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Impact of silver nanoparticles synthesized by green method and microemulsion loaded with the nanoparticles on the development of cress

Natalia E. Sedyakina,*[a] Nataliya B. Feldman,[a] Oksana I. Gudkova,[a] Alexandr L. Rozofarov,[a] Vladimir N. Kuryakov[b] and Sergey V. Lutsenko[a]

[a] *Department of Biotechnology, I. M. Sechenov First Moscow State Medical University, 119991 Moscow, Russian Federation. Fax: +7 499 248 0181; e-mail: nsedyakina@mail.ru*

[b] *Oil and Gas Research Institute, Russian Academy of Sciences, 119333 Moscow, Russian Federation*

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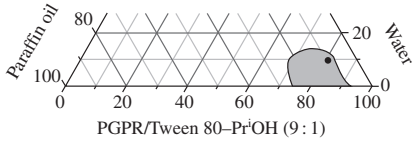
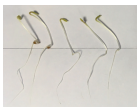
Water-in-oil microemulsion stabilized by PGPR/Tween 80 and isopropyl alcohol was prepared for encapsulation of silver nanoparticles obtained by mycosynthesis. Incorporation into the microemulsion resulted in less phytotoxicity of the particles to seed germination and seedlings development of *Lepidium sativum*.





L. sativum seedlings

Keywords: water-in-oil microemulsion, polyglycerol polyricinoleate, plant protection agent, drug delivery system, silver nanoparticles.

Silver nanoparticles (AgNPs) find an application in textiles, food industry, medicine and cosmetics due to their antibacterial, antifungal, antiviral, anti-inflammatory and antitumor activities.[1–7] However, toxic effects of AgNPs have been also demonstrated to human cells, animals, higher plants, algae and bacteria.[8–14] Mechanism of the adverse action is associated with disruption of cell membrane, the reactive oxygen species (ROS) production and the related oxidative stress as well as damage to proteins and DNA even at non-cytotoxic doses.[15–19] The toxicity of AgNPs including their interaction with higher plants as primary producers and an important component of ecosystem has been investigated.[20,21] The results indicate that the phytotoxicity is caused mainly by oxidative stress and depends on the plant species and age as well as the nanoparticles concentration, type of coating, size, surface morphology, surface charge and the way of preparation.[22,23]

It is known that microemulsions are employed as delivery systems for plant protection agents to reduce their toxic effect on crop.[24–26] In this work, we used a similar carrier to diminish the phytotoxicity of AgNPs, namely water-in-oil microemulsion formulation, which comprised paraffin oil, polyglycerol polyricinoleate (PGPR)–Tween 80 mixture (3.4 : 1 wt) with hydrophilic–lipophilic balance (HLB) of 6.15 as a surfactant as well as isopropyl alcohol as a cosurfactant. The microemulsion composition was optimized through evaluation of its thermodynamic and kinetic stability, then AgNPs sol was incorporated into the microemulsion as an aqueous phase.

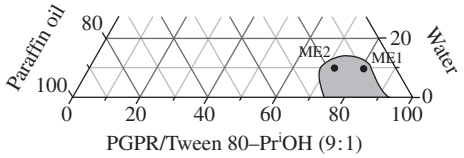


Figure 1 Pseudo-ternary phase diagram for the system consisting of paraffin oil, PGPR/Tween 80–PriOH (9 : 1 wt) mixture and water. The area colored in grey corresponds to the monophasic region. Symbols (•) denote the two compositions ME1 and ME2 chosen for further investigation.

From the known techniques for the AgNPs preparation,[27–29] green synthesis was chosen to avoid dealing with toxic chemical agents. The antimicrobial and phytotoxic effects of the microemulsion loaded with AgNPs in comparison with original AgNPs were evaluated.

The range of components concentrations suitable for the microemulsion was determined in several steps. At first, the phase diagrams for various PGPR–Tween 80 mixtures with HLB values in the range of 5.8–6.25 were constructed.[†] As a result, the mixture with HLB = 6.15 was chosen, because it promoted the formation of microemulsions with lower amount of emulsifier. Then, the pseudo-ternary component systems with different surfactant–Pr^{i}OH ratios, namely 1 : 1, 2 : 1 and 9 : 1 by weight, were tested. The last ratio revealed the best microemulsion forming tendency. The corresponding phase diagram is represented in Figure 1.

