Plasmonic Colloidal Au Nanoparticles in DMSO: A Facile Synthesis and Characterisation

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Research Article

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Abstract

We report a new pathway for the synthesis of plasmonic gold nanoparticles (Au NPs) in a bio-compatible medium. A modified room temperature approach based on the standard Turkevich synthesis, using sodium citrate as a reducing and stabilizing agent, results in highly stable “colloidal” suspension of Au NPs in dimethyl sulfoxide (DMSO). The mean NP size of about 15 nm with fairly low size distribution is revealed by scanning electron microscopy. The stability test through UV-vis absorption spectroscopy indicates no sign of aggregation for months. The Au NPs are also characterized by X-ray photoelectron, Raman scattering, and FTIR spectroscopies. The stabilisation mechanism of the Au NPs in DMSO is concluded to be similar to that of NPs synthesized in water. The Au NPs obtained in this work are applicable as SERS substrates, as proved by common analytes. In terms of bio-applications, they do not possess such side effect as pronounced antibacterial activity, based on the tests performed on non-pathogenic Gram-positive or Gram-negative bacteria.

Introduction

Noble metal nanoparticles show remarkable physical and chemical properties, which determine their extensive use in various fields of optics, electronics, biology, and nanomedicine. Especially valuable is their ability to support localized surface plasmonic resonances (LSPRs) – collective oscillations of free charge carriers, excited with the electric field of light [1]. The applications of LSPR include surface-enhanced Raman scattering (SERS) [2] and related optical sensing techniques [3–7], bio-imaging [8], and photothermal approaches in anti-tumor therapy [9]. Numerous methods of synthesis of colloidal NPs have been developed so far [1, 2, 4, 10, 11], with the facile methods of producing bio-compatible NPs being one of the main research focuses in the community [12–14].

Among the most often used syntheses is the one leading to a colloidal system of spherical gold NPs based on a simple reaction reducing auric acid with either sodium citrate or sodium borohydride [15]. Sodium citrate is particularly interesting, as it is cheap and nontoxic and it does not only act as the reducing agent but its negatively charged carboxylates are also responsible for the charge stabilization of the AuNPs [16].

In addition to imparting specific functionalities to nanoparticles, their dispersion in water or any other biocompatible solvent is crucial for their use in biomedical or biological applications. Therefore, water should be the primary solvent used to disperse functionalized nanoparticles. However, in many cases, the ligands used to protect and provide the functionality to nanoparticles, and the corresponding nanomaterials produced are not soluble or dispersible in water. It would thus be highly beneficial to have a range of other biocompatible solvents, dimethyl sulfoxide (DMSO) being one of them. DMSO is a broadly used solvent, in particular in bio-research and medicine [17, 18], but it has been rarely used as a medium for synthesis of colloidal Ag NPs [19], Au NPs [20, 21], or semiconductor NPs [22, 23].
In this work, we obtained a highly stable “colloidal” suspension of Au NPs in dimethyl sulfoxide (DMSO). A modified room temperature approach based on the standard Turkevich synthesis (Turkevich et al. 1951), using sodium citrate as a reducing and stabilizing agent, results in stable NP colloids with a fairly low size distribution. The possible stabilisation mechanism of the Au NP in DMSO is discussed based on the results of X-ray photoemission spectroscopy (XPS) and Fourier-transform infrared (FTIR) measurements. In view of perspective applications, the Au NPs obtained were tested as SERS substrate and regarding their antibacterial activity.

**Materials And Methods**

**Synthesis of Au NPs**

Hydrogen tetrachloroaurate (III) trihydrate (HAuCl$_4$·3H$_2$O, 99.995% trace metals basis), trisodium citrate dihydrate (HOC(COONa)(CH$_2$COONa)$_2$×2H$_2$O, ≥ 99.0%), dimethylsulfoxide (DMSO, melting temperature of -20 °C) were purchased from Sigma-Aldrich. All these chemicals were used as received. All glassware was cleaned with aqua regia (3 : 1 v/v HCl (37%) :HNO$_3$ (65%) solutions) and then rinsed thoroughly with Milli-Q water before use. Caution: aqua regia solutions are dangerous and should be used with extreme care; never store these solutions in closed containers. Milli-Q water (18 MΩ cm, Millipore) was used to prepare all solutions in the experiments. The aqueous solution of HAuCl$_4$ (1 wt%) was prepared and stored at ca. 4°C before use.

