Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry
2019, 8 (2): 814-820
(International Peer Reviewed Journal)

Gemini-Surfactant Directed Facile Synthesis of Mesoporous Aluminophosphates and their Application in Ferritin Adsorption

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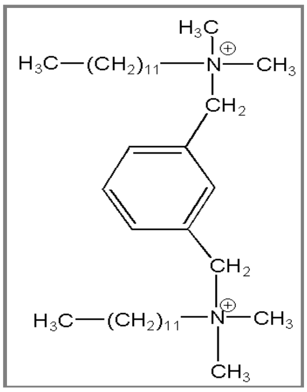
Accepted on 14th March, 2019

ABSTRACT

The usefulness of dicationic Gemini surfactant (DGS) as structure-directing-agent/ template for the facile synthesis of mesoporous aluminophosphates (AlPOs) via both hydrothermal and microwave heating methods was described. An easy extraction of template with ethanolca used a structural transformation in the crystalline phases of AlPOs from lamellar to hexagonal. The morphology, pore-structures, surface properties of as-synthesized and template-free (porous) AlPOs were studied by SEM, XRD, TGA and adsorption-desorption measurements respectively. The local environment around aluminium, phosphate and template in AlPOs were studied by Raman spectroscopy. As the AlPOs contains relatively large pores, immobilization of ferritin protein via adsorption was investigated to design new artificial bio-sensors.

Graphical Abstract



Gemini Surfactant with aryl spacer

Keywords: Gemini Surfactants, Porous Aluminophosphates, Protein Adsorption, Grafter Bio-sensors

INTRODUCTION

Since the discovery of M41S by Mobile company researchers in 1992 [1], the preparation of inorganic mesoporous materials has become a very active research area for catalytic conversions of organic molecules of petroleum industries [2]. These materials have also exhibited good application in protein-adsorption, gas-adsorption and waste water treatment [3]. A variety of inorganic porous materials (metal oxides, metal phosphates, silicates, zeolites) were designed using different organic templates or structure-directing-agents (neutral, cationic and anionic)via ($S^+I^-$and $S^+M^-I^+$), anionic (S^-I^+) or neutral ((S^0I^0) and ($S^0H^+M^-I^+$)) pathways(S = surfactant and I = inorganic) [4]. According to above literature, it appears that the functions of templates and frameworks are not always the same, and mesopores of synthesized products are quite different from each other. In this regard, there is a continual attention has been focused on the study of synthesis and structural characterization of mesoporous aluminophosphates (AlPOs) [5]. The physicochemical properties of these materials are attractive due to their specific framework composed by corner-sharing AlO_4 and PO_4 tetrahedra, which have shown great potential as catalysts for variety industrially important reactions and adsorption for large organic molecules like dyes [6]. Although, according to the International Union of Pure and Applied Chemistry (IUPAC), materials containing pores between 2 and 50 nm are regarded as mesoporous materials, [7] most of the reported AlPOs prepared in the presence of templates such as long chain alkylammonium or alkylamines, or nonionic polymeric surfactants, have exhibited pores below 5 nm in diameter. There are relatively few reports on the synthesis of large pore i.e. >5 nm AlPOs [8] using block copolymer as template under non-aqueous conditions. The synthesis of AlPOs with large pores could be useful for adsorption as well as to process the chemical transformations of certain large organic/bio-molecules. In our efforts, while searching for novel structure-directing agents to produce mesoporous AlPOs, we turned our attention to explore the Gemini surfactants. Previously Gemini surfactants have been served effectively in the generation of mesoporoussilica's and AlPOs [9]. Gemini surfactants (GSs) with two ammonium head groups are known to self-assemble at much lower concentration than their monomeric counterparts, so they should be interesting as structure-directing agents.

This manuscript describes the aptness dicationic GS [10] (figure 1) as structure-directing-agent to produce the mesoporous AlPOs under both conventional hydrothermal and novel micro-wave heating regimes. A special study has been conducted for the removal of template by ethanol and the subsequent structural transformation of AlPOs frame work from lamellar to hexagonal geometries. A detailed description on the characterization of these materials using Scanning electron microscope (SEM), X-ray diffraction (XRD), Raman spectroscopy and N_2 adsorption-desorption techniques is provided. Further, the template extracted AlPOs were examined for ferritin adsorption to spread their reputation in bio-sensing application.

