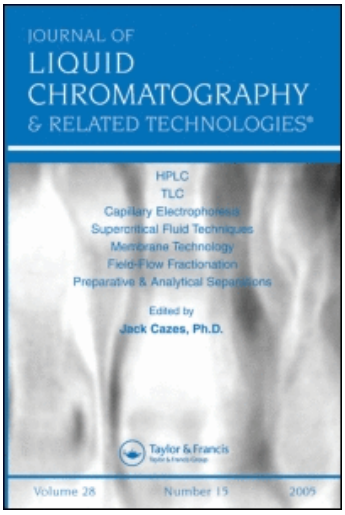

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THE ROLE OF SPACERS IN DISPLACEMENT THIN-LAYER CHROMATOGRAPHY

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Summary

Displacement chromatography generates highly concentrated bands which migrate closely to each other along the stationary phase bed.

Spacer-displacement thin-layer chromatography is a planar method improving the observable resolution by inserting odd compounds (spacers) among the members of displacement train to be separated.

Substances were chromatographed by displacement mode of development using silica plates, chloroform carrier and triethanolamine displacer.

Resolution formula valid for elution chromatography has been adapted to the displacement type of developments. Explanation for the numerical value of required separation is given for various cases of displacement thin-layer chromatography.

Equations are suggested in order to calculate yield, loss and efficiency of displacement chromatography.

Introduction

Displacement chromatography was used and discussed in details in several publications of Tiselius, Claesson, Porath, Holman, Partridge and others (1-5). However, the wide utilization of displacing equilibria in chromatography, for obtaining highly concentrated and definitely separated zones of the components has been playing only minor role for a long dormant period.

In 1981, Horváth et al (6) published a basic study on high-performance displacement chromatography (HPDC). They renewed numerous features of the classical displacement chromatography and also the exploitation of advantages given by the high performance stationary phases. Therefore, the transfer of the diluted sample components into highly concentrated displaced zones, utilization of the majority of the stationary phase bed by the displacement train and the effective separation of zones in a small volume (short length and small diameter) of the stationary phase column can give novel possibilities both in the analytical and preparative scale chromatography.

Practical applications of displacement mode of development for preparative scale separation of substances using columns and instrumentations generally employed for analytical purposes were thereby realized and the load of the chromatographic system in each separation cycle could be increased by 2 - 4 orders of magnitude (3-8). Horváth et al demonstrated the excellent separation power of high performance displacement chromatography for several substance groups as phenoloids (3), polypeptides (7), phenylalkyl amines (8), oligopeptides (9), nucleotides (9, 10), corticosteroids (11), polyethylene glycol oligomers (12) and various amines (13). Recent publication on displacement chromatography has been dealing with preparative scale isolation of oligomycine isomers (14).

Principles of displacement chromatography

Distinct peaks of the sample components move independently from each other in the case of elution

chromatography and even baseline separations can be generally arranged.

Essential constituents of displacement chromatography are the stationary phase, the carrier, the displacer (these latter two are employed instead of the eluent used in elution chromatography), the regenerant as well as the sample to be separated. The initial steps of the displacement chromatography are the equilibration of the stationary phase with the carrier and the introduction of sample into the column. All components of the sample should be adsorbed on the very fore part of the column and they are pushed forward by the displacer. The migration of each component zone in the fully developed displacement train can be characterized by the word "isotachic", this movement is determined by the velocity of the displacer front. The development of the displacement train is completed when the displacer front becomes part of the effluent and then the regeneration can be started. After having finished the supply of the regenerant and that of the carrier, the system is ready for the next cycle (5).

Numerous parameters of the displacement train, including the actual concentrations of the substances in the zones therefore also the migration speed of the zones in the displacement train can be calculated from the adsorbtion isotherms and from the actual concentration of the displacer according to Horváth et al (5). Highly concentrated zones (containing up to several ten mg/ml of the separated sample components) are present in the displacement train (5, 7, 11).

As the dominating interactions are the displacing equilibria between all neighbouring pairs of the system, there is no chance for baseline separation between any two components of a "bona-fide" displacement train if the mass distribution is considered.

Therefore, a basic question of the displacement chromatography has definitely been arisen, how these samples can be aparted from each other. One or a series of odd (spacer) compounds should be inserted between the

adequately displaced zones (16, 17) that the adsorbtion isotherm of the spacers should exist between that of the corresponding pair of substances to be separated (Fig. 1).

The method called spacer-displacement chromatography has resulted in the improvement of separation and/or detection of the neighbouring components (8, 15, 16, 17, 18).

