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SCIENTIFIC PAPER

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OPTIMIZATION OF THE ELECTROSTATIC DROPLET GENERATION PROCESS FOR CONTROLLED MICROBEAD PRODUCTION – SINGLE NOZZLE SYSTEM

The aim of this study was to optimize the electrostatic extrusion process for producing small, spherical and uniform microbeads with different fluid viscosities by varying the operating parameters in very wide ranges. Alginate was used as a model polymer. Since the rheological behavior of the solution is one of the parameters that affects the flow dynamics during extrusion, viscosity measurements of solutions with different alginate content were performed. The results obtained in this study show that an electrostatic droplet generator can be used for the production of spherical microbeads of narrow size distribution from low- and medium- viscous fluids (0.5, 1, and 2% of alginate). The average microbead diameter for low-viscous solutions was less than 100 micrometers. It was possible to obtain beads smaller than 500 micrometers that were very uniform (standard deviations less than 2.5%) and of spherical (the shape distortion was less than 1%) from medium-viscous alginate solution (2%). By reducing the polymer flowrate to less than 1 ml/h, even smaller microbeads were produced with diameters of about 300 micrometers. The particular contribution of this paper is in exceeding limitations regarding the use of high-viscous polymer solutions. Optimization of the operating conditions that included the use of a very small needle (0.15 mm), enlargement of the electrode distance to more than 20 cm and a severe reduction in the polymer flow rate to lower than 5 ml/h (for 3% alginate) or 1 ml/h (for 4% alginate) enabled the production of small, entirely spherical and uniform microbeads with an average microbead diameter lower than 500 and 700 micrometers in the case of 3 and 4% of alginate, respectively.

Key words: Electrostatic extrusion, Alginate, Hydrogel microbeads.

Electrostatic droplet generation is an extrusion technique that uses electrostatic forces to disrupt a liquid surface at the capillary/needle tip forming a charged stream of small droplets. It is used for the production of hydrogel beads that entrap various materials for different applications in the pharmaceutical, chemical or food industry, as well as in agriculture or biotechnology. The electric field induces an electrical charge on the liquid surface and a repulsive outside-directed force. This leads to the production of uniform small-diameter beads (Keshavartz et al., 1992; Bugarski et al., 1994). We have previously investigated the effects of several operating and design parameters on electrostatic extrusion and the resulting microbead size (Poncelet et al., 1999; Nedovic et al., 2002; Bugarski et al., 2004). Certain limitations of the technique regarding the utilization of viscous polymer solutions were realized.

Alginate has been broadly used as a support for cell immobilisation in biochemical processing applications and as a matrix in tissue formation due to its biocompatibility and ease of processing (Melvik and Dornish, 2004). Microbeads with a concentration of alginate in the range of 1- 2% have been used so far for the entrapment of various cell types (Nedovic et al., 2001, 2002; Rosinski et al., 2002). The selection of alginate concentration is important, since it affects the mechanical strength and gelation kinetics of the beads as well as the diffusion rate within the support matrix which can alter cell metabolism. It has been established in previous studies that alginate concentrations up to 2% provide preservation of the viability, cell differentiation and propagation. However, microbeads with high alginate concentrations (3 and 4%) could be advantageous in potential bioreactor applications under special conditions of severe shear stresses. In addition, more concentrated alginate beads are preferable for the encapsulation of flavor molecules for aroma therapy in the food industry, where good flavor retention and controlled release are imperative.

The appropriate bead size is often a compromise of different demands. However, in most biotechnology processes small beads offer advantages due to better diffusion properties within the carrier matrix. The production of microbeads with alginate contents higher than 2% is connected with difficulties with regard to the shape of the beads (poorer roundness with increasing viscosity) and the throughput, which can decrease dramatically. The comparison of different common bead production technologies showed that only electrostatic extrusion and jet-cutters can be used for the production

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of beads from high viscous fluids (viscosities higher than 1000–2000 mPas) at room temperature (Bucko et al., 2004). This study focused on an optimization procedure for producing small, spherical and uniform microbeads with different fluid viscosities by varying the operating parameters in vary wide ranges. Special attention was paid to explore further possibilities of the production of spherical microbeads with 3 and 4% alginate concentrations by electrostatic extrusion.

