The Fallacy of Hyaluronic Acid Binding a Thousand Times its Weight in Water

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Synopsis

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, making 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 (the putative 1,000 times its weight) is a clear, flowing liquid, comparable to a 10 weight % aqueous solution of glycerol. The melting point and melting heat of fusion of the hyaluronic acid solution were indistinguishable from pure water, while both were reduced for the glycerol solution. This is as expected, as colligative properties of aqueous solutions are proportional to concentration, and the polymer is at a much lower molar concentration than glycerol. There is imperceptible freezing point depression by hyaluronic acid, 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.

INTRODUCTION

Hyaluronic acid (HA) is a natural carbohydrate polymer that is a major component of the human extracellular matrix. It is known for diverse physiological roles, including as the lubricant in synovial fluid. In the form of a more widely available bacterial version, HA is used as a humectant which enhances the water-holding capacity of the skin, in personal care and cosmetic products, inter alia. Water is essential to normal skin function, and its retention by the stratum corneum is facilitated by natural hygroscopic agents, including the two compounds studied here, hyaluronic acid and glycerol.[1] They enhance water absorption from the dermis into the epidermis, and are also believed to aid absorption of ambient water by the stratum corneum in humid conditions.[2] Thus, the ability of a humectant to bind water is intrinsic to its utility in cosmetic products, which gives this work its interest to the cosmetic industry.

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The moisturizing power of HA is widely renowned, and many claims appear in various media that HA binds vast amounts of water. Statements that it can bind over one thousand times its weight in water are common, including in peer-reviewed literature ("The unique helical coil conformation of HA allows it to trap up to 1,000-fold of its weight in water"[3], "it retains up to 1,000 times its weight in water"[4]). Similar statements are attributed to dermatologists ("HA binds to one thousand times its weight in water"[5]), including renowned academics ("it is capable of binding over one thousand times its weight in water"[6]). Some commercial product sites make a grander claim that a gram of HA binds 6 L of water ("one gram of hyaluronic acid can hold six litres of H_2O"[7], "a single gram of hyaluronic acid has the impressive ability to hold up to six (yep, *six*) litres of water"[8]). These examples portray HA as the ultimate 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 misimpression that HA has such tremendous water binding properties.

BACKGROUND

One potential source of the belief in the great ability of HA to bind water comes from a review article by Sutherland which states, "the water-binding capacity correlates with the molecular mass and can be up to six litres of water per gram of polysaccharide."[9] However, data to support this statement are not found in this article, and neither is a citation to any literature that made such a measurement. That review might be the original spur for many subsequent erroneous claims. Citing to this paper, Becker states, "one gram of hyaluronic acid can hold up to 6 L of water."[10] While Jegasothy asserts "it can attach and hold large amounts of moisture; approximately 6 L of water in just 1 g"[11], 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.[12] With water's density of 1 g/mL, it is also worth noting these statements can be read to mean that HA binds 6,000 times its weight in water, not the 1,000 times more commonly claimed. Our review of this literature has not found a basis for this change; perhaps later authors were simply motivated to make a more conservative claim.

Several past DSC studies of solutions of HA of various molecular weights have been reported. The DSC method varies the temperature and measures the heat evolved/consumed as solid-liquid phase transitions occur. However, these studies were of much more concentrated solutions than studied here, approximately 1:1 w/w. This enabled observation by DSC of phase transitions of 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 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.
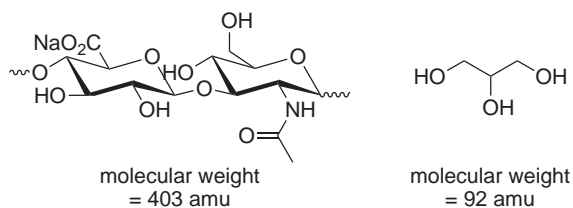
Chart 1. Chemical structures of the hyaluronic acid repeating disaccharide salt and glycerol. Hydrogen bonding to water is possible for each N and O atom. Solvation by water is possible for the Na ion.

