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RESEARCH PAPER

Stability of silver nanoparticles: agglomeration and oxidation in biological relevant conditions

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Abstract Silver nanoparticles (Ag-NP) are the most used nanomaterial in consumer products due to the intrinsic antimicrobial capacity of silver. However, Ag-NP may be also harmful to algae, aquatic species, mammalian cells, and higher plants because both Ag^+ and nanoparticles are responsible of cell damages. The oxidative dissolution of Ag-NP would proceed to completion under oxic conditions, but the rate and extent of the dissolution depend on several factors. This work correlates the effect of the capping agent (albumin and citrate) with the stability of Ag-NP towards agglomeration in simulated body fluid (SBF) and oxidation in the presence of ROS species (H_2O_2). Capping provides colloidal stability only through electrostatic means, whereas albumin acts as bulky ligands giving steric and electrostatic repulsion, inhibiting the agglomeration in SBF. However, citrate capping protects Ag-NP from dissolution to a major extent than albumin does because of its reducing power. Moreover, citrate in solution minimizes the oxidation of albumin-coated Ag-NP even after long incubation times. H_2O_2-induced dissolution proceeds to completion with Ag-NP incubated in SBF, while incubation in citrate leads to an incomplete oxidation. In short, albumin is an excellent capping agent to minimize Ag-NP agglomeration whereas citrate provides a mild-reductive medium that prevents dissolution in biological relevant media as well as in the presence of ROS species. These results provide insight into how the surface properties and media composition affect the release of Ag^+ from Ag-NP, related to the cell toxicity and relevant to the storage and lifetime of silver-containing nanomaterials.

Keywords Spontaneous dissolution · ROS-induced dissolution · Ag^+ release · Agglomeration · Nanotoxicity · Nanomaterials · Health effects

Introduction

Silver nanoparticles (Ag-NP) are the most used nanomaterial in consumer products primarily due to the intrinsic antimicrobial capacity of silver (The Project on Emerging Nanotechnologies 2016; MacCuspie 2011). The antimicrobial mechanism is multifaceted because both Ag^+ and nanoparticles can bind to sulfur-containing proteins on the bacterial cell membrane, inducing changes in membrane permeability and integrity leading to cell death (Ravishankar Rai and Jamuna Bai 2011). Ag-NP also interfere with ATP production, enzyme synthesis, and cell replication by

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binding to bacterial DNA (You et al. 2012). Due to this complex mode of action, Ag-NP are effective agents against broad-spectrum bacteria, viruses, and fungi. Further, it is difficult for the microorganisms to build effective resistance against them. These features have attracted great interest in the last decade to produce Ag-NP-based medical devices and biomaterials in order to prevent bacteria-mediated diseases (Ban and Paul 2016; Chowdhury et al. 2016).

Besides toxicity against microorganisms, Ag-NP may be harmful to algae, aquatic species, mammalian cells, and higher plants (Sharma 2013; Jiravova et al. 2016; Yin et al. 2011). In these cases, it has been also found that both Ag^+ and nanoparticles can be toxic, although its mechanism remains unclear. Several studies suggest that the toxicity is largely explained by released Ag^+ (Kittler et al. 2010; Xiu et al. 2011). However, other works indicate that the generation of reactive oxygen species (ROS) induced by the interaction between Ag-NP and cells is the detrimental cause (Yang et al. 2012; Wei et al. 2015). For instance, the presence of H_2O_2 is thought to be one of the factors contributing to the Ag^+ release in vivo (Wei et al. 2015), as indicated by:

$$2(Ag)_n + H_2O_2 + 2H^+ \rightarrow 2n\ Ag^+ + 2\ H_2O\ \text{(Wei et al. 2015)}.$$

This reaction represents a ROS-induced dissolution process by which Ag-NP are oxidized in the presence of H_2O_2 and Ag^+ released to the medium.

Thermodynamically, the oxidative dissolution of Ag-NP would proceed to completion under oxic conditions. As a result, Ag^+ may be released to the media or may be adsorbed on the Ag-NP and delivered locally to the cells in contact with them. Lok et al. (2007) have proven that the antibacterial capacity of Ag-NP depends on the surface oxidation, since the NPs act as carriers of chemisorbed Ag^+ that cause the antimicrobial activity. The rate and extent of the dissolution process have been correlated to the medium composition, the Ag-NP size, and the presence of capping agents (Xiu et al. 2011; Ma et al. 2012; Levard et al. 2013; Li et al. 2012). These factors may have an effect on the kinetics of the process in such a way to prevent the complete dissolution of Ag-NP. In general terms, particle size may affect not only the oxidative release of ions but also ROS generation that is promoted by nanosized compared to microsized particles (Yang et al. 2012). Medium composition may change the solubility of Ag-NP influencing the bioavailability of Ag^+ to the organisms (Yang et al. 2012; Levard et al. 2013). Finally, capping agents may affect the interaction with serum proteins leading to changes in the antibacterial activity, as well as in cellular viability (Gnanadhas et al. 2013), or may have an impact in the solubility of particles by passivating the surface (Choi et al. 2011).

