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The effect of polymer amount and crosslinker ratio in polymeric hydrogel beads on characterization

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Received: 11 April 2021 / Revised: 21 June 2021/ Accepted: 28 June 2021

ABSTRACT: Today, treating diseases requires increasing the patient's living standard rather than just applying a classical treatment protocol. In this study, it was aimed at facilitating drug intake, reduce the dose of the active substance, reduce the number of daily doses to be taken, reduce or eliminate possible side and/or toxic effects, transport the active substance to the target area and make as much bioavailability from the active substance as possible too. For this purpose, in our study, hydrogel bead formulations with ionotropic gelation technique were developed using ampicillin sodium as a model drug and sodium alginate and HPMC K100 as polymer. $CaCl_2$ was used as a crosslinking agent. While developing a new drug delivery system formulation, size, morphology with SEM, *in vitro* release profiles, release kinetics, encapsulation efficiencies, drug loading capacities, yields, and swelling capacities, FT-IR and XRD analysis were evaluated in the hydrogel beads depending on the amount of the polymer and crosslinking agent. It has been made possible to extend the duration of drug action by changing the amount of polymer and crosslinker ratios in oral drug delivery of ampicillin sodium with alginate beads. Thus, it is likely to increase patient compliance as well as to reduce drug-related side and/or toxic effects with less dosing.

KEYWORDS: Ampicillin sodium; beads; drug delivery system; HPMC; sodium alginate.

1. INTRODUCTION

Today, treating diseases requires increasing the living standard of the patient rather than just applying a classical treatment protocol. In this context, besides the ease of use provided to drugs, it is aimed at reducing the dose of the active substance and the number of doses to be taken daily, reduce or eliminate possible side and/or toxic effects. It is also desirable to deliver the active substance to the target area and to provide as much bioavailability as possible [1]. Nowadays, these desired results are achieved with controlled release systems.

Unlike immediate release dosage forms, controlled release systems are released the active substance at a predetermined rate or over a more extended time to produce a prolonged effect. The release rate and duration depend on the physicochemical and pharmacokinetic properties of the active substance. For example, the plasma level of the drug may rise to the toxic area or fall into an ineffective place for a while after the conventional dosage form is taken. In controlled release systems, the blood plasma profile of the active substance remains between the previously expected and known levels with this case actualized after a specific dose is taken into the body. In this way, the patient's daily drug use amount and frequency are reduced, and the success of the drug with increased bioavailability in treatment is enhanced [1-4]. This situation has been researched and developed for all old and new drug active ingredients in use today. While doing this, we have to use today's technologies and polymer chemistry. Many naturally sourced protein and polysaccharide polymeric biomaterials are used to delay and/or prolong the release of the active ingredient in controlled release systems. These natural biomaterials have advantages such as safe, stable, non-toxic, and easy to process [5, 6].

Hydroxypropylmethylcellulose (HPMC), a synthetic polysaccharide from cellulose derivatives, is a water-soluble biocompatible polymer with very variable molecular weights and viscosity grades. It has unique swelling/erosion properties reflecting its ability to control drug release [7]. We come across many bead formulations prepared using HPMC in the literature [8, 9]. Especially in a study conducted with HPMC K100, it was stated that the active substance not only increased its bioavailability but also reduced its side effects [10]. For this reason, HPMC K100 was used in our study.

How to cite this article: Sevinç Özakar R, Özakar E. The effect of polymer amount and crosslinker ratio in polymeric hydrogel beads on characterization. J Res Pharm. 2021; 25(5): 653-666.

https://dx.doi.org/10.29228/jrp.57

ISSN: 2630-6344

Alginate is a non-toxic, water-soluble anionic polysaccharide naturally derived from brown sea algae. Alginate is biocompatible with blood and body, does not accumulate in any organ, and is excreted by being broken down from the body. There are many studies in the literature regarding its use in biomedical applications, especially in controlled release drug delivery systems, although used in many conventional oral and topical pharmaceutical preparations. Alginate allows the active ingredients to be encapsulated in an aqueous environment. In the prepared beads with alginate, the stable structure of the beads (egg-box model) is formed by ionotropic gelation of spherical drops [11, 12]. The most attractive feature of alginate is that it forms a gel that is simply induced by cross-linking with various divalent cations except the Mg^{+2} ion. Generally, in the literature studies are concentrated on calcium alginate beads. A gel bead structure is formed by dropping a sodium alginate solution into an aqueous solution containing Ca^{+2} ions at a specific concentration. Due to sodium alginate's ability to create a stable and bio-adhesive gel with calcium ions, materials like some sensitive drugs, proteins, and living cells can be transported via beads and used in therapy [13]. Sodium alginate was chosen in our study for these reasons.

Beads are small-sized controlled drug delivery systems that trap the active substance/substances in dispersed or dissolved form and/or can adsorb on them at the same time. They are formed by cross-linking hydrophilic polymers and swell in contact with biological environments, allowing the controlled release of the active substance. The active substance/substances may be in case of solution or crystal form, and they as well as exhibiting extended-release or multiple release profiles [12, 14, 15]. For this purpose, the ionotropic gelation technique is one of the most preferred methods.

Ionotropic gelation is a technique that enables crosslinking of polyelectrolytes and oppositely charged ions. In this method, polyelectrolytes (such as polysaccharides; alginate, gellan, pectin, chitosan) are dissolved in water or a weakly acidic environment and dropped into solutions containing oppositely charged crosslinking ions. While complexing occurs between opposite charges, polysaccharides undergo ionic gelation [16]. Three-dimensional spherical particles (beads) are formed by diffusing polymeric drops around the active substance. Especially low molecular weight crosslinkers are used in ionotropic gelation. Among these, one of the most used is calcium chloride ($CaCl_2$). This method is highly preferred because it allows the use of natural, biocompatible, and biodegradable polymers. It is an essential advantage that it can be prepared by reversible physical crosslinking instead of chemical crosslinking. In addition, since it does not require organic solvents, it eliminates the toxicity and unwanted effects that may occur due to this [11, 12].

