

## Environmental and biological influences on the stability of silver nanoparticles

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Silver nanoparticles (Ag NPs) are easily dispersed and stable, and their application has become widespread. Consequently, concern has arisen regarding their potential effects on the environment and human health. However, the current understanding on the stability of Ag NPs is limited. Therefore, in this study the influence of environmental and biological factors, such as ionic strength, valence, biological molecules, pH and temperature, on the properties of Ag NPs was investigated. The absorption spectra, size, zeta potential, and release of silver ions were evaluated. Ionic strength, valence, Dulbecco's Modified Eagle's Medium (DMEM), without fetal bovine serum (FBS), reduced the stability of the Ag NPs. However, in DMEM with 10% FBS, the Ag NPs maintained their stability and nanoscale characteristics. These findings provide an essential insight into the environmental and biological factors that should be evaluated in a risk assessment of Ag NPs.

**silver nanoparticles, environmental influence, biological influence, stability**

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Nanomaterials have at least one dimension that is 1–100 nm. On this scale, materials have unique physical and chemical properties such as high specific surface area, tunable fluorescence and electron tunneling effect [1–3]. Because of these properties, nanomaterials have potential applications in optical, electronic and medical products [4,5]. Among the different nanomaterials, silver nanoparticles (Ag NPs) are one of the fastest growings. They are used as antibacterial agents, catalysts, medicines, biological sensors and more [6–11]. Ag NPs could potentially be released into the environment from these products, and consequently pose a potential toxicity risk to human health [12,13]. However, little is currently known about the likely adverse effects of Ag NPs on health.

The biological activity of Ag NPs depends on many features, such as their size [14], surface coatings [15] and release of silver ions [16]. Their size and the release of silver

ions are regulated by the Ag NPs stability [17]. A reduction in their stability can result in aggregation of the Ag NPs, which consequently leads to the total or partial loss of their nanoscale properties. The aggregation of Ag NPs also alters their cellular uptake and the response of cells to the NPs [18], and modifies their bioavailability and toxicity [19]. The status of Ag NP aggregation or dispersion determines the magnitude of their toxicity and the mechanism of their environmental and biological effects. Although NP clusters have been observed in some toxicological studies, few of these studies have considered the aggregation kinetics of Ag NPs [20]. Aggregation may change the NP characteristics and lead to different conclusions. Therefore, it is crucial to evaluate the status of Ag NPs in environmental and biological medium prior to determining their environmental risk and toxicity.

In this study, the influence of environmental and biological factors on the properties of Ag NPs, such as absorption peak, size, zeta potential and ion release, was

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investigated. The results of this study are essential for toxicity assessment of Ag NPs.

## 1 Materials and methods

### 1.1 Chemicals and reagents

Ag NPs were purchased from Huzheng Nano Technology Limited Company (Shanghai, China). The particles were purified by centrifugation three times at 12000 r/min for 20 min. The supernatant was discarded and the solid was resuspended in deionized (DI) water and characterized by UV-Vis spectrometry (Beckman Coulter DU 800, USA), nano particle size analysis (N5, Backman, USA), inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500, USA) and transmission electron microscopy (TEM, Hitachi H-7500, Japan). The stock suspension was stored at 4°C in the dark.

Salt reagents (NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, FeCl<sub>3</sub>) were AR grade and purchased from the Beijing Chemical Reagent Company (Beijing, China). High Glucose Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone and Invitrogen (USA), respectively.

### 1.2 Preparation of Ag NPs in different systems

Suspensions of 100 µmol/L Ag NPs were obtained by diluting the stock solution of Ag NPs with either NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> or FeCl<sub>3</sub> at concentrations of 100 µmol/L, 1 mmol/L, 10 mmol/L, and 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 mol/L. The absorption at 400 nm was measured 30 min and 8 h later by UV-Vis spectrometry.

The pH values of the 100 µmol/L Ag NP suspensions were adjusted with 1 mol/L HCl and 1 mol/L NaOH to 4.2, 5.4, 6.4, 7.6 and 8.4. After 30 min and 8 h, the absorption at 400 nm was measured by UV-Vis spectrometry.

The 100 µmol/L Ag NPs suspensions were also prepared in FBS-free DMEM and DMEM with 10% FBS (DMEM+FBS). The absorption at 400 nm was measured 30 min and 8 h later by UV-Vis spectrometry.

