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Drug delivery matrices based on scleroglucan/alginate/borax gels

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Abstract

The aim of this work is to obtain a new drug delivery matrix, especially designed for protein delivery, based on biodegradable and biocompatible polymers, and to describe its main physico-chemical properties. A polysaccharide based semi-interpenetrating polymer network (semi-IPN) was built up, composed by sodium alginate chains interspersed into a scleroglucan/borax hydrogel network. Tablets were obtained by compression of the resulting freeze-dried hydrogel. The different release and physico-chemical properties possessed by the two starting polymers in various aqueous media were combined in the new matrix. In this work, description is given of the in vitro ability of the matrix to deliver in a controlled manner a protein, Myoglobin, in distilled water, simulated gastric fluid and simulated intestinal fluid; the release, simulating a gastric passage, followed by an enteric delivery, was also carried out. Water uptake data, colorimetric experiments and scanning electron microscopy images are given for the characterization of this new solid dosage form; the importance of the borax presence is also discussed.

Keywords: Scleroglucan; Alginate; Solid dosage form; Hydrogel; Modified release; Semi-IPN

1. Introduction

Scleroglucan (Sclg) and alginate (Alg) are well known polysaccharides and their physico-chemical properties, as well as their practical and industrial applications, are extensively described in the literature.

Sclg is a microbial non-ionic polysaccharide; it exhibits a backbone built up by (1 → 3) linked β-D-glc*p* units with single glc*p* side chains linked β-(1 → 6) to every third residue in the main chain (Giavasis et al., 2002). The range of the actual and of the potential applications of the native Sclg and of its derivatives, is very wide; its resistance to hydrolysis and its rheological behaviour made this polymer useful in the secondary oil recovery, in agriculture, in cosmetics, in the field of food additives and in pharmaceutics (Coviello et al., 2005a). As far as this last topic is concerned, native Sclg has been proposed for the preparation of water-swellable matrices for drug delivery (Touitou et al., 1989; Alhaique et al., 1990; Ritz et al., 1994) and as a thickening agent for ocular formulations (Romanelli et al., 1993). On the other side, Sclg crosslinked with aliphatic residues, giving a chemical network (Coviello et al., 1999, 2001), or co-crosslinked with another polysaccharide, giving a physico-chemical gel (Coviello et al., 1998), led to the formation of networks suitable for modified release formulations; furthermore, oxidized Sclg derivatives were able to form physical gels that have been studied for sustained drug delivery systems (Coviello et al., 2005b). Recently, a new matrix based on Sclg/borax hydrogel was developed (Coviello et al., 2003, 2005c); such new system was prepared starting from the freeze-dried gel obtained from the interaction between Sclg and borax, a crosslinker already used with other polysaccharides and synthetic polymers containing hydroxyl groups (Deuel et al., 1948; Ochiai et al., 1981; Rubinstein and Gliko-Kabir, 1995; McLaughin et al., 1997). The tablets, prepared with this polymeric matrix, exhibit a peculiar behaviour, as the water uptake leads to a remarkable anisotropic swelling of the tablets in the direction of the compression (Coviello et al., 2003). The release profiles of model drugs (i.e. Theophylline, Vitamin B12 and Myoglobin) (Coviello et al., 2005c) loaded in the matrices were strongly dependent on the molecular size of the drug: the smaller the van der Waals radius of the guest molecule, the faster the release. Furthermore, for each model drug the delivery followed almost the same profile, irrespective of different release media, i.e. distilled water, simulated gastric fluid (SGF) or simulated intestinal fluid (SIF).

