"DEVELOPMENT OF COLON TARGETED PULSATILE DRUG DELIVERY SYSTEM FOR THE CHRONOTHERAPY OF HYPERTENSION"

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IN

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BIOPHARMACEUTICS

BY

NISARG B. MISTRY, B. PHARM.

UNDER THE GUIDANCE OF

DR. TEJAL A. MEHTA – GUIDE

MR. MAYUR M. PATEL - CO-GUIDE

DEPARTMENT OF PHARMACEUTICS &
PHARMACEUTICAL TECHNOLOGY
INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
SARKHEJ-GANDHINAGAR HIGHWAY
AHMEDABAD-382481
GUJARAT, INDIA
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CERTIFICATE

This is to certify that ***Mr.Nisarg B. Mistry*** *has prepared his thesis entitled "Development of colon targeted pulsatile drug delivery system for the chronotharapy of hypertension", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutics & Pharmaceutical Technology, Institute of Pharmacy, Nirma University.*

Guide

Dr. Tejal A. Mehta
M. Pharm., Ph.D.,
Professor & Head,
Department of Pharmaceutics
& Pharmaceutical Technology,
Institute of Pharmacy,
Nirma University

Co-Guide:

Mr. Mayur M. Patel
M.Pharm.,
Assistant Professor,
Department of Pharmaceutics
& Pharmaceutical Technology,
Institute of Pharmacy,
Nirma University

Forwarded Through:

Dr. Manjunath Ghate
I/c Director
Institute of Pharmacy,
Nirma University

Date: 27th April, 2010

DECLARATION

I declare that the thesis "Development of colon targeted pulsatile drug delivery system for the chronotharapy of hypertension" has been prepared by me under the guidance of Dr. Tejal A. Mehta, Professor, and Mr. Mayur M. Patel, Assistant Professor, Department of Pharmaceutics & Pharmaceutical Technology, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Mr.Nisarg B. Mistry (08mph107)
Department of Pharmaceutics
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 27th April, 2010

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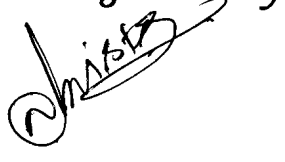
Nisarg B. Mistry



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1. AIM OF PRESENT INVESTIGATION

Chronopharmaceutics, the drug delivery based on circadian rhythm is recently gaining much attention worldwide. Circadian rhythm regulates many body functions in humans, viz., metabolism, physiology, behavior, sleep patterns, hormone production, etc. It has been reported that more shocks and heart attacks occur during morning hour[1]. Various diseases like asthma, hypertension, and arthritis show circadian variation, that demands time scheduled drug release for effective drug action[2].

Blood pressure is also reported to be high in the morning till late afternoon, and then drops off during night[3].Blood pressures are highest during the day, lowest during sleep, and rapidly increase from the low night time values to the higher daytime values during the period between 4:00 AM & 12:00 noon. It is demonstrated during the period of rapid early morning blood pressure gush that the peak incidence of non-embolic stroke and myocardial infarction occurs. Studies in which adequate blood pressure control is obtained during the early morning period have demonstrated a blunting in the early morning incidence of myocardial infarction. This data suggest that effective blood pressure control during the early morning period is valuable.

Most conventional antihypertensive agent formulations are taken in the morning and are at trough levels during the early morning blood pressure gush, providing the least effective blood pressure control at the time that it is most enviable. Antihypertensive formulation taken at night will usually peak prior to early morning blood pressure gush coinciding with the time that blood pressure is physiologically at its lowest level and may potentially coerce blood pressure too low.

That all are the push for development of chronotherapeutic drug delivery system which when dosed at evening (8:00 PM) and release drug during the period between 4:00 AM & 12:00 noon – the period of higher blood pressure and increased risk of a cardiovascular event[4]. Metoprolol, a specific β_1 – adrenoceptor blocker was used as model drug as the absorption of metoprolol is high in colonic region compare to intestinal and stomach region.

Thus the aim of present investigation is to develop pulsatile device which contain sustained release metoprolol loaded microspheres of Eudragit RS/RL 100 in ethyl cellulose coated capsule body, an erodible tablet made up of HPMC and Lactose place at mouth of capsule body and Eudragit L-100 55 coated capsule cap.

Enteric coating on cap prevents exposure of erodible tablet in the stomach and also drug release for first two hrs after dosing at evening (8:00 pm). An erodible tablet prevents release of drug in small intestine for further 3 hr. As device reach in colonic region after 5 hr it start release of metoprolol in colon and sustain for 12 hr to maintain sufficient metoprolol concentration in blood from early morning to late afternoon.

2.1 INTRODUCTION TO CHRONOTHERAPEUTICS

2.1.1 Introduction:

chronotherapeutics is the delivery of medications in the right concentration to the right targeted tissues at the right time to meet biological rhythm-determined needs, e.g., rhythms in the mechanisms of disease, symptom intensity, and/or patient tolerance, to optimize desired and minimize and avert adverse effects.

Many chronic and acute medical conditions exhibit prominent circadian patterns of symptom manifestation and severity. Among the many examples are allergic rhinitis, bronchial asthma, and peptic ulcer disease; all tend to worsen overnight. The symptoms of rheumatoid arthritis are worse in the morning, while those of osteoarthritis are worse at night. The risk of many cardiovascular events, like angina pectoris, myocardial infarction, and thrombotic and hemorrhagic stroke, is greatest in the morning. Abnormally high blood pressure, i.e., **hypertension**, displays different circadian patterns in different patient groups.

Circadian rhythm regulates many body functions in humans, viz., metabolism, physiology, behavior, sleep patterns, hormone production, etc. It has been reported that more shocks and heart attacks occur during morning hours. The level of cortisol is higher in the morning hours, and its release is reported to decline gradually during the day. Blood pressure is also reported to be high in the morning till late afternoon, and then drops off during night. Patients suffering from osteoarthritis are reported to have less pain in the morning than night, while patients suffering from rheumatoid arthritis feel more pain in the morning hours.

Pulsatile effect, i.e., the release of drug as a "pulse" after a lag time has to be designed in such a way that a complete and rapid drug release should follow the lag time.

Pulsatile drug delivery systems are gaining a lot of interest and attention these days. These systems have a peculiar mechanism of delivering the drug rapidly and completely after a "lag time," i.e., a period of "no drug release." Though most delivery systems are designed for constant drug release over a prolonged period of time, pulsatile delivery systems are characterized by a programmed drug release, as constant blood

levels of a drug may not always be desirable ***(figure 1)***. Pulsatile systems are designed in a manner that the drug is available at the site of action at the right time in the right amount. These systems are beneficial for drugs having **high first-pass effect**; drugs administered for diseases that **follow chronopharmacological behavior**; drugs having **specific absorption site** in GIT, **targeting to colon**; and cases where night time dosing is required[5].

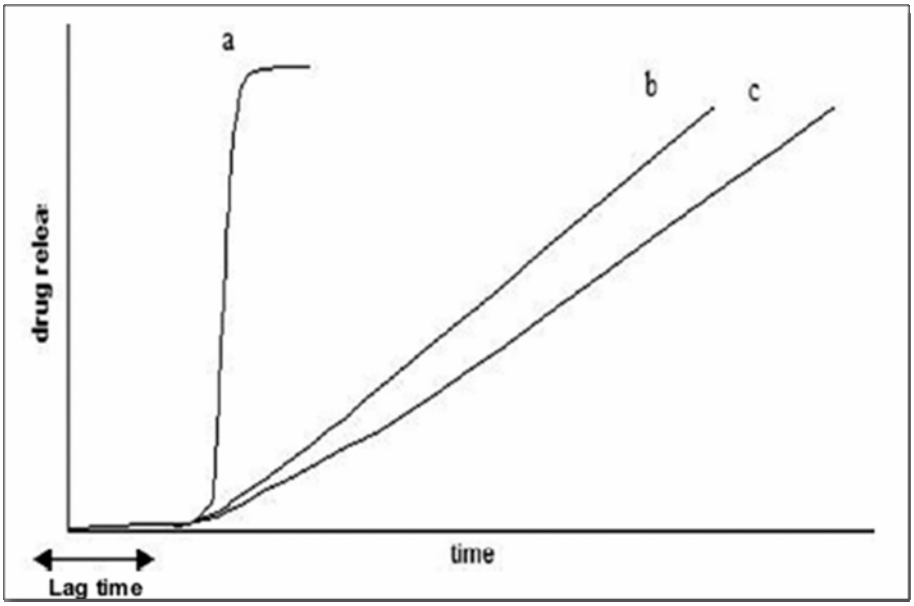


Figure 1 **: Different types of pulse release**

2.1.2 Classification of pulsatile systems:

Pulsatile systems can be classified into single- and multiple-unit systems. Single-unit systems are formulated either as capsule-based or osmosis-based systems. Single-unit systems are designed by coating the system either with eroding/soluble or rupture able coating. In multiple-unit systems, however, the pulsatile release is induced by changing membrane permeability or by coating with a rupturable membrane

Single unit pulsatile systems:

These are sub-classified as capsule-based systems, osmotic systems, delivery systems with soluble or erodible membranes, and delivery systems with rupturable coating.

a) Capsule based systems:

Single-unit systems are mostly developed in capsule form. The lag time is controlled by a plug, which gets pushed away by swelling or erosion, and the drug is released as a "Pulse" from the insoluble capsule body. Pulsincap® was developed by R. P. Scherer International Corporation, Michigan, US, and is one such system that comprises of a water-insoluble capsule enclosing the drug reservoir. A swellable hydrogel plug was used to seal the drug contents into the capsule body[6]. When this capsule came in contact with the dissolution fluid, it swelled; and after a lag time, the plug pushed itself outside the capsule and rapidly released the drug. Polymers used for designing of the hydro gel plug were various viscosity grades of hydroxyl propyl methyl cellulose, poly methyl methacrylates, poly vinyl acetate and poly ethylene oxide. The length of the plug and its point of insertion into the capsule controlled the lag time. Pulsincap® was studied in human volunteers and was reported to be well tolerated[7].

b) System based on osmosis:

The Port® system was developed by Therapeutic system research laboratory Ann Arbor, Michigan, USA, and consists of a capsule coated with a semipermeable membrane. Inside the capsule was an insoluble plug consisting of osmotically active agent and the drug formulation[8]. When this capsule came in contact with the dissolution fluid, the semipermeable membrane allowed the entry of water, which caused the pressure to develop and the insoluble plug expelled after a lag time (*figure 2*). Such a system was utilized to deliver methylphenidate used in the treatment of attention deficit hyperactivity disorder as the pulsatile port system. This system useful to school children.

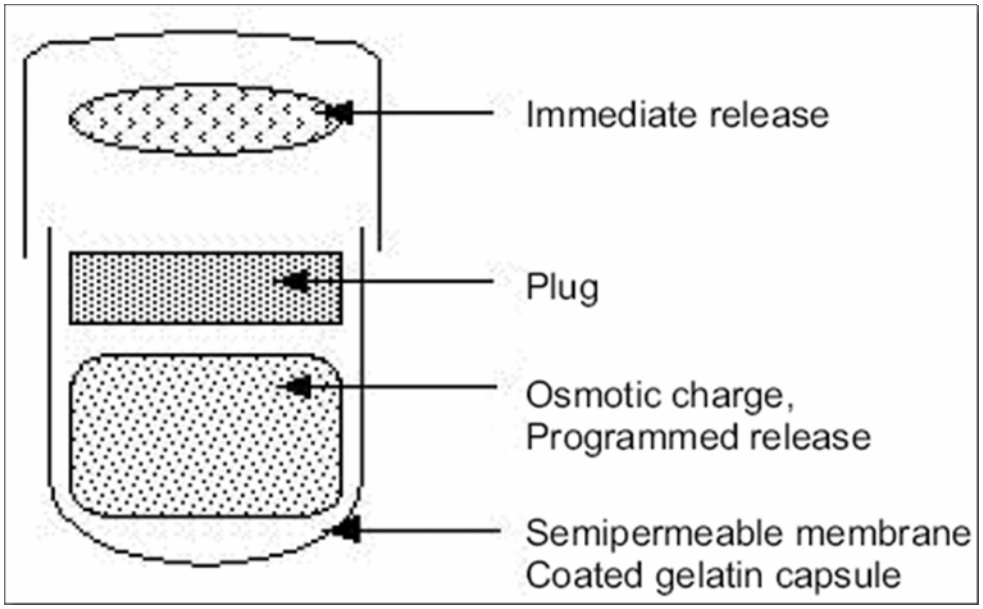


***Figure 3*: Port® system**

c) Drug delivery system with eroding or soluble barrier coating:

Chronotropic® system consists of a core containing drug reservoir coated by a hydrophilic polymer HPMC[9]. An additional enteric-coated film is given outside this layer to overcome intra-subject variability in gastric emptying rates[10]. The lag time and the onset of action are controlled by the thickness and the viscosity grade of HPMC.

The time clock system is a delivery device based on solid dosage form that is coated by an aqueous dispersion[11]. This coating is a hydrophobic-surfactant layer to which a water-soluble polymer is added to improve adhesion to the core. Once in contact with the dissolution fluid, the dispersion rehydrates and redisperses. The lag time could be controlled by varying the thickness of the film. After the lag time, i.e., the time required for rehydration, the core immediately releases the drug. This system has shown reproducible results *in vitro* and *in vivo.* The effect of low calorie and high calorie meal on the lag time was studied using gamma scintigraphy. The mean lag time of drug release was 345 and 333 min respectively.

d) Drug delivery system with rupturable layers/membranes:

These systems are based upon a reservoir system coated with a rupturable membrane. The outer membrane ruptures due to the pressure developed by effervescent agents or swelling agents. Sungthongjeen *et al.* designed a pulsatile drug delivery system where the tablets of buflomedil HCl prepared by direct compression with varying amounts of spray-dried lactose and microcrystalline cellulose were coated with an inner swelling layer using croscarmellose sodium and an outer rupturable layer using ethyl cellulose. It was observed that by increasing the amount of ethyl cellulose coating, the lag time could be prolonged. Ethyl cellulose, being water insoluble, retarded the water uptake. Similar results were obtained with croscarmellose sodium. Increasing the amount of microcrystalline cellulose decreased the lag time substantially[12].

Multiple unit pulsatile systems[13]:

The potential benefits as shown in include:

i. increased bioavailability; predictable, reproducible
ii. generally short gastric residence time;
iii. no risk of dose dumping;
iv. reduced risk of local irritation;

a) Reservoir systems with rupturable polymeric coatings:

Most multiparticulate pulsatile delivery systems are reservoir devices coated with a rupturable polymeric layer. Upon water ingress, drug is released from the core after rupturing of the surrounding polymer layer, due to pressure build-up within the system. The pressure necessary to rupture the coating can be achieved with swelling agents, gas-producing effervescent excipients or increased osmotic pressure. Water permeation and mechanical resistance of the outer membrane are major factors affecting the lag time. Water soluble drugs are mainly released by diffusion; while for water insoluble drug, the release is dependent on dissolution of drug.

b) Reservoir systems with soluble or eroding polymer coatings:

Another class of reservoir-type multiparticulate pulsatile systems is based on soluble / erodible layers in place of rupturable coatings. The barrier dissolves or erodes after a specific lag time followed by burst release of drug from the reservoir core. In general, for this kind of systems, the lag time prior to drug release can be controlled by the thickness of the coating layer. However, since from these systems release mechanism is dissolution, a higher ratio of drug solubility relative to the dosing amount is essential for rapid release of drug after the lag period.

c) Systems with changed membrane permeability:

Sigmoidal release pattern is therapeutically beneficial for timed release and colonic drug delivery, and is observed in coated systems. A sigmoidal release pattern is reported based on the permeability and water uptake of Eudragit RS or RL, influenced by the presence of different counter-ions in the release medium.

d) Low density floating multiparticulate pulsatile systems:

Conventional multiparticulate pulsatile release dosage forms mentioned above are having longer residence time in the gastrointestinal tract and due to highly variable nature of gastric emptying process, may resulted in in vivo variability and bioavailability problems. In contrary, low density floating multiparticulate pulsatile dosage forms reside in stomach only and not affected by variability of pH, local environment or gastric emptying rate. These dosage forms are also specifically advantageous for drugs either absorbed from the stomach or requiring local delivery in stomach.

2.2 INTRODUCTION TO COLON TARGETED DRUG DELIVERY SYSTEM

2.2.1 INTRODUCTION:

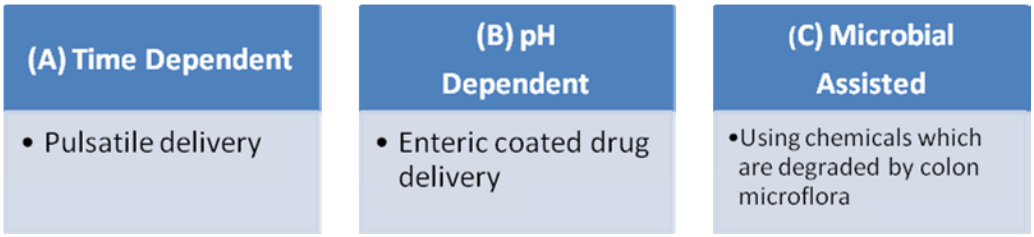
Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome but also for the potential it holds for the systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by peroral delivery, is still deemed to be the ideal site for the delivery of agents to cure the local diseases of the colon [14, 15]. The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine [16].

In order to develop a reliable colonic drug delivery system, the transit time of dosage forms through the gastrointestinal (GI) tract needs to be understood very well. The transit of perorally administered formulation through the GI tract is highly variable and depends on various factors [17, 18, 19]. Gastric transit time of single-unit non-disintegrating dosage forms has been reported to vary from 15 min to more than 3 h[20]. At the same time, the small intestinal residence time is fairly constant and varies between 3-4 h. The maximum mean colonic transit time in humans is reported to be as high as 33 h in men and 47 h in women[21].

Targeting drugs to the colon has proven quite valuable in a variety of disorders, and the colon has proven to be a potential site for local as well as systemic administration of drugs. Colon targeting has proven beneficial for local action in a variety of disease conditions, such as inflammatory bowel disease, irritable bowel syndrome, and colonic cancer. Aminosalicylates, corticosteroids, immunosuppressive agents, cationized antioxidant enzymes, genetically engineered bacteria to produce cytokines, nicotine, and other drugs have exhibited significantly enhanced efficacy when delivered to the colon[22].

2.2.2 COLON TARGETED SYSTEMS:

Basically they are classified in two categories.



2.2.2.1 Time and pH Dependent system

The pH in the terminal ileum and colon (except ascending colon) is higher than in any other region of the GI tract. Thus a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region . And in spite of the limitation of change in luminal pH due to disease state, such pH dependent systems are still very commonly investigated for colon targeting.

Enteric coating has traditionally been used to prevent drug release in the upper GI tract. Enteric coating polymers are reported to have been used as both binders and as coating materials for granules[23].

2.2.2.2 Microbial assisted system

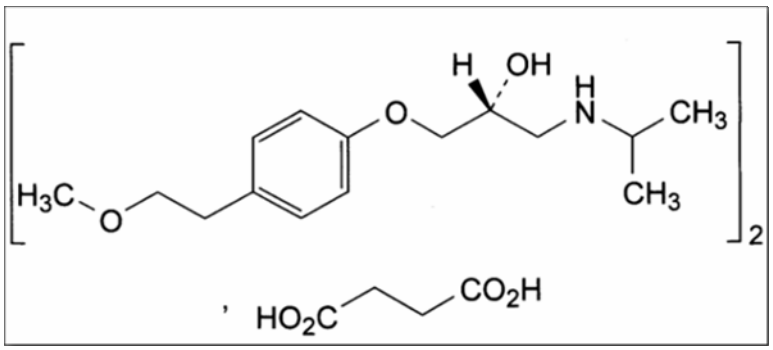
Amongst all the approaches used for colon targeting, a microbially controlled delivery system is the most appealing as it relies on the unique enzymatic ability of the colonic micro flora and enables a more specific targeting, independent of pH variations along the GI tract. Many natural polysaccharides such as chondroitin sulphate,pectin, dextran, guar gum etc. have been investigated for their potential in designing colonspecific drug delivery[24].

TECHNIQUE EMPLOYED	POLYMER(S) USED
pH dependent	Eudragit L100 and S100
	Eudragit S, Eudragit FS, Eudragit P4135 F
	Eudragit L 30 D-55 and Eudragit FS 30 D
Time dependent	Hydroxy propyl methyl cellulose
	Hydroxyethyl cellulose
	Ethyl cellulose
Bacteria dependent	Chitosan
	Pectin
	Guar gum
	Chondroitin sulphate

2.3 INTRODUCTION TO METOPROLOL SUCCINATE[25]

Metoprolol succinate, is a beta1-selective (cardio selective) adrenoceptor blocking agent, for oral administration.

Chemical structure:



Action and use: Beta-adrenoceptor antagonist.

Category: Antihypertensive agent

Chemical name:

(±)1-(isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt)

OR

Bis [(2*RS*) -1-[4- (2-methoxyethyl) phenoxy] - 3 -[(1-methylethyl) amino]propan-2-ol] butanedioate[15].

Molecular formula: $C_{34}H_{56}N_2O_{10}$

Molecular weight: 652.815840 [g/mol]

Official status: Official in I.P, B.P, USP

DOSE: Stated based on the dose equivalent to metoprolol tartrate. 23.75, 47.5, 95 and 190 mg of metoprolol succinate equivalent to 25, 50, 100 and 200 mg of metoprolol tartrate.

