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RESEARCH ARTICLE

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Studies on column size scale-up and flow profile in conical shape liquid chromatographic column of 10° by visualization method

Abstract An improved visualization device made of polymethyl methacrylate (PMMA) was used to study the 3D flow profile inside conical columns of a 10° opening angle packed with C_{18} phase. The outside wall of the conical columns was rectangular in shape in order to improve the transparency property of the column wall and reduce the deformation of the image for better observation of the flow profiles of colored solutes inside the column. The influence of flow rate, particle size and shape on the flow profile of a colored band were studied on a 5-cm-long column and a scaled-up column of four fold in volume. It was found that the flow rates of the mobile phase had little influence on the flat flow profile of the iodine band while the properties of the stationary phase had a certain influence on them. We observed that the flow profiles of the scaled-up column were flat during the whole chromatographic process, and the efficiency and resolution of the column were also increased in accordance with theoretical prediction. The experimental results proved that the 10° conical columns can be proportionally scaled up while still keeping the flat flow profile, sample load per unit volume of packing material, and column efficiency, which are superior to the conventional column.

Keywords conical chromatography column, flow profile, visualization of chromatographic band, column scale-up, preparative liquid chromatography

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1 Introduction

Preparative high performance liquid chromatography has been widely used in phytochemistry, pharmaceutical, and biotechnological processes. Consequently, the improvement of sample loading capacity, separation efficiency, and the consumption of solvent in preparative operation have been of high concern. The direct observation of the dynamic process of sample flow profile in the whole chromatographic process through a proper on-column visualization device [1–8] is useful to the design of the column header, column shape, packing method, packing material, flow rate, and sample injection mode.

Recently, we have developed an on-column visualization method [9–12] using non-poisonous solvent for refractive index matching. In this method, the column wall material was polymethyl methacrylate (PMMA) ($n_D^{20} = 1.49$), and the visualization was improved by submersion of the transparent columns into the cubic box made of PMMA filled with glycerol ($n_D^{20} = 1.47$). This was used to compensate the refractive index difference of the column wall and air to eliminate the cylindrical lens effect during the observation of the column. Cyclohexane ($n_D^{20} = 1.43$) was used as the mobile phase. The flow profiles of the colored iodine solution in the columns were recorded with a digital camera. The technique had been used to study the dynamic flow profiles of a cylindrical column and three conical columns of different opening angles (7°, 10°, and 15°) [13, 14]. Experimental results demonstrated that the flow profile of a chromatographic band in 10° conical columns was flat rather than parabolic. Compared with cylindrical columns having corresponding lengths and volumes, the conical columns with 10º opening angle were superior in column efficiency, sample loadability, and the maximum peak concentration at column outlet; the sample load of the conical column was improved by 30%–40% on injection volume and 50%–60% on sample mass, respectively [15].

In this study, an improved on-column visualization method was developed based on our previous work. We made the transparent chromatography columns with the inner shape conical of 10° and the outer shape rectangular. The flow profiles of colored solutes inside the column can be recorded directly with a digital camera just through the column wall. The definition of the recorded pictures of flow profile was clearer than the previous one. There was no needed of organic solvent such as glycerol to eliminate the cylindrical lens effect. The dynamic flow profiles and separation processes were investigated on a 5-cm-long and scale up column of 10-cm-long 10° conical columns by using this device.

2 Experimental

2.1 Instruments and reagent

The chromatographic system consisted of an HPLC pump (LC-100P, Dalian Johnson Separation Science and Technology Ltd., China) and a variable-wavelength UV-Vis detector (LC-830, Soma Optics Ltd., Japan). The wavelength for iodine was 460 nm and 555 nm for carmine and brilliant blue. The dynamic profiles were recorded by DSC-S85 Cyber-shot digital camera (Sony Corporation, Tokyo, Japan). Chromatographic data were evaluated using KF-98 Chromatographic Station (Ver. 1.10, Dalian Scien-Tech Instruments Ltd., Dalian, China).