Gold nanoparticles were synthesized by citrate reduction of hydrogen tetrachloroaurate (III) trihydrate solution mixed with trisodium citrate dihydrate following the modified Turkevich method (Turkevich et al. 1951). In a typical Au NP synthesis 0.1 mL of 1 M HAuCl$_4$·3H$_2$O was diluted with 10 mL of DMSO and heated until it begins to boil. In order to avoid contamination and evaporation of the solvent during the synthesis, a disposable Petri dish was used to cover the flask. After the HAuCl$_4$ solution reached the boiling point under ambient pressure, a specific volume of HOC(COONa)(CH$_2$COONa)$_2$×2·H$_2$O solution was rapidly injected into the HAuCl$_4$ solution. The molar ratio (MR) of HOC(COONa)(CH$_2$COONa)$_2$×2·H$_2$O to HAuCl$_4$ was the primary factor controlled to achieve the desired particle size. The synthesis was complete when the colour of the suspension no longer changed. Typically, the reaction took 2–5 min depending on the MR. The sample was cooled naturally to room temperature.

**Physical Characterisation**

Optical absorption spectra were recorded from NP solutions in 1 mm wide plastic cuvettes using a StellarNet Silver Nova 25 BWI6 Spectrometer. Scanning electron microscopy (SEM) was performed from NP samples dried from a colloidal solution on a Si substrate using a Tescan Mira 3 MLU. Dynamic light scattering (DLS) measurements were performed with a particle sizer and zeta potential analyzer NanoBrook Omni (Brookhaven Instruments) equipped with a 532 nm laser. XPS measurements were performed with an ESCALAB 250Xi X-ray Photoelectron Spectrometer Microprobe (Thermo Scientific).
equipped with a monochromatic Al Kα (hv = 1486.68 eV) X-ray source. A pass energy of 200 eV was used for survey spectra, 40 eV for Auger spectra, and 20 eV for high-resolution core-level spectra (providing a spectral resolution of 0.6 eV). Spectra deconvolution and quantification were performed using the Avantage Data System (Thermo Scientific). The linearity of the energy scale was calibrated by the positions of the Fermi edge at 0.00 ± 0.05 eV, Au4f7/2 at 83.95 eV, Ag3d5/2 at 368.20 eV, and Cu2p3/2 at 932.60 eV measured on in situ cleaned metal surfaces. To prevent charging, the NP samples were measured using a built-in charge compensation system. Infrared absorption spectra were recorded from NPs dried on double-side polished Si using transmission geometry of a VERTEX 80v FTIR spectrometer (Bruker) equipped with a DLaTGS detector and a KBr beam splitter. Raman spectra were excited with 457, 532, or 671 nm single-longitudinal-mode solid-state lasers, with a power density on the samples of less than 10^3 W/cm^2, in order to preclude any thermal or photo-induced modification of the samples. Dispersion of the spectra was performed using a single-stage spectrometer (MDR-23, LOMO) with a spectral resolution of 6 cm^{-1} (as measured by the peak width of a single crystal Si substrate and the Rayleigh peak). Detection of the spectra was performed with TE-cooled (~ 60°C) CCD detector (Andor iDus 420). At least 4 spots were probed on each sample studied, in order to ensure that the spectra are representative.