CHARCATERS

Appearance - White, crystalline powder

Solubility - Freely soluble in water, soluble in methanol, slightly soluble in alcohol, very slightly soluble in ethyl acetate, acetone, diethyl ether and heptanes.

Storage: Store at room temperature (77 degrees F or 25 degrees C) away from light and moisture.

Dosage :

Hypertension - 25 to 100 mg daily in a single dose

Pediatric Hypertensive - 25 mg daily in single dose

Angina Pectoris - 100 mg daily

STANDARDS[26]

Metoprolol succinate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{34}H_{56}N_2O_{10}$, calculated with reference to the dried substance.

Identification:

To 25 ml of a 0.4 per cent w/v solution add 2 ml of *5 M ammonia*, extract with 20 ml of *dichloromethane*, filter the lower layer through *anhydrous sodium sulphate* and evaporate to dryness. Place in a freezer for a few minutes to congeal the residue and allow to warm to room temperature. On the residue, determine by infrared absorption spectrophotometry. Compare the spectrum with that obtained with *metoprolol succinate RS* treated in the same manner or with the reference spectrum of metoprolol.

Melting point: 136-137 ºc

Mode of action:

Like betaxolol and atenolol, metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Pharmacology:

Metoprolol is a beta1-selective (cardioselective) adrenergic receptor blocking agent. This preferential effect is not absolute, however, and at higher plasma concentrations, metoprolol also inhibits beta2 adrenoreceptors, chiefly located in the bronchial and vascular musculature. Metoprolol has no intrinsic sympathomimetic activity, and membrane-stabilizing activity is detectable only at plasma concentrations much greater than required for beta-blockade. Animal and human experiments indicate that metoprolol slows the sinus rate and decreases AV nodal conduction.

The beta-blocking activity of metoprolol in man, as shown by (1) reduction in heart rate and cardiac output at rest and upon exercise, (2) reduction of systolic blood pressure upon exercise, (3) inhibition of isoproterenol-induced tachycardia, and (4) reduction of reflex orthostatic tachycardia.

The relative beta1-selectivity of metoprolol has been confirmed by the following: (1) In normal subjects, metoprolol is unable to reverse the beta2-mediated vasodilating effects of epinephrine. This contrasts with the effect of nonselective beta-blockers, which completely reverse the vasodilating effects of epinephrine. (2) In asthmatic patients, metoprolol reduces FEV1 and FVC significantly less than a nonselective beta-blocker, propranolol, at equivalent beta1-receptor blocking doses.

Pharmacokinetic:

Absorption – Completely absorbed from the gastrointestinal tract.

Distribution – Peak plasma concentrations vary widely and occur about 1.5 to 2 hr after a single oral dose. It crosses the BBB, the placenta and is distributed in the breast milk. It is slightly bound to plasma protein.

Metabolism – It is extremely metabolized in the liver by oxidation, O-dealkylation followed by oxidation. The rate of hydroxylation to alpha-hydroxymetoprolol is determined by genetic polymorphism. The half life of metoprolol in fast hydroxylators is stated to be 3to 4 hrs, whereas in poor hydroxylators it is about 7 hrs.

Excretion - The metabolites are excreted in the urine with only small amount of unchanged drug.

Indications:

Hypertension - Metoprolol succinate is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents.

Angina Pectoris - Metoprolol succinate is indicated in the long-term treatment of angina pectoris.

Heart Failure - Metoprolol succinate is indicated for the treatment of stable, symptomatic (NYHA Class II or III) heart failure of ischemic, hypertensive, or cardiomyopathic origin.

Adverse effect:

Central Nervous System: Reversible mental depression progressing to catatonia

Cardiovascular: Intensification of AV block

Hematologic: Agranulocytosis, nonthrombocytopenic purpura, thrombocytopenic purpura.

Hypersensitive Reactions: Fever combined with aching and sore throat, laryngospasm, and respiratory distress.

Gastrointestinal: hepatitis, vomiting

Musculoskeletal: arthralgia

Nervous System/Psychiatric: anxiety/nervousness, hallucinations, paresthesia.

Reproductive, male: impotence

Skin: increased sweating, photosensitivity, urticaria

Toxicity:

Over dosage of metoprolol succinate may lead to severe hypotension, sinus bradycardia, atrioventricular block, heart failure, cardiogenic shock, cardiac arrest, bronchospasm, impairment of consciousness/coma, nausea, vomiting, and cyanosis.

PRECAUTIONS

General

Metoprolol succinate should be used with caution in patients with impaired hepatic function. In patients with pheochromocytoma, an alpha-blocking agent should be initiated prior to the use of any beta-blocking agent.

Nursing Mothers

Metoprolol succinate is excreted in breast milk in very small quantities. An infant consuming 1 liter of breast milk daily would receive a dose of less than 1 mg of the drug. Caution should be exercised when metoprolol succinate is administered to a nursing woman.

Marketed products[27]:

Trade name	Dosage form	company
TOPROL-XL®	(metoprolol succinate) Extended-Release Tablets equivalent to 25 mg, 50 mg, 100 mg, and 200 mg of metoprolol tartrate	Astrazeneca Sweden
Seloken- XL®	(metoprolol succinate) Extended-Release Tablets equivalent to 25 mg, 50 mg, 100 mg, and 200 mg of metoprolol tartrate	Astrazeneca pharma limited Bangalore

2.3 INTRODUCTION TO DOSAGE FORM

2.3.1 Introduction to microspheres

1. INTRODUCTION:

The term microspheres are defined as a spherical particle with size varying from 50 nm to 2 mm, containing a core substance. Microspheres are, in strict sense, spherical empty particles. However, the terms microcapsules and micro spheres are often used synonymously. In addition, some related terms are used as well. For example, essentially "micro beads" and "beads" are used alternatively. Spheres and spherical particles are also used for a large size and rigid morphology.

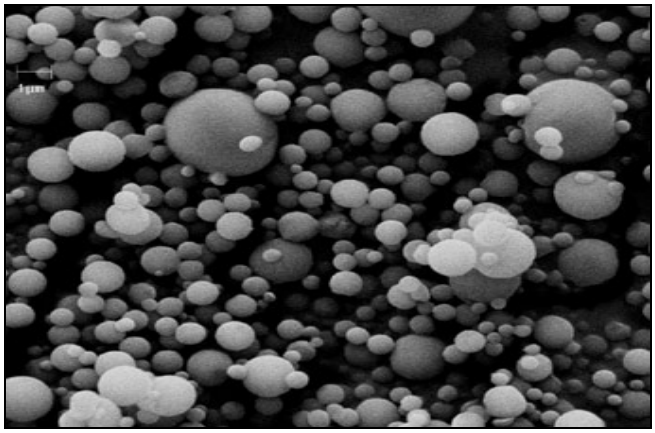


***Figure 4*: SEM image of microspheres**

Advantages of microspheres[28]:

- Microspheres provide an alternative to multiple injections to obtain sustained release of the drug with a single administration.
- Tailoring of drug release rates.
- Protection of fragile drugs.
- Increased patient comfort and compliance.
- Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability and sustained drug release characteristics.

2. POLYMERS USED FOR MICROSPHERES[29]:

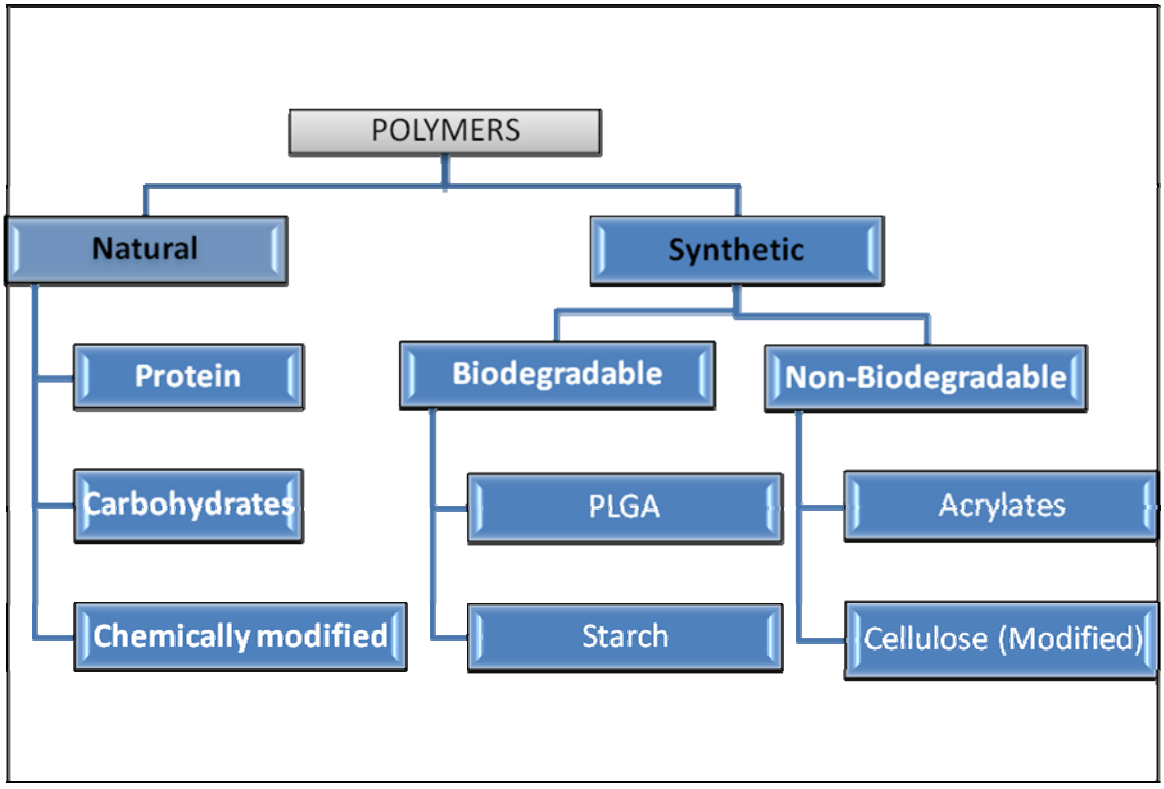
A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of micro spheres. Synthetic polymers employed as carrier materials are methyl methacrylate, acrolein, lactide, glycolide and their copolymers ethylene vinyl acetate copolymer, polyanhydrides, etc. The natural polymers widely used include albumin, gelatin, starch, collagen and carrageenan, etc. Synthetic polymers are now materials of choice for the controlled release as well as targeted micro particulate carriers. Biodegradable carriers, which degrade in the body to non-toxic degradation products, do not posse the problem of carrier toxicity and are more suited for parenteral application[30].

a. *Ideal Requirements for Polymers Used:*

The material utilized for the preparation of microspheres should ideally fulfill the following requirements[31]:

- Longer duration of action
- Control of content release
- Increase of therapeutic efficiency
- Protection of drug
- Reduction of toxicity
- Biocompatibility
- Sterilizability
- Relative stability
- Water solubility or dispersibility

b. Classification of Polymers Used In Microspheres:



3. PREPARATION TECHNIQUES OF MICROSPHERES:

List of Techniques Used For Microspheres Preparation:

1. Single emulsion
2. Double emulsion
3. Polymerization
4. Phase Separation Coacervation
5. Spray drying and Spray congealing
6. Solvent extraction
7. Solvent evaporation
8. Precipitation
9. Freeze Drying
10. Chemical and Thermal cross linking

1. Single emulsion:

The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in the non-aqueous medium e.g., oil. (*Fig: 5*) In the second step of preparation, cross-linking of the dispersed globule is carried out. The cross-linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross-linking agents used include glutaraldehyde, formaldehyde, terephthaloyl chloride, diacid chloride, etc.

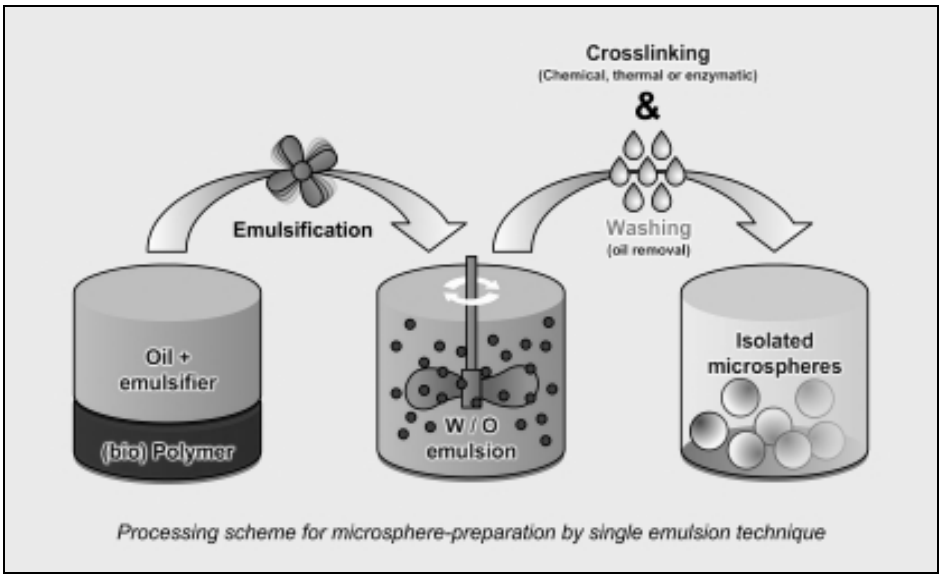


***Figure 5*: Single emulsion technique**

2. Double emulsion:

Briefly, double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w (multiple emulsion) (*Fig. 6*). This method can be used with both the natural as well as the synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is then subjected to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a

double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction process. The solvent evaporation is carried out by maintaining emulsion at reduced pressure or by stirring the emulsion so that the organic phase evaporates out. In the latter case, the emulsion is added to the large quantity of water (with or without surfactant) into which organic phase diffuses out. The solid microspheres are subsequently obtained by filtration and washing[32].

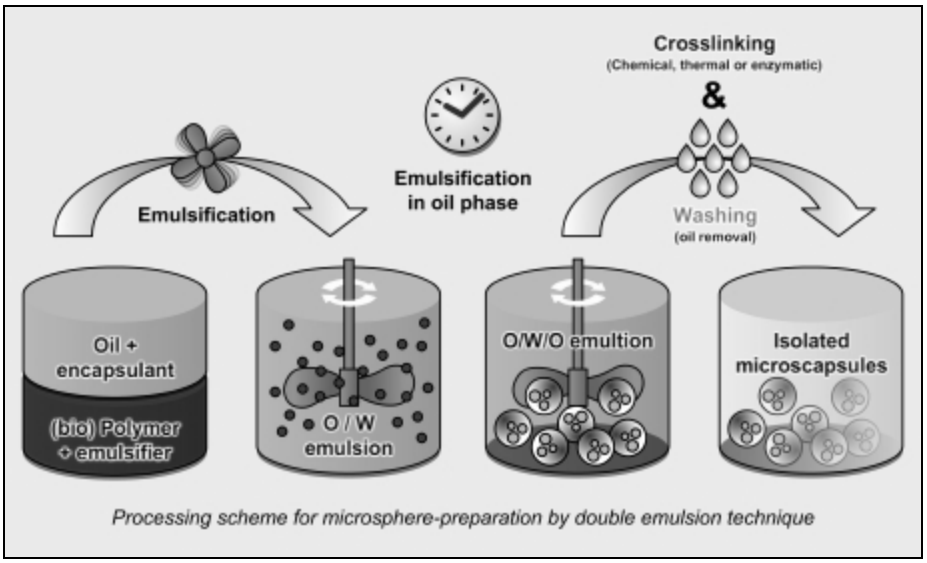


***Figure 6*: Double emulsification technique**

3. Polymerization:

The polymerization techniques conventionally used for the preparation of the micro spheres are mainly classified as:

a. Normal polymerization

b. Interfacial polymerization

Normal Polymerization:

The two processes are carried out in a liquid phase. Normal Polymerization processed and carried out materials using different techniques as bulk, suspension Initiator precipitation, emulsion and micelles polymerization processes. In bulk polymerization, a monomer or a mixture of monomer along with the initiator is usually

heated to initiate the polymerization and carry out the Droplets process. The catalyst or reinitiate is added to the reaction mixture to facilitate or accelerate the rate of Vigorous agitation of the reaction. The polymer so obtained may be polymerization Heat irradiation molded or fragmented as microspheres. For loading of drug, adsorptive drug loading or adding drug during the process of polymerization may be opted. The scheme of the bulk polymerization is represented in the (*Fig 7*).

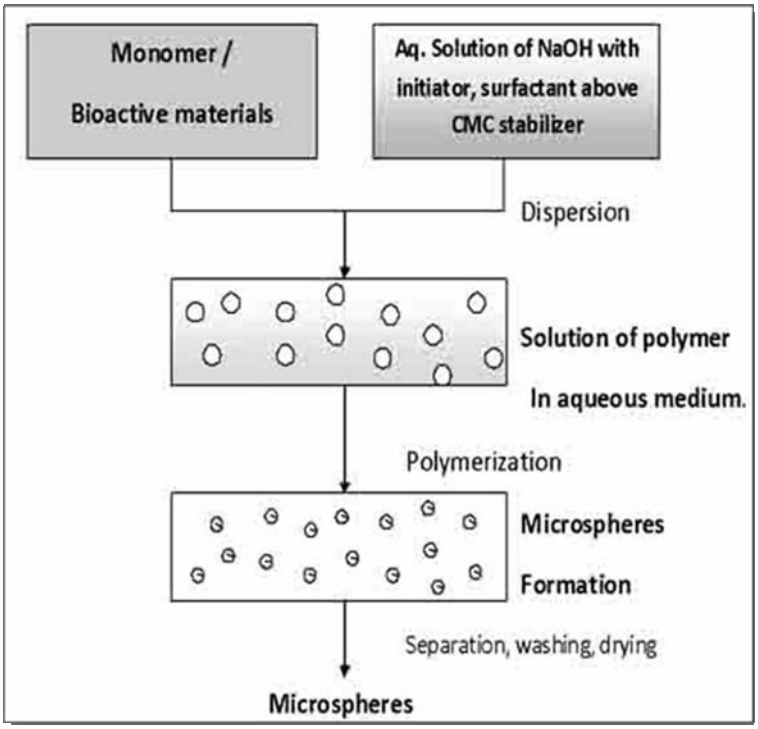


***Figure 7*: Emulsion polymerization technique**

Interfacial polymerization:

Interfacial polymerization essentially proceeds involving reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase. In this technique two reacting monomers are employed; one of which is dissolved in the continuous phase while the other being dispersed in the dispersed phase. The continuous phase is generally

aqueous in nature throughout which the second monomer is emulsified. The monomers present in either phase diffuse rapidly and polymerize rapidly at the interface. Two conditions arise depending upon the solubility of formed polymer in the emulsion droplet. If the polymer is soluble in the droplet it will lead to the formation of the monolithic type of the carrier on the other hand if the polymer is insoluble in the monomer droplet, the formed carrier is of capsular (reservoir) type.

4. Phase Separation Coacervation:

Phase separation method is specially designed for preparing the reservoir type of the system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however, some of the preparations are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids. In matrix type device, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the coacervates. The cooperation can be brought about by addition of the third component to the system which results in the formation of the two phases, one rich in the polymer, while the other one, i.e. supernatant, depleted of the polymer. There are various means and methods, which are effectively employed for coacervation phase separation. The method choice is largely dependent upon the polymer and set of conditions. The methods are based on salt addition, non-solvent addition, addition of the incompatible polymer or change in pH.

In this technique, (*Fig.8*) the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophilic or dissolved in the polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution conditions by using any of the method mentioned above. The process is carried out under continuous stirring to control the size of the micro particles. The process variables are very important since the rate of achieving the coacervates determine the distribution of the-polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension at

suitable speed since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates.

Therefore, the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment[33].

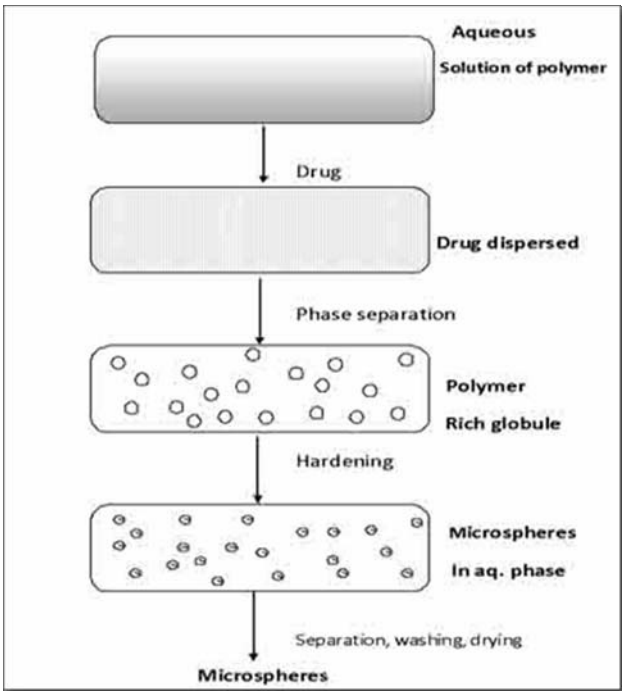


***Figure 8*: Phase separation coacervation technique**

5. Spray drying and Spray congealing:

Spray drying and spray congealing methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or the cooling of the solution, the two processes are named spray drying and the spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the micro spheres in a size range 1-100 µm.

