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Enhanced transport and antifouling properties of polyethersulfone membranes modified with α-amylase incorporated in chitosan-based polymeric micelles

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ABSTRACT

The objective of this study is related to the modification of polyethersulfone membranes with α-amylase incorporated into chitosan-based polymeric micelles in order to reduce the membrane fouling. Amphiphilic chitosan derivatives have been synthesized through grafting of palmitic (PA) and stearic (SA) acids onto low-molecular weight chitosan. CMC values of palmitoyl and stearoyl derivatives of chitosan are equal to $5.7{\cdot}10^{-2}$ mg/mL and $3.9{\cdot}10^{-2}$ mg/mL, respectively. Whereas, the size of polymeric micelles amounted to 707 ± 64 nm and 609 ± 57 nm for Chit-g-PA and Chit-g-SA, accordingly. The obtained micelles with enzyme have been adsorbed onto the surface of polyethersulfonic membranes. Modified membranes are characterized by high stability of the coating over time, thanks to the surface-active properties of chitosan derivatives and electrostatic interactions. The antifouling biocatalytic properties of modified membranes were studied in the process of starch filtration. The unmodified membrane showed the lowest permeability with an increase in the concentration of starch solutions due to dramatically increased cake formation. The permeability of modified membranes has been improved remarkably compared to the pristine one during starch ultrafiltration. The cake resistance of modified membranes decreases 6-fold, compared to the unmodified PES one. Furthermore, the effect of concentration polarization is attenuated owing to starch hydrolysis by α-amylase incorporated into polymeric micelles. The maximum activity of α-amylase immobilized on PES membrane is observed in the pH range of 6.5–7.5 and the higher resistance of enzyme to acidic media compared to the native one has been shown. High stability and reusability of immobilized α-amylase has been demonstrated. The degree of starch conversion reduces by 35% and 30% after 10 cycles for a membrane modified with Chit-g-PA and Chit-g-SA, respectively.

1. Introduction

Self-assembling is a promising approach to obtain nanostructures in a controlled manner [1]. In particular, amphiphilic polymers are widely used for the preparation of micelles and vesicles for intelligent drug delivery of different types of drugs such as antibacterial [2], anti-cancer [3], siRNA [4], and peptide drugs [5]. Thus, the polymeric micelles with various encapsulated active compounds can be used not only for the drug delivery, but also for the protection of enzymes and their stabilization [6,7]. They allow the protection of proteins from degradation, the increasing of pH and temperature stability, and the transporting of substrates and products of enzymatic reaction.

The immobilization of enzymes on membranes is carried out with the aim of heterogeneous biocatalysis in order to provide antifouling or antibacterial properties to membranes, to decompose foulants or to fabricate biosensors. Li et al. modified membranes with laccase and TiO_2 for conducting red X–3B dye degradation by both photo- and biocatalysis [8]. They found out that the immobilized enzyme was characterized by the enhanced pH and temperature stability compared with a native laccase. Cao and co-workers successfully removed bisphenol A from water using membranes modified with laccase and a dopamine layer [9]. Othman et al. also proved the enhanced stability of laccase immobilized on carbon nanotube membranes in the process of Reactive Black decolorization [10]. Xu et al. demonstrated that poly(vinyl

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alcohol)/poly(acrylic acid)/SiO_2 nanofibrous membranes with immobilized horseradish peroxidase were active in the process of paracetamol removal [11]. Duan et al. reported about the preparation of polyethersufone membranes filled with lyzozyme immobilized onto graphene [12]. Such membranes exhibited antibacterial properties towards *E. coli*. Schulze and co-workers performed a covalent immobilization of trypsin onto microfiltration poly(vinylidene fluoride) membranes, which were characterized by self-cleaning and antifouling properties in the process of albumin filtration [13]. Zeuner et al. demonstrated the possibility of formaldehyde reduction to methanol on ceramic membranes with covalently immobilized alcohol dehydrogenase [14]. Moreno-Cortez and co-workers confirmed that papain immobilized on poly(vinyl alcohol) nanofibrous membranes kept its activity and showed good reusability [15]. Furthermore, Qiao and co-workers created membrane enzyme reactor for on-line analysis of proteins based on trypsin immobilized on poly (styrene-co-maleic anhydride) membranes [16].

Amylase as a starch degrading enzyme is widely used in many industries, in particular, for manufacture of fructose, maltose, and oligosaccharide mixtures in the food industry; for starch fermentation to ethanol in fuel production; for starch sizing agent removal in the textile industry; for bleaching in the detergent industry, etc. [17]. Given the variety of applications, the immobilization of amylase is also of interest for immobilization on membranes. Thus, Cloete et al. studied the influence of combined immobilization enzymes on their activity. For this purpose, α-amylase, protease, and β-galactosidase were covalently attached to electrospun poly(styrene-alt-maleic anhydride) nanofibrous membranes [18]. It was shown that α-amylase was characterized by its increased activity in the presence of other enzymes, while protease partially lost its activity. Schulze et al. immobilized pancreatin (a mixture of α-amylase, protease, and lipase) onto polyethersulfone membranes to obtain a stimuli responsive self-cleaning surface [19]. Wong et al. also proved the reduction of the fouling of membranes after the immobilization of an enzyme blend consisting of lipases, amylases, and proteases using them in an anaerobic membrane bioreactor [20]. Previously, we have reported entrapment of α-amylase into micelles based on commercial block-copolymers Tetronics® with their further immobilization onto the surface of polyethersulfone membranes to provide them antifouling and biocatalytic properties [21].

The present study is aimed at the investigation of the transport, antifouling, and biocatalytic properties of polyethersulfone membranes with immobilized α-amylase applied in the process of starch filtration. For this purpose, low molecular weight chitosan has been modified with the palmic and stearic acids to confer self-assembling properties on it. Subsequently, commercial PES membranes have been modified with α-amylase entrapped into obtained polymeric micelles based on chitosan derivatives. The effect of pH on membrane bioactivity was examined and the impact of starch concentration on membrane fouling was studied.

2. Materials and methods

Low-molecular weight chitosan ($M_{\tilde{n}}$~110 kDa) with a degree of deacetylation of 75% and α-amylase from *Bacillus subtilis* were purchased from Fluka (Japan). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was bought from Sigma-Aldrich (USA). Palmitic acid (PA) and stearic acid (SA) were supplied by Enamine (Ukraine). All reagents and solvents were of analytical grade and were used without further purification. Deionized water was used for the preparation of all solutions. Polyethersulfone P030 membranes with a cut-off of 30 kDa were purchased from Microdyn Nadir (Germany). As recommended by manufacturer, membranes were immersed in deionized water for 24 h prior to the modification.

2.1. Modification of chitosan with palmitic and stearic acids

Chitosan was modified with palmitic or stearic acids through the EDC-mediated reaction according to a modified method described elsewhere [22]. A fixed amount of chitosan was added to a 1.0% solution of acetic acid at 20 °C on a temperature-controlled water bath with constant stirring. After that, EDC was added to the above mixture and temperature was increased to 90 °C. 2.5% solution of palmitic or stearic acid in ethanol was added gradually to the mixture at a constant temperature of 90 °C. Furthermore, the solution was left for 2 h at the mentioned temperature. After stirring and cooling down to room temperature, the sample was filtered and precipitated in hexane. The final chitosan derivatives were dried at 40 °C to the constant weight and ground into powder.

2.2. Determination of a substitution degree of chitosan derivatives

The degree of substitution was calculated as the difference of the amount of free NH_2-groups of chitosan and its modified derivatives. For this purpose trinitrobenzene sulfonic acid (TNBS) assay was used as described elsewhere [23]. 1 mL of polymer solution was mixed with 1 mL of 0.1% TNBS solution and 1 mL 4% $NaHCO_3$ solution. The resulting solution was incubated at 37 °C for 2 h, and 1 mL of HCl was added. The amount of amino groups was measured at 344 nm using UV–vis spectrometer.

2.3. Preparation of Chit-g-PA and Chit-g-SA polymeric micelles with incorporated α-amylase

For preparation of polymeric micelles with incorporated α-amylase, a required amount of Chit-g-PA or Chit-g-SA was dissolved in deionized water under sonication during 2 h in order to untangle the polymeric chains. After that, the necessary amount of enzyme was added and the subsequent solution (a resulting concentration of α-amylase was 1 mg/mL) has been mixing at 300 rpm for 24 h.

