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Cell Micro-encapsulation

Methods and Protocols

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Chapter 3

Cell Microencapsulation: Dripping Methods

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Abstract

Microencapsulation processes may be divided into three steps, namely: incorporation of the bioactive substance in the matrix, dispersion of the matrix in droplets, and conversion in microcapsules. This contribution is focused on the second step and more specifically using the dripping approach to form droplets by extrusion of liquid through a nozzle. Different technologies of dripping are described, using as an example the production of alginate beads.

Key words Dripping, Droplet size, Production

1 Introduction

As described in Part 1 of this book, there are many reasons and methods for immobilizing cells. Encapsulation is probably an easier, faster, efficient, reproducible, and cost-effective way to mimic the natural environment and the organization of immobilized cells. Encapsulation may be described in three steps:

1. Mixing the cells with the future capsule core, generally a liquid,
2. Dispersing the resulting suspension as droplets,
3. Converting the droplets in microcapsules.

This contribution will focus on the second step and more specifically on dripping technologies, i.e., forming droplets by extrusion through a nozzle. This approach is still the main method for cell encapsulation [1]. As they are formed, droplets may be converted in beads or capsules by ionic or thermal gelation, coacervation, polymerization, or interfacial cross-linking [2]. The matrix of the particles may be composed of a large range of materials [3] but for simplicity, we will refer in this chapter to the formation of hydrogel beads by extruding drop by drop an alginate solution in a calcium solution.

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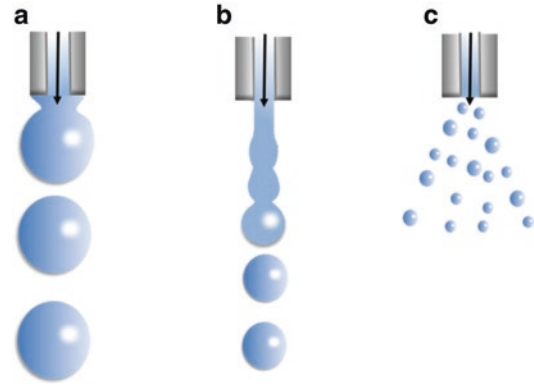

Fig. 1 Dripping regimes: (**a**) Dropwise, (**b**) Jet-breaking, (**c**) Spray regime (E. Martins ©)

Formation of droplets by extrusion of a liquid through an orifice is largely function of the velocity of the extruded liquid. Three regimes may be considered (Fig. 1):

1. For low velocities, droplets are formed at the tip of the orifice and break off one by one: dropwise regime.
2. For intermediate velocities, the liquid exits the orifice as a jet and may break-up as droplets: jet-breaking regime.
3. For large velocities, the jet becomes unstable and breaks in multiple small droplets. This is the spray regime. In the frame of the actual review, this regime will be not treated but it is the basis of spray-drying and spray-cooling technology consisting in spraying the liquid in a large chamber and either drying droplets by warm air or solidifying melt liquid droplets by cool air.

The transition from dropwise to jet-breaking regime is defined by the minimum jet velocity, $u_{,\,\mathrm{min}j}$ [4]

$$u_{\mathrm{j,min}=2\sqrt{\frac{\gamma}{\rho\, d_{\mathrm{j}}}}} \tag{1}$$

Where γ is the liquid surface tension, ρ the liquid density, and dj the internal diameter of the nozzle. The maximum flow rate for the dropwise regime, $F_{\mathrm{max,\ dropwise}}$, may be obtained by multiplying the minimum jet velocity, u_{j}, by the section of the jet (assuming that the jet diameter is equal to the internal nozzle diameter):

$$F_{\mathrm{max,drop\ wise}} = \frac{\pi d_i^2}{2}\sqrt{\frac{\gamma}{\rho d_i}} \tag{2}$$

