Original Article

# Development and Bioavailability Assessment of Ramipril Nanoparticle Formulation

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## ABSTRACT

Ramipril, potent anti-hypertensive agent has been used in the treatment of hypertensive disorders. It has low bioavailability of 28-35% and short biological half-life (i.e. 2-4 hr). Thus this study attempts to evaluate chitosan-alginate nanoparticles as a novel drug delivery for Ramipril to sustain drug release and improve oral bioavailability. Nanoparticles were prepared by ionotropic pre-gelation technique using chitosan and sodium alginate polymers. Total nine formulations (F1 to F9) were prepared by varying the polymer concentrations. The nanoparticles were characterized for particle size, drug content, drug entrapment efficiency, zeta potential, surface morphology (TEM), percentage yield, *in-vitro* diffusion study, *in-vivo* bioavailability studies and stability studies. All prepared formulations were in the nanosize range of 190.5 ± 6.15nm to 361.76 ± 3.32 and with spherical morphology. The drug content and entrapment efficiency was found to maximum for F5 formulation, percentage yield was in the range of 53.72 ± 2.04 to 77.91 ± 0.565% which mainly depends upon polymer concentration. The zeta potential of optimised formulation F5 was found to be -34.2mV, showed good stability of nanoparticles during storage. The *in-vitro* drug release profile showed the suitability of nanoparticles for pH dependent and sustained release of Ramipril for prolonged time. Kinetic modelling revealed that the *in-vitro* drug release followed peppas model and non-fickian diffusion. From the *in vivo* studies it was predicted that oral bioavailability of Ramipril nanoparticles improved 2.17 times more than the pure drug. Stability studies carried out for optimized formulation F5 showed that the nanoparticles are more stable at 5 ± 3°C.

**Key words:** Ramipril, Chitosan, Sodium alginate, Ionotropic pre-gelation technique, Chitosan-alginate polymeric complex, pH sensitive, Oral bioavailability.

## INTRODUCTION

Ramipril, potent anti-hypertensive agent has been used in the treatment of hypertensive disorders. It belongs to Angiotensin-Converting Enzyme (ACE) inhibitor class. It act by selectively suppressing renin-angiotensin–aldosterone system, inhibit ACE, prevents conversion of angiotensin I to angiotensin II, resulting in dilation of arterial and venous vessel. It is highly lipophilic (log p octanol/water, 3.32), poorly water soluble drug with absolute bioavailability of 28-35%. Ramipril has a low bioavailability and also short biological half-life (i.e. 2-4 hr) with fluctuations in plasma concentrations and its significant first-pass metabolism when taken orally.[1] Conventional drug delivery system has been characterized by immediate release and repeated dosing of the drug which might lead to the risk of dose fluctuation.[2] Nanoparticles exhibit enhanced size-dependent properties compared with larger particles of the same material. The main objectives of designing nanoparticles as a drug delivery system are to control particle size, surface properties and to deliver pharmacologically active agents at right place, at the rational rate and dose.[3,4] Chitosan (CS) is a natural cationic

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polysaccharide obtained by the N-deacetylation of chitin, a product found in the shells of crustaceans the primary amine groups provide special properties that make CS very useful in pharmaceutical applications. The addition of CS can not only endow nanoparticles positive surface charge, but also prolong the time for which active ingredients are in contact with the epithelium and thus enhance absorption via the para-cellular transport pathway through the tight junctions.[5-7] Alginate (ALG) is a water soluble linear polysaccharide extracted from brown sea weed and is composed of alternating blocks of 1-4 linked α-L-guluronic and β-D-mannuronic acid residues. Alginates which are a group of hemocompatible polymers have not been found to accumulate in any major organs and have shown evidence of *in vivo* degradation. The guluronic acid residues of alginate in the presence of calcium ion undergo ionic interaction to form gels. The properties of calcium–alginate gel beads make them one of the most widely used carriers for controlled release systems.[6,8] Nanoparticles prepared from alginate alone show low stability and encapsulation efficiency, but these problems can be overcome using cationic polymers such as chitosan. Chitosan-alginate polymeric complex are formed due to ionic interaction between the carboxylate groups of alginate and the ammonium groups of chitosan. The formed nanoparticles are biocompatible, biodegradable, non-toxic and capable to sustain the release of encapsulated materials more efficiently than either alginate or chitosan alone.[9] This necessitated the development of novel chitosan-alginate nanoparticles as novel drug delivery system for Ramipril in order to provide pH dependent, sustained drug release and increase oral bioavailability.

