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Improvement in Flash Chromatography

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Synopsis. Optimum and higher resolution in flash chromatography (FC) has been realized with 20—40 μm silica gel, which gives 1.4—1.9 times larger theoretical plate numbers, than the reported one, 40—63 μm. A new FC tube is proposed and the combination of quartz-made chromatographic tube and silica gel with fluorescent indicator is conveniently used for the preparative separation of colorless but UV absorbing compounds.

Medium-pressure liquid chromatography (MPLC),[1] flash chromatography (FC),[2,3] and vacuum dry chromatography[4] have been used for rapid and preparative separation of organic compounds as routine methods. These chromatographic techniques overcome the defects encountered in traditional gravity column chromatography, which is characterized by its low resolution, long separation time, inferior recovery of sample, and requisition of large amount of solvent and adsorbent. Among these techniques, FC has been accepted as a substantially reliable one from the respect of its simplicity, acceptable resolution, and speed. Still et al. reported the optimum resolution was obtained when FC column was prepared by simple dry packing of 40—63 μm silica gel.[2] During our practical application of the FC technique, we found silica gel with smaller diameter and a slurry packing method resulted in the better resolution than the reported method; we reexamined the optimization of FC with use of several kinds of silica gel with different physical properties. We also propose the practically useful FC system which allows the convenient treatment.

We systematically prepared and used four kinds of silica gel, Nos. 1—4 shown in Table 1, with different particle distribution. For elimination of the specificity of silica gel prepared, the corresponding silica gel from E. Merck with different specific surface area, average pore size, and pore volume was also examined. Optimization studies were carried out under a set of conditions using a mixed sample of toluene and nitrobenzene (1 : 1) on a 20 mm i.d.×25 cm column. In the previous paper,[2] packing method is shown not to affect the separation efficiency. We, however, found slurry packing method gave higher and reproducible column efficiency at the pressure below 1 kg cm^{-2}.[6] Since the applied pressure on silica gel packing sensitively affected the resultant column efficiency, especially for the silica gel with the diameter below 20 μm, all FC columns examined in this report were packed at the pressure below 1 kg cm^{-2}. Resolution (R_s) and separation factor (α) of aromatic components were determined, and theoretical plate number (N) was evaluated from the peak of nitrobenzene.[5] The results are shown in Fig. 1 and the typical chromatograms are illustrated in Fig. 2. The optimum resolution was obtained with the silica gel (15—40 μm and 20—40 μm) and 1.4—1.9 times larger N values for the gel with narrow particle distribution around $D_{p50}\cong 30$ μm were achieved than the reported one (40—63 μm).[2] This difference is obvious from their chromatograms (Fig. 2). Regardless of the difference in the physical properties, three types of the silica gel (Nos. 1—4, 5—8, and 9—11 in Table 1) exhibited the similar trend and column efficiency. Thus, it is concluded the column effciency is predominantly affected by both the particle size and

Table 1. Physical Properties of Various Silica Gel Used in This Work

No.	Silica gel name	Particle distribution/μm	Mean particle diameter, D_{p50}/μm	Specific surface area, S/m^2 g^{-1}
1	LP-20[a]	10—20	14	462
2	LP-40[a]	20—40	30	462
3	LP-60[a]	40—60	45	456
4	C-300[a]	45—75	55	456
5	E. Merck Si 60H[b]	45	25	486
6	E. Merck Si 60[b]	15—40	29	517
7	E. Merck Si 60[b]	40—63	50	516
8	E. Merck Si 60[b]	63	45	508
9	E. Merck LiChroPrep Si 60[b]	5—20	12	506
10	E. Merck LiChroPrep Si 60[b]	25—40	32	490
11	E. Merck LiChroPrep Si 60[b]	40—63	50	515

a) Average pore size P_d=70 Å, pore volume P_v=0.8 ml g^{-1}. b) P_d=60 Å, P_v=0.75 ml g^{-1}.

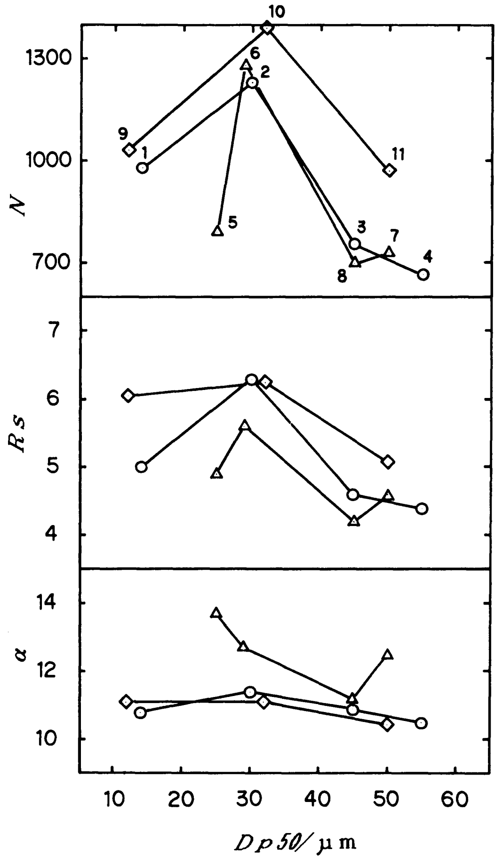

Fig. 1. Mean particle diameter (D_{p50}) vs. parameters of column efficiency, resolution (R_s), theoretical plate number (N), and separation factor (α). Numbers in the figure indicate silica gel employed, see Table 1.

