J Mater Sci (2022) 57:8481–8488

Advanced Nanomaterials

Green preparation and characterization of silver nanocolloids used as antibacterial material in soap

M. Nowak[1], A. Tolińska[1], L. Marciniak[1], M. Skrobańska[1], B. Tylkowski[2], M. Frankowski[1], M. T. Kaczmarek[1], and R. Jastrzab[1,*]

[1] Faculty of Chemistry, Adam Mickiewicz University, Uniwersytetu Poznanskiego 8, 61-614 Poznan, Poland
[2] Centre Tecnològic de La Química de Catalunya, Carrer de Marcelli Domingo, 43007 Tarragona, Spain

Received: 3 August 2021
Accepted: 30 December 2021
Published online: 14 January 2022

ABSTRACT

This study aimed to assess the characteristics, including morphology, physicochemical properties, and antibacterial properties, of silver nanocolloids obtained by D-glucose reduction. Silver nanoparticles were synthesized in accordance with the principles of green chemistry using D-glucose as a reductor. The obtained nanostructures were characterized by UV–vis spectroscopy, transmission electron microscopy, and dynamic light scattering. Stability tests performed after 1 month of storage revealed that the colloids prepared with and without polyvinylpyrrolidone as a stabilizer had the same properties. Distribution of the nanoparticles was tested using inductively coupled plasma mass spectrometry by doping the silver colloids into a natural soap mass. The antibacterial activity of the soap containing silver nanoparticles was tested on dirty hands. The antibacterial activity test demonstrated that the novel green soap materials improved with D-glucose-reduced silver nanoparticles possessed better antibacterial properties than a pure soap, and thus, they could be recommended for quotidian use by dermatological patients.

Introduction

Metal nanoparticles are known to exhibit unique physical, chemical, and biological properties [1–7]. The nanosize of particles determines various parameters such as melting point, optical properties, and thermal, magnetic, and electric conductivities [7–9]. One of the most important advantages of metal nanoparticles is their high area-to-volume ratio. The smaller the diameter of nanoparticles, the more developed is the surface area, which directly influences the reactivity of nanomaterials, their adsorption properties, and antimicrobial activity [5, 10–14]. Silver nanoparticles have been characterized with the strongest antimicrobial activity compared to other nanomaterials [14–16]. Moreover, silver exerts lower toxicity on human cells compared to other metals and

Handling Editor: M. Grant Norton.

Address correspondence to E-mail: renatad@amu.edu.pl

https://doi.org/10.1007/s10853-021-06863-5

is thus considered ideal for the production of antibacterial materials. Indeed, nanosilver has been used as a cover to protect heat-susceptible materials during processes involving high temperatures (e.g., sterilization). A very significant finding is that a wide range of bacteria cannot develop resistance to metal nanoparticles, unlike observed in the case of antibiotics upon prolonged use [12, 17, 18]. It has also been shown that the antibacterial activity of nanoparticles is determined by their size and shape, i.e., the larger the particles, the weaker the antibacterial activity. Nanoparticles in the range of 5–10 nm show the strongest interaction with bacteria [11, 14].

Normal healthy human skin is colonized by numerous microorganisms. The total number of bacterial cells on a human hand ranges from 3.9×10^4 to 4.6×10^6 colony-forming units/cm^2. Bacteria isolated from human hands can be classified into two categories: resident and transient. *Staphylococcus epidermidis*, *Staphylococcus hominis*, and other coagulase-negative staphylococci, as well as coryneform bacteria (propionibacteria, corynebacteria, dermobacteria, and micrococci), are dominant among the resident flora [19–21]. The most common fungus of resident hand skin flora is *Pityrosporum* (Malassezia). Transient flora, on the other hand, results from the contamination of human hands and is responsible for the spread of infectious diseases. Handwashing is the single most effective method for reducing unwanted growth of transitional flora. However, the time of washing is critical. The minimum time for effective hand washing that removes a significant amount of bacteria is 30 s [19–24].

