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FRACTAL PROPERTIES OF COLLAGEN/CHITOSAN/MONTMORILLONITE MEMBRANES

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The aim of this study was to obtain membranes with improved properties from natural, and biodegradable biopolymers with interstratified montmorillonite nanolayers and to apply the fractal concepts to the image profiles of the biopolymeric composite membranes. Organic–inorganic hybrid membranes were prepared starting from collagen (COL), chitosan (CH) and montmorillonite (MMT) with and without addition of plasticizer (PL). The structure of the hybrid membranes was characterized by X-ray diffraction (XRD), SEM (scanning electron microscopy) and fractal analysis. Based on X-ray diffraction measurements, the basal spacing of the polymer/montmorillonite was much larger than that of the natural silicate as a result of intercalating polymer chains into the gallery spaces. The collagen/chitosan/montmorillonite membranes are designed to develop new bionanocomposites.

INTRODUCTION

Recently, nanocomposites that contain layered clay materials such as montmorillonite having silicate layers of 1 nanometer thickness dispersed within a polymeric matrix, have been developed and characterized.[1-4] Biodegradable polymers and bioactive ceramics are being combined in a variety of composite materials for medical applications as scaffolds for tissue engineering.[5,6] Collagens are one of the most abundant families of proteins in animal tissues. Collagen type I is the most abundant and the majority of collagen materials for biomedical applications are based on this type.[7] Chitosan is a natural carbohydrate polymer prepared by the partial deacetylation of chitin which has been found in a wide range of natural sources such as crustaceans, fungi and insects. It has been used in nanocomposites and has been identified as biocompatible, biodegradable, physiologically inert, antibacterial, etc.[8,9] Among clays used to prepare polymer-layered silicate nanocomposites, montmorillonites attract the most attention mainly due to their natural availability and have been a very promising layered silicate material in the fabrication of nanocomposites. Layered silicates are characterised by a periodic stacking of mineral sheet with a weak interaction between the layers and a strong interaction within the layer. The space between the layers is occupied by cations. By cation exchange reactions between the clay and organic cations the layered silicate can be transformed into organically modified clay.[10] Chitosan, a natural biopolymeric polycation, was used to modify montmorillonite for the adsorption of anions.[11] It was found that the easiest and simplest way to modify the structure of MMT is by intercalating various cations or molecules within the interlayer spacing.[12] The linear, cationic polysaccharide chitosan can be introduced into the galleries of silicate layers. Surface properties of biomaterials are important factors that govern in part their biocompatibility.

The surfaces and interfaces of materials are often complex and detailed in structure. The concept of fractal dimension represents an index of the space-filling property of an irregular structure. The fractal dimensions values may be used as a quantitative measure of the degree of heterogeneity and roughness on the membrane surface. The membrane surfaces cannot be expressed by euclidean geometry, because they do not consist of

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perfectly flat and uniform geometrical objects. The characterization of the smoothness of a surface is based on fractal dimension. For a smooth surface (an ideally flat surface) the fractal dimension is equal to 2. For extremely rough surfaces the fractal dimension is approaching to 3. Fractal geometry has emerged recently as an analytical tool suitable for the description of complex structures, such as those found in most porous objects.[13] Avnir and others reported that the surfaces of most solid substances are fractals and are characterized by nonintegral dimensions with values between 2 and 3: $2 < D < 3$.[14,15] Fractal analysis was applied to the electron microscopic images to quantify the surface microstructure complexity. A great impact of the membranes microstructure (internal and surface arrangement of the different content) on surfaces properties was observed. The complexity of the developed structures showed a self-similarity in the analysed scale. The method mostly used in calculating fractal dimensions in the natural sciences is the so-called box counting dimension.

The objective of this work is the intercalation of the cationic biopolymer chitosan and collagen in natrium montmorillonite, providing compact and robust three-dimensional nanocomposites with interesting properties. In our work, montmorillonite was added into the chitosan and collagen solution with and without addition of plasticizer to prepare the organic/inorganic hybrid membranes. The effect of the plasticizer on the morphology of the organic/inorganic hybrid membrane was investigated.