**Study Of Antibacterial Activity**

**Bacterial strains and growth conditions**

Three strains of Gram-positive bacteria (Bacillus subtilis IMB B-7445, Bacillus thuringiensis IFBG 800, Corynebacterium glutamicum IFBG B-216) and four strains of Gram-negative bacteria (Agrobacterium tumefaciens GV3101, Escherichia coli DB31, Rhizobium leguminosarum NRRL B-4403, Bradyrhizobium japonicum IMB B-7538) from the collection of microorganisms of the Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, were used for antibacterial assays. The cultivation media for bacterial stains was solid/liquid Luria-Bertani (LB) bacteria growth media (Sambrook & Russell, 2001). Bacterial cultures were prepared by picking a colony from 24-h-old LB plates and placing them into a liquid LB medium. Cultures were grown overnight and continuously shaken at 100 rpm at 27°C (C. glutamicum, A. tumefaciens, R. leguminosarum, B. japonicum) or at 37°C (E. coli, B. subtilis, and B. thuringiensis). The overnight cultures of each bacteria strain were diluted with LB medium to an optical density OD_{600} = 0.1 and used for disk diffusion assay.

**Antibacterial effect of Au NPs**

The antibacterial test was carried using the disk diffusion method (Klančnik et al. 2010; Buziashvili et al., 2020) with some modifications. For this assay, 1 mL of each bacterial suspension (OD_{600} = 0.1) was uniformly spread on the surface of a solid LB medium in a Petri dish (9 cm in diameter). Four sterile disks of filter paper (d = 5 mm, Whatman®, Sigma-Aldrich, USA) were placed on the surface of each plate with bacteria. Stock solution (SS) of synthesized Au NPs was used to prepare several dilutions (1:0, 1:1, 1:2)
using sterile distilled water. Each disk was loaded with 10 µL of samples: different
dilutions/concentrations of Au NPs (1:2SS, 1:1SS, 1SS or 0.25SS, 0.5SS, 1.0SS or 73.5, 147 and 294 µM,
or 14.45, 28.5 and 57 µg/mL), DMSO (as positive control) and sterile distilled H₂O (as negative control).
Petri dishes with bacteria and loaded disks were incubated for 16 h under appropriate cultivation
conditions. The antibacterial effect was evaluated by measuring the diameter of growth inhibition zones
using ImageJ software (https://imagej.nih.gov/ij/). At least three replicas of each Au NPs concentration
and positive/negative controls were performed.

**Statistical analysis**

The reliability of the results was confirmed with the one-way analysis of variance (ANOVA) test.
Significant differences among means were considered at P-value of $\leq 0.05$ level. Statistical data
processing was performed with the use of Microsoft Office Excel 2010 software.

**Results And Discussion**

As-synthesised Au NPs were first characterised with UV-vis absorption spectroscopy because it is the
most convenient and informative tool to prove the formation of the particles with LSPR [1]. The observed
distinct absorption band at about 518 nm (Fig. 1,a) is characteristic of the LSPR absorption in Au NPs [1],
however, it does not provide a precise estimate of the size of NPs formed. Therefore, a direct method of
size determination was applied, namely scanning electron microscopy (SEM). A representative SEM
image of the samples is shown and prove the formation of nearly spherical NPs with a moderate size
dispersion and an average diameter of 15 ± 5 nm (Fig. 1,b).

Besides the size of the inorganic (metallic) part of the colloidal NPs, it is important to have information
about the molecular layer on their surface, which provides the colloidal stability of the NPs and ligands
and determines their functionality. The method of dynamic light scattering (DLS) delivers the
hydrodynamic size of the NPs, which includes the shell of the ligand bound to the NP surface. The
obtained mean hydrodynamic diameter of our Au NPs, about 30 nm (Fig. 1,c), is notably larger than the
size of the inorganic (metallic) part determined with SEM. Such a large discrepancy can hardly be
explained by the ligand shell alone but it is most likely due to the formation of aggregates of NPs of the
smallest size.