Citrate is a common capping agent employed to prevent Ag-NP aggregation (MacCuspie 2011). However, the agglomeration observed in media of high ionic strength (as culture medium) prevented to study the antimicrobial properties of these dispersions (Ag-NP/citrate) in our previous work (Valenti and Giacomelli 2015). Instead, Ag-NP stabilized by adding albumin during the synthesis procedure (Ag-NP/albumin) showed an enhanced colloidal stability in media of high ionic content as well as in a wide pH range (Valenti and Giacomelli 2015). On the other hand, the presence of citrate as capping agent and/or in solution was found to significantly retard Ag-NP corrosion (Choi et al. 2011; Stoermer et al. 2005). The current work correlates the effect of these capping agents with the stability of Ag-NP towards agglomeration in high ionic strength and complex media, such as simulated body fluid (SBF), and oxidation in the presence of ROS species, as H_2O_2. Ag-NP stability towards agglomeration and oxidation was followed as a function of time from the first minutes after the synthesis up to 13 days of incubation in different media. Such knowledge provides insight into how the surface properties and media composition affect the release of Ag^+ from Ag-NP, directly related to the cell toxicity and relevant to the storage and lifetime of silver-containing nanomaterials. The experimental approach combines surface plasmon resonance (SPR) and dynamic light scattering (DLS) measurements, to evaluate individual nanoscale interactions and size distribution related to the agglomeration and dissolution processes, as well as potentiometric determinations to quantify Ag^+ released from Ag-NP.

Experimental

Material

$AgNO_3$ were obtained from Carlo Erba, $NaBH_4$ from Tetrahedron, albumin (bovine) from Sigma, and trisodium citrate, $NaNO_3$, KNO_3, Na_2SO_4, $NaHCO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, and K_2HPO_4 from J. T. Baker. All

reagents were of analytical grade and used without further purification.

Aqueous solutions were prepared in deionized water (Milli-Q System, Millipore). The pH adjustment was performed by adding NaOH or HNO_3 solutions and the pH measurements were carried out with a glass electrode and a digital pH meter (Mettler Toledo, Seven Compact). All experiments were performed at room temperature (25 ± 2 °C).

The media employed in this work were 7×10^{-4} mol L^{-1} trisodium citrate and 0.15 mol L^{-1} $NaNO_3$ and SBF (Na^+ 142 mmol L^{-1}, K^+ 5 mmol L^{-1}, Mg^{2+} 1.5 mmol L^{-1}, Ca^{2+} 2.5 mmol L^{-1}, Cl^- 147.8 mmol L^{-1}, HCO_3^- 4.2 mmol L^{-1}, HPO_4^{2-} 1 mmol L^{-1}, and SO_4^{2-} 0.5 mmol L^{-1}). It is worth mentioning that, in all the experiments, chloride ions in the formulation were replaced by nitrate ions in order to discard the chemical effect of chloride ions in Ag-NP dissolution (Xiu et al. 2011; Levard et al. 2013; Li et al. 2012). In order to prepare the SBF solution, the following reactants were dissolved in the specified order and concentrations: (1) $NaNO_3$ 136.8 mmol L^{-1}, (2) $NaHCO_3$ 4.2 mmol L^{-1}, (3) KNO_3 3 mmol L^{-1}, 4) $K_2HPO_4 \cdot 3H_2O$ 1 mmol L^{-1}, (5) $Mg(NO_3)_2$ 1.5 mmol L^{-1}, (6) HNO_3 in order to lower the pH to 1.8–2.0, (7) $Ca(NO_3)_2$ 2.5 mmol L^{-1}, (8) Na_2SO_4 0.5 mmol L^{-1}, (9) Tris 50 mmol L^{-1}, and (10) HNO_3 in order to adjust pH at 7.25 at 36.5 °C. During the preparation of the solution, special care was taken in adding the salts in the appropriate order as well as adding the Tris salt slowly in order to avoid local increase in pH of the solution. Then, the solution was stored in a plastic bottle at 4 °C.

Methods

Synthesis of Ag-NP

Ag-NP were synthesized following the method described elsewhere (Valenti and Giacomelli 2015). Briefly, Ag-NP/citrate were synthesized by adding 1 mL of 0.1 mol L^{-1} $AgNO_3$ to 100 mL of 7×10^{-4} mol L^{-1} trisodium citrate solution followed by 1 mL of 0.13 mol L^{-1} $NaBH_4$. The resulting suspension was stirred until no change in the color was observed. In order to synthesize Ag-NP/albumin, 10 mL of 1% *w/v* of albumin solution prepared in 0.05 mol L^{-1} phosphate buffer pH 8.0 was added to the reaction medium before the reduction step. Both reactions lead to the full consumption of Ag^+ ions and allow the preparation of stock dispersions employed for the following experiments. The concentration of the stock dispersions was 9.8×10^{-4} mol L^{-1} for Ag-NP/citrate and 8.9×10^{-4} mol L^{-1} for Ag-NP/BSA.

