Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Robichaud, Michael J. , Sathyagal, Arun N. , Carr, Peter W. , McCormick, Alon V. and Flickinger, Michael C.(1997) 'An Improved Oil Emulsion Synthesis Method for Large, Porous Zirconia Particles for Packed- or Fluidized-Bed Protein Chromatography', Separation Science and Technology, 32: 15, 2547 — 2559
To link to this Article: DOI: 10.1080/01496399708000785
URL: http://dx.doi.org/10.1080/01496399708000785

SEPARATION SCIENCE AND TECHNOLOGY, 32(15), pp. 2547–2559, 1997

TECHNICAL NOTE

An Improved Oil Emulsion Synthesis Method for Large, Porous Zirconia Particles for Packed- or Fluidized-Bed Protein Chromatography

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ABSTRACT

A previously reported oil emulsion technique for the synthesis of large, porous zirconia particles has been modified to overcome problems of nonreproducibility,

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formation of non-spherical or broken particles, and low yield of chromatographically useful particles. The improved technique described here uses a concentrated sol and an in-line mixer to successfully solve these problems. The results indicate that the stirring rate used to maintain the emulsion during drying has a strong effect on the final particle size.

INTRODUCTION

The synthesis of porous, dense ceramic support particles such as zirconia (ZrO_2) for use in fluidized-bed adsorption of biological molecules is important for the development of robust separation media. These particles may be uniquely suited for the direct adsorption of proteins from particulate-containing biological fluids without prior removal of solids (1). Having high effective density and excellent thermal and chemical stability (1–3), zirconia particles have advantages over traditional silica, polymeric, or composite supports. Higher densities allow the use of higher superficial velocities (i.e., throughputs) without entrainment of the support particles, and the use of smaller particles to more rapidly adsorb proteins in the presence of entrained particulate matter such as cells or cell debris. The thermal and chemical stability of zirconia allows the use of harsh clean-in-place and depyrogenation procedures using heat, acids, bases, alcohols, salts, detergents, or combination thereof, such as are commonly used in the pharmaceutical industry.

In this work we describe a technique for the reproducible synthesis of large (50–150 μm) porous zirconia (ZrO_2) particles for packed-bed or fluidized-bed protein chromatography. The technique used is an oil emulsion process related to that reported earlier (1, 4). A stable, aqueous colloidal sol is dispersed as droplets in a hot oil phase; the water in the droplets is extracted into the continuous oil phase to the point where the colloids gel; the resulting aggregates are collected, dried, and sintered. The challenge is to find operating conditions that allow a stable liquid emulsion to be reproducibly generated and maintained and yet allows fast water extraction so as to increase the production rate and minimize broadening of the droplet size distribution due to coalescence and breakage.

Previous experiments to synthesize 50–150 μm porous zirconia particles using the oil emulsion process described in Carr et al. (4) and modified by the addition of a surfactant (1) produced a low yield of 50 μm particles (designated SOM particles) that were useful but not optimal in three respects. First, the yield of spherical zirconia particles from a single batch was low and only shallow beds could be evaluated in small 25 mm diameter

fluidized-bed columns (1). Worse, separate batches were not consistent with one another in particle or pore size distribution, or in density. Worst, separate batches produced using this initial process had a high percentage of broken and nonspherical particles (Fig. 1).

Our objective in this work was to optimize the previously reported oil emulsion process. Specifically, we wanted a reproducible synthesis procedure so that batch-to-batch variation in the properties of the particles is minimized. The technique should also be able to provide a higher yield of spherical zirconia particles in an acceptable size range for fluidized-bed applications (50–150 μm) with minimal broken particles. The process should also be easily manipulated to get a variety of particle and pore sizes. The principal modifications employed in this work are (1) concentration of the sol to reduce drying time and increase yield; and (2) use of an in-line mixer to reproducibly generate the initial drop size distribution.

