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Development of bio-nanocomposite film based on sodium alginate-CuO nanoparticles and essential oils towards medical applications

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Today we are utilizing numerous biopolymers for various purposes owing to its intrinsic biodegradable properties. However, they have exceptionally poor mechanical properties, for example, very less tensile strength. We can utilize nanomaterial in biopolymer matrix to improve mechanical strengths as well as to make the film biodegradable. Nowadays many researches are underway on the improvement of physico-chemical and mechanical properties of bio-nanocomposite. Several research works are utilizing antimicrobial essential oils in biopolymer matrix to improve antimicrobial properties of nanocomposite in view of its medicinal applications. CuO modified essential oils (neem oil, eucalyptus oil, and clove oil) based sodium alginate bio-nanocomposite film was prepared by the solvent casting method. Synthesised bio-nanocomposite films were characterized by using FTIR, XRD, SEM, EDS. XRD analysis had verified that nano CuO has been successfully incorporated into alginate. FTIR uncovers that nano CuO was attached with alginate just by the physical or weak interaction. The EDS analysis reveals the presence of copper, sulphur and other elements. SEM results confirm that the nano particles were dispersed homogeneously in the films. Overall, it is concluded that the alginate/CuO bio-nanocomposite films could be used as material for medical purpose like wound healing by adding essential oils such as neem oil, eucalyptus oil and clove oil.

Keywords: CuO nanoparticle, sodium alginate, bio-nanocomposite, neem oil, eucalyptus oil, clove oil, wound healing, antimicrobial.

Introduction

Enthusiasm for the advancement of the innovative medicinal bandage using biopolymers is growing. Features such as biocompatibility, biodegradation and non-cytotoxicity make this material an excellent candidate for medical applications[1]. The antimicrobial essential oils extracted from natural sources are the alternative for the antimicrobial medical applications[2]. For an environmental friendly material, biopolymer can be used, but there is an issue of poor physical properties[3]. To overcome this issue we are using nanoparticles as a reinforced material to achieve physical strength and other physico-chemical properties[4]. These biopolymers have biodegradability, non-toxicity, biocompatibility, and renewability, so they can be applied to supplant petroleum-based polymers in the preparation of film.

Sodium alginate is a characteristic polysaccharide extracted from brown algae that develops in cold water regions[5,6]. Sodium alginate is a linear ionic polysaccharide made out of alternating blocks of β-(1→4)- connected d-mannuronic acid (M) and α-(1→4)- connected l-guluronic (G) residues[7]. Alginate has great biocompatibility, is less toxic, and low cost.

Metal oxide nanoparticles, for example, Ag, ZnO, and CuO nanoparticles are standing out due to their thermal stability with different functionalities. Of these copper oxide nanoparticles (CuO NP) have pulled immaterial thought on account of the low processing cost, relatively less toxicity, thermal stability and solid antibacterial activity[8,9]. Presently, CuO is used as a reinforcing material to plan bio-nanocomposite for therapeutic applications because of its extraordinary antimicrobial properties.

Experimental

Materials:

Sodium alginate was provided by Akshar Chemical Delhi (Mw = 300000 g/mol), CuO nanoparticles were provided by

Kasliwal Brothers Raipur (Mw = 79.55 g/mol), neem (Azadirachta indic) oil was supplied by Baidyanath Ayurved Ltd., India, eucalyptus oil was procured from Nilgiri Royal and clove oil was obtained from Dabur India Ltd. All other chemicals used were of analytical grade.

Preparation of bio-nanocomposite film:

Sodium alginate/CuO nanocomposite films were synthesized by solution casting technique following Rhim *et al.*[10]. 150 ml of deionized water and 0.4 g of CuO nanoparticles were collected in a beaker and continuous stired for 30 min with magnetic stirrer. After 30 min of stirring, 1.2 g of glycerol as plasticizer was added into the beaker and stirring was continued again for another 20 min. Then 4 g of sodium alginate powder was slowly added so that they didn't coagulate and mix continuously with heating at 900°C until the entire powder had completely dissolved. When the dense solution has started to form, 2 ml of essential oil was added. After having completely dissolved the alginate powder was removed from the beaker. A thick solution was formed, then spread on a glass plate and kept at room temperature to dry for 48 h. The dried films were disrobed from the glass plate.

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis was performed using a total reflection method attenuated in an alpha model of Bruker's infrared spectrometer. The film spectra were acted within the range from 500 to 4000 cm^{-1}. The acute peaks were used to study the functional group present in sample[10,11].

