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Monolithic silica column for in-tube solid-phase microextraction coupled to high-performance liquid chromatography

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Abstract

In-tube solid-phase microextraction (SPME) has successfully been coupled to capillary LC, and further an automated in-tube SPME system has been developed using a commercially available HPLC auto-sampler. However, an open tubular capillary column with a thick film of polymer (stationary phase) is unfavorable because the ratio of the surface area of coating layer contacted with sample solution to the volume of the capillary column is insufficient for mass transfer. A highly efficient SPME column is, therefore, required. We introduced a C_{18}-bonded monolithic capillary column that was used for in-tube SPME. The column consisted of continuous porous silica having a double-pore structure. Both the through-pore and the meso-pore were optimized for in-tube SPME, and the optimized capillary column was connected to an HPLC injection valve for characterization. The results demonstrated that the pre-concentration efficiency is excellent compared with the conventional in-tube SPME. The novel method for both introduction and concentration of the samples was effective, satisfactory and suitable for use in the SPME medium.
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1. Introduction

Miniaturization and automation have been two important trends in the field of high-performance liquid chromatography (HPLC). The main advantages of micro or capillary scale HPLC are: (i) increased efficiency in a shorter time; (ii) decreased solvent consumption; and (iii) enhanced detection performance with the use of concentration-sensitive detectors. In addition, small volumetric flow-rates admit directly to hyphenation, without any flow splitter, of mass-sensitive detectors such as mass spectrometers. However, few reports of capillary HPLC have been found in the literature, due mainly to the smaller injection volume/mass that results in low sensitivity. For maintaining chromatographic efficiency, the maximum injection volume allowed has been theoretically discussed by Vissers et al. [1]. As shown by Vissers et al., the allowable maximum injection volumes were 520 and 46 nl for a 150-mm-long capillary column with 1.0 and 0.3 mm I.D., respectively. Therefore, when analyzing trace amounts of biological, medical or environmental materials, there is frequently lack of detection sensitivity.

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Recently, large-volume injection techniques for capillary HPLC have been investigated to improve sensitivity and determinability. One possible techniques is to use a pre-column for concentration of analyte, which is based on a column switching technique. This method requires an additional pump and injection valve to load sample and flush the pre-column and, therefore, the system tends to be complicated. Another approach is to dissolve analytes in a solvent whose elution strength is weaker than the eluent, which is well known as an on-column focussing technique. In this method, sensitivity can be improved because of the increased injection volume. Moreover, on-line or off-line solid-phase extraction (SPE) is often applied in HPLC, in which an on-line system is very useful because the compounds extracted can be transferred directly into the analytical system in addition to the reduction of solvent consumption. However, special equipment is necessary for on-line SPE. As an improved methodology, Arthur and Pawliszyn [2] developed solid-phase microextraction (SPME). The technique of SPME employs a coated fiber to extract and concentrate analytes, and has been successfully applied in gas chromatographic system. However, there are still some difficulties in interfacing with the HPLC system. In-tube SPME has been developed as an alternative method, in which an open-tubular capillary column is used as the extraction medium. Although the technique has successfully been coupled to capillary HPLC [3,4], the open-tubular column is unfavorable for liquid handling. When using an open-tubular column, the surface area of the coating layer is too small for liquid–liquid extraction. For improvement, it is necessary to repeat draw/eject cycles (generally 10–20 times) of sample solution in extraction process.

Saito et al. [5] reported that a modified in-tube SPME fiber increased extraction efficiency. A stainless steel wire was inserted into the capillary in order to reduce the volume of extraction capillary while the surface area contacted with the sample solution was maintained. The use of stainless steel wires could result in contamination, especially when using biological samples.

In this work, we introduce a C_{18}-bonded monolithic capillary column, which consists of continuous porous silica having a double-pore structure. A monolith-structured column is very suitable for in-tube SPME medium due to its unique features, including low pressure drop, and total porosity higher than particle column. The characteristics of a monolith-structured in-tube SPME column are compared to those of an open-tubular column, and applications in the separation/analysis of pesticides are described.

