Available online at www.sciencedirect.com
ScienceDirect
Polymer 48 (2007) 5484–5485
polymer
www.elsevier.com/locate/polymer

Polymer Communication

Note on the 'Reply to the paper ''Comment on gelation mechanism of agarose and κ-carrageenan solutions estimated in terms of concentration fluctuation'' [Polymer 43 (2002) 5299]'

Madhusudan Roy, Supratic Chakraborty*

Microelectronics Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Kolkata 700 064, India

Received 24 May 2007; accepted 17 June 2007
Available online 22 June 2007

Keywords: Agarose; Gelation; Differential scanning calorimetry

In the reply by Matsuo and co-workers [1], in response to our comment [2], some serious flaws both in observations and interpretations come out for scrutiny once again. As reported in their original paper [3], differential scanning calorimetry (DSC) measurement was carried out on agarose gel by an Exstar 6000 of Seiko Instruments Incorporation with a heating rate of 1 °C min^{-1}. The weight of the sample was 38 mg and the same was scanned over a temperature range of 0–80 °C during heating and 80 to ~12 °C during cooling. The DSC curves for agarose solution of concentration 2.5% by mass exhibit no endothermic peak. Thus they construed that crystallites in the gel were absent. In our comment [2] we explained at length as to why their conclusion regarding the absence of crystallites was erroneous and the same was established on the basis of some recent works [4,5] and our SEM observations (SEM: model: Quanta 200FEG) indicating that there was no morphological changes in the micrographs shown at two different temperatures, namely 24 °C and 80 °C. Nevertheless, before going into a serious debate on this aspect we like to point out that their DSC study was probably not carried out taking the fact that the water content got reduced effectively due to slow heating, namely 1 °C min^{-1}. It was also not amply clear from their reply if the sample pan was sealed properly or not and how much of its volume was occupied by the sample. Keeping all these possibilities in mind we scanned the sample from 5 °C to 80 °C (heating cycle) followed by a cooling cycle from 80 °C to 5 °C using a differential scanning calorimeter (Netzsch, Germany, Model No 204 F1) with heating and cooling rate of 10 °C min^{-1} maintaining an isothermal condition at 80 °C for 1 min. Agarose, after its melting using an indirect heating technique, was poured directly into the sample pan and allowed to cool down to form gel to have a better contact with the bottom surface of the sample pan. Indirect heating technique was employed during melting to avoid any water evaporation that caused change in density of agarose solution. Moreover, the sample pan was poured in by molten agarose to a maximum quantity to minimize the empty space within the pan. It was allowed to cool down the temperature of the pan containing agarose and then sealed the sample pan gently with a lid using a sealing arrangement. We observed an endothermic peak at 16.5 °C as shown by curve (a) in Fig. 1 during heating only. There are two exothermic peaks at 23 °C and 42.7 °C showing transitions. We repeated the experiment with two other concentrations and found endothermic peak in each case during heating only and thus provided a concrete proof to the crystallites present in the gel. We also carried out the same experiment where the sample was prepared separately: after melting of agarose solution, the solution was allowed to cool down to complete gelation process. Once the gel was formed, a portion of it was filled into the sample pan and then scanned. The DSC curves, shown in Fig. 2 did not show any peak as observed by Matsuo *et al.* in Fig. 5 of their paper [3]. To understand further, a thermo-gravimetric analysis (TGA) was carried out using

* Corresponding author.
E-mail address: supratic.chakraborty@saha.ac.in (S. Chakraborty).

0032-3861/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.polymer.2007.06.034

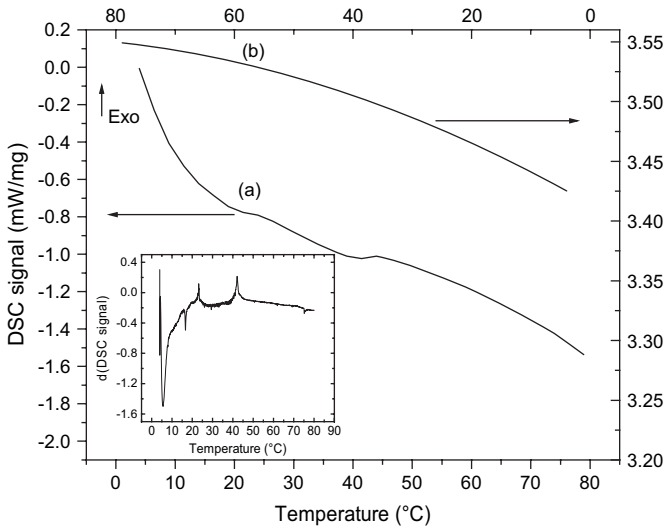

Fig. 1. DSC curves of agarose solution with higher mass of 58.4 mg under heating and cooling conditions: (a) 5 °C to 80 °C and (b) 80 °C to 5 °C. Inset shows the 1st derivative curve (a).