The colloidal stability of the obtained NPs was assessed by storing the suspensions in preset
temperature and humidity conditions for five months. The solution of Au in DMSO, similarly to Au NPs in
water, is a typical electrostatically stabilized colloid so that the electrostatic component is determinant in
securing the stability of the colloid towards aggregation and precipitation. In the literature, Au NPs
synthesized following the Turkevich method are referred to as “citrate-stabilized”. We adhere to this
terminology, although we would like to note that this is a valid terminology as long as the electrostatic
stabilization is taken into account. Due to the fact that the citrate anion acts as both a reducing agent
and stabilizer, its concentration is a critical parameter that affects the reduction rate of the ions and the
growth rate of the NPs. With the citrate used as a reducing agent, the formation of Au NPs is expected to occur by the following reaction:

\[ 2 \cdot \text{HAuCl}_4 + 3 \cdot \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \rightarrow 2 \cdot \text{Au} + 3 \cdot \text{Na}_2\text{C}_5\text{H}_4\text{O}_5 + 3 \cdot \text{CO}_2 + 3 \cdot \text{NaCl} + 5 \cdot \text{HCl} \quad (1) \]

In addition, as a result of the reduction reaction in solution oxidation products may be formed such as \( \text{Na}_2\text{C}_5\text{H}_2\text{O}_4 \) or \( \text{Na}_2\text{C}_5\text{H}_2\text{O}_4 \). However, it should be noted that all these reaction products, except carbon dioxide, can act as a reducing agent of chloroauric acid, so it is difficult to determine at what stage the process stops, and what is the ratio of these components in the final solution.

Citrate is an important stabilizing, reducing, and complexing agent in the wet chemical synthesis of noble metal nanoparticles and other metals [19, 24]. Despite a particularly large number of investigations focused on Cit-Au NPs, the structural details of citrate anions adsorbed on the Au NP surface are still being investigated and discussed [24–26]. Au-citrate coordination in the liquid is supposed to occur mostly bidentate and is simply controlled by its protonation state [16]. In Ref. [24] it was found that, contrary to the common view, the species adsorbed on Ag NPs are, in large part, products of citrate decomposition comprising an alcohol group and one or two carboxylates bound to the surface Ag, and minor unbound carboxylate group. These products may also be mixtures of citrate with lower molecular weight anions. No ketone groups were specified, and very minor surface Ag(I) and Fe (mainly, ferric oxyhydroxides) species were detected. Moreover, the content of adsorbates varied with Ag NPs size and shape. Several forms of citrate bonded to the Ag surface through carboxylate groups were proposed mainly on the base of surface-enhanced Raman scattering (SERS) and DFT simulation [24]. A very informative method regarding the NP-ligand systems is X-ray photoelectron spectroscopy (XPS)[24–28].

The challenge with obtaining XPS data on colloidal Au NPs is twofold. First, as any colloidal NP sample deposited and dried on the substrate for the measurement, it may be undergoing charging during the measurement. The reason is a poor electrical contact between the individual NPs and the substrate, resulting in an uncompensated positive charge in the NPs due to loss of photoelectrons. Secondly, the most convenient substrate for the XPS measurements of colloidal samples is a thin gold film on top of a Si wafer or any other conductive enough material. Gold is preferred because it is chemically inert. Therefore, it does not get oxidised noticeably and does not react with deposited molecules (except sulphur). For measurement of Au NPs, however, the contribution of the substrate overlaps with that of the NPs themselves, requiring additional measurement, with other substrates. Therefore, in our study we performed XPS measurements of Au NPs on Au and Si/SiO\(_2\) substrates. The former is more reliable for avoiding the charging effects, while the latter allows the quantification of Au content in the sample. Although the oxygen from SiO\(_2\) can interfere with the citrate oxygen peaks, this problem can be easily circumvented simply by picking up for analysis only spectra without Si contributions (meaning that there is no contribution of the SiO\(_2\) substrate to the O1s range as well).

The survey XPS spectra of the Au NPs on Au and Si substrates reveal only the elements expected for the samples (Fig. 2,a): Au, C, O, Na (from sodium citrate), Si (from Si substrate), S (DMSO residuals), N (minor
surface contamination from air). The C:O:Na ratio obtained from the survey XPS spectra is 2.5:2.5:1 on Au substrate and 2.6:3:1 on Si.