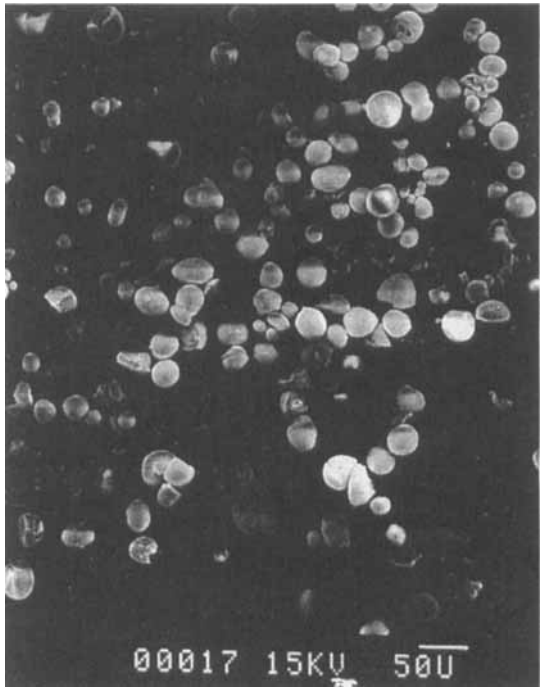

FIG. 1 Scanning electron micrograph of SOM particles synthesized by the previous process.

Such dispersion-type processes have also been used to generate spherical (mainly non-porous) particles of many other materials. For example, Haas and Clinton (5) and Wymer (6) described oil emulsion processes to manufacture pure and mixed oxide particles for nuclear fuel applications. Harris et al. (7) used a reactive dispersion process to form pure and mixed oxide ceramic particles from aqueous salt solutions. Various emulsifying techniques have been used including stirrers (4), two-fluid nozzles (5, 6), vibrating capillaries (5, 6), and pulsing electric fields (7).

EXPERIMENTAL

Aqueous zirconia sol (pH 3.0), containing 20 wt% ZrO_2 particles (90% of the particles having a nominal size of 0.1 μm), was purchased from Nyacol Products, Inc. (Ashland, MA). The colloid-size distribution was determined by photon correlation analysis with a Coulter N4SD particle-size analyzer. Peanut oil (Baker's Secret brand), oleyl alcohol (Eastern Chemical Co.), and concentrated nitric acid (EM Science) were also used.

The procedure used to synthesize the porous zirconia particles (designated FBOM particles) is as follows: The as-received zirconia sol is concentrated to 44.5 wt% zirconia by centrifugation in a Beckman JA-10 centrifuge operated at 17,700*g* for 2 hours. The resulting particle cake is resuspended in pH 3 nitric acid. The amount of nitric acid needed for resuspension is determined through a mass balance using measurements of both the initial density of the sol and the density of the supernatant removed from the pellet. Resuspension is carried out at 37°C using a rotary shaker oscillating at 240 rpm for 12 to 24 hours. The size distribution of the colloids resulting from this concentration procedure is shown in Fig. 2.

A schematic diagram of the experimental apparatus is shown in Fig. 3. The experiments are carried out in a rectangular LDPE vessel with a base of 18.4 cm by 26.0 cm and a height of 30.5 cm. The vessel is initially filled with 3000 mL of a 1:1 volume ratio mixture of peanut oil and oleyl alcohol. This is agitated with a centered, 12.7 cm diameter, 5-bladed propeller-type impeller (Jiffler) rotating at a fixed speed while the oil–oleyl alcohol mixture is preheated to 95°C using a boiling hot water bath insulated with foam. The agitation is just slow enough to avoid vortices. The initial depth of oil in the batch vessel is 9 cm.

An aqueous–oil dispersion is created using 30.7 cm of 0.48 cm O.D. polyacetal in-line Kenics mixers (Cole-Parmer) contained in 0.48 cm I.D. Tygon tubing. The aqueous stream consists of the previously concentrated zirconia sol, and the oil phase stream consists of the peanut oil and oleyl alcohol mixture. The streams are combined using a glass "Y" (Kimax)

