Chromatography of food dyes on Sephadex

Dextran gels ("Sephadex") are known to adsorb aromatic compounds of low molecular weight from aqueous solutions[1], but the separation of food dyes by chromatography on Sephadex has not been reported before. Although cellulose powder has been used with sodium chloride solutions as eluants[2,3], other workers have found more complex systems[4,5] to be necessary for the separation of food dyes.

Experiments in this laboratory have shown that excellent separations of some water-soluble food dyes may be obtained on Sephadex with simple aqueous eluants. In pure water the dyes are not strongly adsorbed, but low concentrations of electrolyte increase the adsorption. The pH of the eluant is not important, but an ammonium acetate buffer can be used if desired. The effect of electrolyte has been observed previously in the chromatography of other anions, and has been attributed to ion-exclusion by carboxylic acid groups in the Sephadex[1]. The best separations of food dyes have been obtained with Sephadex G-25 (superfine grade), since elution is difficult from the less swollen G-10, and adsorption is reduced on the more swollen gels.

The superfine grades of Sephadex may be used for thin-layer chromatography[6], as well as for column chromatography. Thin layers on microscope slides were used to separate dyes rapidly by placing the slides in open beakers containing the eluant. India ink marked the position of the solvent front. The R_F values in Table I were obtained by this method. They are not accurately reproducible, but they illustrate the effect of electrolyte in the eluant, and indicate the possibility of separations on columns.

TABLE I

R_F VALUES OF DYES ON SEPHADEX G-25

Eluants: I = water; II = 0.1 % sodium sulphate solution; III = 4% sodium sulphate solution.

Dye (Colour Index No.[7])	*Eluants*		
	I	*II*	*III*
Blue VRS (42045)	0.48	0.41	0.31
Ponceau SX (14700)	0.47	0.27	0.20
Ponceau 4R (16255)	0.46	0.27	0.21
Tartrazine (19140)	0.42	0.27	0.13
Ponceau 3R (16155)	0.40	0.12	0.06
Indigo carmine (73015)	0.36	0.13	0.06
Amaranth (16185)	0.34	0.15	0.06
Naphthol yellow S (10316)	0.33	0.29	0.16
Carmoisine (14720)	0.27	0.08	0.03
Orange G (16230)	0.25	0.07	0.04

The relative R_F values of the dyes Tartrazine, Indigo carmine and Orange G in 0.1 % sodium sulphate solution corresponded to the relative distances travelled by these dyes on a column of Sephadex, and a mixture of them was completely separated on a 6 cm column by elution with 0.1 % sodium sulphate solution. The recovery of a pure dye from such a column was better than 98 %.

More complex mixtures required longer columns, and separations were best at slow rates of flow. Thus, a commercial "chocolate" colour was applied to a 36 cm

column of Sephadex packed in plastic tubing (1 cm internal diameter). The chromatogram was developed with dilute sodium sulphate solution until the fastest band (yellow) reached the bottom of the column. This band was followed by bands of blue, red, orange and red, all except the third and fourth bands (red and orange) being completely separated. The column was sectioned, and each band was eluted separately with water. The red and orange bands were cut out together, but elution with water removed the red dye before the orange, allowing a complete separation. The spectra of the eluted dyes showed that they were Tartrazine, Indigo carmine, Amaranth, Orange G, and Carmoisine, respectively. Their relative proportions were calculated from their optical densities in a known volume of solution. Attempts to accelerate this separation by pumping were not completely successful because of band-spreading. Nevertheless, a fast flow rate could be used for the rapid separation of some mixtures. Thus, a mixture of Tartrazine, Orange G and Carmoisine was carefully applied to the 36 cm column (syringe injection was unsatisfactory), and was eluted with water containing 10% ethanol. The dyes were collected in the effluent as follows: Tartrazine 5.6–7.2 ml, Carmoisine 7.5–8.9 ml, Orange G 11.4–15.3 ml. This separation was complete in 25 min.

Polyacrylamide gels such as "Biogel P" behave differently from dextran gels, and pH effects are important. Thus dyes are adsorbed by Biogel P from dilute acetic acid, and are eluted by ammonia. Similar behaviour[8] has been reported on powdered polyamides of the nylon type, which have also been used for thin-layer chromatography of food dyes[9,10].

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