Separations of Polysaccharides related to Starch by Electrokinetic Ultrafiltration in Collodion Membranes

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For a series of 'isochemical' molecules, e.g. mixtures of polymeric chain molecules differing only in degree of polymerization (DP) and not otherwise in chemical nature (cf. Brønsted, 1931), the distribution coefficient between two phases at equilibrium changes by a constant factor for each unit added to the molecule. This is the basis for Traube's Rule and for the success of both adsorption and partition chromatography in separating homologues and the lower members of polymeric series (cf. Martin, 1949). However, with larger molecules in such series these methods have not proved successful. Methods used for fractionating polymeric materials have been reviewed by Cragg & Hammerschlag (1946). With the larger molecules it becomes difficult, as Brønsted showed, to find two-phase systems not giving distribution coefficients that are excessively high or low. But there is an additional difficulty due to the porous nature of many of the materials used for chromatography. With such materials as charcoal, silica gel, starch, cellulose or ion-exchange resins, whilst the affinity of the active surfaces or sorbed liquid is greater for the larger molecules, these are often prevented from penetrating the grain or fibre by molecular-sieve effects. Thus, it is usual to find that adsorption affinities increase, reach a maximum and then decrease as a polymeric series is ascended. With increasing DP, slow attainment of equilibrium and irreversibility of adsorption are also increasingly common. A number of examples of such effects in the literature have been referred to by Mould & Synge (1952); see also Deuel, Solms, Anyas-Weisz & Huber (1951), Jenckel & Rumbach (1951), Zamenhof & Chargaff (1951). If for a column consisting of separate pieces of porous material a continuous block of material of rather uniform porosity could be substituted, molecular-sieve and adsorption effects would work together and no longer in opposite senses when liquid containing molecules of different sizes is forced through the block chromatographically. For porous materials at present available, however, the high hydrostatic pressures required to produce reasonable flow through a thick block would cause stresses which would collapse the pore structure. However, electrokinetically promoted flow of liquid (electroendosmosis) does not involve applying

external stresses to the porous structure. These ideas for fractionating larger molecules were discussed for some time by Dr A. J. P. Martin, Professor A. Tiselius and one of us (R. L. M. S.); the suggestion to use electrokinetic flow was prompted by observations on electroendosmotic effects during zone electrophoresis in gels (cf. Synge, 1949; Tiselius, 1949; Tiselius & Flodin, 1953). Schmid (1952) has briefly discussed the possibility of fractionating neutral molecules during electroendosmosis in ion-exchange resins, but without reference to separation according to molecular size or to the need for having the porous material in a single block.

The mixture of linear-chain molecules of different lengths obtained by partial acid hydrolysis of amylose seemed a suitable test mixture for evaluating electrokinetic ultrafiltration procedures, since complications due to differences in charge and chemical nature, such as would arise with the partial hydrolysis products of proteins, would be absent and differences in behaviour would be due to differences in molecular size only. Further, the iodine-staining characteristics of such dextrans depend on molecular size. Early experiments in which hydrolysates of amylose were subjected to zone electrophoresis in agar jelly in the presence of iodine-iodide (intended simply as an indicator) were very encouraging, since a beautiful series of coloured zones was seen to migrate towards the cathode at rates slower than that of the electroendosmosis in the jelly. The blue zones migrated more slowly than the pink and orange zones, presumed due to smaller molecules. This was demonstrated to the Biochemical Society (Synge & Tiselius, 1950). After the published abstract of this demonstration had been submitted, experiments with a different batch of agar showed equally good separation of the zones but most of the material migrated towards the anode, the blue zones migrating fastest. This phenomenon was also demonstrated to the Society and demanded a reassessment of the physicochemical basis of the separation. It became clear during subsequent experiments here and at Uppsala that the basis of the separation observed was not electrokinetic ultrafiltration but electrophoresis of complexes of the polysaccharides with negatively charged triiodide, etc. On omitting iodine, all the

polysaccharide zones moved through the agar at rates approximating to the electroendosmotic flow towards the cathode, which was much greater with the first batch of agar than with the second. However, the zones, as revealed by spraying the jelly with iodine after the run, were not quite devoid of separation; the material of high molecular weight was slightly retarded relative to that of lower molecular weight (Synge & Tiselius, unpublished observations).

The electrophoretic separations based on combination of the dextrans with iodine-iodide proved capable of preparative application and have thrown some light on the nature of the combination of starch and its breakdown products with iodine. Our experiments with these phenomena are described in the two following papers (Mould & Synge, 1954; Mould, 1954).

However, partly in view of the interest of colleagues at this Institute (especially the late Francis Baker) in the microbial breakdown of polysaccharides and in the significance of intermediates in the breakdown of starch, cellulose, etc., for the nutrition of micro-organisms, we tried also to intensify the effects noted by Synge & Tiselius in agar in the absence of iodine, which seemed possibly due to ultrafiltration retardation. Gordon, Keil, Šebesta, Knessl & Šorm (1950; cf. Gordon, Keil & Šebesta, 1949) had shown that a protein of such high molecular weight as haemocyanin can migrate electrophoretically without much hindrance in agar jelly. We accordingly tried to prepare denser gels from electrically charged materials. Experiments using gels made from polymerized formaldehyde-phenolsulphonic acid or glycerol-citric acid mixtures and gels made from carboxymethyl-celluloses were only partially successful. However, promising results were obtained when collodion ultrafiltration membranes of graded porosity were used, and we obtained valuable advice about the preparation of these from Dr W. J. Elford of the National Institute for Medical Research shortly before his death.

The present paper describes experiments using electrokinetic ultrafiltration in collodion membranes. Interpretation of these and of the electrophoretic phenomena described in the following paper was greatly helped by being able to study materials by the two methods in parallel. Demonstrations of them have been given to the Biochemical Society (Mould & Synge, 1951). However, it only became possible to assess our results with any exactness when we later received from colleagues at Bangor samples of linear dextrans synthesized by the action of potato phosphorylase in digests containing glucose 1-phosphate and pure oligosaccharide primers such as maltohexase and maltoheptaose. The products of this reaction are mixtures

of dextrans grouped as to DP fairly compactly around an average which is known from the amount of inorganic phosphate liberated at the time when the synthetic reaction is stopped (Whelan & Bailey, 1954; cf. Bailey & Whelan, 1952). These reference dextrans made it possible to assess the resolving power of the procedure (while at the same time we obtained some information about the heterogeneity of the dextrans themselves), to investigate the physicochemical mechanisms on which the separations depend and to interpret observations made on various natural products related to starch and on derived materials obtained from them by enzymic or chemical treatment.

The present procedure differs in most respects from the 'electro-ultrafiltration' of Bechhold & König which has mainly been used for controlling hydrostatically the electroendosmotic flow across membranes during electrodialysis (see Bechhold, 1929; Stauffer, 1950).

We gave a preliminary account of some of the present work at the International Congress on Analytical Chemistry held at Oxford in September 1952 (Mould & Synge, 1952).

EXPERIMENTAL

Collodion membranes

Preparation. This followed in general the principles given by Elford (1931, 1938). Membranes were cast in a rectangular trough (14 × 52 cm.; 0.6 cm. deep) made by clamping together pieces of plate glass. The starting material was the ethanol-ether solution 'Necol collodion solution 301-261 (356/A9)' as sold by British Drug Houses Ltd., Poole, Dorset. After dilution with the appropriate solvents the collodion solution was poured into the trough and evaporation was allowed to proceed, without special control of room humidity or temperature, until a gel of suitable consistency was obtained. Water was then poured on and the gel was transferred from the trough to a large volume of distilled water, in which it was kept for 24 hr.; the resulting membrane was then transferred to 0.2N acetic acid, under which it was stored until required. Portions were cut from the membranes as required, rejecting parts within 1 cm. of the original edges. The mixtures used for preparing membranes for this work are shown in Table 1.