The obtained value on Si is in perfect agreement with the C:O:Na ratio expected for Na-citrate based on its formula C$_6$H$_5$O$_7$·3Na – 2.6:3:1. Apparently, Na-citrate can be considered as the main stabilizer of the Au NPs synthesized in DMSO. Further details on the molecular species and the mechanism of stabilization of Au NPs can be gained from the high-resolution XPS spectra. The spectrum of carbon (Fig. 2,b), reveals three components that can all be attributed to the carbon atoms in the Na-citrate molecule (inset). The ratio of the components, C$_{285}$:C$_{286}$:C$_{288}$ = 4:1:4, deviates, however, from the one expected for this molecule, 2:1:3, indicating that a minor part of the stabilizer molecules in our samples are products of citrate decomposition, in agreement with previous results on Au NPs stabilized by citrate in water [24].

The commonly characterized core-level spectrum of Au is the 4f doublet, with its 4f$_{7/2}$ component BE at 84 eV for bulk samples [29, 30]. For Au NPs and other types of nanostructures, this characteristic XPS feature was reported both at much higher and much lower BEs. The higher binding energies are related to (contribution of ) oxidized Au species on the surface, Au$^+$, and Au$^{3+}$ [28]. The lower BEs are usually explained by charge transfer from the ligands, substrate, or other environments [31]. Alternatively, the negative core level shift for the surface atoms of bulk Au is explained by an initial state effect due to 6s→5d charge reorganization for less coordinated surface atoms (bulk configuration 5d$^{9.6}$6s$^{1.4}$) [30]. The Au4f$_{7/2}$ peak position of 83.2 eV (Fig. 3,a) indicates that the latter mechanism can be involved. Nevertheless, the charge transfer with the stabilizer molecules cannot be not excluded at the moment.

The spectra measured on Au and Si coincide well for all four elements (Fig. 3), indicating that the continuous area of the NP film was probed, without a noticeable contribution of the substrate, and, on the other hand, the film was thin enough to avoid charging.

(a) (b) (c) (d)

**Figure 3.** High-resolution XPS spectra of Au NPs on Au and Si substrates: Au4f (a), C1s (b), O1s (c), S2p (d).

IR absorption spectra of two Au-cit-DMSO NP samples are shown in Fig. 4.

Typically, the carboxylate group exhibits an asymmetric and a symmetric stretching vibration around 1500–1630 and 1305–1415 cm$^{-1}$, respectively [25, 32]. The contribution of a broad band at 1635 cm$^{-1}$ due to water-bending vibration can be identified in the spectral range of interest by its disappearance during drying of the sample [25]. The IR absorption spectra (acquired in ATR geometry) of purified Cit-AuNPs studied in Ref. [25] showed three distinct peaks, the asymmetric COO$^-$ stretching vibrations, $v_{\text{asy}}$(COO$^-$), at 1611, 1593, and 1558 cm$^{-1}$, and three other peaks assigned to symmetric COO$^-$ stretching vibrations, $v_{\text{sym}}$(COO$^-$), at 1405, 1394, and 1370 cm$^{-1}$. From comparison with the spectrum of pure trisodium citrate (Na$_3$Cit) possessing the $v$(COO$^-$) at 1575 and 1385 cm$^{-1}$ the authors of Ref. [25]
concluded three different configurations of the adsorbed citrate molecule on the Au NPs. The band at 1400 cm$^{-1}$, the same we observe (Fig. 4), was established in Ref. [25] to be related with free citrate molecules, in particular for those with intramolecular hydrogen bonding (of their carboxylate group) with the hydroxyl group (of another citrate molecule). Therefore, we can assume a multilayer aggregation of the citrate molecules around the Au NPs, which partially explains the deviation between the NP size derived from SEM and DLS. However, a peak around 1700 cm$^{-1}$, which is a spectral indication of a hydrogen bonding between free molecules and those bound to the NP surface [25], is not observed in our case.

