Open Tubular Monolith Formation and C18 Ligand Immobilization in Silica Capillary by Microwave Heating for Capillary Electrochromatography

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The methods of C18 modification to silica gel are well described in the literature.[1] Three C18 modification methods are known by which three different types of C18 stationary phases are obtained, respectively-- a brush form, an oligomeric form, and a bulk form. The brush form is composed of separate monomeric ligands chemically attached to the silica surface, and is obtained by reaction with monochlorodimethyloctadecylsilane. The oligomeric form, where a C18 oligomer is bonded to each silanol site of silica surface, is obtained with dichloromethyloctadecylsilane. The bulk form composed of a 3 dimensional polymer-like network, is obtained with trichlorooctadecylsilane. In all cases, endcapping is generally required after C18 modification to prevent the tailing phenomenon in chromatographic separation. The oligomeric and bulk forms are not widely used in the commercial products because preparation of such phases demands drastic and time-consuming reactions at high temperatures. On the other hand, the brush form is easy to make under mild reaction conditions, and is widely used[2]. Spherical porous silica gel is the most common material for the support of C18 modification that gives high separation efficiencies.

Microwave techniques have been used in chemical synthesis[3-5] and derivatization,[6,7] preparation of nano materials,[8-10] extraction,[11-17] sample treatment and dissolution,[18-20] etc. Microwave application has been expanded even to polymerization[21] and immobilization of polymer coating on chromatographic stationary phases.[22,23] Microwave heating is known to often cause fast and good reaction results owing to rapid and uniform heating. However, microwave application in preparation of monoliths or C18 ligand modification has not been reported so far.

Recently, the monolithic column has raised a lot of interest in liquid chromatography.[24-27] The monolithic column is composed of an one-body porous solid structure with nanometer-sized inner pores and micrometer-sized through channels. Particularly, preparation of monolith column is carried out in silica capillary and it is used in capillary electrochromatography (CEC).[28,29] Open-tubular columns have significant advantages over their packed counterparts because of the simplicity in column preparation and hassle-free fritless operation.[30] Column efficiencies of 100 000 theoretical plates per meter are common for open tubular CEC columns of 50 μ ID, and column efficiencies up to half a million theoretical plates per meter have been reported for sol-gel open-tubular CEC columns of 10-13 μ ID.[31]

In this study, the effects of microwave heating have been studied in preparation of open tubular monolithic silica capillary columns and C18 modification in comparison with thermal heating. Three different capillary columns have been prepared by different heating processes-- 1) microwave heating for monolith formation and C18 ligand immobilization; 2) microwave heating for monolith formation and thermal heating for C18 ligand immobilization; 3) thermal heating for monolith formation and C18 ligand immobilization. Their performances in CEC separation have been comparatively studied. The microwave heating method for OT (open tubular) monolith formation was much faster than thermal heating, and the thickness of the monolith layer by microwave heating was much thinner than that by thermal heating.

Experimental Section

Chemicals. Methanol and water were of HPLC grade and purchased from Fisher (Pittsburg, PA, USA) and used without purification. TMOS (tetramethyl orthosilicate), PEG 10,000 (polyethylene glycol, MW 10,000), acetic acid, benzene, toluene, ethylbenzene, propylbenzene, *n*-butylbenzene, amylbenzene, and hexylbenzene were purchased from Aldrich (Milwakee, IL, USA).

CEC. The CEC (Capillary electrochromatography) system used was a Hewlett-Packard HP^{3D} CE (Waldbronn, Germany) instrument. Undeactivated fused capillaries (50 μm ID × 365 μm OD) were purchased from Alltech (Deerfield, IL, USA).

Microwave and SEM instruments. A CEM (Matthews, NC, USA) MAR-5 microwave system was used for microwave heating. The heating power was 300W. A Hitachi (Tokyo, Japan) S-4200 Field emission SEM was used to obtain SEM images.

