



Research Article

DESIGN AND OPTIMIZATION OF CONTROLLED DRUG DELIVERY SYSTEM OF LOSARTAN POTASSIUM

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ABSTRACT

Purpose: The objective of the study was to develop microspheres of Losartan potassium as controlled drug delivery system by using various polymer (HPMC and Ethylcellulose), evaluating the relationship and influence of different content levels of HPMC, and Ethylcellulose, in order to achieve a zero order release of Losartan potassium.

Approach: Microspheres were prepared by solvent evaporation process. Release kinetics was evaluated by using United States Pharmacopoeia (USP) type I dissolution apparatus. The release mechanism of microspheres loaded with Losartan potassium was determined by fitting the data in Korsmeyer peppas equation. The regression coefficient values for Peppas model was found to be high, indicating adequate fitting. The 'n' value was ranged from 0.548 to 0.963 indicating Non Fickian diffusion for all the formulations. Optimization was performed by using desirability function. To validate the model, the optimized formula was subjected to in vitro characterization.

Findings: Release kinetics of Losartan potassium from these microspheres was principally regulated by HPMC K4M and Ethylcellulose. Percentage yield, entrapment efficiency and particle size of optimized formula was found to be 91.42%, 68.01% and 310 μ m. Formulation (F1) release at the end of 12 hours of dissolution studies was found to be 79.81%.

Conclusion: It can be concluded that Losartan potassium loaded microspheres could be successfully formulated by using HPMC 4KM and Ethylcellulose by solvent evaporation method to obtain maximum percentage yield, entrapment efficiency, desired particle size.

Keywords: *Microspheres, Losartan potassium, HPMC K4M, Ethylcellulose, Percentage yield, drug content, Entrapment efficiency*

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INTRODUCTION

The landscape of novel drug delivery system has become highly competitive. Nearly one billion people suffer from high blood pressure and are expected to rise to 1.56 billion people by 2025. Cardiovascular diseases, mainly arterial hypertension is responsible for nearly 20% of all deaths world-wide¹.

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Hypertension is the chronic disease it needs long term treatment. The antihypertensive are having drawbacks like increase in frequency of administration, extensive first pass metabolism and variable bioavailability². Controlled-release products are formulated to release the active ingredient gradually and predictably over a period of 12-hour to 24-hour. These formulations potentially provide for greater effectiveness in the treatment of chronic conditions through more consistent delivery of the medication, reduced side effects, greater convenience and improved patient compliance due to a simplified dosage schedule, compared with those of immediate-

release drugs. Use of biodegradable polymeric microspheres appears to have potential applications in controlled drug delivery. There are various approaches in delivering a therapeutic substance to the target site in a sustained or controlled release fashion. Nanoparticles and microspheres are first and foremost representative frontiers of colloidal drug delivery. Microsphere based drug delivery system have received considerable attention in recent years.⁴ The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 m. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the controlled release of drug.⁵ The main advantage of microsphere based carriers is that they could be injected into the body in a suitable vehicle using a hypodermic needle.³ The aim of present investigation was to design, evaluate and to optimize oral controlled drug delivery system of an antihypertensive drug, Losartan Potassium using biodegradable polymers ethylcellulose and hydroxypropyl methyl cellulose.

Materials and Methods

Losartan potassium and hydroxypropyl methylcellulose were obtained as a gift sample from KAPL, Bengaluru, India. Ethyl cellulose was obtained from Microlab, Bengaluru, as gift sample. Methanol, Dichloromethane, Liquid paraffin, n-hexane, Potassium dihydrogen ortho Phosphate, Sodium hydroxide were purchased from S.D. Fine chemicals Ltd, Bengaluru, India. All other reagents and solvents used were of analytical grade.

Drug- Excipients Compatibility Studies

Fourier transform infrared (FTIR) (Shimadzu 8300) spectroscopy was performed on each of the samples to determine the structure of the organic compound and to identify the presence of specific functional groups present. Spectra were obtained by passing infrared radiation through the sample and determining the fraction of incident radiation absorbed. The energy of a peak in the spectrum corresponds to the frequency of vibration of functional group of the sample. 3-5 mg of composite sample was added to approximately 100 mg of KBr. The mixture was then ground to a fine powder using a mortar and pestle, and transparent discs were formed

by pellet press. The discs were then placed in the FTIR spectrometer and spectra were recorded. The range of the spectra was between 4000-400cm⁻¹

Preliminary Studies

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters in an effort to optimize the formulations for obtaining microsphere with proper physical characteristics and of particle size ranging from 100 – 500µm. The following are the process variables which were studied to standardize the method for preparation of the microspheres.

