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ORIGINAL CONTRIBUTION

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Density and compressibility measurements of a highly viscous polyelectrolyte solution: a study on aqueous solutions of sodium hyaluronate

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Abstract The density of a highly viscous polyelectrolyte solution was measured using both pycnometers and oscillating-tube densimeters in parallel to elucidate the difficulties inevitably involved when an oscillating tube is employed for density measurements of viscous liquids. It was confirmed that the oscillating-tube densimeter gives too high values for viscous liquids, and the deviation increases with the increase in the viscosity of liquids. The analysis of adiabatic compressibilities of sodium hyaluronate (NaHy) solutions, as estimated from density and ultrasound velocity data of the same solution, suggests that the disaccharide unit of the NaHy chain is less hydrated than the sum of its component monosaccharide residues.

Key words Density – Pycnometry – Compressibility – Viscosity – Hydration

Introduction

The oscillating-U-tube densimeter first devised by Kratky et al. [1, 2] is widely used for its measuring speed and the small sample volume required to secure high accuracy. The accuracy, however, is limited to some extent by the proper vibrational frequency of the liquid cell which restricts the acceptable range of the viscosity of liquids [3, 4]. Bernhardt and Pauly [3] have reported that a similar systematic error specific to the liquid cell is involved for different liquids having similar viscosity. They proposed the equation to estimate the amount of error which makes the oscillating-tube densimeter applicable even to very viscous concentrated protein solutions and to give reliable density data. So, it is obvious that we have to take special precautions for density measurements by the oscillating-tube method when the test liquids show highly viscous and non-Newtonian behavior, even if the concentration is relatively low. The purpose of the present study is to emphasize the limitation of the oscillating-tube method for the precise density measurement of viscous aqueous solution of polyelectrolyte solutions. The material used for this study is an aqueous solution of sodium hyaluronate (NaHy), a linear polysaccharide whose molecular structure consists of a repeating disaccharide of sodium D-glucronate and *N*-acetyl-D-glucosamine units. It is well-known that NaHy chains having a molecular weight of several million are entangled with each other and exhibit extremely high shear-dependent viscosity above concentrations of the order of 0.1% by weight. A comparison of the oscillating-tube and pycnometer measurement methods was made for an aqueous solution of NaHy with various molecular weights. The adiabatic compressibility of the aqueous NaHy solution was calculated from the density data obtained by pycnometry together with the ultrasound velocity to consider the hydration behavior of the NaHy chain and was compared with similar data reported by Davies et al. [5, 6] who have measured the density by the oscillating-tube method. The discrepancies between their results and ours are discussed in terms of the density errors inevitably involved due to the viscosity effect.

Experimental

Materials

The sample of NaHy with a molecular weight of 1.4×10^6 was provided by Shiseido Basic Research Laboratory, Yokohama, Japan. The sample was isolated from a strain of bacteria, *Streptococcus Zooepidemicus*, which produces hyaluronic acid extracellularly and was purified according to the method devised by Asakawa et al. [7]. The NaHy of low molecular weight was prepared through the depolymerization of the original sample by means of an ultrasound sonicating bath for 3–15 h. The molecular weight of the sonicated samples was estimated by their intrinsic viscosity $[\eta]$ which was measured using a Ubbelohde capillary viscometer at 25 °C in 0.2 M NaCl solution. The molecular weight of NaHy was calculated according to the relation $[\eta] = 0.0228\ \mathrm{M}^{0.816}$ reported by Cleland and Wang [8]. *N*-Acetyl-D-glucosamine and sodium D-glucuronate were obtained from Sigma. All the solutions studied were prepared gravimetrically using water purified using a Milli-Q system (Millipore). The concentration of NaHy was expressed on the molality basis of the disaccharide unit.

