on

repeated dosing, and accumulation is (1.5- to 2.5)-fold at steady state when Gleevec is dosed once daily. At clinically relevant concentrations of imatinib, binding to plasma proteins in in vitro experiments is approximately 95%, mostly to albumin and α1-acid glycoprotein.

Metabolism and Elimination:

CYP_3A_4 is the major enzyme responsible for metabolism of Imatinib Mesylate. Other cytochrome P_{450} enzymes, such as CYP_1A_2, CYP_2D_6, CYP_2C_9, and CYP_2C_{19}, play a minor role in its metabolism. The main circulating active metabolite in humans is the N-demethylated piperazine derivative, formed predominantly by CYP_3A_4. It shows in vitro potency similar to the parent imatinib. The plasma AUC for this metabolite is about 15% of the AUC for imatinib. The plasma protein binding of the N-demethylated metabolite CGP71588 is similar to that of the parent compound. Elimination is predominately in the feces, mostly as metabolites. Based on the recovery of compound(s) after an oral $_{14}C$-labeled dose of Imatinib Mesylate, approximately 81% of the dose was eliminated within 7 days, in feces (68% of dose) and urine (13% of dose).

Unchanged Imatinib Mesylate accounted for 25% of the dose (5% urine, 20% feces), the remainder being metabolites. Typically, clearance of Imatinib Mesylate in a 50-year-old patient weighing 50 kg is expected to be 8 L/h, while for a 50-year-old patient weighing 100 kg the clearance will increase to 14 L/h. However, the inter-patient variability of 40% in clearance does not warrant initial dose adjustment based on body weight and/or age but indicates the need for close monitoring for treatment-related toxicity.

Drug-Drug Interactions CYP_3A_4 Inhibitors:

There was a significant increase in exposure to Imatinib Mesylate (mean C_{max} and AUC increased by 26% and 40%, respectively) in healthy subjects when Gleevec was co-administered with a single dose of ketoconazole (a CYP_3A_4 inhibitor).

CYP_3A_4 Substrates:

Imatinib Mesylate increased the mean C_{max} and AUC of simvastatin (CYP_3A_4 substrate) by 2- and 3.5-fold, respectively, indicating an inhibition of CYP_3A_4 .

CYP_3A_4 Inducers:

Pretreatment of 14 healthy volunteers with multiple doses of rifampin, 600 mg daily for 8 days, followed by a single 400-mg dose of Gleevec, increased Gleevec oral-dose clearance by 3.8-fold (90% confidence interval = 3.5- to 4.3-fold), which represents mean decreases in Cmax, AUC(0-24) and AUC(0-∞) by 54%, 68% and 74%, of the respective values without rifampin treatment.

***In Vitro* Studies of CYP Enzyme Inhibition:**

Human liver microsome studies demonstrated that Gleevec is a potent competitive inhibitor of CYP_2C_9, CYP2D6, and $CYP_3A_{4/5}$ with Ki values of 27, 7.5 and 8 µM, respectively. Gleevec is likely to increase the blood level of drugs that are substrates of CYP_2C_9, CYP_2D_6 and $CYP_3A_{4/5}$.

CLINICAL STUDIES :

Chronic Myeloid Leukemia Chronic Phase, Newly Diagnosed: An open-label, multicenter, international randomized Phase 3 study has been conducted in patients with newly diagnosed Philadelphia chromosome positive (Ph+) Chronic Myeloid Leukemia (CML) in chronic phase. This study compared treatment with either single-agent (Imatinib Mesylate) or a combination of Interferon-alfa (IFN) plus cytarabine (Ara-C). Patients were allowed to cross over to the alternative treatment arm if they failed to show a Complete Hematologic Response (CHR) at 6 months, a Major Cytogenetic Response (MCyR) at 12 months, or if they lost a CHR or MCyR. Patients with increasing WBC or severe intolerance to treatment were also allowed to cross over to the alternative treatment arm with the permission of the Study Monitoring Committee (SMC).

POLYMER PROFILE

HYDROXY PROPYL METHYL CELLULOSE (Raymond et al., 2003)

Non-proprietary names

Hypromellose (BP), Hypromellosum and Hypromellose(USP)

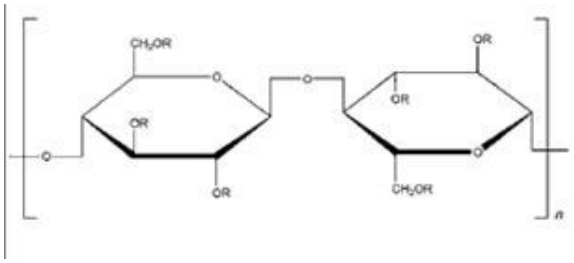
Synonyms

Benecel MHPC, methocel, metolese, pharmacoat, spectracel, tylopur

Functional category

Cooling agent, film former, rate controlling polymer for SR, stabilizing agent, viscosity-increasing agent, tablet binder, suspending agent.

Chemical Structure



Molecular weight

1828

Typical properties

pH	:	5.5-8.0(for a 1% w/v aqueous solution)
Density	:	1.326 g/cm^3
Melting point	:	browns at 190-220°c, chars at 225-230°c
Specific gravity	:	1.26
Incompatabilities	:	with some oxidising agents

Description

Odourless and bland, white or creamy white or granular powder.

Solubility

Soluble in chilly water, growing a viscous colloidal resolution; usually insoluble in chloroform, ethanol (95%) and ether. It is soluble in mixture of ethanol and dichloromethane, and mixture of water and alcohol.

Viscosity

Aqueous solutions are most commonly prepared. Solutions prepared using organic solvents are more viscous.

Stability and storage condition

It is a stable material, although it is a hygroscopic afterward drying. Solutions are stable at pH 3-11. Upon heating and cooling, hypermellose undergoes a sol-gel transformation. Aqueous solutions are liable to microbial spoilage and should be preserved with an antimicrobial preservative. Aqueous solutions may also be sterilized by autoclaving; the coagulated polymer must be redispersed on cooling by shaking.

Powder ought to be stored in well closed containers. In a cool, dry locale.

Applications

Used extensively in oral and topical pharmaceutical formalities. In oral products, it is utilized as tablet binder in film coating and an extended release agent in topical formulations. It can be utilized as an emulsifier, suspending agent and stabilizing agent in topical gels and ointments.

In addition, it is utilized in the producing of capsules, as an adhesive in plastic bandages and as a wetting agent for hard contact lenses. It is extensively utilized in cosmetics and food products (Wade et al., 2006)

Method of manufacturing

A purified form of cellulose is reacted alongside sodium hydroxide solution to produce a swollen mass of alkaline cellulose that is next indulged alongside chloromethane and propylene oxide to produce HPMC. The product is purified and ground to a fine, uniform powder or granules.

Safety

Generally considered as a non toxic and non irritant material, although excessive oral consumption could have a laxative effect.

Handling precautions

Observe normal precautions appropriate to the conditions and number of materials handled.

POLYVINYL ALCOHOL (Raymond et al., 2003)

Non Propreitary Names

USP: Polyvinyl alcohol

Synonyms

Airvol, elvanol, gohsenol, PVA, vinyl alcohol polymer

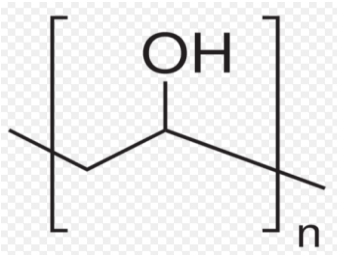
Chemical Name And Cas Registry Number

Ethanol, homopolymer[900-89-5]

Empirical Formula

$(C_2H_4O)_n$

Chemical Structure



Molecular Weight:

20000-200000

Polyvinyl alcohol is a water soluble synthetic polymer represented by formula $(C_2H_4O)_n$. The value of n for commercially available material lies between 500 and 5000, equivalent to a molecular weight range of approximately 20000-200000.

Table:1 Commercially Availble Grades Of Polyvinyl Alcohol

Grade	Molecular Weight
High viscosity	-200000
Medium viscosity	-130000
Low viscosity	-20000

Functional Category

Coating agent, lubricant, stabilizing agent and viscosity increasing agent.

Application on Pharmaceutical Formulation or Technology

Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulation. It is used as a stabilizing agent for emulsions (0.25-3.0%w/v). Polyvinyl alcohol is also used as viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solution for lubrication purpose, in sustained-release formulation for oral administration, and in transdermal patches. Polyvinyl alcohol may be made into microspheres when mixed with a gluteraldehyde solution (Ainley Wade et al., 2006)

Table: 2 Uses of Polyvinyl Alcohol

Use	Concentration
Emulsions	0.5 %
Ophthalmic formulations	0.25-3.00 %
Topical solutions	2.5 %

Description

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

Typical Properties

Melting point:

- 228°C for fully hydrolysed grades
- 18C-190°C for partially hydrolysed grades

Refractive Index

N 25d=1.49-1.53

Solubility

Soluble in water and insoluble in organic solvents. Dissolution requites dispersion (wetting) of the solid in water at room temperature followed by heating the mixture to about 90°C for approximately 5 mins. Mixing should be continued while the heated solution is cooled to room temperature.

Specific Gravity

- 1.19-1.31 for solid at 25°C
- 1.02 for 10% w/v aqueous solution at 25°C

Viscocity (Dynamic)

Table : 3 Viscosity of commercial grades of polyvinyl alcohol

Grade	Dynamic Viscosity of 4% W/V Aqueous Solution at 200°C (Mpas)
High viscosity	40.0-65.0
Medium viscosity	21.0-33.0
Low viscosity	4.0-7.0

Stability and Storage Conditions

Polyvinyl alcohol is stable when stored in tightly sealed container in a cool, dry place. Aqueous solutions are stable in corrosion resistant sealed containers. Preservatives may be added to the solution if extended storage is required. Polyvinyl alcohol undergoes slow degradation at 100°C and rapid degradation at 200°C, it is stable on exposure to light.

Incompatibilities

Polyvinyl alcohol undergoes reactions typical of a compound with secondary hydroxyl groups such as esterification. It decomposed in strong acid and softens or dissolves in weak acids and alkalis. It is incompatible at high concentration at high concentration with inorganic salts, especially sulphates and phosphates, precipitation of polyvinyl 5% w/v can be caused by phosphates, gelling of polyvinyl alcohol solution may occur if borax is present .

Method of Manufacture

Polyvinyl alcohol is produced through the hydrolysis of polyvinyl acetate. The repeating unit of vinyl alcohol is not used as the starting material because it can't be obtained in the quantities and the purity required for polymerization purposes. The hydrolysis proceeds rapidly in methanol, ethanol, or a mixture of alcohol and methyl acetate, using alkalis or mineral acids or catalysis.

Safety

Polyvinyl alcohol is generally considered a non-toxic material. It is non-irritant to the skin and eyes at concentration up to 10% concentration up t 7% are used in cosmetics.

Handling Precautions

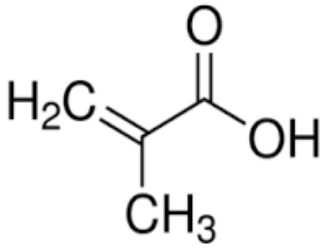
Observed normal precautions appropriate to the circumstances and quantity of material handled. Polyvinyl alcohol dust may be an irritant on inhalation. Handle in well-ventilated environment.

EUDRAGIT

Chemical name: Methacrylic acid

Chemical formula: $C_4H_6O_2$

Chemical structure:



Synonyms:

- 2-(Methoxycarbonyl)-1-propene
- 2-Methyl-2-propenoic acid Methyl ester

Appearance:

Colourless

Odor:

- Unpleasant
- Acrid
- Repulsive

Solubility:

- Soluble in warm water
- Miscible with most organic solvents

Density:

1.02 g/cm^3

Molar mass:

86.06 g/mol

Boiling point:

161°C

Melting point:

15°C

Applications:

- Opthalmic drug delivery
- Buccal and sublingual drug delivery
- Gastrointewtinal drug delivery
- Intestinal drug delivery
- Colon drug delivery
- Transdermal drug delivery
- Vaginal drug Delivery
- Gene delivery
- Vaccine delivery

Eudragit polymer were selected for this studies because they dissolved at p^H > 5.5 and are not soluble in acidic gastric fluid and which can prevent the pre-mature release of the incorporated drug molecule during the preparation before dosing.

Eudragit are sufficiently begin in pre-clinical models to enable *in vivo* safety pharmacology studies where small changes in organ function and physiology are assessed during lead optimization.

MORINGA OLEIFERA (Fuglie et al., 2001)

Scientific classification:

Kingdom: Plantae

Order : Brassicales

Family : Moringaceae

Genus : Moringa

Species : M. oleifera

Synonyms:

- *Guilandina moringa* L.
- *Hyperanthera moringa* (L.) Vahl
- *Moringa pterygosperma*

Solubility:

Soluble in water. Practically insoluble in ethanol, acetone and chloroform.

Loss on Drying:

12.02%

Total ash content:

12%

p^H:

6.8-6.9

Viscosity:

900cps

Traditional medicine and research

The bark, sap, roots, leaves, seeds and flowers are used in traditional medicine. Research has examined how it might affect blood lipid profiles, although it is not effective at diagnosing, treating, or preventing any human diseases.

Extracts from leaves contain low contents of polyphenols which are under basic research for their potential properties. Despite considerable preliminary research on the biological properties of moringa components, there are few high-quality studies on humans to justify its use to treat human diseases.

Potential adverse effects:

Various adverse effects may occur from consuming moringa bark, roots or flowers and their extracts, as these components contain chemicals that appear to be toxic when eaten. Moringa leaves have been used safely in doses up to 6 grams daily for up to 3 weeks.

Uses:

It mainly used for "TIRED BLOOD" (anemia),arthritis and other joint pain and it used for cancer. Moringa destroyed the cancer cell boost of the immune system. It reduces the toxic effect. Sometimes used as an abortifacient and to treat symphilis and rheumatism.

In developing countries, moringa has the potential to improve nutrition, boost food security, foster rural development, and support sustainable landcare. It may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic, and possible adjuvant.

Moringa oleifera leaf powder was as effective as soap for hand washing when wetted in advance to enable anti-septic and detergent properties from phytochemicals in the leaves.

LINSEED

Synonym : Flax seed.

Biological Source:

It consists of the dried fully ripe seeds of *Linum usitatissimum* Linn. Belonging to family *Liliaceae.*

Geographical Sources:

It is cultivated extensively as a source of fibres in Algeria, Egypt, Greece, Italy and Spain; as a source of oil in Afghanistan, India and Turkey; and in Russia (now CIS – countries) for both oil and fibre. It is also found in several temperate and tropical zones.

Preparation The cyanogenetic glycoside :

Linamarin is prepared from the defatted oil meal, seed skins or embryos of flax by standard methods available for glycosides.

Description:

Colour : Reddish brown

Odour : Characteristic odour

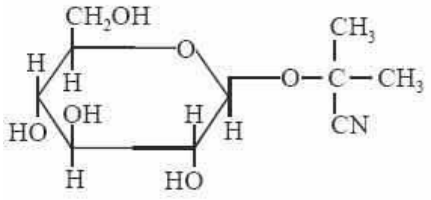
Shape : Oval and strongly flattened

Size : Length = 4-6 mm; Width = 2-3 mm.

Chemical Constituents :

The ripe seeds of linseed contain small quantities of a cyanogenetic glycosides known as *linamarin* (or *phaseolunatin*) as given below:

Interestingly, linamarin evolved HCN with linseed meal only but not with emulsin. However, pure linamarin is a bitter needle like crystalline substance. It is freely soluble in water, cold alcohol, hot acetone, slightly in hot ethyl acetate, ether, benzene, chloroform and practically insoluble in petroleum ether.



Linamarin

Besides, linseed seeds comprise of fixed oil (33-43%) mucilage present in testa (6%), proteins (25%) and an enzyme called *linase*.

Linamarin upon enzytmatic hydrolysis yields HCN which actualy renders the seeds highly poisonous.

Chemical Test :

The mucilage of linseed seed gives a distinct red colour on being treated with Ruthenium Red Solution.

Uses

1. Therapeutically, the linseed oil is mostly recommended for the external applications only; liiments and lotions. Linseed contributes to the normal formation of red blood cells and haemoglobin and to the reduction of tiredness and fatigue. Linseed intract with drug molecule it gives good pharmacological effect for the targeted site.
2. It is employed in the treatment of scabies and other skin disease in combination with pure flowers of sulphur.
3. As the linseed oil has an inherent very high **'**iodine value' it is used mostly in the preparation of non staining 'Iodine Ointment' and several other products such as 'Cresol with Soap'.
4. Commercially, it is one of the most important 'drying oil' and therefore, substantially huge amounts are exclusively used for varnishes and paints.
5. Linseed oil finds its extensive application in the manufacturer of soap, grease, polymer, plasticizer, polish and linoleum.

Production by country

Top ten linseed producers – 2011		
Country	**Production (metric tons)**	**Footnote**
Canada	368,300	
China	350,000	*
Russia	230,000	*
India	147,000	
United Kingdom	71,000	
United States	70,890	
Ethiopia	65,420	
Kazakhstan	64,000	*
Ukraine	51,100	
Argentina	32,170	
World	**1,602,047**	**A**
No symbol = Official data, * = Unofficial figure, A = Aggregate (may include official, semi-official or estimated data)		

Flax seed benefits could help you improve digestion, give you clear skin, lower cholesterol, reduce sugar cravings, balance hormones, fight cancer and promote weight loss… and that's just the beginning! Flaxseeds, sometimes called linseeds, are small, brown, tan or golden-colored seeds that are the richest sources of a plant-based omega-3 fatty acids, called Alpha Llinolenic acid (ALA) in the world.

Nutrition Facts

When you look at the nutritional benefits of flax seeds, there are many things that will catch your attention.

A 1 ounce (3 tbsp) serving of flaxseeds contains:

- Omega-3 (ALA)- 6,338mg
- Fiber- 8g
- Protein- 6g
- Vitamin B_1- 31% RDA
- Manganese **-** 35% RDA
- Magnesium -30% RDA
- Phosphorus **-** 19% RDA
- Selenium **-** 10% RDA
- Also, flaxseeds contain a good amount of vitamin B6, Iron, potassium, copper and zinc.

Flax Seed Benefits

- High in Fiber, but Low in Carbs
- Healthy Skin and Hair
- Weight Loss
- Lower Cholesterol
- Flaxseeds are Gluten-Free
- Flaxseeds are High in Antioxidants (Lignans)
- Digestive Health

Flax Seeds for Cancer

Flax seed benefits have been proven time and time again and even including fighting breast, prostate, ovarian and colon cancer.

The three lignans found in flaxseeds can be converted by intestinal bacteria into enterolactone and enterodiol which naturally balance hormones which may be the reason flax seeds reduce the risk of breast cancer.

Flaxseeds may also reduce the risk of endometrial and ovarian cancer.(Kajila et al., 2015)

High in Omega-3 Fatty Acids

We hear a lot about the health benefits of fish oil or omega-3 fats. Fish oil contains EPA and DHA, two omega-3 fats that are critical for optimal health. Although flaxseeds do not contain EPA or DHA, they do contain ALA, another type of omega-3 fat.

A study published in *Nutrition Reviews* has shown that approximately 20% of ALA can be converted into EPA, but only 5% of ALA is converted into DHA. Also, surprisingly gender may play a big role in conversion where young women had a 2.5-fold greater rate than men.

Regardless of conversion, ALA is still considered a healthy fat and should be included in a balanced diet.(Kajila et al., 2015)

BADHAM

Synonym:

Badam Pisin or Almond Gum is natural edible gum obtained from the sweet almond tree bark. Its scientific name is *Prunus amygdalus* and is different from the Indian almond tree (Scientific name: *Terminalia Catappa*).

Colour &Solubility

Badam Pisin is either white or brown in color and is found in the shape of small rocks. It can be dissolved in water and is used in making jellies.

Badam Pisin Health Benefits and Other Uses:

- It is edible coating on cherries and just 10% is enough to increase the shelf life of the cherries for up to 15 days when stored at 2℃.
- It has great cooling properties and is suitable for people with excessive body heat.
- It helps calm the stomach burns, treats ulcers and reduces the burning sensation in the stomach.
- It is a good cure for people suffering from diarrhoea.
- It is known for its cholesterol lowering properties.
- In North India, Gondh Laddus are famous for its health benefits. It is given to pregnant women for its nutritional and cooling properties. It strengthens the bone and increases body strength.
- It can be used in deserts and beverages after a spicy meal to prevent acidity and stomach burns.
- Being a natural gum, it is free of artificial colors and preservatives. It is therefore ideal for consumption by children.
- It can be used in preparing ice creams and jelly's for children.

- It is used in uncoated tablets and is found to have increased drug release property.
- Since it is a natural gum, it is inexpensive and safe to use.
- When consumed regularly, it helps gain weight. Add badam pisin with milk and drink to see visible result in weight gain.

SPECIAL CONSTITUENTS:

Almond oil:

Scientific Name(s):

Prunus dulcis

Family:

Rosaceae.

Common Name(s):

Almond milk, almond oil, amygdale amara, amygdalin, bittermandel, bitter almond, ku wei bian tao, laetrile, oil of almonds, sweet almond, vitamin B_{17}, volatile almond oil.

Uses:

Almonds are used as a dietary source of protein, unsaturated fats, minerals, micronutrients, phytochemicals, alpha-tocopheral, and fiber, as well as in confectioneries. The efficacy of almonds in altering the lipid profile is weakly supported by the literature; larger, more robust clinical trials of longer duration are required. The almond derivative laetrile/amygdalin has been used as an alternative cancer treatment, but there is no clinical evidence to support this use. Laetrile is banned by the US Food and Drug Administration (FDA) and in Europe for use in cancer therapy.

Chemistry

Almond nuts have a unique fatty acid profile, largely composed of unsaturated fats, some saturated fats, and no cholesterol. In addition to the protein and carbohydrate content, almonds contain large amounts of alpha-tocopherol and arginine, as well as magnesium, potassium, and sodium. Almonds are rich in phytosterols, including beta-sitosterol, stigmasterol, and campesterol. The skin of the almond nut accounts for 4% of the total nut weight and is rich in polyphenols, including hydroxybenzoic acids and aldehydes, flavonol and flavanone aglycones, and glycosides.

Uses and Pharmacology

Cancer

Despite promising *in vitro* experiments, the use of amygdalin as a cancer treatment has not been validated by any clinical trials. The National Cancer Institute sponsored phase 1 and 2 clinical trials in the 1980's but found no evidence to support the use of laetrile in the treatment of cancer. Because a Cochrane review found no clinical trials that met adequate methodological quality, a meta-analysis could not be conducted.

COBALT (II) CHLORIDE

Formula:

$CoCl_2$

Molar mass:

129.839 g/mol

IUPAC ID:

Cobalt(II) chloride

Melting point:

735°C

Density:

3.36 g/cm³

Boiling point:

1,049°C

Soluble in:

Water

IUPAC name

Cobalt (II) Chloride

Other names

Cobaltous chloride

Cobalt dichloride

Muriate of Cobalt

Properties

Chemical formula

$CoCl_2$

Molar mass

129.839 g/mol (anhydrous)

165.87 g/mol (dihydrate)

237.93 g/mol (hexahydrate)

Appearance of blue crystals (anhydrous)

Violet-blue (dihydrate)

Rose red crystals (hexahydrate)

Density

3.356 g/cm^3 (anhydrous)

2.477 g/cm^3 (dihydrate)

1.924 g/cm^3 (hexahydrate)

Melting point

735°C (1.355°F; 1.008 K) (anhydrous)

140°C (monohydrate)

100°C (dihydrate)

86°C (hexahydrate)

Boiling point

1049°C

Solubility in water

43.6 g/100 mL (0°C)

45 g/100 mL (7°C)

52.9 g/100 mL (20°C)

105 g/100 mL (96°C)

Solubility

38.5 g/100 mL (methanol)

8.6 g/100 mL (acetone)

Soluble in ethanol, ether, pyridine, glycerol .

Magnetic susceptibility (χ)

+12,660·10−6 cm3/mol

Structure

Crystal structure

$CdCl_2$ structure

Coordination geometry

Hexagonal (anhydrous)

Monoclinic (dihydrate)

Octahedral (hexahydrate)

Uses:

- Invisible ink
- Cobalt chloride is an established chemical inducer of hypoxia-like responses such as erythropoiesis.
- Cobalt supplementation is not banned and therefore would not be detected by current anti-doping testing.
- It inhibit osteosarcoma development;the mechanism may be related to the hypoxic microenvironment and HIF-1α may be a critical factor and including inhibit proliferation, promoting apoptosis and tumor formation.(Bozhang et al., 2012)
- It is a chemical inducer of hypoxia-inducible factor-1α (HIF-1α). HIF-1α is a transcription factor that has an important role in tumor cell adaptation to hypoxia, and controls the expression of several genes .(Yang et al., 2016)

OBJECTIVE

- To formulate Imatinib Mesylate nanoparticles to treat leukemia, especially Philadelphia positive Chronic Myeloid Leukemia (ph+CML) and Chronic Eosinophillic Leukemia (CEL) using natural and synthetic polymers.
- To formulate Imatinib Mesylate nanoparticles reduce severe side effects such as muscle cramps, stomach pain, musculoskeletal pain.
- To reduce the frequency of dosage form, facilitate the drug targeting and to overcome drug resistance. To check the compatibility between the Imatinib Mesylate nanoparticles with natural polymers (Moringa oleifera, Linseed, Badam) and synthetic polymers (Poly vinyl alcohol, Hydroxy Propyl Methyl Cellulose, Eudragit) by FT-IR.
- To perform the *In vitro* evaluation of formulated Imatinib Mesylate nanoparticles and optimize the formulations.
- To complex the Imatinib Mesylate formulation with anti-proliferative agent in the optimized.
- To perform *In vitro* evaluation of Imatinib Mesylate nanoparticles complexed with Cobalt (II) Chloride.
- To perform the acute toxicity study of Imatinib Mesylate nanoparticles complexation. ($CoCl_2$ NPs) by OECD guidelines 420 using female *wistar rat* model.
- To perform the cancer activity study of $CoCl_2$ NPs using to treat the male *swiss albino mice*(Dalton's Lymphoma Ascities cell line)
- To test the stability of the formulated Imatinib Mesylate nanoparticles.

BACKGROUND OF THE STUDY

Cancer is a class of diseases characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Cancer harms the body when altered cells divide uncontrollably to form lumps or masses of tissue called tumors (except in the case of leukemia where cancer prohibits normal blood function by abnormal cell division in the blood stream). Tumors can grow and interfere with the digestive, nervous, and circulatory systems and they can release hormones that alter body function. Tumors that stay in one spot and demonstrate limited growth are generally considered to be benign.

More dangerous, or malignant, tumors form when two things occur:

1. A cancerous cell manages to move throughout the body using the blood or lymphatic systems, destroying healthy tissue in a process called invasion
2. That cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis.

When a tumor successfully spreads to other parts of the body and grows, invading and destroying other healthy tissues, it is said to have metastasized. This process itself is called metastasis, and the result is a serious condition that is very difficult to treat.

Certain molecular interactions between cells and the scaffolding that holds cancer cells in place (extracellular matrix) cause them to become unstuck at the original tumor site, they become dislodged, move on and then reattach themselves at a new site. (Curtis et al., 2012)

The researchers say this discovery is important because cancer mortality is mainly due to metastatic tumors, those that grow from cells that have travelled from their original site to another part of the body. These are called secondary tumors. Only 10% of cancer deaths are caused by the primary tumors.

Malignant cells can pass more easily through smaller gaps, as well as applying a much greater force on their environment compared to other cells.

Their catalogue will help oncologists detect cancerous cells in patients early on, thus preventing the spread of the disease to other parts of the body.

Cancer is ultimately the result of cells that uncontrollably grow and do not die. Normal cells in the body follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grows out of control.

In India, Cancer is thought to be the great leveler. Whether you are an Indian or a Korean or Icelander, cancer will bring death. But a massive study of 26 million cancer patients over 15 years has shown that survival rates in the 10 most prevalent types of cancer vary hugely across countries. Survival rates in India are quite low for most types of cancer, less than half of the advanced countries in many types. More surprising is the fact that survival rates in India are either stagnating, or inching up very slowly, despite India having some of the better treatment facilities.

