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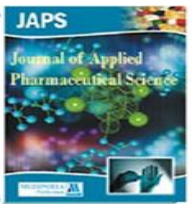

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Effect of Added Brine on Polymer-surfactant interaction

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ABSTRACT

Effect of added brine on the structural transitions of SDS, in different compositions of gelatin has been investigated by viscosity, conductivity, circular dichroism, FTIR Spectroscopy, TGA and DSC. The slow and steady growth of the normal spherical micelles to the higher order aggregates were predicted by viscosity and conductivity measurements. The large negative value for ellipticity observed from CD measurements indicated absence of any conformational change for gelatin. Other measurements were used to study the molecular packing in the micellar aggregates. The complex formed exhibits fantastic properties to be explored in the field of smart gels.

Key words: Micellization; Sphere-to-rod; Surfactant-protein Interaction.

INTRODUCTION

Physicochemical properties of mixtures containing macromolecules and surfactants (or lipids) are relevant and of widespread use in technological applications. Formulation procedures based on the above mixtures may be used to prepare gels for controlled drug release; this is one among many of their appealing applications (Knox and co workers, 1970). Protein–surfactant interactions involve hydrophobic and electrostatic forces of attraction, and therefore, they are closely related to micellization of surfactants. When the surfactant is added to an aqueous solution of gelatin, in consequence of these forces protein macromolecules are modified due to the formation of protein- surfactant complexes. Organized assemblies find application in many fields ranging from detergents to oil recovery technology. In many of the applications, surfactants of different types have been used to interact with different macromolecules. Biopolymer gelatin, a denatured product of the natural protein collagen, has been used extensively in the medical, food, photographic and other industries because of its ability to stabilize colloidal systems. Lipid–protein interaction is an important and inevitable biophysico-chemical phenomenon (Reichow, Gonen, 2009). Lipids, which are the major components of cell membrane, owe their structure to surfactant aggregation. For fundamental understanding, model studies are designed using surfactants/amphiphiles in place of lipids. The application of gelatin modified by surfactants and having new properties, not representative of the individual components, opens up new possibilities of controlling surface activity, the rheological properties of adsorbed layers, structural formation of the dispersional systems, stability of the bilateral films and other surface phenomena.

However, the studies undertaken from, such viewpoints as micellization of surfactant in protein solution are seen to be very few and found to induce conformational changes in proteins (Dikici, and Daunert, 2003). In the present study, we attempt to understand the synergistic effect of brine and gelatin on the micellization behavior of sodium dodecyl sulfate (SDS), an anionic surfactant in aqueous solutions. The presence of a salt (Mesa, 2005) is expected to yield some

useful information in respect of protein–surfactant interactions in aqueous solutions.

Many studies on polymer- surfactant interactions in aqueous solutions have appeared in literature since late 1970. Sodium dodecyl sulphate (SDS), an anionic surfactant is reported to exhibit a variety of structural transitions in aqueous medium on addition of simple salts (Hugonin and co workers, 2008). SDS exhibited electrostatic binding to the charged groups of Gelatin with reduction in hydrodynamic radius(Knox and Parshall, 1970). However, the synergistic effect of salt and gelatin on the micellisation and subsequent structural transition is not yet reported.

In the present paper, we report the effect of NaCl (0.0025-0.5 M) with and without gelatin (0-2.0 wt. %), on the micellization process of aqueous SDS at 35^0C by viscosity, conductivity, Circular Dichroism (CD) spectroscopy, Fourier Transform Infra Red Spectroscopy (FTIR), Thermo gravimetry (TGA), Differential Scanning Calorimetry (DSC) and Rheology measurements. The study showed that in presence of sodium chloride, the sphere –to-rod (s→r) transition of SDS is primarily due to the existence of strong intermolecular interactions, with protein. In addition, however, it was suggested that the probable interactions between the counter ion, Na^+ and gelatin add to the same effect.

EXPERIMENTAL

Materials

Gelatin (LOBA Chemie for bacteriology, India) (Molecular Biology grade) was used as supplied. Sodium dodecyl sulfate (Biochemical grade from BDH) was further purified as described earlier (George and co workers, 2008). Sodium Chloride supplied by Merck, Germany, was also used without further purification.

