Journal of Chromatography, 158 (1978) 227–232

CHROM. 11,066

STUDIES OF OPEN-TUBULAR MICRO-CAPILLARY LIQUID CHROMATOGRAPHY

II*. CHEMICALLY BONDED OCTADECYLSILANE STATIONARY PHASE

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SUMMARY

A chemically bonded octadecylsilane stationary phase was successfully prepared on the inner glass surface of a micro-capillary tube of I.D. 60 μm. Using these capillary columns of length 3–20 m, several aromatic compounds were separated completely with methanol–water or acetonitrile–water as the mobile phase at a linear velocity of 0.3–1.3 cm/sec.

INTRODUCTION

Although the use of open-tubular capillary columns in gas chromatography (GC) is well established[1], few papers have been published on open-tubular capillary liquid chromatography[2–7]. If open-tubular capillary columns could be successfully applied in liquid chromatography (LC), extremely large numbers of theoretical plates and a fine separating ability would be obtained. As the solute diffusivity in the mass transfer process in a liquid mobile phase is nearly 10^4 times lower than that in the gas phase, the column diameter should be much smaller than in GC. In addition, in order to avoid extra-column effects with narrow-bore open-tubular capillary columns, new devices for injection, detection and connections must be developed[3,4,6,7].

Fundamental studies on capillary diameters, which were examined up to 50 μm, for non-retained[4] and retained peaks[6,7] suggested that the smaller the inner diameter of a capillary column the lower is the theoretical plate height (H), as predicted by the Taylor equation[8].

Earlier workers[3] achieved a separation with a wide open-tubular capillary column of I.D. 0.23–0.30 mm, but, owing to the relatively large column diameter, the resolutions obtained were inadequate, even though the separation took about 5 h.

In our first work on open-tubular micro-capillary liquid chromatography

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(OMCLC) we achieved the complete separation of five aromatic compounds using a column of I.D. 60 μm coated with SE-30[6,7]. From this separation, it is not difficult to imagine the potential ability of capillary columns in LC. As SE-30 was coated on the inner wall of a glass capillary, and was not chemically bonded to the glass surface, the mobile phase should always be saturated with SE-30 to avoid the loss of stationary phase.

In this work we have developed a new bonded stationary phase for use in OMCLC.

EXPERIMENTAL

Chemically bonded octadecylsilane (ODS) stationary phase

A borosilicate glass capillary tube, I.D. *ca.* 60 μm, was obtained as described earlier[6,7]. The glass capillary tube was washed with sodium hydroxide solution, hydrochloric acid, water and methanol or only with methanol, and dried at 200° for 1 h with a flow of nitrogen. Then a plug of 20–70 μl of a 4–50% solution of octadecyltrichlorosilane in toluene solution was passed through capillary tube by compressed air in a 250-μl gas-tight micro-syringe. Sometimes the capillary was initially filled with toluene and the plug was subsequently passed through. After the plug had passed through, the capillary was placed in an oven and nitrogen was passed through to remove the solvent and to promote the reaction between the glass surface and the reagents. The temperature of the oven was programmed from room temperature to 130° at 2°/min and kept at 130° overnight. Then nitrogen saturated with water was passed through the column to effect polymerization of unreacted silane reagents under a controlled temperature, which was programmed from 130° to 150° at 4°/min, and the column was again kept at 150° overnight. The column was then dried with nitrogen at 150°.

Whereas most of the experiments were carried out with an untreated glass capillary tube, we also used a surface-modified glass capillary tube, silica "whiskers" being formed inside the capillary according to the method of Onuska *et al.*[9]. Without prior washing of the glass capillary tube, treating the glass wall with a 0.5 or 2.7% solution of NH_4HF_2 in methanol gave an adequate density of whiskers.

Liquid chromatography and extra-column dead volume

The procedure has been described previously in our papers on OMCLC[7] and micro-high-performance liquid chromatography[10]. This system involves splitless injection and a very small detector system with a very small inner volume, although there are other techniques, involving a split injection system and additional liquid purging at the column exit for capillary liquid chromatography[3,5,11]. Fig. 1 shows a schematic diagram of the injection and detection units for a capillary column of I.D. 60 μm. After sucking the sample solution, for example 0.024 μl, into the tube (2) (Fig. 1), the stainless-steel tube (1) is inserted into a PTFE tube, as shown by the arrow, until it was positioned just in front of the capillary column inlet. Then the pump feeds the sample solution and eluent into the capillary column.

