55. savetovanje Srpskog hemijskog društva

KNJIGA RADOVA

55th Meeting of the Serbian Chemical Society

PROCEEDINGS

Novi Sad 8. i 9. juni 2018.
Novi Sad, Serbia, June 8-9, 2018

ISBN 978-86-7132-070-2

Srpsko hemijsko društvo

Serbian Chemical Society

Srpsko hemijsko društvo
Hemijsko društvo Vojvodine

Serbian Chemical Society
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***Izdaje** / Published by*
***Srpsko hemijsko društvo** / Serbian Chemical Society*
Karnegijeva 4/III, 11000 Beograd, Srbija
tel./fax: +381 11 3370 467; www.shd.org.rs, E-mail: Office@shd.org.rs

***Za izdavača** / For Publisher*
***Vesna Mišković STANKOVIĆ**, predsednik Društva*

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Dizajn korica, slog i kompjuterska obrada teksta
Cover Design, Page Making and Computer Layout
Aleksandar DEKANSKI

***OnLine publikacija** / OnLine publication*

*ISBN **978-86-7132-070-2***

Protein-repellent and antioxidative properties of bioactive coatings based on TEMPO oxidized cellulose nanofibrils and chitosan

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In this work, regenerated cellulose (RC) films was coated with TEMPO oxidized cellulose nanofibrils (TOCN) and chitosan (CS) by means of subsequent spin-coated deposition. The bioactivity of coatings was achieved by addition of chitosan. The chitosan was either mixed with the TOCN (TOCN+CS) and deposited on the RC film by spin-coating or deposited on the RC/TOCN bilayer film by pumping its aqueous solution at pH 5.5 over the surface of the film. The pH dependant charging behaviour of the TOCN, TOCN+CS, and CS were evaluated by pH-potentiometric titrations. The protein-repellent properties of investigated coatings were evaluated *in situ* using a continuous flow of bovine serum albumin (BSA) by means of quartz crystal microbalance with dissipation (QCM-D). Antioxidative activity of TOCN, CS and their amphoteric mixture was determined using the 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) radical scavenging assay. Coating with improved protein-repellent properties was obtained using TOCN+CS amphoteric mixture, but on the other hand TOCN+CS amphoteric mixture showed weaker antioxidative properties in comparison to TOCN and CS.

Introduction

Coatings based on cellulose for biomedical application and more specifically medical devices serve numerous purposes and can be found applied to a wide range of surfaces of medical materials [1]. With the emergence and development of nanotechnology a new form of so called nanocellulose, which is described as the products or extracts from native cellulose composed of nanoscale structured material [2], attracted attention as substrate for coating different surfaces. It is a highly reproducible and environmentally friendly nanomaterial suitable for modification of various materials in order to improve existing or to give completely new properties and has been used as thin coating layers for many generic and cutting-edge products [3, 4, 5, 6]. Among various methods, TEMPO oxidation of cellulose and successive mechanical and/or ultrasound treatments is one of the most promising method for obtainig of nanocellulose so called TEMPO oxidized cellulose nanofibrils (TOCN) [4].

In order to achieve bioactivity, many researches focused on the nanocellulose functionalization performed by the use of non-toxic, biodegradable, and environmentally-friendly reagents such as chitosan [7]. Chitosan is a natural amino polysaccharide having multifunctional properties, and wide ranging applications in biomedical and other industrial areas. The positive attributes of excellent biocompatibility, biodegradability, ecological

safety and low toxicity [8] with versatile biological activities such as anti-microbial[9], antiviral[10], antitumor[11], haemostatic[12] and low immunogenicity[8] have provided ample opportunities for further application development. From the standpoint of wound-dressing products, chitosan possesses many interesting properties such as promotion of fibroblast proliferation, collagen synthesis, integrin engagement and expression of cytokines and growth factors that promote wound healing and angiogenesis[13].