EXPERIMENTAL

Protanal LF 20/40 supplied by FMC Biopolymer was used and investigated in this study. Polymer solutions with different alginate concentrations, namely 0.5, 1, 2, 3, and 4%, were prepared by dissolving sodium alginate powder at room temperature in de–ionised water.

Viscosity measurements of alginate solutions of different concentrations were performed on a Rheometrics instrument RMS–605 at 25°C, immediately after the samples were prepared. The viscosities of alginate solutions with low concentrations (0.5 and 1%) could not be measured because of instrument limitations in the viscosity range below 1 Pas.

Alginate microbeads were produced by extrusion of the polymer solution through a blunt edge stainless steel needle using a syringe pump (KD Scientific, Inc., Boston, Massachusetts, Model KDS100) with a 10 ml plastic syringe. The electrode geometry set–up with a (point to plane) positively charged needle and a grounded collecting solution (2% w/w $CaCl_2$) was applied. Gentle stirring was applied during the gelation process by a magnetic stirrer IKA Labortechnik, Model RCTbasic. The potential difference was controlled by a high voltage dc unit (iseg Specialelectronik GmbH, Germany, Model T1CP 300 304p) and the voltage was varied in the range of 3.8 kV to 15 kV. The distance between the needle tip and the hardening solution was varied from 3 to more than 20 cm while the flow rate of the polymer solution was varied in the range from 0.1 to more than 20 ml/h. The needle diameter was varied in the range of 150 to 510 micrometers.

Bead imaging was performed under a stereoscope microscope (Zeiss, Germany, Model STEMI SV8) equipped with a JVC colour video camera (Japan), Model No. TK–1070E. The software analySIS 3.0 (Germany) was used for microbead picture analyses. The average diameter (d), standard deviation (σ) and shape distortion (δ) were determined by analyses of 30 randomly selected beads from each batch. For every set of experimental parameters, three replicates of the measurements were performed. For each particle, the average of the horizontal and perpendicular dimensions was accepted as the mean diameter and the deformation from a sphere (spherical distortion) was calculated as the square deviation of two dimensions (horizontal and perpendicular) from the mean diameter. Beads with spherical distortions lower than 2% (roundness of 98%) were considered as regular spheres. Three batches were produced for each experimental set and the presented data are the average of three replicate measurements.

RESULTS AND DISCUSSION

According to our results of the viscosity measurements presented in Table 1 and the results of the round robin experiment presented in Bucko et al., 2004, an increase in the alginate content from 0.5 to 4% w/w caused a significant increase in the viscosity by about three orders of magnitude. The alginate composition determines its rheological characteristics, i.e. the viscosity (Manojlovic et al., 2005). The high concentrate solution of the low viscous alginate can have a viscosity equal to than of the low concentrate solution of the high viscous type of alginate. Through the selection of alginate type and concentration, the flow characteristics of alginate can be controlled. Another way to improve polymer flowability is to perform the extrusion at higher temperatures since, as a general rule, a temperature increase of 1°C leads to a viscosity drop of approximately 2.5%.

Dealing with high fluid viscosities often cause difficulties in extrusion, accompanied by changes in the mechanism of droplet formation, which affect the distortion of the bead shape and the appearance of irregularities during polymer flow through the needle which causes a dramatic decrease in the productivity of the technique. In order to overcome these drawbacks, a series of experimental runs with different viscosities and under different conditions were performed.

