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Synthesis and characterization of bacterial cellulose/PAni composite for antibacterial and biomedical application

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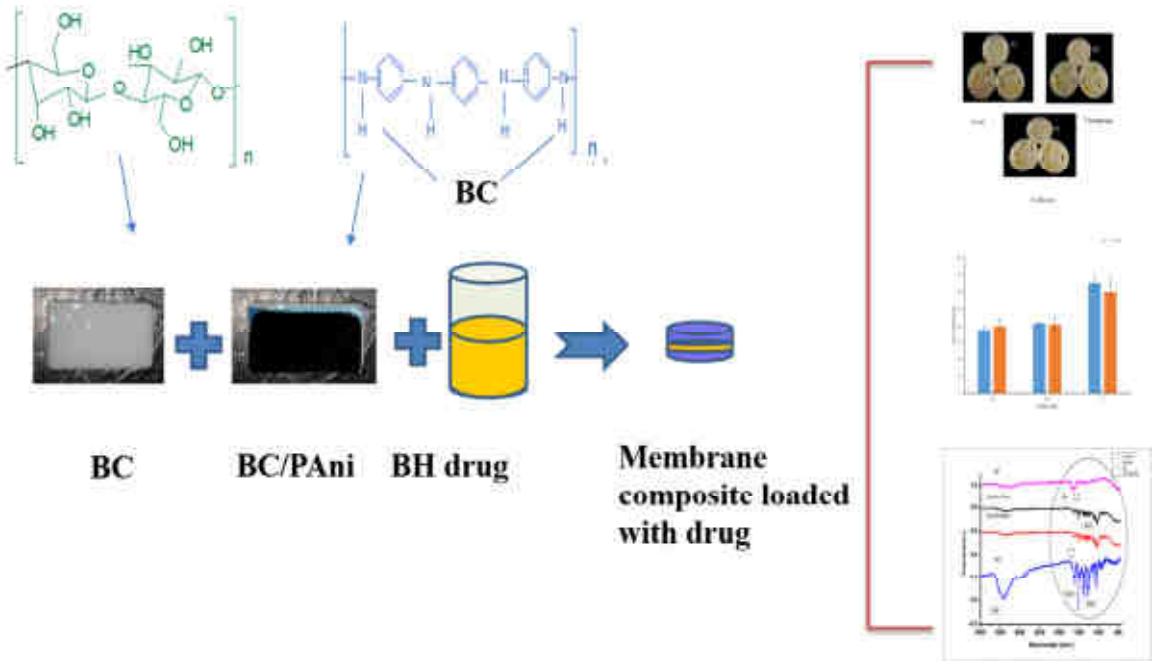
ABSTRACT

In this study, a hydrogel composite based on bacterial cellulose (BC) and polyaniline (PAni) was synthesized and characterized and loaded with berberine hydrochloride (BH) drug through chemical reduction method. The polymerization of aniline on BC fibers occurred *in situ* in the presence of ammonium persulfate (APS) to form BC/PAni composite. BC/PAni composite was characterized for various structural and physico-chemical properties through scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FT-IR), and thermal gravimetric analysis (TGA). According to XRD analysis, the drug was found and covered the composite. SEM images showed the favored shape of drug as triangle which is a benign shape for antibacterial activity. The antibacterial activity of the obtained BC/PAni/BH composite was also evaluated against Gram positive bacteria *Pseudomonas auroginosa* (*P. auroginasa*) and Gram negative bacteria *Escherichia coli* (*E. coli*), and fungi *Candida albicanis* (*C. albicans*) using the paper disk diffusion method. BC/PAni composites loaded with BH were found to be effective against bacteria and fungi compared to pure BC, BC/PAni, and BC/BH. The cytotoxicity test used indirect contact method indicated that BC and BC/PAni hydrogel composite had good biocompatibility.

Key words: Bacterial cellulose; polyaniline; Composite, Antimicrobial activity; cytotoxicity

Graphical Abstract



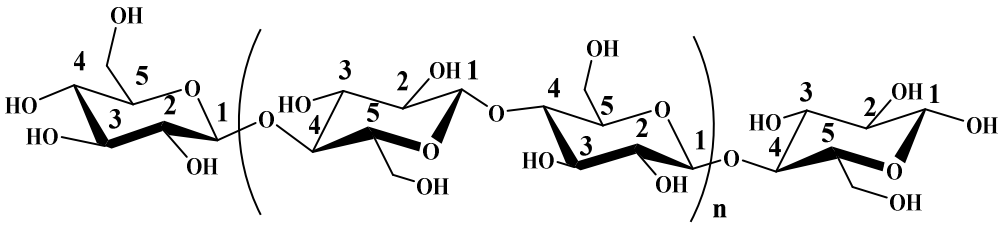
1. INTRODUCTION

Polymer nanocomposite materials have emerged as suitable alternatives to overcome limitations of nanocomposites for biomedical application [**Ray et al., 2003].** In addition to all the advantages, and the application of polymers in medicine also brings relevant to the occurrence of a problem of nosocomial infections. Therefore, a large it has made effort to develop polymers or composites with efficient is antibacterial

properties. In addition to the polymer materials which owns intrinsic antibacterial activity, and these properties can be also it achieved through (a) for coating or adsorption antibacterial agent on the surface of the polymer. (B) Crippling agent antibacterial polymer with ionic or covalent bond, or (c) through direct incorporation of anti-bacterial in the polymer material through in synthesis **(Zdenka Kucekova et al 2013).** In recent years the advances in synthesis techniques and the ability to characterize materials on atomic scale has led to a growing interest in nanometer-size materials.

Bacterial cellulose (BC) (Fig. 1) is mainly produced by various bacterial genera including *Acetobacter, Agrobacterium*, *Rhizobium*, *Pseudomonas*, and *Sarcina* (**Shoda and Sugano 2005**). It is widely used in different fields because of its many excellent characteristics like its biocompatibility, good water holding capacity, slow water release rate, high crystallinity, and high mechanical properties etc. (**Ul-Islam et al, 2012**). Over the past years, it has gained interest in the field of tissue engineering, which is being studied by several research groups as a scaffold for cartilage, dressing the wound, dental implants, and vascular grafts have already been reported [**Alvarez et al., 2004; Sarno, *et al*., 2005; Huber et al., 2006],** especially for medical devices. However, the broad spectrum applications of BC have been limited by several inadequacies associated with BC such as low biocompatibility and lack of bactericidal, antioxidant, conducting and electromagnetic properties etc. These limitations necessitate the development of composites of BC with other materials to improve such properties to BC. To date, composites of BC have been prepared with various materials including polymers such as polyaniline (**Jasim et al., 2017**), gelatin (**Khan et al., 2016**), chitosan (**Ul-Islam et al., 2011**) and nanoparticles such as zinc (**Ul-Islam *et al*, 2014**), titanium dioxide (**Ullah et al., 2016b**), and silver (**Maria et al., 2010**) etc.

