The Fallacy of Hyaluronic Acid Binding a Thousand Times Its Weight In Water

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ABSTRACT

This study re-examined experimental reports and past literature of water binding by the humectant hyaluronic acid, in comparison with another common humectant, glycerol, to critically evaluate the common claim that hyaluronic acid binds a thousand times its weight in water, which makes it especially suited to be a cosmetic moisturizer. Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to study aqueous solutions of glycerol and hyaluronic acid. A 0.1 weight % aqueous solution of hyaluronic acid is a clear, flowing liquid, comparable to a 10 weight % aqueous solution of glycerol. The melting point and melting heat of fusion for the hyaluronic acid solution were effectively the same as pure water, while both were reduced for the glycerol solution. There is imperceptible freezing point depression by HA, whereas that by glycerol is as expected. No experimental evidence was found for any special ability of hyaluronic acid to bind water at the claimed level of a thousand times by weight. The origin of the fallacy that it binds water at that level can be traced to older literature that has been misunderstood for the meaning of binding, as compared to other physical properties such as hydrodynamics.

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1. Introduction

Hyaluronic acid (HA) is a natural carbohydrate polymer that is a major component of the human extracellular matrix. It is known for its diverse physiological roles, including as the lubricant in synovial fluid. In the form of a more widely available bacterial version, it is used as a humectant in personal care and cosmetic products. Its moisturizing power is renowned, and claims appear in various media that hyaluronic binds vast amounts of water. Statements that it can bind over one thousand times its weight in water are common, and the implication is that this makes HA a superior moisturizer. This work critically examines the binding of water by HA in both literature and experimental studies and dispels the notion that it has such remarkable power. Its properties are also compared to a more conventional humectant, glycerol. A thread is traced to early studies that could have led to the mis-impression that HA has such tremendous water binding properties.

One potential source of the erroneous belief about the water-binding capacity of HA comes from an article by Sutherland.[1] "The water-binding capacity correlates with the molecular mass and can be up to six litres of water per gram of polysaccharide." However, data to support this statement are not provided in this review article, and neither is a citation to any literature that made such a measurement. That review might be the original source for many subsequent erroneous claims. Citing to this paper, Becker states: "One gram of hyaluronic acid can hold up to 6 L of water."[2] While Jegasothy[3] states "it can attach and hold large amounts of moisture; approximately 6 L of water in just 1g", no citation to literature nor measurement of this property can be found in this publication. Olejnik et al. state "1 g of HA retains 6 L of water" without literature citation, but likely based on Sutherland's comment.[4] It is also worth noting that each

of these statements can be read to mean that HA binds 6000 times its weight in water, not the 1000 times commonly claimed.

This work experimentally investigated aqueous solutions of HA at the supposedly special ratio of 1000 g of water per gram, as well as a 10% aqueous solution of a more mundane humectant, glycerol (Chart 1). We have also examined the HA literature to discover the root of the fallacy of the special water binding properties of HA, and substantial past experimental studies that show a far more modest water binding property can be supported.
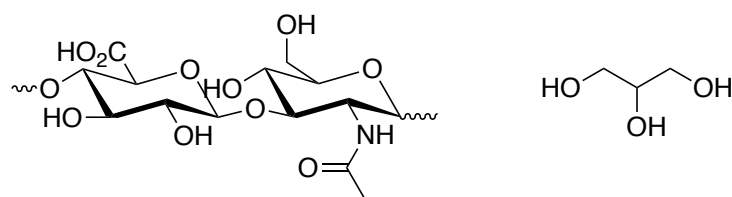

Chart 1. Hyaluronic acid repeating unit and glycerol.

2. Results

Several parameters (freezing heat of crystallization, melting heat of fusion, heat of vaporization, and melting transition peak) were determined by DSC of HA and glycerol solutions and pure water. Results are summarized in Table I and data given in Figures 1-6. These values compare to literature values for water for the specific heat of fusion of 334 J g^{-1} and for the heat of vaporization of 2230 J g^{-1}.

	HA	Glycerol	Water control
Freezing heat of crystallization (J g^{-1})	-251	-222	-294
Melting heat of fusion (J g^{-1})	262	223	320
Heat of vaporization (J g^{-1})	2295	2036	2273
Peak of melting transition (°C)	1.2	-1.6	1.3

Table 1. Parameters for solutions of HA and glycerol determined by DSC

TGA data are collected in Figures 7-9. Profiles for the HA solution show a mass loss of 100% at a temperature of 96.3 °C, and the water control shows a mass loss of 100% at the temperature of 99.6 °C. The mass loss profiles of these two solutions are indistinguishable. The glycerol solution shows a mass loss of 89.4% at 100.6 °C, and the mass loss profile is significantly different from pure water.

3. Discussion

The experimental results are consistent with expectation. Melting point depression by glycerol is as predicted, as its molal concentration is 1.09, comparable to the prediction of 2 °C using water's cryoscopic constant. In contrast, the molal concentration of HA is 1.09×10^{-6}, therefore its effect on the melting point is imperceptible. DSC measurements of HA solutions also show no thermal transitions other than for freezing/melting of free water.