The ICMR has estimated that about 14.5 lakh patients will develop cancer in India during 2016 with the number expected to rise to 17.3 lakh by 2020. Breast cancer is the leading cancer in India, with an estimate of 1.5 lakh cases for 2016, followed by lung cancer that is expected to strike 1.14 lakh patients this year. So, the nanoparticles are used in the treatment of anticancer activity to target only the cancer cells. In this study, nanotechnology is adopted to target the cancer cells with the reduced side effects of Imatinib Mesylate attempted. To study to enhance the anti-cancerous activity of Imatinib Mesylate when complexing with anti-prolifective agent Cobalt (II) Chloride($CoCl_2$ NPs)

MECHANISM FOR THE BIOSYNTHESIS OF COBALT METAL NANOPARTICLES:

Biosynthesis of cobalt nanoparticles in living system is catalysed by various reducing agents or reductase type enzymes present intracellularly or extracellularly. So, it is not easy to claim which compound is exactly responsible for biosynthesis cobalt nanoparticles in the living system. It demands research at molecular level. There are various theories proposed by researchers for the biosynthesis of cobalt metal nanoparticles.

1) Nanoparticles are synthesised by the reduction of metal ions to zero valency atom by various reductase enzyme produced by the cell, like nitrate reductase, which uses NADH as electron donor (Sakthivel et al., 2010).

2) Metal ions are reduced by various reducing sugars present, or synthesised by microbes inside the cell, or secreted extracellularly, into zerovalent metal atom (Philip et al., 2010).

But these agents or enzymes or polymers reduce metal ion into zerovalent atom by the time metal ion concentration levels for the organism. So to reduse the toxicity of high concentration, microorganism starts reducing ions into free zero valent atoms. These free atoms get polarized due to free electrons. Polarization generates attractive force between them and binds them with metallic bond. At an optimum concentration of metal ions, metal nanoparticles are synthesised. If the concentration of added ionic solution is more than the optimum concentration then it will form bigger particles. Generally, due to delocalization of electron, zeta potential generates around the nanoparticles. In physical and chemical methods surfactants or polymer are used to increase the zeta potential of nanoparticles. (Mohanpuria et al., 2008)

SCOPE AND PLAN OF WORK

The field of medicine never dies as long as life exists in this planet. Inventions and discoveries of new drug molecules are increasing from day to day and as a result the field of medicine is the emerging field in today's world. Once the drug molecules are developed the risky phase is their moulding into different formulations and their route of administration. Half life, Bioavailability, firstpass metabolism, adverse drug reactions etc., play a major role in drug development. As a result drug molecules once discovered found new mouldings as the days and years are passed by diseases are increasing from day to day and our nature is a treasure hunt where still many drug molecules are yet to be discovered and developed. The development of drug molecules from the nature results in a decreased level of adverse drug reactions with increased bioavailability.

Recent research efforts throughout the world have resulted in significant development of novel drug delivery systems. Among the systems one of the common methods of controlling the rate of drug release. The resulting nanoparticles have gained good acceptance as a process to achieve the sustained release.

There has been growing interest in the use of natural polymers and synthetic polymers as a drug carrier due to their biocompatibility, biodegradability, and non -toxicity and easy in availability.

The plane of work carried out in period of 10 months (June 2016 to march 2017) which was divided into two phases like

Phase I

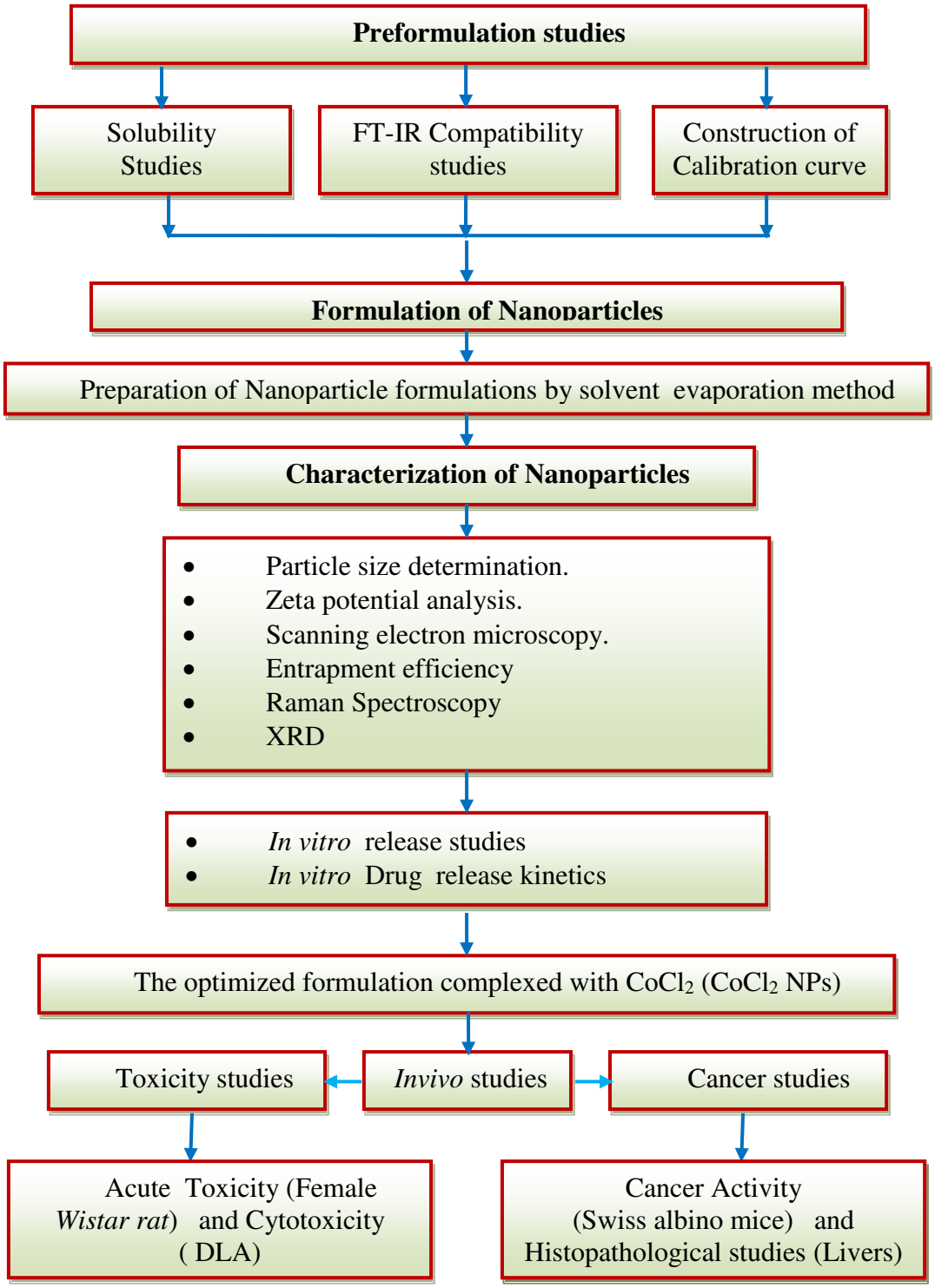
Literature survey

Identification of objective for the current study

Phase II

Preparation of Imatinib mesylate nanoparticles and evaluation.

PLAN OF WORK


Preformulation studies
Solubility Studies
FT-IR Compatibility studies
Construction of Calibration curve
Formulation of Nanoparticles
Preparation of Nanoparticle formulations by solvent evaporation method
Characterization of Nanoparticles
Particle size determination.
Zeta potential analysis.
Scanning electron microscopy.
Entrapment efficiency
Raman Spectroscopy
XRD
In vitro release studies
In vitro Drug release kinetics
The optimized formulation complexed with CoCl2 (CoCl2 NPs)
Toxicity studies
Invivo studies
Cancer studies
Acute Toxicity (Female Wistar rat) and Cytotoxicity (DLA)
Cancer Activity (Swiss albino mice) and Histopathological studies (Livers)

MATERIALS AND EQUIPMENTS

MATERIALS USED

S. No	Material	Source
1	Imatinib Mesylate	Gift sample
2	Hydroxypropyl methyl cellulose	Ozone International, Mumbai
3	Polyvinyl alcohol	SD Fine Chemicals, Mumbai
4	Eudragit	SD Fine Chemicals, Mumbai
5	Moringa oleifera gum	Mothers herbs(P) Ltd, New Delhi.
6	Linseed gum	Green field Agro products, Jabalpur
7	Badam gum	Mothers herbs(P) Ltd, New Delhi.
8	Acetone	SD Fine Chemicals, Mumbai
9	Petroleum Ether	SD Fine Chemicals, Mumbai
10	Liquid paraffin	Thermo Fisher Scientific, Mumbai
11	Cobalt(II) Chloride	SD Fine Chemicals, Mumbai
12	Trypan blue	SD Fine Chemicals, Mumbai

EQUIPMENTS USED

S. No	Equipment	Model/Company
1	Magnetic Stirrer	REMI – 2MLH
2	Optical Microscope	MOTIC B1 SERIES
3	UV Spectrophotometer	JASCO V-530
4	Dissolution Apparatus	ELECTRO LAB TDT – 08L
5	FT-IR Spectrometer	FTIR JASCO – 4100
6	pH meter	PH Tester 1,2 (EUTECH)
7	Zeta Sizer	MALVERN
8	SEM	JSM 6390, Make – JEOL
9	XRD	Rigakuultima IV
10	Raman Spectroscopy	Shimadzu
11	Autmotated analyzer	Orphee Mythic 18

EXPERIMENTAL METHODS

ANALYTICAL METHOD:

A spectrophotometric method based on the measurement of absorbance at 252 nm was used in the present study for the estimation of Imatinib Mesylate in the concentration range of 5-35µg/ml. Calibration curve were constructed.

METHOD DEVELOPMENT

Preparation of standard solution

The standard stock solution of Imatinib Mesylate was prepared by transferring, accurately weighed 100mg of Imatinib Mesylate in 100ml of volumetric flask. The drug was dissolved in 50ml of distilled water under sonication and volume was made up to the mark using distilled water. The standard stock solution (1000µg/ml) was further diluted with distilled water to get the concentration of 10µg/ml. (Wagurkar et al., 2015)

Selection of Analytical wavelength:

The standard solution of 10µg/ml was scanned between 200nm to 400nm in UV spectrophotometer against distilled water as blank after baseline correction. Wavelength range was selected around wavelength maxima (252nm).

Procedure for plotting calibration curve of pure drug

From the standard solution of Imatinib Mesylate was subsequently diluted with water to obtain a series of dilutions containing concentration in the range of 5-35 µg/ml. The absorbance of these solutions was measured at 252 nm UV spectrophotometer (JASCO V530) against corresponding blank. The concentration of Imatinib Mesylate and the corresponding absorbance values are given in table. The calibration curves for the estimation of Imatinib Mesylate were constructed by plotting linear best fit between concentration of Imatinib Meslate and corresponding mean absorbance value are shown in figure:8 (Wagurkar et al., 2015)

IDENTIFICATION OF IMATINIB MESYLATE

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectrum of drug was recorded using a FT-IR Spectra photometer (Shimadzu JASCO 4100). The diffuse reflectance technique was utilized in the mid IR 4000-400 cm^{-1} spectral region. The procedure consist of dispersing the sample in KBr (100mg) using a motor, triturating the materials into a fine powder bed into the holder using compression gauge. The pressure was around 5 tons for 5 min. The pellet was placed light path and the spectrum was recorded. The characteristic peaks of the functional groups were interpreted.(Skoog et al., 2007)

PREPARATION OF NANOPARTICLES BY SOLVENT EVAPORATION METHOD:

Imatinib Mesylate nanoparticles were prepared by using solvent evaporation technique which is an effective technology in preparation of Imatinib Mesylate nanoparticles. Accurately weighed amount of Imatinib Mesylate was completely dissolved in isopropyl alcohol. The obtained drug solution and polymer solution was then injected into the water under stirring at 3000 rpm. Precipitation of solid drug particles occurred immediately upon mixing. The suspension was centrifuged at 10000 rpm for 20 min and washed twice with purified water. The precipitated nanoparticles were dried for 48 hours. The Imatinib Mesylate nanoparticles were formulated using by various synthetic polymers Poly Vinyl Alcohol, Hydroxy Propyl Methyl Cellulose, Eudragit and natural polymers. Moringa oleifera, Badam, Linseed at 1:1,1:2 ratios respectiverly (F1-F12). (Mansouri *et al.,* 2011)

DRUG-EXCIPIENT COMPATIBILITY STUDIES

Before formulating of a drug substance into a dosage form, it is essential that it should be chemically and physically compatibile. Compatibility studies give the information needed to define the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

One of the requirements for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work, a study was carried out by using infrared spectrophotometer to find out if there is any possible chemical interaction between Imatinib mesylate and polymers.

Weighed 3 mg of sample was mixed with 100 mg of potassium bromide (dried at 40-50˚c). The mixture was taken and compressed under 10-ton pressure in hydraulic press to form a transparent pellet. The pellet was scanned from 4000-400cm-1 in IR spectrophotometer.

CHARACTERIZATION OF NANOPARTICLES

Scanning Electron Microscope

A scanning electron microscopic sample holder with a double sided taps and coated with a layer of gold of 150˚A for 2 min using a sputter coater(JSM 6390, Make – JEOL) in a vaccum of 3×10^{-1} atm of organ gas. The samples were then examined using a scanning electron microscope (Kumar et al., 2014).

Determination of particle size

The average mean diameters and size distribution of nanoparticle was found out by photon correlation spectroscopy using Zetasizer (nano ZS90, Malvern Instruments) at 25ºC. The samples were kept in polystyrene cuvette and the readings were noted at a fixed angle (Kumar et al., 2014).

Determination of zeta potential

The electrophoretic mobility (zeta potential) measurements of nanoparticle were made using Zetasizer (Nano ZS90, Malvern Instruments). The samples were placed in a polystyrene cuvette (at 25ºC) and Zeta dip cell was used to find out the zeta potential (Kumar et al., 2014).

Drug Entrapment Efficiency

The prepared Imatinib Mesylate nanoparticles equivalent 250 mg were placed in a known quantity of solvent. After 24 hrs the samples were subjected to centrifugation followed by decantation of the fluid was performed to separate the nanoparticles containing the entrapped drug. The nanoparticles were lysed with isopropyl alcohol and spectrophotometric analysis was carried out at 252 nm. Entrapment efficiency is calculated as percentage (%) (Havanoor et al., 2014).

$$Entrapment\ Efficiency\ \% = \frac{\text{Pr}actical\ \ Drug\ Content}{Theorital\ \ Drug\ Content} X100$$

***IN VITRO* DRUG RELEASE:**

The *In vitro* drug release profile of the prepared Imatinib Mesylate nanoparticles were investigated by USP Type I dissolution apparatus in using distilled water as dissolution medium. At a temperature of 37°C±2°C and the stirrer was rotated at 50 rpm speed. Nanoparticles containing Imatinib Mesylate equivalent 250 mg is introduced into the basket and involved in the dissolution medium transferred into the vessel containing 900 ml of purified water.

At specific intervals, 1 ml aliquot of the dissolution medium was sampled (the pooled sample was replaced with a fresh purified water), filtered by whatmann filter paper (pore size: 0.45 pm) and determine the amount of drug release from Imatinib Mesylate nanoparticle spectrophotometrically at 252nm by using Jasco V530 spectrophotometry from UV absorbance of the sample solution, in comparison with a standard solution having a known concentration of raw drug. (Mansouri et al., 2011)

***In vitro* drug release kinetics**

The drug release kinetics and mechanism of drug release of Imatinib mesylate nanoparticles was determined by fitting *In vitro* release data into various models such as zero order, first order and Higuchi equations Korsmeyer-Peppas model.

Zero-Order Kinetics:

Cumulative amount of drug released was plotted against time.

$$C = K_0 t$$

where K_0 is the zero-order rate constant expressed in units of concentration/time and *t* is the time in hours. A graph of concentration Vs time would yield a straight line with a slope equal to K_o and intercept the origin of the axis. This kinetics describes concentration independent drug release from the formulations.

First order kinetics:

First order as log cumulative percentage of drug remaining vs time. This kinetics describes concentration dependent drug release from the formulations.

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

where C_0 is the initial concentration of drug, *k* is the first order constant, and *t* is the time.

Higuchi's Model:

Higuchi's model as cumulative percentage of drug released vs. square root of time.

$$Q = Kt_{1/2}$$

where *K* is the constant reflecting the design variables of the system and *t* is the time in hours. This model describes the release of drug on the basis of Fickian diffusion as a square root of time dependent process from swellable matrix.

Korsmeyer-Peppas Equations:

The mechanism of drug release, the first 60% of drug release were plotted in Korsmeyer equation log cumulative percentage of drug released vs log time, and the exponent n was calculated through the slope of the straight line,

$Mt / M\infty = Ktn$

Where $Mt/M\infty$ is the fractional solute release, t is the release time, K is a kinetic constant. Characteristic of the drug/polymer system, and n is an exponent that characterizes the mechanism of release of tracers. For cylindrical matrix tablets, if the exponent n = 0.45, then the drug release mechanism is Fickian diffusion, and if $0.45 < n < 0.89$, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release (Balaiah. A et al.,2012).

COMPLEXATION OF OPTIMIZED NANOPARTICLES WITH COBALT (II) CHLORIDE METAL AND ITZ CHARACTERIZATION (F-13)

The optimized Imatinib Mesylate nanoparticles were complexed with Cobalt(II)Chloride($CoCl_2$ NPs). Since Cobalt (II) Chloride posses anticancer activity and useful in radio immune assay, the complexation is carried out.

Experimental Properties

Complex:

Transition elements will form co-ordination compounds (complex) because their d orbitals are not completely filled. In this study ligand was performed between F3 Formulation and cobalt (II) chloride.

The complex is prepared by using Equi molar (0.001M) solution of drugs and Cobalt (II) chloride were taken. To the drug solution was added into the $CoCl_2$ solution slowly until to get the precipitation and again added to the solution to dissolve the precipitation to get clear solution. The clear solution was evaporated. Finally the complexed of crystals were formed. The complexed. The Imatinib Mesylate nanoparticles with Cobalt (II) Chloride ($CoCl_2$NPs) was subjected to characterization, *Invitro* release studies and *In-vivo* studies.

RAMAN SPECTROSCOPY:

Light scattering phenomena may be classically described in terms of electromagnetic radiation produced by oscillating dipoles induced in the molecule by the electromagnetic fields of the incident radiation. The light scattered photons include mostly the dominant Rayleigh and the very small amount of Raman scattered light. The Induced dipole moment occurs as a result of the molecular polarizability α, where the polarizability is the deformability of the electron cloud about the molecule by an external electric field.

In a typical Raman experiment, a laser is used to irradiate the sample with monochromatic radiation. Laser sources are available for excitation in the uv, visible, and near-IR spectral region (785 & 1064 nm). Thus, if visible excitation is

used, the Raman scattered light will also be in the visible region. No energy is lost for the elastically scattered Rayleigh light while the Raman scattered photons loss some energy to the specific vibrational coordinates of the sample. In order for Raman bands to be observed, the molecular vibration must cause a change in the polarizability.

Both Rayleigh and Raman are two photon processes involving scattering of incident light from a virtual state. The incident photon is momentarialy absorbed by a transition from the ground state in to a virtual state and a new photon is created and scattered by a transition from this virtual state. Rayleigh scattering is the most probable event and the scattered intensity is ca.10^{-3} less than that of the original incident radiation. This scattered photon results from a transition from the virtual state back to the ground state and is an elastic scattering of a photon resulting in no change in energy. The $CoCl_2$ NPs was determined by using raman spectroscopy.

X-RAY DIFFRACTION:

X-Ray diffraction is the process by which scattering of X-rays by the atoms of a crystal that produces an interference effect so that the diffraction pattern gives information on the structure of the crystal or the identity of a crystalline substance.

Change in the crystalinity of the drug can be determine by this technique. (Opez et al., 2012)

Powder X-ray diffraction study (Sagar S.Jadhav et al., 2013)

The XRD patterns of pure drug, dummy nanoparticle and optimized nanoparticle are to be measured on a diffractometer. Monochromatic radiation (λ = 1.5418A˚), at 40 kV and 40 mA, were used as the X-ray source. The diffractograms were recorded between 20º and 70º with an increasing step of 0.02º and 2 s as the time for each step.

IN VIVO STUDIES

EXPERIMENTAL ANIMALS:

Female *wistar rats* weighing 200-250 gm were used for acute toxicity studies and male *swiss albino mice* (20-25 mg) were used for anticancer activity. The animals were procured from College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India. The study protocol was approved by the Institutional Animal Ethical Committee (COPSRIPMS/IAEC/PG/PHARAMCEUTICS/01/2015-16) and all procedures were conducted with proper care and use of laboratory animals.

HOUSING AND FEEDING CONDITION:

The animals procured were acclimatized to the laboratory conditions for 7 days prior to the start of the experiment and they were randomized and assigned to various treatment groups. Studies were conducted in controlled environment under the temperature (22±3°C) with 12:12 hour light/dark cycle in polypropylene cages. Animals were provided with standard pellet feed and drinking water *ad libitum*.

ACUTE TOXICITY STUDIES:

Acute toxicity studies was performed following the fixed dose procedure employing the OECD guideline No. 420 (OECD 2002). Healthy young adult female rats are the commonly used laboratory strains. Normally female rats are used because of their sensitivity. Females should be nulliparous and non-pregnant. Animals should be 8-12 weeks old and weight should not deviate from ± 20%. The animal should be fasted for 12 hour prior to the testing with free access to the water only. Due to absence of toxicity information for polymer in the sighting study one animal is administered 5 mg/kg dose and in the absence of toxicity 50mg/kg dose is given to second animal to check for toxicity. If no

mortality is observed for 50 mg/kg dose then the next dose of 300 mg/kg is given to third animal and if no mortality is observed then the fourth animal would administered with 2000 mg/kg dose. If no mortality is observed for upper dose limit of 2000 mg/kg, then the specific dose is selected for main study. In the main study another 5 animals would be dosed with 2000 mg/kg. Animals will be observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Keen observations should be done to determine the onset of signs, onset of recovery and time to death. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, autonomic and central nervous systems and behavioural pattern.

Table:5 Design of acute toxicity studies

STUDY INVOLVED	DOSE(mg/kg)	NO.OF.ANIMALS
Sighting study	5	1
Sighting study	50	1
Sighting study	300	1
Sighting study	2000	1
Main study	2000	5

TUMOR MAINTENANCE AND INDUCTION

The Ascites Dalton's lymphoma (DLA) cell line was obtained from Amala Cancer Research Institute (Thrissur, India). DLA cells would be maintained *in vivo* by serial intraperitoneal transplantation of viable tumor cells to *Swiss* albino mice (in 0.25 mL PBS, *p*H 7.4). For the inoculation of ascites tumors in the mice, DLA cells collected from the donor mouse is suspended briefly in sterile

isotonic saline. Viable cells is counted (Trypan blue assay) and adjusted to a concentration ensuring that each animal receives an Intra Peritoneal (IP) injection of 1×10^6 cells/100µl.

Method for Diluting cell up to 10^6 cells/100 µl PBS

1. Aspirate cell from tumor bearing animal (Volume taken depend upon the no. of animals to be induced, for ex. For 10-15 animals, 1ml of whole cell is sufficient).
2. Wash the cell with PBS (Phosphate Buffered Saline, pH7.2), thrice by slow speed centrifugation.
3. To the cell pellet thus obtained, add thrice volume of PBS and kept this suspension as stock. (If 1 ml cell pellet without blood content, add around 3 ml PBS).
4. Now we should further dilute the stock solution, in order to make it up to 10^6 cells/ 100µl concentration.

This is done by the following method,

a. Take another test tube containing 900µl PBS.
b. To this add 100µl cell suspension from the stock solution.
c. This working solution then counted under microscope, under 10X objective, using heamocytometer.
d. Each corner of the hemocytometer should contain 200 cells (round, transparent individual cells) in order to attain the 10^6 cells/ 100µl concentration. Based on the no. of cells counted from the 100µl working solution, we should further dilute the stock solution.

IN VITRO CYTOTOXICITY STUDIES:

Determination of *in vitro* cytotoxicity of Imatinib mesylate to DLA cells

The tumor cells isolated from peritoneal cavity were washed thrice with phosphate buffer saline. Viable DLA cells (1×10^6 cells in 0.1 mL) were added to tubes containing various concentrations (10, 25, 50, 100 and 200 µg/mL) of Imatinib mesylate and the volume was made up to 1 mL using phosphate buffer saline (PBS). Cell viability was determined by trypan blue exclusion method

$$\% \text{ viability} = \frac{\text{Number of dead cells}}{\text{Number of dead cells + number of live cells}} X100$$

EXPERIMENTAL DESIGN:

The mice would be divided into three groups, with ten animals in each group:

Group 1 : Positive control, tumor-induced treated with pure drug Imatinib Mesylate (20 mg/kg/day)

Group 2 : Tumor-induced mice treated with Cobalt(II)Chloride nano particles ($CoCl_2$ NPs) at low dose (200 mg/kg b.w) in aqueous solution via intra-peritoneal (IP) injection for 15 days

Group 3 : Tumor-induced mice treated with $CoCl_2$ NPs at high dose (400 mg/kg b.w) in aqueous solution via intra-peritoneal (IP) injection for 15 days.

Animals in group I - III would be inoculated with 1×10^6 cells/mouse on day 0, and treatment with standard drug and $CoCl_2$ NPs started 24 hours after transplantation. The tumor control group will be treated with the same volume of 0.9% NaCl solution. The values for control and negative control were taken from PG Pharmacology Department of our college. (Sriram *et al*., 2010)

TUMOR VOLUME AND WEIGHT:

The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. Volume of the fluid was measured by taking it in a graduated centrifuge tube and expressed in milliliter (mL).Tumor weight was measured by taking the weight of the mice before and after the collection of the ascetic fluid from peritoneal cavity and expressed in gram(g) (Dolai *et al*., 2015)

DETERMINATION OF MEAN SURVIVAL TIME AND PERCENTAGE INCREASE IN LIFESPAN

Mean survival time (MST) of each group would be noted. The MST of the treated groups would be compared with that of the control group using the following calculation:

Increase in life span = (T-C/C) × 100

where T = number of days the treated animals survived and C = number of days the control animals survived.

Mean survival time (MST) in days

$$= \frac{\text{day of the first death} + \text{day of the last death}}{2}$$

$$\text{ILS (\%)} = \frac{\text{MST of the treated group}}{\text{MST of the control group}} X100 - 1$$

TUMOR CELL (VIABLE/NONVIABLE) COUNT (Dolai *et al*., 2015)

The ascetic fluid was taken in a WBC pipette and diluted upto 20 times with PBS solution.

Then a drop of the diluted cell suspension was places on the neubauer's counting chamber and the numbers of the cells in the 64 small squares were counted. The viability and non-viability of the cell were determined by trypan

blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and nonviable cells were counted by using the formula.

$$\text{Cell count} = \frac{(\text{Number of cells} \times \text{dilution factor})}{(\text{Area} \times \text{thickness of liquid film})}$$

EUTHANASIA OF EXPERIMENTAL ANIMALS

After completion of the experimental treatment, all the mice would be deprived of food overnight and euthanized by using excess of ketamine-xylazine anaesthesia. The ascitic fluid and blood samples would be collected carefully for various hematologic estimations, respectively.

HEMATOLOGIC ANALYSIS

Blood samples of all treated groups would be collected by retro-orbital puncture following anaesthesia with ketamine-xylazine. The whole blood would be immediately collected in Ethylene Diamine Tetra Acetic-coated vials for the determination of WBC, RBC and platelets. (Sriram *et al.,* 2010)

STATISTICAL ANALYSIS:

The results were statistically analysed by one way –ANOVA followed by Dunnetts test using graph pad instat software. The values were expressed as mean $\pm$SEM. $P<0.05$ were considered statistically significant when compared to standard and negative control.

RESULTS AND DISCUSSION

CONSTRUCTION OF STANDARD CURVE

In the standard curve, linearity was obtained between 5-30 µg/ml concentration of Imatinib mesylate that it obeys Beer Lambert's Law and the regression value was found to be r^2 = 0.9992. The results are shown in table : 6 and Figure : 8 and 9.