These Ag NP suspensions were stored at 4, 20 and 37°C for 8 h. After storage the adsorption at 400 nm was measured by UV-Vis spectrometry.

### 1.3 Particle size analysis and zeta potential measurements

Particle size and zeta potential of Ag NPs in suspensions of 0.2 mol/L NaCl, CaCl<sub>2</sub>, DMEM and DMEM+FBS were measured by laser particle size analyzer (Malvern Nano ZS, Malvern, UK). All experiments were carried out below 20°C.

### 1.4 Assessment of released silver ions

The free silver ions in various Ag NP suspensions were

measured using a Silver Ion-Selective Electrode (Ag-ISE, Jenco, Shanghai, China). A calibration curve was constructed using the diluted AgNO<sub>3</sub> solution. The Ag<sup>+</sup> concentration was calculated from the obtained data using the linear calibration.

### 1.5 Statistics

All statistical analyses were performed using Sigma Plot 10.0 (Systat Software Inc., San Jose, CA). All results are presented as the mean±SD of at least three independent experiments. The statistical significance of the experimental data was analyzed by Student's *t*-test, and a *P* value of <0.05 determined statistical significance.

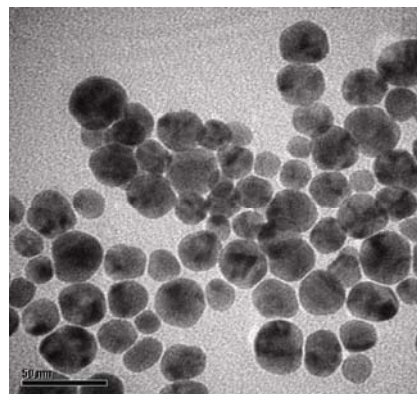
## 2 Results and discussion

### 2.1 Characterization of Ag NPs

TEM imaging showed that the Ag NPs were homogeneously dispersed spheres (Figure 1). The concentration of the stock solution was 4.89 mmol/L and the particle size was 23.8±6.7 nm. An absorption peak typical of Ag NPs was observed at 400 nm.

### 2.2 Influence of ionic strength and valence on stability of the Ag NPs

The absorption peak at 400 nm confirmed that Ag NPs were present in the solution. Consequently, any decrease in the absorption and/or increase in the full width at half maximum (FWHM) could be taken as an indication that the Ag NPs had aggregated and formed bigger particles. Disappearance of the 400 nm peak suggests that the Ag NPs have lost their stability and the particle size has increased to microscale [21]. When the particles were in suspension with 0.5 mol/L NaCl, which has an ionic strength of 0.5 mol/L, the absorption at 400 nm decreased slightly and the FWHM did not change (Figure 2 (a) and (b)). By contrast, in 0.5 mol/L CaCl<sub>2</sub>, which has an ionic strength of 2 mol/L, the



**Figure 1** TEM image of Ag NPs. Magnification: ×80000.

absorption at 400 nm decreased from 1.8 to 0.5, and the FWHM increased significantly (Figure 2(c) and (d)). This finding suggests that increased ionic strength can induce aggregation of the Ag NPs and loss of the stable dispersion. Additionally, it indicates that the higher valence readily induces the loss of the stable dispersion of the Ag NPs. Results from the  $\text{Mg}^{2+}$  suspensions confirmed the effect of cation valence. In addition to the decrease in the absorption at 400 nm, another peak at 500 nm emerged in the  $\text{Mg}^{2+}$  suspensions, which is indicative of the formation of larger particles (Figure 3). Iron had a pronounced effect on the stability of the Ag NPs, and 200  $\mu\text{mol/L}$   $\text{Fe}^{3+}$  solution dissolved all the particles (Figure 4). This might have occurred because of the high cation valence and the oxidative properties of the  $\text{Fe}^{3+}$  ions.

The salt solutions used in this study are common in natural aqueous environmental and biological solutions. These findings indicate that Ag NPs will tend to aggregate in high ionic strength and valence solutions, and lose their nanoscale properties. This observation is consistent with an earlier study on nano- $\text{TiO}_2$  [22].

