An application of molecular complexation to chromatographic separations

The ability of caffeine to complex with a variety of aromatic compounds has been well documented[1-5]. Since two-phase solvent partition studies showed that complex formation can have a profound effect on the distribution of the components[3,5] it seemed likely that this property could be exploited in chromatographic separations. We wish to report preliminary data obtained with silicic acid columns showing that this is indeed the case.

TABLE I

EFFECT OF 7-(2,3-DIHYDROXYPROPYL)-THEOPHYLLINE ON THE ELUTION OF CINNAMIC ACIDS FROM SILICIC ACID COLUMNS

Compound	*Peak effluent volumes*[a] *(ml)*	
	Control column[b]	*Complexation column*[c]
Cinnamic acid	30	55
4-Methoxycinnamic acid	60	110
3,4-Dimethoxycinnamic acid	60	200

[a] The peak effluent volume was defined by MARVEL AND RANDS[7], as that volume of effluent collected while a given compound moves from the top of the column to the bottom and is measured at the point at which the greatest concentration of the compound is eluted.

[b] Control columns were prepared according to BULEN, VARNER AND BURREL[8]. The stationary phase consisted of 8 g silicic acid and 5.5 ml of 0.5 *N* H_2SO_4; the mobile phase was chloroform–cyclohexane (1:3 v/v). Five ml fractions were collected and the cinnamic acids were detected with a Beckman Spectrophotometer, Model DU, by scanning at their high-wavelength maxima. Between 0.5 and 1 mg of each acid was applied as a band consisting of 1 g silicic acid 0.6 ml 0.5 *N* H_2SO_4.

[c] The complexation columns were made up with a 12% solution of 7-(2,3-dihydroxypropyl)-theophylline in 0.5 *N* H_2SO_4 instead of the 0.5 *N* H_2SO_4. In all other respects they were identical to the control columns.

The relative complexing ability of cinnamic acid derivatives with caffeine in aqueous solution has been shown to increase in the following order: cinnamic acid; 4-methoxycinnamic acid; and 3,4-dimethoxycinnamic acid[5]. Attempts to utilize this property for the chromatographic separation of these acids using caffeine in 0.5 *N* sulfuric acid as the stationary phase were not satisfactory due to the partial removal of the caffeine by the elution solvents. This problem was overcome by the use of 7-(2,3-dihydroxypropyl)-theophylline[6] a substance that is comparable to caffeine in complexing ability but possesses more favorable solubility characteristics. Typical results demonstrating the improved resolution obtained when advantage is taken of the difference in complexing abilities of cinnamic acid derivatives are shown in Table I. Neither the recoveries nor the width of the elution bands were adversely affected by the xanthine. Since the solvent used for the separation of the cinnamic acids (Table I) caused the elution of less than 10 mg xanthine per liter, contamination of the fractions by the theophylline derivative does not pose a serious problem.

Additional evidence can be cited to support the claim that the differences in the complexing abilities of the cinnamic acid derivatives listed in Table I, are responsible for the improved resolution. Thus, the peak effluent volumes of dihydrocinnamic acid

and 4-acetoxycinnamic acid—substances which do not form strong complexes with caffeine—are not altered significantly by the 12% solution of 7-(2,3-dihydroxypropyl)-theophylline. Furthermore, when 1,3-dimethyluracil—a poor complexing agent for cinnamic acids—is substituted for the theophylline derivative, in the same molar concentration, the peak effluent volume of 3,4-dimethoxycinnamic acid is not significantly different from that obtained with the control columns.

Other applications of this type of complexation to chromatographic separations are being studied. For example, it has been possible to improve the separation of eugenol and isoeugenol on silicic acid columns with the sodium salt of riboflavin-5'-phosphate. Since isoeugenol has a conjugated double bond, its peak effluent volume is more strongly affected by the riboflavin salt than that of eugenol. We have also been able to influence the elution pattern of caffeine and 1,3-dimethyluracil in a predictable manner by using chlorogenic acid or sodium 2,4,6-trihydroxybenzoate solutions as stationary phases of silicic acid columns.

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Notes

Detection of hypoglycaemic sulphonyl-ureas on paper chromatograms

The determination of antidiabetic agents, such as tolbutamide and carbutamide, in biological fluids, blood, urine and drugs has been reported employing spectrophotometric[1–6], colorimetric[7–13], titration[14,15] (indicator), and anodic chronopotentiometric[16] methods. The colour reactions commonly used are the diazotisation of carbutamide and subsequent coupling with a suitable reagent such as 2-naphthol, etc. In the case of tolbutamide or chlorpropamide, the colorimetric measurement of the yellow dinitrophenyl derivative of the resulting amine has been described[9]. It appears