SIEVING OF ALBUMIN MICROSPHERES WITH SONIFICATION

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To ease the process of sieving for the purpose of obtaining homogeneous size populations of human serum albumin microspheres, a technique was developed that uses sonification of a suspension of microspheres while sieving. This has resulted in populations of microspheres that are almost totally encompassed by the desired size range with little to no outsize contamination.

Measurement of the microspheres was accomplished by photographing the microspheres and a stage micrometer on the same roll of film and at the same magnification. The print of the micrometer was then used to measure the microsphere sizes on the other prints.

The value of microspheres of human serum albumin for the study of the circulation and arteriovenous shunts is well documented (*1–4*). Their value is dependent upon homogeneity of size, which determines what size blood vessel becomes occluded. Sieving to obtain homogeneity was difficult in our laboratory. This problem was studied by using a sonifier* and sieving with the microspheres in suspension.

MATERIALS AND METHODS

Microspheres were prepared essentially by the method of Zolle, et al (*1*) with the following modifications and specifications: (A) 500 ml of cotton seed oil were stirred in a 1-liter 3-necked-round-bottomed flask by a stainless steel paddle stirrer (18 × 18 mm for each of 2 blades); (B) the stirrer was rotated by a variable speed laboratory motor† with the variac setting on 1.50–1.75; (C) 2 ml of 25% human serum albumin were added dropwise through a 20-gage needle; (D) after heat was applied by means of a heating mantle and the temperature reached 130–140°C, this temperature was maintained for 30 min before allowing the mixture to cool with continuous stirring; (E) mixture was then transferred to 250-ml centrifuge bottles and centrifuged at 2,500 rpm (1,000 × G) for 1 hr at 20°C in the PR2 International Refrigerated Centrifuge; and (F) supernatant oil layer was decanted and the sediment was washed with diethyl ether (50-ml portions) at least five times to remove the residual oil.

The dried microspheres were resuspended in 10 ml of saline in a 30-ml beaker. One drop (dispopipette) of Tween 80 was added, followed by sonification for 1 min to break up the clumps. Verification of dispersion was obtained by microscopic examination. The suspension of microspheres was poured into a 60-micron sieve‡. The sieve was rinsed with 500 ml of a solution of 0.1% Tween 80 in saline. The portion that passed through the sieve was then poured into a 30-micron sieve. A Branson sonifier was eccentrically positioned at ⅓ of the diameter of the sieve. The tip of the probe was in the suspending solution 1 cm above the screen. With the liquid level kept above the tip of the probe and the sonifier on low power, the sieve was slowly rotated in a circle to allow full area coverage by the sonifier. The liquid level was maintained by the almost continuous addition of Tween 80-saline from 2 liters of solution. When the saline supply was exhausted, the sonifier was turned off and the fluid remaining on the sieve was pulled through it into the suction flask below. The sieve was then washed with the aid of a spray bottle of absolute ethanol. The sieve was removed from its position and its contents were allowed to dry at room temperature.

* Branson Sonifier Cell Disruptor, Heat Systems Co., 60 Broad Hollow Rd., Melville, N.Y. 11749.

† Model S63 TRI-R Instruments, Rockville Centre, N.Y. 11570. 1/15 H.P., 5,000 rpm capacity.

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‡ The 10-, 20-, 30-, and 60-micron sieves were obtained from the Buckbee-Mears Company, St. Paul, Minn. 55101.

A 20-micron sieve was then placed into position over the vacuum flask, and the washings from the 30-micron sieve were passed through the 20-micron sieve with the aid of the sonifier operating as described above. When the washings had passed through the sieve, 2 liters of Tween 80-saline were put through the sieve as a wash for the microspheres. As the last step, the solids on the sieve were washed with ethanol and allowed to dry.

The 20-micron sieve was replaced with a 10-micron sieve, and the process was repeated with the washings from the 20-micron sieve and 2 additional liters of Tween 80-saline. The microspheres were allowed to dry at room temperature before brushing them from the sieves for weighing and storage.

Measurement of the microspheres was necessary to ascertain the extent of fractionation. This measurement was facilitated by photographing a small representative fraction of resuspended microspheres through a microscope at a preset magnification (100×). A stage micrometer was photographed on the same roll of film at the same magnification so the micrometer prints would be the same magnification as those of the microspheres. These micrometer prints were then used to measure the diameters of the spheres on the other prints in the roll. (Each print thus represents a population of microspheres.) Replicate prints of the same preparation were analyzed for homogeneity and totalled to give a more accurate representation of the population.

