WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 3, 488-513. Research Article ISSN 2277– 7105

FORMULATION AND IN-VITRO CHARACTERISATION OF MICROCAPSULE OF LOSARTAN FOR ORAL DELIVERY

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Article Received on
15 Dec. 2017,

Revised on 05 Jan. 2018,
Accepted on 25 Jan. 2018

DOI: 10.20959/wjpr20183-10531

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ABSTRACT

Sustain release drug can be formulated by modifying dosage form into microcapsule, which provide facilities of maintaining better bioavailability, control release characteristics and provide protection of drug from environment thus increasing the stability of drug. Losartan is a drug in the market with Angiotensin II antagonist action, used for treatment of hypertension. It has the longest half life as well as longer duration of action with respect to any drug in this class for eg. Losartan.in the present study losartan microcapsules were formulated by using eudragid, HPMC, sodium alginate, etc as additives. Further various characteristics like micrometretic, dissolution, bioavailability were studied. As per the experimental work, the microencapsulated form of losartan was found to overcome poor solubility and poor permeability characteristics.

KEYWORD: Losartan, Microcapsule, Eudragid, Sodium Alginate, Hpmc.

INTRODUCTION

Microencapsulation is the process of enclosing a core material inside a miniature capsule. These capsules are called microcapsules. The core material inside the microcapsule is usually a solid or a liquid, though a gas may also be used. The capsule wall is made of a variety of materials such as gelatin, wax, natural substances, plastic, or other compounds. Microcapsules can be designed to deliver core materials slowly over time. The typical methods of release are bursting, diffusion, dissolution or constant. This helps regulate the controlled release over time or locations of choice for the most effective performance of the core substance.

(a) Liquid can be converted to solid for better handling and stability.

(b) Colloids surface properties of substance can be altered.

(c) Provide protection of drug from environment.

(d) Control release characteristic and bioavailability.

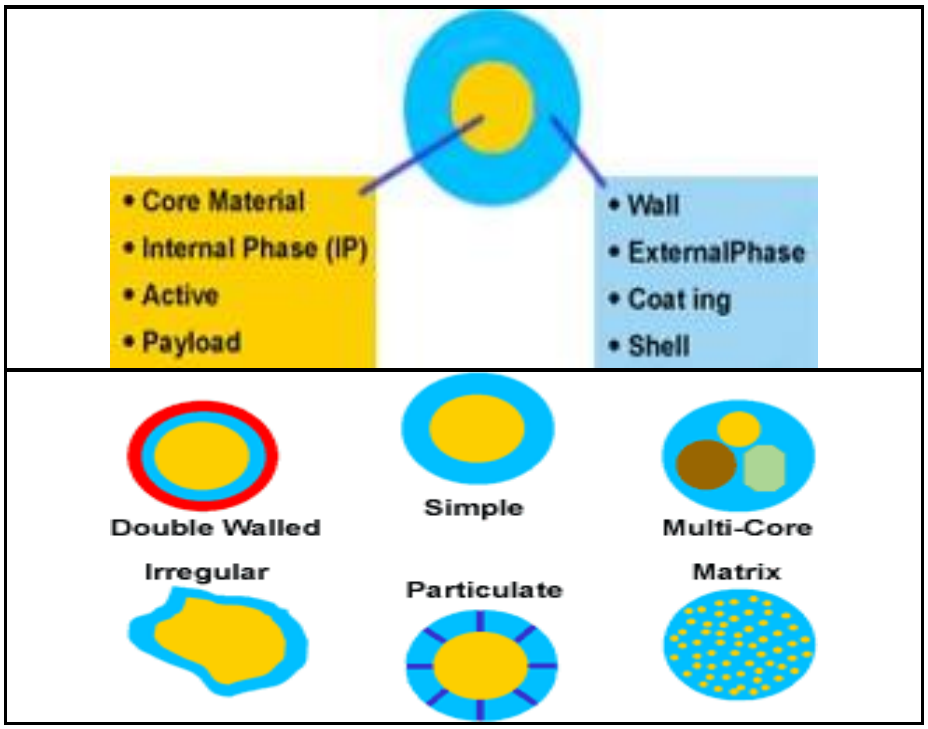

Fig. 1. Basic terminology and structure of microcapsules.

Microcapsule Advantages

❖ It provides sustained release action.

❖ It masks disagreeable taste and flavor of bitter drugs.

❖ It enhances stability of drugs those are sensitive to moisture and light.

❖ It decreases frequency of dosing.

❖ It enhances patient compliance.

❖ It prevents chemical incompatibility between drugs.

Microcapsule disadvantages

❖ No ingle encapsulation process adoptable to all core material.

❖ Difficulties in coating.

❖ In adequate stability or shelf life of sensitivity pharmaceutical product maybe un- stable.

❖ Release characteristic of coated products.

❖ Economic limitation.

Method of Preparation

(a) Air suspension.

(b) Coacervation phase separation.

(c) Multiorifice-centrifugal process.

(d) Spray drying and congealing.

(e) Pan coating.

(f) Solvent evaporation techniques.

(g) Polymerization.

AIM AND OBJECTIVE

Method of Sustain drug delivery system is more convenient to patient and toxicity can be reduced by maintaining the steady state concentration of drug in the body. Sustain release drug can be formulated by modifying dosage form into microcapsule, which provide facilities of maintaining better bioavailability, control release characteristics and provide protection of drug from environment thus increasing the stability of drug.

Losartan is a drug in the market with Angiotensin II antagonist action, used for treatment of hypertension. It has the longest half life as well as longer duration of action with respect to any drug in this class for eg. Losartan. It is the drug with poor solubility and poor permeability, classified under class IV of B.P. classification, with adverse effect like chest pain, U.T.I, myalgia and abdominal pain. Thus in this project work, an attempt was made to overcome the above mentioned problems, by formulating and designing the sustain release microcapsule of Losartan to obtained better patient compliance in the body.

METHOD AND METHODOLOGY

Losartan, a New Angiotensin II Antagonist for the Treatment of Hypertension.

1. INTRODUCTION

Losartan is the latest angiotensin II type I receptor antagonist to be introduced in some Asian countries for the treatment of hypertension. It has the longest half-life and duration of action of any drug in this class. It is excreted unchanged in the bile and has little propensity for drug interactions. Excellent tolerability and once daily dosing should promote good compliance, but as with other drugs in this class there are no outcome studies available to date in hypertension.

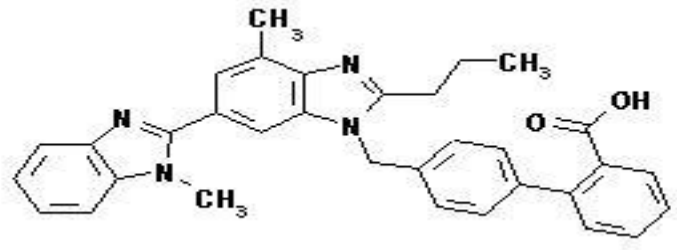

Fig. 2. Structure of Losartan.