## 2.4 Influence of biological molecules on the stability of the Ag NPs

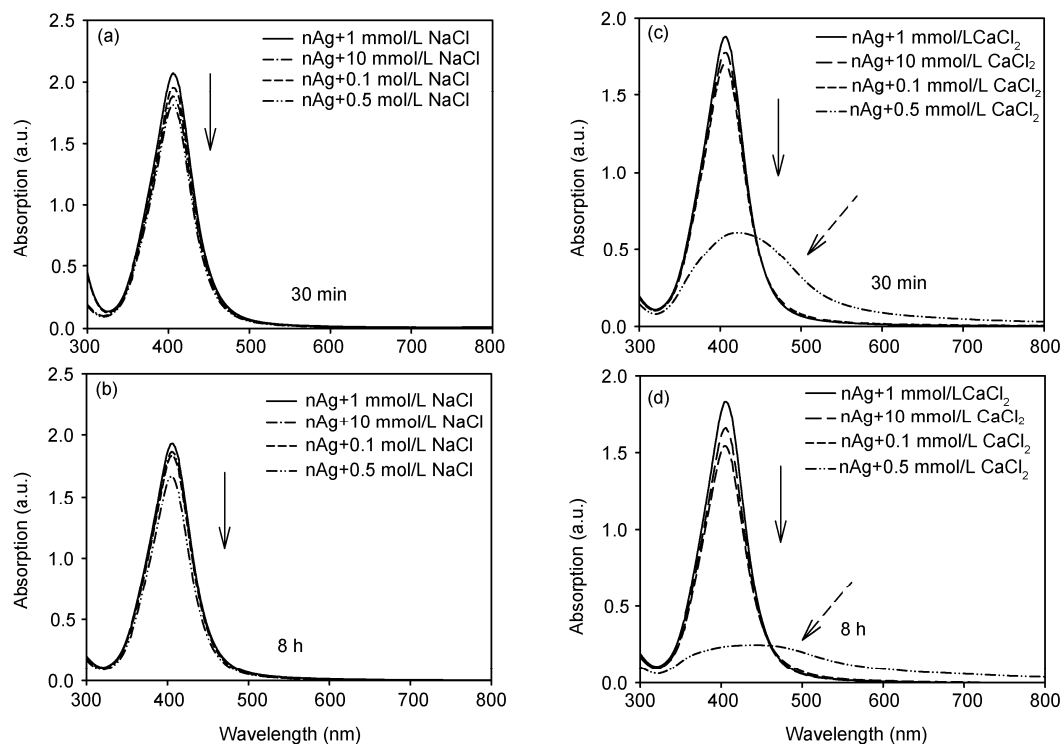
For the *in vitro* experiments, the nanomaterials were suspended in cell culture medium prior to exposure to cells or bacteria. The influence of the cell culture medium on

stability of Ag NPs was evaluated.

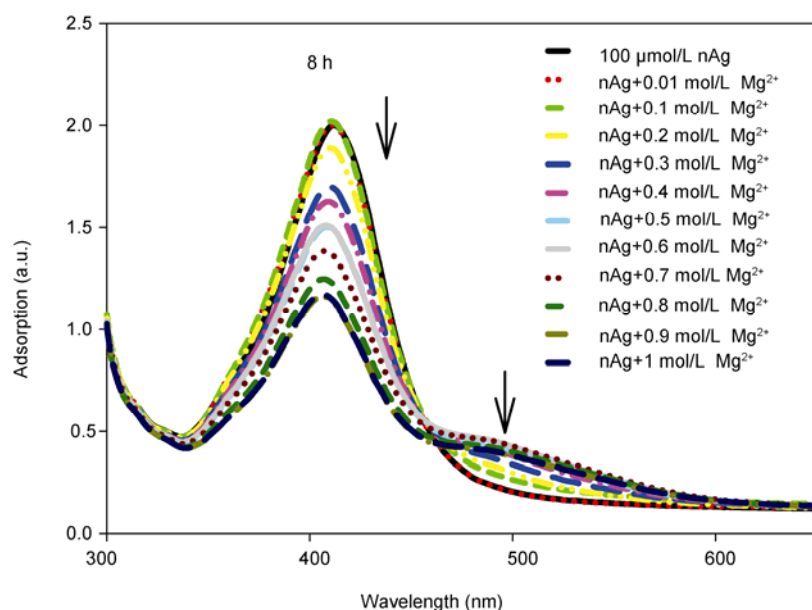
After 8 h of exposure to FBS free-DMEM, which is a common cell culture medium, the specific absorption peak of the Ag NPs disappeared and a peak for the aggregates appeared at 680 nm (Figure 5). This indicates that the components in DMEM (salt and amino acid) led to aggregation of the Ag NPs. However, the addition of 10% FBS kept the Ag NPs in a well-dispersed state, which suggests that FBS could protect the Ag NPs from aggregating. This protective effect was also observed in another common culture medium, RPMI1640 [23]. The protective role of FBS might occur because Ag NPs bind to proteins in the FBS. As a result, the surface of the Ag NPs would be covered in FBS, which could then prevent aggregation [24]. Thus, Ag NPs would remain dispersed in any medium containing FBS. Ag NPs would probably maintain their nanoscale properties under *in vivo* conditions as there are abundant proteins in blood and biological liquids that could act in a similar manner.

