Pharmaceutical Applications of Biocompatible Polymer Blends Containing Sodium Alginate

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ABSTRACT: Biocompatible polymer blends, such as alginate blends, have a widespread use in pharmaceutical and medical applications due to their specific features, such as biodegradation, adhesiveness, and thermo- and pH sensitivity and that can be obtained from the mixture composition. In this work, the use of alginate blends was tested in a novel production methodology of therapeutic dosage forms based on polymeric chain reticulation phenomena induced by exposure to bivalent ions. Two kinds of sodium alginate were used to obtain gel films (structured films) in blends with Pluronic F127®. The blends were considered for applications in gel paving of drug-eluting stents. Sodium alginate was also used in shell–core particle production (structured particles) to obtain shell-barrier reducing drug release in the preparative steps (see wash operations). Both structures, films and particles, were obtained using Cu^{2+} and Ca^{2+} ions, respectively. Film/shell barrier properties were tested in dissolution experiments using vitamin B12 as an active molecule model. Experimental work demonstrated that the alginate composition is a crucial point in defining reticulated structures.
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KEY WORDS: Alginate gel film, Alginate shell–core particles, Biocompatibility, Crosslinking, Hydrogels

Introduction

Hydrophilic biocompatible polymer blends are widely used in many pharmaceutical and medical applications. Among them, dosage formulations for drug delivery have gained a great interest because they can transfer drugs, gene, anticancer chemical drugs, vitamins, and proteins via various administration routes, including oral, nasal, ocular, and intravenous. This large versatility is fundamentally due to some specific features of biopolymers, such as biodegradation, adhesiveness, and thermo- and pH sensitivity.

Alginates, a class of natural polysaccharide, are one of the key components of the biocompatible blends for their capability of forming ionotropic gel, to modulate rheological properties if added in mixtures, for its pH sensitiveness and, finally, to be easily processable using low-cost technologies.

In the following, after a brief introduction on the alginate structure, several literature studies on alginate blends (alginate/chitosan blends, alginate/Pluronic blends, and alginate/chitosan/Pluronic blends) are reported to emphasize how specific formulations can address tailored dosage systems.

Alginate is an anionic linear polysaccharide extracted from brown seaweeds. It consists of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues[1] (the structure is shown in Fig. 1). Composition and extent of the sequences together with molecular weight determine the physical properties of alginate.[2] Alginates can form gels in the presence of bivalent or polyvalent metal ions. Gelation, which can be carried out under a mild environment and using nontoxic reagents, is achieved by the exchange of sodium ions of uronic acids of distinct chains with cations and the stacking of the uronic groups to form an "egg-box" structure. A key parameter for the entrapment of active principles is the average mesh size of the gel polymeric network.

Versatility of alginates has been extensively investigated. Alginate as plain beads, coated beads, and microcapsules has been used to entrap proteins such as melatonin, heparin, hemoglobin, and vaccines.[3] Coated beads and microspheres are found to be the best oral delivery vehicles.[4] In particular, the mucoadhesive nature of alginate makes it especially suitable for biomolecules to mucosal tissues.[5]

Alginate/polymer blends are investigated to enhance the drug-releasing properties. Chitosan is one of these polymers. It is a natural cationic polysaccharide composed of glucosamine and *N*-acetylglucosamine residues derived from partial deacetylation of chitin, which is generally obtained from crustacean shells.[6] Physical and chemical properties of chitosan mainly depend on its molecular weight and degree of deacetylation.[7] In

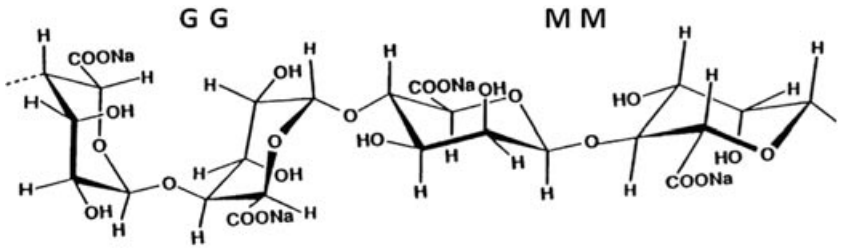

FIGURE 1. Structure of sodium alginate (guluronic (GG) and mannuronic (MM) blocks).

particular, chitosan is used in preparation of mucoadhesive formulations, improving the dissolution rate of the poorly soluble drugs, drug targeting, and peptide absorption.[3]

Simonoska Crcarevska et al.[8] proposed a new dosage form based on chitosan and alginate blends loaded with budesonide, bioadhesive, and controlled release properties in the gastrointestinal tract (colon). In particular, microparticulate systems, based on hydrophilic (chitosan–Ca–alginate) matrix and coated with pH-sensitive Eudragit S 100, were obtained by delivering two solutions, one of $CaCl_2$ and chitosan another of budesonide and alginate, to a two-fluid nozzle spray drier. Chitosan/alginate blends are also used to encapsulate lipid droplets within hydrogel beads, consisting of alginate/chitosan electrostatic complexes, to control their susceptibility to digestion by pancreatic lipase. An example of these applications is reported by Li and McClements,[9] where different methods, fundamentally based on emulsion techniques followed by a reticulation step in the $CaCl_2$ solution, were used to prepare an alginate–chitosan bead carrier.

Others possibilities to determine drug delivery properties based on temperature, composition, and hydrophobicity are suggested by using alginate and Pluronic blends.

Chen et al.[10] proposed a controlled release transdermal system for selegiline (an antidepressant agent also used in Parkinson's and Alzheimer's disease and cocaine addiction treatments) based on an alginate–Pluronic (Pluronic F127, PF127-) composite thermogel. PF127 (or Poloxamer 407) is an amphiphilic synthetic block copolymer of poly(ethylene oxide–propylene oxide–ethylene oxide) (PEO–PPO–PEO), which is transformed from a low-viscosity water solution to a semisolid gel upon heating to body temperatures (at a concentration of >20%). The main drawbacks of PF127 gels are weak mechanical strength, an early temperature gelification (with respect to room temperature), and non-biodegradability. To overcome these problems, Chen et al.[10] developed a polymeric blend with cross-linked alginate. In this way, the resulting blend was kept at a liquid state until it was applied on the skin surface and has also become biocompatible. Both the features are useful for topically selegiline release.

