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Surface characterization of commercial chitosan with SEM and BET techniques

SEM ve BET teknikleri ile ticari kitosanın yüzey karakterizasyonu

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Research Article

Abstract

Chitosan, a natural polymer, has recently been the focus of attention of researchers due to its superior properties such as biocompatible, biodegradable, renewable, and low toxicity. Especially in the fields of chemistry and health sciences, its use as a wound dressing material, adsorbent, resin, drug carrier system, and food packaging is being researched. This study, it is aimed to contribute to the areas where chitosan is used as adsorbent, resin, and wound dressing material by making surface characterization. According to the data obtained from the study, it was determined that chitosan was a non-porous polymer with a membranous surface. In addition, it was determined that there may be calcium particles as a surface impurity on the surface of commercial chitosan samples.

Keywords: Chitosan, SEM and BET techniques, Morphological analysis.

Özet

Doğal bir polimer olan kitosan biyouyumlu, biyobozunur, yenilenebilir ve düşük toksisite gibi üstün özellikleri sebebiyle son zamanlarda araştırmacıların ilgi odağı olmuştur. Özellikle kimya ve sağlık bilimleri gibi alanlarda yara örtü malzemesi, adsorbent, reçine, ilaç salınımı ve gıda ambalajı olarak çalışılmaktadır. Bu çalışmada kitosanın yüzey karakterizasyonu yapılarak onun adsorbent, reçine ve yara örtü malzemesi olarak kullanıldığı alanlara katkıda bulunulması amaçlanmıştır. Çalışmadan elde edilen verilere göre kitosanın gözeneksiz ve zarımsı yüzeye sahip bir polimer olduğu tespit edilmiştir. Ayrıca, ticari kitosan örneklerinin yüzeyinde bir yüzey safsızlığı olarak kalsiyum parçacıklarının da olabileceği saptanmıştır.

Anahtar kelimeler: Kitosan, SEM ve BET teknikleri, Morfolojik analiz.

1. Introduction

Chitin and chitosan polymers are natural amino polysaccharides. These polymers have a unique structure with their multidimensional/directional properties and their application areas are quite wide. They have many positive features such as low toxicity, ecological safety, antimicrobial activity, low immunogenicity, excellent biocompatibility, and biodegradability [1]. In addition to these features, their renewable, low-cost and chemically functional nature have increased the interest in chitin and chitosan, making them the subject of research in many fields such as chemistry, environment, food packing/coating, biotechnology, and medicine.

Chitin is the second most abundant polymer in nature after cellulose. Cellulose is obtained from the cell walls of plants, and chitin is obtained from fungus or the shells of insects or crustaceans. Cellulose and chitin are macromolecules that maintain the structural integrity of plants and animals, respectively. In this respect, it is possible to say that cellulose and chitin are structurally related polysaccharides. In addition, there is a great similarity between chitin and cellulose in terms of molecular structure. Instead of hydroxyl groups (-OH) at the C2 position of cellulose, chitin has acetamide groups (CH_3-(C=O)-NH-), while chitosan has primary amine (-NH_2) groups. It is possible to name chitin and chitosan as renewable polymers, biopolymers, or natural polymers. But chitin is a homopolymer. It consists of N-Acetyl-D-Glucosamine

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monomers as the kit shows in Figure 1. Chitosan, on the other hand, is a copolymer consisting of N-Acetyl-D-Glucosamine and D-Glucosamine monomers [1-3].

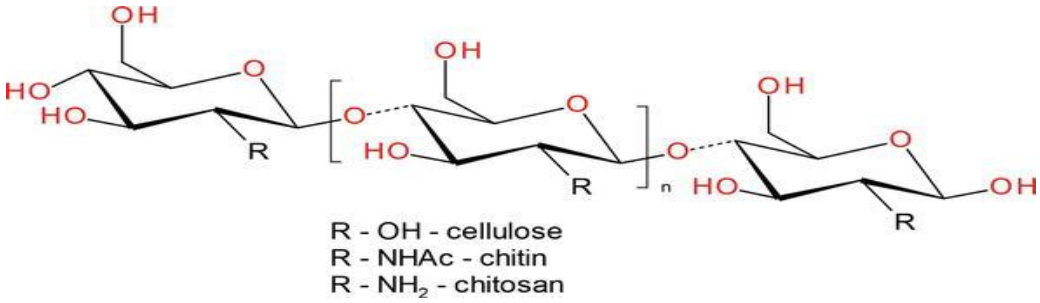

Figure 1. The molecular similarity of chitin, chitosan, and cellulose [4]

