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Analyte preconcentration procedure based on parallel-current open-tubular liquid chromatography

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Abstract

A preconcentration method based on the focusing of the analyte in the tail of the pulse of the retentive layer moving in open capillary was developed. This technique was verified by the preconcentration of aqueous solutions of phenol, 2,4-dinitroaniline, phenanthrene and fluoranthene as model analytes. The pulse of a cyclohexanol layer moved in a fused-silica capillary of diameter 0.2 mm. The effects of the cyclohexanol volume injected and sample volume on the focusing performance were examined. An enrichment factor of up to 30 was achieved.

Keywords: Sample enrichment; Trace analysis; Dinitroaniline; Phenol; Cyclohexanol; Sodium chromate; Phenanthrene; Fluoranthene

1. Introduction

Trace analysis often requires the determination of compounds at concentrations below the detection limit of standard methods. Application of an enrichment technique prior to the determination of trace analytes is frequently used. In gas and liquid analytical systems, enrichment by solid-phase extraction often comes into consideration [1–3]. Liquid–liquid extraction is a routine off-line analytical principle. In flow-injection analysis (FIA), a liquid–liquid extraction can be used as an effective on-line preconcentration method [4–6]. Chromatographic on-line liquid–liquid extraction for enrichment of the trace analytes has so far not been found to be useful.

Chromatographic enrichment techniques are based on the gradient elution principle [7,8]. The retention strength of a chromatographic system is expressed in terms of gradient capacity ratios. An increase in capacity ratios corresponds to an increase in the retention strength of the chromatographic system. At the beginning of the enrichment of the analyte, the retention strength of the system should be as great as possible; the desorption volume of the analyte is indirectly proportional to the decrease in the retention strength of the system.

Parallel-current open-tubular liquid chromatography (PC-OTLC) [9–11] can be regarded as an effective liquid–liquid extraction method. In PC-OTLC, a two-phase flow is generated by the decrease in the solubility of the retentive liquid in the mobile phase. The change in the solubility is generated by the temperature difference between the pumped liquid and the capillary column. The experimental conditions influencing

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important parameters, including the flow ratio of the phases, viscosities of both phases and quality of the inner surface of the capillary column, have been discussed [9–12].

The gradient of retention strength can be generated not only by standard instrumentation techniques [7] but also by injection of a suitable modifier [13] together with the sample [14,15] or by separate injection of the modifier into the mobile phase [16].

Here, injection of the retentive phase into the mobile phase before the sample introduction is described as a method for on-line analyte enrichment using PC-OTLC. The retentive layer is formed by wetting a suitably prepared inner surface with a pulse of immiscible retentive liquid. The thickness and length of the retentive layer decrease owing to dissolution of the retentive liquid in the stream of the mobile phase. Such a process is the background to the formation of the retentive gradient and the enrichment process in a fused-silica capillary described in this paper. The described enrichment method can be expected to show some advantages over standard methods: the retentive liquid can be chosen selectively, the heterogeneous matrix of the sample does not cause blocking of the capillary and the process can be repeated many times. On-line enrichment has potential applications in LC–GC, LC–MS and enrichment of capillary electrophoresis combinations.

2. Experimental

2.1. Instrumentation

Experiments were carried out using laboratory-made apparatus (Fig. 1). The mobile phase was delivered by an HPP 5001 high-pressure syringe pump (Laboratory Works, Prague, Czech Republic). The laboratory-made injector consisted of a six-port valve (1) with an external loop (10–1000 μl) coupled with a three-way injection block (2) made of PTFE. The volume of the high-pressure syringe (3) was 100 μl. The sample was injected using the six-port valve or with the high-pressure syringe. Cyclohexanol was

