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Letter to the Editor

A note on an alternative DSC approach to study hydration of hyaluronan

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Recently, a paper on thermoanalytical investigation of hyaluronan hydration has been published in this journal (Průšová, Šmejkalová, Chytil, Velebný, & Kučerík, 2010). This note remarks upon several problematic inconsistencies in that paper.

First part of the commented paper describes DSC freezing/thawing experiments made on hyaluronan mixed with various amounts of water. Data are evaluated using the procedure used by Liu and Cowman (2000) which tries to distinguish several types of water in water–hyaluronan mixture (free and bound which can be freezing or non-freezing). However, their approach is not unambiguous and is based on assumptions which are not verified in the commented paper. The content of non-freezing water is not, in fact, determined from the fitting line shown in Figure 3 of the commented paper independently but a priori stated to be in parameters of the fitting line. Suggesting more types of water, the same parameter value may give different amount of non-freezing water as shown in the cited work by Liu and Cowman.

The whole approach does not discuss the basic principle of taking data from DSC measurement. The real “input” to DSC measurement is the sample weight and composition and the true output, in fact, is the measured heat (“Joules”). Experimental experience shows that the measured heat is a function of the sample weight and composition. This function can be written $\Delta H = f(m_\mathrm{w}, m_\mathrm{p})$ where ΔH is the measured heat, m_w is the weight of water in the sample and m_p is the weight of (bio)polymer. The most simple explicit formulation of this function is an equation of straight line $\Delta H = k_1 m_\mathrm{w} + k_2 m_\mathrm{p}$ (the line passes through the origin – when both weights are zero, no heat is measured). Normalizing by the polymer weight we obtain $\Delta H/m_\mathrm{p} = k_1 m_\mathrm{w}/m_\mathrm{p} + k_2 \equiv k_1 w_\mathrm{c} + k_2$, i.e. the same straight line as that suggested by Liu and Cowman (2000) and shown in Figure 3 of the commented paper. Thus, these two models are indistinguishable but the model shown here does not include any specification of water types. Physical meaning of parameters of a straight line in $\Delta H/m_\mathrm{p}$ versus w_c coordinates is therefore not clearly stated and proved.

The measured heat is claimed to be due to the water melting. Its normalization only to the weight of dry polymer is therefore unclear. There is no clear reason why not to investigate also its relation to the water content. Fig. 1 in this note shows that for the same data points as given in Figure 3 of the commented paper the dependence of measured heat on the weight of water in sample is close to straight line. This experimental observation was ignored in the paper though it seems to be of primary importance.

Let the straight line indicated in Fig. 1 be written as $\Delta H = k m_\mathrm{w} + q$. Dividing by the (dry) polymer weight we obtain $\Delta H/m_\mathrm{p} = k m_\mathrm{w}/m_\mathrm{p} + q/m_\mathrm{p} \equiv k w_\mathrm{c} + q/m_\mathrm{p}$. This should be the correct dependence of $\Delta H/m_\mathrm{p}$ on w_c following from what was primarily measured. Generally it is not straight line as that shown in Figure 3 of the commented paper. However calculations with the original data show that experiments were designed in such a way that (nonlinear) influence of the nonlinear term (q/m_p) is more or less suppressed (note that also the values of w_c and m_p may be intercorrelated by the design of experiments) and calculated data can be well approximated by a straight line as illustrated in Fig. 2.

It thus seems that the linear dependence shown in Figure 3 of the commented paper and used there to evaluate the non-freezing water content has its origin in the primary measured data shown in Fig. 1 of this note. It is a matter of further analysis to find whether this is a result of improper experimental design or really of some physical cause. It could be further demonstrated (though not shown here) that also the dependence of the measured heat per gram of the sample is a linear function of water mass fraction or that there is a clear dependence of the measured heat per gram of water on the water mass fraction (and linear up to about 70% of water). All this was ignored in the commented paper.

