English Title: Floating Microspheres of Enalapril Maleate as a Developed Controlled Release Dosage Form: Investigation the Effect of Ionotropic Gelation Technique

Turkish Title: Geliştirilmiş Kontrollü Salım Dozaj Formu Olarak Enalapril Maleatin Yüzen Mikroküreleri: İyonotropik Jelleşme Tekniğinin Etkisinin Araştırılması

English Short Title: Floating Microspheres of Enalapril Maleate

Turkish Short title: Enalapril Maleatin Yüzen Mikroküreleri

Abstract

Objective: The purpose of this study is to provide a control drug delivery system through a newly approved work to enhance absorption and bioavailability of enalapril maleate loaded floating microspheres by ionotropic gelation technique using a hydrophilic carrier.

Methods: Over this study, eleven developed formulations of floating microspheres were prepared by ionotropic gelation method using different concentrations of sodium alginate, iota carrageenan**,** sodium bicarbonate**,** calcium chloride, and the drug. Characterization of these microspheres was done using a diversity of parameters like micrometric properties, percentage yield, entrapment efficiency, In-Vitro buoyancy, In-Vitro drug release, and kinetics of drug release. The optimum formula was evaluated and identified for drug-excipients compatibility using Fourier transform infrared spectroscopy (FT-IR), surface morphology, powder X-ray diffraction (PXRD), and differential scanning calorimetry (DSC).

Results: From the results; F4 was selected as an optimum formula since it provides a faster and premium release of drug from the matrix (91.4%). Kinetics of drug release was founded to depend on both diffusion and erosion mechanisms, as the correlation coefficient (R^2) was best fitted with Korsemeyer model and release exponent (n) shown to be between 0.43-0.84. SEM images demonstrate spherical, discrete and freely flowing microspheres with a particle size of 199.4 ± 0.04. Optimum buoyancy properties, percentage yield, and drug entrapment efficiency were achieved. FTIR decided no interaction between enalapril and the polymers. DSC and XRD ascribed the miscibility of the drug with the polymers while maintaining stable crystalline properties of enalapril loaded in the prepared microspheres.

Conclusion: It can be concluded that the developed floating microspheres of enalapril maleate can be considered as a promising controlled drug delivery system; thereby improve patient compliance.

Keywords: Enalapril Maleate, Floating Microspheres, Gastroretentive System, Iota Carrageenan, Sodium Alginate.

ÖZ

Amaç: Bu çalışmanın amacı, bir hidrofilik taşıyıcı kullanılarak iyonotropik jelleştirme tekniği ile enalapril maleatin yüklü yüzen mikro kürelerin emilimini ve biyoyararlanımını arttırmak için yeni onaylanmış bir çalışma ile bir kontrol ilacı verme sistemi sağlamaktır.

Gereç ve Yöntemler: Bu çalışma kapsamında, yüzen mikroküreleri onbir gelişmiş formülasyonu, sodyum aljinat, iotacarrageenan, sodyum bikarbonat, balmumu klorür ve ilacın farklı konsantrasyonlarıkullanılarak iyonotropikjelasyon yöntemi ile hazırlanmıştır. Bu mikroküreleri karakterizasyonu, mikrometrik özellikler, yüzdelik verim, yakalama randımanı, In-Vitro yüzme tekniği, In-Vitro ilaç salımı ve ilaç salımınım kinetiği gibi çeşitli parametreler kullanılarak yapıldı. Optimum formül, Fourier dönüşümü kızılötesi spektroskopisi (FT-IR), yüzey morfolojisi, toz X-ışını difraksiyonu (PXRD) ve diferansiyel taramalı kalorimetri (DSC) kullanılarak ilaç-yardımcı madde uyumluluğu için değerlendirildi ve tanımlandı.

Bulgular: Sonuçlardan; F4 matristen ilacın daha hızlı ve prim salınımını sağladığı için optimum formül olarak seçildi (% 91.4). İyonlaşma kinetiği (R^2) en iyi Korsemeyer modeli ve 0.43-0.84 arasında gösterilen salınım üssü (n) ile donatıldığı için, ilaç salımının kinetiği hem difüzyon hem de erozyon mekanizmalarına dayanmak üzere kurulmuştur.SEM görüntüleri, parçacık boyutu 199.4 ± 0.04 olan küresel, keskin ve serbestçe dönen mikroküreleri gösterir. Optimun yüzdürme özellikleri, yüzdelik verim ve ilaç yakalama etkinliği elde edilmiştir. FTIR enalapril ve polimerler arasında hiçbir etkileşim olmadı. DSC ve XRD, hazırlanan mikro kürelerde yüklü enalapril'instabil kristalin özelliklerini korurken, polimerler ile ilacın karışabilirliğini atfetmiştir.

Sonuç: Enalapril maleatin gelişmiş yüzer mikro küreciklerinin ümit vadeden kontrollü bir ilaç verme sistemi olarak kabul edilebileceği sonucuna varılabilir, böylelikle hasta uyumu geliştirir.

Anahtar Kelimeler: Enalapril Maleatin, Yüzen Mikrosfer, Gastroretentif Sistem, Iota Carrageenan, Sodyum Aljinat.

INTRODUCTION

Conventional drug delivery systems have insufficient control over drug release and concentration at the target site because of amendments in the concentration of bioactive product.[1] As well as, drug absorption in traditional dosing is depended on the body's capability to assimilate the therapeutic molecule.[2] Thus, development of modern administration techniques to maintain steady-state plasma concentration can be achieved through controlled drug release that supports unceasing drug delivery for a programmed period with foreseeable, reproducible kinetics and a drug release mechanism.[3] Once

reached; the effectiveness of drug and patient compliance is enhanced by reducing the frequency of administration.[4]

Microspheres have played a key role in the progress of a controlled release system; as they can encapsulate miscellaneous types of drugs and small molecules, nucleic acids and proteins.[5] They are biocompatible, can deliver superior bioavailability and able to release for longer periods of time.[6] In addition, microspheres have been technologically advanced by numerous techniques comprising of combinations of phase separations or precipitations, emulsion or solvent evaporation and spraying methods.[7]

Floating microspheres are one of the most promising buoyant gastroretentive drug delivery systems. These are free-flowing spherical empty particles without a core, with size varying from 1 to 1000 μm.[8] The gastrointestinal transit-controlled preparations are intended to float on gastric juice with a specific density of less than one, of this property a delayed transit through the stomach occurred.[9] The slowly released drug at a preferred rate, resulting in enhanced gastric retention with abridged fluctuations in plasma drug concentration.[10]

Ionotropic gelation technique is based mainly in our study, however, natural hydrophilic polymers (polyelectrolytes) are used to prepare drug carriers due to their ability to cross-link in the existence of counter ions to form microspheres.[11] These polymers include sodium alginate, gellan gum, hydroxypropyl methylcellulose are used extensively for the encapsulation of drug and act as release rate retardant.[12] For instance, this technique has the virtue of not using organic solvents.[13]

Sodium alginate is, in fact, a water-soluble polymer that becomes a gel in the incidence of polyvalent cations such as calcium chloride, though, is built on the transition of the polymer from a liquid state to a gel.[14] These gels are constituted by dropping a drug-loaded polymeric solution into the aqueous solution of multivalent cations. The cations diffuse into the drug-loaded polymeric drops, creating a three-dimensional lattice of the ionically cross-linked moiety.[15]

Enalapril maleate is a pro-drug employed in the treatment of hypertension. After the oral administration, it becomes hydrolyzed in the liver to release the enalaprilat which acts as an ACE inhibitor. The extent of absorption of enalapril maleate after oral administration is approximately 60% and due to the high hepatic first-pass metabolism of the prodrug (enalapril maleate) to the active form of the drug (enalaprilat) in the gastrointestinal tract before absorption; the bioavailability of enalaprilat becomes approximately 40%.[16]

The objective of this study is to overcome such inherent disadvantages. An attempt is needing to be done to provide a control drug delivery system through this newly approved work for enhancing absorption and bioavailability of Enalapril maleate loaded floating microspheres by ionotropic gelation technique using a hydrophilic carrier.

MATERIALS AND METHODS

Materials

Enalapril maleate (Baoji Guokang Bio-Technology, China), Sodium Alginate (Avonchem, UK), Iota Carrageenan (Provizer pharma, India), Calcium Chloride (Gainland chemical company, UK). All other materials used were of pharmaceutical grade.

Methods

Preparation of Floating Microspheres

Floating microspheres were prepared by ionotropic gelation method using sodium alginate at different concentrations as a primary polymer. To this dispersion, iota carrageenan added in different concentrations and stirring is continued. The required amount of sodium bicarbonate was added to the above solution in a suitable proportion and mixing continued. To this successive solution, the drug was added after cooling. The drug and polymer solution was added dropwise through a syringe with a 31-gauge needle into 100 ml calcium chloride solution and stirred at 200 rpm. The formed microspheres were kept suspended in the solution for 1 h to improve their mechanical strength and then collected with filtration. After that, the floated microspheres were washed with 100 ml of distilled water for 3 times and then dried in the hot air oven for 2 h at 50 ˚C to be stored in a desiccator.[17] The composition of floating microspheres is given in (Table 1).

Characterization of Floating Microspheres

Micrometric Properties

Floating microspheres were characterized by a numerous test to detect their properties which obeys USP standers.

Particle Size Analysis

Floating microspheres were separated into different size fractions by sieving for 10 min through series of standard sieves # 40, # 60, # 80 and # 100 and the particle size of floating microspheres was calculated using an optical microscope (Novel, China) and the mean particle size is calculated.[18]

Bulk Density

Floating microspheres of a weighed quantity of were poured in a graduated cylinder (10 ml). Bulk density was established by a ratio of the mass of floating microspheres to bulk volume.[19]

Bulk density = Mass/Bulk Volume

Tapped Density

Floating microspheres of a weighed quantity were introduced in a graduated cylinder (10 ml) and the cylinder was tapped from a height of 2 cm for 100 standard tapping until there was no more diminution in the density and the volume of the microspheres was calculated.[20]

Tapped Density = Mass/Tapped Volume

Carr's (compressibility) index

Compressibility index of microparticles has been anticipated as a subsidiary measure of bulk density, size, and shape, surface area, moisture content and cohesiveness of materials.[21]

%Compressibility index= Tapped Density – Bulk Density * 100/ Tapped Density

Hausner Ratio

Floating microspheres Hausner's ratio was confirmed by associating the tapped density toward the bulk density as the resulting equation [22]:

Hausner's ratio= Tapped Density/ Bulk Density

Angle of Repose

The flow of floating microspheres was measured by assessing the angle of repose using the funnel method. Prepared microspheres were poured via a funnel fixed at 1 cm above a flat surface till the apex of microspheres pile touched the tip of the funnel.[23] The Angle of repose is calculated by using the following equation:

$\theta = \tan^{-1}(H/R)$

Where, θ = Angle of repose, h = height of pile, r = radius of the pile.