6. Solvent extraction:

Solvent extraction method used for the preparation of micro particles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as is isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the micro spheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal is extraction method depends on the temperature of water, ratio of emulsion volume to the water and the Solubility profile of the polymer.

7. Solvent evaporation:

This is one of the earliest methods of microsphere manufacture. The polymer and drug must be soluble in an organic solvent, frequently methylene chloride. The solution containing the polymer and the drug may be dispersed in an aqueous phase to form droplets. Continuous mixing and elevated temperatures may be employed to evaporate the more volatile organic solvent and leave the solid polymer-drug particles suspended in an aqueous medium. The particles are finally filtered from the suspension.

8. Precipitation:

It is the variation on the evaporation method. The emulsion consists of polar droplets dispersed in a non polar medium. Solvent may be removed from the droplets by use of co solvent. The resulting increase in the polymer drug concentration causes precipitation forming a suspension of micro spheres.

9. Freeze Drying:

This technique involves the freezing of the emulsion. The relative freezing points of the continuous and dispersed phase are important. The continuous phase solvent is usually organic and is removed by sublimation at low temperature and pressure. Finally, the dispersed phase solvent of the droplets is removed by sublimation, leaving polymer drug particles**.**

10. Chemical and Thermal cross linking:

Microspheres made from natural polymers are prepared by a cross linking process; polymers include gelatin, albumin, starch, and dextran. A water/ oil emulsion is prepared, where the water phase is a solution of the polymer that contains the drug to be incorporated. The oil phase is a suitable vegetable oil or oil-organic mixture solvent mixture containing an oil soluble emulsifier. Once the desired w/o emulsion is formed, the water-soluble polymer is solidified by some kind of cross-linking agent.

This may involve thermal treatment or the addition of a chemical cross linking agent such as glutaraldehyde to form a stable chemical cross link as in albumin. If glutaraldehyde is cross-linking agent then residual amounts can have toxic effects. If chemical or heating is the cross linking agent then, the amount of chemical and the time period and the intensity of heating are critical in determining the release rates and swelling properties of the microspheres.

2.3.2 Introduction to PULSINCAP® system:

The Pulsincap® system was developed by R. P. Scherer International Corporation, Michigan, US, and is one of such system that comprises of a water-insoluble capsule body enclosing the drug reservoir. The body is closed at the open end with a swellable hydro gel plug.

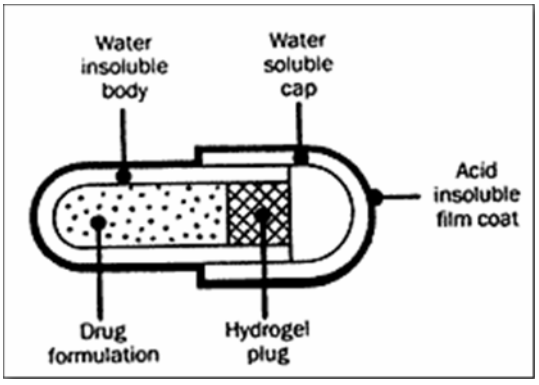


***Figure 9*: Pulsincap® system**

The plug material consists of insoluble but permeable and swellable polymers (eg, polymethacrylates), erodible compressed polymers (eg, hydroxypropylmethyl cellulose, polyvinyl alcohol, polyvinyl acetate, polyethylene oxide), congealed melted polymers (eg, saturated polyglycolated glycerides, glyceryl monooleate), and enzymatically controlled erodible polymer (e.g., pectin). When this capsule came in contact with the dissolution fluid, it swelled; and after a lag time, the plug pushed itself outside the capsule and rapidly released the drug. The dimension or length of the plug and its point or position of insertion into the capsule controlled the lag time[34].

2.4 INTRODUCTION TO POLYMERS (EUDRAGIT RS/RL 100/L 100-55)[35]

1. Nonproprietary Names:

BP: Methacrylic acid–ethyl acrylate copolymer (1 : 1)

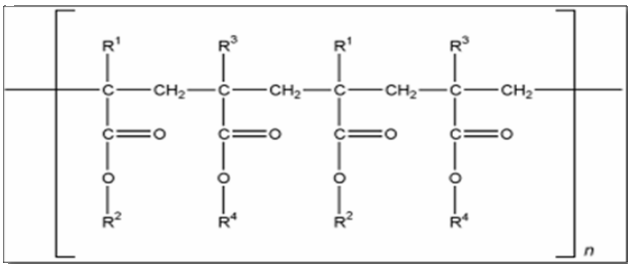
PhEur: Acidum methacrylicum et ethylis acrylas polymerisatum 1 : 1

USPNF: Ammonio methacrylate copolymer

2. Chemical Name and CAS Registry Number:

Chemical name	Trade name	CAS Number
Poly(ethyl acrylate, methyl methacrylate, Trimethylammonioethyl methacrylate chloride) 1 : 2 : 0.1	Eudragit RS 100	[33434- 241]
Poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1 : 2 : 0.2	Eudragit RL 100	[33434-241]
Poly(methacrylic acid, ethyl acrylate) 1 : 1	Eudragit L 100-55	[25212-88-8]

3. Structural Formula:



Polymer	R1	R2	R3	R4
Eudragit RS	H, CH3	CH3, C2H5	CH3	CH2CH2N(CH3)3^{+} Cl−
Eudragit RL	H, CH3	CH3, C2H5	CH3	CH2CH2N(CH3)3^{+} Cl−
Eudragit L 100-55	H, CH3	H	H, CH3	CH3, C2H5

4. Functional Category:

Film former; tablet binder; tablet diluents.

5. Description:

Eudragit RL and *Eudragit RS*, also referred to as ammonio methacrylate copolymers in the USPNF 23 monograph, are copolymers synthesized from acrylic acid and methacrylic acid esters, with *Eudragit RL* (Type A) having 10% of functional quaternary ammonium groups and *Eudragit RS* (Type B) having 5% of functional quaternary ammonium groups.

The ammonium groups are present as salts and give rise to pH-independent permeability of the polymers. Both polymers are water-insoluble, and films prepared from *Eudragit RL* are freely permeable to water, whereas, films prepared from *Eudragit RS* are only slightly permeable to water. They are available as 12.5% ready-to-use solutions in propan-2-ol–acetone (60 : 40). Solutions are colorless or slightly yellow in color, and may be clear or slightly turbid; they have an odor characteristic of the solvents. Solvent-free granules (*Eudragit RL 100* and *Eudragit RS 100*) contain ≥97% of the dried weight content of the polymer.

6. Summary of properties and uses of commercially available Eudragit RS 100/ Eudragit RL 100:

Type	Supply form	Polymer dry weight content	Recommended solvents or diluents	Solubility/permeability	Applications
Eudragit RS 100	Granules	97%	Acetone, alcohols	Low permeability	Sustained release
Eudragit RL 100	Granules	97%	Acetone, alcohols	High permeability	Sustained release
Eudragit L 100-55	Powder	95%	Acetone, alcohols	Soluble in intestinal fluid from pH 5.5	Enteric coatings

7. Solubility of commercially available polymethacrylates in various solvents:

Type	Solvent						
	Acetone and alcohols	Dichloromethane	Ethyl acetate	1 N HCl	1 N NaOH	Petroleum ether	Water
Eudragit RS 100	S	S	S	-	-	I	I
Eudragit RL 100	S	S	S	-	-	I	I
Eudragit L 100-55	S	I	I	-	S	I	I

S = soluble; M = miscible; I = insoluble or immiscible; P = precipitates

8. Pharmacopeial Specifications:

Specifications from USPNF 23

Test	Ammino methacrylate copolymer	Methacrylic acid copolymer
Viscosity		
Type A	≤15 mPa s	50–200 mPa s
Type B	≤15 mPa s	50–200 mPa s
Type C	-	
Loss on drying		
Type A	≤3.0%	≤5.0%
Type B	≤3.0%	≤5.0%
Type C	-	≤5.0%
Residue on ignition		
Type A	≤0.1%	≤0.1%
Type B	≤0.1%	≤0.1%
Type C	-	≤0.4%
Heavy metals	≤0.002%	≤0.002%
Organic volatile impurities	-	+
Limit of monomers	-	≤0.05%
Limit of methyl methacrylate	≤0.005%	-
Limit of ethyl acrylate	≤0.025%	-
Coagulum content	-	-
Assay (dried basis)	Ammonio methacrylate units	Methacrylic acid units
Type A	8.85–11.96%	46.0–50.6%
Type B	4.48–6.77%	27.6–30.7%
Type C	-	46.0–50.6%

9. Incompatibilities:

Incompatibilities occur with certain polymethacrylate dispersions depending upon the ionic and physical properties of the polymer and solvent.

10. Safety:

Polymethacrylate copolymers are widely used as film-coating materials in oral pharmaceutical formulations. They are also used in topical formulations and are generally regarded as nontoxic and nonirritant materials. A daily intake of 2 mg/kg body-weight of *Eudragit* (equivalent to approximately 150 mg for an average adult) may be regarded as essentially safe in humans.

11. Handling Precautions:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Additional measures should be taken when handling organic solutions of polymethacrylates. Eye protection, gloves, and a dust mask or respirator are recommended. Polymethacrylates should be handled in well-ventilated environment and measures should be taken to prevent dust formation.

12. Regulatory Status:

Included in the FDA Inactive Ingredients Guide (oral capsules and tablets). Included in nonparenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

3.1 REVIEW OF WORK DONE ON CHRONOTHERAPEUTICS / PULSATILE DRUG DELIVERY

U Y Nayak[36] et al developed pulsatile capsule dosage form of valsartan for controlled delivery. In the majority of individuals blood pressure rise in the early morning hours, which lead to serious cardiovascular complications. Formulations with constant / programmable delivery rates make it possible to deliver drug at definite time or controlled rate in chronopharmacokinetic studies. The prepared system contained swellable polymer (L- hydroxypropyl cellulose (L-HPC), xanthan gum, polyethylene oxide or sodium alginate) together with drug tablet and erodible tablet (L-HPC or guar gum) in a precoated capsule. Various formulation factors were investigated through series of tests, in-vitro dissolution and ex-vivo continuous dissolution – absorption studies. We found that the type, amount of polymers and erodible tablet influenced the drug release. The formulation containing 200 mg sodium alginate and erodible tablet (150 mg) containing 50% guar gum and 46% lactose showed 5-6 h lag time and 10±2.1% drug release in initial 6 h following rapid release (99±1.7% release in 12 h) of drug was observed.

V.S. Mastiholimath[37] et al investigated of an oral colon specific, pulsatile device to achieve time and/or site specific release of theophylline, based on chronopharmaceutical consideration. The basic design consists of an insoluble hard gelatin capsule body, filled with eudragit microcapsules of theophylline and sealed with a hydrogel plug. The entire device was enteric coated, so that the variability in gastric emptying time can be overcome and a colon-specific release can be achieved. The theophylline microcapsules were prepared in four batches, with Eudragit L-100 and S-100 (1:2) by varying drug to polymer ratio and evaluated for the particle size, drug content and *in vitro* release profile and from the obtained results; one better formulation was selected for further fabrication of pulsatile capsule. Different hydrogel polymers were used as plugs, to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. *In vitro* release studies of pulsatile device revealed that, increasing the hydrophilic polymer content resulted in delayed release of theophylline from microcapsules. The gamma scintigraphic study pointed out the capability of the system to release drug in lower parts of GIT after a programmed lag time for nocturnal asthma.

Programmable pulsatile, colon-specific release has been achieved from a capsule device over a 2–24 h period, consistent with the demands of chronotherapeutic drug delivery.

S Sharma[38] et al developed a multiparticulate floating-pulsatile drug delivery system using porous calcium silicate (Florite RE®) and sodium alginate, for time and site specific drug release of meloxicam. Meloxicam was adsorbed on the Florite RE® (FLR) by fast evaporation of solvent from drug solution containing dispersed FLR. Drug adsorbed FLR powder was used to prepare calcium alginate beads by ionotropic gelation method, using 32 factorial design. Developed formulations were evaluated for yield, entrapment efficiency, image analysis, surface topography, mechanical strength, apparent density, buoyancy studies and dissolution studies. Entrapment efficiency of different formulations varied from 70% to 94%. Formulations show a lag period ranging from 1.9 to 7.8 h in acidic medium followed by rapid release of meloxicam in simulated intestinal fluid USP, without enzymes (SIF). Complete drug release in SIF occurred in less than 1 h from the formulations. The size of beads varied from 2.0 to 2.7mm for different batches. Prepared beads were spherical with crushing strength ranging from 182 to 1073 g. Floating time was controlled by density of beads and hydrophobic character of drug. A pulsatile release of meloxicam was demonstrated by a simple drug delivery system which could be useful in chronopharmacotherapy of rheumatoid arthritis.

P Roy[39] et al developed a programmed delivery of ranitidine hydrochloride from a floating tablet with time-lagged coating. In this study, investigation of the functionality of the outer polymer coating to predict lag time and drug release was statistically analyzed using the response surface methodology (RSM). RSM was employed for designing of the experiment, generation of mathematical models and optimization study. The chosen independent variables, i.e. percentage weight ratios of ethyl cellulose to hydroxypropyl methyl cellulose in the coating formulation and coating level (% weight gain) were optimized with a 3^2 full factorial design. Lag time prior to drug release and cumulative percentage drug release in 7 h were selected as responses. Results revealed that both, the coating composition and coating level, are significant factors affecting drug release profile. A second-order polynomial equation fitted to the data was used to predict the

responses in the optimal region. The optimized formulation prepared according to computer-determined levels provided a release profile, which was close to the predicted values. The proposed mathematical model is found to be robust and accurate for optimization of time-lagged coating formulations for programmable pulsatile release of ranitidine hydrochloride, consistent with the demands of nocturnal acid breakthrough.

A Mohamad[40] et al developed a rupturable, capsule-based pulsatile drug delivery system with pH-independent properties prepared using aqueous coating. The drug release is induced by rupturing of the top-coating, resulting by expanding of swellable layer upon water penetration through the top-coating. Croscarmellose sodium (acdisol) is a preferable superdisintegrant compared to low substituted hydroxypropylcellulose (L-HPC) and sodium starch glycolate (Explotab), because of controlled lag time, followed by a quick and complete drug release. However, due to its anionic character, acdisol showed ph-dependent swelling characteristics (pH 7.4 >0.1 N Hcl) resulting in a pH-dependent lag time. The pH dependency could be eliminated by the addition of fumaric acid to the swelling layer, which allowed to keep an acidic micro-environment. Formation of the rupturable top-coating was successfully performed using an aqueous dispersion of ethylcellulose (Aquacoat ECD), whereby sufficient drying during the coating was needed to avoid swelling of the acdisol layer. A higher coating level was required, when aqueous dispersion was used, compared to organic coatings. However, an advantageous aspect of the aqueous coating was the lower sensitivity of the lag time to a deviation in the coating level.

Hong-Liang Lin[41] et al characterized the influence of core and coating formulations on the release profiles to establish in vitro/in vivo correlations of pulsatile pattern for a pulsatile drug delivery system activated by membrane rupture based on three core tablet formulations (A-core: HPMC 50+4000 cps, B-core: E10M, and C-core: K100M) coated with various thicknesses of a semipermeable ethylcellulose membrane plasticized with HPMC 606 (Pharmacoat 606) at different ratios with/without adding various amounts of water to dissolve it in the coating solution. Drug release behaviors were investigated using apparatus II in four media of pH 1.2 solution, pH 6.8 buffer, deionized water, and a

NaCl solution rotated at 75, 100, and 150 rpm. Pilot studies of the in vivo pharmacokinetics were conducted as well for comparison with the in vitro results. Results demonstrated that drug release from the three kinds of core tablets in deionized water increased with an increasing stirring rate, and decreased with an increasing viscosity grade of HPMC used in the core formulations. A significant promotion of drug release from core tablets was observed for the three levels of NaCl media in comparison with that in deionized water. Results further demonstrated that a slightly slower release rate in pH 1.2 solution and a faster release rate in pH 6.8 buffer than that in deionized water were observed for the A-core and B-core tablets, with the former being slower than the latter. However, similar release rates in the three kinds of media were observed for C-core tablets, but they were slower than those for the A- and B-core tablets. Dissolution of coated tablets showed that the controlling membrane was ruptured by osmotic pressure and swelling which activated drug release with a lag time. The lag time was not influenced by the pH value of the release medium or by the rotation speeds. The lag time increased with a higher coating level, but decreased with the addition of the hydrophilic plasticizer, Pharmacoat 606, and of the water amount in the coating solution. The lag time also increased with a higher concentration of NaCl in the medium. The release rate after the lag time was determined by the extent of retardation of gelation of HPMC in the core tablet based on the ionic strength of the medium. Results of the three pilot crossover studies for the exemplified pulsatile systems indicated that the lag time for the in vivo plasma profile was well correlated with that determined from the in vitro release profile in pH 1.2 solution and the in vivo release rate was better reflected by that performed in pH 6.8 buffer.

J T. McConville[42] et al investigated the variability in the performance of a pulsatile capsule delivery system induced by wet granulation of an erodible HPMC tablet, used to seal the contents within an insoluble capsule body. Erodible tablets containing HPMC and lactose were prepared by direct compression (DC) and wet granulation (WG) techniques and used to seal the model drug propranolol inside an insoluble capsule body. Dissolution testing of capsules was performed. Physical characterization of the tablets and powder blends used to form the tablets was undertaken using a range of experimental

techniques. The wet granulations were also examined using the novel technique of microwave dielectric analysis (MDA). WG tablets eroded slower and produced longer lag-times than those prepared by DC; the greatest difference was observed with low concentrations of HPMC. No anomalous physical characteristics were detected with either the tablets or powder blends. MDA indicated water-dipole relaxation times of 2.9, 5.4 and 7.7 ms for 15, 24 and 30% HPMC concentrations, respectively, confirming that less free water was available for chain disentanglement at high concentrations. In conclusion, at low HPMC concentrations water mobility is at its greatest during the granulation process, such formulations are therefore more sensitive to processing techniques. Microwave dielectric analysis can be used to predict the degree of polymer spreading in an aqueous system, by determination of the water-dipole relaxation time.

S S. Badve[43] et al developed hollow calcium pectinate beads for floating-pulsatile release of diclofenac sodium intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. To overcome limitations of various approaches for imparting buoyancy, hollow/porous beads were prepared by simple process of acid-base reaction during ionotropic cross linking. The floating beads obtained were porous (34% porosity), hollow with bulk density <1 and had Ft50% of 14–24 h. In vivo studies by gamma scintigraphy determined on rabbits showed gastro retention of beads up to 5 h. The floating beads provided expected two-phase release pattern with initial lag time during floating in acidic medium followed by rapid pulse release in phosphate buffer. This approach suggested the use of hollow calcium pectinate microparticles as promising floating-pulsatile drug delivery system for site- and time-specific release of drugs acting as per chronotherapy of diseases.

R Lobenberg[44] et al studied that circadian patterns influence the pharmacokinetics of certain drugs used in the treatment of different diseases. For such drugs, the bioavailability is influenced by the time of administration. The objective of this study was to investigate differences in the pharmacokinetic patterns between a pulsatile drug delivery system using a pulsatile capsule, an immediate release tablet and a controlled

release tablet. Metoprolol was chosen as a model drug because of its high solubility and high permeability pattern throughout the GI tract. The dosage forms were administered to four dogs and the plasma levels were measured using LC-MS/MS. Pharmacokinetic parameters were determined for each dosage form. Fluctuations in the plasma time curves over the observation period indicated that physiological factors like motility have an influence on the drug absorption. The comparison of the plasma time curves of the dosage forms showed that each dosage form caused significant differences in the drug plasma levels. The pulsatile drug delivery capsule caused two defined Cmax values for each dose between 1–1.75 and 2.5–3.5 h. Implications for the use of a pulsatile drug delivery device for chronopharmacotherapy are discussed. Pulsatile drug delivery offers a promising way for chronopharmacotherapy if the time of administration and pulse time are adjusted to the circadian pattern.

A. S Hasan[45] et al The aim of this study was to develop microparticles containing nanoparticles (composite microparticles) for prolonged drug delivery with reduced burst effect in vitro and in vivo. Such composite microparticles were prepared with hydrophobic and biodegradable polymers [poly(e-caprolactone), poly(lactic-co-gly-colic) acid]. Ibuprofen was chosen as the model drug, and microparticles were prepared by the extraction technique with ethyl acetate as the solvent. Nanoparticles and microparticles and an ibuprofen solution (Pedea) were administered subcutaneously at the dose of 1 mg of ibuprofen per kg to overnight-fasted rats (male Wistar). Composite microparticles showed prolonged ibuprofen release and less burst effect when compared to simple microparticles (without nanoparticles inside) or nanoparticles both in vitro (PBS buffer) and in vivo. Moreover, ibuprofen was still detected in the plasma after 96 h with composite microparticles. Consequently, it has been demonstrated that composite microparticles were able to reduce burst release and prolong the release of ibuprofen for a long period of time.

