# Green synthesis of silver nanoparticles using dextran-graft-polyacrylamide as template

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Synthesis of silver nanoparticles (NPs) has been performed using partially hydrolysed star-shaped graft copolymer of polyacrylamide to dextran as auxiliary capping agent. The star-branched copolymer proved to be a very good template for NP synthesis due to high local concentration of functional groups. Good biocompatibility of the copolymer was confirmed by phagocytic index value equal to 84%. In addition to this, the green synthesis approach was ensured using glucose as non-toxic reducing agent. Surface plasmon resonance spectra of the obtained NPs contained two characteristic bands situated at 270–300 and 420–450 nm. Transmission electron microscopy revealed that NPs synthesised in neutral solution (pH = 7) have average diameters equal 10–60 nm with a small portion of NP aggregates. Synthesis carried out at pH = 12 resulted in increased yield of NPs probably due to increased reducing power of glucose in alkaline medium. NPs synthesised in alkaline solution were sized from 20 to 300 nm and contained a large portion of NP aggregates.

**1. Introduction:** Silver nanoparticles (AgNPs) are well known as antimicrobial agents of a wide range of activity [1–3]. Both growth-inhibitory and bactericidal effects of AgNPs are increased when particle size is decreased [4, 5]. In turn, size and shape of AgNPs are dependent generally on synthesis details such as temperature, solution pH, reaction time, reducing agent type and concentration [4–6]. The often used reducing agents such as hydrazine and sodium borohydride [4, 7] are highly toxic substances. For that reason, synthesis procedure should include thorough washing of obtained nanoparticles (NPs) in order to remove reagent residues [4]. Far more convenient reducing agents are non-toxic carbohydrates such as sugar [5], polysaccharides [1, 3] and whole plant extracts [8–10]. NPs synthesised by means of reducing sugars possess good biocompatibility and low cytotoxicity promising wider biomedical applications. Among reducing sugars glucose is well known as good reducing agent [1, 11–15]. An aldehyde group of glucose is oxidised by silver ions and gluconic acid is formed [11–13].

Preparation of AgNPs is usually performed in the presence of a capping agent preventing aggregation of the NPs formed. Typical stabilising agents of low and no toxicity are sodium citrate [4], polyethylene glycol [5], starch [3], dextran [15, 16], hydroxyethyl cellulose [17], carrageenan [18], gelatin [12, 13], polyglutamic acid [14] and so on. Polymers introduce steric hindrances and/or electrostatic repulsions between NPs, thereby preventing from aggregation. Polymers can also play a role of nucleants in the process of Ag NP formation. In this role, branched polymers proved to be more efficient as compared with linear ones [7, 19].

The purpose of this Letter was the preparation of stable AgNPs using green synthesis approach. In this aim, a biocompatible copolymer of polyacrylamide and dextran was used as a host template to control the size and stability of AgNPs. In addition to this, glucose was used as a benign reducing agent to minimise toxic residues.

**2. Experimental:** Analytical grade acrylamide, cerium nitrate, silver nitrate and glucose from Sigma as well as dextran ($M_w = 7 \times 10^4$ g mol$^{-1}$) from Fluka were used. Dextran-g-polyacrylamide (D-g-PAA) copolymer was obtained by free-radical polymerisation of acrylamide in aqueous solution of dextran. Graft polymerisation from dextran mers was initiated by cerium ions generating free radicals sites on the polysaccharide molecule [20]. The number of grafting sites per dextran backbone was pre-determined by molar ratio of acrylamide to cerium ions and it was equal to 20. Details of synthesis and characterisation of the copolymer were described elsewhere [19–21]. Anionic form of the copolymer (referred throughout as a-D-g-PAA) was obtained *via* alkaline hydrolysis of initial graft copolymer during 30 min using 0.25 M NaOH. The selected hydrolysis conditions were proven do not result in macromolecule breakage [21]. The obtained anionic copolymer was purified by means of precipitation with acetone, then freeze-dried and kept in a vacuum desiccator to prevent further hydrolysis. Fraction of acrylamide mers hydrolysed to carboxylate groups was evaluated by potentiometric titration and proven to equal approximately 37%. Biocompatibility of the a-D-g-PAA copolymer was evaluated using cells of murine macrophage J774 d. Phagocytic index was calculated as percentage of cells that have entered in the phagocytosis. Distribution of the cell cycle phases was assessed using a FACSCalibur flow cytometer by the method described in [22].