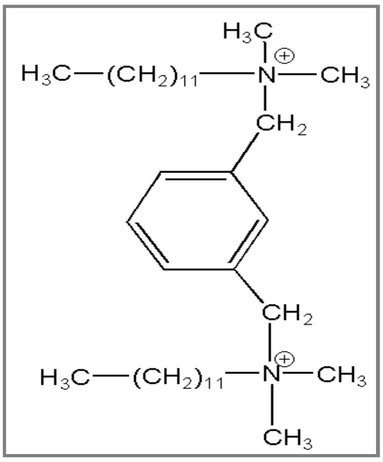


Figure 1. Gemini Surfactant with aryl spacer.

MATERIALS AND METHODS

Materials: The solvents and reagents isopropanol or isobutanol, Al_2O_3, P_2O_5, TMAOH (Tetramethyl ammonium hydroxide), (ALDRICH) and H_3PO_4 (phosphoric acid) 85% (ACROS)are commercially available. The Gemini surfactant (figure 1) was synthesized according to the previously reported method [**10**].

Synthesis of Aluminophosphates: The synthetic gel contains the following molar ratio for hydrothermal and microwave methods.

Hydrothermal synthesis of AlPOs: In a typical hydrothermal synthesis, Al_2O_3 in isopropanol was added to an aqueous solution containing GS (Figure 1), tetramethylammonium hydroxide, H_3PO_4. This mixture was stirred for 1 h at room temperature and then transferred to autoclave and heated for 48 h at 120°C. The solid product obtained was filtered, washed with water, and dried at room temperature.

Microwave synthesis of AlPOs: The sample (0.5 Al_2O_3 : 0.5 P_2O_5: GS: 1TMAOH : 10 ROH : 65 H_2O) was heated batch wise at a power level of 160 W. A total processing time of 10-20 min by exposing the reaction mixture to a microwave electromagnetic field under atmospheric pressure was found to be sufficient for the completion of the synthesis. The strong effect of microwave radiation has been used to progressively reduce the water content in the formulation of product.

Template extraction: The synthesized AlPOs obtained from hydrothermal and microwave methods were then subjected for the extraction of organic GS template with ethanol at temperature of 80°C for a period of 3-4 h to obtain porous AlPOs. After the extraction of organic moiety, the residual inorganic products were washed with water and dried at ambient temperature.

Analytical methods: X-ray powder diffraction (XRD) patterns were obtained using a Philips PW 1710 powder diffractometer. Scanning electron microscopy (SEM) images were obtained on Zeiss EVO 15LS, Nitrogen adsorption-desorption measurements were carried out using the volumetric method with a Coulter SA3100 sorptometer at –196°C. Raman spectra were recorded on a Renishaw RM1000 Raman Spectrometer.

Ferritin immobilization: An iron loaded horse spleen ferritin (Aldrich) at 102 mg mL^{-1} was used without further purification. Some grains of NaN_3 (Merck, 99.0 %) are added to protect ferritin against possible bacterial attacks. An amount of 50 mg of inorganic support i.e. AlPOs with 6.8 mg mL^{-1} ferritin solution was used for the adsorption. The solution pH was stabilized at 6.0 by using 0.05 M phosphate buffer. The reaction mixture was shaken for 6 h moderately and left for 18 h for decantation. The supernatant solution was separated for UV-Vis absorbance test. The amounts of ferritin adsorbed on the supports are based on difference absorbance measurements on a Varian Cary UV-VIS-NIR spectrometer.

RESULTS AND DISCUSSION

SEM analysis: The AlPOs prepared by conventional hydrothermal heating have displayed a sheet-like morphology and the AlPOs obtained under microwave heating also showed sheet-like morphology but the crystals were large and separated (Figure 2).

X ray diffraction study: The XRD patterns of the AlPOs synthesized under hydrothermal heating is shown in figure 3(a). Lamellar mesophases for AlPOs were identified, and the reflections were indexed as (001), (002) and (003).The XRD pattern of as-synthesized AlPOs of microwave synthesis has also appeared like lamellar phases as shown in figure 3(b). These patterns are somewhat similar to the previously reported AlPOs [9b], which were obtained using related Gemini surfactants as structure-directing-agents.