Bacterial cellulose in the medical device unfounded recently gained a great deal of attention for increase tissue engineered interesting products. Cellulose structure of bacteria materials can be engineered over length scales ranging from nano to macro by controlling the biofabrication process which bring a biosynthesis role in in vivo performance of biomaterials. Composites of BC have been developed by coating its nanofibrils with several polymeric matrices or by *in situ* polymerization of monomers on the BC nanofibers **[Gabriel et al., 2013].** To enhance its biocompatibility. Similarly, in order to enhance the electrical conductivity of BC, its nanofibrils have been coated with intrinsically conductive polymers **[Shi, et al., 2014].** For instance, polyaniline (PAni) is a well Polyaniline, an intrinsically conducting polymer, has received great attentions because of its wide potential for various technological applications, such as sensors, electro rheological fluids, anticorrosion coatings and good environmental stability**[Cruz-Silva *et al*., 2006; Sarno *et al*., 2005].** Polyaniline are one of the most used supporting materials for BC created by coating PAni on its surface as core/shell nano composites in order to improve the electrostatic properties of BC. PAni polymers can be prepared by the polymerization of aniline in acidic medium, resulting in the formation of polyaniline (PAni). Many strategies have been developed for the synthesis of such composites **[Steyskal et al., 2002].** Influence of polymerization parameters on the molecular weight of polyaniline has been studied **[Vilnik et al., 1998]** and a new aggregation mechanism for shape and aggregation control of PAni with Nanoparticles was suggested by **[Li et al., 2006].** And Zdenka Kucekova *et al*., 2014 study on to enhance antibacterial activity of polyaniline



Cellulose

Figuer.1 molecular of bacterial cellulose

Although, composites of BC with PAni prepared through different strategies have shown considerable increase to properties of BC, it can be further enhanced by using other ionic possessing to enhance of BC electrostaticaly. For example, Berberine hydrochloride can be doped into the matrix as these possess antiflamation, antibacterial, anticancer, and antidiabetic. Further, recent studies have reported that incorporation of BH into the matrix enhance the antimicrobial properties of the polymer. Similarly, several studies have reported the incorporation of BH into BC using Gamma irradiation treatment could modify the bacterial cellulose surface properties and enhancing its potential for biomedical applications. **[Huang et al., 2013]** has been reported by he used a carrier of the berberine hydrochloride and berberine sulfate to the BC surface to produce new uncensored release system.

The aim of this study is to synthesize a novel hydrogel composite based on berberine hydrochloride coating by BC and PAni polymers. The berberine hydrochloride are impregnated into the hydrogel composite and heating for 30 min, a fast, simple and low cost method. Finally, the antimicrobial property of the obtained composite is evaluated against pathogenic bacteria and fungi by using the agar disk diffusion method. The morphological changes occurring during the polymerization process were monitored by FE-SEM, FTIR, and XRD and TGA analyses. Antimicrobial assay measurement was carried out to investigate the zone of inhibition on pathogenic strains. And biocompatibility of BC, BC/PAni composite membrane in the cell line from human. This combination of BC with berberine hydrochloride and biocompatible polymer (PAni) as excellent antimicrobial assay and biocompatible membrane material for potential applications in biomedical development. To our knowledge, this is the first study to present an interrelation between the morphology, biocompatibility properties and the antimicrobial activity of a BC/PAni composite.

2. MATERIALS AND METHODS

2.1 Materials

Bacterial strain *Acetobacter xylinum* (ATCC53582) was purchased from the American Type Culture Collection (ATCC), *psedomonus auroginosa*, *E. coli*, and *candida albicanis* all the bacterial strains were obtained from the laborato. Hela cells obtained from Prof. Xianqin Zhang's lab, College of Life Science and Technology, Huazhong University of Science and Technology (Wuhan, China).L-02 cell line obtained from Prof. Pin Zhou's lab, Tongji Medical School, Huazhong University of Science and Technology (Wuhan, China) aniline hydrochloride was purchased from sinopharm company china**,** HCl was obtained from Xinyang City Chemical Reagent. Yeast extract and peptone were provided from Beijing Shuangxuan Microbe Culture Medium Products Factory (China). Berberine hydrochloride API Sichuan Guangda Pharmaceutical Co., Ltd. Purity > 98%. Dulbecco modified eagle medium (DMEM), fetal bovine serum (FBS), and trypsin-EDTA (TE) were products of Gibco. Penicillin/ streptomycin mixture was obtained from Gibco. Phosphate buffered saline (PBS) solution was supplied from Hyclone, and CCK-8 from Dojindo.

2.2 Preparation of BC antibiotic disc

Gluconacetobacter xylinus ATCC 53582 (American culture type group, Manassas, VA, USA) was grown on HS medium prepared by dissolving in 2% glucose, 0.5% (w / v), 0.5% (w / v) peptone, 0.27% (w / v) sodium phosphate (Na 2 HPO), and 0.115% (w / v) citric acid for 6 days. BC sheets were prepared in sterilized rectangular containers (5mm) using a static incubated method, where by HS was inoculated with 3 % pre-culture and incubated under static conditions at 30 ^{0}C for 7 days by**[Shi et al., 2014]**. The BC sheets were then treated with 0.1 M NaOH at 121 ^{0}C for 15 min, followed by washing with distilled water. The sheets were stored at 4 ^{0}C before use in composite synthesis.

2.3 Preparation of BC/PAni antibiotic disc composites

The BC hydrogels was immersed in the aniline/ hydrochloric acid solution prepared by mixing 0.02 mol aniline and 0.06 mol hydrochloric acid in 500ml pure water, and 0.1 M KCl in deionized water at ambient temperature for 24h enabling the aniline to fully infiltrate through the network of BC hydrogels. The BC hydrogels were dipped into a 500 ml 0.2 mol/L ammonium peroxydisulfate solution (APS) for 30 time, at room temperature for the loading of APS as well as their in situ and wash several time with D.W to reach ph. 7.

