Self-assembled growth of calcite particles on a tobacco film

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Self-assembled monolayers provide a powerful tool for materials scientists to control the nucleation and growth of ceramic materials. However, the two-dimensional nature of this method greatly limits its application for synthesizing three-dimensional hierarchical materials. Self-assembled three-dimensional protein bodies present in the tobacco leaves have been used in this study as a template for the synthesis of prismatic calcite particles. The trigonal prism-shaped calcite particles replicate the basic two-dimensional triangular structure formed by the nanosized protein rods. Observed three-dimensional assembly of prismatic calcite particles correspond to the underlying ellipsoid conformation of the protein superstructure.

SIZE, morphology, habit of primary inorganic particles and subsequent ordering into hierarchical structure determine the physical and chemical activity of a crystalline phase. Empirically changing the process parameters such as ambient temperature, pH and supersaturation have been proved quite successful in controlling the size of the particles. However, a control over crystal habit, texture and morphology could not be achieved. On the contrary, the templating approach, based on self-assembly and molecular recognition through a preorganized supramolecular structure has been remarkably successful in the creation of advanced inorganic materials with a high degree of sophistication in morphological features as well as the evolution of a hierarchy in the growth[1]. The two different types of templation processes, namely, thermodynamic and kinetic, bear similarity with biomineralization, a matrix-mediated *in situ* process as adopted by nature to create functional biomaterials[2]. Recent studies have established the role of functionalized polymers[3], polymer gels[4], polypeptides[5], biomimetic matrices[6], liquid crystals media[7] and macroscopic architecture of continuous phase microemulsions[8] as active templates to control the formation of crystalline materials. In each case, the control of particle size and morphology is observed. The resulting crystals are randomly arranged with respect to the substrate and lack functional coherency. This limitation directed the materials scientists to design templating substrates which not only control the size and morphology but also induce the assembly of the molecular component into the growing inorganic crystals. Recent studies based on the application of amphiphilic monolayers, formed as a result of the self-assembly of water insoluble amphiphilic molecules at air–water interface, could successfully demonstrate the self-assembly of inorganic crystallites grown on them[9].

In the present study, we report the self-assembly of the trigonal-shaped calcite particles grown *in situ* in the film of TLS (Tobacco Liquor Solution) acting here as a morphology and self-assembly directing agent.

A number of experiments have been carried out involving the preparation of tobacco liquor solution (TLS) and addition of Ca^{2+} ions followed by mixing of sodium carbonate solution to precipitate calcium carbonate particles at various pH, concentration and temperature. However, only the optimum results of the experiments carried out at room temperature have been presented in the following. All the reagents employed in this experiment were of analytical grade.

The tobacco liquor solution (TLS) was prepared by dissolving 4 g of processed and dried tobacco leaves in 100 ml of doubly deionized water. The pH of the TLS stock solution was recorded to be 6.45. A 0.1 (M) solution of $CaCl_2$ and a 0.1 (M) solution of Na_2CO_3 were prepared by dissolving the requisite amounts of the salts in doubly deionized water. The TLS was mixed with the Ca^{2+} salt solution in 1 : 1 volumetric ratio by continuous stirring using a magnetic stirrer. Next, the requisite amount of Na_2CO_3 solution was added slowly at room temperature by continuous stirring to the TLS-Ca^{2+} salt solution. The resulting solution was kept at room temperature for 24 h. This led to the formation of a thin film at air–water interface. This film was carefully transferred on a glass slide and characterized by scanning electron microscopy (SEM) and X-ray diffractometry (XRD).

SEM studies of the unmineralized tobacco liquor film formed at the air–water interface revealed the self-assembled disc shaped clusters having diameter in the range of 10–12 μm (Figure 1 a). A magnified view of this disc exhibited a regular arrangement of the rod-shaped particles, radiating from a common centre and forming a helical trajectory, as indicated by the presence of a hole in the centre (Figure 1 *b*).

