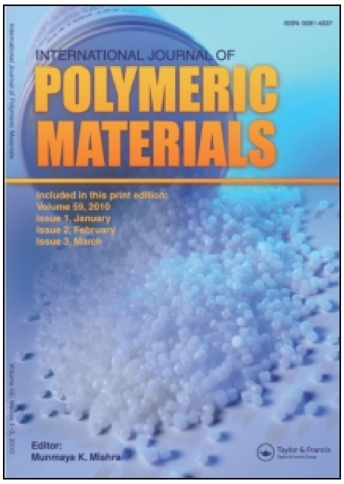

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AFM characterization of chitosan self-assembled films

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AFM CHARACTERIZATION OF CHITOSAN SELF-ASSEMBLED FILMS

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Atomic force microscopy (AFM) was used to examine the surface structure of self-assembled films obtained from water-soluble chitosan rich solution precursor. The film was supported onto functionalized glass slides resulting in a mountain-and-valley structure. This feature is attributed as resulting of the experimental condition where agglomeration of molecules in solution may occur. The film height does not exceed 20 nm with medium pore size of approximately 12 nm. This range of porosity is suitable for nanofiltration applications.

Keywords: AFM, self-assembled, chitosan, thin-film, surface characterization

INTRODUCTION

The self-assembly technique (SA) has been recently used for the fabrication of ultra-thin films and multilayers systems of enzymatic compounds or biopolymers in a controlled fashion [1, 2]. This technique, in concept, refers to a spontaneous attachment that takes place during the contact of molecules dispersed in fluid phase with a solid surface support [3]. In particular macromolecules, such as proteins and polysaccharides are suitable to form films owing to their polar characteristics and charges development in aqueous solution. In SA mechanism, the electrostatic interaction is assumed

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as the driving force to start film assembly on the solid surface. One important feature of SA mechanism is that the process is rather dependent of the solution acidic degree. At specific pHs preferential orientation takes place between the molecules and the solid surface, where the hydrophobic groups will be associated with the surface hydrophilic group and *vice-versa* [4]. That is an important characteristic that can define SA films potential applications.

The water-soluble chitosan (CHI) forms a homogenous solution and is suitable to be used as precursor medium for building thin-films by SA technique. CHI is a natural biopolymer obtained by deacetylation of chitin having broad potential for applications in purification systems, as flocculent agent and for chelation of metal contaminants [5] and in ultrafiltration devices for ionic rinsing in water [6]. CHI in gel form has been also employed in cosmetic formulations and recently used as edible coatings to preserve fruits and vegetables [7]. The purpose of this study is to make use of SA technique to form CHI films on chemically functionalized glass slides. The resulting films morphology was investigated with microscopy of atomic force.

EXPERIMENTAL

We used CHI from Fluka Biochemika (medium molecular weight) and purified as described elsewhere [8]. The slides ($5\,mm \times 10\,mm \times 2\,mm$ optical glass plates) were chemically functionalized by the 'piranha' method [9]. The films were obtained by direct immersion of the treated slides in the CHI solution (10^{-3} M, pH = 3), for three minutes and rinsing in pure water. Three substrates were separately dipped to guarantee test repeatability. After each deposition the samples were allowed to spontaneously dry. AFM images were acquired in non-contact mode (TopoMetrix Discover System) and the images processed by a TOPOSPM software. Random areas of $500\,nm \times 500\,nm$ were scanned. Resulting film thickness was estimated by software analysis on the generated images.

RESULTS AND DISCUSSION

Figure 1 shows the typical topography as found when imaging the glass supporters before solution dipping (a), and after the CHI film formation (b). In Figure 1(c) the drawn box corresponds to a zoomed region presented in (b). The main feature observed in the recorded images is that the surface resulted by CHI deposition, after drying, is comprised by a series of individual clusters that probably are agglomerates of chitosan chains. These are held together to give a film surface with a "mountain-and-valley" structure. Scratches intentionally done indicated stable film structure. Despite image artifacts that could arise from AFM tip scanning, the images

