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Food microencapsulation of bioactive compounds: Rheological and thermal characterisation of non-conventional gelling system

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ABSTRACT

Microencapsulation is a powerful technique commonly used for the protection of a wide range of biomolecules (small molecules and protein) and cells of bacterial, yeast and animal origin. In this work, solutions of mixed biopolymers are investigated as excipients for the formulation of a model system. The influence of the different components is studied from the viscoelastic behaviour of the starting solutions to the thermal characterisation of the gel beads therefrom produced. Rheological characterisation displays an almost regular trend for the several combination of solutes and for the frequency dependence, but some peculiarities emerge when both the model protein lysozyme and the cosolvent ethanol are present in the mixture; for the latter system a delayed melting behaviour of water appears in the gel beads. Changes in the temperature dependence of water evaporation from the beads are taken as an evidence of the rate of release from the beads.

1. Introduction

Microencapsulation is a powerful technique which allows the protection of a wide range of materials of biological interest, from small molecules and protein (enzymes, hormones,...) to cells of bacterial, yeast and animal origin (Thies, 2005). Microencapsulation is based on the embedding effect of a polymeric matrix, which creates a microenvironment in the capsule able to control the interactions between the internal part and the external one. For this reason such versatile technology is widely studied and exploited in the high technological fields of biomedicine and biopharmaceutics, for application ranging from cell therapy to drug delivery (Smidsrød & Skjak-Braek, 1990). The same characteristics make microencapsulation suitable for food industry applications, in particular for the production of high value aliments and nutraceuticals. The industrial production of foods requires the addition of functional ingredients to tailor flavour, colour, texture or preservation properties. Moreover, the inclusion of ingredients with potential health benefits, such as antioxidants and probiotics, is increasing. The preparation of functional foods deals with the control of the bioactive compounds stability during processing and storage, with the prevention of detrimental reactions within the carrier food matrix, and with the fate of the food after the introduction into the human body. Such an example, it is mandatory to ensure the stability of certain compounds (such as probiotics) in the gastrointestinal tract and to allow a controlled release at the appropriate target.

Several microencapsulation methods have been developed and described. The most relevant are spray-coating, spray-drying, extrusion, emulsion and gel-particle technology (Champagne & Fustier, 2007). Each methodology has particular features and characteristics which allow the application to systems based on materials with peculiar mechanical and physico-chemical properties.

Among the existing techniques, microencapsulation in alginate matrices exploits the capability of this biopolymer to form hydrogels in presence of small amounts of divalent cations (such as calcium ions). Alginate is an unbranched polysaccharide of algal and bacterial origin. It is composed by units of β-D-mannuronic acid (M) and α-L-guluronic acid (G) which form homopolymeric block structures along the chains, namely M-blocks and G-blocks interspaced by alternate MG sequences. The interaction between G-blocks and divalent cations results in the formation of interchain crosslinks, according to the "egg-box" model (Rees, 1977), and leads to the formation of the hydrogel 3D-network.

In microencapsulation technology, alginate is often employed together with other materials, in order to combine its gelling

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properties with other characteristics (deriving from the partner) useful for the final encapsulating system. Thus, blends of alginate and other biopolymers have been widely described. Some examples are the production of bioreactors for cell seeding based on the combination of alginate with chitosans or hyaluronic acid (Gerard et al., 2005; Marsich et al., 2008) or the preparation of formulations with controlled release properties based on the association with cellulose derivatives (Lee, Hwang, Park, & Park, 2003).

The encapsulation process gives also the opportunity to combine in the capsule structure several compounds simultaneously. This is of fundamental relevance for the industrial application, since it simplifies the food-enriching process. However, the microenvironment generated by introducing in the encapsulating systems components of different origin, characterised by particular chemical and physico-chemical nature, can strongly influence the gel forming process. This leads to "extreme" situations of non-conventional gelling conditions which affect the formation of the hydrogel matrix.

For example, the introduction of protein into the system is influenced by the possible interaction between protein and polymer (alginate) and from the resulting effect of the complexes on the final properties. The situation is also complicated by the introduction of non-water soluble compounds, such as molecules of lipidic origin. For example the administration of lipoic acid dissolved in ethanol and encapsulated in chitosan hydrogels has been proposed (Weerakody, Fagan, & Kosaraju, 2008).

The examples reported above highlight the relevant need to characterise non-conventional working conditions, in order to be able to associate the most suitable technique for material processing and microcapsule generation.