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As far as the other polymer studied in the present paper is concerned, Alg, there is an extremely vast literature on this subject. The polysaccharide can be extracted from marine brown algae or it can be produced by bacteria. It exhibits a backbone of (1 → 4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues of widely varying composition and sequence. This polymer can be regarded as a true block copolymer composed of homopolymeric regions of M and G, called M- and G-blocks, respectively, interspersed with regions of alternating structure. It was found that the physico-chemical properties of Alg are highly affected by the M/G ratio as well as by the structure of the alternating zone (Draget et al., 1996). The presence of the uronic residues (carboxylic acid moiety on the C-6 of every sugar ring) and the gelling properties in aqueous solution by addition of divalent cations or by lowering the pH, make this polysaccharide very versatile. It is actually widely used for technical applications (e.g. as solution thickener, in textile printing, in paper coating), in food industry (as gelling agent), in biomedicine (e.g. wound dressing, dental impression material, entrapment of cells in calcium Alg beads) (Draget et al., 2002; Rehm, 2002). Alg, both in its sodium salt and in the acidic form of the carboxylic acid moiety, is generally used also in drug delivery systems as binding and disintegrating agent in tablets, as a suspending and thickening agent in water-miscible gels, in lotions and creams, and as a stabilizer for emulsions (Tønnesen and Karlsen, 2002). Furthermore, drug delivery properties of calcium Alg gels (beads and microspheres) have been specifically studied for protein delivery (Dashevky, 1998; Gombotz and Wee, 1998) and cell encapsulation (Brissova et al., 1998; Smidsrød and Skjåk-Bræk, 1990). Alg can also play a significant role in the design of tablets for modified release. The Alg chains generally undergo an almost immediate hydration forming, around the matrices, an imbibition layer of high viscosity, slowing down the diffusion rate of the entrapped drug molecules. This effect is more pronounced when cationic drugs are delivered in acidic medium, in accordance to the anionic nature of Alg and its ability to give gels at low pH values (Hodson et al., 1995; Park et al., 1998). Alg indeed was already proposed as coating material for capsules in order to improve their resistance in the gastric fluid and to deliver the drug into the intestine (Narayani and Rao, 1995). Alg calcium beads coated with another polysaccharide, Chitosan, were also prepared in order to improve their mechanical and drug release properties; in particular protein release was inhibited in the gastric fluid (Coppi et al., 2001; Anal et al., 2003). In this last case the strong interaction between the protonated form of the Chitosan amino groups and the carboxylic moieties of Alg leads to the formation of an electrostatic complex considered as responsible for the delayed delivery (Zhang et al., 2004). Furthermore, semi-interpenetrating polymer networks (semi-IPN) based on Alg chemically or physically crosslinked with Chitosan derivatives were prepared to form pH sensitive hydrogels (Chen et al., 2004; Lin et al., 2005).

In order to prepare an oral dosage form specific for the release in the intestine and capable to protect drugs in the gastric environment, new semi-IPNs based on Alg and Sclg are presented in this paper. Tablets prepared from the gels obtained by dissolution of Alg in the Sclg/borax network exhibit a strong pH dependent release of a model protein (Myoglobin). At the same time also the mechanical properties of the tablets are strongly affected by the environmental pH value. With this new solid dosage form, the different solubilization tendency of Alg in acidic and neutral media together with the Sclg/borax gel properties are exploited in order to modulate the release behaviour of the model drug.

In order to characterize the behaviour showed by this novel matrix, colorimetric experiments and water uptake measurements were performed in different aqueous media. Scanning electron microscopy (SEM) studies were also performed on Sclg/Alg/borax tablets after their immersion in different aqueous media in order to evaluate the effect of pH on the surface of this new oral dosage form.

2. Materials and methods

2.1. Materials

Sclg was a generous gift of Degussa (USA) ($M_w = 10^6$). Alginic acid was provided by Carbomer (USA). The Alg sodium salt form of the Alginic acid (65% L-guluronic acid, 35% D-mannuronic acid content, according to manufacturer) was prepared by titration with NaOH. Myoglobin (MGB) was a Fluka (Italy) product and borax was provided by Carlo Erba (Italy). All the other products and reagents were of analytical grade. Distilled water was always used.

2.2. Hydrogel preparation

The gels were prepared starting always from a Sclg solution obtained by magnetic stirring of the appropriate amount of Sclg in distilled water for 24 h. For the preparation of the Sclg–Alg system, an appropriate amount of Alg was added to the Sclg solution and stirred for, at least, two further hours. According to a modified procedure already described (Coviello et al., 2005c), the hydrogel formation was obtained by addition of a calculated amount of 0.1 M borax solution to the polymers system previously prepared to obtain a molar ratio $r = 1$ (i.e. moles of borax = moles of repeating units of Sclg); the mixture was magnetically stirred for 5 min and then left overnight for gel setting. The final polymer concentration (c_p, w/v) was always 1% for Sclg and 1, 2 or 3%, respectively, for Alg. When needed the gel was loaded with MGB or Methyl Orange dye. For this purpose a given amount of these substances (10% of the total polymer mass in solution) was dissolved in the solution of the polymers before the addition of borax. The resulting hydrogels were always frozen with liquid nitrogen and then lyophilized before use in the experiments.

