

Study on conical columns with different conical angles for semi-preparative liquid chromatography

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Abstract

The dynamic flow profiles and separation performances in conically shaped preparative liquid chromatographic columns (inlet i.d. larger than outlet i.d.) with three different angles (7, 10 and 15°) were studied and compared with cylindrical column of the same length and internal volume. The shapes of dynamic flow profiles were studied by on-column visualization method. The transparent chromatographic columns made of polymethyl methacrylate (PMMA), packed with C₁₈ bonded silica, were immersed into a cubic pool filled with glycerol to eliminate the cylindrical and conical lens effect. The flow profiles of colored iodine solution in the columns were observed clearly using cyclohexane as mobile phase since the refractive indices of C₁₈, column wall and the mobile phase are very close. In the conical column of 15° (20–7 mm i.d.) the mobile phase in the central region migrated slower than in wall region as it moved toward the column outlet, while in the conical column of 7° (17–11 mm i.d.) the mobile phase in the central region migrated faster than in wall region just like in cylindrical column. We found that a plug-like flow profile was generated in the conical column of 10° (18–9 mm i.d.) during the whole migration process. A carmine and brilliant blue mixture was used as a probe to test the separation ability of the columns. The resolutions of the two compounds on the conical column of 7, 10, 15° and on the cylindrical column were 0.6, 1.57, 1.29 and 0.8, respectively.

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1. Introduction

Preparative high performance liquid chromatography is being used for obtaining pure, particularly biologically active compounds. Better separation efficiency and higher loadability for samples can improve throughput and save operating costs. In preparative liquid chromatography large volume of samples are injected onto separation columns in order to achieve high throughput. As the sample band moves through the column, it is gradually diluted by mobile phase. Since the sample load is proportional to the inlet cross-section area (A_{in}) of a column, and the A_{in} of the conical column is larger than that of cylindrical column of the same length and volume, conical columns with larger inlet i.d. than outlet i.d. were considered advantageous in preparative separations. Said [1] showed in his theoretical calculations that the conical column (100 cm × 3 cm i.d. →

1 cm i.d., conical angle 1.1°) was more efficient than the coupled column with equal quantity of stationary phase and identical total length (41.7 cm × 3 cm i.d. + 58.3 cm × 1 cm i.d.). Although the theoretical study has been made two decades ago, conical columns for preparative chromatography were rarely used in practice. According to the literature reported only a plastic conical column with the conical angle of 5.5° [2] has been used in terms of minimizing the band volume occupied by the chromatographic fraction. A conical column (5°) with smaller inlet i.d. than outlet i.d. [3] showed higher column efficiency because of less wall effect, but has the drawback of lower sample capacity than cylindrical column, waste of solvents and packing materials. Pecavar et al. [4] reported a conically shaped analytical column (150 mm × 4 mm i.d. → 2.5 mm i.d., conical angle 0.6°). Nugent [5] invented a 25 mm × 2 mm → 0.5 mm conical column with the conical angle of 3.4°. All the conical columns studied so far, as we found, are low angle columns (≤5.5°). However, these reports did not mention the relationship of conical angle and column efficiency.

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The flow profile inside a column has close relation with the efficiency of the column. The column with a flat flow profile in capillary electro-chromatography or capillary electrophoreses under the electroosmotic drive has higher column efficiency than that with a parabolic flow profile in the conventional preparative liquid chromatography with cylindrical column under pressure drive. In order to observe the flow profile inside a column, a method called on-column visualization method was introduced [6] which could visualize the shape of a solute band during its migration along a cylindrical column. The key parameters in the method are the match of refractive indices n_i between the transparent column wall, the stationary phase and mobile phase. When a colored sample was injected onto a column, the flow profile was observed clearly because the whole column is transparent. To eliminate the cylindrical lens effect, the columns were immersed into a cubic pool filled with solvent of similar refractive index as that of the column wall material [7].