In order to prevent extra-column effects, the extra-column dead volume should be restricted to less than 20% of the total column volume. If the dimensions of the capillary column are, for example, 3 m × 60 μm I.D., the total column volume is

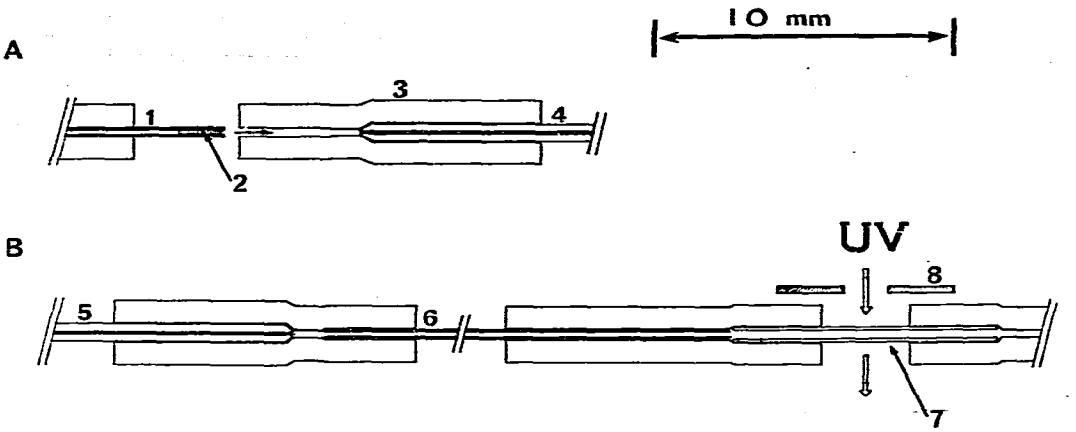


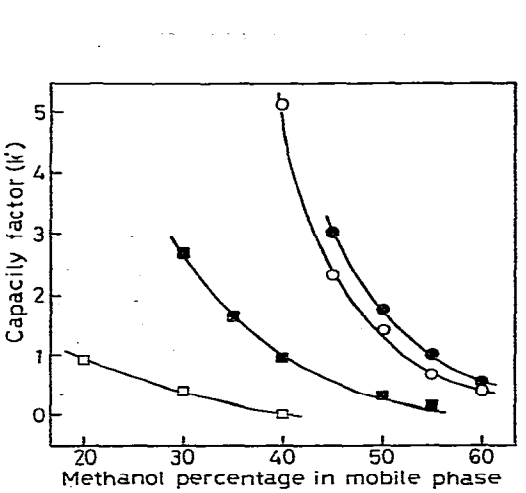
Fig. 1. Schematic diagram of (A) injection and (B) end part of capillary column and detector. (1) Stainless-steel tube, 0.15 mm I.D., 0.3 mm O.D.; (2) sample; (3) PTFE tube, 0.25 mm I.D., 2 mm O.D.; (4) inlet of capillary column, 60 μm I.D., 0.6 mm O.D.; (5) exit of capillary column; (6) stainless-steel tube, 0.15 mm I.D., or PTFE tube, *ca.* 0.06 mm I.D., each of length *ca.* 53 mm; when we used the stainless-steel tube, we usually inserted a stainless-steel rod, 0.1 mm O.D., in it; (7) quartz tube, 0.3 mm I.D., length 5–10 mm; (8) slit, 0.3 mm × 1.5–3.0 mm.

only 7.4 μl and in this instance the extra-column dead volume should be less than 1.5 μl. Our modified technique, which was developed for micro-high-performance liquid chromatography[10], successfully overcame this limitation: the volumes of the total connecting parts and the detector cell in Fig. 1 are about 1.0 and 0.1 μl, respectively.

RESULTS AND DISCUSSION

Silane reagents have been widely used to prepare bonded stationary phases on supports such as silica gel and Chromosorb for chromatography[12–14]. In reactions with the inner surface of glass capillary tubes, silica reagents were used for improving the wettability[15–17] and recently for forming bonded stationary phases[5,18] in glass capillary gas chromatography. Masada *et al.*[5] prepared bonded stationary phases on a wide capillary tube, I.D. 0.25 mm, and applied it in LC, but failed to achieve an effective separation. The work described here is the first successful preparation of a chemically bonded phase in a micro-capillary tube (I.D. 60 μm) for LC.