Determination of Percentage Yield

The calculation of floating microsphere percentage yield was premeditated using the weight of a dried final product regarding the total weight of the drug and polymer measured initially and used for the preparation of microsphere.[24] The percentage yields were calculated as per the formula mentioned below.

Percentage yield = [weight of microspheres obtained / (Weight of drug + polymer)] * 100

Determination of Entrapment Efficiency

Floating microspheres can be assessed for the drug content and it can be approved by dissolving weight amounts of crushed microspheres in 100 ml 0.1 N HCl. Aliquot of 1 ml was taken beside diluted to 10 ml, then the mixture was filtered and analyzed using UV spectrophotometer (Shimadzu 8400S, Japan) at a 219 nm using the calibration curve.[25] Each batch should be examined for drug content in a triplet manner.

% Entrapment efficiency = (Actual drug content) / (Theoretical drug content) *100

Determination of In-Vitro Buoyancy

In vitro buoyancy was carried out to study the floatation behavior of microspheres in the prepared formulations. 50 mg of microspheres were spread in simulated gastric fluid (pH 1.2; 100 mL). The mixture was stirred at 100 rpm in a magnetic stirrer. Afterward 8 h, the buoyant microparticles layer was collected by the pipette method and separated by filtration. The particulate sinking layer particles were separated by filtration. Particles of both types were dried in a desiccator until a constant weight was obtained.[26] The buoyancy percentage was calculated by the following equation:

% Buoyancy = (Weight of floating microspheres) / (Total weight of floating and settled microspheres) * 100

All the determinations were made in triplicate.

In-Vitro Drug Release Study

In vitro-drug release rate from floating microspheres was affirmed using paddle type six-station dissolution test apparatus (Copley-UK). An accurate amount of floating microspheres equivalent to 5, 10 and 20 mg drug was reserved in (0.1 N HCl, 1.2 pH) and the dissolution fluid was maintained at 37± 0.5 ºC at a speed of rotation (50 rpm). Sink condition prevailed during the in-vitro drug release study. 1 ml sample was withdrawn and filtered through a 5µ membrane filter at 5, 10, 15, 20, 30, 60, 120, 180, 240, 300, 360, 480, 600, 720, 1440 min time interval. The initial volume of the dissolution fluid was maintained by adding 4 ml of fresh dissolution fluid after each withdrawal. The samples were analyzed by UV spectrophotometer at 219 nm to determine the concentration of enalapril maleate present in the medium.[27] All experiments were performed in triplicate.

Kinetics of Drug Release

To recognize the mechanism and kinetics of drug release, the result of the in vitro dissolution study of enalapril microspheres were carried out for various kinetic equations.

The kinetics models used were zero order, first order, Higuchi's and Korsmeyer-Peppas models. Correlation coefficient (*R*2) values were calculated for the linear curves obtained by regression analysis.[16]

Drug-Excipients Compatibility Study and Identification

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of pure drug, polymers (sodium alginate and iota carrageenan) and the drug with the polymer (F4 as microspheres) were recorded on a spectrophotometer (Shimadzu 8400S, Japan) using the KBr pellet technique and reported in wave number (cm^{-1}). The scanning range was taken from (4000-450) cm^{-1}. The spectra FT-IR supports in the identification of an existence of the functional groups in the compound. The spectra FT-IR supports in comparing with a standard FT-IR spectrum of the pure drug to detect any physicochemical incompatibility between the drug and different excipients.[28]

Surface Morphology

Scanning electron microscopy (SEM) was used to characterize the surface and cross-sectional morphology of the designed floating microspheres. SEM samples were mounted directly by scattering the powder lightly on a dual adhesive tape fixed to an aluminum stub. Then a gold-palladium coating of stubs to about 20 nm of thickness and underneath an argon atmosphere by a gold sputter module in an evaporator with a high-vacuum. The samples that are coated were then scanned haphazardly and photographs were taken with SEM (TESCAN, VEGA 3 – Czech Republic).[29]

Powder X-ray Diffraction (PXRD)

The PXRD pattern of enalapril alone, sodium alginate, iota carrageenan, and F4 were recorded using XRD (Shimadzu 6000, Japan) with a CuKα line as the source of radiation. Standard runs were executed with a current of 30 mA, a voltage of 40 KV and a scanning rate of 8 deg/min over a θ range of 5-80° using a step size 0.02° second per step. It was therefore used to determine the nature of the pure drug whether it was crystalline or amorphous in nature and to conclude the nature of drug if it was changed or not by using a combination of polymers.[30]

Differential Scanning Calorimetry (DSC)

Thermal analysis was achieved by using Differential Scanning Calorimeter (DSC) instrument (STA PT-1000, LINSEIS – Germany) equipped with argon as an inert gas; to study the drug and microspheres crystalline changeability. Accurately weight of enalapril

alone, sodium alginate, iota carrageenan, and F4 were taken. Weighed samples were put into aluminum pans and hermetically sealed. The samples were heated from 20 °C to 200 °C at a rate of 5 °C per minute under an argon atmosphere with a gas flow rate of 100 ml /min. A covered, empty pan was used as a reference. The results obtained from the heating were recorded.

RESULTS AND DISCUSSION

Characterization of Floating Microspheres

Micrometric Properties

Floating microsphere formulations were evaluated to detect their micrometric properties as following:

Particle Size Analysis

The mean particle size of floating microsphere formulations (F1 – F11) was found in the range of 196.55±0.28 to 520.2±0.09 as showed in (Table 2). Formulations representing an increase of sodium alginate concentration (F1-F3); showed the increase of particle size respectively. This could be ascribed to an increase in relative viscosity at higher concentration of sodium alginate which requires high energy for breaking of droplets and more difficult to disperse due to enhancement of interfacial tension and diminished shearing efficiency that lead to formation of large droplets of floating microspheres during addition of polymer solution to the gelling agent.[32]

In addition, the particle diameter in formulations (F4 and F5) was found to be increased gradually as the concertation of iota-carrageenan increased. This may be attributed for the increasing of gel strength and the formation of strong bridges between anionic iota-carrageenan molecules and cationic $CaCl_2$ salts that led to the helix-helix aggregation of the adjacent spiral chains that contain sulphate groups and formation of a stable three-dimensional network.[33]

Furthermore, formulations (F2 and F6) showed that the particle size of floating microspheres increased as the sodium bicarbonate content increased. On the other hand, a further increase of sodium bicarbonate concentration causes a decrease in the particle size of microspheres and as shown in F7. The suggested mechanism demonstrates that as the concentration of sodium bicarbonate increase; the microspheres expanded and increase in their sizes. An additional increase in sodium bicarbonate concentration will make them burst and decrease in size.[34]

The effect of drug concentration on microsphere size was studied by (F8 and F9); observed an increase in particle size of microsphere as the drug concentration decreased. This factor was related to the solubility of microspheres that reduced with decreasing drug concentration and thus will make these microspheres more rigid and large in comparison to particle size.[35]

The effect of the crosslinking agent ($CaCl_2$) on particle size was detected in (F10 and F11) as the concentration of calcium chloride increased; the mean particle size of microspheres was increased. This is related to the to the availability of a high amount of Ca^{2+} that crosslinked with sodium alginate and iota carrageenan polymers; thereby leading to the formation of larger microspheres.[36]

Rheological Parameters of Floating Microspheres

Rheological studies of Enalapril maleate floating microspheres involved bulk density, tapped density, compressibility index or Carr's index, Hausner's ratio and angle of repose. The formulations were studied for all rheological properties as shown in (Table 2).

Bulk and Tapped Density

The bulk density and tapped density of formulations (F1, F2, and F3) may be triggered by the small difference in flow properties as showed in (Table 2). This is due to the use of low polymer dispersion concentrations in (F1), so the microspheres were not having as good spherical shape as (F2) and had a flattened base at the points of contact with the drying vessels, however, increase in the concentration of sodium alginate dispersion in (F2) tend to make the particles more spherical. This indicates that at low alginate concentrations the particles were composed of loose network structure which collapsed during drying, another hand higher sodium alginate concentration formed dense matrix structure which prevented the collapse of microspheres. As the concentration of sodium alginate in the aqueous dispersion increases in (F3) the relative viscosity of dispersion was increased and it was difficult to transfer polymer dispersion through the needle into the cross-linking agent solution and an increase in concentration moreover found a small tail one end of microspheres which significantly affects the flow properties and particle size distribution.[37]

Floating microspheres prepared with the increased concentration of iota carrageenan (F4 and F5) showed a decrease in bulk and tap density. The reason behind this was the swelling property of the material that absorbs fluid from the surrounding environment in a controlled

manner, making it float above the gastric contents and remain unaffected by the gastric emptying time.[38]

Upon increment of sodium bicarbonate concentration in (F4, F6, and F7), the microspheres were becoming more floated as the density dropped below the 1.0 g/cm^3. This indicated that when the spheres became in contact with the medium (0.1 N HCl, pH 1.2), they start to react and generate a CO_2 gas with upward force and being entrapped within the matrix of sodium alginate and iota carrageenan. This entrapment of CO_2 leads to a decrease in microspheres density thereby become buoyant.[39]

Formulations of floating microspheres representing a decrease in enalapril maleate content (F8 and F9) showed a high level of bulk and tapped density. This is related to the high porosity in the spheres matrix that increases medium flowability directly into these floating microspheres to be denser.[40]

It was evident that bulk and tapped density increased in (F4, F10, and F11) as the concentration of $CaCl_2$ increased. This is due to the higher concentration of crosslinking agent that will lead to the increment of the viscosity thereby increasing the density of microspheres.[41]

