Utilization of Nanometre-order Diameter Columns inside Porous Anodic Alumina for Chromatography Chip System

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Porous anodic alumina (PAA) membranes were used as a nanometre-order diameter column for chromatography chip with acetonitrile–water mobile phase using gradient elution. In a chromatogram, 9-anthracenemethanol (AM), 9-anthracenecarboxylic acid (AC), and dansyl–glycine (DG) showed different retention times, indicating that PAA membranes are applicable to stationary phase for chromatography chip.

Porous anodic alumina (PAA) membranes,[1–5] which consist of a self-assembled honeycomb array of uniformly sized parallel channels, have attracted considerable interest in various areas such as electronics, chemistry, and biomedical science because of their ordered structure, superior resistance to organic solvents, and potentially low cost. The diameter of PAA channels can range from about ten to several hundred nanometers, and membrane thickness is 10 to 100 μm. When solutes permeate through the PAA membrane, the interior of the columnar channels interacts with the solutes molecules. The elution of solutes from the channels depends on the strength of the interaction with the interior.[6] Alumina is widely used for chromatographic matrix in liquid chromatography, and its chromatographic properties have been studied in detail.[7–9] However, few studies have been carried out on the utilization of PAA in liquid chromatography.

A PAA membrane is very thin, which is favorable for reducing the sizes of analysis systems. In this study, we used PAA membranes (Anodisk, 100-nm-diameter pores, 60-μm thick, Whatman) as stationary phase in the chromatography chip and tested whether PAA membranes can function as chromatographic columns in chip system. The chromatography chip, which has been intensively studied,[10–21] is a small tool that can function as chromatographic system. Figure 1 shows a schematic illustration of the experimental setup for chromatographic measurements and a picture of a chip. Five PAA membranes were set in the chip as stationary phase.[24] Glycol-modified polyethylene terephthalate (PETG, Mitsubishi Rayon Co., Ltd.) was used for the chip substrate. As solute molecules, 9-anthracenemethanol (AM), 9-anthracenecarboxylic acid (AC), and dansyl–glycine (DG) were selected. The chromatograms of each solute were presented in Figure 2. The retention times of each solute were different, indicating that PAA membranes can function as chromatographic columns with acetonitrile–water mobile phase.

The retention time reflects the strength of the interaction between solutes and interior of the nanochannels. The strength of the interaction was measured by putting a solution of the solutes to be tested in two bottles, one with one without a PAA mem-

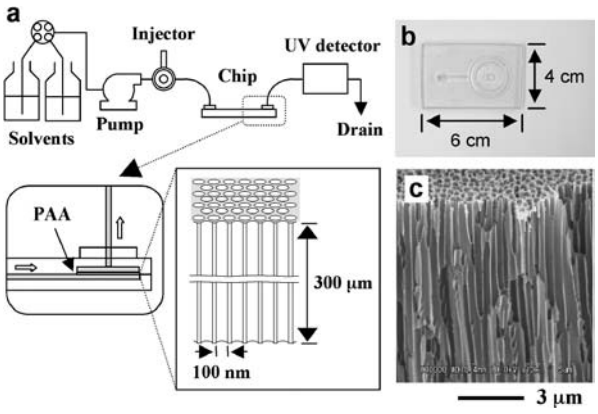

Figure 1. Experimental setup for chromatographic measurement. (a) Schematic illustration of chromatographic system. (b) Picture of chromatography chip. (c) Cross-sectional SEM image of PAA membrane.