Studies of high concentration HA solutions using DSC 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.[13] Molecular dynamics simulations agree with the number of water molecules per disaccharide monomer suggested by Joshi.[14] Ikada found 0.51 g non-freezing H_2O/g hyaluronic acid, which corresponds to 11.5 molecules of water per repeating HA disaccharide.[15] 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.[16] 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.[17,18] One interesting feature of this earlier report is that 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.

Multiple DSC studies of HA hydration used high concentrations which were needed to see thermal transitions associated with bound water. A single study by Cowman used solutions of HA as low as 0.5% concentration,[19] approaching closest to the situation studied herein. Cowman found an anomalously large amount of water associated with HA, calculated from conventional analysis of the thermal analytical data. She explained this finding by the idea that freezing bound water and non-freezing bound water are coincident at low 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 to measure the speed of sound in HA solutions to determine their compressibility. Davies found that in aqueous solution at 25°C, HA is "closely associated with not less than 9 molecules of water of hydration."[20] 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."[21]

While the foregoing reports used different methods to measure the amount of water strongly bound to hyaluronic acid, which produced somewhat different results, they are all similarly in the range of 0.36–0.86 g H_2O/g hyaluronic acid. These results are all obviously quite far from the 1,000 g per g of HA in the commonly made claim.

It is appropriate to consider the genesis of the erroneous claim of water binding by HA in the Sutherland review earlier discussed. Key early studies of HA were conducted by Ogston.[22–25] His work was not primarily aimed at determining the water-binding ability of HA, but rather at its particle size and structure. It used several measurements (centrifugation, viscosity) to measure particle size, finding that HA, despite being a linear polymer that adopts a coiled form in solution, behaves hydrodynamically like a large solvated sphere that encompasses a far larger volume than the molecular volume of the 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.[26] 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,[27] 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 6,000 mL/g HA, 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 statement by Sutherland that HA binds 6,000 times its weight in water, given water's density of 1 g/mL. It also corresponds to the 6 L per gram of HA that was earlier mentioned.

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 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.

EXPERIMENTAL DESIGN

This work examines the binding of water by HA using two experimental methods that have earlier been widely applied to the investigation of the physical chemistry of solutions of carbohydrate polymers. To address the specific claim that HA has exceptional properties as a humectant because of its ability to bind a 1,000 times its weight in water, we revisited past thermal analysis studies of hyaluronic acid solutions conducted at the supposedly special ratio of 1,000 g of water per gram of HA. HA water binding at higher concentrations (using a variety of modalities, including thermal analysis and colligative properties, reviewed above) has been extensively reported so it was unnecessary to study those higher concentrations or the concentration dependence, though that has been done with other spectroscopic methods.[28] Fallacies might also persist among practitioners that HA would form a unique material (such as a solid complex or a gel) at the supposedly special 1,000:1 ratio, which can be dispelled by simple observation of mixtures at this concentration.

The current work experimentally investigated aqueous solutions of HA at the key concentration of interest, as well as a 10% aqueous solution of a more mundane humectant, glycerol. These solutions have comparable viscosity.

MATERIAL AND METHODS

A commercial sample of fermentation-derived, cosmetic-grade HA (specifically, sodium hyaluronate) from Shandong AWA Biopharm (MW 0.92 mDa) (Binzhou City, China) 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. DSC was performed using a Netzsch® DSC 214 Polyma (Selb, Germany). TGA was performed using a Netzsch® TG 209 F1 Libra. DSC is a thermal analytical method where the difference in the amount of heat evolved or required to change the temperature of a sample is measured as a function of temperature. TGA is a method in which the mass of a sample is measured as the temperature is raised. These tools provide information on phase transitions of solutions.

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.

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. The heat uptake/evolution as the sample is cooled from ambient to –40°C and then warmed to a final target temperature is measured, from which the thermal parameters mentioned can be derived. These data can enable various types of water (freezing free, non-freezing bound, etc.) in a solution to be discerned. Results are summarized in Table I and data given in Figures 1–6. The freezing heat of crystallization should be the negative of the melting heat of fusion, measuring the heat absorbed/produced as the solid-liquid phase boundary is transited in each direction. That comparable absolute values are obtained for each parameter for each solution shows there is no hysteresis in this phase change.