The size of nanoparticles can be determined from the color change of their colloids, which can be related to the changes in the optical properties of the particles. It has been reported that gold nanoparticles of red color have a diameter of about 20 nm, while those of orange color are about 80 nm in size [3, 4]. Due to their large surface energy in comparison with macroscopic materials, colloids are unstable and tend to aggregate. To prevent this, they are coated with protective polymers such as polyvinylpyrrolidone (PVP) or ethylene glycol. These polymers also have a positive impact on the formation of nanoparticles. Nanoparticles with sharp edges exhibit higher activity than round-shaped particles [12–15]. Due to their antibacterial and antifungicidal properties, silver nanoparticles could be used in natural cosmetics. In addition, they are chemically stable and can be produced at a low cost. Furthermore, sugars such as glucose, sucrose, fructose, and lactose, which are nontoxic and cheap, could be used in the green synthesis of silver nanoparticles [25–28].

The present study aimed to provide answers to the following questions: Is it possible to obtain natural soap with broad antibacterial and antifungal properties? We assume that a combination of silver nanoparticles and natural soap will be characterized by a complete bactericidal effect, especially against a mixture of bacteria [25, 26]. This paper presents the results of characterization of silver nanocolloids obtained by D-glucose reduction, including the observations of their morphology, physicochemical properties, and antibacterial properties.

Materials and methods

Materials

Silver nitrate, D-glucose, and PVP (Mw 10,000 Da) were purchased from Aldrich and used without further purification. NaOH solutions of concentration 0.08 mol/L and 28% (w/w) were prepared using 50% (w/w) sodium hydroxide (Supelco). Ultrahigh purity water obtained with a Milli-Q system (Millipore Co.) was used for the preparation of all aqueous solutions. The concentration of silver and sodium ions in the initial solution was determined by inductively coupled plasma optical emission spectrometry (ICP OES). Coconut oil, olive oil, shea butter, and castor oil were purchased from OQUEMA Sp. Z o.o. and used without further purification. Yeast extract, peptone (tryptone), glucose, sodium chloride, and agar were purchased from Avantor Performance Materials Poland S.A. and also used without further purification.

Methods

"Green" synthesis of silver nanoparticles

To prepare silver colloid, a solution containing 30.00 mL of 0.001 mol/L $AgNO_3$ and 3.60 mL of 0.012 M D-glucose with or without 0.14 g of PVP was heated up to 85 °C, followed by the addition of 300.00 µL of 0.08 M NaOH [29]. Heating was continued for 30 min, and then, the colloid was stirred for an additional 1 h to allow it to cool to room temperature. All glassware used for colloid synthesis was

previously cleaned with a piranha solution. The prepared colloid was stored at room temperature in an amber glass bottle to protect it from light.

Natural soap synthesis

Natural soap was prepared by applying a low-temperature saponification method (heating to 48 °C). High-quality vegetable raw materials such as coconut oil, olive oil, shea butter, and castor oil were mixed and 28% (v/v) NaOH solution was added to 100.00 g of the prepared fat phase. About 5% of undeprived fat was left in the soap mass. The obtained natural soap was incubated at 42 °C for 12 h and then allowed to mature and dry at room temperature for 8 weeks.

UV–Vis characterization

The spectra were recorded at 20 °C in a quartz cell with 1-cm path length using an Evolution 300 UV–VIS Thermo Fisher Scientific spectrometer equipped with a xenon lamp (range 190–1100 nm, accuracy 0.2 nm, sweep rate 120 nm/min). For UV–vis characterization, the concentration of Ag nanoparticles was reduced twice to reach 0.05 mol/L.

Transmission electron microscopy (TEM) characterization

The size and morphology of the prepared silver colloids were determined by TEM using Jeol 2010 transmission electron microscope operating at 200 kV. For TEM characterization, the concentration of Ag nanoparticles was reduced hundred times to reach 0.001 mol/L.

Dynamic light scattering (DLS) characterization

DLS analysis was performed using a Micromeritics Instruments NanoPlus DLS system (wavelength range 0.1 nm to 12 µm) in glass cuvettes with 1-cm optical path. The synthesized Ag nanoparticles were used as such without modifying their concentration (0.1 mol/L).

Inductively coupled plasma mass spectrometry (ICP-MS) characterization

Soap samples (n = 3) weighing approximately 0.5 g (weighted to the nearest 0.1 mg) were placed in a clean Teflon vessel with 65% HNO_3 and 48% HF (Merck, Germany). The samples were mineralized using a Multiwave PRO microwave reaction system equipped with an 8NXF100 acid digestion rotor (Anton Paar, Austria). The content of Ag was estimated using an ICP-MS 2030 spectrometer (Shimadzu, Japan). The measurement conditions and parameters of ICP-MS have been described previously by Koziol et al. [30].

