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Solubilization of Chitosan by Bipolar Membrane Electroacidification

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Chitosan, a partially deacetylated derivative of chitin, was solubilized by bipolar membrane electroacidification (BMEA). Bipolar/monopolar (anionic or cationic) configuration and chitosan addition mode (single step or stepwise) were examined. Chitosan solubility and electroacidification parameters were monitored during the process to determine the optimal conditions. Bipolar/anionic configuration and stepwise feeding mode led to chitosan solubilization yield of 91% in 60 min at 20 mA/cm^2. In this configuration, chitosan solution had a pH of 2.5, a conductivity of 8.5 mS/cm, and an ash content of 0.2%. Relative energy consumption was 0.05 kWh/L of 1% chitosan solution prepared. Although some chitosan particles were aggregated in the electrodialysis stack, limiting chitosan solubilization, BMEA allowed complete solubilization of chitosan circulating in the system.

KEYWORDS: Chitosan solubilization; electroacidification; bipolar membranes; ionic membranes

INTRODUCTION

Chitosan is a partially deacetylated derivative of chitin, a natural polysaccharide extracted from crustaceans, insects, and certain fungi (*1*). Because chitosan is biodegradable, nontoxic, and biocompatible in animal tissues, it is widely used in the medical industry in products ranging from burn dressings to drug delivery capsules. It is also used as a flocculant, food thickener, paper and textile adhesive, packaging membrane, and chelating agent for metals (*2*). When chitosan, isolated in neutral conditions (i.e., when glucosamine is in the $-NH_2$ form), is dissolved in acidic conditions, it becomes a polyelectrolyte, the intrinsic dissociation constant of the uncharged polymer (pK_0) being 6.5 (*3*). The following equilibrium reaction describes chitosan protonation by an acid HX, the counterion X^- depending on the electrolyte present in the medium and on the acid used:

$$\text{Chit}-NH_2 + HX \leftrightarrow \text{Chit}-NH_3^+, X^- \qquad (1)$$

Chitosan is usually solubilized by the addition of acids such as acetic acid or hydrochloric acid at a concentration of 0.1 M or 1% (*4*). However, chitosan producers are looking for environmentally friendly production processes, because chemical processes generate effluent and require storing and handling capacities. Bipolar membrane electroacidification (BMEA) is an electrochemical process allowing continuous acidification without the addition of acids. Protons are electrochemically generated by water dissociation at the interface of bipolar membranes under an electrical field (*5*). One major advantage of BMEA reported by Bazinet et al. (*6*) is the possibility to acidify and demineralize a stream simultaneously, so that the electroacidified product has a higher purity than a chemically acidified product. In addition, the base generated can be reused at different stages in the process. In the past 10 years, BMEA has been used for the acidification of various food products such as soy protein extracts (*6*, *7*), skim milk (*8*), fruit juices (*9*, *10*), and sweet Cheddar cheese whey (*11*). The present work is part of a study aimed at the optimization of chitosan oligomer production by using acid and base generated by BMEA in a one-step process. Hence, this study will focus on the use of BMEA to acidify electrochemically a chitosan suspension at the laboratory scale. Two feeding modes of chitosan (one step or stepwise) and two BMEA configurations (bipolar/cationic or bipolar/anionic membranes) were examined; indeed, in the latter case, it was demonstrated by Lam Quoc et al. (*9*) that BMEA was more energetically efficient with bipolar/anionic configuration for apple juice inhibition. Finally, soluble chitosan concentration and electroacidification parameters were measured during the process to determine the optimal conditions.

MATERIALS AND METHODS

Chitosan. Low molecular weight chitosan was kindly provided by ISM Biopolymer (Granby, QC, Canada). According to the company, the chitosan was deacetylated at 96%, with a viscosity of 40 cP

