MOBILE PHASE EXCHANGE PROCESS IN STEPWISE ELUTION LIQUID CHROMATOGRAPHY ANALYZED BY CHROMATO-VIDEOSCOPE

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Dynamic aspects of the solvent exchange process in stepwise elution liquid chromatography were investigated using a chromato-videoscope system. The migration velocity of a solute's band changed rapidly at the same flow rate when a second eluent overtook the band. The changing time of the migration velocity is termed an inflective time. Since a plot of the relationship between inflective time and the migration distance up to the overtaking point of each solute band showed a straight line, the solvent front of the second eluent seemed to be sharp during passage through the column. In the case where the content of organic solvent in the mobile phase was changed from higher to lower, there was a delay of the inflective time even though the solvent front of the second eluent seemed to be kept sharp.

KEYWORDS video image analysis; chromato-videoscope; separation process; linear flow velocity; in-column densitogram; stepwise elution

Stepwise elution, one of the popular solvent programmed techniques has been widely used to separate different polar mixtures in high performance liquid chromatography[1]. However, in order to set a chromatographic condition, especially for the choice of eluent and its changing time, the operator's skill is exclusively important. This comes from unclearness in the dynamic aspect of separating solutes and eluent in a column. We have developed a video image analyzing system, named chromato-videoscope, for real-time visualization of separation processes taking place in the liquid chromatography column[2]. In this report, an investigation of the solvent exchange process taking place in stepwise elution liquid chromatography using this system is presented.

The time courses of in-column densitograms under stepwise elution are shown in Fig. 1. By analyzing the densitograms at consecutive intervals, movement of the solute band and the change in band width can be comprehended. For instance, it was found that the migration speed of the DNS-Ala band (peak 3) remained slow in (a) to (d), while thereafter it became obviously fast. Since migration speed of DNS-Gly was slow in spite of a complete change of migration speed of DNS-Ala in (e), resolution of DNS-Gly and DNA-Ala seemed to become worse.

In isocratic elution, the migration distance had a good linear relationship with the migration time and its slope showed the apparent migration velocity[2]. Figure 2 shows the relationship between migration time and migration distance of four dansyl amino acids under stepwise elution mode.

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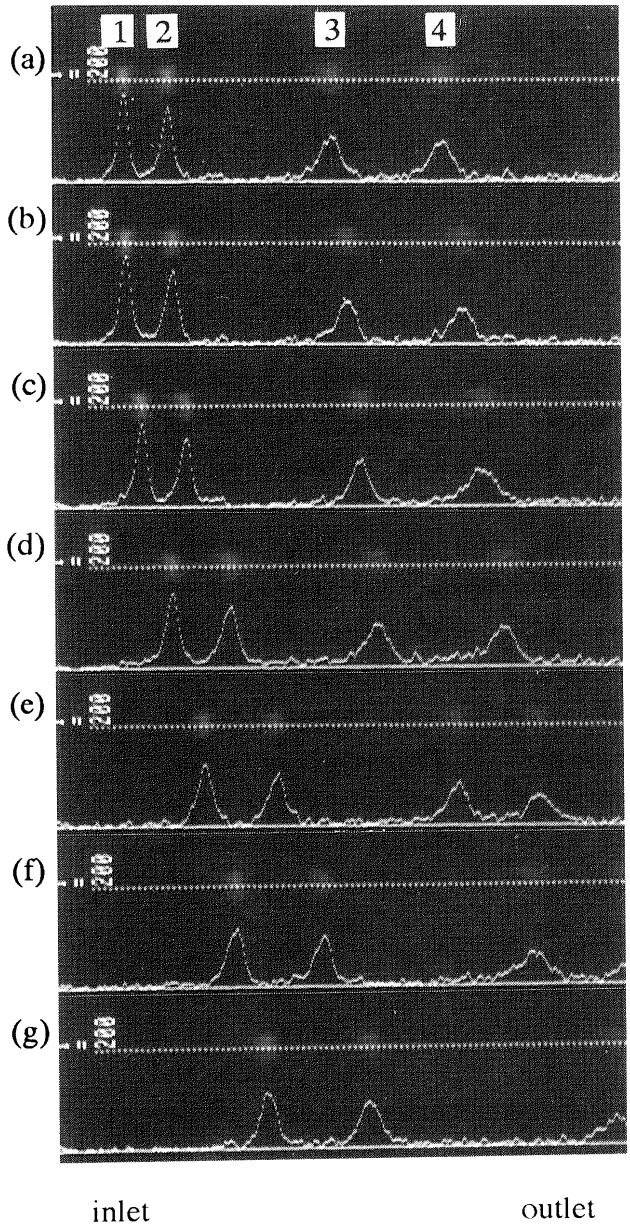

