



Morphosynthesis: high fidelity inorganic replica of the fibrous network of loofa sponge (*Luffa cylindrica*)

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ABSTRACT

High fidelity calcium carbonate and hydroxyapatite (bio) inorganic replicas of the fibrous network of the dried fruit of *Luffa cylindrica* are described, utilizing a facile synthetic route. The loofa sponge is a highly complex macroscopic architectural template, an inexpensive and sustainable resource. In the context of the morphosynthesis, the capability of replication of the loofa sponge opens the possibility of the use of biodiversity in obtaining new materials. We would like to emphasize that the template proposed in this paper, makes possible the preparation of inorganic replicas with a very desirable size, on the centimeter scale. This fact is innovative with respect to inorganic replicas described in the literature, which predominate at the micrometric scale, limited to the original size of the template.

Key words: morphosynthesis, biomimetic synthesis, morphology, calcite, hydroxyapatite.

INTRODUCTION

Morphosynthesis consist of the chemical construction and patterning of inorganic materials with unusual and complex architecture (Ozin 1997, Walsh et al. 1999, Mann 2000). The complex inorganic architectural replication is constructed hierarchically on a length scale from the nanometer to the millimeter level. The controlled synthesis of inorganic materials with specific morphology is an important aspect in the development of new materials in many fields such as catalysis, electronics, nanocomposites, etc. From a materials perspective, biomimetic syntheses have permitted the replication of a range of organized inorganic forms using, as templates, bacterial colonies (Li et al. 2003), insect wings (Cook

et al. 2003), spider silk (Huang et al. 2003) and pollen grains (Hall et al. 2003), for example. Most of the morphosynthesis routes or biomimetic syntheses involve precipitation reactions in self-assembly organic media, such as micelles, surfactants and microemulsions (Mann 2000). Sponge-like microspheres of calcium carbonate (vaterite) were prepared by passive evaporation of the volatile components of a supersaturated water-in-oil microemulsion (Walsh et al. 1999). The biomimetic synthesis of calcium carbonate, one of the most abundant biominerals, has received much attention owing to its wide application in such industrial fields as paper, paint, cosmetics, and ceramics (Yu et al. 2004). Calcite has been reported to be effective in fluoride removal from industrial wastewater and drinking water (Yang et al. 1999, Fan et al. 2003). Recently, the coating of calcium carbonate powder

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with fluoride for wettability enhancement was proposed as a new foaming agent for foamed metal production (Nakamura et al. 2002). Thus, the calcium carbonate waste from water treatment can be recycled as a foaming agent. Calcium carbonate is also a known biomaterial with potential bioactivity (Yu et al. 2004).

The development of advanced materials for biomedical applications is among the most important problems facing modern materials engineering (Kawachi et al. 2000). The greatest potential for bone substitution is shown by materials based on hydroxyapatite, which can develop tight bonding with bone tissue, exhibits osteoconductive behavior, is stable toward bioresorption, and has no adverse effects on the human organism (Finisic et al. 2001, Orlovskii et al. 2002). Synthetic hydroxyapatite precursors are produced by a variety of ceramic processing routes including precipitation, sol-gel and hydrothermal processing. The most important parameters for application of calcium carbonate and hydroxyapatite are specific surface area, particle morphology, chemical and phase composition and microstructure.

However, biomimetic material will only be technologically relevant if inexpensive routes with high product yields can be developed (Walsh et al. 1999). In this context, Hall et al. (2003) described a facile and inexpensive method for replicating the complex surface morphology of flower and tree pollen grains. These replicas consist of silica, calcium carbonate and calcium phosphate minerals.

The aim of this work was to evaluate the capability of the biomimetic synthesis of calcium carbonate and hydroxyapatite inorganic replicas of the fibrous network of loofa sponge (*Luffa cylindrica*, cucurbitaceous family). The loofa sponge is a highly complex macroscopic architectural template, an inexpensive and sustainable resource. The loofa sponge is cultivated, unlike the sponge produced with cellulose that is extracted from trees. The plant is cultivated in many countries, including Brazil, where its cultivation has an increasing economic importance. The fruits of *Luffa cylin-*

drica are smooth and cylindrical shaped. The fibers are composed of 60% cellulose, 30% hemicellulose, and 10% lignin and can be used by industry for many purposes, such as packaging, insulating, or filling materials. The absorbent capacity of the fibers for deionized water is 13.6 g/g (Bal et al. 2004). Traditionally, it is used for bathing and dish washing and, recently, the fibers were used for environmental reclamation (Iqbal and Edyvean 2004).