Analytical grade cyclohexane, methanol, ammonium acetate, and naphthalene were obtained from Shenyang Federal Reagent Factory (Liaoning, China). Mobile phase 1 was analytical grade cyclohexane, which was used in the flow profile observation. Mobile phase 2 was methanol/water (80:20, *V*/*V*) for evaluation of column efficiency. Methanol/0.1 mol/L ammonium acetate (50/50; *V*/*V*) was used as mobile phase 3 for the separation of carmine and brilliant blue. Iodine (obtained from Tianjin Litejier Environmental Protection Institute) was used as a probe sample to study the flow profile inside the column, the concentration of which was 10 g/L. The sample was filtered by a SPE cartridge packed with the same packing material as the test column. Carmine and brilliant blue were purchased from Tianjin Dye Industrial Institute (Tianjin, China). Naphthalene was dissolved in mobile phase 2 and the concentration was 1.2 g/L. Carmine and brilliant blue were dissolved in the mobile phase 3 as sample, the concentrations of which were 2 g/L and 0.15 g/L, respectively. Ultra pure uracil was from Amresco (USA).

2.2 Visualization device

The visualization device is shown in Fig. 1. The transparent columns were machined from a solid block of PMMA. The inner shape was conical with an opening angle of 10° and the outer shape was rectangular. All surfaces were nicely polished. In this design, the flow profiles of colored solutes were recorded directly with a digital camera just through the column wall. The cylindrical lens effect is avoided by changing the outer shape of the conical column from cylindrical to rectangular. Because of the good mechanical character of PMMA, it is possible to machine the column shape of the inside and outside as we wish.

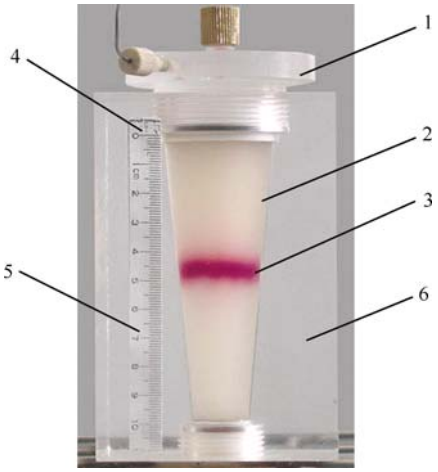

Fig. 1 Photograph of visualization device
1) Inlet; 2) Packed liquid chromatographic column; 3) Flow profile of sample band; 4) Frit and distributor; 5) Ruler; 6) Column wall

Porous stainless steel frits having a pore size of 10–20 μm and thickness of 1.5 mm and 2 mm were obtained from Bejing Antai Science and Technology Ltd., China. Their diameters are the same as the inlet and outlet diameters of the test columns. A paper ruler was fixed beside one vertical side of the test column to allow precise measurement of the position of the sample band inside the column. The packing materials (43–63 μm irregular and 40–60 μm and 20 μm spherical C-Gel C_{18} bonded silica gel) used were from Beijing Jinouya Science and Technology, Ltd., China. The columns were dry packed.

2.3 Preparation of conical column

Two sizes of conical columns were prepared: column 1 was a 50 mm × 18 → 9 mm i.d. and column 2 was 100 mm × 36 → 18 mm i.d. Because PMMA can be easily dissolved in the solvent, stainless columns of the same size as PMMA columns were prepared and used in the evaluation of column efficiency.

A saturated solution of iodine in cyclohexane at the concentration of approximately 10 g/L was used as the probe solute. The injection volume was 80 μL for column 1 and 600 μL for column 2, for the observation of sample flow profile. The injection volume of carmine and brilliant blue solution for column 1 and column 2 was 40 μL and 160 μL, respectively.

3 Results and discussion

3.1 Observation of flow profile

Since the visualization device is the column itself, the axial positions of the inlet and outlet of the inner conical column can be easily allocated from the outside. A paper ruler was fixed to one of the vertical side to allow precise measurement of the position of the sample band inside the column. The zero position was started from the same level as the column inlet. The distance of the colored band from the inlet along the column axis can be measured precisely.

3.1.1 Dynamic flow profiles inside the columns

The dynamic flow profile of column 1 observed through the visualization device and recorded by digital camera is illustrated in Fig. 2. The picture shows that the flow profile was flat during the whole eluting process. The sample band was tailing in column packed with irregular C_{18} phase, as showed in Figs. 2(A) and 3(A), resulting in the decline of column efficiency (refer to Figs. 2(B) and 3(B)). However, there was no obvious tailing of the sample band in the column packed with sphere shape C_{18} phase. The reason was that irregular shape C_{18} phase contains large amounts of pores about 3 nm; while the number of this type of pore was much less in sphere C_{18} phase. Therefore, the residence of iodine in the irregular C_{18}, in the 3-nm-pore in particular, was stronger than that of sphere C_{18} phase.

