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# Electromigration of Chitosan D-Glucosamine and Oligomers in Dilute Aqueous Solutions

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The electromigration behavior of chitosan D-glucosamine and oligomers with a degree of polymerization from 1 to 6 in dilute aqueous systems containing either NaCl or KCl salt at 0.01, 0.05, and 0.1 M at pH values from 2 to 9 was evaluated. The results showed that the electromigration of the chitosan D-glucosamine and oligomers did not change by changing the type of salt in the running medium and that the pH had a significant effect on the direction of migration under an external electric field. In addition, the increase in the ionic strength of the medium caused a significant decrease on the absolute value of the electrophoretic mobility, and the highest values of the electromobility were observed in water. However, the ionic strength had no significant effect on the electrophoretic mobilities at pH 2 in comparison with the other pH values. The dimer showed the highest electrophoretic mobility in the alkaline zone of the pH. At pH values lower than the $pK_a$ of the D-glucosamine, the chitosan D-glucosamine, and oligomers migrated toward the anode, where the amine groups are protonated and carry positive charge. At higher pH values, chitosan D-glucosamine and oligomers migrated toward the anode, even though they did not carry any electric charge. The contribution of the difference in the dielectric constants between the solvent and the solute to this phenomenon was highlighted. It was shown that the glucose moiety contributes to the direction of migration of the chitosan D-glucosamine and oligomers under alkaline conditions and that the difference in the dielectric constant of glucose and the solvent accounts for the direction and the extent of electromobility.

**KEYWORDS: Electrophoretic mobility; chitosan; D-glucosamine; oligomers; dielectric constant**

## 1. INTRODUCTION

Since the early 1990s, glucosamine has been widely promoted as an active molecule for the treatment of osteoarthritis and subjected to placebo-controlled studies. Glucosamine is a bioactive amino sugar that is present in all human tissues and is thought to promote the formation and repair of cartilage and has been shown to reduce the progression of diseases such as osteoarthritis and significantly lessen pain from arthritis (*1*−*5*). This substance is the principal compound of the glucosaminoglycans that form the matrix of the connective tissues. Glucosamine could be combined with other glucosaminoglycans, since it helps to maintain the viscosity in the articulation and stimulates cartilage recovery (*6*). Glucosamine and chitosan oligomers of low molecular weight (with degree of polymerization up to 7) were shown to be absorbed easily into the human intestine (*7*) because of their low molecular weight. Their use as dietary supplement in human food has become quite common, and for this reason, the study of the various characteristics of these biomolecules is of great interest (*8*).

There are two principal methods for the production of chitosan oligomers: acid and enzymatic hydrolysis (*9*, *10*). Acid hydrolysis is nonspecific and leads to the formation of chitosan D-glucosamine and oligomers with a low degree of polymerization. They are generally monomers and oligomers with a low degree of polymerization, as well as polymers of high molecular weights. On the other hand, the enzymatic hydrolysis of the chitosan by an enzyme such as a chitosanase makes it possible to produce oligomers of desirable range of polymerization, and the product of the hydrolysis is a mixture of oligomers of a narrow range of molecular weights (*11*−*13*). Considering the interest for chitosan oligomers of specific molecular weights for food, nutraceutical, and biopharmaceutical industries, effective separation technologies for their production are needed. For this purpose, chromatographic techniques are generally required, but they are expensive. Alternative techniques such as preparative electrophoresis and membrane filtration are at the present time being studied for the production of bioactive

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molecules (*14*, *15*). Hence, membrane techniques could be used on a large scale to selectively separate these bioactive ingredients from complex solutions by exploiting the interactions between the compounds and the membrane surface as demonstrated for peptides (*16*) and for glycine solutions using electrodialysis (*17*). Also, the separation of the mixture of chitosan oligomers can be carried out by exploiting the difference between their electrophoretic mobility. This can be carried out by adjusting the pH and/or the ionic strength of the solutions and the operating conditions of the separation system, which allows separation of small compounds with a high selectivity. An effective separation of chitosan oligomers by electrophoretic techniques requires a good understanding of their behavior under electric field (*15*−*18*). Thus, it is necessary to study the electrophoretic mobility of each oligomer in electrolytic solutions at different pH values and ionic strengths (*19*, *20*). The charged functional groups ($NH_3^+$) in their structures (at some pH values below p$K_a$) would theoretically enable their separation (*21*) by exploiting their potential differential electrophoretic mobilities.

