

Electromigration Behavior of a Mixture of Chitosan Oligomers at Different Concentrations

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In this study, the effect of the concentration of a chitosan oligomer mixture on its electrophoretic behavior was studied as a function of pH and ionic strength added. It was shown that the concentration has a significant effect on the average electrophoretic mobility of the chitosan oligomer mixture and on isoelectric point. At a concentration of 3%, the ionic strength added did not show any effect on the electromigration behavior of the chitosan oligomer mixture. By decreasing the concentration of the chitosan oligomer mixture, ionic strength showed a significant effect on the average electrophoretic mobility but not on the isoelectric point. The highest shift of the isoelectric point was recorded in water at 0.003% concentration of the oligomer mixture. Under these conditions, the isoelectric point was at pH 5 whereas it was at pH 8 at 3% concentration of chitosan oligomer mixture. Electrophoretic measurements were also taken in water/ethanol aqueous medium. By adding ethanol to the medium, the average electrophoretic mobility decreased. This would have been caused by the increase in viscosity of the medium. Increasing ethanol ratio in the running medium, the isoelectric point moved from pH 5 in water up to pH 6–8 dependently on chitosan oligomer mixture concentration and ethanol content of the medium.

KEYWORDS: Chitosan oligomer mixture; concentration; isoelectric point; ethanol; dielectric constant; electrophoretic mobility

INTRODUCTION

Chitosan is the N-deacetylated product of chitin found in the shells of crabs and shrimps and as cell wall components of most fungi, yeasts, and molds. Chitosan has applications in several fields such as biomedical, personal care products, biotechnology, pharmaceutical, nutraceutical, and food. Chitosan oligomers with a degree of polymerization of about 6 are potentially useful as medicinal agents and as food ingredients because of their biological and therapeutic values (1–4). To produce these bioactive oligosaccharides, biocatalytic (enzymatic) and chemical procedures are used (5–7). The yield of the biocatalytic reaction is not high (8). By combining physical methods (shear-force treatment) and acid hydrolysis, the molecular weight of the chitosan oligomer can be decreased (2, 9, 10). Although execution of these physical methods is not difficult, fast degradation rates and random reactions result in product variability (8). In acid hydrolysis, 10% acetic acid is generally used as a solvent, with 5% NaNO₃ added for the deacetylation

reaction. This method can decompose chitosan into units of one to six N-acetylglucosamines, and such products are soluble at pH 7 (11). All these methods can serve to produce a mixture of molecules with various molecular weights.

To separate these chitosan oligomer mixtures to obtain pure or enriched oligomer, it is essential to find a suitable technique. The exploitation of the electric properties of these molecules could offer a solution (12). The chitosan oligomer has one or more amine functional groups depending on the degree of polymerization (13). Under specific pH conditions, these groups are charged because of the protonation of the amine group (NH₃⁺). Therefore, they will migrate under the effect of an external electric field (13). The migration speed of each molecule should be different from others because of differences of their molecular weight, electric charge, and concentration. That would make possible the separation of various chitosan oligomers by exploiting the electrophoretic properties of each molecule. To carry out this objective, it is important to study and understand the electrophoretic behaviors of the chitosan oligomers under various conditions of the medium and different concentrations of the mixture. In a previous study (14), it was shown that electromobility of chitosan D-glucosamine (monomer) and individual chitosan oligomer (dimer, trimer, tetramer,

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pentamer, and hexamer) in dilute media at a concentration of 10 $\mu\text{g/mL}$ originated not only from the electrical charge but also from the glucose moiety through the difference in the dielectric constant of glucose and that of the medium at alkaline pH values.

The aim of this research was to study the electrophoretic behavior of chitosan oligomer mixture at different concentrations under varying conditions of pH, ionic strength, and dielectric constant of the medium.

2. EXPERIMENTAL PROCEDURES

2.1. Chemicals. All chemicals were of analytical grade. Sodium chloride (NaCl) was purchased from EMD Chemicals Inc (Darmstadt, Germany). Hydrochloric acid (HCl) and sodium hydroxyde (NaOH) were purchased from Fisher Scientific (Nepean, Ontario, Canada). Ethanol-95% was purchased from Commercial Alcohols Inc, (Brampton, Ontario, Canada). The mixture of the chito-oligomer (dimers, trimers, tetramers) was obtained from ISM Biopolymer Inc (Quebec, Canada). The water used in all the experiments was of HPLC grade.