Displacement thin-layer chromatography

Displacement thin-layer chromatography (DTLC) facilitated the optimization of conditions of HPDC. The planar arrangement of the stationary phase helped the scouting the adequate carrier, displacer and displacer concentration for preparative scale HPDC of corticosteroids.

The definite features of planar chromatography are as follows:

a. several samples can be separated on the same plate at the same time,
b. thin-layer plates are disposable, no regeneration is required,
c. specific and sensitive color reagents can be used for detection of the separated spots.
d. the separation procedure can be sometimes visually followed,
e. "contact" detection methods as bioautography or autoradiography can be preferably applied.

Spacer substances generally show diverge characteristics in comparison to the original members of the displacement train. Such spacer series were found in the Sudanblack components of the Test Substance II, where definite dark lines were surrounding the white (colorless) spots of the sample components to be separeted. In this case, a series of spacers improve both the separation and the detection of components of interest (16, 17).

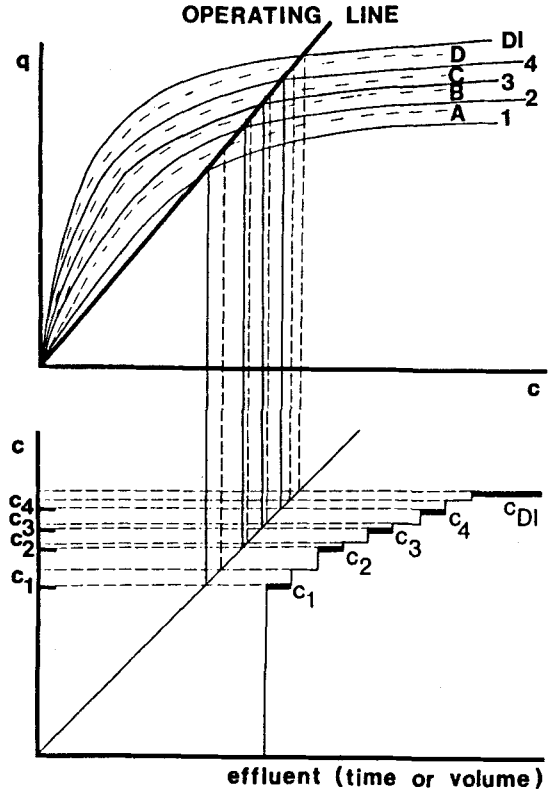
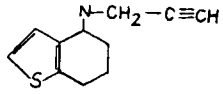


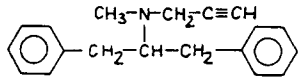
Figure 1.

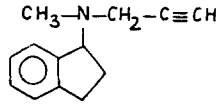
Modification of the displacement train by inserting the spacers. If the adsorbtion isotherm of the spacer is between that of the adequate pairs of the substances to be displaced, the step of the spacer will be also inserted between the displaced compounds.

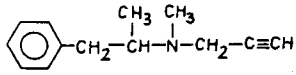
Abbreviations: q and c are the amounts on the stationary and in the mobile phase, respectively. 1, 2, 3, 4 are the sample components to be separated, A, B, C and D are the elements of the spacer series.

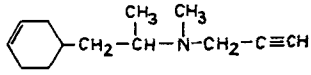
Table 1.
Chemical structures of substances used in the experiments.

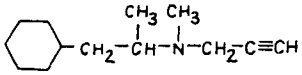
A 

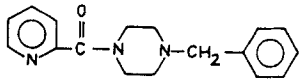
B 

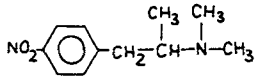
C 

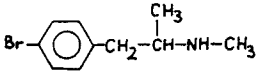
D 

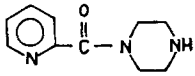
E 

F 

G 

H 

I 

J 

Experimental

All glass thin-layer chromatographic chambers with lids were purchased from Desaga (Heidelberg, F.R.G.).

TLC plates silica gel 60 F_{254}, 20x20 cm in size were obtained from E. Merck (Darmstadt, F.R.G). Triethanolamine, dichloromethane and chloroform were the products of Reanal (Budapest, Hungary). Test Mixture II. was kindly donated by Dr. Janchen (Camag Inc., Muttenz, Switzerland).

The chemical structures of substances displaced are depicted in Table 1.

Unsaturated chambers, that is the chambers with previous-equilibration but without filter paper strip for

the total saturation were used. The substances after their separation were detected either under UV light, or using Berthold scanner or by autoradiography.

Results

Constituents of the Camag Test Mixture II were separated by two directional development using chloroform (1st run) and dichloromethane (2nd run). About 20 components of Sudanblack were succesfully differentiated. All or some of these components can become the member(s) of the displacement train generated by triethanolamine in chloroform.

