

Effect of Surfactants and Polymers on Stability and Antibacterial Activity of Silver Nanoparticles (NPs)

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In this first systematic study, we describe the influence of various surfactants and polymers on aggregation stability and antibacterial activity of silver nanoparticles (NPs) prepared by a modified Tollens process. The surfactant/polymer stabilizing effect was monitored using the newly established method based on a titration of the aqueous dispersion of the silver NPs by aqueous solution of poly(diallyldimethylammonium) chloride (PDDA). The aggregation process was evaluated by the dynamic light scattering (DLS) and UV/vis spectra measurements and finally confirmed by TEM. Among all of the investigated modifiers, two surfactants (sodium dodecyl sulfate-SDS and polyoxyethylenesorbitane monooleate-Tween 80) and one polymer (polyvinylpyrrolidone-PVP 360) exhibit superior stabilization of the silver NP dispersions against the process of aggregation. The differences in the stabilization ability of various tested substances are discussed with respect to their structure and possible mechanism of the surface interaction with the NPs. The antibacterial activity of the modified silver NPs was significantly enhanced especially when modified by SDS where the minimum inhibition concentration (MIC) decreased under the “magical value” of $1 \mu\text{g}\cdot\text{mL}^{-1}$. A correlation was found between the aggregation stability and enhanced antibacterial activity in the system of the silver NPs modified by SDS, Tween 80, and high molecular polymer PVP 360.

Introduction

Silver and its compounds have been used, especially for their antibacterial activity, since the age of ancient Egyptians, and they have been widely used in modern human medicine since the beginning of the 20th century.¹ The invention of penicillin caused a declension of the silver usage in medical applications although development of new bioactive silver compounds has not been suppressed at all. In the 1960s, silver(I) sulfadiazine was introduced and is, at present, the best known and most widely used silver preparative applied in human medicine, the preferred antimicrobial agent for the treatment of serious burns.^{2,3} Silver has also been used for the purpose of antibacterial treatment of surgical instruments (e.g., catheters) and surface modification of endoprosthesis and heart valves.⁴ The end of the 20th century brought an abrupt increase in the bacterial resistance against antibiotics⁵ and consequentially meant at least a partial return toward the usage of silver and its antibacterial properties.

Ionic silver inhibits bacterial growth already at lower concentrations equal to approximately 35 ppb, and at once, it has proven to have significantly low cytotoxicity.⁶ However, the overdose usage of all the forms of silver can lead to complications generally known as argyria.⁷ In comparison to the antibiotics, the bacterial resistance against ionic silver has been observed only rarely and does not constitute any significant complication.^{2,8} Additionally, in the case of silver nanoparticle

(NP) usage as an antibacterial agent, the bacterial resistance has not been observed up to the present moment. This fact is supposedly caused by a difference in the mechanism of the antibacterial actions of the diverse forms of silver.⁹ The fact of the nonexistent bacteria resistance against the destructive effects of the silver NPs, that are observed already at low concentrations (units of milligrams per liter), lead to a recent rapid development in the field of synthesis of the silver NPs conveying the antibacterial activity.^{10–15} The performed research has proven that antibacterial activity of the silver NPs is dependent not only on their size^{10–12} but also on their shape.¹⁴ The antibacterially active silver NPs have been currently applied as disinfecting agents in general practice, for example, for the antibacterial modification of textile materials.¹⁶

The fundamental application problem of silver NPs is connected with the sufficient stability of their dispersions allowing the prevention of the aggregation process because the generation of spacious aggregates leads to a loss of the antibacterial activity.^{15,17} Therefore various surfactants and polymers are commonly applied to stabilize these metal colloids. Although the stability is considered to be a crucial property of the silver NP dispersions, there is still lack of studies employing its tests.

The enhancement of stability of aqueous dispersions of the silver NPs can be obtained via two kinds of protecting mechanisms. The first one is based on the steric repulsion, which displays a stabilizing effect with the assistance of polymers and non-ionic surfactants that are immediately adsorbed at the phase interphase.¹⁸ The balance between the attractive and the repulsive forces is strongly dependent on the thickness of the adsorbed layer,¹⁹ which is, in the case of polymers, dependent

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not only on the chain length but also on its adsorption mode.²⁰ For the purpose of the steric stabilization, there are basically used polyethylene glycols (PEG),²¹ poly(vinylalcohols) (PVA),²² poly(vinylpyrrolidons) (PVP),^{23,24} polyacrylamides,²⁵ and polyurethanes (PU).²⁶ The non-ionic surfactants are, in comparison to the polymers, adsorbed in a more compact mode at the surface of the NP and convey an excellent stabilizing effect.²⁷ Brij, Tween, and Triton X-100²⁸ are probably the most frequently used non-ionic surfactant stabilizers.

The second mechanism of the dispersion system stabilization is based on an electrostatic repulsion. The surface charge of the disperse phase can be enhanced by the ionic surfactant addition providing the electrostatic protection of the NPs to adhere to one another. Sodium dodecyl sulfate (SDS),²⁹ as a representative of the anionic surfactant group, and cetyltrimethylammonium chloride or bromide (CTAC, CTAB),³⁰ as the cationic surfactants, have been employed in several studies and are considered stabilizing agents of immense importance.^{30, 31} In the case of the silver NP interaction with the ionic surfactants, the mechanism of the surfactant adsorption has not been completely clarified. However, Chen et al. suggest a possible mechanism of organization of SDS molecules on the NP surface in which the hydrophilic groups of the surfactant molecules are adsorbed on the silver NP surface and the hydrophobic tails are directed outward to form the first layer. Consequently, a counter-layer is oriented the opposite way resulting in interpenetration of the surfactant hydrophobic tails between the two layers with hydrophilic groups headed outward.³²

The presented study is aimed at the evaluation of the stabilization effects of various polymers as well as non-ionic and anionic surfactants on nearly monodisperse silver NPs prepared by reduction of the $[\text{Ag}(\text{NH}_3)_2]^+$ with D-maltose. As a result, we observed an excellent stability in the systems modified, especially with SDS and Tween 80 surfactants. The mechanism of the surface interaction with silver NPs and the reasons for the extraordinary antibacterial activity of the successfully modified NPs will be discussed.

Experimental Methods

Materials. Silver nitrate (99.9%, Safina), ammonia (25% (w/w) aqueous solution, p.a. Lachema), sodium hydroxide (p.a., Lachema) and D(+)-maltose monohydrate (p.a., Riedel-de Haën) were used for the preparation of silver NPs without any further purification. In order to modify the surface of the silver NPs, the following surfactants, purchased from Sigma-Aldrich, were tested: anionic sodium dodecyl sulfate (SDS; >98%), non-ionic polyoxyethylenesorbitan mono-oleat (Tween 80; >98%), and non-ionic surfactants from the Brij group: polyoxyethylene(23) lauryl ether (Brij 35; >98%), polyoxyethylene(20) cetyl ether (Brij 58; >98%), polyoxyethylene(10) oleyl ether (Brij 97; >98%), and polyoxyethylene(20) oleyl ether (Brij 98; >98%). Besides the above-mentioned surfactants, the following polymers were also tested: polyethyleneglycols (PEG) of different molecular weights equal to 1500, 4000, 10 000, and 35 000 (PEG 1500, PEG 4000, PEG 10000, PEG 35000; p.a., Fluka), and polyvinylpyrrolidone (PVP) of the following average molecular weights; 10 000, 40 000, and 360 000 (PVP 10; PVP 40; PVP 360; Sigma-Aldrich). In order to study the aggregation process of the aqueous dispersions of the silver NPs, cationic polyelectrolyte poly(diallyldimethylammonium) chloride was used (PDDA, 20% (w/w) aqueous solution, Aldrich). All of these chemicals were used without any further purification. All of the used solutions were prepared with deionized water (18 M Ω cm, Millipore).

