Hydration and swelling of amorphous cross-linked starch microspheres

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19 **Abstract**

20 Hydration of cross-linked starch microspheres, commercially available as a medical
21 device, was investigated using a multi-method approach. We found that the uptake of water is
22 accompanied by substantial swelling and changes of the polymer structure. Sorption calorimetry
23 provided information about thermodynamics of water sorption, revealed presence of isothermal
24 glass transition and absence of hydration-induced crystallization, observed in non-cross linked
25 starch material. The changes in the surface and bulk properties of microspheres at different water-
26 starch concentrations were investigated using synchrotron radiation X-ray scattering and analyzed
27 using concept of fractals. The obtained information, combined with the results of differential
28 scanning calorimetry, was used to construct a phase diagram of the studied material. Finally,
29 hydration induced evolution of polymer structure revealed by the X-ray scattering was linked to
30 the changes observed during swelling with optical microscopy.

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1. Introduction

Starch is a biopolymer that is highly abundant in nature where it serves as energy storage in plants. It is synthesized as densely packed granules and consists of the two polysaccharides amylose and amylopectin. Because of the great availability and low price, starch has found a wide range of applications in almost all branches of industry, e.g., in the food, paper, packaging and pharmaceutical industry. Different physical and/or chemical modifications are often employed to tune the properties of starch for practical uses. An example of a modern material obtained by modification of starch is starch microspheres that are used for oral, nasal and intramuscular drug delivery, i.e. for transporting active compounds into the body to achieve a desired therapeutic effect in the target place. Examples of such formulations can be found in the literature and may contain proteins or large molecular weight organic compounds (Edman, et al., 1992, Illum, et al., 1987, Illum, et al., 1990, Pereswetoff-Morath, 1998, Elfstrand, et al., 2006, Rodrigues and Emeje, 2012). Degradable starch microspheres are also in use as a medical device for acceleration of wound healing by promoting hemostasis. The microporous starch particles are applied directly on the wound site where they take up fluid from the blood and create a gel which stops the bleeding (Björnses, et al., 2011, Malmsjö, et al., 2011, Tan and Tope, 2004).

The hydration properties of native starch granules as well as modified starches have been widely investigated since they are very important for most applications (Svensson and Eliasson 1995, Carlstedt 2014, Carlstedt 2015). In native starch granules only a minor part of the polymer is mobile, most of the chains are densely packed and thus isolated from bulk water (Larsen, et al., 2008) while the chains of the amorphous starch microspheres are more accessible for water so their structure resemble hydrogels: hydrophilic three-dimensional networks, held together by

chemical or physical bonds. Some examples of starch-based biodegradable hydrogels may be found in the literature (Elvira, et al., 2002).

An important phenomenon related to hydration is the glass transition in the amorphous parts of starch. Glass transitions of starch can be detected by, e.g., DSC (Thiewes and Steeneken, 1997). They can be caused by increase of temperature and/or addition of plasticizers, i.e. small molecules such as water or glycerol, which shift the glass transition to lower temperatures (Lourdin, et al., 1997). Interestingly, parts of the same chain can be in a crystalline whereas another part in a disordered state in semi-crystalline polymers (Thiewes and Steeneken, 1997).

Another important aspect of hydration is the swelling of the material. In the drug delivery systems release from the microspheres is conducted in the sustained swelling-controlled manner (Fang, et al., 2008). When used for stopping bleeding, it is of particular importance that the starch microspheres are highly absorbing so that they can prevent the blood flow.

In the present work hydration of cross-linked degradable starch microspheres was investigated with a multi-method approach. Methods such as sorption calorimetry, small angle X-ray scattering, gravimetrical swelling, rheology and differential scanning calorimetry were applied to characterize interactions of the amorphous starch and water. The aim of this study is to investigate hydration of the material including its high absorption capacity and swelling properties. In addition, we use X-ray methods to study structural rearrangements that accompany the hydration of the studied material. The obtained data are collected in a temperature vs. composition phase diagram of the starch microspheres – water system.

