

Preparation of Mesoporous Materials and Thin Films It's Application for DNA Sensor

†Seung-Jun Han* · Soon-Young Heo* · Keun-Ho Park* · Soo Lee*
Byung-Kwan Kim* · Jin-Heung Kim**

*Department of Chemical Engineering System,
Changwon National University, Changwon, Kyungnam, 641-773, Korea

**Department of Nano Science,
Ewha Womans University, Seoul, 120-750, Korea

†e-mail : hansj700@korea.com

(Received September 20, 2004 ; Accepted November 16, 2004)

Abstract : Highly ordered pure-silica MCM-41 materials possessing well-defined morphology have been successfully prepared with surfactant used as a template. The fabrication of mesoporous silica has received considerable attention due to the need to develop more efficient materials for catalysis, separations, and chemical sensing. The surface modified MCM-41 was used as an adsorbent for biomolecules. Silica-supported organic groups and DNA adsorption on surface modified MCM-41 were investigated by FT-IR and UV-Vis spectrometer, respectively. The use of MCM-41 as the modification of electrode surfaces were investigated electrochemical properties of metal mediators with biomolecules. The modified ITO electrodes increased peak currents for a redox process of $[\text{Ru}(\text{bpy})_3]^{2+}$ relative to the bare electrode. The electrochemical detection of DNA by cyclic voltammetry when the current is saturated in the presence of the mediator appeared more sensitive due to a higher catalytic current on the MCM-41 supported electrodes modified by carboxylic acid functional groups. The carboxyl or amine groups on the surface of MCM-41 interact and react with the $-\text{NH}_2$ groups of guanine and backbone, respectively. Highly ordered mesoporous materials with organic groups could find applications as DNA sensors.

Keywords : MCM-41, sol-gel, surface modification.

1. Introduction

In 1940's, natural zeolites were used as a softener for hard water, because that zeolites have high surface areas and cation exchange capacity. So, many researchers have been studied to make artificial zeolites. The artificial zeolites were defined by IUPAC in

1990's. There is three classes of materials by pore size ; microporous (pore diameter $\leq \sim 20$ Å), mesoporous (~ 20 -500 Å), macroporous (500 Å \leq).

In 1992, Mobil oil company discovered a new family of silica based mesoporous materials, designated as M41S. MCM-41 is one of the members of this extensive family

of mesoporous sieves, possesses a hexagonal array of uniform mesopores. This material has been synthesized with uniform channels varying from approximately 15 Å to larger than 100 Å in pore size. The surface area of MCM-41 is over 700 m²/g, and hydrocarbon sorption capacities of 0.7cc/g and greater[1].

Mesoporous materials have been extensively investigated[2]. The possible applications of MCM-41 include catalysts, adsorbents, optical materials[3], and biosensor[4]. Biosensors are most interested in application fields using mesoporous materials with widely specific surface area and highly ordered. Biosensors have been developed to detect a variety of biomolecular complexes[4]. The sensors needs smaller substrate, largesurface area and excellent adsorption properties.

To adsorb DNA for biosensor application, MCM-41's surface have to bmodified with active groups. The surface modification of MCM-41, such as the immobilization of catalyrically active sites and electro active species, has been investigated so widely[5,6]. For example, sensors using electrochemically detection methods reports the adsorption of tris(2,2'-bipyridine)-ruthenium(II) ([Ru(bpy)₃]²⁺) complex cations into a mesoporous silica. The exited-state properties of [Ru(bpy)₃]²⁺ have been extensively investigated because of their unique combination of chemical stability of photosensitizing redox reaction, and electrochemical and photophysical properties[6]. [Ru(bpy)₃]²⁺ was adsorbed on MCM-41 and that the states were changed reversibly upon hydration/dehydration. The reversible change in the states of the guest species should be considered for the functions and the applications of the host-guest systems derived from mesoporous silicas[3].

Here in, we describe the synthesis of MCM-41, it's functionalized with carboxyl groups by chemically, and then we propose application of and the resulting hybride

materials have been used as matrixes for the controlled delivery of DNA sensors.

