HYDRATION OF BIOPOLYMERS STUDIED BY DSC AND PERFUSION MICROCALORIMETRY

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Abstract

Hydration is one of the crucial properties for understanding the behavior of any chemical material. Due to the presence of hydrogen bonds together with hydrophobic and other types of intermolecular interactions water sorption influences the properties of the substances. Moreover, the importance of the absorbability is even higher when speaking about biopolymers which are somehow connected with hydrogels applications, potentially used in wound healing. The aim of this work was to study water sorption ability of selected biopolymers (dextran, chitosan, hyaluronan) and humic acid using two thermoanalytical techniques: differential scanning calorimetry (DSC) and relative humidity (RH) perfusion microcalorimetry. These methods which are based on different measurement principle can give a complex overview on the sorption behavior of the sample. The results show expected differences of both temperature of melting and heat of hydration which are caused by many factors such as presence of side functional groups on the biopolymer chain, solubility of the studied biopolymers in water or molecular weight when speaking about hyaluronan.

Keywords: Hydration, biopolymers, DSC, perfusion microcalorimetry

1. INTRODUCTION

Calorimetry is a wide tool for material characterization. There are several kinds of calorimetry which can detect different types of sample changes. This study focuses on two calorimetric techniques - differential scanning calorimetry (DSC) and relative humidity (RH) perfusion microcalorimetry. The motivation of the work was to study the process of water sorption of selected biopolymers using different methods. The most studied material from the sorption point of view is amorphous lactose. Basically, amorphous materials are well studied by perfusion microcalorimetry because it is a nice tool for amorphous phase content determination and therefore it is recommended for characterization of pharmaceuticals [1-4]. Hyaluronan (HYA) is a unique polysaccharide from the point of view of hydration, too. It is a material with a very high water sorption capacity. The physico-chemical properties of hyaluronan are described elsewhere [5,6] and the sorption isotherms of hyaluronan are studied in [7]. The other materials (dextran, chitosan and humic acid (HA)) were chosen due to their different structure, origin or presence of different side functional groups on the main polymer chain which could influence the ability of water sorption.

2. MATERIALS AND METHODS

Hyaluronan of bacterial origin of two molecular weights was purchased from Contipro Group, Ltd., Czech Republic, chitosan (molecular weight 168 kDa) and dextran (molecular weight 425 - 575 kDa) of the best available purity were purchased from Sigma Aldrich and used as received. Humic acid was isolated from lignite in the author's laboratory by a method described elsewhere [8].

The weighted sample powder was put in aluminium pans with small amount of water for dissolving the sample. Humic acid and chitosan were measured as suspensions, other samples were measured as solutions. The experiments were performed under following conditions: temperature range from -50 to 25 °C, heating rate 3°C/min, nitrogen atmosphere. The samples were measured both immediately after preparation and after 24 hours. The experiments were performed using a DSC instrument Q2000 (TA Instruments). The weighted sample powder (approx. 10 mg) was put in the measuring cell and the holder of the cell was inserted in the

calorimeter. The sample in the measuring cell was wetted by nitrogen (gas) flow which relative humidity and flow rate was controlled by the mass flow controllers. The illustration of the perfusion cell is in the **Figure 1**. All the experiments were performed according following conditions: temperature 25 °C, RH constant 10 % for 30 minutes, increasing RH from 10 to 95 % for 48 hours, then 2.5 hours of stabilizing under 95 % RH. The experiments were performed using a modular microcalorimeter TAM III (TA Instruments).