Another section of this film confirmed that self-assembled arrangement of clusters as observed above possesses a three-dimensional ellipsoid morphology (Figure 2 *a*). SEM studies carried out on the reverse side of this film also confirmed a regular arrangement of reverse ellipsoids having a hole in their centre (Figure 2 *b*). A further increase in magnification revealed the formation of these ellipsoids through the self-assembly of two-dimensional planar triangular structures which were evolved again through the self-assembly of porous rod-shaped particles (Figure 2 *c*). These rods, having diameter in the range of 10–20 nm and length ~ 200–500 nm, resemble the morphology and dimensions of tobacco mosaic virus (TMV, 95% protein and 5% DNA), which causes wrinkling of the

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tobacco leaves. It is a classic example of a biomolecule that encompasses molecular recognition, self-assembly and templation[10]. Reports are available about the ellipsoid-shaped bulk protein bodies in tobacco leaves similar to our observations[11]. These protein bodies are characterized by 2130 identical protein subunits having an average molecular weight of 17.5 kDa that are symmetrically spiralled around a single strand of RNA[12]. The major driving forces for the protein bodies in tobacco film to form well-defined aggregates as obtained above are a multitude of non-covalent interactions, such as hydrogen, van der Waals bonds, electrostatic, π–π, and hydrophobic interaction at the hydrocarbon–water interface and the hydrophilic-ionic compression between the head groups. The equilibrium structure of the aggregate is determined by the thermodynamics of the self-assembly process and

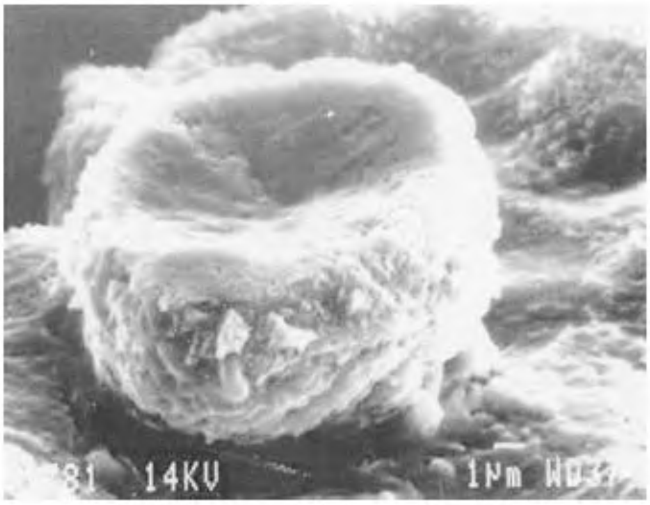

Figure 2 *a*. Three-dimensional ellipsoid morphology of the self-assembled structure in tobacco liquor film.

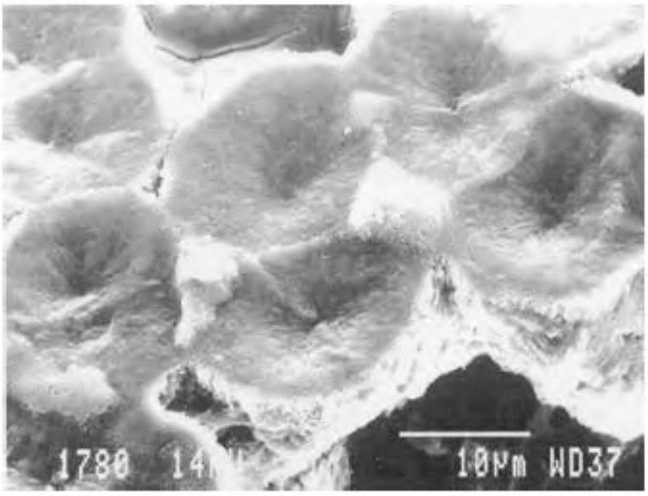

Figure 1 *a*. Microstructure of the top surface of the unmineralized tobacco liquor film exhibiting self-assembly of disc-shaped particles.