2. Materials and methods

2.1. Materials

2.1.1. Starch microspheres

The starch materials were provided by Magle AB (Kristianstad, Sweden). Spray-dried acid hydrolyzed potato starch (maltodextrin) was produced by Lantmännen Reppe AB (Växjö, Sweden). The degradable starch microspheres (DSM) are manufactured by Magle AB (Kristianstad, Sweden) by emulsion crosslinking (polymerisation) of acid hydrolysed potato starch with epichlorohydrin. Prior to measurements the starch material was dried at room temperature in vacuum with 3 Å molecular sieves overnight. The samples were subsequently prepared by equilibration with saturated salt solutions vapor or by adding liquid water.

2.2.2. Salt solutions

Seven different saturated salt solutions were used for setting up relative humidity: LiCl, MgCl₂, Mg(NO₃)₂, NaCl, KCl, KNO₃ and K₂SO₄ with corresponding relative humidity values (RH%) 11.30, 32.80, 52.90, 75.30, 84.30, 93.28 and 97.30 (Greenspan, 1976). Prior to use, the saturated salt solutions were equilibrated for a few weeks at room temperature.

2.2. Scanning Electron Microscopy

The morphology of dry starch microspheres was examined with a scanning electron microscope (Zeiss EVO LS10 SEM). The experiments were performed at 25°C in vacuum at an acceleration potential of 2kV. The material was dried in vacuum prior to examination to ensure minimal moisture content, and then deposited on a graphite covered standard sample holder.

The obtained micrographs were analyzed with respect to morphology and size of the microspheres. The size was established as the mean value of the diameters measured horizontally and vertically for each particle as a distance in pixels. The value was then converted from pixels to µm using the size of the scale bar.

2.3. Sorption calorimetry

Hydration of starch at 25°C was investigated with sorption calorimetry. During the experiment, the water activity a_w and the hydration enthalpy H_w^{mix} are measured simultaneously as a function of water content (Wadsö and Markova, 2002). As all physical and chemical processes are accompanied by release or absorption of heat, sorption calorimetry provides information about the processes occurring within the sample.

The measurements are performed in a two-chamber calorimeter cell inserted in a double-twin microcalorimeter (Wadsö and Wadsö, 1996). The thermal powers from the two chambers are recorded and the thermal power of evaporated water recorded in the vaporization chamber is used to calculate the water activity using an earlier proposed equation (Kocherbitov, 2004)

The partial molar enthalpy of mixing of water is calculated from the relation below:

$$H_w^{mix} = H_w^{vap} + H_w^{vap} \frac{P^{sorp}}{P^{vap}} \quad (1)$$

Where P^{sorp} and P^{vap} are the thermal powers recorded in the sorption and vaporization chambers, and H_w^{vap} is the molar enthalpy of vaporization of pure water (44.0 kJ/mol at 25°C).

2.4. Optical microscopy

An optical microscope (Nikon OptiPhot) was employed to investigate morphological changes and swelling of starch microspheres caused by addition of liquid water. Dry microspheres were placed directly on glass slides and their behavior upon hydration was captured in form of a movie. The recorded movies were subsequently analyzed in terms of changes in size of the microspheres by comparison of the sizes in pixels before and after swelling.

2.5. Gravimetric swelling study

To analyze the swelling limit and water absorption capacity five samples of 1-5 wt% of starch microspheres in water were prepared. After equilibration the suspensions were separated by centrifugation (10000 rpm). The mass of water absorbed by the microspheres $m_{w,a}$ was established

based of the masses of the water used to prepare the suspension and the mass of the supernatant:

$$m_{w,a} = m_w - m_{sup} \quad (2)$$

where m_w - mass of water used to prepare suspension, m_{sup} - mass of the supernatant.

The degree of swelling expressed as the ratio of the absorbed mass to the mass of dried microspheres was calculated based on the swelling study:

$$Q_m = \frac{m_{w,a}}{m_s} = \frac{m_p - m_s}{m_s} \quad (3)$$

where Q_m - degree of swelling, m_p - mass of particles, m_s -mass of starch used to prepare suspension.