In this context, the goal of this study was to investigate the electrophoretic mobility of chitosan D-glucosamine and oligomers with degree of polymerization from 1 to 6 subjected to an electric field. We report here the effects of pH (2.0−9.0), type of salt (NaCl and KCl), and ionic strength (0.0−0.1 M) on the electromigration of chitosan D-glucosamine and oligomers.

## 2. FUNDAMENTAL RELATIONS IN ELECTROMIGRATION

A charged molecule having an effective electric charge, *Z*, placed in an electric field, *E*, is subjected to an electrical force, *F* (*21*):

$$F = ZE \tag{1}$$

The molecule is subjected to acceleration but will not accelerate indefinitely because of the retardation the molecule experiences due to viscous forces that oppose the acceleration until a constant velocity, $\upsilon$, is reached. If the molecule is a sphere of radius *r*, then the frictional force opposing its motion is given by Stokes law (*22*)

$$F = 6\pi r\upsilon\eta \tag{2}$$

where $\eta$ is the viscosity of the medium.

Balancing the electrical force acting on the charged molecule and the resistance force opposing the motion, we obtain (*23*)

$$\upsilon = \mu E \tag{3}$$

From eqs 1−3, the electrophoretic mobility ($\mu$) can be written as

$$\mu = \frac{Z}{6\pi\eta r} \tag{4}$$

where $\mu$ is the electrophoretic mobility of the charged molecule defined as the distance traveled by the charged molecule per unit time under unit electric field. From eq 4, it is evident that the electrophoretic mobility is proportional to its charge and inversely proportional to its size. The mobility is also affected by solvent medium characteristics such as viscosity and the presence of electrolytes, due to an ionic atmosphere surrounding the charged molecule (*24*), and temperature of the medium (*25*−*27*).

## 3. EXPERIMENTAL SECTION

**3.1. Chemicals.** All chemicals were analytical grade. Sodium hydroxide (NaOH), sodium chloride (NaCl), and potassium chloride were purchased from EMD Chemicals Inc. (Darmstadt, Germany). Potassium hydroxide (KOH) was purchased from Laboratoire Mat Inc. (Montreal, Canada). Chitosan D-glucosamine and purified chitosan oligomers (dimer, trimer, tetramer, pentamer, and hexamer) were from Seikagaku Corp. (code number 800105 and purity not less than 98%). A mixture of oligomers composed of dimers, trimers, and tetramers was obtained from ISM Biopolymer, Inc. (Granby, Canada). The water used in all the experiments was of HPLC grade. Ethanol (95%) was purchased from Commercial Alcohols, Inc. (Brampton, Canada).

**3.2. Electrophoretic Mobility Measurements.** The electrophoretic mobility of chitosan d-glucosamine and oligomers was determined using a Zetasizer 2000 system (Malvern Instruments Ltd., Worcestershire, UK) equipped with a Photon Correlation Spectroscopy (PCS) system. The voltage applied to the driving electrodes of the capillary electrophoresis cell was 80 V. The calibration of the Zetasizer 2000 was made using a standard (DTS5050, Malvern instruments) with $\zeta$-potential of $-50 \pm 5$ mV at 25 °C. Before each measurement, the cell of the Zetasizer 2000 was rinsed with HPLC-grade water and dried with an air blast. The measurement temperature was maintained constant at 25 °C. The pH of the solutions was measured and adjusted with a pH meter (SP20 SympHony, VWR). Syringes (2.5 mL) were used for the injection of the chitosan D-glucosamine and oligomers solutions in the Zetasizer 2000.

