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REVIEW

Microencapsulation using vibrating technology

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Abstract

For over a half a century now, microencapsulation has played a very important role in many industries and in the recent decades, this versatile technology has been applied to numerous biotechnology and medical processes. However, successful application in these areas requires a methodology which has the capability to produce mono-dispersed, homogenous-shaped capsules, with a narrow size distribution, using a short production time. The manufacture of capsules using vibrating technology has gained significant interest mainly due to its simplistic approach to produce homogenous microcapsules with the desired characteristics for biotechnological and medical processes. However, certain limitations still exist for this methodology, which include the inability to manufacture microcapsules at large quantities and/or using highly viscous polymers. In this review, a detailed description of the theoretical and practical aspects behind the production of different types of alginate-based microcapsules, for application in biotechnological and medical processes, using vibrating technology, is given.

Keywords: microencapsulation, microspheres/microcapsules, vibrating nozzle encapsulator, alginate, monocentric and concentric nozzles

Nomenclature

v	liquid velocity (m/s)
d_d	droplet diameter (μm)
d_n	nozzle diameter (μm)
σ	surface tension (N/m)
g	acceleration due to gravity (m/s^2)
ρ	fluid density (kg/m^3)
d_{cab}	diameter of calcium alginate bead (μm)
K	overall correction factor
λ_{opt}	optimal wavelength (μm)
d_j	jet diameter (μm)
λ	wavelength of perturbation (μm)
v_j	jet velocity (m/s)
f	vibrational frequency (1/s)
η	fluid viscosity (kg/ms)
We_n	Weber's number
F	liquid flow rate (m^3/s)
v_n	liquid velocity through the nozzle (m/s)
d_m	mean diameter of microcapsule (μm)
d_c	mean diameter of microcapsule core (μm)
M_m	mean size of microcapsule membrane (μm)
M-block (region)	D-mannuronate
G-block (region)	L-guluronate

Introduction

Microencapsulation can be defined as a process, which involves the complete envelopment of pre-selected core material(s) within a defined porous or impermeable membrane (shell) using various techniques, to give miniature-sized particles ranging from 1 to 1000 μm in size (Whilst no set consensus exists for defining capsule size, in this review, capsules with sizes <1 μm are called nanocapsules, 1–1000 μm microcapsules and >1000 μm macrocapsules.).

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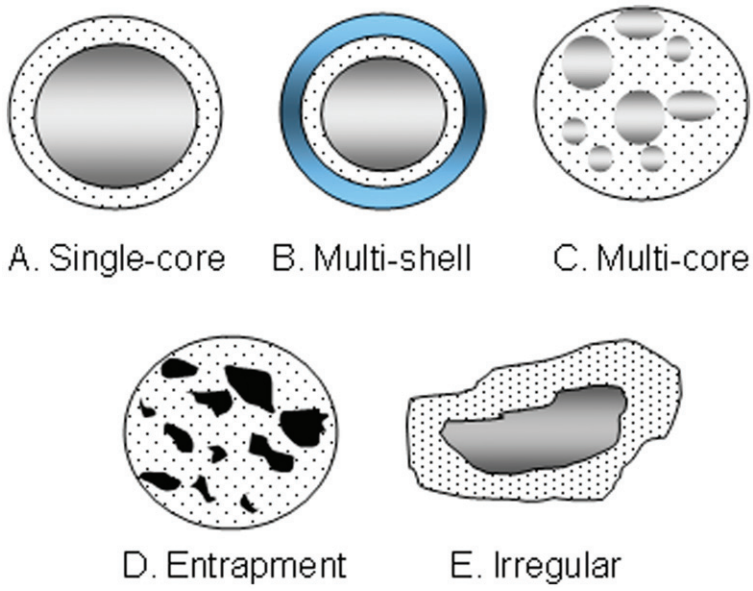

Figure 1. The five main structural forms of microcapsules (adapted from Gibbs et al. (1999), Madene et al. (2006) Arshady (1993), Stark (2001) and Gharsallaoui et al. (2007)). Types C–E can also have multiple shells added to modify stability and permeability of the capsules.

Microcapsules can take many structural forms (Figure 1) and have proven to have many exploitable characteristics for application in many different processes (Table 1). They can be manufactured from a wide range of natural and/or synthetic materials, but are also commonly found in nature, i.e. plant seeds, bacterial spores, egg shells, etc.

The concept of encapsulating a material (also referred to as the encapsulant) within a defined membrane initially dates back to the 1930s in the food industry (Shahidi and Han, 1993), whereby the process was mainly referred to as ''locking'' rather than encapsulation (Arshady, 1993; Graves et al., 2000), in which capsules were produced by a complex coacervation process (Green and Schleicher, 1957).

Early versions were impermeable and had to be broken apart, most often by mechanical means, for the inner ingredients to become active. Examples include controlled release of flavours, aromas, perfumes, drugs, detoxicants, fertilizers and precursors in textiles and printing (Gibbs et al., 1999). In the following years and decades, microencapsulation technology was applied successfully to the cosmetic, printing, chemical, pharmaceutical, agriculture and food industries, where it has become well developed and accepted (Augustin et al., 2001; Heinzen, 2002; Marison et al., 2004). A large number of materials were encapsulated and modification of mass transfer characteristics and reduced interactions of the encapsulant with the environment were the main reasons for performing the process (Stark, 2001).

To date, carbonless copy paper is one of the most significant products utilizing the technology (White, 1998) and in the USA alone, there are several hundred types of capsules being used as food additives (Versic, 1988). They have also been used as extraction aids for various compounds, such as warfarin (Whelehan et al., 2010), hexavalent chromium ions (Ni et al., 1994) and propionic acid (Yoshizawa et al., 1993). In these cases, encapsulation prevented the formation of stable emulsions which subsequently reduces the settling time of an extraction process (Stark, 2001).

In recent times, the technology has been applied to medical and biotechnological disciplines where the encapsulation of recombinant therapeutic proteins (Gombotz and Wee, 1998; Zhou et al., 2002), cell implants (Heald et al., 1994; Park et al., 1998; Visted et al., 2001) and mammalian cells (Seifert and Phillips, 1997; Breguet et al., 2007) has taken place. They have also been applied to bioconversion (Wyss et al., 2005, 2006) and fermentation processes (Stark et al., 2003; Whelehan et al., 2011).

Due to its many existing and potential applications in many diverse areas, microencapsulation has received much attention from both academic and commercial bodies (Senuma et al., 2000), and its further development is of a major interest both from economic and scientific points of view. The growing interest is demonstrated by the exponential increase in the number of publications (non-scientific and scientific articles and patents) reporting on the subject over the decades since the 1950s (Gouin, 2004).

There are six main advantages for encapsulating a material(s) within a membrane compared to its non-encapsulated form, and these are outlined in Table 1, along with examples of where the process was employed. In some cases, envelopment of the core will provide more than one benefit.

Structurally, microcapsules can be classified as five different types (Arshady, 1993; Gibbs et al., 1999; Stark, 2001; Madene et al., 2006; Gharsallaoui et al., 2007), and are shown in Figure 1.

Type A, mononuclear: These are the simplest form of microcapsules, in which the core material (usually a liquid, in which case they can be referred to as liquid-core microcapsules (Wyss, 2005)) is surrounded by a continuous

Table 1. Main reasons for encapsulating a material(s) and examples where the process has been employed.

Reason for encapsulation	Examples	References
Protection or stabilization of the encapsulant from interactions with reactive environments and/or future surroundings	*Cells (Prevention)*	
	Mammalian (immuno-response and cell damage due to agitation and aeration)	Chang et al. (1999), Park et al. (1998) Breguet (2007), Posillico (1986) Krisch and Szajani (1997),
	Yeast (ethanol toxicity)	Norton et al. (1995)
	Enzymes	
	Improved stability and reactivity	Ghanem and Ghaly (2004), Anjani et al. (2007)
	Prevent denaturing	Caruso et al. (2000)
	Food additives and bioactives	
	Off-setting loss and deterioration by:	
	• high temperature food processing	Gibbs et al. (1999)
	• passage through the GI tract	Mokarram et al. (2009)
	• hygroscopicity	Gibbs et al. (1999)
	• evaporation (aroma compounds)	Arshady (1993), Madene et al. (2006)
	• oxidation	Augustin et al. (2001), Chang et al. (1998)
	Recombinant proteins	
	Improved stability and protection	Gombotz and Wee (1998)
Sustained, controlled or timed release of the core material	Agrochemicals (delivery of fertilizers, repellents, herbicides, fungicides, etc.)	Tsuji (1999)
	Folic acid (controlled release)	Madziva et al. (2005)
	Pharmaceuticals (controlled delivery)	Shilpa et al. (2003)
	Antibody (sustained release)	Pelegrin et al. (1998)
	Adhesive (dispensed upon pressure)	Giroud et al. (1995)
	Butan-1-ol flavour (delayed release)	Dickinson et al. (1994)
	Fragrances (dispensed upon pressure)	Versic (1989)
Targeting of encapsulated compounds to specific sites	DNA to phagocytic cells	Walter et al. (2001)
	Vitamin C (oral route)	Esposito et al. (2002)
	Probiotic bacteria (intestine)	Chandramouli et al. (2004)
	Doxycycline for localized treatment of septic arthritis	Haerdi-Landerer et al. (2008)
Enabling core material to be used as an extraction device for product removal including *in situ* product recovery	*Environmental pollutants*	Whelehan et al. (2010)
	Extraction of pharmaceuticals, herbicides/pesticides and heavy metals from water	Wyss et al. (2004a) Ngomsik et al. (2006)
	In situ product recovery	
	Culture environments	Stark et al. (2003)
	Recovery of primary and secondary metabolites	Whelehan and Marison (2011), Whelehan et al. (2011)
	Bioconversion processes	
	Removal of products from hydrolysis of Penicillin G and a lipase-catalysed reaction	Wyss et al. (2006), Wyss et al. (2005)
Improved flow properties of the encapsulant for enhanced handling, usage and storage including Safety	Pesticides (enhanced safety handling and usage)	Tsuji (2001), Scher et al. (1998)
	Biosorbents (improved usage and storage)	Breguet et al. (2008)
	Enzyme (better storage)	Li et al. (2009)
	Bioactives (enhanced handling and storage)	Shahidi and Wanasundara (1995)
	Hydrophobic liquids (new applicability)	Whelehan et al. (2010)
Improved organoleptic properties of the encapsulated product	Shark liver oil (preventing undesirable taste)	Peniche et al. (2003)
	Tea bags (improved appearance)	Dziezak (1988)
	Antibiotic colistin (hiding taste)	Torrado and Torrado (1996)
	Ultra Rice® (preventing undesirable appearance and taste)	Li et al. (2008)

defined membrane. The diameter of the core material and the membrane thickness can vary in size with either occupying between 10% and 90% of total capsule volume. These particles are also termed simple or single-core microcapsules.

