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ORIGINAL PAPER

Comparison of different technologies for alginate beads production

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In memory of our colleague Stefan Rosinski

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This paper describes the results of the round robin experiment "Bead production technologies" carried out during the COST 840 action "Bioencapsulation Innovation and Technologies" within the 5th Framework Program of the European Community. In this round robin experiment, calcium alginate hydrogel beads with the diameter of (800 ± 100) μm were produced by the most common bead production technologies using 0.5–4 mass % sodium alginate solutions as starting material. Dynamic viscosity of the alginate solutions ranged from less than 50 mPa s up to more than 10000 mPa s. With the coaxial air-flow and electrostatic enhanced dropping technologies as well as with the JetCutter technology in the soft-landing mode, beads were produced from all alginate solutions, whereas the vibration technology was not capable to process the high-viscosity 3 % and 4 % alginate solutions. Spherical beads were generated by the electrostatic and the JetCutter technologies. Slightly deformed beads were obtained from high-viscosity alginate solutions using the coaxial air-flow and from the 0.5 % and 2 % alginate solutions using the vibration technology. The rate of bead production using the JetCutter was about 10 times higher than with the vibration technology and more than 10000 times higher than with the coaxial air-flow and electrostatic technology.

Keywords: droplet generation, coaxial air-flow, electrostatic, vibration, JetCutter, alginate beads

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Table 1. Laboratories and applied technologies involved in the round robin experiments for the production of alginate beads

Laboratory	Town	Bead production technology
Slovak Academy of Sciences	Bratislava	Coaxial air-flow enhanced dropping
Polish Academy of Sciences	Warsaw	Electrostatic enhanced dropping
University of Belgrade	Belgrade	Electrostatic enhanced dropping
ENITIAA	Nantes	Vibration
University of Perugia	Perugia	Vibration
Institute Meurice	Brussels	Vibration, JetCutter
vTI / geniaLab GmbH	Braunschweig	JetCutter

Introduction

The encapsulation of various materials and living cells inside beads for different purposes in the pharmaceutical, chemical, or food industry as well as in agriculture, biotechnology, or medicine is of great importance. Despite the broad application of bead-encapsulated materials, the knowledge of the technologies for bead production and their advantages, restrictions, and disadvantages is limited. From literature, it is not easy to extract the best technology available for the special encapsulation purpose as the different technologies have not been compared so far using the same material.

In order to provide a potential user with comparable data on different common bead production technologies, several members of the Working Group 3 within the COST 840 action "Bioencapsulation Innovation and Technologies" within the 5th Framework Program of the European Community carried out the round robin experiment "Bead production technologies" in which beads in a definite diameter range were produced by the most common technologies using the same polymer solutions as the starting material.

Dispersion of a liquid fluid into droplets, which are afterwards solidified by physical or chemical means, is one of the major means of solid beads formation. Despite emulsification (Neufeld & Poncelet, 2004; Poncelet et al., 2002), the main technologies for fluid dispersion into droplets are coaxial air-flow enhanced dropping (Anilkumar et al., 2001; Bučko et al., 2005; Vorlop & Klein, 1983), electrostatic enhanced dropping (Bugarski et al., 2004, 1994; Lewińska et al., 2004; Manojlovic et al., 2006; Nedovic et al., 2002; Poncelet et al., 1999a, 1999b), vibration (Heinzen et al., 2002, 2004), atomization by a rotating disc or rotating nozzles (Ogbonna, 2004), and JetCutter (Prüsse & Vorlop, 2004). Four of these technologies, the coaxial air-flow, electrostatic, vibration, and JetCutter technology, were used for bead production in this round robin experiment. Except the coaxial air-flow technique, each technique was used in at least two different laboratories to obtain further information on the transferability and reproducibility of the results. The participating laboratories and their applied techniques are displayed in Table 1.

In addition, schemes of the technologies used are shown in Fig. 1. Generation of droplets by the coaxial air-flow and the electrostatic technique is based on the formation of single droplets at the nozzle tip. Either by an additional air-flow through an outer concentric nozzle or by an electric field, dropping is enhanced. By variation of the nozzle diameter, the flow rate and the applied air-flow or electric field, respectively, the droplet diameter can be adjusted. Comprehensive descriptions of both technologies can be taken from literature, e.g. Vorlop & Klein (1983) for the coaxial air-flow and Bugarski et al. (2004, 1994), Lewińska et al. (2004), Nedovic et al. (2002), and Poncelet et al. (1999a, 1999b) for the electrostatic technique.

Droplet generation by the vibration technology is achieved applying vibrations on a laminar fluid jet which breaks apart definitely if the right wavelength is applied. The bead size is adjusted mainly by the nozzle diameter and the wavelength although also other parameters, e.g. viscosity, influence the droplet formation (Heinzen et al., 2002, 2004).

Droplet generation by the JetCutter technology is based on a mechanical cut of a liquid jet by rotating cutting wires installed in a cutting tool. The droplet size mainly depends on the nozzle diameter, rotation frequency of the cutting tool, number and diameter of the wires as outlined in detail in (Prüsse et al., 2000, 2002; Prüsse & Vorlop, 2004). During a JetCutter operation, a part of the processed fluid, so-called losses, is slung aside where it can be gathered and recycled. In a usual operation mode, these losses amount to less than 5 % of the processed fluid, under optimized conditions, they can be reduced to less than 1 %. The JetCutter may be operated in two different modes called the normal mode and the soft-landing mode (Fig. 2). In the normal mode, the trajectory of the beads is rather direct from top-down, whereas in the soft-landing mode, it is diagonal from bottom-up. In the soft-landing mode, the velocity of the beads is dramatically reduced near the zenith of the trajectory where they are collected. Hence, the speed at which the beads enter the liquid collection bath is rather low and the risk of bead deformation is reduced.

