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Effect of Process Parameters on Microencapsulation of Active Compounds by Co-Flow Air Assisted Microfluidic Extrusion

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A co-flow microfluidic system consists of two concentric channels inside which flow the inner dispersed phase (sodium alginate) and the outer continuous phase. The size and shape of microbeads depend on the physical-chemical properties and flow rate of fluids and on the process conditions such as diameter, material type of dripping tip and collecting distance. In order to reduce the microbeads size, external forces are used to break up the droplets. These forces could be generated by pressurized air, electrostatic charges and vibration. The technologies presented in literature for matrix encapsulation are based on a liquid-liquid dual immiscible fluid configuration, introducing a series of problems in industrial production, like gelation, oil separation and cleaning which would not arise using air as continuous phase. However, the use of air introduces a more complex fluid dynamics in possibly turbulent flow regime. The aims of this study include: firstly the developing an integrated system pump-microfluidic device; secondly the analysis of the size of Ca-alginate beads at different air flow rates setting the dispersed phase flow rate at 1 ml min^{-1}; and finally the study of the influence of alginate sodium flow rate on beads size distribution and the capsules payload . For these purposes, a 3% (w/v) solution of sodium alginate was used. The air focused the dispersed phase at dripping tip, applying a shear force generating micro-droplets. A 0.2 M solution of calcium chloride dehydrate allowed the extruded drops gelation. Then, the size distribution was investigated using laser diffraction. The results showed that the size of microbeads decreased with increasing air flow rate (from 0.7 to 2.4 l_n min^{-1}).

1. Introduction

Ionotropic alginate microgel particles (microbeads) have been reported to encapsulate a variety of substances, which in their raw form may be sensitive to heat, light, moisture, pH, toxin, oxidation, mechanical shear or pressure. Encapsulation is carried out to prolong the product shelf life, improve/retain the quality of the product, enhance the appearance of the product and/or add value to the product. The microbeads are produced via ionotropic cross-linking reaction between calcium cations and alginate chains. Microencapsulation require simultaneous control and tuning of numerous capsule parameters, including their size, permeability and mechanical properties, which can be difficult to achieve with traditional encapsulation techniques. Instead, double emulsion templates made using microfluidics can offer substantial advantages due its inherent precision and control of a wide range of processing variables such as particle size and its distribution, capsules payload. In fact, microfluidic chips are based on microscale channels where in liquids flow, enabling a high control of their movement and dynamics. Beyond the capsule size control, microfluidics can also be used to produce controlled capsules shapes different from spherical, such as cylindrical shapes with a better bioavailability. In summary, microfluidics are a highly advanced tool for tailoring the physical features microcapsules. One of the most popular ways to produce hydrogel beads is by extrusion dripping. In this method, an alginate solution is delivered through a capillary and then is extruded into droplets which detach from the dripping tip by the influence of gravitational force (natural extrusion). The size of a bead produced by simple extrusion dripping is greater than 1,000 μm bigger than the range size of 200-800 μm desired for most encapsulation applications. Beads with diameters smaller than 1,000 μm could be produced through external forces such as vibration, pressurized air and electrostatic charges (Lee et al., 2013). At the same time, the forces may also increase the microgel production rate. The main innovation of this work relies on the choice to develop a technology based on air assisted microfluidic extrusion that has a number of

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advantages due to easier some post-processing operations like gelation and cleaning. On the other hand the higher velocity and consequently the turbulence flow regime due to high Reynolds numbers involved makes extremely difficult to operate in core-shell configuration (Marra et al., 2017). Moreover, the possible micro pump-micro device integration is up today an open and unresolved problem. The air extrusion method using pressurized air is not complicated and can handle relatively higher viscosity. A typical air extrusion system for alginate microgel formation consists of a pressurized air or gas supply source, an extrusion nozzle and a gelation bath of calcium chloride for gelation process. The use of flow-focusing nozzle to produce microbeads was used in which the inner dispersed phase fluid (sodium alginate) and the outer continuous phase fluid (air) flow inside two concentric channels. The fluids meet into the larger outer channel where the focusing air stream generates shear force (which exceeds the gravitation force) that leads to a concentric stretching of the alginate solution jet in the direction of flow. The shear and stretching effect of the air causes the jet to get attenuated and capillary instability breaks up the jet into homogeneous droplets. The size and the shape of Ca-Alginate beads is influenced by physical properties such as viscosity or surface tension and solution formulation of the alginate and gelling. Dripping tip diameter and collecting distance (between the dripping tip and the gelation bath-air interface) are instead the main factors concerning the experimental set up that may influence the bead size and shape. The principal aims of this work concern (i) the study of a microfluidic device for extrusion of microbeads in matrix configuration by optimising geometric parameters of the device; (ii) analysis of the influence of the process parameters on the microcapsules particle size distribution by varying the dispersed or continuous phase flow rate or the viscosity of alginate solution. In this case the other parameters were keep unchanged.

