0273-2289/89/2021—0561$02.00

Characterization of Alginate Beads Formed by a Two Fluid Annular Atomizer

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ABSTRACT

A two fluid atomizer for the formation of small alginate beads has been described. It produces beads of narrow size distribution, with mean diameters in the range of 50–500 μm. The design of the nozzle allows for quick disassembly for easy cleaning and sterile operation. Empirical correlations for the mean size distributions are also provided.

Index Entries: Alginate beads; atomizer; size distribution; injection; nozzle.

NOMENCLATURE

d_b	average diameter of beads, μm
d_n	external diameter of needle, μm
k	consistency index of solution, $g.s^m/cm$
L	length of the nozzle, cm
m	flow index of solution, dimensionless
Δp	pressure drop across the nozzle, $g.cm/s^2$
R	internal radius of the nozzle, cm
Q_l	liquid flow rate, cm^3/s (cm^3/h when so stated)
v_g	gas velocity around the nozzle at 20°C and 1 atm, m/min

INTRODUCTION

In a number of bioprocesses, formation of the desired product can be effectively decoupled from growth of the cells. In such cases, immobiliza-

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tion of the biocatalyst is often practiced to improve the yield and the volumetric productivity of the processes. Immobilization is commonly achieved by injecting a suspension of cells in an appropriate gelling agent (such as aqueous solutions of sodium alginate (*1*), κ-carrageenan (*2*), or chitosan (*3*) through a nozzle into a suitable salt solution. The suspensions are generally non-Newtonian in nature. Sizes of the beads, thus formed, depend on the design of the nozzle system, operating parameters, and the rheological properties of the solution/suspension being injected.

The simplest procedure is to let individual drops separate from the nozzle tip under gravity. The diameter of the beads is governed by a balance of the interfacial and the gravitational forces. Typically, 2–5 mm diameter beads are formed and the liquid throughput rates are very small (*4*). Smaller beads (0.5–2 mm) and considerably larger liquid flow rates (up to 24 L/h) have been obtained by superimposing resonant vibrations (*5*) on the system and by use of smaller diameter nozzles (*6*). Hulst et al. (*7*) have demonstrated that bead diameters in this range are qualitatively governed by Weber's extension of Raleigh's theory of unstable jets.

For a number of applications, beads of diameters as small as 1 mm are suspected to encounter mass transfer limitations (*8*). For the encapsulation of animal cells, spherical beads in the size range of 100–300 μm are desirable (*9*). These can be obtained by the use of smaller nozzle diameters that unfortunately result in the need for extremely small liquid flowrates and significantly increased clogging frequency of the nozzles. Rehg et al. (*10*) used a two-fluid atomizer design for the formation of small alginate beads. This report describes a two-fluid atomizer design and a quantitative characterization of the sizes of beads formed with it.

MATERIALS AND METHODS

Chemicals and Reagents

High purity Sodium Alginate (Keltone HV, lot #35256A) was obtained from KELCO, Clark, NJ. The alginate powder was dry autoclaved at 250°F for 20 min and then slowly dissolved in distilled water at the concentration levels of 1% (w/v) and 1.5% (w/v). The alginate beads were hardened by reaction with 0.1 *M* $CaCl_2$ (Sigma, St. Louis, MO) solution.

Experimental Setup

The two-fluid atomizer system for injection forming of beads is schematically shown in Fig. 1. It consists of a pressure tank for holding the liquid solution or suspension, an extrusion nozzle, and a collection vessel. A detailed schematic of the extrusion nozzle is shown in part A of Fig. 1. The nozzle assembly is made of brass parts that can be easily disassembled for cleaning or replacement of the hypodermic needle. The