The encapsulation efficiency (EE) was determined by measurement of the enzyme concentration in the supernatant. The amount of non-entrapped α-amylase in the aqueous phase was determined by the Bradford method using bovine serum albumin as a standard [24] after ultracentrifugation of micelles. The EE (%) was calculated from the following equation:

$$EE = \frac{W_0 - W_t}{W_0} \times 100, \quad (1)$$

where W_0 and W_t are the weight of initial enzyme and that of the total amount of protein detected in the supernatant.

2.4. Membrane modification with chitosan derivatives micelles

Commercial polyethersulfone membranes PES-30 with a cut-off of 30 kDa were modified with polymeric micelles *via* adsorption. After soaking in deionized water, they were placed in a dead-end cell and suffused with a solution of Chit-g-A-enzyme micelles for 60 min and then rinsed with deionized water.

2.5. Chitosan derivatives characterization

FTIR spectra of chitosan and its derivatives powders were recorded at a FT-IR spectrometer Bruker Vertex 80v (Billerica, Massachusetts, USA) as KBr pellets or IRAffinity-1 (Shimadzu, Japan) in ATR mode in the range of 4000–400 cm^{-1} at room temperature.

The critical micelle concentration (CMC) values of synthesized chitosan derivatives were determined by the conductometric technique. Conductivity was measured by a digital conductivity meter (Hanna Instruments, USA) at least for 10 different concentrations of samples in

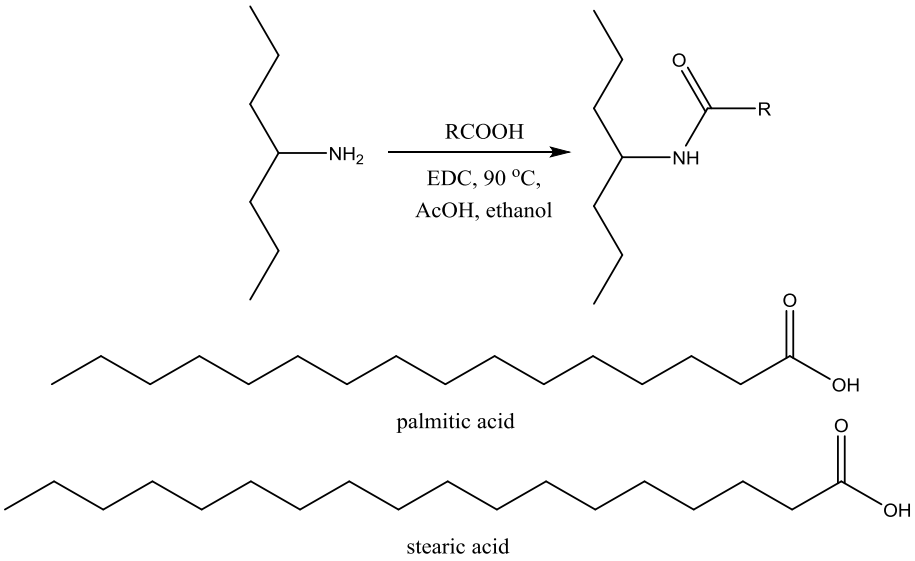

Fig. 1. Scheme of chitosan modification with fatty acids.

0.5 M NaCl for polyelectrolyte effect suppressing.

The hydrodynamic diameters of polymeric micelles were measured by dynamic light scattering DLS (Malvern Zetasizer, Malvern Instruments, UK) at 20 °C. For size measurement Chit-g-PA and Chit-g-SA solutions of five concentrations in the range of 0.1–0.3% were prepared in deionized water.

The uniformity index (UI) was calculated as follow:

$$UI = \frac{D_v}{D_n} \tag{2}$$

where D_v is volume-averaged hydrodynamic diameter [nm]; D_n is a number-averaged hydrodynamic diameter [nm].

2.6. Membrane characterization

Ultrafiltration experiments were conducted in the dead-end mode as described previously [21]. Briefly, a dead-end cell Amicon 8050 (Millipore, USA) with a volume of 50 mL and membrane area of 13.4 cm^2 was used. The ultrafiltration experiments were conducted at operation pressures of 50–300 kPa and at stirring of 300 rpm.

The biocatalytic properties of membranes were evaluated in the process of starch conversion in the range of concentrations 0.01–0.05%. The starch concentration was determined according to the procedure presented elsewhere [25]. The pH optima of immobilized α-amylase were determined at room temperature in various pH of feed solution.

Atomic force microscopy (AFM) images were obtained using NanoScope MultiMode SPM System and NanoScope IIIa and Quadrex controller (Veeco, Digital Instrument, UK). AFM images of dried membranes were recorded over an area of 5 μm × 5 μm in air at room temperature.

Dependence of zeta-potential of membrane surface on pH value was determined using Electrokinetic Analyzer Anton-Paar EKA Streaming Potential Meter (Austria) in KCl solution at a concentration of $1 \cdot 10^{-3}$ M. The pH of electrolyte was controlled by adding of 0.01 N NaOH or HCl solutions.

Darcy's law (Eq. (3)) was used for quantification of membranes fouling during starch ultrafiltration:

$$J_v = \frac{\Delta P}{\mu(R_m + R_c)} \tag{3}$$

where ΔP is the pressure difference across the membrane [Pa]; μ is the dynamic viscosity of solution [Pa·s]; R_m is the membrane resistance [1/m]; R_c is the cake resistance [1/m].

The flux decline ratio (DR) was calculated by using equation (4) [26]:

$$DR = \left(1 - \frac{J_f}{J_w}\right) \tag{4}$$

where J_w is the pure water flux [l/(m^2·h)]; J_f is the flux of feed solution [l/(m^2·h)].

The surface free energy (SFE) of membranes was calculated according to van Oss-Chaudhury-Good method [27] using a system of three equation (5):

$$\begin{cases} \gamma_{l1}(1+\cos\theta) = 2\sqrt{\left(\gamma_s^{LW}\gamma_{l1}^{LW}\right)} + 2\sqrt{\left(\gamma_s^{+}\gamma_{l1}^{-}\right)} + 2\sqrt{\left(\gamma_s^{-}\gamma_{l1}^{+}\right)}; \\ \gamma_{l2}(1+\cos\theta) = 2\sqrt{\left(\gamma_s^{LW}\gamma_{l2}^{LW}\right)} + 2\sqrt{\left(\gamma_s^{+}\gamma_{l2}^{-}\right)} + 2\sqrt{\left(\gamma_s^{-}\gamma_{l2}^{+}\right)}; \\ \gamma_{l3}(1+\cos\theta) = 2\sqrt{\left(\gamma_s^{LW}\gamma_{l3}^{LW}\right)} + 2\sqrt{\left(\gamma_s^{+}\gamma_{l3}^{-}\right)} + 2\sqrt{\left(\gamma_s^{-}\gamma_{l3}^{+}\right)}, \end{cases} \tag{5}$$

where γ^{LW} is the Lishitz-van der Waals component; γ^+ and γ^- are the acidic and basic components; γ_l is the SFE of a measured liquid; θ is the contact angle between the membrane surface and the measured liquid. For determination such liquids as water, glycerin, and hexane were used.

Acid-base component of SFE γ^{AB} of membranes was calculated as:

$$\gamma^{AB} = 2\sqrt{\gamma_s^{+}\gamma_s^{-}} \tag{6}$$

Total SFE was calculated according to equation (6):

$$\gamma_s = \gamma^{LW} + \gamma^{AB}. \tag{7}$$

Work of adhesion was calculated as:

$$W_a = \gamma_l(1+\cos\theta). \tag{8}$$

3. Results and discussion

3.1. Synthesis and self-assembling properties of chitosan derivatives

Chitosan has a lot of free amino groups distributed along the polymer chain that could be modified to change its properties. Acylation is often used for the alkyl moieties attachment in purpose of chitosan hydrophobization [28,29]. This method was applied within this study to modify chitosan with palmitic and stearic acids using EDC as the

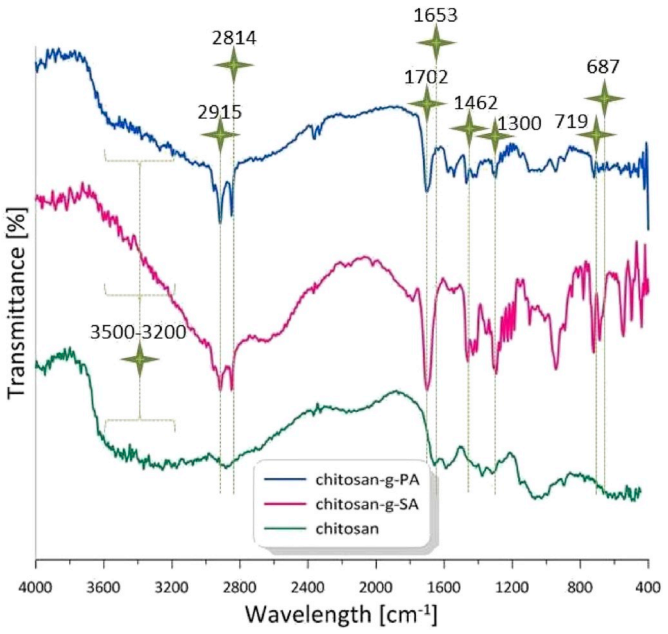

Fig. 2. IR spectra of chitosan and its modified derivatives.

