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# Immobilized Cells



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*Lab Manual*

# Immobilization at Large Scale by Dispersion

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## ■ Introduction

Producing microcapsules at large scale implies first caring about the dispersion processes. The droplet solidification step (gelation, membrane formation ...) generally brings less problems (reader will refer to Chapter 3). In this chapter, four technologies of producing droplet at large scale will be proposed for cell encapsulation purposes:

- jet breaking methods
- LentiKat method
- rotating devices
- emulsification using static mixers

Considering that scale-up is performed in industries, information about large scale equipment remains limited. However, this chapter will try to provide as much complete technical information as possible on the subject.

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## Subprotocol 1

### Jet Breaking Methods

**Principle** While liquid is extruded at large flow (> 30 ml/h) from a nozzle, the formed liquid jet can be broken into droplets of narrow size distribution by two methods: the nozzle resonance method and the jet cutting method. Nozzle resonance method (called prilling in chemistry) involves extruding a liquid through a nozzle as a jet and applying a vibration to the liquid (through a membrane) or the nozzle. This method will be largely described in Chapter 14. Very large production may be reached (tons a day) by using multi-nozzle systems. Size may range from a few micrometers to millimeters. However, for high viscous liquid, damping effects limit the possibilities to reduce the size lower than 800  $\mu\text{m}$ . Commercial devices exist at pilot or even industrial scale (Inotech, Switzerland; Sodeva, France).

Cutting the jet with a little “wheel” has been proposed to overpass the limitation of the nozzle resonance method in regard to the liquid viscosity [Vorlop, 1994]. Inner liquid core of the microcapsules is extruded through a nozzle as a jet. The jet is cut by wire fixed on a rotating wheel (Figure 1a). The falling droplets are collected in solidifying bath (cold water, cross-linking agent solution). The system has been patented [Vorlop, 1994] and is commercialized by geniaLab, Germany (Figure 1c).

### ■ ■ Materials

#### Cutting jet method

The device includes:

- a high speed motor (1000 to 40 000 rpm, in function of the droplet size)
- a rotating wheel (Figure 1a and b)
- an injection system composed of
  - either vessel under pressure or pump without pulsations, and
  - connected to small injectors
- a collecting vessel with a gentle agitation.

The whole system may be incorporated in a close reactor to insure aseptic conditions.

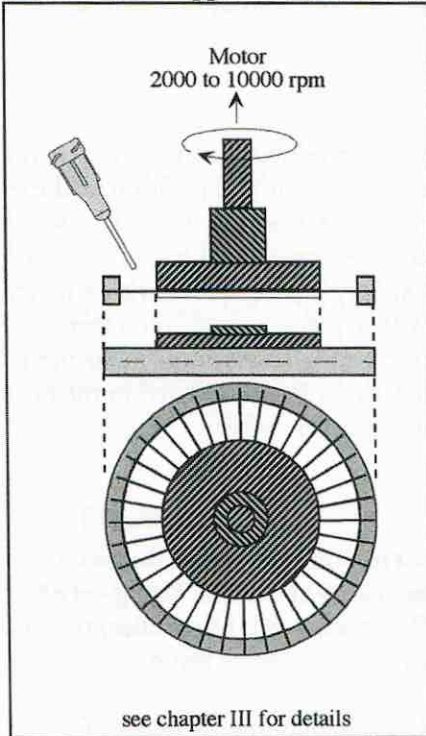
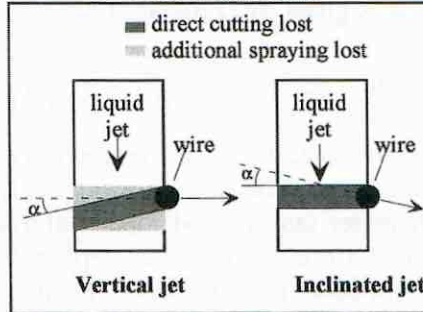
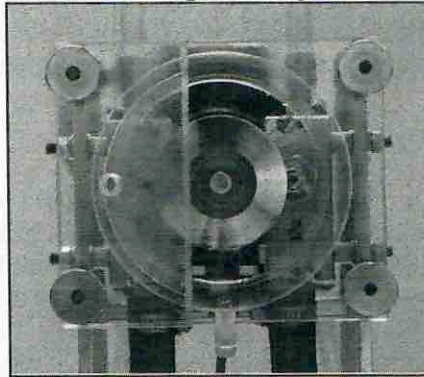
**A. home made apparatus (ENSAIA)****B. Lost evaluations****C. bottom vue geniallab system**