Reduction of Ag ions was performed in aqueous solutions of the polymer template a-D-g-PAA at both neutral (pH = 7) and alkaline (pH = 12) conditions. pH value in the alkaline solutions was adjusted by adding 30% ammonia dropwise. Molar ratio of acrylamide mers to Ag ions was equal to 5. At first 0.1 ml of 0.1 M $AgNO_3$ aqueous solution was added to 5 ml of polymer solution (1 g l$^{-1}$) and stirred during 20 min. Then, 2 ml of 0.1 M glucose solution was added and the obtained aqueous solution was kept at 60°C for 30 min. The obtained Ag sols were stored in cold dark.

Transmission electron microscopy (TEM) images were registered with CM12 microscope (FEI, Netherlands) equipped with a Megaview SIS Camera. Image analysis was carried out using the open-source software ImageJ [23]. Diameter values ($d$) were estimated from area values ($S$) according to the formula: $d = 2\sqrt{(S/\pi)}$. Over a dozen TEM images were processed for size distribution analysis in order to obtain reliable data. UV–vis absorption spectra of the silver sols were recorded by Cary 50 spectrophotometer (Varian) using 1:25 dilutions. Experimental absorbance curves were approximated using




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MieLab software with the assumption the NPs are spherical in shape and monodisperse in size. Theoretical background for scattering computations and MieLab's workflow were described in [24]. The MieLab software may be downloaded from [25].

**3. Results and discussion:** Preliminary synthesis experiments revealed that linear anionic polyacrylamide possesses rather bad stabilising efficiency for AgNPs. Sedimentation of Ag particles was observed already during synthesis and no stable colloid was obtained. Similar results were observed in our previous works using sodium borohydride as reducing agent [7, 19]. The only branched a-D-g-PAA copolymer proved to be good stabiliser for synthesis of AgNPs. The synthesis experiments succeed in quite stable aqueous colloids having reddish brown colour typical for sols of AgNPs [5, 11, 13]. The obtained AgNPs revealed no visible sedimentation over three months.

Formation of AgNPs was confirmed with TEM. Exemplary TEM images of AgNPs synthesised in the presence of the anionic copolymer at pH = 7 are presented in Figs. 1*a* and *b*. The images show that the obtained colloids contain mainly single AgNPs with a small portion of NP clusters. Single AgNPs are both sphere-shaped and polyhedron-shaped (Fig. 1*b*). The NPs are sized from 10 to 60 nm. It should be noted, that TEM image resolution was not sufficient to register very small NPs less than 4 nm in size.

Exemplary TEM images of AgNPs synthesised under alkaline conditions are presented in Figs. 2*a*–*c*. The images revealed a wider variety of the NP sizes as compared to the ones synthesised under neutral conditions. Single NPs nearly spherical in shape are ranged from 20 to 80 nm in size. Multiparticle aggregates sized approximately from 100 to 300 nm are present as well. The clusters comprise 3–7 NPs sized from 20 to 120 nm (Fig. 2*c*).

Distributions of estimated diameters of AgNPs synthesised at both neutral and alkaline conditions are shown in Fig. 3. The diagrams indicate clearly that AgNPs synthesised at pH = 12 have more broad size distribution than those synthesised at pH = 7.

Average values of NP diameters are shown in Table 1. It can be seen that synthesis carried out at pH = 12 resulted in twofold increase of average diameter of AgNPs as compared to the one carried out at pH = 7.

Additional information on the synthesised AgNPs was obtained using UV–vis absorption spectra. It is known that the position and the shape of surface plasmon resonance (SPR) band depend on both the size and shape of NPs [5, 26]. UV–vis spectra of the obtained AgNPs show two well-expressed SPR bands (Fig. 4). The main band with a maximum at wavelengths 415–450 nm is characteristic for AgNPs sized ~10–50 nm [3–5, 11]. The second band situated at 270–300 nm may correspond to both $Ag^+$ ions and very small Ag NPs of 2–4 nm in size. The weak peak at 345 nm may be ascribed to quadrupole part of the SPR band of polygonal AgNPs with size larger than 50 nm [27]. The appearance of the broad shoulder in the range 570–700 nm suggests the presence of NP aggregates with increased size [13, 28].

SPR spectrum of AgNPs synthesised in alkaline medium (pH = 12) proved to have far more intense bands both that at 340–600 and 270–300 nm (Fig. 4). The higher absorbance values suggest that the