In Grassi et al.,[11] a gelling matrix made up by PF127 and alginate blends is proposed as a suitable endoarterial delivery system. PF127 and alginate blends have been selected for the preparation of drug-eluting stents (DES), a therapeutic device applied in percutaneous transluminal angioplasty surgery as a carrier to deliver in situ antiproliferative drugs. The innovative therapeutic device provides, after stent implantation, Pluronic with a drug/alginate aqueous solution on the endovascular surface with the consequent gelation of PF127 due to the body temperature. Subsequently, the inner gel surface is rapidly exposed to a bivalent cation solution, thus inducing formation of an alginate gel film that becomes a barrier for drug delivery in the blood flow.

Moebus et al.[12] formulated and prepared novel hydrogel-based microparticles containing thermogelling poloxamer and cross-linked alginate. Microparticles of alginate/poloxamer blends, obtained by water/oil (w/o) the emulsion external cross-linking method (by $CaCl_2$ solution), were tested for mucosal administration. Because of the aqueous, hydrophilic nature of blends and mild preparation procedures (which offer a suitable environment for peptide and protein drugs), the microparticles loaded with bovine serum albumin showed interesting potential as a controlled protein delivery system.

Das et al.[13] investigated a nanoformulation of curcumin (an antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic molecule characterized by poor water solubility and thus with low bioavailability property) in a tripolymeric composite (alginate/chitosane/Pluronic F127) for delivery to cancer cells. With the aim to encapsulate the

curcumin, an aqueous solution of sodium alginate and curcumin (in ethanol solution) was added to the PF127 water solution, together with an acid acetic solution of chitosan. Alginate and chitosan were selected for their pH sensitivity, biodegradability, and hydrophilicity; PF127 was chosen to improve encapsulation and dispersion of curcumin for its hydrophobic (due to PPO blocks) character and biocompatible properties.

Based on results of Grassi et al.[11,14] and of our previous work[15] on rheological and phase transition properties of alginate/Pluronic F127 systems, this study aims at increasing knowledge of structural features of gel films and release properties of alginate blends. In particular, in this work, the use of alginate blends was tested in a novel production methodology of therapeutic dosage forms, based on polymeric chains reticulation phenomena induced by exposure to bivalent ions. The first goal was to emphasize the effect of the alginate composition on gel films production starting from Pluronic F127®/alginate blends. Two kinds of sodium alginates were used to realize gel formulations to use in DES-paving applications. For this purpose, thin layers of alginate (film structures) were required to act as a barrier on the Pluronic soft gel used as a drug reservoir system.

The second goal was to prepare sodium alginate shell–core particles (particle structures) on the basis of the investigation carried out on films. A reinforced shell of alginate was designed to reduce burst effects and enhance encapsulation effectiveness of active molecules.

Both structures, films and particles, were obtained by using Cu^{2+} and Ca^{2+} (a water solution of copper and calcium) as a reticulating agent, respectively. Film features and shell-barrier properties were tested by ad hoc dissolution experiments using vitamin B12 as a released active molecule model.

Experimental

MATERIALS

Sodium alginate (AL-1) (CAS no. 9005-38-3, Sigma cat. W201502), Pluronic F127 (PF127; Invitrogen, Milan, Italy) flakes (CAS no. 9003-11-6), and vitamin B12 (B12) (CAS no. 68-19-9) were purchased from Sigma-Aldrich (Milan, Italy). Sodium alginate (AL-2) was purchased from FMC BioPolymer (Milan, Italy). All the listed products were used as purchased, without further purification.

$CuSO_4 \cdot 5H_2O$ (CAS no. 7758-99-8) and anhydrous $CaCl_2$ (CAS no. 10043-52-4) were used in water solutions as a cross-linking agent; HCl (CAS no. 47-01-0), NaOH (CAS no. 1310-73-2), and KH_2PO_4 (CAS no. 7778-77-0) were used as buffer solution components. Tween 80 (CAS no. 9005-65-6) was used as a surfactant. All the listed products were purchased from Sigma-Aldrich.

METHODS

Preparation of Alginate/PF127 Blends

Solutions composed of 18% w/w in PF127, 2% w/w in alginate (AL-1 or AL-2), and 80% w/w in distilled water were prepared by adding first alginate powder, then PF flakes in cold distilled water, gently mixing, and allowing the stabilization by keeping them at 4°C overnight. The same procedure was adopted to prepare B12-loaded alginate/PF127 solutions (B12 0.013% w/w).

Alginate Films: Preparation and Characterization

Alginate films were prepared by using a given amount of the AL–PF127 blend into a Petri dish and heating at 37°C to promote the blend gelification (more details on transition phase phenomena are reported in Ref. 15). After 7 min, 5 mL of copper solution (at different concentrations of 1, 2, 3, 4, and 5 g/L) was added, carefully spread on the gel-blend surface, and kept for a given time (contact time, or Cu^{2+} exposure time, t_E: 1, 2, 3, 4, 5 min). An alginate film was produced on the gel-blend surface by the exchange of sodium ions of guluronic acids with the Cu^{2+} cations. The film was then removed, washed, and subjected to thickness measurements by a thickness gauge (Kafer; 0.001–2 mm). Both film preparation (at the given copper solution concentration/contact time) and thickness measurement were performed in triplicate. Fig. 2 shows photographs of the alginate film [a close-up view of the film was obtained by using an optical microscope Leica DM-LP (Leica, Buckinghamshire, UK)].

Alginate/PF127/B12 Film Dissolution Tests

In dissolution tests, alginate films were prepared starting from B12-loaded alginate/PF127 gel blend. In this study, 1 min and 5 g/L were imposed

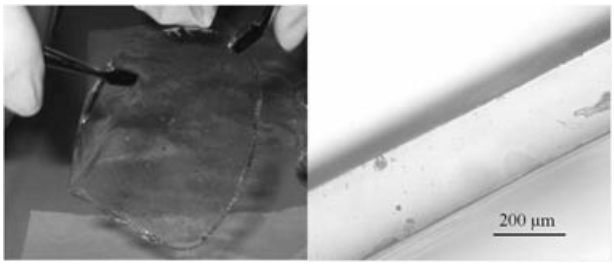

FIGURE 2. Alginate film (on the right, optical microscope image 100×).

as contact time and copper concentration, respectively. After this, excess of solution was removed and the system consisting of the B12-loaded gel blend (contained in a Petri dish) was put in a stirred dissolution bulk (500 mL of buffer solution at pH 7.4 and 37°C), thus exposing only the superficial alginate thin film to the buffer solution environment.