Antibacterial activity test

For the antibacterial activity test, a standard medium was prepared as described in the literature with the following composition: yeast extract (2.50 g/L), peptone (tryptone, 5.00 g/L), glucose (1.00 g/L), sodium chloride (5.00 g/L), and agar (15.00 g/L). The pH of the medium was adjusted to 7 before sterilization [23, 24] by autoclaving (120 °C, 30 min). To prevent contamination, the sterilized medium was poured into Petri dishes in a laminar flow cabinet.

Results

Two types of silver nanoparticles were synthesized using D-glucose as a reducing agent. As illustrated in Fig. 1, one of the colloids was stabilized with a PVP solution (Fig. 1a), which was added at the beginning of the synthesis, before the addition of NaOH solution, while the second colloid (Fig. 1b) was prepared without PVP.

PVP homopolymer contains a polyvinyl skeleton with N and O polar groups which show a strong affinity to silver ions and metal nanoparticles. It has been shown that PVP not only accelerates spontaneous nucleation but also surrounds the formed nanoparticles during colloid synthesis. Thus, it acts as a stabilizer and blocks the aggregation of silver nanoparticles [21, 22]. Figure 2 shows the results of the DLS analysis of the prepared colloids and their TEM micrographs.

TEM micrographs showed that both colloid systems contained well-formed, globe-shaped, and separated silver nanoparticles (Fig. 2). DLS analysis was carried out to analyze the size of the obtained colloids and the size distribution of the nanoparticles. The results showed that silver nanoparticles synthesized with PVP had a diameter ranging between 22 and 184 (± 2) nm. The mean diameter of the nanoparticles was 39 nm, and 90% of the nanoparticles were in the

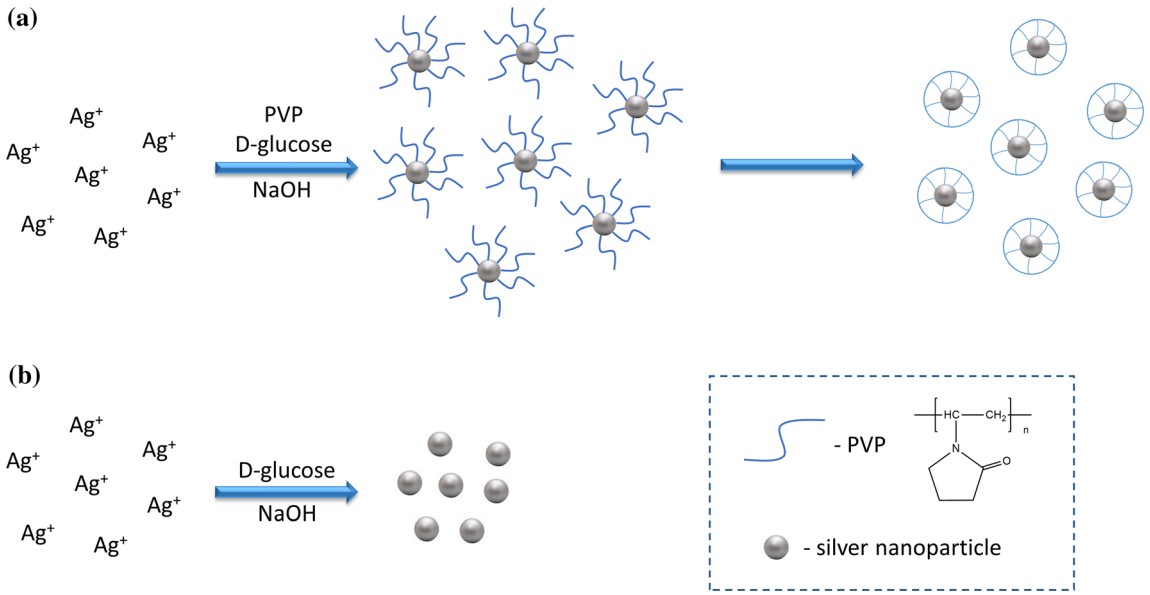

Figure 1 Schematic diagram of colloid synthesis **a** with PVP and **b** without PVP.

