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A Research Work on: Design, Development and In-Vitro Evaluation of Losartan Potassium Loaded Pectinate Microspheres

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ABSTRACT

One of the special and innovative drug delivery strategies is the use of microspheres, which can increase a drug's effectiveness by preventing drug dilution in bodily fluids, allowing drug localization and targeting at a specific site, and maintaining drug concentration between MEC and MTC levels.Microspheres are characterized as roughly spherical, solid particles with sizes between 1 to 1000 µm that are intended to be covered in protective materials such as polymeric or waxy coatings. One of the more innovative methods in the field of oral controlled drug delivery systems is the use of microsphere dosage forms, which target a specific area of the body for a prolonged period of time, allowing for more effective local drug delivery as well as improved systemic drug. The % yield of all the losartan potassium loaded pectinate microsphere formulations (LM1 to LM5) was found within the range from 76.74 ± 3.79 % to 89.06 ± 2.67 %.The % entrapment efficiency and particle size of the prepared losartan potassium loaded pectinate microsphere formulations (LM1 to LM5) was found within the range from 63.68 ± 3.55 % to 84.78 ± 2.27 % and 0.78 ± 0.14 mm to 0.91 ± 0.05 mm respectively. Formulations LM1 to LM5 showed 2.12 % to 12.46 % mucoadhesion up to 6 hours in simulated gastric fluid (pH 1.2, 0.1N HCl) and 74.28 % to 96.04 % mucoadhesion up to 6 hours in simulated intestinal fluid (phosphate buffer, pH 6.8).

KEYWORDS: Losartan Potassium, Pectinate Microspheres, NDDS

INTRODUCTION

- **Potential Advantages of Control Release Dosage Form**

◆ Avoid Patient's compliance problem due to reduced frequency of dosing.

◆ Blood level oscillation characteristics of multiple dosing of conventional dosage form are reduced because a more even blood level is maintained.

◆ Employ a less total drug

➢ Minimize or eliminate local or systemic side effects.

➢ Minimize drug accumulation with chronic dosing.

➢ Obtained less potential of reduction in drug activity with chronic use.

- Improve efficiency in treatment
  - Cure or control condition more promptly.
  - Improve control of condition i.e. reduced fluctuation in drug level.
  - Improve bioavailability of some drugs.
- Make use of special effects, eg. Control release aspect for morning relief of arthritis by dosing before bedtime.
- Economy.
- Overall administration of control release to enable increase reliability of therapy.

Microspheres: The Most Favored Control Release Formulation

Because the microspheres can be specifically designed to satisfy these requirements, they have become the most widely used oral solid dose form. In this formulation, the use of polymers to regulate drug release has become significant. The preferred polymers that can serve as the foundation for the formulation of microspheres for controlled release and oral administration include sodium alginate, chitosan, and guar gum.

These devices have gained a lot of popularity recently for managing the release of medications from solid dosage forms. From an economic and process development perspective, these systems seem to be among the most appealing methods. The component of the formulation mainly in charge of hydration-induced diffusion and erosion-resistant gel layer development is the gelling agent's characteristics. The creation of oral controlled-release dosage forms has garnered a lot of interest; because to their strong compatibility, polymeric hydrogels are being looked into more and more for controlled-release applications. Furthermore, hydrogels are especially well-suited for controlled-release applications because of their capacity to release a medication that has been entrapped in an aqueous medium and to control the release of such drug by cross-linking and swelling control. Drugs and charged solutes that are both hydrophilic and hydrophobic can be released using hydrogels. The drug can be included into a matrix comprising a hydrophilic, rate-controlling polymer to create controlled-release dosage forms. The hydration of the polymer, which creates a gelatinous barrier layer at the matrix's surface through which the contained drug diffuses, regulates the release of drugs from these kinds of systems. Additionally, the viscosity grade of the polymer regulates how resistant such a gel layer is to erosion. Poorly water-soluble pharmaceuticals are mostly released by erosion mechanisms, while water-soluble drugs are released mainly by diffusion of dissolved drug molecules across the gel layer. Water seeping into the matrix causes a glassy-rubbery transition to the polymer, which is the basis for drug release from swellable matrix. The solvent molecules enter the glassy polymer matrix during this step, increasing the polymer chains' mobility and giving the polymer a rubbery (gel-like) texture. In the pharmaceutical industry, one of the most commonly utilized dose forms is microspheres. Here, swelling initiates at the periphery of the surface and forms a gel, which prevents the medication from diffusing (Peppas et al., 1987). Drug release rate is influenced to a greater or lesser extent by a number of formulation variables, although interactions between the drug, polymer, and water are the main determinants in release regulation. Drug release modifiers include drug loading, drug to polymer ratio, polymer particle size, and polymer viscosity grade. In microsphere matrix systems, the polymer and active component make up the majority of the system.

Advantages (Meena et al., 2011):

- Protection of unstable, sensitive materials from their environments prior to use.
- Self-life enhancement by preventing degradative reactions.
- Safe and convenient handling of toxic materials.
- Masking of odor or taste.
- Controlled and targeted drug delivery.
- Handling liquids as solids.
- To improve bioavailability.
- To improve the stability.

Applications:

- Microspheres can be used for controlled and sustained release dosage forms.
- Microsphere can be used to prepare enteric-coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.
- It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. Microsphere does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microsphere.
- Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.
- Microsphere has also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after micro-encapsulation.
- Many drugs have been microencapsulated to reduce gastric irritation.Therapeutic magnetic microspheres are used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.

Different types of microspheres

- Bioadhesivemicrospheres.
- Magneticmicrospheres.
- Floatingmicrospheres.
- Radioactivemicrospheres.
- Polymericmicrospheres.

- Biodegradable polymeric microspheres
- Synthetic polymericmicrospheres.

- **Bioadhesivemicrospheres**

The sticking of drug to the membrane by using the sticking property of the water soluble polymers is called as adhesion. Bio adhesion can be entitled as adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc. A better therapeutic action is produced as these kinds of microspheres depict an extended residence time at the site of application and causes intimate contact with the absorption site.

➢ **Magneticmicrospheres**

A more recent method in the pharmaceutical industry is magnetic microsphere. As an alternative to conventional radiation techniques, which employ extremely penetrating radiation that is absorbed throughout the body, consider using magnetic microspheres. Its toxicity and negative effects restrict its use.

➢ **Floatingmicrospheres**

Floating microspheres are an effervescent drug delivery system that is gastroretentive. Other names for floating microspheres are hollow microspheres, microballoons, and floating microparticles. Floating microspheres are strictly speaking spherical, empty particles without a center.

➢ **Radioactivemicrospheres**

The liver parenchyma and tumor microvasculature both contain radioactive microspheres. The hepatic arteries preferentially vascularize liver tumors, in contrast to the liver parenchyma, where the portal vein provides nearly all of the blood supply.