A Maroni[46] et al evaluated the viability of a time-dependent delivery platform (Chronotopic^{TM}) in preparing an insulin-based system intended for oral colon delivery. The main objectives were to assess the influence of the manufacturing process and

storage conditions on the protein stability. Insulin-loaded cores were manufactured by direct compression and were subsequently coated with hydroxypropyl methylcellulose (HPMC) in a top-spray fluid bed up to increasing weight gains, namely 20%, 60% and 100%. In order to evaluate the impact the operating conditions may have on the protein integrity, insulin and its main degradation products (A21-desamido insulin – A21, Other Insulin-Related Compounds – OIRCs, and High-Molecular Weight Proteins – HMWPs) were assayed on samples collected after each process step by chromatographic methods. Furthermore, long-term (4 °C) and accelerated (25 °C–60% RH) stability studies were carried out on tablet cores and coated systems by assessing insulin, A21, OIRC and HMWP percentages throughout a one-year storage period. In addition, the in vitro release behavior was investigated during the same study period. The overall results indicated that the manufacturing process is not detrimental for insulin integrity and that 4 °C storage temperature alters neither the protein content nor the release performances of the device. It was therefore concluded that insulin-containing systems intended for oral colon delivery can be obtained by the Chronotopic™ technology.

H. N.E. Stevens[47] et al designed Pulsincap™ formulations to deliver a dose of drug following a 5-h delay were prepared to evaluate the capability of the formulation to deliver dofetilide to the lower gastrointestinal (GI) tract. By the expected 5-h release time, the preparations were well dispersed throughout the GI tract, from stomach to colon. Plasma analysis permitted drug absorption to be determined as a function of GI tract site of release. Dofetilide is a well absorbed drug, but showed a reduction in observed bioavailability when delivered from the Pulsincap™ formulations, particularly at more distal GI tract sites. Dispersion of the drug from the soluble excipient used in this prototype formulation relies on a passive diffusion mechanism and the relevance of this factor to the reduced extent and consistency of absorption from the colon is discussed. In these studies the effects of the degree of dispersion versus the site of dispersion could not be ascertained; nevertheless the scintigraphic analysis demonstrated good in vitro–in vivo correlation for time of release from Pulsincap™ preparations. The combination of scintigraphic and pharmacokinetic analysis permits identification of the site of drug

release from the dosage form and pharmacokinetic parameters to be studied in man in a non-invasive manner.

J Binns[48] et al evaluated the tolerability of a 28 day course of twice daily dosage with Pulsincap ™ capsules having the plug set for separation after 6 h. Eight subjects received Pulsincap ™ capsules and four subjects received a matching placebo capsule. There was no active medication in any of the study capsules. Daily assessments were made of adverse events. Laboratory safety data (bloods and urine) and fecal occult blood were monitored weekly. Subjects returned for a post-study medical check during the fortnight following their final treatment session. All subjects completed their dosing schedule satisfactorily. Eleven adverse events were reported by six of the eight subjects in the Pulsincap ™ capsule group and 14 adverse events were reported by the four subjects taking placebo. None of the adverse events in either group was considered to be causally related to treatment. Four males, but no females, had at least one positive test for fecal occult blood, two in the Pulsincap TM capsule group and two in the placebo group. In the single subject for whom the test was positive repeatedly, the occult blood was associated in time with an inter current dental problem and its treatment. Pulsincap ™ capsules were well tolerated by the eight subjects who received them and there was no evidence of adverse events being causally related to this formulation. The results of this tolerability study support the further development of the Pulsincap ™ formulation for delivery of active drugs.

3.2 REVIEW OF WORK DONE ON COLON TARGETED DRUG DELIVERY

A. A. Mohamed[49] et al prepared Acrylic enteric microparticles for oral drug delivery by an oil-in-oil emulsion solvent evaporation process. The novel use of sorbitan sesquioleate as a surfactant produced Eudragit L55, L and S (pH thresholds of 5.5, 6 and 7, respectively) microparticles of good morphology (spherical, smooth surfaced), size (<100_m) and size uniformity. The processwas efficient (yield approximately 90%) and the encapsulated model drug (prednisolone)was in the amorphous form. The Eudragit L and S microparticles showed excellent pH-responsive drug release in dissolution studies (negligible drug release at pH 1.2; rapid drug release above the polymers' pH thresholds). In contrast, Eudragit L55 particles aggregated in fluid and showed poor control of drug release. It was concluded that although the rat is an inappropriate model for the investigation of Eudragit S microparticles, the positive results seen with the Eudragit L microparticles indicate its potential use in pH-targeted drug delivery.

Y. Karrout[50] et al identified novel polymeric films allowing for the site-specific delivery of drugs to the colon of patients suffering from inflammatory bowel diseases. Ethylcellulose was blended with different types of bacteria-sensitive starch derivatives. The water uptake and dry mass loss kinetics of the systems were monitored upon exposure to media simulating the contents of the stomach, small intestine and colon (including fresh fecal samples from Crohn's Disease and Ulcerative Colitis patients). Importantly, ethylcellulose:Nutriose FB 06 and ethylcellulose:Peas starch N-735 films showed highly promising water uptake and dry mass loss kinetics in all the investigated media, indicating their potential to minimize premature drug release in the upper gastro-intestinal tract, and allowing for controlled release once the colon is reached. This can be attributed to the fact that the starch derivatives serve as substrates for the enzymes, which are secreted by the bacteria present in the colon of inflammatory bowel disease patients. Thus, the identified new polymeric films are adapted to the pathophysiological conditions in the gastro-intestinal tract in the disease state. Furthermore, Nutriose is known to provide pre-biotic effects, which can be of great benefit for these patients.

J. Nunthanid[51] et al used Spray-dried chitosan acetate (CSA) and ethylcellulose (EC) as new compression coats for 5-aminosalicylic acid tablets. Constrained axial or radial swelling of pure CSA and EC/CSA tablets in 0.1 N HCl (stage I), Tris–HCl, pH 6.8 (stage II), and acetate buffer, pH 5.0 (stage III), was investigated. Factors affecting in vitro drug release, i.e., % weight ratios of coating polymers, dip speeds of dissolution apparatus or pH of medium or colonic enzyme (b-glucosidase) in stage III, and use of a super disintegrant in core tablets, were evaluated. Swollen CSA gel dissolved at lower pH and became less soluble at higher pH. The system was a dual time- and pH-control due to the insolubility of EC suppressing water diffusion and the swelling of CSA in the stages I and II. The erosion of CSA gel in the stage III induced the disintegration of the coat resulting in rapid drug release. The lower dip speed and higher pH medium delayed the drug release, while a super disintegrant in the cores enhanced the drug release and no enzyme effect was observed.

Z Rahman[52] et al prepare and evaluate the colon-specific microspheres of 5-fluorouracil for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The size of the core microspheres ranged from 22 to 55 μm, and the size of the coated microspheres ranged from 103 to 185 μm. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit S-100 coat ratio of 1:7, and there were no changes in the size, shape, drug content, differential scanning calorimetry thermogram, and in vitro drug release after storage at 40°C/75% relative humidity for 6 months.

N M. Anande[53] et al developed cyst-targeted novel concanavalin-A (Con-A) conjugated mucoadhesive microspheres of diloxanide furoate (DF) for the effective treatment of amoebiasis. Eudragit microspheres of DF were prepared using emulsification–solvent evaporation method. Formulations were characterized for particle size and size distribution, % drug entrapment, surface morphology and *in vitro* drug release in simulated gastrointestinal (GI) fluids. Eudragit

microspheres of DF were conjugated with Con-A. IR spectroscopy and DSC were used to confirm successful conjugation of Con-A to Eudragit microspheres while Con-A conjugated microspheres were further characterized using the parameters of zeta potential, mucoadhesiveness to colonic mucosa and Con-A conjugation efficiency with microspheres. IR studies confirmed the attachment of Con-A with Eudragit microspheres. All the microsphere formulations showed good % drug entrapment (78±5%). Zeta potential of Eudragit microspheres and Con-A conjugated Eudragit microspheres were found to be 3.12±0.7mV and 16.12±0.5 mV, respectively. Attachment of lectin to the Eudragit microspheres significantly increases the mucoadhesiveness and also controls the release of DF in simulated GI fluids.Gammascintigraphy study suggested that Eudragit S100 coated gelatin capsule retarded the release of Con-A conjugated microspheres at low pH and released microspheres slowly at pH 7.4 in the colon.

K. Mladenovska[54] et al prepared Chitosan-Ca-alginate microparticles for colon-specific delivery and controlled release of 5-aminosalicylic acid after peroral administration using spray drying method followed by ionotropic gelation/polyelectrolyte complexation. Physicochemical characterization pointed to the negatively charged particles with spherical morphology having a mean diameter less than 9 _m. Chitosan was localized dominantly in the particle wall, while for alginate, a homogeneous distribution throughout the particles was observed. 1H NMR, FTIR, X-ray and DSC studies indicated molecularly dispersed drug within the particles with preserved stability during microencapsulation and in simulated *in vivo* drug release conditions. *In vitro* drug release studies carried out in simulated *in vivo* conditions in respect to pH, enzymatic and salt content confirmed the potential of the particles to release the drug in a controlled manner. The diffusional exponents according to the general exponential release equation indicated anomalous (non-Fickian) transport in 5-ASA release controlled by a polymer relaxation, erosion and degradation. Biodistribution studies of [131I]-5-ASA loaded chitosan-Ca-alginate microparticles, carried out within 2 days after peroral administration to Wistar male rats in which TNBS colitis was induced, confirmed the dominant localization of 5-ASA in the colon with low systemic bioavailability.

M. Orlu[55] et al designed novel colon specific drug delivery system containing flurbiprofen (FLB) microsponges. Microsponges containingFLBand EudragitRS100 were prepared by quasi-

emulsion solvent diffusion method. Additionally,FLBwas entrapped into a commercial Microsponge® 5640 system using entrapment method. Afterwards, the effects of drug:polymer ratio, inner phase solvent amount, stirring time and speed and stirrer type on the physical characteristics of microsponges were investigated. The thermal behaviour, surface morphology, particle size and pore structure of microsponges were examined. The colon specific formulations were prepared by compression coating and also pore plugging of microsponges with pectin:hydroxypropylmethyl cellulose (HPMC) mixture followed by tabletting. In vitro dissolution studies were done on all formulations and the results were kinetically and statistically evaluated. The microsponges were spherical in shape, between 30.7 and 94.5μm in diameter and showed high porosity values (61–72%). The pore shapes of microsponges prepared by quasi-emulsion solvent diffusion method and entrapment method were found as spherical and cylindrical holes, respectively. Mechanically strong tablets prepared for colon specific drug delivery were obtained owing to the plastic deformation of sponge-like structure of microsponges. In vitro studies exhibited that compression coated colon specific tablet formulations started to release the drug at the 8th hour corresponding to the proximal colon arrival time due to the addition of enzyme, following a modified release pattern while the drug release from the colon specific formulations prepared by pore plugging the microsponges showed an increase at the 8th hour which was the time point that the enzyme addition made. This study presents a new approach based on microsponges for colon specific drug delivery.

3.3 REVIEW OF WORK DONE ON METOPROLOL:

J. Domenech[56] et al studied the absorption of metoprolol from the stomach, small intestine and colon of anaesthetized rats has been evaluated using an in situ technique. Absorption rates were measured in terms of the rate of disappearance of metoprolol fumarate from the lumen between 5 and 30 min after dosing. Adsorption was estimated from the initial rapid fall in luminal content within the first 5 min after drug administration. The rate of drug absorption from the stomach was low or negligible. In the small intestine, the absorption rate constants, ka, at ph 6.2 and 7.5 were 0.66 and 0.81 h-1, respectively. In the colon, the rate of drug absorption at ph 7.5 was faster (ka = 1.21 h-1) than in other segments of the gut. Drug adsorption in the stomach amounted to 11% of the administered dose. In the small intestine adsorption was greater (16-22%), presumably because of the larger surface area in this segment of the gut.

J. W. Fara[57] et al investigated the performance of oxprenolol and metoprolol oros systems have been evaluated in the dog. One study compared in vivo and in vitro release from both systems over 2-14 h. The other compared the systemic availabilities of both drugs after 3 h infusion at a constant rate into the cephalic and hepatic portal veins, and into the lumen of the duodenum and colon. In the in vivo release studies, oros systems were recovered throughout the gut from the stomach to the colon. The amounts of drug remaining in the systems corresponded closely to those measured in a parallel in vitro release experiment. In vitro testing is thus a reliable indicator of in vivo system performance. In the absorption studies, both metoprolol and oxprenolol were shown to be subject to substantial first-pass metabolism. Additionally, for metoprolol the data indicated a significant loss during transport from the gut lumen into the portal circulation. For both drugs the availability from the colon was equal to that from the duodenum. These results provide some justification for the development of oral dosage forms with extended durations of release even for drugs which undergo significant first-pass metabolism.

M. Palanisamy[58] et al developed a sustained release microspheres formulation containing metoprolol Succinate. The various batches of microspheres were prepared by

cross-linking technique using chitosan polymer. The effect of stirring speed and core to coat ratio was studied with respect to entrapment efficiency and micromeritics properties. *In vitro* release study was performed in phosphate buffer (ph 6.8). Shape and surface Analysis are also examined by scanning electron microscope. The microspheres obtained from cross-linking Technique were spherical and free flow in nature. The drug content was ranges from 65.62% -72.06%. Entrapment Efficiency was based on the ratio of polymer present in formulation. This is due to high loss of drug during Formulation. Particle size ranged from 330 to 540μm. Microspheres were spherical in shape and having a smooth Surface as the evident in Scanning electron microscopic photographs. Drug crystals was found outer surface of Microspheres in formulation F1, whereas, it was absent in F4 (1:5 ratio). *In vitro* release study shows a better Sustained effect in F3 (1:3 ratio) over 24hrs. The release data was determined using various kinetic models and Korsmeyer – peppas model showed an acceptable regression value for all composition. Thus, its follows non - Fickians transport mechanism and the drug release was extended up to 96.78% over period of time. This micro Particulate system proves the efficient in the delivering of drug in the system over extended period of time with Minimal dose.

R. V. Nellore[59] et al developed model extended-release (ER) matrix tablet formulations for metoprolol tartrate (100 mg) sufficiently sensitive to manufacturing variables and to serve as the scientific basis for regulatory policy development on scale-up and post approval changes for modified-release dosage forms (SUPAC-MR). Several grades and levels of hydroxypropyl methylcellulose (Methocel K4M, K15M, K100M and K100LV), fillers and binders were studied. Three granulation processes were evaluated; direct compression, fluid-bed or high-shear granulation. Lubrication was performed in a V-blender and tablets were compressed on an instrumented rotary tablet press. Direct compression formulations exhibited poor flow, picking and sticking problems during tableting. High-shear granulation resulted in the formation of hard granules that were difficult to mill but yielded good tablets. Fluid-bed granulations were made using various binders and appeared to be satisfactory in terms of flow and tableting performance. In vitro drug release testing was performed in pH 6.8 phosphate buffer using USP apparatus 2 (paddle) at 50 rpm. At a fixed polymer level, drug release from the higher viscosity

grades (K100M) was slower as compared to the lower viscosity grades (K100LV). In addition, release from K100LV was found to be more sensitive to polymer level changes. Increase in polymer level from 10 to 40% and/or filler change from lactose to dicalcium phosphate resulted in about 25–30% decrease in the amount of metoprolol release after 12 h. The results of this study led to the choice of Methocel K100LV as the hydrophilic matrix polymer and fluid-bed granulation as the process of choice for further evaluation of critical and non-critical formulation and processing variables.

E. Verhoeven[60] et al Mini-matrices with release-sustaining properties were developed by hot-melt extrusion (diameter 3 mm, height 2 mm) using metoprolol tartrate as model drug (30%, w/w) and ethylcellulose as sustained-release agent. Polyethylene glycol or polyethylene oxide was added to the formulation to increase drug release. Changing the hydrophilic polymer concentration (0%, 1%, 2.5%, 5%, 10%, 20% and 70%, w/w) and molecular weight (6000, 100,000, 1,000,000 and 7,000,000) modified the in vitro drug release: increasing concentrations yielded faster drug release (irrespective of molecular weight), whereas the influence of molecular weight depended on concentration. Smooth extrudates were obtained when processed at 40 and 70 °C for polyethylene glycol and polyethylene oxide formulations, respectively. Raman analysis revealed that metoprolol tartrate was homogeneously distributed in the mini-matrices, independent of hydrophilic polymer concentration and molecular weight. Also drug and polymer crystallinity were independent of both parameters. An oral dose of 200 mg metoprolol tartrate was administered to dogs in a randomized order either as immediate-release preparation (Lopresor 100), as sustained-release formulation (Slow- Lopresor 200), or as experimental mini-matrices (varying in hydrophilic polymer concentration). The sustained-release effect of the experimental formulations was limited, and relative bioavailabilities of 66.2% and 148.2% were obtained for 5% and 20% PEO 1,000,000 mini-matrices, respectively.

P. S. Rajinikanth[61] et al prepared Bioadhesive sodium alginate microspheres of Metoprolol tartrate (MT) for intranasal systemic delivery to avoid the first-pass effect, as an alternative therapy to injection, and to obtain improved therapeutic efficacy in the

treatment of hypertension and angina pectoris. The microspheres (Ms) were prepared using emulsification--cross-linking method. The formulation variables were drug loading, polymer concentration, cross-linking agent concentration, and cross-linking time. The Ms were evaluated for characteristics, like particle size, incorporation efficiency, swelling ability, in vitro bioadhesion, in vitro drug release, and in vivo pharmacodynamic performance in rabbits against isoprenaline-induced tachycardia. Treatment of in vitro data to different kinetic equations indicated matrix-diffusion controlled drug delivery from sodium alginate Ms. Polymer concentration, cross-linking agent concentration, and cross-linking time influenced the drug release profiles significantly. In vivo studies indicated significantly improved therapeutic efficacy of MT from Ms with sustained and controlled inhibition of isoprenaline-induced tachycardia as compared with oral and nasal administration of drug solution.

R A. Shabaraya[62] et al formulated metoprolol tartrate microspheres using chitosan by the phase separation emulsification technique. Microspheres of 1:0.5, 1:1 and 1:2 drugs to carrier ratios were prepared and thermally cross-linked. Drug to carrier ratio 1:1 showed maximum percentage yield and highest drug entrapment. The size range of the microspheres varies from 3.5 to 31.5 μm. UV and DSC studies were carried out to confirm the presence and stability of the drug in the microspheres. Short-term stability studies were carried out at different temperatures. In vitro release studies were carried out at different pH for a period of 10 h and compared with the pure drug. The release of metoprolol tartrate from the chitosan microspheres was found to be sustained.

P. Lundborg[63] et al studied a controlled-release formulation containing metoprolol 100 mg and hydrochlorothiazide 12.5 mg. We compared the pharmacokinetics of both substances and the pharmacodynamics of metoprolol with those of a conventional combination tablet. The controlled-release formulation gave less variable plasma metoprolol concentrations, C_{max} 138 nmol·l^{-1} and C_{min} 74 nmol·l^{-1}, whereas for the conventional formulation the mean C_{max} of metoprolol was 629 nmol·l^{-1} and the C_{min} 20 nmol·l^{-1}. Despite lower relative systemic availability (68%) for metoprolol from the controlled-release formulation and a smaller AUC, metoprolol from the controlled-

release formulation produced a greater total effect, calculated as the area under the curve of the effect on exercise heart rate vs. time (303 vs. 259%·h; $P<0.05$). Hydrochlorothiazide was rapidly absorbed from both formulations and the plasma concentration profiles were almost superimposable. Controlled release metoprolol with hydrochlorothiazide combines effective beta $_1$ -adrenoceptor blockade for 24 h without affecting the pharmacokinetics of hydrochlorothiazide.

3.4 REVIEW OF WORK DONE ON EUDRAGIT MICROSPHERES

S. Haznedar[64] et al prepared microspheres by solvent evaporation method using acetone/liquid paraffin system. The influence of formulation factors (stirring speed, polymer:drug ratio, type of polymer, ratio of the combination of polymers) on particle size, encapsulation efficiency and in vitro release characteristics of the microspheres were investigated. The yields of preparation and the encapsulation efficiencies were high for all formulations the microspheres were obtained. Mean particle size changed by changing the polymer: drug ratio or the stirring speed of the system. Although acetazolamide release rates from Eudragit RS microspheres were very slow and incomplete for all formulations, they were fast from Eudragit RL microspheres. When Eudragit RS was added to Eudragit RL microsphere formulations, release rates slowed down and achieved the release profile suitable for peroral administration.

M Yang[65] et al prepared microspheres in liquid system by quasi-emulsion solvent diffusion method, in which the Aerosil was employed as an inert dispersing carrier to improve the dissolution rate of nitrendipine, and Eudragit RS as a retarding agent to control the release rate. The resultant microspheres were evaluated for the recovery, bulk density, average particle size, drug loading, and incorporation efficiency. And the factors affecting the formation of microspheres and the drug-release rate were investigated. It was observed by a scanning electron microscope (SEM) that the microspheres were finely spherical and uniform, and no entire nitrendipine crystals were observed visually. The results of X-ray diffraction indicated that nitrendipine in microspheres was disordered, suggesting that nitrendipine was highly dispersed in microspheres. The drug loading of microspheres was enhanced with increasing the ratio of drug to excipients, and the incorporation efficiency was always >90%. The formation of microspheres was mainly influenced by the amount of bridging liquid and sodium dodecyl sulfate (SDS) in poor solvent. The dissolution profiles could be modulated with adjusting the amount of retarding agent and dispersing carrier formulated.