Compressibility index or Carr's index

The Carr's index represents an indicator for the tendency to form bridges between microspheres. Thus, the values for all formulations were found to be in the range between 2.36 ± 0.1 % and 12.79 ± 0.29% as showed in (Table 2) which displayed an excellent flow of microspheres and excellent compressibility.[42]

Hausner Ratio

Hausner's ratio was measured to indicate the cohesion between microspheres particles. The values of all formulations were founded below 1.25 as showed in (Table 2) and thus indicating good flow properties with easy handling during processing.[43]

Angle of Repose

Values of the angle of repose of all formulations are below 21° as showed in (Table 2) indicating free-flow properties of microspheres. The better flow property of microspheres indicates that the floating microspheres produced were non-aggregated. Similar findings are reported by the novel floating microspheres of metronidazole.[44]

Percentage Yield

The percentage yield of floating microsphere was performed to determine the polymer effect (sodium alginate) on the formulations. The results showed that the percentage yield of formulas F1 to F3 range from 54.5 ± 0.925 to 72.88 ± 0.672 respectively as shown in (Table 3). It is obvious that the increment in the polymer concentration has led to in an accretion in the percentage yield. This effect can be explained by the fact that as the concentration of alginate increase the quantity of polymer becomes adequate to cover enalapril maleate particles completely. In addition to this, the microspheres become well distributed, discrete, spherical and have no clumping thus give a good percentage of yield.[45]

The increment in iota-carrageenan concentration will instantaneously lead to the interfacial cross-linking to take place, followed by a more gradual gelation of the interior, that results in an increase in the percentage yield and as shown in F4. From the results, it was noticed that the viscosity increased dramatically with the further increase in iota-carrageenan concentration that may retard the penetration of the enalapril into the matrix and hence decrease percentage yield as shown in F5.[46]

Formulations prepared with the increased concentration of sodium bicarbonate as a gas forming agent (F6 and F7) was observed to have a decrease in the percentage of yield. The microspheres with a small amount of sodium bicarbonate will have a high dense internal structure of the matrix and they will be able to retain enalapril more effectively as shown in F4. Whereas the porous microspheres with an increment amount of sodium bicarbonate, having a less dense internal structure, resulting in a decrease in the percentage of yield of the drug.[47]

The probable reason for high enalapril percentage of yield in (F4) might be due to a higher water solubility of a freely soluble drug which is always entrapped in a higher ratio, which makes it difficult to diffuse out of the microspheres from the gel surface during the hardening of the carrageenan gel matrix. While this percentage of yield decreased when the drug content reduces and as seen in (F8 and F9).[48]

The effect of increasing $CaCl_2$ concentration in (F10) appear on the degree of cross-linking that will be increased, and so the percentage yield decreased due to the difficulty of drug penetration into the microspheres. Moreover, the percentage of yield in (F11) increased with decreasing $CaCl_2$ concentration. The reason for high percentage yield may be high solubility of the drug that will be more entrapped within the matrix of microspheres.[49]

Entrapment Efficiency

Drug entrapment was referred to the permeation characteristics of polymers used, that could simplify the diffusion of a part of the drug that was entrapped in the besetment medium while preparation of floating microspheres. Drug entrapment efficiency increased with the increment in polymer concentration (F1-F3) and as showed in (Table 3). This is due to the increase in polymer content, so more particles of enalapril would be coated leading to higher encapsulation efficiency.[11]

Entrapment efficiency of iota carrageenan in (F4 and F5) was founded to be decreased with an increment of polymer concentration in the gastric medium. This is because of the increment in the viscosity with the further increase in iota-carrageenan concentration that may retard the penetration of the enalapril into the microspheres and hence decrease entrapment efficiency.[50]

Table 3 showed that as the amount of sodium bicarbonate (as gas forming agent) increased (F4, F6, and F7), a decreased in the entrapment efficiency was observed. Microspheres with low gas forming agents showed high entrapment efficiency as compared to those with high gas-forming agents. This result was attributed with the fact that microspheres with a low gas forming agent have an excessively compact internal structure that was able to keep the drug within its matrix, as compared to the less dense internal structure of the other microspheres which consisted of a high amount of gas forming agents that cause the decrease in drug entrapment. As a result, the formation of more pores on the microspheres networks with an increased amount of sodium bicarbonate will make them having lower drug entrapment efficiency.[51]

Microspheres for formulations of (F8, and F9) were designed with different drug concentrations. The decrease in entrapment efficiency was achieved by decreasing drug concentration. This results from a lower concentration gradient in which the drug may diffuse out of the microspheres matrix to the external medium during preparation, that tends to decrease the encapsulation efficiency.[52]

The effect of $CaCl_2$ on encapsulation efficiency was observed in formulations (F10 and F11). The percentage encapsulation efficiency decreases with the increase in the concentration of calcium chloride as showed in F10. For microspheres cross-linked with the low level of $CaCl_2$ (F11) demonstrated a higher drug encapsulation efficiency in comparison to high levels of calcium chloride. This may be as a result of the immediate gelling of

polymer (sodium alginate) on the addition of $CaCl_2$ and thrust out of the aqueous phase from the gel lattice.[53]

In-Vitro Buoyancy

The percentage buoyancy was calculated for all the formulations and it was found that all formulations were eligible to float on the dissolution medium (0.1 N HCl, pH 1.2) for a period of 24 h. The percentage buoyancy of the microspheres was found to decrease with an increase in n sodium alginate concentration represented by (F1-F3) and as shown in (Table 3). This is because of the elevated viscosity of the polymer solution which in turn is the reason for more dense microspheres and less formation of pores in addition to cavities during preparation.[54]

Moreover, increasing iota carrageenan concentration in (F2 and F4) resulted in an increment of microspheres buoyancy. This was due to the immediate crosslinking of microspheres matrix as an outcome of strongly acidic sulfate groups in iota carrageenan molecule that allows a certain degree of polymer ionization in (0.1 N HCl, pH 1.2) leading to the formation of insoluble gel-like layer of aggregated double-helical segments that form a three-dimensional network by complexation, and consequently slower solvent penetration into the matrices and more controlled CO_2 diffusion was achieved and thus inducing the microspheres to float rapidly.[55] While a further increment of iota carrageenan concentration as shown in F5 will lead to a decrease of buoyancy effect. This was related to the increment of iota-carrageenan viscosity as the concentration raised. Thus, more entrapment of CO_2 gas and less gastric medium penetration into the matrices that will lead to a decrease in buoyancy of enalapril microspheres.

The effect of increasing $NaHCO_3$ concentration on the buoyancy was shown to be non-significant and represented in (F6 and F7). The reason behind this was due to the properties of sodium alginate that form strong crosslinking in the polymer matrix and addition of $NaHCO_3$ decrease in the elasticity of matrix without affecting viscosity, so the buoyancy was not affected.[56]

The effect of drug loading of microspheres was shown in (F8 and F9). From results, as the quantity of drug increased, more drug molecules are available at the surface of microspheres. Also, more solid drug particles will begin to form continuous pores or channels within the matrix. Under these circumstances, the path of least resistance for drug molecules will be diffusion within the channels formed from areas where the drug has

previously leached out from the matrix. Therefore, as the amount of drug content is increased and drug leaches out from the polymer, the matrix becomes more porous and a higher buoyancy occurs. Lower drug contents create fewer pores within the polymeric network; hence a lower rate of drug diffusion was observed, and lower buoyancy was achieved.[57]

The buoyancy was found to decrease with increasing $CaCl_2$ as showed in (F10 and F11). $CaCl_2$ might be responsible to produce a more viscous matrix, which may block the pores on the surface of microspheres. Thus, the higher concentration of $CaCl_2$ can produce a high degree of cross‐linking and thereby decreasing buoyancy from enalapril microspheres.[58]

In-Vitro Drug Release

The effect of different concentrations of sodium alginate on drug release in (F1-F3) represents a significant effect ($p< 0.05$) as showed in Figure 1. The release of enalapril from the prepared alginate microspheres was distinguished by an initial phase of high release (burst effect) followed by the second phase of moderate release. This biphasic manner of release is a distinctive feature of the matrix diffusion kinetics.[59] A significant decrease in the drug release was noticed with the increment in the drug-polymer ratio in the prepared microspheres and is referred to an increment in the density of the polymer matrix and in the diffusional path length that the drug molecules must traverse.[60]

The release profile in Figure 2 represents a significant effect ($p<0.05$) between formulations (F2, F4, and F5). These formulations determine the influence of different concentrations of iota carrageenan on the drug released. Two distinctive release steps could be detected, where the initial rapid release was due to the diffusion while the second step is due to the erosion of the matrix. A faster release in F4 in comparison to F2, because of the presence of high-water content molecules in the matrix. The existence of a higher concentration of iota carrageenan leads to the higher release of the drug. It was suggested that the pore size was higher than F2 results in a higher release.[61] Further increase in iota carrageenan concentration as shown in F5 showed a decrease in drug release. This was ascribed to the gelling property of the polymer that could sustain the drug release from its matrix as well as their ability to wet. The gel matrix will swell and withstand erosion under acidic conditions to maintain a constant diffusion path length; forming a highly crosslinked matrix with minimum porosity.[55]

Moreover, it was noticed from in vitro release study that the drug release rate increased non-significantly ($p>0.05$) with an increase in the proportion of $NaHCO_3$ (F4, F6, and F7) as showed in Figure 3. This was ascribed to the low concentration of $NaHCO_3$, the alginate produces a highly dense internal structure which keeps the drug more promptly, so a minimal amount of drug released but in presence of higher concentrations of $NaHCO_3$ the formulations become more porous and the drug is released at faster manner.