In many medical application, understanding and controling protein adsorption on surfaces is a great challenge. Wherever proteins come into contact with a solid interface they will most likely adsorb to it, *i.e.* it is complicated to avoid protein adsorption[14]. Protein adsorption can trigger adhesion of particles, bacteria or cells possibly promoting inflammation cascades, or fouling processes[15]. In fact there is a huge community seeking for biocompatible and protein-repellent materials applicable to biomedical purpose and a number of recent studies are devoted to understanding and controlling protein adsorption on planar surfaces[16]. Even more, for the new generation of medical products antioxidative properties are very important, since the antioxidative agents can reduce the amount of free radicals that could otherwise damage deoxyribonucleic acid (DNA). Moreover, antioxidative properties may also contribute to anti-inflammatory effects[17]. Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases as well as in the normal process of aging. Reactive oxygen species (superoxide anion, hydroxyl radical and hydrogen peroxide) are generated by normal metabolic process or from exogenous factors and agents and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. These reactive oxygen species (ROS) are capable of damaging a wide range of essential biomolecules[18]. There has been increasing interest for natural antioxidants, since they can protect the human body from free radicals, and retard the progress of many chronic diseases[19].

For the fundamental study of the interaction of polymer solutions with solid matrices, model films with a thickness below 100 nm turned out to be promising since these films have the advantage of a homogeneous surface and morphology. In this study, in order to obtain bioactive coatings based on polysaccharide with improved protein-repellent properties, RC thin film was coated with TOCN and CS, as well as TOCN+CS amphoteric mixture. TOCN was obtained from cotton fibers through 2,2,6,6-tetramethylpiperidine-1-oxyl TEMPO oxidation and successive defibrillation, and RC was obtained through regeneration of trimethysilyl cellulose (TMSC). Protein adsorption on the surface thin films of RC coated with TOCN, CS and TOCN+CS amphoteric mixture, was investigated by QCM-D using bovine serum albumin (BSA) as model solute. Finally, considering targeted biomedical application of RC materials coated with TOCN and CS, antioxidative properties of TOCN, CS and amphoteric TOCN+CS mixture were assessed by inhibition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals.

Materials

Cotton fibers: Russian, I class, 32/33 mm, were used in this study. Chitosan from crab shells with low molecular weight (Aldrich, 448869), 75-85 % deacetylated, was used. Trimethylsilyl cellulose (TMSC) with a degree of substitution (DS) of 2.55 was purchased from Thueringisches Institut fuer Textil- und Kunststoff-Forschung e.V., Germany. Quartz crystal microbalance (QCM-D) sensors coated with a gold layer (QSX303) were purchased from LOT-Oriel, Germany. 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO), sodium

bromide, 13 % sodium hypochlorite solution, bovine serum albumin (BSA-lyophilized powder, ≥96 %), 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), and other chemicals obtained from commercial sources (Sigma-Aldrich, Fluka) were analytical grade and used without further purification.

Working solutions preparation

Chitosan (0.1%, w/v) was dissolved in MQ water set to pH 2.5 using 37% HCl. The solution was stirred overnight at room temperature and filtered through a 5 µm PTFE syringe filter. For the phosphate buffered saline (PBS) solution 8.0 g NaCl, 0.2 g KCl, 1.44 g $Na_2HPO_4{\cdot}H_2O$ and 0.24 g KH_2PO_4 were dissolved in 900 mL of Milli-Q water, the pH was adjusted to 7.4 with 0.1 M NaOH and the solution was filled up to a volume of 1000 mL.
The protein solutions were prepared by dissolving 5 g BSA in 100 mL of PBS-buffer. All solutions were prepared at least 24 h before measurements, using Milli-Q water.