Ampicillin sodium was chosen as a model drug and hydrogel bead formulations were developed using the ionotropic gelation technique. We aimed at examining the size, morphology, *in vitro* release profiles, release kinetics, encapsulation efficiencies (EE), drug loading capacities (LC), yields (Y), and swelling capacity differences depending on changes in the amount of crosslinker and polymer at formulations. In addition, we aimed at detecting changes in the structure of the beads with FT-IR and XRD analyzes. When developing a new delivery system formulation, we wanted to define the necessary parameters for optimum formulation.

2. RESULTS AND DISCUSSION

2.1. Preparation of hydrogel beads formulations

During the formulation development studies, the amount of polymer and the amount of $CaCl_2$ were determined as variable and other parameters were kept constant. The schematic description of the formulations containing ampicillin sodium prepared by ionotropic gelation technique was given below in Figure 1, and the data of the ingredients used in the formulations were given in Table 1.

Table 1. Formulation ingredients and used amounts.

Formulation Code	Ampicillin Sodium (mg)	Sodium Alginate (mg)	HPMC K100 (mg)	$CaCl_2$ (w/v)
F1	250	250	50	10%
F2	250	250	-	10%
F3	250	150	50	5%
F4	250	150	-	5%
F5	250	-	250	10%
F6	250	-	150	5%

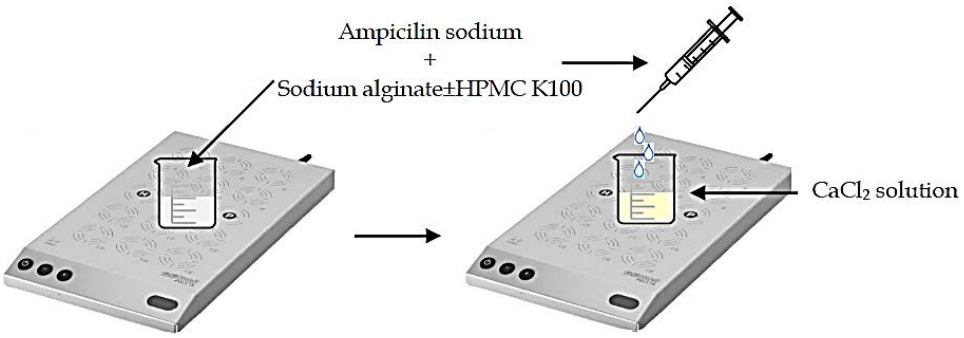

Figure 1. Preparation of ampicillin sodium-containing hydrogel bead formulations.

2.2. EE, LC and Y of hydrogel beads formulations

The data of EE%, LC% and Y% for bead formulations were given in Table 2. The results showed that, EE%, LC% and Y% were found between ~ 30-40%, ~ 15-25% and ~ 32-42%, respectively. The main reason for the low EE was the ampicillin sodium is soluble in water, and the polymer has a low molecular weight. Because the length of the polymer chains of high molecular weight polymers allows more drug encapsulation while slowing down drug release [17]. Considering the formulations, it was significant that the EE% value of the F2 formulation was higher than F1. The difference may be due to the effect of HPMC K100 on ampicillin sodium encapsulation. The fact that the EE% value of the F3 formulation was ~ 25% times higher than F4 was also statistically significant ($p < 0.05$). Here, the amount of HPMC K100 used in proportion to the sodium alginate used in the F3 bead formulation may have been effective in the complexation in the formation of the beads. The same may not have happened for F1 and F2 due to the high amount of alginate. Also, depending on the increase in the amount of polymer (F1, F3), the LC decreased. However, the Y increased significantly with the effect of the increasing amount of polymer F1 and F3 and the HPMC K100 polymer entering the structure compared to F2 and F4 ($p < 0.05$). It has been stated in the literature that increasing the amount of alginate in the formulation increases the EE [18]. In our study, in parallel with the decrease in the amount of sodium alginate between the F2 and F4 formulations, it was observed that there was a significant decrease in the EE. Bead structure could not be obtained for F5 and F6 formulations. Therefore, it has been determined that these formulations could not be designed with HPMC K100 alone. For this reason, studies were carried out on F1-F4 formulations in the next experiments. Results were calculated as mean ($\bar{X}$)±standard deviation (SD).

Table 2. EE%, LC% and Y% data for hydrogel bead formulations (n=3, %±SD).

Formulation Code	EE%	LC%	Y%
F1	34.71 ± 2.24	14.97 ± 1.53	37.51 ± 1.43
F2	40.05 ± 6.90	20.01 ± 2.18	33.22 ± 2.27
F3	39.97 ± 3.14	24.75 ± 1.04	42.46 ± 1.54
F4	29.70 ± 2.91	25.46 ± 2.01	32.37 ± 1.00

2.3. Shape, size and size distribution analysis of hydrogel beads formulations

The shape of the beads, which were spherical in the fresh state, has become both porous and protruding due to the decrease in volume when they were lyophilized. The obtained dimensional data were given in Table 3; images of the freshly prepared formulations and after lyophilization were shown in Figure 2 and Figure 3. Here, there was an increase in size in formulations where HPMC K100 was used. This occurs with an increase in volume as a result of the complexing of the cellulosic structure with sodium alginate. In addition, in the F2 and F4 formulations, it was observed that the decreased amount of sodium alginate significantly reduced the size ($p<0.05$). This situation was similar to a study by İbrahim et al. It has been emphasized that especially the increase in the amount of sodium alginate contributes positively to bead formation and size [13]. The dimensions of both F1 and F2 formulations and F3 and F4 formulations results showed that the decrease in size also could be associated with $CaCl_2$. In the literature, it has been stated that increasing the amount of $CaCl_2$ causes shrinkage in the beads [19]. However, the decrease in the amount of $CaCl_2$ causes flattening and loosening of the bead structure [20]. This was clearly seen in the images were examined of F2 and F4 (5% $CaCl_2$) and F1 and F3 (10% $CaCl_2$).

