ANALELE UNIVERSITĂŢII BUCUREŞTI
Department of Physical Chemistry
4-12 Regina Elisabeta Blvd, District 3, Bucharest
phone: +40-21-3143508; fax: +40-21-3159249
ISSN: 1844-0401

ARS DOCENDI PUBLISHING HOUSE
Sos. Panduri 90, District 5 Bucharest
phone/fax: +40-21-4102575
e-mail: arsdocendi@yahoo.com

USING FRACTAL ANALYSIS TO DESCRIBE COLLAGEN-CHITOSAN MATRICES

Viorica Stănescu, Mihaela Olteanu, Manuela Florea-Spiroiu * and M. Rusu **

abstract: Hydrogels of collagen-chitosan biopolymers have been prepared with different ratio between components. Matrices have been obtained by liophilization from chitosan and collagen hydrogels. The aim of this study is the fractal characterization of biopolymer matrices. The concept of fractal dimension represents an index of the space-filling property of an irregular structure. The fractal dimensions values provide a quantitative measure of the degree of heterogenity on the membrane surface. Modification of collagen matrix with chitosan decrease the fractal dimension, D, as was calculated from Scanning Electron Microscopy (SEM)-image analysis which show a more uniform porous structure

key words: fractal dimension, collagen, chitosan, Scanning Electron Microscopy (SEM).

received: November 17, 2008 *accepted*: November 24, 2008

1. Introduction

A majority of drug delivery systems using natural polymers have been based on proteins (e.g., collagen, gelatin, and albumin) and polysaccharides (e.g., starch, dextran, hyaluronic acid, and chitosan) [1]. Hydrogels from natural polymers, especially polysaccharides have been widely used because of their advantageous properties such as nontoxicity, biocompatibility, and biodegradability [2]. Among these polymers, chitosan, the N-deacetylated product of the polysaccharide chitin, has gained considerable attention. Particularly, chitosan has many properties that have generated interest in its use such as biodegradability, biocompatibility and nontoxic nature [3].

Surface properties of a biomaterial are important factors that govern in part its biocompatibility [4]. Natural and artificial surfaces are often complex and detailed in structure. The properties of based collagen materials are usually modified by organic and inorganic additives [5].

These types of surfaces cannot be expressed by euclidean geometry, because they do not consist of perfectly shaped pyramids or straight lines. Surfaces of most materials, including

* University of Bucharest, Faculty of Chemistry, Physical Chemistry Department, 4-12 Regina Elisabeta blvd., 030018, Bucharest, Romania; *corresponding author e-mail*: oltmi@gw-chimie.math.unibuc.ro
** University of Bucharest, Faculty of Physics, Str. Fizicienilor nr. 1, Magurele, Romania

natural and synthetic, porous and non-porous, amorphous and crystalline are fractal and one way of characterizing surface morphology is by using fractal geometry. Avnir et al. reported that surfaces of most solid substances are fractals and are characterized by nonintegral dimensions with values between 2 and 3 [6]. Characterizing the smoothness of surfaces is based on fractal dimension. For a smooth surface (an ideally flat surface) the fractal dimension is 2. For extremely rough surfaces the fractal dimension approaching to 3.

The fractal property is a physical property expressed at the supermolecular level, at a microscopic scale and at a macroscopic scale. Fractal geometry has been used to characterize the surface structure, characteristics and irregularities of solid materials. Fractal dimensions (D) are numbers used to quantify these properties. Fractal geometry has emerged recently as an analytical tool which is suitable for the description of complex structures, such as those which are found in most porous objects [7]. It has been used to embellish the morphology of highly irregular objects imbedded onto two and three-dimensional space and is defined as two and three-dimensional fractal dimensions [8].

There are several different concepts of the fractal dimension of a geometrical configuration. The most famous method of calculating fractal dimensions in the natural sciences is the so-called box counting dimension. [9]. A basic principle to estimate fractal dimension is based on the concept of self-similarity. The property of self-similarity implies that the form of an object is invariant with respect to scale. In other words, a strictly self-similar object can be thought of as being constructed of an infinite number of copies of itself. The fractal dimension D of a bounded set A in Euclidean n-space, using box counting of a set A in a grey image is given by:

$$D = \lim_{n \to \infty} \frac{\log(N_r)}{\log(1/r)} \qquad (1)$$

where N_r denotes the number of boxes of side length $r = 2^n$, $n = 1, 2, 3, 4 \ldots$ (number of distinct copies of A in the scale). The box-counting method is the most desirable method for image fractal dimension estimation because it can apply fractal patterns with or without self-similarity.

