

## Electronic Supplementary Information

### **Microstructural free volume and dynamics of cryoprotective DMSO-water mixtures at low DMSO concentration**

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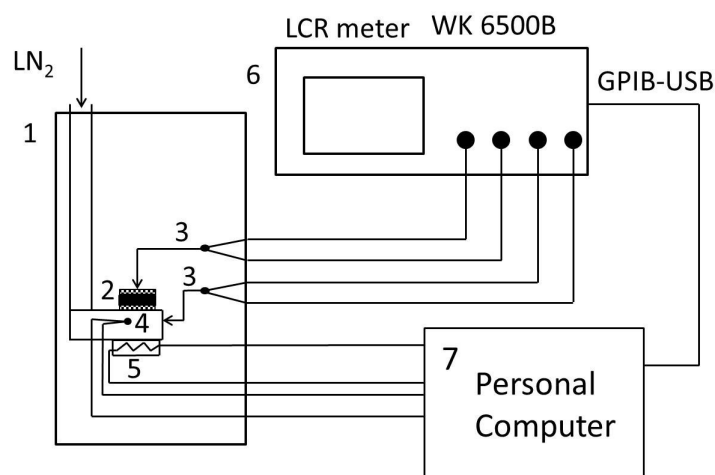
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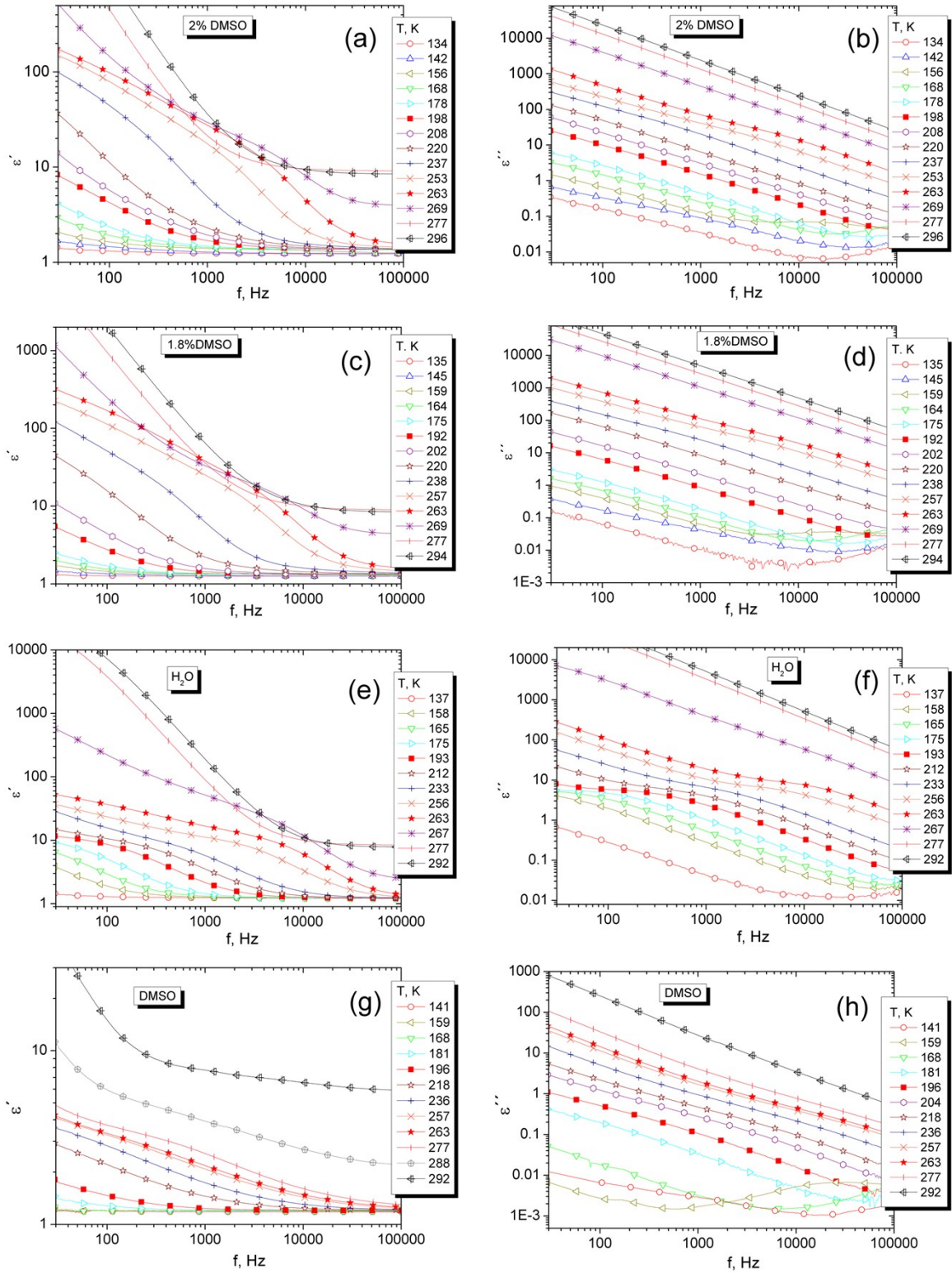
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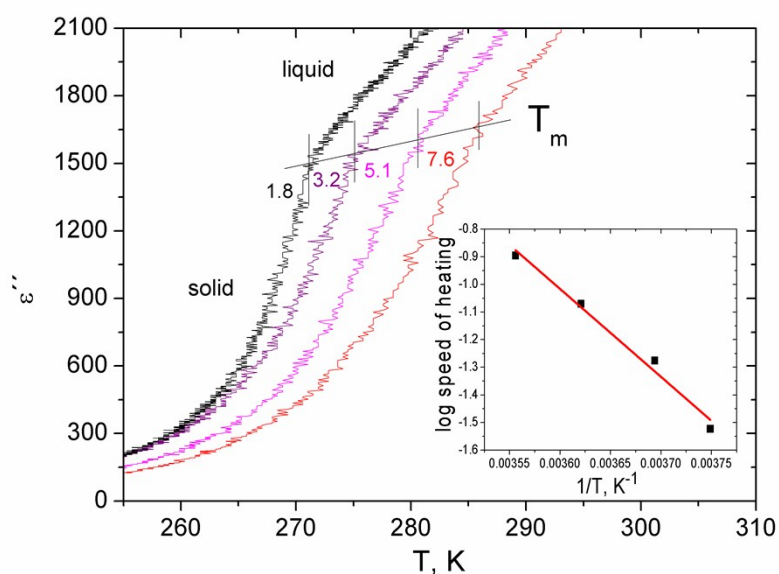
**Figure S1.** Block diagram of the frequency- and temperature-dependent complex permittivity measurement. 1 – cryostat, 2- sample, 3 – probes, 4- thermocouple, 5 – resistor for heating, 6 – LCR meter (WK6500B), 7 – Personal computer with the control circuit and data acquisition, GPIB – USB – connection between 6 and 7, LN<sub>2</sub> – liquid nitrogen.