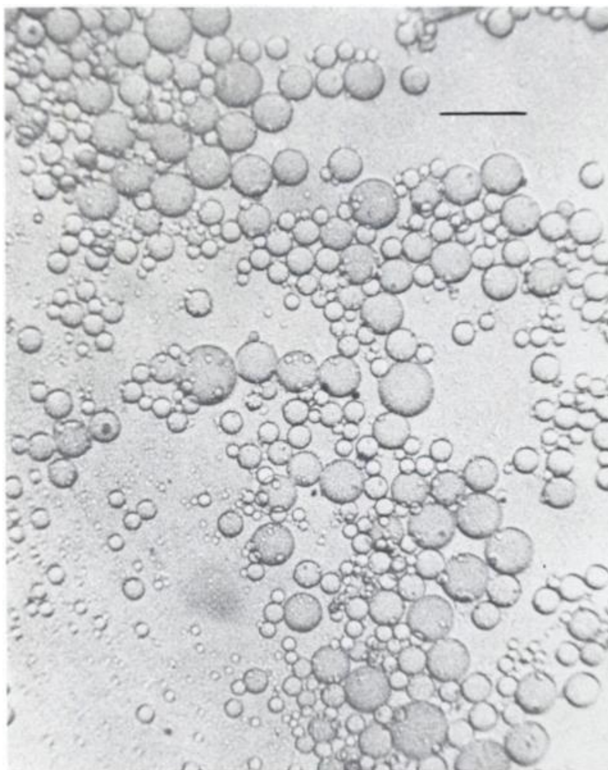

FIG. 1. Preparation of microspheres before fractionation. Size marker is 100 microns.

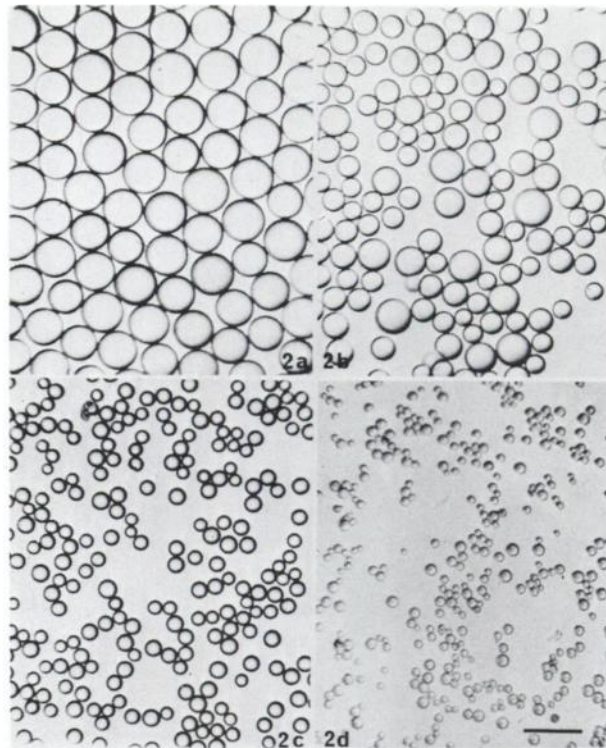

FIG. 2. Microspheres after fractionation by combined sieving and sonification. (A) 58 microns and larger; (B) 28–62 microns; (C) 18–32 microns; and (D) 8–22 microns. Size marker is 100 microns.

Photographs were taken with a 35-mm Leitz camera and Kodak TRI-X or PLUS-X film. The system resulted in a decrease of image size with an image/object ratio of 0.32 to 1.00.

RESULTS

A photograph of the original microsphere preparation is shown in Fig. 1. The inability to focus on all the varied sizes at once created difficulties in measurement; thus, it was not done. However, the composites of Figs. 2 and 3 show the fractions obtained by the sieving process and their degree of homogeneity.

The quantities of each fraction for this preparation are as follows: 60 microns and over, 129 mg; 30–60 microns, 277 mg; 20–30 microns, 66 mg; and 10–20 microns, 35 mg.