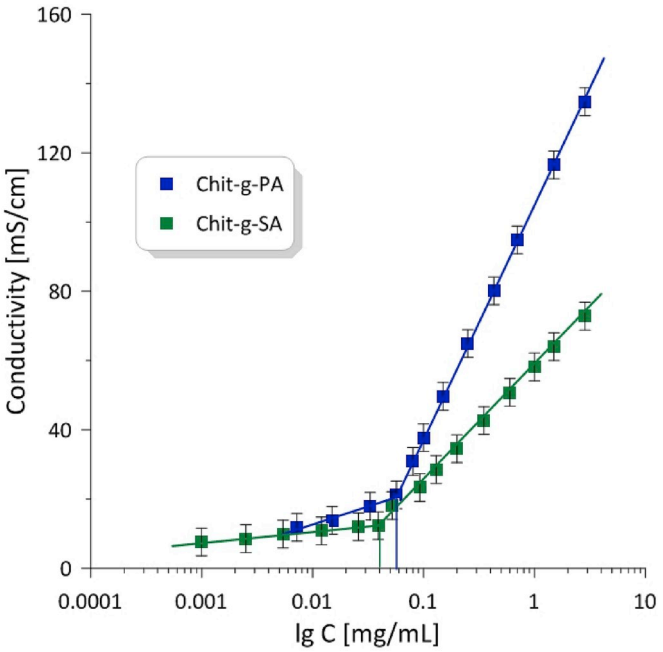

Fig. 3. The relationship of conductivity to the concentration of chitosan derivatives.

coupling agent (Fig. 1). The reaction products are designated as Chit-g-PA and Chit-g-SA for palmitoyl and stearoyl derivatives of chitosan, respectively.

Chitosan derivatives modified with palmitic and stearic acids were obtained as milky flakes in good yields (73% of Chit-g-PA and 71% of Chit-g-SA). The calculated amino-substitution was 5.4 and 5.6% for Chit-g-PA and Chit-g-SA, accordingly. Furthermore, by contrast with chitosan, which is water insoluble, modified polysaccharides were characterized with the ability to dissolve in water at concentrations up to 0.35% giving colourless solutions. This fact could be explained by the weakening of inter- or intra-molecular hydrogen bonds in chitosan by long alkyl moieties [30].

The grafting of palmitic and stearic acids was confirmed by IR-spectroscopy (Fig. 2). For nonacylated chitosan, characteristic bands at 3500-3200 cm^{-1} and 1653 cm^{-1} were observed. Thus, the broad band at 3500-3200 cm^{-1} corresponded to the stretching vibration of N–H bonds of amino groups involved in hydrogen bonds formation, whereas N–H bending vibration of nonacylated amino groups appeared at 1653 cm^{-1}. After modification of chitosan with fatty acids, stretching vibrations of C–H bonds of $–CH_3$ (2913 cm^{-1}) and $–CH_2–$ (2814 cm^{-1}) groups of alkyl moieties were registered. Amide bond formation was confirmed by appearance of bands Amide I, II, III, IV and V in the spectra of modified derivatives. A strong Amide I band associated with $\nu_{C=O}$ was observed at 1702 cm^{-1}. Bending vibrations of N–H bonds of secondary amino groups (Amide II) appeared at 1462 cm^{-1}. An amide III band corresponding to stretching vibrations of C–N bond was found at 1300 cm^{-1}. The out-of-plain bending vibrations of C═O and N–H bonds caused the appearance of bands at 719 cm^{-1} (Amide IV) and 687 cm^{-1} (Amide V), respectively. Moreover, acylation of amino group of chitosan was proved by the disappearance of the bands at 3500–3200 and 1653 cm^{-1} corresponding of $\nu_{N\text{-}H}$ and $\delta_{N\text{-}H}$ vibrations of NH_2-groups of unmodified chitosan, accordingly.

Hydrophobic modified carbohydrate polymers could spontaneously form micelles in aqueous solutions because of inter- and intramolecular hydrophobic interactions [31]. Chitosan, as well, when modified with fatty acids possesses an amphiphilic nature and is expected to self-assemble owing to the difference in the solubility of the hydrophilic polymer chain and grafted hydrophobic segments. Thus, the self-aggregation properties of hydrophobized chitosan derivatives were monitored by the conductometric technique. The relationship of conductivity to the concentration of the samples can be seen in Fig. 3. The inflection point of each curve coincides with the CMC value of polymer. It was found that the CMC value of Chit-g-PA was $5.7{\cdot}10^{-2}$ mg/mL and Chit-g-SA was $3.9{\cdot}10^{-2}$ mg/mL. The obtained results corresponds to the tendency that grafting of longer alkyl chains to polysaccharides causes the decrease in their CMC values [32].

The self-assembled micelles size of chitosan derivatives of different concentrations was determined by the DLS method. Hydrodynamic diameter distributions of micelles in deionized water are shown in Fig. 4 whereas the mean diameter values (D_{mean}) and calculated uniformity indices (UI) are gathered in Table 1. It was noted that the size distributions of both derivatives at lower concentrations (0.15 and 0.20%) possess only one peak, whereas at increasing concentration bimodal distributions were observed which indicate the formation of micelles aggregates. The D_{mean} values of hydrophobized chitosan of 0.15% concentrations were 707 ± 64 and 609 ± 57 nm for Chit-g-PA and Chit-g-SA, respectively. As expected, the size of Chit-g-SA micelles is smaller compared to Chit-g-PA, which was caused by the increasing of the alkyl chain length and, accordingly, hydrophobic interactions allowing more dense polymer arrangement. Micelles derived from modified chitosan of a concentration of 0.15% were characterized by the smallest uniformity indexes, therefore the mentioned concentration was chosen for further α-amylase entrapment and membrane modification.

3.2. PES membrane modification with chitosan derivatives

Commercial polyethersulfone membranes PES-30 were modified by adsorption of Chit-g-PA and Chit-g-SA micelles with and without α-amylase *via* immersing membrane samples in an appropriate solution for 1 h. The encapsulation efficiency of α-amylase into Chit-g-PA and Chit-g-SA polymeric micelles was evaluated as 59.8 and 60.1%, respectively.

IR spectrometry in ATR mode was used to confirm the modification of membrane surface with polymeric micelles with and without enzyme. As can be seen from the obtained IR spectra (Fig. 5), the intensity of bands in the region 3500-3200 cm^{-1} (N–H vibrations), 2900-2800 cm^{-1} (C–H vibrations bond of aliphatic chains), and 1700 cm^{-1} (C═O vibrations) increased after the membrane modification with micelles based