Agglomeration of Ag-NP

Three different media were used to study the agglomeration process of Ag-NP: citrate (synthesis solution), $NaNO_3$, and SBF. As-prepared samples were diluted with the corresponding medium up to 1:5 *v/v* ratio to perform SPR and DLS experiments. SPR spectra were collected in the 350–800-nm range using a UV-1700 PharmaSpec Shimadzu spectrophotometer at a spectral resolution of 0.5 nm. The spectra of each Ag-NP dispersions were collected after a baseline correction performed with the corresponding media. DLS measurements were performed using a Beckman Coulter DelsaNano C equipment. It may be mentioned here that albumin itself has a hydrodynamic diameter around 7 nm (Carter and Ho 1994). However, the scattering from albumin in the absence of Ag-NP was found to be insignificant compared to that from NP. Experiments were conducted in triplicate.

Dissolution of Ag-NP

The Ag-NP dissolution process was also evaluated in the three media at different incubation times up to 13 days, measuring the concentration of released Ag^+ in the absence (spontaneous dissolution) and in the presence of an excess of H_2O_2 (ROS-induced dissolution). The concentration of released Ag^+ was determined by potentiometric measurements using an Ag^+-selective electrode (9416BN, Thermo Scientific Orion), a reference electrode (900200, Thermo Scientific Orion), and a digital potentiometer (Mettler Toledo, SevenCompact). These experiments were performed with different volumes of Ag-NP dispersed in 5 mL of each medium (ranging from 1:100 to 1:10 *v/v* ratios). The potential was determined immediately after reaching a stable value with a drift lower than 10 mV/min. The calibration curve was carried out in a wide range of Ag^+ concentrations (1×10^{-6}–1×10^{-3} mol L^{-1}) performed in SBF or 0.15 mol L^{-1} $NaNO_3$ (to keep the ionic strength constant) in presence and absence of an excess of H_2O_2. Experiments were conducted in triplicate.

The degree of dissolution was determined as the percentage between the concentration of the released Ag^+ ([Ag^+]) in a particular condition and the nominal Ag^+ concentration calculated from the complete dissolution of Ag-NP ([Ag^+]$_{100}$) corrected by the ratio between the surface areas (r_{SA}) of both samples (Ag-NP/citrate and Ag-NP/albumin):

$$\text{Degree of dissolution} = \frac{[\text{Ag}+]}{[\text{Ag}+]_{100} \times r_{SA}} \times 100 \quad (1)$$

Finally, SPR and/or DLS measurements were carried out in parallel with these samples. The dissolution measured in situ (1 spectrum/min) as a function of time (t) is expressed as the percentage of the initial ($t = 0$) intensity value before adding H_2O_2 of the SPR band (I_{SPR}):

$$\%I_{SPR} = \frac{I_{SPR}\ (t)}{I_{SPR}\ (t=0)} \times 100 \quad (2)$$

Results and discussion

Agglomeration of Ag-NP

Ag-NP samples were synthesized using two different methods based on the reduction of Ag^+ ions by $NaBH_4$: (1) the well-known citrate procedure (Ag-NP/citrate) (Lok et al. 2007) and (2) the addition of albumin before the reduction step (Ag-NP/albumin) (Valenti and Giacomelli 2015). The agglomeration behavior of Ag-NP/citrate strongly depends on the dispersion media (citrate, SBF, and $NaNO_3$) and incubation time (Supplementary Information, Fig. 1SI). Ag-NP/citrate dispersed in citrate within 15 min after the synthesis produces a single and intense SPR band at around 390 nm (2 nm hydrodynamic diameter from DLS data). On the other hand, dispersion in SBF or $NaNO_3$ causes a new signal centered at 550 nm together with a strong peak broadening and reduction of the band intensities. These spectral features indicate the depletion of stable Ag-NP and the formation of agglomerates when Ag-NP/citrate are dispersed in SBF or $NaNO_3$ (Chambers et al. 2014). However, DLS data indicate that large particles contributing to these samples are rather minor compared to smaller ones. Although Ag-NP/citrate dispersed in citrate is stable after storing for 24 h, the SPR bands of Ag-NP/citrate incubated in either SBF or $NaNO_3$ vanish and the spectral features related to agglomeration prevail. Moreover, the populations of large particle become more evident on DLS measurements. A completely different behavior is observed with Ag-NP/albumin (Supplementary Information, Fig. 2SI): a single and intense SPR band at around 418 nm appears in the three media and both incubation times. The reasons behind the 30-nm red-shifted spectra of Ag-NP/albumin compared to Ag-NP/citrate can be related to several factors, such as the Ag-NP size, capping agent (MacCuspie 2011; Valenti and Giacomelli 2015), and redox surface sites (Li et al. 2012). Moreover, the lack of broadening and shifting of SPR band in media of high ionic content indicates the absence of agglomeration of single Ag-NP/albumin and changes in size, shape, or coating of the nanoparticles dispersed in these media. As a consequence, high ionic content induces the agglomeration of Ag-NP/citrate, mainly under long incubation time, whereas Ag-NP/albumin keeps the same size, shape, and coating in the three media and incubation times (Valenti and Giacomelli 2015). Citrate capping provides colloidal stability to Ag-NP only through electrostatic means, so the electrolyte concentration at biologically relevant conditions leads to rapid agglomeration of the Ag-NP. On the other hand, albumin molecules act as bulky ligands which give steric and electrostatic repulsion resulting in a significant colloidal stability in media of high ionic content.

