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International Journal of Pharmaceutics 242 (2002) 163–166

international journal of pharmaceutics

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Note

Biopolymeric colloidal carriers for encapsulation or controlled release applications

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Received 24 December 2001; received in revised form 10 January 2002; accepted 16 January 2002

Abstract

Biopolymers represent an interesting alternative to synthetic polymers in order to be used as structured carriers for controlled release and encapsulation applications. In particular, the ability of these carriers to entrap both hydrophilic and hydrophobic drugs may be very promising for many applications. In addition, the absence of chemical compounds and organic solvents used to produce biopolymeric matrices could be very interesting for some industrial applications. Simple or complex coacervation methods involving proteins or protein and polysaccharide mixtures were used to create new matrices dedicated to controlled release applications. Controlled release experiments with model compounds were conducted in order to evaluate the performance of such matrices. An alternative and promising research field deals with particles obtained from hydrogel systems. Totally transparent solid matrices resulting from the dehydration of new protein gels were formed and swelling capacities of these matrices were studied.

Keywords: Coacervation; Encapsulation; Hydrogels; Swelling; Proteins; Arabic gum

In the last decades, micro and nanosized colloidal carriers have received a growing scientific and industrial interest (Thies, 1996). These vectors may be capsules (with liquid core surrounded by a solid shell), particles (polymeric matrices), vesicles or liposomes, multiple or single emulsions and found a wide range of applications. They may be loaded by living cells, enzymes, flavour oils, pharmaceuticals, vitamins, adhesives, agrochemicals, catalysts and offer considerable advantages at use. Liquids can be handled as solids, odour or taste can be effectively masked in a food product, sensitive substances can be protected from deleterious effects of the surrounding environment, toxic materials can be safely handled, and drug delivery can be controlled and targeted (Robinson, 1997).

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00143-6

In the forementioned laboratories, we started with a new strategy based on phase separation in order to prepare natural particles. Simple or complex coacervation methods involving proteins or protein and polysaccharide mixtures (Schmitt et al., 1998) were used to create new matrices dedicated to controlled release applications. The colloidal carriers produced were in the micrometer or nanometer size range depending on the substrates or the methods used.

Wheat proteins, gliadins, were implicated in simple coacervation to produce nanospheres. Nanoparticles were obtained by desolvation of the protein using physiological salt solution as non-solvent. Synperonic PE F68 was used to stabilize the nanoparticles suspension (Duclairoir et al., 1998). Gliadins nanospheres typical size was around 900 nm (Duclairoir et al., 1998). Particles size and polydispersity increased with the increase of the solvent/non-solvent ratio and with the aggregated state of the proteins. Controlled release experiments with model compounds were conducted in order to evaluate the performance of such matrices. Vitamin E-loaded nanoparticles (1 mg) were digested in 10 ml of an ethanol/water mixture (62/38 v/v) at room temperature in the dark. The vitamin E concentration encapsulated in nanoparticles (C_1) and the residual concentration in the supernatant (C_2) were then assayed by HPLC at 290 nm. Empty nanoparticles were treated in the same way and used as references for these determinations. The drug loading (rate) and the entrapment efficiency were calculated according to the equations:

$$\text{Rate } (\%) = \frac{C_1}{m_{\text{gliadins}}} \qquad \text{Efficiency } (\%) = \frac{C_1}{C_1 + C_2}$$

In vitro drug release kinetic was also performed in non-sink conditions using decane as solvent (to prevent drug loss from nanoparticles dissolution) and a laboratory designed release cell. About 10 mg of gliadins nanoparticles (containing 824 μg/g gliadins) were resuspended in 10 ml of decane. Aliquots were collected at successive time intervals and replaced by the same quantity of solvent in order to get a constant volume in the release cell. The samples were analysed by HPLC as described above for encapsulation experiments.

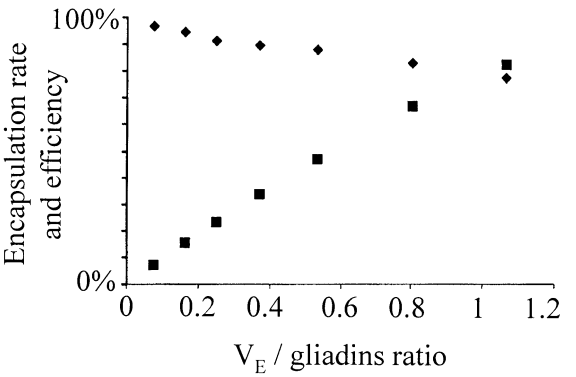

Fig. 1. Vitamin E (V_E) encapsulation rate (■) and efficiency (◆) by gliadins nanospheres as a function of V_E/gliadins ratio.

