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Mutual diffusion of sodium hyaluronate in aqueous solutions

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Abstract

The Taylor dispersion technique has been used for measuring mutual diffusion coefficients of sodium hyaluronate in aqueous solutions at $T = 298.15$ K, and concentrations ranging from $(0.00$ to $0.50)$ g·dm$^{-3}$. The results are interpreted on the basis of Nernst, and Onsager and Fuoss theoretical equations. From the diffusion coefficient at infinitesimal concentration, the limiting ionic conductivity and the tracer diffusion coefficient of hyaluronate ion were estimated. These studies have been complemented by molecular mechanics calculations.

Keywords: Diffusion coefficient; Sodium Hyaluronate Electrolytes; Solutions; Taylor Dispersion; Transport Properties.

1. Introduction
Hyaluronic acid sodium salt (also called hyaluronan or sodium hyaluronate) [1-8] (Na-HA), discovered by Meyer and Palm [1], is a linear polysaccharide \((C_{14}H_{21}NaNO_1)_n\) consisting of a disaccharide repeating sequence. The two saccharide-residues are D-glucuronic acid and N-acetyl-D-glucosamine, which are linked by \(\beta-1,4\) and \(\beta-1,3\) glycosidic bonds with each other (figure 1).

Na-HA is the major macromolecular component of the intercellular matrix of most connective tissues, such as cartilage, eye vitreous humour, and synovial fluid. Playing an important role in the regulation of the transport of fluids and solute in the intercellular processes (e.g. [2]), it is one of the most hygroscopic molecules in nature and when hydrated, it can contain up to 1,000-fold more water than its own weight. This effect is particularly important in the skin for its moisturizing ability which contributes to its application in anti-ageing products. In addition, it is known by its prominent viscoelastic properties, acting as a lubricant and shock absorber in synovial fluid (e.g. [2,9]), as well as by its biocompatibility, biodegradability and non-immunogenicity properties, which allows that polysaccharide to be relevant in pharmaceutical and medical applications [10-12]. However, the understanding of these complex systems has not yet been well established, and consequently, their characterization is very important, helping us to understand their structure and to model them to practical applications, such as pharmaceutical and medicinal applications, as well as cosmetics applications [13,14]). However, few have taken into account their transport behaviour (e.g. [4-6]). Transport properties, particularly mutual diffusion coefficients (also called inter-diffusion), involving coupled fluxes of solutes and solvent molecules driven by concentration gradients, provide a direct measure of the molecular mobility, an important factor in the preservation of biological materials in sugar matrices. We studied the mutual diffusion behaviour of these systems, at therapeutic dosage. As far as the authors know, after careful literature search, no data of mutual diffusion coefficients are available in the literature for aqueous systems containing this polysaccharide.

In the present work, the interdiffusion coefficients of sodium hyaluronate in aqueous dilute solutions at therapeutic dosage, that is, from \((0.00 \text{ to } 0.50) \text{ g dm}^{-3}\) at \(T = 298.15\ K\), were measured using the Taylor technique in aqueous solutions. This technique [15] is based on the dispersion of small amounts of solutes injected into carrier solutions flowing through a capillary tube. The combined action of radial diffusion and convection along the tube axis cause the injected solute samples to spread out,
producing Gaussian concentration profiles. Mutual diffusion coefficients are calculated from refractive-index profiles measured across the dispersed solute peaks at the outlet of the dispersion tube.

The thermodynamic factor values, $F_T$, (attributed to the non-ideality in thermodynamic behaviour) and, the mobility factor, $F_M$, as well as, the equivalent conductance at infinitesimal concentration of the hyaluronate ion and the tracer diffusion coefficient are computed according to Nernst and Onsager-Fuoss equations [15-18]. However, having in mind that in these equations phenomena, such as association between two monomers and/or counter-ion condensation [19, 20] and hydrolysis are not taken into consideration, those values are only estimations. Despite of their limitations, the molecular mechanics studies here presented permitted us to obtain some additional information concerning the probable interactions in this system containing sodium hyaluronate and helped in obtaining a better understanding of the diffusion in these systems.

In conclusion, we intend to contribute not only to a deeper understanding of the fundamental diffusion properties of these solutions, but also to a better understanding of the factors governing the formation of these structures.

