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Amino acid induced fractal aggregation of gold nanoparticles: Why and how

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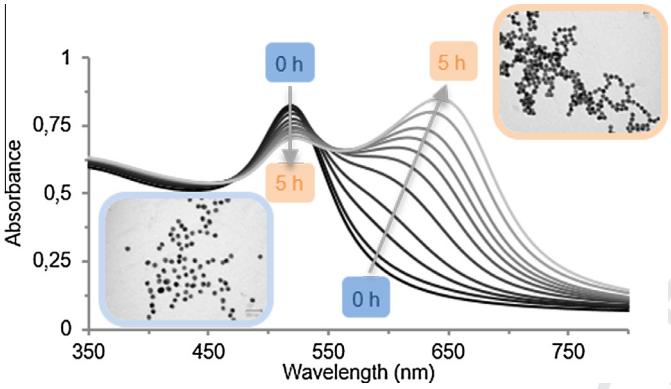
GRAPHICAL ABSTRACT



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ABSTRACT

Gold colloids are the object of many studies as they are reported to have potential biological sensing, imaging and drug delivery applications. In the presence of certain amino acids the aggregation of the gold nanoparticles into linear structures is observed, as highlighted by the appearance of a second plasmon band in the UV–Vis spectra of the colloid. The mechanism behind this phenomenon is still under debate. In order to help elucidate this issue, the interaction between gold colloids and different amino acids, modified amino acids and molecules mimicking their side-chain was monitored by UV–Vis absorption, DLS and TEM. The results show that phenomenon can be rationalized in terms of the Diffusion Limited Colloid Aggregation (DLCA) model which gives rise to the fractal aggregation colloids. The global charge of the compound, which influences the ionic strength of the solution, and the ease with which the compound can interact with the GNPs and affect their surface potential, are, the two parameters which control the DLCA regime. Calculations based on the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory confirm all the experimental observations.

1. Introduction

The unique and tailorable physical, optical, electrical and chemical properties of noble metal nanoparticles are the source of much inspiration for the development of novel devices with varied applications in the biological and biomedical fields [1–4].

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Gold nanoparticles (GNPs) are in this context of particular interest due to their remarkable optical properties [5,6], presumed biocompatibility [7] and ease of surface functionalization.

With a Localized Surface Plasmon Resonance (LSPR) band in the visible range (around 520 nm for GNPs with a diameter of 15 nm) [5,6], GNPs are particularly good candidates for the development of colorimetric sensors [1,2]. The detection of an analyte is based on a change in the LSPR band following the analyte adsorption or recognition at the GNP surface. Studies have been reported with proteins [8–11], DNA [12–16], amino acids [17–19] and metal ions [20–23]. Most often, the adsorption of the analyte is designed to lead to GNPs aggregation as this leads to a drastic change in colour due to the coupling between the surface plasmons of neighbouring nanoparticles [24,25]. In order to develop reliable analytical applications, it is important to understand the origin of the GNP–biomolecule interactions and to control the aggregation step.

For drug delivery [3] or imaging applications [4], GNPs must be stable in biological fluids, a complex environment composed of a dense mixture of molecules such as proteins, lipids, carbohydrates and amino acids. It is known that when nanoparticles come into contact with biological fluids, the biomolecules present can cover their surface forming what is usually called a "*corona*" which defines the "*biological identity*" of the nanoparticle [26–29]. The corona is what the cell "*sees*" and interacts with, and it controls the distribution and fate of the nanoparticles in the organism. It is consequently of also of great importance for *in vivo* applications to know how to control the interaction between GNPs and biomolecules so as to improve their efficiency and safety.

Several studies devoted to the monitoring of the interaction between citrate stabilized GNPs and biological molecules are described in the literature. It has for example been observed, that the interaction between GNPs and some of the natural amino acids, more particularly arginine [30–34], lysine [33–36], histidine [34], cysteine [34,37], homocysteine [38] and methionine [33,39], leads to an aggregation mode which gives rise to a second LSPR band at higher wavelengths. This phenomenon has been described as the consequence of the formation of linear chains of GNPs, leading to the formation of "*rod-like*" structures, as observed by TEM [31,32,37,39]. It is indeed known that nanorods present two bands in their UV–Visible spectrum, one in the 520 nm range, associated to their transverse dimension, and a second, bathochromically shifted, associated to their longitudinal axis [40].