Density measurements

The density measurement was made using both oscillating-U-tube densimeters and pycnometers to compare their accuracies when applied to the density determination of viscous liquids. An Anton Paar DMA 60/602 (Graz, Austria) oscillating-U-tube density meter was used. The density ρ of the sample liquid was calculated from the frequency period τ of the oscillating U-tube filled with the sample liquid using Eq. (1).

$$\rho = (\tau^2 - B)/A, \tag{1}$$

where A and B are the apparatus constants which incorporate the factors of volume, mass, and elastic modulus of the oscillating tube. The oscillating U-tube was calibrated through the measurements of the density of air and distilled water at 25 ± 0.01 °C by determining constants A and B. Equation (1) should be valid provided that the oscillator behaves in an undamped manner over a wide range of fluid densities. The accuracy claimed for the apparatus is $\pm 5 \times 10^{-6}$ g cm^{-3} when the stability of the temperature is ±0.01 °C.

Two Lipkin–Davison type pycnometers, each having a volume of about 40 cm^3, were used. The temperature of the pycnometer containing the sample solution was controlled at 25 ± 0.01 °C in an air thermostat. After thermal equilibrium was attained, the meniscus level was measured by a cathetometer and then the total mass of the pycnometer filled with liquid was weighed using a chemical balance (Mettler H54AR). The calibration of the pycnometers was performed using distilled water. The density thus obtained was estimated to be precise within $\pm 1 \times 10^{-5}$ g cm^{-3}.

Viscosity measurements with a rotational viscometer

A rotational viscometer of the cone-and-plate type (Visconic ELD and Viscorder VDU-3B, Tokimec, Tokyo) was introduced to the present study to measure the flow behaviour of viscous liquids. The sample fluid is placed between a stationary plate and a cone which touches the plate at the apex of the cone. The viscosity of the test fluid was estimated from the observed torque necessary to turn the cone at a required angular velocity. The temperature was kept constant at 25 ± 0.01 °C. The viscometer was calibrated by measuring the viscosity of several calibration oils. The precision of the measurements was better than 2%. The viscosity of the samples was measured at different shear rates from 1 to 400 s^{-1}. The flow curves obtained for aqueous NaHy solutions behaved like non-Newtonian fluids. The viscosity η_0 at zero-shear rate was estimated by extrapolation.

Ultrasonic velocity measurements and calculation of the adiabatic compressibility

The ultrasonic velocity in the solution was measured by a "sing-around pulse method" at 25 ± 0.002 °C using an ultrasound velocity meter (UVM-2, Chou-onpa Kogyou, Tokyo) which worked at a frequency 2 MHz. The accuracy of the sound velocity measurement was ±0.1 m s^{-1}.

Apparent molar volumes ϕ_v and apparent molar adiabatic compressibilities ϕ_k for the NaHy disaccharide unit in water were calculated from the following equations [9, 10].

$$\phi_\mathrm{v} = 1000(\rho_1 - \rho)/(m_2\,\rho\rho_1) + (M_2/\rho) \tag{2}$$

$$\phi_\mathrm{k} = 1000(\beta - \beta_1)/(m_2\,\rho_1) + \beta\,\phi_\mathrm{v} \tag{3}$$

$$\beta = 10^{-3}/(u^2\,\rho) \tag{4}$$

where ρ is the solution density (g cm^{-3}), β the coefficient of adiabatic compressibility (Pa^{-1}) of the solution, M_2 the molecular weight of the disaccharide residue (=401.34), m_2 the molality of the disaccharide residue, and u the sound velocity (m s^{-1}) in the solution. The subscript 1 refers to solvent, i.e., pure water.

Results and discussion

Systematic error of the oscillating-tube densimeter

We started the pycnometric density (ρ_{pyc}) measurement for aqueous solutions of NaHy having a molecular weight ranging from 7.0×10^4 to 1.40×10^6 in the concentration range 0.2–1.0 wt% and found that the density of these samples becomes identical within experimental accuracy, irrespective of the difference in molecular weight as is indicated in Fig. 1. The density of the same NaHy solution was simultaneously measured by using an oscillating-U-tube densimeter. The densities thus measured ρ_{osc} were compared with ρ_{pyc} as shown in Fig. 2A.