Fig. 1. Jet cutting method

## ■ ■ Procedure

From a practical point of view, engineers will first fix the diameter of the droplets,  $d$ . The flow rate,  $F$ , may be computed as the products of the droplet volume times the frequency of cutting (rotational speed,  $\omega$  time the number of wires,  $z$ ):

$$F = \frac{\pi}{6} d^3 (z \omega) \eta \quad (1)$$

where  $\eta$  is the yield (fraction of the extruded liquid recovers as microcapsules). The flow rate may be obviously multiplied by using several injectors.

To evaluate the yield, it is necessary to determine the fraction of the jet lost (ejected) during the cutting (see Pruesse, 1998a et 1998b for complete analysis). While the wire passes through the jet, the jet itself moves (Figure 1b). To minimise the cutting loss, the jet may be inclined in regard to the

Driving conditions

wheel (Figure 1b). The optimal jet-to-wheel angle,  $\alpha$  for minimum cutting loss is given by (Pruesse, 1998 b):

$$\sin \alpha = \frac{u_{jet}}{U_{wires}} = F \frac{\frac{\pi}{4} d_i^2}{2\pi l \omega} = \frac{2 F}{\pi^2 d_i^2 l \omega} \quad (2)$$

where  $u_{jet}$  and  $u_{wires}$  are respectively the linear velocity of the jet and wires,  $l$  the distance between the centre of wheel and the cutting position,  $d_i$  the jet diameter (assimilated to the internal nozzle diameter). The jet-to-wheel angle  $\alpha$  must however be kept in a working range of 45 to 90 °.

In optimum conditions, the loss is mainly a jet cylinder with a height equal to the wires diameter,  $d_w$  (Figure 1d). The fraction of liquid lost ( $1-\eta$ ) is mainly given by the ratio between the cutting lost cylinder and the jet cylinder between two cuts. This ratio may easily be assimilated to the ratio between wire diameter and the distance between wires.

$$1 - \eta = \frac{d_w z}{2\pi D} \quad (3)$$

The main constraint to reduce the lost fraction is the wire diameter. For 0.4 mm wire, lost fraction is ranging between 5 and 10 %. Using very fine wires (0.1 mm), it becomes negligible (less than 2 %). In practice, the loss may be slightly higher. geniaLab in particular observes the need to clean the wheel to remove material glued on it. This phenomena is however difficult to predict as it may vary strongly from material to material.

## ■ ■ Results

The jet cutting method allows to produce beads or capsules ranging from 250  $\mu\text{m}$  (3 l/h) to 2 mm (1500 l/h). The size distribution is relatively small (5 to 10 %) and the yield included between 90 and 100 %.

### Subprotocol 2 Lentikats

#### Principle

Generally, microcapsules are relatively spherical. The Federal Agriculture Research Center of Braunschweig (FAL) has developed a large scale method based on lens-like shape microcapsule production (Figure 2a) [Jekel, 1998]. Droplets are extruded on a flat surface where they solidify in the form of lenses (either internal chemical or thermal gelation). The process is developed by geniaLab (Germany) at lab and industrial scales under the

trademark LentiKats. Lens shape avoids mass transfer while providing good settling properties.

## ■ ■ Procedure

### Materials and Procedure

Figure 2a presents a general view of LentiKats production. Figure 2b shows LentiKats production device for pilot scale. Through a series of nozzles, droplets are extruded on a large moving strip. If required, spraying, heating or cooling system may be placed on top of the strip. At the end of the apparatus, solidified LentiKats are detached by the curvature of the strip (or with help of a cutter).