2.2. Electrophoretic Mobility Measurements. The average electrophoretic mobility of chitosan oligomer was performed using a Zetasizer 2000 system (Malvern Instruments Ltd, Worcs, United Kingdom) equipped with a photon correlation spectroscopy (PCS) system. The calibration of the Zetasizer 2000 was made using a standard (DTS5050, Malvern instruments) with zeta potential of -50 ± 5 mV at 25 °C. Before each measurement, the cell of the Zetasizer 2000 was rinsed with HPLC grade water and was dried with air blast. The temperature was maintained constant at 25 °C. During the measurements, pH of the oligomer solutions was measured and adjusted with a pH meter (Model SP20 SympHony, VWR, United States). Syringes (2.5 mL) were used for the injection of the oligomer solutions in the Zetasizer 2000.

2.3. Protocols. A solution of chitosan oligomer mixture was prepared by dissolving a suitable quantity of the chitosan oligomer mixture (dry form) in HPLC grade water, water/NaCl, and water/ethanol to obtain the desired concentrations of chitosan oligomer mixture. The studied concentrations were 3, 0.3, 0.03, and 0.003%, respectively. The pH of the solutions was adjusted using dilute HCl and NaOH solutions to generate electrophoretic mobility measurements versus pH curves and subsequently the isoelectric point (IEP) for each concentration. The pH values studied were 2–12. The salt used was NaCl at ionic strengths added of 0.01, 0.05, and 0.1 M. To obtain the desired dielectric constant of the medium, water/ethanol ratios studied for these concentrations of chitosan oligomer mixture were 75/25, 50/50, and 25/75. Control measurements in water were also taken. The average electrophoretic mobility of the chitosan oligomer was measured in water at pH values from 2 to 12, then in NaCl at the same pH interval and ionic strengths added of 0.01, 0.05, and 0.1 M, and finally in water/ethanol in pH range from 2 to 12.

2.4. Statistical Analyses. Factorial design of the experimental plan was entirely randomized. The studied variables were concentrations, 4 levels; pH, 11 levels; ionic strength added, 4 levels; and water/ethanol (W/EtOH) ratio, 4 levels. Each treatment was repeated three times. Once all of the experiments had been completed, SAS software (V8.0, SAS Institute Inc., Cary, NC) was used for statistical analysis. A 5% significance level was chosen. The ANOVA procedure was used to analyze the variance. Sigmaplot 8.0 software (SPSS Inc., Chicago, IL) was used to generate the figures in 2D dimensions.

3. RESULTS AND DISCUSSION

3.1. Results. **3.1.1. Electromigration of 3% Chitosan Oligomer Mixture in Water, NaCl, and Water/Ethanol Mixture Media.** **Figure 1a** shows the electromigration behavior of the chitosan oligomer mixture at a concentration of 3% in water (HPLC grade) and NaCl solutions with different ionic strengths added as a function of pH, which had significant effect ($P < 0.0001$). At this concentration, the chitosan oligomer showed the highest electrophoretic mobility at pH 2 and 3 with an average value

of $2.009 \pm 0.105 \times 10^{-6} \text{ m}^2/\text{V}\cdot\text{s}$. At pH 4–6, the oligomer showed a stable electrophoretic mobility but lower than that recorded at pH 2 with an average value of $1.225 \pm 0.051 \times 10^{-6} \text{ m}^2/\text{V}\cdot\text{s}$. At all the previous mentioned pH values, the chitosan oligomer migrated toward the cathode. At pH 7, the electromigration was slower than at the preceding pH values ($0.836 \pm 0.080 \times 10^{-6} \text{ m}^2/\text{V}\cdot\text{s}$). At higher pH values (8–10), the oligomer mixture was motionless. At pH 11 and 12, we noticed a slight negative electromigration (toward the anode) with an average electrophoretic mobility of $-0.331 \pm 0.110 \times 10^{-6} \text{ m}^2/\text{V}\cdot\text{s}$. In NaCl solutions (0.01–0.1 M), electromigration behavior of the chitosan oligomer mixture was quite similar to that in water, and the effect of the ionic strength added compared to the data obtained in water was not significant ($P > 0.971$).

In water mixed with ethanol at different ratios, the behavior of the chitosan oligomer mixture was different from what was observed in NaCl at various ionic strengths added. Indeed, at a 3% concentration of chitosan oligomer mixture (**Figure 1b**) with ethanol addition, we observed a gradual fall of the absolute value of electrophoretic mobility. This fall was gradual. Highest mobilities were recorded in water. In water mixed with ethanol (W/Et–OH) at ratio of 75/25 (v/v), electrophoretic mobility had decreased and had occupied intermediate values between what was observed in water and what was recorded in water with ethanol at ratios of 50/50 and 25/75 (v/v), respectively. Electrophoretic mobilities recorded in these two media (W/Et–OH at ratio 50/50 and 25/75 (v/v)) were not significantly different. The isoelectric point was the same under all conditions (PI = 8). It is the same value which was recorded in the NaCl solutions at various ionic strengths. At pH values above the PI, there was no significant electrophoretic mobility of the chitosan oligomer mixture.