The chromatograms of the two columns under a UV detector are shown in Fig. 3. The result was consistent with the pictures of the flow profile shown in Fig. 2. The above examples demonstrated that real dynamic process of the sample band in the column can be observed.

3.1.2 Effect of flow rate on the flow profile

With the aid of a digital camera and a homemade ruler, the influence of flow rate on flow profile of column 1 was investigated. Figure 4 shows the relationship of flow rate and maximal length of keeping flat flow profile on two different stationary phases. The result demonstrated that the effect of flow rate on flow profile was slight, i.e., the maximal lengths of keeping flat flow profile were invariable in the range of 1–8 mL/min. Comparing the two stationary phases, the maximal length keeping flat flow profile of sphere C_{18} was longer than that of irregular C_{18}. This indicated that the performance of sphere C_{18} was superior to irregular C_{18}.

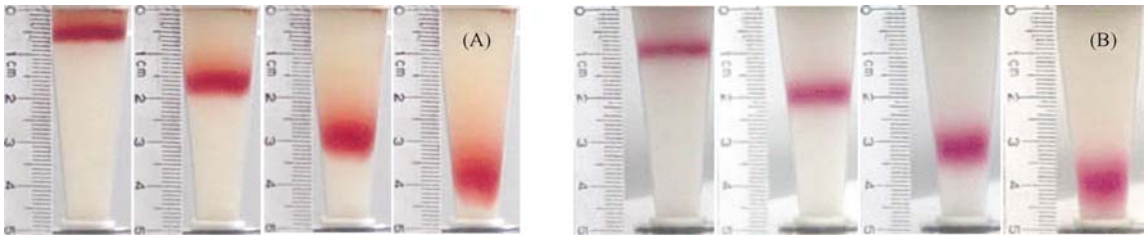

Fig. 2 Photographs of dynamic flow profiles in column 1
(A) Irregular particles C-Gel C_{18}; (B) Spherical particles C-Gel C_{18}

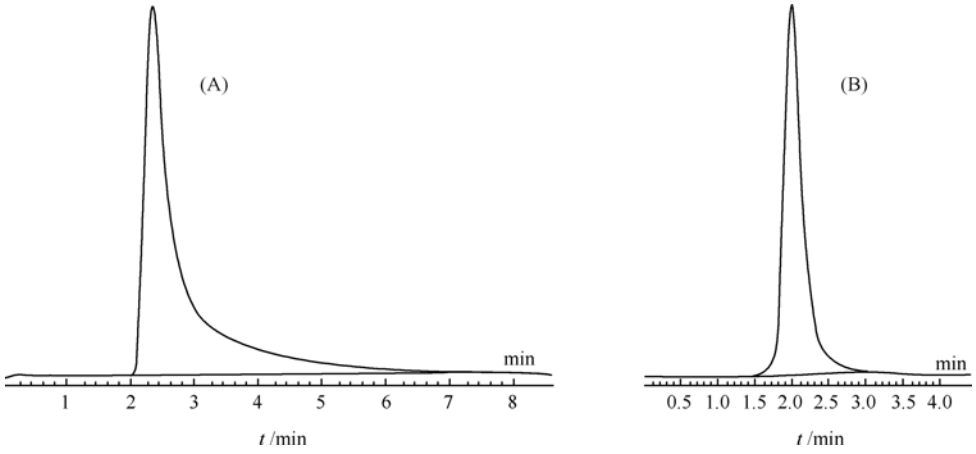

Fig. 3 Chromatograms of the sample from column 1 with UV detection
(A) Irregular particles C-Gel C_{18}; (B) Spherical particles C-Gel C_{18}

