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# Packing of preparative high-performance liquid chromatography columns by sedimentation

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## ABSTRACT

Based on the colloid chemistry of a suspension of ODS packing material, a new packing technique using particle sedimentation has been developed. With this approach, a slurry consisting of the packing and a deflocculating solvent is poured into the column, the bed was formed by sedimentation. The bed is then solidified by a flocculating solvent. Using acetone as the slurry solvent and methanol–water (1:1) as the solidification solvent, high-quality columns (150 mm × 21 mm I.D., 16-$\mu$m $C_{18}$-silica packing) were successfully packed with reduced plate heights of 2.5–3.6 and typical A terms in the Knox plot below 1.0. The normalized sedimentation volume is introduced as a basis for the selection of sedimentation and solidification solvents. Various experimental factors, including slurry concentration, solidification flow-rate programming and temperature, are studied and discussed.

## INTRODUCTION

In the last several years, there has been a considerable increase of interest in preparative liquid chromatography (LC). Many chromatographers are conducting research on the theory and the application of preparative LC, such as overloading and band interactions [1,2], throughput [3], injection [4,5], particle size [6], column design [7] and column performance evaluation [8]. The research of column packing technique still remains one of the important aspects in the development of preparative LC, as evidenced by numerous techniques being developed, patented and commercialized.

The current packing methods may be classified into three groups: dry packing, high-pressure slurry packing and compression slurry packing.

### *Dry packing*

In this technique, the dry packing material is slowly poured into the column and the bed is formed under the action of vibration, rotation, and/or tamping. Dry packing is suitable for large particles with particle diameter ($d_p$) > 30 $\mu$m. For particles with $d_p$ < 20 $\mu$m, this technique usually is poor, because the high surface free energy of

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small particles causes agglomeration and the particles can not be well dispersed by the mechanical action applied in packing. Even for large particles, the column efficiency and its reproducibility often are not very good [9].

*High-pressure slurry packing*

In this technique, the suspension of packing materials, *i.e.*, the slurry, is stored in a reservoir which is connected to the column. A packing solvent is then introduced under high pressure (usually above 5000 p.s.i.) and at high flow-rate into the reservoir to push the slurry into the column to form the bed by filtration. The high pressure slurry packing is the most successful and the most popular method for particles with $d_p < 20$ $\mu$m and columns with 20 mm internal diameter or smaller. Although larger columns can be packed by this method [9,10], the technical requirements (*e.g.*, the mechanical strength of the column and the pump capacity) of this method become more and more difficult.

*Compression slurry packing*

The compression can be axial or radial or both [9,11]. Typically, radial compression is accomplished by applying gas or liquid pressure on a flexible-walled cartridge which is filled with packing material [11,12]. Axial compression is achieved by moving a piston in the column, which is filled with slurry, to force the solvent out of the end frit and make a filtration bed in the column [13,14]. This technique seems perfect for packing large columns because of the low packing pressure required (5–20 atm) and the excellent column efficiency obtained (reduced plate height of about 2) [9]. Annular expension, *i.e.*, compression in both axial and radial directions, is available by insertion of a plunger in the center of a column [11]. The major shortcoming of the compression slurry packing is the requirement of special equipment, which can be expensive.

The selection of solvents in slurry packing methods is very important. From the viewpoint of colloid chemistry, there are two kinds of solvents: flocculating and deflocculating solvents [15,16]. In flocculating solvents, the attraction between particles is stronger than repulsion, causing the particles to agglomerate and to form a loose sedimentation cake. In deflocculating solvents, the repulsion between particles is stronger than the attraction, the particles are well dispersed, and can form a dense sedimentation cake. The properties of the slurry made from flocculating and deflocculating solvents are compared in Table I [16]. Recently, the importance of colloid chemistry and rheology in packing technique has been emphasized and a new high pressure slurry packing method has been developed which combines a deflocculating slurry solvent with a flocculating packing solvent [17–19].