2.4 Drug loading of BC and BC bionanocomposite film

Berberine hydrochloride is an antimicrobial cationic surfactant, which is widely used for commercial antiflamation antibacterial, being effective against Gram positive bacteria. BC does not have an antimicrobial property, but due to its high water holding capacity and porosity, it can absorb and slowly release the antimicrobial solution. The drug loading capacity and steady release of berberine hydrochloride, as well as the antimicrobial capacity of the drug-loaded BC, and BC/PAni composite were tested. BC/PAni composites were prepared by immersing the never-dried BC disk into a drug solution berberine hydrochloride (BH) (0.005 g/ml in phosphate-buffered saline (PBS),) heating for 30 min at followed by freeze drying. Berberine hydrochloride was used as a model drug and loaded to the hydrogels disk (BC and BC/PAni) using the Heating diffusion method. Hydrogel samples were soaked in a 100 mL volume of drug solution (0.005 g/mL) in phosphate-buffered saline (PBS), pH 7.4, for 30 min. The discs of hydrogels were removed from the solution, washed with distilled water, and freeze drying for 48hr.

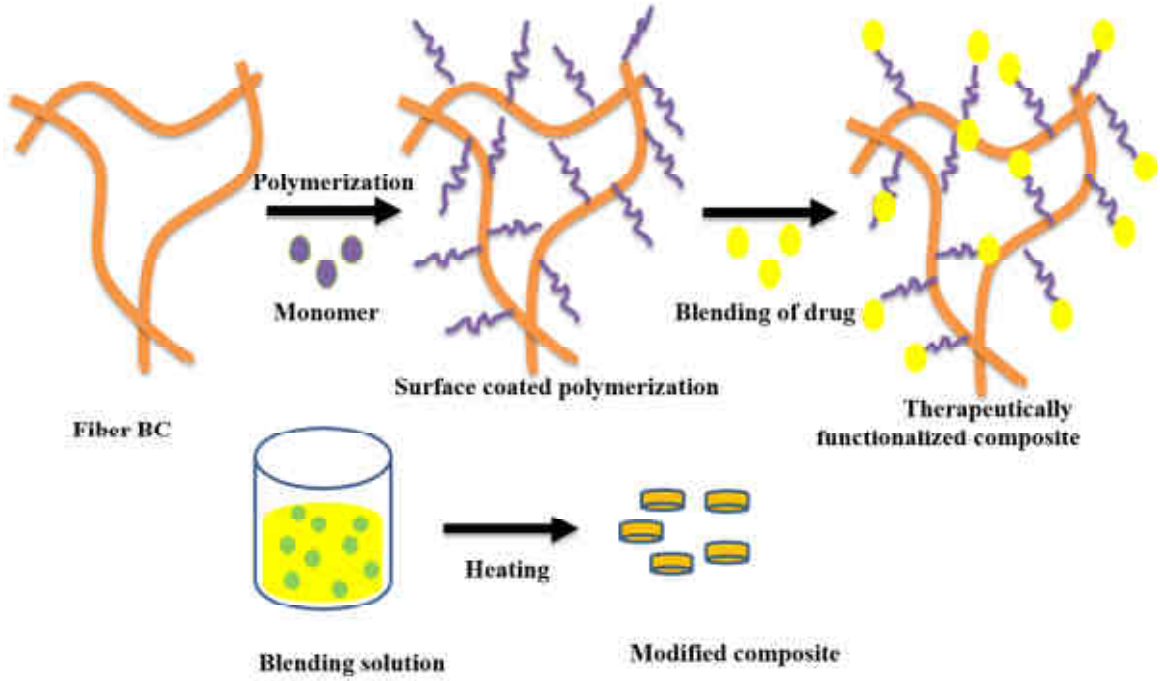


Fig. 3.1 Schematic illustration for the synthesis of the BC/PAni composite. Treated with Drug impregnation method berberine hydrochloride (BH)

2.5 Characterization

The bio composite films were evaluated, SEM and FT-IR techniques, TGA and XRD, Antimicrobial assay, and Biocompatibility.

2.5.1 Fourier Transform Infrared (FT-IR) spectroscopy

Infrared spectroscopy is one of the most powerful analytical tools, which provides the possibility of chemical identification. FTIR spectra of freeze-dried samples was recorded using a Vertex FTIR spectrophotometer (FTIR, VERTEX 70, Germany; spectral range 4000-400cm^{-1}; beam splitter: Ge-coated on KBr; detector: DTGS; resolution: 0. 25 cm^{-1} (step selectable)] to determine the chemical structure of the composite films and possible interactions between their components. The obtained IR data was transferred to PC to acquire spectraspectrum of a chemical substance is a fingerprint for its identification.

2.5.2 Field-emission scanning electron microscopy (FE-SEM)

The surface morphology of freeze-dried samples BC, BC/PAni, and BC/BH/PAni was observed through FE-SEM. Briefly, samples were fixed on a brass holder and coated with gold on a Cu SEM disk and analyzed through a Nova NanoSEM450 FE-SEM (Nova NanoSEM450, FEI, Holand)

2.5.3 X-Ray Diffraction study (XRD)

The XRD pattern of the BC without treated with drug and the BC, and BC/PAni treated with drug samples were recorded using an X-ray diffractometer with an X-ray generator (3 kW) and anode (LFF Cu). The radiation was CuK-α at 1.54 A˚, the X-ray generator tension and current was 40 kV and 30 mA, respectively, and the angle of scanning varied from 0 to 80°.

2.5.4 Thermal Gravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) was performed using a thermal gravimetric analyzer (Shimadzu, TGA-50) on about 5 mg samples. The samples were heated in open alumina pans over 25ºC -650ºC at a heating rate of 10ºC/min under air flow.

