Journal of Chromatography, 520 (1990) 287–293
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1683

Spacer-displacement and carrier spacer-displacement thin-layer chromatography

HUBA KALÁSZ*,[a]
Department of Pharmacology, Semmelweis University of Medicine, Budapest (Hungary)
MÁRIA BÁTHORI
Department of Pharmacognosy, Szent-Györgyi Albert Medical University, Szeged (Hungary)
and
BÉLA MATKOVICS
Department of Biochemistry, József Attila University, Szeged (Hungary)

ABSTRACT

Separations obtained by spacer-displacement and carrier-spacer displacement thin-layer chromatography were compared. Examples are given for preparative carrier spacer-displacement thin-layer chromatography and for using various stationary phases. The separation of a parent drug and its radiolysis product was improved by increasing the amount of spacer.

INTRODUCTION

Whereas elution chromatography is generally based on differences in the migration rates of the components of the sample in the eluent, displacement chromatography assumes that the sample components do not migrate in the carrier (or that their movement in the carrier through the stationary phase bed is very limited) but the components of the sample are adsorbed on the stationary phase. It is the displacer which pushes the components forward, and all components of the sample migrate in front of the displacer front. During migration, there is a competition for the binding sites of the stationary phase. The more strongly adsorbed components displace the less strongly adsorbed components from the column or plate, and the less strongly adsorbed components migrate faster. In a fully developed displacement train, the "isotachic" migration of each band is characteristic. The concentration of sample components in the displaced bands and their concentrations are determined by the crossing of their adsorption isotherms and the operating line.

The displacement mode of development has been widely used to separate biologically active compounds, *e.g.*, amino acids, peptides and fatty acids [1]. High-performance displacement chromatography was introduced by Horváth and co-workers [2–8]. Papers on the theory and application of displacement chromatography

[a] Present address: Department of Pharmacology and Cell Biophysics, University of Cincinnati, 231 Bethesda Avenue, Cincinnati, OH 45267-0575, U.S.A.

0021-9673/90/$03.50

described the basis of self-sharpening fronts [9], the dependence of displacement profile on the conditions of chromatography [10] and practical examples [2,11].

In thin-layer displacement chromatography (TLDC), the well concentrated spots are situated very close to each other, which hampers qualitative and quantitative evaluation of the chromatogram. An essential requirement is to separate the spots from each other, and the inclusion of a series of spacers solves this problem. Application of Sudan Black dye components serves to facilitate detection and separation. In an investigation of the metabolism of phenylalkyl compounds, spacer-displacement thin-layer chromatography (SD-TLC) has been demonstrated to be a useful tool [12–16].

A variation of SD-TLC was called carrier-displacement chromatography. In the case of planar chromatography, the expression "carrier-spacer displacement thin-layer chromatography (CS-DTLC)" characterizes the phenomenon [14,15] fairly accurately.

Preparative applications of either displacement TLC or of spacer-carrier displacement chromatography have not previously been reported.

EXPERIMENTAL

Materials

(−)-Deprenyl, V-111, J-508, amphetamine and methamphetamine were kindly given by Chinoin Pharmaceutical and Chemical Works (Budapest, Hungary). Compound EGYT-475 was supplied by EGIS Pharmaceutical Factory (Budapest, Hungary). Cyclopropylazidomorphine, 3-deoxydihydromorphine, azidomorphine and norazidoethylmorphine were a gift from Alkaloida Pharmaceutical Factory (Tiszavasvári, Budapest, Hungary). The structures of these compounds were given in a previous publication [13]. Test Substance II was obtained from Camag (Muttenz, Schwitzerland); in our earlier experiments, numerous components of Sudan Black were detected in it [12].

The TLC plates used were either silica gel 60 F_{254}, precoated on 200 × 200 mm glass plates, PSC silica preparative layer precoated on 200 × 200 mm glass plates or TLC alumina precoated on 200 × 200 mm glass plates. TLC plates were purchased from E. Merck (Darmstadt, F.R.G.).

The solvents used for extraction and TLC were of the purest grade possible.

Samplex tubes (BioSeparation Technologies, Budapest, Hungary) containing C_{18} silica were used for the removal of the possible contaminating spacer, carrier-spacer and the displacer from the purified sample components.

X-Ray film, 300 mm × 300 mm in size, was purchased from Agfa Gevaert (F.R.G.).

All-glass TLC chambers (Desaga, Heidelberg, F.R.G.) were used for the development of TLC plates.

Methods

The drug MACE was radiolabelled with ^{14}C. Rats were treated subcutaneously with one dose of 10 mg/kg and their urine was collected for 24 h. Urine samples were spotted after their acidic hydrolysis (boiling for 1 h in 1.0 *M* hydrochloric acid) and adjustment to pH 7.0, but without any prepurification.

