

The study of protein - mesoporous SiO₂ nanoparticles interaction

Alba BALMORI¹, Daniela GHEORGHE², Alina BOTEA-PETCU², Aurica PRECUPAS²,
Romica SANDU², Salvador BORROS¹, David SÁNCHEZ¹, Speranta TANASESCU²

¹ Grup d'Enginyeria de Materials (GEMAT), Institut Quimic de Sarrià, Universitat Ramon Llull, Via Augusta 390, 08017 Barcelona, Spain
² "Ilie Murgulescu" Institute of Physical Chemistry of the Romanian Academy, Splaiul Independentei 202, Bucharest, Romania

The aim

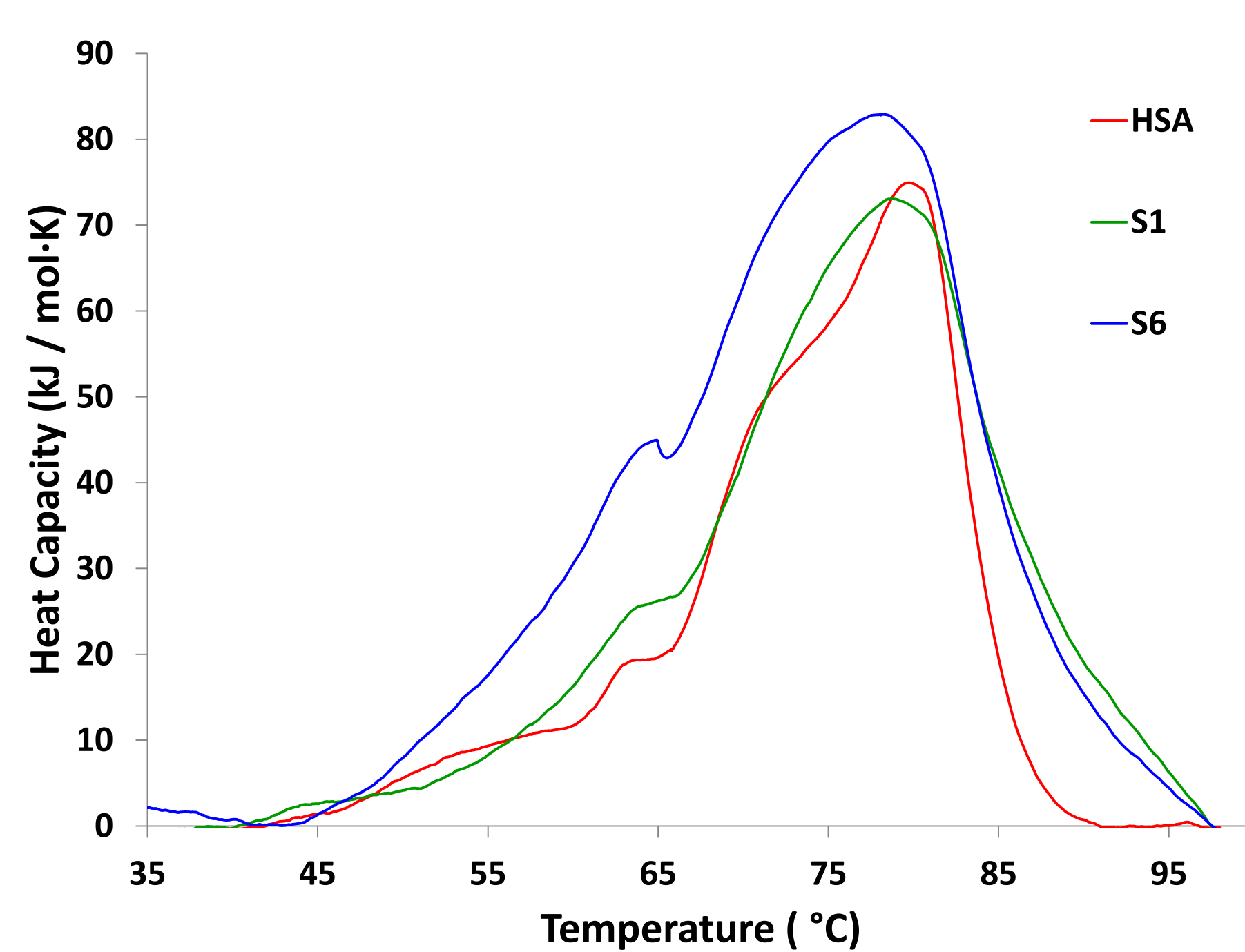
- ❖ Estimation of thermodynamic stability of human serum albumin (HSA) in the presence of mesoporous SiO₂ nanoparticles (MNPs) in sodium buffer phosphate (PBS) at pH 7.4
- ❖ Evaluation the thermodynamics of protein-MNPs interactions and the protein corona (PC) formation in MilliQ water pH 6.3.

