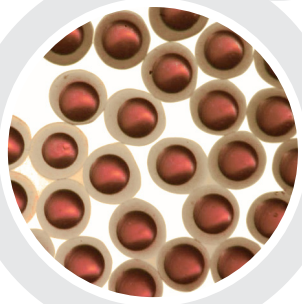
Encapsulator B-390/B-395 Pro
Laboratory Guide



Contents

Part A: Encapsulation Prilling by Vibration

1 Introduction

This laboratory guide gives an overview of encapsulation using the Prilling by Vibration technique and how it can be performed using the BUCHI Encapsulators B-390 and B-395 Pro.

1.1 What is Encapsulation?

Encapsulation can be defined as a process which involves the complete envelopment of pre-selected materials (solid, liquid and gases) within a porous or impermeable matrix and/or membrane using various techniques, to give miniature sized particles ranging from a few hundred nano-meters up to a several millimeters in size [1]. In general particles < 1 µm in size are called nano, 1-2000 µm are termed micro and > 2000 µm are referred to as macro – beads/capsules. Structurally the produced particles can be classified as either beads or capsules (also referred to as core-shell capsule) [2].

Beads: Spherical particles having the encapsulated material distributed throughout matrix structure i.e. has no distinctive core and shell part [Fig. 1(a)].

Capsules: Spherical particles consisting of a defined core (containing the encapsulated material) completely surrounded by a shell (membrane) structure [Fig 1(b)].

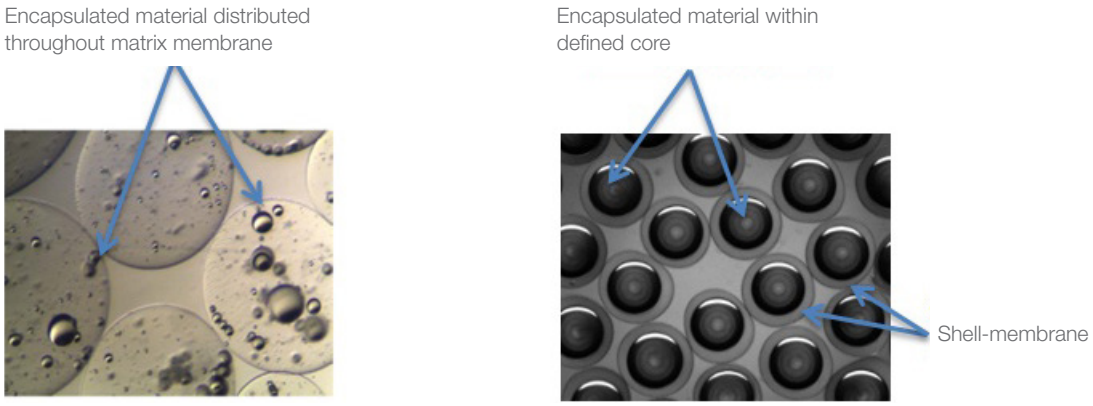


Figure 1: Images displaying the structural differences between (a) beads and (b) core-shell capsules.

1.2 Reasons for encapsulating a material

There are six main benefits for encapsulating a material [1] and are summarized in Figure 2:

· Protection (stabilization) of the encapsulated material from environmental conditions or interactions such as heat, moisture, oxygen etc. and used in food, biotechnology, pharmaceutical and cosmetic industries.

· Controlled or sustained release of the encapsulated material. This type of encapsulation has found a myriad of applications in industries such as food, pharmaceuticals, agriculture, textiles and cosmetics.

· Targeting to specific sites which enables the encapsulated material to be delivered directly to the site where it is required. This has found applications in pharmaceutical, medical and biotechnology sectors.

· To enable the encapsulated material to act as extraction aides for product removal. This is a relatively new application and has found application in agriculture, environmental and biotechnological fields.

· Improved flow properties of the encapsulated material, by converting a liquid into a solid particle which improves handling, usage and storage. Encapsulation also prevents direct contact between the encapsulated material and the handler, hence enabling safer handling of the encapsulated toxic materials.

· Improved organoleptic properties which is a very common reason for using the technology and has being used extensively in the food industry. Encapsulating a material can help mask its unpleasant taste and/or smell, improve its visual appearance as well as its texture.

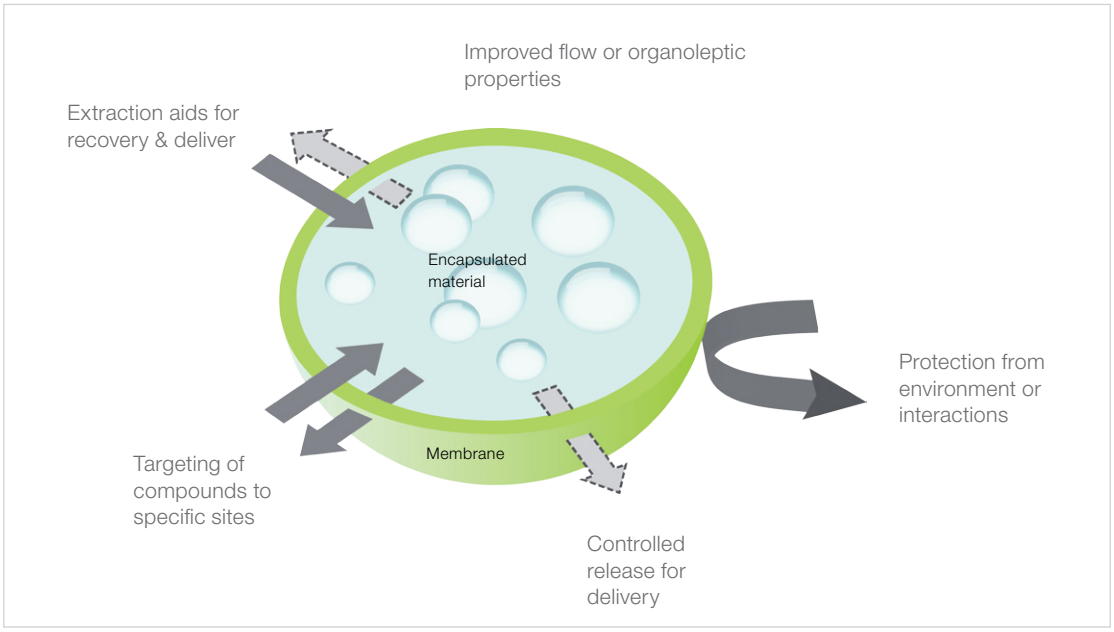


Figure 2: Schematic displaying the main reasons why encapsulation (of a product) takes place.

1.3 BUCHI Encapsulators B-390 and B-395 Pro

The BUCHI Encapsulators B-390 and B-395 Pro (Fig. 3 & 4) are the leading systems for the controlled encapsulation of many different active and functional materials to form beads/capsules for innovative lab-scale R&D work, and are the successor models to the Inotech and EncapBioSystem Encapsulator systems.

These highly innovative and flexible machines enable the concept of encapsulation to be applied to many different segments including; pharmaceuticals, food, feed, cosmetics, agriculture, environmental and biotechnological disciplines [1].

Both the B-390 and B-395 Pro Encapsulators use the same unique operation to produce the required particles with the desired characteristics. The easy-to-use technology works on the principle of laminar jet break up of a liquid stream into equally sized beads/capsules by applying a controlled vibrational frequency to the liquid. In the past this process has being termed vibrating nozzle technique [3, 4] or simply called vibrating technique [1]. However these terms are too ambiguous and do not describe the production process accurately as performed on the BUCHI Encapsulators. The production technique will now be termed “Prilling by Vibration”. Prilling refers to the breaking up of liquid jets into small prills (droplets) which is achieved on the BUCHI Encapsulator by using vibrational frequencies. The easy to adjust production conditions, allow the production of a wide range of pre-selectable particle sizes (80 µm – 4 mm) due to the availability of many different sized nozzles. The reliable and gentle technology also enables the reproducible production of mono-dispersed and homogenous particles, which have a very narrow size distribution (< 5% standard deviation from the average mean size for alginate solutions) using a simple, single-step process, which is also scalable. For these reasons it is one of the most commonly employed techniques to produce beads and capsules at a lab-scale level and is used for the encapsulation of enzymes, drugs, chemicals, flavors & fragrances, vitamins, oils, foods & bioactives, cells or microbes into a wide range of different materials [5].
For a detailed description of the structural and operational differences between both BUCHI Encapsulators B-390 and B-395 Pro see section 4.

Figure 3: The standard set up of the BUCHI Encapsulator model B-390.

Figure 4: The standard set up of the BUCHI Encapsulator model B-395 Pro. The presence of the reaction vessel enables the operator to perform the encapsulation process under fully sterile conditions.

1.4 Applications for Prilling by Vibration Technology

The Prilling by Vibration technique has being used for over 2 decades by scientists to develop new innovative products. The table below highlights some of this work performed on the BUCHI Encapsulators* and also explains the benefits of encapsulating a selected material for application in a particular segment.

Industries	Encapsulated material	Benefit (application)	Ref.
Food & Beverage, Feed	Sunflower oil	Control bioavailability of lipids in food	5
	Folic acid	Improve stability during freeze drying & storage	6
	Probiotics (*Lactobacillus acidophilus*)	Protection of bacteria in gastric conditions	7
	Probiotics (*Lactobacillus fermentum*)	Oral and controlled delivery	8
	Probiotics (*Lactobacillus casei*)	Controlled release (Gastrointesinal (GI) Tract of pigs)	9
	Flavourzyme	Encapsulation of enzyme to improve acceleration of cheese ripening	10
	Avocado oil	Improve storage stability	11
	Olive oil	Improve storage stability	12
	Canola oil	Improve storage stability	13
	Essential oils	Improve storage stability (prevent evaporation)	14
	Iron	Controlled release in the GI Tract	15
	Carvacrol (essential oil)	Controlled delivery (GI Tract of pigs)	16
Pharma	Celecoxib	Controlled release	17
	Furosemide	Enhanced solubility & permeability	18
	Thalidomide	Controlled delivery (Crohn's disease)	19
	Methotrexate	Controlled release	20
	Salicylic acid, propranolol & insulin growth factor I	Controlled release	21
Bio-Pharma	Bacteriophage (Felix O1)	Oral delivery	22
	Sperm (bovine)	Storage and controlled release (artificial insemination)	23
	Vaccine (*Brucella*)	Controlled release	24
	Vaccine (*B. melitensis* vjbR::Tn5 mutant)	Controlled release (treatment of Brucellosis)	25
	Stem cells (human adipose)	Transplantation in vivo for production of growth factors	26
	Mesenchymal stem cells (Whartons Jelly)	In vivo applications	27
	Carbon nanotubes	Controlled delivery	28
	Therapeutic proteins	Targeted and controlled delivery	29

*The BUCHI Encapsulator technology is the successor model to the Inotech and EncapBioSystems devices.

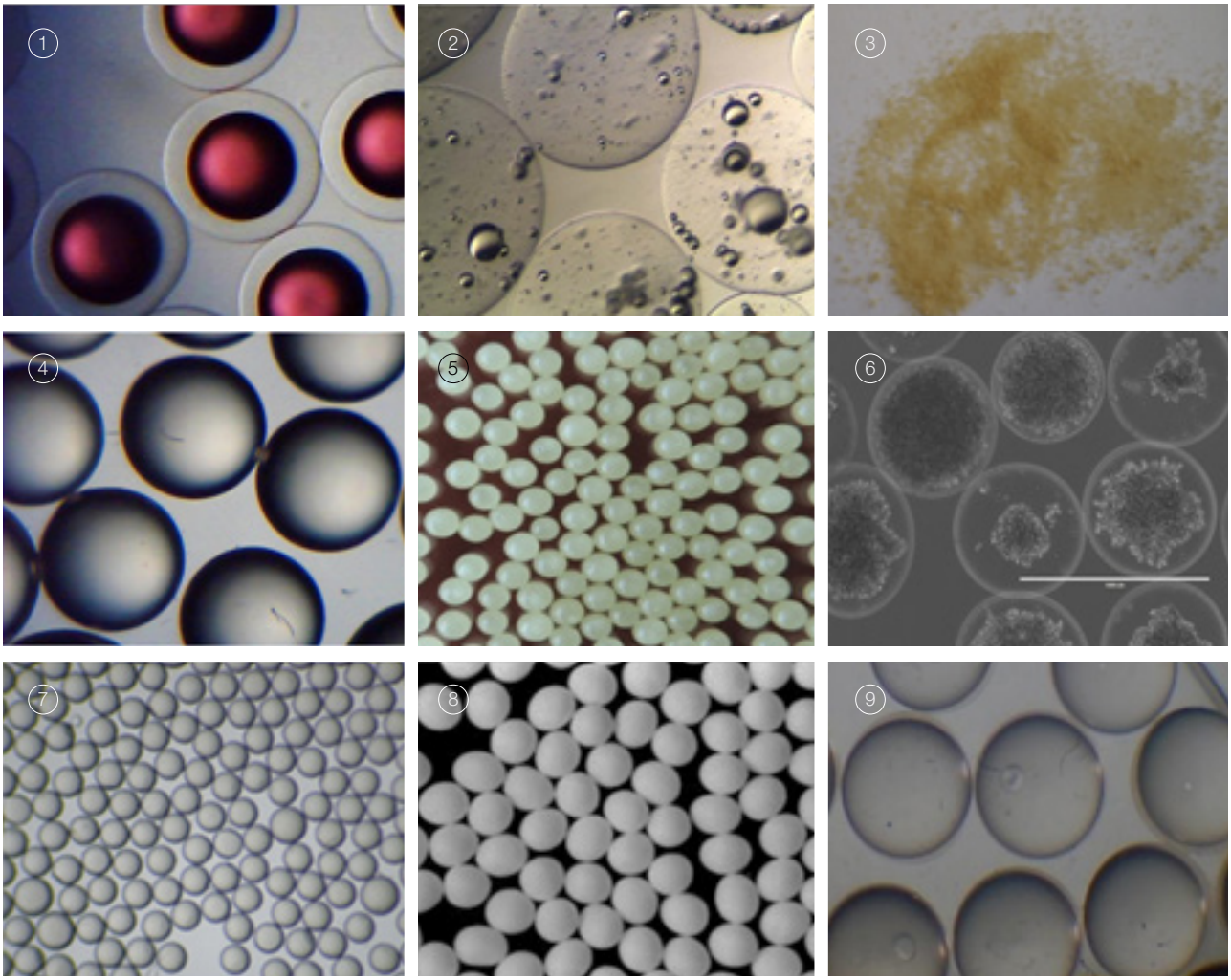


Figure 5: The many different types of beads and capsules which can be produced by the BUCHI Encapsulators and can be used for numerous applications in different industries (see application table).