Characterization. The finer-pored membranes were tough and translucent; the coarser the pores the more opaque and brittle were the membranes. In general the properties of membranes did not change during storage under 0.2N acetic acid for a few months, but some membranes made with mixtures intermediate between those for I and III, although at first giving useful results, after about a week became coarser-pored and lacking in resolving power, suggesting that some change of structure had occurred. Some of the data given below are suggestive of a structural difference between membranes I and III, and it may well be that in membranes cast with solvents of intermediate composition the colloidal phases are in a critical state giving rise to unstable structures (see Elford, 1930). Membrane II is included as an example from this range—although made

from the same solvent mixture as the satisfactory membrane III it appeared to be coarser-pored (perhaps due to difference in the stage of gel formation at which gel was transferred to water) and never at any period during ageing gave compact, well-resolved zones with any of the dextrans tested.

The 'average pore radius' of the membranes was assessed by the rate of passage of 0.2N acetic acid at 25° through a circle of membrane 0.8 cm. in effective diameter, using the method and formula given by Elford (1931, 1938). Thickness of membranes was determined with a screw micrometer. Dry matter was determined on portions of membrane that had been quickly blotted with filter paper and weighed; these were dried to constant weight over H_2SO_4 *in vacuo* at room temperature. The viscosity of 0.2N acetic acid at 25° was taken to be 9.10 millipoises (by interpolation in *International Critical Tables*). The sp.gr. of collodion was taken to be 1.66 (Petitpas, 1948), and the volume of solvent in the membrane was calculated using this figure, the dry matter content and the linear dimensions of the membranes. These results are set out in Table 2.

Apparatus

The electrokinetic ultrafiltration was done in strips of wet collodion membrane surrounded by an electrically insulating liquid to prevent overheating and evaporation. It was necessary that this liquid should not swell or dissolve the collodion. Chlorobenzene (cf. Cremer & Tiselius, 1950) was satisfactory in early experiments, in which a strip of membrane was pushed through a U-tube nearly filled with chlorobenzene so that the ends projected above the surface of the chlorobenzene in each limb of the tube into the anolyte and catholyte respectively, which were small volumes of 0.2N aqueous acetic acid overlying the chlorobenzene. However, this arrangement left some uncertainty as to the point at which the anolyte entered the strip and

also required inconveniently large volumes of anolyte when it was wished to study material only available in small quantity. The arrangement shown in Fig. 1 was finally adopted, using liquid paraffin B.P. that had been equilibrated with a large volume of 0.2N aqueous acetic acid as the insulating liquid. The soft sheet rubber used for the gaskets of the anolyte chamber swelled somewhat in contact with the paraffin and this ensured the absence of leaks; the anolyte was thus able to enter the strip only through its end surface and the anolyte chamber was conveniently small for work with mg. quantities. New gaskets were used for each run.

Procedure

A strip (1.3 × 8.0 cm.) was cut from the membrane and blotted with filter paper to remove adhering liquid. Working quickly to avoid drying the strip, a hole was punched in it near the cathode end and the anode end was mounted in the anolyte chamber which was firmly gripped with a 'bulldog' clip. The strip, held in this way, was then mounted in the test tube so that the hole was hooked by the cathode as shown in Fig. 1. The test tube was then filled to about the level of the top of the cathode with 0.2N acetic acid and then to the level of the junction of the strip and anolyte chamber with paraffin. The sample for analysis (usually 0.5–1.0 mg. polysaccharide) in ca. 0.1 ml. of suitable solution was placed in the anolyte chamber with a micropipette. The anode was then lowered centrally into the anolyte nearly to touch the strip, and a p.d. of 250 v was applied across the electrodes. Currents, which did not vary greatly with the porosity of the membranes, were usually in the range 1.0–2.5 mA. When the anolyte had nearly entered the strip (the time taken for this, determined by the rate of electroendosmosis, varied greatly with the porosity of the membrane used—see below), the anode was removed and cleaned and the anode chamber rinsed out with 0.2N acetic acid; the anode was then

Table 1. *Mixtures used for preparing collodion membranes*

Membrane	Necol solution (ml.)	Ethanol (ml.)	Ether (ml.)	Amyl alcohol (ml.)	Acetone (ml.)	Ethylene glycol monoethyl ether (Cellosolve) (ml.)
I	64	14.2	152	24.8	64	7.1
II and III	95	30	150	25	40	3
IV	95	30	150	25	30	3
V	355 ml. of a mixture of equal parts by wt. of above			—	—	—
VI	The same, allowed to evaporate to a more compact gel			—	—	—

Table 2. *Characterization of collodion membranes*

For experimental details and methods of calculation see text.

Membrane	Thickness (mm.)	Dry matter content (%)	Vol. fraction occupied by solvent (V_L)	Rate of passage of solvent (ml./hr.)	Applied pressure (cm. Hg)	'Average pore radius' (m μ .)	S^*
I	0.53	17.8	0.884	333	19.8	552	y
II	1.07	16.9	0.891	1.99	19.5	61	—
III	0.96	20.6	0.865	0.42	19.3	27	20.4y
IV	0.79	25.9	0.825	0.19	19.3	16.7	33.1y
V	0.76	25.0	0.832	0.059	19.5	9.1	60.8y
VI	0.76	23.8	0.840	0.048	19.3	8.2	67.3y

* See Discussion.

Fig. 1. Apparatus for electrokinetic ultrafiltration in strip of collodion membrane: (a) strip seen from side; (b) strip seen from front; (c) details of sheet-rubber gaskets. The two side gaskets are cut from the piece of rubber used for one of the two main gaskets as indicated by dotted lines. A, platinum-wire cathode sealed through bottom of glass tube; B, collodion strip; C, catholyte (0.2N acetic acid); D, liquid paraffin (see text); E, platinum-wire anode, sealed into glass tube containing mercury; F, reservoir of 0.2N acetic acid; G, capillary siphon for perfusing anode chamber in long runs; H, capillary connected to filter pump for sucking perfusion liquid from anode chamber; I, anolyte; J, sheet-rubber gaskets surrounding anolyte-membrane junction; K, rubber spacing pieces; L, glass microscope slides. The microscope slides are pressed together by a 'bulldog' clip (not shown), causing them to grip the end of the collodion strip and to seal the anolyte chamber.

replaced and the voltage reapplied. When electroendosmosis was slow and the run long the anode chamber was perfused with 0.2N acetic acid admitted slowly by the capillary siphon (Fig. 1) from an adjacent beaker and removed by continuous suction. This prevented accumulation of anodic reaction products; however, perfusion was not necessary with shorter runs, during which the anolyte was replenished at intervals by hand. The time for running in the sample was not allowed to exceed one-sixth of the total running time.

Stains for polysaccharide zones and markers of electroendosmosis. At the end of the run the strip was removed and blotted free of paraffin. The polysaccharides studied in the present work were revealed by spraying the strip lightly on both sides with 0.1N- I_2 in 0.15M aqueous KI. After a few minutes coloration of the zones reached its maximum and measured sketches or colour photographs were prepared. The iodine stain of the polysaccharides of lowest DP was evanescent but that of most of the materials studied was surprisingly permanent during 1–2 yr., although the strips twisted and shrank badly as they dried. The zones were sometimes rather curved in form, having migrated more slowly at the edges than at the centre of the strip (cf. Fig. 4).