The purpose of this work is the study of fractal properties of new collagen-chitosan matrices for generating new applications in medicine.

2. Experimental

Chitosan (a natural polymer derived from chitin with a degree of deacetylation of 85%) was obtained from Fluka. Gel of collagen with native structure, extracted from bovine skin, with molecular weight 300.000 Da at *p*H 2.6 was obtained from National Institute of Research and Development for Textile and Leather, Bucharest, Romania using an original method. Acetic acid is Merck reagent.

Gel of collagen was dilluted and chitosan was disolved in 5% solution of acetic acid in order to obtain 1% biopolymer gels. Collagen, chitosan and collagen/chitosan matrices were prepared by lyophilization.

The obtained membranes were coated with gold and viewed in high vacuum under a SEM (Hitachi S-2600 N).

Using digitized images of the surface of the matrices which have been prepared with different ratio between components, fractal dimensions were measured 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 H. Sasaki [10].

3. Results and discussion

Various proportions of collagen/chitosan matrices (from 1:0 to 0:1) were prepared and evaluated. Collagen/chitosan porous matrices were prepared by lyophilization and morphology observed by SEM (Fig. 1).

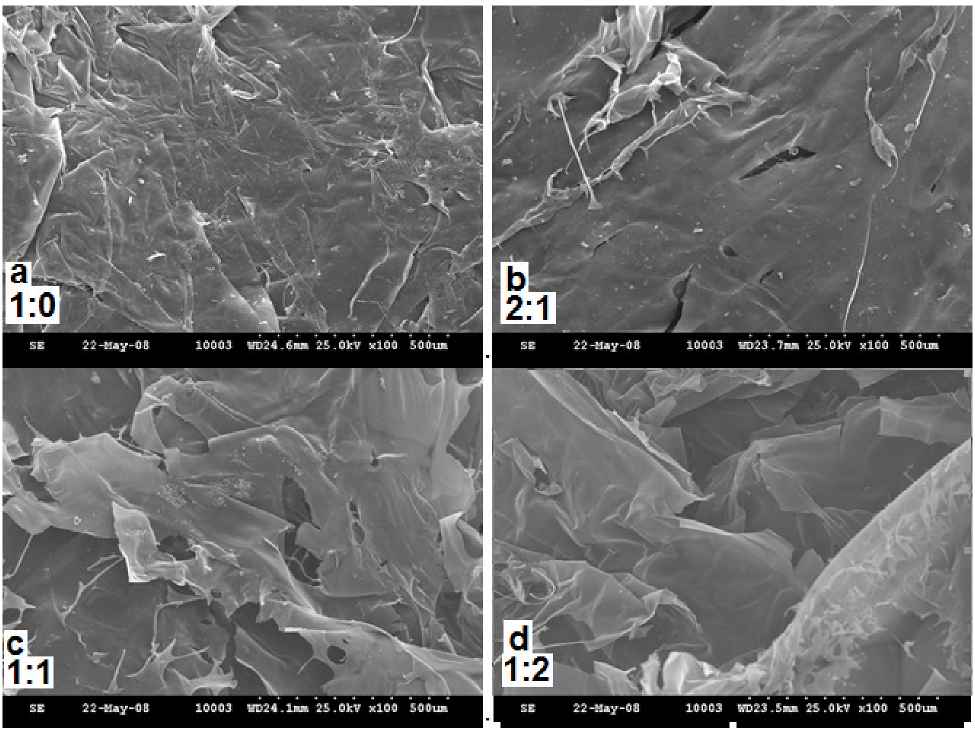

Fig. 1 *Views by SEM* a) collagen:chitosan (1:0); b) collagen:chitosan (2:1); c) collagen:chitosan (1:1); d) collagen:chitosan (1:2)

SEM micrographs indicated good homogeneity between these two materials. The surface roughness a biomaterial can be visualized by SEM analysis of the surface of the samples highlighted the presence of superficial irregularities and protuberances which confer a certain porosity to the membranes.