1. Capsules with a core of sunflower oil (with a red dye) and an alginate shell.
2. Beads containing sunflower oil.
3. Dried alginate beads containing yeast.
4. Wet gelatin beads containing vitamin C.
5. Dried gelatin beads.
6. Encapsulated CHO cells in alginate-PLL-alginate microcapsules.
7. PLGA beads encapsulating Ibuprofen.
8. Wax-based beads
9. Core-shell capsules containing olive oil.

2 Operation of the BUCHI Encapsulators: Prilling by Vibration technique

2.1 Operational principle of the BUCHI Encapsulators

The operation of both BUCHI Encapsulators B-390 and B-395 Pro is based on the principle of laminar liquid jet breakup into droplets by applying a superimposed mechanical vibration onto it (Fig. 6 & 7). The extrusion of a polymer liquid (containing the material to be encapsulated) through a selected nozzle on a BUCHI Encapsulator results in the formation of a laminar flow liquid jet. A controlled, superimposed vibrational frequency at defined amplitude is then imposed onto this jet and causes the jet to break-up into small uniform droplets of equal size, with one droplet formed per hertz of frequency applied [1, 3, 4]. For the BUCHI Encapsulator this force is applied by vibrating the polymer liquid in a chamber (bead producing unit) before it is extruded through the nozzle (Fig. 6). After formation the produced droplets are then converted subsequently into beads or capsules.

To prevent coalescence of the droplets during jet break-up and/or when entering the gelling bath, an electrical charge is induced onto the surface of the droplets using an electrostatic voltage system. This system applies an electrical potential between the nozzle and an electrode, placed directly underneath the nozzle [1, 3, 31]. As droplets fall through the electrode, they are charged and deflected from their vertical position resulting in their impact occurring over a larger area in the hardening solution. This enables mono-disperse beads and capsules with a standard size deviation of ≤ 5% to be produced when using alginate solutions.

The size of the produced droplets and the rate of production are mainly dependent on the nozzle size, the flow rate and viscosity of the extruded liquid, and the vibrational frequency applied. These parameters can all be controlled when using the BUCHI Encapsulators, enabling the operator to pre-determine the size and characteristics of the beads and capsules that are produced. For a detailed description on how each variable parameter effects bead and capsule production see Section 3. For more details on the theoretical aspects behind laminar jet break up (Rayleigh's jet instability) using vibrational frequencies see [1, 3].

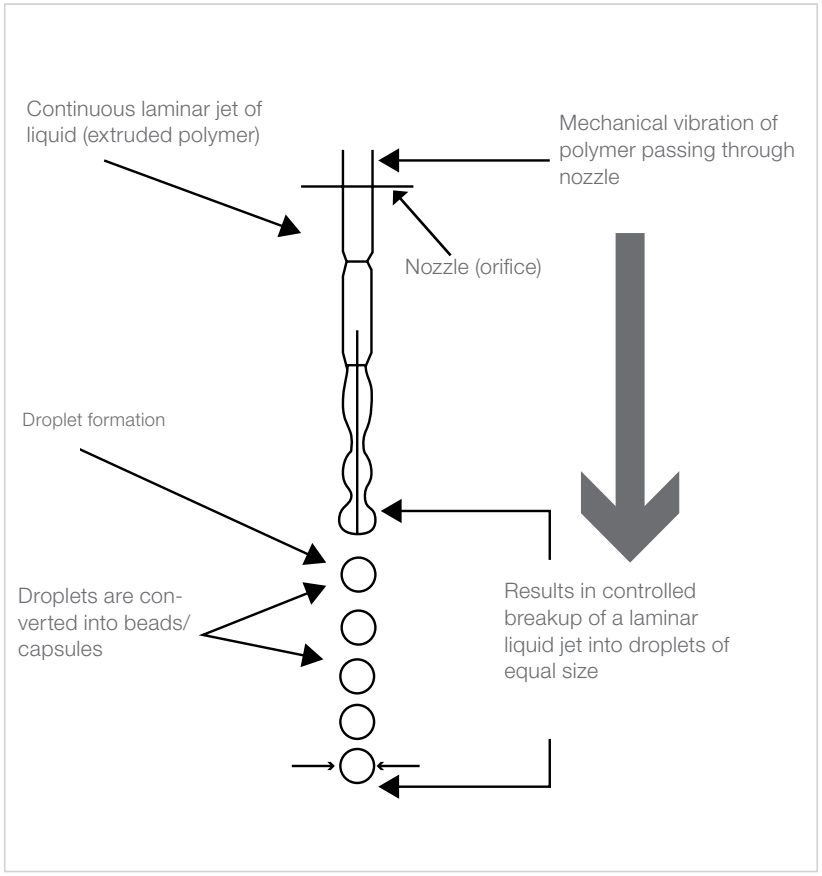


Figure 6: Schematic displaying the operational principle of the BUCHI Encapsulators which uses vibrational frequencies for the controlled breakup of a laminar liquid jet into equally sized droplets [1].

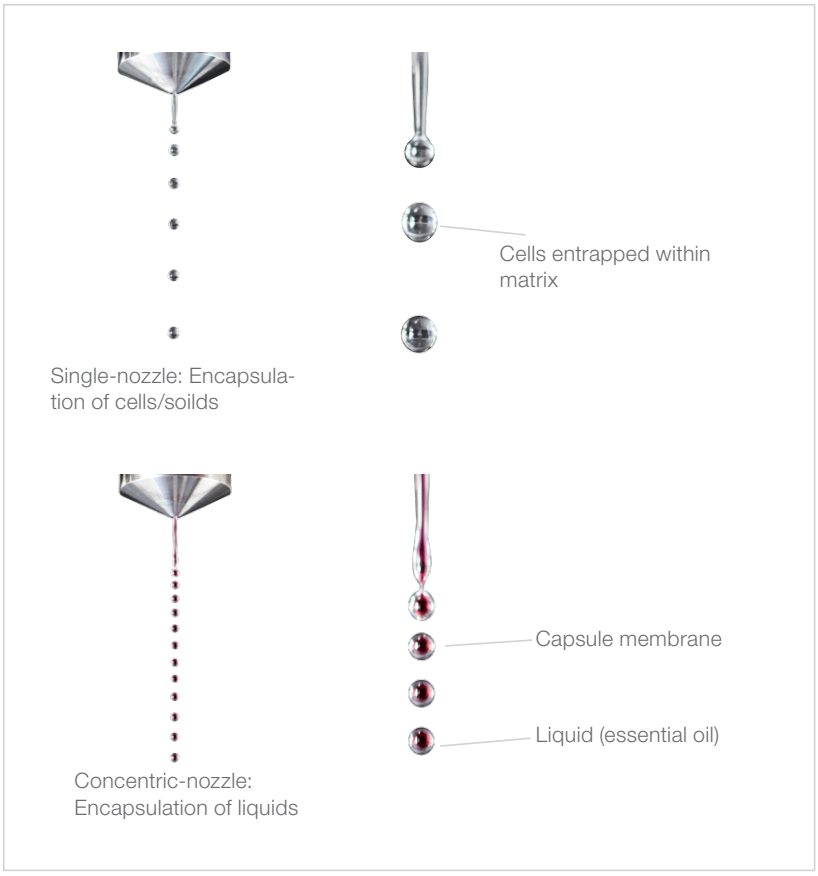


Figure 7: Real-time image of droplets being produced on the BUCHI Encapsulator using prilling by vibration technology. The produced droplets are converted into beads/capsules using different hardening techniques.

2.3 Nozzle configurations

Both BUCHI Encapsulators B-390 and B-395 Pro can work with either one of four different nozzle configurations. The selected nozzle system depends on the type of beads and capsules and the size that are required, as well as the material being encapsulated.

- Single nozzle system: Production of beads with a size of 150 µm – 4 mm.
- Concentric nozzle system: Production of core-shell capsules in a single-step process.
- Flow vibration nozzle: Production of smaller beads (80 µm) from viscous solution.
- Air Dripping nozzle: Encapsulating cell clusters and islets in beads.

2.4 Production of beads – Single Nozzle

The single nozzle configuration is the most commonly employed nozzle system, and is mainly used to produce beads encapsulating hydrophobic liquid(s), solid material(s) and cells, which are encapsulated within a polymer or synthetic matrix. This nozzle system is also employed to produce beads encapsulating no material and these particles can be used as immobilization matrices. The system consists of the bead producing unit incorporating a single removable nozzle (Fig. 8). For the BUCHI Encapsulator 8 different single nozzle sizes are available (diameters of 80, 120, 150, 200, 300, 450, 750 & 1000 µm), which enables the production of beads from 150 µm up to 4 mm in diameter. For applications of the single nozzle system see application notes 9.1, 9.3, 9.4, 9.5, 9.8 and 9.9.

The operation of a BUCHI Encapsulator with a single nozzle system in place (Fig. 8) to produce beads is explained in the following procedure (steps 1-8).

Step 1: The product to be encapsulated is mixed with the polymer material before being placed into the polymer delivery (pumping) mechanism. One of two different pumping systems can be used; either a syringe pump[I] (delivers between 1-60 mL of polymer), or an air pressure regulation system[II] (delivers up to 1 liter).

Step 2: The mixture is then pumped though the nozzle to form a stable jet of liquid.

Step 3: The frequency generator superimposes a vibrational frequency onto the liquid jet.

Step 4: The application of the frequency results in the liquid jet breaking up into equal sized droplets, which are formed one after the other and this is called a droplet chain.

I A syringe pump is not supplied with the Encapsulator B-390 as standard, however an external syringe pump can be purchased from BUCHI or other suppliers – see section 4.3 for more details.
II Present on both Encapsulators B-390 and B-395 Pro.

Step 5: The electrostatic voltage system is turned on and induces an electrical charge onto the surface of each droplet, causing them to repel each other and disperses them into a cone like shape. This prevents coalescence of droplets from occurring, hence enabling mono-dispersed of equal size and shape to be produced.

Step 6: The liquid jet break up process to form droplets can be viewed and monitored in the light of a stroboscopic lamp, which is placed directly behind the droplets. This enables optimal droplet formation to be obtained before landing in the gelling bath.

Step 7: Upon landing in the agitated hardening bath the droplets are converted into beads either by gelation or a polymerization reaction. The encapsulated material is entrapped within the matrix structure of the beads.

Step 8: After production the produced particles can be removed and used as required or pre-treated further.

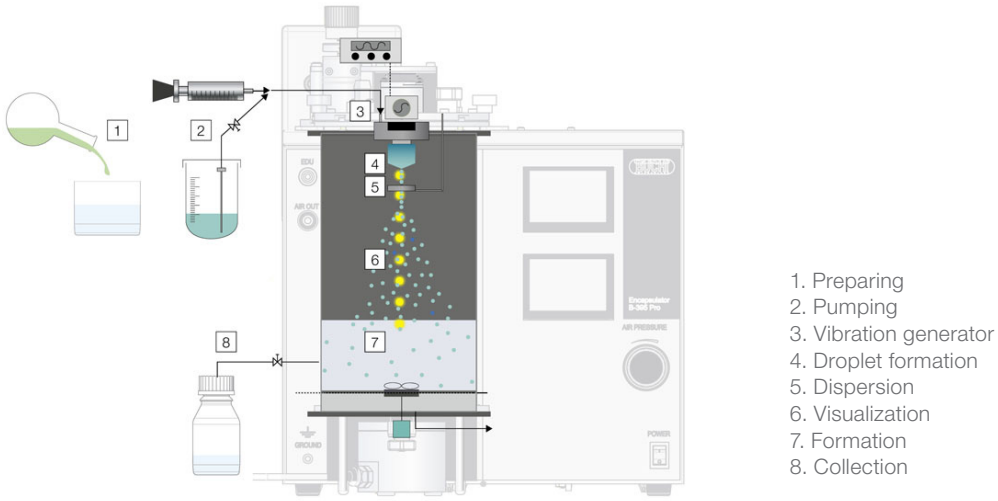


Figure 8: Schematic displaying the operation of the BUCHI Encapsulators B-390/B-395 Pro when using a single nozzle system to produce beads. The numbers 1-8 display the location where each step of the production procedure takes place.

2.5 Production of capsules – Concentric Nozzle

The concentric nozzle is a standard nozzle configuration to produce core-shell capsules in a single step process in the laboratory. The system consists of the concentric nozzle producing unit which holds two nozzles, one termed an inner or core nozzle (one of the nozzles from the single nozzle system), and the second termed an external or shell nozzle (Fig. 9 a). For production of capsules the inner nozzle is placed directly into the shell nozzle and the two nozzles combined are re-

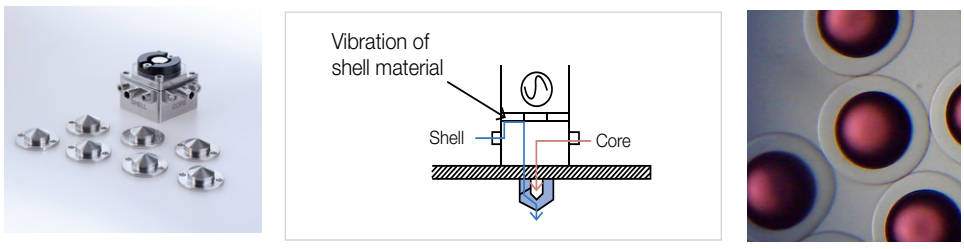
ferred to as the concentric nozzle (Fig. 9b). Seven different diameters for the shells nozzle are available (200, 300, 400, 500, 600, 700 and 900 µm), which enables the production of core-shell microcapsules sizes from 400 µm up to 2.2 mm in diameter. When using the concentric nozzle system it is not only possible to pre-determine the capsule size, but it is also feasible to pre-determine the shell thickness and core diameter. The material to be encapsulated can be in the form of a liquid (core liquid), however if encapsulation of a solid is required this can be achieved by suspending it in the core liquid.

