ES Materials & Manufacturing

RESEARCH PAPER View Article Online

Fabrication of pH-electroactive Bacterial Cellulose/Polyaniline Hydrogel for the Development of a Controlled Drug Release System

Sixiang Li,[1*] Ashwak Jasim,[1*] Weiwei Zhao,[2] Lina Fu,[3] Muhammad Wajid Ullah,[1*] Zhijun Shi,[1*] and Guang Yang[1*]

In the current study, a pH-electroactive hydrogel of bacterial cellulose (BC) and polyaniline (PAni) was fabricated through chemical oxidation polymerization for the development of a controlled drug release system. PAni was densely arrayed along the BC fibers and berberine hydrochloride (BH) was diffused within the hydrogel matrix as confirmed by field emission scanning electron microscope (FE-SEM) and fourier transform infrared (FTIR) analyses. The pH-electroactive drug release behavior of BC/PAni hydrogel was investigated *in vitro*. Variation in pH values from 2.2 to 11 demonstrated different drug release profiles from BC/PAni electroactive hydrogel: fast in alkaline and slow in acidic environments as indicated by a color change between green and dark, respectively. The drug release from BC/PAni hydrogel was stable and continuous which showed a typical pH-electroactive drug release behavior. The drug release curves closely matched the Korsemyer-Peppas kinetic model with free diffusion. Owing to its porous nature, conductive properties, and pH-electroactive drug release behavior, the developed BC/PAni hydrogel system can find potential applications as a controlled drug release system.

Keywords: Bacterial cellulose; Polyaniline; pH-electroactive hydrogel; Controlled drug release

Received 3 September 2018, **Accepted** 10 October 2018

DOI: 10.30919/esmm5f120

1.Introduction

Different strategies developed for controlled and targeted drug delivery over the past few decades have received immense consideration in research and clinical applications.[1–3] Nevertheless, the development of responsive and controlled drug delivery system for active compounds is still a major challenge; therefore, various drug delivery systems have been studied by applying external stimuli such as electric and magnetic fields, ultrasonic waves, light, pressure, and pH for drug release behaviors in a premeditated and controlled fashion. To this end, the electro-stimulated drug release devices (EDRDs) are specially designed to ensure a programmed drug release profile controlled by applying an external voltage or current.[4]

Currently, the design and use of various functional biomaterials for drug loading and release in the development of EDRDs have become a promising trend. The conducting polymers are famous for their applications in the development of supercapacitors, which perform as good as metallic oxides[5,6] and simultaneously possessing good biocompatibility. Therefore, the use of different conducting polymers is receiving immense consideration in the development of EDRDs owing to their potentials to change their redox state upon electrical stimulation, which in turn modulates the delivery, exchange, and the movement of specific ions and molecules in a programmed manner. In one such study, the drug release from an ion gate membrane of polypyrrole (PPy) was controlled by single and stepwise controlled electrical stimulation;[7] however, the amount of drug released was too low for practical therapeutic applications. Therefore, the conducting polymers can be polymerized within the matrix of a polymer with different pore sizes for improved drug loading and release. Lira et al. synthesized a semi-interpenetrating network of polyacrylamide and polyaniline (PAni) that allowed the storage of a large amount of molecules and their release in an electrochemically controlled fashion.[8] In another study, the surface of a gold electrode was coated with the fibers of a biodegradable polymer, poly (lactic-co-glycolic acid) (PLGA), *via* electrospinning and poly (3,4-ethylenedioxythiophene) (PEDOT) nanotubes by electrochemical deposition. The PLGA improved the drug loading capacity while PEDOT nanotubes decreased the impedance and increased the charge capacity of gold electrode. As a result, the release of dexamethasone was precisely controlled *via* external electrical stimulation.[9]

Bacterial cellulose (BC), a natural polymer hydrogel produced by certain microbial strains[10,11] and a cell-free system[12] from different carbon sources,[13] possesses high purity, excellent mechanical properties, high crystallinity, ultrafine fiber network, high water holding capacity and slow water loss rate, biocompatibility, and

[1]*Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China*

[2]*School of Mechanical and Electronic Engineering, Wuhan University of Technology, Wuhan 430070, PR China*

[3]*Department of Surgery-Transplant and Mary & Dick Holland Regenerative Medicine Program, University of Nebraska Medical Center, Omaha, NE 68198, USA*

***E-mail:** wajid_kundi@hust.edu.cn; shizhijun@hust.edu.cn; yang_sunny@yahoo.com.*

moldability into three dimensional structure.[14] These features make BC an ideal material for its broad spectrum applications in regenerative medicines and tissue engineering applications, such as wounding dressing,[15,16] vascular grafts,[17] artificial intervertebral disc,[18] biosensors,[19,20] and various biomedical and electroconductive devices and applications.[21] In our previous study, BC was used as a drug carrier where its fine network was manipulated to maintain the drug concentration in a desired therapeutic range that resulted in extended drug release time.[22] In another study, BC was used as a hydrogel matrix and combined with conductive polymers, PAni and PPy, to fabricate biphasic electroactive hydrogels with integrated electroactivity and biocompatibility.[23]

In the current study, a controlled drug release system based on biocompatible BC and electroactive PAni was developed. The multilayered BC/PAni showed a sandwiched structure with different porosity inside and outside the hydrogel after a controlled chemical oxidation polymerization reaction. The BC/PAni hydrogel was loaded with a drug berberine hydrochloride (BH), the main constituent of the Chinese medicinal herbs *Rhizoma coptidis* (Chinese name: Huang lian) and *Cortex phellodendri* (Chinese name: Huang bai), and evaluated for its ability to stimulate drug release under different pH conditions and electrical stimulation because it is poorly absorbed by oral administration and prohibited through intravenous injection. It was hypothesized that the different porosity of hydrogel inside and outside the hydrogel will allow a controlled and extended drug release under the effect of varying pH and electrical stimulation. The BC/PAni composite was prepared by chemical oxidation polymerization of aniline along BC fibers followed by impregnating BH into its matrix. The developed composite hydrogel showed a "sandwich" structure consisted of two external layers of BC and PAni which showed a good electrochemical property. The morphological changes occurred during the oxidation polymerization process were monitored by field emission scanning electron microscopy (FE-SEM) and fourier transform infrared (FTIR) spectroscopy. The drug release from the composite hydrogel was monitored under different pH and current flow. The developed multilayered BC/PAni sandwiched hydrogel can find potential applications in the development of a controlled drug system due to its different porous nature inside and outside of hydrogel, pH and electro-sensitivity, and conductive properties.

