Use of Bacterial Cellulose as Reinforcement Agent and as Coating Agent in Drug Release Applications

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In this study the use of bacterial cellulose (BC), a biopolymer with remarkable features, as reinforcement agent and as coating agent for drug release applications was investigated. Therefore, poly(vinyl alcohol)-chitosan blends, containing ibuprofen sodium salt as model drug, reinforced with various amounts of BC fibrils uncoated and coated with BC layers were prepared and characterized by FT-IR and SEM. Swelling and drug release studies were performed at pH 1.2 and 7.4. Results revealed that the incorporation of BC fibrils as reinforcement agent affects both swelling and drug release and by its content variation a desirable swelling values and release rates could be obtained. Also, it was observed that coating with BC sheets minimize burst release and provides lower drug release rates. All data suggest that BC is a promising material for drug delivery applications.

Keywords: poli(vinyl alcohol), chitosan, bacterial cellulose, drug release, ibuprofen sodium salt

Bacterial cellulose (BC) is a polysaccharide synthesized by several microorganisms, particularly *Gluconacetobacter xylinus*, and has similar chemical formula to plant cellulose but with ultrafine nanofibrous network structure. The unique nanostructure provides significant benefits over plant cellulose like high mechanical strength, improved crystallinity, high water holding capacity, biodegradability, biocompatibility and the ability to be molded into three dimensional structures during synthesis. BC based composites has been investigated for different applications in pharmaceutical and biomedical field such as artificial skin, artificial blood vessels, tissue engineering, wound-dressing, drug delivery [1-4].

The aim of this paper is to investigate the potential use of BC in drug delivery applications as reinforcing material and as coating agent. To achieve this goal composites based on poly(vinyl alcohol) (PVA) and chitosan were reinforced with BC fibrils (monolayer PVA/Chitosan/BC) and coated with BC never dried membranes (multilayer PVA/Chitosan/BC).

The PVA/Chitosan mixture has been selected after a literature survey because both chitosan and PVA were widely used in various biomedical field. By mixing PVA with chitosan, properties like tensile strength, flexibility, bulk and surface hydrophilicity are improved. These composites have low toxicity, high biocompatibility and are *p*H or/and temperature sensitive materials. Also, this particular combination was intensely investigated for applications like tissue engineering, drug delivery, wound dressing etc. [5-7].

Several studies reported the presence of interactions between chitosan, PVA and BC molecules based on FTIR analysis, NMR spectroscopy and X-ray diffractometry. For example, BC due to its large surface area per unit mass allows extensive hydrogen bonds formation with PVA. The strength of interfaces between both polymers leads to a very strong composite [8, 9].

Taking all the above in consideration, PVA/Chitosan/BC composites and PVA/Chitosan/BC composites coated with BC membranes were characterized with respect to chemical interactions, structure and morphology. Swelling behaviour and drug release studies were performed. As model drug ibuprofen sodium salt (IbuNa) was used, a non-steroidal anti-inflammatory drug with analgesic, antipyretic and anti-inflammatory properties [10, 11].

Experimental part
Materials and methods

Chitosan from crab shells, (85% degree of deacetylation), PVA, (99% hydrolysed and average M_w 85000 – 124000), and ibuprofen sodium salt ($\geq 98\%$) were purchased from Sigma Aldrich (Germany). All other chemicals were analytical grade and were used without further treatment or purification.

Bacterial cellulose membranes were obtained and purified as described elswhere [12] in the Mass Transfer Laboratory of the Chemical and Biochemical Engineering Department of University Politehnica of Bucharest.

Preparation of PVA/Chitosan/BC composites

A chitosan solution (1%) was prepared by dissolving chitosan in aqueous acetic acid solution (1%) under magnetic stirring at 50°C for 4 h. A PVA solution (4%) was prepared by dissolving PVA in hot water under stirring at 90°C for 4 h. Both polymer solutions were cooled down 1 h. The drug was loaded by adding a 0.5% IbuNa aqueous solution into PVA solution with stirring for assuring a better distribution. The prepared polymer solutions were carefully mixed with a mechanic stirrer to obtain homogeneous solutions. The obtained mixture was portioned in 4 parts and then each part was mixed with various amounts of wet ground BC fibrils (corresponding to 0.1 g, 0.2 g, 0.3 g and 0.4 g dry BC respectively).

