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STUDIES ON OPEN-TUBULAR MICROCAPILLARY LIQUID CHROMATOGRAPHY

VI. STYRENE-DIVINYLBENZENE COPOLYMER STATIONARY PHASE

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SUMMARY

Styrene-divinylbenzene copolymer is formed on the inner surface of a glass capillary tube of *ca.* 0.05 mm I.D. and *ca.* 5 m in length, by thermal, catalytic and radiation-induced polymerizations. The polymerization conditions that give good results chromatographically are thermal polymerization for 20 h at 100–260°. These polystyrene glass capillary columns can be employed for separations of aromatic hydrocarbons and phthalates in reversed-phase system. No bleeding of the stationary phase from the column is observed when various organic solvents are used.

INTRODUCTION

Recently, interest has developed in liquid chromatography (LC) with the use of microbore capillary columns that give higher efficiencies in terms of theoretical plate numbers than those used in ordinary high-performance liquid chromatography (HPLC). At present, three types of these columns are available: (1) packed microbore columns^{1–3}; (2) packed microcapillary columns^{4,5}; and (3) open-tubular microcapillary columns^{6–12}.

Columns of length 10 m and I.D. 1 mm packed with commercially available silica gel are commonly employed for packed microbore columns^{1–3}, giving 250,000 theoretical plates, the highest of the three types of column, at the dead volume (retention time 4–6 h). Columns of length 10–40 m and I.D. 0.06–0.08 mm packed with alumina (particle diameter 30 μm)⁴, sometimes followed by the bonding of various silanes so as to obtain different selectivities⁵, have been used for the packed microcapillary columns and 85,000 theoretical plates have been attained for quinoline (retention time 100 min) on a 20 m \times 0.075 mm I.D. alumina-packed microcapillary column⁴. About 10,000–20,000 theoretical plates were attained for aromatic amines (retention time 30 min) on 5 m \times 0.05 mm I.D. glass open-tubular microcapillary column coated with β, β' -oxydipropionitrile (BOP)¹¹.

For open-tubular microcapillary columns the theoretical plate number is inversely proportional to the square of the column diameter, and can therefore be increased by using a narrower bore column. A relatively low inlet pressure (less than 300 p.s.i.) is required in order to pass the eluent at 1 cm/min (linear velocity) into a $5\text{ m} \times 0.05\text{ mm}$ I.D. open-tubular microcapillary column. This suggests that it might be possible to develop longer and/or narrower open-tubular microcapillary columns that have higher efficiencies, if the pumping, sample injection and detection systems could be improved.

We have studied open-tubular microcapillary liquid chromatography (OMCLC) for a few years and have developed several types of columns, such as physically coated^{8,11}, chemically bonded⁹ and support-deposited columns¹³. For the physically coated columns, SE-30⁸, BOP¹¹ and polyethylene glycols (PEG)¹¹ were coated on the surface of glass capillary after appropriate pre-treatments and, if a mobile phase saturated with each stationary phase was employed, long-term stability of the columns was observed.

Octadecylsilane was examined as the stationary phase for the chemically bonded columns⁹ and was found to be more stable than those of physically coated columns.

For the deposited columns, soda-lime glass columns treated with aqueous alkaline solutions were investigated¹³. It was found that silica gel was deposited on the glass surface by treatment with 1 *N* sodium hydroxide solution for 2–6 days at 25–55° and functioned as the adsorbent in a normal-phase system.

In this work, we have examined the formation of polystyrene on the inner surface of a glass capillary and employed it as a stationary phase in a reversed-phase system, while styrene–divinylbenzene copolymer was employed as the packing material for gel permeation and partition chromatography or as the matrix for ion-exchange resins in LC. Thermal, catalytic and radiation-induced polymerizations were tried and polymerization conditions such as temperature, time and proportions of styrene and divinylbenzene were examined, using aromatic hydrocarbons as test samples.

EXPERIMENTAL

All reagents were purchased from Wako (Osaka, Japan). Styrene monomer was of practical grade and the divinylbenzene had a content of about 55%.

The apparatus is the same as that used in previously reported work⁸. A micro-feeder and a 100- μl gas-tight syringe were used for the pumping system and a UVIDEC-100 UV spectrometer (with a modified micro flow cell) (Japan Spectroscopic Co., Hachioji-shi, Japan) for the detection system.

Soda-lime glass was selected as the glass material, as pre-treatment with an alkaline solution was effective for surface modification of this glass, as reported earlier^{11,13}. Glass capillaries, 0.6–0.7 mm O.D. and 0.05–0.06 mm I.D., were prepared with a GDM 1 glass drawing machine (Shimadzu Seisakusho, Kyoto, Japan), followed by pre-treatment with 1 *N* sodium hydroxide solution for 2 days at 25–50°.

