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INDIRECT PHOTOMETRIC DETECTION OF ALCOHOLS IN MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Alcohols were indirectly detected with an ultraviolet spectrophotometer in micro high-performance liquid chromatography. Aromatic hydrocarbons were added in the mobile phase in order to maintain the background signal. The alcohols were detected owing to their perturbation of dynamic equilibrium of the visualization agent between the mobile and the stationary phase. The dynamic reserve achieved in this system was 20 000–26 000, and the limit of detection of the alcohols was sub-nanogram.

INTRODUCTION

Indirect photometric detection of ionic species has been widely applied in ion-pair chromatography and in ion chromatography. Several papers have described indirect photometric detection of non-electrolytes in the reversed-phase mode[1-7]. Gnanasambandan and Freiser used methylene blue in the eluent and achieved indirect detection of aliphatic alcohols[1,3], ketones[1] and monosaccharides[4] with an UV–VIS detector. Parkin[5] added an UV-absorbing species for indirect photometric detection of alkanols and esters. The non-UV-absorbing species were visualized because they perturbed the partitioning characteristics of the UV-absorbing species during their elution. Banerjee[7] described another approach using an eluent saturated with toluene, in which the analytes were detected because of the resulting increased solubility of toluene. Takeuchi and Yeung[8] described laser-based indirect fluorometric detection of non-electrolytes in the reversed-phase mode.

The dynamic reserve (defined as the ratio of the background signal to its noise level), the displacement ratio (defined as the number of the visualization species which are transferred by one analyte species) and the concentration of the visualization agent all play important roles in the sensitivity that can be achieved in indirect detection techniques. The larger the dynamic reserve, the smaller is the limit of detection. Typical dynamic reserves of an UV–VIS detector and a refractive index detector are around $5 \cdot 10^3$ and 10^6, respectively. This explains why interaction between the analyte and the mobile phase component is not required for the latter detection mode.

The displacement ratio is typically 1 in ion-exchange chromatography, but is much smaller in the reversed-phase mode[8]. The concentration of the visualization agent should be as small as possible to achieve higher sensitivity in the ion-exchange mode, providing it does not alter the dynamic reserve, the displacement ratio and the retention time of the analytes. The relationship between these parameters will be discussed in this paper.

On the other hand, the use of microcolumns can improve the mass detectability. A limit of detection of 50 ng was achieved for 1-decanol by using microbore columns with 1 mm I.D. in indirect fluorometry[8]. This paper is concerned with indirect photometric detection of alcohols in the reversed-phase mode on columns of 0.26 or 0.34 mm I.D.

EXPERIMENTAL

An UV spectrophotometer UVIDEC-100V [Japan Spectroscopic Co. (JASCO), Tokyo, Japan] was employed as a detector. A time constant of 1 s was selected. The flow cell was modified for micro high-performance liquid chromatography (HPLC) in our laboratory. It was comprised of fused-silica tubing (1.5 mm × 0.26 mm I.D.) glued to narrow-bore fused-silica tubing (55 μm I.D.; SGE, Melbourne, Australia) by an epoxy-resin adhesive as a low-dead-volume connection to the separation column. The detection volume of the flow cell was 0.08 μl, and the dead volume between the column and the flow cell was $ca.$ 0.3 μl. The separation column was fused-silica tubing packed with 5-μm ODS, SC-01 (JASCO), prepared in our laboratory as reported previously[9]. It was immersed in a laboratory-made waterbath to avoid variations in the ambient temperature. The temperature of water was not regulated in this work. A microfeeder (Azumadenkikogyo, Tokyo, Japan) equipped with a gas-tight syringe MS GAN 050 (Ito Seisakusho, Fuji, Japan) was employed as a pump. An ML-422 micro valve injector (JASCO) was employed, and 18 nl were injected.

Reagent-grade n-alcohols were obtained from Tokyo Chemical Industry (Tokyo, Japan), HPLC-grade distilled water from Wako (Osaka, Japan). The other reagents were of reagent grade (Wako). All the reagents were employed without further purification.

