Formuation, Statistical Optimization and *In-Vitro* Evaluation of Antihypertensive Losartan Potassium Nanoparticles

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Abstract

Present study is focused to formulate, optimize and evaluate polymeric nanoparticles (PNPs) of losartan potassium, to overcome its shortcomings of moderate bioavailability, short terminal half life and potential adverse effects. Drug loaded nanoparticles were prepared by emulsification method followed by solvent evaporation using the polymer ethyl cellulose and PVA as stabilizer. Total seventeen formulations were prepared and analyzed by altering formulation and process variables like concentration of polymer and surfactant, sonication time. Optimization was based on two dependent variables, particle size and entrapment efficiency using design of experiment approach with box behnken design. Optimized nanoparticle formulation was evaluated for particle size, shape, entrapment efficiency and zeta potential. *In-vitro* drug diffusion studies were carried out to understand the drug release behavior. The optimized nanoparticle formulation was found to have the particle size of 423.4 nm, zeta potential -11.4 mV, and entrapment efficiency of 85.57%. SEM analysis showed the smooth, spherical surface of the prepared nanoparticles. Cumulative drug release was 98.42% over a period of 12 hrs. The release pattern followed higuchi model with highest r^2 value of 0.935. Formulation was found to be stable during three months stability studies. Based on the results, the formulated nanoparticles were found to have the great potential for the sustained delivery of antihypertensive losartan potassium.

Keywords: Nanoparticles, box-behnken design, solvent evaporation method, In-vitro drug diffusion, antihypertensive

1. Introduction

Nanotechnology is broadly defined as the study and use of structures in range between 1 to 1000 nanometres in size. It has become an integral part of the twenty-first century. Over the last few years, there has been an outburst of research at both academic and industrial levels - pertaining to ultrafine nanostructures such as nanoparticles and nanofibres due to their high surface area, surface to volume ratio, high porosity, and high drug loading and controlled payload delivering capabilities. These advantages makes nanoparticle to show great potential as drug delivery carriers[1].

Hypertension is one of the key risk factors for all forms of cardiovascular and renal diseases, contributing to CVD death[2]. Treatment of hypertensive depends on how effectively blood pressure can be minimized or reduced. Losartan Potassium, a potent and precise angiotensin II receptor antagonist is the drug of choice for long term hypertension. It has rapid absorption profile, while administered orally, but its bioavailability remains low (33%). Its wide plasma protein binding causes potential adverse effects including headache, gastrointestinal disorders, neutropenia and acute pancreatitis. Short plasma half life of drug (1.5-2.5 hrs) also causes poor patient compliance during long term treatment of hypertension[3]. These side effects can be minimized by formulating the drug into polymeric nanoparticles using rate controlling polymers for sustained action.

2. Materials And Methods

2.1 Materials

Losartan Potassium was obtained as a gift sample from Unimedico Labs, Dehradun (Uttarakhand). Ethyl cellulose and PVA were procured from Leo chem. (Banglore, India) and Otto kemi (Mumbai, India). All the excipients and solvents used in the study were of analytical grade.

2.2 Experimental Design for Optimization of Nanoparticles

For the optimization of polymeric nanoparticles, Box-Behnken statistical design (3^3) and response surface methodology was employed. Polymer concentration, amount of surfactant, and sonication time were chosen as independent variables for the analysis and particle size (nm) and % entrapment efficacy were chosen as dependent or response variables. Multiple regression analysis was performed on the responses to determine the relationship between the factors used and the results obtained. The effect of formulation variables on response variables was statistically evaluated using Design Expert 22.0.2.0 trial version and one-way analysis of variance (ANOVA) at the 0.05 level (Stat Ease, USA). The design was evaluated using a (ANOVA) quadratic model[4]. Independent and dependent variables levels for experimental runs are enlisted in table 1.

Table 1: Independent and dependent variables levels in Box-Behnken design

	Levels		
Independent variables	**-1**	**0**	**+1**
A: Ethyl cellulose (mg)	50	225	400
B: PVA (%)	0.15	0.3	0.45
C: Sonication time (min.)	5	12.5	20
Dependent variables	**Constraints**		
X: Particle size (nm)	Minimize		
Y: Entrapment efficiency (%)	Maximize		

2.3 Preparation of PNPs

Emulsification method followed by solvent evaporation was used for PNPs preparation. Firstly, the polymeric solution (ethyl cellulose) was emulsified in an aqueous solution containing a surfactant (PVA). Then the polymeric solution was evaporated by polymer precipitation. The drug was dissolved in methanol with constant stirring. The organic solution was added drop wise (with the help of syringes) into an aqueous phase containing polyvinyl alcohol (PVA). The emulsion was sonicated for 5-20 minutes to obtain nanosized particles. The organic solvent was then evaporated using constant stirring on a magnetic stirrer for about 4-5 hrs. After centrifugation (30 min, 10000 rpm), the pre-emulsion was then kept for lyophilization for 48 hrs to collect the polymeric nanoparticles. As per experimental design, total 17 formulations were prepared using different proportion of polymers with varying sonication time[5].