Two models are proposed to explain the appearance of this second band. The first model hypothesises that the amino acids partially replace the citrate ions adsorbed at the surface of the GNPs, and that favourable interactions between the zwitterionic heads of adjacent amino acids lead to the formation of amino-acid dense clusters on the surface. This patchy distribution on the surface induces an electronic dipole through the nanoparticle and the alignment of theses dipoles leads to the formation of branched linear structures of GNPs [31,32]. The second proposed model hypothesises that certain amino acids interact with the gold surface specifically via their side chains and that hydrogen bonds between the zwitterionic heads of amino acids adsorbed at the surface of neighbouring GNPs, leads to their aggregation in a linear structures [33,35,37–39].

As the explanations put forward to explain changes in the UV–Visible spectra of GNPs when brought in contact with different amino-acids is currently still controversial, we have decided to investigate these interactions more closely. A better understanding of the mechanism of interaction between GNPs and amino acid would indeed be very helpful for the development of reliable biosensors and could also contribute to the understanding of the fate of gold nanoparticles in biological fluids.

2. Materials and methods

2.1. Synthetis of gold nanoparticles (GNPs)

GNPs were synthesized according to a modified Turkevich method [41,42]. All solutions were prepared with HPLC grade water. Glassware was soaked prior to use with aqua regia (25% HNO_3, 75% HCl) and thoroughly rinsed with milli-Q water. 1 mL of trisodium citrate solution ($Na_3C_6H_5O_7$, Alfa Aesar) was injected into 50 mL of boiling aqueous tetrachloroauric solution ($KAuCl_4$, Sigma Aldrich), both adjusted to pH 7 using concentrated HCl or NaOH. After mixing, the solution was refluxed for five minutes and then allowed to cool to room temperature. Concentrations were chosen so as to achieve a final concentration of 3 mM in $AuCl_4^-$ with a citrate to gold ratio of 1. GNPs were analysed by transmission electron microscope (TEM), Philips CM20-UltraTWIN equipped with a lanthanum hexaboride (LaB6) crystal at 200 kV accelerating voltage.

2.2. Study of the interactions between GNPs and amino acids

GNPs were dialyzed during 24 h in a 1 mM citrate solution (HPLC grade water) using dialysis membrane with a 1 kDa Molecular Weight cut-off (purchased from Orange Scientific) to remove unreduced chloroaurate and oxidation products of citrate present in solution while controlling precisely the citrate concentration. GNPs were diluted using a 1 mM citrate solution to obtain an absorbance of around 0.75. GNP suspension at different concentrations of the following molecules (L configuration), but keeping the citrate concentration constant, were prepared (HPLC grade water): arginine, aspartic acid, glutamine and N-acetyl-arginine (purchased from Sigma Aldrich), asparagine, glutamic acid and arginine methyl ester (purchased from Alfa Aesar), cysteine, glycine, histidine, lysine, threonine and guanidinium (purchased from Merck).

UV–Vis spectra were recorded between 330 and 800 nm at a 480 nm/min scan rate on a Lambda-35 Perkin-Elmer spectrophotometer. Dynamic light scattering (DLS) and zeta potential measurements were recorded with a Malvern Zetasizer Nano ZS equipped with a He–Ne laser (633 nm). GNPs were dispersed in water at 25 °C. A PMMA cuvette for DLS measurements and Zetasizer nanoseries cells for Zeta potential measurements were used as sample container.

2.3. Functionalization of GNPs with mercaptoundecanoic acid

The pH of the GNP suspensions was adjusted to 11 and an ethanolic solution of mercaptoundecanoic acid (Sigma Aldrich, MUA) was added progressively to obtain a final MUA concentration of 1 mM. The solution was then incubated during 60 h at room temperature. The excess of MUA was removed from the GNPs suspension by five rounds of: centrifugation, removal the supernatant and resuspension of the GNPs in water.

3. Results and discussion

The interaction between GNPs and amino acids was studied by time-resolved UV–Vis absorption spectroscopy by following the LSPR band of the colloidal suspension in the presence of different concentrations of amino acids. GNPs used for these studies show a LSPR band at 520 nm and present a size, determined by electron microscopy (TEM), of 14 nm with a standard deviation of 1 nm (see SI – Fig. S1).