**3.3. Protocol.** Stock solutions of each chitosan D-glucosamine and oligomers were prepared by dissolving 5 mg of D-glucosamine or oligomer in HPLC-grade water. Test solutions were prepared as follows: for each measurement, 25 $\mu$L of the stock solution of chitosan D-glucosamine and each chitosan oligomer were dispersed in 2.475 mL of the aqueous solutions of NaCl and KCl, and $H_2O$ was added to obtain a final volume of 2.5 mL. The final solution of the studied molecules that was injected into the Zetasizer 2000 had a concentration of 10 $\mu$g/mL. The different variables studied were the pH (2−9 adjusted with NaOH or KOH), the type of salt added (NaCl or KCl), and the ionic strength added (0.00, 0.01, 0.05, and 0.10 M). The degree of polymerization of the chitosan D-glucosamine, and oligomers was also considered as a variable, with values of 1−6. Each measurement was repeated three times, for a total of 576 runs.

**3.4. Statistical Analyses.** The full factorial design of the experimental plan was entirely randomized. Statistical analysis of the data was performed with SAS software (V8.0, SAS Institute Inc., Cary, NC). A 5% significance level was chosen. The ANOVA procedure was used to analyze the variance. SPSS software (TableCurve 2D V5.01 and TableCurve 3D V4.0) was used to generate the figures in two and three dimensions, respectively.

## 4. RESULTS AND DISCUSSION

**4.1. Results. Figure 1a** shows the electrophoretic mobilities of the chitosan D-glucosamine (monomer) and chitosan oligomers in water. At pH 2, the dimer showed the highest mobility, whereas there was no significant difference between the electrophoretic mobilities of the monomer, trimer, tetramer, pentamer, and hexamer ($P > 0.05$). At pH 3, the monomer showed a slightly lower mobility compared to the other molecules ($P < 0.04$). At pH 4, the electrophoretic mobility of the dimer decreases considerably and its mobility highly increased at pH 5 compared with the others oligomers ($P < 0.001$). The trimer, tetramer, pentamer, and hexamer showed identical but lower mobilities. At pH 6, all oligomers migrated toward the anode. The monomer and the dimer showed the greatest electrophoretic mobilities. The mobilities of the trimer and the pentamer were identical but lower than those of the monomer and the dimer. At this pH, the tetramer and hexamer were quasimotionless. At pH 7, the monomer showed a greater mobility. At pH 8, the dimer was always the most mobile compared to the other oligomers, and the hexamer was quasimotionless. At pH 9, all molecules showed a migration toward the anode except the hexamer, which was motionless. The monomer and the dimer were the most mobile.

The electrophoretic mobilities of chitosan D-glucosamine and oligomers were also measured in both NaCl and KCl aqueous

