Development of microspheres based sustain release formulation on antihypertensive drugs

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Abstract

Polymeric drug delivery systems represent a significant advancement in pharmaceutical technology by improving drug release characteristics and enhancing the bioavailability of both water-soluble and poorly soluble drugs. One of the major challenges in conventional drug therapy is maintaining a consistent therapeutic drug concentration in the bloodstream. Conventional dosage forms such as tablets and capsules often require repeated administration because they release the drug rapidly, leading to fluctuations in plasma drug levels. To achieve the desired therapeutic effect, higher doses are frequently administered, which may increase the risk of adverse effects and toxicity.

Sustained release drug delivery systems have been developed to overcome these limitations. These systems provide a slow and controlled release of the drug over an extended period, maintaining plasma drug concentrations within the therapeutic range for longer durations. As a result, they reduce dosing frequency, minimize side effects, improve patient compliance, and enhance therapeutic efficacy. Sustained release formulations are particularly beneficial in the treatment of chronic conditions such as hypertension, where maintaining a steady drug concentration is essential for effective therapy.

Microspheres are one of the most widely used sustained release drug delivery systems. They are spherical particles ranging in size from 1 to 1000 µm and are composed of biodegradable or non-biodegradable polymers. The drug is either embedded within or encapsulated by the

polymer matrix. Microspheres are characterized by parameters such as particle size, shape, drug loading, entrapment efficiency, surface morphology, and uniformity.

Oral administration remains the most convenient and preferred route of drug delivery. Microsphere-based sustained release systems help maintain therapeutic drug concentrations in the blood for prolonged periods, thereby improving treatment outcomes. Their primary objective is to rapidly achieve the desired therapeutic concentration and then maintain it above the minimum effective level for an extended duration through controlled drug release.

Keywords-Polymeric Drug Delivery System, Sustained Release Drug Delivery, Microspheres,Oral Drug Delivery ,Antihypertensive ,Therapeutic Drug Concentration Plasma Drug Level , Drug Release Profile

Introduction

When the therapeutic agents are introduced in the polymeric drug development system produces a significant technological development providing the better drug release in aqueous environment and the bioavailability of both soluble and insoluble drugs attain its maximum. For maintaining the effective therapeutic concentration of administered drug dosage form complication arises due to introduction of drugs in several times not to fluctuate the drug concentration in plasma. Usually in case of the conventional drug dosages form (tablets and capsules) we have to administer the higher concentration of the drug at the targeted site to maintain the minimum effective concentration, which leads several untoward side effects. But in case of sustained release drug dosages forms the slow and prolonged release of drug is safe in comparison to sudden bulk drug release of conventional drug dosage forms. The drugs which are introduced in the conventional dosages forms produces a varying concentration of drug at the effectors site resulting inappropriate pharmacological response, and it is generally for the drug which are having half-life below three to four hours. Generally, in conventional drug dosage form as the initial loading dose is insufficient to produce the proper therapeutic effect, repeated administration of medicine is required at equal intervals resulting an increase in toxic side effects. Sustained release formulations are therefore advantageous in antihypertensive therapy as they maintain a more uniform plasma drug concentration over an extended period, improve therapeutic outcomes, reduce dosing frequency, and enhance patient adherence to treatment

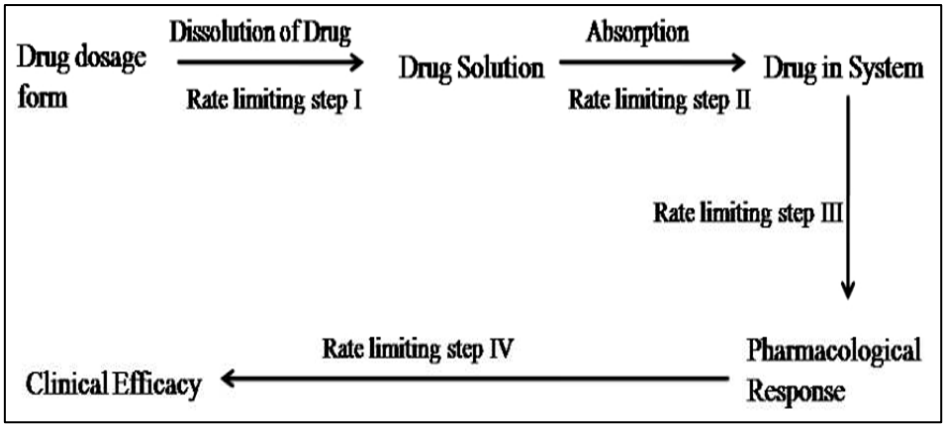


Figure 1. Schematic representation of clinical response of a drug molecule.

Sustained release drug delivery systems have gained considerable importance in the management of chronic diseases that require long-term pharmacotherapy. Hypertension, a chronic cardiovascular disorder characterized by persistent elevation of arterial blood pressure, requires continuous maintenance of therapeutic drug levels to achieve effective control and prevent disease-related complications. Such variations may reduce therapeutic efficiency and patient compliance, particularly during prolonged therapy. Sustained release formulations are therefore advantageous in antihypertensive therapy as they maintain a more uniform plasma drug concentration over an extended period, improve therapeutic outcomes, reduce dosing frequency, and enhance patient adherence to treatment [1,2,3].

1.1 Sustained Release Technology

Oral route is one of the most popular routes of drug delivery because of easy administration, patient compliance, least sterility and flexible design of dosages forms. The most conventional dosage forms for oral drug delivery are tablets, capsules etc. They are formulated to get active compound in the systemic circulation immediately after administration. Nowadays, various modified drug release systems have been developed to deliver therapeutic agent at a controlled rate and sustained manner. controlled drug delivery system delivers the active drug in a designed rate and duration of time to achieve desired plasma drug concentration in the systemic blood circulation [1,2,11].

1.1.1 Advantages of Sustained Drug Delivery System

- The major advantage of the sustained release drug delivery system is its long term efficacy above the therapeutic level with minimum side effects. The frequency of dosing is minimized to avoid the complication in the dosing schedule.

- The effective drug concentration above the therapeutic level is maintained for a longer time which is not found in case of multiple dosing of conventional dosages form.
- Better drug absorption is found with longer time in case of sustained release drug delivery system as small and periodic release for a longer duration is found.
- The drugs having high bioavailability produce a sharp increase in plasma drug concentration which can be controlled by effective administration of sustained release drug delivery.
- The physicians fall in problem during the adjustment of dosages regimen of a sustained release dosages form because it is fitted during the dosages form design.

1.3 The anatomy and physiological characteristics of the gastrointestinal tract

The primary functions of gastrointestinal tract (GIT) are processes of secretion, digestion and absorption. The GI barrier allows passing rapidly into systemic circulation of most nutrients and vitamins by passive diffusion but controls entry of higher molecular weight medicaments and toxins. The oral administration of nutrients, vitamins and medicaments possess into GIT and are distributed into different organs or tissues of the body, then follow to absorption and eliminate from the body and must pass through various location of one or more biological membranes/barriers [37,38]. After orally taken materials are come into the stomach and mixing with gastrointestinal fluid, converted into liquid mass and then pass into the small intestine [37]. The gastrointestinal tract consists of three major anatomical regions: the stomach, the small intestine including duodenum, jejunum, ileum and large intestine. When the administered drug passes through the various regions of the GI-tract and is absorbed at the site of action depending on particular properties of gastrointestinal fluid with respect to pH, enzymes, electrolytes, fluidity and surface features. The stomach is a bag like structure with a smooth mucosa, small surface area and acidic pH 1-3 due to secretion of hydrochloric acid favors absorption of acidic drugs. The small intestine having enormous absorptive area of between 200 and 600 m^2 is the principal site of drug absorption. The perfusion rate of blood to the small intestine is 6 to 10 times more that of stomach and the pH range of 5 to

7.5 is most favorable for sustained or controlled release dosage forms. The properties of small intestine like high permeability, slow peristaltic movement and long transit time provide favorable environment for the absorption of drugs. Thus, these all conditions make small intestine the best site for absorption of most of the drugs [40]. Due to small absorptive area, large intestine plays very little role in the absorption of drugs. Particularly in the case of sustained release preparations, the colonic region of large intestine may play an important

role for the absorption of poorly absorbed drugs because of the long residence time [41].

On the basis of differences in the local environment such as pH, nature of gastric contents, length and surface area, absorptive capacity of three major sections of the GIT, the duration of transit and residence times of a sustained release product in each section, can highly affect the drug release/absorption from oral drug delivery products. On the other hand, some of the other factors such as drug solubility and dissolution rate, particle size, effective surface area, lipophilicity and pKa of the drug, polymorphic nature of the drug can also influence the drug absorption from oral route [40,41].

1.4 Therapeutic Importance of Sustained Release Drug Delivery System in Hypertension

Hypertension is a chronic cardiovascular disorder characterized by persistent elevation of arterial blood pressure and represents one of the major risk factors for cardiovascular morbidity and mortality worldwide. Effective management of hypertension requires long-term administration of antihypertensive drugs to maintain consistent therapeutic plasma concentrations and ensure continuous control of blood pressure. Conventional dosage forms often produce fluctuations in drug levels, which may lead to suboptimal therapeutic response or adverse effects associated with peak plasma concentrations. Sustained release drug delivery systems offer significant advantages in antihypertensive therapy by providing controlled and prolonged drug release, maintaining uniform plasma drug levels, and reducing dosing frequency. Such systems improve patient compliance, enhance therapeutic efficacy, and minimize the risk of sudden hypotensive episodes associated with rapid drug release [2,3,5].

1.5 MICROENCAPSULATION

The theories of micro particles were first observed in the 1960 when it was formulated on silicon rubber [51] and polyethylene [51]. The lack of degradability is the primary reason of its limited application and that leads to surgical removal of polymer from the system. Afterwards degradable polymers were used at random to coat the therapeutic agents [51].

The process of applying relatively thin coating in different types of formulation like solids or droplets of liquid, dispersions and that should be in the size range of 1-1000µm is known as microencapsulation. This is a blend of deep knowledge of pure polymer science and emulsion technology with a proper implementation on drug stabilization. This technology involves entrapment of solids, liquid or gases in a suitable polymeric coating [51]. This is a unique technology of converting liquids into solid with proper release profile inside the body. The ultimate objectives of the microencapsulation drug delivery system is to extend and control the

release of the active drug molecule from the coated particle without attempting to modify the normal biofate of the active drug molecule in the body after administration and adsorption [51].

1.5.1 Advantages of Microencapsulation

- Due to smaller particle size distribution of the drug is wider in the gastrointestinal track.
- The release profile of the drug is fully controlled with incorporation of suitable polymer.
- By changing the polymer ratio variation in drug delivery system change in release profile can be found.
- It is used in taste masking chewable tablet preparation, creams and ointments and aerosols preparations.
- Toxic materials can also be used safely with half of this method.

1.5.2 Disadvantages of Microencapsulation

- Incompatible coating of the core material may be a problem.
- Reproducibility of the medicaments can create another problem.
- Stability of the medicines should be proper or it can produce in self-life.

1.6 MICROSPHERE

Microspheres are spherical particles with size range varying from 1 to 1000µm and made of biodegradable and non-biodegradable coating materials. The active pharmaceutical ingredients are embedded in the matrix of microspheres. Actually each microsphere is the physical mixture of drug and polymeric materials. They are varied by drug content, drug entrapment efficiency, shape and size, quality, surface morphology and particles uniformity [54].

