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D. Poncelet · B. Bugarski · B. G. Amsden
J. Zhu · R. Neufeld · M. F. A. Goosen

A Parallel plate electrostatic droplet generator: parameters affecting microbead size

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Abstract Polymer microbead production by parallel plate electrostatic extrusion is presented. Factors affecting microbead size such as needle gauge, electrostatic potential, distance between needle and collecting solution, and polymer solution concentration and flow rate were evaluated. Smaller microbeads resulted from reduced needle diameter, reduced needle to collecting solution distance, increased electrostatic potential, and reduced polymer solution concentration and flow rate. In terms of process scale-up, it was shown that a multi-needle (20) device could continuously produce relatively uniform beads via electrostatics. The technology was demonstrated to be feasible for cell encapsulation or immobilization as there was no detectable effect of applied potential on *Spodoptera frugiperda* viability.

Introduction

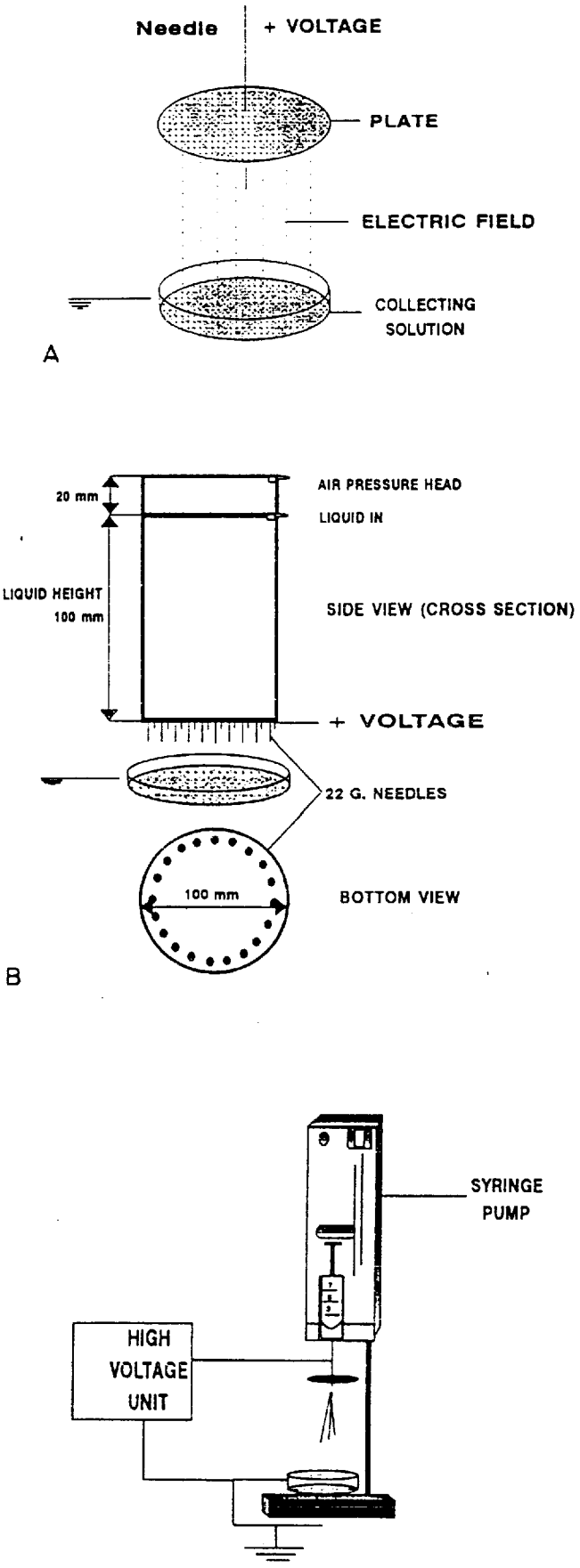
A common procedure for producing gel microbeads involves dispersing a polymer solution into a hardening solution using air jet extrusion technology (Balachandran and Bailey 1982; Fillimore and van Lokeren 1982). Industrial production by such a process implies not only scale-up, but also the need to reproducibly control the microbead properties. Immobilization systems may employ various gel-forming proteins (e.g. gelatine), polysaccharides (e.g. agar, calcium alginate and κ-carrageenan) and synthetic polymers (e.g. polyacrylamide). Relatively large spherical carriers are normally formed by dispersing droplets into the hardening solution using an air jet. A finer dispersion of droplets is possible by emulsifying the polymer in an oil phase followed by internal gelation. Another method involves atomizing the gel solution, with or without cells, through a nozzle, resulting in bead diameters of from 100 to 400 μm (Klein and Vorlop 1983). However, few attempts have been made in the use of electric fields to produce micron-sized polymer beads for cell immobilization (Goosen et al. 1986).

