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PLGA Nanoparticles for Delivery of Losartan Potassium through Intranasal Route: Development and Characterization

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Abstract

Cardiovascular disease caused 2.3 million deaths in India in the year 1990, and this is projected to double by the year 2020. Losartan Potassium and ACE receptor blocker is a drug of choice. The objective of the work was to develop a delivery system that prevents the high first pass metabolism of the antihypertensive drug with sustained release by reducing dosing frequency to effective management of Hypertension.The Two level-Three factor (2^3) optimization model was used to determine the optimized formulation. The independent variables selected for optimization of formulation was Drug: Polymer ratio, Surfactant concentration and Stirring speed. The dependent variables are Particle size and Entrapment efficiency. The formulation was optimized on the basis of dependent variables. The Entrapment Efficiency of optimized formulation was found to be 87 %, Zeta potential of that formulation was -16.6 mV and Particle size was found to be 295.3 nm. The Scanning Electron Microscopy (SEM) image does not exactly shows the Nanoparticle size, but it can be concluded from the image of that particles are of 250 nm. The In-vitro release study was done using Dialysis bag, it releases 84% drug in 48 hours. The release pattern follows Korsemeyer-Peppas Model. The In-vivo fluorescent study was done and presence of green colour confirms that the Nanoparticles of optimized formulation was able to penetrate the nasal epithelium.No major changes in the content of drug was observed at the end of 3 months during Stability study. So data of stability studies revealed that formulation will be stable for longer period of time.

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Key words:
Losartan Postassium, Nanoparticles, Entrapment, SEM, Korsemeyer-Peppas Model.

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INTRODUCTION

Blood transports oxygen and nutrients to the organs. The heart pumps the blood through the blood vessels. This action causes pressure on the blood, the blood pressure.[1]

The normal level for blood pressure is below 120/80. The top number, the systolic blood pressure, corresponds to the pressure in the arteries as the

heart contracts and pumps blood forward into the arteries. The bottom number, the diastolic pressure, represents the pressure in the arteries as the heart relaxes after the contraction.

Cardiovascular disease caused 2.3 million deaths in India in the year 1990, and this is projected to double by the year 2020. Hypertension is the most common cardiovascular disease. It is responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths in India. The prevalence of hypertension in Indian population is about 30-40%.[4]

Hypertension is persistent elevation of systolic BP of 140 mmHg or greater and/or diastolic BP of 90 mmHg or greater.[5]Essential hypertension is a far more common condition and accounts for 95% of hypertension. Essential hypertension develops only in groups or societies that have a fairly high intake of salt, exceeding 5.8 grams daily. Approximately 30% of cases of essential hypertension are attributable to genetic factors.[3,7]The term "secondary hypertension" implies that a patient's blood pressure elevation is the result of an underlying discoverable disease process.

The Renin-Angiotensin-Aldosterone System is one of the major hormonal systems that influence blood pressure. Renin is secreted from the juxtaglomerular apparatus of the kidney in response to glomerular underperfusion, reduced intake of salt, or stimulation from the sympathetic nervous system. Renin results in the conversion of renin substrate (angiotensinogen) to angiotensin I, which is a physiologically inactive substance. A key enzyme, angiotensin converting enzyme (ACE), results in the conversion of angiotensin I to angiotensin II. Angiotensin II is a potent vasoconstrictor that leads to an increase in blood pressure.

The two subtypes are designated bysubscript numbers AT1 and AT2. AT1 is the subtype that mediates the best-known actions of angiotensin.[12]As a rule, the ratio of AT1/AT2 is >1.AT1 is found in almost all parts of body, stimulation of this receptor in kidney, vascular Smooth Muscleand Adrenal gland causes Vasoconstriction and Sodium reabsorption, which leads to BP increase.[16,17]

ARBs are receptor antagonists that block type 1 Angiotensin II (AT_1) Receptors. AT_1 is found in the Heart, Endothelium, Vascular Smooth Muscle, Brain, Platelets, Monocytes, several parts of the Kidney, Adrenal Cortex, Adrenal Medulla, Sperm Cells and Placenta.[6] ARBs include Candesartan, Losartan,Valsartan etc.[15,16,17]