For an appropriate comparison, freeze-dried samples of Alg and borax solutions, as well as Alg and MGB solutions, were also prepared.

2.3. Tablet preparation

Tablets were prepared from the freeze-dried samples, obtained as previously described, and pressed with an IR die (Perkin-Elmer hydraulic press) using a force of 5.0 kN for 30 s.

The weight of the tablets was 230 ± 10 mg, the diameter was 13.00 ± 0.05 mm and the thickness was 1.40 ± 0.1 mm. For an appropriate comparison, tablets of a physical mixture of Sclg, Alg, MGB and borax were also prepared.

2.4. Release experiments from tablets

Release experiments from the model dosage forms were carried out in distilled water (pH 5.4), in simulated gastric fluid (SGF, HCl 0.1N) and simulated intestinal fluid (SIF, phosphate buffer pH 7.4), according to U.S.P XXIV, using the rotating basket apparatus at 37.0 ± 0.1 °C and 100 rpm. Aliquots of dissolution medium were taken at fixed time intervals and the amount of released MGB (M_t) was spectrophotometrically determined at 409 nm, using quartz cells with a path length of 1.0 cm. All the experiments were carried out in triplicate and the obtained values always laid within 10% of the mean. For a better visualization of the trends of the obtained experimental data, error bars have been omitted from the various figures. To test the resistance in acidic medium of the matrices, release experiments were carried out with tablets soaked in SGF for 2 or 24 h and then immersed in SIF for drug release testing.

2.5. Water uptake experiments

Water uptake was evaluated by the relative increase of weight of the tablets, in distilled water, SGF and SIF at 37 °C. To study the influence of the acidic medium on the tablets properties, "ad hoc" water uptake experiments in SIF were performed with tablets previously soaked in SGF for 2 h. Experiments were carried out in triplicate.

2.6. Scanning electron microscopy studies (SEM)

For the SEM study a LEO 1450 VP, LEO Electron Microscopy, Inc., Cambridge, UK, scanning electron microscope, operating at 20 KV and 40 Pa, was used. Secondary electron detection without tablet metallization was also used. SEM photographs of tablets were taken immediately after compression. Photographs of tablets were also taken after soaking them in water or SGF for 5 min, and immediately freeze dried.

3. Results and discussion

In recent years great interest is arisen on hydrogels based on polymer blends as well as on IPN and on semi-IPN because of their peculiar properties suitable for drug delivery (Liu et al., 2005). In these systems the properties of the starting polymers are mixed and new matrices are formed with different improved characteristics.

In order to obtain a drug delivery system able to protect a model protein drug in an acidic medium and to release it into a neutral environment, thus simulating the gastric passage and an enteric delivery, a semi-IPN matrix based on a scaffold of Sclg/borax gel, possessing a controlled porosity, and a "dispersion" of the pH sensitive and hydrophilic Alg was prepared. The starting polymers show a remarkably different behaviour in aqueous media and no experimental evidences are given in the literature concerning drug delivery matrices prepared using the two above-mentioned polysaccharides. Using Sclg based tablets, prepared starting from freeze-dried Sclg/borate gels (Coviello et al., 2005c), is it possible to obtain a sustained delivery; such tablets show no erosion and a dramatic anisotropic elongation along the compression direction when immersed in aqueous environment. The sodium salt form of Alg is freely soluble in water, it does not interact with borax and its tablets swell rapidly for the presence of the carboxylic groups carried by every sugar unit.

The tablets of Sclg/borax/Alg, prepared as described in Section 2, were loaded with MGB as a model protein drug, that was already tested with the matrix obtained from the Sclg/borax gel alone (Coviello et al., 2005c). Sclg concentration was fixed at 1% (w/v) as this is a reasonable low polymer concentration capable to give anyhow a self-sustaining borax gel (the minimum c_p was previously found to be 0.7%) (Coviello et al., 2003).