Note that the two investigated systems (films and particles) have peculiarities, that make them nontestable by conventional methods. Actually, only one side of the films has to be exposed to the dissolution medium; therefore, no commercial diffusion cell may be viable. Moreover, as films are obtained in a (relatively large) Petri dish, they cannot be immersed in a USP Apparatus 2 vessel. Particles are produced in a very low amount (a few milligrams) per test; thus, the conventional USP Apparatus 2 cannot be used because it requires a large volume of dissolution medium.

For the purpose of film dissolution tests, a homemade dissolution arrangement was built with a Petri dish suspended in the dissolution bulk. It is shown in Fig. 3. B12 was spectrophotometrically assayed [by using a Perkin-Elmer Lambda 25 spectrophotometer (Perkin-Elmer Italia, Monza, Italy)] at 361 nm.

All the experimental tests were performed at minimum in triplicate. The error bars refer to the standard deviation (SD).

Preparation of Alginate/B12 Solution

Solutions consisting of 1.5% w/w in alginate (AL-2) in distilled water were prepared by adding alginate powder in water by gently mixing and allowing for the stabilization. The same procedure was adopted to prepare B12-loaded alginate solution (B12 0.05% w/w).

Alginate Particles: Preparation and Characterization

The two different water/AL-2 and water/AL-2/B12 solutions were pumped, under controlled conditions, into a stainless steel coaxial double-channel device (core channel internal diameter: 1.016 mm; shell channel internal diameter: 0.406 mm, see Fig. 4). This device is proposed as a variant of drop generation systems, such as a syringe with a needle or a pipette, typically used to produce large drops. To promote drop generation, a few milliliters of Tween 80 was added in the feed solution. Coarse droplets were then dripped into a stirred water calcium solution (0.9% w/v) to promote alginate chains reticulation. After a given time of exposure to calcium ions, rubbery particles with a shell–core structure were obtained. They were then separated, washed by distilled water, photographed [Canon digital camera, IXUS 850 IS (Canon Italia, San Donato Milanese, Italy)], and subjected to diameter/thickness measurements by image analysis (using the public domain software ImageJ 1.40 g, Wayne Rasband, National Institutes of Health, Bethesda, MD, freely available at http://rsb.info.nih.gov/ij/). Two kinds of feed configurations were investigated:

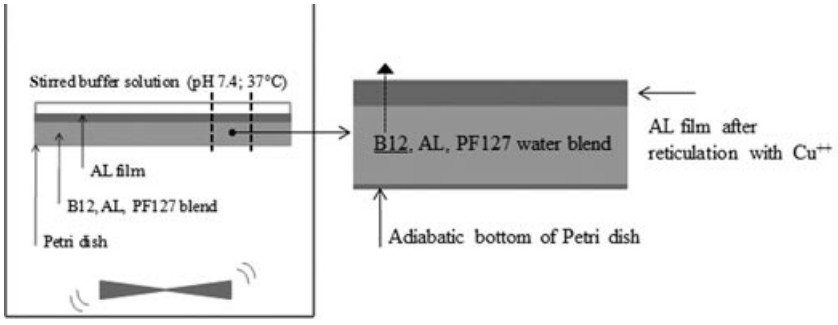

FIGURE 3. A sketch of the dissolution arrangement used in B12 release from films of alginate/Pluronic blends.

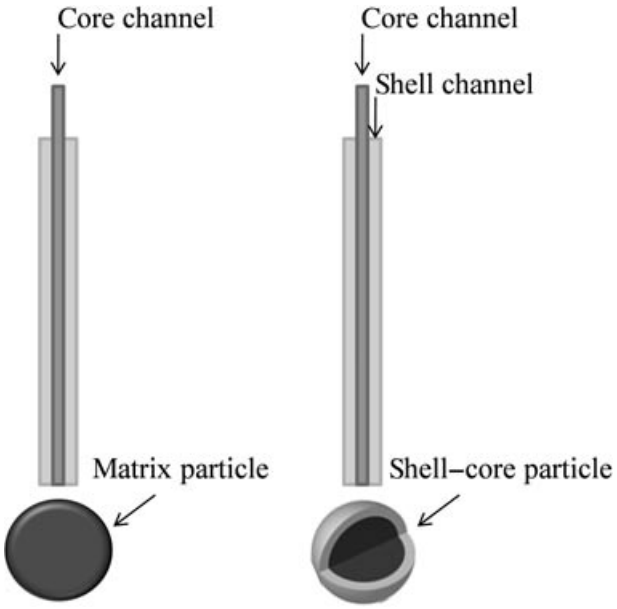

FIGURE 4. A sketch of the coaxial double channel device used to produce matrix and shell–core particles.

water/AL-2/B12 feed solution in only core side (to obtain "matrix particles") and water/AL-2 and water/AL-2/B12 solutions as shell and core feed (to obtain "shell–core particles"), respectively (Fig. 4). In production of shell–core particles, optimal feed conditions were investigated to achieve a good core wrapping. A volumetric rate ratio of 1/6.5 of water/AL-2/B12 to water/AL-2 solutions as a core and shell feed, respectively, was followed.

Alginate Particle Dissolution Tests

All kinds of particles were subjected to dissolution tests to investigate the barrier behavior of alginate walls and to assay the performance of B12 encapsulation. The dissolution tests were carried out by using a given amount of particles in a stirred buffer solution (at pH 7.4 and 37°C). Because of the low concentration, the UV–vis spectrophotometry method was proved to be inaccurate to assay the B12 releases. Thus, a high performance liquid chromatography (HPLC) apparatus [Agilent Technologies, 1200 Infinity Series (Agilent Technologies Italia, Cernusco Sul Naviglio, Milan, Italy)] was used. In particular, an Eclipse Plus C18 column (Agilent Technologies Italia), a hydroalcoholic solution (methanol 30% v/v) as a mobile phase (isocratic method), and 361 nm as a wavelength detector were selected. All the experimental tests were performed at minimum in triplicate. The error bars refer to SD.