For all the experiments GNPS were synthesized by a modified Turkevich method and they are consequently protected by citrate. It has been suggested that GNPS in the presence of citrate are covered by layers of dihydrogen citrate anions: a first layer adsorbed via the central carboxylate group and a second layer that forms hydrogen bonds via their terminal carboxylic acid groups with the adsorbed citrates and which orients their central carboxylate towards the bulk [43]. As citrate influences the ionic strength of the solution, its concentration was controlled with precision.

Fig. 1a displays the evolution as a function of time of the spectrum of a GNP suspension (approximately 3 nM) in the presence of 0.7 mM of arginine at pH 7 and a citrate concentration of 1 mM. The appearance of the second LSPR band around 650 nm is clearly observed, as already reported in the literature for this amino acid [30–34]. This phenomenon is the signature of the coupling between the LSPR bands of the GNPs. This type of evolution in the UV–Vis spectrum was also observed for some other amino acids, but not always under the same experimental conditions, some required much higher concentrations.

The amino acids that have been tested can be classified into three categories according to the concentration required to promote the appearance of the second LSPR band while working at physiological pH, pH at which the head-group of all amino acids is zwitterionic, and with a 1 mM concentration in citrate: amino acids for which low concentrations are required to induce changes (less than 1 mM), those requiring a higher concentration (10–20 mM) and those that do not generate a second absorption band even after 5 h. Histidine falls, like arginine, in the first category while lysine (see Fig. 1b), aspartic and glutamic acid fall in the second one (see SI – Fig. S2). Asparagine (Fig. 1c), cysteine, glutamine, glycine and threonine do not lead to any changes in the LSPR band even at 20 mM and fall into the third category. Fig. 1d presents the time evolution of the absorbance at 650 nm, characteristic of the second LSPR band, for one amino acid representative of each of these three categories. Fig. 2 shows schematically the amino acid concentration required to induce the specific aggregation of GNPs.

The fact that no changes are observed in the UV–Vis spectrum of gold colloids in the presence of certain amino acids is not a definitive proof that they do not interaction with the GNPs. However, zeta potential measurements show that the addition of asparagine, glutamine, glycine and threonine to the suspension do not modify the surface potential of the GNPs, which would be expected if they were adsorbed at the surface of the particles. It is therefore reasonable to assume that they are indeed not interacting with the citrate protected GNPs. In the case of arginine a change in zeta potential is well observed.

From our measurements, only amino acids with a charged side-chain, whether positive or negative, lead to an aggregation mode which gives rise to a second LSPR band. Comparison of TEM pictures of GNPs, taken at the same GNP concentration and under the same experimental conditions, before and after mixing with arginine (Fig. 3), clearly shows that the size of the GNPs does not change but that GNPs form clusters in which some branched linear chains can be observed. DLS measurements showed that, after mixing with arginine, the hydrodynamic diameter of GNPs increases significantly, confirming that GNPs aggregate in solution (Fig. 4).

In both models presented in the literature [31–33,35,37–39], the presence of the zwitterionic head is considered as essential to induce the specific aggregation of the GNPs into linear chains. To further investigate the role played by the zwitterionic head in the aggregation phenomenon, experiments were undertaken with two protected arginines: L-arginine methyl ester where the

