Powder Technology 315 (2017) 250–257

Contents lists available at ScienceDirect

Powder Technology

journal homepage: www.elsevier.com/locate/powtec

Synthesis and characterization of chitosan membranes functionalized with amino acids and copper for adsorption of endoglucanase

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ARTICLE INFO

Article history:
Received 25 August 2016
Received in revised form 28 March 2017
Accepted 3 April 2017
Available online 05 April 2017

Keywords:
Functionalized chitosan membrane
FTIR-ATR
SEM
TGA
DSC
Endoglucanase adsorption

ABSTRACT

Chitosan membranes were obtained and functionalized with amino acids and copper in order to improve adsorption selectivity of endoglucanase. The membranes were characterized by Fourier-transformed infrared spectroscopy with attenuated total reflectance device (FTIR-ATR) to monitor chemical changes. Scanning electron microscopy (SEM) was performed to compare the surface morphology of the membranes. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out in order to analyze thermal stability of functionalized membranes and to identify the differences between functionalized chitosan membranes before and after endoglucanase adsorption. SEM results proved that functionalization had occurred since the surface of the chitosan membrane was modified. FTIR-ATR results confirmed an effective chemical modification of chitosan membranes with amino acids and copper and corroborated endoglucanase adsorption. The characteristic parameters of DSC and TGA also evidenced endoglucanase adsorption. The use of functionalized membranes as adsorbents increased 40-fold the percentage of endoglucanase adsorption as compared to unmodified membranes. Thus, chitosan membranes functionalized with amino acids and copper may represent a novel, low-cost adsorbent to be used in endoglucanase purification from complex systems.

1. Introduction

Cellulase represents approximately 20% of the enzyme global market and it is the world's third largest industrial enzyme by dollar volume [1]. The increasing demand for cellulase is due to its use in the detergent, textile, pulp and paper, medical and pharmaceutical industries [2]. The cellulase system is composed of three enzymes: endoglucanase, exoglucanase and β-glucosidase which acting together, catalyze the hydrolysis of cellulose [3]. Endoglucanase is the most important component for cellulose degradation [4]. However, the high cost of this enzyme represents a challenge to industrial applications. Bioseparation for the recovery of the endoglucanase can account for 50–80% of overall production costs [5].

Conventional methods for endoglucanase separation are ion-exchange chromatography, affinity chromatography, size-exclusion chromatography and precipitation with ammonium sulphate [6–10]. However, chromatography is an expensive method of separation and ammonium sulphate is not environment-friendly; thus, it is necessary to design and develop a process for cellulase recovery which reduces cost and environmental impact.

The adsorption of proteins in batch systems represents an attractive method of protein purification. These processes are cost-effective and simple to operate [11,12]. As a result, the development of low-cost adsorbents with high adsorption capacity and selectivity has been a great challenge. Previous studies have carried out adsorption of cellulase from *Aspergillus niger* on commercial activated carbon [13]; however, activated carbon is considered to be an expensive adsorbent [14], and, therefore, the production of low-cost alternatives has been the aim of researchers in the area.

Chitosan is a linear polysaccharide composed of chains $\beta(1 \rightarrow 4)$ D-glucosamine and *N*-acetyl-D-glucosamine [15,16]. It is produced by deacetylation of chitin, which is a structural element in the exoskeleton of crustaceans and one of the most widely available natural polymers in nature [17,18]. Chitosan exhibits beneficial chemical and biological properties since it is bioactive, biocompatible, biodegradable and contains polycationic properties [19]. In addition, chitosan is economically attractive as it comes from a natural source [20]. Chitosan can be modified by physical or chemical methods to prepare chitosan derivatives. It can be used to synthetize membranes, gel beads or fibers, depending

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http://dx.doi.org/10.1016/j.powtec.2017.04.014
0032-5910/

on its final application [21,22]. Chemical modifications are performed in order to prevent chitosan dissolution in acidic solutions and to improve mechanical resistance and adsorptive selectivity [23,24].

Recently, there has been an increasing interest in polymeric membranes for protein separation [25,26]. Polymeric membranes with functional groups improve adsorption capacity and efficiency. The development of new chemically modified polymeric membranes has been of great scientific concern due to the key role of the adsorbent in the adsorption process [27]. Previous works have reported that chitin matrix modified by L-cysteine has significantly increased the adsorption capacity by introducing free amino groups [28]. In addition, amino acids have been reported to exhibit strong interactions with metals which is important in protein binding [29,30].

Moreover, it has been shown that transition metal ions present affinity for proteins. Hari et al. [31] reported a selective adsorption of human IgG on cellulose membranes immobilized with copper ions.

The aim of this work was to prepare and modify chitosan membranes by functionalizing them with amino acids and copper in order to characterize the functionalized membranes and evaluate the batch adsorption of endoglucanase from *Aspergillus niger*. Chitosan membranes were characterized in terms of physical properties and chemical changes. In order to accomplish this, Fourier-transformed infrared spectroscopy with attenuated total reflectance device (FTIR-ATR), differential scanning calorimetry (DSC) and thermal gravimetric analyses (TGA) were employed. Moreover, the surface morphology of chitosan membranes was monitored by using scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

Chitosan (CHS) in powder form was purchased from Sigma (USA). The CHS source is shrimp shells and has a minimum deacetylation degree of approximately 75% and a molecular weight of 340 g/mol, determined by gel permeation chromatography. Endoglucanase (EG) was purchased from Megazyme (Ireland). All other reagents were of analytical grade. All solutions were prepared with deionized ultrapure Milli-Q water.

