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Preparation and characterization of cellulose-gelatin nanocomposite isolated from jute for biomedical application

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ABSTRACT

Due to their abundance, high strength and stiffness, low weight and biodegradability, nano-scale cellulose fiber materials serve as promising candidates for bio-nanocomposite production. Cellulose nanocrystals (JCNCs) were prepared by sulfuric acid hydrolysis of delignified and bleached jute fibers and were used to reinforce nanostructured biocomposites with gelatin by solution casting method under laminar flow using dimethyl sulfoxide (DMSO) as dispersant. JCNCs and the composite films were characterized with Fourier transform infrared spectrometry, X-ray diffraction, differential scanning calorimetry (DSC), thermo-gravimetric analysis, scanning electron microscopy. Tensile properties such as tensile strength, elongation at break and antimicrobial properties of the composite films were also measured and were compared with pure gelatin film. A significant enhancement of the thermal, mechanical and antimicrobial properties was achieved with a small addition of cellulose nanofibers to the gelatin matrix. The results of thermo-mechanical properties demonstrated that JCNCs containing composites have higher thermal stabilities than that of pure gelatin. The detailed study showed that tensile properties were enhanced up to 65% with 8% JCNCs with gelatin in the composite. Moreover the composite also exhibited high resistance to water and good antimicrobial activities than that of pure gelatin. The outstanding and unusual mechanical properties showed by the composite are due to the formation of a rigid filler network in the gelatin matrix which could be a potential scaffold material for biomedical application such as in wound healing.

KEYWORDS

Biocomposite;
Jute cellulose nanocrystals;
Gelatin;
Nanocomposites;
Biopolymer.

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INTRODUCTION

The production of nano-scale cellulose fibers and their application in composite materials has gained increasing attention due to their high strength and stiffness combined with low weight, biodegradability and renewability . Cellulose nanocomposites may not only show major improvements in mechanical properties but also retain greater optical transparency with respect to other polymer composites[1-7]. In addition, there are abundant and renewable sources of cellulose matter from which micro and nano fibrils can be derived, including jute, flax, hemp, kenaf, cotton, wood, and sisal. Jute, popularly known as the "golden fiber" of Bangladesh, is the cheapest of all natural fibers and has long been used in low-value products such as gunny bags, twine, carpet backing etc. The percentage of crystallinity of jute fibre (73.4%) is considerably higher than other non-woods (52-53%)[8]. The higher crystallinity in jute fiber indicates its suitability in the preparation of micro-/nano-cellulose crystals. It was reported that a controlled acid hydrolysis can produce cellulose nanocrystals with an elastic modulus of 150 GPa, which is higher than that of the S-glass (85 GPa) and Aramid fibres (65 GPa)[9].

Gelatin as a hydrophilic biopolymer specifically interacts with water and undergoes drastic changes of its physico-mechanical properties depending on the moisture content i.e., its poor mechanical properties especially when exposing to wet and humid conditions limit its application in many areas[10,11]. Therefore, gelatin materials for long-term biomedical applications must be submitted to crosslinking using a wide variety of chemical and physical cross-linking techniques, which improves both the thermal and the mechanical stability as well as water-resistant properties of the biopolymer. The traditional aldehyde-based cross-linkers (including formaldehyde, glyoxal, and glutaraldehyde) have shown great efficiency in protein modifications in producing biomaterials, but the toxic nature of these compounds during material biodegradation process prohibits their traditional applications[12-14]. The main focus of this work was to establish an optimum condition for extracting cellulose nanocrystals (JCNCs) from jute fiber. The research also addresses the fabrication of JCNCs reinforced gelatin nano-composites for potential application in biomedical purposes such as tissue engineering in skin substitutes or in wound healing. The details mechanical, thermal, water binding and antimicrobial properties of the composite materials has also been studied and will be discussed in the following sections of this manuscript.

EXPERIMENTAL

Materials

Jute samples were purchased from the local market and were cut into small pieces of about 0.5-1 cm in length, washed thoroughly with the flow of water to remove foreign materials and dried in an oven for 3 hrs at 105±2°C for drying and to remove moisture from it. Gelatin (Type B from cattle bones, Bloom strength-240 g, and Pharmaceutical grade) granules were supplied by the Global Capsules Limited (G.C.L.), Barisal, Bangladesh.