Several past DSC studies of solutions of HA of various molecular weights have been reported. However, these were of much more concentrated solutions than studied here, approximately 1:1 w/w. This enabled the observation of DSC transitions from the different types of water molecules in those solutions. These include non-freezable bound waters, freezable bound waters, and free waters. Non-freezable water refers to water that is directly bound to the polysaccharide rather than free in solution, where it would be available to interact normally with other water molecules and exhibit their conventional behavior, including freezing at the normal temperature. There may also be freezable water associated with the polysaccharide that freezes at a lower temperature. At the low concentration of HA studied in this work, these two types of bound water are also at low concentration and therefore imperceptible by DSC, because it measures a bulk solution property.

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Studies of high concentration HA solutions reached firm and consistent conclusions about the amount of water strongly bound by the polysaccharide (i.e., non-freezing water). Joshi found that up to 21 molecules of water were strongly bound to each hyaluronic acid disaccharide monomer (i.e., repeat unit), and that the amount bound was 0.6 g H_2O/g hyaluronic acid.[5] Ikada found 0.51 g non-freezing H_2O/g hyaluronic acid, which corresponds to 11.5 moles of water per repeating HA disaccharide.[6] He also made one of the few measurements of freezing bound water, which he found to be 0.59 g H_2O/g hyaluronic acid. Yoshida determined a value of 0.5 g H_2O/g hyaluronic acid for strongly bound water, which corresponds to 11 molecules per repeat unit, and also measured the freezing bound water, which he found to be 0.5-1.8 g H_2O/g hyaluronic acid.[7] Kučerík determined there are 17.2-19.1 water molecules bound per polymer repeat unit, and that the amount bound was 0.77-0.86 g H_2O/g hyaluronic acid.[8,9] One interesting feature of this work is that the water binding was studied as a function of the HA molecular weight, and it was found to be approximately the same at most molecular weights.

The past DSC work that approaches closest to the situation studied herein used as low as 0.5% solutions of HA.[10] Cowman explained an anomalously large amount of water associated with the HA calculated from conventional analysis of thermal analytical data by the idea that freezing bound water and non-freezing bound water are coincident at these concentrations. Assuming based on past work 0.6 g H_2O/g HA for non-freezing bound water, the data led her to conclude contributions for freezing bound water are 44 g H_2O/g HA.

Another, early method used to interrogate the amount of water bound by HA used ultrasound. Davies found that in aqueous solution at 25 °C, HA is "closely associated with not less than 9 molecules of water of hydration."[11] The mass of that number of water molecules would be 144 g/mol, which compares to the 403 g/mol molecular weight of the hyaluronic acid disaccharide.

That corresponds to 0.36 g H_2O/g hyaluronic acid. Jouon found that the "amount of non-freezable water associated with the polysaccharide was about 0.7 g H_2O/g dry solids which corresponded to 15 water molecules per disaccharide unit".[12] While each of these reports used a different method to measure the amount of water strongly bound to hyaluronic acid, which produced somewhat different results, they are all similar, in the range of 0.36-0.86 g H_2O/g hyaluronic acid. These results are all obviously quite far from the 1000 g per g of HA in the commonly made claim.

Key early studies of HA were conducted by Ogston.[13] His work was not primarily aimed at determining the water-binding ability of HA, but rather at its particle size and structure. It used streaming birefringence, centrifugation, and viscosity to measure particle size, finding that HA, despite being a linear polymer that adopts a coiled form, behaves hydrodynamically like a large solvated sphere that encompasses a far larger volume of water than polymer. Ogston states "solvent entrained within it will be largely carried with the particle in dynamic measurements" and notes the dynamic entrainment of water in the interstices of a random-coil particle is what makes it behave like a solid spheroid.[14] Such entrained (definition: transport of a fluid by shear-induced turbulent flux) water molecules would not be considered bound in any chemical sense to the polymer. They move with the particle of HA and are neither bound to it by chemical forces nor trapped within it. Ogston amplified this point in later work,[15] stating "the coiled particle carries a great deal of water with it when it moves, through the operation of purely viscous forces." He finds the total volume occupied by the solvated coil is 6000 mL/g, which agrees with the dimensions of the particle measured by light scattering, and notes the coil is permeable to solvent. It seems quite likely that this particular report forms the basis of the

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statement by Sutherland (discussed in the introduction) that HA binds 6000 times its weight in water.

Ogston also states that HA serves to immobilize water in connective tissues, and it "might be said, in a dynamic sense, to 'bind' it." It is important to realize that he is not referring to a chemical binding, under a thermodynamic definition, but merely to restrict its movement within tissue. Hence, this is the basis for him to place the *bind* in his statement within quotations, since he is not referring to binding in any classical chemical sense.