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanospheres are matrix systems in which the drug is physically and uniformly dispersed.[18,19,20]

Nanoparticles are receiving considerable attention for the delivery of therapeutic drugs.[21,22]Particle size is one of the key features in determining performance because it influences circulating Half-life, Cellular Uptake and Biodistribution. The uniformity of the particle population is also a significant factor in performance.[23]

Nowadays many drugs have better systemic bioavailability through nasal route as compared to oral administration. Intranasal drug delivery offers a promising alternative route for administration of Proteins and Peptides as well as drugs shows poor performance with oral route. The interest in intranasal route for therapeutic purposes arises from the Anatomical, Physiological and Histological characteristics of the nasal cavity, which provides rapid systemic drug absorption and quick onset of action.[28,31,33]

Advantages of Nasal Drug Delivery System

- Hepatic first pass metabolism is absent.
- Rapid drug absorption.
- Quick onset of action.
- The bioavailability can be improved by means of absorption enhancer.

- Better nasal bioavailability for smaller drug molecules.
- Drugs which cannot be absorbed orally may be delivered to the systemic circulation through nasal drug delivery system.

The Lactide/glycolide copolymers have had strong success in drug delivery formulations because their degradation can range from 3 weeks to over a year, depending on the composition of the copolymer as well as the method of preparation and formulation. Decreasing the degree of crystallinity will also increase the degradation rate of the resulting polymer. This is evident in the degradation times of 50:50 PLGA, 75:25 PLGA, and 85:15 PLGA being 1-2 months, 4-5 months, and 5-6 months, respectively.

Biodegradation of PLGA:

A three-phase mechanism for PLGA biodegradation has been proposed:

1. Random chain scission process. The M.W. of the polymer decreases significantly, but no appreciable weight loss and no soluble monomer products are formed.
2. In the middle phase, a decrease in M.W. accompanied by a rapid loss of mass and soluble oligomeric and monomer products are formed.
3. Soluble monomer products formed from soluble oligomeric fragments. This phase is that of complete polymer solubilization.

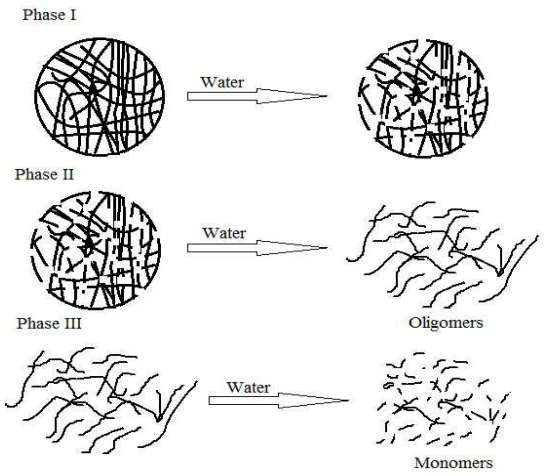


Fig. 2 Degradation of PLGA based Delivery System

It is known that PLGA biodegrades into lactic and glycolic acids. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eliminated as carbon dioxide and water.

PREPARATION AND CHARACTERIZATION

The present work focused on the Development and Characterization of Losartan Potassium loaded PLGA nanoparticles for intranasal delivery of Antihypertensive drug to systemic circulation for the maintenance of hypertension. The polymer is used to sustain the release of drug. The Nanoparticle of water soluble drugs using PLGA as polymer are mostly prepared by Double emulsion solvent evapouration method. In these nanostructures, the drug can be entrapped or attached to the nanoparticle matrix.

Material

Losartan Potassium was obtained as a gift sample from IPCA laboratories ltd., Ratlam. PLGA (50:50) was obtained as gift sample from Torrent pharmaceutical ltd., Ahemdabad. Dichloromethane, Span-20 and Poly Vinyl Alcohol were purchased from Lobachemie, Mumbai. Other reagents and solvents are of analytical grade.

