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FORMULATION AND CHARACTERIZATION OF ENALAPRIL MICROPARTICLES USING GRAFT COPOLYMERIZED HYDROGEL

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Abstract:

The aim of the present study was to formulate and evaluate Enalapril Microparticles through graft copolymerization procedures, utilizing guar gum as the natural polymer and polyacrylamide as the synthetic polymer. The optimal hydrogel formulation was developed as microparticles, loaded with Enalapril, and evaluated using various parameters. The graft copolymer was characterized using FTIR, DSC, and SEM. The preparation conditions of the beads were adjusted by evaluating the % entrapment efficiency, particle size, swelling capacity under varying pH settings, and their release profiles. FTIR studies support the development of grafted copolymers, whereas DSC tests indicate a relatively enhanced thermal stability of these copolymers. The guar gum-g-poly(acrylamide) and sodium alginate (pAAm-g-GG/SA) microparticles exhibited a nearly spherical morphology, as demonstrated by the SEM analysis. The swelling index was highest in phosphate buffer at pH 6.8 with increased concentrations of sodium alginate and guar gum, and lowest with larger concentrations of ethyl cellulose. Microparticles enalapril was discovered to be regulated with an increase in polyacrylamide and sodium alginate content in the microparticles, with a greater release noted in a pH 6.8 medium compared to pH 1.2. The in vitro release kinetics of Enalapril from the polymeric beads adhered to the Zero-order release with drug release as anomalous diffusion. Microparticles of Enalapril based on hydrogel were effectively synthesized with optimal formulations of pAAm-g-GG/SA by a free radical ionization process. All characterization parameters met the acceptance standards.

Keywords: *Hydrogel, Microparticles, Enalapril, Guar gum, Acrylamide, Sodium alginate*

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INTRODUCTION:

A hydrogel-based drug delivery system is the most promising novel approach nowadays for the delivery of a drug for an extended period. Hydrogels are three-dimensional network polymers that swell in aqueous solutions, and in the swollen state, they become soft and rubbery, resembling a living tissue, and some possess excellent biocompatibility. Hydrogel systems possess good stability in surrounding conditions like changes in P^H, ionic strength, temperature, and frequent changes of environment in the GI-tract, which has a variation of environment from the stomach to the intestine [1]. Hydrogel from natural polymers, especially polysaccharides, has been widely used for their advantages over synthetic polymers, such as non-toxic, biocompatible, biodegradable, freely available, able to modify the properties of aqueous environment, capable of thickening, emulsifying, stabilizing, encapsulating, and swelling and forming gels and films. But they can be modified to overcome some drawbacks, like an uncontrolled rate of hydration, microbial contamination, a drop in viscosity on storing, etc. Graft copolymerization is an easier method to modify the structure of natural polymers and make them attractive biomaterials in controlled release applications. The Microparticles are characteristically free-flowing particles consisting of proteins or synthetic proteins or synthetic polymers, which are biodegradable in nature and ideally have a particle size in the micron range capable of releasing drug for a prolonged period of time. Sodium alginate is a salt of alginic acid, which has the capacity to crosslink with other natural or synthetic polymers when it comes in contact with divalent or trivalent cations and the method is called as ionic gelation method. Alginate is a natural carbohydrate polymer commonly used in the drug delivery systems because they are biodegradable, biocompatible and nontoxic. Alginate belongs to a family of linear polysaccharides, produced by brown algae, which contain varying amount of 1, 4-linked β-D-mannuronic acid and α-L-guluronic acid residues. Sodium alginate has been commercially applied and investigated due to its low cost and minimal processing requirement. Alginate gelation takes place when divalent or trivalent cations (usually Ca^{2+}), interacts ionically with guluronic acid residues, resulting in formation of a three-dimensional network which is usually described by egg-box model. Alginate- Ca^{2+} has been widely investigated for oral and nasal drug-controlled release applications. So calcium chloride can be used as a cross linking agent for the preparation of microparticle in ionic gelation method [2].

Hypertension is a prevalent chronic cardiovascular disease, which calls for long-term treatment and continuous monitoring to avoid associated complications. Enalapril is an angiotensin-converting enzyme (ACE) inhibitor that is commonly used in the management of hypertension and heart failure. The drug blocks the conversion of angiotensin I to angiotensin II, which lowers blood pressure and enhances cardiovascular performance. Enalapril has high water solubility, so a sustained release formulation is very important to prolong the release of the drug, decrease the frequency of dosage and improve patient compliance. Thus, the present study aims at development of hydrogel based Enalapril microparticles for efficient and controlled management of hypertension. [3,4].

