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Thermal analysis of aqueous solutions of heparins

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Abstract

The thermal behaviour of both standard and low molecular weight heparins with anticoagulant properties has been studied by DSC after cooling to −100°C. During the rewarming, the endothermic effect that appears between −32 and −12°C is related to the molecular weight (MW) of the heparin fraction: the higher the MW, the higher the endotherm temperature. At high temperatures, the decomposition temperatures of the samples (indicated by an exothermic effect between 241 and 254°C) are in the same relationship as that obtained at low temperatures. The endothermic effect at low temperatures for heparins is similar to that exhibited in the DSC curves of other glycosaminoglycans such as hyaluronate solutions, heparin–dermatan sulphate mixtures or chondroitin sulphate solutions.

Keywords: Aqueous solution; Heparin; TA

1. Introduction

Heparins are a heterogeneous group of straight-chain anionic mucopolysaccharides, called glycosaminoglycans, that have anticoagulant properties. Although others may be present, the main sugars occurring in heparins are: 2 deoxy-2-sulphamino-α-D-glucose 6-sulphate, α-L-iduronic acid 2-sulphate, 2-acetamido-2-deoxy-α-D-glucose, β-D-glucuronic acid, and α-L-iduronic acid. The sugars are joined by glycosidic linkages, forming polymers of varying sizes. Unfractionated standard heparin (UFH)

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samples have a molecular weight (MW) ranging from 5000 to 30000 D (average, 12000–15000 D), whereas low molecular weight heparin (LMWH) derivatives have a size of 4000–6000 D [1–5].

UFH was originally extracted from the liver but is now obtained from animal intestinal mucosa and from bovine lung tissue. UFH is commercially available as the calcium or sodium salt. The LMWH fragments were produced from UFH either by extraction or by depolymerization [6–8] to reduce their average MW to around 5000 D without appreciable destruction of the original material. The molecular heterogeneity of LMWH is a feature of great interest, mainly in relation to the results from the depolymerization and fractionation procedures: commercial products that are apparently similar in size, have MW distributions that are very different and, therefore, pharmaceutical activity profiles that are very distinct.

Because DSC has been successfully applied in earlier aspects of this work, we have designed its application to this study in order to compare different commercial LMWH products with each other and with UFH.

The thermal information obtained for this family of materials will complete our knowledge on the thermal behaviour of polysaccharides in general and glycosaminoglycans in particular [9, 10].

In clinical pharmacology, heparins are used for prophylaxis and treatment of venous thrombosis. Standard heparins inhibit reactions that lead to the clotting of blood mainly by increasing the effects of antithrombin-III which successively accelerates the inactivation of both the activated factor IIa (thrombin) and factor Xa. LMWH fragments do not effectively inhibit factor IIa and their antithrombotic activity is mainly attributed to the inhibition of factor Xa [5].

2. Experimental

2.1. Apparatus

DSC curves were obtained using 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 40-μl sealed aluminium capsules as sample containers. Low-temperature DSC scans were recorded after cooling to −100°C.

2.2. Samples

The standard UFH samples studied were calcium heparin (calciparine) manufactured by Boizot, sodium heparin from Rovi, another sodium heparin preparation made by us using a porcine intestinal mucosa extract obtained from Sigma (H-3125), and a lithium heparin from Biomedics.

The LMWH fragments studied were calcium nadroparine (Cy-216 or Fraxiparine), obtained by ethanol precipitation followed by fractionation according to published procedures from Choay/Sanofi [6], sodium enoxaparin (RP 54563 or Clexane,

and PK 10169 or Decipar, both from Rhône-Poulenc) obtained by debenzilation followed by alkaline depolymerization [7], and sodium dalteparin (K 2165 or Fragmin from KabiVitrum, and Boxol from Rovi), prepared by partial depolymerization with nitrous acid [8].

All these products were supplied in aqueous solution at a concentration of around 200 mg ml^{-1}.

3. Results

Fig. 1 shows the low- and high-temperature DSC scans of commercial heparin solutions. All the curves exhibit an endothermic effect in the temperature range −32–−12°C, and an exothermic effect in the range 241–254°C. In addition, the calcium heparins show a further endothermic effect at around −39°C. The onset and peak temperatures of these common effects are characteristic for each solution, as can be seen in Table 1.