Comparing Figure 3 and Figure 2 of the commented paper, it can be concluded that the Figure 3 is just indirect way how to find water content (w_c) below which no peak can be detected during DSC experiment. The linear model presented here tells that nothing is measured also when the water and biopolymer contributions are compensated: $\Delta H = 0 \Leftrightarrow k_1 m_\mathrm{w} = -k_2 m_\mathrm{p}$.

It should be noted that Pouchlý, Biroš, and Beneš (1979) published a detailed thermodynamic analysis of freezing/thawing experiments and pointed to some other problems in using this approach to determine various types of water in polymer–water systems.

The second part of the commented paper reports on results of DSC measurement of water evaporation from wet hyaluronan.

On the basis of evaporation enthalpy measurements “non-evaporable water” is claimed to be detected and its amount for sodium hyaluronate was found to be 0.31–0.38 g/g of dry biopolymer. This amount corresponds to water content of 24–28% (by weight). Figure 6 in the commented paper shows clear (evaporation) peak even for the water content of 0.08 g/g (7.4%). As far as I know, the producer’s standard limits the moisture content in hyaluronan up to 5–10%. Thus much more water can be evaporated and the determined amount of non-evaporable water is unjustified. The paper does not give any direct proof of existence of this type of water. The paper states: “. . .formation of the ordered hyaluronan structure. . .starts immediately when the hydration water is desorbed from the hyaluronan surface. The concentration at which the

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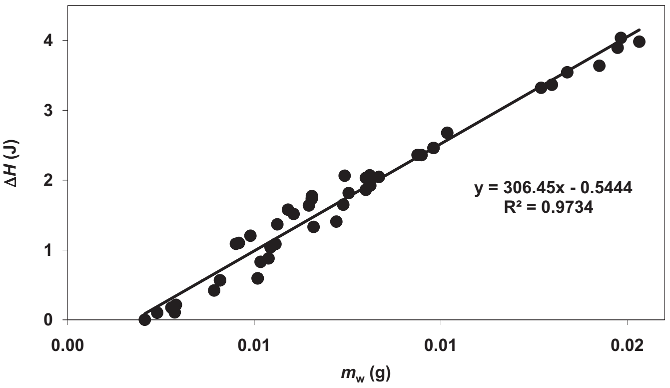

Fig. 1. Measured heat as a function of the weight of water in sample.

process occurred was related to non-evaporable water. . .” which is confusing contradiction.

The evaporation experiments are, in fact, close to drying processes which are well studied and described, e.g., in chemical engineering or silicate industry; only one recent reference, containing references to many other works, is given here as an example (Clausen, Watzke, Hughes, Plummer, & Manson, 2011). All this knowledge is ignored in the paper. The results are presented and evaluated in the form of measured enthalpy divided by the weight of the dry polymer. There is no theory explaining why just this normalization is necessary or why is preferred. Taking into account also the analysis of the measured heat or of the heat per unit water mass is necessary to arrive at more realistic conclusions.

It is well known that in the first stage of drying, when the moisture content is high, the evaporation occurs from the free liquid surface, by a constant rate. Let us assume that above certain concentration of water in the hyaluronan–water mixture, the evaporation is controlled by the free surface evaporation process and the heat observed (measured) is directly proportional to the amount (weight) of water in the sample, regardless the amount of biopolymer: $\Delta H_{\text{obs}} = k_3 m_{\text{w}}$. Dividing by the mass of the dry biopolymer we obtain: $\Delta H_{\text{obs}}/m_{\text{p}} = k_3 m_{\text{w}}/m_{\text{p}} \equiv k_3 w_{\text{c}}$. This is the equation of a straight line going through the origin of coordinate system. Looking at the Figure 7 in the commented paper and taking into account that data for 101 and 1390 kDa hyaluronans are shifted upwards it is seen that the data correspond to this equation for w_{c} higher than 0.3–0.4 (i.e. the water content higher than the content of “non-evaporable water”). The data also show that lines measured for different biopolymer molecular weights are not different as expected for this simple explanation. Intercepts on the y-axis are not exactly zero which can be ascribed to experimental uncertainty.