Methods

Viscometry

Details on the experimental set up for viscosity and CD spectroscopy have been already reported by George and co workers. All solutions were prepared in Milli-Q water having specific resistance 18.2 MΩ cm. The concentrations quoted here are in weight percentage of gelatin.

Differential Scanning Calorimetry (DSC)

DSC traces were recorded in a high sensitivity calorimeter (TA Instruments, DSC Q-10, USA.), in the temperature range -80 to 80^o C, with a heating rate of 5^o C / minute.

Thermo gravimetric analysis (TGA)

TGA was taken from TGA Q 50 (TA Instruments) in the temperature range -80 to 80^o C, with a heating rate of 5^o C / minute.

Conductivity

The conductance of different CTAB+NaCl systems in 1.5% gelatin were measured using Deluxe Conductivity meter, Model 601E (Electronics, India) at 35^0C.

FTIR spectra

FTIR spectra were obtained using a spectrophotometer (Nicolet 380 USA) with transmittance values in the wavelength range of 3200–1000 cm^{-1} wave numbers.

Rheology

The rheological characterizations of the samples were carried out using a controlled stress rheometer (Anton Paar Physica MCR-51) with a cone and plate sensor (40 mm diameter, 3.988^o angle). The sample thickness in the middle of the sensor was 0.050mm. Experiment was performed at 35 $\pm 1^o$C. The viscosity profile of the sample was noted by varying the shear rate from 0.3 to 500 s^{-1}. Frequency sweep measurements were carried out by varying the angular frequency from 0.06 to 100 $rad.s^{-1}$. The experiments were carried out at 1% strain with in the linear viscoelastic range.

RESULTS AND DISCUSSION

The viscosity measurement for micellar solution of mixed SDS + NaCl system was made in absence and presence of gelatin at 35°C in order to study the micelle shape transition induced by the change in ionic strength. The results obtained were quite interesting. (Fig. 1)

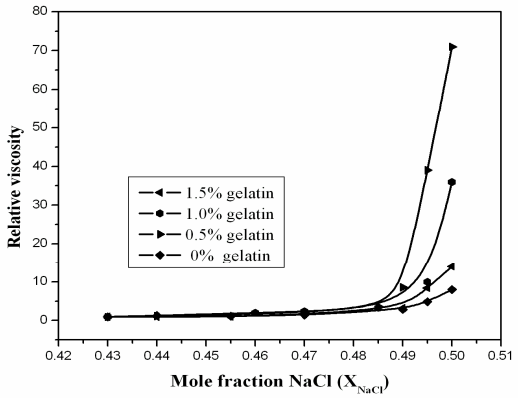


Fig.1. Relative viscosity variations of SDS + NaCl system at varying gelatin concentration.

The relative viscosity of (SDS + NaCl) in biopolymer solution, gelatin does not change significantly up to 0.35 M, while a drastic increase in viscosity is observed at 0.4 M , which is in agreement with the fluorescence probe studies reported by (Gao and co workers, 2011) with regard to surfactant-protein interaction. The steep increase in viscosity suggests the presence of sphere and rod-like micelles. These results are in good agreement with those reported by Hoffmann et al. that adding an ionic surfactant to a zwitterionic surfactant solution cause an initial increase in viscosity. It suggest that anionic surfactants interact with proteins relatively more strongly than cationic surfactants (Otzen,2011). This is explained as arising from hydrophobic bonding between the hydrocarbon chain of the surfactant and hydrophobic amino acid

side chain of relatively large size carried by the positively charged amino acid residues, and is therefore regarded as an indication of surfactant induced protein unfolding. However, no such conformational changes in protein were observed in this particular system by CD measurements, which is a good method for probing the topological changes in proteins. The change in negative values of ellipticity in presence of additive suggests micellar aggregation of higher order. The observed changes can be interpreted in terms of the electrostatic repulsion between charges introduced by the bound SDS. The characteristics of the plots are consistent with the necklace model proposed previously for such complexes in which SDS is bound to a protein polypeptide forming micelle-like clusters and which behave like a flexible polyelectrolyte.