## MATERIALS AND METHODS

### Materials

Ramipril was procured as gift sample from Unichem Laboratories, Goa. Sodium alginate was obtained as a gift sample from Hariharathmaja Chemical Works, Kochi. Chitosan (high viscosity) was purchased from Central Institute of Fisheries Cochin, Kerala. All other reagents used were of analytical grade.

### Experimental MethodsPreparation of Chitosan-Alginate nanoparticles

Chitosan-Alginate nanoparticles were prepared by ionotropic pregelation technique. Briefly specified quantity of sodium alginate and calcium chloride are dissolved in distilled water separately. The pH of the sodium alginate solution was adjusted to 5.1 using 1M hydrochloric acid. Briefly, a known amount of chitosan was dissolved in 1% acetic acid solution and pH was modified to 5.4 using 0.1N NaOH. Calcium chloride, a cross linking agent, was added dropwise to 15 ml of sodium alginate solution (0.06-0.1%w/v) containing Ramipril [initially dissolved in 2ml of ethanol: water (1:1) mixture], under stirring condition. To this 3ml of 0.5% w/v of Pluronic F-68 was added to above solution. Then chitosan solution 3ml of (0.03-0.07%w/v) was added dropwise to resultant solution (Table 1) followed by probe sonication for 30 min and the mixture was kept at room temperature overnight for uniform particle size distribution.[5,10]

### Evaluation of Ramipril chitosan alginate nanoparticles Determination of particle size and Polydispersity index

The size distribution and polydispersity index (PDI) of the formulations was measured by Dynamic Light Scattering Particle Size Analyzer (Nano-flex, Microtrac Inc., USA). The range of the analyzer is 0.02 nm to 2.8 µm. The average diameter and a measure of the distribution width (polydispersity) were determined from the particle size distribution data. Polydispersity index varies from 0.0 to 1.0. The closer to zero the polydispersity value, the more homogenous are the particles. The usual range of PDI values: 0-0.05 (monodisperse standard),

**Table 1: Formulation table (f1 to f9).**

| Ingredients | Formulation code | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
| Ramipril (mg)* | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Sodium Alginate (mg) | 9 | 12 | 15 | 9 | 12 | 15 | 9 | 12 | 15 |
| Chitosan(mg)** | 0.9 | 0.9 | 0.9 | 1.5 | 1.5 | 1.5 | 2.1 | 2.1 | 2.1 |
| Calcium chloride (18mM) (ml) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Pluronics 68 (0.5%) (ml) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Distilled water (ml) | Make up the volume up to 25ml | | | | | | | | |

*Ramipril dissolved in ethanol and water mixture (1:1) ** Chitosan dissolved in 1% acetic acid.

0.05-0.08 (nearly-monodisperse), 0.08-0.7 (midrange polydispersity), > 0.7 (very polydisperse).[11,12]

## Determinations of drug content

A quantity of drug loaded nanoparticles equivalent to 1mg was added to 10ml mixture of methanol and phosphate buffer pH 6.8 (1:10) and stirred continuously for 2 hr and then the final colloidal suspensions were ultracentrifuged at 10000 rpm for half an hour. The supernatant was analysed for drug content by measuring the absorbance at 207 nm using UV spectrophotometer.[13]

## Entrapment efficiency

The encapsulation efficiency of nanoparticles was determined by the separation of drug-loaded

Nanoparticles from the aqueous medium containing non-associated Ramipril by ultracentrifugation at 12,000 rpm at 4°C for 1hr. The amount of Ramipril loaded into the nanoparticles was calculated as the difference between the total amount used to prepare the nanoparticles and the amount that was found in the supernatant. The amount of free Ramipril in the supernatant was measured by UV Spectrophotometer.[14] Entrapment efficiency was then calculated as follows:

Entrapment efficiency was calculated by Eq.1

$$\%EE = \frac{total\ amount\ of\ drug - non\ bound\ drug}{total\ amount\ of\ drug\ added} \times 100 \ldots\ldots Eq1$$

## Percentage yield

Fixed volumes of Ramipril nanosuspension were centrifuged at 9000 rpm for 30 min at 15°C. The obtained sediment was dried and weighed.[11,15] The percentage yield was calculated by Eq.2

$$Perecentage\ Yield = \frac{weight\ of\ nanoparticles\ obtained}{weight\ of\ drug\ and\ excipient} \times 100 \ldots\ldots Eq..2$$