Subsequent to the pre-treatment, the glass capillary was washed with methanol until the eluent became neutral and then with dichloromethane. A mixture of 10% (v/v) of styrene monomer and 0.1–4% (v/v) of divinylbenzene in dichloromethane (with benzoyl peroxide as a catalyst for catalytic polymerization) was coated on the

inner surface of the glass capillary by dynamic coating and dried in a stream of nitrogen for 30 min at room temperature (25–30°). Then both ends of the glass capillary were closed and polymerization was promoted. For the thermal and catalytic polymerizations, coated glass capillaries were placed in an oven and heated to the reaction temperature at the rate of 4°/min and kept at that temperature for 2–20 h. For the radiation-induced polymerization, the glass capillaries were placed 10 cm from the γ -ray source (^{60}Co), where the absorbed dose was $3.4 \cdot 10^5$ rad/h and the temperature was about 25°. With irradiation for *ca.* 23 h, the total absorbed dose was *ca.* 8 Mrad.

Finally, each polymerized column was washed with mobile phase (acetonitrile–water), acetonitrile, dichloromethane, tetrahydrofuran, acetonitrile and mobile phase (*ca.* 100 μl of each).

RESULTS AND DISCUSSION

Effect of pre-treatment with alkaline solution

It was observed previously¹¹ that polar liquid phases such as BOP and PEG were well dispersed on the inner surface of a soda-lime glass capillary treated with 1 *N* sodium hydroxide solution, owing to the deposition of silica gel by that treatment¹³. Therefore, good dispersion of styrene and divinylbenzene on surfaces treated with alkaline solution was expected.

After pre-treatment with 1 *N* sodium hydroxide solution at different temperatures had been carried out, thermal polymerization was promoted. Subsequently, mobile phase was passed into the column. After the baseline had stabilized, the samples were injected and the k' values were measured.

The k' values of biphenyl on columns treated at different temperatures are listed in Table I, including that for a column without pre-treatment. Whereas the k' value without pre-treatment is zero, the higher temperature treatment gives a larger

TABLE I

EFFECT OF PRE-TREATMENT TEMPERATURE ON RETENTION

Pre-treatment: column filled with 1 *N* sodium hydroxide solution for 2 days at different temperatures. Polymerization conditions: thermally polymerized for *ca.* 20 h at 200°. Mobile phase: acetonitrile–water (30:70). Sample: biphenyl.

<i>Treatment temperature (°C)</i>	<i>k' value</i>
No treatment	0
27	0.3
40	1.2
50	1.3

k' value. This suggests that styrene and divinylbenzene are well dispersed on the treated surface. Pre-treatments at temperatures higher than 60° were not examined as no useful results were obtained at these temperatures in the previous work¹³.

As treatments at 40° and 50° gave nearly same results, soda-lime glass capillaries were treated at 40–50° in all subsequent examinations.

Effect of concentration of divinylbenzene in styrene

A dichloromethane solution of styrene containing 0–18% (mole/mole) of divinylbenzene was coated on the column, dried in a stream of nitrogen and thermally polymerized for 20 h at 200°.

The relationship between the retention of a sample and the concentration of divinylbenzene is shown in Fig. 1. As the concentration of divinylbenzene increases, the k' value of biphenyl increases when thermal polymerization is used. In the absence of divinylbenzene, polystyrene was dissolved in the mobile phase and the retention of the sample decreased during a chromatographic run, whereas with polystyrene in the presence of divinylbenzene there was no decrease in retention. In the latter instance, the retention increased after the passage of organic solvents such as acetonitrile, dichloromethane and tetrahydrofuran, which probably resulted from the increase in surface area of the stationary phase due to the elution of unreacted monomers or homopolymers from the column.

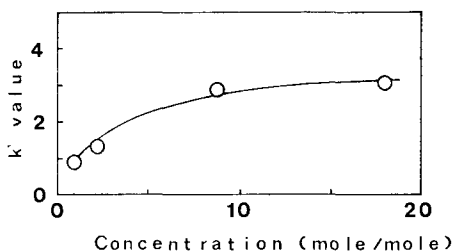


Fig. 1. Effect of concentration of divinylbenzene on retention. Polymerization conditions: thermal polymerization for 20 h at 200°. Mobile phase: acetonitrile–water (30:70). Sample: biphenyl.

Dependence of HETP on concentration of divinylbenzene is shown in Fig. 2. A low HETP value is obtained between 2 and 10% of divinylbenzene. At a concentration of 18%, the peak shape of the largely retained sample ($k' > 1$) was skewed. It is considered that the desirable concentration range of divinylbenzene in styrene is 2–10% (mole/mole).