The most convenient and simple route of the drug administration is the oral route of drug delivery. The sustained release drug delivery system can overcome the problems and maintaine the therapeutic level of drug concentration in blood for the prolonged period of time [54].

In previous days the oral conventional solids dosages forms was used randomly to treat several diseases. But the problem was it could not maintain the plasma drug concentration over the minimum therapeutic concentration for longer period of time. Sustained drug delivery system like microspheres were developed to eradicate this problem so as to the therapeutic

concentration is maintained over a longer period of time and thus therapeutic efficacy of drug was increased [54]. This type of drug delivery system was developed to release the drug in a controlled and sustained manner for a specific time with a certain release profile [54]. Sudden release of drug from conventional dosage forms may lead to fluctuations in plasma drug concentration, which can be minimized by microsphere-based sustained release systems [56].

The aim of sustained/controlled drug delivery system is to deliver the drug above the minimum effective plasma concentration at the required site quickly and after getting desired plasma drug concentration to maintain therapeutic drug concentration at the site of action for prolonged period of time [98].

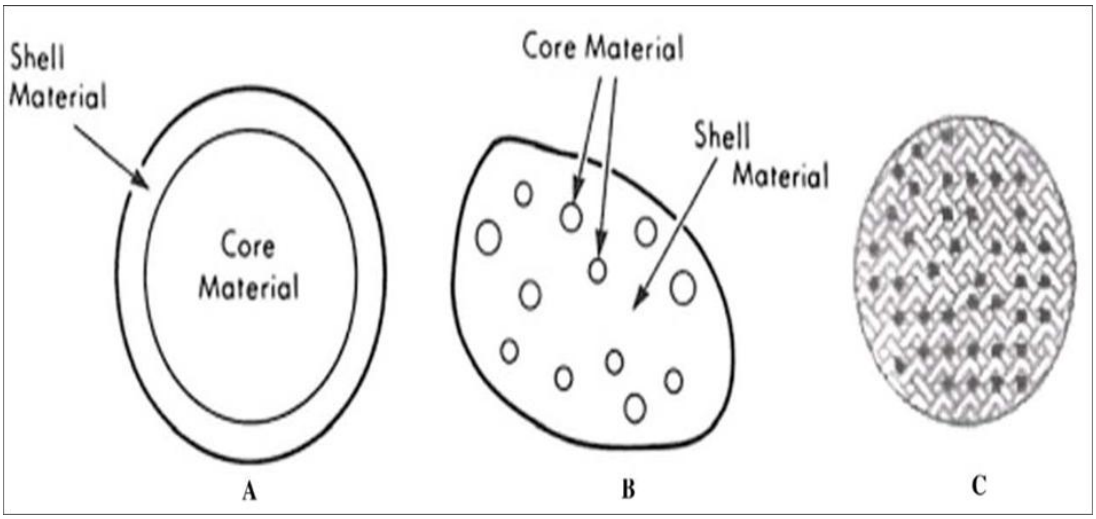


Figure 2: Picture of different type of microparticles

1.7 Microsphere drug delivery system beneficial over the conventional drug delivery system:

- Microspheres maintain prolonged and constant drug action.
- Microspheres provide sustained, controlled and targeted delivery of drug at desired site.
- Microspheres provide more reproducible drug absorption.
- Microspheres spread uniformly in the gastrointestinal tract and help in maintaining uniform plasma drug concentration
- Microspheres minimize the dosing frequency and consequently improve patient compliance.
- In case of microspheres, better therapeutic efficacy may be achieved for the drugs having short half-life (2-8 hrs).
- Microspheres enhance the biological half-life and increase the bioavailability of drug.

- Microspheres help to remove the unpleasant taste and odor.
- Microspheres can avoid the first pass metabolism.
- Microspheres protect the moisture sensitive drug from the environment.
- Microspheres have a low risk of dose dumping.
- Microspheres can be administered easily parenteraly because of their small particle size and spherical in shape.

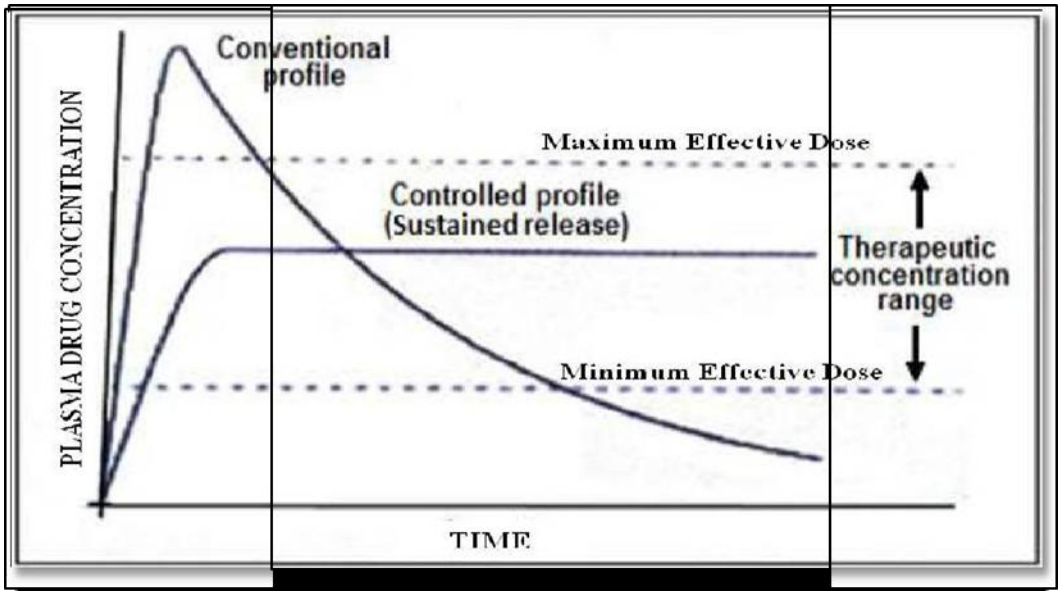


Figure 3: Comparison of conventional dosage form and controlled/sustained release dosage form profiles.

The release of drug from the polymeric microspheres can be regulated by dissolution and degradation of matrix or both of microspheres. In addition, by changing drug and carrier molecule we can modify the in-vitro drug release behavior. The physicochemical properties of carrier molecule can affect the drug absorption, bio-transformation, cellular interaction and clearance kinetics of the drug molecule. The physical mixtures of drug and carrier molecule significantly enhance the therapeutic efficacy of the drug [62].

On considering the release medium the areas of corner are its pH, ionic strength, temperature and its enzyme content. The drug properties to be studied for release kinetics are its solubility, charge distribution stability and most importantly its interaction with the other structural components. Lastly, the particle size distribution has also been seen as important parameters for the evaluation of drug release kinetics [62].

1.8 MECHANISM OF SUSTAINED /CONTROLLED ORAL DRUG DELIVERY

The prime objective of the oral sustained/controlled release system is to deliver the drug into the general blood circulation to perfuse to other body tissue. Thus they follow the dissolution, diffusion or combination of both, to provide slow drug release into the gastrointestinal tract. Following are the mechanism by which the sustained/controlled drug delivery system delivers the drug.

1.9 Dissolution control

The release of drug into the dissolution media from pharmaceutical dosage form is governed by dissolution characteristics of the drug. The drug molecule with slow dissolution will show the sustained release properties. So, decreasing in dissolution rate, the drug can be delivered as sustained manner. This type of drug delivery system is possible by coating the drug molecule with varing coating thikness of material or by dispersing the drug into the polymer matrix. At steady state, the dissolution process as diffusion layer controlled is described by Noyes-Whitney equation:

$Dc/Dt = K_D . A (C_S-C) = D_A (C_S-C)/ \Delta h$

Where, A= Surface area, D= Diffusion co-efficient, Δh=Diffusion layer thickness and (Cs-C) = Concentration difference.

The surface area is most effective parameter for the dissolution process of a dosage form. In addition, to explain dissolution of drug from a dosage form with different shape and size has been proposed by Hoffenberg in 1976. The equation is following

$M_t/M_\infty = 1-[1-K_o t/C_o a]^n$

Where, Mt & M∞ are the amount of drug released at time t and infinite time respectively. ‘n’ is equal to 1 for slab, 2 for cylinder and 3 for a sphere particle.

The dissolution control products can be classified as encapsulation dissolution controlled product and matrix dissolution controlled product.

1.10 Encapsulation dissolution control

The encapsulation of dosage form is done by using slow dissolving polymeric materials. The dissolution rate of drug depends on the thickness and solubility of coating materials.

1.11 Matrix dissolution control

Matrix dissolution control product can be achieved by homogeneous mixing of drug with slow

dissolving or eroding polymer materials. The rate of drug release is regulated by rate of penetration of dissolution media into the polymer matrix. Again, rate if permeation of dissolution fluid is governed by the porosity of the polymer matrix, presence of hydrophobic or hydrophilic excipients and swelling characteristic of dosage form.

1.12 Diffusion controlled release system

Diffusion controlled release of drug depends on the diffusion of drug through the inert polymeric membrane. The process of diffusion can be defined by Fick's Law. Fick's law states that the amount of drug passing through a unit area of dosage form is proportional to the concentration difference across the plane. The equation is given as following:

J= - D dc/dx

Where, J=Flux of drug across the membrane in the direction of decrease in concentration. D= Diffusion constant. And dc/dx = change in concentration(c) with distance(x).

In case of steady state condition, it can be expected as following J= -DKΔC/L

When J can be expresses as the amount of drug release then the rate of drug release is given by dM/dt=ADKΔC/L

Where, A=surface area of dosage form D= diffusion coefficient.

K=partition of drug between polymeric membrane and the drug core. L= Difference in path length.

ΔC = Concentration of gradient across the membrane.

The equation follows the zero order drug release kinetic from a true controlled release system, if all the parameters in the right side of the equation remain constant. With varying one or more variables in above equation, oral sustained/controlled release product can't give the zero order drug release kinetic.

1.13 Matrix device

In matrix devices, the drugs are homogeneously distributed throughout the polymer matrix. Drug present in outer most layer get contact to dissolution medium first and diffuses from the matrix. This process is carried on until the drug become available at the interior of drug –polymer matrix. The system will be diffusion controlled or sustained only when the dissolution of drug is higher than the diffusion of dissolved drug from the polymer matrix. It can be explained by the Higuchi equation as following:

$Q= [D€/T (2A-€C_s) Cst]^{1/2}$

Where, Q=Amount of drug release per unit area.

D= Diffusion Co-efficient of drug in the release medium.

€= Porosity of matrix.

T= the tortuosity of matrix.

Cs= Concentration of drug in polymer matrix (g/ml). Shortly the equation can be represented as following $Q=Kt^{1/2}$

Where K=Constant.

Higuchi drug release model is regulated by various factors like concentration of drug in polymer matrix, porosity of matrix and solubility of drug.

1.14 Diffusion and dissolution control system

In this system, the drug molecules are embedded in a partially soluble polymer matrix. A part of matrix is dissolved and allowed for diffusion of dissolved drug in the polymer coat. The release of drug can be expressed by following equation.

$Q=AD (C_1-C_2)/L$

Where Q=Rate of drug release, A= Surface area, D= Diffusion co-efficient of drug, L=Diffusion path length, C_1= Concentration of drug in core. C_2= Concentration of drug in dissolution media.