MATERIALS AND METHODS

The dried fruit of *Luffa cylindrica* was cut into discs of ca. 10 cm diameter, 3 cm thick, washed with abundant distilled water and then dried at 60°C. Calcium carbonate and hydroxyapatite inorganic replicas were prepared according to a previously described procedure (Hall et al. 2003). Aqueous solutions of CaCl₂ (3.0 mol L⁻¹, pH 8), of Na₂CO₃ (3.0 mol L⁻¹, pH 13) and of Na₂HPO₄ (2.0 mol L⁻¹, pH 10) were first prepared as stock solutions. For both inorganic replicas, the sponge was immersed in CaCl₂ solution for 12 h, at room temperature. After this period, the sponge was removed from the solution and washed with distilled water to remove excess CaCl₂ solution. Calcium carbonate and hydroxyapatite were produced on the fibrous network by reimmersion of the sponge in Na₂CO₃ (12 h) or Na₂HPO₄ (12 h) solutions, respectively. The sponge was again washed with distilled water and submitted to pyrolysis at 600°C for 24 h under air atmosphere after heating rate of 5°C min⁻¹, to produce inorganic replicas.

In order to study the decomposition of the fibrous network of loofa sponge, thermogravimetric analyses were carried out (TGA, Shimadzu 50WS) using an air flow rate of 20 mL min⁻¹ and a heating rate of 10°C min⁻¹. Powder X-ray diffraction (XRD) patterns were obtained using a Shimadzu XRD6000 diffractometer, with Ni filters and CuK α radiation, using 30 kV and 20 mA, calibrated with Si, at a 1° min⁻¹ rate. Infrared spectra (IR) of KBr pellets were measured with a Perkin Elmer 1600 FTIR in the 1400-400 cm⁻¹ range, with a reso-

lution of 2 cm^{-1} . The Raman spectra were recorded on a Renishaw System 3000 Raman Imaging Microscope (ca. $1\mu\text{m}$ spatial resolution) using a He-Ne laser (632.8 nm) and 8 mW of power before the entrance optics. Scanning electron microscopy (SEM) was performed by using a JEOL JSM T-300 Microscope. The BET surface areas were determined by N_2 adsorption using a Micromeritics 2360 instrument.

RESULTS AND DISCUSSION

Fig. 1 shows three mass loss steps in the TGA curve of fibers of the dried fruit of *Luffa cylindrica* with total mass loss around 98.5%. The first one is related to the elimination of water, with a mass loss of approximately 7%. The mass losses for the subsequent events are 91.5%, and can be associated with the pyrolysis of the fibrous network of loofa sponge. The thermal decomposition of the fibrous network of loofa sponge is completed at 446°C in the TGA curve, and yields a fine black powder, related to the presence of residual carbon. Thermal decomposition carried out at 600°C for 24 h yields a fine white powder constituted of potassium carbonate (major component) and calcium carbonate, as determined by energy dispersive X-ray fluorescence spectrometry. For this reason, after production of the calcium carbonate or hydroxyapatite on the fibrous network, the pyrolysis of the vegetable template was carried out at 600°C for 24 h.

Fig. 2a and 2b show the very complex macroscopic morphology of *Luffa cylindrica*. Cross-sectional observation of the *Luffa cylindrica*, by SEM (Fig. 2c and 2d), reveals that the microspungy structure is formed by multicellular fibers bonded together with a large lumen and containing small punctuations along the fibers as interconnections (Bal et al. 2004). Fig. 2e and 2f show the high-fidelity calcium carbonate macroscopic replica of *Luffa cylindrica*. The hydroxyapatite replica is similar. The inorganic replicas preserve the microspungy structure, as illustrated in Fig. 2g and 2h. The capacity of replication of the morphology of the loofa