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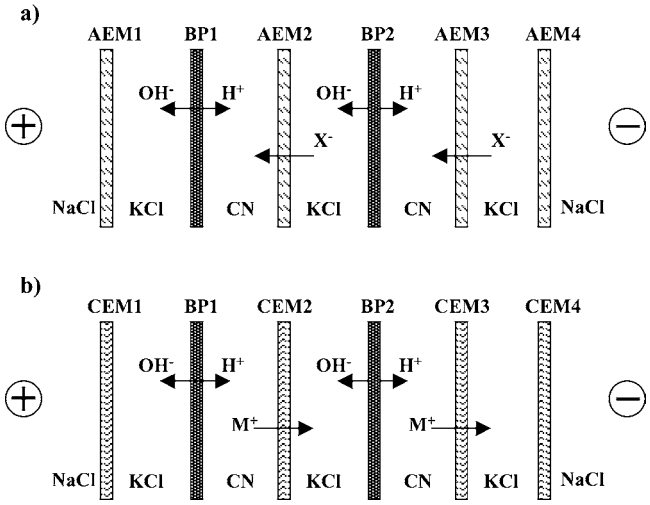

Figure 1. Bipolar membrane electroacidification cell: (**a**) configuration with anionic membranes; (**b**) configuration with cationic membranes. AEM, anion exchange membrane; CEM, cation exchange membrane; BP, bipolar membrane; CN, chitosan suspension; X^-, anions; M^+, cations.

(Brookfield method, 1% solution in 1% acetic acid), a moisture content of 7.1 ± 0.1% (w/w on wet basis), and an ash content of 0.40 ± 0.01% (w/w on dry basis).

Electroacidification Cell. The module used was an MP type cell (100 cm^2 of effective surface) equipped with a dimensionally stable anode (DSA) and a 316 SS cathode from ElectroCell AB (Täby, Sweden). The cell consisted of a structure of seven compartments separated by four AMX-SB anionic membranes **(Figure 1a)** or four CMX-SB cationic membranes **(Figure 1b)** and two Neosepta BP-1 bipolar membranes from Tokuyama Soda Ltd. and purchased from Ameridia (Somerset, NJ). This arrangement defines three circuits containing the chitosan suspension or NaCl electrolyte (3 L), a 2 g/L aqueous KCl solution (6 L), and a 20 g/L NaCl solution (6 L). Each circuit was connected to a separate external reservoir allowing continuous recirculation. The chitosan reservoir was equipped with an overhead stirrer (type RZRI, Caframo Ltd., Wiarton, ON, Canada) to facilitate chitosan homogenization. The anode/cathode voltage difference was supplied by a variable 0−100 V power source. The three electrolytes were circulated using centrifugal pumps (model ECDI-ASAAAYSS, Idex Corp., Rochester, NY). KCl and NaCl flow rates were 3 L/min using flow meters (model F550, Blue White Industry, Huntington Beach, CA); chitosan flow rate was controlled at 2.5 L/min.

Protocol. Two factors were tested in this experiment: (1) the type of monopolar membrane used in combination with BP membranes (i.e., BP/anionic or BP/cationic membranes); and (2) the chitosan feeding mode (i.e., one-step or stepwise addition). In one-step addition mode, chitosan was added at the beginning of the process by dispersing 15 g of chitosan powder in 3 L of aqueous 0.05 M NaCl solution, making a 5 g/L chitosan suspension. In stepwise addition mode, chitosan (15 g) was divided in six aliquots of 2.5 g and added every 10 min, the first aliquot being added to an aqueous 0.05 M NaCl solution (3 L) preacidified at pH 3 by BMEA. The amount of chitosan added for each aliquot (2.5 g) was calculated in order not to increase the pH over 4 after each addition during the process: each aliquot of chitosan corresponds to $\approx 5 \times 10^{-3}$ mol/L (as monomers) to be protonated, whereas the calculated concentration in electrogenerated protons is 7.5×10^{-3} mol/L for each 10 min period of BMEA, so that the chitosan solution would be pH ≈3 before each aliquot addition. Experiments were carried out in triplicates. Electroacidification was performed using a constant current density of 20 mA/cm^2 for a duration of 60 min. BMEA parameters were followed continuously by pH, conductivity, intensity, and voltage measurements. Samples were drawn every 10 min throughout the process, and soluble chitosan and ash contents were determined.

Analysis Methods. *pH.* The pH of the chitosan solution was measured with a pH-meter (model SP 20, VWR International, Montreal, Canada) equipped with an automatic temperature-compensated electrode (no. 14002-778).

Conductivity. The electrical conductivity of the chitosan solution was measured with a YSI conductivity meter (model 3100, Yellow Springs, OH) equipped with a YSI automatic temperature-compensated probe (model 3252) with a cell constant K of 1.0 cm^{-1}.