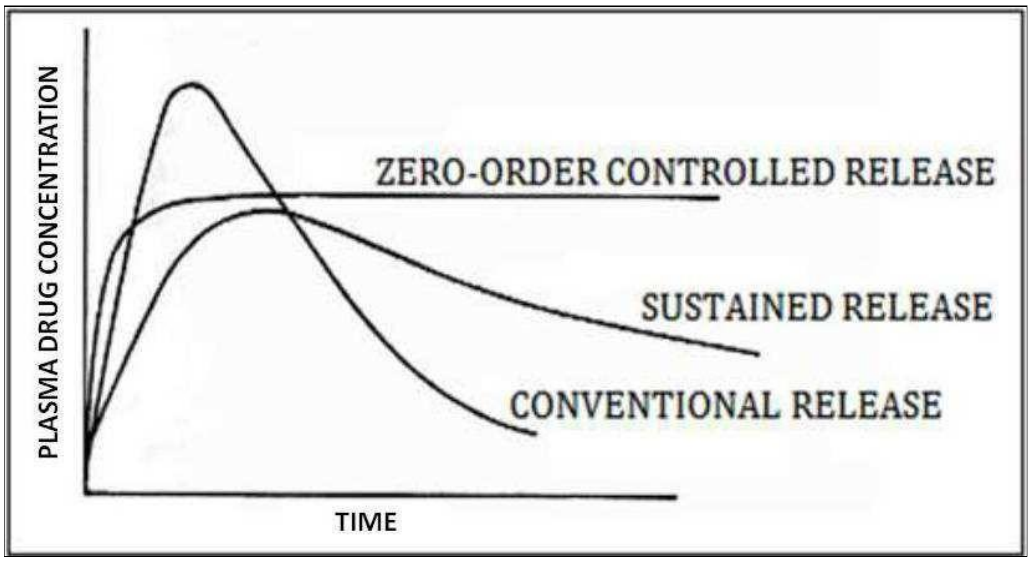


Figure 4: Plasma drug concentration profile for conventional release, a sustained

release and zero order Controlled release formulation

1.15 Mathematical evaluation:

Table 1.1: List of the generally used mathematical models.

Sl. No.	Kinetic Model	Mechanism of Drug Release
1.	Zero Order (Dredan et al., 1961)	Homogeneous dissolution, the release is independent of the amount of drug.
2.	First Order	Coated dosage forms or membrane-controlled dosage form
3.	Higuchi square root time (Karasulu et al., 2003)	Diffusion controlled model, drug is dispersed in a uniform polymer matrix system (Wu *et al., 2003).*
4.	Korsemeyer-Peppas (Lánský and Weiss, 2003)	The diffusion is the main drug release mechanism; n value is used in order to characterize different release mechanisms.
5.	Hixson-Crowell cube root	Water soluble drugs are in porous matrix (Costa and Sousa, 2001), release rate is limited by the drug dissolution rate and not by the diffusion through the polymer matrix.
6.	Baker-Lonsdale (Liu *et al*., 2003)	Drug is dissolved uniformly in the matrix (R. Baker, 1987) ($W_1/O/W_2$ emulsion technique). (Celebi *et al*., 1996)
7.	Hopffenberg (Lánský and Weiss, 2003)	Surface eroding devices with several geometries.

Table 1.2: Interpretation of diffusion release mechanism from polymeric films:

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45 □ n□ 0.89	Non- Fickian Transport	t^{n-1}

0.89	Case II transport	Zero order release
> 0.89	Super case II transport	t^{n-1}

1.16 FUNDAMETAL COSIDERATION

There are some considerations for the preparation of drug loaded polymeric microspheres. These are likely the nature of core and coating materials, their stability, flow property, swelling and drug release characristics and finally the preparation method of microspheres. The physicochemical characteristics and the use of finally prepared microspheres are also being considered [54].

1.17 Core and Coating polymeric materials

Polymers have been accepted as novel versatile compounds in day-to-day life for several years [50]. The combination of polymer science and pharmaceutical science has led to the design and development of novel drug delivery systems (NDDS) [50]. The intimate contact between a drug delivery system and the epithelial cell layer increases the residence time as well as the efficacy of NDDS [50]. As a result, bioadhesive drug delivery and polymeric hydrogel drug delivery systems have been developed to regulate the delivery of bioactive agents. The choice and design of a polymer is a prime criterion with regard to biocompatibility, physicochemical properties and compatibility with drugs and additives used during the preparation of drug delivery systems. Biochemical and preclinical tests are performed to prove its safety.

The physical properties such as permeability and degradability determine the water absorption capacity of the polymer, which influences the hydrolytic degradation and swelling of the polymer. The surface properties of the polymer such as hydrophilicity, smoothness and surface energy regulate the biocompatibility with tissues and blood [50]. Bulk properties like molecular weight and solubility play a significant role in sustained or controlled drug delivery systems [50]. The structural properties of the matrix such as surface morphology and pore size are important factors for controlling the mass transfer of water and drug into and out of the polymer matrix.

Natural polymers are generally biodegradable, biocompatible and non-toxic in nature. A variety of biodegradable polymers have been used to deliver drugs, macromolecules, proteins, cells and enzymes. The acceptability of these polymers can be increased by the incorporation of labile groups such as ester, orthoester, anhydride and carbonate in their backbone [48]. Langer's

group has made a significant contribution in investigating various polymers used for drug delivery and the release of macromolecules from polymers [62].

In the present work, Hydroxypropyl Methylcellulose (HPMC) is used as a core polymer for the preparation of cilnidipine loaded microspheres. HPMC is a well-established, biocompatible, non-toxic and safe polymer widely used in pharmaceutical formulations as a matrix forming and release-retarding material. Due to its excellent swelling property, gel-forming ability and stability, HPMC plays an important role in controlling the drug release from sustained release dosage forms. It is widely used in oral controlled release formulations because of its hydrophilic nature, good film forming ability and compatibility with a variety of drugs and excipients.

Nowadays, Eudragit polymers are widely used in novel drug delivery systems such as microspheres, micro- and nanoparticles, as well as implants. In microsphere preparation, the combination of Eudragit® RS100 and Eudragit® RL100 is commonly used as a coating material for sustained drug delivery systems [51]. These polymers are suitable for matrix structures and provide customized drug release profiles when used in different ratios. The copolymer Eudragit® RS100 is lipophilic, whereas Eudragit® RL100 is relatively more hydrophilic in nature, but the dissolution properties of both polymers are pH independent [65]. In the present study, these polymers are selected due to their biocompatibility, good stability, easy fabrication and insolubility in gastric fluid, which makes them suitable for sustained drug delivery applications [56].

1.18 Solvent Evaporation Technique

To prepare drug loaded microspheres solvent evaporation technique is widely used for the encapsulation of wide Varity of drug molecules. This technique was first developed at late 1970s. A certain amount of drug and polymer are dispersed or dissolved in a suitable vehicle to produce a solution. Polymeric coating material is dissolved in a highly volatile solvent which is immiscible with the external aqueous phase. Primary emulsion is prepared by instilling drop wise the drug- polymer solution in the polymer solution. The primary emulsion is incorporated into the slightly acidic aqueous solution to prepare a $W_1/O/W_2$ emulsion. After continuous stirring the solvent is evaporated and microspheres are formed. The microspheres are collected and separated by filtration and then dried at room temperature.

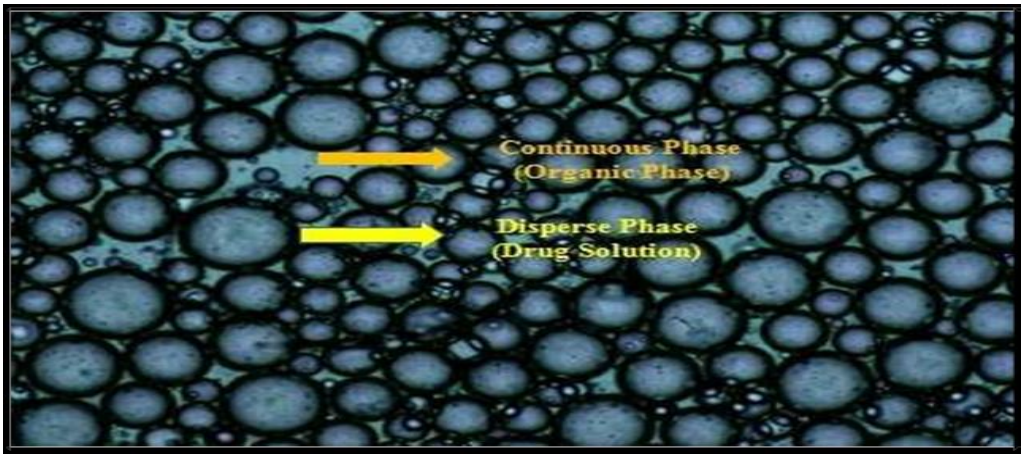


Figure 5: W/O Primary Emulsion system

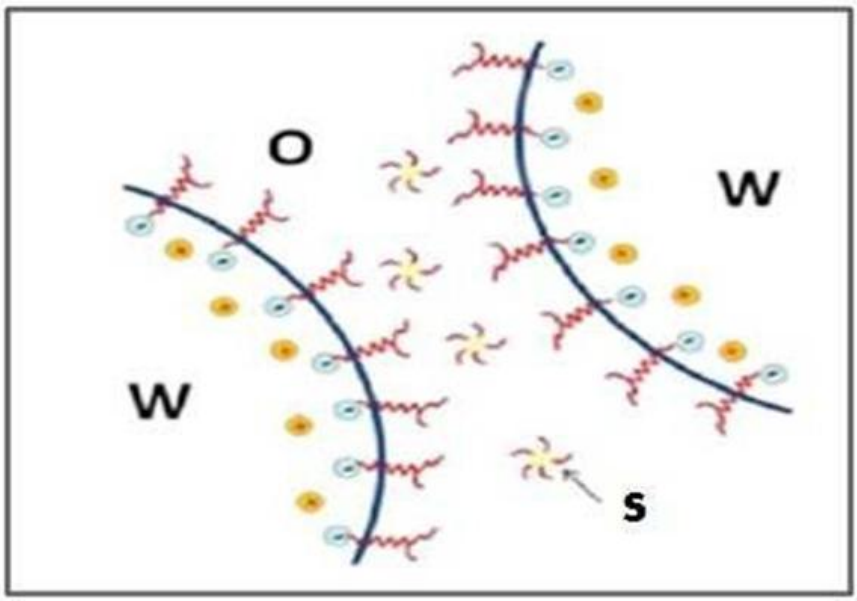


Figure 6: Schematic illustration of interfacial films by which aqueous droplets are stabilized Where W= Aqueous Phase, O= Organic phase and S= Surfactant

A large number of processing variables are involved in the preparation of microspheres by double emulsion solvent evaporation method. There are various factors involved in the manufacturing process of microspheres like the solubility of drug materials, amount of polymer in the inner vehicle, type and concentration of coating materials and stirring speed of mechanical stirrer [51]. Stirring speed is a very important factor to control the size of microspheres although a big number of other factors may also be related with the formulation of microspheres [51]. The multi-emulsion solvent evaporation technique is a simple and practical method to prepare microsphere using different polymeric coating materials [51]. The solvent evaporation technique is better in comparison to spray drying; sonication and homogenization as it require minimum number of equipment and condition like constant

stirring or room temperature [51]. Double emulsion solvent evaporation technique is a unique process of preparation of various types of microspheres using different kind of synthetic as well as biodegradable polymers [51]. Methelene chloride, chloroform and ethyl acetate or combinations of them are used as solvent in the preparation of microspheres although in a limited amount of such as 500 ppm or 50ppm respectively as per USP (XXIII) 1995. The quick evaporation from the bulk phase helps to prepare spherical, uniform microspheres by this technique. It also helps to increase drug entrapment efficiency. The residual solvents can change the nature/character of the polymer resulting change in drug release profile. So, the entrapped solvent should be expelled out from the dosage from [58] and should be followed by the monograph in Pharmacopeia and ICH guideline. The some effective measures for the detection of residual solvent discused are Gas chromatography and other analytical techniques [42]. The solvent selection is a prime criterion for this process which induces the various properties like high volatility, low boiling point, and

poor solubility in the external continuous phase and also the low toxicity of the solvents.