TEMPO-mediated oxidation of cotton fibers and cellulose nanofibrils preparation

In order to decrease energy needed for fibrillation and to prepare cellulose nanofibrils without significant aggregation, cotton fibers were oxidized with NaClO, catalytic amounts of TEMPO and sodium bromide according to a method described previously [20, 21]. In a typical experiment, 10 g of cotton fibers were suspended in water (750 mL) containing TEMPO (0.0250 g) and sodium bromide (0.250 g). A designated amount of the 13 wt% NaClO solution, corresponding to 15 mmol/g cellulose, was added slowly to the cellulose slurry under continuous stirring. The pH of the slurry at room temperature was maintained at 10.5 by addition of 0.5 M NaOH during 3 h. After stirring for a designated time, the oxidation was quenched by adding ethanol (ca. 5 mL). The oxidized cellulose was washed thoroughly with water, subsequently with ethanol and lastly with water on a filter paper set in a Büchner funnel. A slurry of TEMPO-oxidized cotton fibers (0.1, 1 and 2% solid consistency) in water (100 mL) was then passed through a double cylinder-type homogenizer (T 25 digital ULTRA-TURRAX, IKA, Germany) 5 min at 1000 rpm. The obtained gel-like dispersion was further sonicated for 15 min using an ultrasonic homogenizer (WCX 750, SONICS, USA) with a 19 mm diameter probe tip at 20 kHz and 750 W output power. The dispersion was centrifuged at 12,000×g for 15 min to remove a small amount of non-fibrillated and partly fibrillated fraction (<5%) to obtain TEMPO-oxidized cellulose nanofibrils with sodium carboxylate groups (TOCN-COONa) dispersed in water.

1 % (w/v) amphoteric TOCN+CS mixture (TOCN+CS) preparation

50 mL of 2 % (w/v) TOCN dispersion (pH 8.0), 22 mL of 0.5 % (w/v) CS solution (pH 2.5) and 39 mL of Milli-Q water (pH 5.5) were homogenized for 1 min using an ultrasonic homogenizer (WCX 500, SONICS, USA) with a 9 mm diameter probe tip at 20 kHz and 500 W output power. Obtained 1 % (w/v) TOCN and CS mixture had pH 5.5 without additional adjustment.

RC thin film preparation

Prior to spin-coating of the RC thin film, the QCM-D sensors were soaked into a mixture of H_2O/H_2O_2 (30 wt%)/NH_4OH (25 wt%; 5:1:1; v/v/v) for 10 min at 70°C, then immersed in a "piranha" solution containing H_2O_2 (30 wt%)/H_2SO_4 (98 wt%; 1:3; v/v) for 60 s, and then rinsed with MQ-water and finally blow dried with N_2 gas.

RC film was prepared by spin-coating of TMSC, 100 µL of TMSC solution (1 % (w/v)), dissolved in hexamethyldisiloxane (HMDSO), filtered using 5 µm PTFE syringe filter, deposited onto the QCM-D sensors, and rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 rpm s^{-1}. For converting TMSC into regenerated cellulose (RC), the TMSC–coated sensors were placed into a polystyrene Petri dish (5 cm in diameter) containing 3 mL of 10 wt% hydrochloric acid (HCl). The dish was covered with its cap and the TMSC films were exposed to the vapors of HCl for 15 min.

Coating of RC films by TOCN, CS, and TOCN+CS amphoteric mixture

Three different methods were chosen for RC film coating.

Method I: RC film was coated with TOCN by spin-coating of TOCN. 50 µL of the TOCN dispersion (1 % (w/v)) was deposited onto the RC film, rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 rpm s^{-1}.

Method II: RC film was coated with amphoteric TOCN+CS mixture by spin-coating of the amphoteric mixture based on CS and TOCN: 50 µL of 1 % (w/v) amphoteric TOCN+CS mixture was deposited onto the RC film, rotated for 60 s at a spinning speed of 4000 rpm and an acceleration of 2500 rpm s^{-1}.

Method III: RC film was coated with TOCN by spin coating of TOCN, as it is described in Method I, followed by deposition of CS using QCM-D technique. Au sensors coated with cellulose film prepared according to the method I were mounted into the QCM-D flow cell and equilibrated with MQ-water until a constant frequency signal was obtained. Five minutes after purging with MQ-water, CS solution (0.1 %, w/v) was allowed to adsorb onto the cellulose surfaces for 240 min. To remove reversible bound and free chitosan, the film was rinsed with MQ water for 60 min. The measurement was terminated at this stage. The flow rate was kept at 0.1 mL min^{-1} throughout all experiments performed at 21±0.1°C. Chitosan adsorption has been performed at pH value 5.5. The pH of the MQ-water and CS solution was adjusted to 5.5 with 0.1 M NaOH.

List of the samples with description is given in Table 1.