The actual size of each sieve pore is represented by the manufacturer to be within ±2 microns of the given value. Microspheres greater than 58 microns in diam are shown in Figure 2A. Of a total of 116 microspheres counted from four fields, one smaller sphere was seen (Fig. 3A). The major population of microspheres is in the 59–96 micron range, which is 98% of the total, with one outsize sphere appearing at 109 microns.

In the 30–60-micron fraction (actually, 28–62 microns, according to the manufacturers tolerances) (Fig. 2B) there are a total of 686 microspheres in the ten fields examined. The size range extends from 28 through 62 microns with no outsize microspheres (Fig. 3B).

The 20–30-micron fraction (actually 18–32 microns) is shown in Fig. 2C and has a total measured population of 671 microspheres. The range of sizes extends from 20 to 40 microns (Fig. 3C) with 667 microspheres (99.4%) in the acceptable range of 18–32 microns. No spheres appeared below 18 microns whereas 4 were above 32 microns. This represents a contamination of 0.6%.

The 10–20-micron fraction (actually, 8–22 microns) is shown in Fig. 2D and has a total measured population of 1,393 microspheres in the four fields examined. The range of sizes extends from 9 through 22 microns (Fig. 3D) with all of them in the acceptable range of 8–22 microns.

The microspheres that passed through the 10-micron sieve were collected by centrifugation but they were not measured for size and distribution of size.

DISCUSSION

A procedure is described that has simplified for us the problem of fractionation of a raw population of microspheres prepared from human serum albumin. The method involves sieving with the aid of a sonifier.

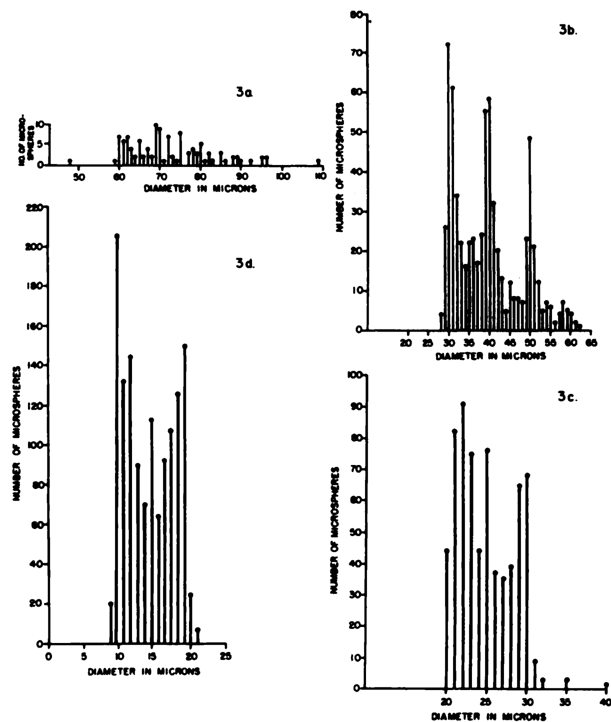

FIG. 3. Size distribution of microspheres in each fraction. (A) 58 microns and larger; (B) 28–62 microns; (C) 18–32 microns; and (D) 8–22 microns.

The sonifier creates a force that pushes the smaller microspheres through the pores of the sieve and prevents these pores from becoming clogged. This sonic force also keeps the suspension of microspheres in constant agitation, which prevents the larger microspheres from settling and blocking the pores of the sieve and inhibits their aggregation.

We use only stainless steel sieves and avoid using high power. It is possible that we are solubilizing or suspending very small amounts of metal from the surface of the screen that we are not able to detect. We recommend sonifying new sieves with saline alone to remove particles of dirt and loose metal that might be on the sieve.

The sources of technical problems include the extent of suspension of the microspheres before sieving, the washing of the smaller microspheres through the sieves, leaks in the sieves, and inadequate washing of the apparatus between the sieving of each fraction.

A commercial preparation of microspheres is composed of a population of sizes 5–50 microns; 90% of this population is in the 15–30-micron range. The procedure cited herein gives tighter control of microsphere sizing. Microspheres of sizes other than those commonly used for circulation studies have been utilized for special purposes. For example, Scheffel, et al (*5*) have used microspheres of approximately 1-micron particle size to study the reticuloendothelial system. Microspheres above 30-micron particle size have been used for studies of arteriovenous anastomoses (*6*). In as much as the anastomoses themselves can be of different sizes, microspheres of varied sizes are needed to show the required blockage size.

ACKNOWLEDGMENT

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