➢ **Polymericmicrospheres**

The polymeric microspheres are classified into two types:

a) Biodegradable polymeric microspheres:

Since natural polymers like starch are biodegradable, biocompatible, and consequently bioadhesive, they are used in the preparation of these kinds of microspheres. Gel formation is the outcome of biodegradable polymers' high degree of swelling characteristic when combined with an aqueous media. These microspheres' ability to swell increases their residence time when they come into touch with mucosal membranes. The concentration of the polymer used to produce the microsphere controls the rate and amount of drug release, and the release pattern is sustained. One significant disadvantage of biodegradable microspheres is their complex drug loading efficacy, which makes it challenging to regulate drug release.

b) Synthetic polymeric microspheres:

They serve as medication delivery vehicles, embolic particles, fillers, and bulking agents. These microspheres are compatible and safe. These microspheres' primary drawback is their propensity to move from the injection site, which increases the risk of embolism and further organ damage.

Different Methods for Fabrication of Microspheres:

- Coacervation method.
- Wax coating and hot melt method.
- Solvent evaporation method.
- Spray drying method.
- Emulsification technique
- Freeze drying method.
- Chemical and thermal cross-linking method.
- Ionic gelation method.

Coacervation phase seperation technique:

5 Phase Separation/Coacervation Method. Proteins and other hydrophilic molecules are encapsulated using the coacervation process. Using this method, homogeneity and particle size can be adjusted by varying the amount and molecular weight of polymer utilized, as well as the viscosity of the non-solvent, etc.

Complex coacervation-

- First the core material (usually oil) is dispersed into a polymeric solution (eg; cationic aqueous polymer, gelatin).
- The second polymer (anionic, water soluble, gum arabic) solution is added to the prepared dispersions.
- Deposition of the shell material onto the core material occurs when the two polymers form acomplex.
- This process is triggered by the addition of salt or by changing pH, temperature or by dilution of themedium.
- Finally, the prepared microspheres are stabilized by cross-linking with formaldehyde, dissolvation or thermal treatment.
- Complex coacervaton is used to produce microspheres containing fragment oil, new crystals, flavours, dyes or inks as a corematerial.

Steps

a. Solution of the cell material in water eg copolymer, coating gum, Arabic solution 20-30% and gelatin solution 20%.

b. The core material will be added to the solution and dispersed by agitation .the particle size will depend upon stirring speed, stirrer shape, suface tension and viscosity of the shell material solution.

c. Coacervation is triggered by the addition of non solvent or by changing thepH, temperature or by dilution of the medium this results reduction of the solubility of the dispersed phases.

d. The shell material (coacervate) starts to precipitate from thesolution.

e. The shell material forms a continuous coating around the coredroplets.

f. The shell material is cooled down to harden and form the final capsule. Hardening agents like formaldehyde may be added to the process.

g. The microcapsules are now stable, in the suspension and ready to be dried.

Wax Coating and Hot Melt Method:

The waxy materials are used to coat the core particles or encapsulate the drug. This can be achieved either by dissolution or dispersion of core material in molten wax. The waxy solution or suspension is usually mixed by high speed mixing into cold solution, such as cold liquid paraffin and then the mixture is churned for at least one hour. The external phase (liquid paraffin) is then decanted and the microspheres are suspended in an immiscible solvent and allowed to air dry. Carnauba wax and beeswax are most commonly used waxes for this method.

Solvent Evaporation Method:

In this method the drug and the polymer selected for encapsulation are required to be soluble in organic

solvent. The solution containing polymer and drug is usually dispersed in an aqueous phase to form droplets. Sometimes the influential parameters such as mixing and elevated temperatures are employed to evaporate solvents and leave the solid polymer-drug particles suspended in an aqueous medium, which are then filtered out.

Spray Drying Method:

It is a closed-system process that is applicable to wide variety of materials, including heat-sensitive materials. It is a single step process where the drug and polymeric coating materials are dissolved in suitable solvent (aqueous or non-aqueous) or is suspended in the polymer solution or is dissolved or suspended within an emulsion or coacervate system. Various parameters control the size of the microsphere such as the rate of spraying, the feed rate of the polymer-drug solution, the nozzle size, the temperature of drying and collecting chambers.

Example: Biodegradable polylactide microspheres can be prepared by dissolving the drug and the polymer in methylene chloride.

Emulsification technique:

Oil water emulsion:

a. In a polymeric solvent system the drug substance is either dispersed or dissolved.
b. By continuous agitation it is added to the aqueousphase.
c. Agitation continues till solvent partitions into aqueous phase and is removed byevaporation.
d. This causes hardening ofmicrospheres.
e. Microspheres are washed anddried.

Water oil emulsion:

a. Polymer mostly dicholoromethane is dissolved in organic phase. In this organic phase the aqueous drug solution is emulsified using high sped homogeniser.
b. To external aqueous phase containing surfactant the primary emulsion is added. And then stirred to allow evaporation of dichloromethane.
c. The microspheres obtained are collected by ultracentrifugation, filtration and thenlyophilisation.

Multiple emulsification:

a. Drug used should be watersoluble.
b. The solution of drug is prepared in distilled water and is emulsified with solution of polymer in dichloromethane or chloroform with vigorousstirring.
c. Water in oil emulsion is prepared, this primary emulsion is again emulsified with surfactant solution and forms o/w/oemulsion.
d. The double emulsion is then subjected to stirring until most of the organic solvent evaporates leaving solidmicrospheres.
e. Microspheres are then washed anddried.

Chemical and Thermal Cross-Linking Method:

This process involves formulating of microspheres from natural polymers by a cross-linking process. The different polymers used comprise of gelatin, albumin, starch and dextran. Usually a water-oil type

emulsion may be prepared, where the water phase acts as solution of polymer which helps to carry the drug and the oil phase. Once the desired water-oil emulsion is formulated, the water soluble polymer is solidified by thermal treatment or by addition of a chemical cross-linking agent such as glutaraldehyde to form a stable chemical cross-link.

Ionic Gelation Method:

This method is based on the ability of polymers to cross-link in the presence of counter ions to form hydrogels. This technique has been widely used for the purpose of encapsulation of drugs or cells (Lim & Sun, 1980) by using various polymers such as alginates, gellan gum and carboxy methyl cellulose etc. These polymers form a meshwork structure by combining with the polyvalent cations and induce gelation by binding mainly to the anion blocks. The microspheres are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuse into the drug-loaded polymeric drops and forms ionically cross linked particle.

Classification of Polymers:

Hydrophilic Polymers*:*

These are the water-soluble polymers that swell indefinitely in contact with water and eventually undergo complete dissolution, e.g.methylcellulose, hydroxyethyl cellulose, hydroxy propyl methyl cellulose, sodium carboxy methyl cellulose, carbomers, chitosan and plant gums etc.

Hydrogels:

These are water swellable materials, usually a cross-link polymer with limited swelling capacity, e.g. poly (acrylic acid co acrylamide) copolymers, carrageenan, sodium alginate, guar gum and modified guar gum etc.