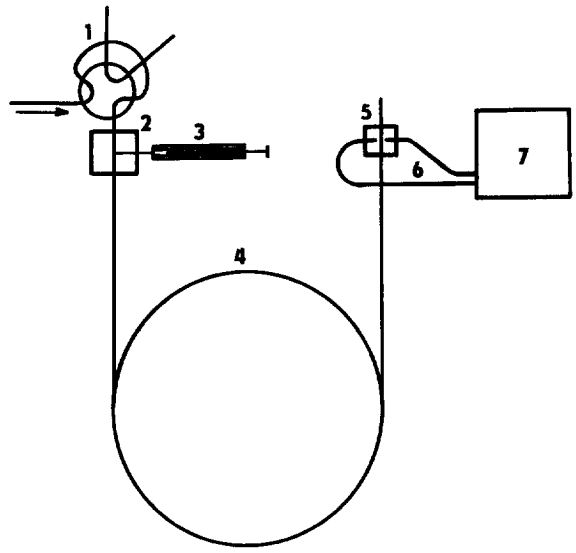

Fig. 1. Experimental arrangement of the chromatograph with UV detection. 1 = Six-port valve; 2 = injection block made of PTFE; 3 = 100-μl high-pressure syringe; 4 = fused-silica capillary (0.2 mm I.D.); 5 = detection cell; 6 = optical fibres; 7 = variable-wavelength UV detector.

injected with the high-pressure syringe. Fused-silica capillaries (9500 mm × 0.2 mm I.D.) were purchased from Lachema (Brno, Czech Republic). The internal surface of the capillaries was persilylated with octamethylcyclotetrasiloxane (D_4 reagent) [10]. The procedure was carried out twice. The capillary (4), which was connected directly to the injection block (2), was immersed in a water-bath maintained at 50°C by a U8 thermostat (MLW Prüfgeräte-Werk, Medingen/Sitz Freital, Germany). On-column UV detection with optical fibres as described previously [17] was used. A variable-wavelength UV detector (7) (LCD 2082; ECOM, Prague, Czech Republic) was optically connected by means of spherical quartz lens and optical fibres (6) (Polymicro Technologies, Phoenix, AZ, USA) to the detection cell (5). The geometry of the detection spot was defined by the dimensions of the fused-silica capillary used (0.2 mm I.D., 0.36 mm O.D.) and by the core diameter of the optical fibre (0.2 mm). The effective volume of the on-column detection cell was less than 5 nl and did not influence shape of the peaks. The detector signal

was monitored with TZ 4100 line recorder (Laboratory Instruments, Prague, Czech Republic).

2.2. Chemicals and model analytes

The pumped liquid was 0.01 mol l^{-1} sodium acetate in water. Based on preliminary experiments, measurements were carried out at a linear velocity of the mobile phase of 26 mm s^{-1}. The model analytes were sodium chromate (marker of the dead time, t_0), phenol, 2,4-dinitroaniline, phenanthrene and fluoranthene. The test solutes and other chemicals used were purchased from Lachema. D_4 reagent was obtained from VCHZ Synthezia (Kolín, Czech Republic).

2.3. Determination of the distribution constant K_i

The solute distribution constant, K_i, between co-existing cyclohexanol and aqueous phases for phenol, $K_i = 16.7$ at 50°C, was taken from Ref. [9]. The value for 2,4-dinitroaniline, $K_i = 90.2$ at 50°C, was determined by the method described previously [18]. The logarithm of the octanol–water distribution constant of phenanthrene and fluoranthene is reported to be 4.45 [19] and 4.95 [20], respectively; a similar value is expected for the cyclohexanol–water system.

2.4. Calculations

The linear velocity of the mobile phase was determined from the retention time of sodium chromate (t_0) and the capillary length. The retention factor, k_f^*, was determined through the equation

$$k_f^* = (t_r - t_0)/t_0 \quad (1)$$

where t_r is the retention time of the solute in the gradient of the concentration pulse of retentive liquid. The optimum time for solute injection into the capillary was found to be within the time delay, Δt, after the injection of cyclohexanol segment, $2 < \Delta t < 10$ s, which was found based on the highest k_f^* obtained.

The enrichment factor, F, was calculated from the time-based standard deviation of the eluted peak of the solute, σ_t^f, and from the time-based length of the injected sample plug, V_t, with use of the relationship for the standard deviation of a square-wave profile [21]:

$$F = V_t/\sqrt{12}\sigma_t^f \quad (2)$$

3. Results and discussion

3.1. Model description

The process of sample focusing according to the suggested model is illustrated schematically in Fig. 2A–D. Prior to analysis the capillary is continuously flushed with mobile phase. The preconcentration run is started by injection of a short plug of retentive liquid, shown as a black rectangle. Then, at time $t = 0$ (see Fig. 2A), the sample is injected by valve 1 (see Fig. 1). The volume of the sample shown as the double-hatched rectangle can be substantially large than volume of retentive liquid injected. For simplicity, the sample contains only two analytes, one with very small K_i, which can simulate the hydrophilic sample matrix, and the other with reasonably high K_i, which models the compound of interest. After some time, $t < t_0$, the injected pulse of retentive liquid is transformed into a moving film of the retentive phase, similarly as in PC-OTLC. The average film velocity, u_R, is smaller than the velocity of mobile phase, u_M, $u_R < u_M$. However, the rear end of the zone of retentive liquid is continuously dissolving in the mobile phase so that a tailing profile can be expected (see Fig. 2B). According to the principle of liquid–liquid chromatography, the decrease in the film of retentive liquid leads to a decrease in the analyte retention. Thus, in the region of the tail of the zone of retentive liquid the conditions of gradient elution [7] are adjusted. The just described gradient has the features of injection-generated gradients [13,14]; the magnitude of analyte retardation on the column is proportional to amount of compound injected which generates the analyte retention. The analyte is focused at the gradient position with a

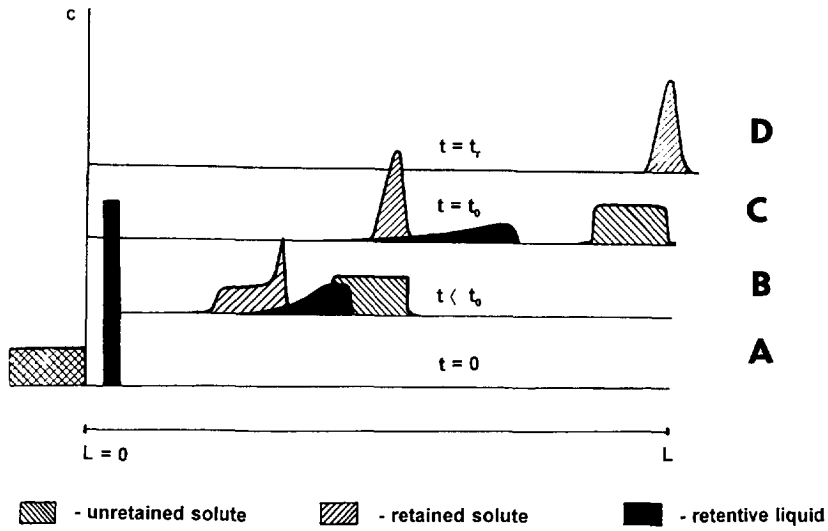

Fig. 2. Principle of focusing on pulse of retentive liquid. (A) Concentration profile of the model analytes after injection of the cyclohexanol segment and with time of the injection of a volume pulse of a mixture of dead-time marker and solute ($t = 0$). (B) Total volume of the retained solute is not fully focused ($t < t_0$). (C) Retained solute is focused in cyclohexanol ($t = t_0$). (D) Retained solute in the mobile phase at that moment after dissolution of the cyclohexanol layer ($t = t_r$). c = Total concentrations of cyclohexanol and analytes; L = length of the capillary column.

characteristic retention strength which is bound to a certain thickness of the retentive film. Since the thickness of the retentive film can decrease from a few micrometres down to molecular dimensions, a dynamic range of the gradient characterized in terms of the change in the capacity ratio can be expected up to several orders of magnitude. The components of the sample are eluted from the capillary according to the increase in their K_i values. Recalling our two-component model sample, the unretained analyte is eluted in the dead time, t_0 (see Fig. 2C), in the shape of the injected profile modified by dispersion in the capillary. The component of interest is eluted focused at time t_r (see Fig. 2D). The suggested model function was examined by the experiments described below.

3.2. Method operation

Elution of single components

In order to choose suitable settings of the instrument, concentrations and volumes of sampled solutions, single components were injected. Some results are shown in Fig. 3. Fig. 3a shows the result obtained after the injection of a solution of sodium chromate. This salt was used as an unretained component of the sample and marker of the dead time. As expected, the response to the injection of 10 μl is a Gaussian profile (peak 1), while injection of 1 ml gives a nearly rectangular zone (peak 2). In Fig. 3b, the profiles corresponding to injection of 10–1000 μl of 2,4-dinitroaniline solution (zones 1–5) are shown. This compound is one of the model analytes used for verifying the model. Again, with increase in the volume injected the length of the recorded zone increases. The injection of cyclohexanol (Fig. 3c) should not given any appreciable response in the UV–Vis detector. Indeed, at 336 nm (see line 2 in Fig. 3c) and at 233 and ca. 400 nm no response was observed after injection of 20 μl of cyclohexanol. However, an appreciable signal was obtained at 254 nm (see line 1 in Fig. 3c). This can be explained by the presence of some impurity absorbing at this wavelength. The peak at the end of time interval t_z (see Fig. 3c), is probably due to focusing of this impurity in the tail of cyclohexanol pulse by the process described in the paragraph above. The height of

