STUDIES ON MICRO HIGH-PERFORMANCE LIQUID CROMATOGRAPHY

V. DESIGN OF A MICROSCALE LIQUID CHROMATOGRAPH AND ITS APPLICATION TO CATION-EXCHANGE SEPARATION OF ALKALI METALS

D. ISHII, A. HIROSE, K. HIBI and Y. IWASAKI

Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya (Japan)

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SUMMARY

A microscale liquid chromatograph has been designed and optimized by estimating band broadenings. The high-performance cation-exchange resin, Hitachi No. 2610, was packed into the column, and applied to the separation of alkali metals by radioactivity detection. Four alkali metals were separated within 26 min, using only 208 μl of 0.7 N hydrochloric acid as carrier solution.

INTRODUCTION

The efficiency of a chromatographic column is evaluated by the Plate Theory, especially in terms of plate height (HETP). Plate height is affected by various operational parameters, so that it is usually measured under fixed conditions. To evaluate the effect of column diameter (d_c) on the efficiency, plots of the HETP against d_c have been made[1,2]. Knox and Parcher[1] have shown, in their investigation of the effect on peak dispersion of the ratio of column diameter to particle diameter, that the HETP tended to decrease with decreasing column diameter. However, Wolf[2] found that the HETP *increased* with decreasing column diameter. In neither investigation, however, was the microcolumn practically examined, and the contradictory results give the impression that a microcolumn cannot be used to obtain effective separations.

Recently, we have designed microcolumns of less than 0.5 mm diameter and studied practical separations and the effect of column diameter on efficiency. We found that the microcolumns gave satisfactory separations and plate heights similar to those obtained using conventional columns. The microscale liquid chromatograph was successfully applied to various chromatographic separations in our laboratory[3–7]. This paper reports the design of the microscale liquid chromatograph and its application to cation-exchange separation of alkali metals.

DESIGN OF THE MICROSCALE LIQUID CHROMATOGRAPH

Basic equations

In contrast to the ordinary liquid chromatograph, extra-column band broadening is a serious problem when using a microcolumn. Therefore, it is important to design the microscale column by evaluating and minimizing the broadening contributions due to injection, column, connecting tube and detection cell.

The band broadening registered on the recorder is given by[8-11] $\sigma^2 = \sigma_i^2 + \sigma_c^2 + \sigma_t^2 + \sigma_d^2$ where the sigma terms express the broadening due to injection (σ_i), column (σ_c), connecting tube (σ_t) and detection cell (σ_d). When a flow-through cell is used, the broadening also depends on the time taken for the solute band to pass through the cell. Therefore, the total band broadening is given by eqn. 1, expressed in terms of volume:

$$Vw = \sqrt{(Vw_i^2 + Vw_c^2 + Vw_e^2)} + V_d \tag{1}$$

where Vw_i and Vw_c are the broadening contributions due to injection and column, respectively, and V_d is the volume of the detection cell. Vw_e is given by

$$Vw_e^2 = Vw_{t_1}^2 + Vw_{a_1}^2 + Vw_{a_2}^2 + Vw_{t_2}^2 + Vw_d^2 \tag{2}$$

where Vw_{t_1} and Vw_{t_2} are the broadenings due to the connecting tubes between injection and column and between column and detection cell, respectively, Vw_{a_1} and Vw_{a_2} are those due to the quartz wool plugs, and Vw_d is that due to the detection cell.

The band broadening in the column is given by eqn. 5, which is obtained from the Plate Theory (eqns. 3 and 4):

$$N = 16 \left(\frac{V_r}{Vw_c}\right)^2 \tag{3}$$

$$k' = \frac{V_r - V_0}{V_0} \tag{4}$$

thus

$$Vw_c = \frac{4 V_0 (1 + k')}{\sqrt{N}} \tag{5}$$

where N is the theoretical plate number, V_r is the retention volume, V_0 is the volume of mobile phase in the column, and k' is the capacity ratio.

