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Behaviour of wetting films of sodium hyaluronate saline solutions

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Abstract

The behaviour of wetting films of sodium hyaluronate (NaHA) saline solutions, at physiological conditions, is investigated using interferometry. Concentrations in the range of dilute and semi-dilute regimes were chosen to assess the role of bulk molecular interactions on the surface forces. The transition from dilute to semi-dilute solutions is known to occur at $c^* = 0.59\ \mathrm{mg\,mL^{-1}}$. Dilute solutions form stable films whose behaviour is explained by van der Waals interactions. For semi-dilute solutions, the disjoining pressure vs film thickness isotherms show branches attributed to stepwise thinning or film stratification, which is interpreted in terms of a network as predicted by the theory of semi-dilute polyelectrolyte solutions. The distance between branches is 16 nm for the NaHA concentration of $2\ \mathrm{mg\,mL^{-1}}$ and 12 nm for the concentration of $3.5\ \mathrm{mg\,mL^{-1}}$, in agreement with the calculated correlation lengths of the polymeric network. Since no experimental evidence exists for the presence of a stable network in the bulk, this tendency for self-association should be the result of the liquid confinement. To our knowledge, the existence of oscillatory disjoining pressure isotherms in the presence of excess salt was never observed for other polyelectrolyte solutions.

Keywords: Sodium hyaluronate; Wetting films; Disjoining pressure isotherms; Interferometry; Polyelectrolytes

1. Introduction

Sodium hyaluronate (NaHA) is the sodium salt of hyaluronic acid (HA), a high molecular mass polysaccharide found in vitreous humor, synovial fluids and many other living substrata. HA consists of repeating disaccharide units, negatively charged, whose structure is shown in Fig. 1.

HA has attracted much attention in the biomedical field due to the great number of biological functions attributed to this molecule. For example, NaHA solutions are used in ophthalmology and eye surgery to substitute the vitreous fluid and, as lubricants, in orthopaedic joints.

The broad applications of HA led to intensive research on its physical-chemical properties both in aqueous saline solutions and in the solid state. In the excellent review of Cowman et al. [1] a number of experimental studies on HA together with the theoretical framework of data interpretation are reported. They include viscometric, osmotic pressure, light scattering and diffusion coefficient analysis as well as studies of local conformation of HA molecules either in solution or on the surfaces.

The biological functions of hyaluronic acid derive from the viscoelastic properties of its solutions which are due to the open, random coil structure of the HA molecule. However, the conformation of HA in solution depends on the local environment, including, among other factors, ionic strength, specific ions interactions, local dielectric constants and the presence of

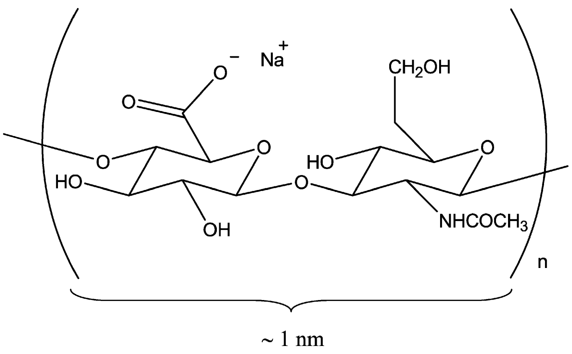

Fig. 1. Structure of NaHA.

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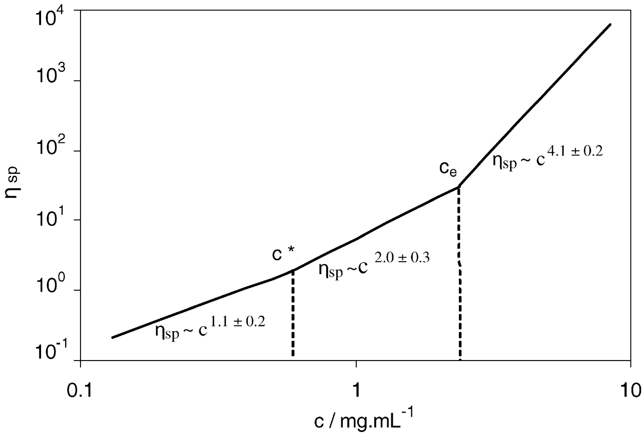

Fig. 2. Specific viscosity, η_{sp}, of NaHA saline solutions as a function of concentration. Adapted from Fig. 3 of Ref. [2].

