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Thermochimica Acta, 242 (1994) 153–160

thermochimica acta

Thermal behaviour of aqueous solutions of sodium hyaluronate from different commercial sources

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(Received 10 November 1993; accepted 25 January 1994)

Abstract

The thermal behaviour of some commercial sodium hyaluronate solutions used in ophthalmology has been studied by DSC after cooling to −100°C. During the rewarming, the endothermic effect that appears at around −22°C is associated with the molecular weight (MW) of the fractions of sodium hyaluronate: the higher the MW, the higher the endotherm temperature. At high temperatures, the dehydration temperatures of the samples (onset at around 110°C) are in a sequence that is the inverse of that obtained at low temperatures. Both effects, low-temperature phase transition and dehydration, have been related to intramolecular and intermolecular hydrogen bonding capabilities and to hydration and crosslinking effects.

Keywords: Cross link; Dehydration; DSC; Hydrogen bond; Ophthalmology; Phase transition; Polymer; Sodium hyaluronate; Viscoelastic

1. Introduction

Hyaluronic acid (HA) was isolated in 1934 by Karl Meyer. Sodium hyaluronate (NaHA) occurs in a comparatively pure form in the extracellular matrix of connective tissue in animals and humans. It is mainly present in the vitreous and

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SSDI 0040-6031(94)01733-W

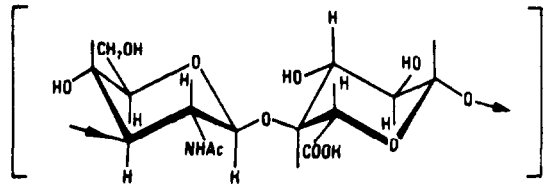

Fig. 1. Hyaluronic acid.

aqueous humour of the eye, and is also found in synovial fluid, in the skin and in Wharton's jelly, the principal component of the umbilical cord. Its role is evidently less structural than one of lubrication, in the widest sense. HA is a heteropolymer, with the sugar derivatives *N*-acetylglucosamine and D-glucuronic acid arranged in an alternating sequence (Fig. 1). Structurally, the molecule is a β-(1 → 4)-linked disaccharide polymer with the interdisaccharide unit linked β-(1 → 3) (Fig. 1). It has a very high relative molecular mass, probably in excess of 12×10^6 and seems to behave in solution as a random coil. It is estimated that 1 g of polymer occupies about 12 l of solvent. This solution therefore has a high viscosity. However, HA has anomalous viscosity, i.e. its viscosity alters with the rate of shear to which it is subjected (Fig. 2). HA offers almost infinitely high viscosity at very low shear rates, because the polymer chains remain stubbornly intertangled. At high shear rates the viscosity collapses and the polymer lines up with the streamlines of flow. It is known that in addition to the "chitinous" structure of the β-(1 → 4)-linked *N*-acetylglucosamine residues, the insertion of the β-(1 → 3)-linked glucuronic acid residues endows the HA molecule with marked polyelectrolyte behaviour, by virtue of the charged groups incorporated into the polymer. This means that it could bind cations, behaving in the extracellular environment as a soluble ion-exchange resin. The polyelectrolyte character, its massive volume occupancy in solution, and its enormous molecular length, all contribute to the function of this remarkable lubricant [1].

NaHA 1% viscoelastic solutions are used as a surgical aid in anterior segment procedures, including cataract extraction and intraocular lens implantation. High molecular weight fractions of NaHA in solution maintain the normal position of the vitreous face, thus preventing formation of a post-operative flat chamber. Therefore, differences in molecular weight of NaHA fractions in commercial products are of interest for ophthalmologists. This paper shows how information on this subject can be obtained by DSC.

2. Experimental

2.1. Apparatus

DSC curves were obtained with a Perkin-Elmer DSC 7 in dynamic N_2 ($20\ cm^3\ min^{-1}$), at a heating rate of $10°C\ min^{-1}$, and with capsules of aluminium as sample containers.