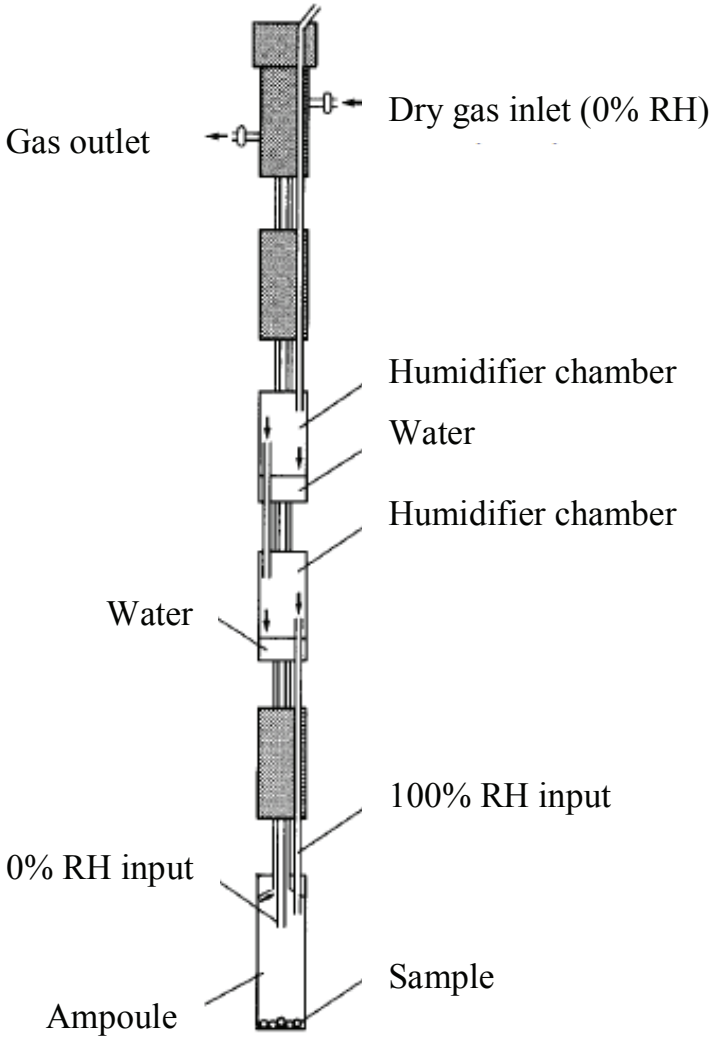

Figure 1 A schematic of the RH perfusion cell [9]

3. RESULTS AND DISCUSSION

3.1. DSC

DSC records have two peaks, exothermic above the baseline and endothermic under the baseline. The exothermic peak corresponds to solidification and crystallization of possible amorphous phase and the endothermic corresponds to a melting process. The area under the peaks is proportional to amount of heat released or absorbed during the process and the high of the peaks is proportional to the temperature gradient during the reaction. For this purpose, only the parameters of the endothermic peak were analyzed - temperature onset and the height of the peak. These parameters are connected with the degree of sample hydration. The lower is the onset temperature, the higher is the hydration ability. In other words, the melting temperature of the sample decreases with increasing water content.

