Journal of Chromatography, 92 (1974) 77–85

CHROM. 7242

STUDIES ON THE INSTRUMENTATION OF COUNTERCURRENT CHROMATOGRAPHS*

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(First received May 2nd, 1973; revised manuscript received November 16th, 1973)

SUMMARY

A new type of countercurrent chromatograph for pre-processing has been developed. The principle of this instrument is based upon the concept developed by Ito and Bowman of the National Institutes of Health, U.S.A. The system and method of liquid–liquid chromatography are adopted. The stationary phase is held in the column by a planetary motion of the latter, that is, rotation combined with revolution, while the mobile phase is continuously pumped in and out of the column. It is possible to separate the substance by use of the differential partition coefficient between the two phases. The present report concerns instrumentation and experimental results.

INTRODUCTION

The principle of countercurrent chromatography is illustrated in Fig. 1 (refs. 1

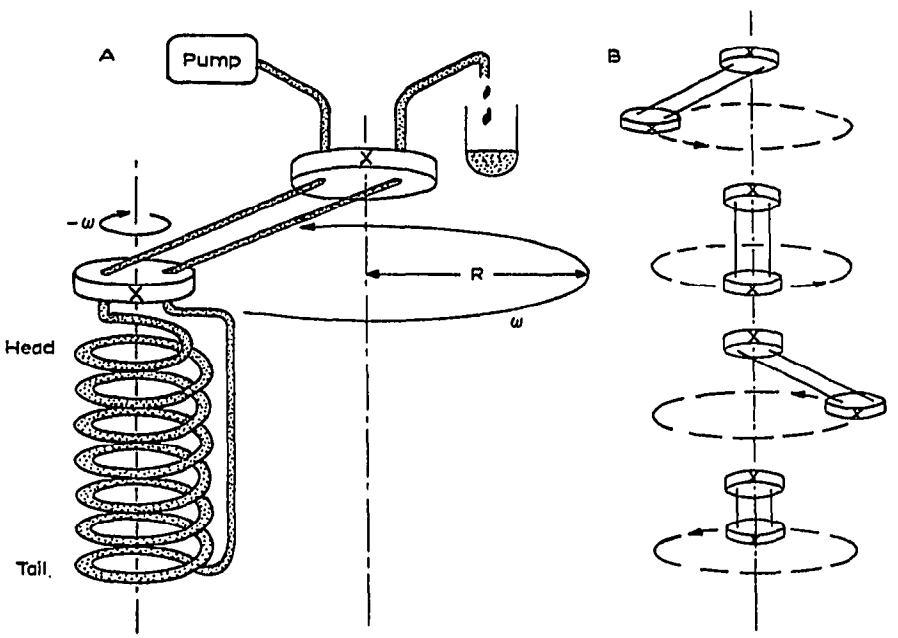

Fig. 1. Schematic illustration of the general principle of countercurrent chromatography.

* Presented at the I. International Symposium on Column Liquid Chromatography, Interlaken, May 2–4, 1973. The majority of the symposium papers has been published in *J. Chromatogr.*, Vol. 83 (1973).

and 2). The column (a coiled tube) revolves around a central axis at angular velocity ω and at the same time rotates around its own axis at angular velocity $-\omega$, through the gear mechanism. This is called planetary motion. The column holds one of two fluids, the upper or lower phase of an equilibrated mixture, as the stationary phase. Then, with the column in planetary motion, the sample is injected and the mobile phase pumped in. In the column, the two phases are arranged alternately, and the components of the sample are distributed between them and flow out in order of decreasing solubility. In this case, as the ratio of angular velocity of column revolution to that of rotation is 1 : 1, it never happens that the inlet and outlet tubes intertwine. For this reason, the countercurrent distribution proceeds continuously.