TABLE I

PROPERTIES OF FLOCCULATING AND DEFLOCCULATING SLURRY

| Deflocculating slurry | Flocculating slurry |
|---|---|
| Particles are well dispersed | Particles agglomerate |
| Sedimentation rate is slow | Sedimentation rate is high |
| Sediment is dense | Sediment is loose |

In this research, a new packing technique, sedimentation packing, based on the colloid chemistry properties of the suspension of ODS packing material, is developed. Various experimental factors are studied and discussed.

## EXPERIMENTAL

### *Instrumentation*

A Waters Prep LC 3000 chromatograph (Millipore, Milford, MA, U.S.A.) was used for packing and conditioning of columns. The chromatograph used for column evaluation was composed of a HP-1084A liquid chromatograph (Hewlett-Packard, Boblingen, F.R.G.), a Lambda-Max Model 481 spectrophotometer (Millipore), and a Waters 740 data module.

The column hardware (150 mm and 500 mm × 21 mm I.D. columns) was supplied by PQ (Conshohocken, PA, U.S.A.).

### *Packing material and chemicals*

The irregular $C_{18}$-silica with average particle size 16 $\mu$m and the test compounds uracil, caffeine and phenol were supplied by PQ. The test compound propiophenone was obtained from Aldrich (Milwaukee, WI, U.S.A.), the solvent isooctane from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.), other solvents from Fisher Scientific (Fair Lawn, NJ, U.S.A.).

### *Sedimentation volume measurement*

Typically, 1.5 g of packing material were put into a 10-ml graduated cylinder, manually bounced on the table to make a dry packing bed, and its volume was recorded. Then 8–9 ml of solvent were added and a suspension was made by hand shaking. After a few minutes, the total volume was adjusted to 8.6 ml (concentration 17%) by removal of the supernatant. Then, the suspension was reshaken, allowed to settle and the volume of the sediment was recorded until no change was noted.

### *Packing procedure*

New and reused packing materials were filter-washed with acetone or methyl ethyl ketone and dried under reduced pressure at room temperature. For 150 mm × 21 mm I.D. column, 37 g silica was put into a 500 ml erlenmeyer flask with about 200 ml of a slurry solvent, acetone (AT) or methyl ethyl ketone (MEK). Fines were removed by sonicating the content for 1–2 min and decanting the supernatant after settlement of particles. This operation was repeated 2–3 times.

The 150-mm column was terminated with an end-fitting and frit, and was then connected to a reservoir, *i.e.*, a 500-mm column with the same internal diameter. The suspension prepared above was poured into the column, and allowed to sediment overnight.

After sedimentation, the supernatant was poured off, and the reservoir was filled with methanol. The inlet was sealed with an end-fitting, and then a solution of methanol–water (1:1) was passed through the assembly to solidify the column.

Soon after sodification, the column was disconnected gently from the reservoir and was sealed with an end-fitting and a frit. The column was conditioned with methanol–water (1:1) at 50 ml/min for 20–30 min with recording of the pressure drop.

*Column evaluation*

The test mixture was composed of uracil (capacity factor, $k' = 0$), caffeine, phenol and propiophenone ($k' = 6$) in the individual concentrations of 0.05–0.4 mg/ml (20 $\mu$l injected). The mobile phase was methanol–water (1:1). The column performance was evaluated by the propiophenone at a flow-rate of 10 ml/min (reduced velocity, $v = 24$) in terms of reduced plate height $h$, asymmetry factor $b/a$, flow resistance $\phi$ and the Knox plot (see ref. 20).

The peak width and the asymmetry factor were manually measured at 10% peak height from the chromagrams. The column efficiency was calculated using eqn. 1:

$$N = 18.5(t_R/w_{0.1})^2 \quad (1)$$

where $N$ is the theoretical plate number, $t_R$ is the retention time and $w_{0.1}$ is the peak width at 10% peak height. The standard deviation of measurement is $\pm 50$ for $N$ (corresponding to $\pm 0.09$ for $h$ when $n = 2300$), $\pm 0.05$ for $b/a$.