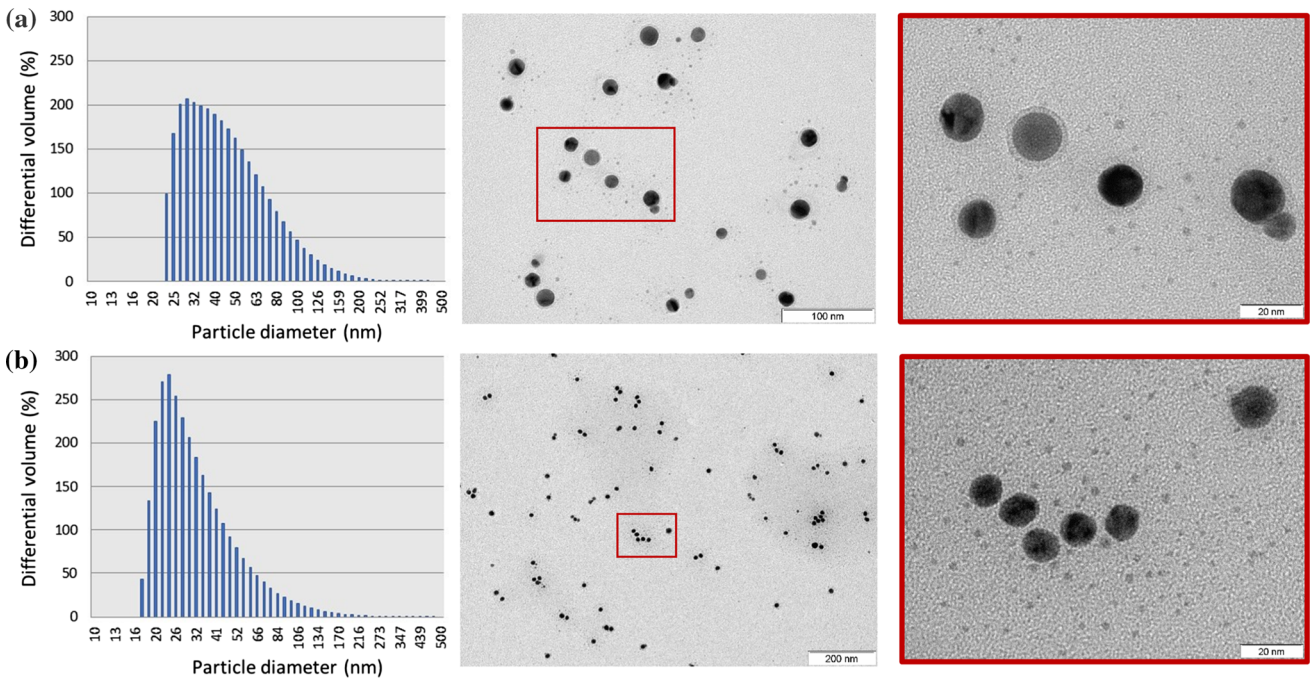

Figure 2 DLS and TEM characterization of the colloids **a** with PVP and **b** without PVP.

range of 25–169 nm. In the case of nonstabilized system, the diameter of the nanoparticles ranged between 18 and 141 nm with a mean value of 29 nm. The size distribution of the nanoparticles in the nonstabilized system appeared narrower, as 90% of the nanoparticles were in the range of 20–128 (± 2) nm. Analyses of TEM images using Image-ProPlus5 software showed that the sizes of the particles observed in the images were in good agreement with the values determined by DLS. The marginally larger size of the PVP-stabilized nanoparticles could be attributed to the presence of the polymer coating. Figure 2a also shows that stabilized nanoparticles are surrounded by a 2-nm PVP coating layer.

The maximum absorption of the obtained silver colloids without and with PVP was 412 and 422 nm,

respectively (Fig. 3). This indicates the monodispersity and structural uniformity of both colloids [16].