2.2. Preparation of chitosan membranes

A solution of 2.5% w/v was prepared by dissolving 15 g of chitosan in 600 mL of acetic acid solution 3% v/v. The solution was kept under mechanical stirring at 3000 rpm and then stored at 4 °C.

The solution was spread on Petri dishes. Drying was carried out at 60 °C until the mass was 50% of initial mass. Chitosan membranes were immersed in 1 M NaOH for 24 h at 25 °C. After addition of 1 M NaOH, chitosan membranes have sufficient mechanical strength to be easily removed without breaking. Finally, they were washed exhaustively with Milli-Q water until all alkali was removed and stored in Milli-Q water at 4 °C.

2.2.1. Functionalization of chitosan membranes

Functionalization of chitosan membranes was performed following the method proposed by Sano and Murase [32].

Firstly, epichlorohydrin and the amino acid were mixed in a 1:1 proportion in 2 M NaOH for 4 h at 60 °C: 0.95 mL of epichlorohydrin was added to each flask containing 2.66 g of L-aspartic acid (Asp), 2.94 g of L-glutamic acid (Glu), 3.10 g of L-histidine (His), and 2.50 g of L-taurine (Tau), respectively. The solution was then cooled to 0 °C and NaOH was added under constant stirring. Chitosan membranes were added to the solution containing the amino acid and epichlorohydrin. The reaction was conducted at 65 °C for 16 h. Finally, the membranes were washed repeatedly with Milli-Q water in order to remove residues. The following membranes were obtained: CHS-Asp, CHS-Glu, CHS-His, CHS-Tau, respectively.

Functionalization with copper was carried out by placing the functionalized membranes with amino acids in 2.6 mM $Cu(NO_3)_2$ solution under constant stirring for 60 h. The membranes were preserved intact after shaking. Finally, the membranes were washed with Milli-Q water.

2.3. Endoglucanase assays

Assay for the activity of endoglucanase was performed as follows:

Carboxymethylcellulose (CMC, 1%) solution was prepared in 50 mM sodium citrate buffer (pH 5.3) and incubated with supernatant samples of batch adsorption at 50 °C for 10 min. After adding 1 mL of 3,5-Dinitrosalicylic acid (DNS) reagent, it was further incubated at 100 °C for 10 min and absorbance was read at 560 nm. The reducing sugar concentration generated from the enzymatic reaction was then measured and used to calculate endoglucanase activity.

2.4. Endoglucanase adsorption

Chitosan membranes were immersed in a 5% v/v endoglucanase solution. Adsorption was carried out for 3 h at 25 °C and 200 rpm. The membranes were separated from the solution.

The percentage of endoglucanase adsorption was calculated by comparing the final activity of the adsorption with the initial activity of endoglucanase as follows:

$$EG\ adsorption(\%) = ((Ai - Af)/Ai) \times 100$$

where *Ai* (U/L) and *Af* (U/L) are endoglucanase activities before and after adsorption experiments, respectively.

The experiments were carried out in triplicate.

2.5. Membrane characterization

All membranes, before and after adsorption, were lyophilized to remove the water present in the pores without destroying them. A Labcomco equipment (Freeze Dry System/Freezone 4.5, Brazil) was used under vacuum of 35×10^{-3} bar and at a temperature of −65 °C.

2.5.1. Scanning electron microscopy

The lyophilized membranes were analyzed by scanning electron microscopy. Samples were coated with a 200 Å - thick gold layer using an EMITECH K450 sputter coater (Kent, United Kingdom). Micrographs were obtained in a scanning electron microscope with Energy Dispersive X-ray detector, (Leo 440i, EDS 6070, SEM/EDS: LEO Electron Microscopy/Oxford Cambridge, England).

2.5.2. Fourier transformed infrared spectroscopy

FTIR spectra were obtained in ATR mode on a Nicolet 6700 Fourier transform infrared spectrometer (Thermo Scientific, Madison, USA) equipped with Smart Omni-Sampler. Spectral scanning was acquired in a wavenumber range from 4000 to 675 cm^{-1} at 4 cm^{-1} resolution.

2.5.3. Thermogravimetric analysis

TGA analyses were performed using a SHIMADZU TGA-50 M thermogravimetric analyzer (Kyoto, Japan) under a nitrogen atmosphere. Samples were placed on an alumina cell, and heated from 22 °C to 900 °C at a heating rate of 10 °C/min.

2.5.4. Differential scanning calorimetry

Lyophilized membranes (4–5 mg) were used in order to analyze thermal properties. Differential scanning calorimetry measurements were performed with a Mettler Toledo DSC1 (Zürich, Switzerland). Samples were heated from −80 °C to 350 °C at a heating rate of 10.0 K/min, under nitrogen atmosphere at a flow rate of 50 mL/min.