This could be due to uneven distribution of electric field and temperature across the strip and possible drying at the edges. All measurements of zones were made in the midline of the strip.

When it was wished to measure electroendosmosis, 10% (w/v) of fructose was incorporated in the solution taken for analysis. It had been shown (see below) that fructose is not adsorbed or retained by the collodion. At the end of the run the strip was divided along its midline; half was stained with iodine and the other half was divided along its length into about 20 segments, each of which was placed in 1 ml. of water in a test tube for 1 hr. At the end of this time the 'rapid furfural' test (Cole, 1933) was done on each of the eluates, thus revealing the position of the fructose zone. Several of the usual colour reactions for sugars by direct spraying of the strip were tried, but did not seem to work well on collodion; the most encouraging was spraying with sat. ethanolic urea oxalate (suggested by Dr P. C. Arni; cf. Hirst, McGilvray & Percival, 1950), but the subsequent heating at 105° which revealed the fructose as a dark zone shrank and distorted the strip. N-2:4-Dinitrophenyl-ethanolamine, which we have found useful as a coloured neutral marker for electroendosmosis in other electrophoretic work (e.g. Mould & Synge, 1954), was useless as it was retained by the collodion, forming an immobile zone at the anode end of the strip.

Polysaccharides

To avoid trouble from retrogradation all solutions were prepared immediately before each experiment.

Amylose. Preparations by the alumina-thymol procedure (Hobson, Pirt, Whelan & Peat, 1951) from potato starch were given to us by Dr P. N. Hobson and by Dr W. J. Whelan. For electrokinetic ultrafiltration runs, samples (2 mg.) were dissolved with heating in 0.1 ml. 0.04N-NaOH. On cooling, 0.1 ml. 0.2N acetic acid was added and 0.1 ml. of the resulting solution was used for the run. Some runs were done with undried thymol complex brought into solution by Dr Hobson as follows. Wet suspension (1 g., calculated to contain 50 mg. amylose) was heated for 5 min. at 100° with 10 ml. of solution 0.02N in sodium acetate and 0.18N in acetic acid. This gave an opalescent solution, which on cooling became cloudy. This was centrifuged and the clear supernatant, which gave a strong blue stain with iodine, was used for the experiments.

Partially hydrolysed amylose. Amylose (80 mg.) was dissolved in a minimum of 10N-HCl and kept for 10 min. at room temperature. The mixture was then evaporated to dryness *in vacuo* not allowing the temperature to exceed 30° or the time of evaporation to exceed 15 min. Water was added, and evaporation repeated 2–3 times to decrease the concentration of HCl. The residue was dissolved in 4 ml. water and neutralized to pH 4 with sodium acetate. This procedure gave a representative mixture of dextrans suitable for many tests on methods. The mixture stained purple with iodine. If a mixture having a rather narrower range of DP was desired, the hydrolysis was effected with salivary α -amylase. The degree of hydrolysis was controlled by observing the change in 'blue value' (B.V.) of the polysaccharide when it is stained with iodine under the conditions specified by Bourne, Haworth, Macey & Peat (1948). Human saliva was centrifuged to remove mucin and the clear supernatant used as the enzyme source. The α -amylase activity was not standardized. Potato amylose (250 mg.)

was wetted with ethanol and dispersed in 20 ml. N -NaOH at 100° for 5 min., neutralized with N -HCl to pH 5, 50 ml. 0.4M sodium acetate buffer (pH 4.7) added and the solution made up to 250 ml. at pH 4.8. The salivary α -amylase preparation (0.5 ml.) was added. Samples (1 ml.) were taken during hydrolysis and made up to 100 ml. with I_2 (2 mg./100 ml.) and KI (20 mg./100 ml.) for determination of B.V. For extended hydrolysis a further 0.5 ml. of saliva preparation was added. Enzymic hydrolysis was terminated by heating to 100° ; the denatured protein was then removed by centrifuging. A blue-purple coloration with iodine corresponded to B.V. 0.56.

Phosphorylase-synthesized linear dextrans. These were preparations provided by Dr W. J. Whelan, made from pure amylohexaose or amyloheptaose and having average DP as specified (Whelan & Bailey, 1954). However, one preparation studied, having average DP 75.5 before dialysis, had been made from a heterogeneous primer mixture. This primer mixture was obtained by acid hydrolysis of potato amylose (1%, w/v) in 0.25N- H_2SO_4 at 100° for 15 min. After cooling and neutralizing (6N-NaOH) the average DP was measured by determination of reducing power (Whelan, Bailey & Roberts, 1953) and found to be 6.0. In using this material for amylose synthesis, it was assumed that all molecules present acted as primers. This assumption is incorrect in so far as any glucose and maltose present are devoid of priming activity. In consequence the true average DP must have been greater than 75. Samples were dissolved as described above for amylose. As noted by others (cf. Whistler & Johnson, 1948), samples having DP above 50 were considerably less easy to dissolve in the alkali than potato amylose. However, heating never exceeded 10 min. at 100° and experiments were done with whatever material had dissolved under these conditions.

Electrophoretic fractions of partially hydrolysed amylose. These were worked up after an electrophoretic run as described by Mould & Synge (1954). For electrokinetic ultrafiltration experiments they were dissolved as described above for amylose.

Amylopectin. A sample of potato amylopectin (B.V., 0.164) prepared by the method of Hobson, Pirt, Whelan & Peat (1951) was provided by Dr Whelan. It dissolved readily, as above, for the runs without heating.

Potato amylopectin β -limit dextrans. These were freeze-dried dextrans prepared by the method described by Hobson, Whelan & Peat (1950) from a thymol amylopectin and from a thymol amylopectin which had been purified by adsorption of the last traces of amylose on a cellulose column. They dissolved readily without heating.

Products of action of 'debranching' enzyme. Hobson, Whelan & Peat (1951) have described the isolation of an enzyme, 'R-enzyme', from broad beans and potatoes which effects the scission of the branch linkages in amylopectin and amylopectin β -dextrin. The isolation, on a large scale, of the products of action of this enzyme ('R-dextrans') which have been used in this work will be described in detail in a forthcoming paper (Peat, S., Hobson, P. N., Whelan, W. J. & Thomas, G. J., to be published), but relevant details are given here. A thymol-amylopectin from potato starch was further purified by fractionation with methanol (Hobson, Pirt, Whelan & Peat, 1951) and from part of the product a β -dextrin was prepared as described by Hobson *et al.* (1950). The amylopectin and β -dextrin were treated with bean R-enzyme on a large scale and the products separated into fractions diffusible and indiffusible through cellophane. The

indiffusible fractions were used here. In each case the total recovery was 90% of the amount of polysaccharide started with and the indiffusible fraction in the case of the amylopectin R-dextrin was 84.0% of the starting material and in the case of the β -dextrin R-dextrin 63.5%. The B.V.'s of the dextrans were 0.320 and 0.265 respectively. The R-dextrin from the β -dextrin of waxy maize starch was prepared in a similar manner, the indiffusible portion representing 49.0% of the initial β -dextrin, and the B.V. of the R-dextrin being 0.285. The materials dissolved readily as above in the cold or with gentle warming.

