

Aminopolycarboxylic acids and alginate composite-mediated green synthesis of Au and Ag nanoparticles

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Abstract Nanosized particles of gold and silver were produced in aqueous solution as well as in calcium alginate gel beads by simple addition of aminopolycarboxylic acids (APCAs) and alginate. It is shown that the composite of alginate and APCAs served as a reductant and stabilizer. The influence of experimental parameters on the formation of nanoparticles was investigated via UV–visible spectroscopy, X-ray diffraction, transmission electron microscopy, and selected area electron diffraction analyses. Further, using sorption experiments it has been demonstrated that the loading of gold nanoparticles on calcium alginate beads is much more as compared to that of Ag nanoparticles. The as-prepared metal sols and solid-phase biopolymer-based nanoparticles show excellent stability.

Keywords Green synthesis · Gold nanoparticles · Silver nanoparticles · Aminopolycarboxylic acids · Alginate beads

Introduction

Various biopolymers are used in many different ways to entrap or incorporate inorganic nanoparticles [1, 2]. Polysaccharides are most commonly used in the preparation of bio-nanocomposites. They are classified according to their ionic character as neutral, anionic, and cationic. Their

combination with nanoparticles provides a tool to impart/induce novel properties and chemical functionalities to nanocomposites. Polysaccharides are derived from natural sources and have advantages in terms of biodegradability, low toxicity, and low cost. Polysaccharide-based composites have characteristic properties that make them an interesting option as composite materials [1, 2].

The utmost important property of polysaccharides is their biocompatibility. They are incorporated into various nanocomposites to improve their biocompatibility. Polysaccharides are hydrophilic and generally non-toxic when used in approved conditions. Heparin, a polysaccharide with anticoagulant property, is used as an effective coating agent to increase the biocompatibility of several materials including carbon nanotubes [3] and metal nanoparticles [4].

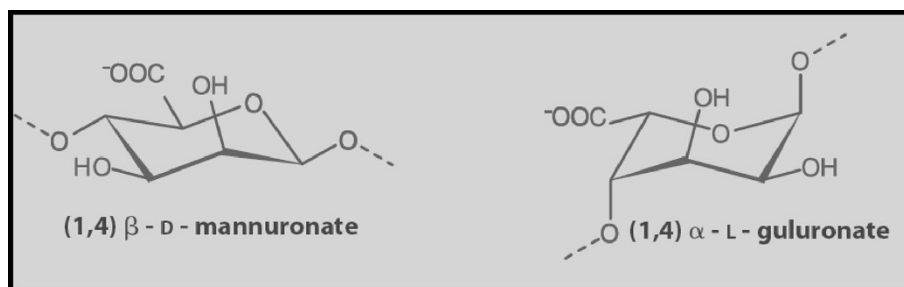
Most polysaccharides form gels that respond to physiological changes with change of temperature and pH or even to the mechanical stress. This property has been explored in the preparation of composite systems for remotely triggered applications including bio-adhesives and drug delivery, among others. Alginate [5] and chitosan [6] were used in the preparation of magnetic composite hydrogels for magnetically triggered drug release. On applying a high-frequency magnetic field an increase in the local temperature of the embedded magnetic nanoparticles occurs that induces structural changes in the polysaccharide matrix, thus leading to a controlled and enhanced release of an encapsulated drug.

Alginic acid is a copolymer of two uronic acid units/monomers: mannuronic acid and guluronic acid. The commercially available alginates are in salt form hence these are also termed as mannuronate (M) and guluronate (G) (Scheme 1). A single molecule of alginate can have about 100–3,000 monomer units linked into a flexible chain-like structure containing varying compositions of

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Scheme 1 Two uronic acid monomers present in alginate, mannuronic acid and guluronic acid [salt forms are called mannuronate (M) and guluronate (G)]



three types of monomer blocks, viz. M–M–M, G–G–G–, and M–G–M. Chemical and physical properties of alginate molecules are decided by the composition of these building blocks with respect to their proportion, distribution, and length of blocks. G blocks will govern the gel-forming potential of the molecule, while the flexibility of the uronic acid chains depends on MM and MG units. Like all natural products, properties of alginates are influenced by various factors like place of origin, seasonal effects, and production method. Sometimes, even the alginates obtained from different parts of same plant differ in composition.