3.1.2. Electromigration of 0.3% Chitosan Oligomer Mixture in Water, NaCl, and Water/Ethanol Mixture Media. **Figure 2a** shows the electromigration behavior at a concentration of 0.3% of the chitosan oligomer mixture in water and NaCl solution at different ionic strengths added as a function of pH. The effect of pH was significant ($P < 0.001$). In water, in the pH interval from 2 to 5, the electromigration of the chito-oligomer was stable and was toward the cathode with a mean value of $1.651 \pm 0.209 \times 10^{-6} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$. A decrease of the electromobility was recorded at pH 6. At pH 7, the chitosan oligomer mixture was at the isoelectric point. By increasing the pH, the electromigration was toward the anode. In the interval of pH from 8 to 12, the electrophoretic mobility of the mixture was relatively stable and showed an average electrophoretic mobility of $-1.029 \pm 0.091 \times 10^{-6} \text{ m}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$. By adding salt to the medium (**Figure 2a**), the electromigration behavior of the chitosan oligomer was affected ($P < 0.027$). At ionic strength added up to 0.01 M, a significant decrease of the electrophoretic mobility was recorded between pH 2 and 5, and increasing the ionic strength added up to 0.05 or 0.1 M did not change the electromigration behavior of the chitosan oligomer mixture.

At a concentration of 0.3% of chitosan oligomer mixture (**Figure 2b**), the highest mobilities were recorded in water. In this case, the isoelectric point was moved compared to that recorded at a concentration of 3%, and at pH values above the isoelectric point (IP), negative electrophoretic mobilities were observed. By adding ethanol, at pH values below the isoelectric point, the same phenomenon was observed as in the case with a concentration of 3%. Electrophoretic mobility decreased gradually. At pH values above the isoelectric point, no significant difference was observed. The values recorded in water mixed with ethanol at ratio of 75/25 (v/v) were intermedi-