3. Antimicrobial assay

The antimicrobial activity of berberine hydrochloride loaded BC, BC/PAni disc was tested against of Gram-positive *Psedomonus auroginosa* and Gram-negative bacteria *(E. coli)* and yeast *(Candida albicans)* on Muller hinton (ML) agar plates by the disc diffusion method. The Mueller-Hinton agar plates were spread with a test culture suspension and the berberine hydrochloride loaded BC, BC/PAni discs were placed on the plates. The

discs were slightly pressed and kept for diffusion at 4^0C in the refrigerator for 30 min. The plates were examined for a possible clear zone of growth inhibition after incubation at 37 ^{0}C for 24 h.17 [**Bhavana et al., 2014].**

4. Cytotoxicity Study.

4.1 Cell culture

Both Hela cells and L-02 cells were cultured using DMEM containing 10% fetal bovine serum(fbs) and 1% antibiotics (100 mg/ml streptomy and 100 U/ml penicillin) and were maintained at 37℃in a humidified atmosphere with 5% CO_2 They were subculture every 2 days when they reached 90% confluence by digestion with trypsin/EDTA .

4.2 CCK-8 assay

The cytotoxicity test were used indirect method according to ISO 10993-5 .The BC/PAni composites and pure BC were sterilized by atouclaving and then used to prepare the extract. Five pieces of small round sterile BC / PAni and pure BC with diameter around 7 mm immersed in cell culture medium at 37℃ for 72 h. The precipitates were removed after centrifugation at 1000r rpm for 5 min and then the supernatant was filtrated with 0.22μm filter membrane.CCK-8 assay was followed with the manufacturer's instruction. Briefly, cells were seeded 1×10^4 cells/well in 96-well plate and incubated at 37℃ for overnight. The medium was replaced with the extract, using DMEM as a control. After incubation for 24, 48 and 72 h, the cells were treated with 10 μl/well of CCK-8 solution under shaking for 5 min and incubated for another 30 min. The OD values were measured in triplicate at wavelength of 450 nm using a microplate reader (Multiskan Go,Thermo Fisher,USA). The cell viability was calculated by the following equation:

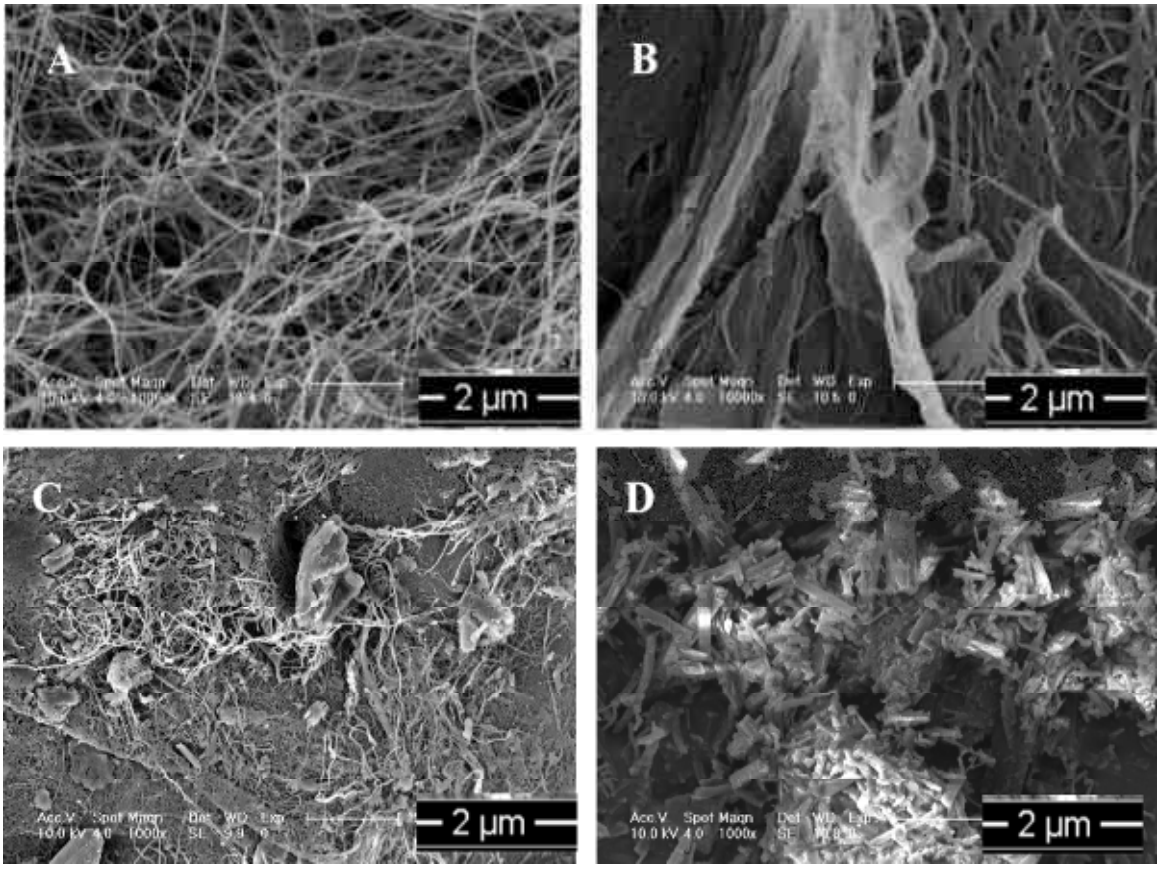
Cell viability= $\frac{OD_S - OD_B}{OD_N - OD_B} \times 100\%$

5. RESULTS AND DISCUSSION

Synthesis and structural characterization of the BC, and BC/PAni composites during cultivation, *G. xylinum* were used to synthesize BC-PAni composite membranes produces a homogeneous and moldable cellulose pellicle, which has a highly swollen fiber network and an extensive interior surface area. The three-dimensional porous structure of the BC pellicle makes it a hydrophilic hydrogel, **(Shi et al, 2016; Klemm et al. 2001).** Therefore, we propose that dipping the cellulose pellicle into PAni will allow the multiple layers of adsorbed water molecules surrounding the polyglucosan chains to be replaced by new bonds between the cellulose and PAni to test this, we use morphological test by

5.1 Morphological analysis of pure BC and BC/PAni treated with drug.

Field emission FSEM (FEI Nova Nano-FSEM 450).450, HUST Analysis Center) observation was carried out to analyze the morphology of the constructs and confirm the incorporation of PAni into the BC network to enable the morphology characterization BC, BC/PAni in terms of shape, matrix and roughness. Typical micrographs of the BC and BC/PAni after treated with drug are shown in Figure 3.



Figuer.3. Field-emission scanning electron microscopy images of (A) pure BC, (B) BC/PAni composite hydrogel: BC/BH/PAni (C), and berberine hydrochloride powder (D).