To compare the temperatures of the thermal effects of the UFH samples with those of LMWH, it is observed that the former temperatures are always higher than the latter. This observation is common to the endothermic effect at low temperatures and to the exothermic effect at high temperatures. Thus, the temperatures of both effects can be considered as molecularity degree markers. This result is partially in accordance with that already reported for hyaluronate solutions with different molecular weights: the first endotherm temperatures are shifted to higher temperatures as the MW increases [10]. Nevertheless, two main differences separate the thermal behaviour of hyaluronate and heparin solutions. Firstly, whereas the former exhibit an endothermic peak at 110°C attributed to dehydration which is absent in the DSC curves of heparins, the latter show an exothermic peak at around 240°C, which is missing in the DSC curves of the hyaluronate solutions. Secondly, whereas the dehydration temperatures of the samples of hyaluronate are in a sequence that is the inverse of that obtained at low temperatures, the decomposition temperatures of the heparin solutions follow the same relationship as that reported at low temperatures.

Concerning the two sodium LMWH types reported in this study (Table 1), it is observed that the thermal effects of sodium enoxaparin (Clexane and Decipar) occur at higher temperatures than those of sodium dalteparin (Fragmin and Boxol). According to earlier considerations, we have associated this feature with the average MW of their respective mean fractions and with the MW of their respective higher MW fractions, which were always lower in Fragmin and Boxol than in Clexane and Decipar. Thus, in molecular size aspects, the sodium dalteparin results are more separated from the precursor sodium UFH than those of sodium enoxaparin.

For the high-temperature exotherms, it is observed (Table 1) that within a group with the same cation, the enthalpy changes of the decomposition enthalpy process decrease as MW decreases. Also it is noteworthy that the decomposition enthalpy change values from LMWH with different cations are closely grouped around 139.3 J g^{-1} (except Clexane, with $\Delta H = -180.9$ J g^{-1}).

4. Discussion

The main endothermic effect exhibited in the DSC scans of heparins is the same as that shown in the DSC scans of hyaluronate solutions [10], and as that registered in the DSC curves of both a mixture of heparin–dermatan sulphates and a chondroitin