Thermoplastic Polymers:

These polymers include the non-erodible neutral polystyrene and semi crystalline bioerodible polymers, which generate the carboxylic acid groups as they degrade, e.g.polyanhydrides and polylactic acid. Various synthetic polymers used for the formulation of microspheres are polyvinyl alcohol, polyamides, polycarbonates, polyalkylene glycols, polyvinyl ethers, esters and halides, polymethacrylic acid, polymethylmethacrylic acid, methylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose and sodium carboxymethylcellulose etc.

Mucoadhesive Microspheres

Bioadhesion is a process in which out of the two materials at least one is biological in nature and are held together by means of interfacial forces. Mucoadhesion here is defined as adhesion of the polymers with the surface of the mucosal layer. The absorption and the bioavailability of the drugs are further more improved by incorporating mucoadhesive properties to the microspheres. Intimate contact with the mucous layer as well as drug targeting to the absorption site by antibodies and various ways are enhanced by mucoadhesive microspheres. They provide localised as well as controlled release of drug by adhering to the mucosal tissue in eye, nasal cavity, urinary and gastrointestinal tract.

Advantages

- Prolong therapeutic effect isachieved.
- Frequency of administration is reduced thereby improving patientcompliance.
- Due to mucoadhesive property the drug release is more hence the absorption is facilitate and hence improves the bioavailability of the drugs, and therefore reduces thetoxicity.
- A controllable variability in degradation and drug release is permitted by the morphology ofmicrospheres.

Classification of mucoadhesive polymers

The mucoadhesive polymers are divided into 2 types

Synthetic Polymer	Natural Polymers
Hydroxy propyl methyl cellulose (HPMC)	Chitosan
Poly polymers	Sodium alginate
Polyvinyl pyrollidon (PVP)	pectin
Poly vinyl alcohol (PVA)	Guar gum

Mechanism of mucoadhesion:

Mucoadhesion can be defined as the linking of the drug with suitable carrier of the mucosal layer .it is a complex phenomenon which involves wetting, absorption and inter penetration of polymer chains.

Steps

Swelling phenomenon-There should be intimate contact between mucoadhesive delivery system and mucosal membrane.

Inter Penetration – Penetration of delivery system into the tissue or into the surface of mucous membrane.

Theories of mucoadhesion (Lee et al., 2000):

Different theories involved are as follows:-

- ❖ Electronictheory.
- ❖ Wettingtheory.
- ❖ Absorption theory.
- ❖ Diffusion theory.
- ❖ Mechanical theory.
- ❖ Cohesivetheory

Electronictheory-

According to this theory, electron transfers occur upon contact of adhesive polymer with a mucous glycoprotein network because of difference in their electronic structure. This results in the formation of electrical double layer at the interface. Example- interaction between positively charged polymer chitosan and negatively charged mucosal surface which become adhesive on hydration and provides an intimate contact between a dosage form and absorbing tissue.

Wetting theory-

This theory is applicable for liquids. The wetting theory postulates that if the contact angle of liquids on the substrate surfaces lower, then there is a greater affinity for the liquid to the substrate surface. If two substrate surfaces are brought in contact with each other in the presence of liquid, the liquid may act as an adhesive among the substrate surface.

Absorption theory-

According to this theory, after an initial contact between two surfaces, the material adheres because of surface force acting between the atoms in two surfaces. Two types of chemical bond resulting from these forces can be distinguished as primary chemicals bonds if covalent nature and secondary chemical bond having many different forces of attraction, including electrostatic forces, Vander wall forces, hydrogen forces and hydrophobic bonds.

Diffusion theory-

According to this theory the polymer chains and the mucus mix to a sufficient depth to create a semi-permanent adhesive bond. The exact depth to which the polymer chain penetrates the mucus depends on the diffusion coefficient and the time of contact. The diffusion coefficient in turn depends on the value of molecular weight between crosslinking and decreases significantly as the crosslinking density increases.

Mechanical theory-

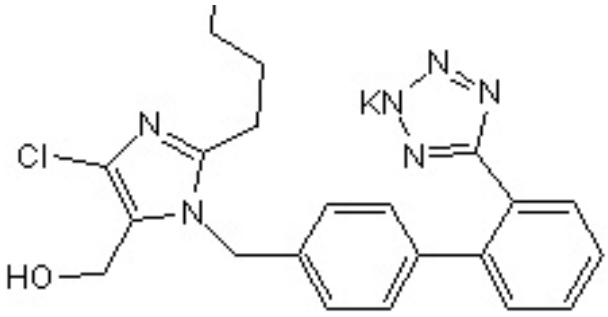
The theory explains the formation of an interlock structure by the diffusion of the liquid adhesives into the micro cracks and irregularities present on mucoadhesive substrate resulting in mucoadhesion.

DRUG PROFILE

LOSARTAN POTASSIUM:

- **Formula:**
  - **Empirical:**C22H22ClKN6O
  - **Structural:**

Figure-1. Chemical Structure of Losartan



IUPAC Name: 2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-yl-phenyl)benzyl]imidazole-5-methanol mono potassium salt.

- **Molecular Weight:**461.01
- **Appearance:**White to off white powder crystalline powder.
- **Physical Properties:**
  - Melting Point:265 °C.

- Solubility:Losartan potassium is freely soluble in water, phosphate buffers, alcohols, DMSO and DMF but has slight solubility behavior with common organic solvents like acetonitrile.

- **Use:**

The drug finds active utilization in treatment of hypertension and chronic heart failure when treatment with ACE inhibitors is not appropriate due to incompliance. The drug also finds usage in patients suffering from renal diseases and type -2 diabetes mellitus with protein urea to control hypertension in case of these patients.

- **Mechanism of Action:**

Angiotensin II is a potential vasoconstrictor that is formed due to catalytic reaction caused by angiotensin converting enzyme, kininase II on angiotensin I which is responsible for pathology of hypertension. The drug and its metabolites bind to the AT1 receptor and selectively block binding of angiotensin II to the site thus influencing hypertension effect. Moreover the inhibition of aldosterone secretion may also increase elimination of sodium and water while decreasing the rate of elimination of potassium as result it effectively reduces blood pressure. Losartan, is thus used in treatment of essential hypertension, left ventricular hypertrophy and diabetic nephropathy.

- **Dosage and Administration:**

Losartan potassium is usually administered orally with varied strength of 25 mg, 50 mg, 100 mg depending on severity of the diseased state.

Pharmacokinetics:

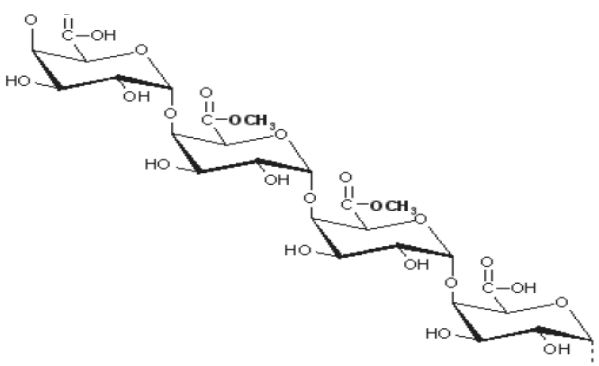
- Absorption:Losartan potassium is well absorbed and has the systemic bioavailability of 33% approximately. The peak plasma concentration is attained within 1 hour for losartan in its parent form while for its metabolite it is 3 to 4 hours after oral administration
- Metabolism:Losartan undergoes significant first pass metabolism by CYP-450 2C9 and 3A4 enzymes of hepatic system and gets converted in to a 5-carboxylic acid derivative. Out of total administered oral dose about 14% of the drug gets converted to the active metabolite form and is accountable for angiotensin II receptor antagonist activity.
- Distribution: The drug is highly protein bound (99.7 %).
- Elimination: Losartan has a renal clearance value of 75ml/min. while the metabolite has a clearance rate of 25ml/min. after oral administration. The biological half life of losartan is 2 hours.