In the present work the behaviour of the mixed system was studied preparing matrices with different ratios of the two polymers and using different release media, i.e. water, SGF and SIF. In Fig. 1 the release profiles in distilled water of the different systems prepared at a fixed Sclg concentration and varying the Alg content are reported: as the Alg percentage was increased, a corresponding increase of MGB release rate was obtained. In the same figure MGB release from Sclg/borax and from Alg/borax tablets are reported as references. The release profiles of the tested mixed systems lie between those obtained with Alg/borax and Sclg/borax systems, respectively, showing a quasi-cooperative effect by increasing the Alg concentration. Furthermore, the presence of Alg contributes to a complete release of the model drug in 24 h, while only 70% of the total MGB content is released from the Sclg/borax tablets after the same time. It has to be pointed out that the presence of Alg (even at 1% concentration) leads to a disaggregation of the tablets. In fact, as soon as they are soaked in water, all tablets start to absorb the solvent and a shell of imbibed polymer around

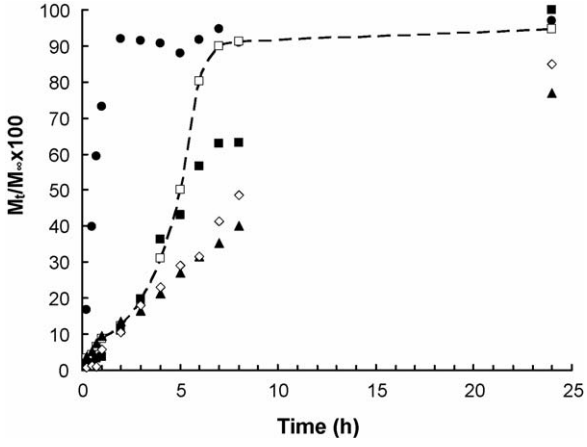

Fig. 1. Release profile of MGB in distilled water from tablets of Alg3%–borax (●), Sclg1%–borax (▲), Sclg1%–Alg1%–borax (◊), Sclg1%–Alg2%–borax (■) and Sclg1%–Alg3%–borax (SA3) (□). Experiments were carried out in triplicate and the obtained values always laid within 10% of the mean.

the tablets is formed. While the Sclg/borax tablet behaved as a monolithic matrix, elongating in an anisotropic way, thus leading, after 24 h, to a cylindrical gel that can be recovered from the dissolution apparatus, Sclg/borax/Alg tablets swell in an isotropic manner and after the 24th hour only small-sized particulate matter could be recovered in the case of 1 and 2% Alg, while a complete dispersion was obtained in the presence of 3% Alg. On the other side, tablets prepared with only Alg/borax swelled and rapidly dissolved just in a few hours. The quasi-cooperative behaviour, particularly evident for the 3% Alg system (see the continuous line of Fig. 1), can be ascribed to the balance between two opposite forces; the first one related to the Alg tendency to dissolve in the medium, while the second one is related to the strong cohesion of the Sclg/borax gel system. This dynamic equilibrium produces a transition between the two reference systems as a function of Alg concentration (i.e. from Sclg/borax to Alg/borax). In order to test the behaviour of the new Sclg/borax/Alg delivery system the matrix with a 1:3 ratio Sclg:Alg (SA3) was chosen for further experiments. The release of MGB was then performed in SIF and the obtained results are reported in Fig. 2. As it is possible to observe, and as expected, MGB delivery from Alg/borax tablets is again very rapid, while the delivery from Sclg/borax is quite similar to that observed in water (about 70% after 24 h). Release rate of MGB from SA3 is, within the first 8 h, slightly lower than that obtained with Sclg/borax, while the delivery is almost complete after 24 h because of the erosion process that takes place because of the presence of Alg; anyhow the trend is remarkably different from that obtained in water because the salts present in solution are able to screen the carboxylate groups of Alg thus reducing the water uptake of the system (Dautzenberg et al., 1994).

The behaviour of the tablets changed dramatically when the release medium was SGF. In this case, while Sclg/borax is still able to swell and release the test drug, MGB is not released at all from SA3—at least within the limit of detection of the analytical method (results not shown). This last result can be interpreted

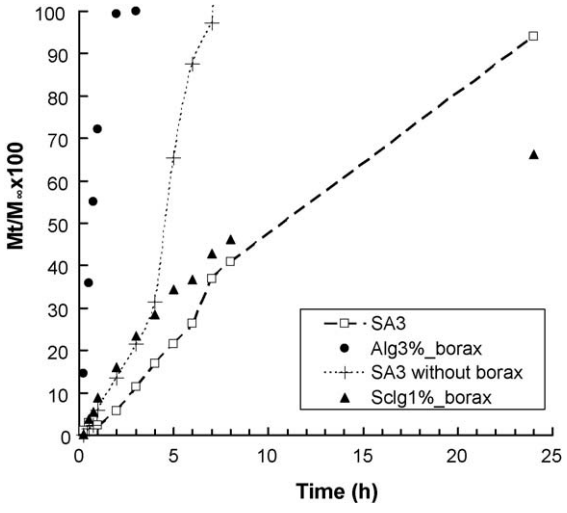

Fig. 2. Release profile of MGB in SIF from tablets of Alg3%–borax (●), Sclg1%–borax (▲), SA3 without borax (+) and SA3 (□). Experiments were carried out in triplicate and the obtained values always laid within 10% of the mean.