3. Results and discussion

3.1. Membrane characterization

3.1.1. Membrane surface morphology

SEM technique was performed in order to compare the surface morphology of the chitosan membranes.

Fig. 1 shows scanning electron micrographs of unmodified chitosan membrane and of chitosan membranes functionalized with amino acids and copper. According to SEM images, after functionalization with the ligands, all membranes exhibited their surface modified especially regarding their pores. The chitosan membrane presented large-size pores, some of them being larger than 30 μm (Fig. 1a). However, pore diameter was reduced after functionalization. The CHS-Asp-Cu membrane (chitosan membrane functionalized with aspartate and copper) presented a smoother surface morphology than the others (Fig. 1b to e). The CHS-Glu-Cu membrane (chitosan membrane functionalized with glutamate and copper) exhibited pores of about 720 nm diameter. The pore width of the CHS-His-Cu membrane (chitosan membrane functionalized with histidine and copper) varied within a range from 500 nm to 1 μm. The CHS-Tau-Cu membrane (chitosan membrane functionalized with taurine and copper) presented pore sizes up to about 857 nm in diameter. These results may prove that functionalization has occurred, since the surface of the chitosan membrane has been modified.

3.1.2. FTIR results

FTIR-ATR spectra of chitosan membranes functionalized with amino acids and copper before and after endoglucanase adsorption were obtained in order to identify changes in chemical interactions that may have taken place after functionalization of chitosan membranes and endoglucanase adsorption (Fig. 2).

In general, it was observed that the major changes in FTIR-ATR spectra after endoglucanase adsorption were similar in all the analyzed membranes, suggesting that the mechanism of endoglucanase adsorption onto the four types of membranes was essentially the same.

The peak found at around 3440 cm^{-1}, which was attributed to the presence of hydroxyl groups (−OH), overlapped with the NH group-stretching vibration at 3360 cm^{-1} [33]. The bands at 2910–2870, 1730, 1655, 1590, 1560, 1415, 1377, 1255, 898 cm^{-1} corresponded to asymmetrical and symmetrical stretching vibrations of CH_2 groups in the pyranose ring, carbonyl group vibration, axial C═O stretching in amide group, NH_2 in the amino group, NH-bending vibration in the

