Spherical Beads as Filters: Separation and Size Determination of Coccidia[1] (36544)

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Columns of uniform spherical beads (1) may be used as filter beds providing pore openings proportionate to the bead size. When tightly packed, the filter openings in these columns equals the maximum spherical space between three beads which was trigonometrically computed to be 15.46% of the diameter of the beads. Particulates percolate through the column or are retained in the bed according to size.

Materials and Methods. Several sizes of spherical copolymer particles (Micules) were obtained from Sondell Scientific Instruments Inc. Glass beads (Superbrite, Class B) were obtained from Minnesota Mining and Manufacturing Company, Minneapolis, MN. Both types were washed in 95% ethanol and water. The glass beads were additionally slurried through U.S. standard sieves (W. S. Tyler, Mentor, OH) to obtain relatively uniform bead sizes with narrow size distributions. Beads passing through each sieve were successively passed through sieves with smaller pore openings. The beads retained by each sieve were checked microscopically for maximum, minimum and mode diameters (Table I).

Preparation of column. Distinct sizes of glass beads were transferred into 10 ml glass syringe barrels to make columns 5 cm tall over an 80 μ screen and glass wool support. Micule columns, 1 to 2 cm high, were packed in glass tubes, the micules supported by a 74 μ screen held in place between Teflon rings.[2] The columns were covered with saline containing 0.1% Tween 80. By tapping the wall of the cylinder and by applying suction, tight packing was accomplished. Particulate matter to be filtered was added, suction was applied, and the top centimeter of beads was agitated to reduce prefiltering effects of retained material. Washing was repeated two or three times. The effluent was collected in graduated tubes; portions of the filtrate were examined in a hemocytometer chamber, and the rest was transferred to the next smaller filter bed. When desired, most of the retained material could be recovered from the filter bed by suspending the beads with saline and drawing off the slower settling material in the supernatant.

Application and correlation of column fractions with infectivity. To illustrate their use, two separations are described. Fecal forms of *Toxoplasma* had been thought to be associated with *Toxocara* eggs (2). However, on filtration, the *Toxocara* eggs (60–80 μ) were retained by a 44 μ U.S. standard sieve, whereas the *Toxoplasma* infectivity passed through this filter (3). Complete separation required preliminary separation through 80 μ sieves to remove fungal mycelia and assorted fecal debris. In order to identify the morphologic representation of *Toxoplasma* infectivity, we filtered the suspension through micule bead column of 115, 60, and 50 μ diameters with pore ratings of 18, 9 and 8 μ. *Toxoplasma* infectivity passed through the two larger filter beds, but not the smallest (Table II). Isosporan-type oocysts followed the same pattern. This determination helped identify *Toxoplasma* as an isosporan oocyst measuring 9–11 $\times$ 11–14 μ (4).

Separation of Toxoplasma *from other coccidia of cats.* A formalinized fecal suspension containing *Toxocara* ova (60–80 μ), *Isospora*

[1] Supported by grant AI-7489 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

[2] Microsphere Filter, Pat. No. 3,463,320.

TABLE I. Fractionation of Glass Beads by U.S. Standard Sieves.

Rated sieve size (μ)	Retained beads (μ)		Computed spherical filter space (μ)	
	Size range	Mode diameter	Range	Mode
250	244–285	265	38–44	41
210	194–244	224	30–38	35
180	184–208	194	29–32	30
106	102–122	112	16–19	17
90	83–112	98	14–17	15
63	63– 78	75	9.7–15	12
53	51– 62	57	7.1–9.6	8.8
45	46– 55	50	7.1–8.5	7.7
37	27– 46	43	4.0–7.1	6.7
Not retained	19– 39	31	2.9–6.0	4.8

