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STUDIES OF OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY

III. β,β′-OXYDIPROPIONITRILE AND ETHYLENE GLYCOL STATIONARY PHASES

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SUMMARY

Capillary columns coated with β,β′-oxydipropionitrile and ethylene glycols have been applied successfully in open-tubular microcapillary column liquid chromatography. These stationary phases were spread out well on a soda-lime glass surface treated with alkaline solution. The capillary columns obtained have efficiencies of about 1500 theoretical plates per metre. Xylenol isomers, aromatic amines and phthalates were separated in a normal-phase system.

INTRODUCTION

A problem in liquid chromatography is the achievement of high efficiencies in terms of the number of theoretical plates when using microbore columns. At present there are three types of column available: (1) open-tubular microcapillary columns[1–5]; (2) packed microcapillary columns[6]; and (3) packed microbore columns[7,8]. Scott and Kucera[7] produced a 10-m microbore column by connecting in series 1 m × 1 mm I.D. columns that were packed with Partisil 20 (particle diameter 20 μm) for exclusion chromatography, and achieved 250,000 theoretical plates at the dead volume (retention time 4.6 h). Tsuda and Novotny[6] produced a 29 m × 75 μm I.D. alumina (particle diameter 30 μm) packed microcapillary column and achieved 85,000 theoretical plates for quinoline (retention time 100 min). With open-tubular microcapillary columns, the highest theoretical plate number achieved was 10,000 for anthracene (retention time 70 min) using an ODS capillary column (20 m × 56 μm I.D.). Using a 3 m × 60 μm I.D. ODS open-tubular microcapillary column 1000–3000

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theoretical plates were obtained for aromatics with retention times of less than 30 min[4]. Although the highest theoretical plate numbers are at present given by method (3), the potential of method (1) is high. Method (1) would operate at a relatively low pressure compared with methods (2) and (3). The history of gas chromatography, in which open-tubular capillary columns have the highest efficiency[9], suggests that capillary column liquid chromatography should be tried.

There are several publications[1–5] on the basic feature and applications of open-tubular capillary columns. Although secondary flow in the capillary tube apparently causes a large diffusion coefficient[1,5], this effect becomes important in the relatively high flow velocity region. As the square of the column radius is inversely proportional to the HETP (H), a narrower capillary column is more favourable if technical and detection problems can be overcome.

We have solved these problems for capillary columns with diameters up to *ca.* 60 μm by using modified injection, detection and connection systems that were developed for micro-scale high-performance liquid chromatography[10]. The detector, with a very small cell, operates well without any loss of sensitivity. Our earlier work on open-tubular microcapillary liquid chromatography (OMCLC)[3,4] dealt with columns coated with SE-30 or bonded with octadecylsilane, and separations were performed in a reversed-phase system. In this paper, we describe the use of a normal-phase system. β,β'-Oxydipropionitrile (ODPN) and ethylene glycols are employed as stationary phases. As solute diffusion in the mobile phase (isooctane or *n*-hexane) is greater than in methanol–water or acetonitrile–water and also solute diffusions in these stationary phases is greater than in SE-30, because of their relatively low viscosities, a better column efficiency can be expected in this system.

EXPERIMENTAL

The apparatus used in this experiment was the same as in our previously reported work[3,4]. Home-made flow cells of I.D. 0.17 mm and length 1.5 mm were set in Jasco Uvidec 100-II UV detector (Japan Spectroscopic Co., Hachioji-shi, Japan). Isooctane or *n*-hexane was used as the mobile phase, and was saturated with the liquid of the stationary phase. Soda-lime glass tubes (6 mm O.D. and 0.5–0.3 mm I.D.) were drawn out with a Shimadzu GDM-1 drawing machine; the coil diameter was 11 cm. The composition of the glass tube was SiO_2 72%, Na_2O 12.4%, CaO 11.4%, Al_2O_3 2.4%, K_2O 1.4%, SO_3 0.28% and Fe_2O_3 0.05%. Unless specified otherwise the experiments were carried out using a column coated with β,β'-oxydipropionitrile.