2. Experimental

2.1. Materials

Silica sources were used tetraethyl orthosilicate (TEOS, Aldrich Chemical Co.), cetyltrimethylammonium bromide (CTAB, C₁₆H₃₁(CH₃)₃NBr) were obtained from Aldrich as quaternary ammonium cation surfactant compound, trimethylammonium hydroxide (TMAOH, 25% aqueous solution, Aldrich Chemical Co.) as base catalyst, 3-triethylsilyl butyronitrile (TEBN, Aldrich Chemical Co.)and 3-aminopropyl triethoxysilane(APTES, Aldrich Chemical Co.) as surface modification agents, methanol (Duksan Chemical Co., anhydrous), hydrochloric acid (Duksan Chemical Co., 35%), toluene (Duksan Chemical Co., reagent grade) and sulfuric acid (95%, Duksan Chemical Co.) were used as solvent. Calf-thymus DNA was obtained from Aldrich as DNA source. Sodium phosphate, dibasic (98%, Oriental Chemical Industries) and sodium phosphate, monobasic (98%, Wako Pure Chemical Industries Ltd.) were used to make buffer solution.

2.2. Instruments

X-ray powder diffraction (XRD) data were obtained with Phillips X'pert PW 1840/00 (Cu target) and microstructure were observed with scanning electron microscopy (SEM, JEOL, JSM-5610). Electrochemical detection (cyclic voltammetry method) were carried out by potentiostat/galvanostat (EG&G Instrument, Model 263A).

2.3. Preparation of pure silica MCM-41

CTAB were added in double distilled deionized water and then the solution was stirred. TMAOH were added as the catalyst. And then, TEOS was added into this solution by droplet with stirring. Total molar ratio of

the mixture solution was Si:CTAB:TMAOH:H₂O=1:0.12:0.14:35.71. This mixture was placed in a static autoclave at 165°C for 12~24hr. After cooling to room temperature, the solid product was recovered by filtration on a Buchner funnel, washed with water, and dried in a dry oven at 70°C for overnight. As-synthesis product was then extracted with methanol and hydrochloric acid.

2.4. Surface modification of pure silica MCM-41

As-synthesized MCM-41 and MCM-41 removed surfactant suspended in toluene, and to the suspension, TEBN was added. The mixture was refluxed for 4hrs under stirring, cooling to the room temperature, washed with chloroform and dichloro methane, and finally dried in a drying oven at 60°C for overnight.

2.5. Preparation of electrodes

Polystyrene had to be added in order to obtain films that adhered well to the conductive substrates and were robust enough for handling. Polystyrene has been used extensively in the preparation of zeolite-modified electrodes[7]. MCM-41 modified electrodes used in this work consisted of MCM-41 or surface modified particles dispersed in polystyrene and THF (tetrahydrofuran). And then the mixture was well mixed with a stirrer. This mixture was spread on a 1×1cm piece of ITO (Indium-doped tin oxide coated glass, Delta technologies). The solvent was evaporated.

2.6. Electrochemical measurement

The electrochemical measurement were taken EG&G 263A Potentiostat in three-electrode cell. The reference electrode was a Ag/AgCl, counter electrode was a Pt, and working electrode was MCM-41 coated with ITO. The buffer in this study was 50mM Na₂/PO₄ in deionized distilled water. The mediator was Ru(bpy)₃²⁺ in buffer.

3. Results and Discussion

3.1. Preparation of pure silica MCM-41

MCM-41 prepared by hydrothermal synthesis was characterized by XRD and infra-red spectroscopy (FT-IR). The samples that were removed surfactant by extraction were investigated by FT-IR. Fig. 1 shows the saturated carbon bonds from surfactant in fully treated MCM-41 structure. The surfactant is the template role of MCM-41 synthesis.

In Fig. 1, saturated carbon bond could be found in 2500~3000cm⁻¹. On (a) sample with surfactant, there is a double peak at about 2854 and 2923cm⁻¹, respectively. These peaks appeared by the surfactant. But we can not find any peaks in same area on (b) sample. The surfactant entirely removed by extraction and calcinations process.

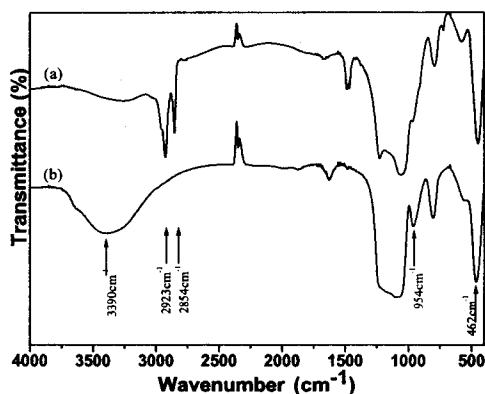


Fig. 1. FT-IR spectra of before (a) and after (b) removing surfactant by extraction.