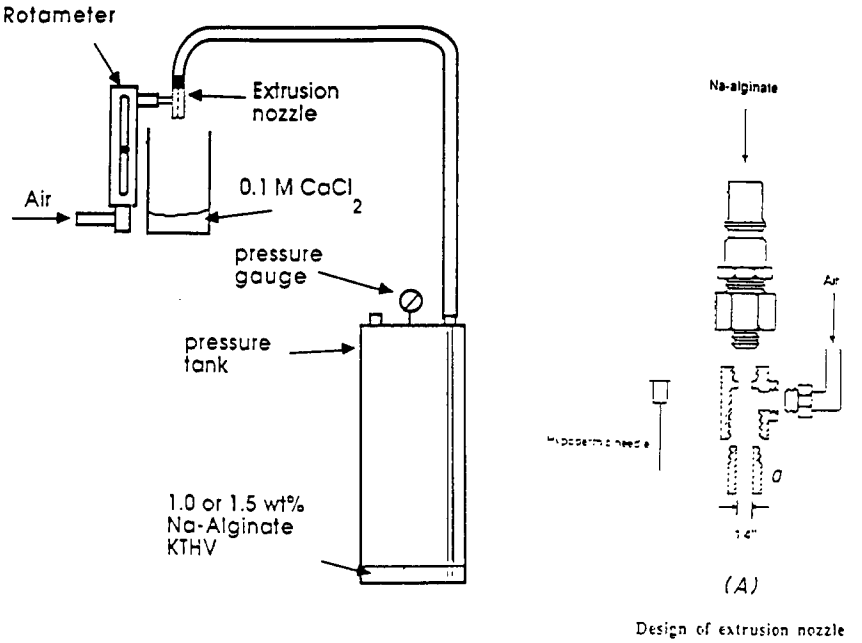

Fig. 1. Experimental setup.

whole assembly can be sterilized and a quick-connector has been used on the solution side in order to permit sterile connections to the cell suspensions. The length of the bottom tube (a) was adjusted such that its end corresponded to the tip of the needle. This design permitted use of needles of different lengths as well as formation of a manifold of several needles.

Hypodermic needles of 22 gage (od 710 μm, id 424 μm, length 1″) and 25 gage (od 445 μm, id 302 μm, length 1.5″) were used in this work. Needles of smaller diameters were found to clog easily. The tips of the needles were not flattened as flattening caused wider size-distributions and the formation of a significant number of nonspherical beads. Flowrates of the alginate solutions through the nozzle assembly were varied by adjusting the pressure in the pressure tank. Air was blown concentrically around the needle and its flowrate was measured with a rotameter. The air flowrate as well as the pressure in the pressure tank were carefully controlled as operating parameters.

The distance between the tip of the needle and the surface of $CaCl_2$ solution in the collection vessel was found to be crucial for the shape of the beads. For the gas velocities used in this work, a distance of 8″ or greater resulted in spherical beads as observed under a microscope. Since the beads are dispersed in a conical pattern from the nozzle, larger distances require the use of a big trough as a collection vessel. The collection vessel used in this work was a 2-L glass beaker.

All experiments were conducted in a controlled environment at 20°C. The beads were allowed to harden for 1 h before their size distributions

were measured under a microscope fitted with a micrometer. Sizes of at least 100 beads were measured for each operating condition. When beads were allowed to stay longer in the $CaCl_2$ solution, agglomerates were formed. Upon transfer into isotonic solutions, these agglomerates quickly dispersed into individual beads without affecting their size distribution.

Rheological Properties

Dry autoclaving of alginate powder results in changes in the rheological properties of alginate solutions. Therefore, these were estimated by using the present setup as a capillary viscometer. Flowrates of alginate solutions were measured for different values of pressure drops across the capillary, and these data were fitted into the following expression for the flow of power law fluids through a capillary

$$Q_1 = (\Delta p)^{1/m} [1/(2Lk)]^{1/m} [m/(3m + 1)] R^{(3m + 1)/m} \quad (1)$$

The 1 and 1.5% solutions were found to follow the power law model very well. For the 1% solution, the flow index (m) was 0.67 and the consistency index (k) was 5.32 $g.s^m/cm$. For the 1.5% solution, these values were 0.62 and 18.4 $g.s^m/cm$, respectively. Surface tensions were also measured using a drop-measurement method and were found to have the same values as water at 20°C.

