Gellan microgels as encapsulating matrix: Study of microchannels design

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Abstract*: Microgels were obtained from the droplets gelation of a water-in-oil emulsion containing gellan (0.6% w/w) in the aqueous phase and calcium acetate (2.0% w/w) in the oily phase. A potential use of gellan microgels as encapsulating matrix of active compounds was evaluated by adding a hydrophilic dye, Rhodamine B, in the aqueous phase using two strategies. The microgels exhibited uniform and spherical shape. In addition, results showed good retention capacity of the dye and stability over time of storage, indicating that microgels obtained by microfluidics technique may be used for the encapsulation of hydrophilic compounds, including those sensitive to temperature and pressure, as well as in drug delivery.*

Key-Words*: calcium acetate, hydrophilic compound, dye, stability, microfluidic*

Introduction: Microfluidics is an emerging technique that can generate emulsions with small size droplets and low polydispersity. This technique has an additional advantage of producing droplets with a lower energy demand than conventional methods (Shah et al., 2008), minimizing the degradation of sensitive compounds to temperature and pressure. Microgels can be produced from the gelation of the emulsion droplets, promoting enhanced mechanical resistance and stability (Helgeson et al., 2011). The possibility of using gellan for the formation of hydrogels is interesting since this polysaccharide shows high resistance to low pH values (Moritaka et al., 1995), allowing that these gels can pass intact through the stomach to be disintegrated only in the gut. Thus, this work aimed to study two different microchannels design for the encapsulation of a hydrophilic dye in gellan microgels produced by the external gelation method using planar microfluidic devices.

Experimental: The use of gellan microgels as an encapsulating matrix of active compounds was evaluated using two different strategies. In the first configuration (Design 1), a hydrophilic dye, Rhodamine B, was added to the 0.6% (w/w) gellan solution before being introduced into the channel (inlet 1), while the oily phase, composed by soybean oil, 4% (w/w) PGPR and 0.5–2.0% (w/w) calcium acetate, was introduced into the lateral channels (inlet 2). The microchannel design shown in Fig. 1B was used in the second strategy (Design 2). The dye (inlet 1) and the 0.6% (w/w) gellan solution (inlet 1′) were introduced separately and collided each other at the microchannel junction, followed by the immediate droplet detachment. The oily phase, composed by soybean oil, 4% (w/w) PGPR and 2.0% (w/w) calcium acetate, was introduced into the channel (inlet 2) and the flow rate of the dispersed (Q_d) and continuous (Q_c) phase was fixed at 1.5 and 6.0 μL/min, respectively.

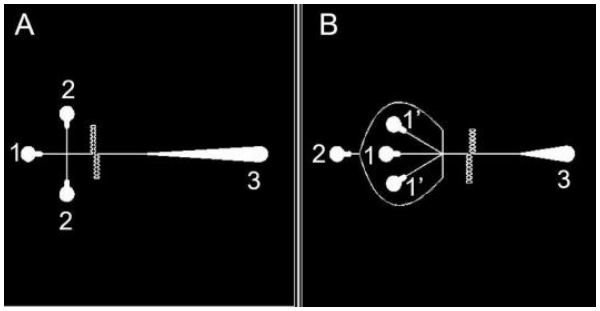

Figure 1. Design of the flow-focusing microchannels. A) Design 1 (inlet 1: aqueous phase (gellan solution); inlet 2: oily phase (soybean oil, PGPR and calcium acetate) and inlet 3: droplets collection chamber) and B) Design 2 (inlet 1: hydrophilic dye; inlet 1′: gellan solution; inlet 2: oily phase (soybean oil, PGPR and calcium acetate) and inlet 3: droplets collection chamber).

Results and discussion: The dye was added to the gellan aqueous solution before its injection into the microchannels in Design 1. Encapsulation of Rhodamine B was carried out by inserting the gellan gum solution and Rhodamine B in different channels in Design 2, i.e., the fluids were mixed within the device. However, in both conditions was observed a good retention and a high intensity of Rhodamine B dye onto the interface of particles (Fig. 2A and B1). Fig. 2C shows the droplet formation using Design 2 and the Rhodamine B aqueous solution (fluorescent) flowing inside the central microchannel. The particles average diameter ($D_{32,0}$) was 106.6 ± 0.4 μm (CV = 0.4%) and 106.8 ± 2.9 μm (CV = 2.7%) using Design 1 and 2, respectively. The particles average diameter were similar and despite of the increase in the CV in Design 2, this value is even smaller than coefficients of variation of droplets generated by conventional methods of emulsions production. This result suggests that the microgels produced by both configurations could be used in the encapsulation of hydrophilic compounds, especially those temperature-sensitive ones, such as vitamins and probiotics, and those carrying active biological structures, such as enzymes and cells. The optical microcopy of individual particles shows the microgel surface changes over time during about 2 min (Fig. 6D). A rough surface layer was observed just few seconds after preparation, but this characteristic became more intense over time. Moreover a phase separation between the oily phase and microgels was observed after one day of storage. Microgels were stable during 10 days of storage, maintaining the average size almost unchanged during this time, with values of $D_{32,0}/D_{32,f} > 0.92$ and narrow size distribution (CV = 3.3%) (Fig. 2E and F1). In addition, the high intensity of Rhodamine B dye onto the interface of freshly particles was observed even after 10 days confirming the good retention and stability of dye over time (Fig. 2F2).

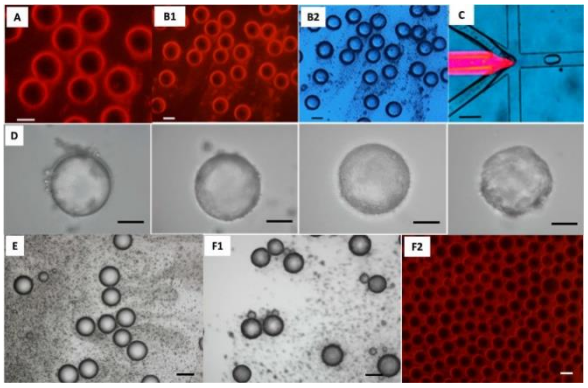

Figure 2. Gellan microgels produced by Design 1 (A) and Design 2 (fluorescent (B1) and optical microscopy (B2)). Microchannel optical microscopy and fluorescence microscopy of Rhodamine B solution by Design 2 (C); scale bar: 500 μm. Gellan microgel surface over time during about 2 min (D). Optical microscopy of gellan microgels freshly prepared (E) and after 10 days of storage (optical (F1) and fluorescent microscopy (F2)); scale bar: 100 μm. Red areas represent Rhodamine B retention within the particles. Process conditions: Q_d = 1.5 μL/min and Q_c = 6.0 μL/min.

Conclusion: The visual characteristics of the gellan microgels obtained by the addition of a hydrophilic compound into the gellan solution or in separated channels were similar. Gellan microgels were stable during 10 days of storage, maintaining the average size almost unchanged during this time. Therefore, the choice of the encapsulation design of hydrophilic compounds in microfluidic devices will be a function of the properties of the biopolymer and the disperse phase, as well as the particle applications and process conditions.

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