2. Materials and Methods

2.1 Materials

The chemical reagents including glucose, yeast extract, peptone, disodiumphosphate, citric acid, phosphate buffer saline (PBS), and berberine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrated hydrochloric acid, sodium hydroxide, aniline, and ammonium peroxydisulfate (APS) [$(NH_4)_2S_2O_8$] of analytical grade were purchased from Sinopharm Chemical Regents Co., Ltd. (Shanghai, China). All reagents were used without further processing.

2.2 Production of BC hydrogels

The BC hydrogels were produced by *Gluconacetobacter xylinum* (ATCC53582) and purified according to a previously described method.[24] Briefly, *G. xylinum* was cultured in a Hestrin and Schramm (HS) medium (pH 5.0) containing glucose 20 g/L, yeast extract 5 g/L, peptone 5 g/L, disodium phosphate 2.7 g/L, and citric acid 1.5 g/L and incubated statically for 10 days at 30℃. The BC hydrogel produced at the air-medium interface was harvested and dipped in distilled water for 2 days to remove excess medium residuals. Thereafter, it was treated with 0.3 N NaOH at 121℃ for 15 min to disrupt and dissolve any living cells and cell debris. Afterwards, the BC hydrogel was purified by washing several times with distilled water until its pH become neutral and stored at 4℃ for further use.

2.3 Preparation of BC/PAni composite hydrogels

BC/PAni hydrogels were prepared according to a previously reported method.[25] Briefly, the BC hydrogels were immersed in aniline hydrochloride solution prepared by mixing 0.02 M aniline and 0.06 M HCl in 100 mL distilled water and incubated at ambient temperature for 24 h to allow the infiltration of aniline into the BC hydrogel matrix. Thereafter, the BC hydrogels were dipped in 100 mL of 0.2 mol/L APS solution for different time intervals at room temperature to allow loading and *in situ* polymerization of PAni with BC fibers. The BC/PAni composites were rinsed several times with distilled water to remove APS and accessory substance until its pH become neutral. The sandwiched structure of BC/PAni was obtained by removing the edges and cutting into 1 cm 1 cm squares. Finally, the fabricated multilayered BC/PAni hydrogels were immersed in PBS (pH 7.4) and stored in 4℃.

2.4 Drug loading in electroactive BC/PAni hydrogels

The model drug BH was loaded into the BC/PAni composite hydrogel through simple diffusion method. Briefly, equally cut pieces of BC/PAni hydrogel were added into 0.5% (w/v) BH solution prepared in PBS (pH 7.4) and stirred at 150 rpm for 30 min. The BC/PAni hydrogels loaded with BH were removed from the solution and washed three times with distilled water to remove excess or surface adsorbed BH.

2.5 Characterization of BC, BH, BC/PAni, and BC/PAni/BH hydrogel

The synthesized BC/PAni electroactive hydrogels before and after loading the BH were characterized for various features through different techniques. The thickness of the layers in hydrogel was measured by micrometer in the wet state. The surface morphology of freeze-dried samples was observed through FE-SEM. Briefly, the samples were fixed on a brass holder and coated with gold on a Cu SEM disk and analyzed through a Nova Nano SEM450 FE-SEM (FEI, Holland). FTIR spectra of the freeze-dried samples were recorded using a Vertex FTIR spectrophotometer (Germany; spectral range 4000-400 cm^{-1}; beam splitter: Ge-coated on KBr; detector: DTGS; resolution: 0. 25 cm^{-1} (step selectable)) to determine the chemical structure of hydrogels and possible interactions between the components. The obtained IR data was transferred to PC to acquire spectra. The cyclic voltammetry measurements were carried out at room temperature with CHI660D electrochemical station (CHI Instrument) in the potential range of -1.2 V to 1.2 V and a series of scan rate (25, 50, 75 and 100 mV/s) were measured. Measurements on the hydrogel were carried out between two SnO_2F coated glass electrodes. The BC/PAni hydrogel was immersed in PBS buffer solution at different pH values ranging from 1.7 to 14.5 to study its electrical properties.

2.6 Drug release behavior of BC/PAni hydrogel

under applied voltage at different pH

Different pH and voltages (0-0.5V) were applied to determine the drug release behavior of BC/PAni hydrogel by measuring the amount of released drug using the gravimetric method. Briefly, a specialized device shown in Fig. 1 was designed where BC/PAni hydrogel was placed in an insulated framework and fixed between two conductive glass electrodes. Several pores (R = 1 mm) were drilled in one of the conductive glass electrode for the drug release. The BH loaded BC/PAni hydrogel was added into 80 mL PBS solution at 37℃ and pH varying from 2.2 to 11.6. At specified time intervals, a 1.0 mL PBS solution was taken out from the system for drug release analysis while maintaining its total volume by adding 1.0 mL of fresh PBS solution. The amount of released BH was determined after every 1, 2, 4, 6, 8, 12, and 24 h for 3 consecutive days using a UV spectrophotometer by measuring the absorbance at 341 nm. The amount of total released drug was determined by a cumulative release curve. The results were presented as cumulative percentage (%) release as a function of time using the following formula:

$$\text{Cumulative drug release (\%)} = \frac{W_I}{W_D} \times 100\% \quad (1)$$

Where W_I is the amount of cumulative BH released until a given time while W_D is the total amount of loaded BH in the hydrogel.

The release mechanism of BH from electroactive BC/PAni hydrogel was determined using Higuchi order (Eq. 2), Zero order (Eq. 3), and Korseymer-pappas (Eq. 4):

$$Q = Q_0 + kt^{\frac{1}{2}} \quad (2)$$

where Q is the cumulative amount of released drug at a given time, k is the Higuchi constant, and t is the drug release time. Higuchi order gives a cumulative percentage of released drug versus square root of time and was used to investigate the drug release from the matrix based on Fickian diffusion.

$$Q_t = Q_0 + kt \quad (3)$$

where Q is the amount of released drug, Q_0 is the initial amount of drug in the solution, and k is the zero order release constant. Zero order gives cumulative drug release versus time and was used to investigate the concentration-independent drug release rate from the formulation.

$$F = \left(\frac{M_t}{M}\right) = \frac{M_0}{M} + kt^n \quad (4)$$

where F is the fraction of drug released at time 't', M_0 is the burst release at the beginning; M_t is the amount of drug released time 't', M is the total loaded drug, 'k' is the kinetic constant, and 'n' is the release constant. Korsemyer-Peppas gives absolute cumulative amounts of drug release at time 't'.