The BUCHI Encapsulator using the concentric nozzle system has a set up that is similar to the single nozzle system as seen in Fig. 8; the main difference being the replacement of the single nozzle system with the concentric one, with the latter nozzle requiring two liquid feeds, one for the outer nozzle (the shell material) and one for the inner nozzle (the core material).
Core-shell capsules are produced on the BUCHI Encapsulators using a similar procedure for producing the beads (Fig. 8 steps 1 - 8), however there are changes to steps 1-2 and they are as follows:

Step 1 (for concentric nozzle): The core and shell material are placed into their respective delivery (pumping) mechanisms. For the Encapsulator B-390 model the air pressure regulation systems is used to deliver both the core and the shell material to the nozzle. However an external syringe pump(s) can also be used to deliver the core and/or shell material. For the Encapsulator B-395 Pro model a syringe pump (mounted onto the Encapsulator) and the air pressure regulation system are available as liquid delivery mechanisms. When operating the Encapsulator B-395 Pro, the concentric nozzle system is set up in such a way that the syringe pump is used to directly supply the core material, and the air pressure system is used to supply the shell material system (See section 5.6 Operation Manual Encapsulator B-395 Pro).

Step 2: The core and shell liquids are pumped though their respective nozzles at the set flow rates and meet at the tip to form a stable co-extruded concentric liquid jet composed of the shell and core material.

Step 3-8: As performed in section 3.2.1
For more details on producing core-shell capsules using concentric nozzle see application note 9.2.



(a) (b) (c)

Figure 9: Concentric nozzle for producing core-shell capsules. (a) Nozzle system with 7 shell nozzles. (b) Schematic of the internal structure and setup of a complete concentric nozzle system and (c) example of core-shell capsules produced using the concentric nozzle.

2.6 Production of small beads - Flow Vibration Nozzle

The Flow Vibration Nozzle (FVN) system uses vibration frequencies in combination with a controlled flow of air to produce beads. This allows the nozzle system to produce smaller beads (as small as 80 µm) in comparison to the single nozzle system when the same nozzle sizes are being used. The use of air also enables more viscous solutions to be processed into beads. The FVN (Fig. 10) is a similar set up to the concentric nozzle system, as the producing unit also contains two inlets and holds a concentric nozzle consisting of an inner nozzle (for pumping the bead producing material), and an outer nozzle (for air).

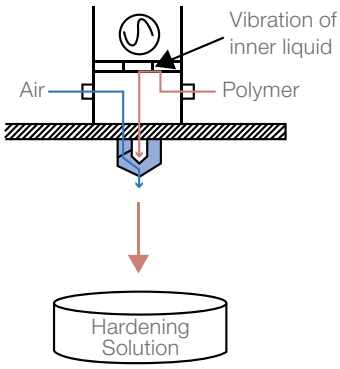


Figure 10: Schematic showing the operation of the flow vibration nozzle.

During operation air is pumped through the external nozzle and the polymer containing the material to be encapsulated is pumped through the inner nozzle (Fig. 10). The air flows around the liquid emerging from the tip of the inner nozzle and has two functions. Firstly it facilities the formation of a liquid jet by immediately removing the polymer liquid from tip of the inner nozzle when it is been pumped through it (hence enabling more viscous solutions to be used). Secondly it also allows a thinner liquid jet to be produced from this extruded liquid. The liquid jet is then vibrated causing it to break up into equal sized droplets.

These produced droplets can be up to 40% smaller in diameter than droplets produced when no air stream is used. When using alginate solutions on the FVN a general rule of thumb is that the final bead size is slightly larger than the size of the nozzle which is used. For example when using a nozzle size of 150 µm, it is possible to produce beads with a diameter as low as 175 µm. Like the single nozzle system, the size of the beads produced with the flow vibration nozzle is a function of the nozzle size, the vibration frequency and liquid flow rate used, however it is also a function of the air flow rate employed, with higher (air) flow rates producing smaller beads.

Note: The standard concentric nozzle system cannot be used as a flow vibration nozzle system.

2.7 Encapsulation of cell clusters – Air Dripping Nozzle

The Air Dripping Nozzle is specially designed for encapsulating different types of cell clusters (e.g. islets of Langerhans) in beads with sizes between 0.5 to 0.8 mm. However it can produce beads up to 2 mm in diameter if required. In addition the system is ideal when only a very small volume of cells are available/required for encapsulating, as the system generates minimal dead volume (< 0.2 mL). The system uses a controlled flow of air around the core nozzle to produce beads from a poly-

mer extruded through the core nozzle.
When encapsulating animal or stem cells into beads; the final particle size should be <1 mm in diameter and must be strictly adhered too. Sizes above 1 mm can limit the mass transfer (diffusion) of nutrients and Oxygen throughout the bead structure resulting in nutrient limitation for the encapsulated cells, hence causing cell death. This is especially true for the inner most cells of the cluster.

The system is made up of two nozzles [Fig. 11(a)]; a core nozzle which has an extended tip and inner diameter of 0.4 mm, which passes out through the (second) external air nozzle. The encapsulation liquid (polymer) containing the cells is slowly pumped (1 to 3 ml/min) through the core nozzle and accumulates at the nozzle tip where it is removed by an air stream flowing along the nozzle shaft.

The removal of liquid at the extended tip results in the formation of dispersed droplets [containing the cells – Fig. 11(b)] which are subsequently converted into beads by a hardening technique. The bead size can be controlled by the air flow rate with higher rates enabling the production of smaller beads.

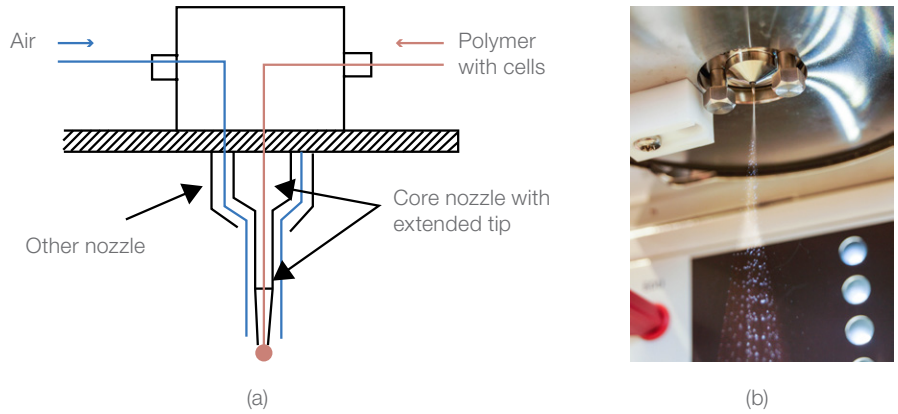


Figure 11 (a): Schematic displaying the operation of the Air Dripping Nozzle. (b) Real-time image displaying operation of Air Dripping Nozzle for encapsulating cells.

2.8 Summary of nozzle configurations

Table 1: Summary of different nozzle configurations available for BUCHI Encapsulators B-390 and B-395 Pro.

Nozzle config.	Encapsulated material	Particles produced	Size range (µm)	Special features	Industrial Applica-tions
Single	Solid materials & liquids	Beads	150 - 4000	Encapsulation of solids and liquids into beads in a one-step process	All segments*
Concentric	Solid materials & liquids	Core-shell capsules	400 - 2200	Core-shell capsules produced in one-step process	All segments*
Flow Vibration	Solid materials & liquids	Beads	80 - 2000	Produce smaller beads from more viscous solutions	All segments*
Air Dripping	Cell clusters & agglomerates	Beads	500 - 2000	Encapsulation of cell clusters & agglomerates in beads as small as 500 µm	Medical & biotech

* Food & beverage, feed, pharma, biopharma, biotech, medical, cosmetics, enviromental, agriculture and textile industries.

3 Process Parameters for Optimized Production

The parameters influencing the production of beads and capsules (and their effects on production) from alginate solutions when using the single and concentric nozzle systems are outlined below. To obtain optimized production operators should familiarize themselves with the information.

3.1 Process parameters influencing production

· Nozzle size(s*).
· Vibration frequency.
· Flow rate of the extruded mixture(s*).
· Viscosity of the extruded mixture.

*The plural value refers to the concentric nozzle system only.

3.2 Rules for producing beads/capsules

· Larger nozzle sizes result in higher production rates.
· Higher frequencies generate smaller beads/capsules.
· Lower flow rates generate smaller beads/capsules.
· A too low flow rate results in nozzle wetting.
· A flow rate too high results in uncontrolled dropled formation.
· The dispersion of smaller droplets requires lower voltages.
· Higher amplitudes are needed for liquids with high viscosities.
· Higher amplitudes are needed for larger nozzle sizes.

3.3 Rules for bead production only

· The bead diameter is around twice the nozzle diameter size.

3.4 Rules for capsule production only

· The final outer diameter of core-shell capsules is around twice the size of the external nozzle.

· Higher flow rates of the core material will generate large core diameters.

· Higher flow rates of the shell material will generate thicker capsule membranes.

Grid highlighting the parameters influencing production and their effect on bead size and productivity

Process parameters influencing bead size and productivity

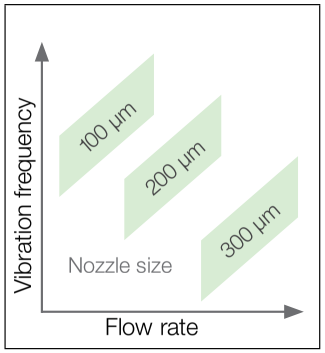
Parameter / Result	Nozzle size ⇈	Vibration frequency ⇈	Flow rate ⇈	Electrode voltage ⇈	Viscosity ⇈
Bead size	↑	↓	↑	–	↑
Productivity	↑	↑	↑	–	–

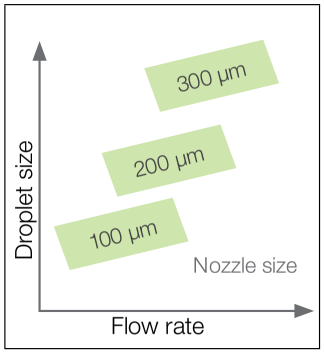
Adaption of process parameters for higher viscosity solutions

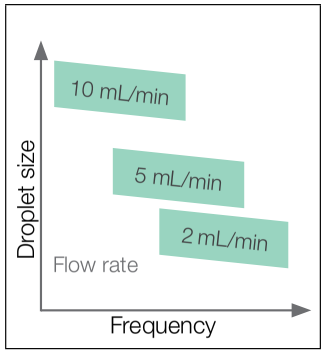
Viscosity ⇈				
	⇑	⇓	⇑	⇑

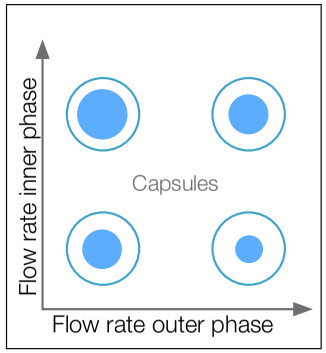
major influence	↑
moderate influence	↑
minor influence	↑
no influence	–
parameter variation	⇈
adaptation direction	⇑

Optimizing process parameters









4 Structural differences between the Encapsulator B-390 and B-395 Pro

There are three main structural differences between the Encapsulator B-390 and Encapsulator B-395 Pro models. These differences can affect the characteristics of the produced particles and can also influence the types of materials used to make the beads and capsules.

The three structural differences between both Encapsulator B-390 and B-395 Pro and their functions are described as follows:

· Temperature controlled carrier plate and heating block: For increasing/maintaining temperature of extruded polymers by heating up the bead/capsule production unit and the nozzle(s) – available on the Encapsulator B-390 model only.

· Auoclavable reaction vessel: Encloses the entire bead/capsule production area of the Encapsulator and enables the process to be performed under full sterile conditions, if required - available on the Encapsulator B-395 model only.

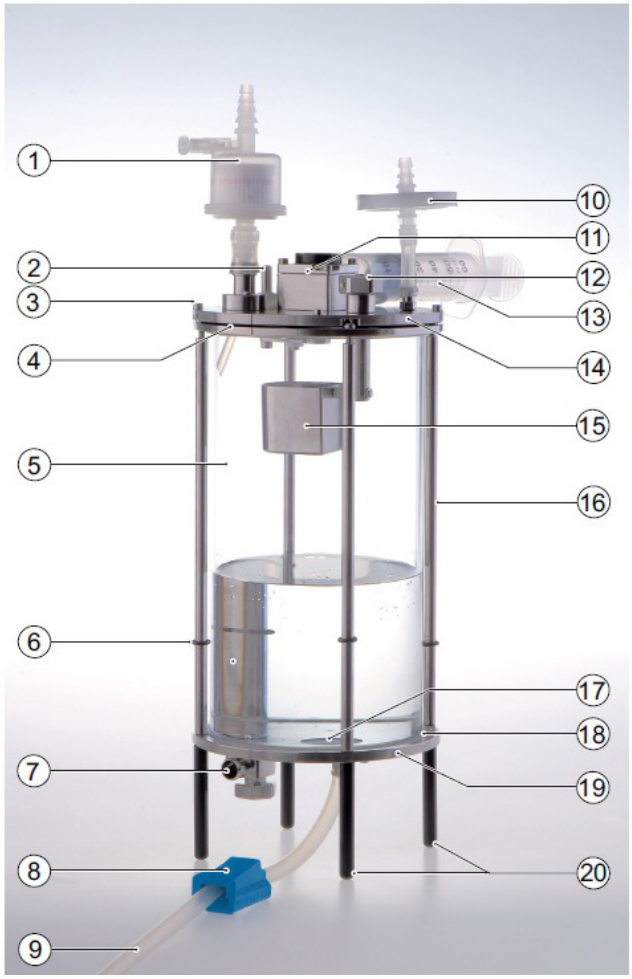
· Syringe pump: For volumetrically defined deliver of the polymers/liquids to the nozzle – Available on the Encapsulator B-395 model only. However an additional external syringe pump can be obtained from BUCHI and used on the Encapsulator B-390 model. An extra syringe pump can also be used on the Encapsulator B-395 Pro model when using the concentric nozzle system.

4.1 Temperature dependent polymer solutions

The Encapsulator B-390 has a temperature controllable carrier plate connected to a heating block and is incorporated onto the Encapsulator (Fig. 12). The carrier plate can heat up the nozzle and the pulsation chamber to the desired temperature (up to 80 °C), and works by conducting heat from the heating block. This device is very important when using polymers and materials which gel within a certain temperature range. A prime example is the use of the polymer gelatin which remains in a liquid state above 35 °C and gels below 35 °C. The temperature controlled carrier plate enables the temperature of the polymer to remain > 50 °C when it is being pumped through the production unit and nozzle, hence keeping it in a liquid form and facilitating pumping, and preventing it from gelling and blocking the system. This type of production is referred to as Hot-Prilling.
Maintaining the temperature of the polymer > 50 °C during pumping through the nozzle also allows more viscous solutions to be used and produced into beads and capsules. High viscous polymer solutions cannot be extruded through the nozzle orifice especially when using nozzle diameters of ≤ 300 µm. For applications of this device see application notes 9.4 & 9.7.