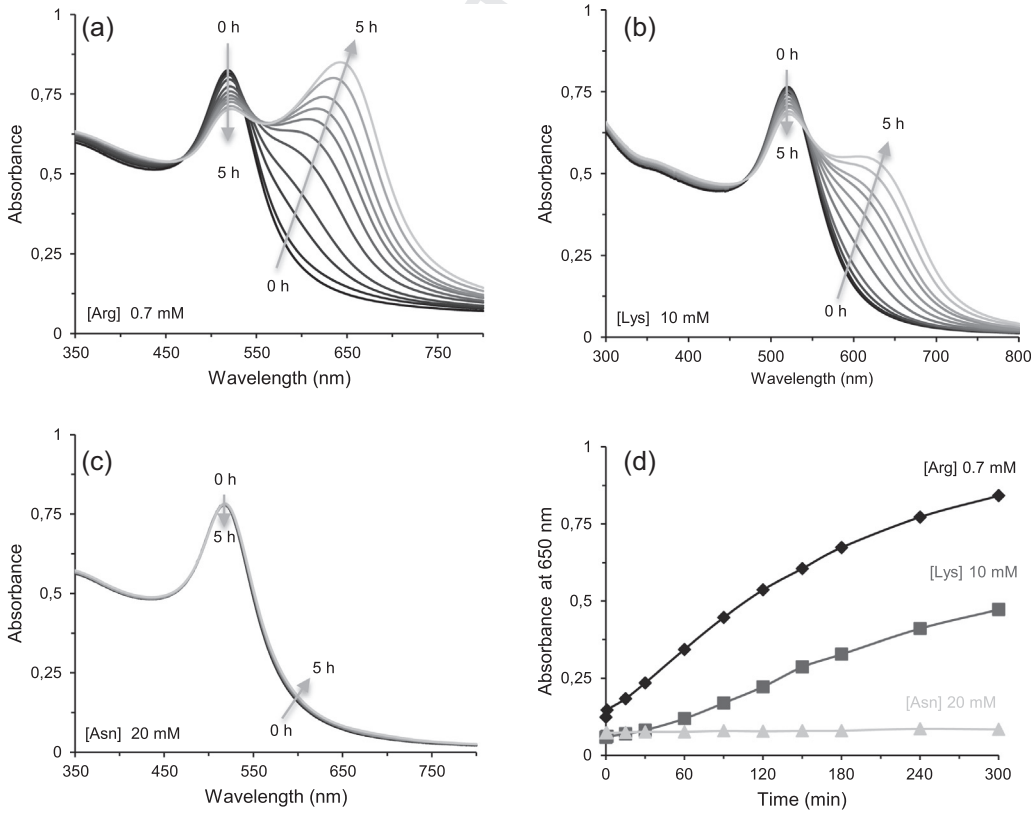

Fig. 1. UV–Vis absorption spectra of a GNPs suspension recorded as a function of time at pH 7 and 1 mM citrate in the presence of (a) 0.7 mM arginine; (b) 10 mM lysine; (c) 20 mM asparagine at pH 7 and 1 mM in citrate and (d) evolution of the absorbance at 650 nm in the presence of different amino acids ([Arg] 0.7 mM; [Lys] 10 mM and [Asn] 20 mM).

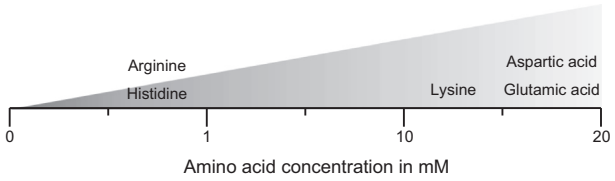

Fig. 2. Concentration of amino acids requested to aggregate GNPs.

carboxyl group is protected by a methyl ester (called Arg-$COOCH_3$) and N-acetyl-L-arginine where the amine group is transformed into an amide (called Arg-NH_2COCH_3). Fig. 5 displays the evolution of the absorbance at 650 nm as a function of time of a suspension of GNPS in the presence of these two forms of arginine. The second LSPR band appears in all cases and, for Arg-$COOCH_3$, even at lower concentrations than with arginine. The precipitation of aggregates is observed in less than one hour for a concentration in Arg-$COOCH_3$ approximately 20 times lower than that of arginine.

We also studied the behaviour of GNP colloids in the presence of compounds corresponding to the side-chains of the amino acids which lead to the aggregation. Experiments were undertaken with guanidinium (arginine side chain), imidazolium (histidine side-chain), propylamine (lysine side chain) and acetate (aspartate and glutamate side-chains). To our great surprise, these molecules also lead to the second LSPR band (see SI – Fig. S3).

These experiments with protected amino acids and with molecules mimicking the amino acid side chains seem to show that the mechanisms proposed in the literature to explain the appearance of a second absorption band in presence of amino acids does not provide a complete picture of the phenomenon as it is clearly possible to obtain the same aggregation mode without the presence of zwitterionic heads. These experiments also highlight that it is the amino acid side-chains which interact with the GNPs.

