

NMR study of interactions of silver nanoparticles with biothiols

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INTRODUCTION

Introduction of nanoparticles (NPs) to biological environments results in the formation of the so-called “biomolecular corona” – a layer of adsorbed biomolecules on the surface of NPs.¹ The composition and nature of corona and its impact on pharmacodynamics and pharmacokinetics of thusly-modified NPs is a major question in nanomedicine.² It is found that large plasma proteins passivate only a small fraction of the NP surface, and such coating layer is highly permeable for adsorption of organosulfur compounds, including endogenous sulfur biomolecules (cysteine, homocysteine, and glutathione).³ In this study, we employed nuclear magnetic resonance (NMR) to evaluate interaction of silver NPs with glutathione (GSH) and cysteine (CYS) in ultrapure water.

MATERIALS AND METHODS

AgNPs were synthesised by reducing AgNO₃ with NaBH₄ in the presence of cysteine (CYS) or glutathione (GSH) as stabilising agents. The final concentrations of reagents were: 5.6 mM AgNO₃, 5.6 mM coating and 56 mM NaBH₄ (1:1:10). The reaction mixture was stirred vigorously at room temperature for 2h. Size distribution and surface charge were obtained by dynamic and electrophoretic light scattering, respectively.

The ¹H spectra in H₂O WITH 10% D₂O were recorded with a Varian INOVA 400 spectrometer, at 399.6 MHz. Chemical shifts in the ¹H NMR spectra are expressed in parts per million (ppm) vs. TMS as the external standard. ¹H NMR spectra were recorded immediately after mixing the reagents, and subsequently every 20 minutes until AgNPs formation finished. A sample of free CYS and the reaction mixture of CYS- AgNPs at 40min was alkalized with NaOH to the pH 12 and measured.

Table 1. Hydrodynamic diameters (d_H) and corresponding volume percentages and zeta potential (ζ) for CYS-AgNP and GSH-AgNP in ultrapure water.

Nanoparticle	d _H (nm)	% Volume	ζ (mV)
CYS-AgNP	8,0 ± 0,9	99,7	-56,9 ± 7,5
GSH-AgNP	6,0 ± 1,2	99,9	-50,9 ± 2,3

RESULTS

Chemical shifts of signals in the NMR spectra, along with the loss of resolution and peak broadening, confirm the interaction of CYS/GSH with AgNP surface. Both CYS and GSH AgNP spectra show the separation of peaks corresponding to protons adjacent to the thiol group. This indicates that CYS and GSH bind to the NP surface primarily through the thiol. Furthermore, the intermediate spectra were captured, with distinct peaks corresponding to both bound and unbound biothiols. The addition of NaOH to the reaction mixture causes a significant upfield shift of signals of unbound CYS, while the signals of bound CYS remain relatively unaffected.

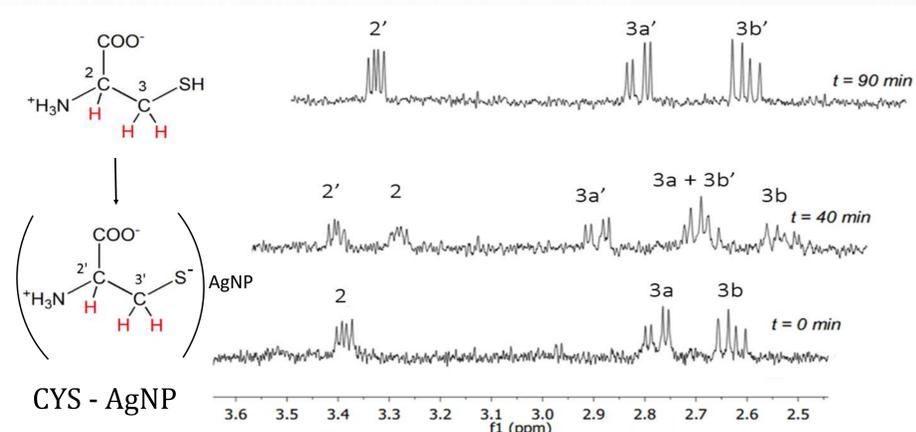


Figure 1. ¹H NMR spectra of CYS-AgNPs in ultrapure water. The reaction was tracked in 20min intervals.

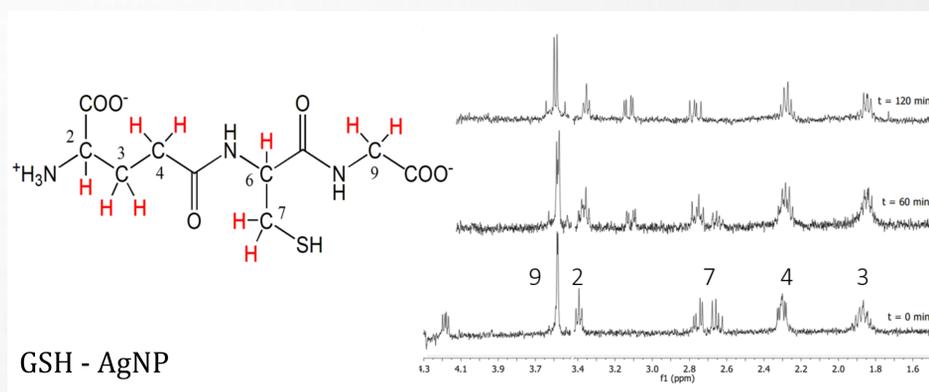
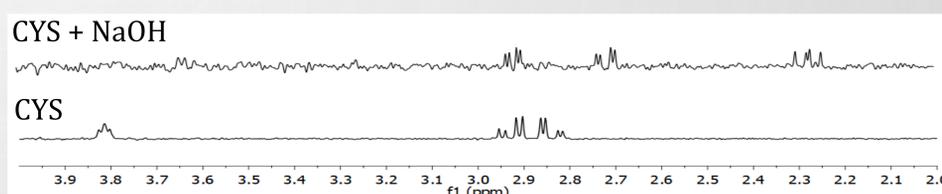


Figure 2. ¹H NMR spectra of GSH-AgNPs in ultrapure water. The reaction was tracked in 20 min intervals.

a)



b)

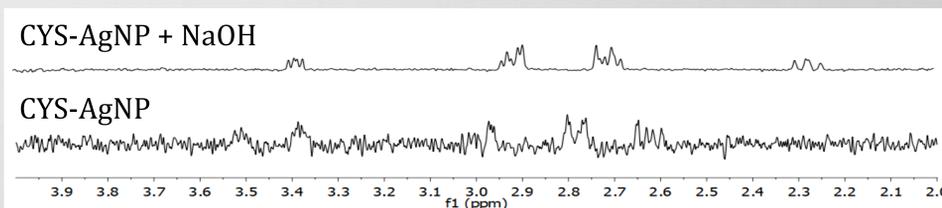


Figure 3. ¹H NMR spectra of a) free CYS in ultrapure water before and after the addition of NaOH b) reaction mixture of CYS-AgNPs at 40min of stirring, before and after the addition of NaOH.

REFERENCES:

- (1) Fleischert C.C., and Payne C.K. Acc. Chem. Res (2014) 47(8), 2651–2659.
- (2) Lohse, S. E., and Murphy, C. J. J. Am. Chem. Soc. (2012), 134, 15607–15620.
- (3) Vangala K. et al. J. Phys. Chem. C (2012) 116, 3645–3652.

ACKNOWLEDGEMENT

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