The band broadening in the connecting tube (empty) is given by eqn. 6 when $Q/DL \ll 1$:[12]

$$Vw_t = \sqrt{\left(\frac{0.212\, S\, Q\, V_t}{D}\right)} \tag{6}$$

where L, S and V_t are the length, cross-section and volume of the connecting tube, respectively, Q is the volumetric flow-rate, and D is the diffusion coefficient; eqn. 6

shows that the broadening due to the tube decreases with decreasing tube diameter. In the present work, a very short tube was used, so the condition $Q/DL \ll 1$ was not satisfied. In such cases, the actual broadening may be more or less than the calculated value. It is considered, however, that any such error would have little bearing on the design, since the broadening contribution due to the short tube would be much smaller than that due to the column.

The detection cell is also an empty tube, so that the contribution of Vw_d in eqn. 2 can be estimated from eqn. 6. However, the full volume of the cell cannot affect Vw_d; took half the volume as the effective figure.

The band broadening due to the quartz wool plug was investigated experimentally and found to be given approximately by

$$Vw_q \approx 0.5 \, Vw'_t \approx \sqrt{\left(\frac{0.212 \, S \, Q \, V_q}{4D}\right)} \tag{7}$$

where V_q is the volume of the plug.

The effect of Vw_i on Vw in eqn. 1 was studied and the following approximation was obtained:

$$Vw_i^2 \approx 2.43 \, V_i^2 \tag{8}$$

where V_i is the injection volume of sample. Eqn. 8 was derived according to the following investigations where the band broadenings were examined by changing the injection volume.

Experiment 1. Sampling tube, 0.3 mm I.D.; connecting tube between injection and detection cell, 220×0.5 mm I.D.; detection cell, 0.73 μl; sample, methyl violet; carrier, ethyl alcohol; flow-rate, 25/3 μl/min; detection, UV detector (254 nm).

The results obtained are shown in Fig. 1. The smallest band broading recorded was 15.34 μl, when $V_i = 0.2 \, \mu$l. Under these conditions, $Vw_c^2 = (15.34-0.73)^2$; hence from eqn. 1, $Vw_i^2 = 2.2-2.6 \, V_i^2$. However, some of the injection volumes in this experiment were considerably larger than those used in practice. Therefore, a second experiment was performed on a practical scale.

Experiment 2. Sampling tube, 0.39 mm I.D.; column, 121×0.5 mm I.D., packed with Permaphase ODS; quartz wool plugs, 0.5 mm $\varnothing \times 0.3$ mm; connecting tubes between column and detection cell, 12×0.39 mm I.D. and 85×0.15 mm I.D.;
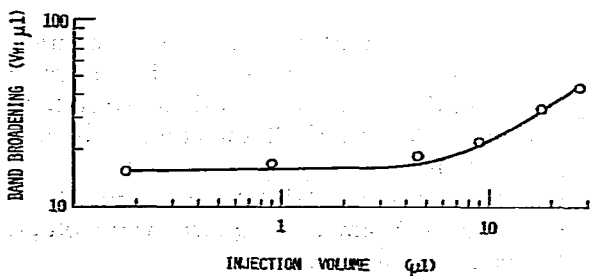

Fig. 1. Band broadening as a function of injection volume. The points are experimental values, and the line represents theoretical values.

detection cell, 0.09 μl; sample, benzene; carrier, ethyl alcohol; flow-rate, 25/3 μl/min; detection, UV detector (254 nm).

   The results obtained are shown in Fig. 2. The data were treated as in experiment 1, and we obtained $Vw_c^2 + Vw_e^2 = (2.06)^2$ and $Vw_i^2 = 2.3$–$2.5\ V_i^2$. As expected from eqn. 1, the factor 2.3–2.5 increases with increasing V_i, so we applied the result obtained from the 1.87-μl injection to determine the factor exactly and arrived at eqn. 8. Based on this conclusion, the theoretical band broadenings for experiments 1 and 2 were calculated, and are shown by the continuous lines in Figs. 1 and 2, from which it can be seen that the experimental values are in good agreement with the theoretical ones; the errors are smaller than the reproducibilities on the practical scale experiment, as shown in Fig. 2.
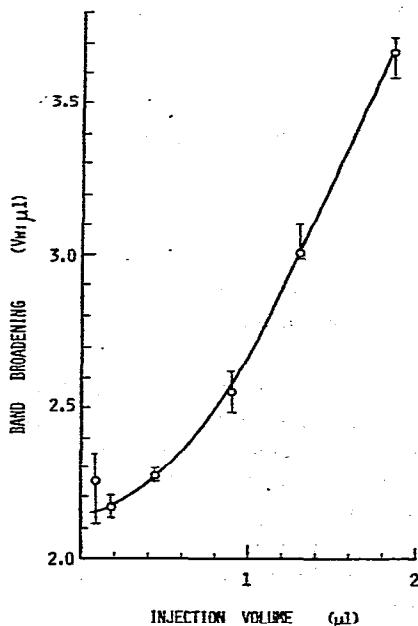

Fig. 2. Band broadening as a function of injection volume. The points and bars show the reproducibility and average of experimental values in four runs, and the line represents theoretical values.