Next, two microemulsion formulations, namely ME1 and ME2, (see Figure 1, Table 1) were chosen in the monophasic region for investigation of thermodynamic and kinetic stability. Both

[†] For construction of each phase diagram, paraffin oil (Hansen & Rosenthal KG) as well as a blend of PG-3-PR (Gobiotics BV) and Tween 80 (Sigma–Aldrich) with various HLB and surfactant–cosurfactant weight ratios were mixed carefully. Each mixture was gradually titrated with distilled water. The time for equilibration between the additions of successive aliquots was 1–24 h. The monophasic region corresponding to homogeneous, clear, optically transparent and fluid water-in-oil microemulsion was detected by visual observation.[30] Each composition was tested in triplicate.

Mean droplet size (particle diameter) for the microemulsion samples was measured using a Photocor Complex multi-angle dynamic and static light scattering instrument (Russia) with an avalanche photodiode photon counting system at 25 °C at the scattering angle of 90°.

Viscosity of the microemulsions was determined using an SV-10 vibro-viscometer (A&D). Each experiment was carried out in triplicate.

Table 1 Composition and characteristics of the microemulsion formulations.

Micro-emulsion sample	Paraffin oil (wt%)	PGPR/Tween 80–Pr^{i}OH (wt%)	Aqueous phase (wt%)	Droplet size/ nm	η/Pa s
ME1	9	81	10[a]	88 ± 24	2.4 ± 0.3
ME2	18	72	10[a]	–	–
MEAgNPs	9	81	10[b]	74 ± 8	2.1 ± 0.4

[a] Distilled water. [b] AgNPs sol.

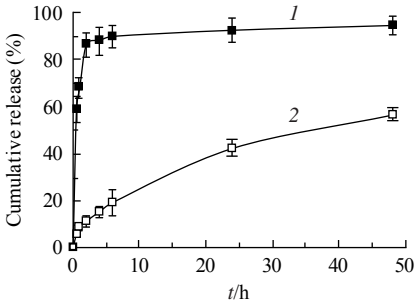


Figure 2 Release of MB *in vitro* in PBS at 37 °C from (*1*) free dye solution and (*2*) dye-loaded microemulsion, data represent the mean ± SD (n = 3).

compositions had yellow color as well as were homogeneous and optically isotropic. The formulation ME1 sustained freeze–thaw and heating–cooling cycles as well as demonstrated kinetic stability for two months, whereas phase separation was detected for the ME2 formulation. Thus, for the composition ME1 the mean droplet size and viscosity were determined for further exploration (see Table 1).

Methylene blue (MB) dye as a model of water-soluble drug was incorporated into the ME1 microemulsion, and *in vitro* release of the dye in PBS (pH 7.4) was followed for 48 h compared with free MB solution as a control (Figure 2).

The dye-loaded microemulsion batch revealed no burst release and ~60% of MB was liberated after 48 h, compared with 86% for control after 2 h. The sustained release of the dye originated from its slow diffusion through the layers of surfactants adsorbed at the surface of the dispersed phase droplets and from the viscous nature of continuous phase in the water-in-oil microemulsion.

AgNPs used in this work were synthesized by the known green-type mycosynthesis[31] and exhibited a particle size of 26 nm and a zeta potential value of −41.6 mV (for details, see Online Supplementary Materials). The AgNPs sol was introduced as an aqueous phase into the mixture corresponding to microemulsion ME1, resulting in AgNPs-loaded microemulsion (MEAgNPs) with overall concentration of AgNPs 50 μg ml^{-1} (see Table 1) and no remarkable difference in parameters compared with the pristine ME1 microemulsion.