Dissolution of Ag-NP

Figure 1a shows the SPR spectra of Ag-NP/albumin dispersed in SBF as a function of the incubation time (the arrow indicates the direction in which time proceeds). Although the normalized spectra merge in a master curve (Supplementary information, Fig. 3SI), the SPR band intensity (I_{SPR}) diminishes as a function of time. This spectral change is easily observed in Fig. 1c where % I_{SPR} (Eq. 2) is displayed as function of time. Agglomeration can be discarded as the cause of such a diminution, due to the absence of any spectral changes as well as the unaffected hydrodynamic diameter in media of high ionic content (Supplementary information, Fig. 2SI). Therefore, the diminution in I_{SPR} already observed in the first minutes of the incubation in SBF indicates that the concentration of single Ag-NP/albumin decreases. This experimental result can only be due to a process of spontaneous Ag-NP/albumin dissolution in SBF. Consequently, the protein capping protects the Ag-NP from agglomeration but it does not

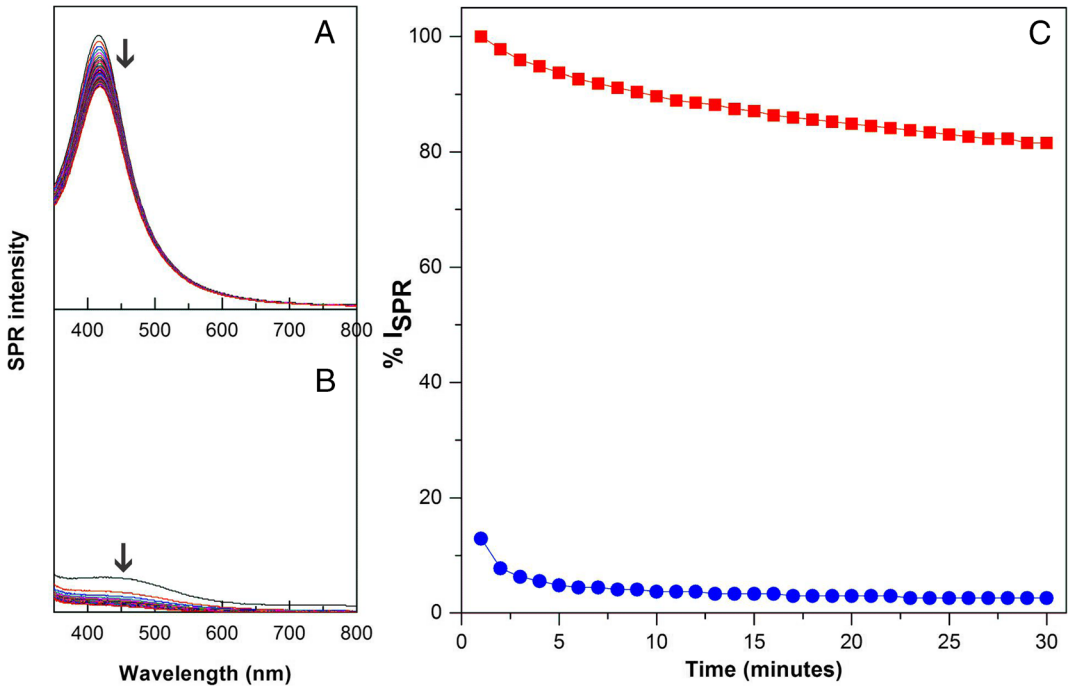


Fig. 1 SPR spectra of Ag-NP/albumin dispersed in SBF at 1/20 dilution ratio as a function of time, in the absence (**a**) and in the presence (**b**) of H_2O_2 ($[H_2O_2] = 9 \times 10^{-3}$ mol L^{-1}). The *arrows* indicate the direction in which time proceeds. Intensity of the SPR bands as a function of time in the absence (*red*) and in the presence (*blue*) of H_2O_2, calculated from Eq. 2 (**c**)

prevent some dissolution in high ionic, complex medium. Figure 1 also collects the spectra (b) of Ag-NP/albumin dispersed in SBF in the presence of H_2O_2 as a function of the incubation time and displays the corresponding % I_{SPR} (c). Clearly, the strong disappearance of the SPR band in the first minute in the presence of H_2O_2 indicates the fast dissolution of the Ag-NP/albumin under this oxidative condition. These results leave several open questions regarding the Ag-NP stability towards dissolution upon changing the capping agent, the media composition, and the incubation time. These issues are further discussed following both the spontaneous and ROS-induced oxidation processes.