The results obtained in this study show that an electrostatic droplet generator can be used for the production of spherical microbeads (Figure 1) of narrow size distribution from low– and medium– viscous fluids (0.5, 1, and 2% of alginate). The average microbead diameter for low–viscous solutions was lower than 100 micrometers under conditions of up to 9 kV applied voltage and using a needle of 150 micrometers internal diameter. Further increase in the applied potential caused electric sparking between the needle tip and collecting solution. For medium viscous alginate solution (2%), it was shown that it was possible to obtain beads smaller than 500 micrometers (Figure 2) that were very uniform ($\sigma < 2.5\%$) and spherical (the shape distortion

Table 1. Dynamic viscosity of the alginate solutions at various concentrations

Na–alginate concentration in % w/w	Dynamic viscosity η in mPas (shear rate 1Hz)
2	2092
3	7048
4	17400

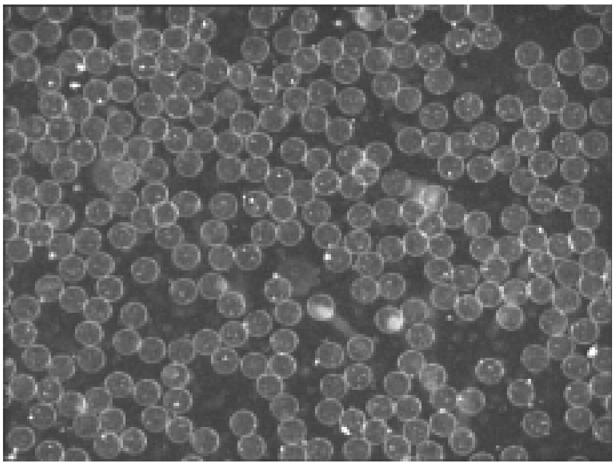

Figure 1. Microbeads obtained by the electrostatic generation of 1% alginate – the size of microbeads is around 270 micrometers, narrow size distribution (6 kV applied potential, 1 ml/h flowrate, 21.5 cm electrode distance, 0.15 mm needle diameter)

was less than 1%). By reducing the polymer flowrate to less than 1 ml/h, even smaller microbeads were produced with diameters of about 300 micrometers.

After the extrusion of 1% alginate solution under certain conditions, beads with a multimodal size distribution were produced. It was reported in previous studies that a multimodal size distribution was the outcome of harmonic needle oscillations under applied voltages geater than 6 kV (0.45 mm needle). Consequently, fragmentation of the forming filament at the needle tip resulted in a bimodal size distribution (Bugarski et al, 1994). The described phenomenon was not observed with larger needles (Bugarski et al, 1994). In our investigations, very light (thin and short) needles were tested (0.15 to 0.51 mm). It is surprising that a decrease of the needle diameter from 0.25 mm to 0.15 mm led to the disappearance of a multimodal size distribution (Figure 3) of the 1% alginate solution. It should be emphasized that the described results fit to a very high electrode distance (more than 20 cm). The multimodal size distribution here cannot be 'a priori' attributed to needle oscillations and a detailed examination of the mechanism of droplet formation is needed to fully rationalize the obtained results.

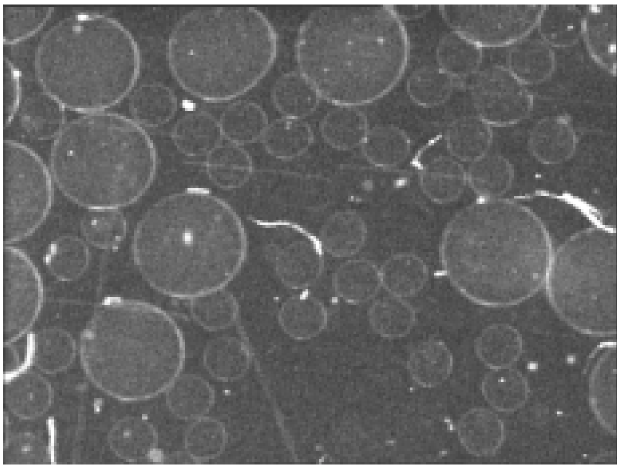

Figure 3. Microbeads obtained by the electrostatic generation of 1% alginate – the size of the smaller microbeads is around 290 micrometers and the size of the larger microbeads around 680 micrometers, bimodal size distribution (6 kV applied potential, 1 ml/h flowrate, 21.5 cm electrode distance, 0.15 mm needle diameter)