2. Experimental

2.1. Reagents

4-Methylphenol, 4-ethylphenol, 4-propylphenol, 4-butylphenol, and 4-pentylphenol were purchased from Wako (Tokyo, Japan). Acetophenone, benzene, toluene, and naphthalene were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Pesticide standards were obtained from GL Sciences (Tokyo, Japan). Acetonitrile used for the eluent was of HPLC grade and purchased from Kishida (Osaka, Japan). Deionized water was prepared with a Milli-Q system (Nihon Millipore Kogyo, Tokyo, Japan).

2.2. Chromatographic conditions

All capillary-scale chromatographic experiments were performed with a Model MP-680 Micro-Flow pump (GL Sciences), a Model SM2-010A low volume static mixer (GL Sciences), and a Model UV-702 UV–Vis detector (GL Sciences) equipped with a capillary flow-cell holder for on-column detection. The mobile phase was prepared using acetonitrile and water, and the flow-rate was typically 5 μl/min. Chromatographic separation was performed on an Inertsil ODS-3 column (150 mm×0.3 mm I.D., 3 μm particle size; GL Sciences). A Model 8125 valve (Rheodyne, CA, USA) and a Model CI4 valve (Valco, TX, USA) were used as the sample injector. Data acquisition and processing were performed using EZChrom Elite (GL Sciences).

2.3. In-tube SPME experiments

The C_{18}-bonded monolithic silica column (450 mm×200 μm I.D., silica skeleton size of ~3.0 μm), with a through-pore size of 10 μm and a meso-pore

size of 12 nm, was prepared by in situ hydrolysis and polycondensation of alkoxysilane by a method described previously [6,7].

The column was cut to 150 mm length for use in in-tube SPME, and mounted in the position of sample loop of a six-port injector. A zero dead-volume union (Valco) was employed for the connections of capillary tubing.

In the first step, the capillary column was washed and conditioned by first by methanol and then by pure water prior to extraction. The extraction of analytes into the capillary column was performed by manual injection (or using several repeated draw/eject cycles in the case of conventional in-tube SPME). After the valve was switched to the ''Inject'' position, the extracted analytes were desorbed from the capillary column with mobile phase, and transported to a UV–Vis detector equipped with a separation column.

3. Results and discussion

3.1. Characteristics of monolithic column for in-tube SPME medium

Sample capacity is one of the important characteristics for in-tube SPME process. If there is not enough capacity, the analytes will pass, without being trapped, through the pre-column during extraction. For evaluating the monolith column, breakthrough volume was measured by pumping acetonitrile–water (30:70, v/v) solution containing 7.7 mg/l biphenyl. After conditioning the column with acetonitrile, the acetonitrile–water solution was continuously delivered to the detector equipped with a 150-mm monolithic column. An abrupt increase in absorbance would be caused by any breakthrough of biphenyl contained in the solution. Under the conditions of 5-μl/min flow-rate, the breakthrough time appeared to be 28.5 min, so that the sample capacity could be calculated with both the breakthrough volume and the concentration. In the cases of 10-, 15- and 50-μl/min flow-rates, sample capacities were also calculated as shown in Table 1. The results indicated that the biphenyl sample with 50-μl injection volume and 7.7-ppm concentration could be completely trapped in the monolithic column under

Table 1
Breakthrough volumes of 150-mm-long monolithic silica used as in-tube SPME medium

Flow-rate (ml/min)	Breakthrough time (min)	Sample capacity (ng)	Pressure drop (MPa)
5	28.5	1097	0.2
10	13.8	1063	0.4
15	9.0	1040	0.7
50	2.6	1001	2.4

the conditions of 5-μl/min flow-rate and acetonitrile–water (30:70, v/v) solution. If using a decreased concentration of organic solvent, a larger trapping amount may be achieved.