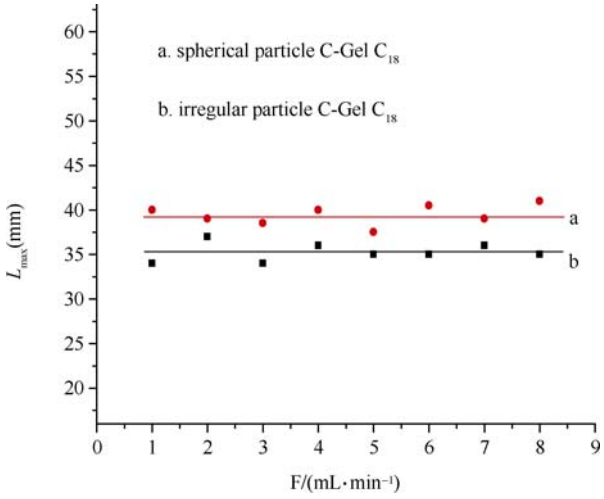

Fig. 4 Effect of mobile phase flow rate on flow profiles for column 1

3.1.3 *Effect of the particle size of C_{18} phase on flow profile*

We evaluated the effect of the particle size of the C_{18} phase on the flow profile. Instead of (50 ± 10) μm sphere C_{18}, we packed column 1 with 20-μm sphere C_{18} and recorded the flow profile at a flow rate of 2 mL/min, as shown in Fig. 5. It shows that the flow profile was keeping flat in the whole chromatographic process. We concluded that the particle size of the stationary phase had little effect on the flat flow profile in a conical column of 10°. However, the width of the sample band in the column packed with 20-μm sphere C_{18} was narrower than that of (50 ± 10) μm sphere C_{18} at the same injection volume and flow rate condition. Figure 6 shows the corresponding chromatograms on the columns packed with different C_{18} phases. The peak half width in Fig. 6(A) and Fig. 6(B) were 0.26 min and 0.19 min, respectively. This result was consistent with the pictures of the flow profile obtained from the on-column visualization device. The on-column visualization device is a valuable tool for direct measurement and evaluation of column performances of preparative liquid chromatography.

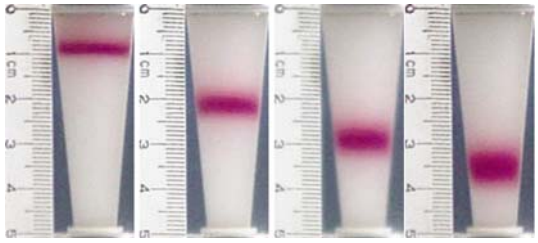

Fig. 5 Photographs of dynamic flow profiles of column 1 packed with 20 μm spherical particles C-Gel C_{18}

3.1.4 *Effect of column size on flow profile*

We proportionally scaled up column 1, and obtained a 10-cm-long conical column 2. A sample distribution disk was added on top of column 2. The flow profile of iodine was flat during the whole chromatographic process, as shown in Fig. 7. The sample was distributed uniformly within the column inlet part, as shown in the first picture of Fig. 7, indicating that the design of the column header and the distributor was successful.

3.2 Effect of column dimension on the column efficiency and resolution

Figure 8 demonstrates the relationship between average linear velocity of the mobile phase and the effective plate height of columns for naphthalene (mobile phase 2) on 10° conical columns with different dimensions. The theoretical plate number of column 1 was 524/5 cm and the reduced plate height ($h = H/D_p$) was 1.91; the theoretical plate number of column 2 was 1 171/10 cm and the reduced plate height was 1.71. The column efficiency of column 2 was improved by 10% compared with that of column 1. Because the column inlet cross-sectional area of column 2 was four times larger than that of column 1, the injection volume of column 2 (160 μL) was also four times larger than column 1 (40 μL). The results showed that the column loading increased proportionally with the column volume as the column

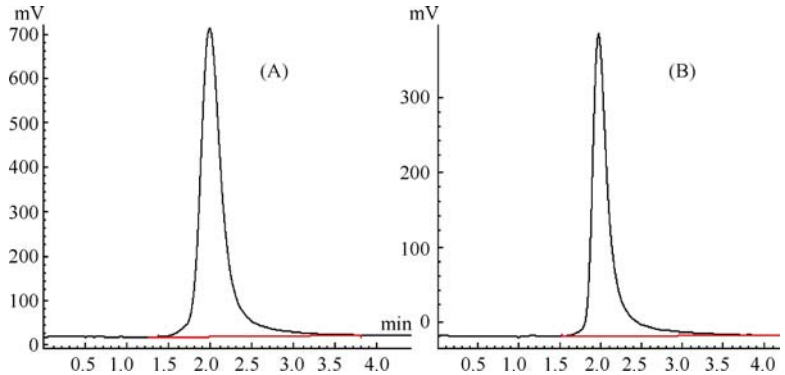

Fig. 6 Chromatograms of sample from column 1 with UV detection
(A) Spherical particle C-Gel C_{18}, 40–60 μm; (B) Spherical particle C-Gel C_{18}, 20 μm

