ELSEVIER

Journal of Chromatography A, 695 (1995) 155–159

JOURNAL OF CHROMATOGRAPHY A

Short communication

Adsorption chromatography on cellulose XII. General effects of aqueous solutions of α-cyclodextrin as eluent

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

First received 25 March 1994; revised manuscript received 28 November 1994; accepted 2 December 1994

Abstract

Adsorption chromatography on cellulose was examined for compounds that form complexes with cyclodextrins using aqueous solutions of α-cyclodextrin as eluent. Interesting effects were observed with methyl orange and similar diazo dyes and with aromatic nitro compounds.

1. Introduction

In a previous paper in this series we described the improvement of chiral separations of substituted tryptophans when aqueous solutions of α-cyclodextrin solutions were used as eluents [1]. R_F differences of 0.21 could be obtained in some instances whereas without α-cyclodextrin the R_F differences were 0.06–0.10. It was also noted that some substituted tryptophans increased their R_F values considerably in the presence of α-cyclodextrin, e.g., L-6-methyltryptophan from 0.24 to 0.58, but others much less, e.g., L-4-fluorotryptophan from 0.30 to 0.44, although in both instances the improvement in the chiral separation was almost identical. We felt that a general survey of the effect of α-cyclodextrin in an aqueous eluent would be interesting and report our findings here.

A preliminary survey of the compound groups that separate well on cellulose with aqueous solvents was disappointing. A random selection of alkaloids (berberine), acridine dyes (acriflavine), inks of felt pens, mercurochrome and some pH indicators such as methylene blue, α-naphthyl red, methyl red, bromocresol green and eosine, all of which yield good chromatograms on cellulose, do not notably change their R_F values when α- or β-cyclodextrin is added as the eluent. When we considered compounds known to form complexes with cyclodextrins, we were more successful in finding interesting chromatographic behaviour. In this paper we describe results obtained with methyl orange and similar diazo compounds and with nitrophenols, all of which have been extensively studied [2].

2. Experimental

Standard paper and thin-layer chromatographic techniques were used as reported previously

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01193-1

[3]. The cyclodextrins were obtained from Fluka (Buchs, Switzerland).

3. Results

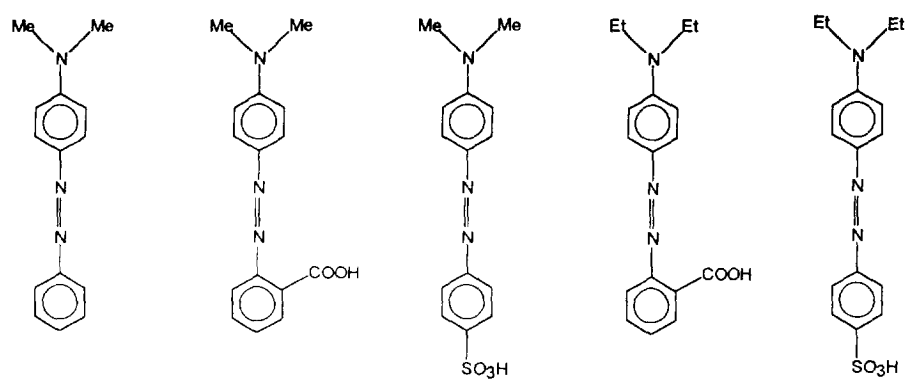
3.1. Methyl orange and similar diazo compounds

The α-cyclodextrin complex of methyl orange has been studied extensively and its crystal structure has been investigated [4].

Our first chromatograms showed a large increase in R_F value when α-cyclodextrin was added to the aqueous eluent. We therefore chromatographed a number of similar dyes (see formulae) together (the pH ranges given were those for which these compounds are used as indicators).

In chromatographing these compounds, not only the complexation with α-cyclodextin but also the degree of ionization must be taken into account. As shown in Table 1, pH values from 1 to 10 were examined. There remained the question of whether the anion of the buffer solution could play a role in so far as ion pairs could form between the cationic form of the compounds and the anions of the eluents.

We found there were only minor differences between 1 *M* $HClO_4$, 1 *M* HNO_3, 1 *M* HCl and 1*M* H_2SO_4, so that ion pairing could be considered of little importance. The R_F values in 2 *M* acetic acid were different, but it has a higher pH and exhibits considerable organic solvent prop-



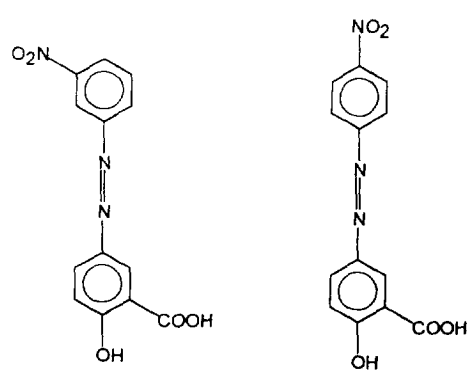


Table 1
R_F values of methyl orange and similar diazo compounds on microcrystalline cellulose thin layers (Merck No. 5577) with aqueous eluents in presence and in absence of α-cyclodextrin