Figure 4 shows a bimodal size distribution with peaks at 430 and 120 μm using a 0.25 mm needle and under an applied potential of 6 kV (10 ml/h flowrate and 21.5 cm electrode distance), while microbeads with a mean diameter of 300 micrometers were obtained (under the same conditions) using a thinner needle (0.15 mm), although the size deviation was still at on a high level (±10%). In order to improve the uniformity of the beads, further optimization led to a change of the alginate flowrate in both directions (higher than 10 ml/h

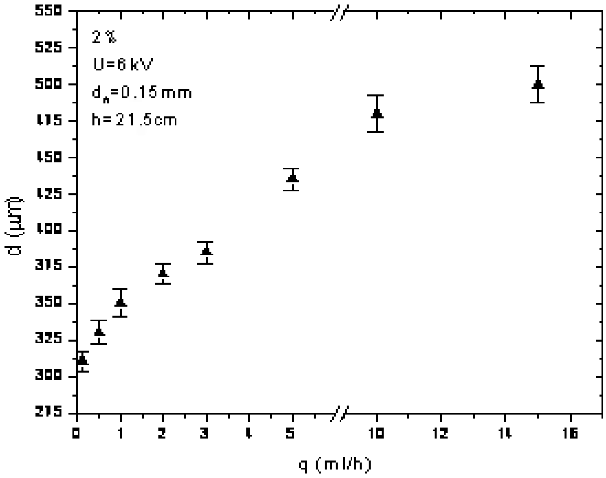

Figure 2. Mean microbead diameter as a function of the flowrate for 2% alginate (6 kV applied potential, 0.15 mm needle diameter, 21.5 cm electrode distance)

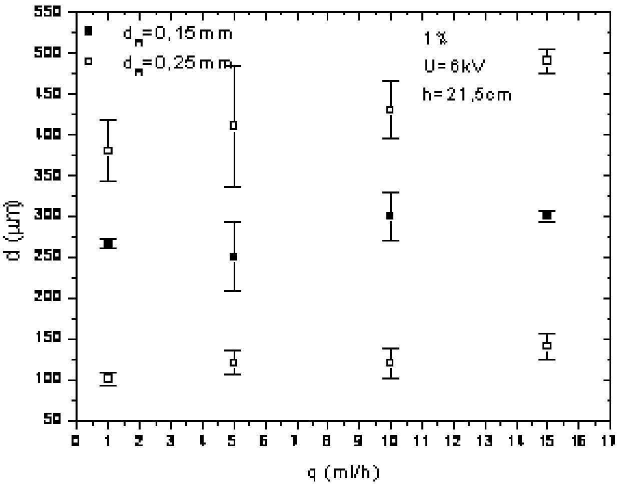

Figure 4. Mean microbead diameter as a function of the alginate flowrate (1%) for two needle diameters: 0.15 and 0.25 mm (6 kV applied potential, 21.5 cm electrode distance)

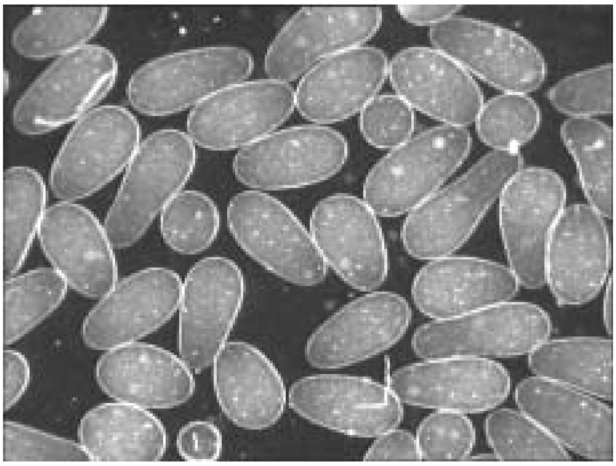

Figure 5. Microbeads obtained by the electrostatic generation of 3% alginate – irregular shapes (6 kV applied potential, 10 ml/h flowrate, 16 cm electrode distance, 0.15 mm needle diameter)

and lower than 5 ml/h). An increase in the flowrate to 15 ml/h caused a reduction of the small bead fraction, which contributed to an improvement of the uniformity. However, the best results were achieved with flowrates in the range from 0.5 to 5 ml/h: very nice, small (around 200 micrometers) and uniform (~±1%) microbeads were obtained.