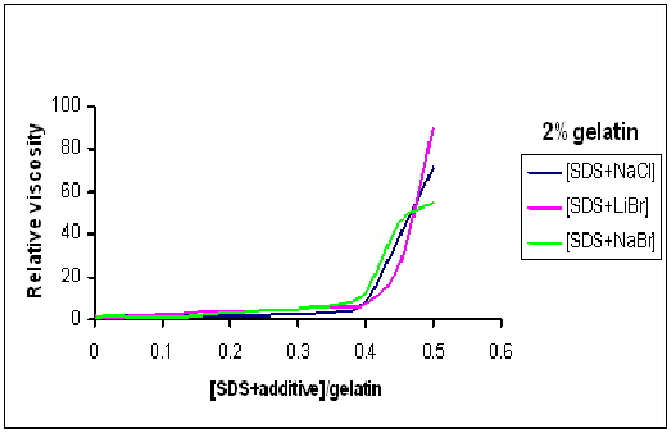


Fig.2. Relative viscosity variation of SDS + NaCl/LiBr/NaBr system at varying gelatin concentration

Considering the ionic nature of the surfactant, the structure seems to be modified in presence of salt (KBr, NaBr and NaCl; Fig.2) due to the repulsive intermicellar interactions which can lead to an increase in the diameter leading to the transition at high concentrations. It is of interest to note that SDS–gelatin concentration dependence in the presence of salt, NaCl/LiBr/LiCl follows similar trend with increasing concentrations. Thus, the observed change in apparent size arises from decrease of intermicellar interaction and growth of the micelles. In view of the hydrophobic and electrostatic contributions towards protein–surfactant interactions, structural consequences of intermolecular interactions between SDS and NaCl might be of great relevance to interpret this trend. Hydrophobic interaction between surfactant–surfactant as well as surfactant–protein is expected to suffer relatively more significantly in presence of salt. Considering large changes in the relative viscosity of the solution a prolate ellipsoidal or rod-like growth of the micelles can be envisioned.

Gelatin surface bears polar as well as hydrophobic sites (Thomas and co workers, 1991). At gelatin concentrations <0.34%, SDS binding to gelatin is, therefore, suggested governed by both electrostatic and hydrophobic interactions. Figure.1.clearly depicts that above 0.34 % (w/v) gelatin, no free SDS molecules are expected left to interact with gelatin, which suggests stacking of gelatin molecules probably through intermolecular hydrophobic contribution.

Thermal behavior

Fig.3 and 4 depict the DTA and TGA of SDS–NaCl–gelatin. The peak temperature (Fig.3.b.) was shifted towards higher temperature (about 9°C). The presence of the shoulder observed on the low temperature side of the peak transition (Fig.b) obtained with concentration higher than 0.35M is possibly due to the denaturation of the different species suggesting either partial loss of tertiary structure of Gelatin molecules upon SDS binding at room temperature and/or exothermic surfactant/protein interactions(Slade and Levine, 1995). Tryptophan or histidine residues may also be involved in the binding process. The increase in peak temperature with surfactant concentration is characteristic of a hydrophobic binding process, which is accompanied, at high surfactant concentration.

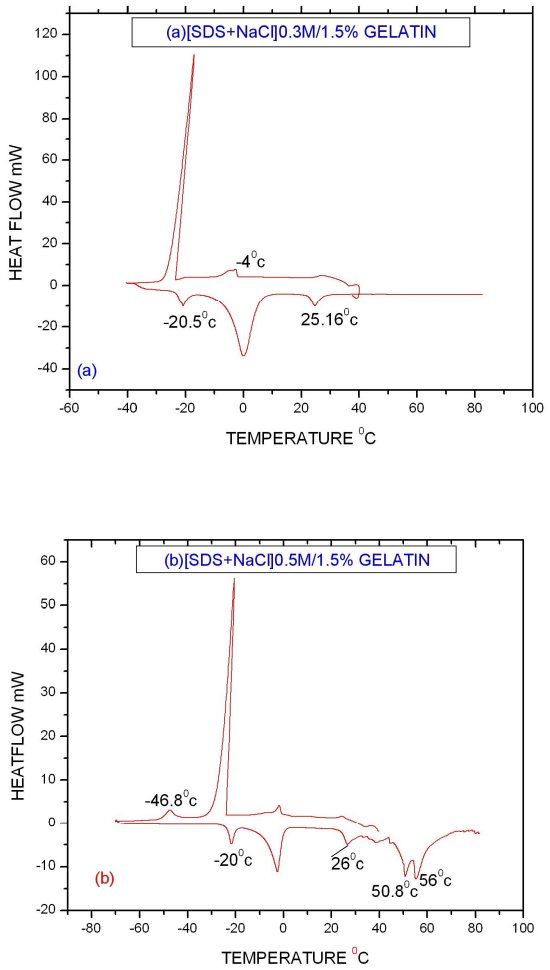
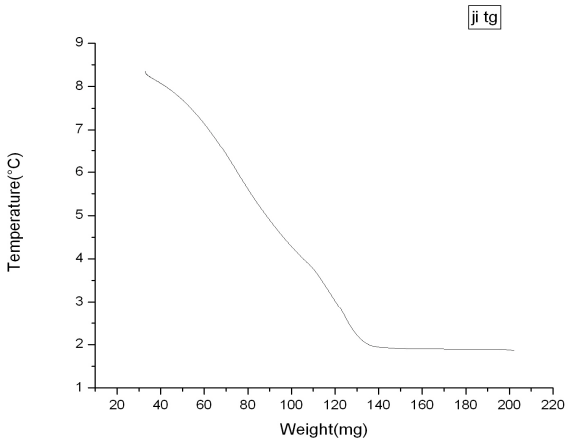