Method

Preparation of Nanoparticles

Double Emulsion Solvent Evapouration method is the most suitable method for the preparation of Nanoparticles of hydrophilic drugs. So, the PLGA Nanoparticles entrapping Losartan potassium was prepared by using double emulsification method. The primary emulsion between the internal aqueous phase and the organic phase (polymer solution in DCM) will be prepared by Vortexing. Organic to aqueous phase volume ratio during primary emulsification step will be 1:4. Secondary

emulsification will be carried out in varying concentration of PVA solution using mechanical stirrer to obtain Nanoparticles. The prepared nanoparticles will then be centrifuge and washed with water three times to remove the excess of surfactant from the nanoparticles.

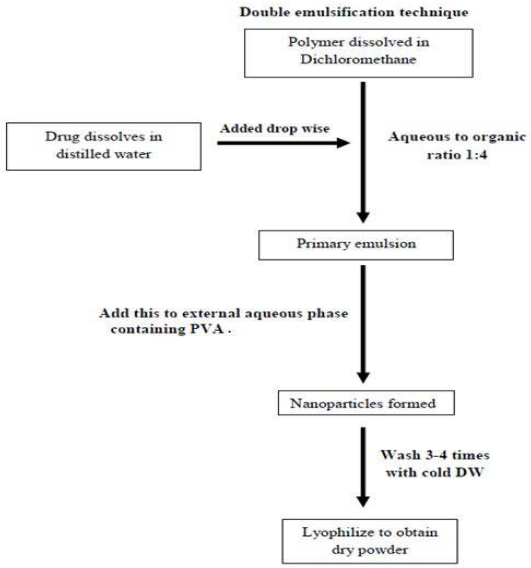


Fig. 3 Procedure for Nanoparticle Preparation

Design of Experiment

A two level, three-factor factorial design (2^3) was selected to study the main effects and interaction of three factors on entrapment efficiency and particle size. The independent factors investigated were the Drug-Polymer ratio, Surfactant concentration and Stirring speed. The parameter level selection was based on the literature. The two levels of independent factors for experiment domain of each variable are summarized in Table 4 and Table 5.

Variable	Levels	
	Low	High
Drug-Polymer ratio	1:2	1:4
Surfactant (PVA) (%W/V)	1	2
Stirring speed	2000	4000

Table 11: Variable Values of 2^3 Factorial Design for Nanoparticles

S. No.	Batch No.	Drug-Polymer ratio	Surfactant Conc. (%w/v)	Stirring Speed	% Entrappment Efficiency	Particle Size (nm)
1	**LPN-1**	1:2	1	2000	65.38 % ± 0.23	430
2	**LPN-2**	1:2	1	4000	41.7% ± 0.08	240
3	**LPN-3**	1:2	2	2000	67.5% ± 0.20	310
4	**LPN-4**	1:2	2	4000	61.8 % ± 0.11	184
5	**LPN-5**	1:4	1	2000	71.7% ± 0.03	480
6	**LPN-6**	1:4	1	4000	64.4% ± 0.025	346
7	**LPN-7**	1:4	2	2000	87 % ± 0.31	295.3
8	**LPN-8**	1:4	2	4000	83% ± 0.14	197

Values are expressed in mean ±SD, n=3

Table 12: Full Factorial 2^3 Design Layout for Losartan Potassium Loaded Nanoparticles and Effectof PVA Concentration, Stirring Speed and Drug:Polymer Ratio

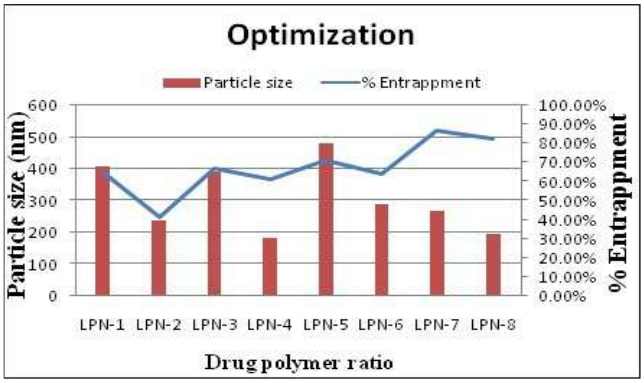


Fig. 4 Effect of Independent Variables

The nanoparticle formulation was optimized using two level-three factor factorial design (2^3). The independent variable shows various effects on the dependent variables i.e. Particle size and Entrappment Efficiency. The nanoparticles size is found to icrease with increase the concentration of polymer and decrease with increase the concentration of surfactant. The stirring speed also affects the particle size i.e. the particle size decrease with increase the speed. The Entrapment Efficiency found more with decrease in stirring speed and increase with increase in polymer concentration. On the basis of following observations the formula given in table 13 was taken as optimized formula for getting the required properties of Losartan Potassium loaded nanoparticles.