For the purpose of the antibacterial assays of the silver NPs, there was used a cultivation medium (Mueller-Hinton broth), which was supplied by Difco (Bedston Dickinson). As the standard reference strains (labeling according to Czech Collection of Microorganisms, Czech Republic), the following were used: *Staphylococcus aureus* CCM 3953, *Enterococcus faecalis* CCM 4224, *Escherichia coli* CCM 3954, and *Pseudomonas aeruginosa* CCM 3955. Further, the following bacterial strains isolated from human clinical material at the Teaching Hospital, Palacký University in Olomouc, Czech Republic were used: *Pseudomonas aeruginosa*, methicillin-susceptible *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and ESBL-positive *Klebsiella pneumoniae*.

Methods. Silver NPs, with the diameter of approximately 26 nm, were synthesized by the reduction of the complex cation $[\text{Ag}(\text{NH}_3)_2]^+$ with D-maltose, as referred previously.³³ The initial concentrations of the reaction components were 1×10^{-3} mol·L⁻¹ and 1×10^{-2} mol·L⁻¹ for AgNO₃ and the reducing sugar, respectively. The concentration of the used ammonia was 5×10^{-3} mol·L⁻¹. Sodium hydroxide solution was added to the reaction system to adjust the value of pH at about 11.5, as well as to achieve a reaction time of several minutes. The as-prepared aqueous dispersion of the silver NPs was used for subsequent experiments without any additional modifications. All of the measurements were performed at laboratory temperature (~25 °C). The study of the aggregation stability of the silver NP represents a newly established method performed by consequential additions of PDDA solution and will be discussed later (in section results) to a greater extent.

The size of the individual well-dispersed silver NPs as well as the size of the agglomerates formed during the aggregation process was determined by a dynamic light scattering method (DLS) using a Zeta Plus analyzer (Brookhaven). All of the presented results of the particle sizes are listed as average values from three independent measurements. The Zeta Plus analyzer was also used for the Zeta potential measurements. TEM observations of the silver NPs were performed with a JEM 2010 (Jeol) electron microscope at 100 kV of the acceleration voltage. UV/vis absorption spectra of the silver NP dispersions were acquired by using a Specol S 600 (Analytic Jena AG) spectrophotometer.

The surface tension of the studied systems, necessary for the determination of the dynamic contact angles, was measured by the deNoüy method (using standard Pt/Ir ring with the diameter 19.1 mm) with a Lauda TD-1 tensiometer. Final values of the surface tension were gained after a correction, which is given by the following relation: $\gamma_{\text{corr}} = f \cdot \gamma_{\text{uncorr}}$ where $f = 0.8759 + 0.9188 \times 10^{-4} (\gamma_{\text{uncorr}}/\rho_{\text{sol}})$ and γ_{uncorr} is the experimentally determined uncorrected value of the surface tension in mN·m⁻¹; ρ_{sol} is the value of the solution density in grams per cubic centimeter. The dynamic contact angles of the studied solutions on the silver surface (Ag wire with the diameter 1.0 mm and purity of 99.9%, Safina) were performed by a Cahn DCA315 tensiometer at the speed of the Ag wire motion equal to 24 $\mu\text{m} \cdot \text{s}^{-1}$ in two cycles. The values of the dynamic contact angles for advancing liquid surface v_{adv} and receding surface v_{rec} were determined from the experimentally obtained curves using the WinDCA32 software.

The antibacterial activity of the modified and unmodified aqueous silver NP dispersions as well as the reference samples involving "pure" solution of ionic silver (AgNO₃) and "pure" solutions of the used modifiers were tested using a standard

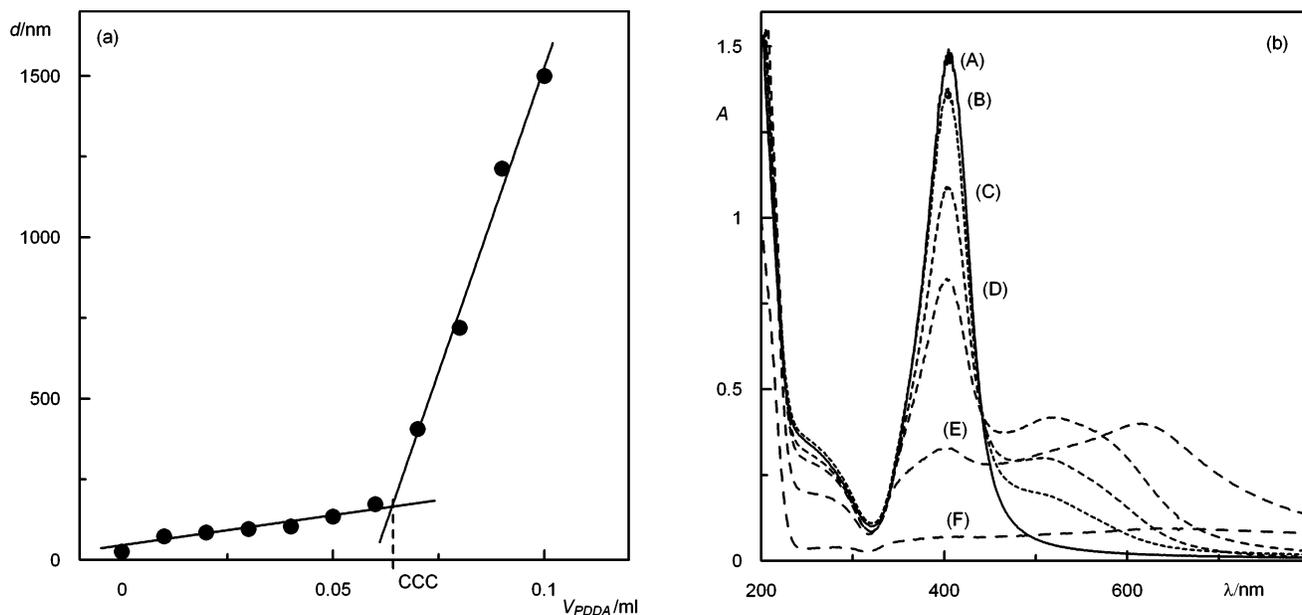


Figure 1. Size changes obtained from DLS (a) and the changes in UV/vis spectra (b) in the course of the titration of the aqueous dispersion of the unmodified silver NPs by the PDDA solution in the amount of 0 (A), 20 (B), 40 (C), 60 (D), 80 (E), and 100 μL (F).