The FEG-SEM micrographs of a dried BC and BC/ PAni composite surfaces are shown in Fig. 2. The BC membrane was formed by a network structure of long cellulose nanofibers with high aspect ratio (Fig. 3A) as described in [**Mu ller et al., 2011].** In both BC/PAni composites the presence of PAni monomer entangled with cellulose nanofibers can be observed (Fig. 3B). The micrographs revealed that PAni was constituted of particles with mean size of 10 nm merged together to form a uniform layer that completely coated the nanofiber surface. The polymer coating adhered to the nanofiber and a continuous conducting network was formed which was responsible for the high electrical conductivity values. Additional in the figure 3B,C shows the SEM images of BC/PAni hydrogel before and after treated with drug (BH). SEM images indicate that BC/PAni surface morpholgy was changed as BC. There are very small fiber in BC/PAni film surface in the image of (C) but after treated with drug, fibers had become thinner fibers, as shown in. Fig3(C) with drug. In addition, these fibrous structures may be an indication of the interaction between BC and PAni and drug.

5.2 FTIR spectroscopy characterization

The chemical interactions between unmodified BC and BC/PAni before and after treated with drug berberine hydrochloride were explored by FTIR technique (Fig. 4). The pure BC sample showed typical peaks of bacterial cellulose, with distinctive peaks of the cellulose chains at 3347 cm $^{-1}$ (hydroxyl groups and inter- and intra-molecular hydrogen bonds)**,[(Ul-Islam et al., 2013; Ullah et al., 2016]** 1635cm^{-1} (carbonyl groups of the glucose), 1053 cm^{-1} (C-O-C stretching), and 899 cm-1 (β- glycosidiclinkages). For the drug powder (BH) the spectra of the drug display several characteristic peaks, at 1500 and 1600 cm^{-1} **[Ifuku et al., 2007]** , associated with the benzene ring, and at 1380 cm^{-1}, due to the C-H_3 bond stretching vibrations, which are absent from the pure BC. In the composites BC/BH, no additional peaks attributable to the formation of a complex appeared, but variations in the relative intensities of the characteristic peaks for cellulose and the drug can be observed **[Li et al., 2013**]. The spectra for the sample of BC/PAni treated with drug were analyzed using FT-IR and the spectra were compared as seen Figure (**4.1.2.2).** The polyaniline was incorporated into the BC., was showed three peaks at 1000 cm^{-1}, 1460 cm^{-1} and1560 cm^{-1}, which were assigned to quinoin group and penzyle group, respectively and there is It is important to remember how PAni we observed that the intensity for the 3490 cm^{-1} band, which

is attributed to the hydroxyl group (OH), was disappeared in BC/PAni the absorption spectra of BC/PAni composites did not show any N-H stretching absorption. Thus, we can said that BC may have reacted via dehydration condensation with PAni, leading to cause the disappearance of O-H and N-H bonds absorption as reported previously **[Shi et al., 2012].** Thus, BC may have reacted via dehydration-condensation with PAni and may result in the disappearance of O-H and N-H bonds The FT-IR spectra of the sample of BC/BH/PAni the absorption characteristic bands of BC, PAni and BH presented in figure were compared with spectra of pure BC hydrogel of PAni, BC and PAni presented elsewhere . The spectrum of all five samples of composites showed three peaks at 1560 cm^{-1} and 1160 cm^{-1}, which were assigned to quinine and penzyle, respectively (Q represents quinonoid and B represents benzenoid moieties.

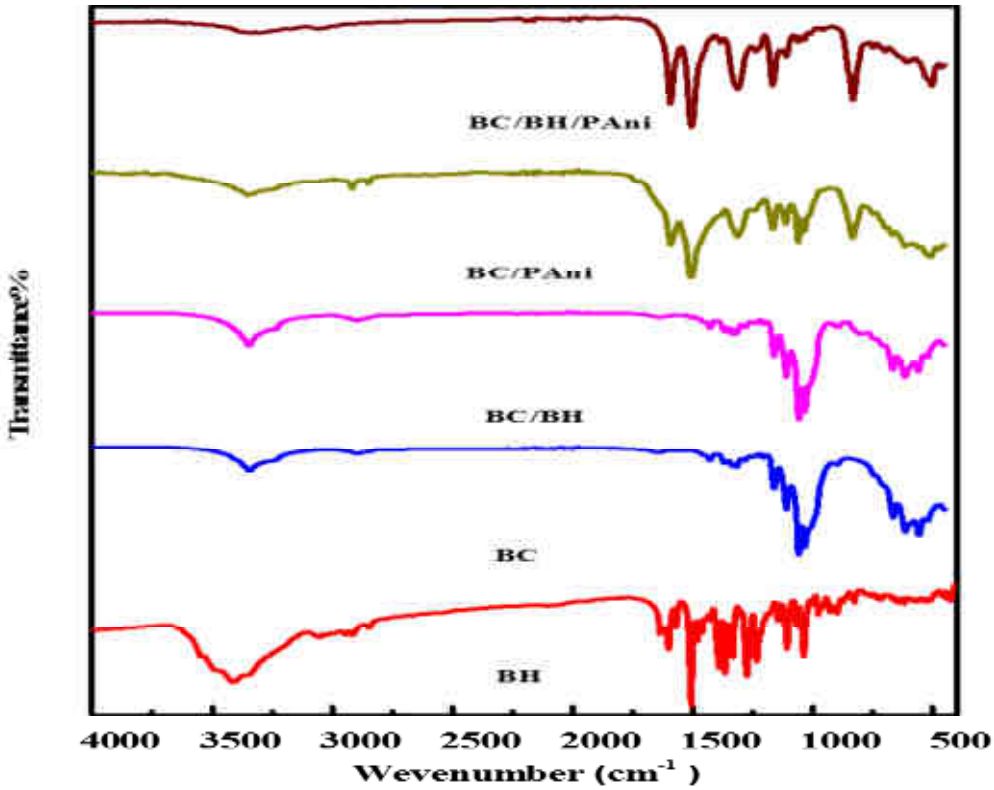


Figure 3. FTIR spectra of Drug loaded BC and polyaniline hydrogel; BC, Drug berberine hydrochloride (BH), Drug loaded BC (BC/BH), BC/PAni; and Drug loaded BC/PAni (BC/PAni/BH) at PH 7.