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Table 3. Sizes of the freshly prepared and lyophilized hydrogel beads (n=3, X̄±SD).

Formulation Code	Freshly prepared (mm)	Lyophilized (mm)
F1	4.34 ± 0.138	1.89 ± 0.142
F2	3.93 ± 0.177	1.39 ± 0.200
F3	4.06 ± 0.122	1.86 ± 0.179
F4	3.63 ± 0.176	1.24 ± 0.187

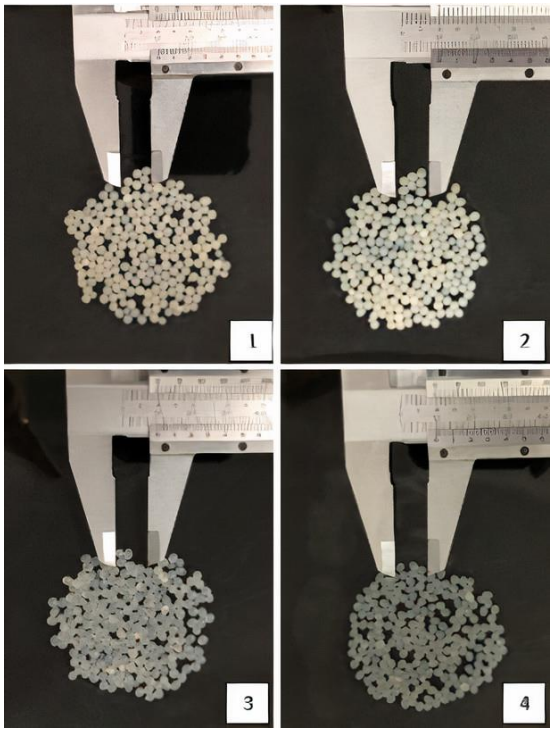

Figure 2. Images of freshly prepared hydrogel bead formulations (1-F1, 2-F2, 3-F3 ve 4-F4).

Figure 3. Images of lyophilized hydrogel bead formulations (1-F1, 2-F2, 3-F3 ve 4-F4).

https://dx.doi.org/10.29228/jrp.57
J Res Pharm 2021; 25(5): 653-666

2.4. *In vitro* release study of hydrogel beads formulations

The *in vitro* release study of the bead formulations was initiated in a pH 1.2 HCl buffer release medium for 2 hours and then the beads were transferred into a pH 6.8 phosphate buffer medium. The release study was carried out for a total of 24 hours. The *in vitro* release study results were given in Figure 4.

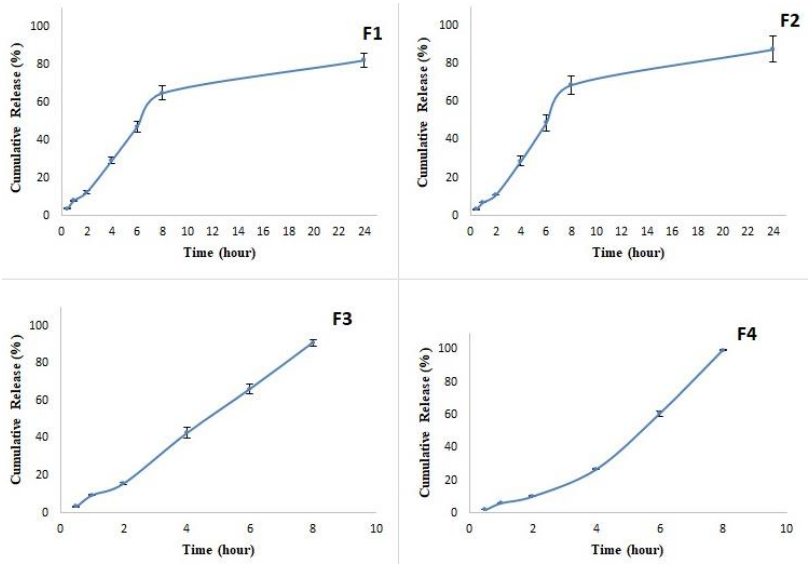

Figure 4. *In vitro* release study of hydrogel bead formulations.

The results showed that the release due to the amount of crosslinker in F1 and F2 formulations was 2 times higher compared to F3 and F4, and the release was extended up to 24 hours. However, in F3 and F4, where less crosslinker was used that almost 100% of ampicillin sodium was released at the end of about 8 hours. Especially between F3 and F4, there appear to be a delay in release in both pH 1.2 HCl and pH 6.8 phosphate buffer environments due to not using HPMC K100 in the F4 formulation. It was understood that HPMC K100 increases hydrophilicity and therefore accelerates the release. This difference did not see between F1 and F2 due to the amount of HPMC K100 in the formulation and the use of excess crosslinker. In the literature, it has been reported that active substance release and release rate decrease due to the increase in the amount of alginate [17, 19].

The graphical results indicated that there was no rapid release with any burst effect. This was important that ampicillin sodium does not allow a large amount of immediate release, and a prolonged controlled release was achieved. Because observed the immediate release with the burst effect was generally considered as a negative effect when long-term drug delivery systems were considered. This burst effect can lead to adverse consequences such as toxicity from high drug concentrations, requires more frequent dosing therapeutically, and economically results in drug waste. In addition, surface cracks caused by drying the beads lead to flash release with the burst effect as they facilitate polymer erosion. It could be clearly seen that the resulting calcium alginate reduces the flash release behavior with the burst effect. This reduction was due to the decrease of cavities in the alginate polymer network. The low cavity areas in the polymer network reduce the swelling of the beads and thus prevent burst release [17]. In similar studies in the literature, the effect of polymer concentration and cross-linking time on the cumulative percentage of drug release was examined. And it was found that the percentage of drug release decreased with an increase in polymer concentration and cross-linking time. The increase in cross-linking time adds more rigidity to the structure. Thus, the drug was slowly released through the highly cross-linked polymer matrix [21]. This was probably due to the increased amount of $CaCl_2$; more cross-linking with sodium alginate results in a decrease in the release of the active substance from the tight alginate matrix [19].