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 2,230 J g^{-1}. The experimental values for water differ from literature by 2–4%, suggesting this is the error in these measurements. The reduction of melting heat of fusion and heat of vaporization in the glycerol solution are far greater than the HA solution, which is as expected owing to the higher molar concentration of glycerol. Both solutions give data significantly different from water.

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 10% glycerol solution shows a mass loss of 89.4% at 100.6°C, and the mass loss profile is significantly different from pure water.

Table I
Parameters for Solutions of HA and Glycerol Determined by DSC

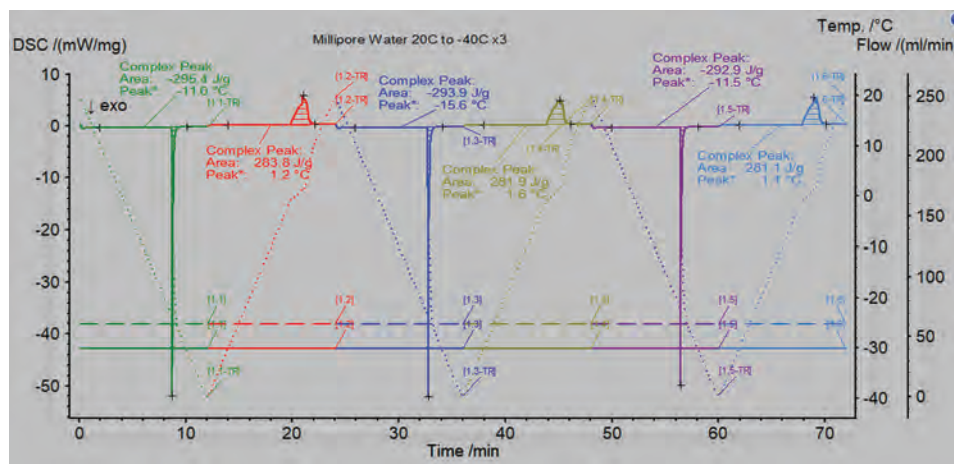|	HA	Glycerol	Water control
Freezing heat of crystallization (J g^{-1})	−251	−222	−294
Melting heat of fusion (J g^{-1})	262	223	320
Heat of vaporization (J g^{-1})	2295	2036	2273
Peak of melting transition (°C)	1.2	−1.6	1.3

Figure 1. DSC data for pure water scanned from 20 to –40 to 20°C in triplicate (control).
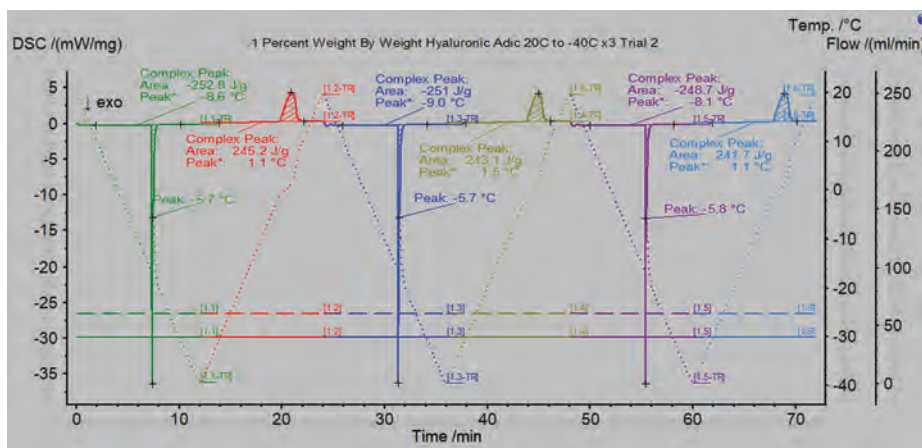

Figure 2. DSC data for 0.1% (w/w) solution of hyaluronic acid scanned from 20 to –40 to 20°C in triplicate.

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.