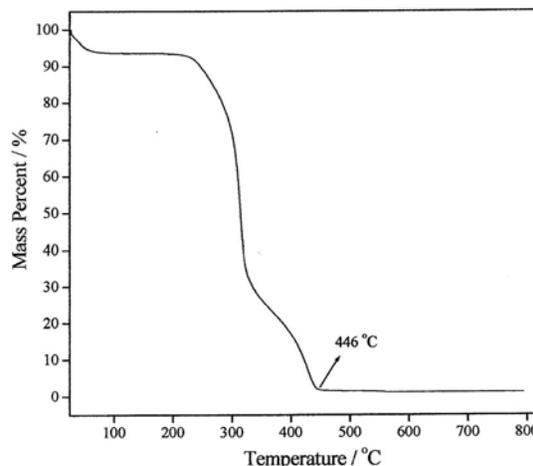


Fig. 1 – Thermogravimetric curve of fibers of the dried fruit of *Luffa cylindrica*.

sponge is due to microspungy fibrous system that offers good accessibility to fluid associated with the high retention capacity of the fibers for aqueous solutions. Both inorganic replicas present a reduction in the volume between 30% and 40% in relation to the original template and they are fragile, although, sufficiently resistant to permit handling.

Powdered samples of calcium carbonate and hydroxyapatite replicas exhibit specific surface areas with $1.10\text{ m}^2\text{ g}^{-1}$ and $2.75\text{ m}^2\text{ g}^{-1}$, respectively. Recently, experiments for fluoride removal from aqueous solutions were carried out with calcium carbonate and hydroxyapatite with specific surface areas of $0.057\text{ m}^2\text{ g}^{-1}$ and $0.052\text{ m}^2\text{ g}^{-1}$, respectively (Fan et al. 2003).

The three common polymorphs of calcium carbonate (CaCO_3) are vaterite, aragonite and calcite, in order of increasing thermodynamic stability. Because of their different crystal structures the three phases can be discriminated using a vibrational technique, i.e. infrared and Raman spectroscopy (Kontoyannis and Vagenas 2000, Dickinson and McGrath 2001, Vagenas et al. 2003). The stronger and more highly resolved bands at $\sim 1085\text{ cm}^{-1}$ are unable to be used in the analysis of the mixed systems due to extensive overlap between the three polymorphs in this region. The Raman active bands

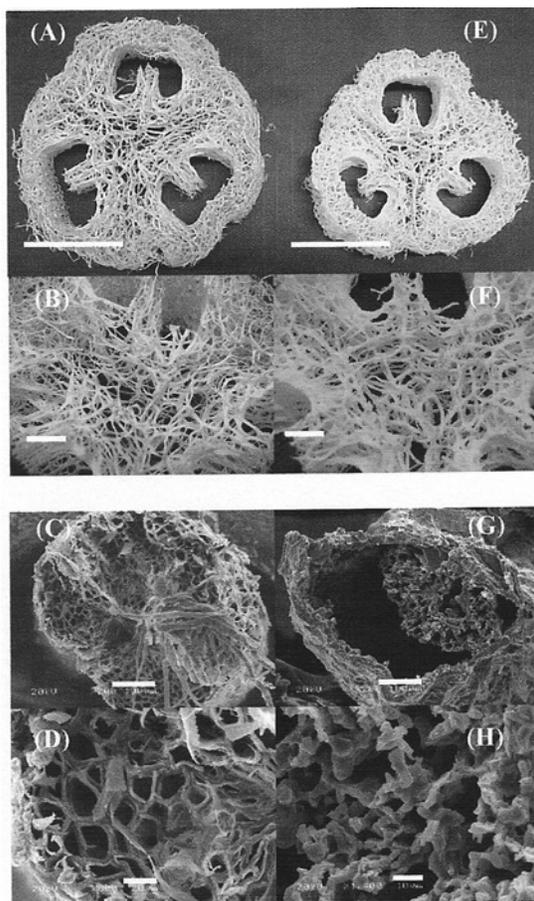


Fig. 2 – Photographs of the fibrous network of the *Luffa cylindrica* fruit (A, B; scales 5 cm, 1 cm). Cross-sectional SEM micrographs of the fibers of the *Luffa cylindrica* (C, D; scales 100 μm , 20 μm). Photographs of the calcium carbonate inorganic replica (E, F; scales 5 cm, 1 cm). Cross-section SEM micrographs of the calcium carbonate inorganic replica (G, H; scales 100 μm , 10 μm).