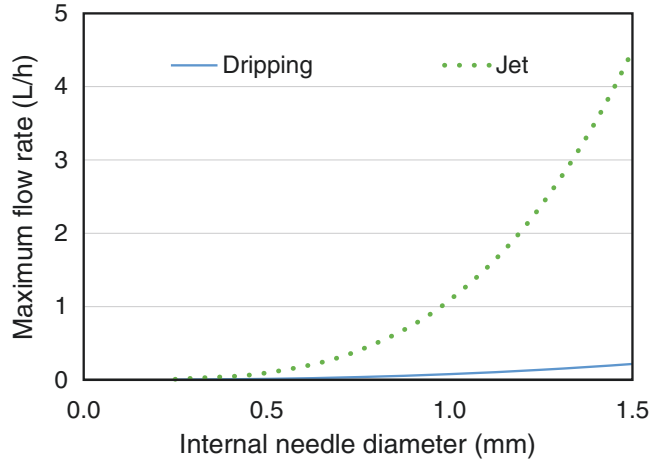

Fig. 2 Influence of the droplet diameter on the maximum productivity

The jet velocity must be limited to the terminal drop falling velocity, *ut*, to avoid collision between the jet and the droplet (reference):

$$u_t = \sqrt{\frac{4gd_d}{3\rho C_D}} \tag{3}$$

where d_d is the droplet diameter, *CD* is the drag coefficient equal to 0,44 [5]. The maximum flow rate for jet-breaking regime, $F_{max,\ jet\text{-}breaking}$ is again found by multiplying by the jet section:

$$F_{max,jet\ breaking} = \frac{\pi d_i^2}{4}\sqrt{\frac{4gd_d}{3\rho C_D}} \tag{4}$$

Figure 2 shows the flow rate or productivity in function of the internal diameter diameter. In jet-breaking regime, the droplet diameter is approximately twice the internal jet diameter (see later),

It is clear that the dropwise regime will be suitable only for small productions (50–250 mL/h), while the jet-breaking, especially using multi-nozzle systems, may produce quite large amounts (up to hundreds L/h for large droplets). On another side, Fig. 2 shows that the maximum productivity for both regimes is mainly proportional to the square of the droplet diameter. Reducing by two the droplet/capsule diameter thus decreases by four the productivity.

1.1 General Equipment

Figure 3 presents two assemblings for producing alginate beads by extrusion/dripping.

1. Figure 3a presents an installation based on a syringe pump, which allows easy fix precise flow rate. However, this setting is mainly adequate for dropwise regime, at low production (30–60 mL) and is limited to low viscosity (200 mPa.s).

