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A Technique for Partition Chromatography on Starch

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In the last years the principle of counter current liquid-liquid distribution has gained interest because of the realisation of its potential high capacity in the resolution of mixtures of substances with differing distribution coefficients in a pair of immiscible solvents. The principle has been employed methodically along two somewhat different lines. In the method of Craig [1, 2] the resolution of the mixture is achieved through systematic, consecutive distributions of the substances to be resolved between two immiscible solvents. The essential feature in the method of Martin, Synge [3] and collaborators is that one of the solvent phases is finely dispersed on a supporting medium and in that way immobilized whereas the other phase flows in intimate contact over the immobile phase. The immobile phase is usually water and as a support silica gel, cellulose or starch have been used. The partition chromatography, as the latter method has been called, has an exceedingly high resolving capacity. It can be performed in a column essentially in the same way as an ordinary chromatographic procedure.

Potato starch has proved to be a superior support for the immobile phase [4, 5]. A difficulty, however, arises because of the slow rate of flow of liquid through a potato starch column with the consequence that one fractionation may take several days. This makes an automatic collector of the effluents almost indispensable. In this paper is described a technique for starch chromatography which has been used successfully for a long time.

EXPERIMENTAL

Pretreatment of the starch. Commercial potato starch contains contaminants which must be removed before the starch can be used for a chromatogram. Unless this is done disturbances will appear in the development of the chromatogram. The nature of these impurities is unknown except that they are

water soluble and can be removed by means of thorough washings of the starch with water. The practice has been to stir up the starch in five volumes of water, let it settle and decant the water. This process is repeated four times with tap water and finally two times with distilled water. The starch is sucked as dry as possible on a Buchner funnel and then spread and dried in air. The lumps of dry starch are broken up and the powder passed through a sieve, mesh 20/cm. For many purposes this treatment is sufficient. In certain instances, however, it was found desirable to defat the starch and to remove material absorbing in the ultraviolet. This was accomplished through extraction of the starch in a Soxhlet apparatus for 24 hours with dioxane or methyl alcohol containing 20 % water. Afterwards the organic solvent was removed from the starch by means of repeated washings with water and the starch then treated in the way already described. It is advisable to work up as large a batch as possible since there may be individual variations in properties between different batches. When the starch is stored in closed glass containers no changes in properties have been observed. One batch has been used with reproducible results for more than two years.

Preparation of the column. A suitable tube for the column can be made from a Buchner funnel with a sintered glass filter to which is sealed a glass tubing of the same diameter as the funnel so that the height of the funnel will be about 40 cm from the filter to the upper rim. A funnel made of American Pyrex glass with a plane filter of porositles C or M has been found most suitable.

The starch is dispersed in two volumes of the water-saturated solvent which will later be used for the development of the chromatogram and immediately transferred to the tube. The tube is inverted carefully several times to ensure an even dispersion of the starch in the solvent. Care should be taken that no air bubbles are introduced into the slurry. The tube is then placed in exactly the vertical position and the starch left to settle. Fresh solvent is constantly siphoned into the tube by means of the arrangement seen in Fig. 1. After 4—5 hours the starch column will have attained its final height and should present as mooth and rather firm upper surface. It is essential that this period is not shortened since the starch requires time to attain equilibrium with the water-saturated solvent. The period given has been found minimal when *n*-butanol is used as a solvent.

The chromatographic procedure. The solvent above the starch is now left to drain. At the moment when the solvent meniscus disappears into the starch the solution of the sample to be analyzed is introduced on the top of the column, care being taken not to disturb the surface of the starch. The sample should preferably be dissolved in the smallest possible volume of the water-