Figure 2a shows the concentration of released Ag^+ ($[Ag^+]$) from Ag-NP/albumin and Ag-NP/citrate dispersed in SBF at different dilution ratios measured within 15 min after the synthesis, in the absence (spontaneous oxidation) and presence (ROS-induced oxidation) of H_2O_2. The lines indicate the nominal $[Ag^+]$ calculated from the complete dissolution of Ag-NP ($[Ag^+]_{100}$). First of all, two processes are clearly observed with both samples at the different dilution ratios: a complete dissolution of Ag-NP caused by the presence of H_2O_2 and a partial dissolution due to the spontaneous oxidation in SBF that occurs to a similar extent for both samples. This is a surprising result considering the agglomeration state of each sample (single nanoparticles in Ag-NP/albumin while small agglomerates in Ag-NP/citrate) which is also evident from the SPR spectra displayed in Fig. 2b, c. Hence, the dissolution processes take place even in the presence of agglomerates. Secondly, both processes linearly depend on the dilution ratios indicating that the Ag-NP concentration does not affect the dissolution extent. However, the measured $[Ag^+]$ during the spontaneous process does not correspond to an equilibrium state because it depends on the amount of the solid. Finally, to calculate the degree of dissolution (Eq. 1), $[Ag^+]$ was corrected to account for the effect of the surface area on the dissolution process (Dobias and Bernier-Latmani 2013) by the ratio between them. This ratio was calculated from the hydrodynamic diameters (4 nm for Ag-NP/albumin and 2 nm for Ag-NP/citrate, assuming that during the agglomeration most of the surface area is available for heterogeneous reactions (Liu and Hurt 2010)). The degree of dissolution decreases by changing the capping agent from albumin