Correlation between the injection volume and the response was established for large-volume injection by injections of 2, 15, 50 and 100 μl standard alkylphenol solution (0.1 mg/ml, in 50% CH_3CN). The standard sample was 10-fold diluted with pure water. As shown in Fig. 1, good linearity was acquired with a correlation coefficient of 0.999 for each over a range of 2–100 μl.

3.2. Comparison of open-tubular capillary column and monolithic silica column

Many reports related to conventional in-tube SPME, which were based on open-tubular columns, indicated that multiple inject/eject cycles (generally 10–20 times) are required to achieve sufficient
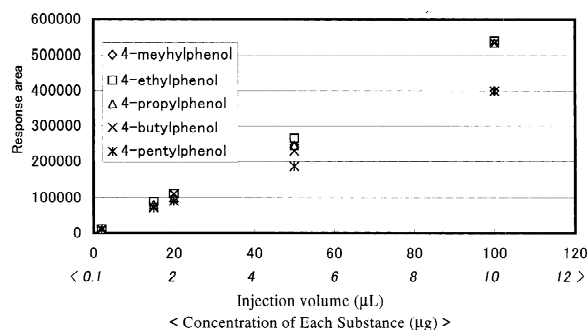

Fig. 1. Relationship between the linearity and the injection volume. Sample: alkylphenol (C_1–C_5), each 0.1 mg/ml in 