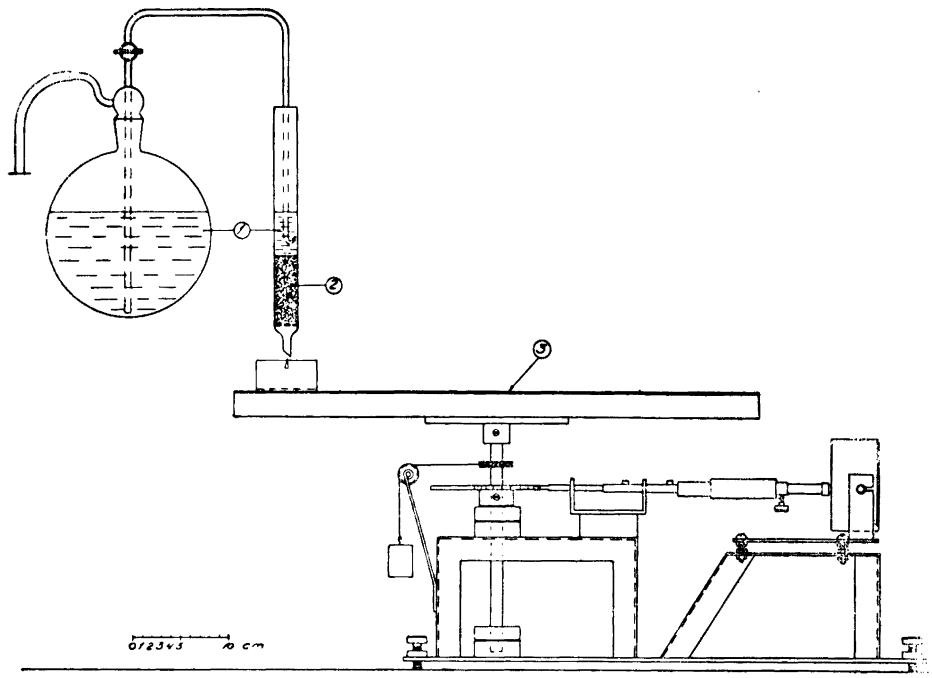

Fig. 1. Assembled chromatographic setup. 1. Solvent. 2. Potato starch. 3. Disk supporting the vessels.

saturated solvent since a small initial zone width is desirable. After the sample has drained into the column it is followed by an equal volume of the same solvent which is also left to drain into the column. Thereafter solvent is siphoned from the large storage bottle (Fig. 1) to the column. The rate of flow through the column is adjusted by raising or lowering the level of the liquid above the starch. By means of this arrangement it is easy to keep the flow of solvent at a fairly constant rate throughout a whole fractionation.

The rate of flow of the solvent requires some further consideration. In several cases investigated, the zones have been found to move at a rate which is directly proportional to the flowrate of the solvent. This relationship held true up to the highest rate of flow employed namely 4 ml per hour per cm^2. The sharpness of the zones were not appreciably impaired at this rate of flow. It is conceivable, however, that the maximal rate of flow in other instances may be lower.

In principle the same solvents as those used in paper chromatography can be employed in starch chromatography although for various reasons some of them are less suitable. Their use, however, in starch chromatography requires