2.6. Rheology

Viscosities of suspensions of microspheres with concentrations ranging from 0.5 to 2 wt% were determined with a capillary viscometer calibrated with miliQ water. A measurement is performed by first pumping up the suspension into a glass capillary. Then the time required for the liquid to flow, due to gravity, between two points marked on the capillary was measured. The viscosity was calculated based on the Hagen-Poiseuille equation which relates the pressure drop in the fluid flowing through the tube to the viscosity of the fluid (Hagen, 1839, Poiseuille, 1841)

$$\Delta P = \frac{8\eta L \Delta V}{\pi r^4 \Delta t} \quad (4)$$

Where: η – viscosity, ΔP - the pressure drop, ΔV - volume of the fluid, Δt – time required for the liquid to flow between marked points, L - length of the capillary, r –radius of the capillary.

For each suspension an average of 10 measurements was calculated and compared with the value obtained for pure water which has a known viscosity.

2.7. Differential Scanning Calorimetry

Several concentrations of starch – water mixtures were analyzed with a DSC (DSC 1 Mettler Toledo). Samples were placed in 40 μ l aluminum crucibles, hermetically sealed and after equilibration scanned in temperature ranges between -80°C and 160°C at a scanning rate of either 1°C/min or 10°C/min. Indium was used as calibrant and an empty aluminum crucible (40 μ l) was used as a reference. A dry nitrogen gas flow of 80 ml/min was applied in the furnace chamber of the DSC instrument.

2.8. *Small-angle X-ray Scattering (SAXS)*

Structural aspects of the microspheres were studied using small-angle X-ray scattering (SAXS). The experiments were performed at the MAX IV Laboratory, Lund, at beamline I911-SAXS (Labrador, et al., 2013). The wavelength of the beam was 0.91 Å and the sample to detector distance was 1340 mm. The software Fit2d was used for data evaluation (Hammersley, 1997). Background subtraction (empty solid sample holder) was performed using program written in Matlab.

3. Results

3.1. *Scanning electron microscopy*

SEM micrographs reveal the variety of shapes and sizes of the investigated starch microspheres (Figure 1). Most of the microspheres appear as solid, non-damaged, round shaped particles with sharp contours and coarse surface, whereas a few appear to have a smoother surface. The starch microspheres do not form aggregates.

Figure 1. SEM pictures of dry starch microspheres at various magnifications.

3.2. Sorption calorimetry

Sorption of water by the starch microspheres was studied with sorption calorimetry and compared to our earlier data for acid hydrolyzed starch – an intermediate material used in the production (Carlstedt, et al., 2014). The sorption isotherms show the relation between water activity and amount of water absorbed by the sample, see Figure 2.

The enthalpy plot presented in Figure 2 shows that the hydration of starch is initially an exothermic process. Moreover, a thermal event in form of a step in the enthalpy plot between 17.2 and 21.1 wt% of water is visible.

Figure 2. Water sorption isotherm (a) and enthalpy of hydration (b) of starch microspheres (black) and acid hydrolyzed potato starch (red) obtained with sorption calorimetry at 25°C.

3.3. Optical microscopy

Optical microscopy was used to visualize the microspheres in the presence of different amounts of water and to track the swelling of the individual particles. It was observed that the microspheres in the presence of water change their shape, size and general appearance: dry particles are dark while hydrated are transparent (Figure 3.). The structural changes observed upon hydration of the microspheres are discussed in section 4.4.

Figure 3. Starch microspheres in the presence of water. From left top corner: 10, 20, 30, 41, 50, 59, 73, 82, 92 and 100 wt% of starch.

Moreover, in the presence of liquid water the microspheres undergo rapid swelling (Figure 4). The change of size is substantial: comparison of the sizes before and after contact with water made for 20 particles showed that the diameter increase on average 2.1 ± 0.2 times. No further swelling was observed after 16 s.

Figure 4. Swelling of starch microspheres captured by optical microscopy. Some additional microspheres were transferred into the observed area by water.

3.4. Gravimetric swelling study

To further evaluate the swelling process, a gravimetric swelling study was performed as described above. A linear correlation between the mass of absorbed water and the mass of starch used for preparation of the suspension was found (Figure S1 in supplementary information). It should be noted that the line obtained by linear regression does not cross the (0,0) point on the graph because of an experimental error that is caused by some amount of water on the phase border that cannot be completely separated from the sediment. The water trapped between the swollen

particles is dependent on the amount of starch, i.e. the more starch there is the larger the error. Thus the constant in the linear equation may be attributed only to the experimental error brought by the separation method. According to the results, the swelling degree is equal to 10.6 g/g.

