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In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads

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ABSTRACT

In recent years, the use of swelling polymeric matrices for the encapsulation and controlled release of protein drugs has received significant attention. The purpose of the present study was to investigate the release of albumin, a model protein from alginate/hydroxypropyl-methylcellulose (HPMC) gel beads. A hydrogel system comprised of two natural, hydrophilic polymers; sodium alginate and HPMC was studied as a carrier of bovine serum albumin (BSA) which was used as a model protein. The morphology, bead size and the swelling ratio were studied in different physical states; fully swollen, dried and reswollen using scanning electron microscopy and image analysis. Finally the effect of different alginate/HPMC ratios on the BSA release profile in physiological saline solution was investigated. Swelling experiments revealed that the bead diameter increases with the viscosity of the alginate solution while the addition of HPMC resulted in a significant increase of the swelling ratio. The BSA release patterns showed that the addition of HPMC increased the protein-release rate while the release mechanism fitted the Peppas model. Alginate/HPMC beads prepared using the ionic gelation exhibited high BSA loading efficiency for all formulations. The presence of HPMC increased the swelling ability of the alginate beads while the particle size remained unaffected. Incorporation of HPMC in the alginate gels also resulted in improved BSA release in physiological saline solution. All formulations presented a non-Fickian release mechanism described by the Peppas model. In addition, the implementation of non-parametric tests showed significant differences in the release patterns between the alginate/HPMC and the pure alginate beads, respectively.

1. Introduction

Nowadays there is an enormous demand to develop delivery systems for protein and peptide drugs since they are becoming a very important class of therapeutic agents. However, there are several problems associated with the administration of the protein dugs. Such drug agents present short in half lives degrade by enzymes and poorly pass through biological barriers due to their diffusivity and low partition coefficient (Lee, 1988). For these reasons the entrapment of protein drugs within various delivery systems, using different biodegradable and biocompatible polymers has been studied. The most referred delivery platforms are the preparation of microspheres through microencapsulation methods (Arshady, 1991; Couvreur, Blanco-Prieto, Puisieux, Roques, & Fattal, 1997; Heller, 1993; Jalil & Nixon, 1990; Langer, 1990; Langer & Folkman, 1976; O'Donnell & McGinity, 1997; Zhou & Wan Po, 1991) or superporous hydrogels (Dorkoosh, Borchard, Rafiee-Tehrani, Verhoef, & Junginger, 2002; Dorkoosh et al., 2001; Dorkoosh, Verhoef, et al., 2002).

Alginates are natural polysaccharides extracted from brown sea weed, composed of linear chains of the the α-L-guluronic acid (G) and the β-D-mannuronic acid (M). Alginates are anionic compounds and one of their most important features is the capability to form hydrogels in the presence of divalent cations like Ca^{2+} (Bajpai & Sharma, 2004; Ouwerx, Velings, Mestdagh, & Axelos, 1998).

Alginate hydrogels are considered biocompatible materials (Klock et al., 1997) with mucoadhesive properties (Gombotz & Wee, 1998) and have been found applicable in several pharmaceutical and biotechnological systems. For instance, they have been widely used in controlled delivery of proteins or drug molecules (Bodmeier & Paeratakul, 1989; Fernández-Hervás, Holgado, Fini, & Fell, 1998; Gombotz & Wee, 1998; Hari, Chandy, & Chandra, 1996; Rasmussen, Snabe, & Pedersen, 2003; Sezer & Akbuga, 1999) cell encapsulation systems (Reyes, Rivas-Ruiz, Domínguez-Espinosa, & Solís, 2006), and scaffolds for tissue or organ regeneration (Seal, Otero, & Panitch, 2001).

Hydroxypropyl-methylcellulose (HPMC) has been extensively used in oral drug delivery systems as a hydrophilic carrier. It presents several characteristics such as high swellability and surface activity (Chang & Gray, 1978; Siepmann & Peppas, 2001). The first characteristic has an important effect on the drug release kinetics since the contact with water or biological fluid results in drug

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diffusion into the medium leading to polymer chain relaxation with volume expansion (Brannon-Peppas, 1990). The latter caused polymer adsorption onto the drug surface (Brannon-Peppas & Peppas, 1990). In particularly, cellulose ethers containing methoxyl or hydroxypropyl groups are adsorbed onto hydrophobic drug surfaces (Rasenack, Müller, & Hartenhauer, 2003). HPMC has been successfully introduced as a rate controlling polymer in solid dispersions (Meshali & Gabr, 1992; Ohara, Kitamura, Kitagawa, & Terada, 2005; Serajuddin, 1999; Won, Kim, Lee, Park, & Hwang, 2005) of numerous drugs. Recently the alginate/HPMC mixture was used as an in situ gelling vehicle to enhance ocular bioavailability and patient compliance (Liu et al., 2006).

