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Mini Review on the Morphological and Microanalysis Characterization of Chitosan Nanocomposites Using Scanning Electron Microscopy

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ABSTRACT

The deacetylation of chitin, leads to the production of chitosan, which is the second-most abundant natural polymer on earth today. Some of its unique appealing properties such as non-toxicity, chemical versatility, biodegradability, biocompatibility, high adsorption capacity makes them to stand out among other polymers used in formulation. Application of chitosan cuts across different spheres such as biomedical applications for wound dressing, drug delivery, biosensors, and dietary supplement. However, as a biomaterial, low mechanical strength and poor thermal stability are some of the limitations associated with chitosan. With the advent of nanotechnology over the years, chitosan has garnered immense interest as a matrix of nanocomposites that have led to the advancement in its effectiveness, eventually expanding its application areas. Chitosan nanocomposites are promising bio-based polymeric nanocomposites with exceptional biological and physicochemical properties that offer great potential for the delivery along with the controlled release of drugs and active compounds, thus functioning as a superior nanocarrier system. The application of chitosan in biomedical research such as bioimaging, cancer therapy, dentistry, and wound healing, among others has been recorded.

Keywords: Chitosan, Polymer, Formulation, Scanning Electron Microscopy, Drug Delivery.

INTRODUCTION

Chitosan: An overview of its properties

Chitosan which is a derivative of chitin, belongs to the family of linear

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polysaccharides which is composed of different amounts of (β 1-4) linked residues of N-acetyl-2-amino-2-deoxy-D-glucose (glucosamine) and 2-amino-2-deoxy-D-glucose residues [1]. Chitin and chitosan interest, lies in the different biological and technological properties exhibited by these polymers. The tight relationship that exists in the physicochemical properties of these polymers is majorly in the molecular weight and acetylation degree [2]. It is mandatory to carry out a good polymer characterization when working with both chitin and chitosan.

There is a great interest among researchers in the polymers of biological origin due to the environmental impact of synthetic polymers [3]. Due to the emergence of various classes of materials, there is need to carry out proper characterization on these materials in order to understand the complex structure and morphology which will assist in their ideal applications [4]. One of the multifunctional techniques that has been used over the years in the in-depth analysis and understanding of materials over a large-scale magnification range is the microscopy technique [5]. Microscopy entails the detailed observation of materials and their properties in the range of millimeters to nanometers. At such a high magnification, there is a guarantee to access the physical, chemical and structural information of such materials. With the help of the microscopic technique, the image of the material can be acquired and subsequently analyzed both at the millimeters and nanometers levels [6].

Over the years in the area of material research, microscopy technique has been useful in numerous discoveries. A lot of characterization techniques are available that are used to analyze and characterize materials. They assist by providing a thorough knowledge of the structure and property relationships [7]. Table 1 summarizes the scanning electron microscopy (SEM) and energy dispersion x-ray spectroscopy (EDS), their application and relevant biopolymers.

Table 1. Scanning electron microscopy (SEM) and energy dispersion x-ray spectroscopy (EDS), their application and relevant biopolymers

Technique	Application	Biopolymers	References
Scanning electron microscopy	Fiber diameter and surface modification, cystal alignment, failure behaviour	Gum Arabic, gum karaya, kondagogu gum, celulose nanocrystals, gelatin/maltodextrin	[8-10]
SEM + energy dispersive x-ray spectroscopy	Elemental composition	Cellulose	[11,12]

Scanning electron microscopy (SEM) is one of the most popular techniques that is commonly used in the industry and institutions [13]. The mode of operation, is based on its ability to use a high magnification and resolution image. The electron beam from the electron gun interacts with the electrons on the sample and produces certain signals about the surface topography. The images from the SEM are obtained by analyzing the signals from the secondary and backscattered electrons, which contain information regarding the sample [14]. The SEM can provide detailed information concerning the surface morphology, crystallinity and elemental composition of a sample [15]. Three major conditions that could be deployed in the analysis of samples under SEM. They include the high vacuum, low vacuum and wet conditions [16]. In the analysis of samples using SEM, they are usually frozen under liquid nitrogen and subsequently coated to avoid charging and metal shadowing [17]. In biopolymers, SEM is used to determine the surface topography, homogeneity and phase separation [17]. For chitosan scaffolds, that have porous structures, SEM could be used to determine the pore size, structure and density [17-19]. In the characterization of nanocomposites, SEM could be deployed in assisting to determine the dispersion and distribution of the fillers in the polymer matrix [20,21].

