AGAROSE MICROCAPSULES PRODUCTION

INTRODUCTION

Microcapsules comprising double emulsions are some of the most useful microcarriers that remain incredibly difficult to fabricate at laboratory and industrial scales. Double emulsion-based microcarriers are used across:

- Pharmaceutical applications such as controlled and targeted drug delivery of oil-based API.
- Bioavailability enhancement.
- Supplements delivery e.g. solubilisation of oil-based vitamins, such as Vitamins B,D, and taste masking of unpalatable formulations.
- Cosmetics such as delivery of active components, such as vitamin A, in aqueous formulations.

Across all of these applications, the value of the double emulsions is determined by:

- Loading ability, the quantity of oil within a single droplet of the double emulsion.
- Encapsulation efficiency of the oil-based active, i.e. reduction of lass factor during the encapsulation.
- Absence of empty shells as well as the absence of doublets and triplets.
- High degree of monodispersity (uniformity of emulsion).
- Run-to-run reproducibility. [1-6]

Microfluidic techniques enable the precision manipulation of individual droplets. To date, however, devices capable of generating double emulsions were difficult to fabricate and use. The Raydrop™ (developed and manufactured by Secoya) is the first easy-to-use device that enables the robust production of double emulsion. It doesn't rely on double coating treatment of PDMS/glass in planar chips [10] or the alignment of two round capillaries in a third square tubing, as in lab-made glass capillary microfluidic devices [11].

The controllable double emulsions can be used as excellent templates for synthesizing microcapsules with well-tailored internal structures, such as highly monodisperse core–shell structures and multicompartmental structures [5].

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 soft

tissues. Agarose is a particularly attractive hydrogel as the manufacture of microbeads does not require chemical gelation and relies on controlled temperature profiles instead. As a result, agarose microbeads are widely used as scaffold structures in tissue engineering, as high-yield material in cell growth and in biomolecule immobilization [13].

This application note describes agarose microcapsule production using the complex emulsion production platform, which was developed in collaboration with Secoya. The complete platform is a robust system for producing outstanding monodispersed double emulsion in one single device. The system uses Fluigent's LineUP microfluidic pumps and the RayDrop™ Double Emulsion device, which is a technology breakthrough for high monodispersed and stable double emulsion.

MATERIALS AND METHODS

Materials

Reagents:

Core phase:

- MCT 1: MCT oil

Shell phase:

- Ag1: MiliQ water with 1% w/w TWEEN 80 and 1%w/w Agarose

OR

- Ag2: MiliQ water with 1% w/w TWEEN 80 and 2%w/w Agarose

OR

- Ag3: MiliQ water with 1% w/w TWEEN 80 and 3%w/w Agarose

Continuous phase:

- MCT 2: MCT with 2% w/w PGPR (E576)

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. Three Flow Units M are used here to monitor and control the flow rates of the dispersed 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. This allows to generate highly monodispersed emulsions with any kind of fluids including agarose.

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).

Figure 1: Complex emulsion production platform.