Alginate is one of the few important naturally occurring polyelectrolyte polymers used in preparation of hydrogel matrix. The beads made up of alginate hydrogel are very popular due their adaptability and utility. Alginate is biocompatible and forms gels at room temperature in the presence of divalent cations. Sodium alginate when dissolved in water forms a reticulate structure. This reticulate structure has an ability to cross link on addition of polyvalent cations to form insoluble meshwork. This process is also called ionotropic gelation. The calcium and zinc ions are widely used as cross-linkers in the gelation process. To form gel the alginate should contain sufficient proportion of G monomers in a block to react with the calcium ion. It is known that calcium ion fits into the cavity of the G blocks like an egg fits in an egg box. Since calcium is divalent ion the same calcium ion can bind with the G block of other alginate molecule/chain to form junction zones that result into gelation.

The water molecules are physically trapped into the interstitial sites of the gel matrix due to capillary forces. The gelation involves expulsion of water from the gel matrix, which means as degree of gelation increases more water is expelled from the interstitial cavities of the gel matrix [7, 8]. The ionotropic gelation is a unique property of the alginate gels as gel formation does not involve temperature changes unlike common gelling agents. Thus, thermo-sensitive samples can be explored in alginate gel. The gels can be made firm, brittle, soft, and pliable as desired by adjusting the formulation. The gelation occurs over a wide pH range from acidic to neutral. The general

technique for gelation is diffusion of metal ions either at acidic or neutral pH. The alginate gels also have film-forming ability that can be explored for varied applicability. Thus, the gelation reaction can be used to convert the aqueous sol into a gel matrix, beads and into thin films of the alginate hydrogel. This is a reversible gelation and can be reversed by replacing calcium ions with other cations by adjusting the ionic equilibrium of the solution.

Various reports exist in literature in which the entrapment of particles in gel matrix is reported [9–14]. It is shown that gold nanoparticles incorporated in chitosan and alginate nanocomposites exhibit good ability as glucose-sensing platforms [15, 16]. Generally, chemical reduction or photolytic reduction methods were used for the entrapment of nanoparticles in the gel [9–12]. Aminopolycarboxylic acids (APCAs) are being used in many applications [17] including food industry. As APCAs have affinity toward metal ions it is worthwhile to investigate the incorporation of metal nanoparticles in the alginate gel in their presence. In this report, we have made an attempt to prepare metal nanoparticles in a mixed aqueous media composed of APCA and sodium alginate without using any external reducing agent. It is shown that APCAs and alginate serve as reductants and stabilizer, respectively. Entrapment of gold nanoparticles in the alginate beads is also shown.

Experimental

Materials

Sodium alginate (HF-120) was purchased from FMC Biopolymer. Silver nitrate (99.9 %) was obtained from Aldrich. Iminodiacetic acid (IDA), nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), *N*-(hydroxyethyl) ethylenediaminetriacetic acid (HEDTA), diethylenetriaminepentaacetic acid (DTPA), and triethylenetetraminehexaacetic acid (TTHA) were purchased from Fluka. Solutions were prepared in Millipore water (conductivity $0.06 \mu\text{S cm}^{-1}$). All solutions were wrapped in aluminum foil to protect from light.

Characterization

Absorption measurements of the samples were taken on Jasco V-560 spectrophotometer. The UV–visible absorption spectra were recorded at room temperature using a 2-mm and 1-cm quartz cuvette. Samples for transmission electron microscopy (TEM) were prepared by placing a drop of the colloidal solution on a copper grid coated with a thin amorphous carbon film. Samples were dried and kept under vacuum in a desiccator before putting them in a sample holder. TEM characterization was carried out using a JEOL JEM-2000FX electron microscope. Particle sizes were measured from the TEM micrographs. The particle size was calculated by taking average of at least 100 particles.

Synthesis of Au and Ag metal nanoparticles in mixed solutions of APCAs and biopolymer sodium alginate

HF-120 type of alginate is composed of 45–55 % mannuronic acid and 45–55 % guluronic acid. Alginate undergoes hydration in the aqueous solution. This process takes time and it depends on the concentration of the solution. A 2 % solution requires about 6 h to fully hydrate and forms a clear solution. Sample solutions were prepared from stock solutions of APCAs (0.1 M), sodium alginate (2 % by wt.), $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.01 M), and AgNO_3 (0.01 M).

