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Optimization of the encapsulation and release of β-lactoglobulin entrapped poly(DL-lactide-co-glycolide) microspheres

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Abstract

The goal of the present paper was to optimize the encapsulation of β-lactoglobulin (BLG) within poly(lactide-co-glycolide) (PLGA) microparticles prepared by the multiple emulsion solvent evaporation method. The role of the pH of the external phase and the introduction of the surfactant Tween 20, in the modulation of the entrapment and release of BLG from microparticles, was studied. Reducing the solubility of BLG by decreasing the pH of the external phase to a value close to the p*I* of BLG resulted in a better encapsulation with, however, a larger burst release effect. By contrast, Tween 20 was shown to increase the encapsulation efficiency of BLG and reduce considerably the burst release effect. In fact, Tween 20 was shown to be responsible for removing the BLG molecules that were adsorbed on the particle surface. In addition, Tween 20 reduced the number of aqueous channels between the internal aqueous droplets as well as those communicating with the external medium. Thus, the more dense structure of BLG microspheres could explain the decrease in the burst release. These results constitute a step ahead in the improvement of an existing technology in controlling protein encapsulation and delivery from microspheres prepared by the multiple emulsion solvent evaporation method.

Keywords: Biodegradable microspheres; Poly(DL-lactide-co-glycolide); β-Lactoglobulin; Tween 20; Encapsulation; Release kinetic

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1. Introduction

Poly(DL-lactide-co-glycolide) (PLGA) microspheres have been studied extensively as protein/peptide delivery systems. The biodegradable and biocompatible nature of PLGA makes this polymer a suitable candidate, among other applications, for the systemic and oral administration of proteins (Couvreur et al., 1997). However, when entrapped in microspheres prepared by the multiple emulsion solvent evaporation method, proteins that display amphiphatic properties accumulate at the interface of the droplets of both the first and the second emulsion. Therefore, the loss of the protein in the external aqueous phase during the process of preparation of the microspheres is a major drawback. In addition, because of their particular location in the microspheres, the proteins are generally released very quickly into the external media, displaying the so-called burst release effect (Blanco-Prieto et al., 1996). In this study, using a model protein, β-lactoglobulin (BLG) known to adsorb at oil–water interfaces, we have attempted using either pH modifications or surfactants to improve the entrapment of BLG and to reduce the burst release effect from PLGA microspheres.

2. Methods

PLGA microspheres containing BLG were prepared by a W/O/W solvent evaporation technique. BLG was dissolved in 0.4 ml TRIS buffer (20 mM, pH 7.5) at different concentrations. This aqueous solution was emulsified with 2.5 ml MC (O) containing 250 mg of PLGA using an Ultraturrax® at 13 500 rpm for 2 min to form a primary W/O emulsion. This emulsion was then poured into 20 ml of an aqueous solution. When evaluating the effect of the pH of the external aqueous phase on microsphere preparation, a simple solution of PVA (2% w/v) (pH 6.5) or PVA(2% w/v)–PBS (pH 5.6 or 5.2) were used. When Tween 20 was introduced into the inner emulsion, its concentration ranged from 0 to 26 mg/ml of a PVA–PBS solution (2% w/v, pH 6.5). Emulsification occurred over 2 min at 13 500 rpm. The multiple W/O/W emulsion was stirred at 1000 rpm for 3 h at room temperature to allow solvent removal and microsphere formation. Microspheres were collected after centrifugation (4000 rpm for 10 min), washed three times with distilled water, freeze-dried and stored at 4°C. The protein content in the microspheres was determined according to the method described by Hora et al. (1990). Freeze-dried microspheres were dispersed in a 0.1 M NaOH solution containing SDS (1% w/v) to give a final concentration of 5.0 mg of particles per ml. The resulting suspension was kept under stirring at room temperature for 24 h. The samples were centrifuged (4000 rpm for 5 min.), and the BLG concentration was measured in the supernatant by a Bio-Rad DC microassay. The encapsulation efficiency was expressed by the ratio between the actual and the theoretical BLG loading × 100.

Microspheres diameter and size distribution were measured using a Coulter Multisizer‘II (Coultronics, France). For microsphere surface charge measurements, freeze-dried microspheres were introduced into a 10 ml glass vial and dispersed in distilled water. The ζ potential of the suspensions was determined using a Zetasizer 4 (Malvern, France), and the measurements were carried out at 25°C.

For the burst release studies, freeze-dried microspheres corresponding to 0.7 mg BLG were accurately weighed in test tubes and dispersed using a vortex in 4 ml TRIS buffer (20 mM, pH 7.5) at 37°C. At 2 min after dispersion, the suspension was centrifuged (4000 rpm for 5 min) and the BLG concentration was determined in the supernatant by a Bio-Rad DC protein microassay.

3. Results and discussion

The loading efficiency was determined as a function of the pH of the external phase. Under the different experimental conditions, the diameter of the microspheres was always between 6.6 to 7.3 μm. For all tested values, it was found that the actual loading of BLG in the microspheres increased by increasing the initial amount of BLG

(Table 1). However, for a given pH value, the encapsulation efficiency decreased when increasing the initial amount of BLG (Table 1). This was explained by the decreased stability of the first emulsion due to an increase in the viscosity of the internal phase as the concentration of BLG increased (Leo et al., 1998). Nevertheless in an attempt to reduce the solubility of BLG in the external phase, the pH of this phase was reduced to the p*I* of BLG (5.2). The pH of the internal phase was kept constant at the optimal solubility of BLG. As the pH diminished, the encapsulation efficiency increased significantly (Table 1). However, in vitro release experiments displayed a strong burst release effect when the pH was adjusted to 5.2 (Table 1). This results suggest that the protein was strongly adsorbed on the surface when reducing its solubility in the external phase. This phenomena was confirmed by adsorption experiments which have shown that adsorption was stronger as the pH was reduced to 5.2 (Leo et al., 1998). In conclusion, it was important to adopt a new strategy consisting in both stabilizing the interfaces and also displacing BLG from the interfaces. This is the reason why Tween 20 was used for this purpose.