3.5. Rheology

The viscosities of the dilute suspensions of starch microspheres were determined with a capillary viscometer. A linear relation between the relative viscosity and the concentration of starch between 0.10 and 1.55 wt% was observed (Figure S2). To estimate the volume fraction of swollen starch particles in dilute suspension, the Einstein equation for viscosity of a dilute suspension of hard spheres was applied (Einstein, 1906):

$$\eta_r = 2.5\phi + 1 \quad (5)$$

where: $\eta_r = \frac{\eta_{solution}}{\eta_{solvent}}$ – relative viscosity, ϕ – volume fraction of swollen spheres. Obtained values were plotted against the concentrations in Figure S2.

3.6. Differential Scanning Calorimetry

In order to investigate thermal events in the starch-water system differential scanning calorimetry (DSC) was employed. Melting of water was observed in samples containing up to 70 wt% of starch microspheres; an example of the melting peak is presented in Figure 5(a). For samples with starch concentrations ranging from 20 to 70 wt%, the glass transition step was observed before the ice melting peak, while in more concentrated samples it was observed as a single step (Figure 5(c)) or together with an additional endothermic peak (Figure 5(b)).

Figure 5. DSC scans for samples of different concentrations of starch microspheres (a) 40.6 wt% scanned at 1°C/min, (b) 81.69 wt% at 1°C/min, (c) 89.30 wt% at 1°C/min, (d) 92.45 wt% scanned at 10°C/min, 1st (red) and 2nd scan (black).

In low moisture samples (above 80 wt% of starch) an additional endothermic peak may be observed (Figure 5(b,c,d)). This peak is not present in the second scan performed directly after first scan (Figure 5(d)); however, it generally reappears upon storage at room temperature. We assign this peak as originating from a sub-T_g transition (see discussion section).

The dependence of the enthalpy of melting of ice on the concentration of water has a pronounced linear character (Figure S3 in supplementary information). Based on this linear dependence, the amount of non-freezing water in starch microspheres was determined to be 27.4 wt% or 0.38 g/g, which corresponds to on average 3.4 water molecules for each repeating unit of starch.

3.7. Small-angle X-ray scattering (SAXS)

Small-angle X-ray scattering (SAXS) was used to investigate structural aspects of the starch microspheres. Figure 6 shows curves obtained for mixtures of starch and water with different concentrations. No clear Bragg peaks were detected in the whole concentration range, however, the shapes of the curves change with concentration, which will be further discussed in the Discussion section below.

Figure 6. SAXS curves for samples of 10 to 40 (a), 50 to 70 (b) and 82 to 100 wt% of starch microspheres (c).

Samples were studied at 25, 40 and 70°C. As presented in Figure 7, for the samples of 82 and 85 wt% a decrease of intensity was observed after heating above 40°C. It was also noticed that for the scans performed after cooling the sample back to 25°C, the curves coincided with the high temperature curves and not with the initial ones at 25°C, suggesting an irreversible change.

Figure 7. Temperature dependences of SAXS curves for 65 (a), 82 (b), 85 (c), and 89 (d)

wt% of starch.

4. Discussion

4.1. Water sorption

The sorption isotherms and enthalpies of hydration of starch microspheres and acid hydrolyzed starch are presented in Figure 2. The enthalpy plot shows that the hydration of the starch microspheres is initially an exothermal process. It should be noted that the enthalpy of hydration at zero water content is close to -18 kJ mol^{-1} , a value found for several biopolymers (Kocherbitov, et al., 2004, Kocherbitov, et al., 2008, Kocherbitov, et al., 2010, Znamenskaya, et al., 2012). Moreover, for starch microspheres a step in the enthalpy plot was noted. It occurs at a water activity of 0.85, which corresponds to 18.9 wt% of water and indicates a glass transition - a transition of a polymer from a glassy, rigid into a flexible state and is caused by the addition of water, which acts as a plasticizer (Chung, et al., 2002, Chung, et al., 2004, Zavareze and Dias,

2011). Although, the glass transition is very seldom so clearly visible in sorption calorimetry, it is also present in the acid hydrolyzed starch (red curve in Figure 2). For that material it appears at lower water concentration and is followed by an exothermic peak, which has been attributed to hydration-induced crystallization (Carlstedt, et al., 2014).