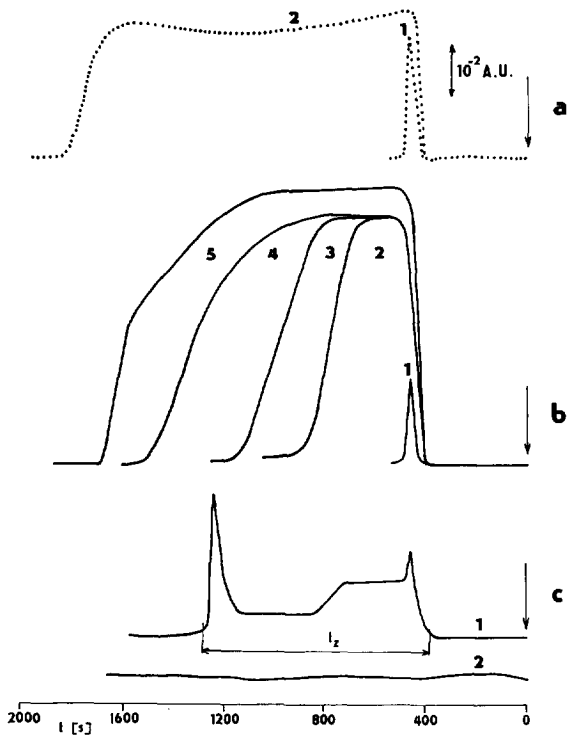

Fig. 3. Elution profiles of single components. Conditions: 9500 × 0.2 mm I.D. fused-silica capillary modified using D_4 reagent; column temperature, 50°C; pumped liquid, 10 mmol l^{-1} CH_3COONa in water; linear mobile phase velocity, u_M = 26 mm s^{-1}; UV detection. (a) 1, 2 = Zones of the sodium chromate solution (dead-time marker) (336 nm) after injection of 10 and 1000 μl, respectively, of the solutions; (b) 1–5 = zones of 2,4-dinitroaniline (336 nm) after injection of 10 μl of 10^{-4} mol l^{-1} and 250, 500, 750 and 1000 μl of 10^{-6} mol l^{-1} solutions, respectively; (c) 1, 2 = responses on the injection of the segment of the cyclohexanol (V_{CHOH} = 20 μl) at 254 and 336 nm, respectively. t_z (s) = Response on dissolution of the concentration pulse of the retention layer of cyclohexanol.

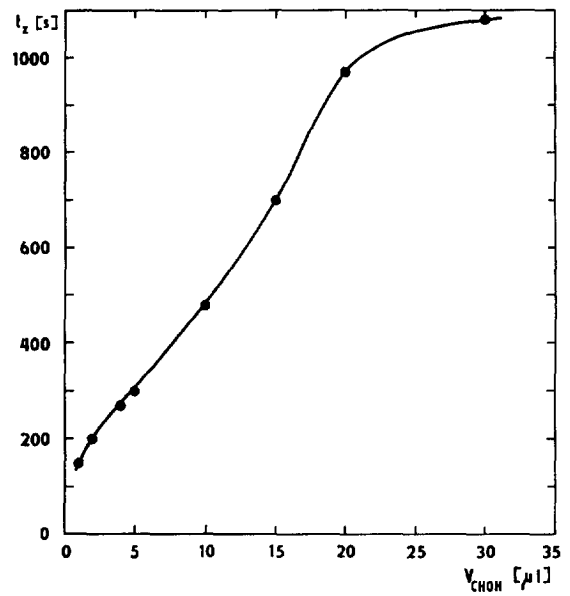

Fig. 4. Dependence of t_z on the volume of injected segment of cyclohexanol, V_{CHOH}. Conditions and designations as in Fig. 3.

this peak increases with increasing amount of cyclohexanol injected. Although the signal at 254 nm may complicate the detection of some analytes, it enables one to estimate the maximum volume of cyclohexanol which can be injected into the capillary. It was found to be 20–26 μl under the conditions used (see Fig. 4, where the time interval t_z is plotted against the volume of cyclohexanol injected). That a larger amount of retentive liquid leads to breakthrough of droplets into the detection cell was indicated also by the extensive noise, similarly as was observed previously [11,12]. From comparison of the lengths of the profiles of individual components, the maximum volume of the injected sample which can be focused was estimated as 750 μl. The part of the sample volume which is above this value is eluted later than the tail of the cyclohexanol pulse and cannot be focused.