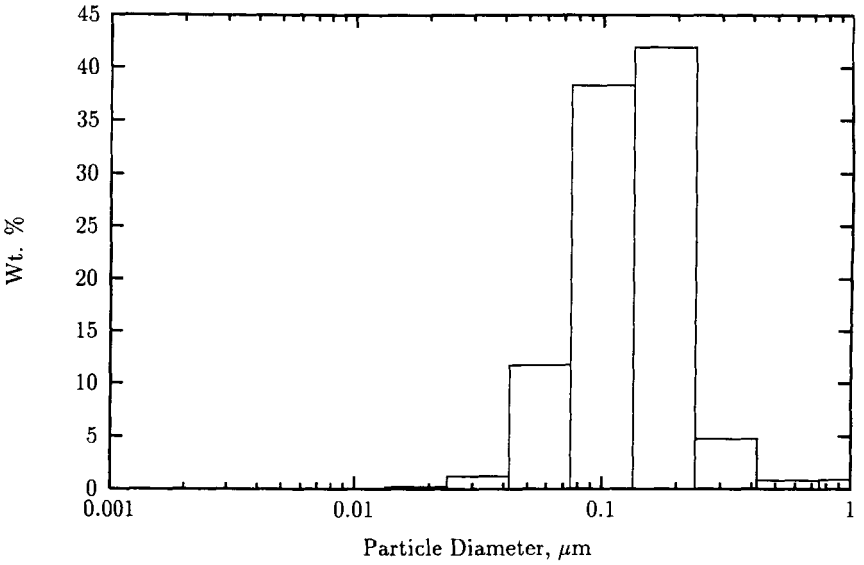

FIG. 2 Size distribution of zirconia colloid obtained after centrifugation and resuspension.

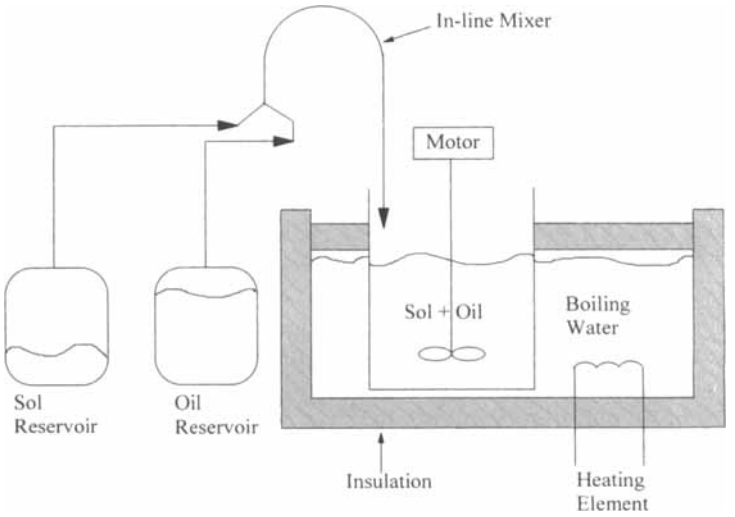

FIG. 3 Schematic diagram of the experimental apparatus used to prepare the FBOM particles.

and flow through the in-line mixer at a 1:3 sol:oil volume ratio. Pumping is accomplished using peristaltic pumps. Temperatures of the sol and oil are ambient (~22°C). All of the sol is able to be completely pumped through the in-line mixer without air bubbles by adding ~50 mL of the oil phase to the top of the sol and pumping for an extra minute to clear the lines of sol. The addition of the sol–oil dispersion causes the batch temperature to drop to 76°C and the batch depth to increase to ~13.2 cm (~5160 mL total oil plus sol). As the depth increases, the agitation rate can be increased while still avoiding vortices. The agitation rate is gradually increased to its final value over a time period of about 8 minutes.

The batch temperature increases to a steady-state temperature of 90°C after about 60 minutes, and the heating and stirring is continued in order to remove water from the sol droplets. As the water from the emulsion is eliminated, the sol droplets densify, and ultimately the colloidal particles within the droplets aggregate to form solid, stable particles with little water present (~81 wt% zirconia as collected). The typical batch time is approximately 90 minutes. During this time the status of the droplets/particles is monitored periodically by examining samples under an optical microscope at 200× magnification. In addition, particle stability is also tested by simulating the wash protocol on a small sample and examining it under the microscope. The particles are considered stable to the solvent wash when the largest particles remain intact during the wash and can support a microscope cover slip without breaking.