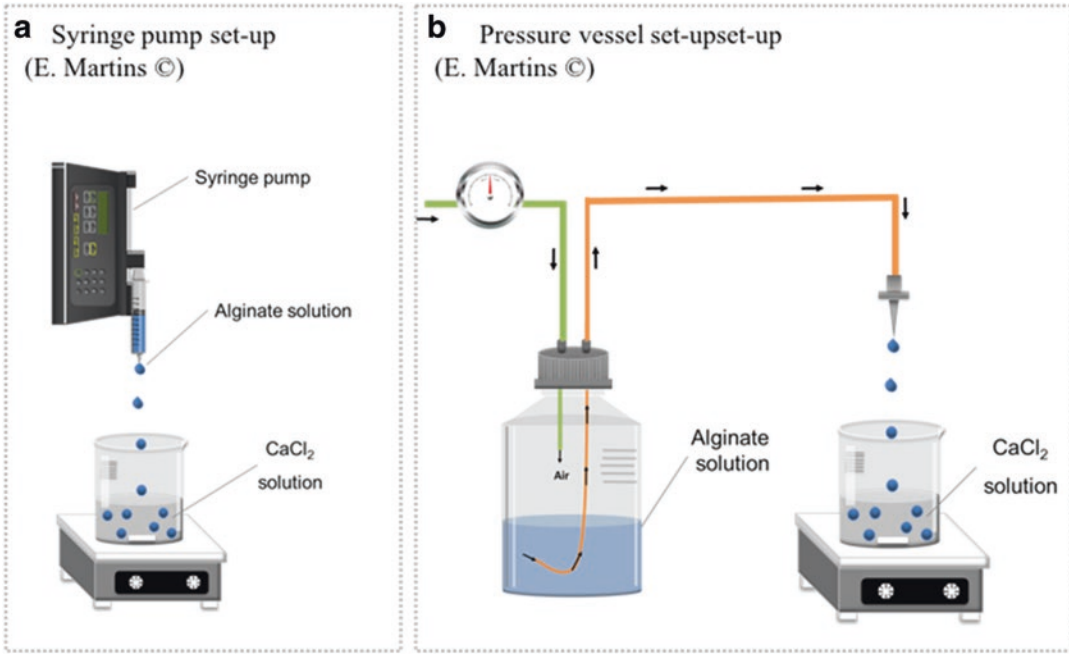

Fig. 3 Assembling for extrusion/dripping production of alginate beads

2. The system presented in Fig. 3b is based on pressure vessel. Normally, the flow rate is proportional to the applied pressure. However, any variation in the liquid viscosity may modify the flow rate making this system more difficult to control. This solution is mainly required for break-jet regime.

In dropwise regime, needles are generally used as nozzles (Fig. 4a). Smaller is the internal diameter and longer is the needle, higher are the pressure needed to reach a certain flow rate and the risk of needle blockage. In practice, it is advisable to select needles and cones with an internal diameter larger than 0.4 mm. Using cones (Fig. 4b) in place of needles reducing the pressure by a factor 5 to 10. Some models are compatible with Luer-Lock connections (Fig. 4c), allowing to alternatively use needles or cones on the same setup.

In jet-breaking regime, liquid must form a linear and vertical jet, avoiding turbulence. Most commercial equipments have a specific design and nozzles manufactured by high technologies such as laser. For in-house setup, one would prefer nozzles specifically developed to produce high-quality jet (Fig. 4d) in place of needles or plastic cones.

1.2 Protocol

The following generic protocol will be applied for the following sections, with some specific remarks linked to the methods.

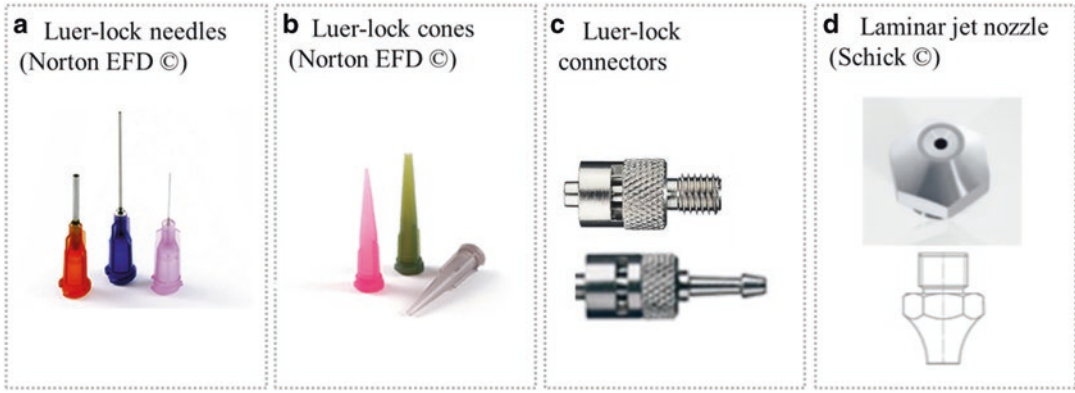

Fig. 4 Nozzles and connectors

1. An alginate solution (18 g/L) is produced by adding slowly 3, 6 g sodium alginate powder on 200 mL of distilled water under gentle agitation. Let stand for 1 h until complete dissolution.
2. Either
   (a) Fill with the alginate solution a syringe equipped with a needle or a cone, and place it on the syringe pump, on top of the collecting bath.
   (b) Fill the pressure vessel with the alginate solution, connect the vessel to the nozzle support.
3. Prepare the collecting bath by filling a beaker with 200 mL of calcium chloride solution (50 mM), place the beaker on a magnetic stirrer and fix the agitation to get a low vortex; droplets must penetrate the liquid at half distance between the beaker wall and center.
4. Start the syringe pump or fix the vessel pressure to get adequate flow rate.
5. After alginate solution extrusion, let stand the beads 15 min in the collecting bath.
6. Filter the beads on a 50–100 μm mesh, rinse the beads, and resuspend them in appropriate media for future application.