MATERIALS AND METHODS:

Materials

Enalapril was received as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India; Guar gum was obtained from Girijan Cooperative Corporation, Hyderabad, India. Acrylamide was obtained from Nice Chemicals Pvt. Ltd., Mumbai, India. Calcium chloride ($CaCl_2$) was purchased from the Central Drug House (P) Ltd., New Delhi, India. Acetone and potassium dihydrogen phosphate were purchased from Sara Fine Chemicals, Baroda, India. All chemicals and reagents used in the study were of analytical grade. Double-distilled water prepared in the laboratory was used in the experimental work.

Synthesis of graft copolymer of guar gum-Acrylamide

The graft copolymerization was conducted in a specifically engineered jacketed reaction vessel equipped with an inlet and output port. The inlet port was linked to the nitrogen gas provided by the nitrogen cylinder (AR grade). The grafted copolymer of guar gum and acrylamide was synthesized using the free radical polymerization method. A designated quantity of Guar gum was dissolved in 75-150 ml of water and allowed to hydrate overnight with stirring in a 250 ml round-bottom flask. The designated quantity of Acrylamide was dissolved in 20 ml of water and incorporated into the GG solution, stirred thoroughly for one hour, and thereafter transferred to the reaction vessel. Ceric Ammonium Nitrate (CAN) was included into this solution at varying quantities to optimize the formulation and enhance production. Polymerization was conducted at 60 °C with continuous nitrogen gas purging for 6 hours in a magnetic stirrer operating at 100 rpm. Upon the completion of polymerization, an enough quantity of acetone was introduced to precipitate the grafted copolymer. The grafted polymer was subsequently filtered and dried in a hot air oven at 40°C for 24 hours [5,6,7,8].

The % yield was calculated as

Percentage yield = (mass of the co-polymer after drying/ mass of the total polymer) × 100

The graft co-polymerization method was optimized for the optimum graft yield using different proportions of Guar gum and polyacrylamide and using different concentration of initiator (CAN). The results are shown in table-1 indicating different proportion of Guar gum and polyacrylamide with different concentration of CAN and % yield of co-polymer. The composition of polymers & initiator used for different batches in copolymer synthesis was depicted in table 1.

Table 1: Composition of polymers & initiator for different batches in copolymer synthesis.

Batch	*Wt. of Guar gum (gm)*	*Wt. of AAm (gm)*	*Ratio of polymer (GG:AAm)*	*Concentration of CAN (M)*	*% Yield*
B_1	1	3	1:3	0.167	86%
B_2	1	3	1:3	0.128	88%
B_3	1	3	1:3	0.121	91%
B_4	1	3	1:3	0.091	93%
B_5	1	3	1:3	0.055	94%
***B*$_6$**	**1**	**3**	**1:3**	**0.036**	**99%**
B_7	1	3	1:3	0.005	-
B_8	1	2	1:2	0.036	81%
B_9	1	1	1:1	0.036	72%
B_{10}	2	1	2:1	0.036	64%
B_{11}	3	1	3:1	0.036	52%
B_{12}	1	4	1:4	0.036	83%

Preparation of pAAm-g-GG/SA Microparticles

A designated quantity of dried hydrogel was processed in a mortar and pestle with hot water until a homogeneous paste was achieved. A specific quantity of sodium alginate was combined with 25 ml of water and thoroughly crushed to create a gel-like substance, which was then blended with a homogenous paste-like mass of hydrogel using the trituration method. The paste-like substance was introduced dropwise into 200 ml of 0.054 M CaCl2 solution using a syringe fitted with a 0.1 mm needle. The formed spongy globules were subsequently filtered, rinsed multiple times with distilled water, blotted with soft filter paper, and dried in a hot air oven at 40°C for 24 hours. The various ratios of hydrogel and sodium alginate utilized are presented in Table 2.

Loading of Enalapril in microparticles

Prepared microparticles from different formulations were loaded with drug by soaking in an aqueous solution containing 10% (w/v) of Enalapril. Soaking was carried out for nearly 24 hours to insure complete equilibrium. The formulations were filtered and the surface adhered drug solution was removed by washing and blotting with soft filter paper, dried in air, and stored in a desiccator until further use.