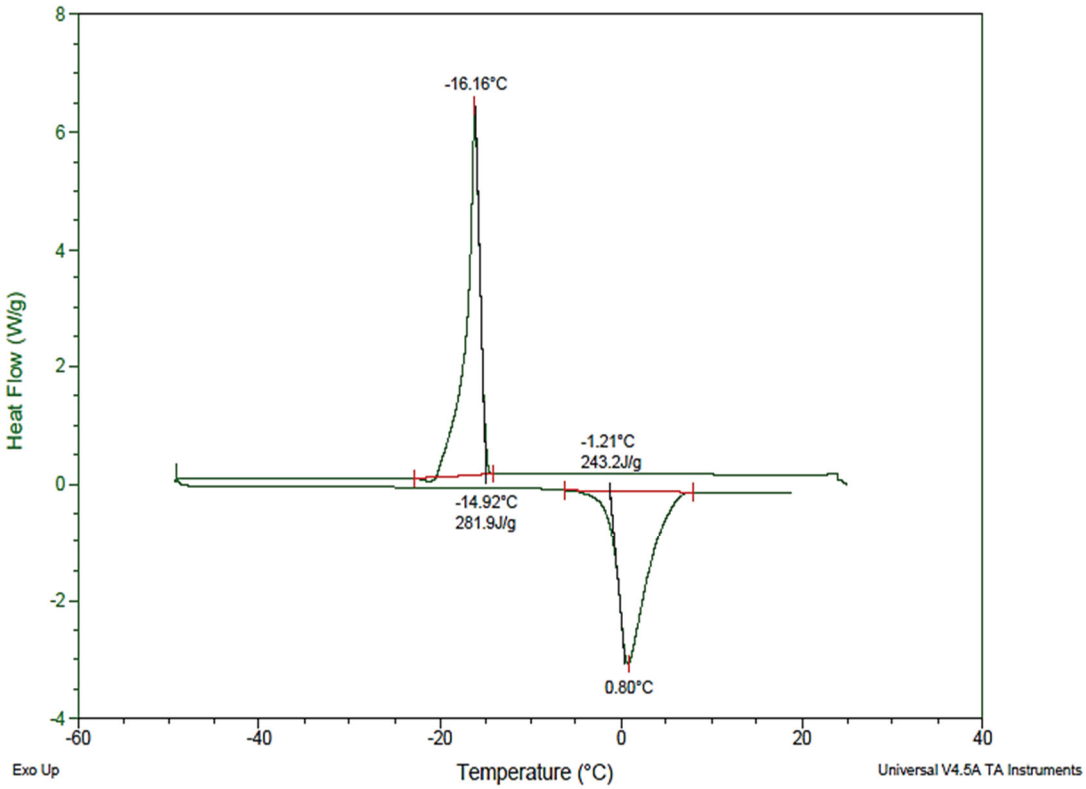

Figure 2 DSC curves of chitosan measured after preparation

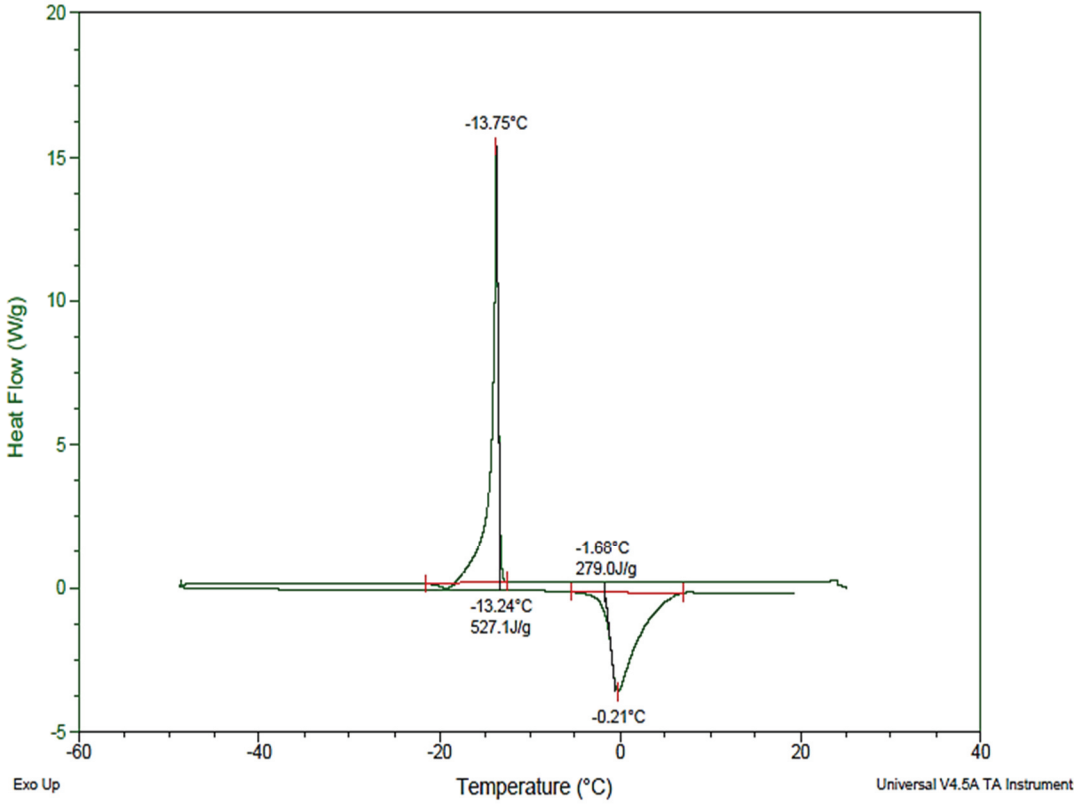

Figure 3 DSC curves of chitosan measured within 24 hours of preparation

The selected results for chitosan are presented in **Figure 2** and **Figure 3**. The determined offset temperatures, temperatures of melting as well as heat of melting for all measured samples are summarized in **Table 1**.

Table 1 The evaluated results from DSC measurement, where Q_m is heat of melting, T_{onset} is onset temperature and T_m is melting temperature.