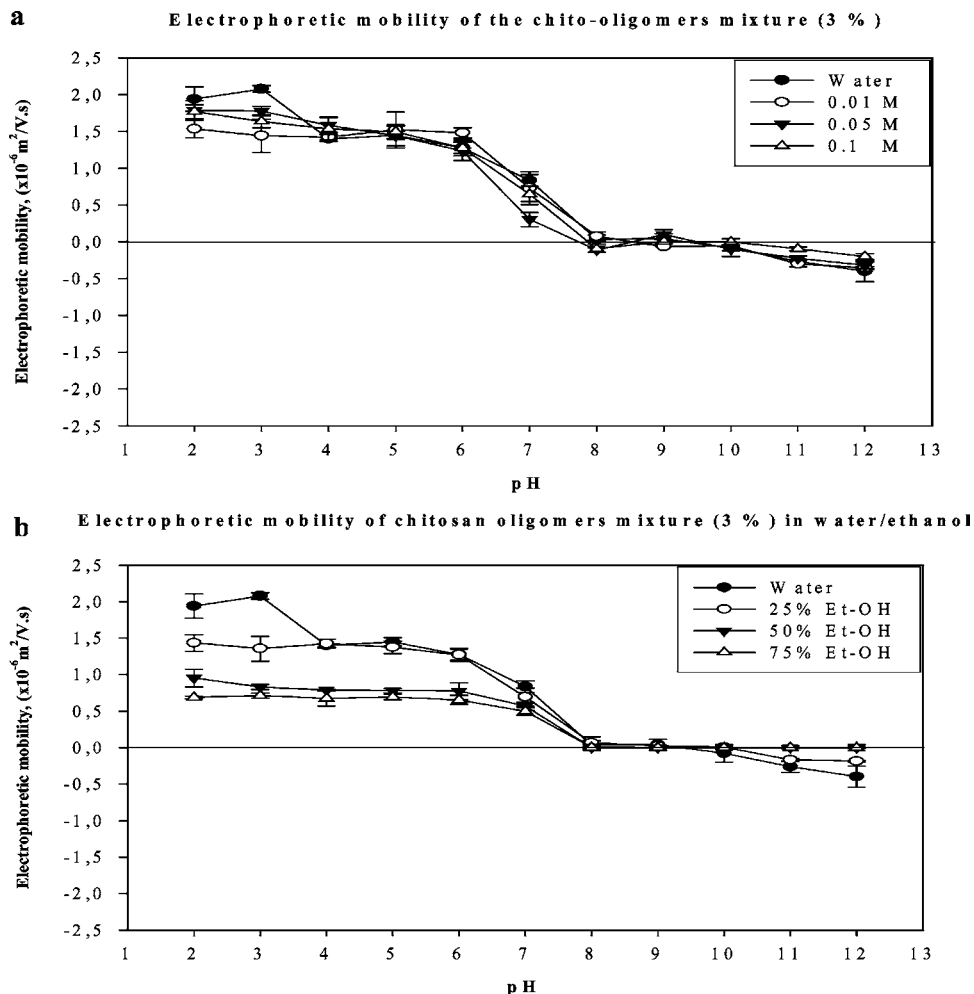


Figure 1. Electrophoretic mobility of the chitosan oligomer mixture at a concentration of 3%: (a) in water and water with NaCl at different ionic strengths added and (b) in water and water/ethanol mixture at different ratios (v/v).

ate between those observed in water (the highest) and those recorded in water with ethanol at ratios of 50/50 and 75/25 (c/v), respectively. Mobilities in these two cases were the lowest. No significant difference was observed between electrophoretic mobilities in these two media. As regards the isoelectric point (IP), it moved up to pH 7.5 in water/ethanol at ratio of 75/25 (v/v) and up to pH 8 in water/ethanol at ratios of 50/50 and 25/75 (v/v), respectively.

3.1.3. Electromigration of 0.03% Chitosan Oligomer Mixture in Water, NaCl, and Water/Ethanol Mixture Media. **Figure 3a** illustrates the electromigration behavior of the chitosan oligomer mixture at concentration of 0.03% under different conditions. In all cases, the effect of pH was significant ($P < 0.001$). In water, the mixture showed the greater electrophoretic mobility at pH 2–4 with a mean value of $1.779 \pm 0.227 \times 10^{-6} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. By increasing the pH up to 5, the electrophoretic mobility decreased. At all these pH values, the migration was toward the cathode. The isoelectric point was recorded at pH 6. By increasing the pH, the oligomer mixture completely changed its behavior and migrated toward the anode. At pH 7, the recorded electrophoretic mobility was $-0.866 \pm 0.245 \times 10^{-6} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. In the pH interval between 8 and 12, the electromobility of the oligomer reached a plateau with an average value of $-1.386 \pm 0.112 \times 10^{-6} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. Data analyses showed that ionic strength influenced the electromigration behavior of the mixture depending on pH range. Between pH 2 and 4, ionic strength added had a significant effect on the magnitude of the electrophoretic mobility ($P < 0.0001$), where

the electrophoretic mobility decreased by increasing ionic strength to the medium. In the range of pH between pH 5 and 7, the ionic strength added had no effect on the electrophoretic mobility of the chitosan oligomer mixture ($P > 0.877$), and the isoelectric point was situated in this pH range. In this pH interval, the mixture was closer to the isoelectric point. Between pH 8 and 10, the electrophoretic mobility decreased by the addition of salt to water. In the pH range between pH 11 and pH 12, ionic strength added did not show any effect on the electrophoretic mobility ($P > 0.160$).

At a concentration of 0.03% of chitosan oligomer mixture (**Figure 3b**), the highest electrophoretic mobilities were recorded in water. This was valid at pH values below and above the isoelectric point (IP). By adding ethanol to the medium, electrophoretic mobility of the chitosan oligomer mixture decreased gradually. The PI was observed at pH close to 6 in water and then it passed up to pH close to 8 in water mixed with ethanol at different ratios.

3.1.4. Electromigration of 0.003% Chitosan Oligomer Mixture in Water, NaCl, and Water/Ethanol Mixture Media. **Figure 4a** shows the electrophoretic behavior of the chitosan oligomer in water at a concentration of 0.003% and NaCl solution at different ionic strengths added as a function of pH. The effect of pH was significant at all conditions ($P < 0.001$). In water, the isoelectric point was recorded at pH 5. The cationic character of the chitosan oligomer was measured at pH 2–4, and the electromigration was toward the cathode. In the pH interval