## Shape and Surface Morphology

### *Transmission electron microscopy (TEM)*

External morphology of Nanoparticles was determined using Transmission electron microscopy (TEM) at Punjab University, Chandigarh. Samples of Nanoparticles were prepared by placing one drop on a copper grid, without being stained before being examined using TEM.[16]

## Zeta potential

The zeta potential value of optimized Ramipril loaded chitosan- alginate (CS-ALG) nanoparticle formulation was measured with the Zetasizer. To determine the zeta potential, optimized formulation was diluted with double-distilled water and placed in an electrophoretic cell.[17]

## *In vitro* drug release

The release of Ramipril from nanoparticles was evaluated using USP type II paddle apparatus over 24 hr, dialysis membrane was loaded with nanoparticle formulation containing 10 mg equivalent of drug, which was suspended initially for 2hrs in 900 ml of 0.1NHCl buffer of pH 1.2 and then in pH 6.8 phosphate buffer upto 24 hr maintained at 37±0.5°C and 50 rpm. At regular intervals aliquots of 1 ml of the sample were withdrawn and replaced with the same volume of the respected fresh buffer solution. The amount of released drug was assessed by UV-1700 analysis at 207 nm (Shimadzu UV-1700, Japan) after dilution.

## *In vivo* studies

Healthy male wistar rats weighing 180-200 gm were housed in polypropylene cages and maintained at room temperature under 12 hr dark/light cycles. They were fed with standard pelleted diet and water. The animals were acclimatized for one week under laboratory conditions before experiments on the animals. Ethical clearance was obtained from the Institutional Animal Ethics Committee (Resolution No: KLECOP/IAEC/Res.20-09/08/2014) prior to the beginning of research work. This study was aimed mainly to estimate the amount of drug in the blood withdrawn from rats at various time intervals.[18] Dose for animal

Dose conversion formula from human dose to animal dose (Rat)

$$Rats\ Dose = Human\ Dose \times Factor(0.018)\ Eq\ \ldots.3$$

The animals were divided into 2 groups

Group 1: Received pure drug Ramipril 1.08mg for 6 animals in normal saline through oral route.

Group 2**:** Received nanoparticles containing equivalent to 1.08 mg of Ramipril for 6 animals in normal saline through oral route.

After 0.5, 1, 2, 4, 8, 12 and 24 hr 0.5-1ml blood was collected from eye by retro-orbital puncture. Blood was taken in Eppendorf containing 0.1ml of 4%w/v of sodium citrate solution and centrifuged at 5000rpm for 10 min supernatant plasma was collected and acetonitrile was added for precipitating the plasma proteins and vortexed for 1 min and then centrifuged at 13,000rpm for 15 min and supernatant solution was collected and analyzed in HPLC.

## HPLC method development for analysis of Ramipril in plasma samples

### *Instrument*

A Shimadzu prominenece HPLC system (Shimedzu, Kyoto, Japan) equipped with LC-20AD quartenary

pump, DGU-20A5 online prominence degasser, SPD-M20A photodiode array detector, SIL-20AC HT Autosampler, Rheodyne injection valve with 20µl loop and CTO-10ASVP-column oven. Software-LC solution V.1.25.

### Chromatographic Conditions

Sheseido column $C_{18}$ column 250 x 4.6mm, 5 µ diameter was used for separation. The mobile phase containing 0.2% orthophosphoric acid: acetonitrile in the ratio of 50:50(v/v) was delivered at a flow rate 0.6 ml/min and the elution was monitored at 205 nm. Injection volume was 20µl. Pressure of 65kg/cm$^2$ and temperature 28°C.

### Preparation of 0.2% orthophosphoric acid

Measure 2000 µl of orthophosphoric acid in 1000 ml clean dry beaker and make up volume to 1000ml with HPLC water.

### Preparation of mobile phase

Mix a mixture of above solution i.e. 0.2% orthophosphoric acid 500ml (50%) and 500 ml of Acetonitrile HPLC (50%) and degas in ultrasonic water bath for 5 min. Filter through 0.45 µ filter under vacuum filtration.

### Standard solutions and spiked samples

Accurately weigh and transfer 10 mg of Ramipril in 10ml clean dry volumetric flask add about 5ml. Acetonitrile, sonicate to dissolve it completely and make volume up to the mark with 0.2% orthophosphoric acid. Further pipette out 10 µl, 20 µl,30 µl,40 µl,50 µl and 60 µl of the above stock solution into a 2ml eppendorf tube and dilute up to the mark with mobile phase to obtain workingstock solutions of concentration 5µg/ml, 10 µg/ml,15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml. Standard solutions (1 µg/ml,2 µg/ml, 3 µg/ml,4 µg/ml,5 µg/ml and 6 µg/ml) were prepared by spiking 200 µl of working solution(5µg/ml, 10 µg/ml,15 µg/ml, 20 µg/ml, 25 µg/ml and 30 µg/ml) into 200 µl of blank plasma and 600 µl acetonitrile. These standards were used to construct calibration curve in plasma.