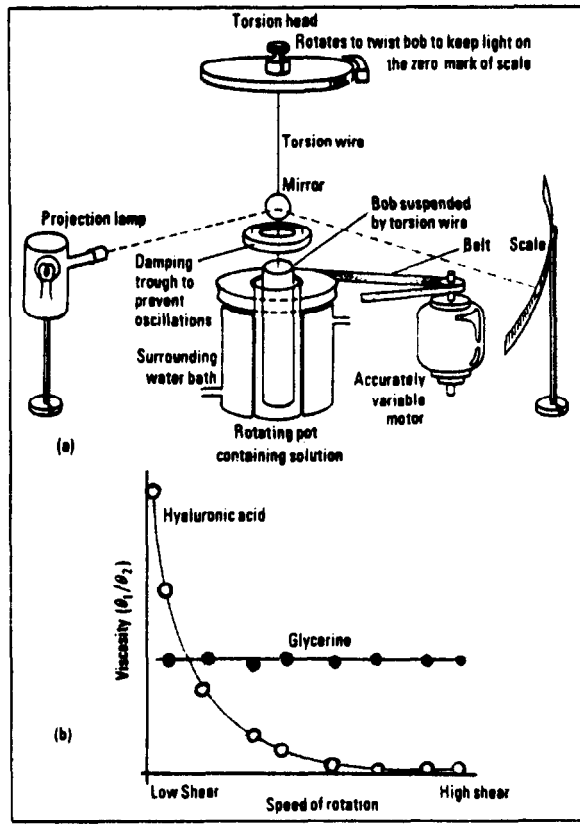

Fig. 2. (a) Couette viscometer. Viscosity is simply determined by determining the angles rotated by the bob with the solution (θ_1) and with the solvent alone (θ_2). (b) Shear and viscosity properties of glycerine and hyaluronic acid.

2.2. Samples

NaHA in Healon G.V. (Pharmacia AB), Amvisc (MedChem Products Inc.), Healon (Kabi Pharmacia AB) and Vitrax (Allergan Medical Optics) was extracted from avian tissues, whereas in Biolon (BioTechnology General, Israel) it was extracted from bacterial cells. The chemical information for NaHA in Viscoat (Alcon Surgical, Inc.) does not include data on its preparation. In all these products, NaHA is supplied in disposable glass syringes, dissolved in physiological sodium chloride–phosphate buffer (pH 7.0–7.5). The concentration of NaHA in each ml is 14 mg in Healon G.V., 12 mg in Amvisc, 10 mg in Healon and Biolon, and 30 mg in Vitrax and Viscoat. In Viscoat, each ml also contains 40 mg of sodium chondroitin sulphate. The osmolarity of all these solutions is 320 ± 40 mOsm and their viscosity is $40\,000 \pm 20\,000$ cP (at a shear rate of $2\ s^{-1}$).

Both Occucoat (Storz Ophthalmics, Inc) and Coatel (Opsia Pharma), two non-NaHA viscoelastic solutions also used in ophthalmic surgery, are compared

with the above natural solutions in this study. Each ml of either Occucoat or Coatel provides 20 mg of hydroxypropylmethylcellulose with a high molecular weight greater than 80 000 daltons in a pH 7.2 ± 0.4 buffered salt solution. The osmolarity of both solutions is 285 ± 32 mOsm and their viscosity is 4000 ± 1500 cP.

The human aqueous humour, used as reference in this study, was obtained in the course of cataract surgery.

3. Results

3.1. Low-temperature DSC curves

Figure 3 shows the low-temperature DSC scans of commercial NaHA solutions together with that of the aqueous humour of the eye and those of non-NaHA

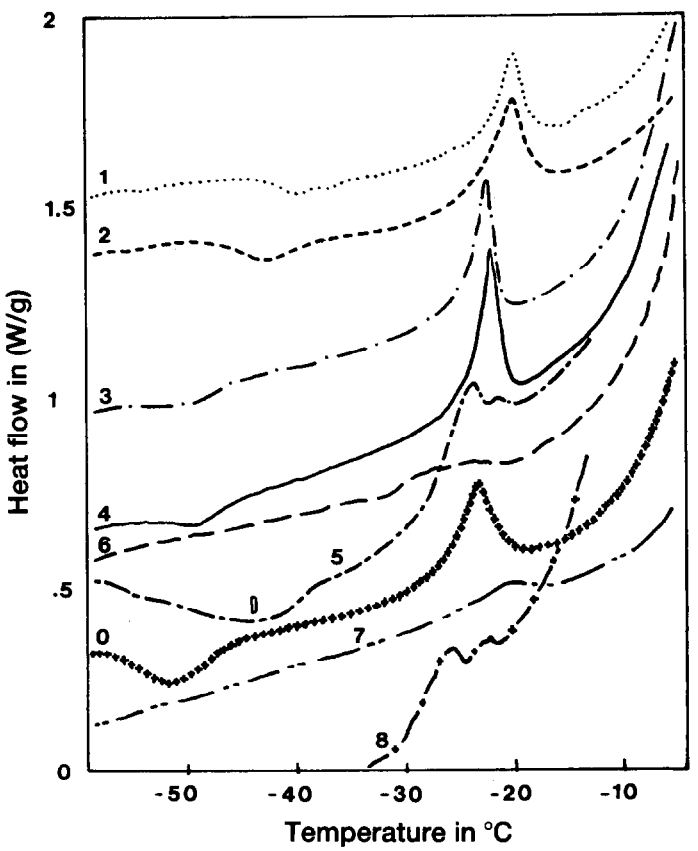

Fig. 3. DSC curves for the aqueous humour of the eye (0) and for commercial solutions used as surgical aids in ophthalmology: 1, Healon G.V.; 2, Amvisc; 3, Healon; 4, Biolon; 5, Vitrax; 6, Viscoat; 7, Occucoat; and 8, Coatel.