The spectrum of “bulk” citrate recorded in Ref. [33] exhibited bands at 1079, 1157, 1194, 1269, 1279, 1399, and 1591 cm$^{-1}$. For citrate bound to Au NPs in Ref. [33] the latter two features shifted to 1382 and 1638 cm$^{-1}$ and notably weakened in intensity, while stronger peaks at 1261 cm$^{-1}$ (sharp), 1098 cm$^{-1}$ (broad), and 804 cm$^{-1}$ (sharp) appeared. A rough estimation performed in Ref. [33] for 24 nm Au NP gave 3000 citrate molecules per NP after purification, which corresponds to only half surface coverage. For the (not purified) Au NP samples, which contained an excess of citrate, an intermediate situation was observed — the latter three peaks were present along with peaks at 1399 and 1591 cm$^{-1}$ [33]. This spectral pattern is closer to that observed in our work for NPs in DMSO (Fig. 4), compared to other works [25, 32], if we assume the correspondence between the peaks at 840 cm$^{-1}$, 1137 cm$^{-1}$, and 1260 cm$^{-1}$ in our work with 804 cm$^{-1}$, 1098 cm$^{-1}$, and 1261 cm$^{-1}$ observed in [33]. The latter differences in the spectra of Au NP synthesized in DMSO compared to those reported in the literature for citrate-stabilized Au NPs in water can be caused by the effect of the solvent on the stabilization of the NPs by citrate molecules. In Ref. [25] it was shown that the pH of the solution can have a significant effect on the citrate adsorption geometry on the Au NP surface. From the XPS spectra, we can conclude that a small quantity of DMSO molecules remains in the sample after drying the solution on the substrate. At a closer look at the 1137 cm$^{-1}$ peak in our spectra, it contains shoulders at 1083 and 1112 cm$^{-1}$ (Fig. 4). Therefore, the three vibrations in this range can be due to the contribution of free and differently bound citrate molecules.

As one of the main applications of Au NPs is surface-enhanced Raman scattering of low quantities of organic molecules [1] and inorganic nanostructures [34], we performed a preliminary test of the new synthesized NPs as a SERS substrate for detection of a low concentration of popular analytes. In particular, we observed enhancement for crystal violet (Fig. 5,a) and mercapto benzoic acid (Fig. 5,b) molecules mixed in a solution with colloidal Au NPs and dried on the Si substrate. Note that the fact of enhancement in case of MBA can be inferred based on an order-of-magnitude stronger intensity of the substrate (Si) peak, indicating that much smaller molecular coverage in the case of MBA with Au NPs gives a comparable signal intensity to the thick pure MBA film.

Since the synthesized Au NPs will be used for further development of a SERS-nanoplatform for the detection of different bacterial species and microbial contaminants, it is important to establish the presence or absence of possible side effects, namely the toxic effect of Au NPs on certain bacteria. For this an antibacterial activity of Au NPs against seven different Gram-positive ($B. subtilis$, $B. thuringiensis$, $B. subtilis$, $B. thuringiensis$,
C. glutamicum) and Gram-negative (A. tumefaciens, E. coli, R. leguminosarum, B. japonicum) bacteria strains was studied using the disk diffusion method. Even though this method is not appropriate for the identification of the minimum inhibitory concentration (MIC) of the substance, it allows quick and accurate estimation of antibacterial activity of preparations on multiple bacterial specimens (Balouiri et al., 2016).

The results of the disk diffusion assay show that Au NPs do not affect Gram-positive B. subtilis and B. thuringiensis, and slightly inhibit the growth of A. tumefaciens GV3101, E. coli DB31, R. leguminosarum NRRL B-4403, and B. japonicum IMB B-7538 compared with the positive control (DMSO) (Fig. 6, Fig. 7 shows Au NPs effects on two species only). Thus, it was found that for the Gram-positive bacterium C. glutamicum and all tested Gram-negative species the growth inhibition zones for Au NPs ranged from 6.8 to 7.4 mm in diameter, whereas for DMSO the growth inhibition zones were within 6.4–6.7 mm (Fig. 6, Fig. 7).