dilution micromethod. This method is based on the determination of the minimum inhibitory concentration (MIC) leading to inhibition of tested bacterial strains growth. The precise description of this method was presented in previous work.¹² Disposable microtitration plates were used for the tests. The MIC was read after 24 h of the incubation at 37 °C as the concentration of the tested substance that inhibited the growth of the bacterial strain. The silver NP dispersions were used in the form in which they had been prepared and modified. The concentration of silver in the prepared aqueous dispersion and in the reference sample of ionic silver was 108 $\mu\text{g}\cdot\text{mL}^{-1}$. For the purpose of the antibacterial assays, the samples were consequently diluted, according to the geometric progression from 2 to 128 times with 100 μL of Mueller–Hinton broth inoculated with the tested bacteria in the concentration of approximately 10^5 – 10^6 CFU $\cdot\text{mL}^{-1}$. The obtained concentrations of silver were in the range from 54 $\mu\text{g}\cdot\text{mL}^{-1}$ to 0.84 $\mu\text{g}\cdot\text{mL}^{-1}$. Furthermore, there was also performed the antibacterial test of all samples, containing silver, that were diluted 256 times, and while they did not show any antibacterial activity at all, this dilution was abandoned. The used reference samples of 1% (w/w) solutions of the modifiers were tested according to the previously described dilution micromethod and the obtained concentration range was from 0.5% (w/w) to 7.8×10^{-3} % (w/w) in this case.

Results and Discussion

Methodological Approach to the Evaluation of the Aggregation Stability. The standard study of the aggregation stability, using the inorganic polyvalent cations with a simple structure, cannot be performed in the tested systems because these cations would be hydrolyzed at the pH of the silver NP dispersion (equal to 11.5). By considering this point of view, there was introduced a new approach of stability testing. This procedure is based on the titration of the silver NP dispersions using a cationic polyelectrolyte poly(diallyldimethylammonium) chloride (PDDA) which is suitable for the aggregation study of the negatively charged particles. PDDA solution significantly destabilizes the tested dispersions of the silver NPs already at very low concentration, which enabled the study of the aggregation process under the conditions of the minimal concentration changes of the tested dispersions.

The aggregation process, caused by the addition of PDDA solution, was monitored by DLS measurements through the changes of the sizes of the particles, and ultimately their aggregates. In the non-stabilized system, a gradual increase in agglomerate size with the PDDA volume appears, the fact indicating the formation of agglomerates from the originally well-dispersed particles (Figure 1a discussed in the next section). In an ideal case, the stabilization of the system is manifested by a constant size of the particles according to the DLS measurements, independent of the added PDDA volume. The gradual changes in the particle, and the aggregate sizes, dependent on the increasing volume of the PDDA solution, were reflected also in the UV/vis absorption spectra (Figure 1b). The surface plasmon of the silver NPs, located at the wavelength of approximately 400 nm, indicates the presence of the well-dispersed NPs while the fully aggregated silver NP system gave evidence by a complete disappearance of this characteristic peak. Finally, TEM observations were applied to explore the aggregation process and to consider the possible stabilization of the silver NP using various surfactants and polymers.

In more details, the aqueous PDDA solution (0.01% (w/w)) was step-by-step added into the tested silver NP dispersions (25 mL) in the 10 μL amounts, and the course of the process under the conditions of the minimal concentration changes of the tested dispersions aggregation process was monitored by the DLS and UV/vis measurements performed 5 min after each PDDA addition. The modifiers were added to the dispersion of the silver NPs prior titration in the final amount of 1% (w/w). As the reference experiment, the same approach was applied to evaluate the aggregation stability of the unmodified aqueous dispersion of the silver NPs.

Unmodified Silver Nanoparticles. The silver NPs prepared by the mentioned reduction route reveal an average diameter of 26 nm, the spherical shape and very narrow size distribution with polydispersity of 2.3%. The surface plasmon, characteristic of the well-dispersed silver NPs of this size, is located at the wavelength equal to 405 nm. In the aqueous dispersion, silver NPs exhibit a long-term stability due to the ζ potential equal to -25 mV. However, they undergo a slow aggregation process when the pH of the system is lowered toward the acid range or at the enhanced ionic strength performed by inorganic electro-

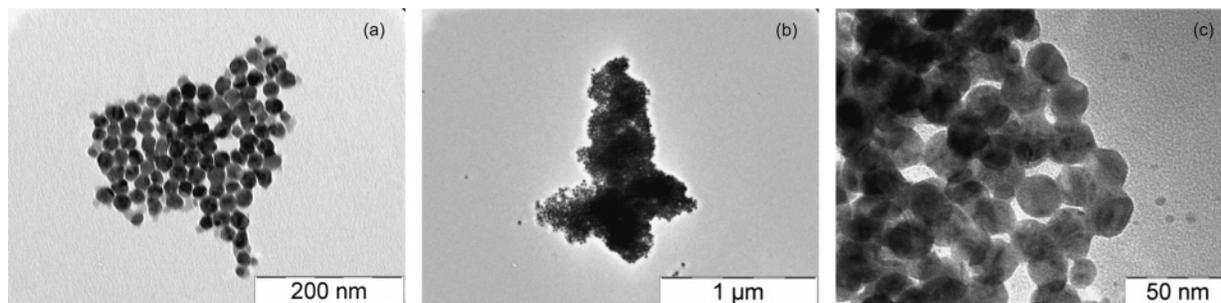


Figure 2. TEM images of the originally unmodified silver NPs (a); the aggregate of the silver NPs generated by addition of 80 μL of the PDDA solution (b); a detailed illustration of the arrangement within the silver NPs agglomerate (c).

lytes. The aggregation process occurring in the system of unmodified silver NPs was monitored by the DLS and UV/vis measurements performed after the step-by-step addition of the PDDA destabilizing solution (Figure 1a,b, respectively). In the range from 10 to 70 μL of the added PDDA solution, slow increases were observed in the sizes of the aggregates, which were followed by a steep growth (at the volume of 70 μL) of the aggregate sizes and the consequential additions (in total amount 100 μL) produced agglomerates of micrometric diameters in the system (Figure 1a). These observations indicate a probable formation of the primary assemblies of the NPs in the dimensions of several tens or a few hundreds of nanometers before the massive agglomeration is started. Such course of the aggregation process was also confirmed by the UV/vis spectra displaying a decrease in intensity of the absorption maxima of the surface plasmon with increasing volume of the added PDDA solution (Figure 1b).

Moreover, another absorption peak emerges at the wavelength approximately equal to 505 nm after addition of 20 μL of PDDA. This absorption peak is gradually shifted toward higher wavelengths, even to 615 nm after addition of 80 μL of PDDA solution. Moreover, this secondary peak increases slightly in intensity with the added PDDA volume along with the simultaneous lapse in intensity of the original surface plasmon at 405 nm. The secondary absorption peak would be ascribed to the primarily formed assemblies (aggregates) of the NPs having the nanodimensional character and increase in size with the added PDDA volume. After the final addition of 100 μL of the PDDA solution into the dispersion of the silver NPs, the specific absorption peaks have completely vanished, which is clear evidence of the complete agglomeration of the NPs. The character of the originally well-dispersed NPs and the formation of the micrometer-sized aggregates, induced by an addition of 80 μL of the PDDA solution, are clearly illustrated by the TEM images in Figure 2.

