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Short communication

Adsorption chromatography on cellulose XIII. Chromatography with aqueous solutions of carbohydrates as eluents

Thi Kieu Xuan Huynh, M. Lederer*, E. Leipzig-Pagani

Institut de Chimie Minérale et Analytique, Université de Lausanne, Boîte Postale 115, Centre Universitaire, CH-1015 Lausanne 15, Switzerland

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Abstract

The effect of adding carbohydrates to the aqueous eluent in adsorption chromatography on cellulose was investigated. No R_F shifts were obtained when sucrose or linear dextrans were added to the eluent. R_F changes occurred with soluble starch, but these are smaller than those with α-cyclodextrin and discriminate less between enantiomers (tryptophans) or between positional isomers (azo dyes). They could be obtained only in neutral or alkaline solutions.

1. Introduction

There is an extensive literature on the application of cyclodextrins in chromatography [1–3]. Cyclodextrins have been used as part of the stationary phase or as a constituent of the mobile phase. The forces involved were considered and Menges and Armstrong [4] concluded that hydrophobic interactions and hydrogen bonding in addition to specific steric effects due to the ring structure could account for the phenomena observed. Adsorption on cellulose was found to be essentially due to the same effects and all three interactions, hydrophobic, hydrogen bonding and chiral effects, were found to differ between native and microcrystalline celluloses [5].

When cellulose thin layers or papers are developed with aqueous solutions of cyclodextrins, good separations of enantiomers [6] and diazo dyes [7] were obtained. In these systems we thus have a stationary and a mobile carbohydrate phase and solute molecules interact with both phases in a similar manner.

To our knowledge, so far there have been no studies of adsorption on cellulose with carbohydrates other than cyclodextrins in the eluent. Adducts between saccharides and other compounds have been reported. Weichert [8] obtained a sucrose–tryptophan adduct that exhibited chiral specificity. We therefore felt that it might be rewarding to study a number of carbohydrates dissolved in an aqueous mobile phase.

In this work, we concentrated on the behaviour of substituted tryptophans and on diazo dyes similar to methyl orange (for structures of the azo dyes and the pH ranges for which they are used, see Ref. [7]), as both give substantial effects with α-cyclodextrin [6,7]. It is possible

* Corresponding author.

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that these two compound groups are not representative, but at least we could use them for a comparison with α-cyclodextrin, with which they exhibit very marked effects.

2. Experimental

Standard ascending paper and thin-layer chromatographic techniques were employed.

The starches were obtained from Fluka (Buchs, Switzerland), except for Amidon soluble pour analyses, lot 86022, which was supplied by Prolabo (Paris, France). Dextran samples were obtained from Fluka.

Tryptophans were detected either with 1% ninhydrin in acetone or with iodine vapour. No reagents were needed for the azo dyes.

3. Results

3.1. Sucrose in the eluent

Weichert [8] reported adducts with sucrose obtained by evaporation, i.e., at very high sucrose concentrations. In this work, no effect was observed when 8% sucrose in 1 *M* NaCl was used as the eluent for diazo dyes, as shown in Fig. 1. No changes in enantiomeric separations were noted when sucrose was added to the eluent in the separation of substituted tryptophans. Perhaps one reason for the lack of effects is the high molecular mass of sucrose (342.30). An 8% solution is thus only 0.23 *M*. Higher concentrations tend to give very viscous solutions, which are impractical as eluents.

3.2. Starch in the eluent

We examined mainly two soluble starches, namely Amidon soluble pour analyses, Prolabo lot 86022, and Starch from potatoes, Fluka lot 85642. Both yield clear solutions when dissolved in 1 *M* NaCl.

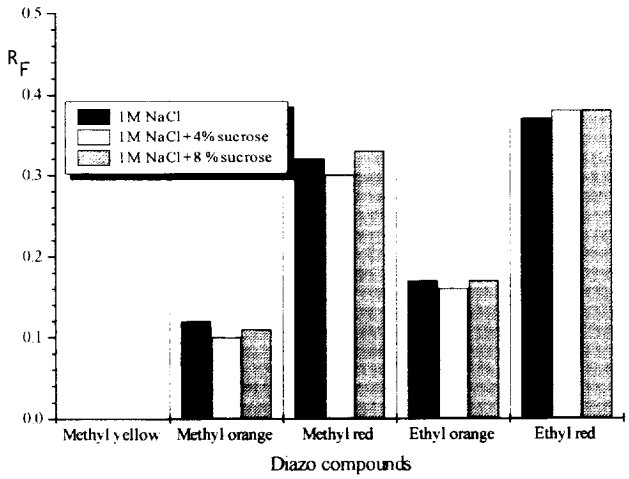

Fig. 1. Effects of different concentrations of sucrose in the eluent on the R_F values of azo dyes on Merck No. 5577 microcrystalline cellulose thin layers.