Fig. 2 shows the XRD patterns of synthesized MCM-41 samples. We observe that the typical for MCM-41 hexagonal array on all of samples. The observation of 3~4 peaks which can be indexed on a hexagonal lattice is typical of MCM-41 materials[1]. These results mean that the hexagonal arrays are well ordered.

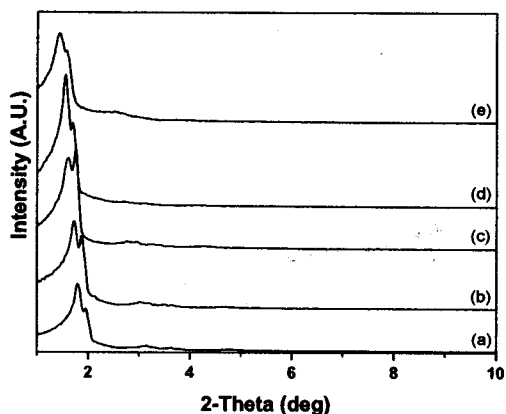


Fig. 2. Powder X-ray diffraction patterns of MCM-41 samples after extraction of surfactant. The reaction time is (a) 12 hrs, (b) 24 hrs, (c) 48 hrs, (d) 60 hrs, (e) 72 hrs.

3.2. Surface modification of pure silica MCM-41

The preparation of organically modified silica bearing amine or carboxyl groups *via* the sol-gel method has been known for some time, but their application as simple catalysts and reagents for organic synthesis is much less developed[8-11].

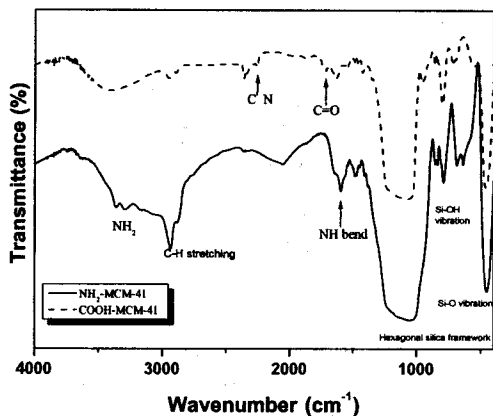
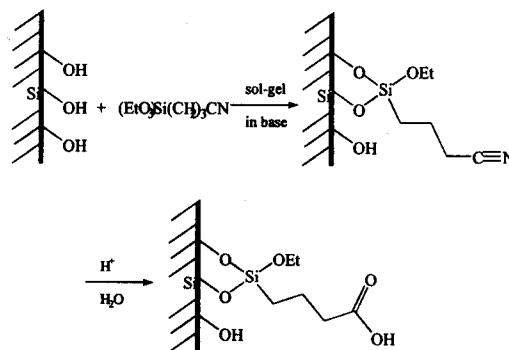


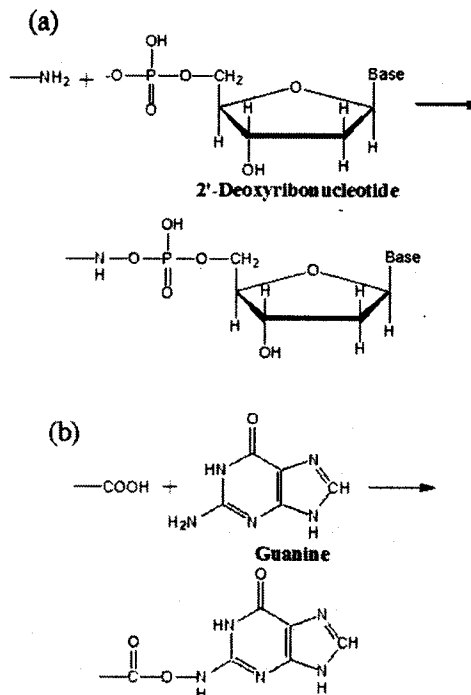
Fig. 3. FT-IR spectra of COOH-MCM-41 (dash line) and NH₂-MCM-41 (solid line).

To immobilize DNA on surface of

MCM-41, in a first step MCM-41 were dispersed in TEBN solution. Subsequent hydrolysis by aqueous sulfuric acid afforded the MCM-41 anchored carboxylic acids (Scheme 1). Fig. 3 shows surface modified MCM-41 with different organic group.



