nitrophenol. This solvent system is simple to use and would appear to have some potential for the paper chromatographic separation of other nitrophenols.

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1 J. GASPARIC, *J. Chromatog.*, 13 (1964) 459.
2 D. ROBINSON, J. N. SMITH AND R. T. WILLIAMS, *Biochem. J.*, 50 (1951) 221.
3 M. LEDERER, *Australian J. Science*, 11 (1949) 208.
4 A. G. LONG, J. R. QUAYLE AND J. R. STEDMAN, *J. Chem. Soc.*, (1951) 2197.

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Separation of acid polysaccharides by starch gel electrophoresis*

The acid mucopolysaccharides (APS) have differing charge densities, molecular sizes and configurations. These differing properties serve as the basis of identifying unknown APS samples with starch gel electrophoresis. This method has the advantage of simplicity, speed of operation, economy, and is sensitive to 50 μg of APS.

Materials and methods

Preparation of gel. Citric acid buffer (0.01 *M*) is adjusted to pH 3.5 with saturated sodium hydroxide. Approximately 13 g of dry starch** is added to each 100 ml of 0.01 *M* citric acid buffer in a 2-l Pyrex vacuum flask. The flask contents are heated to boiling with constant vigorous swirling. Initially, the mixture is very viscous and opaque; after 3 min of heating, it becomes less viscous and appears translucent. At this point, heating is stopped and negative pressure applied. After the mixture has boiled approximately 2 more min, it is poured into a previously prepared mold. A preparation of 200 ml is adequate for a mold 20.5 × 8 × 0.75 cm. After cooling for 1 h at 4°, the gel is ready for use[1,2].

Preparation of chamber. The electrophoretic chambers are each filled with 500 ml of 0.1 *M* citric acid buffer, pH 3.5. The prepared gel is trimmed to 7.5 cm × 20.5 cm and is placed on a glass plate of the same size on the supports of the chamber (Fig. 1). Wicks (12 cm × 8 cm) made of 2 layers of Whatman No. 3 mm paper extend from the buffer in the chamber to the top of the slab of gel. The APS sample solutions (concentrations 2 mg/ml) are placed inside wells cut in the gel. These depressions are made with a small, stainless steel spatula, are 3 mm on each side, penetrate the entire thickness of the gel, and are set a minimum of 1 cm apart.

After the samples are applied to the gel, the chamber cover is sealed. A direct current*** of 10 mA (approximately 45 V) is applied for 8 h at room temperature. After electrophoresis, the gel is placed in a tray containing a mixture of acetone

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** Connaught Laboratories, Toronto, Canada.
*** Spinco Duostat power supply, Beckman Industries, Belmont, California.

(technical grade) and glacial acetic acid (24:1, v/v) for 15–30 min. This fixes the polysaccharides in the gel. The opaque gel is then placed in 0.02 % toluidine blue o* in a 0.5 % acetic acid solution. The APS are usually adequately stained after 15 to 20 min.

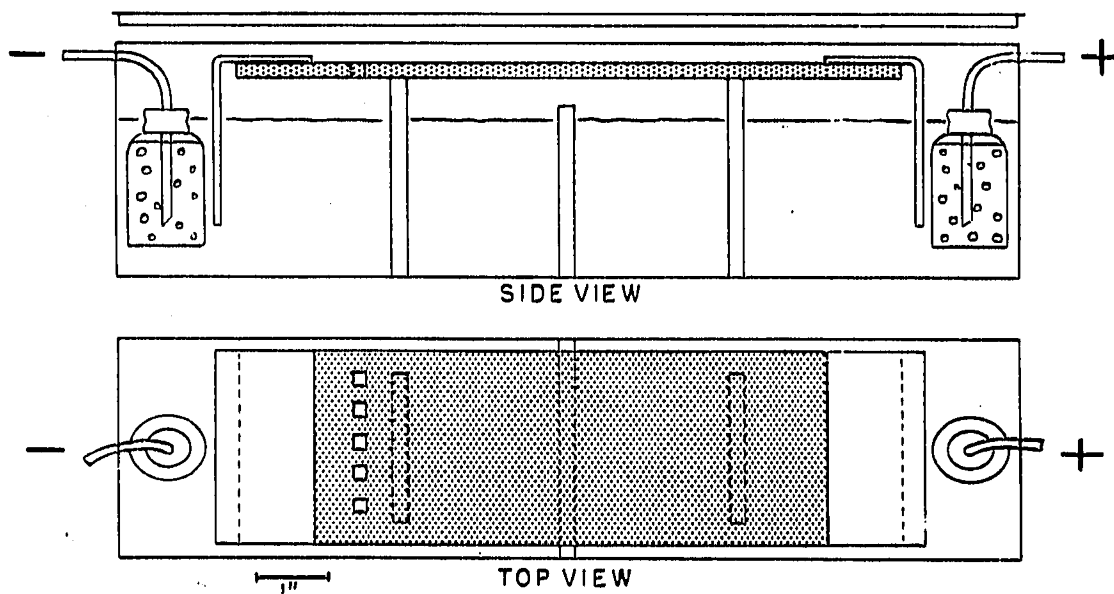

Fig. 1. Diagram of the apparatus used in the method.