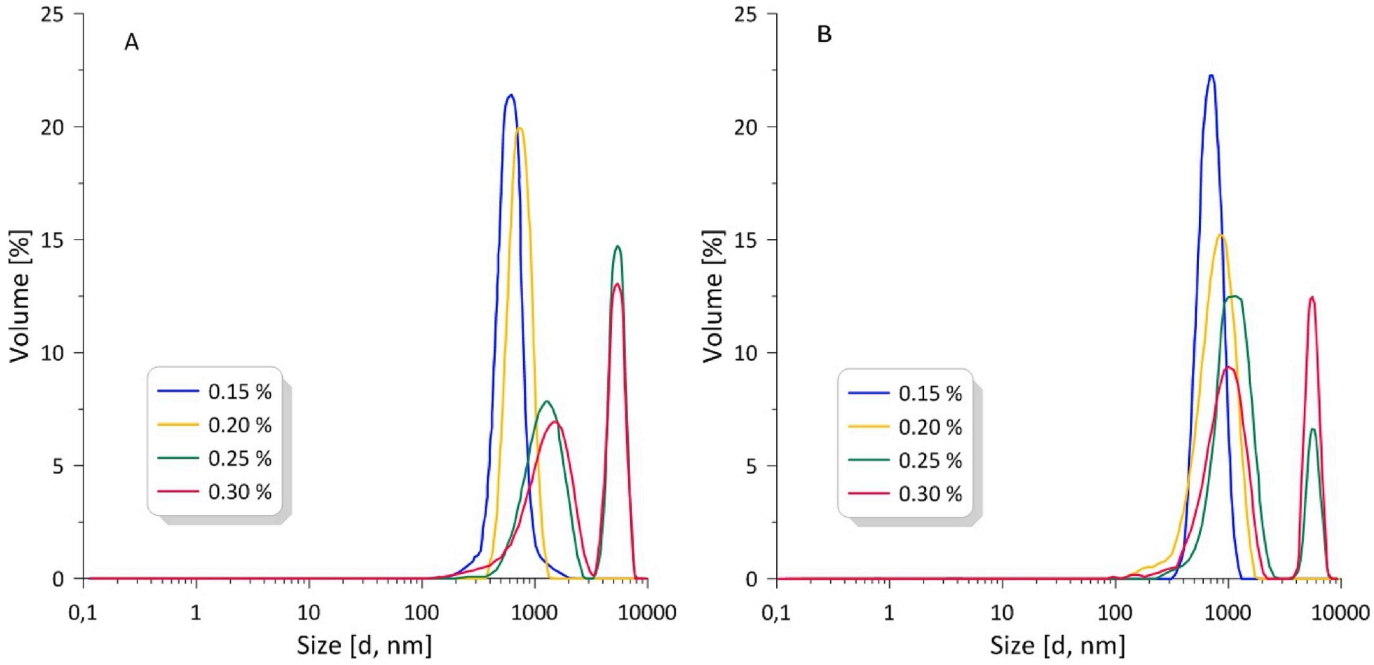

Fig. 4. Volume-based hydrodynamic diameter distribution of (A) Chit-g-PA and (B) Chit-g-SA derivatives in deionized water.

Table 1
Characteristics of micelles of chitosan derivatives.

	Chit-g-PA		Chit-g-SA	
Concentration, %	D_{mean}, nm	UI	D_{mean}, nm	UI
0.15	707 ± 64	1.17	609 ± 57	1.19
0.20	870 ± 71	1.27	742 ± 69	1.23
0.25	1159 ± 68; 5682 ± 127	1.56	1274 ± 74; 5316 ± 112	1.35
0.30	1022 ± 109; 5502 ± 231	1.59	1461 ± 129; 5379 ± 214	1.38

on chitosan derivatives. Moreover, the entrapping of α-amylase into the micelles caused a shift of maximum of the bands to a lower wavelength which can be attributed to the strengthening of the hydrogen bonding interactions.

The AFM was conducted to study the membranes topography. The three-dimensional and two-dimensional AFM images of the pristine membrane and membranes with immobilized micelles are shown in Fig. 5. The unmodified membrane was characterized by a smooth surface with a root mean square (RMS) value of 4.0 ± 0.3 nm (Fig. 6, A.1, A.2). The difference between the roughness parameters of the unmodified membrane and membranes modified with micelles was negligible,

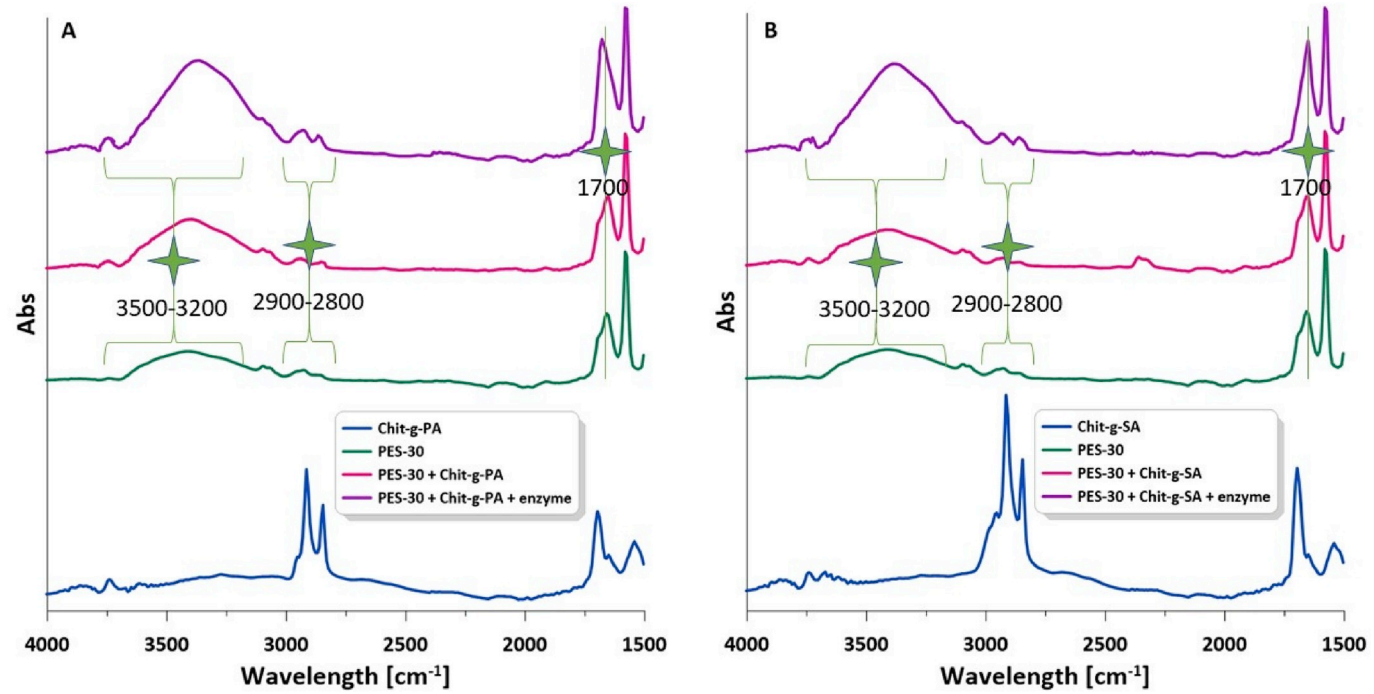

Fig. 5. IR spectra of chitosan derivatives and membrane surface after modification with polymeric micelles with and without α-amylase.