Compound	0.5 *M* HCl (pH ≈ 0)	0.5 *M* HCl + 1% α-CD	1 *M* NaOAc + 1 *M* HOAc (pH ≈ 4.5)	1 *M* NaOAc + 1 *M* HOAc + 1% α-CD	1 *M* Na_2CO_3 (pH ≈ 10)	1 *M* Na_2CO_3 + 1% α-CD
Methyl yellow	0.31	*0.73*	0.11	*0.22*	0	*0.40*
Methyl red	0.19	0.17	0	0.09	0.11	0.14
Methyl orange	0.38	*0.77*	0.10	*0.43*	0.01	*0.72*
Ethyl orange	0.78	*0.97*	0.35	*0.69*	0.04	*0.73*
Ethyl red	0.37	0.35	0.26	0.28	0.17	0.23
Alizarin yellow 2G	0	0	0.08	*0.19*	0.01	*0.51*
Alizarin yellow R	0	*0.22*	0.03	*0.10*	0.01	*0.43*

erties. Therefore it cannot be compared with the other acids.

In Table 1, important R_F increases (≥0.10) on addition of α-cyclodextrin to the solvent are indicated in italics. Methyl red and ethyl red with a carboxylic group in the *ortho* position do not seem to interact with α-cyclodextrin. Ethyl orange, although much less adsorbed than methyl orange, shows very strong desorption in the presence of α-cyclodextrin. Alizarin yellow 2G and alizarin yellow R show the greatest effects in alkaline medium. The sulphonic group on methyl orange and ethyl orange is not of importance for complex formation, as shown by the fact that methyl yellow is desorbed fairly well in presence of α-cyclodextrin.

With this group of compounds we have the largest effects due to α-cyclodextrin so far encountered, and although most of them have both an acidic and an amino group, the effect is present in both acidic and alkaline media.

The effect of the α-cyclodextrin concentration was examined for 1 *M* NaCl as eluent, as shown in Table 2, and with 0.5 *M* HCl, as shown in Fig. 1. Fig. 1 shows that the plot of R_F versus concentration of α-cyclodextrin is an S-shaped curve. However, methyl orange and methyl yellow give parallel curves. Without data on the activities of the various constituents, it is difficult to draw conclusions. A methyl orange–α-cyclodextrin complex of ratio 1 : 2 has been characterized [4]. The S-shaped curve suggests the transition from a 1 : 1 to a 1 : 2 complex in solution. Methyl yellow exhibits behaviour very similar to that of methyl orange. In both compounds the remarkably high R_F increase due to α-cyclodextrin may be due to the formation of a very stable 1 : 2 complex.

Table 2
R_F values of methyl orange and similar diazo compounds on microcrystalline cellulose thin layers (Merck No. 5577) with 1 *M* NaCl as eluent and various concentrations of α-cyclodextrins

Compound	0% α-CD	1% α-CD	2% α-CD	4% α-CD	1β-CD
Methyl yellow	0	0.66	0.66	0.73	0
Methyl red	0.08	0.08	0.09	0.08	0.12
Methyl orange	0.10	0.90	0.75	At solvent front	0.60
Ethyl orange	0.44	0.86	0.87	At solvent front	0.72
Ethyl red	0.12	0.11	0.11	0.23	0.38
Alizarin yellow 2G	0.03	0.52	0.57	0.77	0.24
Alizarin yellow R	0	0.38	0.48	0.65	0.11

