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Colloidal stability of zwitterionic polymer-grafted gold nanoparticles in water

Céline Durand-Gasselin [a], Régis Koerin [a], Jutta Rieger [b], Nicolas Lequeux [c], Nicolas Sanson [a,*]

[a] Sciences et Ingénierie de la Matière Molle, PSL Research University, ESPCI ParisTech, Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7615, 10 rue Vauquelin, 75231 Paris Cedex 05, France
[b] Laboratoire de Chimie des Polymères, Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 7610, 3 rue Galilée, 94200 Ivry, France
[c] Laboratoire de Physique et Etudes des Matériaux, PSL Research University, ESPCI ParisTech, Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 8213, 10 rue Vauquelin, 75231 Paris Cedex 05, France

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ABSTRACT

We investigate the colloidal stability of gold nanoparticles (AuNPs) coated with zwitterionic sulfobetaine polymers in aqueous solution. Zwitterionic polymers with different molar masses, synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization of N,N′-dimethyl(methacrylamido propyl)ammonium propanesulfonate (SPP) exhibit a well known Upper Critical Solution Temperature (UCST) in water, i.e., phase separate at low temperature. The colloidal stability of gold nanoparticles grafted with PSPP was studied as a function of the temperature. The effects of the molar mass of the grafted polymers, the salt concentration, and the presence of free polymer chains in solution were investigated. UV–vis spectroscopy and dynamic light scattering measurements show that whatever the molar mass of the grafted polymer, the nanoparticles never aggregate at low temperature in pure water. However, a reversible thermal-driven aggregation process of the gold nanoparticles is observed in presence of free polymer chains in solution and explained by a depletion process.

1. Introduction

During the last decade, metallic nanoparticles and especially gold nanoparticles (AuNPs) have received tremendous interest in diverse application fields such as biomedical labeling [1,2], drug delivery [3,4], photothermal therapy [5] and catalysis [6], mainly due to a combination of their unique physical and chemical properties. For most of these applications, a grafted polymer layer is required which act as a protecting shell against aggregation processes, non-specific interactions and which can be functionalized for targeting. Among ligands used to stabilize gold nanoparticles, zwitterionic polymers which are composed of an equal number of positive and negative charges appear to be hopeful [7]. This interest for this type of ligands lies in the remarkable anti-fouling properties, i.e., resistance to cell, bacteria and protein adsorption, exhibited by zwitterionic functions as a result of their strong hydration [8]. These excellent properties actually make zwitterion-based materials the ideal candidate for biomedical applications maybe better than well-known poly(ethylene glycol) [9] or oligo(ethylene glycol) [10]. However, some hydrophilic

zwitterionic polymers tend to precipitate in water as the temperature decreases, a property related to the UCST (Upper Critical Solution Temperature) behavior, which comes from Coulombic interactions between positive and negative charges located either on the same repeat unit or between different repeat units of the polymer chain (intra or interchain interactions) [11–13]. Such behavior mainly depends on the structure, molar mass and concentration of the zwitterionic polymer. One may wonder if the thermoresponsive property of zwitterionic polymers used as ligands for nanoparticles may be disturbing for colloidal stability.

In the literature, most of the studies regarding the properties of thermosensitive polymer-coated gold nanoparticles reported to date concern polymers exhibiting a LCST (Lower Critical Solution Temperature) behavior such as poly(N-isopropylacrylamide) poly(-NiPAM) [14], poly(N-vinyl caprolactam) poly(VCL) [15], ethylene oxide oligomers of different lengths [16], or statistical copolymers based on ethylene oxide and propylene oxide groups [17]. In many cases, it was observed that, when the temperature increases above the phase transition temperature, the decrease of the solvent quality induces a collapse of the polymer leading to an aggregation of the polymer-coated AuNPs and a shift of the Surface Plasmon Resonance SPR towards longer wavelengths. In general, the thermoresponsive behavior is totally reversible and the influence of many

* Corresponding author. Fax: +33 (0) 140 794 640.
  E-mail address: nicolas.sanson@espci.fr (N. Sanson).

parameters on the phase transition temperature such as AuNPs concentration and size, ligand composition, has been studied.

On the other hand, very few studies on the influence of the temperature on the stability of UCST polymer-coated nanoparticles (including gold) have been reported. Dong et al. studied the effect of the temperature, salt, concentration and solvent on the behavior in water of silica nanoparticles coated by poly(*N,N′*-dimethyl(methacrylate ethyl)ammonium propanesulfonate) (PSPE) [18]. They found that PSPE-coated silica nanoparticles aggregate when the temperature decreases meaning that the SPE polymers exhibit an UCST behavior. They also observed that the aggregation–dissociation process of poly(SPE)-grafted silica particles is not totally reversible, meaning that the thermal-driven processes of aggregation and dissociation are distinct, difference explained by a possible rearrangement of the polymer-coated silica particles due to intra and interchain interactions. Using the same zwitterionic polymers on polystyrene latex particle, Polzer et al. observed a solubility change of the polymer corona as a function of the temperature and salt establishing the UCST behavior of PSPE polymers in aqueous solution. However, no temperature induced aggregation is reported in their study [19]. Housni et al. reported the preparation of gold nanoparticles coated with a block copolymer composed of poly(ethylene oxide) and poly(*N,N′*-dimethyl(methacrylate ethyl)ammonium propanesulfonate) [20]. The zwitterionic polymer block which constitutes the inner layer on the AuNP surface exhibits a phase transition with the temperature. On the other hand, no aggregation occurs due to the steric protection of the poly(ethylene oxide) outer layer.