The stability of silver colloids prepared with and without PVP did not differ. Thus, in line with the principles of green chemistry, silver colloid prepared without PVP was chosen for use as an antibacterial agent in soaps. In the next step of the investigation, the colloid without PVP was used for the fabrication of the antibacterial soap. The pH of the synthesized colloid was about 5; however, due to the high basicity of the soap mass, the colloid was tested at a high pH value. The UV–Vis spectra of the colloid were obtained at high pH to characterize the behavior of the silver nanoparticles. As shown in Fig. 4, at high pH, the nanoparticles tended to aggregate [31], and thus, during the preparation of silver-doped soap the colloid was rapidly added to the soap mass and mixed continuously with a magnetic stirrer at 1000 rpm to prevent the aggregation of silver nanoparticles.

The recipe of natural soap bar was developed for the purpose of application of silver nanocolloids in cosmetic products. Synthesis was carried out with high-quality materials using a low-temperature method. It was ensured that the ingredients chosen for the recipe had optimal physicochemical properties such as pH, hardness, foaming, and cleaning and were skin-friendly. Furthermore, the procedure applied for the synthesis does not result in waste.

The obtained silver nanocolloids were added to natural soap. (When stirring the soap mass, 3.5 mL of silver colloid per 100 g of fat phase was added exactly at the 8th minute.) The soap was left for 1 month to mature. The ICP-MS analysis of the samples obtained from three different areas of the soap showed that silver nanoparticles were equally distributed in the soap bar (Table 1).

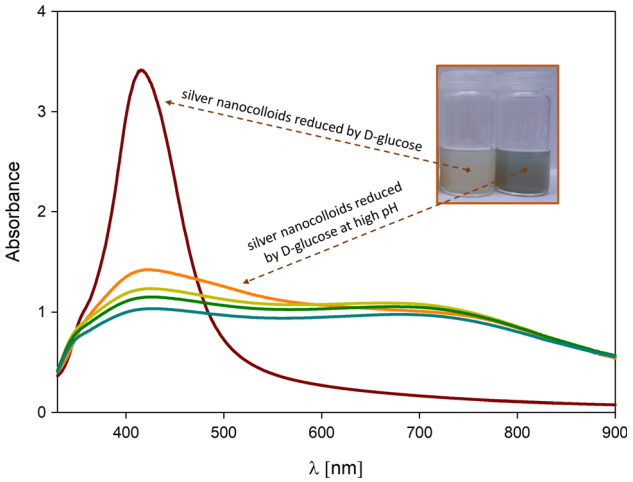

Figure 4 Comparison of UV–Vis extinction spectra of silver colloids at different pH.

The antibacterial properties of the soap with and without silver nanoparticles were tested against bacterial pathogens collected from the palm skin (Fig. 4). The antimicrobial activity of the soap mass with or without silver nanoparticles was also tested. To determine the colonization of palm skin with bacterial and fungal pathogens, four Petri dishes containing the same amount of standard medium handprint were made. On the first Petri dish, a dirty hand palm was

