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Short Communication

Antibacterial Activity of Polypyrrole-Chitosan Nanocomposite: Mechanism of Action

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Abstract

Polypyrrole-chitosan nanocomposite (PPy-CTN) was synthesized by dispersion polymerization of PPy in presence of CTN. Preparation of spherical PPy nanoparticles that were successfully covered with a thin layer of chitosan was confirm by transmission electron microscopy (TEM) technique. Characterization techniques also revealed the presence of CNT in the polymer matrix and its presence was confirmed by the shift of absorption band in fourier transform infrared spectroscopy (FTIR). The prepared polymer was then tested for the antibacterial properties against Gram-negative bacteria; Escherichia coli (E. coli) bacteria. The antibacterial property was assessed by disk diffusion method. The results showed clearly that, PPy-CTN nanocomposite strongly inhibit the growth of wild-type E. coli (20 ± 0.5 mm) followed by PPy (11 ± 0.5 mm) and CTN (2 ± 0.5 mm). The PPy-CTN nanocomposite exhibits a significant enhancement in antibacterial activity better than that of pristine PPy and CTN. In particular mechanisms of antibacterial action in PPy-CTN nanocomposite were discussed.

Keywords: *Polypyrrole, Chitosan, Antibacterial mechanism, Escherichia coli.*

1. INRODUCTION

Conjugated polymers have attracted enormous attentions from both science and technology as semiconductors and electroactive materials for their wide range commercial and technological applications, such as rechargeable batteries [1], sensors and indicators [2], catalysts [3], digital memory devices [4], supercapacitors [5], electromechanical actuators [6], antistatic [7] and anticorrosion coatings [8], fuel cells [9] and recently antibacterial agent [10]. Unique features of polypyrrole (PPy) such as simple and low cost synthesis, adequate level of conductivity, electrochemical and optical properties and excellent environmental stability, captivate the imagination of those involved in intelligent materials research [11, 12]. However, the use of conducting polymers in biological applications is often limited because of their insolubility in common solvents, thereby making the processing difficult [13]. In general, the composites obtained by incorporating a rigid conducting polymer (such as PPy) into flexible matrix of biopolymers can combine the good processability of the matrix and the electrical conductivity of the conductive polymer. Chitosan (CTN), an N-deacetylation product of chitin as one of the most promising natural polymers is a soluble cationic stabilizer in acidic media. Much attention has been paid to CTN in the green synthetic strategy of nanoscale materials due to its excellent properties such as biocompatibility, biodegradability, and nontoxicity [14]. CTN is used in a wide range of applications such as wastewater treatment, separation membrane, food packaging, wound healing, and a drug

delivery system [15]. Polymeric antibacterial agents have the advantages that they are nonvolatile, chemically stable, and find it difficult to permeate through the skin of man or animal and may enhance the efficiency of some existing antibacterial agents and minimize the environmental problems accompanying the residual toxicity of the agents in addition to prolonging their lifetime [16-19, 6, 11]. Therefore, the use of polymeric materials with antibacterial properties gains an increasing interest from both academic and industrial point of view. Conducting polymers have become one of the most attractive subjects of antibacterial polymers investigation in recent years [20, 21, 10]. In recent studies, there are a few researches in the literature have been reported on the synthesis of PPy-chitosan composites and investigation the antibacterial properties. Cabuk et al. [6] reported polypyrrole grafting on to chitosan chemically and the antibacterial activity of the PPy-chitosan copolymer. The antibacterial activity of PPy-chitosan copolymer was stronger than pristine chitosan and PPy alone. Zare et al. synthesized PPy-dextrin nanocomposites and analyzed it for antibacterial activity against Gram-positive and Gram-negative bacteria. The results indicated that the nanocomposites are effective against all of studied bacteria and nanocomposite [22]. In the present work, we report synthesis of PPy-CTN nanocomposite as an antibacterial polymer. Morphology and chemical properties of PAn PPy-CTN nanocomposite was characterized by a number of techniques including scanning electron microscope (SEM), transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FTIR). The biological activity of PPy-CTN nanocomposite was explored against Gram-negative bacteria; *E. coli* (PTCC 1398).