APPARATUS AND MATERIALS

Design of gyration machine

In this system, centrifugal force holds the stationary phase in the coiled tube, while rotation speed affects the separation efficiency between stationary and mobile phases. As a trial, the gyration radius was set at 200 mm and the revolution rate at 0–1000 rpm. The maximum centrifugal acceleration of the column was 220 *g*. A d.c. series motor allowing any rotation speed was used. In order to stabilize the rotation speed, the input a.c. voltage for the motor was regulated to 100 V ± 1.5% by a stabilizer. As a safety measure, the outside of the rotor was covered with iron plate of 4.5-mm thickness. It was also arranged that the main switch turned off when abnormal amplitude, overspeed, motor overload current, etc., oc-

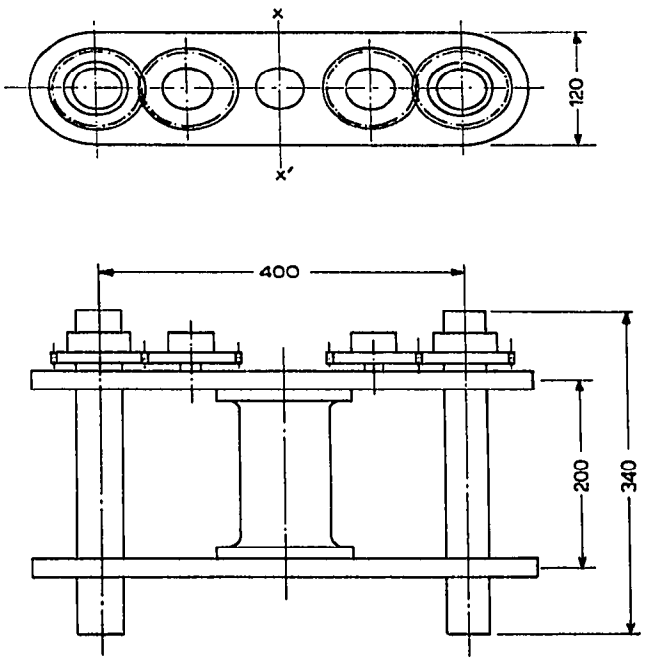

Fig. 2. Schematic diagram of rotor.

curred. The rotor and the protector are mounted on the base through a vibration-preventing rubber pad, absorbing the vibration due to rotor imbalance. The motor turns the rotor and at the same time puts the column holder into rotation with a speed ratio of 1 : 1 through the gear mechanism.

Design of rotor. Fig. 2 shows a schematic diagram of the rotor. The rotor consists of a flange, rotor arm, column holder, bearing, gear, etc., and has a symmetrical construction. The results of a numerical analysis of the strength and life-time of various components of the rotor will be described below.

Load of ball bearing and strength of column holder. The bearing load consists of uniform load due to the centrifugal force on the column holder and the concentrated load on the gear. These loads produce a bending moment on the column holder. This bending moment should not exceed the allowable bending load of the material. The model used for computation is illustrated in Fig. 3 shows the bending moment diagram obtained by a numerical calculation on the basis of given parameters.

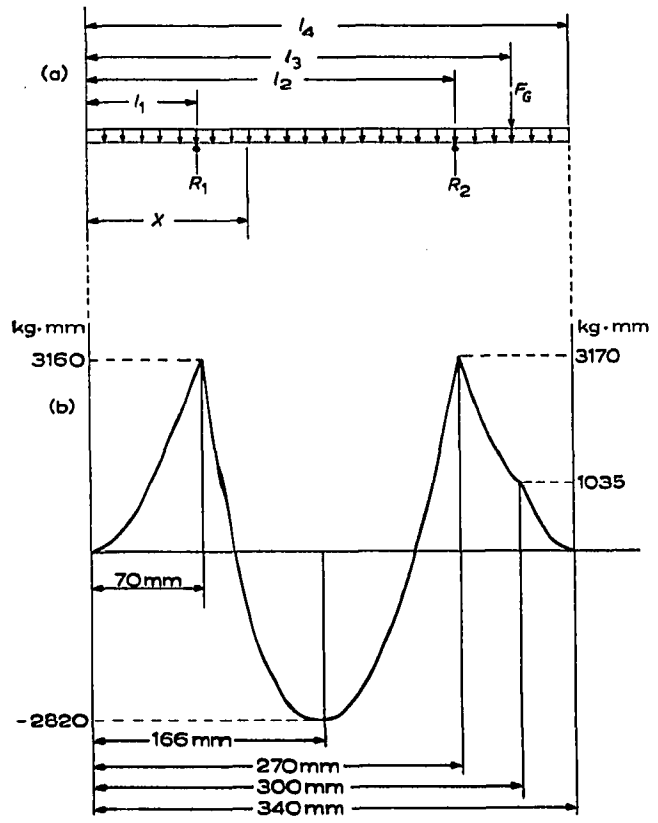

Fig. 3. (a) Column holder model. (b) Bending moment diagram.