Substituted enantiomeric tryptophans give a measurable increase in R_F differences with 8% Amidon, and this seemed greatest with tryptophans substituted in the 4-position (4-methyl and 4-fluoro). The differences were about 0.1, but the effect is usually less than that with the same concentrations of α-cyclodextrin [7].

On microcrystalline cellulose plates, there is a considerable effect when methyl orange and related diazo compounds are chromatographed in the presence of starch (Fig. 2). Similar results were obtained on Whatman 3MM paper. This behaviour is different from that obtained earlier with α-cyclodextrin [7]: α-cyclodextrin interacts also at acidic pH (0.5 *M* HCl), whereas starch does not (Fig. 2a); α-cyclodextrin does not interact with methyl red and ethyl red [7], whereas starch does (fig. 2b and c); and the R_F shifts are less with starch than with α-cyclodextrin but they give linear R_M versus log[starch] plots (Fig. 3), which is not the case with α-cyclodextrin.

Complexes between azo dyes and amylose have already been studied by spectral methods by Sensse and Cramer [9] and it was suggested that the addition could be explained by the helical structure of amylose. In soluble starch, however, there are also branched structures of

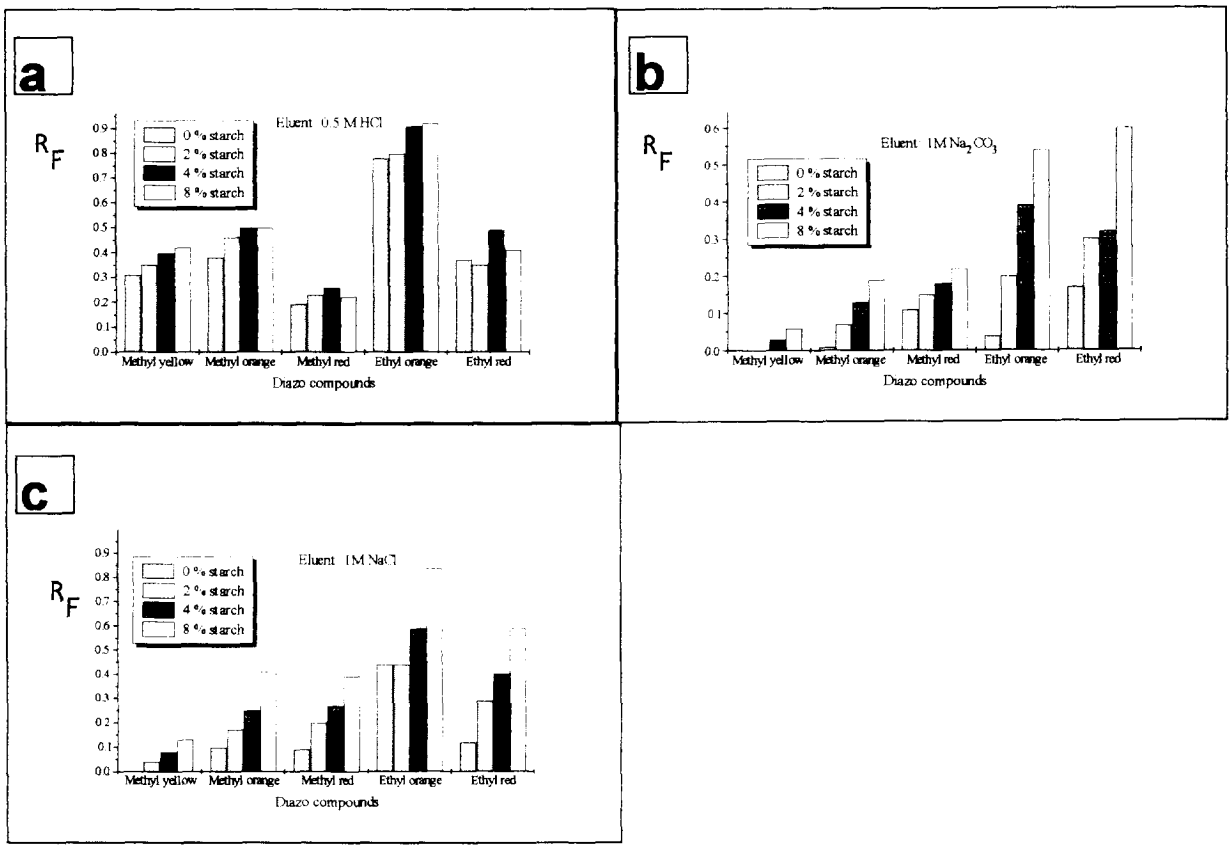

Fig. 2. Effects of starch in the eluent on the R_F values of some azo dyes on Merck No. 5577 microcrystalline cellulose thin layers with eluents (a) 0.5 *M* HCl, (b) 1 *M* Na_2CO_3 and (c) 1 *M* NaCl.

the amylopectin, which may also have a similar action. Table 1 shows a comparison of potato starch with wheat and maize starch. The latter two are only poorly soluble (less than 1%) and saturated solutions were prepared. It can be seen that no great difference exists between them once concentrations are taken into account.

