



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 $0.50)$ $\text{g} \cdot \text{dm}^{-3}$. 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.

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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 $(\text{C}_{14}\text{H}_{21}\text{NaNO}_{11})_n$ consisting of a disaccharide repeating sequence. The two saccharide-residues are D-glucuronic acid and N-acetyl-D-glucosamine, which are linked by β -1,4 and β -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 conse-

quently, 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 $(0.00$ to $0.50)$ $\text{g} \cdot \text{dm}^{-3}$ 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

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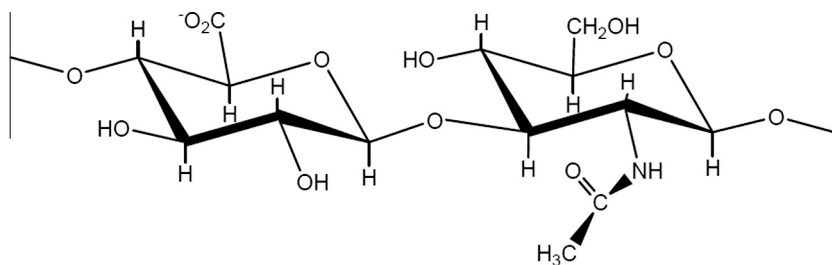


FIGURE 1. The monomeric unit of hyaluronate anion [5].

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 min 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 $(3.2799 \pm 0.0001) 10^3$ cm, in agreement with less-precise check measurements using a good-quality measuring tape. The radius of the tube, (0.05570 ± 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 $0.17 \text{ cm}^3 \cdot \text{min}^{-1}$ was maintained by a metering pump (Gilson model Minipuls 3) to give retention times of about $8 \cdot 10^3$ s. The dispersion tube and the injection valve were kept at $T = 298.15 \text{ K}$ and $T = (303.15 \pm 0.01 \text{ K})$ 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

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

to the detector voltages. The additional fitting parameters were the mean sample retention time t_R , peak height V_{\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 IU-PAC-recommended 2 and 4 pH buffers. From the pH meter calibration, a zero- pH of (5.080 ± 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

TABLE 1

Provenance and mass fraction purity of the sample.

Chemical name	Source	Purity
Hyaluronic acid sodium salt	Sigma-Aldrich <i>Streptococcus equi</i> CAS number 9067-32-7, code 53747	Mass fraction purity $\geq 0.99\%$

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