Drug also loaded during the preparation of microparticles by the trituration method. The required quantity of drug was mixed with a small quantity of distilled water, and the drug solution was mixed with a semisolid mass produced by trituration of hydrogel and sodium alginate.

Table-2: Composition of different formulations of microparticles

Formulations	*Ratio of Polymers in Hydrogel (GG:AAm)*	*Ratio of Hydrogel : SA in microparticles*	*% Yield*
ENM1	1:3	3:1	96.4%
ENM2	1:3	2:1	97.5%
ENM3	1:3	1:1	96.4%
ENM4	1:3	1:2	95.3%
ENM5	**1:3**	**1:3**	99.4%
ENM6	1:2	1:3	95.2%
ENM7	1:1	1:3	96.5%
ENM8	2:1	1:3	96.4%
ENM9	3:1	1:3	95.6%
ENM10	1:4	1:3	92.5%

CHARACTERIZATION OF pAAm-g-GG HYDROGEL AND ENALAPRIL-LOADED MICROPARTICLE [8,9]

Drug-polymer compatibility study by Fourier Transform Infrared (FTIR) spectroscopy

Guar gum grafted copolymer and the cross-linked drug-loaded microparticles, along with individual polymers, were analysed by FTIR spectroscopy to confirm grafting and cross-linked reactions. The polymer samples were crushed with potassium bromide to make pellets. Spectra were taken on a FTIR spectrophotometer in a range of 400-4000 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis

DSC is a thermo-analytical method in which the amount of heat needed to increase the temperature of a sample as a function of temperature is measured. Throughout the experiment, the temperature is maintained almost the same for the sample and the reference. It is a method in which it measures the physical properties of the sample, i.e. when the sample is cooled or heated, it measures the energy absorbed or released. During heating or cooling, the sample may undergo one or more phases. The DSC Studies were carried out for pure drug and optimised formulation. The sample was heated from room temperature to 600 ^{0}C at a heating rate of 10^{0}C per min. The obtained peaks were compared, which usually indicated the melting point of the sample.

Particle shape and surface morphology

Scanning Electron Microscopy (SEM) was performed to investigate the surface morphology of microparticles. Pharmaceutical formulations and raw materials' nanoscale surface topography, size, morphology, and elemental content are all examined using an electron beam in SEM research. It exceeds the limitations of traditional optical microscopes in terms of resolution. Lyophilized microparticles were applied to double-sided adhesive tape that was attached to an aluminum stub in order to create samples. A gold coating, approximately 300 angstroms thick, was applied using a sputter coater. A scanning electron microscope (LEO 435 VP, Eindhoven, Netherlands) with an acceleration voltage of 30 kV was used to evaluate the samples and take photomicrographs. The Indian Institute of Science Education and Research (IISER) in Bhopal, Madhya Pradesh, India, is where the SEM imaging was carried out.[21-22]

Swelling Studies

The equilibrium water absorption of the cross-linked drug-loaded microparticles was assessed by evaluating the degree of swelling of the matrices in phosphate buffer at pH 6.8. To achieve complete equilibrium, the samples were permitted to swell for 24 hours. An electronic microbalance was employed to measure the expanded microparticles with an accuracy of 0.01 mg, following the removal of excess liquid droplets adhered to the surface using filter paper. Subsequently, the hydrogel microparticles were subjected to drying for five hours at 60 degrees Celsius in an oven until the mass of the samples stabilized. [23–24]

The percentage of equilibrium water uptake was computed as follows:

(Mass of swollen microparticles – Mass of dry microparticles / Mass of dry microparticles) × 100

Estimation of Drug entrapment efficiency of microparticles

Since hydrogel microparticles cannot be fully dissolved in organic solvents or acids, the drug entrapment effectiveness of produced microparticles was determined by mechanically crushing the microparticles in the presence of phosphate buffer pH. The solution was then gently boiled for 2 hours to extract the medications fully and filtered to analyze the amount of Enalapril entrapped by using a UV-Visible spectrophotometer at 225 nm.