Two typical columns were conditioned by recirculation with methanol–water (1:1) or pure methanol at 50 ml/min for 109 h in order to observe their stability. The solvent volume passed during this conditioning was 6300 column volumes.

## RESULTS AND DISCUSSION

*Selection of solvents*

Particle interactions play a very important role in determining the bed structure, and these interactions are strongly controlled by solvents. The principle of sedimentation packing is simple: firstly, the particles in a deflocculating solvent freely participate to form a uniform and dense bed in a column; and secondly, a flocculating solvent is passed through the column to consolidate the bed. Therefore, solvent selection plays the predominant role in sedimentation packing.

The flocculation property of a solvent can be measured by the sedimentation volume SV [16] at equilibrium

$$\text{SV} = \text{volume of sediment/volume of suspension} \quad (2)$$

or the sedimentation quotient SQ [17,18]

$$\text{SQ} = 1000(\text{height of sediment/height of slurry}) \quad (3)$$

However, SV and SQ values only make sense if each suspension has the same concentration, and such relative values can not be used to predict the real density of the sediment. Therefore, a normalized sedimentation volume is introduced:

$$\text{SV}_n = \text{volume of sedimentation bed/volume of dry bed} \quad (4)$$

The $\text{SV}_n$ value is relative to the real density of the sediment and does not depend on the concentration of the suspension. The $\text{SV}_n$ values of some solvents commonly used in packing are tabulated in Table II.

From Table II it can seen that both strongly polar (methanol, acetonitrile and

TABLE II

NORMALIZED SEDIMENTATION VOLUME OF SOME SOLVENTS

MA–W = methanol–water (75:25); MA = methanol; ACN = acetonitrile; IPA = isopropanol; AT = acetone; EA = ethyl acetate; THF = tetrahydrofuran; OCT = isooctane; MEK = methyl ethyl ketone.

| | Solvent | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MA–W | MA | ACN | IPA | AT | EA | THF | OCT | MEK |
| $SV_n$ [a] | – | 1.29 | 1.24 | 1.19 | 1.02 | 1.00 | 1.00 | 1.18 | 1.05 |
| $SV_n$ [b] | 1.46 | 1.36 | 1.32 | 1.21 | 1.02 | 1.00 | 0.98 | 1.13 | 1.11 |

[a] Packing material lot p89-146-1.
[b] Packing material lot p87-183-2.

isopropanol) and non-polar (isooctane) solvents have high $SV_n$ values (1.18–1.36), that is, they are flocculating solvents and are not suitable as the slurry solvent, but may be good as the solidification solvent. The addition of water to methanol makes the solvent more flocculating. In contrast, acetone, ethyl acetate and tetrahydrofuran are deflocculating solvents, and their $SV_n$ values are almost equal to unity, *i.e.*, the sedimentation bed formed in such solvents is as dense as that formed in dry packing. According to this research, solvent with $SV_n$ values smaller than 1.05 were suitable as the slurry solvents.

Besides the $SV_n$ value, the particle settling rate also should be considered in order to save settling time. It can be seen in Fig. 1 that acetone has a higher settling rate than that of either ethyl acetate or tetrahydrofuran, and is therefore chosen as the slurry solvent although the three solvents have a similar $SV_n$ value.

*Slurry concentration*

The $SV_n$ value of acetone slurries was measured in the concentration range 9–31% (w/v), and there was no change in the $SV_n$ values measured. However, the