The sorption isotherms of both materials initially follow the same dependence but at a water activity around 0.2 they start to diverge and the starch microspheres absorb more water. Additionally, a step in the sorption isotherm of acid hydrolyzed starch should be noted. It appears in the same water activity as the endothermic peak and is thus attributed to crystallization. In summary, our data show that the introduced cross-links in the starch microspheres prevent the material to crystallize and enable a higher water absorption capacity.

4.2 Swelling in liquid water

Optical microscopy revealed that the sorption of water results in a rapid and substantial swelling of the starch microspheres. Quantitative evaluation of this process is a non-trivial task; therefore, three different methods were used to quantify swelling: optical microscopy, gravimetric analysis and viscosimetry.

The swelling starts immediately when starch is in contact with high humidity or liquid water and, when fully hydrated, the diameter of the particle becomes 2.1 ± 0.2 times larger than the dry particle, which results in 9.3 times larger volume if spherical shape is assumed. Based on these numbers, the degree of swelling (mass of water per mass of dry starch) is about 5.2. The details of calculation are shown in Supplementary materials. It should be noted that there is some inaccuracy in the size determination of the dry particles that introduces some error to the method. This comes from the fact that a microscope image of a dry particle is a projection of this particle that includes the most outstanding points in all cross sections. Therefore, the dry microspheres are

in fact smaller than the measured value, the swelling is thus larger and as a result the swelling factor should be higher.

Another method used in this work to evaluate the water uptake was gravimetric analysis, based on a linear relation between the amount of starch and the mass of absorbed water. The obtained degree of swelling was 10.6.

The third method used in this work to calculate the degree of swelling was based on the rheology data. As described above, the relative viscosity may be recalculated to volume fraction of particles according to Einstein's equation (eq. 5). From the volume fraction of particles, the degree of swelling is estimated to be 10.7 (details of calculations are in Supplementary materials).

Summing up, the swelling is substantial and based on optical microscopy, the gravimetric swelling study and viscosimetry, the degree of swelling is 5.2, 10.6 and 10.7 g/g respectively. The first of the three values is less reliable because of difficulties in measuring the size of dry particles. The literature data suggest that the degree of swelling is higher for porous starches and is dependent on the degree of cross-linking (Gao, et al., 2014). Thus, the high water absorption capacity can be considered as high porosity in the presence of water. Considering the swollen particle as a gel made up of starch and water we can calculate the volume porosity of this structure:

$$porosity = \frac{V_p - V_s}{V_p} = \frac{m_p d_p - d_s m_s}{m_p d_p} = 87\% \quad (6)$$

where: V_p – volume of the swollen particles, V_s – volume of dry starch used for preparation of the suspension, m – mass, d – density (subscripts have same meanings as for volumes). It should, however, be stressed that this value represents porosity induced by water, in the dry state the porosity is much lower.

4.3 The phase diagram

The DSC data allow calculation of the amount of non-freezing water that cannot easily crystallize due to presence of polymer chains. An analysis of the enthalpies of melting of ice shows that the amount of non-freezing water for starch microspheres is equal to 0.38 g of non-freezing water per 1 g of starch (3.4 water molecules per repeating unit of starch). This data was compared with values established for native and acid hydrolyzed potato starches (0.35 and 0.34 g/g respectively) showing that the amorphous material contains higher fraction of non-freezing water than those that were reported to be partially crystalline (Carlstedt, et al., 2014).

For the concentrated samples the glass transition temperature shows a strong dependence on the composition of the sample which results in a very steep line when plotted. For the samples below 70 wt% of starch, the glass transition occurs in the water melting region and in the phase diagram is represented by a horizontal line at -12°C (the midpoint of the transition, the onset is at -18°C). It corresponds to a boundary between two two-phase regions consisting of ice + flexible polymer and ice + glassy polymer. The glass transition temperature in this region is independent on the amount of water since the composition of the polymer phase does not change. While going along this line on the phase diagram the ratio between the two phases changes but not the phases composition.