Table 1
Thermal data for the main endothermic peak at low temperatures in DSC scans from commercial viscoelastic preparations for ophthalmology

Viscoelastic material	T_{onset}/°C	T_{peak}/°C	ΔH/(J g^{-1})
Sodium hyaluronate			
Healon G.V.	−21.5	−19.3	3.7
Amvisc	−21.9	−19.5	4.4
Healon	−24.0	−22.1	5.5
Biolon	−24.3	−22.5	5.1
Vitrax	−28.6	−26.2	1.3
Viscoat	−30.3	−26.7	1.3
Hydroxypropylmethylcellulose			
Occucoat	−21.8	−20.8	0.5
Coatel	−28.5	−26.4	1.0

viscoelastic solutions. All the curves exhibit an exothermic effect at around −47°C, followed by two endothermic effects at around −22 and 10°C, respectively (the last is not represented). The onset and peak temperatures and the enthalpy changes of these common effects are characteristic for each solution. In Table 1, it can be observed that such thermal data vary considerably. The peak temperatures of Healon G.V. and Amvisc solutions are approximately equal, higher than those of Healon and Biolon solutions (in turn, quite closely grouped), and much higher than those of Vitrax and Viscoat.

These results can be associated with the molecular weight (MW) of the NaHA fractions in aqueous solution: whereas Healon G.V. and Amvisc contain very high MW fractions, and Healon and Biolon were prepared with high MW fractions, Vitrax and Viscoat appear to contain medium MW fractions.

DSC curves for Occucoat and Coatel indicate barely perceptible endothermic peaks at temperatures under 0°C. The onset and peak temperatures for the endothermic from Occucoat are closer to those of Healon G.V., Amvisc, Healon and Biolon NaHA viscoelastics than to those of Coatel, which are, however, near those of Vitrax.

We must emphasize that in the DSC scans for Biolon and Healon the onset and peak temperatures of the endotherm under consideration are almost the same as those of the corresponding endotherm for the aqueous humour of the eye (onset temperature, −25.7°C; peak temperature, −22.5°C; ΔH, 2.54 J g^{-1}).

3.2. High-temperature DSC curves

The samples that were heated in sealed pans suddenly "decomposed" at temperatures between 122 and 147°C. However, the DSC thermal effects of this decomposition did not display the constant onset and peak temperatures necessary for characterization purposes.

When sample heating was carried out in open capsules, the samples did not undergo decomposition, but rather selective dehydration with useful DSC onset

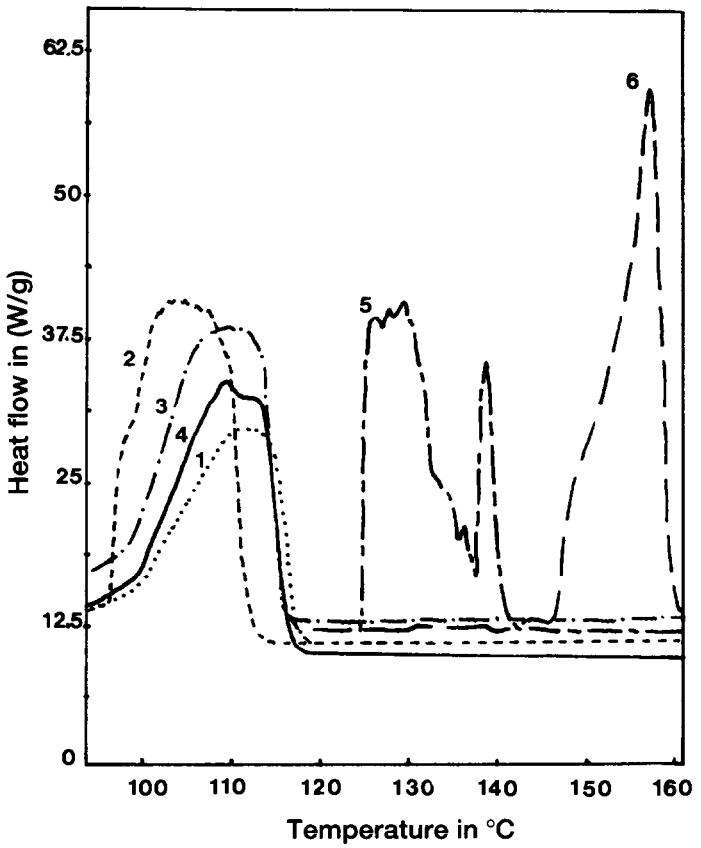

Fig. 4. High-temperature DSC curves for commercial sodium hyaluronate solutions. (For identification, see the numerical assignations in Fig. 3.)