In the experiments with high viscous fluids (3 and 4% of alginate) microbeads with irregular shape were obtained in several cases (Figure 5). However, after some process optimizations, we succeeded to obtain very nice microbeads of narrow size distribution (Figure 6) with an average microbead diameter lower than 500 and 700 micrometers in the case of 3 and 4% of alginate, respectively. For 3% alginate the optimization method comprised of using a very small needle (0.15 mm), increasing the electrode distance to morethan 20 cm and severely reducing the polymer flow rate to less than 5 ml/h. Figure 7 shows the bead diameter and bead shape distortion as a function of the flowrate for 3% alginate. It is obvious that the increase in the 3% alginate flowrate from 0.1 to 15 ml/h led to larger beads (from 345 μm for 0.1 ml/h to 850 μm for 15 ml/h) and to an increase in both size deviation (from ±1.4% to ±14%) and shape distortion (from 1.3% to 26%). A significant increase in the distance between the electrodes and a reduction in the flow rate allowed the falling droplets enough time to form regular spherical particles and caused the polymer to outflow from the needle in a continuous stream, which contributed to the uniformity of the beads. These extreme conditions led to very nice beads, although such low throughputs indicate possibilities for bead production only for lab applications.

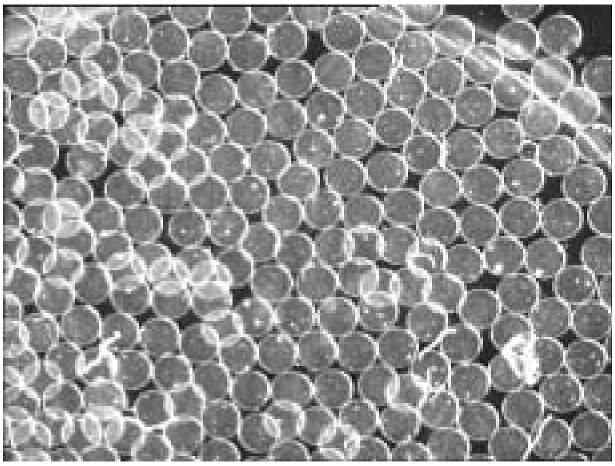

Figure 6. Microbeads obtained by the electrostatic generation of 3% alginate – the size of the microbeads is around 400 micrometers, narrow size distribution (6 kV applied potential, 1 ml/h flowrate, 21.5 cm electrode distance, 0.15 mm needle diameter)

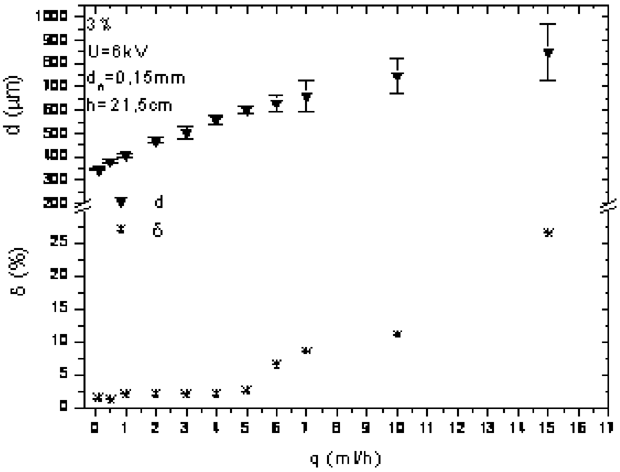

Figure 7. Mean microbead diameter and shape distortion as a function of the flowrate for 3% alginate (6 kV applied potential, 0.15 mm needle diameter, 21.5 cm electrode distance)