POLYMER PROFILE

PECTIN:

- **Structure:**

Figure-2. Chemical Structure of Pectin



- **Synonyms:**Poly (1, 4-alpha-D-galacturonide), Methoxypectin, Methyl pectinate, Pectinate, Pectinic acid.
- **Chemical Name:**Pectin.
- **Molecular Weight:** 30,000 to 100,000 g/mole.
- **Functional Category:**Pectin is used as gelling agent, thickener, emulsifier, viscosity-enhancer agent and colloidal stabilizer.
- **Description:**Pectin is an odourless olfactory property. It is usually a white or light yellowish to yellowish colour powder.
- **Physical Properties:**
  - Moisture content: Not more than 8 %.
  - Esterification degree: 65 to 70 %.
  - Solubility:Pectin exhibits partial solubility profile with water but dissolves more faster if wetted previously with alcohol, glycerol, sugar syrup or homogenized with 3 or more parts of sucrose. The solubility of pectin with other solvents like alcohol, dilute alcohol and organic solvents is found to be in category of insoluble substance.
- **Stability and Storage Conditions:**

The polymer needs to be stored in a cool and well ventilated environment in an air tight container.

- **Incompatibilities:**

Pectin demonstrates incompatible reaction in presence of alkalis, heavy metals, salicylic acid, tannic acid and strong alcohols.

- **Safety:**

Normally this polymer is considered to be non toxic its modified citrus pectin from if administrated at high doses may precipitate gastric intolerance so it is advised that the used of this should be avoided in case of pregnant women and nursing mothers.

- **Applications:**
- Pectin can be used as binding agent in tablet formulations.
- Pectin polymer can be utilized to develop microsphere by emulsification method.

Pectin is widely used as controlled-release matrix former in tablet.

EXPERIMENTAL PROCEDURE

Analytical Methods Used for Estimation of Losartan Potassium

A UV-visible spectrophotometric method based on the measurement of absorbance at 252 nm in distilled water was used in the present study for the estimation of losartan potassium.

An accurately weighed quantity (100 mg) of losartan potassium was dissolved in 100 ml of distilled water to give 1 mg/mL solution. The above solution was subsequently diluted with distilled water to obtain a series of dilutions containing 2, 4, 6, 8, and 10 µg/ml of losartan potassium solution. The absorbance of the above dilutions was measured by UV-visible spectrophotometer (UV-1700 Shimadzu, Japan) at 252 nm using distilled water as blank. The absorbance values were plotted against the concentration of losartan potassium as shown in Figure-1**.** The linear relationship between the concentration of losartan potassium and the corresponding absorbance values was shown by,

$$Y=0.0225\ X$$

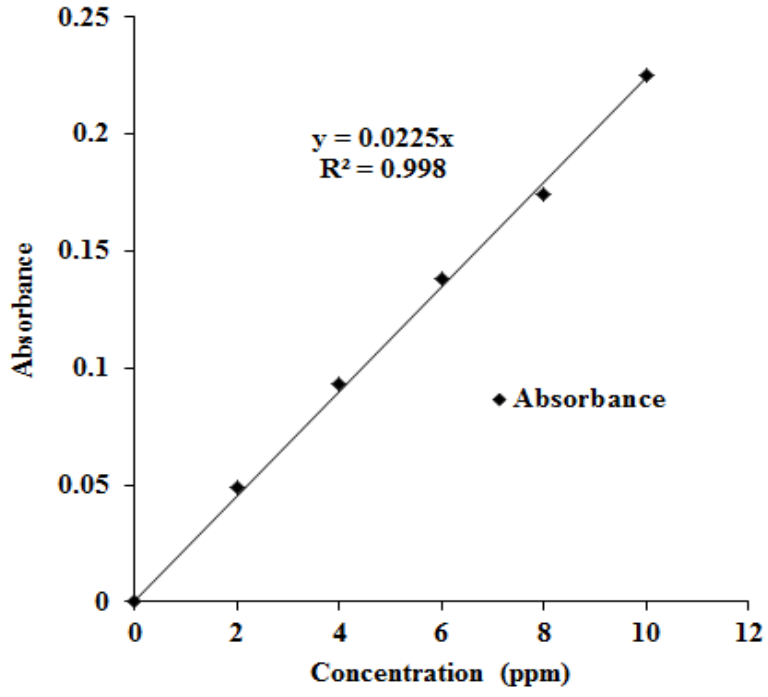
Where, Y = absorbance, and X = concentration of losartan potassium (µg/ml)

A correlation between the concentration of losartan potassium and the corresponding absorbance values was observed between 2 to 10 µg/ml (coefficient of determination, r^2 = 0.998). The amount of losartan potassium in either microspheres or the dissolution fluids was calculated using the linear relationship as given above or directly from the standard graph as shown in Figure-1**.**

Table-1. Calibration Table of Losartan Potassium in Distilled Water.

Concentration(µg/ml)	Absorbance
0	0
2	0.049
4	0.093
6	0.138
8	0.174
10	0.225

Graph-1. Calibration Curve of Losartan Potassium.



Fabrication of Losartan Potassium loaded Pectinate Microspheres:

Pectinate microspheres containing losartan potassium were prepared by ionic gelation method. Pectin was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution. After that required quantity of losartan potassium was added to the polymer solution and mixed thoroughly to form a smooth viscous dispersion. The resulting dispersion was then added drop-wise in 500 ml of 5 % calcium chloride solution (Formulation LM1 to LM5) using a 24 G needle at a constant rate and under

continuous stirring. The stirring was continued for 30 minutes for complete reaction. Then the mixture was filtered and product was dried at 40 °C for 6 hour. The microspheres along with coat composition are listed below (Table-2).

Table-2. Formulation of the Losartan Potassium loaded Pectinate Microspheres.