RESULTS AND DISCUSSION

Morphology and properties of chitosan/ collagen, chitosan and collagen nanocomposites with and without plasticizer have been studied and compared. Scanning electron microscopy, energy dispersive spectrometry, X-Ray diffraction analysis and fractal analysis are used to investigate the changes in morphology and structure of the membranes prepared from biopolymers and silicate.

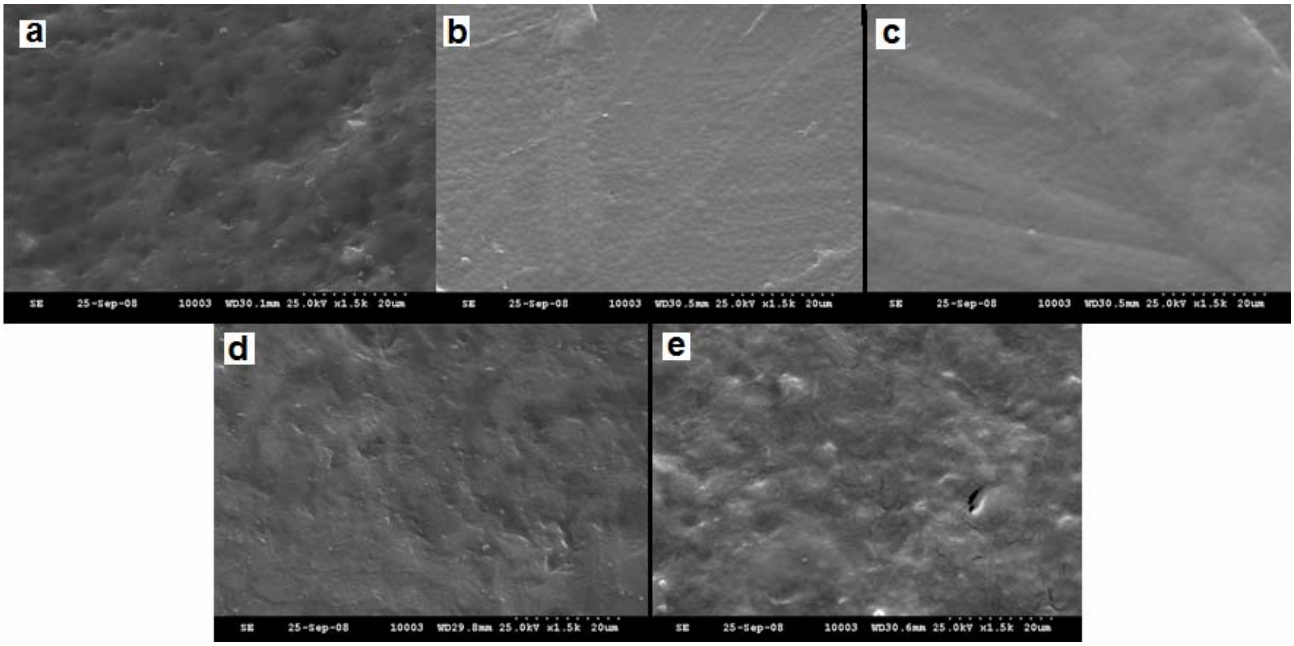

Fig. 1 – SEM micrographs of the COL/CH/MMT/PL (a), COL/CH/MMT (b), CH/MMT/PL (c), CH/MMT (d), COL/MMT/PL(e).

Scanning electron microscopy (SEM) coupled with energy dispersive X-ray analysis has been used in studying the membrane surface morphologically and examining the global chemical composition of the membranes. SEM images of cross-sections demonstrate local uniformity and the homogeneous distribution of MMT particles on membrane surface was observed (figure 1). The biopolymer chains diffuse from the bulk polymer melt into the galleries between the silicate layers.

The X-ray diffraction (XRD) is a powerful tool for identifying crystalline structures on the nanoscale. The intercalation of the polymer into clay layers was confirmed with wide-angle X-ray diffractometry (Figure 2).