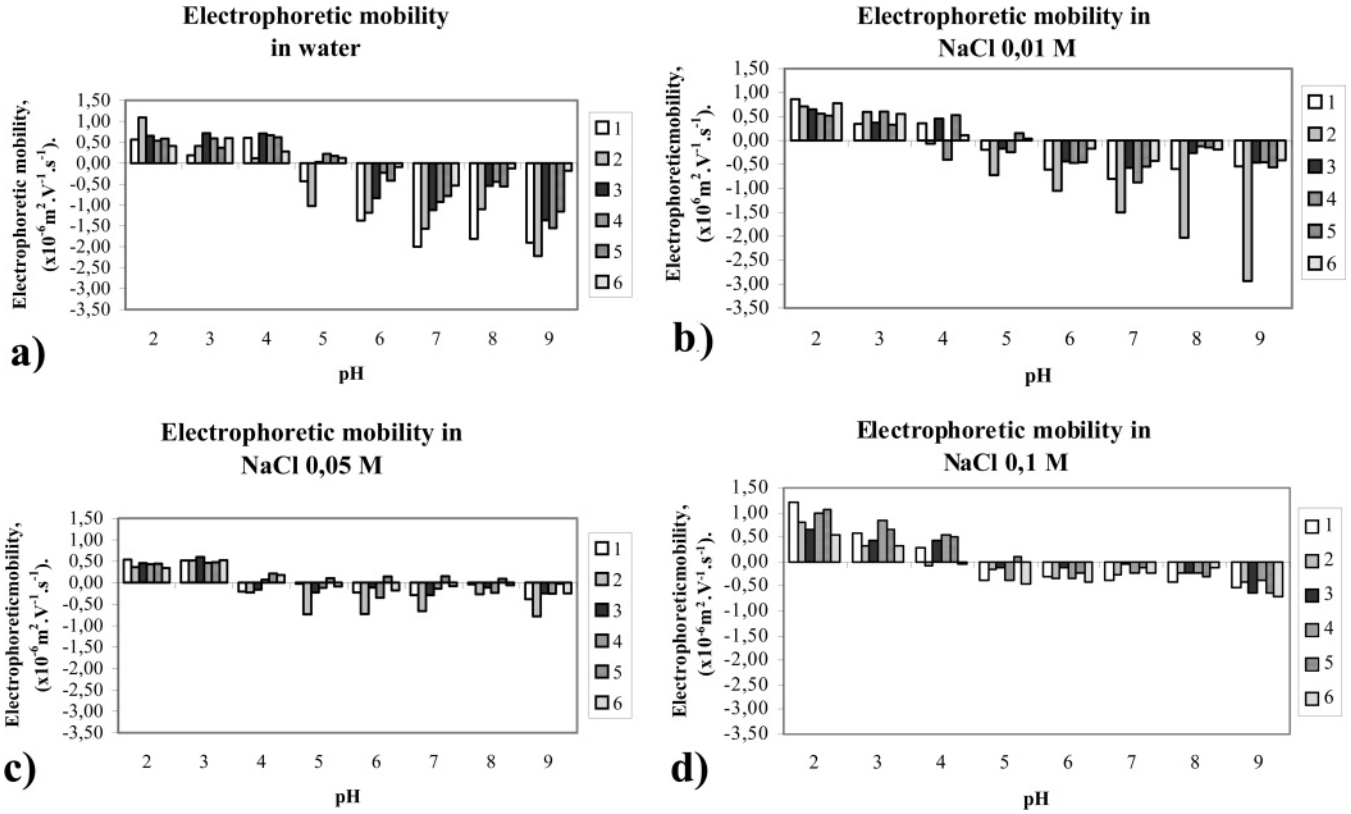


**Figure 1.** Electromigration behavior of chitosan D-glucosamine and oligomers in water (**a**) and aqueous solutions of 0.01 M NaCl (**b**), 0.05 M NaCl (**c**), and 0.1 M NaCl (**d**): 1, monomer; 2, dimer; 3, trimer; 4, tetramer; 5, pentamer; 6, hexamer.