***In Vitro* drug release study**

The *in vitro* drug release from the pAAm-g-GG/Sodium alginate hydrogel microparticles was carried out in a USP-I rotating basket dissolution apparatus at a rotation speed of 100 rpm at 37 ºC. A proper simulation of gastrointestinal (GIT) condition was maintained by altering the pH of dissolution medium at different time intervals following two step dissolution conditions. To simulate the physiological conditions of GIT, first 2 hour of dissolution was carried out in 900 ml of simulated gastric fluid HCl buffer pH 1.2 and the rest of the time in 900 ml of simulated intestinal fluid phosphate buffer pH 6.8 at regular intervals of time, the aliquots were withdrawn and analysed for drug using the UV-Visible spectrophotometer at 225 nm. After each sampling an equal volume of fresh dissolution media was added to the dissolution medium. All the dissolution studies were repeated six times [12, 16].

Kinetics of release profile

To know the kinetic characteristics and drug release profile of optimised formulation, the in vitro release data was fitted to zero-order, first-order, Higuchi and Korse-Meyer Peppas kinetic model and graph was plotted.[18-19]

Stability Studies

Stability testing in these investigations was conducted over a duration of up to 90 days, or 3 months, for optimized microparticle formulations. The chosen formulation was evaluated for the in vitro release research and its physical appearance. The accelerated testing was conducted under storage conditions of 40°C±2°C and 75%±5% relative humidity, with the findings for appearance and entrapment efficiency compared to those of fresh formulations. [26, 30]

RESULTS AND DISCUSSION:

Optimization of graft co-polymerization techniques

The present study aimed to attempt graft copolymerization of GG with Acrylamide by using Ce(IV) catalyzed free radical reaction. The chelate complex formed between –OH group of GG decomposes to generate the free radical site, facilitating grafting to occur at the active site of GG with Acrylamide monomer. The reaction is shown in **figure 1.** Graft copolymerization process was optimized with concentration and ratio of polymer used by comparing batch to batch yield. It was observed that at concentration greater than 0.1276 M, the hydrogel formed was sticky in nature and deposited at the bottom with less yield. But by using concentration below 0.1276 M and upto 0.0182 M, the hydrogel formed was non-sticky in nature and that floated on the surface. At polymer ratio of 1:3 (GG:pAAm) and with CAN concentration of 0.0364 M, maximum yield was obtained [11,13].

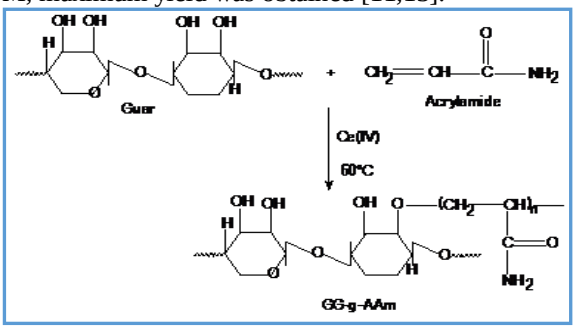

Fig. 1: Showing structural representation of GG-g-AAm

Formulations of GG-pAAm hydrogel/sodium alginate Enalapril microparticles

Hydrogel obtained from different ratio of Guar gum and pAAm with optimised concentration of CAN were taken. Then different ratio of hydrogel and sodium alginate were used for preparation of microparticles. It was observed that when hydrogel:SA ratio was 3:1, the microparticles formed couldn't retain it's spherical shape after drying. By increasing SA ratio, the spherical shape of microparticles could be retained and it was optimum when hydrogel: SA ratio is 1:3 having GG:pAAm in hydrogel was 1:3 also.

FTIR characterization of graft copolymer and drug polymer compatibility study in microparticles

To ascertain the compatibility of the drug and polymers utilized in various formulations, FTIR spectra of the pure drug, polymers, and the physical mixing of the drug and polymer were conducted. Upon comparison of the spectra of Enalapril maleate and the microparticles of the improved formulation, it is observed that the distinct peaks present in Enalapril maleate at 3525 cm-1, 2878 cm-1, 2618.62 cm-1, 1701 cm-1, and 1607 cm-1 are likewise evident in the microparticles of the optimal formulation. Consequently, it is evident that all characteristic peaks observed in the spectra of the pure pharmaceuticals are replicated in the spectra of the optimized formulation of Enalapril maleate microparticles, indicating the absence of interaction between the drug and the polymers. The FTIR spectra of the medication are presented in **figure 2.**