One of the objectives in cell encapsulation is to provide protection from shear forces prevailing in a bioreactor or during handling procedures. However, microbead or microcapsule formulation itself involves dispersive forces. Equipment for the generation of liquid droplets must then be designed to minimize the shear applied to the cells, while achieving a homogeneous distribution of the cells within the carriers. The encapsulation of reagents such as drugs and pesticides is presently conducted on a large scale, often in batches of several cubic metres using processing operations such as atomization, batch emulsification and centrifugal techniques (Poncelet et al. 1989). These methods are often not suitable for live cell encapsulation due to shear effects.

An alternative technique that may have application for cell immobilization is electrostatic droplet generation. In electrostatic droplet generation, a polymer solution is extruded through a charged needle. The collecting solution directly beneath the needle is either grounded or has a charge opposite to that of the needle. As the polymer solution passes through the needle it accumulates charge and droplets formed at the end of the needle are pulled off by the electrostatic attractive force between the needle and the collecting solution. The result is a charged stream of fine droplets. Electrode geometry is also important. In the case of a parallel plate electrode, the electric field between the needle and collection solution is uniform in direction and strength, resulting in a uniform force pulling the drop let off the end of the needle (Fig. 1A) (Bugarski et al. 1994a).

D. Poncelet · R. Neufeld
Department of Chemical Engineering, McGill University, Montreal, Quebec, Canada

B. Bugarski · B. G. Amsden · J. Zhu · M. F. A. Goosen (✉)
Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada



The production of small polymer microbeads using parallel plate electrostatic extrusion methods has been investigated. Parameters such as needle size, potential difference and distance between needle and collecting solution. and polymer solution concentration and flow rate were examined. The use of a large-scale (multiple needle) droplet generator was also assessed.

Materials and methods

Chemicals

Sodium alginate of low (S170) medium (S550) and high viscosity (S1160) was purchased from Sanofi (Paris, France). Calcium chloride was obtained from BDH (Toronto, Canada). Needles of 16, 22, 22s, and 26 gauge were purchased from Chromatographic Specialities, Brockville, Canada.

Electrostatic droplet generation

In the experimental set-up, a parallel-plate electrode system was employed to produce a uniform electric field in a vertical direction (Fig. 1A). The upper electrode was brought to a positive potential and the polymer droplets were formed from a 90° blunt needle protruding 0.5 cm through a small aperture at the centre of the disc electrode. A petri dish containing collecting solution [1.5% (w/v) $CaCl_2$] having the same dimensions as the upper grounded plate was placed beneath the extrusion assembly. Sodium alginate solution (2%, w/v) was forced out of the needle by a syringe pump (2–16 cm^3/h). The needle tip was mounted at a specified distance above the surface of the collecting solution. The needle tip was connected to a high voltage DC unit (Model 30R. Bertan Associates, Canada) capable of 30 kV and a maximum current of 0.4 mA. Calcium alginate beads were generated at various needle sizes, flow rates of 2 to 16 cm^3/h, electrode distances of 2.0 and 4.8 cm, and applied voltages of between 9 and 14 kV.

For scale-up purposes, a 1.5-l cylindrical reservoir (15 cm high × 10 cm in diameter) with multiple needles for continuous production of polymer beads was designed and constructed. The sodium alginate solution flow rate was controlled at 0.350 l/h (16 ml/h per needle) by adjusting the air pressure above the polymer solution. A grounded collecting dish of $CaCl_2$ solution was placed 2 cm below the needles. Twenty 22-gauge stainless steel needles, 1.2 cm radially apart, were mounted to the reservoir containing 1 l of polymer solution and connected to the high-voltage unit with the positive electrode connected to the needle plate. Beads were generated at voltages ranging from 8 to 14 kV (Fig. 1B).