Compounds with a wide variety of their chemical structures were displaced by triethanolamine displacer in the chloroform carrier.

Thin-layer chromatogram series in Fig. 2 demonstrate that any member of the displacement train can serve as spacer between other components.

An outdated sample of a radiolabeled drug was investigated and its main byproduct was separated from the original drug using a spacer, which was phenylethylamine (Fig. 3).

Discussion

Resolution of elution chromatography has been widely discussed in basic text books on chromatography and one of its generally used form is as follows:

$$R_{s(elut.comp.a,b)} = \frac{2\,(t_b - t_a)}{w_b + w_a} \qquad \text{(eq. 1)}$$

where R_s, t and w are the resolution, time of elution of the peak and width of the peaks belonging to *a* and *b*, respectively. In some cases, the peak width is substituted with the half peak width or double of the standard deviation

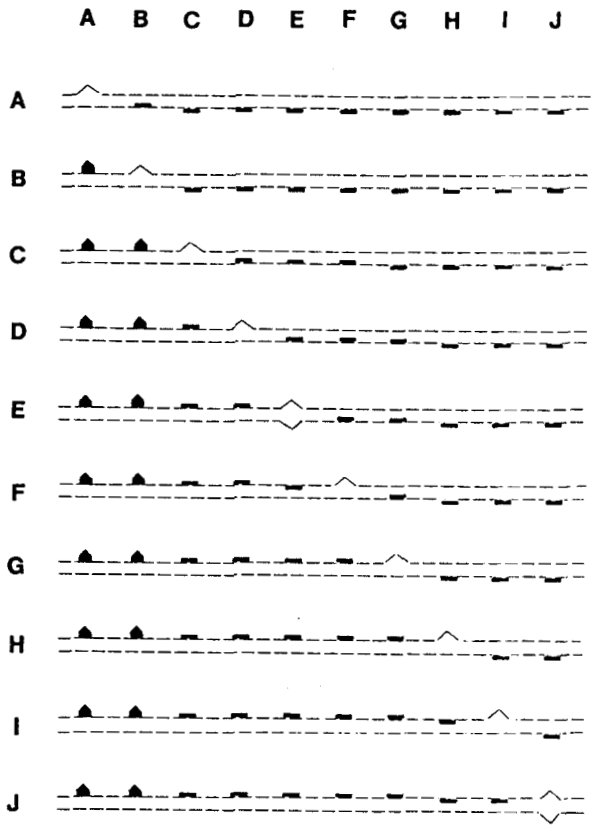


Figure 2.
Displacement trains which were constructed from A, B, C, D, E, F, G, H, I and J compounds spotted and one of them (as spacer) lined in the start.

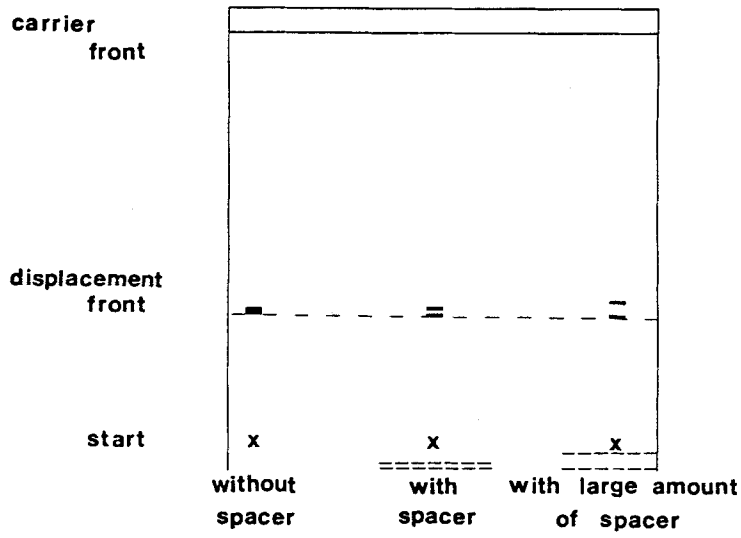


Figure 3.
Separation of two closely situated bands of the displacement train. Displacement thin-layer chromatography without spacer does not resulted in the observable differentiation of the components, the spacer facilitated the improvement of separation, and the large amount of spacer further increased the distance between the spots.

of the peak (distance between the two inflection points of the Gaussian type of peak). Naturally, in these cases the multiplication factor, 2 should be eliminated from equation 1.