S. No.	Optimum parameter	Optimized value
1	Drug :polymer ratio	1:4
2	Internal aquous phase	1 ml
3	Span 20	2.5%
4	External aq. Phase	50 ml
5	PVA	2 % w/v
6	Stirring speed	2000 rpm
7	Stirring time	2 min

Table 13: Optimized Formulation

Characterization of Nanoparticles

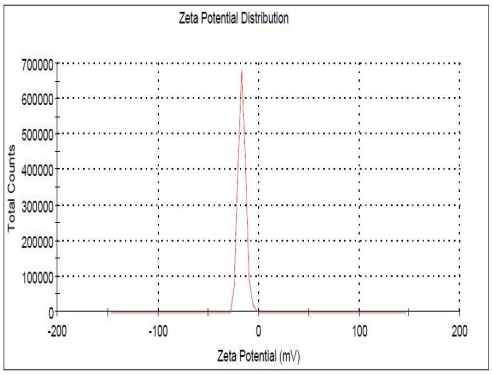
Particle Size and zeta potential

Nanoparticles size and size distribution were determined using photon correlation spectroscopy (PCS) (Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a fixed angle of 90^0 at 25^0C using sample appropriately diluted with filtered water (0.2 µm filter, Minisart, Gottrgen, Germany).

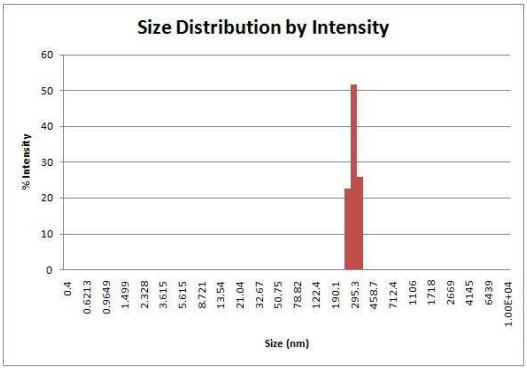
Zeta potential was measured using photon correlation spectroscopy (PCS) (Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK) using a disposable zeta cuvette. For each sample, the mean diameter/ Zeta potential + standard deviation of three determinations were calculated applying multimodal analysis. Thumb rule describes the relation between zeta potential determination responses of the suspension being tested, particularly hydrophobic colloids.

S. No.	Formulation	Zeta potential	Size (nm)	PDI
1	Nanoparticles	-16.6	295.3	0.280

Table 14: Zeta Potential, Size and PDI of Nanoparticles



(A)



(B)

Fig. 5: Optimized Nanoparticles Formulation (A) Zeta Potential,(B)Particle Size Distribution

The Zeta potential was measured using photon correlation spectroscopy (PCS) (Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK) using a disposable zeta cuvette. The Zeta potential of the optimized formulation was found -16.6 mV, which shows good stabilityof the formulation in a suspension form.

The size of nanoparticles was determined using Photon Correlation Spectroscopy. The size of the optimized formulation was obtained 295 nm with a polydispersity index 0.280. This is a suitable size range for intranasal delivery to systemic circulation.

8.2.3.2 Determination of Drug Entrapment Efficiency (%EE)

Entrapment of Losartan Potassium in nanoparticles was determined by direct method. The nanoparticle suspension was centrifuged at 15000 rpm and supernatant was decanted off, washed three times with distilled water. The small amount of Dichloromethane was added to nanoparticles allowed to dissolve the polymer, then add 10 ml of water and centrifuge again. The supernatant was then analysedfor unentrapped drug after suitable dilution by UV spectrophotometer at 205 nm.