While the documentary evidence discussed above makes clear how a misunderstanding about hydrodynamic measurements led the water-binding properties of hyaluronic acid to be misconstrued, a first principles analysis also makes clear that the "one thousand times its weight" claims are unreasonable. Multiplying the molecular weight of the disaccharide repeating unit by 1000 and dividing by the molecular weight of water gives the number of water molecules that would necessarily be bound to each polymer subunit, which is 22,389. The obvious way water would bind to a carbohydrate polymer is through hydrogen bonding. However, each hyaluronic acid disaccharide repeating unit has only 12 atoms capable of hydrogen bonding, and each of those can only bind to a maximum of three water molecules, for a theoretical maximum of 36 hydrogen-bonded water molecules per disaccharide. Experimental measurements of the number of water molecules bound per disaccharide repeating unit discussed above gave ca. 14 ($\pm$5) waters.

While the foregoing seriously compromises the idea that HA binds a thousand times its weight in water, there are other, synthetic polymers that bind large amounts of water per unit weight. For example, poly(acrylate) sodium salts are well known and found in various personal care products. These polymers are typically used in a dry state, are envisioned to convert into their

hydrated forms that sequester water, and would not be considered useful ingredients for cosmetic moisturizers. Given past claims about hyaluronic acid, one might imagine that the special composition of HA and a thousand times its weight in water would give a hydrated form with special properties, such as a stable gel or solid form. However, the 0.1% hyaluronic acid solution prepared in this study is a free-flowing liquid that looks and behaves like water. Thus, that amount of water is dissolving HA as it would any other solute to form a dilute solution.

It is appropriate to contemplate the water-binding properties of HA in relation to glycerol. While the theoretical maximum hydrogen-bonding capacity of glycerol is 9 water molecules, experimental studies have shown the actual hydrogen bonding by glycerol in dilute aqueous solution is 6.3 ± 1.4.[16] Translating that value into the vernacular used in this study, that would be glycerol binding 1.23 g H_2O/g, significantly exceeding what has been found for hyaluronic acid.

The commonly made claim for hyaluronic acid is that it can bind 1000 times its weight in water. How this became a standard statement in the popular (and sometimes scientific) literature compared to the "6000 times its weight in water" statement in Ogston's work based on a misunderstanding of the early scientific literature is a topic for further literature research. It is apparent that neither is supportable in scientific studies, however.

A role of humectants in cosmetic products is to impede water loss by evaporation via binding to it. A chemical force must necessarily be involved in that process. Some of the other ways in which water might associate with hyaluronic acid have been found in detailed scientific studies but may not play a useful role in the more mundane world of moisturizers.

4. Experimental

https://doi.org/10.26434/chemrxiv-2023-r728q **ORCID:** https://orcid.org/0000-0003-4585-8353 Content not peer-reviewed by ChemRxiv. **License:** CC BY-NC-ND 4.0

A commercial sample of fermentation-derived, cosmetic-grade HA (specifically, sodium hyaluronate) from Shandong AWA Biopharm (MW 0.92 mDa) was heated and dried under high vacuum to remove all traces of water of hydration. Glycerol was ACS certified reagent grade. Solutions of these materials (w/w%) were prepared in water with resistance >50 MΩ from an in-lab water purification system. Differential Scanning Calorimetry (DSC) was performed using a Netzsch DSC 214 Polyma. Thermal Gravimetric Analysis (TGA) was performed using a Netzsch TG 209 F1 Libra.