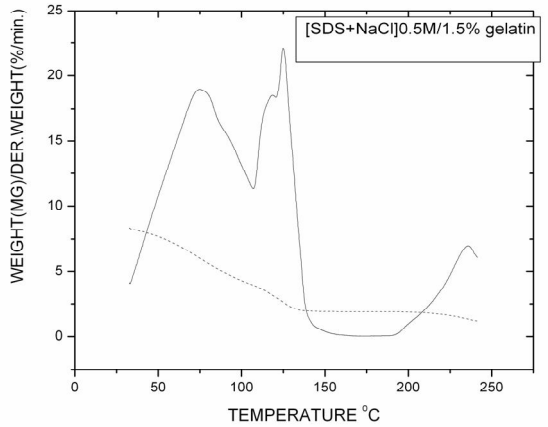
Fig. 3. The DSC heating profiles of SDS-NaCl systems containing 0.3M [SDS+NaCl] at 5 ◦C/min (fig. a) and for 0.5M (fig.b).

The endothermic peak in (Fig. 4a) at -20°C corresponds to desorption of water. Fig. 4b indicates the effective surface modification by NaCl-Gelatin. The broad peak at ~50 °C in Fig.

4b corresponds to the multi-step degradation and pyrolyzation of the complex helical structure of gelatin. However, in the present case the endothermic peak at 50-56 °C indicates that the hydrophobicity is retained at much higher temperatures.



(a)



(b)

Fig.4.a and b-TGA curve for SDS-NaCl systems containing 0.5M [SDS+NaCl] in 1.5 % Gelatin.

Another interesting feature revealed by the thermal analysis, DSC, indicates a prominent phase transition above 35^0C. There is striking difference between the data obtained at high and low concentrations observed in the presence of gelatin; at temperatures >35 °C, concentration of (SDS+NaCl) is found to be sensitive to temperature. This peculiar behavior in aqueous gelatin solution appears to represent some unusual state of SDS–gelatin association, with respect to temperature, in presence of the salt. This indicates some conformational change induced in the structure of gelatin at temperatures above 35 °C. i.e., gelatin begins to unfold above 35 °C. This can be explained on the basis that on increasing the temperature, the rod-like micelles do form small micelles but they preferred to stay near each other forming a network. For the gel, the observed endothermic peak was large and rather well defined. The maximum of the peak was located at above 35°C, in agreement with the viscosity data predictions. This transition can be attributed to the melting of the gelatin helical structures. When salt +SDS was added to gelatin, the peak was shifted to higher temperatures (for example, the maximum of the peak was around 49 °C for the low conc. system.