They are revealed as elongated oval red purple metachromatic spots against a blue background. After staining, the gel is returned to the initial fixative solution in order to clear the blue background. Comparisons are made between unknown and standard solutions run on the same gel. The spots may be preserved by placing the gel in a transparent (Saran) wrapping and storing at 4°. Other methods may be used to preserve, photograph, and scan the gel[2]. A minimum of approximately 50 μg of sulfated acid polysaccharides can be revealed by this method.

Results

The acid polysaccharides studied included: heparitin sulfate**, chondroitin sulfate A***, chondroitin sulfate B (prepared according to MEYER *et al.*[3]), hyaluronic acid sulfate§, and hyaluronic acid§. Heparitin sulfate migrates the most rapidly of the compounds tested. The results with other compounds can be accordingly expressed in relation to it (Fig. 2). All fractions of heparitin sulfate studied contained 2 fractions. The upper limit of the top spot served as a (relative) 100 % mark. The top of the second fraction migrates about 54 % of the total distance. These values are usually consistent on subsequent runs to within $\pm$5 % providing that the current and other variables involved remain the same. Advantages of this method of separation over others include both simplicity of operation and economy (each run costs less than one dollar).

* National Aniline, 40 Rector Street, New York 6, New York.
** Upjohn Company, Kalamazoo, Michigan; repurified in our laboratory.
*** Sigma Chemical Company, Missouri; repurified in our laboratory.
§ Searle and Company, Chicago, Illinois.

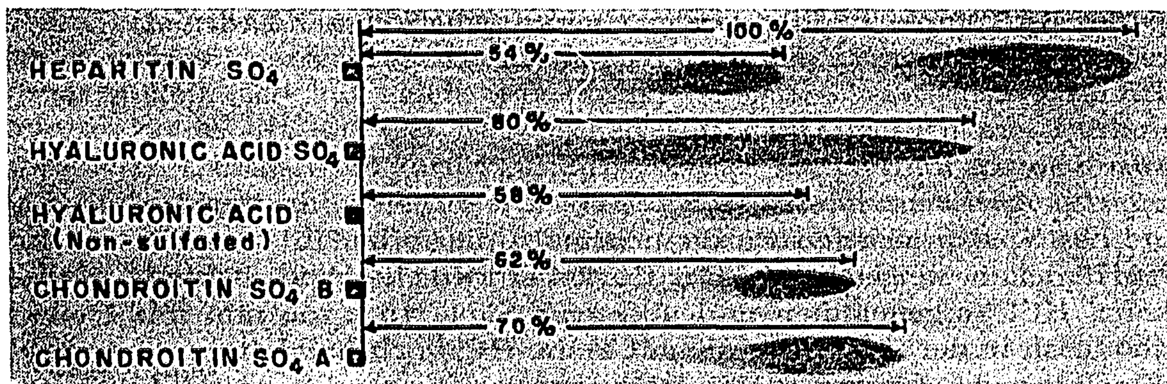

Fig. 2. Typical separations of various acid polysaccharides.

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1 O. SMITHIES, *Biochem. J.*, 61 (1955) 629.
2 J. BODMAN, in I. SMITH (Editor), *Chromatographic and Electrophoretic Techniques*, Vol. II, Interscience, New York, 1960, pp. 91–157.
3 K. MEYER, E. DAVIDSON, A. LINKER AND P. HOFFMAN, *Biochim. Biophys. Acta*, 21 (1956) 506.

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Die Isolierung von trägerfreiem Strontium-89 und Barium-140 aus kurzzeitig bestrahltem Uran durch Ionenaustauschchromatographie

Der Zweck der vorliegenden Untersuchungen war es, trägerfreies Strontium-89 und Barium-140 von etwa 100 μC in grösstmöglicher Reinheit herzustellen. Als Ausgangsmaterial diente im Reaktor kurzzeitig bestrahltes Uranoxid und Uranmetall.

Die Ionenaustauschmethoden bieten die Möglichkeit, mit einem relativ geringen Arbeits- und Zeitaufwand zum Ziel zu gelangen. In einigen früher beschriebenen Veröffentlichungen[1–5] wurde über die Isolierung von Strontium, Barium und Lanthan untereinander und von Uran und den Spaltprodukten berichtet. Auf Grund der von KRAUS UND NELSON gegebenen Daten[6] über den Anionenaustausch von zahlreichen Metallen in salzsauren Lösungen wird die Trennung der Erdalkalimetalle und Seltenen Erden von Uran und den anderen Spaltprodukten durchgeführt. In der anschliessenden Chromatographie an einem Kationenaustauscher unter Verwendung