4.2 Sterile production and applications

The Encapsulator B-395 Pro can be used to produce bead and capsules under fully sterile conditions. Sterility is achieved by using a glass reaction vessel (Fig. 13) which fits which around and completely encloses the bead/capsule production unit. The reaction vessel is autoclavable along with all other parts which come into contact with the beads/capsules and the solution used to produce them, ensuring fully sterile conditions are obtained. This additional feature further advances the application portfolio of the technology, by enabling it to be applied to numerous biotechnological and medical processes and other fields, which require sterile conditions, and more importantly allows the technique to be integrated into a GMP process.



1. Liquid filter
2. Connection to electrode
3. Screw M4x10
4. Flange
5. Glass cylinder
6. O-Ring (6x2) for gap control
7. Harvesting valve
8. Plastic clamp
9. Silicon tube (6x9) of drain line
10. Air filter
11. Bead producing unit
12. Bypass knob
13. Syringe
14. Cover plate
15. Bypass cup
16. Support bar
17. Filter of drain line
18. Flat silicone fitting
19. Base plate
20. Foot

Figure 13: Autoclavable reaction vessel for Encapsulator B-395 Pro which provides a completely sterile environment for the bead/capsule production process.

4.3 High precision delivery of polymer/liquids

The Encapsulator B-395 Pro has a high precision syringe [Fig. 14 (a)] mounted on top of the unit which can be used to deliver polymers to the nozzle unit at pre-selectable, defined and easily controllable flow rates. However this syringe pump is not available on the Encapsulator B-390 model which instead uses an air pressure system to deliver the polymer [Fig. 14 (b)]. This air pressure system is also available on the Encapsulator B-395 Pro model, where it can be used in conjugation with the syringe pump when making core-shell capsules, or where it is required to deliver up to 1 liter of polymer during a single production run. Note: When using the air pressure system to deliver the polymer to the nozzle it is highly recommended to use the liquid flow regulation valve (Figure 14b) to precisely control the flow rate of the polymer, otherwise the flow will be too hard to accurately control. The exact flow rate generated when using the air pressure system can be determined by collecting the extruded polymer in a graduated cylinder over a defined time period.
While a syringe pump is not available on a Encapsulator B-390 model, an external syringe pump [Fig. 14(c)] is available and can be used to pump material to the nozzle. In this case it is recommended to use the Legato 100 syringe pump model (KD Scientific) and can be obtained from BUCHI[III].

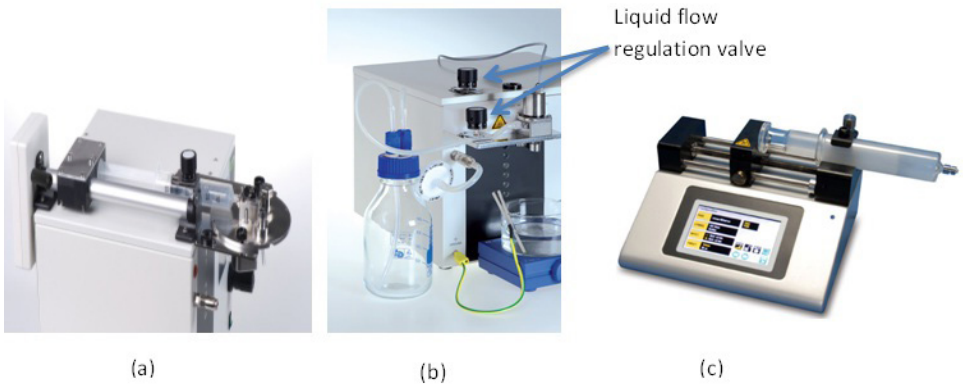


Figure 14: Different mechanisms to delivery polymers and liquids to the BUCHI Encapsulators. (a) High precision syringe pump mounted onto the Encapsulator B-395 Pro. (b) Air pressure regulation system for the Encapsulator B-390 (also available on Encapsulator B-395 Pro) and its set up during operation and (c) recommended external syringe pump (from KD Scientific model – Legato 100), which can be used for both Encapsulator models.

Table 2: Comparison of Encapsulators B-390 and B-395 Pro

	Encapsulator B-390	Encapsulator B-395 Pro
Particle size	80 – 4000 µm	80 – 4000 µm
Particle type	Beads & capsules	Bead & capsules
Hot-Prilling	Yes	No
Syringe pump	No	Yes

III For order information on the external syringe pump contact our local office or distributor.

Sterile work	No	Yes
Aseptic work*	Yes	Yes
Dead volume	~ 2 mL	~ 0.25 mL
Options	External syringe pump 4 nozzle configurations	External syringe pump 4 nozzle configurations

*Achieved by placing instrument under a laminar flow cabinet and following aseptic technique.

5 Quick-start guide for the Single Nozzle system

Table 3 displays some pre-determined parameters used to produce beads in the size ranges outlined when alginate solutions were used for the single nozzle system. These values can act as a reference or starting value for the operator if using a similar alginate type[IV] and concentration. An empirical approach is then used (beginning with the reference value) to determine the optimal production parameters.

Table 3: Parameter range to produce Ca-alginate beads (in the sizes ranges outlined) when using the single nozzle system on the Encapsulator B-390 and B-395 Pro.

Nozzle (µm)	Flow rate range (ml/min)* (Production)	Air pressure (bar)	Optimal frequency range (Hz)**	Amplitude	Size range of produced beads (µm)
80	1.1	0.5 - 0.7	1300 - 3000	1 - 4	120 - 200
120	1.5 - 1.8	0.5 - 0.7	1000 - 2500	1 - 4	200 - 300
150	2.3 - 2.8	0.4 - 0.6	800 - 1800	1 - 3	260 - 350
200	3.5 - 4.5	0.4 - 0.6	600 - 1200	1 - 3	350 - 450
300	6.0 - 8.0	0.3 - 0.5	400 - 800	1 - 3	550 - 700
450	11 - 15	0.3 - 0.5	200 - 500	1 - 4	700 - 1150
750	19 - 25	0.3 - 0.5	40 - 300	6-9	1150 - 1800
1000	30 - 40	0.3 - 0.6	40 - 220	6-9	1600 - 2400

* This flow rate value can be obtained using the high precision syringe pump which comes separately or is mounted onto the Encapsulator B-395 Pro.**Frequency range was determined when using the 2% low viscosity grade alginate solution for the 750 and 1000 µm nozzle sizes, 1.5% alginate solution for 150 – 450 µm nozzle sizes and 1.2% alginate solution for the 80 and 120 µm nozzle sizes.

IV Recommended to use BUCHI Alginate. Contact our local office or distributor for order information.

Before beginning production

· Decide on the required bead size or size range and chose the appropriate nozzle size from Table 3.

· Choice the polymer to produce the beads and the material to be encapsulated. Note: If alginate is not used to produce the beads, the operator can still use the values in Table 3 as a reference point and make changes accordingly, after performing initial tests.

· Chose a frequency and polymer flow rate value (for the Encapsulator B-390 use the air pressure values displayed) from the range recommended in Table 3 for the selected nozzle size. Table 3 displays a large range of values for both the frequency and flow rate, so the operator should select an average value displayed.

· Select an amplitude value, again selecting the average. Note: Amplitude has minimal effect on the production process except when using 750 and 1000 µm nozzles.

Beginning production

· Firstly pump the polymer material to the nozzle using either the syringe pump (Encapsulator B-395 Pro) or air pressure system (Encapsulator B-390). Note: When using the air pressure system to deliver the polymer to the nozzle it is highly recommended to use the liquid flow regulation valve [Figure 14(b)] to precisely control the flow rate of the polymer.

· Increase the pumping speed until a stable liquid jet has formed [Fig. 15(a)]. For the B-395 Pro the operator can increase the speed by pressing the turbo button (on the syringe pump control panel) and leaving at this speed for 3-5 seconds. After formation of a stable jet, turn off the turbo function and the liquid jet will stabilize to the set flow rate value[V] . For the air pressure system, after obtaining a stable liquid jet, slightly decrease the pumping speed using the liquid-flow regulation valve. Note: When using the air pressure system to deliver the polymer to the nozzle it is highly recommended to use the liquid flow regulation valve (Figure 14b) to precisely control the flow rate of the polymer, otherwise the flow will be too hard to accurately control. The exact flow rate generated when using the air pressure system can be determined by collecting the extruded polymer in a graduated cylinder over a defined time period.

V The turbo function is necessary as it breaks the initial surface tension of the extruded polymer at the nozzle tip resulting in liquid jet formation, otherwise the polymer passes through the nozzle in the form of droplets.

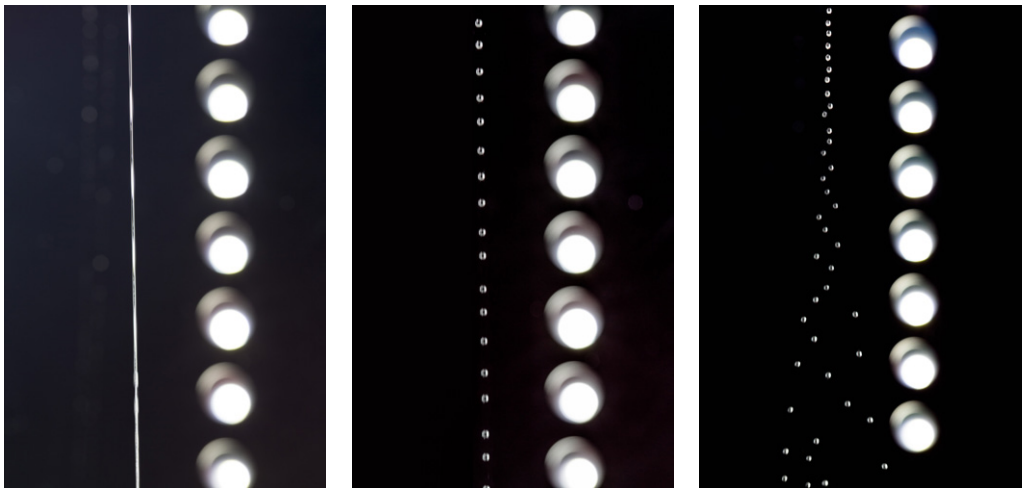
· Turn on the frequency at the set value and obtain a stable droplet chain [Fig. 15(b)]. If a droplet chain is not obtained, firstly increase/decrease the flow rate in increments of 5%, making sure to observe the effects for each new value. The operator can also increase/decrease frequency in increments of 5% to help obtain a stable droplet chain.

· Turn on the electrostatic charge and increase/decrease until a circular dispersion is obtained (Fig. 15c). The optimal value should enable a circular dispersion of the droplets of around 3 to 5 cm in size.

Note: When using the 750 and 1000 µm nozzle sizes the electrostatic charge will not be able to disperse the droplets into a circular dispersion due to the large droplet size. However it will help stabilize the drops in the chain, which will prevent coalescence occurring and allow the formation of mono-dispersed particles of equal size and shape with a standard size deviation of ≤ 5%.

· Collect beads and allow hardening for appropriate time (depends on polymer used).

· Record any new process parameters and results in the template provide in Section 5.1. Process parameters can also be stored on the Encapsulators for future use.



(a) (b) (c)

Figure 15: Production of Ca-alginate beads using the BUCHI Encapsulators B-390 and B-395 Pro. (a) Production of a stable liquid jet. (b) Production of a stable droplet chain and (c) circular dispersion of the droplets using the electrostatic charge to prevent collision.

5.1 Template for recording process parameters and results

Parameters

Type of nozzle(s) and size(s)	
Bead/capsule-membrane material	
Encapsulated material	
Liquid-core material CN*	
Hardening solution	
Mechanism of pumping polymer (syringe or air-pressure)	
Flow rate of bead/capsule-membrane material (ml/min)	
Flow rate of liquid-core material (ml/min) CN*	
Frequency (Hz)	
Amplitude (%)	
Voltage (kV)	
Length of gelling (min)	
Washing Solution	

Results

Mean diameter size of beads (µm) and St. Dev (%)	
Mean diameter size of capsule (µm) and St. Dev (%)	
Diameter of liquid core Avg. (µm)	
Diameter of Polymer shell Avg. (µm)	
Shape of beads/capsules	

* CN: Concentric Nozzle

5.2 Characteristics of the beads/capsules produced using the BUCHI Encapsulators B-390 and B-395 Pro and the characteristics of the production technique

Bead/capsule characteristics	Characteristics of the production technique

· Mono and singly dispersed
· Homogenous and spherical shape
· Size ranges:
  · Bead diameter 80 µm - 4 mm
  · Capsule diameter 400 µm - 2.2 mm
  · Shell thickness 100 µm – 500 µm
  · Core diameter 150 µm – 1.5 mm
· Narrow size distribution (< ± 5% from the mean size)
· Can be produced from a range of different polymers and core liquids (including hydrophobic liquids)
· Can be produced from temperature dependent polymer solutions such as gelatin, waxes etc.
· Sterile particles (Encapsulators B-395 Pro only)
	· Relatively easy set up and simple operation
	· Gentle technique (encapsulation of cells)
	· Fully controllable operation
	· Low operating (and servicing) costs
	· High efficiency
	· Produce a range of different sized capsules in separate batches
	· Ability to extrude viscous solutions (FVN)
	· Repeatable results
	· Operate under sterile conditions (Encapsulators B-395 Pro only)
	· GMP compliant
	· One step process to produce beads & capsules

6 Large Scale Production

The BUCHI Encapsulators are especially designed for performing innovative R&D work for many different sectors, and can also be used in industry for producing small quantities of beads and capsules for commercial purposes. In the latter case they are mostly used to encapsulate high-value low-volume materials such as specialized drugs or stem/animal cells. The production rate of a BUCHI Encapsulator is mainly dependent on the diameter of the nozzle being used, with increasing diameters resulting in higher production rates. Table 3 shows the production rates which are obtainable, and show that with the largest nozzle size a production rate of 40 mL/min (2.6 L/hr) can be reached. However, these rates fall well short of the quantities (up to tons/day) required by most industries planning to manufacture encapsulated products for the market places.