Prepared chitosan/collagen membranes have uniform porous structure whence can be determinate from the fractal analysis because fractal dimension is a measure of the space-filling property of an irregular object or a fractal pattern. Fractal analysis based on micrographs electronic microscopy SEM showed nanostructure features with self-similar properties. Fractal dimensions calculated from SEM-image analysis represented realistic values from approximately 2.39 to 2.59 (Fig. 2). A decrease in fractal dimension with increase in chitosan concentration is expected and observed [11]. The D values of the surface of biopolymeric matrices indicate that chitosan determines the porosity of mixed matrices.

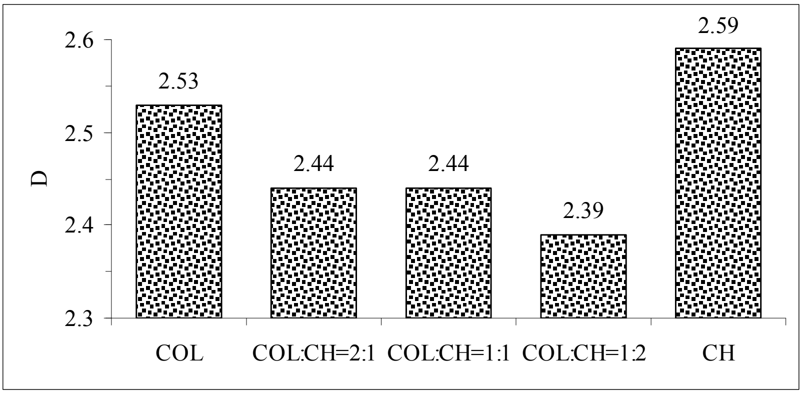

Fig. 2 *The fractal dimensions (D) of the collagen (COL), chitosan (CH) and collagen/chitosan surface matrices calculated by fractal analysis from SEM micrographs.*

The fractal dimension decreases when chitosan is added to collagen matrix, revealing a synergetic effect, not an additive one. The synergetic effect can be determined by electrostatic interactions between positive amino groups of chitosan and negative charge of collagen which diminish the roughness and the porosity of the matrices. For three-dimensional objects with fractal surfaces, the obtained results confirm that fractal dimension is between 2 and 3.

4. Conclusions

The new matrices prepared from chitosan and collagen at different ratio were characterized by fractal geometry from SEM images. The porosity of collagen membrane can be controlled by chitosan content.

The fractal properties of these matrices were found to be mainly dependent on the collagen/chitosan ratio presenting a maximum synergetic effect at a 1:2 collagen/chitosan ratio.

The calculation of fractal dimension for surface microgeometry offers a great opportunity for characterization of microscopic images transformed in pixels matrix.

REFERENCES

1. Park, J.H., Ye, M. and Park, K. (2005) *Molecules* **10**, 146-61.
2. Peppas, N.A. (1997) Hydrogels and drug delivery, *J. Microbiol. Methods* **2,** 531-37.
3. Varma, A.J., Deshpande, S.V. and Kennedy, J.F. (2004) *Carbohydr. Polym.* **55**, 77-93.
4. Chappard, D., Degasne, I., Huré, G., Legrand, E., Audran, M. and Baslé, M.F. (2003) *Biomat.* **24**(8), 1399-407.
5. Olteanu, M., Achimescu, D., Vuluga, Z. and Trandafir, V. (2008) *Rev. Roum. Chim.* **53**(2), 157–63.
6. Avnir, D., Farin D. and Pfeifer P. (1985) *J. Colloid Interface Sci.* **103**(1), 112-23.
7. Rouquerol, J., Avnir, D., Fairbridge, C.W., Everett, D.H., Haynes, J.H., Pernicone, N., Ramsay, J.D.F., Sing, K.S.W. and Unger, K.K. (1994) *Pure & Appl. Chem.* **66**(8), 1739-58.
8. Chang, Y., Liu, Q. and Zhang, J. (2005) *J Zhejiang Univ SCI.* **6B**(10), 1038-44.
9. Narine, S.S. and Marangoni, A.G. (1999) *Food Res. Int*. **32**, 227-48.
10. Sasaki, H. http://cse.naro.affrc.go.jp/sasaki/fractal /fractal-e.html.
11. Stănescu, V., Olteanu, M., Florea-Spiroiu, M., Vuluga Z. and Trandafir, V. (2008) *Proceedings of International Conference of Physical Chemistry*, Sept. 3 - 5, Bucharest, Romania, 117.