For this round robin experiment, the production of calcium alginate beads from sodium alginate solutions was chosen as the model system as alginate beads are

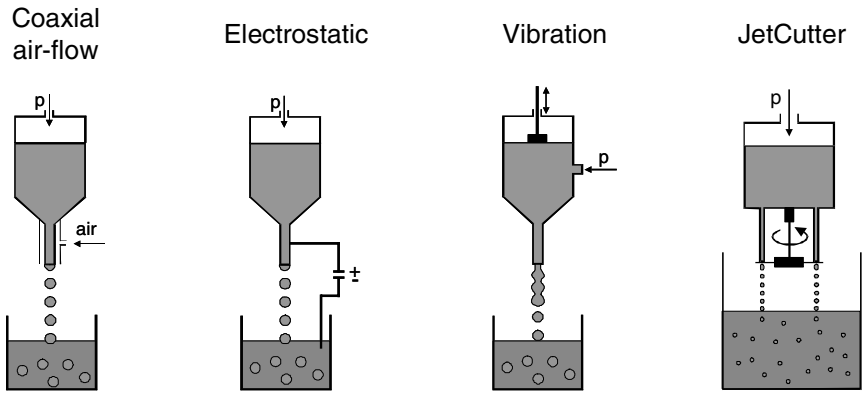

Fig. 1. Scheme of the bead production technologies involved in the round robin experiment for the production of alginate beads.

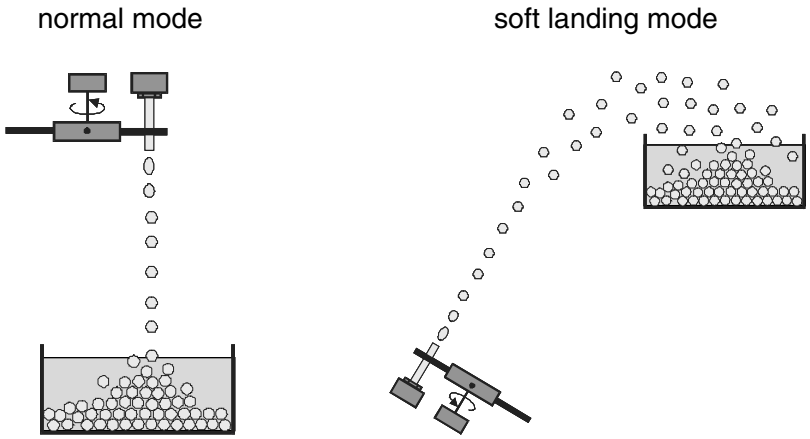

Fig. 2. Scheme of the two JetCutter modes for bead production.

still one of the most widely used encapsulation matrices (Melvik & Dornish, 2004; Strand et al., 2004). Each of the applied techniques is capable of processing alginate solutions under aseptic conditions, e.g. Lacík et al. (1998) for the coaxial air-flow technique, Rosinski et al. (2002) for the electrostatic technique, Heinzen et al. (2002) for vibration, and Schwinger et al. (2002) for the JetCutter. However, no aseptic conditions were applied in the present study. In particular, alginate solutions with various sodium alginate concentrations up to 4 mass % were processed to check the ability of the technologies to process fluids of different viscosity. (800 ± 100) μm was the targeted bead size as in beads between 500–1000 μm, a low mass-transfer limitation with good separation properties are combined. The resulting beads were characterized with regard to their roundness and bead size distribution. Further on, the different bead production technologies were compared regarding the production rate of the beads.

Experimental

A dry powder of sodium alginate, type Protanal LF20/40 purchased from FMC BioPolymer, Drammen, Norway, was used for the preparation of the alginate solutions. In order to ensure comparable conditions, portions of 200–300 g of the sodium alginate powder were taken from the same sample and were shipped to each laboratory taking part in this round robin experiment. In each laboratory, solutions of 0.5 mass %, 1.0 mass %, 2.0 mass %, 3.0 mass %, and 4.0 mass % of sodium alginate were prepared by dissolving the alginate powder in distilled/deionized water at room temperature. Only freshly prepared alginate solutions were used for the determination of viscosity and for the production of beads.

In various laboratories, dynamic viscosity η of freshly prepared sodium alginate solutions was determined at 30 °C (Bratislava, Warsaw, Nantes, and Belgrade) and at 35 °C (Brussels). The following rheometers were used for the viscosity measurements: Brookfield DV-II+ (Brookfield, USA) in Bratislava, Rheomat RM 180 (Mettler, Germany) in Warsaw, Viscotester VT 500 (Haake, Germany) in Nantes, Viscosimeter RI:2:L (Rheology International, Ireland) in Brussels, and Rheometrics RMS-605 (Rheometric Scientific, USA) in Belgrade.

Droplets of the sodium alginate solution generated by different technologies were allowed to enter a stirred

solution consisting of 2 mass % of calcium chloride in distilled/deionized water, which is further referred to as the collection bath. In the calcium chloride solution, gelation of sodium alginate droplets to calcium alginate hydrogel beads occurred (Melvik & Dornish, 2004; Strand et al., 2004). Both the droplet generation and the gelation were carried out at room temperature. After beads were produced from the different sodium alginate solutions, they were stored in a refrigerator for two days in the same calcium chloride solution before being characterized with regard to bead size and shape. The two-day storage procedure ensured that the gelation process was finished and no further shrinkage of the beads occurred. The low storage temperature prevented microbiological degradation of the beads.