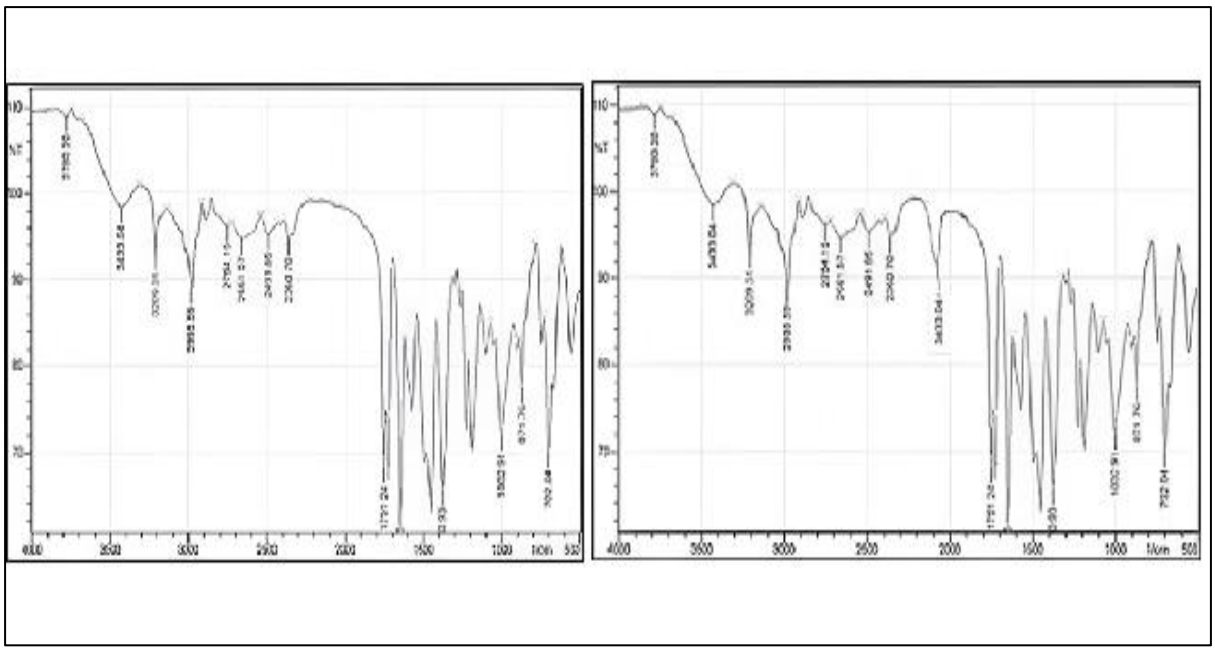

Fig. 2: Comparative studies of FTIR spectra of pure Enalapril and Microparticles

DSC study

The DSC thermogram of the improved formulation of Enalapril microparticles was prepared to assess any potential thermal interactions between the drug and the polymer. This study presents the endothermic peaks observed in both pure medication and the optimized formulation of microparticles. The endothermic peak was noted at 170.3 °C for both Enalapril and the improved formulation of microparticles. The DSC studies indicated that the formulation is thermodynamically stable, as it necessitated slightly more heat than the pure drug due to the presence of various excipients with the drug. No transition of peaks from endothermic to exothermic was observed. The DSC thermograms for pure medication and the improved formulation of microparticles are presented in figure 3.