While the documentary evidence discussed in the background section 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 1,000 and dividing by the molecular weight of water gives the number of water molecules that would necessarily be bound to each polymer subunit,
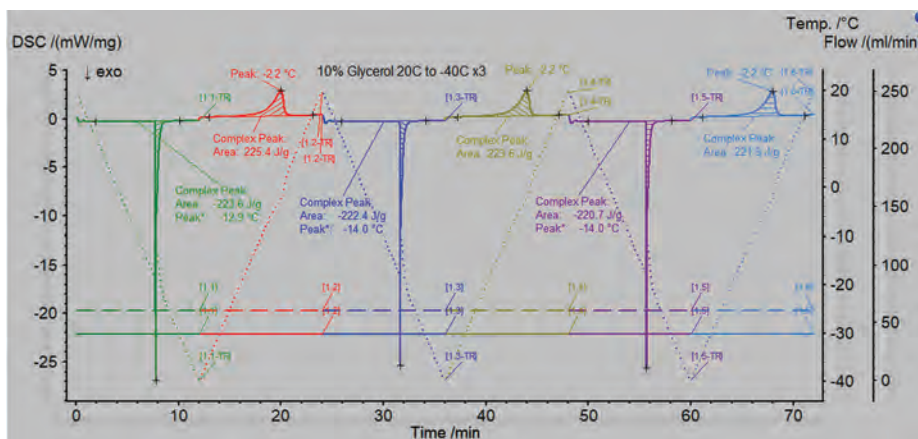
Figure 3. DSC data for 10% solution of glycerol scanned from 20 to −40 to 20°C in triplicate.
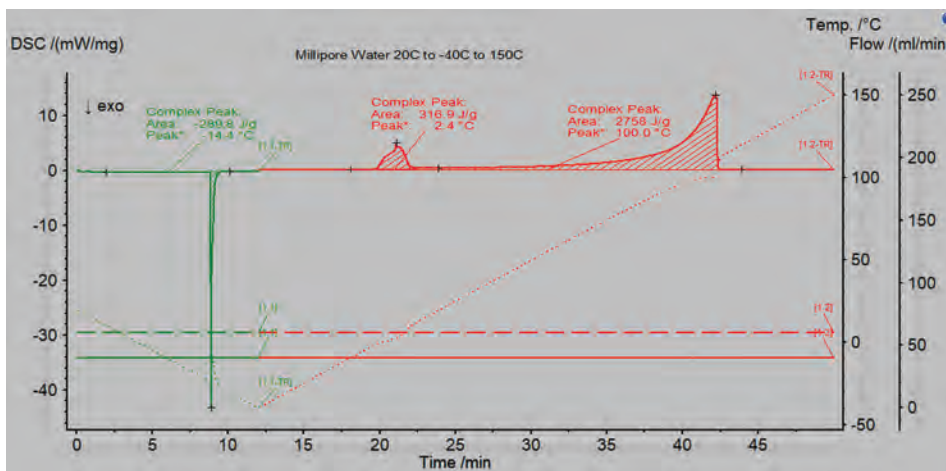

Figure 4. DSC data for pure water scanned from 20 to −40 to 150°C (control).

which is 22,389. Experimental measurements of the number of water molecules bound per disaccharide repeating unit discussed above gave ca. 14 ($\pm$ 5) waters per disaccharide repeat unit. The only 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 3 water molecules, for a theoretical maximum of 36 hydrogen-bonded water molecules per disaccharide. Because the molecular weight of water is 18 and that of the repeating unit in the HA polymer (N-acetylglucosamine-D-glucuronic acid) is 403, the theoretical mass ratio for a water-HA hydrogen-bonded complex would be $36 \times 18 = 648$ per 403 amu. That ratio works out to be 1.61 times the weight of the HA polymer.