2. Experimental

2.1 Materials

Hyaluronic acid sodium salt, from *Streptococcus equi* (table 1) was used as received. The solutions for the diffusion measurements were prepared in calibrated volumetric flasks using bi-distilled water and were freshly prepared and de-aerated, by using a Sonorex RK106 ultrasonic bath, for about 30 minutes before each set of runs.

2.2 Mutual diffusion coefficients, $D$, measurements

The theory of the Taylor dispersion technique is well described in the literature [21-28] and consequently the authors only point out some relevant points concerning such method on the experimental determination of binary diffusion coefficients and ternary diffusion coefficients, respectively.
Dispersion methods for diffusion measurements are based on the dispersion of small amounts of solution injected into laminar carrier streams of solvent or solution of different composition, flowing through a long capillary tube. The length of the Teflon dispersion tube used in the present study was measured directly by stretching the tube in a large hall and using two high quality theodolites and appropriate mirrors to accurately focus on the tube ends. This technique gave a tube length of \((3.2799 \pm 0.0001) \times 10^3\) cm, in agreement with less-precise check measurements using a good-quality measuring tape. The radius of the tube, \((0.05570 \pm 0.00003)\) cm, was calculated from the tube volume obtained by accurately weighing (resolution 0.1 mg) the tube when empty and when filled with distilled water of known density.

At the start of each run, a 6-port Teflon injection valve (Rheodyne, model 5020) was used to introduce 0.063 cm\(^3\) of solution into the laminar carrier stream of slightly different composition. A flow rate of 0.17 cm\(^3\)·min\(^{-1}\) was maintained by a metering pump (Gilson model Minipuls 3) to give retention times of about \(8 \times 10^3\) s. The dispersion tube and the injection valve were kept at \(T = 298.15\) K and \(T = (303.15 \pm 0.01)\) K in an air thermostat.

Dispersion of the injected samples was monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, \(V(t)\), were measured at accurately timed 5 s intervals with a digital voltmeter (Agilent 34401 A) with an IEEE interface. Binary diffusion coefficients were evaluated by fitting the dispersion equation

\[
V(t) = V_0 + V_1 t + V_{\text{max}} (t_R/t)^{1/2} \exp[-12D(t - t_R)^2/r^2t]
\]

(1)

to the detector voltages. The additional fitting parameters were the mean sample retention time \(t_R\), peak height \(V_{\text{max}}\), baseline voltage \(V_0\), and baseline slope \(V_1\).

Measurements of \(pH\) were carried out with a Radiometer pH meter PHM 240 with an Ingold U457-K7pH conjugated electrode; \(pH\) was measured in fresh solutions and the electrode was calibrated immediately before each experimental set of solutions using IUPAC-recommended 2 and 4 \(pH\) buffers. From the \(pH\) meter calibration, a zero-

\(pH\) of \((5.080 \pm 0.030)\) and sensitivity higher than 98.7 % were obtained.
2.3 Molecular mechanics studies

Energy minimization was obtained in Hyperchem 8 (Hypercube, Inc.; USA) using the molecular mechanics MM+ force field, under a conjugated gradient (Polack-Ribiere) with a final RMS gradient of 0.1 kcal/mol, in vacuum and in a cage of water molecules. The calculations were performed in a HP-Z620 workstation under Windows 7 (Microsoft, Inc.; USA).

3. Results and discussion
3.1 Measurements of diffusion coefficients

3.1.1 Concentration dependence of mutual diffusion coefficient, $D$, at infinitesimal and finite concentrations

Table 2 gives the average $D$ value at infinitesimal concentration for each injection solution determined from 4 to 5 profiles generated by different injecting samples in water. $D^0$ is obtained by extrapolated values obtained from the $D$ least-squares for total number of injections (that is, $D^0 = 1.184 \times 10^{-9}$ m$^2 \cdot$s$^{-1}$).

Tables 3 and 4 give the average $D$ value for finite concentrations at two carrier solutions (0.25 g cm$^{-3}$ and 0.5 g cm$^{-3}$), determined from 4 to 5 profiles generated by injecting samples in those solutions (i.e., $D = 0.717 \times 10^{-9}$ m$^2 \cdot$s$^{-1}$ and $D = 0.600 \times 10^{-9}$ m$^2 \cdot$s$^{-1}$, respectively). Table 5 show all results, including the $D$ value obtained for 0.3 g cm$^{-3}$, but determined from 4 profiles generated by different injecting samples more or less concentrated than the carrier solution. Good reproducibility was, in general, observed, within ±2 %.