R. A. Kendall[66] et al prepared microspheres by an oil-in-oil emulsion solvent evaporation process. The novel use of sorbitan sesquioleate as a surfactant produced

Eudragit L55, L and S (pH thresholds of 5.5, 6 and 7, respectively) microparticles of good morphology (spherical, smooth surfaced), size (<100_m) and size uniformity. The process was efficient (yield approximately 90%) and the encapsulated model drug (prednisolone)was in the amorphous form. The Eudragit L and S microparticles showed excellent ph-responsive drug release in dissolution studies (negligible drug release at ph 1.2; rapid drug release above the polymers' ph thresholds). In contrast, Eudragit L55 particles aggregated in fluid and showed poor control of drug release. In vivo in rats, Eudragit L microparticles released their drug load rapidly (*T*max < 1 h) and the *C*max and AUC were higher than those of a control suspension of prednisolone. Drug absorption from Eudragit S microparticles was low which was attributed to the fact that the threshold ph of Eudragit S was not reached in the rat intestine and drug releasewas therefore incomplete. It was concluded that although the rat is an inappropriate model for the investigation of Eudragit S microparticles, the positive results seen with the Eudragit L microparticles indicate its potential use in ph-targeted drug delivery.

P. Trivedi[67] et al microencapsulated the anti-inflammatory drug to provide controlled release and minimizing or eliminating local side effect by avoiding the drug release in the upper gastrointestinal track. The drug was targeted to colon and their aligned area for their local effect. Acceclofenac was microencapsulated with eudragit (S 100,RL 100, RS 100), using o/w emulsion solvent evaporation technique. Prepared microspheres were subjected to micromeritic properties. Microspheres were subjected to drug loading, in-vitro drug release as well as for scanning electron microscopy. The drug- polymer concentration of dispersed phase influences the particle size and drug release properties.

B. C Behera[68] et al prepared microspheres using polymethacrylate polymers (Eudragit® RS 100 and RL 100) by solvent evaporation method and characterized for their micromeritic properties and drug loading, as well as by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy. In vitro release studies were performed in phosphate buffer (ph 7.4). The purpose of the present investigation was to formulate and evaluate microencapsulated glipizide produced by the emulsion – solvent evaporation method.

P. Wu[69] et al prepare and evaluate the sustained release of potassium chloride formulations. Eudragit RS and/or RL loaded with potassium chloride microspheres were prepared by a solvent evaporation method. The effect of sustained release of Eudragit microspheres was evaluated by an in vitro dissolution test and in vivo oral absorption study, and the results were compared to a commercial product (Slow-K). The results showed that Eudragit microspheres loaded with potassium chloride can be easily prepared and satisfactory results obtained considering the size distribution and shapes of microspheres by incorporating aluminum stearate. The encapsulation efficiency and loading capacity were about 84–90% and 27%, respectively. Moreover, the Eudragit RS (30–45 mesh) and Eudragit RS/RL (20–30 mesh) microspheres showed a similar sustained release effect of commercial product via in vitro dissolution and in vivo oral absorption study.

R R Parsuram[70] et al developed delayed release microspheres using cellulose acetate phthalate. The effect of various other enteric polymers such as hydroxypropyle methyl cellulose phthalate, eudragit S 100, eudragit L 100 on the release aceclofenac from the cap microspheres have been evaluated. The microspheres characterized for particle size, scanning electron microscopy, percentage yield, drug entrapment and for in-vitro release kinetic. The shape of microspheres was found to be spherical by SEM. The drug entrapment efficiency of microspheres was found to be ranging from 75.62 to 96.52.%w/w. The result revealed that the HPMCP exhibits positive influence whereas eudragit L 100 and eudragit S 100 exhibits negative effect on the drug release rate of CAP microspheres. In- vitro drug release from all formulations followed the first order release kinetic and erosion plot. Formulation with drug:CAP:HPMCP ratio of 1:8:2 was considered best because it showed delayed release.

M.L. Lorenzo-Lamosa[71] et al studied chitosan (CS) microcores entrapped within acrylic microspheres. Sodium diclofenac (SD), used as a model drug, was efficiently entrapped within CS microcores using spray-drying and then microencapsulated into Eudragit L-100 and Eudragit S-100 using an oil-in-oil solvent evaporation method. The size of the CS microcores was small (1.8–2.9 mm) and they were efficiently encapsulated within

Eudragit microspheres (size between 152 and 223 mm) forming a multireservoir system. Even though CS dissolves very fast in acidic media, at pH 7.4, SD release from CS microcores was delayed, the release rate being adjustable (50% dissolved within 30–120 min) by changing the CS molecular weight (MW) or the type of CS salt. Furthermore, by coating the CS microcores with Eudragit, perfect pH-dependent release profiles were attained. No release was observed at acidic pHs, however, when reaching the Eudragit pH solubility, a continuous release for a variable time (8–12 h) was achieved. A combined mechanism of release is proposed, which considers the dissolution of the Eudragit coating, the swelling of the CS microcores and the dissolution of SD and its further diffusion through the CS gel cores. In addition, infrared (IR) spectra revealed that there was an ionic interaction between the amine groups of CS and the carboxyl groups of Eudragit, which provided the system with a new element for controlling the release. In conclusion, this work presents new approaches for the modification of CS as well as a new system with a great potential for colonic drug delivery.

R.D. Kale[72] et al prepared microspheres using an enteric polymer and emulsification solvent-evaporation method. The microspheres remained buoyant continuously over the surface of acidic media containing surfactant for a period of 8-12 h *in vitro*. Differential scanning calorimetry and X-ray diffraction studies showed that drug incorporated in the outer shell of the polymer was completely amorphous. Scanning electron micrographs indicated that the microsphere is perfect sphere with an internal hollow cavity enclosed by a rigid shell of polymer. The micromeritic properties of microspheres were found to be much improved compared with original drug crystals. The *in vitro* drug release behavior of the floating microspheres was characterized as an enteric property. Polymer being soluble above pH 7.0, the drug release rates from microspheres changed dramatically above and below pH 7.0. At intestinal pH the drug release was faster and continuous as compared to the amount released at gastric pH.

A. Paharia[73] et al evaluated Eudragit-coated pectin microspheres for colon targeting of 5-fluorouracil (FU). Pectin microspheres were prepared by emulsion dehydration method using different ratios of FU and pectin (1:3 to 1:6), stirring speeds (500-2000 rpm) and

emulsifier concentrations (0.75%-1.5% wt/vol). The yield of preparation and the encapsulation efficiencies were high for all pectin microspheres. Microspheres prepared by using drug:polymer ratio 1:4, stirring speed 1000 rpm, and 1.25% wt/vol concentration of emulsifying agent were selected as an optimized formulation. Eudragit-coating of pectin microspheres was performed by oil-in-oil solvent evaporation method using coat: core ratio (5:1). Pectin microspheres and Eudragit-coated pectin microspheres were evaluated for surface morphology, particle size and size distribution, swellability, percentage drug entrapment, and in vitro drug release in simulated gastrointestinal fluids (SGF). The in vitro drug release study of optimized formulation was also performed in simulated colonic fluid in the presence of 2% rat cecal content. Organ distribution study in albino rats was performed to establish the targeting potential of optimized formulation in the colon. The release profile of FU from Eudragit-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of FU.

S K. Jain[74] et al developed for delivery of albendazole specifically into the colon. The effects of polymer concentration, stirring rate, and concentration of emulsifier on particle size and drug loading were studied. A comparative *in vitro* drug release study of the optimized formulation was carried out in simulated colonic fluid, with and without 2% rat cecal material.

Z. Rahman[75] et al evaluated the colon-specific microspheres of 5-fluorouracil for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and in vitro drug release studies. The outer surfaces of the core and coated microspheres, which were spherical in shape, were rough and smooth, respectively. The

size of the core microspheres ranged from 22 to 55 μm, and the size of the coated microspheres ranged from 103 to 185 μm. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit S-100 coat ratio of 1:7, and there were no changes in the size, shape, drug content, differential scanning calorimetry thermogram, and in vitro drug release after storage at 40°C/75% relative humidity for 6 months.

F. Atyabi[76] et al prepared pectinate beads containing trimethyl chitosan chloride (TMC) as an absorption enhancer were prepared using Coomassie Brilliant Blue G 250 (CB) as a relatively high molecular weight water-soluble model drug. Effects of different formulation variables, such as cross-linker type, cross-linking time, cross-linker concentration, TMC: pectin ratio, pectin concentration and voltage of the bead generator, were assessed on in vitro bead characteristics by release and swelling–erosion studies. The bead formulation was optimized by factorial design. Some measures were taken to improve the bead characteristics and prolong their integrity during the gastrointestinal transit, such as biomineralization of the beads or coating them with high-methoxy pectin (PHM) or Eudragit L30-D 55 (EU). Possible CB-TMC complexation was investigated by Job's Plot method. The suitable system was obtained by coating the optimized core formulation with PHM or EU. TMC was found to form a complex with CB as a model anionic drug. Therefore, TMC-drug interactions can be used to modify the release characteristics of dosage forms.

M S Crcarevska[77] et al prepared Eudragit S 100 coated chitosan–Ca–alginate microparticles efficiently loaded with budesonide (BDS), with bioadhesive and controlled release properties in GIT. Microparticles were spherical with mean particle size of 4.05–5.36 lm, narrow unimodal distribution and positive surface charge. A greater extent of calcium chloride limited the swelling ratio of beads, while swelling behavior of coated beads was mainly determined by properties of enteric coating. Comparing the release profiles of formulations, under different pH conditions, influence of polymer properties

and concentration of cross-linker on the rate and extent of drug release was evident. Coating has successfully sustained release of BDS in buffers at pH 2.0 and 6.8, while providing potential for efficient release of BDS at pH 7.4. Release data kinetics indicated influence of erosion and biodegradation of polymer matrix on drug release from microparticles. Prepared formulations were stable for 12 months period at controlled ambient conditions.

T Oosegi[78] et al investigated chitosan-succinyl-prednisolone conjugate microspheres (Ch-SP-MS) coated with Eudragit L100 and Eudragit S100. Sonication was utilized to prepare finer Ch-SP-MS, and the addition ratio of Eudragit was reduced to yield Eudragit-coated Ch-SP-MS with higher drug content. Ch-SP-MS and Eudragit-coated Ch-SP-MS had mean sizes of 1.3 lm and approximately 30 lm, respectively, and showed prednisolone (PD) contents of 4.6% (w/w) and approximately 3% (w/w), respectively. Morphological changes of all the types of microparticles in different pH media were observed by scanning electron microscopy and confocal laser scanning microscopy. Both methods gave similar results. Both types of Eudragit-coated Ch-SP-MS protected Ch-SP-MS from morphological change at pH 1.2, and regenerated Ch- SP-MS fast at pH 6.8 and 7.4. For all types of microparticles, release of PD was suppressed at pH 1.2, but caused gradually at pH 6.8. These particle characteristics and in vitro behaviors demonstrated that the present Eudragit-coated Ch-SP-MS were considered potentially suitable for in vivo or practical application as a specific delivery system of PD to IBD sites.

Y. S. Tanwar[79] et al evaluated floating microspheres of verapamil hydrochloride for improving the drug bioavailability by prolongation of gastric residence time. Cellulose acetate, acrycoat S100 and eudragit S100 microspheres loaded with verapamil hydrochloride were prepared by solvent diffusion evaporation method. The microspheres had smooth surfaces, with free-flowing and good-packing properties. The yield of the microspheres was up to 70.51% and cellulose acetate microspheres entrapped the maximum amount of the drug. Scanning electron microscopy confirmed their hollow structures with sizes in the range 251.80 to 350.75μm. The prepared microspheres exhibited prolonged drug release and remained buoyant for more than 12 h. Radiographic

images of dog stomach revealed that cellulose acetate microspheres loaded with barium sulphate floated on the gastric fluid for about 3.2 h. In vitro release studies demonstrated non-Fickian diffusion of drug from the microspheres.

Plan of Work:-

4.1. Identification of the drug

4.2. Establishment of calibration curve of metoprolol succinate (MTS) in different buffer medium and solvent

4.3. Microspheres preparation and optimization

4.4. Scanning electron microscope (SEM) image, FT-IR spectroscopy study and DSC study

4.5. Studies of drug release rate kinetics using different pharmacokinetic model fitting

4.6 Optimization of Erodible tablet

4.7 Optimization of ethyl cellulose coating on capsule body and eudragit L-100 55 coating on capsule cap

4.8 3^2 Factorial design

4.1 IDENTIFICATION OF DRUG:

4.1.1 Melting point determination of Metoprolol succinate (MTS):

The thiel's tube method of melting point determination in liquid paraffin was used in the present investigation. The melting point of MTS was found to be 135ºC. The reported standard value of melting point of MTS is 136-137ºC with decomposition. The observed value is closer to the standard value. This indicated good purity of drug sample.

4.1.2 UV absorbance Spectra of MTS:

Powder of MTS equivalent to 50 mg of metoprolol tartrate was accurately weighed and transferred into the 250 ml volumetric flask and then volume was made using pH 6.8 phosphate buffer and resultant solution (200µg/ml) was scanned in the range of 200 nm to 400 nm using Shimadzu double beam UV/Visible spectrophotometer. The absorption maximum was found to be 274 nm and the absorption value was 0.9803**.**

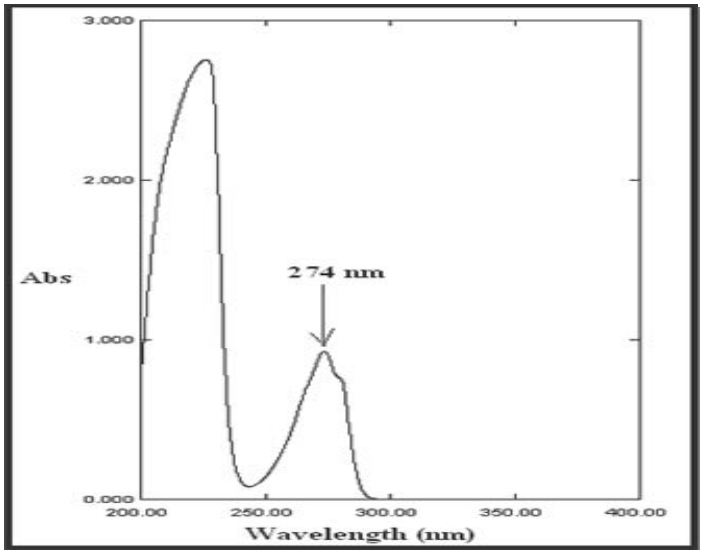


Figure 4.1 : UV absorption maxima of MTS

4.1.3 FT IR spectra of MTS[80]

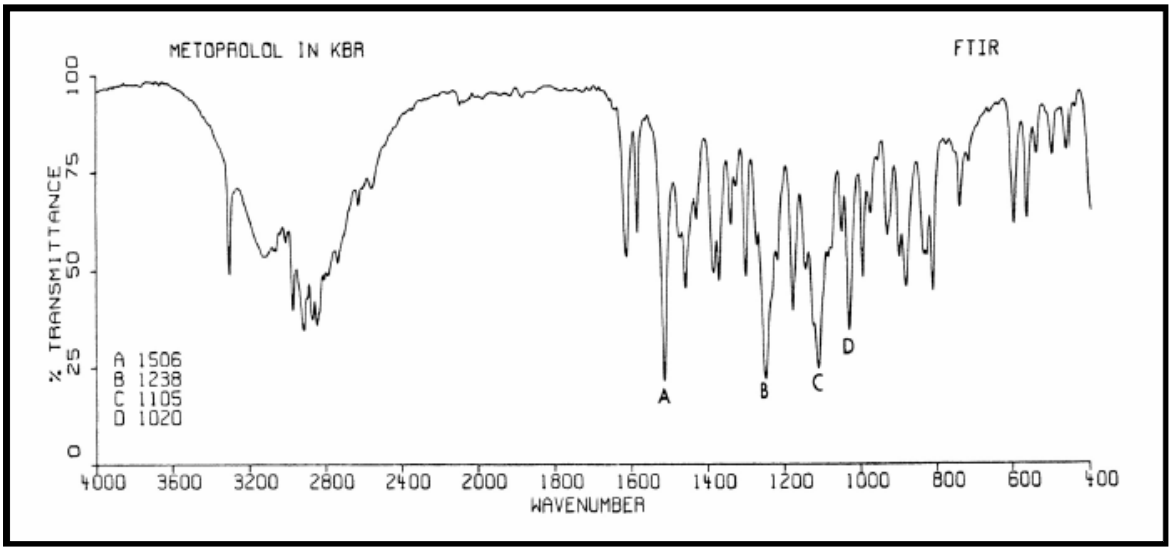


Figure 4.2 : FT IR spectra of reference MTS

TABLE 4.3 Characteristic peak of MTS

SR NO.	Functional Group	Standard Absorbance Peak	Obtained Absorbance Peak
1	Carboxylic acid salt	1580 cm^{-1}	1564.32 cm^{-1}
2	secondary amines	>3000 cm^{-1}	3554.93 cm^{-1}
3	alcohols	3100-3300 cm^{-1}	3171.8 cm^{-1}
4	Isopropyl group	1180 cm^{-1}	1186.26 cm^{-1}

Results :

From the data shown in Table 4.3 it was confirmed that sample drug is metoprolol succinate and having high purity.

4.2 Establishment of calibration curve of MTS in different buffer medium and solvent:

4.2.1 Establishment of calibration curve of MTS in 0.1 N hydrochloric acid

Powder of MTS equivalent to 50 mg of metoprolol tartrate was accurately weighed and transferred into the 250 ml volumetric flask and than volume was made using 0.1 N HCL having pH 1.2. From this stock solution (200 µg/ml), different standard solutions with the concentration ranging from 40 µg/ml to 180 µg/ml were prepared. The absorbance was measured at λ_{max} 274nm using Shimadzu double beam UV/Visible spectrophotometer in triplicate and the plot of average absorbance vs. concentration was established.

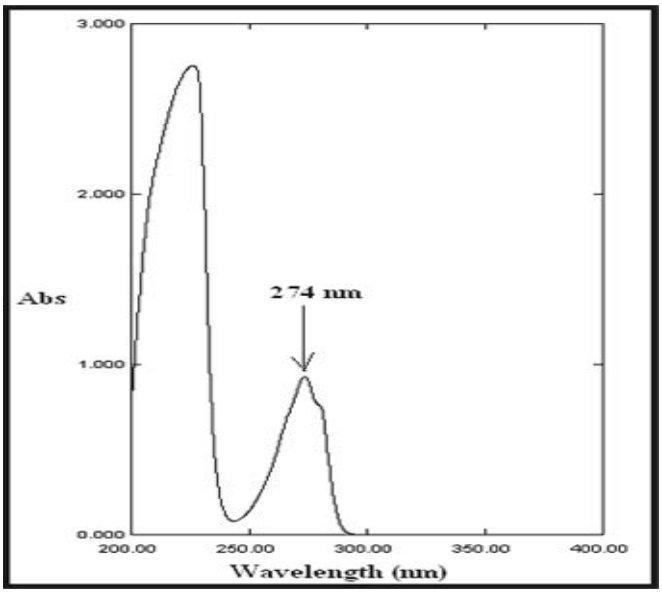


Figure 4.4 UV spectra of MTS in 0.1 N HCL pH 1.2

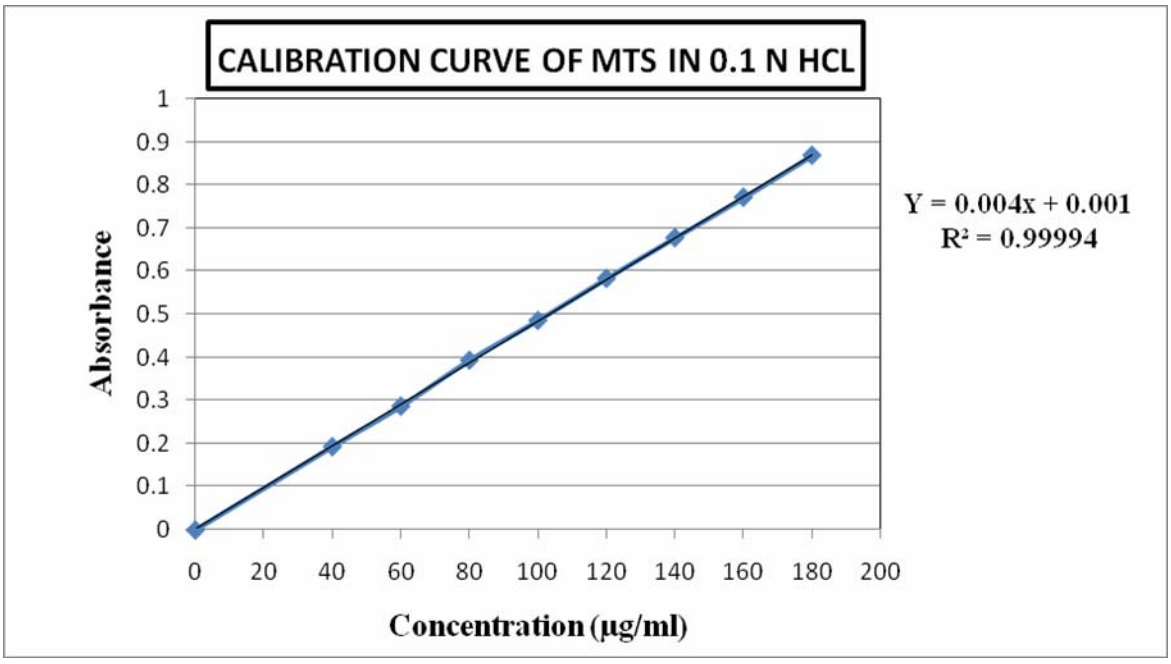


Figure 4.5: Calibration curve of MTS in 0.1 N HCL

According to Beer–Lambert law, the linearity range was found in between 0 to 180 µg/ml and R^2 value was near to 1.