2.4 Evaluation of polymeric nanoparticles

2.4.1 Particle size

For nanoparticle characterization, particle size is one of the most important parameter. Malvern Zeta Sizer was used to measure the average particle size of formulated polymeric nanoparticles. The dispersions were diluted with millipore filtered water to an appropriate scattering intensity at 25°C. Disposable sizing cuvette was used to place the sample[6].

2.4.2 Zeta potential

Movement velocity in an electric field and the particle charge of the nanoparticles was determined by measuring the zeta potential. Polymeric nanoparticles were diluted 10 times with distilled water, sonicated for 5-15 minutes and then analyzed by Zetasizer Malvern instrument for the measurement of zeta potential[6].

2.4.3 Entrapment efficiency

Drug -loaded polymeric nanoparticles were centrifuged at 15,000 rpm for 30 minutes (using REMI ultra centrifuge). Supernatant drug (free drug) concentration was determined using UV spectrophotometer[7]. Entrapment efficiency was calculated using the given formula:

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc.*100

2.4.4 Scanning Electron Microscopy (SEM)

Surface morphology of optimized PNPs was examined via SEM analysis. PNPs were coated with a thin layer of gold and the specimen was then bombarded with an electron beam. The electrons scattered at 90° were detected from the particle's surface and further processed for obtaining surface topography images[7].

2.4.5 *In-vitro* drug release study

Optimized PNPs were studied for *in-vitro* drug release using dialysis bag diffusion method. Firstly losartan potassium loaded nanoparticles were dispersed into dialysis bag and the bag was then placed in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was kept over a magnetic stirrer (100 rpm) and the whole assembly was maintained at a constant temperature (37 ± 1 °C) throughout the experiment. 2 ml of samples were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. Suitable dilutions were made and analyzed using UV–Visible spectrophotometer at 206 nm[8].

2.4.6 Drug release kinetics study

In-vitro drug release data were analyzed and fitted to various kinetic models to describe the release kinetics. Zero order kinetic states that rate of drug release is independent of its concentration while first order is the concentration dependent kinetic. Higuchi model describes the drug release that follows Fick's law of diffusion and Korsemeyer-Peppas model explains Quasi Fickian diffusion. Best fit model was selected by plotting the release data with different modes of data treatment[8].

2.4.7 Accelerated stability study

For stability study, PNPs were subjected to temperature and humidity conditions of 40±1°C/ 75% RH as per ICH guidelines to study the effect of different formulation additives on drug stability. Under accelerated storage conditions, physical and chemical stability of the optimized formulation was determined by withdrawing the samples at the end of 0, 30, 60, and 90 days and analyzing/evaluating them for active drug content, particle size, % entrapment efficiency and appearance[7].

3. Results And Discussion

3.1 Experiment Design and Formulation Optimization

Total 17 confirmatory runs were developed using Box-Behnken design (Table 2) for the optimization of losartan potassium PNPs by taking 3 independent and 2 dependent variables in consideration. Selection of working method was done on the basis of particle size and drug entrapment.

Table 2: Formulation trials as per Box–Behnken design

Formulations	Run	Factor 1: Ethyl cellulose (mg)	Factor 2: PVA (%)	Factor 3: Sonication time (Min.)	Response 1 Particle size	Response 2 Entrapment efficiency
F1	1	400	0.3	5	900.5	75.83
F2	2	225	0.45	20	965.3	85.21
F3	3	225	0.3	12.5	894.4	84.11
F4	4	225	0.45	5	692.4	84.28
F5	5	50	0.3	20	819.4	77.09
F6	6	50	0.3	5	300	82.54
F7	7	50	0.15	12.5	541.9	79.84
F8	8	225	0.3	12.5	414.1	86.52
F9	9	225	0.15	5	382.9	84.59
F10	10	400	0.3	20	369.9	74.01
F11	11	225	0.15	20	326.9	85.32
F12	12	50	0.45	12.5	301.3	84.53
F13	13	225	0.3	12.5	422.1	89.14
F14	14	400	0.15	12.5	299.8	74.38
F15	15	225	0.3	12.5	425.2	86.56
F16	16	225	0.3	12.5	452.8	80.49
F17	17	400	0.45	12.5	744.9	75.6