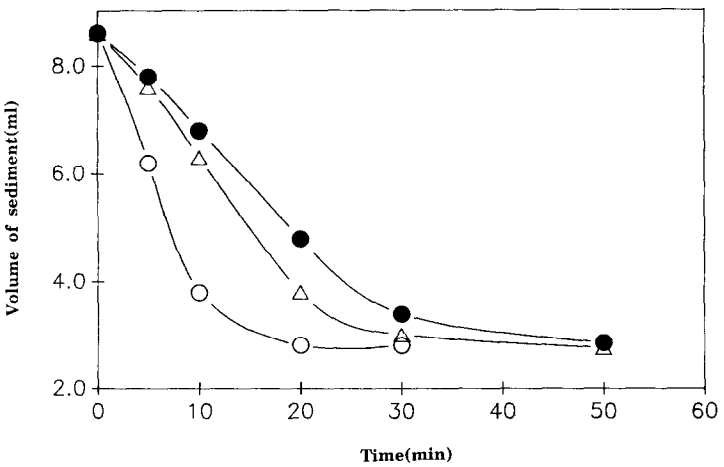


Fig. 1. Sedimentation rate of slurry made of acetone (○), tetrahydrofuran (△) and ethyl acetate (●).

TABLE III

EFFECT OF SLURRY CONCENTRATION ON COLUMN PERFORMANCE

| Column[a] | Concentration | $N$ | $h$ ($v = 24$) | $b/a$ | $\phi$ |
|---|---|---|---|---|---|
| 32AT | 26% (w/v) or 49% (v/v) | 2130 | 4.4 | 0.82 (leading) | 1100 |
| 27AT | 17% (w/v) or 31% (v/v) | 2650 | 3.5 | 0.85 (leading) | 1070 |
| 33AT | 9% (w/v) or 17% (v/v) | 1850 | 5.1 | 0.96 (leading) | 1070 |

[a] Used packing material; AT = acetone used as slurry solvent.

column performance was affected by the slurry concentration. The columns listed in Table III were packed using the acetone slurry and the best solidification condition (see below). The highest column efficiency was obtained with a slurry concentration of 17% (w/v); either increasing or decreasing the concentration lowered the column efficiency.

*Solidification condition*

The settled bed is not ready to be used. Firstly, the settled bed is not dense enough to give very good column performance. Secondly, the bed is not stable enough to maintain the column performance. The purpose of solidification is to increase the attraction between particles. The solidification solvents should be strongly flocculating solvents such as methanol and water in order to encourage particle-particle interactions. The selection of the condition includes the flow-rate profile, the final flow-rate, the gradient and the total volume of solvent passed through the bed.

*Flow-profile.* Three flow-rate profiles were studied. Program I is a constant flow-rate in the range of 50–100 ml/min for 10–60 min. Using program I, columns gave either a very low column efficiency (see Table IV) with low conditioning flow-rate or split peaks with conditioning flow-rate greater than 70 ml/min.

The flow-rate in program II is started at 10 ml/min for 30 min, then is increased to 100 ml/min via a linear gradient of 5 (ml/min)/min and is held for 10 min. Using program II, the column efficiency is slightly improved. The average reduced plate height of five columns is 6.6 at $v = 24$ (see Table IV), but the peaks are obviously tailed with $b/a$ values 1.3–1.6.

TABLE IV

EFFECT OF FLOW-RATE PROGRAM ON THE COLUMN PERFORMANCE

| Slurry | Flow-rate program[a] | $N$ | $h$ ($v = 24$) | $b/a$ | $\phi$ |
|---|---|---|---|---|---|
| MEK (1 column) | I | 1280 | 7.3 | 1.17 | – |
| MEK (5 columns) | II | 1420 ± 68 | 6.6 ± 0.3 | 1.3–1.6 | 750 ± 98 |
| MEK (4 columns) | III | 2380 ± 190 | 3.9 ± 0.3 | 0.84–0.95 | 1140 ± 86 |
| AT (5 columns) | III | 2590 ± 250 | 3.6 ± 0.3 | 0.82–0.98 | 1090 ± 28 |

[a] Program I: methanol–water (1:1), 70 ml/min, 20 min.
Program II: same solvent, 10 ml/min 30 min, 5 (ml/min)/min to 100 ml/min, hold 10 min.
Program III: same solvent, 1–5 (ml/min)/min from 1 to 50 or 75 ml/min, hold 5–20 min.