RESULTS AND DISCUSSION

In indirect photometry, non-UV-absorbing analytes can be detected sensitively from the variation in the background signal if they displace, transfer or interact with the visualization species. When the concentration of the analyte in the detector is C_s, its peak volume is V_p, the concentration of the visualization agent is C_m and one analyte molecule transfers R molecules (or ions) of the visualization agent from the analyte band, the variation in the concentration of the visualization agent, ΔC_m, can be expressed as

$$\Delta C_m = \frac{V_p}{V_p + C_s V_p V_s - R C_s V_p V_m} \cdot \frac{C_m V_p - R C_s V_p}{V_p} - C_m \tag{1}$$

$$\Delta C_m = \frac{-C_s(R + C_m V_s - RC_m V_m)}{1 + C_s(V_s - RV_m)} \tag{2}$$

where R is the displacement ratio, V_s is the volume of 1 mol of the analyte and V_m is the volume of 1 mol of the visualization agent. In order to distinguish the variation in the background due to the analyte, $C_m/|\Delta C_m|$ must be smaller than the dynamic reserve, D_R:

$$\frac{C_m}{|\Delta C_m|} = \frac{C_m[1 + C_s(V_s - RV_m)]}{C_s(R + C_m V_s - RC_m V_m)} < D_R \tag{3}$$

Rearrangement of eqn. 3 for C_s yields:

$$C_s > \frac{C_m}{D_R(R + C_m V_s - RC_m V_m) + C_m(RV_m - V_s)} \tag{4}$$

The limit of detection can be estimated from eqn. 4.
    When $R = 1$ and assuming that $V_m = V_s$, eqn. 4 becomes:

$$C_s > C_m/D_R \tag{5}$$

Eqn. 5 is generally valid for the ion-exchange mode when the valences of the analyte and visualization ions are same. The limit of detection of the analyte is proportional to the concentration of the visualization species and inversely proportional to the dynamic reserve. This means that we should decrease the concentration of the visualization species in the mobile phase as much as possible in order to improve the detectability. The important point is whether we can maintain both the retention time and the dynamic reserve independent of the concentration of the visualization agent, otherwise these may cancel the improvement in detectability. In indirect photometry, use of a visualization agent possessing a large extinction coefficient is recommended.
    When $R = 0$ eqn. 4 becomes:

$$C_s > \frac{1}{(D_R - 1)V_s} \tag{6}$$

Eqn. 6 is valid when the analytes only dilute the mobile phase. In this case, the limit of detection is independent of the concentration of the visualization agent, and it is inversely proportional to the dynamic reserve because $(D_R - 1)$ is approximately equal to D_R.
    The linearity of the response was examined by using anthracene dissolved in acetonitrile. Fig. 1 shows a plot of the absorbance *versus* the concentration of anthracene. A linear relationship is observed up to an absorbance of *ca.* 0.2. The deviation from linearity at higher concentrations is related to the structure of the flow cell, *viz.*, a cylindrical cross-flow cell. We prepared a slit *ca.* 0.2 mm in width using an aluminium tape. The linearity can be improved by carefully modifying the structure of the slit.
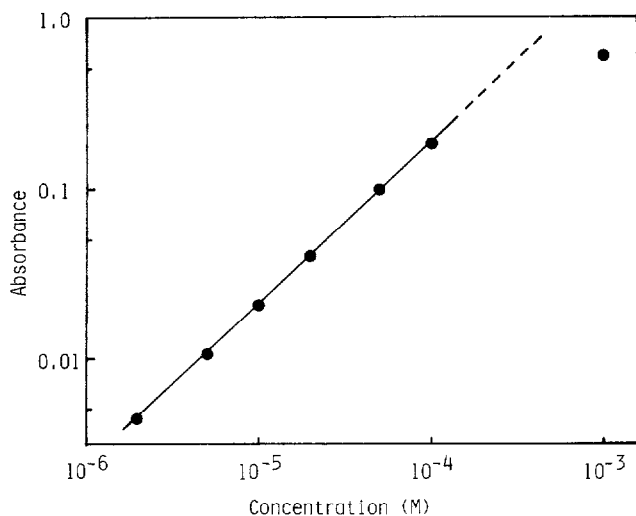
Fig. 1. Plot of absorbance *versus* concentration of anthracene. The sample was dissolved in acetonitrile. Wavelength of UV detection: 251 nm. Flow-cell volume: 0.08 μl.

