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Templated growth of mesoporous aluminum phosphate tubules using protein molecules of tobacco

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Template-based approaches involving the co-operative organization of organic-inorganic assemblies as intermediates are emerging as a promising conceptual basis for future developments of porous inorganic materials. This field has been fuelled by new synthetic strategies such as "supramolecular templating" that have enabled precise engineering of pore size, shape and connectivity on the mesoscopic scale [1]. Randomly oriented layers of $AlPO_4$ were synthesized and used as chemical sensors and low dielectric constant materials [2] earlier, but now, attention is being directed towards the formation of highly oriented films of metallic phosphates [3]. Development of $AlPO_4$ based materials, in particular, has extended the compositional and structural diversity of the molecular sieves. Anisotropy induced by biomolecules leads to orientation and many exotic properties associated with the hierarchical structures and make them suitable for a range of applications including catalysis, ion-exchange separation, nuclear medicine and optics [4]. Though the synthetic templates namely latex fibers, polyurethane foams, starch gels, carbon nanotubes and organic molecules with long chains are being routinely employed for the synthesis of hierarchical inorganic frameworks [5], mineralization of biomolecular templates like tobacco mosaic virus (TMV) is a relatively new concept [6]. It leads to the formation of porous structures at nanolength scales that are strictly controlled by the presence of protein superstructures. Moreover, these biomolecular templates offer unusual symmetry and mesoscale ordering in the porous structures. Abundance and stability of the protein molecules present in tobacco leaves and their tendency to undergo self assembly has motivated us to use it directly as templates to synthesize self supporting hybrid films at liquid-air interface. The experiment is based on the time dependent surface gelation phenomenon as exhibited by an aqueous solution of biomolecules that results in precipitation of self assembled inorganic particles exhibiting a high degree of sophistication in microstructural features. The present manuscript demonstrates and discusses the reproducible synthesis of the self-assembled highly oriented mesoporous tubules of $AlPO_4$ in tobacco liquor solution. This process has an inherent potential to be scaled up unlike the more common Langmuir-Blodgett monolayer formation.

Nanosized $AlPO_4$ particles were *in situ* synthesized in the film of tobacco liquor solution (TLS) formed at the air-water interface. A 4% TLS was prepared by dissolving the weighed amount of dried and processed tobacco leaves in 100 ml of deionized water. pH of this solution was recorded to be 6.54. 20 ml each of 0.1 M aluminum nitrate (LR, s.d. fine-chem. Ltd.) solution and the above TLS solution was mixed gently until complete mixing was ensured and the mixture was incubated at 40 °C for 96 h. This led to the formation of a thick film at the air-water interface. The underlying solution was carefully decanted, the film was washed with de-ionized water at room temperature and soaked in 20 ml of 0.033 M phosphoric acid (AR, Qualigens) solution for a maximum period of 4 days. After 2 days the sample was periodically withdrawn and dried at room temperature under controlled atmosphere and characterized by scanning electron microscopy (SEM), X-ray diffractometry (XRD) and infrared (IR) spectroscopy. The proteins present in TLS were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Tobacco mosaic virus is a classic example of biomolecules that encompass molecular recognition, self-assembly and templation. It is characterized by linear tubular structure (length 300 nm, width 18 nm) that self-assemble in the form of parallel channels or radial spherulites in the presence of specific cations [6]. Results reported in Plant Science confirm the presence of rod shaped protein aggregates in the tobacco leaf [7]. Our previous studies have proved that unmineralized TLS film is characterized by self assembled rod shaped particles (dia 10–20 nm, length 100–200 nm) that form spherulitic structures [8]. SDS-PAGE of TLS showed the presence of protein bodies with an average molecular weight of 60 kDa (Fig. 1). This matches well with the reported range of average molecular weight of the proteins molecules (10–120 KDA) found in tobacco leaves [9]. These protein bodies have limited solubility in water and hence undergo phase segregation with hydrophobic and hydrophilic components