The targeted size of the calcium alginate beads after complete shrinkage was (800 ± 100) μm.

Bead production by the coaxial air-flow technology (Bratislava)

A home-made coaxial air-flow device equipped with a stroboscopic light for droplet quality control was applied in Bratislava. The same single coaxial nozzle was used for the production of beads from all alginate solutions. It consisted of an inner needle, 0.4 mm in inner diameter and 0.7 mm in outer diameter, and an outer needle, for compressed air flow, 1.5 mm in inner diameter. The alginate flow was adjusted by applying pressure. The distance between the nozzle and the collection bath was approximately 120 mm.

Bead production by the electrostatic technology (Warsaw, Belgrade, Braunschweig)

The electric field was installed between the stainless steel needle and the collection bath employing a high-voltage unit of proprietary construction operating in a pulse mode (Warsaw). Regulated electric parameters included: applied voltage U (in the range of 0–25 kV, stepwise), duration time of the voltage impulse τ (in the range of 1–9 ms, stepwise) and frequency of impulse application f (in the range of 1–200 Hz, stepwise). The nozzle was additionally equipped with a thin stainless steel plate (diameter of about 4 cm) to increase the uniformity of the static electric field between the nozzle tip and the gelation bath. For the production of beads from the different alginate solutions, the inner needle diameter ($\varnothing_{\mathrm{in}}$) and the applied voltage (U) were optimized to obtain the desired bead size. For solutions of 0.5 mass % of sodium alginate the settings were: $\varnothing_{\mathrm{in}} = 0.44$ mm, $U = 8$ kV, $f = 10$ Hz; for 1 mass % of sodium alginate: $\varnothing_{\mathrm{in}} = 0.44$ mm, $U = 8$ kV, $f = 5$ Hz; for 2 mass % of sodium alginate: $\varnothing_{\mathrm{in}} = 0.44$ mm, $U = 13$ kV, $f = 50$ Hz; for 3 mass % of sodium alginate: $\varnothing_{\mathrm{in}} = 0.33$ mm, $U = 17$ kV, $f = 5$ Hz; and for 4 mass % of sodium alginate: $\varnothing_{\mathrm{in}} = 0.28$ mm, $U = 15$ kV and $f = 5$ Hz. The duration time of the voltage impulse τ was adjusted to 5 ms for all experiments, except the last one. For the solution containing 4 mass % of alginate, $\tau = 6$ ms was applied. The alginate flow was adjusted by applying pressure. The distance between the nozzle and the collection bath was adjusted to 20 mm.

The experiments employing the electrostatic technology were also carried out partly in Belgrade and partly in Braunschweig. The electric field was installed between the needle and the collection bath by a high voltage unit Model 30R (Bertan Associates, USA) for 0.5 % and 1 % alginate solutions in Belgrade or Model T1CP 300 304p (iseg Spezialelektronik, Germany) for 2 %, 3 %, and 4 % alginate solutions in Braunschweig. For the production of beads from the different alginate solutions, the inner needle diameter ($\varnothing_{\mathrm{in}}$) and the applied voltage (U) were optimized to obtain the desired bead size. For alginate solutions of 0.5 mass % of sodium alginate: $\varnothing_{\mathrm{in}} = 0.41$ mm and $U = 4.6$ kV, for the 1 % alginate solution: $\varnothing_{\mathrm{in}} = 0.41$ mm and $U =$ 5 kV; for solutions containing 2 mass % and 3 mass % of alginate: $\varnothing_{\mathrm{in}} = 0.25$ mm and $U = 6$ kV; and for 4 % alginate solution: $\varnothing_{\mathrm{in}} = 0.15$ mm and $U = 4$ kV. The alginate flow during experiments was adjusted by a syringe pump Model R100-E (Razel Scientific Instruments, USA) in Belgrade and Model KDS100 (KD Scientific, USA) in Braunschweig. The distance between the nozzle and the collection bath was between 20–30 mm, except for the production of beads from the 4 mass % alginate solution, where it was adjusted to 8 mm.

Bead production by the vibration technology (Nantes, Perugia, Brussels)

Bead production by the vibration technology was performed in Nantes on an Inotech Encapsulator IER-20 (Inotech, Switzerland) using a nozzle with the inner diameter of 0.4 mm and frequency of 300 Hz or 400 Hz from the 0.5 % and 1 % alginate solutions, respectively. No beads could be produced from more concentrated alginate solutions. During the beads production, an electric charge of 1 kV was additionally applied to the nozzle in order to minimize the droplet coalescence during falling as a result of the electrostatic repulsion of the equally charged droplets. The distance between the nozzle and the collection bath was adjusted to 200 mm.

In Perugia, bead production was carried out on an Inotech Encapsulator (Medical IEM-40, Inotech, Switzerland) using a nozzle with the inner diameter of 0.3 mm and frequency of 700 Hz from the 0.5 %, 1 %, and 2 % alginate solutions. No beads could be produced from more concentrated alginate solutions. No additional electric charge was applied and the distance between the nozzle and the collection bath was maintained at 800 mm.

Table 2. Dynamic viscosity η and applied shear rate D for the viscosity measurements of alginate solutions at 30 °C in different laboratories

Alginate content	Bratislava		Warsaw		Belgrade		Nantes		Brussels[a]	
	η	D	η	D	η	D	η	D	η	D
mass %	mPa s	s^{-1}	mPa s	s^{-1}	mPa s	s^{-1}	mPa s	s^{-1}	mPa s	s^{-1}
0.5	33	100	24	233	–	–	26	300	32	12
1.0	140	10	92	233	–	–	83	300	155	11
2.0	1232	1	667	129	1129	6.3	424	300	1334	1
3.0	5501	0.5	2008	23	5236	2.5	–	–	4632	20
4.0	10516	0.1	10560	10	11251	2.5	–	–	12187	10

a) Measurements carried out at 35 °C.