Determination of microbead size distribution

Volumetric (volume of microspheres in each diameter class) and cumulative bead size distributions were determined by laser light scattering, using a 2602-LC particle analyser (Malvern Instruments) according to the log-normal distribution model. This provides a mean value estimate of the diameter at 50% of the cumulative volume fraction. The mean diameter and the arithmetic standard deviation were calculated from the resulting cumulative distribution

Fig. 1A–C Schematic representation of the experimental apparatus. **A** Single-needle parallel plate arrangement. **B** Multi-needle arrange-

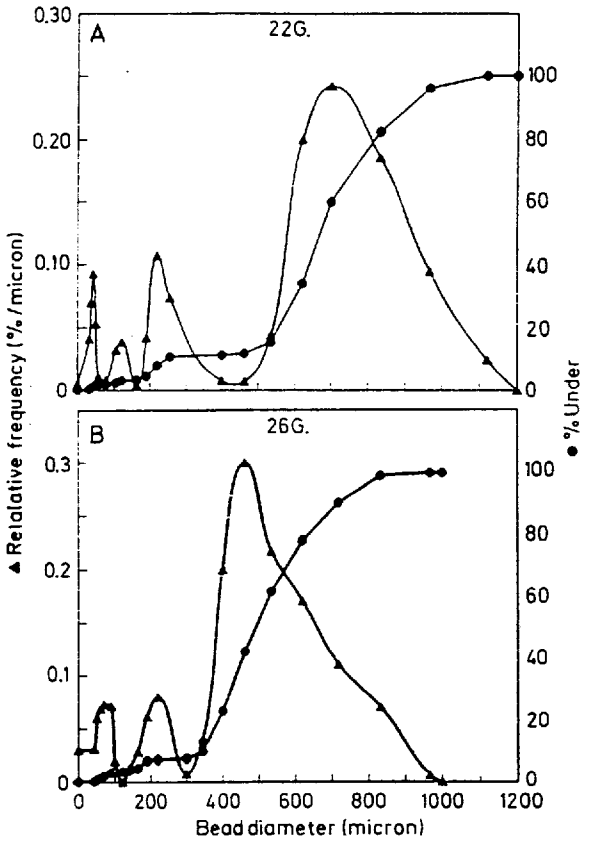


Fig. 2A, B Microbead size distribution from S170 alginate, 12 kV potential, 12 cm^3/h flow rate, and electrode spacing of 4.8 cm. **A** 22-Gauge needle (*22G*). **B** 26-Gauge needle (*26G*).

curve (Neufeld et al. 1991; Poncelet et al. 1989). A measure of the spread of the distribution is given by the span, which is the difference between the diameters at the 10% cumulative volume frequency and at the 90% cumulative volume frequency, divided by the mean diameter.

Extrusion of animal cell suspensions using electrostatics

The insect cells were cultured in shake flasks in IPL41 medium with 5% serum for 2–3 days prior to testing (Wu et al. 1990). After passing the cell suspension (*Spodoptera frugiperda*) through the droplet generator assembly, cell viability was assessed by staining the cells with trypan blue dye. All solutions and apparatus were sterilized in an autoclave under standard conditions.

Results and discussion

Investigation of parameters affecting droplet size

The size distribution curves obtained by plotting relative frequencies versus bead diameter, typically resulted in a continuous function symmetrical about the mean value (Fig. 2). For instance, the mean bead size distribution was found to vary about a mean of 750 μm for the gauge needle (Fig. 2a) and 480 μm for the 26-gauge needle (Fig. 2b), respectively. Satellite peaks as a result