After this time the agitation is turned off and the batch is allowed to settle for 10 minutes. After decanting the oil the particles are resuspended in five 1-L portions of isopropanol. Each portion is filtered under vacuum with fresh fast-flow filter paper (Baxter Healthcare) lining a 10.5-cm Buchner funnel. Finally, the particles in the filter cake are rinsed with isopropanol and dried under vacuum for 45 minutes.

The particles are transferred to a porcelain boat large enough that the depth of particles is no more than 2.5 cm and a staged heating process is used to sinter the particles. The particles are first heated for 2 hours at 100°C to drive off water and isopropanol, followed by 2 hours at 375°C to burn remaining organics. The temperature is then increased to 750°C for 6 hours to burn off surface carbon and nitrogen, and finally the particles are sintered for 3 hours at 900°C to improve their mechanical strength. Sintering at higher temperatures (975°C, 1050°C) did not significantly reduce the pore size but decreased the surface area by 22.5 and 40%, respectively (data not shown). A 40°C/min temperature ramp in a programmable furnace (NEY, Model 6-525) is used to reach each temperature.

After sintering, the particles are screened with U.S. Standard sieves to select particles with diameters between 38 and 75 μm. The screened parti-

cles are then washed with 0.5 M NaOH and 0.5 M nitric acid to establish a consistent surface chemistry to prepare the particles for chromatographic use. The base and acid wash begins by adding the particles to enough carbonate-free double-distilled water to saturate and cover the particles. The water and particles are alternately swirled and sonicated under vacuum for 15 minutes to eliminate air from the particle pores. The supernatant is decanted and the particles are washed and gently rocked in excess carbonate-free 0.5 M NaOH on a shaker table overnight (8–14 hours). The supernatant is decanted and the particles are rinsed with double-distilled water. The particles are then washed and gently rocked in 0.5 M nitric acid on a shaker table overnight (8–14 hours). Again, the supernatant is decanted and the particles are rinsed with copious amounts of double-distilled water. The particles are finally dried under vacuum at 100°C for 8 hours. The washed particles are then elutriated with water in a 25 mm × 60 cm column to remove colloid that becomes loosened, and to remove hollow or low density particles and fragments. The elutriation process is also used for size classification of the particles by controlling the upward flow rate of water.

Nitrogen adsorption isotherms of the particles were collected on a Micromeritics Sorptometer. Scanning electron micrographs of the particles were taken with a Hitachi 450 scanning electron microscope as previously described (1). The apparent density of the particles was measured by weighing a volumetric flask before and after loading the sample. The sample was loaded in a minimum of four increments; after each, the flask was tapped for at least 30 seconds to assist in uniform packing of the particles.

RESULTS AND DISCUSSION

An electron micrograph of the particles synthesized in a typical batch is shown in Fig. 4. The micrograph shows that the synthesis technique produces spherical particles with minimal clumping and minimal breakage. The size distribution, determined by sieving, for the batch shown in the micrograph is shown in Fig. 5. Approximately 75% of the particles (by weight) are in the size range of 38–74 μm diameter, a size useful for fluidized-bed applications. The pore size distribution of these particles obtained by nitrogen adsorption and desorption is shown in Fig. 6. The particles have a fairly narrow range of pore sizes.

A major concern with the previous oil emulsion technique to synthesize porous particles was the variability in particle characteristics with experiments conducted under identical conditions. The modifications described in this work were tailored to improve the reproducibility of the process. The characteristics of the particles obtained in repeat experiments con-