Capillary columns with a chemically bonded ODS phase were prepared with good reproducibility and showed good stability and efficiency. There were no detectable changes with solvents such as hexane, methanol and dichloromethane. The speeds of the plug of ODS solution were varied between 1 and 10 cm/sec, the most favorable speed being *ca.* 4 cm/sec. The four lines in Fig. 2 correspond to four different capillary columns that were treated dynamically with 4–50% solutions of ODS in toluene. As the capacity factor increases as the ODS concentration increases at a constant mobile phase composition, the thickness of the ODS layer, which is chemically bonded, also increases. However, there is little variation between a 20% and a 50% solution. As, for chromatographic development, the mobile phase should preferably contain more than 40% of methanol in order to give an adequate sol-



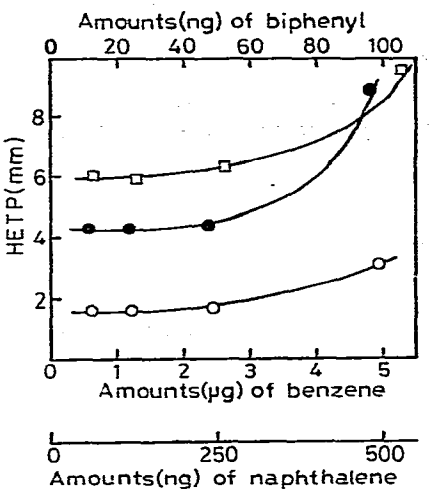


Fig. 2. Capacity factor *versus* ODS concentration of plug. ODS concentration: 4% (□), 16% (■), 20% (○) and 50% (●). Plug velocity, *ca.* 4 cm/sec; mobile phase, methanol–water; ODS capillary column, length 333–336 cm I.D. 59–63 μm; sample, 0.024 μl of a 0.024% solution of biphenyl in methanol; flow-rate, *ca.* 1.3 cm/sec.

Fig. 3. Effect of amount of sample on column efficiency. Glass capillary column of chemically bonded ODS, 336 cm × 60 μm I.D. Amount injected was kept constant at 0.024 μl. Flow-rate, 1.3 cm/sec; mobile phase, methanol–water (52:48); sample, methanolic solution of a mixture of benzene (○), naphthalene (●) and biphenyl (□).

ubility of aromatic compounds, we selected a 20% solution of ODS in toluene for further work.

Overloading of samples on the ODS capillary columns begins at 50 ng of biphenyl, 250 ng of naphthalene and 2.5 μg of benzene, as shown in Fig. 3. As the ODS layer is very thin, the capacities of these samples are not high, as expected. The relationship between plate height and flow velocity is linear, as shown in Fig. 4. Within the velocity range 0.3–1.3 cm/sec, a long column cannot be used if the total analysis time is to be kept constant. However, it is not recommended to use a very short capillary column, because extra-column effects become larger and make the separation difficult. The technique described here can be used with capillary columns longer than 3 m if the inner diameter is 60 μm. The relationship between plate height and linear velocity is unknown for values of the latter over 1.5 cm/sec. If the plate height does not increase in a first-order manner relative to linear velocity (this is usually observed in high-performance liquid chromatography), there is the possibility that long capillary columns (over 20 m) will give a high efficiency with a moderate separation time.

Three typical chromatograms for aromatic compounds are shown in Figs. 5–7. The volume flow-rate of 1 μl/min for a 60-μm I.D. capillary column is equivalent to a linear velocity of 0.59 cm/sec. The plate number ofr biphenyl is 250 per metre (Fig. 5). At a low linear velocity, such as 0.3 cm/sec, this value increases to 600 plates per metre. These values are about one-fifth to one-tenth of those for ordinary glass capillary columns in GC. This relatively low efficiency might be due mainly to mass transfer processes in or on the stationary phase, and especially to the slow rate con-

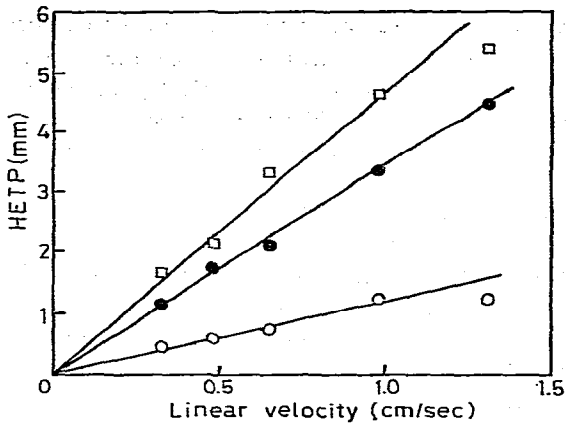


Fig. 4. Plate height *versus* linear velocity. Column and mobile phase as in Fig. 3. Amount injected, 0.024 μl of a solution of a mixture of 2.5% benzene, 0.15% naphthalene and 0.045% biphenyl in methanol.