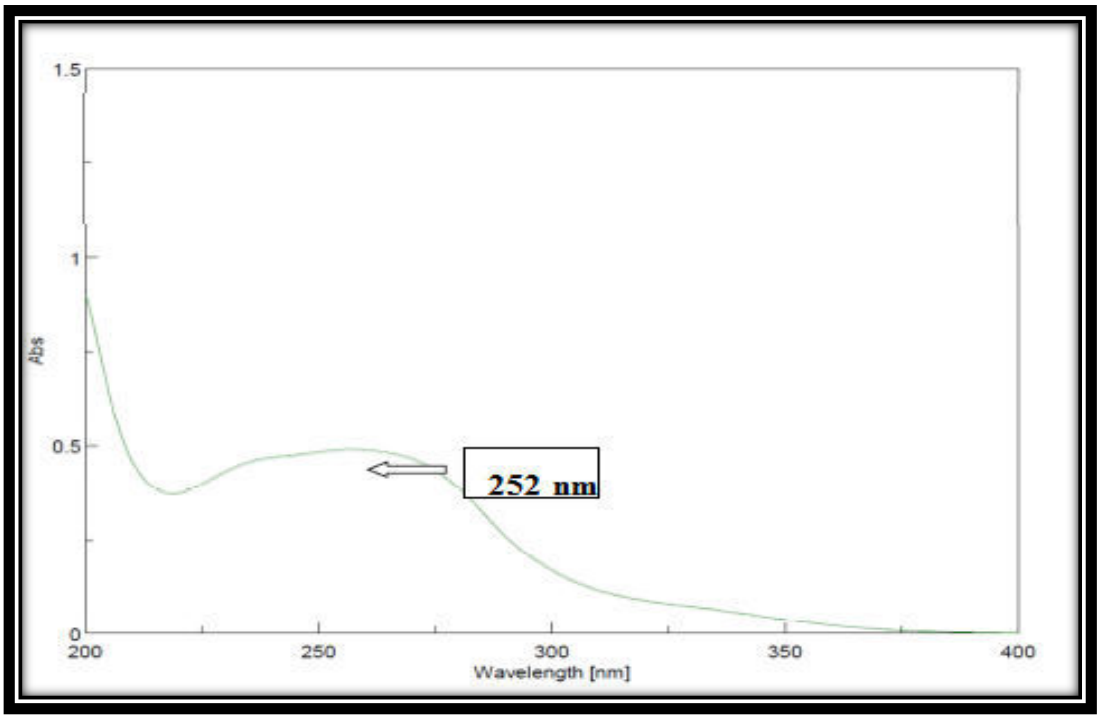


Figure : 8 UV spectra of Imatinib Mesylate

λmax of the Imatinib Mesylate was determined at 252 nm and the UV spectra was determined.

Table:6 Concentration of absorbance values for the estimation of Imatinib Mesylate at 252 nm

S.No	Concentration(µg/ml)	Absorbance at 252 nm
1	5	0.0038
2	10	0.0075
3	15	0.0112
4	20	0.1580
5	25	0.0192
6	30	0.2101

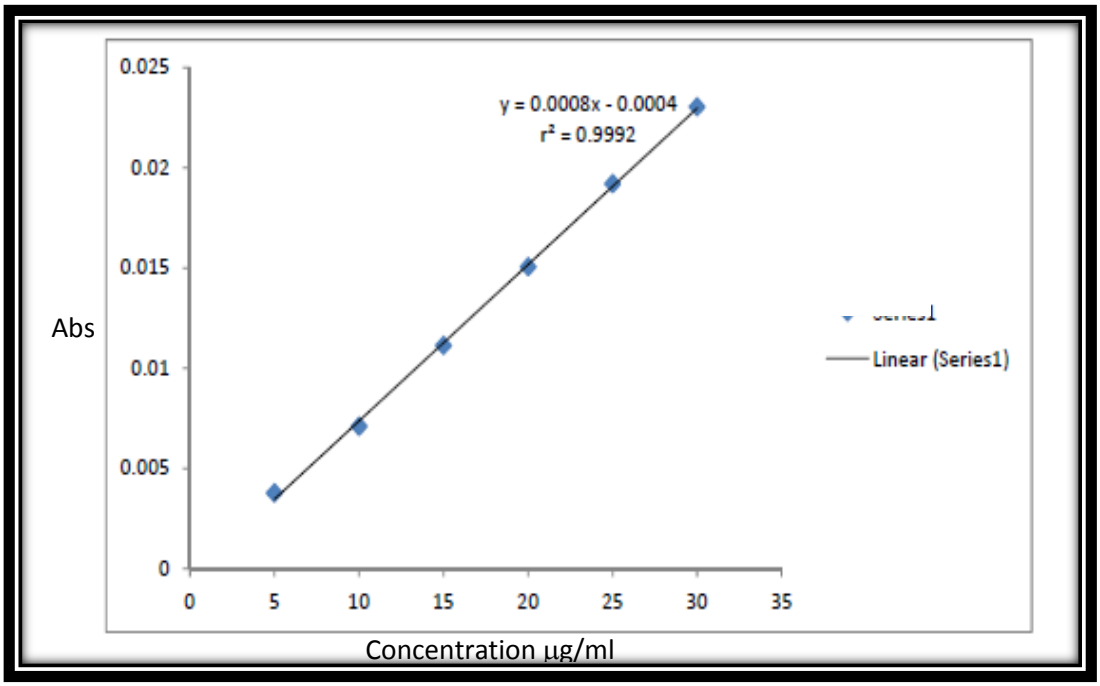


Figure: 9 Calibration curve measured at the absorption of 252 nm

INFRA RED ANALYSIS

Compatibility studies

FT-IR spectroscopy

FTIR spectrum of all formulation is given Figure : 10-254 and table 7-22.

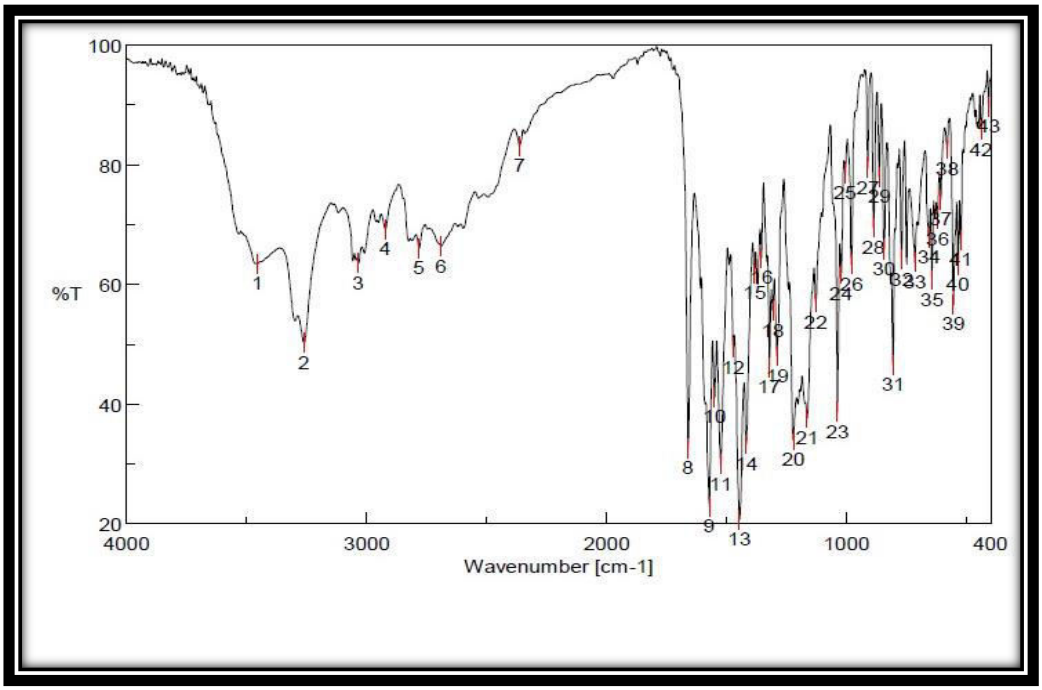


Figure :10 FTIR spectrum of pure drug – IMATINIB MESYLATE

Table:7 FTIR interpretation of pure drug

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
Imatinib mesylate	3000-3700	3454.85	OH stretching
	1500-1700	1524.45	NH bending
	2700-3300	2781.81	CH stretching
	1300-1500	1321.96	CH bend in plane
	700-900	807.063	NH rocking

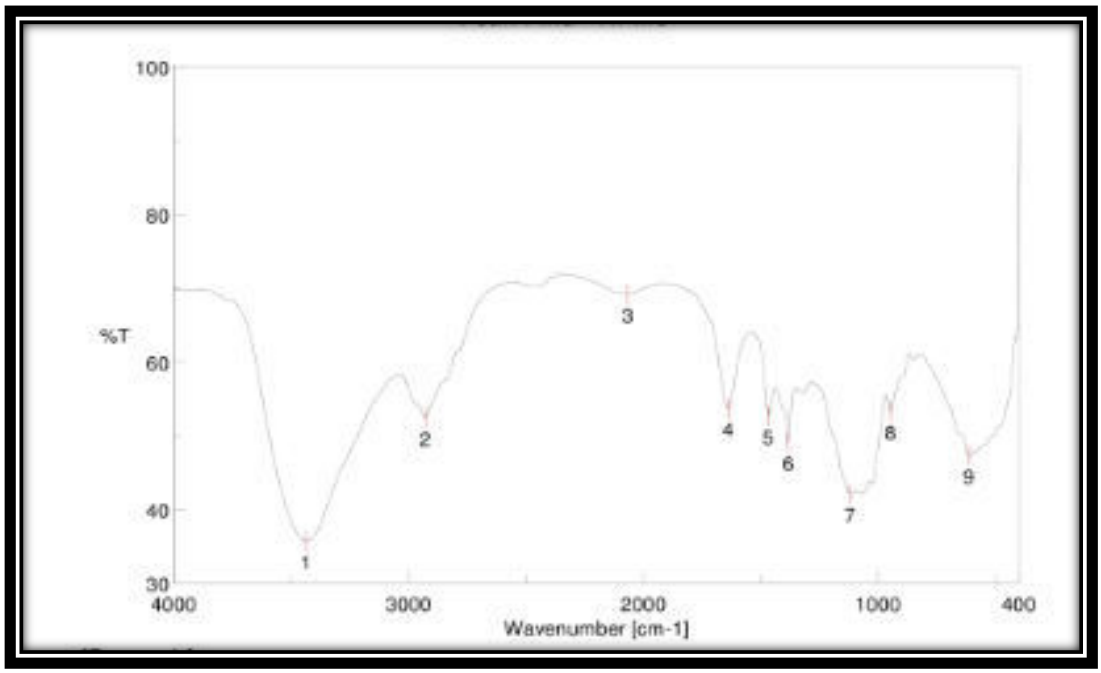


Figure: 11 FTIR spectrum of HPMC

Table:8 FTIR interpretation of HPMC

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
HPMC	3300-3600	3437.49	OH streaching
	2850-2970	2926	CH_3 streaching
	1500-1760	1637	COOH streaching
	1050-1300	1118.51	C-O streaching
	600-900	613.252	C-H rocking

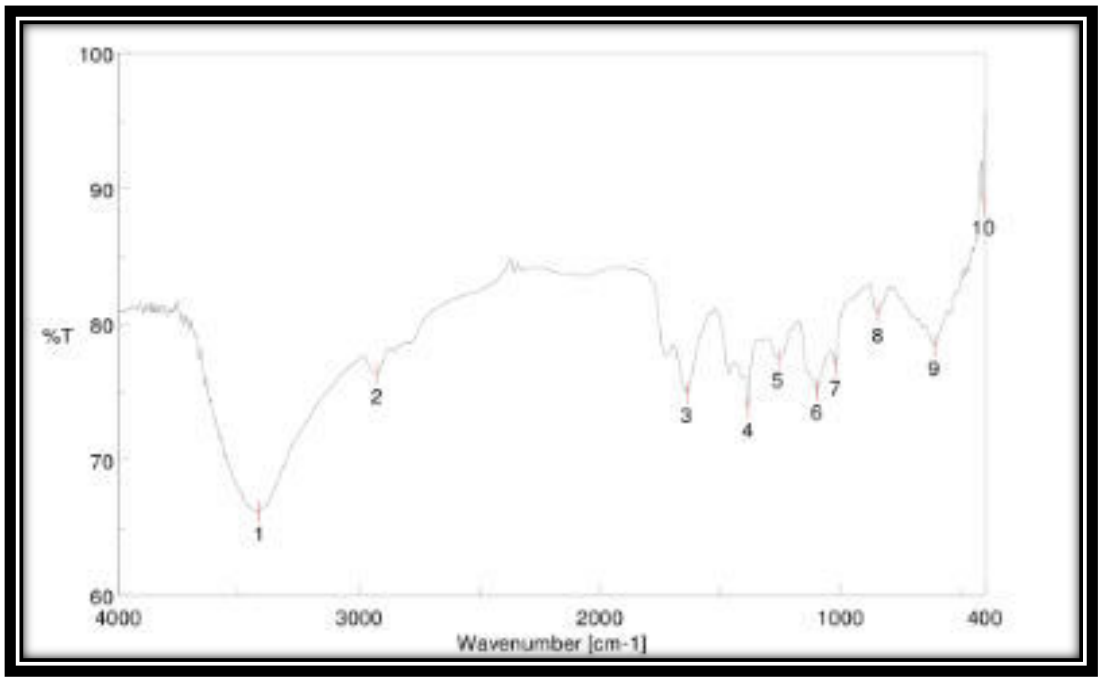


Figure:12 FTIR spectrum of PVA

Table:9 FTIR interpretation of PVA

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
PVA	3300-3600	3415.31	OH Stretching
	2850-2970	2924.52	CH_3 stretching
	1500-1760	1638.23	COOH
	1340-1470	1383.23 1259.29	Alkanes bending
	1050-1300	1044.23	C-O stretching
	600-900	843.704 611.324	C-H rocking

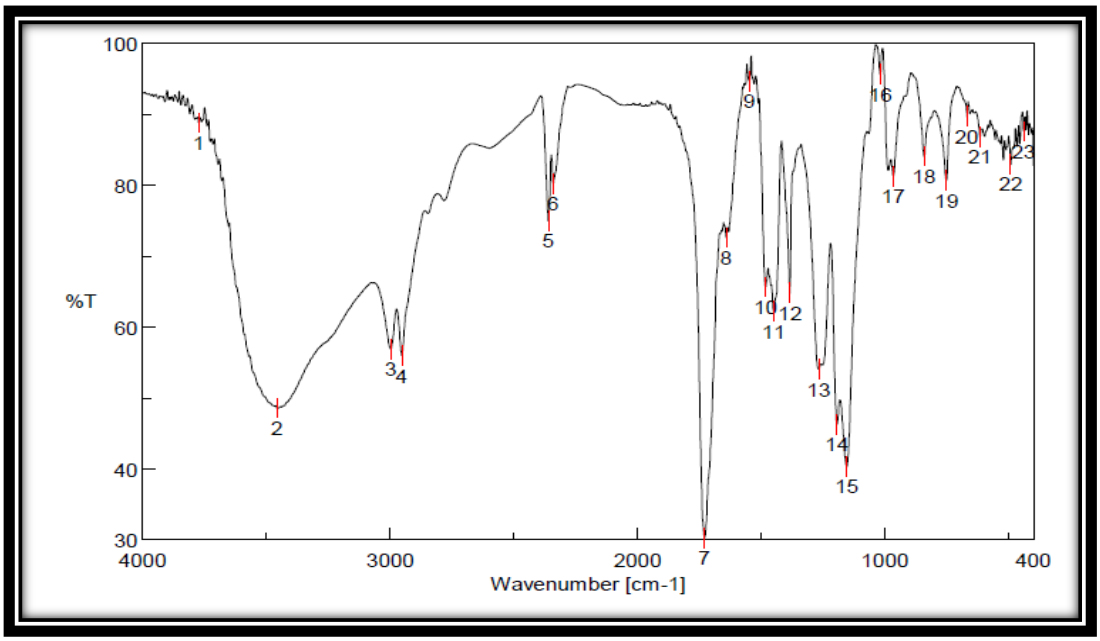


Figure:13 FTIR spectrum of EUDRAGIT

Table:10 FTIR interpretation of EUDRAGIT

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
EUDRAGIT	3000-3700	3455.83	OH stretching
	1500-1700	1548.56	NH bending
	2700-3300	2939.95	CH stretching
	1300-1500	1384.64	CH bending
	900-1300	966.162	C-O stretching

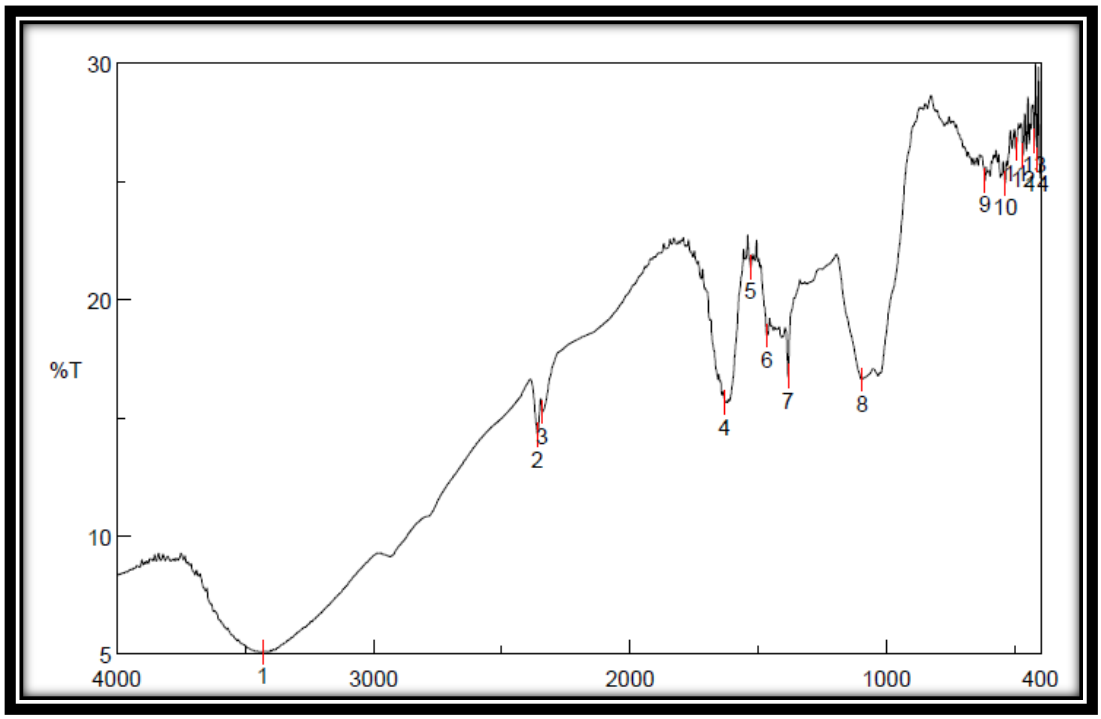


Figure: 14 FTIR spectrum of MORINGA OLEIFERA

Table:11 FTIR interpretation of MORINGA OLEIFERA

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
MORINGA OLEIFERA	3000-3600	3431.71	NH stretching
	1500-1700	1529.27	NH bending
	1300-1500	1384.64	CH stretching
	600-900	616.145	CH rocking
	800-1200	817.23	C-C stretching

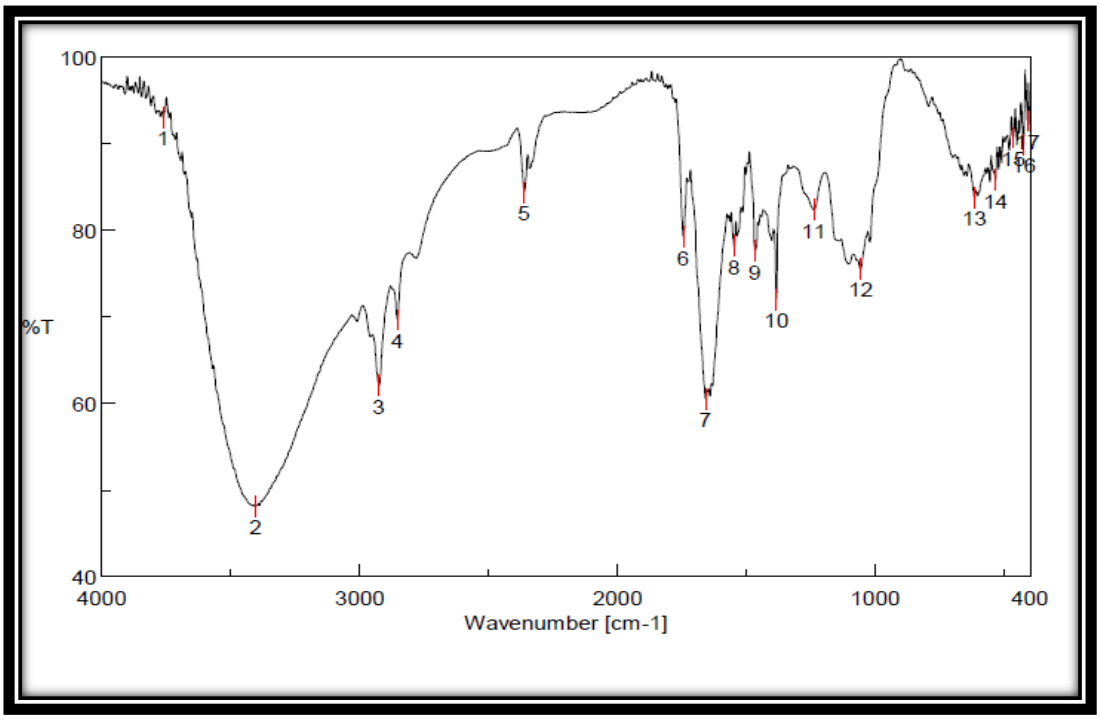


Figure:15 FTIR spectrum of LINSEED

Table :12 FTIR interpretation of LINSEED

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
LINSEED	3000-3700	3404.71	OH stretching
	1500-1700	1547.59	NH bending
	1300-1500	1465.63	CH bending
	600-900	616.145	CH rocking
	1600-1900	1657.52	C-O stretching

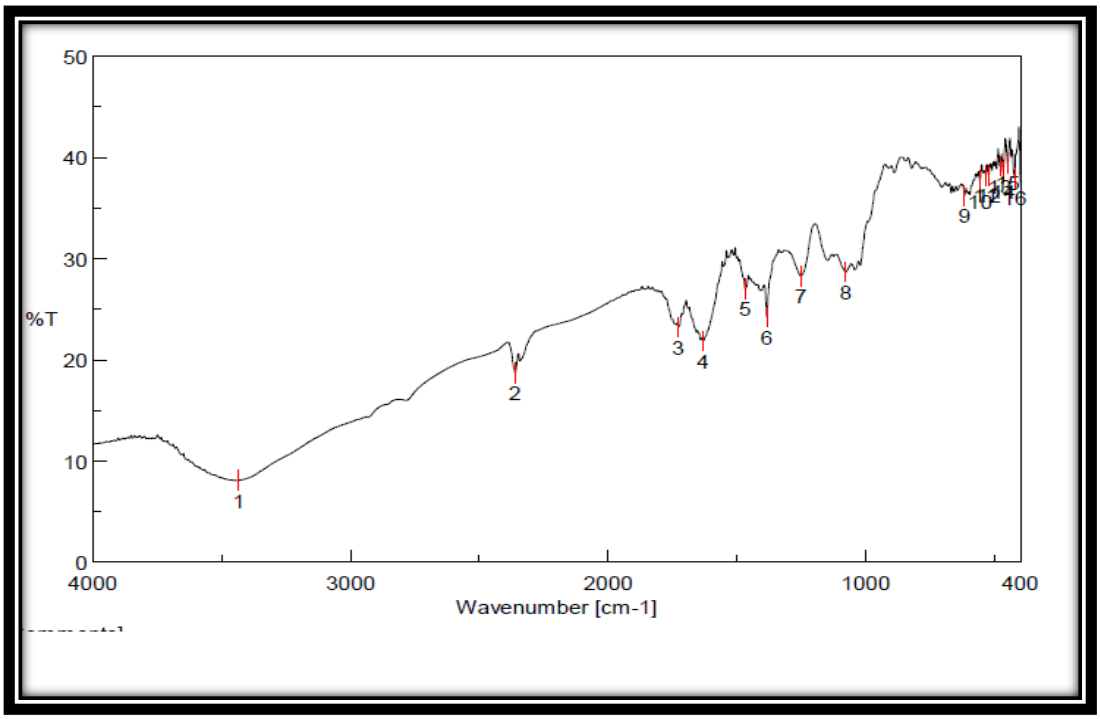


Figure:16 FTIR spectrum of BADAM

Table:13 FTIR interpretation of BADAM

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
BADAM	3000-3700	3433.64	OH stretching
	1500-1700	1630.52	NH bending
	1300-1500	1383.68	CH bending
	600-900	616.145	CH rocking
	1600-1900	1657.52	C-C stretching

z

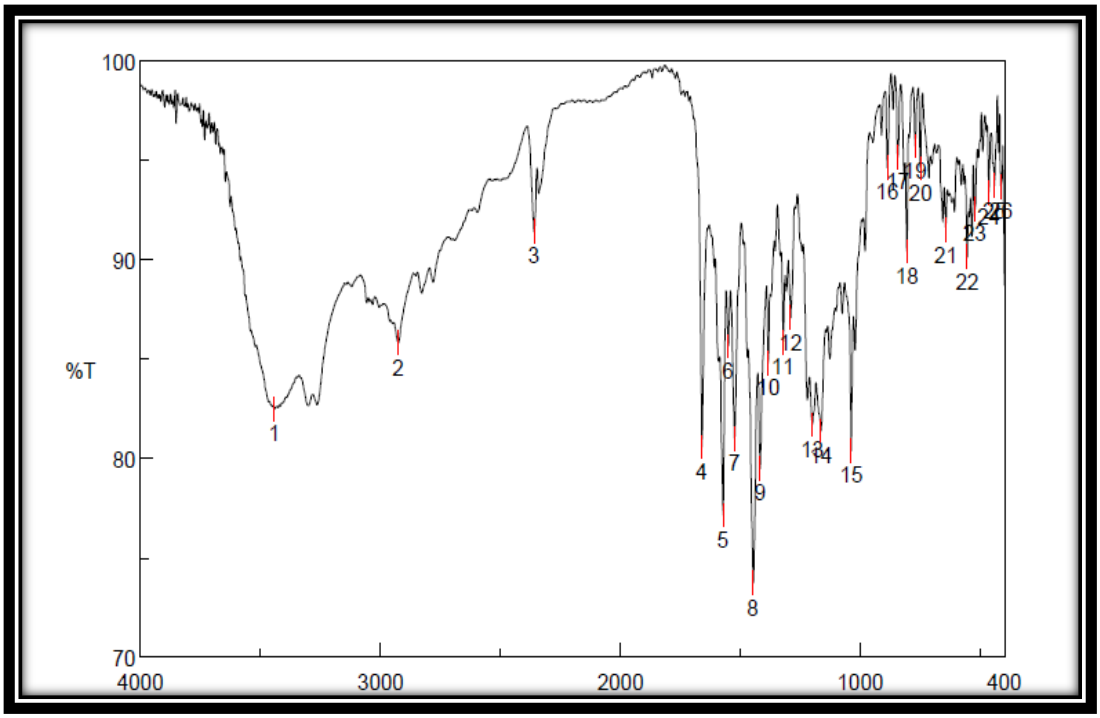


Figure:17 FTIR spectrum of DRUG+HPMC

Table:14 FTIR interpretation of DRUG+HPMC

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+HPMC	3000-3700	3442.31	OH stretching
	1500-1700	1571.7	NH bending
	1300-1500	1418.39	CH bending
	600-900	888.059	CH rocking
	1600-1900	1660.41	C-C stretching