Type B, double/multi-shells (walls): Usually mononuclear microcapsules in which a second shell or multiple shells are added to the original capsule. The extra shells are added to modify the original stability and/or permeability characteristics of the microcapsules (Stark, 2001; Heinzen et al., 2004)

Type C, polynuclear (multi-core): Microcapsules containing two or more separate cores (Atkin et al., 2004; Kim et al., 2004) and are usually formed from emulsions. Traditionally, microcapsules are seen as spherically shaped particles with a well-defined shell and core structure (types A–C) but other forms do exist.

Type D, microbeads/microspheres: This is the most common type of capsule. They contain particles (including cells) entrapped within a solid matrix and do not possess a distinctive membrane (Strand et al., 2004). Whilst these structures can be termed capsules, they are usually referred to as microbeads (Strand et al., 2004) or microspheres (Bowersock et al., 1996; Raymond et al., 2004). In this review, both terms are used interchangeably. They can be converted into type A by the addition of an outer shell and

the subsequent liquefaction of the core, and this is the basis for the production of the classical poly-L-lysine-alginate microcapsules as described by Lim and Sun (1980). These particles can also be manufactured with no entrapped material and can be used as immobilization matrices.

Type E, irregular or non-spherical shaped capsules. These particles can be mononuclear, polynuclear or solid particle entrapment. These are the most common type of capsules used in industry, but will not be discussed here.

This review provides a detailed overview of the production of different types of microcapsules using vibrating technology. Initially, it discusses the theoretical aspects behind droplet formation at an orifice as well as their formation in liquid jets (streams) of varying velocities and how the latter can be controlled using vibrational technology. A detailed description of the practical know-how which enables the production of different types of microcapsules (A–D) for application in biotechnological and medical processes using vibrational technology (on an Inotech encapsulator) is then given. Finally, the challenges facing implementation of vibrating technology for the production of large quantities of microcapsules for application in the aforementioned areas are discussed.

Production of microspheres/microcapsules: requirements for application in medical and biotechnological processes

Many different techniques for the production of microspheres/microcapsules have been described (Li et al., 1988; Heinzen et al., 2004). For simplicity, the methods can be categorized as chemical, physicochemical or mechanical processes (Marison et al., 2004) and include the following examples:

- chemical; *in situ* polymerization (Berg et al., 1989) and interfacial polymerization (Kulkarni et al., 2000),
- physiochemical; complex coacervation (Weinbreck et al., 2003), and
- mechanical; spray-drying (Gharsallaoui et al., 2007) and extrusion-based methods (Serp et al., 2000).

As suggested by Ubbink and Kruger (2006) and Madene et al. (2006), the technique selected for encapsulation should always depend on the end-use (which is governed mainly by the core material and to a smaller degree the shell material) of the encapsulated product. This is in contrast from normal practice, whereby the technique is usually chosen before the core and polymer materials as companies usually have set equipment already in place.

Successful application and performance of microcapsules in medical and biotechnological applications requires a methodology capable of producing small (<200 μm), mono-dispersed, homogenous and spherically shaped spheres/capsules, with a narrow size distribution, using a short production time, under mild and simple conditions and low costs, with high encapsulation efficiencies (% of product encapsulated) and production rates, from highly viscous solutions, allowing different production sizes and the ability to produce microcapsules of types A–D (Figure 1), which can all be performed under sterile conditions if required.

Mechanical procedures

Mechanical techniques are one of the most common types of mechanisms used for producing microspheres/microcapsules for medical and biotechnological applications and one such technique will be the focus of this review. These operations use mechanical procedures rather than a well-defined physical or chemical phenomenon to produce the desired particles (Li et al., 1988). They are based on the principle of generating a droplet(s) from a polymer extruded through a nozzle (orifice) and work using mechanical means (i.e. cutting or vibration forces) to increase the normal dripping process at the orifice, or they break up the extruded liquid stream produced by the polymer when it is passed through the nozzle. After production, the droplets are immediately solidified to spheres/capsules by either physical, e.g. cooling or heating, or chemical means, e.g. gelation. Various coating and spray-drying methods are often used in industry, whereas the extrusion of a polymer through a nozzle(s) is used mainly at a lab scale (Stark, 2001; Wyss, 2005). Some of the main mechanical technologies for fluid dispersion into droplets and subsequent conversion into capsules are: (1) coaxial air-flow; (2) electrostatic extrusion; (3) rotating disc; (4) jet-cutting; (5) spray-drying and (6) vibrating nozzle. Since all these methods are primarily based on droplet formation at an orifice, or in a liquid jet, an understanding of these processes is required to enable an adequate understanding of each system, and this phenomenon will be discussed in the following section.

Theoretical aspects behind the formation of droplets by liquid extrusion through a nozzle

The extrusion of a liquid through an orifice results in one of five different droplet formation processes occurring at the discharge point of the nozzle (Figure 2), with the active droplet mechanism being dependent on the velocity v of the extruded liquid (Muller, 1985), as well as gravitational, surface tension, impulse and frictional forces (Heinzen, 1995). At very low velocities ($v < v_I$), the extruded liquid

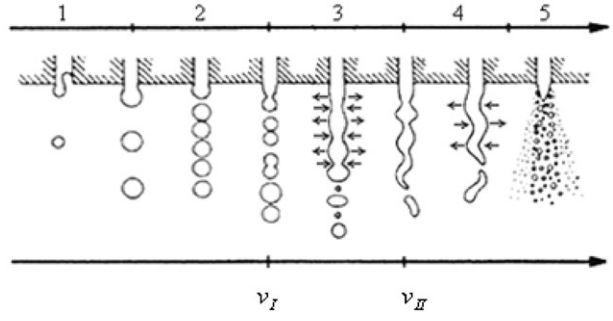

Figure 2. Different mechanisms of droplet formation as a function of jet velocities. Reproduced from Heinzen et al. (2004) with permission.

sticks to the edge of the nozzle until the gravitational force is high enough to overcome the surface tension, resulting in the release of a drop (mechanism 1). A small rise in the velocity increases the number of droplets formed, whilst further escalation amplifies droplet formation (mechanism 2), and can result in coalescence of the droplets occurring, reducing mono-dispersity. The diameter of the droplet formed (d_d) during mechanisms 1 and 2 can be estimated from Equation (1) (Poncelet et al., 1999; Stark, 2001; Heinzen et al., 2004; Xie and Wang, 2007), which approximates the balance between the two main forces present; gravitational force pulling the drop down and the force of surface tension holding the drop pendant to the tip at the instant of drop detachment (Chan et al., 2009), and is given as follows:

$$d_d = \sqrt[3]{\frac{6d_n\sigma}{g\rho}} \quad (1)$$

where d_n is the diameter of the nozzle, σ the surface tension of the extruded liquid, g the acceleration due to gravity and ρ the density of the fluid.

Mechanisms 1 and 2 are commonly used at a lab scale where only small volumes of droplets are required and this process is commonly known as dripping (Fundueanu et al., 1999; Chan et al., 2009). As seen in Equation (1), the size of the droplets produced here is mainly dependent on the nozzle diameter.

However, for systems producing alginate drops which are subsequently gelified into beads by landing in a bath of calcium chloride, it must be noted that

$$d_d \neq d_{cab} \quad (2)$$

in which d_{cab} is the diameter of the produced calcium alginate bead.

Recently, Chan et al. (2009) extended Equation (1) further by adding in an overall correction factor (K) which enables a good approximation of d_{cab} to be obtained.

$$d_d = \sqrt[3]{\frac{6d_n\sigma}{g\rho}} \cdot K \quad (3)$$

In this study (Chan et al., 2009), it was calculated that the overall correction factor varies from 0.73 to 0.85 under the conditions examined which correlated well with their experimental data, resulting in an average absolute size deviation of <5%.

Increasing the velocity further causes the formation of an uninterrupted laminar jet (continuous stream), which eventually breaks up into droplets by axial symmetrical vibrations and surface tension (mechanism 3). An additional escalation of the jet velocity ($v > v_{II}$), leads to statistical distribution of the droplet size, which is caused by either spiral symmetrical vibrations (mechanism 4) or by the high frictional forces that are present, when the jet is sprayed (mechanism 5). Whilst mechanisms 2 and 3 enable the formation of mono-dispersed droplets, only the latter is used at an industrial level, as it enables the production of sufficiently higher quantities of equal-sized droplets (subsequently produced into spheres/capsules), if a vibrational frequency is applied to the jet. This is the basis behind microcapsule production using vibrating technology and will be discussed in the following section.

Vibrating-jet (nozzle) technique

The vibrating-jet technique, which is more commonly known as the vibrating nozzle technique or prilling (Hulst et al., 1985; Stark, 2001; Del Gaudio et al., 2005), is one of the most widely used methods for the production of microspheres and microcapsules (Senuma et al., 2000). The method is based on the principle of laminar jet break-up by the application of a vibrational frequency with defined amplitude to the extruded jet. As discussed in the previous section, when a liquid is extruded through a nozzle at certain flow rates, it produces a laminar jet (mechanism 3), which can break up freely into short lengths by natural irregular disturbances (provided these perturbations reach a threshold; otherwise, little or no break-up occurs). These segments then form spherical droplets due to the force of surface tension. However, natural break-up by axial symmetrical vibrations can be irregular and is not possible to fully control, hence resulting in the formation of droplets which are not of equal size and shape (Haas, 1992).

Lord Rayleigh demonstrated that controlled break-up of laminar jets into uniform droplets of equal size can be achieved simply by applying a permanent sinusoidal force at defined frequencies to the jet, resulting in the formation of one droplet per hertz of frequency applied (Figure 3). This highly regular and reproducible break-up occurs only at vibrational frequencies that are near the natural frequency for the break-up of the jet itself (Haas, 1992). The characteristics of the drops formed are dependent on the nozzle diameter, the flow rate of the laminar jet, the size of the frequency at defined amplitude and the viscosity of the extruded liquid (Serp et al., 2000) and will be discussed in detail in the next section.