For the system to be in equilibrium the following relations should be satisfied:

$$R_1+R_2=wl_4+F_G$$

$$R_1l_1+R_2l_2=\tfrac{1}{2}wl_4^2+F_Gl_3$$

$$F_G=\left(\frac{2\pi}{60}\right)^2\frac{W_G}{g}rn^2$$

$$F_C=wl_4=\left(\frac{2\pi}{60}\right)^2\frac{W_C+W_{CH}}{g}rn^2$$

If the distance from the origin on the left to any desired point is denoted by X, then

$$0\leqq X\leqq l_1 \qquad M_X=\tfrac{1}{2}wX^2$$

$$l_1\leqq X\leqq l_2 \qquad M_X=\tfrac{1}{2}wX^2-R_1(X-l_1)$$

$$l_2\leqq X\leqq l_3 \qquad M_X=\tfrac{1}{2}wX^2-R_1(X-l_1)-R_2(X-l_2)$$

$$l_3\leqq X\leqq l_4 \qquad M_X=\tfrac{1}{2}wX^2-R_1(X-l_1)-R_2(X-l_2)+F_G(X-l_3)$$

where

R = reaction of bearing (kg)
w = load per unit length (kg/mm)
l = length (mm)
F_G = centrifugal force due to gear (kg)
F_C = centrifugal force due to column and column holder (kg)
W_C = column weight (kg)
W_G = gear weight (kg)
W_{CH} = column holder weight (kg)
r = radius of gyration (cm)
n = revolution rate (rpm)
g = acceleration due to gravity (cm/sec^2)
X = distance from the origin (mm)
M_X = bending moment at point X (kg·mm)

From the numerical calculation, the following values were obtained:

$$R_1=215 \text{ kg}$$

$$R_2=258 \text{ kg}$$

$$M_{max.}=3170 \text{ kg·mm}$$

The maximum bending stress acting on the column holder, which is a stainless-steel (SUS 32) tube, is given by

$$\sigma_B=M_{max.}/Z$$

$$Z=\pi(D^4-d^4)/(32D)$$

where

σ_B = maximum bending stress (kg/mm^2)
$M_{max.}$ = maximum bending moment (kg·mm)
Z = sectional modulus (mm^3)
D = outer diameter of tube (mm)
d = inner diameter of tube (mm)

If a tube of $D=53.5$ mm and $d=50$ mm is used, $\sigma_B=0.9$ kg/mm^2. This value is well below the allowable bending load of the material.

Tensile strength of rotor arm. The centres of the upper and lower rotor arms are subject to the centrifugal force due to column, column holder and gear as well as to that due to the weight of the bearing and the rotor arm itself. These centrifugal forces act as a tensile load on the rotor arm. The tensile stress at the cross-section XX′ at the centre of the rotor arm shown in Fig. 2 is given by

$$\sigma_t = F_0/A$$

$$F_0 = R + F_B + F_R$$

where

σ_t = tensile stress at the centre of the rotor arm (kg/mm^2)
F_0 = tensile load at the centre of the rotor arm (kg)
A = cross-section at the centre of the rotor arm (mm^2)
R = bearing reaction, or bearing load (kg)
F_B = centrifugal force due to bearing (kg)
F_R = centrifugal force due to rotor arm (kg)

From these calculations, with $F_0=640$ kg and $A=7.28$ cm^2, the resultant tensile stress, σ_t, was 0.9 kg/mm^2.

As the rotor arm was required to be made of materials of light weight and great tensile strength, forged anticorrosion aluminum alloy was used. This material was chosen to eliminate the effects of strength reduction by chemicals and uneven quality of materials. Taking a safety factor of 10 into consideration, the allowable tensile stress of this material is 1.3 kg/mm^2.

Columns

The column may be either of rocule (chambered) type or of spiral type. In the rocule type, PTFE collars and glass spacers are alternately packed in a Junflon tube*. In the spiral type, the Junflon tube is wound around a plastic rod to form a coil. The spiral tube columns were manufactured in two versions, 0.8 mm I.D.×48 m tube length and 2 mm I.D.×10 m tube length.

As the vibration of the column affects the separation efficiency unfavourably, the column was fixed with epoxy resin on an aluminium cylinder of O.D. 50 mm, I.D. 46 mm and length 340 mm.

Operation

Fig. 4 shows the flow diagram of a countercurrent chromatograph. The operational procedures are described below.