BLG-loaded microparticles were prepared using a concentration of BLG of 50 mg/ml and various concentrations of Tween 20 (molar ratio: protein/surfactant of 0–8). Average particle size, protein loading, entrapment efficiency and burst release were determined (Table 2). All batches of BLG microspheres displayed a mean diameter from 6.2 to 7.2 μm. Tween 20 did not modify the microsphere size characteristics. However, the amount of surfactant affected both loading and encapsulation efficiency. The introduction of Tween 20 in the inner aqueous phase improved the encapsulation of BLG. Nevertheless, the protein entrapment efficiency was improved only from a molar ratio of protein/Tween 20 of 1:0.018. Below this ratio, the surfactant is below its critical micellar concentration (CMC: 0.06 mg/ml). In addition, the aqueous solubility of the BLG in the presence of Tween 20 (at 25°C and at a molar ratio protein/Tween 20 of 1:8) increased by 25%. The solubilization of BLG by micelles formed within the inner aqueous phase may be responsible for reducing the protein to protein or protein to polymer interactions. Consequently, this process could increase the amount of protein located within the aqueous globules. Moreover, the formation of micelles makes possible an homogeneous distribution of BLG into microspheres. Tween 20 also induced the formation of more homogeneous internal aqueous globules than those obtained in the absence of surfactant as shown by freeze-fracture electron microscopy (Rojas et al., 1999). In vitro release experiments with different molar ratios of BLG/Tween 20 showed that microspheres prepared in the absence of Tween 20 displayed the highest burst effect

Table 1
Effect of pH on particle size, loading and encapsulation efficiency and burst release after 2 min incubation when measured[a]

BLG concentration (mg/ml)	pH of the external aqueous phase	Average size (μm)	Loading efficiency (mg BLG/100 mg MS)	Encapsulation efficiency (%)	Amount of BLG released after 2 min incubation (%)
50	6.5	6.6	1.5	23.8	14
50	5.6	7.2	3.8	55.5	12
50	5.2	6.7	4.1	62.0	23
125	6.5	6.9	2.5	16.9	33
125	5.6	7.3	7.0	42.0	35
125	5.2	6.6	8.3	57.3	40
250	6.5	7.3	3.8	18.3	39
250	5.6	6.7	7.8	39.1	25
250	5.2	7.0	12.6	58.3	49

[a] The theoretical loading was 7.4 mg BLG/100 mg MS, ($n = 3$).

Table 2
Effect of Tween 20 on particle size, loading and encapsulation efficiency and burst release after 2 min incubation when measured[a]

Protein:Tween 20 (molar ratio)	Average size (μm)	Loading efficiency (mg BLG/100 mg MS)	Encapsulation efficiency (%)	Amount of BLG released after 2 min incubation (%)	ζ Potential (mV)
1:0	6.6	2.4	32	23	−8.9
1:0.01	6.8	2.5	33	N.D.	−10.1
1:0.018	6.7	3.4	46	N.D.	N.D.
1:0.1	7.0	3.4	46	N.D.	N.D.
1:0.5	6.9	3.7	50	N.D.	N.D.
1:1	7.0	4.1	55	N.D.	−15.5
1:4	7.2	5.9	80	16	−14.5
1:8	6.2	6.7	91	1	−22.3
0:8	6.3	–	–	–	−25.6
0:0	6.4	–	–	–	−24.8

[a] Theoretical loading was 7.4 mg BLG/100 mg MS, ($n = 3$); N.D., not determined.

(23%). As the amount of Tween 20 was increased the burst release was reduced: at a molar ratio of 1:4, the burst effect was 16% and at 1:8, only 1% (Table 2). The large burst effect observed for the microspheres without Tween 20 was explained by the presence of a high amount of BLG on the microsphere surface (Rojas et al., 1999) and also from its release through pores and channels identified within the microspheres (Rojas et al., 1999). On the contrary, as Tween 20 was able to migrate from the first emulsion to the outer surface of the microspheres, this made possible a reduction in the amount of BLG adsorbed onto the microsphere surface and a reduction in the presence of pores and channels (Rojas et al., 1999). Reduction of adsorption of BLG in the presence of microspheres prepared with Tween 20 was also confirmed by adsorption experiments (Rojas et al., 1999). The influence of Tween 20 on the microsphere surface was also evaluated by measuring the particle ζ potential. Unloaded microspheres displayed a potential of −24.8 mV, and BLG microspheres prepared without Tween 20 displayed a potential of −8.9 mV (Table 2). However, as the amount of Tween 20 increased, the microsphere ζ potential diminished until it reached a value that was very close to that of the unloaded microspheres.

4. Conclusions

BLG microspheres made of biodegradable PLGA were prepared by either adjusting the pH of the external aqueous phase or introducing a non ionic surfactant in the internal phase. It was shown that using an adequate quantity of Tween 20 was able to displace protein from the microsphere surface and increase the encapsulation efficiency. Reduction of the burst effect could improve the control of drug release constituting a strategy for the delivery of proteins from PLGA microspheres.

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