### Plasma sample preparations

100 µl of plasma containing unknown concentration of drug (withdrawn from rats at different time interval) were pipetted using micropipette into 2 ml eppendorf tube. To this 200 µl of acetonitrile was added to precipitate protein present in the plasma. This solution was capped and mixed by vortexing at high speed for 1min. Next all tubes were centrifuged at 13,000 rpm for 15min in eppendorf minispin centrifuge, After centrifugation the upper organic layer was filtered through 0.2 µm membranes into clean vials and a volume of 20 µl was injected into HPLC system.

### Short term stability studies

Optimized formulation was chosen to perform short term stability studies. Samples were stored in glass vials for 3 months at 5±3°C in freeze and at 30±2°C/65±5%RH. After 30, 60 and 90 days samples were observed for particle size, % entrapment efficiency and drug release were carried out for optimized formulation at every one month interval.[19]

## RESULTS AND DISCUSSION

### Preparation of chitosan–sodium alginate nanoparticles

The chitosan-alginate nanoparticles are successfully prepared by ionotropic gelation technique. Preparation is simple, rapid and reliable. A number of trials were carried out inorder to obtain appropriate concentration range of polymers so as to allow the formation of turbid solutions and not the aggregates. The final concentration range selected 0.03- 0.07% w/v and 0.06–0.1% w/v for CS and ALG, respectively. Chitosan–alginate (CS–ALG) polyionic complexes are formed through the ionic gelation via interactions between the carboxyl groups of alginate and the amine groups of chitosan.[5,8] The effect of pH on the nanoparticles formulation was studied by Douglas and Tabrizian. It was found that size of nanoparticle was smaller when alginate solution of pH 5.3 was combined with chitosan solution pH 5.5. This may be explained by the fact low solubility of chitosan in water at neutral and alkaline pH hence the chitosan solution is prepared in acidic conditions. Due to this there are chances of chitosan precipitating upon addition of alginate solution with higher pH resulting in less Chitosan available for nanoparticles formation. Also as the pKa of Chitosan is reported to be 6.5, addition of alginate solution with neutral pH would result in the majority of amine groups of CS being unprotonated and, therefore, unable interact with ALG. Thus using alginate at slightly lower pH (5.0–5.3) will overcome this problem allowing stronger interaction between chitosan and alginate. Motwani K S *et al.* observed that if alginate and chitosan solution were used in more acidic ranges results in larger size particles and smaller nanoparticles when both polymers have a pH range of 5.1-5.7. Within this range, the amine groups of the chitosan are protonated and the carboxyl groups of the alginate are ionised, which is most important for optimum interaction and the polyionic complex formation.[3]

**Table 2: Particle size, pdi, drug content, % yield and % ee values of ramipril nanoparticles formulations F1 to F9.**

| Formulation | Particle size (nm) | PDI | % Yield | Drug content | % EE |
|---|---|---|---|---|---|
| F1 | 190.5±6.15 | 0.500±0.02 | 53.72±2.04 | 88.93±1.84 | 43.75±0.62 |
| F2 | 206.73±5.94 | 0.447±0.051 | 57.61±0.71 | 90.16±4.304 | 52.5±0.40 |
| F3 | 237.9±3.78 | 0.719±0.04 | 59. 7±1.10 | 88.52±2.28 | 62.5±0.81 |
| F4 | 248.9±6.45 | 0.207±0.071 | 62.60±0.69 | 93.44±1.22 | 74±0.40 |
| F5 | 258.76±4..36 | 0.149±0.036 | 68.05±1.50 | 96.72±0.853 | 86.7±0.52 |
| F6 | 302.36±6.03 | 0.269±0.04 | 70.88±1.22 | 89.75±4.10 | 69.7±1.08 |
| F7 | 319.86±7.04 | 0.723±0.74 | 73.20±0.45 | 95.49±6.97 | 77.5±1.03 |
| F8 | 349.4±3.35 | 0.149±0.307 | 76.01±0.98 | 94.6±1.25 | 64.5±0.62 |
| F9 | 361.76±3.32 | 0.570±0.08 | 77.91±0.56 | 93.0±0.012 | 62±0.40 |

