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INDIRECT DETECTION OF NON-ELECTROLYTES IN LIQUID CHROMATOGRAPHY WITH ADDITIVE SATURATED ELUENTS

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SUMMARY

The eluent in a reversed-phase liquid chromatographic column was saturated with an additive to which the detector was tuned. Analytes of greater mobility than the additive in the system were generally found to solubilize the additive from the stationary phase and to induce positive signals. In one example, simple alkanes were detected at 275 nm through use of *p*-terphenyl as the additive. Analytes of lower mobility than the additive induced negative signals through a displacement mechanism, as demonstrated with a number of chlorinated compounds. Sensitivity increased as the retention of analyte and additive converged, but decreased if the additive concentration was lowered below saturation.

INTRODUCTION

Several procedures for the indirect detection of non-electrolytes by liquid chromatography have recently been described[1–4]. All of these require the perturbation of a detectable eluent component by the analyte(s) of interest. In earlier work[4], we introduced an indirect detection procedure where a reversed-phase column was equilibrated with a toluene-saturated eluent. When an analyte such as methylene chloride was injected, it solubilized toluene from the stationary phase into the eluent, and thereby induced a signal from the detector which was tuned to toluene. Analytes that were strongly retained with respect to additive provided negative signals presumably through displacement of additive. In this paper we extend these findings, propose a mechanism for the detection process, and relate our results to analogous work on ion pairs.

EXPERIMENTAL

The chromatographic system consisted of a Varian 5560 instrument coupled to a Varian UV 200 detector containing a cell of 3-mm path length, a Hewlett-Packard 3390A integrator and a 30 (or 100) × 2.1 mm column from Brownlee Labs., containing C_{18} material of 5 μm particle size. The flow-rate of the pump was within

3% of nominal values. Injections were made through a Rheodyne 7125 valve fitted with a 10- or 100-μl loop.

Eluents were prepared from solvents of HPLC quality. Other materials were of reagent grade and were used as received. The mobile phase was saturated with the additive by stirring for about 36 h. Eluents containing *p*-terphenyl were filtered just before use. Those containing toluene were allowed to phase separate through standing. A layer of toluene was maintained over the eluent to ensure continued saturation of the latter. The most intensely absorbing eluent used was 30% methanol in water saturated with toluene, which had an absorbance of about 0.1 AU at 260 nm. The analytes used were quite volatile, and they were prepared in methanol–water mixtures of higher methanol content than the eluent in order to minimize evaporative loss.

RESULTS AND DISCUSSION

Consider a situation where an eluent such as methanol–water is saturated with an additive such as toluene, and the resulting solution is pumped through a reversed-phase column. At equilibrium, an uniform layer of additive is deposited on the stationary phase, and the column effluent is saturated with additive. It is convenient to separate analytes into those that are weakly retained with respect to additive, and those that are retained strongly, since a different mechanism of detection applies to each case.

Weakly retained analytes

When a weakly retained analyte is injected, it is unable to displace additive from the column, since the additive–column interaction is stronger than that between analyte and column. As a result, the retention of analyte is primarily governed by its sorption to the additive coated stationary phase rather than to the stationary phase itself. The difference is significant since the additive coating may alter the retention characteristics of the analyte. For example, in an earlier study[4], we found that the retention of ethyl acetate decreased substantially when the stationary phase was coated with toluene.

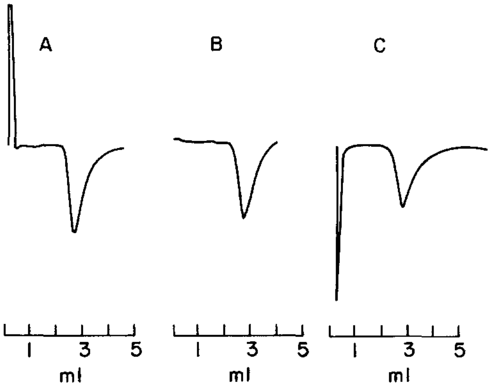


Fig. 1. Chromatograms of (A) 40, (B) 20 and (C) 10% methanol in water obtained at 273 nm at a flow-rate of 0.4 ml/min with a 30 × 2.1 mm column and an eluent of 20% methanol in water saturated with toluene.

The presence of analyte may disturb the distribution of additive between stationary and mobile phase. An extreme situation is illustrated in Fig. 1 where a 20% (v/v) solution of methanol in water saturated with toluene was pumped through a C_{18} column into a photometric detector tuned to toluene. Fig. 1A, B and C derive from injections of 40, 20 and 10% methanol in water, respectively. As the injected materials did not contain toluene, they were expected to dissolve toluene from the stationary phase, with the amount of toluene dissolved increasing with increasing methanol content. The solution injected in Fig. 1A was richer in methanol than the eluent, and as a result, a peak for toluene was observed when the injected band eluted from the column. The toluene deficiency created on the column appeared as a system vacancy at the retention volume of toluene.