Bead production in Brussels was performed on an Inotech Encapsulator Research (Inotech, Switzerland) using a nozzle with the inner diameter of 0.5 mm and frequency of 240 Hz or 310 Hz from the 0.5 % and 1 % or 2 % alginate solutions, respectively. No beads could be produced from more concentrated alginate solutions. During experiments, an electric charge of 1 kV was additionally applied to the nozzle. The distance between the nozzle and the collection bath was adjusted to 110 mm.

Bead production by the JetCutter technology (Brussels, Braunschweig)

Bead production in Brussels was also performed on a JetCutter TO203-2 (geniaLab, Germany) in the normal mode (Fig. 2) using a nozzle with the inner diameter of 0.5 mm for all processed alginate solutions. The cutting tool was equipped with 48 wires of 90 μm in diameter. The rotation of the cutting tool was adjusted to 4500 min^{-1}, except for the production of beads from the 4 % alginate solution when it was adjusted to 5000 min^{-1}. The distance between the nozzle and the collection bath was between 1 m and 1.5 m depending on the deflection of the beads.

In Braunschweig, bead production was performed on a JetCutter JCS (geniaLab, Germany) in the soft-landing mode in which the collection bath was placed at the height of approximately 2 m (Fig. 2). A nozzle with the inner diameter of 0.4 mm was used for all alginate solutions. The cutting tool was equipped with 48 wires of 90 μm in diameter. The rotation of the cutting tool was adjusted to 3900 min^{-1} for the 0.5 % alginate solution, to 5000 min^{-1} for the 1 %, 2 %, and 3 % alginate solutions, and to 4500 min^{-1} for the 4 % alginate solution. The distance between the nozzle and the collection bath was between 2.5 m and 3.5 m depending on the deflection of the beads.

Characterization of the bead size and shape

In all laboratories, the beads were characterized with regard to their size and shape. Therefore, pictures of the bead samples were taken using optical microscopes. The mean bead diameter was calculated either automatically using an image analysis software or manually. In doing so, at least 20 beads of each sample were taken into account.

Results and discussion

Viscosity of alginate solutions

As outlined above, dynamic viscosity of the freshly prepared sodium alginate solutions was determined in different laboratories. Due to the non-Newtonian, pseudoplastic behavior of the sodium alginate solutions, the value of dynamic viscosity has to be connected to the shear rate at which the viscosity was measured. Thus, in Table 2, both the viscosity values and the applied shear rates are listed.

Although the values of the dynamic viscosity scatter to some extent, mainly due to the different shear rates applied, all laboratories obtained comparable results. The exponential increase of the dynamic viscosity with the increasing sodium alginate concentration is clearly visible. In this study, solutions with the sodium alginate content of up to 2 mass % will be regarded as low-viscosity solutions and those of 3 mass % and 4 mass % as high-viscosity ones.

Beads produced by the different technologies

Photographs of the beads produced by the technologies in the different laboratories under the conditions outlined in the Experimental are shown in Figs. 3–10.

Fig. 3 shows beads prepared from the different sodium alginate solutions by the coaxial air-flow technique in Bratislava. Using this technique, all chosen alginate solutions could be processed. The beads prepared from the low-viscosity sodium alginate solutions, i.e. with alginate contents of 0.5–2 mass %, are uniform in their size and they are very round. The beads prepared from high-viscosity alginate solutions, i.e. 3 mass % and 4 mass % of alginate, also show a uniform size but they are deformed. The beads originating from the 3 % alginate solution are slightly

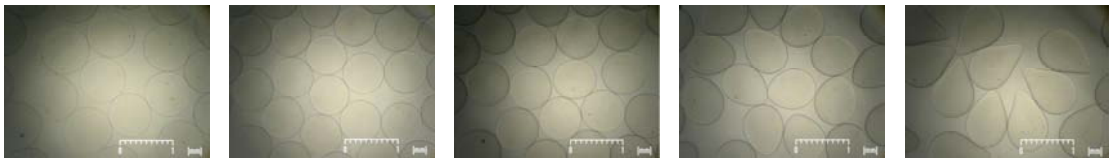

Fig. 3. Photographs of alginate beads produced in Bratislava by the coaxial air-flow technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

Fig. 4. Photographs of alginate beads produced in Warsaw by the electrostatic technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

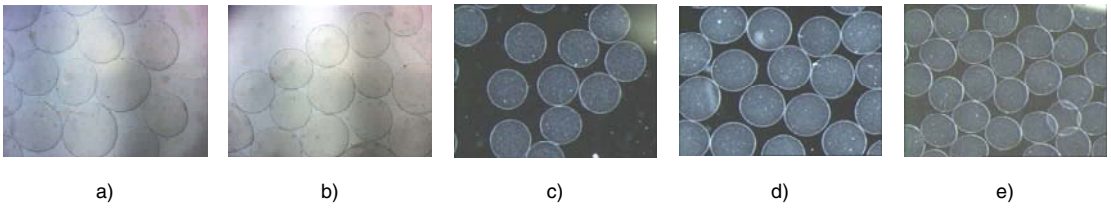

Fig. 5. Photographs of alginate beads produced in Belgrade/Braunschweig by the electrostatic technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

egg-shaped and those from the 4 % alginate solution exhibit a pronounced drop-like shape. Deformation of the beads is caused mainly by two effects, both of them originating from the higher viscosity of the alginate solutions used:

i) Due to the higher adhesion forces of the more concentrated alginate solutions at the nozzle tip, the droplets generated are already deformed to a greater extent than those formed from less concentrated solutions.

ii) Droplets which are slowly formed at the nozzle tip are stretched by the bottom-down directed air flow surrounding the nozzle. When such a stretched droplet is torn away from the nozzle, it is further accelerated by the air flow. As the surface tension driven contraction of the deformed droplets, which leads to the formation of spherically shaped beads, becomes slower with the increasing alginate solution viscosity, the time of falling of the droplets into the gelation bath is no more sufficient to reshape the deformed droplets to a sphere.