*Data are expressed as mean ±S.D. ($n$=3)

## Particle size and Size distribution

The mean particle size for formulations F1 to F9 varied in range of 190.5±6.15 to 361.76±3.32 (Table 2). It was observed that mean particle size increases with the increase in the polymer concentration upto a level. Further increase in the polymer concentration above the concentration range mentioned resulted in the aggregation of the particles. The mean polydispersity index values for the Ramipril loaded chitosan alginate nanoparticle formulations F1 to F9 are in the range of 0.1499- 0.723 as shown in (Table 2). The results of PDI can be simultaneously checked with particles size analysis. A monodisperse sample indicates PI value nearer to 0. However, PDI < 1 indicates polydisperse samples. Therefore, PI measurement was essential to confirm the size distribution of the particles.[8,9]

## Drug content

Drug content varies in the range of 88.52±2.28 to 96.72±0.853 and was determined using the UV spectroscopic analysis at 207 nm.

## Entrapment efficiency

Encapsulation efficiency of the nanoparticles was found to vary between 43.75±0.62 and 86.7±0.52. Motwani S K *et al*. suggested that at intermediate concentration of chitosan and sodium alginate the encapsulation of drug was maximum. The percent entrapment efficiency increases from F1 to F5 and then decreases from F6 to F9. This is because increase in the polymer concentration causes increase in the drug entrapment upto a level, further increase in the polymer concentration leads to decrease in the entrapment efficiency which can be explained on the basis of the fact that at higher concentrations of the two polymers, it is polymers that make the bulk of the nanoparticles matrix and less volume is available for drug encapsulation. Formulation F5 (86.7±0.52) show maximum entrapment efficiency. Based on entrapment efficiency and drug content formulation F5 was taken as optimized formulation.[3,9]

## Percentage yield

Percentage yield was found to be 53.72% to 77.91 % for formulation F1 to F9 (Table 2). Percentage practical yield depends on the concentration of polymer added, as the concentration of polymer increases there is increases in the % yield. Maximum yield obtained is 77.91% for formulation F9.

## TEM Analysis

Optimized formulation F5 containing chitosan (0.05%) and sodium alginate (0.08%) was subjected to TEM with different resolution. In the magnification of 10000× particles were small, spherical (Figure 1) and discrete while the same formulation at 30000× magnification the surface was seen to be smooth and spherical with the size range of 245nm and 253nm.

## Zeta potential

Zeta potential is an important parameter to analyze the long-term stability of the nanoparticles. It refers to the surface charge of the particles. Zeta potential of nanoparticles is of significance on stability in suspension through the electrostatic repulsion between the particles. Zeta potential of the optimized formulation F5 was found to be -32.2mV (Figure 2). The zeta potentials of about -34.2 mV indicate good stability of formulation. This might be attributed to surfactant which decreases the electrostatic repulsion between the particles and sterically stabilizes the nanoparticles by forming a coat around their surface.[20]

## *In vitro* drug release

*In vitro* drug release from the nanoparticles was initially carried out in 0.1N HCl buffer pH 1.2 for 2hrs followed by Phosphate buffer pH 6.8 upto 24 hr, it was