2. EXPERIMENTAL

2.1. Materials

Pyrrole (Py), iron (III) chloride ($FeCl_3$), hydrochloric acid (HCl), sodium hydroxide (NaOH), nitric acid (HNO_3) were phurchased from Merck, Germany. Chitosan (CTN, $C_{12}H_{24}N_2O_9$) with a degree of deacetylation of 75% was prepared from shrimp shells phurchased from Sigma–Aldrich, USA. Py monomer was purified by simple distillation and stored in refrigerator prior to use. All chemicals were of reagent grade.

2.2. Bacteria

The Gram-negative bacteria; *E. coli* (PTCC 1398) provided by the Babol University of Medical Sciences, were used as a test bacterium in the experiments on the antibacterial activity of PPy-CTN nanocomposite. Microorganisms were incubated at 37°C for 24 h on a nutrient agar plate before use.

2.3. Synthesis of PPy-CNT Nanocomposite

PPy-CTN nanocomposite was prepared by the chemical polymerization of Py in the presence of a CTN in a hydrochloric acid aqueous solution (CTN is not soluble in aqueous or alkali solvents and it is soluble in acidic solvents). In a typical experiment, 0.5 g CTN was added to 100 mL of hydrochloric acid (1M) and then uniform solution was resulted by using magnetic mixer for 15 minutes. Then, 5 g of FeCl3 was added to solution and 1 mL fresh distilled pyrrole monomer was added to stirred solution after 10 minutes. The reaction was carried out for 5 h at room temperature. After that, the solution was centrifuged for 30 min at 6000 rpm and precipitation was washed with deionized water and the washing procedure was repeated until the polymerization solution became colorless.

2.4. Characterization

In this study, a scanning electron microscope (SEM) (model XL30) was used to characterize the surface of polymers at very high magnification at an accelerating voltage of 25 KV. Samples were coated with gold and palladium by a sputter coater with conductive materials to improve the quality of micrograph.

The image of transmission electron microscope (TEM) was obtained with a Zeiss - EM10C. Acceleration voltage for TEM was 80 kV. In the sample preparation of TEM characterizations, small amount of the sample

powder was dispersed in an ethanol and was sonicated for 20 minutes. A small drop of this solution was dropped on a Carbon coated copper grid Mesh 300 and was used for TEM characterization.

A study on the chemical structure of polymers was investigated by Fourier Transform Infrared spectrometer (FTIR) (Shimadzu model 4100, Japan). Spectra were collected with a spectrometer using KBr pellets. The ratio of the sample to KBr was 1:100. In each case, 1.0 mg of dried sample and 100 mg of KBr were homogenized using mortar and pestle thereafter pressed into a transparent tablet at 200 kgf/cm^2 for 5 min. The pellets were analyzed with a FTIR Spectrometer in the transmittance (%) mode with a scan resolution of 4 cm^{-1} and 64 scans by spectrum, over the 4000 −500 cm^{-1} region.

2.5. Antibacterial Activity Test

The antibacterial property was measured by powder inhibition zone method against Gram-negative bacteria. *E. Coli*, which is widely applied as a biological index for pollution and contamination in water and instrumentation, was chosen as the standard bacterium to determine the antibacterial properties of PPy-CTN nanocomposite. The agar medium was poured into on sterile Petri plates. After solidification, three wells per dish were made in the agar medium which had 200 µL inoculums fairly well-distributed. Then 100 mgr each of PPy, CTN and PPy/CTN nanocomposite powder was loaded into the wells separately. The Petri dishe was incubated at 37◦C for 24 h. After incubation, the zone of inhibition was measured and expressed as zone of inhibition in millimeter diameter.

Area of zone of inhibition is used as a criterion to ascertain the biocidal activity. According to this criterion, 20 mm zone of inhibition represent significant activity while 10–12 mm inhibition activity is good, 7–9 mm is low, and an inhibition zone below 7 mm would represent non-significant activity.

3. RESUT AND DISCUSSION

3.1. Morphology of Nanocomposite

Figure 1(a–b) show typical SEM and TEM images of PPy-CTN nanocomposite. The SEM image in Figure 1(a) reveals that the resulting product is composed of a large number of spherical PPy nanoparticles that were successfully covered with a thin layer of chitosan. TEM image in Figure 1(b) shows well defined particles which contained the light contrast CTN shell and the dark contrast PPy core. Through TEM observation it can be confirmed that the 50±5 nm PPy nanosphere was covered with very thin CTN shell while the mean thickness of the chitosan shell is 5 ± 1 nm.