2. Materials and Methods

Experimental Set Up. The microdevice geometry was created using CAE software and printed in methacrylate photopolymer resin with a 3D printer for rapid prototyping (FORMLABS 1+, USA). The device consisted of a support for the extrusion nozzle and an air chamber. In particular the extrusion nozzle consisted of a fused silica polyimide coated capillary with a tip diameter, d_d = 100 µm (MOLEX, USA). This was inserted into a hypodermic needle which ensured the capillary to be always properly centered and aligned. A 700 µm (d_c) inner diameter pipettor tip was used for conveying the air flow, thus focusing the flow of the dispersed phase. The collecting distance between dripping tip and gelation bath was fixed at 5 cm. The co-flow device adopted in the experiments is described in Figure 1.

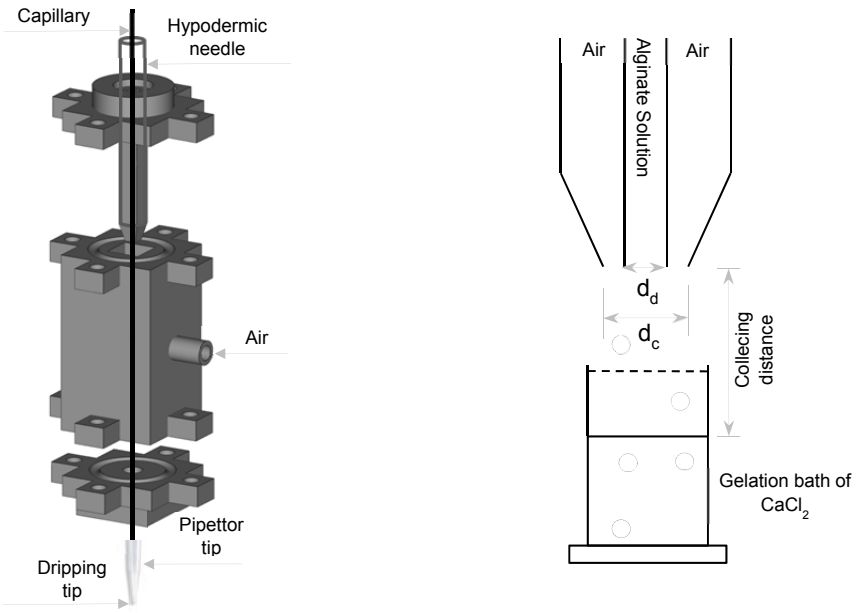

Figure 1: Microdevice design (a) and scheme of extrusion dripping (b).

Materials and Sample Preparation. Sodium alginate and calcium chloride dehydrate was purchased from the Sigma-Aldrich (Milano, Italy). Sodium alginate solutions with different concentrations (2.0 %, 2.5%, 3.0%, 3.5% w/v) were prepared by dissolving a proper amount of powder in 100 mL deionized water; the solutions

were mixed by a magnetic stirrer for 24 hours to facilitate degasation and then were transported by a syringe pump (Aladdin 300-220, WPI). The gelation bath was made up of $CaCl_2 \cdot 2H_2O$ in the concentration 0.2 M (Cerverò et al., 2011).
Air flow was controlled by mass flow meter (MASS-VIEW Bronkhorst, The Netherlands) connected to a pressurized air. The microbeads were recovered with a filter and then washed with deionized water.