Field emission scanning electron microscopy (FESEM) is deployed in the provision of higher resolution images and a greater energy range. Its advantage over SEM is that FESEM uses a field emission gun as an electron generation system. Environmental scanning electron microscope (ESEM) has a better advantage than ordinary SEM due to the fact that the specimen to be examined does not require special sample preparation like coating. In addition, the specimen can be examined at different regimes. The application of ESEM in the area of crystallization wetting, swelling, drying, melting and

freezing has been documented [22-25].

Energy dispersive x-ray (EDX) is added to SEM as an additional accessory. Its function is to determine the elemental composition or chemical characterization of a sample [26]. Its function is based on the ability of each element to showcase a unique set of peaks on its x-ray spectrum which corresponds to a unique atomic structure. A high-energy incident beam excites an electron in an inner shell, and it will be ejected from the shell creating a hole in its place. Another electron from a higher energy outer shell will fill this hole and the energy difference between the shells will be released in the form of x-rays which will be characteristics of the atomic structure of the emitting element [26]. In biopolymer systems, the application of EDS is also extended to the elemental composition, impurities, chemical modifications and sample functionalization [27-30]. Researchers find EXD analysis useful as it helps to determine the presence of heteroatoms (chlorine and sulphur) [27]. The application of EDX in determining the distribution of carboxymethyl chitosan and calcium alginate in a composite has been determined.

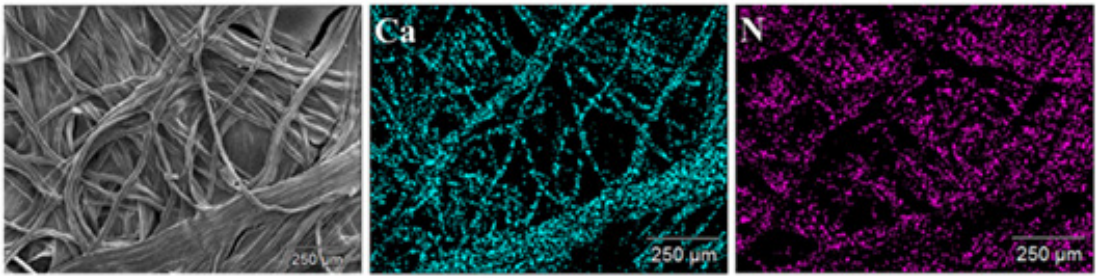

Figure 1. Energy dispersive x-ray (EDX) mapping of carboxymethyl chitosan and calcium alginate composite dressings [28].

Scanning electron microscopy is useful in determining the elemental composition and could also assist in analyzing surface structure. The operation is based on its ability to shine a monochromatic electron beam with a wavelength ranging from some ev to 50Kev onto the specimens' surface which results in the creation of an image. With the help of the electron gun, the beam is formed by this lens. Unique properties associated with SEM include: flexibility, various imaging modes, ease of sample preparation, spectroscopy and diffraction capabilities and ease of image interpretation.

SEM characterization technique for chitosan and its based nanocomposites

Over the years, chitosan nanocomposites have gained versatile physic-chemical characteristics and properties. SEM is a valuable tool which is used to determine the surface visualizations, in depth study of functionalized/agglomerated chitosan nanoparticles (NPs) and estimates the sample composition via energy dispersive x-ray spectroscopy EDX. SEM uses an energized electron beam (typically 1-30 eV) which scans the surface of the sample in a raster pattern, wherein the secondary emitted electrons or backscattered electrons are detected, thereby achieving resolutions in the nanometer range [29].