2.5. Determination of release kinetics of hydrogel beads formulations

Information of release kinetics is essential for the efficient use of the drug delivery system. The release of the drug from the delivery system depends on many properties of the drug and the carrier structure such as porosity, surface roughness, chemical composition, molecular weight, rate of degradation, particle size, matrix interaction, the amount of pharmaceutical dosage form, and the physicochemistry of the drug. Drug

release from the polymeric system includes three different mechanisms: (i) release due to polymer erosion, (ii) diffusion through the swollen matrix, and (iii) release from the polymeric system surface. In most cases, drug release is controlled by more than one mechanism. The release from the polymeric system surface causes a burst release effect, and the drugs adsorbed on the surface or trapped in the surface fissures are released with a rapid release. Crosslinking could be avoided by washing the beads with the appropriate solvent or reducing the amount of crosslinker. However, this method reduces the EE of the drug. Besides the three mechanisms described above, the chemically controlled drug release mechanism is also possible in the polymer hydrogel system. In this case, the release of the drug is determined by chemical reactions that occur between the polymer network and the drug [17].

Zero-order release explains that the drug releases with a release mechanism independent of its concentration. The Higuchi model defines the release of drugs from an insoluble matrix environment as the square root of a time-dependent process based on the diffusion principle of 'Fick'. The Korsmeyer-Peppas model is used to describe the release of drugs from pharmaceutical dosage forms when the mechanism of drug release from the system is not fully known or when more than one type of release phenomenon is in question [22]. In order to determine the release kinetics, the slopes of the dissolution rate profiles and the R^2 (determination coefficient) values were calculated using the graphical method using a software. The results were given in Table 4. R^2 was taken into account in model selection and results approaching 1 were evaluated [23].

Table 4. Release kinetics data for hydrogel bead formulations (R^2).

Formulation Code	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n value
F1	0.995	0.918	0.952	0.990	1.013
F2	0.990	0.929	0.938	0.986	1.077
F3	0.996	0.881	0.959	0.983	1.186
F4	0.953	0.931	0.870	0.983	1.292

The release kinetics of the formulations results indicated that F1, F2, and F3 comply with the zero-order release kinetics. It appears that the polymer matrix controls the release regardless of the amount of drug. It was understood that F4 conforms to Korsmeyer-Peppas kinetics. This may be due to the fact that F4, unlike F3, did not have HPMC K100 in its structure and was treated with half the crosslinker compared to F1 and F2. The formulations exhibited a controlled release over time. According to the release kinetics results of the formulations, "n" values greater than 0.45 when the release mechanism was in the form of super case II transport (n>0.89). This indicates that all formulations were released by the non-normal non-Fick diffusion principle (super case-II transport mechanism). It means that drug molecules diffuse through the highly hydrated polysaccharide matrix, which was involved in the dissolution or relaxation of polymer chains [24].

2.6. Determination of swelling capacity of hydrogel beads formulations

The process of swelling (water absorption) and dissolution in alginate hydrogel beads is highly complex, and the osmotic pressure gradient between the bead and the medium plays an important role in the swelling process. The swelling phenomenon usually begins with the loosening of the polymer network at high osmotic pressure. Although the swelling of the calcium alginate beads in an acidic environment is insignificant, the penetration of the medium into the dense gel structure in the beads is due to the proton-calcium ion-exchange created with alginic acid. However, beads begin to gain weight with hydration of hydrophilic groups, possibly due to the penetration of water through the pores on the bead surface and show marginal swelling even in an acidic environment [25].

The swelling capacity results of lyophilized bead formulations and comparative graphical data were given in Table 5 and Figure 5, respectively. With the effect of HPMC K100, which was included in F1 and F3 bead formulations, they absorbed 300-400% water in the first half-hour compared to their dry weight, and their weight increases rapidly. Also, the swelling degree was determined as 70-80% in beads prepared with sodium alginate alone (F2 and F4) in the first half-hour. The water absorption was approximately 1.5 times higher in the F3 formulation due to the 2 times more crosslinking treatment compared to F1 in the evaluation of formulations in terms of crosslinkers. This situation could be interpreted as the effect on the passage of water molecules that were in the structure of the F3 formulation prepared with less crosslinker. The excess crosslinker in F1 may have kept the structure tighter and prevented it from swelling by taking in more water.

Similarly, when evaluated in terms of F2 and F4 crosslinkers, it was seen that the increased crosslinker in the structure did not reveal a significant difference. Thus, HPMC K100 was certainly an important parameter that reveals the effect of crosslinkers for our formulations. Although an equal amount of HPMC K100 was used in F1 and F3 formulations, it was observed that the water absorption capacity of F1 decreased by 1.5 times compared to F3 due to the increasing crosslinker. In similar studies in the literature, it has been emphasized that increasing the crosslinker concentration was inversely proportional to the absorption of water and swelling capacity [26, 27]. The increase in hydrophilicity in the structure (HPMC K100) significantly affected the swelling capacity. Especially F1 and F3 were compared, only one-sixth of the total polymer amount in F1 was HPMC K100, while this ratio corresponded to one in four in F3. Even the difference in these rates showed how much HPMC K100 affects the swelling capacity. In a similar study in the literature, it was observed that swelling capacity increased in bead formulations due to the using HPMC [25].

Table 5. Swelling capacity datas of hydrogel bead formulations (n=3, %±SD).