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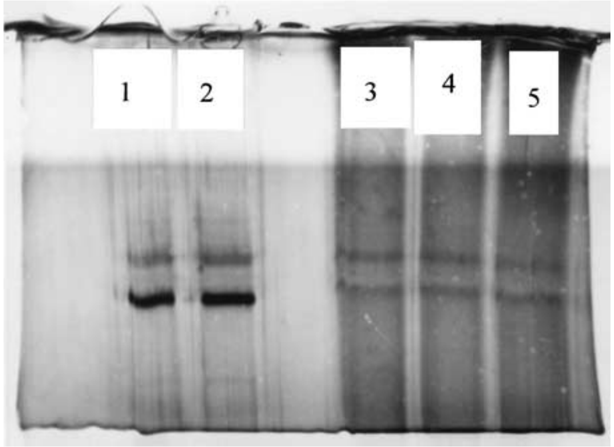

Figure 1 SDS-PAGE analysis of TLS showing the average molecular weight of 60 kDa of the protein molecules where lanes 1 and 2 have the TLS and lanes 3, 4 and 5 are varying concentrations of the BSA marker.

on nanometer and micrometer scale at the air-water interface.

SEM studies of the mineralized film exhibited 200–300 nm long and 10–15 nm wide aluminum phosphate nanoparticles that self assembled to form a hollow tubule with a diameter of (1–1.5 μm) (Fig. 2a). A prolonged soaking of the film in the solution containing phosphate ions resulted in highly anisotropic growth of the precipitated particles along the major axis of the tubule (Fig. 2b). One may notice that the tubule diameter, which forms an open channel, remains invariant; also obvious is the formation of multiwall tubule or cocentric tubules. Surface of the cocentric tubules are separated from each other, thus indicating the formation of higher order linear porous structure. Further soaking revealed the growth of self assembled micron sized continuous tubules of aluminum phosphate (Fig. 2c). The exact mechanism of such an orderly oriented growth using tobacco protein molecules is not well established, the results indicate a linear self assembly of the rod shaped protein molecules that form tubule like structure. Formation of such structures may be attributed to the partial solubility of the protein macromolecules in water forming micelles. Spacing between the parallel protein rods along the tubular axis intercalates the aluminum and phosphate ions leading to the precipitation of nanosized aluminum phosphate particles. A high diffusion rate at the air-water interface of the particles leads to the formation of a continuous mesoporous tubular structure.

XRD studies of the sample established the presence of an amorphous phase (Fig. 3a). This result is in agreement with Davis's recent observation that except ordered, hexagonal mesoporous benzene-silicate composites, ordered mesoporous materials are not crystalline [1]. The reasons of the amorphous nature of the nanosized mesoporous materials are not known, however, it may be understood on the basis of correlation between framework density (FD, no. of atoms per nm^3) and structural features of the porous materials. EDX analysis of the sample confirms the presence of aluminum as well as phosphorous in the precipitated particles.