3. Results and discussion

3.1 Development and morphological analysis of electroactive BC/PAni hydrogel

An electroactive hydrogel was developed through a chemical oxidation polymerization of PAni along BC fibers for the development of a controlled drug release system (Fig. 2). During the chemical polymerization, the aniline salt diffused out of the monomer treated BC hydrogel while the APS diffused into the hydrogel network forming two gradients. Thereafter, PAni formation started at the surface along the BC fibers and grew inwards. With the polymerization of PAni and the consumption of the reactant, the pores of hydrogel narrowed and the reaction rate decreased. Thus by controlling the different reaction time, the sandwiched BC/PAni hydrogel with different PAni thickness was achieved.

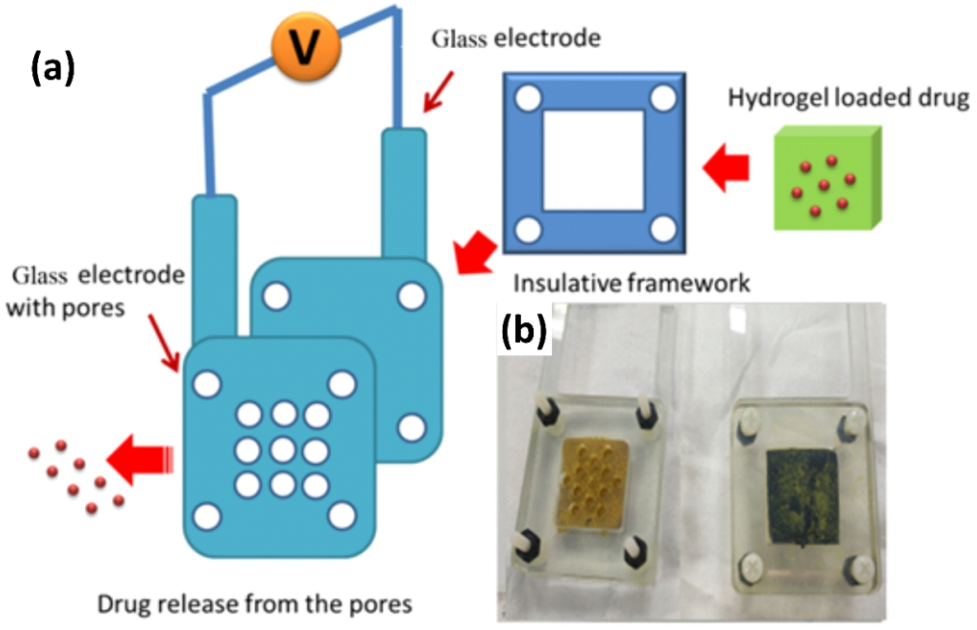

Fig. 1 (a) Schematic illustration and (b) photograph of conductive glass electrode.

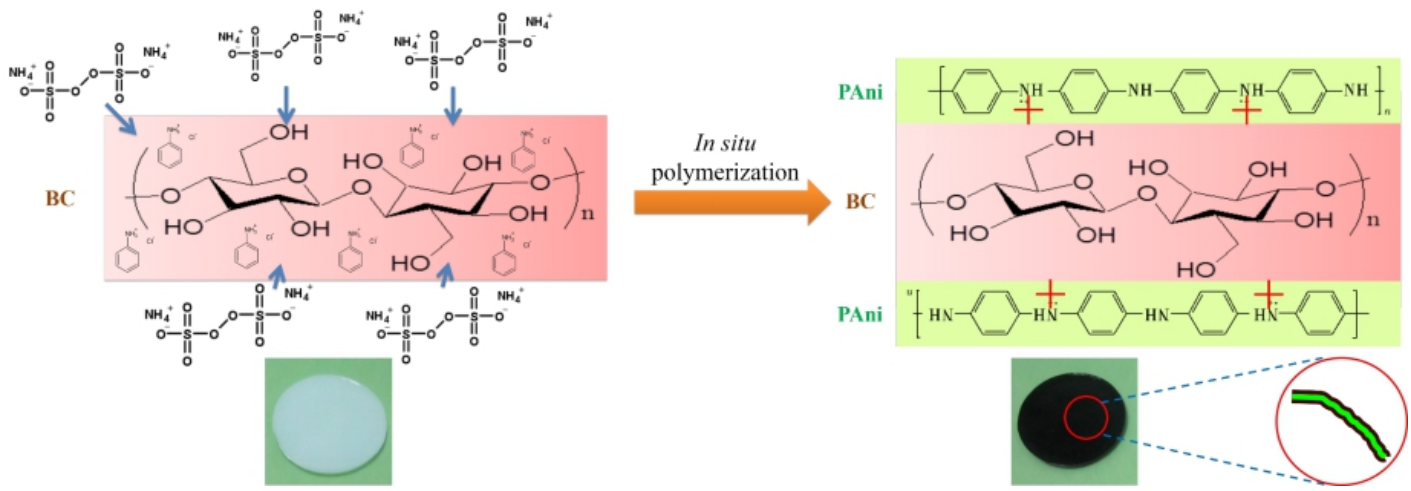

Fig. 2 Schematic illustration of chemical oxidation polymerization of aniline along the BC fibers during the preparation of BC/PAni sandwich hydrogel structure.

The multilayered BC/PAni hydrogel, variation in its thickness, and structural morphology are shown in Fig. 3. The naked-eye observation revealed the synthesis of differently colored five-layered hydrogel containing the top dark and green layers (PAni), middle white layer (BC), and lower green and dark layers (PAni) (Fig. 3a). The thickness analysis revealed that it varied differently for these layers in a time-dependent manner (Fig. 3b). For instance, the thickness of top dark layer increased slightly with time and reached to a maximum of 30% of the total thickness of hydrogel after 70 min. This indicates the occurrence of polymerization of PAni along BC fibers present at the top surface. In contrast, the thickness of top green layer was nearly constant after 70 min indicating the uniform polymerization of BC fibers present here. A similar trend was observed in the lower green and dark layers whose thicknesses were increased and constituted 18% and 16.5%, respectively of the total thickness of the hydrogel. Interestingly, the thickness of middle layer