Table 1. Sample denotations and descriptions

Sample denotation	Sample preparation
RC/TOCN	RC ultra-thin film coated with 1 % TOCN dispersion (method I)
RC/TOCN+CS	RC ultra-thin film coated with 1 % amphoteric TOCN+CS mixture (method II)
RC/TOCN/CS	RC ultra-thin film coated with 1 % TOCN dispersion after adsorption/desorption CS (method III)

Methods

pH-potentiometric titrations

The pH dependent potentiometric titration was performed on 0.1% (w/v) TOCN dispersion, 0.1 % (w/v) CS solution and 1 % (w/v) amphoteric TOCN+CS mixture. Samples were prepared by dilution using Milli-Q water (a very low carbonate ion content, $c<10^{-6}$ mol L^{-1}). Samples were titrated in a forward (from acidic to alkaline) and backward (alkaline to acidic) manner from pH 2 to pH 11 using 0.1 M HCl and 0.1 M NaOH. The KCl concentration of samples was set to 0.1 M using potassium chloride. The titrants were added to the system in a dynamic

mode using a double burette Mettler Toledo T70 automatic titration unit, and the pH value was measured using a Mettler Toledo DG-111-SC combined glass electrode. Details on the measurements as well as the calculation of the charge and content of functional groups are described in literature [22].

Protein-repellent characterization

The quartz crystal microbalance with dissipation (QCM-D) as a nanogram-sensitive balance is powerful tool for studying polymer adsorption on thin films. In this study, a QCM-D device (model E4, Q-Sense, Gothenburg, Sweden) was used to investigate the protein adsorption on polysaccharide films, samples listed in Table 1. The QCM-D instrument determines changes in frequency (f) of an oscillating quartz crystal caused by deposition of mass whereas negative frequency shifts (Δf) indicate a deposition of mass.

The change in frequency can be directly related to the adsorbed mass using the Sauerbrey equation [23]:

$$\Delta f = -C\frac{\Delta m}{n}$$

where Δf represents the frequency shift, Δm is the mass change, C is the Sauerbrey constant (C=17.7 ng Hz^{-1} at the frequency of 5 MHz), and n is the number of the overtone (n= 1, 3, 5, *etc.*) of oscillation.

Sauerbrey equation is only applicable for thin and rigid films, which fully couple to the oscillation of the quartz sensor. The model investigated films in this study were treated as a rigid extension of the quartz crystal when estimations were done using the Sauerbrey equation.

For the data analysis in this study, the change in the third overtone's frequency (f_3) was used. For all QCM-D experiments, sensors coated with films were mounted into the QCM-D flow cell and the initial resonance frequency of the sensor was measured. Afterwards, the films were equilibrated with Milli-Q water followed by rinsing with the background solution (PBS buffer was used for protein adsorption experiments) until a constant frequency was established.

In each QCM-D experiment, before protein injection tested films were equilibrated with MQ-water until a constant frequency signal was obtained. All frequencies were set to zero and the measurement was started. Five minutes after purging with MQ-water, PBS-buffer was purged through the cells for 10 min and subsequently the BSA solution with a concentration of 50 mg mL^{-1} for 15 min. The films were rinsed with PBS-buffer in the cells for 10 min to desorb reversibly bound protein. The measurement was terminated at this stage. The flow rate was kept at 0.1 mL min^{-1} throughout all experiments. All measurements were performed at 21±0.1°C and physiological pH 7.4. The amount of adsorbed protein was determined as the overall change in frequency of the third overtone of the oscillation frequency (f_3) after the desorption step with PBS-buffer. Mean values and standard deviations were calculated from three individual measurements.

ABTS radical scavenging assay

The antioxidative activity of CS solution (1 % w/v), TOCN dispersion (1 % w/v) and TOCN+CS amphoteric dispersion (1 % w/v) was measured in order to get the antioxidative profile of all used components of investigated coatings. The antioxidative activity was assessed as

described below. Experiments were performed on the Cary 60 spectrophotometer (Agilent technologies, UK) fitted with peltier temperature control.

Preformed radicals of ABTS were generated by the oxidation of ABTS (7.0 mM) with potassium persulfate (2.75 mM $K_2S_2O_8$) for 12 h in the dark at room temperature. After generation, ABTS was diluted with PBS buffer to an appropriate concentration (absorbance of 0.70 ± 0.02 at 734 nm), affording the working solution. The absorbance of a mixture of a 0.1 g of sample and a 3.9 mL of working solution was measured 15 and 60 min after beginning of reaction, at 734 nm.