DSC was performed under nitrogen with two different temperature programs. The first began at 20 °C, chilled the sample to -40 °C, and then returned the sample to 20 °C. The second began at 20 °C, chilled the sample to -40 °C, and then heated the sample to 150 °C. All temperature gradients were 5 K/min. The sample volume was ca. 10 μL. TGA was performed beginning at 20 °C with heating of the sample to 150 °C at 5 K/min. The sample volume was ca. 30 μL. All runs were performed in triplicate.
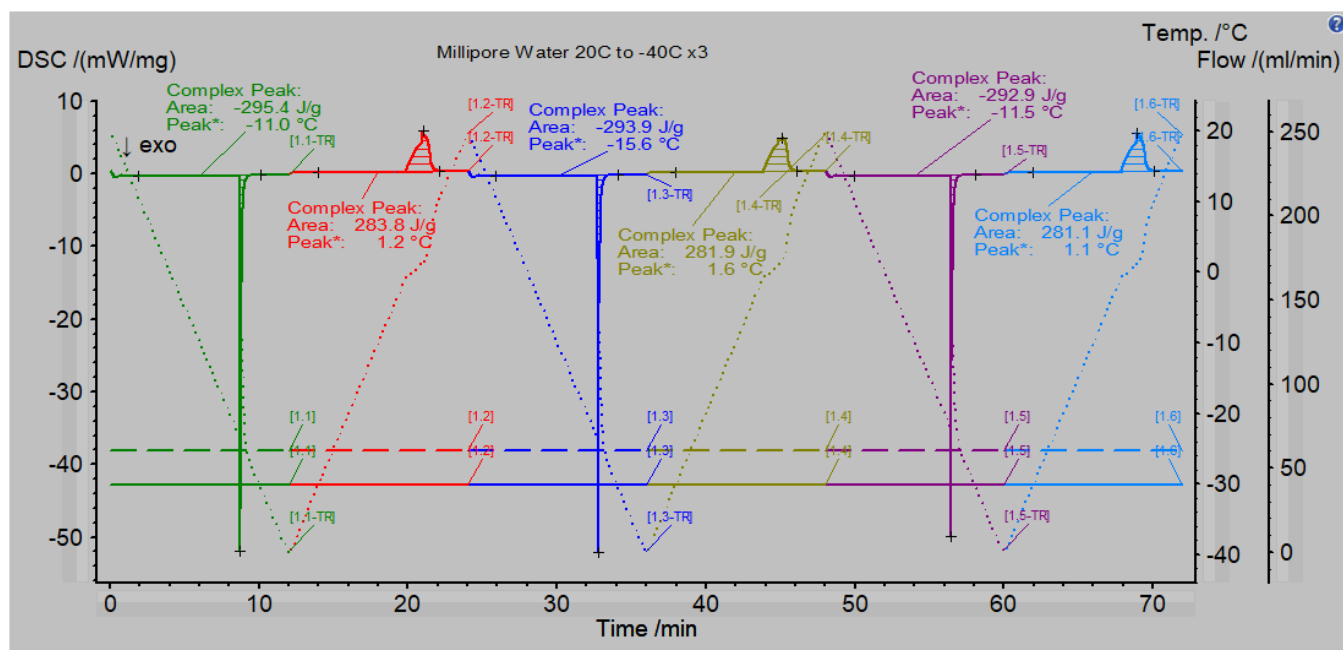
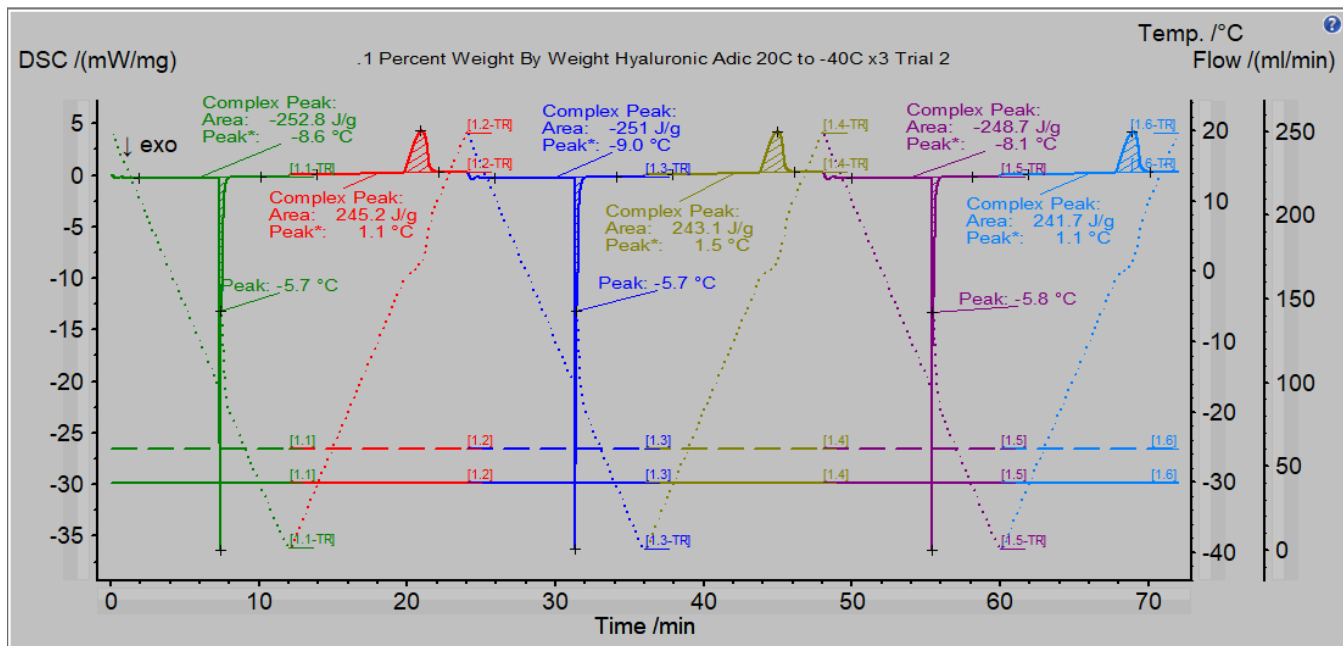Figure 1. DSC data for pure water scanned from 20 → -40 → 20 °C in triplicate (control).