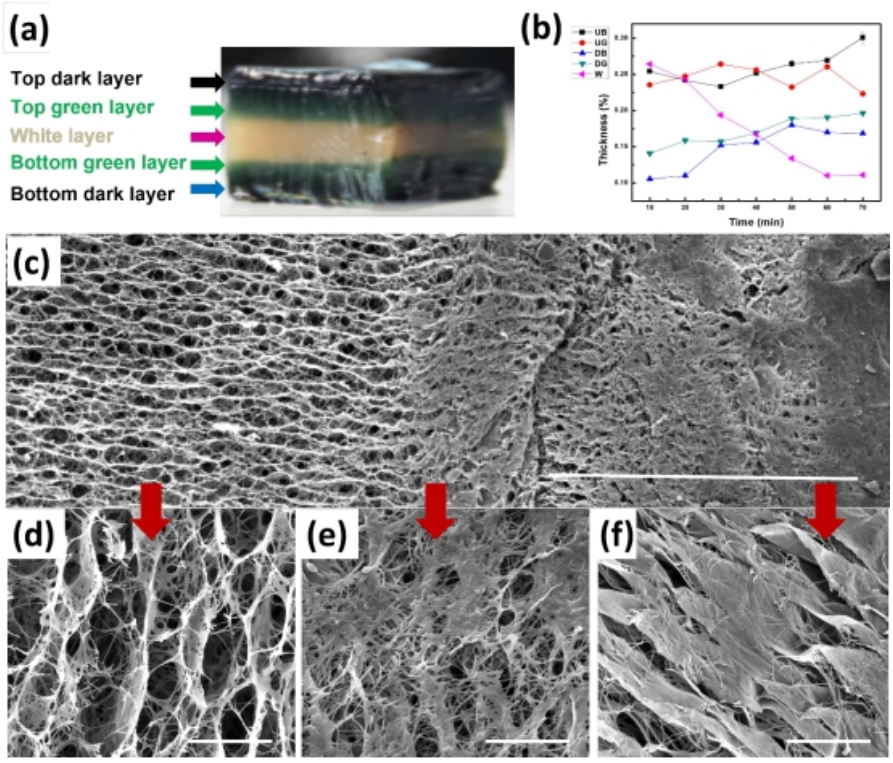

Fig. 3 Illustration of (a) synthesis of a multilayered sandwich structure of BC/PAni hydrogel, (b) thickness-time correlation curves, and SEM micrographs of (c) porous 3D nanostructure of BC/PAni hydrogel, (d) middle white layer, (e) top and bottom green layer, and (f) top and bottom dark layer. The scale bar for (c) is 100 μm and (d), (e), and (f) is 10 μm.

(BC) was decreased significantly from 26% to 12% after 70 min indicating that polymerization of PAni occurred along the BC fibers. A cross-sectional analysis of electroactive BC/PAni hydrogel *via* SEM showed a porous 3D nanostructure of BC network (Fig. 3c), which can potentially allow the impregnation of different size and shape materials through physical diffusion or chemical polymerization. The polymerization of PAni along the BC fibers is shown in Fig. 3d-f, which demonstrate that PAni was densely arrayed along BC fibers at the top and the bottom surfaces. Fig. 3d shows that BC fibers were hardly polymerized in the middle layer. In contrast, the top and bottom green layers indicated lower polymerization of PAni along the BC fibers (Fig. 3e) compared to those of the top and the bottom dark layers (Fig. 3f), which indicate that polymerization of PAni along BC fibers took place gradually from outer to inner matrix.

The SEM micrographs were analyzed using Sigma Scan Pro 5 software for determination of pore size in different layers (Table 1). The results showed that compared to non-polymerized BC (middle layer) (51.54%), the porosity of BC/PAni hydrogel at the top and bottom surfaces significantly decreased to 12.85% and 7.72%, respectively which is in accordance with the SEM micrographs. A similar trend was observed for the maximum, minimum, and mean pore sizes of the top, bottom, and middle layers indicating the successful polymerization of PAni and expansion of BC fibers present at the top and the bottom surfaces of BC/PAni hydrogel.

Table 1. Porosity analysis of different layers in the BC/PAni sandwich hydrogel structure.

Property	Middle white layer	Top-bottom green layer	Top-bottom dark layer
Porosity(%)	51.54	12.85	7.72
Mean poresize(nm)	42.9	26.6	15.7
Maximum poresize(nm)	560	141	180
Minimum poresize(nm)	10	10	6.8

3.2 FTIR characterization of BC/PAni electroactive hydrogel

FTIR is an important spectroscopic technique to investigate the presence of different functional groups in a molecule and nature of chemical bonds and functional groups linking them together on the surface a molecule, polymer, or an electroactive hydrogel.[26] Therefore, FTIR characterizations of BC, BH, BC/PAni, and BC/PAni/BH hydrogel were carried out and their FTIR spectra were comparatively analyzed to verify the basic chemical structures of BC and BH and investigate the nature of chemical interactions between BC, PAni, and BH in the developed BC/PAni drug delivery system. The combined spectra of BC, BH, BC/PAni, and BC/PAni/BH hydrogel are shown in Fig. 4, which reveal the characteristic peaks for different functional groups in BC, PAni, and BH and respective peak shift and intensities after the chemical oxidation polymerization of BC and PAni and loading of BH in the BC/PAni electroactive hydrogel.

The FTIR spectrum of pure BC showed characteristic peaks at 3347 cm^{-1} and 2895 cm^{-1} due to stretching vibration of OH and CH functional groups, respectively. The presence of CH stretching vibration was further supported by the presence of several peaks between 1450 cm^{-1} and 1200 cm^{-1}. Further, two additional characteristic peaks at 1443 cm^{-1} and 1417 cm^{-1} represent the symmetric deformation and bending vibration of CH functional group in pure BC. The peak for glucose carbonyl group (CO) appeared at 1673 cm^{-1}. The peak due to C-O-C stretching vibrations in BC appeared at 1067 cm^{-1}. The FTIR spectrum of BH showed characteristic peaks at 1600 cm^{-1} and 1500 cm^{-1} for quaternary iminium ion (C=N) and stretching vibration of aromatic ring (C=C), respectively, which is in agreement with a previous study.[27] Other major absorption bands between 1000 cm^{-1} and 1116 cm^{-1} represent free primary amino group (NH_2). A characteristic peak for CH_3 stretching vibration appeared at 1380 cm^{-1} in the BH spectrum, which was absent in the FTIR spectrum of pure BC. In the FTIR spectrum of BC/PAni, the intensity of OH functional group at 3347 cm^{-1} was significantly reduced, indicating the chemical oxidation polymerization of PAni along the BC fibers. It was further confirmed by the appearance of two additional strong peaks at 1460 cm^{-1} and 1560 cm^{-1}, which were assigned to quinone and benzene rings stretching oscillation, respectively, which is in agreement with previous studies.[19,25] In conductive PAni, the quinonoid and benzenoid units are generally present in equal ratio, thus intensities of their respective bands appeared at same position in the FTIR spectrum.[28] The presence of equal intensity bands for quinonoid and benzenoid units in the FTIR spectrum of BC/PAni indicates that these possess a similar structure to that of the conductive PAni, thus the composite is expected to possess high electrical conductivity. The FTIR spectrum of BC/PAni did not show NH stretching absorption which is in agreement with a previous study[25] indicating the reduction of BC *via* dehydration-condensation with PAni and resulted in disappearance or reduction of OH and NH bonds. The FTIR spectrum of BC/PAni/BH showed different absorption bands compared to pure BC, BH, BC-BH, and BC/PAni hydrogel. These results suggest successful chemical oxidation polymerization of BC and PAni and incorporation of BH into the hydrogel matrix through the synthesis of polyelectrolyte complex between BC and PAni.