To study the effect of formulation variables on particle size and entrapment efficiency, the results of formulations as per the design when fitted into various models, a linear model was found to be significant with F values 3.44 and 3.99 and P values 0.0414 and 0.0408 respectively for particle size and entrapment efficiency. The model equations for particle size and entrapment efficiency were: +544.34 + 44.06 A +144.05 B + 25.71 C + 171.42 AB – 262.50 AC + 82.22 BC (for

particle size), +85.36 -3.02A +0.6863 B – 0.7013 C – 0.8675 AB + 0.9075 AC + 0.0500 BC (for entrapment efficiency). The effect of both the factors A and B was explained with the help of the 3D response surface plot as shown in above Figure 1(a) and 1(b).

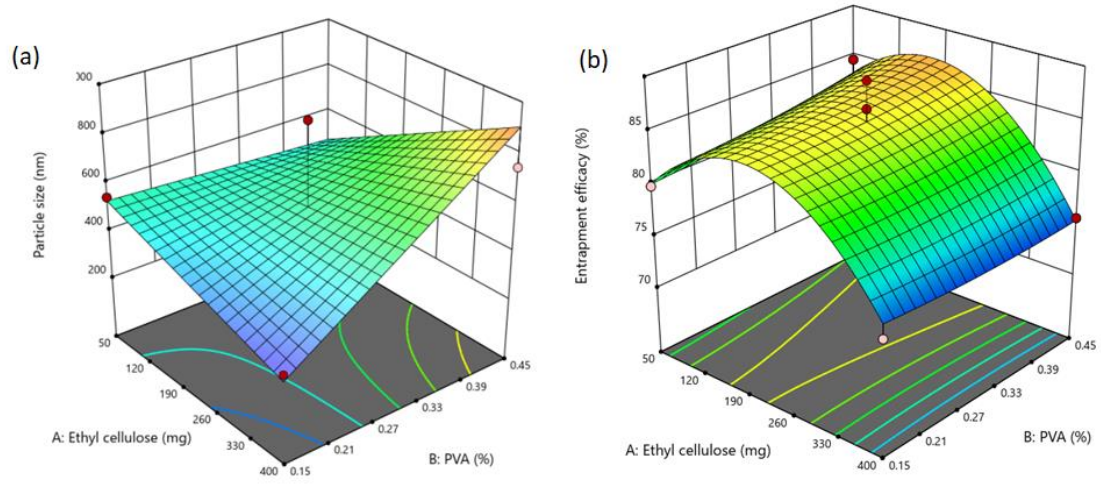

Figure 1: Response surface plots for the effect of ethyl cellulose (mg) and PVA (%) on particle size in nm (a) and % entrapment efficiency (b) of formulated polymeric nanoparticles

As the p-value was found to be less than 0.05% and f value was also within the limit, that was significant for in case of the quadratic model, such that every model terms was significant; hence factor (A, B and C) were found to be significant. The results when analyzed and optimized had generated around 100 numerical optimized solutions based on this experimental design and out of them, a solution was selected randomly, coded and considered as optimized nanoparticle formulation that consists of drug (100mg), ethyl cellulose (225mg) and PVA (0.45 %).

The optimized nanoparticle formulation was developed and characterized for particle size and % % entrapment efficiency. The results of predicted observation and actual experimentation confirmed the closeness of the predicted results with that of the observed results. Experimental values for the responses X (particle size) 423.4 nm and Y (% drug entrapment) 85.57% of optimized formulation were found in close agreement with the predicted valued for responses generated by the software and thus assures the validity of the experimental design used[9].

3.2 Particle size, entrapment efficiency and zeta potential

Particle size analysis illustrated the average particle size of polymeric nanoparticles (Figure 2a), that was found to be 423.4 nm with PI value 0.322. Optimized nanoparticles possessed high drug entrapment efficiency (85.57%), while mild variation was due to the changes in the polymer concentration. Zeta potential of optimized formulation was found to be -13.4 mV with peak area of 100% intensity indicates the good stability of formulated nanoparticles (Figure 2b).