Fig. 1. Time Course of Typical In-Column Densitograms in Stepwise Elution Mode These densitograms were obtained at -30s(a), 0s(b), 30s(c), 60s(d), 90s (e), 120s(f), 150s(g) after solvent change. Chromatographic conditions : Mobile phase was changed from acetonitrile-0.1M acetic acid(20:80) to acetonitrile-0.1M acetic acid(40:60). Flow rate was 0.8 ml/min through the experiment. Column was 11.5 cm x 5 mm i.d. glass column packed with ODS. Samples were dansyl(DNS)-leucine(Leu)(1), DNS-valine(Val)(2), DNS-alanine(Ala) (3) and DNS-glycine(Gly)(4). Fluorescence of DNS-amino acids under UV light were detected by CCD video camera, and video images were analyzed and converted to densitograms by image analyzer.

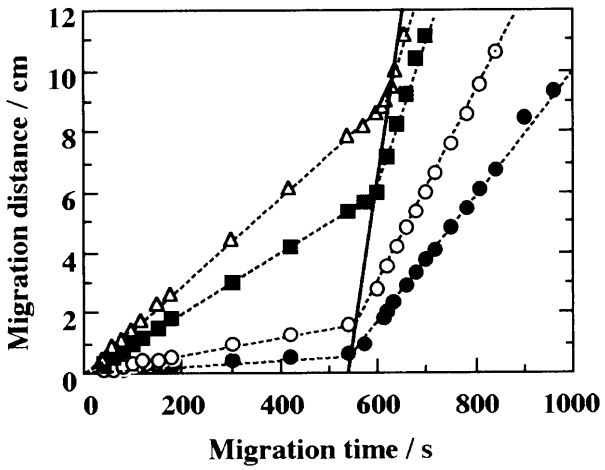

Fig. 2. Relationship between Migration Time and Migration Distance in Stepwise Elution Mode Chromatographic conditions : Mobile phase was changed from acetonitrile-0.1M acetic acid(20:80) to acetonitrile-0.1M acetic acid(40:60) at 540s. Samples are DNS-Leu(●), DNS-Val(○), DNS-Ala(■) and DNS-Gly(△). Dotted lines were obtained by linear least square method before and after inflective points. Bold line shows the fitted line by linear least square method of each inflective point. Other conditions are the same as described in the legend of Fig. 1.