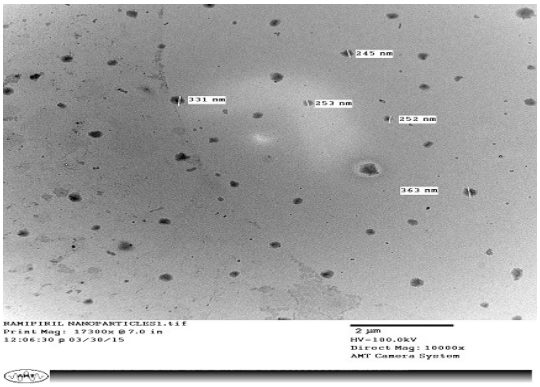


**Figure 1: TEM of optimized formulation F5.**

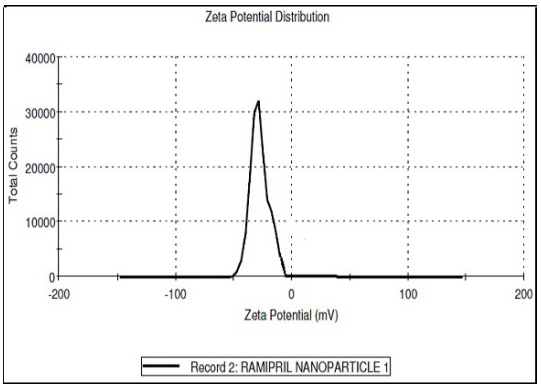


**Figure 2: Zetapotential of optimized formulation (F5).**

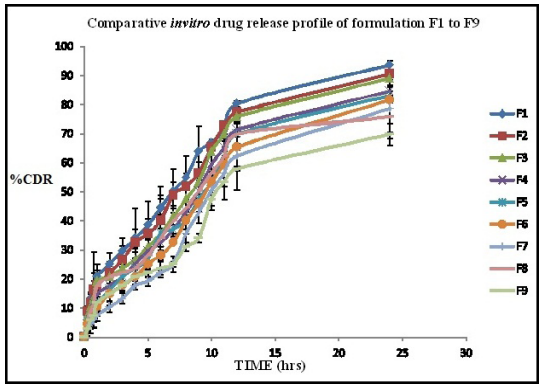


**Figure 3: Comparative *in vitro* drug release profile of F1 to F9.**

performed using dialysis bag diffusion technique. The drug release at the end of 2 hrs in 0.1N HCl pH 1.2 for F1-25.14%, F2-22.25%, F3-20.71%, F4-17.74%, F5-16.2%, F6-14.7%, F7-10.35%, F8-20.7%, F9-14.7% and at the end of 24 hrs the mean cumulative drug release was F1-93.63%, F2-90.6%, F3-89.12%, F4-84.6%, F5-83.14%, F6-81.62%, F7-78.6%, F8-75.7%, F9-69.7% (Figure 3). It was observed that at low polymer concentration i.e. F1 showed maximum release at the end of 24hr. As the polymer concentration increases from F1 to F9 the release decreases, this is mainly due to higher

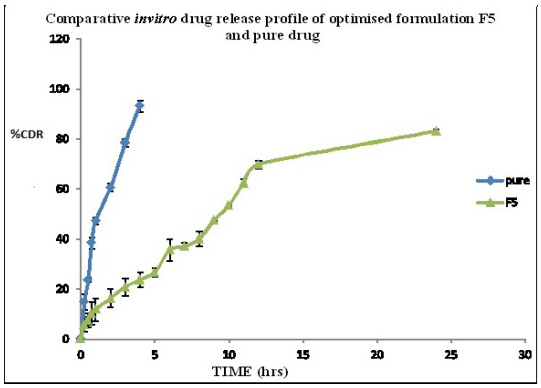


**Figure 4: Comparative *in vitro* drug release profile of optimized formulation F5 and pure drug.**

**Table 3: Pharmacokinetic parameter of pure ramipril and optimized formulation f5.**

| Formulations | $C_{max}$ (mcg/ml) | $T_{max}$ (h) | $AUC_{0-t}$ (mcg/ml.h) |
|---|---|---|---|
| Pure Ramipril | 7.9768 | 0.5hrs | 114.874 |
| Optimized formulation F5 | 12.482 | 8hrs | 249.77 |

polymer concentration the drug will take longer time to diffuse out.

The initial release of the drug at the end of 2 hr may be due to the drug present on the surface of Nanoparticles. Whereas the drug which is present in the matrix inside the nanoparticles have additional barrier due to the polyelectrolyte complex which remains intact at acidic pH and when the nanoparticles are exposed to phosphate buffer pH 6.8 the polyelectrolyte complex weaken and there is slow release of drug for a prolong period of time.[21]

Evaluation of the release profiles of pure drug showed that almost all the Ramipril was released immediately during first 4 hrs, suggesting that the developed nanoparticles can be used as an important platform for sustained drug release. (Figure 4)

Among the models tested, the drug release profiles for formulations (F1 to F9) were best fitted with Korsmeyer- Peppas model based on regression coefficients (0.9923, 0.9890, 0.9810, 0.9856, 0.9888, 0.9890, 0.9843, 0.9828, 0.9827 respectively) and n being greater 0.5 suggesting non-Fickian diffusion process.[12]

## *In vivo* studies

The study was carried out on male wistar albino rats to compare plasma concentration of Ramipril nanoparticles (optimised formulation F5) with that of pure drug given orally in normal saline.