In Fig. S1, the sample 2 was placed in the hermetic closed chamber with two mutually isolated flat electrodes. The gap between them was 0.5 mm, the diameter of electrodes was 20 mm. Isolation of the chamber was performed by teflon. The chamber was hermetically closed after filling of liquids. The temperature measurement was performed by thermocouple type T with connection to DAQ card NI PCI6229. Cold junction compensation was performed and the electronic circuit was made at the Institute of Physics. The electronic circuit was used according to Analog Devices's recommendation. Measured data from LCR meter was acquisitioned by GPIB – USB module connected to the computer 7.

Fig. S2 shows the frequency dependences of real and imaginary part of complex permittivity for the selected temperatures above 140 K up to liquid state. Despite the low concentrations of DMSO in water in the investigated mixtures, the effect of DMSO on suppressing the relaxation, seen in DS spectra for pure water, was seen. Due to the position of the peaks and the similar character of the spectra to pure water, it can be assumed that these relaxation in the mixtures are associated with the present crystalline phase. Also in pure DMSO, an ordered (crystalline) phase below the melting point can be observed in accordance with another work.<sup>1</sup>



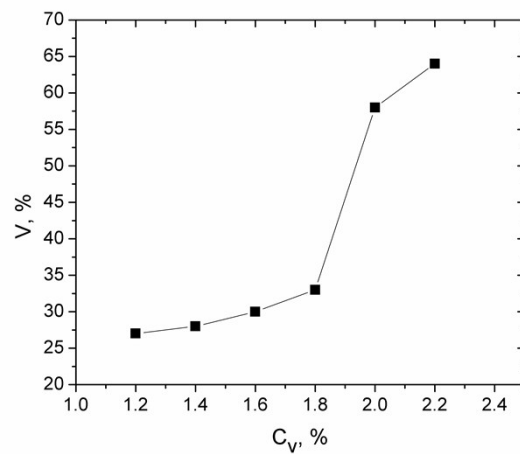
**Figure S2.** Dielectric spectra, the real (left figures) and imaginary (right figures) part of complex permittivity for the DMSO concentrations of 2% (a, b), 1.8% (c, d), pure water (e, f) and pure DMSO (g, h) for selected temperatures.



**Figure S3.** The imaginary portion of relative permittivity  $\epsilon''$  versus temperature for different heating rates for 10% DMSO measured at 1 kHz. The curve number indicates the heating rate in K/min. Temperature  $T_m$  indicates the change of dynamics at melting. The embedded image is an Arrhenius graph.

Fig. S3 shows the evolution of the imaginary part of the relative permittivity  $\epsilon''$  versus temperature for the different heating rates (1.8-7.6 K/min) in the temperature range of the system dynamic changes from the solid to the liquid phase. The transformation temperature  $T_m$  was determined at the bend point in  $\epsilon''(T)$  dependence and it moves in the range 267-281 K. Based on Arrhenius dependence, the activation energy  $E_a = 26.4$  kJ/mol of the structural changes during heating was determined. This can be understood as dissolution of the complex structure consisting of ice and molecular clusters of DMSO-water type. Since hydrogen bonds between molecules are responsible for the formation of different molecular structures, this is a manifestation of break down of H-bonds in investigated sample, between water and DMSO molecules probably. It is indicated by the calculated  $E_a$  which is found close to value of the hydrogen bond energy of 23 kJmol<sup>-1</sup> for the water<sup>2</sup> or 28 kJmol<sup>-1</sup> for bound water on the protein surface in water solutions.<sup>3</sup>

The step-dependent cell viability on DMSO in water as a cryoprotective medium for cell deposition at liquid nitrogen temperature is in Fig. S4. The large difference in cryoprotective effects at low concentration changes is evident.



**Figure S4.** Cell viability  $V$  in % of surviving cells according to data from<sup>4</sup> versus volumetric concentration of DMSO in water  $C_v$ .

## References

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- 3 N. Miura, Y. Hayashi, N. Shinyashiki, S. Mashimo, *Biopolymers* 2000, **54**, 388.
- 4 J. Lakota and P. Fuchsberger, *Bone Marrow Transplant.*, 1996, **18**, 262.