5.3 X-ray Diffraction study (XRD)

The crystallographic structure of BC and BC/PAni hydrogel films treated and untreated with drug were determined by XRD. As presented in Figure.4 the XRD pattern of BC, BC/PAni hydrogel composite are shown in Figure3. The XRD result confirms the formation of nanocomposites. Belonging the present XRD pattern to the BC/PAni composites. By analysing the BC hydrogel, the three main characteristic peaks for pure BC, at namely 14.18o; 16.74o and 22.60 which correspond to the (110), (110) and (200) cellulose diffraction planes respectively, are visible **[French, 2014].** Regarding the BC/PAni composites it is visible that the BC peaks disappear almost completely, becoming much more tenuous on the XRD pattern. Overall, the hydrogel corresponds to an amorphous material, having some similarity to the PAni pattern (to the exception of the broad peak at 26.61o being unnoticeable). This reinforces the conclusion that PAni are coated cellulose fiber **(Wang et al., 2013).** And after treatment with drug we can conclude the physical morphology can explain that the drug is molecularly dispersed in the polymer matrix or occur as an ionic interaction complex. The berberine hydrochloride has crystalline peaks which are indicated by the presence of sharp intense peaks. The polymers used also have sharp peaks, indicating the presence of their semi crystalline nature. The binding of berberine hydrochloride in the hydrogel composite still shows the presence of sharp peaks as seen in the XRD for the

BC/PAni/BH hydrogel composite. We can thus conclude that the berberine hydrochloride is present in its crystalline state in the composite hydrogel.

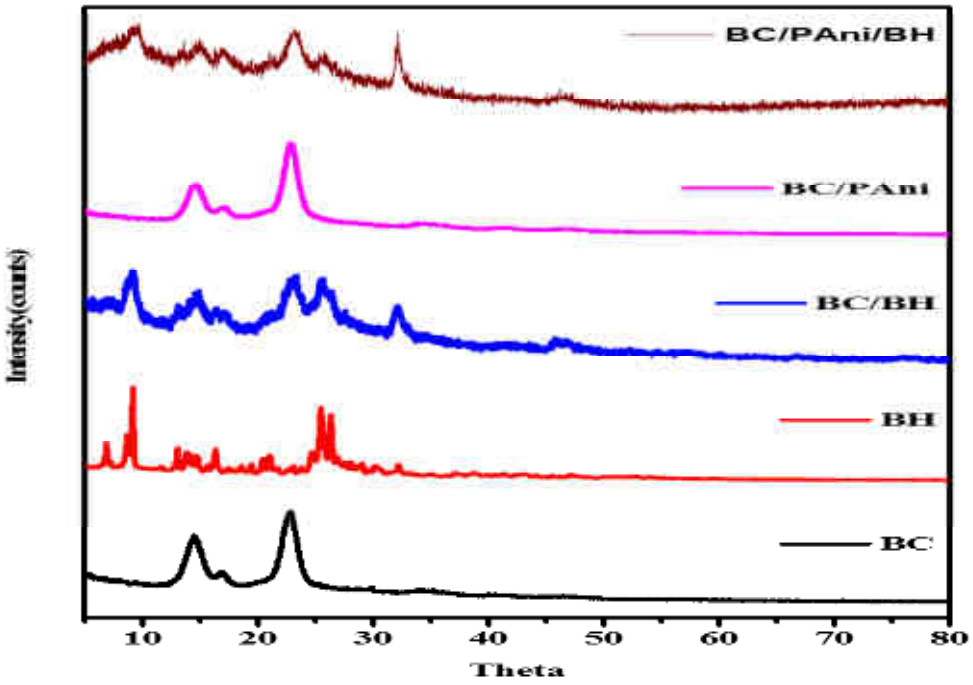


Figure 4. X-ray diffraction patterns of BC and BC treated with drug (BC/BH), BC/PAni hydrogel and BC/PAni treated with drug(BC/PAni/BH), sample were produced under static conditions at 30°C and pH 7.0.

5.4 TGA analysis

. The TGA of the BC, and BC/PAni hydrogels was determined to evaluate the effect of grafting on the thermal degradation behavior of the hydrogels. The Figures 5 and 6 show (TGA) thermograms of BC and BC/PAni composite before and after loaded with drug. Respectively. The curves show that BC/PAni has slightly higher thermal resistance than BC. TGA curve for BC has a softening temperature at 200o C and degradation initiation under 210oC and for BC/PAni degradation initiation glycosyl units, and the last by the formation of a charred residue. The TGA spectra of the different samples are presented in fig.5. Two thermal degradation phases could be registered, from room temperature to about 230 C, **[Sotica et al 2013; Li, et al., 2014]** and 240 C to 380 C. **[Figueiredo et al., 2013].** The first phase was attributed to the loss of water and the 2nd phase to the degradation of the main sample components (BC and BC/PAni). In the curve of composite BC/PAni indicates as can be observed in Figure 5, the TG curve of BC/PAni composite shows that weight loss occurred in three different stages and higher than BC. During the initial stage from room temperature to 100ºC there is a weight loss assigned to the evaporation of the water present in the composite. From 210º to 400ºC the samples undergo a strong weight loss, which can be explained by the burning of BC composite. The final step in weight loss from 400 to 650ºC can be attributed to the thermal oxidative degradation of polyaniline (PAni). This thermal behavior is in concordance with results obtained by [**Mo et al., 2009; Stejskal *et al*., 2008; and Hu *et al*., 2011]** Indicating that the thermal stability of the composites BC/PAni loaded and non-loaded with drug is larger than that of pure BC and BC treated with drug.

