Kinetics and Mechanism of Green Synthesis of Silver Nanoparticles Using the Rhamnolipid of Natural Origin

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Abstract. Widespread use of synthetic antimicrobial drugs leads to the development of antibiotic resistance of pathogenic strains of microorganisms. Therefore, today the researchers are very interested in drugs based on nanoparticles of metals, in particular silver, which has antibacterial, antifungal and antiviral activity. A critical analysis of the results of research performed in recent decades has shown that despite the large number of references devoted to the study of properties, use and development of methods for obtaining the silver nanoparticles [1-3], the influence of kinetic parameters on their characteristics is insufficiently studied. In presented work silver nanoparticles (AgNPs) have been synthesized by "green synthesis" method using the silver nitrate as precursor and surface-active rhamnolipid (RL-1) of natural origin, which represents by itself the product of microbial synthesis of the strain *Pseudomonas* sp. PS–17 and was used as reducing agent and stabilizer. The kinetics of green synthesis of AgNPs via reduction of Ag^+ ions by RL-1 was studied in details using the UV-vis spectroscopy as well as obtained products were studied using IR-spectroscopy and transmission electron microscopy..

Influence of synthesis conditions on the rate of reduction of Ag^+ by RL-1

As it was mentioned in Abstract, for the green synthesis of AgNPs was used rhamnolipid RL-1 (Fig. 1) which contains both carboxyl and sugar functional groups, as well as long hydrocarbon chains, and therefore can simultaneously act as a ligand for silver ions, reducing agent Ag^+, as well as a surfactant. i.e. a stabilizer of AgNPs.
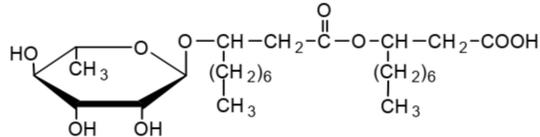

Fig. 1. Structural formula of RL-1.

In order to optimize the methods of obtaining the components of bactericidal drugs based on silver nanoparticles, the influence of synthesis conditions (initial concentrations of OH^-, Ag^+ and RL-1, as well as temperature) on the kinetics of formation of colloidal solutions of silver was studied using the UV-vis spectroscopy (Fig. 2).
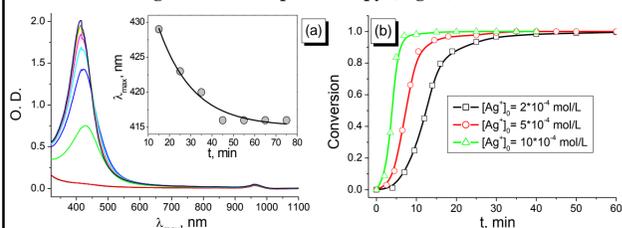

Fig. 2. Evolution of the absorption spectra of working solutions during the synthesis of AgNPs (initial concentrations of Ag^+ and RL-1 are equal to 0.2 mM and 1 g/L respectively, temperature 70 °C, insertion is the changing of the position of the PPR band over time) (**a**) and kinetic curves of AgNPs formation at different concentrations of Ag^+ (**b**).

It was found that in the all of studied experimental conditions:

✓ the spectra of the solutions after the completion of the synthesis are characterized by a clear absorption maximum, which may indicate the spherical shape of the obtained AgNPs;

✓ during the synthesis there is a shift of the absorption maximum in the short-wavelength region (Fig. 2) due to the position of the surface plasmon resonance (PPR) band is significantly influenced by the value of the dielectric constant of the medium, and in the case of surfactants that are adsorbed on the particle surface by the interaction between surfactant molecules and surface atoms of the growing silver particle; with increasing of particle size during synthesis the fraction of surface atoms that interact with surfactant molecules decreases, which, accordingly, affects the position of the PPR band of the AgNPs solutions;

✓ due to the optical characteristics of AgNPs solutions may be used for the evaluation of kinetics of their formation [4, 5], the values of the maximal absorbance were used for the plotting of the kinetic curves of AgNPs formation and it was found that all kinetic curves are sigmoidal that suggest the autocatalytic nature of the process;

✓ the rates of nucleation and growth of AgNPs linearly depend on the initial concentration of RL-1 and Ag^+ that is means the first order of the processes of nucleation and growth of AgNPs on RL-1 and Ag^+.