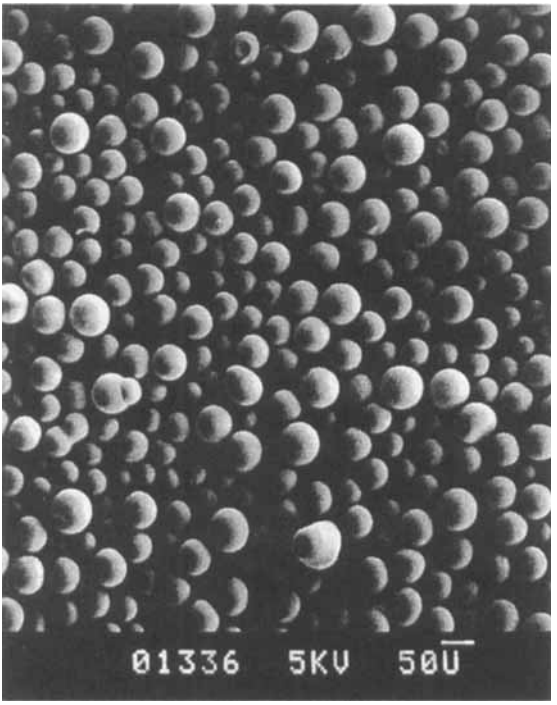

FIG. 4 Scanning electron micrograph of representative FBOM particles prepared by the improved process.

ducted under identical conditions are compared in Table 1. As shown in the table, many of the characteristics of the particles obtained in the two experiments are nearly identical.

We have studied the effect of varying the stirrer speed and the flow rate of the dispersion through the in-line mixer on particle characteristics. A test batch was also generated without an in-line mixer. The average particle sizes obtained are compared in Table 2. In spite of these variations, greater than 65 wt% of the particles formed in these experiments were within one standard sieve size on either side of the average particle size, resulting in a reasonably sharply peaked distribution.

Table 2 indicates that the stirrer speed has a greater effect than the flow rate through the in-line mixer on the final particle size. For example, the particle size increases sharply with a large decrease in the stirrer speed. The difference in the flow rates through the in-line mixer in these two

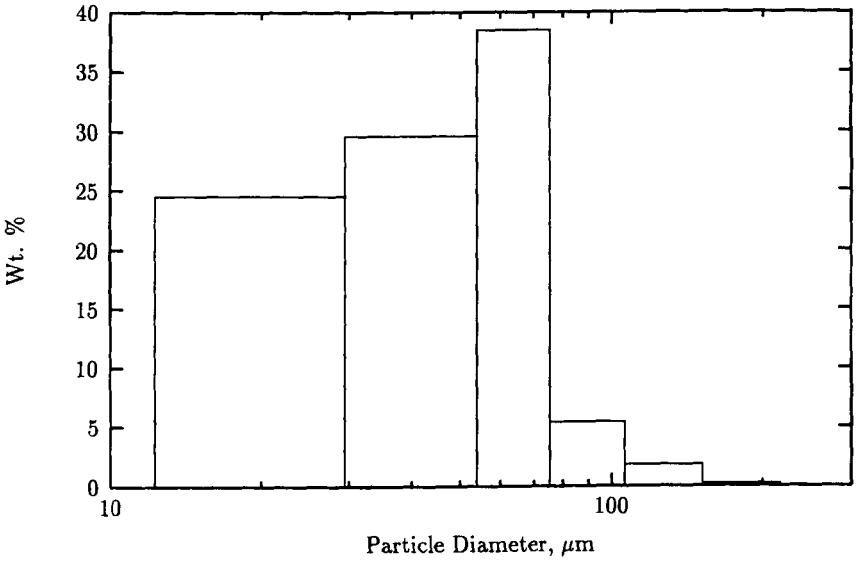

FIG. 5 Size distribution of FBOM particles.

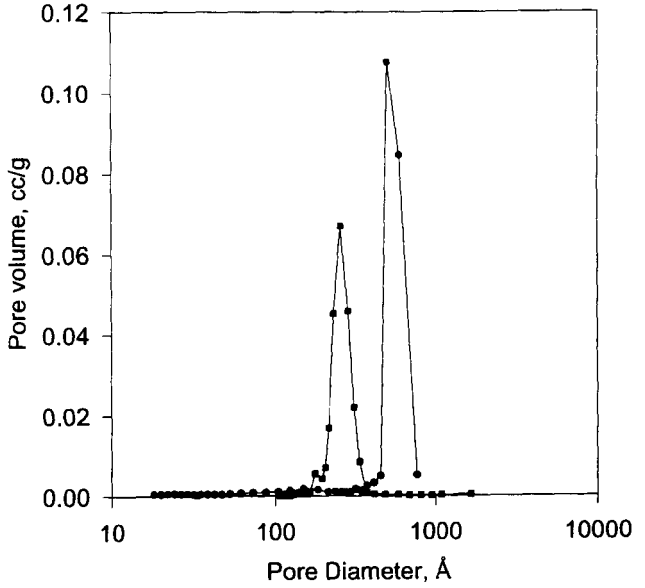

FIG. 6 Adsorption (●) and desorption (■) pore size distributions of FBOM particles obtained via nitrogen porosimetry.