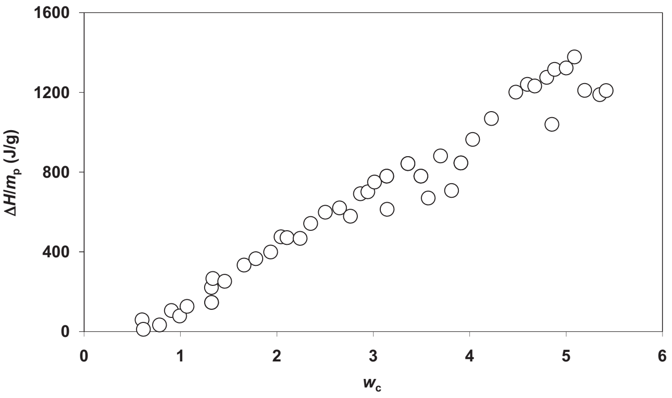

Fig. 2. Recalculated heat per unit hyaluronan mass as a function of the relative water content in sample.

When the water concentration is so low that the free surface no more can be formed water evaporates (releases, better speaking) from different states, e.g., thin layers covering solid particles, from pores or capillaries, from adsorbed state. Let us further suppose that below the water concentration representing the lower limit for free surface forming, evaporation occurs primarily from the interstitial space and thin layers on biopolymer particles (this is the governing evaporation mechanism) and that the observed heat is determined by the amount of biopolymer forming the interstitial space and bearing the water layers (regardless the actual water content): $\Delta H_{\text{obs}} = k_4 m_{\text{p}}$. Dividing by the mass of the dry biopolymer we obtain: $\Delta H_{\text{obs}}/m_{\text{p}} = k_4$, i.e. the observed enthalpy normalized by the weight of dry biopolymer is constant and does not depend on the water concentration (w_{c}) – exactly what is shown on Figure 7 in the commented paper for low values of w_{c}.

Thus, the data measured for water evaporation from sodium hyaluronate–water mixture can be explained also by simple ideas on drying process with no need for unjustified hypothesis on additional heat evolution process. The problem is that the paper does not distinguish between the total enthalpy measured by DSC and the recalculated (specific?) enthalpy per unit polymer mass. Once again, analysis of primary measured quantities is missing.

Data for the acid form of hyaluronate (the inset in Figure 7 of the commented paper) show similar behavior – perhaps the first point (for the lowest water content) indicates yet another stage of water evaporation because it does not rest on (constant) straight line with the successive two or three points; more data points would be necessary in this concentration region.

Another crude estimate could be made. Let us suppose, as a first approximation, spherical shape of (dried) biopolymer particles and ignore the differences in density. It is well known that the free space (volume) between spheres depends on their packing and is between 25.9 and 47.6% which would correspond to the content of interstitial water from 0.35 to 0.92 g/g. This estimate could be specified more precisely using the actual shape and density. Nevertheless, the “non-evaporable water” content reported in the commented paper falls within this range.

Use of thermoanalytical investigation of water evaporation is neither original nor new approach in study of biopolymers hydration. For example, Hatakeyama and Hatakeyama (1998) gave a general overview of thermogravimetric study of hydration water, Muffett and Snyder (1980) applied “evaporative” DSC to study soy protein hydration (and normalized the measured heat to the weight of water which is much more appropriate) and De Andres-Santos, Velasco-Martin, Hernández-Velasco, and Martín-Gil (1994) used “evaporative” DSC just for hyaluronan.

To summarize briefly:

(1) Freezing/thawing experiments in Průšová et al. (2010) seem to result only in determining the water content at which no melting peak can be detected but causes of not melting were not clarified. Some important and interesting experimental outcomes have been ignored.

(2) Evaporation experiments in Průšová et al. (2010) very probably determined only the end of evaporation from the free surface (the lowest water content at which this stage of evaporation occurs). The concept of the “non-evaporable water” is unjustified.

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