Scheme 1. The reaction mechanism of carboxylation

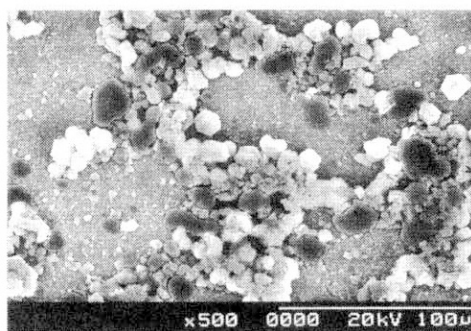


Scheme 2. Schematic diagrams showing binding of DNA (a) onto the aminated silica surface and (b) the carboxylated silica surface.

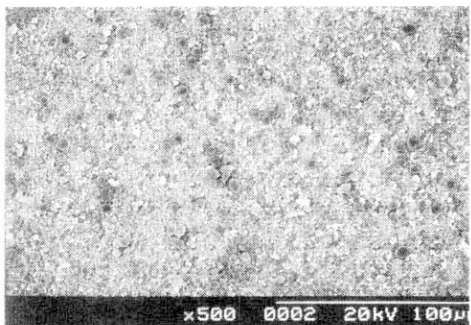
Surface modified MCM-41 with carboxyl group is well adsorbed on amino groups of DNA and with amine group is well attached on PO_4^- group in back-bone of DNA (Scheme 2). The amine groups in DNA anchored with carboxyl groups in surface of MCM-41. These reactions were called to self-assembled monolayers (SAM's)[12].

3.3. Preparation of electrode

Fig. 4 shows the scanning electron micrographs of MCM-41 with polystyrene modified electrode. The coverage of the MCM-41 particles on ITO substrate was not completely with load coated sample, however, MCM-41 particles with spin-coated ITO were uniform throughout, relatively. So, we used in this work all electrodes were spin coated.



(a)



(b)

Fig. 4. Scanning electron micrographs of a 1cm^2 piece of ITO coated MCM-41 in polystyrene. (a) load-coating, (b) spin-coating

Fig. 5 shows cyclic voltammograms taken in a buffer solution containing $[\text{Ru}(\text{bpy})_3]^{2+}$. Curves show the voltammogram obtained with bare or coated ITO electrode. Curve (a) shows the voltammogram obtained from polystyrene coated ITO. A small voltammetric wave for the oxidation of $[\text{Ru}(\text{bpy})_3]^{2+}$. But MCM-41 coated ITO's curve (c) shows were indicated bigger than polystyrene coated or bare ITO's one.

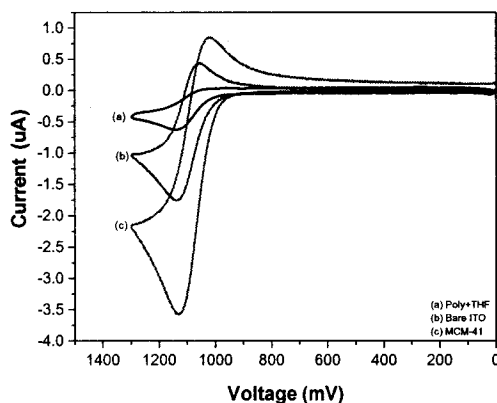


Fig. 5. Cyclic voltammograms recorded in $[\text{Ru}(\text{bpy})_3]^{2+}$; (a) polystyrene coated ITO (b) bare ITO, (c) MCM-41 coated ITO

3.4. Electrochemical measurement

Fig. 6 shows cyclic voltammograms taken in a buffer solution containing $[\text{Ru}(\text{bpy})_3]^{2+}$ and DNA. These quasi-reversible cyclic voltammogram indicate that how many guanine in calf thymus DNA were oxidized by $[\text{Ru}(\text{bpy})_3]^{2+}$. Bare ITO and pure MCM-41 coated ITO shows small oxidation curve than surface modified MCM-41 coated ITO. It means that MCM-41 coated ITO has more wide surface area. And surface modified with carboxyl group MCM-41 shows bigger than pure MCM-41 coated and bare ITO. It means carboxyl group well accept the amine group in DNA.