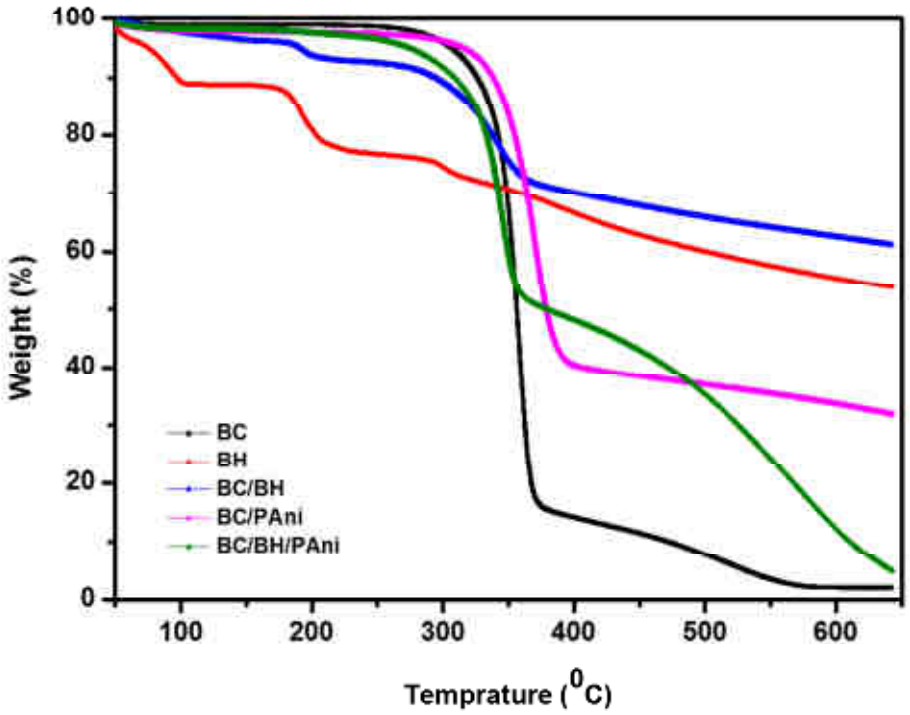


Figure 5 TGA curves of pure BC, BC-PAni, Drug loaded BC /polyaniline hydrogel; BC, Drug berberine hydrochloride (BH), Drug loaded BC (BC/BH), BC/PAni; and Drug loaded BC/PAni (BC/BH/PAni) at PH 7.

5.5 *In-vitro* antimicrobial activity of drug loaded BC, and BC/PAni discs

Demonstrates a qualitative antibacterial activity against *E. coli* and *P. auroginosa*, and *candida albicanis*. During the disc diffusion method, the impregnated berberine hydrochloride immediately begin to diffuse outwards from the nanocomposite disc. The BC/PAni with higher berberine hydrochloride concentration (0.005g/ml) exhibited antimicrobial activity against *E. coli, P. auroginosa* and *candida albicanis*. Based on the diameter of the inhibition zone, a higher antimicrobial activity was remarked against C *albicanis and E. coli* as showed in (table. 1). No inhibitory zone was observed on BC, BC/PAni non treated with drug on any type of bacteria Hence, it could be concluded that the inhibitory activity was attributed to berberine hydrochloride as use BC and BC/PAni as carrier for drug the disc did not produce any inhibition zone and the composites from BC/PAni and BC .On the other hand, clear inhibition zones or areas of no growth were produced by the BC/PAni.

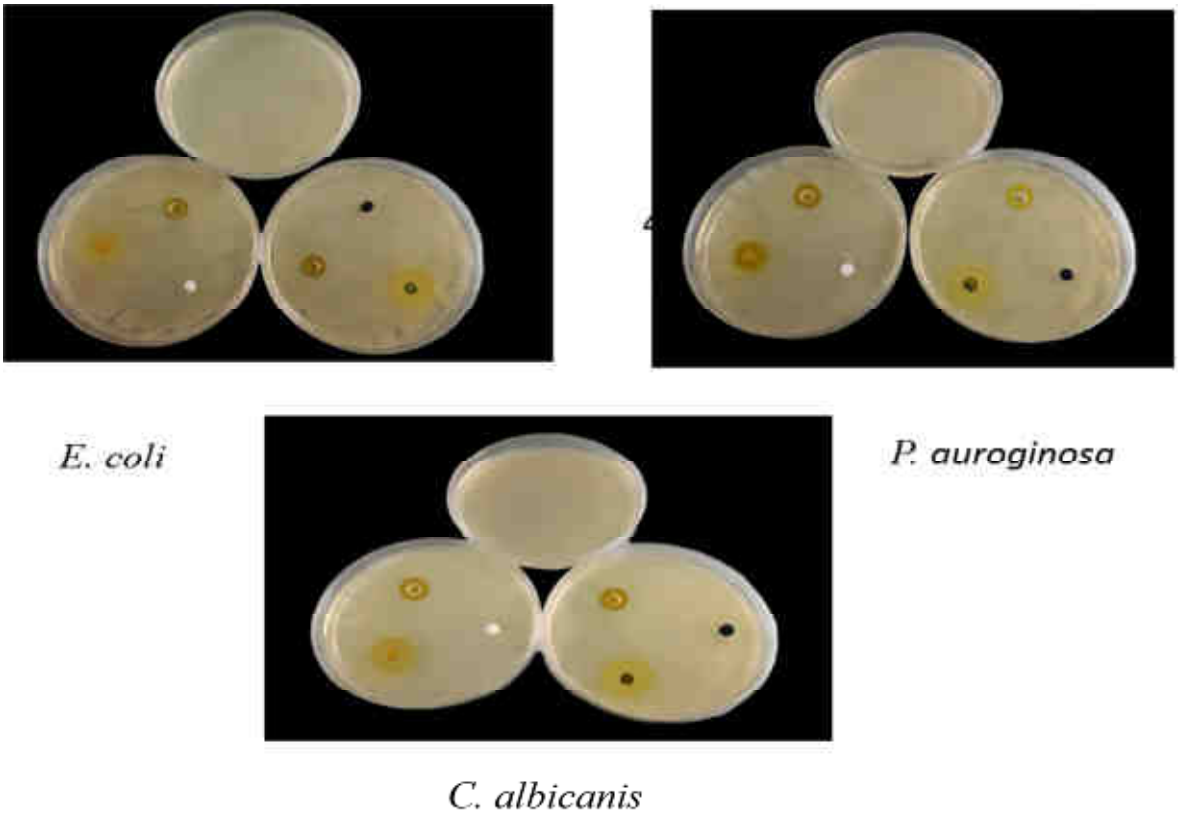


Figure 7. Antimicrobial activity of berberine hydrochlorid loaded BC, BC/PAni disc against *P. auroginosa, E. coli, and C albicanis,* berberine hydrochloride solution (1), BC (5), BC/ berberine hydrochloride (4), BC/Polyaniline (2), and BC/ berberine hydrochloride/Polyaniline (3).

Table.1. Zone inhibition of microbial assay.