Ethylcellulose and HPMC K4M

Four different batches namely 1 to 4 were formulated with varying the amount of drug: polymer ratio (Ethylcellulose and HPMC K4M). Concentration of surfactant (span 80, 0.5%w/v) and Stirring speed (1200 rpm) were kept constant. The obtained microspheres were evaluated for % drug entrapment efficiency and physical characteristics.

Surfactant

Three different formulations namely 1, 2 and 3 were prepared by varying the surfactant (Span 80) concentration from 0.2%, 0.3%, 0.4 to 0.5% w/v respectively. The prepared microspheres were evaluated for % drug entrapment efficiency

Formulation Design

Based on the results of preliminary investigation, the different process parameters like effect of amount of Ethylcellulose and HPMC K4M, concentration of surfactant and stirring speed were optimized and final formulations were designed by varying polymer to drug ratio. Losartan potassium microsphere was prepared by adopting emulsion-solvent evaporation technique. Ethyl cellulose and HPMC K4M were dissolved in a mixture dichloromethane and methanol. These dispersions was slowly poured into 100 mL of light and heavy liquid paraffin (1:1) containing 0.5% (w/v) span 80 and was emulsified by vigorous stirring using a three-blade mechanical stirrer at 1200 rpm. Stirring was continued until Dichloromethane and Methanol has evaporated, the formed microspheres were allowed to settle, washed with n-hexane to remove any oil residue and kept for overnight drying in hot air oven at 40 °C. The different ratio of drug: polymers used in study are shown in Table 1.

Table 1: Different ratio of drug: polymer used in study.

Formulation Code	Drug: Polymer Ratio	Polymer : Polymer Ratio (Ethylcellulose : HPMC K4M)	Drug (gm)	EthylCellulose (gm)	HPMC K4M (gm)
F1	1:6	4:2	0.5	2	1
F2	1:8	5:3	0.5	2.5	1.5
F3	1:6	5:1	0.5	2.5	0.5
F4	1:6	3:3	0.5	1.5	1.5
F5	1:4	3:1	0.5	1.5	0.5
F6	1:7	4:3	0.5	2	1.5
F7	1:5	3:2	0.5	1.5	1
F8	1:5	4:1	0.5	2	0.5
F9	1:7	5:2	0.5	2.5	1

Yield of microspheres⁶

- Theoretical yield: Theoretical yield was calculated based on the amount of solid added to solvent to prepare a solution.
- Practical yield: The dried product from the hot air oven was weighed.
- Percentage yield: It can be calculated by using the formula

$$\% \text{ Yield} = \frac{\text{Practical yeild (microspheres)}}{\text{Theroretical yeild (drug+polymer)}} \times 100$$

Flow properties⁷

a) Bulk density (Db):

It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in g/ml.

$$D_b = M/V_b$$

Where, M = Mass of the powder & V_b = Bulk volume of the powder.

b) Tapped density (Dt):

It is the ratio of total mass of powder to the tapped volume of powder. The tapped volume was measured by tapping the powder to constant volume. It is expressed in g/ml and is given by

$$D_t = M/V_t$$

Where, M = Mass of the powder & V_t = Tapped volume of the powder.

c) Carr's compressibility index (CI):

It indicates the ease with which a material can flow. It is expressed in percentage.

$$CI = (D_t - D_b / D_b) \times 100$$

d) Hausner's ratio:

The Carr's index and Hausner's ratio are measures of the propensity of a powder to be compressed.

$$\text{Hausner's ratio} = D_t / D_b = V_t / V_b$$

e) Angle of repose:

The maximum angle which is formed between the

surface of a pile of powder and horizontal surface is called the angle of repose.

$\theta = \tan^{-1} h/r$ Where θ = angle of repose, h is the height and r is the radius of pile.

The powder mixture was allowed to flow through the funnel fixed to a stand at a definite height. Height and radius of the heap of powder formed was noted and angle of repose was reported.