We can see that the oscillating-tube method gives too high values for the solution density except for the sample with the lowest molecular weight of 7.0×10^4, and the deviation increases with the increase in concentration. These results strongly suggest that the major cause of errors induced in density measurements by the oscillating tube is related to the viscosity of the sample solutions. The difference $\Delta\rho$ between ρ_{osc} and ρ_{pyc} is plotted against the viscosity at zero-shear rate η_0 in Fig. 2B. The qualitative tendency that $\Delta\rho$ increases with viscosity could be seen, but any universal relationship between $\Delta\rho$ and η_0 was not confirmed. We may interpret the above observation as a result of damping the oscillation of the U-tube. The flow of internal fluid in the tube is assumed to have little effect on the resonant vibration of the tube in oscillating-tube densimetry. It is very likely, however, that the energy loss induced by the internal friction of the highly viscous liquid in the tube leads to a reduction in the resonance frequency of the U-tube which increases $\Delta\rho$ with η_0 as indicated in Fig. 2B.

Moreover, it is to be noted here that the NaHy solution behaves as a non-Newtonian and viscoelastic fluid [11, 12]; therefore for the quantitative analysis of the damping effect, further studies concerning the flow pattern of the internal solution in the U-tube which oscillates at a frequency of about 3 kHz, and the shear-rate dependence of viscosity and elasticity of NaHy solutions will be necessary.

Apparent molar volume and compressibility of NaHy in water

The concentration dependence of the ultrasound velocity u for aqueous solutions of NaHy of various

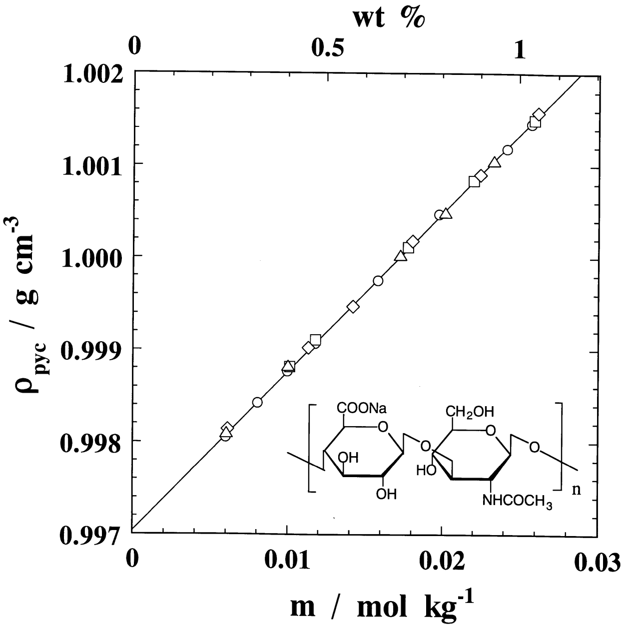

Fig. 1 Concentration dependence of the density of sodium hyaluronate (*NaHy*) aqueous solutions measured pycnometrically. Molecular weights of NaHy samples are 1.40×10^6 (○), 6.0×10^5 (□), 1.5×10^5 (◇), and 7×10^4 (△). *Inset*: molecular structure of a repeating disaccharide unit of NaHy