Based on the theory of hydrodynamics, we postulated that the angle of conical columns should affect the flow profile of mobile phase, and hence the column efficiency. We have studied the dynamic flow profiles and column efficiencies in conically shaped semi-preparative liquid chromatographic columns of two angles (7 and 15°) [8]. Different from that in the conical column of 7° and in the cylindrical column, the sample band in the conical column of 15° migrated slower in the central region than in the wall region, which indicated that a flat flow profile might be realized in a conical column with a conical angle between 7 and 15°. In this paper, we studied the flow profiles and separation performances of conical columns with angles of 7, 10 and 15°. The results demonstrated that a flat flow profile (plug-like flow profile) in semi-preparative liquid chromatographic columns could be realized in the conical column of 10° under our experimental condition.

2. Experimental

2.1. Mobile phases and chemicals

Analytical grade cyclohexane, used as mobile phase in flow profile observation, was purchased from Shenyang Solvent Plant (Liaoning, China). Analytical grade glycerol and iodine (99.9%) were purchased from Shenyang Federal Reagent Factory (Liaoning, China). Glycerol was used to compensate the refractive index difference of the column wall and air in order to eliminate the cylindrical or conical lens effect. Iodine was used as sample probe for study the mobile phase flow profile. The samples were filtered by a solid phase extraction column packed with the same packing material as the test column, and then through a 0.45 μm filter before usage.

Methanol/0.1 M ammonium acetate (50/50, v/v) was used as mobile phase for the separation of a sample containing two components-carmine and brilliant blue. Analytical grade

ammonium acetate was purchased from Shenyang Reagent Factory. Carmine and brilliant blue were purchased from Tianjin Dye Industrial Institute (Tianjin, China) and were dissolved in water as sample. The mobile phase was filtered through a 0.45 μm filter before usage.

C-Gel C₁₈ (diameter 40–63 μm , irregular particle, pore size 90 Å) from CU Chemie Uetikon AG (Switzerland) was used as the chromatographic stationary phase.

2.2. Columns

The conical columns and the cylindrical column were machined from solid block of polymethyl methacrylate (PMMA). For polishing a column, a metal mold with conical shape was machined. Then cover the mold with very fine grain and rotate it in the PMMA column. After that a polishing column can be obtained. The factory that machined the PMMA columns did this procedure. The lengths of both conical columns and the cylindrical column were identical, 50 mm long. The internal volumes of the columns were similar. In our experiment, three different angles of conical columns were used: (a) the 7° column with the inside diameter varied from 17 to 11 mm, internal volume of 7.8 ml; (b) the 15° column with the inside diameter varied from 20 to 7 mm, internal volume of 7.7 ml; (c) the 10° column with the inside diameter varied from 18 to 9 mm, internal volume of 7.7 ml. The cylindrical column for comparative study was 50 mm \times 14 mm i.d. with the inner volume of 7.7 ml. All columns were filled with about 5 g of the same stationary phase, and were prepared by a dry packing technique. The headers of the columns were designed for on-column injection, and were made of transparent PMMA for observation of the sample distribution at the column inlet. Porous stainless steel frits with pore size 10–20 μm and thickness of 1.5 mm (Beijing Antai Science and Technology Ltd., China) were used. The diameters of the frits were the same as the inside diameters of the columns. Frits were soaked in methanol and sonicated for 30 min for removal of dust and deposit before usage. Because PMMA can be dissolved in acetone, tetrahydrofuran and halide hydrocarbon etc., the mobile phase and samples should contain no such solvents.

2.3. Equipment

The chromatographic system consisted of an HPLC pump (LC-100P, Dalian Johnson Separation Science and Technology Ltd., China), a variable-wavelength UV-Vis detector (LC-830, Soma Optics Ltd., Japan) and the self-made columns. Data were evaluated with KF-98 Chromatographic Station (Ver. 1.10, Dalian Scien-Tech Instruments Ltd., Dalian, China).