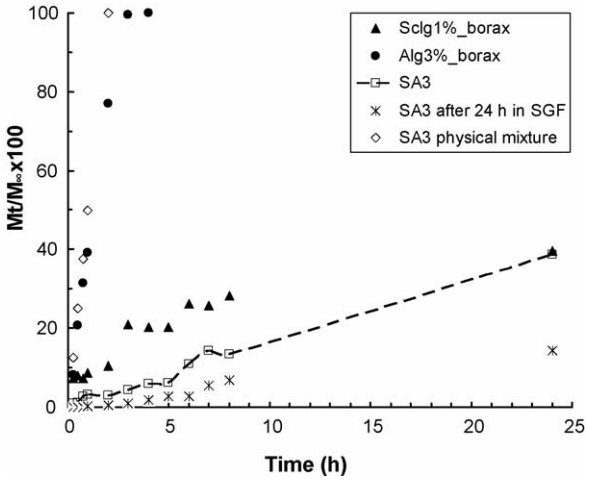

Fig. 3. Release profile of MGB in SIF from tablets of Alg3%–borax (●), Sclg1%–borax, and SA3 (□), after 2 h soaking of all tablets in SGF. Data relative to MGB release from SA3 (*) tablet after 24 h soaking in SGF and relative to a physical mixture of Sclg, Alg, MGB and borax in which the component ratios are the same of SA3 (◊) are also shown. Experiments were carried out in triplicate and the obtained values always laid within 10% of the mean.

if we consider that the presence of the acidic medium strongly influences the ability of the tablet to release drugs, because of the H^+ interaction with the Alg carboxylic groups. According to these data, release from tablets was also tested in SIF after soaking the matrices for 2 or 24 h in SGF. The obtained data, that are reported in Fig. 3, need some remarks:

- Alg shows a release profile similar to that observed when tablets were directly immersed in SIF;
- SA3 shows a release rate slower than that the obtained with Sclg/borax tablets;
- after 24 h the amount of MGB released from the SA3 matrix, previously immersed in SGF, is remarkably lower than that observed when the tablets were directly immersed in SIF;
- the release of MGB in SIF is affected by the time of the previous soaking in SGF: the longest the immersion time (e.g. 24 h instead of 2 h) the slowest the release rate that leads to a lower overall amount of drug released after 24 h (i.e. about 40% instead of 100%).

Furthermore, also the role of the presence of borax in the network was studied and the results are shown in Fig. 4, where the release profile of SA3 is compared with that obtained using the matrix prepared with Sclg and Alg at the same concentration of SA3 but without borax. The resulting trends are remarkably different, indicating that the borax, inducing a specific connectivity between the Sclg chains in the overall system, is able to form a network that slows down the release. It is worth to notice that a similar behaviour was observed in release experiments in SIF: as shown in Fig. 2, MGB was completely released from Sclg1%–Alg3% system (i.e. the SA3 composition without borax) within 8 h, while 24 h were needed for the complete MGB delivery from SA3.

Furthermore, the importance of role played by the borax in the semi-IPN, built up by a network of Sclg/borax interpenetrated

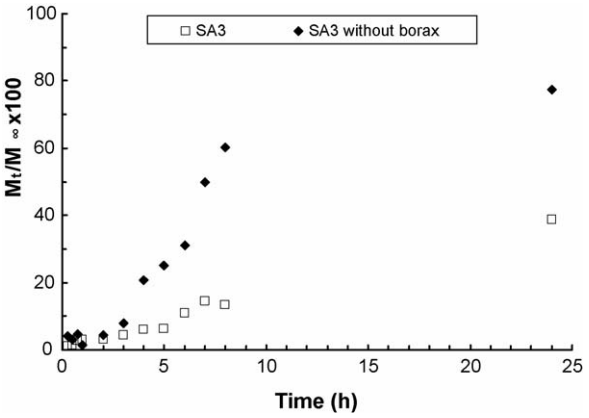

Fig. 4. Release profile of MGB in SIF from tablets of SA3 (□) and the same system without borax (◆) after 2 h soaking in SGF. Experiments were carried out in triplicate and the obtained values always laid within 10% of the mean.

with Alg chains, can be clearly evidenced by the release curves reported in Fig. 3. SA3 and the simple physical mixture of its components, in the same ratio and compression conditions, show a remarkably different profile due to the absence, in the latter case, of the formation of Sclg/borax network and of the inter-entanglements between such network and the Alg chains.