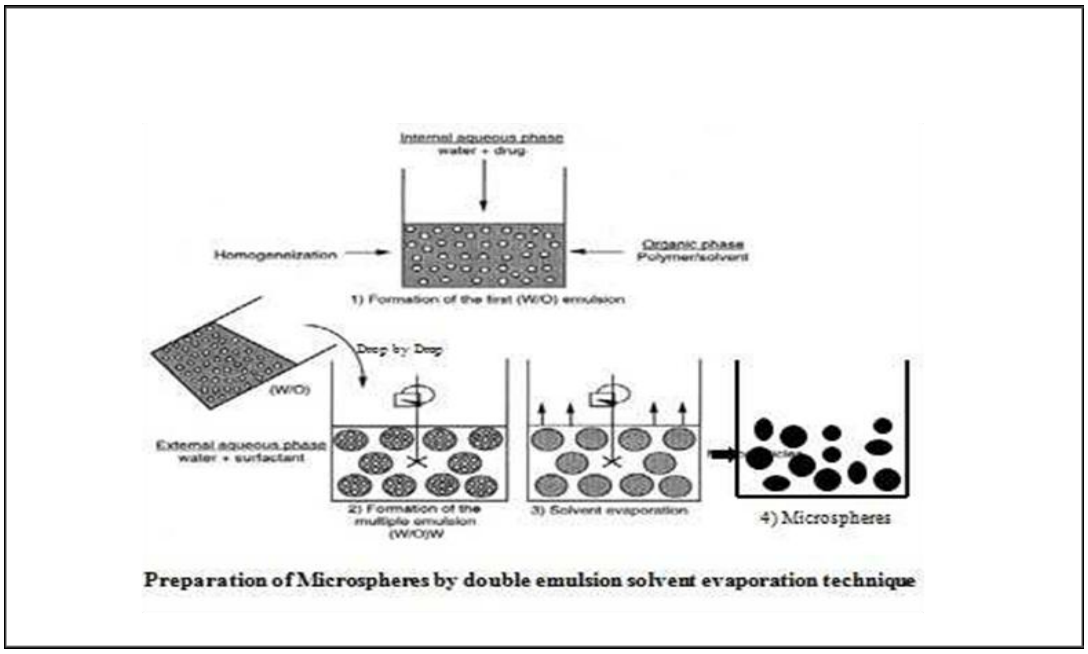


Figure 7: Microspheres preparation by double emulsion solvent evaporation Technique.

In the preparation of the microspheres, the selection of the appropriate organic solvent is one of the most important leading factors. The ideal organic solvent has low toxicity and diffuses quickly into the external aqueous phase, thereby ensuring uniform solidification of the polymer. Rapid solidification of the globules is a key step, because this avoids coalescence of the droplets and drug outflow from them [51]. Dichloromethane (DCM) is a good solvent for

the Eudragit polymers. Its water solubility is 1.961% (vol/vol), and it has a low boiling point (39.8ºC) [42]. It has been shown that aggregated microspheres can very easily form when this solvent is used [51].

There are various methods to deliver a therapeutic agent to the target site in a proper manner by the sustained drug delivery approach. Polymeric microsphere may be a useful carrier for drugs and medicines. Microsphere can be introduced as an effective tool of sustained drug delivery system and they can be designed with proper release profiles with a suitable target oriented drug delivery system. Biodegradable and non-biodegradable polymers can be used to prepare various types of microspheres depending on their final application.

1.19 Factors governing the design of the formulation of oral sustained release Drug delivery system

The design of a sustained drug delivery system is subjected to different variables. The variables are the route of drug delivery, type of the drug delivery system, type of the diseases being treated, the condition of the patients, and duration of the drug therapy and the different properties of the drug. The properties of the drugs have the greatest effect on the behavior of the drug delivery from the dosages form in the body.

1.20 The biological factors influencing the oral sustained release dosages form are following: Absorption

Absorption behavior of a drug can affect its ideality as a sustained release product. The rate, extent and uniformity of absorption of a drug are important factors where the formulations are considered as a controlled sustained release system. The rate of drug release from a sustained release product is its rate limiting step because it is the slowest process. It is the most essential that the rate of drug release is much slower that the rate of drug absorption (K_r □□□ K_a) [40]. If we consider the transit time of a dosages form through the absorption area of gastrointestinal tract is between 9 and 12 hours, the maximum absorption half-life should be approximately 3-4 hours. The minimum absorption rate constant (K_a of 0.17 to 0.23 hr^{-1}) is required for the amount of 80-90% absorption over a 9 to 12 hours transit time.

1.21 Biological Half Life

The drugs with short half-life are good candidate for the sustained release formulation. Large amount of drugs with very short half-life will be required in each dosages unit to maintain the sustained properties of the dosages form. Therapeutic compound with relatively long hafe life

more than 8hours are not suitable for the sustained release form. The limited residence time of any dosages form in the gastrointestinal tract is 8 to 12 hours [1].

1.22 Metabolism

Drugs that are significantly metabolized in small intestine can show minimum bioavailability for the sustained release dosages form as it is saturated by enzyme system.

1.23 The physicochemical factors influencing the oral sustained release dosages form are following:

1.23.1 Aqueous Solubility

Solubility is the thermodynamic property of a compound. It can be defined as the amount of compound that present in the solution in a known volume of solvent having the non-dissolved materials. The fraction of drug is absorbed into the portal blood circulation. It is a function of amount of drug come into solution of gastrointestinal tract (G.I.T) that is known as the intrinsic permeability of the drug. Most of drugs are weak acids or weak bases. Drugs with very low water solubility and slow dissolution rate to incorporate into sustained drug delivery system. For such drug delivery system, the diffusion of drug through the polymer matrix is the rate limiting state in drug release. The driving force for diffusion is the concentration of drug in the polymer matrix or solution. This concentration is low for the drug with low water solubility. For a drug with very high water solubility and rapid dissolution rate is often difficult to decrease its dissolution rate and slow its absorption. The aqueous solubility of weak acids or weak bases depends on the pK_a of the substances and the pH of the medium. The pH partition hypothesis states that the absorption of weakly acidic drugs (unionized form) will be high in stomach (pH= 1-2). But the absorption of weakly basic drugs (ionized form) will be poor. The pH dependent solubility in the physiological range would be another problem for the oral sustained drug delivery system because of variation in pH throughout the gastrointestinal tract (G.I.T) and variation also in dissolution rate of drugs [3].The Biopharmaceutical Classification System (BCS) allows the estimation of concentration of three major governing factors likely solubility, dissolution and the gastrointestinal permeability which affects the absorption of drug from the drug delivery system. According to BCS, the classification of drugs is following as

Class- I: High solubility –High permeability Class-II: High solubility –Low permeability

Class-III: Low solubility – High permeability Class-IV: Low solubility – Low permeability

1.23.2 Partition Coefficient

Partition coefficient is defined as the fraction of drug in an oil phase to that of an adjacent aqueous phase. Mathematically it can be expressed as $K= C_o/C_w$

Where, Co= Equilibrium concentration of all form of the drug in an organic phase at equilibrium, C_w = Equilibrium concentration of all forms in an aqueous phase. Higher value of K indicates that the drugs are more lipids soluble and will partition into the biological membrane. Its effect not only the permeation of drugs across the biological membrane but also the diffusion across the rate controlling membrane or matrix between the time when the drug is taken and when it is removed from the body. The drug must diffuse through a variety of biological membranes that acts as biphasic barrier. The relationship between permeation and partition coefficient of a drug can be explained by the Hansch correlation. This correlation describes a parabolic relationship between the logarithm of the activity of a drug or its ability to be absorbed and the logarithm of its partition coefficient [52]. So the drugs that have lower partition coefficient are not suitable for oral sustained drug delivery system. Because the will remain localized in the first aqueous phase it contacts. And the drugs with larger value results in poor aqueous solubility but high lipid solubility are not suitable for such drug delivery system because they will not partition out of the lipid membrane once it gets in the membrane [52].

1.23.3 Molecular Size and Diffusivity

The drug molecules must passes through a variety of biological membrane during its time course in the body. In extended release drug delivery system, drug must diffuse though a rate controlling polymeric matrix or membrane. Diffusivity of drug depends on the shape and size of the cavity of the membrane. The ability of a drug to diffuse in the polymeric matrix is a function of its diffusivity (Diffusion coefficient, D). It is also a function of its molecular size (Molecular weight, M). For the most of polymers, Log D correlates empirically to some function of molecular size as mentioned following:

$$\mathbf{logD = - S_v log V + k_v = -S_M log M + k_m}$$

Where, V = Molecular Volume, S_v, S_M, k_v, k_m = Constants

The value of D, related to shape and size of the cavities of the polymer as well as the shape and size of drug molecule. Generally, The diffusion coefficient of intermediate molecular weight for drug is 150-400 Daltons through flexible polymers range from 10^{-6} to 10^{-9} cm^2/sec. But the

value of flexible polymer is 10^{-8} that is mostly common [3]. For the drug having molecular weight greater than 500 Da, their diffusion coeffienct in many polymers are very less (less than 10^{-12} cm^2/sec). So, drugs having high molecular weight should be expected to show very slow release kinetics in extended release devices using diffusion through polymeric matrix of membrane as a release mechanism [1].

Drug Stability

The important factors are acid/base hydrolysis, enzymatic degradation and metabolism in the

G.I.T when the drug is administered through the oral route. The relative bioavailability of a drug can be significantly improved by releasing the drugs in a specific area of G.I.T when they are stable. The drugs that are unstable in the stomach, the best suitable dosage form would be one that releases its content only in the intestine. Similarly, the drugs unstable in the environment of intestine, the most appropriate controlling unit would be one that releases its content only in the stomach. So the stability problem of drugs in the specific area of G.I.T is less suitable for sustained release formulations those deliver the contents uniformly over the whole G.I.T.

1.23.4 Drug pKa and Ionozation Constant

The pKa is a measure of the strength of an acidic or a basic. The pKa indicates the charge distribution on a drug molecule at any given pH. Drugs molecules are active in unionized state and able to cross the lipoidal biological membrane rapidly than the ionized state. The amount of drug remains unionized form is a function of dissociation constant of a drug and pH of the biological fluid at the absorption site. For the absorption of the drug molecules, it should be unionized form at the absorption site. Absorption of the unionized drugs is good but permeation of ionized drugs is negligible because the absorption rate of ionized drugs is 3-4 times less than unionized drugs. The pKa range for acidic drugs whose ionization is pH sensitive is around 3.0-7.5 and for basic drugs is around 7.0-11.0. These are the ideal condition for the optimum positive absorption [3 7] . So, the drugs those are in ionized form at the absorption site are the poor candidate for the oral sustained release drug delivery system [37].

1.24 The parameters influencing the physicochemical properties of microspheres

Stirring speed: the micro-fine and homogeneous emulsion is prepared by homogenizer at higher speed (50000rpm) for 5-7 minutes but good stable w/o type emulsion is formed by

applying stirring speed of magnetic stirrer (1000rpm) for 8-10 minutes and the prepared microspheres are larger in size. The drug entrapment efficiency of microspheres is increased with increase in stirring speed of mechanical stirrer during the secondary emulsification process. It is stated that there is no significant relationship between the drug entrapment and stirring speed of the mechanical stirrer [51].