Formulation	Drug : Polymer	Losartan Potassium (g)	Polymeric blend composition (g)	Cross-linking agent (w/v)
LM1	1:1	0.5 g	Pectin, 0.5 g	CaCl2, 5%
LM2	1:2	0.5 g	Pectin, 1 g	CaCl2, 5%
LM3	1:3	0.5 g	Pectin, 1.5 g	CaCl2, 5%
LM4	1:4	0.5 g	Pectin, 2 g	CaCl2, 5%
LM5	1:5	0.5 g	Pectin, 2.5 g	CaCl2, 5%

Characterization of Prepared Microspheres

Yield (%):

The percentage yield of prepared batches was calculated on weight basis with respect to the weight of starting material. All experiments were carried out in triplicate.

$$\text{Yield (\%)} = \frac{\text{weight of the final formulation}}{\text{weight of the initial raw materials used in the fomulation}} \text{ X } 100$$

Entrapment Efficiency:

The entrapment efficiency of the prepared pectinate microspheres formulations was determined by the method of extraction of the drug present in the microsphere. The dried microspheres (100 mg) were taken and extracted in 100 ml of distilled water for 4 hours. Then the dispersion of microspheres was vigorously stirred for 30 min and the solution was filtered through a 0.45 µm filter. Finally, the polymeric debris was washed twice with distilled water to extract any adhering drug. The drug content of filtrate and washings was determined spectrophotometrically at 252 nm (UV-1700, Shimadzu, Japan). (Agnihotri et al, 2006).

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

Particle Size Study:

Sieve analysis technique has been used in order to study the particle size of prepared pectinate microspheres. Firstly separation of the prepared pectinate microspheres into different size fractions or % weight fraction were done by sieving for 10 min. using standard sieves having nominal mesh apertures of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm (sieve no. 12, 14, 16, 18 and 22). The particle size distributions of the microspheres were

determined and the mean particle size of microspheres was calculated using the following formulae,

$$\text{Mean particle size} = \frac{\sum(\text{mean paricle size of the fraction X weight fraction})}{\sum\text{weight fraction}}$$

Mucoadhesion Study:

Simulated intestinal fluid (phosphate buffer, pH 6.8) and simulated gastric fluid (pH 1.2) were used to study mucoadhesive property of prepared pectinate microspheres. Freshly excised pieces of goat intestinal mucosa were mounted onto glass slides using cyanoacrylate glue. About 50 microspheres of each batch were spread onto each wet rinsed tissue specimen and immediately thereafter the slides with suitable support were hung onto the arm of a USP tablet disintegrating test apparatus. The tissue

specimen was given a slow, regular up and down movement in 900 ml of the test fluid at 37˚C. At different time intervals (up to 6 hours), the number of microspheres still adhered to the tissue specimen was counted (Lehr et al, 2000).

$$\text{Adhesion (\%)} = \frac{\text{Number of microspheres adhered}}{\text{Number of microspheres applied}} \text{ X } 100$$

***In-Vitro* Dissolution Study:**

Release of losartan potassium from the prepared pectinate microspheres (weight equivalent to 100 mg of the drug) was studied in phosphate buffer pH 6.8 (900 ml) using an USP 8 station dissolution (LAB INDIA, DISSO 8000) rate testing apparatus with a rotating paddle at 50 rpm and at 37 ± 0.5 ºC temperature. Samples of 5 ml were withdrawn at different time intervals and diluted using pH 6.8 phosphate buffer. After suitable dilutions the absorbance was measured at 252 nm using UV-visible spectrophotometer against a blank. The dissolution study was conducted in triplicate.

Kinetics of Drug Release:

The rate and mechanism of release of losartan potassium from the prepared pectinate microspheres were analyzed by fitting the dissolution data into the zero-order equation,

$$Q = k_0 t$$

where Qis the amount of drug released at time t and k0 is the release rate constant.

First order equation,

$$\ln (100 - Q) = \ln 100 - k_1 t$$

where k1 is the release rate constant.

The dissolution data was fitted to the Higuchi's equation (Higuchi, 1963),

$$Q = k_2 t^{1/2}$$

where k2 is the diffusion rate constant.

To compare the dissolution profiles, several release models were tested, such as Higuchi's equation, which can provide information about drug particles dispersed in a matrix. The drug release data was further analyzed by Peppas equation (Korsmeyer et al., 1983),

$$\frac{M_t}{M_\infty} = kt^n$$

Where n = diffusional exponent, Mt = amount of drug released at time t, M∞ = amount of drug released at time ∞, K is the kinetic constant.

Thus M_t / M_∞ is the fraction of drug release at time t, a measure of the primary mechanism of the drug release and n characterizes the mechanism of drug release from the formulations during dissolution process.

n values	Mechanism of diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Non-Fickian diffusion
1	Case II transport
> 1.0	Super case II transport

Field Emission Scanning Electron Microscopy (FE-SEM):

Shape and surface morphology of the prepared pectinate microspheres were studied by using field emissionscanning electron microscope. The sample was spread on stub and the stub was then coated with conductive gold with sputter coater attached to the instrument. Then the stub containing the sample

was placed in the field emissionscanning electron microscope (Carl Zeiss, SUPRA 55, Germany) chamber at acceleration voltage of 10 kV and chamber pressure of 0.6 mm Hg and the photographs were taken using field emission scanning electron microscope.

Fourier Transform Infrared Analysis (FTIR):

Fourier Transform Infrared Analysis (FTIR) of pure drug (losartan potassium), pure polymer and optimized microsphere formulations were obtained using FTIR analyzer (PerkinElmer, Spectrum 400, FT-IR/ FT-FIR Spectrometer.). The pellets were prepared on KBr-press (Kimaya Engineers, India) under hydraulic pressure of 5 Ton. The samples were scanned over the wave number ranges between 4400 to 400 cm^{-1} at the ambient temperature.

The samples that were analyzed for FTIR are namely as follows,

- Pure losartan potassium.
- Pure pectin.
- Optimized microsphere formulation.

Differential Scanning Calorimetric Analysis (DSC):

Differential Scanning Calorimetric (DSC) thermograms of pure drug (Losartan Potassium), pure polymer and losartan potassium-loaded microsphere formulations were obtained using a Differential Scanning Calorimeter (Diamond DSC, PYRIS, Perkin Elmer, USA). Indium standard was used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in perforated aluminum pans and heated at constant rate over a temperature range of 50 ºC to 400 ºC. The system was purged with nitrogen gas at the rate of 100 mL/min to maintain inert atmosphere.

The samples that were analyzed for DSC are namely as follows,

- Pure losartan potassium.
- Pure pectin.
- Optimized microsphere formulation.

X-Ray Diffraction (XRD) Studies:

X-Ray Diffraction (XRD) studyof pure drug, pure polymer and losartan potassium loaded optimized microsphere formulations were assessed for crystallinity by X-Ray Diffractometer (X'Pert Pro, Panalytical, Nertherlands) using monochromatized Cu K α-1 radiation (λ = 1.54 A) at a voltage of 45 kV and current of 40 mA. Measurements were carried out in the angular scan range from 5° to 40° (2θ).

- Pure losartan potassium.
- Pure pectin.
- Optimized microsphere formulation.