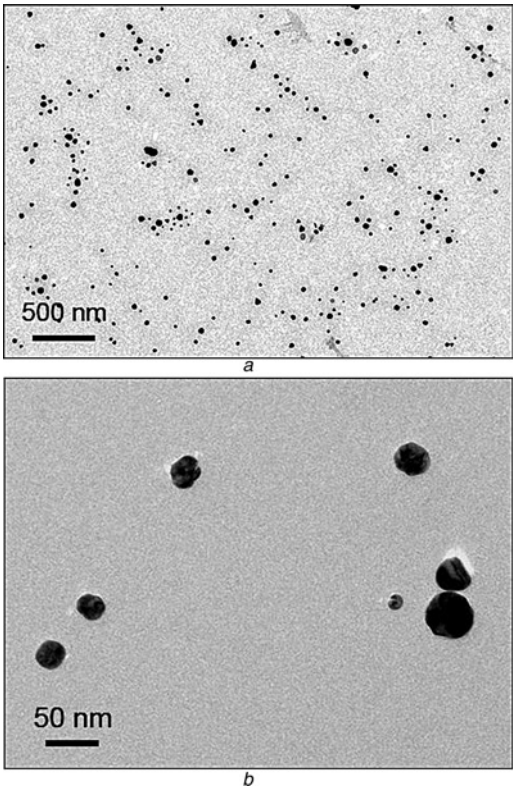


**Fig. 1** *TEM images of AgNPs synthesised at pH = 7*
*a*, *b* – images taken at different magnifications

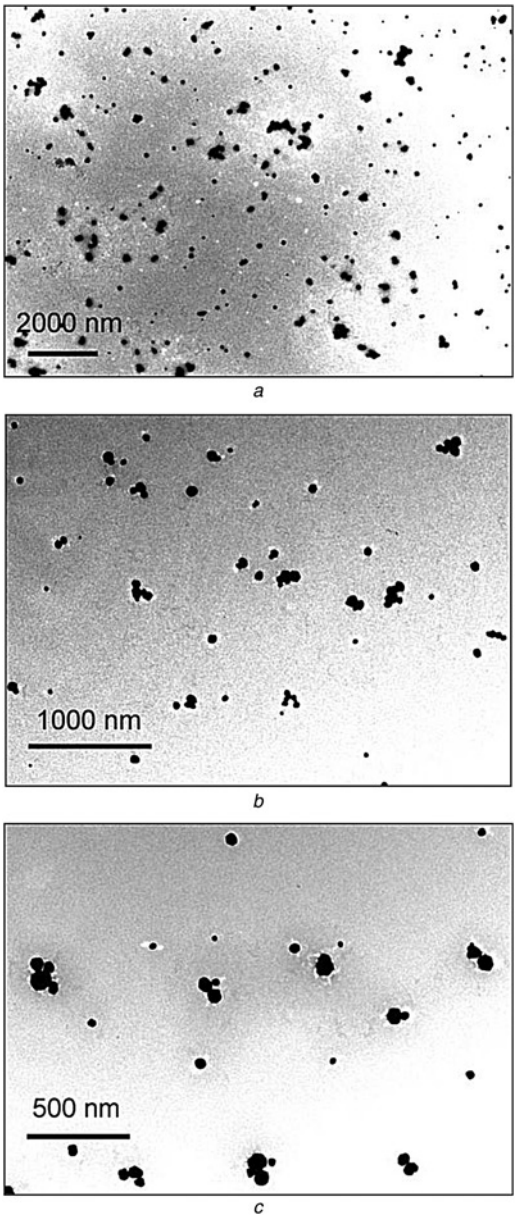


**Fig. 2** *TEM images of AgNPs synthesised at pH = 12*
*a*, *b*, *c* – images taken at different magnifications

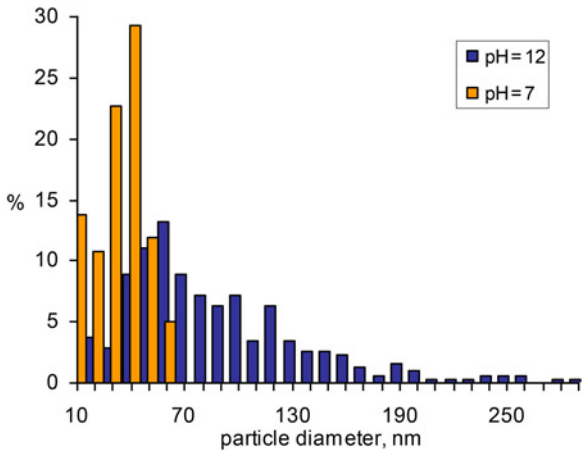


**Fig. 3** *Size distribution of AgNPs synthesised at different pH*

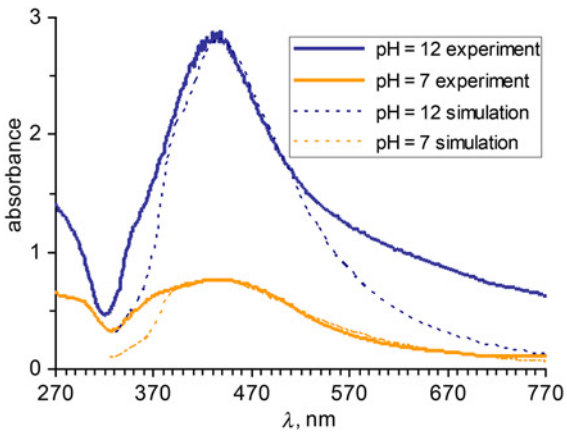


**Fig. 4** *Experimental SPR spectra and Mie simulations of the ones*

**Table 1** Average diameter (nm) of AgNPs evaluated from TEM image analysis and UV–vis band shape

| Synthesis medium | TEM | UV–vis |
|---|---|---|
| neutral | 37 | 36 |
| alkaline | 61 | 73 |

