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Silica monolithic membrane as separation medium Summable property of different membranes for high-performance liquid chromatographic separation

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Abstract

We studied an applicability of a silica monolithic membrane as separation medium for high-performance liquid chromatography (HPLC). We prepared porous monolithic silica membranes having a three-dimensional network structure to cut and shape into a membrane separation medium. We evaluated chromatographic properties of a variety of solutes using a column containing the membranes with HPLC to elucidate summable property of the membrane separation media. In addition, we made brief study on separation of HbA1c in whole blood with the "stacked" membranes having different surface characteristics in one column, which is a membrane column. We confirmed that the membrane column was able to separate HbA1c from other matrix in whole blood to some extent, and it also had an excellent ability for hydrophobic and ion exchange adsorption.
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1. Introduction

To analyze some target molecule in complicated matrix, the separation medium having specific molecular recognition ability for the target molecule should be required. Molecularly imprinted polymer (MIP) might be one good example for those purposes, but MIP is not always versatile especially for analysis of low-concentrated target molecule in complicated matrix, because lots of interferences might be also retained on MIP to disturb desired detection for the target molecule.

Usually, the separation medium having different retention abilities are preferably utilized for these complicated separations; in addition, multi-dimensional separation has been recently utilized for the complicated separations such as protein separations as well as DNA separations. However, separation media for these purposes cannot be prepared very easily and multi-dimensional separation should require lots of instrumentations.

Among those, commercially available, convective interaction media (CIM) disk monolithic columns are mainly intended for very fast analyses, in-process control, and laboratory purification. It is also reported that separations of complex protein mixtures can be carried out within just a few seconds while maintaining a flow-unaffected high dynamic binding capacity. The benefits of CIM disk monolithic column are particularly noticeable when used for the separation or purification of target biomolecules such as peptides, proteins, and nucleotides [1–3]. It is probably because the network structure of CIM media is not well controlled for small molecule. This phenomenon was clearly showed by some pictures on their leaflet. At this moment, CIM media do not have enough efficiency for separation of small molecules.

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In this study, we wish to briefly report that possibility of separation medium based on silica monolithic membrane to achieve relatively complicated separation. We prepared and evaluated new silica monolithic membranes based separation medium in terms of summable separation properties of "stacked" membrane columns.

2. Experimental

2.1. Materials

Most of the solvents and reagents were purchased from Nacalai Tesque (Kyoto, Japan). Toluene employed for the modification of silica-based membranes was dried over molecular sieves. Tetramethoxysilane (3-methacryloxypropyl)trimethoxysilane were purchased from Shin-Etsu Chemical Co. (Tokyo, Japan). Methacrylic acid 3-sulfopropyl ester potassium salt was purchased from TCI (Tokyo, Japan) and used without further purification. Acetic acid was purchased from Nacalai Tesque. Poly(ethylene oxide) was purchased from Aldrich to be used in preparation of silica monolithic membranes.

2.2. Preparation of porous monolithic silica membranes

We prepared porous monolithic silica membranes having a three-dimensional (3D) network structure with sol–gel transition of a metal alkoxide solution [4–8] as is shown in Fig. 1. The sol–gel transition of it is generally caused by two kinds of reaction, i.e., hydrolysis and poly-condensation, of course, this method has been reported previously [4–8]. The detailed method is as follows: tetramethoxysilane (24 mL) was added to the solution of poly(ethylene oxide) (4.2 g, M_W = 10,000) in 0.01 M acetic acid (50 mL), and the mixture was stirred at 0 °C for 30 min. The resulting mixture was poured into a PTFE-coated mold and allowed to react overnight at 40 °C. Silica membranes thus formed were washed with 50% ethanol in water and then treated with aqueous ammonium hydroxide solution. After drying at 50 °C for 3 days, the membranes were treated at 1050 °C for 5 h and modified to become a desired size.

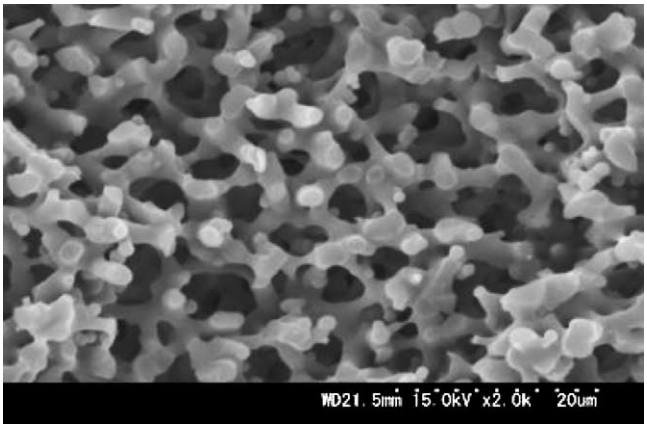

Fig. 1. Scanning electron micrograph of porous silica membranes.