*In-vivo* study revealed that formulation F5 showed greater bioavailability than that of the pure drug. The

HPLC peaks of pure Ramipril and optimized formulation F5 at 1hr and at 12 hr are shown in Figure 5, Figure 6, Figure 7 and Figure 8 respectively. AUC of 249.77 µg /ml hr, $C_{max}$ of 12.48 µg and $T_{max}$ of 8 hr was observed for formulation F5 given orally whereas pure drug showed AUC of 114.874 µg/ml hr, $C_{max}$ of 7.95 µg and $T_{max}$ 0.5hr calculated by applying trapezoidal method. (Table 3)

$AUC°_{-t}$ of pure drug 114.874 µg/ml hr was increased up to 249.77µg /ml hr by Ramipril nanoparticle formulation through oral route. Figure 9 shows the comparative in vivo release profile of optimized formulation F5 and pure drug (Ramipril) after oral administration. Ramipril nanoparticles lead to enhancement of oral bioavailability by 2.17 times than that of pure drug.[22]

## Short term stability studies

Stability studies were carried out on the optimized formulation F5 as per ICH guidelines for 90 days. By comparing this data with initial data it was observed that there was an slight decrease in the percentage entrapment efficiency and increase in particles size due to degradation of polymer and aggregation of particles. (Table 4) There was not much change in the drug release. Formulation stored at (4 ± 2°C) showed bet-

**Table 4: Effect on particle size, % Entrapment efficiency, % drug release during stability studies.**

| | | OPTIMISED FORMULATION F5 | | | | | |
|---|---|---|---|---|---|---|---|
| | Initial | Final at 5 ± 3ºC | | | Final at 30±20C/65±5%RH | | |
| | | 30 days | 60 days | 90 days | 30days | 60 days | 90days |
| Particle size (nm) | 257.9±2.89 | 260.5±3.8 | 263.65±9.1 | 270.35±4.8 | 268.4±5.2 | 273.9±0.9 | 278.7±3.8 |
| % Entrapment Efficiency | 86.7±0.001 | 85.65±5.3 | 83.62±13.2 | 79.84±1.3 | 83.76±8.0 | 81.45±3.2 | 74.84±2.3 |
| % Drug Release | 83.2±0.01 | 81.7±1.54 | 80.25±4.58 | 77.34±4.1 | 79.54±6.8 | 75.78±1 | 70.29±1.3 |

*Data are expressed as mean ±SWWW.D. ($n$=3)