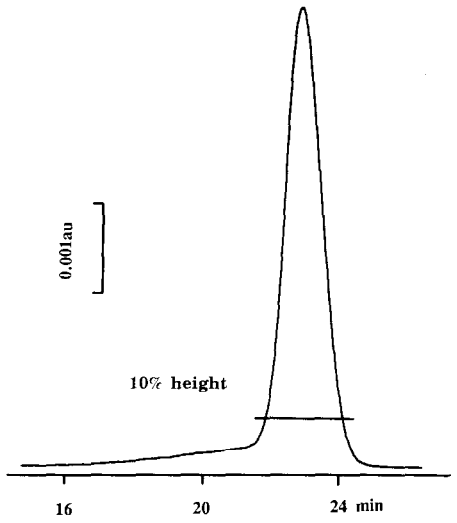


Fig. 2. Leading peak (propiophenone).

In program III, the flow-rate is started at 1 ml/min, then is increased to a final value ranging from 30 to 90 ml/min via a linear gradient varied from 1 to 10 (ml/min)/min with a final hold for 5–30 min. Using program III with suitable parameters (discussed below), very good columns are obtained. The average reduced plate height is 3.9 for the methyl ethyl ketone slurry and 3.6 for the acetone slurry at $v = 24$ (see Table IV).

When using program III to consolidate columns, a unusual leading peak often appears. Fig. 2 shows a typical leading peak. It is not a normal fronting peak but looks like a combination of a normal peak and a drifting baseline. The inflection point is usually below 10% height. In this paper, the term "leading peak" refers to that observed as in Fig. 2. The cause of such peak is currently unknown, but it may be due to short channels between the packing bed and the column wall. Columns packed with reused packing material almost always produce such leading peaks.

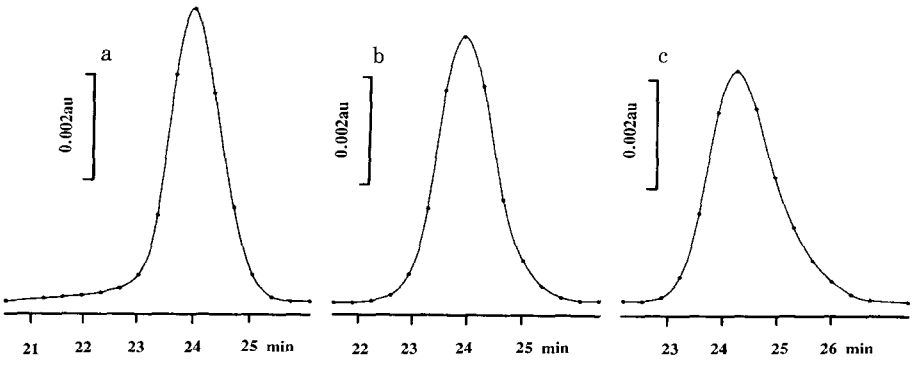


Fig. 3. Effect of final flow-rate on the peak shape (propiophenone, replotted and normalized). (a) 70 ml/min; (b) 50 ml/min; (c) 30 ml/min.

TABLE V

EFFECT OF FINAL FLOW-RATE ON COLUMN PERFORMANCE

| Column[a] | Final flow-rate (ml/min)[b] | $N$ | $h$ ($v$ = 24) | $b/a$ | $\phi$ |
|---|---|---|---|---|---|
| 5AT | 90 | 1780 | 5.3 | 0.94 (leading) | 1030 |
| 2AT | 70 | 2730 | 3.4 | 1.04 (leading) | 1070 |
| 3AT | 50 | 2060 | 4.6 | 1.21 | 920 |
| 4AT | 30 | 1460 | 6.4 | 1.57 | – |

[a] New packing material.
[b] Program III, gradient 2 (ml/min)/min.