1.25 Concentration gradient between internal and external phase

The concentration gradient between W1 phase and W2 phase in the preparation of multi-emulsion system is a governing factor for the drug entrapment in microspheres formulation. If the concentration of W2 phase is higher in comparison to W1 phase, the water of W1 phase can migrate through the polymer solution phase into the W2 phase. As a result, the prepared microspheres are small in particle size and drug entrapment will be decreased [51]. In addition of buffer to the inner phase enhance the migration of water from the external phase to internal phase due to the concentration gradient. The salt addition to the external phase promotes the formation of a dense and homogeneous polymer matrix. The presence of salt in external aqueous phase reduces the water solubility of organic solvent as a result precipitation soluble polymer on the surface of the microspheres [51].

1.26 Volume and pH of internal phase

The volume and pH of internal phase of $W_1/O/W_2$ emulsion affect significantly on the viscosity of internal phase, solidification and particle size of fabricated microspheres. The viscous and concentrated solution is difficult to broken into the smaller particles, resulting formation of larger particles and porous matrix of the particles [55]. The increased in volume of external phase and decreased in pH lead to increase in both particle size and drug entrapment efficiency of microspheres. It may be cause of reduced mixing or dispersing efficacy during the preparation of double emulsion. Generally there is a limitation for the increasing the fraction as W1-Φ1 and W2-Φ2. The existing fraction value is within the range of $0.60 \leq \Phi 1 \leq 0.75$ for the inner phase and $0.60 \leq \Phi 2 \leq 0.80$ for the external phase. Otherwise, the emulsion (W/O) will be highly viscous to be disperse or may be negative in result.

1.27 The type of evaporating solvent

The selection of suitable organic solvent or solvent mixture is important processing parameters for the preparation of drug loaded microspheres. The formation of microspheres wall is regulated by the rate of extraction into the external phase and rate of evaporation of the organic solvent from the external phase. The rate solvent extraction depends on the water

solubility and rate of evaporation is limited by its boiling point.

1.28 Temperature

Another process variable, temperature has a significant role on the formation of microspheres with high drug entrapment efficacy and desired drug release profile. If the preparation of microspheres is performed at low temperature than room temperature, then the extraction and evaporation of organic solvent will be slow [55]. When the formation of microspheres are exposed to higher temperature($T \square T_g$), the rubbery state of polymer will be changed which is more fluent and flexible, resulting the polymer can move through the matrix and fill up the pores. It also forms a coating on the existing drug crystal that is popularly known as in situ-micro-coating [56].

1.29 DRUG CANDIDATE

Hypertension is a chronic cardiovascular disorder characterized by persistent elevation of arterial blood pressure and is considered one of the major risk factors for cardiovascular morbidity and mortality worldwide. It is associated with serious complications such as coronary artery disease, stroke, heart failure, and renal dysfunction if not adequately controlled. The prevalence of hypertension has increased significantly due to changes in lifestyle, dietary habits, stress, and aging population, making it a major public health concern. Effective management of hypertension requires long-term pharmacological intervention aimed at maintaining blood pressure within normal physiological limits. Continuous control of blood pressure is essential to prevent disease progression and reduce the risk of cardiovascular complications, thereby necessitating dosage forms capable of providing prolonged therapeutic action and improved patient compliance [5,4].

1.30 Need for Sustained Release Drug Delivery System in Hypertension

Hypertension requires long-term and continuous pharmacological management to maintain blood pressure within the normal physiological range and to reduce the risk of cardiovascular complications. Conventional immediate release dosage forms often produce fluctuations in plasma drug concentration, resulting in periods of sub-therapeutic effect or excessive drug levels that may increase the incidence of adverse effects. Such variations may compromise therapeutic efficacy and reduce patient compliance, particularly in chronic conditions requiring lifelong treatment. Sustained release drug delivery systems are designed to release the drug at a controlled rate over an extended period, thereby maintaining relatively constant plasma drug concentrations and minimizing peak–trough fluctuations. This controlled drug release

improves therapeutic effectiveness, reduces dosing frequency, enhances patient adherence to therapy, and provides better overall management of hypertension [1,2,3].

Past Studies on HPMC as a Drug Release Retarding Material

Hydroxypropyl methylcellulose (HPMC) is widely used as a hydrophilic polymer in pharmaceutical formulations because of its excellent gel-forming ability, swelling characteristics and ability to control drug release. Several studies have reported the successful application of HPMC as a drug release retarding material in sustained and controlled release dosage forms.

Singh I et al. (2010) formulated matrix tablets of tramadol using HPMC as a release modifier. The prepared matrix tablets were evaluated for physicochemical parameters such as hardness, friability, tensile strength, drug content and drug release characteristics. In-vitro drug release studies were performed and the release data were fitted to different mathematical kinetic models to understand the drug release mechanism. The results showed that HPMC formed a strong gel layer upon hydration, which effectively controlled the drug release. The release mechanism was mainly governed by diffusion and polymer relaxation. The Korsmeyer–Peppas model showed an ‘n’ value indicating anomalous (non-Fickian) diffusion, suggesting that both diffusion and polymer swelling contributed to the drug release process. The study concluded that HPMC is a suitable polymer for sustained drug delivery systems due to its excellent swelling and gel-forming properties [48].

Bharariraja B et al. (2011) developed a controlled drug delivery system for ibuprofen using HPMC as a matrix-forming polymer. Matrix tablets containing different concentrations of HPMC were prepared by the wet granulation method. The prepared tablets were evaluated for hardness, drug content, swelling index and in-vitro drug release studies. Dissolution studies

were carried out in simulated gastric fluid and intestinal fluid to understand the release behavior of the drug. The results indicated that the drug release profile depended on the concentration of HPMC present in the formulation. Higher polymer concentrations produced stronger gel barriers, resulting in prolonged drug release. The release kinetics were best fitted to the Korsmeyer–Peppas model and followed anomalous transport mechanisms. The authors concluded that HPMC-based matrix tablets are effective for achieving controlled and sustained drug delivery [48].

AIM AND OBJECTIVES

AIM: To develop microsphere based sustain release formulation of anti-hypertensive drug

Objectives:

- To reduce dosing frequency.
- To minimize plasma drug level fluctuations.
- To enhance therapeutic efficacy
- To reduce dose related side effect
- To improve bioavailability
- To develop stable and reproducible formulation
- To evaluate the prepare microsphere
- To study drug release kinetics

DRUG PROFILE

Cilnidipine

Cilnidipine is a newer generation dihydropyridine calcium channel blocker widely used in the management of hypertension. It produces its antihypertensive effect by blocking both L-type and N-type calcium channels, leading to relaxation of vascular smooth muscle and reduction in peripheral vascular resistance, thereby lowering arterial blood pressure [87]. Inhibition of N-type calcium channels also reduces sympathetic nerve activity, which contributes to improved blood pressure control and decreased cardiovascular workload [88]. Cilnidipine is

clinically effective in the treatment of mild to moderate hypertension and is associated with improved tolerability compared to conventional calcium channel blockers [89].

Cilnidipine possesses poor aqueous solubility and exhibits a relatively short biological half-life, which may result in fluctuations in plasma drug concentration when administered through conventional dosage forms [90]. These variations can affect therapeutic response and patient compliance during long-term therapy. Development of a sustained release formulation of cilnidipine is therefore considered beneficial for maintaining uniform plasma drug levels over an extended period. Incorporation of the drug into polymeric microspheres may provide controlled drug release, reduce dosing frequency, and improve overall therapeutic efficacy in the management of hypertension [91].

Physicochemical Properties of Cilnidipine

Name: Cilnidipine

Molecular Formula: $C_{27}H_{28}N_2O_7$

Molecular Weight: 492.52 g/mol

Chemical Class: Dihydropyridine calcium channel blocker

Description: Cilnidipine is a yellow to pale yellow crystalline powder belonging to the dihydropyridine group of calcium channel antagonists [92].

Melting Point: Approximately 109–112°C

pKa: Approximately 2.7

Solubility: Practically insoluble in water and freely soluble in organic solvents such as methanol, ethanol, and chloroform. The poor aqueous solubility of cilnidipine contributes to its dissolution-limited absorption and supports its suitability for sustained release formulation [92].

Partition Coefficient (Log P): High lipophilicity, which favors incorporation into polymeric matrices for controlled drug release [90].

Protein Binding: Highly protein bound (>95%) [90].

Biological Half-Life: Approximately 2–5 hours [90].

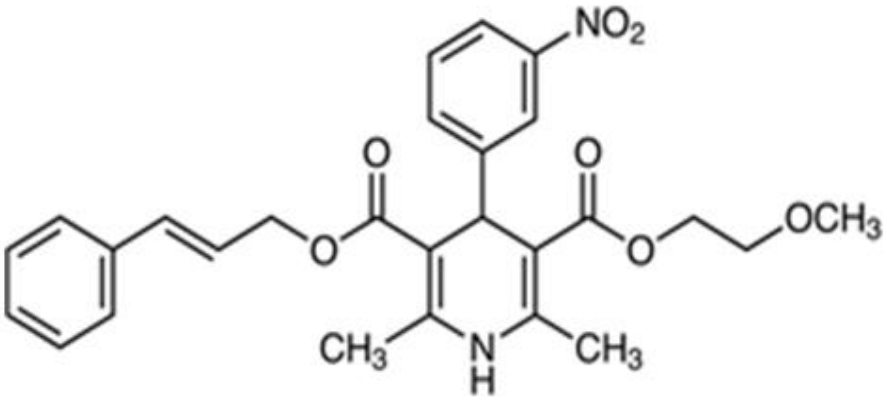


Figure 8: Structure pf Cilnidipine

The physicochemical characteristics of cilnidipine, particularly its poor aqueous solubility, lipophilic nature, and relatively short biological half-life, make it a suitable candidate for formulation into sustained release microspheres in order to achieve prolonged therapeutic action and improved patient compliance.

Pharmacokinetics of Cilnidipine

Absorption:

Cilnidipine is absorbed from the gastrointestinal tract following oral administration. Being a highly lipophilic dihydropyridine derivative, its absorption is influenced primarily by its dissolution rate and gastrointestinal transit time [90]. Due to its poor aqueous solubility, cilnidipine exhibits dissolution-limited absorption, which may result in variability in oral bioavailability under conventional immediate-release conditions [92].

The drug is absorbed mainly through passive diffusion across the intestinal epithelium owing to its high lipophilicity and favorable membrane permeability characteristics (Takano et al., 2006). Peak plasma concentrations (Tmax) are generally achieved within 1–3 hours following oral administration. However, rapid absorption may contribute to fluctuations in plasma drug levels, particularly when administered in conventional dosage forms. This pharmacokinetic characteristic supports the rationale for developing sustained release formulations to achieve controlled and prolonged absorption [92].

Distribution:

Cilnidipine is widely distributed throughout body tissues after systemic absorption. It exhibits a high degree of plasma protein binding, exceeding 95%, predominantly binding to serum

albumin [90]. High protein binding contributes to prolonged retention in systemic circulation and influences its pharmacodynamic effect.

The lipophilic nature of cilnidipine facilitates effective penetration into vascular smooth muscle cells, where it blocks L-type and N-type calcium channels to exert its antihypertensive action [87]. Its distribution characteristics also reduce rapid clearance from the circulation, thereby contributing to sustained pharmacological activity despite its relatively short elimination half-life.