Examples of analyte focusing

In Fig. 5, examples of chromatograms obtained with suggested procedure are shown. In both cases 18 μl of cyclohexanol were injected, followed by 250 μl (Fig. 5a) or 500 μl (Fig. 5b) of 10^{-5} mol l^{-1} 2,4-dinitroaniline as a model analyte and sodium chromate as a model of an unretained sample matrix. The time delay between the injection of cyclohexanol and the sample was Δt = 2 s. It can be seen that zone of chromate is not focused and it is eluted as a nearly rectangular profile, similarly to Fig. 3a. The analyte with an appreciable distribution constant, K_i = 90, is both retained and focused as expected according to the model suggested. The sample volume of 500 μl (see Fig. 5b) is close to

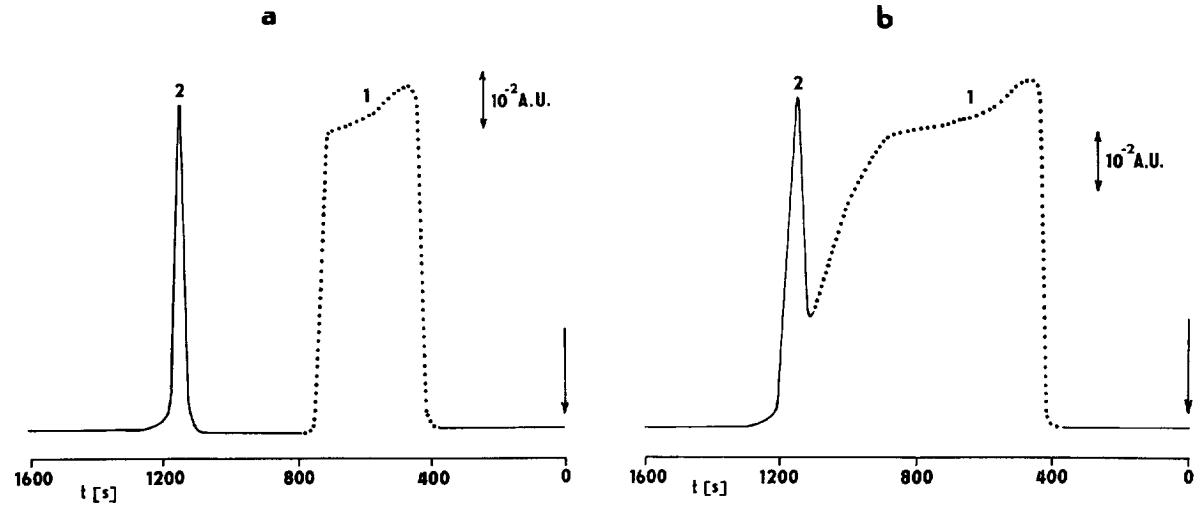

Fig. 5. Focusing of 2,4-dinitroaniline under different experimental conditions. Conditions and designations as in Fig. 3; $\Delta t = 2$ s; $V_{CHOH} = 18$ μl. (a) Injection of 250 μl solution of sodium chromate (1) and 10^{-5} mol l^{-1} solution of 2,4-dinitroaniline (2); (b) volume of the injected solution was 500 μl.

the maximum which gives separated zones of the matrix and analyte under the conditions used. Based on Eq. 2, a maximum enrichment factor, F, in the range 25–30 can be achieved for injection of 500–750 μl of 2,4-dinitroaniline (see Table 1). The lower F for phenol found with injection of 250 μl of sample can be explained by the lower K_i of the analyte.