2.3. Preparation of hydrophobic membranes

Hydrophobic membranes have been prepared with octadecylsilica (ODS) whose monolithic diameters are on the order of 2.0–2.5 μm. The membranes were converted to C_{18} phase by applying a toluene solution of octadecyldimethylchlorosilane at 110 °C, followed by a trimethylsilylation with hexamethyldisilazane.

2.4. Preparation of ionic membranes

Ionic membranes have been prepared, with (3-methacryloxypropyl)trimethoxysilane (MOP) and methacrylic acid 3-sulfopropyl ester potassium salt (MASK) whose monolithic diameters are on the order of 2.0–2.5 μm. The membranes were converted into MOP phase by applying a toluene solution of (3-methacryloxypropyl)-trimethoxysilane at 110 °C, followed by ionization with methacrylic acid 3-sulfopropyl ester potassium salt.

3. Results and discussion

3.1. Chromatographic properties of a membrane column

3.1.1. Summable hydrophobic property

We wish to confirm whether a porous monolithic silica membrane column potentially has applicability to HPLC. Firstly, we studied the characteristics of porous monolithic silica membranes by using liquid chromatographic method, or not. As described before, we modified those membranes into forms suitable for a commercially available empty column for the disk separation media [9,10]. First of all, we evaluated chromatographic properties of the membranes modified with octadecyl functionality (ODS) using hydrophobic solutes and others with HPLC. The membrane column consisted of ODS-modified membranes. The membrane columns were also evaluated with alkylbenzenes by changing total length or the number of silica membranes in piles as is shown in Figs. 2 and 3.

A membrane column of couple of piled membranes provided the increase of separation abilities where as the pressure drop was still in acceptable range. Silica membranes with the longer total length provided longer retention time and greater theoretical plate number, while it is of course low because of short column length. Since many piled membranes caused high-pressure drop, this is not suitable for easy use. From these results, we confirmed that a porous monolithic silica membrane was able to be used as separation media for HPLC and the membrane column containing a couple of membranes had an ability to retain hydrophobic materials under the condition of low-pressure drop with acceptable summable

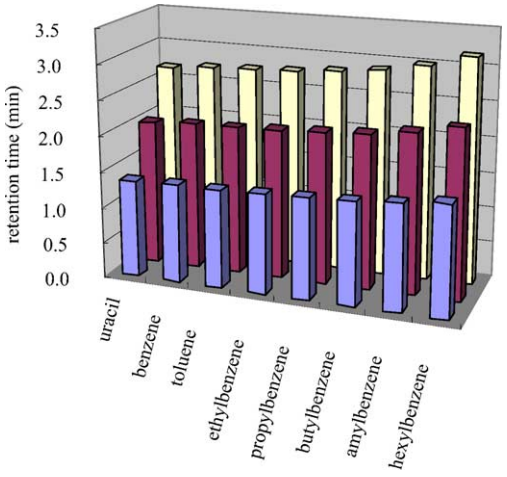

Fig. 2. The bar diagram of effect of retention time on changing the number of silica membranes. The lowest bar is retention time when we selected one membrane (total thickness; 3.75 mm), and the middle bar is retention time when we selected two membranes (total thickness; 7.20 mm), the highest bar is retention time when we selected three membranes (total thickness; 10.75 mm). HPLC conditions: mobile phase, acetonitrile–water (60:40, v/v); flow rate, 0.4 ml/min; column, membrane column (3.75–10.75 mm × 12.0 mm i.d.); detection, UV 210 nm; injection volume, 2 ml.

properties of the membranes. Additionally, to compare the ability of separation of silica monolithic membrane column with that of CIM monolithic membrane column, the former was superior to the latter. Because, since CIM monolithic column has an excellent ability to adsorb hydrophobic materials and has very broad peak, it unfit for the purpose of separation. As mentioned before, the 3D network structure of CIM is not well controlled for small molecules. This is an unavoidable problem of polymer-based monolithic separation media at this moment. Therefore, the silica monolithic membrane we prepared has lots of advantages compared with CIM polymer-based media.