Relative Energy Consumption and Electrical Resistance. Relative energy consumption was calculated to measure the efficiency of the electroacidification process using eq 2

$$E_r = \int \frac{UI\,dt}{36CV} \quad (2)$$

where E_r is relative energy (kWh/L of chitosan 1% w/v), U is voltage (V), I is current (A), t is time (s), C is soluble chitosan concentration in the suspension (g/L), and V is the volume of chitosan solution (L).

Resistance (R) was calculated according to Ohm's law ($R = U/I$). The current intensity (I) was read on the control panel and maintained constant at 2.0 A. The voltage difference (U) was measured by a Mastercraft digital multimeter purchased from Canadian Tire, Quebec, Canada.

Soluble Chitosan Concentration. The soluble chitosan concentration in BMEA samples was measured using a colorimetric method adapted from that of Muzzarelli (*12*). Oven-dry chitosan powder (0.5 g) was suspended in demineralized water (50 mL); after 30 min of stirring, 0.5 mL of glacial acetic acid (Fisher Scientific, Ottawa, ON, Canada) was added to dissolve chitosan. The solution was made up to 100 mL with demineralized water and filtered through a Whatman no. 1 filter paper to remove trace impurities. The resulting solution was further diluted 10-fold to a concentration of 0.5 g/L. Glycine−HCl buffer solution was prepared by dissolving 1.87 g of glycine (Bio-Rad, Richmond, CA) and 1.46 g of sodium chloride (laboratoire MAT, Québec, QC, Canada) and made up to 250 mL with demineralized water. Aliquots (81 mL) of this solution were made up to 100 mL with 0.1 M HCl. Cibacron brilliant red 3B-A was obtained from Sigma (Oakville, ON, Canada). A dye solution was prepared by dissolving the powder (150 mg) in demineralized water (100 mL). Aliquots of the dye solution (6 mL) were made up to 100 mL with 0.1 M glycine−HCl buffer. The final dye concentration was 0.090 g/L. Different concentrations of chitosan solutions were prepared by dilution of the 0.5 g/L chitosan solution with buffer. Three hundred microliters of the resulting diluate was added to 3 mL of dye solution. After mixing, absorbance values were measured at 579 nm with a HP 8453 spectrophotometer (Mississauga, ON, Canada) against the blank solution being buffer (0.3 mL) and dye (3 mL). Samples of electroacidified solutions (1.5 mL) were centrifuged at 11180*g* for 10 min at 20 °C (Eppendorf centrifuge, Hamburg, Germany). Soluble chitosan concentration was determined in the supernatants as described for standards.

Ash Content. Crucibles were washed beforehand in 3 N nitric acid, rinsed with deionized water, dried at 100 °C, and cooled in a desiccator. About 5 g of chitosan solution sample was dried in an oven and ashed at 600 °C for a minimum of 18 h. The samples were cooled in a desiccator and weighed.

$$\text{ash (\%)} = [(\text{wt of residue, g})/(\text{sample wt, g})] \times 100$$

Linear Regression. Data of conductivity, ash, and solubility were subjected to linear regression using SigmaPlot software (version 8.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

pH Profile. The two system configurations consisted of the use of cation exchange (CEM) and anion exchange (AEM) membranes in combination with bipolar membranes (**Figure 1**). For one-step addition, pH curves were sigmoidal, starting at pH 7.7 ± 0.2 and ending at pH 2.6 ± 0.1 for CEM configuration and at pH 2.3 ± 0.1 for AEM configuration **(Figure 2)**. Lower pH values were observed for AEM than CEM because more H^+ were retained in the chitosan compartment with AEM, whereas H^+ could cross the cationic membrane. The