## 2.5 Effect of temperature on the stability of the Ag NPs

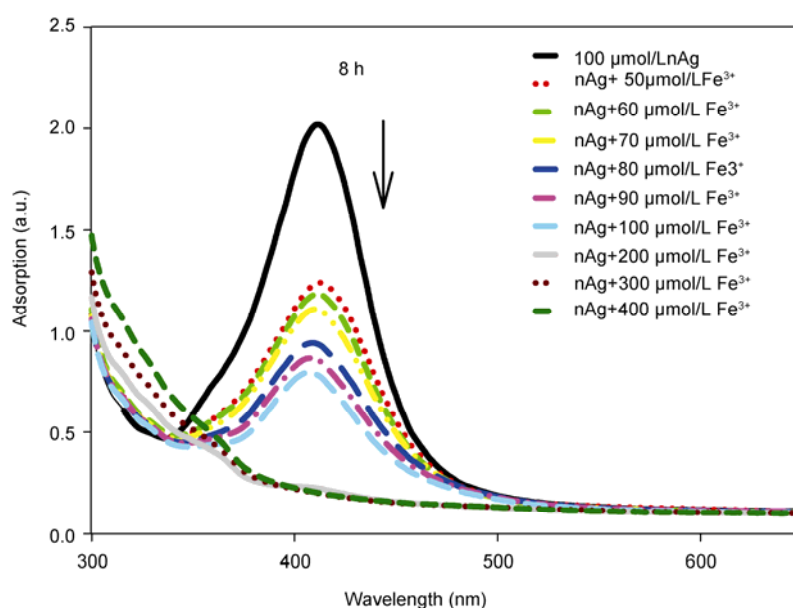
Temperature is one of the important environmental factors that influence the stability, activity and chemical characteristics of materials. The effect of temperature on the stability of the Ag NPs was investigated at 4, 20 and 37°C. The Ag NPs in DI water remained intact at all temperatures tested (Figure 6). In the salt solutions and DMEM, the



**Figure 2** The UV-Vis adsorption spectra of Ag NPs diluted in NaCl and  $\text{CaCl}_2$  solutions. Solid arrows indicate the typical absorption peak of Ag NPs, and the dotted arrows indicated where the absorption is decreasing and the FWHM is increasing.



**Figure 3** The UV-Vis adsorption spectra of Ag NPs diluted in  $\text{MgCl}_2$  solution. Arrows show the typical absorption peak of Ag NPs and the peak of larger particles at 500 nm.



**Figure 4** The UV-Vis adsorption spectra of Ag NPs in the  $\text{FeCl}_3$  solution. The arrow indicates the decrease in the absorption at 400 nm.

increase in temperature facilitated aggregation of the Ag NPs. However, the particles still showed the typical absorption peak at 400 nm, even in DMEM with 10% FBS at 37°C.