	After preparation			After 24 hours		
Sample	Q_m (J/g)	T_{onset} (°C)	T_m (°C)	Q_m (J/g)	T_{onset} (°C)	T_m (°C)
HA	173	-2.12	0.70	268	-1.33	-0.08
Chitosan	243	-1.21	0.80	279	-1.68	-0.21
HYA 300 kDa	227	-5.03	-1.34	195	-7.69	-2.92
HYA 1400 kDa	211	-4.72	-0.82	187	-6.55	-1.25
Dextran	206	-3.87	-0.97	184	-4.78	-1.29

As expected, all the samples show lower temperatures of melting after 24 hours which corresponds to higher water content compared to fresh samples. The differences in the heat of melting increases after 24 hours for HA and chitosan (suspensions) and decreases for HYA and dextran (solutions).

3.2. RH perfusion

The results from RH experiments show similar trends for all the samples. The first part of the curve with a relatively small signal change is followed by the relaxation part before the increase of the signal which corresponds to the most intensive water sorption by the sample. The sharp decrease of the signal at the constant RH 95 % will be studied in details in the future experiments.

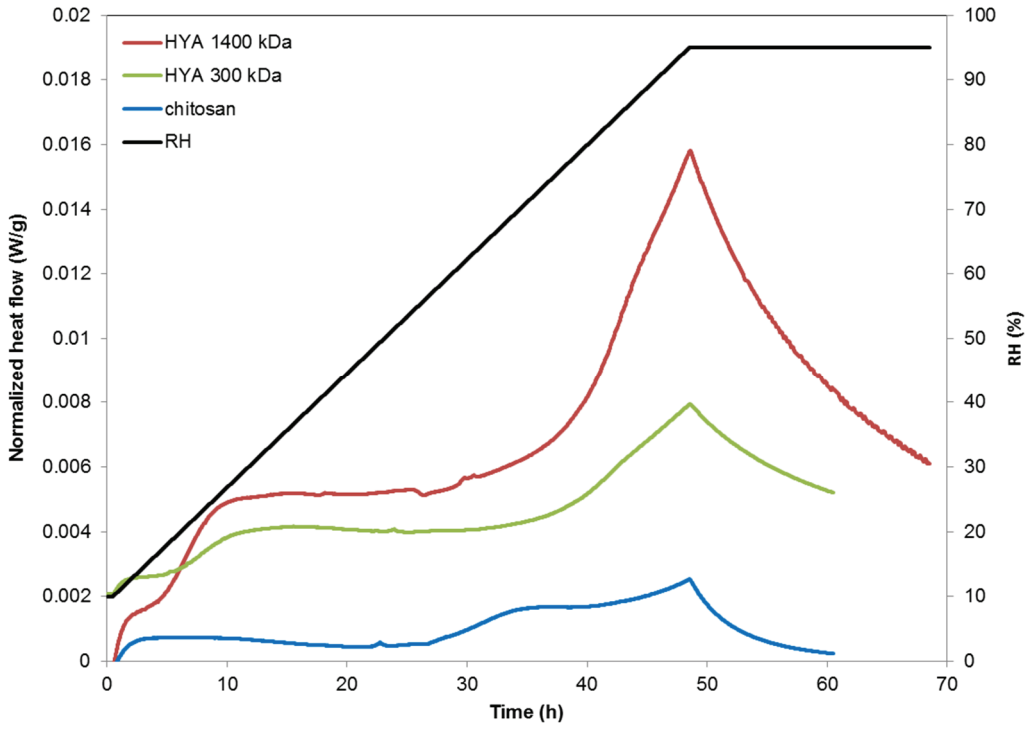

Figure 4 The calorimetric responses to the adsorption of water vapor on chitosan and both hyaluronans in the RH perfusion cell. The heat flow data are connected with the left y-axis, the RH values for the black curve are displayed to the right y-axis

The selected results for chitosan and both hyaluronans are shown in **Figure 4**. The evidence of the interaction between samples and added water vapour (wetted nitrogen) is expressed in increasing (exothermic) signals of all the studied samples. The intensity of the signal corresponds to the greater sorption ability. The less intensive signal was observed for chitosan, the most intensive signal is shown for hyaluronan of higher molecular weight which was expected due to the well-known sorption properties of this polysaccharide.

CONCLUSION

The presented results show that the measuring conditions should be optimized for future measurements to obtain the whole sorption curve when the signal is back close to the baseline also at the end to the experiment. But evidently, hyaluronan of both molecular weight showed greater sorption ability (from RH perfusion results) than the other samples which corresponds to DSC where hyaluronan show big differences between T_m of fresh and samples measured after 24 hours.

ACKNOWLEDGEMENTS

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