SUPPORTING INFORMATION

ENCAPSULATION OF BSA/ALGINATE WATER-IN-WATER EMULSIONS BY POLYELECTROLYTE COMPLEXATION

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A) Optical microscopy observations of emulsions

BSA-in-Alginate water-in-water emulsions were difficult to observe by optical microscopy, due to the small difference in refractive index between disperse and continuous phases, resulting in low contrast when observing by conventional optical microscopy. Therefore, preliminary assays were performed to find the most appropriate conditions for observations. Illustrative examples are shown in Fig. S2. Differential interference contrast (a) provided acceptable images, whereas fluorescence did not allow observing droplets of fluorescein-labelled BSA (b), probably because of the small size of droplets and the presence of BSA in both phases, resulting in a low contrast. The best images were captured using dark field optical microscopy, illuminating the specimen with an intense light guided through optical fiber (c).

Fig. S1. Optical microscopy images of the emulsion prepared with 2.5 wt% BSA / 2 wt% ALG, at pH $\approx$ 7, using different observation techniques: (a) differential interference contrast (DIC); b) Fluorescence using fluorescein-labelled BSA; (c) Dark field optical microscopy with an intense light source. All scale bars indicate 20 μ m.

B) Rheological properties

Simple visual observations indicated that BSA-in-alginate emulsions were more viscous than alginate solutions without BSA. These observations have been confirmed by determining rheological flow curves of (a) 2 wt% alginate in water and (b) BSA-in-alginate emulsion prepared with 2.5 wt% BSA and 2 wt% alginate (Fig. S3). These flow curves were determined by rheological measurements by using a stress-controlled rheometer TA Instruments AR-G2 with a 20 mm plate-plate geometry at $T=25^{\circ}\text{C}$. The measurements were carried out by triplicate and in the next figure the three flow curves for each solution are plotted: (a) alginate 2 wt% and (b) 2.5 wt% BSA / 2 wt% alginate. The results confirm that indeed the emulsion (4.03 Pa.s) is more viscous than the solution (2.87 Pa.s).

Fig. S2. Flow curves of alginate 2 wt% solution (a) and 2.5 wt% BSA / 2 wt% alginate emulsion (b) at 25°C .

C) Colloidal stability of emulsions

The stability of emulsion with the composition 2.5 wt% BSA / 2 wt% alginate was studied at 25°C for 3h to make sure that these emulsions stable enough for their encapsulation. Fig. S3 shows the optical micrographs at times 0, 1, 2 and 3h. The images illustrate that emulsion had virtually no variation of droplet size during this period of time. Then, these emulsion droplets can be considered to be stable enough, and are suitable to be encapsulated into alginate/cation capsules.

Fig. S3. Images by optical microscopy, showing the stability of 2.5 wt% BSA / 2 wt% Alg emulsions as a function of time: (a) $t=0h$; (b) $t=1h$; (c) $t=2h$ and (d) $t=3h$. No differences were observed within 3 hours, and it was concluded that the emulsions are stable enough to be encapsulated. All scale bars indicate 10 μm .

D) Formation of capsules

As described in the experimental section, emulsions were encapsulated by dropping them into aqueous solutions of CaCl_2 , FeCl_3 or chitosan dissolved in acetic acid solutions. The capsules were kept into the bath during certain periods of time, and finally were extracted and rinsed with purified water. Fig. S1 shows some examples of capsules, as seen after 30 minutes of formation.

Fig. S4. Illustrative examples of capsules of 2.5 wt% BSA / 1.5 wt% alginate emulsion obtained at pH 13, in 2 wt% Fe^{3+} (A) 10 wt% Ca^{2+} (B) and 1 wt% chitosan (C). The beaker bottom had a diameter of approximately 4.2 cm.

TABLE

pH of external solutions

Table S1. pH of receptor baths, before and after encapsulation.

	CaCl_2 10wt%	FeCl_3 2wt%	Chitosan 1wt%
Before encapsulation	6.95	1.68	4.02
After encapsulation	6.68	1.79	4.10