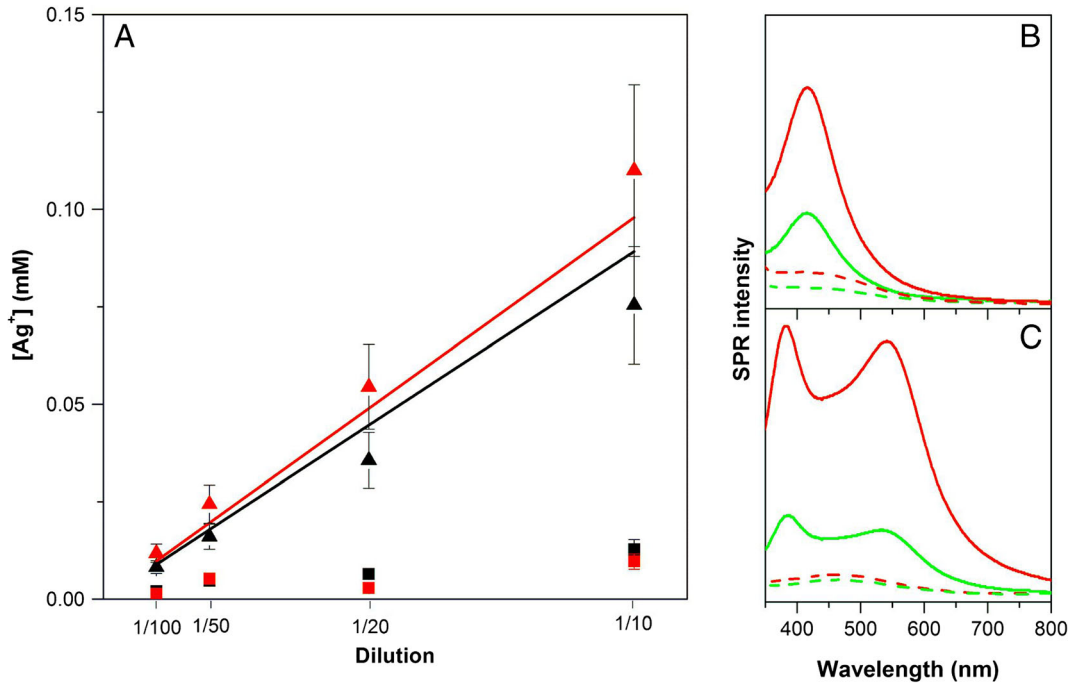


Fig. 2 Concentration of released Ag^+ ($[Ag^+]$) from Ag-NP/albumin (*black*) and Ag-NP/citrate (*red*) dispersed in SBF at different dilution ratios measured within 15 min after the synthesis, in the absence (*squares*) and in the presence (*triangles*) of H_2O_2 ($[H_2O_2]$ between 2×10^{-3} and 2×10^{-2} mol L^{-1}) (**a**). The *lines* indicate the nominal $[Ag^+]$ calculated from the complete dissolution of the Ag-NP. The *symbols* represent the media of three repeats and the *error bars* its standard deviation. SPR spectra of Ag-NP/albumin (**b**) and Ag-NP/citrate (**c**) dispersed in SBF at different dilution ratios (1/20 *red* and 1/50 *green*), in the absence (*solid*) and in the presence (*dashed*) of H_2O_2

(17 ± 7%) to citrate (5 ± 1%), suggesting that it may be considered as an intrinsic property of each Ag-NP sample. Consequently, the extent of the spontaneous process is mainly controlled by the capping agent; it is particularly related to the adsorbed citrate molecules on Ag-NP that act as a reducing agent of the oxide layer on the Ag-NP surface, decreasing the solubility (Li et al. 2012). Additionally, citrate may bind Ag^+ through the carboxylic groups, lowering the amount of released ions (Liu and Hurt 2010). Thereby, surface citrate exerts a protective effect against oxidation when they are capping agents, whereas albumin mostly acts as a bulky ligand which imparts steric and electrostatic repulsion capable of hinder agglomeration in media of high ionic strength.

Figure 3 compares the spontaneous dissolution and the agglomeration processes of Ag-NP/albumin and Ag-NP/citrate in SBF to those measured in $NaNO_3$ after 15 min of incubation. Figure 3a shows the degree of dissolution in the absence of H_2O_2 (spontaneous oxidation) and the SPR spectra are compiled in Fig. 3b (Ag-NP/albumin) and Fig. 3c (Ag-NP/citrate). Recalling that the ionic strength of SBF was simulated in the $NaNO_3$ solution, the results shown in Fig. 3 allow understanding the causes behind the agglomeration and the spontaneous dissolution. Clearly, both media produce the same degree of dissolution from each sample. Further, either SBF or $NaNO_3$ does not affect the SPR band of Ag-NP/albumin while they change the spectral features of Ag-NP/citrate. All together, these results confirm that both processes are induced by the high ionic strength of SBF. Finally, Fig. 3 compares the agglomeration and the spontaneous dissolution processes of Ag-NP/albumin and Ag-NP/citrate in SBF at short (15 min) and long (24 h) incubation times. The degree of dissolution increases between two and three times at 24 h of incubation. As indicated previously, the measured $[Ag^+]$ within 15 min does not represent an equilibrium condition, more than a day is necessary to achieve such a state. In addition, the value measured from Ag-NP/albumin is higher than that observed for Ag-NP/citrate, highlighting the protective effect of adsorbed citrate against spontaneous