## ■ ■ Results

LentiKats have usually 3 to 4 mm in diameter and 200 to 400  $\mu\text{m}$  in thickness. Assuming 20 nozzles and a strip speed of 1 cm per second, the productivity is 20 kg per hour. Such a process implicates the use of encapsulating material of very good mechanical strength. Currently, LentiKats are made from polyvinyl alcohol (PVA) which shows very good mechanical stability. Several months of continuous stirring didn't cause a decrease in stability.

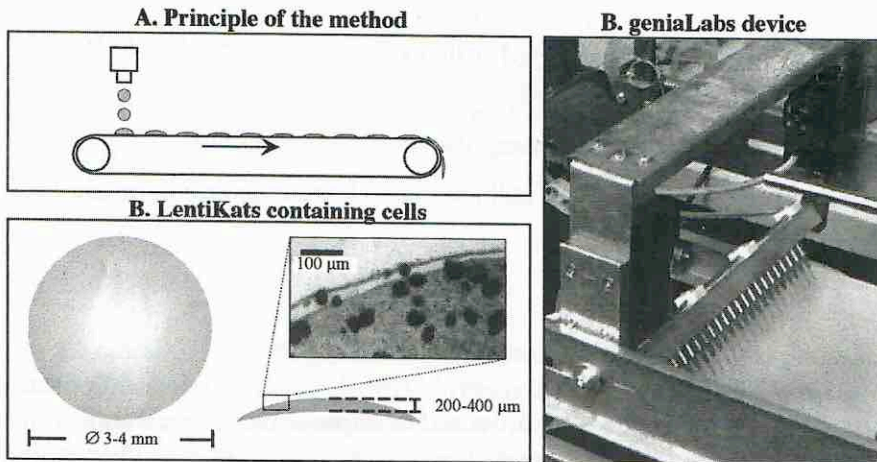


Fig. 2. LentiKat process

### Subprotocol 3

#### Rotating Devices

**Principle** While a liquid is flowed onto a rotating disk, it is ejected by centrifugation as droplets, ligaments or continuous film in function of the rotating speed, disk size, liquid flow rate and liquid properties. Film breaks into ligaments, ligaments into droplets. The flow rate is generally high (hundreds of litres per hour) and rotating devices are very promising for very large flow rates.

Unfortunately, the theories are quite complex and could not be summarized here. Moreover, due to missing information a complete description of the apparatus is not possible. As an example, most authors do not give the droplet ejection distance. Most settings of the system are given as adimensional numbers which don't allow the return to real apparatus dimensions.

#### ■ ■ Materials

In all cases, the system will comprise:

- an injection system composed of
  - a vessel under pressure or unpulsed pump
  - connected to an injector
- a rotating device which must avoid vibration as much as possible. Four devices have been proposed:
  - simple disk
  - rotating cup (Figure 3a) [Hinze, 1950]
  - vibrated disk (Figure 3b) [Chicheportiche, 1993]
  - nozzle fixed on a rotating cylinder (Figure 3c) [Schlameus, 1993]
- a collecting device containing the solidifying solution.

The complete system may be included in a closed chamber to insure sterility.

#### ■ ■ Procedure

#### Running description

The simple disk allows limited control of the flow characteristics. The three other systems have then been proposed to improve the characteristic of the droplet formation. Rotating cup allows improvement of flow characteristics and reduction of the droplet ejection distance [Hinze, 1950]. In the