Formulation Code	Time (Hour)					
	0.5	1	2	4	8	24
F1	322.6±10.02	328.87±14.01	325.13±10.72	346.67±27.80	350.53±10.58	364.27±24.93
F2	179.53±10.40	172.47±18.06	163.87±8.89	159.80±7.94	172.13±7.71	163.20±20.90
F3	448.13±16.94	480.93±30.10	479.07±4.97	491.13±2.50	529.40±21.72	574.60±4.34
F4	188.73±17.42	179.73±27.85	177.07±23.87	178.60±30.26	182.07±23.20	164.93±23.36

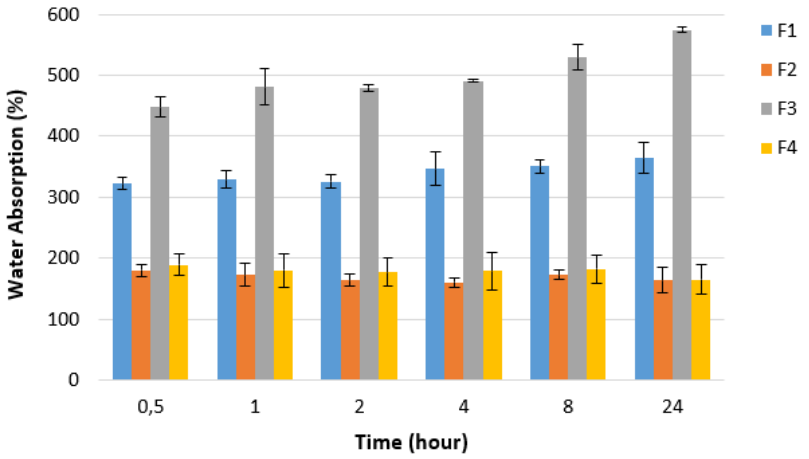

Figure 5. Swelling (water absorption) capacities of hydrogel bead formulations.

2.7. Analysis of hydrogel beads formulations by SEM

SEM images of the lyophilized bead formulations were given in Figure 6. The SEM image results showed that the difference of HPMC K100 in F1 and F2 formulations and F3 and F4 formulations were reflected approximately 10% on the size. The effect of an increase in total polymer amount on the size was examined. The size increased due to the increasing amount of polymer in F1-F3 and F2-F4. The close-up SEM images were examined of the beads formulated with HPMC K100 (F1 and F3). The beads were examined more spherical with fewer pores. While the prepared beads with only sodium alginate (F2 and F4) had much larger pores, and the structure was not spherical. Here, it was observed that HPMC K100 was characterized by a tighter polymer lattice and smaller pore structures, as well as keeping the structure in spherical form as a result of polymeric complexation with sodium alginate.

2.8. FT-IR analysis of hydrogel beads formulations

Infrared (IR) spectroscopy examines vibrations caused by IR rays, stresses of bonds between atoms, and changes in bond angles. When molecules absorb this energy, vibrational energy transitions occur in the IR region. Given that the molecule absorbs only certain frequencies by IR rays due to its own structure, the vibration frequency is associated with a particular bond type. Therefore, each vibration frequency is unique to that region of that molecule. Consequently, it is a crucial analysis technique in detecting changes in molecular structure [28].

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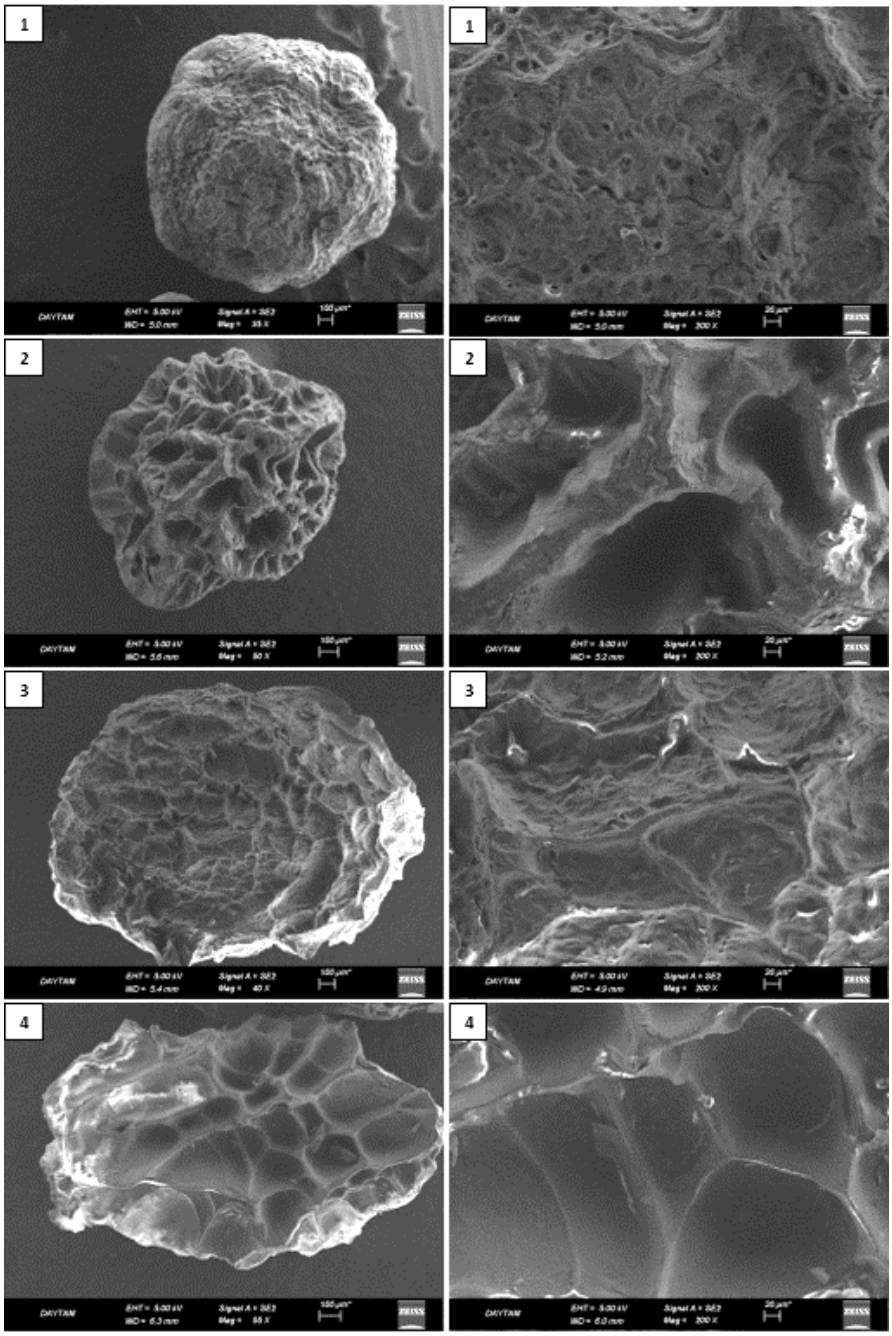

Figure 6. SEM images of lyophilized hydrogel bead formulations (1-F1, 2-F2, 3-F3 ve 4-F4).