Methods

Preparation of micro and nano crystals from jute fibers

Dried samples were soaked in absolute ethanol (5 mL/g jute) for nearly 12 hours and then washed with distilled water. The washed sample was again dried at 100±2°C in an oven for 2 hours. Wax and fat free dried samples were treated with 10% (w/v) NaOH solution at 60ºC to remove lignin. The black liquor containing lignin was removed by frequent washing with distilled water until all the alkali was removed. The dried sample was bleached with 5% NaOCl (5 mL NaOCl/g delignified jute sample) by constant stirring till the sample became white. Bleaching not only make the sample white but also removed any lignin if remain after delignification. The sample was then frequently washed with distilled water and dried in an oven at 100±2°C for 2 hours. The bleached jute sample was hydrolyzed by 64% sulphuric acid solution at 55-60°C for 5 hrs (9 mL acid solution/g bleached jute samples). After hydrolysis, the sample was frequently washed with distilled water by centrifugation at 10000 rpm for 45 mins to remove free acid. Centrifugation was repeated 5 times. Finally precipitated was collected from the vial of centrifuge and dried in a freeze drier and JCNCs were obtained. The dried JCNCs samples were characterized by FTIR/ATR (Model-01831, Shimadzu Corp. Japan).

Fabrication of JCNC reinforced gelatin nanocomposite

JCNCs were dispersed in DMSO at a concentration of 5-15 mg/mL by ultrasonication. Nanocomposites were fabricated by blending the desired amounts of JCNCs (1-10%) and gelatin solution (90-99%). The mixture was ultrasonicated for 30 minutes for homogeneous dispersion before casting under laminar air flow on silicon paper followed by drying in a vent oven at 50°C. Pure gelatin film without JCNCs will be considered as control. Before various characterizations tests, the resulting films were kept in conditioning desiccators of 43% relative humidity (RH) for one week at room temperature to ensure the equilibration of the water content in the films. The dried films were analyzed by ATR.

Thermogravimetric and differential thermal analysis (TG/DTA)

Thermal degradation measurements of gelatin-JCNCs nanocomposites with various JCNCs contents were carried out using a TG/DTA 6300 (Seiko Instrument, Inc. Japan) system controlled by an EXSTAR 6000 station. Samples of about 2.5 mg kept into aluminum cell were heated in the temperature range of 30-600 °C at a scanning rate of 20 °C/min under nitrogen atmosphere. Differential scanning calorimetry (DSC) measurements were done to understand thermal behavior of gelatin and JCNCs reinforced biocomposites 2-4 g was taken in an aluminum pan and pellet was formed in sample preparation machine under pressure. The pellet was then placed in Differential Scanning Calorimeter (Model: DSC-60, Shimadzu Corp., Japan). The temperature range was maintained from 30 °C to 500 °C and the temperature was increased at a rate of 10 °C/min. the flow rate of nitrogen gas was 20 ml/min. Thus the differential scanning calorimetry of pure gelatin, JCNCs reinforced gelatin composites & JCNCs were observed.

Tensile properties

The tensile properties of the composites were measured by using a Universal Testing Machine (model: H50KS-0404, Hounsfield Series S, UK) with 5000 N load range at a crosshead speed of 20 mm/min. The specimens were cut into dog-bone-shape and then conditioned at 25 °C and 65% relative humidity for 10 days before performing the test. Specimens, type IV (ASTM-D638) had a rectangular cross section of 6 mm and a gauge length of 40 mm with a thickness of 0.3 mm. Values were averaged including standard deviation of at least five specimens.

Water binding capacity (WBC)

WBC of gelatin was measured using a modified method described by Wang and Kinsella[15]. WBC was initially carried out by weighing a centrifuge tube containing 0.5 g of sample, adding in 10 ml of water, and thoroughly mixed in a vortex mixer (VM-2000, Digisystem Laboratory Instruments Inc., Taiwan) for 1 min. The contents were left at ambient temperature for 30 min with intermittent shaking for 5s and then centrifuged at 3,500 rpm for 25 min (Z383K, HERMLE-National Labnet Company, Woodbridge, NJ. USA). The supernatant was decanted, the tube was weighed again. WBC was calculated using following equation: WBC (%) = [water bound (g) / initial sample weight (g)] × 100.

X-ray studies

Wide-angle X-ray diffraction (XRD) data was collected using Bruker D8 Advance Diffractometer D5000, rotating anode X-ray generator from 5-70° of 2θ (Scanning angle), at a rate of 1°/min using Cu Kα radiation generated at 30 mA and 30 kV. XRD was used to study the crystallinity of the pure JCNCs and the composites.