CD spectral analysis

The effect of SDS at various concentrations on the secondary structure of gelatin in the presence of NaCl was studied using CD (Fig. 5). The random coil structure found in gelatin gives rise to a characteristic negative peak at around 220 nm in the CD spectrum which changes in ellipticity upon addition of [SDS+NaCl]. The general shape and peaks of the spectrum does not show much change when treated with [SDS+NaCl] of varying concentrations except for the magnitude of ellipticity, which is maximum for the highest concentration. The gelatin treated with [SDS+NaCl] displays negative peaks with lower intensity compared to the gelatin, which implies that the gelatin has no conformational change at 35^0C. The lower ellipticity indicates the alteration or disturbance in the order or periodicity in the structure of surfactant ie.aggregation or disruption as predicted from other measurements.

The decrease in molar ellipticity at 220nm for the system is indicative of aggregated structures of surfactant/gelatin in the presence of salt and is very much pronounced at higher concentrations of salt. At higher concentrations due to the loss of secondary structure, no clear CD spectra were observed. This shows that the unfolding of gelatin is closely related to [SDS+NaCl] concentrations. This study suggests that surfactant+salt have an important role for stabilizing gelatin secondary and tertiary conformation.

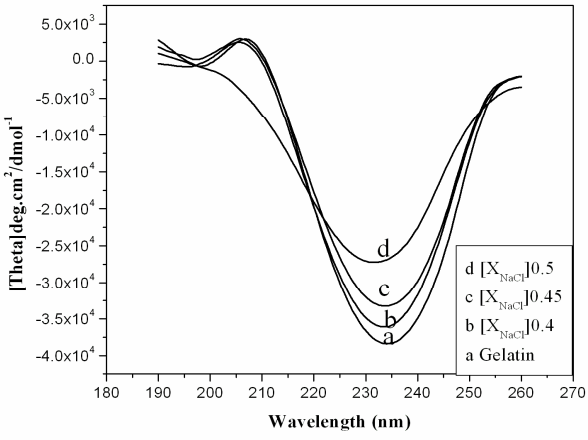


Fig. 5. CD spectrum of (1) gelatin alone, (2) gelatin + 0.5 M [SDS+NaCl], (3) gelatin + 0.4 M [SDS+NaCl] and (4) gelatin + 0.3 M [SDS+NaCl].

Conductivity measurement

The specific conductance measured at 35°C (fig.6.) indicates the release of ions into solutions with increasing concentrations in gelatin solution. FTIR method provides a route to observation of effectiveness of bond formation. The results are

shown in Fig. 7. The surfactant +salt show the characteristic band for the CO-NH bond in the range of 1613–1743 cm^{-1}. After addition of more (surfactant +salt) two bonds at 2852 and 2945cm^{-1} were observed. These bonds again are indicating the surface modification of the system and are showing that salt is bound in different environments inside the micelle. In the outer coordination sphere of water the hydrogen bonds are weakened most due to the interaction with the surfactant and therefore this region probably corresponds to the band at the lowest wave number.

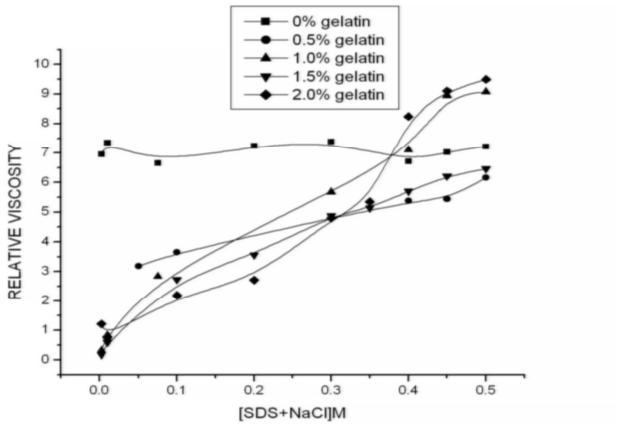


Fig. 6: The variation of the specific conductivity (κ) against [SDS + NaCl] at different wtpercentage of gelatin at 35°C.