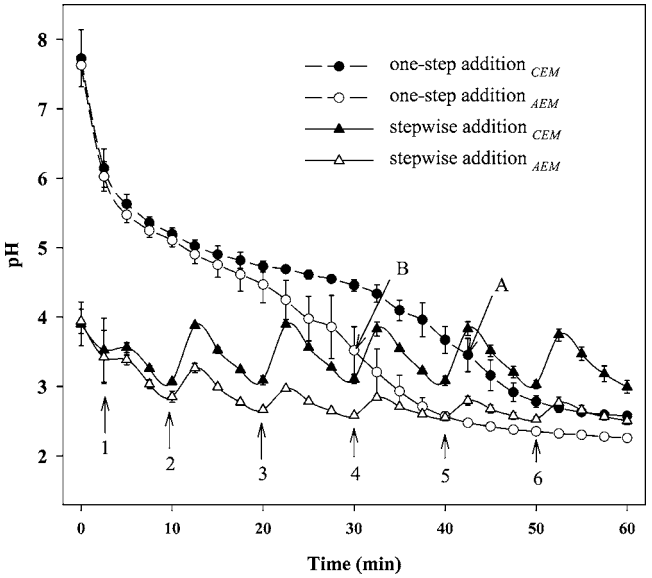

Figure 2. pH evolution during chitosan electroacidification. Arrows 1–6 indicate chitosan introduction for stepwise addition. A and B indicate inflection points in pH curves for, respectively, CEM and AEM one-step-addition configuration.

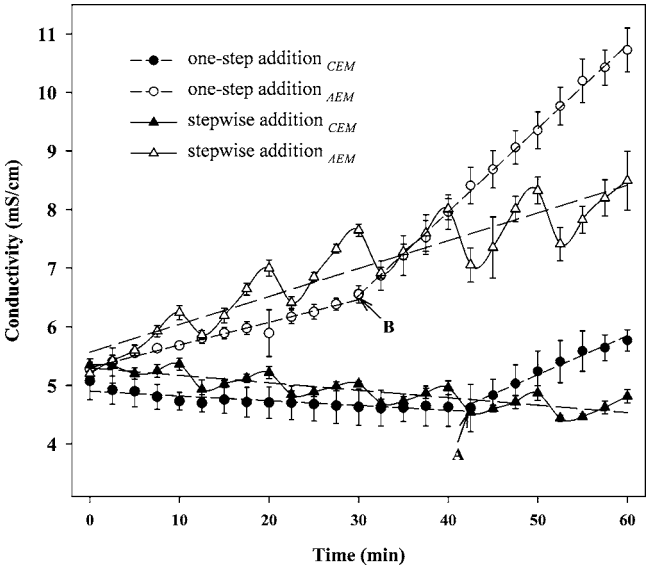

Figure 3. Conductivity evolution during chitosan electroacidification. A and B indicate inflection points in conductivity curves for, respectively, CEM and AEM one-step-addition configuration.

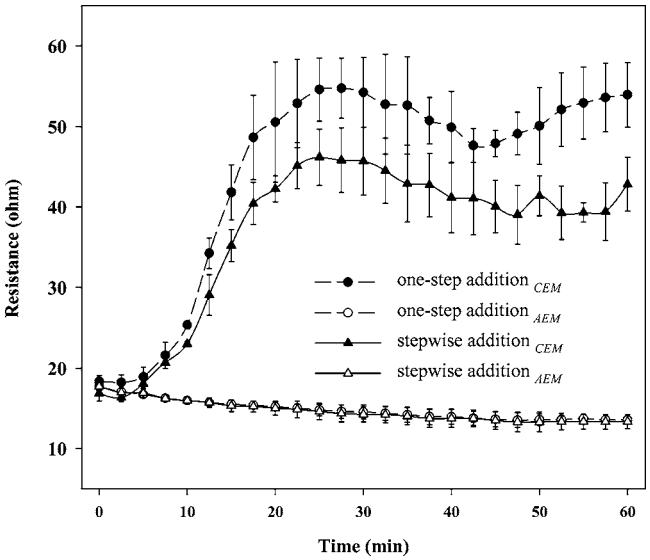

Figure 4. Overall resistance during chitosan electroacidification.

sigmoidal shapes are consistent with chitosan potentiometric titration (*13*), where two inflection points usually indicate the beginning and the end of amino group protonation. Inflection points appear at point A (42 min, pH 3.5, CEM configuration) and point B (30 min, pH 3.5, AEM configuration), indicating the end of chitosan protonation by electrogenerated H^+. In stepwise addition, pH values fluctuated between 3.9 ± 0.3, which is the pH of preacidified water, and 2.5 ± 0.1. During the process, each addition of chitosan was immediately followed by a pH increase due to proton absorption by chitosan; then the pH decreased because the free proton concentration increased in the medium. With AEM configuration, pH fluctuation decayed with each addition, reaching a quasi-steady-state value, while the pH value remained lower than the ones with CEM configuration throughout the process. The difference between the two configurations is that the proton gradient is higher in the chitosan compartment with AEM than with CEM, facilitating accelerated protonation of chitosan. Moreover, chitosan solubilization is higher in stepwise mode than in one-step mode due to the possibility of maintaining acidic conditions (pH <4) during the process, because the pK_a of chitosan is 6.5.