2.4. Visualization device

In on-column visualization method, the choice of stationary phase and mobile phase was very important. The

refractive index n_D^{20} of the stationary phase should be close to that of the mobile phase so as to obtain a transparent column bed. We compared the influence of methanol ($n_D^{20} = 1.33$) and cyclohexane ($n_D^{20} = 1.43$) as mobile phase on

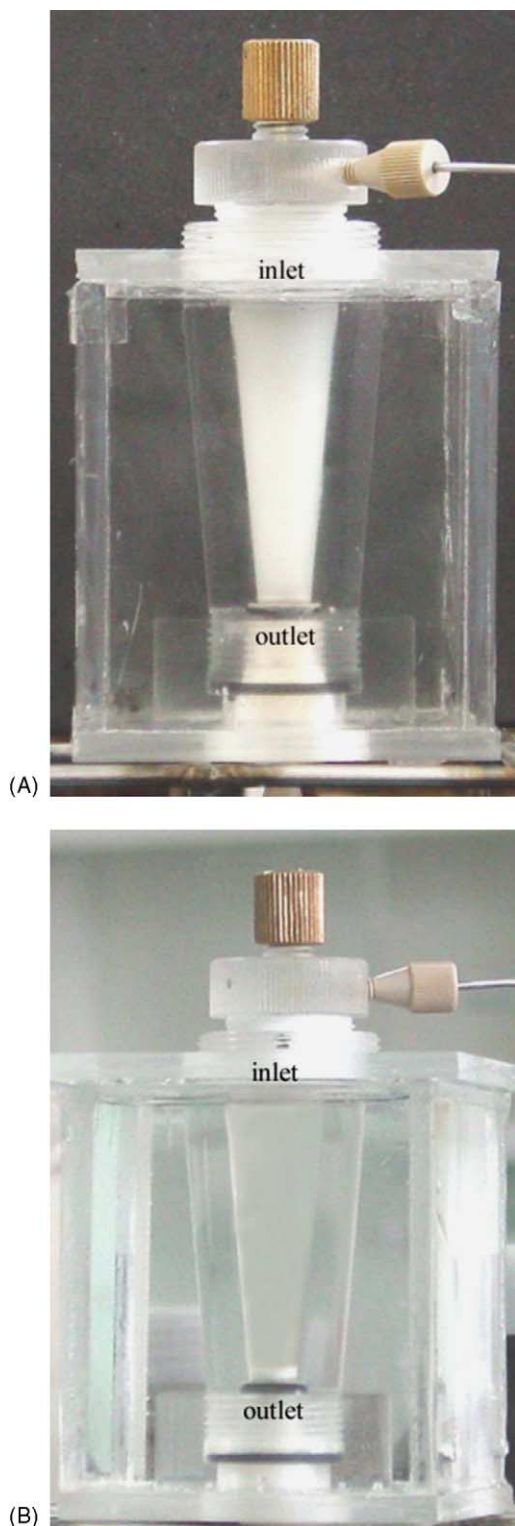


Fig. 1. Transparency of column bed with methanol and cyclohexane as mobile phase. (A) Mobile phase: methanol. (B) Mobile phase: cyclohexane.



Fig. 2. Photograph illustrating the cylindrical lens effect using the reservoir half filled with glycerol.

transparency of column bed (see Fig. 1). Stationary phases used were both C_{18} with refractive index n of 1.46.

Visualization was improved by submersion of the transparent columns into a cubic box made of PMMA ($n_D^{20} = 1.49$) filled with a glycerol ($n_D^{20} = 1.47$). The use of glycerol eliminated the cylindrical lens effect during the observation of the column, and avoided the use of poisonous chlorinated hydrocarbons (used in reference [6]). Fig. 2 illustrates the cylindrical lens effect using the reservoir half filled with glycerol. The photographs of flow profile of sample band were taken by DSC-S85 cyber-shot digital camera (Sony Corporation, Tokyo, Japan).

2.5. Conditions

2.5.1. Sample injection

2.5.1.1. Single component. A saturated solution of iodine in cyclohexane at concentration of approximately 0.01 g/ml was used as sample probe. Eighty microliters of the sample was injected onto the column by on-column injection method. Under this condition, the solute band was highly colored which allowed for the observation and recording of the flow profile.