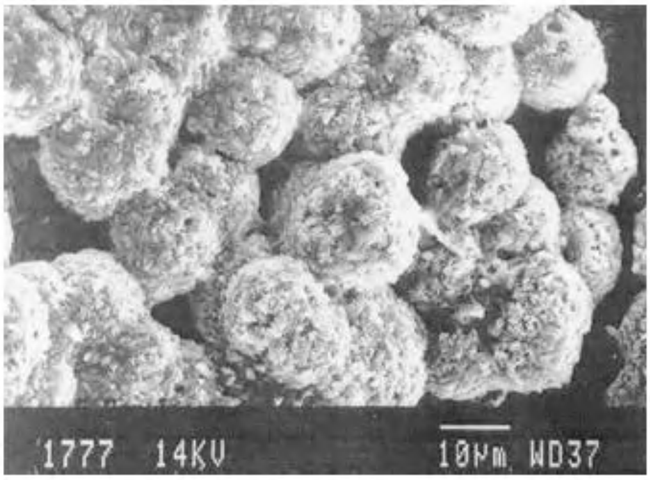

Figure 2 *b*. Microstructure of the surface of tobacco liquor film in contact with aqueous medium showing regular arrangement of reverse ellipsoids with a hole in centre.

Figure 1 *b*. Magnified view of the disc revealing the helical arrangement of rod-shaped particles radiating from a common centre.

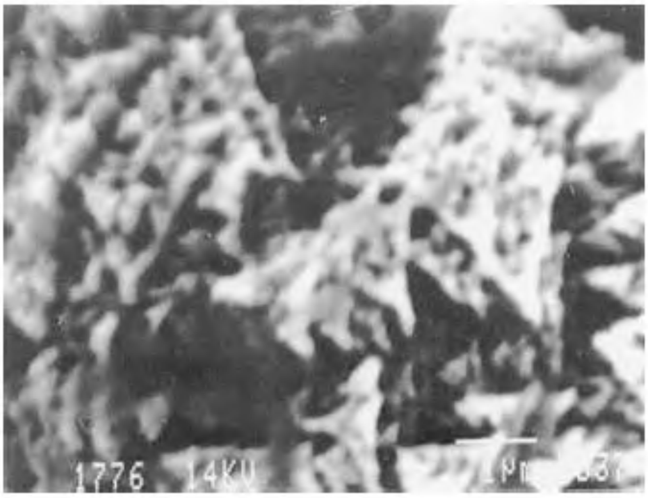

Figure 2 *c*. Magnified view of the apex of ellipsoid revealing the self-assembled structure made of nanorods forming isosceles triangles.

their inter- and intra-aggregate structures. The ordered helical growth leading to a spiral structure (Figure 1 *b*) may find a strong resemblance with the structure of the sunflower head that is known to possess an underlying Fibonacci sequence[13]. The Fibonacci sequence is very common in the growth of several topological structures characterized by icosahedral or other similar shape possessing 5- and 10-fold rotational symmetry. At this stage, we have only obtained visual resemblance between the structures obtained by us and the available reports. Samples have been sent for complete biochemical characterisation. The detailed results will be published subsequently.

Transportation of calcium ions in the tobacco extract film did not change its original morphology. Calcium carbonate particles, formed by the incorporation of carbonate ions in Ca^{++} ion induced tobacco liquor film, were structurally characterized as calcite phase by XRD (Figure 3).

Samples, properly washed and dried, after 48 h of soaking, manifested the growth of prism-shaped interlocked calcite particles forming a self-assembled cluster (Figure 4 *a*). Development of this morphology is due to the nucleation along (001) plane as a result of the rapid growth of the oriented nuclei along the (001) and ⟨11.0⟩ direction. The formation of the pyramidal structure may be attributed to the growth from the calcium binding site of tobacco layers, of the symmetry related rhombohedral faces of {104} type, which intersected to produce a clearly defined apex. A five times magnified view of these pyramidal-shaped calcite particles revealed that in fact they were not single particles as seen at a lower magnification, but were formed as a result of the self-assembly of smaller triangular calcite particles having shape and size in correspondence with the planar structures formed by the protein rods (Figure 4 *b*). This clearly depicts the effect of organic macromolecule on the size and shape of the calcite particles. A prolonged soaking of calcium ion induced tobacco liquor film in sodium carbonate solution for 96 h resulted in the formation of self-assembled globular cluster of calcium carbonate having diameter around 12 μm, made up of ~ 2 μm prismatic calcite clusters (Figure 4 *c*). This inorganic cluster replicates the size and morphology of the bulk protein bodies present in the unmineralized tobacco liquor film.