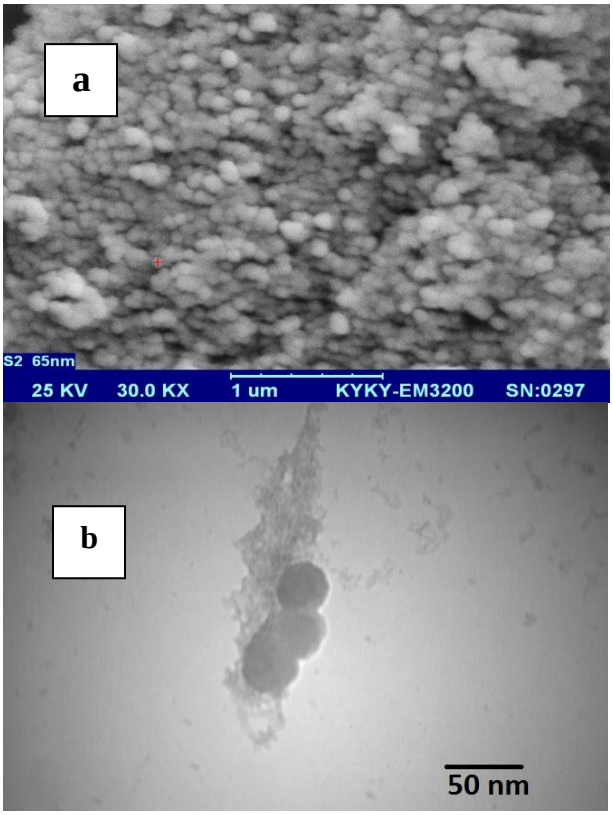

Figure. 1*. Micrograph of PPy-CTN. nanocomposite, a: SEM, b: TEM.*

3.2. Structural Characterization of the Nanocomposite FT-IR Analysis

The chemical structure of the PPy, CTN and PPy-CTN nanocomposite were determined by FTIR spectroscopy, which has provided valuable information regarding the formation of nanocomposite. FTIR spectra of all the materials are depicted in Figure 2 (a–c). CTN showed expected distinctive peaks of typical saccharide absorptions [11, 6]. The

peak at 1630 cm^{-1} corresponds to the C=O stretching vibration of –NHCO– in CTN. The broad peak at around 3438 cm^{-1} belongs to O–H and N–H stretching vibration and the peak at 3031 cm^{-1} corresponds to C–H stretching vibration of the polysaccharide. The peaks at 1564 and 1349 cm^{-1} could be attributed to C=C and C–N asymmetric and symmetric stretching vibrations of the pyrrole ring, respectively. The peak at 830 cm^{-1} belongs to C–H ring-wagging vibration [12, 23] of the aromatic pyrrole rings. In the FTIR spectrum of PPy-CTN characteristic adsorption bands for both CTN and PPy were observed. The peaks corresponding to C–N stretching and C–H ring-wagging vibrations shifted from 1349 cm^{-1} to 1364 cm^{-1} and from 830 cm^{-1} to 867 cm^{-1}, respectively, which could be attributed to the bonding between CS and PPy chains.