Materials

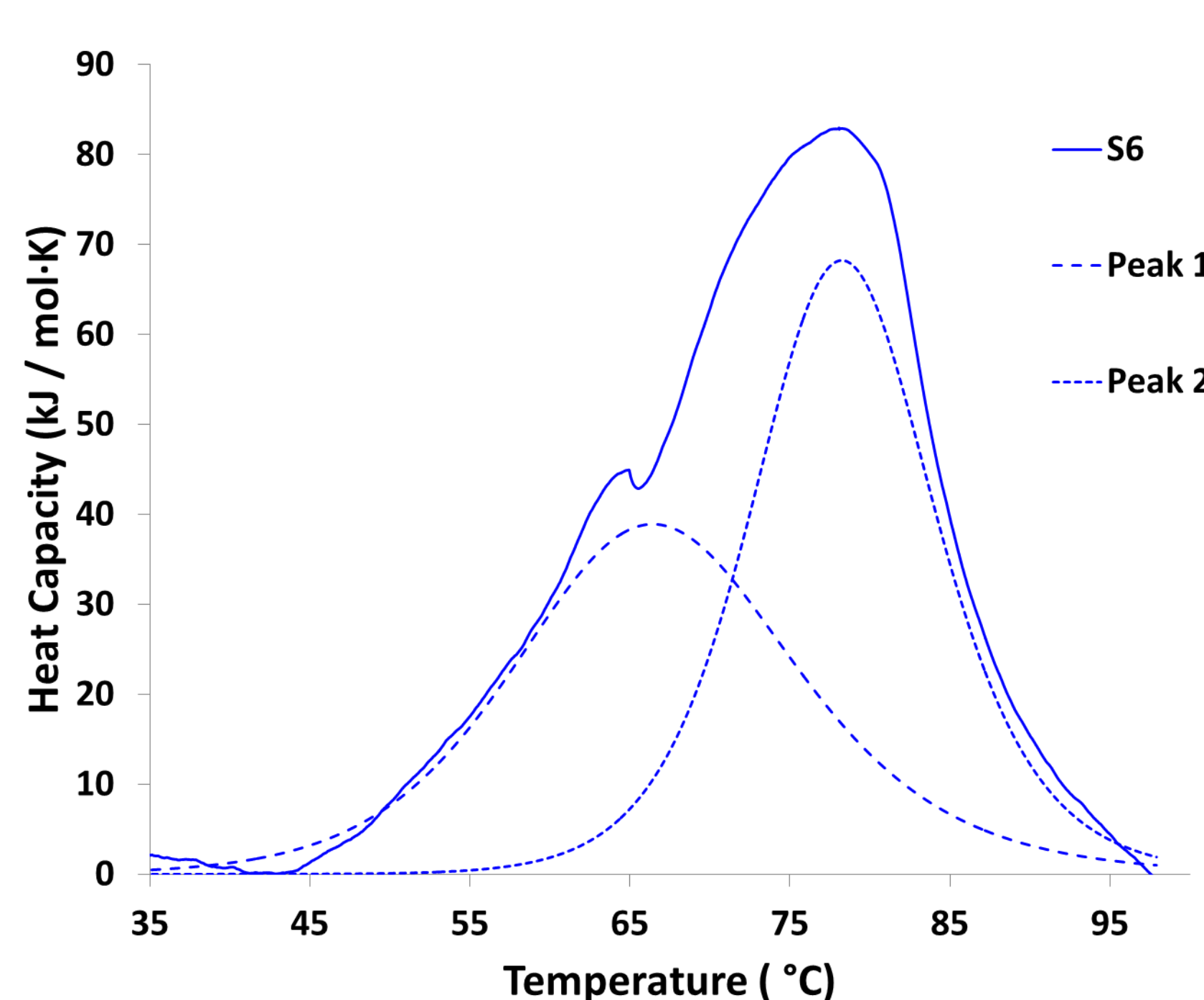
- The chemical and structural characterization of the mesoporous SiO₂ (MNPs) with particle size of 300 nm (S1) and 270 nm (S6) was realized by IR, BET surface area, BJH pore size and volume analysis, SEM (scanning electron microscopy), DLS (dynamic light scattering) size distribution and zeta potential.
- Human serum albumin (HSA Fraction V) was purchased from Sigma Aldrich.
- Water- or buffer-dispersion of SiO₂ MNPs at different concentrations and solutions of HSA were prepared by weighting and by adding MilliQ water or PBS at pH 7.4.