at 711 cm^{-1} , 700 cm^{-1} and 750 cm^{-1} for calcite, aragonite and vaterite, respectively, were used for quantitative analysis. IR bands chosen for differentiation between polymorphs occur at 713 cm^{-1} for calcite, at 700 and 713 cm^{-1} for aragonite, and at 745 cm^{-1} for vaterite. Analysis by powder X-ray diffraction uses the reflection peak, in 2θ , at 29.5° for calcite, 25.0° for vaterite, and 45.9° for aragonite (Kontoyannis and Vagenas 2000, Dickinson and McGrath 2001). The calcium carbonate replica is

featured by the presence of a Raman band at 708 cm^{-1} (Fig. 3a) and an IR band at 713 cm^{-1} (Fig. 3b), assigned to in-plane bending vibration modes of CO_3^{2-} in the crystalline lattice of calcite. In the XRD pattern (Fig. 4), the presence of a peak at 29.3° (2θ) confirms the occurrence of the calcite polymorph of calcium carbonate.

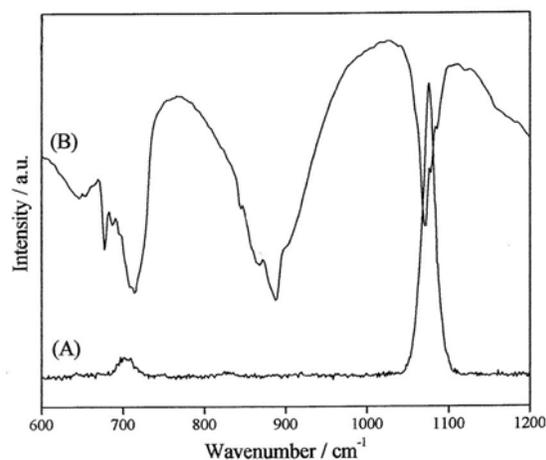


Fig. 3 – (a) Raman and (b) IR spectra of the calcium carbonate inorganic replica.

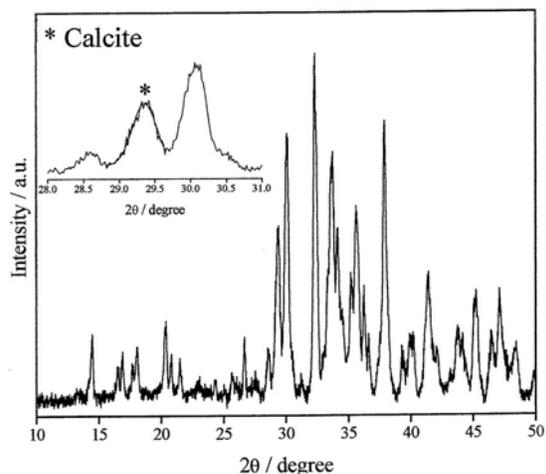


Fig. 4 – Powder XRD patterns of the calcium carbonate inorganic replica.

The Raman spectrum of the calcium phosphate replica (Fig. 5a) exhibits a very strong band at 962 cm^{-1} , unequivocally attributed to the totally

symmetric $\nu_1(\text{PO}_4)\text{A}_1$ stretching mode of 'free' tetrahedral phosphate ion in the hydroxyapatite phase, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$. The Raman bands observed in the frequency regions $400\text{--}460\text{ cm}^{-1}$, $570\text{--}625\text{ cm}^{-1}$, and $1010\text{--}1095\text{ cm}^{-1}$ are assigned, respectively, to ν_2 -, ν_4 - and ν_3 -type internal PO_4^{3-} modes (Aza et al. 1997, Silva and Sombra 2004). The synthesis of hydroxyapatite as a major phase in the calcium phosphate replica is also confirmed by the XRD pattern (Saeri et al. 2003) (Fig. 6). The calcium phosphate replica presents two minor phases: $\beta\text{-Ca}_3(\text{PO}_4)_2$ (identified by XRD in Fig. 6) and $\beta\text{-Ca}_2\text{P}_2\text{O}_7$. The occurrence of this latter phase was confirmed by the presence of the IR band at 725 cm^{-1} (Fig. 5b), which is characteristic of the $\beta\text{-Ca}_2\text{P}_2\text{O}_7$ $\nu(\text{P-O-P})_{\text{sym}}$ mode (Waal and Hutter 1994).