The sinusoidal force can be applied by either vibrating the nozzle (vibrating nozzle technique), pulsating the polymer in a chamber before passing through the nozzle (vibrating chamber technique), or periodic changes of the nozzle/orifice diameter during extrusion (Stark, 2001; Heinzen et al., 2004; Wyss, 2005). Whilst no set agreement exists, the authors suggest that collectively these different methods of applying the sinusoidal force to the laminar jet be termed the ''vibrating-jet techniques''. The choice of method used to administer the vibrational force is dependent on the system it is being applied to. For example, in liquid–liquid systems, it has been proven that the pulsation of the liquid is the optimal method, whilst for microsphere formation in a gas phase, all three techniques can be employed successfully (Heinzen et al., 2004).

Production of droplets by Raleigh's jet instability (theoretical aspects behind jet break-up)

At the end of the nineteenth century, Lord Raleigh analysed theoretically the aspects behind the instability of liquid jets and their ability to break up into droplets due to axial symmetric disturbances (Rayleigh, 1879). Using a linearized

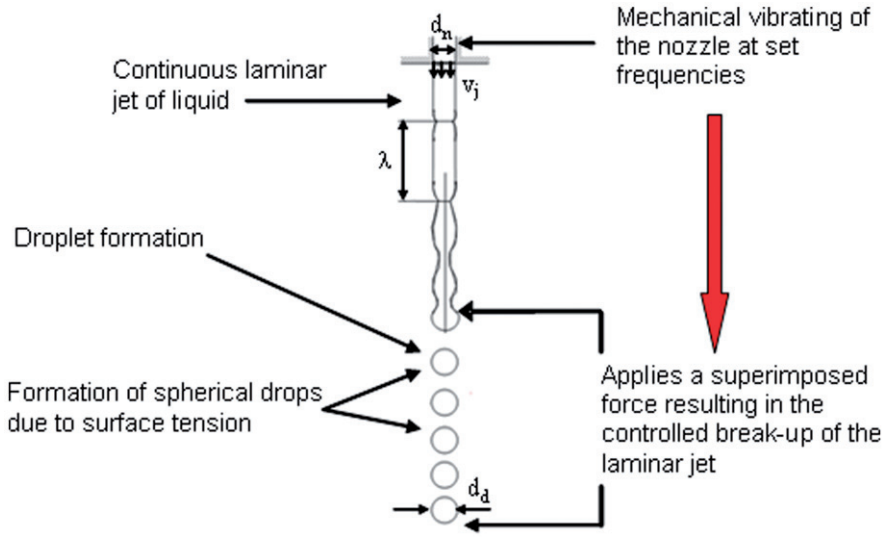

Figure 3. Controlled break-up of a laminar liquid jet into droplets of equal size. This is achieved by applying a sinusoidal force to the extruded jet, in this case by mechanically vibrating the nozzle at a set frequency with a defined amplitude. Reproduced from Heinzen et al. (2004) with permission.

stability analysis, Rayleigh showed that for wavelengths smaller than the circumference of the jet, no break-up occurred (Brandenberger et al., 1999). For longer wavelengths, the disturbances grow exponentially and cause break-up. He proposed a mathematical description (Equation (4)) for inviscid and Newtonian fluids relating the optimal wavelength of the disturbance as a function of the jet diameter.

$$\lambda_{\text{opt}} = \pi\sqrt{2} \cdot d_{\text{j}} \tag{4}$$

where λ_{opt} represents the optimum wavelength for break-up of a jet of diameter d_{j}.

The frequency is related to the wavelength and the jet velocity by the following equation:

$$\lambda = \frac{v_{\text{j}}}{f} \tag{5}$$

where v_{j} represents jet velocity and f the frequency vibration.

In 1931, Weber extended the equation further and included the effects of the physico-chemical properties of the fluid on determining the optimal wavelength for jet break-up and proposed the following equation (Weber, 1931):

$$\lambda_{\text{opt}} = \pi\sqrt{2} \cdot d_{\text{j}} \sqrt{1 + \frac{3 \cdot \eta}{\sqrt{\rho \cdot \sigma \cdot d_{\text{j}}}}} \tag{6}$$

where η is the fluid viscosity. The size determination of the jet diameter is based on different assumptions such as:

$$d_{\text{j}} = d_{\text{n}} \tag{7}$$

as proposed by Schneider and Hendricks (Schneider and Hendricks, 1964).

However, Brandenberger and Widmer (1998) showed that for nozzles using a precision-drilled sapphire stone as an orifice, the relationship between both d_{j} and d_{n} is a function of Weber's number of the nozzle (We_{n}), given by the following equation:

$$\frac{d_{\text{j}}}{d_{\text{n}}} = 4.33 \cdot We_{\text{n}}^{-0.337} \tag{8}$$

where

$$We_{\text{n}} = \frac{v_{\text{n}}^2 \cdot \rho \cdot d_{\text{n}}}{\sigma} \tag{9}$$

where v_{n} is the velocity of the liquid in the nozzle.

Since one droplet is generated by each hertz of vibration, the drop diameter, d_{d}, can be calculated by the following simple mass balance equation (Serp et al., 2000):

$$d_{\text{d}} = \sqrt[3]{6\frac{F}{\pi \cdot f}} \tag{10}$$

where F is the flow rate of the extruded liquid.

The vibrational frequency is itself linked to the wavelength by the following equation:

$$f = \frac{F}{\lambda} \cdot \frac{4}{d_{\text{j}}^2 \cdot \pi} \tag{11}$$

This implies that the droplet diameter, as a function of wavelength and the jet diameter, is given by the following equation:

$$d_{\text{d}} = \sqrt[3]{\frac{3}{2} d_{\text{j}}^2 \lambda_{\text{opt}}} \tag{12}$$

Equations (4)–(12) imply that for a given nozzle diameter, there are two main parameters to be determined to achieve optimal droplet formation: vibrational frequency and jet velocity. These two parameters trigger the production conditions and have to be optimized within a certain range (Heinzen et al., 2004). The equations also suggest that a range of frequencies exist around f_{opt}, whereby uniform-sized droplets can be obtained, and is dependent on the

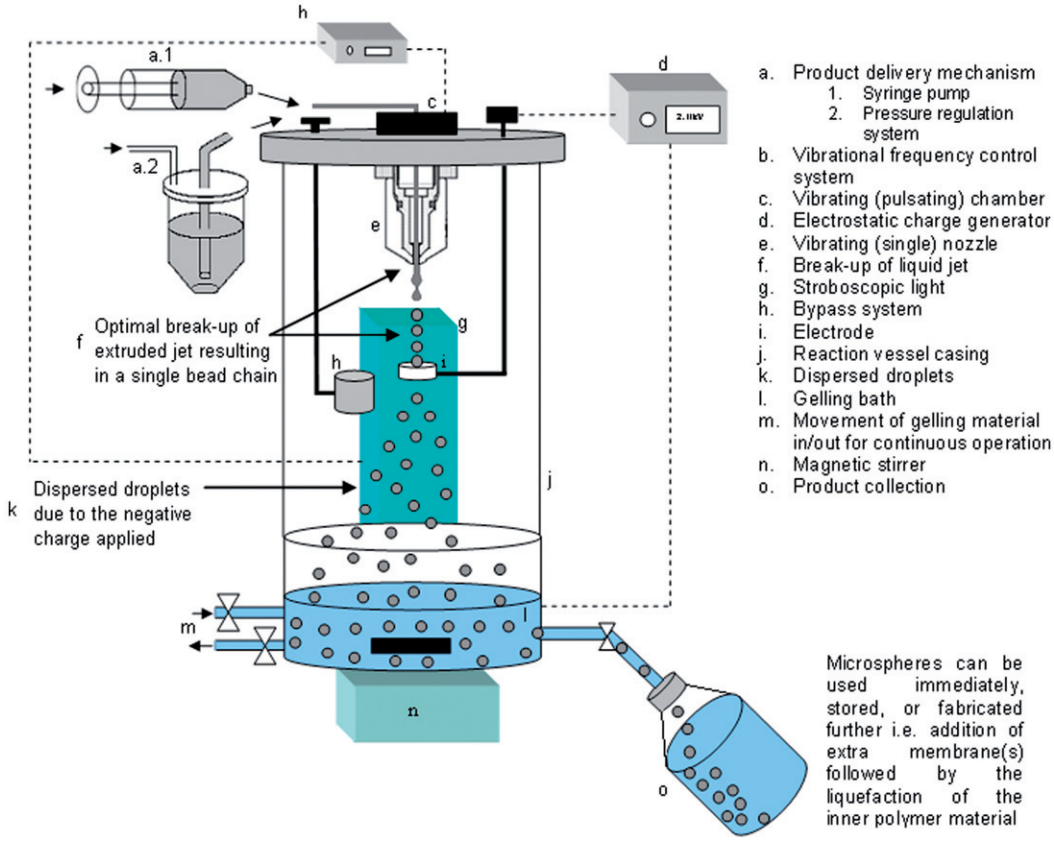

Figure 4. Schematic representation of an encapsulation device based on the principle of laminar jet break-up by vibrational frequencies. The schematic represents the Inotech encapsulator IE-50R and has a single-flow nozzle system (e) in place.

nozzle diameter, rheology of the fluid and the surface tension (Stark, 2001). Whilst this vibration system is theoretically based on liquids with Newtonian fluid dynamics, it can also be applied to non-Newtonian liquids such as alginate (Serp et al., 2000), to make uniform drops, which can be produced into microspheres by methods such as ionotropic gelation.

The equations can be used to give an approximation of the frequency and flow rates needed to break up the polymers into the desired particles (Heinzen et al., 2004). However, in most cases, the required values for a given nozzle diameter are determined experimentally using an empirical approach for each system, in which the calculated value is employed as a reference (starting) point (Serp et al., 2000; Stark, 2001; Heinzen et al., 2004).

For most polymers, it must be again noted, as in Equation (2), that the size of the droplet does not necessarily equal the size of the fabricated sphere/bead.

