

## Dynamic equilibrium in CTAB gold nanoparticle bilayer, and the consequent impact on the exposure to biological fluids and on the formation of the nanoparticle protein corona.

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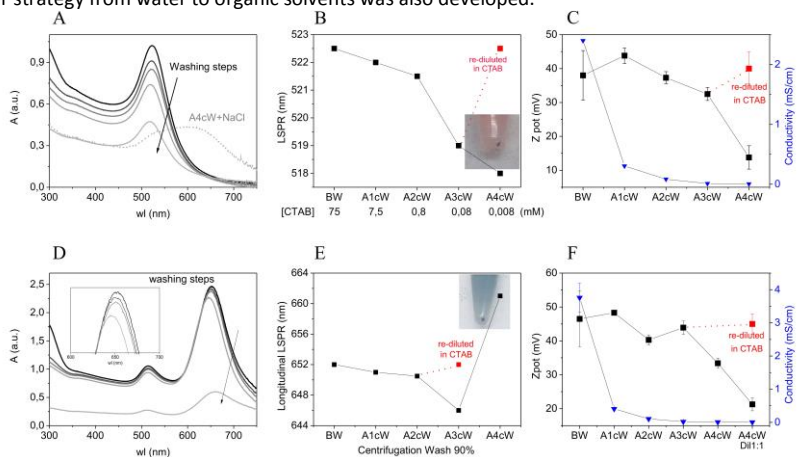
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Gold nanoparticles in solution are usually surrounded by stabilising molecules that avoid aggregation and determine their surface properties, highly influencing their behaviour. Study the surfaces natures is of paramount importance in the development of nanoparticles applications. A widely used stabilizer is the cationic surfactant CTAB, mainly because allowed the controlled synthesis of several shapes of gold nanoparticles, moreover the resultant particles are commonly used as cationic model in nano-safety studies. The physicochemical properties of the evidenced CTAB bilayer coating the surface of the particles has been largely investigated, but not its static vs dynamic nature. The present work was aimed to shed light on this question and to understand its impact on nanoparticles evolution in biological and environmental scenarios. A systematic physicochemical study of CTAB bilayer of gold nanospheres and nanorods was carried out, exploring the role of the surfactant in excess in solution on the nanoparticles surface properties and colloidal stability. The results highlighted the presence of a dynamic equilibrium between the CTAB present in the bilayer and the free surfactant in solution, resultant in the existence of multiple natures of the surfactant bilayer depending on the whole CTAB concentration. The dynamics of the bilayer entail that this dispersions always contain free CTAB, was underlined that cannot be prepared a colloiddally stable sample that present in the bilayer a concentration of surfactant higher than the free one. The impact of the dynamic equilibrium on the particle exposition to biological fluids and on the formation of nanoparticles protein corona was also studied, highlighting a different nanoparticles behaviours depending on their purification grade. During this work a simple and low-cost gold nanoparticle phase transfer strategy from water to organic solvents was also developed.



**Figure 1:** LSPR,  $\zeta$  potential and colloidal stability changes observed along NPs centrifugation washing steps. (A, D) UV-Vis spectra of AuNS and AuRod after several purification by centrifugation steps, in each stage 90% of the supernatant volume was removed and the samples were filled with an equal volume of water. (B-E) LSPR value of AuNPs after each purification step; the photos represent the samples after 4 washing steps and it is possible to notice that the NP pellets were not totally re-dispersable. (C, F)  $\zeta$  potential (■) and conductivity (▼) of AuNPs after each purification step.