A Simple Procedure for the Preparation of Single Crystals of Orthorhombic Mercury(II)oxide

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The procedures described in the literature for the preparation of single crystals of orthorhombic mercury(II)oxide¹ have been found to be rather uncertain and give poor results. In connection with studies on the hexagonal modification of this substance a simple method was found for the synthesis of beautiful crystals of the orthorhombic form. One part of 0.1 M K_2HgI_4 was mixed with two parts of 10–13 M NaOH and poured into a gold tube which was covered with a gold lid. The tube was slid into a pyrex glass tube which was sealed and placed in an oven at 100–175°C for about 70 h.

The crystals appeared in the form of light red rods with lustrous faces of up to 2 mm extension. Amply exposed powder patterns obtained in a Guinier focusing camera did not show any reflexions not belonging to the pattern for orthorhombic mercury(II)oxide.

This convenient method has been discovered in connection with work on mercury compounds which has been financially supported by the *Swedish Natural Science Research Council*.

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Separation of Acid Mucopolysaccharides by Paper Electrophoresis

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The separation of acid mucopolysaccharides from various sources by means of paper electrophoresis has been reported

by several investigators¹⁻⁴. The conditions for the electrophoresis as well as the methods of locating the separated mucopolysaccharide fractions have differed in each particular case. For the purpose of investigating the mucopolysaccharide contents of mucous membranes a number of methods were tested, but no particular procedure was found to be entirely satisfactory. However, a system was developed which allowed a distinct separation of hyaluronic acid and chondroitin sulphate. This system represents a combination of some of the methods referred to above.

Methods. A box, similar to that described by Grassmann⁵, was constructed from parts cut out of 0.5 cm thick Perspex acrylic sheet to serve as support for the paper.

The buffer solution employed was made by combining 100 ml of 0.9 M sodium chloride with 50 ml of 0.2 M phosphate buffer (pH 7.0) and diluting to a total volume of one liter with distilled water.

The paper used was Whatman No. 3 MM filter paper for chromatography.

The electrophoresis was carried out at 160 V for 18 h at room temperature.

Prior to electrophoresis, a paper strip, 57 cm in length and 4 cm wide, was moistened with buffer from both ends to within 1 cm from the midline and blotted dry with filter paper. The paper was next placed in the box, and the vertical ends of the paper were dipped into the buffer. The horizontal stretch of the paper strip in position in the box was 40 cm. Buffer equilibration was allowed to proceed for 30 min. From 50 to 100 μ l of a crude mucopolysaccharide mixture dissolved in the buffer were then applied along the midline to the wet paper. Another 30 min were allowed to pass before the current was connected.

Following electrophoresis, the paper strip was dried at 110°C for 30 min. Acid mucopolysaccharide fractions were next located in the manner described by Bera *et al.*² In order to obtain satisfactory results, however, it was necessary subsequent to the immersion of the paper in Cetavlon to wash it in hot running tap water for 45 min. The paper was then dried at 110°C for one hour, sprayed with the bromocresol purple reagent, and again dried at 110°C for 15 min.

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Fig. 1.

In order to identify the separated fractions, two paper strips were run at the same time. Following electrophoresis, one paper was developed with Cetavlon/bromocresol purple². The areas of the second paper corresponding to the spots on the first paper were cut out and eluted in the manner described by Rienits¹. Concentrated hydrochloric acid was added to the eluates in amounts sufficient to make the solution 7 N in terms of the acid. Hydrolysis was carried out in sealed test tubes at 100°C for 4 h. The solutions were treated with Norit A for a few minutes, centrifuged, and the supernates were taken to dryness by lyophilization. The residues were dissolved in appropriate amounts of distilled water and paper chromatography of these solutions were carried out by the method of Stoffyn and Jeanloz⁶ for the identification of the aminosugars present.

In cases where larger amounts of mucopolysaccharide mixtures were applied to paper strips for electrophoretic separation, milligram amounts of each fraction could be obtained after elution by precipitating with ethanol in the presence of sodium acetate. Following hydrolysis, the mucopolysaccharides obtained in this fashion were shown by paper chromatography and paper electrophoresis to be of a satisfactory purity.

Results and discussion. Fig. 1 represents a typical result of paper electrophoresis of a crude mucopolysaccharide mixture obtained from human gingiva, followed by staining with Cetavlon/bromocresol purple.

By means of the method described, it was shown that the slowest moving component contained glucosamine as the only aminosugar, whereas the fastest moving component contained galactosamine, but no glucosamine.

Paper electrophoresis of a number of crude mucopolysaccharide mixtures extracted in different manners from various tissues and of widely different purities

were carried out. The results obtained showed that acid mucopolysaccharides could readily be separated and identified in the manner described, and the results were entirely reproduceable. Amounts of 5–6 mg of crude mixtures could be applied on the paper for electrophoresis. When amounts of a particular acid mucopolysaccharide were below 100 μ g the spot produced by the staining reaction was very weak.

None of the other staining reagents tested gave as distinct results as those obtained with the Cetavlon/bromocresol purple method.

The separation of hyaluronic acid and chondroitin sulphate in the manner described compared favorably to all of the other systems tested.

The above described system provides a simple tool for a comparatively rapid demonstration of the presence of acid mucopolysaccharides in biological materials and for a ready separation of hyaluronic acid and chondroitin sulphate from a mixture.

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