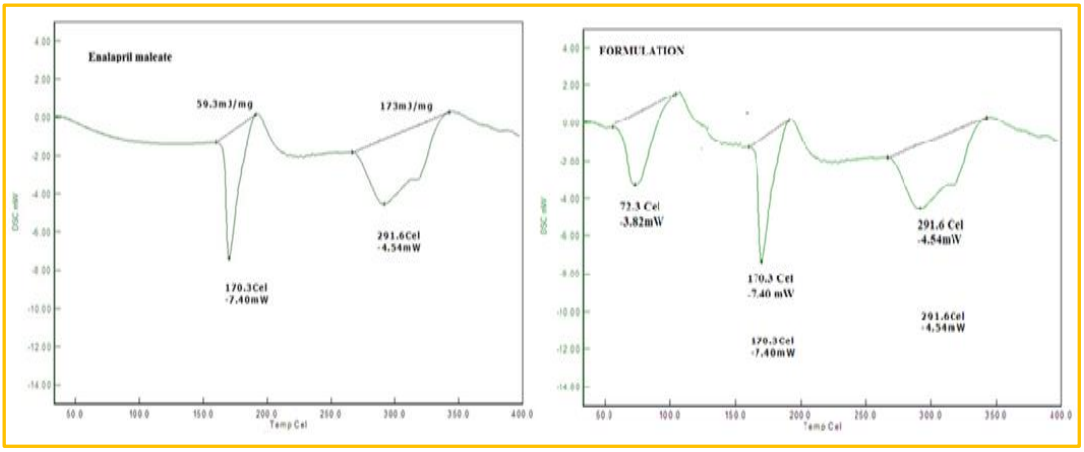

Fig. 3: DSC thermogram of pure Enalapril and Formulation

Particle shape and surface morphology

The SEM investigation demonstrated that the optimized microsphere formulation exhibited a spherical morphology and a smooth surface, as depicted in figure 4. Particles demonstrate remarkable uniformity and constancy in their sizes. circular with uninterrupted, non-aggregated, and impermeable surfaces. The precise dimensions of the microparticle varied between 15 µm and 27 µm.

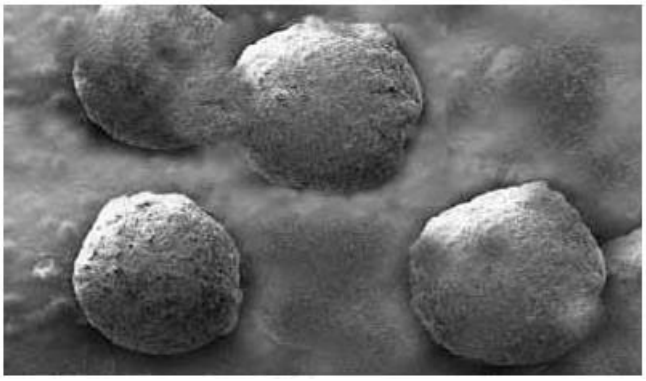

Fig. 4: SEM photomicrograph of Empagliflozin loaded microparticles

Drug entrapment efficiency

The microparticles were prepared by incorporating the drug during the preparation process and the entrapment efficiency of different formulations was determined. The prepared microparticles showed high drug entrapment efficiency from 88.45% to 97.82% for different batches. The high entrapment efficiency may be attributed to the efficient polymeric network of the hydrogel system, which retained the drug inside the microparticles efficiently. Among all formulations, the drug entrapment efficiency was highest (97.82%) for ENM_{10} with effective drug incorporation and homogenous distribution in the polymer matrix. The results of drug entrapment efficiency are presented in the corresponding histogram (Fig. 5) representing the percentage drug content of different formulations.