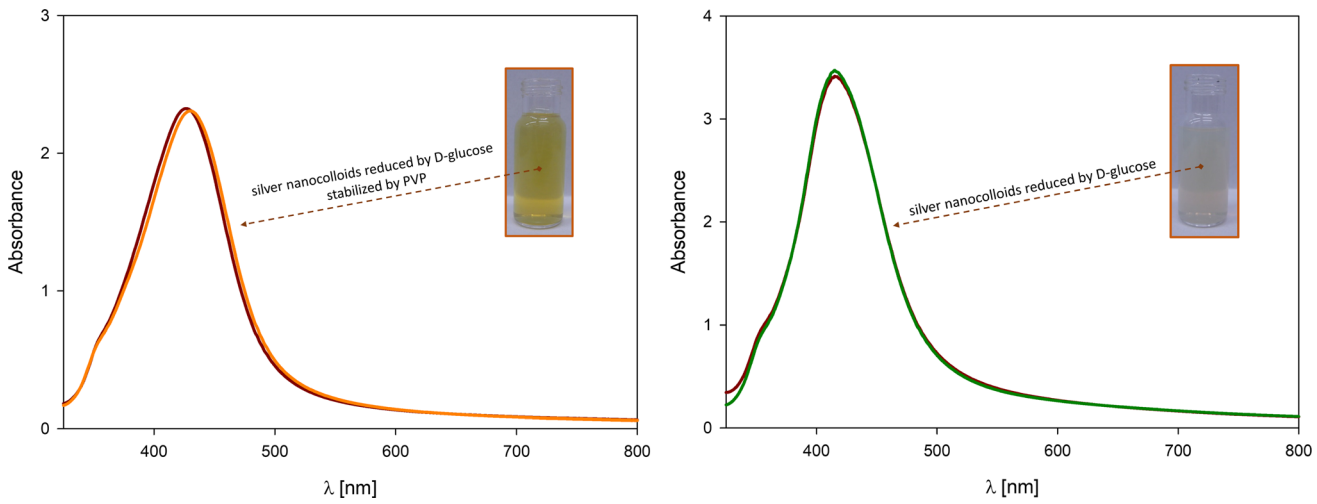

Figure 3 UV–Vis extinction spectra of silver nanocolloids stabilized with PVP (yellow solution; left spectra) and silver nanocolloids without PVP (light yellow solution; right spectra). Black curves—on the day after the synthesis; colorful curves (orange or green)—2 weeks after the synthesis.

Table 1 ICP-MS analysis of soap samples for the presence of silver

Soap with nanosilver	Silver content (μg/g)	Relative standard deviations RSD (%)
Probe 1	1.927	2.01
Probe 2	1.881	1.69
Probe 3	1.653	0.83

handprinted. On the second Petri dish, a dirty hand palm that was washed with a natural soap was handprinted. On the third Petri dish, a dirty hand palm that was washed with a natural soap containing D-glucose-reduced silver colloid was handprinted. The fourth Petri dish containing only standard medium was used as a control. All samples were left for 24 and 48 h, respectively (Fig. 5). After incubation, no changes were observed in the control.

Discussion

The obtained results showed that the presence of PVP polymer in the colloid system did not have any significant impact on the nanoparticle size. Besides, the nonstabilized system also possessed well-separated silver nanoparticles. According to the literature, the range of wavelengths in which the maximum absorbance of silver colloids occurs is 390–420 nm [7, 8, 32, 33]. A detailed analysis of the UV–Vis spectrum allowed determining the quality of the obtained colloid. The spectra with relatively high absorbance obtained for silver colloid without stabilization indicated its slightly smaller size in comparison with colloid with PVP. The process of aggregation of nanocolloids could be observed as a decrease in absorbance, with a shift of λ_{max} toward lower energies and widening of the spectrum [32]. The stability in time of the obtained nanoparticles was controlled at the first step by UV–Vis spectroscopy. After 2 weeks, no changes in λ_{max} and absorbance of spectrum were observed (Fig. 3).

In the Petri dish handprinted after hand washing with the soap containing silver nanoparticles, a significantly lower bacterial growth was found. Silver