The methanol fraction in the solution injected in Fig. 1B was equal to that in the eluent, and significantly, this mixture did not induce a signal upon elution. Clearly, the injected mixture was completely saturated with toluene during its brief contact with the stationary phase, and the only visible outcome of the injection was the system vacancy. The solution used in Fig. 1C contained proportionately less methanol than the eluent, and was unable to dissolve toluene to the same extent as the mobile phase. Its elution was therefore indicated by a negative signal, and this was followed by the system vacancy.

In order to confirm that saturation of the injected band was maintained under differing chromatographic conditions, we used water saturated with toluene as the eluent, and injected a solution of 20% methanol in water into a 30 × 2.1 mm column under varying conditions of eluent flow-rate and injection volume. The resulting intensity data are provided in Table I. If the injected band follows a Gaussian profile, then the methanol concentration C_M at the detector is given by eqn. 1[5]

$$C_M = C_0(V_S/V_R)(N/2\pi)^{0.5} \quad (1)$$

where C_0 is the initial methanol concentration (20%) and V_S and V_R are volume of sample and retention volume respectively. The plate count N is given by eqn. 2[6]

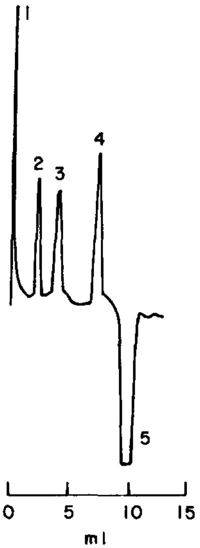
$$N = 2\pi(tH/A)^2 \quad (2)$$

TABLE I

DEPENDENCE OF INTENSITY ON CHROMATOGRAPHIC PARAMETERS

$n = 2$.

Eluent flow-rate, F (μl/min)	*Injection volume, V_S (μl)*	*Peak height, H (arbitrary units)*	*Peak area, A (arbitrary units)*	V_S/FA
10	10	2.33	6.26	0.160
20	10	1.95	3.21	0.156
40	10	1.38	1.44	0.174
80	10	1.44	0.883	0.142
160	10	1.23	0.431	0.145
320	10	1.07	0.234	0.134
640	10	1.03	0.111	0.141
100	100	10.1	8.36	0.120



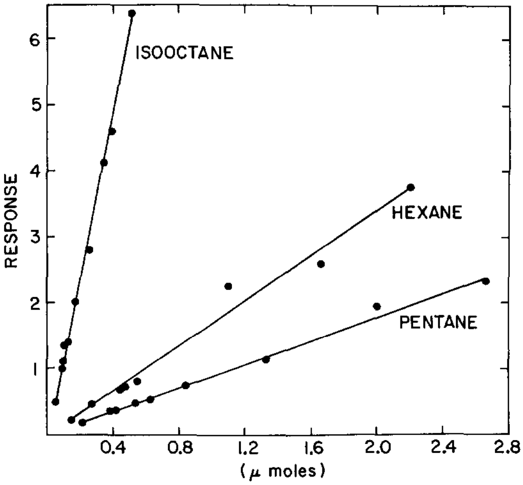


Fig. 2. Chromatogram obtained at 275 nm at a flow-rate of 0.5 ml/min with a 100 × 2.1 mm column and an eluent of 70% methanol in water saturated with *p*-terphenyl. Signals: 1 = the methanol injection solvent, 2 = pentane (1.33 μmol), 3 = hexane (1.10 μmol), 4 = isooctane (0.261 μmol), 5 = the system vacancy.

Fig. 3. Response curves of alkanes obtained under conditions used in Fig. 2.

where t is retention time, and H/A is the height:area ratio of the signal. Combining eqn. 1 with eqn. 2 yields eqn. 3:

$$C_M = C_0 V_S H/FA \qquad (3)$$

where F is eluent flow-rate.

The peak height H should be a measure of the amount of toluene solubilized at the detector. The relationship between C_M and H is expected to be independent of chromatographic conditions, *i.e.* V_S/FA should be constant for a given value of C_0. This is indeed the case (within a standard deviation of 11%) for the data in Table I over a 64-fold variation in F and a 10-fold change in V_S.

An application of the method to general chromatographic detection through solubilization is illustrated in Fig. 2, where alkanes were detected at 275 nm with *p*-terphenyl as the additive. A similar observation has been made by Vigh and Leitold[3], where the additive concentration was well below saturation. The response curves provided in Fig. 3 show that sensitivity improves as the retention of analyte converges with that of additive. This convergence is indicative of increasing compatibility between analyte and additive, and the sensitivity will improve as the two species grow closer in their solubility properties.

Finally, we determined the extent to which detection was attenuated by incomplete saturation of eluent. The saturated eluent was mixed at the pump with corresponding additive-free solution, and the response of the alkanes used above were determined as a function of eluent saturation. The results provided in Table II show

TABLE II

EFFECT OF DEGREE OF SATURATION OF ELUENT ON RESPONSE

Chromatographic conditions were those used for Fig. 2.