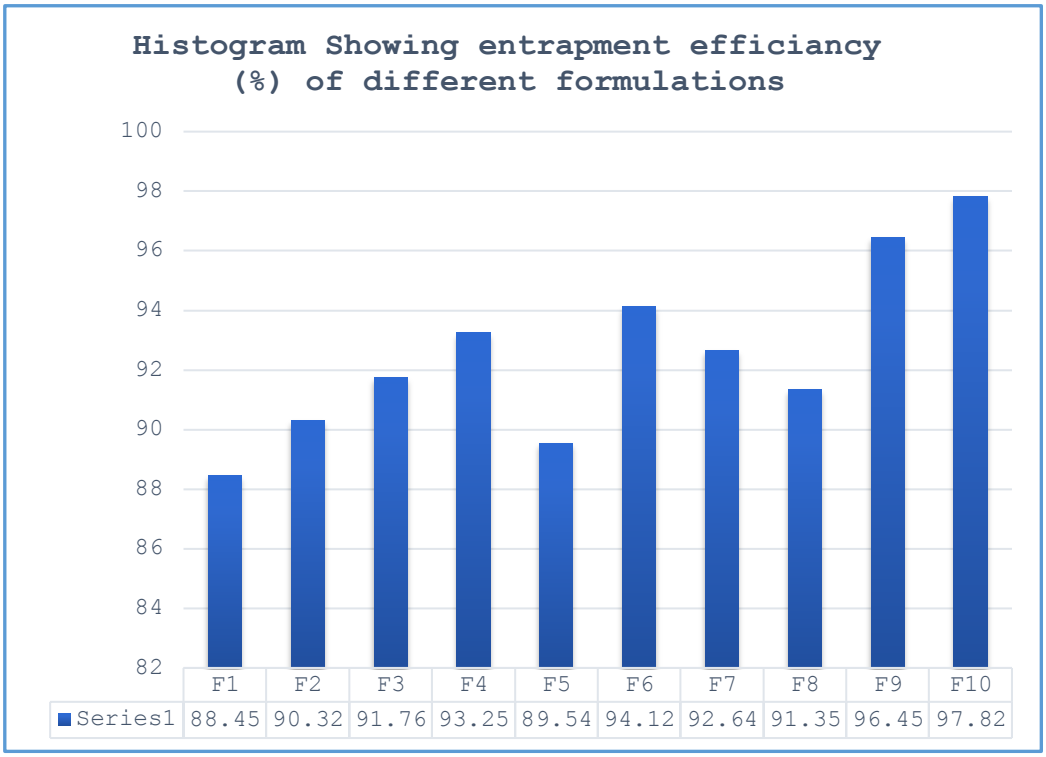

Fig 5. Histogram showing the entrapment efficiency (%) of different formulations

Swelling studies

For evaluating the fluid uptake behaviour of the hydrogel system, the prepared Enalapril microparticles were subjected to swelling studies in phosphate buffer pH 6.8. The microparticles' swelling ability increased gradually with time due to absorption of dissolution medium into the polymeric network. The swelling index was varied for different formulations depending on the concentration and composition of polymers used. Out of all the formulations, ENM_{10} showed the highest swelling index of 100 after 10 hours, indicating better hydration and controlled swelling behaviour. The corresponding histogram shows the swelling profiles for all the formulations.

***In vitro* drug release study**

The *in vitro* drug release studies were carried out in pH progressive media to determine the release behaviour of Enalapril from the formulated microparticles under gastrointestinal conditions. The study was carried out using HCl buffer pH 1.2 for the first 2 hours and then phosphate buffer pH 6.8 for the remaining time. The rate and extent of drug release depended on the concentration and ratio of polymers used in the formulations. An increase in the concentration of sodium alginate resulted in the rapid release of the drug. But an increase in concentration of ethyl cellulose retarded the release due to its hydrophobicity. Among all formulations, ENM_{10} showed a sustained and controlled drug release profile up to 12 h with 99.6% cumulative drug release. In contrast, formulations with lower polymer concentration showed a relatively higher drug release. The results demonstrate that an optimum combination of polymers could control the release of Enalapril from the hydrogel microparticles effectively. The *in vitro* release characteristics of all formulations are represented in figure 6.