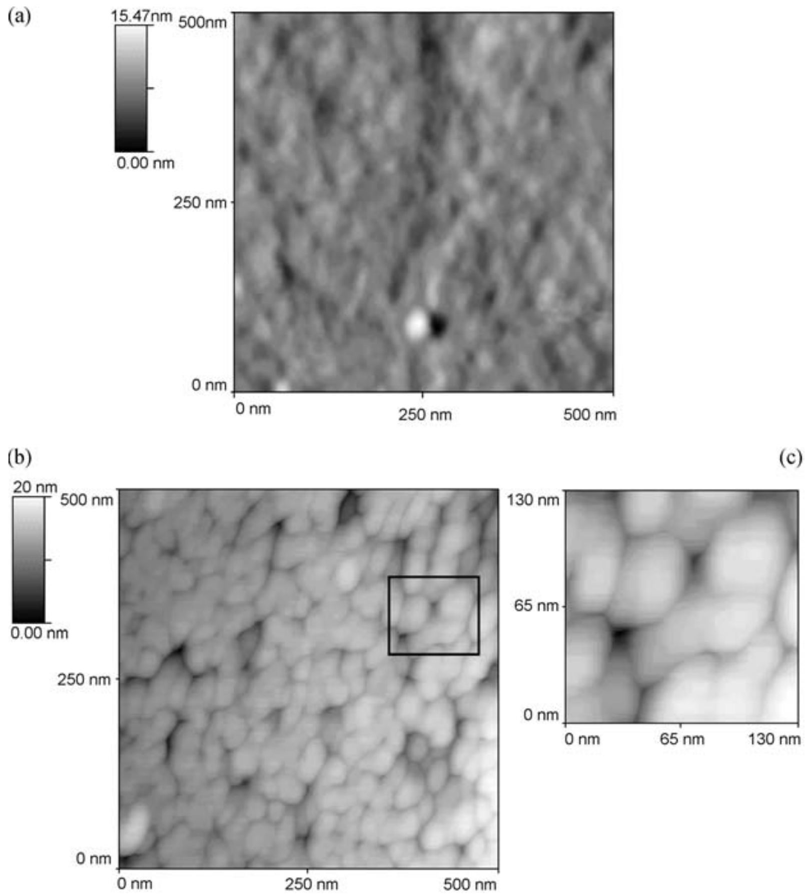

FIGURE 1 AFM recorded images of the surface morphology of a glass slice before dipping (a) and after 10-minutes immersion in CHI solution (b). In (c) a zoomed region where the CHI clusters formation is better visualized.

suggested layer formation by mechanisms as nucleation and coalescence of submonolayers islands. Another possibility is the formation of such clusters by molecules aggregation while they are still in solution. CHI molecules can assume variable configuration at different pHs. CHI is a linear polyelectrolyte and at $pH = 3$ it is expected that due to the intermolecular electrostatic repulsion between adjacent groups in an individual molecule, the polymeric chain assumes a stretch-conformation. These molecules however, are available to interact among similar ones in solution, configuring agglomeration in a defined volume. Since there is an amino group on each glucose ring of CHI structure, at low pH solution, positive charges

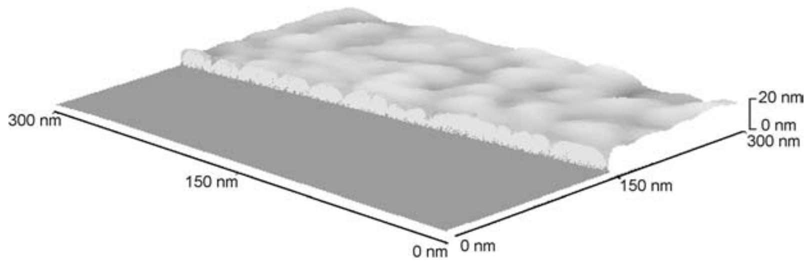

FIGURE 2 Artificial 3D image of CHI film onto glass slide. This allows good estimative of film formation and thickness.

will be predominant, available to be attracted by opposite charge present on the solid surface. CHI behavior at different acidic degrees has been subjected to tests, and low pHs were found to obtain better results in the homogeneities of SA film built up [10]. The clusters in the film are of regular dimensions, on the order of 30 nm diameter, and almost cover the entire surface. The apparent layer thickness does not exceed 20 nm. Artificial cross-section image in the scanned films can be performed, as presented in Figure 2.

