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VACANT PEAKS IN LIQUID CHROMATOGRAPHY

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SUMMARY

The origin and properties of vacant chromatographic peaks that occur most often when a two-component mobile phase is used and that do not belong to any component of the mixture under analysis are described.

INTRODUCTION

Peaks are sometimes found on chromatograms in liquid chromatography that cannot be ascribed to any component of the mixture under analysis. This phenomenon appears most often when a two-component mobile phase and a non-selective detector, such as a refractometer, capacitance detector or micro-adsorption detector, are used. The above phenomenon has also appeared when an apparently single-component mobile phase was used that contained impurities or was saturated with a stationary liquid phase. It can similarly be expected that the phenomenon will also appear when a selective detector is used, for example with the use of a UV detector, assuming the mobile phase contains impurities that absorb energy at the frequency used in the detector. These peaks, which can have both positive and negative responses, appear most often at the beginning of the chromatogram in the elution time which is close to a dead elution time. These peaks have usually been ascribed to a further non-identified component in a number of instances and are indicated as an impurity in the sample. These peaks are often drawn incompletely on chromatograms and also possible overlapping with some of the peaks belonging to the mixture under analysis makes the interpretation of the chromatograms difficult.

In this work, correlations were investigated between the retentions of the compounds under analysis, the properties of a chromatographic system with a two-component mobile phase and sample size on the one hand, and the size and character of the peak that did not belong to the compound under analysis on the other hand. Typical chromatograms which were found are shown in Fig. 1. If a third compound (sample) is injected into a two-component mobile phase, a peak appears on the chromatogram in the elution time of the moderator (the mobile phase component that is adsorbed the more strongly). This vacant peak^{1,2} is caused by a local change in the concentration of the moderator in comparison with the equilibrium composition of the mobile phase. This change, depending on the retention of the sample, can mean either an increase or a decrease in the moderator concentration in the mobile phase.

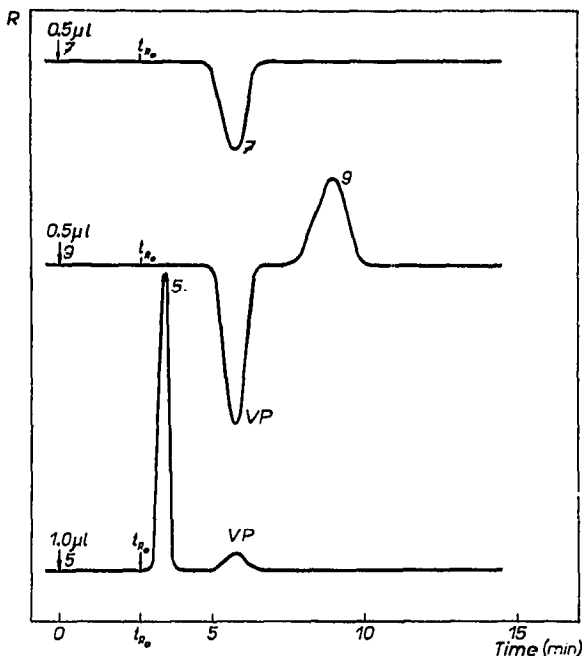


Fig. 1. Examples of the vacant peaks (VP). The numbers of the peaks correspond to the compounds listed in Table I. Mobile phase: cyclohexane + 2% diethyl ether. Detector: LDC Model 1103 refractometer.

EXPERIMENTAL AND RESULTS

A liquid chromatograph was used that consisted of a reservoir of the mobile phase, a pump (Orlita, Giessen, G.F.R.), a pressure pulse damping device according to Locke⁵, a pre-column packed with activated charcoal, a membrane sampling device and a stainless-steel column (500 × 1.8 mm). A differential refractometer of the Fresnel type (LDC Model 1103, Laboratory Data Control, Riviera Beach, Fla., U.S.A.) was used as a non-selective detector. The flow-rate of the mobile phase was measured by means of a graduated capillary and a stop-watch.

A two-component mobile phase, cyclohexane-diethyl ether, containing 0.1–2.0% (v/v) of diethyl ether, was selected for the studies. These two components differ considerably in their adsorption properties on the sorbent that was used and also their refractive indices are sufficiently different for even small changes in the composition of the mobile phase to be recorded.