Production of microcapsules (type A, C and D) using the vibrating-jet technique

This section will discuss how microcapsules of types A, C and D are produced from a device based on the vibrating-jet technique, and emphasis will be based on the ability of the process to produce the required microcapsules (criteria set out previously) for medical and biotechnological processes (Overcoming the practical problems for producing the required microcapsules will be discussed later, along with possible solutions.). All experiments, unless specified, were performed on a lab-scale Encapsulator model IE-50R from Inotech Biotechnologies, Basel, Switzerland (now EncapBioSystem, Greifensee, Switzerland) using low-viscosity sodium alginate (Keltone LV and was obtained from Inotech) as the shell (polymer) material at concentrations of 2% (w/v) and a gelling (hardening) bath consisting of 3.2% (w/v) calcium chloride. Alginate stock solutions were prepared by dissolving alginate powder in 0.209% (w/v) MOPs buffer and mixing overnight. After solubilization, the alginate solution was purified by filtering through a 0.22 μm filter membrane under pressure (Whelehan et al., 2010).

All images of microcapsules were taken using a camera attached to a light microscope, interfaced to a PC, at a magnification of 40×, unless stated differently. This encapsulator is designed to work with either a monocentric (single flow) (Figure 4(e)) or concentric nozzle (two flow) (Figure 8(a) and (b)) system. The chosen nozzle system depends on the type of microcapsules required (Heinzen et al., 2004).

General components of a vibrating nozzle encapsulator

Whilst different encapsulation devices, based on vibrating technology, are available in the market, e.g. Nisco Engineering AG, Inotech Biotechnologies AG and Brace Technologies, most consist of several central elements

and can be assembled simply, at a low cost, to make a lab-scale encapsulator. These elements include:

- pump mechanisms to feed the polymer(s) and/or core material to the nozzle(s),
- nozzle(s) to create laminar liquid jet,
- vibrating device and control system using signal frequency and amplitude to enable controlled break-up of liquid jet,
- stroboscopic light to allow visualization of droplet chain and tuning of frequency,
- agitated gelling bath to enable controlled gelification/polymerization of droplets to form microspheres/microcapsules, and
- collection device to enable easy recovery of produced particles.

In order to obtain the desired capsules at the required volumes, additional parts can be added to improve the process, i.e. increase the number of nozzles, which will be discussed later on in greater detail (large-scale production/scale-up). Figure 4 shows a schematic representation of a lab-scale encapsulator with additional equipment and is based on the Inotech encapsulator IE-50R. This system can be used to directly produce type A, C and D microcapsules using a single-flow or a concentric nozzle system.

Monocentric nozzle system

The single-flow nozzle system can be used to directly produce microcapsule/microspheres of types C and D, but is mainly used to produce the latter in which cells or other solid particles are encapsulated within a polymer matrix. It consists of a single orifice (Figure 4(e)) in which the extruded polymer material passes through. The Inotech encapsulator uses a precision-drilled sapphire stone as the orifice on the tip of a stainless steel cone (Brandenberger and Widmer, 1998), although other materials have been used to create the orifice, but they usually suffer from limitations caused by imperfections in nozzle geometry (Brandenberger and Widmer, 1998). Nozzle diameters of the range 50–1000 μm are available, which enable the production of particles in the size range 100–2000 μm. The production process can be carried out under sterile and non-sterile conditions, the former being performed by the simple addition of a glass casing around the apparatus (Figure 4(j)) followed by sterilization.

Production of microbeads/microspheres using the single-flow nozzle system

The product to be encapsulated is mixed with the polymer matrix before being placed in the delivery apparatus (Figure 4(a.1) and (a.2)). A very steady flow of the polymer through the nozzle at controllable rates is required to obtain the optimal break-up of the extruded liquid jet into droplets and enable the production of mono-dispersed microspheres with a very narrow size distribution. The flow of polymer to the nozzle can be achieved by one of the two different systems, the selected one usually being dependent on the quantities required. For the first mechanism, a pulsation-free high-precision syringe pump (a.1) is used for the extrusion of volumes of range 1–60 mL of polymer through the nozzle. For the second, an air pressure regulation system (a.2) is used. In this system, compressed air is supplied to a vessel (which can be agitated if required) containing the polymer at a head pressure between 0.1 and 2 bar and the desired flow rate is set using a pressure reduction valve. This system enables very large volumes to be delivered in a single process. It also produces more uniform and steadier flow rates of the polymer solution compared to the syringe pump, allowing a narrower size distribution to be obtained for the produced particles. For the air pressure system, standard size deviations from the mean diameter of 1–1.5% have been achieved compared to 1.5–3.0% for the syringe pump system. Other pumping systems do also exist; for example, Nisco Engineering uses a Lineasepta™ pumping bag system to deliver the polymer.

The polymer, pumped through the nozzle, must be at a flow rate that is high enough to overcome the viscosity and/or the surface tension of the solution, so that it can be extruded through the nozzle to form a continuous stable laminar liquid jet (mechanism 3, theoretical aspects behind the formation of droplets by liquid extrusion through a nozzle). However, the flow rate must not be too high, which could prevent jet break-up in a controllable manner and/or could result in the formation of a spray (mechanism 5). High jet velocities will also increase the impact forces on the droplets when entering the gelification/collecting bath, resulting in their deformation (Heinzen et al., 2004; Prusse et al., 2008), whilst also increasing the occurrence of coalescence (Haas, 1992). The size of this impact force on the droplets can be limited by reducing the distance between the nozzle and the impact site or decreasing the jet velocity, but this leads to reduced production volumes. A simpler method is to add a surfactant such as Tween 80, which does not affect the sphere/capsule formation process (Whelehan et al., 2010) and can significantly reduce surface tension (Ogbonna, 2004), preventing or reducing the occurrence of deformation and/or coalescence.

The extruded liquid jet is broken up into droplets of equal size by the application of a vibrational frequency with defined amplitude to the laminar jet (Serp et al., 2000). For the Inotech encapsulator, the sinusoidal force is applied by passing the polymer through a pulsation chamber (Figure 4(c)) before reaching the nozzle. This method enables more reproducible results to be achieved compared to vibrating the nozzle itself (Vibrating nozzle experiments have been performed on an Inotech encapsulator IEM, results are not shown.). The size of the applied frequency to obtain optimal break-up of the jet can be estimated using Equations (5) and (6), for a given nozzle diameter and flow rate (Heinzen et al., 2004). The calculated frequency can be ±20–40% of the required frequency for optimal jet break-up, which is determined using an

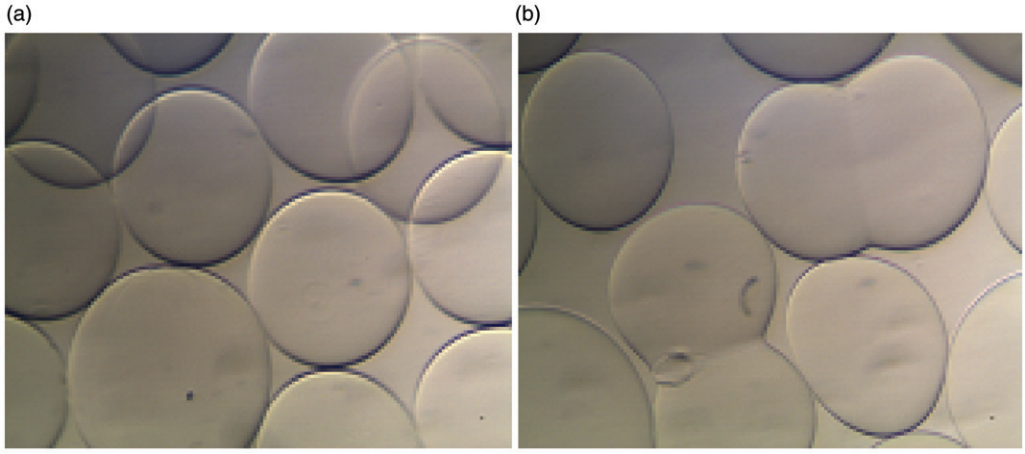

Figure 5. Light microscope images displaying droplet coalescence after jet break-up: (a) in the air, prior to reaching the hardening bath, resulting in double-volume calcium alginate microbeads and (b) during entry into the gelling bath, resulting in the formation of doublets.

empirical approach as discussed previously. The value estimated using Equations (5) and (6) acts as a starting point and as mentioned previously, a range of frequencies exist (around f_{opt}) whereby uniform-sized droplets can be produced. As seen in Equation (12), the droplet diameter formed here is mainly dependent on the jet diameter, which is directly related to nozzle diameter (Equations (8) and (9)). A general rule of thumb for producing alginate microspheres (alginate concentration 1.5–2.0%) using the Inotech encapsulators (IE-50R and IEM) is that the final bead diameter is approximately twice that of the nozzle diameter. Nevertheless, this size can be varied within a certain range by increasing/decreasing the frequency of the vibration and/or the flow rate of the polymer with higher frequencies and lower jet velocities, enabling the generation of smaller droplets (Equation (10)). However, application of higher frequencies can increase the occurrence of droplet coalescence and in the case of lower frequencies cause the formation of one or more small droplets between the main ones called satellites (Serp et al., 2000; Stark, 2001; Heinzen et al., 2004), with both affecting the overall standard size deviation. Yuen (1968) could explain the formation of these satellites by extending Rayleigh's linerized theory to a third order (Yuen, 1968).

Optimal break-up of the jet results in the formation of a single bead chain (Figure 4(f)) which can be monitored using a stroboscopic lamp (Figure 4(g)) placed directly behind the chain. The frequency of the lamp is directly synchronized to the frequency applied to the jet, which enables individual drops to be observed during break-up as they pass in front of the lamp (stroboscopic effect). The formation of droplets can be so regular that they may appear to be slowed down, completely stopped or in reverse motion whilst being viewed through the synchronized lamp (Haas, 1992). A video camera may also be installed to observe the drops and allow them to be measured.

A bypass system (Figure 4(h)) is used to prevent polymer droplets, which may not be the required size or shape, from entering the gelling bath. It also enables the retrieval of this polymer, which can be then re-used for future experiments. The apparatus consists of an electronic or manually controlled cup, which can be positioned directly underneath the nozzle when required. The bypass system is usually operated during initial priming (setting optimal flow rate and frequency) of the nozzle with the polymer solution.