The effect of the global charge of the amino acids was also investigated. Fig. 6 shows that the interaction between arginine and GNPs at pH 10.5, pH at which the amine group of the arginine head is deprotonated and the amino-acid globally neutral, does not lead to any changes at the level of the LSPR band, even after 3 h. If the pH value is decreased to 7.9, pH at which the amino-acid is positively charged, the second LSPR band appears. This shows that in the absence of a net charge, no aggregation of GNPs occurs.

Adding charged amino acids to a solution increases its ionic strength (zwitterions do not influence the ionic strength [44]). It is known that an increase in ionic strength can lead to colloid aggregation by screening the electrostatic repulsion between particles. The effect of amino-acid concentration on the appearance of the second LSPR band, at constant ionic strength, was monitored. Fig. 7 shows the evolution with time of the absorbance at 650 nm of GNP colloids in the presence of different arginine concentrations at constant ionic strength (kept constant by the addition of NaCl). Arginine concentration accelerates the appearance of the second LSPR band, highlighting that its appearance is not simply due to an increase in the ionic strength of the colloid. The decrease observed in the absorbance after approximately 4 h for the 1 mM and 1.3 mM arginine solutions is simply due to the precipitation of the aggregates that have reached a critical size.

In order to establish if the amino acids with a positively charged side-chain (arginine, lysine, histidine) interact with the surface of GNPs or with the negative layer of the stabilizing agent (citrate), GNPs were functionalized with mercaptoundecanoic acid (MUA). As the MUA binds covalently to the GNPs via its thiol group, the GNPs are surrounded by a layer of grafted carboxyl groups that cannot be displaced by the simple addition of amino acids in solution. The 6 nm redshift of the maximum of the LSPR band after the addition of MUA confirms that the successful functionalization (see SI – Fig. S4a). These GNPs can be centrifuged and re-suspended in milli-Q water repeatedly which is not the case for citrate protected particles, and this also confirms that the functionalization has been successful. The addition of arginine to a suspension of the MUA functionalized GNPs does not induce any modification of the LSPR band (see SI – Fig. S4b) even at high amino-acid concentrations (20 mM) and a 5 h wait. From this it is possible to conclude that the positively charged amino acids interact directly with the gold surface and are not just adsorbed on top of the negatively charged citrate layer. This will be discussed in more detail later.

It has been reported in the literature that colloidal particles aggregate following one of two distinct rate limiting regimes: a Reaction Limited Colloidal Aggregation regime (RLCA) when there is a substantial, but not insurmountable potential barrier that particles have to overcome to come in close contact and a Diffusion Limited Colloidal Aggregation regime (DLCA), when repulsive forces are negligible and the aggregation rate that is solely limited by the time taken for nanoparticles to encounter each other by diffusion [45,46]. With the RLCA mode, dense clusters are formed but, in the case of the DLCA, the aggregates form fractal clusters. Simulations of the absorption spectra of growing GNP fractal clusters, reported by Taylor et al. show that the few almost-linear chains present in these complex structures dominate their plasmonic behaviour and lead to the appearance of the second plasmonic band [47]. It can be concluded that the appearance of the second LSPR band observed when GNPS are in contact with certain amino-acids is the signature that they aggregate following the DLCA regime. It is possible to estimate theoretically the potential energy between particles as a function of the inter-particle distance using the theory developed by Derjaguin, Landau, Verwey and Overbeek (DLVO) [48–50]. This theory considers that the potential energy, V_T, between two particles can be expressed as the sum of two contributions: the van der Waals attraction, V_{vdW}, and the electrostatic double layer repulsion, V_E.

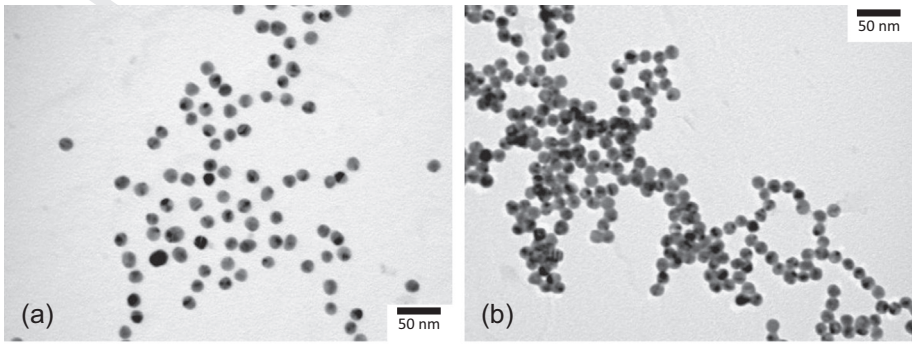

Fig. 3. TEM pictures of GNPs (a) before and (b) after addition of arginine in the solution.