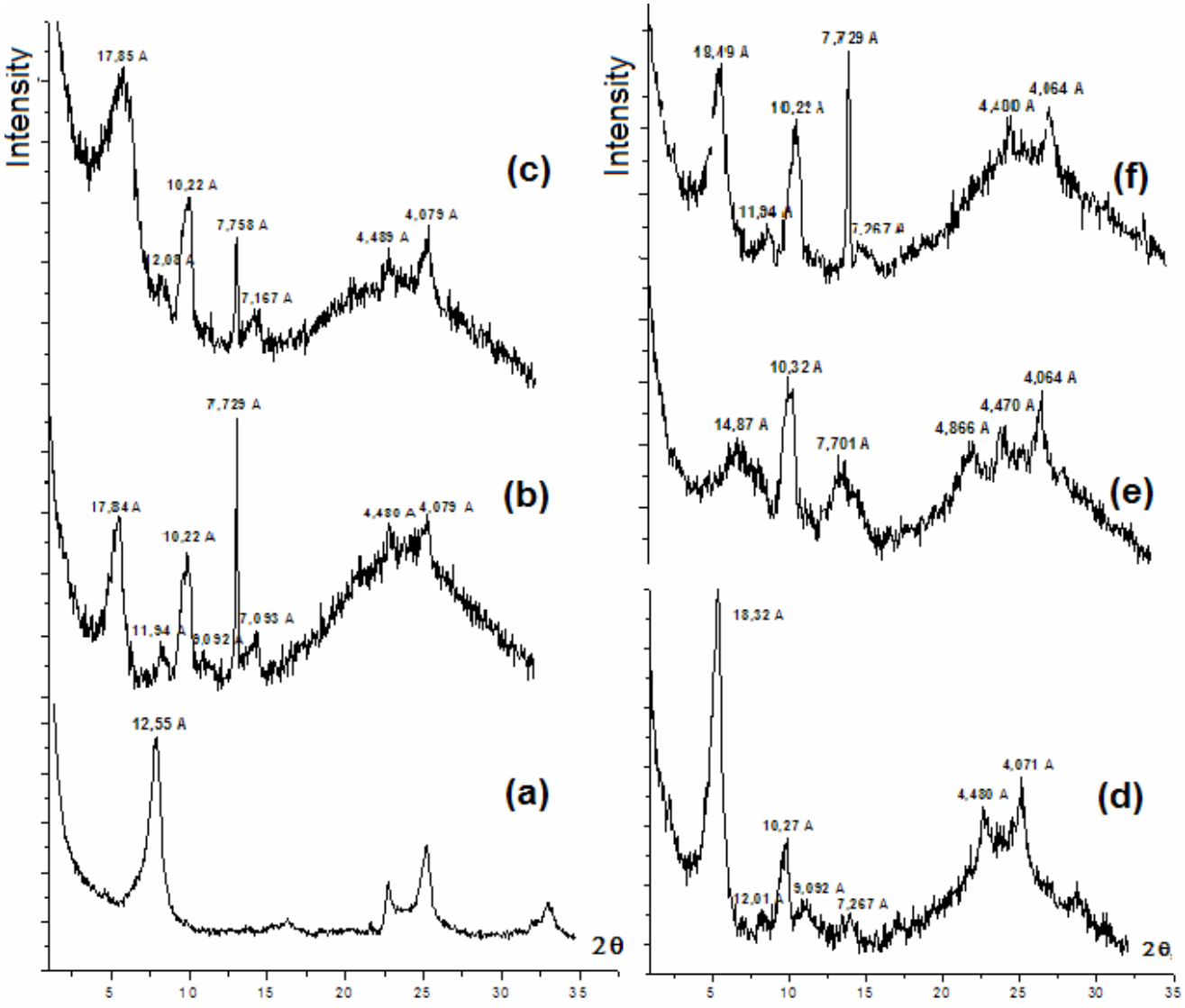

Fig. 2 – The XRD diffractogram of MMT (a); COL/CH/MMT/PL (b); COL/CH/MMT (c); CH/MMT/PL (d); CH/MMT (e); COL/MMT/PL (f).