X-Ray diffraction:

X-Ray diffraction was performed using the Xpert PRO analytical PAN model XRD which operates at 40 kV and 30 mA. The radiation was generated from a Cu Kα source (wavelength = 1.540598 Å). The diffraction was carried out from 2θ values from 5° to 80°, where θ is the angle of incidence of the X-ray beam of the sample under testing. The size of scanning step size performed was 0.026261[12].

Scanning Electron Microscopy:

The surface morphology and the pore size of the samples were analyzed by using scanning electron microscopy (ZEISS EVO series Scanning Electron Microscope Model EVO 18). A sample of bio-nanocomposite of alginate/CuO with essential oils was cut in a 10 mm square piece and for the first time it was used to cover with gold cathode spray coating and analyzed under SEM.

Energy Dispersive X-ray Spectroscopy (EDS):

The Energy Dispersive X-ray Spectroscopy (EDS) was performed by the Oxford-Energy Dispersive X-ray system (INCA 250 EDS with X-MAX 20 mm Detector).

Results and discussion

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectra of sodium alginate/CuO and various essential oils based film are shown in Fig. 1. Film spectra based on neem oil, clove oil and eucalyptus oil were recorded and compared. At 846.92 cm^{-1} a weak peak was observed on clove oil based BNC and this was due to presence of C=C (alkene group) bonds and the same in 914.22 and 916.98 cm^{-1} was observed in clove and neem oil based BNC, respectively. At 1029.1 cm^{-1} a strong peak was observed on neem oil based bio-nanocomposites (BNCs) due to the presence of C=O stretching bond and also at 1029.05 and 1030.55 cm^{-1} medium peak were observed in eucalyptus and clove oil based BNCs, respectively due to presence of C=O and

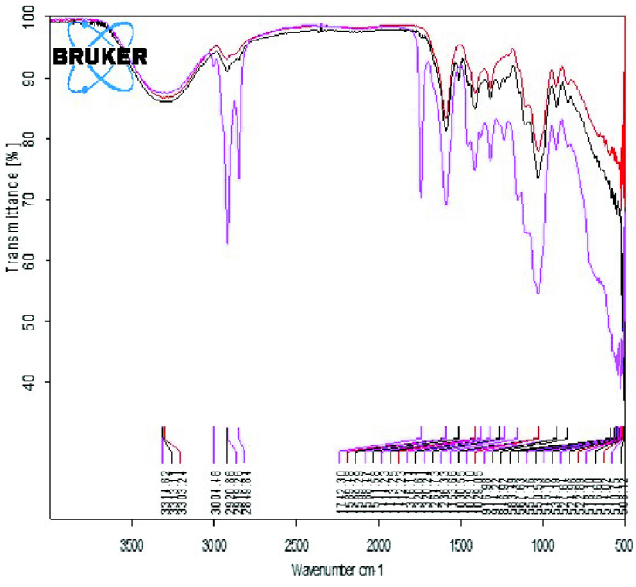

Fig. 1. Red – Sodium alginate/CuO and eucalyptus oil based BNC, Black – Sodium alginate/CuO and clove oil based BNC, Purple – Sodium alginate/CuO and neem oil based BNC.

Table 1. Crystal size of different film prepared using different essential oils

Alg./CuO BNC based on neem Oil		Alg./CuO BNC based on clove oil		Alg./CuO BNC based on eucalyptus oil	
2θ	D size in NM	2θ	D size in NM	2θ	D size in NM
31.7056	28.2222	31.7086	28.2196	31.6903	28.2355
35.5562	25.2492	35.5571	25.2485	35.5355	25.2634
45.4341	19.9466	45.4438	19.9591	45.4274	19.9494
48.8007	18.6463	48.7868	18.6668	48.7384	18.6687
56.4609	16.2847	56.465	16.2971	56.4541	16.2865
61.5506	15.0545	61.5626	15.0643	61.5347	15.0581
66.2345	14.0989	65.8478	14.184	65.7932	14.1827
68.1063	13.7563	68.0969	13.7693	68.0852	13.76