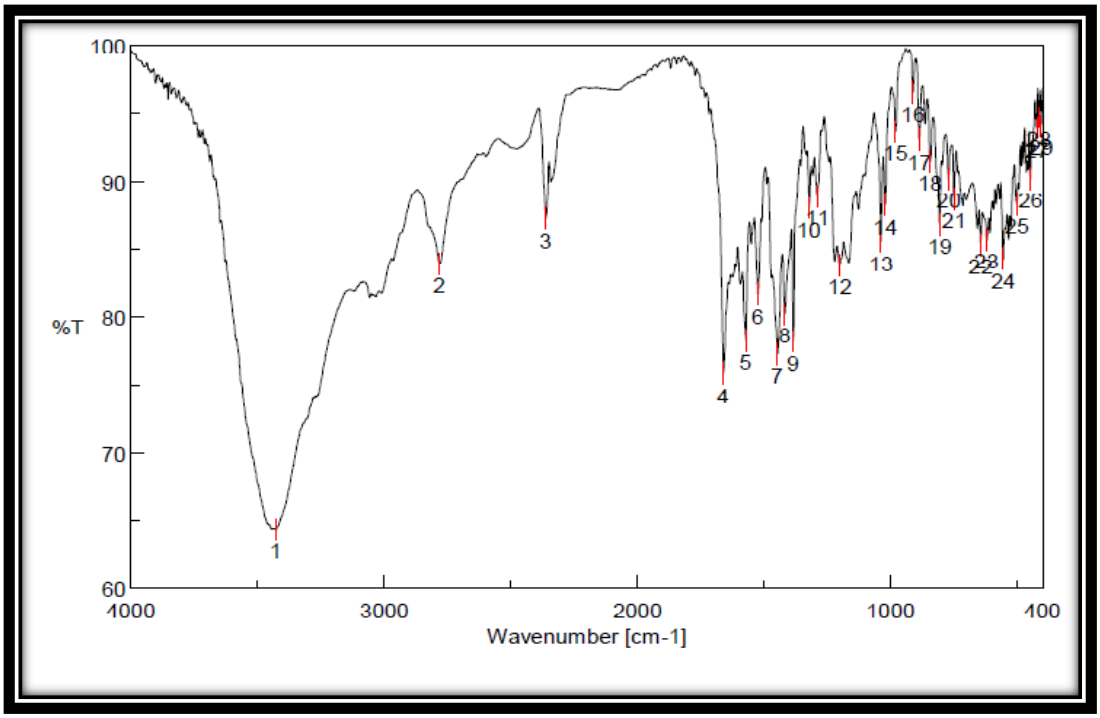


Figure:18 FTIR spectrum of DRUG+PVA

Table:15 FTIR interpretation of DRUG+PVA

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+PVA	3000-3700	3427.85	OH stretching
	1500-1700	1571.7	NH bending
	1300-1500	1384.64	CH bending
	600-900	843.704	CH rocking
	1600-1700	1662.41	C-C stretching

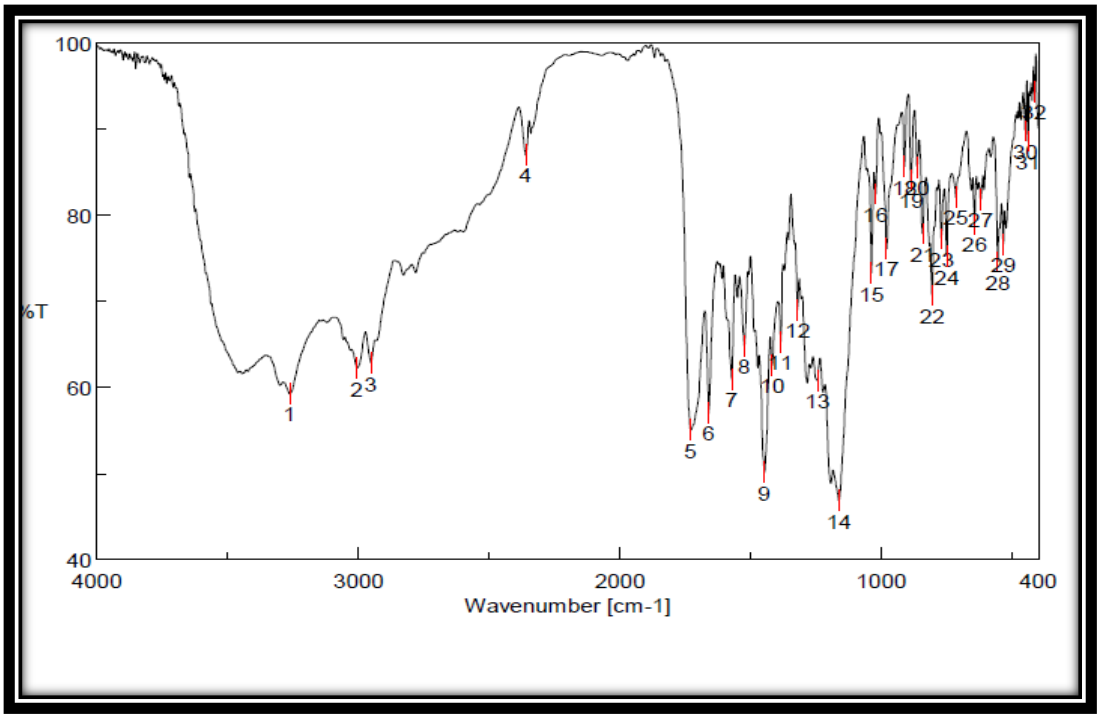


Figure:19 FTIR spectrum of DRUG+EUDRAGIT

Table:16 FTIR interpretation of DRUG+EUDRAGIT

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+EUDRAGIT	3000-3700	3262.97,3004.55	OH stretching
	2700-3300	2952.48	CH bending
	1300-1500	1447.31	CH bending
	600-900	863.953	CH rocking
	1600-1900	1657.52	C-O stretching

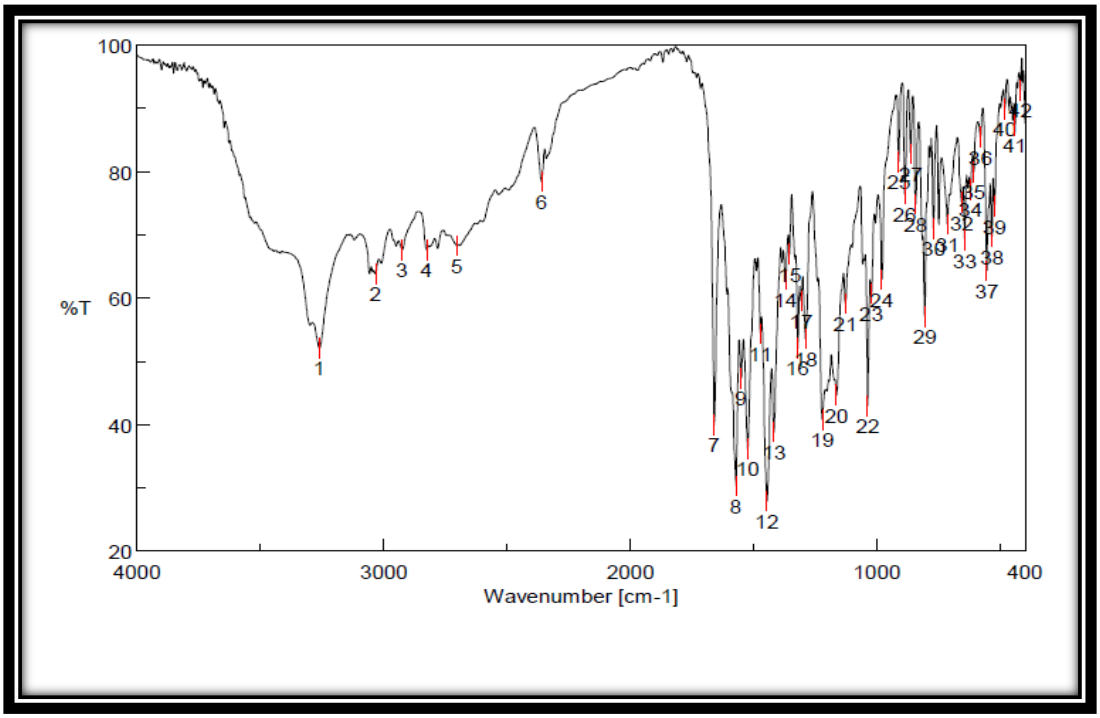


Figure:20 FTIR spectrum of DRUG+MORINGA OLEIFERA

Table: 17 FTIR interpretation of DRUG +MORINGA OLEIFERA

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+MORINGA OLEFERAE	3000-3700	3261.04	OH stretching
	2700-3300	2924.52	CH bending
	1300-1500	1473.35	CH bending
	600-900	843.704	CH rocking
	1600-1900	1660.41	C=O stretching

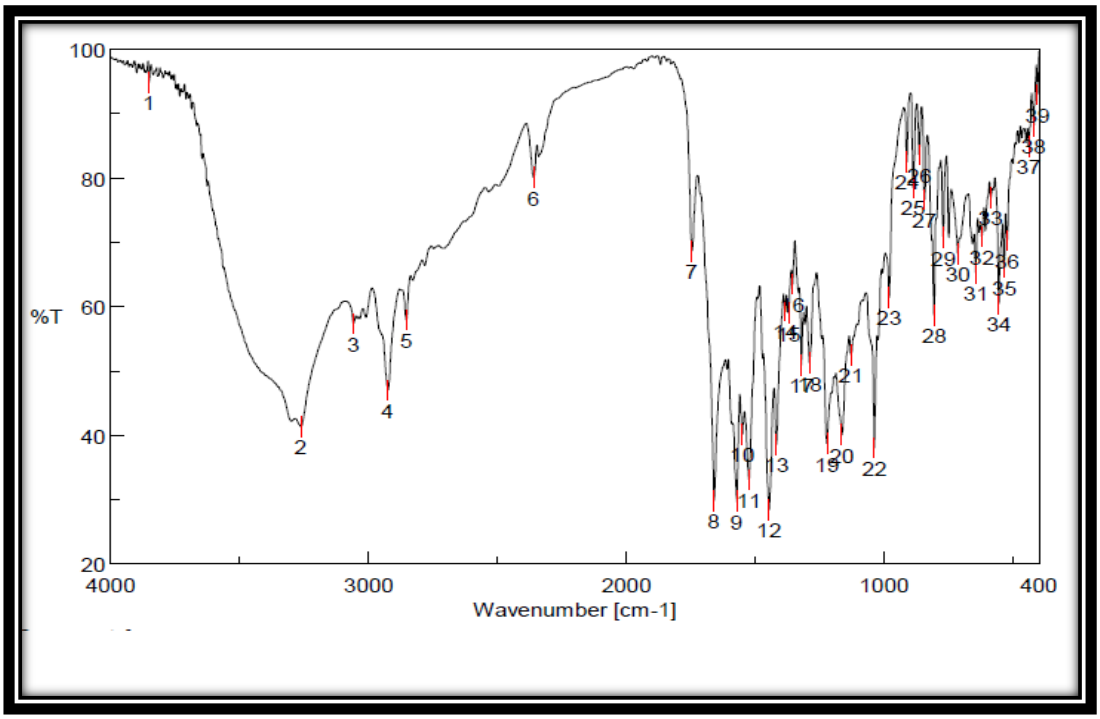


Figure:21 FTIR spectrum of DRUG+LINSEED

Table:18 FTIR interpretation of DRUG+LINSEED

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+LINSEED	3000-3700	3057.58	OH stretching
	1500-1700	1571.70	NH bending
	1300-1500	1372.1	CH bending
	600-900	807.063	CH rocking
	1600-1900	1746.23	C=O stretching

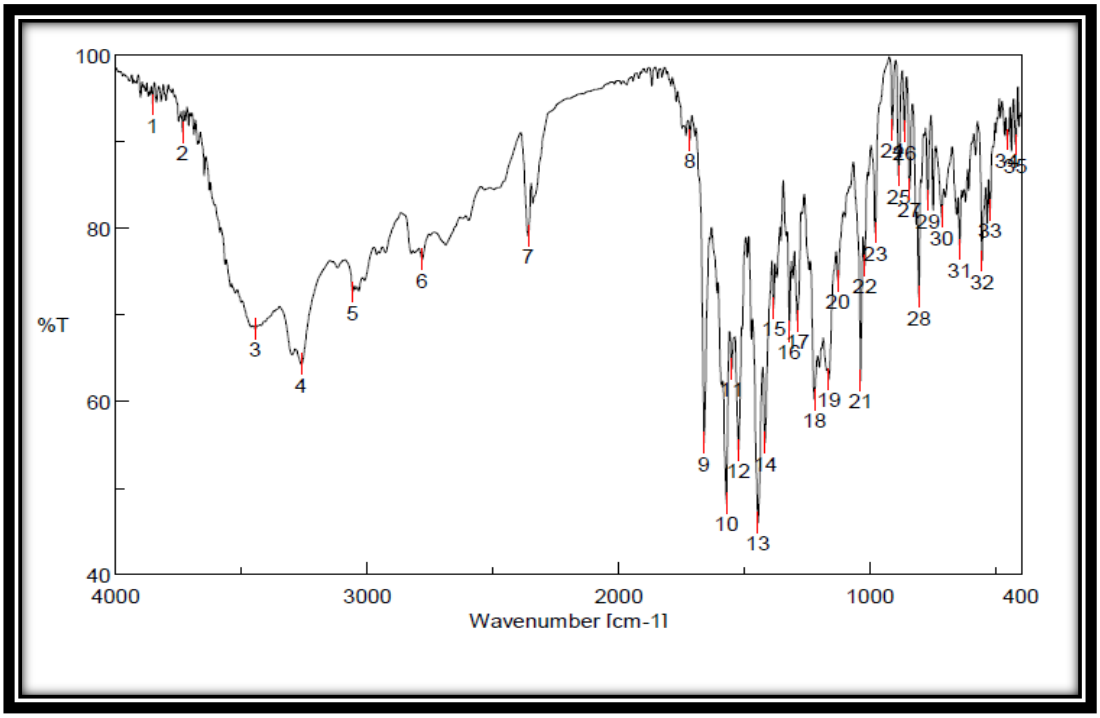


Figure:22 FTIR spectrum of DRUG+BADAM

Table:19 FTIR interpretation of DRUG+BADAM

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+BADAM	3000-3700	3734.48	OH stretching
	1500-1700	1571.7	NH bending
	1300-1500	1384.64	CH bending
	600-900	715.461	CH rocking
	1600-1700	1660.41	C-C stretching

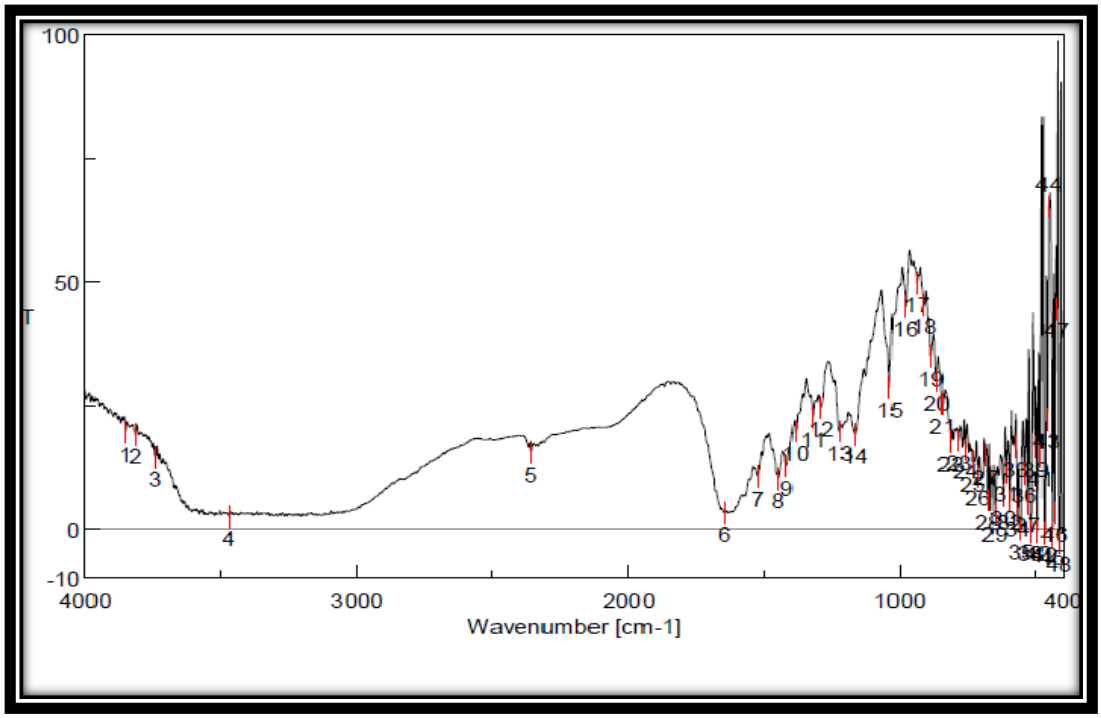


Figure:23 FTIR spectrum of DRUG+COBALT CHLORIDE(II)

Table: 20 FTIR interpretation of drug+cobalt chloride(II)

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
DRUG+COBALT CHLORIDE(II)	3000-3700	3470.28	OH stretching
	1500-1700	1525.42	NH bending
	1300-1500	1321	CH bending
	600-800	620.966	C-CI stretching
	1600-1700	1644.98	C-C stretching

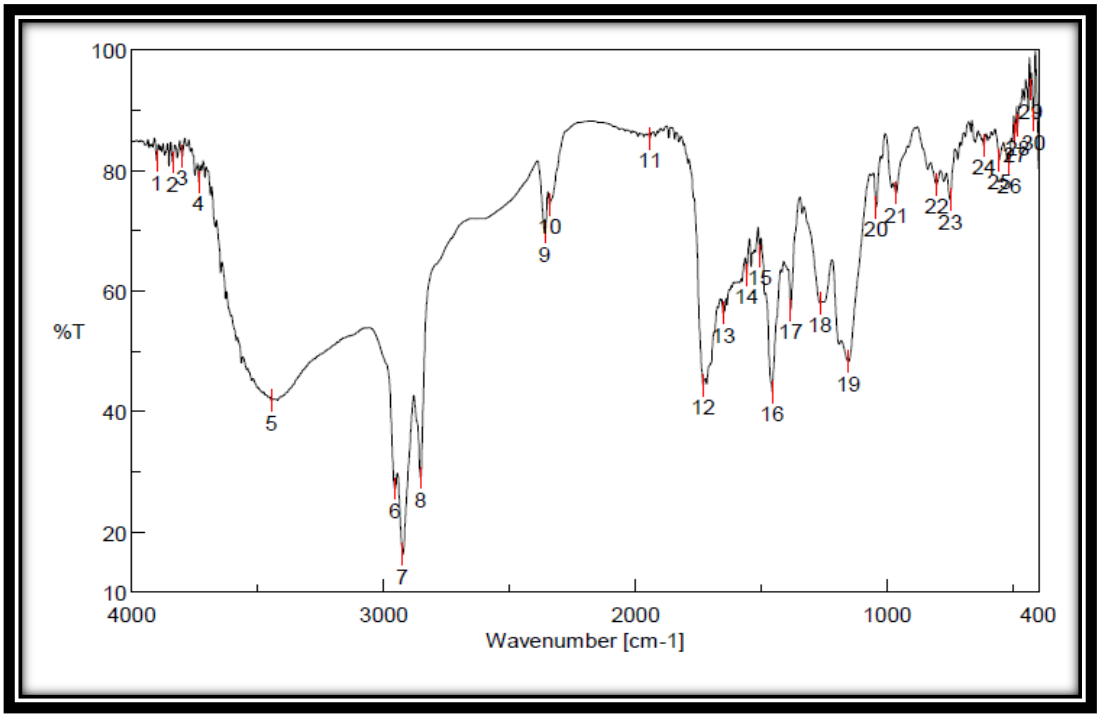


Figure:24 FTIR spectrum of F3 FORMULATION (NANOPARTICLES)

Table: 21 FTIR interpretation of F3 FORMULATION(NPs)

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
F3 NANOPARTICLES	3300-3600	3446.17	C=O stretching
	1500-1700	1507.1	NH bending
	1300-1500	1384.64	CH bending
	600-900	617.109	CH rocking
	1600-1900	1652.70	C-C stretching

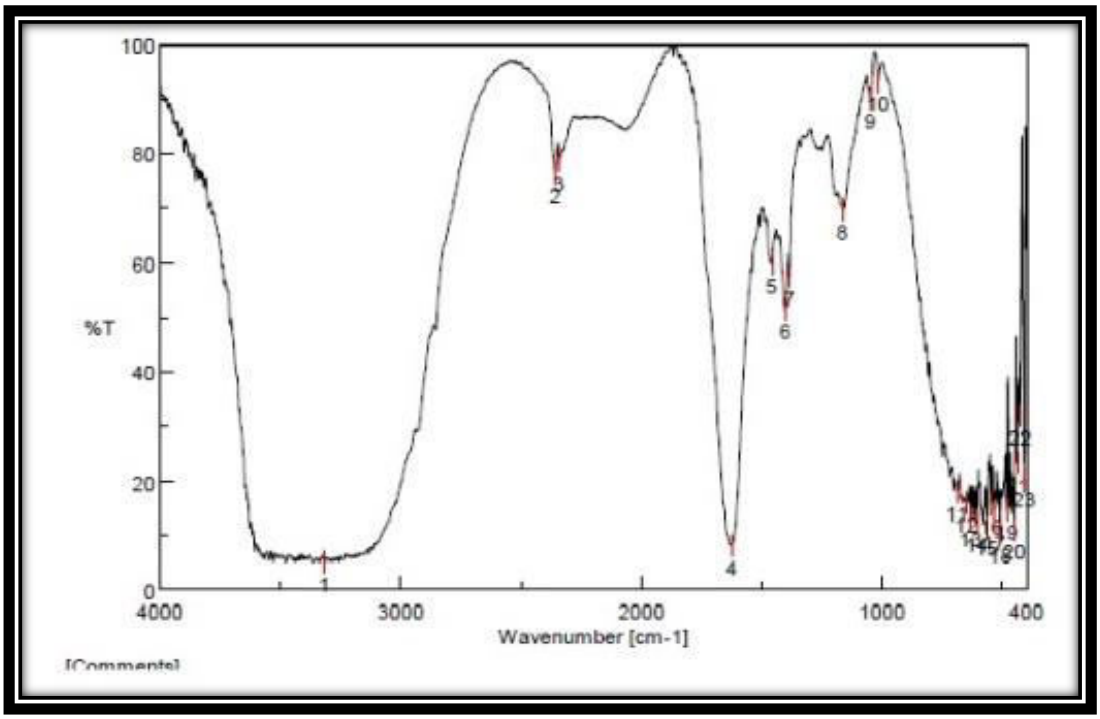


Figure:25 FTIR spectrum of F13 formulation ($CoCl_2$ NPs)

Table:22 FTIR interpretation of $CoCl_2$ NPs(COMPLEX)

Materials	Standard wave Number (cm-1)	Test wave Number (cm-1)	Functional group assignment
($CoCl_2$ NPs)	3000-3700	3315.03	OH stretching
	1500-1700	1623.77	NH bending
	1300-1500	1384.64	CH bending
	600-800	606.50	C-CI stretching
	1600-1700	1657.52	C-C stretching

From the spectra's it can be concluded that the drug and excipient don't have any incompatibility since the peaks present in the drug can also be visualized in the drug and excipient spectra. Hence these excipients can be chosen for the preparation of nanoparticles and for further ananlysis.

FORMULATION OF NANOPARTICLES

Total eight formulations (F1- F12) of Imatinib mesylate nanoparticles with various synthetic polymers Poly Vinyl Alcohol. Hydroxy Propyl Methyl Cellulose, Eudragit and natural polymers Moringa oleifera, Badam, Linseed in this ratios were formulated and it is given in table no: 23

Table:23 Formulation of Imatinib Mesylate nanoparticles

S.no	Formulation code	Drug	Polymer	Drug:Polymer
1	F1	Imatinib Meylate	HPMC	1:1
2	F2		HPMC	1:2
3	F3		Eudragit	1:1
4	F4		Eudragit	1:2
5	F5		PVA	1:1
6	F6		PVA	1:2
7	F7		Badam	1:1
8	F8		Badam	1:2
9	F9		Linseed	1:1
10	F10		Linseed	1:2
11	F11		Moringa oleifera	1:1
12	F12		Moringa oleifera	1:2

Table:24 Formulations of Imatinb Mesylate nanoparticles by solvent evaporation technique with various natural and synthetic polymers

S. No	Formulation code	Weight of drug (mg)	Weight of polymers (mg)
1	F1	500	500
2	F2	500	1000
3	F3	500	500
4	F4	500	1000
5	F5	500	500
6	F6	500	1000
7	F7	500	500
8	F8	500	1000
9	F9	500	500
10	F10	500	1000
11	F11	500	500
12	F12	500	1000

SCANNING ELECTRON MICROSCOPE ANALYSIS

The SEM images were seen in different magnification starting from 1000 X to 10000 X Surface morphology of formulated Imatinib mesylate nanoparticles were determined by SEM analysis which showed the small spherical shaped, particles without aggregation and smooth texture in surface morphology. In this, the formulations F1- F12 were analyzed and given in figure:26-29

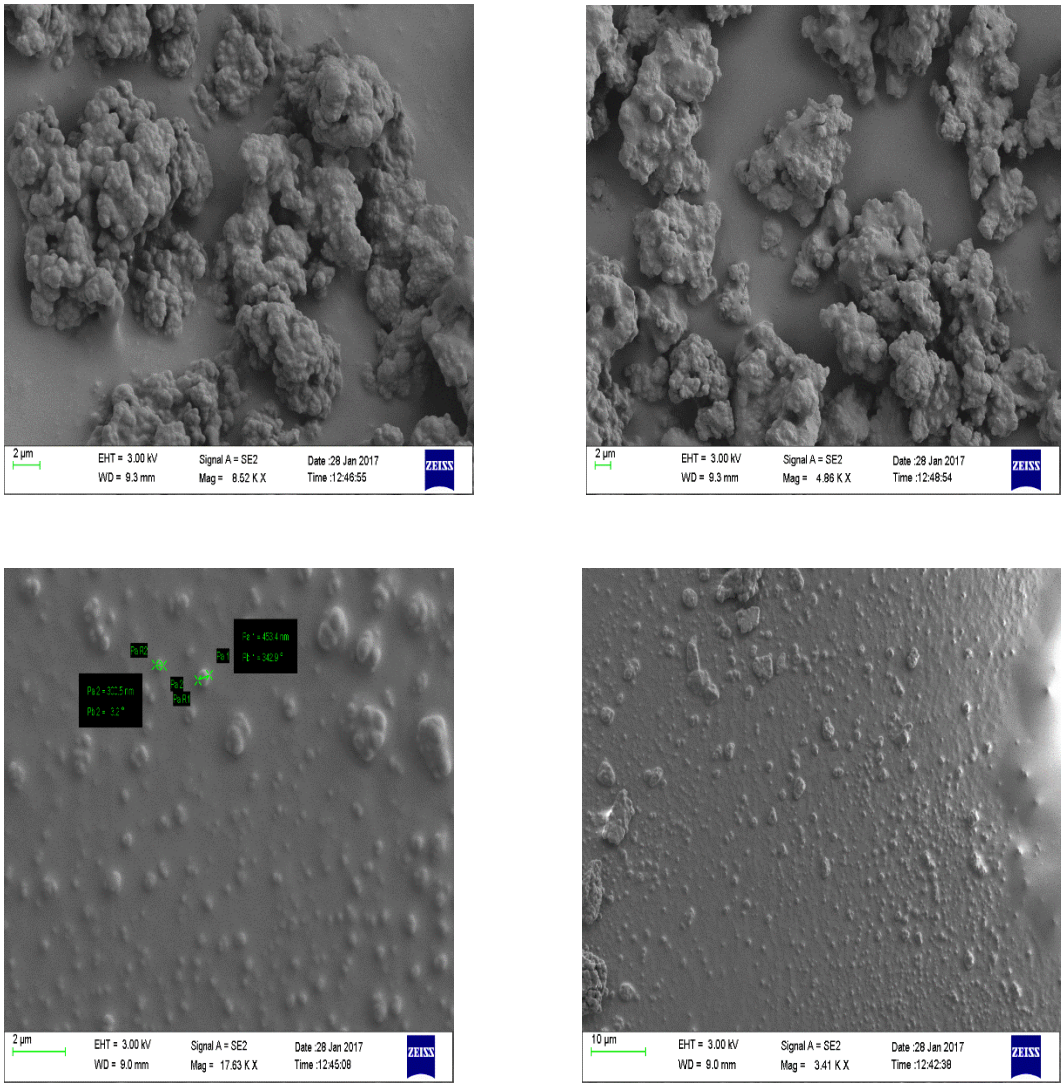


Figure: 26 SEM Photograph of Imatinib Mesylate nanoparticle (formulation code F1- F4)