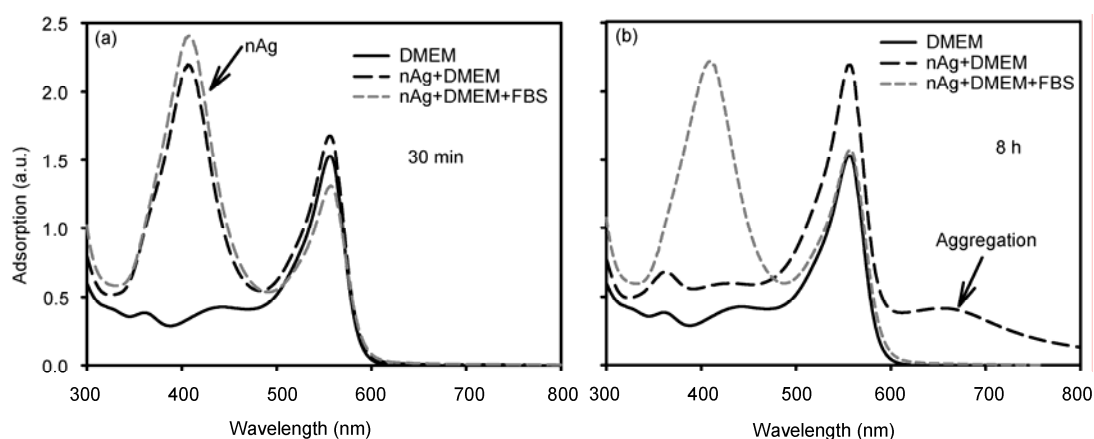
## 2.6 Influence of pH on the stability of the Ag NPs

In an earlier study, a large change in the pH of the solution greatly reduced the stability of the Ag NPs [25]. In the current study, we demonstrated that in a typical environmental and over the biological pH range of 4.2–8.4, changes in the pH did not alter the stability of the Ag NPs (Figure 7).

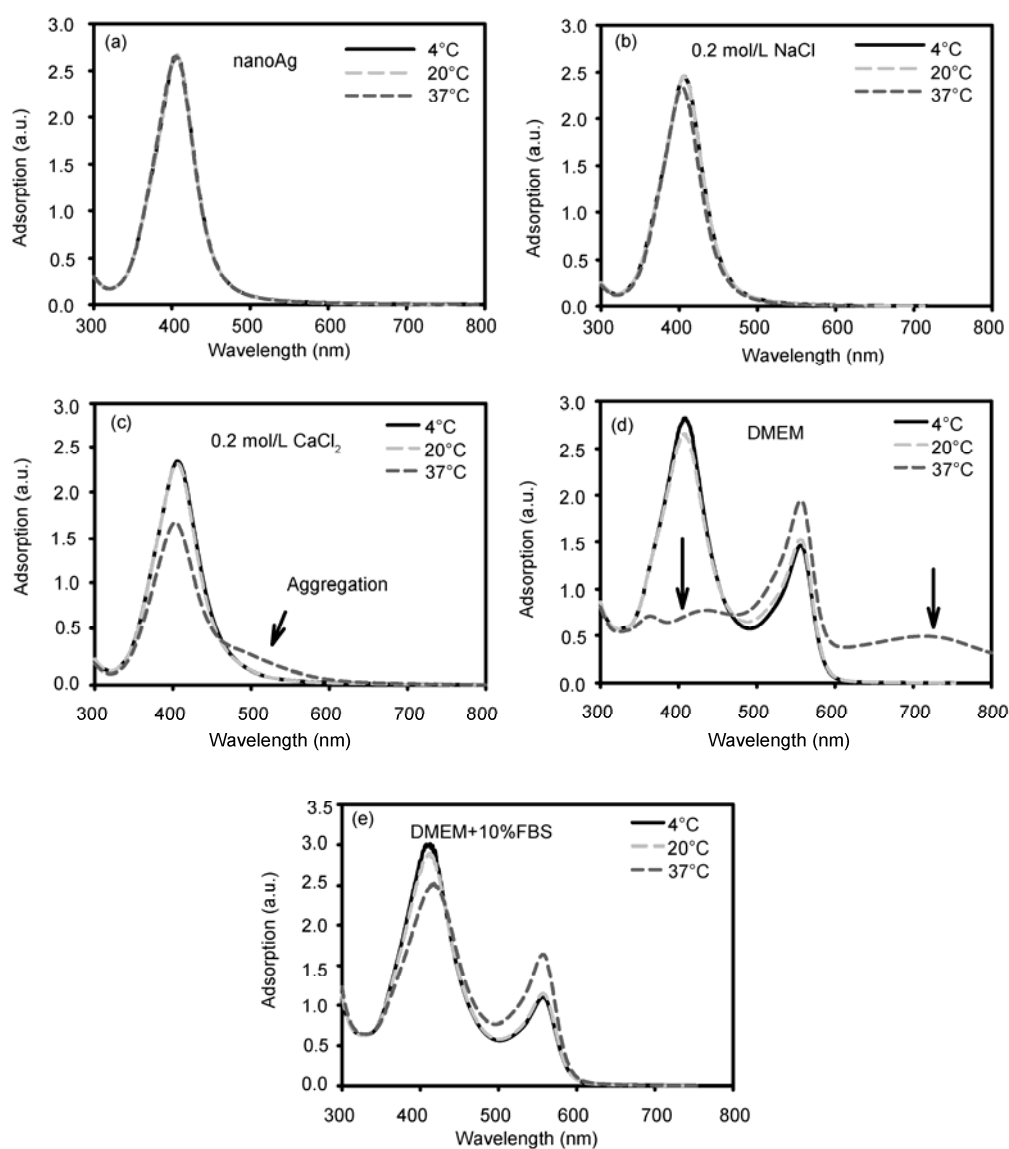
## 2.7 Influence of environmental and biological factors on size and Zeta potential of Ag NPs