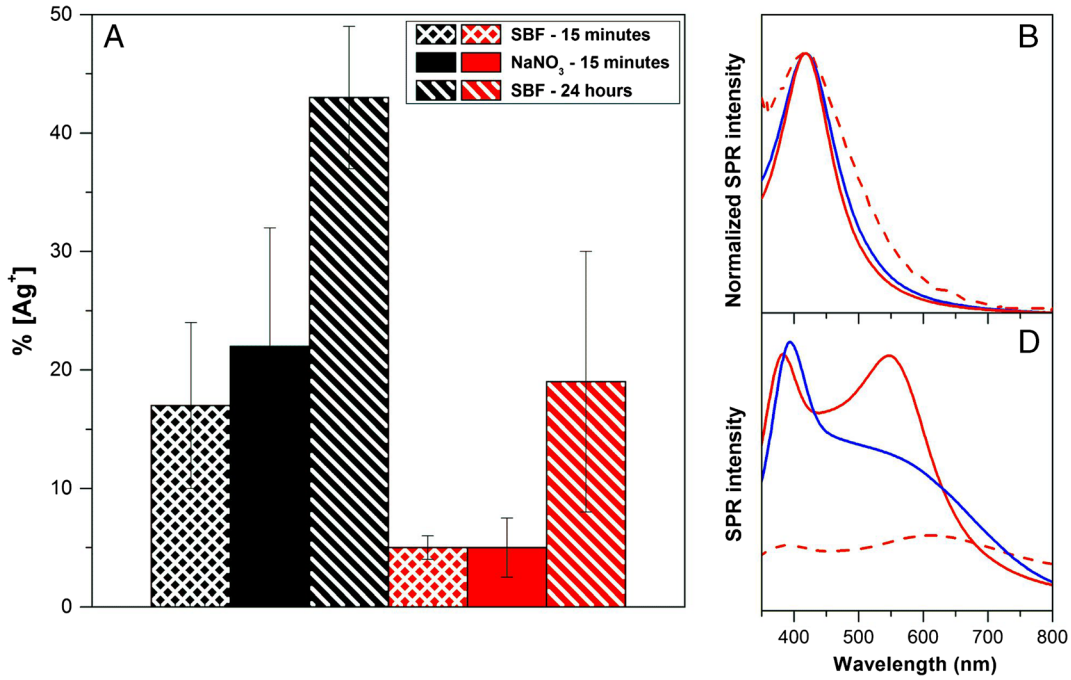


Fig. 3 Spontaneous dissolution of Ag-NP/albumin (*black*) and Ag-NP/citrate (*red*) incubated in SBF (*patterns*) or $NaNO_3$ (*solid*) for different times, calculated from Eq. 1 (**a**). The *error bars* indicate the standard deviation of three repeats. SPR spectra of Ag-NP/albumin (**b**) and Ag-NP/citrate (**c**) dispersed in SBF (*red*) or $NaNO_3$ (*blue*) measured at short (*solid*) and long (*dashed*) incubation times. Dilution ratio = 1/20

oxidation. The spectral features of Ag-NP/albumin and Ag-NP/citrate after 24 h of incubation in SBF reinforce the mentioned results.

Figure 4 compares the spontaneous dissolution processes of Ag-NP/albumin incubated in SBF or citrate at different incubation times. Figure 4a shows the degree

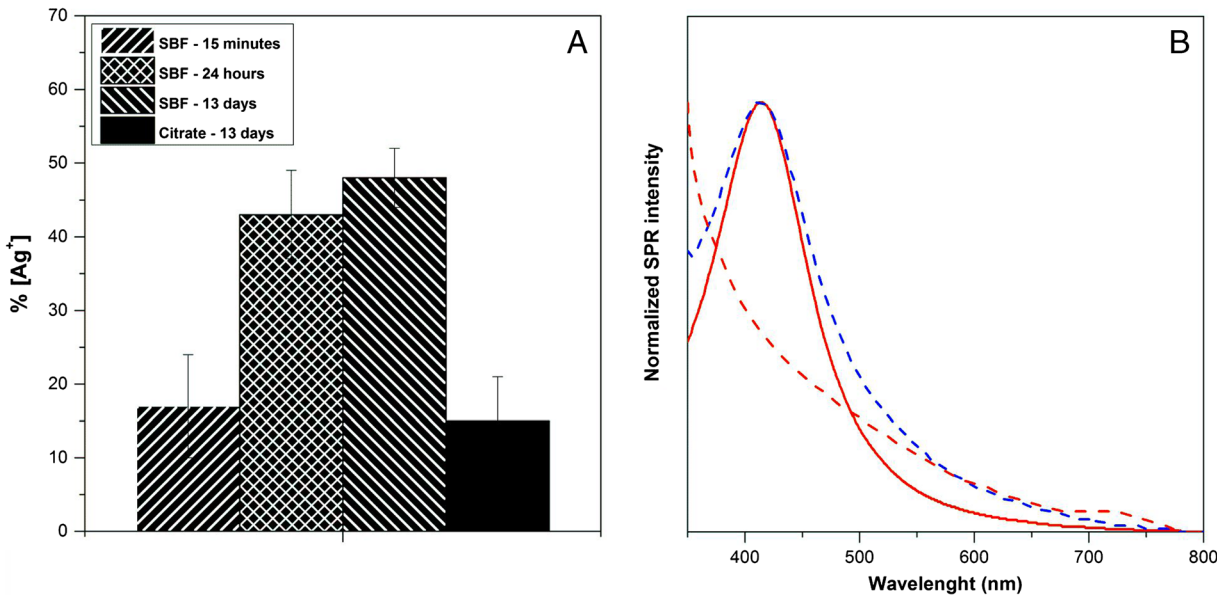


Fig. 4 Spontaneous dissolution of Ag-NP/albumin incubated in SBF (*patterns*) at different times or citrate (*solid*) at long incubation time, calculated from Eq. 1 (**a**). The *error bars* indicate the standard deviation of three repeats. SPR spectra of Ag-NP/albumin (**b**) dispersed in SBF (*red*) or citrate (*blue*) measured at short (*solid*) and long (*dashed*) incubation times. Dilution ratio = 1/20