The purpose of the present study was to investigate the release of albumin, a model protein from alginate/HPMC gel beads. Particularly, this study focuses on the effect of different alginate/HPMC formulations on the release behavior within physiological saline solution. In addition, the swelling behavior and the bead size of different alginate/HPMC formulations have been examined.

2. Materials and methods

2.1. Materials

Low-viscosity (250 cps of 2% solution) alginic acid sodium salt (NaAlg), and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), were purchased from Sigma–Aldrich (Athens, Greece). Hydroxypropylmethyl cellulose (HPMC, Viscosity 3 cp, Pharmacoat 603) was purchased from Shin–Etsu Chemical Co., Ltd. (Tokyo, Japan) while Bovine Serum Albumin (BSA, Fraction V, pH: 7) from Serva.

2.2. Preparation of Alg/HPMC beads

Sodium alginate (NaAlg) gels in ultrapure water (conductivity <0.1 $\mu S\,cm^{-1}$) containing HPMC or/and BSA were prepared by weighting the respective solids. The gels were prepared under magnetic stirring and finally they were introduced into an ultrasonic water bath for 10 min to remove bubbles.

The formation of the hydrogel beads was based on the ionic gelation technique. Using a 10 ml syringe the NaAlg was transferred dropwise from a distance of 10 cm into a solution of $CaCl_2 \cdot 2H_20$ (0.2 M) which was under magnetic stirring. Hydrogel beads were formed instantly and they were left in contact with the solution for 30 min in order to complete the gelation. Finally they were rinsed gently with ultrapure water and dried at 37 °C. Seven different formulation were prepared, with various NaAlg:HPMC ratios and constant BSA concentrations (1%). The prepared formulations are summarized in Table 1.

2.3. Morphological studies

The size and the morphology of the produced beads were studied using digital photography and scanning electron microscopy. To determine the mean bead diameter, each bead formulation was photographed using a digital camera at three different states: swollen state (immediately after preparation), dry state and reswollen state (in physiological saline for seven hours). Digital photographs were analyzed with the Image Tool Version 3.0 (Wilcox, Dove, McDavid, & Greer, 2002) program by measuring at least 30 beads of each formulation. Detailed morphological analysis was performed by using scanning electron microscopy (SEM, Jeol JSM-5200). Representative samples of each formulation were dehydrated through a series of ethanol/water mixtures varying from 25% to 100% ethanol and finally covered with a thin gold layer.

2.4. Swelling studies

Swelling studies were conducted in dry beads after remaining in physiological saline solution for seven hours. The beads were removed from the solution using a stainless steel grid wiped gently with a tissue paper and weighted on an analytical balance. The swelling ratio Q_s was expressed by their ability to absorb water and calculated using the following equation.

$$Q_s = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

where W_d is the is the weight of the bead dry state and W_s the weight in the swollen state.

2.5. Drug release experiments

The *in vitro* release studies were performed in physiological saline solution (0.9% w/v NaCl) at 37 ± 0.1 °C. Accurately weighted amounts of beads were placed in covered glass vials containing 25 ml of physiological saline solution. Samples of 50 μl were taken from the release medium at specific time intervals for a total period of 7 h and they were replaced with the same amount. Each sample was treated with the Bradford reagent (Sigma–Aldrich, Athens, Greece) and measured at 595 nm in a spectrophotometer (Hitachi U-2800). BSA concentration in the unknown samples was measured using a calibration curve created by known BSA concentration solutions.

The drug encapsulation efficiency was determined using the following equation.

$$\text{Drug encapsulation efficiency}(\%) = \frac{M_i - M_d}{M_i} \times 100\% \quad (2)$$

where M_i is the initial amount of BSA dissolved in the alginate solution and M_d the amount of alginate mass measured in the gelling media ($CaCl_2 \cdot 2H_20$ solution) plus the water used to wash the beads after their preparation.

