ALGINATE MICROCAPSULES PRODUCTION

INTRODUCTION

Microcapsules are used in food, pharmaceutics, cosmetics, agriculture, and environmental conservation applications. In pharmaceutical applications, they assist in the protection of encapsulated active ingredients and the controlled release of the encapsulated drugs. They are also used as biomimetic microreactors, interpreting and duplicating the ability of living organisms to synthesize materials.

Microcapsules are used for bioavailability enhancement and supplement delivery (e.g solubilisation of oil-based vitamins). They allow the release of active ingredients, such as vitamin A or vitamin C, in aqueous formulations in cosmetics [1-4].

One of the most promising biomaterials is **alginate** due to its non-toxicity and excellent biocompatibility [5]. Alginate is a hydrophilic natural polysaccharide derived from brown seaweeds and is composed of □-D-mannuronic acid and □-L-guluronic acid.

In **alginate microcapsule production**, the alginate can form **hydrogels** rapidly with multivalent cautions, such as Ca2+, Ba2+, or Fe3+ in mild conditions via cross-linking. Hydrogels are a preferred material for **microencapsulation** across a wide range of applications due to their fully natural origins, chemical inertness, and ability to mimic tissues [6,7].

Microcapsule behavior is dependent upon the mechanical properties and the permeability of the shell-tunable by the microcapsule size, shell thickness, and composition. For controlled release, an encapsulation system that provides fine control over these parameters is required. **Uniform sizes** and **structures** are important for the microcapsules to encapsulate active-ingredients quantitatively and to release them at controlled rates. Consequently, the value of the double emulsions can be determined by the loading ability, the encapsulation efficiency of the oil-based active, the absence of empty shells and doublets and triplets, and the run-to-run reproducibility [8].

Despite numerous approaches and materials available to produce microcapsules, control over microcapsule dimensions and chemical composition combined with high encapsulation efficiencies is often difficult to achieve in a one-step process.

Alginate microcapsule production usually requires two processes: formation of emulsion templates and ion cross-linking. Traditionally, the emulsions are prepared by mechanical stirring or high shear mixing, and then transformed to alginate microcapsules via external, internal or inverse gelation [9].

An alternative approach to fabricating **alginate microcapsules** with narrow size distributions is the extrusion-dripping technique, in which the oil phase and the alginate solution are extruded out from a nozzle to create small droplets periodically under electrostatic potentials or centrifugal forces [10]. However, the gelling process usually deforms the shape of the droplets, leading to non-spherical morphology of the alginate microcapsules.
Double emulsions using microfluidics have been shown to overcome this difficulty by enabling fine control of the template dimensions and offering high flexibility with materials that can later be used to form the capsule shell. Microfluidic techniques enable the precise control of droplet formation [8].
The **Raydrop™** (developed and manufactured by Secoya) is the first easy-to-use device that enables the reliable production of double emulsions. It doesn't rely on double coating treatment of PDMS/glass in planar chips [11] or the alignment of two round capillaries in a third square tubing, as in lab-made glass capillary microfluidic devices [12]. Its specific design allows for multiple liquid type emulsification within the same device with no coating needed.

The Microfluidic Complex Emulsion Platform is a system for producing a **monodispersed double emulsion** in a single device. The system uses **Fluigent's LineUP microfluidic pumps** and the **RayDrop™ Double Emulsion** device.

MATERIALS AND METHODS

Materials

Reagents:

Core phase:

- MCT 1: MCT oil

Shell phase:

- Al1: MiliQ water with 1% w/w TWEEN 80 and 1%w/w Alginate and 2:1 molar ratio of CaEDTA

OR

- Al2: MiliQ water with 1% w/w TWEEN 80 and 2%w/w Alginate and 2:1 molar ratio of CaEDTA

OR

- Al3: MiliQ water with 1% w/w TWEEN 80 and 3%w/w Alginate and 2:1 molar ratio of CaEDTA

Continuous phase:

- MCT3 (MCT with 2% w/w PGPR (E576) and 5% acetic acid).

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Products/Instrument:

» **Microfluidic flow controller:** The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control pressure or flow rate. It can be used without a PC. Three Flow EZ with 2 bar of full scale pressure are used in the setup presented here

» **Flow sensor:** The Flow Unit is a flow sensor that allows real time flow rate measurement. By combining a Flow Unit with the Flow EZ, it is possible to switch from pressure control to flow rate control, allowing for the generation of highly monodispersed droplets over a long period of time. Two Flow Units M and one Flow Unit L are used here to monitor and control the flow rates of the core phase, shell phase and continuous phase during the run.