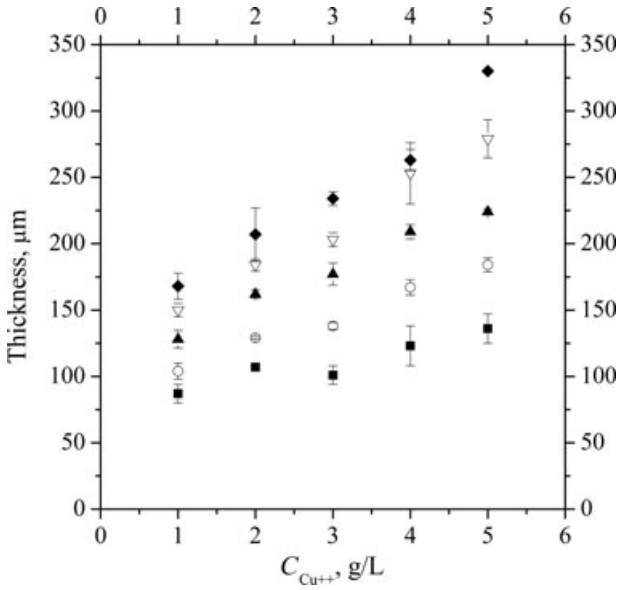

FIGURE 5. Alginate (AL-1) film thickness as a function of the Cu^{++} concentration, at different times to exposure to reticulating solutions (■: 1 min; ○: 2 min; ▲: 3 min; ▽: 4 min; ♦: 5 min).

Results and Discussion

ALGINATE FILM CHARACTERIZATION

Figures 5 and 6 show thickness measurements of alginate gel films, obtained by gelling with a bivalent cation of copper. A film thickness is reported as a function of two operative parameters: the copper concentration ($C_{Cu^{2+}}$, in abscissa) and the time of exposure (t_E, parametric values) to the binding ions. Copper cations were selected in gel films as calcium cations cannot be used in DES applications for coronary diseases, which is the final goal of the study.

In all the examined cases, the gel film thickness increases with the $C_{Cu^{2+}}$ and t_E, whereas the thickness range depends on the kind of alginate used. Films obtained using AL-1 in FP127/water blends showed thickness in the range of 87 μm (1 g/L; 1 min) to 330 μm (5 g/L; 5 min). Films prepared using AL-2 were characterized by lesser values and in the range 41 μm (1 g/L; 1 min) to 270 μm (5 g/L; 5 min). The effect of thickness is shown in Fig. 7: The thickness of AL-1 and AL-2 films after t_E = 1 min at different bivalent cation concentrations is compared. As reported in the Introduction, alginate gels are obtained when egg-box architectures are

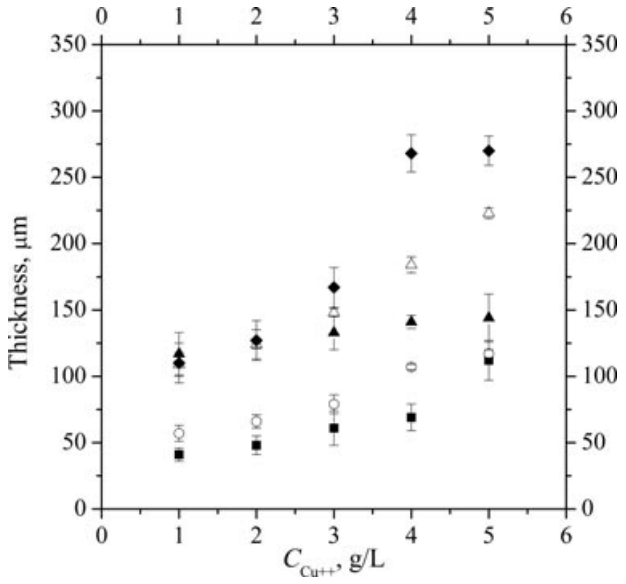

FIGURE 6. Alginate (AL-2) film thickness as a function of the Cu^{++} concentration, at different times to exposure to reticulating solutions (■: 1 min; ○: 2 min; ▲: 3 min; ▽: 4 min; ♦: 5 min).

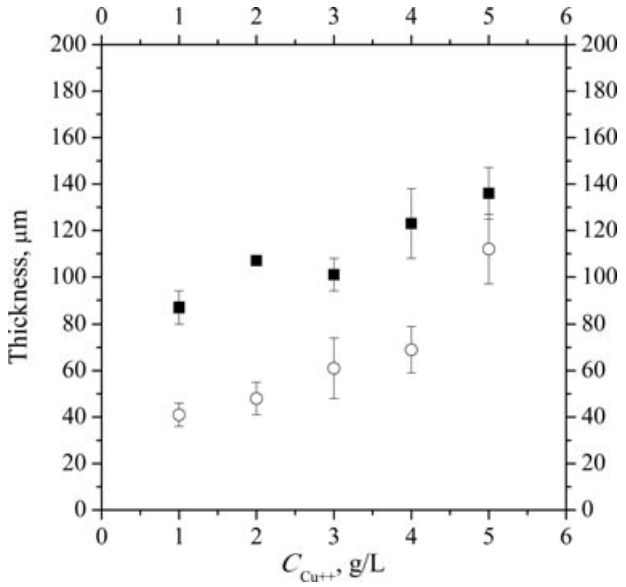

FIGURE 7. AL-1 (■) and AL-2 (○) film thickness as a function of the Cu^{++} concentration and after 1 min of exposure to reticulating solutions.

developed as a result of the exchange of sodium ions of uronic acid residues with multivalent cations. So, a reticulation phenomenon holds and insoluble alginate structures are produced. The ability to develop an egg-box architecture starting from alginate flexible coils in a water solution depends on the relative proportions of three types of blocks that characterize the alginate polymer chain: homopolymeric sequences of mannuronate (MM) and guluronate (GG) residues, and regions where the two residues (MG) are alternated. Many researchers[16–18] have shown that gelling properties of alginates mainly depend upon the M/G ratios. The difference in binding among the three types of blocks was discussed, speculating on alginic acid secondary structure.[16] Guluronate residues are arranged in a bucklet structure and showed deep cavities where metal ions can be allocated; mannuronate residues are structured in a flat ribbon-like configuration and showed shallow cavities. Thus, egg-box structures were mainly promoted by GG segments, which can offer more stable structures, fundamentally due to the strong electrostatic interaction between negatively charged carboxylate groups in the guluronate/cations buckle, enhanced by the small distances in the conformational structure. It is worth to note that the size (ion radius), loading of metal ions, and ease of packing of the alginate chains around the metal ions are crucial to the alginate reticulation (the "egg-box model" was first referred as the GG/calcium bivalent ions binding). Because a mechanism of progressive carboxylate group site saturation occurs, a binding with MM blocks is the final step. Nevertheless, MM bindings are characterized by less ionic strength (the flatter structures entail a larger distance between the charged units and carboxylate groups/copper ions). Conformational structures of GG and MM blocks in the presence of divalent ions have thus a great influence on the final properties of the alginate hydrogels. Alginates with a larger GG content allow production of gels characterized by higher stability and mechanical resistance but also by higher rigidity and fragility. Alginates rich in MM blocks allow production of weak gels, but with an elastic structure.[2]

In summary, it is possible to assert that the final features of the alginate gel are strongly affected by the binding extension between GG and MM segments with metal ions.