Antibacterial and antifungal activity of the formulations against *Escherichia coli* ATCC 8739 and *Fusarium sporotrichioides* T11 VKPM F-902 was investigated in undiluted form as well as after two-, four- and eightfold dilutions. The activity was compared with aqueous AgNPs sol and pristine ME1 microemulsion at the same dilutions (Table 2). Undiluted MEAgNPs exhibited less inhibition than the aqueous AgNPs sol with an equal silver concentration, probably due to the difficulty of particles diffusion from the microemulsion into agar. However, after dilution of the microemulsion two times, its activity increased and then gradually decreased with further dilutions. In this case, two concentric zones were detected, namely (i) the diffusion zone of the microemulsion and (ii) a small increase in the diameter of suppressed microbial growth zone due to the diffusion of AgNPs from the microemulsion to agar (Figure S1, Online Supplementary Materials).

Table 2 Growth inhibition of *E. coli* and *F. sporotrichioides* by the formulations synthesized.

	E. coli inhibition zone/mm[a]				*F. sporotrichioides* inhibition zone/mm[a]			
Dilution of sample	–	2	4	8	–	2	4	8
ME1	9	0	0	0	0	0	0	0
AgNPs[b]	18.5	–	–	–	18.8	–	–	–
MEAgNPs	12.2	14.5	13.4	10.6	11.5	15.1	0	0

[a] RSD less than 10% (n = 3). [b] Aqueous sol.

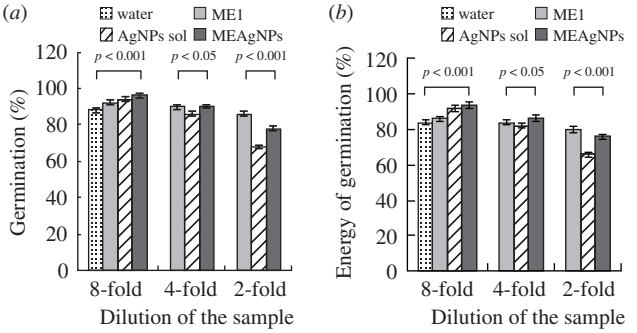


Figure 3 Effect of the formulations synthesized on (*a*) germination and (*b*) the energy of germination for *L. sativum* seeds.

Evaluation of the effect of the formulations synthesized on seed germination and plant growth was carried out using curled cress (*Lepidium sativum* L. var. *crispum*) as a test organism (Figure 3).‡ ME1 microemulsion at the largest eightfold dilution had a weak stimulating effect on the germination of cress seeds. The values of germination and the energy of germination for fourfold dilution of the microemulsion were comparable with those of control, and for twofold dilution they were slightly lower than the control. The same trend was observed for the AgNPs sol, namely the concentration of 6.25 μg ml^{-1} had a stimulating effect, while the concentration of 25 μg ml^{-1} had a pronounced inhibitory action.

When the MEAgNPs in the largest eightfold dilution (6.25 μg ml^{-1}) were tested, synergistic effect was observed: germination and the energy of germination were higher than for exposure to the corresponding components in equal concentrations. At the lowest twofold dilution (25 μg ml^{-1}), the composition had a significantly less pronounced inhibitory effect on seed germination (78%) compared with the AgNPs sol in equal concentration (66%), possibly due to a decrease in toxicity of AgNPs included in the microemulsion.

To determine the effect of the formulations on further plant development, the length of root and hypocotyl for seedlings was measured on the day 7 of germination (Figure 4). ME1 microemulsion had a significant inhibitory effect with weakly expressed dose-dependent growth of the root and, conversely, stimulating effect to the growth of hypocotyl. The AgNPs sol demonstrated pronounced dose-dependent inhibition of the hypocotyl growth. With regard to development of the main root, the sol revealed significant inhibitory effect with root length 42% of control in

‡ Germination was carried out with 100 seeds × 3 groups in each run. Before germination, the seeds were incubated for 1 h with 2-, 4- and 8-fold dilutions of aqueous AgNPs sol, the unloaded ME1 microemulsion and MEAgNPs, while control groups of seeds were soaked in water. After incubation, the seeds were germinated in Petri dishes over a wet filter paper at 20 °C in the dark. The energy of germination after 3 days, germination after 5 days, length of hypocotyls after 7 days and length of the main root after 7 days were evaluated (Figure S2), the results were expressed as mean ± SD. The ANOVA method was used for comparison of groups, the difference was considered to be significant at $p < 0.05$.