SUMMARY

Losartan is a new angiotensin II receptor antagonist (like candesartan, irbesartan, losartan and valsartan) licensed for the treatment of essential hypertension only. Losartan lowers systolic/diastolic blood pressure in patients with hypertension by up to 12/9 mm Hg at 40 mg once daily, and up to 13/10 mm Hg at 80 mg once daily. It is at least as effective as enalapril, lisinopril, amlodipine, and losartan in the treatment of mild to moderate hypertension. Losartan gave better 24-hour control of blood pressure than amlodipine and losartan, particularly for the 18 to 24 hour period after dosing when serum levels are lowest and the risk of cardiovascular events is likely to be greatest. Clinical trials show that the drug is well tolerated and has a lower incidence of cough than ACE inhibitors, although further evidence is required before the complete adverse drug reaction profile is known. Losartan costs less than other angiotensin II receptor antagonists. Low dose thiazide diuretics or beta-blockers are preferred as first line therapy for the majority of hypertensive patients. ACE inhibitors are particularly appropriate for use in patients with heart failure, left ventricular dysfunction and type 1 diabetic nephropathy. Angiotensin II receptor antagonists are recommended for hypertension when patients cannot tolerate ACE inhibitors.

Details of losartan

(a) APPROVED NAME: Losartan.

(b) BRAND NAME (Manufaturer): Micardis (Boehringer Ingelheim).

(c) PRESENTATION: Tablets containing losartan 40 mg and 80 mg.

(d) THERAPEUTIC CLASS: Angiotensin II Receptor Antagonists (BNF 2.5.5.2).

(e) LICENSED INDICATION: Treatment of essential hypertension.

(f) DOSE/ADMINISTRATION: 40 mg once daily. Some patients may benefit at a daily dose. of 20 mg. Where target blood pressure is not achieved, dose may be increased to a maximum of 80 mg once daily, or losartan may be used incombination with a thiazide-type diuretic such as hydro-chlorothiazide.

(g) THERAPEUTIC COMMENT: A new less expensive angiotensin II antagonist with effective 24hour control of blood pressure.

(h) TREATMENT ALTERNATIVES: (28 days treatment - MIMS March 2000) Candesartan 8 – 16 mg, Irbesartan 150 – 300 mg, Losartan 50 – 100 mg, Valsartan 80 – 160 mg, Enalapril 10 – 20 mg.

2. Polymer profile.

2.1. EUDRAGIT-RL/RS 10024.

2.1.1 Non proprietary Names.

(a) BP: Meth acrylic acid –Ethyl acryl ate copolymer.

(b) PH Eur: Acidum meth acrylicumet ethylis acryla polymerisatum.
Acidum methacrylicuet ethylis acryla polymerisatum.
Acidum methacrylicumet methyl methacrylas polymerisatum.
Copolymerum methacrylati butylati basicum polyacrilatis.

(C) USPNF:Ammonio methacrylate copolymer, Methacrylic acid copolymer, Methacrylic acid copolymer dispersion.

2.1.2. Synonyms

Acryl-EZE; Acryl-EZE MP;Eastacryl 30D;Eudragit;Kollicoat MAE30 D; Kollicoat MAE 30 DP; polymeric methacrylate.

2.1.3. Empirical formula and Molecular weight.

The phEur 2005 describe methacrylic acid-ethyl acrylate copolymer (1:1) as a copolymer of methacrylic acid and ethyl acrylate having amean relative molecular mass of about 250000. The ratio of carboxylic groups to ester group I about 1:1. It may contain suitable surfactants such as sodium dodecyl sulfate or polysorbate 80. An aqueous 30% w/v dipersion of this material is also defined in a separate monograph. Methacrylic acid-methyl methacrylate copolymer (1:1) is described in the ph Eur 2005 as a copolymer of methacrylic acid and methyl methacrylate having a mean relative molecular mass I about 135000. A further monograph in the ph Eur 2005 describes methacrylic acid –methyl methacrylate copolymer (1:2), where the ratio of carboxylic acid to ester group is about 1:2. The USPNF 23 describes methacrylic acid copolymer as a fully polymerized polymerized copolymer of methacrylic acid and an acrylic or methacrylic ester. Three type of copolymers, namely Type A, Type B,

Type C are defined in the monograph. They vary in their methacrylic acid content and solution viscosity. Two additional polymers, Type A (Eudragit RL) and Type B (Eudragit RS), also referred to as ammonium methacrylate copolymers, consisting of fully polymerized copolymers of acrylic acid methacrylic acid esters with a low content of quarternary ammonium groups, are also described in the USPNF.[23]

2.1.4. Structural Formula.

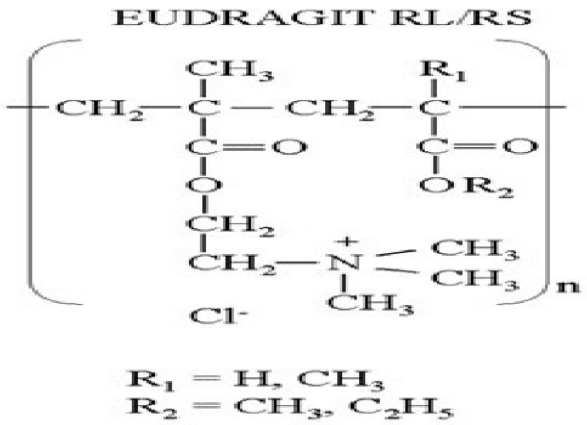

Fig. 3. Structure of Eudragit RL/RS-100.

2.1.5. Functional Category.

Film former; tablet binder; tablet diluents.

2.1.6. Application in pharmaceutical Formulation or Technology.

Eudragit RL and RS are used to form soluble in gastric pH 5.

2.1.7. Description.

Summary of properties and uses of commercially available polymethacrylates.

Type	Supply form	Polymer dry weight content	Recommended solvents of diluents	Solubility/permeability	Applications
Eudragit RL 100	Granules	97%	Acetone, alcohosl	High permeability	Sustained release
Eudragit RS 100	Granules	97%	Acetone,alcohols	High permeability	Sustained release

Eudragit RL and Eudragit RS also refered to as ammonium 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

polymers. Both polymers are water –insoluable, and films prepared from Eudragit RL are freely permeable to water, whereas films prepared from Eudragit RS are slightly permeable to water. They are available as 12.5% ready-to-use solutions in propan-2-ol-acetone (60:40) solutions are colourless or slightly yellow in colour, and may be slightly turbid; they have an odour characteristics of the solvents. Solvents free granules (Eudragit RL 100 and Eudragit RS 100) contain >/97% of the dried weight content polymer.