The antioxidative activity of CS solution (1 % w/v), TOCN dispersion (1 % w/v) and TOCN+CS amphoteric dispersion (1 % w/v) was measured as the decrease in absorbance of the ABTS and was expressed as percent inhibition of ABTS radicals calculated according to the following equation:

$$\text{Inhibition, \%} = \frac{Abs_0 - Abs_1}{Abs_0} 100$$

where Abs_0 is the absorbance measured at the initial concentration of ABTS, Abs_1 is the absorbance measured at the residual concentration of ABTS.

Result and discussion

Preparation TOCN from cotton fibers

TOCN dispersions of different concentrations were prepared by fibrillation of the TEMPO-oxidized cotton fibers. During the disintegration treatment, significant amounts of sodium carboxylate groups (0.83 mmol/g cell) and aldehyde groups (0.09 mmol/g cell) in the cotton fibers formed by oxidation allowed the nanofibrils within the fibers to separate more easily due to the repulsive forces between the ionized carboxylates, which overwhelmed the hydrogen bonds holding them together [6].

Charging behaviour of the TOCN, TOCN+CS, and CS

For polyelectrolytes such as TOCN, CS and their mixture used in this work, the charging behaviour is an important surface property with respect to adsorption and swelling. Results of the pH dependent potentiometric titrations performed on 0.1 % (w/v) TOCN dispersion, 0.1 % (w/v) CS solution and 1 % (w/v) amphoteric TOCN+CS mixture are shown in Figure 1a c. Increasing negative surface charges of TOCN with increasing pH values can be seen since all carboxyl moieties are uncharged at pH below 2 and exhibit the highest surface charge at pH≥7 (full deprotonation of carboxylic groups). Potentiometric charge titration of CS shows an increasing positive surface charge with reducing pH values due to the fact that all amino moieties are uncharged at pH≥ 8 and exhibit the highest surface charge at pH ≤ 4 (Figure 1b). Potentiometric charge titration of TOCN+CS amphoteric mixture shows typical amphoteric curve with zero charge at pH 7 (Figure 1c) *i.e.* when the pH equals 7 the numbers of negative and positive charges are in balance resulting in a net neutral mixture. At lower pH conditions the mixture is positively charged whereas at higher pH conditions the mixture is negatively charged due to pH dependent protonation/deprotonation of weak acids/base. The content of anionic functional groups present in TOCN (COO^-) and cationic functional groups present in CS (NH_3^+) is 0.83 mmol g^{-1} and 4.28 mmol g^{-1}, respectively. For TOCN+CS amphoteric mixture the content of anionic (COO^-) and cationic (NH_3^+) functional groups is equal (0.45 mmol g^{-1}) *i.e.* total quantity of negative surface charge from TOCN is

completely neutralized with same quantity of positively charged ammonium groups from CS. From the results, it can also be seen that by preparation of TOCN+CS mixture electrostatic interaction between anionic charged TOCN and cationic charged CS are established.

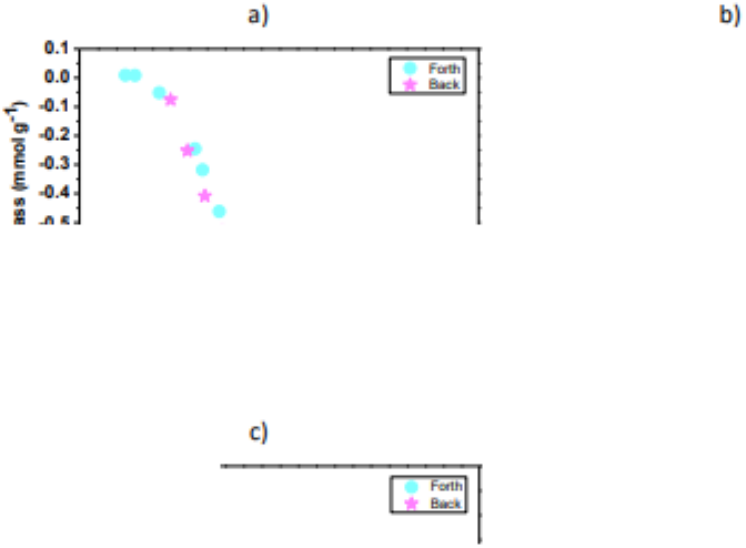

Figure 1. *Potentiometric charge titration values of TOCN dispersion (a), CS solution (b) and TOCN+CS amphoteric mixture (c) as a function of pH*