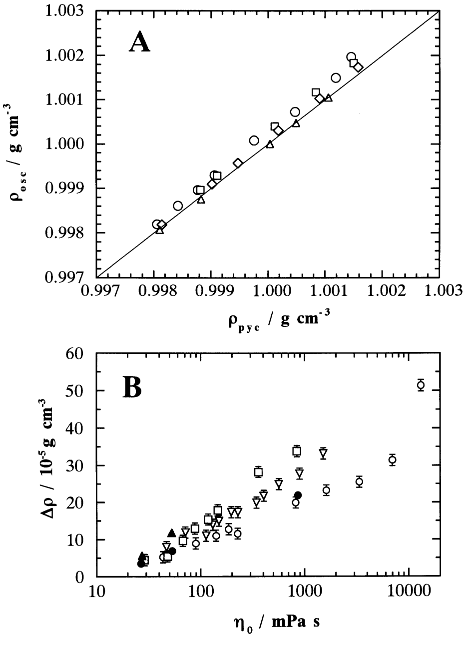

Fig. 2 **A** Plots of the oscillating densimeter reading, ρ_{osc}, versus densities determined by pycnometry, ρ_{pyc} for aqueous solutions of NaHy. Symbols are the same as in Fig. 1. The deviation from the diagonal line indicates the instrumental error of the densimeter. **B** Experimental density deviation, $\Delta\rho$ ($= \rho_{\mathrm{osc}} - \rho_{\mathrm{pyc}}$), as a function of the zero-shear viscosity, η_0, for aqueous solutions of NaHy with molecular weights 1.40×10^6 (○), 8.1×10^5 (▽), and 6.0×10^5 (□), respectively. Furthermore, five data points obtained for aqueous solutions of glycerol (●) and sucrose (▲), which behave as Newtonian fluids, are shown

molecular weighs is shown in Fig. 3A. An approximate linear relationship is seen to exist between the sound velocity and NaHy concentration, however, they appear to be molecular-weight-independent within experimental error. Figure 3B shows the concentration dependence of the adiabatic compressibility β which is calculated from Eq. (4) using the density data obtained by pycnometry. It is confirmed that our β values coincide with the data (dashed line) reported by Davies et al. [6], although they measured density by an oscillating-U-tube method. The agreement is contrary to what we expected if it were not the exceptional profit given to their oscillating glass tube when it was blown [3]. In any case, for the precise density measurements of viscous liquids using an oscillating densimeter, in general, it is very important to check the effect of viscosity of the sample to its own densimeter [3] and to confirm the limitation of the apparatus.

According to Eqs. (2) and (3) the apparent molar volume ϕ_v and the apparent molar compressibility ϕ_k for a NaHy disaccharide unit were calculated and plotted against molality, and the values at infinite dilution were obtained by an extrapolation procedure (see Fig. 4). The value of $\lim_{m\to 0}\phi_v$ was 229 cm^3 mol^{-1} (=0.570 cm^3 g^{-1}: the same value as the partial molar volume of the NaHy disaccharide unit for samples dialyzed against 0.2 M NaCl [13]), and $\lim_{m\to 0}\phi_k$ was -6.4×10^{-8} cm^3 mol^{-1} Pa^{-1}. For the individual constituent monosaccharide units of NaHy, on the other hand, we obtained the values of $\lim_{m\to 0}\phi_v = 149.3(97.1)$ cm^3 mol^{-1} and $\lim_{m\to 0}\phi_k = -0.83(-8.65) \times 10^{-8}$ cm mol^{-1} Pa^{-1} for *N*-acetyl-D-glucosamine (sodium D-glucuronate), respectively (data not shown here). Comparing the apparent molar volumes of the disaccharide unit and the sum of constituent monosaccharides, the former is 17 cm^3 mol^{-1} smaller than the latter. This

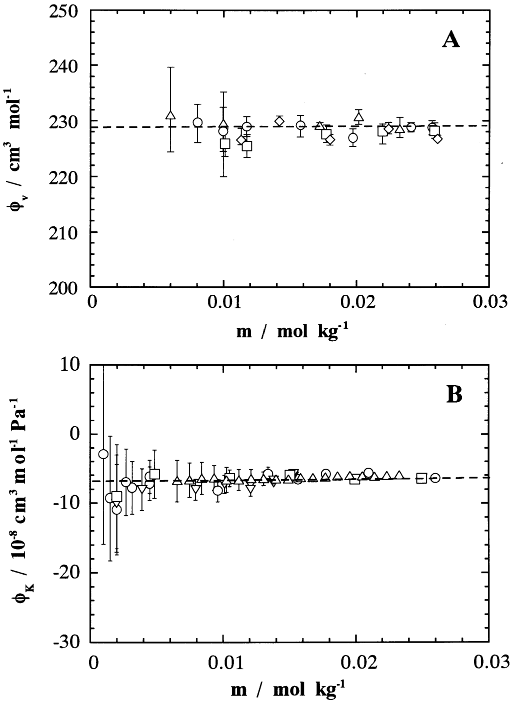

Fig. 4 **A** Concentration dependence of the apparent molar volume and **B** the apparent molar adiabatic compressibility for the NaHy disaccharide unit at 25 °C. Symbols are the same as in Fig. 3