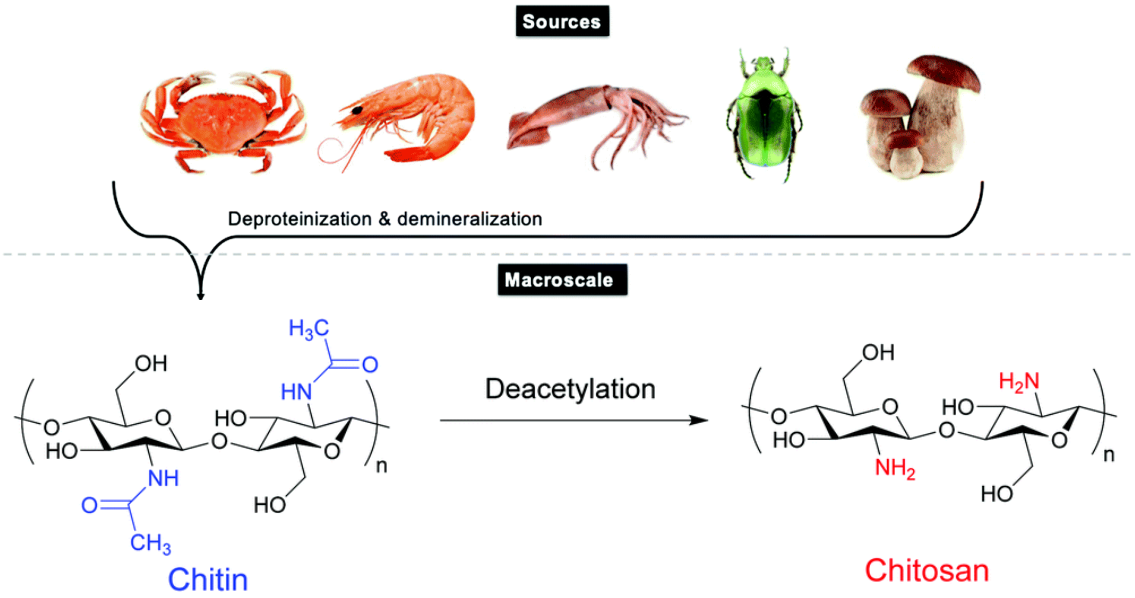

Figure 2. Chemical structures of chitin and chitosan via deacetylation [5]

Chitin is produced industrially from mushrooms or the shells of shellfish such as shrimp. Chitosan is the primary derivative produced by the alkaline deacetylation of chitin (Figure 2). The production of chitosan from shellfish waste occurs in three basic stages. These basic steps can be listed as de-mineralization (DM), deproteinization (DP), and de-acetylation (DA). At the end of the DM and DP processes, chitin is produced from waste shells. With the DA process, which is the last step, the conversion of chitin into chitosan is achieved [3, 6].

There are three important physicochemical properties used to describe the chitosan macromolecule. These are the degree of deacetylation (DD), molecular weight (Mw), and solubility. The most obvious difference between chitin and chitosan is that their solubility properties are different. Because chitin has strong intramolecular and intermolecular hydrogen bonds, it is insoluble in conventional solvents, including organic solvents and mineral acids. The low solubility of chitin may limit its use. Therefore, there is a need for more easily soluble derivatives such as chitosan. Acetic acid, lactic acid, oxalic acid, formic acid, and pyruvic acid can be used as solvents for chitosan. The most commonly used solvent for chitosan is dilute acetic acid solution. The dissolution of chitosan occurs through the protonation of amine groups. The percentage of primary amine groups in the polymer chain of chitosan relative to the acetamide groups is defined as the degree of deacetylation. The de-acetylation degree and molecular weight of chitosan can vary depending on the reaction conditions in the chitosan production process [1, 3, 7, 8].