It is known that the antibacterial properties of Au NPs mainly depend on their concentration, size, shape, surface modifications, presence of impurities (Au⁺ and Au³⁺ ions, reducing reagents or surface stabilizers), and tested microorganisms. The choice of bacteria used in our research was due to the fact that they are representatives of the ecosystem, and are also used in various research and biotechnological developments: industrial species used for the synthesis of aminoacids and enzymes (B. subtilis, C. glutamicum) (Martinez, 2013; Pavkov-Keller et al., 2011), B. thuringiensis producing insecticide and nematicide Bt-proteins, the product of expression of cry genes (Ibrahim et al., 2010), symbiotic nitrogen fixing bacteria (R. leguminosarum, B. japonicum) (Beeckmans & Xie, 2015; Muimba-Kankolongo, 2018), and model bacteria A. tumefaciens and E. coli frequently used in biotechnology and molecular biology. Moreover, B. subtilis and B. thuringiensis are components of soil microbiome (Ibrahim et al., 2010; Martinez, 2013), A. tumefaciens is a plant pathogenic bacterium, R. leguminosarum and B. japonicum are nitrogen fixing symbiotic bacteria important for plant growth and soil remediation (Beeckmans & Xie, 2015; Muimba-Kankolongo, 2018).

In our study, the difference found in sensitivity to Au NPs between Gram-positive and Gram-negative bacteria could be due to the structure of their cell walls and because of their species (Pavkov-Keller et al., 2011). In general, the results obtained are in agreement with the data of other similar researches. Most of the studies report the low antibacterial properties (about 10 times lower than that of silver NPs) of naked Au NPs or functionalized with inert chemical compounds such as polyvinyl pyrrolidone (PVP) (Zhang et al., 2015; Tao, 2018; Chen et al., 2020). At the same time, there is a report that shows the antibacterial activity of Au NPs against multiple drug resistant (MDR) bacteria such as bacteria of the «ESKAPE» group (Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter sp.), which are the most common nosocomial pathogens (Okkeh et al., 2021). Such Au NPs were produced for possible complex therapy against MRD bacteria, for the detection of markers of different diseases, etc.
Thus, here we show for the first time that Au NPs show little or no activity towards bacteria such as *R. leguminosarum, B. japonicum, B. subtilis, C. glutamicum, B. thuringiensis, A. tumefaciens*. In the previous works, the antimicrobial activity of only Ag NPs or NPs of metal oxides (ZnO, TiO$_2$, MgO, etc.) on soil microbiome was thoroughly studied in the context of soil contamination (Grün et al., 2019; Tian et al., 2019).

In addition, surface stabilizers are another key factor that determines the antibacterial activity of Au NPs. Despite the favorable chemical properties of dimethyl sulfoxide (DMSO), this reagent is used as a surface stabilizer of NPs including Au-based NPs (Maksymovych et al., 2010; Feng et al., 2015; Shoua et al., 2018). In our work, DMSO was used also for Au NPs synthesis as a stabilizing agent. Recently it was shown that DMSO influences different cellular processes such as apoptosis, autophagy, cell cycle, differentiation, lipid metabolism, etc. (Tunçer et al., 2018). At high concentrations, it enhances cell permeability causing deformation of membranes and disrupting their integrity (Gironi et al., 2020). Numerous studies are devoted to the influence of different concentrations of DMSO on the growth of bacteria cultures. For example, in a work of Wadhwani et al. (2008) it was shown that low DMSO concentrations (1–3%) do not affect significantly the growth of 5 different bacterial species (*S. epidermidis, P. oleovorans, Vibrio cholerae, Shigella flexneri, S. paratyphi*); the level of bacterial growth was at 93–97% compared to that of the control. Recently, Dyrd et al. (2019) showed that the treatment with 20% DMSO resulted in the absolute loss of viability of the cells of *E. coli, B. subtilis*, and *Saccharomyces cerevisiae*. In our study, the treatment with 100% DMSO lead to insignificant growth inhibition of *C. glutamicum, A. tumefaciens, E. coli, R. leguminosarum, B. japonicum* only, both *Bacillus* species, *B. subtilis* and *B. thuringiensis* were insensitive to DMSO.