The noise level was not markedly dependent upon the intensity of the background, being around 10^{-5} absorbance units. This means that the higher the background, the larger is the dynamic reserve. When the background is 0.2 and its noise level is 10^{-5} (both in absorbance units, the dynamic reserve is $2 \cdot 10^4$.

Fig. 2 shows the stability of the baseline using $2 \cdot 10^{-5}$ M anthracene. When the column is at ambient temperature, the baseline is unstable. This is due to the variation in column temperature, which in turn changes the distribution coefficient of anthracene between the mobile and the stationary phase. The heat capacity of a microcolumn is so small that column temperature is easily affected by variations in the temperature of the surroundings. On the other hand, when the column was immersed in water, the baseline was stabilized. Although the temperature of the water was not regulated, the column temperature was constant owing to the apparent increase in heat capacity.

Fig. 3 demonstrates the indirect photometric detection of alcohols using anthracene as a visualization agent. A positive signal was obtained for the solutes eluted before the system peak, while the solute eluted after the system peak gave a negative peak. This indicates that all the solutes in Fig. 3 transferred anthracene from the
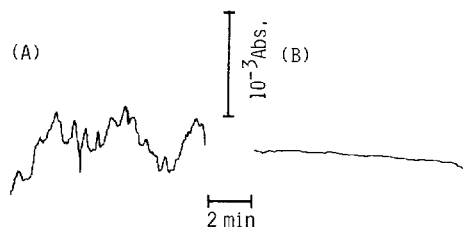

Fig. 2. Stability of the baseline. (A) At ambient temperature; (B) column immersed in water. Column: 5-μm ODS, 150 mm × 0.34 mm I.D. Mobile phase: acetonitrile–water (80:20) including $2 \cdot 10^{-5}$ M anthracene. Flow-rate: 4.2 μl/min. Detections as in Fig. 1.

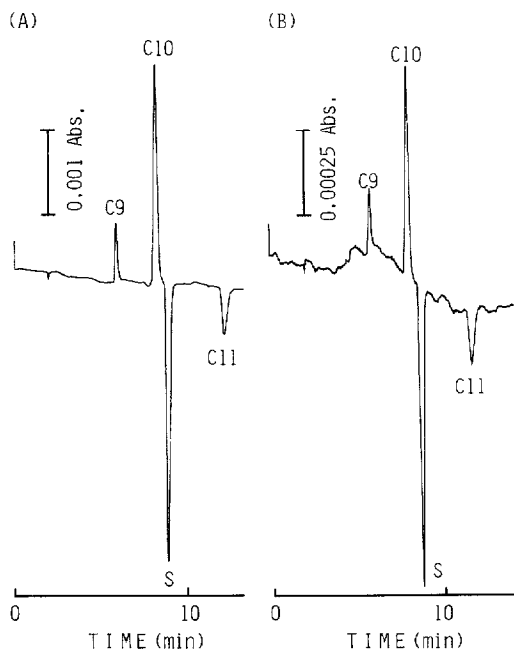Fig. 3. Indirect photometric detection of alcohols using anthracene as a visualization agent. Column: 5-μm ODS, 150 mm $\times$ 0.34 mm I.D. Mobile phase: acetonitrile–water (75:25) including $8 \cdot 10^{-5}$ M anthracene. Flow-rate: 4.2 μl/min. Wavelength of UV detection: 250 nm. Samples: C9 = 1-nonanol; C10 = 1-decanol; S = system peak; C11 = 1-undecanol; (A) each 37 ng; (B) each 9.3 ng.

stationary phase to the mobile phase. The displacement ratio was calculated from the background signal, peak height and peak volume[8], viz., $7.6 \cdot 10^{-4}$ for 1-nonanol, $3.6 \cdot 10^{-3}$ for 1-decanol and $1.5 \cdot 10^{-3}$ for 1-undecanol, respectively. In other words, 1300 molecules of 1-nonanol, 280 molecules of 1-decanol or 660 molecules of 1-undecanol transferred one anthracene molecule. The background signal and its noise level are 0.18 and $7 \cdot 10^{-6}$ absorbance units, respectively. The dynamic reserve is then calculated to be 26 000. The limits of detection were 0.7 ng for 1-nonanol, 0.2 ng for 1-decanol and 0.8 ng for 1-undecanol. These values are about 200 times smaller than those obtained by laser-based indirect fluorometry using microbore columns (1 mm I.D.)[8]. Thus, the detectability will be markedly improved in ion chromatography because the displacement ratio is much larger than that observed in the reversed-phase mode.