The entrapment efficiency was calculated using following equation.

$$\% \text{ Entrapment} = \frac{\text{Total Amt.of Drug added} - \text{Total Amt. of Drug in Supernatant}}{\text{Total Amt. of Drug added}} \times (100)$$

S. No.	Formulation	Entrapment efficiency
1	Optimized Nanoparticles	87%

Table 15: Entrappment Efficiency of Optimized Formulation

The % Entrapment Efficiency of the optimized formulation was determined in the supernatant remains after centrifugation and it was found to be 87 % which is highest than other formulations.

8.2.3.3 Surface Morphology

Surface morphology was determined using Scanning electron microscope. The samples for SEM were prepared by lightly sprinkling the SLN powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å using a sputter coater at an acceleration voltage of 2.0 kV. All samples were examined under a scanning electron microscope (JEOL 5400, Japan) and photomicrographs were taken at suitable magnification.

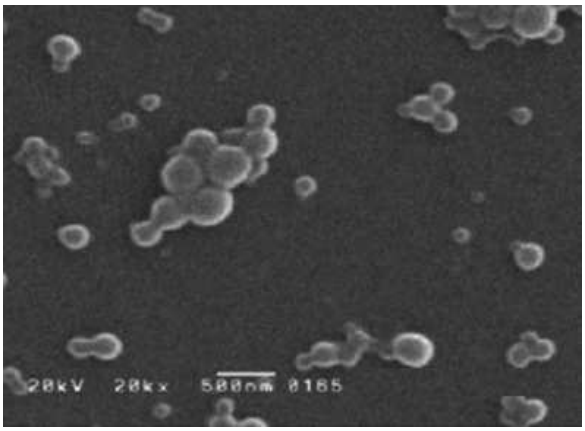


Fig. 6 SEM Image of Optimized Formulation

8.2.3.4 In-vitro release studies

The dialysis bag diffusion technique was used to study the In-vitrodrug release of Losartan Potassium loaded nanoparticles. 5 ml of nanoparticle suspension were placed in a dialysis bag with a membrane of molecular weight cut off size of 10,000 Da and immersed in to 50ml of PBS (7.4). The entire system was kept at 37°C with the continous stirring on Magnetic Stirrer at 150 rpm. Samples were withdrawn from the receptor compartment at predetermined intervals and replaced by fresh medium. The amount of drug dissolved was determined with UV Spectrophotometry at 205 nm.

S. No.	Time (In hrs.)	% Drug release	% Cumulative Drug Release
1	0.5	3.97±0.08	3.97
2	1	4.35±0.10	4.59
3	2	6.54±0.22	6.97
4	3	9.93±0.18	10.58
5	4	13.18±0.12	14.15
6	5	18.06±0.18	19.37
7	6	20.26±0.10	22.06
8	7	24.86±0.06	26.88
9	8	27.4±0.14	29.87
10	9	31.32±0.16	34.06
11	10	34.19±0.19	37.31
12	11	38.01±0.13	41.42
13	12	44.21±0.09	48
14	24	59.15±0.12	63.56
15	48	78.12±0.21	84.01

Values are expressed in mean ±SD, n=3

Table 16: In-vitro Drug Release

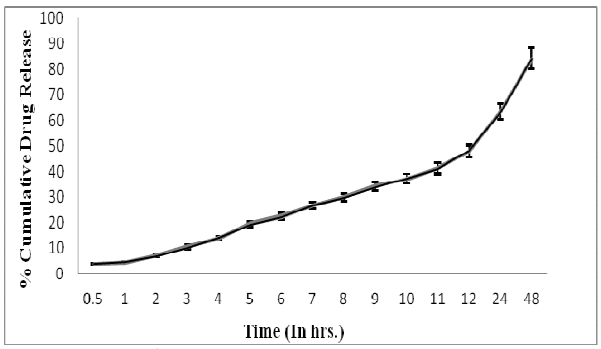


Fig. 7: In-vitro Drug Release

The In vitro release study was performed using Dialysis method. The receiver compartment was phosphate buffer which has similar pH as that of blood. The volume of receiver compartment was 50 ml and the rotation speed was set at 150 rpm. The nanoparticle preparation follows controlled release. 84% of the total drug was released in 48 hrs at room temperature.