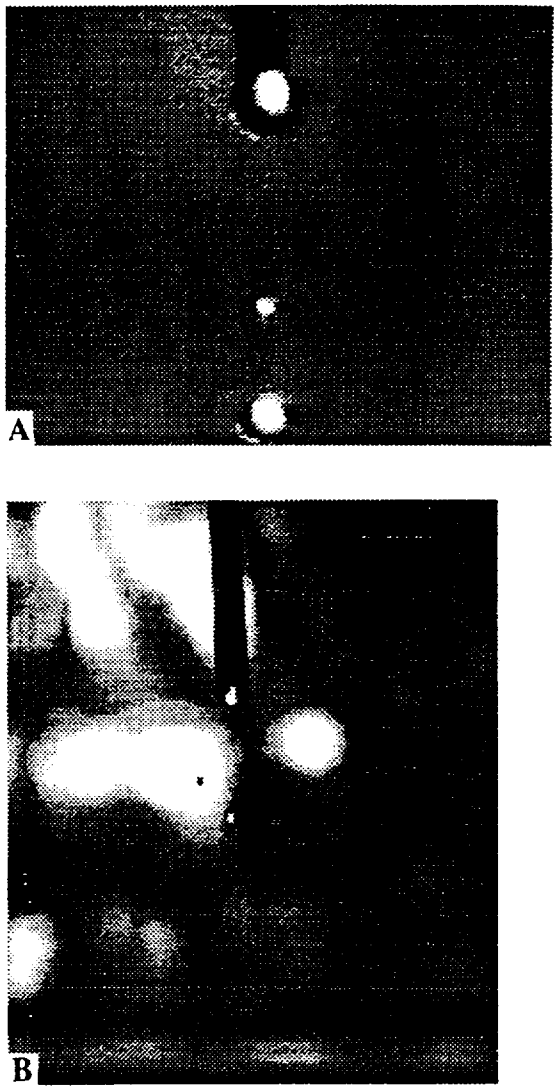


Fig. 3A, B Photographs illustrating effect of needle gauge on microbead diameter. **A** 22-Gauge needle. Microbead size distribution corresponds to Fig. 2A. **B** 26-Gauge needle. Microbead size distribution corresponds to Fig. 2B

of the filament bursting effect (Bugarski et al. 1994a) (Figs. 2 and 3), were more pronounced with the 22-gauge needle even though the total fraction of small beads occupied only 10% of the overall bead volume.

Varying the sodium alginate solution flow rate, while keeping the potential difference and needle diameter constant at 12 kV and gauge 22, respectively, had a significant effect on bead size. A 50% decrease in bead diameter was observed when the flow rate was decreased from 16 ml/h to 4 ml/h (Table 1). Bead diameter could also be controlled by the needle size. For example, at the same voltage, electrode distance, and flow rate the bead diameter was reduced by a factor of two when the needle size was reduced from gauge 16 to 26 (Table 2). The bead diameter was also dependent on the sodium alginate solution concentration. Using

Table 1 Effect of sodium alginate flow rate on average gel bead diameter using S170 alginate, 22-gauge needle, 12 kV applied and an electrode distance of 4.8 cm

Alginate flow rate (cm^3/h)	Mean bead diameter (μm)	Span
4	596	1.1
8	582	0.5
12	757	0.6
16	1091	0.8

Table 2 Effect of needle size on mean bead diameter using S170 alginate, 12 kV applied voltage, flow rate 12 cm^3/h, and an electrode distance 4.8 cm

Needle gauge	Mean bead diameter (μm)	Span
16	1080	0.7
22	757	0.6
26	490	0.6

Table 3 Effect of alginate type on mean gel bead diameter using 12 kV applied voltage, flow rate 12 cm^3/h, and an electrode distance 4.8 cm

Alginate	Needle gauge	Mean diameter (μm)	Span
S170	22	757	0.7
S550	22	1032	0.6
S1600	22	1120	0.6
S170	26	361	0.9
S550	26	420	0.7

Table 4 Effect of applied electrostatic potential and electrode distance on mean gel bead diameter using S170 alginate, 22-gauge needle, and a flow rate 12 cm^3/h

Voltage (kV)	Distance (cm)	Mean diameter (μm)	Span
9	4.8	947	0.8
12	4.8	757	0.7
9	2.0	537	0.9
12	2.0	401	0.6
14	2.0	410	0.7

different molecular mass sodium alginate resulted in a 20% decrease in calcium alginate bead diameter at the same applied voltage (Table 3) (Bugarski et al. 1994a).

The effect of electrode spacing and applied potential (i.e. electric field strength) on gel bead size is shown in Table 4. As the potential between the electrodes increased from 9 to 12 kV the average bead diameter decreased from 947 μm to 757 μm. Reducing the electrode distance resulted in even smaller bead sizes. For example, at a constant potential difference of 9 kV, reducing the distance from 4.8 cm to 2.0 cm resulted in a decrease in gel bead diameter from 947 μm to 537 μm. Increasing the potential difference above 14 kV while simultaneously decreasing electrode distance did not result in smaller bead diameters. This result was probably due to a charge discharge between the plates accompanied by arcing as a result of air ionization in the space between the electrodes (Bugarski et al. 1994a).