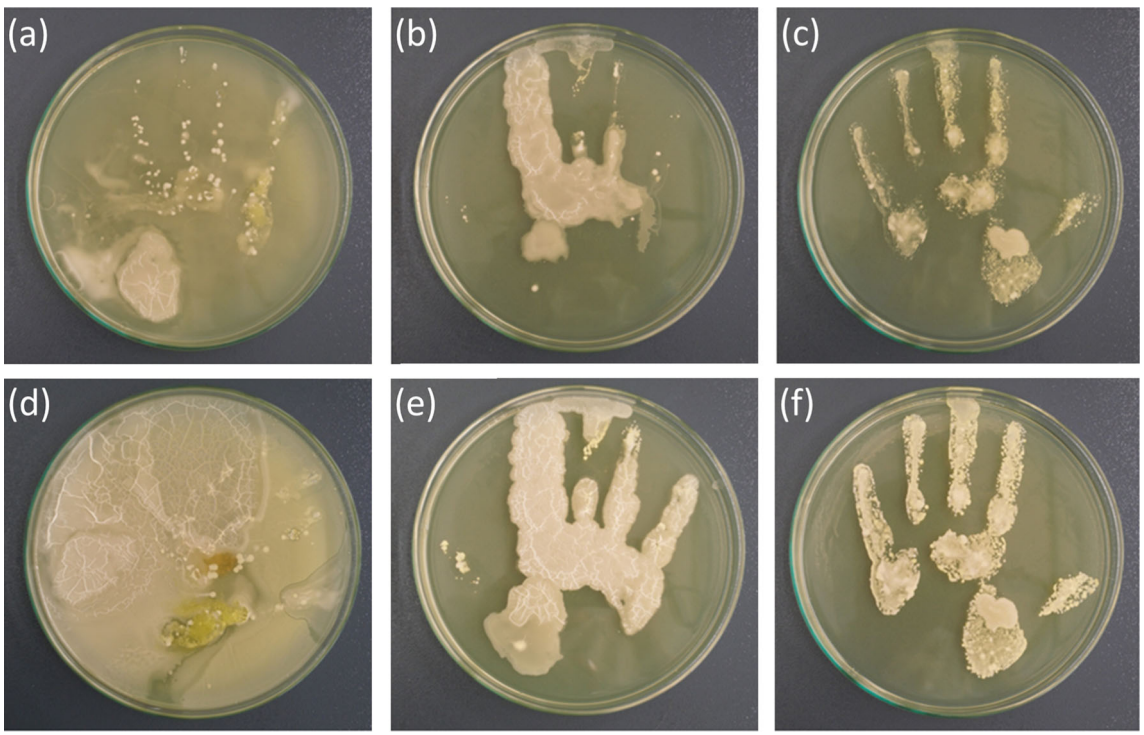

Figure 5 Growth of bacteria and fungi on agar medium: after 24 h—**a** without washing, **b** washing with standard soap, and **c** washing with soap containing nanoparticles; after 48 h—**d** without washing, **e** washing with standard soap, and **f** washing with soap containing nanoparticles.

nanoparticles with a size of about 20 nm can pass through bacterial cell walls, resulting in cell death. This clearly indicates that nanoparticles obtained from natural ingredients have potential application as an antibacterial agent in soaps and products containing these nanoparticles can be recommended to dermatological patients.

Conclusions

Time-stable silver colloids were synthesized by D-glucose reduction of silver nitrate in the presence and absence of PVP. The prepared silver colloids were stable for 1 month and had an average size of 20 nm. In line with the principles of green chemistry, silver colloid obtained without PVP was used as an antibacterial additive for the preparation of soap. The soap mass obtained using natural ingredients was characterized by optimal pH, hardness, frothiness, and washing as well as skin-friendly care properties. It was also found that soaps containing the prepared silver colloids exhibited antibacterial and antifungal properties, as confirmed by less bacterial growth and a reduced number of bacterial colonies in the antibacterial activity test. Thus, the results showed that soaps containing silver colloids obtained by D-glucose reduction, without any harmful chemical additives, can be recommended particularly to people with dermatological problems.

Acknowledgements

We would like to express our gratitude to Center for Advanced Technologies in Poznan for granting permission to perform DLS analysis in their laboratory.

Funding

Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

[1] Sun Y, Xia Y (2012) Shape-controlled synthesis of gold and silver nanoparticles. Science 298:2176–2179

[2] Wang R, Yao Y, Shen M, Wang X (2016) Green synthesis of Au@Ag nanostructures through a seed-mediated method and their application in SERS. Colloids Surf A 492:263–272

[3] Jain PK, Lee KS, El-Sayed IH, El-Sayed MA (2006) Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. J Phys Chem B 110:7238–7248

[4] Zuber A, Purdey M, Schartner E, Forbes C, van der Hoek B, Giles D, Abell A, Monro T, Ebendorff-Heidepriem H (2016) Detection of gold nanoparticles with different sizes using absorption and fluorescence based method. Sensors and Actuators B 227:117–127

[5] Panyala NR, Pena-Mendez EM, Havel J (2008) Silver or silver nanoparticles: a hazardous threat to the environment and human health? J Appl Biomed 6:117–129

[6] Chen D, Qiao X, Qiu X, Chen J (2009) Synthesis and electrical properties of uniform silver nanoparticles for electronic applications. J Mater Sci 44:1076–1081

[7] Das M, Shim KH, An SSA, Yi DK (2011) Review on gold nanoparticles and their applications. Toxicol Environ Health Sci 3:193–205

[8] Shobha G, Vinutha M, Ananda S (2014) Biological synthesis of copper nanoparticles and its impact—a review. Inter J Pharm Sci Invent 3:28–38

[9] Maruyama M, Matsubayashi R, Iwakuro H, Isoda S, Komatsu T (2008) Silver nanosintering: a lead-free alternative to soldering. Appl Phys A 93:467–470