Depending on the nature of the sample (chitosan nanomaterials), the electronic interaction with the sample varies, thus subsequently resulting in various types of emitted electrons which could occur at the sample surface. The detected electrons of different energies are processed and displayed as a pixel in the monitor, thereby visualizing 3D images or composition of nanomaterials as shown in Figure 2. The field emitter scanning electron microscopy (FE-SEM) is useful for beaming electrons under a high electric field [30-32].

There are some metals that are normally used to improve the electrical conductivity and contrast of nanomaterials. They include gold, silver and platinum [33]. In 2010, Kumirska et al. reported the synthesis of poly (lactic acid)/chitosan PLA/CS NPs using emulsion and solvent evaporation techniques [34]. The images of the PLA/CSNPs are displayed in Figure 3a. In 2013, Hosseini et al. prepared the essential oil encapsulated chitosan NPs using a two-step process of oil-in-water emulsion preceded by ionic gelation [35]. The morphology of the prepared chitosan NPs found to be spherical in nature which is nicely intact and had a clear distribution among themselves as shown in Figure 3b.

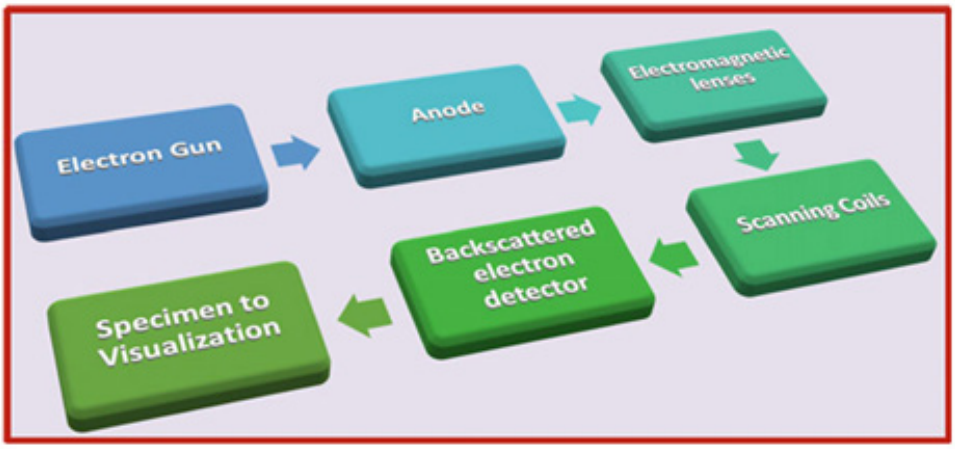

Figure 2. Flowchart representing an overview of the SEM analysis process [32].

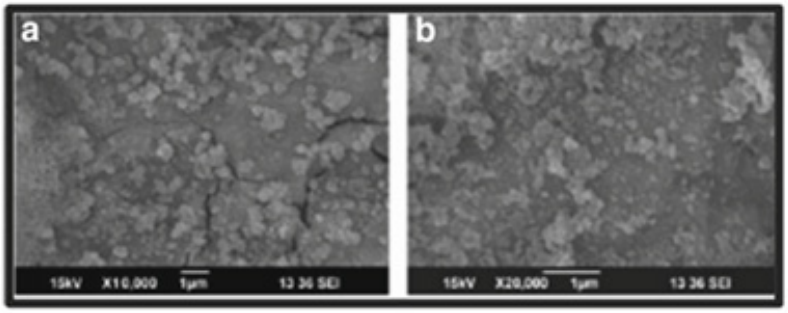

Figure 3. SEM images of a poly-lactic acid/chitosan PLA/CS NPs [32].

CONCLUSION

Chitosan characterization is critical since its structure determines its qualities, which in turn serves to define its wide potential application in industry. Chitosan in the form of pure matrix material may display poor mechanical and thermal properties. Due to this reason, chitosan as a nanocomposite base matrix seems like a rather preferable option because of its high availability in nature, low cytotoxicity, low cost, and high versatility, as well as biocompatibility.