Methods: **Agarose microcapsules production**

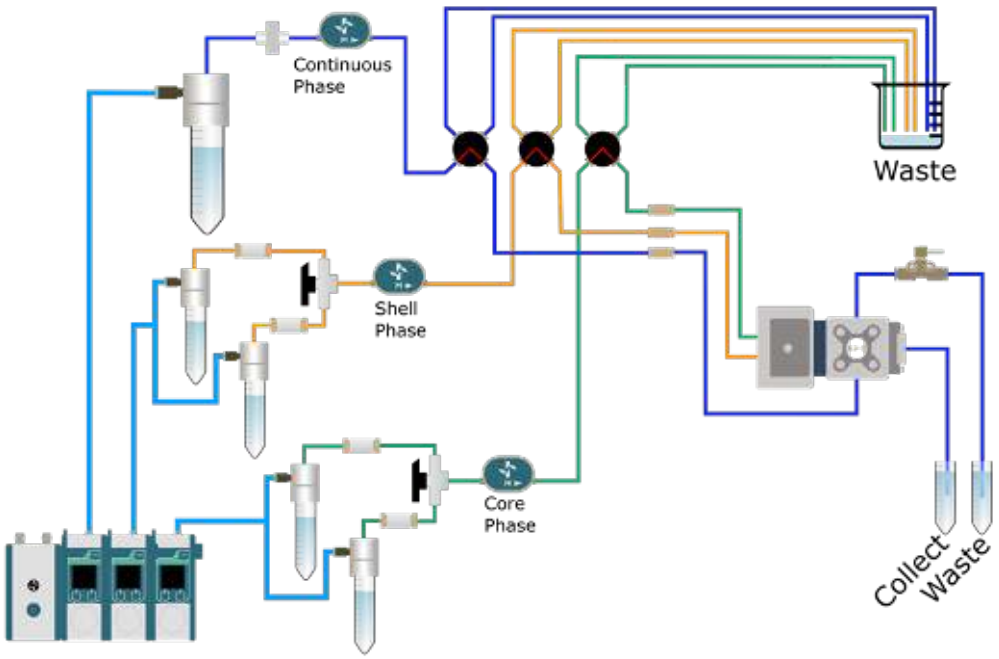

Figure 2: Schema of the setup of Agarose microcapsules production.

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

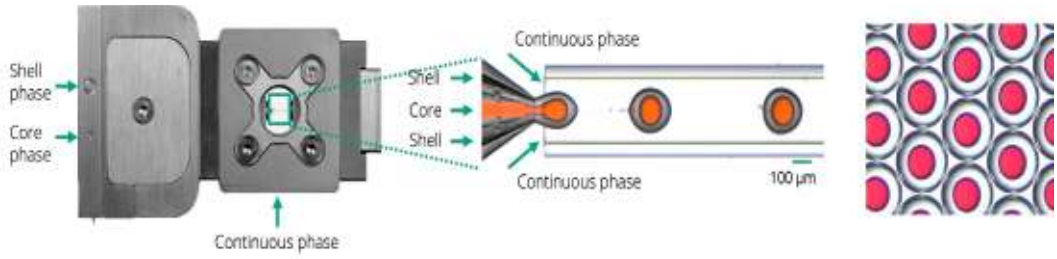

Figure 3: RayDrop™ Double Emulsion

As described on Figure 3, 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.

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Experiment sequence:

» Before the experiments, the dispersed phase was heated to 50 °C and maintained at that temperature. The Raydrop™ chip was placed in a stainless-steel casing and then placed on a hotplate set to 80 °C. The temperature of the Raydrop consistently measured at 57 °C. All leading to and out of the chip was maintained at an elevated temperature by circulating preheated air. The tubing was also heated with a heat gun to prevent the agarose from precipitating and blocking the channels.
» It's recommended to filter all liquids, in order to avoid clogging (pore size 0.2 micrometers).
» It's also important to degas 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 and closed again.
» A stable continuous pressure was maintained to fill back with the continuous phase first, and then the shell channel and the core channel by opening the manual valves
» This results in the fluidic lines to the RayDrop and to the waste being wetted with the continuous phase and free of bubbles.

Stage 1: Shell Phase Simple Emulsion

» Starting with first producing a single emulsion of the shell phase in the MCT 2 oil.
» 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 is perfectly 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. Then, the valve was turned in the position from the reservoir 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

» Once double emulsion droplets were formed, they were allowed to age in the MCT oil working solution for at least 15 minutes. The formed microcapsules could remain in MCT oil solution indefinitely.
» After gelation is complete, the microcapsules were collected on a filter/sieve and washed with excess water and resuspended in MCT oil working solution.
» Gelled 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 Agarose were used (Ag1, Ag2, Ag3), targeting thin and thick shell microcapsules.