*Final flow-rate.* When using program III, the final flow-rate is very important in the control of column efficiency and peak shape. The effect of the final flow-rate can be seen from Fig. 3 and Table V. At high final flow-rate (70 ml/min, corresponding to 1400–1500 p.s.i.), the column efficiency is very good, but the peak is leading (2AT). At low final flow-rate (30 ml/min, 400–500 p.s.i.), the column efficiency is low (4AT, $h$ = 6.4 at $v$ = 24) and a seriously tailing peak appears. The best final flow-rate is 50 ml/min (corresponding to 800–900 p.s.i.). Similar trends were obtained using the methyl ethyl ketone slurry except a best final flow-rate of 75 ml/min (1400–1500 p.s.i.) was ascertained.

*Flow-rate gradient.* The major effect of flow-rate gradient is on the peak shape as can be seen from Table VI. As the flow-rate gradient is increased from 2 to 5 and then to 10 (ml/min)/min, the peak shape is changed from slightly tailing (3AT) to almost symmetric (6AT) to leading (14AT), respectively. The column efficiency shows minor changes. Although the 5 (ml/min)/min gradient can give good columns, this gradient seems to be a critical point and a slightly leading peak sometimes appears using the 5 (ml/min)/min gradient. Therefore, the 2 (ml/min)/min gradient is prefered.

*Total solvent volume.* The total solvent volume passed through the column in solidification seems not to be very important. By changing the hold-time at final flow-rate (see Table VII), the total solvent volume was altered from 750 to 1250 ml (6AT, 8AT) or from 1500 to 2600 ml (53MEK, 50MEK), and no significant change in column performance was observed. However, when the hold-time was increased to 30 min, the column efficiency deteriorated (7AT).

Finally, the best solidification condition established for columns packed with the

TABLE VI

EFFECT OF FLOW-RATE GRADIENT ON COLUMN PERFORMANCE

| Column[a] | Gradient [(ml/min)/min][b] | $N$ | $h$ ($v$ = 24) | $b/a$ | $\phi$ |
|---|---|---|---|---|---|
| 3AT | 2 | 2060 | 4.6 | 1.21 | 920 |
| 6AT | 5 | 2390 | 3.9 | 0.95 | 1020 |
| 14AT | 10 | 2460 | 3.8 | 0.87 (leading) | 1020 |

[a] New packing material.
[b] Program III, final flow-rate 50 ml/min.

TABLE VII

EFFECT OF TOTAL SOLVENT VOLUME ON COLUMN PERFORMANCE

| Column | Final flow-rate hold time (min) | Total solvent volume (ml)[a] | $N$ | $h$ ($v$ = 24) | $b/a$ | $\phi$ |
|---|---|---|---|---|---|---|
| 6AT | 10 | 750 | 2390 | 3.9 | 0.95 | 1020 |
| 8AT | 20 | 1250 | 2270 | 4.1 | 1.13 | 1020 |
| 7AT | 30 | 1750 | 1330 | 7.0 | 1.02 (leading) | 1050 |
| 53MEK | 5 | 1500 | 2150 | 4.4 | 0.84 (leading) | 1070 |
| 50MEK | 20 | 2600 | 2300 | 4.1 | 0.91 (leading) | 1070 |

[a] Program III.

acetone slurry and the methanol–water (1:1) solidification solvent was: program III, start at 1 ml/min, 2 (ml/min)/min gradient to 50 ml/min, then hold for 10 min. Fig. 4 presents a chromatogram obtained from a typical column (35AT) packed according to these conditions and the reduced plate height is 3.1 ($v$ = 24). This column provides an optimum reduced plate height of 2.5 at $v$ = 12 (flow-rate 5 ml/min), with asymmetry factor 1.00 and flow resistance 990.

To the best of the authors' knowledge, the best preparative columns obtained with high-pressure slurry packing gave a reduced plate height of 1.8 [9,10]. In terms of reduced plate height, although sedimentation packing does not yet equal high-pressure slurry packing, these preliminary results are satisfactory. Moreover, according to Dewaele *et al.* [8], the column efficiency at one flow-rate is affected not only by packing procedures, but also by the properties of the packing materials and chromatographic system. Therefore, measurement of efficiency *vs.* flow-rate is necessary (see below).