solutions at different ionic strength. Since there was no significant difference between the types of salt, whatever the ionic strength, only results obtained with NaCl are reported here. **Figure 1b** shows the electromigration behavior of the chitosan D-glucosamine and oligomers in aqueous solution of 0.01 M NaCl. At pH 2 and 3, all molecules showed identical electrophoretic mobilities ($P > 0.05$). At pH 4, the monomer, trimer, and pentamer migrated toward the cathode without significant difference between their mobilities, while the dimer and hexamer did not show any mobility. The tetramer migrated toward the anode with higher mobility than the other oligomers ($P < 0.001$). At pH 5, only the dimer migrated toward the anode, and at pH 6, the dimer showed a higher mobility than the hexamer ($P < 0.005$). At pH 7, the dimer showed the highest electrophoretic mobility. By increasing the pH up to 9, the same phenomenon was observed. All the molecules migrated toward the anode and the dimer was the most mobile. The other chitosan oligomers migrated with identical electrophoretic mobilities ($P > 0.173$). **Figure 1c** shows the electromigration of the chitosan D-glucosamine and oligomers in aqueous solution of 0.05 M NaCl. At pH 2 and 3, D-glucosamine and all oligomers migrated with identical electrophoretic mobilities ($P > 0.778$). At pH 4, the monomer, dimer, and trimer migrated toward the anode without any difference between them. At this pH, the electrophoretic mobilities of these molecules were significantly reduced. The tetramer, pentamer, and hexamer migrated toward the cathode with identical but lower mobilities. At pH 5, the monomer, tetramer, and pentamer were motionless while the dimer and trimer migrated toward the anode. At pH 6, the dimer showed the highest mobility. At pH 7, the pentamer showed a cationic behavior, whereas the other molecules migrated toward the anode. At this pH, the dimer was the most mobile. At pH 8, chitosan D-glucosamine and all the chitosan oligomers did not show any mobility, and at pH 9, the dimer was more mobile

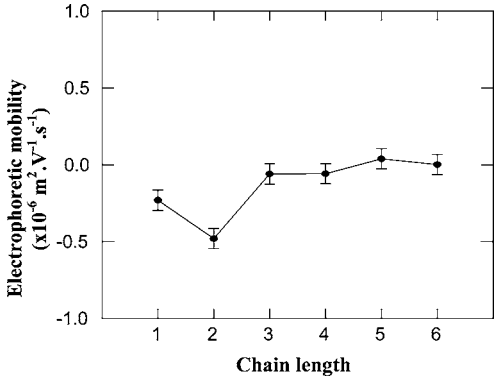


**Figure 2.** Effect of chain length on the chitosan D-glucosamine and oligomers electrophoretic mobility.

and only the monomer showed mobility somewhat closer to that of the dimer. A significant decrease of the mobilities was recorded for all oligomers. In aqueous solution of 0.1 M NaCl (**Figure 1d**), all the studied molecules showed identical mobilities at pH 2 and 3, whereas at pH 4, the dimer was motionless. At this same pH, the monomer, trimer, tetramer, and pentamer showed a cationic behavior with higher mobilities compared with that of the dimer. With increasing pH (5−9), chitosan D-glucosamine and all the chitosan oligomers showed identical electrophoretic mobilities.

The chitosan oligomers' chain length had an effect on electromigration ($P < 0.013$). In general, by increasing the degree of polymerization, the mobility decreased. The mobility of the monomer was different from that of the dimer, and their mobilities were significantly different from those of the other oligomers. Data analysis by the least squares means (**Figure 2**) showed that there was no difference between the mobilities of the trimer, tetramer, pentamer, and hexamer. This means that

the chain length had no impact on electromobility of the chitosan oligomers above the degree of polymerization of 3. Since the charge density is equal for all the oligomers, it is plausible that the mobility decreases with an increase in the molecule size. However, the dimer showed the greatest mobility.

**4.2. Discussion.** The data did not show any difference between the effect of NaCl and KCl, presumably because the mean effective ionic diameter of $Na^+$ and $K^+$ are about the same (*28*). In contrast to the type of salt, the ionic strength of the running medium affected the migration of the solutes. The electrophoretic mobility decreased as the ionic strength of the medium was increased. As the ionic strength of the medium is increased, the number of counterions around the migrating molecules increases. In the presence of an external electric field, migrating molecules move in one direction and the counterion atmosphere moves in the opposite direction, each carrying solvent molecules along with them. As result, the migration of the molecule is retarded by the screening effect of the counterions. This phenomenon was stronger when the chitosan oligomers migrated toward the anode with $Na^+$ and $K^+$ as counterions, which are hydrated, but not with $Cl^-$ counterions, which are not hydrated when the electromigration was toward the cathode. The highest values of the electrophoretic mobilities of the chitosan D-glucosamine and oligomers were obtained in water because the screening effect of the counterions was lowest, followed by those recorded in aqueous solutions with an ionic strength of 0.01 M. The lowest values of the electrophoretic mobility were recorded in the aqueous solutions of salt with ionic strengths of 0.05 and 0.1 M, respectively, because the counterion screening effect is expected to be stronger under these conditions. This is in good agreement with the literature data (*20*, *29*).