Typical examples

   For analytical purposes, it may be essential that the total extra-column broadening contributions are less than 10%; i.e. $Vw/Vw_c = 1.1$. We should thus have $V_i/Vw_c = 0.15$, $Vw_e/Vw_c = 0.3$ and $V_d/Vw_c = 0.03$. Let us now consider the following typical examples of how the injection volume, detection cell and connecting tube parameters are determined using these "ideal" figures.

   Consider the following experimental conditions: HETP = 0.1 mm; $D = 1.0 \times 10^{-5}$ cm^2/sec; $Q = 8\ \mu$l/min; column, 100×0.5 mm I.D.; quartz wool plugs, 0.5 mm $\varnothing \times 0.5$ mm; packing ratio, 60:40 (particles:vacancy).

   The injection volume, detection cell and connecting tube parameters are estimated as follows:

For $k' = 1$ solute: injection volume, 0.3 μl; detection cell, 1.9 × 0.2 mm I.D. (0.06 μl); connecting tube between column and detection cell, 1.5 × 0.2 mm I.D.

For $k' = 3$ solute: injection volume, 0.6 μl; detection cell, 1.7 × 0.3 mm I.D. (0.12 μl); connecting tube, 6.4 × 0.3 mm I.D.

In order to achieve this instrumentation, it is necessary to (1) inject the sample by the on-column method, (2) detect the separated solutes by the on-cell method, (3) reduce the diameter of the connecting tube as much as is practicable. The lengths of connecting tubes between injection and column and between column and detection cell can be reduced to zero and 1.5 mm, respectively, and the diameter to 0.1 mm.

Microscale liquid chromatograph for the separation of alkali metals

The microscale system used in the present work is specified in Table I. It was designed for the $k' = 9.3$ solute, namely the Na^+ ion, which has the smallest k' value of the alkali-metal ions in 0.7-N HCl. Some basic parameters, such as HETP, V_0 and k', were preliminarily determined; when such parameters are available in the literature on ordinary-scale liquid chromatography, they may be used to design a microscale column, at least to a first approximation.

TABLE I

THE MICROSCALE LIQUID CHROMATOGRAPH DESIGNED FOR CATION-EXCHANGE SEPARATION OF ALKALI METALS

Basic parameters: $H = 0.12$ mm; $V_0 = 5.7$ μl (packing ratio: 60/40); k' (Na^+ in 0.7-N HCl) = 9.3; $Q = 8$ μl/min; $D = 1.35 \times 10^{-5}$ cm²/sec (value in infinite dilution)[13].

		Broadening contribution
Injection volume of sample	0.5 μl*	0.6%
Connecting tube between injection and column	zero	—
Quartz wool plug	0.5 mm $\varnothing$ × 0.5 mm	0.1
Column	72 × 0.5 mm I.D.	94.1
Quartz wool plug	0.3 mm $\varnothing$ × 0.5 mm	0.01
Connecting tube between column and detection cell	20 × 0.25 mm I.D.	1.0
Detection cell	8 × 0.25 mm I.D., 0.4 μl	4.2**

* Designed as $Vw/Vw_c = 1.05$ ($V_i/Vw_c = 0.05$, $Vw_c/Vw_c = 0.12$, $V_d/Vw_c = 0.04$); thus the injection volume can be increased to 2 μl ($Vw/Vw_c = 1.1$, $V_i/Vw_c = 0.21$) if necessary.
** $V_d = 4.0\%$, $Vw_d = 0.2\%$.

In our system, the longer connecting tube between the column and the detection cell was used because when radioactivity is applied during detection, the cell must be shielded with an acrylic acid resin plate of 10 mm thickness; the 20-mm connecting tube gave more effective shielding than the 10-mm tube[7].