*Stability and Knox plot*

The column performance of two columns (8AT and 25AT) was examined before and after 109 h of conditioning. Figs. 5 and 6 are the Knox plots obtained from the 8AT and 25AT columns, respectively. According to the plots, the coefficients of the Knox equation (see ref. 20)

$$h = B/v + Av^{0.33} + Cv \tag{5}$$

TABLE VIII

COEFFICIENTS OF KNOX EQUATION

| Column | Status | $B$ | $A$ | $C$ | $r^2$ |
|---|---|---|---|---|---|
| 8AT | New | 9.80 | 0.90 | 0.05 | 0.997 |
| 8AT | After conditioning[a] | 10.80 | 0.75 | 0.05 | 0.995 |
| 25AT | New | 15.39 | 0.84 | 0.04 | 0.97 |
| 25AT | After conditioning[b] | 12.31 | 1.00 | 0.02 | 0.95 |

[a] Methanol–water (1:1), 50 ml/min for 109 h.
[b] Methanol, 50 ml/min for 109 h.

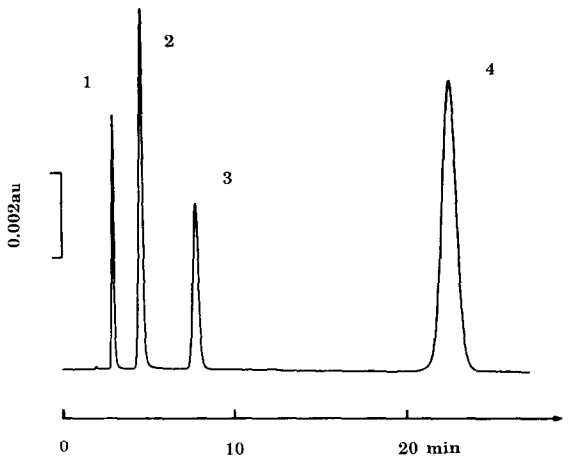


Fig. 4. Separation of test mixture. Column, 150 mm × 21 mm I.D. (35AT), methanol–water (1:1), 10 ml/min ($v$ = 24). Peaks: 1 = uracil; 2 = caffeine; 3 = phenol; 4 = propiophenone.

are calculated and listed in Table VIII. From Figs. 5 and 6, it can be seen that the column performance of both columns is stable, with no obvious change after 6300 column volumes of solvent conditioning. For 8AT which was conditioned by the same condition of solidification [methanol–water (1:1), 50 ml/min], the long time of conditioning even leads to a slight improvement of the column performance. According to Dewaele *et al.* [8], the $A$ term in eqn. 5 is the best parameter to justify the packing quality, and a well-packed column should have an $A$ value < 2 and the lower the better. In Table VIII, the $A$ terms of columns 8AT and 25AT are 0.75–0.90 and 0.84–1.0, respectively, indicating the two columns are packed to an excellent degree by sedimentation packing technique.

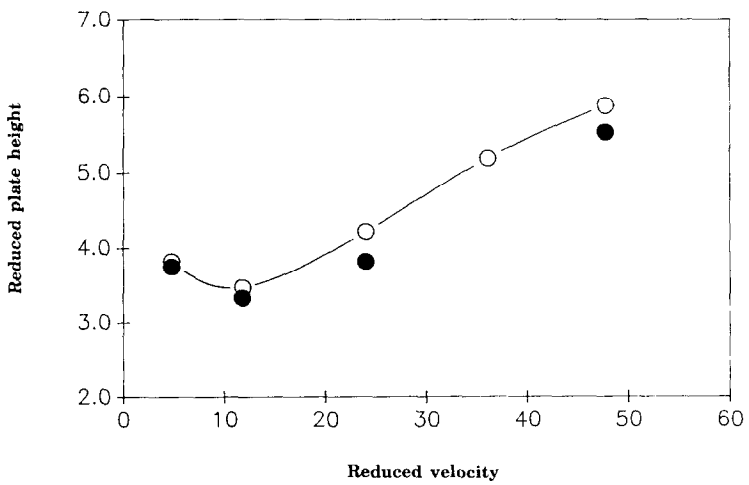


Fig. 5. Knox plot from 8AT before (○) and after (●) conditioning (see text).

