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THE INVESTIGATION OF POROUS BIOMATERIALS THROUGH FRACTAL GEOMETRY METHODS

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Rezumat. *În ultimii ani, rezoluţia înaltă a microscopiei electronice şi analiza fractală s-au dovedit a fi un mijloc important pentru analiza structurii biomaterialelor. Scopul cercetării noastre este caracterizarea fractală a unor membrane de biopolimeri din colagen şi chitosan cu diferite rapoarte între componente. Pentru evaluarea imaginilor obţinute prin microscopie electronică de baleiaj am aplicat analiza fractală. Dimensiunile fractale au fost estimate prin metoda "numărării cuburilor" (box counting). Rezultatele obţinute indică faptul că membranele de biopolimer sunt fractali şi sunt caracterizate de dimensiuni fracţionare cu valori între 2 şi 3 (2 < D < 3).*

Abstract. *In recent years, high resolution electron microscopy and fractal analysis has proved to be an important tool for analysing the structure of biomaterials. The purpose of our research is the fractal characterization of biopolymer membranes with collagen and chitosan with different ratio between components. For the evaluation of the Scanning Electron Microscopy (SEM) images we applied fractal analysis. Fractal dimensions were estimated by the box counting technique. The results indicated that the biopolymer membranes are fractals and are characterized by non-integer dimensions with values between 2 and 3 (2 < D < 3).*

Keywords: Fractal dimension, Image analysis, Scanning Electron Microscopy (SEM), biomaterials

1. Introduction

New membranes based on collagen and chitosan biopolymers hydrogels doped with silver nanoparticles were fabricated and characterized to be used for medical applications. The new membranes were characterized by Scanning Electronic Microscopy (SEM) and fractal analysis. After Mandelbrot [1] a mathematical fractal is an object which has a very fragmented or irregular shape, presents self-similarity (or self-affinity), and presents scale-invariance. The familiar Euclidean dimension of the physical world is expressed as an integer such as "2" in 2-dimensional space for a flat surface or "3", for 3-dimensional space, geometrical solid object. We expect that for our collagen materials the fractal dimension D to be between 2 and 3. Based on fractal dimensional analysis, the morphological properties such as aggregate porosity or density can be correlated to fractal characteristics, which can be determined using image analysis. The value of the fractal dimension reflects the degree of irregularity of membrane pores. Different values of D show different types of membrane pores and perhaps their different formation mechanisms. The results obtained are useful for the investigation of the

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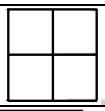
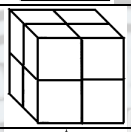
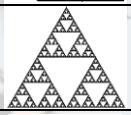
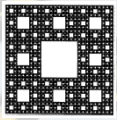
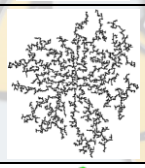
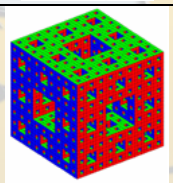
relationship between membrane function and morphology, and for the study of the mechanisms of membrane pore formation. In Euclidean geometry, dimensions are integers or whole numbers (e.g., 0 for the point, 1 for lines, 2 for areas and 3 for volumes). The fractal dimension, denoted as D, is a central construct of fractal geometry. It is called fractal dimension because it is a fractional (or non-integer) number. Any fractal structure can have any value between 0 and the Euclidean dimension in which the object is embedded. The Menger sponge provides an illustrative example of a three-dimensional fractal shape with a dimension between 2 and 3. The Menger sponge is a ideal fractal embedded in 3-dimensional space and is a porous body with a fractal surface area.