Preparation of capillary column coated with β,β'-oxydipropionitrile

The best column was obtained by means of the following procedure. PTFE tubes (0.5 mm I.D., 1 mm O.D. and 10 cm long) were connected at both ends with glass capillary tubes (*ca.* 0.6–0.7 mm O.D.) and PTFE tubes of slightly larger bore (1 mm I.D., 2 mm O.D. and 10 mm long) were fixed over these connecting parts for strengthening by using a micro-burner. Then the glass capillary was filled with a 1 N alkaline solution, such as lithium, sodium or potassium hydroxide solution, using a micro-syringe. The capillary tube was kept for 2–3 days

at 20–30°, then washed with methanol (*ca.* 100 μl) until neutral. The capillary was washed again and filled with dichloromethane (*ca.* 100 μl), then coated with 14% (w/w) ODPN solution by the dynamic coating method. Sodium tetraphenylborate, was usually added to the solution of ODPN in methanol–dichloromethane (1:20) at a concentration of 1% (w/w). Nearly the same column efficiency was obtained without the addition of this surface-active reagent, using a solution of ODPN in dichloromethane.

After the plug of ODPN solution (20–30 μl) had been sucked into a PTFE tube (0.5 mm I.D. and 30 cm long), this tube was connected to the PTFE tube attached to the capillary tube of the column by using a small piece of stainless-steel tubing (0.55 mm O.D., 0.3 mm I.D. and 2 cm long). The other end of the capillary was connected to an extra capillary tube (50 μm I.D. and 5 m long). Then, by using nitrogen at 20 atm pressure, the plug was forced into the capillary at the rate of about 4 cm/sec. Just after the plug had passed through the capillary tube of the column, the PTFE tube connection between the capillary tube and the nitrogen cylinder was cut off. After dynamic coating, the capillary was dried at room temperature for 0.5–1.0 h and kept at 52° for 3–5 h under a flow of nitrogen. The experiments were carried out using a soda-lime glass capillary, unless specified otherwise.

The other pre-treatments were as follows. With 6 *N* hydrochloric acid, the capillary was filled with the acid and both ends sealed, then it was kept at 150–200° for 2–4 h. After cooling, it was washed with water, methanol and dichloromethane. With chromic acid, the capillary was filled with the acid and kept at 100° for 4 h, then it was washed with water, methanol and dichloromethane.

Preparation of mono-, di-, tri- and tetraethylene glycol phases

Ethylene glycols were supplied from Tokyo Kasei Kogyo (Toyoshima, Tokyo, Japan). The procedures for the preparation of ethylene glycol phases were the same as that of β,β′-oxydipropionitrile except that the drying temperature was 43°.

RESULTS AND DISCUSSION

ODPN was coated on the inner wall of the capillary tube using the dynamic method. The effects of pre-treatment of borosilicate or soda-lime glass on the column efficiency are illustrated in Table I. Pre-treatment with alkaline solutions gives better column efficiencies than that with acidic solution; also, soda-lime glass is better than borosilicate glass. Thus, soda-lime glass columns pre-treated with alkaline solution show much better column efficiencies than the others. Typical columns are shown in Fig. 1. In Fig. 1A, ODPN is not spread uniformly over the bare surface of the borosilicate glass and formes large droplets. Fig. 1B shows that the droplets become very small and spread well over the surface of soda-lime glass treated with 1 *N* potassium hydroxide solution for 2 days. The latter column gives about four times as many theoretical plates as the column shown in Fig. 1A. When we examined with an electron microscope the surface of soda-lime glass treated with alkaline solution we found a very rough surface. This roughness might be very important in obtaining a uniform stationary phase. Treatment with alkaline solution is also effective with ehtylene glycol stationary phases.