Fig. 4 shows beads prepared from the different sodium alginate solutions by the electrostatic technique in Warsaw. With this technique, all the alginate solutions could be processed. The beads prepared from all sodium alginate solutions are uniform in size and they are very round. Very similar results were obtained in Belgrade/Braunschweig (Fig. 5), where the same electrostatic technique but with a different experimental set-up was applied. It is demonstrated that the results obtained using the electrostatic technique are highly transferable and reproducible.

Figs. 6–8 show beads prepared using the vibration method in Nantes, Perugia, and Brussels, respectively. In each laboratory, round beads with a narrow size distribution could be prepared only from the 1 % sodium alginate solutions. The beads prepared from the 0.5 % alginate solution were either slightly oval in shape (Figs. 6 and 8) or they were not uniform in size (Fig. 7). From the 2 % alginate solution, beads could be produced only in Perugia (Fig. 7) and Brussels (Fig. 8). In both laboratories, the size distribution of the resulting beads was rather broad and the roundness of the beads produced in Brussels was, in addition, very poor. In none of the three laboratories using the vibration technology, beads from the high-viscosity alginate solutions could be prepared. According to the theory of droplet formation by a vibrational jet break-up (Heinzen et al., 2002, 2004), the droplet diameter was proportional to the viscosity of the processed fluid. Accordingly, with a constant nozzle diameter, beads of a definite diameter could only be pro-

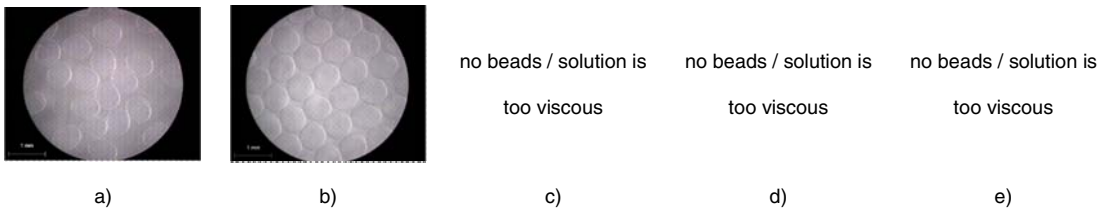

Fig. 6. Photographs of alginate beads produced in Nantes by the vibration technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

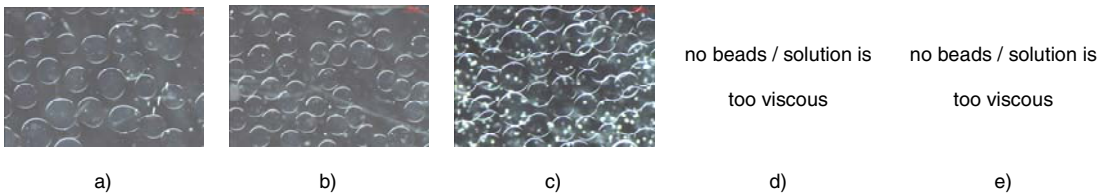

Fig. 7. Photographs of alginate beads produced in Perugia by the vibration technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

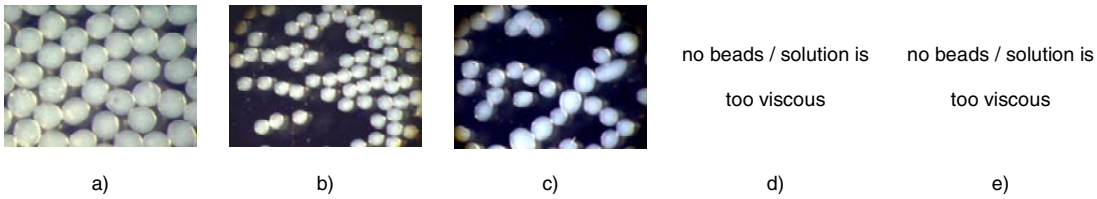

Fig. 8. Photographs of alginate beads produced in Brussels by the vibration technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

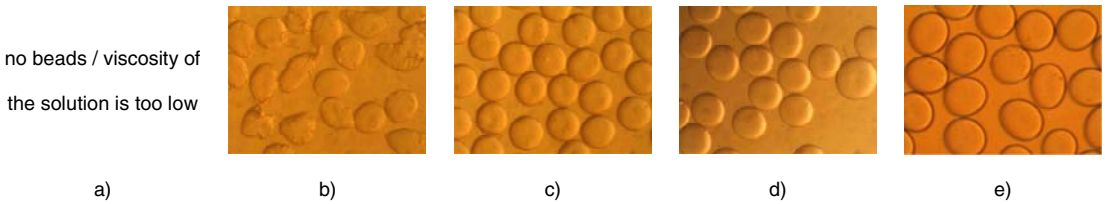

Fig. 9. Photographs of alginate beads produced in Brussels by the JetCutter technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

duced up to a corresponding upper viscosity value. For the sodium alginate system studied, this viscosity value was exceeded while using the 3 % and 4 % alginate solutions.