Table 1. Dependency of the kinetic parameters of AgNPs formation on the reaction conditions

pH	$[Ag^+]_o$, mM	RL-1, g/L	t, °C	t_{ind}, min	dC/dt, min⁻¹
8	0.2	4	70	45	0.008
9				7	0.054
11				3	0.09
10	0.2	4	70	5.0	0.08
	0.5			3.3	0.13
	1			1.8	0.25
10	0.2	1	70	9	0.04
		2		7	0.06
10	0.2	4	50	∞	0
			60	40	0.01
			80	2.5	0.14

Size and shape of obtained AgNPs

Obtained AgNPs were characterized using TEM. It was found (Fig. 3) that the obtained AgNPs are mostly irregular in shape. At the same time, a detailed analysis of microphotographs obtained at a high magnification (~ 100,000) revealed that large particles are surrounded by a large number of small (1–4 nm) particles (Fig. 3, insertion).
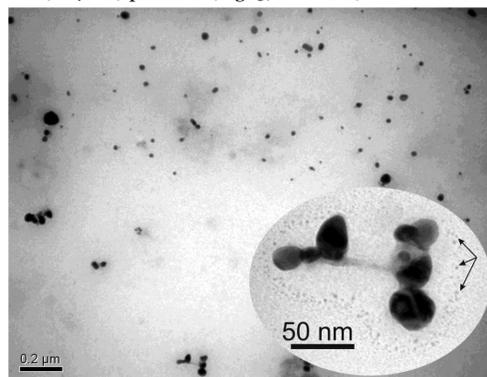

Fig. 3. TEM image of AgNPs obtained at 70 °C at initial concentrations of Ag^+ and RL-1 equal to 0.2 mM and 4 g/L respectively .

Such pattern was observed for the all tested samples and can be caused by the actual method of preparation of samples for TEM, namely: for TEM investigations the drop of liquid sample was dropped to a suitable substrate and dried in air. Thus, the concentration of AgNPs increased slowly; fluctuations of the concentrations of small particles could gradually be formed on the inhomogeneities of the substrate, which led to their uneven coagulation and, accordingly, to the formation of large (~ 10–40 nm) particles of irregular shape. Such assumption is well confirmed by the histograms of particle size distribution (Fig. 4).
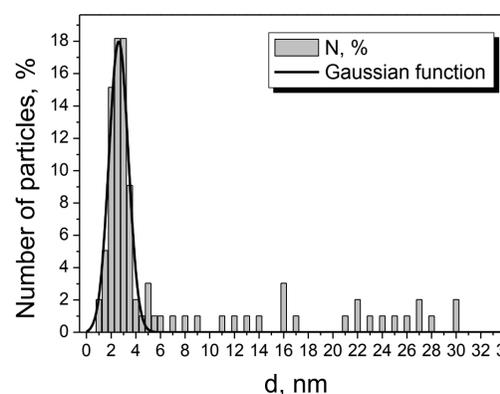

Fig. 4. Histogram of particle size distribution of AgNPs obtained at 70 °C at initial concentrations of Ag^+ and RL-1 equal to 0.2 Mm and 4 g/L respectively.

Table 2. Dependency of the size of obtained AgNPs on the reaction conditions

$[Ag^+]_o$, mM	RL-1, g/L	d±Δd, nm	The fraction of particles with a size within 1 ... 4 nm, %
0.2	4	2.6±1.4	70
0.5		2.6±1.3	70
1		2.6±1.2	75
0.2	1	4.8±4.3	40
	2	2.6±2.2	60

As it can be seen from the presented in Table 2 data, increasing of the concentration of silver ions has almost no effect on the size of the resulting AgNPs: the values of their average diameter (d) and polydispersity (Δd) are close. At the same time, with a decrease of the RL-1 concentration to 2 g/L, an increase of Δd AgNPs is observed, and with a further decrease of the stabilizer concentration, together with Δd, the value of the average particle diameter also increases.

Spectral characteristics of AgNPs stabilized by RL-1

In order to establish a possible mechanism of interaction of Ag^+ with RL-1, the absorption spectra of RL-1 and the product of its interaction with silver ions (AgNPs_RL) were studied using IR-spectroscopy (Fig. 5, Table 3).
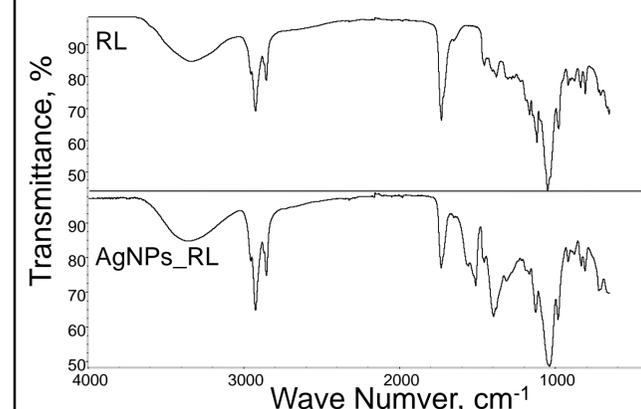

Fig. 5. IR-spectra of RL-1 and AgNPs_RL.