The Menger Sponge has been used to model the porous structure. Conner and Bennett have determined the experimental data for the characterization of pore structure and compare these data to the properties of idealized fractals (a Menger sponge, an idealized three-dimensional fractal) [2]. The Menger sponge is the three-dimensional analogous of the two-dimensional Sierpinski carpet. Some common examples of the Euclidean and fractal objects and their fractal dimension are presented in the Table 1. The fractals theory has been increasingly applied in the field of materials science and engineering. Surface properties of a biomaterial are important factors that govern in part its biocompatibility [3]. Natural and artificial surfaces are often complex and detailed in structure. These types of surfaces cannot be expressed by Euclidean geometry, because they do not consist of perfectly regular Euclidean shapes. Surfaces of most materials, including natural and synthetic, porous and non-porous, amorphous and crystalline are fractal on a molecular scale and are characterized by non-integer dimensions with values between 2 and 3: $2 < D < 3$ [4] [5]. Characterizing the smoothness of surfaces is based on fractal dimension. A higher fractal dimension indicates a more compact structure. The fractal property is a physical property expressed at the molecular 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 [6]. Because fractal dimension is a measure of the space-filling property of an irregular object or a fractal pattern, it can be used as a quantitative measure of the spatial pattern heterogeneity. It has been used to embellish the morphology of highly irregular objects imbedded onto two and three-dimensional space and is defined here as two and three-dimensional fractal dimensions [7]. The calculation results for various bi- and multi-disperse porous solids have demonstrated that the scale-dependent nature of the surface fractal dimension is ubiquitous [8].

Table 1. Geometrical and fractal dimension

Name (object)	Illustration	(exact value)	(value)
Line		$\frac{\log(2)^1}{\log(2)}$	1
Square		$\frac{\log(2)^2}{\log(2)}$	2
Cube		$\frac{\log(2)^3}{\log(2)}$	3
Sierpinski triangle		$\frac{\log(3)}{\log(2)}$	D ≈ 1.58
Sierpinski carpet		$\frac{\log(8)}{\log(3)}$	D ≈ 1.89
Diffusion Reorganized Aggregation (DRA) model [9]. (Sapoval, 2005)			D ≈ 175
Menger sponge		$\frac{\ln(20)}{\ln(3)}$	D ≈ 2.73

Membrane physical and physicochemical properties are dependent on features such as particle-size distribution, structural order and shape and fabric. These properties determine the potential applications as a biological and medical material. The specific properties of biomaterials based on collagen, both in terms of physical and chemical characteristics, have a direct impact on cellular adhesion, spreading, and proliferation rates, and ultimately on the rate and extent of new extra cellular matrix formation *in vitro* or *in vivo* [10]. The formation of pores at the surface of the collagen/chitosan matrices is a result of complex mixture of these two materials. The porosity of the manufactured solid is subject to change and depends on its composition and the processing technique. The porosity is controlled by a range of methods and the resulting surface structures can be investigated by microscopy and analyzed using fractal methods. A comparison of the fractal dimension of a membrane prepared at different ratios of collagen/chitosan revealed a possible correlation between the physical behaviour of these surfaces and their surface geometry. Fractal geometry has been applied to

such diverse fields as material science, meteorology, ecology, urban landscapes, economics and finance, soil sciences, and medical imaging. Papadopoulos and co. [11] working at the pore–particle scale, describe the use of the slit island method with image analysis to calculate fractal dimensions of soil pore perimeters.

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: $D = \lim_{n\to\infty} \frac{\log(N_r)}{\log(1/r)}$, where N_r denotes the number of boxes of side length $r = 2^n$, $n = 1, 2, 3, 4...$ (number of distinct copies of A in the scale).

2. Experimental details

New matrices based on collagen and chitosan biopolymers hydrogels doped with silver nanoparticles were obtained and characterized. Chitosan (a natural polymer derived from chitin with a degree of deacetylation of 85%) and collagen have been used for the preparation of the membrane. Gel of collagen with native structure, extracted from bovine skin, at pH 2.6 was obtained from National Institute of Research and Development for Textile and Leather, Bucharest, Romania. Collagen/Chitosan membranes were doped with silver nanoparticles and prepared by lyophilization. Both chitosan and silver nanoparticles have been added to collagen membranes and matrices to achieve a better antibacterial activity. The fabricated membrane were coated with gold and viewed in high vacuum under an SEM (Hitachi S-2600 N). Using digitized SEM images of the membrane surface of collagen/chitosan, fractal dimensions were measured. The box-counting method was used for the fractal analysis of grayscale images using the fractal analysis software written by H. Sasaki [12].