Summarizing from the data obtained we can conclude that the AuNPs produced by us do not possess such side effect as pronounced antibacterial activity on the tested non-pathogenic Gram-positive or Gram-negative bacteria as *B. subtilis* and *B. thuringiensis, C. glutamicum, A. tumefaciens, E. coli, R. leguminosarum* and *B. japonicum*.

**Conclusions**

A new method of aqueous synthesis of plasmonic Au NPs was developed. This room temperature approach is based on the classical Turkevich synthesis, using sodium citrate as a reducing and stabilizing agent, resulting in highly stable “colloidal” suspensions of Au NPs in dimethyl sulfoxide (DMSO). The mean NP size of about 15 nm with fairly low size distribution is revealed by scanning electron microscopy. The stability test through UV-vis absorption spectroscopy indicates no sign of aggregation for months. Based on the spectral data of X-ray photoelectron and FTIR spectroscopies, the stabilisation mechanism of the Au NP in DMSO is concluded to be similar to that of NPs synthesized in water. The tiny residuals of DMSO in dried samples (detected by XPS) do not hinder the detection of organic analytes by SERS. The Au NPs do not possess such side effect as pronounced antibacterial activity, based on the tests performed on non-pathogenic Gram-positive or Gram-negative bacteria as *B. subtilis* and *B. thuringiensis, C. glutamicum, A. tumefaciens, E. coli, R. leguminosarum* and *B. japonicum*. 
Declarations

Author Contributions

SP, OK, OY - Methodology, Investigation, Data Curation; AB - Investigation, Writing; YP and VD - Project administration, Funding acquisition, Review; AY and VY - Conceptualisation, Editing, Critical Remarks; DRTZ – writing, review, and editing. All authors have approved the final manuscript version.

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Conflicts of Interest

The authors declare that they have no competing interests.

References


Figures

Figure 1

UV-vis absorption spectrum of Au NPs in DMSO (a), SEM image (b), and DLS size distribution (c).
Figure 2

(a) Survey XPS spectra of the Au NPs on Au and Si substrates (normalized to Au peak intensity). (b) High-resolution XPS spectrum of carbon (C1s), with deconvolution into components. The inset shows the schematic of the Na-citrate molecule and the expected contribution of bonds to the XPS C1s spectrum.
Figure 3

High-resolution XPS spectra of Au NPs on Au and Si substrates: Au4f (a), C1s (b), O1s (c), S2p (d).
Figure 4

Infrared transmission spectrum of Au NPs.

Figure 5

(a) $\lambda_{\text{exc}} = 457\text{ nm}$

10$^{-6}$ M CV on glass

10$^{-6}$ M CV @ AuNPs

(b) $\lambda_{\text{exc}} = 457\text{ nm}$

AuNPs:MBA = 1:1

MBA @ AuNPs

Si
An illustration of the plasmon-enhanced Raman spectra (SERS) of crystal violet (a) and mercapto benzoic acid (b) by Au NPs synthesized in this work.

![Graph showing antibacterial effect of different concentrations of Au NPs on various bacteria](image)

**Figure 6**

Antibacterial effect of different concentrations of Au NPs on *B. subtilis, B. thuringiensis, C. glutamicum, A. tumefaciens, E. coli, R. leguminosarum, B. japonicum* detected by the disk diffusion assay.
Figure 7

Zones of inhibition assay for antimicrobial activity of DMSO and Au NPs (stock solution) for Gram-positive (*B. thuringiensis*) and Gram-negative (*E. coli*) bacteria.