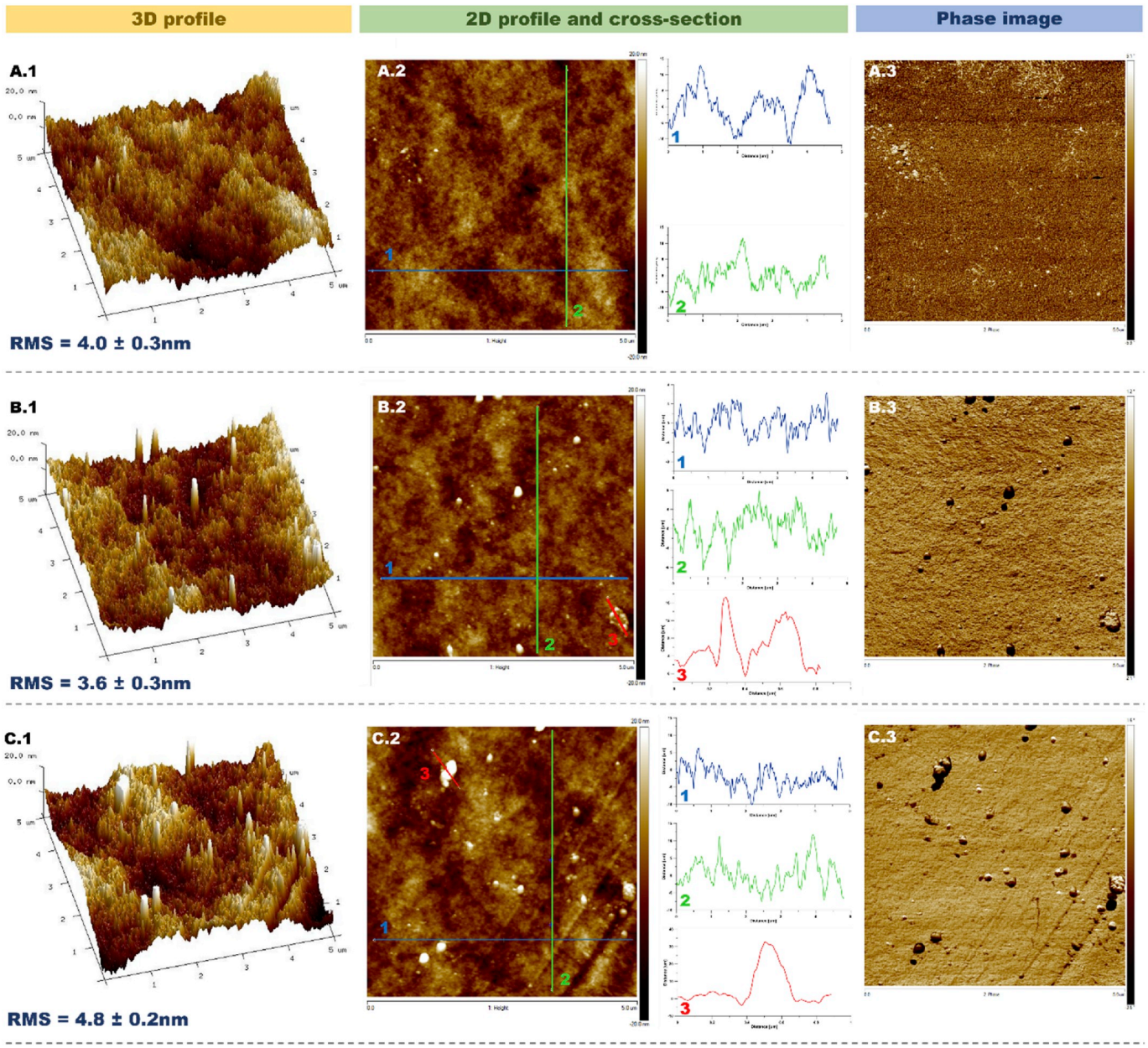

Fig. 6. AFM images of membranes: A – the unmodified PES-30 membrane; B – PES-30 membrane modified with Chit-g-PA-α-amylase micelles; C – PES-30 membrane modified with Chit-g-PA-α-amylase micelles: 1 – 3D profile; 2 – 2D profile and cross-section; 3 – phase images.

Table 2
Surface energy parameters.

Membrane	γ^{LW}, mN/m	γ^{-}, mN/m	γ^{+}, mN/m	γ^{AB}, mN/m	γ_s, mN/m	W_a (H_2O), mJ/m^2
Unmodified PES-30	28.17	1.09	21.26	9.63	37.8	106.98
Chit-g-PA	28.45	10.87	23.15	31.73	60.18	131.70
Chit-g-PA-α-amylase	28.58	13.06	22.70	34.44	63.02	133.86
Chit-g-SA	28.52	7.95	25.53	28.49	57.01	129.38
Chit-g-SA-α-amylase	28.64	13.30	22.98	34.95	63.90	134.54

but micelle adsorption caused changes in the surface profiles (Fig. 6, B.2, B.3). Thus, RMS values were 3.8 ± 0.3 nm and 4.8 ± 0.3 nm for membranes modified with Chit-g-PA and Chit-g-SA micelles, respectively. It should be noted that the needle-like structures were observed on the surfaces of modified membranes, which reduces the contact area of the surface with foulants and reduces their adhesion to membranes [33].

Surface energy data calculated for the membrane surface are summarized in Table 2. As can be seen, modification of membranes did not influence on the Lishitz-van der Waals (γ^{LW}) and acidic (γ^{+}) components of SFE, while it caused an increase of the basic component and as a result the acid-base component (γ^{AB}) and total surface energy (γ_s). The work of adhesion is higher for the modified membranes compared to the pristine membrane suggesting higher predisposition of the functionalized membrane surfaces to interact with water and hydrate the micelles that leads to the repulsion of moderately hydrophobic molecules of starch.

Surface zeta-potential was evaluated using streaming potential measurement in the range of pH values from 2.5 to 9.6 to confirm the successful immobilization of polymeric micelles onto membrane surfaces (Fig. 7). The pristine membrane was negatively charged in the whole range of pH values. Its zeta-potential was changed from -12.1 ± 0.6 mV at pH 2.8 to -16.3 ± 0.3 mV at pH 9.6. Modified

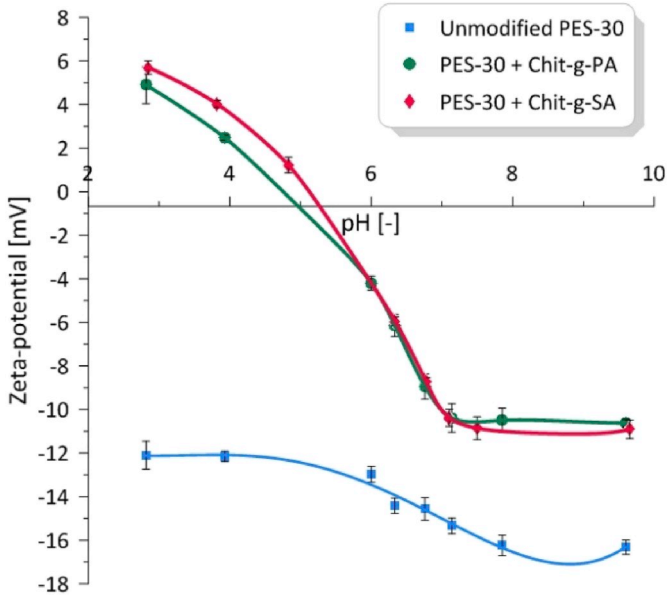

Fig. 7. Dependence of zeta-potential of unmodified membrane and membranes modified with chitosan derivatives on pH values.

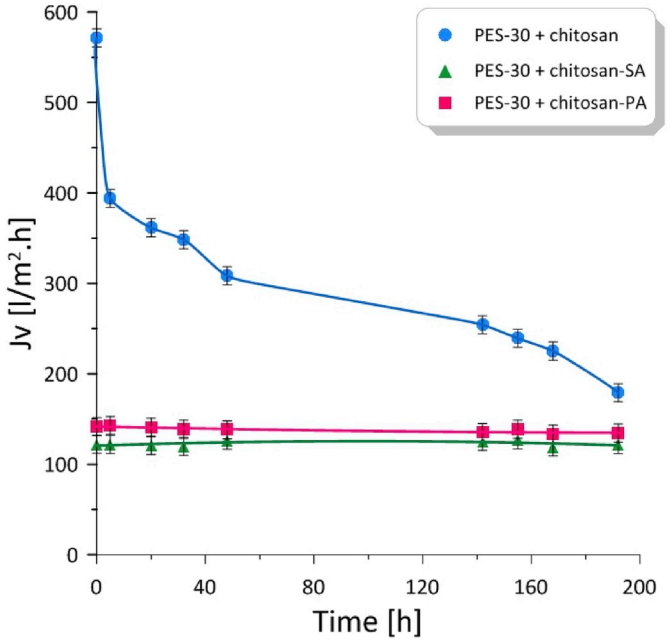

Fig. 8. Stability of membrane volumetric fluxes with time: ΔP = 200 kPa.

membranes possessed less negative zeta-potential values and significant changes of surface charge under the influence of pH variation. Thus, modified membranes were positively charged in the acidic medium (up to 4.8 ± 0.8 and 5.7 ± 0.3 mV at pH 2.8 for Chit-g-PA and Chit-g-SA, accordingly) and negatively charged in the alkaline one (up to 10.6 ± 0.2 and -10.9 ± 0.4 mV at pH 9.6 for Chit-g-PA and Chit-g-SA, accordingly) with the isoelectric point at 4.7 and 5.0 for Chit-g-PA-enzyme and Chit-g-SA-enzyme membranes, respectively. Such behavior could be explained by protonation and deprotonation of unsubstituted amino groups of chitosan derivatives and implied that membrane surfaces were covered with layers of micelles.