The aim of this work is the understanding of the properties of a multi-component model system of interest in pharmaceutical encapsulation and for food applications. The system is characterised by an alginate matrix combined with a cellulose-based excipient (hydroxypropyl-methyl-cellulose, HPMC) which controls the matrix releasing properties, with a model protein of food and pharmaceutical relevance (lysozyme) and ethanol as solvent for non-water soluble compounds. In the first part of the work a rheological characterisation has been carried out in order to determine the properties and the resulting behaviour of the materials employed in the formulation. The second part deals with the preliminary investigation of the morphological and structural properties of hydrogel beads of various compositions used in this work. Then, a thermal characterisation of the system, in terms of both the raw materials and the complete hydrogel structure has been performed. The influence of each component on the overall system response to freezing and dehydration phenomena has been investigated.

2. Materials and methods

2.1. Materials

Sodium alginate (from brown algae, Mw 5.3×10^5, $F_G = 0.30$, Qingdao Bright Moon Seaweed Group Co. Ltd., China); HPMC, (hydroxypropyl-methyl-cellulose, Hercules Doel, Beveren, Belgium), lysozyme (E.C.3.2.1.17; Sigma–Aldrich Co., St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation and characterisation of the encapsulating model system

2.2.1. Bead-forming solutions

The multi-component encapsulation system developed is based on two bead-forming solutions in which alginate (with final concentration of 1% and 2% w/v respectively) is blended with the other components. The influence of each component has been determined by studying the behaviour of solutions in which one or more components of the original preparation have been removed. The composition of the set of these starting solutions employed is reported in Table 1. Alginate and alginate-lysozyme solutions were prepared by dissolving the powders in deionized water. HPMC was dissolved either in water or hydro-alcoholic solution (ethanol 40% v/v). The final blends have been obtained by mixing equal volumes of alginate (and alginate-lysozyme) solution and HPMC solution, under vigorous stirring.

2.2.2. Rheological measurements

Rheological characterisation has been carried out on the original bead-forming solutions and on the solutions reported in Table 1, in order to fully characterise the system. Continuous and oscillatory shear tests were performed with a rotational controlled stress rheometer (Rheostress RS150, Haake GmbH, Germany), equipped with a parallel plate geometry ($d = 35$ mm, gap = 1 mm) with serrated surfaces in order to avoid wall slippage effects. The apparatus was computer controlled (RheoWin software program) and the temperature was maintained at 25 ± 0.1 °C (Thermostat TC500, Haake GmbH, Germany). The flow properties were determined applying an increasing sequence of constant stress values in the range 1–1000 Pa and measuring the corresponding shear rates. Each stress value was maintained constant until the corresponding time variation of the shear rate satisfied the following constraint, $(\Delta\dot{\gamma}/\dot{\gamma})\Delta t \leqslant 0.05\%$, or at most for 90 s. A pre-shearing step at constant shear rate ($30\ s^{-1}$ for 180 s) was applied to each sample before the stress sequence in order to ensure controlled and repeatable initial conditions.

Oscillatory stress sweep tests were performed at 1 Hz to individuate the upper limit of the linear viscoelasticity range. All the frequency sweep measurements were performed at ω ranging from 100 to 0.1 Hz within the linear range.

2.3. Beads characterisation

2.3.1. Beads formation

Calcium hydrogel beads were prepared by dripping the polymer blend from a plastic syringe (equipped with a 21-gauge needle) into a gelling solution ($CaCl_2$ 50 mM) under gentle stirring, at room temperature. The formed beads were cured for 30 min, allowing the hardening of the hydrogel structure and then rinsed with deionized water prior to use.

Table 1
Composition of the solutions employed in this study.

Sample	Alginate % (w/v)	HPMC % (w/v)	Lysozyme % (w/v)	Ethanol % (v/v)
A1	1	0.2	0.5	40
A2	2	0.2	0.5	40
B1	1	0.2	0.5	–
B2	2	0.2	0.5	–
C1	1	–	0.5	40
C2	2	–	0.5	40
D1	1	–	0.5	–
D2	2	–	0.5	–
E1	1	0.2	–	40
E2	2	0.2	–	40
F1	1	0.2	–	–
F2	2	0.2	–	–
G1	1	–	–	40
G2	2	–	–	40
H1	1	–	–	–
H2	2	–	–	–