After finding the procedure to improve the resolution of displacement mode of development, it is crucial to define the concept of resolution for displacement chromatography.

Interpretation of resolution for displacement chromatography can be very similar to that of the elution chromatography. However, the terms of elution time and peak

width have to be converted to the formal requirements of the displacement train. Therefore, elution time of the Gaussian peak should be replaced by the mean effluent time of the displacement trapezoid or displacement mean time (t_x) and half of the peak width should be substituted by trapezoid mean width (w_x).

$$R_{s\ (displ.comp.\ a,b)} = \frac{t_b - t_a}{w_a + w_b} \qquad \text{(eq. 2)}$$

where $R_{s(displ.)}$ t_a, t_b, w_a and w_b are the resolution between the displaced band pair, the mean effluent time of a component, the mean effluent time of b component, the mean width of a component and the mean width of b component, respectively (Fig. 4). In the case of a fully developed displacement train, the resolution between the well separated trapezoids is 0.5. Any further increase of resolution is limited by the bare fact that the displacing interaction between the individual trapezoids (or substance zones) is the essential procedure of displacement chromatography.

This is the point where any consideration concerning the analytical type of displacement chromatography - or displacement thin-layer chromatography for analytical purposes essentially diverges from the preparative scale displacement chromatography. At analytical separation, the mass distribution can differ from the detector signal, as it is depicted in Fig. 5. If one of the components of the displacement train does not reveal a characteristic which is the basis of the detection, this substance-band or substance-trapezoid will serve as spacer from the point of view of detection. Therefore, the resolution between the monitored components of the displacement train will be increased. Supposing the equality (or similarity) of the mean width of the trapezoids, the resolution will be 1.0 (Fig. 5, bottom).

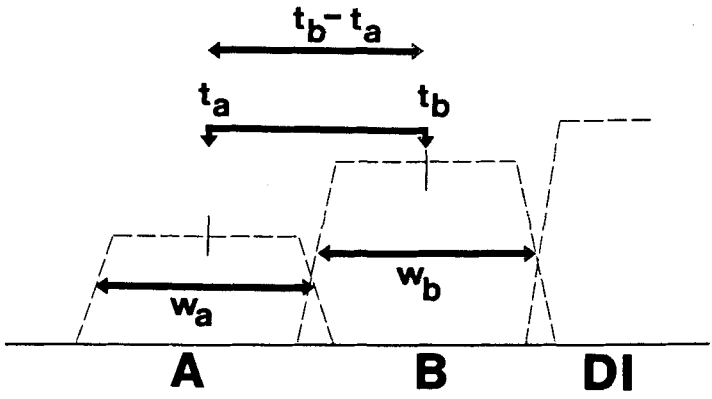


Figure 4.
Resolution of displacement separation can be calculated from the distance and widths of the displacement trapezoids, where the displacement mean times (t_a, t_b) and the trapezoid mean widths (w_a, w_b) have to be considered. A, B and DI are the 1st, the 2nd components to be separated and the displacer, respectively.

From technical point of view, the spacer(s) should be lined onto the plate (16, 17) as it was demonstrated in Fig. 3.

For visual observation of the one-dimensional thin-layer displacement chromatogram, this resolution about 1.0 seems to be far enough for reliable differentiation (Figs. 3 and 5). Additional improvement of resolution can be required when instrumental evaluation of the displacement thin-layer chromatogram needs a certain minimum of distance between the spots. The increase of the amount of the spacer spot will proportionally increase its width. Further increase of separation can be achieved by using two-dimensional (elution - spacer-displacement) thin-layer chromatography (16, 17).