Microscopy

The freeze dried jute cellulose nanocrysatls (JCNCs) and JCNCs reinforced gelatin biocomposites were coated by platinum coating using a JEOL JFC-1600 Auto Fine Coater and the internal structures were inspected at an accelerating voltage of ~25kV using a JEOL JSM- 6490 LA Analytical Scanning Electron Microscope. The optical microscopy images of gelatin/CNWs were taken by an Olympus CKX 41 microscope, Japan.

Antimicrobial activity

Antimicrobial activity of gelatin and JCNCs reinforced gelatin biocomposites against *Escherichia coli* were investigated by the disc diffusion method. This

method was performed in Muller Hinton medium. The media used for antimicrobial activity was poured into sterile petri plate and was allowed to be cooled. Then the test culture (*Escherichia coli*) was inoculated properly onto the media. JCNCs were autoclaved for 2 h 10 m to remove any bacterial contamination. The discs (6-7 mm diameter) were diffused into the pure gelatin and nanocomposite solution with different nano loading and then placed on the *Escherichia coli*-cultured agar plate. Subsequently, these plates were incubated overnight at 37 °C and the inhibition zone was measured for the evaluation of antimicrobial activity of gelatin and JCNCs reinforced gelatin biocomposites.

Statistical analysis

Five replicates were carried out in each experiment of mechanical properties. All data were analyzed by SPSS software, version 15 using one-way ANOVA analysis. The level of statistical significance was set at 5% ($p < 0.05$).

RESULTS AND DISCUSSION

FT-IR and XRD analysis

FT-IR spectra of JCNCs, gelatin and the nanocomposite are shown in Figure 1. The presence of absorption band at 1062 cm^{-1} for C-O stretching of the cellulose component in JCNCs was also observed in the nanocomposite thus indicating the reinforcement of JCNCs with gelatin matrix[16,17]. The addition of JCNCs into the gelatin matrix has slight decreases the intensity of O-H stretching. This may be due to the fact that -OH groups of the surface of JCNCs interacts with the adjacent -COOH groups of gelatin thus ester linkage has formed which appears at 1750 cm^{-1} in the composite films.

The XRD analysis of the JCNCs and the nanocomposites was done to obtain further insight into the materials crystallinity (as shown in Figure S1 in the supporting information). Two prominent peaks are observed at 2θ values of 17.2, 26.4° and less prominent peak at 40.4° were observed in the curve (a) for JCNC which corresponds to the highly crystalline nature of the JCNCs. With the addition of JCNCs in the gelatin matrix, some diffraction peaks appear in the diffractograms. With an increase of the JCNCs content

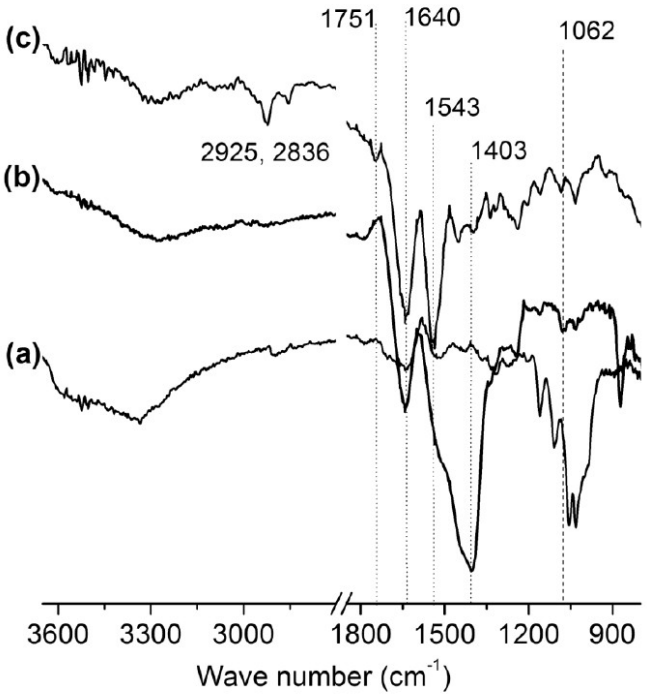

Figure 1 : FT-IR spectra of (a) JCNC, (b) gelatin and (c) JCNC-gelatin composite.

in the films (curve c-e), the peaks become more significant. When the JCNCs content increases to 6-10 wt%, four peaks at 2θ = 11.6, 22.8, 34.2 and 37.4° are observed, which corresponds to the typical crystal pattern of cellulose I.