Conductivity Profile. For one-step-addition CEM, conductivity linearly decreased from 5.1 ± 0.3 to 4.6 ± 0.4 mS/cm with a negative slope of 0.009 and then increased to 5.8 ± 0.2 mS/cm with a slope of 0.066 (**Figure 3**). For one-step-addition AEM, conductivity linearly increased from 5.3 ± 0.1 to 6.6 ± 0.2 mS/cm with a slope of 0.037 and then increased to 10.7 ± 0.4 mS/cm with a slope of 0.143. In stepwise mode, conductivity curves were also fitted with linear regressions: for cationic configuration, values of conductivities are in the range of 4.4−5.4 mS/cm with a negative linear regression slope of 0.013; for anionic configuration, the conductivity range was 5.2−8.5 mS/cm with a linear regression slope of 0.047. Conductivity values tend to decrease for cationic configuration because of partial demineralization caused by CEM configuration (*6*, *11*) and tend to increase for anionic configuration because of partial mineralization caused by migration of anions from KCl solution (*9*). For one-step addition, two inflection points, A and B, confirm that full protonation occurred at 42 and 30 min for, respectively, CEM and AEM configuration, in agreement with titration curves. After the inflection points, the slopes of conductivity curves increased because chitosan was fully protonated, allowing free protons to increase quantitatively in the solution.

Electrical Resistance. The initial resistance of the BMEA system was 17.7 ± 0.6 Ω. For one-step-addition CEM, it increased to 55 ± 4 Ω at 25 min and then maintained itself above 48 ± 2 Ω (**Figure 4**). For multiple-step-addition CEM, the resistance increased to 46 ± 3 Ω at 25 min and then maintained itself above 39 ± 4 Ω. For anionic configurations, whether chitosan was added in one or multiple steps, resistance decreased to a final overall resistance of 13.5 ± 0.7 Ω. The increase in resistance observed for cationic configuration can be explained by the conductivity decrease in the acidified compartment due to the migration of free cations (*11*) and also by possible fouling of cation-exchange membranes by positively charged chitosan. In fact, Urano et al. (*14*) observed that cationic membranes could be fouled by positively charged compounds such as iron(III); the cause of the increase of membrane electrical resistance was concomitant with the accumulation of the iron(III) ions. For anionic configuration, the decrease of

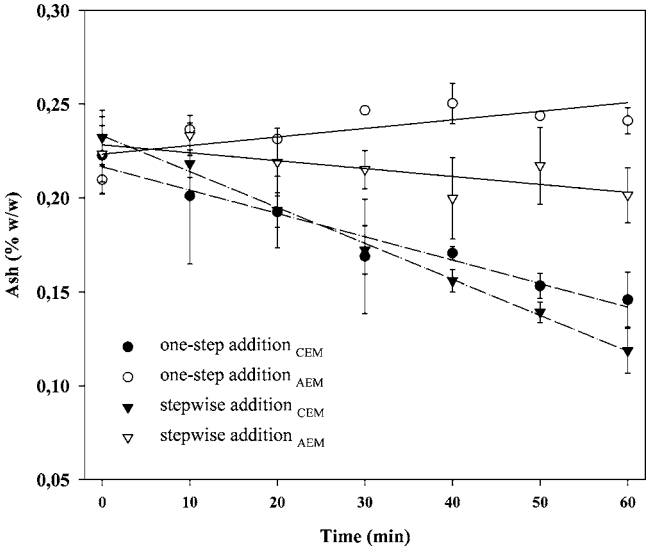

Figure 5. Ash content during chitosan electroacidification.

overall resistance can be explained by an increase of conductivity in the acidified compartment due to migration of anions from the KCl solution and also because anionic membranes are less fouled by positively charged chitosan.