RESULTS AND DISCUSSION

Bead size distributions were measured for different combinations of gas velocity (23.8–38.6 m/min), liquid flowrate (3.1–92.0 mL/h), needle size (22 and 25 gage), and alginate concentration (1 and 1.5% w/v). These were used to calculate the average bead diameters, standard deviations, and the coefficients of skewness (*11*). The results are presented in Table 1. Several distributions were measured repeatedly and excellent reproducibility of the data was confirmed. The qualitative nature of the results clearly shows that the average bead diameter is very sensitive to the gas velocity. It is not affected by liquid flowrate within the operating range reported here. For flowrates higher than 90 cm^3/h with the 22 gage needle, the distributions of drop sizes were very broad, even though small beads were still formed. The nozzle diameter has only a moderate influence on bead size distributions. The bead size is one to two orders of magnitude smaller, and the standard deviations are comparable to those made by Hulst et al. (*7*). Figure 2 shows a typical bead size-distribution on a normal probability plot and it can be concluded that these distributions are *normal*. This conclusion can be justified for most of the measurements reported in Table 1.

The average bead diameters have been plotted in Figs. 3 and 4 as a function of gas velocity around the needles. The measurements for 1.5% alginate solution show a linear reduction in bead diameter with increas-

Table 1

Alginate conc. w/v	Needle, gage	v_g, m/sec	Q_1, cm^3/h	Average diameter, μm	Standard deviation, μm	Coeff. of skewness
1.0	22	32.8	0.5	38.6	7.1	0.25
		29.8		59.1	6.7	0.77
		26.8		88.4	6.0	−0.75
		23.8		423.0	11.1	−0.07
1.0	22	32.8	4.7	66.7	7.6	0.13
		29.8		82.4	7.7	0.22
		26.8		104.0	7.7	0.50
		23.8		442.0	17.0	0.15
1.0	22	32.8	11.0	63.9	9.6	0.49
		29.8		86.4	10.1	0.68
		26.8		107.0	7.6	0.31
		23.8		439.0	15.5	0.46
1.0	22	32.8	38.0	65.9	9.4	0.27
		29.8		87.0	11.2	0.38
		26.8		110.7	9.3	0.19
		23.8		442.7	15.2	0.58
1.0	22	32.8	62.0	72.0	11.3	0.58
		29.8		90.9	9.6	0.51
		26.8		115.4	11.2	0.45
		23.8		444.7	13.6	0.55
1.0	22	32.8	89.0	72.3	10.0	0.66
		29.8		93.6	11.1	0.36
		26.8		119.0	11.1	0.32
		23.8		446.7	13.4	0.60
1.0	25	32.8	1.3	56.9	6.0	0.19
		29.8		62.6	6.4	0.60
		26.8		311.0	11.1	−0.59
		23.8		402.0	8.4	0.55
1.0	25	32.8	2.2	49.6	6.2	0.48
		29.8		60.6	9.7	0.36
		26.8		328.0	11.5	0.40
		23.8		406.0	8.7	1.62
1.0	25	32.8	3.3	48.1	6.7	0.09
		29.8		63.7	12.2	0.85
		26.8		282.0	15.1	−0.22
		23.8		401.0	9.0	−0.03
1.5	22	32.8	6.5	150.0	11.1	−0.26
		29.8		309.0	13.4	−0.54
		26.8		447.0	13.5	−1.18
		25.3		540.0	15.4	−0.98
		23.8		609.0	7.6	−0.35
1.5	25	32.8	14.1	103.0	9.3	0.14
		29.8		252.0	15.1	−0.28
		26.8		377.0	13.2	−0.55
		25.3		423.0	15.1	−0.22
		23.8		541.0	11.5	0.80