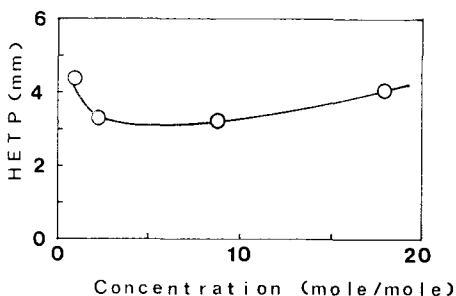


Fig. 2. Effect of concentration of divinylbenzene on HETP. Polymerization conditions as in Fig. 1. Linear velocity: *ca.* 1 cm/min. Sample: biphenyl ($k' = 1$).

Effect of thermal polymerization temperature

A styrene solution containing 8.8% of divinylbenzene was polymerized between 50° and 260° and k' values of the samples were measured. The results are shown in Fig. 3. Nearly same retentions are observed for columns polymerized at 100–260°, whereas biphenyl is not retained on columns polymerized at less than 80° by the use of 30% (v/v) acetonitrile in water as the mobile phase; nor are samples retained in the latter instance by the use of 20% (v/v) acetonitrile in water as the mobile phase, which indicates that the thermal polymerization has hardly been promoted.

The relationship between column efficiency and thermal polymerization temperature is shown in Fig. 4. Nearly the same HETP values are obtained in the region between 100° and 260°.

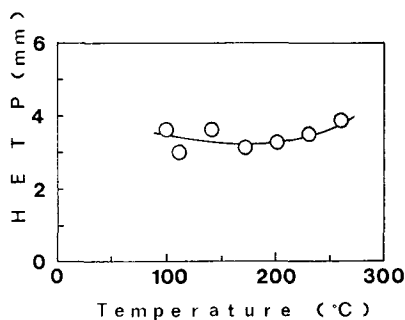
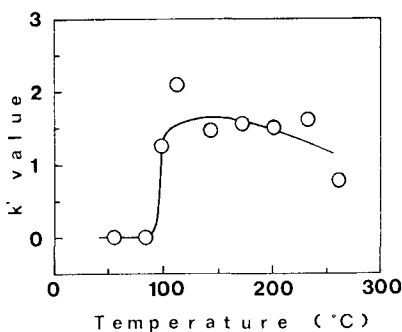


Fig. 3. Effect of polymerization temperature on retention. Polymerization conditions: thermal polymerization for 20 h. Concentration of divinylbenzene: 8.8%. Mobile phase: acetonitrile-water (35:65). Sample: biphenyl.

Fig. 4. Effect of polymerization temperature on HETP. Polymerization conditions as in Fig. 3. Flow-rate: 1.7 μ l/min. Sample: biphenyl ($k' = 1$).

Influence of polymerization time

The influence of polymerization time on the retention of samples was examined for thermal and catalytic polymerization. Divinylbenzene (8.8%, mole/mole) was added to styrene for both polymerizations, and 1% (w/v) of benzoyl peroxide was included for the catalytic polymerization. A temperature of 110° was adopted for the thermal polymerization and 80° for the catalytic polymerization. The relationship between the retention of the sample and the polymerization time is shown in Fig. 5. In both instances, as the polymerization time increases the k' values of biphenyl increase. A larger k' value is obtained for the thermal polymerization than for the catalytic polymerization. In addition, it can be concluded that the catalytic polymerization is predominantly promoted in the latter instance as the thermal polymerization is hardly promoted at 80°, as discussed in the preceding section.

Comparison of polymerization methods

At present, the best columns can be obtained by thermal polymerization. The k' values of samples for catalytic and radiation-induced polymerization are lower than that for thermal polymerization, as shown in Table II. It is considered that

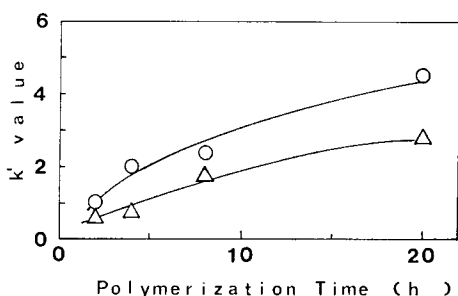


Fig. 5. Relationship between retention of sample and polymerization time. Thermal polymerization at 110° (○); catalytic polymerization at 80° (△). Mobile phase: acetonitrile-water (30:70). Sample: biphenyl.

the polymerization reactions are not adequately promoted in the catalytic and radiation-induced polymerization under the conditions used. Sometimes clogging was caused on passing water into the column, especially when using catalytic and radiation-induced polymerizations.

TABLE II

COMPARISON OF k' VALUES ON COLUMNS PREPARED BY DIFFERENT METHODS
Mobile phase: acetonitrile-water (30:70). Sample: biphenyl.