Alginate droplets contract during gelation; the resulting beads have a diameter of around 80% of the droplet size.

2 Dropwise Regime Under Simple Gravity

In the dropwise regime, the liquid flow through the nozzle forming a droplet which grows until the gravity forces are larger than the force which maintain the droplet on the device (Tate's law):

$$mg = \pi d_e \gamma \tag{5}$$

where m represents the mass of the droplet, g the gravity constant, d_e the external diameter of the nozzle and γ the surface tension. The mass of the droplet is given by:

$$m = \frac{\pi}{6} d^3 \rho \tag{6}$$

where ρ is the density of the extruded solution. Combining Eqs. 1 and 2 allows to define the droplet diameter, d_d

$$d = \sqrt[3]{\frac{6 d_e \gamma}{g \rho}} \tag{7}$$

Figure 5 shows that the size of the droplets will be ranging between 3 to 4 mm, relatively independent to the nozzle diameter.

2.1 Equipment

Developing in-house a module for maintaining the needle/cone is not too complex. As an alternative, equipment may be purchased from Nisco Swiss company, either with a single or multi-nozzle setup (http://nisco.ch/) (Fig. 6). The company provides also a single nozzle with coaxial liquid system allowing to make core/shell capsules

2.2 Protocol

The protocol is identical to the generic one presented above. The flow rate will simply be adjusted to a value inferior to the minimum jet flow rate (100–200 mL, mainly depending of the liquid viscosity).

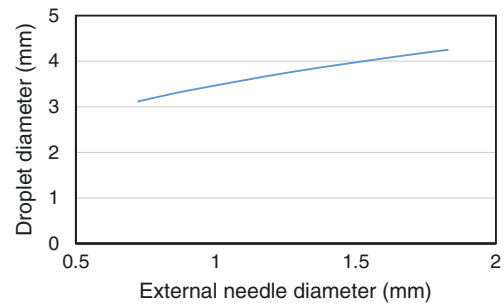

Fig. 5 Size of the droplet, d_p, versus the external needle diameter, d_e (surface tension of the extruded solution 73 mN/m at 20 °C)

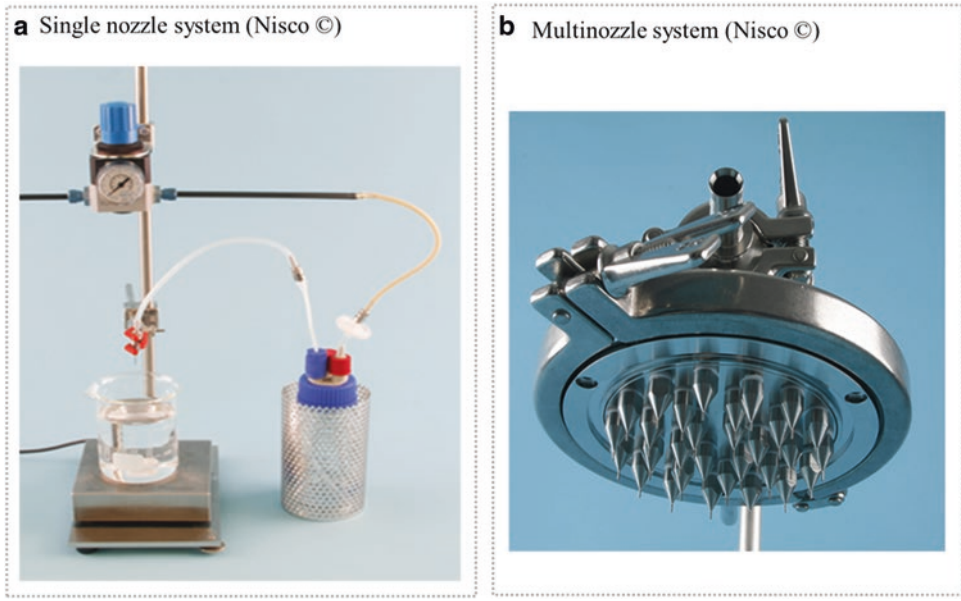

Fig. 6 Assembling for dripping under dropwise and simple gravity regime

3 Dropwise Regime Under Electrostatic Potential: Electro-dripping

Under simple gravity, only large droplets/beads can be produced. By applying an electrostatic potential to the needle, ions migrate to the surface of the droplets [6]. This results in some repulsion at the surface of the droplet, which interferes and reduces the surface tension [6]:

$$\gamma = \gamma_o \left(1 - \frac{U^2}{U_c^2}\right), \tag{8}$$

where γo is the surface tension without applied electrostatic potential, U the applied electrostatic potential, Uc, the critical electrostatic potential. As a result of combining Eqs. 7 and 8, the Fig. 7 gives a typical evolution of the droplet size in function of the electrostatic potential applied to produce alginate beads. As the electrostatic potential U increases, the droplet size decreases until a minimum value, reached while the electrostatic potential is equal to the critical electrostatic potential, Uc. At higher electrostatic potential the liquid will exit the nozzle as jet. This jet may separate into droplets whose size is independent of the applied voltage.

Different factors may affect the critical electrostatic potential Uc as well as the minimum droplet size, including the diameter of the nozzle, sign of the electrostatic potential, composition of the solution, flow rate [4].

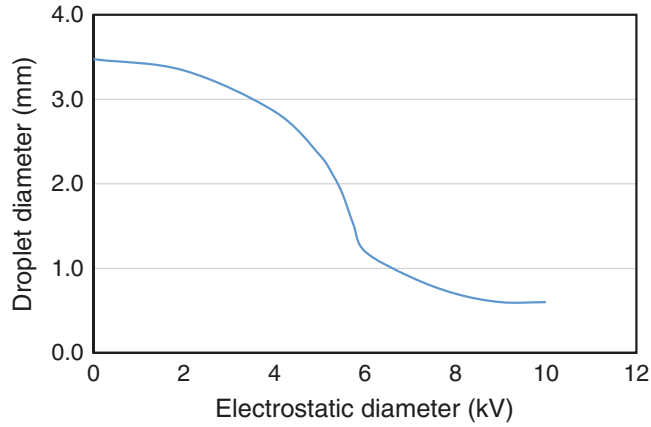

Fig. 7 Droplet size versus the electric potential

3.1 Equipment

The electrostatic potential may be applied between the needle and the collecting bath (Fig. 7a) or on a metallic ring placed under the needle (Fig. 7b). The electrostatic potential must be applied using a high-voltage power supply delivering a very low current intensity (Bertan, USA). The collecting vessel or the ring will be preferably connected to the ground. Distance between the needle and either the collecting solution or the ring should be at least one centimeter per 10 kV applied to avoid electric arch. The risk of electrocution is very limited but the electric discharge may be painful. Always turn off the power supply and wait a few seconds before to manipulate the system.

It exists at least one commercial electro-dripping device developed by the Nisco Swiss company (http://nisco.ch/, Fig. 8c).

3.2 Protocol

Follow the generic protocol. As the liquid is extruded as droplets, turn on the power supply and increase the electric potential until you reach the expected droplet size. When the solution is extruded, decrease the electric potential, turn off the power supply, and wait a few seconds before manipulating the system.

4 Dropwise Regime with Coaxial Air Flow

To decrease the size of droplet, air flow can be applied coaxially around the needle. The air drag force will support the gravity force. Establishing equations to predict the size of the droplets in function of air flow rate is challenging. However, depending on the design and the air flow, size as low as a few micrometers could be obtained. As expected, the productivity of the system decreases quickly as the droplet size decreases.

They are two configurations for the coaxial air flow:

1. The needle tip extends outside of the air flow tube (Fig. 9a). The air simply applies on the forming droplet a drag force leading to

