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Short communication

Modeling of the reticulation kinetics of alginate/pluronic blends for biomedical applications

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ABSTRACT

In this work, blends of alginate/pluronic (F127) for biomedical applications were investigated. In particular, the kinetics of alginate chain reticulation by bivalent cations was studied by experimental and modeling approaches. Two kinds of sodium alginate were tested to obtain hard gel films. The thicknesses of the reticulated alginate films were measured as function of the exposure time and of the reticulating copper (Cu^{2+}) solution concentration. The kinetics was described by a proper model able to reproduce the experimental data. The model parameters, evaluated based on the measurements of thicknesses as function of Cu^{2+} concentration and exposure time, were further validated by comparing the prediction of the model with another set of independent measurement; here, the depletion of Cu^{2+} ions in the conditioning solution above the reacting gel is measured as function of time. The tuned model could be used in the description of the future applications of the blends.

1. Introduction

Biocompatible aqueous blends of pluronic and alginates are of great interest in pharmaceutical and biomedical applications for their unique properties: the pluronic, once exposed at physiological temperatures, gives a soft gel, producing a framework for scaffolding or for drug delivery; the alginates, once reticulated, give a hard gel which can be useful to protect the pluronic soft gel and/or to improve the blend mechanical characteristics. Therefore, proper mixtures of these two polymers can be used to obtain gels of tailored features [1,2].

The two classes of the abovementioned polymers were widely used in biomedical applications, even if usually not in the form of their blends. For example, the alginates are commonly and widely used in protein and cell delivery and encapsulation [3–5], or in general, as drug delivery carriers [6,7]. Similarly, the use of pluronic in drug delivery system formulation and in other biomedical applications has been investigated [8–14]. Of course, a large amount of work has been devoted to material characterization. Rheology of pluronic solutions [15], micellization kinetics [16], and gelation thermodynamics and kinetics [17–20] have been investigated by several methods, including dielectric measurements [17,21]. Alginate nature (chemical composition) and conformation (structural arrangement) have been studied, for example by NMR [22]. An exhaustive analysis of the related literature goes well beyond the scope of this work. On the other hand, the blends of alginate and pluronic are not well known. Potential applications of these blends have been recently proposed for example in the drug eluting stent covering [23,24]. Their use in drug delivery requires adding to the blends also drugs, in free form [1,25,26] or embedded in suitable vectors [27], even based on enteric particles for oral administration [28,29].

In particular, the use of pluronic–alginate blends has been suggested in order to provide a novel gel-paving system for covering stents inserted in the artery during interventions of PCA (percutaneous coronary angioplasty) [23,24]. Following the suggested approach, a layer of soft gel, located close to the artery wall, is needed in order to provide the reservoir for the drug to be able to reduce the growth of muscular smooth cells, and a layer of hard gel, on the side of the blood stream, is needed in order to limit the erosion due to the blood and to reduce the release of the drug in the hematic stream. The soft gel layer can be obtained by the thermal gelation of the pluronic solution (injected as a cold liquid, 4–5 °C, solutions of proper concentrations give soft gel once exposed to the body normal temperature, 37 °C); the layer of hard gel can be obtained exposing the alginate to a solution of bivalent ions (ionotropic gelation). The best candidate as bivalent cation for the ionotropic gelation would be the calcium, Ca^{2+}, which is biocompatible and non-toxic. However, a sudden release of calcium in the cardiac region (where the interventions are carried out) can have dramatic side effects (since the heart normal function requires a given calcium–potassium balance); therefore the copper, Cu^{2+} has been selected as a reticulating agent, which is more toxic than calcium in other regions of the body, but does not affect the cardiac function. Furthermore, the

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total amount of copper expected to be used in this application, even if fully released in the blood stream by accident, will give a total copper concentration below the toxic threshold.

The two fundamental steps in the preparation of systems based on pluronic–alginate blends are the thermal gelation due to the pluronic fraction and the reticulation due to the alginate fraction. Whatever the application, the knowledge of "how much" (the thermodynamic) and of "how fast" (the kinetics) the gelation takes place, is necessary in order to describe and to manage the applications of these biomedical gels. The first step has been already studied working with pluronic solutions [17], and the results were proved to be valid also on pluronic–alginate solutions (i.e., the presence of alginate does not influence the thermodynamics and the kinetics of thermal gelation of pluronic).

The aim of this work is to propose and to validate a model useful in the description of the reticulation of pluronic–alginate blends.

2. Materials and methods

2.1. Materials

Sodium alginate (AL-1) (CAS no. 9005-38-3, Sigma cat. W201502), Pluronic F127 (PF127) flakes (CAS no. 9003-11-6), and $CuSO_4\ 5H_2O$ (CAS no. 7758-99-8) (to be used in water solutions as reticulating or cross-linker agent) were purchased from Sigma Aldrich s.r.l., Milano IT. Sodium alginate (AL-2) (FMC) was purchased from FMC Biopolymer, Milano IT.

The two alginates used in this work to prepare the blends are practically of the same molecular weight ($MW_{AL\text{-}1} = 396$ kDa, $MW_{AL\text{-}2} = 419$ kDa, estimated through intrinsic viscosity measurements and using the MHS, Mark–Houwkind–Sakurada, coefficients suggested in literature [30]), but they have very different mannuronic/guluronic ratio ($M/G_{AL\text{-}1} = 1.49$, $M/G_{AL\text{-}2} = 0.56$, estimated by NMR [1]). The M/G ratios identify AL-1 as obtained from the algae *Macrocystis pyrifera*, and AL-2 as obtained from the algae *Laminaria hyperborea*. Alginates with large amounts of M fraction, such as AL-1, once reticulated give weak and elastic gels; alginates with large amounts of G fraction, such as AL-2, once reticulated give hard and fragile gels. Therefore, the selection of different alginates could be of use to obtain tailored blends for different applications.