In the sum, quite comparable results were obtained with the vibration technology, but their transferability and reproducibility are lower than those of the electrostatic method.

The beads produced by the JetCutter technology are displayed in Fig. 9 for the normal mode applied in Brussels and in Fig. 10 for the soft-landing mode applied in Braunschweig. When the JetCutter was used in the normal mode, no beads could be produced from the 0.5 % alginate solution and only very badly shaped and distributed beads/particles were obtained from the 1 % alginate solution. From the more concentrated alginate solutions, beads with a narrow size distribution were produced. The roundness of the beads originating from the 2 % and 3 % alginate solutions was good, whereas the majority of beads from the 4 % alginate solution exhibited a slightly oval shape.

Using the JetCutter in normal mode (Fig. 2), droplets falling on a direct trajectory from the nozzle into the gelation bath, is obviously not suitable for the production of beads from low-viscosity fluids. A prerequisite for bead production by the JetCutter technology is that the solid fluid jet pressed out of a nozzle is maintained till it is cut by the rotating cutting wires (Prüsse et al., 2002; Prüsse & Vorlop, 2004). This can only be achieved at a high fluid veloc-

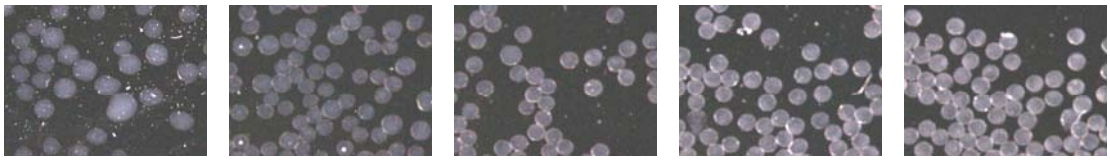

Fig. 10. Photographs of alginate beads produced in Braunschweig by the JetCutter technology from alginate solutions with the concentration of a) 0.5 %, b) 1 %, c) 2 %, d) 3 %, and e) 4 % of sodium alginate in water.

Table 3. Qualitative description of the size distribution and shape, i.e. roundness, of the beads produced from alginate solutions by the different technologies

Alginate concentration	Qualitative description of bead size distribution / bead shape				
	Coaxial air-flow	Electrostatic	Vibration	JetCutter	
mass %				Normal	Soft-landing
0.5	+ / +	+ / +	o / o	no beads	o / o
1.0	+ / +	+ / +	+ / +	– / –	+ / +
2.0	+ / +	+ / +	o / o	+ / +	+ / +
3.0	+ / o	+ / +	no beads	+ / +	+ / +
4.0	+ / –	+ / +	no beads	+ / +	+ / +

Quality: good (+), medium (o), bad (–).

ity. Hence, the droplets generated by the technology also possess such a high velocity typically ranging from 5 m s^{-1} up to 30 m s^{-1}. In these particular experiments the fluid velocity was 10 m s^{-1}. As the droplets are decelerated by the air resistance only slightly, they enter the gelation bath at a much higher velocity than if prepared by any other technology studied here. Accordingly, droplets obtained from low-viscosity fluids exhibiting only a minor cohesion burst on the surface of the gelation bath when they try to enter it, whereas droplets of high-viscosity fluids stay intact when entering the gelation bath due to their higher cohesion.

Bead deformation caused by this effect can be overcome if the JetCutter is used in the soft-landing mode (Fig. 2). In such case, the JetCutter is placed in some distance and considerably below the gelation bath, which results in a diagonal bottom-up trajectory of the droplets decelerating them by the gravitation force. Near the zenith of the droplet trajectory at which the gelation bath is placed, the velocity of the droplets is considerably lower when entering the gelation bath compared to the conditions when the JetCutter is working in normal mode. Hence, applying the soft-landing mode, beads from all alginate solutions could be produced as displayed in Fig. 10. The roundness and size distribution of the beads were very good for the beads produced from all alginate solutions. Only those prepared from the 0.5 % alginate solution exhibited a slightly broader size distribution and some minor deformation (egg-shape).

The uniformity of the produced beads, i.e. the size distribution, and the shape of the beads with regard to their roundness are compared in a qualitative manner in Table 3. Three of the technologies, i.e. coaxial air-flow, electrostatic, and JetCutter in soft-landing mode, were found to be capable to produce beads from all chosen alginate solutions. With regard to the size distribution and the shape, the electrostatic technology performed the best, followed by the JetCutter one in soft-landing mode, and the coaxial air-flow technique. No beads could be produced from very low-viscosity solutions by the JetCutter applied in normal mode and from high-viscosity solutions employing the vibration technique.

The average size of the beads produced in the different laboratories is shown in Table 4. The aimed average bead size within the round-robin experiment was (800 ± 100) μm. The size of the beads produced in Brussels was not measured but the values calculated according to the theoretical description of the vibration and JetCutter technologies are displayed in Table 4. As for both technologies, a well-defined and reliable theoretical model is available, the real sizes should not vary significantly from the calculated values. Except in Perugia, where slightly smaller beads were produced, the aimed bead size was always reached in all laboratories and with all techniques whenever bead production was possible.

Comparison of production rates of the different technologies

Beads are usually produced for a definite purpose, e.g. for immobilization of cells or enzymes, for encap-

Table 4. Average size of the beads produced by the different technologies

Alginate concentration	Mean bead diameter/mm							
	Coaxial air-flow	Electrostatic		Vibration			JetCutter	
							Normal	Soft-landing
mass %	Bratislava	Warsaw	Belgrade/Braunschweig	Nantes	Perugia	Brussels	Brussels	Braunschweig
0.5	0.85	0.82	0.81	0.77	0.70	0.8*	no beads	0.85
1.0	0.78	0.80	0.77	0.78	0.56	0.8*	0.77*	0.76
2.0	0.82	0.82	0.79	no beads	0.64	0.8*	0.77*	0.80
3.0	0.78	0.79	0.78	no beads	no beads	no beads	0.77*	0.80
4.0	0.80	0.80	0.68	no beads	no beads	no beads	0.77*	0.80

*Calculated value.