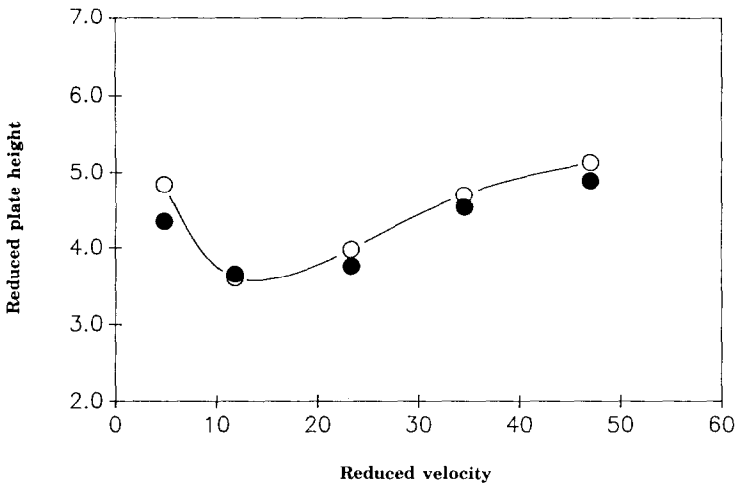


Fig. 6. Knox plot from 25AT before (○) and after (●) conditioning (see text).

*Temperature effect*

In order to obtain high column efficiency in sedimentation packing, the sedimentation temperature and the solidification temperature should be under control. The temperature effect on column performance can be seen from Table IX. The highest column efficiency is obtained when the sedimentation temperature is 3–5°C higher than that of solidification (19AT). The reason for this temperature effect is unclear at present, but the thermal effect of solvent mixing may play an important role. The bed seems to need to be kept at constant temperature during the sedimentation and the solidification. However, the mixing of methanol and acetone is endothermic, and the mixing of methanol and water is exothermic. During solidification, the bed temperature will decrease first (methanol + acetone) and then increase [methanol–water (1:1) + methanol]. Therefore, a warm bed will compensate somewhat the temperature change created by solvent mixing. In addition, the sedimentation temperature also should be stable.

TABLE IX

EFFECT OF TEMPERATURE ON COLUMN PERFORMANCE

| Column[a] | Temperature control | $N$ | $h$ ($v$ = 24) | $h/a$ | $\phi$ |
|---|---|---|---|---|---|
| 19AT | 25°C sedimentation<br>22°C solidification | 2990 | 3.1 | 0.91 (leading) | 1140 |
| 20AT | 20°C sedimentation<br>24°C solidification | 2050 | 4.6 | 0.86 (leading) | 1100 |
| 21AT | 24°C sedimentation<br>24°C solidification | 2430 | 3.9 | 0.89 (leading) | 1100 |

[a] Used packing material, solidification gradient 5 (ml/min)/min, final flow-rate 50 ml/min and hold 15 min.

## CONCLUSION

Based on the colloid chemistry properties of the suspension of ODS packing material, the sedimentation packing technique has been developed. Using acetone as the slurry solvent and methanol–water (1:1) as the solidification solvent, high-quality columns (150 mm × 21 mm I.D.) were obtained with reduced plate heights of 2.5 – 3.6 and stable column performance. The typical $A$ term in the Knox plot was below 1.0. Although the best columns produced using sedimentation packing do not equal the best columns produced with high-pressure slurry packing in term of reduced plate heights, the new method shows obvious promise because of its simplicity. The normalized sedimentation volume was introduced in order to easily select sedimentation and solidification solvents. Various experimental factors were studied, such as the slurry concentration, the flow-rate profile in solidification, the final flow-rate, the flow-rate gradient, the total solvent volume and the temperature effect. These studies provide valuable guidelines for the preparation of packed large bore columns.

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