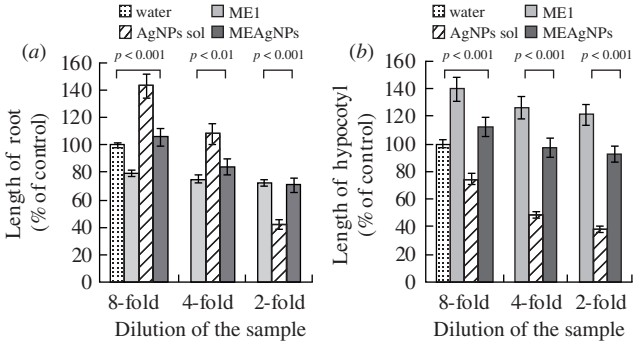


Figure 4 Effect of the formulations synthesized on (*a*) the length of main root and (*b*) hypocotyl of *L. sativum* seedlings after 7 days of germination.

the highest concentration and stimulating effect (143% of the control) in the lowest one.

The use of MEAgNPs significantly smoothed these effects in contrast to individual components. At the greatest dilution, slight growth stimulation was manifested for both the main root and hypocotyl. At the largest concentration, MEAgNPs inhibited growth of hypocotyl by 8% and the main root by 29%, whereas for the AgNPs sol inhibition of hypocotyl and root was by 62 and 58%, respectively.

Thus, there was a significant reduction in toxic effect of AgNPs due to their incorporation in the microemulsion. In summary, a new water-in-oil microemulsion stabilized by PGPR/Tween 80 and isopropyl alcohol as surfactant and co-surfactant, respectively, was prepared. The AgNPs obtained using green-type mycosynthesis were incorporated into the microemulsion. AgNPs in their aqueous sol and incorporated forms were tested for effect on germination and the energy of germination of cress seeds as well as on the length of the main root and hypocotyl of the cress seedlings. As a result, AgNPs incorporation into the microemulsion resulted in less phytotoxicity compared with the initial AgNPs sol. This approach is promising for development of efficient microemulsion-based delivery systems for prolonged release of AgNPs for further agrochemical application.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.05.011.