The electrophoretic mobility experiments on the chitosan D-glucosamine and oligomers revealed that the pH had a significant effect on the behavior of the molecules when they are subjected to an external electric field. The pH determines the charge of the molecule, and consequently, the direction of migration will be toward the cathode when the molecule carries positive charge and the migration will be toward the anode if the molecule carries negative charge. Generally, the absolute electrophoretic mobility of a charged particle submitted to an external electric field is directly proportional to the charge/mass ratio (eq 4). With increasing medium pH, the electrophoretic mobility of the oligomers decreased and passed through zero value, which corresponds to the isoelectric point of each molecule in that medium. Chitosan D-glucosamine and 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 D-glucosamine and oligomers migrated toward the anode at some pH values near the p$K_a$ of glucosamine (pH <7), where the amine groups were always protonated, and at pH values greater than the glucosamine p$K_a$ value, where the amine groups are unprotonated and the chitosan D-glucosamine and oligomers did not carry any electric charge.

It was hypothesized initially that the anionic character acquired by the chitosan D-glucosamine and oligomers may originate from the glucose moiety. To confirm that the glucose moiety contributes to the electromigration of chitosan D-glucosamine and oligomers, electrophoretic measurements were carried out in various aqueous media (**Figure 3**). Measurements of the electrophoretic mobility of the glucose were carried out under the same conditions as previously for chitosan D-glucosamine and oligomers. Glucose showed a cationic behavior at pH 2 and 3 in water (**Figure 3**), with higher mobility at pH 2. At pH 4, glucose did not show any mobility. Above pH 4, this molecule migrated toward the anode. By adding salt to the medium, glucose showed migration toward the cathode only at pH 2 and 3. As in water, with increasing pH, glucose migrated toward the anode. Also by increasing the ionic strength of the medium, the absolute value of the electrophoretic mobility of the glucose decreased considerably. This phenomenon was greater at the alkaline pH values.

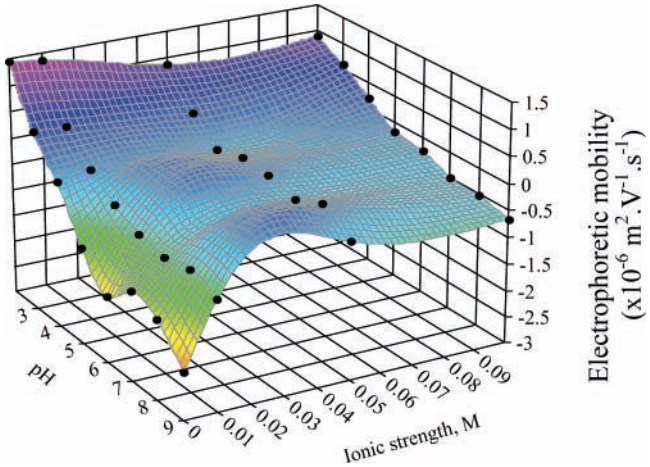


**Figure 3.** Electrophoretic behavior of D-glucose in water and aqueous solution of NaCl as function of pH and ionic strength.