The concentration dependence of the measured diffusion coefficients can be represented by the polynomial equation,

$$D/(10^{-9} \text{ m}^2 \cdot \text{s}^{-1}) = 1.174 \cdot 0.845 \cdot c^{1/2} \quad (R^2 = 0.992)$$  (2)
permitting us to calculate values of diffusion coefficients at specified concentrations within the range of the experimental results shown in the table 4. The goodness of the fit (obtained with a confidence interval of 98 %) can be assessed by the excellent correlation coefficients, \( R^2 \) and the low percentage of standard deviation (< 1 %).

The pH measurements were made on some of the sodium hyaluronate in aqueous solutions to assist interpretation of these results for \( c = 4 \text{g·dm}^{-3} \) and \( T = 298.15 \text{K} \), the pH value was 6.33.

The interpretation of the diffusion behaviour of this aqueous system (sodium hyaluronate) can be made on the basis of the Onsager-Fuoss model (equation 3), suggesting that \( D \) is a product of both kinetic, \( F_M \) (or molar mobility coefficient of a diffusing substance) and thermodynamic factors, \( F_T \) (\( F_T = c \partial \mu / \partial c \)), where \( \mu \) represents the chemical potential of the solute. Thus, two different effects can control the diffusion process: the ionic mobility and the gradient of the Gibbs energy,

\[
D = F_M \times F_T \quad (3)
\]

where

\[
F_M = \frac{1}{M} \left( \frac{|z_1 + z_a|}{z_c z_a} \right) \frac{RT}{c} = (D^0 + \Delta_t) \quad (4)
\]

and

\[
F_T = \left( 1 + c \frac{\partial \ln y_\pm}{\partial c} \right) \quad (5)
\]

\( y_\pm \) represents the thermodynamic activity coefficient of the solute, \( D \) is the mutual diffusion coefficient of the electrolyte in \( \text{m}^2 \text{s}^{-1} \), \( R \) is the gas constant in \( \text{J·K}^{-1}·\text{mol}^{-1} \), \( T \) is the absolute temperature, \( z_1 \) and \( z_a \) are the algebraic valences of a cation and of an anion,
respectively, and the last term in parenthesis is the activity factor, with $y_\pm$ being the mean molar activity coefficient, $c$ the concentration in mol·m$^{-3}$, and $\bar{M}$, in mol$^2$·s·m$^{-3}$·kg$^{-1}$, given by

$$\bar{M} = \frac{1}{N_A^2 \varepsilon_0^2} \left(\frac{\lambda^0_c \lambda^0_a}{\nu_a z_a + z_c \nu_c} \right) \left( c + \Delta M' \right) \quad (6)$$

In equation 6, the first-order electrophoretic term, is given by

$$\Delta M' = -c \frac{\left( z_a \lambda^0_c - z_c \lambda^0_a \right)^2 \nu_c \nu_a}{N_A \left( z_c \nu_c \lambda^0_c + z_a \nu_a \lambda^0_a \right)^2} \frac{k}{6 \pi \eta_0 (1+ka)} \quad (7)$$

where $\eta_0$ is the viscosity of the water in N·s·m$^{-2}$, $N_A$ is the Avogadro’s constant, $e_0$ is the proton charge in coulombs, $\nu_c$ and $\nu_a$ are the stoichiometric coefficients, $\lambda^0_c$ and $\lambda^0_a$ are the limiting molar conductivities of the cation and anion, respectively, in m$^2$·mol$^{-1}$·Ω$^{-1}$, $k$ is the “reciprocal average radius of ionic atmosphere” in m$^{-1}$ (see e.g., Harned & Owen, 1964 [16]), $a$ is the mean distance of closest approach of ions in m, ($a = 5.1 \times 10^{-10}$ m).

The values of the $\Delta_1$, indicated in table 5, are very small and, consequently, $F_M$ is almost constant for the concentration range. In fact, the values of the $\Delta_1$ for the studied interval of concentrations contribute only around 0.1 % to the decreasing of $D^0$.