2.5.1.2. Two components. Forty microliters carmine and brilliant blue mixture solution at concentration of 0.002 g/ml each was used as sample.

2.5.1.3. *Wavelength choice.* We selected the wavelength of 460 nm for iodine [8] and 550 nm for carmine and brilliant blue detection.

3. Results and discussion

3.1. Flow profile of unretained component

Many factors influence the flow profile in cylindrical columns [9], such as the viscosity of mobile phase and the

solvent used in diluting sample [10,11], the porosity and diameter of frits in the header of columns [12,13], injection modes [7], methods of column packing [14] and temperature gradient [15], etc. In order to avoid interferences from other factors, all the conditions in our experiment were kept identical both in the cylindrical and the conical columns except the column shape.

The flow profile of iodine solution embodied that of mobile phase, since the retention factor of iodine solution was very low (0.14) in our experiment. The visualized dynamic flow profiles of iodine band in the cylindrical

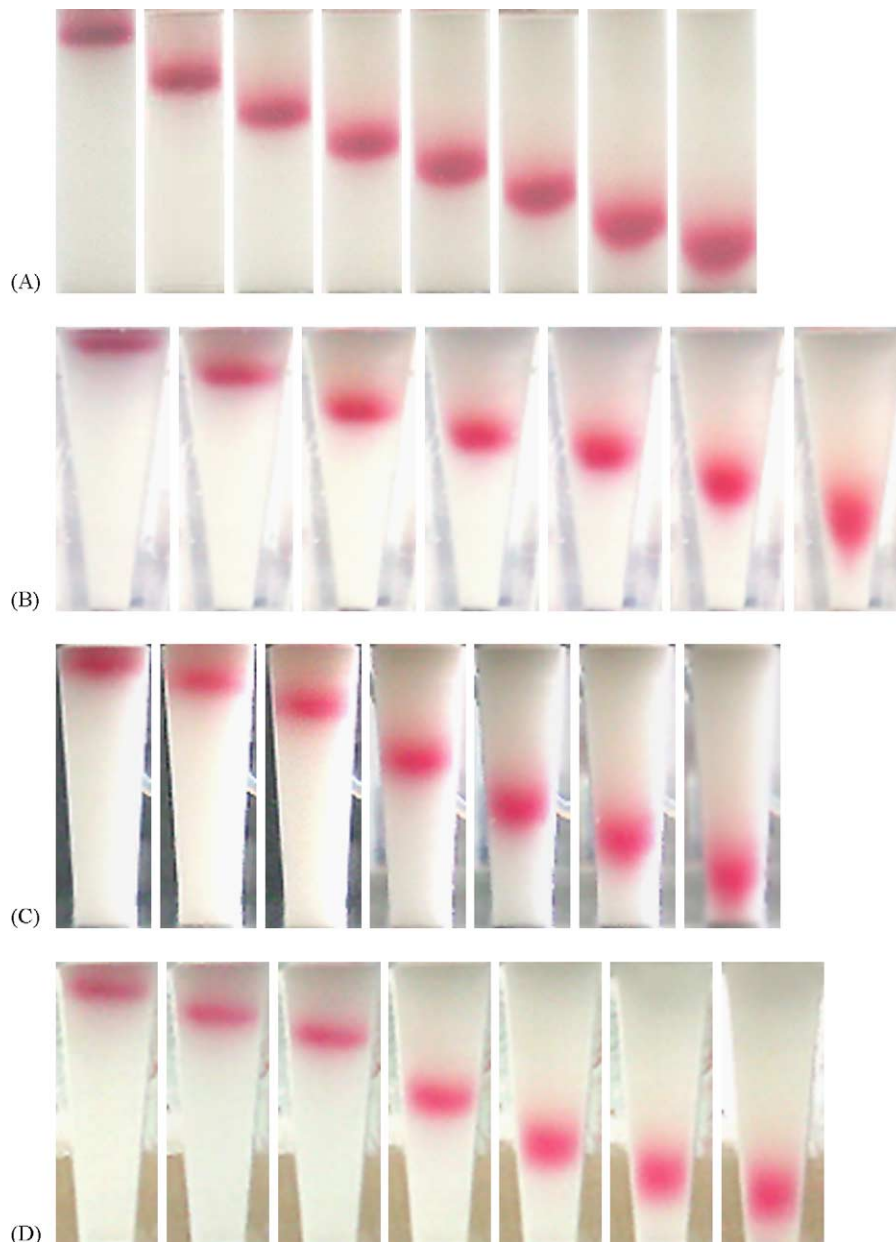


Fig. 3. Photographs of the flow profiles in cylindrical column and conical columns at different moment. The top of the column is inlet and the bottom is outlet. Conditions—flow-rate: 2 ml/min; mobile phase: cyclohexane; sample: 80 μ l of 0.01 g/ml iodine solution in cyclohexane; detection wavelength: 460 nm. (A) Photographs of the flow profiles in cylindrical column at different moments. (B) Photographs of the flow profiles in the conical column of 15° at different moments. (C) Photographs of the flow profiles in the conical column of 7° at different moments. (D) Photographs of the flow profiles in the conical column of 10° at different moments.

and the conical columns at the flow-rate of 2 ml/min are demonstrated in Fig. 3. At initial moment the flow profiles in the four columns were all flat at the exit of the inlet frits, proving that the choice of the frits was satisfactory and the

distribution of samples on the column inlet was uniform. In the cylindrical column the mobile phase in the central region migrated faster than in wall region (shown in Fig. 3A) as it moved toward the column outlet. While in the conical