The effect of enalapril concentration on drug release profile in (F4, F8, and F9) represents the non-significant effect ($p>0.05$) as showed in Figure 4. It was noted that at a higher drug concentration in F4, less than 92% of the drug was released in 24 h as compared to 100% drug release from microspheres with lower drug concentrations in F8 and F9. In contrast to the low enalapril-loaded microspheres which exhibited smooth surfaces, in the highly loaded enalapril microspheres showed rippled and rough surfaces. No enalapril crystals were observed embedded or attached firmly to the surface of the low enalapril loaded microspheres. Thus, this surface structure should be attributed to possible molecular interactions between the coating polymer and the enalapril rather than to an excess of incorporated drug which might result in recrystallization of enalapril within the microspheres as was the case with the high enalapril loading microspheres.[62]

The results of in vitro drug release study indicated that $CaCl_2$ concentration effect on drug release (F4, F10 and F11) significantly ($p<0.05$) as showed in Figure 5. It was shown that the drug release decreased with the increase in the concentration of calcium chloride. This is attributing to the formation of a tight junction between the MM/GG residues of sodium alginate with calcium ion which in turn decreases the swelling capacity of the microspheres. Therefore, enalapril cannot be readily released from the microspheres, as the surface roughness and porosity increased as well as the steric entanglements comprise a strong barrier, thus a poor entry of dissolution medium into the polymer matrix may be delayed drug release.[63]

Kinetic Assessment of Dissolution Data

The release pattern of enalapril in gastric fluid (0.1 N HCl, pH 1.2) from all formulations of floating microspheres (F1 to F11) followed Higuchi matrix model as showed in Table 4. The effervescent floating systems obeyed the Higuchi model indicating drug release via a diffusion mechanism. In addition, formulations (F1, F6, and F7) were observed to have (n value of 0.43 or less, the release mechanism follows “Fickian diffusion”.[27] While,

formulations (F5, F8, F9, and F11) were noticed to have a high n value of >0.84, thus the mechanism of drug release is regarded as super case II transport. The value of release rate exponent (*n*) of Korsmeyer–Peppas release model for F2, F3, F4, and F10 was founded as $0.43 < n < 0.84$ for mass transport which follows a non-fickian model (anomalous transport). Therefore, it can be concluded that F4 was selected as an optimum formula and the drug release was mainly following Anomalous Transport that corresponds to diffusion, erosion and swelling mechanism or mixed-order kinetics.[64]

Drug-excipients Interaction Study and Identification

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was carried out to establish the compatibility of enalapril with polymers after the preparation of microspheres. Individual FT-IR scanning of the pure drug powder (Figure 6A), polymers [Sodium alginate, iota carrageenan] as showed in (Figure 6B and 6C) respectively; in addition to enalapril microspheres (F4) as showed in (Figure 5D).

The FT-IR spectrum for pure enalapril was distinguished by the principal absorption bands at 1753/cm due to C=O stretching (ester), 1649/cm due to N-H bending and at 1444/cm due to C-H (alkanes) bending [65]. While the FT-IR spectrum of sodium alginate was characterized the absorption bands at 2933, 1610, 1421, 1033/cm due to stretching of -CH, -COOH, -CH and -C-O-C, respectively [66]. In addition, the FT-IR spectrum of iota carrageenan was observed with the characteristic bands at 1228/cm for the ester sulfate group, 918/cm for the 3,6-anhydrogalactose, 846/cm for the galactose-4-sulfate, and 771/cm for the 3,6-anhydrogalactose-2-sulfate.[67]

The IR spectra pattern for microspheres [F4] were compared with the IR spectrum of the pure drug for confirmation of major functional groups. All the characterized bands of enalapril with polymers have appeared and indicated no significant variation in the peaks, suggesting that the drug and excipients were compatible. Moreover, it shows that no interaction between enalapril pure powder and the used polymers. Subsequently, it can be decided that the drug is chemically stable in the polymer matrix and can release with ease from microspheres.

Surface Morphology

The surface morphology of microspheres represented by the particle size and a characteristic shape was determined by SEM (Scanning Electron Microscope). The SEM images of microspheres taken at different magnifications were shown in (Figure 7). It was

noticed in SEM images that the microspheres were spherical, discrete and freely flowing. In addition, the surfaces were slightly rough and drug crystals were also present on the surface of microspheres. These drug crystals were responsible for the burst release of drug from the microsphere.[68]

Powder X-ray Diffraction (PXRD)

The X-ray diffraction of pure enalapril, sodium alginate, iota carrageenan, and F4 was demonstrated in (Figure 8). It was noticed that the pure enalapril powder exhibited a highly intense, sharp distinctive and narrow diffraction peaks at 2θ of 10.48, 20.92, 24.89, 31.57, indicating that the drug was a highly stable crystalline. However, when the drug was incorporated into the polymer matrix, the principal peaks of the drug diffractogram were observed with lower intensity. This could be ascribed to the crystalline state of the drug in the microsphere.[69]

Differential Scanning Calorimetry (DSC)

DSC studies were performed to investigate the physical state of the drug in the microspheres because this aspect could influence the in vitro and in vivo release of the drug from the systems. Figure 9 shows the DSC thermogram of pure enalapril powder, sodium alginate, iota carrageenan and F4 as microspheres. Pure powder of enalapril showed a sharp endothermic peak at 147.6 °C corresponding to its melting point. While sodium alginate showed a broad endothermic peak at around 87 °C which was mainly due to loss of water. The endothermic peak of the iota carrageenan was observed at 134 °C; that has narrower peak compared with sodium alginate, which may be attributed to lower relative numbers of hydrophilic OH groups (anhydride bridge). The appearance of a broader peak in F4 with minimum shifting in position, indicate the presence of the crystalline drug in the microsphere samples at the least in the particle surface level. Therefore, it could be concluded that F4 has shown the miscibility of the drug with the polymers while maintaining stable characteristic properties of enalapril loaded in the prepared microspheres.[70, 71, 72]

CONCLUSION

The present study established the prepared enalapril maleate microspheres through ionotropic gelation technique; to provide better therapeutic efficacy, as a result of the continuous availability of the drug. In-vitro release studies show a significant decrease in the drug release with an increment of sodium alginate concentration, calcium chloride concentration and higher concentrations of iota carrageenan. At the same time, the effect

of sodium bicarbonate and drug concentration increment on the drug release were non-significant. FTIR spectra suggested that drug and excipients were compatible. Surface morphology discipline spherical shape with rough surface microspheres. While, X-ray diffraction showed the crystalline state of the drug. The DSC studies displayed miscibility of the drug with the polymers. The overall result offers promising pharmaceutical dosage form of enalapril maleate microspheres.

ACKNOWLEDGMENT

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad-Iraq for its support in the present work.

Conflict of Interest: No conflict of interest was declared by the authors.

References:

1. Anusha K, Krishna SA. Preparation and Evaluation of Mefenamic Acid Loaded Microspheres Using Synthetic and Natural Polymers. Der Pharmacia Lettre. 2016;8:197-205.

2. Kamel R. Multiparticulate Carriers for Controlled Oral Drug Delivery. Pharm Sci Tech. 2016;1:1-3.

3. Swapna N, Jithan A. Preparation, Characterization and in vivo Evaluation of Parenteral Sustained Release Microsphere Formulation of Zopiclone. J Young Pharm. 2010;2:223–228.

4. Varde NK, Pack DW. Microspheres for Controlled Release Drug Delivery. Expert Opin Bio Ther. 2004;4:35–51.

5. Berkland C, King M, Cox A, Kim K, Pack DW. Precise Control of PLG Microsphere Size Provides Enhanced Control of Drug Release Rate. J Control Release 2002;82:137–147.

6. Zhang H, Xu J. Enhanced Oral Bioavailability of Salmeterol by Loaded PLGA Microspheres: Preparation, in vitro, and in vivo evaluation. Drug Deliv. 2014;23:248-253.

7. Freitas S, Merkle HP, Gander B. Microencapsulation by Solvent Extraction/Evaporation: Reviewing the State of the Art of Microsphere Preparation Process Technology. J Control Release. 2005;102:313–332.

8. Mukund JY, Kantilal BR, Sudhakar RN. Floating Microspheres: A review. Brazilian J Pharm Sci. 2012; 48(1): 17–30.

9. Shashank C, Prabha K, Sunil S, Vipin Kumar A. Approaches to Increase the Gastric Residence Time: Floating Drug Delivery Systems- A Review. Asian J Pharm Clin Res. 2013;6:1–9.
10. Chugh C, Nanda A. Gastroretentive Drug Delivery Systems- A Review. Int J Pharm Bio Sci. 2017;8(1):62–68.
11. Prakash S, Bhandari A, Mishra R, Sharma PK. Development and Optimization of Floating Microspheres of Gliclazide. Int J Pharm Sci Res. 2015;6:807-817.
12. Aly Kassem M, El Assal MIA, Al-Badrawy AA. Preparation and Evaluation of Certain Hydrophilic Drug-Loaded Microspheres. Int Res J pharm. 2012;2:82-90.
13. Miladi K, Ibraheem D, Iqbal M, Sfar S, Fessi H, Elaissari A. Particles from Preformed Polymers as Carriers for Drug Delivery. Exp Clin Sci Int J 2014;13:28-57.
14. Patil P, Chavanke D, Wagh M. A Review on Ionotropic Gelation Method: Novel Approach for Controlled Gastroretentive Gelispheres. Int J Pharm Pharm Sci. 2012; 4:27–32.
15. Pahwa R, Neeta, Bhagwan S, Kumar V, Kohli K. Floating Microspheres: An Innovative Approach for Gastric Retention. Der Pharmacia Lettre. 2010;2:461-475.
16. Nanjwade BK, Patel UD, Kadam VT, Idris NF, Srichan T. Formulation and Evaluation of Enalapril Maleate Biodegradable Microspheres. J Pharm Sci Pharmacol. 2014;1:200–210.
17. Gadad AP, Naik SS, Dandagi PM, Bolmal UB. Formulation and evaluation of Gastroretentive floating microspheres of Lafutidine. Indian J Pharm Edu and Res. 2016;50: S76–S81.
18. Sammour OA, El-Ghamry HA, El-Nahas HM, Barakat W. Development and Characterization of Controlled Release Ketoprofen Microspheres. J App Pharm Sci. 2012;2:60–67.
19. Wasnik S, Parmar P, Singh D, Ram A. Preparation and Characterization of Floating Drug Delivery System of Azithromycin. Acta Poloniae Pharmaceutica - Drug Res. 2012; 69(3): 515–522.
20. Bhardwaj P, Chaurasia D, Singh R, Swarup A. Development and Characterization of Novel Site Specific Hollow Floating Microspheres Bearing 5-Fu for Stomach Targeting. Sci World J. (2014):1-11.