From our measurements of diffusion coefficients, $D$, and considering equation (3), we have estimated the thermodynamic factor values within the interval of concentrations studied (table 5). The decrease of the diffusion coefficients, $D$, and also, of the gradient of the Gibbs energy with concentration, $F_T$, leads us to conclude that this behaviour of the sodium hyaluronate in aqueous solutions at $T = 298.15$ K appear to be affected by the presence of aggregated species (fact that is confirmed by molecular mechanics calculations), having a lower mobility than hyaluronate monomers due to
their size. Considering our experimental conditions (i.e., dilute solutions), and consequently, assuming that the parameters such as viscosity, dielectric constant, hydration and association or complexation, (factors not taken into account in this model) do not change with concentration, we can conclude that the variation in $D$ is due mainly to the variation of $F_T$ (attributed to the non-ideality in thermodynamic behaviour), and, secondary, to the electrophoretic effect in the mobility factor, $F_M$ (table 5).

### 3.1.2 Estimation of tracer diffusion coefficient of the hyaluronate ion

By using the Nernst-Hartley equation \[15, 18\]

$$D^0 = \frac{RT \left| Z_c \right| + \left| Z_a \right|}{F^2 \left| Z_c \times Z_a \right|} \frac{\lambda_c^0 \lambda_a^0}{\lambda_c^0 + \lambda_a^0}$$

where $D^0$ value is the diffusion coefficient for the sodium hyaluronate at infinitesimal concentration (table 2), $Z_c$ and $Z_a$, and $\lambda_c^0$ and $\lambda_a^0$ represent the algebraic valences and the equivalent conductance at infinitesimal concentration of Na$^+$ and HA, respectively, and taking the limiting ionic conductivity of sodium ion as equal to 50.10 x 10$^{-4}$ S·m$^2$·mol$^{-1}$ \[29\], we estimated $\lambda_a^0$, being equal to 40.05 x 10$^{-4}$ S·m$^2$·mol$^{-1}$.

From this value for limiting ionic conductivity of the hyaluronate ion, the corresponding limiting tracer diffusion coefficient, $D_T^0$, can be estimated through the Nernst equation (equation 9)

$$\lambda_a^0 = \frac{D_T^0 Z_a F^2}{RT}$$

giving a value of 1.066 ×10$^{-9}$ m$^2$·s$^{-1}$.

Taking into account that the $\lambda_a^0$ represents the limiting equivalent conductance of the hyaluronate anions in unit of sodium hyaluronate, and not the limiting ionic conductivity.
conductance of polymeric HA-anions, one would expect us to achieve a higher value for the tracer diffusion coefficient, $D_T^0$, compared to what would be predicted.

Our calculation shows that the mutual diffusion coefficient of this system at infinitesimal concentration is significantly larger (1.1 times) than that the corresponding tracer diffusion coefficient. This increase characterises the electrostatic dragging effect of sodium ions on hyaluronate ions.

3.2. Molecular mechanics in vacuum and in water

Our computational studies were designed to evaluate two experimental situations. The first one was the mobility of the Na$^+$ cation over the polymer backbone. The second was the probability of aggregation between two independent polymer units.

To do the evaluation of the mobility of the Na$^+$ cation we run several calculations of geometry optimization based in energy minimization. The first interesting observation was that the Na$^+$ ion placed near de -COO$^-$ group in the starting basis, after geometry optimization based in energy minimization shifts quickly to the region between the -COO$^-$ group and the nearby hydroxyls 15 and 12. That shows the Na$^+$ cations have a noticeable tendency to coordinate with the hydroxyls present along the polymer backbone and therefore have a reasonable mobility around the polymer. Similar results were obtained in a cage of 500 water molecules. That, is some way, can be interpreted in terms of the ion condensation over polyelectrolytes described by the Manning theory, despite more detailed calculations need to be made to support these preliminary findings.

To evaluate the probability of the aggregation between two independent polymer units we performed several geometry optimization calculations by docking two polymer repeating units (PRU) side by side, both head-to-head and head-to-tail. All calculations gave similar results in terms of energy of the systems. Also the calculations in vacuum or in a cage of 1700 water molecules gave similar results in terms of the geometry, pointing for solvent independent interactions. The calculations were made assuming a pH of 7, which discards the possibility of amide hydrolysis but allows for the existence of zwitterions, resulting from the intramolecular protonation of the amide by the carboxylic acid. Using MM+ geometry minimization, all those chemical species were evaluate for global energy variations and geometry similarities and all showed very
similar results in terms of energy and geometry, both in vacuum and in water solutions, with deviation of less than 5% between them. But despite the energetics of those systems being similar the docking process showed several close contacts between the paired PRUs that may be responsible for association processes. In the case of the paired zwitterionic species this close contacts reached very short values, near $3 \times 10^{-10}$ m, and may be responsible for the aggregation process observed up on higher concentration of hyaluronic acid sodium salt experiments. That distance is even shorter than the distance between the carboxylic acid and the Na$^+$ cation ($5.1 \times 10^{-10}$ m), pointing indeed to a relevant electrostatic interaction between the polymeric chains, that may induce a considerable aggregation between hyaluronic acid chains.