Experimental

- The changes in the thermal behaviour of HSA (both free in solution and adsorbed to nanometer-sized oxide particles) were studied by using NanoDSC-TA Instruments equipment. Measurement conditions: pressure 2 atm, temperature range 298 - 378 K, scanning rate of 1 Kmin⁻¹. Once the conditions have been optimized, the stability of the protein corona formed after the nanoparticles are incubated at 37°C in PBS. The calorimetric data were corrected using a sigmoidal baseline in NanoAnalyze software.
- For the study of HSA-MNPs interaction, isothermal calorimetric measurements in water at 310 K were performed using an ITC200 microcalorimeter.



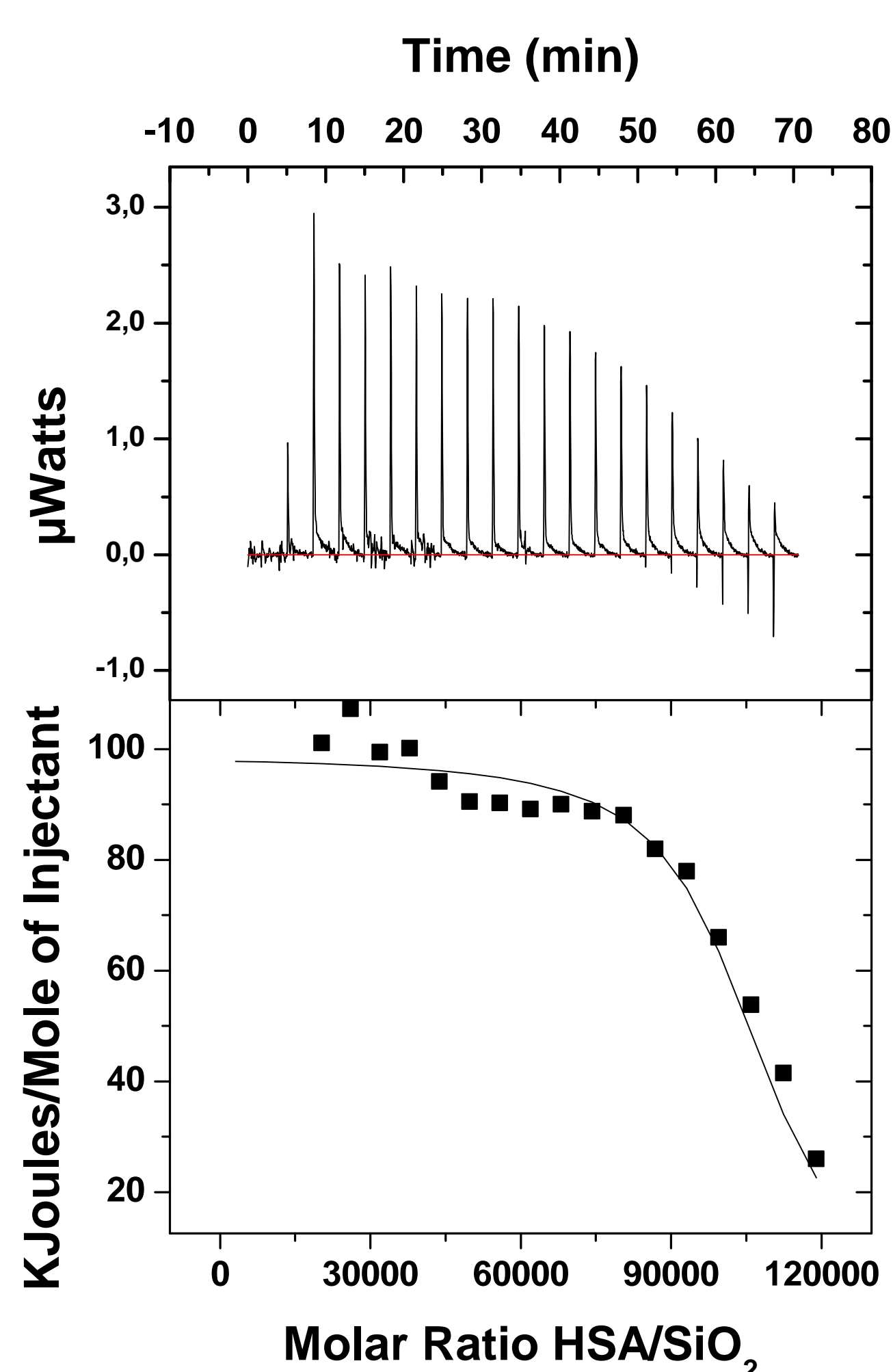
The heat capacity versus temperature profiles for the thermal denaturation of HSA (c=2 mgml⁻¹) in the presence of SiO₂ (c=2 mgml⁻¹) MNPs in PBS at pH 7.4