AUTHORSHIP CONTRIBUTION

Ezegbe Chekwube Andrew: Writing, review, supervision, **Okorafor Ezinne Chinemerem:** Writing, Review, Ogbonna **Emmanuel Emeka:** Writing, review.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Ghormade V, Pathan EK, Deshpande MV. (2017). Can fungi compete with marine sources for chitosan production? Int J Biol Macromol. 104(Pt B):1415-1421.
2. Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B, Acosta N, et al. (2009). Functional characterization of chitin and chitosan. Curr Chem Biol. 3(2):203-230.
3. Cacciotti I, Mori S, Cherubini V, Nanni F. (2018). Eco-sustainable systems based on poly(lactic acid), diatomite and coffee grounds extract for food packaging. Int J Biol Macromol. 112:567-575.
4. Garavand F, Rouhi M, Razavi SH, Cacciotti I, Mohammadi R. (2017). Improving the integrity of natural biopolymer films used in food packaging by crosslinking approach: A review. Int J Biol Macromol. 104(Pt A):687-707.

5. Venkateshaiah A, Cheong JY, Habel C, Wacławek S, Lederer T, Cerník M, et al. (2020). Tree Gum–Graphene Oxide Nanocomposite Films as Gas Barriers. ACS Appl Nano Mater. 3:633-640.
6. Babu RP, O'Connor K, Seeram R. (2013). Current progress on bio-based polymers and their future trends. Prog Biomater. 2(1):8.
7. He M, Lu A, Zhang L. (2014). Advances in Cellulose Hydrophobicity Improvement. American Chemical Society: Washington, DC, USA. pp. 241-274.
8. Zhang Y, Xu Y, Xi X, Shrestha S, Jiang P, Zhang W, et al. (2017). Amino acid-modified chitosan nanoparticles for Cu2+ chelation to suppress CuO nanoparticle cytotoxicity. J Mater Chem B. 5(19):3521-3530.
9. Majoinen J, Kontturi E, Ikkala O, Gray DG. (2012). SEM imaging of chiral nematic films cast from cellulose nanocrystal suspensions. Cellulose. 19(5):1599-1605.
10. Rizzieri R, Baker FS, Donald AM. (2003). A study of the large strain deformation and failure behaviour of mixed biopolymer gels via in situ ESEM. Polymer. 44(19):5927-5935.
11. Karhale S, Bhenki C, Rashinkar G, Helavi V. (2017). Covalently anchored sulfamic acid on cellulose as heterogeneous solid acid catalyst for the synthesis of structurally symmetrical and unsymmetrical 1,4-dihydropyridine derivatives. New J Chem. 41(12):5133-5141.
12. Shankar S, Oun AA, Rhim JW. (2018). Preparation of antimicrobial hybrid nano-materials using regenerated cellulose and metallic nanoparticles. Int J Biol Macromol. 107(Pt A):17-27.
13. Mekonnen T, Mussone P, Khalil H, Bressler D. (2013). Progressinbio-based plastics and plasticizing modifications. J Mater Chem A. 1(43):13379-13398.
14. Dufresne A, Thomas S, Pothen LA. (2013). Biopolymer Nanocomposites. John Wiley and Sons, Inc. Hoboken, NJ, USA.
15. Spence JCH. (2013). High-Resolution Electron Microscopy. Oxford University Press: New York, NY, USA.
16. Yu X, Arey B, Chatterjee S, Chun J. (2019). Improving in situ liquid SEM imaging of particles. Surf Interface Anal. 51(13):1325-1331.
17. Naranda J, Sušec M, Maver U, Gradišnik L, Gorenjak M, Vukasović A, et al. (2016). Polyester type polyHIPE scaffolds with an interconnected porous structure for cartilage regeneration. Sci Rep. 6:28695.
18. Zhou FL, Parker GJ, Eichhorn SJ, Hubbard Cristinacce PL. (2015). Production and cross-sectional characterization of aligned co-electrospun hollow microfibrous bulk assemblies. Mater Charact. 109:25-35.
19. Yin HM, Qian J, Zhang J, Lin ZF, Li JS, Xu JZ, et al. (2016). Engineering Porous Poly(lactic acid) Scaffolds with High Mechanical Performance via a Solid State Extrusion/Porogen Leaching Approach. Polymers (Basel). 8(6):213.
20. Mafirad S, Mehrnia MR, Zahedi P, Hosseini S-N. (2017). Chitosan-based nanocomposite membranes with improved properties: E ect of cellulose acetate blending and TiO2 nanoparticles incorporation. Polym Compos. 39(12):4452-4466.
21. Zhang ZH, Han Z, Zeng XA, Xiong XY, Liu YJ. (2015). Enhancing mechanical properties of chitosan films via modification with vanillin. Int J Biol Macromol. 81:638-643.
22. Daio T, Bayer T, Ikuta T, Nishiyama T, Takahashi K, Takata Y, et al. (2015). In-Situ ESEM and EELS Observation of Water Uptake and Ice Formation in Multilayer Graphene Oxide. Sci Rep. 5:11807.
23. Jenkins LM, Donald AM. (1997). Use of the environmental scanning electron microscope for the observation of the swelling behaviour of cellulosic fibres. Scanning. 19:92-97.
24. Podor R, Ravaux J, Brau HP. (2012). In Situ Experiments in the Scanning Electron Microscope Chamber, Scanning electron Microscopy. London, UK: IntechOpen.
25. Jansson A, Nafari A, Sanz-Velasco A, Svensson K, Gustafsson S, Hermansson AM, et al. (2013). Novel method for controlled wetting of materials in the environmental scanning electron microscope. Microsc Microanal. 19(1):30-37.