Since the membranes were modified by the physical adsorption, it was important to determine the stability of the coating over time. For this purpose, the membranes were kept in deionized water with constant

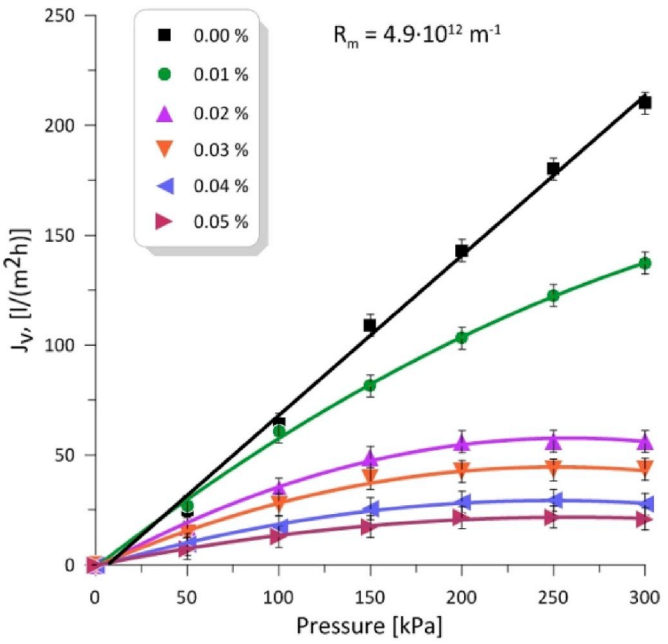

Fig. 9. The volumetric flux – the applied pressure curves for the pristine PES-30 membrane during the process of starch ultrafiltration at different feed concentrations.

stirring, periodically measuring their permeability. The membrane modified with chitosan was used as a control. Obtained dependencies are shown in Fig. 8. For the control membrane, the dramatic decline in permeability was observed after 2 h and the volumetric flux decreased in 3 times during 8 days. It could be explained by the leaching out of chitosan chains and blocking the pores of the membranes. In contrast to the control membranes, membranes modified with chitosan derivatives possessed stable volumetric fluxes during the experiment. It proved that Chit-g-PA and Chit-g-SA polymers were characterized by active surface-active properties due to a grafted alkaline moiety.

To control the stability of enzyme-chitosan derivative complex on the membrane surface, the protein amount in permeate and retentate after flux measurement was performed by the Bradford method. The obtained results confirmed the stability of formed polyelectrolyte complex Chit-A-enzyme, since leaking of α-amylase into the solution during the ultrafiltration experiment was not observed.

3.3. Permeability and antifouling properties of PES membranes modified with chitosan derivatives in the process of starch ultrafiltration

The process of the ultrafiltration of polysaccharide solutions is characterized by a flux decline due to fouling of the membrane surface. This phenomenon is caused by a convection flow through the membrane, which leads to direct contact of the foulants with the membrane surface and their adsorption and accumulation due to acid-base, electrostatic and hydrophobic-hydrophobic interactions.

Herein, the pure water volumetric flux and antifouling properties of membranes were investigated in the dead-end mode using water-soluble starch as model foulant. The ultrafiltration experiments were conducted at applied pressures from 50 to 300 kPa with starch solutions in the range of concentrations of 0.01–0.05%. The dependence of the volumetric flux on the pressure could be seen in Fig. 9. As expected, the unmodified PES membrane was characterized by poor antifouling properties. A significant flux decline was observed at a starch concentration of 0.02%. The shape of curves indicates the occurrence of concentration polarization phenomenon.

Dependences of the volumetric flux on the transmembrane pressure for membranes modified with chitosan derivatives with and without

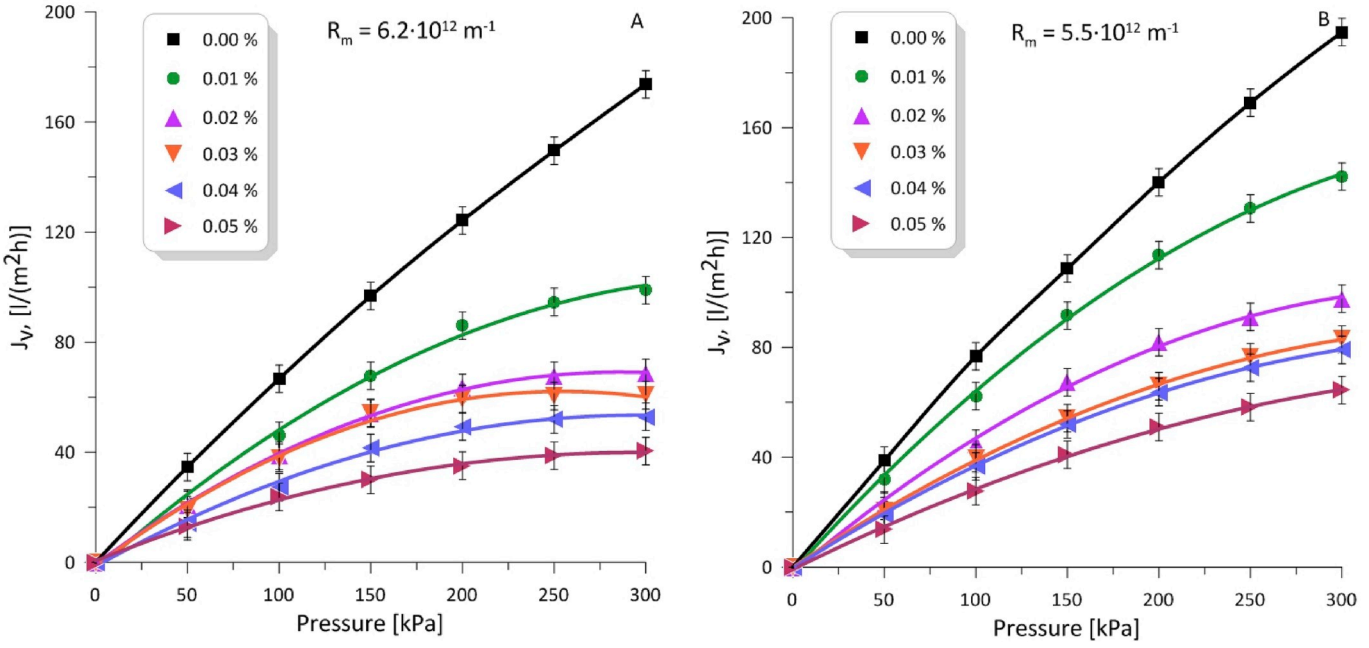

Fig. 10. The volumetric flux as a function of the applied pressure for membranes during the process of starch ultrafiltration at different feed concentrations: A – membrane modified with Chit-g-PA micelles without α-amylase; B – membrane modified with Chit-g-PA micelles and α-amylase.

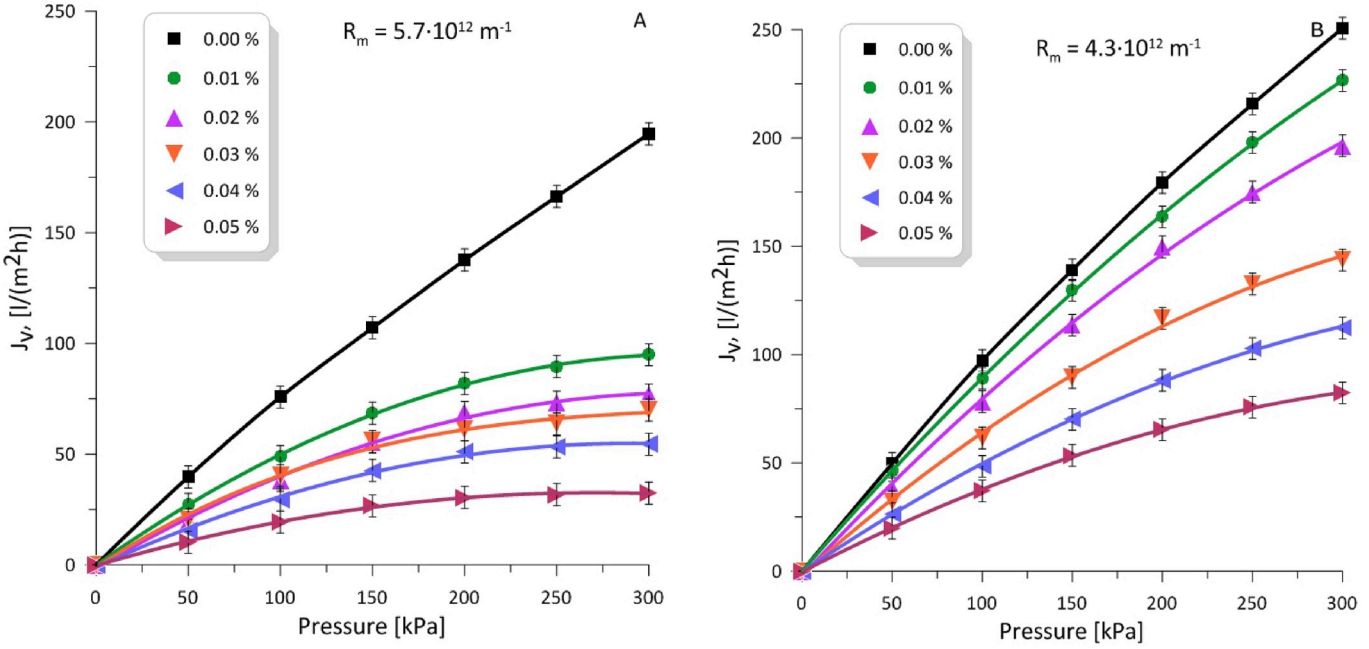

Fig. 11. The volumetric flux as a function of the applied pressure for the membranes during the process of starch ultrafiltration at different feed concentrations: A – membrane modified with Chit-g-SA micelles without α-amylase; B – membrane modified with Chit-g-SA micelles and α-amylase.