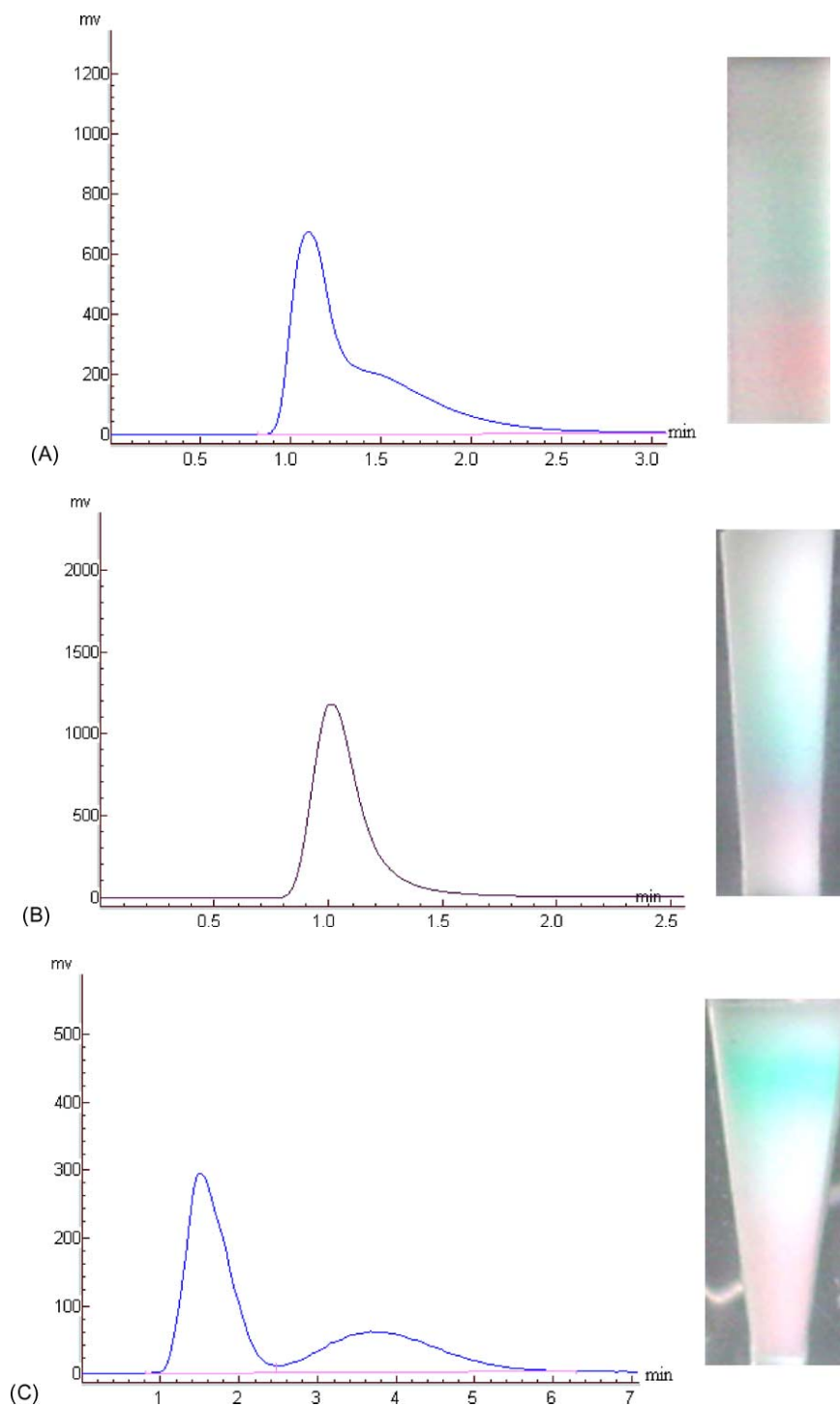


Fig. 4. Flow profile and chromatogram of carmine and brilliant blue in the columns. (A) Flow profile and chromatogram of carmine and brilliant blue in the cylindrical column; $R = 0.8$. Conditions—flow-rate: 3 ml/min; mobile phase: methanol/0.1 M ammonium acetate (50/50) (v/v); sample: 40 μ l of 0.002 g/ml carmine and brilliant blue solution; detection wavelength: 550 nm. The amount of sample injected was not overloaded. (B) Flow profile and chromatogram of carmine and brilliant blue in the conical column of 7°; $R = 0.60$. Conditions as in (A). (C) Flow profile and chromatogram of carmine and brilliant blue in the conical column of 15°; $R = 1.29$. Conditions as in (A). (D) Flow profile and chromatogram of carmine and brilliant blue in the conical column of 10°; $R = 1.57$. Conditions as in (A).

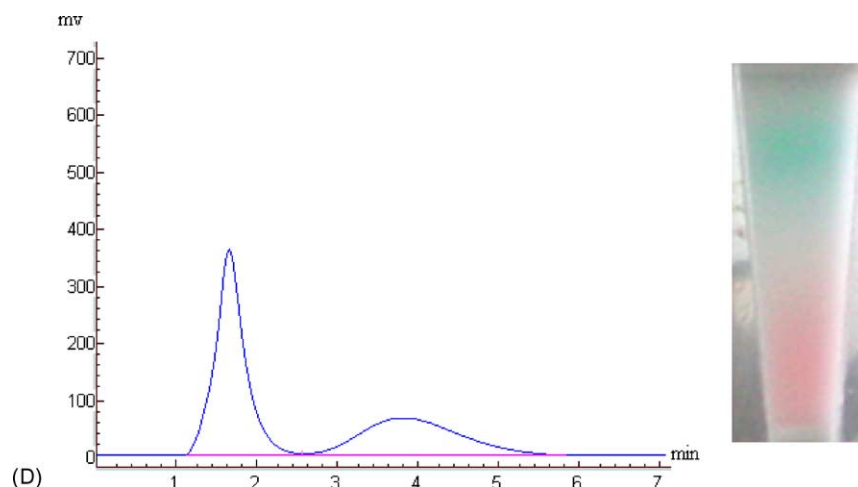


Fig. 4. (Continued).

cal column of 15° the mobile phase in the central region migrated slower than in wall region (shown in Fig. 3B) as it moved toward the column outlet. In the conical column of 7° (shown in Fig. 3C) the flow profiles were similar to that in the cylindrical column. These results indicated that there existed an optimal angle, which is larger than 7° and less than 15° , a conical column with that 'angle' could generate a flat flow profile. The above-mentioned phenomenon led us to make a new conical column with angle of 10° used to test. A flat flow profile was generated during a whole elution process, from the inlet of the column till the outlet, under the same conditions as other columns (shown in Fig. 3D). In the columns packed with smaller size of packing material (spherical $20\ \mu\text{m}$ C_{18}) similar flow profiles can be found except for narrower longitudinal width.