21. Jagtap Y, Ranade A, Ranpise N, Bhujbal R. Effect of Various Polymers Concentrations on Physicochemical Properties of Floating Microspheres. Indian J Pharm Sci. 2012;7: 512-520.
22. Kusuma D, Krishnan KS, Sri S, Sree V. Formulation and Evaluation of Floating Microspheres of Acebutolol. Int J Pharm Sci Rev Res, 2017;46:31-36.
23. Dhanalakshmi G, Lakshmikanth M, Veena R. Design and Characterization of Itopride HCl Floating Microspheres. Int J Invent Pharm Sci. 2013;1:70–80.
24. Rane BR, Gujarathi NA, Patel JK. Preparation and In-Vitro Characterization of Floating Microspheres of Nateglinide. Int J Pharm Sci Res, 2012;3(11): 4306-4313.
25. Negi M, Shukla VK, Easwari T.S. Preparation and Evaluation of Ofloxacin Sustained Released Gastro Retentive Floating Microspheres. UK J Pharm Biosci. 2014;2:19-24.
26. Pandya N, Pandya M, Bhaskar VH. Preparation and in Vitro Characterization of Porous Carrier-Based Glipizide Floating Microspheres for Gastric Delivery. J young pharm. 2011;3:97–104.
27. Jani P, Vadalia K, Bagdai H, Dedania R, Manseta P. Formulation and Evaluation of Controlled Release Floating Microspheres of Tolperisone Hydrochloride. Asian J Pharm. 2012;6:190-7.
28. Maraie NK, Alhamdany AT, Mahdi ZH. Application of the New Oroslippery Technology in the Preparation of Enteric Slippery Coated Tablet of Naproxen. Int J Pharm Pharm Sci. 2017;9,198–204.
29. Sharma M, Kohli S, Dinda A. In-vitro and In-vivo Evaluation of Repaglinide Loaded Floating Microspheres Prepared from Different Viscosity Grades of HPMC polymer. Saudi Pharm J. 2015;23:675–682.
30. Aute SM, Payghan SA, Mali SS, Patrekar PV. Development of Floating Microspheres of Anti-ulcer Drug as a Gastroretentive Drug Delivery System. Der Pharmacia Lettre. 2015;7:364-377.
31. Aute SM, Kate VK, Payghan SA. Formulation of Floating Microspheres of Nizatidine: Investigation of Effect of Solvent Evaporation and Spray Drying Technique. Inventi Impact: NDDS. 2015;3:85-100.
32. Chouhan M, Chundawat AVS, Chauhan CS. Development and Characterization of Floating Microspheres of Esomeprazole Magnesium Trihydrate by Solvent Evaporation Method. Int J Pharm Sci Res. 2017;8:686-697.

33. Gasperini L, Mano JF, Reis RL. Natural Polymers for the Microencapsulation of Cells. J Royal Society Interface. 2018;15(145):1-19.

34. Leemsuthep A, Nayan NAM, Zakaria Z, Lan DNU. Effect of Sodium Bicarbonate in Fabrication of Carbon Black-Filled Epoxy Porous for Conductive Application. Macromol Symposia. 2017;371:44–49.

35. Vaidya A, Jain A, Khare P, Agrawal RK, Jain SK. Metronidazole Loaded Pectin Microspheres for Colon Targeting. J Pharm Sci. 2009;98:4229–4236.

36. Patil SB, Sawant KK. Development, Optimization and In Vitro Evaluation of Alginate Mucoadhesive Microspheres of Carvedilol for Nasal Delivery. J Microencapsulation. 2009;26:432–443.

37. Pravallika YV, Rajyalakshmi K. Formulation and Evaluation of Gastroretentive Hydrochlorothiazide Floating Microspheres: Statistical Analysis. Pharma Tutor. 2011;4:28-36.

38. Yegappan R, Selvaprithiviraj V, Amirthalingam S, Jayakumar R. Carrageenan Based Hydrogels for Drug Delivery, Tissue Engineering and Wound Healing. Carbohydrate Polymers. 2018;198:385–400.

39. Venkateswarlu K, Preethi JK, Kiran BSS. Formulation Development and *In-vitro* Evaluation of Floating Tablets of Ciprofloxacin HCl. Asian J Pharm. 2016;10:271-278.

40. Garcia JG, Ghaly ES. Preliminary Evaluation of Glipizide Spheres and Compacts from Spheres Prepared by Cross-Linking Technique. Puerto Rico Health Sci J. 2001;20:25-30.

41. Semalty A, Adhikari L, Pandey M. Development and Evaluation of Alginate Microspheres of Paracetamol: Effect of Different Concentrations of Crosslinking Agent and Coating. Int Res J Invent Pharm Sci. 2014;2: 28-32.

42. Vinodbhai PK, Gohel MC, Parikh RK, Bariyac S, Suthar RN. Sustained Release Floating Microspheres of Acyclovir: Formulation, Optimization, Characterization And *In Vitro* Evaluation. Int J Drug Develop Res. 2011;3:242-251.

43. Lohani A, Singh G, Bhattacharya SS, Hegde RR, Verma A. Tailored-Interpenetrating Polymer Network Beads of k-carrageenan and Sodium Carboxymethyl Cellulose for Controlled Drug Delivery. J Drug Del Sci Tech 2016;31:53-64.

44. Farooq U, Khan S, Shahid Nawaz S, Ranjha NM, Haider MS, Khan MM, Nawaz A. Enhanced Gastric Retention and Drug Release Via Development of Novel Floating

Microspheres based on Eudragit E100 and Polycaprolactone: Synthesis and In Vitro Evaluation. Designed Monomers and Polymers. 2017;20: 419-433.

45. Shadab Md, Ahuja A, Khar RK, Baboota S, Chuttani K, Mishra AK, Ali J, Gastroretentive Drug Delivery System of Acyclovir-Loaded Alginate Mucoadhesive Microspheres: Formulation and Evaluation. Drug Deliv. 2011;18:255-264.

46. Sankalia MG, Mashru RC, Sankalia JM, Sutariya VB. Physicochemical Characterization of Papain Entrapped in Ionotropically Cross-Linked Kappa-Carrageenan Gel Beads for Stability Improvement Using Doehlert Shell Design. J Pharm Sci. 2006;95:1994–2013.

47. Jassem NA, Rajab NA. Effect of Effervescent Agents on the Formulation of Famotidine Loaded Sodium Alginate Floating Beads. Kerbala J Pharm Sci. 2012;4:166-176.

48. Sipahigil O, Dortunc B. Preparation and In Vitro Evaluation of Verapamil HCl and Ibuprofen Containing Carrageenan Beads. Int J Pharm. 2001;228:119–128.

49. Patil NN, Patil KP, Pawar SP, Tadvi SA. Formulations and Evaluations of Metformin Microspheres by Ionotropic Gelation Technique. World J Pharma and Pharm Sci. 2017; 6:1473-1486.

50. Selvakumaran S, Muhamad II. Optimization of Formulation of Floating Hydrogels Containing Gas Forming Agent Using Response Surface Methodology. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6:526-530.

51. Selvakumaran S, Muhamad II, Razak SIA. Evaluation of Kappa Carrageenan as Potential Carrier for Floating Drug Delivery System: Effect of Pore Forming Agents. Carbohydrate Polymers. 2016;135:207-214.

52. Maravajhala V, Dasari N, Sepuri A, Joginapalli S. Design and Evaluation of Niacin Microspheres. Indian J Pharm Sci. 2009;71(6):663-669.

53. Nagpal M, Maheshwari DK, Rakha P, Dureja H., Goyal S, Dhingra G. Formulation Development and Evaluation of Alginate Microspheres of Ibuprofen. J Young Pharm. 2012; 4:13-6.

54. Ghareeb MM, Issa AA, Hussein AA. Preparation and Characterization of Cinnarizine Floating Oil Entrapped Calcium Alginate Beads. Int J Pharm Sci Res. 2012;3:501-508.

55. Alhamdany AT, Maraie NK, Msheimsh BR. Development and In Vitro/In Vivo Evaluation of Floating in Situ Gelling Oral Liquid Extended Release Formulation of Furosemide. UK J Pharm Biosci. 2014;2:1-11.

56. Ozdemir O, Çelik MS, Nickolov ZS, Miller JD. Water Structure and its Influence on the Flotation of Carbonate and Bicarbonate Salts. J Colloid Interface Sci. 2017;314:545–551.
57. Dinarvand R, Moghadam SH, Mohammadyari-Fard L, Atyabi F. Preparation of Biodegradable Microspheres and Matrix Devices Containing Naltrexone. AAPS PharmSciTech 2003;4:1-10.
58. Moganti M, Shivakumar HN. Formulation and Evaluation of Gastroretentive Floating Multiparticulate System of Lisinopril. Indian J Health Sci Biomed Res. 2017;10:50-56.
59. Lemoine D, Wauters F, Bouchend S, Preat V. Preparation and Characterization of Alginate Microspheres Containing a Model Antigen. Int J Pharm. 1998;176:9-19.
60. Rajinikanth PS, Sankar C, Mishra B. Sodium Alginate Microspheres of Metoprolol Tartrate for Intranasal Systemic Delivery: Development and Evaluation. Drug Deliv. 2003; 10:21-28.
61. Varghese JS, Nisha C, Fathima NN. Gelatin-Carrageenan Hydrogels: Role of Pore Size Distribution on Drug Delivery Process. Colloids and Surfaces B: Biointerfaces. 2014; 133:346-351.
62. Benita S, Barkai A, Pathak YV. Effect of Drug Loading Extent on the in-vitro Release Kinetic Behaviour of Nifedipine from Polycrylate Microspheres. J Control Release. 1990; 12:213-222.
63. Manjanna KM, Pramod Kumar TM, Shivakumar B. Effect of Manufacturing Conditions on Physico-chemical Characteristics and Drug Release Profiles of Aceclofenac Sodium Microbeads. Drug Invent Today. 2009;1:98-107.
64. Ansary J, Chaurasiya AK, Bashirul Huq KM. Formulation and Evaluation of Metformin HCl Floating Microspheres. Asian J Med Bio Res. 2015;1:396-405.
65. Kameswara RS, Yusuf MD, Saraswathi P, Rao CRR, Murali P, Vijayakumar V. Formulation and Evaluation of Orodispersible Enalapril Maleate Tablets: A Comparative Study on Natural Super Disintegrants and Synthetic Super Disintegrants. Int J Adv Sci Res. 2015;1:313-321.
66. Wang K., He Z. Alginate-Konjac Glucomannan-Chitosan Beads as Controlled Release Matrix. Int J Pharm. 2002;244:117-126.
67. Volery P, Besson R, Schaffer-Lequart C. Characterization of Commercial Carrageenans by Fourier Transform Infrared Spectroscopy Using Single-Reflection Attenuated Total Reflection. J Agri Food Chem. 2004;52:7457-7463.