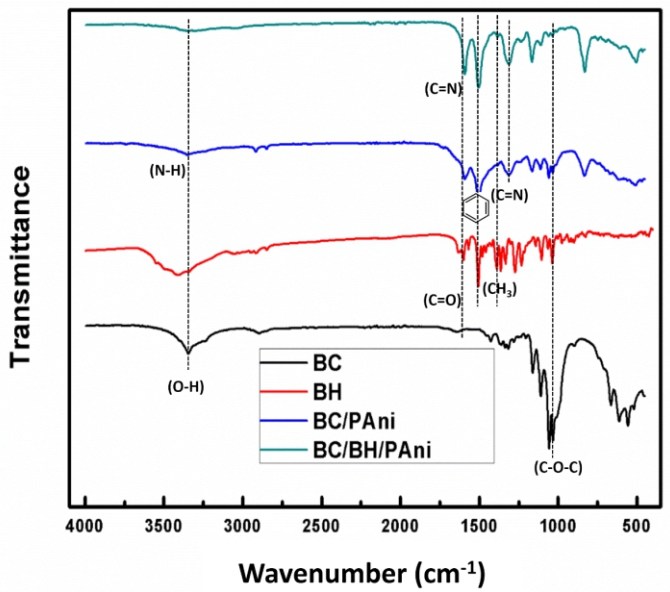

Fig. 4 FTIR spectra of pure BC, drug BH, BC/PAni, and drug loaded BC/PAni/BH hydrogel at pH 7.4.

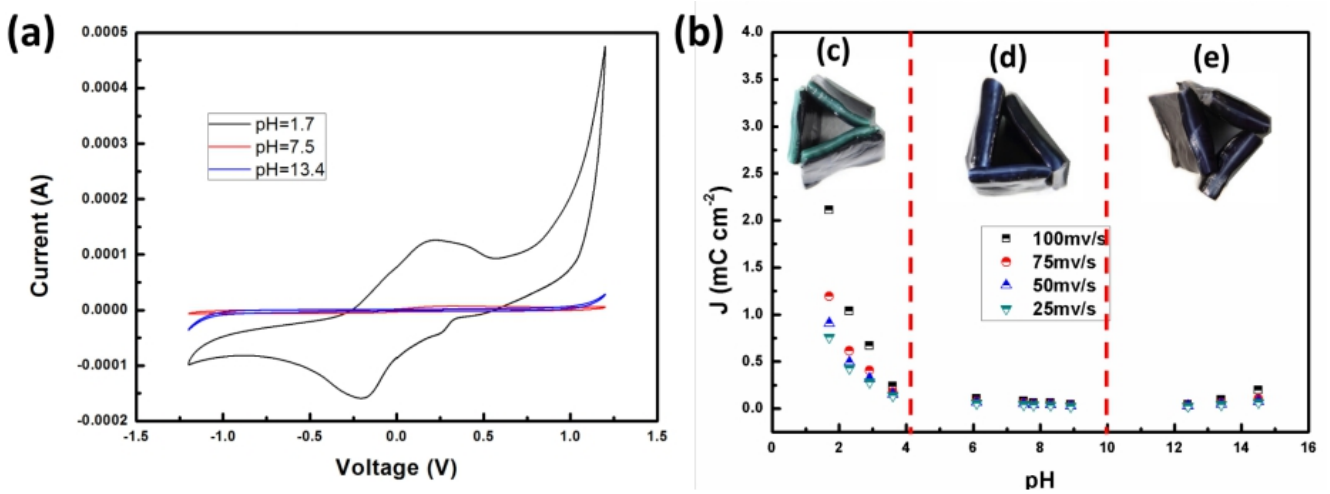

Fig. 5 (a) Cyclic voltammograms curves of BC/PAni at different pH (1.7, 7.5 and 13.4), (b) surface current density under different pH conditions calculated from cyclic voltammograms, and demonstration of color variations from (c) green to (d, e) dark when environment was changed from acidic to neutral/alkaline.

3.3 Cyclic voltammogram analysis

The redox activity determined *via* cyclic voltammogram (CV) analysis showed that BC/PAni possesses better conductivity in the acidic solution due to doping of proton acid. CV voltammograms of BC/PAni hydrogel at different pH values ranging from 1.7 to 14.5 are shown in Fig. 5a and Figure S1 using which the surface current density (J) was calculated (Table S1 and Fig. 5b). The results showed that, at pH<4, the doping of proton acid made BC/PAni hydrogel more electrochemical because of the formation of radical cations; whereas, the positive charges on the polymer chain are compensated by the imide ions due to the acidic environment. At this stage, PAni was present in the form of emeraldine salt (ES) which showed better conductivity, wherein the H^+ ions and the substituted cations doped on the polymer chain can transfer electrons under electrical stimulation. This process made the BC/PAni hydrogel to possess high surface current density (J) (2.11 mC/cm^2 at scan rates of 100 mV s^{-1} when pH = 1.7). In contrast, at pH>4, the PAni in BC/PAni hydrogel was translated to eigenstate form due to loss of proton acid; thus decreased the surface current density (J) (Table S1). These results demonstrated that PAni retained its pH sensitivity even after it was combined with BC as demonstrated by the color variation from green to dark in acidic to neutral/alkaline conditions (Fig. 3a, b and c) in a reversible way.