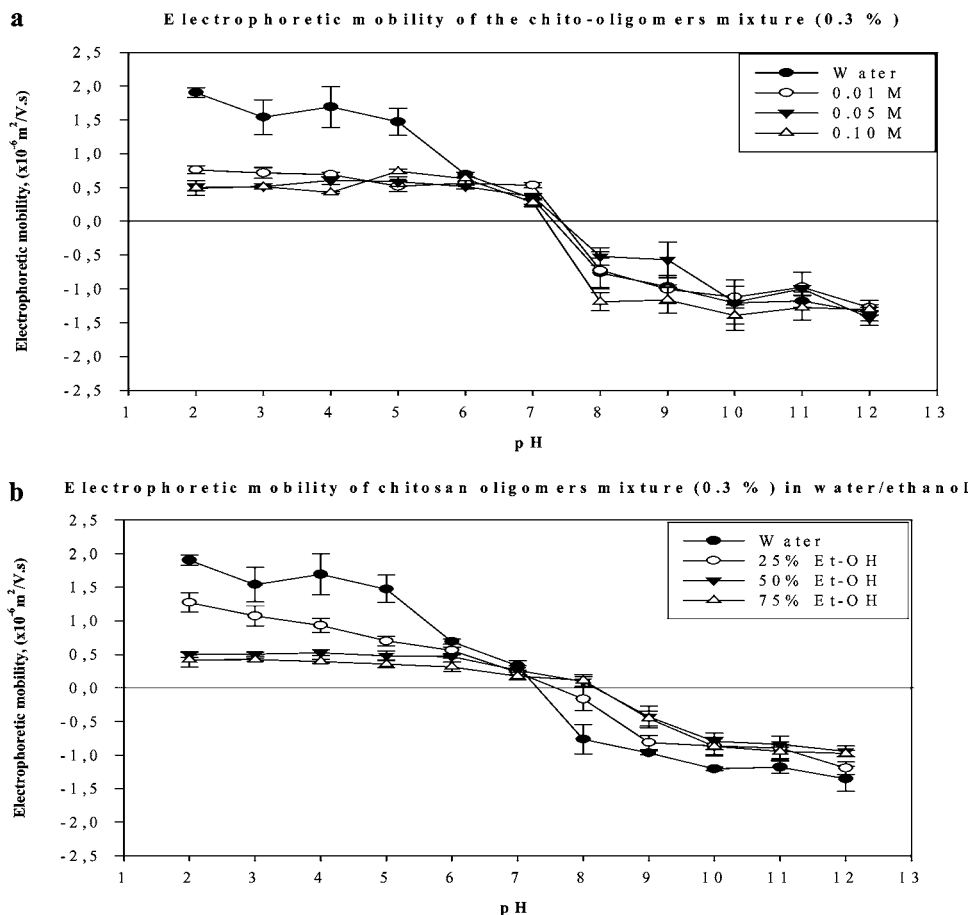


Figure 2. Electrophoretic mobility of the chitosan oligomer mixture at a concentration of 0.3%: (a) in water and water with NaCl at different ionic strengths added and (b) in water and water/ethanol mixture at different ratios (v/v).

between 6 and 12, the migration was toward the anode. The highest electromobility was at pH 12 with an average value of $-1.555 \pm 0.224 \times 10^{-6} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. In NaCl solutions, data analysis showed three intervals for the effect of ionic strength added. At pH between 2 and 6, the ionic strength had no significant effect on the electrophoretic mobility of the chitosan oligomer mixture ($P > 0.465$). At pH between 7 and 9, ionic strength had a significant effect on the absolute value of the electrophoretic mobility ($P < 0.003$). As seen in **Figure 4a**, the chitosan oligomer mixture was more mobile in water and by increasing the ionic strength added, the electrophoretic mobility decreased. The effect of the ionic strength added of 0.01 M was different from that of 0.05 or 0.1 M. In the pH range between 10 and 12, there were no significant differences between electrophoretic mobilities ($P > 0.622$).

At a concentration of 0.003% of chitosan oligomer (**Figure 4b**), no significant difference was observed between electrophoretic mobilities below the isoelectric point (IP). The IP was at pH 5 in water. By adding ethanol to the medium, the isoelectric shifted up to pH 6 in water/ethanol at ratio of 75/25 (v/v) and up to pH 7 in water/ethanol at ratios of 50/50 and 75/25 (v/v), respectively. At pH values above the isoelectric point, the highest electrophoretic mobilities were observed in water. By adding ethanol to the medium, electrophoretic mobility decreased gradually.