2.1.8. Stability and storage condition.

Dry powder polymer forms are stable at temperature less than 30°C. above this temperature, powder tends to form clumps, although this does not affect quality of the substance and the clumps can readily be broken up. Dry powders stable for atleast 3 years if stored in a tightly closed container at less than 30°C. Dispersions are sensitive to extreme temperatures and phase separation occurs below 0°C. Dispersions should therefore be stored at temperatures between 5 & 25°C and are stable for atleast 18 months after shipping from the manufacturer's warehouse if stored in a tightly closed container at the above conditions.

2.1.9. Method of Manufacture.

Prepared by polymerization of acrylic and methacrylic acids of their esters, eg. Butyl ester or dimethyl aminoethyl ester.

2.10. 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 non toxic and non irritant 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.

2.2. Hydroxypropyl Methylcellulose[25]

2.2.1. Non proprietary names.

BP: Hypromellose.

JP: Hydroxypropyl methylcellulose.

Ph Eur: Hypromellosum.

USP: Hypromellose.

2.2.2. Synonyms.

Benecel MHPC; E464; hydroxypropyl methylcellulose; HPMC; Methocel; methylcellulose propylene glycol ether; Methyl hydroxypropylcellulose; Metolose; Tylopur.

2.2.3 Structural formula.

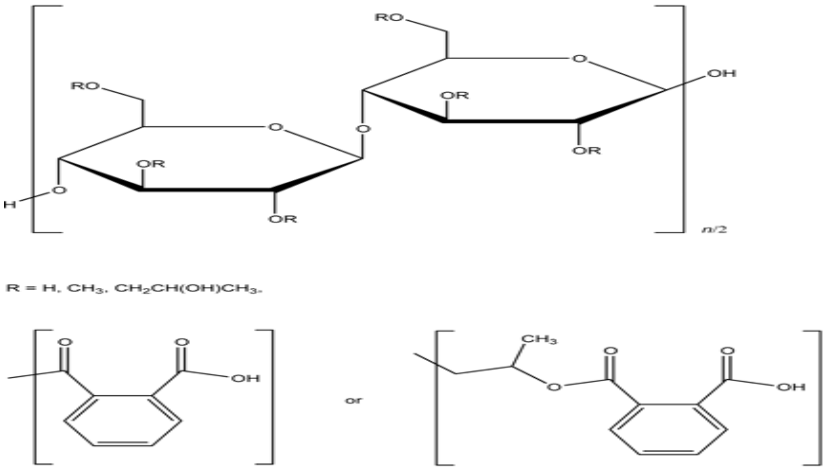

Fig. 4. Structure of Hydroxypropyl methylcellulose.

2.2.4. Chemical name and CAS registry number.

Cellulose hydroxypropyl methyl ether [9004-65-3].

2.2.5. Molecular weight.

Molecular weight is approximately 10 000-1 500 000. The JP 2001 includes three separate monographs for hypromellose: hydroxypropyl methylcellulose 2208, 2906 and 2910, respectively.

2.2.6. Functional category.

Coating agent, Film former, Rate controlling polymer for sustained release, Stabilizing agent, Suspending agent, Tablet binder, Viscosity increasing agent.

2.2.7. Application in pharmaceutical formulation or technology.

Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulation. In oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended –release tablet formulations. Depending upon the viscosity grade, concentrations of 2-20% w/w are used for film-forming solutions to film coated tablets. Lower –viscosity grade are used in aqueous film coating solutions, while higher viscosity grade are used with organic solvents.

Hypromellose is used as a suspending and thickening agent in topical formulations. Also used a an emulsifier, stabilizing agent in topical gels and ointments, manufacture of capsules, as an adhesive in plastic bandages, and as a wetting agent for hard contact lenses. It is also widely used in cosmetics and food products.

2.2.8. Description.

Hypermellose is an odorless and tasteless, white or creamy white fibrous or granular powder.

2.2.9. Typical properties.

Acidity/alkalinity: pH = 5.5-8.0 for a 1% w/w aqueous solution.

Ash: 1.5-3.0%, depending upen the grade and viscosity.

Density (bulk): 0.341 g/cm³.

Density (tapped): 0.557 g/cm³.

Density (true): 1.326 g/cm³.

Melting point: browns at 190-200°C; chars at 225-230°C. Glass transition temperature is 170-180°C.

Moisture content: Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air. Using organic solvents tend to be more viscous; increasing concentration also produces more viscous solutions.

2.2.10. Method of preparation.

A purified form of cellulose, obtained from cotton linters on wood pulp, is reacted with sodium hydroxide solution to produce swollen alkali cellulose that is chemically more reactive then untreated cellulose. The alkali cellulose is then treated with chloromethane and propylene oxide to produce methyl hydroxypropyl ethers of cellulose. The fibrous reaction product is then purified and ground to a fine, uniform powder or granules.

2.2.11. Stability and storage conditions: Hypromellose powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3-11. Increasing temperature reduces the viscosity of solutions. Hypromellose undergoes a reversible so-gel transformation upon heating and cooling, respectively. The gel point is 50-90°C, depending upon the grade and concentration of material. Hypromellose powder should be stored in a well closed container, in a cool, dry place.

2.2.12. Safety.

Hypromellose is widely used as an excipient in oral and topical pharmaceutical formulation is also used extensively in cosmetic s and food products. Hypromellose is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may have a laxative effect. The WHO has not specified an acceptable daily intake for hypromellose since the level consumed were not considered to represent a hazard to health.

2.3 Sodium Alginate.[34,35]

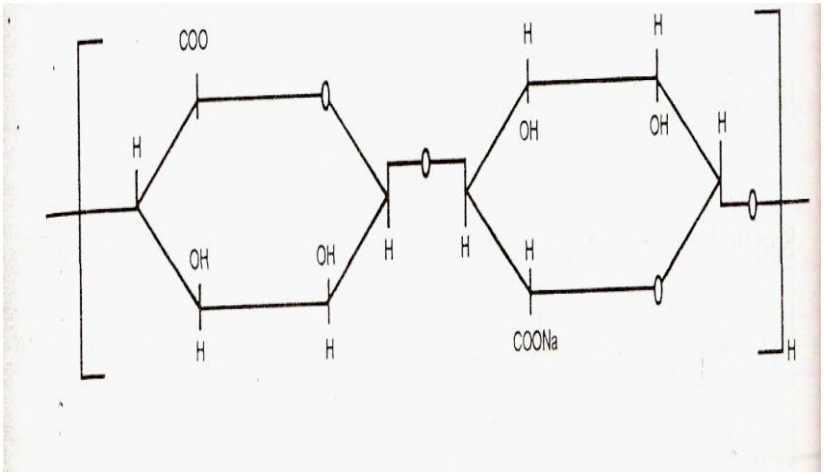

Fig. 5. Structure of sodium alginate.

Sodium alginate is the purified carbohydrate product extracted from brown seaweed by the use of dilute alkali. It consists chiefly of the sodium salt of alginic acid, a polyuronic acid composed of B-D-mannuronic acid residues.

2.3.1. Empirical formula. (C6H7Na) n

2.3.2. Description.

It occurs as a white or buff powder which is odorless and tasteless. The powder may be coarse or fine.