In summary, a helical arrangement of self-assembled protein rods, formed as a result of hydrophobic interactions, not only templated the pyramidal growth of the triangular calcite particles but also assembled them in a well-defined manner replicating the growth mechanism of the template itself.

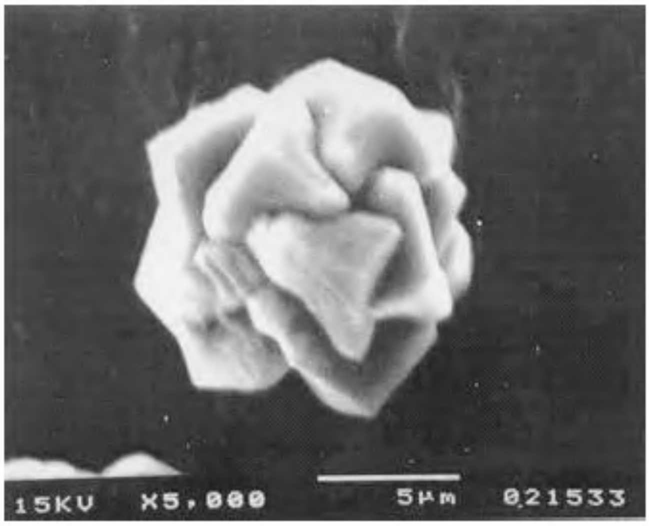

Figure 4 *a*. Scanning electron micrograph of the self-assembly of prismatic calcite particles showing mutual interlocking.

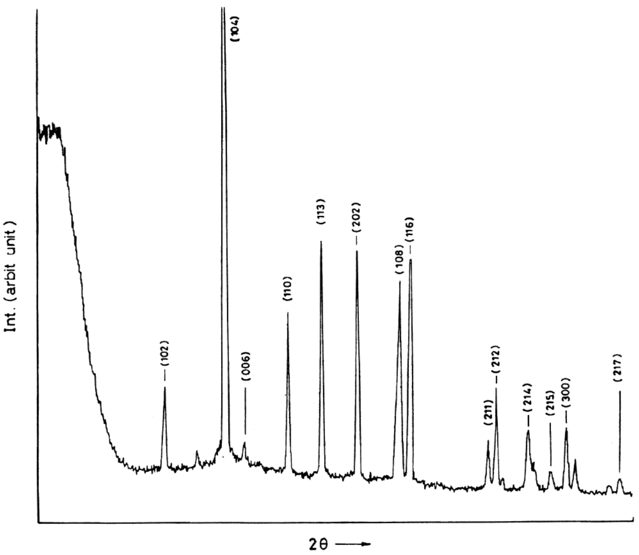

Figure 3. XRD of the calcium carbonate particles precipitated on tobacco liquor film.

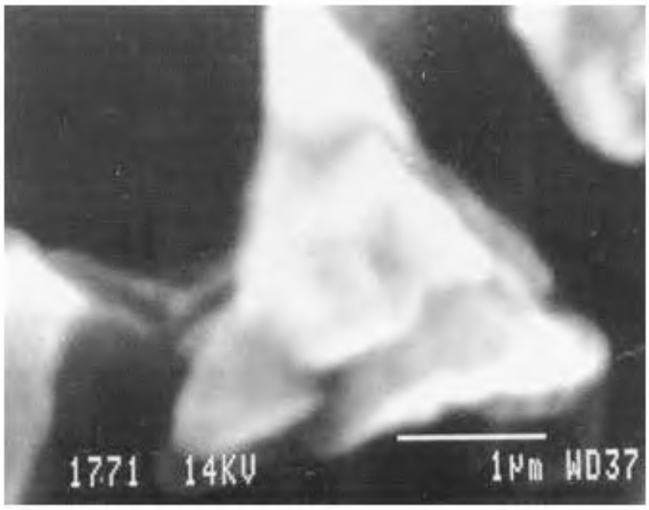

Figure 4 *b*. Magnified view of prismatic calcite particle revealing its formation due to the self-assembly of smaller-sized calcite particles of triangular shape: an evidence of induced hierarchy.