The two kinds of alginates used in this work present a different M/G ratio (in Table I, several properties are summarized) with almost equal molecular weight, so that only the effect of composition, in terms of the GG–MM block, was investigated. On the basis of the different GG dimer content, the gel films resulted in different thicknesses. In particular, AL-2 gel films showed a more compact structure due to the more richness in GG segments: Thin films were thus achieved. The thickness

TABLE I
Several Properties of Alginates and B12 Used in Experimental Tests

Component	Molecular weight (Da)[a]	M/G[a]	Diffusion coefficient[b]	Molecular radius
AL-1 - Sodium alginate (Sigma Aldrich)	396.000	1.49		
AL-2 - Sodium alginate (FMC)	419.000	0.56		
Vitamin B12	1355.4		3.8×10^{-10} m^2/s	0.86 nm

[a]Molecular weight and M/G determinations of alginates were performed by viscosity and NMR measurements, respectively, on commercially purchased products.
[b]In water at 37°C.

increase with the copper concentration was due to the progressive saturation of carboxylic groups interacting with metal ions to give more extended bindings, whereas the thickness increase with the time exposure was correlated with the binding site saturation as well as deeper diffusion of the copper ions through the gel layer. As far as gel are reticulated, ion diffusion became more difficult. Therefore, a gel based on alginate AL-2, which is richer in GG segments, produced a hard gel with a thickness less than the gel based on alginate AL-1.

In DES applications, the thickness of alginate films has a substantial role because, as reported in the Introduction, the films must act as a barrier. Alginate film is deputed to confine active molecules loaded in Pluronic soft gel at both ends: to ensure the drug release at the targeted arterial wall and reduce Pluronic washout by blood flux. The structure of a DES based on alginate/Pluronic blend is shown in Fig. 8. Alginates rich in GG segments can give the desired performance.

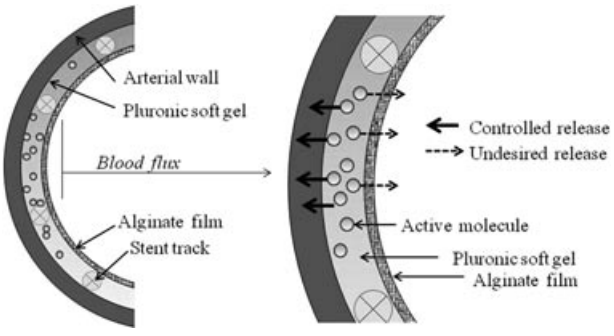

FIGURE 8. A sketch of the DES structure composed by the alginate/Pluronic blend loaded by an active molecule.

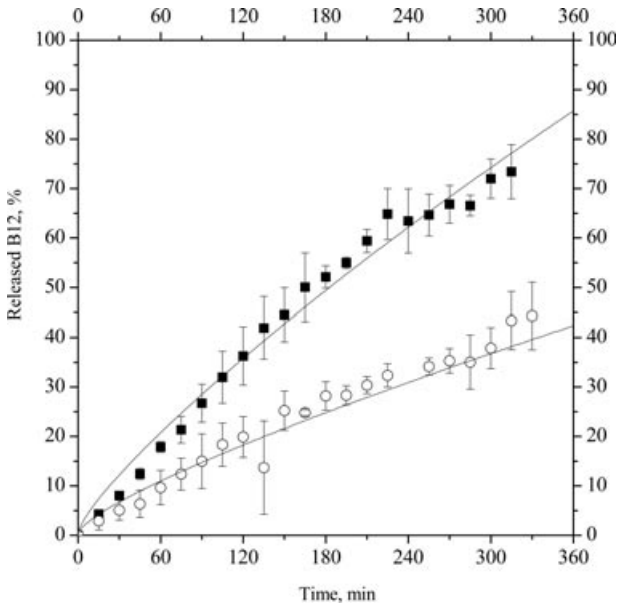

FIGURE 9. Release profiles of B12 loaded in AL-1/PF127 (■) and AL-2/PF127 (○) blends.

Alginate/PF127/B12 Film Dissolution Tests

In Fig. 9 (symbols: experimental data; curves: fitting model), results of dissolution tests are reported. Using the setup described in the Methods, films of AL-1 and Al-2 were produced starting from alginate/PF127 blends loaded with B12. Because of the low content of B12, alginate reticulation was supposed to occur by the same phenomenon investigated in preparation of gel film. Thus, using 5 g/L and 1 min as the copper concentration and exposure time, respectively, AL-1 gel-films of 135 μm in thickness and AL-2 gel-films of 110 μm in thickness were obtained. As can be seen in Fig. 9, owing to the different structural arrangements of the

alginate/copper network, different percentages of B12 release through AL-1 and AL-2 gel films, at a given time, were obtained. The spectrophotometric analysis proved to be an affordable technique (fast, repeatable, economic, not disruptive) to assay released B12.

B12 release was due to phenomena of diffusion and erosion; the former was correlated with the mesh size in an alginate/copper network, whereas erosion was strictly associated with swelling and hydrodynamic effects. B12 was selected as a model drug because of its relatively large molecular size (Stokes radius of 0.86 nm[19]), because smaller molecules (theophylline[20]) were found to be too small to be entrapped within the gel network. Most of the drugs that are to be used as antiproliferative and anticancer drugs should be of larger molecules; therefore, their behavior should be closer to the one observed for B12 than the behavior observed for theophylline. To verify the main hypothesized phenomena occurring during film dissolution tests, that is, drug diffusion through the gel layers and erosion of the gels themselves, a nonconventional setup (as shown in Fig. 3) was realized. The adiabatic bottom of the Petri dish allowed B12 to pass from a gel blend to dissolution bulk only through alginate films by diffusive and erosion phenomena. Indeed, both the phenomena contribute to the drug release kinetics, in particular diffusion at first, then erosion.