26. Girão AV, Caputo G, Ferro MC. (2017). Application of Scanning Electron Microscopy–Energy Dispersive X-Ray Spectroscopy (SEM-EDS). Compr Anal Chem. 75:153-168.

27. Singh P, Chauhan K, Priya V, Singhal RK. (2016). A greener approach for impressive removal of As(iii)/As(v) from an ultra-low concentration using a highly e cient chitosan thiomer as a new adsorbent. RSC Adv. 6(69):64946-64961.

28. Gao Y, Zhang X, Jin X. (2019). Preparation and Properties of Minocycline-Loaded Carboxymethyl Chitosan Gel/Alginate Nonwovens Composite Wound Dressings. Mar Drugs. 17(10):575.

29. Azmana M, Mahmood S, Hilles AR, Rahman A, Arifin MAB, Ahmed S. (2021). A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications. Int J Biol Macromol. 185:832-848.

30. Kravanja G, Primožič M, Knez Ž, Leitgeb M. (2019). Chitosan-based (Nano)materials for Novel Biomedical Applications. Molecules. 24(10):1960.

31. Bagheri-Khoulenjani S, Taghizadeh SM, Mirzadeh H. (2009). An investigation on the short term biodegradability of chitosan with various molecular weights and degrees of deacetylation. Carbohydr Polym. 78(4):773-778.

32. Moreno JAS, Mendes AC, Stephansen K, Engwer C, Goycoolea FM, Boisen A, et al. (2018). Development of electrosprayed mucoadhesive chitosan microparticles. Carbohydr Polym. 190:240-247.

33. Husain S, Al-Samadani KH, Najeeb S, Zafar MS, Khurshid Z, Zohaib S, et al. (2017). Chitosan Biomaterials for Current and Potential Dental Applications. Materials (Basel). 10(6):602.

34. Kumirska J, Weinhold MX, Thöming J, Stepnowski P. (2010). Biomedical activity of chitin/chitosan based materials-influence of physicochemical properties apart from molec ular weight and degree of N-Acetylation. Polymers. 3(4):1875-1901.

35. Ghanbari B, Shahhoseini L, Hosseini H, Bagherzadeh M, Owczarzak A, Kubicki M. (2018). Synthesis of Pd(II) large dinuclear macrocyclic complex tethered through two dipyridine bridged aza-crowns as an efficient copper- and phosphine-free Sonogashira catalytic reaction. J Organomet Chem. 866:72-78.