felis (35–50 μ), *Isospora rivolta* (20–30 μ), and *Toxoplasma gondii* (10–14 μ) oocysts, was available. As before, large fecal particles were removed by an 80 μ sieve and *Toxocara* ova by a 44 μ sieve (Table III). Three filter beds were prepared having 194, 112 and 57 μ average bead diameters, providing pore ratings of 30, 17 and 8 μ. The sequential removal of the oocysts in each filter was observed and tabulated as total counts retained in the filter, or washed through into the filtrate (Table III). The 194 μ beads with 30 μ pore rating retained all of the *Isospora felis*, about 10% of the *Isospora rivolta*, and about 10% of the *Toxoplasma* oocysts. The next column with a pore size of 17 μ, retained all of the remaining *Isospora rivolta* and about 20% of the *Toxoplasma* oocysts. The smallest, 57 μ bead filter with 8 μ pores, retained all of the *Toxoplasma* oocysts. Repeated washings of this filter followed by resuspension of the filter bed produced a clean suspension of the *Toxoplasma* oocysts, free of the other larger feline isosporan oocysts and most smaller particulates. The same procedure was used successfully also to separate nonformalinized, live preparations.

TABLE II. Filtration of *Toxoplasma* Infectivity and Isoporan Oocysts Through Filter Beds Composed of Copolymer Spheres.[a]

Av bead size (μ)	Pore rating (μ)	Infectivity	No. of oocysts
115	18	+	40
60	9	+	3
50	8	—	0
Unfiltered	—	+	214

[a] Micules, Sondell Scientific Instruments Inc. Infectivity was determined by injecting 40% of the filtrate into each of two mice, and oocysts were microscopically counted in 10% of the filtrate.

Discussion. Glass beads have been used for fractionating leukocytes, lymphoid cells (5), and protozoan sporozoites (6). In these instances, single columns consisting of relatively large beads (200 μ) were used and the interstices (31 μ) were ample for the cells or sporozoites to pass. The sporozoites were separated from contaminating elements by utilizing differences in settling speed during gravity flow; all the material passed through the column, however, in different fractions. For leukocyte separation, the property of certain cell types to adhere to glass was utilized; in some instances the cells retained in the column were recovered by elution with Versene (7).

As used here, copolymer and glass beads, easily separated and grouped by sieving, were used, (as suggested by Mr. J. A. Patterson Sondell Scientific Instruments Inc.), to construct a series of filters with pores of about 15% of the separating sieves. Suction instead of gravity flow was used. Filter beds, which should allow the passage of smaller oocysts, tended to retain a minor percentage, probably due to prefiltration effects of the larger retained particulates blocking many of the pore channels. However, the absolute cutoff points were quite sharp, allowing the com-

TABLE III. Sequential Filtration of Feline Helminth Ova and Isoporan Oocysts Through Graded Filter Beds Composed of Glass Beads.[a]

Av bead size (μ)	Pore rating (μ)	Total counts $\times 10^6$							
		Filtrates				Retained			
		Toxoplasma	Rivolta	Felis	Toxocara	Toxoplasma	Rivolta	Felis	Toxocara
(sieve)	80	1610	110	120	10	—[b]	—	—	—
(sieve)	44	1520	103	112	0	—	—	—	—
194	30	1340	98	0	0	176	12	100	0
112	17	938	0	0	0	225	75	0	0
57	8.8	0	0	0	0	936	0	0	0

[a] Particulates in a mixed formalinized suspension were microscopically counted in a hemocytometer. 0 indicates absence of particular oocyst which was retained by this or prior filter.

[b] No determination attempted from sieves.

plete removal of larger oocysts and the subsequent recovery of relatively pure suspensions of *Toxoplasma* oocysts.

Spherical copolymer beads were used for the size characterization of *Toxoplasma* fecal infectivity prior to direct morphological observation. These filters of uniform bead size were most useful in concentrating the search for the infectious particle at a size range between 8 to 20 μ. For isolative filtration, the availability and economy of the less uniform glass beads, which may be sieved into narrow uniform distributions, also provided outstanding separations. It is theoretically possible to further extend this technique to isolate the excysted sporozoites of this parasite by choosing the proper beads for filters. These techniques may also be applied to the separation and isolation of other small particles.

Summary. Copolymer and glass beads were separated by sieving into uniform sizes. When packed in columns, they provided sieves with pore spaces of about 15% the diameter of the beads. Sharp cutoff points allowed for the removal of larger particulates and the isolation of those of a chosen size range. Differential filtration of cat coccidia varying in size provided a characterization of the filter's utility in the 8 to 40 μ range.

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Received Feb. 23, 1972. P.S.E.B.M., 1972, Vol. 140.