After developing and optimizing a bead/capsules production process at lab scale (using a BUCHI Encapsulator), the next step for most companies will be to scale up the process. The main goal will be to produce higher quantities of the particles without incurring significant changes in their properties. Scale up (increased production) of the Prilling by Vibration technique can be successfully achieved by increasing the number of nozzles used for polymer extrusion, and the increased productivity rate is a direct function of the number of nozzles used. Provided the nozzle size, vibrational frequency, amplitude and flow rate are similar to the values used at lab-scale (as well as being constant across all nozzles); beads and capsules with similar characteristics can be obtained at much high quantities.

BUCHI offers a new multi nozzle Encapsulator which is a scaled up version of its Encapsulator B-390. This is the device of choice for further scale up of encapsulation process developed using BUCHI Encapsulator at lab scale and offers low to median production levels. The device has 6 nozzles held on a steel plate, and enables a 6-fold increase in the quantity of beads. For this device further increases in production volumes can be achieved by adding more nozzles (in multiples of 6) to the machine. While used for pilot-scale production and tests, this device can also be for industrial production. For further information on the BUCHI Multi-Nozzle Encapsulator contact your local office/distributor.

Some companies now also offer large scale Encapsulators for industrial production, with one such company being Brace GmbH (www.brace.de). The Brace large scale Encapsulators can have hundreds of nozzles fit onto the one device and have the capability to produce tons of encapsulated material per day. These Encapsulators work on the same principle as the BUCHI Encapsulators and are seen as the machines of choice for further scaling up encapsulation production process developed using the BUCHI one-nozzle and multi nozzle Encapsulator.

7 Operational trouble-shooting

Common operating problems encountered when using the BUCHI Encapsulators B-390/B-395 Pro.

Problem	No extrusion of polymer solution through nozzle			
Possible cause	Pump speed is too low	Filter within bead producing unit is blocked	Polymer solution is too viscous	Bead producing unit/nozzle is blocked
Suggested Solution	Increase pump speed	Change filter (Manual section 5.4)	Dilute polymer solution or use Flow vibration Nozzle (section 2.6)	Clean bead production unit/nozzle: For alginate, soak the unit in 0.1 M NaOH for 1 hr and rinse with water, or place in a sonication bath for 5 min. For gelatin soak in hot water (> 60 °C) and flush, or sonicate as above.

Problem	Inadequate or no dispersion of droplets when using electrostatic charge		
Possible cause	Droplets are too big	Droplets are repelled from landing in gelling vessel	Droplets are hitting the wall of the gelling vessel
Suggested Solution	For Nozzle sizes ≥ 750 µm the electrostatic device will not disperse droplets	Place grounding hook into gelling solution	Charge is too high, decrease appropriately
Possible cause	Electrode is not turned on	Electrode is not connected properly (B-390 model)	The electrode is not cleaned
Suggested Solution	Turn on electrode	Connect properly (Manual section 5.6)	Clean electrode

Problem	Non-homogenous bead-size-distribution		
Possible cause	Pump speed is too high	The frequency is too low/ high	Inadequate dispersion of the droplets by the electro-static charge (nozzle ≥ 300 µm)
Suggested Solution	Decrease pump speed	Adjust frequency	Increase charge

Problem	Droplets are not visible in strobe light			
Possible cause	The vibration unit is not in place	The vibration is not turned on	The frequency is too high/low	The viscosity of the polymer solution is too high
Suggested Solution	Place vibrating unit on bead producing unit	Turn on vibration	Increase/decrease frequency	Dilute the polymer solution

Problem	Unstable droplet chain			
Possible cause	Frequency is too low/high	Pump speed is too low/high	Polymer solution is too viscous	The nozzle is not cleaned properly
Suggested Solution	Increase/decrease frequency	Increase/decrease pump speed	Dilute polymer solution	Clean nozzle (as described Pg 27)

Problem	Unstable liquid jet		
Possible cause	Pump speed is too low/high	Polymer solution is too viscous	The nozzle is not cleaned properly
Suggested Solution	Increase/decrease pump speed	Dilute polymer solution	Clean nozzle (as described Pg 27)

8 References

[1] Whelehan, M. & Marison I.W. Journal of Microencapsulation. 2011;28:669-88.
[2] Gibbs, B.F. et. al. International Journal of Food Sciences and Nutrition. 1999;50:213-24.
[3] Serp, D. et. al. Biotechnology and Bioengineering. 2000;70:41-53.
[4] Heinzen, C. et. al. Fundamentals of cell immobilisation technology. Dordrecht: Kluwer Academic Publishers; 2004:257 - 75.
[5] Whelehan, M. The present role of microencapsulation. Eurolab. 2012:16-17.
[6] Hoad, C. et. al., Food Hydrocolloids. 2011;25:1190-1200.
[7] Madziva, H. et. al., Journal of Microencapsulation. 2005;22:343-51.
[8] Chandramouli, V. et. al., Journal of Microbiological Methods. 2004;56:27-35.
[9] Bhathena, J. et. al., Journal of Medicinal Food. 2009;12:310-319.
[10] Lyer, C. et. al., Letters in Applied Microbiology. 2005;41:493-497.
[11] Anjani, K. et. al., International Dairy Journal. 2007;17:79-86.
[12] Sun-Waterhouse, D. et. al., Food Bioprocess Technology. 2012;5:3090-3102.
[13] Sun-Waterhouse, D. et. al., Food Chemisty. 2011;126:1049-1056.
[14] Wang, W.et. al., Food Research International. 2013;54:837-851.
[15] Soliman, E.A. et. al., Journal of Encapsulation & Adsorption Science. 2013;3:45-55.
[16] Perez-Moral, N. et. al., Food Hydrocolloids. 2013;31:114-120.
[17] Wang, Q. et. al., Journal of Applied Microbiology. 2009;107:1781-1788.
[18] Zvonar, A. et al., Journal of Microencapsulation. 2009;26:748-759.
[19] Zvonar, A. et al., International Journal of Pharmaceutics. 2010;388:151-158.
[20] Metz, T. et. al., Cell Biochemistry & Biophysics. 2005;43:77-85.
[21] Genc, L. & Butuktiryaki, S. Pharmaceutical development & technology. 2014;19:42-47.
[22] Wenk, E. et. al., Journal of Controlled Release. 2008;132:26-34.
[23] Ma, Y. et. al., Applied & Environmental Microbiology. 2008;74:4799-4805.
[24] Weber, W. et. al., Journal of Biotechnology. 2006;123:155-163.
[25] Arenas-Gamboa, A.M. et. al., Journal of Wildlife diseases. 2009;45:1021-1029.
[26] Arenas-Gamboa, A.M. et. al., Infection & Immunity. 2008;76:2448-2455.
[27] Paul, A. et. al., Cell Transplantation. 2012;21:2735-2751.
[28] Penolazzi, L. et. al., Tissue Engineering Part C-Methods. 2010;16:141-155.
[29] Kulamarva, A. et. al., Nanotechnology. 2009;20:1-7.
[30] Fluri, D.A. et. al., Journal of Controlled Release. 2008;131:211-219.
[31] Brandenberger H; Nussli D; Piech V; Widmer F. Journal of Electrostatics. 1999;45(3):227-38.

Part B: Application Notes

For more application notes please visit http://www.buchi.com/en/applications/finder

9.1 Production of Ca-alginate beads

Encapsulator B-390 / B-395 Pro: Production of Ca-alginate Beads with a size of 560 - 680 µm

9.1.1 Introduction

Ca-alginate micro-beads can be used to encapsulate a wide variety of materials. This method enables the encapsulated substance (termed encapsulant) to be protected from many different environmental conditions such as oxygen, heat, pH etc., and can help prolong its shelf life. In addition encapsulation also enables the controlled release of the encapsulant.

The following example substances can be encapsulated using this method:

- Cells (yeast, bacteria and animal cells)
- APIs (hydrophobic)
- Essential oils & hydrophobic liquids
- Flavors & fragrances
- Vitamins & minerals
- Bioactive materials
- Enzymes
- Detergents
- Cosmetics

By using the BUCHI Encapsulator B-390/B-395 Pro, with a 300 µm nozzle in place, it is possible to control the size of the micro-beads within the range of 550 µm to 700 µm, while obtaining a narrow size distribution (± 5%).
With the use of one of the additional nozzles (eight in total) provided with the Encapsulator it is possible to create particles in the size range of 150 to 2000 µm.

Aim: To produce Ca-alginate micro-beads using the Encapsulator B-390/B-395 Pro with the 300 µm nozzle

9.1.2 Equipment

- Instrument: Encapsulator B-390/B-395 Pro
- Set up: Single-flow nozzle system – 300 µm nozzle
- Pumping: Syringe pump/air pressure system
- Blender: Any kind of blender can be used (see Fig. 9.1-1)

9.1.3 Chemicals and Materials

- Polymer: 1.5% (w/v) Na-alginate (BUCHI) dissolved in H_2O using a blender
- Gelling sol: 100 mM $CaCl_2$
- Deion. water: Dissolving the Na-Alginate
- Encapsulant: Not used in this method (materials listed in section 9.1.1 can be used with a max. conc. of 20%)

9.1.4 Procedure and Parameters

Procedure

Add 4.0 g of Na-alginate powder into 200 mL of water. Use the blender to dissolve the Na- alginate completely (Fig. 9.1-1). Let the solution sit until it is clear and has released all the air within it. The air bubbles can be removed quicker by placing in a sonication bath or placing under vacuum. If a material is to be encapsulated within the beads, add the material to the alginate solution and mix again.

Figure 9.1-1:
Polymer preparation

Begin production (hardening) after obtaining a stable droplet chain (Fig. 9.1-2a). Use electrostatic charge to disperse droplets and prevent collision (Fig. 9.1-2b). Deliver 10 mL of alginate through nozzle into hardening/gelling bath containing 100 mL of $CaCl_2$. Allow to harden for 30 min (T=0, when last drop lands in gelling bath). Wash with water and examine under a microscope.

The following process parameters may vary slightly and are dependent on the type of alginate used.

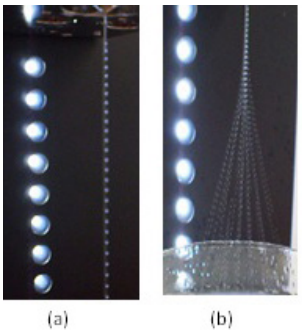


Figure 9.1-2: (a) Alginate droplet chain produced using set out parameters. (b) Dispersion of the droplets using electrostatic charge.

Process parameters I

- Flow rate 7.0 mL/min
- Frequency 600 Hz
- Pressure 0.5 bar
- Amplitude 2
- Charge 1000 - 2500 V

Process parameters II

- Flow rate 7.0 mL/min
- Frequency 750 Hz
- Pressure 0.5 bar
- Amplitude 2
- Charge 1000 - 2000 V

Process parameters III

- Flow rate 7.8 mL/min
- Frequency 600 Hz
- Pressure 0.5 bar
- Amplitude 2
- Charge 1000 - 2500 V

Hint: Increasing / decreasing micro-bead diameter

After the initial production of micro-beads and size determination, the operator can increase/decrease the diameter of the produced micro-beads in additional production runs (using the same nozzle size) by varying certain key parameters.

General rule:
For any given nozzle size there are two main parameters which will affect size during production – the frequency and the flow rate.

- Higher frequencies generate smaller microbeads.
- Lower polymer flow rates generate smaller microbeads.

The production of bigger/smaller micro-beads will have an effect on the amount of the charge required to adequately disperse the droplets and prevent coalescence from occurring.

General rule:
Smaller microbeads require lower electrostatic charges to adequately disperse the droplets and prevent colliding.

9.1.5 Results

Results I

- Amount extruded 10 mL
- Yield > 99%
- Morphology Spherical
- Size 602 µm
- Std. Dev. ± 0.9%

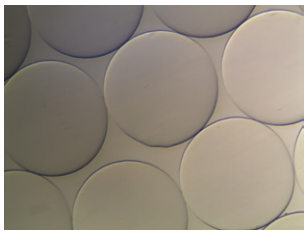


Figure 9.1-3: Light microscope image at 40X displaying microbeads produced by the BUCHI Encapsulator B-390.

Results II

- Amount extruded 10 mL
- Yield > 99%
- Morphology Spherical
- Size 560 µm
- Std. Dev. ± 0.9%

Results III

- Amount extruded 10 mL
- Yield > 99%
- Morphology Spherical
- Size 680 µm
- Std. Dev. ± 1.45%

9.1.6 Conclusion

The BUCHI Encapsulator B-390 / B-395 Pro are able to produce spherical alginate micro-beads in the size range of 560 - 680 µm with the 300 µm nozzle. The particles achieved have an extremely narrow size distribution.

General rule: Final micro-bead diameter is about twice the size of the selected nozzle size, however this can vary and depends on the encapsulated material, which can cause shrinking and swelling effects.

9.1.7 References

[1] Whelehan and Marison (2011). Microencapsulation using vibrating technology. Journal of Microencapsulation 28:669-688.

9.2 Production of core-shell capsules

Encapsulator B-390/B-395 Pro: Production of core-shell microcapsules for encapsulation of hydrophobic liquids and oils

9.2.1 Introduction

Hydrophobic liquid-core microcapsules play a very important role in numerous industries and have being mainly used in the perfume, cosmetic, paper and agricultural industry, but recently they have found application in drug and bioactive (food industry) delivery.

The method allows most oils or hydrophobic liquids to be encapsulated and the following substances are common examples:

- Perfume oils
- Essential oils
- Fatty acids
- Flavors and fragrances
- Omega 3 oils
- Hydrophobic APIs
- Hydrocarbons
- Detergents
- Cosmetics

This method enables the encapsulated substance (termed encapsulant) to be protected from many different environmental conditions such as oxygen, heat, pH etc., and can help prolong its shelf life. In addition encapsulation also enables the controlled release of the encapsulant.

In comparison to encapsulation of oils and hydrophobic liquids in microbeads (Section 9.3) the use of core-shell capsules has the following advantages:

- Higher loading of encapsulant - up to 40%
- No encapsulant on the surface of the capsules (complete envelopment)
- Greater protection of the encapsulant
- Release profile: burst or delayed

With the different nozzles supplied with the BUCHI Encapsulator it is possible to obtain capsules sizes between 400 - 2200 µm, while obtaining a narrow size distribution (± 5%).

Aim: Production of sunflower oil-core microcapsules with a Ca-alginate membrane (Fig. 9.2-3).