Saturation (%)	*Relative response*		
	Pentane	*Hexane*	*Isooctane*
10	1	1	1
20	2.34	2.06	2.64
30	3.83	3.67	4.19
40	5.15	4.87	5.47
60	7.55	6.80	7.73
70	9.46	8.10	9.18
80	11.1	10.7	10.5
100	13.3	12.6	12.4

that detection is possible, although with reduced sensitivity, even in systems which are quite far removed from saturation.

Strongly retained analytes

Analytes that are strongly retained with respect to additive tend to displace additive from the stationary phase, since their binding to the stationary phase is stronger than that of the additive. An additive deficient band is therefore created on the stationary phase, and the displaced additive is taken up in the injection solvent. This displaced material replaces some of the additive that would otherwise be dissolved by the injection solvent. As a result, the system vacancy which is caused by removal of additive by the injected solvent is reduced in intensity.

Consider the chromatograms in Fig. 4 where chlorinated compounds were detected via toluene. The system signal 2 in Fig. 4A is negative since the amount of additive displaced is less than that required to fully saturate the injection solvent. The intensity of the system signal drops as the amount of analyte increases. At high analyte levels, the signal may even reverse sign as shown in Fig. 4B. It would seem that the amount of additive displaced exceeds that required to saturated the injection solvent, and supersaturation occurs to a small extent. However, supersaturation is incompatible with an equilibrium model[7,8], and we speculate that full equilibration does not occur at the microscopic level in the injection zone.

In other respects, the behavior of the analytes in Fig. 4 was comparable to that described for the weakly retained materials in Fig. 2. Response was linear in the mid to high nanomole range, and the detection limit for chloroform was about 8 nmol. As before, sensitivity decreased as analyte and additive diverged in their retention properties. The effect of incomplete saturation of eluent on response was similar to that observed for the alkanes in Table II.

In summary, analytes eluting prior to the system signal will tend to be positive and will originate from a solubilization mechanism. Those trailing the system signal will usually be negative, and will be induced by a displacement effect. However, exceptions may occur; for example, the initial signal in Fig. 1C is negative even

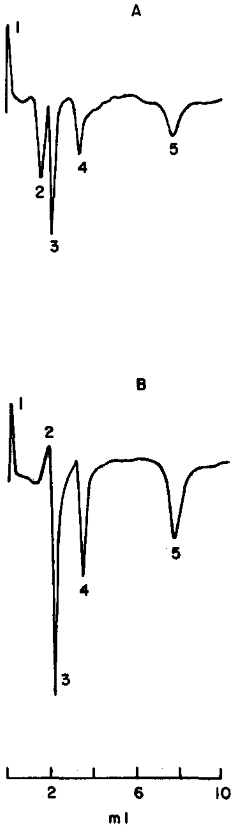


Fig. 4. Chromatograms obtained at 260 nm at a flow-rate of 0.5 ml/min with a 30 × 2.1 mm column and an eluent of 30% methanol in water saturated with toluene. (A) Signals: 1 = injection solvent [methanol–water (1:1)], 2 = system vacancy, 3 = chloroform (45.2 nmol), 4 = vinylidene chloride (47.0 nmol), 5 = 1,1,1-trichloroethane (68.2 nmol). (B) Signals: 1 = injection solvent (as above), 2 = system peak, 3 = chloroform (90.4 nmol), 4 = vinylidene chloride (94.0 nmol), 5 = 1,1,1-trichloroethane (136 nmol).

though it precedes the system vacancy, since the injected material decreases the solubility of the additive.

Although our study is the first to utilize saturated eluents, several earlier workers have used indicator additives at concentrations well below saturation. For example, Parkin[1] used micromolar quantities of benzaldehyde and benzoate esters as indicators to detect alcohols and esters at the micromole level. Vigh and Leitold[3] obtained similar results with benzyl alcohol as the indicator. Neither study focussed on the mechanism of detection other than to attribute the indirect signal to a disturbance of the steady-state additive concentration. Our contribution is to establish that two mechanisms, namely solubilization and displacement are responsible for the disturbance. The lower sensitivity obtained by Parkin[1,2] and Vigh and Leitold[3] is an outcome of their use of unsaturated conditions, in keeping with our observation in Table II that sensitivity decreases with increasing departure from saturation.

The relationship between response and retention observed for non-electrolytes follows the pattern first established by Schill and co-workers[9,10] in their elegant work with ion pairs. For example, Denkert *et al.*[9] have shown that the response factor increases with increasing k', goes through a maximum when k' for analyte and additive are about equal, and then levels off at higher k'. That the same behavior is observed for non-electrolytes despite the difference in mechanism is remarkable. However, it seems reasonable to expect interaction between additive and analyte to be best when the two species most resemble each other, which will occur when their k' values are similar. Thus, response–k' relationship of Denkert *et al.*[9,10] would seem to apply to any indirect detection scheme regardless of mechanism.

Finally, we point out that the utility of the technique for general chromatographic detection is limited by its inability to accommodate solvent gradients, and by its need for saturated eluents. Sensitivity is largely dependent upon the nature of the additive, which has to be carefully selected with regard to its retention and response properties.

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