A type of interaction of the PDDA molecules with the silver NP surface can be deduced from the following simplified calculations of the effective charge of the PDDA molecule inducing the aggregation process from the critical coagulation concentration (CCC) value. The DLVO theory predicts the value of CCC based on the consideration of the repulsive electrostatic interactions and the attractive van der Waals forces. This prediction can be expressed in the form of approximate equation:³⁴

$$\text{CCC} = 87 \times 10^{-40} / (z^6 A^2) \quad (1)$$

where z represents the charge of counterion of the added electrolyte and A stands for Hamaker constant in Joules for CCC in moles per liter.

The CCC value equal to $2.4 \times 10^{-5} \%$ (w/w) of PDDA was determined from a plotted dependency of the size of the

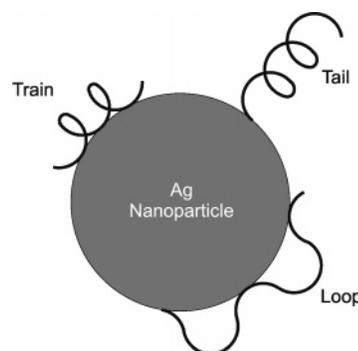


Figure 3. Drafts of possible modes of how polymers can be bonded on the silver NP surface.

aggregates on the PDDA addition as the intersection of its linear parts (Figure 1a). As the best estimation of the value of Hamaker constant for interacting pair of Ag–Ag atoms, the value of $39 \times 10^{-20} \text{ J}$ was determined experimentally by the AFM method.³⁵

On the basis of the value of CCC equal to 0.94 nM (for the mean molecular weight of PDDA polymer equal to 275 kDa), the effective charge of the PDDA cation could be calculated as 20 elementary charges for the interaction with the tested silver NP dispersion. This value of the effective charge represents only about 1% of the net charge of the PDDA polymer molecule, which is in a good agreement with the expected mode of the interaction of the PDDA molecules with the silver surface.²⁰ In this so-called tail mode, the polymer molecule interacts with the solid particle surface only via the end of the polymer chain (Figure 3), which represents a typical interaction mode for the polymer of a high molecular weight having a long linear polymer chain that is not branched.

Modification by Ionic Surfactant. From the point of view of the above-described aggregation study, the anionic surfactant, sodium dodecyl sulfate (SDS), proved to be the most effective stabilizer of the aqueous dispersion of the silver NPs among all of the tested modifiers. Silver NPs stabilized by addition of 1% (w/w) of SDS did not reveal any tendency to aggregate in the course of the titration up to the amount of 100 μL of the PDDA solution while the unmodified system was already completely aggregated. This fact has been verified both by the DLS measurements (Figure 4a) and the UV/vis absorption spectra of the aqueous dispersion of the SDS modified silver NPs (Figure 4b). The DLS data give clear evidence for the complete suppression of the aggregation process as documented by nearly the same size of the particles, independently on the added volume of the PDDA solution. Similarly, the UV/vis spectra do not show any change in intensity of the surface plasmon until an addition of 5 mL of the PDDA solution when a reasonable fall in the peak intensity (approximately 17%) can be observed, which is evidently caused only by the dilution of the SDS modified dispersion (dilution factor is 0.83). The

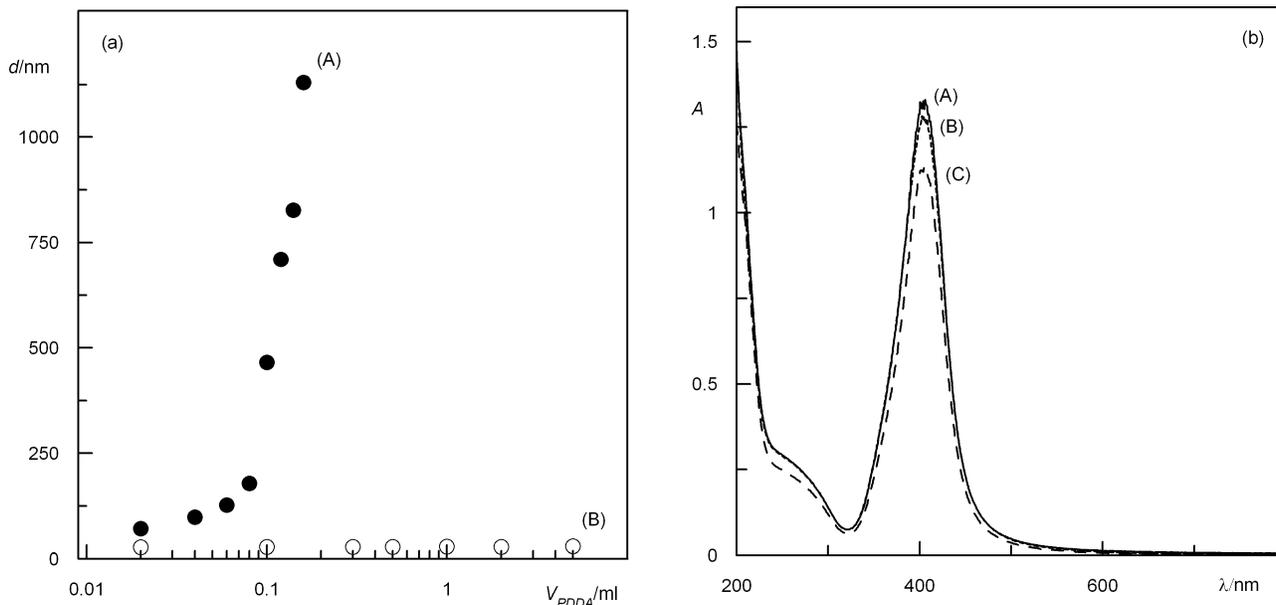


Figure 4. Size changes obtained from DLS (a) for unmodified (A) and SDS modified (B) silver NPs; the changes in UV/vis absorption spectra (b) in the course of the titration of the aqueous dispersion of the SDS modified silver NPs by the PDDA solution in the amount of 0 (A), 0.5 (B), and 5 mL (C).

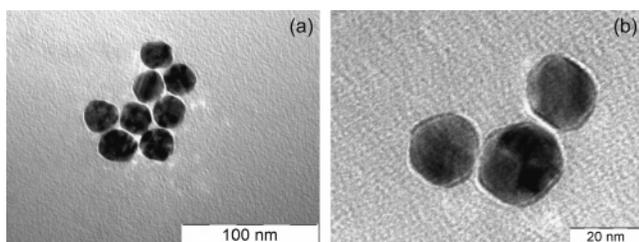


Figure 5. TEM image of the silver NPs modified by SDS after the addition of 100 μ L of the PDDA solution (a); a detailed demonstration of the SDS shell on the particle surface (b).

extraordinary aggregation stability of the silver NPs modified by SDS was definitely confirmed by the TEM images that display the well-separated particles having the same size as those in the unmodified dispersion even after the addition of 100 μ L of the PDDA solution (Figure 5a). The detail in Figure 5b shows the SDS shell on the surface of silver NP. The extraordinary stabilizing action of SDS is supposedly connected with two effects. Following the DLVO theory, the stability is enhanced because of a significant increase in the absolute value of the NP surface charge reflected in the nearly doubled value of ζ potential equal approximately to -50 mV. Although this electrostatic stabilization is of a key importance, the steric effect of the proposed double layer structure should also be taken into account.³² These combined effects of SDS resulted in an exceptional stabilization of the silver NP dispersions that even at such addition as 5 mL of the PDDA solution did not initiate the aggregation process.

