Image Analysis of Separation Processes. I. Development of a Video Image Analyzing System and Its Fundamental Application to the Direct Observation of a Separation Process in a Column

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In order to analyze the separation processes taking place in liquid chromatography, a video image analyzing system was developed. A video camera, video tape recorder and an image processor were linked to a microcomputer. The distribution of fluorescent solutes in a glass column was measured as a densitogram which was regarded as a chromatogram inside the column. The apparent migration rate, which was the migration distance for a unit migration volume, was determined by this method and was found to have a good linear relationship with the capacity factor *k'*. Band broadening during separation was also estimated at various flow rates. It was concluded that the band broadening was mainly due to multiple-path diffusion.

Keywords HPLC; video image analysis; separation process; capacity factor; apparent migration rate; inside column chromatogram

A number of investigations have been carried out to test various theories of the dynamic aspects of chromatography.[1-5] However, the separation processes in liquid chromatography columns have not been observed directly and investigators have used data from chromatograms following detection after elution from the column. If a glass column, detectable solutes (dyes, fluorescent solutes, *etc.*) and a video detection system are used, one can get a real-time image and the time course of the separation processes in a column. In this report, a video image analyzing system was developed for direct observation of the migration of solutes in a column and applied to analyze the dynamic aspects of the separation processes.

Experimental

Chemicals Dansyl (DNS) amino acids were purchased from Seikagaku Kogyo (Tokyo, Japan). All other chemicals were of guaranteed high quality.

Apparatus A block diagram of the video image analyzing system is shown in Fig. 1. The HPLC apparatus consisted of an HPLC pump (880-PU, JASCO, Japan), an injection valve (7125, Rheodyne, U.S.A.), and a 115 mm × 5 mm i.d. glass column (Pharmacia, U.S.A.) which was packed with Chemcosorb ODS-H (9 μm, Chemco, Japan) resin in our laboratory. A video camera with a charge-coupled device (DXC-930, SONY, Japan) was used as a detector and the video image was recorded using a standard video recorder (AG-7355, Panasonic, Japan) or a time-lapse video recorder (AG-6720A, Panasonic, Japan). The image data were analyzed by means of an image processor (PIP-4000, ADS., Japan), linked to a microcomputer (PC-98, NEC, Japan) and the analyzed video image was printed out by a video printer (CP-11, Mitsubishi, Japan).

HPLC Process The mixture of DNS-Gly, DNS-Ala, DNS-Val and DNS-Leu derivatives were separated with acetonitrile–0.01 M acetic acid (40 : 60) as the mobile phase and the separating bands were detected by their fluorescence under UV light.

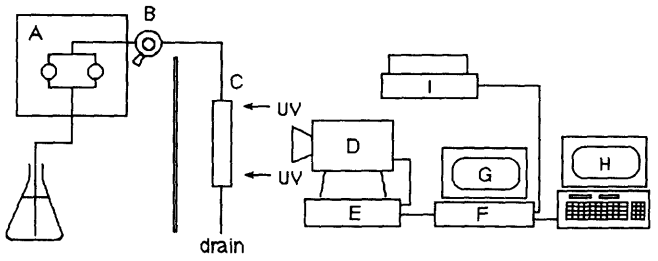

Fig. 1. Schematic Diagram of the HPLC Video Image Analzing System

A, pump; B, injector; C, column; D, video camera; E, video recorder; F, image processor; G, monitor; H, personal computer; I, video printer.

Results and Discussion

Figure 2 shows the video image of the separating solutes in the column. The fluorescent intensity of this image was converted by an image processor to a densitogram represented by the dotted curve in Fig. 2. We will call the densitogram hereafter the inside column chromatogram (ICC). The migration distance, band width, peak height and peak area of each band were evaluated from this ICC profile. In order to evaluate the performance of this system, a fundamental aspect of the separation process in a column was analyzed.