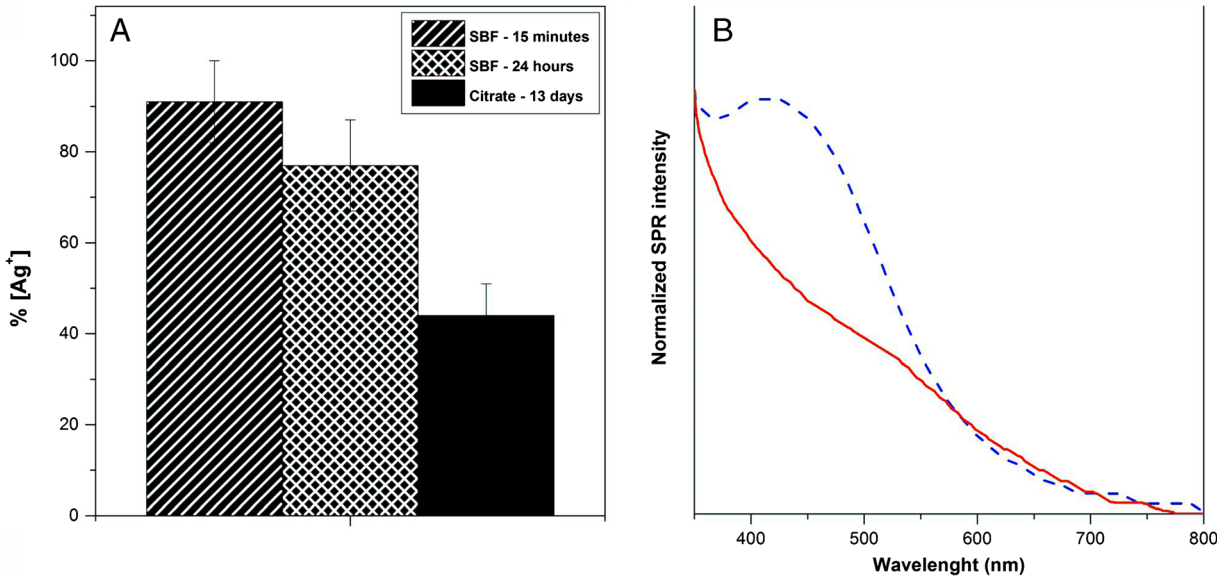


Fig. 5 ROS-induced dissolution of Ag-NP/albumin incubated in SBF (*patterns*) at different times or citrate (*solid*) at long incubation time, calculated from Eq. 1 (**a**). The *error bars* indicate the standard deviation of three repeats. SPR spectra of Ag-NP/albumin (**b**) dispersed in SBF (*red*) or citrate (*blue*) measured at short (*solid*) and long (*dashed*) incubation times. Dilution ratio = 1/20

of dissolution, while the SPR spectra are compiled in Fig. 4b. Although the oxidation produced by SBF is enhanced with time, a high percentage of the quantity detected after 13 days is reached within the first 24 h of incubation. In accordance with these results, the incubation in SBF for 13 days dissolves Ag-NP/albumin until the disappearance of the SPR band. Therefore, the spontaneous oxidation of Ag-NP/albumin in SBF proceeds to completion in the course of days. In contrast to this behavior, the incubation of Ag-NP/albumin in citrate during 13 days hampers the release of Ag^+, reaching a value similar to that observed in SBF during the first 15 min. In fact, the spectrum of Ag-NP/albumin incubated in citrate for 13 days only evidences a slight spectral change, just as the incubation in SBF for short periods of time does. Hence, citrate in solution behaves as a mild-reductive medium that minimizes the spontaneous oxidation of Ag-NP/albumin even after long incubation times.

Figure 5 compares the ROS-induced oxidation processes of Ag-NP/albumin incubated in SBF or citrate at different incubation times. Figure 5a shows the degree of dissolution, while the SPR spectra are compiled in Fig. 5b. The dissolution is complete in the samples incubated in SBF, whereas 13 days of incubation in citrate leads to an incomplete oxidation by H_2O_2. This effect is also evident from the spectra: the SPR band of Ag-NP/albumin incubated in SBF disappears in the presence of ROS, whereas the incubation in citrate for 13 days leads to a detectable SPR band. This piece of evidence highlights the protective effect of citrate against oxidation even under oxidative conditions. Therefore, the presence of citrate in the storage solution coming from the Ag-NP synthesis, or added after it, enhances the protection against corrosion. It has been postulated (Stoermer et al. 2005) that citrate may compete effectively with various oxygen species for the surface binding sites or may itself react with oxygen species adsorbed on the Ag-NP. In short, any produced Ag^+ will be re-reduced by the excess of citrate.

Conclusion

The capping agent plays a crucial role in determining the Ag-NP antibacterial properties because it controls both the size in simulated biological fluid and the dissolution in oxidative conditions. Our works provide insight into the effect of two capping agents on the colloidal stability, antimicrobial properties, and partial oxidation of Ag-NP. Albumin is an excellent capping agent to minimize Ag-NP agglomeration, whereas citrate provides a mild-reductive medium that prevents dissolution. All together, these results indicate that albumin-coated Ag-NP stored in citrate is a promising system to be used in medical devices and biomaterials in order to prevent bacteria-mediated diseases and to improve the storage

conditions and lifetime of silver-containing nanomaterials. Finally, our experimental evidence is helpful to rationally design the synthesis route of Ag-NP based on the specific application determined by the medium in which silver ions need to be released.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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