The characteristic band stretching of ampicillin sodium (Figure 7) was observed that the band of 1350-1400 cm^{-1} for the N-C aromatic bond, 1600-1650 cm^{-1} for aromatic C = C bond, 1750-1800 cm^{-1} and 3200-3250 cm^{-1} for C = O and O-H bonds of carboxylic acid, 2000-2100 cm^{-1} for S-C bond tensions and 3450-3500 cm^{-1} for amine groups [29]. IR spectrum of sodium alginate (Figure 7) showed that the asymmetric and symmetric stress bands of the carboxyl groups at 1600-1650 cm^{-1} and 1400-1450 cm^{-1}. Vibration at 3400-3500 cm^{-1} was characteristic bands of O-H bonds of sodium alginate [30]. Also, the crosslinking of sodium alginate with the crosslinker (Ca^{+2}) was seen with a decrease in the wavenumber of the carbonyl peak and the intensity of the peaks associated with the carboxylate groups. The O-H peak of calcium alginate formed was associated with a higher wavenumber than sodium alginate. This was thought to be due to the effect on bond formation containing adjacent hydroxyl groups as a result of possible conformational changes of sodium alginate after it reacts with Ca^{+2} [30, 31]. The IR spectrum of HPMC K100 (Figure 7) showed that the characteristic vibrational stresses of O-H bond at 3400-3500 cm^{-1}, C-H bond alkanes at 2900-3000 cm^{-1}, and aliphatic C-O bond at 1100-1150 cm^{-1} [32].

https://dx.doi.org/10.29228/jrp.57
J Res Pharm 2021; 25(5): 653-666

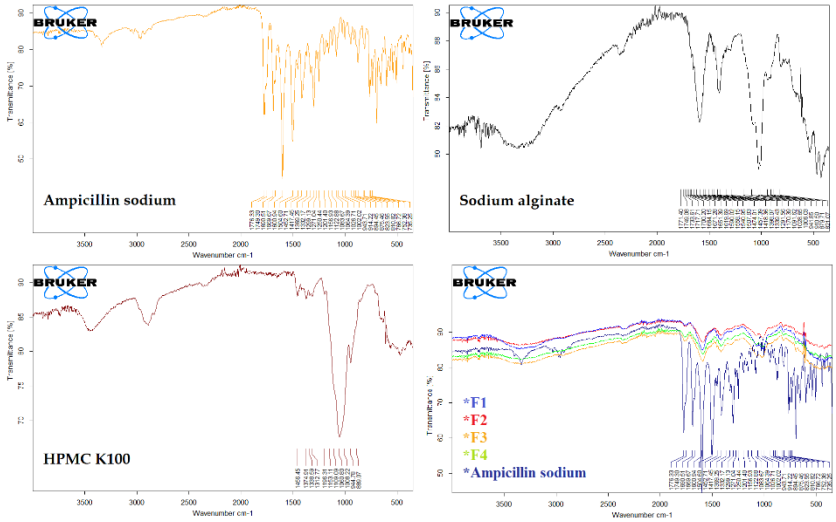

Figure 7. IR spectrums of ampicillin sodium, sodium alginate, HPMC K100 and hydrogel bead formulations.

The IR spectra of the formulations showed that the band voltages of ampicillin sodium, sodium alginate, and HPMC-K100 were suppressed, some of them disappear, but there was no change in the characteristic band voltages. In this case, it could be said that there was no undesirable interaction between the drug and the polymers, and the band tensions were reduced by encapsulating ampicillin sodium into the bead formulations. This situation was observed in similar studies in the literature. Huei et al. prepared carboxymethyl cellulose-gelatin beads containing ibuprofen and later suggested that the sharp band vibrations of ibuprofen in the IR spectra they received were suppressed due to the confinement of chitosan-gelatin beads [33]. Del Gaudio et al. had prepared alginate beads loaded with ketoprofen and had received IR spectra to study the polymer interaction with ketoprofen. It was concluded that the sharp band vibrations of ketoprofen in the spectra were reduced in bead formulations, the bonds in the ketoprofen molecule did not interact adequately with IR beams, and thus ketoprofen was encapsulated [34]. Similarly, Abdelmalek et al. interpreted the variations in band tension in the encapsulated ampicillin formulations in this direction [29].

2.9. XRD analysis of hydrogel beads formulations

X-ray diffraction analysis, Cu anode K alpha conditioned at 45 kV and 40 mA, X rays with a wavelength of 1.541874 Å were transmitted. XRD diffractograms of ampicillin sodium and formulations were given in Figure 8. While ampicillin sodium showed a dominant crystal phase with sharp and long diffractions in the diffractogram, this situation decreased in formulations. It was observed that ampicillin sodium was sharpened by throwing beads and suppressing long diffractions. From this, it could be concluded that ampicillin sodium was confined to beads and was not crystalline but converted to amorphous. This may have occurred during the preparation of the beads by the ionotropic gelation method. Iswandana et al. prepared chitosan beads containing tetrandrine and they attributed them to the suppression of XRD diffraction of tetrandrine in bead formulations and the reason for this being trapped in effective bead formulations [35]. Elsewhere, Mandal et al. studied alginate beads containing trimetazidine dihydrochloride and attributed the suppression of sharp refractions of the crystal structure of trimetazidine dihydrochloride in XRD diffractograms and its entrapment in the beads they prepared [19]. The same observations have been found in similar studies in the literature [21, 36-38].