* PTFE tubing made by Junko-sha Ltd., Tokyo, Japan.

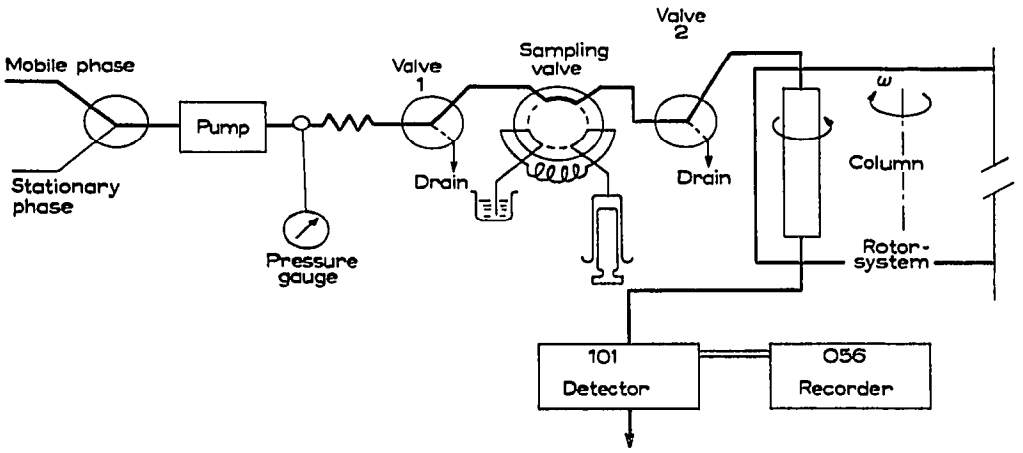

Fig. 4. Flow diagram of countercurrent chromatograph.

Mix the solvents required for the separation thoroughly and, after equilibrating, allow the upper and lower phases to separate; one of these will be the stationary phase and the other the mobile phase.

Pump the stationary phase into the column through the supply valve. Run sufficient fluid to wash out the flow path and the column.

Turning the selector valve 1 to drain side, set the supply valve so as to feed mobile phase through the pump to wash out the flow path. This drains off any stationary fluid trapped in the pressure gauge.

Turning the selector valve 2 to drain and the selector valve 1 to sampling, run the mobile phase to wash out the flow path. At the same time, the sample is fed into the sampling coil by a syringe.

Putting the column into rotation, switch the selector valve 2 to the column side and turn the sampling valve so that the sample is carried by the mobile phase to allow sampling and separation to begin.

The components separated in the column are removed in order of decreasing solubility in the mobile phase and are detected by the detector. The detected signals are recorded by the recorder. It is possible to remove sample components separately while observing the recorded signal. For the experiment a reciprocating piston pump, a Hitachi Model 101 spectrophotometer and a Hitachi Model 056 table-top recorder were used.

RESULTS AND DISCUSSION

HETP and separation factor

Analytical conditions. Chloroform, glacial acetic acid and 0.1 *N* hydrochloric acid (2:2:1) were mixed, agitated, equilibrated and left until the upper and lower phases separated. These were used as mobile and stationary phases, respectively. A 0.5-mg amount each of DNP amino acid derivatives (L-aspartic acid and L-cystine derivatives) was dissolved in the mobile phase and a 20-μl sample was taken. A 0.8-mm-I.D. Junflon tube was coiled around a 6-mm-O.D. plastic rod; the Junflon

tube was 360 cm in length and 167 turns were used for each column. The measuring wavelength was 360 nm.

Under these conditions, the revolution rate was 200, 300, 400 or 600 rpm and the flow-rate was also changed. HETP and the separation factor were calculated from the chromatogram values of two components using the following formulae:

$$H = L/N$$

$$N = \left(\frac{4 \cdot \Delta V_R}{W_2 - W_1}\right)^2$$

where

H = height equivalent to a theoretical plate
L = length of column (cm)
N = number of theoretical plates
V_R = distance between peaks 1 and 2 (mm)
W_1 = width of peak 1 on baseline (mm)
W_2 = width of peak 2 on baseline (mm)

The above formula was derived using the assumption that the two adjacent peaks 1 and 2 had nearly identical theoretical plate numbers. When the theoretical plate number is obtained for peak 1, it is greater than the value calculated from the formula shown above.

$$\text{Resolution} = \frac{OM}{OP_1} \times 100\ (\%)$$

where

OM = distance from the baseline to the trough of the chromatogram curve (mm)
OP_1 = height of peak 1 (mm)

The results are illustrated in Figs. 5 and 6.