Amylose β -dextrin. The dextrin was prepared by Dr Gwen J. Thomas as follows. Potato amylose (B.V., 1.40; 2.922 g. dry wt.) was placed in a 2 l. standard flask and moistened with ethanol (5 ml.). Water (500 ml.) and 6N-NaOH (14 ml.) were added and the flask was heated in a boiling-water bath for 5 min. The flask was cooled, the contents were diluted to 1800 ml. with water and neutralized (6N- H_2SO_4). 0.2M Sodium acetate buffer (pH 4.8; 50 ml.) and crystalline sweet-potato β -amylase suspension (5000 units; Hobson *et al.* 1950) were added and the digest was diluted to 2 l. During incubation at 35° the course of reaction was followed by measurement of intensity of iodine stain. After 26 hr., when the reaction appeared to be complete and the intensity of stain had fallen to the usual level, the digest was heated in a boiling-water bath for 10 min. and the cooled solution dialysed against running tap water for 2 days to remove salts and maltose. The solution was concentrated under reduced pressure to 100 ml. and freeze-dried. The yield of dextrin was 0.953 g. having a B.V. of 1.23. The dextrin dissolved incompletely after heating for 5 min. at 100° in the NaOH. However, the supernatant after acidification gave a deep-blue stain with iodine.

Determination of adsorption equilibria in collodion membranes

Equilibration by diffusion. (i) *Fructose.* Square portions of a collodion membrane similar to VI (Tables 1 and 2), blotted with filter paper and having wet weight 0.17–0.23 g. were placed in tared stoppered weighing bottles and weighed. Approximately 0.8 ml. of solutions of D-fructose in 0.2N acetic acid ranging from 0.1 to 10% (w/v) were then added to each weighing bottle; the bottles were again weighed. After equilibrating for 24 hr. at room temperature, weighed portions of the supernatants (0.2–0.3 g.) were transferred to tared tubes, evaporated to dryness *in vacuo* over H_2SO_4 at room temperature and the dried residues were weighed. Similar portions of the original fructose solutions were analysed gravimetrically in the same way. Eight different concentrations of fructose were used and a control portion of membrane was kept in 0.2N acetic acid only. The control supernatant contained 0.27 mg. dry matter/g. which had passed into solution from the collodion. On deducting this from the concentrations of dry matter found in the experimental supernatants, we calculated the total remaining dissolved dry matter, assuming it to be evenly distributed between the supernatant liquid and the liquid inside the pores of the membrane (cf. Table 2). We thus obtained weights of unadsorbed fructose not significantly different from those taken initially for each experiment (Table 3). This proves that fructose at the concentration of 10% (w/v) and slightly less, as used in the marker experiments, is not significantly adsorbed on the collodion of the membranes

Table 3. *Equilibrium adsorption of fructose on collodion membrane*

For experimental details and calculations see text.

Approx. final fructose concentration (% w/v)	Fructose taken (mg.)	Fructose found in liquid (mg.)	Difference (adsorbed fructose) (mg.)
10	95.10	96.10	-1.00
5	45.70	45.80	-0.10
2	18.45	18.38	0.07
1	8.94	9.30	-0.36
0.5	4.95	4.78	0.17
0.2	2.19	2.11	0.08
0.1	1.25	1.03	0.22

and is therefore a reliable marker for electroendosmotic flow in the membranes.

(ii) *Linear dextrans*. Wet collodion membrane (1 g.) was cut into small squares (approx. 3×3 mm.) and added to 10 ml. of a solution of polysaccharide in 0.2N acetic acid. The flask containing this was shaken continuously for 24 hr. at room temperature. Then 0.5 ml. of supernatant was removed, diluted to 25 ml. and this solution was made 0.001M in respect of KI. One ml. 0.001N- I_2 in 0.001M-KI was added and after 7 min. the light absorption at the absorption peak was measured in a Beckman DU spectrophotometer. The polysaccharide concentration was found by interpolation in a calibration curve made using dilutions of the original polysaccharide solution. Shaking of the flask was continued, samples being taken at 48 and 96 hr., but it was found that the increase in adsorption after 24 hr. was not significant.

Equilibration of polysaccharides by mechanical ultrafiltration. A circular section of collodion membrane (effective diam. 4.6 cm.) was placed between rubber gaskets above the perforated plate of a Seitz filter. The filter was filled with 25 ml. of polysaccharide solution in 0.2N acetic acid and a positive pressure of N_2 (1 kg./cm.²) was applied. Fractions of filtrate (0.5 ml.) were collected with a small fraction collector and the polysaccharide concentration in each was determined spectrophotometrically as above. The rate of flow of solution through the membrane was also measured. The course of an experiment using electrophoretic fractions of an amylose hydrolysate is shown in Fig. 2. The shape of the curves and the fact that the polysaccharide content of the filtrate approaches that of the original solution and does not decrease shows that adsorption proceeds until equilibrium is reached and that there is no sieving action or blocking of the pores (Elford, 1933). The different adsorption of the different polysaccharides was calculated from the retention volumes found by integrating the curves, and was expressed as mg. polysaccharide/mg. dry collodion for the given polysaccharide concentration.

RESULTS

Rate of electroendosmosis

The electroendosmotic permeability D_I , (i.e. the liquid volume (ml.) electroendosmotically transported in 1 sec. by a current of 1A, across 1 cm.² cross-section of membrane) measured in the course of the experiments for the different membranes is plotted in Fig. 3 against the pore radius of the

membranes (cf. Table 6). Absolute rates of electroendosmosis ranged from 0.2 cm./hr. for the finest membranes to 6 cm./hr. for the coarsest.

R_F values of phosphorylase-synthesized polysaccharides (homogeneous primers)

Values for these were determined on the various membranes by developing until the fructose zone had nearly approached the cathode end of the strip. More accurate estimates of R_F for the slower-moving bands could be obtained by running for longer and measuring the rate relative to some zone other than fructose; incorporation of such data does not materially alter the results. Breadths of bands and R_F values are set out in Table 4. Since all the zones appeared reasonably symmetrical, the R_F value of the centre of the band has been assigned to the polysaccharide component having the average DP for each fraction as determined from the phosphate-liberation data. Where bands resolved well from one another, several R_F values could be determined in one strip. Fig. 4 is a sketch of a developed strip

Fig. 2. Retention of electrophoretically fractionated linear dextrans dissolved in 0.2N acetic acid during flow through collodion membrane IV: Δ , red-staining fraction (0.548 mg./ml.; rate of flow, 0.13 ml. hr.⁻¹ cm.⁻²); $\circ$, blue-staining fraction (0.387 mg./ml.; rate of flow, 0.29 ml. hr.⁻¹ cm.⁻²). For further details see text.

Fig. 3. Electroendosmotic permeability of the collodion membranes in relation to average pore radius (see text and Table 2). The vertical lines give the extreme range of values of D_I observed with each membrane.

Table 4. *Ranges and mean values for R_F of linear dextrans in different collodion membranes*
The values in the extreme right-hand column are arithmetical means of the two adjoining values.