Release Kinetic Model

There are number of kinetic models, which described the overall release of drug from the dosage forms. Because qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. Model dependent methods are based on different mathematical functions, which describe the dissolution profile. The model dependent approaches included Zero order, First order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas.

S. No.	Time (in hrs.)	% CDR	Log time	Log % CDR	Sq. root time	Log % CD Remaining	Cube root %D Remaining
1	0.5	3.97	-0.301	0.5987	0.707	1.982	4.5793
2	1	4.59	0	0.6618	1	1.979	4.5732
3	2	6.97	0.301	0.8432	1.414	1.968	4.5381
4	3	10.58	0.477	1.0244	1.732	1.951	4.4825
5	4	14.15	0.602	1.1507	2	1.933	4.4279
6	5	19.37	0.698	1.2871	2.236	1.906	4.3434
7	6	22.06	0.778	1.3436	2.449	1.891	4.3041
8	7	26.88	0.845	1.4294	2.645	1.864	4.2197
9	8	29.87	0.903	1.4752	2.828	1.845	4.1716
10	9	34.06	0.954	1.5322	3	1.819	4.0952
11	10	37.31	1	1.5718	3.162	1.797	4.0373
12	11	41.42	1.041	1.6172	3.316	1.767	3.9576
13	12	48	1.079	1.6812	3.464	1.716	3.8210
14	24	63.56	1.380	1.8031	4.898	1.561	3.4440
15	48	84.01	1.681	1.9243	6.928	1.203	2.7969