To prevent coalescence of the droplets from occurring, which results in the loss of mono-dispersity and an increase in the standard size deviation (Brandenberger et al., 1999), a strong negative charge is induced onto their surface during break-up using an electrostatic voltage system (Figure 4(d)). Coalescence in the air can lead to the formation of particles with at least double the volume of other beads present (Figure 5(a)) or upon impact during entry into the hardening solution resulting in the production of irregular-shaped particles known as doublets (Figure 5(b)). The voltage system applies an electric potential between the nozzle and an electrode placed directly beneath the nozzle (Figure 4(i)). As droplets fall through the electrode, the induced charge on their surface causes them to repel one another, resulting in dispersal of the chain into a cone-like shape (Figure 4(k)) due to Coulomb forces (Brandenberger et al., 1999). Deflection of the droplets during break-up from their vertical position prevents them impacting one another in the air, and also results in entrance into the hardening solution over a larger area, hence enabling the formation of mono-dispersed, homogenous and spherical microspheres (Figure 6).

The size of the charge required for adequate separation of the jet is mainly dependent on jet velocity, droplet diameter and concentration of the polyelectrolyte used, with higher values required for larger jet velocities and low polymer concentrations. The encapsulator IE-50R is capable of applying an electrical potential of 0–2.15 kV between the nozzle and the electrode. The main advantages of the system is that it enables higher frequencies to be used to produce smaller ($\sim$100 μm) mono-dispersed beads for a given nozzle size (Brandenberger et al., 1999) as well as allowing higher jet velocities for increased production capacity. The addition of a higher potential (>2.15 kV) is

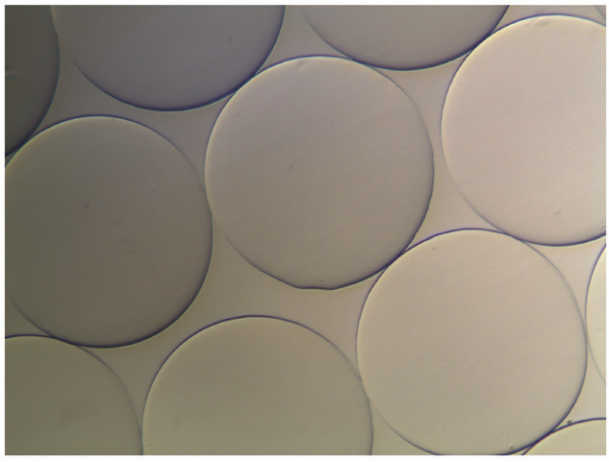

Figure 6. Light microscope image showing microspheres produced after the application of an electrostatic charge to the droplet chain to prevent coalescence. These calcium alginate microbeads were produced under the same conditions as those in Figure 5 and have a mean diameter of 763 μm ± 1.89%.

not advised and can result in unstable droplets which can deform and/or burst (Davis and Bridges, 1994; Brandenberger et al., 1999).

Upon landing in the agitated bath, the alginate droplets are hardened by ionotropic gelation to form microspheres. A very critical point for the droplet is entry into the gelling solution. When a solution with a high surface tension is used, such as calcium chloride (Ogbonna, 2004), the polymer droplets can be held back briefly at the surface during entrance, resulting in the formation of non-spherical droplets which are gelified before regaining their original shape (Figure 12(a) shows the outcome of this process for the production of type A microcapsules using the concentric nozzle system.). As mentioned previously, the problem of the surface tension can be overcome by the addition of a surfactant, which enables the formation of spherical microcapsules (Figure 12(b)).

The encapsulator may also be used for the continuous production of microspheres. In this system, high-precision peristaltic pumps are used to control the movement of fresh and utilized hardening solution to and from the reaction vessel (Figure 4(m)), as well as the removal of newly formed microspheres to a collecting device (Figure 4(o)). The pumps keep the volume of the reaction vessel at a constant level to ensure the impact height (force) for all droplets is equal. After being given sufficient time to harden, microbeads are removed and used immediately, or modified further to produce the desired particles.

Once the optimal parameters have been obtained for production, a standard operating procedure should be defined on the parameters used, with slight tuning if necessary, to enable the repeated production of the required microspheres on the encapsulator IE-50R. Provided all other parameters are kept constant (i.e. alginate concentration, impact height, etc.), the size of the alginate particles produced in each run will be in general ±2.0% of the original mean size, with a small amount of tuning of the frequency and/or flow rate enabling this to be reduced even further. Figure 7(c) displays an example of type D microcapsules produced using the vibrating-jet technique and involved the entrapment of *Streptomyces hygroscopicus* cells in a hydrogel matrix.

Production of mononuclear and multi-core microcapsules using the single-flow nozzle system

After production, type D microspheres (Figure 7(c)) can be converted into type A by the addition of an outer membrane followed by liquefaction of the now inner matrix incorporating the encapsulated product to produce type A (Breguet et al., 2007). A well-known example of this encapsulation technique is the production of alginate-poly-L-lysine microcapsules for the immobilization of mammalian cells and has been used since the 1960s (Chang, 1964; King et al., 1987; Breguet et al., 2007). Capsules produced using this approach and incorporating mammalian cells can be seen in Figure 7(a).

Type C microcapsules (Figure 7(b)) can be produced on the Inotech encapsulator IE-50R by gently mixing the polymer with a hydrophobic substance such as oleic acid. For the capsules in Figure 7(b), 1 mL of oleic acid was mixed with 49 mL of alginate (although much higher amounts of fatty acid can be used). The mixture is then produced into microcapsules using the same procedure used for the production of type D microcapsules. The main advantage of this technique is that it enables very high percentages of a product to be encapsulated (>95%). However, the main disadvantage is the difficulty in obtaining equal amounts of oil within each capsule. This predicament can be reduced by agitating the mixture at higher speeds for longer periods, followed by immediate extrusion of the liquid through the nozzle.

Figure 7(d) also shows type D macrospheres incorporating the anti-inflammatory drug diclofenac.

Concentric nozzle system

The Inotech encapsulator IE-50R employing the concentric nozzle system has a set-up that is similar to the single-flow nozzle system seen in Figure 4; the main difference involves the replacement of the single-flow nozzle with a concentric system (Figure 8(a)), which itself requires two feeds, one for the outer shell and the other for the inner core. The concentric nozzle system consists of two single nozzles termed an internal and an external nozzle, in which the inner nozzle is placed directly into the outer one (Figure 8(b)). Both can also be used on the encapsulator as a single-flow system. The encapsulant is usually in the form of a liquid but if the encapsulation of a solid is required; this can be achieved by suspending the solid in a liquid, which is then extruded through the central orifice. For the Inotech system, diameters of range 100–1000 μm are available for the outer nozzle and 50–900 μm for the

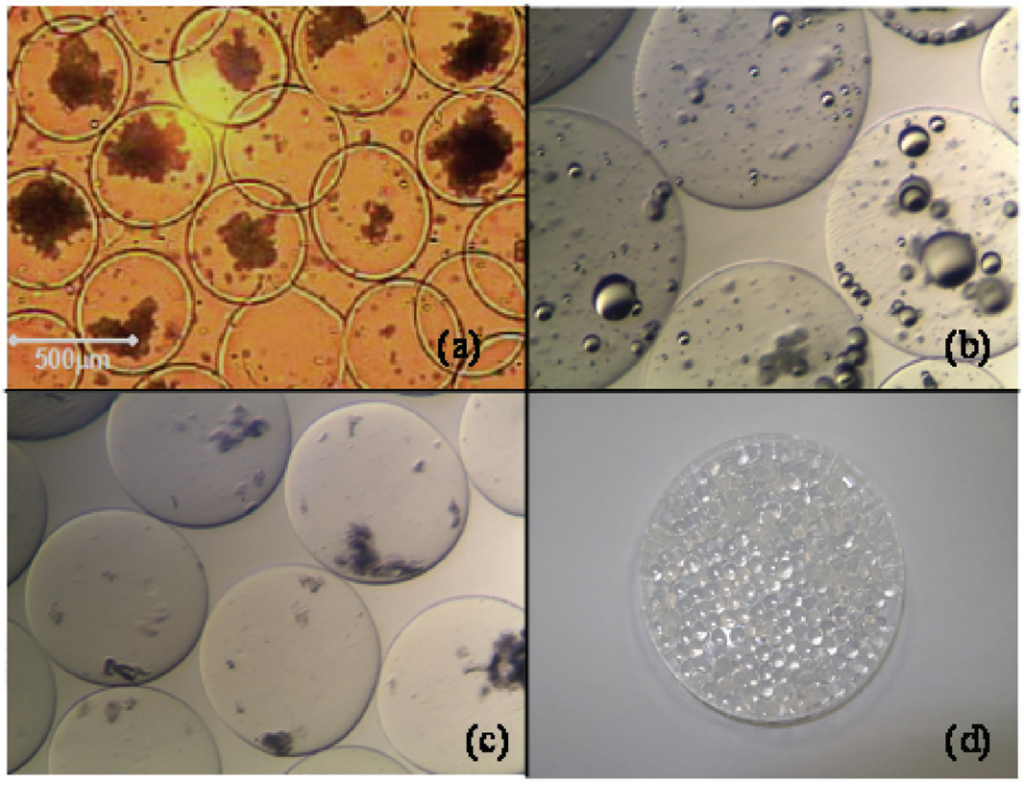

Figure 7. Light microscope images of microcapsules of types A, C and D produced using a single-flow nozzle system on an Inotech encapsulator IE-50R or directly produced from these microcapsules: (a) type A microcapsules produced from type C ones. The capsules contain CHO cells and are enveloped in a poly-L-lysine-alginate membrane and have a diameter of around 500 µm. Picture reproduced from Breguet et al. (2007) with permission; (b) type C microcapsules, with a mean diameter of 953 µm ± 1.96%, containing multi-cores of oleic acid; (c) type D microspheres containing cells of *S. hygroscopicus* var. geldanus, which can also be produced into type A if required. Displayed microspheres had a mean diameter of 681 µm ± 2.51%; and (d) type D macrospheres with a mean diameter of 2.5 mm ± 2.01%, containing the anti-inflammatory diclofenac. This picture was taken to scale using a digital camera.