Average DP of polysaccharide preparation	No. of expts.	R_F				
		Extreme values		Mean values		Centre of zone
		Leading edge	Trailing edge	Leading edge	Trailing edge	
Membrane I						
47.5	2	—	—	—	—	1
103	2	1	0.53	1	0.58	(0.79)
205	1	0.73	0.45	—	—	0.59
250	1	0.66	0.21	—	—	0.43
Membrane III						
34	3	0.79	0.61	0.79	0.61	0.70
77	3	0.50	0.21	0.44	0.28	0.36
103	1	0.46	0.21	—	—	0.33
160	2	0.16	0.08	0.15	0.08	0.115
Membrane IV						
34	2	0.93	0.55	0.85	0.60	0.72
103	3	0.37	0.12	0.35	0.14	0.245
160	1	—	—	—	—	(0.1)
Membrane V						
34	4	0.56	0.38	0.54	0.40	0.47
47.5	1	0.50	0.31	—	—	0.405
77	4	0.25	0.10	0.22	0.11	0.165
103	1	0.18	0.09	—	—	0.135
160	1	—	—	—	—	Zero
Membrane VI						
34	4	0.54	0.29	0.52	0.32	0.42
77	3	0.22	0.09	0.21	0.10	0.155
103	1	—	—	—	—	Zero
160	1	—	—	—	—	Zero
205	1	—	—	—	—	Zero
250	1	—	—	—	—	Zero

showing three such resolved bands. The polysaccharides with average DP 34 and 47.5 showed colour play within the zones, the leading parts staining pinker with iodine and the trailing parts bluer. The other polysaccharides all gave a uniform blue reaction throughout the zones.

Adsorption results for linear polysaccharides of known DP: calculation of expected R_F values

Adsorption results with membrane IV for a 'blue' electrophoretic fraction of hydrolysed amylose having DP approx. 40–90 (see below; Mould & Synge, 1954) were obtained by the diffusion and ultrafiltration procedures each at two different concentrations and are shown in Fig. 5. The results by the two different methods are seen to lie reasonably close to the same postulated isotherm. Similar isotherms were obtained for a 'red' electrophoretic fraction (DP ca. 25–40; see below) with membranes IV and VI and with an enzyme-synthesized preparation (average DP 77) with membrane VI. Points on these isotherms (as shown in Table 5) were used for calculating the R_F values expected,

assuming that adsorption is the only factor retarding the polysaccharides. The calculation was done as follows. The formula of Consden, Gordon & Martin (1944) gives

$$R_F = \frac{A_L}{A_L + \alpha A_S},$$

where A_L = cross-section of moving phase, A_S = cross-section of stationary liquid phase, α = partition coefficient = $\frac{[\text{solute}]_{\text{stationary phase}}}{[\text{solute}]_{\text{moving phase}}}$. For adsorption chromatography and considering volume rather than cross-section, we may rewrite the equation thus:

$$R_F = \frac{V_L}{V_L + \alpha M_s}, \tag{1}$$

where V_L = volume of moving phase in unit volume of chromatogram (see Table 2), M_s = mass of adsorbent in unit volume of chromatogram (see Table 2), α = slope of adsorption isotherm = $\frac{\text{mass of solute adsorbed/unit mass of adsorbent}}{\text{concentration of solute in moving phase}}$.

The R_F values calculated using equation (1) from direct determinations of adsorption are compared in Table 5 with those found in electrokinetic experiments (Table 4 and below under 'Electrophoretic fractions'). It is seen that the former, while exhibiting the same trends, are consistently lower than the latter.

Enzyme-synthesized polysaccharide from heterogeneous primer

This material, which gave a clear blue stain with iodine, scarcely entered membranes (similar to V

Fig. 4. Resolution of phosphorylase-synthesized linear dextrans (average DP of preparations 34, 77 and 160) on membrane III. Portions (1 mg.) of each preparation were heated at 100° in 0.2 ml. 0.04N-NaOH. After cooling, 0.2 ml. 0.2N acetic acid was added. This mixture was run into the strip for 30 min. and the run continued with 0.2N acetic acid as anolyte for a further 450 min. The DP 160 preparation dissolved poorly, the corresponding band being weaker than the other two.

and VI) that were freely penetrated by material of DP 77 made from homogeneous primer. On membrane I it had R_F 0.57–0.80, but the trailing edge of the zone was sharp while the leading edge was diffuse. This suggests a DP range 110–200, with a predominance of the higher polymers.

Amylose hydrolysates

These were much used for orienting experiments but were not subsequently studied in detail. HCl-hydrolysed amylose gave broad continuous zones and, in the finer-pored membranes, showed a brilliant series of changes of iodine colour in the parts of the zone corresponding with DP below about 40, the blue colour being preceded by a narrow purple region, then pink, then orange; the orange colour was not permanent, but disappeared rather rapidly as iodine evaporated from the strip. Partial hydrolysates made with saliva showed the same play of colours, but their zones did not cover such a wide range of R_F values. In each case increasing the extent of hydrolysis increased the R_F value for the trailing edge of the zone and increased the concentration in the red- and orange-staining part of the zone.

Fig. 5. Adsorption isotherm for electrophoretically fractionated dextrin (DP 40–90) on membrane IV. +, Equilibration by diffusion; ■, equilibration by mechanical ultrafiltration. For details, see Experimental section.

Table 5. Adsorption data for polysaccharide preparations on collodion membranes related to R_F values

For details see text.

Membrane			Adsorption data				R_F	
No.	V_L	M_s (g./ml.)	Polysaccharide	Polysaccharide (mg.) adsorbed/ g. dry collodion	Concn. polysac- charide (mg./ml.)	α (ml./g.)	Calc. from adsorption data	Found in electro- kinetic experiments
IV	0.825	0.29	Electrophoretic 'blue'	3.8	0.225	16.9	0.14	0.48*
IV	0.825	0.29	Electrophoretic 'red'	3.8	0.548	6.9	0.29	0.73*
VI	0.840	0.265	Enzyme-synthesized (av. DP 77)	4.0	0.125	32.0	0.09	0.155
VI	0.840	0.265	Electrophoretic 'red'	4.5	0.400	11.3	0.22	0.43

* By interpolation—see Table 4 and Fig. 7.

Electrophoretic fractions

'Orange' fractions could not conveniently be studied, owing to the low sensitivity and impermanence of the iodine staining. Experiments were done with 'red' and 'blue' fractions from a salivary-amylase hydrolysate of potato amylose (B.V. 0.56). In membrane V the 'red' material had R_f 0.40–0.58 and gave a uniform pink coloration. From Fig. 7 this would indicate DP range 25–40. The front of the zone of 'blue' material, which showed a slight purplish tinge, had R_f 0.40, and the zone, the rest of which was blue, extended back to R_f 0.07, corresponding to DP approx. 120. A mixture of the two fractions gave a single zone with no gap in the region R_f 0.40.

A second specimen of 'red' material (amylose hydrolysis to B.V. 0.42) tested on membrane VI had R_f 0.37–0.49, indicating DP range 27–42, while pooled 'blue' fractions from a number of salivary hydrolysates had R_f 0.06–0.38 suggesting DP range 40–90. Appearances in this experiment were the same as in the previous one, and a mixture of the 'red' and 'blue' fractions also behaved in the same way.

The significance of these observations is discussed in the following paper (Mould & Synge, 1954).

Amylose

This did not enter the finer membranes, forming a skin of blue-staining material on the anode end, which could be removed by gentle scraping. In the coarse membrane I an intense blue zone extended from the origin to R_f 0.06, preceded by pale-blue coloration as far as R_f 0.4. This suggests that the substances of lowest molecular weight present had DP ca. 250 and that most of the preparation was of substantially higher DP. Results were the same with dried material brought into solution with alkali and the undried thymol complex brought into solution without alkali. A second preparation of amylose by the alumina-thymol procedure gave rather more material with R_f 0.06–0.4.

Amylose β -dextrin

With membrane I this gave a picture not different from those obtained with amylose above.

Amylopectin

This did not enter the finer membranes. In I there was an intense zone from the origin to R_f 0.05, a weaker region extending to R_f 0.2 and traces of staining up to R_f 0.4. All the staining had a uniform purple colour.