2.6. Dissolution data analysis

Albumin release kinetics was analyzed by various mathematical models, which were applied considering the amounts of drug released from 0 to 120 min. For that reason SigmaPlot 10.0 software (Systat Software Inc., Germany) was implemented. Table 2 presents the models tested. GraphPad Instat (GraphPad Software Inc., San Diego, USA) was used to compare the dissolution profiles

Table 1
Composition of the prepared Alg/HPMC formulations and their BSA encapsulation efficiency

Formulation	NaAlg (%) (w/v)	HPMC (%) (w/v)	Encapsulation efficiency (%)
(2–0)	2	0	68.27
(2–2)	2	2	68.21
(3–0)	3	0	69.10
(3–1)	3	1	67.45
(3-3)	3	3	65.46
(4–0)	4	0	67.85
(4–4)	4	4	69.16

Table 2
Applied release models

Model	Equation
Baker–Lonsdale	$\frac{2}{3}\left[1-\left(1-\frac{F}{100}\right)^{2/3}\right]-\left(\frac{F}{100}\right)=kt$
First order	$F = 100(1-e^{-k_1 t})$
Higuchi	$F = k_H\sqrt{t}$
Hixson–Crowell	$F = 100\left[1-(1-k_{HC}t)^3\right]$
Peppas	$F = k_p t^n$

F, amount of drug released in time t, k_{LB}, k_1, k_H, k_{HC}, k_P release rate constants, n release exponent.

of the solid dispersions and pure drugs. Comparisons were performed using the Mann–Whitney, non-parametric two-tailed *P* test.

3. Results and discussion

3.1. Bead size and morphology

Fig. 1 shows a series of representative digital images of NaAlg/HPMC beads, categorized by the composition and the swelling state (fully swollen, dry and reswollen). It can be seen that in the fully swollen state, the beads of all systems are semitransparent with almost perfect spherical shape. Reswollen beads were opaque and presented smaller particle size with less spherical shape in contrast to the fully swollen state. Similarly, dry beads showed less spherical shape and a noticeable reduced size. The mean diameter of the prepared beads is shown in Fig. 2. It can be seen that addition of HPMC in the formulations has not significant effect on the mean particle size of all physical states. The only significant difference was observed in 4–0 and 4–4 preparations in the reswollen state.

Careful examination of representative beads of each formulation using SEM microscopy revealed more detail information regarding their external and internal morphological features. As it can be seen from the SEM images (Fig. 3) the beads presented a rough surface with characteristic large wrinkles and micropores. The presence of HPMC in Figs. 3e and 3f has not any impact on the external morphology of the beads and no specific localizations of the HPMC or BSA were observed in the matrix.

