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THE DIFFERENT CHROMATOGRAPHIC BEHAVIOUR OF HIGH- AND LOW-MOLECULAR-WEIGHT SUBSTANCES ON NITROCELLULOSE MEMBRANES

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SUMMARY

High- and low-molecular-weight polyethyleneglycols have shown a different behaviour when submitted to membrane chromatography on nitrocellulose ultrafilters, the former remaining at the start and the latter migrating with the front in aqueous solutions. This was a good analogy with the behaviour of proteins and amino acids and peptides, respectively. An interpretation of the mechanism of adsorption to nitrocellulose was proposed, especially emphasizing the simultaneous multivalent binding between the macromolecules and nitrate dipoles.

Nitrocellulose membrane filters have recently been shown to be suitable carriers for some specific and simple chromatographic and electrophoretic separations, identifications and estimations, especially in the ultra-micro analysis of proteins and nucleic acids. It was the strong and rather specific adsorption of high-molecular-weight proteins to nitrocellulose membranes which prompted us to develop a series of rapid microtechniques using this carrier, for example: the simple microdeproteinization of biological fluids[1] and the quantitative chromatographic microestimation of proteins[2,3] on intact nitrocellulose membranes; electrophoresis and immunoelectrophoresis[4,5] or chromatography and immunochromatography[6,7] on membranes impregnated with neutral detergents or with proteins; the use of wedge-compressed membranes[8,9] for filtration chromatography etc. It was also possible to achieve a rapid separation of serum albumin[10] from other proteins, and a separation of proteins from nucleic acids[11], as well as different types of nucleic acids and proteins from one another[12]. A brief review of our results is given in refs. 13 and 14 and a more detailed review is now in preparation[15].

The chromatographic behaviour of proteins on nitrocellulose membranes differed in many respects from that on acetylcellulose or cellulose carriers. This seems to be caused amongst other factors by the presence of many relatively strong and easily accessible nitrate dipoles on the surface of the nitrocellulose membranes which can bind and fix high-molecular-weight proteins by simultaneous electrostatic interactions on a large number of sites[16]. In contrast, the binding of the chemically analogous, but low-molecular-weight substances (*e.g.* amino acids or peptides) which can only be bound to one or to a few dipoles of the carrier at the same time, is not sufficient to immobilize those substances on nitrocellulose membranes during chromato-

graphy. A more detailed study dealing with the probable mechanism of the binding of proteins under different experimental conditions is presented in ref. 16.

Quite recently we tested the chromatographic behaviour of polyethyleneglycols with various molecular weights in aqueous solutions on nitrocellulose membranes and we showed again that there was a dependence on the molecular weight. Polyethyleneglycols with a mean mol. wt. lower than about 1,500 migrated from the start without any visible adsorption, whereas those of a higher mol. wt. were adsorbed to the membranes in a manner similar to high-molecular-weight proteins or the Tweens. This made possible a simple binary group separation of polyethylene glycols of different molecular weight. However, we have not yet been successful in finding suitable conditions for the separation of more complex mixtures of polyethyleneglycols into individual fractions by chromatography on nitrocellulose membranes.

We used polyethyleneglycols of the following molecular weights: 200, 400, 600, 1,500, 4,000, 6,000, 20,000 in the following developing solutions: water, Michaelis buffer pH 7.3 and 3.8, 0.1*N* NaOH, 0.1*N* HCl. NaCl up to a 10% concentration was added to the solutions. No difference in the chromatographic behaviour of the polyethyleneglycols was observed in the solutions tested. The polyethyleneglycols were detected by Dragendorff's reagent and by $KMnO_4$ (*cf.* ref. 16).

It can be concluded that the different behaviour of some homologous substances of different molecular weight during chromatography on nitrocellulose membranes is most probably due to the different amounts of binding sites on the surface of the molecules. Further extension of the practical use of this phenomenon is under investigation.

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