» **Droplet generator:** The RayDrop™ is used to control the generation of alginate droplet. The RayDrop is based on the alignment of two capillaries immersed in a pressurized chamber containing the continuous phase. The dispersed phase exits one of the capillaries through a 3D-printed nozzle, placed in front of the extraction capillary for collecting the droplets. This non-embedded implementation of an axisymmetric flow-focusing is referred to co-flow-focusing. The advantage lies in his geometry which is leading the droplet formation and then remove all wettability issues that could appear in other microfluidic chip.

To make things easier, we use the RayDrop platform to hold all components in one place. This helps keep a nice overview, keeps the RayDrop vertically to drain the air to the top and enables the continuous monitoring thanks to its horizontal microscope.

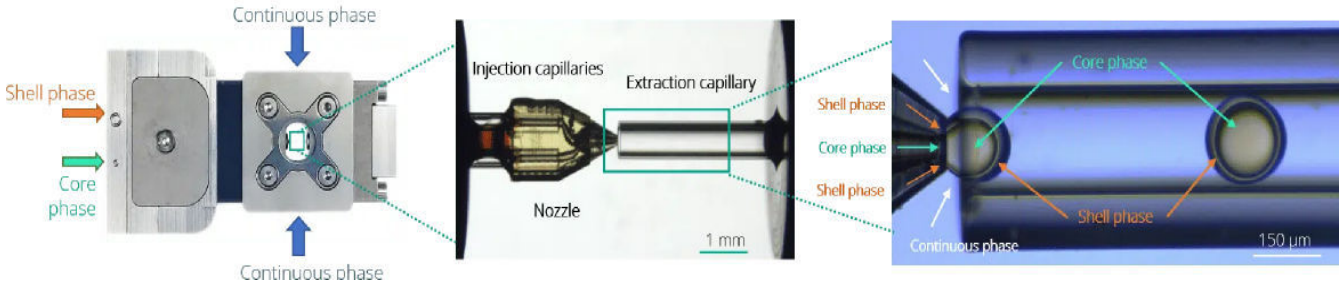


Figure 1. RayDrop Double Emulsion

Figure 2: Complex emulsion production platform.

Methods: **Alginate microcapsules production**

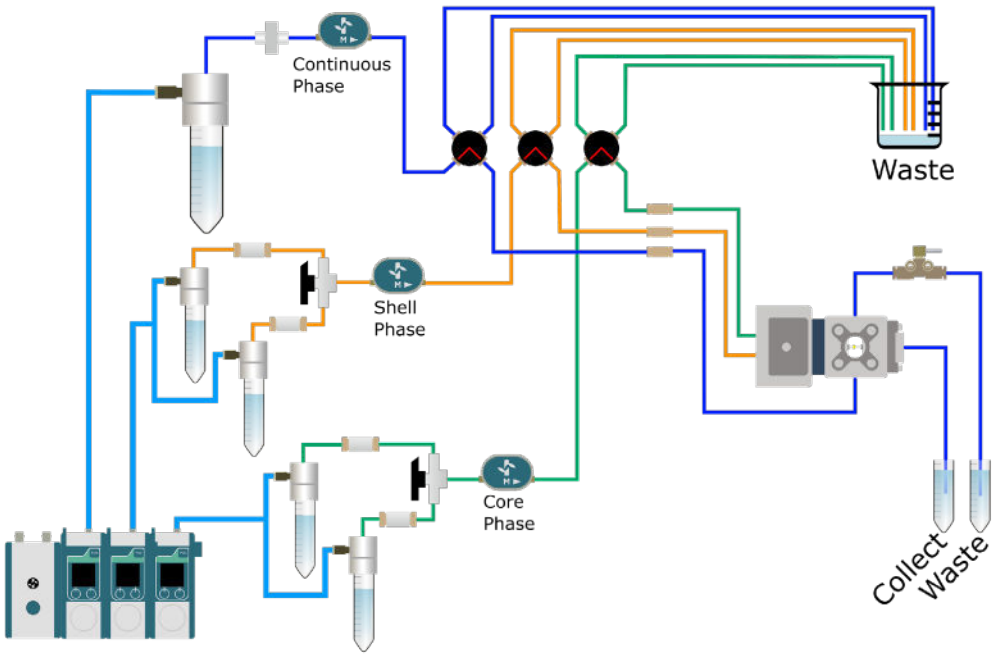
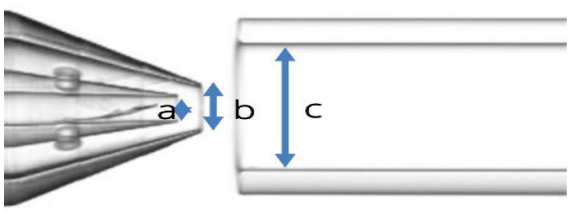


Figure 3: Schema of the setup of Alginate microcapsules production.