The possible reason for different flow profiles in the conical columns is as follows. Supposing the inside diameter of a conical column is $2r$, the linear velocity of mobile phase (\mathbf{u}) is: $\mathbf{u} = F/(\varepsilon_T \pi r^2)$, where F is the volume flow-rate of the mobile phase, ε_T is the total porosity. Since the value of r is changing from large (inlet) to small (outlet) along the column while the volume flow rate of the mobile phase is constant at any cross-section of the column, the linear velocity of the mobile phase is increasing gradually, according to the above equation, along the column axis till the column outlet. However, in the region near the conical column wall, there exists inclined plane where the composition of forces generated by mobile phase flow and the wall is different from that in the central region of the column. The acceleration speed of the velocity in the wall region can be faster than that in the central region when the angle of the conical column exceeds certain value. The difference of the acceleration speed in the wall region and that in the central region changes with the conical angle. Therefore, at different angle, the sample band in the wall region migrates either faster (15°) or slower (7°) than in central region, or the same as

in central region (10°), resulting in different flow profiles in the conical columns.

3.2. Separation of carmine and brilliant blue

Considering the iodine had weak retention on C_{18} packing material with cyclohexane as mobile phase, we chose a sample containing two components- carmine and brilliant blue. Brilliant blue had stronger retention than carmine. We examined the separation abilities of the four columns for carmine and brilliant blue and studied flow profiles of them on the columns. The flow profiles and chromatograms of the samples in the cylindrical column, the conical column of 7° , 15° and 10° at flow rate of $3\ \text{ml/min}$ are shown in Fig. 4.

The resolution (R_s) is determined by three methods according to the separation performance. When the two components were separated completely, the equation was used, $R_s = (t_2 - t_1)/[(1/2)(W_1 + W_2)]$, where t_1 and t_2 are the retention time for the first and the second peak, respectively. W_1 and W_2 are the width at the baseline for the first and the second peak, respectively. When they were not separated completely and the half peak width could be determined, the other equation was used, $R_s = 1.18(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the width at half peak height for first and the second peak, respectively. When the two components overlapped seriously, R_s was estimated by standard resolution curves, which can be found in professional books on chromatography. R_s of the two components on the conical columns of 15° , 10° , 7° and the cylindrical column were 1.29, 1.57, 0.6 and 0.8, respectively. These results demonstrate the conical column with plug-like flow profile has the best separation performance in the tested four columns. Since the transparency of column bed was not very satisfactory when methanol/0.1 M amine acetic acid (50/50, v/v) was used as mobile phase, the flow profiles were not very clear. However, the separation status on the four columns for the two components can be observed clearly.

4. Conclusions

We studied the dynamic flow profiles inside the conical columns with the aid of on-column visualization technique. The fascinating change of the flow profiles from flat to parabolic shape in conical columns at different angles was observed. It was concluded that a plug-like flow profile could be realized in a conical column of appropriate angle. A carmine and brilliant blue mixture was used as a probe to test the separation ability of the columns. The resolutions of the two compounds on the conical columns of 10 and 15° with better column efficiencies were higher than that on the cylindrical column. These results demonstrated that the conical column has its advantages in separation technique. Since the fabrication of the conical columns is not complicated and expensive, the application of this shape of columns can be extended. The current chromatographic theory is based on the assumption that the linear velocity of mobile phase in a certain axial position along a cylindrical column is constant, while the flow velocity varies with radial position in the column. However, linear velocity in a certain axial position along a conical column we studied is not constant, that is, the acceleration speed of linear velocity is larger than zero. More complicated theory describing the chromatographic behavior of conical column should be developed. The conical column with flat flow profile was proved to be the best for separation. We are currently working on the theoretical explanation behind the phenomena.

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