This result is accompanied by a very different behaviour of the tablets with respect to their mechanical properties, according to the release medium that is used. In fact, while in water or SIF tablet fragmentation occurs during the solvent uptake, in SGF the tablets behave as rather compact monolithic matrices and swell only in a limited extent.

In Fig. 5a the solvent uptake by SA3 in different media are compared. The highest uptake was registered in water; in SIF a relevant solvent uptake was also measured, but in a lesser extent, due to the shielding, exerted by the ions in solution on the Alg carboxylate groups. On the other side, tablet solvent uptake in the SGF environment showed, after 120 min, an increase of only two times with respect to the initial weight (in water such increase after the same interval time was more than five times), thus indicating a reduced ability of the system to absorb water in such conditions.

In accordance to this data also the mechanical properties of the tablets were very different; instead of the tablet fragmentations observed when the dosage form are immersed in water or SIF, in SGF the tablets did not break down and only a thin swelled layer appeared around the dosage form as it will be better discussed later illustrating Fig. 6.

The solvent uptake behaviour is also related to the presence of borax as it is shown in Fig. 5b, where the water uptake of the system without borax is reported. In this case the system is less sensitive to the ionic strength, being almost the same the water uptake recorded in water and in SIF. Furthermore, the overall water uptake is lower with respect to that obtained with the system in the presence of borax; also in this case the water uptake in SGF after 120 min leads to an increase of about two times the initial weight of the tablet.

Swelling in SIF was also determined after immersion in SGF: in this case the swelling of the SA3 and SA3 without the addition

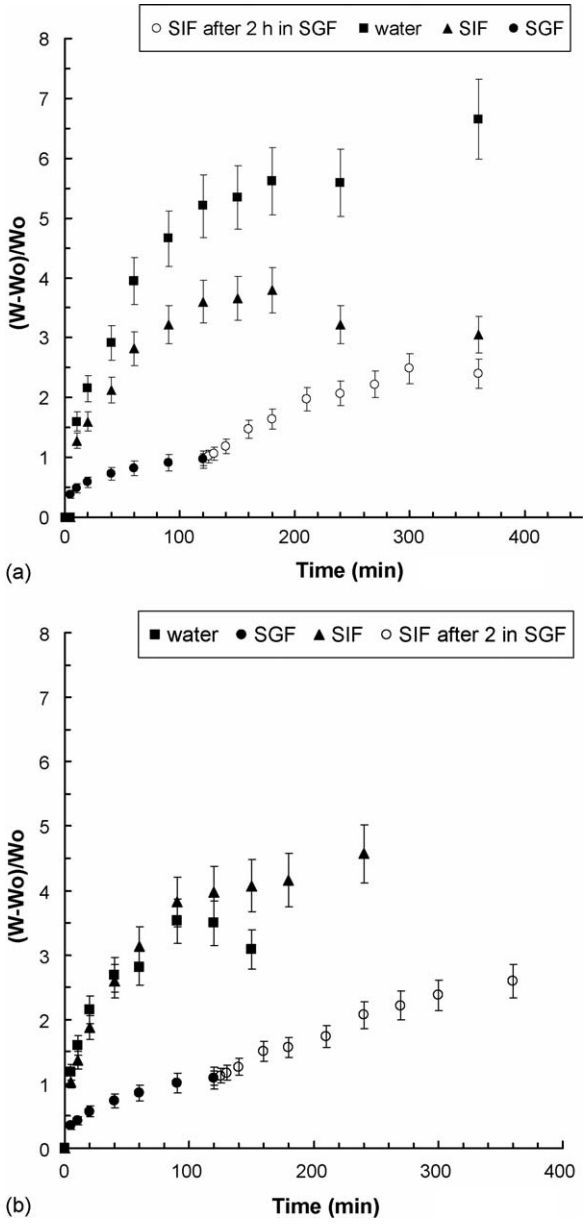

Fig. 5. (a) Water uptake of tablets of SA3 in different solvents: water (■), SIF (▲), SGF (●) and SIF after soaking 2 h in SGF (◯). (b) Water uptake of tablets of Sclg1%–Alg3% (SA3 without borax) in different solvents: water (■), SIF (▲), SGF (●) and in SIF after soaking 2 h in SGF (◯).