4.2.2 Establishment of calibration curve of MTS in phosphate buffer pH 7.4

Powder of MTS equivalent to 50 mg of metoprolol tartrate was accurately weighed and transferred into the 250 ml volumetric flask and than volume was made using phosphate buffer having pH 7.4. From this stock solution (200 µg/ml), different standard solutions with the concentration ranging from 40 µg/ml to 180 µg/ml were prepared. The absorbance was measured at λ_{max} 274nm using Shimadzu double beam UV/Visible spectrophotometer in triplicate and the plot of average absorbance vs. concentration was established.

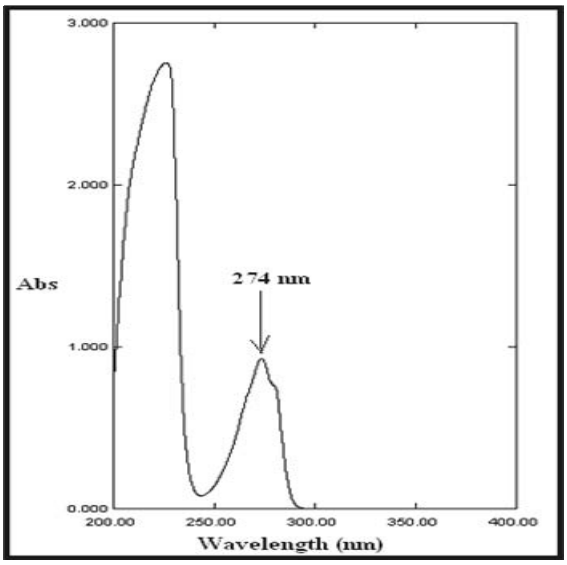


Figure 4.6 UV spectra of MTS in phosphate buffer pH 7.4

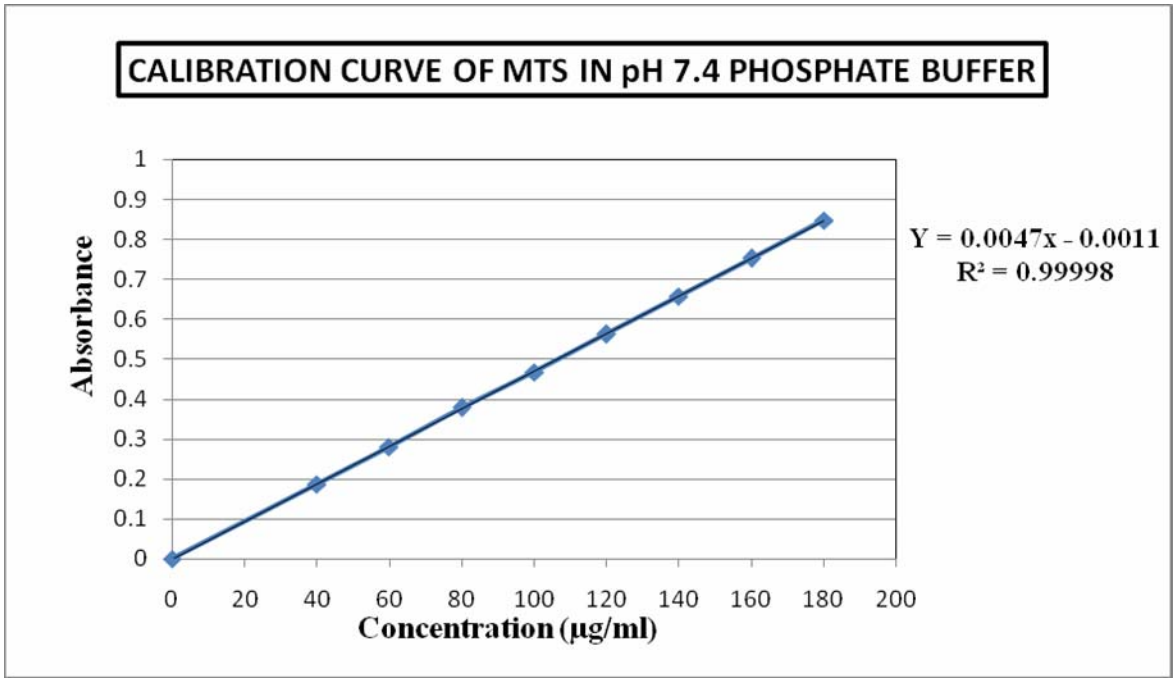


Figure 4.7: Calibration curve of MTS in phosphate buffer pH 7.4

According to Beer–Lambert law, the linearity range was found in between 0 to 180 µg/ml and R^2 value was near to 1.

4.2.3 Establishment of calibration curve of MTS in pH 6.8 phosphate buffer

Powder of MTS equivalent to 50 mg of metoprolol tartrate was accurately weighed and transferred into the 250 ml volumetric flask and than volume was made using pH 6.8 phosphate buffer. From this stock solution (200 µg/ml), different standard solutions with the concentration ranging from 40 µg/ml to 180 µg/ml were prepared. The absorbance was measured at λ_{max} 274nm using Shimadzu double beam UV/Visible spectrophotometer in triplicate and the plot of average absorbance vs. concentration was established.

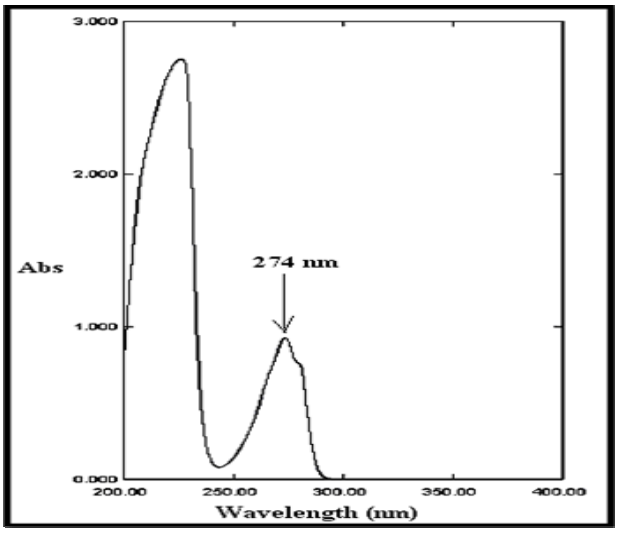


Figure 3.8: UV spectra of MTS in phosphate buffer pH 6.8

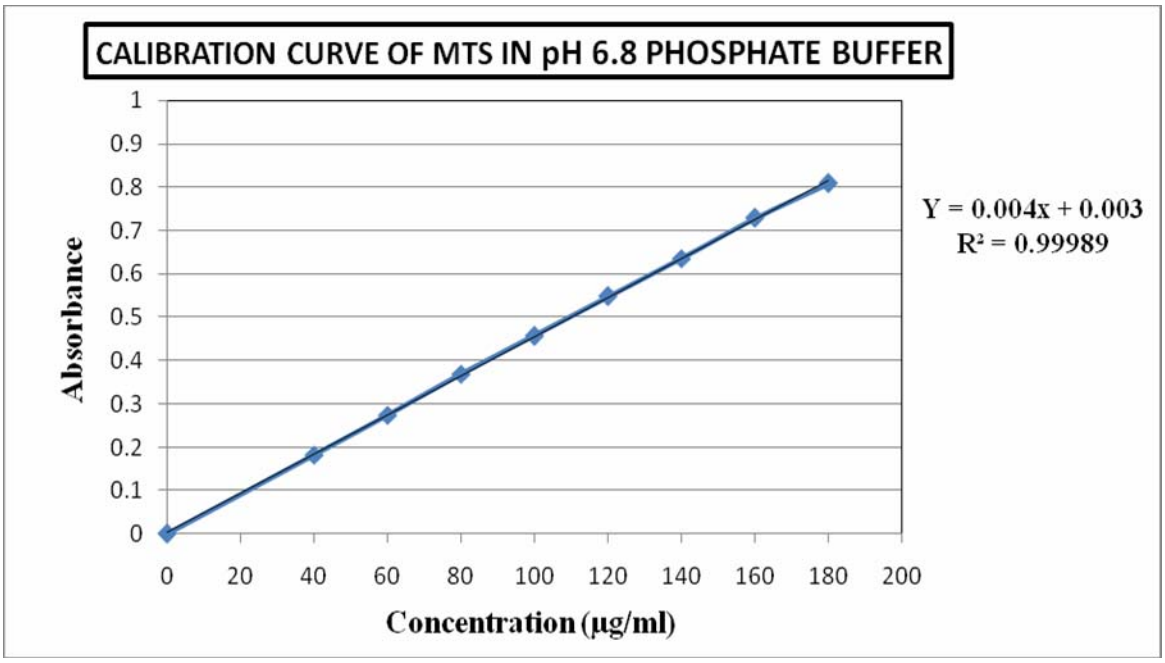


Figure 4.9: Calibration curve of MTS in pH 6.8 phosphate buffer

According to Beer–Lambert law, the linearity range was found in between 0 to 180 µg/ml and R^2 value was near to 1.

4.2.4 Establishment of calibration curve of MTS in solvent B :

Powder of MTS equivalent to 50 mg of metoprolol tartrate was accurately weighed and transferred into the 250 ml volumetric flask and than volume was made using solvent B. From this stock solution (200 µg/ml), different standard solutions with the concentration ranging from 40 µg/ml to 180 µg/ml were prepared. The absorbance was measured at λ_{max} 276 nm using Shimadzu double beam UV/Visible spectrophotometer in triplicate and the plot of average absorbance vs. concentration was established.

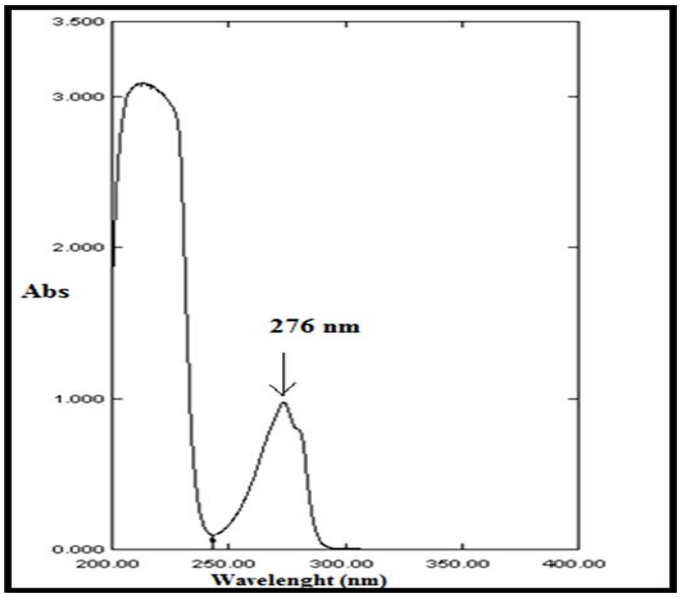


Figure 4.10 UV spectra of MTS in solvent B

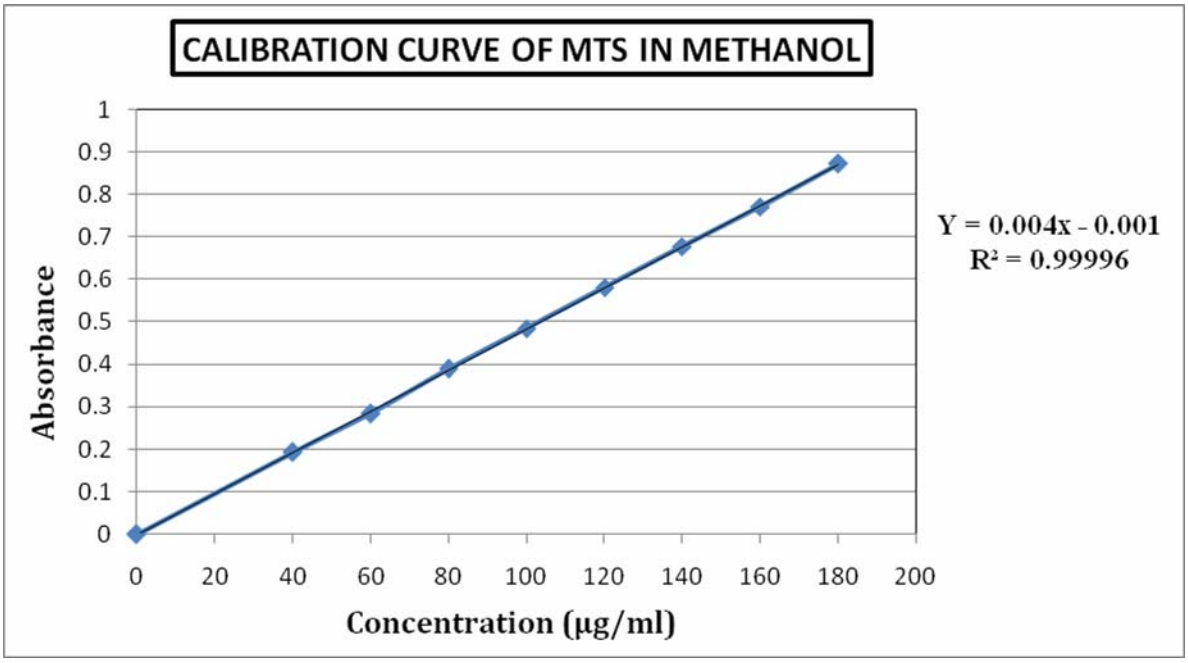


Figure 4.11: Calibration curve of MTS in Solvent B

According to Beer–Lambert law, the linearity range was found in between 0 to 180 µg/ml and R^2 value was near to 1.

4.3 Preparation & Optimization of Microspheres:

4.3.1 Material used:

Drug : Metoprolol succinate (MTS)

Polymers : Eudragit RS 100, Eudragit RL 100

Emulsifiers : Surfactant A

External Phase : Liquid paraffin B

Internal Phase : Solvent A/solvent B mixture

4.3.2 Method of Preparation:

Microspheres were prepared by *single emulsion solvent evaporation* method. Accurately weighed amount of MTS was dissolved in appropriate volume of solvent B. Simultaneously Eudragit (RS 100/RL 100) was dissolved in appropriate volume of solvent A. The drug-polymer solution was sonicated for 15 minutes till the solution became clear. The resulting dispersion was then poured into a Beaker containing the mixture of appropriate volume of liquid paraffin. Stirring was continued until solvent evaporated completely. Filtered the resulting mixture using vacuum filtration technique and remove adhered liquid paraffin from microspheres by washing it with appropriate solvent. Microspheres obtained were dried in oven at 45ºC.

4.3.3 Evaluation Parameters for Microspheres:

1. Particle Size:

Particle size analysis was carried out using optical microscopic method. A calibrated stage micrometer was used for measuring the size of microspheres. Microspheres were spread on glass slide and observed under microscope. About 100 particles were counted and mean particle size was calculated for each batch.

2. Drug Entrapment Efficiency:

Accurately weighed 100 mg microspheres were taken in 100 ml volumetric flask containing solvent B. The final volume was made up and one ml of resulting solution diluted ten times. The solution was filtered through whatmann no.44 filter paper and the filtered solution was analyzed spectrophotometrically for determining the amount of drug entrapped in the microspheres.

The Percentage Entrapment Efficiency *(PEE)* was calculated from the following formula.

$$\text{PEE} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of drug used in formulation}} \times 100$$

3. % Drug Loading:

Same procedure had followed as for determining PEE. Then % drug loading was calculated from the following formula.

$$\%\ \text{Drug Loading} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres formed}} \times 100$$

4. In-Vitro Drug Release Study of Microspheres:

In-Vitro drug release study was performed using USP Dissolution Apparatus Type-1 (Rotating Basket). Samples of microspheres equivalent to 50 mg drug were tested using 500 ml 6.8 pH phosphate buffer as dissolution medium. The rotating basket containing samples of microspheres were covered with the muslin cloth and the rotational speed was set at 100 rpm. 7ml of sample solution was withdrawn at predetermined time intervals, filtered through whatman filter paper and analyzed spectrophotometrically. An equal amount fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using beer–Lambert equation.

5. 4. In-Vitro Drug Release Study of pulsatile device :

In-Vitro drug release study was performed using USP Dissolution Apparatus Type-2 (Rotating Paddle). Pulsatile devices containing microspheres equivalent to 50 mg of drug were kept at bottom of the bowl using sinkers to prevent floating. They were tested in the presence of 500 ml of dissolution medium using 0.1 N hydrochloric acid for first 2 hr (gastric transit time), phosphate buffer pH 7.4 for 3 hr (intestinal transit time) and phosphate buffer pH 6.8 for 12 hr at rotational speed of 100 rpm. 7ml of sample solution was withdrawn at predetermined time intervals, filtered through whatman filter paper and analyzed spectrophotometrically. An equal amount fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percentage drug dissolved at different time intervals was calculated using beer–Lambert equation.

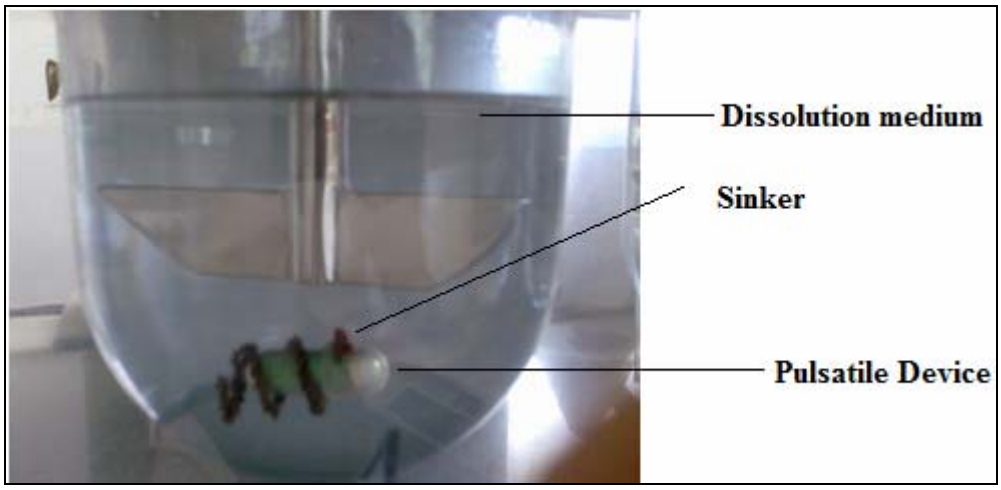


Figure 4.12: In-Vitro Drug Release Study of pulsatile device

5. Scanning Electron Microscopy (SEM):

Scanning electron microscopy was done at SICART (Vallabh Vidhyanagar) to confirm the sphericity of microspheres.

6. IR Spectroscopy:

IR spectroscopy of MTS alone and mixture were done for confirmation of MTS entrapment in Eudragti RS / RL 100.

4.3.4 Trials for Microspheres preparation:

The objective of this work was to formulate microspheres having good PEE, drug loading, sphericity, sustained drug release up to 12 hrs and least aggregation.

4.3.4.1 Blank Microspheres:

- Selection of external phase
- Selection of stirrer position
- Selection of amount of Non-Solvent

4.3.4.2 Drug Loaded Microspheres:

- Selection of Solvent Combination
- Optimization of drug to polymer ratio
- Optimization of amount of Surfactant A
- Optimization of Stirring Speed
- Optimization Hardening Time
- Optimization of Eudragit RS 100 to Eudragit RL 100 ratio

4.3.4.1 Blank Microspheres :

Selection of External Phase

Formula:

Eudragit RS 100 : 10 % w/v in solvent B
Surfactant A : 0.2 %
Hardening time : 5 hrs
Stirring speed : 1000 rpm
External phase : Batch LP-1:- Liquid Paraffin B
: Batch LP-2:- Liquid Paraffin A
: Batch LP-3:- Liquid Paraffin (A:B) (1:1)

Results and Discussions:

TABLE 4.12 Selection of External phase

Batch	Remarks
Batch LP-1	Spherical microspheres formed
Batch LP-2	Stable emulsion formed
Batch LP-3	Flakes were formed

The microspheres were not formed when liquid paraffin(A) and the ratio of light and heavy liquid paraffin was used. But with liquid paraffin(B), spherical microspheres were formed. Thus liquid paraffin (B) was selected as an external phase for preparation of microspheres.

Selection of Stirrer Position

Formula:

Eudragit RS 100 : 10 % w/v in solvent B
Surfactant A : 0.2 %
External phase : liquid paraffin B
Hardening time : 5 hrs
Stirring speed : 1000 rpm

Stirrer position : Batch STP-1: Stirrer at position a
: Batch STP-2: Stirrer at position b
: Batch STP-3: Stirrer at position c

Results and Discussions:

Microspheres of batch STP-1 had irregular particle size and were smaller than other batches. Microspheres of batch STP-3 were spherical in shape and were larger than other batches. While microspheres of the batch STP-2 exhibited better sphericity and were of average particle size. Thus stirrer was kept in position b for further studies.

