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

Argyrios Nochos [a], Dionysios Douroumis [b], Nikolaos Bouropoulos [a,*]

[a] Department of Materials Science, University of Patras, Patras GR-26504, Greece
[b] Medway School of Science, Department of Pharmaceutical, Chemical and Environmental Sciences, University of Greenwich, Chatham Maritime ME4 4TB, Kent, United Kingdom

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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.

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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

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 $CaCl_2 \cdot 2H_2O$ (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.

$$Q_s = \frac{W_s - W_d}{W_d} \times 100 \tag{1}$$

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.

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

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 ($CaCl_2 \cdot 2H_2O$ 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	$\frac{2}{3}\left[1 - (1 - \frac{F}{100})^{2/3}\right] - (\frac{F}{100}) = kt$
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.