Modification by Non-Ionic Surfactants. In the case of the silver NPs modified by the non-ionic surfactants, the stabilization was considered less effective when compared with the system stabilized by SDS; however, its effects on antibacterial activity has not been studied yet. Nevertheless, Tween 80 revealed also a remarkable stabilizing effect with a considerable enhancement of the silver NP stability in comparison with the unmodified system and to all other tested non-ionic surfactants from the Brij group. Although the slow aggregation process was observed in the DLS measurements also for the Tween 80 modified system (Figure 6a), no drastic increase in size of the

agglomerates was registered even after addition of 100 μ L of the PDDA solution when the micrometer-sized agglomerates are formed in the unmodified system. This fact strongly indicates stabilization of the silver NPs by Tween 80. The preservation of the nanodimensional character of the system modified by Tween 80, with limited interparticle interactions, is also evident from the UV/vis absorption spectra (Figure 6b) exhibiting relatively intense surface plasmon peak even after addition of 100 μ L of the PDDA solution. In the UV/vis spectra of the silver NPs modified by Tween 80, there emerges the secondary maximum around the wavelength equal to approximately 500 nm, which corresponds to the formation of weakly interacting NP assemblies. Such weak interaction among silver NPs modified by Tween 80 demonstrating the efficacious stabilization is clearly observable in the TEM images (Figure 7a,b). It is worth mentioning that the enhancement of the aggregation stability of the NP dispersion modified by Tween 80 is exclusively connected with the steric stabilization. The electrostatic stabilization effect, playing the key role in the SDS modified system, cannot be taken into account as the zeta potential of the silver NPs modified by Tween 80 remained nearly unchanged, that is, -24 mV. This steric mechanism of the stabilization can be attributed to the formation of the surface structure comparable with the SDS double layer (discussed previously).

From the tested non-ionic surfactants of the Brij group, only Brij 58 and Brij 98 proved to convey a weak stabilizing effect of the silver NP dispersion, which can be quantified by the increase in the CCC value from 2.4×10^{-5} % (w/w) of PDDA (for the unmodified dispersion) to 2.8×10^{-5} % (w/w) for Brij 58 and 3.2×10^{-5} % (w/w) of PDDA for Brij 98. The slight stabilizing effect of these substances can be also detected from the hindered growth of the aggregates (Figure 8a) as can be consulted with the UV/vis absorption spectra (Figure 8b) and with TEM images (Figure 7c). Considering the system modified by Brij 97 and Brij 35, the effect of the surface modification on the stabilization of the aqueous dispersions of the silver NPs proved to be negligible as also confirmed by UV/vis absorption spectra (not shown). Such insignificantly stabilizing effect, performed by the Brij group surfactants, can be explained by a

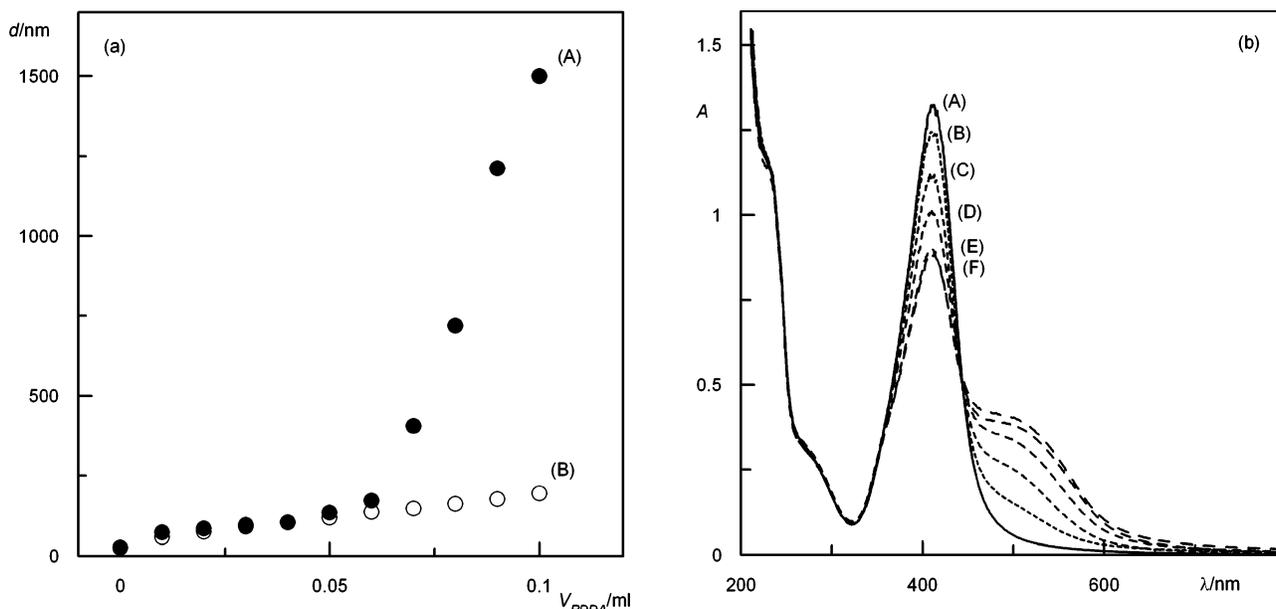


Figure 6. DLS measured size changes induced by the PDDA titration of the aqueous dispersion (a) of the unmodified silver NPs (A) and NPs modified by Tween 80 (B); the changes in the UV/vis absorption spectra of the silver NPs stabilized by Tween 80 (b) involving 0 (A), 20 (B), 40 (C), 60 (D), 80 (E), and 100 μ L (F) of the PDDA solution.