3.2. Discussion. **3.2.1. Effect of pH.** Electrophoretic mobility of the studied oligomer showed that the pH has a significant effect on the behavior of the molecules when they are subjected to an external electric field (14, 15). The chitosan oligomers are positively charged in acid medium since the amine groups

are protonated and their migration toward the cathode is expected. However, it was interesting to see that the chitosan oligomer showed an electromigration toward the anode at some pH values near the pK_a of glucosamine (pH < 7), where the amine groups were always protonated and at pH values greater than the pK_a value, where the oligomer did not carry any electric charges. In a previous study reported by Aider et al. (14), it was demonstrated that the anionic character acquired by the oligomer originates from glucose moiety and that the origin of the charge might arise from the difference in dielectric constant of the medium and the migrating molecule (**Table 1**). At 50% and higher of ethanol content of the solvent, the oligomer did not show any mobility at pH 7. This was caused by the fact that ethanol has a dielectric constant lower than that of water (16), and the fall of the dielectric constant of the medium caused the disappearance of this phenomenon.

3.2.2. Effect of the Ionic Strength Added. As the ionic strength added of the medium was increased, the ionic atmosphere composed of counterions around the migrating molecules increased. Under the effect of the applied external electric field, migrating chitosan oligomer moves in one direction and the counterion atmosphere moves in the opposite direction, each carrying solvent molecules along with it. As a result of this phenomenon, the electromigration of the molecule is retarded by the atmosphere of counterions, that is, the screening effect of these counterions increases. In dilute aqueous medium, this phenomenon was stronger when the chitosan oligomer migrated toward the anode with Na^+ as counterions which are hydrated (solvated), while the Cl^- counterions are not hydrated when the electromigration was toward the cathode. The highest values

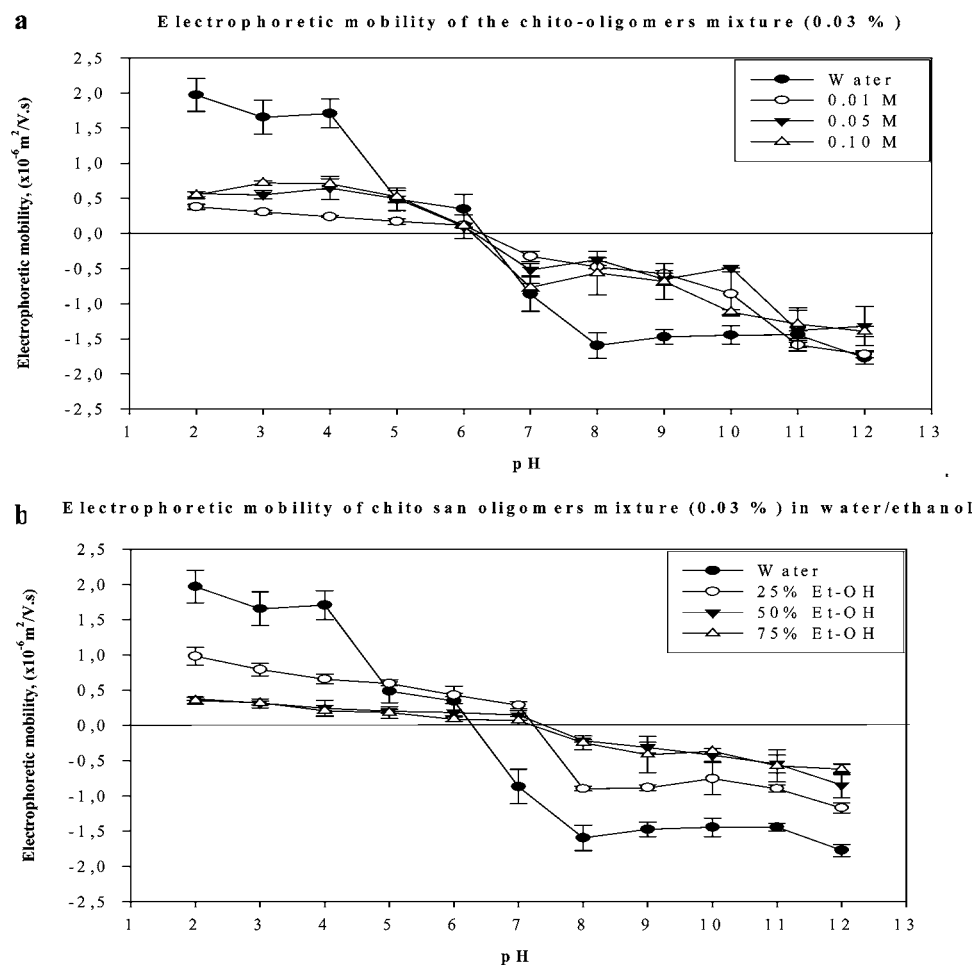


Figure 3. Electrophoretic mobility of the chitosan oligomer mixture at a concentration of 0.03%: (a) in water and water with NaCl at different ionic strengths added and (b) in water and water/ethanol mixture at different ratios (v/v).