Metal colloid was prepared by the reduction of metal salts in a mixed solution of APCAs and sodium alginate. The final concentration of APCAs and metal salts in the solutions was optimized to 0.0005 and 0.001 M, respectively. Alginate 1 % (by weight) solution was used as stabilizer for the metal nanoparticles. To study the effect of concentration of APCAs on the Au nanoparticles, concentrations of APCAs were varied from 0.0005 to 0.05 M. Equal measure of APCA solution was mixed with alginate solution on a vortex mixer. Then, Au ions were introduced into the solution and gently mixed. The solution was kept undisturbed till the appearance of a stable red to purple coloration sol. A similar procedure was followed for the preparation of Ag nanoparticles. The sols so obtained were highly stable for a month in ambient conditions and for ~4 months in refrigerator.

Preparation of metal nanoparticles-alginate hydrogel beads

The metal nanoparticless synthesized in APCA/alginate mixtures were in form of a colloidal solution. To form beads the required amount of solution was taken in a burette. The flow rate was adjusted to 20 drops per minute. In another beaker a concentrated solution of calcium chloride (0.02 M) was taken on a magnetic stirrer

assembly. The concentration of the calcium ions in solution was high enough for immediate bead formation even in the presence of the APCAs up to 0.001 M. The drops from the burette were allowed to fall into the calcium chloride solution while the solution was stirred very gently. The volume of calcium chloride solution was kept high (50 ml) to avoid hindrance and clashing during the bead formation. A glass-coated stirring bead was used to stir the solution. The distance between the tip of burette and the liquid surface was typically 5 cm. The beads were formed as soon as the gold sol came into contact with the calcium chloride solution. Optically transparent colored beads of about 3 mm size were obtained by this method. The color of beads was the color of the parent metal sol used and it varied from ruby red, pink to blue. The beads were allowed to equilibrate for 12 h followed by 3–4 times washing with Millipore water. The formed beads can be stored under water for weeks in refrigerator. When required the beads were dried in an oven at 60 °C. The dried beads can be stored without refrigeration. Beads containing silver nanoparticles were typically bright yellow in color.

Results and discussion

Formation of Au nanoparticles in sol

It was observed that on addition of aqueous solution containing APCA (5×10^{-4} M) at pH 10.5 to an aqueous solution containing Au^{3+} ions (1×10^{-4} M) the color of the solution changed to violet indicating the formation of the particles. The induction time for the formation of the particles was ~3 h. However, it was observed that within few minutes the particles got precipitated due to agglomeration. TEM results showed the presence of agglomerated particles (results not shown). Due to this an attempt was made to stabilize nanoparticles in the presence of water-soluble anionic biopolymer sodium alginate.

It was observed that on addition of aqueous solution containing IDA (5×10^{-4} M) at pH 10.5 to an aqueous solution containing Au^{3+} ions (1×10^{-4} M) and alginate 1 % by weight the color of the solution changed to violet indicating the formation of the particles. The formed particles showed broad surface plasmon absorption band having maxima at 540 and 780 nm (Fig. S1). The broad absorption band could be due to the aggregation and/or due to the formation of anisotropic particles. TEM results showed the presence of agglomerated particles (Fig. S2). Somewhat similar results were obtained for NTA. However, in the presence of EDTA, HEDTA, DTPA, and TTHA, the formed particles showed ellipsoid shapes. This indicates that as the complexing ability of the APCA is



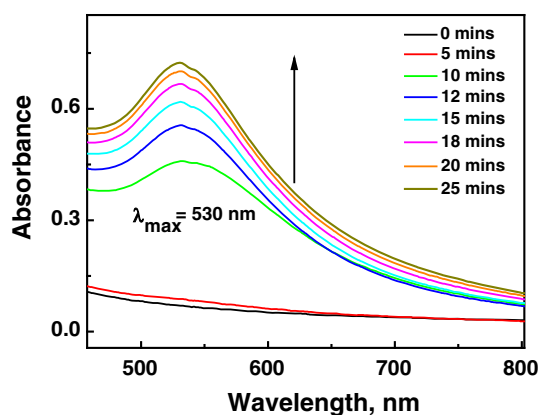


Fig. 1 UV-vis absorption spectrum of gold nanoparticles obtained on mixing TTHA ($5 \times 10^{-4} \text{ M}$) at pH 10.5 to an aqueous solution containing Au^{3+} ions ($1 \times 10^{-4} \text{ M}$) and alginate 1 % by weight. Path length of cell 2 mm