3.4 pH-sensitive controlled release behavior of BH-loaded BC/PAni hydrogels

The mechanism of BH release from the electroactive BC/PAni hydrogel was investigated under different physiological media of simulated gastric fluid (SGF) (pH 2.2), simulated intestinal fluid (SIF) (pH 6.8), and pH 11 (Fig. 6a). In our previous study, BC membranes were used as carrier which demonstrated an extended released time for two model drugs, berberine hydrochloride and berberine sulphate. The results showed up to 80% release of BH after 20 h, 8 h, and 10 h at pH 2.1, 6.8, and pH 12, respectively.[22] In the current study, the electroactive BC/PAni hydrogel showed 37%, 45%, and 69% drug release at pH 2.2, 6.8, and 11, respectively after 59 h (Fig. 6b). Compared to pure BC hydrogel, the multilayered

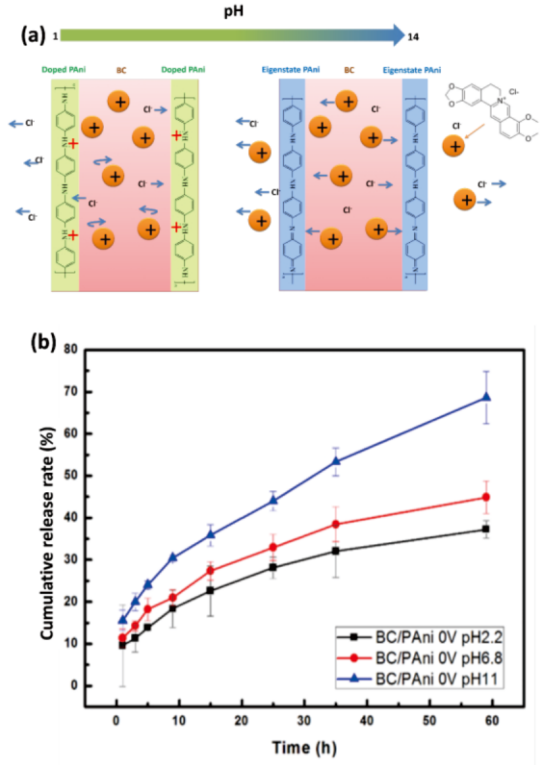

Fig. 6 (a) Illustration of drug release profiles from BC/PAni hydrogels at different pH values. (b) The cumulative amount of BH released from BC/PAni hydrogels at time 't' (h) under different pH values at 37 °C.

sandwich microstructure of BC/PAni hydrogel showed an extended drug release profile of BH, which could be attributed to the different porosity, 12.85% and 7.72%, at the top and the bottom surfaces, which significantly reduced the exchange rate of molecules.

The schematic of drug release behaviors of BH loaded in BC/PAni hydrogels at pH 2.2–11 is shown in Fig. 6a. It was

observed that BH was released fast under alkaline conditions and slowly in the acidic environment. At low pH values (<4), the PAni in BC/PAni hydrogels was doped by protonic acid. The outer layer of BC/PAni was positively charged as a smart charge filter to separate ions, and the cations migrated through the network leaving behind anions (berberine^{+}) in the BC network; thereby resulted in an extended anions drug release time. In contrast, at pH>4, the PAni in BC/PAni from doped PAni was translated to eigenstate PAni form; thus both cations and anions moved freely across the outer layer of BC/PAni hydrogel which resulted in an increased drug release rate. The BC/PAni drug release system showed a typical pH sensitive release behavior; i.e. fast in alkaline and slow in acid environments. The hydrogels could protect the drug in an acidic environment (SGF) (pH 2.2) before releasing it into the small intestine (SIF) (pH 6.8) for drug absorption into the blood circulation system.

3.5 Electro-responsive release behavior of BH-loaded BC/PAni hydrogels

The release behavior of BH from BC/PAni hydrogels with and without applied voltage at pH 2.2, 6.8, and 11 is shown in Fig. 7. The results showed that BH released from BC/PAni hydrogel after 3 h at 0 V was 11%, 14%, and 19% at pH 2.2, 6.8, and 11, respectively. With further increase in time up to 59 h, the BH released from BC/PAni hydrogel at 0V was found to be 37%, 44%, and 68% at pH 2.2, 6.8, and 11, respectively. In contrast, the BH released from BC/PAni hydrogel was increased to 12%, 15%, and 22% at pH 2.2, 6.8, and 11, respectively with voltage (0.5 V) input. With further increase in time up to 59 h, the BH released from BC/PAni hydrogel was 44%, 47%, and 74% at pH 2.2, 6.8, and 11, respectively with voltage (0.5 V) input. These results demonstrated that the released BH was significantly increased upon voltage input indicating the BC/PAni system under a consideration as an electroactive hydrogel. In the current system, the anions including berberine^{+} preferred to escape and moved to cathode while cations migrated to anode upon application of an electrical stimulus. In conclusion, the electrical stimulation accelerated the directed release of ionic drug BH (Fig. 7a). A comparison of berberine derivative release behavior of current electroactive BH/PAni sandwiched hydrogel with previously reported drug release systems is given in Table 2.

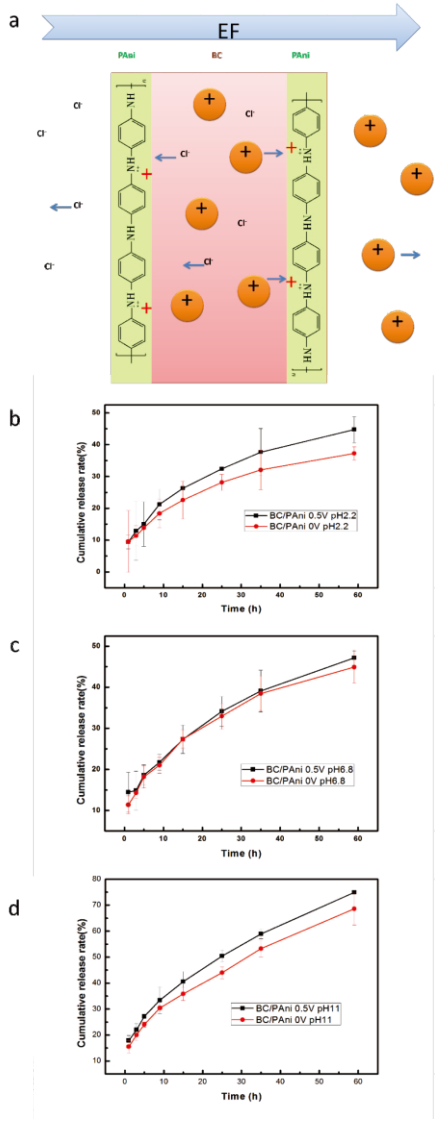

Fig. 7 (a) Illustration of drug release profiles from BC/PAni hydrogels with voltage input. Cumulative release profile of BH at (b) pH 2.2, (c) pH 6.8, and (d) pH 11 is demonstrated. The release of BH from BC/PAni with and without voltage input over a period of 59 h.