Metabolism:

Cilnidipine undergoes extensive hepatic metabolism following absorption. The drug is primarily metabolized through oxidative biotransformation mediated by cytochrome P450 enzymes, particularly CYP3A4 [91]. These metabolic processes convert cilnidipine into inactive metabolites, which lack significant pharmacological activity.

First-pass metabolism in the liver contributes to moderate systemic bioavailability. Variations in hepatic enzyme activity may influence plasma concentration levels, which further emphasizes the importance of controlled release formulations to maintain steady drug exposure during chronic therapy.

Excretion:

Cilnidipine and its metabolites are eliminated predominantly via the biliary route and excreted in feces. A smaller fraction of metabolites is excreted through the renal pathway in urine [90]. The elimination half-life of cilnidipine ranges approximately between 2–5 hours, depending on patient variability and metabolic status.

The relatively short half-life and rapid clearance profile justify the need for sustained release drug delivery systems to maintain therapeutic plasma concentrations for extended durations. By providing gradual drug release, sustained formulations may reduce dosing frequency and minimize peak–trough fluctuations associated with conventional dosage forms.

Surfactant:

The surfactants are mostly used in the preparation of emulsion. It decreases the surface tension of the continuous phase and prevents agglomeration of drop lets in continuous phase and finally stabilized the emulsion. The selection of suitable surfactant is creditable factor which regulate the stability of emulsion and size and desired drug release characteristics from the microspheres. Non- ionic surfactant has no charge. The increases in concentration of the

surfactant decrease the size of microspheres [55]. The amount of surfactant controls the pore formation of microspheres. It is also indicated that the drug release profile and initial burst drug release is depended upon the concentration of emulsifier [103].

Name: Polysorbate 80

Synonyms: Tween 80

IUPAC name: Polyoxyethylene (20) sorbitan monooleate.

Molecular weight: 1310 g/mol Chemical formula: $C_{64}H_{,24}O_{26}$ CAS number:9005-65-6

Physical properties

State: Oily liquid

Odor: Fatty

Appearance: Amber colored viscous liquid

Density: 1.06-1.09 g/mL

Boiling point: >100°C

Melting point: -20.556°C

Specific gravity: 1.06-1.10

Solubility: Easily soluble in cold water and hot water. Soluble in methanol.

Soluble in: Toluene, alcohol, cottonseed oil, corn oil, ethyl acetate. Insoluble in mineral oil.

Flash point: Closed cup: >148.89°C (300°F).

Viscosity: 300-500 centistokes (25°C) Critical micelle

Concentration: 0.012 mM in pure water.

Hazards identification

Potential health Effects: Slightly hazardous in case of skin contact(irritant), of eye contact (irritant), of ingestion, of inhalation.

Carcinogenic Effects: Not available.

Mutagenic Effects: Not available.

Teratogenic Effects: Not available.

Storage: Under dry condition, at max. 8°C, sealed under inert gas.

Storage: Stored at -20°C further improves the shelf life.

Name:

Structure of Sorbitan monooleate

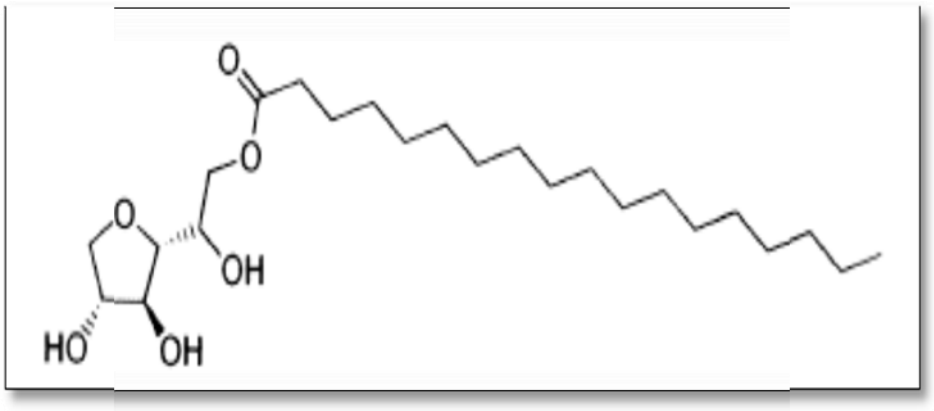


Figure 9: Structure of Sorbitan monooleate

Synonym: Sorbitan oleate; Sorbitan (Z)-mono-9-octadecenoate IUPAC name: 1,4-arthy dro-6-O- [(9Z)-octadec-9-enoy 1] -D-glucitol Physical Appearance: clear yellow viscous liquid

Storage: room temperature

Store a Molecular Weight: 428.60

Molecular Formula: $C_{24}H_{44}O_6$ CAS Number: 1338-43-8

Form: Liquid

Density: 0.99 g/mL at 20 °C

Description: Partial oleate ester of sorbitol and its mono- and Dianhydrides

Boiling Point: > 100°C Solubility in water: Very soluble

Viscosity: 300-500 centistokes at 25°C.

Eudragit RS100 and Eudragit RL100

Eudragit polymers are copolymers of acrylic and methacrylic acid or their esters. Their specific properties are determined by different functional groups R1 to R3 and different ratios of acrylic to methacrylic acid on the one hand, and free acids to esters on the other hand [103].

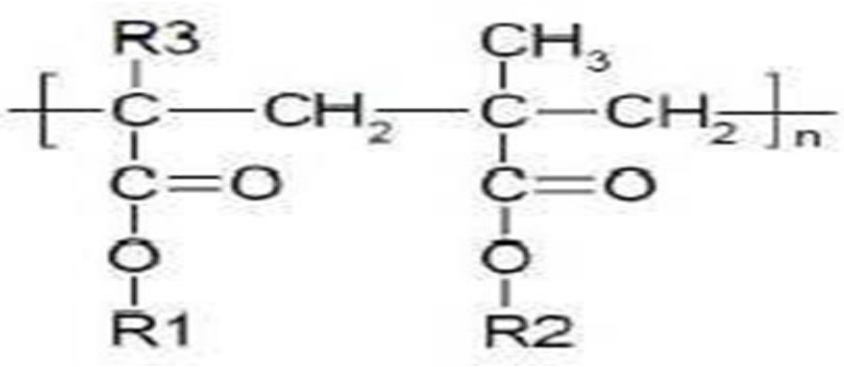


Figure10 : Basic structure of Eudragit polymers.

Eudragit RL 100 and Eudragit RS 100 are copolymers of ethyl acrylate, methyl methacrylate and a low content of a methacrylic acid ester with quaternary ammonium groups (trimethyl ammonia ethyl methacrylate chloride). The ammonium groups are present as salts and make the polymers permeable. The average molecular weight is approximately 150,000 Polymethacrylates Eudragit RL and RS are chemically known as (poly [ethylpropenoat- co methyl- 2-methylpropenoat- co- 2-(trimethyl ammonium) ethylpropenoat] - chloride). Eudragit RL has basic chemical structure similar to Eudragit RS and only differs in the relative number of quaternary ammonium groups. While Eudragit RS contains about 5 % w/w trimethylammonio- ethyl methacrylate chloride groups; Eudragit RL contains approximately 10 % w/w [103].

Appearance	Colorless to light yellow liquids of low viscosity, clear to slightly cloudy. The odor is characteristic of the solvents.
Solubility	Both are miscible with methanol, ethanol and isopropyl alcohol (containing approx. 3 % water), as well as with acetone, ethylacetate and methylene chloride in a ratio of 1:1. The polymer is precipitated from Eudragit RL and Eudragit RS when mixed with petroleum ether in a ratio of 1:1.
Viscosity at 20°C	15 mPas

MATERIALS AND METHODS

6.1 Materials

Cilnidipine and HPMC was obtained as a gift sample from Fleming Laboratories Limited Medak district, Andhra Pradesh, India. Eudragit RS100 and Eudragit RL100 were purchased

from the Yarro Chem Products Mumbai (India), Dichloromethane, Tween 80 and Span 20 (Merck, India), and all other reagents were of analytical grade obtained commercially and used as received.

Table 1: List of Materials/Drugs

Sl. No.	Material Name	Category	Purpose in Study
1	Cilnidipine	Active Pharmaceutical Ingredient	Antihypertensive drug
2	HPMC	Polymer	Sustain Release polymer
3	Sodium Alginate	Natural Polymer	Matrix former
4	Chitosan	Natural Polymer	Polymer matrix
5	Compritol	Lipid Polymer	Core material
6	Magnesium Stearate	Lubricant / Stabilizer	Droplet stabilizer
7	Span 20 / Span 80	Surfactant	Emulsifier
8	Calcium Chloride	Crosslinking Agent	Ionotropic gelation
9	Ceric Ammonium Nitrate (CAN)	Initiator	Grafting agent
10	Epichlorohydrin	Crosslinking Agent	Polymer crosslinking
11	Sodium Diclofenac	Model Drug (Literature)	Encapsulation study reference

Table 2: List of Chemicals

Sl. No.	Chemical / Solvent	Role in Formulation
1	Dichloromethane (DCM)	Organic solvent

2	Chloroform	Organic solvent
3	Acetone	Organic solvent
4	Ethyl Acetate	Organic solvent
5	Liquid Paraffin	External oil phase
6	Methanol	Analytical solvent
7	Ethanol	Analytical solvent
8	Hydrochloric Acid	pH adjustment
9	Phosphate Buffer (pH 7.4)	Dissolution medium
10	Simulated Gastric Fluid (pH 1.2)	In-vitro release medium
11	Simulated Intestinal Fluid	In-vitro release medium
12	Distilled Water	Aqueous phase

Table 3: List of Instruments and Equipment

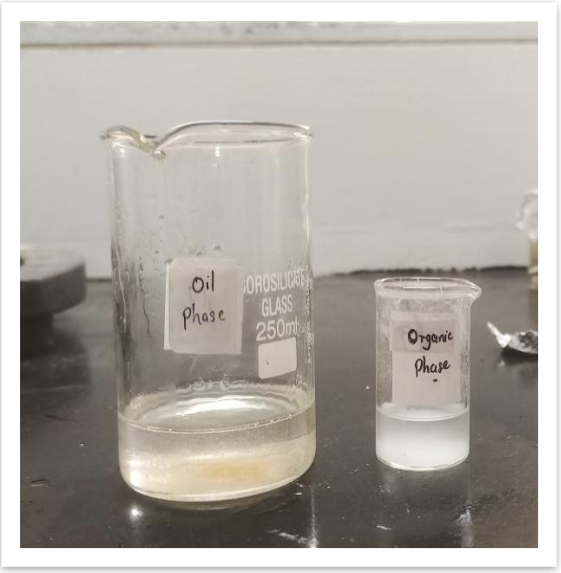
Sl. No.	Instrument	Purpose
1	UV-Visible Spectrophotometer	Drug estimation
2	Magnetic Stirrer	Emulsion preparation
3	Mechanical Stirrer	Secondary emulsification
4	Homogenizer	Primary emulsion formation
5	Optical Microscope	Particle size analysis
6	Scanning Electron Microscope (SEM)	Surface morphology

7	Fourier Transform Infrared Spectroscopy (FTIR)	Drug-polymer compatibility
8	X-Ray Diffraction (XRD)	Crystallinity study
9	Dissolution Test Apparatus (USP Type I/II)	In-vitro release study
10	pH Meter	pH measurement
11	Hot Air Oven	Drying
12	Analytical Balance	Accurate weighing

6.2 Methods

6.2.6 Method of preparation –Non solvent evaporation method

Preparation of Cilnidipine Microspheres with Hydroxypropyl Methyl Cellulose (HPMC k750pH) Polymer HPMC (HPMC k750pH) was used as the polymer and the method employed was non-aqueous-solvent evaporation method. Drug and the polymer were mixed in acetone. The slurry was slowly introduced into 50 ml of liquid paraffin containing 1% span 80 as emulsifying agent while being stirred at 600 rpm by a mechanical stirrer equipped with a three bladed propeller at room temperature. The solution was stirred for 2hr to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed thrice repeatedly with petroleum ether until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in a desiccator [106].