Ash Content. The initial ash content of the chitosan suspension was 0.22 ± 0.01% (w/w), mostly due to NaCl addition to increase conductivity of the suspension. The evolution of ash content as a function of acidification time was fitted by linear regression (**Figure 5**). After 60 min of BMEA, the ash content decreased by 35% for one-step CEM configuration, by 48% for stepwise CEM configuration, and by 11% for stepwise-addition AEM, whereas it increased by 13% for one-step-addition AEM. As expected, ash content decreased for cationic configurations because of decationization of the solution during the process; this phenomenon is well-known and is due to the removal of cations in order to maintain electroneutrality, as protons are produced by the bipolar membrane (*15*). For the anionic configuration, a 13% mineralization occurred in one-step addition due to migrations of anions Cl^- from the KCl solution. On the other hand, an 11% demineralization occurred in multiple-step addition with AEM. This phenomenon may be attributed to migration of Cl^- anions through ionic leakage in the bipolar membrane (*16*).

Soluble Chitosan Concentration. Chitosan solubilization yield was calculated from the ratio (final solubilized chitosan/total chitosan added) × 100. The total chitosan added was 4.6 g/L, because 5 g/L of raw chitosan powder was used that contained 7.5% humidity and 0.4% ash. For single-step-addition CEM, soluble chitosan concentration increased and stabilized at 2.7 ± 0.3 g/L after 40 min, which represents 59% of total added chitosan (**Figure 6 a**). For single-step-addition AEM, the concentration increased and stabilized at 3.3 ± 0.3 g/L after 30 min, which represents a 72% chitosan solubilization yield. In single-step addition, chitosan solubilization exhibited a lag phase followed by a linear phase for both configurations, with a higher rate of solubilization with AEM. The lag phase is attributable to disaggregation of chitosan, preceded by protonation. For stepwise addition, the soluble chitosan concentration increased linearly at 3.6 ± 0.3 and 4.2 ± 0.1 g/L for CEM and AEM configurations, respectively, after 60 min of BMEA (**Figure 6b**). Chitosan solubilization rates were 78 and 91% for CEM and AEM with stepwise addition, respectively. For single-step-addition mode, the soluble chitosan concentration reached a maximum value and then stabilized; at saturation, chitosan available in circulation is fully protonated, and the maximum soluble chitosan concentration was reached. However, the amount of initial chitosan was not completely solubilized because part of the added chitosan formed a gel on the electrodialysis (ED) compartment stack and, thus, was not easily available for the protonation process. The presence of large hydrodynamic particles at the beginning of BMEA appears to contribute to gel formation. On the other hand, stepwise addition allowed higher solubilization rates than one-step addition, because it would nearly diminish the lag phase and reduce the gel formation; therefore, more chitosan is available in circulation. In all cases, BMEA allowed a complete solubilization of chitosan circulating freely in the ED system, resulting in the production of a clear and homogeneous chitosan solution in the mixing tank.

Relative Energy Consumption. At the end of the processes, relative energy consumptions were calculated and expressed as energy consumption (in kilowatt hours) necessary to prepare 1 L of aqueous 1% chitosan solution. In cationic configurations, the relative energy consumption was 0.24 ± 0.04 kWh/L for one-step addition and 0.14 ± 0.02 kWh/L for stepwise addition. In anionic configurations, the relative energy consumption was 0.06 ± 0.01 kWh/L for one-step addition and 0.05 ± 0.01 kWh/L for stepwise addition. Cationic configurations consumed 2−5-fold more energy than anionic configurations because of membrane and stack fouling by chitosan aggregates that created high resistance in the ED system, as evidenced previously for