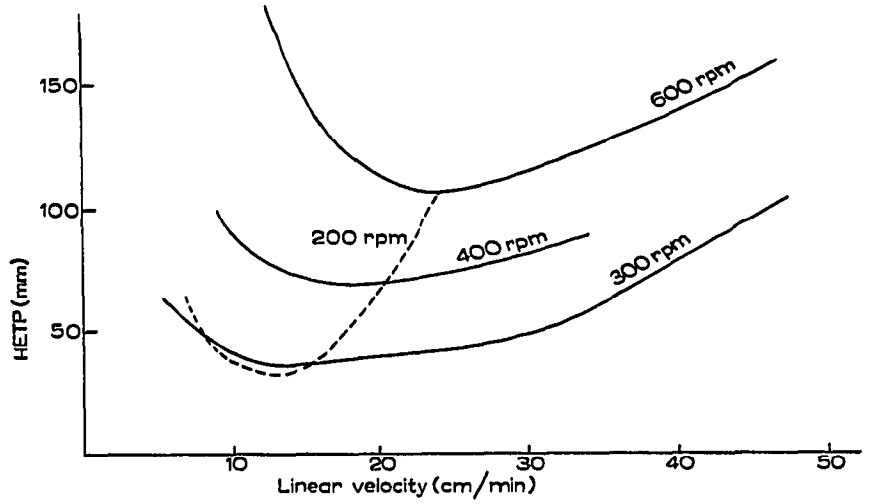

Fig. 5. HETP *vs.* flow-rate curve at various revolution rates.

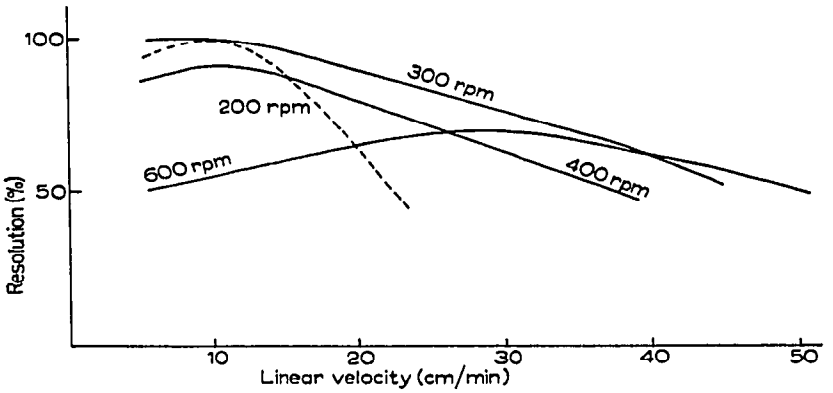

Fig. 6. Resolution *vs.* flow-rate curve at various revolution rates.

Separation of DNP amino acid derivatives

Analytical conditions. The analytical conditions, solvent system and measuring wavelength are the same as above. A 1-mg amount of each DNP amino acid derivative was dissolved in 1 ml of mobile phase and a 20-μl sample taken. A 22-m Junflon tube of I.D. 0.8 mm was coiled in 1000 turns around six plastic rods of O.D. 6 mm. The revolution rate was 300 rpm. The flow-rate of the mobile phase was 10 ml/h.

The chromatogram of the DNP amino acid derivatives in this experiment is shown in Fig. 7.

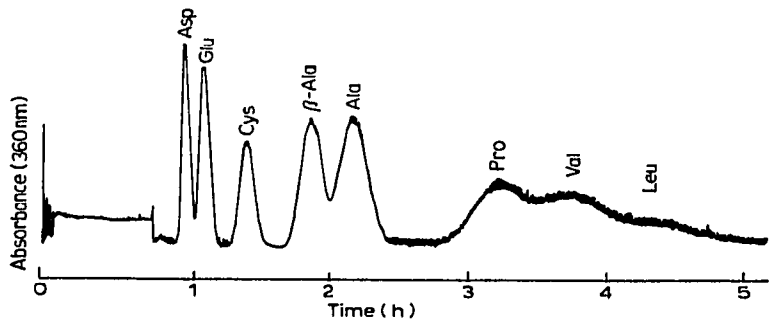

Fig. 7. Chromatogram of DNP amino acids.