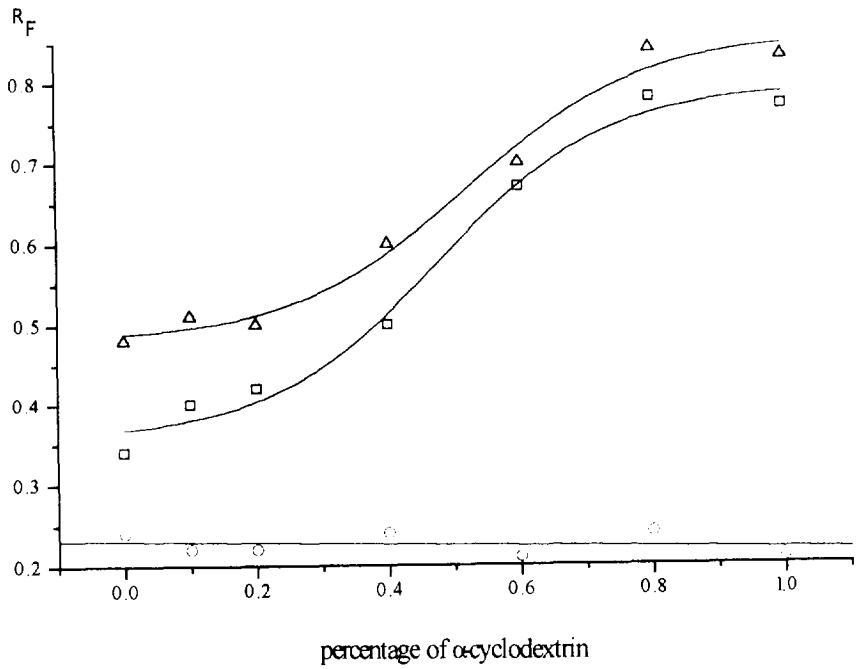


Fig. 1. Graph of R_F values of (□) methyl yellow, (○) methyl red and (△) methyl orange plotted against concentration of α-cyclodextrin in the eluent on Merck No. 5577 microcrystalline cellulose thin layers with 0.5 *M* HCl as eluent.

In considering the results in Table 2 and Fig. 1, the movement of the azo dyes depends on their adsorption on cellulose and their complexation with cyclodextrin. The forces involved in both interactions are believed to be mainly hydrophobic interactions and hydrogen bonds.

In the case of α-cyclodextrin, methyl yellow, methyl orange, ethyl orange, alizarin yellow 2G and alizarin yellow R all seem to favour complexation over adsorption on cellulose whereas methyl red and ethyl red favour adsorption on cellulose. When eluted with β-cyclodextrin the situation is not the same: methyl yellow is not eluted, ethyl red is more eluted than with α-cyclodextrin and alizarin yellow 2G and alizarin yellow R are not as strongly eluted. The structure of the cyclodextrin seems to play an important role.

3.2. Nitrophenols

Complexes between *p*-nitrophenol in its non-ionized and ionized forms and α-cyclodextrin have been characterized and the dissociation constants measured [2]. Here 2-, 3- and 4-nitrophenol, 2,4-dinitrophenol and picric acid were examined. These compounds give intermediate R_F values in various eluents (acidic, neutral and alkaline) on cellulose and their R_F values increase when α-cyclodextrin is added as the eluent. Care has to be taken with 2-nitrophenol: as it is fairly volatile it can be lost from the chromatogram.

There is an increase in the R_F values of all the compounds examined when α-cyclodextrin is added (Table 3). Comparison with the results obtained with methyl orange and other diazo compounds seems to suggest that all of them react with α-cyclodextrin, irrespective of the pH of the solution. Several methyl-*p*-nitrophenols

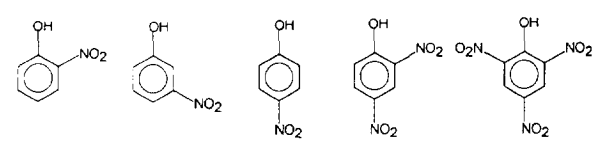


Table 3
R_F values of nitrophenols on microcrystalline cellulose thin layers (Merck No. 5577) with acidic, neutral and alkaline eluents containing α-cyclodextrin

Nitrophenol	1 *M* Na_2CO_3	1 *M* Na_2CO_3 +4% α-CD	40% sat. $(NH_4)_2SO_4$	40% sat. $(NH_4)_2SO_4$ +5% α-CD	1 *M* H_2SO_4	1 *M* H_2SO_4 + 4% α-CD
2-Nitrophenol	0.60	0.69	0.38	0.75	0.54	0.80
3-Nitrophenol	0.53	0.68	0.38	0.59	0.51	0.64
4-Nitrophenol	0.43	At solvent front	0.38	0.73	0.51	0.70
2,4-Dinitrophenol	0.33	0.78	0.25	0.71	0.51	0.59
Picric acid	0.33	0.53	0.24	0.53	0.44	0.55

have been reported to form complexes [2], so it is not surprising that the compounds listed in Table 3 also show R_F increases when α-cyclodextrin is added to the eluent.

4. Conclusions

Many compounds do not change their behaviour when α- or β-cyclodextrin is added to the eluent. Substantial effects could be obtained with compounds known to form inclusion complexes such as methyl orange and similar diazo compounds and nitrophenols. Increases in R_F values (in some instances from 0.1 to 1.0) could be obtained and were usually observed with neutral, acidic and alkaline solvents. This effect could provide an interesting diagnostic method for detecting cyclodextrin inclusion compounds. However, its limitation is that it could only be used for compounds that adsorb on cellulose.

References

[1] T.K.X. Huynh and M. Lederer, *J. Chromatogr.* 659 (1994) 191.
[2] M.L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, 1978; and references cited therein.
[3] T.K.X. Huynh and M. Lederer, *J. Chromatogr.*, 645 (1993) 185.
[4] K. Harata, *Bull. Chem. Soc. Jpn.* 49 (1976) 1493.