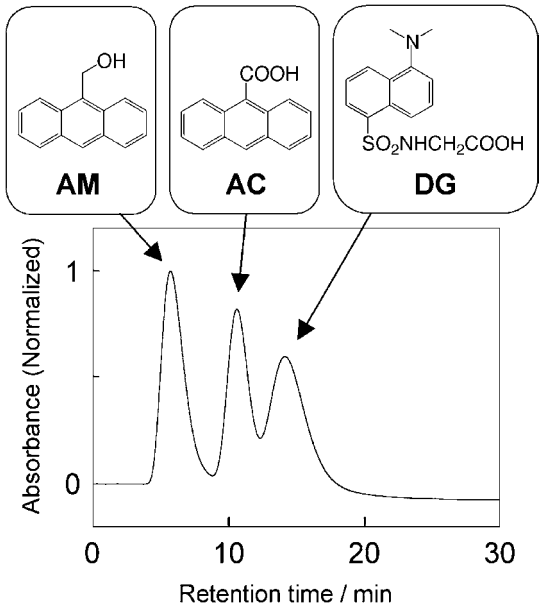

Figure 2. Chromatogram of mixture of AM, AC, and DG by chromatography chip. A flow-rate of 0.4 mL/min was used over 30 min with the following gradient: 0 min, 4% water and 96% acetonitrile; 20 min, 40% water and 60% acetonitrile (linear gradient). The injection volume was 5 μL, and concentration of each solutes was 5×10^{-4} M. The mobile phase was degassed by helium during measurements. The detection wavelength was 340 nm.

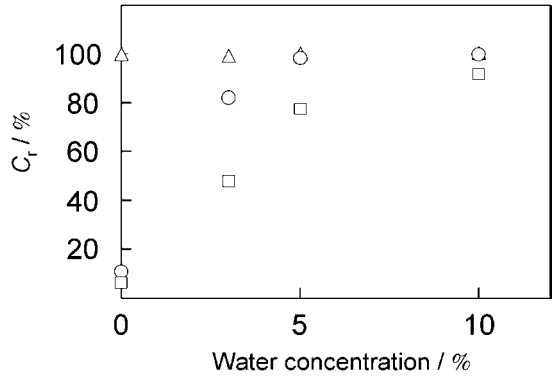

Figure 3. C_r for AM, AC, and DG versus water concentration: triangle, circle and square show AM, AC, and DG, respectively. C_r is defined as $C_r = C_e/C_0$,where C_0 is the initial concentration of solutes before immersing the PAA membrane, and C_e is the concentration at adsorption equilibrium after immersing PAA membrane.

brane (Figure 3). After 8 h of shaking, the concentrations of solutes in the two bottles, C_e and C_0, respectively, were measured. The residual amount of solute in solution was defined as $C_r = C_e/C_0$, so that C_r is inversely proportional to the strength of the adsorption. AC and DG adsorbed to the PAA membrane, whereas AM did not adsorb. The main difference between these solutes is their functional group; AC and DG have a carboxyl group, and AM has a hydroxy group. The C_r values of AC and DG decrease when the water concentration is low, which shows that AC and DG adsorbed to the PAA membrane. The order of adsorption was AM < AC < DG, which agreed with the order of retention time. This correspondence indicates that the retention of solutes mainly depends on adsorption. Therefore, it can be concluded that carboxyl groups interact more strongly with the alumina surface than hydroxy groups in this solvent condition. The absorbed amount of AC and DG decreased with increasing water concentration in the solvent. This decrease is thought to be a result of both solvation of solutes and the interaction between water and alumina. Carboxyl groups can form a hydrogen bond with water molecules,[22] which reduces the interaction between the alumina surface and solutes. In addition, water molecules are also known to interact with the alumina surface.[23] These interactions are competitive. Therefore, when the water concentration increases, the interaction between solutes and the alumina surface is reduced by not only solvation of solutes but also interactions between alumina and water molecules. The amount of DG that adsorbed was larger than the amount of AC. This difference is probably due to the number of functional groups and the pK_a of the carboxyl group. DG has carboxyl, dimethylamino, and imino groups. On the other hand, AC has only a carboxyl group. Thus DG can interact with alumina more strongly than AC. The pK_a of each solute is different, which may also affect the adsorption.

The order of retention time in Figure 2 can be explained in the same way. In the HPLC experiment, a linear gradient starting with 4% water–96% acetonitrile and increasing to water to 40% was applied within 20 min at a flow rate of 0.4 mL/min. Initially, the water concentration of the mobile phase is low, and AC and DG were retained in the nanochannels. As the water concentration increased during the gradient, AC and DG were eluted from the nanochannels via solvation and competitive interaction. Since DG was retained more strongly than AC, DG was eluted after AC. AM had the smallest retention of the three solutes because of its weak interaction with nanochannels.

In summary, we have shown that PAA membranes can be utilized for a chromatographic column in chromatography chip with acetonitrile–water mobile phase. We expect that PAA membranes will be a powerful tool in analytical science and microchip technology.

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