RESULTS AND DISCUSSION

Physico-Chemical Characterization of Pectinate Microspheres:

The % yield of the prepared losartan potassium loaded pectinate microspheres was found within the range from 76.74 ± 3.79 % to 89.06 ± 2.67 % (Table 3). The particle size of losartan potassium loaded pectinate microsphere formulations was found from 0.78 ± 0.14 mm to 0.91 ± 0.05 mm while the encapsulation efficiency was found to be 63.68 ± 3.55 % to 84.78 ± 2.27 % (Table 3). It was observed that particle size as well as entrapment efficiency of all the prepared microspheres were increased as the concentration of pectin was increase. It may due to the higher concentration of polymer increases the viscosity of the medium and greater availability of calcium binding sites in the polymeric chains which increases the degree of cross-linking as well as particle size and entrapment efficiency.

Table-3.Characterization of Losartan Potassium loaded Pectinate Microspheres.

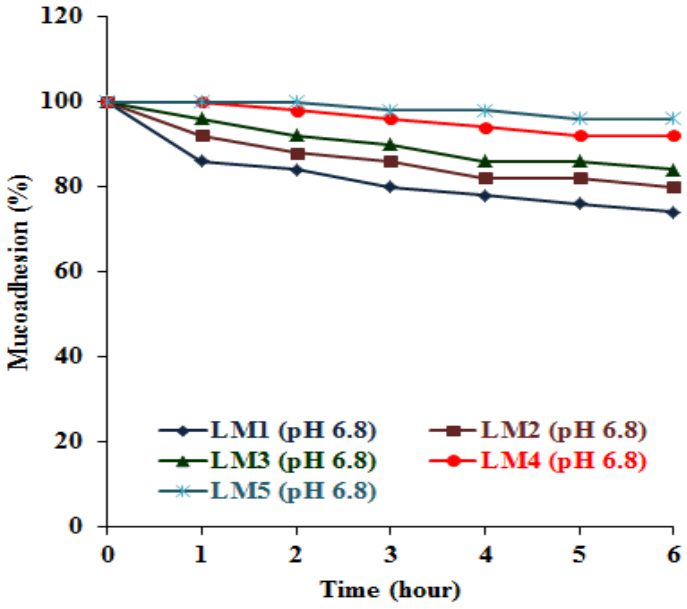
Formulation	Yield(%)	Entrapment Efficiency (%)	Particle size(mm)
LM1	79.99 ± 2.88	63.68 ± 3.55	0.78 ± 0.14
LM2	76.74 ± 3.79	74.57 ± 1.79	0.82 ± 0.07
LM3	89.06 ± 2.67	81.24 ± 2.51	0.85 ± 0.03
LM4	84.75 ± 1.83	83.52 ± 3.06	0.86 ± 0.12
LM5	81.37 ± 2.58	84.78 ± 2.27	0.91 ± 0.05

Mean ± SD, n = 3.

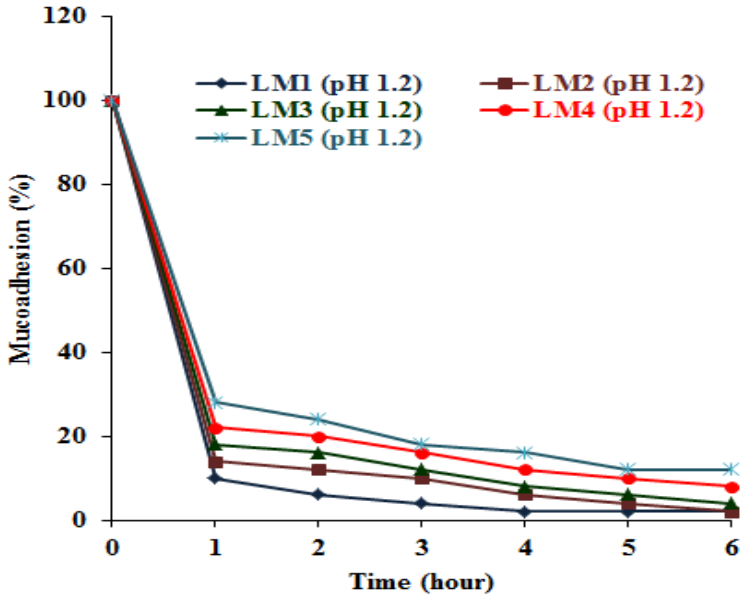
Mucoadhesion Study:

Mucoadhesion studies of the prepared losartan potassium loaded pectinate microsphere were studies by *in-vitro*wash-off test using phosphate buffer (pH 6.8) and 0.1 N HCl (pH 1.2). It was also observed that, all the formulations exhibited more adhesion in phosphate buffer (pH 6.8) as compare to 0.1 N HCl (pH 1.2) indicating that mucoadhesion was pH dependent. All the formulations exhibited 2.12 % to 12.46 % mucoadhesion up to 6 hours in simulated gastric fluid (pH 1.2) (Figure 2b) and 74.28 % to 96.04 % mucoadhesion up to 6 hours in simulated intestinal fluid (pH 6.8) (Figure 2a). The results indicate that the pectin was practically insoluble in aqueous acidic solution, whereas in phosphate buffer its solubility, hydration and mucoadhesivity property was increased due to ionization of carboxylic acid group and other functional groups present in the polymer. This ionization of functional groups in simulated intestinal fluid helps to increase polymer solubility in turn to produce a viscous gel which increases the mucoadhesion property (Chakraborty et al., 2010).

Graph 2a. Mucoadhesive Study of Pectinate Microspheres (Phosphate Buffer, pH 6.8).



Graph 2b. Mucoadhesive Study of Pectinate Microspheres (0.1 N HCl, pH 1.2).



In-Vitro Dissolution Study:

In-vitro drug release study was performed by using USP dissolution rate test apparatus II using 900 ml of phosphate buffer, pH 6.8 (Table 4, Figure 3). Formulation LM1, LM2 and LM3 (drug : pectin ratio 1:1, 1:2 and 1:3 respectively) were able to sustain the drug release up to 4, 5 and 7 hours respectively. Formulation LM1 shows 92.34 % drug release at 4 hours, formulation LM2 shows 90.95 % drug release at 5 hours and formulation LM3 shows 91.25 % drug release at 7 hours.

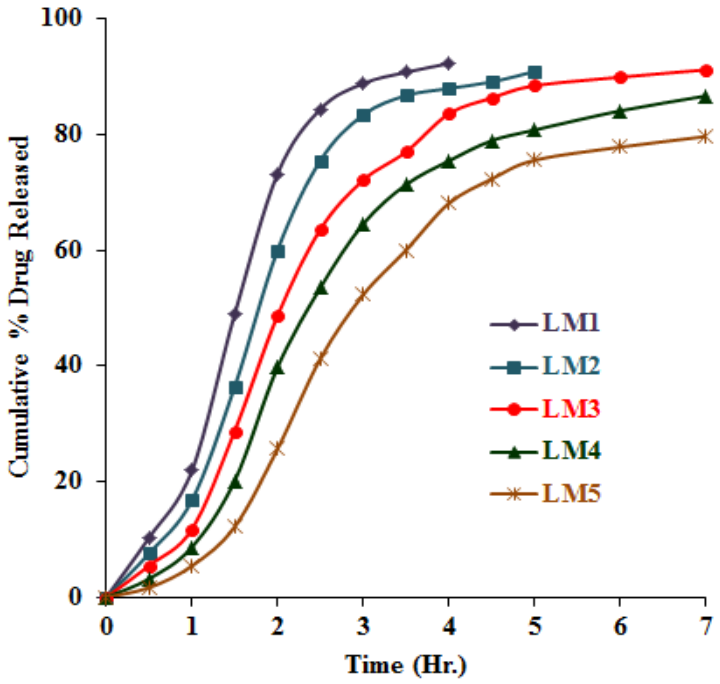
Table-4. *In-Vitro* Dissolution Profile of Losartan Potassium loaded Pectinate Microspheres.