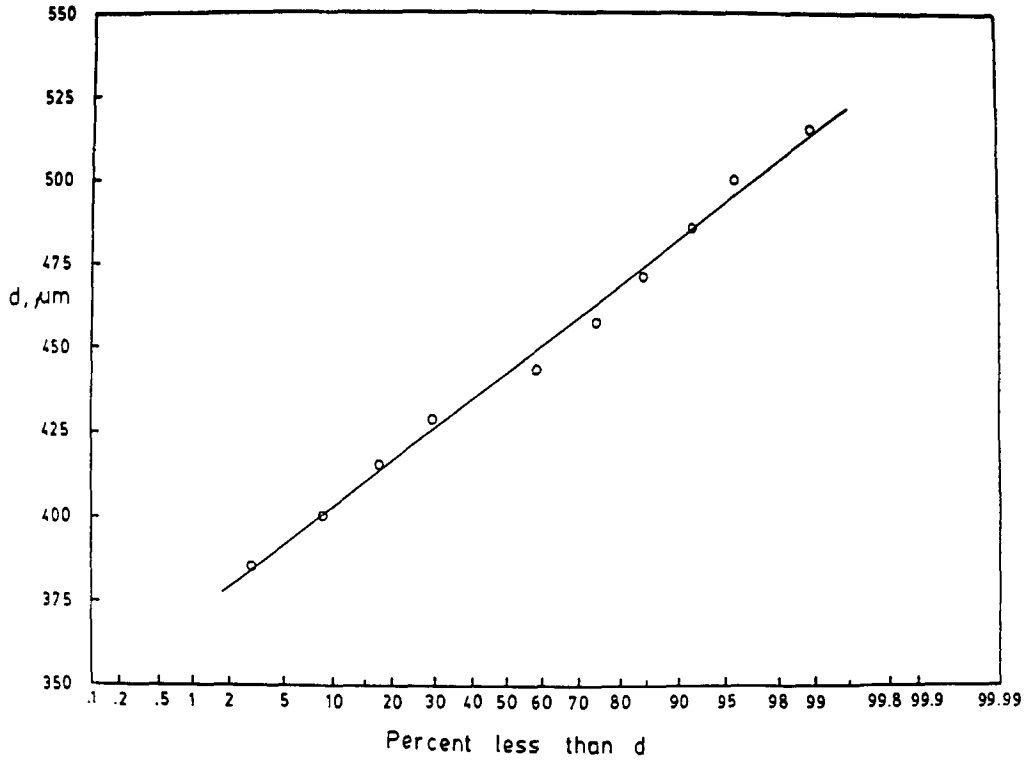

Fig. 2. Bead size distribution data for 22 gage nozzle, gas velocity = 23.8 m/s, liquid flowrate, $Q_l = 4.7$ cm^3/h.

ing gas velocity. The data for 1% alginate solution have a complex dependence on gas velocity. Unlike the experience of Rehg et al. (*10*), an attempt to explain these data with the help of the Nukiyama and Tanasawa (*12*) correlation for two fluid atomizers was not successful. The gas velocities in the present work were many fold higher than those used by Rehg et al. (*10*), which may be the primary reason for the failure of the correlation. This correlation has been used by Laskowski and Ranz (*13*) to predict their data for the spraying of low viscosity liquids.

A visual observation of bead-formation (1% alginate solution) at the needle-tip with the help of a cathetometer (20x magnification) resulted in the following description of the phenomena. At low gas flowrates, the solution exiting the needle forms a drop at the tip, which is then sheared away. The solution wets the outer perimeter of the needle. The magnitude of bead diameters in this situation are of the order of the size of needle, d_n. As the gas flowrate is increased, the phenomenon changes to one in which a continuous flow of liquid takes place to the very pointed tip of the needle where small drops are sheared off. At very high liquid flowrates where a continuous stream of liquid can be seen to flow out of the nozzle, destabilization of liquid jet by turbulence induced by the air stream results in formation of beads whose sizes have a relatively wide distribution. This