While the foregoing seriously compromises the idea that HA binds 1,000 times its weight in water, there are other synthetic polymers that do bind large amounts of water per unit
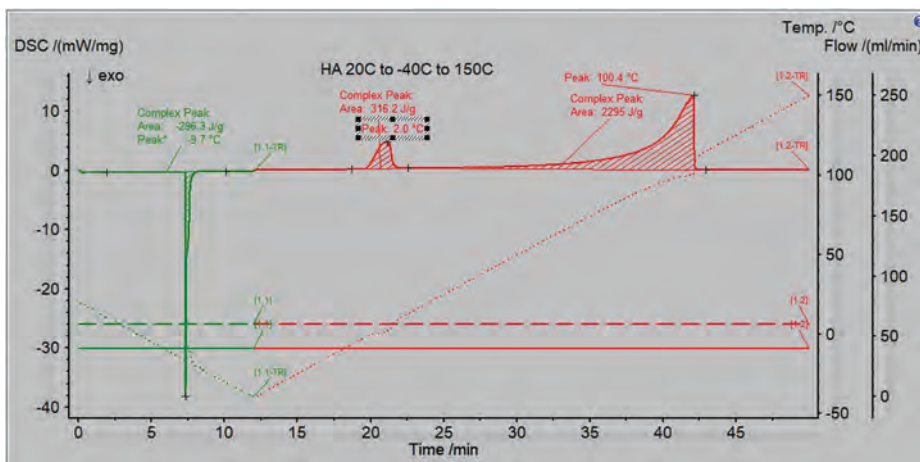
Figure 5. DSC data for 0.1% (w/w) solution of hyaluronic acid scanned from 20 to –40 to 150°C.
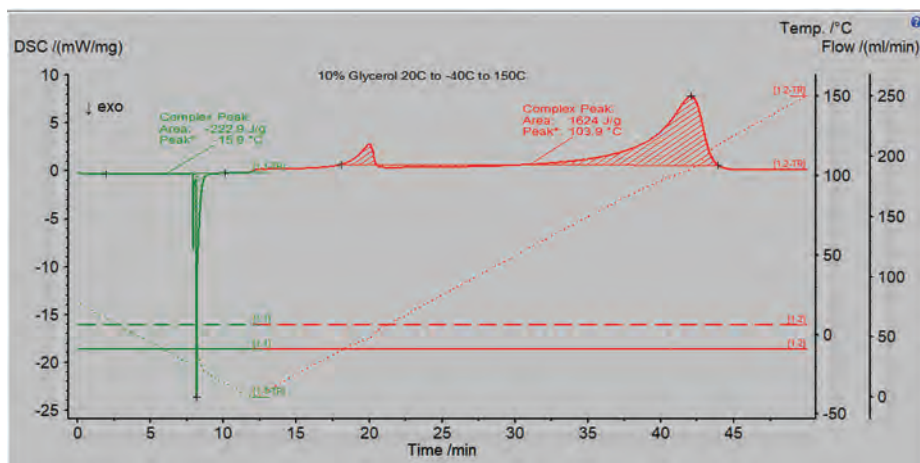

Figure 6. DSC data for a 10% solution of glycerol scanned from 20 to –40 to 150°C.