The relationship between the migration volume and migration distance is shown in Fig. 3, where a linear relationship can be seen between migration volume and migration distance. This result shows that no stirring processes, channeling, abnormality due to over-loading, *etc.*, were observed in this case. The apparent migration

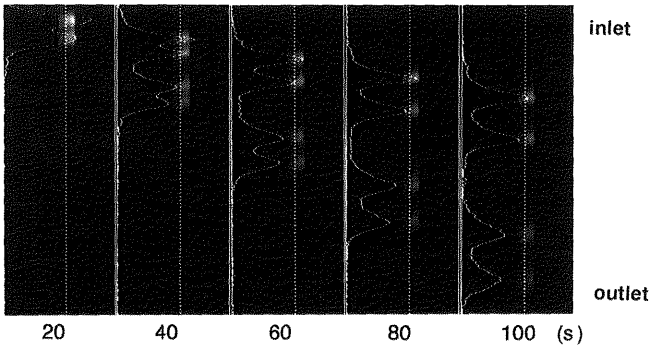

Fig. 2. Separating Bands in a Glass Column and Inside Column Chromatograms Analyzed by an Image Processor

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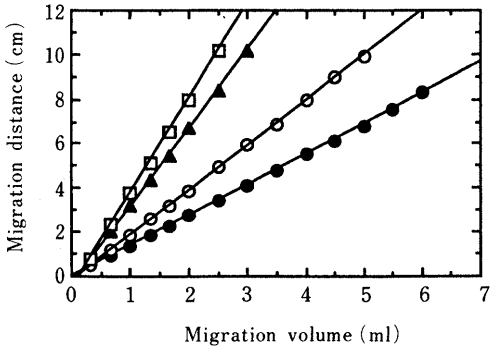

Fig. 3. Relationship between Migration Volume and Migration Distance

Sample: □, DNS-Gly; ▲, DNS-Ala; ○, DNA-Val; ●, DNS-Leu. Chromatographic conditions: flow rate, 0.8 ml/min; other conditions as described in the experimental section.

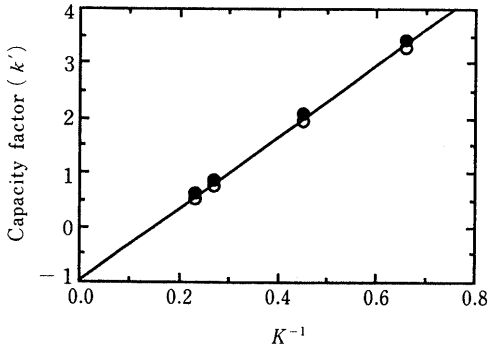

Fig. 4. Relationship between k' and K^{-1}

○, $k'_{calc.}$; ●, $k'_{exper.}$.

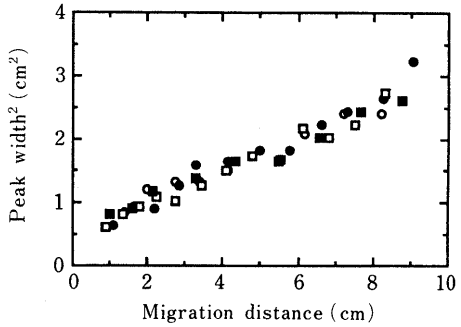

Fig. 5. Relationship between Migration Distance and the Square of the Band Width

Sample, DNS-Leu; chromatographic conditions, flow rate; ○, 1.5 ml/min; ●, 1.2 ml/min; □, 1.0 ml/min; ■, 0.8 ml/min, other conditions as described in the experimental section.

rate (K), defined as the migration distance for a unit migration volume, was determined by the slope of this line in Fig. 3.

In conventional chromatography, the capacity factor (k') has often been used to describe the chromatographic conditions. It has been found that k' is related to the apparent migration rate in the following way. R, which is defined as the ratio of the amount of solute molecules existing in the mobile phase to the total amount of solute molecules,[6] is related to K by the equation:

$$R = (\text{apparent migration rate of solute})/(\text{migration rate of solvent}) = K/K_0 \quad (1)$$

and K_0 is defined as,

$$K_0 = L/V_0 \quad (2)$$

where L is the column length and V_0 is the void volume. The capacity factor is described as,

$$k' = N_s/N_m \quad (3)$$

where N_s and N_m are the amounts of solute molecules in the solid and mobile phases, respectively.
R can be also described by,