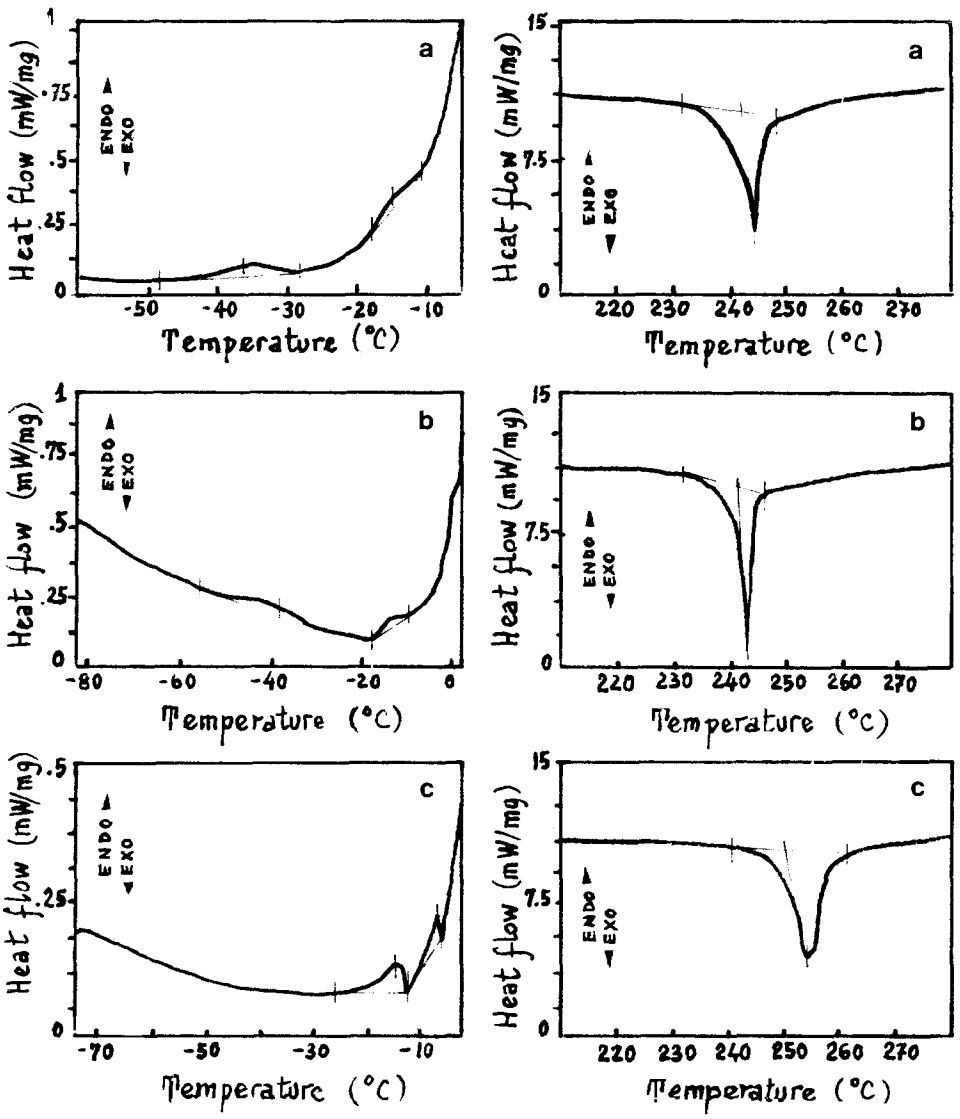

Fig. 1. Low- (left) and high- (right) temperature DSC curves for: (a) unfractionated calcium heparin from Boizot; (b) calcium nadroparine from Sanofi/Choay; (c) standard sodium heparin; (d) sodium enoxaparin from Rhône-Poulenc as Decipar; (e) sodium enoxaparin from Rhône-Poulenc as Clexane; (f) sodium dalteparin from KabiVitrum (Fragmin); and (g) sodium dalteparin from Rovi (Boxol).