Encapsulation of vitamin E (V_E) into gliadins nanospheres revealed that the rate and efficiency decreased with the decrease of the V_E/gliadins ratio (Fig. 1). For a V_E/gliadins ratio of 1, an encapsulation rate of 824 μg/mg gliadins and an efficiency of 77% were obtained. The V_E release kinetic from loaded particles was displayed Fig. 2. The experimental points were fitted with the following equations:

$$\frac{V_E}{\text{initial } V_E} = 6\sqrt{\frac{\tau}{\pi}} \quad \text{(short time)}$$

$$\frac{V_E}{\text{initial } V_E} = 6\sqrt{\frac{\tau}{\pi}} - 3\tau \quad \text{(intermediate time)}$$

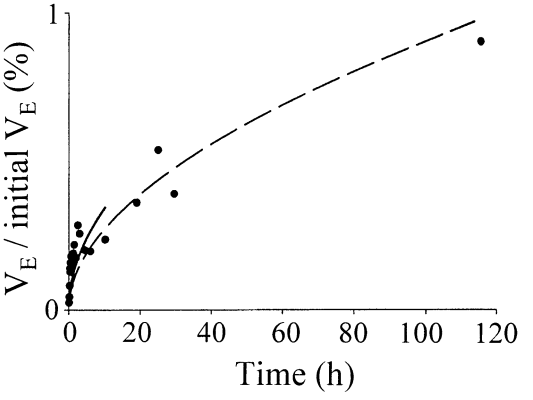

Fig. 2. Vitamin E (V_E) release kinetic by gliadins nanospheres as a function of time (h). See text for equations used in the fitting procedure: (●) released V_E (%); (—) fit (short time); (----) fit (intermediate time).

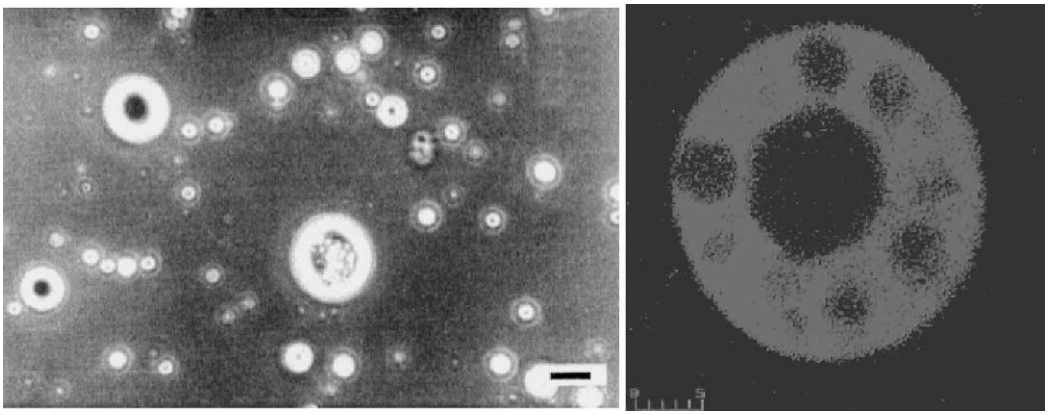

Fig. 3. (Left side) Phase contrast optical micrograph of 1 wt.% β-lactoglobulin/Arabic gum mixtures at pH 4.2 ratio 1:1. Scale bar represents 20 μm. (right side) Confocal scanning laser micrograph of a coacervate obtained with a 1 wt.% β-lactoglobulin/Arabic gum mixture at pH 4.2 ratio 1:1. Scale bar represents 5 μm.

with $\tau = Dt/r^2$, D being the diffusion coefficient of the entrapped vitamin E and r, the radius of the nanoparticles.

The kinetic profile was thus interpreted by a burst effect coupled with a drug diffusion process through the particle modelled as a homogeneous sphere. The drug diffusion coefficient was considerably reduced when entrapped in the carrier: $D_{VE} = 1.1\ \ 10^{-20}$ m^2/s compare to $D_{VE} = 10^{-9}$ m^2/s in solution. The encapsulation of different drugs into nanospheres showed that carriers had more affinity for hydrophobic drugs and that the ζ-potential of the particles was directly related to the nature of the drug (Duclairoir, 2000).