In this work, the behavior and the colloidal stability of sulfobetaine polymer-coated gold nanoparticles in aqueous solution was investigated. First, a series of zwitterionic polymers, poly(*N,N′*-dimethyl(methacrylamido propyl)ammonium propanesulfonate) (PSPP), with different molar masses were synthesized by RAFT polymerization using 4-cyanopentanoic acid dithiobenzoate as RAFT agent. The advantage of the RAFT polymerization is twofold: (i) it allows to control the molar mass of the synthesized polymers which is an important parameter for the study of thermosensitive behavior of polymer solutions. (ii) The end-group of 4-cyanopentanoic acid dithiobenzoate allows, after the cleavage of the dithiobenzoate function to obtain a thiol function which constitutes a good anchor for the gold nanoparticle surface. In the first stage, the phase diagrams of the zwitterionic polymers were determined in pure water before their grafting on gold nanoparticles. Then, the behavior of zwitterionic polymer-coated AuNPs (AuNP@PSPP), prepared by ligand exchange, was investigated in aqueous solution. The effects of the average molar mass of the grafted polymers, the salt concentration, the temperature and the presence of free polymer chains in solution were studied. We found that the AuNP@PSPP do not exhibit a thermal-driven aggregation in pure water but show a reversible aggregation in presence of an excess of free polymer chains in solution. With these results, we aimed at a deeper understanding of the colloidal behavior of gold nanoparticles coated by zwitterionic polymer chains in aqueous solution.

2. Experimental part

2.1. Materials

N,N′-Dimethyl(methacrylamido propyl)ammonium propanesulfonate (SPP) was donated from RASCHIG GmbH, Germany. 2,2′-Azobis(2-amidinopropane) dihydrochloride (V50), hydrogen tetrachloroaurate(III) (HAuCl₄), sodium citrate tribasic dehydrate, sodium borohydride (NaBH₄) were purchased from Sigma–Aldrich. All products were used as received. The RAFT agent, 4-cyanopenta-

noic acid dithiobenzoate, was synthesized as reported in Ref. [21]. Ultrapure deionized water (MilliQ, Millipore, France) was used in the experiments.

2.2. Synthesis

2.2.1. Zwitterionic polymers

The zwitterionic polymers, PSPP, were synthesized by RAFT polymerization in water at 70 °C in the presence of 4-cyanopentanoic acid dithiobenzoate as RAFT agent. In a typical experiment (Table 1, PSPP40K), SPP monomers (2 g, 6.9 mM) and RAFT agent (14 mg, 0.05 mM) were dissolved in 150 mL of water in a 500 mL round-bottom flask. The content was stirred under N₂ atmosphere during 40 min. Then, V50 (500 μL, 2 g/L) was added and the reaction was performed during 16 h at 70 °C. The polymers were purified by dialysis in Spectra Por membranes (cutoff 12–14 kg/mol) against pure water during one week and finally freeze dried.

2.2.2. Gold nanoparticles (AuNPs)

Citrate stabilized AuNPs of diameter 17 nm were synthesized using the standard citrate reduction method. Briefly, 1 mL of a HAuCl₄ solution (25 mM) was added to 100 mL of water. The solution was boiled and then 5 mL of trisodium citrate solution at 1% in weight was added under vigorous stirring. After 20 min of boiling, the solution was allowed to cool to room temperature. Water volume was then completed to 100 mL. Citrate AuNPs solutions were concentrated 4 times by centrifugation and stored at 4 °C.

2.2.3. Zwitterionic polymer-protected gold nanoparticles (AuNP@PSPP)

Zwitterionic polymer-protected gold nanoparticles were prepared by ligand exchange procedure. 2 mL of PSPP aqueous solution at 1 wt% (1 eq) was mixed, under stirring, with a freshly prepared NaBH₄ solution (3 eq) during 30 min in order to cleave the dithiobenzoate end group and obtain thiol group at the end of the polymer chain. The disappearance of the characteristic pink color of the RAFT agent was observed during this reaction. Then, 2 mL of citrate stabilized gold nanoparticles (∼4 nM) were added into the functionalized polymers under stirring for 1 h at 25 °C. First, the zwitterionic polymer-grafted gold nanoparticles were purified three times by centrifugation/redispersion cycles (18,600*g*, 10 min) at 40 °C to remove the polymer excess. Afterwards, they were purified by dialysis in Spectra Por membranes (cutoff 12–14 kg/mol) against pure water until the conductivity of the solution reached 6–7 μS/cm. They are noted AuNP@PSPP followed by the weight-average molar mass of PSPP used.

2.2.4. Mixture of AuNP@PSPP and free polymer chains

The mixtures of AuNP@PSPP and free PSPP polymer chains were directly prepared in the UV–vis spectrophotometer at 50 °C under stirring (400 rpm). For instance, 1 mL of AuNP@PSPP with a nanoparticle concentration of about 2 nM was mixed with 1 mL of a 2 wt% aqueous polymer solution in order to obtain a solution of AuNP@PSPP in 1 wt% of free PSPP chains.