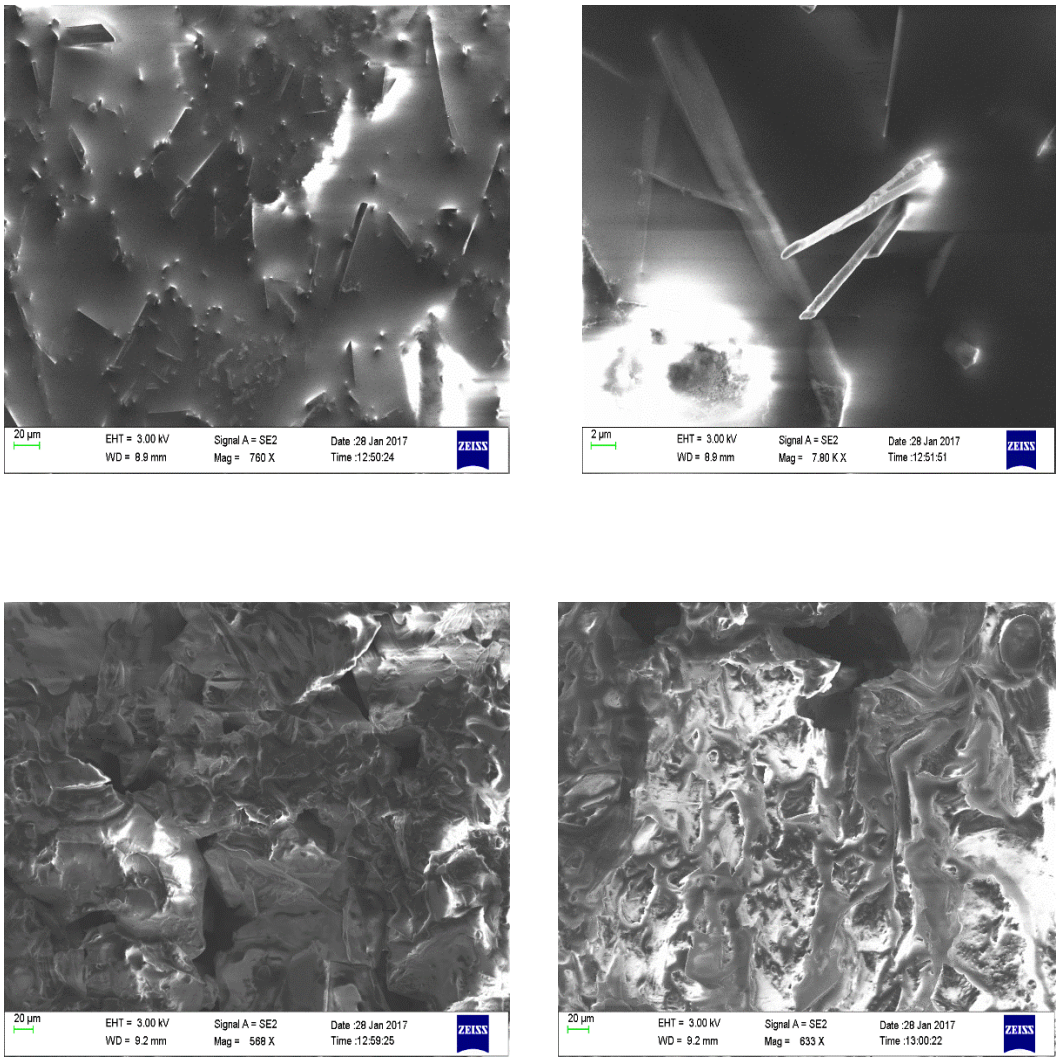


Figure:27 SEM Photograph of Imatinib Mesylate nanoparticle (formulation code F5-F8)

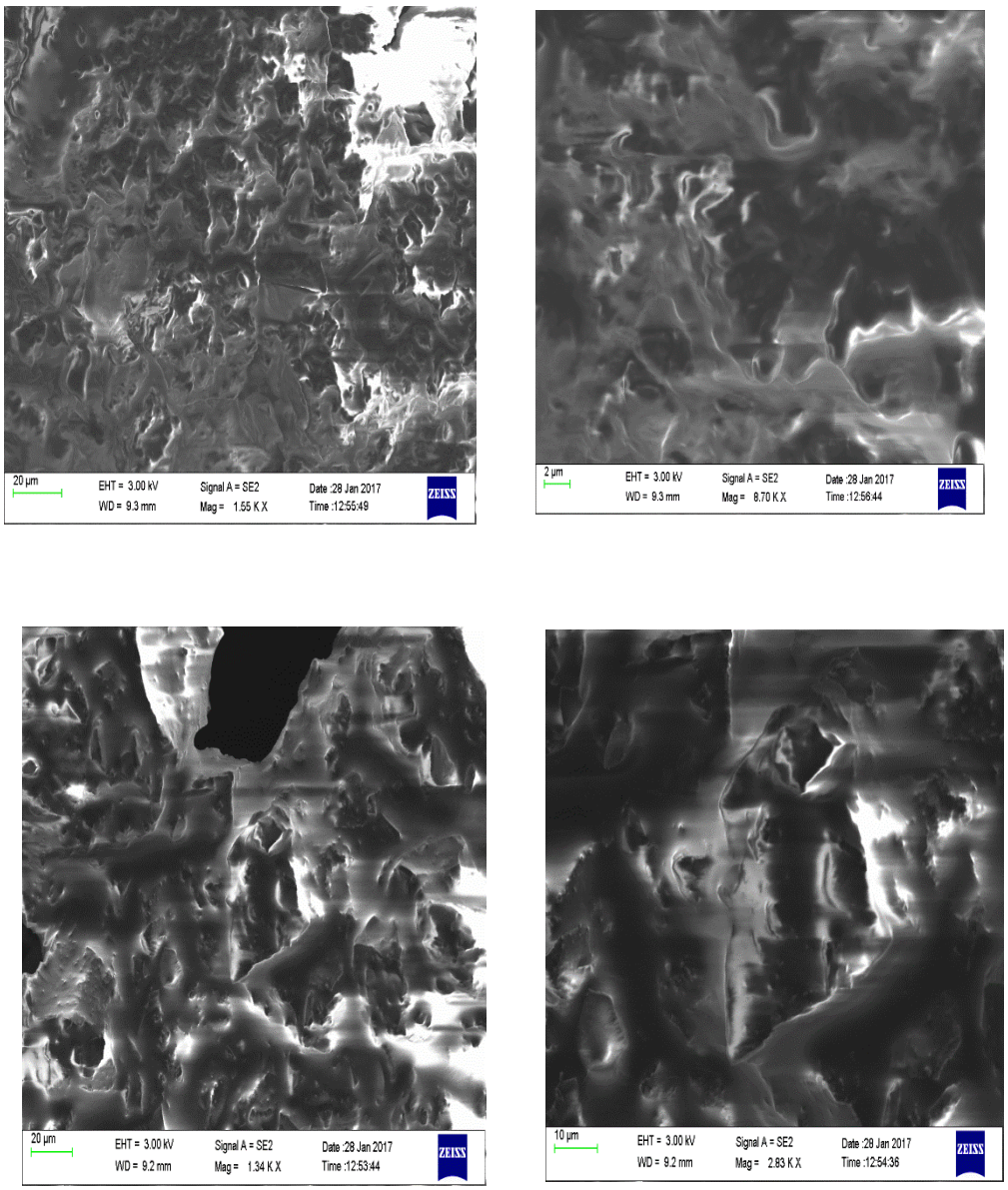


Figure: 28 SEM Photograph of Imatinib Mesylate nanoparticle (formulation code F9- F12)

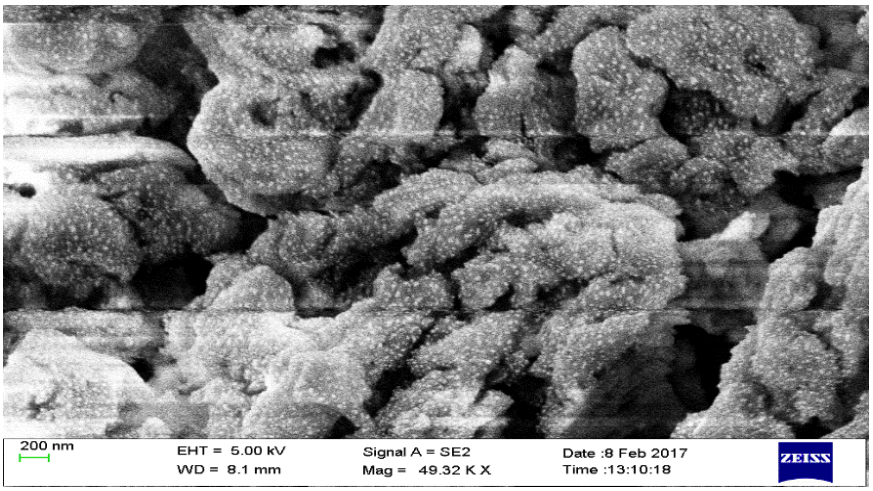


Figure: 29 SEM Photograph of Imatinib mesylate nanoparticle with Cobalt(II) Chloride Complexation ($COCL_2$ NPs).(F-13)

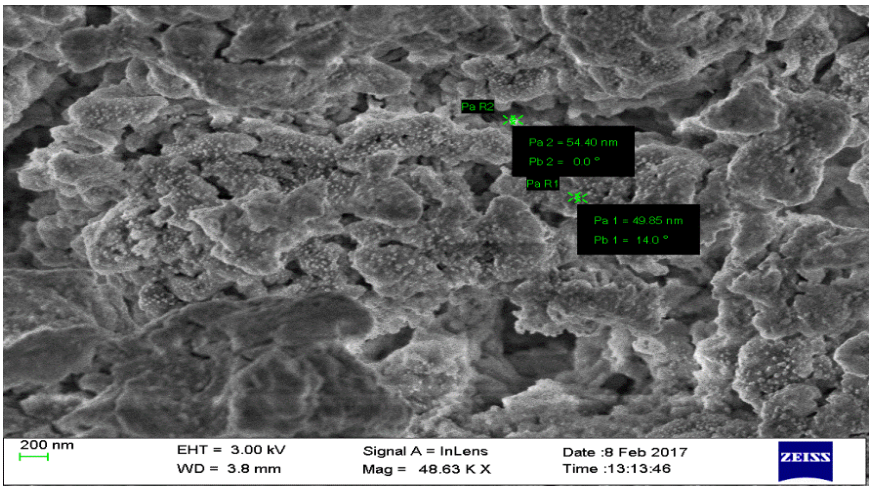


Figure: 30 SEM Photograph of Imatinib mesylate nanoparticle with Cobalt(II) Chloride Complexation ($COCL_2$ NPs).(F-13)

The surface morphology of formulated Imatinib Mesylate nanoparticles were determined by SEM analysis which showed the small spherical shaped, particles without aggregation and smooth texture in surface morphology.

PERCENTAGE YIELD ANALYSIS

Percentage yield analysis was calculated by weighing the formulated Imatinib meesylate nanoparticles by using the following formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} X100$$

Table :25 Percentage yield analysis of different formulations F1-F12

S.No.	Formulation Code	% Yield
1	F1	78
2	F2	72.29
3	F3	92
4	F4	88.34
5	F5	70.2
6	F6	69.4
7	F7	72.4
8	F8	74.52
9	F9	76.86
10	F10	72.82
11	F11	56.62
12	F12	53.40

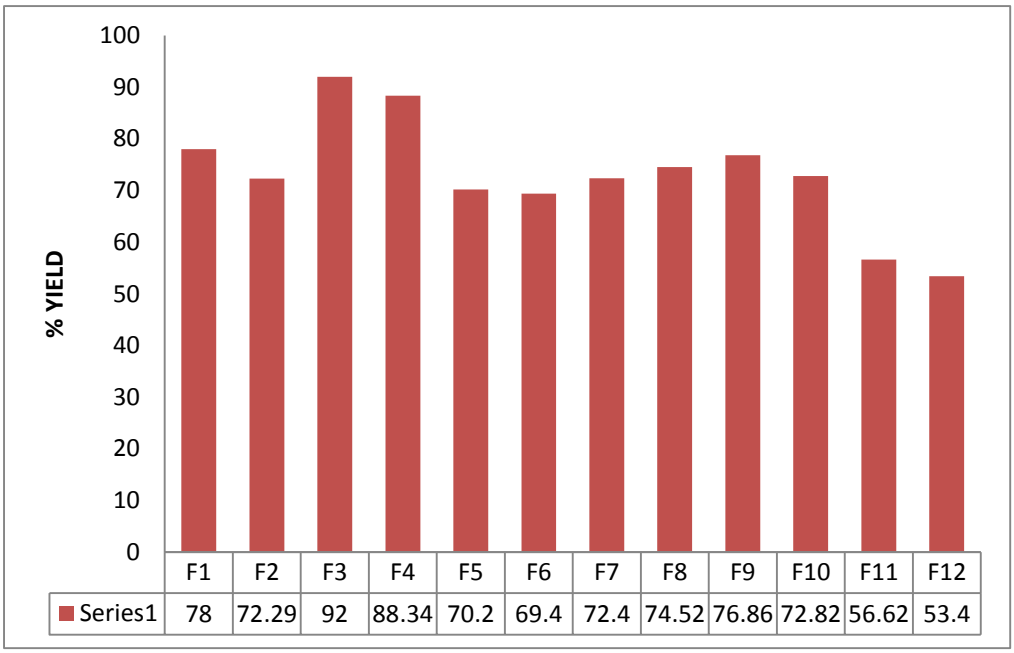


Figure: 31 Percentage yield ananlysis of different formulations F1-F12

The percentage yield was minimum for formulation F12 (53.40%) and maximum for formulation F3 (92%).As the concentration of polymer increases percentage yield also increases.

DETERMINATION OF ENTRAPMENT EFFICIENCY

Centrifugation followed by decantation of the fluid was performed to separate the nanoparticles containing the entrapped drug. The nanoparticles were lysed with isopropyl alcohol and spectrophotometric analysis was carried out at 252 nm. Entrapment efficiency is calculated as percentage (%).

$$Entrapment\ Efficiency\ \% = \frac{\text{Practical Drug Content}}{\text{Theoritical Drug Content}} X100$$

Entrapment Efficiency of the prepared formulation are given in Table: 26 And Figure:32-34

Table:26 Entrapment Efficiency of various formulations

s. no	Formulation code	EE (%)
1	F1	87.53
2	F2	88.36
3	F3	96.78
4	F4	93.60
5	F5	77.23
6	F6	81.20
7	F7	69.56
8	F8	67.76
9	F9	80.72
10	F10	76.19
11	F11	69.70
12	F12	65.82

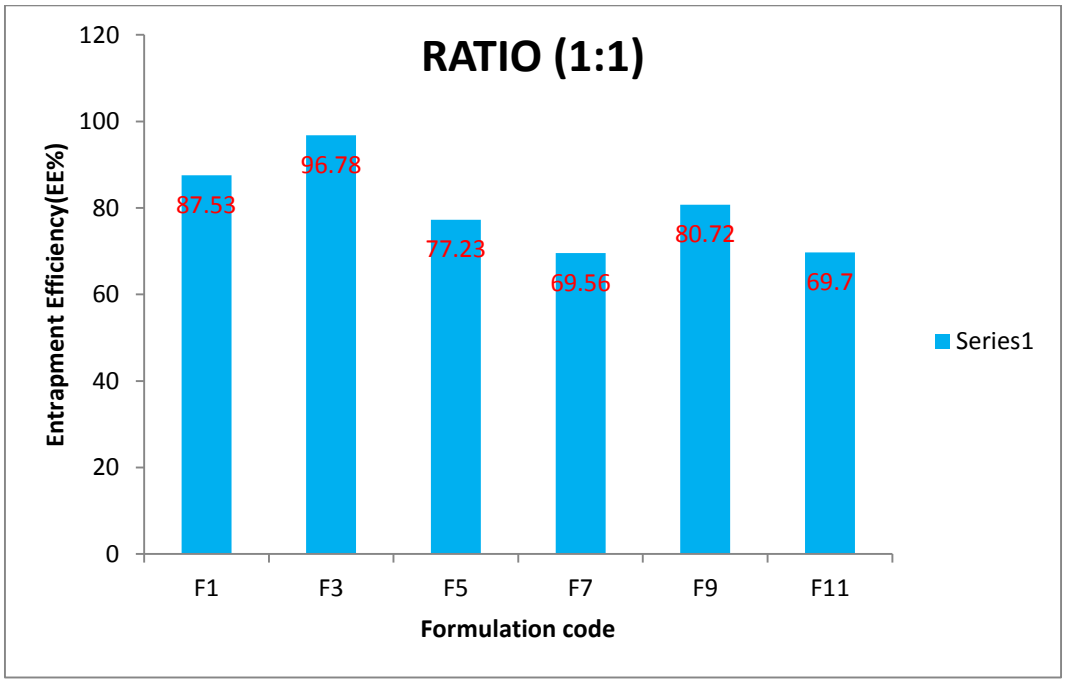


Figure:32 Entrapment Efficiency of various formulations ratio(1:1)

The Entrapment Efficiency was found to be highest for F3 formulation (Imatinib Mesylate: Eudragit ratio 1:1) which is 96.78% and the lowest entrapment of drug was found for the F7 formulation (Imatinib Mesylate: Badam ratio 1:1) having 69.56%.

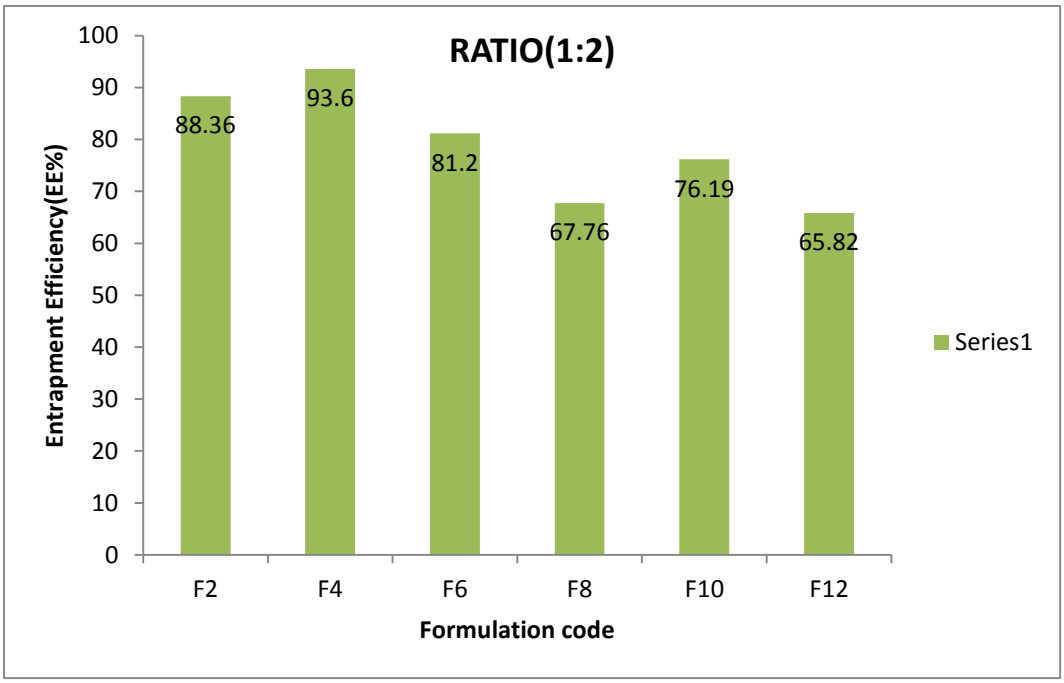


Figure:33 Entrapment Efficiency of various formulations ratio(1:2)

The Entrapment Efficiency was found to be highest for F4 formulation (Imatinib Mesylate: Eudragit ratio 1:2) which is 93.6% and the lowest entrapment of drug was found for the F12 formulation(Imatinib Mesylate: Moringa oleifera ratio 1:2) having 65.82%.

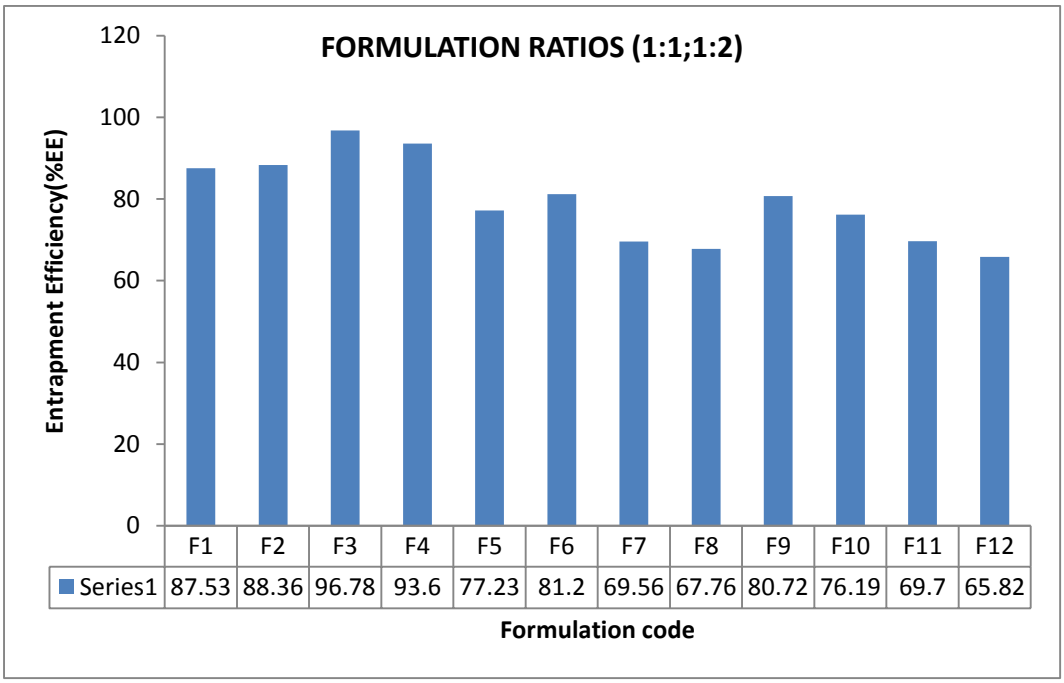


Figure:34 Entrapment Efficiency of Imatinib Mesylate nanoparticles

The Entrapment Efficiency was found to be highest for F3 formulation (Imatinib Mesylate: Eudragit ratio 1:1) which is 96.78% and the lowest entrapment of drug was found for the F12 formulation (Imatinib Mesylate: Moringa oleifera ratio 1:2) having 65.82%.

PARTICLE SIZE ANALYSIS

Zeta size analysis of formulated Imatinib Mesylate nanoparticles are given in figure: 35-47

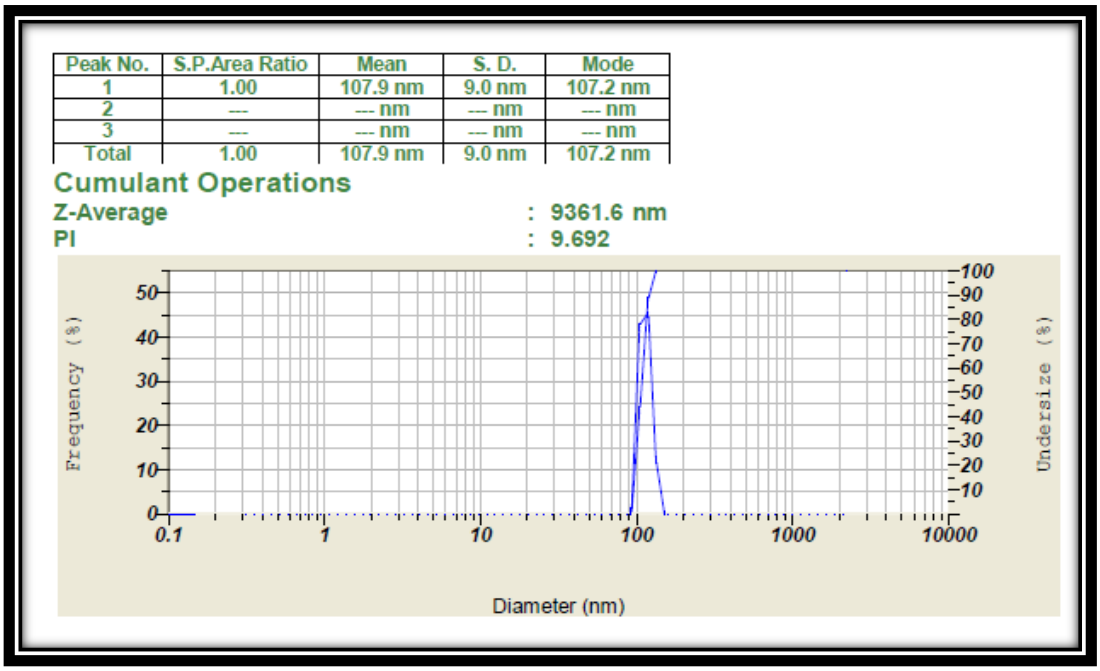


Figure: 35 Zeta size distribution of F1 formulation

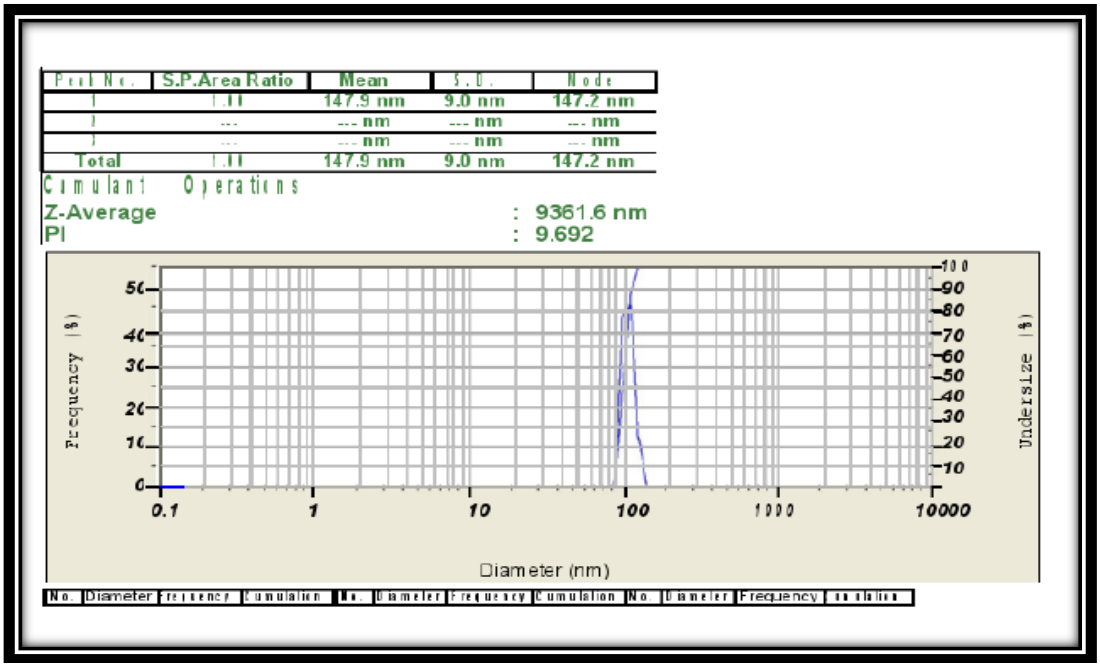
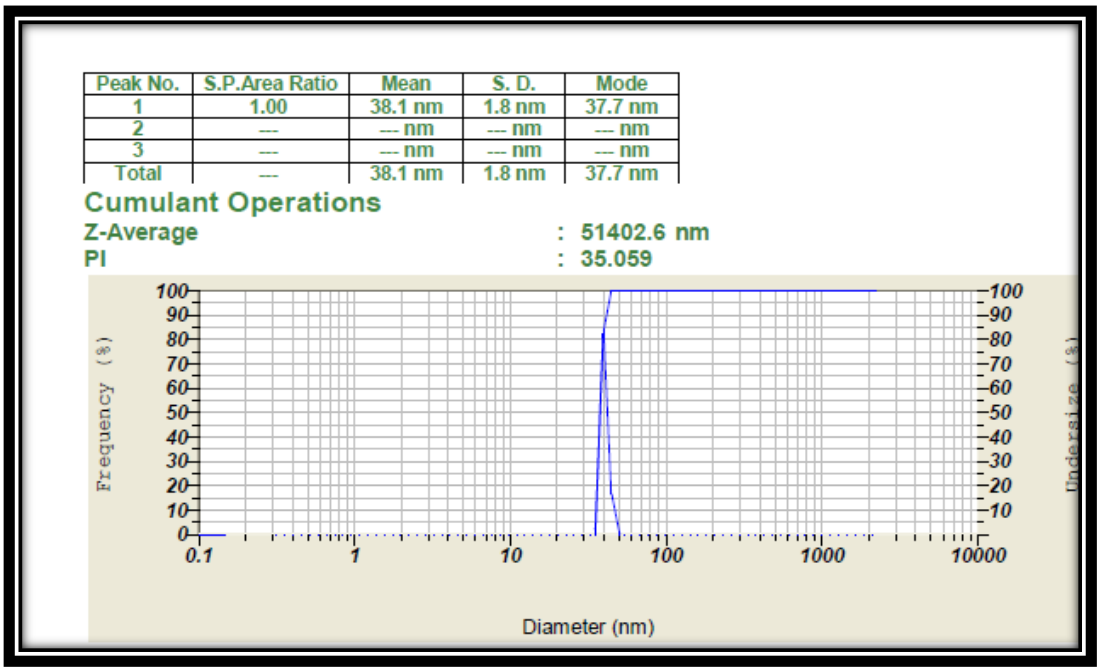