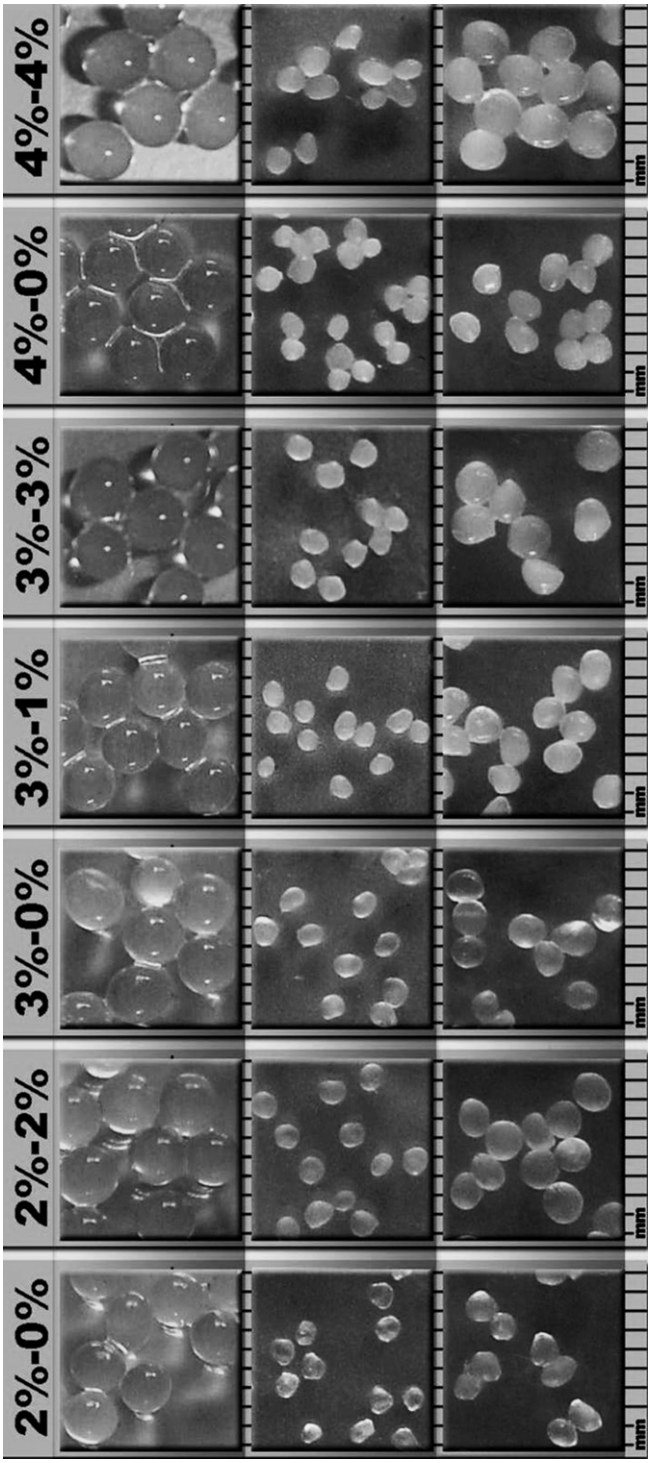

Fig. 1. Representative digital photographs of the seven NaAlg: HPMC formulations in the three different states (from left to right): swollen, dry and reswollen.

3.2. Swelling studies

Fig. 4 show the swelling degree of pure calcium alginate and alginate/HMPC beads after incubation in physiological saline solution for 7 h. It was observed that pure calcium alginate beads exhibit a swelling degree ranging from 118% to 151%. Swelling of the wet beads can be explained due to the absorption of free or bulk water that fills the void regions of the polymer network and/or the centre of larger pores and macropores (Hoffman, 2002). Alginate/HPMC beads exhibited additional swelling in saline solution than pure alginate beads. With an exception to the 2–2 and 2–0 preparations it was clear that the presence of HPMC increases the swelling degree of the prepared beads. This was obviously observed for the 4–4 and 3–3 compositions, with increased HPMC concentration, in comparison to the 4–0 and 3–0. The above behavior can be explained due to the high hydrophilicity and swellability of HPMC. It has been reported that HPMC is the dominant hydrophilic polymer that swells to a significant extent upon contact with water (Siepmann & Peppas, 2001).

3.3. Drug encapsulation efficiency

The amount of BSA encapsulated in the beads was approximately the same for the prepared systems varying from 65.4%

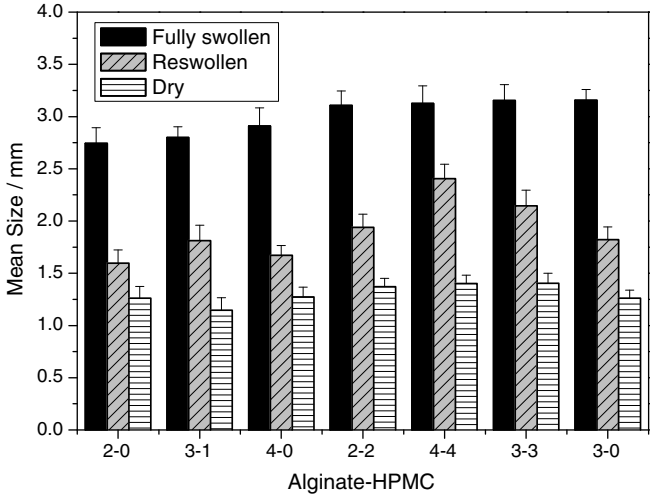

Fig. 2. Measured bead gel size in three different states prepared using the formulations shown in Table 1.