References

1 X.-F. Zhang, Z.-G. Liu, W. Shen and S. Gurunathan, *Int. J. Mol. Sci.*, 2016, **17**, 1534.
2 J. Pulit-Prociak, J. Chwastowski, A. Kucharski and M. Banach, *Appl. Surf. Sci.*, 2016, **385**, 543.
3 O. Suvorov, G. Balandin, L. Shaburova, D. Podkopaev, S. Volozhaninova and G. Ermolaeva, *Mater. Today: Proc.*, 2017, **4**, 6863.
4 D. Macocinschi, D. Filip, M. F. Zaltariov and C. D. Varganici, *Polym. Degrad. Stab.*, 2015, **121**, 238.
5 S. Kokura, O. Handa, T. Takagi, T. Ishikawa, Y. Naito and T. Yoshikawa, *Nanomedicine*, 2010, **6**, 570.
6 D.-l. Su, P.-j. Li, M. Ning, G.-y. Li and Y. Shan, *Mater. Lett.*, 2019, **244**, 35.
7 S. S. Nogueira, A. R. de Araujo-Nobre, A. C. Mafud, M. A. Guimarães, M. M. M. Alves, A. Plácido, F. A. A. Carvalho, D. D. R. Arcanjo, Y. Mascarenhas, F. G. Costa, P. Albuquerque, P. Eaton, J. R. de S. de A. Leite, D. A. da Silva and V. S. Cardoso, *Int. J. Biol. Macromol.*, 2019, **135**, 808.
8 R. Vazquez-Muñoz, B. Borrego, K. Juárez-Moreno, M. García-García, J. D. M. Morales, N. Bogdanchikova and A. Huerta-Saquero, *Toxicol. Lett.*, 2017, **276**, 11.
9 A. I. Perfileva, O. A. Nozhkina, I. A. Graskova, A. V. Sidorov, M. V. Lesnichaya, G. P. Aleksandrova, G. Dolmaa, I. V. Klimenkov and B. G. Sukhov, *Russ. Chem. Bull., Int. Ed.*, 2018, **67**, 157 (*Izv. Akad. Nauk, Ser. Khim.*, 2018, 157).
10 A. Barbasz, M. Oćwieja and M. Roman, *Colloids Surf., B*, 2017, **156**, 397.
11 E. E. Elsharkawy, M. A. El-Nasser and H. F. Kamaly, *Environ. Toxicol. Pharmacol.*, 2019, **70**, 103194.
12 H. Liu, X. Wang, Y. Wu, J. Hou, S. Zhang, N. Zhou and X. Wang, *Environ. Pollut.*, 2019, **246**, 414.
13 S. Zheng, Q. Zhou, C. Chen, F. Yang, Z. Cai, D. Li, Q. Geng, Y. Feng and H. Wang, *Sci. Total Environ.*, 2019, **660**, 1182.
14 A. Cox, P. Venkatachalam, S. Sahi and N. Sharma, *Plant Physiol. Biochem.*, 2016, **107**, 147.
15 T. Zhang, L. Wang, Q. Chen and C. Chen, *Yonsei Med. J.*, 2014, **55**, 283.
16 R. Kannaujia, C. M. Srivastava, V. Prasad, B. N. Singh and V. Pandey, *Plant Physiol. Biochem.*, 2019, **142**, 460.
17 A. A. Dayem, M. K. Hossain, S. B. Lee, K. Kim, S. K. Saha, G.-M. Yang, H. Y. Choi and S.-G. Cho, *Int. J. Mol. Sci.*, 2017, **18**, 120.
18 A. El-D. H. Sayed and H. A. M. Soliman, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2017, **822**, 34.
19 P. Cvjetko, A. Milošić, A.-M. Domijan, I. V. Vrček, S. Tolić, P. P. Štefanić, I. Letofsky-Papst, M. Tkalec and B. Balen, *Ecotoxicol. Environ. Saf.*, 2017, **137**, 18.
20 R. M. Galazzi, C. A. Lopes, Jr., T. B. de Lima, F. C. Gozzo and M. A. Z. Arruda, *J. Proteomics*, 2019, **191**, 88.
21 P. Cvjetko, M. Zovko, P. P. Štefanić, R. Biba, M. Tkalec, A.-M. Domijan, I. V. Vrček, I. Letofsky-Papst, S. Šikić and B. Balen, *Environ. Sci. Pollut. Res.*, 2018, **25**, 5590.
22 Y. S. El-Temsah and E. J. Joner, *Environ. Toxicol.*, 2012, **27**, 42.
23 L. Yin, B. P. Colman, B. M. McGill, J. P. Wright and E. S. Bernhardt, *PLoS One*, 2012, **7**, e47674.
24 S. Engelskirchen, R. Maurer, T. Levy, R. Berghaus, H. Auweter and O. Glatter, *J. Colloid Interface Sci.*, 2012, **388**, 151.
25 P. Leng, Z. Zhang, Q. Li, M. Zhao and G. Pan, *PLoS One*, 2014, **9**, e109580.
26 J. Hu, N. Akula and N. Wang, *PLoS One*, 2016, **11**, e0150433.
27 S. H. Lee and B.-H. Jun, *Int. J. Mol. Sci.*, 2019, **20**, 865.
28 R. R. Fazleeva, G. R. Nasretdinova, Yu. N. Osin and V. V. Yanilkin, *Russ. Chem. Bull., Int. Ed.*, 2019, **68**, 1525 (*Izv. Akad. Nauk, Ser. Khim.*, 2019, 1525).
29 S. M. Mousavi, S. A. Hashemi, Y. Ghasemi, A. Atapour, A. M. Amani, A. S. Dashtaki, A. Babapoor and O. Arjmand, *Artif. Cells, Nanomed., Biotechnol.*, 2018, **46**, S855.
30 N. E. Sedyakina, A. F. Krivoshchepov, A. Ya. Zasypko, A. G. Demchenko, A. L. Rozofarov, V. N. Kuryakov, N. B. Feldman and S. V. Lutsenko, *Mendeleev Commun.*, 2019, **29**, 320.
31 P. C. Nagajyothi, T. V. M. Sreekanth, J.-i. Lee and K. D. Lee, *J. Photochem. Photobiol., B*, 2014, **130**, 299.

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