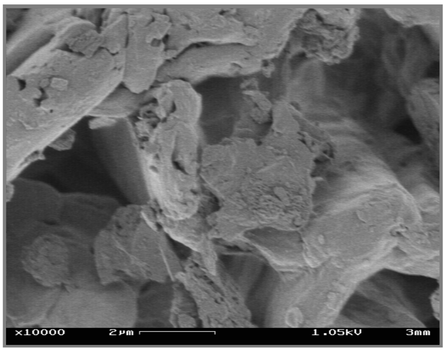


Figure 2. SEM images of as-synthesized AlPOs obtained *via* microwave synthesis.

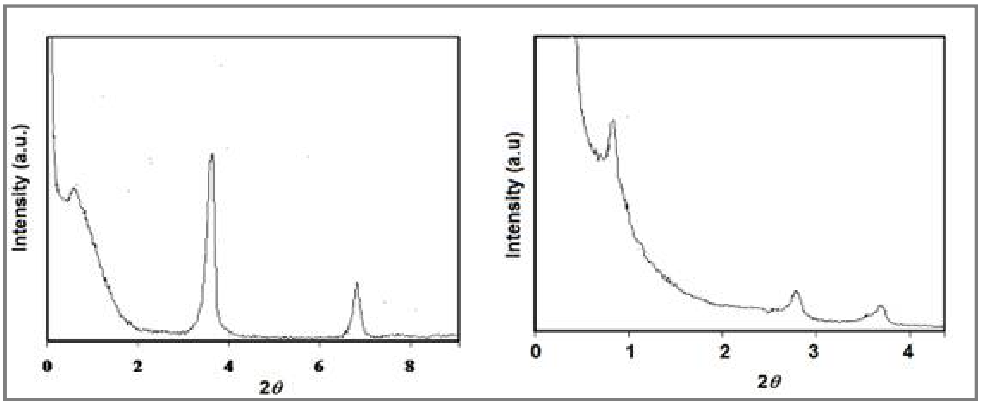


Figure 3. XRD Patterns of as-synthesized AlPOs **(a)** hydrothermal synthesis **(b)** microwave synthesis.

Raman spectra of aluminophosphate framework: Normally the structural form of AlPOs can be described as a frame work built by sharing of alternating $[PO_4]^{3-}$ and $[AlO_4]^{5-}$. The AlPOs frame work will have characteristic the P–O and Al–O bond characteristic vibrations. The experimental wave number values of the free PO_4^{3-} ion in aqueous solution are v_1=938, v_2=420, v_3=1017, and v_4=567 cm^{-1}respectively [11]. However, the normal modes of the $[PO_4]^{3-}$ ion can be shifted to higher or lower wave numbers in the solid state. According to the literature, the solid materials containing a $[PO_4]^{3-}$ unit have displayed the stretching and bending vibrations in an average range of~ 900–1200 and ~ 400–600 cm^{-1}, respectively [12]. In our work, the as-synthesized AlPOs materials show several vibrations in between 400-1200 cm^{-1} due to the presence of organic GS along with frame works. However, we made appropriate assignments for P-O and Al-O vibration by comparing the Raman spectra of as synthesized AlPOs (Fig 4(a)) with the template extracted AlPOs (Figure 4(b)). A discussion on the method of extraction of template given in the following sections).The Raman vibrations corresponding to organic GS/template are clearly absent in extracted AlPOs. The P-O vibrations for both as-synthesized and extracted AlPOs prepared under hydrothermal and microwave methods were observed in the range ν_1= 945-955cm^{-1}, ν_3=1042–1055 cm^{-1}, ν_2 = 570-580 and ν_4 = 451-464 cm^{-1}. The Raman bands are experienced both blue and red-shifting after the extraction. Figure 4(a) and 4(b) provides a representative example of the Raman spectra of as-synthesized and extracted AlPOs of hydrothermal synthesis. The vibrations associated with aluminum can be seen at 710 cm^{-1} and 620 cm^{-1}. Further, the additional vibrations observed in between 800-1500 assignable to various stretching, bending, scissoring and rocking vibrations of organic surfactant moiety are strongly coupled. However, the specific vibrations of the C=C of aryl ring are found in between 1500-1650cm^{-1} and the C-H vibrations are found in between 2900-3100 cm^{-1}.