Figure:36 Zeta size distribution of F2 formulation



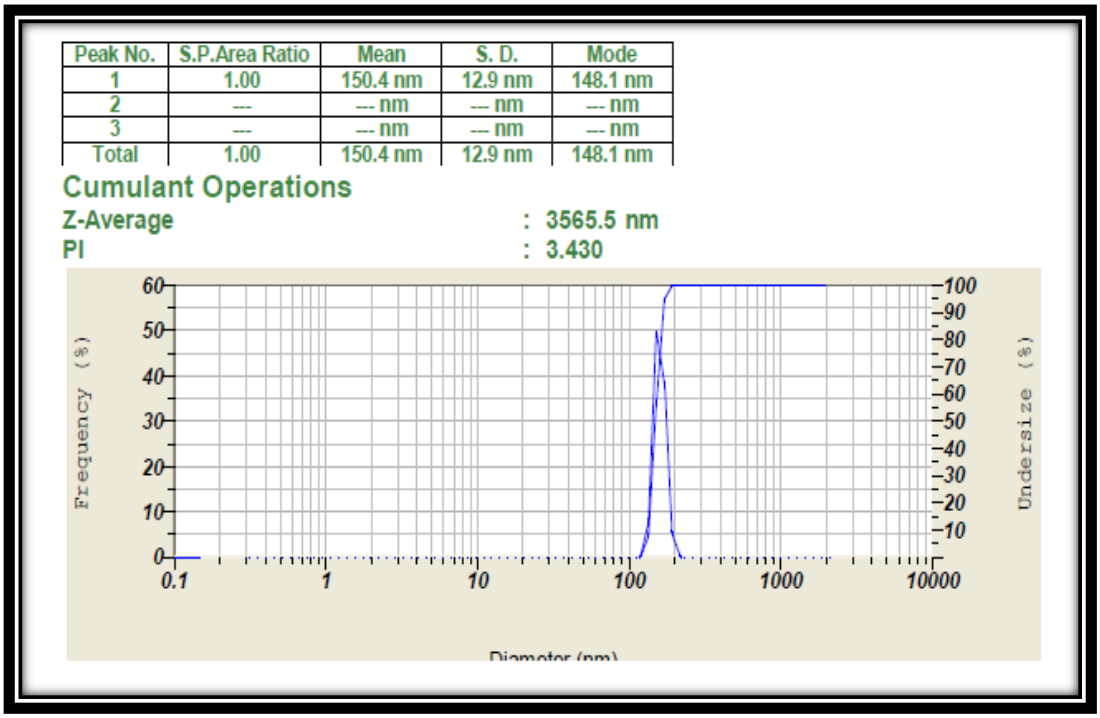
Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	38.1 nm	1.8 nm	37.7 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	---	38.1 nm	1.8 nm	37.7 nm

Cumulant Operations

Z-Average : 51402.6 nm

PI : 35.059

Figure:37 Zeta size distribution of F3 formulation



Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	150.4 nm	12.9 nm	148.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	150.4 nm	12.9 nm	148.1 nm

Cumulant Operations

Z-Average : 3565.5 nm

PI : 3.430

Figure:38 Zeta size distribution of F4 formulation

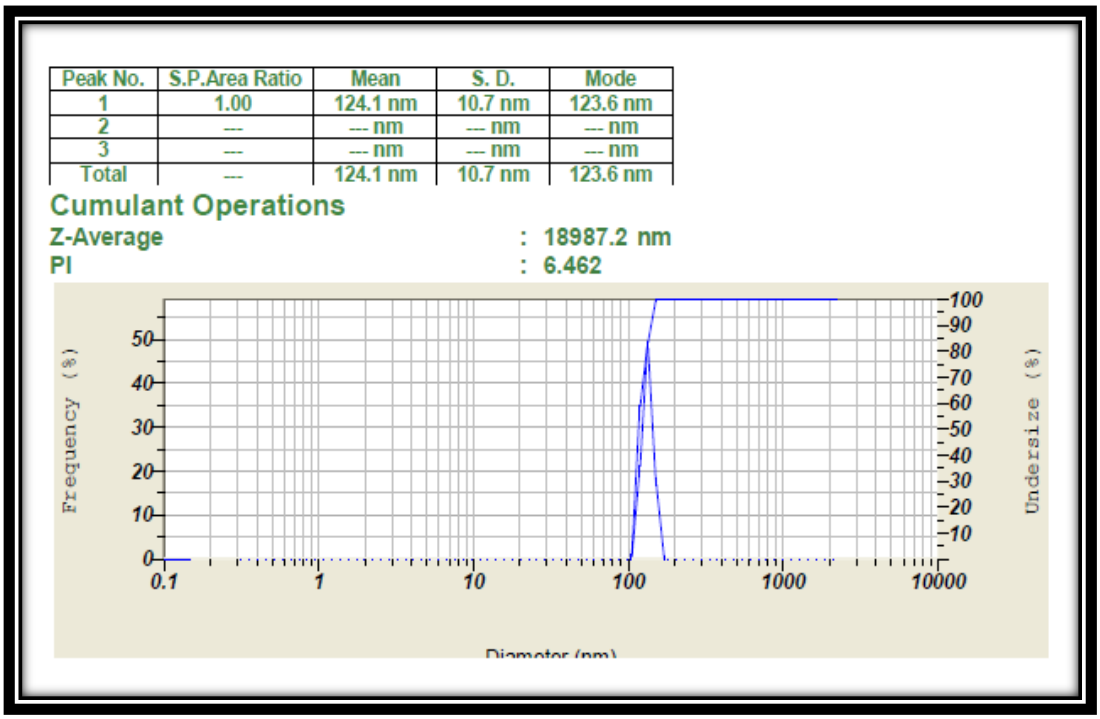


Figure:39 Zeta size distribution of F5 formulation

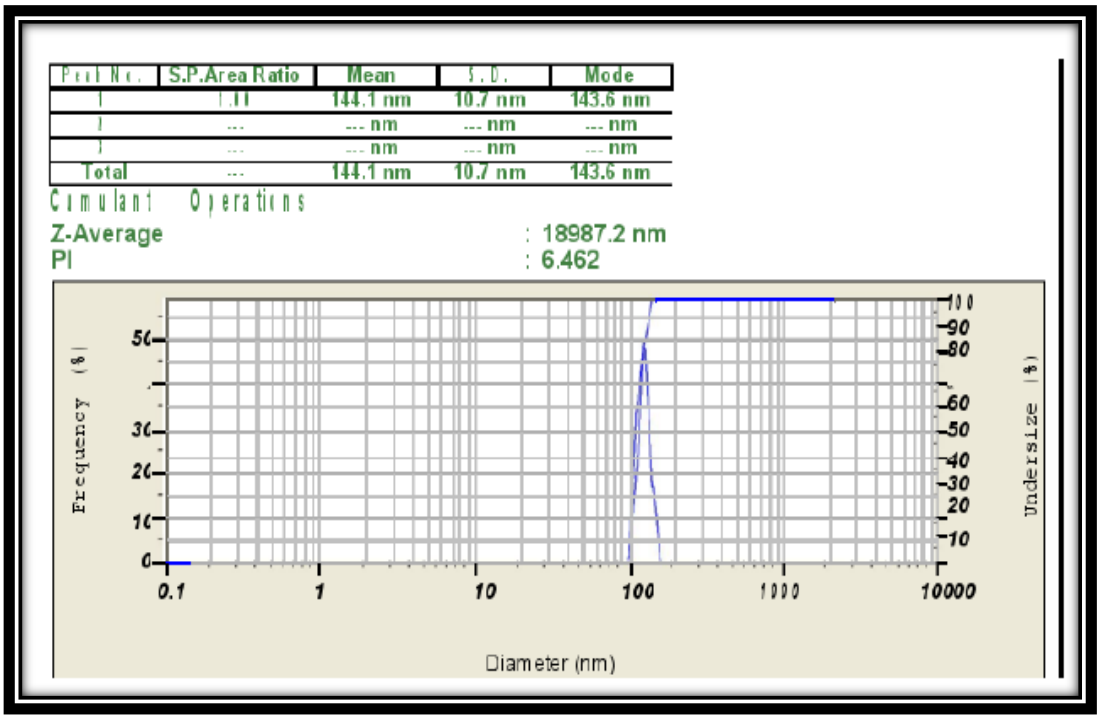


Figure:40 Zeta size distribution of F6 formulation

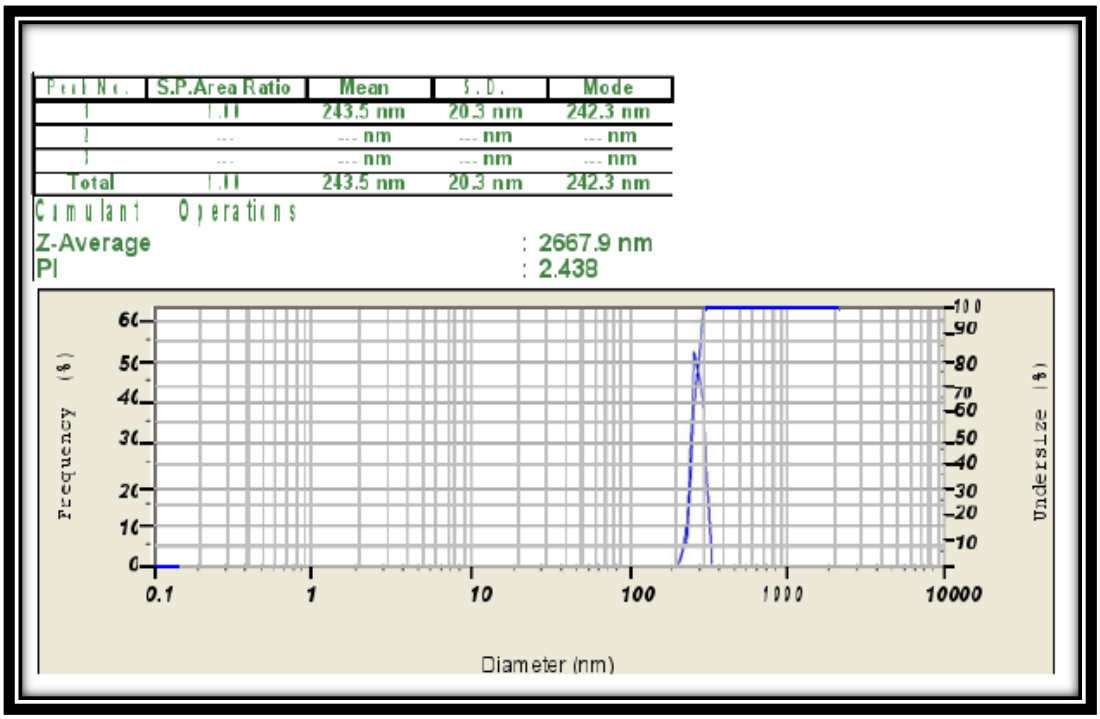


Figure:41 Zeta size distribution of F7 formulation

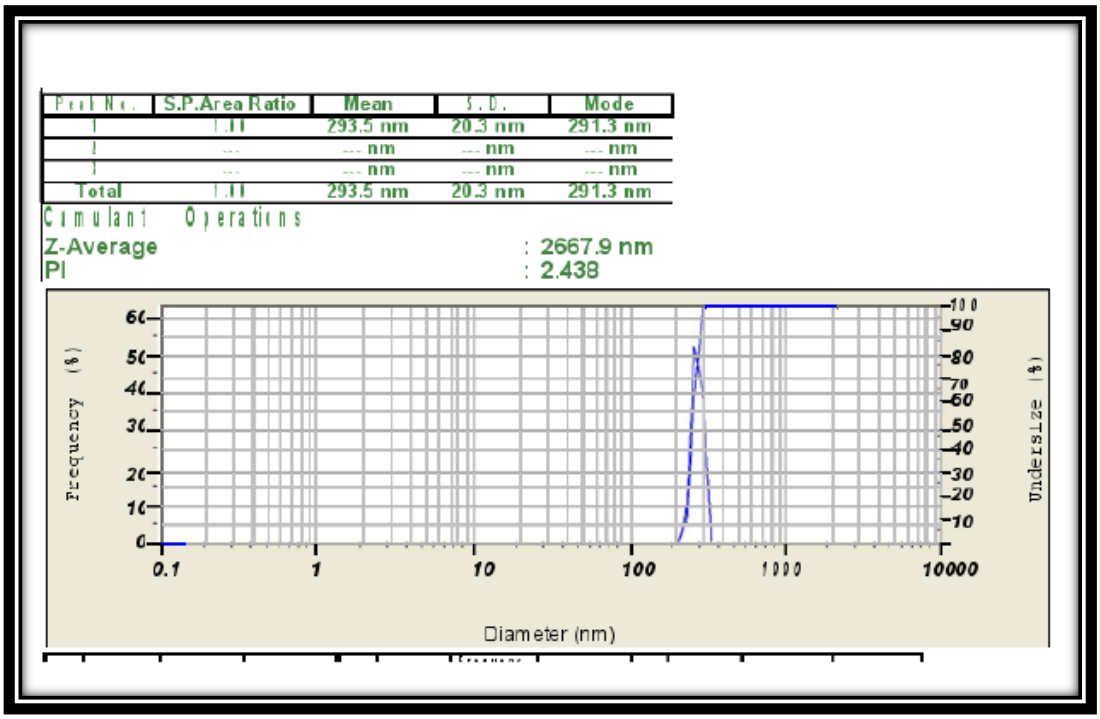


Figure:42 Zeta size distribution of F8 formulation

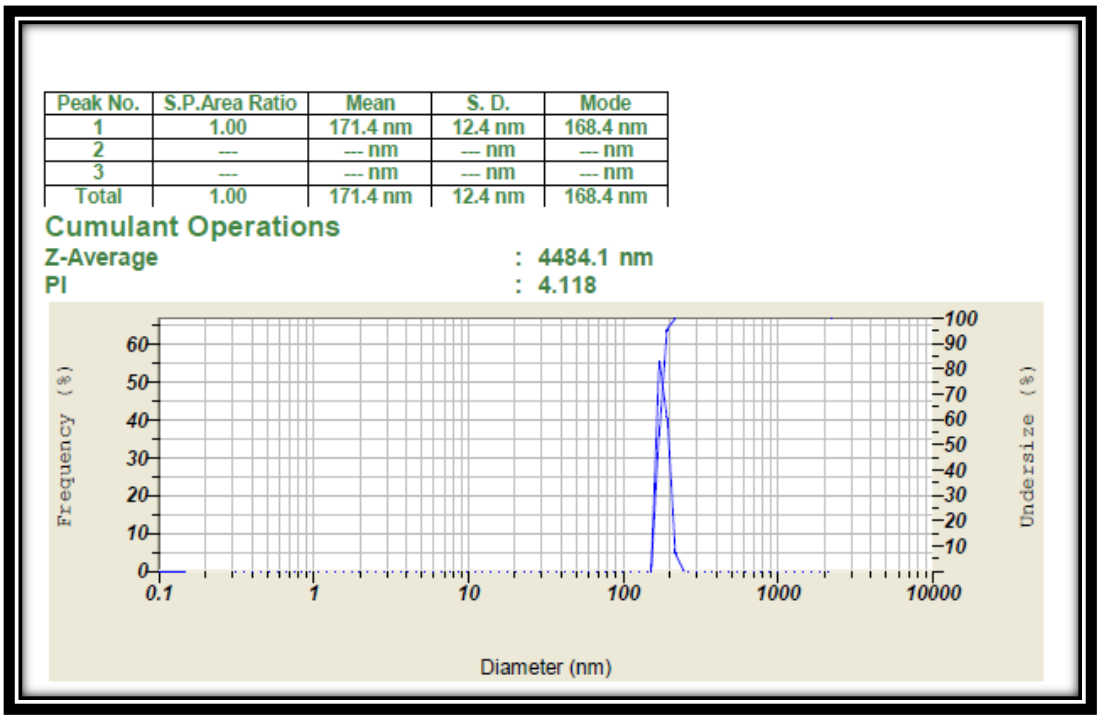


Figure:43 Zeta size distribution of F9 formulation

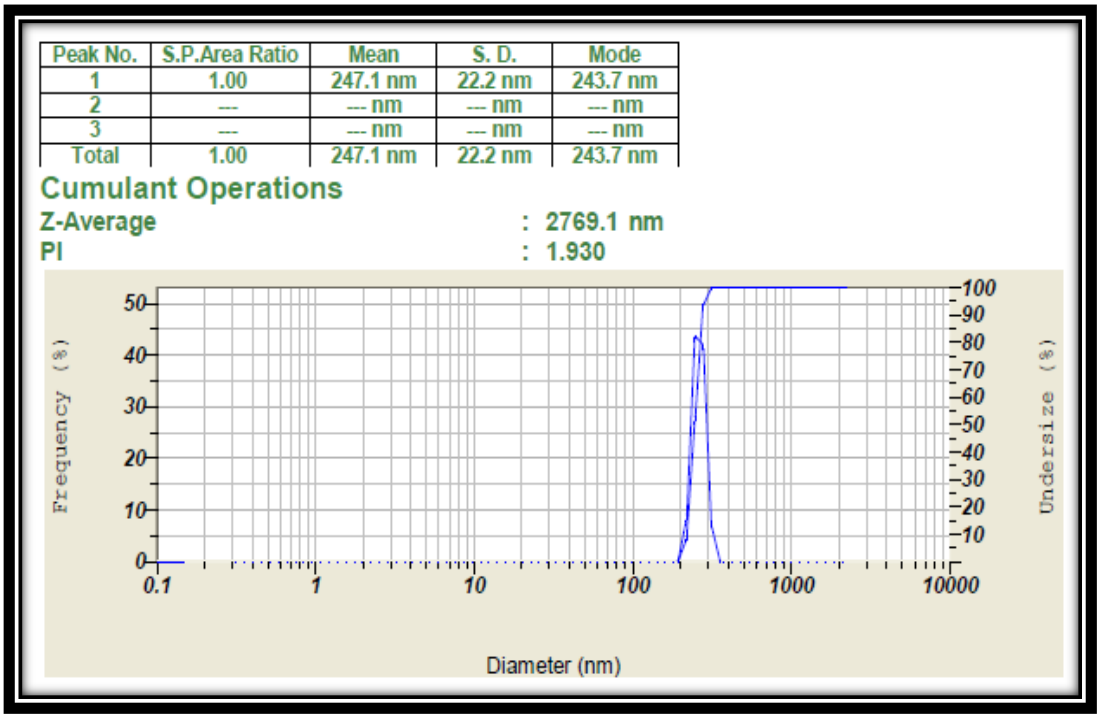


Figure:44 Zeta size distribution of F10 formulation

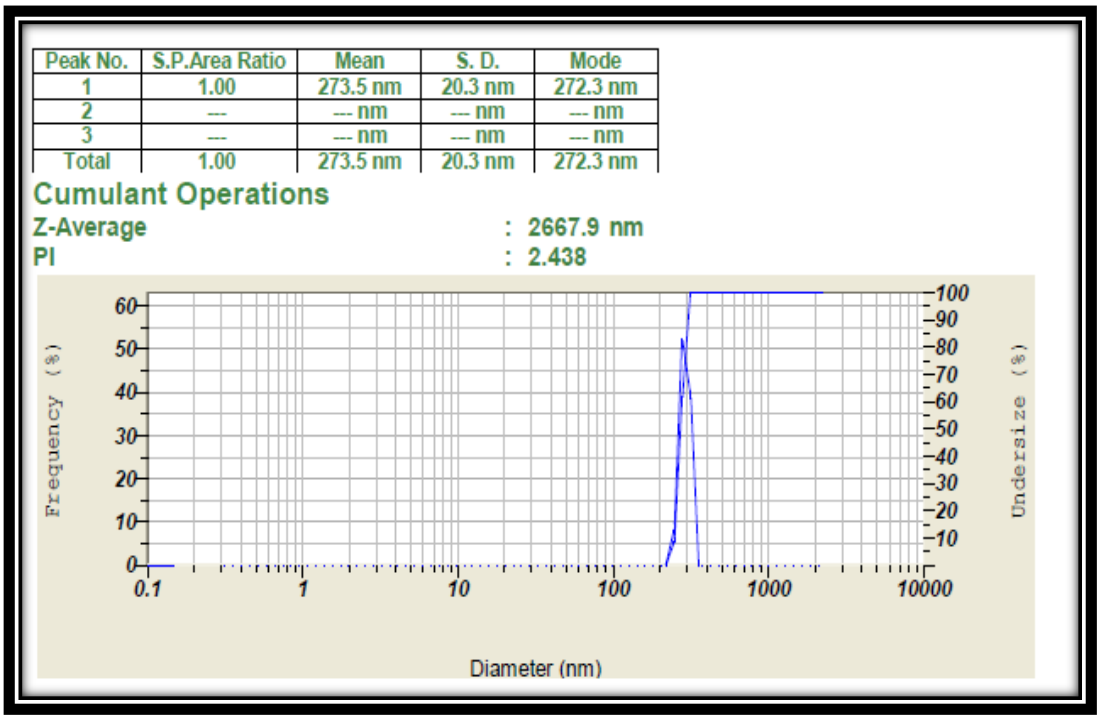


Figure:45 Zeta size distribution of F11 formulation

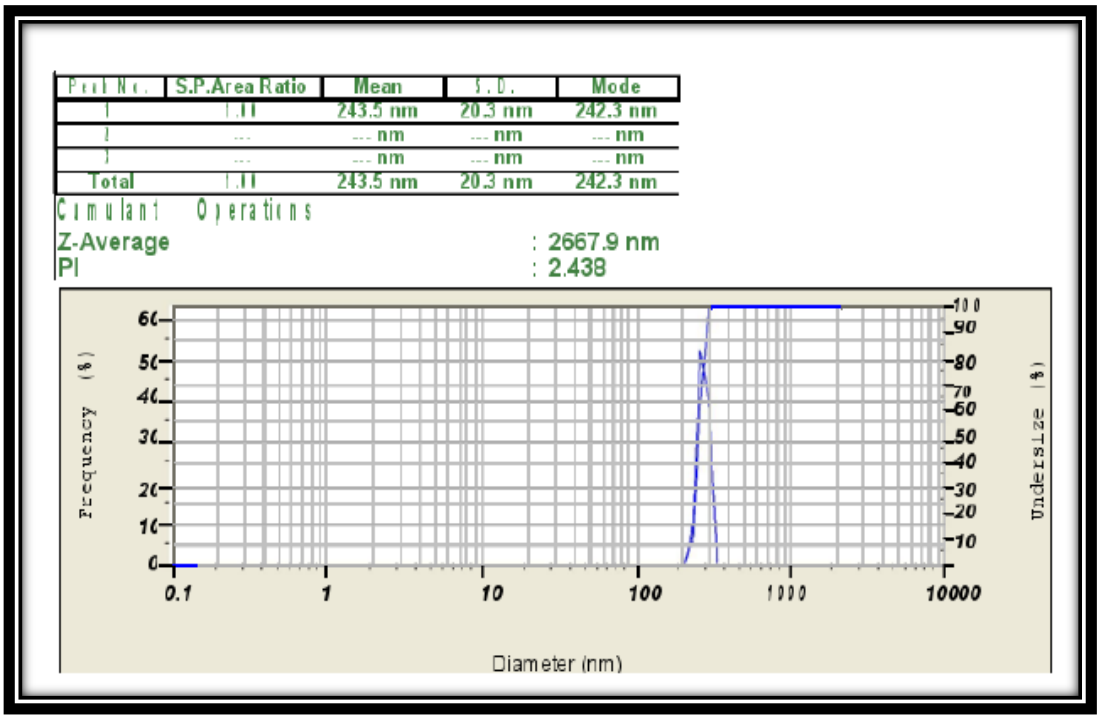


Figure:46 Zeta size distribution of F12 formulation

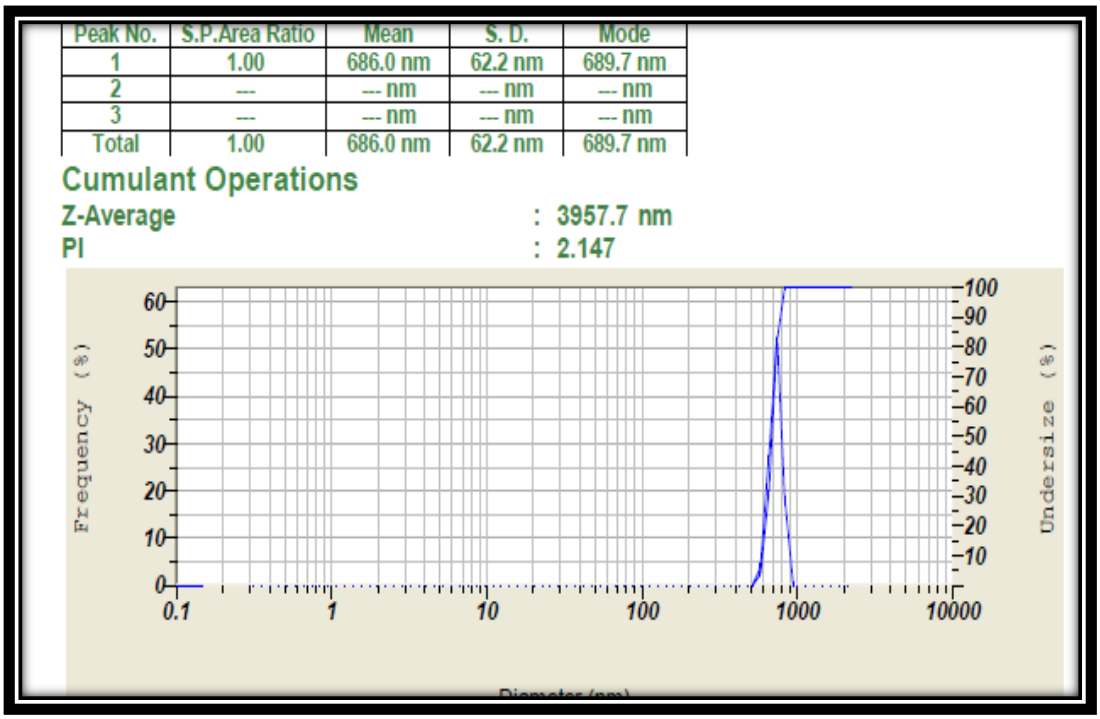


Figure:47 Zeta size distribution of F13 formulation ($CoCl_2$ NPs)

Table:27 Zeta size distribution of Imatinib Mesylate nanoparticles formulations

S. No	Formulation code	Size (d.nm)
1	F1	107.9
2	F2	147.9
3	F3	38.1
4	F4	150.4
5	F5	124.1
6	F6	144.1
7	F7	243.5
8	F8	293.5
9	F9	171.4
10	F10	247.1
11	F11	273.5
12	F12	243.5
13	F13($CoCl_2$ NPs)	686

DETERMINATION OF ZETA-POTENTIAL

Zeta Potential was determined using Malvern zeta-sizer instrument. Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. Zeta potential analysis was done by producing a potential difference between the two electrodes. In the electrical forces are also acting. The ionized solid particle is surrounded by liquid ions charged oppositely to the surface particle. During the movement of the solid particle through the liquid, a liquid layer surrounding the particle will move together with it. The potential difference between the external surface of that shell and the liquid as a whole is called electro kinetic potential difference is recorded. The particle size of the $CoCl_2$ NPs formulations was found. The measurement of ions on slipping plane of the vesicles. The value was obtained from the Figure plotted as apparent zeta potential vs. total counts.