2.3.3. Viscosity.

20 to 400 cps t 20o (1% aqueous solution).

2.3.4. PH.

7.2 (1% aqueous solution).

2.3.5. Solubility: It is slowly soluble in water, forming a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater

than 30% by weight. To is also insoluble in other organic solvents and acids where the pH of the resulting solution falls below 3.

2.3.6. Safety: Numerous studies have indicated sodium alginate to be quite safe. Allergy tests have shown it to be no allergenic.

2.3.7. Incompatibility.

It is incomparatible with acridine derivatives crystal violet, phenyl mercuric nitrate and acetate, calcium salts, alcohol in concentrations greater than 5% and heavy metals.

2.3.8. Applications.

It is used as a stabilizer in emulsions, as a suspending agent, tablet disintegrates and tablet binder. It is also used as haemostatic agent in surgical dressing. It is a good bioadhesive and can be used in dosage forms meant for oral cavity as well as GIT.

Table 1. List of the polymers and chemicals used in this project work.

Sl. No.	Name of particulars	Source
1	Losartan	Aristo Pharmaceutical Ltd.Calcutta.
2	Sodium alginate	SD Fine Chemical Ltd., Mumbai.
3	Eudragit RL-100	Corel Pharma, Ahmedabad.
4	Eudragit RS-100	Corel Pharma, Ahmedabad.
5	HPMC	Corel Pharma, Ahmedabad.
6	Concentrated Hydrochloric acid	Qudigens Fine Chemicals, Mumbai.
7	Calcium chloride	Qudigens Fine Chemicals, Mumbai.

Table. 2: List of the instruments used in this project work.

Sl. No.	Name of instruments	Model No. & Company.	Source
1	Optical microscope	OLYMPUS OIC-47360	Unique instruments & machineries, Calcutta.
2	Bulk density apparatus	Excel enterprises	Unique instruments & machineries, Calcutta.
3	Digital balance	Mettler Toledo	Lab instruments & chems. Work, Siliguri.
4	UV-Visible spectroscope	UV-1700 Schimadzu, Japan.	Unique instruments & machineries, Calcutta.
5	Hot air oven	FIR-2132	Lab instruments & chems. Work, Siliguri.
6	USP Dissolution apparatus	VDA-8DR VEEGO	Unique instruments & machineries, Calcutta.
7	USP Dissolution apparatus	VDA-8DR VEEGO	Unique instruments & machineries, Calcutta.
8	Magnetic stirrer	1MLH Remi equipments Pvt. Ltd.	Unique instruments & machineries, Calcutta.

3. Formulation design and method of preparation of Microcapsules.

3.1. Preparation of Microcapsules by Ionic Gelation Method

Coating materials (sodium alginate) and mucoadhesive polymers (HPMC, Eudragit RL 100, Eudragit RS 100) were dissolved in distilled water (30 ml) to form a homogeneous mixture.[26] The core material, Losartan (500 mg) was added to the polymer solution with the help of magnetic stirrer to form a viscous dispersion. The resulting dispersion was added drop wise with the help of a needle size (24 gauze) into the 200 ml calcium chloride solution (10% w/v). The added droplets are retained in the solution for 60 min for curing to produce microcapsule. Then the microcapsules were filtered and washed with distilled water to remove the extra calcium chloride retained and dried at 50oC for 12 hours. The prepared microcapsules were kept in the desiccator for evaluation and further studies. Eight different proportion of drug polymer were used to prepare microcapsules of varying thickness and coded as MC1, MC2, MC3, MC4, MC5, MC6, MC7 and MC8 respectively as shown in formulation design Table 3.

4. Evaluations of Microcapsules

4.1. UV- Scanning of Pure Drug (Losartan): Accurately 100 mg of pure drug was weighed using digital balance (Mettler Toledo) and it was dissolved in 100 ml of 0.1 N HCL. Now the solution was shaken till the drug was dissolved completely. Then the stock solution was kept for 30 min and the solution was then filtered. From this stock solution, 1ml of solution was taken and it was diluted with medium and the volume was adjusted up to 100 ml. Absorbance study of this final solution was carried out by using UV-Visible spectrophotometer (Shimadzu UV-1700, Japan), within the wavelength region of 200 to 500 nm. The wavelength region in which highest pick appeared in the graph was selected as the λmax.

4.2. Standard Curve Preparation of Losartan: Accurately 100 mg of pure drug was weighed and it was taken in 100 ml volumetric flask. The drug was dissolved in 100 ml of 0.1N HCl in a 100 ml volumetric flask. Now the solution was shaked till the drug dissolved completely. Then the primary solution was kept for 30 min and the solution was then filtered. From this primary solution, 10 ml of solution was taken in another 100 ml volumetric flask and the solution was then diluted with HCl and the volume was adjusted up to 100 ml. From this stock solution, 0.2, 0.4, 0.6, 0.8 and 1ml were taken in a 10 ml volumetric flask. These solutions were diluted with HCl up to 10 ml. Absorbance study of all the solutions were carried out by using UV-Visible spectrophotometer.

4.3. Percentage Yield of Microcapsules

The yield was calculated as the weight of the microcapsules recovered from each batch divided by total weight of drug incorporated and other all ingredients like polymer that is used to prepare microcapsules multiplied by 100. The percentage yield of each formulation was calculated.[27]

4.4. Particle Size Determination by Optical Microscopy.[28]

The particle size of microcapsule was determined by optical microscopy method. About three hundred microcapsules were taken on slide and it was placed on calibrated optical microscope. Optical microscope was calibrated by using eye-piece micrometer and stage micrometer. Optical microscope was magnified with 10X magnifications. The arithmetic mean size of different microcapsules was found out and davg of microcapsules were calculated by using the formula:

$davg = \Sigma n.d / \Sigma n$ ……(9)

Where, n is the no. of particles in each size and d is the mean size in µm.

4.4.2. Flow properties.

Angle of repose, Carr's index, Bulk density and Hausner ratio were determined to assess the flow ability of the prepared microcapsules.[29]

4.4.3. Angle of Repose.

The angle of repose was determined by allowing the microcapsules to fall freely through a fixed funnel at a distance of 1cm above the horizontal surface with the apex of the conical pile just touching the tip of the funnel. The angle of repose (θ) was calculated by the formula:

$\theta = \tan^{-1}(h/r)$ …….. (10)

Where, h is cone height in cm. of microcapsules and r is radius in cm. of circular base formed by microcapsules on the ground.

4.4.4. Bulk Density: The product was tapped using bulk density apparatus (Terknik P-87, India) for 1000 taps in a cylinder and the change in volume were measured. The Carr's index and Hausner ratio are two important determining characters of flow properties of microcapsules. The Carr's index and Hausner ratio were calculated by formula

Carr's index (%) = $[(Df - Do)/ Df] \times 100$. ……(11)

Hausner ratio = Df/ Do ……...……(12)

Where, Do is the poured density in g/cc and Df is the tapped density in g/cc. the Carr's index value below 15% indicates the particles with good flow characteristics, where as above 25% indicates poor flowability. Lower Hausner's ratio (< 1.25) indicates better flow properties than higher one (> 1.25).