50%CH_3CN, was 10-fold diluted with water; in-tube SPME–capillary LC: SPME column, monolithic silica column (150 mm× 0.2 mm I.D., skeleton size of ~3.0 μm); gradient condition: CH_3CN–water 70/30 to 100/0 in 25 min.
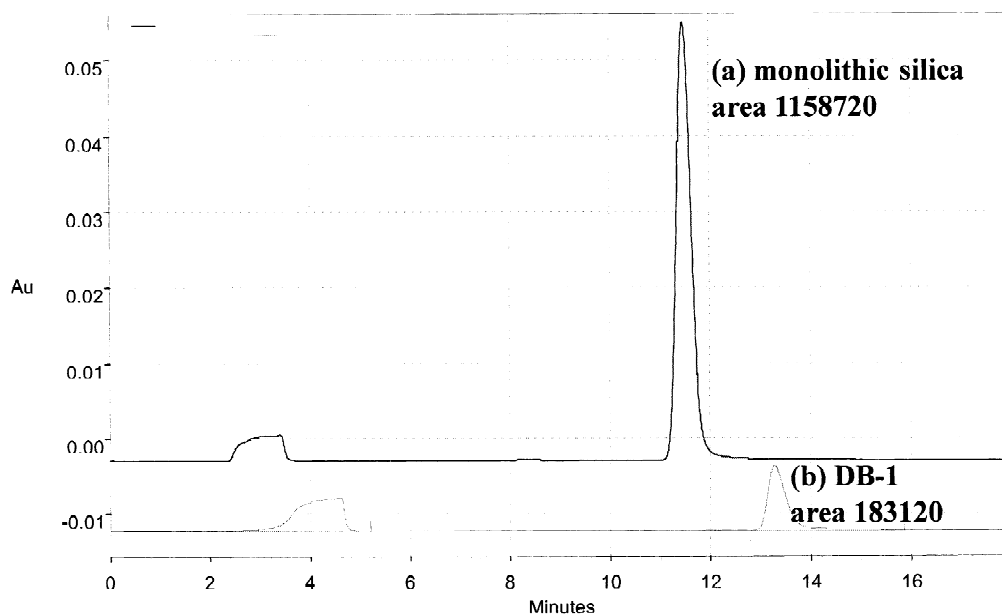
Fig. 2. Chromatograms of biphenyl extracted by different conditions. (a) Monolithic silica column (150 mm×0.15 mm I.D., silica skeleton size of ~3.0 μm); (b) in-tube SPME with open-tubular DB-1 column (150 mm×0.25 mm I.D., 0.25 μm thick); and (c) in-tube SPME with monolithic silica column. Sample: 0.05 m*M* biphenyl was 10-fold diluted with water; column used in capillary LC system: C_{18} bonded (150 mm×0.3 mm I.D., particle size 3 μm); isocratic elution condition: CH_3CN–water (75/25); 5 μl/min; detection, UV 254 nm.
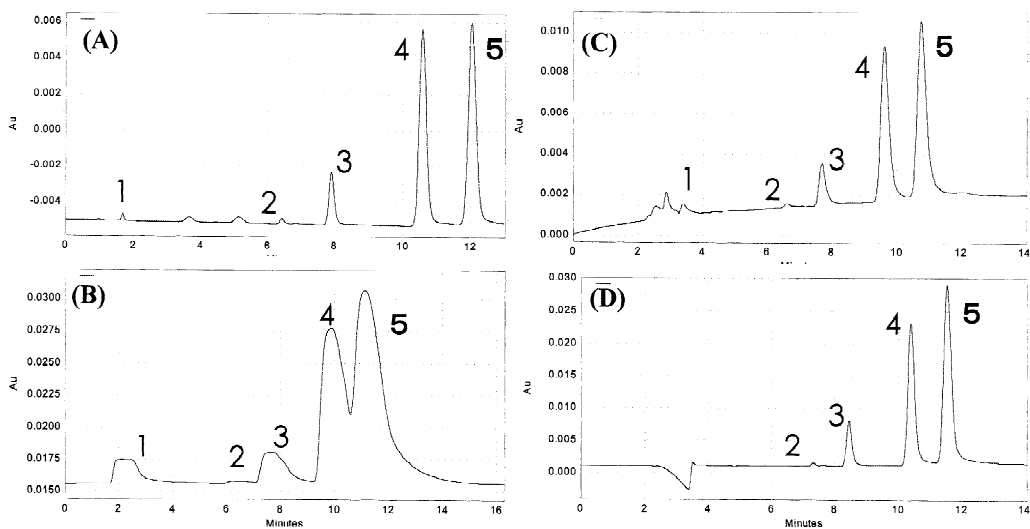