9.2.2 Equipment

- Instrument: Encapsulator B-390/B-395 Pro
- Set up: Concentric nozzle system: Shell 400 µm & core nozzle 150 µm
- Pumping: Air pressure (shell) & syringe pump/air pressure (core)
- Blender: Any kind of blender can be used (see Fig. 9.2-1)

Figure 9.2-1: Polymer preparation

9.2.3 Chemicals and Materials

- Polymer: 2.0% (w/v) Na-alginate (BUCHI) dissolved using a blender
- Gelling sol: 100 mM $CaCl_2$ containing 0.1% Tween 80
- Material: Sunflower oil
- Deion. water

9.2.4 Procedure and Parameters

Add 4.0 g of Na-alginate powder into 200 mL of water. Use the blender to dissolve the Na-alginate completely (Fig. 9.2-1). Let the solution sit until it is clear and has released all the air within it. The air bubbles can also be removed by placing in a sonication bath or under vacuum.
Dissolve 1.47 g of $CaCl_2$ (dihydrate) and 0.1 mL of Tween 80 in 100 mL of water. The Tween 80 is added to reduce the surface tension of the gelling solution, which prevents capsules bursting during their entry into the solution.
The alginate should be first pumped through the shell nozzle. After obtaining a stable droplet chain, the pumping of the sunflower oil through the core nozzle should begin. Slight adjustment (to the set up values) of both flow rates will be required to obtain a stable chain of mono-centric droplets (Fig. 9.2-2), which produce the liquid-core microcapsules (Fig. 9.2-3) after landing in the gelling bath. Allow the particles to harden in the $CaCl_2$ bath for 20 min. Wash the capsules with plenty of water.

Note: When using the air pressure system to deliver the polymer to the nozzle it is highly recommended to use the liquid flow regulation valve (Figure 14b) to precisely control the flow rate of the polymer, otherwise the flow will be too hard to accurately control. The exact flow rate generated when using the air pressure system can be determined by collecting the extruded polymer in a graduated cylinder over a defined time period.

9.2.5 Process parameters

- Flow rate 10 (shell) & 1.5 (core) mL/min
- Frequency 600 Hz
- Pressure 0.5 bar
- Amplitude 3
- Charge > 2000 V

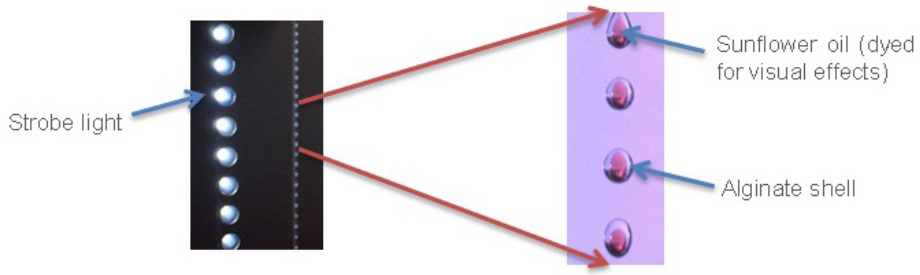


Figure 9.2-2: Stable chain of droplets which consist of sunflower oil core enveloped within an alginate shell.

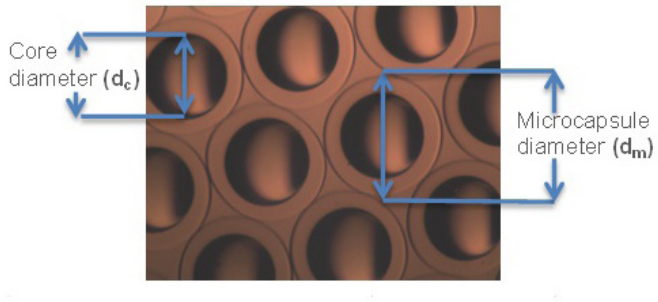


Figure 9.2-3: Image at 40X of sunflower oil core microcapsules produced using the BUCHI Encapsulator B-395 Pro.

· Yield of oil	> 95%
· Morphology	Spherical
· Loading	15%
· Std. Dev.	± 2.5%

Oil loading %

The % loading (make-up) of the microcapsules with sunflower oil (liquid) can be calculated from; % loading = Vc/Vm ×100. Where Vc = microcapsule vol. & Vm = liquid-core vol. The vol. of the microcapsule and liquid-core can be calculated from the vol. of sphere equation $V = 4/3\ \pi r^3$

9.2.6 Conclusion

The formation of core-shell capsules with Na-alginate can be done with a high number of different hydroscopic liquids and materials. The loading of liquid within the capsules can be up to 40%. With the different nozzle sizes available for the Encapsulator the capsule size can be chosen in the range of 400 - 2200 µm.

9.2.7 References

[1] Whelehan and Marison (2011). Microencapsulation using vibrating technology. Journal of Microencapsulation 28:669-688.

[2] Whelehan and Marison (2011). Capsular perstraction as a novel methodology for the recovery and purification of geldanamycin. Biotechnology Progress 27:669-1077.

9.3 Encapsulation of hydrophobic liquids within microbeads

Encapsulator B-390/B-395 Pro: Encapsulation of hydrophobic liquids and oils within Ca-alginate beads

9.3.1 Introduction

A simple way to encapsulate hydrophobic liquids and oils is to mix them with polymers such as alginate (or another polymer which is used to make the micro-bead) and subsequently extrude the formed emulsion through the single nozzle system of the Encapsulator. This results in the formation of alginate droplets incorporating the oil, which are subsequently solidified in a gelling bath to form Ca-alginate beads encapsulating the hydrophobic liquid.

The method allows most oils or hydrophobic liquids to be encapsulated and the following substances are common examples:

- Perfume oils
- Essential oils
- Fatty acids
- Flavors and fragrances
- Omega 3 oils
- Hydrophobic APIs
- Hydrocarbons
- Detergents
- Cosmetics

This method enables the encapsulated substance (termed encapsulant) to be protected from many different environmental conditions such as oxygen, heat, pH etc., and can help prolong its shelf life. In addition encapsulation also enables the controlled release of the encapsulant.
The advantage of this technique compared to producing core-shell capsules using the concentric nozzle (Section 9.2) is that it is a simpler technique. However the maximum loading is only 20% and some of the encapsulated liquid can be found on the surface of the beads, and is exposed to environmental conditions.

Aim: Production of Ca-alginate micro-beads incorporating sunflower oil (Fig. 9.3-2 & 9.3-3).

9.3.2 Equipment

- Instrument: Encapsulator B-390/B-395 Pro
- Set up: Single nozzle system – 300 µm nozzle
- Pumping: Syringe pump/air pressure system
- Blender: Any kind of blender can be used (see Fig. 9.3-1)

9.3.3 Chemicals and Materials

- Polymer: 2.0% (w/v) Na-alginate (BUCHI) dissolved using a blender
- Gelling sol: 100 mM $CaCl_2$
- Encapsulant : Sunflower oil
- Deion. water

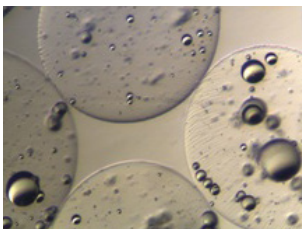
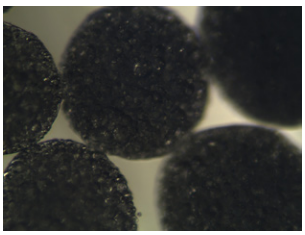


Fig. 9.3-2: Image at 40X displaying sunflower oil (at a conc. of 2% of the total capsule volume) encapsulated within Ca-alginate microbeads of size 953 µm.



Picture 9.3-3: Image at 40X displaying sunflower oil (at a conc. of 20% of the total capsule volume) encapsulated within Ca-alginate microbeads of size 1085 µm.

9.3.4 Procedure and Parameters

Procedure

Add 4.0 g of Na-alginate powder into 200 mL of water. Use the blender to dissolve the Na- alginate completely. Add the required amount of sunflower oil to Na-alginate solution (max. 20% of sunflower oil). Use the blender to emulsify the oil in the alginate solution. Let the solution sit until it is clear and released all the air within it. The air bubbles can be removed quicker by placing in a sonication bath or placing under vacuum.

Dissolve 1.47 g of $CaCl_2$ (dihydrate) and 0.1 mL of Tween 80 in 100 mL of water. The Tween 80 is added to reduce the surface tension of the gelling solution, which prevents capsules bursting during their entry into the solution.
Begin production (hardening) after obtaining a stable droplet chain. Use electrostatic charge to disperse droplets and prevent collision before entering the hardening/gelling bath. Allow to harden for at least 30 min (T=0, when last drop lands in gelling bath). Wash microbeads with copious amount of deionized water to remove any un-reacted material.

Process parameters

- Flow rate 7.5 - 8 mL/min
- Frequency 600 Hz
- Pressure 0.5 bar
- Amplitude 3
- Charge > 1000 V

9.3.5 Results

Results I

- Amount extruded 20 mL
- Yield > 99%
- Morphology Spherical
- Size 953 µm
- Std. Dev. ±1.8%
- Loading 2%

Results II

· Amount extruded	20 mL
· Yield	> 99%
· Morphology	Spherical
· Size	1085 µm
· Std. Dev.	4.5%
· Loading	20%

9.3.6 Conclusion

The method of encapsulating (emulsified) oil into a Ca-alginate matrix is possible with various concentrations of oil (recommended ≤ 20% of oil). With the different nozzle sizes available for the Encapsulator the particle size can be chosen in the range of 150 - 2000 µm.

9.3.7 References

[1] Whelehan and Marison (2011). Microencapsulation using vibrating technology. Journal of Microencapsulation 28:669-68.

9.4 Production of hard fat capsules

Encapsulator B-390: Encapsulation of water and water-soluble materials within hard-fat based capsules

9.4.1 Introduction

Long chain triglycerides can be employed as a unique structural material to produce beads and capsules for the successful encapsulation and retention of water and water-soluble materials. At temperatures below 40 °C these fats form solid structures which can entrap the material present. Above these temperatures the solid fats melt and release the encapsulated material.

The advantage of this method is that it is able to encapsulate hydrophilic substances. The following example substances can be encapsulated using this method:

- Perfume oils
- Detergents
- Cosmetics
- APIs (hydrophilic and hydrophobic)
- Vitamins and minerals
- Flavors and fragrances
- Bioactive materials
- Many other aqueous based materials

This method enables the encapsulated substance (termed encapsulant) to be protected from many different environmental conditions such as oxygen, heat, pH etc., and can help prolong its shelf life. In addition encapsulation also enables the controlled release of the encapsulant.

9.4.2 Equipment

- Instrument: Encapsulator B-390
- Set up: Concentric nozzle system: Shell 300 µm & core nozzle 150 µm
- Pumping: Air pressure

9.4.3 Chemicals and Materials

- Polymer: Vegetal fat
- Gelling sol: Ethanol maintained at 10 °C
- Encapsulant: Water
- Deionized water

9.4.4 Procedure and Parameters

Figure 9.4-1: Image displaying the produced capsules containing a water core surrounded by a hard fat membrane. The capsules have a size of between 550 to 800 µm.

Melt 200 g of fat with a heating plate and keep the fat at 55 °C.
Switch on the nozzle heater of the Encapsulator B-390 and set it to 60 °C. Wait until the nozzle has reached the temperature.

Pump the liquidized fat solution through the heated shell nozzle to form a liquid jet which is broken up into droplets by the vibrational frequency. The fat solution can be maintained at a temperature of 55 °C by placing the solution on a hot plate with magnetic stirring. After obtaining a stable droplet chain with the shell material, the pumping of the water through the core nozzle should begin. Slight adjustment (to the set up values) of both flow rates will be required to obtain a stable chain of mono-centric droplets.

The distance between the tip of the nozzle and the surface of the cooling ethanol should be at least 50 cm to allow the fat-based shell of the droplet to cool enough before landing in the cooling ethanol, otherwise the droplet will lose its spherical shape. The distance between the surface and bottom of the cooling solution should also be at least 30 cm to allow the bead to harden sufficiently before hitting the bottom of the vessel. Allow to harden for 30 minutes in the ethanol and make sure this liquid remains below 10°C during production process. After production remove excess ethanol by filtration.

Note: Ethanol was chosen as the hardening solution as its density is lower than that of the fat material used in this study. This ensures that the produced capsules sink to the bottom of the vessel after entering. This prevents incoming droplets from colliding with these particles, which may occur if capsules float on top of the surface, hence enabling the production of spherical and mono-disperse capsules. Produced capsules should be stored under 30°C to prevent any melting and sticking.

Process parameters

- Flow rate 6-8 (shell) & 0.5 - 1 (core) mL/min
- Frequency 500 - 700 Hz
- Pressure 0.5 bar
- Amplitude 3
- Charge > 2000 V

9.4.5 Results

- Yield of aq. liquid > 95%
- Morphology Spherical
- Loading 15%

9.4.6 Conclusion

The formation of hard fat based microcapsules for the encapsulation of water and other aqueous based liquids can be performed using the Encapsulator B-390 due to the temperature controlled nozzle, which helps maintain the temperature above the solidification point of the fat. The loading of the core material within the capsules can be up to 30%. With the different nozzle sizes available for the Encapsulator the capsule size can be chosen in the range of 400 - 2200 µm.

9.4.7 References

This application was developed in-house by BÜCHI Labortechnik AG. For more information please contact www.buchi.com.

9.5 Producing large Ca-alginate microbeads

Encapsulator B-390/B-395 Pro: Production of large Ca-alginate micro-beads with a size of > 1500 µm

9.5.1 Introduction

Ca-alginate micro-beads can be used to encapsulate various kinds of materials. This method enables the encapsulated substance (termed encapsulant) to be protected from many different environmental conditions such as oxygen, heat, pH etc., and can help prolong its shelf life.

The following example substances can be encapsulated using this method:

- Cells (yeast, bacteria and animal cells)
- APIs (hydrophobic)
- Essential oils & hydrophobic liquids
- Flavors & fragrances
- Vitamins & minerals
- Bioactive materials
- Enzymes
- Detergents
- Cosmetics

Aim: To produce Ca-alginate microbeads using the Encapsulator B-390/B-395 Pro with a size > 1500 µm.

General rule: Final micro-bead diameter is roughly twice the size of the selected nozzle size.

9.5.2 Equipment

- Instrument: Encapsulator B-390/B-395 Pro
- Set up: Single nozzle system – 1000 µm nozzle
- Pumping: Syringe pump/air pressure system
- Blender: Any kind of blender can be used

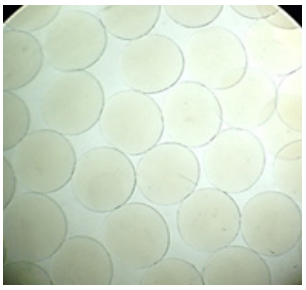


Figure 9.5-2: Microscopic image at 40X displaying the Ca-alginate micro-beads produced by the BUCHI Encapsulator B-390 using the described procedure.