Table 17: Values for Release Kinetics

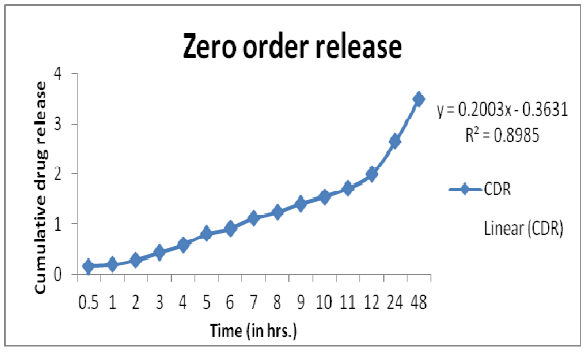


Fig. 8 Zero Order Release Kinetics

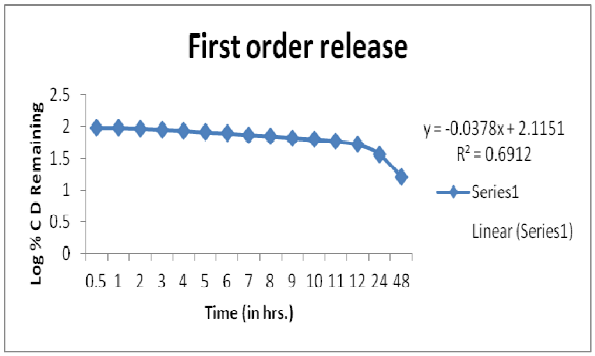


Fig. 9 First Order Release Kinetics

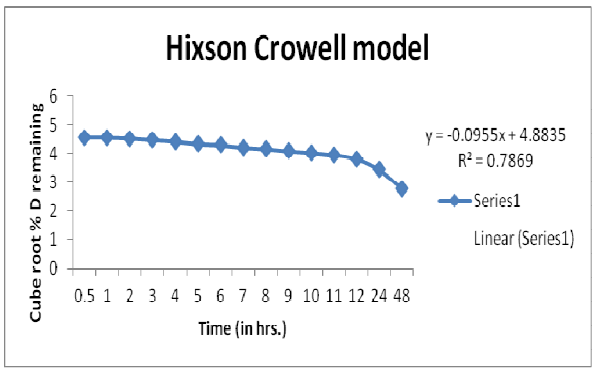


Fig. 10 Hixson Crowell Model of Release Kinetics

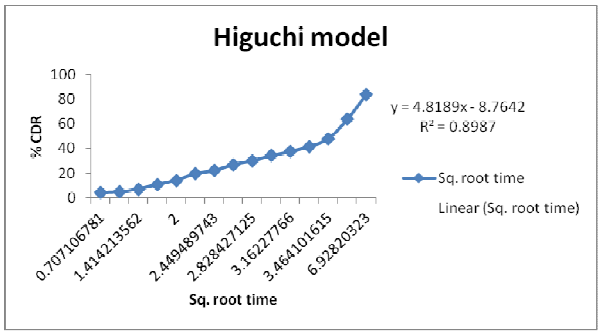


Fig. 11 Higuchi Model of Release Kinetics

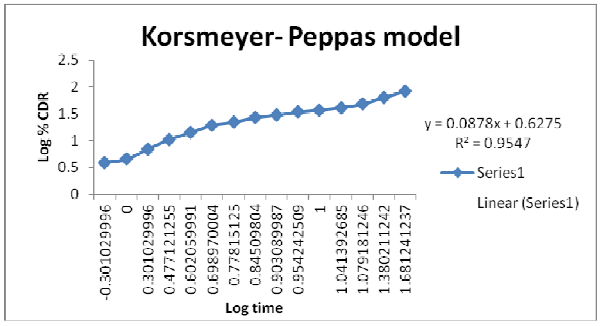


Fig. 12 Korsmeyer- Peppas Model of Release Kinetics

Fluorescence microscopy

Fluorescence microscopy will be performed to confirm the nasal epithelium penetration ability of nanoparticle formulation. The fluorescent labeling will be carried out by preparing the optimized Losarta Potassium loaded formulation with fluorescence marker calcein indicator. Calcein loaded formulation will be applied using a syringe to spray nanoparticle suspension in nasal cavity of rat. After 3 hours of application the rats will be sacrificed and nasal epithelium will be removed, cut into small pieces, fixed into fixative solution (3:1, absolute alcohol: chloroform) for 3 hours. The organs will be first transferred to absolute alcohol for 0.5 hour and then in absolute alcohol and xylene for 1 hour. The wax scrapings will be added in this solution until saturation and will be kept for 24 hours. After 24 hours, the paraffin blocks will be made by embedding the tissue in hard paraffin, matured at 62°C. The sections of 5µm will be cut using microtome and examined under a fluorescence microscope.

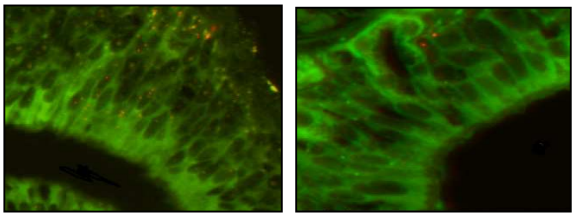


Fig. 13 TheGreen Colourin the Nasal Epithelium Shows the Calcein Loaded Nanoparticles

Stability Study

Stability is defined as, the capacity of a product to remain within specification established to ensure its identity, strength, quality and purity. The purpose of stability testing is to provide evidence on how the quality a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and to establish a re-test period for the drug substance or a shelf for the drug product (ICH Guidelines 2003). The stability of study of optimized formulation was carried out as per ICH guidelines at 5±3°C and at 25±2°C (Room temperature) for three months. Samples were withdrawn monthly and were determined for drug content by the method discussed previously in Entrapment Efficiency section.

S. No.	Time (In Days)	Drug Content (%) at 5±3°C	Drug Content (%) at 25±2°C (Room Temperature)
1	0	100±0.00	100±0.00
2	30	99.76±0.11	99.43±0.19
3	60	99.17±0.24	98.74±0.36
4	90	98.92±0.32	98.13±0.21

