OBTAINING OF ELEMENTAL SILVER NANOPARTICLES (SNPs) WITH USING PLANT AND ANIMAL PRODUCTS

Oleg V. Mikhailov

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The data on the specifics of synthesis of elemental silver nanoparticles (SNPs) having the shapes of various geometric shapes (pseudo spherical, prismatic, cubic, trigonal-pyramidal, etc.), obtained by using various plant and animal substrates, and fields of their possible use, have been systematized and generalized. The review covers mainly articles published in the current XXI century.

*Corresponding Author
E-Mail: olegmkhlv@gmail.com
[a] Kazan National Research Technological University, K. Marx Street 68, 420015 Kazan, Russia

INTRODUCTION

In recent years, there has been a very pronounced increase in interest in both fundamental and applied branches of science associated with nanoparticles' synthesis, especially nanoparticles of elemental metals, the study of their properties and practical applications. Progress in this area is primarily due to the rapid development of experimental and synthetic methods for the preparation and study of materials based on nanoparticles, which have high hopes in connection with their possible application in various industries of anthropogenic activity, in particular optics, microelectronics, digital technologies, etc., also, in pharmacology, medicine and light industry. As is well known, the smaller the particle size of the substance, the greater part of them is in the surface layer of the material; this circumstance leads to the fact that nanoparticles in general and nanoparticles of elemental metals, in particular, have a number of unique properties that are not observed for macroparticles and even for microparticles. The use of elemental metal nanoparticles took place in ancient times – at least, since the II century, when so-called "Holy Water", which is not exposed to infection by microorganisms and spoilage for many months and years, became known; moreover, it has a very detrimental effect on a wide variety of pathogenic microorganisms. However, only at the end of the XIX century, the phenomenon “oligodynamia” – the silver bactericidal effect on the cells of microorganisms by Ag^+ ions was found by the Swiss botanist K. Nägeli.[1] However, a more detailed study about the antibacterial activity of "Holy Water," carried out in the second half of the XX century, showed that it is connected with both the presence of Ag^+ ions and the presence of silver nanoparticles (in further, SNPs).[2] Herewith, among elemental metal nanoparticles, namely elemental silver has the strongest bactericidal effect,[3,4] which is a direct consequence of the optimal ratio of their surface areas and volumes. Now Silver ions showed bactericidal, bacteriostatic, antiviral, antifungal and antiseptic effects of silver ions and SNPs suspensions on more than 500 pathogenic microorganisms, yeast fungi and viruses. Moreover, their antibacterial and antiviral activity is even more pronounced than the effect of penicillin, biomycin and other "classic" antibiotics.[5,6] The low probability of certain mutations with the result of resistance to SNPs becomes extremely important in the struggle of microbiologists with an ever-growing assortment of pathogenic bacteria and viruses that are resistant to traditional antibiotics. This important circumstance, relatively low toxicity and allergenicity of SNPs, and its good tolerance by patients, contributed to the increased interest in SNPs in many countries of the world and the creation of various medical preparations anti-inflammatory, antiseptic and bactericidal action on their basis. It should be noted in this connection that, in small concentrations, elemental silver nanoparticles are safe for cells of mammalian organisms, but they are detrimental to the cells of the vast majority of microscopic fungi and bacteria, as well as viruses.

The study of the mechanism of antibacterial activity of SNPs showed that this property is due to morphological and structural changes in bacterial cells.[2] A priori, it is obvious that this effect's degree should very significantly depend on the size and shape of SNPs. On the other hand, in many studies of recent decades, the size, morphology, stability, and both chemical and physical properties of elemental metals nanoparticles, including silver, were very dependent on the parameters of the processes in which they are formed. These parameters are directly related to the specifics of the given processes, reactions of the metal ions interaction with reducing agents, and sorption processes of stabilizing agents on nanoparticles, which prevent their aggregation with each other[7]. In general, control of the shape, size, and distribution of the resulting SNPs is achieved by varying the synthesis methods, reducing the influence of fluctuations and stabilizing factors affecting the above parameters of nanoparticles.[8-12] By varying the conditions for the synthesis of SNPs, characteristics, such as color, melting point, magnetic properties, Ag(I) / Ag redox potential, etc., can be changed and controlled in a fairly wide range.[13-18] Comprehensive coverage of SNPs problems was presented in reviews.[17-20] However, the main focus was on the synthesis of SNPs using various physicochemical methods. Another synthetic method, in which some objects of biological origin are used to obtain elemental silver nanoparticles, has become increasingly popular. This approach has certain advantages compared to traditional physicochemical methods. The possibilities of its implementation for SNPs' production are not only not exhausted, but even not completely identified. The given review is dedicated to this issue.