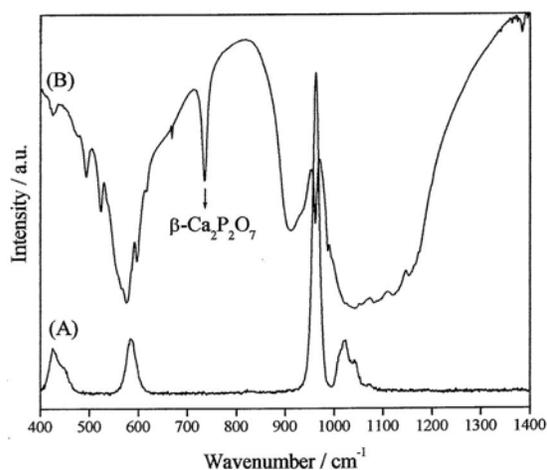


Fig. 5 – (a) Raman and (b) IR spectra of the hydroxyapatite inorganic replica.

The SEM, IR, Raman and XRD results confirm the morphosynthesis of high fidelity inorganic replicas of the fibrous network of the dried fruit of *Luffa cylindrica*. As emphasized previously, the controlled synthesis of inorganic materials with specific morphology is an important aspect in the development of new materials. In this context, the capability of replication of the loofa sponge is consistent with the possible use of biodiversity, with special prominence for Brazilian biodiversity, in the obtain-

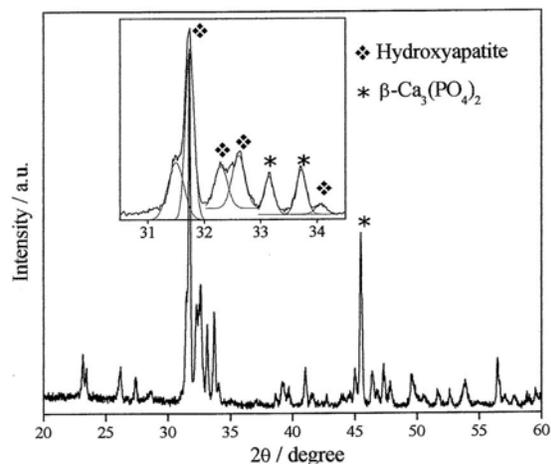


Fig. 6 – Powder XRD patterns of the hydroxyapatite inorganic replica.

ing of new materials. We would like to emphasize that the template proposed in this paper, shows the possibility of preparation of inorganic replicas with desirable sizes, on the centimeter scale. This fact is innovative with regard to inorganic replicas described in the literature, in which the micrometric scale predominates, being limited by the original size of the template.

CONCLUSION

High fidelity calcium carbonate and hydroxyapatite replicas of the fibrous network of the dried fruit of *Luffa cylindrica* are described, utilizing a facile synthetic route and an inexpensive and sustainable resource template. The capacity of replication of the morphology of the loofa sponge is due to its microspungy fibrous system that offers good accessibility to fluid associated with the high retention capacity of the fibers for aqueous solutions. In the context of the morphosynthesis, the capability of replication of the loofa sponge presents the possibility of use of the biodiversity in the obtaining of new materials.

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RESUMO

Réplicas (bio) inorgânicas de carbonato de cálcio e de hidroxiapatita, com elevada fidelidade à morfologia fibrosa do fruto seco da espécie *Luffa cylindrica* são descritas, utilizando uma rota de síntese simples e de baixo custo. A esponja vegetal é um molde macroscópico com arquitetura altamente complexa, de baixo custo e de fonte renovável. Dentro do contexto da morfossíntese, a capacidade de replicação da *Luffa cylindrica* acena com a possibilidade de uso da biodiversidade na obtenção de novos materiais. Enfatizamos que o molde proposto neste trabalho possibilita a preparação de réplicas inorgânicas com o tamanho desejado, em uma escala de centímetros. Este fato é inovador em relação as réplicas inorgânicas descritas na literatura, nas quais predominam a escala micrométrica, limitadas ao tamanho original do molde.

Palavras-chave: morfossíntese, síntese biomimética, morfologia, calcita, hidroxiapatita.

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