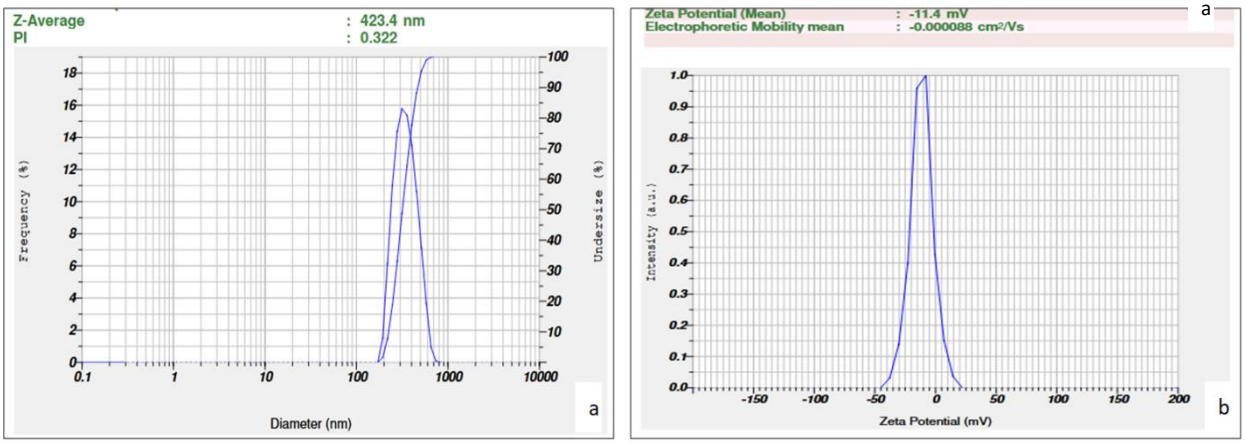

Figure 2: Particle size (a) and zeta potential (b) of optimized losartan potassium nanoparticles

3.3 Scanning Electron Microscopy (SEM)

Microscopic characters i.e., shape and morphology of optimized PNPs was determined by performing SEM analysis at 51.66 KX magnification. Result of the study confirmed the spherical shape and smooth surface morphology as clearly observed in the SEM image (Figure 3).

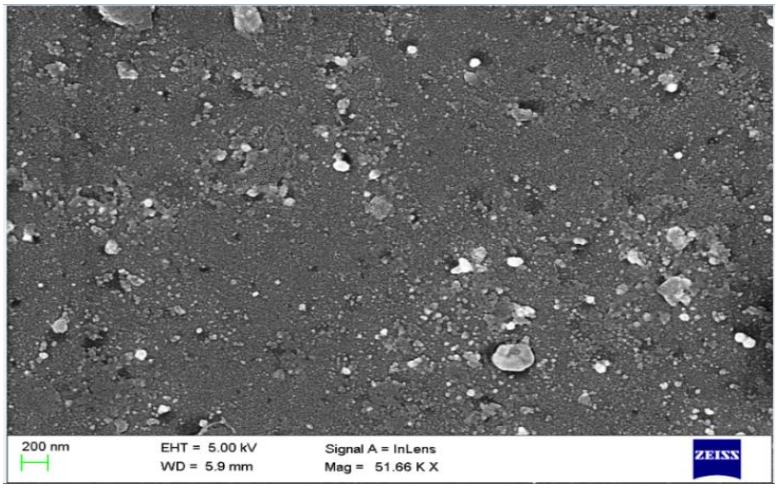

Figure 3: SEM image of optimized losartan potassium nanoparticles

3.4 In-*vitro* drug release and release kinetics study

For *In*-vitro drug diffusion stuties, dialysis bag method was used. The cumulative percentage drug release data for optimized formulation were shown in Table 3. These data were fitted to various kinetic models to study the release kinetics of optimized PNPs. Drug release kinetics results are summarized in Table 3. These release data were plotted for different kinetic models (zero order, first order, higuchi and korsmeyer peppas model) in order to get line of the best fit and determination coefficient (R^2) value that was depicted in Table 4. On the basis of highest R^2 (0.935) value, it was concluded that the optimized PNPs formulation followed the Highuchi kinetic model[10].

Table 3: Cumulative drug release (%) & release kinetics study of optimized formulation

S. No	Time (Hr)	Cumulative % drug release	% drug remaining	Square root of time	log Cumulative % drug remaining	log time
1.	0	0	100	0.000	2.000	0.000
2.	2	15.84	84.16	1.414	1.925	0.301
3.	4	23.76	76.24	2.000	1.882	0.602
4.	6	31.87	68.13	2.449	1.833	0.778
5.	8	53.23	46.77	2.828	1.670	0.903
6.	10	69.82	30.18	3.162	1.480	1.000
7.	12	76.19	23.81	3.464	1.377	1.079
8.	16	85.04	14.96	4.000	1.175	1.204
9.	22	98.42	1.58	4.690	0.199	1.342

Table 4: Correlation value (R^2 value)