Stationary phase was scraped from the PSC silica preparative layer, stirred with ethanol–water (9:1, v/v), filtered and dried. The residue was dissolved in a small portion of ethanol and the solution was subjected to analysis.

The solvent systems used for TLC were (1) chloroform–triethanolamine (95:5, v/v) and (2) chloroform–triethanolamine (95:5, v/v) + deprenyl (1 g per 100 ml).

Radiolabelled components were detected by using X-ray film with an exposure time of 2 days or by using a TLC Radioscanner (Berthold, Wildbad, F.R.G.).

RESULTS

The equations that have served for the calculation of efficiency and resolution in elution-type developments need to be modified to the requirements of displacement chromatography. The resolution (R_s), yield (Y), loss (L) and efficiency (E) can be characterized by the following equations [13,14]:

$$R_s = \frac{t_a - t_c}{w_a + w_c} \tag{1}$$

$$Y = \frac{W}{w} \tag{2}$$

$$L = \frac{w - W}{w} \tag{3}$$

$$E = \frac{h}{w - W} \tag{4}$$

where t_a, t_c and h are the times required for the appearance of the centre of the displaced zones of components a and c, w is the mean peak width, W is the peak width at the top and h is the height of the trapezoidal form of the displaced band.

Some drugs were subjected to TLC using various types of displacement mode of development. Fig. 1A shows the well defined zones of amphetamine, V-111, methamphetamine, deprenyl, azidomorphine, EGYT-475 and J-508. The coloured bands of spacer improved the direct observation even during the separation process and in the evaluation of separations. At the same time, the compounds belong to the category "displaced by the displacer". Fig. 1B, C and D indicate that additional zones of (−)-deprenyl and V-111 define the subclasses: subclass 1 was not displaced either by V-111 or deprenyl; subclass 2 was not displaced by V-111 but was displaced by deprenyl; and subclass 3 was displaced by both V-111 and deprenyl.

Metabolites of a radiolabelled drug (MACE) were purified by using thin-layer carrier-spacer displacement chromatography on a preparative silica layer. The separated bands (together with the silica) were scraped from the glass support plate and the extracts were also investigated by TLC. The well separated spots confirmed the effective separations (Fig. 2).

Using various stationary phases, the order of the components of the displacement train could be changed. This phenomenon is demonstrated in Fig. 3, which shows