Entrapment Efficiency:

Microspheres equivalent to 100 mg of drug were accurately weighed and triturated using mortar and pestle and transferred to 100 ml volumetric flask. To this flask, 5ml of methanol and 95 ml of phosphate buffer pH 6.8 was added to dissolve the microsphere completely by using ultrasonicator for 30 min. filter the solution and pipette out 1 ml from filtrate solution and transferred to 100 ml volumetric flask. The absorbance was measured at 206 nm using phosphate buffer pH 6.8 as blank.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theroretical drug content}} \times 100$$

Particle size⁸

Particle size of the prepared microspheres was determined by optical microscopy. The optical microscope was fitted with an ocular micrometer and a stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of more than 300 microspheres were measured randomly by optical microscope.

Calibration of Eyepiece Micrometer:

One division of the stage micrometer = 0.01 mm = 10 μ m

$$\mu = (SM / EM) \times 100$$

Where, μ – Correction factor, SM – reading of stage micrometer, which coincides, with reading of eye piece micrometer (EM)

Formulation Development:

Design of Experiment: Central Composite Design⁹

The most popular Response Surface Method (RSM) design is the Central Composite Design (CCD). A CCD has three groups of design points:

- Two-level factorial or fractional factorial design points
- Axial points (also called "star" points)
- Centre points CCD's are designed to estimate the coefficients of a quadratic model. All point descriptions will be in terms of coded values of the factors. The example points of a Central Composite Circumscribed design with two input parameters.

1. Factorial Points: The two-level factorial part of the design consists of all possible combinations of the +1 and -1 levels of the factors. For the two factor case there are four design points: (-1, -1) (+1, -1) (-1, +1) (+1, +1)

2. Star or Axial Points: The star points have all the factors set to 0, the midpoint, except one factor, which has the value +/- Alpha. For a two factor problem, the star points are: (-Alpha, 0) (+Alpha, 0) (0, -Alpha) (0, +Alpha). The value for Alpha is calculated in each design for both rotatability and orthogonality of blocks. The experimenter can choose between these values or enter a different one. The default value is set to the rotatable value.

3. Centre Points: Centre points, as implied by the name, are points with all levels set to coded level 0 - the midpoint of each factor range: (0, 0).

Variables:

Independent variables:

X1 = Ethyl cellulose ratio

X2 = HPMC K4M ratio

Dependent variables (responses):

Y1 = Percent of cumulative drug release for 12 hr

Y2 = % of Drug entrapment efficacy

Y3 = Particle size (µm)

In Vitro Dissolution Studies:

Dissolution test was performed using a USP type-2 paddle apparatus at $37 \pm 0.5^\circ \text{C}$ in 900 ml of Phosphate buffer pH 6.8 with a speed of 50 rpm. Samples (5ml) were withdrawn at 1 hour time intervals over a period of 12 hour and medium was replenished with fresh dissolution fluid. The

samples withdrawn were analyzed UV spectrophotometrically at 206 nm.

In Vitro Drug Release Kinetics¹⁰

The release data obtained was fitted into various mathematical models using PCP Disso – V2.08 software. The parameters 'n' and time component 'k', the release rate constant and 'R', the regression coefficient were determined by Korsmeyer – Peppas equation to understand the release mechanism. To examine the release mechanism of Losartan potassium microsphere formulations, the release data was fitted into Peppas equation,

$$M_t / M_\infty = K t^n$$

Where,

M_t / M_∞ = the fractional release of drug,

t = release time,

K = A constant incorporating structural and geometrical Characteristics of the device,

n = the diffusional exponent and characterize the type of release mechanism during the release process.

RESULTS AND DISCUSSION

FTIR Compatibility Study

FTIR spectra of the drug, excipients and the optimized formulation were recorded in the range of 500-4000 cm^{-1} . Losartan potassium showed some prominent and characteristic peaks at 3196, 3021, 1651, 1575, 759 cm^{-1} , which corresponds to the vibration of O-H bending, C-H bending, C=N, C-C stretches and 1-4- Di substituted aromatic groups respectively. In IR spectra of drugs with polymers, all characteristic peaks were same which indicated no strong interaction between the drug and excipients. FTIR spectra of the drug, excipients and the optimized formulation are shown in Fig 1 to 4.