The observed phenomena are depicted by a physical and mathematical model, with the aim of better understanding of their role and quantification. For this purpose, the mass balance of the drug within the gel layer can be written because diffusion takes place only along the layer thickness. The transient mass balance is

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1}$$

where C is the drug concentration, D is its diffusivity within the gel, and x is the thickness. Under the hypothesis of a similar mesh structure in the two different layers, the diffusivity can be taken as a constant and therefore Eq. (1) can be used once the initial and boundary conditions are defined. They can be stated as

$$I.C.\ \forall x@t = 0 \quad C = C_0 \tag{2}$$

$$B.C.\ 1\ \forall t@x = L_0 \quad C = C_1 \tag{3}$$

$$B.C.\ 2\ \forall t@x = -L_0 \quad C = C_1 \tag{4}$$

These conditions imply that in the region $x < L_0$ (with L_0 the initial overall thickness of the gel layer), the film is initially loaded by a homogeneous drug concentration C_0. The asymmetric system (Petri dish wall/gel layers/dissolution medium) was ideally replaced with a symmetric system with the same physical behavior, that is, a layer of gel of double thickness ($2L_0$), exposed to a dissolution medium from both sides. It is well known that a layer of thickness $2L_0$, in which diffusion and erosion take place, with the two opposite surfaces kept at the constant concentration, behaves exactly as a layer of thickness L_0 (one half of the first system), if one surface is kept at the constant concentration and interested by the erosion phenomenon, whereas the other one is adiabatic (i.e., the mass flux is zero as much as the symmetrical plane in the double thickness layer is characterized by a mass flux equal to zero). Therefore, the boundary conditions (3) and (4) mean that at the interface between gel and dissolution medium the drug concentration is C_1. Under these conditions, the system[1–4] has a well-known solution,[21] as given by Eq. (5):

$$C(t,x) = C_0 + (C_1 - C_0)\cdot\left[1 - \frac{4}{\pi}\sum_{i=0}^{\infty}\left[\frac{(-1)^{\mathrm{n}}}{2\mathrm{n}+1} \times \exp\left[-(2\mathrm{n}+1)^2\pi^2\frac{tD}{4L_0}\right] \times \cos\left[\frac{(2\mathrm{n}+1)\pi x}{2L_0}\right]\right]\right] \tag{5}$$

The fractional drug release can be obtained once the drug concentration profile within the polymer layer has been calculated by integrating

$$R(t) = 1 - \frac{m(t)}{m(0)} = 1 - \frac{1}{m_0}\rho A\int_{-L_0}^{L_0} C\,dx \tag{6}$$

where ρ is the polymer blend density and A is the transverse area of the polymer layer. Equation (5) gives the drug concentration profiles, and Eq. (6) gives the fractional drug release for a constant thickness gel layer,L_0. Solving the balance equation under the further hypothesis of a constant erosion velocity, v, which causes the gel layer to reduce its thickness according to a linear law, $L(t) = L_0 - vt$, the fractional drug release will be given by Eq. (7), obtained by solving Eq. (1) and then integrating the drug

concentration profile according to Eq. (6):

$$R_M(t) = 1 - \frac{L(t)}{L_o} + \frac{(C_1 - C_0)}{C_0}\,\frac{L(t)}{L_0} \times \left[-1 + \sum_{n=0}^{N}\left[\frac{8}{(2n+1)^2\pi^2} \times \exp\left[-(2n+1)^2\pi^2\frac{Dt}{4L_0}\right]\right]\right] \quad (7)$$

Equation (7) is therefore the model equation that is able to describe the drug release due to simultaneous diffusion and erosion phenomena. The model was fitted to the experimental data, and the results are reported in Fig. 9. Both experimental data series, in the two systems investigated, were nicely described by the model. Each data curve was fitted by regression of two parameters: the B12 diffusivity through soft and hard gel layers, supposing D to be uniform, and the erosion front velocity, v. Water diffusivity of vitamin B12 is $D_0 = 3.8 \times 10^{-10}$ m^2/s.[19] With reference to the blend containing the alginate AL-1, the diffusivity and the erosion velocity fitted values are $D_{\text{AL-1}} = 7.3 \times 10^{-11}$ m^2/s and $v_{\text{AL-1}} = 1.9 \times 10^{-7}$ m/s. For the blend containing the alginate AL-2, the fitted values are $D_{\text{AL-2}} = 3.7 \times 10^{-11}$ m^2/s and $v_{\text{AL-2}} = 7.6 \times 10^{-8}$ m/s. These values are used to obtain the curves in Fig. 9 on the basis of Eq. (7). The ratio D/D_0 for the two systems therefore assumes the following values: $D_{\text{AL-1}}/D_0 = 0.192$ and $D_{\text{AL-2}}/D_0 = 0.098$.

Lustig and Peppas[22] proposed, and Amsden[23] extensively tested, a relationship obtained on the basis of the volume free theory:

$$\frac{D}{D_0} = \left(1 - \frac{r_s}{\xi}\right) e^{\left(-Y\frac{\varphi}{1-\varphi}\right)} \quad (8)$$

where r_s is the Stokes radius (0.86 nm for B12), φ is the polymer volume fraction that is about the same of the mass fraction = 0.20, and ξ is the network mesh size. According to Lustig and Peppas, the coefficient Y can be taken as unity and this allows to calculate the mesh size of $\xi_{\text{AL-1}} = 1.15$ nm and of $\xi_{\text{AL-2}} = 1.00$ nm. However, Amsden pointed out that $Y = 1$ is not a good estimate and, under the further hypothesis of $r_s \ll \xi$, he found that $Y = k_2 r_s^2$. For B12 in a network made of polyvinyl alcohol (PVA), Amsden calculated $k_2 = 6.1$ nm^{-2}. Assuming that this value is also suitable for B12 in the network as investigated inthis work, $Y = 4.4$, $\xi_{\text{AL-1}} = 2.05$ nm, and $\xi_{\text{AL-2}} = 1.20$ nm. The method of Amsden was also successfully used in a recent work dealing with different polymers and gels.[24] It should be noted that the method depends both on the value of k_2, which was estimated for a system different from the present one, and the value of the ratio D/D_0, obtained by fitting the experimental data with a simplified model. Nevertheless, this is a simple and effective method to estimate the order of magnitude of the mesh size.