Figure 2. DSC data for 0.1% (w / w) solution of hyaluronic acid scanned from 20 → -40 → 20 °C in triplicate.
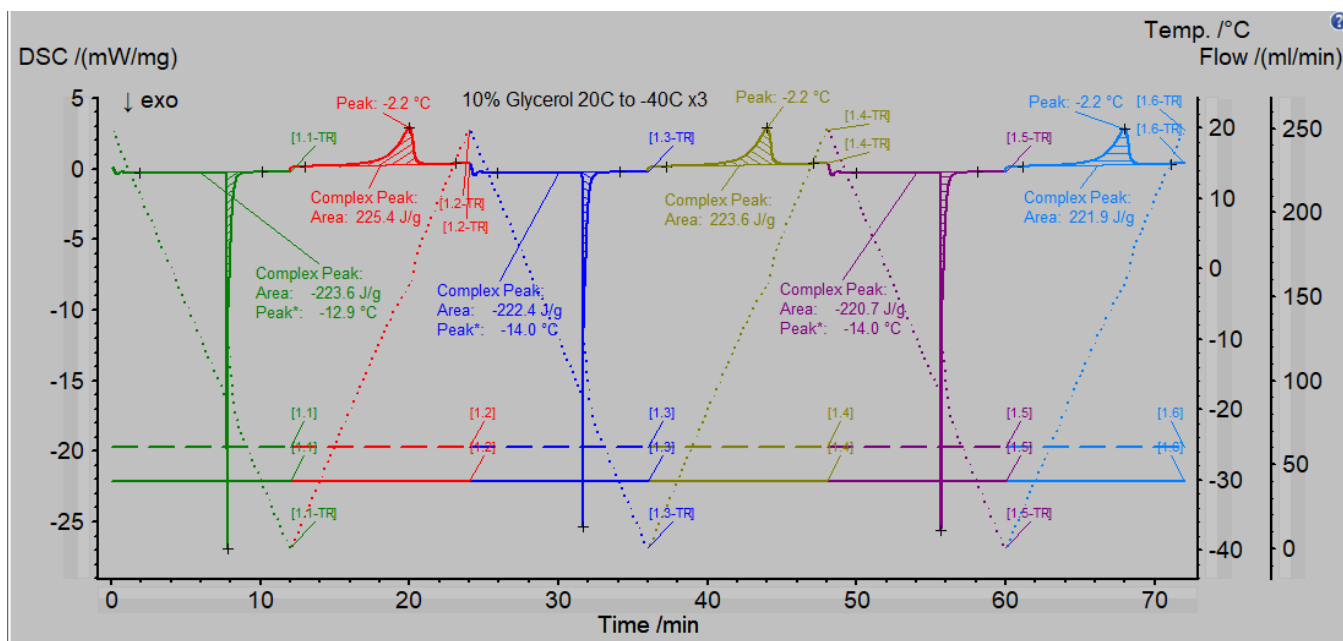

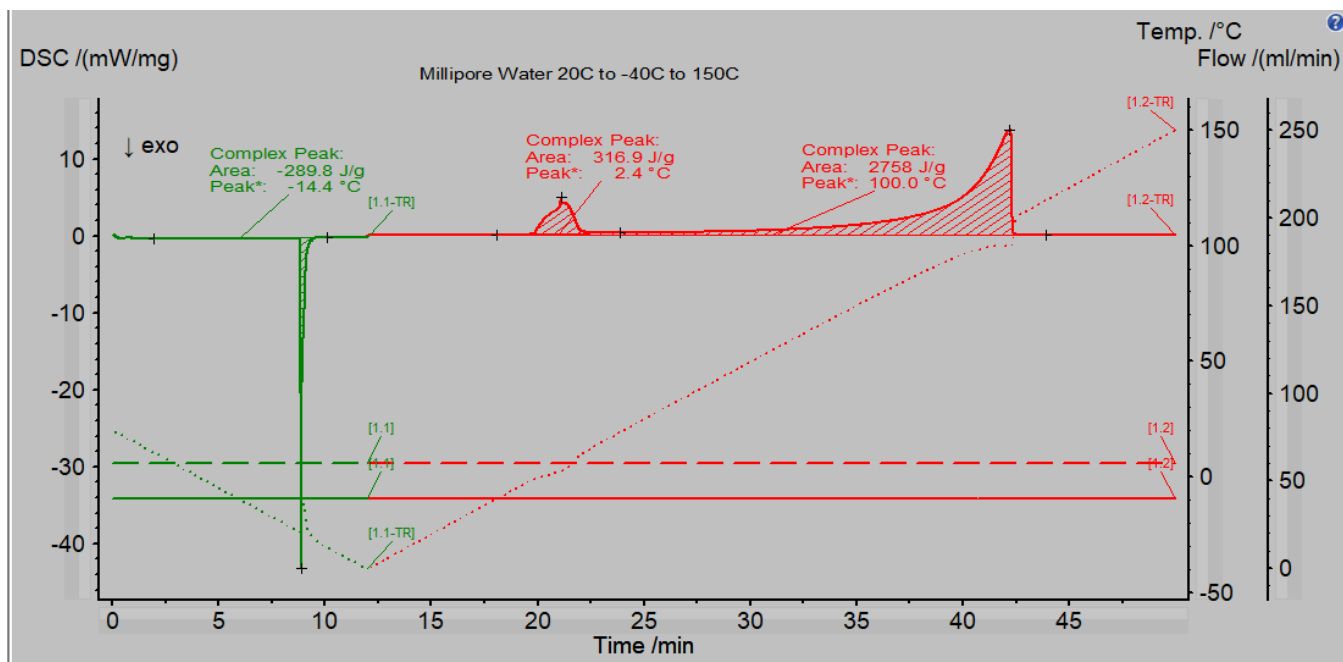Figure 3. DSC data for 10% solution of glycerol scanned from 20 → -40 → 20 °C in triplicate.