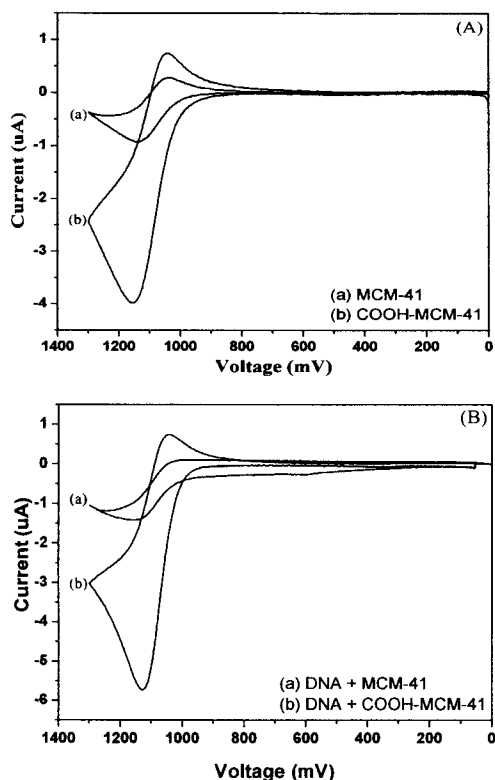


Fig. 6. Cyclic voltammograms of COOH-MCM-41; (A) $[\text{Ru}(\text{bpy})_3]^{2+}$, (B) DNA.

4. Conclusions

The results of this work indicate that highly ordered mesoporous MCM-41 could be synthesized through hydrothermal synthesis via sol-gel process. Research on post synthesis grafting is mostly focused on MCM-41, with its simple hexagonal arrangement of parallel, one-dimensional channel. Anchoring of functional groups by post-synthesis grafting with $-\text{NH}_2$ or COOH functionalities was successful using sol-gel method without destroying MCM-41's structure. Highly ordered mesoporous materials with organic group can be used in DNA sensor in electrochemical part with $\text{Ru}(\text{bpy})_3^{2+}$ as mediator.

References

1. J. S. Beck; J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T-W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, and J. L. Schlenker, A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates, *J. Am. Chem. Soc.*, **114**, 10834 (1992).
2. N. K. Raman, M. T. Anderson, and C. J. Brinker, Template-Based Approaches to the Preparation of Amorphous, Nanoporous Silicas, *Chem. Mater.*, **8**, 1682 (1996).
3. M. Ogawa, T. Nakamura, J.-I. Mori, and K. Kuroda, Luminescence of Tris(2,2'-bipyridine)ruthenium(II) Cations ($[\text{Ru}(\text{bpy})_3]^{2+}$) Adsorbed in Mesoporous Silica, *J. Phys. Chem. B.*, **104**, 8554 (2000).
4. S.-Y. Victor, K. Motesharei, K.-P. S. Dancil, M. J. Sailor, and M. R. Ghadiri, A Porous Silicon-Based Optical Interferometric Biosensor, *Science*, **278**, 840 (1997)
5. K. Moller and T. Bein, Inclusion Chemistry in Periodic Mesoporous Hosts, *Chem. Mater.*, **10**, 2950 (1998).
6. X. Feng, G. H. Fryxell, L.-Q. Wang, A. Y. Kim, J. Liu, and K. M. Kemner, Functionalized Monolayers on Ordered Mesoporous Supports, *Science*, **276**, 923 (1997).
7. G. Villemure and T. J. Pinnavaia, Cyclic voltammetry of Tris(2,2'-bipyridine) ruthenium(II) Cations Adsorbed in Electrodes Modified with Mesoporous Molecular Sieve Silicas, *Chem. Mater.*, **11**, 789 (1999), reference there in.
8. S. Abramson, M. Lasperas, A. Galarnau, D. Desplandier-Giscard, and D. Brunel, Best Design of Heterogenized β -aminoalcohols for Improvement of Enantioselective Addition of Diethylzinc to Benzaldehyde, *Chem. Commun.*, 1773 (2000).

9. N. Bellocq, S. Abramson, M. Lasperas, D. Brunel, and P. Moreau, Factors Affecting the Efficiency of Hybrid Chiral Mesoporous Silicas used as Heterogeneous Inorganicorganic Catalysts in the Enantioselective Alkylation of Benzaldehyde, *Tetrahedron Asymmetry*, **10**, 3229(1999).
10. D. Brunel, Functionalized Micelle-templated Silicas (MTS) and Their use as Catalysts for Fine Chemicals, *Microporous Mesoporous Mater*, **27**, 329(1999).
11. M. Lasperas, N. Bellocq, D. Brunel, and P. Moreau, Chiral Mesoporous Templated Silicas as Heterogeneous Inorganic-organic Catalysts in the Enantioselective Alkylation of Benzaldehyde, *Tetrahedron Asymmetry*, **9**, 3053 (1998).
12. A. Ulman, Formation and Structure of Self-Assembled Monolayers, *Chem. Rev.*, **96**, 1533 (1996).