S=O (sulfate group) bonds. Peaks at 1153.66 and 1236.33 cm^{-1} were observed in neem oil based BNC which was due to the presence of C-O stretching bond. Medium sharp peak at 1264.73 cm^{-1} was observed in clove oil based BNC due to the presence of C-N stretching bond (aromatic amine group). A low peak at 1320.24 and 1320.61 cm^{-1} was seen in neem and clove oil based BNC due to presence of a C-N stretching bond (aromatic amine group). At 1376.14 cm^{-1} medium sharp peak of neem oil based BNC was seen because of O-H bending (phenol group). Medium sharp peak was observed at 1412.23 cm^{-1}, 1413.25 cm^{-1} and 1414.23 cm^{-1} in eucalyptus, clove, and neem oil based BNCs because of presence of S=O stretching (sulfate group). A weak peak at 1511.58 cm^{-1} was observed in clove oil based BNC because of the presence of N-O stretching (nitro group). Very strong peak at 1588.47 cm^{-1} of neem oil based BNC and a medium sharp peak at 1589.29 cm^{-1} and 1589.48 cm^{-1} was seen in clove and eucalyptus oil based BNCs, respectively due to presence of N-H stretching bending (amine group). The exceptionally sharp peak at 1742.3 cm^{-1}, 2849.84 cm^{-1} and 2919.26 cm^{-1} were seen in neem oil based BNC due to presence of C=O stretching (ester group), N-H stretching (amine salt) and C-H stretching (alkane group), respectively. And also weak peak at 2920.86 cm^{-1} was observed in clove oil based BNC due to presence of O-H stretching (alcohol group).

X-Ray diffraction:

The X-ray diffraction pattern was observed and the crystal size was calculated in nano meter for different values of 2θ for alginate/CuO/essential oil based bio-nanocomposite film.

The X-ray diffraction of alginate/CuO nanocomposite films containing neem oil is shown in the following Fig. 2. The net diffraction peaks at 2θ = 31.7056, 35.5562, 38.7329 and 45.4341 for the cross-linked alginate film affirmed the crystalline structure of sodium alginate film. The X-ray diffraction of alginate/CuO nanocomposite films containing clove oil is shown in Fig. 2. The sharp diffraction peaks at 2θ = 31.7086, 35.5571, 38.7235 and 45.4438 for the cross-linked alginate film affirmed the crystalline structure of sodium alginate film. The X-ray diffraction of alginate/CuO nanocomposite films containing eucalyptus oil is depicted in Fig. 2. The sharp diffraction peaks at 2θ = 31.6903, 35.5355, 38.6944 and 45.4274 for the cross-linked alginate film confirmed the crystalline structure of sodium alginate film.

In the XRD of sodium alginate(SA)/CuO nanocomposite films, diffraction peaks relating to alginate/CuO based on neem oil were observed. The 2θ estimations of 31.7056,

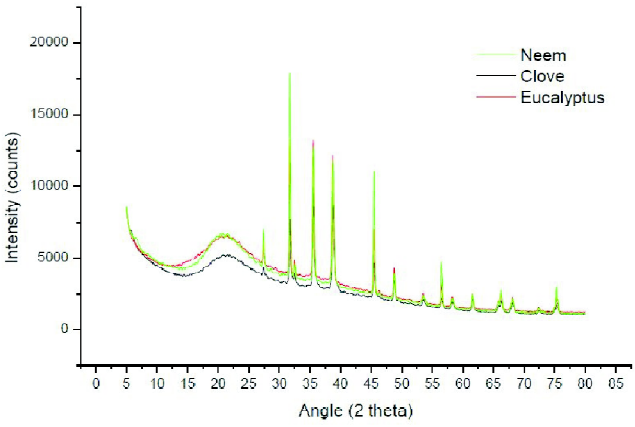

Fig. 2. XRD pattern of sodium alginate/CuO bio-nanocomposites.