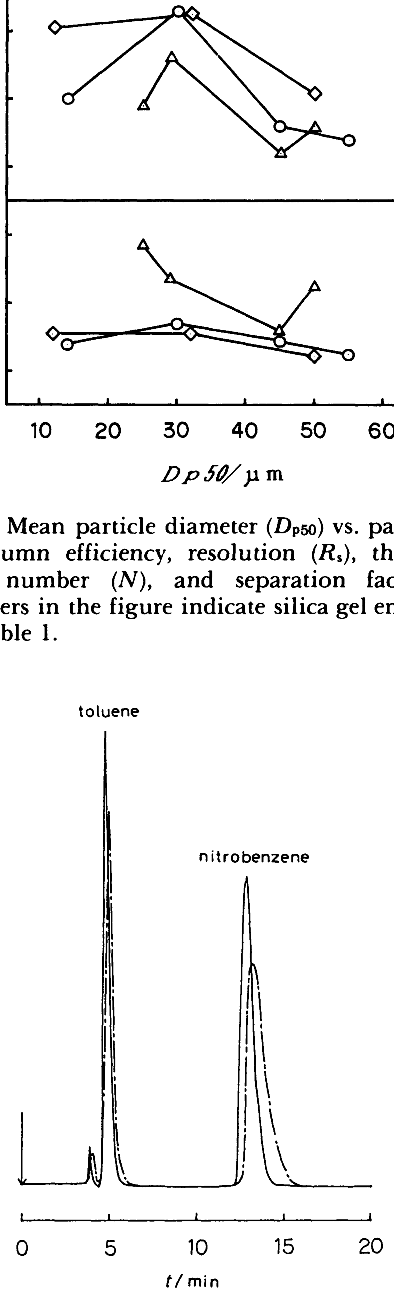

Fig. 2. Chromatograms of toluene–nitrobenzene mixture with the No. 2 silica gel (——) and the No. 3 silica gel (–––). For chromatographic conditions, see experimental section.

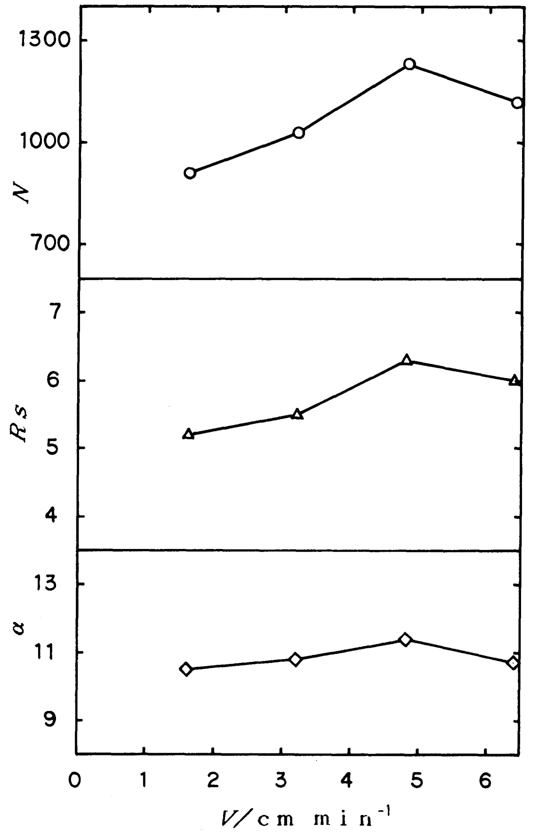

Fig. 3. Flow rate (V) vs. parameters of column efficiency. The No. 2 silica gel (LP-40) was used.

its distribution.

For the No. 2 silica gel in Table 1, effect of eluent flow rate on the resolution was examined (Fig. 3) and its optimum value was about 15 ml min^{-1} (linear velocity ca. 5 cm min^{-1}).

Improvement of FC Column. Since the use of silica gel with finer particles under the optimum separation conditions requires increased gas pressure for elution, the reported FC apparatus[2,3] turned to be not suitable for the present purposes, e.g. compressed gas leakage from the glass joint, leakage of adsorbent from the lower end of column, and difficulty in disposal of used gel. We made a new type of glass column apparatus as shown in Fig. 4. The column head **D** can be installed on the column **G** through fluoro-rubber O-ring **F** with clamp **E**, which makes possible quick installation and removal and holds them without any observable leakage of compressed gas below 2 kg cm^{-2}. The sintered glass filter **H** (G-3) effectively holds the applied adsorbent during the chromatographic operation: as long as No. 2 silica gel was used, the particles did not clog pores of the filter. The combination of the gas-tight O-ring joint and the snap-on clamp makes possible to prepare the larger internal diameter FC columns up to 50 mm and this system has practically and repeatedly been used without any problem.