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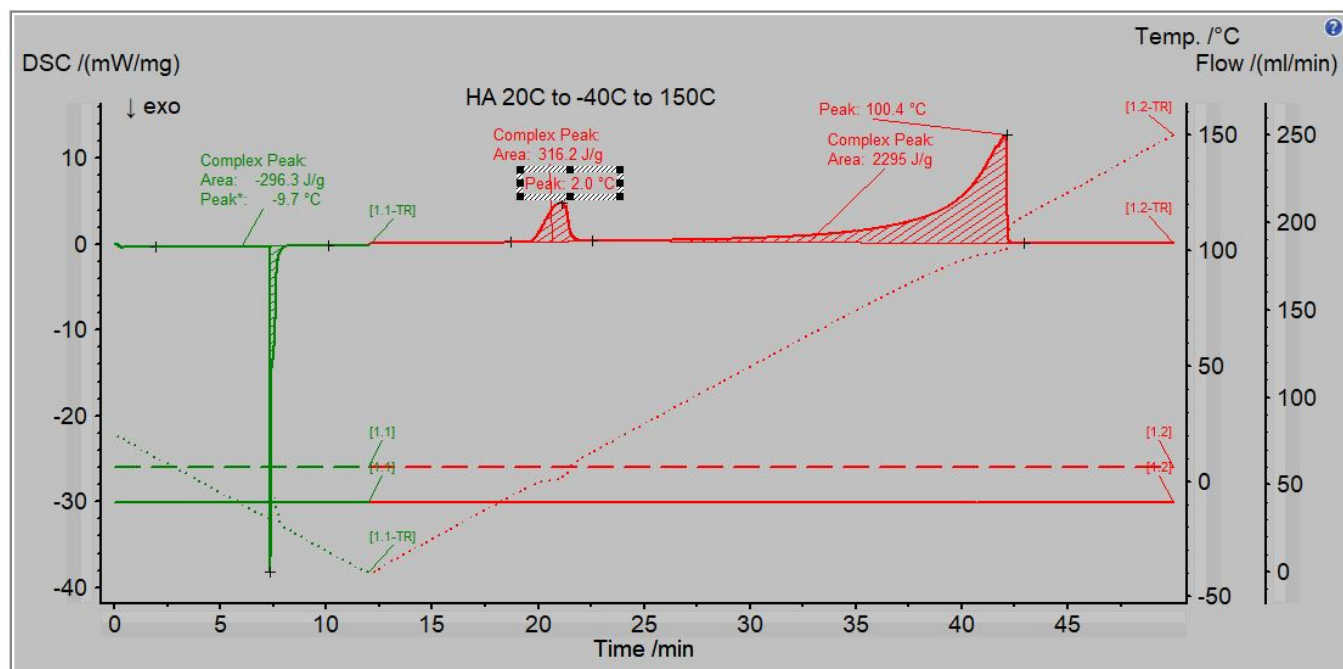Figure 4. DSC data for pure water scanned from 20 → -40 → 150 °C (control).

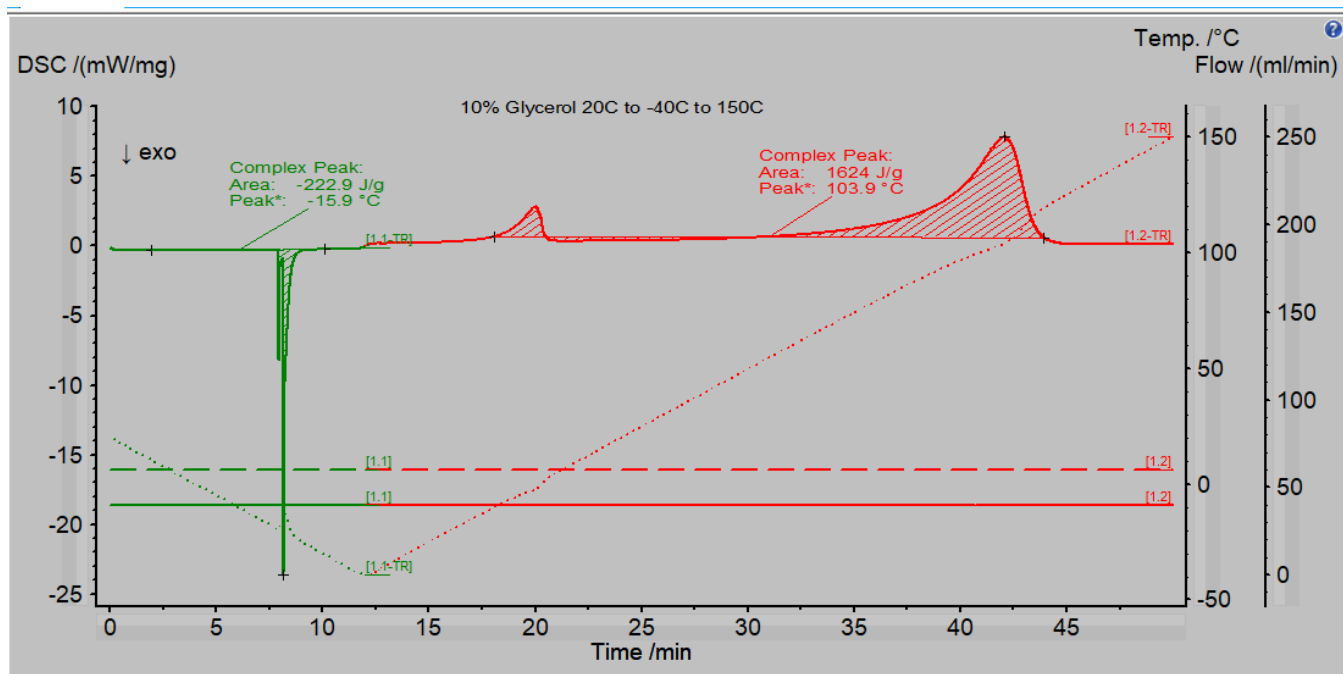Figure 5. DSC data for 0.1% (w/w) solution of hyaluronic acid scanned from 20 → -40 → 150 °C.