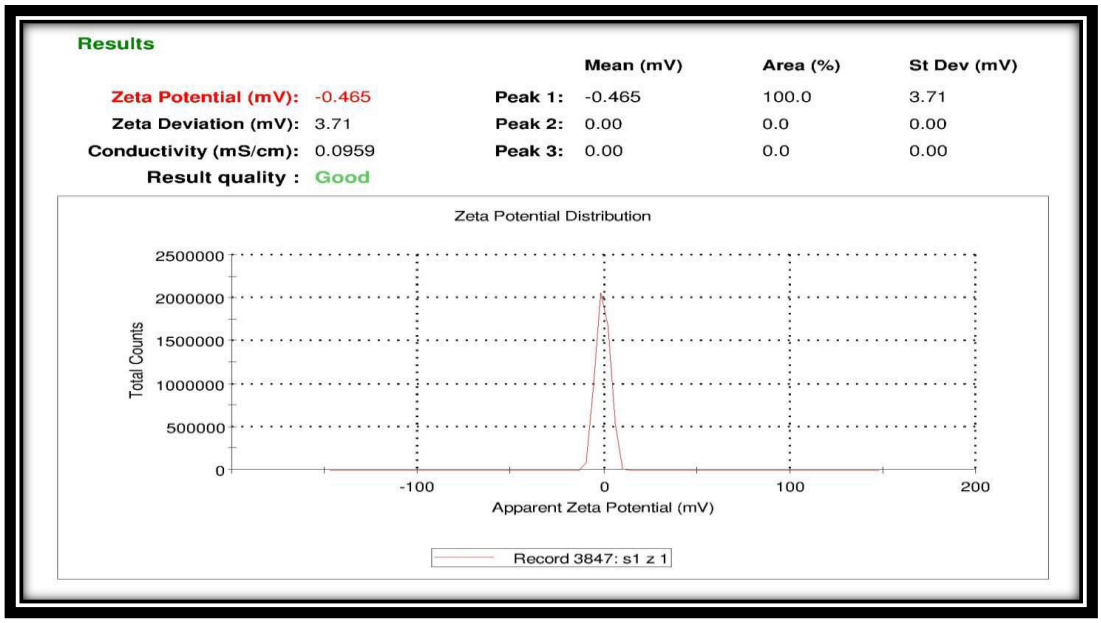


Figure:48 Zeta Potential for $CoCl_2$ NPs

The zeta potential measurement allows predictions about the stability of formulations. In common, particle clumb is less likely to happen for charged particles due to electric repulsion. The zeta potential values of the prepared nanoparticle revealed that they are stable.

IN VITRO DRUG RELEASE STUDIES

In vitro drug release profile data of all formulated nanoparticles are given in Table:28-30 and Figure: 49-51.

Table:28 *Invitro* Release profile of Imatinib Mesylate nanoparticles(F1-F6)

S.No	Time (hrs)	Cumulative % release of drug					
		F1	F2	F3	F4	F5	F6
1	0	0	0	0	0	0	0
2	4	18	18	23.4	18	25.2	18
3	7	23.4	27	27	27	30.6	23.4
4	24	45	41.4	46.8	41.4	39.6	46.8
5	28	50.4	54	55.8	50.4	48.6	54
6	32	63	66.6	66.6	54	55.8	59.4
7	48	90	90	91.2	64.8	68.4	63

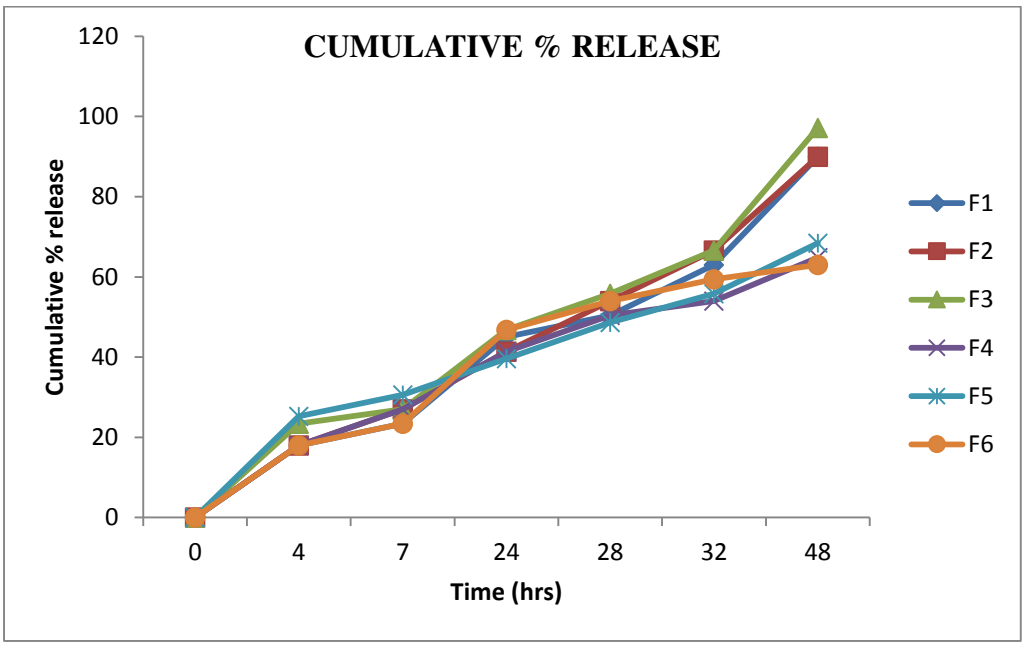


Figure:49 Release profile of Imatinib Mesylate nanoparticles(F1-F6)

From the *in-vitro* release data, it was noticed that all the prepared formulations had a release of 15-98% by the end of 48th hour. Initially ther was a fast release obtained where as a sustained effect was observed after 24th hour. The release data shows that the drug release was found to be approximately linear for the formulations (F1-F6), we can also notice that after the 32nd hour till the 48th hour, the release of the drug is in a sustained manner, which can be studied from the figure:49

It was also found from the release data that formulations F1, F2, F3 showed the best release of 90%, 90%, 91.2% when compared with other formulations. It was also noticed that for all the two types of polymers used in the study, the concentration of the polymer at two ratios to the was optimum and obtained maximum release in each category. The lowest release or sustained effect was observed for F4, F5, F6 formulations having a release of 64.8%, 68.4% and 63% respectively.

Table:29 *Invitro* Release profile of Imatinib Mesylate nanoparticles(F7-F12)

S.No	Time (hrs)	Cumulative % release of drug					
		F7	F8	F9	F10	F11	F12
1	0	0	0	0	0	0	0
2	4	18	9	18	18	18	9
3	7	27	21.6	27	23.4	27	18
4	24	45	39.6	41.4	45	36	32.4
5	28	54	45	50.4	50.4	39.6	43.2
6	32	57.6	55.8	54	55.8	46.8	45
7	48	73.8	75.6	63	66.6	55.8	61.24

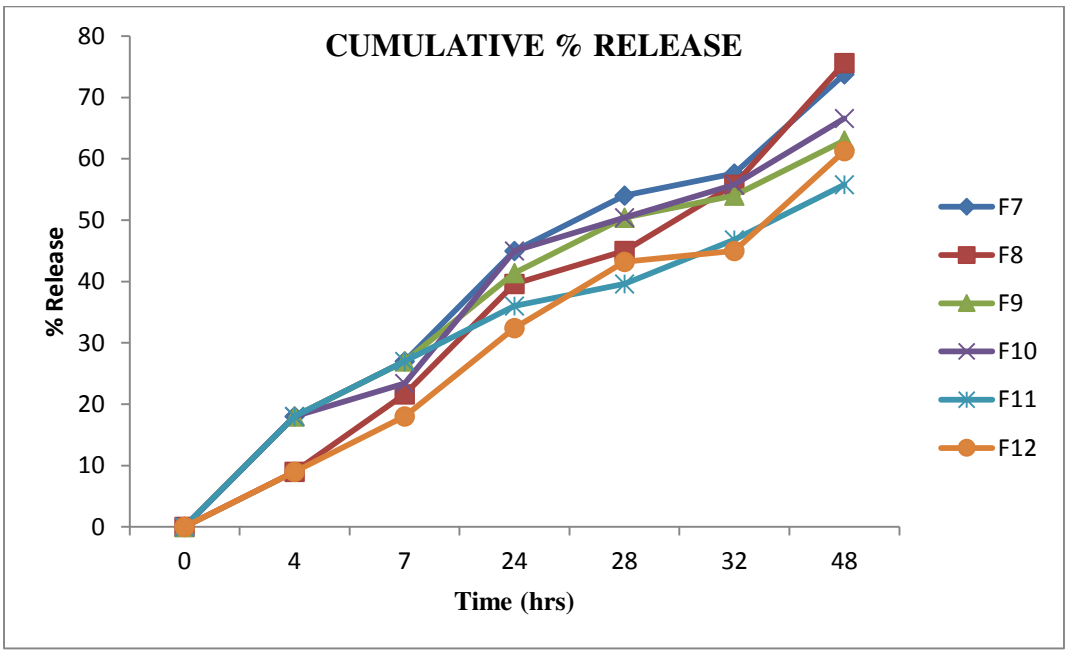


Figure:50 Release profile of Imatinib Mesylate nanoparticles(F7-F12)

From the *in-vitro* release data, it was noticed that all the prepared formulations had a release of 45-65% by the end of 48th hour. Initially ther was a fast release obtained where as a sustained effect was observed after 24th hour. The release data shows that the drug release was found to be approximately linear for the formulations (F7-F12), we can also notice that after the 32nd hour till the 48th hour, the release of the drug is in a sustained manner, which can be studied from the figure:

It was also found from the release data that formulations F7, F8, F10 showed the best release of 73.8%, 75.6%, 66.6% when compared with other formulations. It was also noticed that for all the two types of polymers used in the study, the concentration of the polymer at two ratios to the was optimum and obtained maximum release in each category. The lowest release or sustained effect was observed for F9, F11, F12 formulations having a release of 63%, 55.8% and 61.24% respectively.

Finally, all the formulations cumulative release are noticed till 48th hour. It was also found from the release data that formulation F3 showed the best release of 91.2% when compared with other formulatios. The lowest release was observed for F11 formulation having a release of 55.8% respectively.

Table: 30 *In vitro* release profile of formulation F13 ($CoCl_2$ NPs)

S. No	Time (hrs)	Absorbance (252 nm)	Con.from Graph (µG/ml)	Con. (µG/900 ml)	Con. (Mg)	Cumulative % Release
1	0	0	0	0	0	0
2	4	0.0126	1.2	108000	108	21.6
3	7	0.0148	1.5	135000	135	27
4	24	0.0280	2.7	243000	243	48.6
5	28	0.0340	3.2	288000	288	57.6
6	32	0.0258	3.8	342000	342	68.4
7	48	0.0458	5.3	477000	477	95.4

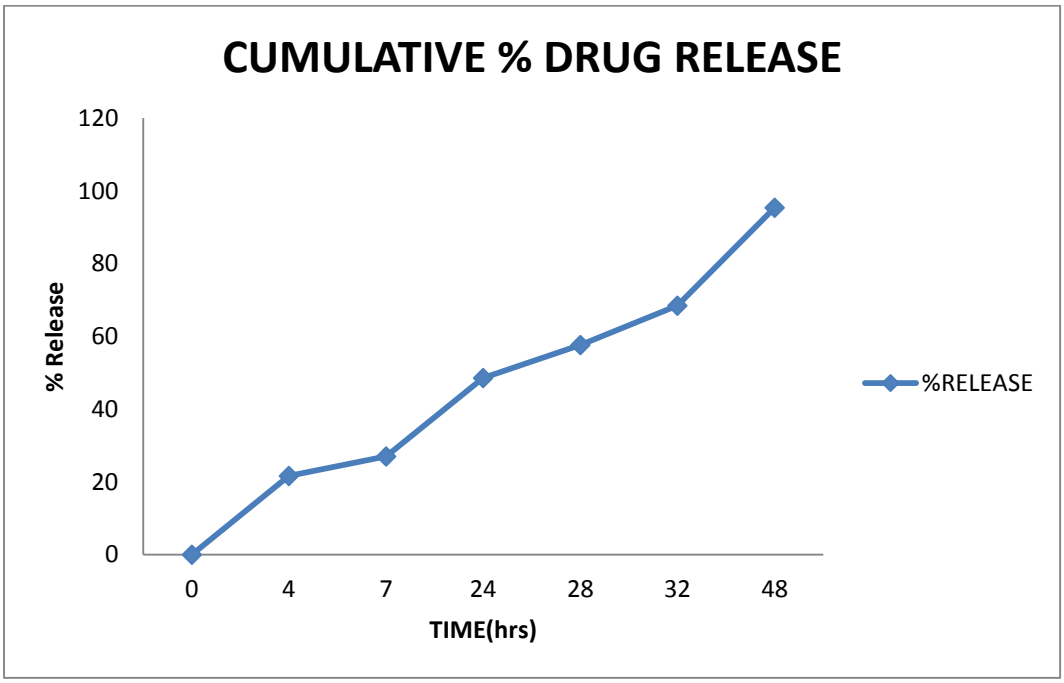


Figure: 51 Release profile of formulation F13 ($CoCl_2$ NPs)

Initial cumulative percentage release for formulation F13 ($CoCl_2$NPs) was 21.6% at 4 hours time and increased up to 95.4% by the end of 48th hour.The total release percentage by the end of 48th h was found to be 95.4%.

RAMAN SPECTROSCOPY:

$CoCl_2$

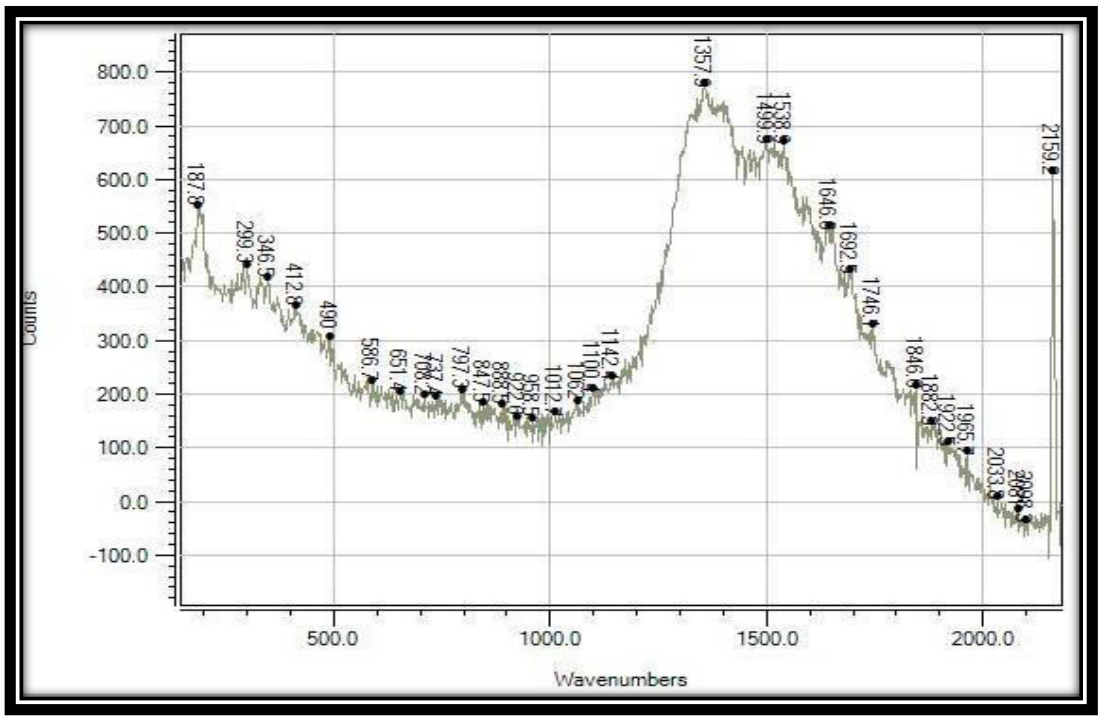


Figure: 52 Raman Spectroscopy

From the spectra's it can be concluded that the drug and excipient don't have any incompatibility since the peaks present in the drug can also be visualized in the drug and excipient spectra. Hence these excipients can be chosen for the preparation of nanoparticles and for further ananlysis.

X-RAY DIFFRACTION

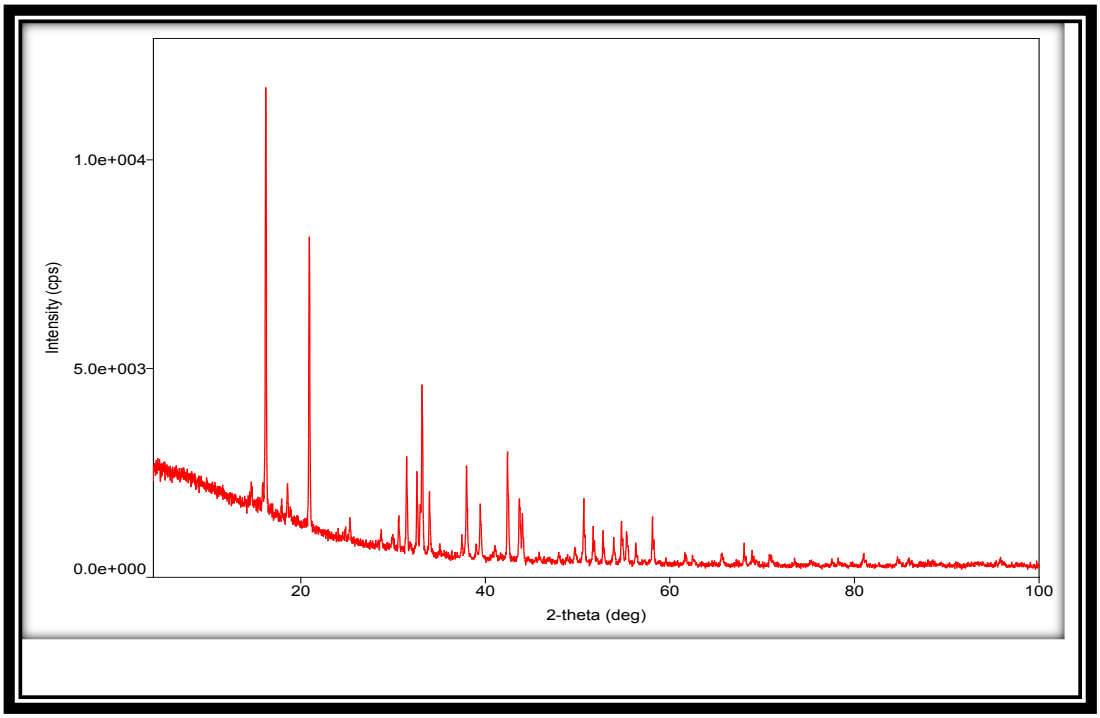


Figure: 53 X- Ray Diffraction

X-Ray	40 kV , 30 mA	Scan speed / Duration time	5.0000 deg./min.
Goniometer		Step width	0.0200 deg.
Attachment	-	Scan axis	2theta/theta
Filter		Scan range	4.0000 - 100.0000 deg.
CBO selection slit	BB	Incident slit	2/3deg.
Diffrected beam mono.	D/teX Ultra Monochro.	Length limiting slit	-
Detector	D/teX Ultra	Receiving slit #1	2deg.

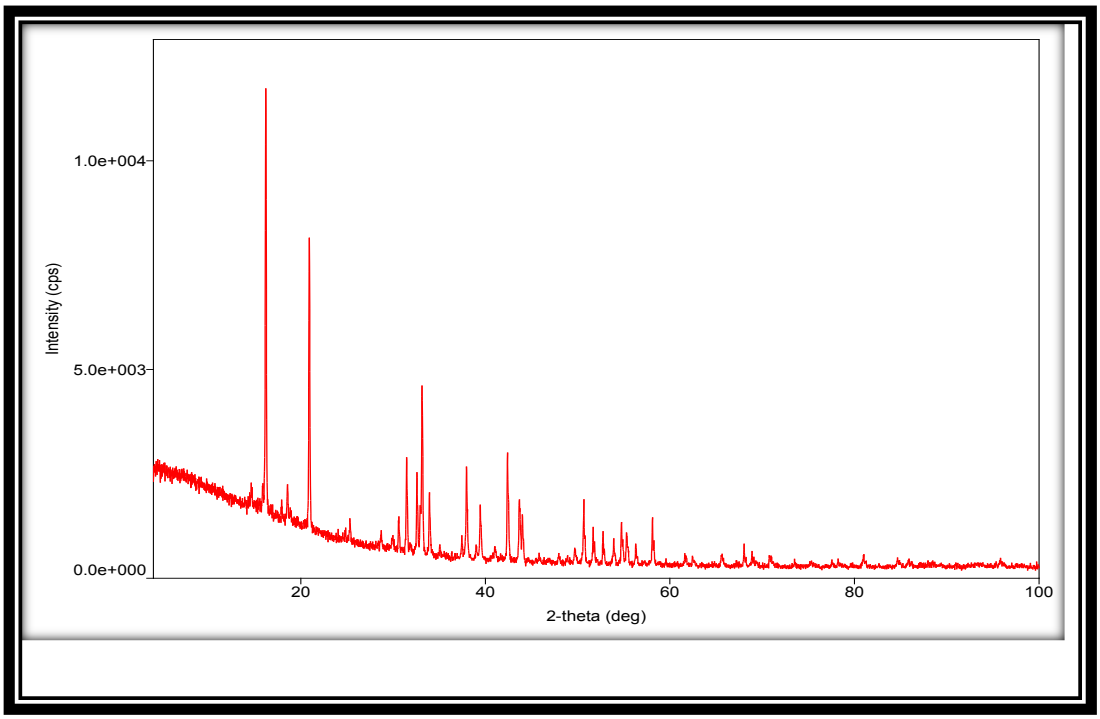


Figure: 53 (a) Measurement Condition

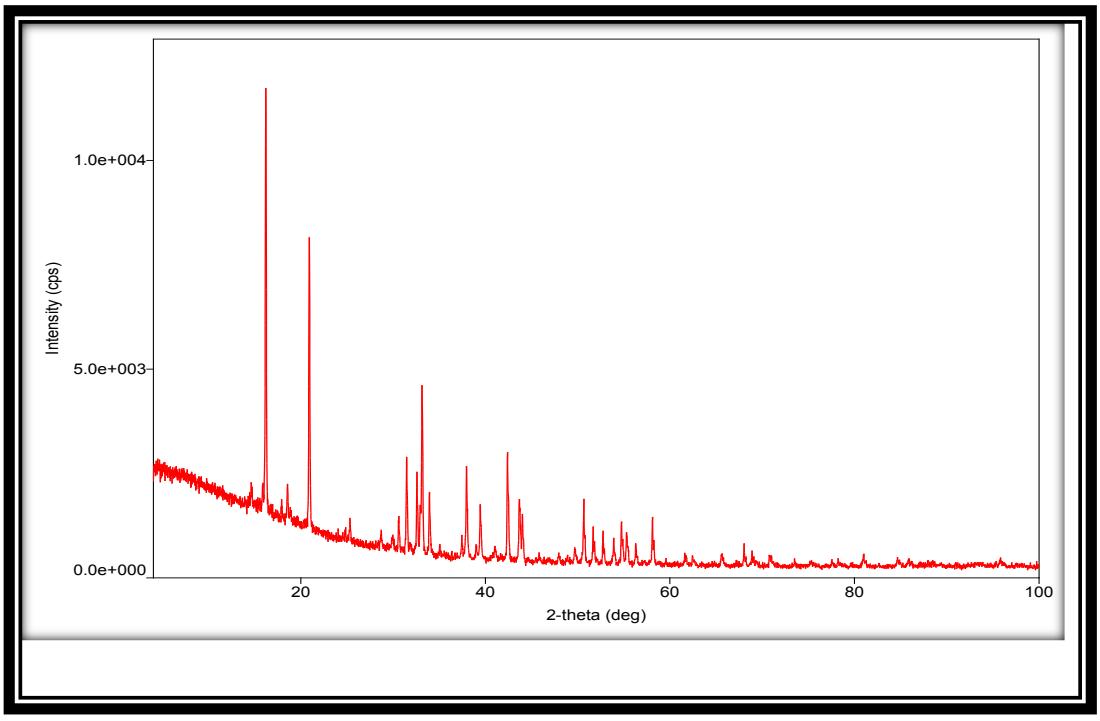


Figure: 53 (b) Dendrogram

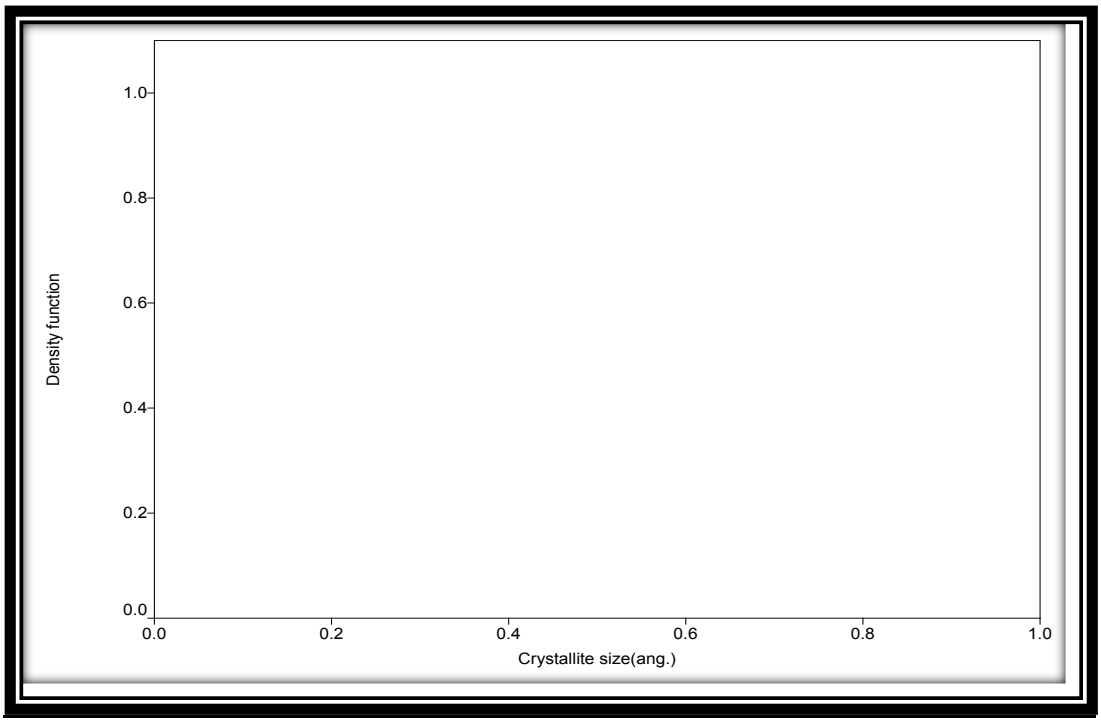


Figure: 53 (c) Crystallite size distribution

IN-VITRO DRUG RELEASE KINETICS

From the results of *in-vitro* drug release kinetics it is apparent that the first order plots of different preparations shows linearity, as shown by their high regression values. The data indicates a lesser amount of linearity when plotted by the zero order equation. Hence, it can be concluded that the major mechanisam of drug release follows first order kinetics. Understanding the mechanism of drug release is possible by configuring the data into mathematical modeling of Korsmeyer-Peppas plots. Plotting of graph using percentage release vs. square root of time, revealed linearity. Data based on the first order models usually provide evidence to the diffusion mechanism of drug release from sustained release delivery system.

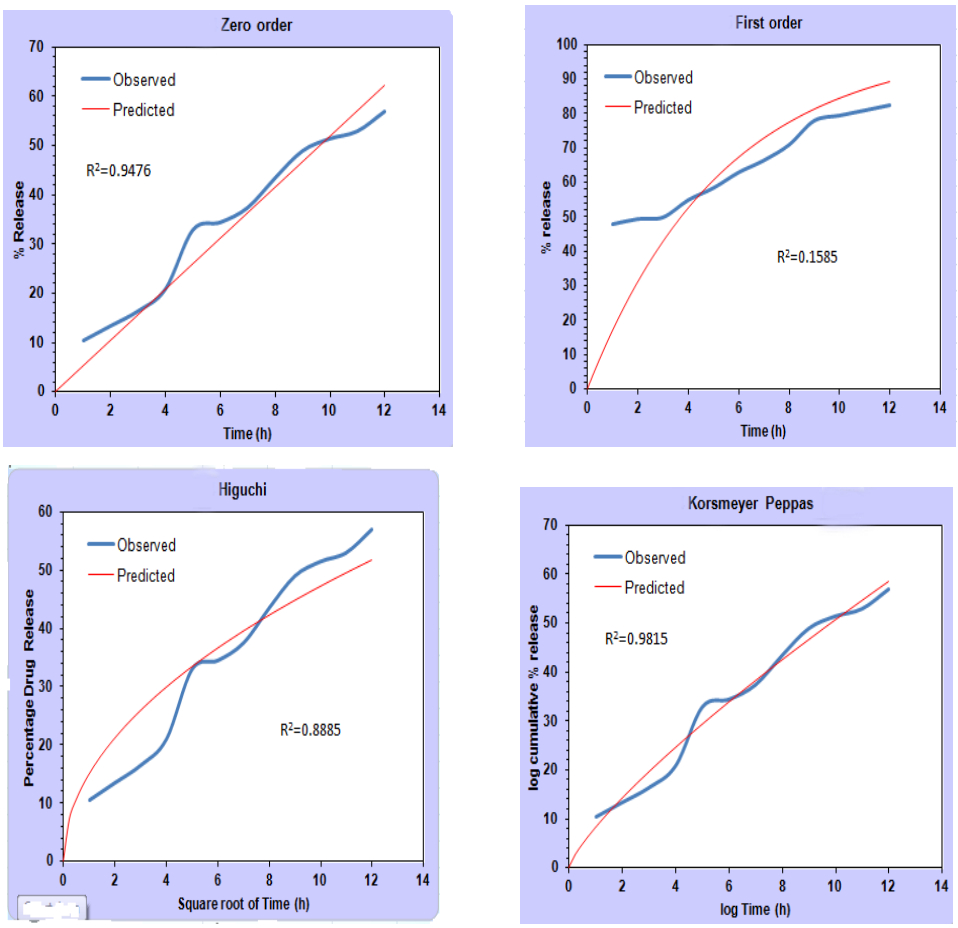


Figure:54 Drug release data of Formulation F13 ($CoCl_2$ NPs) fitting to various kinetic models .