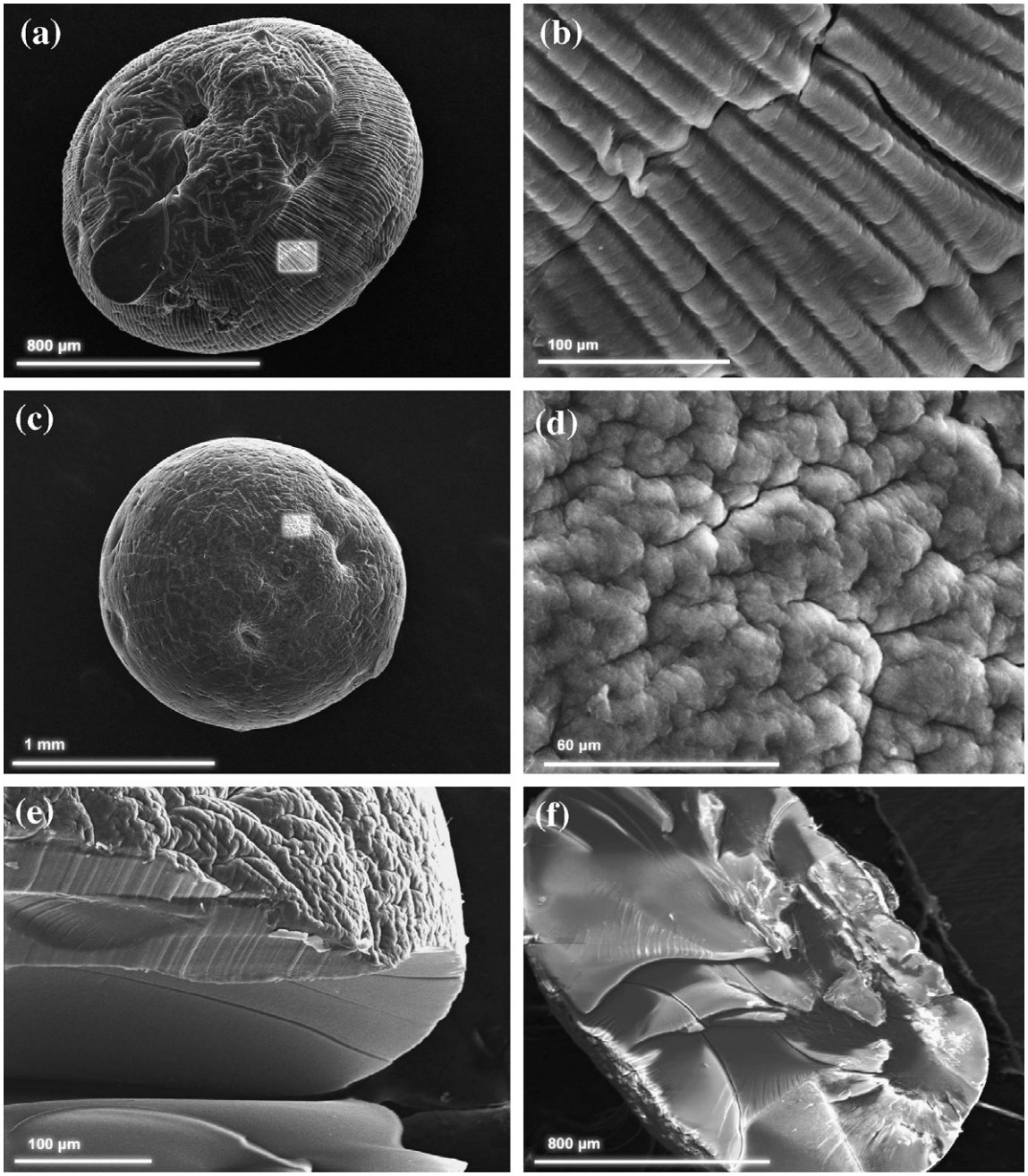

Fig. 3. SEM photographs: (a) calcium alginate bead prepared from 2 to 0 formulation (b) magnification of the area indicated in picture (a and c) beads prepared using the 3–0 formulation (d) Magnification of the area indicated in picture (c and e) cross section and surface details of a bead prepared from 2 to 2 formulation and (f) section of a bead made using 4–4 formulation.

to 69.1% as shown in Table 1. These findings indicate that the encapsulated efficiency depends on the alginate content from 2% to 4% and also on the alginate/HPMC ratio. High encapsulation efficiency of BSA in alginate matrices was observed also by Lemoine, Wauters, Bouchend'homme, and Préat (1998). BSA was encapsulated in calcium alginate microspheres prepared by an emulsification technique. The encapsulation efficiency of BSA varied from 61% to 92% depending on the BSA initial loading. In another study, Coppi, Iannuccelli, Leo, Bernabei, and Cameron (2002) demonstrated that BSA loading at a pH value lower than the protein isoelectric point (pI) was higher than that at a pH similar to the pI due to an electrostatic interaction between the charged protein and the polyanionic alginate. Furthermore, the encapsulated efficiency at pH values higher than the isoelectric point of the protein is related to the capacity of polymeric chains to entrap the protein. The same authors also showed that another parameter that affects the protein encapsulation is the alginate/BSA ratio. In particular, when the initial solution the percentage of alginate is increased or equivalently the percentage of BSA is decreased then a greater amount of protein is entrapped. Especially for very large values of the aforementioned ratio the drug loading efficiency has been reported to reach almost 90%. In another study using the ionic gelation method alginate/chitosan microspheres were found to encapsulate more than 98% of the water-soluble drug sodium diclofenac and the encapsulation efficacy was neither affected by the alginate amount nor the cross-linking ion used (Gonzalez-Rodrıguez, Holgado, Sanchez-Lafuente, Rabasco, & Fini, 2002).