Protein adsorption

In this study, using QCM-D technique, protein-repellent properties, *i.e.* adsorption and desorption of BSA from surface of RC/TOCN and RC/TOCN/CS films, were compared. Additionally, considering fact that proteins tend to adhere more strongly to charged then to uncharged substrates[24], 1 % amphoteric dispersion based on TOCN and CS with low charge density at pH 7.4 (Figure 1c) was also used for coating RC surfaces (method II for preparing RC/CS+TOCN film). Figure 2 shows representative plots of Δf_3 versus time for RC/TOCN, RC/TOCN/CS and RC/TOCN+CS films switched from H_2O, PBS buffer, BSA solution and back to PBS buffer. The amount of adsorbed BSA on the surface of investigated films is shown in Figure 3.

Change in the frequency of -33 Hz between BSA adsorptive and PBS buffer rinse step (Figure 2) clearly shows that adsorption of BSA on the surface of RC/TOCN film occurs. Isoelectric point of BSA is around 4.7-4.9[25], so at higher pH conditions BSA is negatively

charged. Actually, at pH 7.4 the net surface charges of both BSA and TOCN are negative, so it is possible that there is an electrostatic repulsion between them. Therefore it is suggested that the interaction of BSA with TOCN may be very specific, in accordance with the anionic groups of polysaccharides [25].

Figure 2. *QCM-D change in frequency for the adsorption of BSA on a) RC/TOCN, b) RC/TOCN/CS and c) RC/TOCN+CS films*

In the case of RC/TOCN/CS film, amino groups of chitosan are minorly protonated at pH 7.4 (Figure 1b) so it is possible that there is an electrostatic attraction between them and BSA. Figure 2 shows that frequency shift of -39 Hz between BSA adsorptive and PBS buffer rinse step for RC/TOCN/CS film is bigger than frequency shift of -33 Hz for RC/TOCN film *i.e.* deposited chitosan has contributed to the deterioration of protein-repellent properties. If we analyze these results together with the TOCN and CS surface charge at pH 7.4 (Figure 1a-b), which amounts -0.8 mmol g^{-1} and +0.23 mmol g^{-1}, respectively, it is logical that the amount of adsorbed BSA increases as a consequence of the surface negative charge decrease and decreased electrostatic repulsion. Namely, a significant portion of the carboxyl groups is covered with the bound CS molecules and unavailable for electrostatic repulsion with BSA molecules. Additionally, bounded positively charged CS molecules can electrostatically attract negatively charged BSA molecules.

In the case of both films (RC/TOCN and RC/TOCN/CS), considerable adsorption of BSA was recorded (Figure 3). Due to the high surface charge of TOCN as well as CS, we get the idea to make film with amphoteric character of its surface and with low surface charge at pH 7.4. For that purpose amphoteric dispersion based on TOCN and CS i.e. polyanion/polycation complex was prepared. For this complex, at pH 7.4 anionic charge is -0.04 mmol g^{-1} (Figure 1c). As expected, frequency change of 7 Hz observed for BSA adsorption on RC/TOCN+CS film was significantly smaller than in the case of both RC/TOCN and RC/TOCN/CS films (Figure 2) confirming on such way a weak adsorption of BSA on the RC/TOCN+CS film (Figure 3).

Upon rinsing with PBS buffer an increase in frequency is detected due to the reversible protein adsorption. We may assume that the weak adsorption of proteins to a surface only

occurs on the top of the swollen film and the weakly bound protein rapidly dissociates from the surface when the solution of protein is replaced with buffer. With respect to the fact that TOCN, CS and their amphoteric mixture are different substrates, as well as their possibility to interact by various and still incompletely researched mechanisms with BSA[24], it is very hard to give detailed explanation for obtained results.