proteins. According to Colby et al. [2] the rheology of NaHA in phosphate-buffered saline is typical of flexible polyelectrolytes in the high-salt limit. No strong associations between NaHA chains under physiological conditions exist and the concentration dependence of viscosity, relaxation time and terminal modulus are consistent with observations on neutral polymers in good solvents, as predicted by scaling models for polyelectrolytes with excess salt. The viscosity data presented in Fig. 2, adapted from Colby et al. [2], shows that the solutions of NaHA in physiological conditions (PBS, 138 mM NaCl, 2.7 mM KCl) may have the regimes: dilute, semi-dilute unentangled and semi-dilute entangled. The transition from dilute to semi-dilute unentangled occurs at $c^* = 0.59\ \text{mg}\,\text{mL}^{-1}$ while the entanglement concentration is $c_e = 2.4\ \text{mg}\,\text{mL}^{-1}$. These data have been sometimes misinterpreted as evidence for the existence of stable networks in the bulk. Experimental results from diffusion coefficients analyses confirmed that the overlapping of the domains of the individual molecules does not lead to the formation of a stable network, typical of a gel [1,3]. Instead, the formation of transient networks characterised by correlation lengths described according to de Gennes's theory of electrolytes has been assumed [3].

The conformation of the HA molecules on a surface is quite different from that in neutral aqueous solution. Atomic force microscopy studies of HA deposited on mica or on graphite, from aqueous solutions, showed that HA has a tendency to self-associate and form networks of fibers. The structure of the aggregates formed on the surface depend on the interactions among HA molecules and between HA and the surface. Jacoboni el al. [4] found that on hydrophilic mica HA molecules formed monolayered sheets, 0.3 nm thick, while on hydrophobic graphite the HA molecules form a less ordered network with non-uniform height which does not correspond to a monolayer.

Different results were reported by Cowman et al. [5] who claim that HA molecules interact more strongly with graphite than with mica. The reason for this discrepancy was suggested by the authors as being the difference in the concentration of the HA solutions. Cowman used lower concentrations than Jacoboni and suggested that, at higher concentrations, the HA–HA interactions can mask the hydrophobic aspects of the HA structure and minimize the interaction with hydrophobic graphite.

In spite of the numerous studies of NaHA solutions reported in the literature, there is one aspect, which remains almost unknown: the behaviour of these solutions in confined geometries. In many biomedical applications, e.g. in biological lubrication, the NaHA solutions form liquid films on solid surfaces and the knowledge of the surface forces is very important to predict the conditions for film stability. In equilibrium, the force of interaction between the interfaces of the film with the solid substrate and the gas phase equals the disjoining pressure acting within the film. From the shape of the disjoining pressure isotherms, which represent the dependence of the disjoining pressure on the film thickness, the stability of the liquid films may be inferred.

Sodium hyaluronate is a polyelectrolyte and the issues of polyelectrolyte confinement between surfaces and of forces that arise from this confinement have been addressed by many authors [6–14]. However, most studies concerned foam films and those concerning thin wetting films of polyelectrolytes are rare [6,14]. Furthermore, solutions of polyelectrolytes containing film-stabilising surfactants, which do not form complexes with the polyelectrolytes, are generally used as film formers [7–9,12]. It is believed that the surfactant stabilises the foam film through adsorption at the air-water interface and does not disturb the polyelectrolyte behaviour inside the film.

The objective of the present work is to investigate the behaviour of wetting films of NaHA saline solutions, at physiological concentrations of NaCl, through the determination of disjoining pressure isotherms of the films formed between an air bubble and a glass substrate. A classical interferometric technique using the captive bubble method was used to measure both the film thickness and the disjoining pressure [15–17].

The experimental results are compared with theoretical predictions based on the application of the existing theories of surface forces in confined polymeric aqueous solutions. Interactions between surfaces in thin wetting films are van der Waals, electrostatic and steric and/or structural forces [18]. The relative importance of each component depends on the nature of the system. Electrostatic forces, known to be predominant in water films, become negligible at the high salt concentrations typical of physiological conditions. In the presence of polymer-like structures, long-range oscillatory structural forces play an important role. These oscillatory forces are responsible for film stratification that has been observed in films of polyelectrolyte solutions.

In this work, films formed with solutions of three different concentrations of NaHA were investigated. For the lowest concentration, in the dilute regime, the disjoining pressure isotherm is typical of a stable film stabilised by van der Waals interactions. However, in the semi-dilute regime, either below or above the entanglement concentration, the NaHA solutions form films with stratification identified by the presence of jumps in the disjoining pressure isotherms. The jump size is close to the correlation length of the polymeric network. This result is new because the oscillatory structural forces occur despite the high salt concentrations. Such behaviour was never reported on