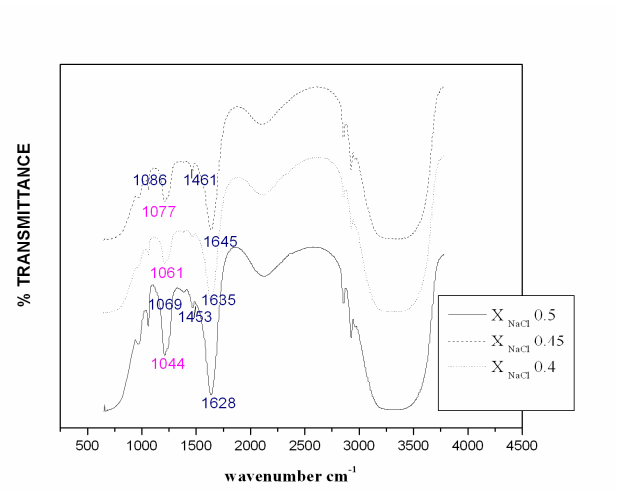
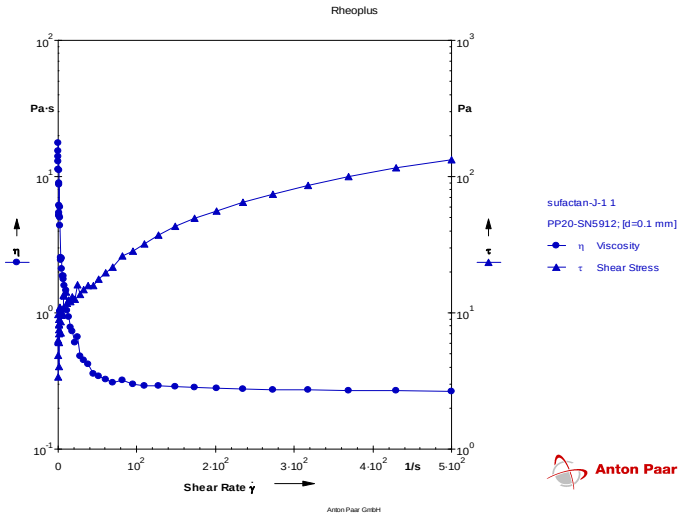


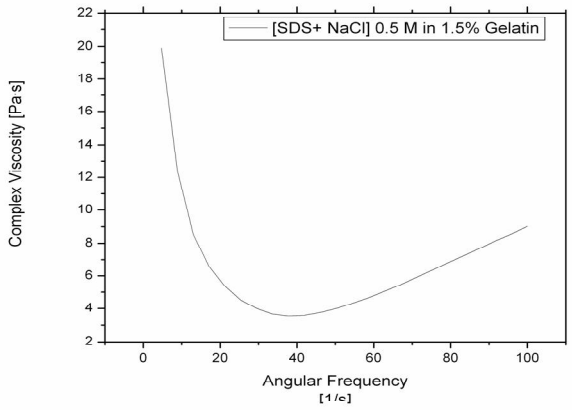
Fig.7: FTIR spectra of 0.5 M/0.35M/0.01M [SDS+NaCl] in1.5% gelatin at 35 ^{0} C.

Shear thinning behavior

Shear thinning is often a consequence of high molecular weight molecules in solution becoming untangled and orientated during steady shear flow. It is conventionally represented by a decrease in viscosity with an increase in shear rate. However, for most polymers in the linear viscoelastic region there is a direct analogy between the shear rate in shear flow and the frequency in dynamic oscillatory measurements (Chhabra and co workers, 2001). The complex viscosity profiles for many polymer solutions have been shown to decrease with increase in frequency. An AFM study of viscoelastic thin films of polydimethylsiloxane (PDMS) by Friedenberg and Mate also indicates shear thinning behaviour with increasing frequency that is, reduction in complex viscosity with frequency.



(a)



(b)

Fig.8.a.and b. Shearthinning behavior of system depicted by rheology

CONCLUSION

We have shown that anionic surfactants are capable of binding to the positively charged gelatin molecules, at very low surfactant concentrations. With its cationic amino acid, residues it shows strong electrostatic interaction with oppositely charged SDS surfactant. The conformational stabilization and aggregation mechanisms are strongly dependent on the nature of the surfactant and the binding ratio, which correspond to the type of interaction between the surfactant and protein. The mixed surfactant system of SDS + NaCl with and without gelatin showed strong synergisms and sphere–rod–sphere transitions. Further experimentation is being performed in our laboratories to attempt to detect effects of

heat denaturation on the interactions with salt/gelatin and consequent effects on surface properties. CD and FTIR measurements on the system support the viscosity results by showing change in magnitude of ellipticity, peak shift respectively. The dependence on temperature is revealed from DSC and TG measurements.

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