The long-term stability of columns coated with ODPN was examined by

TABLE I

EFFECT OF PRE-TREATMENT ON COLUMN EFFICIENCY FOR ODPN CAPILLARY COLUMNS

Column		*Glass*	*Pre-treatment*	*Capacity factor of β-naphthylamine*	*Column efficiency*	
I.D. (μm)	*Length (cm)*				*Plate number per meter*	*Resolution of aniline and β-naphthylamine*
50	399	Borosilicate		0.76	407	3.2
53	398		6 *N* HCl	0.88	166	2.1
53	361		Chromic acid	1.00	198	2.4
56	396		1 *N* NaOH	0.53	737	3.3
53	403		1 *N* KOH	0.74	258	2.5
52	507	Soda-lime		0.51	472	2.8
49	498		6 *N* HCl	0.53	368	2.5
48	535		1 *N* NaOH	1.06	1528	7.6
49	504		1 *N* KOH	1.25	1385	8.1
48	466		1 *N* LiOH	1.48	1440	7.9

repeated analyses. Five injections of sample were made after each passage of 1000 μl of mobile phase. The results are shown in Table II. The retention and resolution decrease slightly after the passage of about 2000–3000 μl, but this decrease is only 20% at 5500 μl of effluent used compared with the initial values. This suggests that

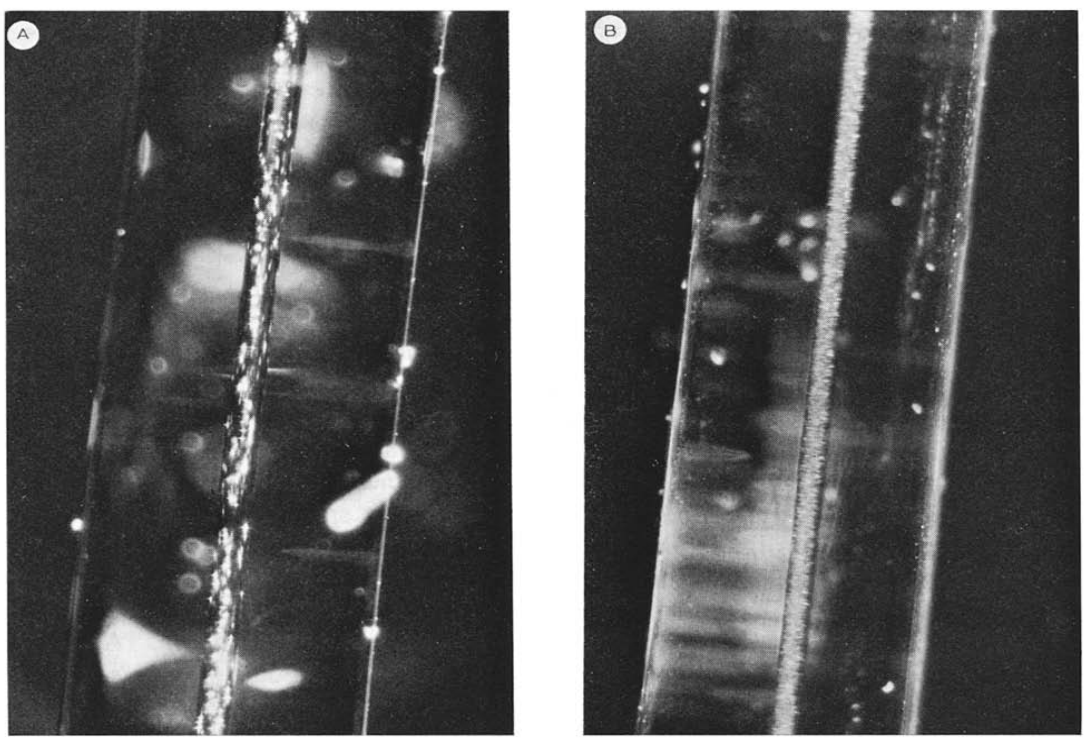


Fig. 1. Microphotographs of capillary column coated with ODPN. (A) Coating on borosilicate glass surface without any pre-treatment; (B) coating on soda-lime glass surface with pre-treatment with 1 *N*-potassium hydroxide solution.

TABLE II

DURABILITY OF ODPN COLUMN

Column: 507 cm × 52 μm I.D. Mobile phase: isooctane saturated with ODPN, 1.4 μl/min.