KEY PRINCIPLES OF SNPs OBTAINING

The basis of all chemical and physicochemical methods for the synthesis of SNPs is the idea of a specific increase in the area of their faces in the presence of certain chemical reagents. Biological methods base on similar ideas, but biological objects (microorganisms, products of their vital activity, extracts of plants, etc.) are used to form SNPs. As a rule, SNPs is obtained as a result of the reduction of certain Ag(I) compounds (usually $AgNO_3$), under the influence of various reducing agents – inorganic (e.g.., hydrazine N_2H_4, sodium tetrahydridoborate(III) $Na[BH_4]$), as well as organic (e.g., ethylene glycol, ascorbic acid). The process of reducing Ag(I) compounds to elemental silver according to the Ag(I)→Ag scheme can occur in a variety of reaction media; at the same time, specific chemical reagents (most often polyvinylpyrrolidone) are used to stabilize the resulting nanoparticles (to prevent their aggregation). The formation of SNPs, according to data,[17,21,22] begins with the incipience of a decahedral "embryo" formed by five tetrahedral clusters (Figure 1*a*) that have common faces. Since the dihedral angle in the tetrahedron is ~ 70.5°, namely the decahedron, consisting of five tetrahedrons (Figure 1*b*), is that structural element from which the most thermodynamically stable forms of SNPs.

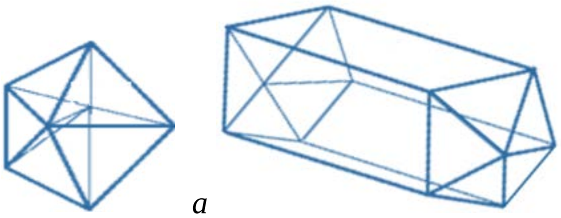


Figure 1. Scheme of growth of SNPs; *a* – "starting" decahedral "embryo", *b* – SNPs composed of decahedral "embryos" and having pentadecahedron shape.

It should be noted that the dihedral angle in the tetrahedron indicated above is slightly smaller than the angle in the above decahedron (72°); therefore, tetrahedrons cannot be perfectly packed into a decahedron without gaps (which leads to the formation of structural defects).[17] The scheme in Figure 1 displays only the simplest variant of SNPs formation, which leads to the formation of elemental silver nanocrystals in the form of nanorods or "nanowires" (Figure 1*b*). In reality, this process often turns out to be significantly more complex and interesting, and as a result, other geometric forms – tabular prisms, cubes, octahedrons, pyramids, etc., can be realized.[17,21] As it was shown in various studies, the shape and size of the resulting SNPs depend on many experimental parameters. It can be the temperature, the concentration of the compound Ag(I), the pH of the solution, the molar ratio between the stabilizing agent and the precursor containing Ag(I) ($AgNO_3$, AgCl, et al.), the degree of chemical interaction of the molecules of the stabilizing agent with various crystallographic planes of elemental silver.[22] The nature of the reducing agent of Ag(I) compounds (citric acid, L-ascorbic acid, $Na[BH_4]$ et al.) and the method used to produce SNPs (chemical, physical or biological) play a significant role, too. Using particles with a specific morphology (for example, polyhedral or twin) as a "primer", it is possible under certain synthesis conditions to purposefully control the final "architecture" of SNPs.[17,23,24]

The chemical and physicochemical methods used to produce SNPs are usually quite expensive; in addition, toxic chemical compounds are often used in them. Biological methods are practically devoid of these disadvantages. Of course, they are also not ideal: it should be noted that the SNPs synthesis results' reproducibility is worse than in the case of physicochemical methods. Despite this, the number of works about SNPs biosynthesis has a pronounced tendency to annual growth. In modern literature, SNPs biosynthesis is often called "green synthesis".

The three key types of biological objects used for this purpose – extracts of various plants, various microorganisms, and animal products, can be distinguished in SNPs biosynthesis literature. It should be noted that many works devoted to this problem were published either in biological journals or in medical journals. Most of these publications devoted not only to SNPs biosynthesis but also the possibility of using silver nanoparticles as antibacterial agents. This aspect of SNPs characterization is very important and interesting, and it will also be analyzed in this review, along with a discussion of their synthesis.

Now there are a lot of papers in the field of the SNPs "green synthesis". In this regard, a difficult question arises as to how to systematize the available material on the given problem. The “catchiest” characteristic of nanoparticles and SNPs is undoubtedly their shape because it largely determines their properties (including the properties of the materials in the composition of these nanoparticles). For this reason, available literally material was systematized by the types of biological objects used for the synthesis of SNPs and by the shape of elemental silver nanoparticles formed in this process.

Getting a little ahead in the course of the presentation, we would like to note that such geometric shapes of SNPs, which were obtained using chemical and physicochemical methods, apparently, could also be obtained using biological methods, although not all of these possible shapes were received experimentally.