Monolayer composites (coded as C1, C2, C3 and C4) were obtained by casting the solutions on glass plates, followed by solvent evaporation for 7 days in open air. Multilayer composites (coded as C1a, C2a, C3a and C4a) were obtained from previously dried monolayer composites

by coating them on each side with a BC membranes (approximately 90% humidity) and allowed to dry 3 days at room temperature (25°C).

FT-IR analysis

Possible interactions between the components of the composite were studied by Fourier transform infrared (FT-IR) spectroscopy. The FT-IR spectra (50 scans recorded at a resolution of 4 cm^{-1}) were recorded using a Jasco FT/IR 6200 with Intron μ Infrared microscope with ATR-1000-VZ objective in the range of 4000 to 500 cm^{-1} with a DLATGS detector.

Morphology analysis

Surface morphology was visualized by scanning electron microscopy (SEM) using a HITACHI S2600 N scanning electron microscope (HITACHI Science Systems Ltd., Tokyo, Japan) operating at 15-25 kV at a magnification of 5000-60000K.

Swelling behaviour

Samples were immersed in 20 mL buffer solution of *p*H 1.2 at room temperature (25°C) for 2 h and then transferred in buffer solution of *p*H 7.4 for 7 h. At fixed moments, composites were removed from solutions, wiped quickly with filter paper for free surface water removal and then weighed. The swelling ratio (SR) was calculated gravimetrically using equation (1).

$$SR = (W_t - W_d)/W_d \qquad (1)$$

where W_t is the weight of swollen hydrogel, W_d is the weight of dried hydrogel.

Drug release study

In vitro drug release profiles were obtained by immersing films in 20 mL buffer solution of *p*H 1.2 for 2 h and then samples were transferred in 20 mL buffer solution of *p*H 7.4 for 7 h. Samples were kept at room temperature (25°C) under moderate stirring. At fixed intervals, the amount of IbuNa released was determined using an UV-VIS spectrophotometer (CINTRA 6, GBS-Australia) at 221 nm wavelength according to the standard calibration curve. The cumulative release of IbuNa was calculated with equation (2):

$$Cumulative\ release = (M_t/M_\infty) \times 100 \qquad (2)$$

where M_t is the amount of ibuprofen released at time *t* and M_∞ is the amount of ibuprofen released at infinite time.

Results and discussions
FT-IR analysis

In figure 1 an example of the FT-IR spectra of PVA/Chitosan/BC monolayer composite C1 is given. The absorption characteristic bands of chitosan, PVA and BC presented in figure were compared with spectra of pure films of PVA, BC and chitosan presented elsewhere [13]. The peaks at 913 cm^{-1},1085 cm^{-1} and 1413 cm^{-1} are characteristic to PVA, the majority compound in the composition of the prepared composites, for CH$_2$ rocking, C-O stretching and CH$_2$ bending.

In several papers is stated that for composites overlapping and/or shifting of absorption peaks are possible. Chitosan characteristic peak for –NH$_2$ observed usually at 1560 cm^{-1} in the composite spectrum C1 is shifted at 1556 cm^{-1}. Also, the peak at 1333.5 cm^{-1} could be attributed to chitosan for vibration of C-H bond. Although BC is used in
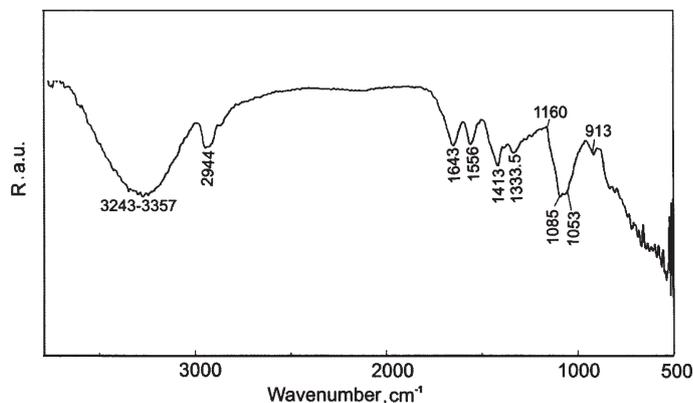