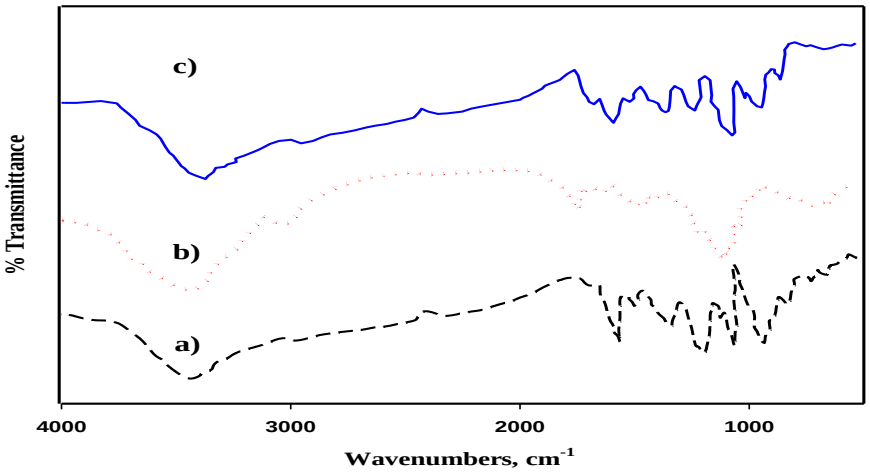

Figure 2. *FTIR spectra of: (a) PPy, (b) CTN and (c) PPy-CTN nanocomposite.*

3.3. Antibacterial Activity

Figure 3 shows the antibacterial activity results of PPy, CTN and PPy-CTN nanocomposite (inhibition zone diameter does not includes the diameter of sample.). White, hazy areas indicate bacterial growth, whereas the more transparent circles surrounding the wells, in the agar, indicate bacterial-free regions, i.e. zones of inhibition. PPy-CTN nanocomposite have showed good zone of inhibition compared with pristine PPy and CTN. Based on result of powder inhibition zone method, antibacterial activity against *E. coli* are in the following order: PPy-CTN nanocomposite > PPy > CTN. PPy-CTN nanocomposite showed maximum antibacterial activity against *E. coli* (20 ± 0.5 mm) followed by PPy (11 ± 0.5 mm) and CTN (2 ± 0.5 mm).

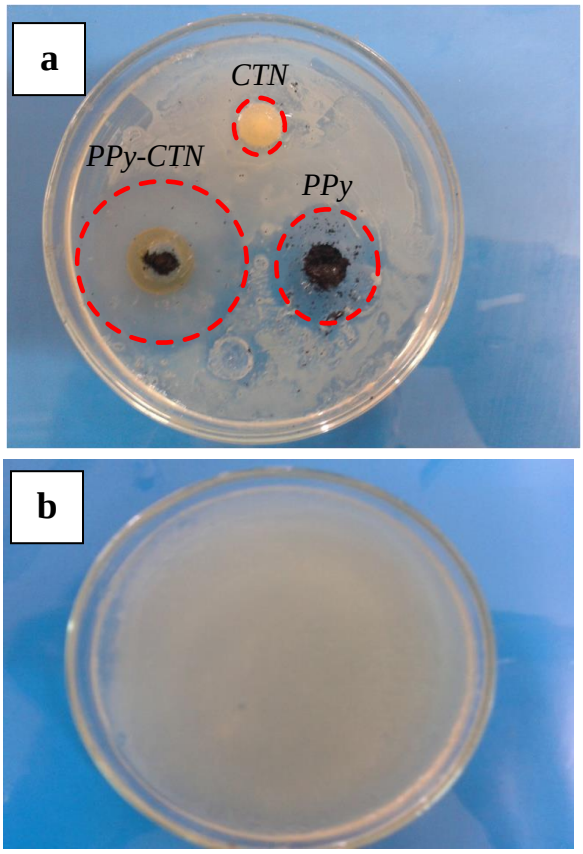

Figure 3. *a) Inhibition zones of PPy, CTN and PPy-CTN.nanocomposite against E. coli, b) conrol sample.*

3.4. Bactericidal Mechanism

It is generally accepted that the mechanism of the bactericidal action of the polycationic biocides involves destructive interaction with the cell wall and/or cytoplasmic membranes [24]. Antibacterial effect of PPy-CTN nanocomposite could be explained by "phospholipid sponge effect" hypothesis [25]. According to this hypothesis, the biocidal action is triggered by the interaction between the negatively charged phospholipids in the *E. coli* cellular membranes and the positively charged surface of PPy-CTN nanocomposite. Mechanisms of antibacterial action of PPy-CTN nanocomposite are depicted in Figure 4. As Figure 4 shows, in step 1 PPy-CTN nanocomposite via electrostatic contact, made direct binding to cell wall components and subsequently at step 2, leads to disruption of the *E. coli* cell wall membrane and leakage of critical cell contents and cell death.