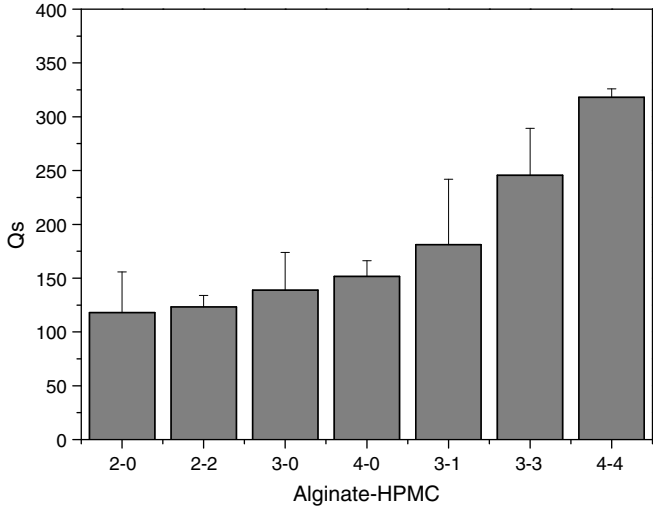

Fig. 4. Swelling ratio of wet beads after immersion in physiological saline solution for 7 h.

3.4. Drug release experiments

Fig. 5 shows the cumulative BSA release profiles for the formulations with total alginate and HPMC solid mass of 4%. Only 5% of the entrapped BSA was released after 7 h from the 4–0 system while about 42% was released from the 2–2 system indicating that the presence of HPMC substantially increases the release rate.

The same effect was also observed when the alginate percentage was increased in parallel with the HPMC percentage while the ratio of the two polymers remained constant (1:1). This can be clearly observed in Fig. 6 where the increase of the HMPC ratio led to enhanced release rates for different combinations.

The overall release studies showed that the incorporation of HPMC, even at small ratio, in the alginate solution altered the release of albumin. This can be attributed to the high swellability of HPMC. In terms of the structure of the polymeric network this is associated with the interactions between alginate, HPMC and the protein. The polymeric network is dominated by physical entanglements between the albumin and alginate or HPMC chains rather than electrostatic interactions. BSA loading was prepared at

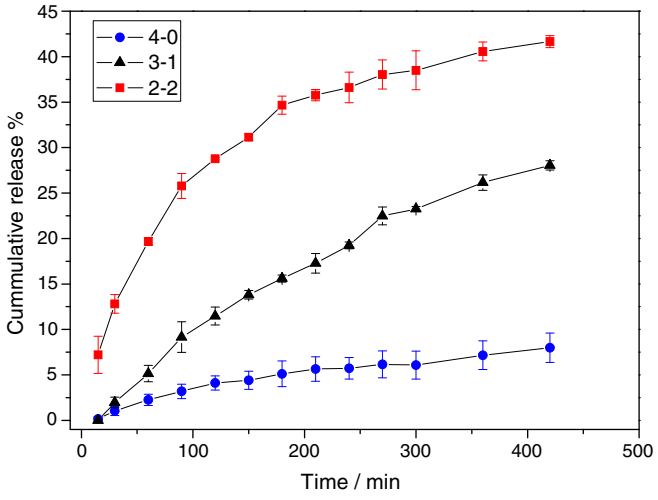

Fig. 5. Cumulative release profiles of BSA in physiological saline solution, from preparations with total solid mass of 4%.

