Bolaamphiphile Surfactants As Nanoparticle Stabilizers : Application To Reversible Aggregation Of Gold Nanoparticles

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I) Materials and methods

Materials.

Sodium borohydride (NaBH₄), hydrogen tetrachloroaurate (III) trihydrate, triethylamine and solvents (HPLC grade) were purchased from Aldrich. Water was purified through a filter and ion exchange resin (resistivity ≥16 MΩ.cm), using a Purite device. Hexadecan-1,16-dioic acid and L-Alanine methyl ester hydrochloride were obtained from Fluka. Oxalyl chloride and sodium carbonate were purchased from Alpha Aesar and Prolabo respectively.

Characterization.

¹H NMR spectra were recorded on a Bruker ARX 500 equipped with a cryosonde.

UV-visible spectra of the colloidal gold aqueous suspensions were measured by a diode array (HP 8452A, Hewlett Packard) or a double beam (Cary 400 bio, Varian) UV-visible spectrophotometer, equipped with a temperature control system and magnetic stirring.

Transmission Electron Microscopy (TEM). AuNps were observed using a HITACHI HU12 microscope operating at 70 keV. A drop of AuNp suspensions was deposited on a TEM carbon grid and dried in air. TEM micrographs were analyzed using Image J software (http://rsb.info.nih.gov/ij/) (more than one thousand particles were counted and measured for each sample).
II) Gold Nanoparticle Synthesis

The synthesis of the gold Nps was adapted from literature data\textsuperscript{1}. To 4 mL of a 2.10\textsuperscript{-3} mol.L\textsuperscript{-1} HAuCl\textsubscript{4} solution were added 38 mL of distilled water and 600 µL of a 0.1 mol.L\textsuperscript{-1} aqueous solution of sodium hydroxyde. pH was then adjusted (if necessary) around 7.5-8. 400 µL of a freshly made 2.10\textsuperscript{-2} mol.L\textsuperscript{-1} NaBH\textsubscript{4} solution were then added under stirring. The solution immediately turned into red, indicating the formation of gold Nps. Typical TEM analysis of these Nps is given in the following figure S1.

![Average Diameter = 4.7 ± 1.8 nm
Counted Nps = 1540](image)

**Figure S1**: Representative TEM image and analysis of the gold Nps.
III) Bolaamphiphile synthesis

The synthesis and characterization of the bolaamphiphiles Bola-C\(_n\) with \(n=10, 12\) and \(20\) have been already described\(^2\). Similarly, the compounds Bola-C\(_n\) with \(n=14\) and \(16\) have been synthesized according to the following scheme S1.

**Scheme S1**: Synthesis of the bolaamphiphile Bola-C\(_n\)

Typical experimental procedure:

Under argon atmosphere, 6.5 mL (13 mmol) of a solution of oxalyl chloride (2 mol.L\(^{-1}\) in dichloromethane) were added to a solution of the dicarboxylic acid HOOC-(CH\(_2\))\(_n\)-COOH (4.2 mmol in 25 mL of dichloromethane). The mixture was heated under reflux during 2 hours. The reaction was followed by IR. When complete, the solvent is evaporated under reduced pressure (!!! Add a chemical trap !!!) and the white residue is washed with 5 mL of dichloromethane.

Under argon atmosphere, the white solid and 1.18 g (8.45 mmol) of L-alanine methyl ester hydrochloride were dissolved in 20 mL of dichloromethane. 2.35 mL of freshly distilled triethylamine (16.9 mmol) were then added dropwise, followed by 8 mL of DMF. The mixture is then maintained under reflux during three days. After cooling, 20 mL of chloroform were added and the organic phase was washed three times by 20 mL of water, dried on anhydrous MgSO\(_4\). After solvent evaporation under reduced pressure, a beige solid was obtained.
The beige solid was dissolved in 66 mL of methanol. 33 mL of water in which were dissolved 4.3 g of Na₂CO₃ (42 mmol) were then added. The mixture was then heated under reflux during 90 min. After cooling, it was filtered and the solid was washed with warm ethanol. The solution was evaporated under reduced pressure then lyophilized leading to a white solid.

Characterization of the Bola-C₁₄:

Yield: 90%.