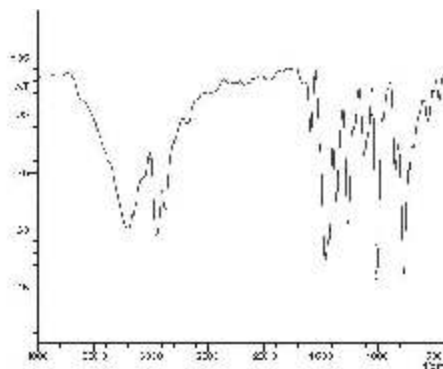


Fig 1: IR Spectra of Losartan potassium.

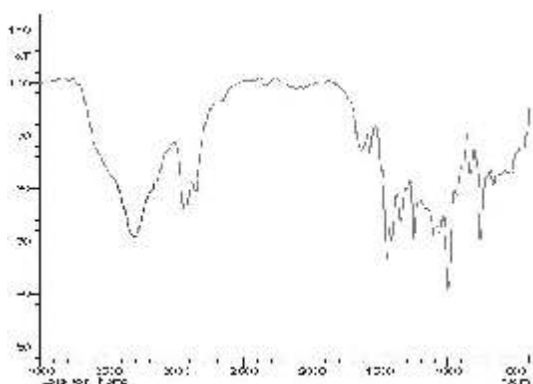


Fig 2: IR Spectra of binary mixture of Losartan potassium and HPMC K4M.

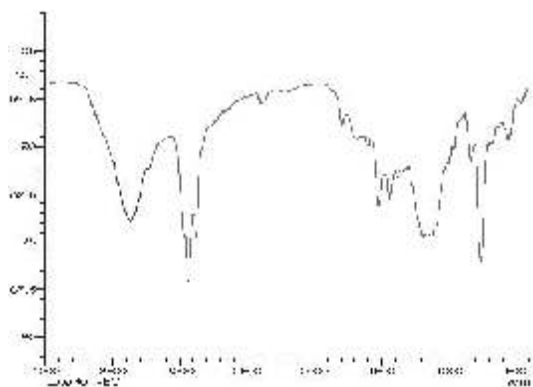


Fig 3: IR Spectra of binary mixture of formulation. Losartan Potassium and Ethyl cellulose.

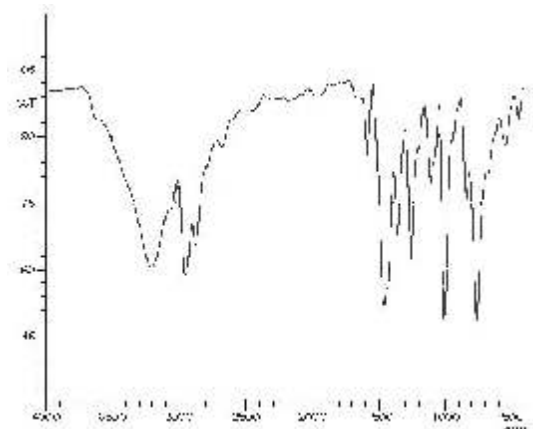


Fig 4: IR spectra of optimized

Formulation and Development

The microspheres of Losartan potassium were prepared by emulsion solvent evaporation method by using methanol: dichloromethane (1:1) ratio. The microspheres were found to be discrete spherical and free flowing. All formulations showed uniform particle size in the range of 285 to 345µm. As per the preliminary studies, it was observed that increase in core: coat ratio, increases the particles and exhibited

controlled release for a longer duration of time. 1:1, 1:2, 1:3 showed quick release within a period of 3 hrs but microspheres were fine and round, without irregularities. Based on preliminary studies, total 9 formulations were formulated with drugs: polymer ratios of 1:4 to 1:8 and factorial design were employed to study the effect of variables i.e. Ethyl cellulose: HPMC4KM ratio on release of drug capabilities. All formulation showed the good yield within range of 86 to 94.45%. The encapsulation efficacy was within the range of 60 to 77.64%.

SURFACE MORPHOLOGY:

The surface morphology of microspheres loaded with the drug was examined by using a scanning electron microscopy. Spherical shaped microspheres were obtained and were obtained are shown before and after dissolution in Fig 5 & 6.