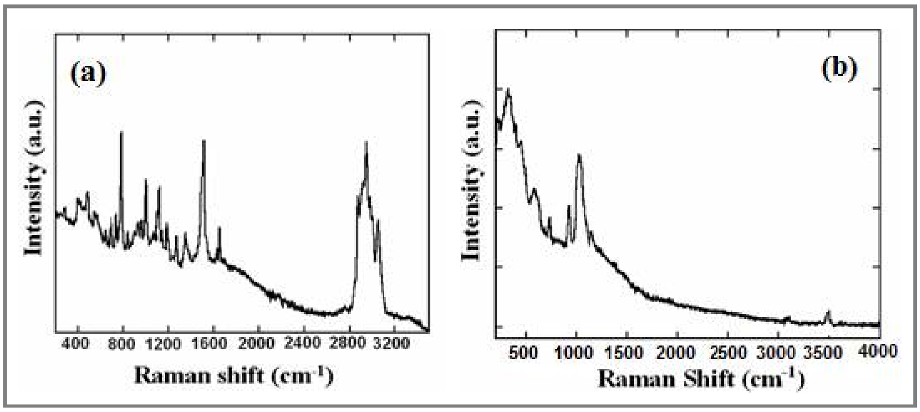


Figure 3. Raman Spectra of AlPOs **(a)** as-synthesized, **(b)** extracted AlPOs.

Surfactant removal and structural transformations: Since the as synthesized AlPOs of this work have shown mixed crystalline phases, we have decided to observe the changes in the crystalline nature after the removal of organic template. The removal of organic GS (Structure-directing agent) through calcinations led to the decomposition of the crystalline structure of as-synthesized AlPOs. To overcome this problem, we have followed extraction with ethanol to remove the organic moiety. Refluxing with anhydrous ethanol solution for 1 h was resulted in the removal of ~ 95% of surfactants as observed from TGA. Once the internal organic moieties are eliminated by extraction, the frameworks are composed of only inorganic units of $[AlO_4]^{5-}$ and $[PO_4]^{3-}$. Specifically, the absence of organic moiety was clearly noted in the Raman spectra of the extracted AlPOs, which did not show the vibrations belonging to the organic environment. The P-O vibrational bands only were observed, which have become more prominent after the extraction (Figure4(b)).

The extracted AlPOs were studied again by the XRD to investigate the nature of crystallinity. Interestingly, the extracted AlPOs of both hydrothermal and microwave synthesis have adopted a structural transformation. As shown in figure 5 the XRD pattern shows a low angle reflection for the first main peak of 2θ= ~1.2 and 1.5°, which was assigned mainly to hexagonal crystal phase. Since the first major peak of as-synthesized AlPOs is shifted to further lower angles upon extraction, the

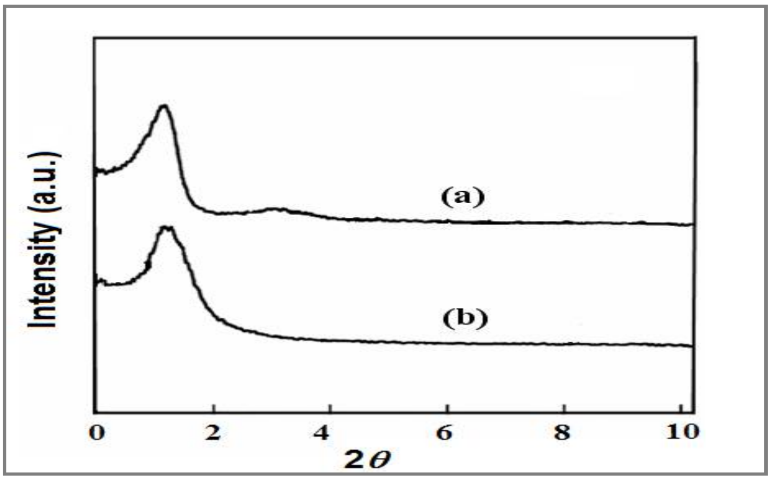


Figure 5. XRD patterns of extracted AlPOs **(a)** hydrothermal **(b)** microwave synthesis.