Preparation of monolithic coating in silica capillary. The silica capillary was filled with 0.1 M sodium hydroxide for 24 h at room temperature to activate the inner surface and convert its siloxane groups into silanols. Next, the capillary was flushed with 0.01 M HCl, water, and acetone in sequence and dried. The preparation of open tubular monolithic silica gel in the capillary tubing was carried out as follows: TMOS (104 μL) was added to a solution of PEG 10,000 (108 mg) in acetic acid (0.01 M, 1 mL) and stirred for 30 min at 0 °C. A much smaller amount of TMOS (0.7

M) was used to secure formation of open tubular monolith than had been used (2.7 M) in formation of bulk monolith.[27] The resultant homogeneous solution was filled into a 50 μm ID (365 μm OD) silica capillary. The gel was subsequently aged in the capillary for 24 h at 40 °C, 24 h at 110 °C, and 24 h at 200 °C in the thermal heating method or was aged in water for 10 min at 40 °C and 10 min at 120 °C in the microwave heating method. The capillary column was washed with ethanol to remove residual reagent and PEG 10,000.

C18 modification. The process of C18 ligand modification is as follows. First, the silica capillary with the monolith film was washed with xylene, and filled with 50/50 (v/v %) chlorodimethyloctadecylsilane/xylene with a syringe. The reaction was taken place by thermal heating for 24hr at 110 °C or by microwave heating for 10 min at 110 °C. The open tubular capillary column was thoroughly washed with xylene, THF, MeOH, 50/50 (v/v %) MeOH/H_2O, and MeOH, in sequence.

Results and Discussion

The open tubular monolith structure was confirmed by the SEM images of the monolithic columns (50 μm ID, 365 μm OD) (Figure 1). The SEM images of the capillary column made by thermal heating (Figure 1a) are compared with the SEM image of the capillary column made by microwave heating (Figure 1b). It is hard to measure the thickness of the monolith film because of the scattering of the film images, but at least it is clear that the film thickness of the monolith made by thermal heating is far larger than that made by microwave heating. It should be noted that a very long reaction time (24 h at 40 °C, 24 h at 110 °C, and 24 h at 200 °C in sequence) was required for monolith formation by thermal heating while a very short time (10 min at 40 °C followed by 10 min at 120 °C) was enough for formation of monolith by microwave heating. The capillary was immersed in water or other solvent at a given temperature in the case of microwave heating while it was placed in an electric oven in the case of thermal heating. When a shorter time (a few hours each step for example) was used in thermal heating, monolith formation was unsuccessful. On the other hand, no better results were obtained for expanded reaction time in microwave heating. It is likely that the monolith formed in a long time (thermal heating) should be thicker than the monolith formed in a short time (microwave heating). In addition, it seems that there should be differences in structure and density of monoliths between thermal and microwave heating. The wave pattern of the surface winding for the monolith prepared by thermal heating looks loose with larger waves (Figure 1a) while the surface wave pattern for the monolith prepared by microwave heating looks compact with minimized ruggedness (Figure 1b). With such a compact monolith structure, formation of thick monolith will not help in improvement of chromatographic separation, thus the capillary monolith column prepared by microwave