Maximum sample feeding (I)

Analytical conditions. The solvent system and measuring wavelength are the same as above. A 4-mg amount each of the DNP derivatives of L-aspartic acid ($k=3.8$) and L-glutamic acid ($k=1.9$) was dissolved in 4 ml of mobile phase and 1 ml was sampled. A 48-m Junflon tube of I.D. 0.8 mm was coiled around twelve plastic rods of O.D. 6 mm. The revolution rate was 350 rpm. The flow-rate of the mobile phase was 5 ml/h.

The result is shown in Fig. 8a.

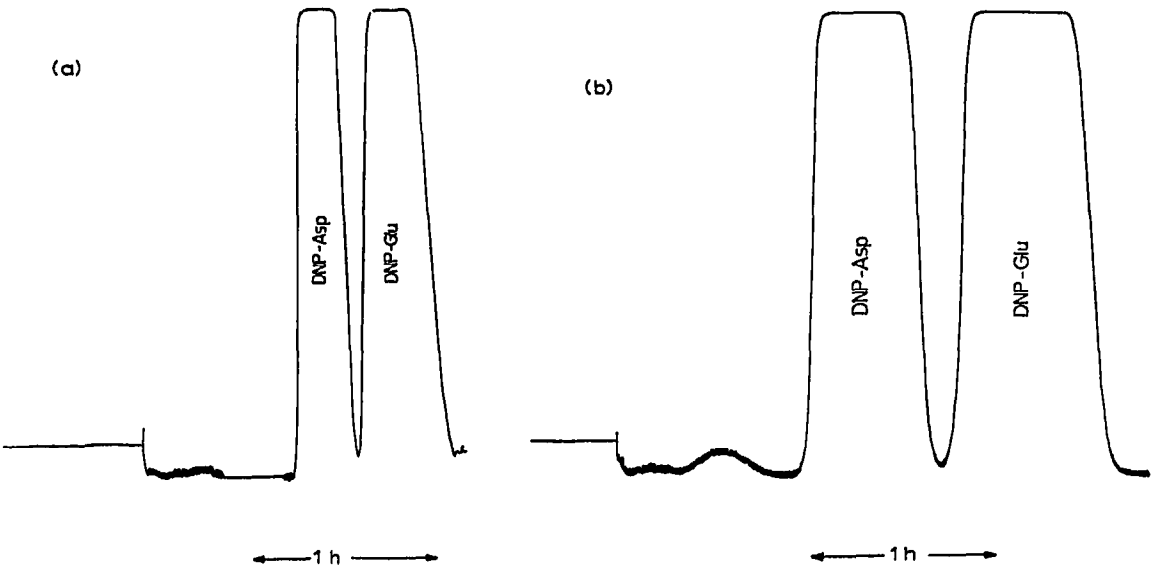

Fig. 8. Chromatograms for sample feeding. (a) 1 mg, (b) 2 mg of each sample.

Maximum sample feeding (II)

Analytical conditions. The solvent system, measuring wavelength and revolution rate of the column are the same as above. A 4-mg amount each of the DNP derivatives of L-aspartic and L-glutamic acids was dissolved in 2 ml of mobile phase and 1 ml of the solution was sampled. The flow-rate of the mobile phase was 3 ml/h.

The result is shown in Fig. 8b.

CONCLUSION

A series of experiments was carried out with the present trial equipment. From the result of these experiments, it may be concluded that adequate equipment performance is obtained and column vibration is sufficiently low.

The large quantity sampling which is the purpose of designing this equipment seems to be achieved by increasing the diameter of the column tube at the expense of the separation factor. At present, the use of 1 mm × 40 m or 1.5 mm × 25 m columns is under consideration. When using samples of large quantity and good separation, it seems necessary to use long columns of large diameter. If revolution rate, flow-rate of mobile phase and column construction are revised and improved hereafter, it may be expected that the theoretical plate number will be increased and large-quantity sampling assured.

REFERENCES

1 Y. Ito and R. L. Bowman, *Science*, 173 (1971) 420.
2 Y. Ito and R. L. Bowman, *Anal. Chem.*, 43 (1971) 69A.
3 K. Ojima and S. Ganno, *Proc. 16th Liquid Chromatography Meeting in Japan, Feb. 1973, Kyoto*, 14 (1973) 35.