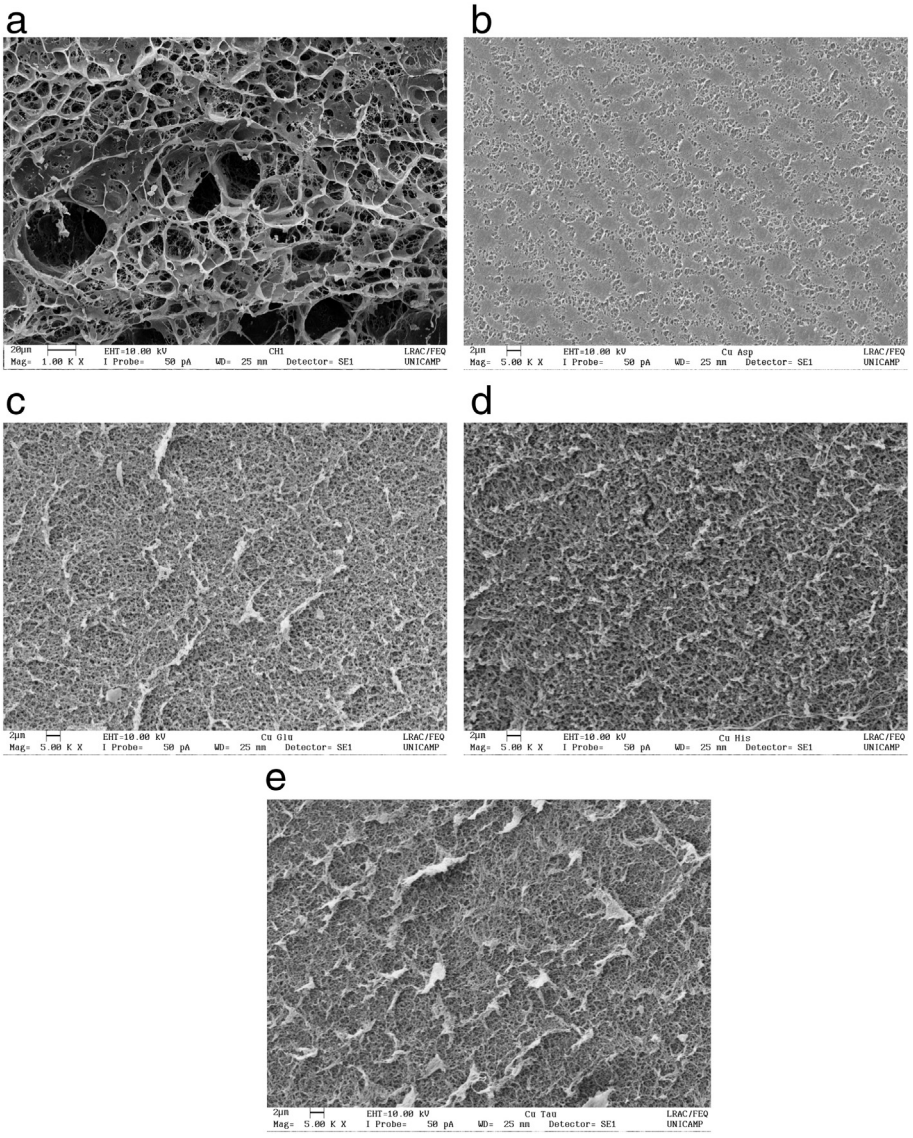

Fig. 1. SEM micrographs of chitosan membranes: (a) surface morphology of the surface of chitosan membrane (b) surface morphology of the surface of chitosan membrane functionalized with aspartate and copper (c) surface morphology of the surface of chitosan membrane functionalized with glutamate and copper (d) surface morphology of the surface of chitosan membrane functionalized with histidine and copper (e) surface morphology of the surface of chitosan membrane functionalized with taurine and copper.

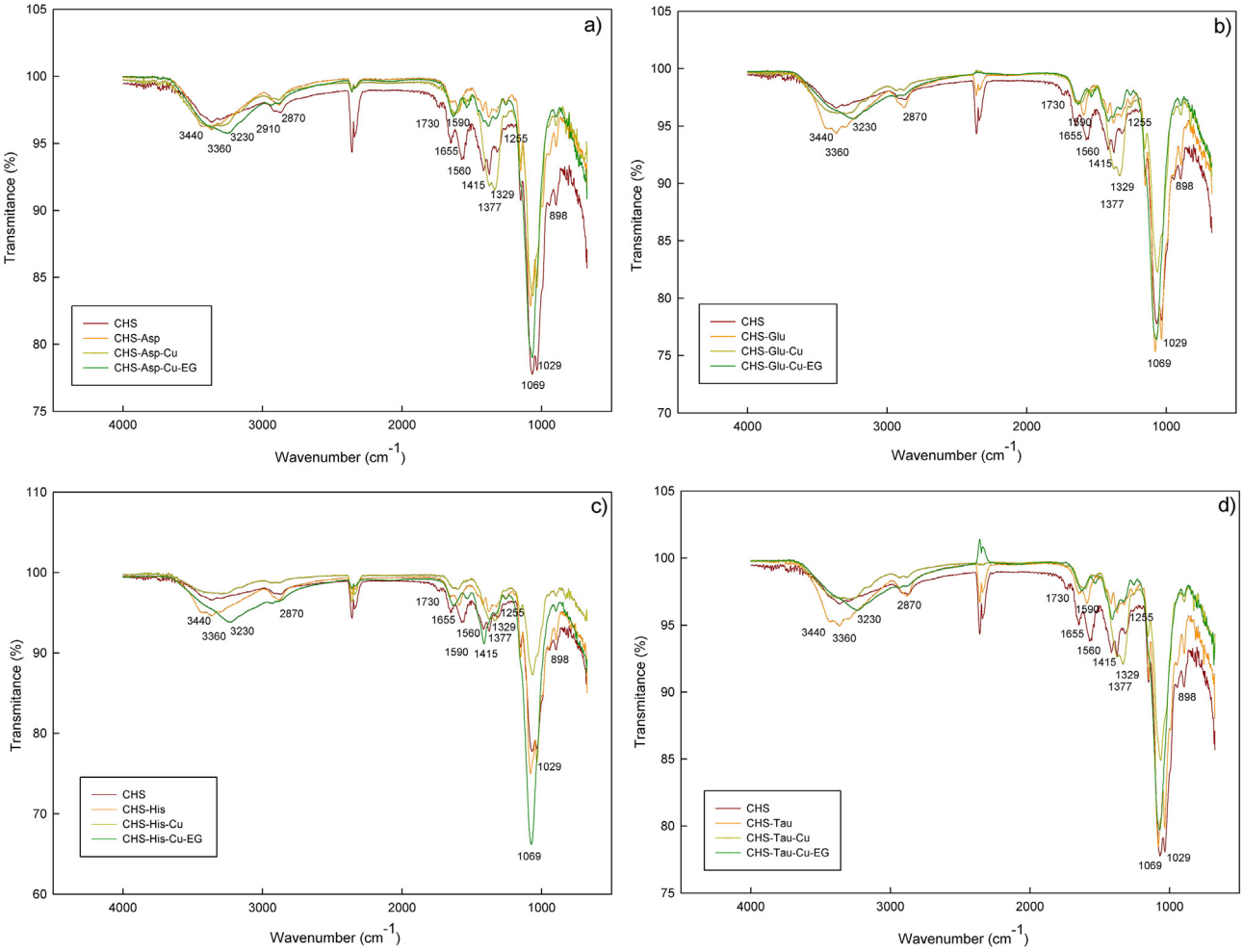

Fig. 2. FTIR-ATR spectra of chitosan membranes functionalized with amino acids and copper before and after endoglucanase adsorption. FTIR-ATR spectra of chitosan membranes functionalized with aspartate and copper (a). FTIR-ATR spectra of chitosan membranes functionalized with glutamate and copper (b). FTIR-ATR spectra of chitosan membranes functionalized with histidine and copper (c). FTIR-ATR spectra of chitosan membranes functionalized with taurine and copper (d).

amide group, vibration intensity of OH in the pyranose ring, CH_3 symmetrical deformation, C—O group, stretching vibration of CH_3COH group, respectively [33–35]. Variations in the intensity of these peaks were observed after functionalization with amino acids and copper and after endoglucanase adsorption.