number of obtained AgNPs is much larger as compared to the synthesis in neutral medium (pH = 7). Taking into account that all experimental conditions of the syntheses were identical besides pH value, one can infer that it is alkaline medium that enhances efficiency of reduction reaction. This observation agrees well with the literature data indicating alkali are good accelerators of AgNP formation when glucose is used as reducing agent [11–13]. The most probable cause of the observed increase in NPs yield is increased reductive efficiency of glucose at high pH values. It is well known that several different tautomeric forms of glucose are present in solution at dynamic equilibrium. Reducing agent for silver ions is fleeting open-chain form (aldose). Hydroxide ions promote ring opening, thereby promoting silver ion reduction. In addition, alkaline medium facilitates formation of gluconic acid converting it to sodium gluconate [17].

Fig. 4 presents also the best fits to the experimental SPR spectra computed according to the Mie theory. Unfortunately, significant deviations of the theoretical curves from the experimental ones are observed for both neutral and alkaline medium. The most probable cause is the presence of non-spherical aggregates not considered under Mie simulations. Average diameters of AgNPs were estimated from the theoretical curves as well. The estimated values proved to be close to the ones evaluated from TEM images (Table 1).

One more visible difference in SPR spectrum of AgNPs synthesised at high pH is increased proportion of side band at 520–770 nm (Fig. 4). The wide band suggests that the synthesised sols contain a significant fraction of NP aggregates [5]. This inference agrees well with microscopy data presented in Figs. 2*b* and *c*. To discuss probable causes of NP aggregation, molecular structure of the polymer template should be taken into consideration. The used polymer has 20 polyacrylamide arms grafted to dextran backbone. The side groups on the grafted arms are amide and carboxylate ones. The carboxylate moieties are present mainly as carboxyl groups because of hydrolysis. Dextran part has compact random coil conformation because of cross-linking with cerium ions used as initiator at grafting stage [19]. In fact, poly(acrylic acid) chains are grafted onto surface of fluffy dextran core. As a result, the D-g-PAA copolymer has star-like molecular architecture with 20 arms. Due to the confined space, the grafts have more extended conformation near their tethering point. Far from this point, the grafted chains recover a random conformation similar to linear polyacrylamide. Just due to increased local concentration of functional groups the branched copolymers are very good templates for NP synthesis [7, 19]. Solution pH has great effect on the structural peculiarities of the polymer template. First, conformation of grafted chains becomes more stretched in alkaline medium. The cause is increased degree of ionisation of side carboxylate groups resulting in increased repulsion between the side chains. As a result, the macromolecular chains become more stretched and rigid macromolecular structure is formed. Second, carboxylate anions formed in alkaline medium result in the increased attraction of silver cations to polymer chains. Carboxylate groups bearing silver ions are good nucleants for AgNPs formation. For that reason formation of very small AgNPs is promoted as confirmed by increased peak at 270–300 nm (Fig. 3). On the other hand, the rigid macromolecular structure restricts moving out the small NPs formed. For that cause NP aggregation is enhanced in alkaline medium as confirmed both by TEM images (Figs. 2*b* and *c*) and increased spectral shoulder at 520–770 nm (Fig. 4). One can summarise that alkaline medium promotes both yield and aggregation of NPs. In other words, to decrease aggregation and polydispersity of AgNPs the synthesis should be carried out at neutral conditions.

Finally, biocompatibility tests revealed that macromolecules of a-D-g-PAA were well absorbed by macrophages. The phagocytic index value was determined to equal 84%. The good biocompatibility of a-D-g-PAA copolymers permits to use them for preparing NPs aimed for biomedical applications.

**4. Conclusion:** Stable sols of AgNPs were synthesised using glucose as reducing agent and a-D-g-PAA copolymer as template. The obtained sols were quite stable in time with no visible changes over three months. Rigid macromolecular structure of the used copolymer favour formation of very small AgNPs. Strong alkaline medium under synthesis (pH = 12) favours formation of NP aggregates. In order to minimise aggregation of AgNPs with anionic dextran-g-polyacrylamide as template, synthesis should be carried out in neutral solutions. The excellent biocompatibility of the copolymer suggests the prepared silver NPs may be used for biomedical applications.

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