temperatures were observed (Fig. 4): 146°C for Viscoat, 124°C for Vitrax, 97.5°C for Biolon, 96.9°C for Healon, and 96.3°C for Amvisc and Healon G.V. Although the temperature differences are small, it is clear that the NaHA preparations with lower MW fractions (Viscoat and Vitrax) show dehydration temperatures higher than those of the higher MW fractions (Healon G.V. and Amvisc). Interestingly, the above order is the inverse of that obtained at around −22°C and is in agreement with previous observations at low and high temperatures for bacterial lipopolysaccharides [2].

4. Discussion

The interpretation of DSC curves of biological materials in aqueous solutions at low temperatures is not straightforward. The "state" of freezable and non-freezable water and the biomaterial–water interactions are complex phenomena, the knowl-

edge of which is fraught with uncertainties. Whereas freezable water has been associated with free water, non-freezable water has been identified with the immobilized, fully or partially bound, water content of solutions and gels. It is believed that ageing causes the water to be released from the gel state (vitreous) and to accumulate as free water [3, 4].

The existence of a glassy phase can be evidenced in our DSC scans by an inflexion at around −80°C, which results from a change in the specific heat of the sample. According to Simatos et al. [5], this change is associated with a second-order transition, consisting of the transformation from one type of glass to another. A fraction of the vitreous water can crystallize in the rewarming, as indicated by the exothermic effect at around −47°C (D in Fig. 3). Such occurrences have been described for various simple aqueous solutions including those of glycerol [6–8], polyvinyl pyrrolidone [9], ethylene glycol and sugars [7], as well as for complex systems such as plasma [10].

The endothermic effects at around −22°C and 10°C correspond to an incompletely elucidated phase transition and to the melting of ice, respectively. The first endotherm has been observed previously by us in DSC curves for chitin, chitin–glucans and cellulose [11], and for lipopolysaccharides (LPSs) of Gram-negative bacteria [2]. Also, we have observed this effect in DSC scans for beef muscle and egg white [5]. For chitins, we have shown that this effect corresponds to a phase transition between a distorted structure, poor in interchain hydrogen bonding, and an undistorted structure, rich in interchain hydrogen bonding (in chitin, it seems that the phase transition occurs from the β- to the α-form). For LPSs, we have also postulated a phase transition between structures with different hydrogen bonding networks to explain the observed thermal effect but, in addition, we have associated this effect (in an inverse relationship) with that of depolymerization at temperatures above 120°C [2].

At present, we believe that the event occurring at around −22°C can be mediated by the rearrangement of both water and solution molecules in the polysaccharide–water glass and that the endothermic peak temperature can be useful as a marker for the ageing state [4]. Thus, the lower the peak temperature of this endotherm, the higher the number of water and solute molecules that remain bound. Inversely, the higher the peak temperature of this endotherm, the higher the crosslinking between the solute molecules (aggregation by hydrogen bonding) and the higher the water mobility.

With regard to the viscoelastic solutions of this study, the fact that the preparations with the highest MW fractions (Healon G.V. and Amvisc) both have the higher temperatures of the endotherm at around −22°C and the lowest temperatures of the dehydration effect, indicates an increased number of junction zones (higher viscosity) and a higher content of freezable water. If instead of high viscosity (a desirable property when an almost solid matrix is required in the ocular cavities, as in anterior chamber surgery), we need somewhat less viscosity and more favourable flow facilities (to safeguard endothelial cells during cataract surgery), then Biolon, Healon or Vitrax are the pharmaceuticals to be recommended. The close analogy of the DSC curves of Biolon and Healon with that of the aqueous humour of the eye must always be taken into account.

Finally, for an explanation of the anomalous viscosity of NaHA we can speculate that (a) at low shear rates, NaHA shows high viscosity due to the polymer chains being crosslinked by hydrogen or covalent bonding, which also occurs when very high MW fractions are predominant; (b) at high rotation speeds, the viscosity suddenly decays because the interchain bonding is broken and substituted by intrachain hydrogen bonding. In this latter condition, the material could be described as a conjunct of solute chains that, instead of being linked one to another, are principally bound to water molecules. Structural studies are required to test all our interpretations.

Acknowledgement

The authors thank technician N. Chebib-Abuchala for valuable laboratory assistance.

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