a thermo-gravimetric analyzer (Netzsch, Germany, model: STA 449C) under N_2 flow at a heating rate of 10 °C min^{-1} and indicated in Fig. 3. It was evident from the figure that there was a loss in mass due to release of water content from the agarose with temperature. It is attributed to the fact that the water molecules leave voids and interstitials of agar gel quite regularly during heating. Like Matsuo *et al.*, we also did not notice any peak as shown by the curve (b) in Fig. 2 during cooling phase probably due to the absence of water in the sample content. We suspect that water present was liberated from agarose during heating cycle that changed the gel-like property of agarose. So, our submission to their observation [1] that 'no weight change between before and after the measurements was confirmed' is highly erroneous. To explain the discrepancy so arisen lies in the fact that there is a gradual

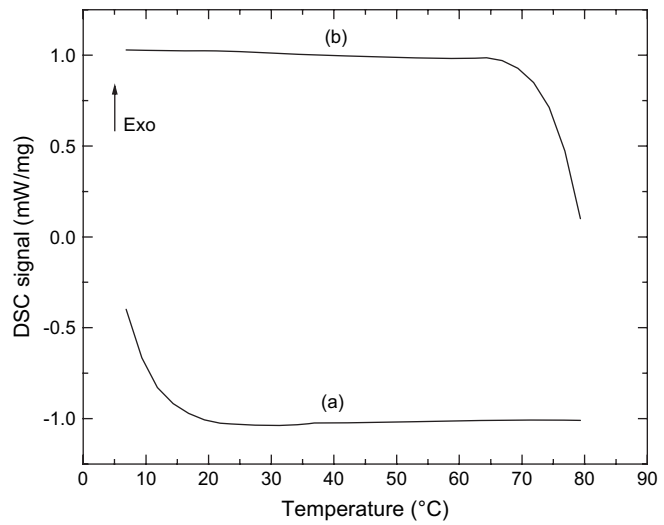

Fig. 2. DSC curves of agarose with a mass of 39.3 mg under heating and cooling conditions: (a) 5 °C to 80 °C and (b) 80 °C to 5 °C.

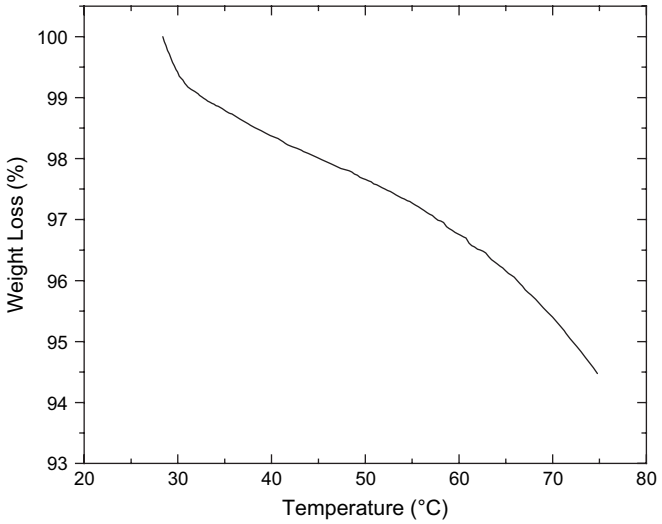

Fig. 3. Thermo-gravimetric data of mass loss of agarose with temperature.

loss in water content and it becomes critical if proper care is not adopted besides adopting a new methodology as proposed in the last paragraph of our comment [2]. In fact use of large volume of sample, maintenance of relatively higher heating rate and sealing of sample may be useful to minimize the water loss and thus be able to have a closer accurate observation for this kind of agarose-like hydrogels when subjected to a temperature change.

Our observation did not contest that agarose will not melt as fictitiously argued by Matsuo and co-workers in the second paragraph of their reply [2]. We only stated that the DSC curves as shown by them were taken under improper conditions and thus misled them to conclude that crystallites in gel were not present. In our comment, we explained the discrepancy on the basis of SEM observation [2] and now on the basis of DSC curve we concluded that use of large volume of sample, maintenance of relatively higher heating rate and sealing of sample are the necessary criteria to minimize the water loss to have a proper DSC data for agarose-like hydrogels. The observation was substantiated by thermo-gravimetric analysis. However, we are still in the opinion that an instrument to investigate the temperature dependent properties of agarose-like hydrogel is to be equipped with an indirect heating arrangement so as to minimize loss of water content. Finally, we conclude that ensuring bulk heating by increasing volume by filling the sample pan with agarose gel to a maximum quantity and sealing it properly, one might get better observations of hydrogels.

References

[1] Matsuo M. Polymer 2005;46:3538.
[2] Roy M, Chakraborty S. Polymer 2005;46:3535.
[3] Matsuo M, Tanaka T, Lin M. Polymer 2002;43:5299.
[4] Bulone D, Giacomazza D, Martorana V, Newman J, San Biagio PL. Phys Rev E 2004;69:041401.
[5] Kanaya T, Ohkura M, Kaji K, Furusaka M, Misawa M. Macromolecules 1994;27:5609.