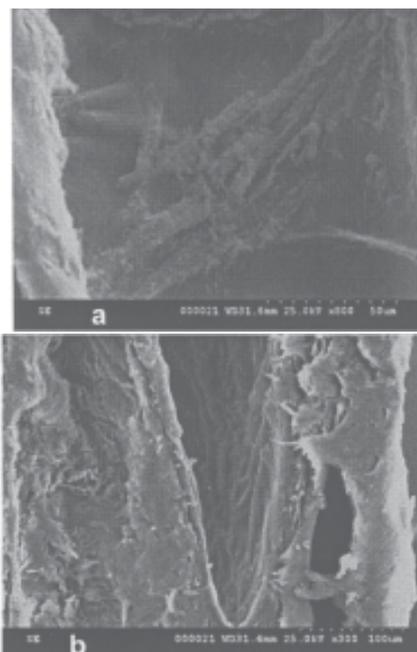Fig.1. FTIR spectra of PVA/Chitosan/BC monolayer composite C1.

Fig.2. Morphology of PVA/Chitosan/BC composite C1- surface (a) and cross- section (b).

small quantities, the absorption peaks at 1053 cm^{-1}, 1160 cm^{-1} and 1644 cm^{-1} could be also assign to its presence for C-O-C pyranose ring skeletal vibrations, C-O-C asymmetric stretching, C-H deformation and H-O-H bending of the adsorbed water, respectively. The broad band in the region 3200-3400 cm^{-1} observed for C1 sample is an overlapping example due to the fact that all components presents characteristic absorption peaks in this domain. Therefore, the peaks in this aria could be attributed to intermolecular hydrogen bonding and –OH stretching vibration characteristics for PVA and BC, but also could be attributed to –NH and –OH stretching vibration specific for chitosan. Also, the band at 2940 cm^{-1} is common peak for all the three polymers attributed to –CH stretching.

Morphology analysis

Surface and cross-section morphology of PVA/Chitosan/BC monolayer composite C1 are presented in figure 2. The cross-section view revealed that the composite exhibits porous structure which is responsible for swelling. In surface view crystals could be observed, attributed to the model drug, IbuNa, entrapped in the polymer matrix.

Swelling behaviour

In order to simulate the behaviour of the drug release system in the gastrointestinal tract, the samples were transferred from the simulated stomach conditions (*p*H
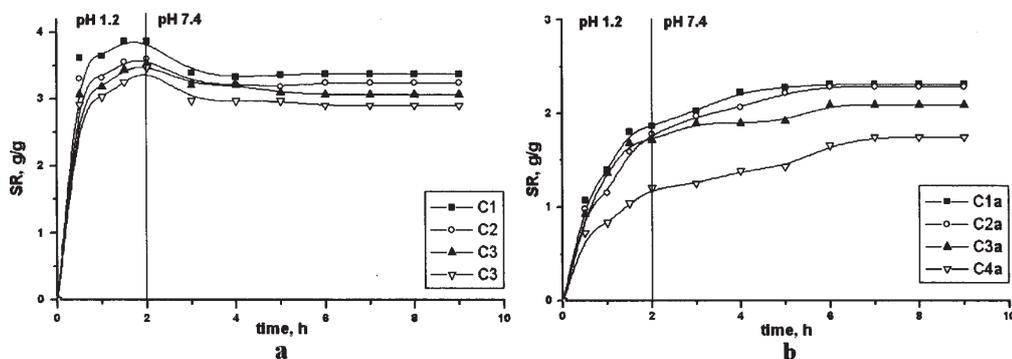
Fig.3. Swelling behaviour of PVA/Chitosan/BC monolayer composites C1-C4 (a) and multilayer composites C1a-C4a (b).
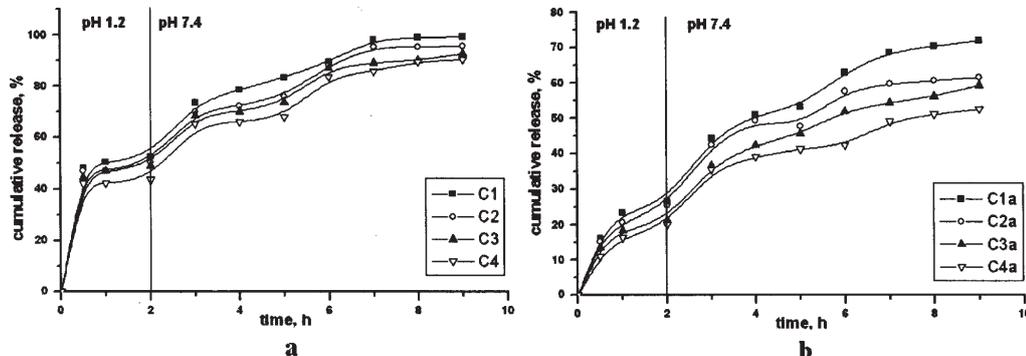

Fig.4. Release of ibuprofen from PVA/Chitosan/BC monolayer composites C1-C4 (a) and multilayer composites C1a-C4a (b).