Table 5. Production rate of the beads produced from alginate solutions by the different technologies

Alginate concentration	Bead production rate/(g s^{-1})				
	Coaxial air-flow	Electrostatic	Vibration	JetCutter	
				Normal	Soft-landing
mass %					
0.5	0.007	0.008	0.13	2.0	1.1
1.0	0.007	0.004	0.17	2.0	1.5
2.0	0.007	0.005	0.18	2.0	1.4
3.0	0.0008	0.001	–	2.0	1.4
4.0	0.0003	0.0001	–	2.0	1.3

sulation of ingredients in foods or cosmetics, or for controlled-release applications in pharmaceutical industry. Regardless of the application, the beads shall not only be produced in the desired round shape and in the desired size with a narrow size distribution but shall also be produced in the desired amount. Hence, the production rate at which the beads are produced is another important parameter for comparison of the different technologies.

Typically in research, small amounts of beads are sufficient, whereas for any kind of industrial application, only such technologies are valuable which are capable of producing large amounts of beads. However, as research is usually aimed at subsequent industrial application of its results, also in research the bead production techniques exhibiting or enabling scale-up to larger production amounts without posing additional problems should be preferred.

The rates at which the beads were produced employing the different technologies are compared in Table 5. These rates refer to the mass of the processed alginate solution per second and are valid for one single nozzle, although none of the devices was operated with more than one nozzle. Further on, the production rates refer to the production of (800 ± 100) μm beads and would be higher for each technology if larger beads were to be produced. When one technology was used in different laboratories, the average value of the applied rates is given. The production rates determined by the different laboratories varied with a maximum of about ± 20 % of this average value.

Production rates of the coaxial air-flow and electrostatic technologies are very similar. The rates were very low already when beads from the low-viscosity alginate solutions were produced, and they further decreased by about one order of magnitude when the high-viscosity solutions were used. The following calculation helped to get a better feeling of the rates for single-nozzle devices. For instance, it takes approximately one hour to process only one gram of the 4 % alginate solution with the coaxial air-flow and approximately three hours with the electrostatic technique. Accordingly, processing of one kilogram of alginate solution takes about 40 days with the coaxial air-flow technique and about four months with the electrostatic technology, assuming the device being permanently in operation.

The production rate of the vibration technology is about 20–40 times higher than those of the coaxial air-flow or electrostatic technologies. However, only low-viscosity alginate solutions up to 2 mass % can be processed by this technology. When processing was possible, comparable rates were used for the alginate solutions. Processing one gram of a low-viscosity alginate solution took about six seconds and one kilogram could be processed in less than two hours.

The production rate of the JetCutter is about 10 times higher than that of the vibration technique. The

applied rates are independent of the alginate solution viscosity. Processing one gram of alginate solution took less than one second and one kilogram could be processed in about ten minutes. Compared to the coaxial air-flow or electrostatic techniques, production rates of the JetCutter are about 200–500 times higher for low-viscosity alginate solutions and about 1400–20000 times higher for high-viscosity alginate solutions.

The production rates shown in Table 5 refer to the use of one single nozzle and have to be multiplied if multi-nozzle systems are used. Such multi-nozzle systems have been described for all of the four techniques used in the round robin experiment ((Vorlop & Klein, 1983) for the coaxial air-flow technique, (Poncelet et al., 1994) for the electrostatic technique, (Brandenberger & Widmer, 1998) for the vibration technology, and (Prüsse et al., 2000) for the JetCutter technology). However, it should be kept in mind that a 20–40 nozzle coaxial air-flow or electrostatic device has to be used to reach the production rates of a single-nozzle vibration system or a 200–20000 nozzle device to reach the production rates of a single nozzle JetCutter.

Hence, the JetCutter was found to be the best technology for large-scale/industrial applications although it is not limited to such scales and can also be used for small/lab-scale applications. The vibration technology can also be used in any scale although the lower throughputs, compared to the JetCutter, require multi-nozzle devices for larger scale applications. The coaxial air-flow and the electrostatic technologies are limited to small/lab-scale applications.

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References

Anilkumar, A. V., Lacík, I., & Wang, T. G. (2001). A novel reactor for making uniform capsules. *Biotechnology and Bioengineering*, *75*, 581–589. DOI: 10.1002/bit.10077.

Brandenberger, H., & Widmer, F. (1998). A new multinozzle encapsulation/immobilisation system to produce uniform beads of alginate. *Journal of Biotechnology*, *63*, 73–80. DOI: 10.1016/S0168-1656(98)00077-7.

Bučko, M., Vikartovská, A., Lacík, I., Kolláriková, G., Gemeiner, P., Pätoprstý, V., & Brygin, M. (2005). Immobilization of a whole-cell epoxide-hydrolyzing biocatalyst in sodium alginate-cellulose sulfate-poly(methylene-co-guanidine) capsules using a controlled encapsulation process. *Enzyme and Microbial Technology*, *36*, 118–126. DOI: 10.1016/j.enzmictec.2004.07.006.

Bugarski, B., Li, Q. L., Goosen, M. F. A., Poncelet, D., Neufeld, R. J., & Vunjak, G. (1994). Electrostatic droplet generation: Mechanism of polymer droplet formation. *AIChE Journal*, *40*, 1026–1031. DOI: 10.1002/aic.690400613.