of the electrophoretic mobilities of the chitosan oligomer were obtained in water because the screening effect of the counterions was lowest, followed by those recorded in solutions with an ionic strength added of 0.01 M. The lowest values of the electrophoretic mobility were recorded in the salt solutions with ionic strengths added of 0.05 and 0.1 M. All these observations are in good agreement with previous results in the literature (13, 14, 17). Also, by decreasing concentration of the chitosan oligomer mixture, the oligomer molecules are separated and the screening effect is reduced. The network formed between these oligomers was lower because the interactions between chitosan oligomer molecules decreased (interaction by means of the hydrogen bonds).

The above given explanation is not valid in the case of chitosan oligomer solution of 3% concentration. Ionic strength added did not have any effect on the electrophoretic mobility of the chitosan oligomer mixture. At this concentration (3%), the hydrogen bonds formed between the molecules contribute to the formation of a network between the oligomer, and thus the screening effect of the ionic strength was negligible. By decreasing the concentration, the ionic strength affected the electrophoretic mobility of the oligomer.

3.2.3. Effect of Ethanol. With the addition of ethanol to the medium (water), the absolute value of the electrophoretic mobility decreased. This was the result of the increase in viscosity of the running medium (water/ethanol mixture) (18). At the same time, the addition of ethanol to the medium had caused a decrease of the medium dielectric constant. This decrease of the medium dielectric constant decreased the

interaction between the solvent (water/ethanol mixture) and the solute (19, 20). The reason for this behavior is that the applied external electric field caused a reorientation of the dipoles of the migrating molecules and the ionic atmosphere around the oligomer composed of hydroxyl ions (OH^-) contributed by medium dielectric constant diminishes as the dielectric constant of the solvent (water/ethanol) is decreased because of the low dielectric constant of ethanol.

The dependence of the electrophoretic mobility on the concentration of chitosan solution was also reported by López-León et al. (21). The shift of the isoelectric point of the chitosan oligomer mixture in water/ethanol media was lower than it was in water because the deprotonation of the amine group in water/ethanol solvent was lower.

3.2.4. Effect of the Concentration on Electrophoretic Behavior of Chitosan Oligomer Mixture. As can be seen (Table 1), the isoelectric point and, consequently, the electrophoretic behavior of the chitosan oligomer depend on the concentration. It was at pH 8 for a concentration of 3% and then decreased by decreasing the concentration. At a concentration of 0.03% and below 0.003%, the isoelectric point was lower than the pK_a of D-glucosamine. This indicated that the isoelectric point and, in general, the electrophoretic behavior of chitosan oligomer mixture depend on the concentration.

The dependence of the electrophoretic behavior of the chitosan oligomer mixture on the concentration could be explained as follows: at a concentration of 3%, the interactions between the molecules by the intermediary of the hydrogen bonds are stronger and the network is not highly hydrated. The