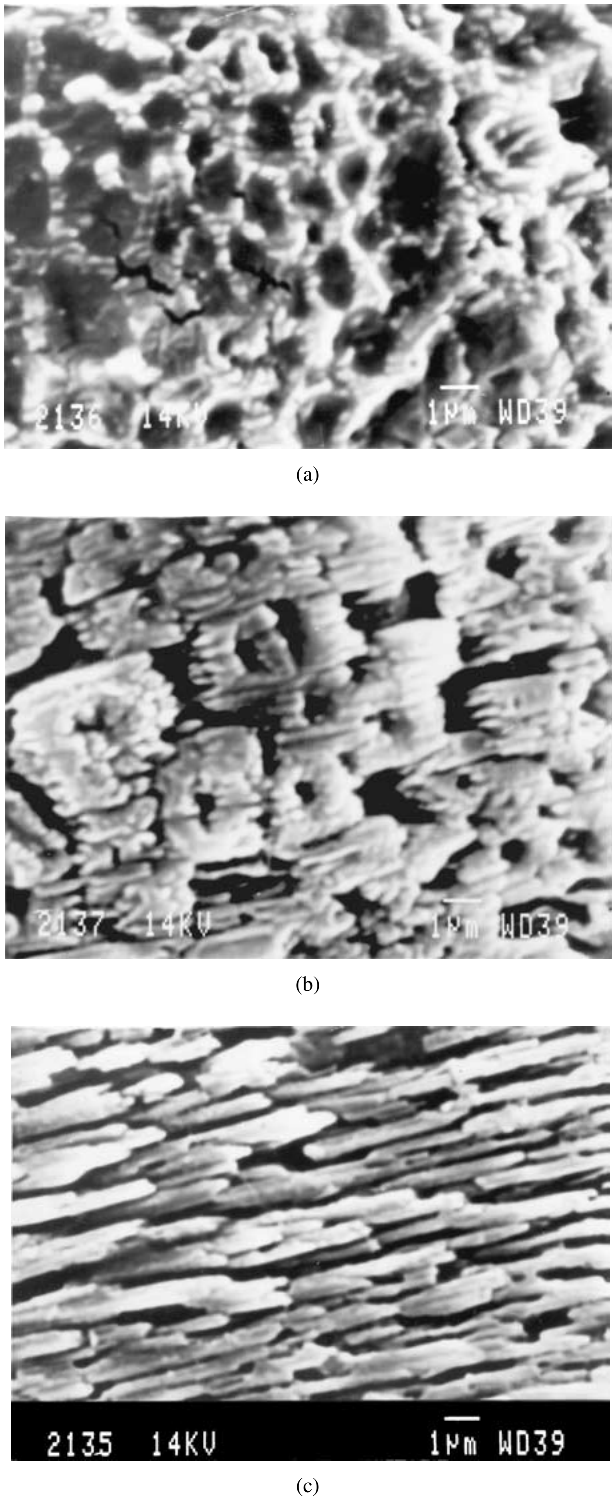

Figure 2 (a) Mineralized TLS film exhibiting the self assembly of precipitated aluminum phosphate nanoparticles forming a hollow tubules. (b) Anisotropic growth of the aluminum phosphate particles along the major axis of the tubule on soaking in phosphoric acid for 48 h. (c) Growth of self assembled micron sized continuous tubules of aluminum phosphate on soaking in phosphoric acid for 96 h.

IR spectra of the *in situ* precipitated aluminum phosphate in TLS film revealed the presence of the characteristic bands corresponding to both the metal phosphate and the underlying biomolecular matrix (Fig. 3b). A strong band at 3449 cm^{-1} is attributed to the symmetric N–H stretching vibration of the secondary amide of the protein polypeptide chain [10]. The band of medium intensity at 1640 cm^{-1} corresponds to the amide $-$C=O