Since chitosan is biocompatible, biodegradable, and has low toxicity, it has been the focus of interest in medical studies. In this context, chitosan has started to be used in the development of wound dressing materials, gene therapy, and the development of controlled drug release systems [7, 9, 10]. In addition, the fact that chitosan is the second most abundant polymer in nature and has active chemical groups such as amine and hydroxyl in its chemical structure has made it the focus of attention in the field of chemistry. It is widely used especially as an adsorbent and resin. It is possible to come across many studies in the literature in which chitosan or modified chitosan derivatives are used as sorbents [2, 11]. A sorption process is a surface event that takes place through the pores or active groups on the surface of the sorbent. Due to the widespread use of chitosan and its derivatives as sorbent, it has become a necessity to investigate the surface properties. Thus, determining the surface characteristics of commercial chitosan with BET and SEM techniques has been the focus of this study.

2. Material and Method

2.1. Material supply

The chitosan used in the experiments was obtained from Adaga Food and Consulting Joint Stock Company operating in Antalya and is of commercial purity (Lot No: 070891). It was determined DD:80-85% and molecular weight:530-600 kDa by the manufacturer.

2.2. Surface characterization with SEM-EDX technique

Commercial chitosan samples were dried in an oven at 50 °C for 1 day before SEM analysis. Then, the gold-plated chitosan samples were analyzed in the ZEISS brand EvoMA10 model device.

2.3. Porosity analysis with BET technique

The surface porosity of chitosan samples was analyzed as a result of adsorption/desorption experiments carried out under a nitrogen atmosphere with a BET device (Micromeritics ASAP 2020). During the BET analysis, de-gas was applied to the chitosan sample at 60 °C for 12 hours. Thus, all volatile components including moisture in the content of chitosan were removed from the structure.

3. Results and Discussion

SEM images of the commercial chitosan sample are given in Figure 3. According to the 75 times magnified image, it is understood that commercial chitosan has a layered structure in the macro sense. However, in some parts of these layer fragments, a whitish sheen was noted. The presence of calcium (Ca) was detected in the structure as a result of the EDX analysis performed in a region that will include these whitish sheen. According to the spectrum obtained from the EDX analysis performed on the selected region in Figure 3(a), it was determined that the chitosan sample consisted of 32.5% C, 46% O, and 21.5% Ca. When looking at the 5000 times magnified image in the region where the white region is concentrated, it is clearly understood that the chitosan surface is covered with white particles. According to the result of EDX analysis performed in a narrower region in this image, the elemental composition was determined as 15.5% C, 48.5% O and 36% Ca. The obtained data show that there is a significant amount of Ca compound or mineral on the chitosan surface. The C content of chitosan decreased to 15% in regions where Ca concentration increased.

After the chitosan samples were washed three times with distilled water, they were dried in an oven at 50 °C and SEM images were examined again. The resulting image is given in Figure 4. According to this image, as a result of washing with pure water, it was noticed that the calcium particles on the chitosan surface decreased significantly and the chitosan surface was seen more clearly. According to the elemental composition obtained from the EDX spectrum, chitosan was determined to consist of 26.5% C, 46.5% O and 27% Ca. Elemental composition data also confirmed that the amount of Ca in the washed samples decreased and the C content increased.

Finally, while the chitosan samples were washed with distilled water, a few drops of diluted HCl solution was added to the washing water. It was similarly washed three times with acidified water and dried at 50 °C, and SEM images were taken again. According to the data obtained, it was observed that the calcium on the chitosan surface was completely removed as a result of pre-washing with acidified water. The data obtained from the EDX spectrum also confirmed that there was no residual Ca on the surface. In this context, the elemental composition was determined as 50.2% C, 44.5% O, and 5.3% N.