The droplet generation set-up is illustrated in the previous figure. 3 Flow EZ's were connected to core phase, shell phase and continuous phase.
Oil-core agarose shell emulsions are manufactured using a **RayDrop™ 90-160-450** due to the flexibility and stability in water-based double emulsions.



- a = 90 µm
- b = 160 µm
- c = 450 µm

Figure 4: RayDrop™ Double Emulsion 90-60-450

As described on Figure 2, the Raydrop™ relies on the use of a 3D-printed injection nozzle carrying two fluids, the core and the shell phases. This is positioned in front of an extraction capillary in a cavity filled with the third (continuous) phase. Fine control of the fluid flows leads to defined capsule and shell dimensions.
Using the double emulsion Raydrop™, controlled double emulsification is achieved by flowing the core fluid into an immiscible shell fluid, which is then encapsulated by the third fluid.

Methods: **How does microfluidic alginate production work?**

Experiment sequence:

- Filter all liquids to avoid clogging (pore size 0.2 µm).
- De-gas solutions to minimize the apparition of air bubbles inside the system.

Stage 0: Preparation

» All fluidic lines of the setup (figure 2) are sequentially filled with liquid. The manual valve was used to first close the core and shell phase and open the continuous line. The upper port of the RayDrop was opened for 3 seconds to evacuate the air.

» A stable pressure was maintained to fill the device back with the continuous phase followed by the shell channel and the core channel by opening the manual valves » This results in wetting the fluidic lines to the RayDrop and the waste, with the continuous phase leaving it free of bubbles.

Stage 1: Shell Phase Simple Emulsion

» A single emulsion of the shell phase in the MCT oil was produced.

» The core phase remains closed, and the shell phase is turned to the waste in order to wet the tubing from the reservoir to the valve (air and bubbles are pushed to the waste).

» The pressure of the continuous phase was set to enable a flow rate. Then, the valve of the shell was turned on to the RayDrop position and increased the pressure until an optimal flow rate was established.

» Pressure was increased until jetting mode was reached to thoroughly flush the shell nozzle until the flow was stable.

Stage 2: Double Emulsion

The valve of the core phase was turned around to again push the air and the bubbles to the waste. The valve was then turned to the RayDrop.

Pressure is adjusted until an optimal flow rate is reached. The rate is maintained for 5 minutes to flush the nozzle properly until the core flow is perfectly stable. Then, the flow rate is reduced and double emulsion production begins.

Stage 3: Droplet collection and microcapsule precipitation (crosslinking).

- Double emulsion experiments were performed for 60 minutes.
- Once the droplets were formed, the cross-linking procedure was performed. The cross-linking procedure consists of collecting the output of the RayDrop reactor into a 50 ml stationary reservoir filled with working solution MCT 3.
- This step will generate the polymerization process and recover microcapsules. By decreasing the pH of the alginate droplet using acetic acid in the collection reservoir (MCT 3), the calcium-EDTA dissociates and releases CA2+ ions allowing for the crosslinking of alginate chains.
- The collected material is allowed to reside in MCT 3 solution for a period of at least 180 minutes after the completion of the experiment. This enables complete crosslinking of the alginate shell.
- After cross-linking is complete, the microbeads are collected on a filter/sieve, washed with excess water and resuspended in a MCT 1 working solution.
- After the crosslinking procedure, microcapsules were tested via physical agitation.

Stage 4: Production run

- Oil core - hydrogel shell microcapsules were produced at gram quantity. The system was left running for 30 minutes to determine the long-term stability and ability to withstand clogging.
- Experimentation will be repeated using Ag1, Ag2, and Ag3 precursor solutions

RESULTS

During these runs, three concentrations of Alginate were used (Al1, Al2, Al3), targeting thin and thick shell microcapsules.

Al1 and Al2 resulted in the successful formation of oil core–alginate shell microbeads. The core to droplet ratio has altered, resulting in larger cores. This could be due to the interaction of alginate with the oil phase or the retardation of shell formation owing to the viscosity of alginate.

As can be seen in Table 1, Al3 (3% alginate solutions) did not result in stable droplet formation as the concentration was too viscous. Instead, sticking was observed at the point of droplet formation, and the experiment was stopped to prevent the clogging of the Raydrop chips.

In alginate microcapsules production, the outer diameter of the emulsions, and thus the capsule size, can be selected by changing the size of the collector capillary and the nozzle tip dimensions. This is easily achieved by a change of the two inserts.

