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Zone Electrophoresis on Sponge Rubber

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▶ **Foam rubber is a highly satisfactory supporting material for the preparative separation of small amounts of proteins and other electrolytes by zone electrophoresis.**

For several years this laboratory has used foam rubber sponge as a convenient supporting medium for zone electrophoresis of proteins and other substances. The principal advantage of this material rests on the ease with which samples can be recovered after ionophoresis—simply by squeezing the sponges. In the authors' experience proteins do not absorb appreciably on rubber. Very little modification of equipment is needed to use sponge rubber blocks in the place of starch paste, silica gel, glass powder, or similar materials (*4*).

APPARATUS

The sponge-containing compartment is a trough 2 cm. deep, 2 cm. wide, and 40 cm. long, and it carries a cooling jacket on the bottom side for circulation of ice water. Except for the bottom of the trough, which is $^1/_8$ inch thick, the apparatus is constructed of $^1/_4$-inch Lucite. The two open ends of the sponge trough extend through one wall of each of two Lucite boxes, 12 × 12 × 12 cm., which constitute the buffer reservoirs and electrode compartments. The electrodes are made of 5-cm. lengths of platinum wire (No. 22) mounted on Lucite baffle plates in the end compartments. A second pair of perforated baffle plates is placed between the electrodes and the ends of the sponge trough. The two ends of the sponge trough are closed (watertight) by clamping pieces of dialysis tubing across the openings. This prevents free flow of buffer in or out of the sponge compartment.

PREPARATION OF FOAM RUBBER SPONGES

Strips of foam rubber (standard flat stock, medium, U. S. Rubber Co., or other uniform sponge) 7 mm. wide are cut from a $^1/_2$-inch sheet with a paper cutter and then into 21-mm. lengths with a sharp razor blade. These sponge rubber blocks are then cleaned thoroughly by boiling briefly and successively in 0.1*M* sodium hydroxide, 0.1*M* hydrochloric acid, 50% ethyl alcohol, and distilled water. After several washings the sponges are saturated with buffer and packed into the trough with the $^1/_2$-inch edges vertical and the $^1/_2$-inch × 21-mm. faces in close contact with each other. The top and bottom of the rubber stock differ in pore size and sponges should be packed the same side up. Corners and edges can be straightened with a needle when necessary. Excess buffer is removed from the top of the sponges with a syringe, and the sponges are covered with a Lucite strip that fits inside the trough and lies directly on the sponges. After the end vessels have been filled with cold buffer to the level of the top of the sponges, ice water is circulated in the cooling jacket for at least 30 minutes to permit equilibration of the apparatus. All operations are carried out in a cold room.

Table I. Recoveries of Cytochrome c, Lysozyme, and Arabinose after Ionophoresis on Sponge Rubber

Conditions. Seven hours at 300 volts and 13 ma. in tris(hydroxymethyl)aminomethane hydrochloride buffer, pH 7.2. Cooling with ice water. Mixture was placed in trough on sponge number 0.

	Sponge Number												
	−10	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2
	% Found in Each Sponge												
Cytochrome *c*	0	0	20	29	26	15	3	0	0	0	0	0	0
Lysozyme	0	0	0	0	0	52	47	0	0	0	0	0	0
Arabinose	0	0	0	0	0	0	0	0	0	40	54	0	0

OPERATION

Following temperature equilibration, the Lucite strip is removed from the top of the sponges, and one sponge is extracted from the trough. The buffer is squeezed out, and the sample contained in 1.2 to 1.4 ml. of buffer is absorbed in the sponge. This is then carefully reinserted into its original position between two tight-fitting, thin pieces of celluloid. The celluloid pieces are removed, sponge corners are straightened, and current from a variable voltage power unit is applied.

After completion of ionophoresis, fractions are obtained by removal of the sponges starting at one end of the trough. The buffer and dissolved materials contained in each sponge are obtained by squeezing between finger tips (rubber gloves) or by squeezing in a large syringe.

AN ILLUSTRATIVE EXPERIMENT

Cytochrome *c* (Sigma, 8.5 mg.), lysozyme (Nutritional Biochemicals, 6 mg.), and arabinose (5 mg.) were dissolved in 1.2 ml. of buffer [tris(hydroxymethyl)aminomethane hydrochloride, 0.03*M*, pH 7.2]. The sample was taken up in one sponge and inserted into an equilibrated trough (10° C.) as described above. After application of a voltage of 300 (measured across the electrodes) for 7 hours, sponges were removed and their contents were expressed each into a separate test tube by use of a 10-ml. syringe. The sponges were extracted only once in the experiment described. Cytochrome *c* was determined spectrophotometrically by measurements of absorbances at 420 mμ (*2*). Lysozyme was determined by turbidimetric estimations of its action on bacteria (*1*) and arabinose was determined colorimetrically by the standard orcinol reaction (*3*). Results are summarized in Table I. The calculated total recoveries of the three substances are cytochrome *c*, 93%; lysozyme, 99%; arabinose, 94%.

DISCUSSION

The experimental example illustrates the usefulness of foam rubber as a supporting material for ionophoresis. In other experiments up to 98% recoveries of samples have been obtained by three extractions of each sponge, a fact which indicates that the sponge rubber contains very few "dead" spaces. In the experiment given the high recovery of lysozyme is probably due to the large errors inherent in the method of analysis and the single-extraction recovery is more likely around 93%. This value is of particular interest here, because a minimum dilution is frequently desirable in enzyme purifications and is most easily attainable by use of sponge rubber.

Solutions with a high content of dissolved solids tend to sink and spread from the original sponge. This can be alleviated to a considerable extent by addition of a nonelectrolyte such as mannitol to the buffer solution contained in the sponge trough.

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