Rᵣ = 0.55 (Eluent: dichloromethane/ethanol 95/05)

¹H NMR (CDCl₃, δ ppm):
1.20 (m, 20H, CH₂) ; 1.34 (d, J= 9Hz, 6H, CH₃βCOO) ; 1.57 (tt, J= 6Hz, 4H, CH₂βCONH) ; 2.15 (t, J= 6Hz, 4H, CH₂αCONH) ; 3.70 (s, 6H, CH₃αCOO) ; 4.55 (q, J= 9Hz, 2H, CH) ; 5.92 (d, J= 9Hz, 2H, NH).

J-modulated ¹³C NMR (CDCl₃, δ ppm):
18.60 (s, CH₃βCOO) ; 25.60 (s, CH₂) ; 29.42 (m, CH₂βCONH) ; 36.60 (s, CH₂αCONH) ; 47.89 (s, CH) ; 52.47 (s, CH₃αCOO) ; 172.72 (s, COO) ; 173.78 (s, CONH).

Mass (ESI<0) m/z = 213.4 (C₂₂H₃₈N₂O₆₂⁻) ; m/z = 427.6, (C₂₂H₃₈N₂O₆⁻).

Anal calc (%) for C₂₂H₃₈N₂O₆Na₂, C 55.92 ; H 8.11 ; N 5.93 Found C 57.60 ; H 8.81 ; N 4.61.

Characterization of the Bola-C₁₆:

Yield: 46%.

Rᵣ = 0.55 (Eluent: dichloromethane/ethanol 95/05)

¹H NMR (CDCl₃, δ ppm):
1.19 (m, 28H, CH₂) ; 1.35 (d, J= 9Hz, 6H, CH₃βCOO) ; 1.58 (tt, J= 6Hz, 4H, CH₂βCONH) ; 2.16 (t, J= 6Hz, 4H, CH₂αCONH) ; 3.72 (s, 6H, CH₃αCOO) ; 4.56 (q, J= 9Hz, 2H, CH) ; 5.93 (d, J= 9Hz, 2H, NH).

J-modulated ¹³C NMR (CDCl₃, δ ppm):
18.58 (s, CH₃βCOO) ; 25.57 (s, CH₂) ; 29.39 (m, CH₂βCONH) ; 36.57 (s, CH₂αCONH) ; 47.86 (s, CH) ; 52.44 (s, CH₃αCOO) ; 172.69 (s, COO) ; 173.75 (s, CONH).

Mass (ESI<0) m/z = 227.4 (C₂₄H₄₄N₂O₆₂⁻) ; m/z = 455.8 (C₂₄H₄₄N₂O₆⁻) ; m/z = 477.9 (C₂₄H₄₂N₂O₆Na⁻).

Anal calc (%) for C₂₄H₄₂N₂O₆Na₂, C 57.58 ; H 8.46 ; N 5.60 Found C 58.81 ; H 8.88 ; N 5.23.
IV) Determination of the pK\textsubscript{a} of bolaamphiphile heads

Due to the low solubility of the acid form in water (10\textsuperscript{-3} mol.L\textsuperscript{-1}) and to avoid any micelles or vesicles formation in the solution (that will modify the final value of pK\textsubscript{a}), this study was performed at low concentrations (10\textsuperscript{-4} mol.L\textsuperscript{-1}). To accurately measure the pK\textsubscript{a} value, \textsuperscript{1}H NMR spectroscopy equipped with a cryosonde was then used. We followed the evolution of the chemical shift of the methyl group in \(\beta\) of the carboxylic group as a function of pH.

Practically, the determination of pK\textsubscript{a} was realized as followed: to a 100 \(\mu\)L D\textsubscript{2}O solution of BolaC10 (10\textsuperscript{-3} mol.L\textsuperscript{-1}) different volumes of a HCl solution (0.1 mol.L\textsuperscript{-1}) was added and the total volume adjusted to 1 mL. So we obtained solution with pH from 1.2 to 8 (measured with a microelectrode from Bioblock). Chemical shifts where then measured with \textsuperscript{1}H NMR.