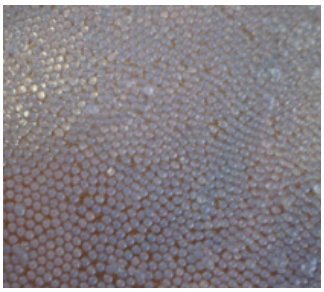


Figure 9.5-3: Digital camera image displaying the produced Ca-alginate micro-beads.

9.5.3 Chemicals and Materials

- Polymer: 2% (w/v) Na-alginate (BUCHI)
- Gelling sol: 100 mM $CaCl_2$
- Deion. water

9.5.4 Procedure and Parameters

Remove pre-filter from bead producing unit. Add 4.0 g of Na-alginate powder into 200 mL of water. Use the blender to dissolve the Na- alginate completely (Fig. 9.5-1). Let the solution sit until it is clear and has released all the air within it. The air bubbles can be removed quicker by placing in a sonication bath or placing under vacuum.

Begin production (hardening) after obtaining a stable droplet chain. Use electrostatic charge to disperse droplets and prevent collision (coalescence) before they enter the gelling bath. Deliver 50 mL of alginate through nozzle into gelling bath. Allow to harden for 30 min (T=0 when last drop lands in gelling bath). Wash the beads with plenty of water.

Process parameters

· Flow rate	30 - 35 mL/min
· Frequency	50 - 70 Hz
· Pressure	0.5 bar
· Amplitude	9
· Charge	> 2000 V

9.5.5 Results

· Amount extruded	32 mL
· Yield	> 99%
· Morphology	Spherical
· Size	1870 µm
· Std. Dev.	± 5.2%

9.5.6 Conclusion

Due to the parameters employed, very large droplets are produced using this procedure. These large particles can be observed in the strobe light during jet break up.
In most cases the electrostatic charge employed will not be able to disperse the droplets in the chain due to their large size. On the other hand it will help stabilize the drops in the chain, which will prevent coalescence occurring. After turning on the charge the jet will appear to be deflected off center, however this is very common occurrence and is due to the applied charge. This deflection is helpful in obtaining mono-dispersed particles of equal size and shape.

9.5.7 References

This application was developed in-house by BÜCHI Labortechnik AG. For more information please contact www.buchi.com.

9.6 Producing cellulose sulphate microcapsules

Encapsulator B-390/B-395 Pro: Production of cellulose sulphate-polyDADMAC microcapsules for application within medical, biotech and pharmaceutical fields

9.6.1 Introduction

Cellulose sulphate is a biocompatible ester and microcapsules are obtained by dripping solutions of the polymer into a precipitation bath containing the synthetic polycation poly diallydimethylammonium chloride (polyDADMAC). This results in a rapid electrostatic interaction at the interface between the two oppositely charged polymers, cumulating in the formation of a mechanically stable hydrogel membrane in a single step process. These microcapsules have excellent mechanical properties due to strongly interacting sulphate groups and do not initiate immune response. In addition membranes have a homogenous structure and a narrow pore size, and the latter can be pre-determined.
The method is mainly used for the encapsulation of animal and stem cells for medical and biotechnological applications, and has being used in clinical trials for transplantation of cells within humans to treat many diseases. It can also be used for encapsulating API's.

Aim: To produce cellulose sulphate-polyDADMAC microcapsules using a single step process compared to other cell encapsulation process (alginate-poly-L-lysine) which require multiply steps.

9.6.2 Equipment

- Product: Encapsulator B-390/B-395 Pro
- Set up: Single-flow nozzle system – 300 µm nozzle
- Pumping: Syringe pump/air pressure system
- Blender

9.6.3 Chemicals and Materials

- Polymer: 2% (w/v) Cellulose sulphate (Biorefinary, Germany)
- Gelling sol: 4% PolyDADMAC (Mw 35 kDa) (Biorefinary, Germany)
- Deion. water

9.6.4 Procedure and Parameters

Remove pre-filter from bead producing unit.

Dissolve 2 g of cellulose-sulphate in 100 mL of water. Use the blender to dissolve the cellulose sulphate completely. Let the solution sit until it is clear and has released all the air within it. The air bubbles can be removed quicker by placing in a sonication bath or placing under vacuum.

Add 8 g of PolyDADMAC (Mw 35 kDa) into 200 mL of water and stir until it is completely dissolved.

Materials which are to be encapsulated are added to the cellulose sulphate after it has been mixed. During capsule production these materials become entrapped (encapsulated) within the cellulose sulphate core, which is completely enveloped with the polyDADMAC membrane.

Use 20 mL of the Cellulose-sulphate solution. Begin production (hardening) after obtaining a stable droplet chain of droplets. Use electrostatic charge to disperse droplets and prevent collision. After cellulose sulphate droplets have landed in polyDADMAC bath, allow to harden for at least 30 min (T=0, when last drop lands in gelling bath). Wash capsules with copious amount of water to remove any unreacted polyDADMAC, which may be present around the particles.

9.6.5 Process parameters

- Flow rate 8 mL/min
- Frequency 400 - 600 Hz
- Pressure 0.5 bar
- Amplitude 5
- Charge > 1000 V

9.6.6 Result

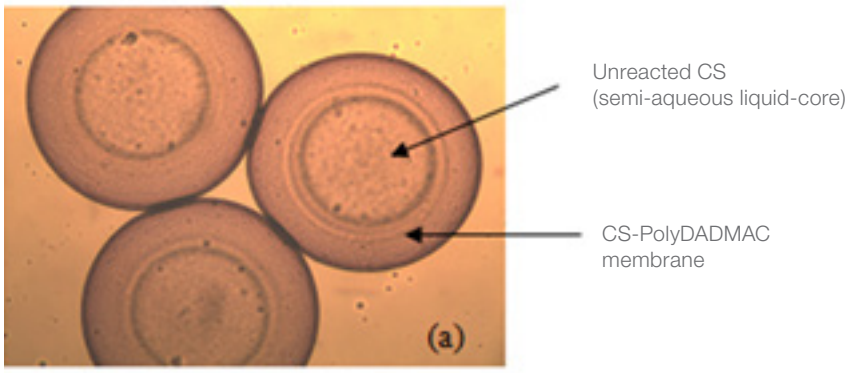


Figure 9.6-6: Cellulose sulphate-polyDADMAC semi-aqueous liquid-core microcapsules produced by dropping cellulose sulphate droplets into a solution of polyDADMAC.

- Amount Extruded 20 mL
- Yield > 99%
- Morphology Spherical
- Size 745 µm
- Std. Dev. ± 2.3%

9.6.7 Conclusion

The Encapsulator B-390 and B-395 Pro are able to produce spherical cellulose sulphate-polyDADMAC microcapsules in a single step process with the produced particles having an extremely narrow size distribution. These capsules can also be produced under sterile conditions by using the reaction vessel of the B-395 Pro for encapsulation of animal and stem cells for bio-medical applications.

Many studies have shown that these types of capsules function more optimally compared to alginate-poly-L-lysine capsules system as they do not initiate immune response and form a more stable structure.
With the different nozzle sizes available for the Encapsulator the cellulose sulphate capsule size can be chosen in the range of 400 - 2500 µm and capsule size can also be varied by using different molecular weight polyDADMAC.

9.6.8 References

Dautzenberg, H. et al. (1999). Development of cellulose sulphate-based polyelectrolyte complex microcapsules for medical applications, P. 46-63. In D. Hunkeler, et al., (Ed). Bioartifical Organs II: Technology, Medicine and Materials. New York Acad Sciences, New York (1999).

9.7 Encapsulation within gelatin beads

Encapsulator B-390: Encapsulation of water soluble materials within gelatin microbeads in a single step production process

9.7.1 Introduction

Gelatin is a natural organic protein that can be used to create beads and capsules. It dissolves in warm water (> 35 °C) and forms a homogenous solution. Upon cooling the liquid gelatin hardens to form an elastic gel. Gelatin is one of the most important polymers for the production of beads and capsules for application in the pharmaceutical industry where it is used to encapsulate many active ingredients. The material is also used abundantly in food (including nutraceuticals) and feed industries for encapsulating many different ingredients including, bioactives, vitamins, minerals and other supplements.
Aim: To produce gelatin beads encapsulating water soluble materials by using the Encapsulator B-390.

Note: The main advantage of the BUCHI Encapsulator compared to other production techniques is that it can produce gelatin beads incorporating an active (ingredient) in a single-step process. For other production techniques, a two-step process is usually used, where firstly the gelatin beads are produced, and in a second step they are filled with the material.

9.7.2 Equipment

- Instrument: Encapsulator B-390
- Set up: Single-flow nozzle system
- Pumping: Air pressure system

9.7.3 Chemicals and Materials

- Polymer: 30% (w/v) Gelatin from porcine skin
- Gelling sol: Median chain triglyceride cooled to 10 °C
- Deion. Water

9.7.4 Procedure and Parameters

Dissolve 60 g of gelatin powder in water to a final volume of 200 mL at 65 °C. Stir in a water bath until the gelatin is completely dissolved and do not allow the solution to cool down after the gelatin has dissolved. The material to be encapsulated is added to the gelatin solution by mixing. This material becomes encapsulated within the bead structure during cooling step of the production process.

Switch on the nozzle heater of the Encapsulator B-390 and set it to 70 °C and wait until it heats up. Place the medium chain triglyceride (cooling oil) into a beaker, precool to 10 °C and maintain at this temperature during production. The oil is used to catch and cool the warm gelatin droplets and produce gelled elastic beads. Pump the gelatin solution, which is kept at 60 – 65 °C (by placing the bottle containing the solution into a water bath), through the heated nozzle to form a liquid jet, which is broken up into droplets by the vibrational frequency. The produced droplets will land into the cooling oil bath below which should be agitated at a high speed (vortex size > 2 cm) using a magnetic stirrer. The distance between the surface and bottom of the cooling oil should be at least 10 cm to allow the gelatin bead to harden sufficiently before hitting the bottom of the vessel used to hold the cooling oil.

Allow the gelatin beads to harden for 30 min in the cooling oil and make sure this liquid remains below 10 °C during the production process. Remove excess oil by filtering and/or with a paper towel. The gelatin beads can be either dried over a period of 24 - 48 h by leaving to air dry at ambient conditions or within 8 - 24 h using a BUCHI Rotavapor to evaporate the water. During the beads should be constantly rotated to prevent sticking and clumping.

9.7.5 Process parameters

- Nozzle size 1000 µm
- Flow rate 30 - 35 mL/min
- Frequency 40 Hz
- Pressure 0.4 – 0.7 bar
- Amplitude 9
- Charge ≥ 2000 V

9.7.6 Results

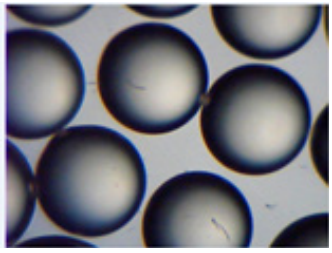


Figure 9.7-1: Image displaying wet gelatin beads of 1.9 mm which contain vitamin C.

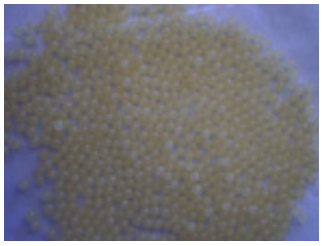


Figure 9.7-2: Image displaying the gelatin beads after drying in which their size decreases to 1 mm.

- Amount extruded 200 mL
- Yield > 99%
- Morphology spherical
- Size (wet/dry) 1900/1000 µm
- Std. Dev. ± 5%

9.7.7 Conclusion

The formation of gelatin microbeads for the encapsulation of hydrophilic materials in a single step process is possible using the Encapsulator B-390. The heated nozzle helps maintain the temperature above the solidification point of gelatin. With the different nozzle sizes available for the Encapsulator, the bead size can be chosen in the range of 200 - 2000 µm.

9.7.8 References

This application was developed in-house by BÜCHI Labortechnik AG. For more information please contact www.buchi.com.

9.8 Encapsulation of probiotics in whey protein beads

Encapsulator B-390/B-395 Pro: Encapsulation of probiotic bacteria within whey protein microbeads for probiotic protection and controlled delivery within the intestine

9.8.1 Introduction

This study [1] evaluated the efficacy of whey protein isolate as an encapsulation matrix to produce microbeads for the maintenance of probiotic bacteria (Lactobacillus rhamnosus GG) viability in simulated gastro-intestinal conditions. Liquid whey is the principle by-product of the cheese manufacturing process and contains lactose, minerals and proteins. It can be filtered to obtain a whey protein isolate which contains high concentrations of lactalbumin. Heat denaturing of the ß-lactalbumins within the whey protein isolate triggers hydrophobic interactions with other proteins and leads to the formation of soluble gel structure, which can be used to produce beads and capsules. In recent years numerous studies have demonstrated the ability to encapsulate drugs, nutrients, bioactive peptides and probiotic bacteria within whey-based bead/capsules to provide protection and enhance delivery.

9.8.2 Equipment

- Instrument: Inotech Encapsulator*
- Set up: Single-flow nozzle system – 150 µm nozzle
- Pumping: Syringe pump

*Precursor model to BUCHI Encapsulator models B-390/B-395 Pro

9.8.3 Chemicals and Materials

- Polymer: 11% w/v whey protein isolate (Davisco Foods International)
- Gelling sol: Na-acetate buffer (0.5 M) pH 4.6 at 35 °C
- Encapsulant: Lactobacillus rhamnosus GG
- Deion. water

9.8.4 Procedure and Parameters

Dissolve 11 g of whey protein in 100 mL of water, heat the mixture to 78 °C for 45 min under agitation to dissolve the protein completely. Cool on ice and store at 4 °C overnight before use. Before encapsulation experiments add Lactobacillus rhamnosus GG at a concentration of 109 cfu/ml into the prepared whey protein solution. This solution will then be extruded through the nozzle to form the whey protein beads.

Begin production after obtaining a stable chain of whey droplets containing the probiotic bacteria and use the electrostatic charge to disperse the droplets and prevent coalescence. After droplets have landed in gelling bath (Na-acetate buffer) allow to harden for 30 min. Wash the beads twice with distilled water to remove any unreacted material.