For convenient monitoring of separation process during chromatography, we used quartz FC chroma-

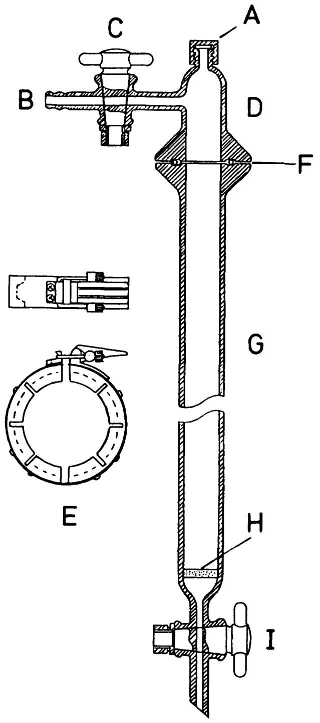

Fig. 4. Improved flash chromatography apparatus and some specifications. **A**, screw cap for pressure release; **B**, compressed gas inlet; **C**, gas stopcock; **D**, column head; **E**, O-ring joint clamp; **F**, fluororubber O-ring; **G**, column; **H**, glass sintered filter; **I**, stop cock.

tographic tubes and silica gel with fluorescent indicator in combination. When the applied solute can be detectable by TLC[7] under the illumination of UV light, this mixed fluorescent material is applicable to most of solvent systems except aromatics.[8] This method is also useful for the optimization of solvent systems during chromatography.

Experimental

Material. Irregular-shaped silica gel (Nos. 1—4 in Table 1) was sorted by pneumatic classifier, Nisshin Eng. Co., Turbo Classifier TC-15N. The particle size distribution and the specific surface area were determined with Coulter Counter, Model ZB and Yuasa Battery Co., Monosorb Surface Area Analyzer, respectively. Three types of silica gel with different distribution of particle size and average particle diameter D_{p50} were prepared (Nos. 1—3 in Table 1). The silica gel used was activated at 110 °C for 2 h. Silica gel with fluorescent indicator was prepared according to the reported method.[8] Three kinds of fluorescent materials, $Sr_2P_2O_7/Sn$, Zn_2SiO_4/Mn, and YVO_4/Eu, were mixed in a 20:5:1 ratio, respectively, and 10 wt% of the mixture was added to silica gel. As one of the silica gel frequently applied for medium pressure chromatography, Wakogel C-300 (No. 4 in Table 1) was used as a reference. For elimination of the specificity of silica gel caused by the difference of its physical properties, Merck silica gel 60 and Merck LiChroPrep (Nos. 5—11 in Table 1) were used. Physical properties of various silica gel used in this work were summerized in Table 1.

Chromatographic tubes made of Pyrex or quartz were assembled as shown in Fig. 4. Solvents for HPLC grade were used in these experiments.

Evaluation of Column Efficiency. Silica gel was charged to 20 mm i.d.×25 cm chromatographic tube by a slurry packing method. Each silica gel (60 g) was suspended in hexane (300 ml) and transferred to the hexane-filled tube at the flow rate of 80—100 ml min^{-1} keeping the pressure below 1 kg cm^{-2}. The net amount of the charged silica gel was 35—40 g. A mixture of toluene (50 g) and nitrobenzene (50 g) was diluted to 100 ml with heptane and used as a stock solution of the samples. After substitution of the column with 1 vol % acetonitril in hexane, the stock solution (250 μl) was loaded to the silica-gel column and eluted with the solvent at the flow rate 15 ml min^{-1} (5 cm min^{-1}). The effect of the flow rate on separation efficiency was examined on the range 5—20 ml min^{-1} (1.6—6.4 cm min^{-1}).

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References

1) A. I. Meyers, R. K. Smith, and C. E. Whitten, *J. Org. Chem.*, **44**, 2247 (1979).

2) W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).

3) L. J. Crane, M. Zief, and J. Horvath; *Am. Lab.*, **13**, 128 (1981); M. Zief, L. J. Crane, and J. Horvath, *ibid.*, **14**, 144 (1982).

4) E. J. Leopold, *J. Org. Chem.*, **47**, 4592 (1982).

5) Parameters of separation efficiency were defined as follows;[6]

$$R_s = \frac{2(t_{R2} - t_{R1})}{W_1 + W_2}, \quad \alpha = \frac{K_2'}{K_1'}$$

$$K' = \frac{t_R - t_0}{t_0}, \quad N = 5.54 \times [\frac{t_{R2}}{W_2/2}]^2,$$

where t_{Ri} and W_i are the retention time and the peak width of i compornent, respectively.

6) C. Liteanu and S. Gocan, "Gradient Liquid Chromatography," Ellis Horwood, Chichester (1974), pp. 55—90.

7) TLC plates with fluorescent indicator, e.g. silica gel 70 FM Plate-Wako, was applied.

8) Z. Tamura, *Talanta*, **19**, 573 (1972).