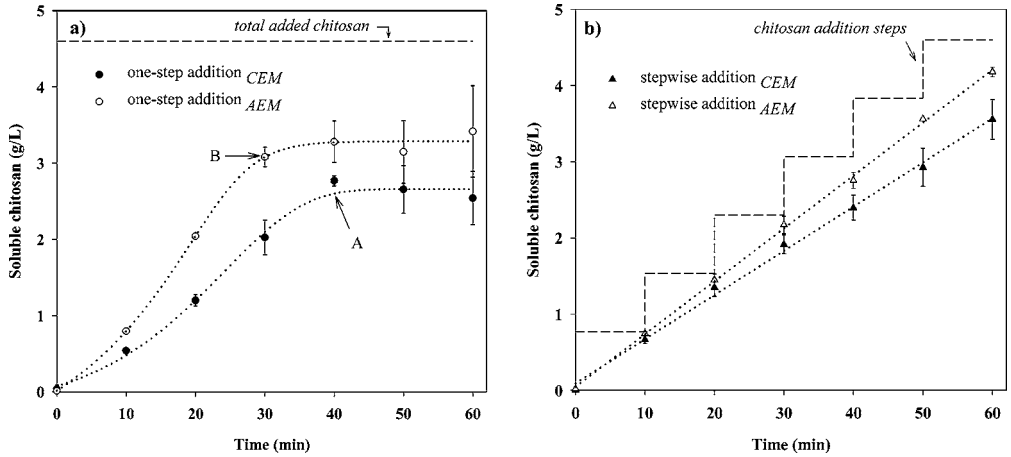

Figure 6. Soluble chitosan concentration during (**a**) single-step BMEA and (**b**) stepwise BMEA. A and B indicate inflection points in soluble chitosan curves for, respectively, CEM and AEM one-step-addition configuration

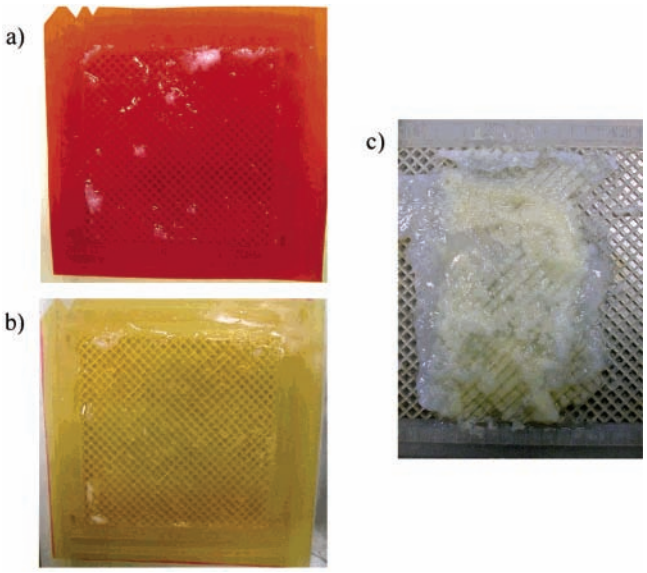

Figure 7. Anion exchange membrane (**a**), cation exchange membrane (**b**), and chitosan fouling in acidified compartment spacer (**c**) after 60 min of BMEA for single-step chitosan addition.

cationic configurations. Indeed, small quantities of chitosan deposits remained on the ionic membranes (**Figure 7a**,**b**), and they could be washed off with water. Fouling of the spacers was observed for all configurations and appeared as an alkaline gel (**Figure 7c**). Thus, optimization of the process of chitosan solubilization by BMEA would consist of the minimization of fouling phenomenon, particularly in the spacers. To achieve that goal, several options can be considered: (1) modify the design of the spacers to improve the circulation of the chitosan particles in suspension; (2) reduce any contamination of the acidified compartment by hydroxyls ions, for instance, by controlling the pH of the adjacent compartment at a pH closer to the pK_a of chitosan. Finally, the electrode reactions are responsible for a large part of the energy consumption. Surely, the calculated values for energy consumption would be smaller with a filter-press configuration involving several units cell.

Conclusion. Electrochemical solubilization of chitosan by BMEA depends on the type of monopolar ionic membranes and on the introduction mode of chitosan in the ED system. The best configuration identified in this study consisted of anion exchange membranes and stepwise feeding mode, which led to a chitosan solubilization of 91%. Anionic configurations were less energy-consuming in comparison with cationic configurations, whereas stepwise addition mode improved chitosan disaggregation in the BMEA system for better protonation. Further experiments will focus on minimizing chitosan aggregation in the electroacidified compartment, which can be an effective means of improving chitosan solubilization. Bipolar membrane electroacidification has potential as an alternative to acid methods for the preparation of soluble chitosan.

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