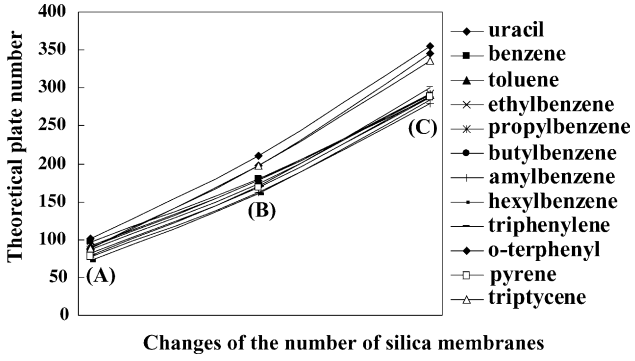

Fig. 3. The line diagram of effect of theoretical plate number on changing the number of silica membranes. Plot A is theoretical plate number when we selected one membrane (total thickness; 3.75 mm), and plot B is theoretical plate number when we selected two membranes (total thickness; 7.20 mm), plot C is theoretical plate number when we selected three membranes (total thickness; 10.75 mm). HPLC conditions: mobile phase, acetonitrile–water (60:40, v/v); flow rate, 0.4 ml/min; column, membrane column (3.75–10.75 mm × 12.0 mm i.d.); detection, UV 210 nm; injection volume, 2 ml.

3.1.2. Ionic properties

The silica membranes can be easily modified with hydrophobic as well as ionic functionalities, and applied for HPLC. We prepared different types of the membranes and applied them to the separation of HbA1c in human whole blood. Since it was reported that HbA1c was separated by mainly ionic interactions as well as some of hydrophobic interaction [11], we selected HbA1c as a target of the evaluation to investigate ion-exchange and hydrophobicity. HbA1c is one of the fractions of glycosylated hemoglobin. The measurement of HbA1c is found to be a convenient and effective way for the diagnosis. HbA1c represents mean value of glucose concentration during last 1–3 months, not reflect temporary fluctuation of physiological conditions. The measurement of HbA1c is a major diagnosis for diabetes due to its highly positive correlation with the severity of diabetes.

We determined HbA1c in human whole blood using the membrane column as a stationary phase of HPLC as is shown in Fig. 4. A membrane column consisted of two different types of membrane such as hydrophobic and ion exchange membranes. To elucidate the detailed properties, we utilized relatively long separation time and we confirmed that the membrane column was able to separate HbA1c from the matrix in human whole blood to some extent.

3.1.3. Investigation for the phenomenon of "aging"

Generally, HbA1c cannot be eluted from HPLC column until actual whole blood is injected many times when a new column is applied. Due to investigation of this phenomenon, we revealed that it occurred due to the hydrophobic adsorption of proteins into the meso-pore sized in 5 nm of diameter. Consequently after saturation of the adsorption sites for

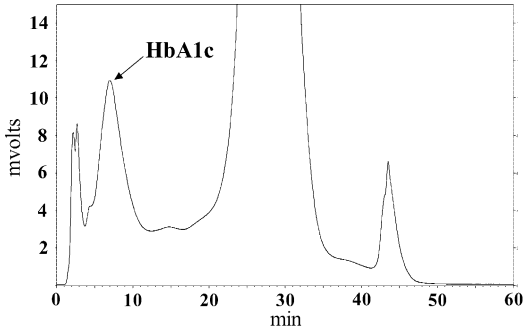

Fig. 4. Chromatogram of human whole blood. HPLC conditions: flow rate, 0.4 ml/min; column, membrane column; detection, 415 nm; injection volume, 3 ml; mobile phase, (elution A) 20 mM MES, 20 mM HEPES, 0.01% NaN_3, pH 5.20 (elution B) 20 mM MES, 20 mM HEPES, 400 mM NaCl, 0.01% NaN_3, pH 7.00; gradient conditions, starting at 18% elution B, gradient elution to 19% elution B at 10 min, 23% elution B at 16 min, 26% elution B at 17 min, 28% elution B at 35 min, 80% elution B at 36 min, 80% elution B at 38 min, 18% elution B at 39 min, stop run at 50 min.

the proteins is made by the repeated injections, the peak of HbA1c can be observed. This number of ageing time is found to be in direct proportion to the length of ODS-modified silica monolithic membranes in the column. In other words, summable property was also found for this aging phenomenon in HbA1c separation.

In conclusion, these results demonstrated that a membrane column had excellent abilities for hydrophobic and ionic adsorption. In our investigation for the ability of hydrophobic separation, a membrane column of a couple of piled membranes provided the increase of separation abilities whereas the pressure was still in acceptable range. Additionally, a membrane column was able to separate HbA1c from the matrix in whole blood to some extent and retain HbA1c for a long time, which had ionized groups. These results suggested that a membrane column was useful as separation medium for some complicated medium.

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