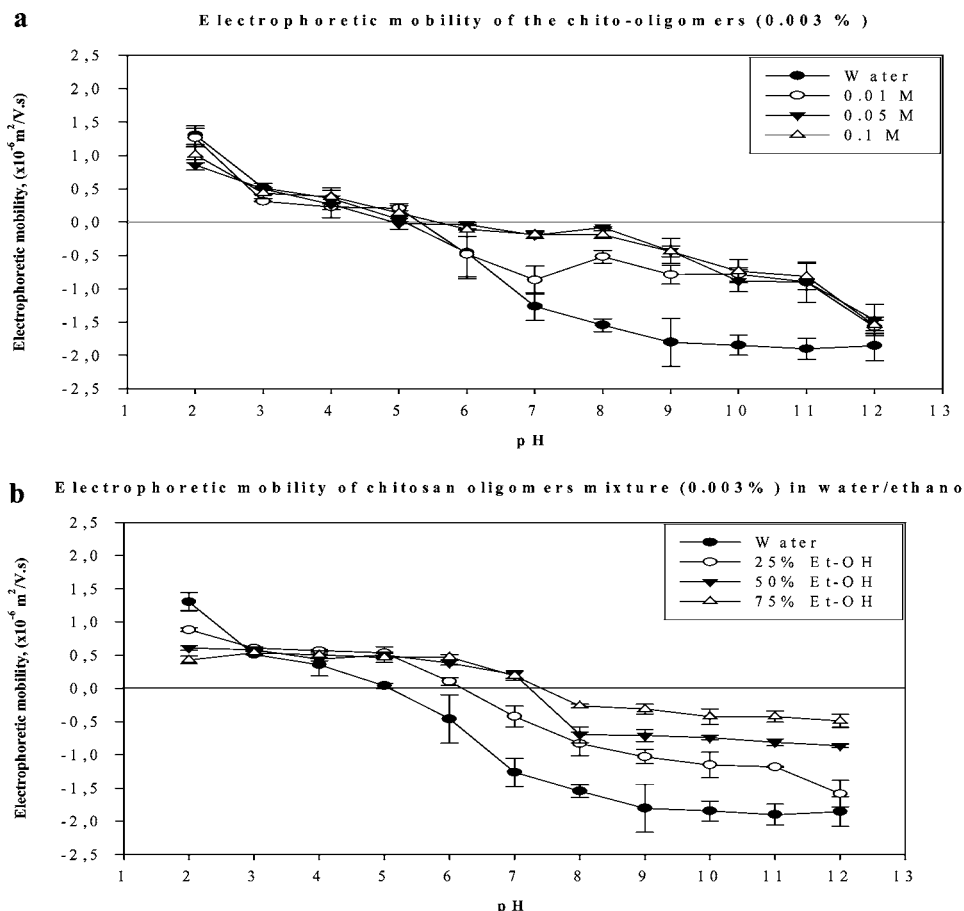


Figure 4. Electrophoretic mobility of the chitosan oligomer mixture at a concentration of 0.003%: (a) in water and water with NaCl at different ionic strengths added and (b) in water and water/ethanol mixture at different ratios (v/v).

Table 1. Isoelectric Point of Chitosan Oligomer Mixture at Various Concentrations in Different Media^a

solvent	chitosan oligomer mixture concentration,%			
	3	0.3	0.03	0.003
water	8	7.2	6.3	5
0.01 M NaCl	8	7.4	6.3	5
0.05 M NaCl	8	7.5	6.3	5.2
0.10 M NaCl	8	7.5	6.3	5.2
W/Et-OH 75/25 (v/v)	8	8	7.8	6
W/Et-OH 50/50 (v/v)	8	8	8	7.8
W/Et-OH 25/75 (v/v)	8	8	8	8

^a Dielectric constant of the solvent (water/ethanol) could be calculated from the relation $\epsilon_m = \epsilon_w f_w + \epsilon_e f_e$, according to Seedher and Bhatia (22), where $\epsilon_w = 78.36$, $\epsilon_e = 24.30$, and ϵ_m are the dielectric constants of water (W), ethanol (Et-OH), and the mixture (W/Et-OH), respectively; f_w , f_e are the volume fractions of water and ethanol, respectively, in the final mixture.

molecules form a more compact network and, consequently, the effect of the medium or more exactly the friction between the medium and the solute was less significant. On the other hand, by decreasing the concentration of the chitosan oligomer mixture, the network formed by the molecules becomes weak and the dielectric friction with the medium becomes stronger. This friction has caused the attraction of the chitosan oligomer molecules toward the anode as if they are anions. This phenomenon caused the displacement of the isoelectric point (IP) to pH values lower than the isoelectric point recorded at a concentration of 3% (Figure 1a). The deprotonation of the

amine group was stronger in dilute media, and thus the shift of the chitosan oligomer mixture isoelectric point was more pronounced.

3.3. Conclusion. In this study, it was demonstrated that the concentration has a significant effect on the isoelectric point and the electrophoretic behavior of the chitosan oligomer mixture. By decreasing the concentration of the chitosan oligomer mixture from 3% to 0.003%, the isoelectric point was reduced from pH 8 to pH 5. The isoelectric point of the chitosan oligomer mixture is thus linearly contingent on the free hydronium (H^+) ion concentration present in the medium. The interactions with the medium become increasingly significant by decreasing the concentration. The contribution of the difference in dielectric constant between the solute and the solvent was also demonstrated. In dilute medium, this phenomenon led to the migration of the chitosan oligomer toward the anode. Moreover, this study also made it possible to observe the effect of the ionic strength added on the electrophoretic mobility at various concentrations of the chitosan oligomer mixture. At a concentration of 3%, no effect on the electrophoretic mobility of the ionic strength added was shown. However, by decreasing the concentration, the effect of the ionic strength added became significant. The pH of the medium showed a significant effect on the electrophoretic behavior of the chitosan oligomer mixture at all concentrations because it determines the charge of the molecules and thus the direction of the migration.

These results are actually the basis for electroseparation of

chitosan oligomer mixture by an electro dialysis-ultrafiltration (EDUF) system.

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