Table 3. Some characteristic absorption peaks of functional groups of RL-1 and AgNPs_RL

Functional group	Wave number, cm⁻¹		
	RL	AgNPs_RL	RL [6]
–O–H	3335	3355	3391
C–H (stretching)	2923	2924	2925
C–H (stretching in methyl and methylene groups)	2855	2854	2854
C=O	1730	1732	1739
?	–	1560	–
?	–	1510	–
C–H (bending)	1456	1456	1460
	1378	1396	1382
C–O	1048	1038	1045

As it can be seen from Fig. 5 and presented in Table 3 data, the peaks of absorption of RL-1 and AgNPs_RL are quite similar excepting the section within 1600 ... 1500 cm⁻¹. In the section within 1600 ... 1500 cm⁻¹ of the AgNPs_RL spectrum two maxima at 1560 and 1510 cm⁻¹ are observed. We assume that these bands can be attributed to the valence oscillations of the C=O group in the oxidized rhamnose ring (ketoramnose) similarly to that observed in β-diketones [7] .

Thus, due to the extreme dependence of the rate of reduction of Ag^+ by rhamnolipid RL-1 on pH and temperature, we can assume that the reducing agent for silver ions is a rhamnose ring, and its oxidation occurs through the formation of alkoxides in an alkaline environment [8] with the subsequent formation of ketoramnose:
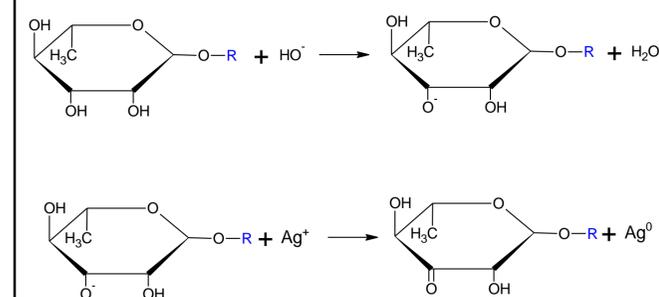

Fig. 6. Probable oxidation scheme of RL-1 with silver ions.

However, this scheme requires a more substantiated justification with the involvement of additional theoretical and experimental research methods.

REFERENCES
(1) Kuntyi O, Mazur A, Kytsya A, Karpenko O, Bazylyak L, Mertsalo I, Pokynbroda T, Prokopalo A (2020) Electrochemical synthesis of silver nanoparticles in solutions of rhamnolipid. Micro Nano Lett 15(12):802-807. https://doi.org/10.1049/mnl.2020.0195;
(2) Bazylyak L, Kytsya A, Karpenko O, Prokopalo A, Pokynbroda T, Mazur A, Kuntyi, O (2020) Synthesis of silver nanoparticles using the rhamnolipid biocomplex of microbial origin. Visn Lviv Univ Ser Chem 61(2):404-413. https://doi.org/10.30970/vch.6102.404;
(3) Bazylyak L, Kytsya A, Karpenko I, KarpenkoO (2020) Synthesis and antimicrobial activity of silver nanoparticles stabilized by citrate anions. Proc Shevchenko Sci Soc Chem Sci LX:127-135. https://doi.org/10.37827/ntsh.chem.2020.60.127;
(4) Sandoe HE, Watzky MA, Diaz SA (2019) Experimental probes of silver metal nanoparticle formation kinetics: Comparing indirect versus more direct methods. Int J Chem Kinet 51:861-871. https://doi.org/10.1002/kin.21315;
(5) Kytsya A, Bazylyak L, Simon P, Zelenina I, Antonyshyn I (2019) Kinetics of Ag300 nanoclusters formation: The catalytically effective nucleus via a steady-state approach. Int J Chem Kinet 51(4):266-273. https://doi.org/10.1002/kin.21249;
(6) Kumar CG, Mamidyala SK, Das B, Sridhar B, Devi GS, Karuna MS (2010) Synthesis of biosurfactant-based silver nanoparticles with purified rhamnolipids isolated from Pseudomonas aeruginosa BS-161R. J Microbiol Biotechnol 20(7):1061-1068. https://doi.org/10.4014/jmb.1001.01018;
(7) Тарасевич, Б. Н. (2012). ИК спектры основных классов органических соединений. М.: МГУ;
(8) Gomes JF, Garcia AC, Ferreira EB, Pires C, Oliveira VL, Tremiliosi-Filho G, Gasparotto LH (2015) New insights into the formation mechanism of Ag, Au and AgAu nanoparticles in aqueous alkaline media: alkoxides from alcohols, aldehydes and ketones as universal reducing agents. Phys Chem Chem Phys 17(33):21683-21693. https://doi.org/10.1039/C5CP02155C.