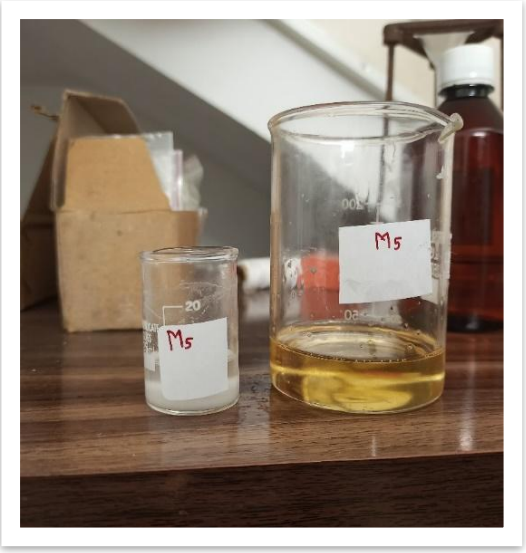


M1

M2

M3

M4



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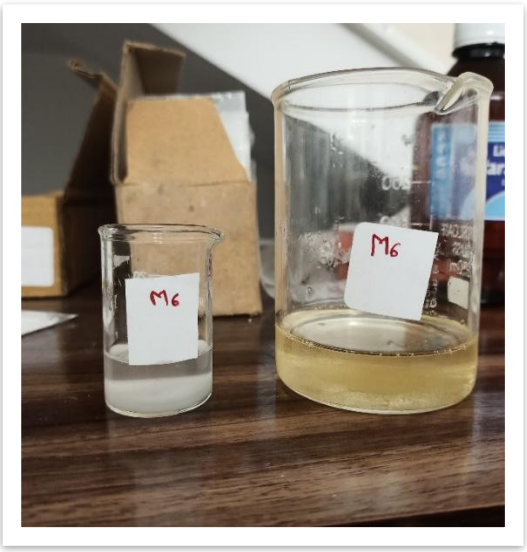

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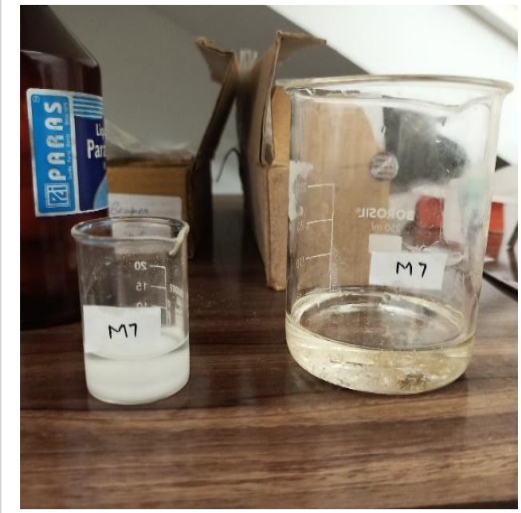

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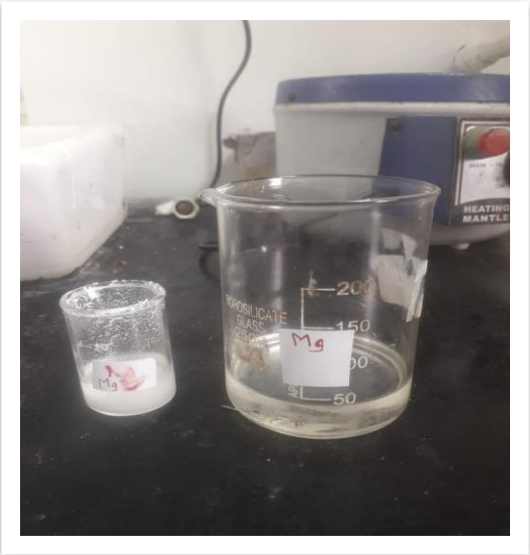
M8



M9

M10

M11

M12

M15

Figure 11: Method of preparation –Non solvent evaporation method

Formulation and optimization using central composite design (CCD) [8]

In the current study, a Response Surface Methodology (RSM) known as Central Composite Design (CCD) was employed using Design Expert® software (Version 13.0). The CCD was applied with three independent variables: the amount of HPMC k750pH (A), Span 80(%) (B), and Mechanical stirring time (hrs.) (C).The dependent variables under investigation in this study are Entrapment efficiency (%) and Drug content (%). This CCD design incorporated factorial points, a center point, and axial points, resulting in a total of 15 runs. The details of the independent variables, their coded levels, and the scheme matrix of the CCD can be found in the provided table.

Table 4: Levels of independent variables with their transformed values in the preparation of microsphere.

Independent Variables	Unit	Low Actual	High Actual	Low Coded	High Coded
HPMC k750pH (A)	mg	250	800	-1.000	1.000
Span 80 (B)	%	1	2	-1.000	1.000
Mechanical stirring time (C)	Hrs.	2	4	-1.000	1.000

Table 5: Model fitting with Entrapment efficiency and drug content (%) of microsphere.

Response	Name	Units	Obs.	Analysis	Minimum	Maximum	Mean	Std. Dev.	C.V. %	Model
Y1	**Entrapment efficiency**	%	15	Polynomial	28.8	94.84	59.77	13.72	22.95	2F1
Y2	**Drug content**	%	15	Polynomial	44.68	96.48	77.49	11.28	14.55	Linear

Table 6: Doe suggested and experimental batches

Formulation code	Cilnidipine (mg)	HPMC k 750pH(mg)	Span 80 (%)	Acetone (ml)	Liquid paraffin (ml)	Mechanical stirring time
M1	250	250	1	10	50	2
M2	250	525	1.5	10	50	3
M3	250	525	0.6591	10	50	3
M4	250	250	1	10	50	4
M5	250	525	2.3409	10	50	3
M6	250	250	2	10	50	2
M7	250	800	1	10	50	4
M8	250	250	2	10	50	4
M9	250	525	1.5	10	50	1.31821
M10	250	62.507	1.5	10	50	3
M11	250	987.493	1.5	10	50	3
M12	250	800	2	10	50	2
M13	250	800	1	10	50	2
M14	250	800	2	10	50	4
M15	250	525	1.5	10	50	4.68179

6. Preformulation studies-

Preformulation study is standardization of drug and excipients before going to formulation development. The drug and excipient should be free from harmful impurities. All should be compatible with each other. Preformulation study described as phase of research and development process where the physical properties of the drug and excipients used are characterized to developstable and effective dosage forms. [11, 37,38]

6.1 Determination of melting point

The melting point of Cilnidipine drug was determined using a Thiele's tube apparatus containing liquid paraffin. A capillary tube sealed at one end and filled with a small amount of the drug was utilized. The capillary tube was secured to the thermometer with a thread and suspended in the tube. Heat was applied using a burner, and the temperature at which the Cilnidipine melted was recorded [9,10].

6.2 Determination of λ max

The maximum absorption wavelength (λ max) of Cilnidipine in Distilled water was determined by scanning a solution of Cilnidipine at a concentration of 10 g/ml in the range of 200-400 nm using a UV-visible spectrophotometer. The wavelength corresponding to the peak of the spectrum was recorded [10].

6.3 UV analytical method development

1. Standard Calibration Curve of Cilnidipine in Methanol-

Calibration curve study was performed by using UV visible spectroscopy. Standard stock

solutionwas prepared by taking 50 mg drug Cilnidipine in 50ml methanol. The concentration of solution is 1000µg/ml. From prepared standard stock solution, 10 ml was pipette out and was diluted up to 100 mlwith methanol. From the 100 µg/ml. The resulting solutions were made to obtain concentration range of 2-12 µg/ml.

The resultant solutions were then scanned in a UV spectrophotometer from 400 to 200 nm. From the resulting spectra λ max for Cilnidipine were calculated. The absorbance vs. concentration graph was constructed [10,39].

2. Standard Calibration Curve of Cilnidipine in Distilled water-

Calibration curve study was performed by using UV visible spectroscopy. Standard stock solution was prepared by taking 50 mg drug Cilnidipine in 50ml distilled water. The concentration of solution is 1000µg/ml. From prepared standard stock solution, 10 ml was pipette out and was diluted up to 100 mlwith distilled water. From the 100 µg/ml. The resulting solutions were made to obtain concentration range of 0.5-3 µg/ml.

The resultant solutions were then scanned in a UV spectrophotometer from 400 to 200 nm. From the resulting spectra λ max for Cilnidipine were calculated. The absorbance vs. concentration graph was constructed [10,39].

3. Standard Calibration Curve of Cilnidipine in ethanol

Calibration curve study was performed by using UV visible spectroscopy. Standard stock solutionwas prepared by taking 50 mg drug Cilnidipine in 50ml ethanol. The concentration of solution is 1000µg/ml. From prepared standard stock solution, 10 ml was pipette out and was diluted up to 100 ml with ethanol. From the 100 µg/ml. The resulting solutions were made to obtain concentration range of 2-12 µg/ml.

The resultant solutions were then scanned in a UV spectrophotometer from 400 to 200 nm. From the resulting spectra λ max for Cilnidipine were calculated. The absorbance vs. concentration graph was constructed [10,39].

4. Standard Calibration Curve of Cilnidipine in phosphate buffer pH 6.8.

Calibration curve study was performed by using UV visible spectroscopy. Standard stock solutionwas prepared by taking 50 mg drug Cilnidipine in 50ml phosphate buffer ph 6.8. The concentration of solution is 1000µg/ml. From prepared standard stock solution, 10 ml was pipette out and was diluted up to 100 ml with phosphate buffer ph 6.8. From the 100 µg/ml. The resulting solutions were made to obtain concentration range of 2-10 µg/ml.

The resultant solutions were then scanned in a UV spectrophotometer from 400 to 200 nm. From the resulting spectra λ max for Cilnidipine were calculated. The absorbance vs. concentration graph was constructed [10,39].

5. Standard Calibration Curve of Cilnidipine in phosphate buffer pH 7.4

Calibration curve study was performed by using UV visible spectroscopy. Standard stock solutionwas prepared by taking 50 mg drug Cilnidipine in 50ml phosphate buffer ph 7.4. The concentration of solution is 1000µg/ml. From prepared standard stock solution, 10 ml was pipette out and was diluted up to 100 ml with phosphate buffer ph 7.4. From the 100 µg/ml. The resulting solutions were made to obtain concentration range of 1 -6 µg/ml.

The resultant solutions were then scanned in a UV spectrophotometer from 400 to 200 nm. From the resulting spectra λ max for Cilnidipine were calculated. The absorbance vs. concentration graph was constructed. (Indian pharmacopeia volume III)

3. Solubility estimation

Cilnidipine is practically insoluble in water but freely soluble in methanol and ethanol. Ethanol were taken in five different conical flask and added in it. Conical flasks were stirred for 24 hours on mechanical rotary shaker.