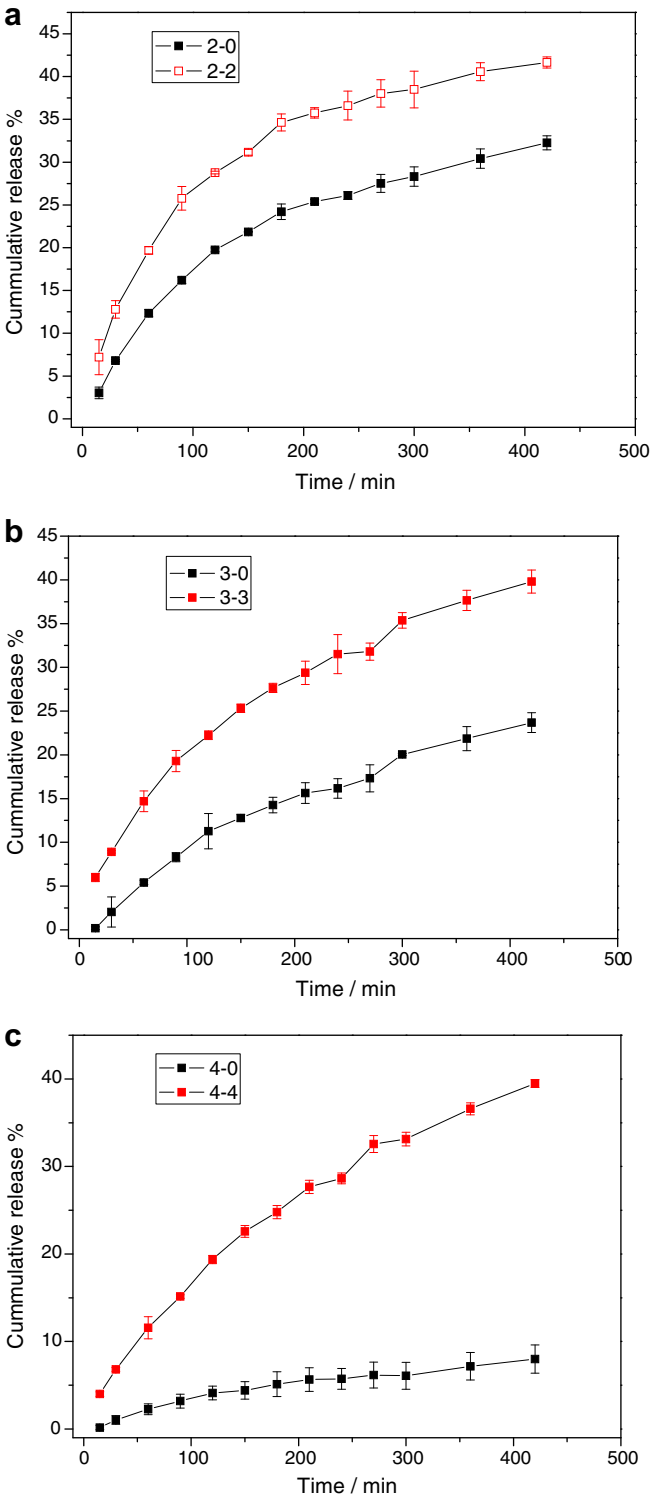

Fig. 6. Cumulative release of BSA in physiological saline solution from pure calcium alginate beads and the corresponding alginate/HPMC beads at a ratio of 1:1.

values higher than the isoelectric point of the protein. For example in the case of the 2–0 formulation the pH of the medium before the crosslinking with calcium was measured at 7.1 and was not affected by the presence HPMC or albumin. At pH 7.0 albumin is negatively charged since has an isoelectric point of 4.7. Furthermore, at the same pH alginates are in the form of polyanion while HPMC is non-ionic suggesting that electrostatic interactions are weak (Liu

et al., 2004; Wells & Sheardown, 2007). When the alginate/HMC matrix is exposed to the dissolution medium it swells rapidly resulting enhanced drug diffusion outside the gel matrix. The albumin release (Figs. 5 and 6) was also improved with decreased polymer amounts of alginate–HP2MC mixtures or pure alginate, respectively.

Protein release from pure alginate or alginate reinforced matrices depends on many factors such as pH, ionic strength, dissolution media and counterion concentration. BSA and Interleukin-2 release from three different formulations of 2% w/w alginate beads was studied by Liu et al. (1997). The results showed that protein-release rate can be controlled by changing the counterion used. In particular, albumin release was dominated by a large initial burst and nearly 100% BSA was released from the alginate/$CaCl_2$ microspheres in about 6 h while 75% was released from the alginate/polylysine microspheres in 6 h, followed by release of the remaining protein in 24 h. Furthermore, albumin release from alginate/chitosan microspheres was prolonged compared to the other two formulations and was characterized by a 45% release in 6 h followed by a steady release for about 4 days (Liu et al., 1997). The slower release profiles observed in the present work compared to the aforementioned study should be attributed to the different release medium since PBS medium was used and to the porous structures of the prepared beads resulted from the lyophilization of the formed gel. Phosphate containing media lead to degradation of alginates while porous structures provide more surface area and hence exhibit higher release capacity.

3.5. Drug release kinetics

The drug release kinetics from alginate beads were estimated by using the equations presented in Table 1 with the non-linear regression model of SigmaPlot 10.0. The parameters calculated by these models and the determination coefficients (R^2) obtained are summarized in Table 2.

Based on these estimations the fit of each model was predicted. Considering the R^2 values, the calculated Peppas model (Siepmann & Peppas, 2001) successfully fitted the HPMC–alginate formulations. In spherical matrices, if $n \leqslant 0.43$, a Fickian diffusion (case-I), $0.43 \leqslant n < 0.85$, a non-Fickian transport and $n \geqslant 0.85$, a case-II transport (zero order) drug release mechanism dominates (Ritger & Peppas, 1987; Varshosaz & Keihanfar, 2001). An anomalous (non-Fickian) mechanism became apparent for all formulations. However, the addition of HPMC increased the diffusivity of albumin in the matrix in the medium without altering the release mechanism. All the other models such as first order (Wagner, 1969; Wagner, 1971), Higuchi (Higuchi, 1961; Higuchi, 1963), Baker–Lonsdale (Baker & Lonsdale, 1974) as well as the Hixson–Crowell model (Hixson & Crowell, 1931; Niebergall, Milosovich, & Goyan, 1963) were not able to fit the albumin release profiles. As it can be seen in Table 3 there are some formulations where the Higuchi and Baker–Lonsdale also fitted the release profiles. In this case the selection of an adequate model was based on comparisons of the following features of each model: higher determination coefficient; smaller standard error of model parameters; and smaller residual mean square (Yuskel, Kanik, & Baykara, 2000). On the basis of these comparisons, Peppas model fitted best for all release profiles respectively. The significance of HPMC in the release patterns was examined by comparing the three selected release couples (2–0/2–2, 3–0/3–3 and 4–0/4–4). Comparisons were performed using the Mann–Whitney, non-parametric two-tailed P test. In each case the P value ($p < 0.05$) indicated significant differences between the HPMC–alginate mixtures and the pure alginate beads.