Time (Hr.)	LM1	LM2	LM3	LM4	LM5
0	0	0	0	0	0
0.5	10.33	7.61	5.48	3.22	1.69
1	21.9	16.78	11.79	8.67	5.44
1.5	48.99	36.45	28.77	20.17	12.22
2	73.24	60.03	48.72	39.79	25.68
2.5	84.46	75.56	63.65	53.56	41.26
3	88.89	83.45	72.16	64.59	52.33
3.5	90.81	86.79	77.04	71.37	60.04
4	92.34	88.02	83.62	75.48	68.12
4.5	-	89.16	86.38	78.99	72.35
5	-	90.95	88.48	80.83	75.61
6	-	-	89.98	84.17	77.88
7	-	-	91.25	86.73	79.72

Further increase in pectin concentration in formulation LM4 and LM5, very less amount of drug was released within 7 hours (i.e. 86.73 % and 79.72 % respectively) (Table 4). It was found that the polymer concentration of the prepared microspheres increased; result in decrease the drug release

proportionately. It may be due to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increases the diffusion path length, which the drug molecules have to travel. Among all the formulations, LM3 showed the best drug release profile (i.e. more than 90 % released). So, formulation LM3 was selected as an optimized formulation for further use.

Graph 3. Drug Release Profile of Losartan Potassium loaded Pectinate Microspheres.



Kinetics of Drug Release:

The drug release data obtained from in-vitro dissolution study were evaluated kinetically by zero order, first order, Higuchi and Korsmeyer model. According to the coefficient of determination (R^2) and release exponent (n) values, drug release data of all the microsphere formulations were best characterized by Higuchi model followed by non-Fickian diffusion mechanism. The coefficient of determination (R^2) and release exponent (n)values of all the formulations were shown in Table 5.

Table-5. Drug Release Kinetics of Losartan Potassium loaded Pectinate Microspheres.

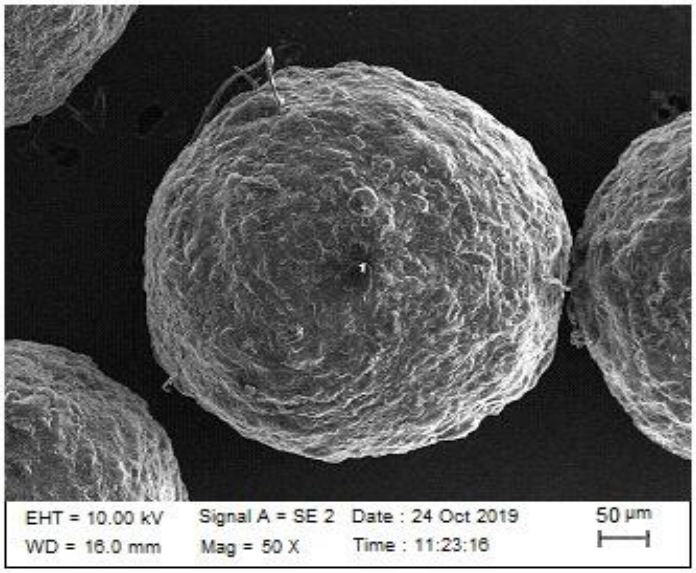
Formulations	Coefficient of determination (r^2)				Release exponent (n)
	Zero Order	First Order	Higuchi Model	Korsmeyer Model	
LM1	0.910	0.891	0.985	0.916	0.53
LM2	0.888	0.873	0.990	0.922	0.61
LM3	0.856	0.794	0.994	0/928	0.59
LM4	0.875	0.834	0.991	0.951	0.62
LM5	0.907	0.856	0.987	0.943	0.74

* Analyzed by the regression coefficient method.

Field Emission Scanning Electron Microscopy (FE-SEM):

The surface morphology was studied through field emissionscanning electron microscope. The FE-SEM of optimized losartan potassium loaded pectinate microspheres is shown in Figure 4. The SEM results revealed that optimized microspheres were discrete and almost spherical in shape.

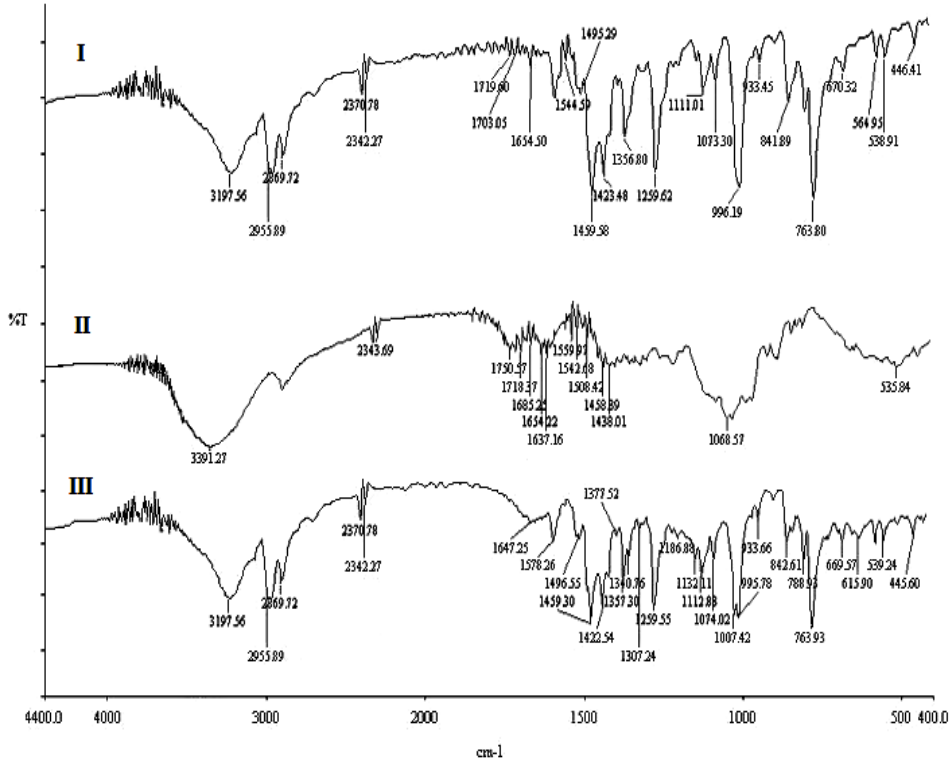
Figure 3. FE-SEM Photomicrograph of Optimized Formulation (LM3).