Thermal analysis

Thermogravimetric (TGA) and differential thermogravimetric analysis (DTG) were carried out in order to investigate the thermal properties of the nanocomposites. The results of the TGA and DTG are presented in Figure 2, which shows residual weight vs. temperature for JCNCs, gelatin and the composite materials. It was obvious that all materials were thermally stable in the region below 250°C. Thus both JCNCs and nanocomposites were able to maintain more than 90% of their original weight at this temperature. It was also observed a slight decrease of weight for all materials below 120°C was due to the evaporation of the moisture. The weight loss of gelatin occurred in steps and the first stage, continuing up to about 120 °C, was related to the evaporation of the absorbed and bound water as well as NH_3 and CO_2 gases from the biopolymers[18,19]. The results also showed that gelatin and the nanocomposites degrade earlier than JCNCs and thermal stability of the nanocomposites was in-

creased after incorporating JCNCs as reinforcing with the matrix gelatin. The weight reduction of the JCNCs seemed to occur more stepwise than the nanocomposites and the JCNCs had higher residual weight at 550°C. It is believed that the main degradation for JCNCs occurred between 200 °C and 280 °C was due to depolymerization, dehydration, and decomposition of glycosyl units followed by the formation of a char, while the degradation above 325 °C could be ascribed to the oxidation and breakdown of the char to lower molecular weight gaseous products. For the nanocomposite film, they showed similar behavior with gelatin in the range of 200-280°C. But it showed that weight loss decreased with increased amount of JCNCs in the range of 120-200°C. From the figure it is also clear that minimum weight loss occurred for the nano film with 6% JCNCs which ensured the higher stability followed by the composite obtained from 8% JCNCs.

The difference in the thermal decomposition behavior of gelatin, JCNCs and nanocomposites can be interpreted more clearly from the DTG curves as shown in small window in Figure 2. From DTG analysis data it has seen that, the rate of mass loss in the temperature range of 120-200°C were 19.8 µg/min for pure gelatin and 15.8 µg/min for JCNCs. But, the curved showed a

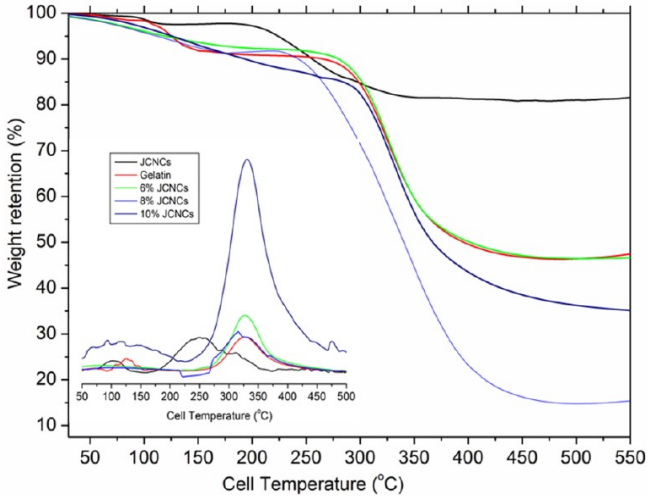

Figure 2 : TGA and DTG thermogram of JCNC and JCNC-gelatin nanocomposites.

difference for the composites than pure gelatin film. With the increment of JCNCs in the composites, the rate of mass loss decreased. The minimum mass loss for 8% JCNCs-gelatin composite film was 4.25 µg/min followed by the composite prepared from 6% JCNCs 5.45 µg/min.