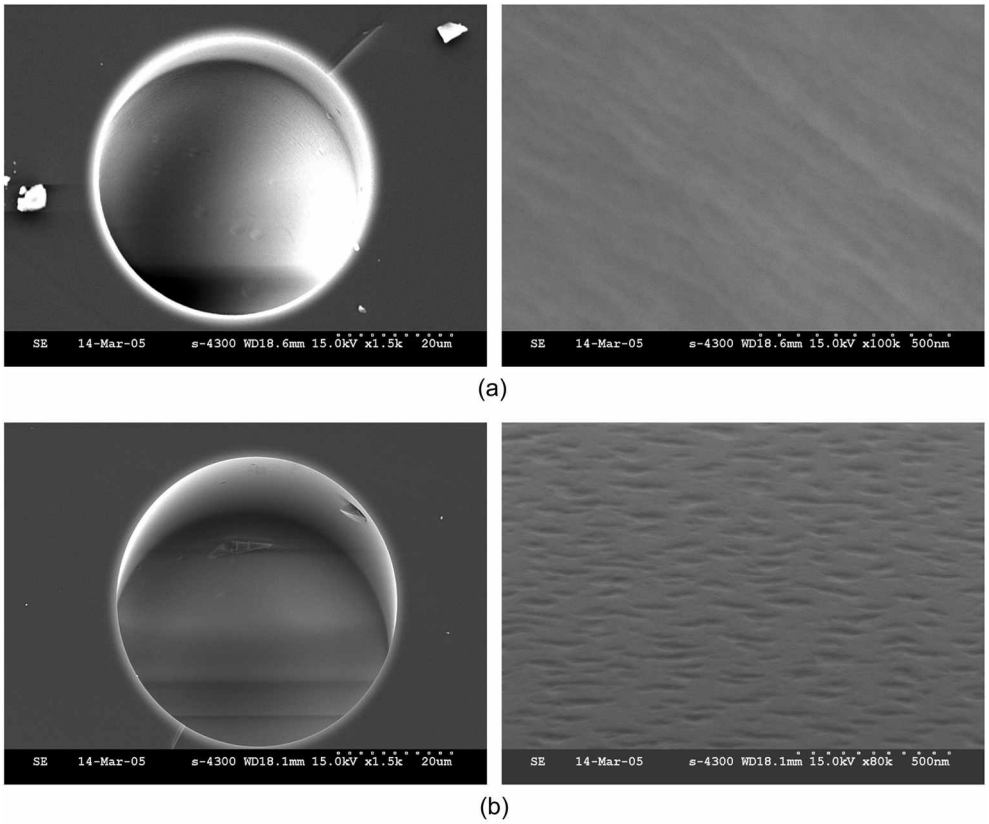

Figure 1. The SEM images (capillary cross-sections and expanded inner surfaces) of the open tubular monolith columns made by (a) the thermal heating method, and (b) the microwave heating method.

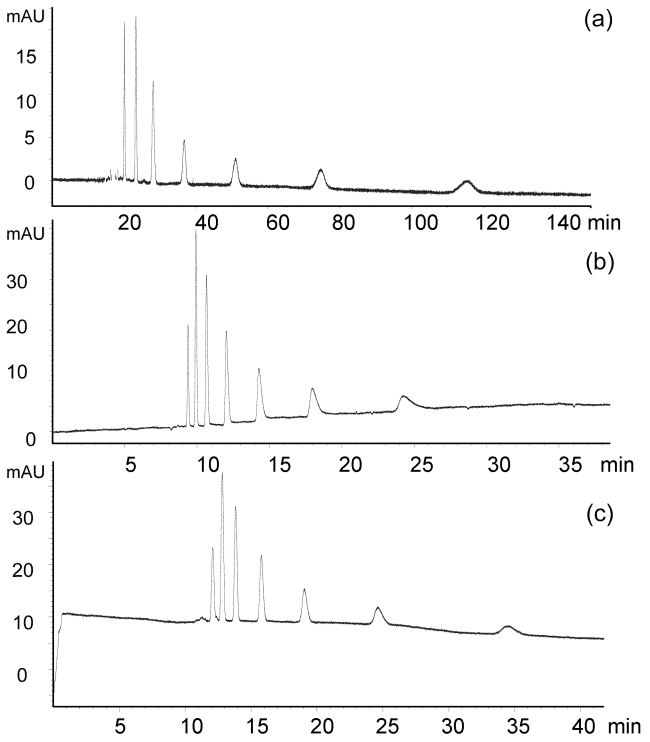

Figure 2. CEC separation of alkylbenzenes (benzene-hexylbenzene) on the open tubular monolithic column made by (a) oven heating for monolith formation and ligand attachment (b) microwave heating for monolith formation and oven heating for ligand attachment (c) microwave heating for monolith formation and ligand attachment <Conditions> monolith OT column: 43/50 cm × 50 μm ID, Buffer: 50/50(v/v%) MeCN/50 mM TRIS pH 8, Injection: 5 kV, 5 sec. Applied voltage: 15 kV.

heating for longer time did not show better separation efficiency.

Three different capillary columns were prepared by different heating processes-- 1) microwave heating for monolith formation and C18 ligand immobilization; 2) microwave heating for monolith formation and thermal heating for C18 ligand immobilization; 3) thermal heating for monolith formation and C18 ligand immobilization. Their separation performances in CEC were comparatively examined in separation of alkylbenzenes from benzene to hexylbenzene (Figure 2).