3. CONCLUSION

Ampicillin sodium-containing sodium alginate/sodium alginate-HPMC K100 beads were successfully prepared by the ionotropic gelation method. It has been observed that HPMC K100 added to the formulations increases hydrophilicity and significantly changes water absorption and swelling capacity. Accordingly, due to the increase in the amount of $CaCl_2$, a decrease in bead size and a slowdown in the *in vitro* release rates were observed. As the amount of cross-linker entering the structure increased, the release of ampicillin sodium was delayed, and thus a prolonged release was obtained. It is predicted that by changing the amount of copolymer and cross-linker ratios to be used in oral transport of drugs with alginate beads, the duration of drug action will be extended and the drug-related side and/or toxic effects to be used less frequently, as well as its contribution to patient compliance, will be important.

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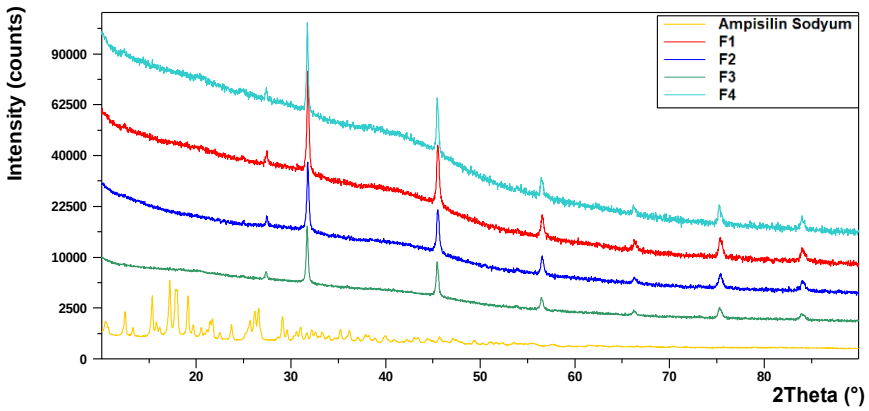

Figure 8. XRD diffractograms of ampicillin sodium and hydrogel bead formulations.

4. MATERIALS AND METHODS

4.1. Materials

Ampicillin sodium and HPMC K100 were a gift from İ.E. Ulagay İlaç Sanayii A.Ş. (Turkey) and Santa Farma İlaç Sanayii A.Ş. (Turkey). Sodium alginate, $CaCl_2$, HCl, KH_2PO_4 and NaOH were purchased from Alfa Aesar® (Germany), J. T. Baker (Holland), Isolab® (Germany), Sigma Aldrich (Germany) and Merck (Germany), respectively. All chemicals used were of analytical or pharmaceutical grade. Ultrapure water was used in all studies (Merck Millipore Direct-Q™ 3, Germany).

4.2. Preparation of hydrogel beads formulations

Ampicillin sodium-containing sodium alginate/sodium alginate-HPMC K100 hydrogel beads were prepared by the ionotropic gelation method. $CaCl_2$ was used as the crosslinking agent. For each formulation, ampicillin sodium-containing sodium alginate/sodium alginate-HPMC K100 dispersions were prepared by mixing at 500 rpm with the addition of ultrapure water. Variable amounts of $CaCl_2$ solutions were also prepared by adding ultrapure water at 500 rpm. After both mixture groups were prepared separately, polymeric mixtures with ampicillin sodium were added dropwise to $CaCl_2$ solutions at the same level and speed with the help of a 22G injector. After the dropping process was completed, it was mixed for a short time to harden the beads. The beads were then filtered. The formulations were washed twice with ultrapure water, and excess $CaCl_2$ was removed [16, 39]. Subsequently, the beads frozen at -20 °C overnight were dried by lyophilization (Lyophilizer, Martin Christ, Alpha 1-4 LD plus, Germany) under -55 °C and 79 mTorr pressure for 24 hours [40]. The obtained lyophilized beads were stored in a desiccator for further analysis and experiments. During the experiments, a minimum of six batches were studied in the dark conditions.

4.3. EE, LC and Y of hydrogel beads formulations

For each formulation, 100 mg of lyophilized beads were powdered in a mortar and mixed in 50 mL of pH 1.2 HCl buffer (USP30-NF25) for 24 hours at 750 rpm in a multipoint mixer (2mag Mix 15 Eco, Germany). Subsequently, it was passed through a mechanical homogenizer (IKA®, T-18 Digital Ultra-Turrax, Germany) at 13000 rpm for 3 minutes. After releasing ampicillin sodium from the beads, the bead residues were filtered through 0.45 µm membrane filters. The obtained supernatant was analyzed using a validated method with UV-VIS spectrophotometer (n=3; Shimadzu UV 1800, Japan). Dilutions were made with pH 1.2 HCl buffer. After the results were obtained, EE% (Equation 1), LC% (Equation 2), and Y% (Equation 3) were calculated as X̄±SD using the following equations. Also, validation parameters were given at Table 6.

$$EE\% = \frac{Theoretical\ ampicillin\ sodium\ amount - Experimental\ ampicillin\ sodium\ amount}{Theoreticalampicillin\ sodium\ amount} x\ 100\ \% \quad \text{(Eq. 1)}$$

$$LC\% = \frac{Theoretical\ ampicillin\ sodium\ amount - Experimental\ ampicillin\ sodium\ amount}{Obtained\ bead\ amount} x\ 100\ \% \quad \text{(Eq. 2)}$$

$$Y\% = \frac{Obtained\ bead\ amount}{Theoretical\ total\ formulation\ ingredients\ amount} x\ 100\ \% \quad \text{(Eq. 3)}$$

https://dx.doi.org/10.29228/jrp.57
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Table 6. Validation parameters of analytical method.

	pH 1.2 HCl buffer	pH 6.8 Phosphate buffer
Equation	y=0.0462x+0.0162	y=0.0523x-0.0222
R^2	0.9998	0.9998
λ	205	203
LOD (µg/mL)	0.627	0.282
LOQ(µg/mL)	1.901	0.855

* Accuracy and precision calculations were found to be less than ±2% in both buffer media.
** Recovery (%) calculations were not found less than 98% in both buffer media.