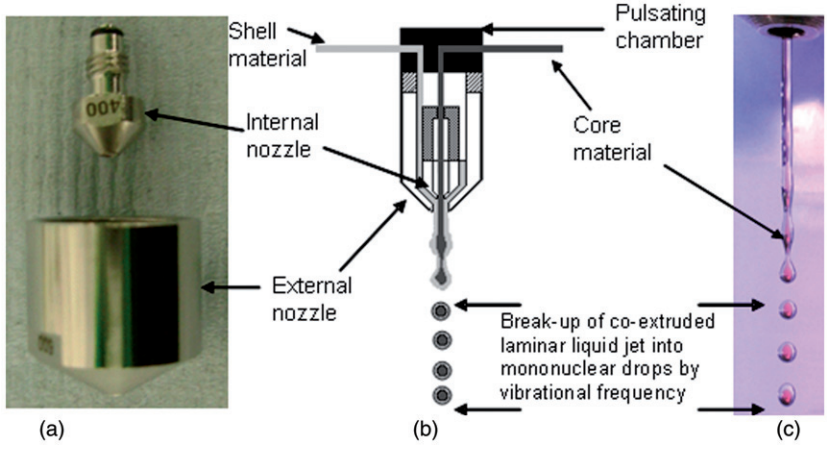

Figure 8. Images displaying the two-flow nozzle system used to directly produce type A microcapsules on the Inotech encapsulator IE-50R: (a) concentric nozzle with an internal diameter of 400 µm and an external diameter of 600 µm; (b) schematic representation of the internal structure of the two-flow nozzle system; and (c) real-time image showing the laminar jet break-up of a co-extruded jet into mononuclear droplets. Reproduced from Heinzen et al. (2004) with permission.

inner one, enabling production of capsules in the size range 200–2000 µm. Conventional wisdom tells us that the external orifice aperture must always be larger than the internal one to obtain the desired microcapsules. The external nozzle can be anywhere between 50 and 900 µm wider compared to the internal nozzle, which enables the membrane size to be controlled (Membrane size can also be determined by varying the volume of the shell/core

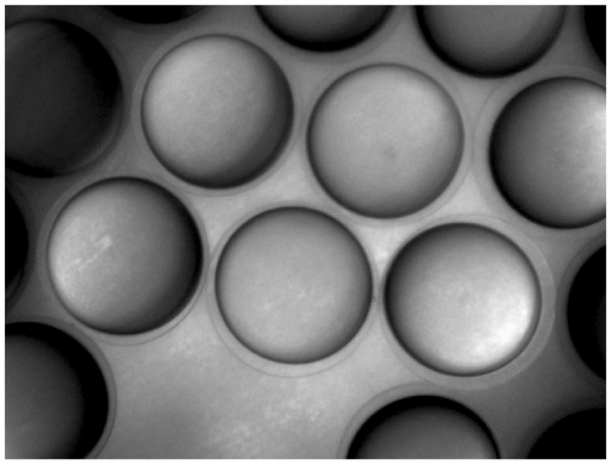

Figure 9. Light microscope image of mono-dispersed and uniform mononuclear microcapsules (type A) produced using the concentric nozzle system. The capsules displayed contain dibutyl sebacate as the liquid-core material and have a d_m of 616 μm ± 1.52%, a d_c of 551 μm ± 1.23% and a M_m of 32 μm ± 5.88%.

material and will be discussed in the next section.). Type A microcapsules can be described as having an average diameter, (d_m), which is the sum of the diameter of the core and the shell material (for microcapsules of types B, C and D, d_m is also taken as the diameter of the whole capsule) and an average internal diameter (d_c) which consists solely of the measurement of the core diameter. The average size (thickness) of the microcapsule membrane (M_m) can then be obtained from the following equation:

$$M_m = \frac{d_m - d_c}{2} \quad (13)$$

Production of mononuclear microcapsules using the two-flow nozzle system

For the concentric system, the two liquids required for production of the microcapsules can also be supplied to the nozzle using either a syringe pump or a pressure regulation system or a combination of both. The simultaneous supply of the two feeds to the concentric nozzle results in the formation of a co-extruded laminar liquid jet, which is subsequently broken up into mononuclear drops by the application of a vibrational frequency (Figure 8(b) and (c)). The produced droplets are then gelled into the desired mononuclear microcapsules. The effect of the two liquid flows on producing liquid-core microcapsules using the Inotech encapsulator IE-50R in comparison to producing microspheres will be discussed in this section.

The capsule diameter is mainly dependent on the diameter of the outer nozzle and like the single-nozzle system; the size can be varied within a certain range by increasing/decreasing the applied flow rate and vibrational frequency. The diameter of the internal nozzle and the flow rate of the material will also affect the final capsule size with increasing diameters and volumes resulting in larger core volumes, hence bigger sized capsules. A general rule of thumb for the production of type A microcapsules (using alginate at a concentration of 1.5–2.0%, for the shell material) is that the final d_m can vary between 1.5 and 2.5 times the size of the external nozzle diameter, with decreasing sizes resulting from higher frequencies and lower flow rates of the shell and core material. The diameter of d_c can vary between 1.0 and 2.5 times the diameter of the inner nozzle, with increasing flow rates resulting in larger diameters. This implies that the thickness of the membrane material can be predetermined and controlled within a certain range for a given system by varying the ratio of the flow rate (volume) of the shell material to the volume of the core material in the extruded concentric liquid jet during the production process. For example, an external nozzle with a diameter which is 100 μm greater than the internal nozzle will generally produce capsules with a membrane thickness size of around 100 μm. However, by increasing/decreasing the core/shell material volumes, the membrane thickness can be changed to greater or less than 100 μm in diameter, as seen in Figure 9. The membrane thickness can be a very important characteristic as it has the ability to significantly affect the egress/ingress of compounds from microcapsules (Wyss et al., 2004a; Whelehan et al., 2010; Whelehan and Marison, 2011).

Unlike the monocentric nozzle system, no equations presently exist to help determine close values and/or an approximation of the optimal flow rates and vibrational frequency for the system and the values must be determined using an empirical approach. Similar to the single-flow nozzle system, a range of frequencies around the f_{opt} value exist for the break-up of the jet into uniform droplets. In general, to obtain good production conditions, the flow rate of the shell material is usually at least twice that of the core liquid; however, this value can be reduced to obtain thin-membrane microcapsules as mentioned previously. Depending on the diameters of the internal and external nozzles and the flow rates of the two materials, it is possible to obtain mononuclear capsules with a membrane diameter smaller than 50 μm and greater than 500 μm.

The application of frequencies below the f_{opt} can again lead to the production of miniature-sized satellites (Figure 10(a) and (b)). For the concentric nozzle system, these structures can have the following effects on the production processes: (1) they can form smaller mononuclear capsules during gelification, which are completely separate and independent of the larger desired microcapsules (Figure 10(a)), which will significantly increase the overall standard size deviation and (2) the small independent particles may become incorporated into the membrane of the larger capsules. This may occur when small satellites collide with the larger droplets either during jet break-up and/or when entering the gelling bath. This results in the formation of a smaller independent core adjacent to the larger liquid-core and/or in some cases, it joins onto the core material itself (Figure 10(b)).

A charge can also be applied to the mononuclear droplets to enable their dispersion and prevent coalescence

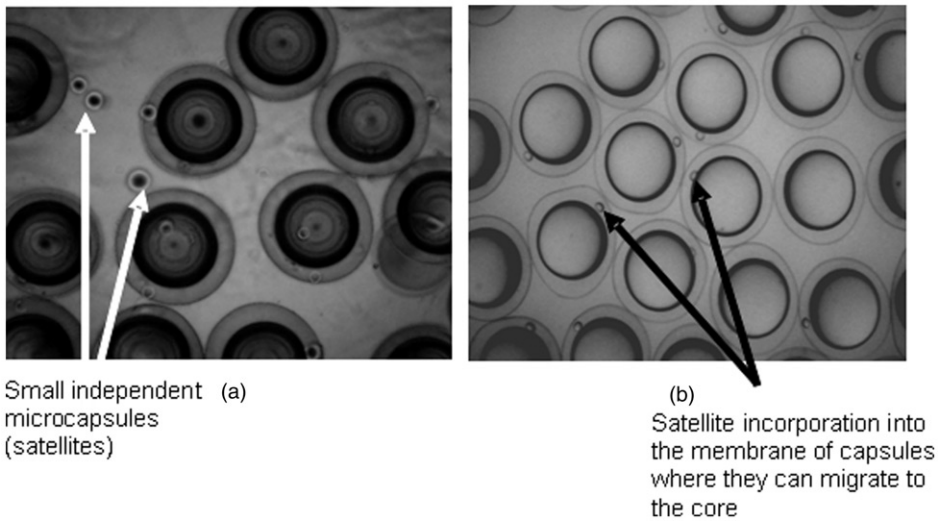

Figure 10. Light microscope images displaying the different types of satellites formed during the production of Type A microcapsules using the concentric nozzle system: (a) small independent microcapsules with a d_m of 123 μm ± 19.78% and a d_c of 85 μm ± 22.6%. The larger microcapsules have a d_m of 751 μm ± 4.29% and a d_c of 567 μm ± 2.7% and (b) satellite incorporation into the capsule membrane and/or the core material.

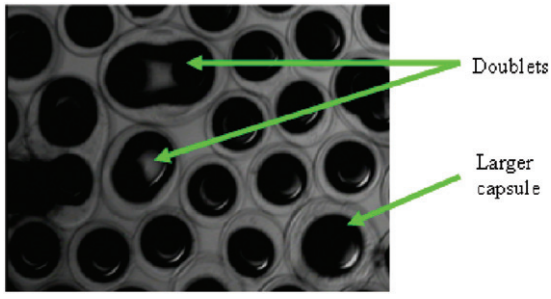

Figure 11. Light microscope image showing the effects of droplet coalescence after jet break-up and during entry into the hardening bath when using the concentric nozzle system. This can result in the formation of doublets and larger sized capsules.

occurring in the air and/or upon impact, resulting in the formation of duplets and/or larger microcapsules (Figure 11). This charge must be applied at higher values compared to the monocentric nozzle system to enable similar droplet dispersion to be achieved, due to the smaller percentage of polyelectrolyte present in the droplet because of the core material. This problem can be overcome by increasing the alginate concentration; however, as mentioned previously, this will increase viscosity, which can prevent extrusion and/or jet break-up.