Values are expressed in mean ±SD, n=3

Table 18: Effect of Temperature on % Drug Content of Optimized Nanoparticles

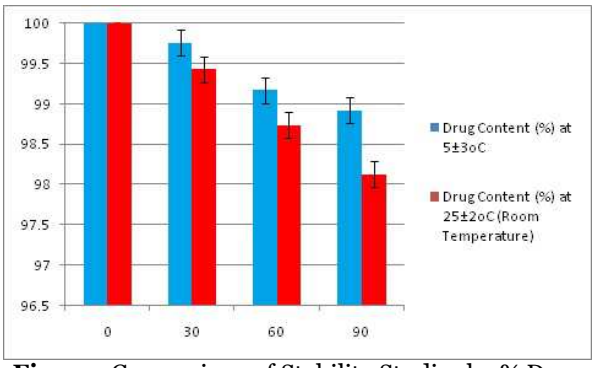


Fig. 14: Comparison of Stability Studies by % Drug Content of Optimized Nanoparticles

RESULTS AND DISCUSSION

The Two level-Three factor (2^3) optimization model was used to determine the optimized formulation. The independent variables selected for optimization of formulation was Drug: Polymer ratio, Surfactant concentration and Stirring speed. The dependent variables are Particle size and Entrapment efficiency. The results of the optimization parameters were shown in table 12. The Particle size of Nanoparticles was found to be decreased with increase of concentration of Surfactant and stirring speed where as the increased concentration of polymer results in increase in particle size. The Entrapment Efficiency of Nanoparticles found to increase with increase in polymer concentration and Surfactant concentration. The Stirring speed has not shown any significant effect on the entrapment of Losartan Potassium. On the basis of that observations the optimized formulation was selected (Table 13).

The optimized formulation of Losartan Potassium loaded Nanoparticles was characterized for Entrapment Efficiency, Zeta Potential, Particle Size, Scanning Electron Microscopy (SEM), In-vitro Drug Release and In-vivo Penetration Study.

The Entrapment Efficiency of Losartan Potassium in optimized formulation was found to be 87 % (Table 15), Zeta potential of that formulation was -16.6 mV (Table 14). Particle size was determined using Photon Correlation Spectroscopy (HAS 3000; Malvern Instruments, Malvern, UK) and it was found to be 295.3 nm (Fig.21). The Scanning Electron Microscopy (SEM) image does not exactly shows the Nanoparticle size. But it can be concluded from the image that theparticles are of 200 nm (Fig.22).

The In-vitro release study was done using Dialysis bag, the volume of the Phosphate buffer solution (pH 7.4) used was 50 ml with stirring speed of 150 rpm at room temperature. The formulation show controlled release of Losartan Potassium and release 84% in 48 hours. Various release kinetic models are applied to describe the suitable dissolution profile. The release pattern follows Korsemeyer-Peppas Model.

The In-vivo fluorescent study was done to determine the extent penetration of Nanoparticles from the nasal epithelial membrane. The presence of green colour (fig.28) shows the calcein loaded PLGA Nanoparticles in Nasal epithelium, which confirms that the Nanoparticles of optimized formulation was able to penetrate the nasal epithelium.

The Stability study of optimized Nanoparticles was performed for 3 months. No major changes in the content of drug were observed at the end of 3 months. So data of stability studies revealed that formulation will be stable for longer period of time.

CONCLUSION

The present work was an attempt to develop nanoparticles of Losartan Potassium using Poly [D,L-Lactide-co-Glycolide] (PLGA). On the basis of literature review, which reveals that the nanoparticles with size range 250-500 nm will easily permeate the nasal epithelial membrane.

The independent factors investigated were Drug-Polymer ratio, Surfactant concentration and Stirring speed. Concentration of PLGA and Surfactant had significant effects on entrapment efficiency and particle size. Entrapment of Losartan Potassium was found to be increase with increase with increase the Polymer and surfactant concentration. The particle size of the nanoparticles was found to be decreased

with increase Surfactant concentration and Stirring speed. The release kinetic profile of nanoparticle formulation showed that the drug release follow Korsmeyer-peppas model.The study of nanoparticles show good penetration through the nasal epithelium which is the most important factor for the intra-nasally delivered nanoparticle formulations.

Thus it can be concluded that Nanoparticles represent a promising particulate carrier having sustained drug release, potential to permeate through nasal epithelium and release the drug in systemic circulation for longer time, which is the prime requirement for the management of hypertension.

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