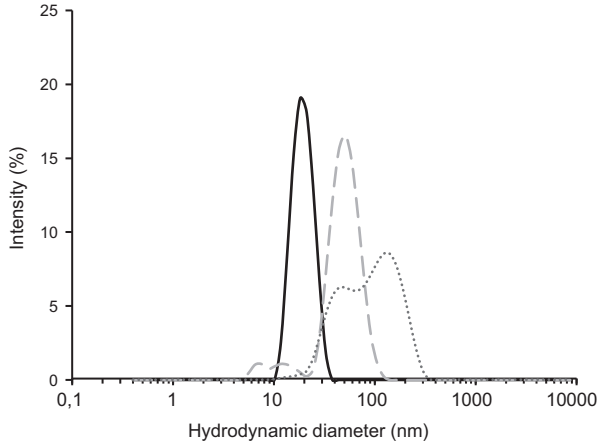

Fig. 4. Hydrodynamic diameter of a GNPs suspension in absence (bold line) and in presence of 0.7 mM in arginine at pH 7 (1 mM in citrate) after 90 min (dashed line) and 180 min (dotted line).

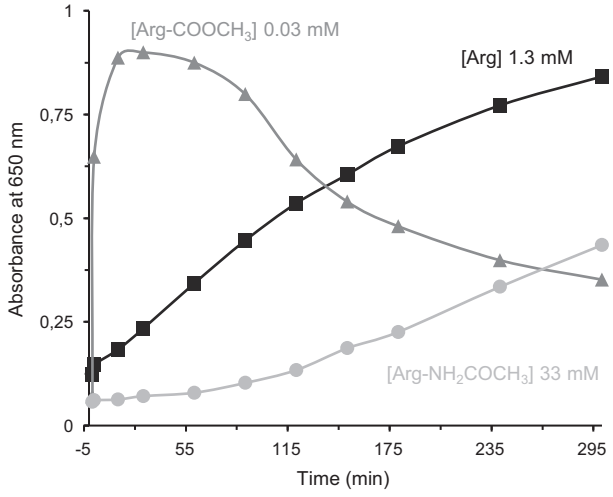

Fig. 5. Evolution of the absorbance at 650 nm as a function of time for GNPs in the presence of different forms of arginine ([Arg] 0.7 mM (square) at pH 7, [Arg-$COOCH_3$] 0.03 mM (triangle) at pH 6 and [Arg-NH_2COCH_3] 33 mM (solid circle) at pH 7 (all 1 mM in citrate).

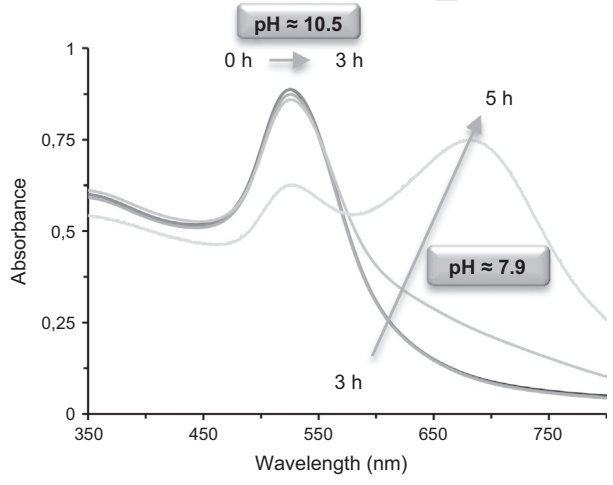

Fig. 6. UV–Vis absorption spectra of a GNP suspension (0.01 mM in citrate) in the presence of 0.7 mM arginine at pH 10.5 (monitored during 3 h) after which the pH was reduced to 7.9 (monitored for an extra 2 h).