Upon landing in the gelling bath, drops can be again held back momentarily due to the high surface tension, which can again result in the formation of oval-shaped capsules (Figure 12(a)). In some instances, this delay can cause the droplet to burst, releasing the core material before gelification takes place (Figure 13). It is hypothesized that this bursting is caused by the movement of the core liquid out through the pre-hardened membrane material when capsules are being held back briefly, and hence results in the release (bursting) of the core liquid. Bursting of the droplet can also be caused by high impact forces when the pre-gelified droplet hits the solution, which can again be reduced by the addition of a surfactant and/or by slightly heating (50–60°C) the gelification solution. This enables quicker entry of the drop into the solution, preventing its deformation and resulting in instantaneous wetting of the particle. Under ideal conditions a standard size deviation of <2.5% and an encapsulation yield >90% for the encapsulant can be obtained for alginate-membrane microcapsules produced by this process, and depending on several variables, 200–2000 capsules/s can be produced. Most solvents can be encapsulated using the co-extrusion laminar jet break-up technique, provided a difference exists between the viscosity/surface tension of the membrane polymer and the liquid-core material (Whelehan et al., 2010).

Challenges facing the vibrating nozzle system: possible solutions

As discussed and shown in the previous section, the jet break-up technique based on vibration technology has shown the capability to produce microcapsules with the required characteristics for application in medical and biotechnological processes. However, it is still susceptible to the following commonly known problems:

1. small production yields of microspheres/microcapsules, and
2. limited availability of polymers for producing the desired microcapsules for biotechnological and medical applications (over-emphasizes on alginate).

These issues need to be addressed and overcome to enable further development of the process, which will help facilitate its application towards a relevant industrial process. This section discusses the two main challenges facing the technology and describes possible solutions to these problems.

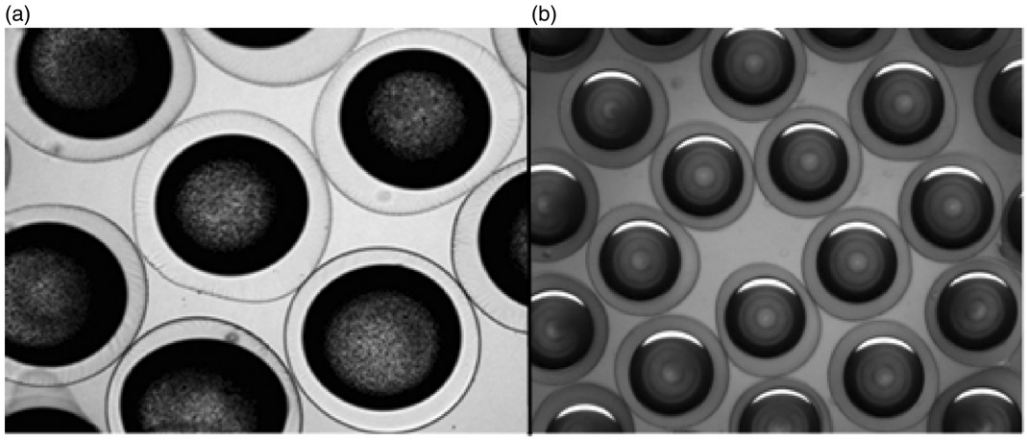

Figure 12. Light microscope image of: (a) non-spherical capsules formed due to the high surface tension of the gelling bath and (b) improved structure and spherical shape as a result of heating the hardening solution which also contained the surfactant Tween 80.

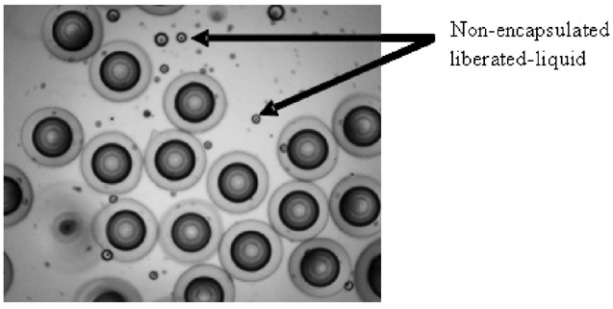

Figure 13. Light microscope image showing the release of the core liquid from type A microcapsules due to bursting of the capsules when entering (impacting) the gelling bath.

Large-scale production/scale-up

The vibrating technique using a monocentric or concentric nozzle system is usually limited to relatively small production yields of microspheres/microcapsules, as it only produces single droplets, one after another at any given time. The production flow rate is mainly dependent on the nozzle diameter with increasing diameters resulting in higher production volumes. However, even for the largest orifice diameters, very low production volumes are still achieved. For example, using the single-flow nozzle system on an Inotech encapsulator IE-50R, with an orifice diameter of 500 μm, only yields a maximum flow rate of 1.25–1.35 L/h. For the concentric system (external nozzle of 600 μm and internal of 400 μm), a maximum flow rate of only around 2.1–2.7 L/h can be achieved. It must also be noted that these flow rates do not equal production volumes, as losses will be incurred during initial priming of the system. Whilst these volumes are sufficient for lab-scale research, they fall well short of quantities (tons or cubic metres per day) required at an industrial level.

As stated by Heinzen et al. (2004), increasing the production rates of a vibrating-jet encapsulator can be obtained by simply increasing the number of nozzles on the machine. This was shown by Brandenberger and Widmer (1998), when they increased microsphere output by adding more nozzles (from 1 to 13) to the nozzle plate of their encapsulator and obtained a vibrating-jet monocentric multi-nozzle encapsulator. Figure 14 shows a schematic of an eight-nozzle encapsulator, which represents a system supplied by EncapBioSystems, and is set-up similar to that used by Brandenberger and Widmer (1998), the main difference being the presence of an electrostatic device on the EncapBioSystems model (discussed later). For this system, nozzle diameters between 50 and 1000 μm are available for monocentric systems.

The flow rate can be kept constant on the multi-nozzle encapsulator by pumping the polymer through a concentric split (Figure 14) placed before the nozzles. When using this spilt, Brandenberger and Widmer (1998) were able to obtain a relative flow difference of <2.0% between all 13 nozzles on their encapsulator. Before extrusion, the polymer solution passes through a vibrating chamber (Figure 14) which transmits a disturbance onto the solution, hence resulting in the break-up of all jets into droplets of equal size. Whilst differences in droplet size can be obtained, this can be attributed to small differences in the diameters of the nozzles (Brandenberger and Widmer, 1998). Subjecting all liquid jets with the same sinusoidal force would pose a difficult engineering challenge and this is another reason for using a pulsating chamber to exert the perturbation onto the extruded jet, compared to vibrating each nozzle individually.

Using the multi-nozzle system with a nozzle diameter of 200 μm, Brandenberger and Widmer (1998) obtained a semi-continuous production capacity of up to 5 L/h (0.385 L/h per nozzle). This low production level was achieved due to the 1 L capacity of the delivery apparatus for the polymer, but can easily be overcome by increasing the volume of the delivery mechanism using larger pressurized vessels to control the flow. Due to the stop–start nature of production, initiated by the low delivery volumes, a clean-in-place process using steam and filtered water was applied to the multi-nozzle system to prevent nozzle

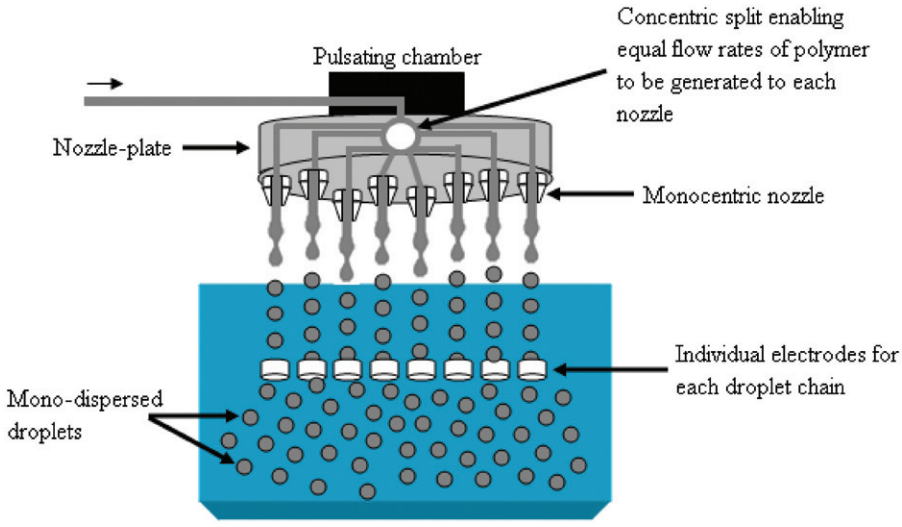

Figure 14. Monocentric multi-nozzle encapsulator representing a device supplied by EncapBioSystems, in which eight nozzles are present on the nozzle plate.

clogging by gelled alginate. The use of larger polymer volumes reduces the need for this cleaning procedure, hence reducing the number of steps, which add cost and complexity to the process.

Using Brandenberger and Widmer (1998) system as an example and the previously mentioned flow rates and nozzle diameters, it is estimated that this system incorporating a nozzle diameter of 500 μm, should be capable of reaching a production capacity of around 17 L/h for the monocentric system, and for the concentric set-up, a capacity of around 27–35 L/h could be obtained. These quantities are still below what is required for industrial applications, but as discussed previously by Heinzen et al. (2004), provided the vibrational frequency, amplitude and flow rate are kept constant across all nozzles, larger quantities can be obtained by a further increase in the number of nozzles, which should still enable the production of spherical and equal-sized capsules. At present, the authors are unaware of any published research discussing the maximum number of nozzles which can be placed on the same nozzle-plate for optimal production.

The multi-nozzle system used by Brandenberger and Widmer (1998) did not employ an electrostatic voltage system to separate the droplets during jet break-up, which is a major problem and it resulted in coalescence occurring and increased the overall standard size deviation of the microspheres produced. However, the newly developed multi-nozzle encapsulator supplied by EncapBioSystems can exert a charge to the individual chains to separate the droplets and can be applied by individual electrodes placed directly underneath each nozzle (Figure 14). Unlike other parameters, the charge affixed to each jet does not have to be the equal and needs only be applied at a minimum value, which adequately disperses the chain (results not shown).

The multi-nozzle system has also shown the capability to be operated under sterile conditions (Brandenberger and Widmer, 1998; Heinzen et al., 2004) and can be used to produce microspheres/microcapsules of types A, C and D.