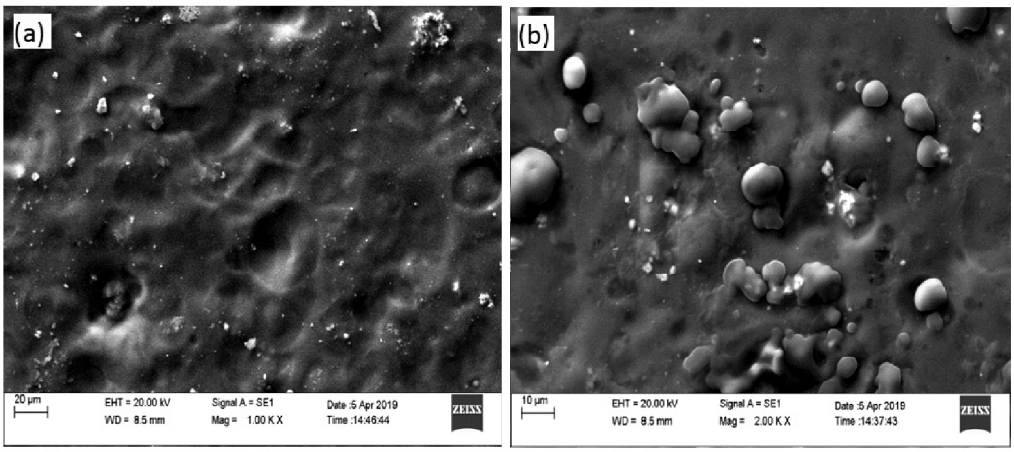

Fig. 3. SEM images of sodium alginate/CuO BNC using neem oil.

66.2345 were comparing to the 2θ estimation of sodium chloride. 2θ estimation of 35.5562 relate to the 2θ estimation of iron oxide. 2θ esteem 45.4341 relate to the 2θ estimation of silicon oxide. 2θ estimations of 48.8007, 56.4609, and 61.5506 compare to the 2θ estimations of calcium carbonate.

In the XRD of SA/CuO nanocomposite films, diffraction peaks were observed corresponding to SA/CuO based on clove oil. 2θ values of 31.7086, 45.4438, and 56.465 correspond to the 2θ value of sodium chloride. 2θ value of 35.5571 correspond to the 2θ value of iron oxide. 2θ value 65.8478 correspond to the 2θ value of silicon oxide. 2θ values of 48.7868, 61.5626 correspond to the 2θ values of calcium carbonate.

In the XRD of SA/CuO nanocomposite films, diffraction peaks were observed corresponding to SA/CuO based on eucalyptus oil. 2θ values of 31.6903, 56.4541, and 45.4274 which correspond to the 2θ value of sodium chloride. 2θ value of 35.5355 correspond to the 2θ value of iron oxide. 2θ value of 65.7932 correspond to the 2θ value of silicon oxide. 2θ values of 61.5347, 48.7384 correspond to the 2θ values of calcium carbonate.

All peaks in all the three samples were observed similar to each other. Also compound observed were same in all the samples.

Scanning Electron Microscopy:

Sodium alginate and CuO particles based all nanocomposite films were smooth, flexible and free. Surface morphology of the alginate-based composite films was detected by SEM and the outcomes appeared in the Fig. 3. In the SEM pictures, the alginate film showed a smooth and homogeneous surface. Notwithstanding, alginate/CuO composite films demonstrated rough surfaces with CuO particles embedded in the film matrix or superficially. As should be obvious the porous structure in the SEM images of BNC based on SA/CuO. Therefore, all films are crystalline in nature and can also be demonstrated by EDX scanning of the composite film. The particles homogeneously dispersed over the film. Also, the formed crystals are clearly appeared in the Figure.

Energy Dispersive X-ray Spectroscopy:

In the EDX analysis of SA/CuO BNC using clove oil; the presence of C, O, Na, Al, Cl, Ca and Cu were confirmed as delineated in the Fig. 4. The amount of C, O, Na, Al, Cl, Ca

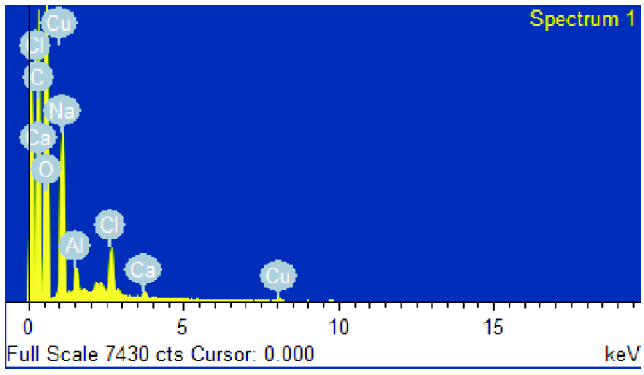

Fig. 4. EDX of SA/CuO/clove oil BNC.

and Cu in the prepared thin film was obtained 44.77, 44.55, 7.13, 0.70, 1.79, 0.28 and 0.78 wt.%, respectively. In the EDX analysis of SA/CuO BNC of using eucalyptus oil; the presence of C, O, Na, Cl, Ca and Cu was confirmed as shown in Fig. 5. The quantity of C, O, Na, Cl, Ca and Cu in the prepared thin film was obtained 42.93, 38.09, 10.99, 7.18, 0.11 and 0.71 wt.%, respectively.