phenomenon indicates a structural change of maximum portion from lamellar to hexagonal. It appears from the XRD spectrum that the pore structure of the new hexagonal phase is partially disordered. As mentioned before, the as-synthesized AlPOs have been showing the existence of some portion in the hexagonal phase. Literature survey reveals that transformation of lamellar structure to hexagonal and hexagonal to lamellar is possible in porous structured materials during the removal of organic structure-directing agent [13]. We anticipate that the presence of alcohol moiety could influence the packing of GS during the heat treatment and responsible for structural changes.

N_2-adsorption properties of AlPOs: The nitrogen adsorption–desorption isotherms of extracted AlPOs have been measured at liquid nitrogen temperature. The isotherm of a typical Type IV (Langmuir type) was obtained typical of mesoporous materials [14]. The BET surface area of AlPOs of hydrothermal synthesis was 212 $m^2 g^{-1}$ and microwave synthesis was 150 $m^2 g^{-1}$. The pore volume was ~0.21 $cm^3 g^{-1}$. The pore size distribution curve shows pore diameter of 5.7-8.4 nm for hydrothermal AlPOs and 4.9-7.5 nm for microwave AlPOs, calculated from the adsorption branch of the isotherm using the BJH method. However, there was also some secondary pore-size distribution between 20 and 30 nm in the extracted AlPOs.

Immobilization of Ferritin on AlPOs: The AlPOs of hydrothermal synthesis was investigated as a support to immobilize the ferritin protein via adsorption. Because, the AlPOs of present work are water-stable, possesses relatively large pores, so they should be interesting supporting materials not only for surface adsorption, but also able to entrap the proteins in the available large pores. The details of the adsorption procedure are given experimental. In general, the adsorption measurements performed at a pH 6.0 using phosphate buffer were suitable for this study. The presence of various charged species on the surface of ferritin and in AlPOs are responsible for electrostatic interactions between the support and the protein and other interactions are not excluded during the adsorption phenomenon. The quantification of immobilized or adsorbed ferritin protein is monitored by UV-Visible spectroscopy, which exhibits a characteristic absorption at 280 nm due to $\pi \rightarrow \pi^*$ electronic transitions from aromatic residues. Based on the concentration dependent UV-Visible absorption in solution, a maximal ferritin loading of 74 mg g^{-1} support was detected on AlPOs support of hydrothermal synthesis, after 6 hours of soft agitation and 18 h of decantation. Further, the Raman spectrum of ferritin immobilized AlPOs exhibits the characteristic bands at ~1000-1010 cm^{-1}, assignable for ferritin's biomineralized core of FeOOH. Another Raman band observed at ~940 cm^{-1} was assigned to the phosphate present in the iron core of the protein. The ferritin immobilized AlPOs were found to be stable at pH 6 at 4°C for some weeks without any leaching.

APPLICATION

The manuscript entitled "Gemini-surfactant directed facile synthesis of mesoporous alumino phosphates and their application in ferritin adsorption" describes the usefulness of Gemini surfactants as structure-directing-agents in synthesizing the 'mesoporous aluminophosphates *via* hydrothermal and microwave methods and the application of newly synthesized mesoporous aluminophosphates in the adsorption of ferritin. This study is useful in the creation of novel grafted biosensors.

CONCLUSION

The dicationic Gemini surfactants are efficient structure-directing agents to obtain mesoporous AlPOs with relatively large pore structures. The use of ethanol to remove GS-template could influence its packing in the AlPOs frame-works and favored a structural transformation. Previous works on template free microwave synthesis gave microporous AlPOs [15], while our results demonstrate that a little concentration of Gemini surfactant is enough to afford mesoporous AlPOs. Preliminary results on the immobilization of ferritin suggest that the mesoporous AlPOs of present work are good supporting materials in the fabrication of grafted bio-sensors.