Morphology

Figure 3 shows the SEM images of freeze dried JNCs, gelatin and the nano-composites. Rod like struc-

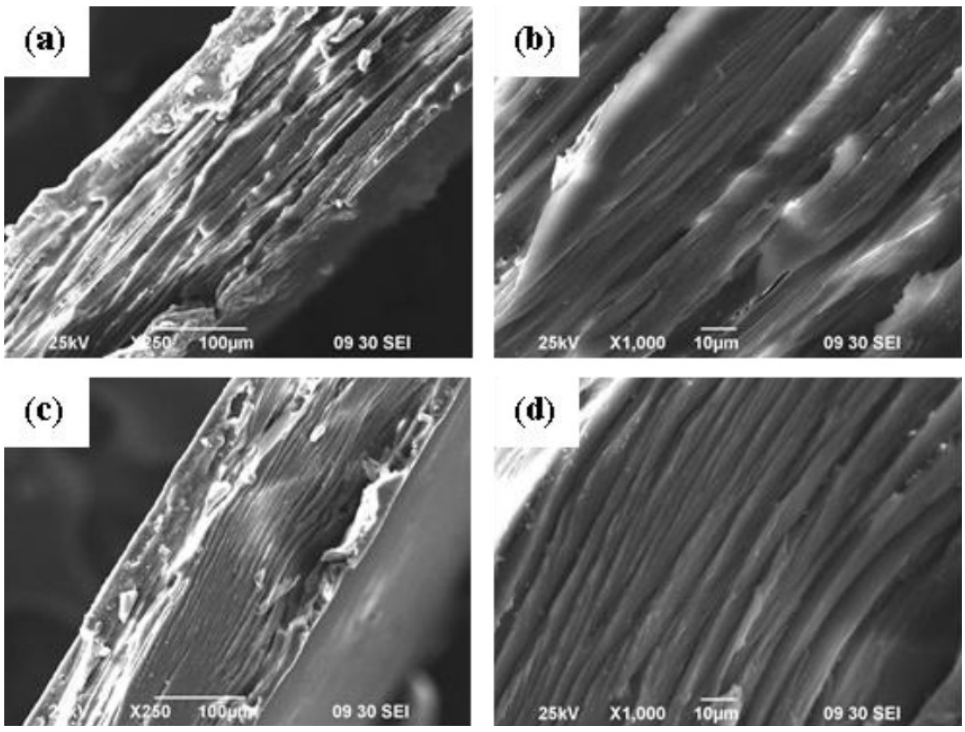

Figure 3 : SEM images of fractured surface of (a) and (b) gelatin and (c), (d) JCNC-gelatin nanocomposite.

ture was obtained for crystalline cellulose on the other hand porous, with a three-dimensional interconnected microstructure are observed for gelatin. In the fracture surface of nanocomposites it was observed that nanocomposites the cellulose crystals are uniformly distributed with the matrix which is essential to obtain good mechanical performance.

The JCNCs appeared white dots in the nanocomposites and its concentration is a direct function of the cellulose content in the composite. The shiny dots correspond to the transverse sections of the cellulose whiskers. It is worth noting that the filler was more or less evenly distributed within the gelatin matrix but some whiskers were not fully individualized and form small aggregates. It could be ascribed to a higher degree of crystallinity of the material and/or strong interactions between the filler and the matrix leading to a fracture path through nanoparticles. The optical micrograph images also showed the slight aggregation of JCNCs for higher loading (more than 4% JCNCs in the composite).

Tensile properties of the nanocomposite

The tensile strength of the pure gelatin film and the nanocomposites were measured and are shown in TABLE 1. It was observed that TS increases with increasing JCNCs content in the composites which indicated good dispersion and strong interfacial actions between the polymer and the JCNCs. Due to the aggregation of the JCNCs within the matrix at higher concentration, above 8% JCNCs in the nanocomposite decreases the TS value. It was observed that only gelatin film showed TS 35.2 MPa however 8% JCNCs in the composite yielded 51.3MPa and the corresponding elongation at break (%) was reduced from 12.394 to 3.83% respectively. The tensile test results indicated that the mechanical properties of the gelatin/JCNCs nanocomposites were substantially superior than that of neat gelatin probably due to (i) the reinforcement effect from the finely dispersed high performance JCNCs nanofillers throughout the biopolymer matrix and (ii) strong interaction between JCNCs and the gelatin matrix due to the possible ester linkages between the –OH group of cellulose and –COOH of gelatin. Gelatin, a hydrophilic biopolymer and it possesses two kinds of functional groups, amino and carboxylic groups and JCNCs contain hydroxyl groups; a strong hydrogen-bond also formed between gelatin and JCNCs. The compatibility and strong interaction between JCNCs fillers and the matrix greatly enhanced the dispersion as well as the interfacial adhesion, thus significantly increases the mechanical properties of the nanocomposites.