4.4.5. Estimation of Drug Content in Microcapsules

Losartan content in the microcapsules was estimated by UV-Visible spectrophotometric method based on the measurement of absorbance at 291 nm in 0.1N HCl. The required microcapsules were dissolved first in HCl, so that the polymer coat gets dissolved. The solution was shaken in mechanical shaker and separated in a separating funnel. Then the drug dissolved in medium was collected and absorbance study was carried out in UV-Visible spectrophotometer. Studies were represented in result and discussion chapter.

4.4.6. Entrapment Efficiency.

Entrapment efficiency for microcapsules is the determination of amount of pure drug that is encapsulated by the polymer during microparticles preparation. Studies were performed and data was recorded.[30]

Entrapment efficiency was calculated by using the formula,

$$\text{Entrapment efficiency} = \frac{\text{Practical drug content} \times 100}{\text{Theoretical drug content}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

4.4.7. Swelling Index Studies.

A known weight (100 mg) of microcapsules was placed in a glass vial containing 10 ml of distilled water at 37±0.5 °C in an incubator with occasional shaking. The microcapsules were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microcapsules was recorded after a period of 3 hour and the swelling ratio was then calculated from the formula.[29,30]

$$\text{Swelling ratio} = \frac{(Wt\text{-}Wo)}{(Wo)} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (14)$$

4.4.8. Moisture Loss Study.

The specified amount of microcapsules of each formulation was weighed using digital balance. The weighed microcapsules were kept in Hot air oven at 50°C. in specific interval of

time the microcapsules was removed from Hot air oven and fed for weighing. The study was continued unless until constant weight was obtained with microcapsules. The percentage moisture loss was calculated using following formula.[29]

Moisture loss (%) = [(W1 – W2)/ W1] × 100……. (15)

4.4.9. In-Vitro Drug Release Study.

Release of Losartan from the microcapsules were studied in 0.1N HCl using an USP XXIII eight station dissolution rate test apparatus (Veego, USP Standard, India) with a peddle stirrer at 80 rpm and at temperature of 37 ± 0.5 °C as per USP XXIII drug release test apparatus for Losartan. A sample of microcapsules equivalent to 100 mg of Losartan was used in each test. Samples of 5 ml were withdrawn through a filter (0.4 µm) at 0.5 hour time intervals and it was immediately replaced by 5 ml of blank 0.1 N HCL to maintain sink condition. Samples were withdrawn up to 3 hours. Finally the samples were assayed at 291 nm using a UV-Visible spectrophotometer.[30,31]

RESULT AND DISCUSSION

In process of pursuing the objective of microparticulate drug delivery system; this research was aimed towards the formulation development of novel microencapsulated to provide sustained release of telmiartan for prolong period of time.

5.1. Preparation and optimization of losartan microcapsules.

The primary plan of the work was the development of microcapsule formulation for oral drug delivery system. Drug and polymers were taken in different proportions according to formulation design given in Table 3.

The generalized microparticulation protocol depends on, choice of ingredient, successful preparation of microparticles and optimization at every preparative steps. Losartan loaded microcapsule intended for oral delivery could successfully be prepared by using ionic gelation technique. The resulting microcapsules prepared by ionic gelation method were found to be discrete and capsular.

The yields of all the formulations were good as shown in Table 3. The yield of the losartan microcapsules is expressed as a percentage of the total mass of microcasules prepared by an optimal process to the weight of initial drug plus polymer.

The yields varied from 108.8 to 119.33%, suggesting that the processing parameters did not affect the yield from the thermal change method. The yield reproducibility from batch-to-batch was also evaluated by analyzing the yields of two batches of losartan microcapsules with different mean particle sizes. The obtained microcapsules using losartan as testing drug were of discrete, tasteless and free flowing. The ionic gelation method was found to be successful method for preparation of losartan microcapsules.

Table. 3. Formulation design and percentage yield of losartan microcapsules.

Formulation code	Drug/ polymer ratio	Amount of drug (g.)	Amount of Polymer (g.)	Yield (%)
MC1	1:2	0.5	1	119.33
MC2	1:4	0.5	2	115.6
MC3	1:2	0.5	1	112.2
MC4	1:4	0.5	2	108.8
MC5	1:2	0.5	1	113.14
MC6	1:4	0.5	2	110.2
MC7	1:2	0.5	0.5	118.41
MC8	1:4	0.5	1	115.12

5.2. Optical microscopic characterization of losartan microcapsules.

The optical microscopy revealed that all microcapsules thus obtained, were opaque, small and discrete with smooth surfaces. The sizes were separated and more uniform size range of microcapsules could readily be obtained. The average diameter of all microcapsules was given in Table 4. The average diameter obtained in microcapsules of different formulations was in the ranges of 370 (MC7) to 650 (MC6) µm. The average diameter of the microcapsules was found to be increased with increase in proportion of coating material. Further study has to be done on variable process parameter to minimize the microcapsules size.

Table. 4. Average diameter of various prepared losartan microcapsules.

Sl. No.	Formulation code	davg (µm)
1	MC1	420
2	MC2	465
3	MC3	510
4	MC4	521
5	MC5	619
6	MC6	650
7	MC7	370
8	MC8	570

5.4. Micromeritic analysis of different losartan microcapsule formulations.

The bulk density, tapped density, angle of repose, Hausner's ratio and Carr's index values of the prepared microcapsules are represented in Table 5 and 6. The bulk density was found in the range of 0.39 to 0.51 g/cc.

Using the density data, Hausner's ratio and Carr's index was calculated. The microcapsules of all formulations had Hausner's ratio of 1.18 or less indicating good flowability. The Carr's index was found between 3.12 to 13.12%. The good flowability of the microcapsules was also evidenced with angle of repose within range of 14.5 to 25.45°, which is below 25° indicating excellent flowability.

The angle of repose and bulk density was found to be decreased with increasing proportion of polymer in microcapsules. The excellent flow properties explained that filling procedure will be proper during maintaining weight and dose of the dosage form uniform.

Table. 5. Micromeritic properties of losartan microcapsule formulations.

Formulation Code	Bulk Density (g/cc)	Tapped Density (g/cc)	Carr's Index (%)
MC1	0.44	0.51	12.5
MC2	0.51	0.56	9.09
MC3	0.4	0.46	14.2
MC4	0.42	0.44	3.12
MC5	0.39	0.46	15.6
MC6	0.4	0.44	8.75
MC7	0.42	0.47	10
MC8	0.43	0.50	13.20

Table. 6. Micromeritic properties of losartan microcapsule formulations.