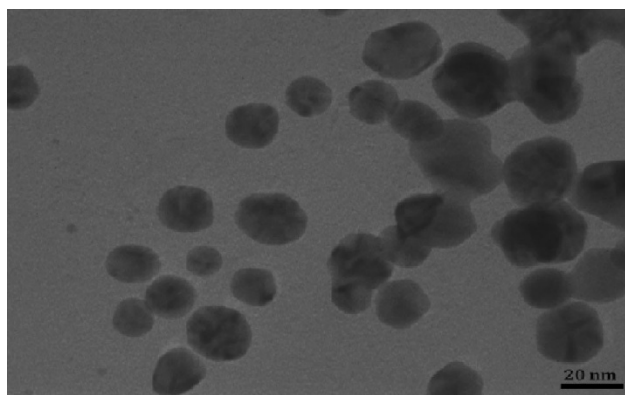


Fig. 2 TEM images of Au nanoparticles. Other conditions are same as in Fig. 1

increasing the agglomeration of the particles decreases. In case of TTHA the surface plasmon absorption band showed only one maximum at 530 nm (Fig. 1). The corresponding TEM image is shown in Fig. 2. It can be seen that the formed particles are in the range of 15–20 nm. Alginate itself can act as a reducing agent. Hence, the experiments were carried out at identical conditions but without IDA. No sign of reduction was observed till 3 days. Thus, any contribution of alginate in the beginning of the particle formation can be neglected.

The effect of concentration of APCAs on the shape of the particles was also studied. It was noticed that as the concentration of APCAs was increased the rate of reduction also increased. Figure 3 shows surface plasmon absorption band for Au sol obtained at 0.05 M EDTA concentration. It can be seen that the surface plasmon absorption band shows only one sharp band at 523 nm. The corresponding TEM image for the formed

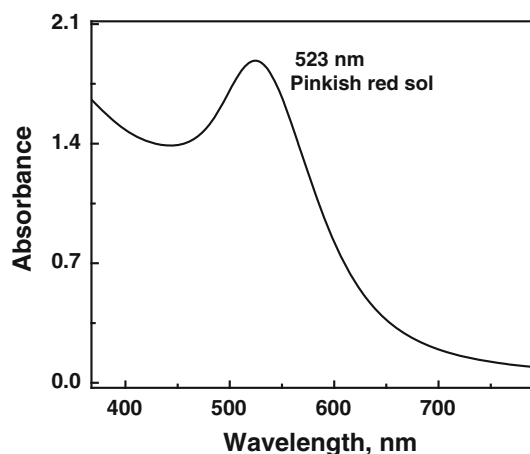


Fig. 3 UV-vis absorption spectrum of gold nanoparticles obtained on mixing EDTA ($5 \times 10^{-2} \text{ M}$) at pH 10.5 to an aqueous solution containing Au^{3+} ions ($1 \times 10^{-4} \text{ M}$) and alginate 1 % by weight. Path length of cell 10 mm

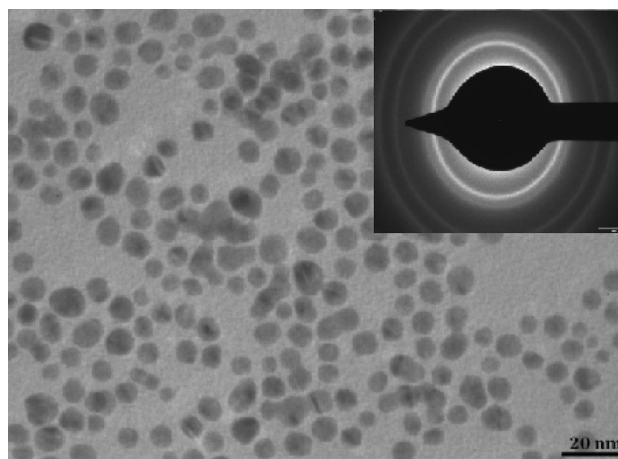


Fig. 4 TEM images of Au nanoparticles. Other conditions are same as in Fig. 3. *Inset* selected area electron diffraction pattern of Au nanoparticles

particles is shown in Fig. 4. It can be seen that the particles become more or less spherical with the increase in the concentration of EDTA. This is due to the fact that as the rate of reduction increases the nucleation and growth steps get separated. This leads to the formation of monodisperse spherical particles. However, it was noticed that when the concentration of alginate was increased up to 2 % it resulted into slowing down of the reduction reaction rate and increased reaction time without any substantial enhancement in the absorbance yield of the final metal sol. The obtained effect could be due to the increase in viscosity of the medium. Hence, the concentration of alginate was optimized at 1 % by wt.