As can be seen in Table 1, Ag3 (3% agarose solutions) did not result in stable droplet formation. This was primarily due to the to the high viscosity of the solution.

Ag1 and Ag2 have resulted in the successful formation of oil core–agarose shell microcapsules. Premium quality microcapsules were successfully obtained. Droplet formation was stable enough to enable long-term production of the oil core – agarose shell sample over the course of 180 minutes.

In agarose microcapsules production, the outer diameter of the emulsions and thus the capsule size can be selected within a broad range 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 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 215µm to 300µm can be easily produced (see Figure 4 and Figure 5). The shell thickness of microcapsules can also be varied by changing the ratio of flow rates of the shell and core phases. Here, core thickness varies from 80µm to 170µm.

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
Ag1	20	60	100	225	85	224
Ag1	30	80	150	281	119	158
Ag1	50	20	100	215	145	224
Ag1	50	40	150	254	119	175
Ag2	20	60	100	235	83	196
Ag2	30	80	150	275	89	168
Ag2	50	20	100	241	139	159
Ag2	50	40	150	257	169	169
Ag3	-	-	-	-	-	No droplets
Ag3	-	-	-	-	-	No droplets
Ag3	-	-	-	-	-	No droplets
Ag3	-	-	-	-	-	No droplets

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

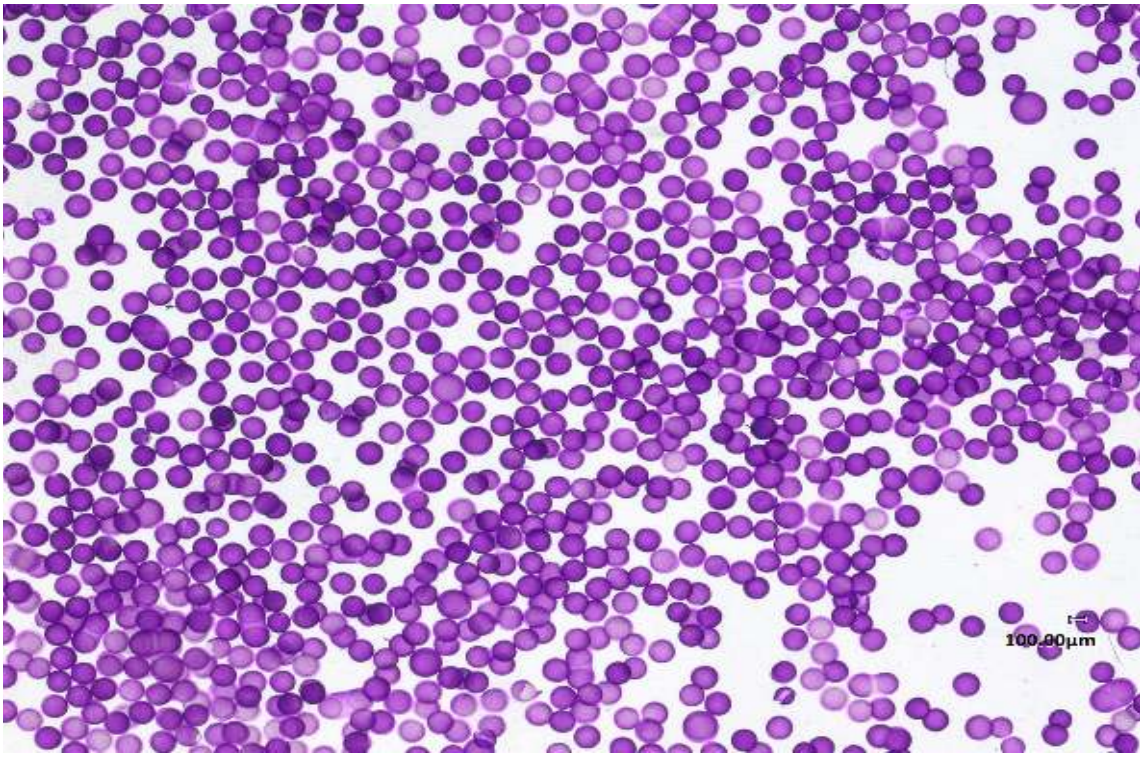

Figure 4. Agarose microcapsules in suspension.