Amylopectin β -limit dextrin

This was not distinguishable from amylopectin in its distribution on the strips, although it gave a pinker staining reaction.

Debranched preparations

The preparations both from potato amylopectin and from its limit dextrin were sharply separated, on the coarse membrane I, into a fast-running zone (R_f 0.8–1.0) and a slow-running zone (R_f 0–0.04). With debranched amylopectin the fast zone stained blue-purple and the slow zone purple. With the debranched limit dextrin the fast zone stained blue-purple but had a pink leading edge, while the slow zone stained pink-purple. The product from waxy maize starch gave a similar picture.

To characterize more closely the fast-moving fractions the debranched products were studied on membrane IV. Again in each case there was purple-pink staining material which scarcely entered the membrane. With the debranched potato amylopectin, the material entering the membrane gave a zone having R_f 0.14–0.56, staining blue, the front part more intense and having a slight purple tinge. With the product from the corresponding limit dextrin the faster zone had R_f 0.26–0.75 and had the characteristic orange-pink colour play towards its leading edge. The debranched limit dextrin from waxy maize starch likewise showed much material unable to enter the membrane and a zone R_f 0.42–0.9 with characteristic colour play and a concentration of orange-staining material at its leading edge.

These results suggest that, if the faster moving products of the enzyme action are linear amylopectins, they lie, for debranched potato amylopectin in the range DP 60–140 and for the corresponding debranched limit dextrin in the range DP 25–110 (Fig. 7). The products from the debranched limit dextrin from waxy maize starch have an upper DP limit ca. 75, but the lower limit cannot easily be determined owing to the poor staining with iodine. In each case there seems to be a preponderance of material towards the lower end of the DP range.

DISCUSSION

Physicochemical mechanisms determining the separations

As explained in the introduction to this paper, it was hoped, by the procedure adopted, to make use of molecular-sieve effects in so far as they could help the desired fractionations and at the same time to prevent them from interfering with any separations due primarily to adsorption. We feel that this object has been achieved; however, it remains to ascertain the parts played by the two effects. The fact that with the enzyme-synthesized polysaccharides compact zones with definite R_f values could be obtained indicates that any adsorption was according to approximately linear isotherms and that any retardation by molecular-sieve action was

for each solute a constant proportion of the electroendosmotic flow velocity irrespective of solute concentration.

The results on adsorption of linear dextrans obtained either by diffusion or by determining retention volumes during mechanical ultrafiltration across the membrane gave mutually consistent values for the adsorptions. This agreement, the form of the 'frontal analysis' curves from the mechanical ultrafiltrations (Fig. 2) and the fact that the effluent concentration rises to that of the original solution strongly suggests that with these dextrans that can penetrate the membranes only simple adsorption on the collodion surfaces need be postulated to explain the retardation. In fact, an explanation is demanded as to why the apparent adsorption should be less under electrokinetic ultrafiltration conditions than otherwise.

It should be noted that in the finer membranes the electrokinetic flow was 10–100 times greater than the mechanical flow, and would give rise to a correspondingly greater shear. Further, under the electrokinetic conditions the shear gradient is probably steeper near the collodion surfaces than during mechanical flow, giving rise to greater absolute shears at the surfaces. The work of Silberberg & Kuhn (1952) on disturbance of equilibria by shear in liquid-liquid systems is relevant in this connexion, and one could well imagine a similar effect of shear in modifying adsorption equilibria at solid surfaces. However, the adsorption found during free diffusion and mechanical ultrafiltration may well have been increased by irreversible adsorption or retrogradation, since it proved impossible by continuous washing of the collodion with 0.2N acetic acid to remove completely iodine-staining material after the experiments. Such irreversible phenomena cannot have occurred in the electrokinetic work, since the migrating zones left no tail of iodine-staining material. The high shear perhaps also acted to check retrogradation. This problem requires further study with materials which, unlike these linear dextrans, are in more stable equilibrium in the dissolved state.

As to the actual rates of electroendosmotic flow of 0.2N acetic acid in the membrane, it is seen (Fig. 3) that this varies with the pore radius of the membranes, increasing approximately linearly with

pore radius and then falling off, presumably to approach the constant velocity independent of pore radius required by the Helmholtz-Smoluchowski equation. The similarity of the whole curve to that obtained from the extensive data of Manegold & Solf (1931) indicates that the general electroendosmotic behaviour of the membrane in a direction parallel to its surface is the same as in the more usually investigated transverse direction. The theoretical treatment of Schmid (1951), applied by him to the data of Manegold & Solf, is probably only applicable up to a pore radius of 10 μ . (i.e. our membranes V, VI), the thickness of the double layer being of this order for 0.2N acetic acid. The hydrostatic permeability D_H , i.e. the volume (cm.³) of 0.2N acetic acid flowing through 1 cm.² of membrane/sec. under a pressure of 1 cm. water, can only be measured for flow across the membrane. D_H cannot therefore be strictly compared with D_I without making an arbitrary assumption as to the probable effect of the difference in thickness of the membranes under the two experimental conditions. The values of D_I , D_H , D_I/D_H are shown in Table 6. Assuming a constant factor for the thickness effect it can be seen, however, that D_I/D_H is approximately constant for the smallest pored membranes and then markedly decreases with increasing pore radius, in agreement with the data of Manegold & Solf and the theoretical treatment of Schmid. O. N. Grigorov (quoted by Zhukov, 1943) obtained similar results.

Further tests were applied to see whether surface adsorption on the membrane structure could give an adequate explanation of the R_p values observed. As all the membranes were made from the same commercial collodion, the amount of a given polysaccharide adsorbed at given concentration should be directly proportional to the adsorbing surface area in unit volume of membrane irrespective of the porosity. Although the 'pore radii' calculated as above cannot be simply related to the complicated actual structure of the collodion membrane, if it is assumed that the pore structure of each membrane has a similar geometry, differing only in scale, then these dimensions will be related linearly to the 'pore radius' (cf. Sullivan & Hertel, 1942). Surface area of the membrane structure/unit volume of membrane thus varies inversely with the first power of

Table 6. Comparison of electroendosmotic and hydrostatic permeabilities of membranes

For explanation see text.

Membrane	Pore radius (m μ .)	D_I (cm.sec. ⁻¹ A ⁻¹)	D_H ($\times 10^6$)	D_I/D_H ($\times 10^{-4}$)
I	552	0.836	685	0.122
II	61	0.240	4.1	5.85
III	27	0.116	0.903	12.8
IV	16.7	0.185	0.395	46.7
V	9.1	0.055	0.124	44.4
VI	8.2	0.049	0.102	48.0

the 'pore radius'. (Altering the linear dimensions of a given structure by the factor x changes its surface area by the factor x^2 and its volume by x^3 . The surface area now present in the original volume is therefore x^2/x^3 , i.e. $1/x$.)

Equation (1) may be rewritten substituting bS for aM_s where b is the slope of the adsorption isotherm redefined in terms of area as

$$\frac{\text{mass solute adsorbed/unit area of adsorbing surface}}{\text{concentration of solute in moving phase}},$$

and S is area of adsorbing surface/unit vol. of membrane. Rearranging,

$$b = \frac{V_L}{S} \left(\frac{1}{R_p} - 1 \right). \quad (2)$$

For membrane I an arbitrary value y is assigned to S . The values of S for the other membranes then vary inversely as pore radius and are shown in Table 2. Substituting these and the appropriate values for V_L and R_p in (2), values of b in terms of y can be calculated for the data in the last column of Table 4. Values of $\log_{10} by$ for each polysaccharide and membrane are plotted against DP in Fig. 6. The values for a given polysaccharide are fairly independent of membrane, confirming that surface adsorption can explain the phenomena observed. Furthermore, all the points lie fairly close to a single straight line, showing that the adsorption affinity increases with DP according to Traube's Rule. However, the three points for membrane I might be regarded as departing from this line and a second line has been drawn for them only. This deviation may reflect a change in structure of the membranes between I and III, which is in keeping with the idea of a critical region postulated for II and inter-

mediate solvent mixtures between those for I and II (see above).