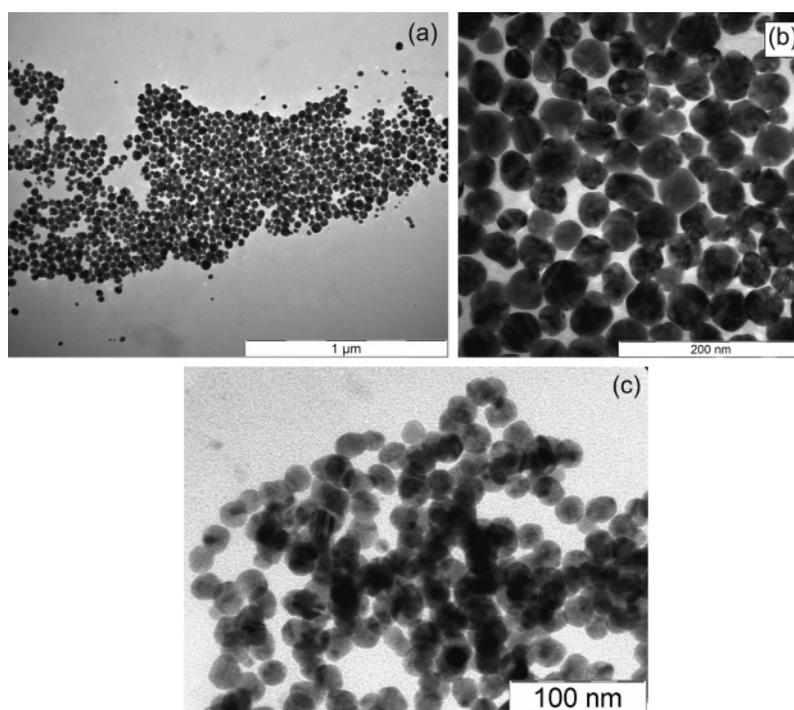


Figure 7. TEM images of the assemblies of silver NPs generated in the course of the PDDA titration. The Tween 80 modified silver NPs (a) and their detailed image (b); the Brij 98 modified silver NPs (c) after the addition of 80 μ L of the PDDA solution.

weak adsorption on the surface of the silver NPs. The surfactant molecules are therefore easily substituted for the molecules of the cationic electrolyte (PDDA) in the course of the titration. Insignificant differences among the surfactants from the Brij group can be related to the variation in their structure, that is, in the balance between the polar and the nonpolar part of their molecules. In the case of Brij 58 and Brij 98, the weak stabilizing effect can be therefore ascribed to structure of the molecule reflected in the value of hydrophilic–lipophilic balance (HLB),³⁶ which is approximately 15 (as well as for Tween 80). On the contrary, the HLB value is higher for Brij 35 (approximately 17) and lower for Brij 97 (approximately 13) and therefore conveys less effective stabilization.

Modification by Polymers. For the purpose of this study, there were tested two groups of polymers, based on polyethyleneglycol (PEG) and polyvinylpyrrolidone (PVP) that are commonly used as stabilizers of the aqueous NP dispersions. Considering the structural and chemical differences between both groups, a distinctively different bonding strength of the polymer molecules on the surface of the silver NPs was expected. The polymers of the PVP group are bonded stronger on the silver NP surface through the nitrogen atom in their molecule, while PEGs are weakly bonded via the oxygen atom. Because of the weak interactions with the particle surface, the PEG molecules can be, in contrast to the PVP molecules, quite easily substituted by the PDDA molecules, which undoubtedly

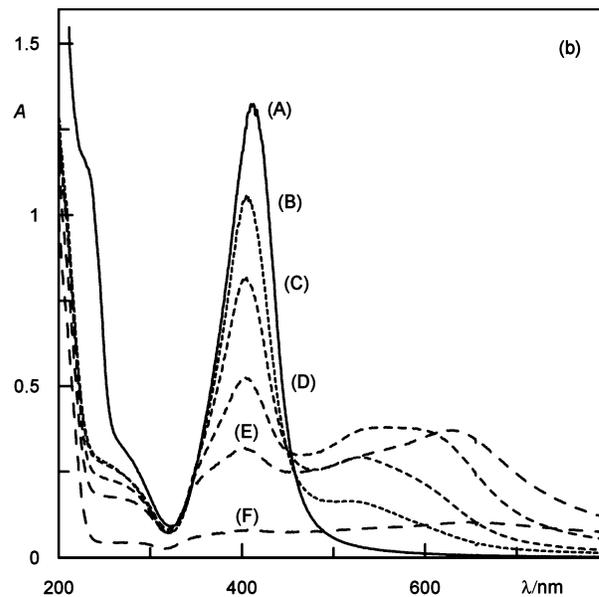
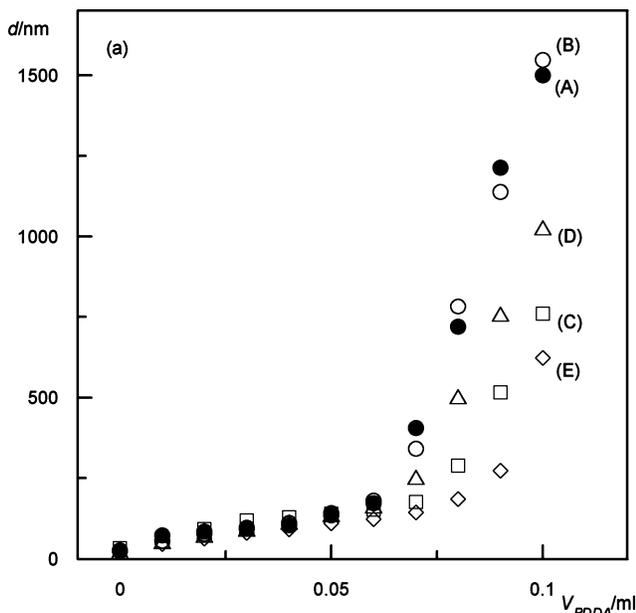


Figure 8. Comparison of the change in size (a) of the unmodified silver NPs (A) and silver NPs modified by polymers of the Brij group: Brij 35 (B), Brij 58 (C), Brij 97 (D), and Brij 98 (E) modified systems in the course of the titration by PDDA solution; and the changes in the UV/vis absorption spectra of the silver NPs stabilized by Brij 98 (b) involving 0 (A), 20 (B), 40 (C), 60 (D), 80 (E), and 100 μL (F) of the PDDA solution.

TABLE 1: Values of the Surface Tension γ and Contact Angles ν (Degrees) of the Silver Surface for the Solutions of the Polymer Substances That Were Used for the Silver NP Modification

solution ^a	γ^b (mN/m)	1. cycle ^{b,c}		2. cycle ^{b,c}	
		ν_{adv}	ν_{rec}	ν_{adv}	ν_{rec}
unmodified system	69.0/62.9	80.0/62.0	18.5/9.5	49.5/32.0	19.0/9.0
PEG1500	59.2/60.8	70.5/71.0	0.0/13.0	36.5/38.0	0.0/10.5
PEG10000	59.6/61.3	59.0/56.0	0.0/10.5	27.0/28.5	0.0/9.5
PVP10	57.1/63.5	76.0/59.5	23.5/17.5	31.5/36.0	24.0/16.5
PVP360	63.7/64.5	78.0/60.5	15.0/11.0	37.5/21.5	18.0/9.5

^a The tested solutions correspond by their pH and their content of ammonia with the composition of the aqueous dispersion of the silver NPs but without the content of the reducing agent and the silver NPs as such. The other solutions were entitled by the abbreviation of the particular polymer used for the purpose of the surface modification in the concentration equal to 1% (w/w). ^b Values of the surface tension and contact angles for tested solutions without PDDA/with PDDA solution. ^c ν_{adv} and ν_{rec} represent the advancing and receding dynamic contact angles.

leads to the easy destabilization of the system. These differences in the strength of the adsorption can be well-illustrated by the contact angle measurements that were performed on the silver surface (Table 1).