2.3. Characterization

2.3.1. SEC

The number-average molar mass (M_n), the weight-average molar mass (M_w), and the dispersity ($Đ = M_w/M_n$) were determined by size exclusion chromatography (SEC) in 0.5 M NaNO₃ aqueous solution at 25 °C and at a flow rate of 1 mL/min using a Viscotek SEC system equipped with three SHODEX OHpack columns SB-806M HQ (13 μm, 300 × 8 mm). All polymers were injected at a concentration of 2 mg/mL after filtration through a 0.2 μm pore-size membrane. The absolute molar masses of the samples were

Table 1
Characteristics of the zwitterionic polymers, PSPP.

Sample	M_n (g/mol)[a]	M_w (g/mol)[a]	$Đ$[a]	R_h (nm)[b]	dn/dc (mL/g)[a]	UCST (°C)[c]
PSPP40K	35,600	40,600	1.14	–	0.158	18
PSPP70K	50,800	72,700	1.43	4.8	0.167	21
PSPP100K	73,600	106,100	1.44	6.4	0.170	26
PSPP230K	136,000	227,700	1.67	7.2	0.161	33

[a] Average molar mass, M_n and M_w, $Đ$ and average dn/dc values determined by SEC in water (+0.5 M NaNO$_3$).
[b] Hydrodynamic radii, R_h, of the polymers determined by dynamic light scattering in water at 35 °C.
[c] UCST determined by turbidity experiments using UV–vis spectrophotometer.

determined by the three detectors in line (refractometer, viscometer and light scattering), coupled with a calibration based on poly(ethylene oxide) standards.

2.3.2. UV–vis spectroscopy

Absorbance measurements were carried out at different temperatures with a UV–vis Hewlett-Packard 8453 spectrophotometer using a quartz cell, in a wavelength range from 200 to 1100 nm and equipped with a temperature controller (±0.1 °C). For UV–vis experiments, the nanoparticle concentration was about 1 nM.

2.3.3. Phase diagrams and UCST determination

UV–vis spectroscopy allowed us to build the phase diagram of the zwitterionic polymers in the concentration range from 0.04 to 20 wt% depending on the average molar mass of the polymer. For this, we collected the transmitted intensity as a function of the temperature. For a given concentration, we defined the cloud point, i.e., the phase transition temperature as the temperature where the transmitted intensity reaches 50% of the incident intensity.

2.3.4. Dynamic light scattering

Dynamic Light Scattering (DLS) was carried out on a CGS-3 goniometer system equipped with HeNe laser illumination at 633 nm and an ALV/LSE-5003 correlator. All samples were initially filtered through 0.2 µm Millipore syringe filters. The samples were stabilized at constant temperature for 10 min prior to measurement. The data were collected by monitoring the light intensity at different scattering angles. The hydrodynamic size distribution was obtained using CONTIN algorithm and represented in percentage of the diffused intensity.

2.3.5. Total Organic Carbon (TOC)

A Shimadzu TOC-V CSH was used to measure the carbon content of the purified AuNP@PSPP which allows the determination of the polymer grafting density. AuNP@PSPP aqueous dispersion, with a known concentration of particles, was directly injected in the apparatus, and the measurement was repeated twice.

2.3.6. Microscopy

TEM images were performed with a JEOL 2010 field electron gun microscope operating at an acceleration voltage of 200 kV. Samples were prepared by spreading a drop of sample on an ultra-thin 300 mesh Formvar/carbon-coated copper grid and dried in air. The particle size distributions were determined using ImageJ software.

3. Results

### 3.1. Behavior of zwitterionic polymers in water

Before investigating the behavior of polyzwitterion-grafted gold nanoparticles, the characteristics of the synthesized polymers alone in water were examined. RAFT polymerizations of

zwitterionic monomers, SPP, in presence of 4-cyanopentanoic acid dithiobenzoate as RAFT agent were performed in water at 70 °C (Scheme 1). Zwitterionic polymers with an average molar mass from 40 to 230 kg/mol were obtained. After purification, the chemical structure of PSPP was confirmed by ^{1}H NMR (Fig. S1 in Supporting Information). The average molar masses, M_n and M_w, together with the dispersity, $Đ$, were determined by aqueous size exclusion chromatography (Fig. S2 in Supporting Information) and reported in Table 1. Fig. 1 reports the phase diagrams of PSPP of different average molar masses in water determined by turbidity experiments. For all synthesized polymers, the phase diagrams exhibit an usual dumbbell shape characteristic of UCST polymers. As predicted by Flory's theory, the critical temperature, T_c, corresponding to the maximum of the phase diagram curve, depends on the number-average polymerization degree, X_n [22]. This dependence allows us to determine the theta temperature, θ, which is defined as the critical temperature for a polymer having an infinite molar mass. The theta temperature was determined by plotting T_c for each polymer as a function of the number-average polymerization degree, $1/T_c = f\left(1/X_n^{1/2} + 1/2X_n\right)$, (Fig. S3 in Supporting Information). We found a theta temperature θ for PSPP in water at 49 °C. The value of the theta temperature is very close to the critical temperature of 46 °C for a PSPP with a weight-average molar mass of 1000 kg/mol found by Mary et al. [23].

3.2. Behavior of zwitterionic polymer-grafted gold nanoparticles

Gold nanoparticles (~17 nm in diameter) protected by zwitterionic polymers with different average molar masses, AuNP@PSPP, were prepared by the ligand exchange procedure as shown in Scheme 1. The use of 4-cyanopentanoic acid dithiobenzoate as RAFT agent allows, after cleavage using NaBH$_4$ as reducing agent, to obtain a zwitterionic polymer with a thiol function which is an excellent anchor for the gold surface. The AuNP@PSPP were intensively purified by centrifugation/redispersion cycles and dialyzed in order to remove both the excess of polymer chains and salt, components which can disrupt the thermosensitive behavior in aqueous solution. TEM images of grafted particles do not show evidence of aggregation (see Fig. S4 in Supporting Information). The grafting density was calculated from total organic carbon measurements and estimated at around 0.2 chains per nm^2.