Rheological characterization. Rheological properties of sodium alginate solutions were performed by using a Rotational rheometer (Kinexus lab+, Malvern Instruments). The measurements were carried out at 25 °C using a cone plate configuration (CP4/40) with a 40 mm diameter and a 4 ° cone angle, the range of shear rate from 1 to 1,000 s^{-1} were applied, viscosity ($\boldsymbol{\eta}$) was recorded as a function of shear rate ($\dot{\boldsymbol{\gamma}}$).

Particle Size Analysis. The particle size distributions of microbeads were measured via a Malvern Mastersizer 3000E Hydro, (Malvern Instruments, Worcestershire, U.K.). Each individual particle size measurement was determined from the average of three readings made per sample. Mastersizer was used with a laser obscuration greater than 10%, experimental test were repeated three times for each run, and micro-beads were characterized as milky particles with particle adsorption index equal to 1.51 and particle refraction index equal to 1.36. Small variation of optical indices did not show significant variation of results. Although the non-spherical option was used, a certain amount of size distribution spread is related to the relative orientation of the particles passing by in the detection area respect to laser light scattering detection sensors. Results were averaged using at least 20 independent measures.

3. Results and Discussion

Steady-shear flow measurements

Understanding of the rheological behaviour of sodium alginate aqueous solutions at different concentrations is fundamental to evaluate the influence of viscosity on Ca-alginate beads production in terms of monodispersion, size and shape. In fact, it is generally known that the viscosity of the discontinuous phase is one of the key factors for determining the transition from the dripping regime to the jetting regime. Moreover, the droplets produced in the dripping mode exhibit a more uniform size distribution than ones produced in jetting regime (Moon et al., 2014). The viscous properties for alginate solutions were determined using steady-shear flow test over the range of shear rate ($\dot{\gamma}$) from 1 to 1,000 s^{-1}. The results describe the relationship between viscosity (η) and shear rate ($\dot{\gamma}$) of sodium alginate at different concentrations ranging from 2.0 to 3.5 % (w/v). From the shapes of the flow curves depicted on Fig. 2, there was an initial Newtonian plateau region as the shear rate approaches zero. The viscosity exhibited by a material at a shear rate inclining to zero is defined as the zero shear viscosity (η_0) and indicated that the material flows (like liquid) at rest. Above a critical shear rate, all solutions showed a power law region (shear thinning behaviour) where the viscosity decreased with the increase of shear rate. Furthermore, this critical shear rate shifted towards lower values of shear rate as the concentration of sodium alginate increased. According to Ma et al. (2014) the shear data were analysed by fitting the Cross model to the data:

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1 + (k\dot{\gamma})^m}$$

where η is the viscosity at any specific shear rate $\dot{\gamma}$, η_0 and η_∞ are the viscosity at zero and infinite shear rates, respectively, k is a shear-dependent time constant and its reciprocal 1/ k, corresponds to a critical shear rate that provides a useful indicator of the onset of the shear thinning region. The exponent (m) gives the degree of thinning (0 = no thinning, that is, Newtonian behavior and 1 = maximal thinning). In summary oscillatory frequency sweep test proved that the material is dominated by a viscose component in rest conditions. Based on this dynamic viscoelastic measurements, a Cross model has been chosen to accurately fit the rheological data. The Cross model parameters for the different concentrations of sodium alginate solutions were summarized in Table 1.
As shown in Table 1, the curve at 2.0 % had a determination coefficient R^2 lower than 0.99, which indicated the Cross model was not suitable. In fact, the lowest-concentration solution viscosity was independent of the rate of shear (Figure 2) proving the Newtonian behavior of the fluid. Figure 2 also showed that the viscosity increased with the increase of sodium alginate concentrations. The increase in η could be attributed to the increased intermolecular interactions between polymer molecules.