For the initial gel layer thickness of $L_0 = 0.5$ cm, the time required to fully erode the gel is $t_{\text{AL-1}} = L_0/v_{\text{AL-1}} = 438$ min (7.3 h) and $t_{\text{AL-2}} = L_0/v_{\text{AL-2}} = 1100$ min (18.3 h). All these findings agree with the fact that an alginate with a low M/G ratio (in this case AL-2, see Table I) easily produces the egg-box aggregates, that is, a more structured gel, which is, in turn, more difficult to be eroded and offers a higher resistance to drug diffusion (lower diffusivity). Furthermore, the observed time for the complete erosion of the gel layers agrees well with the predicted values. Therefore, the modeling confirms that

1. the main phenomena have been correctly identified and quantified and
2. the more structured nature of the gel obtained using alginate with a lower M/G ratio has been confirmed by the reduction in diffusion and erosion rates.

Alginate Particle Characterization

Alginate particles are produced through the same reticulating method applied to obtain films. AL-2 alginate was selected on the basis of the observations on thin slab structures, that is, to have reduced diffusional and erosion rates.

Figure 10 presents photographs of matrix and shell–core particles. Loaded B12 was visible due to its dark pink color (gray shadows in the photographs). In particular, on the left side, the entrapped B12 within the core side in the shell–core

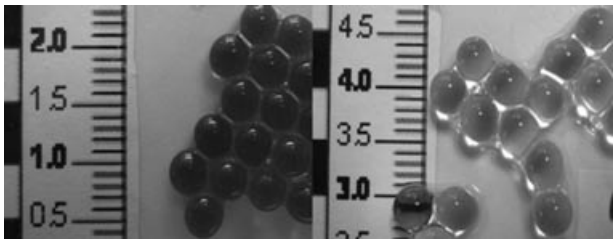

FIGURE 10. Photographs of alginate/B12 matrix (on the left side) and shell–core (on the right side) coarse particles obtained by using the dripping method.

particles was well evident (as reported above, in shell–core particles production, water/AL/B12 solutions were pumped through the core channel).

Both coarse matrix and shell–core drops were cured to obtain particles using a calcium solution as reticulation medium, as reported in the Methods. Calcium bivalent ions are the most diffused choice in the alginate reticulation process in food,[25,26] biopharmaceutical,[18] and pharmaceutical applications (see references in the Introduction), mainly due to the biocompatibility and rheological features of the produced gel. Calcium ions, as other alkaline earth ions, show, in particular, binding preferences for GG blocks rather than MM,[16] so rigid particles are expected to be produced mainly for the alginate richer in GG blocks (AL-2). Of course, the wall thickness is affected by the ion concentration and curing time in the calcium solution; even these effects were not investigated in this work. A behavior similar to those observed with the gel layers reticulated using copper solutions is expected.

Both coarse matrix and shell–core particles showed a fully developed spherical shape and a diameter size of 3.8 mm with a very narrow size distribution; a mean value of 700 μm was measured as wall a shell–core particle thickness. The diameter size and wall thickness were measured after an overall time of 2 min (dripping time) and 5 min (reticulation time lapse) under the conditions previously reported.

Alginate Particle Dissolution Tests

The given amount of alginate particles was dissolved in a buffer solution at pH 7.4, under controlled conditions, with the aim of investigating B12 encapsulation performance and release profiles of both matrix- and shell–core-prepared particles.

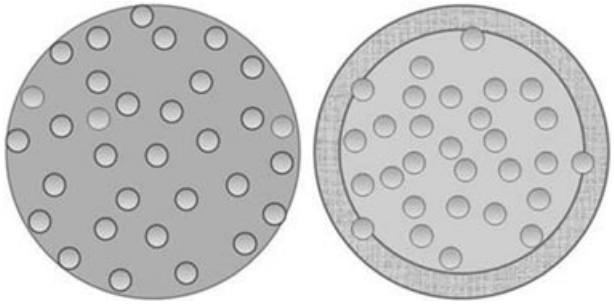

FIGURE 11. A sketch of B12-loaded alginate particles with a matrix (on the left side) and a shell–core (on the right side) structure (circles: B12 distribution).

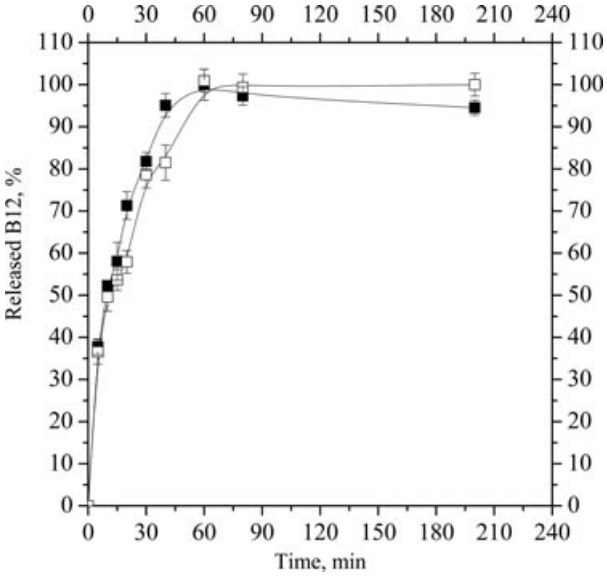

FIGURE 12. B12 release profiles from alginate particles (■: matrix particles; □: shell–core particles).

Because of the different structural arrangements of coarse alginate drops (Fig. 11 shows matrix and shell–core particles), distinct behaviors are expected. In Figs. 12 and 13, results of dissolution tests are reported.