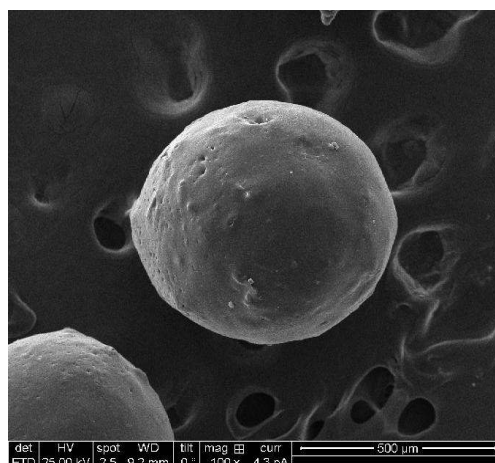


Fig 5: SEM of microspheres of optimal formula (F1) before dissolution

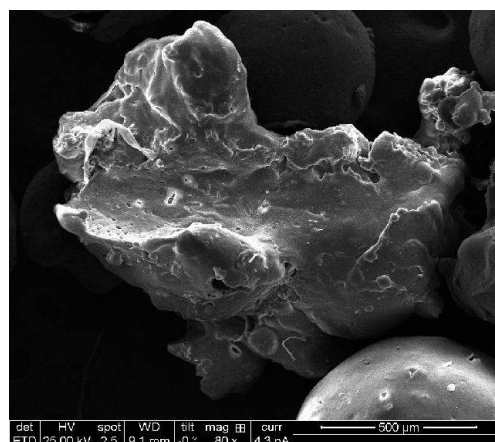


Fig 6: SEM of microspheres of optimal formula(F1) after dissolution

Design of Experiment: Effect of Formulation Variables

Central composite design was used to study the effect of formulation variables such as drug to polymer ratio of Ethylcellulose (X1) and polymer to polymer ratio of HPMC K4M (X2) as two independent variables. The percentage drug release at 12hr %CDR (Y1), % Drug entrapment efficiency (Y2) and particle size (Y3) were selected as dependent variables. A factor is considered to influence the response if the effects significantly differ from zero and the p-value is less than 0.05. A backward elimination procedure was adopted to fit the data into different predictor equations. The final equations of the responses are given below:

$$Y1 = +79.99 - 2.32A + 4.26B + 3.67AB - 4.55A^2 + 0.19B^2$$

$$Y2 = +67.58 + 0.16A + 1.31B + 0.88AB + 10.80A^2 - 5.10B^2$$

$$Y3 = +309.38 + 11.17A + 6.33B + 2.25AB + 24.10A^2 - 6.40B^2$$

Non-significant terms were omitted and only significant terms of the model were retained in the further explanation. The coefficients of X1, X2 were found to be significant $P < 0.05$, hence they were retained in the reduced model. ANOVA and multiple regression analysis were done using Stat-Ease Design Expert 8.0.4.1 trial software. 3D Response surface plot and Contour plot showed the effect of proportion of Ethylcellulose and HPMC 4KM as independent variables. Dependent variables are %CDR, % drug entrapment efficiency and particle size.

Response Y1: Percentage of drug release at 12 hour

The quadratic model was found to be significant with a probability value of 0.0098 indicating adequate fitting to the model. The Model F-value of 19.47 implies the model is significant.

$$Y1 = +79.99 - 2.32A + 4.26B + 3.67AB - 4.55A^2 + 0.19B^2$$

The quadratic model revealed that the selected factors influenced the release of drug from microspheres. The response surface plot and counter plot illustrated 75 to 80% drug release after a period of 12 hrs and increased in HPMC ratio, release of drug was also increased. As ethylcellulose content is increased that resulting decreased in the release rate. The counter plot for % CDR for 12 hr (Fig 7) showed considerable curvature indicating that the selected factors are significant.

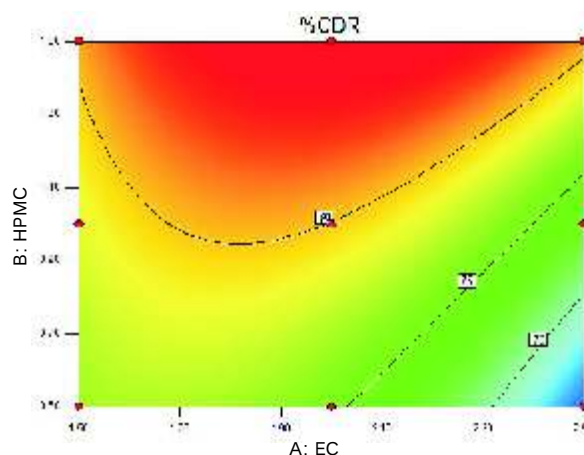


Fig 7: Contour plot for % CDR for 12 hr.