Influence of volume of cyclohexanol injected

It can be expected from the model suggested that the magnitude of analyte focusing is proportional to the amount of injected retentive liquid and to the distribution constant of the analyte. Since the retention of phenol in the water–cyclohexanol system was described previously [9–12], the dependence of the peak shape of phenol on the amount of cyclohexanol injected was examined. Examples of chromatograms obtained after injection of 250 μl of 10^{-5} mol l^{-1} phenol solution are shown in Fig. 6a. As expected, the retention times of the peak maxima increase with increasing amount of retentive liquid injected. For the smallest volume of cyclohexanol injected, only part of the sample volume is focused (see Fig. 6a, peak 1). The increase in retention of the phenol peak is also shown graphically in Fig. 6b as a plot of k_f^* against V_{CHOH}. It follows from the graph that the retention enhancement is less pronounced at larger injected volumes of retentive liquid. Simultaneously, the front of the phenol peak becomes broader (see Fig. 6a, peaks 3 and 4). This effect can be explained by the low K_i of phenol in the system used ($K_i = 16.7$), which may result in the phenol peak moving at the transition between a uniform moving film and a gradient of film thickness. Symmetrical peaks were obtained in similar experiments with 2,4-dinitroaniline ($K_i = 90$) (see also Fig. 5).

Table 1
Dependence of the enrichment factor, F, on the volume of the solution of the analyte injected, V_s [a]

Analyte	V_s (μl)	c_s (mol l^{-1})	F
2,4-Dinitroaniline	10	10^{-4}	0.5
	250	10^{-6}	14
	500	10^{-6}	31
	750	10^{-6}	33
Phenol	250	10^{-5}	7.0

[a] $V_{CHOH} = 18$ μl; $\Delta t = 10$ s.

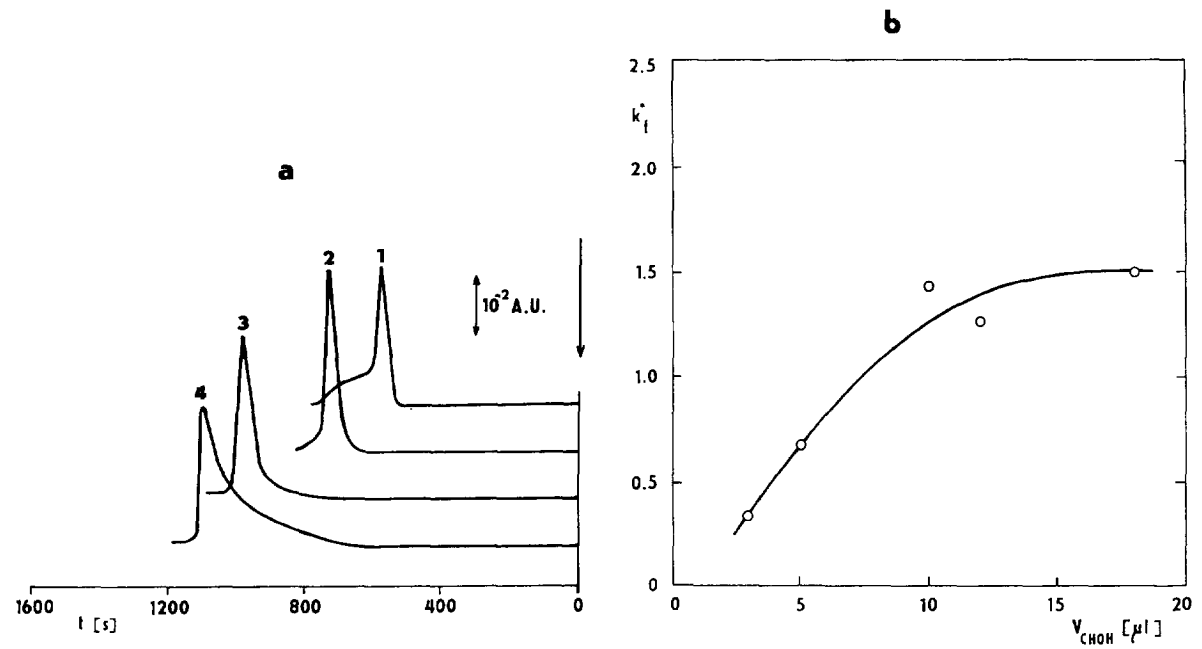

Fig. 6. Dependence of phenol retention on cyclohexanol volume, V_{CHOH}. Conditions and designations as in Fig. 3; $\Delta t = 2$ s; volume of 10^{-5} mol l^{-1} solution of phenol injected, 250 μl; detection wavelength, 283 nm. (a) Peaks 1–4 correspond to $V_{CHOH} = 3$, 5, 12 and 18 μl of cyclohexanol, respectively; (b) dependence of the retention factor, k_f^*, on V_{CHOH}.