Total volume of mobile phase used (μl)	*Capacity factor, k'*			*Resolution*	
	Aniline	*α-Naphthylamine*	*β-Naphthylamine*	*Aniline and α-naphthylamine*	*α- and β-naphthylamine*
500	0.52	0.79	1.07	3.4	2.5
1500	0.50	0.75	1.02	3.2	2.5
2500	0.54	0.84	1.14	2.8	2.1
3500	0.43	0.67	0.90	2.5	1.8
4500	0.43	0.66	0.89	2.4	1.8
5500	0.42	0.64	0.86	2.4	1.9

these capillary columns endure more than 100 analytical cycles without any unfavourable effects (one analytical cycle needs about 25 μl of effluent). ODPN stationary phase is stable for more than 3 months.

The influence of injection volume on H was examined in the range 0.01–0.25 μl using isooctane solutions of β-naphthylamine (0.007%) and aniline (0.03%). No increase in H was observed with the former, and a slight increase above 0.2 μl with the latter. The effects of the amount of sample on H were also examined. H remained constant over a wide range of amounts, *e.g.*, as 0–40, 0–200 and 0–300 ng for β-naphthylamine, aniline and N,N-diethylaniline, respectively.

Table III shows the variation of k' with different ethylene glycol stationary phases. As the ethylene chain length increases, the polarity of the stationary phase decreases and k' increases. The greatest effect is observed with β-naphthylamine. H values using ethylene glycol stationary phases are in the range 0.5–2.0. These values are large compared with that of β,β'-oxydipropionitrile, which is 0.5–1.0 as shown in Figs. 3 and 4. The stability of mobile phases saturated with ethylene glycols is poor. They are very sensitive to moisture and become cloudy unless kept absolutely dry. Therefore, careful handling of these mobile phases is essential. Mobile phases saturated with β,β'-oxydipropionitrile are very stable.

TABLE III

COMPARISON OF k' VALUES WITH DIFFERENT ETHYLENE STATIONARY PHASES

Column: 466–535 cm × 48–53 μm I.D. Mobile phase: isooctane for ethylene and diethylene glycol and *n*-hexane for tetrathylene glycol.

Sample	*Stationary phase*		
	Ethylene glycol	*Diethylene glycol*	*Tetraethylene glycol*
β-Naphthylamine	0.42	0.71	1.93
3,4-Dimethylphenol	1.40	1.89	3.02
m-Cresol	3.02	3.51	4.69

Fig. 2 shows the relationship between column efficiency and linear velocity of the mobile phase. In previous papers[3,4], we demonstrated that the relationship was linear at relatively low linear velocities (less than 1.5 cm/sec) as also derived from

theory in OMCLC and described in a previous paper[3]. Fig. 2 shows that H also increases linearly with increasing linear velocity up to 7 cm/sec. In other words, the secondary flow effect is not noticeable at this linear velocity. The flow pattern in the coiled capillary (coil diamater 11 cm) is a strictly laminer flow.

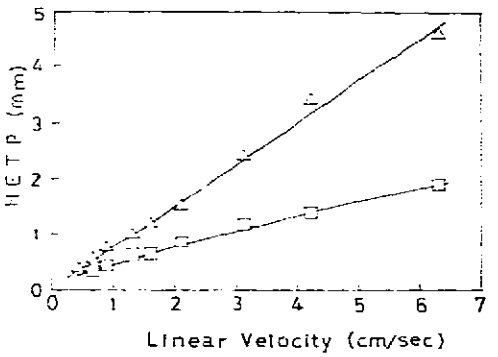


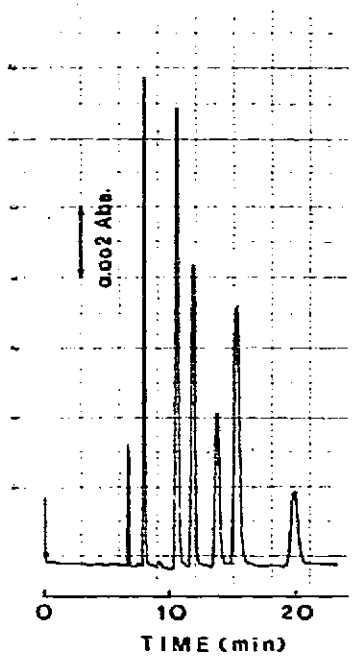
Fig. 2. Relationship between HETP (H) and linear velocity. Column: 507 cm × 52 μm I.D. glass capillary coated with ODPN. Mobile phase: isooctane saturated with ODPN. Amount of sample: 0.02 μl of isooctane solution containing 2 ng of N-phenyl-α-naphthylamine (□) and 1 ng of β-naphthylamine (△) ($k' = 0.14$ and 1.0, respectively).