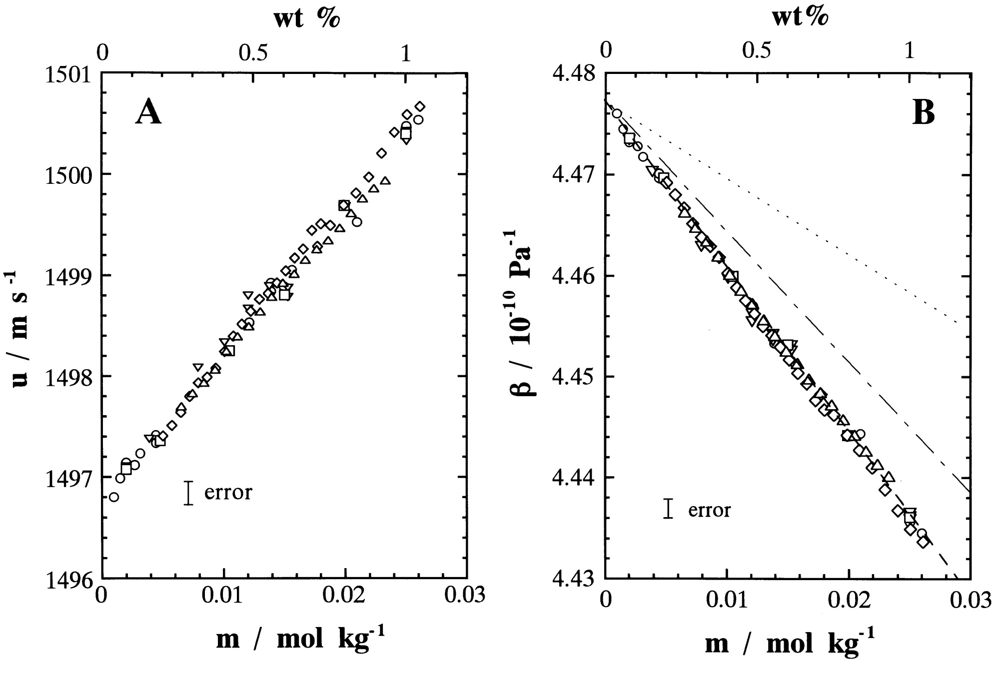

Fig. 3 **A** Concentration dependence of the ultrasound velocity *u* and **B** the adiabatic compressibility β for NaHy aqueous solutions. Molecular weights of NaHy samples are 1.40×10^6 (○), 8.1×10^5 (▽), 6.0×10^5 (□), 1.5×10^5 (◇), and 7×10^4 (△). Also indicated in **B** are the reported adiabatic compressibility data for aqueous solutions of NaHy (*broken line*), sodium D-glucronate (*chain line*), and *N*-acetyl-D-glucosamine (*dotted line*) [6]

difference appears to be quite reasonable when we take into account the two glycoside linkages in one disaccharide unit. It is obvious, however, that the absolute compressibility value of a disaccharide unit is considerably smaller than the sum of constituent sugars: the former is only 68% of the latter. This result clearly suggests that the disaccharide unit in the NaHy chain is less hydrated that the sum of its component monosaccharide residues. So, it may be said that although the molecular domain of the NaHy chain occludes a large amount of solvent [14] in aqueous solution it is not however, an excessively hydrated molecule [6].

A possible explanation for the reduced hydration of the disaccharide unit may be that owing to the formation of interglycosidic linkages in NaHy chains, the water structure around hydrated C-1 and C-3 (C-1 and C-4) hydroxy groups of *N*-acetyl-D-glucosamine (sodium D-glucuronate) should be disrupted.

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