Method

Samples of pure substances (listed in Table I) were injected into a stream of mobile phase of known composition on the chromatographic column. The dependence of the capacity ratio of the compounds under analysis and the vacant peak on the composition of the mobile phase was investigated at a constant sample size and flow-rate of mobile phase. The dependences found for some compounds are shown in Fig. 2. Further, the dependence of the area of the vacant peak on the size of the sample

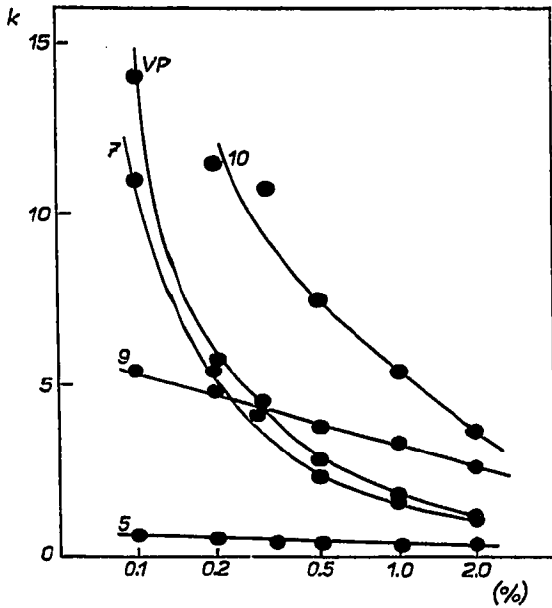


Fig. 2. Dependence of the capacity ratio of the compounds listed in Table I and the vacant peaks (VP) on the concentration of the moderator (diethyl ether) in the mobile phase (cyclohexane).

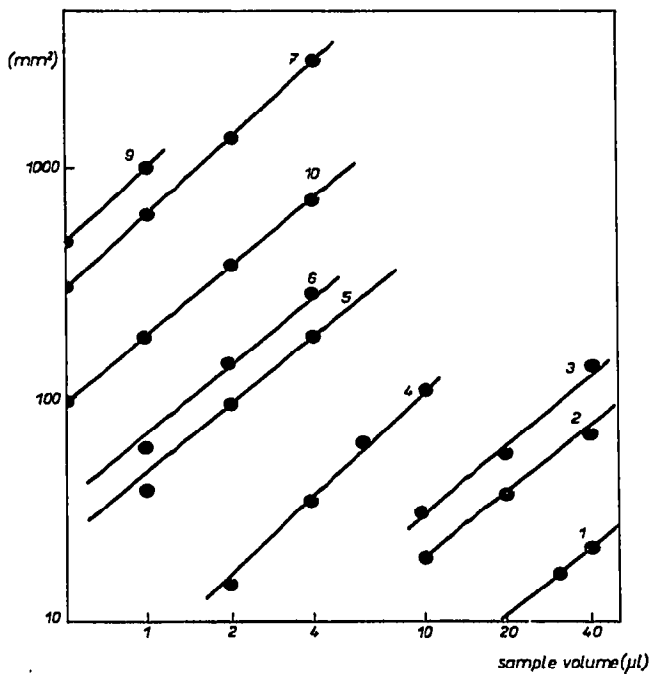


Fig. 3. Dependence of the response of the compounds listed in Table I on the sample volume. Mobile phase: cyclohexane + 2% diethyl ether. Detector: LDC Model 1103 refractometer.

injected was studied at a constant composition of the mobile phase. These relative measurements were carried out at one selected detector and recorder sensitivity. The dependences found with the use of the mobile phase comprising cyclohexane plus 2% (v/v) of diethyl ether are shown in Fig. 3. The volume of diethyl ether that corresponds to the area of the vacant peak was read from the calibration dependence obtained by the injection of pure diethyl ether. The relationship between the retention times of the compound under analysis and the vacant peak and that between the retention times of the compound under analysis and the increase or decrease in the concentration of the moderator corresponding to the vacant peak could also be evaluated in this way. Hence, the ratio between the volume of diethyl ether, corresponding to the vacant peak, and the volume of the substance injected was calculated. The values calculated for this ratio (P) are given in Table I.

TABLE I

RATIO OF THE VOLUME OF DIETHYL ETHER CORRESPONDING TO THE VACANT PEAK TO THE SAMPLE VOLUME

No.	Sample	Capacity ratio	P
1	Squalane	0.00	-0.0010
2	Cyclohexane	0.00	-0.0032
3	Isooctane	0.00	-0.0055
4	Tetrachloromethane	0.24	-0.018
5	Benzene	0.30	-0.080
6	Chloroform	0.34	-0.12
7	Diethyl ether	1.2	(+1.0)
8	Nitromethane	1.9	+0.28
9	Nitrobenzene	2.4	+1.30
10	Benzyl alcohol	3.4	+0.33
11	Diethyl phthalate	9.0	+0.31
12	Benzylcyanide	19	+0.40
13	<i>n</i> -Butanol		+0.38
14	Acetone		+0.68
15	Dimethyl sulphoxide		+0.22

DISCUSSION

It is obvious from Figs. 1 and 2 that the capacity ratio of the vacant peak follows the capacity ratio of diethyl ether, which suggests that this peak is caused by a vacancy, *i.e.*, by a local change in the composition of the mobile phase in comparison with the equilibrium state.