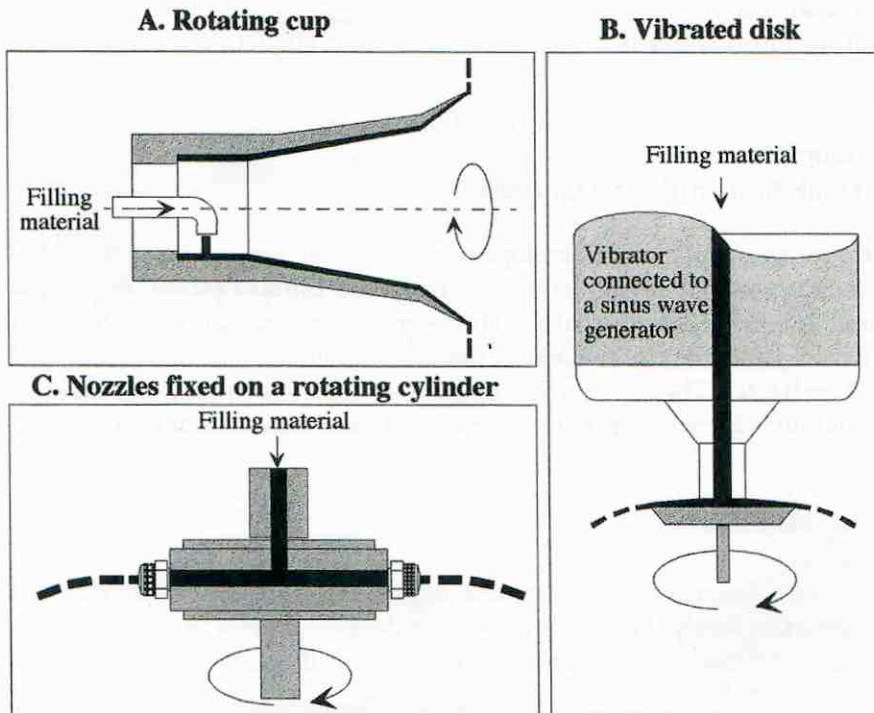


Fig. 3. Rotating devices

ligament formation state, controlled vibration may be applied to the disk and ligaments will break in quasi monodispersed droplets [Chicheportiche, 1993]. Although the theory is more complex, the principle is similar to the nozzle resonance method (Chapter 14). Finally, flowing the liquid through a nozzle on a rotating cylinder allows us to form a jet of controlled characteristics. It may be possible to combine such technology with the nozzle resonance technology [Schlameus, 1993].

## ■ ■ Results

In all cases, the size distribution is narrower with rotating disk (standard deviation as low as 5 %) than with pressure atomisers (30 to 50 %). It allows us to reach a large scale (tons per hour). The ejection diameter may however be quite large (up to 10 m), limiting the square meter productivity and requiring very large collecting bath. In such conditions, sterility may be



difficult to maintain. Vertical collectors have been proposed (Figure 3e) but no information is available to prove their efficiency.

#### Subprotocol 4 Emulsification Using Static Mixer

**Principle** Several encapsulation technologies are based on the emulsification of an aqueous phase in an oil phase (see Chapter 3). Emulsification may be realized in turbine reactor (batch). However, at large scale, the production of microcapsules may be realized by passing both phases through a static mixer (Figure 4c). The advantages comprise continuous processing, small installation, easy sterilizing, low shear, narrower size distribution.

#### ■ ■ Materials

Figure 4a schematises a complete device for producing microcapsules using static mixer. Figure 4b presents a detailed system including the injection and the static mixer. The material will then include:

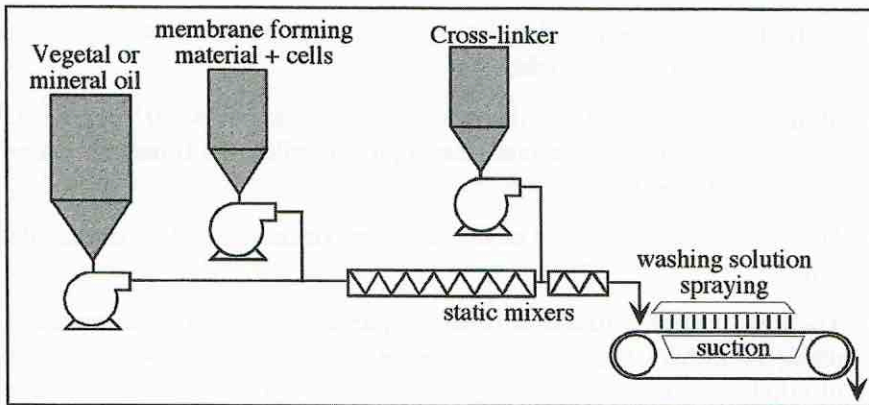
- three tanks (dispersed phase, continuous oil phase, solidifying solution)
- three pumps (the process is relatively insensible to small pulses. Internal phase is often viscous, screw or moineau pump, PCM Pumps, France, allow to pump it )
- the emulsification device (Figure 4b) containing the static mixer (Sulzer, Switzerland) (Figure 4c)
- the collecting device (see below)