The use of n-hexane as the mobile phase gave a 20–30% higher efficiency than isooctane, because of the difference in the diffusion of the solute in these two liquids.

If the extra-column effects on H are negligible, H is equal to $H_m + H_s$ (the subscripts indicate the mobile and stationary phase, respectively)[3]. If the diffusion coefficient of β-naphthylamine in isooctane at 20° is $1.6 \cdot 10^{-5}$ cm^2/sec, H_m is calculated to be 2.4 mm at a linear velocity of 3.1 cm/sec, $k' = 1.02$ and the column radius is 26 μm. The experimental value of H is 2.5 mm. Then the calculated H_s value is 0.1 mm. The H values of xylenols (Fig. 3) are between 0.45 and 1.0 mm. Calculated H_s values are between 0.1 and 0.2 mm for a linear velocity of 1.3 cm/sec and $k' = 0.19$–1.5. In both instances the contribution of H_s to H is very small. H is mostly due to Taylor diffusion in the mobile phase. In other words, the conditions with ODPN stationary phase are nearly ideal and mass transfer from or to the liquid phase is fast enough.

Fig. 3 shows a typical separation of xylenol isomers and m-cresol. Fig. 4 shows the rapid separation of six amines. Columns coated with ethylene glycols give slightly different chromatograms to those shown in Figs. 3 and 4.

Columns coated with ODPN and ethylene glycols have nearly the same separative ability as ordinary packed columns. These phases look very similar to glass capillary surfaces treated with alkaline solution. Fig. 1B shows very fine droplets, not a "thin film", so that there is the possibility of obtaining even better capillary columns. To work with much narrower capillary columns, with I.D. around 20 μm, more sensitive detectors and other developments will be necessary. This work suggests that OMCLC is valid method for the analysis of trace amounts of samples and could be used in combination with other spectrometric methods such as mass spectrometry. When using a 3 m × 60 μm I.D. capillary column the inlet



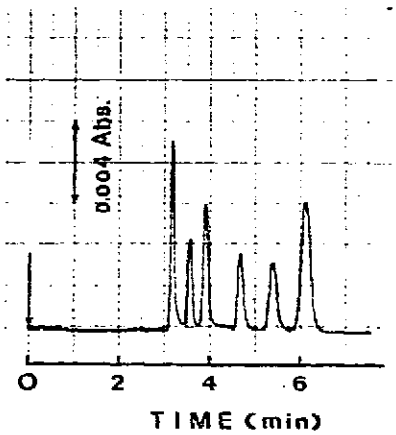


Fig. 3. Chromatogram of xylenol isomers and *m*-cresol. Column as in Fig. 2. Mobile phase: *n*-hexane saturated with ODPN, 1.7 μl/min. Wavelength of detection: 280 nm. Amount injected: 0.02 μl of isooctane solution containing 40 ng of 2,6-, 42 ng of 2,4-, 42 ng of 2,3-, 28 ng of 3,5- and 32 ng of 3,4-xylenol and 22 ng of *m*-cresol, eluted in that order.

Fig. 4. Rapid separation of six aromatic amines. Column: 504 cm × 49 μm I.D. glass capillary coated with ODPN. Mobile phase: *n*-hexane saturated with ODPN, 3.3 μl/min. Wavelength of detection: 235 nm. Amount injected: 0.02 μl of isooctane solution containing 11 ng of N,N-diethylaniline, 1.5 ng of N-phenyl-α-naphthylamine, 2 ng of N-phenyl-β-naphthylamine, 5 ng of aniline, 1 ng of α-naphthylamine and 1 ng of β-naphthylamine, eluted in that order.

pressure is *ca.* 10 atm. Therefore, if we want to use a long capillary column and keep the inlet pressure below 200 atm, a 60-m capillary column will be required. If we can handle a 10-μm capillary column, it will be possible to achieve high-efficiency capillary column chromatography by using a short column (under 1 m).

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