The retention times of the analytes were not affected by the concentration of anthracene up to 10^{-4} M. In addition, their peak areas were well correlated with the reciprocal of the resolution between the analyte and the system peak. It is not certain why the sum of the areas of positive peaks did not coincide with that of negative peaks. The theoretical treatment concerning the signal intensity requires further examination.

As in Fig. 3 of this paper and in previous work[8], the solute eluting close to the system peak gave an higher peak. This means that the detectability can be improved by selecting a visualization agent which has a retention time very similar to that of the analyte of interest. Fig. 4 demonstrates the indirect photometric detection of
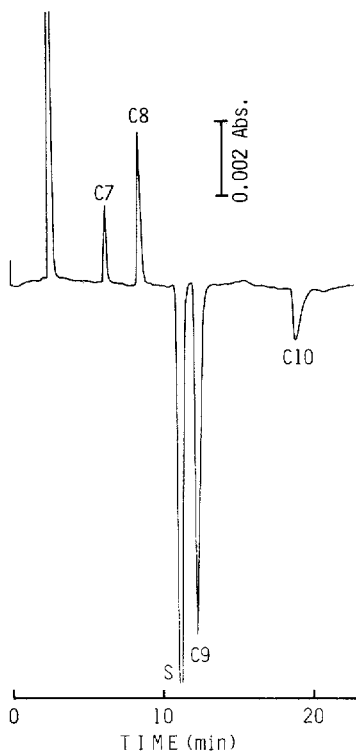
Fig. 4. Indirect photometric detection of alcohols using naphthalene as a visualization agent. Column: 5-μm ODS, 150 mm × 0.26 mm I.D. Mobile phase: acetonitrile–water (60:40) including 1.4 · 10^{-4} M naphthalene. Flow-rate: 2.1 μl/min. Wavelength of UV detection: 221 nm. Samples: C7 = 1-heptanol; C8 = 1-octanol; S = system peak; C9 = 1-nonanol; C10 = 1-decanol; each 75 ng.
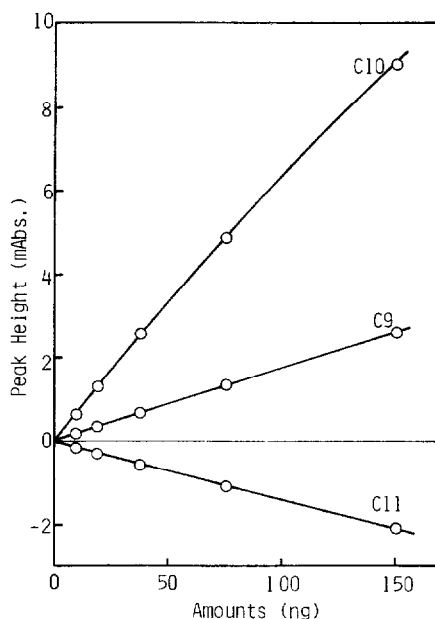
Fig. 5. Plots of peak height *versus* the amounts of the analytes injected. Operating conditions as in Fig. 3 except for the sample concentrations.

alcohols by using naphthalene as a visualization agent. The system peak was eluted close to 1-nonanol, and the detectability of 1-nonanol was improved compared with that achieved by using anthracene as a visualization agent.

Fig. 5 shows the relationships between the peak heights and the amounts of the analytes injected. Linear relationships were observed up to 75 ng, indicating that this method gives quantitative information.

CONCLUSIONS

Mass detectability of alcohols was improved by indirect photometric detection in micro HPLC. The limit of detection was at the subnanogram level. Detectability of the analyte of interest was also improved by appropriate selection of the visualization agent. This system can improve the mass detectability of ions because the displacement ratio is usually in ion chromatography, much larger than that observed in the reversed-phase mode. It is important to find a visualization ion possessing a

large extinction coefficient (*ca.* 10^5) because the detectability is proportional to the concentration of the visualization agent and because the pathlength of an UV detector in micro HPLC is shorter than that in conventional HPLC.

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