#### ■ ■ Procedure

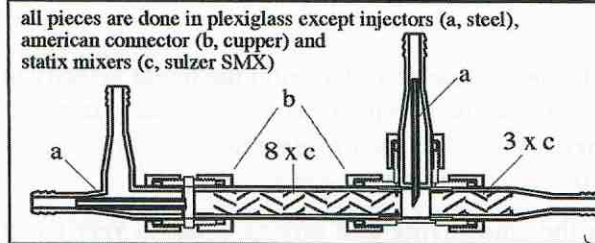
Regarding the building of the device the following advice may be given:

- Dispersed phase (microcapsule core) must be injected at the centre of the tube.
- Dispersed solution injector diameter will be designed to insure equal linear velocity of the dispersed and continuous phase (50 % of external tube for 25 % dispersed phase of total flow).

### A. Principle of the method



### B. Laboratory scale device (ENSAIA)



### C. Sulzer SMX

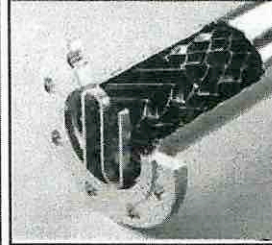


Fig. 4. Static mixer systems

- Selection of the static mixer depends on the solution properties, flow rate. Sulzer SMX mixers provide good emulsification with limiting shear capacity.
- Static mixer must not move in the tube but not be fixed to allow easy washing (in case of accidental solidification in the static mixer).
- Flow rate of the solidifying solution must be limited (maximum 10 % of the total flow rate).
- Tubing after homogenization may be prolonged (as spiral) to insure complete solidifying.
- Microcapsules may be collected in a tank and separated from oil later. A better solution would involve collecting microcapsules on a vibrating moving strip with bottom aspiration and top washing solution spraying.

From the point of view of the driving conditions, it must be pointed out that:

- Static mixers allow low droplet size (as low as 100  $\mu\text{m}$ ) to be attained without requiring emulsifier.
- Standard deviation of size distribution may be as low as 30 % in case of low viscosity dispersed phase (less than 100  $\text{mPas} \cdot \text{s}$ ). It may be necessary to heat the solution (30 to 40°C).
- Dispersed phase must also be limited to maximum 25 to 30% of the total flow.
- The bead size is a function of many parameters. As a first approach, using 10  $\text{mPas} \cdot \text{s}$  dispersed phase and continuous phase composed of ethyl cocoate (20  $\text{mPas} \cdot \text{s}$ ), a SMX static mixer (1 cm) was found whose size correlated to:

$$d = 45 u^{-1.2} \quad (4)$$

where  $d$  (mm) is the droplet diameter and  $u$  (m/s) the linear velocity in the static mixer. This law may be independent of the static mixer diameter. Other experiments show that the droplet size is mainly proportional to the inverse of the continuous phase viscosity.

- the residence time in the emulsifying and mixing zones is very small (0.1 to 0.5 s). To succeed to get nice capsules, it is then very important to realise the solidification step in a very short period. Assuming that the process of solidification it-self is fast, this may be achieved by:
  - introducing chemical cross-linkers soluble in continuous oil phase or
  - cooling the droplets directly by addition of cold oil after emulsification

## ■ ■ Results

In the absence of emulsifiers, microcapsules ranging from 100  $\mu\text{m}$  to 1 mm may be obtained. For 1 cm diameter SMX Sulzer static mixer, productivity ranges from 10 to 25 kg/h (respectively 250 to 600 kg/h for 5 cm mixer), allowing very large production.

## ■ References

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## ■ Suppliers

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