The studied polymers from the PEG group accomplish a marginal influence on the aggregation stability of the aqueous silver NP dispersion, possibly excluding PEG 1500 (Figure 9). The slightly higher stabilizing effect of the PEG 1500, having the lowest molecular weight among the tested PEG polymers, could be probably connected with the fact that the used concentration (1% w/w) of this modifier, recalculated to the molar concentration, is reasonably the highest (6.6 mM) and therefore can generate a more compact surface layer on the silver NP surface. As a result, the PDDA molecules can penetrate the adsorption layer with more difficulties than they do in systems modified by the other tested PEG polymers. Recalculated molar concentrations of other PEGs are reasonably lower (2.5 mM for PEG 4000, 1 mM for PEG 10000, 0.3 mM for PEG 35000) which is consequently reflected in a less perfect surface layer. Simultaneously, because of the adsorption mode, such compact-

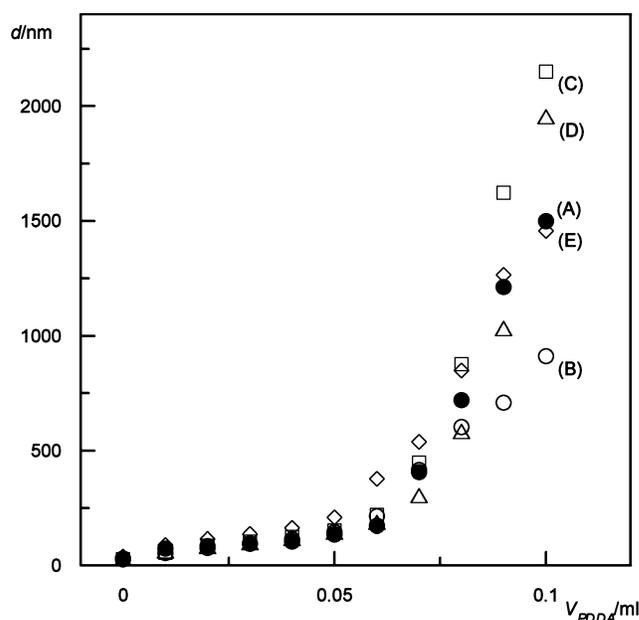


Figure 9. Comparison of the change in size of the unmodified silver NPs (A) and silver NPs modified by polymers of the PEG type-PEG 1500 (B), PEG 4000 (C), PEG 10000 (D), and PEG 35000 (D) modified systems in the course of the titration by the PDDA solution and monitored by DLS.

ness of the adsorption layer is not achieved as in the case of PEG 1500, which is mainly adsorbed in the train mode (Figure 3). These considerations can be supported by the measurements of the silver surface wettability performed with the solutions of these polymers. In the "pure" silver NP-polymer systems (without PDDA), containing polymers from the PEG group, a low wettability in the first cycle of the dynamic contact angle measurements was observed at the advancing mode. In the adverse mode, the receding contact angle was rapidly changed to a zero value, which signifies that the silver surface is covered with the PEG molecules that are of a hydrophilic character. When the mixture of the PDDA and PEG solutions was used, a fundamental change in the wetting behavior of the whole system was registered. Already in the first cycle, the receding

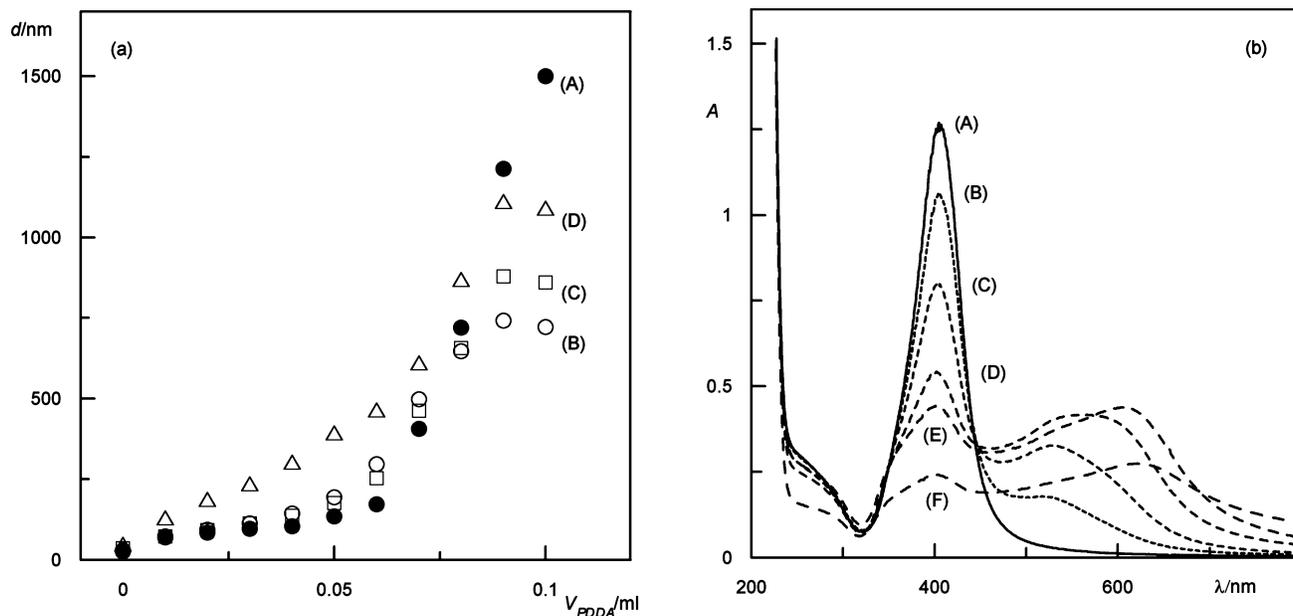


Figure 10. Comparison of the change in size of the original silver NPs (A) and silver NPs that were modified by polymers of the PVP type: PVP 10 (B), PVP 40 (C), and PVP 360 (D) modified systems in the course of the titration by PDDA solution (a); and the changes in the UV/vis absorption spectra of the silver NPs stabilized by PVP 360 (b) involving 0 (A), 20 (B), 40 (C), 60 (D), 80 (E), and 100 μL (F) of the PDDA solution.

contact angle changed to the value corresponding to the surface covered with the adsorbed PDDA molecules (Table 1). Additionally, in the second cycle, the advancing contact angle values approached the values for the “pure” PDDA solution. This fact can be interpreted as an effect of the PEG molecules elimination from the adsorption layer by the PDDA molecules. In the case of PEG 1500, the value of the advancing contact angle and also the value of the hysteresis of the contact angle in the second cycle are not influenced to such an extent by the PDDA solution as for the other PEGs.