Water binding capacity

The water binding capacity (WBC) of gelatin and JCNCs reinforced nanocomposites were monitored and are shown in TABLE 2. The increase of the weight of the sample was due to immersion in water and one can see that incorporation of JCNCs provided a stabililization effect to the matrix. This phenomenon can be ascribed to the presence of a three dimensional cellulosic network that strongly restricts the dissolution of the polymeric matrix in water. Water absorption by gelatin film is significantly higher than the reinforced nanocomposites due to the formation of hydrogen bonds between $–NH_2$ groups of gelatin and water molecules. As the concentration of JCNCs in the composites was

TABLE 1 : Tensile strength and elongation at break (%) of the nanocomposites at different JCNCs loading

JCNCs (%) in the nanocomposites	Tensile strength (MPa)	Standard deviation	Elongation at break (%)	Standard deviation
Pure gelatin (0% JCNCs)	35.206	±3.14	12.394	±1.86
2% JCNCs	40.578	±2.57	8.326	±2.82
4 % JCNCs	44.106	±3.9	6.162	±1.45
5% JCNCs	46.296	±1.6	5.168	±1.02
6% JCNCs	49.578	±0.94	4.23	±0.98
8% JCNCs	51.258	±0.44	3.83	±0.90
10% JCNCs	43.476	±2.92	5.56	±1.29

All data were analyzed by SPSS software, version 15 using one-way ANOVA analysis. The level of statistical significance was set at 5% ($p < 0.05$).

increased, water binding capacity was found to decrease. As discussed earlier, it was observed that the end groups of the gelatin reacted with the cellulose nanocrystals as a result; hydrogen bond forming capacity was reduced to the significant extent. This capacity became lower as the amount of JCNCs is increased in the composites. So, it was observed that the composite for which tensile strength was maximum, water binding capacity was found to minimum. The water binding capacity of pure gelatin film is 18.073% however 8% JCNCs reinforced composite yielded 6.78% WBC.

TABLE 2 : Water binding capacity of gelatin and nanocomposite films

JCNCs (%) in the nanocomposites	Initial weight of (mg)	Final weight (mg)	WBC (%)
Pure gelatin (0% JCNCs)	200	3614.6	18.1
2% JCNCs	200	3060	15.3
4 % JCNCs	200	2920	14.6
5% JCNCs	200	2464	12.3
6% JCNCs	200	2236	11.2
8% JCNCs	200	1804	9.0
10% JCNCs	200	1356	6.8

however the composite obtained from 6 & 8% JCNCs showed better antimicrobial properties than others and the nanocomposites can induce the prohibition of the bacterium growth.

CONCLUSION

The performance of the nanocomposites derived from natural macromolecular proteic materials (gelatin) can be tuned by reinforcing with cellulose nanocrystals for specific needs.

The research described here leads the following conclusion:

Cellulose nanocrystals have been successfully extracted from jute (locally called the golden fiber of Bangladesh) which showed rod like structure in SEM observation. The reaction conditions were optimized to 40% H_2SO_4 (for acid hydrolysis) stirring in a mechanical stirring for 5 hrs at 60°C.

The nanocomposites were fabricated with gelatin by reinforcing JCNCs (2-10%) by weight and various

Antimicrobial activities

Antimicrobial activity of gelatin and JCNCs reinforced gelatin biocomposites against *Escherichia coli* were investigated by the disc diffusion method. This method was performed in Muller Hinton medium. After incubation, the plate which showed an inhibition zone around the hole which was made in the middle of the media was considered that its sample has antimicrobial activity. The results of inhibition zone are shown in TABLE 3.

The results showed that the antimicrobial activities are almost same for the gelatin and the nanocomposites

TABLE 3 : Inhibition zone of *Escherichia coli* for gelatin and JCNCs reinforced nanocomposites

Nanocomposites	Inhibition zone (mm)
Pure gelatin	9
2% JCNCs	10
4% JCNCs	8
6% JCNCs	7
8% JCNCs	7
10% JCNCs	11

physic-mechanical, thermal, antimicrobial properties have been evaluated to find out its potential application in biomedical purposes.

It was observed that the nano-composite derived from 8% JCNCs have showed better mechanical properties, antimicrobial activities, thermal stability than the composites prepared from other JCNCs loading.

As gelatin is a hydrophilic polymer, the introduction of hydrophobic JCNCs reduces the water binding or water absorption properties of gelatin thus makes it suitable for biomedical purposes such as body tissue, scaffold/bandage etc.

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