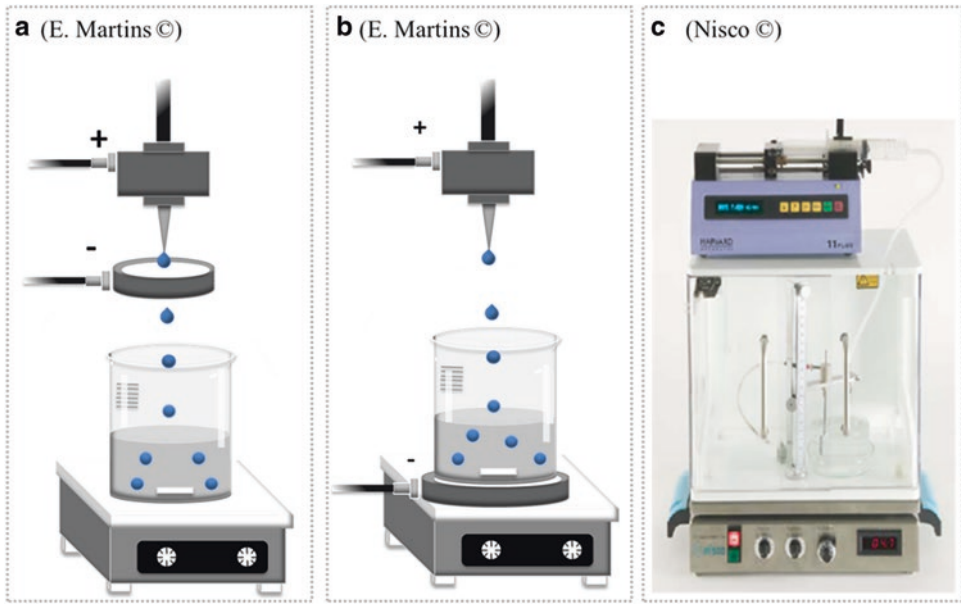

Fig. 8 Assembling for production of alginate beads by electro-dripping

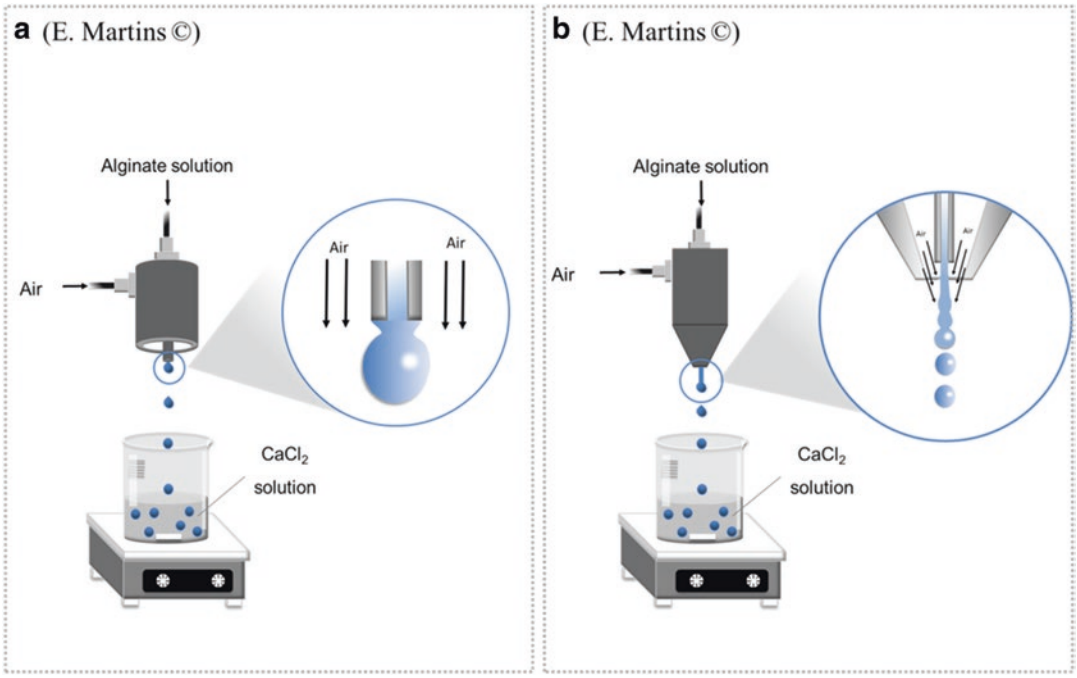

Fig. 9 Assembling for alginate bead production using coaxial air flow

smaller size. The droplet diameter generally may be reduced to 800 μm without forming a spray.

2. The needle tip is inside the air flow tube (Fig. 9b). The air compresses the liquid, generally leading to a jet, which breaks into droplets down to a few micrometers.