The Traube's Rule relationship illustrated in Fig. 6 holds, however, only for those substances which have finite R_p values. R_p fell to zero with increase of DP sooner than could be expected from Traube's Rule and molecular-sieve effects must be postulated to explain this. They can be the only explanation where solute was actually scraped off the anode end of the membrane. We were rather surprised that these molecular-sieve effects set in over such a comparatively narrow range of DP—we had expected they would manifest themselves more gradually, and Barrer (1949) held the same opinion for ultrafiltration membranes made from organic materials. This seems to be a further testimony to the uniformity of pore size of this type of collodion membrane.

It is interesting that the converse adsorption of dissolved collodion on to starch grains has been used by Brooks & Badger (1950) for the chromatographic fractionation of collodion.

Range of DP in the enzyme-synthesized polysaccharide preparations

By plotting the data of Table 4 curves can be constructed showing variation of R_p with DP for the different membranes. The breadth of the zones can then be used to define the range of DP represented in each polysaccharide preparation. This has been done in Fig. 7. The DP ranges found with the different membranes are shown synoptically in Table 7. Although some of the experiments were rather crude, agreement is reasonable where experiments were repeated several times with different membranes.

Fig. 7. Relation of R_p to DP for the polysaccharides in the different membranes. The points are the data of Table 4 for zone centres. The slope of the appropriate curve in the neighbourhood of each experimental point has been used for converting the R_p values of the zone edges (Table 4) to the DP ranges given in Table 7. Membranes represented as follows: $\square$, I; ∇ , III; $\triangle$, IV; $\circ$, V; $\bullet$, VI. Graphical conversion of the data for the preparation having average DP 77 on membrane III is illustrated as an example.

Fig. 6. Relation of adsorption on internal surfaces of collodion membranes with DP of the polysaccharides. The full line relates to the data for all membranes (16 points), the dotted line to the data for membrane I only. For details of calculation see text. Membranes represented as follows: $\square$, I; ∇ , III; $\triangle$, IV; $\circ$, V; $\bullet$, VI.

Table 7. *Ranges of DP judged to include 95 % of molecules in enzyme-synthesized polysaccharide preparations*

See Fig. 7 and text.

	DP (glucose residues/molecule)						
	Average DP of preparation						
	34	47.5	77	103	160	205	250
Membrane I	—	—	—	—	—	140-250	170-320
Membrane III	21-46	—	62-92	75-130	150-170	—	—
Membrane IV	20-49	—	—	75-128	—	—	—
Membrane V	27-42	35-62	63-90	89-115	—	—	—
Membrane VI	22-46	—	67-85	—	—	—	—
Extreme range for all membranes used	20-49	35-62	62-92	75-130	150-170	140-250	170-320
Mean range for all membranes used	23-46	35-62	64-89	80-124	150-170	140-250	170-320
Spread of DP in mean range	23	27	25	44	20	110	150

The zones will be wider than ideally on account of diffusion, heteroporosity, etc., and also because the material was applied as a zone of finite width. However, the finite sensitivity of the iodine-staining reaction works in the opposite direction. We do not think it unreasonable to assume that 95 % of the molecules in each preparation lie within the limits of DP shown in Table 7, and this forms the basis of the discussion of the heterogeneity of the preparations in an accompanying paper (Whelan & Bailey, 1954). It is manifest that not all the width of the bands can be attributed to deviations from ideal chromatographic behaviour, since the colour play in the zones given by polysaccharides with average DP 34 and 47.5 implies some separation of molecules according to DP within the zone. However, the results obtained with membrane I (last two columns of Table 7) show a bigger spread of DP than the results with the other membranes, and we are inclined to attribute this to a greater departure of the membrane from ideal behaviour, which is in agreement with other anomalies noted above for membranes of this kind. Results with this membrane are accordingly disregarded in the discussion by Whelan & Bailey (1954). These authors also consider our results with the polysaccharide synthesized enzymically from heterogeneous primer.

Applicability of the method

Starch and related polysaccharides. The present results show that the method as so far developed is useful for the characterization of linear dextrans according to their molecular weight; the results with debranched materials suggest that they can simultaneously be separated from substances of higher molecular weight or degree of branching. The range of dextrans of DP 20-250 is satisfactorily handled and the method should therefore be useful in connexion with many problems of enzymic degradation and synthesis in the starch family. It is probably only the evanescence of the colour with iodine which

has prevented study of dextrans having DP < 20. Carroll & Van Dyk (1952) have suggested the use of congo red with dextrans in this DP range. Given a suitable detection method and, if necessary, finer-pored membranes, the present method should be useful at and above the DP at which charcoal displacement chromatography and paper partition chromatography cease to be effective.

With the membranes used by us, there were no great differences in the behaviour of amylose and amylopectin, both of which only slowly entered the coarsest membranes. This throws some light on the inconclusiveness of the data of Fouard (1908*a*, *b*, *c*, *d*), who ultrafiltered dispersions of native starch made with boiling water through collodion membranes and eventually concluded that starch is chemically homogeneous but has physical properties which are very sensitive to environmental changes. (See also Taylor & Iddles, 1926.)

Our results seem to establish that potato amylose has a DP considerably greater than 250, since a linear dextrin having this average DP migrated freely away from the origin. They are thus more consistent with the data of Potter & Hassid (1948*a*, *b*, 1951) than with lower estimates.

The ' β -dextrin' from potato amylose did not behave significantly differently from the parent amylose. Dr W. J. Whelan writes: 'Crystalline sweet-potato β -amylase converts only 70 % of potato amylose into maltose (Peat, Pirt & Whelan, 1952), and therefore, as in the case of amylopectin, the amylose molecule would seem to contain anomalous linkages which act as barriers to the end-wise progression of β -amylase along the molecule. In amylopectin these anomalous linkages are situated, on average, in the centre of each chain of α -1:4-linked glucose units, and the limit β -dextrin has half the molecular weight of amylopectin. In amylose, however, it would seem that the anomalous linkage is situated at or near the non-reducing chain end, preventing any substantial β -amylolytic

degradation, but in order to account for the degree of conversion into maltose this can apply only to 30 % of the molecules; the remainder must be considered free from the anomaly.'