68. Chavan M.S., Sarode S., Bhushankumar S., Vadnere G.P. Formulation and Evaluation of Sustained Release Microspheres of Acebutolol Hydrochloride. World J Pharm Pharm Sci. 2014;3:636-646.
69. Sethi RK, Barik BB, Sahoo SK. Preparation and Determination of Drug-Polymer Interaction and In-vitro Release of Didanosine Microspheres Made of Cellulose Acetate Phthalate or Ethylcellulose Polymers. Int J Drug Develop Res. 2013;5:341-353.
70. Jelvehgari M, Hassanzadeh D, Kiafar F, Loveymi BD, Amiri S. Preparation and Determination of Drug-Polymer Interaction and *In-vitro* Release of Mefenamic Acid Microspheres Made of cellulose acetate Phthalate and/or Ethylcellulose Polymers. Iranian J Pharm Res. 2011;10:457-467.
71. Rao KM, Rao KSVK, Sudhakar P, Rao KC, Subha MCS. Synthesis and Characterization of Biodegradable Poly (Vinyl caprolactam) Grafted on to Sodium Alginate and its Microgels for Controlled Release Studies of an Anticancer Drug. J Applied Pharm Sci. 2013;3:61-69.
72. Mohamadnia Z, Zohuriaan-Mehr MJ, Kabiri K, Jamshidi A, Mobedi H. Ionically Cross-linked Carrageenan-Alginate Hydrogel Beads. J Biomaterial Sci, Polymer Edition. 2008;19: 47–59.

Table 1. Formulation of Enalapril Maleate Floating Microspheres

Formulations	Drug (mg)	Sodium Alginate (mg)	Iota carrageenan (mg)	Sodium Bicarbonate	$CaCl_2$ (%)
1	20	500	100	200	2
2	20	1000	100	200	2
3	20	1500	100	200	2
4	20	1000	200	200	2
5	20	1000	400	200	2
6	20	1000	200	400	2
7	20	1000	200	800	2
8	10	1000	200	200	2
9	5	1000	200	200	2
10	20	1000	200	200	4
11	20	1000	200	200	1

Table 2. Micrometric Properties of Enalapril Maleate Floating Microspheres

Formula Code	Particle size (µm)	Bulk Density (g/cc)	Tapped Density (g/cc)	Compressibility Index (%)	Hausner's Ratio	Angle of repose
F1	196.55 ± 0.28	0.463 ± 0.12	0.493 ± 0.09	6.08 ± 0.05	1.06 ± 0.03	13.52 ± 0.34
F2	242.10 ± 0.17	0.497 ± 0.04	0.537 ± 0.11	7.44 ± 0.03	1.08 ± 0.07	15.90 ± 0.22
F3	520.2 ± 0.09	0.908 ± 0.23	0.99 ± 0.17	8.28 ± 0.02	1.09 ± 0.09	18.4 ± 0.16
F4	199.4 ± 0.04	0.812 ± 0.02	0.860 ± 0.07	5.58 ± 0.01	1.06 ± 0.05	17.64 ± 0.11
F5	429.3 ± 0.09	0.785 ± 0.14	0.837 ± 0.12	6.21 ± 0.09	1.07 ± 0.11	18.9 ± 0.24
F6	403.2 ± 0.13	0.777± 0.08	0.891± 0.32	12.79 ± 0.29	1.14 ± 0.15	16.74 ± 0.09
F7	372.6 ± 0.18	0.763 ± 0.16	0.871 ± 0.26	12.39 ± 0.33	1.14 ± 0.08	13.21 ± 0.31
F8	347.4 ± 0.21	0.812 ± 0.25	0.840 ± 0.27	3.33 ± 0.17	1.03 ± 0.04	18.1 ± 0.17
F9	421.2 ± 0.06	0.799 ± 0.22	0.820 ± 0.21	2.56 ± 0.11	1.02 ± 0.06	19.32 ± 0.13
F10	458.4 ± 0.14	0.880 ± 0.31	0.900 ± 0.05	6.38 ± 0.08	1.07 ± 0.12	20.22 ± 0.25
F11	298.8 ± 0.15	0.704 ± 0.09	0.721 ± 0.03	2.36 ± 0.1	1.02 ± 0.06	16.5 ± 0.17

Table 3. Percentage Yield, Drug Entrapment Efficiency, and In-vitro Buoyancy

Formula Code	Percentage Yield	Entrapment Efficiency	Buoyancy (%)
F1	54.5 ± 0.92	73.4 ± 0.07	80.22 ± 0.21
F2	67.07 ± 0.87	81.5 ± 0.05	76.12 ± 0.53
F3	72.88 ± 0.67	84.3 ± 0.15	68.89 ± 0.44
F4	91.18 ± 0.24	92.3 ± 0.04	92.41 ± 0.21
F5	84.2 ± 0.36	88.7 ± 0.09	70.17 ± 0.37
F6	69.3± 0.46	76.15 ± 0.11	72.21 ± 0.77
F7	68.8 ± 0.21	72.15 ± 0.08	71.43 ± 0.55
F8	75.9± 0.23	78.2 ± 0.06	82.95 ± 0.41
F9	82.7± 0.35	72.8 ± 0.12	74.10 ± 0.34
F10	72.38 ± 0.23	80.5 ± 0.17	50.92 ± 0.74
F11	85.4± 0.27	83.5 ± 0.03	78.54 ± 0.82

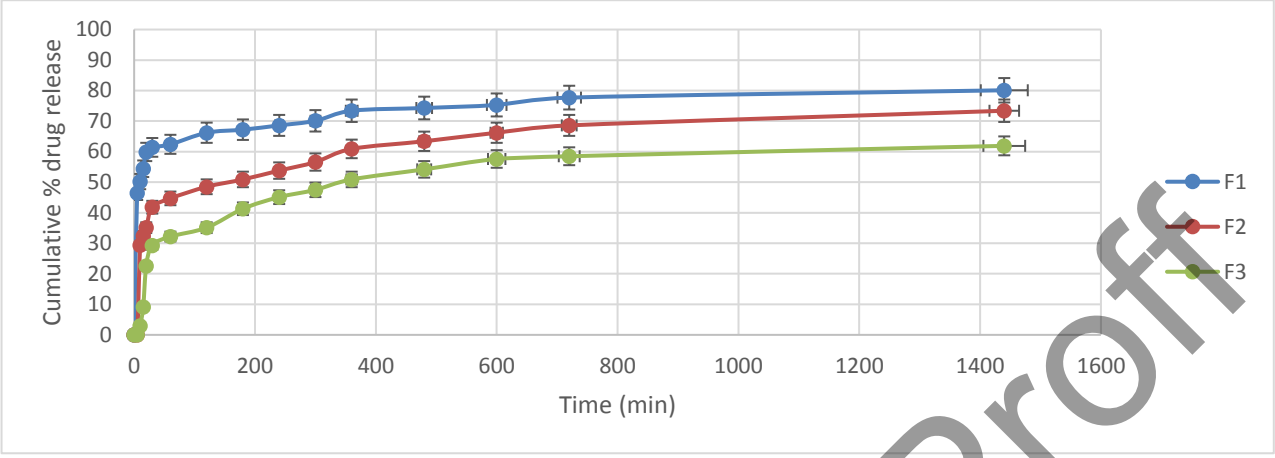

Figure 1. Dissolution Profile of Enalapril from Floating Microspheres Containing Different Concentrations of Sodium Alginate (F1-F3), data given in mean±SD, n=3.

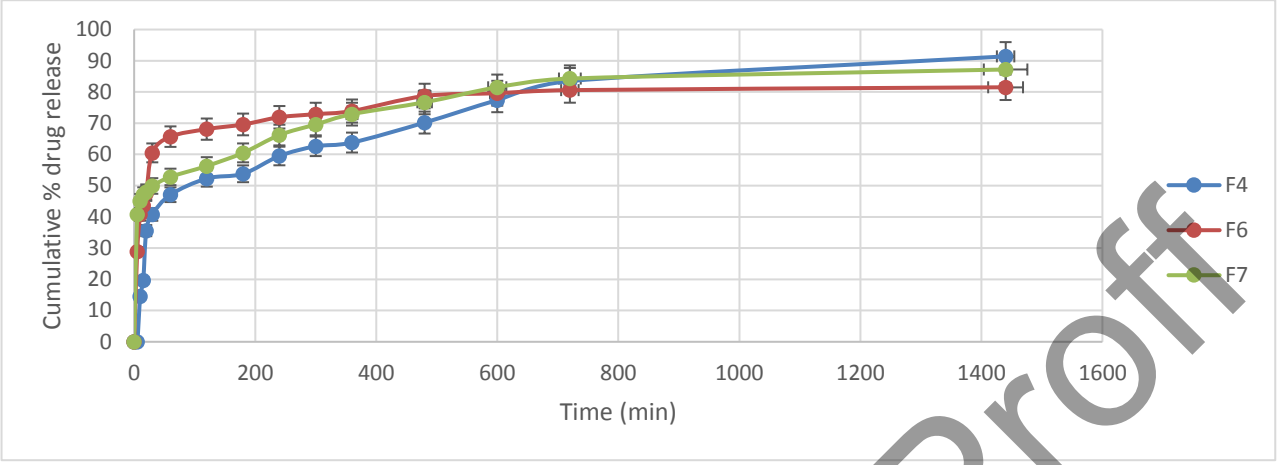

Figure 2. Dissolution Profile of Enalapril from Floating Microspheres Containing Different Concentrations of iota Carrageenan (F2, F4, and F5), data given in mean±SD, n=3.