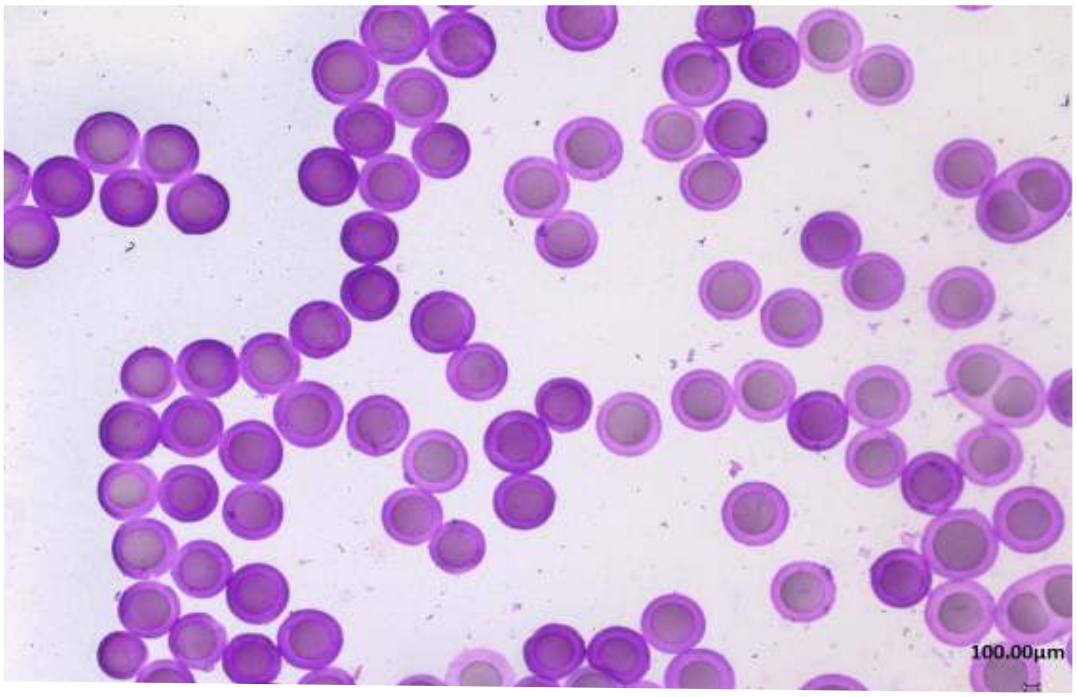

Figure 5. Agarose microcapsules in suspension.

CONCLUSION

In this application note, made in collaboration with Small Biotechnologies, the complex emulsion production platform generated agarose microcapsules with a size between 215 and 300 µm with standard RayDrop™ configuration (Nozzle of 90µm and outlet capillary 150µm) using agarose solution at 1% in water. Other concentrations of agarose such as 2%, which is widely used in biological application, have also been successfully tested by following the same process.

The alteration in the continuous phase does not produce a significant change in droplet formation rate or size – this adds to the stability of the system. In addition, long-term agarose microcapsule production (over 180minutes) was successfully undertaken.

Furthermore, we have demonstrated that microcapsule size variation can be successfully obtained by altering the flow rates of the microcapsule phases and/or the size of the Raydrop™ chips. Besides, we have also demonstrated that core-shell ratio can be successfully adjusted by altering the relative flow rates of core and shell phases.

This confirms that the Raydrop™, in combination with Fluigent pumping technology, are one of the most competitive double emulsion systems available due to the flexibility (changing capillary size can be easily done to target different droplet sizes), the ease of use, the breadth of accessible chemical systems, the long-term performance stability and the lack of coatings (e.g. sigma coat).

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