Fourier Transform Infrared Analysis (FTIR):

The IR spectra of losartan potassium, pectin and optimized losartan potassium loaded pectinate microspheres (LM3) were scanned over a wave number range of 4400-400 cm^{-1} using FTIR analyzer and shown in Figure 5. FTIR spectra revealed that pure losartan potassium exhibited different shoulders at 3197.56 which represent the presence of NH stretching, 2955.89 for stretching vibration of CH of aromatic hydrocarbon chromophore, 1544.59 for C-C multiple bond stretching, 1459.58 and 1423.48 represent CH bending vibration of CH3, 1259.62 for stretching vibration of C-N, 841.89 for adjutants H atom in aromatic ring and 763.80 for stretching vibration due to presence of C-Cl. It was observed from the FTIR study that all the major shoulders of losartan potassium were almost intact in the optimized microspheres formulation (LM3) which leads to the conclusion that there is no interaction between pure drug and polymer.

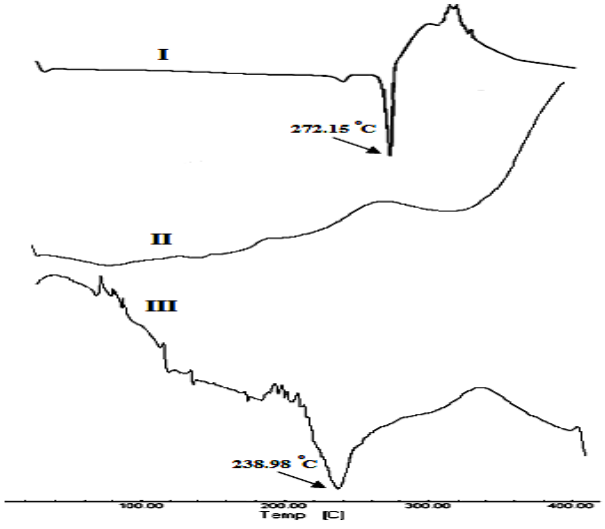
Graph 5. FTIR Spectra of Losartan Potassium (I), Pectin (II)and Optimized Microsphere Formulation (LM3) (III).



Differential Scanning Calorimetric Analysis (DSC):

In the present investigation, DSC thermograms of pure drug, pure polymer and optimized microspheres (formulation LM3) were taken and shown in Figure 6. It was observed that pure losartan potassium exhibited prominent melting endotherm at 272.15 °C. But in the optimized formulation, the intensity and sharpness of the endothermic peak corresponding to the melting point of losartan potassium was significantly decreased (238.98 °C). So it was concluded that in the optimized formulation (LM3), losartan potassium was present in relatively amorphous state, dissolved or molecularly dispersed state.

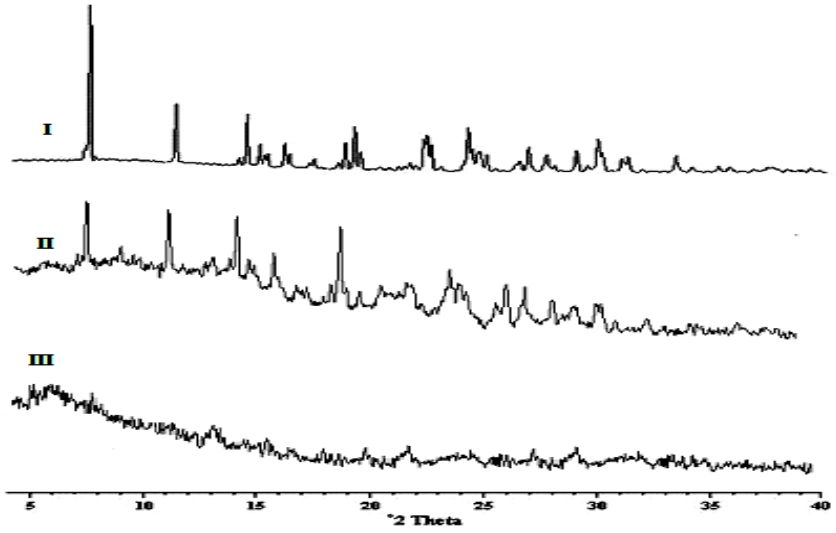
Graph 6. DSC Thermodram of Losartan Potassium (I), Pectin (II)and Optimized Microsphere Formulation (LM3) (III).



X-Ray Diffraction (XRD) Studies:

The XRD spectra of pure losartan potassium depicted distinctive peaks at 2θ values of 7.45°, 11.22°, 14.36°, 18.67°, 19.08°, 22.27°, 24.06°, 29.79° etc., indicating the presence of crystalline losartan potassium. But in the optimized microsphere formulation (LM3), no crystalline peaks of losartan potassium were observed. This result presumed that the drug moleculewas dispersed at the molecular level and the crystallinity of the drug was not shown by X-ray diffraction study (Figure 7). So it is concluded that losartan potassium is present as an amorphous form in the losartan potassium loaded pectinate microsphere formulation.

Graph 7. XRD Spectra of Losartan Potassium (I), Pectin (II)and Optimized Microsphere Formulation (LM3) (III).



CONCLUSION

1. The % yield of all the losartan potassium loaded pectinate microsphere formulations (LM1 to LM5) was found within the range from 76.74 ± 3.79 % to 89.06 ± 2.67 %.
2. The % entrapment efficiency and particle size of the prepared losartan potassium loaded pectinate microsphere formulations (LM1 to LM5) was found within the range from 63.68 ± 3.55 % to 84.78 ± 2.27 % and 0.78 ± 0.14 mm to 0.91 ± 0.05 mm respectively.
3. Formulations LM1 to LM5 showed 2.12 % to 12.46 % mucoadhesion up to 6 hours in simulated gastric fluid (pH 1.2, 0.1N HCl) and 74.28 % to 96.04 % mucoadhesion up to 6 hours in simulated intestinal fluid (phosphate buffer, pH 6.8).
4. Mucoadhesion was pH dependent and increase in polymeric concentrations led to increase in % mucoadhesion.
5. As the concentration of pectin increased, the drug release from the micro-matrix was decreases proportionately.
6. Formulation LM3 was chosen as an optimized losartan potassium loaded pectinate microsphere formulation (more than 90% drug was release within 7 hours).
7. The kinetic data of all the pectinate microsphere formulations (LM1 to LM5) shows best fit in Higuchi model followed by non-fickian diffusion mechanism.
8. FE-SEM of optimized microsphere formulation (LM3) revealed that the microspheres were discrete and almost spherical in shape.

9. FTIR study revealed that there was no interaction between the drug and polymer in the prepared microsphere formulations.
10. DSC revealed that in the optimized microsphere formulation (LM3), losartan potassium was present in relatively amorphous state, dissolved or molecularly dispersed state.
11. X-ray diffraction study stated that losartan potassium is present as an amorphous form in the losartan potassium loaded pectinate microsphere formulation.

From the above research it may be concluded that pectin can be used as a suitable polymer for sustain release drug delivery system to deliver losartan potassium in a sustained manner for longer duration.

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