As can be seen in figure 2 the calculated isopotential surface generated for two PRU side by side in a head-to-tail arrangement in the zwiterionic form shows several well defined positive (lighter) and negative (dark) zones that can be responsible for the observed aggregation of hyaluronic acid polymeric chains and may help in the understanding of the experimental behaviour observed.

**Conclusions**

Based on these measurements of diffusion coefficients of sodium hyaluronate in aqueous solutions, and on the molecular mechanics calculations, we conclude that the diffusion of this polysaccharide in aqueous solutions, is strongly affected by the presence of new different species resulting from various equilibria (e.g. aggregation) and, consequently, to the decreasing of the diffusion coefficients with the increasing of concentration. The effect of aggregation on the diffusion of Na-HA, confirmed by analysis of the dependence of diffusion on concentration as well as by molecular mechanics calculations is due mainly to the variation of $F_T$ (attributed to the non-ideality in thermodynamic behaviour), and, secondarily, to the electrophoretic effect in the mobility factor, $F_M$.
Diffusion coefficients measured for aqueous solutions of sodium hyaluronate provide transport data necessary to model the diffusion in pharmaceutical and engineering applications.

**Acknowledgments**

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**References**


Figure 1
The monomeric unit of hyaluronate anion [5]
Figure 2
Isopotential surface generated for two polymer repeating units, side by side in a head-to-tail arrangement in the zwitterionic form (positive (lighter) and negative (dark) zones)
Table 1. Provenance and mass fraction purity of the sample.

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Source</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid sodium salt</td>
<td>Sigma-Aldrich</td>
<td>Mass fraction purity ≥ 0.99 %</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus equi</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAS number 9067-32-7, code 53747</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mutual diffusion coefficients, $D$, of Na-HA in aqueous solutions at infinitesimal concentrations at $T = 298.15$ K and the standard deviations of the means, $S_D$

<table>
<thead>
<tr>
<th>$c$ /($\text{g} \cdot \text{dm}^{-3}$)</th>
<th>$D^{\text{a})} \pm S_D$ /($10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.000</td>
<td>0.997 $\pm$ 0.003</td>
</tr>
<tr>
<td>2.500</td>
<td>0.947 $\pm$ 0.003</td>
</tr>
<tr>
<td>3.000</td>
<td>0.887 $\pm$ 0.002</td>
</tr>
<tr>
<td>3.500</td>
<td>0.855 $\pm$ 0.004</td>
</tr>
<tr>
<td>4.000</td>
<td>0.805 $\pm$ 0.003</td>
</tr>
</tbody>
</table>

$D^{\text{b})} = 1.184 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$

$^a$ $D$ is the mean diffusion coefficient value from 4-6 experiments and $S_D$ is the standard deviation of that mean. $^b$ Extrapolated values obtained from the $D$ least-squares for total number of injections, that is, $D/10^{-9} \text{ m}^2 \cdot \text{s}^{-1} = 1.184 - 0.095c$ ($R^2 = 0.993$).
Table 3. Mutual diffusion coefficients, $D$, of Na-HA at $T = 298.15$ K in aqueous solutions at concentrations 0.25 g·dm$^{-3}$ and 0.50 g·dm$^{-3}$