9.8.5 Process parameters

- Flow rate 2.3 - 2.8 mL/min
- Frequency 800 - 1800 Hz
- Pressure 0.5 bar
- Amplitude 1 - 3
- Charge 1500 V

9.8.6 Results

The production of mono-dispersed whey protein microbeads encapsulating probiotic bacteria was developed in this work [1]. Optimization of encapsulation conditions generated self-supporting microbead structures (200 ± 1.2 µm) with an individual loading capacity of 2.7 x 104 cfu / microbead. The most stable structures were obtained within the acetate buffer at pH 4.6.

9.8.7 Conclusion

In this study [1] it was demonstrated that the technique employed had no detrimental effect on cell viability during bead production. Following production the probiotic-containing microbeads were incubated under simulated gastro-intestinal conditions. It was subsequently shown that incubation of the microbeads under conditions associated with the stomach (pH 1.8; 37 °C) demonstrated acid-stability and peptic-resistance of the beads, which helped maintain viability of the encapsulated bacteria under these conditions. However incubation under intestinal conditions resulted in the rapid breakdown (due to enzyme activated intestinal conditions) of the bead structure, hence enabling the controlled release of the probiotic bacteria into the surrounding environment.

9.8.8 References

[1] Doherty S.B., et. al. (2011). Development and characterization of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocolloids 25:1604-1617.

9.9 Encapsulation of Methotrexate in alginate and hyaluronic acid microcapsules for controlled release in cancer treatment

9.9.1 Introduction

In recent years it has being shown in numerous studies how microbeads produced by the BUCHI Encapsulator can be used as an effective delivery system for the controlled release of drugs and bioactives [1,2,3].

In the presented study of Genc & Butuktiryaki, Methotrexate (MTX) was encapsulated in alginate-hyaluronic acid microbeads which were prepared using the Inotech Encapsulator (precursor of the BUCHI Encapsulators) [4]. MTX is an anti-metabolite and anti-folate drug used in treatment of cancer, autoimmune diseases, ectopic pregnancy, and for the induction of medical abortions. It acts by inhibiting the metabolism of folic acid.

Encapsulation of MTX provides a mechanism for the controlled release of the drug; enabling the release of the required dose while also preventing interaction of the drug with healthy tissue (reduces toxic side-effects) [4]. Encapsulation also protects the drug from oxygen, pH changes and other unfavorable conditions which can cause the drug to degrade before reaching its target area.
The effect of different nozzle diameters and MTX concentrations on encapsulation efficiencies and the characteristics of the produced capsules were examined as well as the ability of the microcapsules to effectively deliver MTX to a cancer cell line (5RP7).

Aim: Development of a microencapsulation system for the controlled delivery of MTX to cancer cells

9.9.2 Equipment

- Instrument: Inotech Encapsulator IE-50R*
- Set up: Single nozzle system
- Pumping: Syringe pump

*Precursor model to the BUCHI Encapsulator models B-390/B-395 Pro.

9.9.3 Chemicals and Materials

Chemicals:

- Polymer: Sodium alginate and hyaluronic acid in deion. water
- Gelling sol: 100 mM $CaCl_2$
- Encapsulant: Methotrexate

9.9.4 Procedure and Parameters Procedure

Suspend the required amount of MTX in water and then add to a solution of 1.5% sodium alginate and 100 ppm hyaluronic acid. This solution is pumped and extruded through the selected nozzle size to produce droplets, which are converted into MTX-loaded microbeads after landing in a solution of $CaCl_2$.

Begin bead production after obtaining a stable chain of droplets and use the electrostatic charge to disperse the droplets and prevent coalescence. After droplets have landed in gelling bath allow to harden for 30 min. After hardening remove from CaCl2 and wash several times with HPLC.

Process parameters

- Nozzle sizes 200 & 400 µm
- Flow rate -
- Frequency 400 - 900 Hz
- Amplitude 1 - 4
- Charge 1000 - 2500 V

9.9.5 Results

MTX-loaded microcapsules

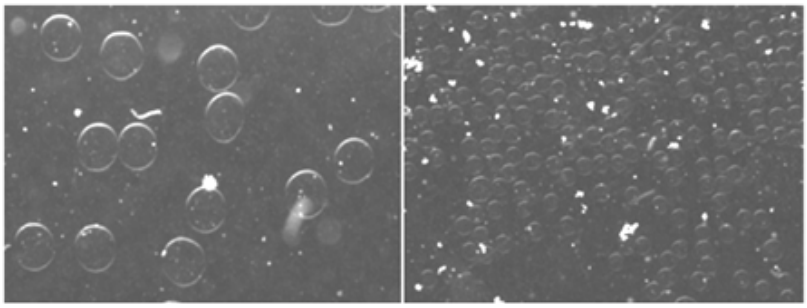


Figure 9.9-1: Light microscope image displaying MTX-loaded microbeads which were produced in this study [4].

- Sizes (nozzle size) 400 (200) & 800 (400) µm
- Morphology Spherical
- Encapsulation efficiency 76-89%

Encapsulation efficiency

The encapsulation efficiency (% of drug remaining in capsule after production) of MTX in microcapsules as a function of nozzle size and initial drug concentration was examined to determine optimal conditions to obtain maximum drug loading. From Fig. 9.9-2 it can be observed that the best encapsulation efficiencies for MTX loading were achieved using bigger nozzles and higher concentrations of MTX. For the biggest nozzle size (400 µm) used in this study it was possible to achieve an encapsulation efficiency off up to 89% in the alginate/hyaluronic acid microcapsules.

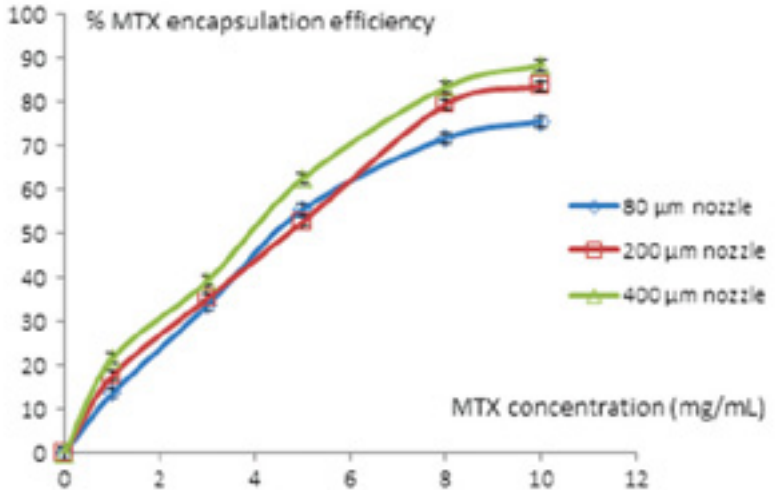


Figure 9.9-2. MTX-loading efficiencies in microbeads as a function of nozzle size and initial drug concentration [4].

In vitro release of MTX

The in vitro release profile of encapsulated MTX from microcapsules was examined and the results are shown in Fig. 9.9-3. From the results it can be seen that it was possible to control the release of MTX from microcapsules over 30 hours, which wasn't possible for un-encapsulated MTX. Using the developed encapsulation system it was possible to deliver between 68-79% of the encapsulated drug in a controllable manner, with bigger particles delivering higher amounts.

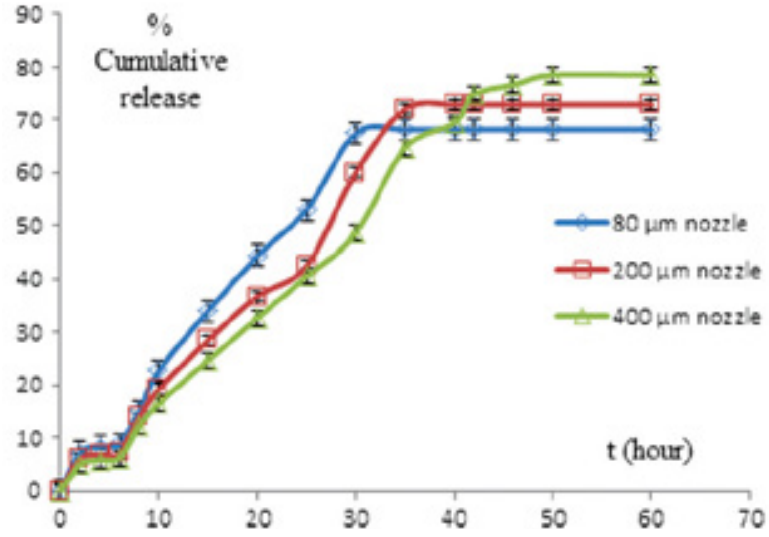


Figure 9.9-3. In vitro release of MTX from microcapsules as a function of size [4].

Cytotoxicity on a cancer cell line

In vitro cytotoxicity effects of MTX-loaded microbeads formulations on 5RP7 (rat fibroblast cancer cell line) was also examined. From the results it was observed that increasing the dose of microcapsules resulting in higher cell death of the cancer cell line.

9.9.6 Conclusion

This study has shown how microencapsulation can be used to control the release of MTX to cancerous cells and reduce its toxic effect on healthy tissue. Using the microcapsule system developed on the Encapsulator it was possible to obtain an MTX encapsulation efficiency of up to 89% while also effectively controlling the release rate of the drug from the microbeads over a 30 h period. In cell culture studies delivery of the encapsulated MTX reduced cancer cell viability by up to 88.5% compared to 49.7% for un-encapsulated MTX.

9.9.7 References

[1] Doherty S.B., et. al. (2011). Development and characterization of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocolloids 25:1604-1617.

[2] Dorati, R et. al. (2013). Microencapsulation of a hydrophilic model molecule through Vibration nozzle and emulsion phase inversion technologies 30(6):559-570.

[3] Hoad, C. (2011) Encapsulation of lipid by alginate beads reduces bio-accessibility: An in vivo 13C breath test and MRI study. Food Hydrocolloids 25:1190-1200.

[4] Genc, L. & Butuktiryaki S (2014). Preparation and characterization of methotrexate-loaded microcapsules. Pharmaceutical development & technology 19(1): 42-47.

Acknowledgments
The author would like to express his gratitude his colleagues Philipp John, Marco Meuri, Dr. Raphael Plüss, Dr. David Rütti and Dr. Simon Diezi for their invaluable inputs, suggestions and proof-reading of the manuscript. Additional thanks are conveyed to Dr. Raphael Plüss and Dr. David Rütti for their scientific input and expertise. Christophe Waeber and Sanami Bruderer from BUCHI Global Communications department are acknowledged for preparing the printed version of the manuscript.

Dr. Micheal Whelehan

Imprint

Author	Dr. Micheal Whelehan
Publisher	BÜCHI Labortechnik AG, CH-9230, Flawil, Switzerland
Copyright	© 2014 by BÜCHI Labortechnik AG, CH-9230, Flawil, Switzerland

Quality in your hands

BUCHI Affiliates:

BÜCHI Labortechnik AG
CH – 9230 Flawil 1
T +41 71 394 63 63
F +41 71 394 65 65
buchi@buchi.com
www.buchi.com

BUCHI Italia s.r.l.
IT – 20010 Cornaredo (MI)
T +39 02 824 50 11
F +39 02 57 51 28 55
italia@buchi.com
www.buchi.it

BUCHI Russia/CIS
United Machinery AG
RU – 127787 Moscow
T +7 495 36 36 495
F +7 495 981 05 20
russia@buchi.com
www.buchi.ru

Nihon BUCHI K.K.
JP – Tokyo 110-0008
T +81 3 3821 4777
F +81 3 3821 4555
nihon@buchi.com
www.nihon-buchi.jp

BUCHI Korea Inc
KR – Seoul 153-782
T +82 2 6718 7500
F +82 2 6718 7599
korea@buchi.com
www.buchi.kr

BÜCHI Labortechnik GmbH
DE – 45127 Essen
Freecall 0800 414 0 414
T +49 201 747 490
F +49 201 747 492 0
deutschland@buchi.com
www.buechigmbh.de

BÜCHI Labortechnik GmbH
Branch Office Benelux
NL – 3342 GT
Hendrik-Ido-Ambacht
T +31 78 684 94 29
F +31 78 684 94 30
benelux@buchi.com
www.buchi.be

BUCHI China
CN – 200052 Shanghai
T +86 21 6280 3366
F +86 21 5230 8821
china@buchi.com
www.buchi.com.cn

BUCHI India Private Ltd.
IN – Mumbai 400 055
T +91 22 667 75400
F +91 22 667 18986
india@buchi.com
www.buchi.in

BUCHI Corporation
US – New Castle,
Delaware 19720
Toll Free: +1 877 692 8244
T +1 302 652 3000
F +1 302 652 8777
us-sales@buchi.com
www.mybuchi.com

BUCHI Sarl
FR – 94656 Rungis Cedex
T +33 1 56 70 62 50
F +33 1 46 86 00 31
france@buchi.com
www.buchi.fr

BUCHI UK Ltd.
GB – Oldham OL9 9QL
T +44 161 633 1000
F +44 161 633 1007
uk@buchi.com
www.buchi.co.uk

BUCHI (Thailand) Ltd.
TH – Bangkok 10600
T +66 2 862 08 51
F +66 2 862 08 54
thailand@buchi.com
www.buchi.co.th

PT. BUCHI Indonesia
ID – Tangerang 15321
T +62 21 537 62 16
F +62 21 537 62 17
indonesia@buchi.com
www.buchi.co.id

BUCHI do Brasil
BR – Valinhos SP 13271-570
T +55 19 3849 1201
F +41 71 394 65 65
latinoamerica@buchi.com
www.buchi.com

BUCHI Support Centers:

South East Asia
BUCHI (Thailand) Ltd.
TH-Bangkok 10600
T +66 2 862 08 51
F +66 2 862 08 54
bacc@buchi.com
www.buchi.com

Latin America
BUCHI Latinoamérica Ltda.
BR – Valinhos SP 13271-570
T +55 19 3849 1201
F +41 71 394 65 65
latinoamerica@buchi.com
www.buchi.com

Middle East
BUCHI Labortechnik AG
UAE – Dubai
T +971 4 313 2860
F +971 4 313 2861
middleeast@buchi.com
www.buchi.com

BÜCHI NIR-Online
DE – 69190 Walldorf
T +49 6227 73 26 60
F +49 6227 73 26 70
nir-online@buchi.com
www.nir-online.de

We are represented by more than 100 distribution partners worldwide.
Find your local representative at: www.buchi.com

11595052 en 1408 A /Technical data are subject to change without notice/Quality Systems ISO 9001.
The English version is the original language version and serves as basis for all translations into other languages.