of borax are almost the same. The data suggest that, according to the model proposed for the Scgl/borax system (Coviello et al., 2003), borax induces a more open structured network than that present in the solution containing only the entangled polymers. The experimental data indicate that such system results capable to uptake an higher amount of solvent.

It is worth to underline that water uptake experiments were performed only for the first few hours in order to avoid possible interferences due to dissolution and/or fragmentation of the samples. Thus it can be asserted that solvent uptake experiments confirmed the release data: the presence of Alg in acidic medium yields a more soluble matrix, influencing the delivery of MGB as well as the swelling of the matrix.

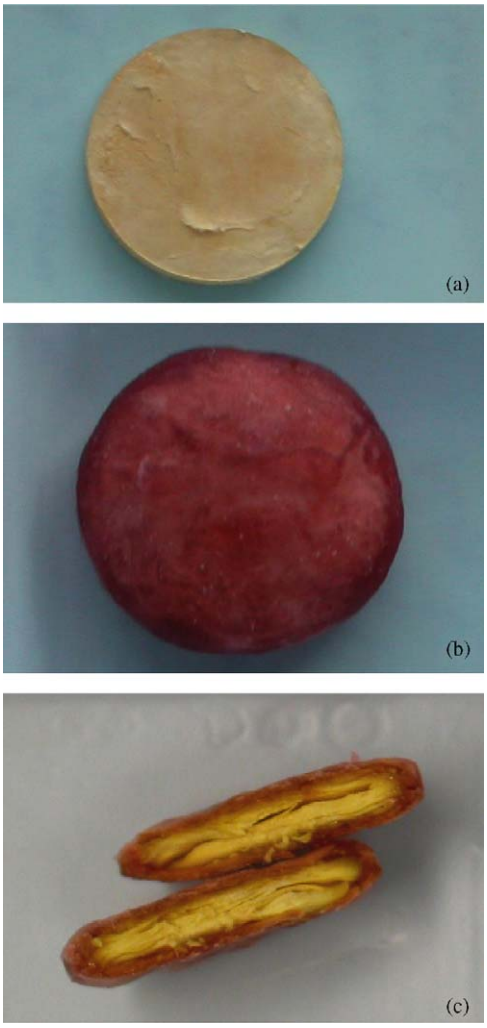

Fig. 6. Pictures of tablets of SA3 loaded with Methyl Orange dye; tablet loaded with Methyl Orange before (a) and after (b) soaking 2 h in SGF. (c) Internal view of the tablet in (b).

