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Preparation and characterization of biocompatible silver nanoparticles using pomegranate peel extract

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ABSTRACT

The potential application of any nanoparticles, including silver nanoparticles (AgNPs), strongly depends on their stability against aggregation. In the current study, an aqueous extract of pomegranate peel was used as a stabilizer during synthesis of AgNPs. Nanoparticles have been prepared by the chemical reduction method from an aqueous solution of silver nitrate in the presence of sodium borohydride as a reducing agent. The AgNPs were characterized by dynamic light scattering (DLS), zeta-potential measurements, UV–Vis spectroscopy and transmission electron microscopy (TEM). The antibacterial efficiency of AgNPs against *Escherichia coli* was investigated. The size, polydispersity index, FWHM, and colloidal stability of nanoparticles in dispersion depends on the extract concentrations. In the presence of pomegranate peel extract, the nanoparticles suspension shows colloidal stability at least for a week. Our studies show that synthesized AgNPs with the above described procedure were stable at pH = 3–12 and in the temperature range of 25–85 °C. Additionally, AgNPs exhibit antibacterial properties, especially at the lowest amount of extract to silver ratio ($K_{Extract/Ag}$).

1. Introduction

Nanoparticles play an important role in pharmaceutical, industrial and biotechnological applications. In particular, silver nanoparticles (AgNPs) are proved to have antibacterial, antifungal, antiplasmodial and larvicidal properties [1–5]. It was found that among all metals with antimicrobial properties, silver has the most effective antibacterial action and the lowest toxicity to animal cells [6]. Numerous methods have been applied to synthesize AgNPs, such as laser ablation [7], chemical reduction [8–10], photo-chemical or radiation-chemical reduction [11], metallic wire explosion [12] and sonochemical method [13]. From the practical point of view, chemical reduction from aqueous solutions is preferred approach to obtain nano-sized silver particles [8]. Nanoparticle preparation by chemical reduction basically relies on the chemical reduction of metal salts. Control over the growth of primarily formed nanoclusters and their agglomeration is an important task, which is mainly the management of using a variety of stabilizers, in the form of donor ligands, polymers and surfactants [14]. A number of chemicals have been used as a protecting agent in the synthesis of AgNPs such as sodium citrate [15], CTAB (cetyltrimethyl ammonium bromide) [16], SDS (sodium dodecyl sulfate) [10], PVA (polyvinyl alcohol) [17] and PMVE (poly (methylvinylether)) [18]. However, some of chemical stabilizers are toxic and able to pollute the environment. Extensive efforts to find an eco-friendly method to synthesis nanoparticles have been directed toward the usage of natural components as a stabilizer in the synthesis process such as gelatin [19], chitosan [20], starch [21], dextran [22] and plant extracts [23, 24]. Hydroxyl groups in natural polyphenols components can act as both reducing agents for silver nitrate and stabilized AgNPs [25]. Pomegranate (*Punicagranatum* L.) belongs to the family Punicaceae which grows in all warm countries of the world. The pomegranate peel contains a considerable amount of flavonoids and tannins [26]. Punicalagin, the main ingredient of pomegranate peel, is a high molecular weight polyphenolic compound which has shown remarkable pharmacological activities attributed to the presence of dissociable OH groups [27]. In the current study, AgNPs have been prepared by the chemical reduction method from an aqueous solution of silver nitrate. An extract of pomegranate peel was used as a stabilizing agent in the presence of sodium borohydride as a reducing agent. The most important feature of using pomegranate peel is the fact that it is widely present in biomass wastes and use as a natural colorant in textile dyeing process. Such cheap source of the material gives an opportunity to a cost-effective and eco-friendly preparation of AgNPs.

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2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$ extra pure, > 99.8%) was used as a precursor of AgNPs, and sodium borohydride ($NaBH_4$, 99.9%), as a reducing agent, purchased from Merck (Frankfurt, Ludwigshafen, Germany). The pH of each solution was adjusted to 8 with 0.1 N NaOH (Sigma Aldrich).

2.2. Synthesis of AgNPs

Extracted component of pomegranate peel was used as a biocompatible agent for stabilizing AgNPs. 10 g pomegranate peel powder was weighed in a round-bottom flask. A magnetic stirring-bar and a 400 ml methanol/water solution at the ratio 8:2 (*v*/v) were added to the flask and placed on a heater with a stirrer and refluxed for 15 min to boil. The extracts were then filtered using Whatman No. 541, 22 µm and kept in a Petri dish at 45 °C for 24 h to obtain the powder extract. The powder was kept in a refrigerator for further experiments. A required amount of this powder was mixed with water to achieve the desired concentration and final volume of 15 ml. The preparation of AgNPs was carried out by adding a 25 ml of 600 ppm silver nitrate solution to a 15 ml prepared extract solution while stirring (approximately 1000 rpm). Then, 10 ml from 30% (*w*/w) fresh reducing-agent solution was immediately added to the solution. In this method, $K_{Extract/Ag}$ indicates the ratio of extracted component to silver ion concentration. The value of this ratio for all samples is presented in Table 1. In order to obtain small nanoparticles [28], the concentration of the reducing agent was chosen twice as high as that of the precursor. The extracted components were acidic, and mixing different amounts of these components with water resulted in different pH-values. To avoid the effect of different pH-values on the synthesis reaction, water, silver nitrate and extract solution was separately adjusted to pH = 8 before mixing together. The synthesized AgNPs were used at further characterization step without any purification process.