incorporated α-amylase are presented in Figs. 10 and 11. It should be noted that adsorption of polymeric micelles onto a membrane surface slightly affects membrane permeability and resistance. Consequently, it could be argued that the adsorption of the micelles onto the membrane surface did not block or overlap its pores. Thus, membrane permeability of Chit-g-PA, Chit-g-PA-enzyme, and Chit-g-SA membranes decreased by 10–20% compared to the unmodified PES membrane. Meanwhile, for the membrane modified with Chit-g-SA-enzyme 20% flux increase was observed. This property of membranes could be explained by the formation of denser micelles in the case of grafting stearic acid with a longer aliphatic chain to chitosan in comparison with palmitic acid.

The permeability of membranes during starch ultrafiltration was improved remarkably, especially for the membrane modified with Chit-g-SA micelles. This effect was caused by the creation of a hydration barrier of polysaccharide micelles on the surface that reduced the interaction forces between starch molecules and the membrane surface. In addition, incorporated enzymes also prevented membrane fouling due to starch splitting in the boundary layer.

To quantify the fouling of membranes during starch ultrafiltration, membrane permeability, L_p, cake resistances, R_c, and the flux decline ratio, DR were calculated. Dependences of L_p and R_c on the concentrations of starch solutions are shown in Fig. 12. The unmodified membrane

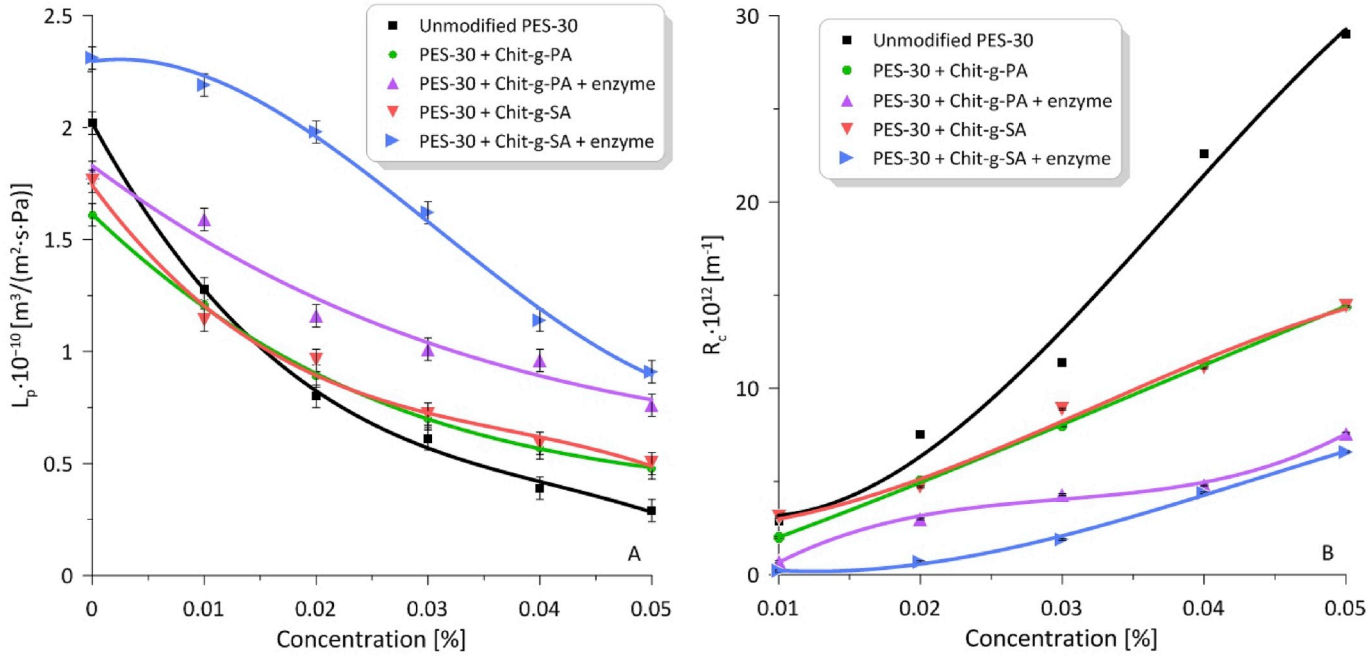

Fig. 12. Dependence of membrane permeability L_p (A) and the cake resistance R_c (B) on the concentration of feed solutions.

Table 3
Calculated flux decline ratios for membranes in the process of starch ultrafiltration under ΔP = 300 kPa.

Starch concentration, % Membrane	0.01	0.02	0.03	0.04	0.05
Unmodified PES-30	0.346	0.732	0.793	0.869	0.901
Chit-g-PA	0.432	0.604	0.696	0.651	0.768
Chit-g-PA-α-amylase	0.270	0.499	0.574	0.594	0.670
Chit-g-SA	0.512	0.606	0.641	0.720	0.833
Chit-g-SA-α-amylase	0.960	0.217	0.427	0.552	0.672

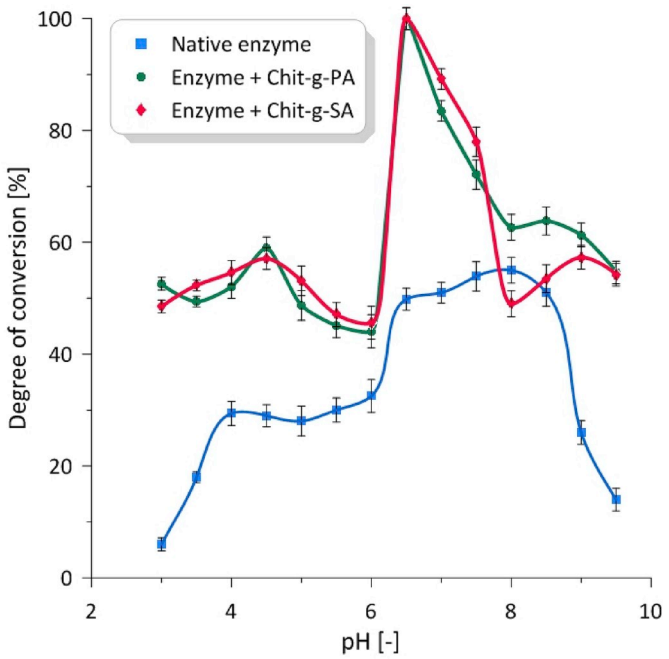

Fig. 13. The influence of the pH of the feed solution on the degree of starch conversion for PES membranes with immobilized α-amylase: ΔP = 200 kPa; starch concentration – 0.05%; recovery – 70%.