Selection of volume of Non-Solvent

Formula:

Eudragit RS 100 : 10 % w/v in solvent A
Surfactant A : 0.2 %
External phase : Liquid paraffin B
Stirring speed : 1000 rpm
Volume of Hexane : Batch NS-1: 10 ml
: Batch NS-2: 20 ml
: Batch NS-3: 30 ml

Results:

TABLE 4.13 Selection of volume of Non-solvent

Batch	% Yield	Hardening time (min)	Sphericity
Batch NS-1	89	285	++++
Batch NS-2	84	255	++++
Batch NS-3	72	230	++

+ Poor, ++ Average, +++ Good, ++++ Very good

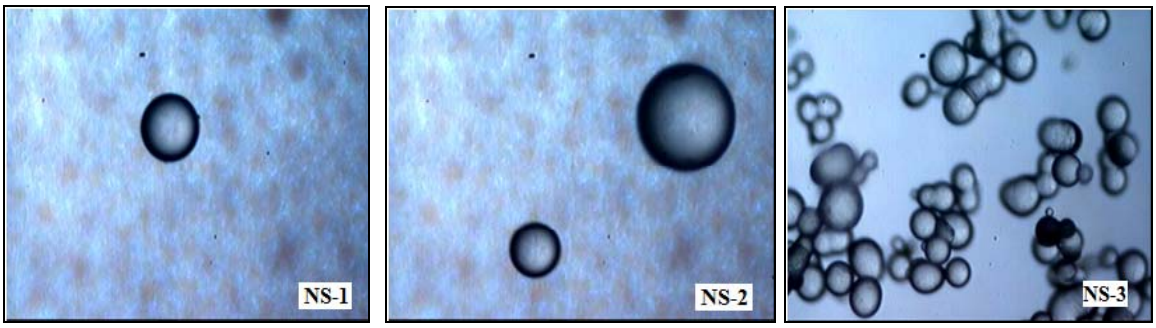


Figure : 4.14 Microspheres for optimization of volume of non-solvent

Discussions:

Results suggested that as the volume of non-solvent increases there was decrease in hardening time due to increase in diffusivity of solvent A:solvent B from the external phase (Liquid paraffin B oil). But if we increase above 20 ml, it showed decrease in yield of microspheres as well as sphericity.

4.3.4.2 Drug Loaded Microspheres:

(A) Selection of Solvent Combination

As metoprolol succinate practically insoluble in solvent A so there was a need to use another solvent in which drug is soluble. Different solvents were tried in combination with solvent A to formulate microspheres.

TABLE 4.14 Selection of solvent combination

Batch	Solvent A	Solvent B	Solvent C	Total Volume
SC-1	5	5	-	10
SC-2	5	-	5	10
SC-3	5	2.5	-	7.5
SC-4	5	-	2.5	7.5
SC-5	5	2.5	2.5	10

Results:

TABLE 4.15 Effect of solvent combination

Batch	% Yield	PEE	Sphericity	Hardening Time (hr)
SC-1	88	79	+++	10
SC-2	89	84	+++	16
SC-3	91	89	+++	8
SC-4	87	83	+++	12
SC-5	90	82	+++	11

+ Poor, ++ Average, +++ Good, ++++ Very good

Discussions:

Results showed that all the batches have high percentage entrapment efficiency, percentage yield and sphericity. But when we compared the stirring time the batch SC-3 required lowest hardening time (8 hr) in comparison to other batches. Thus 5 ml of solvent A and 2.5 ml of solvent B mixture (total 7.5 ml) used for further optimization of various parameters.

(B) Optimization of drug to polymer ratio

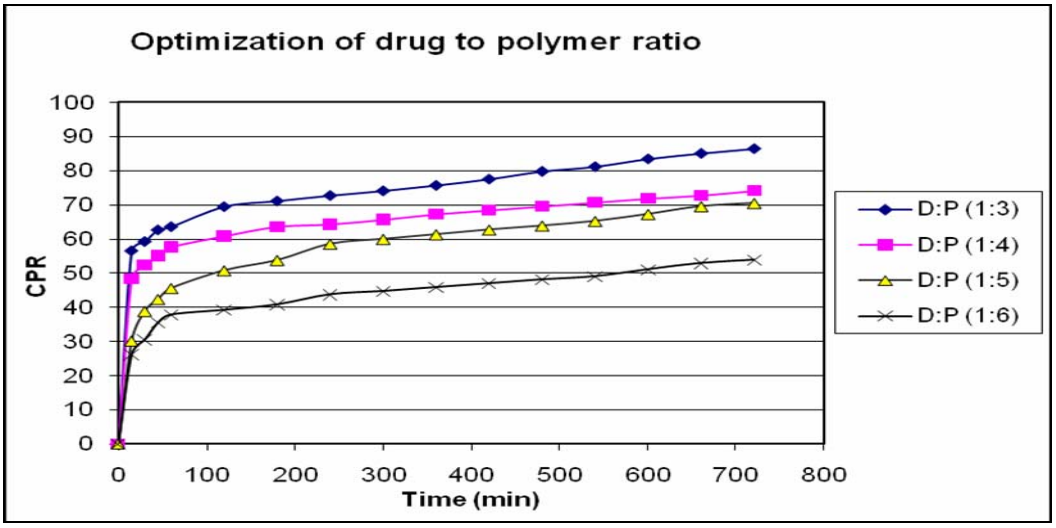


Figure: 4.16 In-vitro drug release profile of batches with different drug to polymer Ratio

Result & Conclusion :

Drug to polymer ratio had a significant effect on entrapment efficiency. The PEE of batches with different drug to polymer ratios shown in table. As the polymer ratio was increased there was increase in the PEE. All the batches had almost more than 80 % of yield and entrapment efficiency.

Drug release profiles of all batches had compared as shown in figure which indicated that the drug release could be more sustained at higher polymer ratio. The rate of release of Metoprolol succinate from the Eudragit microspheres followed ***biphasic kinetic mechanism***: an initial burst effect, which was probably due to release of the non entrapped surface adhered drug, followed by slow release which controlled by rate of permeation of the dissolution medium through polymer matrix.

Microspheres were not obtained using drug : polymer ratio of 1:1 and 1:2. Batches DP-5 and DP-6 had shown good and excellent sphericity respectively, while batches DP-3 and DP-4 exhibited average sphericity.

Although drug release of batch DP-3 and DP-4 was 86.49 and 74.16 percentage respectively within 12 hour, they showed very high burst release effect up to 56.60 and 48.38 percentages respectively which was not desirable. Batch DP-5 showed 70.51 percentage drug release. Thus drug to polymer ratio 1:5 was considered for the further study.

(C) <u>Optimization of surfactant amount</u>

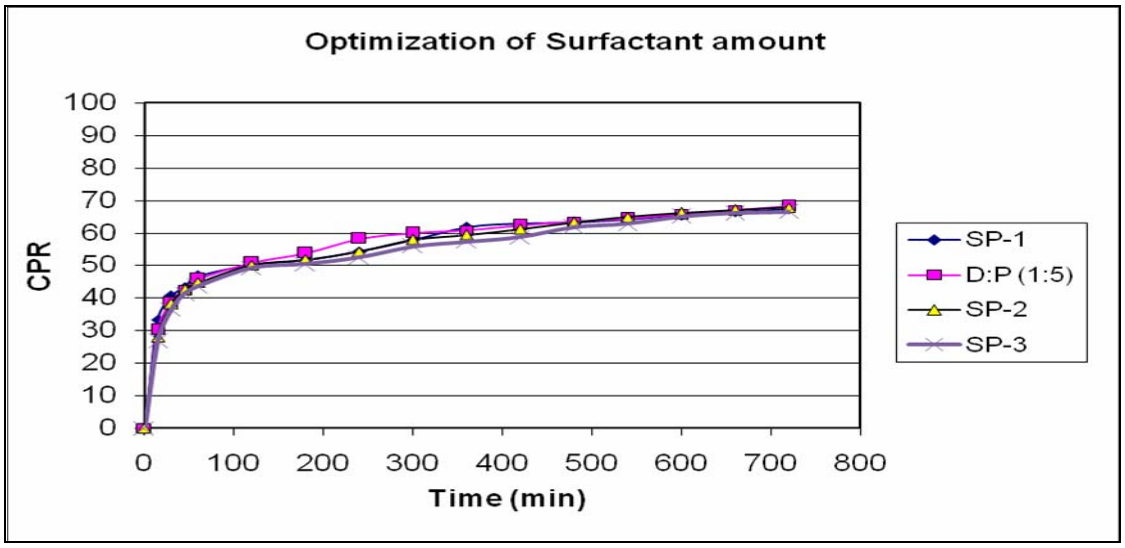


Figure : 4.17 In-vitro drug release profile of batches with different % of surfactant

Results and Discussions:

Surfactant A was used in different concentration to find its optimal amount to produce the microspheres having excellent sphericity. Amount of Surfactant A exhibited no significant effect on the PEE, drug release.

Sphericity of microspheres was found to be increased with an increase in the amount of Surfactant A. Surfactant A is a nonionic lypophilic surfactant mainly responsible for the sphericity of the microspheres.

Although all Batches had similar drug release up to almost 70 % in 12 hr. But as the percentage of Surfactant A increases there was increase in the sphericity of microspheres. Batches SP-2 and SP-3 have almost similar percentage drug release, percentage entrapment efficiency, drug loading and sphericity. So the batch SP-2 having 0.3 % Surfactant A chosen for further optimization.

(D) Optimization of stirring speed

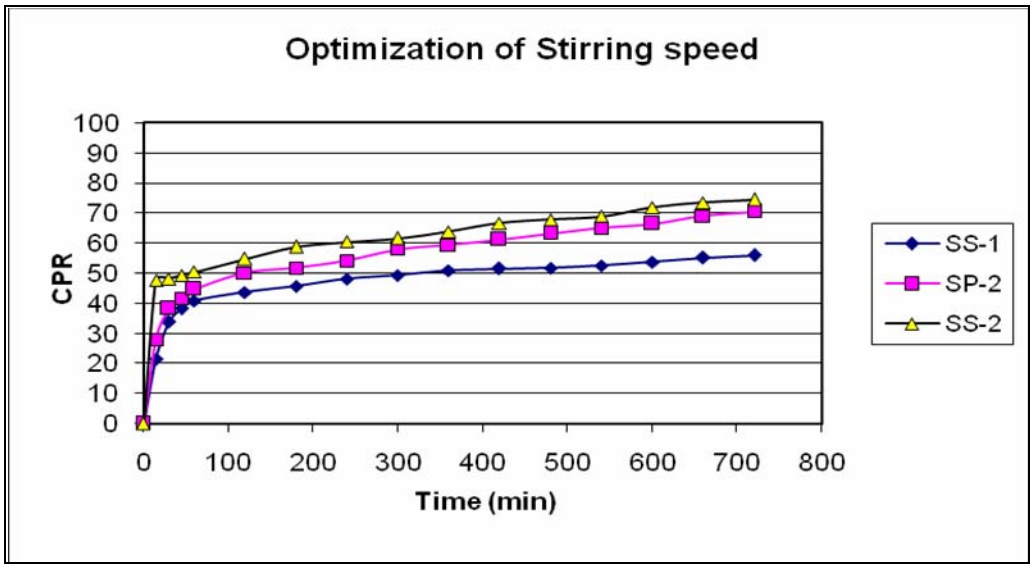


Figure : 4.18 In-vitro drug release profile of batches with different stirring speed

Result & Discussion:

Drug release from the microspheres was found to be highly affected by the stirring speed. Batches with higher stirring speed had shown faster drug release. This could be explained on the basis that at higher stirring speed, smaller particles produced which resulted in an increased surface area and improved drug dissolution and hence drug release.

Batch SS-2 had shown higher drug but it showed initial burst release up to 47.71 % in first 15 min which is not beneficial. Batch SP-2 showed higher drug release up to 67.77 % in 12 hr compare to SS-1 batch.

(E) Optimization of hardening time

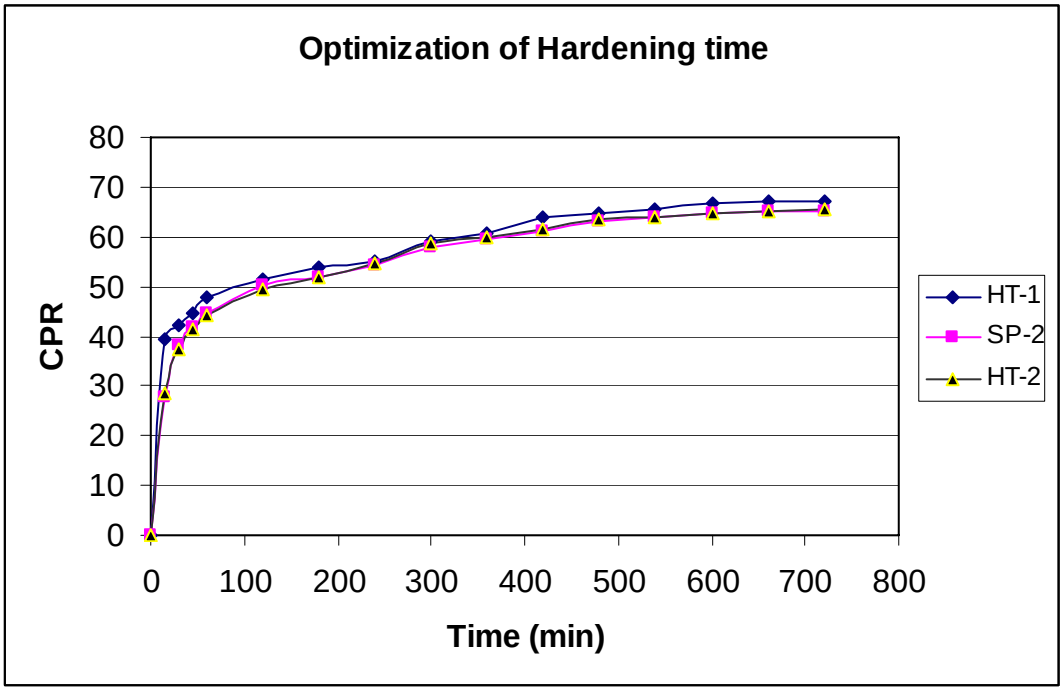


Figure : 4.19 In-vitro drug release profile of batches with different Hardening time

Result & Discussion:

Duration of hardening time was affected release of the drug from the polymeric structure. Drug release was found to be increased with a decrease in hardening time. This might be due to that at lower hardening time, drug was not properly entrapped in polymeric matrix and got easily released. Thus initial burst release up to 39.27 % within 15 minute was observed in batch HT-1.

While in case of batch SP-2 and HT-2, microspheres was properly hardened and became dense, so drug release was found to be sustained. Drug released from both batches observed were sme. Thus the hardening time with 8 hr used for the further optimization of microspheres.

(F) Microspheres preparation using combination of polymer (Eudragit RS 100 & Eudragit RL 100)

Microspheres obtained with the use of Eudragit RS 100 alone showed maximum drug release up to 68% till 12 hour. So If we increased the permeability of polymer matrix by incorporation of highly permeable polymer we can get higher percentage of drug release.

Eudragit RL 100 classified under the same category as that of Eudragit RS 100 and it has higher amount of amino group present so it has higher permeability compare to the Eudragit RS 100. So various ratios of Eudragit RS 100 to Eudragit RL 100 were tried to get 100% drug release in 12 hours.

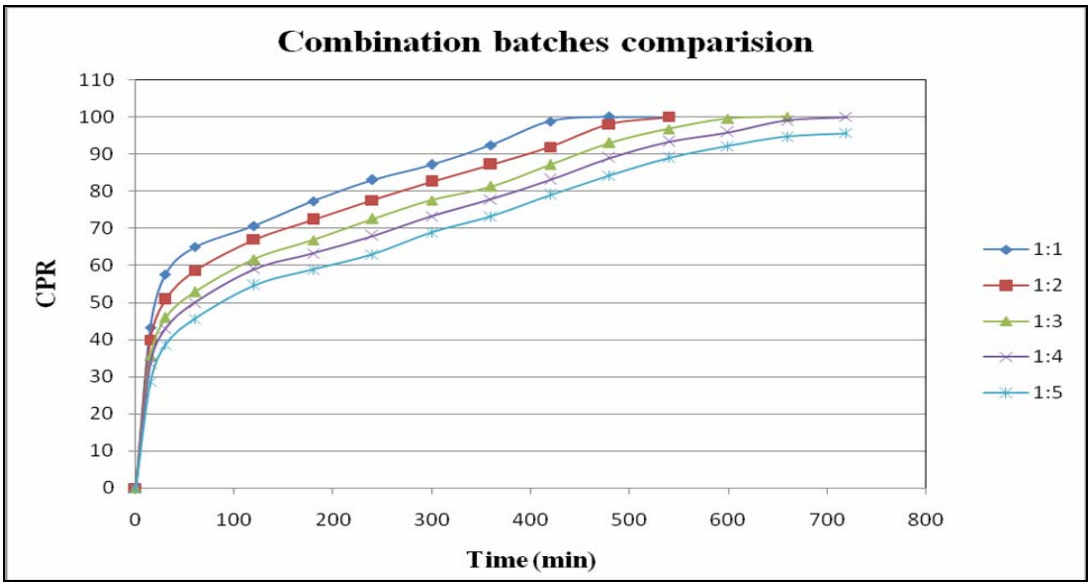


Figure : 4.20 In-vitro drug release profile of Eudragit RS/RL combination batches

Result & Discussion:

Different ration of Eudragit RL 100 to Eudragit RS 100 were tried to get maximum drug release up to 12 hr with minimum initial burst release. It showed as the amount of Eudragit RL 100 increased there was increase in percentage drug release due to high permeability nature of Eudragit RL 100. But simultaneously increase in initial burst release was observed.

When we compared dissolution profile of all five batches it showed that batch CB-4 having Eudragit RL:Eudragit RS 100 ratio (1:4) gave 100 % drug release within 12 hour. This batch was further used for development of programmable drug delivery system.

4.5 STUDIES OF DRUG RELEASE RATE KINETICS[81]

Release of drug from the insoluble matrix is extremely highlighted in the literature. To obtain the dissolution rate constants of drug from matrices, various models are reported. Various models were tried to fit the optimized batch to determine the mechanism of drug release.

Zero order model:

In many of the modified release dosage forms particularly controlled or sustained release dosage form is zero-order kinetics.

$$M = k*t$$

Where k is zero order rate constant, m is % drug unreleased (or released) and t is time. The plot of % drug unreleased (or released) vs. time is linear.

First order model:

Most conventional dosage forms exhibit this dissolution mechanism. Some modified release preparations, particularly prolonged release formulations, adhere to this type of dissolution pattern.

$$M = e^{a} * e^{-bt}$$

Where, a is intercept and b is slope.

It assumes that the drug molecules diffuse out through a gel like layer formd around the drug during the dissolution process. A plot of log % drug released vs time is linear.

Higuchi model:

A large number of modified released forms contain some sort of matrix system. In such instances, the drug dissolves from this matrix. The dissolution pattern of the drug is dictated by water penetration rate (diffusion controlled) and thus the following relationship applies.

$$M = (100-q)* \text{sqrt of time}$$

Where, q is the Higuchi constant (% per square root of time)

In Higuchi model, a plot of % drug unreleased (or released) vs. sqrt of time is linear.

Korsmeyer-Peppas model:

$$Mt/M = k*t^n$$

Where, Mt/M is the fraction of drug released at time 't'.

n is diffusion exponential;

If n = 1, the release is of zero order,

N = 0.5, release best explained by Fickian diffusion,

0.5<n<1, release is through anomalous diffusion or case-II diffusion.

A plot of log fraction of drug release vs. log t is linear.

Hixon-Crowell model:

Some dosage forms contain many particles of the same size and shape or their agglomerates that dissolve evenly. In such instances the cube root law can express the dissolution process.

$$M = (100^{1/3} – (k*t))^3$$

Where, k is the Hixon-crowell constant (mass/time) $^{1/3}$.

In this model, the % drug unreleased vs. cube root of time is linear.

Weibull distribution model:

When applied to the dissolution data, the Weibull equation expresses the accumulated fraction of material in solution at time by;

$$M = 1 – \exp (- (t-ti)^{b/a})$$

Where, a = scale parameter which defines the time scale of the process. It is location parameter which represents the lag period before the actual onset of dissolution process (in most cases, ti=0) and b is the shape parameter.

Plot of log time vs. ln(l-m) is linear.

Table 4.40 RESULTS OF MODEL FITTING

Models	SSR Value	F value
Zero order	1849.245193	142.2496302
First order	11036.44585	848.9573728
Higuchi	498.7441602	38.3649354
Korsmeyer-Peppas	98.96815193	8.247345994
Weibull	804.1881	67.01567
Hixon-Crowell	328.8603	27.40502

RESULTS AND DISCUSSION:

Goodness of fit test for optimized batch (CB-4) was conducted using various models like Zero order, First order, Higuchi, Korsmeyer-Peppas, Weibull and Hixon-Crowell.

Release of optimized batch best fitted to **Korsmeyer-Peppas** equation showing least SSR and F value. Thus, it could be concluded that release of MTS from microspheres was diffusion controlled.

4.6 Optimization of formula for Erodible tablet:

Each size 0 capsules were filled with 300 mg of colored granules made from lactose using wet granulation technique with 5% PVP in isopropyl alcohol (IPA) to determine the end point (time required to color dissolution medium) in USP dissolution apparatus at 100 rpm in presence of phosphate buffer pH 7.4 (simulating intestinal fluid).