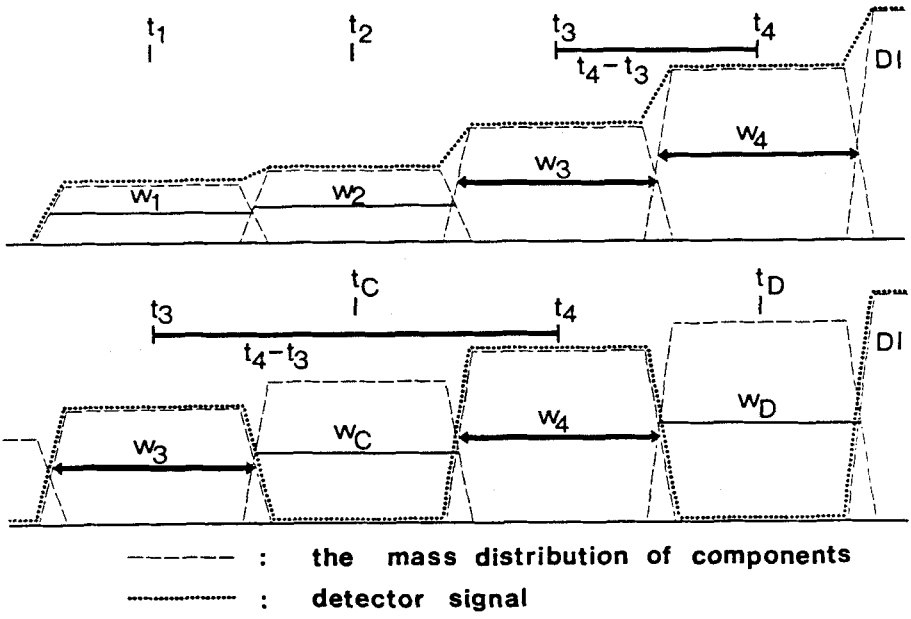


Figure 5.
Displacement (upper part of the figure) and spacer displacement (bottom part of the figure) chromatograms for analytical purpose.

Several other characteristics of the displacement type of development can be also calculated, as the yield (or efficacy) of the separation ($\underline{Y}$), the loss of the separation ($\underline{L}$) and efficiency ($\underline{E}$). The basic condition is that the overlapping part(s) of the trapezoid series of the displacement train is (are) the side-segment(s). Thereby, the real meaning of w and $\underline{w}$ are the width of the trapezoids of interest with and without overlapping the neighbouring trapezoids, respectively (Fig. 6).

$$Y = \frac{\underline{w} \times h}{w \times h} = \frac{\underline{w}}{w} \qquad \text{(eq. 3)}$$

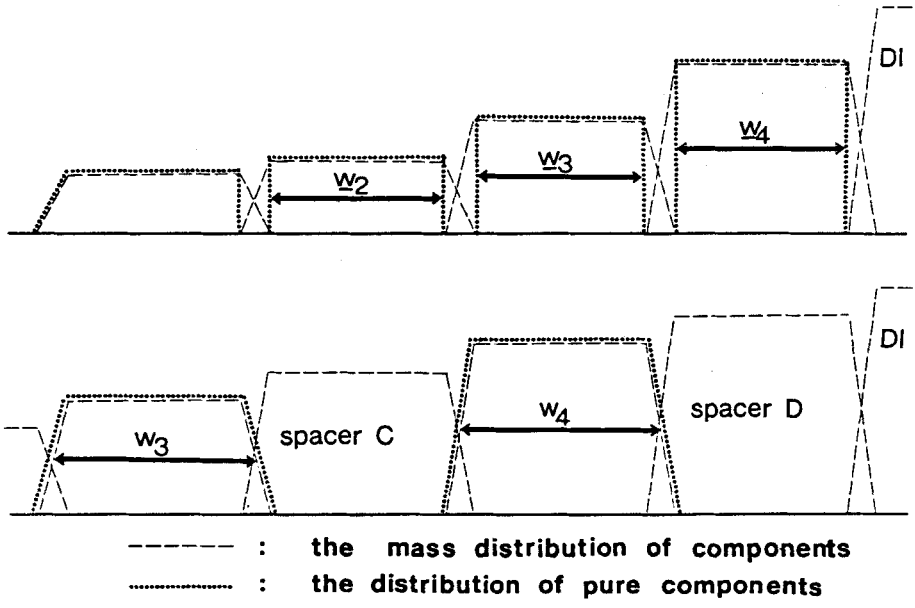


Figure 6.
Displacement (upper part of the figure) and spacer displacement (bottom part of the figure) chromatograms for preparative separation. From the trapezoid mean widths (w_3 and w_4) and trapezoid widths without contamination ($\underline{w}_3$ and $\underline{w}_4$) yield, loss and efficiency can be calculated for displacement and spacer displacement chromatography.

$$L = \frac{w \times h - \underline{w} \times h}{w \times h} = \frac{w - \underline{w}}{w} \qquad \text{(eq. 4)}$$

$$E = \frac{Y}{L} = \frac{\frac{\underline{w}}{w}}{\frac{w - \underline{w}}{w}} = \frac{\underline{w}}{w - \underline{w}} \qquad \text{(eq. 5)}$$

Using spacers for increase of the virtual separation, the situation changes basically, as there is not any overlapping part between the trapezoids following each other in the displacement train. Therefore, other meaning of the trapoezoid width at its top should be considered, that is, w is the trapezoid width without disturbance (or contamination) from the neighbouring substance trapezoid (Fig. 6).

In a well performed spacer displacement chromatogram, the yield will approximate 1.00, and the loss will converge 0.00.

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