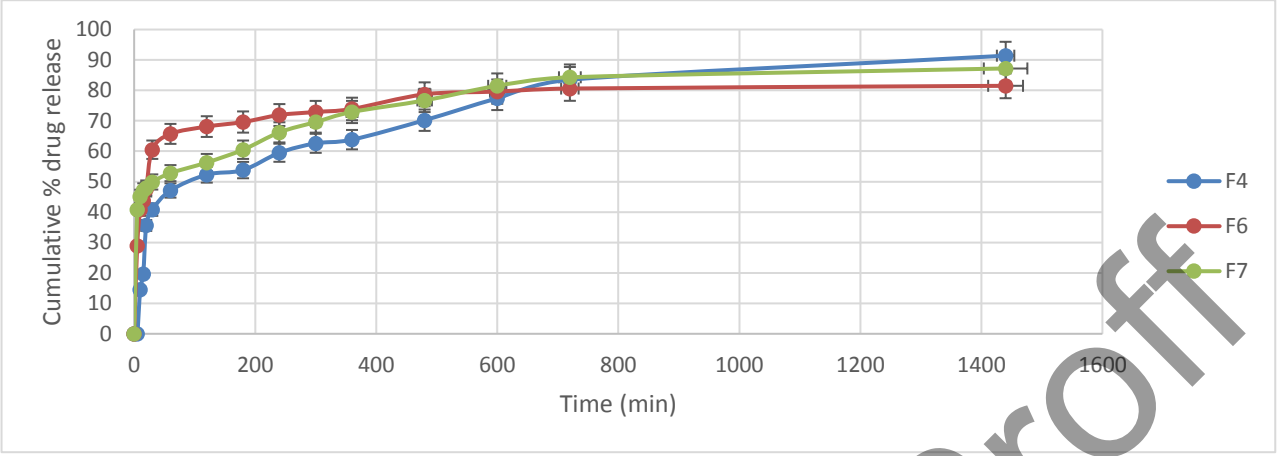

Figure 3. Dissolution Profile of Enalapril from Floating Microspheres Containing Different Concentrations of sodium bicarbonate (F4, F6, and F7), data given in mean±SD, n=3.

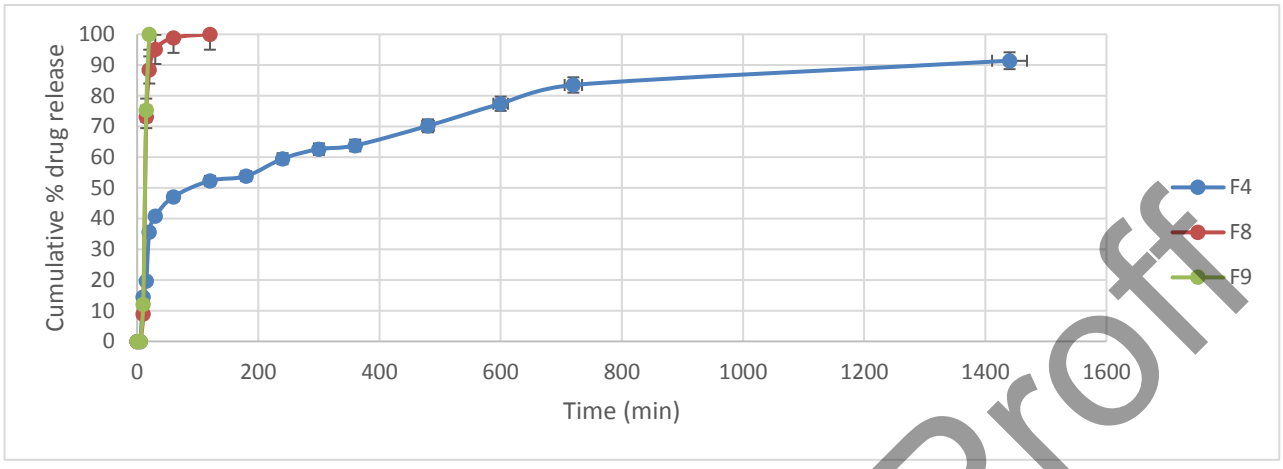

Figure 4. Dissolution Profile of Enalapril from Floating Microspheres Containing Different Concentrations of Enalapril (F4, F8, and F9), data given in mean±SD, n=3.

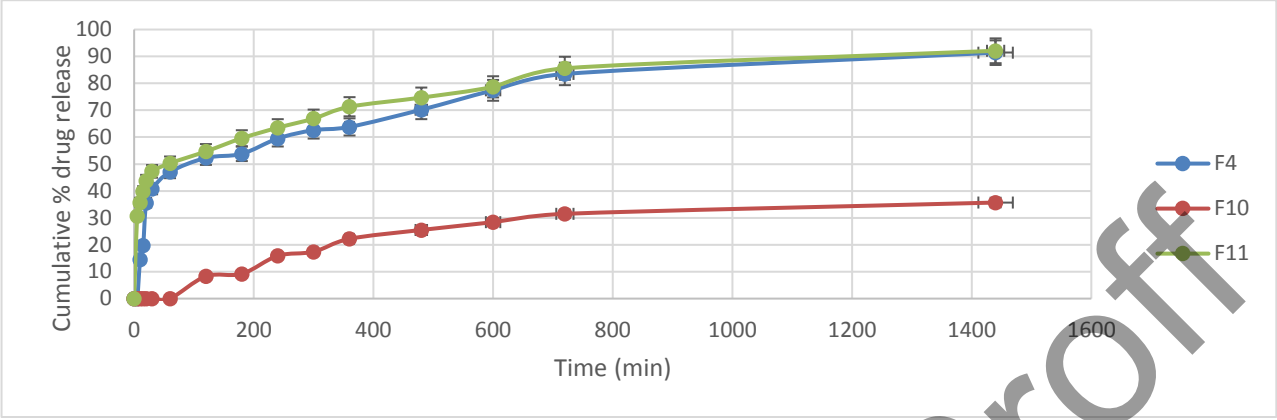

Figure 5. Dissolution Profile of Enalapril from Floating Microspheres Containing Different Concentrations of $CaCl_2$ (F4, F10, and F11), data given in mean±SD, n=3.

Table 4. Kinetic Data Models for the Prepared Enalapril Microspheres

Formulation	Zero-order		First-order		Higuchi-order		Korsmeyer-Peppas		
	K_0(mg h^{-1})	R^2	K_1(h^{-1})	R^2	K_H($h^{-1/2}$)	R^2	n	K_{kp}($h^{-1/3}$)	R^2
F1	0.0203	0.6177	-0.0003	0.7375	0.8844	0.8443	0.3509	1.0278	0.4676
F2	0.0359	0.5419	-0.0003	0.7234	1.5826	0.7563	0.5372	0.4572	0.6573
F3	0.0392	0.5623	-0.0003	0.671	1.7546	0.8089	0.6158	0.1383	0.7955
F4	**0.0983**	**0.7108**	**-0.0009**	**0.8214**	**2.7776**	**0.8462**	**0.5789**	**0.4298**	**0.6399**
F5	0.0357	0.4806	-0.0002	0.5361	1.6643	0.7502	0.8606	-0.5975	0.7932
F6	0.0545	0.6268	-0.0007	0.7741	1.7369	0.7963	0.176	1.4368	0.8926
F7	0.072	0.9510	-0.0008	0.985	1.72	0.9887	0.13	1.5117	0.9627
F8	1.6325	0.5322	-0.057	0.803	42.927	0.7805	1.2111	-0.0982	0.7582
F9	7.264	0.9373	-0.0607	0.8185	44.375	0.8114	1.6255	-0.3657	0.7534
F10	0.0302	0.7975	-0.0002	0.8338	1.2281	0.9486	0.8423	-0.9491	0.8966
F11	0.0731	0.8792	-0.0008	0.9601	1.9878	0.9703	0.1827	1.3785	0.9859