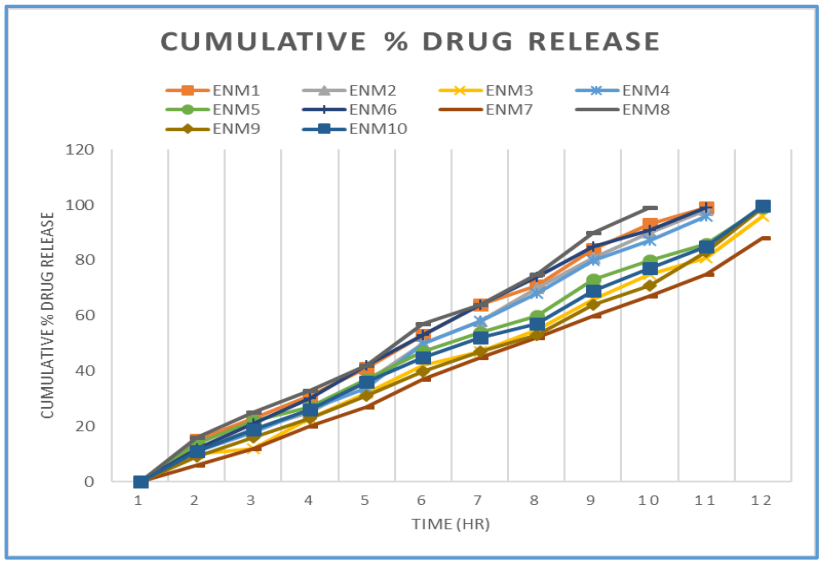

Fig 6: Showing *in vitro* drug release study of Enalapril loaded microparticles

Release kinetic and mechanism of drug release

The *in vitro* dissolution data of the optimal Enalapril microparticle formulation were analyzed using various kinetic models, including zero order, first order, Higuchi, and Korsmeyer-Peppas, and the corresponding graphs were generated. The zero-order plots exhibited linearity, as seen by their superior regression values, indicating optimal formulations. The release exponent 'n' for optimized formulations ranged from 0.5 to 1 ($0.5 < n < 1$), suggesting a connection of the diffusion and erosion mechanisms, referred to as anomalous diffusion. In the current investigation, the in vitro drug release kinetics of the optimal formulations adhered to zero-order release kinetic models, with the drug release mechanism characterized by anomalous diffusion combined with erosion. Figure 7 illustrates the release kinetic graphs.

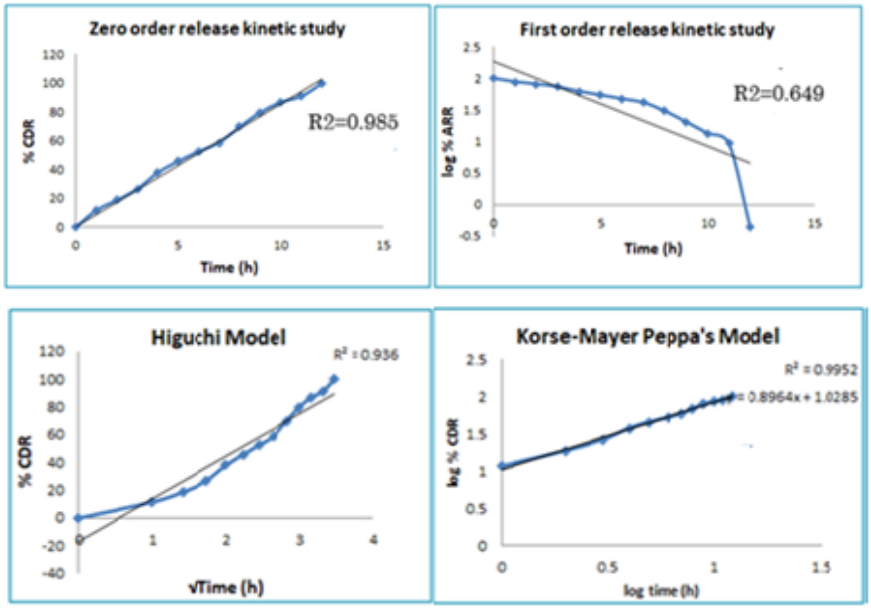

Fig 7: I*n vitro* release kinetic plot of best formulation

Stability studies

The improved Enalapril microparticles underwent accelerated stability testing at 40°C ± 2°C / 75% ± 5% RH for three months, in accordance with ICH recommendations. Post-storage, only negligible fluctuations in drug content were detected, with no substantial alterations in physical appearance noticed. The results demonstrated that the formulated microparticles exhibited physical and chemical stability under accelerated storage conditions [26, 27].

CONCLUSION:

Enalapril-loaded hydrogel microparticles were prepared by the ionotropic gelation technique with polymers such as sodium alginate, ethyl cellulose, and other polymers. To obtain a sustained drug release profile, different formulations were

developed by varying the concentration and ratio of polymers. The prepared microparticles showed high drug entrapment efficiency in the range of 88.45% to 97.82%, which indicates the effective incorporation of drug in the polymeric matrix. FTIR studies have indicated that there was no significant interaction between Enalapril and polymers used in the formulation. Swelling studies showed that the microparticles absorbed fluid slowly, and this affected the drug release profile. The swelling index was found to differ between formulations in accordance with polymer concentration and composition. In vitro drug release studies were performed in 0.2 N HCl, followed by phosphate buffer pH 6.8. The formulations with a higher concentration of ethyl cellulose showed more sustained drug release due to its hydrophobic nature. Among all formulations, ENM_{10} presented the best controlled release profile with almost total drug release in 12 hours. Release kinetics studies showed that the optimized formulation followed zero-order kinetics with an anomalous diffusion mechanism involving diffusion and erosion. The stability studies revealed that the optimized formulation was stable for three months under accelerated storage conditions with no significant change in drug content and physical appearance. In conclusion, the prepared Enalapril-loaded hydrogel microparticles could be a potential sustained-release drug delivery system for the management of hypertension.

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