Table 3
Release rate constants and determination coefficients of the produced formulation

Dissolution models		2–0	2–2	3–0	3–1	3–3	4–0	4–4
Baker–Lonsdale	K_{BL} (min^{-1})	$5.2 \times 10^{-5} \pm 2.2 \times 10^{-6}$	$1.0 \times 10^{-4} \pm 5.0 \times 10^{-6}$	$2.1 \times 10^{-5} \pm 1.8 \times 10^{-6}$	$2.8 \times 10^{-5} \pm 2.8 \times 10^{-6}$	$7.8 \times 10^{-5} \pm 2.0 \times 10^{-6}$	$2.3 \times 10^{-6} \pm 1.4 \times 10^{-7}$	$6.7 \times 10^{-5} \pm 3.7 \times 10^{-6}$
	R^2	0.9686	0.9525	0.913	0.8828	0.9876	0.9452	0.9568
First order	k_1 (h^{-1})	$1.2 \times 10^{-3} \pm 8.2 \times 10^{-5}$	$1.8 \times 10^{-3} \pm 2.0 \times 10^{-4}$	$7.0 \times 10^{-4} \pm 2.6 \times 10^{-5}$	$9.0 \times 10^{-4} \pm 2.8 \times 10^{-5}$	$1.5 \times 10^{-3} \pm 9.1 \times 10^{-5}$	$2.0 \times 10^{-4} \pm 1.2 \times 10^{-5}$	$1.4 \times 10^{-3} \pm 5.7 \times 10^{-5}$
	R^2	0.7394	0.5203	0.9456	0.9789	0.8057	0.8498	0.9456
Higuchi	k_H (% min^{-1})	1.66 ± 0.033	2.31 ± 0.060	1.07 ± 0.04	1.24 ± 0.06	2.0 ± 0.02	0.37 ± 0.01	1.87 ± 0.04
	R^2	0.9676	0.9257	0.9222	0.8958	0.9905	0.9472	0.9701
Hixson–Crowell	k_{HC} ($min^{-1/3}$)	$4.0 \times 10^{-4} \pm 2.6 \times 10^{-5}$	$6.0 \times 10^{-4} \pm 4.9 \times 10^{-5}$	$2.0 \times 10^{-4} \pm 8.9 \times 10^{-6}$	$3.0 \times 10^{-4} \pm 7.4 \times 10^{-6}$	$5.0 \times 10^{-4} \pm 2.9 \times 10^{-5}$	$7.6 \times 10^{-5} \pm 4.0 \times 10^{-6}$	$4.0 \times 10^{-4} \pm 1.9 \times 10^{-5}$
	R^2	0.6953	0.4211	0.9358	0.9722	0.7588	0.8442	0.9034
Peppas	k_p (min^{-n})	1.64 ± 0.34	3.86 ± 0.70	0.33 ± 0.08	0.25 ± 0.06	1.85 ± 0.20	0.18 ± 0.04	0.98 ± 0.11
	R^2	0.9676	0.957	0.9796	0.9844	0.9909	0.9731	0.9929
	n	0.50 ± 0.04	0.51 ± 0.03	0.72 ± 0.05	0.79 ± 0.04	0.51 ± 0.02	0.63 ± 0.04	0.62 ± 0.02

4. Conclusions

In concluding the present study, the ionic gelation method was used to prepare calcium alginate beads with or without the incorporation of HPMC. The beads exhibited high BSA loading efficiency for all formulations. The presence of HPMC increased the swelling ability of the alginate beads while the particle size remained unaffected. Incorporation of HPMC in the alginate gels also resulted in improved BSA release in physiological saline solution. All formulations presented a non-Fickian release mechanism described by the Peppas model. In addition, the implementation of non-parametric tests showed significant differences in the release patterns between the alginate/HPMC and the pure alginate beads, respectively.

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