Fig. 3. Typical chromatograms obtained by different injection methods. Sample: (1) uracil, (2) toluene, (3) naphthalene, (4) biphenyl and (5) fluorene. In-tube SPME conditions: extraction capillary monolithic silica column (150 mm×0.15 mm I.D., silica skeleton size of ~3.0 μm). Column used in capillary LC system: 150 mm×0.3 mm I.D., 3 μm C_{18}. Isocratic elution condition: CH_3CN–water (75/25); 5 μl/min; UV 254 nm.

extraction efficiency. This is due to the fact that mass transfer speed is not great enough for an on-flow extraction system. Fig. 2 shows the typical chromatograms of biphenyl by in-tube SPME using a conventional open-tubular column (DB-1, 0.25 mm I.D., 0.25 μm thick; Agilent Technologies, USA) and a monolithic column as extraction materials. As shown in Fig. 2, although these are very simple procedures, the peak height obtained by the monolithic column was far higher than the DB-1 column where a 10-times inject/eject extraction process was carried out. Also, the difference in the retention times is due to the fact that the DB-1 column has a larger internal

volume than the monolithic column. It is clear that SPME with a monolith-structured column exhibits a powerful ability for pre-concentration of trace samples in an aqueous matrix.

3.3. Capability of large volume injection by in-tube SPME technique using monolithic column

The capability and efficiency of large-volume injection by in-tube SPME was investigated using standard 0.5-mM sample solutions including uracil, toluene naphthalene, biphenyl and fluorene. The chromatograms in Fig. 3A–D were obtained under
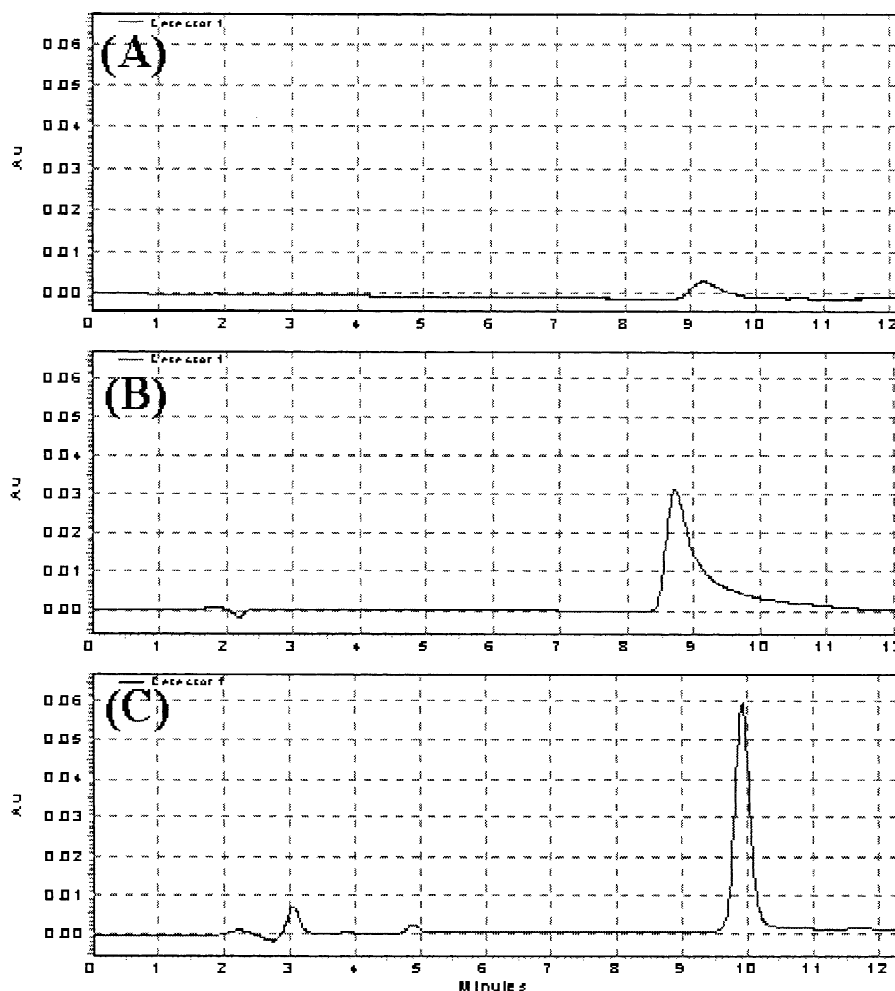

Fig. 4. Typical chromatograms of warfarin obtained by different injection methods. (A) Injection (0.1 μl) of 2.5 mM warfarin. (B) Injection (0.1 μl) of 0.25 mM warfarin. (C) Injection (0.1 μl) of 0.25 mM warfarin by in-tube SPME.

the following conditions: (A) 0.1-µl volume of a
0.5-m*M* sample dissolved in mobile phase (acetoni-
trile–water, 70:30, v/v) was introduced into injection
loop; (B) 1-µl volume of a 0.5-m*M* sample diluted
in acetonitrile–water (70:30, v/v) was introduced
into injection loop; (C) 6-µl volume of 0.083-m*M*
sample diluted in water was introduced into mono-
lithic column that was installed instead of the
injection loop; and (D) 10-µl volume of a 0.05-m*M*
sample diluted in water was introduced into mono-
lithic column. Chromatogram (B) exhibited
broadened peaks owing to an excess of sample over
column capacity; other chromatograms (C) and (D)
showed excellent results. Fig. 4A and B gives the
results of warfarin analysis by conventional capillary
LC and in-tube SPME. Chromatogram (C), which is
based on in-tube SPME using a monolithic column,
shows sensitive response and peak shape without
tailing.