TABLE 1
Comparison of FBOM Particles Obtained with Repeat Experiments Compared to SOM Particles Synthesized Using the Previous Method Described in Griffiths et al. (1)

Particle characteristics	SOM	FBOM Experiment 1	FBOM Experiment 2
Mean particle size, μm	43 ± 13	50 ± 23	48 ± 22
Porosity	0.54	0.57	0.56
Average pore diameter, Å	366	320	321
Surface area, m^2/g	21.5	28.9	27.9
Apparent density, g/cm^3	1.7	1.63	1.63
Particle yield in 38–74 μm range, %	81	75.1	73.0

experiments is quite small and cannot entirely account for the effect observed on particle size. A comparison of the experiments done at the two stirrer speeds (300 and 370 rpm) with the same flow rate through the in-line mixer also shows that particle size increases with a decrease in the stirrer speed. The magnitude of the variation in particle size with stirrer speed is comparable to the change observed in average drop size with stirrer speed in agitated liquid–liquid dispersions (8–10). However, using the in-line mixer does seem to have an effect on the particle size as evidenced by the increase in particle size when no in-line mixer is used.

There have been numerous studies on the effect of flowing a liquid–liquid mixture through an in-line mixer. For example, Middleman (11), Haas (12), and Berkman and Calabrese (13) reported the average drop sizes and the drop size distributions obtained on flowing a two-phase liquid mixture through a Kenics in-line mixer. Haas (12), in particular, studied the dispersion of aqueous drops in an organic phase, and the flow rates used in his

TABLE 2
Comparison of Average Particle Sizes Obtained Under Different Experimental Conditions

Flow rate through in-line mixer (mL/s)	Stirrer speed (rpm)	Average particle size (μm)
4.22	450	33 ± 17
4.00	260	88 ± 83
2.48	370	49 ± 22
2.48	300	63 ± 25
No in-line mixer	300	78 ± 38

study are comparable to those used in this work. Using the correlation for the average drop size developed by Haas (12), and using typical values for the physical properties of the liquids (assuming that the two liquid viscosities are comparable, and that the interfacial tension is approximately 15 dynes/cm), the average sol drop size exiting the in-line mixer in our experiments varies from approximately 300 μm (at 4.22 mL/s flow rate) to 1650 μm (at 1.35 mL/s flow rate). These sizes are much higher than the final particle sizes reported (Table 2) even accounting for the shrinkage of the drops due to the removal of water (using the initial colloid concentration in the sol and the final particle porosity, the drops should shrink in diameter by approximately 33%). This indicates that the sol drops exiting the in-line mixer are broken by the action of the impeller into smaller drops while they dehydrate and solidify.

The modification introduced in this work of dispersing the sol with an in-line mixer before adding it to the stirred vessel does offer some advantages. By dispersing the sol into droplets before entering the stirred vessel, we allow the sol drops to be broken more consistently by the stirrer. Without the use of the in-line mixer, the sol is initially in the form of large drops (mm scale) which can be broken by the impeller in an inconsistent manner. This could lead to the formation of some large drops, hence increasing the average particle size, and this would also explain the batch-to-batch variation in particle sizes observed in the previous oil emulsion process (1). The variability in mixing behavior when the dispersed phase is poured into the stirred vessel has also been reported in the liquid–liquid mixing literature. Sathyagal et al. (14), in their studies on drop breakage, stirred the dispersion slowly on addition of the dispersed phase to gently break up the large dispersed phase drops before increasing the stirrer speed to the final value. By following this procedure they were able to obtain reproducible results between experiments. As mentioned by Godfrey (15), an advantage claimed for the in-line mixer is that of predictable and repeatable mixing performance. This indicates that, under the same experimental conditions, the size distribution of drops entering the stirred vessel from the in-line mixer should be similar, resulting in a more reproducible oil emulsion.