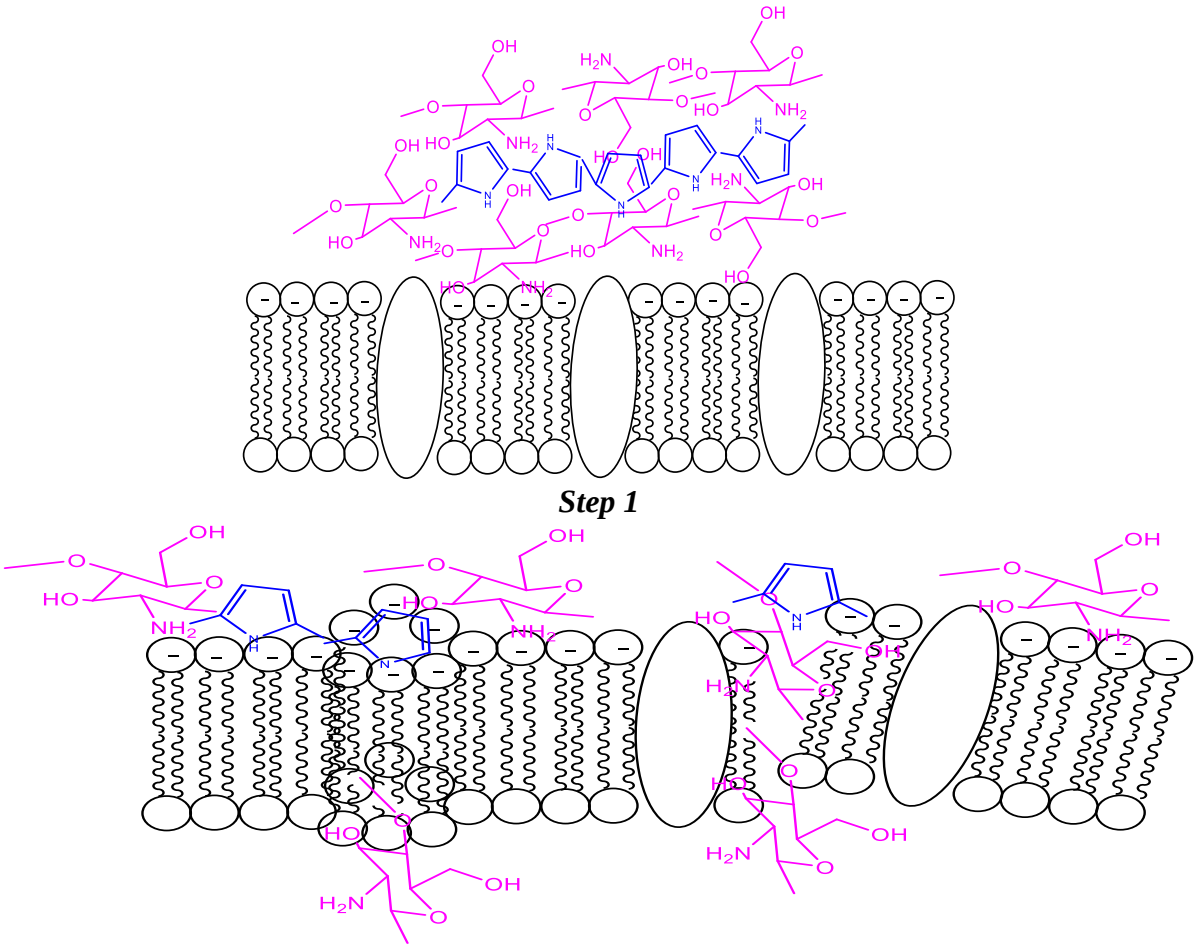

Figure 4. *Mechanisms of antibacterial action of PPy-CTN nanocomposite.*

4. CONCLUSION

In this paper, we reported the syntheses of PPy-CTN nanocomposite by chemical in-situ polymerization. The product polymer was characterized by a number of techniques including FTIR, SEM and TEM. Characterization techniques confirm preparation of PPy-CTN nanocomposite. Characterization techniques revealed the presence of CTN in the polymer matrix and its presence was confirmed by the shift of absorption band in FTIR. The antibacterial property was assessed by disk diffusion method. PPy-CTN nanocomposite has showed good zone of

inhibition against *E. coli* microorganisms. The PPy-CTN nanocomposite exhibits a significant enhancement in antibacterial activity better than that of pristine PPy and CTN. Finally, it is anticipated that the prepared biocompatible antibacterial nanocomposite would be of great help in the field of biomedical applications and biological water treatment.

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