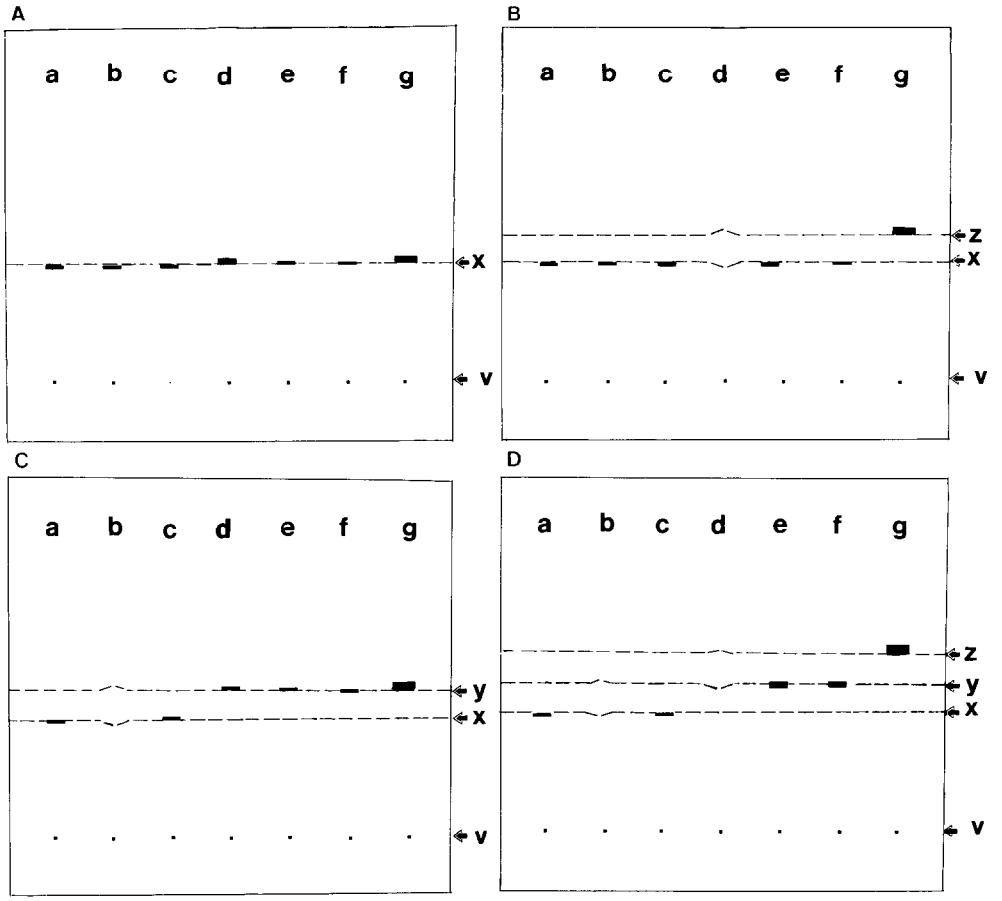

Fig. 1. (A) Displacement thin-layer chromatogram of (a) amphetamine, (b) V-111 (*p*-bromomethamphetamine), (c) methamphetamine, (d) (−)-deprenyl, (e) EGYT-475, (f) azidomorphine and (g) J-508 using silica stationary phase and solvent system No. 1 as mobile phase. At the start (v), Camag Test Dye Mixture was spotted; the position of the displacer front is indicated by x. (B) Carrier spacer displacement chromatography of the same compounds using solvent system 2. Camag Test Dye Mixture was applied as above; z indicates the front of the carrier-spacer (deprenyl). (C) Spacer displacement chromatography of the same compounds using solvent system 1. V-111 spacer (2 mg) was spotted at the start together with the Camag Test Dye Mixture; y indicates the front of the spacer (V-111). (D) Carrier-spacer displacement chromatography of compounds using solvent system 2 and V-111 spacer (2 mg). The solution of V-111 was spotted at the start together with the Camag Test Dye Mixture; x indicates the front of the displacer. The fronts of the carrier-spacer (deprenyl) and spacer (V-111) are indicated by y and z, respectively.

how the order of components was chaged in the displacement train when alumina instead of silica as the stationary phase was used.

An outdated sample of radiolabelled MACE (1 mg sample in each experiment) was subjected to displacement chromatography using a spacer present in various amounts. The by-product of radiolysis of the drug was further and further removed from that of the parent drug as the amount of the spacer was increased (Table I).

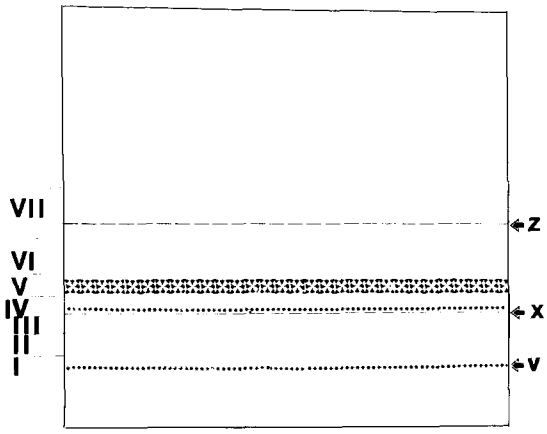

Fig. 2. Preparative displacement TLC of 2 ml of rat urine containing the metabolites of MACE. Mobile phase and spacer as in Fig. 1C. The radiolabelled spots were visualized by autoradiography. Zones were scraped as marked.

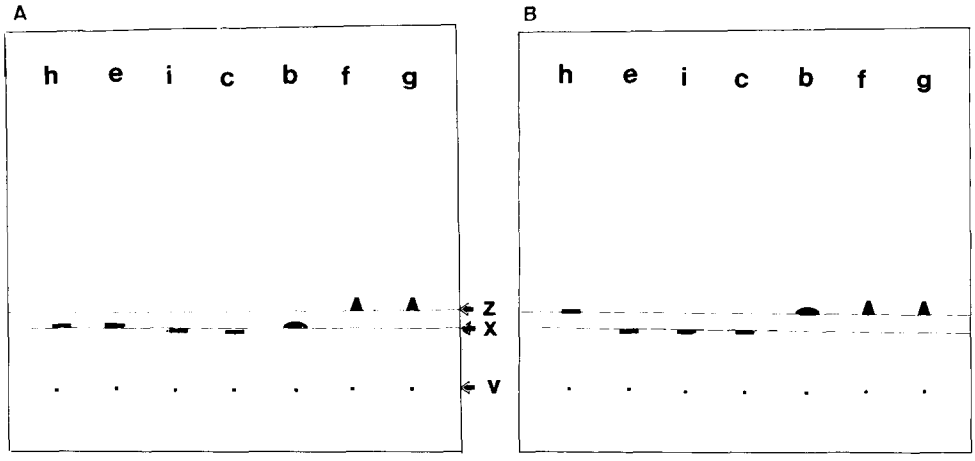

Fig. 3. (A) Spacer displacement chromatography of compounds as in Fig. 1 using silica stationary phase and solvent system 1. Cyclopropylazidomorphine spacer (1.5 mg) was spotted at the start together with the Camag Test Dye Mixture; x indicates the front of the spacer and h and i indicate 3-deoxydihydromorphine and norazidoethylmorphine, respectively. (B) Spacer displacement chromatography of the same compounds using alumina stationary phase and solvent system 1. Other conditions as in (A).