Polymerization conditions			Concentration of divinylbenzene (%)	k' value
Method	Temperature (°C)	Time (h)		
Thermal	110	20	8.8	4.5
Catalytic	80	20	8.8	2.8
Radiation-induced	25	23 (7.8 Mrad)	18	0.2

Effect of amount of sample injected

The effect of amount of sample injected on column efficiency is shown in Fig. 6. An increase in HETP is observed when amounts larger than 10 ng are used.

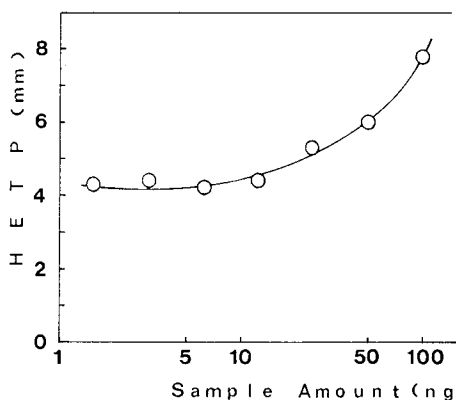


Fig. 6. Effect of amount of sample injected on HETP. Column: 5.4 m × 58 μm I.D. glass capillary. Stationary phase: styrene-divinylbenzene copolymer thermally polymerized for 20 h at 200°. Mobile phase: acetonitrile-water (40:60). Flow-rate: 2.2 μl/min. Sample: biphenyl.

Also, as the amount of sample injected increases, a decrease in k' values is observed. Although the capacity of this polystyrene column is lower than those of SE-30⁸ and ODS column⁹, the column efficiencies are comparable.

Typical separations of aromatic hydrocarbons and phthalates are shown in Figs. 7 and 8, respectively.

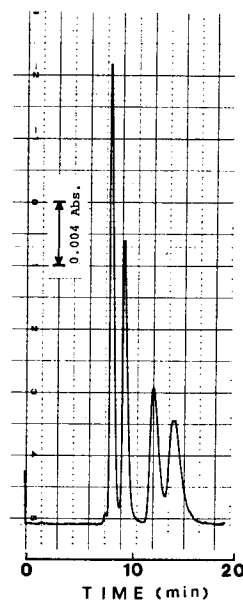
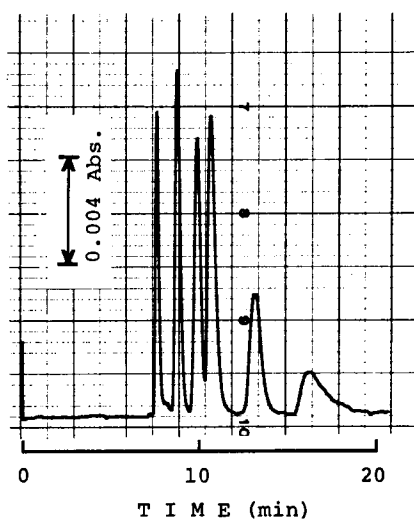


Fig. 7. Separation of aromatic hydrocarbons. Column: $5.4 \text{ m} \times 52 \mu\text{m}$ I.D. glass capillary. Stationary phase: styrene-8.8% divinylbenzene copolymer thermally polymerized for 20 h at 110° . Mobile phase: acetonitrile-water (50:50). Flow-rate: $1.7 \mu\text{l}/\text{min}$. Sample: acetonitrile solution containing 180 ng of benzene, 17 ng of naphthalene, 3 ng of biphenyl, 5 ng of fluorene, 0.5 ng of anthracene and 4 ng of pyrene, eluted in that order. Wavelength of detection: 254 nm (UV).

Fig. 8. Separation of phthalates. Column: $5.4 \text{ m} \times 53 \mu\text{m}$ I.D. glass capillary. Stationary phase: styrene-8.8% divinylbenzene copolymer thermally polymerized for 20 h at 170° . Mobile phase: acetonitrile-water (32:68). Flow-rate: $1.7 \mu\text{l}/\text{min}$. Sample: acetonitrile solution containing 21 ng of dimethyl, 21 ng of diethyl, 21 ng of diisopropyl and 19 ng of di-*n*-propyl phthalate, eluted in that order. Wavelength of detection: 235 nm (UV).

CONCLUSION

Styrene-divinylbenzene copolymer polymerized on the surface of a soda-lime glass capillary treated with 1 *N* sodium hydroxide solution can be employed as the stationary phase in a reversed-phase system. The optimal polymerization conditions are thermal polymerization for 20 h at 100 – 260° in the presence of 2–10% of divinylbenzene as the cross-linking material. This stationary phase is useful for the separation of aromatic hydrocarbons and phthalates, and is stable towards organic solvents. It is expected to be possible to introduce ion-exchange groups into this copolymer.

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