A change in the particle size is another indicator of reduced stability of the Ag NPs. In 0.2 mol/L  $\text{CaCl}_2$  or FBS-free DMEM, the particle size increased up to a microscale, especially at 37°C. But in 0.2 mol/L NaCl and DMEM with 10% FBS, there were no large changes in the particle size (Figure 8). This observation is consistent with the results from UV-Vis spectroscopy (Figures 1, 2, 5 and 6).

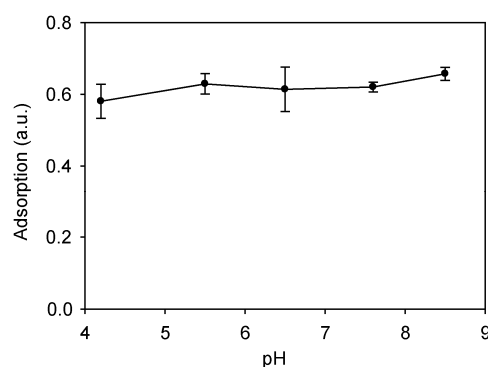
The Zeta potential was measured to investigate the



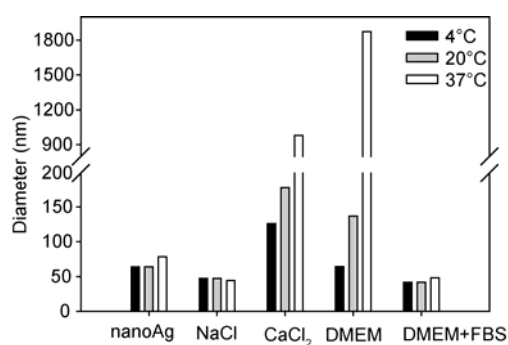
**Figure 5** UV-Vis adsorption spectra of Ag NPs exposed to DMEM and DMEM+FBS. Peak at 550 nm is the typical absorption peak of phenol red.



**Figure 6** The UV-Vis adsorption spectra of Ag NPs suspended in various solutions at 4, 20 and 37°C.



**Figure 7** The absorbance of the Ag NPs at 400 nm from pH 4.2–8.4.



**Figure 8** Influence of ionic strength, valence, temperature, and FBS on the size of Ag NPs.

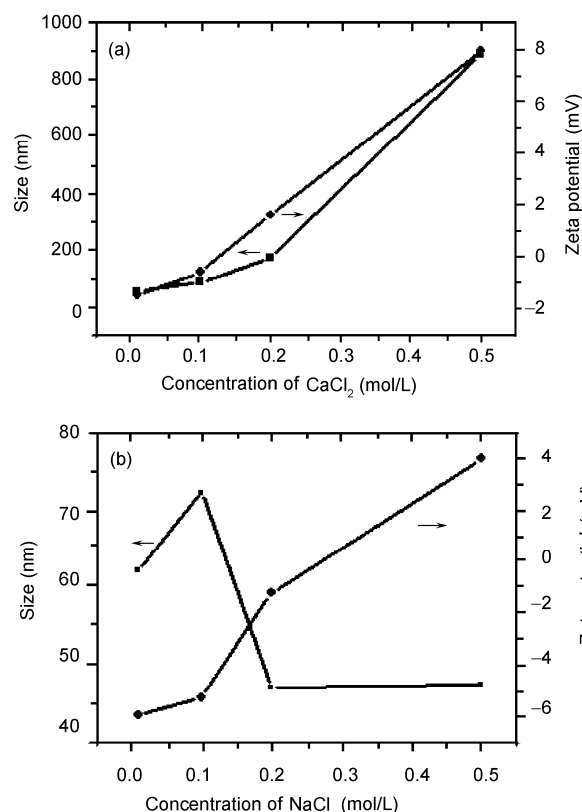
influence of various solutions on the surface charge of the Ag NPs. In 0–0.5 mol/L  $\text{CaCl}_2$  or  $\text{NaCl}$  solution, the zeta potential of the Ag NPs increased from negative to positive (Figure 9). As biological media could not be assessed by this method, no data is presented for the zeta potential of the Ag NPs in DMEM and DMEM+FBS.