$$R = N_m/(N_s + N_m) \quad (4)$$

from Eq. 3 and Eq. 4,

$$k' = (1-R)/R \quad (5)$$

using Eq. 1 and Eq. 5,

$$k' = K_0 \times K^{-1} - 1 \quad (6)$$

and using Eq. 2 and Eq. 6,

$$k' = L/V_0 K^{-1} - 1 \quad (7)$$

In our experiment, the V_0 of this column was estimated as 1.76 ml using acetone. Since L was 11.5 cm, the intrinsic column factor (K_0) was calculated as 6.53 cm/ml from Eq. 2. From Eq. 6, the relationship between $k'_{calc.}$ and K was

$$k'_{calc.} = 6.53 \times K^{-1} - 1 \quad (8)$$

In addition, the value of K^{-1} was obtained from the apparent migration rate in Fig. 3. The value of $k'_{exper.}$ was also estimated experimentally from the conventional chromatogram. Thus, the relationship between $k'_{exper.}$ and K^{-1} for each solute was plotted in Fig. 4 and its equation was formed to be

$$k'_{exper.} = 6.61 \times K^{-1} - 0.904 \quad (9)$$

The right sides of Eqs. 8 and 9 are in good agreement, within 10% in this experiment. These results show that k' can be represented by the intrinsic column factor (K_0) and the apparent migration rate (K).

It is well known that a solute with a large k' value exhibits a broader chromatographic peak. This peak broadening is considered mainly due to molecular diffusion, multiple-path diffusion and the delay in mass transfer. The video image analyzing system was used to investigate the mechanism of peak broadening in HPLC. When the flow of mobile phase was stopped in the course of migration through the column, the time courses of peak broadening of the solute bands were measured. However, the peak widths did not change for 30 min. Thus, molecular diffusion was not effective in this case. The relationship between the migration distance and the band width at various flow rates was plotted in Fig. 5. A good linear correlation between the migration distance and the square of the peak width was observed. This means that the theoretical plate number (N) is proportional to the column length, because N is proportional to the square of the peak width. The degree of peak broadening was nearly the same at flow rates from 0.5 to 1.5 ml/min. In other words, peak broadening depends not on the migration time but the migration distance (migration volume). This shows that

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mass transfer is sufficiently fast in this experiment and, in fact, it has been said that the delay in mass transfer is almost negligible in HPLC.[2] Thus, multiple-path diffusion increases with column length or migration distance and band broadening in liquid chromatography is mainly due to multiple-path diffusion, as shown in this experiment.

Conclusion

A video image analyzing system was developed to investigate the migration processes of separating solutes in liquid chromatography, and applied to analyze the fundamental aspects of the separation processes taking place in the column. The apparent migration rate K (migration distance of a solute/its migration volume) was correlated with the capacity factor k', such that $k' = (K_0/K) - 1$ and there is a good linear relationship between K and k'. It was also confirmed by this system that peak broadening in HPLC is not influenced by either simple molecular diffusion or the delay in mass transfer but depends mainly on multiple-path diffusion, unlike in gas chromatography. This video image analyzing system can be applied to the analysis of the dynamics of separating processes in the column and is a useful tool for investigating a variety of aspects of column chromatography.

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References

1) J. C. Giddings, "Dynamics of Chromatography," Marcel Dekker Inc., New York, 1965.
2) F. Geiss, "Die Parameter der Dünnschicht-Chromatographie," Friedr. Vieweg & Sohn, Braunschweig, 1972.
3) G. Guiochon, A. Siouffi, H. Engelhardt, I. Halász, *J. Chromatogr. Sci*, **16**, 152 (1978).
4) A. M. Siouffi, F. Bressolle, G. Guiochon, *J. Chromatogr.*, **209**, 129 (1981).
5) P. Valentin, *J. Chromatogr.*, **556**, 25 (1991).
6) S. Hara, S. Mori, T. Hanai (eds.), "Kuromatoguraphi Bunri Sisutemu (in Japan.)," Maruzen, Tokyo, 1981, p. 103.

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