EXPERIMENTAL

Apparatus

The JRR-4 reactor at the Japan Atomic Energy Research Institute was used to activate the alkali metals. It has a thermal-neutron flux of 1.8×10^{13} n/cm²/sec at the irradiation position.

An end-window type GM-counting tube connected to a scaler was used to record the radioactivity.

A micro-feeder (Model MF-2, from Azumadenkikogyo, Tokyo, Japan) was used to drive the carrier and sample solutions through the ion-exchange bed and detection cell. The micro-feeder consists of a small synchronous motor, gears and screws, by which the flow-rate can be changed stepwise. By combining a 250 μl syringe and a 0.31 mm I.D. sampling tube with the micro-feeder, 0.1–2.0 μl of sample could be collected with an accuracy of ± 0.01 μl.

Reagents

Ion-exchange resins. TSK LS-212 (obtained from Toyo Soda, Tokyo, Japan), Hitachi No. 2610 (Hitachi, Tokyo, Japan), Shodex HC-125s, Shodex HP-175 (Showa Denko, Tokyo, Japan) and Dowex 50W-X8 (200–400 mesh; Dow Chem., Midland, Mich., U.S.A.) cation-exchange resins were used. The particle sizes are *ca.* 6, 14, 12.5, 17.5 and 70 μm in diameter, respectively, and the ion-exchange capacities *ca.* 4 mequiv./g. Each of these ion exchangers has the sulphonic acid ionic group in styrene–divinyl benzene matrix, and are porous polymer particles except for Dowex 50W-X8. They were conditioned with carrier solution before use.

Radioactive alkali-metal solutions. Sodium and potassium nitrates and rubidium and caesium sulphates were wrapped in aluminium foil, irradiated in a reactor for 1 h, and then dissolved in small amounts of water. The sample solution containing the four alkali metals was prepared by mixing the solutions. The following radioactive nuclides were produced: ^{24}Na ($T_{\frac{1}{2}}$, 15 h, β_{max}, 1.39 MeV); ^{42}K (12.5 h, 3.52 MeV); ^{86}Rb (18.7 day, 1.78 MeV); and ^{134}Cs (2.1 yr, 0.66 MeV)[14].

Radioactive ^{131}I solution. Carrier-free ^{131}I$^-$ solution was obtained from the Japan Radioisotopes Assoc., and diluted to 29 μCi/μg-I and 0.25 μg-I/μl by adding potassium iodide and water; the solution was used to measure the volume of mobile phase in the column.

All the reagents used were of analytical grade, and deionized water was used throughout.

Procedure

0.5 mm I.D. (1.5 mm O.D.) PTFE tubing was used for the column material, in which the high-performance cation-exchange resin, Hitachi No. 2610, was loaded to 72 mm in length by the slurry-packing technique[3]. Each end of the column was sealed with quartz wool. The top 2 mm of the column was left unpacked for use in the sample injection and carrier solution connections.

A 1300 $\times$ 0.5 mm I.D. PTFE tube was connected to a 250-μl syringe on the micro-feeder unit, and a glass capillary sampling tube, 15 $\times$ 0.31 mm I.D. (0.51 mm O.D.), was linked to the other end of the PTFE tube. Benzene was drawn through to protect the metallic parts of the syringe from corrosion, then 0.7 N HCl carrier solution. Finally, 0.5 μl of sample solution was drawn into the sampling tube, which was then inserted into the ion-exchange column inlet. The sample and carrier solutions were driven through the ion-exchange bed at a flow-rate of 8 μl/min.

The separated alkali-metal ions were driven into the detection cell through the connecting tube. The connecting tube and detection cell were made by elongating the end of the ion-exchange column to 0.25 mm I.D. $\times$ 20 mm and 4 mm, respectively, by

heating with a gas burner. The detection cell was placed close to the GM-tube window through a 0.7 mm ∅ hole bored through the acrylic acid resin shield, and the eluent was collected on filter papers through another hole[7]; hence fractionation is possible, if necessary. The measurement of radioactivity was followed by reading the integrated counts on the scaler every 15 sec. Therefore, the differential elution profile curve could be obtained by subtraction.