The colloidal state of AuNPs grafted with zwitterionic polymers as a function of the temperature, from 50 to 10 °C, was investigated using combined UV–vis spectroscopy and dynamic light scattering experiments. The results for AuNP@PSPP70K are shown in Fig. 2. All UV–vis spectra superimpose with a SPR centered at 525 nm without any red shift, indicating an absence of aggregation of the gold nanoparticles when the temperature decreases (Fig. 2a). This result is correlated with dynamic light scattering experiments where the hydrodynamic diameter of AuNP@PSPP70K does not change in the temperature range (Fig. 2b). Similar observations were made for all AuNP@PSPP whatever the average molar mass of the grafted zwitterionic polymer.
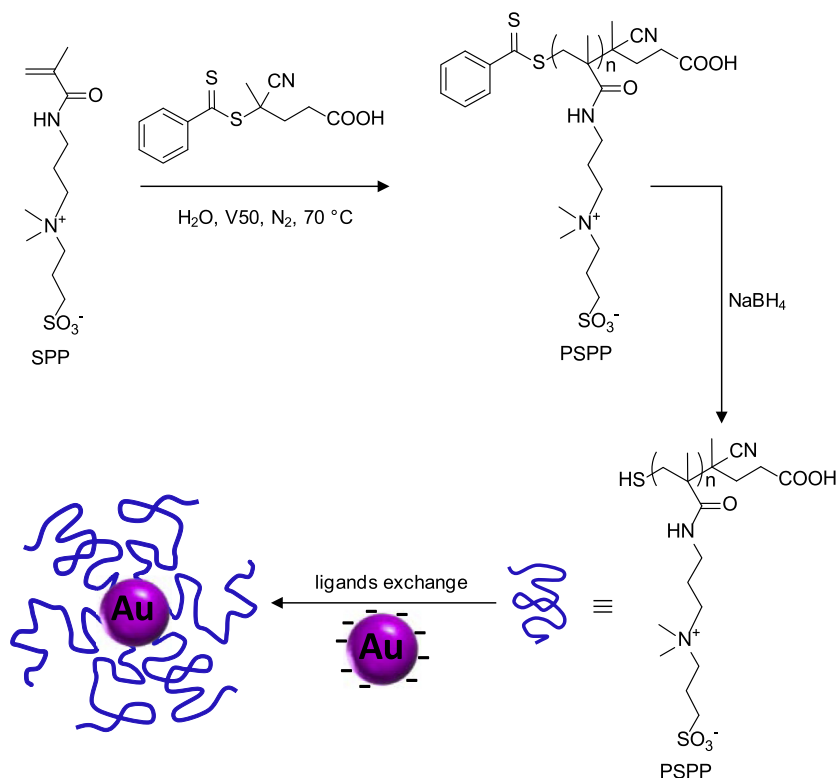
Scheme 1. Schematic representation of the synthesis of gold nanoparticles coated with zwitterionic polymers, AuNP@PSPP. In the first step, PSPP were synthesized by controlled radical polymerization using a RAFT agent. After purification using dialysis, the PSPP polymers were freeze-dried. Then, the end-function of the polymers was cleaved using NaBH$_4$ as reducing agent. Gold nanoparticles capped with zwitterionic polymer chains were obtained by a ligand exchange procedure on citrate-stabilized gold nanoparticles.
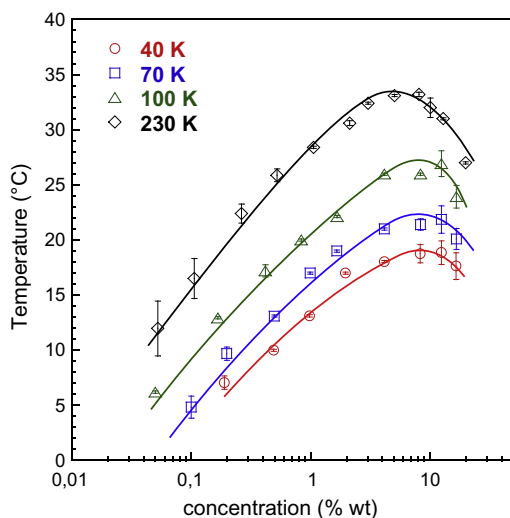

Fig. 1. Phase diagrams in deionized water of polyzwitterions, PSPP, with different weight-average molar masses: (○) PSPP40K, (□) PSPP70K, (△) PSPP100K, (◇) PSPP230K. The lines are guides for the eye.