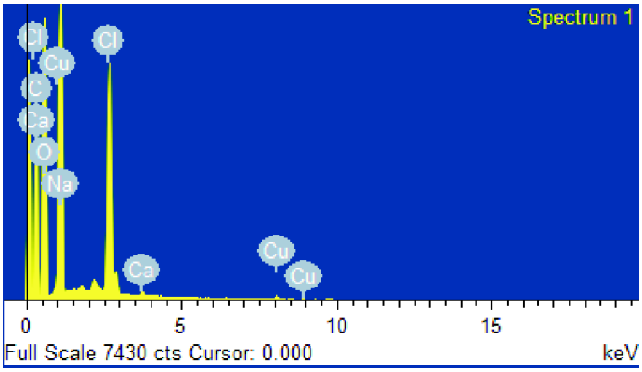

Fig. 5. EDX of SA/CuO/eucalyptus oil BNC.

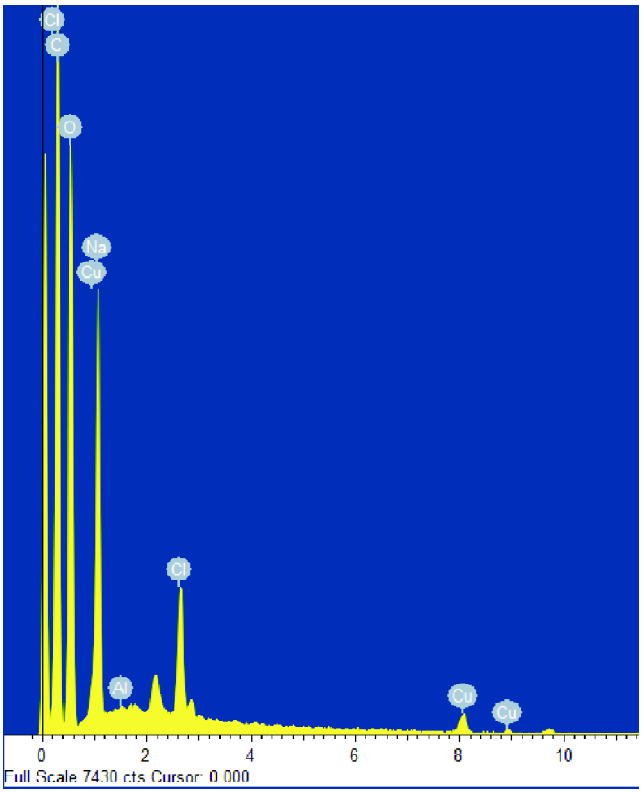

Fig. 6. EDX of SA/CuO/neem oil BNC.

Table 2. Element composition in (a) clove oil based BNC and (b) eucalyptus oil based BNC

(a) Clove oil based BNC			(b) Eucalyptus oil based BNC		
Element	Weight%	Atomic%	Element	Weight%	Atomic%
CK	44.77	53.88	CK	42.93	53.75
OK	44.55	40.02	OK	38.09	35.81
NaK	7.13	4.48	NaK	10.99	7.19
AlK	0.70	0.38	ClK	7.18	3.05
ClK	1.79	0.73	CaK	0.11	0.04
CaK	0.28	0.10	CuK	0.71	0.17
CuK	0.78	0.18			

Also in EDX analysis of the SA/CuO BNC using neem oil; the presence of C, O, Na, Al, Cl and Cu was confirmed as depicted in Fig. 6.

Conclusions

In this study the sodium alginate (SA)/CuO based bio-nanocomposite (BNC) films using essential oils (clove oil, neem oil, and eucalyptus oil) were successfully synthesized by solvent casting and characterized towards its potential for medicinal applications. The mechanism of formation of composite, morphology, crystallite size and functional group of the SA/CuO BNC were investigated and results were interpreted. The XRD study showed its crystalline nature and this was also proven by SEM based morphological analysis. The surface morphology showed homogeneous dispersion of CuO nanoparticles in BNC films. Based on analysis of different result it may be concluded that the prepared films may be used in biomedical fields, such as medical bandages, after assuring its antimicrobial properties, and biocompatibility.

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