TABLE I

EFFECT OF AN INCREASE IN THE AMOUNT OF SPACER

Amount of spacer (mg)	R_s	Y	L	E
0.0	1.0	0.5	0.5	1.0
0.1	1.5	0.9	0.1	9.0
1.0	4.0	1.0	0.0	

DISCUSSION

The zones of the separated sample components displace each other, so the displaced components are very closely situated to each other. By the use of one (or a series) of easily removable substances in the displacement train, neither of which gives any signal during detection, the detection of the substances and also the resolutions and yields will be improved.

Fig. 1 shows the increase in the resolution of several compounds. With displacement chromatography (Fig. 1A), the tiny bands of Sudan Black improved the visual observation of the displaced components. When deprenyl was applied either as a spacer or as part of the carrier–spacer–displacer system (Fig. 1B), not only the visualization but also differentiation were facilitated. Similar results were obtained by using V-111 as spacer (Fig. 1C). However, other bands became situated before and behind the band of the spacer. The most successful displacement separation was achieved when both a spacer (V-111) and a carrier-spacer (deprenyl) were employed (Fig. 1D).

Preparative TLC using the displacement mode of development made it possible to spot 2 ml of rat urine containing the metabolites of MACE, and the metabolites of the radiolabelled drug were visualized by autoradiography using X-ray film (Fig. 2). The scraped-off zones contained the various radiolabelled metabolites. Their homogeneity (with respect to the radiolabelled components) was demonstrated by their TLC analysis. The removal of the possibly contaminating spacer, carrier-spacer or displacer can preferably be done by extraction using the SAMPLEX procedure, which will be published separately [16].

In the separation of various components on either silica or alumina, the order of substances and spacer can be varied. Hence displacement chromatography on various stationary phases can serve both analytical and preparative purposes (Fig. 3).

The effect of an increase in the amount of spacer is given by the numerical results (calculated on the basis of eqns. 1–4) given in Table I.

ACKNOWLEDGEMENT

This work was financially supported by the grant from the Hungarian Academy of Sciences, OTKA-SzEüMin. No. 1892.

REFERENCES

1 A. Tiselius, *Ark. Kemi Mineral. Geol.*, 16A (1943) 1.
2 Cs. Horváth, A. Nahum and J. F. Frenz, *J. Chromatogr.*, 218 (1981) 365.
3 H. Kalász and Cs. Horváth, *J. Chromatogr.*, 215 (1981) 295.
4 H. Kalász and Cs. Horváth, *J. Chromatogr.*, 238 (1982) 423.
5 Cs. Horváth, J. F. Frenz and Z. El Rassi, *J. Chromatogr.*, 255 (1983) 273.
6 Z. El Rassi and Cs. Horváth, *J. Chromatogr.*, 266 (1983) 319.
7 A. W. Lita, Z. El Rassi, D. M. LeMaster and Cs. Horváth, *Chromatographia*, 24 (1987) 881.
8 J. Frenz, P. Van der Schrieck and Cs. Horváth, *J. Chromatogr.*, 330 (1985) 1.
9 D. D. Frey, *J. Chromatogr.*, 409 (1987) 1.
10 M. W. Phillips, G. Subramanian and S. M. Cramer, *J. Chromatogr.*, 454 (1988) 1.
11 Cs. Horváth, in F. Bruner (Editor), *The Science of Chromatography* (*Journal of Chromatography Library*, Vol. 32), Elsevier, Amsterdam, 1985, p. 179.

12 H. Kalász, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1984) 39.
13 H. Kalász, *J. Liq. Chromatogr.*, 7 (1988) 1371.
14 H. Kalász, M. Báthori and J. Nagy, *Chromatographia*, 27 (1989) 253.
15 H. Kalász, J. Nagy and M. Báthori, *J. Planar Chromatogr.*, 2 (1989) 39.
16 H. Kalász and J. Varga, *Liquid Chromatography: New Developments*, Akadémiai Kiadó, Budapest, in press.