Finally, the region found between 1150 and 1024 cm^{-1}, corresponded to deforming vibrations of —C—O—C— groups in glycosidic linkage. In this region, intense peaks were observed at 1069 cm^{-1} and 1029 cm^{-1} for the chitosan membrane but they significantly decreased after functionalization with copper.

These results suggested an effective chemical modification of chitosan membranes with amino acids and copper. In addition, a new band, attributed to N—Cu—O, arose at 1329 cm^{-1} after functionalization with copper [36]. Besides, the peaks found at 1560 cm^{-1} associated with —NH groups became weaker for all spectra after the functionalization of the chitosan membrane. This decrease in the band at 1560 cm^{-1} in all the modified chitosan membranes and the simultaneous appearance of the peak at 1329 cm^{-1} attributed to N—Cu—O confirmed that functionalization with copper occurred, probably due to a chelation ion exchange process between copper and amino groups [37].

After endoglucanase adsorption was performed, the emergence of the band at around 3230 cm^{-1}, related to N—H stretching vibration in the endoglucanase molecules [38], evidenced the effective adsorption of the enzyme on the modified chitosan membranes.

3.1.3. TGA results

Thermal degradation of chitosan membranes functionalized with amino acids and copper was studied by means of thermogravimetric analysis (TGA). Fig. 3 displays curves, in the form of weight loss versus temperature for chitosan membranes functionalized with taurine and copper. Similar patterns were obtained for the other membranes.

TGA characteristic parameters are: the temperature at which thermal degradation begins (T onset), the temperature at which thermal degradation is maximum (T peak), the temperature at which the process is complete (T end set) and the percentage of weight loss during each stage (W%). Comparative data regarding thermal degradation are shown in Table 1. The decomposition curves of chitosan membranes functionalized with amino acids and copper were divided into two stages. The first degradation process that starts below 100 °C is attributed to the loss of bound water. The second stage observed at a temperature range of 223–362 °C may be related to chitosan decomposition [39].

The chitosan membranes functionalized with amino acids and copper without attached endoglucanase showed slightly reduced thermal stability over the same membranes with adsorbed endoglucanase. Table 1 shows that T onset in stage I for the initial weight loss increased when functionalized chitosan membranes were linked to endoglucanase. This greater thermal stability in membranes with adsorbed endoglucanase is probably due to a decrease in membrane hydrophilicity, resulting in lower water retention. However, in stage II thermal degradation started at a lower temperature for functionalized chitosan membranes linked to

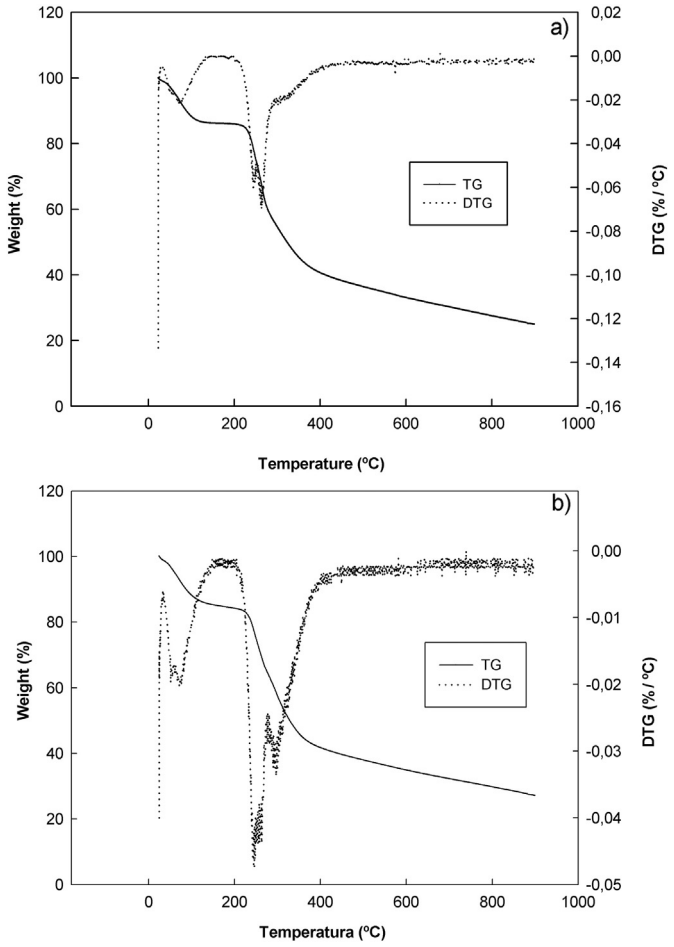

Fig. 3. TGA curves for chitosan membranes functionalized with Tau and copper: (a) without adsorbed endoglucanase; and (b) with adsorbed endoglucanase.

endoglucanase. In stage II, a lower T onset was observed for functionalized membranes linked to endoglucanase compared to functionalized membranes without adsorbed endoglucanase. This indicates that adsorption of endoglucanase makes the functionalized chitosan membrane less stable at this temperature range. A previous study made by Tirkistani (1998) reported that free amino groups have a stabilizing effect on the polymer. Therefore, the adsorption of endoglucanase may occur through amino groups, leaving the matrix less stable as it is observed in stage II temperature range.