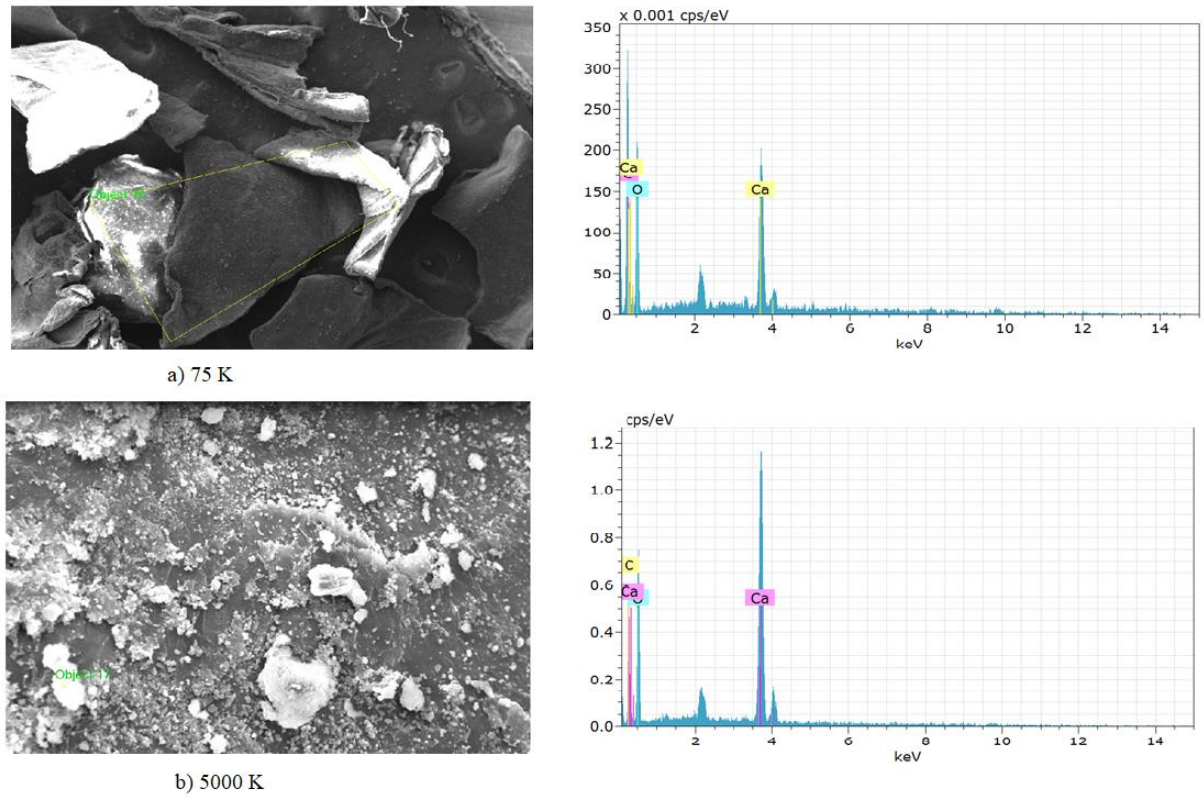

Figure 3. SEM images and EDX spectra of commercial chitosan

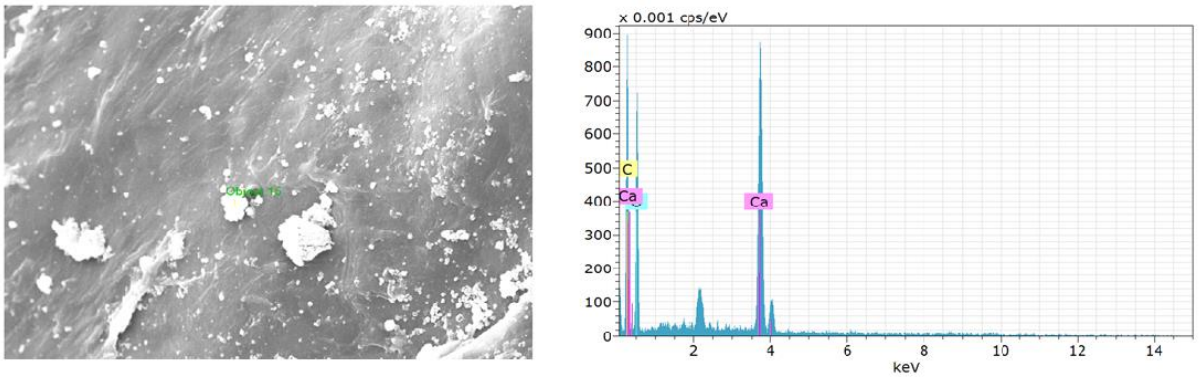

Figure 4. SEM image (x5000) and EDX spectrum of the chitosan sample washed with distilled water