4.1 Equipment

Building the first configuration is not too complex but again Nisco company proposes a ready-to-use unit. For the second configuration, it is best to buy a commercial equipment. Two companies offer such a system: Nisco (http://nisco.ch/) and Cellena (http://www.cellena.net/en/).

4.2 Protocol

The protocol is identical to the generic protocol, except that you have to set up the air flow rate to get the desired droplet size. Some specific instructions may be provided with the commercial equipment.

5 Jet-Breaking Regime Using Vibrating Nozzle

When the flow rate increases over the minimum jet velocity conditions, the liquid will exit the nozzle as a jet. In practice, a flow rate corresponding to two or three time the minimum jet velocity is needed to get a stable jet. As pointed above the flow rate may be limited to avoid collision between the jet and the droplets but also to remain in laminar flow conditions. The jet will break into droplets due to Rayleigh instability. By avoiding any vibration but applying an optimum frequency f, the droplet size will be mainly mono-dispersed [7]:

$$f = \frac{u_j}{\lambda} \text{ and } \lambda = 4.0458 \mathrm{d_j} \tag{9}$$

Where λ is the wavelength but also the length of the jet portion created by the vibration, and u_j is the jet linear velocity. This jet portion will take the form of a sphere of the same volume that the cylinder:

$$\frac{\pi}{6} d_{\mathrm{d}}^3 = \left(\frac{\pi}{4} d_j^2\right)\left(4.058 d_{\mathrm{j}}\right) \text{ or } d_{\mathrm{d}} = 1.89 d_{\mathrm{j}} \tag{10}$$

The droplet size is thus about twice the internal nozzle diameter. The real value is function of the liquid viscosity, and the mono-dispersion may be obtained for wave lengths ranting from 3.5 to 7 the internal diameter of the nozzle.

The breakage of the jet is due to resonance, assuming that the vibration is moving inside the jet. High viscosity has a damping effect, and this process will correctly work only for solution of viscosity lower than 200 mPa.s

5.1 Equipment

This technology is relatively difficult to develop in-house. At our knowledge, three companies propose such equipment: Buchi (http://www.buchi.com/), Nisco (http://nisco.ch/) and Brace (http://www.brace.de/), the last one provides also equipment for

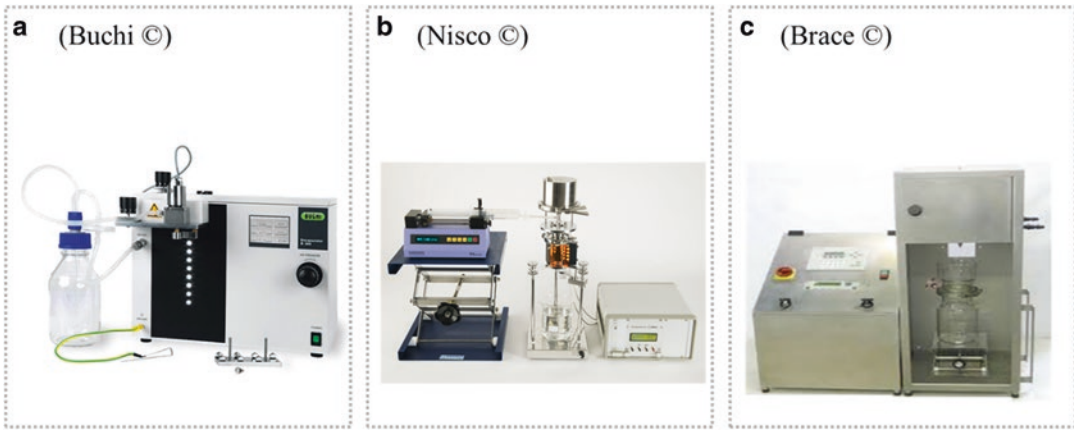

Fig. 10 Commercial equipment for producing alginate beads by vibrated nozzle jet breaking

industrial production. Nisco and Brace offer multi-nozzle equipment. All of them also propose a coaxial system (Fig. 10) allowing production of core/shell capsule.

