

The use of Sephadex for the desalting of dyes

Sephadex has been widely used for the desalting of albumins and macromolecular substances. The principle of desalting is based on the fact that the low-molecular substances penetrate easily into the Sephadex gel but the macromolecules do not. If the mixture of macromolecular and low-molecular substances, *e.g.* the salt, moves through the Sephadex column, the salt is slowed down due to its diffusion into the gel and the macromolecular substances which move faster down the column are thus separated from the salt¹.

Some aromatic compounds are also adsorbed on the Sephadex column and may thus be separated. Some mixtures of amino acids have been separated in this manner^{2,3}. For example, dinitrophenylaspartic acid placed on the top of a Sephadex G 25 column in a mixture with sodium chloride is concentrated during the movement through the column and accumulates at the end of the gradient between the salt and the distilled water forming the mobile phase³. The isolation of conjugated oestrogens from other low-molecular substances present in urine is based on the principle of adsorption and ion exclusion and was described by BELING⁴.

Salting-out with a saturated solution of sodium chloride is used as the final step of isolation in the preparation of some phthalein dyes such as bromosulphophthalein. Even when the dye is extracted with the absolute ethanol, small amounts of sodium chloride appear in the final product and its removal is a very difficult problem.

We thought that adsorption of the dye on the Sephadex column might divide it from the salt and that sodium chloride would move down the column faster than the dye.

Method

A Sephadex G 25 column, 8 × 500 mm, thoroughly soaked in distilled water for 24 h and then decanted, was used for the separation of up to 200 mg of bromosulphophthalein, chlorosulphophthalein and iodosulphophthalein from sodium chloride in varying amounts, sometimes even higher than the total amount of the dye. The K_d value found on the column, from the simultaneous introduction of

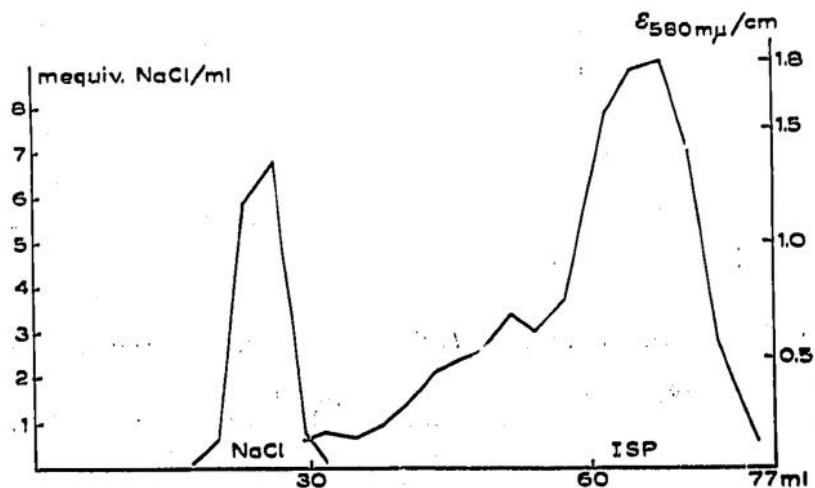


Fig. 1. The separation of NaCl from iodosulphophthalein (ISP) on a Sephadex G 25 column.

Indian ink and sodium chloride in the mixture with the sulphophthalein, is about 1.56, which indicates adsorption of this dye on Sephadex. The separation of iodosulphophthalein from sodium chloride is quoted as an example.

The initial weight of the mixture was 79.8 mg. The sharp separation during the movement through the column is seen in Fig. 1. The total amount of chloride found by titration with 0.01 *N* AgNO₃ was 43.36 mg; the content of pure dye measured photometrically was found to be 36.1 mg. The extinction value of the dye solution, concentration 1 mg/100 ml 0.1 *N* NaOH, was 0.247 before the treatment and after chromatography 0.545 (measured on Unicam SP 500 in 10 mm cuvettes (580 m μ)). Extinction of chlorosulphophthalein was 0.69 before desalting and 0.975 afterwards, and of bromosulphophthalein 0.40 before desalting and 0.82 afterwards.

Discussion

Our findings showed the possibility of using Sephadex G 25 for the removal of salt from some aromatic dyes. Since the adsorption of some dyes, particularly the phthaleins on ion exchange resins, is so strong that they cannot be eluted, the use of ion exchange resin can sometimes be disadvantageous; this phenomenon has been used *e.g.* for labelling Dowex with phenolphthalein in the chromatography of acids⁵. It is possible that Sephadex can be used for the desalting of other aromatic compounds.

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Chromatographic separation and quantitative estimation of isomeric dinitrobenzenes

In the present work we were interested in examining the possibilities of applying TLC to the separation of isomeric dinitrobenzenes. At the same time an attempt was made to develop a method for the quantitative estimation of these isomers in a mixture, which might be of interest for the explosives industry.

FRANC¹ in 1955, as well as PRAY AND KABIL² in 1956, succeeded in separating only *ortho*- from *meta*-dinitrobenzene by reversed phase paper chromatography, using an acidic solvent system. ETTTEL, POSPIŠIL AND DEYL³, in a very comprehensive study of the chromatographic behaviour of nitro compounds, found, however, that

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