1.2) to colon conditions (pH 7.4). The investigation of swelling behaviour and drug release in these conditions is very important, so this method was applied for both swelling and drug release.

The swelling behaviour of PVA/Chitosan/BC monolayer and multilayer composites is presented in figure 3. One can observe that the swelling ratio of all monolayer samples exhibited fast high values in buffer solutions of pH 1.2 due to the protonation of amino groups in acidic media. The protonation in acidic media of amino groups increase electrostatic repulsions, dissociation of hydrogen bonds and the relaxation of the macromolecular chains, all finally leading to an increase of swelling ratio. After 2 h the composites were subsequently transferred into buffer solutions of pH 7.4 and it can be seen clearly in figure 3a that swelling fast decreased at the contact with this environment due to the deprotonation of amino groups in neutral media. Materials with this kind of pH sensitivity were previously mentioned in several papers [14, 15].

Also, it can be observed that BC as reinforcement agent influence swelling behaviour; the increase of BC concentration decreases the flexibility of network structure and the hydration ability of composites, leading to lower swelling values.

The multilayer composites present different swelling profiles than monolayer composites as seen in figure 3b. All swelling values of these samples were lower compared with values for monolayer composites Also the pH change does not influence swelling values of multilayer composites. These results could be explained by the BC additional layers that minimize the contact area of the composite matrix with the medium, thereby these layers limit swelling. These types of results have been previously mentioned [16, 17].

In vitro drug release study

The profiles of cumulative release of IbuNa from PVA/Chitosan/BC monolayer and multilayer composites were presented in figure 4. As presented in figure 4a, the cumulative release profiles exhibit a low release of IbuNa at pH 1.2 and an increase release when the samples were transferred into an environment of pH 7.4. Also, for all samples the cumulative release for monolayer composites

exhibited a burst effect in acidic medium indicating a rapid diffusion into the network composites. The burst effect could be explained by the high swelling values displayed for these samples.

The cumulative release profiles of multilayer composites presented in figure 4b exhibit the same tendency as monolayer composites, a low release of IbuNa at pH 1.2 and an increase release at pH 7.4. These profiles present also differences compared with those of monolayer composites, such us: burst release in acidic medium is strongly reduced, better differentiated profiles and lower cumulative release values were obtained. Results could be explained by the coating effect which minimize the contact area of the composite matrix with release medium and by decreasing swelling values.

Also, it can be observed that a decrease of BC content in all composites leads to an increase in cumulative release. Therefore, the composite with the higher BC content (C4) showed lowest cumulative release values. An explanation of these results could be the fact that BC as reinforcement agent affects the water uptake and network flexibility, all finally influencing drug release.

All data obtained suggest that BC is a promising biomaterial which could be used as reinforcement agent and also as coating agent in order to improve swelling and drug release of composite materials.

Conclusions

Poly (vinyl alcohol) -chitosan composites, loaded with ibuprofen sodium salt as model drug, reinforced with various amounts of bacterial cellulose fibrils uncoated or coated with BC never dried membrane were prepared in order to investigate the potential of BC use as reinforcement agent and as coating agent in drug release applications. The results of FT-IR spectra confirmed a good compatibility of all components of PVA/Chitosan/BC composite. The morphological analysis revealed that these composites have a porous structure. Swelling and drug release studies were performed at pH 1.2 and 7.4, simulating the behaviour of the drug release system in the gastrointestinal tract. The incorporation of BC as reinforcement agent affects swelling properties and drug release, highest swelling value and highest release rate was obtained for PVA/Chitosan/BC film

with lowest BC content. The results for composites coated with BC displayed better characteristics than those obtained for uncoated composites like well differentiated profiles and lower cumulative release values with reduced burst release in early stages.

All results suggest that BC as reinforcement agent and as coating agent is a promising material for controlled drug delivery systems.

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