![Figure S2. A) Representative spectra of Bola-C10 at pH 1.72, 2.88, 4.11 and 7.77 ([Bola-C10] = 10\textsuperscript{-3} mol.L\textsuperscript{-1}). B) Evolution of the chemical shift (black arrow in Figure S2 A) of the methyl group in \(\beta\) of the carboxylic group as a function of pH.](image)

Points where then fitted with the following equation where \(\delta\)\textsubscript{RCOOH} and \(\delta\)\textsubscript{RCOO\textsuperscript{-}} are the chemical shifts of the methyl group for the carboxylic and carboxylate forms respectively:

\[
\delta_{\text{obs}} = \frac{\delta_{\text{RCOO\textsuperscript{-}}} - \delta_{\text{RCOOH}}}{\delta_{\text{RCOO\textsuperscript{-}}} - \delta_{\text{RCOOH}}} + \delta_{\text{RCOOH}}
\]

This leads to the estimate value of pK\textsubscript{a}=2.6 (the pK\textsubscript{a} value of the carboxylic function in alanine was found equal to 2.3). The number of methylene groups in the
hydrophobic chain of the bolaamphiphiles studied was large enough to assume that the pKₐ values of the two heads were similar. The previous value was estimated for isolated bolaamphiphile molecules.
V. Stabilisation properties of gold nanoparticles embedded by bolaamphiphile with various chain lengths (from 10 to 20 CH₂ groups).

**Figure S3.** Evolution of the solutions of gold NPs stabilized by alanine (Ala), without stabilizer (Blank) or by a bolaamphiphile with 10, 12, 14, 16 and 20 CH₂ groups depending on the pH value: initially at pH 8, then after addition of a 0.1 M HCl solution to reach a pH value of 2 (5 min and 2 hours after addition of HCl) and finally addition of NaOH.
after addition of 0.1 M NaOH solution to get a pH value of 8 ((HAuCl₄) = 0.18 mol.L⁻¹ and (Stabilizer) = 80 µmol.L⁻¹).
VI. Free bolaamphiphile titration by NMR

**Preparation of gold nanoparticles in D$_2$O.** To 4 mL of a 3.17 $10^{-3}$ mol.L$^{-1}$ HAuCl$_4$ solution in D$_2$O were added 38 mL of D$_2$O and 500 µL of a 0.1 mol.L$^{-1}$ solution of NaOH in D$_2$O (pH of the solution was found around 8). Then, 4mL of a freshly made NaBH$_4$ solution in D$_2$O (3.16 $10^{-3}$ mol.L$^{-1}$) were then added under stirring. As described previously in water, gold NPs of average diameter 5 ± 2 nm were obtained.

**Preparation of Nps/bolaforme solutions.** A series of solutions with increasing quantity of Bola-C$_n$ were then prepared in D$_2$O as followed: to a 2 mL solution of gold NPs were added from 0 to 400 µL of a Bola-C$_n$ solution (10$^{-3}$ mol.L$^{-1}$ in D$_2$O), 100 µL of a DMSO solution (4.59 $10^{-4}$ mol.L$^{-1}$ in D$_2$O) and then completed with D$_2$O to 2.5 mL (final concentrations: [DMSO]=1.83 $10^{-5}$ mol.L$^{-1}$, [Au(0)]=2.18 $10^{-4}$ mol.L$^{-1}$, [Bola-C$_n$] = 0 to 1.60 $10^{-4}$ mol.L$^{-1}$). $^1$H NMR spectrometer equipped with a cryosonde was then used to record spectra (from 32 to 512 scans depending on the bolaamphiphile concentration, delay between two pulses D1 = 2s, T = 298.3 K). A typical spectrum is given in the Figure S4.

![Figure S4. $^1$H NMR spectrum of a solution of Bola-C$_{20}$ in presence of gold NPs ([Au(0)]=[NaBH$_4$]=2.97 $10^{-4}$ mol.L$^{-1}$) with DMSO as an internal standard.](image)

The integration of the peaks relatively to the bolaamphiphile Bola-C$_n$ were compared with the integration for the internal standard DMSO (6H) to find out how much bolaamphiphile was free in the solution. The concentration of the bounded bolaamphiphile was then deduced by subtracting the concentration of the free bolaamphiphile from the known total amount of bolaamphiphile. The analysis was done using the peak at 1.3 ppm (CH$_3$ group) or at 1.1 ppm (CH$_2$ groups).
References