weight. For example, poly(acrylate) sodium salts are well-known, found in various personal care products, and may absorb 300 times their weight in water (from 30–60 times their own volume). 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 1,000 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 nine water
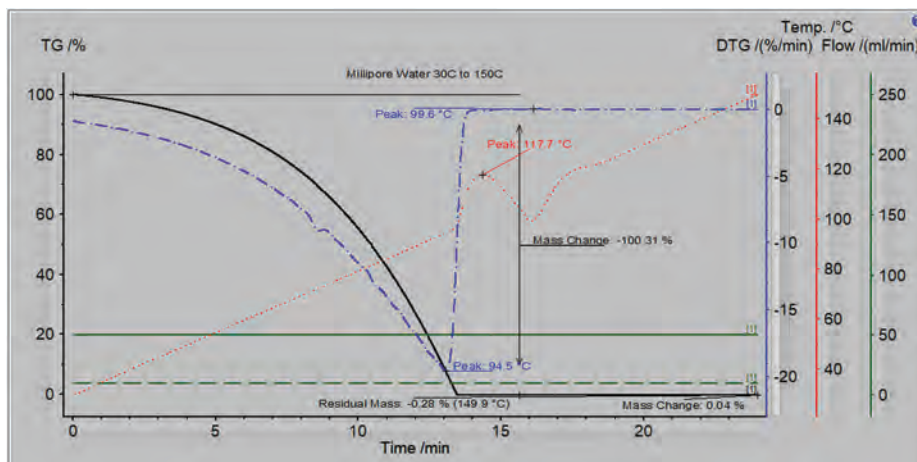
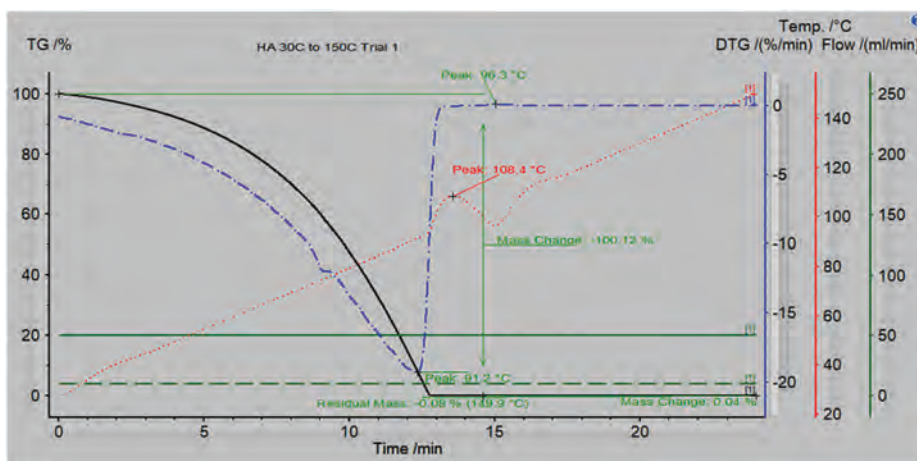**Figure 7.** TGA data for pure water from 30 to 150°C (control).

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

molecules, experimental studies have shown the actual hydrogen bonding by glycerol in dilute aqueous solution is 6.3 ± 1.4.[29] 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 experimentally found for HA. This finding agrees with the assertion that glycerol is the most effective humectant.[30]

CONCLUSIONS

This work dispels any notion that HA has remarkable water-binding power that makes it a superior humectant. A role of humectants in cosmetic products is to impede water loss by evaporation via binding to it. That process inherently requires the involvement of
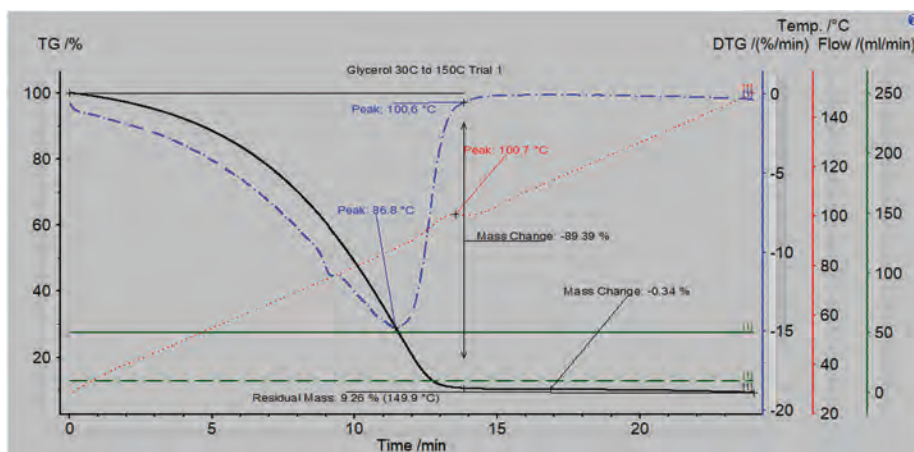
Figure 9. TGA data for a 10% solution of glycerol from 30 to 150°C.

a chemical force. Some of the other ways in which water might associate with hyaluronic acid have been found in detailed scientific studies (such as hydrodynamics) but may not play a useful role in the world of moisturizers. In fact, glycerin has the best performance as a humectant. HA may have other properties based on its native functions that still make it desirable to use in cosmetic products, however. The HA literature provides some hints about the roots of the fallacy of the special water binding properties of HA. Substantial past experimental studies combined with our experimental results show that a far more modest water-binding property for HA can be supported. Like any scientific misconception, it will be difficult to overcome the long-standing habits of thought in this field that attribute special water-binding properties to hyaluronic acid.

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