3.4. Application to pesticides

In-tube SPME–capillary HPLC using a monolith-
structured column was applied to the separation/
analysis of pesticides. Fig. 5 shows typical chromato-
grams of five pesticides. The upper chromatogram
was obtained by direct injection of 0.1-µl sample
into the sample loop, and the lower chromatogram

used a monolithic material instead of the injection
loop. Although concentration is lower, the technique
based on in-tube SPME using a monolithic column
exhibited excellent separation and peak shape as
compared with 0.1-µl direct injection. It is very
obvious that SPME with a monolith-structured col-
umn has a powerful ability for the pre-concentration
of trace samples in a complex matrix.

4. Conclusion

A novel pre-concentration technique using mono-
lithic material as the in-tube SPME medium was
investigated. Monolith-structured columns improve
upon the shortcomings of conventional in-tube
SPME in which open-tubular capillary columns are
used. For trace analysis of environmental or bio-
logical samples, the large-volume injection technique
using the in-tube SPME/on-column focusing tech-
nique led to higher sensitivity (~50 times) than that
published using capillary LC techniques. In cases
using the same amount of sample, this capillary LC
system using in-tube SPME showed higher perform-
ances of separation and concentration than the above
direct injection. The in-tube SPME using a mono-
lithic column could be carried out by manual in-
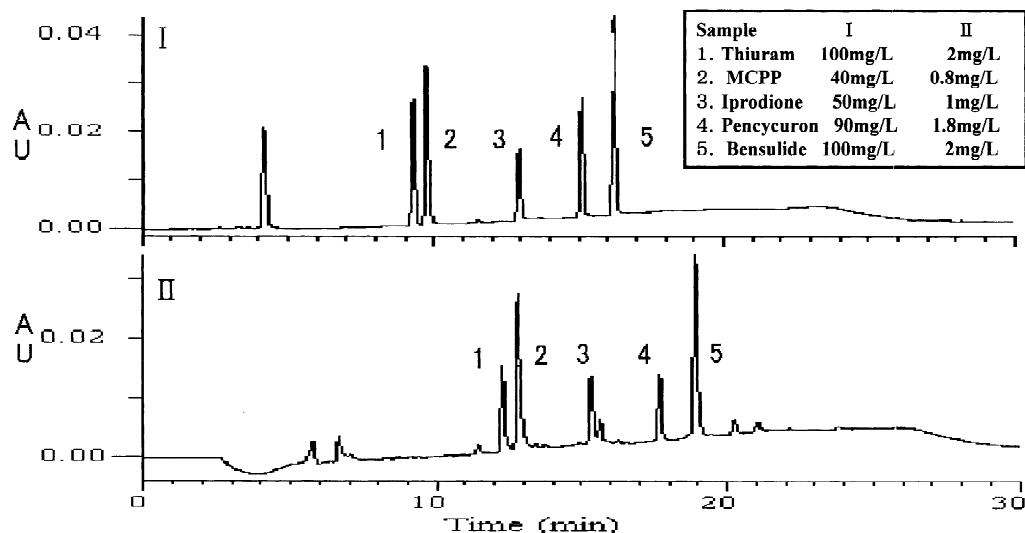jection because of its low pressure drop. Low

Sample	I	II
1. Thiuram	100mg/L	2mg/L
2. MCPP	40mg/L	0.8mg/L
3. Iprodione	50mg/L	1mg/L
4. Pencycuron	90mg/L	1.8mg/L
5. Bensulide	100mg/L	2mg/L

Fig. 5. Chromatograms of pesticides: (I) 0.1-µl injection, and (II) 50-µl injection.

column pressure drop allows the use of high flow-rate to achieve a high throughput. Further optimization of the silica structure, including the skeleton and through-pore size, would achieve high efficiency for in-tube SPME medium.

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