Optimization of Microspheres Formulation by a Co-extrusion Method

Henry N., Buchala L., Gueraud F. and Poncelet D*.
GEPEA – UMR CNRS 6144, Oniris, Nantes, France (Nina.henry@oniris-nantes.fr)

INTRODUCTION AND OBJECTIVE

Cancer colon may be initiated by actives molecules metabolised from the food, during the gastro-intestinal tract. To evaluate the toxicity of these molecules, it is proposed to release them directly into the colon. Usually, to reach this target, the coating in fluidised bed is the preferred method but the smaller batches are still quite large (minimum 100g). Due to the cost of the molecule (100€/mg) a new encapsulation method has to be developed, allowing producing very small amount of capsules, able to release the active principle ingredient (API) in the colon. Thus, the micro-encapsulation is a very interesting approach.

The literature review has led us to select an extrusion method and more precisely a co-extrusion technique. The co-extrusion encapsulation permits the production of microspheres with different materials for the shell and the core. Thus, it is possible to modulate the release properties of the beads and thereby, reach specific sites for the active molecule liberation.

In a preliminary study, the microspheres formulation has been optimised using alginate material for the core and the shell. Afterwards, the materials may be modified to optimise the microspheres formulated.

MATERIALS AND METHODS

Materials

Inotech Encapsulator® Research IER-20 (Figure 1a). The technique is based on the principle that a vibration can cut a laminar jet into homogeneous sized dropplets (Figure 1b).

Alginate Algogel 3001 (Cargill), Alginate Algogel 5541 (Cargill) and Alginate Satialgine S 60 NS (Cargill), Calcium Chloride (Sigma Aldrich). The alginate viscosity values presented thereafter have been mesured by a viscosimeter Haake G (Fisons).

Formation of the beads

Selected alginate was added gradually to deionized water under high stirring until getting a homogeneous solution. The stirring velocity was then reduced to evacuate the incorporated air bubbles.

Alginate solution was extruded using the Inotech Encapsulator® Research IER-20 into a calcium chloride gelation bath.

An inner nozzle of 400µm and outer nozzle of 500µm were used. It has been established that a total flow rate of 22 ml/min and a vibrating frequency of 384 Hz were the best parameters and have been used for all the following experimentations.

After each experiment, the average size of the microspheres, the sphericity (smaller/larger diameters ratio) and their general aspect were evaluated at room temperature by an observation on a microscope.

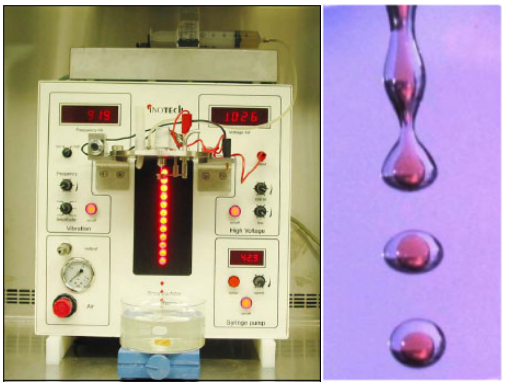


Figure 1: Inotech Encapsulator® (a) allows the ruptre of a laminar jet by vibration (b)

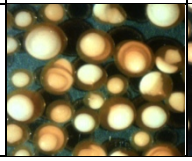
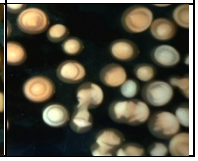
RESULTS AND DISCUSSION

Three different alginates were tested as well as various concentrations and inner/outer flow rates. As we use alginate for both phases, the core is coloured with titanium dioxide.

Influence of the alginate nature

The influence of the alginate nature has been studied by testing three different alginates (Table 1). For these experiments the concentration of the solutions were fixed at 2% and the flow rates at 7 ml/min for the core and 15 ml/min for the shell.