The dependence of the character of the change in the moderator concentration, corresponding to the vacant peak, on the relationship of the retention times of the compound under analysis (t_{R_s}) and moderator (t_{R_m}) is obvious from Fig. 3 and Table I. Provided that $t_{R_m} > t_{R_s}$, the vacant peak corresponds to the decrease in the concentration of the moderator in the mobile phase. However, if $t_{R_m} < t_{R_s}$, then the increase in the moderator concentration in the zone of the vacant peak is involved. The effect of the vacancy of the less adsorbed component on the mobile phase in the above instance is in question. However, the direction (polarity) of the response is decided

also by the relationship between the refractive indices of cyclohexane and diethyl ether, which is given by the character of the detection used.

The conclusion can also be drawn from Figs. 1 and 2 that the retention time of the vacant peak is always the same at a given concentration of the moderator, regardless of the sense of the change in the moderator concentration.

The increase in the vacant peak height with increase in the retention time of the compound injected, provided that $t_{R_m} > t_{R_s}$, is evident from Fig. 3 and Table I. If $t_{R_m} < t_{R_s}$ holds, a simple correlation between the retention time of the compound under analysis and the value of the change in the concentration of the moderator in the vacant peak zone is not evident. More complicated adsorption phenomena, which occur during the adsorption of three components when different sorption mechanisms of individual components appear (Fig. 2), are involved. This aspect will be investigated in further studies of the above phenomenon.

It follows from the measurements carried out with nitrobenzene injections at various concentrations of the moderator, which were selected in such a way that $t_{R_m} \approx t_{R_s}$, that the vacant peak does not converge to zero. The peaks in the chromatogram are considerably distorted (see Fig. 4), apparently due to overlapping of the vacant peak with the peak of the solute as a result of insufficient separation on the chromatographic column.

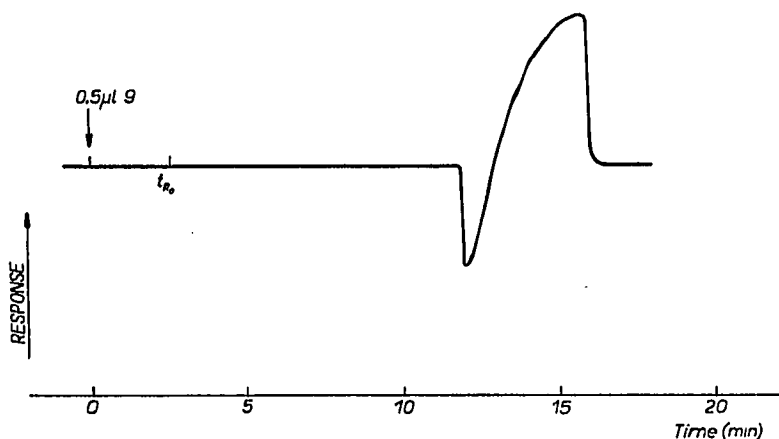


Fig. 4. Vacant peak for the solute with a retention time similar to that of the moderator.

The injection of an entirely different solute, as far as possible of a pure compound, can therefore be recommended as the most simple experimental verification of whether the vacant peak is really concerned. The vacant peak will keep its retention time and only the polarity of its response will change according to whether the solute introduced into the column has a shorter or longer retention time than that corresponding to the retention time of the vacant peak. As already stated, the vacant peaks can originate even if admixtures are present in a single-component mobile phase. This phenomenon was also used for the analysis of these admixtures in both gas¹ and liquid³ chromatography.

CONCLUSION

The origin of the vacant peak is evident if the above chromatographic system with a two-component mobile phase is used with a non-selective detector, as shown by the experiment. The vacant peak is caused by a local change in the moderator concentration in the mobile phase. The relationship between the retention times of the sample and the moderator is decisive for the magnitude and sense of the change in the composition of the mobile phase.

The ratio of the retention characteristics of the compounds under separation can be substantially affected by changing the composition of the mobile phase⁴. Therefore, the optimal separation of the mixture under analysis can often be achieved by selecting a suitable moderator and its concentration without the requirement for a high column efficiency.

In order to avoid complications in the record of the chromatographic analysis, it is useful, in addition to the requirement of the optimal separation (in the minimal time), to observe also one of the following conditions:

(1) To change the moderator or its concentration in such a way that $t_{R_m} > t_{R_s}$ for the most strongly adsorbed component. The vacant peak is then shifted behind the chromatographic profile, where it is so strongly broadened that it does not appear significantly.

(2) To change the moderator or its concentration in such a way that $t_{R_m} < t_{R_s}$ for the component being adsorbed least. The vacant peak precedes the chromatographic profile and is usually broadened sufficiently little not to overlap with the other components of the mixture.

(3) To replace the detector with a selective detector that does not respond to changes in the composition of the mobile phase.

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