Figure 12 shows release profiles from alginate particles and no relevant difference in release behaviors has been observed. With the lapse of time

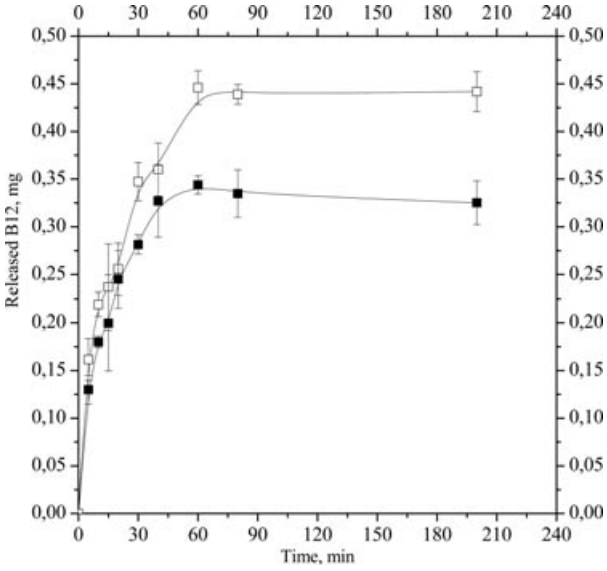

FIGURE 13. B12 mass release evolution with time from alginate particles (■: matrix particles; □: shell–core particles).

of about 90 min, all the loaded B12 was released; just a little increase in the "barrier effect" was detected in shell–core particles release profiles. In spite of these results, an interesting observation of the B12 mass release evolution with time is shown in Fig. 13. In shell–core particles, the highest B12 loading was achieved. Starting from the same volume of B12/AL-2 dripped solution, an encapsulation performance of 60% in shell–core particles was noticed compared with 46% noticed in matrix particles. As expected, the shell barrier reduces the loss of B12 because of the hindered diffusion through the reticulated network during washing operations. It is worthwhile to note that an increment in loading was achieved only by using a double-channel device without any variation in chemical composition feed.

Conclusions

In this work, alginate blends were used with the aim of studying alginate reticulated gel features. Two kinds of sodium alginate were tested to obtain gel films; they are used as key components in biocompatible polymer blends of Pluronic investigated as potential DES gel paving. Thin layers (film structures) were produced using two commercial alginates with different M/G ratios and copper (bivalent cations) solution as reticulating medium. Gel films of FMC alginate, as a result of the richness of GG segments, showed a more compact structure than the Sigma–Aldrich alginate films, resulting in a thin layer with good barrier features. This suggests that the alginate composition plays a crucial part in the reticulated structural arrangements.

Thin FMC alginate was then used in shell–core particle development (particle structures). These particles were prepared by dripping in the calcium solution. Dissolution tests of B12-loaded particles showed that the presence of an additional thin layer of reticulated alginate (i.e., the shell structure) reduces losses during production, such as washing operations.

References

1. Yu, W.; Song, H.; Zheng, G.; Liu, X.; Zhang, Y.; Ma, X. J Membr Sci 2011, 377, 214–220.
2. Gombotz, W.; Wee, S. Adv Drug Del Rev 1998, 31, 267–285.
3. Dash, M.; Chiellini, F.; Ottenbrite, R. M.; Chiellini, E. Prog Polym Sci 2011, 36, 981–1014.
4. George, M.; Abraham, T. E. J Control Rel 2006, 114, 1–14.
5. Tønnesen, H.; Karlsen, J. Drug Dev Ind Pharm 2002, 28, 621–630.
6. Rudzinski, W. E.; Aminabhavi, T. M. Int J Pharm 2010, 399 (1–2), 1–11.
7. Wang, J. J.; Zeng, Z.; Xiao, R.; Xie, T.; Zhou, G.; Zhan, X.; Wang, S. Int JNanomed 2011, 6, 765–774.
8. Simonoska Crcarevska, M.; Glavas Dodov, M.; Goracinova, K. Eur J Pharm Biopharm 2008, 68, 565–578.
9. Li, Y.; McClements, D. J. Food Hydrocolloids 2011, 25, 1025–1033.
10. Chen, C.-C.; Fang, C.-L.; Al-Suwayeh, S. A.; Leu, Y.-L.; Fang, J.-Y. Int J Pharm 2011, 415, 119–128.
11. Grassi, G.; Crevatin, A.; Farra, R.; Guarnieri, G.; Pascotto, A.; Rehimers, B.; Lapasin, R.; Grassi, M. J Colloid Interface Sci 2006, 301, 282–290.
12. Moebus, K.; Siepmann, J.; Bodmeier, R. Eur J Pharm Biopharm 2009, 72, 42–53.
13. Das, R. K.; Kasoju, N.; Bora, U. Nanomed: Nanotechnol Biol Med 2010, 6, 153–160.
14. Grassi, G.; Noro, E.; Farra, R.; Guarnieri, G.; Lapasin, R.; Grassi, M.; Matricardi, P.; Coviello, T.; DalCortivo, A.; Alhaique, F. J Control Rel 2006, 116, e85–e87.
15. Barba, A. A.; d'Amore, M.; Grassi, M.; Chirico, S.; Lamberti, G.; Titomanlio, G. J Appl Polym Sci 2009, 114, 688–695.
16. DeRamos, C. M.; Irwin, A. E.; Nauss, J. L.; Stout, B. E. Inorg Chim Acta 1997, 256, 69–75.
17. Kawarada, H.; Hirai, A.; Odani, H.; lida, T.; Nakajima, A. Polym Bull 1990, 24, 7.
18. Thu, B.; Gåserød, O.; Paus, D.; Mikkelsen, A.; Skjåk-Bræk, G.; Toffanin, R.; Vittur, F.; Rizzo, R. Bipolymers 2000, 53, 11.
19. Grassi, G.; Lapasin, R.; Grassi, M.; Colombo, I. Understanding Drug Release and Absorption Mechanisms: A Physical and Mathematical Approach; CRC Press: Boca Raton, FL, 2007.
20. Cascone S.; Lamberti G.; Titomanlio G.; Barba A. A.; d'Amore M. Drug Dev Industrial Pharm. In press. doi: 10.3109/03639045.2011.653814.
21. Crank, J. The mathematics of diffusion, 2nd Ed. Clarendon: Oxford, UK, 1975.
22. Lustig, S. R.; Peppas, N. A. J Appl Polym Sci 1988, 36, 735–747.
23. Amsden, B. Macromolecules 1998, 31, 8382–8395.
24. Barba, A. A.; d'Amore, M.; Cascone, S.; Chirico, S.; Lamberti, G.; Titomanlio, G. J Pharm Sci 2009, 98, 4100–4110.
25. Gómez-Díaz, D.; Navaza, J. M. J Food Eng 2003, 56, 387–392.
26. Olivas, G. I.; Barbosa-Canovas, G.V. LWT—Food Sci Technol 2008, 41, 359–366.