Table 1: Cross model fitting parameters of sodium alginate at different concentrations ranging from 2.0 to 3.5 % w/v.

concentration (% w/v)	η_0 (Pa·s)	η_∞ (Pa·s)	k	m	R^2
2.0	0.0373	0.0358	0.0433	0.8034	0.8475
2.5	0.0605	0.0497	0.0126	0.2467	0.9976
3.0	0.0948	0.0733	0.0042	1.2320	0.9998
3.5	0.1506	0.1009	0.0034	0.9282	0.9998

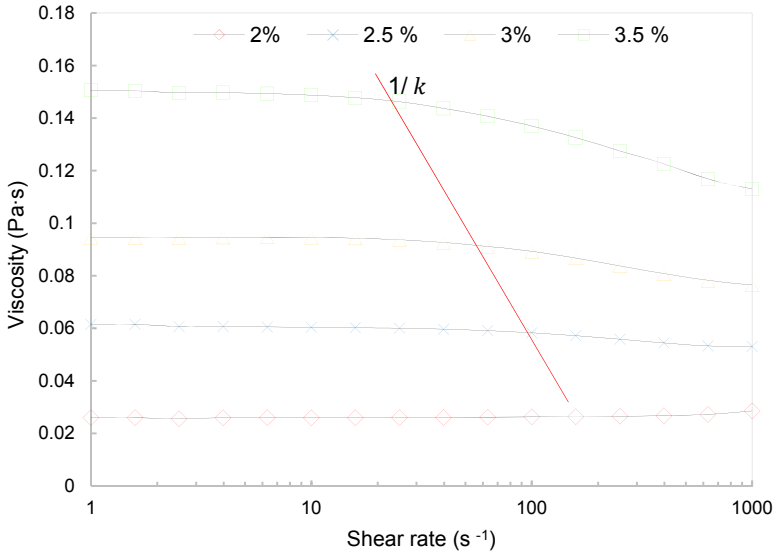

Figure 2: Flow curves of sodium alginate aqueous solutions at different concentrations ranging from 2.0 to 3.5% (w/v).

3.2 Influence of continuous phase flow rate on size distribution of Ca-alginate beads

Setting flow rate of dispersed phase at 0.0185 ml min^{-1} (concentration 3 % w/v), the production of Ca-alginate beads at different air flow rates, ranging from 0.7 to 2.2 l_n min^{-1} has been investigated. The first tests were carried out at the continuous phase flow rate lower than 0.7 l_n min^{-1} where the shear force generated by air was not enough to obtained desired dripping regime. Starting from 0.7 to 1.6 l_n min^{-1}, size distribution data of the different microbeads samples were depicted both as a density distribution (upper portion of the graph) and a cumulative volume curve (lower portion of the graph) as shown in Figure 3. The cumulative curve describes how many percentages of samples are below a certain size of particles. The D10, D50 and D90 is defined as the size value corresponding to cumulative size distribution at 10%, 50%, or 90%, which represents the size of particles below which 10%, 50%, or 90% of the sample lies. The median diameter D50 was used to compare the results obtained at different air flow rates because it was less affected by presence of secondary populations of particles (secondary peaks). As shown in Figure 3a, the values of D50 decreased with increasing flow rate of continuous phase ranging from 0.7 to 1.6 l_n min^{-1}. The D50 of samples 0.7, 0.9, 1.1, 1.3 and 1.6 l_n min^{-1} was 87.5, 47.3, 45, 38.1 and 27.1 μm, respectively. This was attributed to the higher shear provided by pressurized air as its flow rate increased. Furthermore, secondary peaks could be explained by misalignment of the capillary that affects dripping regime during extrusion. With increasing air flow rate above 1.6 l_n min^{-1} there was no sharp decrease in particle size distributions of microbeads because the interaction between a high speed air jet with an alginate coaxial jet that makes much more difficult to predict and control the extrusion phenomena, including the formation of satellites drop and the effect of “coalescence”. More specifically, the discontinuous flow could tend to transit from dripping to jetting regimes as the flow rate of the continuous phase increased. As a result at the end of the jet, the main drop with jet diameter is followed by satellite drops with lower diameters (double whipping). In some instances, air pressure could make faster the drop closer at the tip of the needle than another drop that has detached earlier. As a result, coalescence between the two drops could occur (merging). (Marra et al., 2017).