The release data obtained from the in-vitro dissolution study of prepared nanoparticles was fitted into models of Zero order, First order, Higuchi and Kormeyer-Peppas. Shows the respective model fitted data obtained for all the eight formulations studied with R^2 values and exponential factor 'n' values. The overall data fitting showed that the drug release from the prepared nanoparticles follows Higuchi, korsmeyer-Peppas.

ACUTE TOXICITY STUDIES AND SELECTION OF DOSE FOR *IN VIVO* STUDIES :

Acute toxicity was determined as per OECD guidelines (420) employing fixed dose procedure for selecting the dose for biological activity. For acute toxicity studies female *Wistar* rats weighing 200-250g were taken and they were fasted overnight before the experimental day. Overnight fasted rats were weighed and body weight determined for dose calculation and the test $CoCl_2$NPs was administered orally. Sighting study was conducted with a lower dose of 5 mg/kg using $CoCl_2$NPs. After administration of this dose the animal was observed for signs of toxic effects. No toxic effect was observed and after sufficient interval of time (2-3 days) the second animal was administered with 50 mg/kg dose of test flavonoids. Similar observations were made as before and since the dose was non-toxic the third animal was administered with 300 mg/kg. This dose also did not exhibit any toxicity or morbidity and therefore the last and highest dose in fixed dose procedure of 2000 mg/kg was administered to fourth animal. No toxicity was noticed for highest dose. Signs and symptoms of toxicity and death if any were observed individually for each rat at 0, 0.5, 1, 2, 3 and 4 h for first 24 h and thereafter daily for 14 days (Table 13 & 14). Diet was given to the animals after 4 h of dosing. The animals were observed twice daily for 14 days and body weight changes, food and water consumption etc., were noted. In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg of body weight. There were no changes in normal behavioural pattern and no signs and symptoms of toxicity and mortality were observed (Table 14). Based on the results of acute toxicity the $CoCl_2$NPs formulation was considered non-toxic and $1/10^{th}$ and $1/20^{th}$ dose was used for the biological evaluation (anti-cancer activity) and the studies were conducted at dose levels of 100 and 200 mg/kg body weight.

Table : 31 Observations done for the Acute Oral Toxicity Study with $CoCl_2$NPS

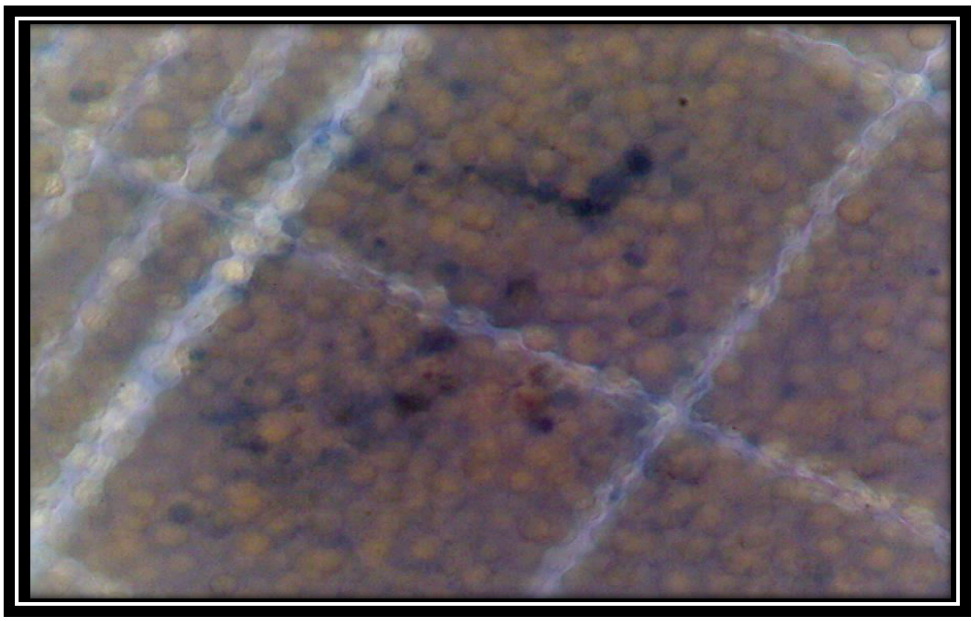
Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Respiratory	Dyspnea	-	-	-	-	-	-	-	-	-	-	-	-
	Apnea	-	-	-	-	-	-	-	-	-	-	-	-
	Nostril discharges	-	-	-	-	-	-	-	-	-	-	-	-
Motor activity	Tremor	-	-	-	-	-	-	-	-	-	-	-	-
	Hyper activity	-	-	-	-	-	-	-	-	-	-	-	-
	Hypo activity	-	-	-	-	-	-	-	-	-	-	-	-
	Ataxia	-	-	-	-	-	-	-	-	-	-	-	-
	Jumping	-	-	-	-	-	-	-	-	-	-	-	-
	Catalepsy	-	-	-	-	-	-	-	-	-	-	-	-
	Locomotor activity		-	-	-	-	-	-	-	-	-	-	-
Reflexes	Corneal reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Pinna reflex	-	-	-	-	-	-	-	-	-	-	-	-
	Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	Tonic and clonic convulsion	-	-	-	-	-	-	-	-	-	-	-	-
Muscle tone	Hypertonia	-	-	-	-	-	-	-	-	-	-	-	-
	Hypotonia	-	-	-	-	-	-	-	-	-	-	-	-
Ocular sign	Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-
	Miosis	-	-	-	-	-	-	-	-	-	-	-	-
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	-
	Ptosis	-	-	-	-	-	-	-	-	-	-	-	-

Parameters observed		0 h	0.5h	1 h	2 h	4 h	Day 2&3	Day 4&5	Day 6&7	Day 8&9	Day 10&11	Day 12&13	Day 14
Skin	Edema	-	-	-	-	-	-	-	-	-	-	-	-
	Skin and fur	-	-	-	-	-	-	-	-	-	-	-	-
	Erythema		-	-	-	-	-	-	-	-	-	-	-
Cardiovascular signs	Bradycardia	-	-	-	-	-	-	-	-	-	-	-	-
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	Contraction of erectile tissue of hair	-	-	-	-	-	-	-	-	-	-	-	-
Gastro intestinal signs	Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-

Live phase animals	Observations
1. Body weight every day	Normal
2. Food consumption daily	Normal
3. Water consumption daily	Normal
4. Home cage activity	Normal

Note: Acute toxicity study of $CoCl_2$ compound was performed and it was non-toxic up to 2000 mg/kg dose.

CELL VIABILITY ASSAY



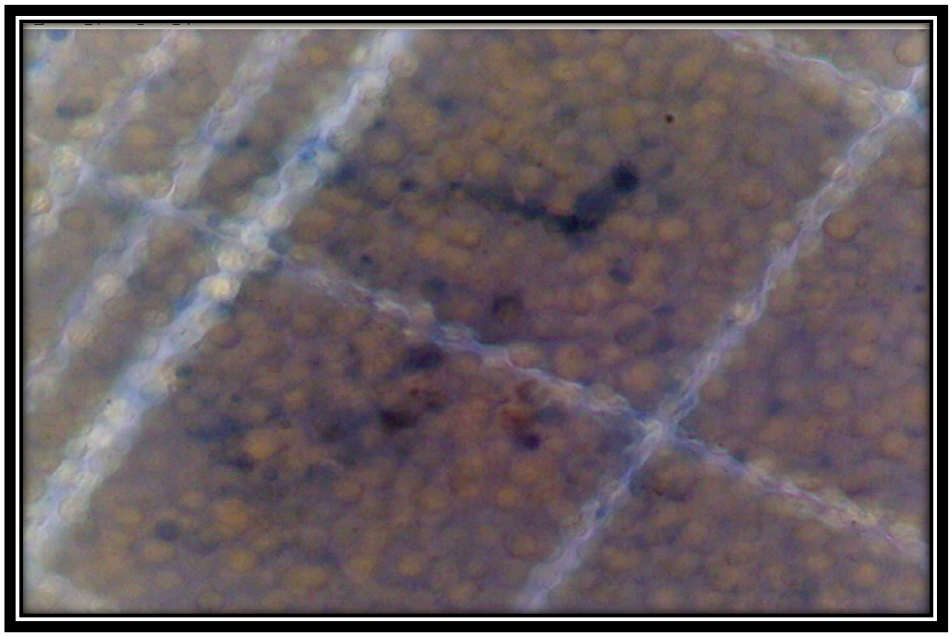


Figure : 55 Viable Cell Count of DLA Washing

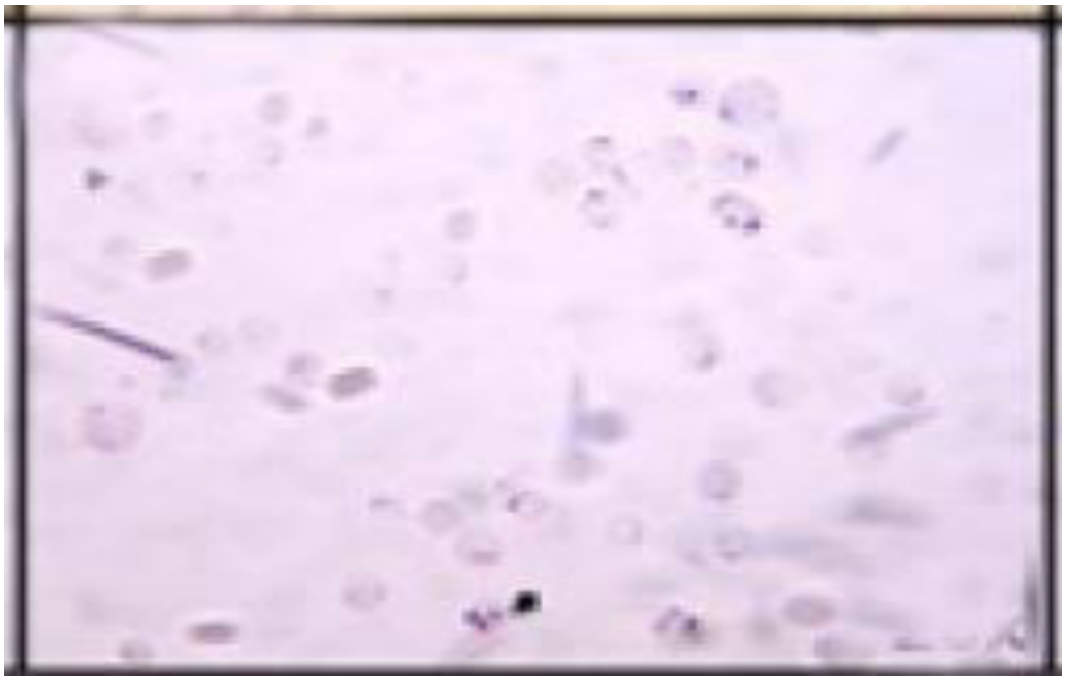


Figure : 56 Viable Cell Count after drug treatment

Table : 32 Cell Viability Assay

S.No.	Concentration(µg/ml)	% Cytotoxicity of DLA cells
1	5	25
2	10	50
3	20	78.57
4	40	100
5	80	100

In vitro cytotoxicity of $CoCl_2$ NPs was studied at concentration of 5, 10, 20, 40 and 80µg/ml using trypan blue dye exclusion method. A concentration dependent decrease in percentage viability was observed. At concentration of 10µg/ml CoCl2 NPs the percentage activity was found to be 50% and at 20µg/ml the percentage decrease in cell viability was found to be 78.57% and at 40 and 80µg/ml concentration 100% decrease in cell viability noticed and IC50 value was found to be 10µg/ml of $CoCl_2$ NPs.

Table 33 : Effect of $CoCl_2$ on Ascitic Tumor volume, body weight and percentage increase in body weight

S.No.	GROUP	Treatment	Body weight(before)	Body weight(after)	% increase in body weight	Tumor volume(ml)
1	Control	0.9% saline (10ml/kg)	22.2 ±1.25*	23. 4±0.76*	**-**	-
2	Negative control	0.9% saline (10ml/kg)	22.5±0. 64*	37.75±1. 10*	-	23.92±0. 6598*
3	Standard	Imatinib Mesylate (20mg/kg)	27. 84±2. 073#	18.44 ±1. 92#	3.044±10. 33#	10.28±4. 36#
4	Low dose	$CoCl_2$ NPs(200mg/kg)	23.25±1.518#	21.326±1.48#	8.39±0.5034#	2.126±0.299#
5	High dose	$CoCl_2$ NPs(400mg/kg)	23.75±1.82#	22.426±1.738#	5.58±0.8591#	1.00±0.8591#

Table : 34 Determination of mean survival time and percentage increase in lifespan

S.No	GROUP	Treatment	MST	%ILS
1	Negative Control	0.9% saline	12.33±0.55	60
2	Standard	Imatinib mesylate (20mg/kg)	14.23±0.76	33.3
3	Low dose	CoCl2 NPs(200mg/kg)	12.33±1.02	66.7
4	High dose	CoCl2 NPs(400mg/kg)	14.26±0.52	75

The values are expressed as mean ±SEM of 10 mice in each group *p<0.001 when compared to control, #represents p<0.01 when compare to negative control and standard drug treatment. Significance of the difference among various group was evaluated by oneway ANNOVA followed by Dunnett's test.

The DLA control group there was significant change in body weight was observed between 10-12 days of treatment and on 12th day the percentage change in body weight was found to be 37.75±1.10 and this increase in body weight might be due to increase in tumor volume when compared with 0 day treatment 22.5±0.64.

Treatment with the standard drug Imatinib Mesylate the tumor volume was found to be 10.28±4.360 and treatment with $CoCl_2$ NPs low dose (200mg/kg) drastically reduced the tumor volume to 2.126±0.299ml and with high dose (400mg/kg) further substantially reduced the tumor volume to 1.00±0.8591ml. So the results uptained suggest that $CoCl_2$ NPs at both doses were able to reduce tumor load compared to the standard drug and hence prove the effectiveness of $CoCl_2$ NPs.

A similar decrease in body weight was observed in standard, low dose, high dose treatment with $CoCl_2$ NPs. The results obtained with percentage change in body weight suggests that a decrease in tumor volume might have contributed to the reduction in body weight.

The mean survival time of tumor control was found to be 12.33±0.55 and standard as found to be 14.23±0.76 and treatment with low dose of $CoCl_2$ NPs(200mg/kg) was fond to be 12.33±1.02 and high dose of $CoCl_2$ NPs(400mg/kg) was found to be 14.26±0.52.

The percentage increase life pan of standard was found to be 33.3% and treatment with low and high dose was found to be 66.7% and 75%. The result of this study suggests that high dose treated $CoCl_2$ NPs increased life span compared to low dose $CoCl_2$ NPs.

Table : 35 (a) Effect of $CoCl_2$ NPs on Hematological Parameters

Treatment	Dose	WBC ($x10^9$ / L)	Lym%	Mon%	Gra%	RBC ($X10^{12}$/L)	Hgb%
Control	10 ml/kg Normal saline-i.p	2.96 ±0.18	66.8 ±0.58	6.86 ±0.33	24.54±0.679	7.18 ±0.33	13.3 ±0.242
Negative control	10 ml/kg Normal saline-i.p	11.25 ±1.63	49.25±6.00	1.77 ±0.14	45.5 ±8.026	4.47 ±0.31	11.77 ±0.69
Low dose ($CoCl_2$ NPs)	$CoCl_2$ NPs(200mg/kg)	8.25 ±0.94	89.72±2.06	4.2 ±0.73	6.07 ±1.47	8.33 ±0.32	13.7 ±0.38
High dose ($CoCl_2$ NPs)	$CoCl_2$ NPs(400mg/kg)	4.15 ±0.43	81.7 ±0.16	4.42 ±0.07	12.8 ±0.23	6.85 ±0.48	16.06 ±4.19
Standard (Imatinib mesylate)	Imatinib mesylate(20mg/kg)	62.8 ±23.9	58.8 ±8.80	14.57 ±2.86	26.54±6.036	8.15 ±0.28	12.12 ±0.335

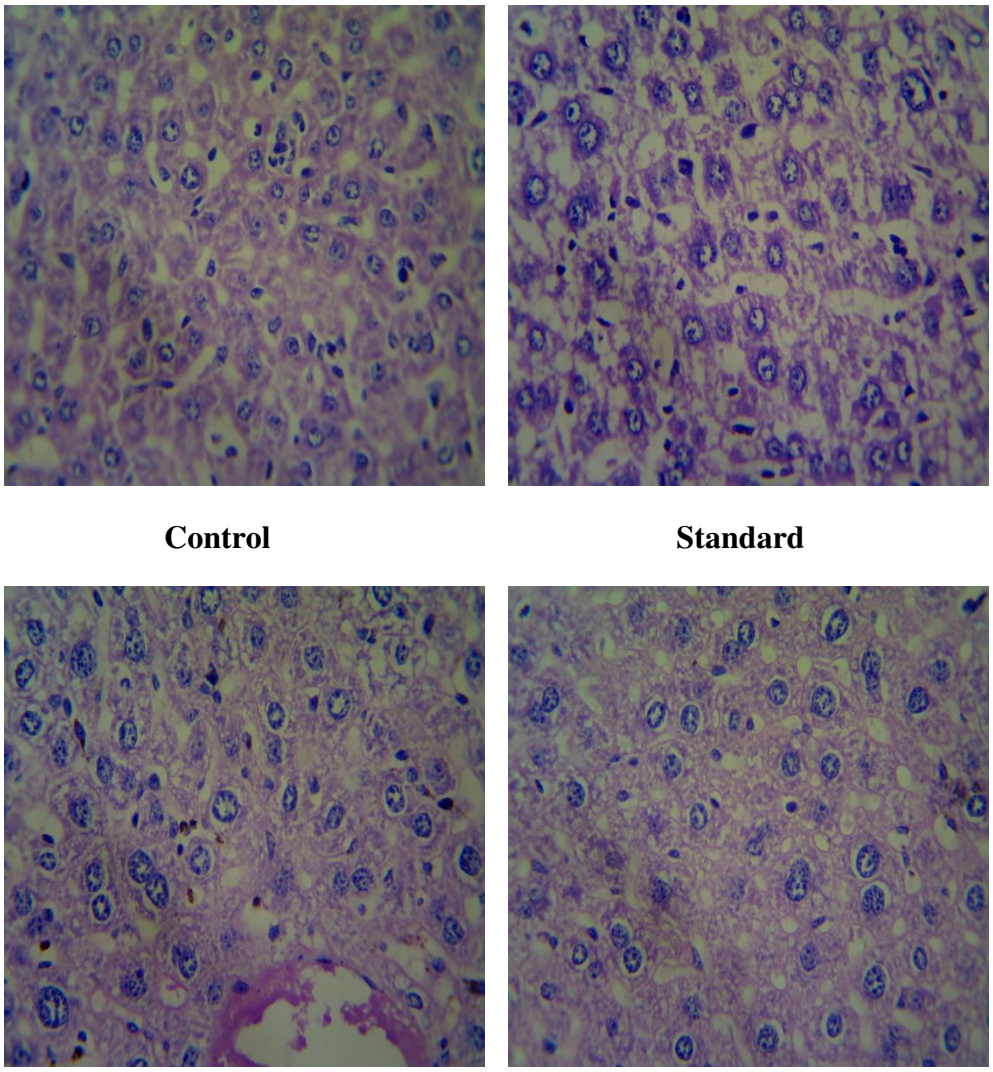
Table : 35 (b) Effect of COCL2 NPs On Hematological Parameters

Treatment	Dose	HCT (%)	MCV (μm X 3)	MCHC(g/dL)	MCH (pg)	PLT (X 10^9/L)
Control	10 ml/kg Normal saline-i.p	35.9±0.58	48.02±0.866	35±0.46	16.28 ±0.65	741.8 ±3.35
Negative control	10 ml/kg Normal saline-i.p	38.1±0.42	47.8±0.73	38.1±0.7	-	-
Low dose ($CoCl_2$ NPs)	$CoCl_2$ NPs(200mg/kg)	44.76±1.12	53.92±1.22	30.58±0.33	16.45±0.22	781 ±31.19
High dose ($CoCl_2$ NPs)	$CoCl_2$ NPs(400mg/kg)	35.88±1.86	56.62±1.03	23.26±3.4	15.37±0.21	484 ±96.40
Standard (Imatinib mesylate)	Imatinib mesylate(20mg/kg)	41.68±0.9836	51.66±1.581	29.14±0.6539	14.87±0.15	821 ±64.4

Values are expressed mean ± SEM of six mice in each group; low dose and high dose are compared with standard drug and negative control; it denotes P<0.01 when compared to standard significance of the difference among various groups was evaluated by one-way ANOVA followed by Dunnett's test.

The effect of $CoCl_2$NPs on various haematological parameters are shown in table 35: treatment with low dose and high dose of $CoCl_2$ NPs reduced the level of WBC and significantly increased the level of RBC and heamoglobin when compared to tumor control group. Treatment with standard drug significantly decreased the level of WBC and increased the RBC and heamoglobin when compared with the tumor control group. Similarly the agranular leukocytes levels such as lymphocytes and monocytes and granular leucocytes (neutrophils, eosinophils and basophils) levels were found to decreased in tumor control mice when compared to treatment groups.(low and high dose $CoCl_2$ NPS and standard drug).

HISTOPATHOLOGY OF LIVER IN TUMOR BEARING MICE:



Control **Standard**

Low dose **High dose**

Figure : 57 Histopathology of liver

Control - Normal lobular architecture

Standard - Imatinib Mesylate (20mg/kg) – Altered lobular architecture

Low dose (200mg/kg of $CoCl_2$NPs) - Altered lobular architecture with interface hepatitis

High dose (400mg/kg of $CoCl_2$NPs) – Mild Altered lobular architecture

Histopathology of in control mice shows normal lobular architecture with normal hepatocytes. There was no central vein dilation and congestion and with normal sinusoids.

The liver of standard drug treated group showed altered lobular architecture. The individual hepatocytes shows few cytoplasmic vacuolation and mild hepatitis and portal triad shows mild periportal inflammation with dilatation of central vein and sinusoids.

Treatment with low dose of $CoCl_2$ NPs showed altered globular architecture in liver .The hepatocytes were found to contain cytoplasmic vaculation with no significant pathology of portal triads. The central vein and sinusoids shows mild dilatation and congestion.

Treatment with high dose of $CoCl_2$ depicts almost near normal lobular architectcure with very mild cytoplasmic vacuolation, binucleation and reactive atypia in the hepatocytes. The central vein and cynocytes shows very mild dilatation compared with low dose and standard drug treatment group.

SUMMARY AND CONCLUSION

SUMMARY

Main objective of in this study to formulate Imatinib Mesylate nanoparticles to treat leukemia, especially Philadelphia positive Chronic Myeloid Leukemia (ph+ CML) and Chronic Eosinophillic Leukemia (CEL) using natural and synthetic polymers. Imatinib Mesylate nanoparticles reduce severe side effects such as muscle cramps, stomach pain, musculoskeletal pain and reduce the frequency of dosage form, facilitate the drug targeting and to overcome drug resistance.

So the present work was aimed to formulate a novel approach to enhance the delivery of drug by encapsulating it into the nanoparticle system whereby the dose of the drug administered can be lowered to control the night time seizures. The present work aimed to formulate stable Imatinib Mesylate nanoparticles by using various synthetic and natural polymers Poly Vinyl alcohol, Hydroxy Propyl Methyl Cellulose, Eudragit, Moringa oleifera, Badam, Linseed. The polymer were found to be economic, biocompatible and easily available. Imatinib Mesylate nanoparticles were prepared by using solvent evaporation method without the addition of surfactants and charge inducing agents, having vesicle size in the range of 400-800 nm.

FTIR and UV spectral studies showed that the spectra obtained with sample drug were matched with standard spectra of pure drug. This shows that the obtained drug sample was Imatinib Mesylate.

The FTIR spectra of pure drug and mixture of drug polymer shows that no appearance of additional new peak and disappearance of existing peaks of the drug. This indicates that there is no interaction between the drug and polymers used in the present study.

Scanning electron micrograph of the prepared nanoparticles F1-F12 are shown at a magnification ratio of X100,X6000. The surface morphology of the prepared nanoparticles was shown to be swollen and spherical.

Vesicle size determination was carried out using Zeta-sizer F3 has the lowest size of 38.1 nm and F8 has maximum size of 293 nm.

The zeta potential that was calculated and it was found that $CoCl_2$NPs had the highest zeta potential of 3.71. The charge of the nanoparticles dosage form must be above 25 mV so as to get a stable formulation. Since no charge inducing substances were added to the formulation, the zeta potential that is calculated solely depends upon the formulation alone.

Entrapment efficiency of 96.78% for the formulation F3. Where as for formulation F12 the entrapment efficiency is found to be 65.82% of it was observed that when polymer concentration was increases entrapment efficiency will decrease.

The sustained release patterns was observed for the prepared nanoparticles (F1-F13) clearly exhibiting an increase in the polymer concentration.

The present work aimed to form stable nanoparticles, which was prepared using solvent evaporation method without the addition of surfactants and charge inducing agents, having vesicle size in the range of 400-800 nm. Drug entrapment was highest for F3 formulation was 96.78%. As no charge inducing agent was added, the charge of the nanoparticles were below 25 mV and the highest charge was found to be -23mV. Finally F3 formulation complexed with $CoCl_2$NPs .Maximum release was obtained for the drug release kinetics for the prepared formulations (F13) showed Zero order R^2 =0.9476 and Higuchi release with R^2 value 0.8885 and Korsmeyer-Peppas with for (F13) R^2 value 0.9815 .

CONCLUSION

Imatinib Mesylate nanoparticles have been successfully prepared by solvent evaporation method.

HPMC, PVA, Eudragit, Moringa oleifera, Linseed, Badam were used as polymers for the preparation of Imatinib Mesylate nanoparticles.

Imatinib Mesylate nanoparticles were prepared by solvent evaporation method using various synthetic and natural polymers ratio of 1:1,1:2 respectively.

Based on this physiochemical characterization *in vitro* drug release kinetics of Imatinib Mesylate nanoparticles showed 91.2% of drug at the end of 48^{th} hrs. The F3 formulation complexed with Cobalt (II) Chloride ($CoCl_2$ NPs) (F13 gives *in vitro* drug release for 95.4%

The present study was carried out to evaluate the anti-tumor effect of $CoCl_2$NPs in DLA- bearing mice. The CONPs treated animals at dose of 200 and 400 mg/kg significantly inhibited the tumor volume, and brought back the haematological parameters to more or less to normal levels. In tumor control group, an increase in the volume of ascetic fluid was observed. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in volume of ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. Treatment with $CoCl_2$NPs increased the percentage the tryphan blue positive dead cells in tumor bearing mice. The reliable criteria for assessing the value of any anti-cancer drug are the prolongation of the life span of animals. The $CoCl_2$NPs decreased the ascites fluid volume, viable cell count and increased the percentage of life span of animals. Thus, it may be concluded that the reason for decreasing the nutritional fluid volume and arresting the tumor growth, might be due to increase in life span of DLA- bearing mice.

The major problem encountered with anti-cancer therapy are myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin levels and this may be due to iron deficiency or hemolysis of RBC. After the treatment with $CoCl_2$NPs, the hematological parameters RBC hemoglobin and WBC counts were altered and this may be due the protective action of the $CoCl_2$NPs on the haemopoitic system.

Based on the encouraging results, the Imatinib Mesylate ($CoCl_2$NPs - F13) were found to possess good sustained release property and was able to target DLA cell with strong anticancer property.

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