ACKNOWLEDGEMENTS

The authors acknowledge the support from Indo-South Africa Science and Technology inter governmental joint research project (No.**DST/INT/SA/P-15/2011, 10-01-12.**).

REFERENCES

[1]. G. E. Putri, S. Arief, N. Jamarun, F. R. Gusti1, A. Fisli, Zilfa and U. Septiani, Synthesis and Microstructural Characterization of Modified Nano-Cerium Silica Mesoporous by Surfactant-Assisted Hydrothermal Method, *J. Applicable Chem*., **2017**, 6, 1058.

[2]. H. K. D. Nguyen and P. V. Pham, Biodiesel Synthesis from Vegetable Oil Deodorizer Distillate Over Mesoporous Superacid Oxo-Phosphated Sulfated Zirconia Catalyst, *J. Applicable Chem.*, **2017**, 6, 265.

[3]. S. Nagappan, S. S. Park, B. K. Kim, D.-G. Yoo, N.-J. Jo, W.-K. Lee and C.-S. Ha, Synthesis and functionalisation of mesoporous materials for transparent coatings and organic dye adsorption, *New J. Chem*., **2018**, 42, 10254.

[4]. D. Levy, M. Zayat, *in 'The Sol-Gel Handbook;* Synthesis, Characterization, and Applications, 3 Volume Set, John Wiley and Sons, **2015**.

[5]. K. Sivakami, K. Muthuraja, C. Kannan, Adsorptive removal of anionic dyes from aqueous solution using nanoporous magnesium aluminophosphate material, *Materials Today: Proceedings*, **2018**, 5, 8812.

[6]. A. V. Vijayasankar, S. Deepa, B. R. Venugopal, N. Nagaraju, Amorphous Mesoporous Iron Aluminophosphate Catalyst for the Synthesis of 1,5-Benzodiazepines, *Chin. J. Catal.*, **2010**, 31, 1321.

[7]. J. Rouquerol, D. Avnir, C. W. Fairbridge, D. H. Everett, J. M. Haynes, N. Pernicone, J. D. F. Ramsay, K. S. W. Sing, K. K. Unger, Recommendations for the Characterization of Porous Solids, *Pure and Applied Chem.*, **1994**, 66,1739.

[8]. T. Kimura, Oligomeric Surfactant and Triblock Copolymer Syntheses of Aluminum Organophosphonates with Ordered Mesoporous Structures, *Chem. Mater.*, **2005**, 17, 5521.

[9]. (a) K. Czechura, A. Sayari, Synthesis of MCM-48 Silica Using a Gemini Surfactant with a Rigid Spacer, *Chem. Mat.* **2006**, 18, 4147, (b) S. Kinane, C. S. Vasam, P. P. Knops-Gerrits, **2006**, 219, 279-298, Fluid Transport in Nanoporous Materials *NATO Science Series-II.* Chapter *Vibrational Spectroscopy to Monitor Synthesis, Adsorption and Diffusion in Micro- and Mesoporous Metal Phosphates.*

[10]. A. Laschewsky, K. Lunkenheimer, R. H. Rakotoaly, L. Wattebled, Spacer effects in dimeric cationic surfactants, *Colloid Polym Sci.*, **2005**, 283, 469.

[11]. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, John Wiley and Sons, New York, **1986** and references there in.

[12]. R.L. Frost, T. Kloprogge, P. A. Williams, W. Martens, T. E. Johnson, P. Leverett, Vibrational spectroscopy of the basic copper phosphate minerals: pseudomalachite, ludjibaite and reichenbachite, *Spectrochim. Acta, Part A*, **2002**, 58, 2861.

[13]. Neraj, C. N. R. Rao. Phase transformations of mesoporous zirconia, *J.Mater.Chem.*, **1998**, 1631.

[14]. F. J. Sotomayor, K. A. Cychosz, M. Thommes, Characterization of Micro/Mesoporous Materials by Physisorption: Concepts and Case Studies, *Acc. Mater. Surf. Res.*, **2018**, 3, 34.

[15]. K. Kunii, K. Narahara and S. Yamanaka, Template-free synthesis of AlPO4-H1, -H2, and -H3 by microwave heating, *Micropor. Mesopor. Mater*, **2002**, 52, 159.