Table 1
Thermal parameters obtained from thermogravimetric analysis of chitosan membranes functionalized with amino acids and copper.

Membranes	Stage	T onset (°C)	T end set (°C)	T peak (°C)	W(%)
CHS—Asp—Cu	I	37.24	80.63	50.08	14.72
	II	230.96	362.29	263.70	72.69
CHS—Asp—Cu—EG	I	58.33	113.42	72.88	18.41
	II	224.20	333.12	293.40	29.39
CHS—Glu—Cu	I	59.30	106.60	74.32	15.26
	II	230.95	298.96	259.70	35.52
CHS—Glu—Cu—EG	I	67.27	112.42	79.20	18.11
	II	223.02	345.79	240.50	38.23
CHS—His—Cu	I	61.36	96.69	71.53	12.77
	II	235.59	306.88	262.10	36.85
CHS—His—Cu—EG	I	82.86	106.52	77.05	16.06
	II	225.82	347.09	242.40	41.04
CHS—Tau—Cu	I	77.46	106.99	73.26	16.40
	II	231.48	313.46	263.70	40.62
CHS—Tau—Cu—EG	I	98.83	105.98	74.53	15.24
	II	223.91	343.78	245.70	37.03

3.1.4. DSC results

DSC curves are consistent with the results of TGA analysis.

The DSC thermograms of chitosan membranes functionalized with amino acids and copper are shown in Fig. 4. In these figures, an endothermic peak close to 100 °C can be attributed to loss of water [40]. According to Sakurai et al. (2000), thermal degradation of chitosan begins at about 250 °C. The second thermal event, i.e., exothermic peaks, around 221–269 °C, may be associated to the decomposition of amine residues of chitosan (Fig. 4).

Table 2 displays the endothermic and exothermic peaks and the enthalpies related to each peak for chitosan membranes functionalized with amino acids and copper with adsorbed and unadsorbed endoglucanase. All membranes presented an endothermic peak due to loss of water associated to hydrophilic groups, whereas the exothermic peak corresponds to chitosan decomposition [41].

Besides, when T onset in the second thermal event of each functionalized chitosan membrane adsorbed with endoglucanase was compared to the T onset obtained from the unadsorbed membranes, it was observed that the former started at a lower temperature probably due to the presence of endoglucanase (Table 2). This degradation of the endoglucanase present in functionalized chitosan membranes was also observed in the TGA analysis.

The results of DSC and TGA analyses obtained in this study support the existence of a structural difference between the membranes before and after endoglucanase adsorption, confirming the presence of endoglucanase molecules in the membranes.

3.2. Endoglucanase adsorption

Chemically modified chitosan membranes were obtained in order to increase the selectivity of enzyme adsorption.

The adsorption of enzymes depends on hydrophobic interactions and hydrogen bonding. In addition, electrostatic interactions between protein molecules and the modified groups on the chitosan surface could also play a role in this process [42].

No buffer solution was used since counter-ions can interfere in the adsorption process by interacting with the adsorbent support [29,37].

The pH values of solutions were controlled and maintained within the 5.40 to 6.66 range. These pH values fall within the range of optimal and stability endoglucanase pH [43].

Fig. 5 shows the percentage of endoglucanase adsorption for different functionalized chitosan membranes. It is to be noted that functionalization of chitosan membranes improved endoglucanase adsorption. When unmodified chitosan membranes were used, a percentage of adsorption of just 1.1% was achieved. The CHS-Tau-Cu membrane presents the maximum percentage of adsorption reaching 40% while the other functionalized membranes exhibit values close to 30%.

It has been reported that copper forms complexes with amino acids [44].

Asp and Glu are α-amino acids which contain an α-amino group which is in the protonated form ($-NH_3^+$) under working conditions, an α-carboxylic acid group which is in the deprotonated form ($—COO^-$) under working conditions, and a side chain with a negatively charged carboxylic acid group.

Besides, Asp and Glu chelate metal ions weakly via the amino nitrogen and carbonyl oxygen. It has been reported that strong chelation occurs upon amide nitrogen bound hydrogen by some metal ions such as Cu^{2+} [45].

L-histidine is an interesting ligand because it presents nitrogen groups as α-amino group and imidazole nitrogens where copper binding may occur. Furthermore, if the carboxylic group is found deprotonated, it can also interact with cations [37].