In low moisture samples an endothermic peak was observed for temperatures between 40 and 60°C. This event was not present in a second scan performed immediately after the first scan. Although the peak was not present in a the second scan, it re-appeared upon storage at room temperature, indicating relaxation towards equilibrium of a glassy state (Chung, et al., 2004), so it might be interpreted as the sub- T_g endotherm known from other starch materials (Thiewes and Steeneken, 1997). This phenomenon has been referred to as enthalpy relaxation, enthalpy recovery or structural relaxation of the glassy-amorphous regions inside the starch and can be related to

physical aging of the material (Elfstrand, et al., 2006, Vinu, et al., 2003, Yalkowsky and Dannenfelser, 1992). Another explanation is based on the starch-water interactions (Appelqvist, et al., 1993). Additionally, the temperature and enthalpy of this peak in the present system expressed a dependence on starch content (see phase diagram below and Figure S4, Supplementary Information).

Based on the data obtained by DSC and sorption calorimetry a phase diagram was constructed, see Figure 8. The water melting temperature was calculated by extrapolating the DSC data to zero scan rate. The border between the one and the two phase regions was estimated based on the swelling studies and the short vertical line for the water melting was established based on the amount of non-freezing water.

Figure 8. The phase diagram for the starch microspheres – water system, • -glass transition, ○ - glass transition from sorption calorimetry, Δ-sub-Tg, ∇- melting of ice.

4.4 Structural changes

Small-angle X-ray scattering (SAXS) was used for structural studies of starch microspheres. No peaks were found which indicates absence of crystalline structure. However, the curves clearly show several regimes. Four regimes were selected within the plots (Figure 6), their extensions vary with starch concentration. The slopes of the linear regimes were evaluated to obtain the fractal dimensions. For surface and mass fractals respectively, scattering intensities scale in the following way (Roe, 2000):

$$I(q) \propto q^{-(6-d_s)}$$

$$I(q) \propto q^{-d_f} \quad (7)$$

where: I – intensity, q - scattering vector, d_s – surface fractal dimension, d_f - mass fractal dimension.

The first regime at low q values is linear. This regime is clearly pronounced in samples with low moisture (Figure 6(c)). This region can be attributed to the characteristics of the surface of the particles. The second regime is a plateau; its position and length varies between the samples. At higher q there is another regime with a pronounced slope. It is clearly visible for concentrations below 50 wt% (Figure 6(a)) and the slope characterizes the state of the polymer chains. Finally, the last regime ($q > 4 \text{ nm}^{-1}$) represents scattering from individual glucose units of starch chains (Carlstedt, et al., 2014).

The slope in the first regime (surface regime) depends on starch concentration (Figure S6a). For dry substance the values of the slopes are close to -4 and the surface fractal dimension is 2, at 70 wt% the slope changes up to approx. -3 (fractal dimension is 3) and continues changing at lower concentrations. The change of the surface fractal dimension indicates an increase in roughness in the nm scale with addition of water. In the dry state the chains collapse and form a smooth surface.

In higher humidity a thin water layer around the surface provides some mobility to those chains so they may protrude from the surface resulting in some roughness. In the presence of substantial amounts of water the whole structure starts to change resulting in changes in the overall shape of the surface (cf. Figure 3).

The change in the intensities in the first regime finds explanation in the different interfaces and contrasts. For dry particles the starch-air interface provides good contrast and high intensities in the surface regime. At higher hydration levels, when the system approaches the glass transition, the particles may undergo structural rearrangements at the surfaces leading to decreased surface area, which decreases the scattering intensity.

A transition was noted in the SAXS plots for low moisture samples as a change in the plot shape between 70 and 85 wt% of starch. The change is also visible in the temperature scans between 40 and 70°C (Figure 7) and corresponds to the temperature range at which both the glass transition and the sub-T_g occur. Moreover, the curves do not regain their original shape when cooled and in the surface regime shift to lower intensities without change in shape. This might indicate that this transition is related to the sub-T_g. However, the absence of this event in more concentrated samples indicates rather a glass transition. That may be also supported by the phase diagram presented above. However, the glass transitions do not cause changes in the structure so they should not be visible in SAXS. Therefore, the observed changes in the scattering pattern correspond to structural changes that accompany the glass transition. The glass transition makes structural changes possible because it triggers the mobility of the material. As the change involves decreased intensity and the scattering occurs on the surface, the structural change is attributed to the decrease of surface area. This rearrangement is driven by minimization of the free energy by minimizing the surface area and it is possible only when the chains are in the flexible state.