RESULTS AND DISCUSSION

Selection of ion exchanger

In order to compare the ion exchangers, the plate heights obtainable were measured and compared at various flow-rates, using radioactive ^{137}Cs solution, 3-N HCl as carrier, and 55–70 × 0.5 mm I.D. columns. The results are shown in Fig. 3. As expected, Dowex 50W-X8 gave high plate heights at fast flow-rates, so that it cannot be used in high-performance chromatography. Similarly, Shodex HP-175 is somewhat inadequate, but TSK LS-212, Hitachi No. 2610 and Shodex HC-125-s gave satisfactory plate heights. In the present work, all the subsequent investigations were carried out with the Hitachi No. 2610.
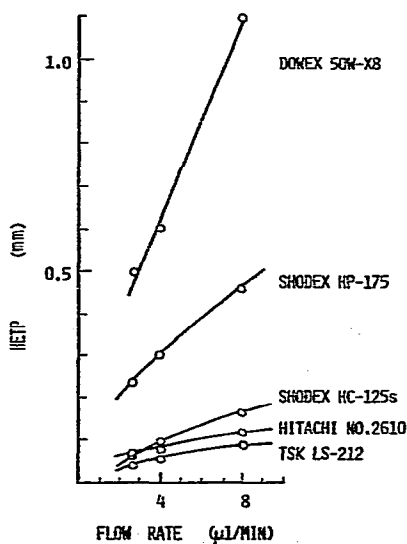

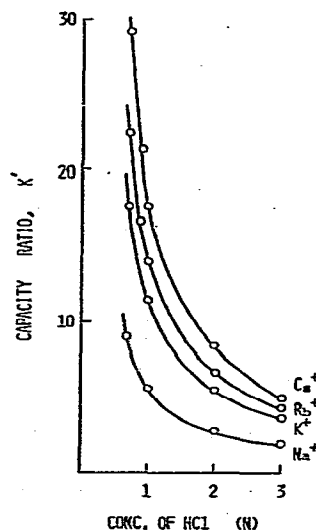Fig. 3. The height equivalent to a theoretical plate, HETP, at various flow-rates.

Fig. 4. Capacity ratios of alkali-metal ions in hydrochloric acid media.

Separation of alkali-metal ions

The capacity ratios, k', of alkali metals in various concentrations of hydrochloric acid were measured and calculated from eqn. 2. The results are shown in Fig. 4. In this case, the volume of mobile phase in the column, V_0, was measured using the radioactive ^{131}I solution, and the value of 5.7 μl for the 72 × 0.5 mm I.D. column was obtained. Thus, the packing ratio was estimated to be *ca.* 60:40.

The chromatographic separation of alkali-metal ions was carried out with 0.7 N HCl as carrier solution. The elution profile curve is shown in Fig. 5, from which it can be seen that the separation of four alkali-metal ions was satisfactory. The resolutions were estimated to be *ca.*: Na/K, 4.7; K/Rb, 1.2; Rb/Cs, 1.4.

When the 80 × 0.5 mm I.D. column was used at a flow-rate of 16 μl/min, the separation time required was reduced to 15 min, and the resolutions were about: Na/K, 4.0; K/Rb, 1.0; Rb/Cs, 1.3.
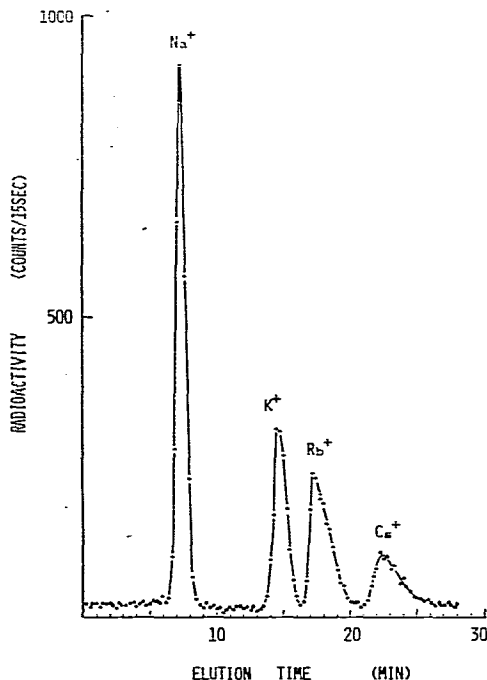

Fig. 5. Elution profile curve for cation-exchange separation of alkali-metal ions: sodium, 0.07 μg; potassium, 0.2 μg; rubidium, 3.5 μg; caesium, 6.5 μg.

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