CONCLUSIONS

In this study it was shown that an electrostatic droplet generator can be used for the production of very small beads of narrow size distribution from low–, medium– (0.5 to 2% of alginate), as well as high–viscous (3 and 4% of alginate) solutions. The average microbead diameters obtained under conditions of high electric field (9 kV) and using small needles (0.15 mm internal diameter) were lower than 100 μm with low alginate solutions (viscosity lower than 1000 mPas) and lower than 500 and 700 μm in the case of high viscosity solutions, 7048 and 17400 mPas, respectively. The optimization procedure included an incerease of the electrode distance and a reduction in the polymer flow rate, which contributed to the production of small beads with a narrow size distribution, even with high viscous solutions. In order to explain all the obtained findings with the aim of fully understanding the electrostatic extrusion process, the effects of each parameter should be individually considered and the mechanism of its influence should be examined, as well as their mutual influence on the overall extrusion process.

Electrostatic droplet generation is a very suitable technique for lab–scale applications, in which the throughput of the technique is of minor importance. Scale up of the technique is necessary for higher throughputs. A multi nozzle device should be constructed and tested to enable an increase of the productivity of the technique. Further adjustment and optimization of the system with a multi nozzle head should be the next step in the development of the electrostatic extrusion technique.

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IZVOD

OPTIMIZACIJA ELEKTROSTATIČKOG GENERISANJA ČESTICA U CILJU KONTROLISANJA NJIHOVIH MIKRONSKIH VELIČINA – SISTEM SA JEDNOM MLAZNICOM

(Naučni rad)

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Elektrostatička ekstruzija je novija ekstruziona metoda koja se zasniva na primeni električne sile koja deluje na površini meniskusa tečnosti na vrhu igle, usled čega dolazi do generisanja velikog broja kapljica. U predhodnim istraživanjima je utvrđeno da je veličina čestica funkcija više parametara, kao što su električni potencijal, prečnik kapilare, rastojanje između elektroda, protok i fizičko–hemijske osobine polimera (površinski napon, viskoznost, koncentracija). Takođe, utvrđeno je i da postoje određena ograničenja koja se odnose na ekstruziju veoma viskoznih rastvora. Ovaj rad je posvećen optimizaciji metode radi dobijanja što sitnjih i uniformnijih čestica. S obzirom da je viskoznost rastvora jedan od parametara koji utiče na dinamiku isticanja polimera kroz kapilaru, najpre su izvršena merenja viskoznosti rastvora alginata različitih koncentracija. Utvrđeno je da povećanje koncentracije rastvora sa 2 do 4% dovodi do povećanja viskoznosti sa oko 2000 na 17000 mPas na 21°C. Dobijeni rezultati su pokazali da je elektrostička ekstruzija vrlo povoljna za dobijanje sferičnih čestica uskog opsega raspodele veličina koristići nisko– i srednje– viskozne rastvore polimera (0,5, 1 i 2% alginata). Sa nisko–viskoznim rastvorom alginata, pod određenim uslovima, dobijene su čestice čiji je srednji prečnik čak manji i od 100 μm. Sa srednje viskoznim alginatnim rastvorom dobijene su čestice prečnika ispod 500 μm, koje su bile vrlo uniformne (srednje kvadratno odsupanje manje od 2,5%) i sferične (deformacija oblika manja od 1%), a smanjenjem protoka rastvora, moguće je dobiti čestice i manje od 300 μm. Poseban doprinos rada je u prevazilaženju ograničenja koja se odnose na ekstruziju veoma viskoznih rastvora. Optimizacija procesnih parametara koja je podrazumevala primenu vrlo tanke igle (0,15 mm), povećanje rastojanja između elektroda iznad 20 cm i smanjenje protoka ispod 5 ml/h (za 3% alginat), odn. 1 ml/h (za 4% alginat), omogućila je dobijanje malih, potpuno sferičnih i uniformnih mikročestica čiji je srednji prečnik bio ispod 500 i 700 μm za 3 i 4% alginatni rastvor, respektivno.

Ključne reči: Elektrostička ekstruzija, Alginat, Mikročestice hidrogela.