The changes of the interlayer distance in different nanocomposites were estimated from XRD by monitoring the position, shape and intensity of the basal reflections corresponding to the silicate layers. The intercalated nanocomposites are obtained when the diffraction peak is shifted toward small angles. The exfoliated nanocomposites are obtained when the XRD diffractograms do not contain anymore the diffraction peak of the silicates. The existence of the first peak explains the intercalation of the polymer between the gallery spaces to form nanohybrids. The increase in d-spacing of MMT implies the intercalation of grafted chitosan and collagen biopolymer chains into silicate galleries. The X-ray diffractograms exhibited the larger interlayer spacing of MMT in the biopolymer/MMT composite membranes in comparison to MMT. It is significant to observe that the increase in the intensity of the original main peak of the nanoclay and the increase of d-spacing indicate the formation of nanocomposite structure with intercalation of biopolymer in the gallery of the silicate layers of MMT. In the X-ray diffraction pattern for the MMT, the 2θ value was 7.55° (Figure 2.a), which corresponded to a basal space of 12.55 Å according to the Bragg equation. The XRD patterns showed an increase in the basal spacing from 12.55 Å to 17.84 Å for COL/CH/MMT/PL (figure 2.b), to 17.85 Å for COL/CH/MMT (figure 2.c), and shifting the diffraction peak toward lower values (about of 2θ = 5°). For the modified silicate prepared with chitosan, in the interlayer region, an increase in the

MMT basal spacing from 12.55 Å to 18.32 Å for CH/MMT/PL (figure 2.d) and 14.87 Å for CH/MMT (figure 2.e) was observed. The experimental results indicated that the presence of the plasticizer was in favor of the biopolymer intercalation. The left shift of the XRD curve indicated that more polymers (and/or plasticizers) entered the clay gallery and the clay platelets were forced further apart (the basal interlayer spacing increases). XRD showed an increase in the basal spacing from 12.55 Å to 18.49 Å for COL/MMT/PL (figure 2.f).

In the present study, the X-ray diffraction studies reveal the formation of intercalated and disordered intercalated nanostructure. Figure 3 shows a schematic structure of unmodified clay (MMT) and polymer-intercalated MMT when the basal spacing increases. The intercalation of the polymer chains increases the interlayer spacing of the clay over that of pure clay, shifting the diffraction peak toward lower values of 2θ (figure 2).

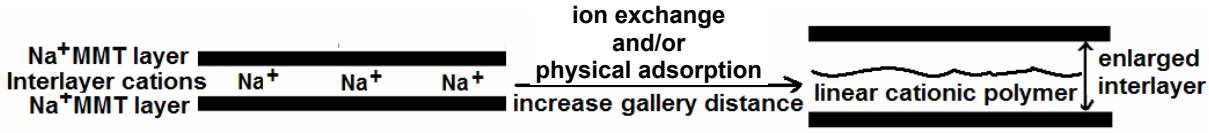

Fig. 3 – Schematic structure of unmodified clay (MMT) and polymer-intercalated MMT.

The SEM images were used for the computation of dimensions after conversion to a bitmap image file. Fractal dimension was applied to study the cross-sections of surface roughness. Fractal dimension is a measurement of disorder, corresponding to the degree of irregularity and complexity of the space-filling capacity. A perfectly smooth surface has a low fractal dimension value while a highly rough, disordered surface has a high fractal dimension. Fractal dimension was calculated with the method of box-counting of grayscale image after pre-processing, using the fractal dimension calculator software for the digitized images of the surface of the five membranes. Hence the molecular structure here is somewhat more "open", or less space filling, than a uniform solid. The fractal dimension for these five materials calculated from gray-scale image analysis of the polymer/silicate membranes are all in the range of 2.29 to 2.39. This implies that the material is not space filling. Hence the biopolymer/silicate structure is slightly "open" compared to a uniform, three–dimensional solid (which would correspond to a fractal dimension of 3.0). Figure 4 indicates that formulations no. 1 and no. 3 have a smoother surface with fewer irregularities while formulation no. 2, no. 4 and no. 5 have a more irregular surface. The addition of a plasticizer decreases the fractal dimension. The lowest values for formulations no. 1 and no. 3 (samples with plasticizer) confirm the SEM observation and XRD patterns, and suggest a lower degree of irregularity than that appreciated by direct vision.