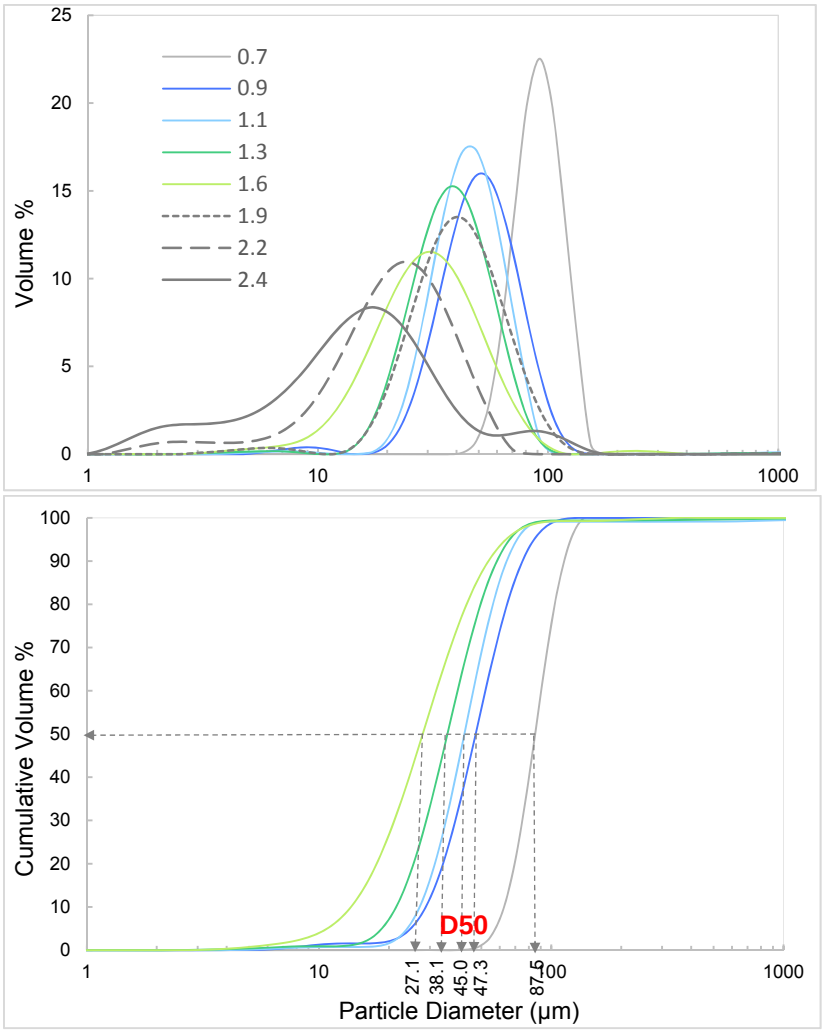

Figure 3: Particle volume distribution of the samples obtained at different flow rates of air, 0.7, 0.9, 1.1, 1.3, 1.6, 1.9, 2.2 and 2.4 l_n min^{-1}(figure above). *Cumulative volume at 0.7, 0.9, 1.1, 1.3 and 1.6 l_n min^{-1}*(figure below).

Effect of dispersed phase flow rate

Size distributions of droplets generated at different flow rates of the dispersed phase (7, 11, 15 and 19 µl min^{-1}) are shown in Figure 4. The flow rate of the continuous phase was kept at 1.6 l_n min^{-1} based on the results obtained previously because the device exhibited the best performance in terms of capillary stability. There were no significant differences in the D50 of the samples collected at different flow rate of alginate revealing that the droplet formation was primarily affected by the flow rate of the continuous phase.

Effect of alginate solution concentration

The tests were carried out considering four alginate solution concentrations, ranging from 2 to 3.5% (w/v). It was not possible to produce beads using concentration of 2 % (w/v) because the viscous forces within the droplet were unable to compete with the drag forces exerted when the drop hit and entered the gelling bath. Moreover, low viscosities of alginate solution results in higher drop deformation upon impact with the surface of the gelation bath. It has been reported that the viscosity (or concentration) of alginate solution significantly affects the spherical-shaped of the microbeads (Chan et al., 2009). Figure 5 shows results obtained varying alginate concentration (2.5, 3.0 and 3.5 % w/v) by maintaining constant the value of dispersed phase and air flow rates (values of 7 µl min^{-1} and 1.6 l_n min^{-1}, respectively). The median diameter (D50) of beads decreased slightly (from 43.5 to 29.4 µm) as the concentration increased from 2.5 to 3.0 % w/v. Alginate solution with a concentration of 3.5 % w/v was not suitable for this extrusion method due to difficulties in the bead forming process (pumping and obstruction at the dripping nozzle) (Gombotz and Wee, 1998). This could explain the generation of a bimodal population of beads in correspondence to the alginate concentration of 3.5 % w/v.