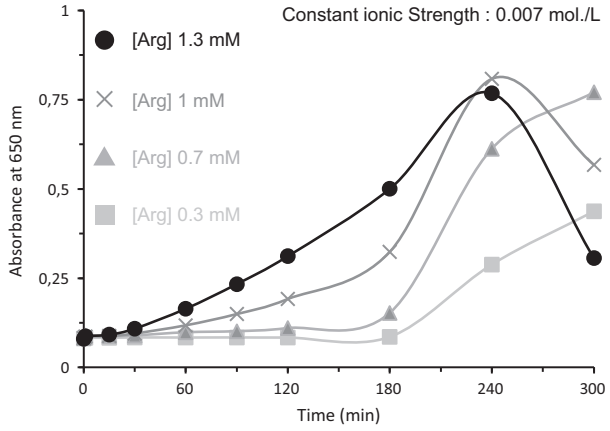

Fig. 7. Time evolution of the absorbance at 650 nm of GNPs at pH 7, with 1 mM citrate and at constant ionic strength (0.007 mM) in the presence of different arginine concentrations: 0.3 mM (square); 0.7 mM (triangle); 1 mM (cross) and 1.3 mM (solid circle).

$$V_T = V_{vdW} + V_E \quad (1)$$

In the case of two spherical particles of same size and separated by a distance d, the van der Waals potential can be estimated by Eq. (2) [51]:

$$V_{vdW} = \frac{-A_H}{6}\left(\frac{2r^2}{d^2+4rd} + \frac{2r^2}{d^2+4rd+4r^2} + \ln\frac{d^2+4rd}{d^2+4rd+4r^2}\right) \quad (2)$$

where r is the radius of nanoparticles and A_H is the Hamaker constant, that is equal to 3×10^{-19} J for GNPs in water [52]. The electrostatic potential between two GNPs can be described by Eq. (3) [51,53].

$$V_E = \frac{64\pi k_B T n_0 r}{\kappa^2}\tanh\left(\frac{z\psi_s e}{4k_B T}\right)^2 \exp(-\kappa d) \quad (3)$$

where k_B is the Boltzmann constant, T is the temperature, z is the charge of the solvated ion, e is the electron charge and n_0 is the number density of ion (molecules/L) far from the nanoparticle surface. The electrostatic potential is dependent on the inverse Debye length, κ, and on the surface potential, ψ_s. The inverse Debye length can be expressed by Eq. (4) and is directly dependent on the ionic strength of the solution, I (Eq. (5)).

$$\kappa = \frac{1}{\lambda_D} = \sqrt{\frac{e^2 N_a 2I}{\varepsilon_r \varepsilon_0 k_B T}} \quad (4)$$

$$I = \frac{1}{2}\sum_i C_i z_i^2 \quad (5)$$

where ε_0 is the permittivity of free space, ε_r is the relative permittivity of water, z_i is the valence of the ion of type i and C_i its concentration expressed in mol/L.

Considering the above, amino acids in solution could influence the interaction potential between GNPs by modifying the surface potential of GNPs and/or the ionic strength of the solution. The surface potential of GNPs will change when specific amino acids interact with the GNPs and replace the negatively charged citrate anions. The zeta potential, considered in first approximation to be equal to the surface potential, increases from −55 mV to −30 mV when 0.7 mM of arginine are added in a GNP suspension.

The interaction potentials between two GNPs in the absence and in presence of arginine in solution, computed using Eqs. (1)–(5) are shown in Fig. 8. In the presence of the amino acid the potential barrier becomes negligible, confirming the DLC aggregation

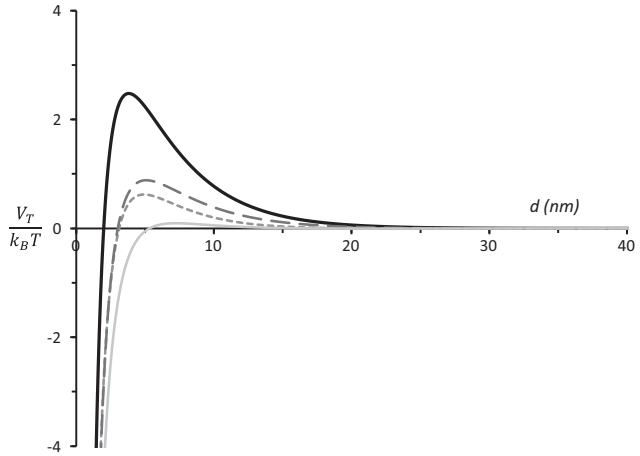

Fig. 8. Variation of the interaction potential between two 13.5 nm GNPs in a 1 mM citrate solution upon addition of 0.67 mM of arginine bearing a net positive charge in the absence (bold line) and presence (grey line) of arginine. The dotted and hashed lines represent the interaction potential for the influence of the ionic strength (dotted) and of the surface potential (hashed) considered separately.