It is envisaged that in the future, encapsulating devices (based on the vibrating-jet technology) applied at an industrial level could possibly contain several hundred nozzles, which would enable the required quantities of tons/day to be produced.

Polymer choice

A vast range of polymer materials are available to manufacture microspheres/microcapsules using various production techniques. These include natural materials such as proteins (whey proteins), carbohydrates (starch and maltodextrin), lipids (hydrogenated fat), acacia (Arabic) gums, cellulose and synthetic polymers (Risch and Reineccius, 1988; Gibbs et al., 1999; Madene et al., 2006). The vibrating-jet break-up technique, like a lot of other capsule production processes, has mainly focused on using alginate as the encapsulation matrix for application in biotechnological and medical domains. However, other polymers have been successfully used by this method to produce the desired capsules (Table 2).

Alginates are composed of unbranched homopolymeric regions of two sugars: D-mannuronate (denoted as M-blocks) and L-guluronate (denoted as G-blocks) separated by regions of alternating M- and G-blocks, the exception being alginates synthesized by *Pseudomonas,* which lack G-blocks (Skjakbraek et al., 1986). Their use in biotechnological and medical processes is limited on account of their naturally low mechanical stability (which will be discussed first) and also their sensitivity towards chelating compounds such as phosphate, citrate, EDTA and lactate, or other anti-gelling cations like Na^+ or Mg^{2+} (Willaert and Baron, 1996), which are commonly found in biological and bioprocessing environments (Wyss et al., 2004b). The presence of these compounds can reduce the mechanical

Table 2. Polymers used to produce microspheres/microcapsules by vibrating-jet technique and advantages/disadvantages of employing the material as an encapsulating matrix.

Polymer	Advantages	Disadvantages
Chitosan (Gugerli, 2003)	Stronger and more biocompatible in comparison to poly-L-lysine when used to make beads/capsules with alginate (Gaserod et al., 1998, 1999)	Weak mechanical properties, usually combined with other polymers to form desired capsules (Riddle and Mooney, 2004). Forms very viscous solution when used at high concentrations
Cellulose sulphate (Gugerli, 2003)	Can be used to make very strong microcapsules by a single-step process in combination with PolyDADMAC* (Pelegrin et al., 1998; Zhang et al., 2005)	Large batch-to-batch variation in product between different suppliers (due to different degrees of sulphation), which affects reproducibility. High cost to produce polymer (Yao, 2000).
Gelatin (Marison et al., 2004)	Abundant and cheap protein which can form hard or soft stable microcapsules (Gold et al., 1997)	Very viscous solution at room temperature

Note: *polydimethyldiallylammonium chloride.

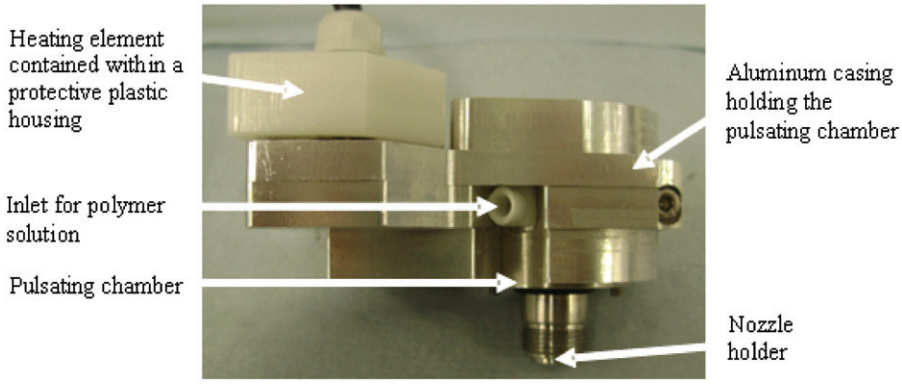

Figure 15. Image of the heating nozzle/pulsating-head device for the Inotech encapsulator IE-50R. The nozzle holder displayed belongs to a monocentric system.

stability of the hydrogel, and in some cases cause complete dissolution of the gel network structure, releasing the encapsulant itself.

As shown in numerous studies, increased alginate concentrations can result in microspheres with considerably improved mechanical properties. However, exponential increases in viscosity are obtained with increasing concentrations of alginate (Del Gaudio et al., 2005). In some cases, depending on the source and purification procedure used, doubling the concentration will cause a 10-fold increase in the viscosity of the alginate solution (Stark, 2001; Del Gaudio et al., 2005). High viscosities can prevent extrusion of the alginate through the nozzle, especially when syringe pumps are used, on account of their limited power. This problem can be resolved by forcing the solution under high pressure through the nozzle, but the high jet velocities and/or viscosities can again prevent the break-up of the jet (Wyss et al., 2004b). Using the Inotech encapsulator, it was possible to extrude and break up alginate solutions between concentrations of 2.5% and 3% (w/v). However, concentrations $\geq$3% (w/v) could not be extruded and/or caused unstable jet break-up, leading to the production of hetero-disperse beads (Breguet et al., 2005). These results are somewhat in contradiction to results obtained by Prusse et al. (2008), in which it was observed that microspheres could not be produced at alginate concentrations, in some situations, at and above 2% (w/v). This study also claimed then when production did occur at 2%, large standard size deviations were obtained and the spherical structure of the particles was compromised. This is again in contradiction to results obtained by our research group and is probably due to the use of different alginate solutions having higher viscosity/molecular weight range.

This problem of only being able to use solutions containing low concentrations of alginate, which enables the production of particles with limited mechanical strength, can be resolved by reducing the viscosity of the solution. This can be achieved by increasing its temperature during extrusion through the nozzle(s). Recently, an apparatus has been developed (by EncapBioSystems), for heating and/or maintaining polymer solutions at controllable temperatures as they pass through the pulsation chamber, before extrusion and break-up. This apparatus, termed a heating nozzle/pulsating-head device (Figure 15), consists of a temperature-controlled unit connected to a heating element, placed adjacent to a circular aluminium casing, which conducts the heat from the element itself. The pulsating chamber (monocentric/concentric) with the nozzle holder attached sits within the heating device and is

completely enveloped. The casing can be fastened tightly to the nozzle housing to improve heat transfer and enables a more precise control of temperature. The heating apparatus can supply a controllable temperature of up to $60^{\circ}C \pm 1^{\circ}C$ to the chamber, which subsequently heats and/or maintains the temperature of the polymer during extrusion and jet break-up. The shell and/or core liquid can be heated in their reservoirs to the desired temperature using standard water baths before being pumped along insulted tubing to the pulsating chamber. At present, only preliminary experiments have been preformed in regard to using this device and as of yet, no concrete conclusions can be made. However, initial experiments have been performed and have shown the device to enable concentrations of alginate of $\geq 4\%$ (w/v) to be successfully produced into microspheres which showed considerable improvements in mechanical strength compared to lower concentrations of alginates.

Whilst increasing alginate concentration can significantly improve mechanical strength, it does not, however, overcome its sensitivity towards chelating agents and other anti-gelling cations. In response to this predicament, numerous attempts have been made to help stabilize alginate gels using various hardening techniques. These include complexation with ions other than calcium, i.e. barium (Quintana and Dalton, 1999) or additional complexation with other polymers (Bartkowiak and Hunkeler, 1999). Other methods include covalent cross-linking of alginate and alginate–polyethyleneimine mixtures with glutaraldehyde (Bahulekar et al., 1991; Yeom and Lee, 1998), acrylamide (Wyss et al., 2004b) and epichlorohydrin (Moe et al., 1993). Glycol alginate has itself been cross-linked with proteins (Levy and EdwardsLevy, 1996). Whilst the stability and strength of the gel structure improved significantly in some instances, the gels did, however, still show sensitivity towards chelating agents, or in some cases, the hardening step was not suitable for the encapsulated particles (i.e. cells), or the cross-linking component was itself affected when applied to the new environment, i.e. hydrolytic enzymes (Levy and EdwardsLevy, 1996). Long-term stability is still a major problem and must be overcome to enable use of alginate for encapsulation of artificial organs and for incorporation in biotechnological processes (Thu et al., 1996).

Another sizable problem with using alginate relates to structural differences. The overall composition and the sequence of the M- and G-regions of the polymer (Figure 14) can vary extensively between different batches of alginate and is mainly dependent on the species of origin, location of alginate in the plant and the time of year in which harvesting was performed. This variable composition has many negative affects on reproducibility of experiments (especially for cell encapsulation) and is still one of the limiting factors for large-scale use of alginate at an industrial level (Orive et al., 2004). However, certain bacterial strains do produce alginate homogenously. Bacterial alginate fermentations mainly use *Azotobacter vinelandii* as the producing organism but are not usually available commercially due to low production volumes (around 4 g/l), which make the product commercially non-viable (Rehm and Valla, 1997; Remminghorst and Rehm, 2006). Nonetheless, research has been undertaken to control and increase the production of alginate by certain bacteria (Rehm and Valla, 1997). Provided high enough concentrations can be manufactured, this type of research could enable the continuous production of alginates with a constant structure, which could potentially solve the consistency problems plaguing applicability in biotechnological and medical applications.

At present, very few polymers seem to be available to make the desired capsules according to the criteria previously mentioned, when the technique described in this study/review is employed, and some of these polymers are highlighted in Table 2. However, the heating device previously mentioned has the potential to increase this number of polymers by enabling very viscous polymers and gums, which only form liquids above room temperature to be extruded (i.e. gelatin) to produce microspheres/microcapsules. However, subjecting the polymer to high temperatures (up to $60^{\circ}C$) has the potential to adversely affect the encapsulant which could be counterproductive to the processes. This certainly would be the case for the encapsulation of most animal cell lines, probiotic bacteria as well as the entrapment of heat-sensitive pharmaceuticals. Most aroma compounds are themselves very volatile and the process could result in evaporation of large amounts during the production process.

Whilst alginate has proven to be a very versatile and interesting compound to work with and has provided the encapsulation community with a vast amount of interesting results and ideas for nearly 50 years, the authors feel that in the coming decades, for encapsulation technology to reach its full potential in biotechnological and medical processes, a shift from the extensive research on alginate is required towards other polymers.

Declaration of interest

To their knowledge, the authors have no conflict of interest with any statements/points made in this review.

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