It is well known that the solubility of the zwitterionic polymers is promoted by salt addition [23–26]. Indeed, when the salt concentration increases, the Coulombic interactions between opposite charges are screened inducing a dissociation of the charges and a swelling of the polymer chains, phenomenon generally called "antipolyelectrolyte effect". Consequently, the presence of salt tends to decrease the phase transition temperature of zwitterionic polymers [23]. Fig. 3 shows the variation of the hydrodynamic diameter of two zwitterionic polymer-coated AuNPs,
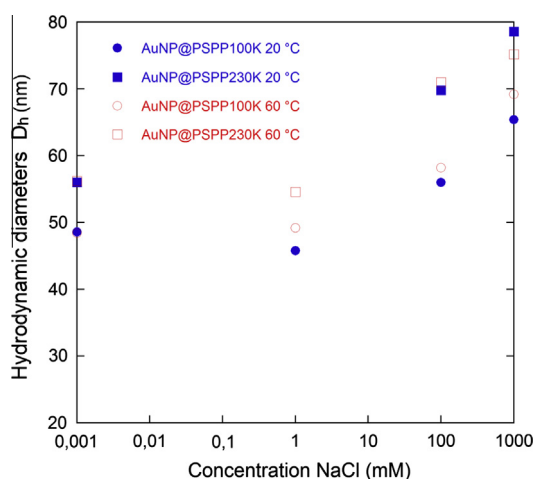
AuNP@PSPP100K and AuNP@PSPP230K, as a function of NaCl concentration at 20 and 60 °C. For both samples, the diameter increases significantly for more than 15 nm from 0 to 1 M NaCl concentration (Fig. S5 in Supporting Information). The effect of the temperature in different salt solutions was investigated at 20 and 60 °C and no significant difference in size at both temperatures was revealed.

3.3. Behavior of AuNP@PSPP in presence of free polymer chains

The behavior of zwitterionic polymer-grafted gold nanoparticles, AuNP@PSPP, in presence of an excess of free polymer chains in solution was investigated. Fig. 4a shows the UV–vis spectra of AuNP@PSPP100K (1 nM) in presence of 1 wt% of free polymer chains PSPP100K by decreasing the temperature from 50 to 20 °C. During the temperature decrease, two phenomena were observed. In the first part of the ramp, all UV–vis spectra superimpose up to 40 °C indicating that AuNP@PSPP100K remain well dispersed. Then, at 38 °C, the absorbance at the SPR decreases together with an increase of the absorbance at longer wavelength and a red shift of the λ_{SPR} from 527 to 567 nm meaning that the AuNP@PSPP100K start to aggregate (Fig. 4b). We define the aggregation temperature, T_{agg}, as the first temperature where a SPR red shift is observed. By continuing to decrease the experiment temperature (Fig. 4b), we observed a fast turbidity increase starting at 22 °C corresponding to the phase separation of the free zwitterionic polymer in solution in agreement with the phase diagram previously determined for this polymer (Fig. 1). The aggregation of zwitterionic polymer-coated AuNPs in presence of free polymer chains is totally reversible and can be repeated many times over consecutive cooling–heating cycles above the turbidity tempera-
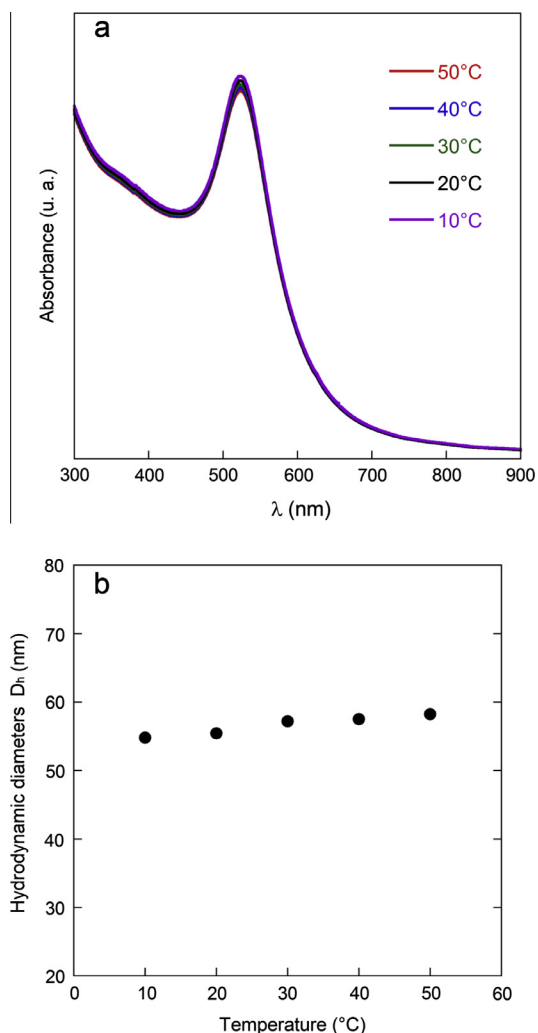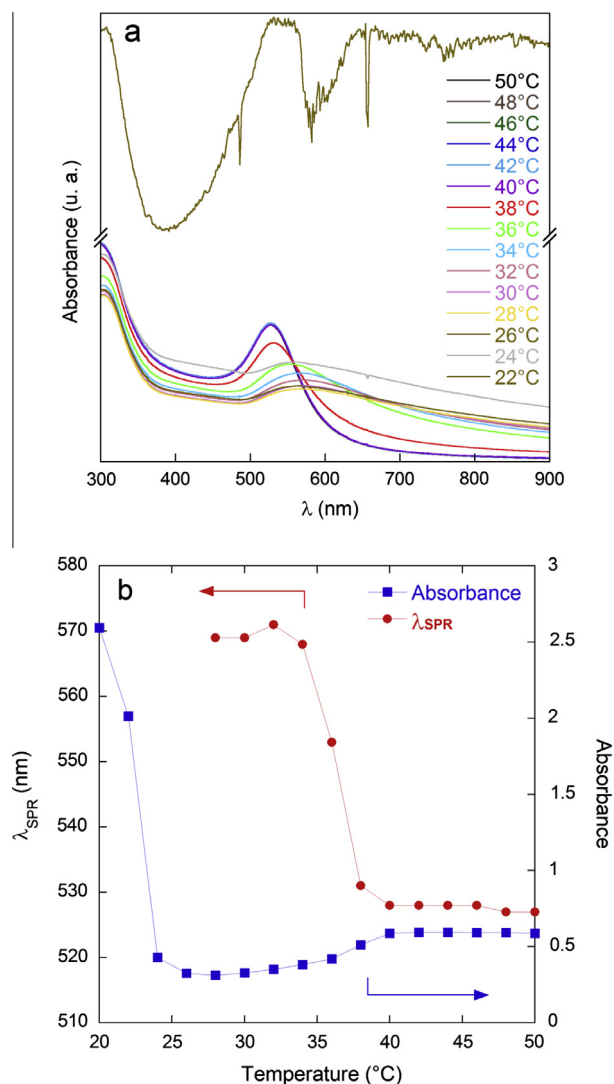
Fig. 2. Influence of temperature from 50 to 10 °C on a solution of AuNP@PSPP70K (1 nM) (a) UV–visible absorption spectra; (b) hydrodynamic diameters D_h.