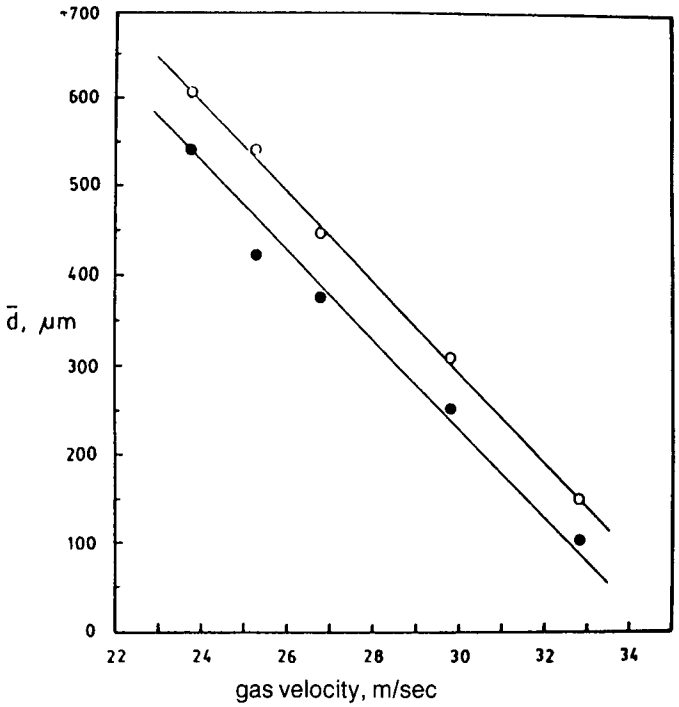

Fig. 3. Average bead diameter as a function of gas velocity. 1% alginate solution. ○: $Q_1 = 0.5$ cm^3/h, △: 11.0, □: 38.0, ●: 1.3, ▲: 2.2, ■: 3.3.

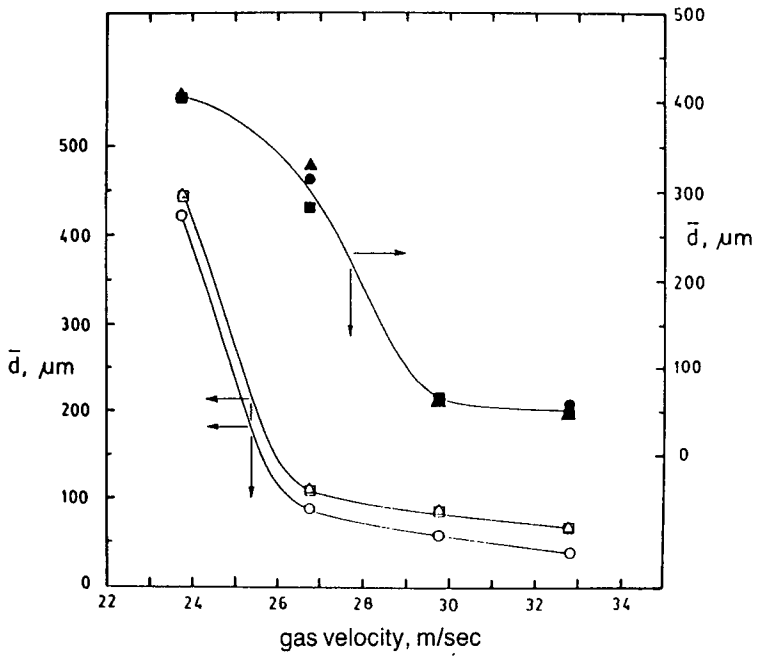

Fig. 4. Average bead diameter as a function of gas velocity. 1.5% alginate solution. Open symbols are for 22 gage needle and closed symbols are for 25 gage needle.

range is not interesting from the viewpoint of formation of beads with narrow size distribution. Thus, the data in Table 1 can be split into two regions, depending on the gas flowrates. At low gas flowrates, $d_b \sim d_n$ and

$$d_b = \exp(11.3)\, v_g^{-2}\, d_n^{1/6} \tag{2}$$

For higher gas flowrates were $d_b << d_n$

$$d_b = \exp(9.7)\, v_g^{-2.5}\, d_n^{0.5} \tag{3}$$

For the 1.5% alginate solution, no such clear change in the phenomenon was evident, and the beads apparently formed at the very pointed tip of the needles. For these data, the following empirical correlation was found

$$d_b = \exp(13.3)\, v_g^{-3.2}\, d_n^{0.5} \tag{4}$$

These correlations should be used cautiously as the phenomenon of atomization with pointed needles is not clearly understood (*10,14*). When used within the range of experimentation, predictions within $\pm 10\%$ can be expected. There is a need to conduct a fundamental fluid-mechanical analysis of the formation and shearing of drops of viscous non-Newtonian fluids at the nozzles in two-fluid atomizer systems.

CONCLUSIONS

A two-fluid atomizer has been shown to be capable of producing small alginate beads in the range of 50–500 μm mean diameter. The distributions are as narrow as those obtained with other comparable methods producing considerably larger beads. Empirical expressions have been presented relating the measured data to operating conditions.

ACKNOWLEDGMENT

High purity sodium alginate powder used in this work was supplied by Kelco, Div. of Merck & Co., Inc., NJ, which is gratefully acknowledged.

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