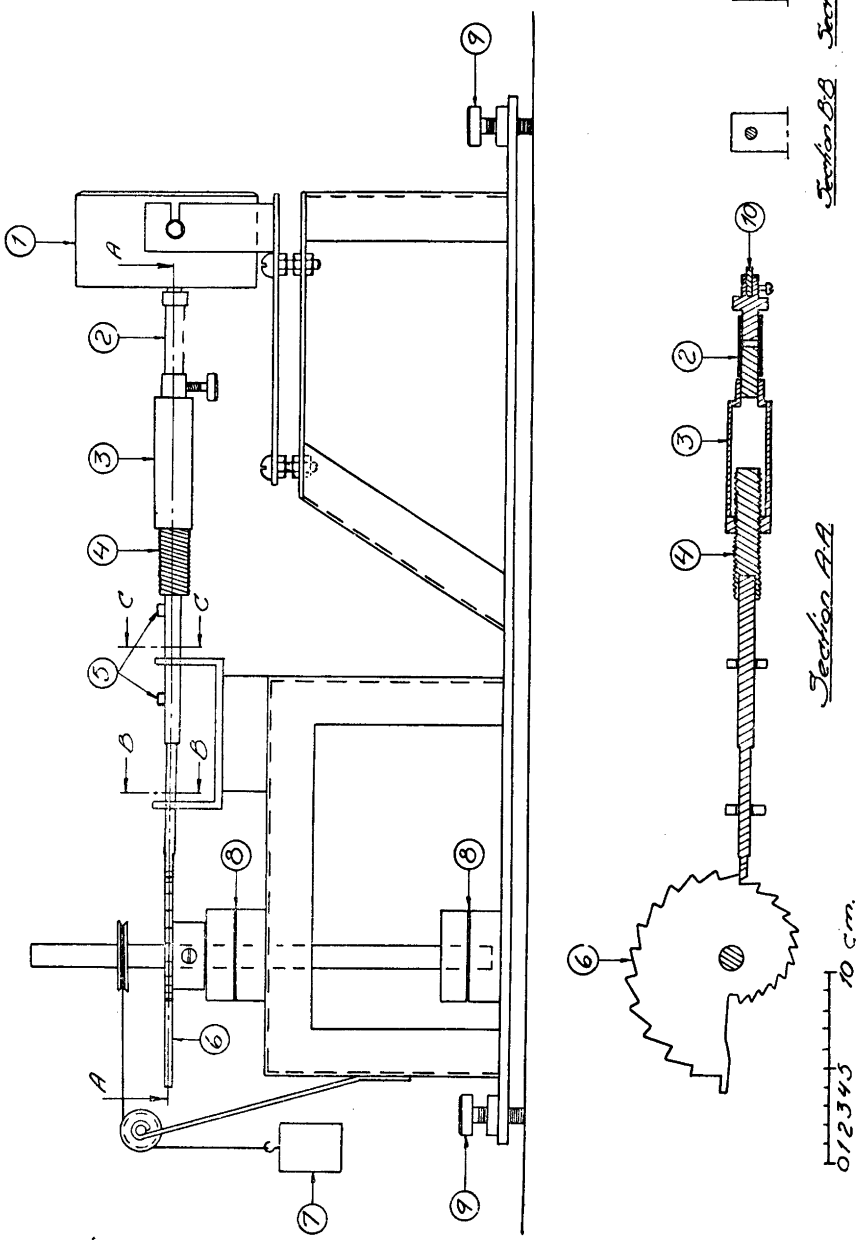

Fig. 2. Automatic collector (except the disk). 1. Clock work. 2. Rubber tubing. 3. Nut. 4. Screws. 5. Stop screws. 6. Notched wheel. 7. Weight. 8. Ball bearing. 9. Levelling screw 10. Axis of the clock.

a more rigorous purification. It is preferable not to saturate the organic solvent completely with water in order to avoid the formation of emulsions.

The automatic collector. This machine (Figs. 1 and 2) shifts the receiving vessel at the regular time interval of one hour and it is designed to collect 24 samples *. The operation of the machine is regulated by a clock work (1 in Fig. 2). To the axis of the clock work is attached a nut (3). The nut turns on a screw (4) connected to a rod. In this way the rod is made to retract from the notches of a spiralformed wheel (6). Every time a notch is released the disk (3 in Fig. 1) supporting the receiving vessels will move 1/24 of a complete revolution bringing a new receiver under the column. The disk should be made from a material which will not warp and should be covered with a rubber mat to prevent the receivers from sliding. It is essential that the levelling screws (9 in Fig. 2) are so aligned that the disk is exactly in the horisontal plane. The position of the clock work should be so aligned that its axis (10) will coincide as close as possible with the axis of the rod although the soft rubber connection (2) will take care of any strain resulting from a small deviation. All moving parts should be freely greased. The apparatus, once started, requires no attention for 24 hours.

As receiving vessels low beakers have been found very convenient. When a fractionation is finished the beakers with their contents are transferred to a vacuum oven and the solvent evaporated at a temperature of around 40° C. For subsequent analyses the dry residues are dissolved in a suitable medium.

The technique described here has been employed successfully for the separation of peptides [6], purines [7], and nucleosides [8].

SUMMARY

A technique is given for the performance of partition chromatography on starch. The pretreatment of the starch, the preparation of the column, the chromatographic procedure and an automatic collector of the effluents are described.

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* The apparatus is manufactured by Lindmark & Holm, Nybrogatan 76, Stockholm.