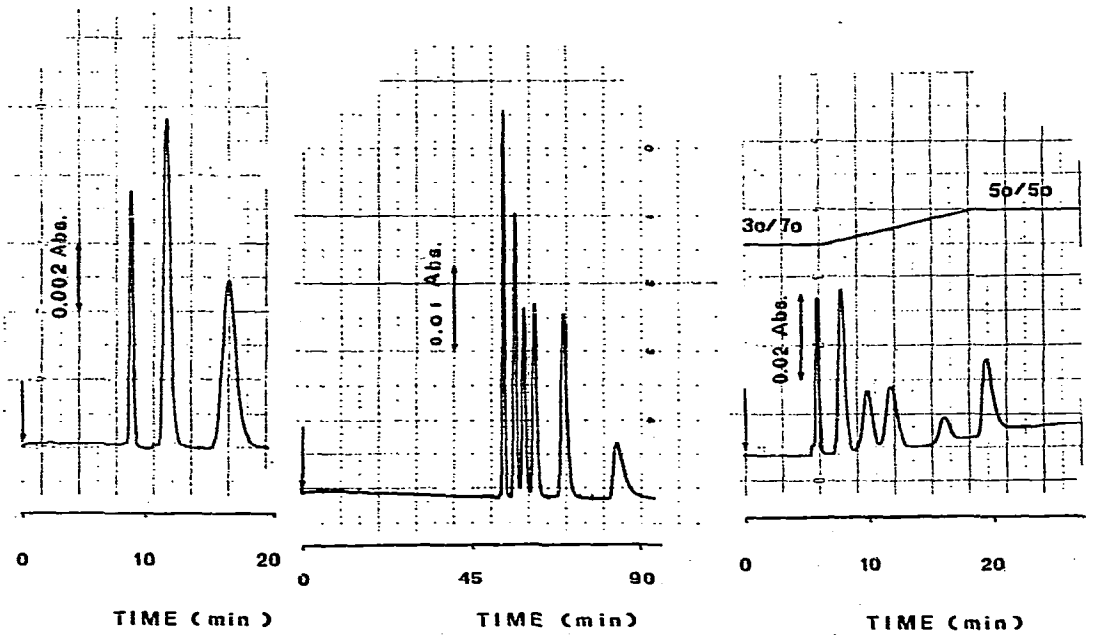


Fig. 5. Chromatogram of aromatics on chemically bonded ODS capillary column with "whiskers". Column: 384 cm × 60 μm I.D., modified surface with 0.5% NH_4HF_2 following Onuska *et al.*[9], and then treated with 20% ODS–toluene solution. Mobile phase, acetonitrile–water (25:75); flow-rate, 2.22 μl/min; amount injected, 0.024 μl of a solution of a mixture of 1.6% benzene, 0.15% naphthalene and 0.034% biphenyl, eluted in that order.

Fig. 6. Chromatogram with 20-m column. Capillary column of chemically bonded ODS, 20.88 m × 56 μm I.D. Mobile phase, acetonitrile–water (40:60); flow-rate, 1 μl/min; amount injected, 0.03 μl of an acetonitrile solution of a mixture of 4.1% benzene, 0.37% naphthalene, 0.05% biphenyl, 0.08% fluorene, 0.01% anthracene and 0.1% pyrene, eluted in that order.

Fig. 7. Chromatogram with gradient elution. Column as in Fig. 3. Mobile phase, acetonitrile–water varied from 30:70 to 50:50, following the line shown. Amount injected, 0.036 μl of an acetonitrile solution of a mixture of 3.3% benzene, 0.35% naphthalene, 0.045% biphenyl, 0.05% fluorene, 0.007% anthracene and 0.088% pyrene, eluted in that order.

stant for desorption[19]. In order to improve this mass transfer process, the preparation of a very thin stationary phase, such as a monolayer, is worth considering. Alternatively, capillary LC can be carried out at high temperature. Another problem in capillary LC is that the capacity factor (k') is relatively small; the largest value that we have obtained is about 2. It is necessary to have a value of at least 10 for the analysis of complex mixtures.

The advantages of OMCLC are as follows. (1) With the open column one does not need a high pressure to feed the eluent, for example a pressure of 10–20 atm suffices for a 3 m × 60 μm I.D. column at a linear velocity of 1.3 cm/sec. (2) Direct connection with a mass spectrometer is feasible, as the flow-rate of the mobile phase is only about 1 μl/min, which is one-thousandth of that in high-performance liquid chromatography. (3) As capillary LC can be applied to micro-amounts of sample, it is easy to apply this method in microanalysis.

In this work, the inner diameter of the capillary column was about 60 μm, although a smaller value would be more favorable. We are now investigating this problem and those mentioned above, and also attempting to prepare capillary columns with polar stationary phases.

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