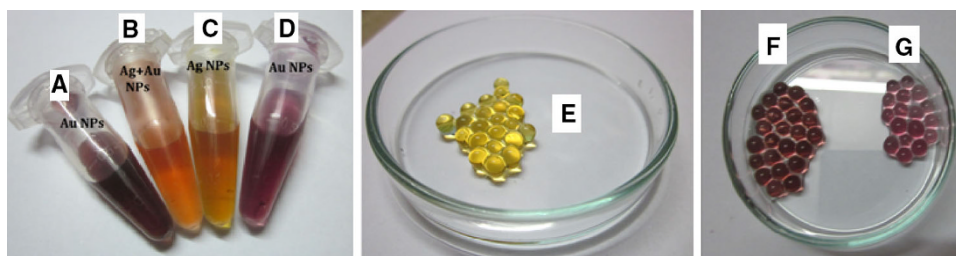


Fig. 5 A–D Photographs of the Au and Ag nanoparticle colloidal sols obtained using EDTA/alginate reaction media. E Alginate beads made from yellow colored Ag nanoparticles shown in eppendorf labeled as

C. F Beads prepared from the ruby red Au sol shown in eppendorf labeled as A. G Beads prepared from bright magenta pink Au sol shown in eppendorf labeled as D

Formation of Ag nanoparticles in sol

The initial experiments on reduction of silver ions in the APCA/alginate mixed media indicated that the reduction of silver was quite difficult. The blank experiment of silver with alginate showed no trace of particle formation for weeks unlike gold. Gold is softer than silver hence the reduction of gold ions occurs at higher potentials than silver. Thus, the softer reducing agents will not be able to reduce silver ions effectively. However, in the presence of APCAs the silver ions were reduced completely in 4–6 h. A typical case for Ag NPs prepared in the presence of TTHA/alginate is shown in Figs. S3 and S4. The UV–visible spectra of Ag nanoparticles prepared in the presence of other APCAs and alginate showed a similar wide absorption band with maxima stretching from 400 to 500 nm. The colors of all the sols were mostly yellow with pale orange tinge in it. As the reduction of silver ions was very slow the formed nanoparticles showed wide range of size and lower particle density. It can be seen that the formed particles were of ellipsoid shape and polydispersed. There was no significant change in the yield and dispersion of Ag nanoparticles on increasing the concentration of APCAs.

Entrapment of Au and Ag nanoparticles in the alginate beads

Figure 5 shows image of beads prepared with different samples. The samples A–D are the images of metal sols prepared in APCA/alginate media. Samples A and D correspond to the Au nanoparticles sol prepared containing 0.05 and 0.0005 M concentration of EDTA, respectively. As mentioned earlier that the sample A has a deep ruby red color, high particle density and smaller particle size as compared to sample D that has a wine pink color with lower particle density and larger sized nanoparticles. The beads prepared from samples A and D are labeled as F and G. The beads have color similar to its precursor colloidal sol and there is no visible change in color of sol after gelation. It is pertinent to mention here that the preparation of the beads from a highly concentrated Au sol (sample A)

involves addition of some neat alginate solution to the metal sol along with increased concentration of the calcium ions. When calcium ions are added to the APCA/alginate medium, the alginate molecules bind to the calcium ions resulting into gelation. Optimum gelation requires complete hydration and homogenous distribution of the alginate molecules in the solution. Thus, it is inevitable to stir the alginate solution on a vortex mixer after complete hydration of the sample is achieved. The sequestering agents are generally used in formations to delay gelation after addition of the calcium ions. Hence, it is to be noted that it is obvious that the amount of calcium required for gelation in the presence of APCAs will be slightly higher than in their absence as APCAs have affinity for the calcium ions. In fact, this property can be used for controlled release of nanoparticles from the entrapped beads. Hypothetically some of the calcium ions involved in the gelation are expected to slowly sequestered by the APCAs (e.g. EDTA has greater affinity for calcium ion) in the beads and may result into reversal of gelation at some places and can lead to release of the entrapped metal nanoparticles. This aspect is yet to be studied in detail. The transparency of the bead is not affected due to high concentration of the Au nanoparticles and is comparable to the beads prepared from sample with lower concentration. The sample C is yellow in color and contains pure silver NPs. The beads prepared from this sample are labeled as E. The beads have bright yellow color of its parent sol.

Conclusions

The silver and gold nanoparticles were synthesized successfully using APCA/alginate mixtures as a reaction medium. Alginate serves as the stabilizing agent to support reducing ability of the APCAs in the mixed medium to obtain stable suspensions of metal nanoparticles. Further, calcium ion-induced ionotropic diffusion setting gelation technique showed that it is possible to successfully entrap/immobilize the metal nanoparticles prepared in the APCA/alginate medium.

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