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Figure 6. DSC data for a 10% solution of glycerol scanned from 20 → -40 → 150 °C.
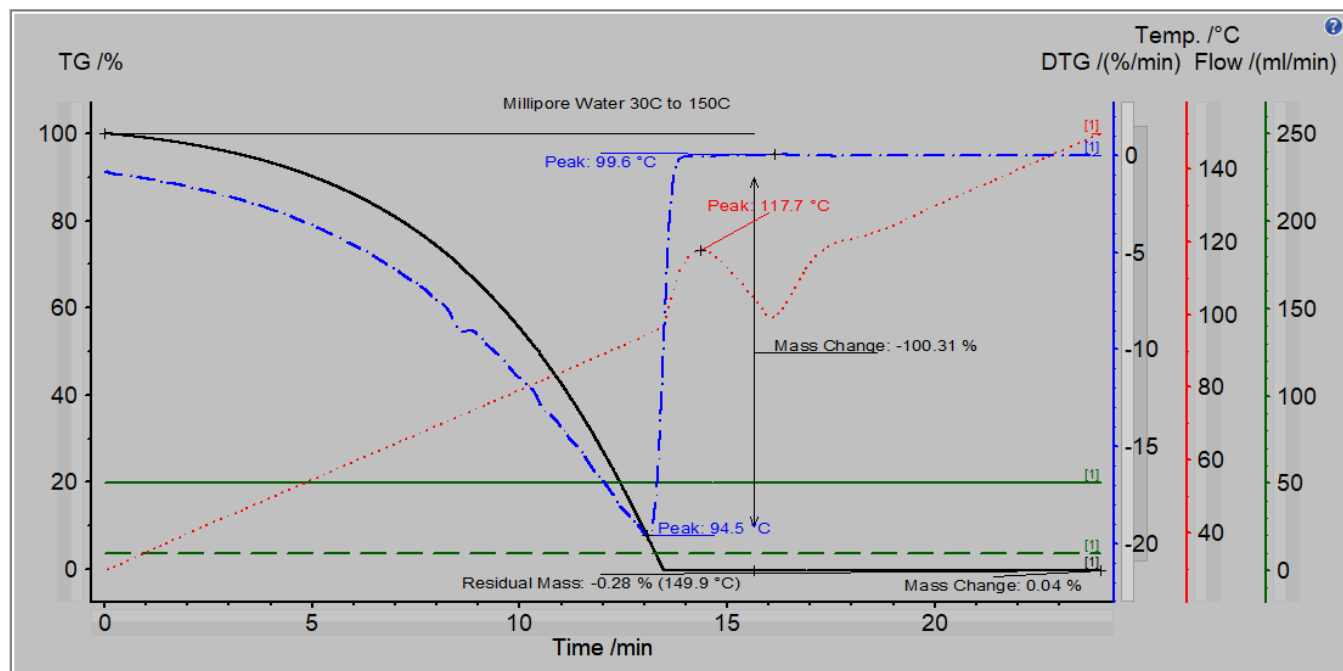