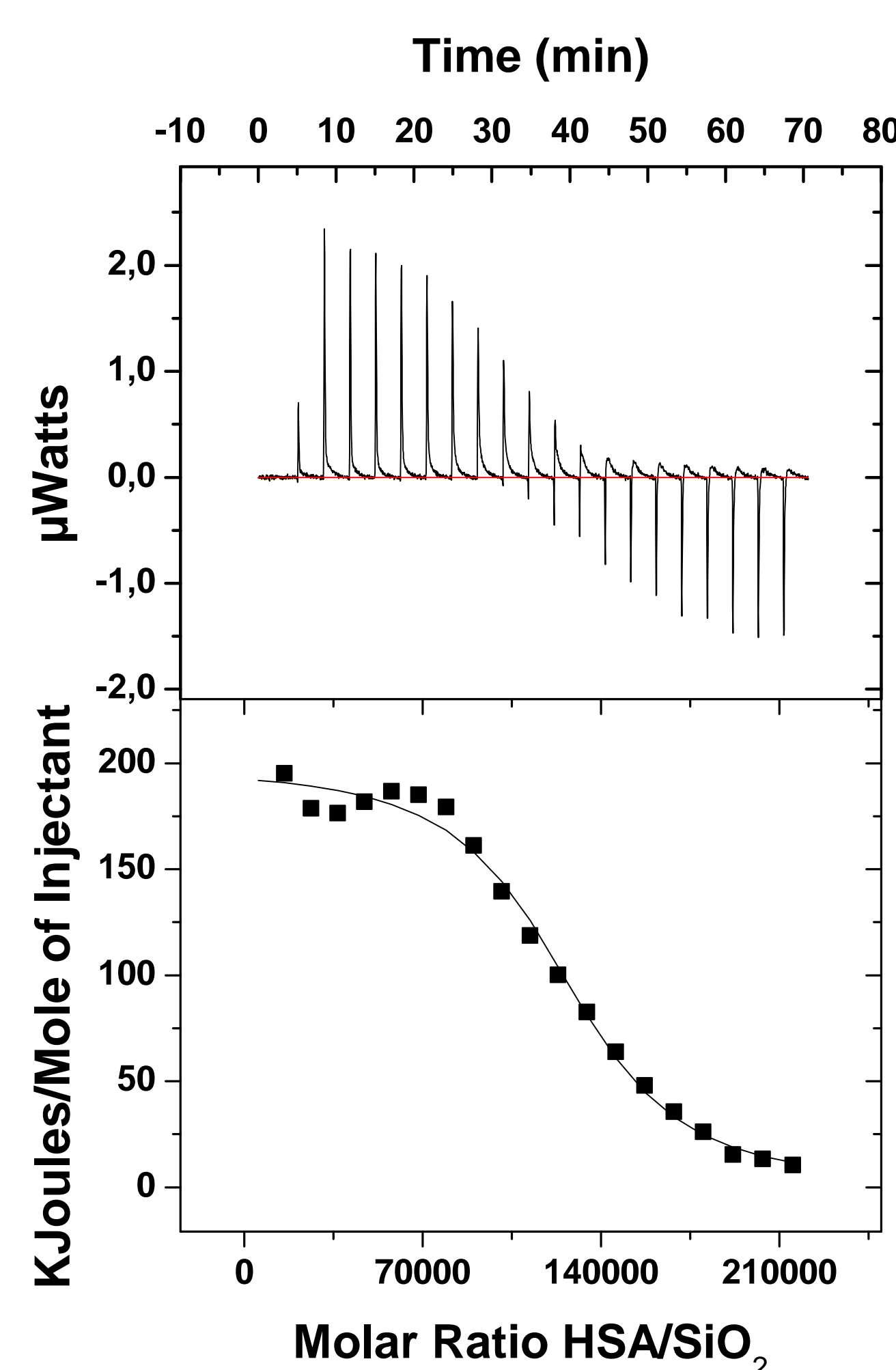
Decomposition of the thermal denaturation signal of HSA (c= 2 mgml⁻¹) in the absence and in the presence of SiO₂ MNPs (c=2mgml⁻¹) in PBS