regime. The interaction potential was also computed considering only the effect of the change in ionic strength (dotted line) or only the effect of the change in surface potential (dashed line). The results suggest that both contributions, increase of ionic strength and decrease of surface potential (in absolute values), are needed to promote DLC aggregation, as neither contribution is sufficient to annul the potential barrier. In this context, it is interesting to point out that cysteine, which is known to form covalent bonds with the gold surface via its thiol group [54] does not promote aggregation. The binding of cysteine will change the surface potential of the GNPs but will not influence the I.S as it is zwitterionic at physiological pH. When working with 3.3 mM cysteine and doubling the I.S. via NaCl addition, DLC aggregation occurs (see SI – Fig. S5) confirming the importance of both the I.S and the surface potential for this to occur.

All charged amino acids will contribute identically to the modification of the ionic strength of the solution but they will however not have the same influence on the surface potential of the GNPs which certainly explains the differences observed in the concentration or rate at which the second LSPR band appears. Molecules which can interact with the gold surface, will replace the negatively charged citrate molecules and lead to a change in the surface potential. If a sufficiently large decrease (in absolute terms) in surface potential is induced, the GNPs will aggregate.

The interaction between gold surfaces and carboxylates, via electrostatic interactions [43], or amines, via covalently bonding with under-coordinated gold atoms [54,55], is well established in the literature. This can explain the observed interaction between GNPS and glutamic acid, aspartic acid and lysine and the molecules corresponding to the side-chains. Our results with arginine and histidine and their corresponding side-chains, show that the interaction is also possible via other nitrogen containing functional groups where the non-bonding doublet of the nitrogen can lead to the formation of a covalent bond with the gold surface. The presence of this doublet is however not necessarily sufficient to lead to DLC aggregation as the I.S must also be considered as shown above in the case of cysteine.

Molecules that are able to bind more efficiently to the GNP surface, i.e. with a larger affinity for the surface, or leading to a more important change in the surface potential will require a lower concentration to promote DLC aggregation of the GNPs. Arginine or histidine have the most pronounced effect on the GNPs as polyanionic citrate is replaced by a cationic species which furthermore form a covalent bond with the gold surface. With aspartic and glutamic acid higher concentrations are required as the change in surface charge is less significant and the amino acids only interact via electrostatic interactions. Regarding the results obtained with Arg-$COOCH_3$ and Arg-NH_2COCH_3, as Arg-$COOCH_3$ bears two positive charges it will have a more significant influence on both the ionic strength and the surface potential than arginine that bears only one net positive charge or Arg-NH_2COCH_3 which is zwitterionic and only leads slowly to the aggregation of GNPs at a very high concentration.

4. Conclusions

In order to gain insight into the fractal aggregation of citrate stabilized GNPs induced by amino acids, the interaction between gold colloids and various amino acids, modified amino acids and molecules mimicking amino acid side chains was monitored by UV–Vis, DLS and TEM.

The results highlight that amino acids can be classified into three categories according to the concentration at which they induce the appearance of the second LSPR band: amino acids that require low concentrations (arginine and histidine), those requiring a higher concentration (10–20 mM: lysine, aspartic acid and glutamic acid) and those that do not generate a second LSPR band (asparagine, cysteine, glutamine, glycine and threonine). Experiments with modified arginines and amino acid side-chains show that the zwitterionic head is not required to induce the aggregation phenomenon. The presence on the interacting molecule of a global charge and of a chemical group able to bind to the gold surface are the essential parameters to consider. The Diffusion Limited Colloid Aggregation (DLCA) of GNPs can indeed be induced by the modification of the ionic strength, influenced by the charge of the molecules, and of the surface potential of the GNPs, induced by the replacement of citrates initially adsorbed on the surface.

Calculations of the interaction potential between GNPs based on the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory confirm all the experimental observations and our conclusions.

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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.2015.11.017.

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