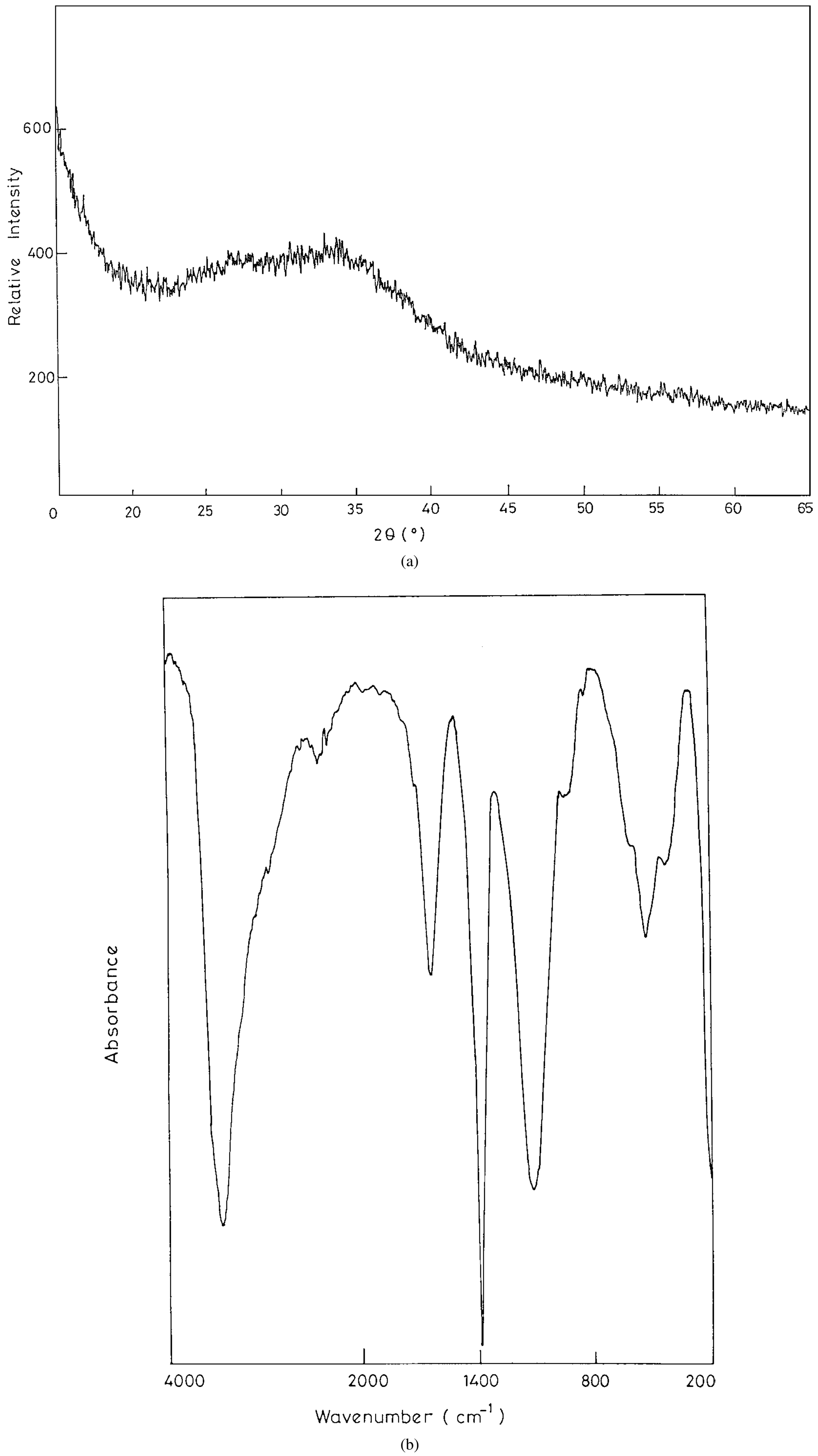

Figure 3 (a) XRD of the *in situ* precipitated aluminum phosphate particles in TLS film. (b) IR spectra of the *in situ* precipitated aluminum phosphate in TLS film.

stretching [10] while a strong band at 1384 cm^{-1} is due to the $-\text{CH}_2$ asymmetric bending of the parent chain constituting the protein molecules of the underlying matrix. A small hump at $\sim$920 cm^{-1} corresponds to Al—OH bending mode [11] due to absorption of water molecules on the surface the mineralized film. Two bands, one of strong intensity at 1115 cm^{-1} and another of medium intensity at 528 cm^{-1} respectively is attributed to the stretching and bending modes of the P—O bond in the phosphate anion of the precipitated metal phosphate.

In summary, the self assembly of 60 kDa protein molecules present in TLS at air-water interface has been used as growth directing agents for precipitation of highly oriented mesoporous tubules of aluminum phosphate. Due to limited solubility, these protein bodies self assemble in aqueous medium and form hollow long tubules that intercalate aluminum and phosphate ions. In the organic environment, this finally leads to formation of multiwall, cocentric mesoporous tubules of aluminum phosphate that are crystallographically amorphous in nature.

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