These equipments are supplied with LEDs flashing at the same frequency that the vibration applied on the liquid. When the conditions are optimum, the jet breaks into mono-dispersed droplets and light flashes happen at the exact stage of droplet detachment. The droplets seem immobile. If the droplets seem to move, the flow rate or the frequency has to be modified until droplets become apparently immobile.

5.2 Protocol

General protocol may be applied here, adjusting the parameters as follow:

1. Select a nozzle with an internal diameter half of the requested droplet diameter.
2. Set the frequency and the flow rate, according either to the Eqs. 9 and 11, or to supplier recommendations.
3. If the flow of droplets seems to move up or down, modify either the frequency or the liquid flow rate until droplets seem immobile. This step is critical and often not understood by the operator. Supplier recommendations are based on a specific solution and must be adjusted in function of the physical properties of the real used liquid.

6 Jet-Breaking Regime Using a Rotating Wheel

If the solution viscosity is too high, the jet may be broken using a wheel equipped with very fine wires: jet-cutter (Fig. 11). This system was developed by Vorlop's group in Germany [8] and exploited

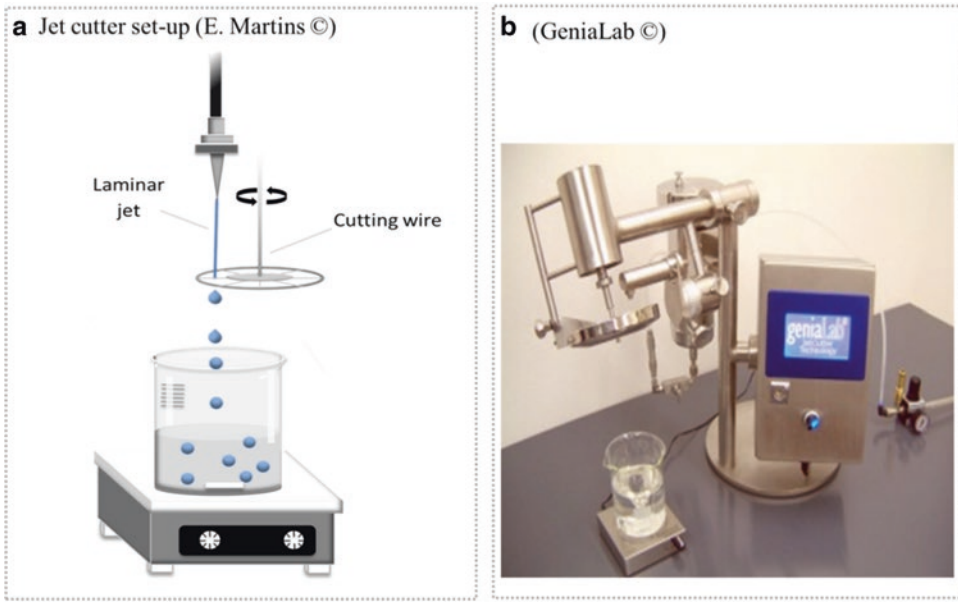

Fig. 11 Production of alginate beads using a jet-cutter

by the start-up GeniaLab. The diameter of the droplets may be predicted by:

$$d_{\mathrm{d}} = \sqrt[3]{\frac{6}{\pi}\frac{F}{N'}} \tag{11}$$

where F is the extruded liquid flow rate, N the number of wires on the wheel and Ω the rotational speed of the wheel. The liquid jet velocity must be high, similar to the maximum jet flow rate (Fig. 2) to favor a correct cutting. Some part of the liquid jet may be expelled by the wire crossing the jet. The liquid lost is mainly proportional to the wire diameter, but it may be minimized by tilting the wheel [6]. The wire diameter should preferably be lower than 80 μm (50 μm) to get yields higher than 95 % (98 %).

6.1 Equipment

GeniaLab (http://genialab.de/) sales (or sold) laboratory equipments. Recently, Nisco (http://nisco.ch/) started to propose similar equipment.

6.2 Protocol

The generic protocol may be applied. Select a nozzle diameter half the size of the expected droplets, fix the flow rate, and compute the corresponding jet velocity, fix the rotational speed to get a wire velocity at the position of the cutting similar to the jet velocity.

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