2.3. Characterization of AgNPs

The AgNPs were characterized by dynamic light scattering (DLS), using a Zetasizer Nano HPPSv420 (Malvern Instruments, Ltd., Malvern, UK) at 25 °C. The hydrodynamic diameter (z-average), polydispersity index (PDI), and width distribution of the particles were determined. The UV–Vis absorption spectra for different samples were taken at room temperature on a Cary 100 Bio spectrophotometer (Varian, USA) with a 1-nm resolution. The spectrum wavelengths were between 350 and 800 nm. A glass cuvette with a 1 cm optical path was used. The stability of colloidal AgNPs was studied by taking the UV–Vis absorption spectra of all samples after 1 and 7 days. For UV–Vis spectroscopy measurements, a reference solution was made by mixing extract powder and water to obtain the same concentration of the extract in each sample. Transmission Electron Microscopy (TEM) observations were performed using a Libra 200 (Carl Zeiss SMT). Samples were prepared by placing 2 µL droplets of AgNPs colloidal dispersion on the carbon-coated copper TEM grids and followed by slowly evaporating at room temperature.

Table 1
Composition of sample solution.

Sample	$K_{Extract/Ag}$	[Extract](ppm)	[Ag^+] (ppm)	[NaBH4] (ppm)
1	0	0	300	600
2	0.1	30	300	600
3	1	300	300	600
4	10	3000	300	600

2.4. Stability of AgNPs at Different pH and Temperatures

To check the colloidal stability of AgNPs at different pH-values, the hydrodynamic diameter and zeta potential of nanoparticles was measured during titration of the dispersion in acidic and basic region using the Zetasizer Nano HPPSv420 (Malvern Instruments, Ltd., Malvern, UK) at 25 °C. 0.1 M HNO_3 and 0.1 M NaOH were used for titration. Moreover, the hydrodynamic diameter and polydispersity index of synthesized AgNPs at different temperatures were measured between 25 and 85 °C every 10 °C. The average of the three measurements at each pH or temperature is reported.

2.5. Antibacterial Assay

To check the influence of different amounts of $K_{Extract/Ag}$ on the antibacterial efficiency of synthesized AgNPs, 40 ml of synthesized AgNPs dispersion was mixed in sterile tubes with a bacterial suspension at a final concentration of 10^8 CFU/ml. Tubes were shake at 37 °C for 10, 20, 30, 40 and 120 min, followed by incubation of 5 µl from each tube without dilution on CASO agar medium for 24 h at 37 °C. The test was repeated for 3 times. *Escherichia coli* cells (AATCC 11229) were used for testing.

3. Results and Discussion

3.1. TEM and DLS Results

TEM images of synthesized AgNPs are presented in Fig. 1. The AgNPs that were not stabilized by extract solution were easily agglomerated and linked to each other and created large particles (Fig. 1-a). In presence of extract solution ($K_{Extract/Ag}$ = 1), the spherical AgNPs (Fig. 1-b) with a size of 3–13 nm and the mean value of 8.3 nm were synthesized.

The effect of various amounts of $K_{Extract/Ag}$ on Z-average and Zeta potential of AgNPs is demonstrated in Table 2. The zeta potential of AgNPs is sufficiently high (−37 to -32 mV) for electrostatic stabilization [30]. Moreover, an increase of the extract concentration resulted in smaller AgNPs. These trends can be interpreted with the role of the extract component as a stabilizer. Increases of the stabilizer concentration are also expected to control the nanoparticles growth more reliable which results in smaller nanoparticles. Moreover, the extract concentration influences the polydispersity index of synthesized AgNPs. The polydispersity index is dimensionless and values larger than 0.7 indicating that the sample has a very broad particle size distribution [28]. The lowest extract concentration of pomegranate reveals in the lowest polydispersity index (0.25). However, all stabilized AgNPs revealed a polydispersity index value lower than 0.7, indicating a narrow particle size distribution.

3.2. UV–Vis Spectroscopy Results

An UV–Vis absorption spectrum of the prepared sample without stabilizer is presented in Fig. 2. After 30 min, a single extinction peak is centered at 387 nm, which confirms the formation of spherical shaped AgNPs [28, 31]. After 1 day, the peak disappeared because of instability of AgNPs in the solution while the UV–Vis absorption spectrum of AgNPs was prepared in presence of extract solution did not change even after 7 days (Fig. 3). The UV–Vis absorption spectrum for AgNPs in dispersion with $K_{Extract/Ag}$ = 0.1 (Fig. 3-a) was relatively stable, but less compared to $K_{Extract/Ag}$ = 1 (Fig. 3-b). It can be concluded that the extract component acts as a stabilizer and a decrease in extract concentration has resulted in less stability. It seems that $K_{Extract/Ag}$ = 1 is the optimum concentration to have the maximum stability because a further concentration increase toward $K_{Extract/Ag}$ = 10 results in a less stable UV–Vis absorption spectrum (Fig. 3-c). This decrease in the stability could be related to an agglomeration of extract molecules or