**Table 1.** Electrophoretic Mobility of Glucose, Glucosamine, and a Mixture of Chitosan Oligomers (dimer, trimer, and tetramer) in Different Media at pH 7

| medium | analyte | $\mu$ ($10^{-6}$ m$^2$ V$^{-1}$ s$^{-1}$) |
|---|---|---|
| water | glucose | –1.340 |
| water/ethanol (50%, v/v) | glucose | 0.001 |
| 0.01 M NaCl | glucose | –0.830 |
| 0.01 M NaCl + ethanol (50%, v/v) | glucose | –0.002 |
| 0.01 M NaCl | monomer | –0.800 |
| 0.01 M NaCl degassed with $N_2$ | monomer | –0.610 |
| 0.01 M sodium phosphate buffer | monomer | –0.888 |
| 0.01 M NaCl + ethanol (50%, v/v) | monomer | 0.002 |
| 0.01 M NaCl | mixture | –0.868 |
| sodium phosphate buffer | mixture | –0.400 |
| degassed sodium phosphate buffer | mixture | –0.436 |
| 0.01 M NaCl + ethanol (50%, v/v) | mixture | 0.003 |

While the results confirmed that a glucose moiety may contribute to the electromigration of chitosan D-glucosamine and oligomers toward the cathode under lower pH conditions (since glucose can form oxonium ion at low pH conditions) and toward the anode under higher pH conditions, it was not clear regarding the origin of its migration toward the anode. It was further hypothesized that glucose migration toward the anode may arise from the difference between the dielectric constant of the medium and the migrating molecule. To verify this possibility, the electromigration of glucose, D-glucosamine (monomer), and a mixture of chitosan oligomers composed of dimers, trimers, and tetramer (1:1:1) was determined in water without salt added and in solutions of 0.01 M NaCl and 0.01 M NaCl/ethanol in the ratio of 50:50 (v/v) at pH 7.0. **Table 1** shows the results obtained for these analyses. Without ethanol addition, the monomer, glucose, and chitosan oligomers mixture migrated toward the anode at pH 7 (**Table 1**). But the addition of 50% (v/v) of ethanol to the medium eliminated its migration toward the anode. Under these conditions, monomer (glucosamine), glucose, and oligomers were motionless.

The addition of ethanol to the medium, whose dielectric constant is lower than that of water (*30*, *31*) caused disappearance of electromigration toward the anode at pH 7.0. Since the addition of ethanol decreases the dielectric constant of the medium, the difference between the dielectric constant of the solvent and the solute is lowered. Molecules with permanent dipole moments are oriented under the effect of external electric field and can have induced dipoles. The oriented highly polar solvent molecules may exert an electrophoretic effect on less polar solute molecules, leading to their electromigration. Ethanol addition lowers the dielectric constant of the solvent medium and diminishes the electromigration of glucose and chitosan oligomers.

At low pH values, while the amine functions of the oligomers are protonated, electrophoretic mobilities of these molecules were not significantly different. This would be probably due to the charge/mass ratio, which is the same one for all the oligomers (eq 4). At higher pH values, the dimer showed the greatest electrophoretic mobility in water and in electrolytic solution with an ionic strength of 0.01 M. The higher oligomers showed comparable mobility. Under low potential field, convective diffusion can occur and contribute to overall mobility of the smaller molecules. This is much apparent in alkaline medium where the amine function was uncharged. This is probably due to the fact that the hydroxyl ions of the medium are able to reach the dimer more easily than the other oligomers, and consequently, the ionic atmosphere around the dimer is greater in comparison with the others oligomers.

In conclusion, the results of this study on the electrophoretic mobility of the chitosan D-glucosamine and oligomers in dilute aqueous media showed that pH, ionic strength, chain length, and dielectric constant of the medium have a significant influence on this property. We have shown that the pH played a principal role in the migration of the chitosan D-glucosamine and oligomers. The ionic strength of the medium influenced the absolute value of the electrophoretic mobility of the chitosan D-glucosamine and oligomers. There was no difference between the effects of NaCl and KCl on the electromigration behavior of the chitosan D-glucosamine and oligomers in aqueous solutions. Monomer and dimer displayed the higher mobilities in general, but the dimer was the more mobile one. The mobility was not affected by chain length beyond the degree of polymerization of 3. The glucose moiety contributes to the electromigration behavior of the uncharged chitosan D-glucosamine and oligomers in dilute alkaline medium because of the difference between the dielectric constants of the solvent (water) and the glucose.

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