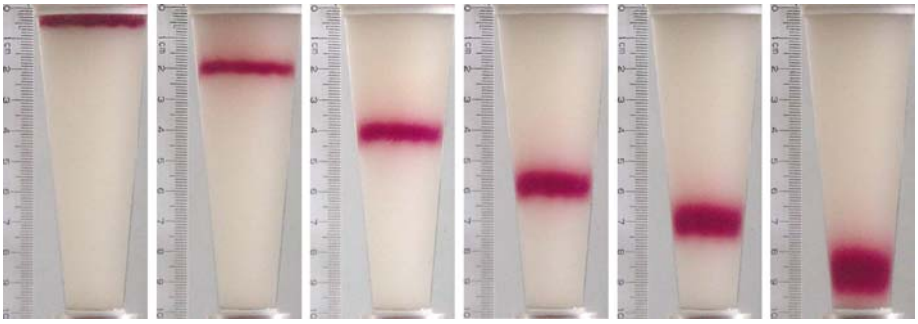

Fig. 7 Photographs of dynamic flow profiles in column 2

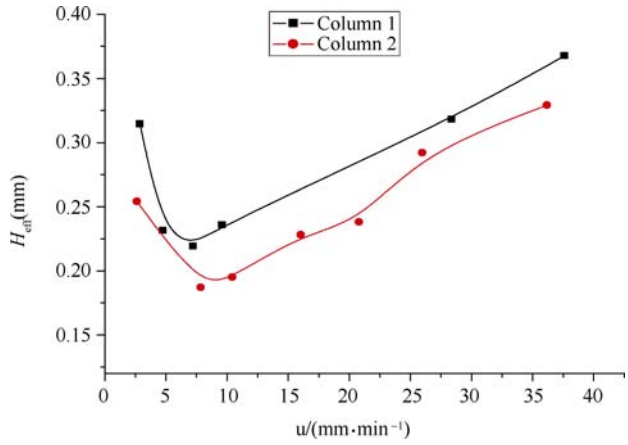

Fig. 8 Effect of flow rate on plate height

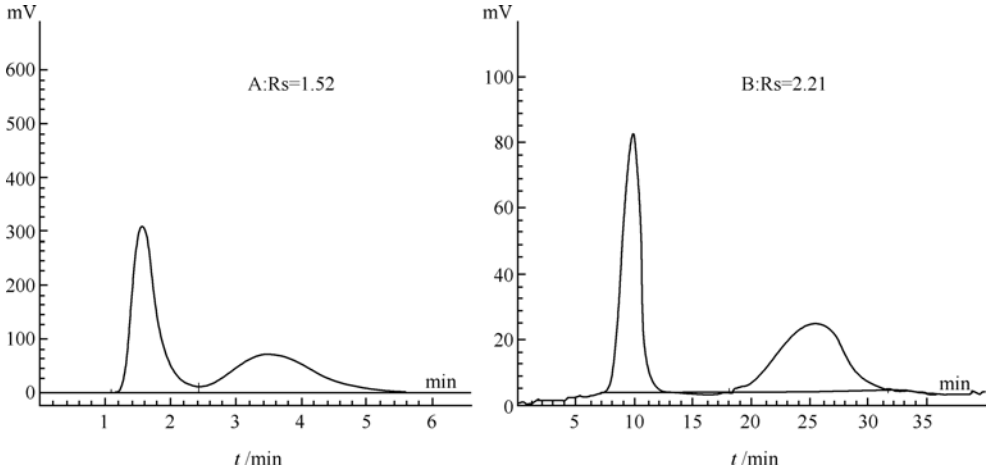

Fig. 9 Chromatograms of carmine and brilliant blue separated on column 1 (A) and column 2 (B).
Conditions: Mobile phase: methanol/0.1 mol/L ammonium acetate (50/50)(*V*/*V*)； Flow-rate: 4 mL/min；
Sample: 2g/L carmine and brilliant blue solution； Detection wavelength: 555nm.

size scaled up, and the column efficiency was improved further due to the decrease of extra-column effect.

We also investigated the effect of column size on resolution by using carmine and brilliant blue as the test samples. They were separated on column 1 and column 2 using the same packing material and mobile phase (mobile phase 3). Figure 9 shows the resulting chromatograms with UV detection. The resolutions of carmine and brilliant blue for column 1 and column 2 were 1.52 (R_{s1}) and 2.21 (R_{s2}), respectively. The value of $R_{s1}/R_{s2}=1.45$ was larger than $\sqrt{2}$, indicating that the resolution was improved when the 10° conical column was scaled up. We did not find the reason for the additional improvement on resolution.

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