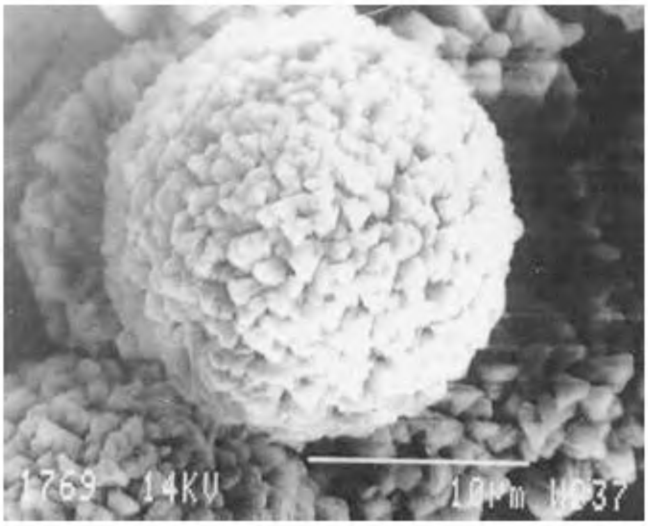

Figure 4 *c*. A globular self-assembled cluster of prismatic calcite particles as obtained after 96 h of soaking in sodium carbonate solution.

The SEM studies illustrated the role of self-assembled protein layers in film of the tobacco liquor solution (TLS) as a template which not only induces the nucleation and growth of the calcite particles but also provides an active motif for their self-assembly. Analysis of results revealed the presence of hierarchy at two different length scales in the growth of pyramidal calcite crystals.

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SPOT VEGETATION multi temporal data for classifying vegetation in south central Asia

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Satellite remote sensing has enabled the acquisition of land use/land cover and vegetation information at different spatial and temporal scales. Vegetation instrument on-board Spot 4 satellite with four spectral bands – blue (0.43–0.47 μm), red (0.61–0.68 μm), infrared (0.78–0.89 μm) and short wave infrared (1.58–1.75 μm) at a spatial resolution of 1 km and temporal resolution of 1 day meets the requirement of vegetation mapping at a continental scale. This study focuses on the use of multitemporal SPOT VEGETATION data for vegetation mapping in south central Asia. The basis of classification is temporal dynamics, i.e. the pattern of change of Normalized Difference Vegetation Index (NDVI) values through a temporal domain which reflects the phenology of vegetation, crop cycle and the cropping system of agricultural practices. The temporal profile of NDVI facilitates the discrimination between different vegetation types and different types of cropping pattern.

OVER the past two decades, data from earth observation satellites has become important in mapping the earth's features and infrastructure, managing natural resources and studying environmental changes. Remote sensing and Geographic Information Systems (GIS) provide tools for advanced ecosystem management. The collection of remotely sensed data facilitates the synoptic analyses of earth-system function, patterning, and change at local, regional, and global scales over time. Coarse spatial resolution (1 km resolution), combined with high temporal resolution component would allow for the early production of an actual global forest/non-forest status map. This effort could provide a first hand data, which can be used in conjunction with other data sets available to define the extent of deforestation. In the present study, SPOT VEGETATION data has been used to prepare a land use/land cover map of south central Asia. This initiative is part of the on-going global land cover-mapping project (GLC 2000) to which Indian Institute of Remote Sensing is a collaborator with Joint Research Center (European Commission) for mapping in the south Asia. The project aims to explore the possibilities of SPOT VEGETATION data sets for vegetation analysis.

Land cover information is time sensitive. The identification of crops, for instance, may require imaging

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