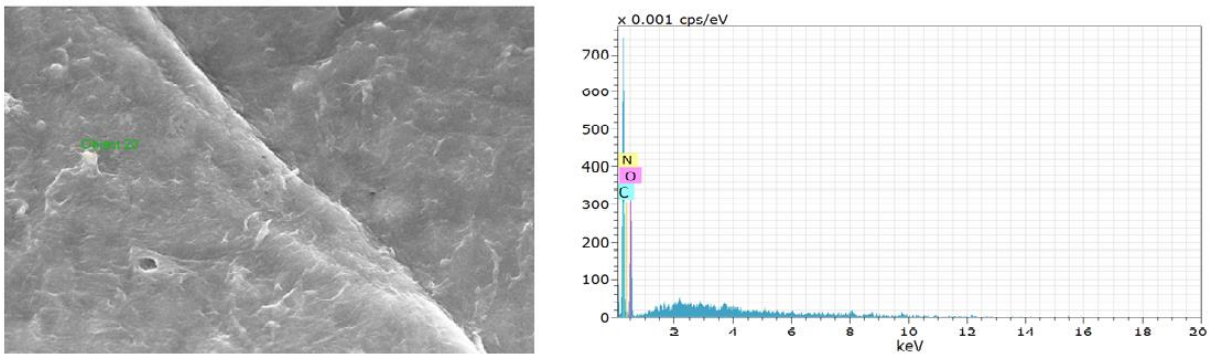

Figure 5. SEM image (x5000) and EDX spectrum of chitosan samples washed with acidified water

Commercial chitosan which was provided by the *Adaga Food and Consulting Joint Stock Company* in this study was produced from shrimp shells. It is possible to encounter minerals such as Ca and Mg in the shells of sea creatures such as shrimp. Although the demineralization step with HCl is applied in the production process of chitosan from these shells, some Ca remain in the structure of chitosan, since the demineralization efficiency is not 100%. In this study, pre-washing with dilute HCl solution can be considered as a second de-mineralization step. Thus, Ca, etc. minerals in the chitosan structure or surface can be completely removed.

The dissolution of chitosan in the acetic acid solution can be thought of as an acid-base reaction. Because chitosan can give all the reactions of amines by acting as an amine compound due to the active amine ($-NH_2$) groups in its structure [12]. Thus, the acetate salt of chitosan is formed as a result of the dissolution in the acetic acid medium. If there are mineral impurities such as Ca etc. on the chitosan surface, the formation of calcium acetate salt will be inevitable during the dissolution process. This situation causes both solvent loss and adverse effects in the modification process of chitosan with techniques such as intercalation and cross-link. Because the modification of chitosan takes place in the solution medium. In addition to these, in cases where chitin or chitosan is used directly as a sorbent, mineral impurities on the surface pass into the solution medium and cause contamination of the sorption medium due to the sorbent. All these reasons emphasize that mineral impurities such as Ca etc., which are a result of the low efficiency in the demineralization process, should be removed from the chitosan surface.

According to the SEM image in Figure 5, it is possible to say that the chitosan surface is non-porous, smooth, and membranous (like a membrane). The fact that chitosan is a non-porous material indicates that it performs its sorption efficiency, not through porosity, but through active amine and hydroxyl groups in its structure.

As a result of the adsorption/desorption experiments of chitosan samples under a nitrogen atmosphere with a BET device, the surface area was determined as 0.0632 m^2/g and pore volume as 0.001255 cm^3/g. Moussout et al., (2018) [13] found the surface area of the chitosan sample, which has a DD of 82.5%, to be 0.1 m^2/g in their study. The data obtained are in agreement with the literature.

In addition, the detection of the surface area as a very small value of 0.0632 m^2/g also confirms the SEM images. The very low surface area due to porosity can be considered as another proof that chitosan is non-porous.
The data obtained within the scope of this study revealed that chitosan is a non-porous polymer. It also showed that there may be mineral layers such as calcium on the surface of commercially pure chitosan samples. If chitosan is to be used as an adsorbent in adsorption applications such as water softening, heavy metal removal, and sulfate removal, it is very important to pre-wash by using distilled water acidified with dilute HCl solution.

4. Acknowledgment

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5. Author Contribution Statement

In the study, Author 1 contributed to forming the idea, the analysis of the results, provision of the materials and examination of the results; Author 2 contributed making the design and literature review; Author 3 contributed to checking the spelling and checking the article in terms of content.

6. Ethics Committee Approval and Conflict of Interest

There is no need for any an ethics committee approval in the prepared article. However, funding support was received from TÜBİTAK.

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