[10] Huang CM, Chen CH, Pornpattananangkul D, Zhang L, Chan M, Hsieh MF, Zhang L (2011) Eradication of drug

resistant Staphylococcus aureus by liposomal oleic acids. Biomaterials 32:214–221

[11] Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB (2009) The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Ag 34:103–110

[12] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16:2346–2353

[13] Verran J, Sandoval G, Allen NS, Edge M, Stratton J (2007) Variables affecting the antibacterial properties of nano and pigmentary titania particles in suspension. Dyes Pigm 73:298–304

[14] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. Nanomedicine 3:95–101

[15] Pal S, Tak YK, Song JM (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gramnegative bacterium Escherichia coli. Appl Environ Microb 73:1712–1720

[16] Agnihotri S, Mukherji S, Mukherji S (2014) Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv 4:3974–3983

[17] Larson EL, Hughes CA, Pyrek JD, Sparks SM, Cagatay EU, Bartkusal JM (1998) Changes in bacterial flora associated with skin damage on hands of health care personnel. Am J Infect Control 26:513–521

[18] Wilson M (2005) Microbial inhabitants of humans: their ecology and role in health and disease. Cambridge University Press, New York, NY

[19] De Angelis G, Lohmeyer FM, Grossi A, Posteraro B, Sanguinetti M (2021) Hand hygiene and facemask use to prevent droplet-transmitted viral diseases during air travel: a systematic literature review. BMC Public Health 21:760

[20] Hay RJ (1993) Fungi and fungal infections of the skin. In: Noble WC (ed) The skin microflora and microbial skin disease. Cambridge University Press, Cambridge, UK, pp 232–263

[21] Rayan GM, Flournoy DJ (1987) Microbiologic flora of human fingernails. J Hand Surg Am 12:605–607

[22] Kokura S, Handa O, Takagi T, Ishikawa T, Naito Y, Yoshikawa T (2010) Silver nanoparticles as a safe preservative for use in cosmetics. Nanomedicine 6:570–574

[23] Lee YL, Cesario T, Lee R, Nothvogel S, Nassar J, Farsad N, Thrupp L (1994) Colonization by *Staphylococcus* species resistant to methicillin or quinolone on hands of medical personnel in a skilled-nursing facility. Am J Infect Control 22:346–351

[24] Borchgrevink CP, Cha JM, Kim SH (2013) Hand washing practices in a college town environment. J Environ Health 75:18–24

[25] Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD (2019) Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. RSC Adv 5:2673–2702

[26] Sharma VK, Yngard RA, Lin Y (2009) Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 145:83–96

[27] Panigrahi S, Kundu S, Ghosh S, Nath S, Pal T (2004) General method of synthesis for metal nanoparticles. J Nanopar Res 6:411–414

[28] Bansod SD, Bawaskar MS, Gade AK, Rai MK (2015) Development of shampoo, soap and ointment formulated by green synthesized silver nanoparticles functionalised with antimicrobial plants oils in veterinary dermatology: treatment and prevention strategies. IET Nanobiotechnol 9:165–171

[29] Osorio-Roman IO, Ortega-Vasquez V, Vargas VC, Aroca RF (2011) Surface-enhanced spectra on D-gluconic acid coated silver nanoparticles. Appl Spectrosc 65:18–23

[30] Koziol K, Uszczyk A, Pawlak F, Frankowski M, Polkowska Ż (2021) Seasonal and spatial differences in metal and metalloid concentrations in the snow cover of hansbreen, Svalbard. Frontiers in Earth Science, vol. 8. Article number 538762

[31] Kvítek O, Siegel J, Hnatowicz V, ŠvorIík V (2013) Noble metal nanostructures influence of structure and environment on their optical properties. J Nanomater 2013:743684

[32] Kneipp K, Kneipp H, Manoharan R, Hanlon EB, Itzkan I, Dasari RR, Feld MS (1998) Extremely large enhancement factors in surface-enhanced raman scattering for molecules on colloidal gold clusters. Appl Spectrosc 52:1493–1497

[33] Suyal G (2003) Bimetallic colloids of silver and copper in thin films: sol-gel synthesis and characterization. Thin Solid Films 426:53–61