Table 2. Comparison of different drug release systems for berberine derivatives

Drug release system	Control method	Release time at 40%	Ref.
Spray-dried muco-adhesive micro particles	---	<10 min	29
Self-nanoemulsifying	---	10 min	30
MgAl hydrotalcite	---	20 min	31
PEG–lipid–PLGA NPs	pH	16 h	32
PAMAM	Conjugation	1-8 h	33
Pellets	Pelletsize	30-90min	34
BC/PAni	pH, voltage	59 h	This work

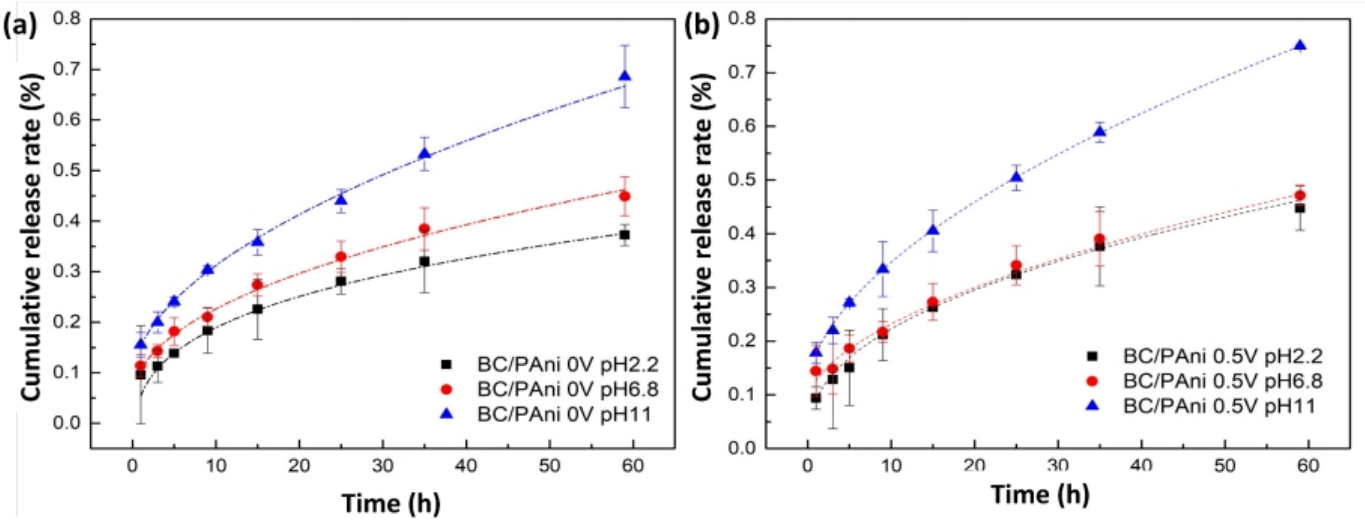

Fig. 8 Korsmeyer–Peppas model for the mechanism of BH release at different pH values (a) without (0V) and (b) with voltage (0.5V) input at 37℃.

3.6 Release kinetic analysis of BC/PAni hydrogels

The various release kinetic models developed are supposed to reason different release mechanisms.[35,36] Therefore, in the current study, the zero-order release equation, Higuchi order equation, and Korsemyer-Peppas were used to analyze the *in vitro* released data. The adjusted correlation coefficients of Zero-order, Higuchi equation, and korsemyer- Peppas kinetic models used in the current study are shown in the supplementary Table S2. The *in vitro* drug release profiles of BH at pH 2.2, 6.8, and pH 11 were well-fitted with Korsmeyer-Peppas model (Fig. 8). According to this model, a value of n<0.5/0.45/0.43 for a slab/cylinder/sphere indicates a diffusion controlled drug release. When n>1/0.89/0.85 for a slab/cylinder/sphere, the drug release behavior is pH-electro sensitive, which is also called case-II transport. In contrast, when 0.5/0.45/0.43<n<1/0.89/0.85, the drug release behavior is classified as a combination of both phenomenon and is referred to anomalous transport. In the current study, the slab shape samples were prepared with critical n-values as 0.5 and 1. The results in supplementary Table S3 suggested that BH release was more diffusion-based at pH 2.2 (n=0.290) and 6.8 (n=0.464) than anomalous type at pH 11 (n=0.532). In an acidic environment, there were affluent H^+ ion doping into the PAni which formed a positively charged protective layer, thus these slowed down the diffusion rate of cationic BH compared to neutral condition at pH 6.8. At an ambient pH 11, the drug release was rapid due to the precipitation tendency of BH and OH^-. At pH 6.8, the BH release was more likely to be Fickan diffusion without impeding or driving forces mentioned above. Therefore, BC/PAni hydrogel loaded with cationic drug showed a pH-sensitive release behavior, which can be described as suppressive, normal, and induced while in acidic, neutral, and alkaline ambient environments, respectively.

Upon applied voltage (0.5 V), the BH followed diffusion-based release at pH 2.2 (n=0.430) and anomalous type at pH 11 (n=0.561); however, it changed to non-Fickan diffusion at pH 6.8 (n=0.549). The release model change proved that the electrical stimulation played a part during the release process. To be more specific, the voltage input created an electric field between outer BC/PAni layers, which promoted the movement of charged BH towards the outer layer of hydrogel which in turn increased the BH release rate, thus the fit exponent n-value was increased. The voltage-drive increased release was observed in all experimental groups. At pH 2.2, the exponent 'n' increased from 0.290 to 0.430, closed to the critical value which demonstrated that the electrical stimulation possesses the increasing function against acidic force which still took the main influence during the drug release. The release rate was slightly increased at pH 11 which further verified that the voltage input accelerated the whole drug release process even at different pH values. In summary, the BC/PAni hydrogel could be used as cationic drug carrier, which can show different release rates at different pH and can promote the drug release under an electrical stimulation.

4. Conclusions

A pH-electroactive drug release system was developed through the chemical oxidation polymerization of BC and PAni for the release of precise quantities of ionic drug. Through a controlled polymerization reaction time, the BC/PAni hydrogel system demonstrated multilayered sandwiched structure where PAni was densely arrayed along the BC fibers; thus resulted in different porosity of BC/PAni hydrogel at the top and bottom surfaces, which significantly extended the drug release time and thus confirmed the proposed hypothesis. Further, the pH-electro sensitive hydrogel demonstrated slow drug release in acid condition and fast release in neutral and alkaline environments. In acidic condition, PAni was doped by protonic acid whereas the outer layer of BC/PAni hydrogel was positively charged as a smart charge filter to separate ions. The cations migrated through the BC network leaving behind anions ($berberine^+$); thereby extending the anions drug release time. In neutral and alkaline environments, PAni was present in eigenstate form. Both cations and anions moved freely through the outer layers BC/PAni which resulted in an increased drug release rate. Upon application of an electrical stimulus, the anions including $berberine^+$ preferred to escape and moved to cathode while cations migrated to anode. The current system demonstrated rapid and directional drug release with voltage input and demonstrated Korsemyer-Peppas kinetic model with free diffusion. The developed pH-electroactive BC/PAni hydrogel with different porosities at the top and the bottom layers can find potential applications in the development of a

controlled drug system.