3. Results

Various proportions of collagen/chitosan mixtures were prepared to form sponges membranes and evaluated. Collagen/chitosan porous membranes were prepared by lyophilization and morphology observed by Scanning Electron Microscopy (SEM) (Figure 1). SEM analyses of these collagen membranes indicate that a microporous architecture was created by modulating the processing of the membrane. SEM micrographs also indicated good homogeneity between these two materials. SEM analysis of the surface of the samples highlighted the presence of superficial irregularities and protuberances which confer certain porosity to the matrices. Fractal analysis based on micrographs electronic

microscopy SEM showed nanostructure features with self-similar properties on a large scale. Fractal dimensions calculated from SEM image analysis represented realistic values from approximately 2.50 to 2.38. A decrease in fractal dimension with increase in chitosan concentration is expected and observed. Using digitized images of the surface of the three membranes which have been prepared with different ratio between components, fractal dimension was calculated by the method of box-counting of grayscale with fractal analysis software. The fractal nature of the surface was revealed for this membranes and was obtained fractal dimensions of 2.50 for collagen: chitosan (1:0); 2.39 for collagen: chitosan (2:1); 2.38 for collagen: chitosan (1:1). Modification collagen matrix with chitosan decreases the fractal dimensionality revealing change complexity of hybrid material surface.

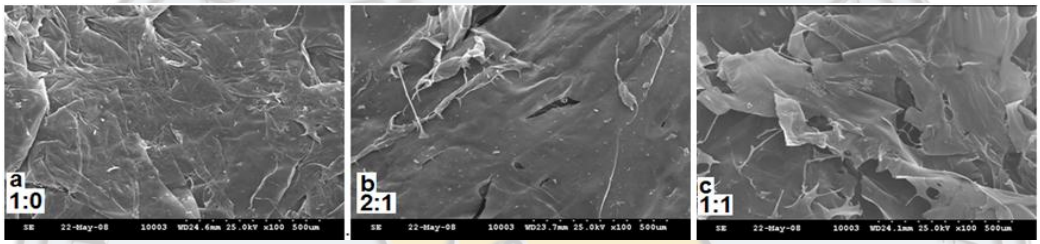


Figure 1. Views by SEM
a) collagen: chitosan (1:0); b) collagen: chitosan (2:1); c) collagen: chitosan (1:1).

We have seen modification of the fractal dimension of the TEM images of silver nanoparticles prepared by chemical reduction with sodium citrate using different $AgNO_3$ concentrations which control the aggregation processes. At the beginning an increase in $AgNO_3$ from 1×10^{-3} M $AgNO_3$ to 3×10^{-3} M $AgNO_3$ increased fractal dimensions from 2.59 to 2.70 corresponding to the presence aggregates of silver nanoparticles. The fractal dimension increases with the size and the complexity of the aggregate having a more compact morphology. Values approaching 3 for a three dimensional indicates a high degree of compaction whilst values approaching 1 indicates a very loose and open structure.

4. Conclusions

In this work we pointed out the importance of the concept of fractal structure of the fractal structure concept in physical characterization of materials. Fractal dimensions of the studied materials were found to be in the range of $2 < D < 3$. Fractal analysis based on micrographs electronic microscopy SEM images shows nanostructure features with self-similar properties on a large scale. The porosity of collagen matrix can be controlled by chitosan content; as the content of chitosan was increased, the fractal dimension decreased. Scanning electron microscopy indicated that the addition of chitosan greatly influences structure and change collagen fibres cross-linking, reinforcing the structure and increasing pore size.

Materials with a higher fractal dimension are less porous than those with a lower fractal dimension. New methods of investigation and a new method to determine the fractal dimension for biomaterials are necessary in the future in order to understand how the porosity of these organic materials is connected to the fractal dimension.

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