4.4. Shape, size and size distribution analysis of hydrogel beads formulations

Digital images of freshly prepared and lyophilized beads were taken with a camera. For the determination of size, randomly selected 100 beads from all bead formulations (blank and ampicillin sodium-containing), both in wet and lyophilized form, were measured individually using a manual caliper. With the data obtained, the mean particle size was calculated arithmetically (X̄±SD) [19].

4.5. *In vitro* release study of hydrogel beads formulations

In vitro release study of ampicillin sodium-containing lyophilized bead formulations (equivalent to 250 mg ampicillin sodium) was evaluated in 50 mL pH 1.2 HCl buffer (USP30-NF25) for 2 hours and then 50 mL pH 6.8 phosphate buffer (USP30-NF25) for 22 hours. This study was run at 50 rpm in a horizontal shaker water bath (Memmert WNB 14, Germany) set at 37±0.5 °C (n=6) [25]. At the time intervals determined for each formulation group (0.5, 1., and 2. hour for pH 1.2 HCl buffer; 4., 6., 8. and 24. hour for pH 6.8 phosphate buffer), 2 mL samples were taken, and each of filtered through 0.45 µm membrane filter. Then the samples were analyzed spectrophotometrically at 205 nm for pH 1.2 HCl buffer and at 203 nm for pH 6.8 phosphate buffer. By adding fresh medium at the same temperature as the amount of sample taken, it was ensured that sink conditions were maintained. Dilutions were made with *in vitro* release mediums (pH 1.2 HCl buffer or pH 6.8 phosphate buffer).

4.6. Determination of release kinetics of hydrogel beads formulations

In vitro release data require different equations and kinetic models to explain the kinetics of the release [41]. Release kinetics are important in understanding the absorption, distribution, metabolism, and excretion of the active substance after ingestion [42]. Some mathematical operations and equations are needed to explain the mechanism of drug release from beads. In order to determine the appropriate kinetic model, R^2 values were taken as the basis. The release profile results of all ampicillin sodium-containing bead formulations in pH 1.2 HCl buffer (USP30-NF25) and pH 6.8 phosphate buffer (USP30-NF25) were applied to Microsoft Office Excel program, and it has been determined which of them comply with zero order, first order, Korsmeyer-Peppas, and Higuchi models.

4.7. Determination of swelling capacity of hydrogel beads formulations

The swelling capacities of the ampicillin sodium-containing hydrogel beads were performed using 50 mL of 1.2 HCl buffer (USP30-NF25) in a horizontal shaker water bath (Memmert WNB 14, Germany) at 37 °C and 100 rpm. Fully weighed 50 mg bead formulations were evaluated. At certain time intervals (0., 0.5., 1., 2., 4., 8. and 24. hours), the changes in the weight of the beads taken by filtering from the swelling medium were recorded. Thus, their water absorption (swelling) capacities were found. The experiment was run at least three times (mean % ± SD). The weights of the beads before the experiment were compared with the weights after 24 hours, and the swelling capacity was determined as a percentage (Equation 4)[17, 35].

$$\text{Swelling capacity (\%)} = \frac{(W2 - W1)}{W1}\ x\ 100\ \% \qquad \text{(Eq. 4)}$$

W_1 = Beads weight before the experiment
W_2 = Beads weight after the experiment

4.8. Analysis of hydrogel beads formulations by SEM

The surface properties and morphology of lyophilized beads (blank and ampicillin sodium-containing) were investigated using SEM (Zeiss Sigma 300, Germany). Information was obtained about the size of the beads, surface porosity, and general morphology. Since the samples were non-conductive, they were covered with approximately 100 Å thick gold before measurement [21].

4.9. FT-IR analysis of hydrogel beads formulations

It is a characterization technique used to elucidate the composition of the chemical structure of substances and the arrangements of chemical bonds. It is carried out to determine whether there is an interaction between the active substance and the excipients and/or the method used [43]. FT-IR spectra in the wavenumber range 4000-400 cm^{-1} (Bruker VERTEX 70v, Germany) were taken from direct samples of pure ampicillin sodium, polymers, and all prepared bead formulations.

4.10. XRD analysis of hydrogel beads formulations

X-ray diffraction (XRD) analysis is one of the characterization techniques performed to determine whether there are changes in the crystal structure of the active substance after the preparation of the formulation [44]. X-ray diffractograms were made to identify changes in the crystal form of ampicillin sodium during the preparation of ampicillin sodium-containing beads. X-ray diffractograms of the prepared pure ampicillin sodium and all the formulations were taken from the known amount of dry powder samples (PANalytical Empyrean XRD, Netherlands).

4.11. Statistical analysis

The statistical measurements were calculated as mean and standard deviation. Also, the statistical evaluations between the samples in the analyses and the experiments were evaluated with the "Mann-Whitney U" test and the "One-Way Analyses of Variance (ANOVA)" test (according to the homogeneity of the variances and the size of the population). Results at the $p < 0.05$ level were considered significant.

Acknowledgements: Authors would like to thank to East Anatolian High Technology Research and Application Center (DAYTAM) of Ataturk University for SEM, FT-IR and XRD study.

Author contributions: Concept – R.S.Ö., E.Ö.; Design – R.S.Ö., E.Ö.; Supervision – R.S.Ö., E.Ö.; Resources – R.S.Ö., E.Ö.; Materials – R.S.Ö., E.Ö.; Data Collection and/or Processing – R.S.Ö., E.Ö.; Analysis and/or Interpretation – R.S.Ö., E.Ö., R.S.Ö., E.Ö.; Literature Search – R.S.Ö., E.Ö.; Writing – R.S.Ö., E.Ö.; Critical Reviews – R.S.Ö., E.Ö.

Conflict of interest statement: The authors declared no conflict of interest.

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