Conflict of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by National Natural Science Foundation of China (51603079, 31270150, 21774039), China Postdoctoral Science Foundation (2015M572132, 2016M602291), the Doctoral Program of Higher Education of China (20070487096), Open Research Fund of State the Key Scientific Research Foundation of Huazhong University of Science and Technology (HUST-SRF no.0125170002), the Fundamental Research Funds for the Central Universities (HUST2014XJGH009), and the Science and Technology Support Plan in Jiangsu Province of China (BE2014684).

References

1. F. H. Meng, Z. Y. Zhong and J. Feijen, *Stimuli-Responsive Polym. Program. Drug Deliv.*, 2009, **10**, 197–209.
2. Z. Shi, X. Gao, M. W. Ullah, S. Li, Q. Wang and G. Yang, *Biomaterials*, 2016, **111**, 40-54.
3. S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, 2013, **12**, 991–1003.
4. A. Guiseppi-Elie, *Biomaterials*, 2010, **31**, 2701–2716.
5. D. Bin Jiang, B. Y. Zhang, T. X. Zheng, Y. X. Zhang and X. Xu, *Mater. Lett.*, **215**, 23-26.
6. S. Zhu, L. Li, J. Liu, H. Wang, T. Wang, Y. Zhang, L. Zhang, R. S. Ruoff and F. Dong, *ACS Nano*, **12**, 1033-1042.
7. K. Kontturi, P. Pentti and G. Sundholm, *J. Electroanal. Chem.*, 1998, **453**, 231–238.
8. L. M. Lira and S. I. Córdoba De Torresi, *Electrochem. commun.*, 2005, **7**, 717–723.
9. M. R. Abidian, D. H. Kim and D. C. Martin, *Adv. Mater.*, 2006, **18**, 405–409.
10. M. W. Ullah, M. Ul Islam, S. Khan, N. Shah and J. K. Park, *Korean J. Chem. Eng.*, **34**, 1591–1599.
11. Z. Shi, Y. Zhang, G. O. Phillips and G. Yang, *Food Hydrocoll.*, 2014, **35**, 539–545.
12. M. W. Ullah, M. Ul-Islam, S. Khan, Y. Kim and J. K. Park, *Carbohydr. Polym.*, 2015, **132**, 286–294.
13. M. U. Islam, M. W. Ullah, S. Khan, N. Shah and J. K. Park, *Int. J. Biol. Macromol.*, 2017, **102**, 1166–1173.
14. Z. Shi, X. Shi, M. W. Ullah, S. Li, V. V. Revin and G. Yang, *Adv. Compos. Hybrid Mater.*, **1**, 79-93.
15. Z. Di, Z. Shi, M. W. Ullah, S. Li and G. Yang, *Int. J. Biol. Macromol.*, **105**, 1638-644.
16. L. Fu, J. Zhang and G. Yang, *Carbohydr. Polym.*, **92**, 1432-1442.
17. Y. Li, K. Jiang, J. Feng, J. Liu, R. Huang, Z. Chen, J. Yang, Z. Dai, Y. Chen, N. Wang, W. Zhang, W. Zheng, G. Yang and X. Jiang, *Adv. Healthc. Mater.*, **6**, 1601343.
18. J. Yang, L. Wang, W. Zhang, Z. Sun, Y. Li, M. Yang, D. Zeng, B. Peng, W. Zheng, X. Jiang and G. Yang, *Small*, **14**, 1702582..
19. A. Jasim, M. W. Ullah, Z. Shi, X. Lin and G. Yang, *Carbohydr. Polym.*, 2017, **163**, 62–69.
20. B. Zhang, J. Zhou, S. Li, X. Zhang, D. Huang, Y. He, M. Wang, G. Yang and Y. Shen, *Talanta*, **131**, 243-248.
21. M. Ul-Islam, S. Khan, M. W. Ullah and J. K. Park, *Biotechnol. J.*, 2015, **10**, 1847–1861.
22. L. Huang, X. Chen, T. X. Nguyen, H. Tang, L. Zhang and G. Yang, *J. Mater. Chem. B*, **1**, 2976-2984.
23. Z. Shi, Y. Li, X. Chen, H. Han and G. Yang, *Nanoscale*, 2014, **6**, 970–977.
24. M. Ul-Islam, W. A. Khattak, M. W. Ullah, S. Khan and J. K. Park, *Cellulose*, 2014, **21**, 433–447.
25. Z. Shi, S. Zang, F. Jiang, L. Huang, D. Lu, Y. Ma and G. Yang, *RSC Adv.*, 2012, **2**, 1040–1046.
26. M. W. Ullah, M. Ul-Islam, S. Khan, Y. Kim and J. K. Park, *Carbohydr. Polym.*, **136**, 908-916.
27. S. Ifuku, M. Tsuji, M. Morimoto, H. Saimoto and H. Yano, *Biomacromolecules*, 2009, **10**, 2714–2717.
28. D. W. Hatchett, M. Josowicz and J. Janata, *J. Phys. Chem. B*, **103**, 10992-10998.
29. C. Godugu, A. R. Patel, R. Doddapaneni, J. Somagoni and M. Singh, *PLoS One*, **9**, e89919.
30. Z. Ke, Z.-P. Zhu, Z.-Y. Xu, C. Fang and S.-Q. Hu, *Trop J Pharm Res Trop. J. Pharm. Res. May J. Cit. ReportsScience Ed.*, **14**, 747-752.
31. M. A. Djebbi, Z. Bouaziz, A. Elabed, M. Sadiki, S. Elabed, P. Namour, N. Jaffrezic-Renault and A. B. H. Amara, *Int. J. Pharm.*, **506**, 438-448.
32. F. Yu, M. Ao, X. Zheng, N. Li, J. Xia, Y. Li, D. Li, Z. Hou, Z. Qi and X. D. Chen, *Drug Deliv.*, **24**, 825-833.
33. L. Gupta, A. K. Sharma, A. Gothwal, M. S. Khan, M. P. Khinchi, A. Qayum, S. K. Singh and U. Gupta, *Int. J. Pharm.*, **528**, 88-99.
34. Y. Gao, X. Jin, Y. Sun, F. F. Xu and M. Zhang, *Adv. Powder Technol.*, **29**, 682-691.
35. S. Dash, P. N. Murthy, L. Nath and P. Chowdhury, *Acta Pol. Pharm.*, **67**, 217-223.
36. P. C. Wu, M. J. Tsai, Y. Bin Huang, J. S. Chang and Y. H. Tsai, *Int. J. Pharm.*, 2002, **243**, 119–124.