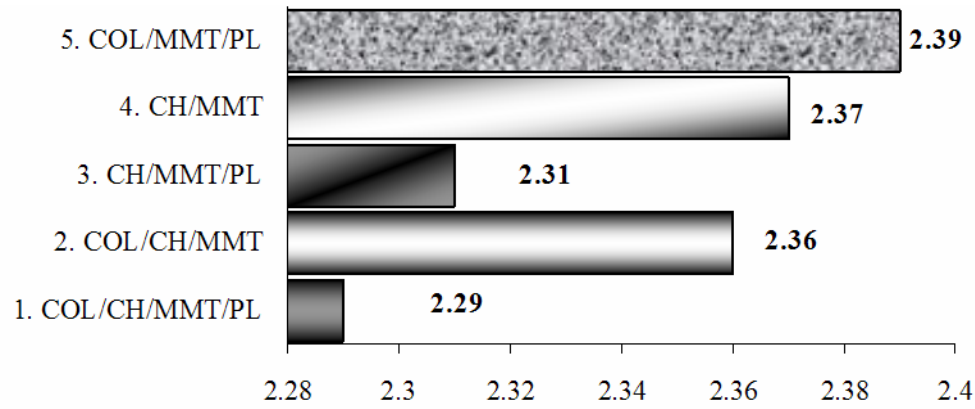

Fig. 4 – The fractal dimensions (D) of the surface membranes: 1. COL/CH/MMT/PL; 2. COL/CH/MMT; 3. CH/MMT/PL; 4. CH/MMT; 5. COL/MMT/PL (calculated by fractal analysis from SEM micrographs).

EXPERIMENTAL

Chitosan (a natural polymer derived from chitin with a degree of deacetylation of 85%) was obtained from Aldrich. Acetic acid is Merck reagent. Gel of collagen with native structure, extracted from bovine skin, with molecular weight 300.000 Da at pH 2.6 was obtained from National Institute of Research and Development for Textile and Leather, Bucharest, Roumania.[16] Montmorillonite was obtained by bentonite purification (Bentonite of Valea Chioarului-Romania). Collagen, chitosan and collagen/chitosan membranes were prepared by lyophilization with silicate. A series of five different membranes were successfully prepared: 1. COL:CH:MMT with weight ratios at 2:2:1 with plasticizer, 1%, by weight relative to the total weight of the composition; 2. COL:CH:MMT with weight ratios at 2:2:1 without plasticizer; 3. CH:MMT with weight ratios at 4:1 with plasticizer, 1%, by weight relative to the total weight of the composition; 4. CH:MMT with weight ratios at 4:1 without plasticizer; 5. COL:MMT with weight ratios at 4:1 with plasticizer, 1%, by weight relative to the total weight of the composition. The obtained membranes were coated with gold for increased conductivity and viewed in high vacuum under a Scanning electron microscope Hitachi S-2600 N. The major elements were detected by energy dispersive spectrometry (EDS), coupled to the SEM operating at 20 keV, using a Si(Li) detector with a thin beryllium window. X-Ray diffraction quantitative analysis (XRD) was performed using a diffractometer DRON-2. Samples were scanned in the range of diffraction angle 2θ =1 - 35°. The interbasal spacing was calculated based on the Bragg law ($n\lambda = 2d \sin\theta$, where n is an integer; λ is the wavelength of X-ray; d is the spacing between the planes in the atomic lattice; and θ is the angle between the incident ray and the scattering planes). Using digitized images of the surface of the membranes, fractal dimensions were obtained with a fractal analysis software. The box-counting method was used for the fractal analysis of grayscale images using the software equipment to perform fractal analysis of digitised images written by Sasaki.[17]

CONCLUSIONS

In this work, the importance of fractal structure concept in physical characterization of biomaterials was pointed out. Fractal geometry, a vocabulary of irregular shapes, can be useful in a quantitative description of the biomaterial surface architecture and can become a useful tool for analyzing morphological formation. By adding small amounts of plasticizer a decrease of the fractal dimension was observed in the membranes prepared from chitosan, collagen, and montmorillonite, which indicates that the surface is smoother and more regular. The values between 2.29 and 2.39 of the fractal dimension are in accordance with the experimental XRD data. The calculation of fractal dimension for surface microgeometry offers a great opportunity for the characterization of microscopic images transformed in pixels matrix. New methods of investigation for membrane surfaces are necessary in future in order to understand how the roughness of these organic-inorganic materials is related to the fractal dimension.

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