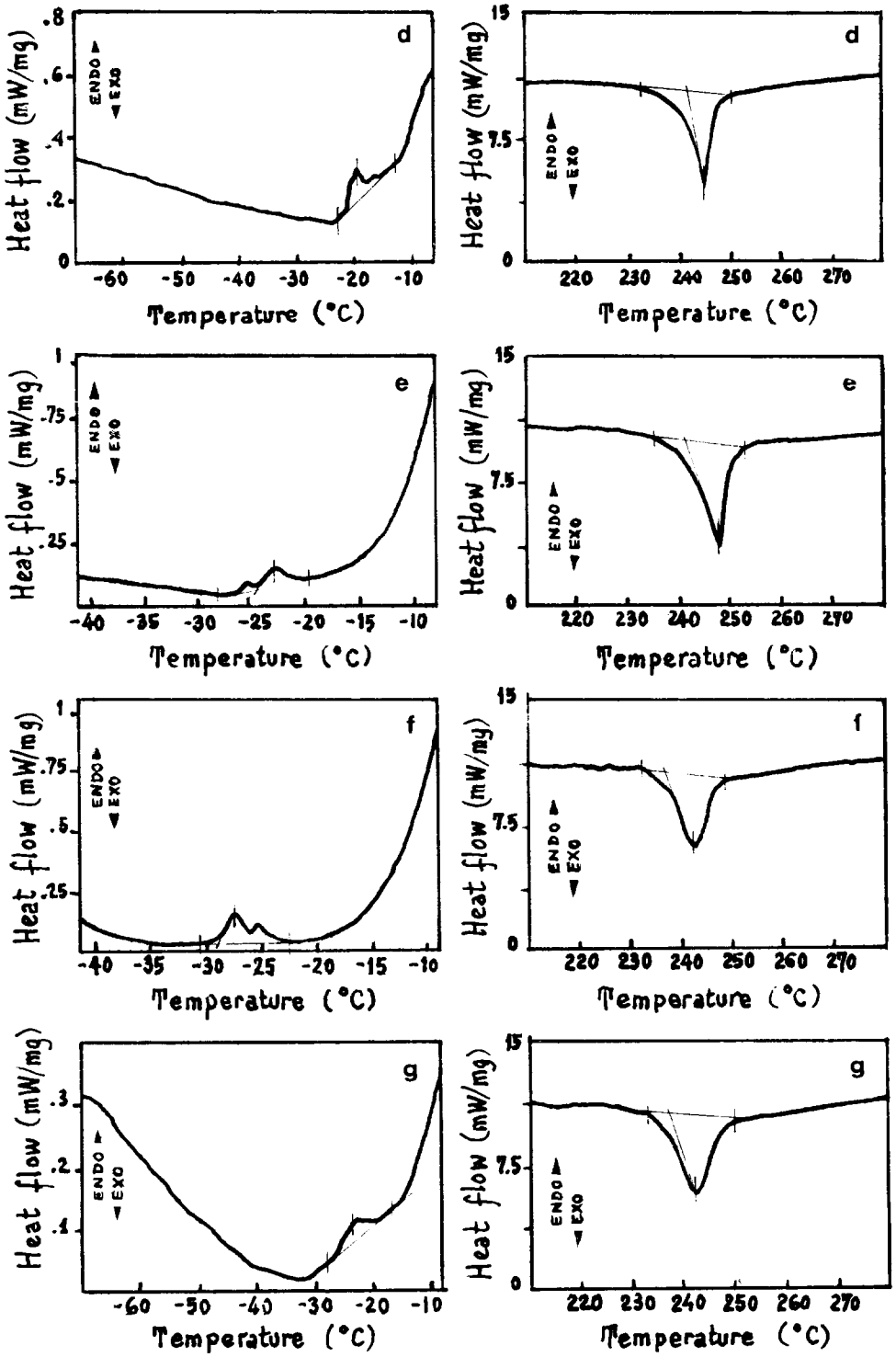

Fig. 1 *(continued)*

Table 1
Thermal data for the endothermic effects at low temperature and for the exothermic effect at high temperature in the DSC scans of heparin solution

Heparin solution	T_{onset}/°C	T_{peak}/°C	ΔH/(J g^{-1})	T_{onset}/°C	T_{peak}/°C	ΔH/(J g^{-1})	T_{onset}/°C	T_{peak}/°C	ΔH/(J g^{-1})
UF calcium heparin									
Boizot, MW ~ 12000 D	−48.8	−36.3	1.5	−14.4	−11.9	0.2	242.4	244.9	−150.2
Calcium nadroparine									
Sanofi/Choay, MW ~ 5000D	−53.2	−39.5	1.0	−16.7	−15.0	0.2	241.7	243.4	−139.5
UF sodium heparin									
Prolabo MW ~ 20000 D					–		250.6	254.4	−206.7
Sigma [a]				−19.6	−15.0	1.3		–	
Sodium enoxaparin									
Rhône-Poulenc									
Decipar^{R}, MW ~ 5000 D				−21.0	−19.2	1.6	241.3	245.4	−139.7
Clexane^{R}, MW ~ 5000 D				−22.9	−20.2	1.0	240.9	244.9	−180.9
Sodium dalteparin									
KabiVitrum									
Fragmin^{R}, MW ~ 5000 D				−26.1	−24.2 −21.7	2.1	236.9	242.5	−139.4
Rovi									
Boxol^{R}, MW ~ 5000 D				−26.0	−23.4	0.7	236.8	242.0	−139.1
Lithium heparin									
Biomedics [a]				−33.9	−31.7	0.2	232.2	241.8	−265.2

[a] Mean MW unknown.

sulphate solution [11]. It can be concluded that this effect is common to all the glycosaminoglycans. This endotherm has been associated with an incompletely elucidated phase transition, possibly mediated by the rearrangement of both water and solution molecules in the polysaccharide–water glass [10].

There is considerable controversy over the advantages of different LMWH. Both antithrombin and antithrombotic activities are involved in the discussions. Molecular size seems to be important in the antithrombin activity. Only the high-MW fractions moderately accelerate the rate at which antithrombin- III neutralizes thrombin (factor IIa) by complexing stoichiometrically with it. The low-MW fractions cannot form such ternary complexes and do not accelerate the inhibition of thrombin by antithrombin-III [12]. Thus, the low-MW fractions are less anticoagulant than the high-MW fractions. The antithrombotic activity of LMWH is mainly attributed to the inhibition of activated coagulation factor Xa and the molecular size does not seem to be important in this mechanism.

Our observations on sodium LMWH suggest that, in reality, sodium dalteparin has an average molecular size that is slightly lower than that of sodium enoxaparin. So, the anti-IIa activity in the preparations Fragmin and Boxol might be lower than in Clexane and Decipar, resulting in a slightly lower effect on coagulation (a relatively favourable property) for the former. In this study, we did not come to any conclusion concerning the relationship between calcium and sodium LMWH and their anti-IIa activities because the different influence of the cations on the thermal activity does not allow accurate comparisons.

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