Sample	Zone of inhibition (mm)		
	E. coli	*P. auroginosa*	*C. albicanis*
BH	4	3	4
BC	---	---	---
BC/BH	5.6	4	5.8
BC/PAni	---	---	---
BC/BH/PAni	7	5.8	7.3

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5.6 Cytotoxicity Study

In vitro indirect cytotoxicity of BC and BC/PAni was evaluated with different cell lines including Hella cells and L-02 cells using CCk-8 assay .Cell viability was calculated using the equation described in material part 5.2 and the result are shown in the (Figure 8.a, b).The viability of almost all cases indicated a high level cell viability of 80%.The growth tendency is different between Hella and L-02 two cell lines .For Hella cells ,the cell viability is higher at the time point of 24 h than other time points, as to L-02 is opposite. The possible reason for this result is that L-02 (liver cell) is more sensitive than tumor cells .However, the effect to cell of BC-PAni has no significant difference compared to pure BC group. In sum, BC/PAni hydrogel composites has no cytotoxicity and good biocompatibility.

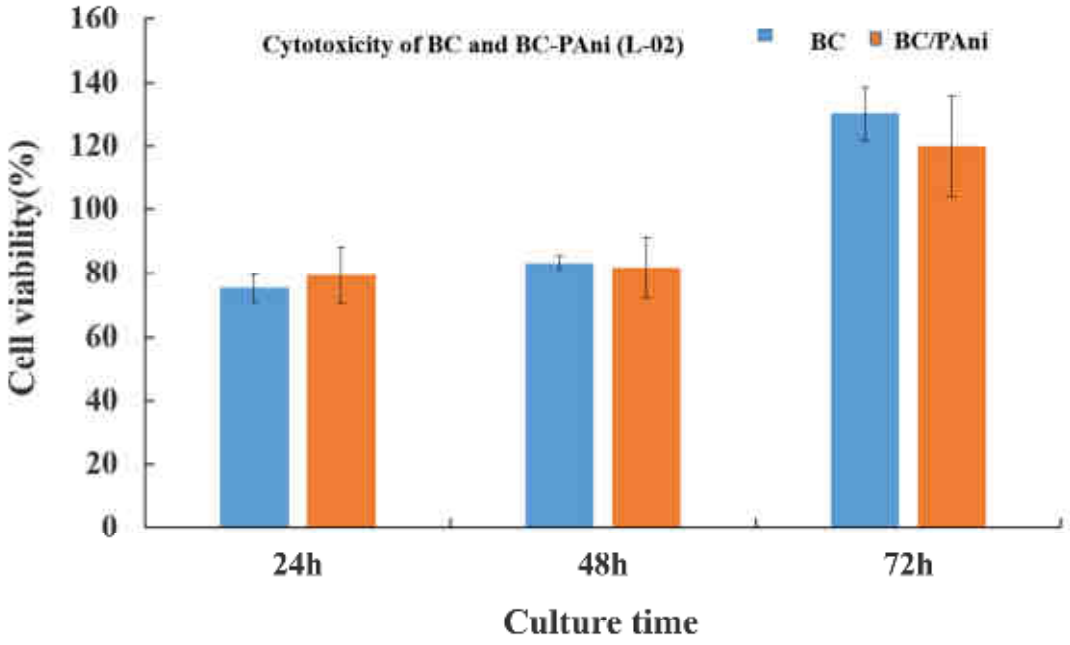

Figure 8.a Cytotoxicity of pure BC and BC-PAni composite with L-02 cells.

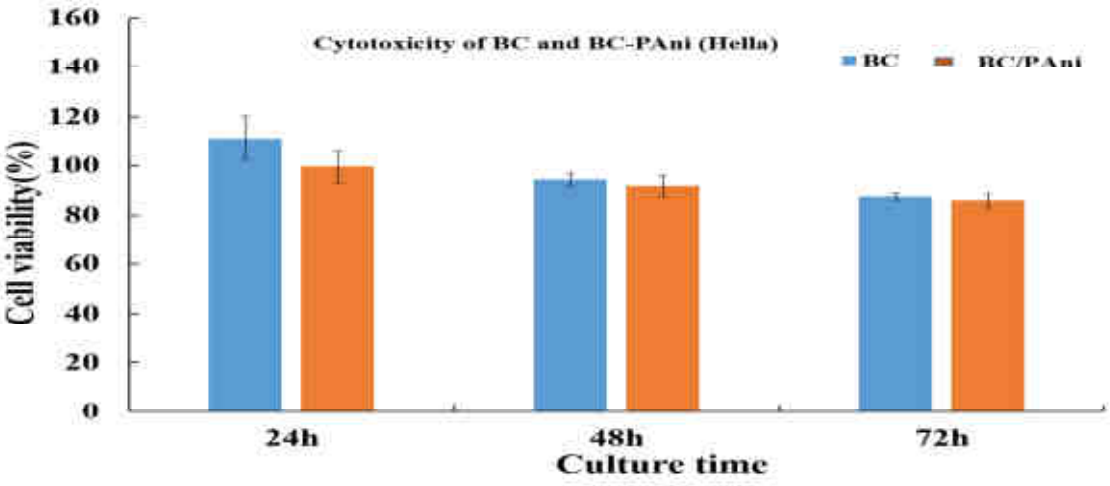

Figure 8.b Cytotoxicity of pure BC and BC-PAni composite with Hella cells.

6. Conclusions

A BC/PAni composite was synthesized using simple in situ penetration of aqueous drug berberine hydrochloride (BH) into the BC, and BC/PAni composite was characterized with FE-SEM, FTIR, XRD, TGA, antimicrobial activity and cytotoxicity was evaluated against human cell line. The enhanced biocompatibility, BC/PAni hydrogel composite were synthesized using hella cell line. BC/BH/PAni Nano composites were prepared with concentrations of drug 0.005mg/ml. The antibacterial activity of BC/BH/PAni Nano composites was shown a strong antibacterial activity against Gram-positive, bacteria and antifungal. These results were shown that the antibacterial of drug berberine hydrochloride in Nano composites could be modified according to zone of inhibition depend on pathogenic strain. Further studies should be focused on the investigation of antimicrobial

effect of BC/BH/PAni Nano composites against different types of pathogenic strains for potential widening of their applications, such as in antibacterial activity. Moreover, the XRD analysis peaks for berberine hydrochloride were in good agreement with those of BC, and BC/PAni structure. SEM images were shown an appropriate distribution of berberine drug onto the polymer, effect of Nano composite, offer promising possibility of using these materials in biomedical applications.

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