Response Y2: Drug encapsulation efficiency
The Quadratic model was found to be significant with a probability value of 0.0414 indicating adequate fitting to the model. The Model F-value of 10.36 implies the model is significant.

$$Y2 = +67.58 + 0.16A + 1.31B + 0.88AB + 10.80A^2 - 5.10B^2$$

The model indicated that the factors showed significant influence on encapsulation efficacy the 3D plot showed that as HPMC K4M proportion is increased, the encapsulation efficacy also got increased. Formulations F3, F5 and F8 showed low encapsulation efficiency 61.32 to 71.4%. The counter plot for percentage entrapment efficacy showed (Fig 8) describes that the factors are significant, as curvature is significant.

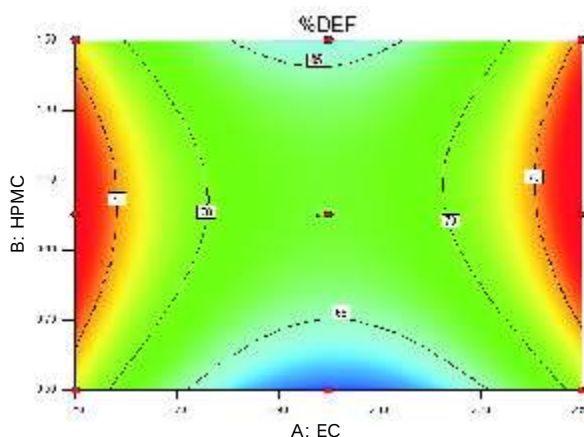


Fig 8: Contour plot for Drug entrapment efficiency.

Response Y3: Particle size

The Quadratic model was found to be significant with a probability value of 0.0343, indicating adequate fitting to the model. The Model F-value of 11.88 implies the model is significant.

$$Y3 = +309.38 + 11.17A + 6.33B + 2.25AB + 24.10A^2 - 6.40B^2$$

The 3D plot (Fig 9) illustrated that the particle size showed an increase in particle size as Ethyl cellulose content is increased. Formulation F2, F3 and F9 showed higher particle size in the range of 333 to 345 μm . The counter plot showed significant curvature, indicating that the factors are significant. Based on factorial design and in vitro release studies and fitting into release kinetics, formulation F1 with Ethyl cellulose and HPMC K4M(2.5:1) at drug: polymer ratio of 1:6, was optimized, with particle size of 310 μm and drug release of 79.81% at 12hrs release study.

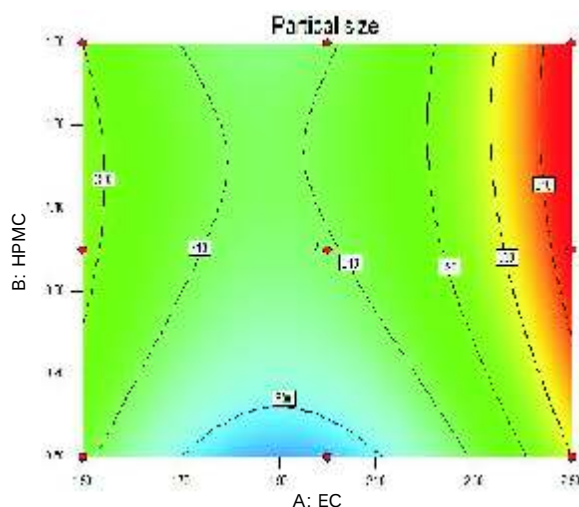


Fig 9: Contour plot for drug entrapment efficacy.