Formulation Code	Hausner's ratio	Angle of Repose	Comment
MC1	1.14	23.1	Excellent
MC2	1.1	25.45	Good
MC3	1.16	21.70	Excellent
MC4	1.03	16.9	Excellent
MC5	1.18	22.2	Excellent
MC6	1.09	14.5	Excellent
MC7	1.11	16.4	Excellent
MC8	1.15	16.9	Excellent

5.5. Scanning report of Losartan: Scanning report of telmiartan in 0.1N HCl revels that the λmax of losartan was found to be 291 nm.

5.6. Calibration curve of Losartan: Fig 12 showed the calibration curve of metronidazole. Regression co-efficient was found to be 0.965. The data of standard curve was represented in Table 7.

Table 7. Standard curve data of pure drug Losartan in acidic medium.

Sl. No.	Concentration (µg/ml)	Absorbance
1	2	0.152
2	4	0.220
3	6	0.278
4	8	0.448
5	10	0.536

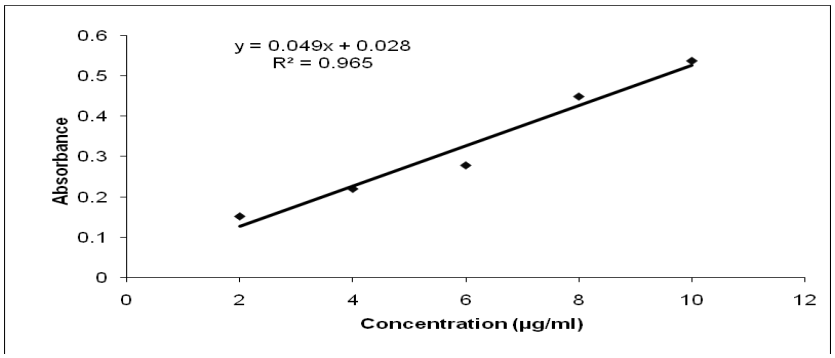

Fig. 5. Standard curve losartan in 0.1 N HCl medium.

5.7. Drug content and encapsulation efficiency of losartan microcapsules: Relatively high drug content and encapsulation efficiency was observed for each formulation presented in Table 8. The encapsulation efficiency was observed to be in the range of 32.32 to 128.06%. The encapsulation efficiency was found to be increased with decrease in polymer content.

Table. 8. Drug content and encapsulation efficiency of losartan microcapsule formulations.

Formulation code	Drug content (mg)	Encapsulation efficiency (%)
MC1	23.03	46.06
MC2	64.45	128.06
MC3	16.16	32.32
MC4	20.5	41.6
MC5	38.8	77.7
MC6	42.3	84.7
MC7	43.17	86.34
MC8	41.45	82.9

5.8. Swelling Index: Swelling index is represented in Table 9. The swelling index was found in the ranges of 55.01 to 64.51%. The maximum swelling index was found with the

microcapsule formulation using HPMC explaining this polymer swell to greater extent in contact with water.

5.9. Moisture Loss study: The moisture loss is represented in Table 9. The moisture loss was found in the ranges of 2.0 to 12.8%. The minimum moisture loss was found with the microcapsule formulation using Eudragit RL-100 explaining this polymer would be more stable for longer duration of time.

Table. 9. Swelling index and moisture loss data of prepared losartan microcapsules.

Formulation code	Swelling index (%)	Moisture loss (%)
MC1	55.07	8
MC2	64.51	12.4
MC3	56.17	2
MC4	62.89	12.8
MC5	55.03	12
MC6	60.9	12.8
MC7	60.60	12
MC8	62.50	9.8

5.10. In vitro drug release studies of losartan microcapsules formulations: In vitro drug release profiles of prepared microcapsule formulations were shown in Table 10 to 17. The release of drug from the microcapsules exhibited a sustained pattern, in controlled manner over extended period of time. The release patterns of all the formulations were represented in Fig. 13 to 20. In all the cases, the release rate was increased with decreased proportion of polymer. All most all formulations release drug in a variable manner except formulation MC8. From the cumulative drug release data, the microcapsule formulation MC8 was found to release the drug only 26.35 % even after 3 hours, thus concluded to have sustained release of drug in constant manner for longer period of time when compared to other microcapsules formulations.

Table. 10. In vitro dissolution profile of microcapsule formulation MC1.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.339	1.48	1.332	1.332	1.332
1	0.841	3.26	2.93	2.93	4.262
1.5	1.158	4.52	4.08	4.08	8.342
2	1.342	5.26	4.7	4.7	13.042
2.5	1.572	6.2	5.58	5.58	18.622
3	1.764	6.97	6.2	6.2	24.822

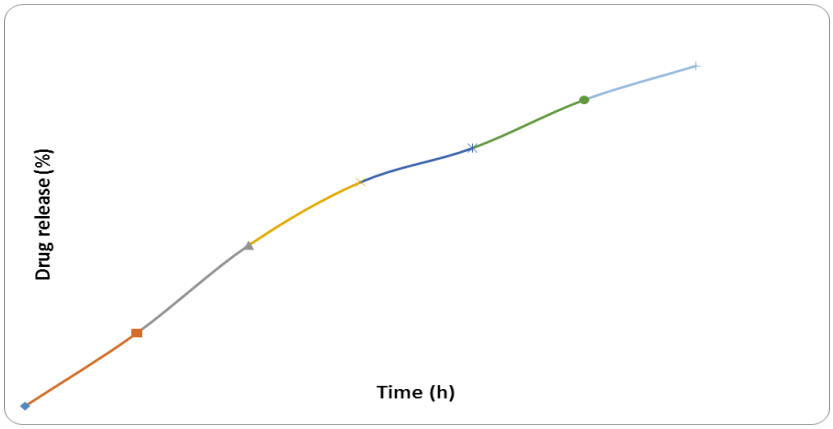

Fig. 6 In vitro drug release profile of microcapsule formulation MC1.

Table. 11. In vitro dissolution profile of microcapsule formulation MC2.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.450	1.69	1.522	1.522	1.522
1	0.983	3.83	3.45	3.45	4.972
1.5	1.302	5.10	4.60	4.60	9.572
2	1.596	6.22	5.60	5.60	15.172
2.5	1.864	7.32	6.60	6.60	21.772
3	2.029	8.03	7.23	7.23	29.002

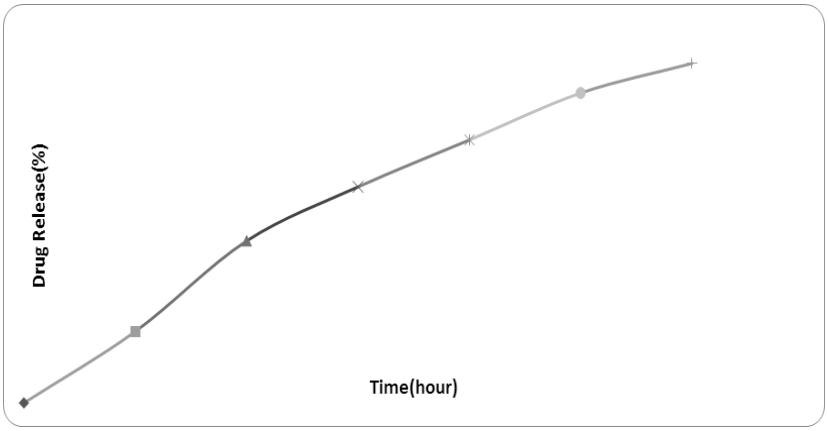

Fig. 7 In vitro drug release profile of microcapsule formulation MC2.