The suspensions were filtered through whatmann filter paper No 1 and dissolved amount of Cilnidipine were drug quantitated each buffer by using UV visible spectroscopy at 283nm [12].

4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of Cilnidipine confirmed the presence of characteristic functional groups corresponding to its chemical structure. A prominent absorption peak observed in the range of 1700–1750 cm^{-1} corresponds to the ester carbonyl (C=O) stretching vibration, indicating the presence of ester functional groups in the molecule. The aromatic C=C stretching vibrations were observed in the region of 1450–1600 cm^{-1}, confirming the presence of an aromatic ring system in the structure. Additionally, characteristic absorption bands in the range of 1500–1600 cm^{-1} are attributed to the nitro ($–NO_2$) group stretching vibrations, further supporting the structural integrity of Cilnidipine.

The absence of any significant shift or disappearance of these characteristic peaks in the physical mixture and optimized formulation indicates no chemical interaction between Cilnidipine and HPMC K750PH polymer, confirming the compatibility of the drug with the selected excipients [39].

5. XRD

The data acquired from XRD (Bruker D8 Advance Series 1) was employed to assess whether the newly formed compounds are crystalline or amorphous. The measurement conditions included target metals Cu, filter K, an applied voltage of 40 kV, and a current of 30 mA. Nilotinib was scanned over a two-degree range of 10–90 °C with a 0.2 °C phase scale

[105].

EVALUATION AND CHRACTERIZATION MICROSPHERE FORMULATION-

1. EVALUATION

1. Drug content (%)

A formulation comprising 10 microspheres was prepared by dispersing them in 10 ml of methanol, followed by sonication for 15 minutes. Absorbance measurements were then conducted using a UV-visible spectrophotometer (Jasco V-630) [54].

2. Entrapment efficiency (EE) determination

10 mg microsphere powder in 5 ml methanol and entrapment efficiency of Cilnidipine within Microsphere formulation was estimated using ultracentrifugation technique. In brief, Cilnidipine Microsphere were subjected to rigorous centrifugation at 17,000 rpm for 30 min at 4C to separate the un-entrapped drug. The drug concentration in the supernatant was quantified at 283 nm using a UV–Vis spectrophotometer (Jasco V-630) [54,56].

$$\textbf{Entrapment efficiency}(\textbf{EE}\%) = \frac{\textbf{Entrapped drug}}{\textbf{Total drug}} \times \textbf{100}$$

3. Drug Loading (%) [11]

10 mg of microspheres were dissolved in 10 mL of methanol and agitated at 1000 rpm for 30 min. The Cilnidipine content in the sample solution was measured using a UV spectrophotometer (Jasco V-630) at an absorbance wavelength of 283 nm [108].

$$\textbf{Drug Loading}(\%\textbf{DL}) = \frac{\textbf{Weight of terbinafine in microparticles}}{\textbf{Weight of dry microsphere}} \times \textbf{100}$$

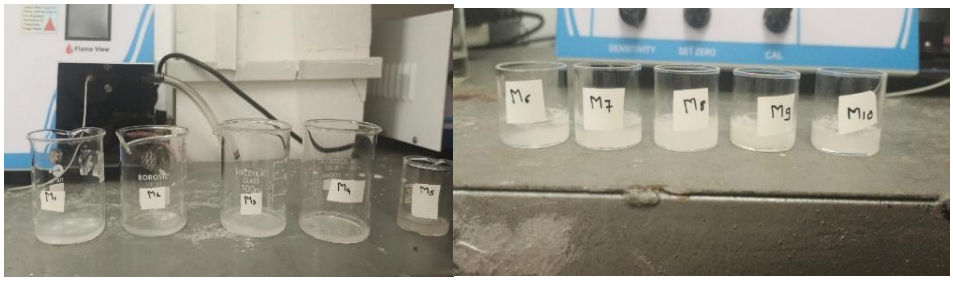
4. Percent yield (%) [12]

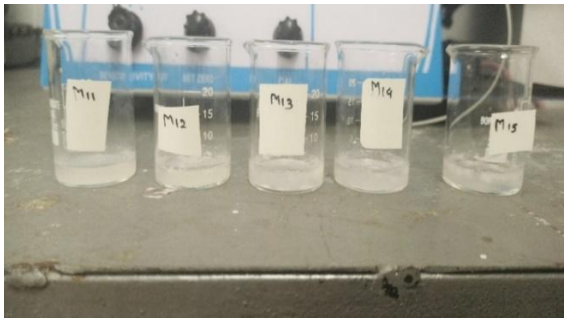
Percentage yield of the prepared microsphere was calculated using the following equation [108].

$$\% \textbf{ Yield} = \frac{\textbf{Weight of dried microsphere}}{\textbf{Amount of drug} + \textbf{Amount of polymer}} \times \textbf{100}$$

5 .Equilibrium swelling degree

The swelling degree of microspheres (Sw) was determined by introducing 100 mg Cilnidipine loaded HPMC microspheres in a glass beaker. Then 4 ml PBS pH 7.4 beakers was added and kept aside for 24 hrs. Then microspheres were filtered and weighed. The following equation was used for computing the swelling degree [108].





2. CHRACTERIZATION

1 .Particle size and zeta potential

A 3-5mg sample of microsphere formulation was taken and mixed with distilled water, then subjected to sonication for 30 minutes. The analysis was conducted at a temperature of 25°C. The same procedure was repeated for measuring the zeta potential. The prepared formulations were characterized for zeta potential to assess the stability of the formulations [56].

2. Fourier Transform infrared (FTIR) spectroscopy (FTIR)

FTIR spectroscopy is a technique that measures the absorption of infrared light by molecules, providing information about the functional groups and chemical bonds present in a sample. The principle behind FTIR spectroscopy is based on the fact that different types of chemical

bonds absorb infrared radiation at specific frequencies, resulting in characteristic peaks in the FTIR spectrum. FTIR spectra of Cilnidipine, physical mixture (Cilnidipine: HPMC k750ph) and optimized formulations (M13) were recorded using infrared spectrophotometer (Shimadazu 1800)**.** Infrared spectroscopy was carried out using FTIR Spectrophotometer (Jasco 1800) over the range of between 500to 4000 cm-1 [39].

3. In vitro dissolution study

According to USP, a dissolution study was carried out for formulated batches M1–F13.Cilnidipine microsphere powder from various batches were added to a jar containing 900 ml phosphate buffer pH 7.4 for the dissolving test. The device ran for 12 hours at 37±0.5°C at 50 rpm. At intervals of 1, 2, 3, 4, 5, 6, 7,8,9,10,11 and 12 hours, samples were removed, replaced with new medium, and UV spectroscopy (Jasco V-630) was used to determine absorbance [8,3]. This technique assesses the release profile and dissolving behavior of the

Discussion

The present study was conducted to develop and evaluate Cilnidipine-loaded HPMC microspheres as a sustained-release drug delivery system. The primary objective was to overcome the limitations of conventional oral dosage forms, including frequent dosing, fluctuating plasma drug concentrations, and poor patient compliance. HPMC was selected as the polymer due to its excellent biocompatibility, swelling properties, and ability to provide controlled drug release.

The prepared microspheres were subjected to various evaluation and characterization studies to assess their physicochemical properties and sustained-release potential. Drug content analysis confirmed the successful incorporation and uniform distribution of Cilnidipine within the microsphere matrix. Uniform drug distribution is essential for dose accuracy, reproducibility, and consistent therapeutic performance. The UV spectrophotometric method employed provided reliable quantification of the drug content.

Entrapment efficiency studies demonstrated the ability of HPMC to effectively encapsulate Cilnidipine within the polymer matrix. High entrapment efficiency indicates minimal drug loss during formulation and reflects the suitability of the selected preparation method. Efficient

drug encapsulation contributes significantly to prolonged drug release and improved therapeutic efficacy.

Drug loading studies further supported the successful incorporation of the drug into the microspheres. Adequate drug loading ensures that a sufficient amount of active pharmaceutical ingredient is available for sustained release while maintaining the structural integrity of the microspheres. The formulation variables used in this study were found to be effective in producing microspheres with satisfactory drug-carrying capacity.

The percentage yield of the prepared microspheres was found to be satisfactory, indicating efficient recovery of the final product and minimal material loss during preparation. High production yield is desirable for industrial-scale manufacturing as it enhances process economy and reproducibility.

The equilibrium swelling study revealed that the HPMC microspheres absorbed phosphate buffer and exhibited controlled swelling behavior. The hydrophilic nature of HPMC promotes water uptake, leading to the formation of a gel layer around the microspheres. This gel barrier plays a critical role in controlling drug diffusion and achieving prolonged drug release. Therefore, swelling behavior directly influences the sustained-release characteristics of the formulation.

Particle size analysis demonstrated the formation of discrete and uniformly sized microspheres suitable for oral administration. Appropriate particle size distribution is important because it influences drug release kinetics, surface area, and formulation stability. Zeta potential measurements indicated adequate surface charge, suggesting good physical stability and reduced tendency for particle aggregation during storage.

FTIR spectroscopy studies confirmed the compatibility between Cilnidipine and HPMC K750PH. The characteristic peaks of the drug were retained in the optimized formulation without significant shifts or disappearance, indicating the absence of chemical interactions and confirming the stability of the drug within the polymer matrix.

The in vitro dissolution study demonstrated controlled and prolonged release of Cilnidipine over a period of 12 hours in phosphate buffer pH 7.4. The sustained release profile confirmed the effectiveness of HPMC in regulating drug diffusion and maintaining therapeutic drug levels

for an extended period. Overall, the optimized formulation (M13) exhibited desirable physicochemical characteristics and sustained-release behavior, indicating its potential as an effective oral drug delivery system for improving the therapeutic efficacy and patient compliance of Cilnidipine therapy.

CONCLUSION

The present study successfully demonstrated the potential of HPMC polymer for the development of sustained release microspheres of cilnidipine. Comprehensive pre-formulation studies confirmed the suitability of cilnidipine for microencapsulation, establishing its physicochemical stability and compatibility with selected polymers. The formulation of microspheres using the $W_1/O/W_2$ double emulsion solvent evaporation technique resulted in spherical, stable, and reproducible microstructures with satisfactory yield, entrapment efficiency, and controlled particle size distribution.

The incorporation of HPMC, in combination with Eudragit polymers, significantly contributed to sustained drug release behavior. The swelling and gel-forming characteristics of HPMC provided a hydrophilic diffusion barrier, while the permeability-modulating properties of Eudragit polymers regulated drug transport through the matrix. The synergistic interaction between natural and synthetic polymers ensured controlled and prolonged release of cilnidipine, minimizing burst release and promoting extended drug availability.

Drug release kinetic analysis suggested diffusion-controlled and anomalous transport mechanisms, confirming that both polymer swelling and matrix relaxation played important roles in regulating drug release. The sustained release profile achieved in the present study highlights the effectiveness of combining hydrophilic natural polymers with hydrophobic synthetic polymers to achieve multi-mechanistic control over drug delivery.

From a therapeutic perspective, the developed sustained release microsphere system may offer significant clinical advantages in hypertension management by reducing peak–trough plasma fluctuations, decreasing dosing frequency, and potentially improving patient compliance. The findings support the feasibility of utilizing **hydroxypropyl methylcellulose (HPMC)** as a promising polymer for controlled drug delivery applications due to its excellent swelling, gel-forming ability and compatibility with pharmaceutical formulations.

Overall, the developed cilnidipine-loaded microsphere formulation demonstrates strong potential as a sustained release oral drug delivery system, providing a safe, stable, and effective strategy for long-term antihypertensive therapy.

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