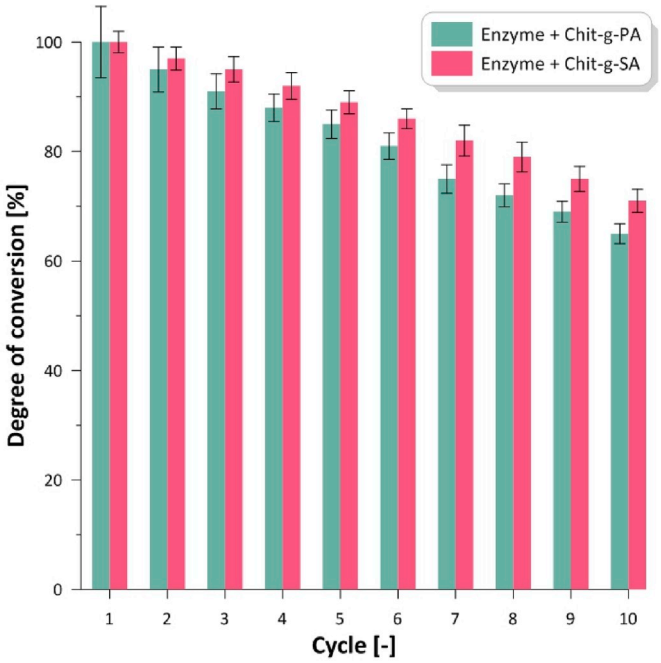

Fig. 14. Activity of α-amylase entrapped in polymeric micelles immobilized onto PES membranes over 10 cycles: pH 6.5; ΔP = 200 kPa; starch concentration – 0.05%; recovery – 70%.

revealed the lowest permeability with an increase in the concentration of starch solutions due to dramatic increase in the cake resistance.

The permeabilities of membranes modified with Chit-g-PA and Chit-g-SA micelles almost did not differ from the unmodified membrane, while the cake resistances decreased twice. Thus, even the modification of membranes with polymeric micelles improved the antifouling properties of membranes and reduced the impact of concentration polarization effect. Moreover, incorporation of the enzyme into the micelles significantly improved the permeability of the membranes. The cake resistance of such membranes decreased 6-fold compared to the

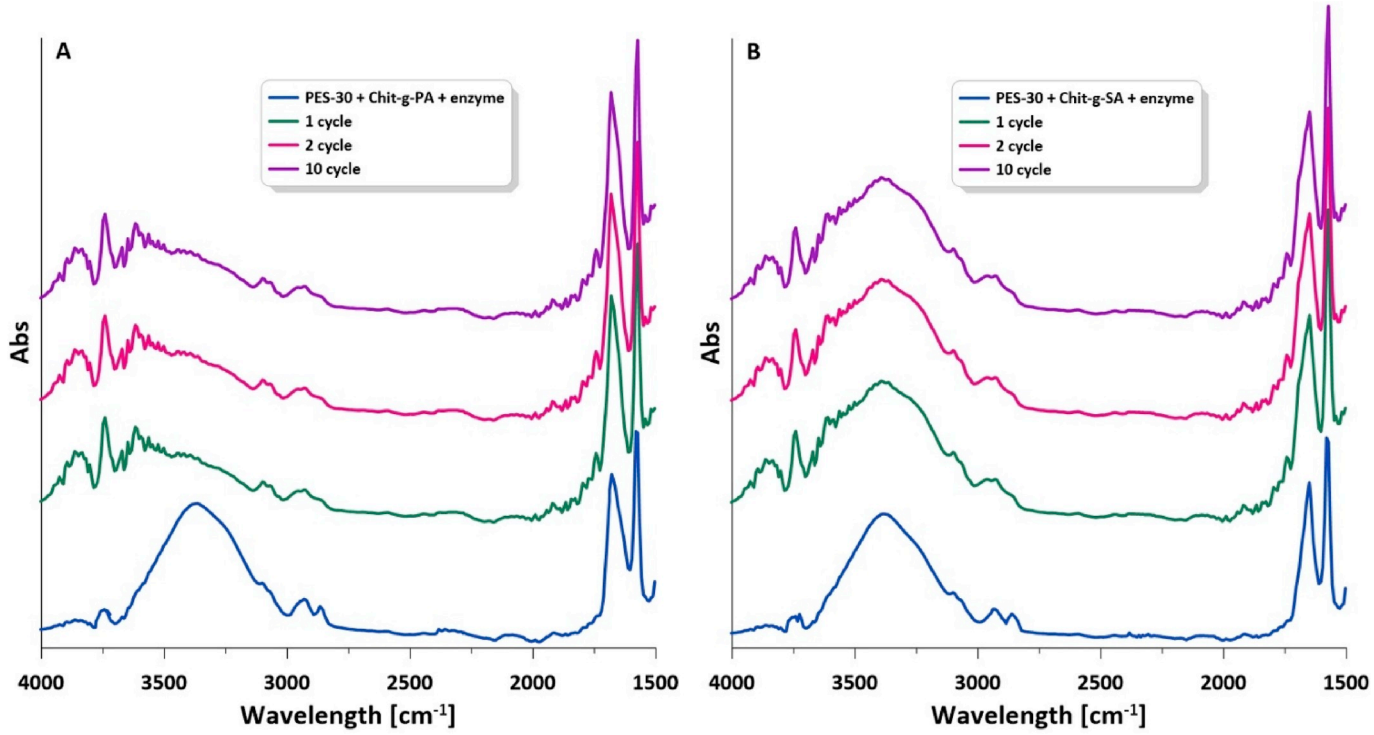

Fig. 15. IR spectra of modified membranes before and after cycles of starch ultrafiltration.

unmodified PES one.

The calculated flux decline ratios are listed in Table 3. The flux of the pristine membrane declined by about 70–90% for different starch concentrations compared to water flux. Membranes modified with micelles with entrapped enzyme showed a lower flux decline of 20–70% due to biocatalytic degradation of starch. It was concluded that Chit-g-SA-enzyme membrane showed better fouling resistance compared to all of the analyzed membranes.

The influence of pH on the enzyme activity was studied in the range of pH 3.0–10.0. A membrane with adsorbed α-amylase without polymeric micelles was used as a control. The obtained results are presented in Fig. 13.

The maximum activity of native α-amylase immobilized on PES membrane was observed in the pH range of 7.5–8.0. α-Amylase entrapped in Chit-g-PA and Chit-g-SA micelles was characterized with maximum activity at pH 6.5. It should be noted that α-amylase in polymeric micelles demonstrated higher resistance to acidic media.

In order to study the reusability and stability of immobilized α-amylase, 10 cycles of starch conversion using 50 mL of fresh feed solution for each were conducted. Results are presented in Fig. 14.

Membranes with enzyme entrapped in polymeric micelles were characterized by the high stability of α-amylase activity. The degree of starch conversion was reduced by 35% and 30% after 10 cycles for the membrane modified with Chit-g-PA and Chit-g-SA, respectively.

IR spectra of membranes after cycles of the starch ultrafiltration (Fig. 15) also confirmed the stability of the active layer on the membrane surface. The increasing in intensity of the band corresponding to N–H bonds vibrations and the decreasing in intensity of the broad band in the range of 3500–3200 cm^{-1} after the first cycle of ultrafiltration could be explained by partial washing of polymeric micelles from the surface. The possible solution to this problem can be a short-term UV irradiation of membranes after modification. This technique has been already tested on Pluronics® micelles, which were easily washed out during the ultrafiltration experiment [21]. As it was shown, UV irradiation within 3–5 min allowed for the micelles attachment to the surface of membranes and did not affect the enzyme activity.

4. Conclusions

The research demonstrated the possibility of modifying polyethersulfone membranes with polymeric micelles with entrapped α-amylase in order to improve membrane antifouling properties. Through grafting palmitic and stearic acids to low-molecular weight chitosan, amphiphilic chitosan derivatives have been successfully synthesized. Palmitoyl and stearoyl derivatives of chitosan could self-assemble in water with CMC values of $5.7 \cdot 10^{-2}$ mg/mL and $3.9 \cdot 10^{-2}$ mg/mL, respectively. It was found that α-amylase was entrapped into polymeric micelles, the size of which was 707 ± 64 nm and 609 ± 57 nm for Chit-g-PA and Chit-g-SA, respectively. Obtained micelles with enzyme were adsorbed onto the surface of polyethersulfonic membranes. Modified membranes were characterized by the high stability of the coating over time due to the surface-active properties of chitosan derivatives and electrostatic interaction.

The unmodified membrane showed the lowest permeability with an increase in the concentration of starch solutions due to increased cake resistance. The permeability of modified membranes during starch ultrafiltration was improved remarkably compared to the pristine one. The cake resistance of such membranes decreased 6-fold compared to the unmodified PES one. Furthermore, the effect of concentration polarization is attenuated owing to starch hydrolysis by α-amylase incorporated into polymeric micelles.

The maximum activity of native α-amylase immobilized on PES membrane was observed at the pH 6.5 and it was shown a higher resistance of enzyme to acidic media compared to native one. The starch conversion degree was reduced by 35% and 30% after 10 cycles for the membrane modified with Chit-g-PA and Chit-g-SA, respectively.

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