The retention times in the monolith column made by microwave heating (Figure 2b) for monolith formation are much shorter than those in the monolith column made by thermal heating (Figure 2a) as we may expect based on the relative monolith thickness, considering that the same thermal heating was used for C18 ligand modification of both columns. It is interesting that the retention times in the column made by thermal heating for C18 modification (Figure 2b, column B) are shorter than those in the column made by microwave heating (Figure 2c, column C). The same microwave heating was used for monolith formation of column B and C. It implies that more C18 ligands were chemically attached to the monolith surface by microwave heating than by thermal heating even though the heating

Table 1. The numbers of theoretical plates per meter (N/m) of alkylbenzenes obtained by three CEC capillary columns

	oven/oven[a]	mw/oven[b]	mw/mw[c]
benzene	99000	193000	101000
toluene	52000	139000	78600
ethylbenzene	48900	102000	67500
propylbenzene	23100	58100	47900
butylbenzene	20700	32000	40500
pentylbenzene	17300	19700	26500
hexylbenzene	13300	12400	14900

[a]Thermal heating for monolith formation and C18 modification. [b]Microwave heating for monolith formation and thermal heating for C18 modification. [c]Microwave heating for monolith formation and C18 modification.

time of microwave heating (10 min at 110 °C) was much shorter than the time of thermal heating (24 hr at 110 °C). This observation confirms that microwave heating causes a very fast reaction rate and high reaction yield.

However, better separation efficiency was in general observed for the column prepared by thermal heating for C18 modification (Table 1). We guess that chemical attachment of too much C18 ligands for the thin and compact monolith was caused by microwave C18 modification compared to thermal C18 modification. Further reduction of reaction time of microwave heating caused poor reproducibility of C18 modification.

The number of theoretical plates of column B for benzene is 193,000/m. The separation efficiency of the column with thermal C18 modification (column B) was better than those (column C) with microwave C18 modification when the solutes are benzene, toluene, ethylbenzene, and propylbenzene. However, as for long-retained solutes (butylbenzene, amlybenzene and hexylbenzene), the situation is reversed.

The column efficiency obtained for benzene in this study is comparable to the best column efficiencies obtained with other OT CEC columns of 50 μ ID so far. Better column efficiencies would be obtained if OT CEC columns of 10-25 μ ID could be prepared. We have some technical problems in preparing such OT CEC columns at present, and keep improving our techniques to get OT CEC columns of 10-25 μ ID prepared by microwave heating.

Our experimental results show that a compact thin monolith was formed by microwave heating in a very short time (for 10 min at 40 °C and 10 min at 120 °C) while a much thicker monolith was formed by thermal heating in a long time (for 24 h at 40 °C, 24 h at 110 °C, and 24 h at 200 °C). The better separation efficiency of the monolith made by microwave heating is primarily owing to much thinner film of monolith. Such thin thickness of the stationary phase enables fast mass transfer and high separation efficiency. That thin monolith cannot be formed by thermal heating since thermal heating for a reduced reaction time cannot create monolith structure. Thus, it is clearly favorable to use microwave heating instead of thermal heating for formation of open tubular monolith in view of both reaction time and separation efficiency.

Our experimental results also show that too much C18 ligands were attached to the monolith film when microwave heating was used for C18 modification even though the reaction time (for 10 min at 110 °C) was very short. Further reduction of reaction time caused poor reproducibility of C18 modification. Thus, thermal heating for C18 modification is rather recommended instead of microwave heating.

Conclusion

A thin and compact silica monolith was formed on the inner surface of silica capillary by microwave heating. Monolith formation by microwave heating was fast and effective, and its C18 modified CEC open tubular column showed very good separation efficiency. Analysis time of the open tubular monolithic capillary column made by microwave heating was much shorter than that of the monolithic column made by thermal heating while the separation efficiency was better. C18 modification by thermal heating, however, rather than by microwave heating, showed better results. Monolith formation by microwave heating coupled with C18 modification by thermal heating is recommended to make a good open tubular CEC column that enables rapid and efficient separation.

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