2.2. Methods

Blends composed by 18 wt.% in PF127, 2 wt.% in alginate (AL-1 or AL-2) and 80 wt.% in distilled water were prepared by adding first alginate powders then PF flakes in cold distilled water, gently mixing, and allowing the stabilization by keeping them at 4 °C overnight.

Films were prepared putting given amounts of the AL-PF127 blend into a Petri dish and heating at 37 °C to promote the thermal gelation of the pluronic fraction. After 7 min, 25 mL of copper solution (in experiments of type "a", working at different concentrations: 1, 2, 3, 4 and 5 g/L) or 7 mL of copper solution (in experiments of type "b", working at concentration of 1 g/L) were added, carefully spread on the gel-blend surface and kept for given times (contact time, or Cu^{2+} exposure time, t_E: 1, 2, 3, 4, 5 min). Alginate film was produced on the gel-blend surface by 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 mm–2 mm). Both the film preparation (at given copper solution concentration/contact time) and the thickness measurement were performed in triplicate.

In the experiments of type "a", the reticulation solution was poured on the soft gel film in large excess (25 mL), in order to keep the copper concentration practically constant. In the experiments of type "b", the aim of which was to measure the depletion of copper from the solution, a lesser amount of copper solution (7 mL) was added to produce reticulation. Finally, to further check the reliability of the model, a given amount of hard gel was dissolved and the copper contained in the gel was thus quantified (experiments of type "c").

The copper content in solutions was assayed by UV spectrometry (PerkinElmer LAMDBA 25 spectrophotometer) working at $\lambda = 282$ nm. It is well-known that copper sulfate solutions absorb in the visible range (being clear blue, its maximum absorption wavelength is 802 nm), but the use of UV peak allows the measurement of high concentrations avoiding the absorbance saturation effect and making unnecessary the cumbersome diluting procedures. Furthermore, in order to point out a method (for future uses) usable in simultaneous measurements [31] of copper and drugs (which usually absorb in the UV range), the measurements were performed not reading directly the absorbance and tuning the Lambert–Beer law (which is the traditional method), but fitting the spectra in the range of wavelength 250–300 nm with a Gaussian curve and thus obtaining the copper concentration. The curve and the parameters, obtained by a tuning procedure, are (C = copper concentration in mg/mL, λ = wavelength in nm):

$$A(\lambda) = k_\lambda C \exp\left[-4\ln(2)\left(\frac{\lambda-\lambda_c}{w_\lambda}\right)^2\right] = (0.0587)C\exp\left[-4\ln(2)\left(\frac{\lambda-282.02}{49.684}\right)^2\right]. \quad (1)$$

3. Modeling

The hard gel based on alginate fraction is due to structures known as "egg-box", which were formed by interactions mainly by the guluronic units (G) [1] of the alginate chains with cations. The egg-boxes give to the network an increased mechanical modulus with respect to the soft gel due to pluronic fraction. Therefore, the main phenomena which take place during the reticulation are the diffusion of cations within the gel, and their reaction with the guluronic units to give the egg-box [32]. A simple attempt to model the full process could be easily proposed looking at Fig. 1, in which the process itself was schematized in terms of profiles of concentrations for diffusing ions and egg-box. Then, being Q the concentration of diffusing ("free") cations in the gel, and S the concentration of egg-box in the gel, the specie mass balances in transient and along the single diffusion direction, x, can be written as:

$$\frac{\partial Q}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial Q}{\partial x}\right) - k''' Q \quad (2)$$

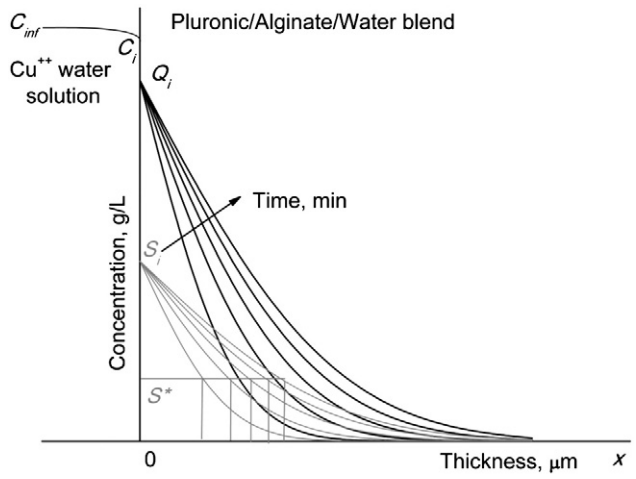

Fig. 1. A schematic of the reticulation process summarizing the modeling simplifications adopted. On the left, the copper water solution is reported, with the meaningful concentrations C_{inf} (the bulk concentration) and C_i (the interface concentration). On the right (the positive x-values), the time evolution of free-cations, Q, and egg-box, S, concentration profiles are drawn (black lines are the free cation concentration profiles, gray lines are the egg-box concentration profiles). The horizontal line at the level S^* (the critical value which indicates when the hard-gel is obtained) allows the identification of the thicknesses of the hard-gel layer obtained for different exposure times.