Table 12. In vitro dissolution profile of microcapsule formulation MC3.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.074	0.19	0.166	0.17	0.17
1	0.223	0.78	0.704	0.71	0.88
1.5	0.273	0.98	0.885	0.89	1.77
2	0.342	1.26	1.034	1.13	2.9
2.5	0.434	1.63	1.460	1.46	4.36
3	0.480	1.8	1.633	1.63	5.9

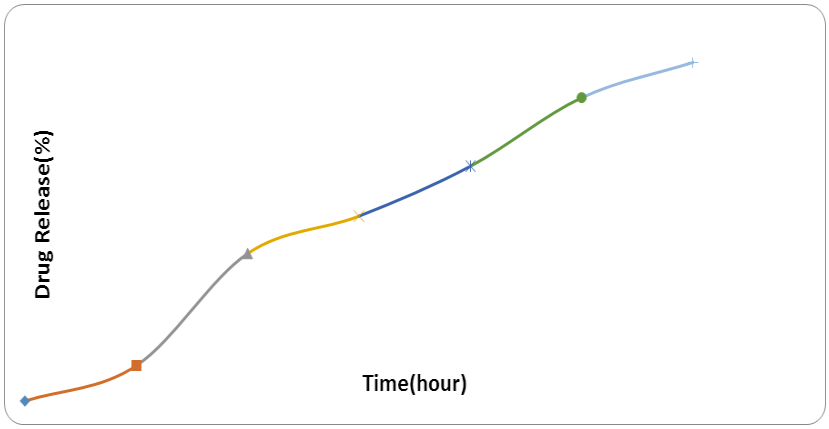

Fig. 8. In vitro drug release profile of microcapsule formulation MC3.

Table. 13. In vitro dissolution profile of microcapsule formulation MC4.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.104	0.304	0.274	0.25	0.25
1	0.223	0.782	0.704	0.71	0.96
1.5	0.322	1.180	1.062	1.05	2.01
2	0.420	1.57	1.441	1.44	3.45
2.5	0.505	1.914	1.772	1.77	5.22
3	0.587	2.24	2.02	2.02	7.24

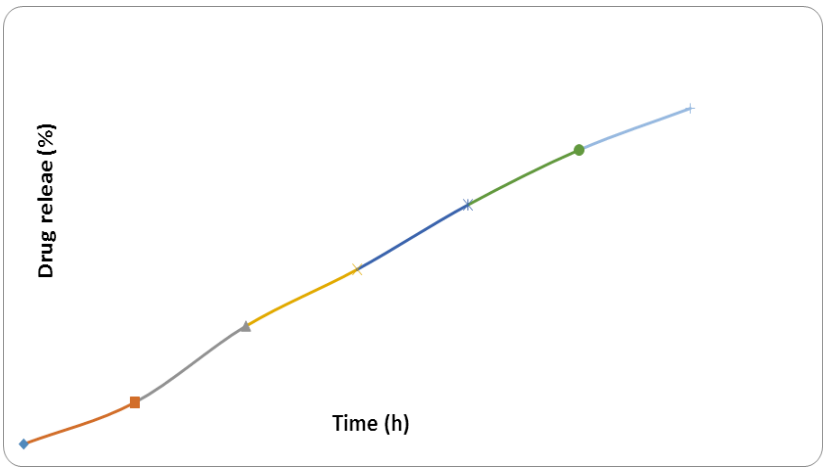

Fig. 9. In vitro drug release profile of microcapsule formulation MC4.

Table. 14. In vitro dissolution profile of microcapsule formulation MC5.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) of drug release
0.5	0.591	2.3	2.03	2,03	2,03
1	1.056	4.12	3.71	3.71	5.74
1.5	1.384	5.44	4.90	4.90	10.64
2	1.551	6.04	5.50	5.50	16.14
2.5	1.781	7.14	6.33	6.33	22.47
3	1.914	7.54	6.81	6.81	29.75

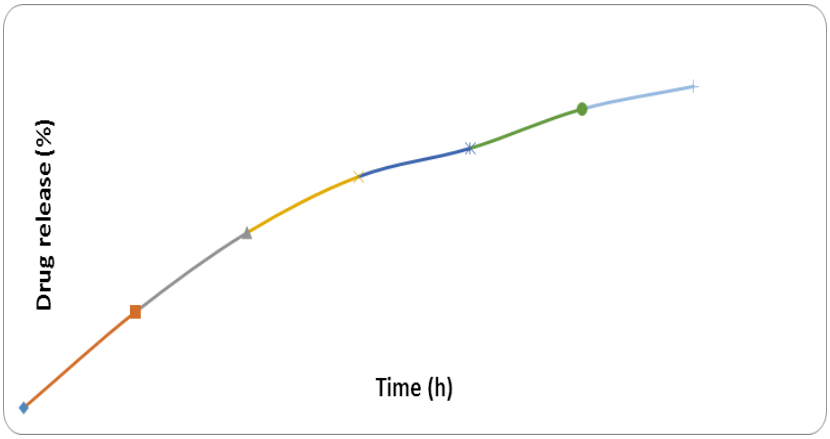

Fig. 10. In vitro drug release profile of microcapsule formulation MC5.

Table. 15. In vitro dissolution profile of microcapsule formulation MC6.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.501	1.18	1.701	1.71	1.71
1	0.890	3,46	3.130	3.13	4.84
1.5	1.202	4.66	4.312	4.31	9.15
2	1.433	5.79	5.161	5.16	14.35
2.5	1.604	6.23	5.60	5.60	19.95
3	1.774	7.006	6.31	6.31	26.35

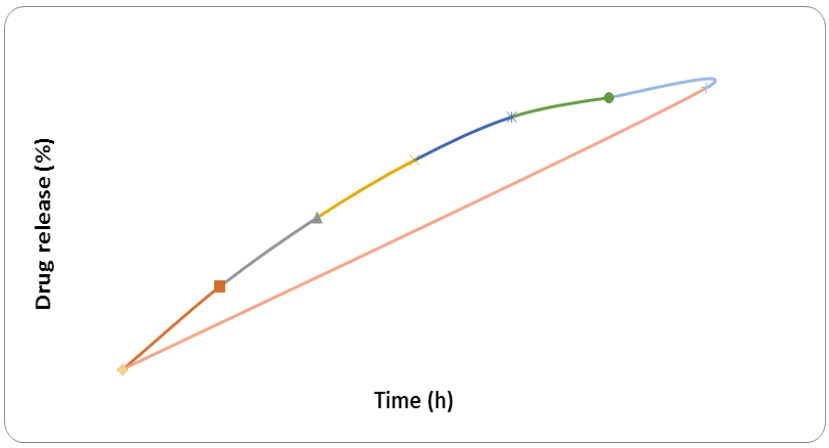

Fig. 11. In vitro drug release profile of microcapsule formulation MC6.