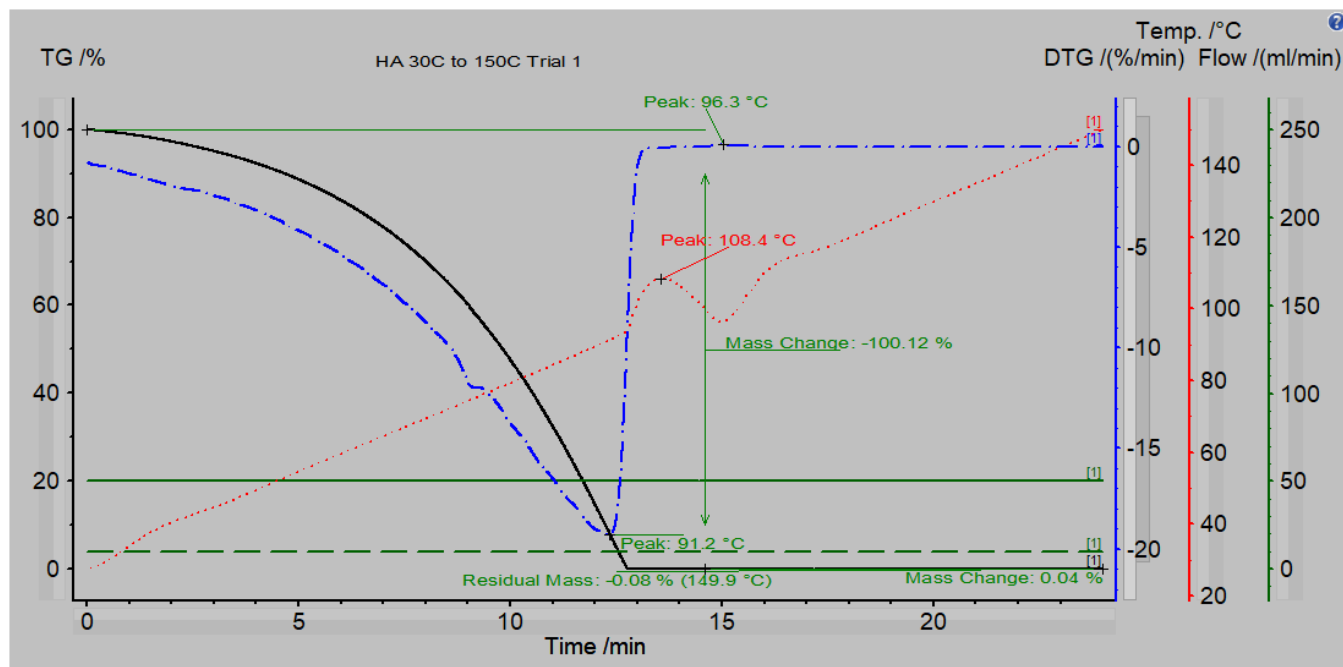Figure 7. TGA data for pure water from 30 → 150 °C (control).

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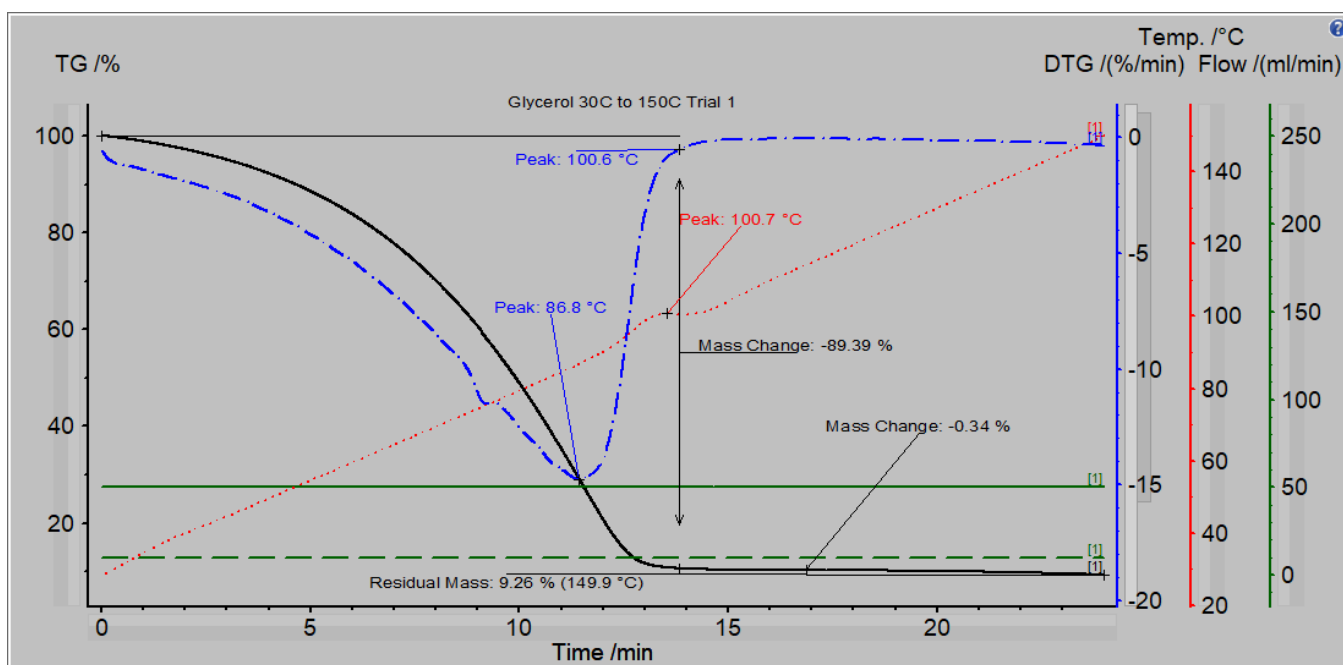Figure 8. TGA data for 0.1% (w/w) solution of hyaluronic acid from 30→150 °C.

Figure 9. TGA data for a 10% solution of glycerol from 30→150 °C.

5. Acknowledgments

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