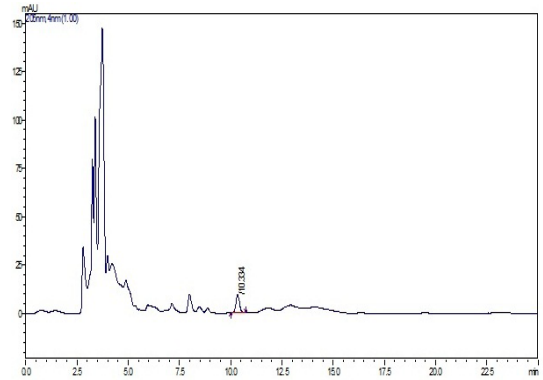

**Figure 5: HPLC peak of Ramipril at 1hr (pure drug).**

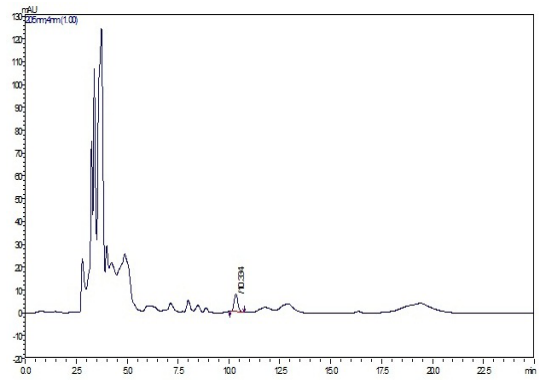

**Figure 7: HPLC peak of Ramipril at 12hrs (pure drug).**

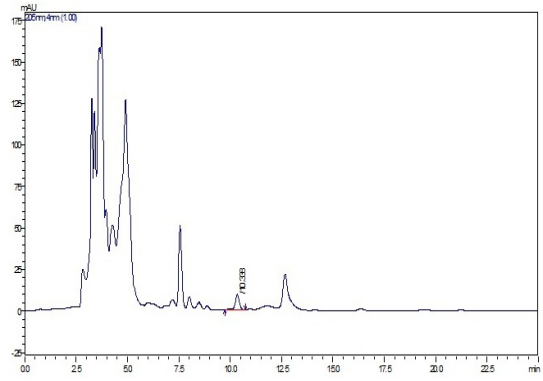

**Figure 6: HPLC peak of Ramipril at 1hr (Optimized formulation F5).**

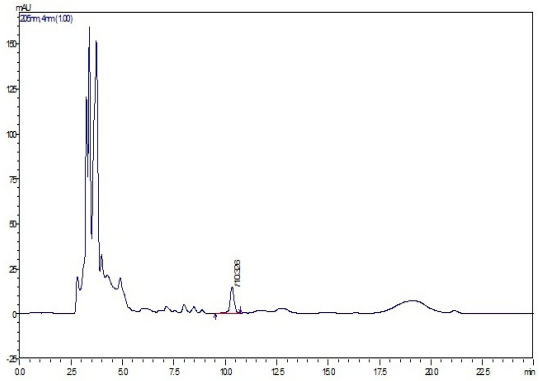

**Figure 8: HPLC peak of Ramipril at 12hrs (optimized formulation F5).**

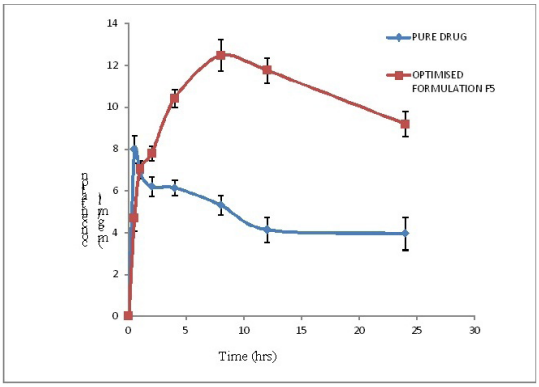


**Figure 9: Comparative *in vivo* release profile of optimized formulation F5 and pure drug (Ramipril) by oral administration.**

ter stability as compared to the formulation stored at 30±2°C/65±5%RH.

Ramipril chitosan- alginate nanoparticles can be successfully prepared by ionotropic pregelation technique. *In vitro* release study showed that chitosan- alginate nanoparticles showed pH dependent and sustained release of drug for a prolong period of time. The oral bioavailability of Ramipril when formulated into pH sensitive chitosan alginate nanoparticles was improved by 2.17 times the pure drug.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**ACE:** Angiotensin-Converting Enzyme inhibitor; **TEM:** Transmission electron microscopy; **HPLC:** High performance liquid chromatography; **ALG:** Alginate; **CS:** Chitosan; **PDI:** Polydispersity index.

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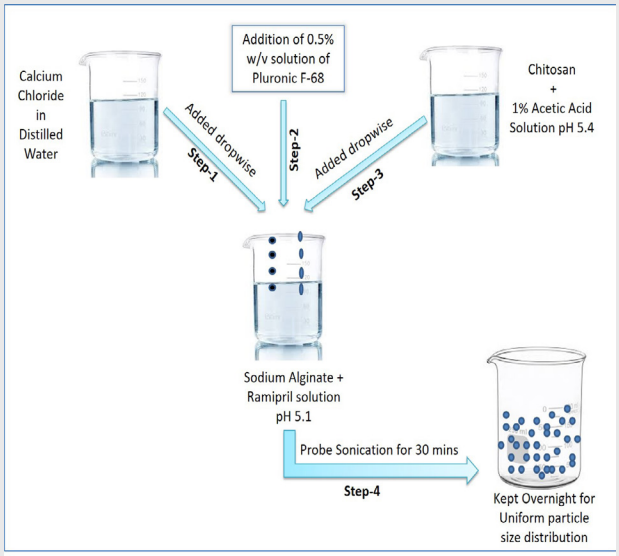
## PICTORIAL ABSTRACT




## SUMMARY


- A total of nine formulations (F1-F9) were formulated by varying the concentration of chitosan (0.003%-0.07%) and sodium alginate (0.06-0.1%) using ionotropic pregelation technique and the various parameters were evaluated.
- The size of nanoparticles were in nanosize range, spherical and discrete, particle size increase with increase in polymer concentration. Entrapment efficiency increased with increasing the polymer concentration to certain level and then decreased.
- F5 formulation was considered as optimized formulation based on higher entrapment efficiency, drug content and good Zeta potential of-32.4mV.
- *In vitro* release study showed initial burst effect, this may be due to drug present on the surface of nanoparticles followed by sustain release of drug.
- The oral bioavailability of Ramipril when formulated into pH sensitive CS-ALG nanoparticles was improved by 2.17 times more than that of pure drug. From the stability studies, it can be found that 5 ± 30 0C is the ideal temperature for storage of nanoparticles.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