In Fig. 7, the dependence of the retention of the 2,4-dinitroaniline peak on the volume of cyclohexanol injected is shown for several volumes of sample injected. The general trend of an increase in retention with increase in the volume of cyclohexanol injected is maintained, but there is some scatter of the retention values with sample volume. The points shown lie between the top line (1) for injection of 10 μl of sample and the bottom line (2) for injection of 1000 μl of sample. The simultaneous influence of the volume of retentive liquid, the sample volume and the analyte concentration on the described focusing process is probably complex and needs more detailed study.

Influence of analyte nature on focusing

The retention of analytes in the suggested procedure is expected to depend on the magnitude of the distribution constant of compound of interest. This influence was examined by focusing several analytes with different K_i values. In Fig. 8, the chromatogram shown was obtained from focusing solutions of single analytes under identical conditions. A similar record was also obtained when all analytes were included in a single injection, but unresolved peaks were observed. It is apparent that the peaks of retained analytes are eluted in a short time interval despite the considerable difference in their K_i values. The ratio of the K_i values of fluoranthene (the last peak, 5) to phenol (the first focused peak, 2) is more than three orders of magnitude. Such a finding indicates a short gradient of thickness of the cyclohexanol layer in the capillary. Hence the process suggested can be advantageously used for focusing and group separation in a single step.

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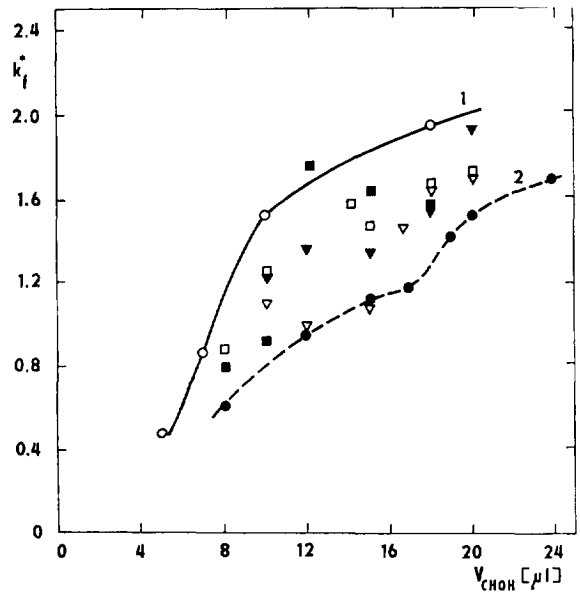

Fig. 7. Dependence of the retention factor, k_f^*, of 2,4-dinitroaniline on the cyclohexanol volume, V_{CHOH}. Conditions as in Fig. 3; $\Delta t = 2$ s; detection wavelength, 336 nm. Volume and concentration of the analyte: line 1, ○ = 10 μl of 10^{-4} mol l^{-1} solution; line 2, ● = 1000, ▽ = 750, ▼ = 500 and ■ = 250 μl of 10^{-6} mol l^{-1} solution and □ = 500 μl of 10^{-5} mol l^{-1} solution.

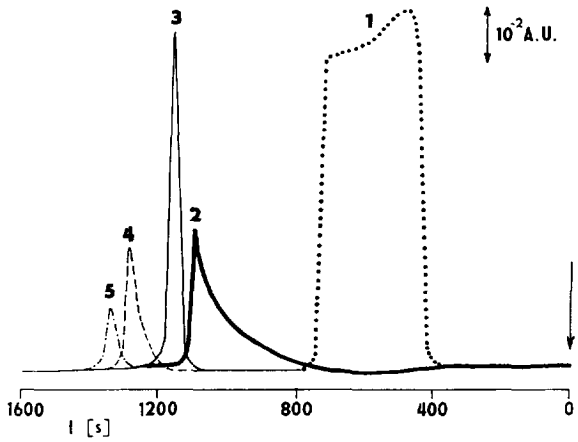

Fig. 8. Focusing of several analytes on pulse of retentive liquid. Conditions and designations as in Fig. 3; $\Delta t = 2$ s; $V_{CHOH} = 18$ μl. Analytes: 1 = sodium chromate; 2 = phenol (250 μl of 10^{-5} mol l^{-1} solution, 283 nm); 3 = 2,4-dinitroaniline (250 μl of 10^{-5} mol l^{-1} solution, 336 nm); 4 = phenanthrene (250 μl of 10^{-6} mol l^{-1} solution, 235 nm); 5 = fluoranthene (250 μl of 10^{-6} mol l^{-1} solution, 235 nm).

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