Fig. 4. Influence of temperature on a solution of AuNP@PSPP100K (1 nM) in presence of 1 wt% of PSPP100K. The temperature was decreased from 50 to 20 °C at a ramp 0.3 °C/min: (a) UV–visible absorption spectra; (b) evolution of maximum wavelength (λ_{SPR}) and absorbance at the λ_{SPR}.

Fig. 3. Salt dependence of the hydrodynamic diameters, D_h, of AuNP@PSPP100K (circles) and AuNP@PSPP230K (square) at 20 °C (filled symbols) and 60 °C (empty symbols). NaCl concentration in starting purified water solution was arbitrarily estimated at 10^{-3} mM.

ture of the free polymer, between 25 and 50 °C (see Fig. S6 in Supporting Information). The aggregation temperature mainly depends on both the free polymer amount in solution and the average molar mass as shown in Fig. 5 and in Fig. S7 in Supporting Information. Note that the systems were always formulated considering the same polymer grafted on gold nanoparticles and free in solution. For instance, in the case of experiment with free PSPP100K in solution, T_{agg} first increases with increasing the polymer concentration from 0.01 to 1 wt% and decreases when the free polymer concentration is higher than 1 wt% (Fig. 5). For AuNP@PSPP70K and AuNP@PSPP100K in presence of respective free polymers, the T_{agg} curves exhibit a bell shape similar to the phase diagrams of the polymers alone (see Fig. 1). However, the curves are shifted both to lower polymer concentration and to higher temperature. For AuNP@PSPP230K, at a concentration of polymer higher than 1 wt%, the nanoparticles immediately aggregate during mixture at 60 °C, but an aggregation temperature can be measured for lower polymer concentration. On the other hand, the addition of NaCl has a dramatic effect on the aggregation behavior: at salt concentration as low as 10 mM, the nanoparticle aggregation phenomenon totally disappears whereas polymer phase transition temperature is slightly shifted by only few degrees (from 20 to 16 °C for PSPP100K) (see Fig. S8 in Supporting Information).
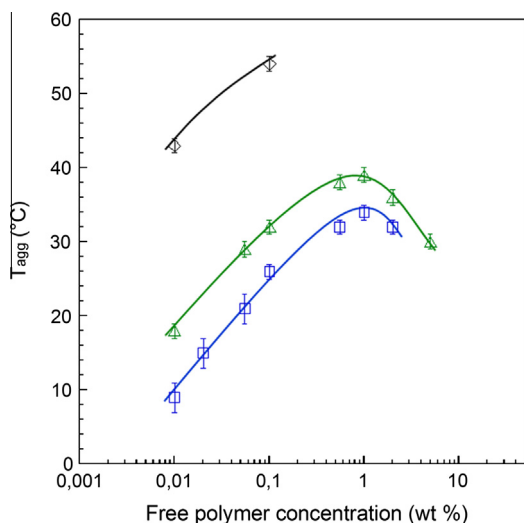
Fig. 5. Aggregation temperature, T_{agg}, as a function of the free polymer concentration for different studied systems: (□) AuNP@PSPP70K/PSPP70K, (△) AuNP@PSPP100K/PSPP100K, (◇) AuNP@PSPP230K/PSPP230K. The lines are guides for the eye.

4. Discussion

We have shown that zwitterionic polymers, PSPP, of different average molar masses, synthesized by RAFT polymerization, exhibit a UCST behavior in water (see Fig. 1), phenomenon which is attributed to the Coulombic interactions between charged ammonium and sulfonate groups of same (intra) or different (inter) polymer chains. On the other hand, when these thermosensitive polymers were grafted on gold nanoparticle surface, no AuNP@PSPP aggregation was observed with the temperature in relation to the UV–vis and light scattering experiments, with or without the presence of salt (Figs. 2 and 3). This result is quite surprising compared to several papers in the literature concerning the behavior of thermosensitive polymer-grafted gold nanoparticles where a thermal response is observed as the polymer alone. From experimental data of gold nanoparticle hydrodynamic radius and grafting density, the local polymer concentration in the corona around the gold nanoparticle core can be approximated. For instance, for AuNP@PSPP100K, we found a local polymer concentration value close to 60 wt%. At this concentration, the tethered SPP polymer chains, forming a gel around the AuNP core, are always soluble in the studied temperature range as observed in the phase diagram in Fig. 1. Compared to our results, Dong et al. observed a thermal-induced aggregation of silica nanoparticles grafted with SPE polymer chains (from 50 to 130 kg mol^{-1}) with a similar grafting density (0.35 chains nm^{-2}) [18]. The discrepancy can be only explained by the chemical nature of the polymers. Indeed, for polymers with the same average molar mass, PSPP precipitates at lower temperature than PSPE as showed by Mary et al. [23]. This solubility difference between the two polymers can explained why nanoparticles grafted with PSPP exhibit a higher stability at low temperature than one grafted with PSPE.