Since the film is formed by deposition of individual or molecular agglomeration from aqueous media, isolated islands on the surface and the voids between these features configure a porous structure. The porosity of CHI SA film is very important parameter for devising practical applications. This porous structure is better assessed through cross sectional analyses of the scanned regions. Figure 3 illustrates a region of the film where two large-size pores in film structure are highlighted. The quantitative pore-size measurements were made by cross sections analysis, where 40 lines were taken into consideration. Dark regions in the topographic images identify the pores in the deposition. The depth of the pores appears to be related by the dimensions and deposition characteristics of the isolated clusters. The pores were found to be just slightly shorter than the average clusters diameters, *i.e.*, the pore's depths do not exceed 20 nm high, which can be related to the film thickness. The pore-size distribution obtained from the analyzed curves is plotted in Figure 4. The medium pore size in the dried film was found by means of statistical treatment as being approximately 12 nm.

Artifacts inherent in the AFM scanning system, such as convolution and tips imaging effects evidently should be taken into account for a correct interpretation. Certain criteria were assumed for the present analysis, as considerations introduced by Villarrubia [11] and Tesini *et al.* [12]. Under the experimental conditions adopted in this work, we can affirm that the resultant chitosan film has porosity that corresponds to the range used in

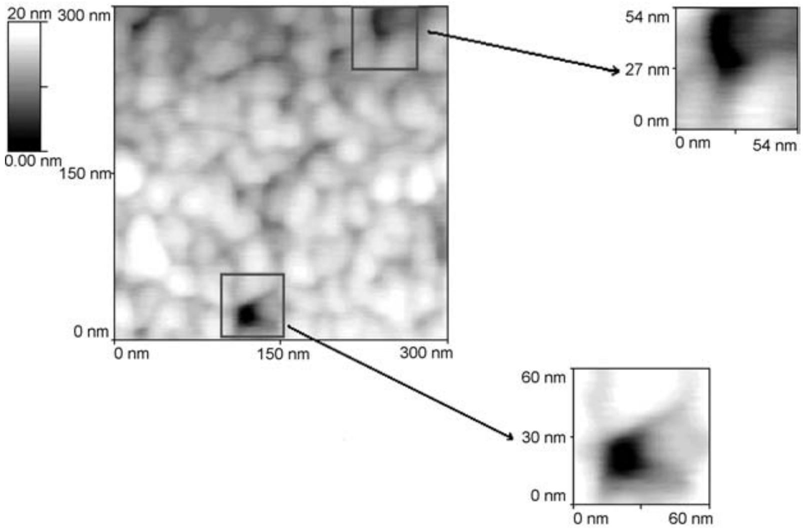

FIGURE 3 Image of film structure on glass slide. Zoomed regions illustrate large-size pores.

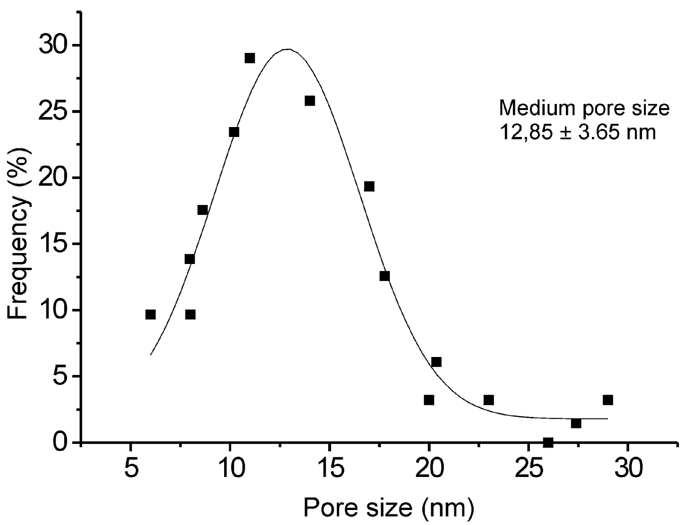

FIGURE 4 Pore-size distribution curve.

nanofiltration applications and allows easy gas permeation. The porosity of the final film is the most important parameter in chitosan protective coatings and it is understood in the process as the result of the deposition mechanism.

CONCLUSION

Self-assembled CHI film is easy to obtain and its final structure can define technological applications. In the process, many other factors such as pH, concentration rates, immersion time, *etc*., will undoubtedly determine the degree of homogeneity of the film. Throughout, the AFM technique presents an advantage for polymeric film characterization in the measurements that covers many orders of magnitude and acquires three-dimensional data. Additionally, the evaluation of the porosity of thin deposited film is not always possible by conventional SEM technique. The necessity of sputtering metallic deposition on biologic surface for electric conduction greatly interferes in the integrity of the biologic film, degrading and/or masking significantly the surface features.

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