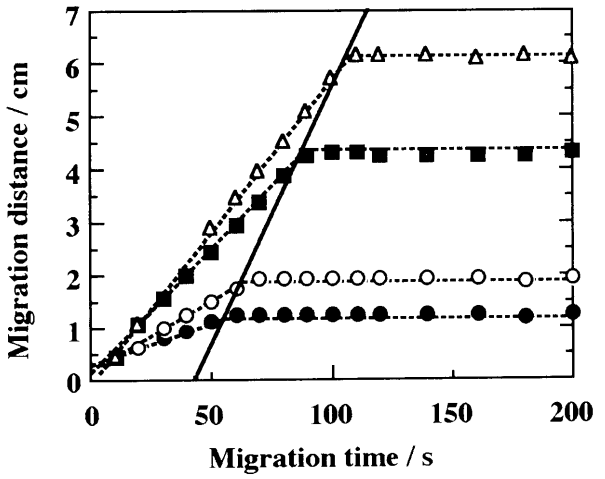

Fig. 3. Relationship between Migration Time and Migration Distance in Reversed Type Stepwise Elution Mode Chromatographic conditions : Mobile phase was changed from acetonitrile-0.1M acetic acid (40:60) to 0.1M acetic acid at 20s. Samples are DNS-Leu(●), DNS-Val(○), DNS-Ala(■) and DNS-Gly(△). Dotted lines were obtained by linear least square method before and after inflective points. Bold line shows the fitted line by linear least square method of each inflective point. Other conditions are the same as described in the legend of Fig. 1.

The bent line was observed for each solute and gave a clear inflective point. The value of the correlation coefficient for each linear dependence with a least square method (before and after

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inflective point) was at least 0.99 or better. Thus, the round shape at each bend point was not found for slow migrating solutes like DNS-Leu and DNS-Val nor for fast migrating solutes like DNS-Ala and DNS-Gly. This shows that migration velocity changed not gradually but sharply for all solute bands through a whole column length.

A plot of the relationship between inflective time and migration distance up to the overtaking point of each amino acid shows a straight line (Fig. 2 by solid). The time of the intercept of this line to the time axis was 538s, which is consistent with the experimental time on solvent change from first eluent to second one (540s). Therefore, this line was the movement of solvent front of second eluent, and velocity of solvent front of second eluent seemed to be kept constant on moving through the column. The velocity of mobile phase is preferably specified as the liner flow velocity μ (cm/min) rather than volume flow rate F (ml/min). The μ can be calculated from a dead volume or a free cross section of packed column. However, evaluation of μ is difficult in liquid chromatography because it is not established to estimate a dead volume or a free cross section of packed column. By this experiment, the μ of the mobile phase was calculated as 6.51 cm/min without any other complicated parameters like a dead volume or a free cross section of packed column.

In a reversed phase stepwise elution mode, mobile phase is usually changed from containing lower concentration of organic solvent to higher concentration of organic solvent in order for loosely retained solute to be eluted faster and for strongly retained solute to be eluted later. When mobile phase was changed from containing 40% acetonitrile in 0.1M acetic acid to only 0.1M acetic acid, migration of solute bands was stopped as shown in Fig.3. There are also two straight line functions without round shape at inflective point, and it was found that the change of migration velocity was very sharp also in this case. However, the intercept of the fitting line to time axis (45s) showed delay from the experimental time (20s). Similar delay was also observed when the mobile phase was changed from acetonitrile-0.1M acetic acid(40:60) to acetonitrile-0.1M acetic acid(20:80). Therefore, this delay was caused by the fact that organic solvent in the mobile phase was decreased stepwise. Then, this delay supposed that the lipophilic layer over the ODS-packings was not easily exchanged when the composition of acetonitrile in the mobile phase was decreased, while in the case that composition of acetonitrile in the mobile phase was increased, the layer which has more lipophilic character was smoothly changed.

In conclusion, it was found that the migration velocity of solute band in stepwise elution mode was changed rapidly, whenever the solvent front of second eluent seemed to be kept sharp during movement through the column. However, some delay was observed in reversed pattern even though inflective points were on a straight line. This delay supposed that the lipophilic layer over ODS-packings was not easily exchanged. In this report, our discussion is concerned only with change of migrating band velocity. However, many parameters are obtained by chromato-videoscope; for example, change of band shape and band broadening. These results are under further investigation from various points of view for further understanding of chromatography.

ACKNOWLEDGEMENT This study was partly supported by a Grant-in-Aid for Scientific Research (B) (04454525) and (06804043), from the Ministry of Education, Science and Culture, Japan.

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(Received August 4, 1994; accepted October 12, 1994)

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