The further decrease of intensity is attributed to elimination of a part of the interface as the flexible swollen tightly packed particles adjust shapes to each other (see Figure 3). Finally, in the most dilute samples the particles are fully swollen and become round again but it is not reflected in the scattering curves as a consequence of low contrast at the water – hydrated particle interface.

The slopes of the third regime also vary substantially, showing positive dependence on concentration of starch. The scattering arises from the polymer chains and the mass fractal dimension is related to the Flory exponent:

$$v = \frac{1}{d_f} \quad (8)$$

where: v - Flory exponent, d_f -mass fractal dimension. The Flory exponent gives information about the conformation of the polymer in the solution, as it relates the molecular mass of the polymer to its radius of gyration:

$$R_g \propto M^v \quad (9)$$

where: R_g - radius of gyration, M – molecular mass. Typical conformations of a polymer in solution include stiff rod ($v=1$), random coil ($1 > v > 1/3$) and compact globule ($v=1/3$)(Flory, 1949). A Flory exponent equal to 0.5 reflects a random walk polymer conformation, while the value of 0.58 corresponds to self-avoiding chains.

For the most dilute samples the slopes are close to -1.7, which corresponds to Flory exponent of 0.59. The slope value increases (becomes less negative) with starch concentration indicating that the chains become straighter with increasing starch concentration. This behavior may be attributed to intrinsic tensions in the particles, introduced by the cross-links, causing straightening of the chains upon drying.

Correlations between SAXS and optical microscopy results can be seen. As shown, at room temperature the glass transition occurs in samples that contain at least 18.9 wt% of water. As Figure

3 shows, the dry particles are round and at concentrations below 80% they appear slightly brighter and they adhere to each other which may correspond to the rearrangements discussed based on the SAXS data. Subsequently, a change in the appearance of the particles occur around 50 wt% when they swell and appear as separate particles. The changes were also visible on macroscopic scale; the samples went from lumps into paste. It should be noted that in this concentration range a border between two types of behaviors in SAXS was observed. Finally, the hydrated microspheres are clustered close to each other and adjust shapes to adjacent particles which leads to the discussed lack of contrast.

To sum up, the dry starch microspheres have a fully amorphous structure that changes in the aqueous environment. In the dry state the material forms spheres with smooth surfaces. During hydration, the chains undergo glass transition followed by decrease of surface area. At higher water contents the chains start to protrude from the surface, which increases the roughness on a nm level. Further increase of water contents changes chain conformation in the bulk from stretched to self-avoiding random chains.

5. Conclusions

Employing a multi-method approach, we have examined the properties of starch microspheres and the effects of their hydration and found that:

- Starch microspheres swell rapidly in contact with water and increase their volume substantially. The degree of swelling at room temperature determined with different methods is 10.6

- The hydration of starch at 25°C is initially an exothermic process, and the enthalpy plots suggest that the glass transition occurs at a water activity of 0.85, which corresponds to 18.9 wt% of water.
- The glass transition temperature is -12°C at starch concentrations 0-70 wt%; above that concentration it increases with a steep dependence on starch concentration.
- The sub-Tg endotherms were detected in DSC curves for low-moisture samples at temperatures between 45 and 63°C; the dryer the sample the higher the sub-Tg endotherm temperature.
- The amount of non-freezing water in starch microspheres is 0.38 g/g which corresponds to 3.4 water molecules per repeating unit of starch, which is higher than for starch materials that include some crystalline structure.
- The starch microspheres contain no crystalline fraction, which was confirmed by SAXS. Comparison of the hydration plot of acid hydrolyzed starch and starch microspheres suggests that formation of crystallites can be prevented by introducing crosslinks to the material.
- SAXS provided information on evolution of the surface and chain properties as function of hydration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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