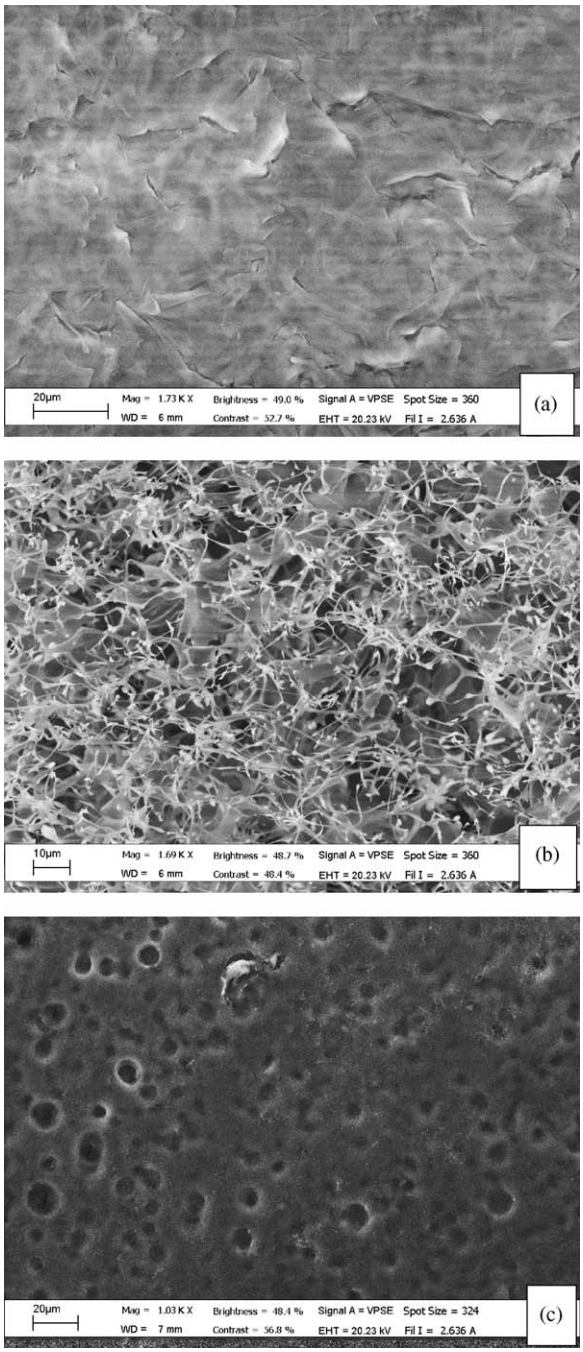

Fig. 7. SEM images of tablets of SA3 (a), after 5 min of soaking in water (b) and after 5 min of soaking in SGF (c).

In addition, in order to visualize water (and H^+ ions) penetration within the bulk of the tablet, colorimetric experiments were also performed. For this purpose, SA3 tablets were loaded with Methyl Orange dye (yellow at pH > 7; red at pH < 7). The dosage forms were immersed in SGF for 2 h and then they were broken in order to see the extension of tablet imbibition by the surrounding medium. While in water (or SIF) the swelling process proceeds very rapidly from the surface of the tablets toward the core, in SGF the colorimetric assay indicated that only an outer shell of the tablet was penetrated by the solvent (Fig. 6) as confirmed also by the release of only 5% of the dye from the SA3 system after 2 h (data not reported).

For a further characterization of this new semi-IPN system, SEM pictures were taken. In Fig. 7 the surface of the SA3 tablet before (Fig. 7a), and after 5 min immersion in water (Fig. 7b) and SGF (Fig. 7c), are shown. In water the surface rapidly swells leading to a loose arrangement of the polysaccharidic chains. On the other side, the interaction with an acidic medium (i.e. reduced swelling) leaves the surface almost intact, as it was before immersion. This type of surface allows the water to diffuse into the tablet in a lesser extent and prevents MGB dissolution in the surrounding medium.

4. Conclusions

In this work MGB delivery from the new polysaccharidic matrix based on Alg chains interspersed into a Sclg/borax system is described. The delivery was tested in various aqueous solvents; the data obtained indicate that the presence of Alg leads to a modulation the release profile: the delivery rate increases as Alg concentration in the solid dosage form is increased. After

24 h the tablets containing 3% Alg and 1% Sclg/borax (SA3) are completely disaggregated in water and in SIF, allowing the complete delivery of the MGB.

On the other side, the Alg chains are able to prevent the release of the tested protein in simulated gastric conditions; indeed, when the SA3 was immersed in SGF, no MGB was delivered, because of:

- ionic interaction between carboxylate ions carried on Alg chains and the protonated form of the protein, and
- lack of dissolution of Alg in acidic media.

In the experiments that simulate in vitro the gastric passage, tablets, previously immersed in SGF, were able to deliver MGB when immersed in SIF. The release profile is clearly modulated by the presence of the Sclg/borax network.

The presence of Alg molecules modify the physico-chemical properties of the tablets with respect to the corresponding dosage form prepared with only Sclg/borax; in fact Alg modulates the water uptake process according to the various aqueous solvents that have been investigated.

The mechanical properties are also influenced by the presence of the Alg chains: SA3 tablets disaggregate in neutral aqueous solvents, at least after 24 h, while Sclg/borax tablets behave as monolithic matrices elongating in a peculiar anisotropic manner. Furthermore, from a mechanical point of view, SA3 tablets are more resistant to the acidic environment and their surfaces, when immersed in SGF, appear as more compact in comparison to tablets immersed in water.

The overall data described in the present work indicate that the new Sclg/borax/Alg matrix is capable to protect the model protein drug in acidic media, allowing, in vitro experiments, its transit through an acidic medium and its subsequent release in a modulated manner into a neutral environment.

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