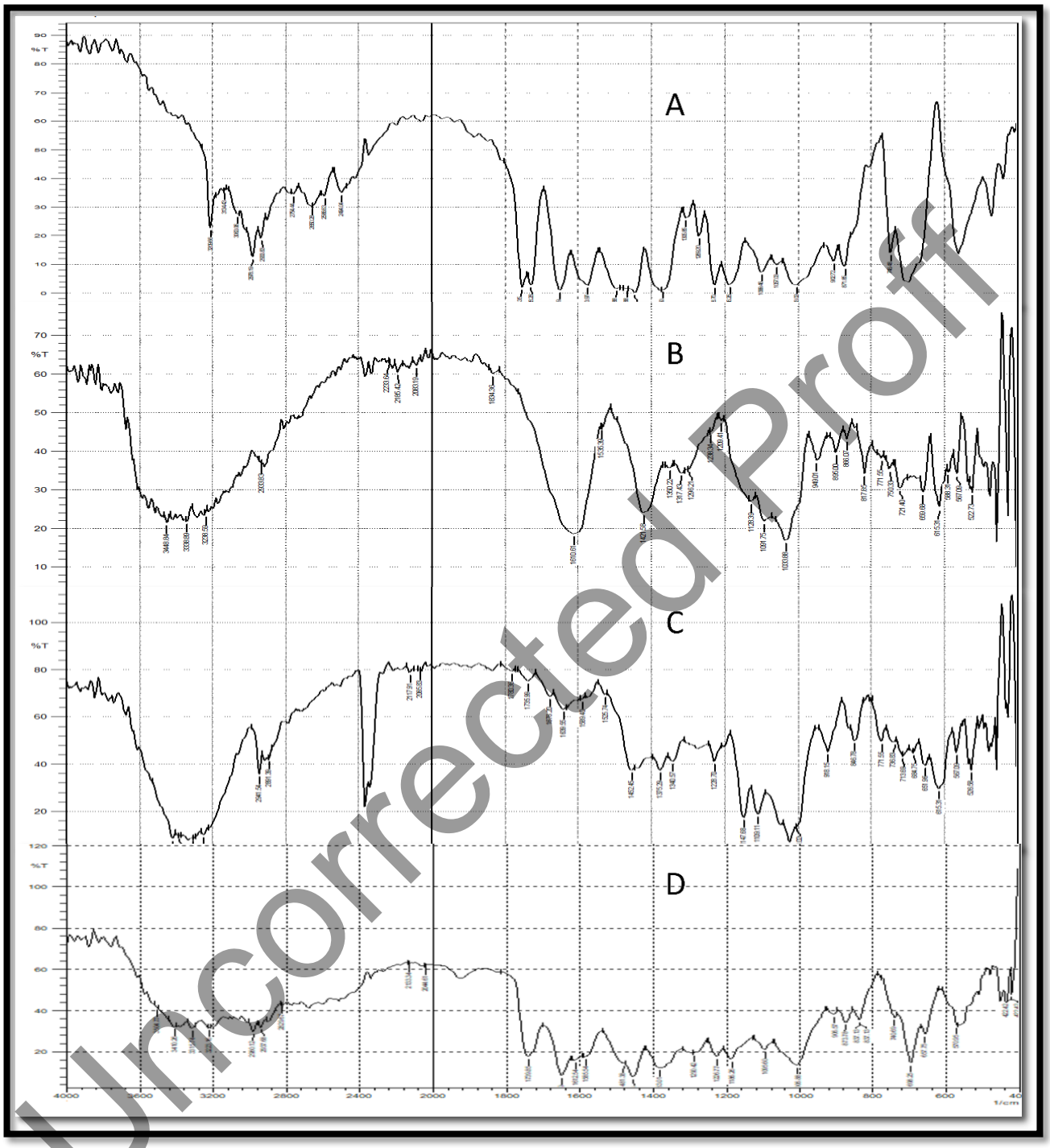

Figure 6. The FT-IR Spectra for (A) Pure enalapril; (B) Sodium alginate; (C) Iota carrageenan; (D) Enalapril microspheres [F4]

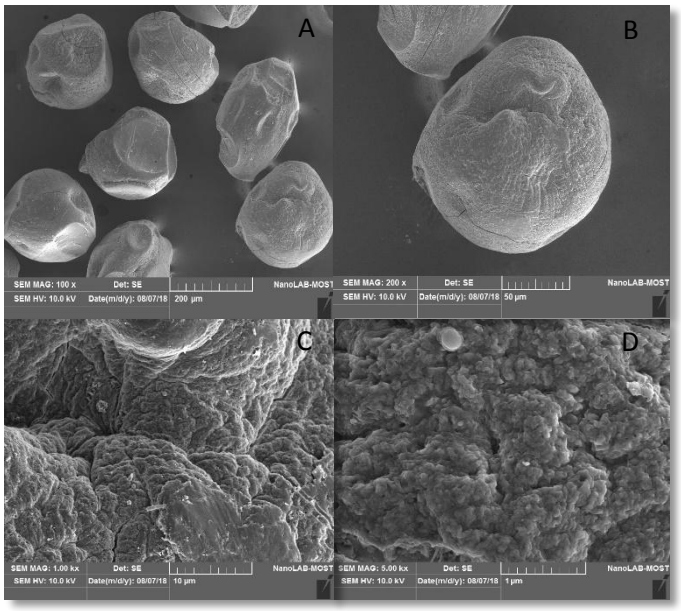

Figure 7. Scanning Electron Microscopy for F4 Represents Different Magnifications A) 100X; B) 200X; C) 1K; D) 5K

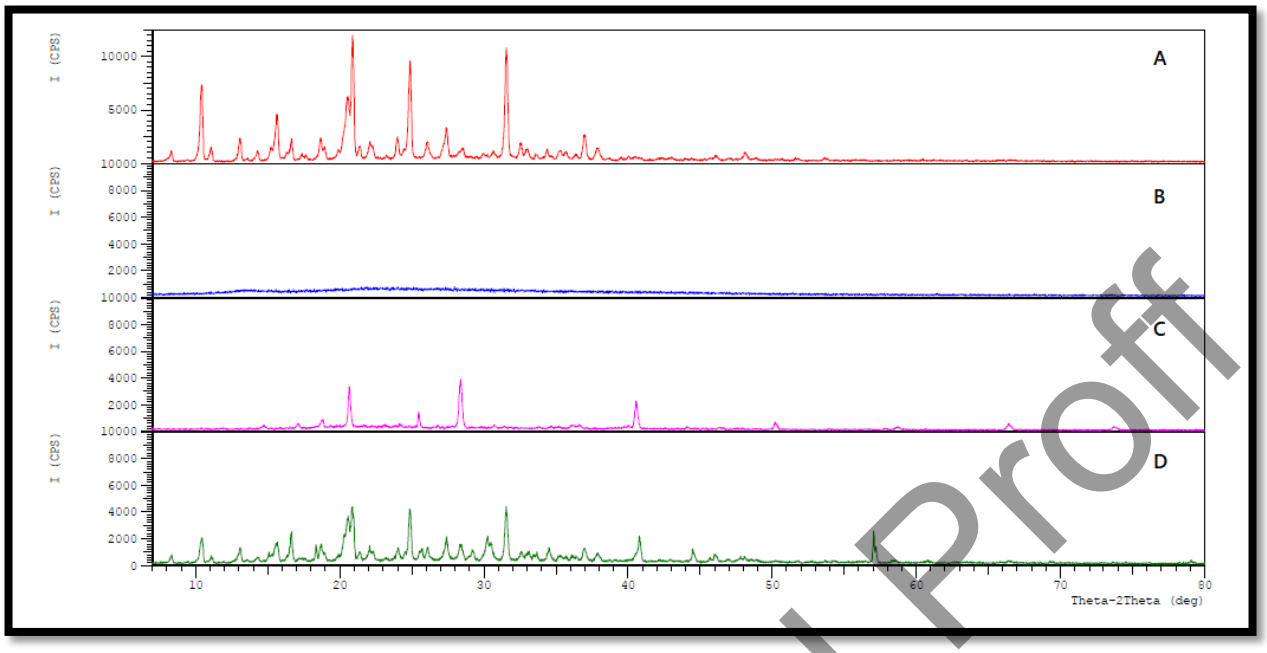

Figure 8. X-Ray Diffraction (XRD) Patterns of A) Pure Enalapril Powder; B) Sodium Alginate; C) Iota Carrageenan; D) F4.

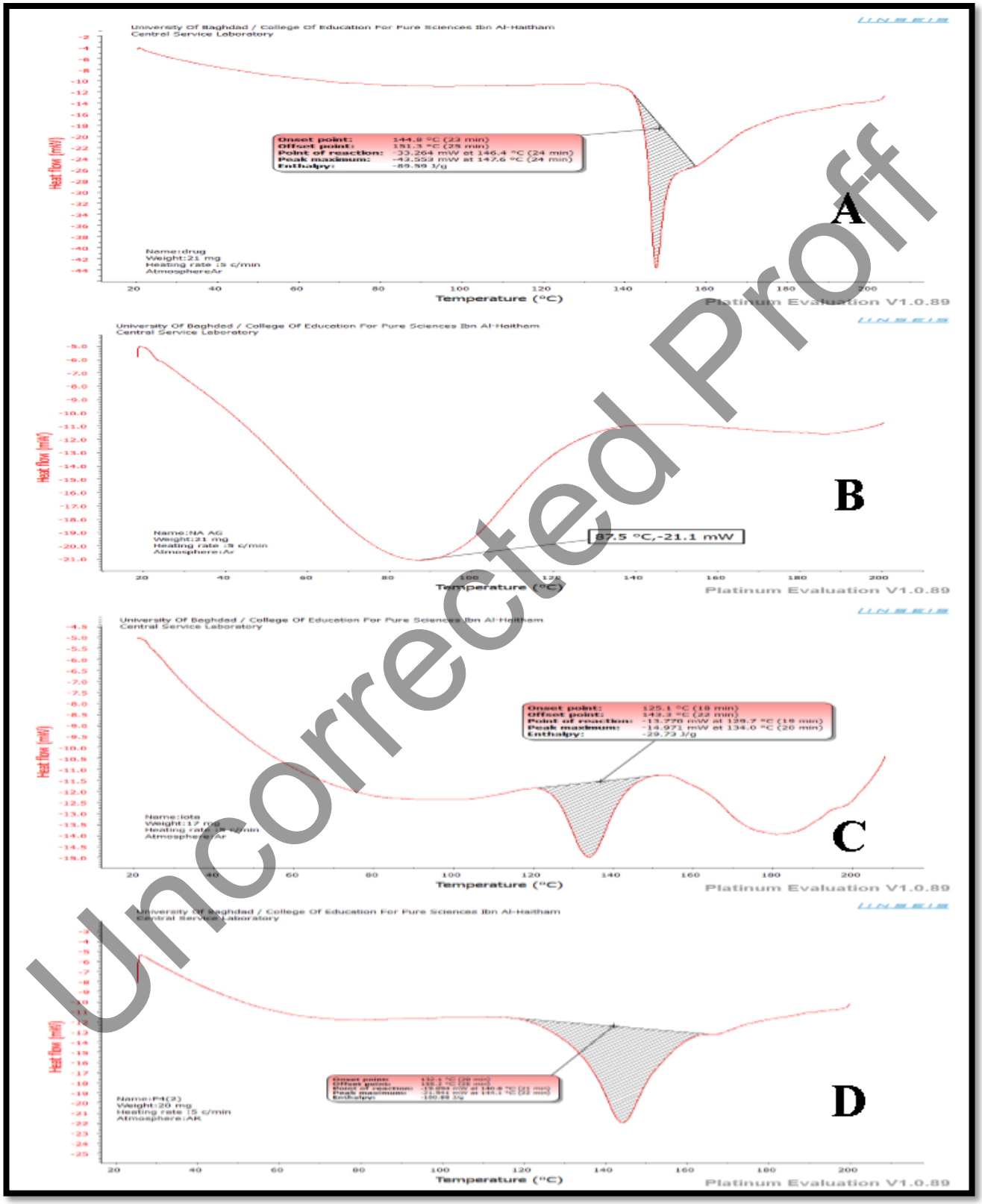

Figure 9. Differential Scanning Calorimetry (DSC) Patterns of A) Pure Enalapril Powder; B) Sodium Alginate; C) Iota Carrageenan; D) F4.