Neither amylopectin nor limit dextrans derived therefrom by the action of β -amylase entered our membranes at any appreciable rate. The products of action on these of R-enzyme gave in all cases a purple-staining fraction unable to enter the membrane rapidly and a freely migrating fraction behaving similarly to the linear dextrans studied. Since in electrophoretic experiments (Mould & Synge, 1954) these preparations also gave material behaving in the same way as linear dextrans, it seems justifiable, pending a study of the behaviour of dextrans having only a few branching points in the molecule, to assume that the migrating materials are in fact linear dextrans. On this assumption their DP distributions have been assessed and throw some light on the structures of the parent polysaccharides and the mode of action of the enzyme. Dr P. N. Hobson writes: 'It was postulated by Hobson, Whelan & Peat (1951) that R-enzyme has a purely hydrolytic function, hydrolysing the α -1:6 branch linkages of amylopectin without the subsequent joining up of the liberated glucose chains by α -1:4 linkages. The results of electrokinetic ultrafiltration analysis are in accord with this hypothesis and also with the finding that R-enzyme does not completely "debranch" amylopectin or limit β -dextrin, as a portion of the products remains unhydrolysable by β -amylase. The structure of amylopectin (and, consequently, the β -dextrin) probably corresponds more closely to the arborescent formula of Meyer (cf. Meyer & Bernfeld, 1940) than to the regular formula of Haworth (cf. Haworth, Hirst & Isherwood, 1937). For discussion see, for example, Peat, Whelan & Thomas (1952). The partial debranching of an arborescent structure would result in some short-chain material (represented by the diffusible fraction of the R-dextrin), linear dextrans of varying chain length, and a molecule containing branch linkages inaccessible to the R-enzyme, which is probably the material which scarcely entered the ultrafiltration membranes. The R-dextrin from a β -dextrin would be expected to yield debranched molecules of lower average chain length than those of the amylopectin R-dextrin, and this is confirmed by the R_p values of the dextrans.'

Finally, since adsorption is presumed to play a part in the separations observed, it should be noted that starch and related polysaccharides have been fractionated on adsorbents by other workers. Cellulose selectively adsorbs amylose from mixtures with amylopectin (e.g. Tanret, 1914; Samec, 1940; Pacsu & Mullen, 1941; Kerr & Severson, 1943; Ashford, Evans & Hibbert, 1946). In view of this,

we had hoped that cellulose adsorption chromatograms would fractionate the partial hydrolysis products of amylose. Indeed, paper chromatograms of HCl hydrolysates of amylose developed with 0.2N acetic acid gave long streaks staining pinker with iodine furthest away from and bluer nearest the origin. However, the enzyme-synthesized linear dextrans, now shown to be of rather uniform DP, gave almost equally long streaks on paper under the same conditions. The ineffectiveness of cellulose for chromatography of this series is presumably for the reasons given in the introduction to this paper.

Ulmann (1950*a, b*, 1951*a, b*) has obtained fractionation of starches on alumina columns. Amylose seems to be selectively adsorbed by ordinary alumina and amylopectin by acid-treated alumina. Similar effects have been described by Fischer & Settele (1953). Ionic interaction of the positively charged adsorbent with the phosphoric ester groups of the amylopectin may play a part. (A similar interaction may enhance the coprecipitation effect with alumina used by Bourne, Donnison, Peat & Whelan, 1949.) The behaviour of the fractions on Ulmann's chromatograms seems to reveal changes in the starting materials not readily detectable by other means. Ling & Nanji (1923) obtained at least partial separation of amylose from amylopectin by adsorption on alumina or colloidal iron. Koval'skii (1947, 1948) has obtained interesting fractionations of glycogens by chromatography on calcium carbonate (see also Koshtoyants & Yanson, 1950; Yanson, 1951).

General. The method has not so far been used preparatively. Difficulties are foreseen in increasing the scale by the use of thicker membranes; satisfactory membranes would be difficult to prepare and temperature differences within them might cause trouble. However, up to 1 mg. polysaccharide has been handled on the present strips (1.3 cm. broad) and some increase of scale could be achieved simply by increasing breadth. It would be well to arrange for fractions to be isolated to run out of the strips electrokinetically, in view of the difficulties noted above in removing polysaccharide from the collodion by mechanical ultrafiltration or diffusion.

Given suitable means of detection, the method should be useful for studying other soluble polysaccharides such as dextrans and fructosans and also intermediate stages in the synthesis and degradation of polysaccharides in general. Substances that do not show a tendency to retrograde from solution are likely to be easier to handle than those here studied. The method should similarly be able to handle other water-soluble neutral polymers, and offers the prospect of obtaining samples of very uniform DP which will be valuable reference substances for physicochemical studies of compounds

of high molecular weight. We are interested also in the possibilities with non-isochemical polymers, especially peptides.

With charged compounds, electrophoretic effects will be superimposed on movement due to the electrokinetic flow. With collodion, only positively charged compounds will enter the membranes. If the compounds are very highly charged, ion-exchange binding in the membrane may be troublesome. For negatively charged substances membranes made from anion-exchange resins or collodion membranes with reversed charge (made in some of the many ways that have been suggested) may prove useful. To extend the method to the large number of polymers that are only soluble in organic solvents which would destroy collodion membranes, it seems promising to try denitrating the collodion (cf. discussion of Mould & Syngé, 1952; Fuoss & Mead, 1943; Zhukov, Poddubnyi & Lebedev, 1948; Bulgakova & Shafershtein, 1951).

SUMMARY

1. In order to circumvent difficulties commonly encountered in chromatographic separation of members of polymeric series, particularly difficulties due to molecular-sieve effects, a procedure has been devised for conducting chromatography inside a strip of collodion ultrafiltration membrane. Flow of liquid in the membrane is produced electrokinetically.

2. The procedure has been evaluated using a series of linear dextrin preparations of known average degree of polymerization (DP) (Whelan & Bailey, 1954). Information has been gained as to the range of DP represented in the various preparations.

3. The separations effected can be explained in terms of normal adsorption of the polysaccharides on the inner surfaces of the membranes. However, ultrafiltration effects prevent molecules greater than a certain size from entering the membrane. Comparisons of adsorption during free diffusion, mechanical ultrafiltration and electrokinetic ultrafiltration suggest that adsorption equilibria at the surfaces may be modified under electrokinetic-flow conditions.

4. Results are reported on velocity of electrokinetic flow in relation to the pore size of the membranes.

5. Several polysaccharide preparations related to or derived from starch have been studied by the new procedure, and estimates of DP range have been made for the cases of linear polymers.

6. The new procedure is discussed in relation to previous work on the chromatography of polysaccharides.

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The Electrophoretic Mobility and Fractionation of Complexes of Hydrolysis Products of Amylose with Iodine and Potassium Iodide

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This paper describes some experiments aimed at elucidating the electrophoretic behaviour of polysaccharides related to starch in the presence of iodine and iodide. It has been explained in the previous paper (Mould & Synge, 1954) how these effects came to be observed. The role of iodide in the combination of starch polysaccharides with iodine was studied potentiometrically by Gilbert & Marriott (1948) and by Higginbotham (1949), who give references to earlier work. It has, however, been neglected by most other workers despite the presence of iodide in nearly all of the systems used for studying the starch-iodine reaction. Our experiments also aimed at preparative fractionation of these polysaccharides, and for this purpose the continuous electrophoretic apparatus of Svensson & Brattsten (1949; cf. Grassmann & Hannig, 1950) was used with some modifications in design and operating procedure. Potentiometric and spectro-

photometric studies of the behaviour of the isolated fractions with iodine and iodide are described in the following paper (Mould, 1954).

EXPERIMENTAL

Experiments in agar-agar gels

Agar-agar. As explained in the preceding paper (Mould & Synge, 1954) the velocity of electroendosmosis greatly varied with different specimens of agar-agar although the relative velocities of the zones did not differ. No attempt has been made to relate electroendosmosis with composition or origin of the agar-agar; all the later experiments reported here were done with a batch of strip agar-agar, presumably Japanese, supplied before 1939 by British Drug Houses Ltd.

Apparatus and general procedure. This was according to Consden, Gordon & Martin (1946, Method B). Gels were set in an enclosed trough 3.8 cm. broad and 0.7 cm. deep, the inlaid zone being 1 cm. broad. The agar was dissolved in boiling water at double the desired final concentration and