Analysis of electric field effects

A recent study using a similar method of calcium alginate microbead formation (Keshavarz 1992) showed that the minimum microbead size achieved varied between 600 μm and 1000 μm. In this study, a 2% sodium alginate solution was extruded through a 23-guage needle with a potential difference of 5 kV applied across the electrodes. In our study, with a higher potential difference (i.e. 12 kV) and with a larger needle (i.e. 22-gauge), it was shown that it is possible to achieve an average microbead diameter that is slightly lower than that reported by Keshavarz (400 μm compared to 600–1000 μm) (Table 4). The different results obtained by Keshavarz may have been due to a different equipment arrangement (i.e. positive voltage connected to the needle tip versus the parallel plate electrode arrangement used in our investigation).

The forces acting on the solution at the needle tip are the forces of gravity and the electric field force acting downward, while opposing these forces is the surface tension of the solution. The electric field force, F_e, is the product of the strength of the electric field, E, and the charge on the droplet formed, q. Consider the electric field effects on droplet formation using the positively charged needle arrangement and the parallel plate electrodes. In both cases, the force due to the presence of an external electric field and surface charge was responsible for the instability of the droplet (Taylor 1966). The charges distributed on the liquid surface repel each other and cause a force opposing the surface tension. In the case of the positively charged needle, the conducting liquid and needle are in close contact, bearing approximately the same potential difference. The electric field very close to the meniscus, E, is therefore,

$$E = \frac{q}{4\pi\varepsilon_0 r^2} \quad (1)$$

in which ε_0 is the permissivity of the air, and r is the radius of the droplet (roughly equal to the needle radius). The charge is applied to the whole needle, which can be considered a cylinder. Therefore the charge density on the needle, σ, is approximately,

$$\sigma = \frac{q_T}{2\pi r L} \quad (2)$$

in which q_T is the total applied charge, and L is the needle length. The effective charge on the droplet

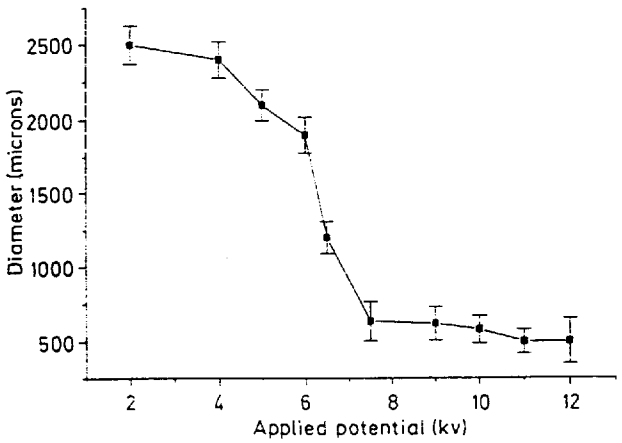


Fig. 4 Effect of applied potential on microbead diameter from multi-needle apparatus

would then be,

$$q = \frac{r}{2L} q_T \quad (3)$$

For the parallel-plate arrangement, the electric field strength is given as,

$$E = \frac{\Delta V}{x} \quad (4)$$

in which ΔV is the potential difference, and x the distance, between the plate and collecting solution. The effective droplet charge would be, in this case,

$$q = \left(\frac{r}{R}\right)^2 q_T \quad (5)$$

where R is the radius of the plate.

An examination of these relationships leads to the following conclusion. Due to the small area available for charge distribution (needle tip) the overall surface charge would be higher for the directly charged needle than for a parallel plate electrode, given the same potential difference. Therefore a much higher potential difference is required to build a sufficiently large charge on the plate to produce the same effect as that of a single positively charged needle.

Large-scale electrostatic droplet generation

The multiple needle device was designed to be identical in set-up to the parallel-plate, single-needle electrode arrangement. The results obtained were comparable to those obtained from the single needle. At a 2-cm electrode distance, increasing the potential difference from 6 to 14 kV resulted in a decrease in bead diameters from 900 ± 100 μm to 500 ± 150 μm (Fig. 4).

Effect of electrostatic droplet generation on cell viability

To assess the effect of an electrostatic field on animal cell viability, an insect cell suspension was extruded through the electrostatic droplet generator set-up. No detectable change in cell viability was observed. The initial cell density was 3.5×10^6 cells/ml whereas the post-electrostatic cell density was 3.4×10^6 cells/ml after application of 10 kV. Prolonged cultivation of cells previously exposed to a high voltage did not show any loss of secretion function (Bugarski et al. 1994b). This is a promising result as it demonstrates that the technique is amenable for cell immobilization and encapsulation.

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