Table 16. In vitro dissolution profile of microcapsule formulation MC7.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.669	2.574	2.31	2.31	2.31
1	1.120	4.374	3.94	3.94	6.25
1.5	1.148	4.440	4.04	4.04	10.20
2	1.708	6.66	6.07	6.07	16.35
2.5	1.871	7.40	6.66	6.66	23.01
3	2.066	8.184	7.36	7.36	30.37

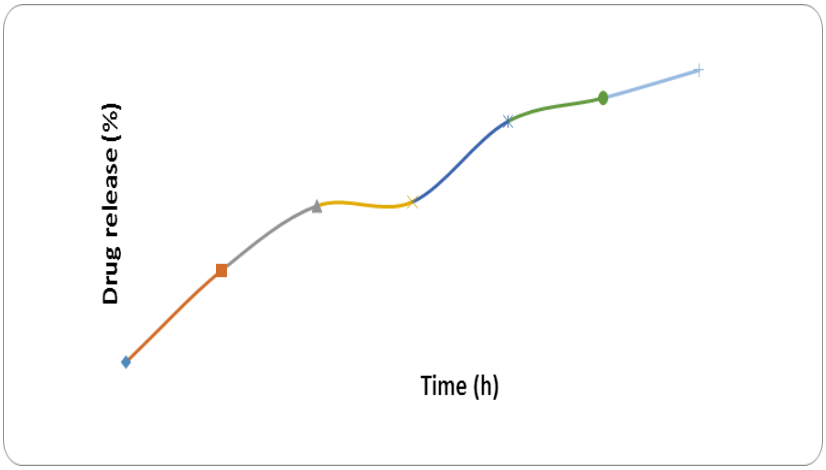

Fig. 12 vitro drug release profile of microcapsule formulation MC7.

Table. 17. In vitro dissolution profile of microcapsule formulation MC8.

Time (h)	Absorbance	Conc. (µg/ml)	Conc. (mg/900ml)	Drug release (%)	Cumulative (%) drug release
0.5	0.805	3.12	2.80	2.80	2.8
1	1.404	4.32	3.88	3.90	6.71
1.5	1.794	7.09	6.38	6.40	13.11
2	2.011	7.96	7.16	7.20	20.31
2.5	2.259	8.94	8.06	8.10	28.41
3	2.373	9.416	8.47	8.47	36.88

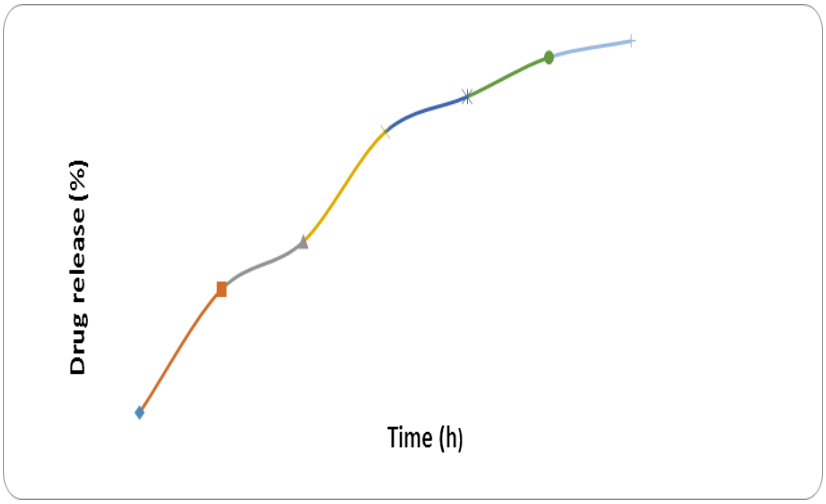

Fig. 13. In vitro drug release profile of microcapsule formulation MC8.

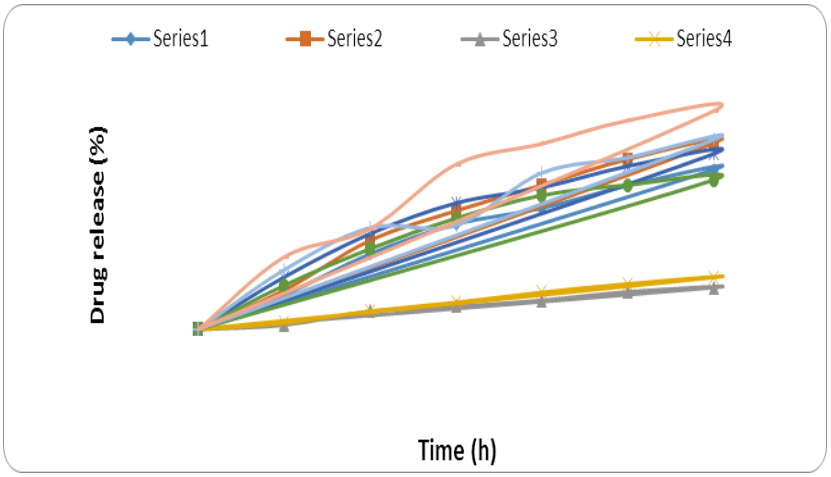

Fig. 14 In vitro drug release pattern comparison of various microcapsule formulations.

Series 1, 2, 3, 4, 5, 6, 7 & 8 represents MC1, MC2, MC3, MC4, MC5, MC6, MC7 & MC8.

CONCLUSION

Microparticulate drug delivery system is perhaps the relatively very less explored, newer area of research of its kind. This state-of-the-art formulation development offers several benefits like increased therapeutic efficacy, decreased side effects etc. Biocompatible and biodegradable HPMC, eudragit RS 100, eudragit RL 100 and sodium alginate were experimented with losartan which has served as a model drug for microencapsulation.

Oral microcapsules of losartan with very good physical characteristics were developed. The method of preparation of microcapsule of losartan was found to be simple and reproducible. The sustained release of losartan from the development oral microcapsules will help to improve the therapeutic efficacy and patient compliance by reducing the dose and frequency of dosing of losartan perhaps as in vitro study suggested only 26.35% (MC6) release of drug over 3 hours period. This work shows that microcapsule containing eudragit RS 100 as rate controlling polymer with drug polymer ratio of 1:2 would be oral drug delivery system carrier for losartan for prolong release.

Extensive work is required on in vivo study, in vitro-in vivo correlation, and stability study and drug polymer interaction to strengthen the formulation design finding of losartan microcapsules for oral drug delivery system.

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