More beneficial results, in comparison with the polymers from the PEG group, were obtained with the polymers from the PVP group although the stabilization they perform is far less effective than the one ensured by SDS or Tween 80. By considering the DLS measurements, no significant stabilizing effect was observed on the aggregation stability of the NP dispersions modified by the PVP type polymers (Figure 10a). However, the obtained UV/vis absorption spectra (Figure 10b) do not verify the results from DLS measurements especially for PVP 360. This disagreement reflects the real behavior of the system where the silver NPs are interconnected via the particular parts of the molecular chains of the polymers weakly bonded in the tail mode.²⁰ In comparison with the unmodified system, the UV/vis absorption spectra confirm a significant stabilizing effect. In the case of the modification performed by the high-molecular PVP 360, the immediately started aggregation of the silver NP dispersion can be subscribed to the phenomenon of flocculation.³⁷ The obtained data are in good agreement with the previously published results concerning modification of the silver NP performed by polymers, especially using the polymers from PVP group.²⁴

Influence of the Surface Modification on the Antibacterial Activity. The above-mentioned results proved a positive stabilizing effect in the silver NP systems modified especially by SDS, eventually by Tween 80 and PVP 360. The following part of this study was devoted to the reflection of these most effective stabilizers in the antibacterial activity of the modified silver NP systems. In order to recognize their effect, antibacterial tests of the modified silver NPs and of the pure 1% (w/w)

solutions of SDS, Tween 80, and PVP 360 were performed. All of these antibacterial activity assays were performed according to the above-mentioned dilution micromethod. The obtained results revealed that none of the “pure” solutions of modifiers, at the tested concentration, showed any antibacterial effect. However, the antibacterial activity of the modified silver NPs was considerably enhanced as confirmed by MIC values ranging from 6.75 down to 0.84 $\mu\text{g}\cdot\text{mL}^{-1}$ (Table 2) in comparison with the previously published antibacterial activity assays of the unmodified silver NPs having MIC in the range from 13.5 to 1.69 $\mu\text{g}\cdot\text{mL}^{-1}$ strongly dependent on the tested bacterial strains.¹² Therefore, there can be found twice enhanced antibacterial activity of the silver NPs and break through the concentration limit of 1 $\mu\text{g}\cdot\text{mL}^{-1}$, which is corresponding with the value of the bests antibacterial agents.

In the case of the silver NP modification performed by the anionic SDS surfactant, the best surface stabilization is in a direct relation to the observed highest antibacterial activity. Beside the extraordinary stabilization effect, SDS can contribute to the enhancement of the antibacterial activity of the silver NPs due to its ability to increase the permeability of the cell wall or to disrupt the cell wall especially of gram-positive strains,³⁸ although “pure” SDS solution, in the used concentration, did not prove this effect. By using SDS, we gained the antibacterial activity results comparable with the effects of ionic silver, tested as reference sample (Table 2), which bears the highest antibacterial activity of all silver compounds. Therefore, it should once again be emphasized that, for some bacterial strains, the MIC value decreased even under the “magical value” of 1 $\mu\text{g}\cdot\text{mL}^{-1}$ of Ag. The synergic antibacterial effect of SDS and the silver NPs was proven against only one gram-negative *Pseudomonas aeruginosa* CCM 3955 and all of the tested gram-positive strains except for methicillin-resistant *Staphylococcus epidermidis*. Interestingly, there were previously published results indicating the decrease in the antibacterial activity of the SDS modified silver NPs³⁹ that cannot be considered reliable especially when consulting with the results, showing the synergic effect of both components, presented in this study.

TABLE 2: Minimal Inhibition Concentration (MIC) of the Silver NPs Prepared by the Reduction of the $[\text{Ag}(\text{NH}_3)_2]^+$ Complex Cation with D-maltose and Consequentially Modified by Addition of the Chosen Surfactant in Concentration of 1% (w/w)

tested strain	minimal inhibition concentration ^a ($\mu\text{g}\cdot\text{mL}^{-1}$ Ag)				
	Ag ⁺	unmodified dispersion	surfactant/polymer modifier		
			SDS	Tween 80	PVP 360
<i>Enterococcus faecalis</i> CCM 4224	1.69	6.75	3.38	6.75	6.75
<i>Staphylococcus aureus</i> CCM 3953	1.69	3.38	1.69	3.38	3.38
<i>Escherichia coli</i> CCM 3954	0.84	1.69	1.69	1.69	3.38
<i>Pseudomonas aeruginosa</i> CCM 3955	0.84	3.38	1.69	3.38	1.69
<i>Pseudomonas aeruginosa</i>	0.84	3.38	3.38	1.69	1.69
<i>Staphylococcus epidermidis</i> (methicillin-susceptible)	0.84	1.69	0.84	1.69	1.69
<i>Staphylococcus epidermidis</i> (methicillin-resistant)	0.84	1.69	1.69	1.69	1.69
<i>Staphylococcus aureus</i> MRSA	0.84	3.38	1.69	3.38	1.69
<i>Enterococcus faecium</i> VRE	1.69	6.75	3.38	3.38	3.38
<i>Klebsiella pneumoniae</i> ESBL	1.69	6.75	6.75	3.38	6.75

^a Values of the enhanced antibacterial activity of the modified silver NPs are written in bold.

Further tested modifier, non-ionic surfactant Tween 80, revealed also the enhancement of the antibacterial activity when compared with the unmodified silver NPs; however, this effect was not as significant as for the SDS-modified system. The explanation of the lower antibacterial activity of the Tween 80-modified system is supposedly connected not only with its lower stabilization effect but also with the expected structure of the adsorption layer on the surface of the silver NPs restraining a direct contact of the silver NP with the cell wall. This contact is necessary in order to provide the destructive effect of the silver NPs on the tested bacteria. Therefore, aside from the positive stabilizing effect of Tween 80 on the aggregation stability of the silver NPs, the antibacterial activity is not enhanced in such an extent as in the case of the anionic SDS surfactant.

PVP 360, as the most effective polymer stabilizer, enhanced the antibacterial activity of the silver NPs in a significant extent. This positive influence can be attributed to the ability of the polymer to stabilize the NPs against aggregation. In spite of the flocculation process, the silver NPs are separated from each other by the chain of the polymer molecule. Therefore, the nonaggregated NPs are able to interact strongly with the cell wall because of their high surface energy and mobility that are not lowered by the formation of spacious aggregates. However, the stabilization effect of this polymer is comparable with the effect ensured by Tween 80 and even the enhancement of the antibacterial activity of the silver NPs was found within the same range. Therefore, SDS modification can still be considered the most effective, both from the stability and from the antibacterial activity enhancement point of view.

Conclusions

The uniformly sized silver NPs were synthesized by the modified Tollens process using D-maltose as a reducing agent and consequently modified by a variety of surfactants and polymers in order to achieve the enhancement of their stability and antibacterial activity. The new experimental approach including a titration of the aqueous dispersion of the silver NPs by an aqueous solution of poly(diallyldimethylammonium) chloride was established to evaluate the aggregation stability of the silver NPs. Among the large scale of various surfactants and polymers, sodium dodecyl sulfate (SDS), polyoxyethylene-sorbitan monooleate (Tween 80), and polyvinylpyrrolidone (PVP 360) were found to act as the best stabilizers resulting also in the considerable enhancement of the antibacterial activity of the modified silver NP systems.

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