Table 1: Influence of the Alginate nature

	Algogel 5541	Algogel 3001	Satialgine S 60 NS
Viscosity (mPa.s)	1515	345	175
Sphericity	-	0,952 ± 3,8%	0,932 ± 6%
Average size (mm)	-	1,538 ± 18%	1,516 ± 15%
Aspect of the micro-spheres	No micro-spheres		

When the viscosity is too high (as for algogel 5541 at 2% concentration) no microspheres could be made. When the viscosity is lower, the apparatus is able to cut the laminar jet to form microspheres. With the Alginate 3001 the microspheres formed are the most spherical and the cores are well defined. Thus, all the further experimentations have used this alginate.

Influence of the alginate concentration

The influence of the concentration was then evaluated by testing solutions from 1% to 3% of the algogel 3001. The flow rates used are still 7 ml/min for the core and 15 ml/min for the shell.

Table 2: Influence of the Alginate concentration

	1,5%	**2%**	**2,5%**
Viscosity (mPa.s)	143	345	591
Sphericity	0,944 ± 5%	0,952 ± 3,8%	0,957 ± 2,7%
Average Size (mm)	1,415 ± 20%	1,538 ± 18%	1,816 ± 16%
Aspect of the micro-spheres			

At concentrations below 1,5%, the beads formed weren't spherical and the core wasn't homogeneous. For 3% concentrations and above, the solutions were too viscous and no microspheres could be realized (Data no presented).

At concentrations between 1,5% and 2,5% spherical microspheres were made (Table 2). The experiments realized with the 2,5% alginate concentration led to the most spherical microspheres, homogeneous shell and well-defined cores. With the two other concentrations, the core isn't spherical nether centred.

Thereby, the 2,5% concentration is chosen for the following experimentations. Indeed, in addition to the better-formulated microspheres, having a more concentrated shell will lead, after drying, to a less porous and stronger outer membrane.

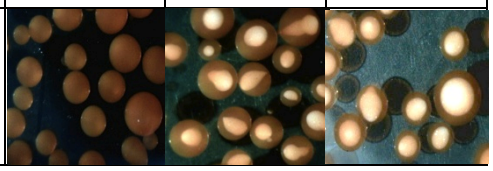
The concentration, and consequently the viscosity, of the solution used is a very important point to optimize on the Inotech Encapsulator® while carrying out co-extrusion.

Influence of the core/shell flow rates ratio

Finally, the influence of the core/shell flow rates ratio was studied. The experiments were made with a 2,5% concentration of the Algogel 3001 alginate solution and a total flow rate of 22 ml/min (Table 3). Three flow rates were tested for both the core and the shell of the microspheres.

Setting a higher flow for the shell will lead to obtain a less porous and more resistant membrane after drying. Thus, we have always used an external flow superior to the internal flow.

Table 3: Influence of the flow rates ratio

Core/Shell Flows (ml/min)	**2/20**	**5/17**	**7/15**
Sphericity	0,974 ± 2,3%	0,980 ± 2,5%	0,957 ± 2,7%
Average size (mm)	1,790 ± 15%	1,819 ± 17%	1,816 ± 16%
Aspect of the micro-spheres			

When the core flow is very low (=1/10 of the shell's rate) the microspheres do not have any core. When increasing the inner flow rate, the core is more and more present. At 7/15 ml/min for the core/shell outflow, the core is spherical with a well-defined contour.

Besides the concentration and viscosity, the flow of each phase has an importance to realize spherical and homogeneous microspheres.

CONCLUSIONS

As presented in this contribution, microspheres of alginate have been made by a co-extrusion method using the Inotech Encapsulator®. They are spherical and homogeneous, with a well-defined core. Nevertheless, a certain optimization is still needed in order to reduce both the average size of the beads to 400 µm, and the dispersion size.

Subsequently, the core and shell materials will also have to be adjusted in order to reach the best microsphere possible and obtain a controlled release allowing a specific colon delivery. As the shell will probably need to be reinforced, we have planned to incorporate Shellac or cellulosic derivate in this phase. Regarding to the core material, chitosane, oils and glycerol distearate are scheduled for the following tests.

Finally, the release properties of the microspheres will be evaluated *in vitro* before being administrated to rats to analyze the toxicity of the API encapsulated.

ACKNOWLEDGMENTS

I would like to acknowledge the Cargill Company for the samples they have provided.