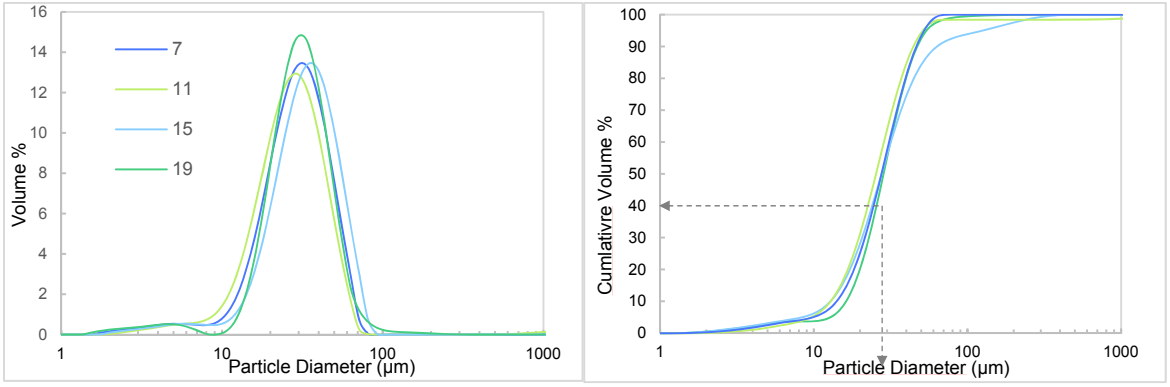

Figure 4: Particle volume distribution of the samples obtained at different flow rates of alginate, 7, 11, 15 and 19 µl min^{-1} (a) and cumulative volume (b).

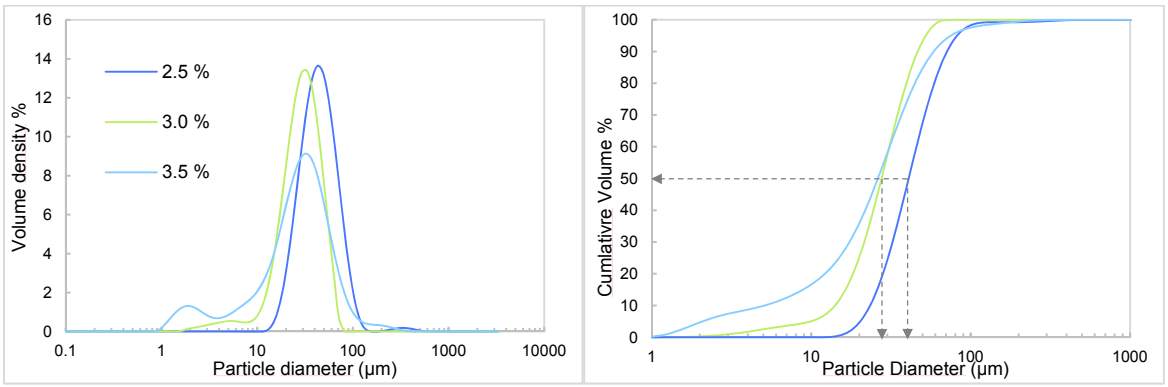

Figure 5: Particle volume distribution of the samples obtained at different concentration of alginate solution, 2.5, 3.0 and 3.5 % (w/v) (a) and cumulative volume (b).

4. Conclusions

Experimental test reported in this work demonstrated that size distribution of microbeads is affected by process parameters not only in terms of D50 size, but also in terms of diameter distribution shape. A major effect is played by the flow rate of the continuous phase. Viscosity effects are also quite important, generating a possible change in drop formation pattern when viscosity increase. A more extended experimental set of process parameters is required to fully characterize the possible envelope of microbeads size and characterize it in term of adimensional numbers.

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