Bugarski, B., Obradovic, B., Nedovic, V., & Poncelet, D. (2004). Immobilization of cells and enzymes using electrostatic droplet generation. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 277–294). Dordrecht: Kluwer Academic Publishers.

Heinzen, C., Berger, A., & Marison, I. (2004). Use of vibration technology for jet break-up for encapsulation of cells and liquids in monodisperse microcapsules. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 257–275). Dordrecht: Kluwer Academic Publishers.

Heinzen, C., Marison, I., Berger, A., & von Stockar, U. (2002). Use of vibration technology for jet break-up for encapsulation of cells, microbes and liquids in monodisperse microcapsules. *Landbauforschung Völkenrode*, *SH241,* 19–25.

Lacík, I., Briššová, M., Anilkumar, A. V., Powers, A. C., and Wang, T. (1998). New capsule with tailored properties for the encapsulation of living cells. *Journal of Biomedical Materials Research*, *39*, 52–60. DOI: 10.1002/(SICI)1097-4636(199801)39:1<52::AID-JBM7>3.0.CO;2-H.

Lewińska, D., Rosiński, S., & Weryński, A. (2004). Influence of process conditions during impulsed electrostatic droplet formation on size distribution of hydrogel beads. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology*, *32*, 41–53. DOI: 10.1081/BIO-120028667.

Manojlovic, V., Djonlagic, J., Obradovic, B., Nedovic, V., & Bugarski, B. (2006). Investigations of cell immobilization in alginate: rheological and electrostatic extrusion studies. *Journal of Chemical Technology and Biotechnology*, *81*, 505–510. DOI: 10.1002/jctb.1465.

Melvik, J. E., & Dornish, M. (2004). Alginate as a carrier for cell immobilisation. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 33–51). Dordrecht: Kluwer Academic Publishers.

Nedovic, V., Obradovic, B., Poncelet, D., Goosen, M. F. A., Leskosek-Cukalovic, I., & Bugarski, B. (2002). Cell immobilisation by electrostatic droplet generation. *Landbauforschung Völkenrode*, *SH241*, 11–17.

Neufeld, R. J., & Poncelet, D. (2004). Industrial scale encapsulation of cells using emulsification/dispersion technologies. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 311–325). Dordrecht: Kluwer Academic Publishers.

Ogbonna, J. C. (2004). Atomisation techniques for immobilisation of cells in micro gel beads. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 327–341). Dordrecht: Kluwer Academic Publishers.

Poncelet, D., Babak, V. G., Neufeld, R. J., Goosen, M. F. A, & Bugarski, B. (1999a). Theory of electrostatic dispersion of polymer solutions in the production of microgel beads containing biocatalyst. *Advances in Colloid and Interface Science*, *79*, 213–228. DOI: 10.1016/S0001-8686(97)00037-7.

Poncelet, D., Bugarski, B., Amsden, B. G., Zhu, J., Neufeld, R., & Goosen, M. F. A. (1994). A parallel-plate electrostatic droplet generator – Parameters affecting microbead size. *Applied Microbiology and Biotechnology*, *42*, 251–255. DOI: 10.1007/BF00902725.

Poncelet, D., Neufeld, R. J., Goosen, M. F. A, Burgarski, B., & Babak, V. (1999b). Formation of microgel beads by electric dispersion of polymer solutions. *AIChE Journal*, *45*, 2018–2023. DOI: 10.1002/aic.690450918.

Poncelet, D., Teunou, E., Desrumaux, A., & Della Valle, D. (2002). Emulsification and microencapsulation: State of art. *Landbauforschung Völkenrode*, *SH241*, 27–31.

Prüsse, U., Dalluhn, J., Breford, J., & Vorlop, K.-D. (2000). Production of spherical beads by JetCutting. *Chemical Engineering & Technology*, *23*, 1105–1110. DOI: 10.1002/1521-4125(200012)23:12<1105::AID-CEAT1105>3.0.CO;2-V.

Prüsse, U., Jahnz, U., Wittlich, P., Breford, J., & Vorlop, K.-D. (2002). Bead production with JetCutting and rotating disc/nozzle technologies. *Landbauforschung Völkenrode*, *SH241*, 1–10.

Prüsse, U., & Vorlop, K.-D. (2004). The JetCutter technology. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 295–309). Dordrecht: Kluwer Academic Publishers.

Rosiński, S., Lewińska, D., Migaj, M., Wozniewicz, B., Weryński, A. (2002). Electrostatic microencapsulation of parathyroid cells as a tool for the investigation of cell's activity after transplantation. *Landbauforschung Völkenrode*, *SH241*, 47–50.

Schwinger, C., Koch, S., Jahnz, U., Wittlich, P., Rainov, N. G., Kressler, J. (2002). High throughput encapsulation of murine fibroblasts in alginate using the JetCutter technology. *Journal of Microencapsulation*, *19*, 273–280. DOI: 10.1080/02652040110105328.

Strand, B. L., Skjak-Braek, G., & Gaserod, O. (2004). Microcapsule formulation and formation. In V. Nedovic, & R. Willaert (Eds.), *Fundamentals of cell immobilisation biotechnology*, Vol. *8A* (pp. 165–183). Dordrecht: Kluwer Academic Publishers.

Vorlop, K.-D., & Klein, J. (1983). New developments in the field of cell immobilization - Formation of biocatalysts by ionotropic gelation. In R. M. Lafferty, & E. Maier (Eds.), *Enzyme technology* (pp. 221–235). Berlin: Springer.