In vitro Release Studies And Release Kinetics

The release study indicates that all formulations released the drug of 71.4 ± 1.7 to $83.8 \pm 1.8\%$ at 12 hrs. The release data were fitted into kinetic models like zero order, first order, Higuchi and Korsmeyer Peppas. The results indicate that the drug release from F1-F9 followed zero order model. Except F7 and F9, this showed first order model and Korsmeyer Peppas model showed that formulations follow non Fickian diffusion (the critical value of $n=0.57-0.81$). Formulation F7, revealed erosion mediated release, which is supported by the fact that the combinations of polymer swelling, drug dissolution and matrix

erosion. The drug release from swellable matrixes, either on a macroscopic or on a molecular level. Formulations F6, F7 and F9 showed initial burst release in the range of 19 to 22% in first hours. Formulation F1 with drug to polymers ratio 1:6 showed good controlled release with correlation coefficient of 0.9962 than other formulations and with release of $79.81 \pm 15\%$. The dissolution profiles of microspheres F1 to F9 are shown in Fig

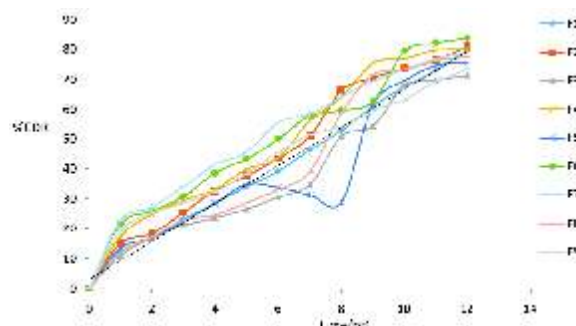


Fig 10: Dissolution profile of microspheres F1 to F9

Comparative Dissolution Profiles with Marketed Product:

The dissolution profile of marketed product (Losacar50 and Covance 50) were carried out, in case of Losacar50 the drug release was observed $96.9 \pm 53.18\%$ at the end of 2 hours of dissolution. Whereas, in case of Covance 50 the drug release was observed $98.8 \pm 2.8\%$ at the end of 8 hours of dissolution. The dissolution profile for optimized formulation (F1), Covance and Losacar are shown in Fig 11.

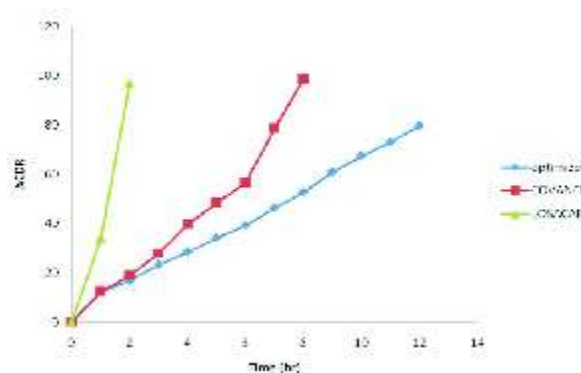


Fig 11: Dissolution profile for optimized formulation (f1), Covance and Losacar.

Stability Studies¹¹

The stability studies of optimized formulation at room temperature and $40 \pm 20^\circ\text{C}$ / $75\% \pm 5\%$

RH, showed no significant change in physical appearance of the microspheres, entrapment efficiency, and in vitro dissolution studies in phosphate buffer pH 6.8. Hence the optimized formulation was stable over a period of study and releases the drug in controlled manner without dose dumping.

CONCLUSION

Microspheres of Losartan potassium were formulated at various drug: polymer ratio ranging from 1:4 to 1:8 and optimized using a 3^2 factorial design. The influence of polymer to polymer (EC: HPMC K4M) on physical characteristic of microspheres (size, entrapment efficacy and percentage release) was investigated. The quantitative effect of these factors at different levels on the release ratio could be predicted by using polynomial equation. The quadratic response surface methodology studied for the release rate helped in understanding the effect of Ethylcellulose: HPMC K4M ratio on three different responses. The FTIR studies showed no interaction between drug and polymers. All formulation except F1, F9 showed slow and controlled release up to a period of 12 hrs. Formulation F1 was optimized which is formulated at drug: polymer ratio 1:6, and at Ethylcellulose: HPMC K4M (4:2) with correlation coefficient of 0.970 for zero order for drug release study. The optimized formulation was subjected to the stability studies, and based on the results, it was concluded that the optimized formulation was found to be stable for long period. Thus the objective of envisaged work of optimization and evaluation of control drug delivery system of model antihypertensive drug of Losartan potassium by using optimization technique has been achieved with success.

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