The presence of salt in solution enhances the colloidal stability of AuNP@PSPP due to an increasing of the polymer layer thickness (Fig. 3). These observations are in good agreement with those of Polzer et al. who also report the increasing size of the zwitterionic polymer corona upon salt addition for a system composed of a polystyrene latex particle surrounded by a PSPE corona [19].

In the present work, the aggregation of AuNP@PSPP as a function of the temperature was only observed in the presence of free PSPP chains in solution (Figs. 4 and 5). Before studying the effect of the free polymer concentration on T_{agg}, we can ask us if the thermal-driven aggregation of AuNP@PSPP is actually induced by a bridging of the AuNP@PSPP by free PSPP. To respond to this question, we performed UV–vis experiments as a function of the temperature on AuNP@PSPP40K in presence of 1 wt% of polyethylene oxide, PEO (35 kg/mol), which does not interact with PSPP polymers (Fig. S9 in Supporting Information). The results showed that AuNP@PSPP40K with 1 wt% of PEO aggregate at 36 °C indicating that the AuNP@PSPP aggregation is not induced by a bridging phenomenon.

The phenomenon of particle aggregation in presence of free non-adsorbing polymers in solution named "depletion flocculation" is now well established since the 1950s with the first quantitative analysis by Asakura and Oosawa [27,28]. Thereafter, Jones and Vincent have extended this theory to polymer-coated spherical particle in presence of free polymer chains without the compression of the grafted polymer layer [29–31]. For these systems, they established an equation for the depletion potential:

$$V_{dep} = 2\pi R \Pi_{osm} \left(\Delta + \delta - p - \frac{h}{2} \right)^2 \qquad (1)$$

where R is the radius of the particle, Π_{osm} is the osmotic pressure of the polymer solution, Δ is the depletion layer, δ is the thickness of the grafted polymer layer, p is the depth of penetration of the free polymer into the grafted polymer layer and h is the surface–surface separation distance between the particles. This expression can be reduced considering the polymer-coated particles in contact, i.e., when $h = 2\delta$.

$$V_{dep,min} = 2\pi R \Pi_{osm} (\Delta - p)^2 \qquad (2)$$

For a complete description of our system, a steric stabilization must be taken into account as well as attractive Van der Waals interactions which are no negligible for gold nanoparticles.

At 50 °C, starting temperature for UV–vis experiments, grafted and free polymers are in theta solvent condition, so no depletion occurs due to the partial interpenetration of polymer chains. As the temperature decreases below T_c, the water becomes a poor solvent for zwitterionic polymers and the grafted polymer layer becomes impenetrable to the free polymer chains inducing depletion aggregation [29]. This depletion process inducing the aggregation (T_{agg}) occurs at higher temperature than the phase transition temperature of the free polymer chains.

We have shown that the aggregation temperature of AuNP@PSPP is dependent of the free polymer concentration (Figs. 5 and S7). At low free polymer concentration, T_{agg} increases with increasing the polymer concentration. In the light of the Eq. (2), the minimum of the depletion potential is proportional to the osmotic pressure of the free polymer chains and consequently proportional to the free polymer concentration. As the polymer concentration increases, T_{agg} reaches a maximum and then decreases. This behavior is explained by the fact that the depletion potential and then T_{agg} is maximum at the overlap concentration of the free polymer, C^* [29]. Finally, when the free polymer concentration exceeds C^*, the depletion potential decreases due to a decrease of the depletion layer Δ in semi-dilute regime explaining the bell-shaped curve. We can notice that the maxima of T_{agg} for the systems AuNP@PSPP70K and AuNP@PSPP100K in presence of PSPP70K and PSPP100K respectively, are reached for C^* values of about 1 wt% (Fig. 5), value which is less than the C^* of the polymer alone in water (Fig. 1). The shift of the overlap concentration for PSPP70K and PSPP100K is in agreement with an increase of the solubility of the UCST polymer chains with increasing the temperature, inducing a lower overlap concentration [32].

The behavior of zwitterionic polymers in water is highly dependent of the salt concentration which increases their solubility

[18,19]. Indeed, 10 mM of NaCl solution decreases the phase transition temperature of PSPP100K from 22 to 16 °C and totally suppress the depletion-induced aggregation (see Fig. S8). In the case of AuNP@PSPP, the salt screens a part of the attractive interactions which promote the solubility of the zwitterionic polymer corona surrounding the gold nanoparticles. It leads to an increase of the steric potential protecting the nanoparticles against aggregation. Another effect is the possible dissymmetric adsorption of the positive and negative ions on the opposite charges of the zwitterionic unit repeat leading to the formation of charged polymer which makes the stabilization of the gold nanoparticle more efficient [33].