The other significant modification introduced here was that of concentrating the sol before using it to form particles. For the current process the sol was concentrated to 44.5 wt% zirconia in nitric compared to approximately 15 to 20 wt% used in previous methods. This concentration was chosen because it was near the highest concentration of zirconia possible at ~pH 3 without the sol becoming unstable due to flocculation. Concentrating the sol allowed the amount of particles produced to be increased greatly without a large increase in the amount of associated

water. The reduction in water content of the sol also reduced the time required for drying. The reduction in drying time by concentrating the sol from the process means that the droplets/particles have to spend less time in the stirred environment, and the particles thus have less opportunity to break when they are in a brittle state and to form chopped, flattened, or broken particles. Overall, concentrating the sol allowed the total batch size to be increased from 80 g of SOM particles with previous processes (1) to 360 g of FBOM particles with the improved process reported here.

Although the use of urea and surfactant in oil emulsion processes have been reported (1, 4), neither were used in this process.

CONCLUSIONS

We have successfully modified the previous oil emulsion process (1) to overcome the problems of batch-to-batch variability, low yield, and chopped, nonspherical particles. By concentrating the sol before it is used to form particles, we have reduced the drying time and increased the amount of particles obtained from each experiment. By introducing the sol into the stirred vessel through an in-line mixer, we have significantly improved the reproducibility of the process. We have studied the effects of varying stirrer speed and flow rate through the in-line mixer on the average particle size. The results indicate that the stirrer speed has a greater effect than the flow rate of the dispersion through the in-line mixer on the final particle size. However, the in-line mixer does have some effect on the particle size, as omitting it altogether increases the particle size under similar stirring conditions.

It must be emphasized that there can be other ways to modify the oil emulsion process to especially improve its reproducibility. For example, in a dilute dispersion (to minimize drop coalescence), a high stirrer speed could be initially used to break up the dispersed phase droplets and the impeller speed could later be reduced to keep the drops suspended while they dry and solidify.

REFERENCES

1. C. Griffith, J. Morris, M. Robichaud, M. J. Annen, A. V. McCormick, and M. C. Flickinger, *J. Chromatogr. A*, Accepted for Publication, 1997.
2. J. A. Blackwell and P. W. Carr, *J. Chromatogr.*, *549*, 59–75 (1991).
3. M. H. Glavanovich and P. W. Carr, *Anal. Chem.*, *66*, 2584–2589 (1994).
4. P. W. Carr, E. F. Funkenbusch, M. P. Rigney, P. L. Coleman, D. A. Hanggi, and W. A. Schafer, US Patent 5,015,373 (1991).
5. P. A. Haas and S. D. Clinton, *Ind. Eng. Chem., Prod. Res. Dev.*, *5*, 236–244 (1966).

6. R. G. Wymer, in *Sol-Gel Processes for Ceramic Nuclear Fuels*, International Atomic Energy Agency, Vienna, 1968, pp. 131–172.
7. M. T. Harris, T. C. Scott, and C. H. Byers, *Mater. Sci. Eng.*, *A168*, 125–129 (1993).
8. H. T. Chen and S. Middleman, *AIChE J.*, *13*, 989–995 (1967).
9. C. A. Coulaloglou and L. L. Tavlarides, *Ibid.*, *22*, 289–297 (1976).
10. R. V. Calabrese, C. Y. Wang, and N. P. Bryner, *Ibid.*, *32*, 677–681 (1986).
11. S. Middleman, *Ind. Eng. Chem., Prod. Res. Dev.*, *13*, 78–83 (1974).
12. P. A. Haas, *AIChE J.*, *33*, 987–995 (1987).
13. P. D. Berkman and R. V. Calabrese, *Ibid.*, *34*, 602–609 (1988).
14. A. N. Sathyagal, D. Ramkrishna, and G. Narsimhan, *Chem. Eng. Sci. 51*, 1377–1391 (1996).
15. J. C. Godfrey, in *Mixing in the Process Industries*, 2nd ed. (N. Harnby, M. F. Edwards, and A. W. Nienow, Eds.) Butterworth-Heinemann, Oxford, 1992, pp. 225–249.

Received by editor October 17, 1996
Revision received February 1997