Taurine possesses a sulfonic group in its chemical structure and it could be ionized under the pH conditions used due to its low pKa value. Besides, it has been reported that Taurine binds to copper [46]. The porosity of the CHS-Tau-Cu membrane observed in the previous

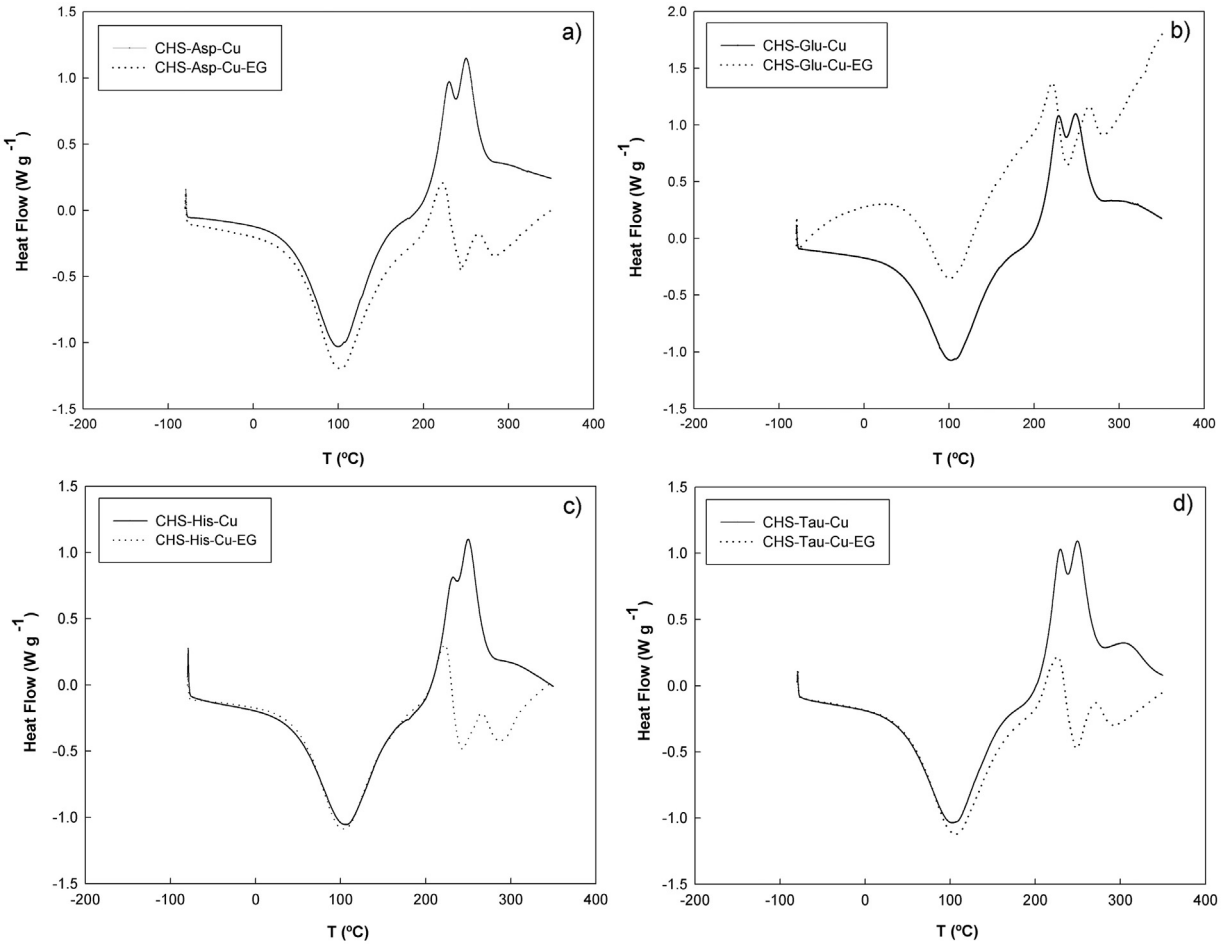

Fig. 4. DSC thermograms of chitosan membranes functionalized with copper and with the following amino acids: a) Asp b) Glu c) His d) Tau.

Table 2
Peak temperatures and enthalpy changes in the DSC thermograms for chitosan membranes functionalized with amino acids and copper.

Membranes	Temperature(°C)			ΔH (J/g)
	T onset	T end set	T peak	
CHS—Asp—Cu	45.28	154.66	100.17	−400.76
	221.71	271.02	250.11	244.06
			230.23	
CHS—Asp—Cu—EG	49.15	157.78	101.12	−417.72
	195.50	243.63	223.22	100.13
	246.98	280.57	263.78	28.46
CHS—Glu—Cu	66.01	106.39	102.15	−333.25
	209.35	256.30	228.93	240.18
			248.95	
CHS—Glu—Cu—EG	53.65	150.37	102.60	−278.76
	198.32	234.08	221.69	95.85
	242.44	277.96	263.66	45.90
CHS—His—Cu	49.59	158.24	106.79	−319.73
	217.65	271.48	249.81	270.57
			232.22	
CHS—His—Cu—EG	54.05	151.00	101.64	−276.58
	191.52	237.32	222.74	152.60
	252.95	282.18	265.44	30.32
CHS—Tau—Cu	47.68	157.43	102.98	−349.02
	220.18	270.04	229.43	256.20
			249.62	
CHS—Tau—Cu—EG	49.53	170.04	106.11	−422.88
	201.15	243.00	227.23	88.32
	251.72	288.21	269.63	36.27

SEM results and the ionization of taurine sulfonic group may favor endoglucanase adsorption on this membrane. The synergy of all these factors could promote an increase in endoglucanase adsorption on the CHS-Tau-Cu membrane. Besides, previous works have reported that surface functional groups of adsorbents not only affect sorption behavior, but also dominate the sorption mechanism. Adsorbents with sulfonic groups on the surface could interact with adsorbates through ion exchange [47].