In the case of complex coacervation, the β-lactoglobulin/Arabic gum couple was tested. The mechanism of formation and the structural properties of coacervates were first highlighted in order to better control the stability of these systems. Coacervation process was established at pH 4.2 in water where the two biopolymers interact electrostatically (Schmitt et al., 2000). The structure of the coacervates was explored using both phase contrast and confocal scanning laser microscopies. β-lactoglobulin/Arabic gum spherical vesicular coacervates revealed by microscopy were the hallmark of these dispersions (Fig. 3). Large 'foam-like' coacervates induced by partial coalescence of single coacervates were visible especially at the 2:1 protein to polysaccharide ratio (Schmitt et al., 2001). Increasing dispersions stability was reached by increasing protein to polysaccharide ratio or by decreasing total biopolymer concentration. Another alternative to increase the stability is to produce composite dispersions containing both protein aggregates embedded in protein–polysaccharide coacervates and free coacervates (Schmitt et al., 2000). These systems could thus be used as multifunctional reservoirs for applications in microencapsulation.

An alternative and promising research field deals with particles obtained from hydrogel systems. The hydrogels may be sensitive to environmental stimuli such as pH, ionic strength, electric/magnetic fields, light and temperature depending on the substrate used. Totally transparent solid matrices resulting from the dehydration of new protein gels, revealed variable swelling capacities that depend on the solvent used and physical chemical conditions. The protein hydrogels were formed at pH 8 in 50% ethanol solutions (Renard et al., 2000). Swelling kinetics of cylindrical gels in both hydrated ($m_0 \sim 4.5$ mg) and dehydrated ($m_0 \sim 1.5$ mg) forms were followed in different solvent conditions. The swelling kinetics of β-lactoglobulin hydrogels showed that the increase of mass was the highest in water for both hydrated and dehydrated gels (Fig. 4). The capacity of swelling (or not) depended on the solvent conditions and would allow a controlled

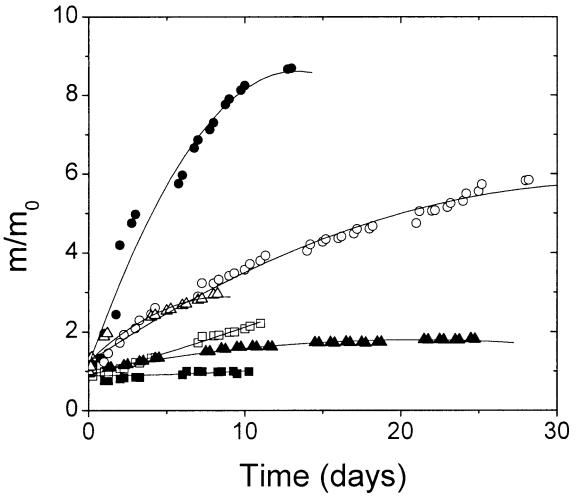

Fig. 4. Swelling kinetics of β-lactoglobulin hydrogels both in dehydrated and hydrated forms for different solvent conditions: (■) ethanol; (□) water/ethanol 50/50 (v/v); (●) water; (○) 0.1M NaCl; (▲) water/ethanol 50/50 (v/v) (hydrated gel); (△) water (hydrated gel).

release of both hydrophilic and hydrophobic drugs.

A new dispersion/gelation method was developed to produce micro-beads having potential applications in the encapsulation field. Basically, the dispersion of a β-lactoglobulin pre-gel in an apolar phase produced gelled droplets. These droplets were then washed and dehydrated under vacuum in order to produce particles of 500 μm mean diameter (Fig. 5). Such protein matrices were totally transparent and could be used to encapsulate large molecules or microorganisms.

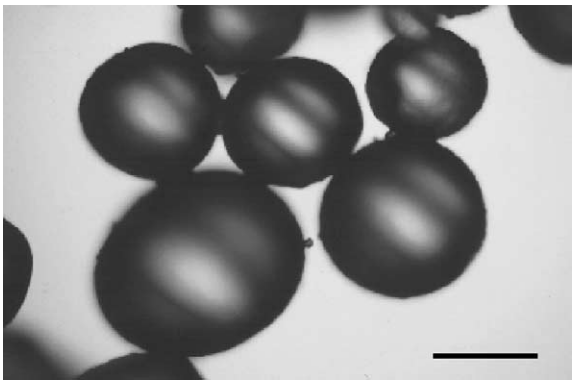

Fig. 5. Optical micrograph of β-lactoglobulin beads obtained by a gelation/emulsification method. Scale bar represents 500 μm.

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