## 2.8 Release of silver ions in various suspensions of Ag NPs

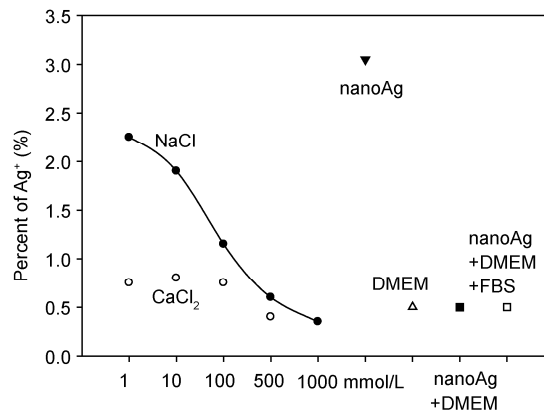
The toxicity of Ag NPs arising from the release of free silver ions has been well documented [26]. The release of silver ions is also a reflection of the stability of the Ag NPs. In suspensions of 0–1 mol/L  $\text{CaCl}_2$  and  $\text{NaCl}$ , DMEM or DMEM+FBS, the release of silver ions decreased from 3.0% to 0.5% (Figure 10), which suggests that the activity of the Ag NPs reduced. This might occur because of the aggregation or protein binding of the Ag NPs (Figure 11).

## 3 Conclusion

Ionic strength, valence, temperature, and some biological molecules were found to affect the stability of Ag NPs. The addition of DMEM and an increase in the ionic strength and valence all resulted in aggregation of the Ag NPs. The aggregation process was also promoted by an increase in the

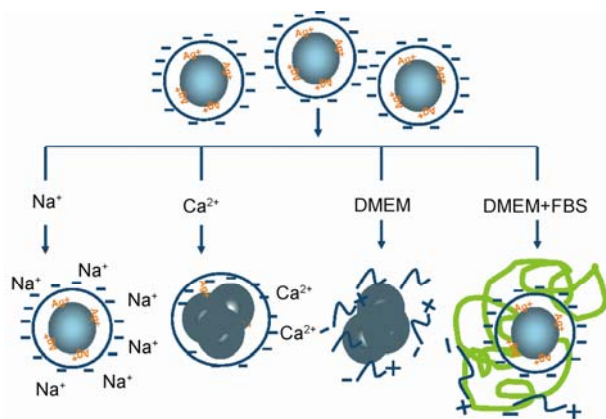


**Figure 9** The Zeta potential of Ag NPs in 0–0.5 mol/L  $\text{CaCl}_2$  (a) and  $\text{NaCl}$  (b).



**Figure 10** The release of silver ions in various suspensions of Ag NPs.

solution temperature. However, the presence of FBS in the cell culture medium helped maintain the stable dispersion of the Ag NPs. The stability of the Ag NPs was minimally affected by a change in pH within a range of 4.2–8.4. All tested solutions reduced the release of silver ions from the Ag NPs. These findings are summarized in Figure 11. The electrical double layer of the Ag NPs was not destroyed in weak ionic solutions or when the NPs were coated in proteins. This resulted in stable dispersions of the Ag NPs. By contrast, in stronger ionic solutions or those that lacked proteins, the electrical double layer collapsed and this led to



**Figure 11** Summary of the influence of various factors on the stability of Ag NPs.

aggregation of the Ag NPs.

In the typical conditions of *in vitro* culture media, the Ag NPs could maintain their nanoscale features. Therefore, they should remain stable under *in vivo* conditions. These results will allow further investigation of the *in vitro* and *in vivo* toxicity of Ag NPs.

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