Figure 3. *The amount of adsorbed BSA on surface of the RC/TOCN, RC/TOCN/CS and RC/TOCN+CS films*

ABTS radical scavenging assay

Antioxidative activity is one of the well-known functions of CS, but according to our best knowledge this function still is not studied for TOCN and TOCN/CS mixture. Figure 4 shows the antioxidative inhibition percentages of ABTS radical by CS solution (1 % w/v), TOCN dispersion (1 % w/v) and TOCN+CS amphoteric mixture (1 % w/v). In this test, an anti-oxidative molecule reduces the ABTS radical to 2,2'-azino-bis-3-ethylbenzothiazoline-6-sul-fonic acid.

Figure 4. *Scavenging ability of 1% CS solution, 1% TOCN dispersion, and 1% TOCN+CS amphoteric mixture*

The extent of discoloration will depend on the hydrogen-donating ability. For all investigated samples, inhibition increases with the progress of time. According to the

results, after 60 min CS shows 18.34 %, and TOCN 13.38% of inhibition of ABTS radicals. Many studies have shown that CS inhibits the ROS and several mechanisms about the antioxidative action of CS have been proposed[26]. CS can scavenge free radicals or chelate metal ions from the donation of a hydrogen or the lone pairs of electrons[27]. The hydroxyl groups and amino groups in CS are the key functional groups for its antioxidative activity, but can be difficult to be dissociated due to the semi-crystalline structure of chitosan with strong hydrogen bonds[27]. At the same time, amphoteric TOCN+CS mixture shows significantly lower inhibition (2.23%) in comparison with CS and TOCN which could be due to the interactions between CS and TOCN i.e. their H-atom donating groups.

Conclusion

In this work, a new approach to obtain protein-repellent and antioxidative coatings based on TEMPO oxidized cellulose nanofibrils and chitosan was proposed. Coating with improved protein-repellent properties was obtained using TOCN+CS amphoteric mixture with almost zero charge (-0.04 mmol g^{-1} at pH 7.4) considering the fact that proteins tend to adhere more strongly to charged than to uncharged surfaces. The amount of adsorbed BSA on surface RC/TOCN+CS is significantly lower than on surfaces RC/TOCN and RC/TOCN/CS (TOCN and CS are highly surface charged at pH 7.4 (-0.8 mmol g^{-1} and +0.23 mmol g^{-1}, respectively)). On the other hand, TOCN+CS amphoteric mixture shows significantly weaker antioxidative properties in comparison to TOCN and CS, which could be due to the interactions between CS and TOCN i.e. their H-atom donating groups. Obtained results are good starting point to create upgraded coatings of TOCN and CS that will show both properties simultaneously.

Acknowledgments: *The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support through the project OI 172029.*

Protein-odbijajuća i antioksidativna svojstva bioaktivnih prevlaka na bazi TEMPO oksidisanih celuloznih nanofibrila i hitozana

U ovom radu, filmovi od regenerisane celuloze (RC) su naslojeni sa TEMPO oksidisanim celuloznim nanofibrilima (TOCN) i hitozanom (CS) metodom rotirajućeg diska. Bioaktivnost prevlaka postignuta je dodavanjem hitozana. Hitozan je ili pomešan sa TOCN (TOCN+CS) i naslojen na RC film tehnikom rotirajućeg diska ili nanesen na RC/TOCN dvoslojni film propumpavanjem njegovog vodenog rastvora, pH vrednosti 5,5, preko površine filma. Naelektrisanje TOCN, TOCN+CS i CS u zavisnosti od pH je okarakterisano pH-potenciometrijskim titracijama. Protein-odbijajuća svojstva ispitivanih prevlaka određena su in situ, u kontinualnom toku goveđeg serumskog albumina (BSA) primenom kvarc-kristal mikrovage sa praćenjem disipacije (QCM-D). Antioksidativna svojstva TOCN, CS i TOCN+CS amfoterne mešavine su određena metodom inhibicije radikala 2,2'-azino-bis(3-etil-benzotiazolin-6-sulfonske kiseline). Prevlaka sa poboljšanim protein-odbijajućim svojstvima je dobijena upotrebom TOCN+CS amfoterne mešavine, ali sa druge strane TOCN+CS amfoterna mešavina je pokazala slabija antioksidativna svojstva u poređenju sa TOCN i CS.

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