5. Conclusions

From the physico-chemical studies of zwitterionic sulfobetaine polymer-coated gold nanoparticles, AuNP@PSPP, as a function of the temperature, salt concentration and the presence of free polymer chains in solution, several conclusions can be drawn. First, the synthesis of zwitterionic polymers using a RAFT agent is a good way to produce *stimuli*-responsive hybrid nanoparticles. The study of synthesized zwitterionic polymers, with molar masses from 40 to 230 kg/mol, alone in pure water is consistent with a UCST behavior. Compared to free polymer chains which precipitate at low temperature, AuNP@PSPP remain perfectly dispersed at any temperature even at low ionic strength. This stability can be explained by the presence of a dense polymer layer around the gold nanoparticle which acts as a swollen gel.

On the other side, an aggregation of AuNP@PSPP as a function of the temperature is observed in presence of free polymer chains in solution. All observations of the temperature and concentration dependence are consistent with a depletion model. The depletion is induced by a conformation change of the polymer corona which becomes dense and impenetrable below the theta temperature. Salt completely inhibits the thermal-driven depletion process due to an enhancement of the solubility of the grafted zwitterionic polymers making these type of ligands ideal to stabilized nanoparticles in various conditions of temperature and salt concentration.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2014.07.048.

References

[1] R.A. Sperling, P. Rivera gil, F. Zhang, M. Zanella, W.J. Parak, Chem. Soc. Rev. 37 (2008) 1896–1908.
[2] R. Wilson, Chem. Soc. Rev. 37 (2008) 2028–2045.
[3] M. De, P.S. Ghosh, V.M. Rotello, Adv. Mater. 20 (2008) 4225–4241.
[4] A. Llevot, D. Astruc, Chem. Soc. Rev. 41 (2011) 242–257.
[5] Z.P. Qin, J.C. Bischof, Chem. Soc. Rev. 41 (2011) 1191–1217.
[6] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293–346.
[7] E. Muro, T. Pons, N. Lequeux, A. Fragola, N. Sanson, Z. Lenkei, B. Dubertret, J. Am. Chem. Soc. 132 (2010) 4556–4557.
[8] S.Y. Jiang, Z.Q. Cao, Adv. Mater. 22 (2010) 920–932.
[9] Z.G. Estephan, P.S. Schlenoff, J.B. Schlenoff, Langmuir 27 (2011) 6794–6800.
[10] E. Ostuni, R.G. Chapman, R.E. Holmlin, S. Takayama, G.M. Whitesides, Langmuir 17 (2001) 5605–5620.
[11] S. Kudaibergenov, W. Jaeger, A. Laschewsky, Adv. Polym. Sci. 201 (2006) 157–224.
[12] J. Seuring, S. Agarwal, Macromol. Rapid Commun. 33 (2012) 1898–1920.
[13] J. Seuring, S. Agarwal, ACS Macro Lett. 2 (2013) 597–600.
[14] J. Shan, M. Nuopponen, H. Jiang, E. Kauppinen, H. Tenhu, Macromolecules 36 (2003) 4526–4533.
[15] M. Beija, J.D. Marty, M. Destarac, Chem. Commun. 47 (2011) 2826–2828.
[16] C. Boyer, M.R. Whittaker, M. Luzon, T.P. Davis, Macromolecules 42 (2009) 6917–6926.
[17] C. Durand-Gasselin, M. Capelot, N. Sanson, N. Lequeux, Langmuir 26 (2010) 12321–12329.
[18] Z. Dong, J. Mao, M. Yang, D. Wang, S. Bo, X. Ji, Langmuir 27 (2011) 15282–15291.
[19] F. Polzer, J. Heigl, C. Schneider, M. Ballauff, O.V. Borisov, Macromolecules 44 (2011) 1654–1660.
[20] A. Housni, Y. Zhao, Langmuir 26 (2010) 12933–12939.
[21] Y. Mitsukami, M.S. Donovan, A.B. Lowe, C.L. McCormick, Macromolecules 34 (2001) 2248–2256.
[22] A.R. Shultz, P.J. Flory, J. Am. Chem. Soc. 74 (1952) 4760–4767.
[23] P. Mary, D.D. Bendejacq, M.-P. Labeau, P. Dupuis, J. Phys. Chem. B 111 (2007) 7767–7777.
[24] D.N. Schulz, D.G. Peiffer, P.K. Agarwal, J. Larabee, J.J. Kaladas, L. Soni, B. Handwerker, R.T. Garner, Polymer 27 (1986) 1734–1742.
[25] M.B. Huglin, M.A. Radwan, Polym. Int. 26 (1991) 97–104.
[26] M.B. Huglin, M.A. Radwan, Makromol. Chem. – Macromol. Chem. Phys. 192 (1991) 2433–2445.
[27] S. Asakura, F. Oosawa, J. Chem. Phys. 22 (1954) 1255–1256.
[28] S. Asakura, F. Oosawa, J. Polym. Sci. 33 (1958) 183–192.
[29] B. Vincent, J. Edwards, S. Emmett, A. Jones, Colloids Surf. 18 (1986) 261–281.
[30] A. Jones, B. Vincent, Colloids Surf. 42 (1989) 113–138.
[31] A. Milling, B. Vincent, S. Emmett, A. Jones, Colloids Surf. 57 (1991) 185–195.
[32] M. Rubinstein, R.H. Colby, Polymer Physics, Oxford University Press, New York, 2003.
[33] R. Kumar, G.H. Fredrickson, J. Chem. Phys. 131 (2009) 104901–104916.