<table>
<thead>
<tr>
<th>$c_{\text{inj}}$ / (g·dm$^{-3}$)$^a$</th>
<th>$D \pm S_D$$^b$ / ($10^{-9}$ m$^2$·s$^{-1}$)</th>
<th>$D \pm S_D$$^b$ / ($10^{-9}$ m$^2$·s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(c = 0.25 g·dm$^{-3}$)</td>
<td>(c = 0.50 g·dm$^{-3}$)</td>
</tr>
<tr>
<td>2.000</td>
<td>0.622 ± 0.026</td>
<td>0.523 ± 0.020</td>
</tr>
<tr>
<td>2.500</td>
<td>0.591 ± 0.015</td>
<td>0.505 ± 0.021</td>
</tr>
<tr>
<td>3.000</td>
<td>0.570 ± 0.020</td>
<td>0.487 ± 0.025</td>
</tr>
<tr>
<td>3.500</td>
<td>0.551 ± 0.025</td>
<td>0.466 ± 0.026</td>
</tr>
<tr>
<td>4.000</td>
<td>0.520 ± 0.023</td>
<td>0.447 ± 0.016</td>
</tr>
</tbody>
</table>

$D = 0.717 \times 10^{-9}$ m$^2$·s$^{-1}$$^c$ $D = 0.600 \times 10^{-9}$ m$^2$·s$^{-1}$$^d$

$^a$ $c_{\text{inj}}$ represent the concentration of the injection solutions. $^b$ $D$ is the mean diffusion coefficient value from 4-6 experiments and $S_D$ is the standard deviation of that mean. $^c$ Extrapolated values obtained from the $D$ least-squares for total number of injections, that is, $D/10^{-9}$ m$^2$·s$^{-1} = 0.717 - 0.049 c$ ($R^2 = 0.992$). $^d$ Extrapolated values obtained from the $D$ least-squares for total number of injections, that is, $D/10^{-9}$ m$^2$·s$^{-1} = 0.600 - 0.038 c$ ($R^2 = 0.999$).
Table 4. Mutual diffusion coefficients, $D$, of Na-HA at $T = 298.15$ K in aqueous solutions at infinitesimal and finite concentrations, $c$.

<table>
<thead>
<tr>
<th>$c$</th>
<th>$D$ a()</th>
<th>(\text{/(g\cdot dm}^{-3}))</th>
<th>(\text{/(10}^{-9}\text{ m}^2\cdot s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.184 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.717 *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>0.641 b*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.600 *</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* $D$ is the mean diffusion coefficient value from 4-6 experiments and $\delta_D$ is the standard deviation of that mean. \* Value obtained from the $D$ least-squares for different injecting samples more and less concentrated than the carrier solution ($\Delta c = \pm 0.8$).
Table 5
Thermodynamic coefficients of sodium hyaluronate, $F_T$, calculated from our experimental $D$ values and from equation (4) at $T = 298.15 \text{ K}$

<table>
<thead>
<tr>
<th>$c$ (mol dm$^{-3}$)</th>
<th>$D/10^{-9}$ m$^2$ s$^{-1}$</th>
<th>$(\Delta_1)/10^{-9}$ m$^2$ s$^{-1}$</th>
<th>$(\Delta_1)/10^{-9}$ m$^2$ s$^{-1}$</th>
<th>$F_M/10^{-9}$ m$^2$ s$^{-1}$</th>
<th>$F_T/10^{-9}$ m$^2$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>1.184</td>
<td>0.000</td>
<td>0.000</td>
<td>1.184</td>
<td>1.000</td>
</tr>
<tr>
<td>0.250</td>
<td>0.717</td>
<td>-1.677 x 10$^{-4}$</td>
<td>-2.105 x 10$^{-5}$</td>
<td>-1.100 x 10$^{-5}$</td>
<td>1.184</td>
</tr>
<tr>
<td>0.400</td>
<td>0.641</td>
<td>-2.980 x 10$^{-4}$</td>
<td>-6.720 x 10$^{-5}$</td>
<td>-2.125 x 10$^{-5}$</td>
<td>1.184</td>
</tr>
<tr>
<td>0.500</td>
<td>0.600</td>
<td>-2.370 x 10$^{-4}$</td>
<td>-7.512 x 10$^{-5}$</td>
<td>-2.376 x 10$^{-5}$</td>
<td>1.184</td>
</tr>
</tbody>
</table>

$a)$ $F_M = (D' + \Delta_1 + \Delta_2)$, where $(\Delta_1 + \Delta_2)$ represent the electrophoretic corrections (p. 11). These parameters are estimated by using the equations (8) to (10).

$b)$ $F_T = D_{exp}/F_M$. 
Binary diffusion coefficients for the systems containing sodium hyaluronate.
Influence of the aggregation on diffusion of the sodium hyaluronate in the aqueous media.
Estimation of the thermodynamic and mobility factors from mutual diffusion.