3.3. Dextrans in the eluent

Dextrans are linear α-1–6-linked D-glucose polymers of bacterial origin and are available in a range of molecular mass fractions. Unlike starch, there are no R_F changes worth discussing either in enantiomeric separations of substituted tryptophans or in the separation of diazo dyes.

3.4. Differences between microcrystalline cellulose thin layers and Whatman 3MM paper

In this work we noted for azo dyes that when 1 *M* NaCl is used as the eluent different sequences are obtained with microcrystalline cellulose and Whatman 3MM paper, whereas in all other systems that we have investigated [5–7] there is little difference between these two supports.

The problem is illustrated in Table 2 (the values in italics indicate R_F differences >0.10). Here we compare also R_F values in alkaline and acidic media, which show the expected agreement. Whatman 3MM paper must be assumed to be rich in calcium and even to contain $CaCO_3$ on the surface owing to its manufacture with very hard water in the UK. For this reason, Whatman

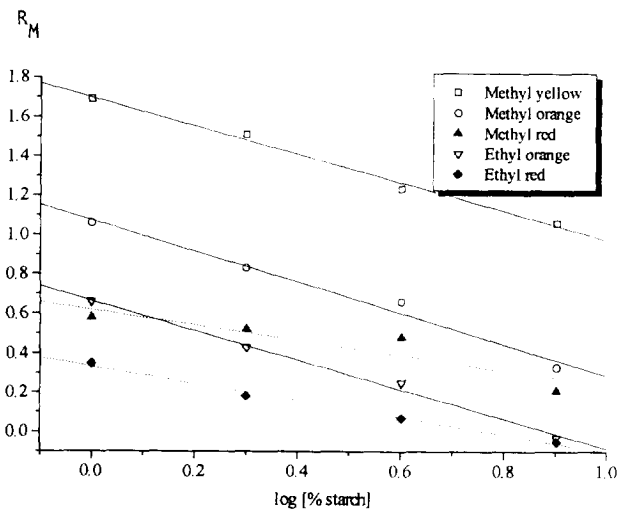

Fig. 3. Relationship between R_M values and percentage of added potato starch for some azo dyes chromatographed on Whatman 3MM paper using 1 *M* NaCl as eluent.

papers must be washed with acid for separations involving phosphate (see the last column in Table 2). Chromatography on acid-washed papers was tried and the same sequence as on the thin layer was obtained (Table 2). In presence of 8% of starch essentially the same chromatogram was obtained as on unwashed paper.

4. Discussion

After observing substantial R_F shifts when α-cyclodextrin was added to aqueous solvents in adsorption chromatography on cellulose [6,7], we wanted to investigate whether other polysaccharides also exhibited similar behaviour. Sucrose and linear dextrans did not produce any R_F shifts with substituted tryptophan enantiomers or with azo dyes whereas soluble starch showed measurable effects. However, the interactions seem to be different from those observed with cyclodextrins, as they do not occur in acidic medium and they seem to be less discriminatory with respect to molecular shapes. We did not observe essential differences between starches of different origins.

Table 1
R_F values of azo dyes on Whatman 3MM paper developed with 1 *M* NaCl containing different starches

Compound	Eluent: 1 *M* NaCl		
	+1% potato starch	Saturated with wheat starch	Saturated with maize starch
Methyl yellow	0.02	0.02	0.02
Methyl orange	0.17	0.09	0.09
Methyl red	0.36	0.31	0.32
Ethyl orange	0.30	0.16	0.18
Ethyl red	0.49	0.38	0.40

Table 2
Comparison of R_F values on Merck No. 5577 microcrystalline cellulose thin layers (TL) and Whatman 3MM paper with different eluents

Compound	0.5 *M* HCl		1 *M* NaCl		1 *M* Na_2CO_3		1 *M* NaCl
	Whatman 3MM	Merck TL	Whatman 3MM	Merck TL	Whatman 3MM	Merck TL	Acid-washed Whatman 3MM
Methyl orange	0.59	0.50	0.12	0.10	0.03	0.01	0.20
Methyl red	0.30	0.25	*0.32*	*0.08*	0.17	0.11	0.07
Ethyl orange	0.78	0.78	*0.17*	*0.43*	0.05	0.04	0.67
Ethyl red	0.44	0.37	*0.37*	*0.12*	0.24	0.17	0.12

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