For a configuration with the nozzle and output capillaries (respectively 90µm, 160□m and 450µm) as presented in this note, adjusting the flow rates of the fluids allows for fine control of the capsule dimensions. With this setup, microcapsules from 212µm- 245µm can be easily produced (see Figure 5). The shell thickness of microcapsules can vary by changing the ratio of the flow rates of the shell and core phases. Here, core thickness varies from 75µm to 173µm.

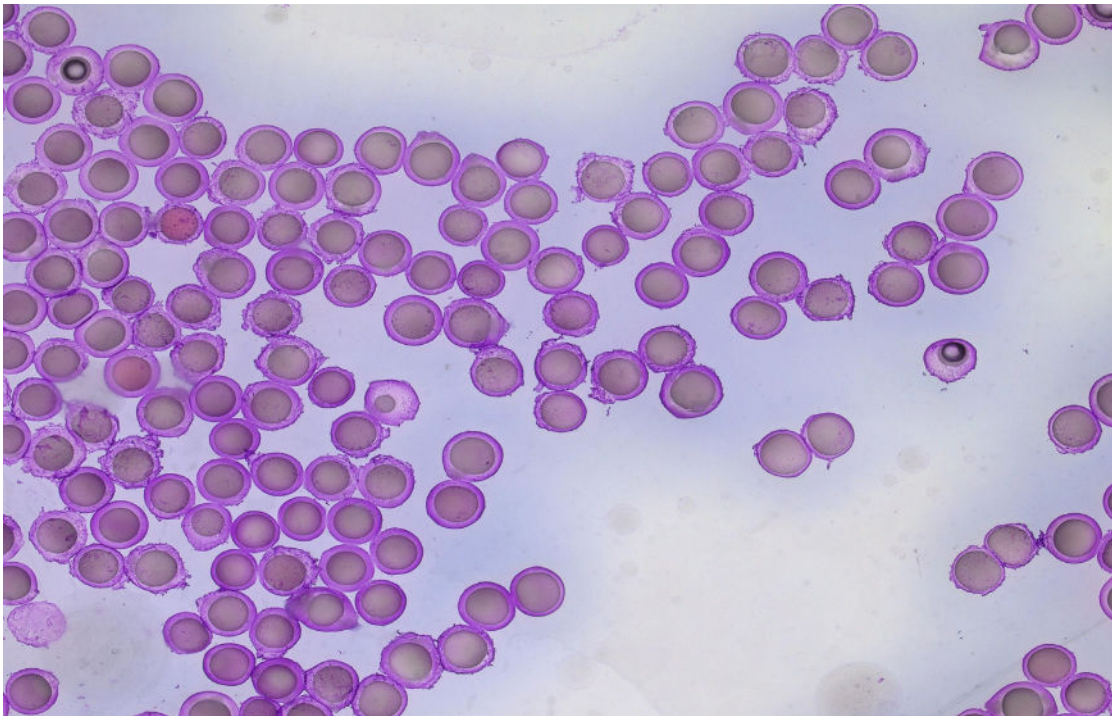


Figure 5. Alginate microcapsules in suspension.

Working solution	Core flow rate µL/min	Shell flow rate µL/min	Continuous flow rate µL/min	Droplet size /µm	Core size /µm	Droplet formation rate /Hz
Al1	20	60	100	221	75	236
Al1	30	80	150	271	124	176
Al1	50	20	100	222	152	204
Al1	50	40	150	234	127	273
Al2	20	60	100	215	77	256
Al2	30	80	150	245	85	238
Al2	50	20	100	212	141	234
Al2	50	40	150	239	173	210
Al3	-	-	-	-	-	No droplets
Al3	-	-	-	-	-	No droplets
Al3	-	-	-	-	-	No droplets
Al3	-	-	-	-	-	No droplets

Table 1. Representation of core, shell and continuous flow rate; droplet and core size, and the droplet formation rate.

CONCLUSION

Microfluidic technology adaptation for alginate microcapsule production has seen significant growth in varied application fields. The benefits of reproducibility, real-time control and reduction of waste are leading users to switch from conventional batch methods to microfluidics.

In this application note, made in collaboration with Small Biotechnologies, the Complex Emulsion Production platform generated highly monodispersed alginate microcapsules with a size between 212 and 245 µm with standard RayDrop™ configuration (Nozzle of 90µm-160µm and outlet capillary 450µm) using an alginate solution of 1% and 2% in water. Other concentrations of alginate such as 2%, which is widely used in the delivery of small chemical drugs, protein delivery, and wound dressings, have been successfully tested by following the same process.

In this alginate microcapsule production application, we have demonstrated that microcapsule size variation can be successfully obtained by altering the flow rates of the phases and/or the size of the Raydrop™. We have also demonstrated that core-shell ratio can be successfully adjusted by altering the relative flow rates of core and shell phases.

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