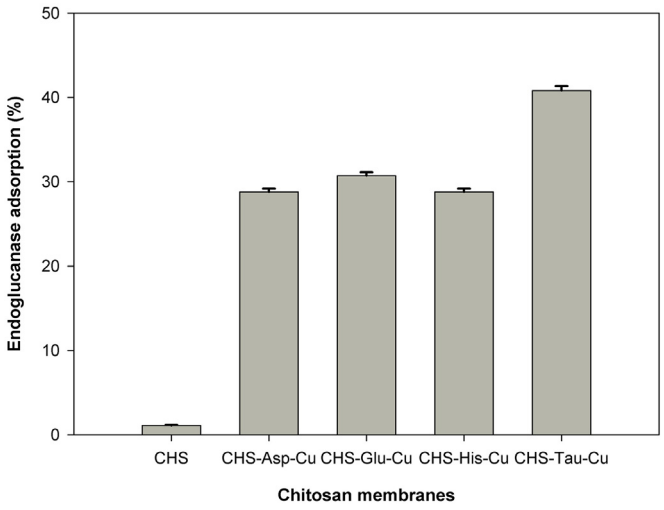

Fig. 5. Percentage of endoglucanase adsorption on different chitosan membranes.

In this work, a new chitosan biosorbent modified with amino acids and copper was prepared and employed for the adsorption of endoglucanase. Immobilized Metal-Affinity (IMA) has been reported to be an adequate separation technique for the purification of proteins. It uses covalently bound chelating compounds on solid supports to entrap metal ions, which are used as affinity ligands for different proteins. In IMA the adsorption of proteins is based on the coordination between an immobilized metal ion and electron donor groups from the protein surface [48]. Therefore, in this work, copper ions which can be considered to be Lewis acids would be interacting with an electron-donor group present on the endoglucanase surface. Besides, it has been reported that glycoside hydrolases with endoglucanase activity present copper binding sites required for enzyme activity [49]. In addition, it has been reported that the endoglucanase enzyme of *Aspergillus niger* contains 19 negatively charged residues (aspartate and glutamate) [4] which could also interact with copper through electrostatic interactions.

Other authors have used chromatographic techniques for the purification of endoglucanase from different fungal strains [50,51]. However, these methods are more expensive and require more steps. Besides, the use of a large number of operations might generate low yield of the product of interest due to losses at each separation step. Previous works [51,52], have reported purification of endoglucanases by sequentially using several techniques, such as ammonium sulphate precipitation, ultrafiltration, ion-exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography and columns packed with poly-buffer exchanger. The endoglucanase activity recovered after applying these techniques was <10%, which is at least four times lower than that retained by the functionalized chitosan membranes used in this work. In addition, there is a risk of loss of conformation of the enzyme due to such extensive processes.

Finally, the adsorption process proposed in this work could reduce costs, as it can replace several extractive steps and the use of high cost techniques such as chromatography.

Thus, these novel supports could be a powerful industrial tool for use in larger scale for the purification of endoglucanase from complex biological systems such as fungal culture extracts, due to their low cost and high specificity for endoglucanase.

4. Conclusions

Chitosan membranes functionalized with amino acids and copper were obtained and used to improve adsorption selectivity of endoglucanase. SEM results indicated that functionalization occurred since the surface of the chitosan membrane was modified. FTIR results showed an effective chemical modification of chitosan membranes with amino acids and copper, as revealed by the appearance of new peaks and the intensity variation of others after functionalization. DSC and TGA were successfully used to characterize chitosan membranes functionalized with amino acids and copper. The characteristic parameters of these thermal methods confirmed endoglucanase adsorption on functionalized membranes. The functionalization of chitosan membranes significantly increased the percentage of endoglucanase adsorption. The highest percentage of endoglucanase adsorption achieved was 40% when CHS-Tau-Cu membrane was used.

Due to the importance of cellulase in industry, it is convenient to develop an inexpensive, clean and simple process in order to separate endoglucanase from complex systems. The batch adsorption process using chitosan membranes functionalized with taurine and copper may represent a low-cost, non-polluting and selective method for the endoglucanase purification from complex systems, with potential use in the fermentation process for cellulase production.

Acknowledgements

This work was supported by CONICET (PIP 112 2011 01 00578) and by Program for Scientific and Technological Cooperation between the Ministry of Science, Technology and Productive Innovation of Argentina (MINCYT) and the Coordination of Improvement of Higher Education Personnel (CAPES) of Brazil (BR/13/11). Authors would like to thank the Staff from the English Department (Faculty of Biochemical and Pharmaceutical Sciences, National University of Rosario), for correcting the language of this manuscript.

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