Parameters obtained from deconvolution TwoStateScaled Model of DSC signals of HSA (c= 2 mgml⁻¹) in the absence and in the presence of SiO₂ MNPs (c=2mgml⁻¹) in PBS

	HSA	Sample1	Sample6
ΔH (kJ/mol)	1173.7	1417.5	1925.4
ΔS (kJ/(mol·K))	3.3	4.0	5.5
T_{max} (°C)	79.7	78.5	78.1
A_w	3.3	2.42	5.57
T_{max} (°C)	72.4	66.1	66.8
ΔH (kJ/mol)	248.9	165.9	163.8
A_w	0.6	4.72	3.87
T_{max} (°C)	80.3	78.9	78.4
ΔH (kJ/mol)	595.0	242.5	269.0



1	Concentration [SiO ₂]= 1.645·10 ⁻¹⁰ M [HSA]= 1.11079·10 ⁻⁴ M	N (sites) K (M ⁻¹) ΔH (kJ/mol) ΔS (kJ/(mol·K))	1.04·10 ⁵ ± 516 7.09·10 ⁶ ± 8.91·10 ⁵ 9.860·10 ⁴ ± 1108 449
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6	Concentration [SiO ₂]= 8.2972·10 ⁻¹¹ M [HSA]= 1.0141·10 ⁻⁴ M	N (sites) K (M ⁻¹) ΔH (kJ/mol) ΔS (kJ/(mol·K))	1.26·10 ⁵ ± 1.71·10 ³ 2.88·10 ⁶ ± 4.24·10 ⁵ 1.985·10 ⁵ ± 3821 764
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ITC thermograms for the interaction of HSA with SiO₂ MNPs in water

Conclusions

- ❖ Compared to the thermal denaturation of free protein in sodium buffer phosphate at pH 7.4, lower values of the transition temperature for adsorbed protein were observed, indicating that the stability of the protein when it forms the corona is lower than that of free albumin.
- ❖ Furthermore, enthalpy (ΔH), and heat capacity (C_p) values for the adsorbed protein on MNPs are bigger than the corresponding values in the MNP absence, due to the presence of water molecules when the protein corona is formed. Within the studied samples the one that has a higher protein corona (262 µg HSA/mg MNP), and at the same time more surface area (827.3 m²/g) and a higher pore (38.8 Å), has less increase comparing with the free albumin and the MNP that has the lower protein corona - (81 µg HSA/mg MNP), less surface area (780.7 m²/g) and pore (36.0 Å).
- ❖ The binding of HSA on SiO₂ MNPs' surface is a complex proces. At the beginning of the titrations, the endothermic effect indicates the dominance of hydrophobic interactions. It is followed by an exothermic effect implying a predominant role of polar interactions, e.g. ionic and/or hydrogen bond formation. The less ΔH and the high K correspond to higher PC.

Acknowledgements

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