Formulation	Model	Kinetic parameter values
Polymeric Nanoparticle	Zero Order	$R^2 = 0.927$
	First Order	$R^2 = 0.770$
	Higuchi	$R^2 = 0.935$
	Korsmeyer peppas	$R^2 = 0.864$

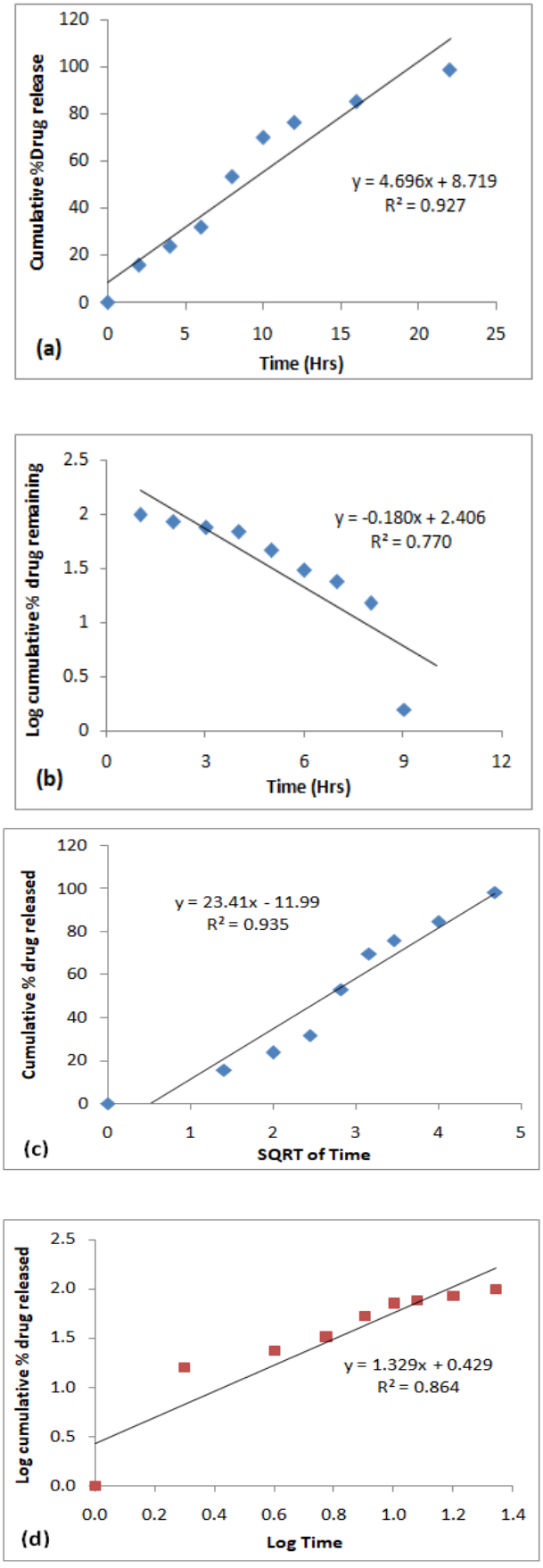

Figure 4: Cumulative drug release kinetic models (a) Zero order, (b) First order, (c) Higuchi, (d) Korsmeyer peppas models

3.5 Accelerated stability study

Optimized PNPs were subjected to stability testing for three months to check any possible drug degradation or instability issue in the formulation. All the physical characteristics of formulation remained unchanged and there was no significant difference in drug content confirmed the stability of the formulation under given conditions.

4. Conclusion

Losartan potassium loaded polymeric nanoparticles using ethyl cellulose as rate controlling polymer were prepared and optimized using 3^3 factorial Box-Behnken design. The dependent variables (responses) particle size and drug entrapment efficiency for various concentrations of independent variables i.e., polymer, surfactant and sonication time were determined experimentally and the values were found to fit the quadratic model. On the basis of experimental design, proposed optimized formulation consisting of 225 mg ethyl cellulose and 0.45% PVA and sonication time of 5 minutes was developed successfully. Formulated polymeric nanoparticles were found to have average particle size of 423.4 nm, 85.57% entrapment efficiency with smooth and uniform surface. Optimized formulation was found to be stable and showed sustained release behavior over a prolonged period. The drug release kinetic followed Korsmeyer-Peppas model with R^2 value of 0.864. Thus, the results revealed that formulation of losartan potassium polymeric nanoparticles can be a promising approach for sustained release for longer time period for the effective management of hypertension. Optimized PNPs can be further evaluated for in-vivo bioavailability studies and also present a scope to be formulated into nanosystem based drug delivery systems other than oral route to confiscate the entire obstacles of conventional oral administration.

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