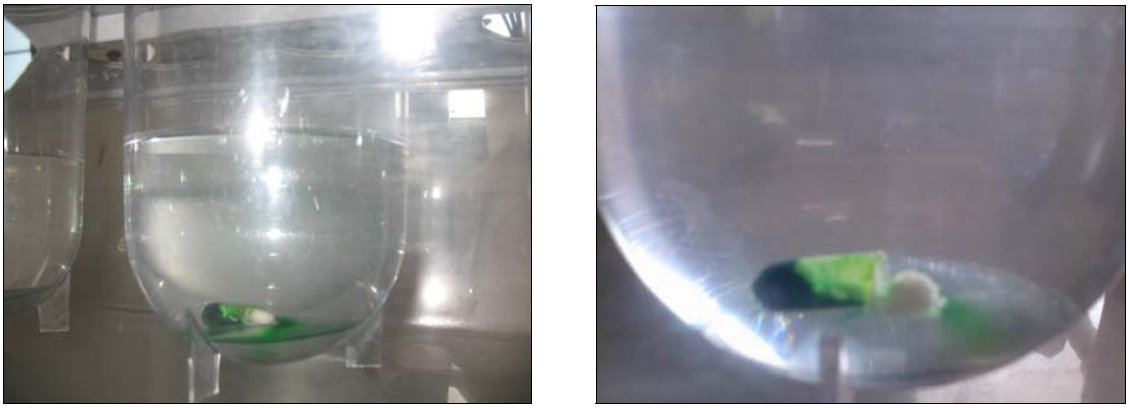


Figure : 4.28 Determination of lag time using colored granules

Formation of erodible tablets:

Tablets were prepared by direct compression as well as wet granulation technology using different grades of Hydroxypropyl methyl cellulose and observed for lag time.

Wet granulation technique

Various grades of HPMC were mixed with lactose in different proportion in mortar and pestle and granules were prepared using 5% PVP in isopropyl alcohol. Resulting mass was passed through 22# sieve to get uniform size granules. Granules were compressed to get tablet of 120 mg weight. They all were observed for lag time.

Hardness of all tablets were restricted between 3-4 kg/cm^2 .

Dry granulation technique

Various grades of HPMC were mixed with lactose in different proportion in mortar and pestle and mixed it with 5% PVP. Resulting powder was passed through 44# sieve to get uniform size powder. Powder was compressed to get tablet of 120 ± 10 mg weight. They were observed for lag time.

Hardness of all tablets were restricted between 3-4 kg/cm^2.

Results:

From the results it was concluded that dry compression technique gives small range of lag time compare to wet granulation technique due to absence of enough binding force between the particles.

4.7 Optimization of ethyl cellulose coating on capsule body and eudragit L-100 55 coating on capsule cap :

Coating of capsule body:

Size 0 capsules were coated with 5 % dispersion of ethyl cellulose in solvent A using dip coating method. 10 % weight gain was enough to make capsule body insoluble.

Enteric coating of capsule caps:

Coating was done using 10 % dispersion of Eudragit L 100-55 in solvent A by dip coating method to prevent premature release in stomach. Various percentage of

Eudragit RL 100-55 coating were applied on capsule cap surface and check for it's strength to prevent rupturing in 0.1 N hydrochloric acid (simulated gastric fluid) until 2 hr.

Dissolution profile of programmable device:

Dissolution study of whole programmable device was carried out in 0.1 N hydrochloric acid (gastric fluid pH) for 2 hr (gastric emptying time) than in phosphate buffer pH 7.4 (intestinal pH) for 3 hr (intestinal transit time) and lastly in phosphate buffer pH 6.8 (colonic pH) for 12 hrs using USP tablet dissolution apparatus type 2 at 100 rpm and 500 ml of dissolution medium.

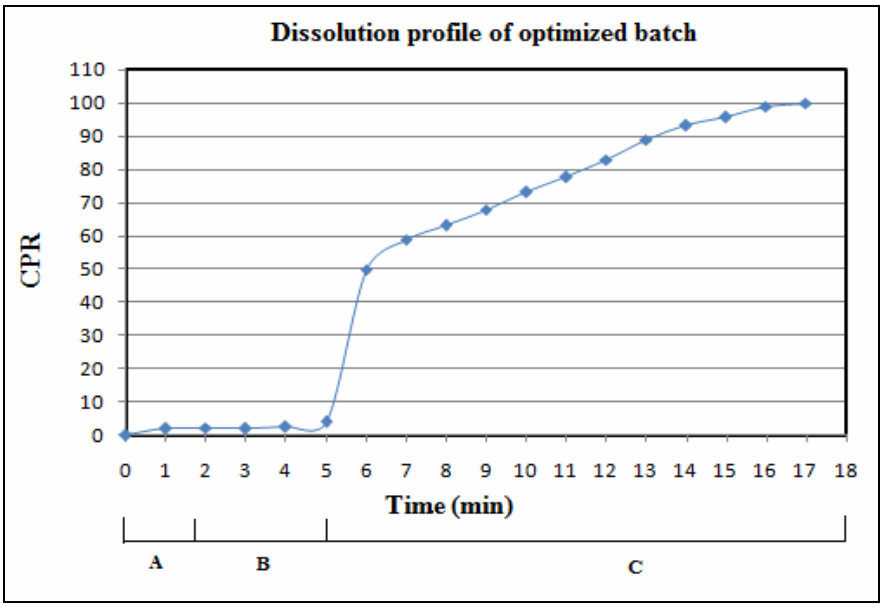
Formulation Ingredients:

Microspheres – Batch CB-4 (Eudargit RL 100 to Eudragit RS 100 Ratio 1:4)

Erodible tablet – BatchEW-1

Capsule body – 10 % Ethyl cellulose coating

Enteric coated cap - 10 % Eudragit L 100-55 coating



A-0.1 N hydrochloric acid, B- phosphate buffer pH 7.4, C- phosphate buffer pH 6.8

Figure : 4.32 In-vitro drug release profile of optimized pulsincap device

Discussion :

Optimized batch showed negligible release in 0.1 N hydrochloric acid for 2 hr and pH 7.4 phosphate buffer for 3 hr. After 5 hr of dissolution there was initial burst release of metoprolol followed by gradual release up to 12 hr. Initial burst release of metorpolol may be due to presence of high surface adhered drug molecule.

So, it is beneficial to prevent early morning rise in blood pressure and maintained it in normal value till late afternoon.

4.8 INTRODUCTION TO FACTORIAL DESIGN[82]:

The design of an experiment can be simply defined as the plan that governs the plan that governs the performance of an experiment.

It is the best interest of pharmaceutical scientist to understand the theoretical formulation and the target processing parameter and the formulation development should be done in the shortest possible time, using minimum number of men's hours and quantity of raw material. The developed formula is then tried at the pilot scale-up therefore, it is very essential to study the formulation from all the perspective at laboratory levels.

In addition to the art of the formulations, a statistical technique is available that can aid in the pharmacist's choice of formulation components, which can optimize one or more formulation attributes.

The traditional experiments require greater efforts and time, especially where complex formulations are to be developed. A very efficient way to enhance the value of research and to minimize the process development time is through design experiments.

Factorial designs are used in experiments when the effects of different factors or conditions, on experiment results are to be elucidated. Factors may be qualitative or quantitative. The levels of an each factor are the value or designation assigned to combination of all levels of all factor. The effect of a factor is the change in response caused by varying the levels of the factor.

The full factorial design is designated by following nomenclature;

$N=L^K$

Where; K = number of variables, L = number of variables levels, N = number of the experimental trials.

The objective of the factorial design is to characterize the effect of changing the levels of the factor or combination of factors on the response variable. Predictions based on the results of an undesired experiment will be more variable than those, which could be obtained in a designed experiment, in particular factorial design.

The optimization procedure is facilitated by construction of an equation that describes the experimental results as a function of the factor levels. A polynomial equation can be constructed, where the coefficients in the equation are related to the effects and interaction of the factors. The equation constructed form 3^n factorial experiment is in the following form.

$$\mathbf{Y= B_O +B_1X_1+B_2X2\ldots\ldots B_nX_n+ B_{12}X_1X_2+ B_1X_1^2 + B_{22}X_2^2 \ldots B_{mn}X_n^2 \ldots\ldots (1)}$$

Where,

Y= the measured response,

X_i = level of i^{th} factor

Bi,Bj,Bij= the coefficients from the response of the formulation in design,

B_o= Intercept

The magnitudes of the coefficients represent the relative importance of each factor. Once the polynomial equation has been established, an optimum formulation can be found out by grid analysis. With the use of computer a grid method can be used to identify optimum regions, and response surfaces may be depicted. A computer can calculate the response based on equation at many combinations of factor levels. The formulation whose response has optimal characteristics based on the experimenter's specification is then chosen.

3^2 FULL FACTORIAL DESIGNS

The three- level design is written as a 3^2 factorial design. It means that 2 factors are consider, each at 3 levels which are usually referred to as low, intermediate and high levels. These levels are numerically expressed as -1 , 0 and +1. It is a simplest three level design. It has 2 factors each at 3 levels.

- **Advantages of factorial design:**

1. Minimum number of trials per independent variable is required.
2. Factorial designs have maximum efficiency in estimating main effects.
3. They form the basis for several other designs (like fractional factorial, composite etc.)
4. More information is obtained with less work.
5. They can be used as building block to define a large response surface.
6. The effects are measured with maximum precision.
7. Both quantitative and qualitative variables can be examined and results can be easily interpreted.

- **Applications:**

1. To help and interpret the mechanism of an experimental system.
2. To recommend or implement , a practical procedure or a set of condition, in an industrial manufacturing operation.
3. As a guidance for further experimentations.

4.7.1 COMPOSITION OF 3^2 FULL FACTORIAL DESIGN:

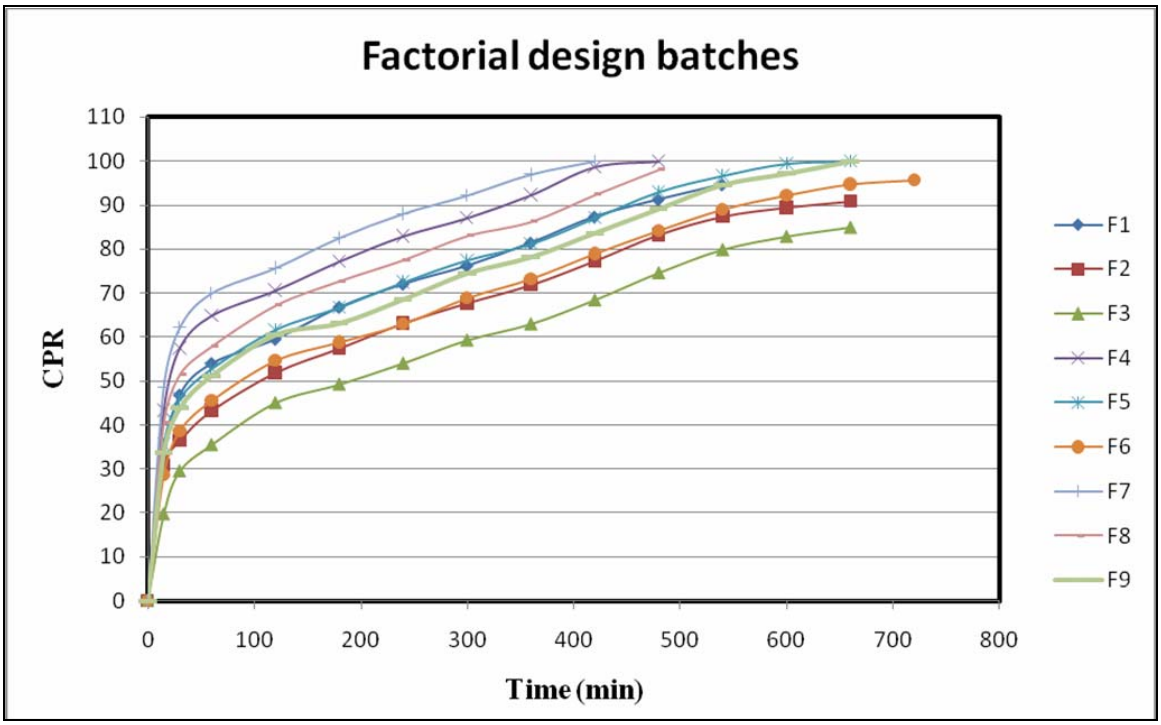


Figure: 4.29 In-vitro drug release profile of factorial batches

4.7.2 STATISTICAL ANALYSIS OF FACTORIAL DESIGN BATCHES:

A. SUMMARY OF REGRESSION ANALYSIS AND ANOVA FOR Y_1 (Particle size in µm):

The main effect (X_1 and X_2) represents the average result of changing one factor at a time from its low value to its high value. The interaction (i.e X_1X_2) shows how the response values (i.e. Y_1, Y_2) changes when two factors are simultaneously changed.

Where X1= Stirring speed, X2= Eudragit RL to Eudragit RS ratio

The fitted equation relating to the response Y_1 (Particle size in µm) to the transformed factors is shown in equation

$Y_1 = 356.5556 - 225.833\ X_1 + 0.5\ X_2 - 1.25\ X_1X_2 - 3.83333\ X_1^2 - 2.83333\ X_2^2$ ---- (1) Full factorial

The coefficients B_1, B_2 were found to be significant at P is less than .05 and thus, were retained and B_{11}, B_{12}, B_{22} were removed in reduced model.

$Y_1 = 352.8 - 226.867\ X_1 - 0.5333\ X_2$ ..... (2) Reduced factorial design

From the equation (1) & (2), it was concluded that both the variable X_1 and X_2 have effects on particle size but higher value of B_1 indicates that X_1 affect significantly.

B. SUMMARY OF REGRESSION ANALYSIS AND ANOVA FOR Y_2

(Time required to release 80% drug release)

The fitted equation relating to the response Y_2 (Time required to release 80 % of drug) to the transformed factors is shown in equation

$Y_2 = 330.6667 - 57.333\ X_1 + 111\ X_2 + 1X_1X_2 - 2\ X_1^2 - 5X_2^2$ ---------- (3) Full factorial

The coefficients B_1, B_2 were found to be significant at P is less than .05 and thus, were retained and B_{11}, B_{12}, B_{22} were removed in reduced model.

$Y_2 = 326.0667 - 57.433\ X_1 + 110.9\ X_2$ ---------- (4) Reduced factorial design

From the equation (3) & (4), it was concluded that both the variable X_1 and X_2 have effects on particle size but higher value of B_2 indicates that amount of X_2 affect significantly.

4.7.4 FORMULATION OF CHECK POINT BATCHES

Check point batch was prepared in order to validate factorial design model.

PREDICTED RESPONSES				OBTAINED RESPONSES	
Full factorial		Reduce factorial			
Y_1 (µm)	Y_2(min)	Y_1 (µm)	Y_2(min)	Y_1 (µm)	Y_2(min)
468	414	469	410	462	422
356	384	353	382	353	386
242	356	239	353	239	342

Results and discussion:

Table 4.55 revealed that practical responses matched well with the predicted theoretical responses derived from the individual equation. Hence we can conclude that our statistical model is mathematically valid.

It is concluded that by adopting systemic formulation approach, one can reach to an optimized point in shortest time with minimum efforts. The other advantage is if by chance some change is done in formulation on manufacturing floor, it will be feasible for researcher to predict its influence on the performance of the tablets.

RESPONSE SURFACE PLOT

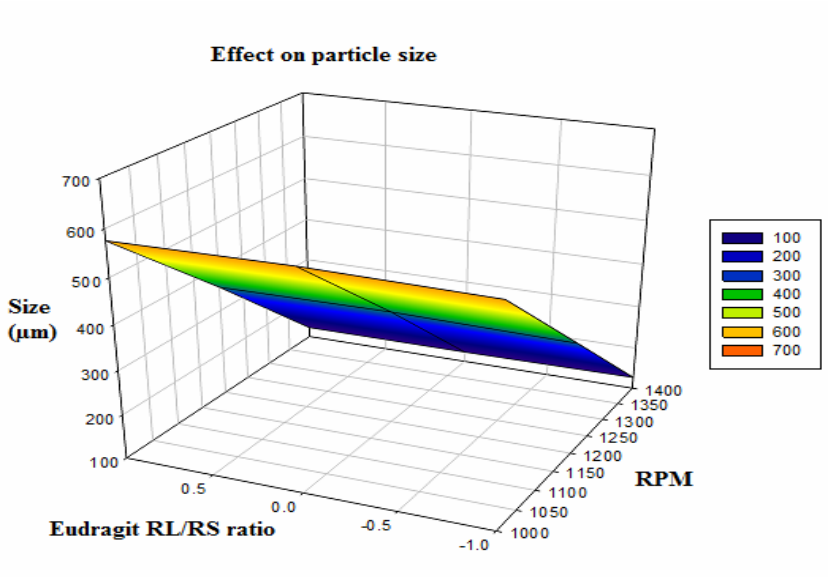


Figure: 4.30 Effect of stirring speed and polymer ratio on particle size

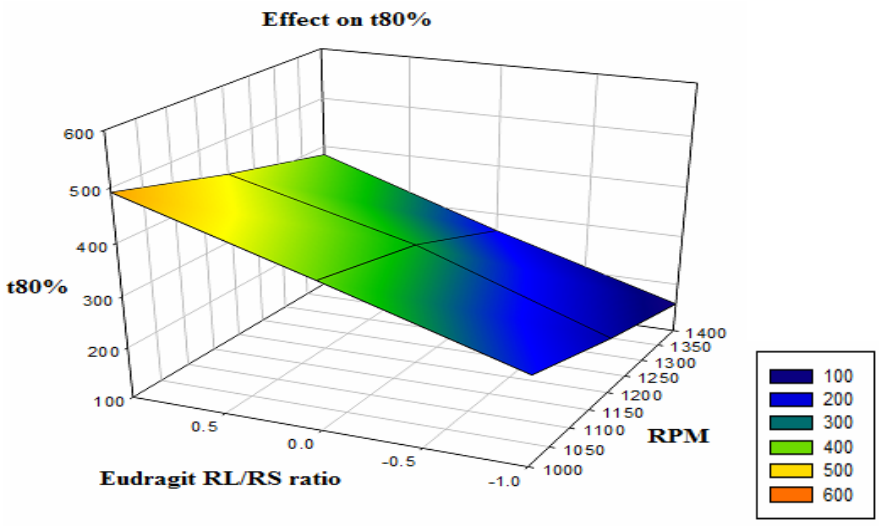


Figure: 4.31 Effect of stirring speed and polymer ratio on $t_{80\%}$

Discussion :

Figure 4.30 and figure 4.32 showed that as there is increased in stirring speed there is greater decrease in particle size. While the ratio of Eudragit RS to RL 100 have no significant effect on particle size of microspheres.

Figure 4.31 and figure 4.33 clearly indicate that there is increase in time required to release 80 % of drug as there is decrease in stirring speed and increase Eudragit RL to RS 100 ratio.

Finally we can concluded that there was large influence of stirring speed on particle size as well as time required to release 80% of drug, While Eudragit RS to RL 100 ratio have large influence on the time required to release 80% of drug and there was no any significant effect on particle size.

Summary

Successful delivery of pulsatile capsule to the patient of early morning hypertension can be possible using technology like PULSINCAP®. But here we used erodible tablet instead of swellable polymer plug. Lag time of pulsaitle device was set for 5 hr which prevent the release of drug before reaching to the colon and also it may maintain better therapeutic concentration of metoprolol for 12 hrs after lag time. This device may administered to the patient at evening (8:00 pm) and its will start release after 5 hrs (1:00 am) and will reach to effective blood plasma concentration after 3-4 hr (around 4:00 am) which is the period of early morning hypertension (4:00 am to 12 nooon) and maintained effective blood concentration for 12 hrs (up to 1:00 pm).

Microspheres were filled in the insoluble HPMC capsule body. Mouth of capsules were fitted with erodible tablet made from HPMC and lactose and capsule bodies were fitted with cap which was previously enteric coated with Eudragit L-100 55.Sustained release of metoprolol up to 12 hrs was achieved by microsphers that were prepared using Eudragit RS 100 and Eudragit RL 100 polymer combination and single emulsion solvent evaporation technique. Microspheres prepared were optimized for formulation parameters and process parameters. Batches with a range of 1:3 to 1:6 of drug to polymer ratio,0.1% to 0.4 % of Span 80, 8 hr to 12 hr of hardening time and 900 rpm to 1400 rpm of stirring speed were prepared for optimization of drug to polymer ratio, Span 80 concentration, duration of hardening and Stirring speed respectively. Batch CB-4 showed release up to 12 hr with average particle size of 353 µm.

A study of drug release rate kinetics was performed by fitting various models to release profiles of optimized batch. Values of Sum of Square of Residuals (SSR) & F-value were found for models like Zero order, First order, Higuchi, Korsmeyer-Peppas, Weibull and Hixon-Crowell respectively. Release of optimized batch fitted to Korsmeyer-Peppas equation showing least SSR and F- value of 98.96815193 and 8.247345994 respectively as compared to other batches. Thus, it could be concluded that release of metoprolol from microspheres was diffusion controlled.

10 % of ethyl cellulose coating on capsule body was sufficient to make capsule body insoluble. 10 % of Eudragit L 100-55 required to prevent exposure of erodible tablet before capsule reach to the intestine and also to prevent release of drug in stomach. Erodible tablet of batch showed promising lag time of 3.27 hr which is sufficient to prevent drug release in intestine.

3^2 Factorial design was applied to see the effect of factors like stirring speed (X_1) and Eudragit RS to RL 100 ratio (X_2) on responses like particle size (Y_1) and time required to release 80 % of drug release (Y_2). The reduced model equation $\mathbf{Y_1 = 352.8 - 226.867\ X_1 - 0.5333 X_2}$ and $\mathbf{Y2 = 326.0667 - 57.433\ X_1 + 110.9\ X_2}$ best describe the effect of factors on response. They also graphically represented using response surface and contour plot successfully.

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