SNPs OBTAINING WITH USING PLANTS PRODUCTS

Plant extracts obtained from leaves, stems, roots, etc., as the result of exposure to various liquid solvents – extractants. Water, ethanol, dimethyl ether, plant oil, etc., can act as extractants. Plant extracts are complex in composition, containing various chemical compounds arising in the process of plant life and transferring into solution during extraction. In the process of the synthesis of SNPs according to the general scheme Ag(I)→Ag, the chemical compounds contained in the extract can fulfill three functions: 1) act as a reducing agent of silver(I) compounds to elemental silver, 2) act as an agent that has a specific influence on the formation of a certain shape and size of SNPs due to inhibition of the growth of certain faces of the nanocrystals of these nanoparticles and 3) to act as a stabilizer formed during the synthesis of SNPs, preventing their self-association and (or) aggregation with each other. Due to the fact that the composition of the original plant material depends significantly on the type of a particular plant, the qualitative and quantitative composition of the resulting extracts can vary widely. Moreover, even for the same plant species, it does not remain constant and in some cases, depends significantly on the conditions of its growth. Therefore, it is likely that the technology of synthesis of SNPs using extracts of the same plant, but in laboratories located in different regions of our planet, can lead to very different final results (we mean the shape and size of elemental silver nanoparticles). Thus, the reproducibility of SNPs biosynthesis results will not be too good a priori. It should be noted that despite the very large number of works devoted to the SNPs biosynthesis using plant extracts, among them there are not even two such works in which extracts from the same plant species were used, but grown in different geographical, climatic and soil conditions.

An extremely large number of publications have been devoted to the "green synthesis" of SNPs using biological objects of this type.[25-108] In all of them, only $AgNO_3$ was used as a precursor containing Ag(I) for the SNPs synthesis. To obtain these extracts can be used in various parts of the plants. The leaves were used as raw materials for the preparation of extracts[25-74] in the vast majority of cases; much less often, they were obtained from fruits,[75-82] roots,[43,69,83-88] flowers,[53,69,89-92] underground shoots (tubers),[93-95] seeds and pods,[43,102] resinous secretions (gum).[101] Sometimes, plants as a whole were used for this purpose, namely algae and seagrasses [103,105,107,108] or young shoots of some plants.[104,106] In most of the experiments described in the literature, the particles having a spherical and/or oval (ellipsoidal) shape were shown; shapes differed from those were observed in the experiment much rarely.[64-74,82,86-88,92,95,98] In this connection, it should be noted that, spherical and oval shapes are conglomerates of smaller "embryonic" particles of elemental silver, which are complex combinations of two "starting" geometric forms shown in Figure 1.

To some extent, this can be confirmed by SEM images of spherical SNPs at high resolution,[24] shown in Figure 2. In this connection, it would be better to call such SNPs pseudospherical nanocrystals. Nevertheless, we will continue to use the generally accepted term to refer to these objects (i.e., spherical SNPs).

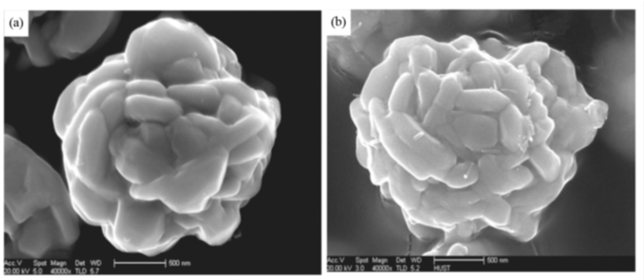


Figure 2. Typical SEM images of pseudo-spherical SNPs at high resolution**[24]**.

Among the earliest works of the XXI century devoted to the production of silver nanoparticles using plant extracts is the publication of Shankar, Ahmad and Sastry,[25] where Geranium *Pelargonium graveolens* leaf extract was used to synthesize SNPs. The spherical SNPs particles were obtained with the size varied in the range of 16–40 nm. Similar results were achieved by Chandran et al., [26] in which *Aloe Vera* leaf extract was used to synthesize SNPs. The spherical SNPs particles, the size of which varied in the range (15.2+4.2) nm, were shown. The authors of work[27] got spherical silver nanoparticles using *Capsicum annuum* L. extract with sizes from 50 to 70 nm (Figure 3). The results of the given work allow affirming that silver nanoparticles synthesized from such a method show antibacterial activity against *Escherichia coli*. Cruz et al.[28] obtained spherical SNPs with an average diameter of 15-30 nm, using leaf extract *Lippia citriodora* (Lemon Verbena).

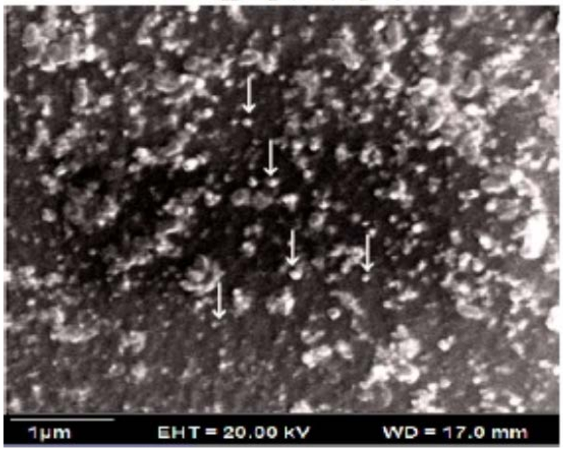


Figure 3. SEM images of spherical SNPs are described in the article.[27]

Close-sized SNPs were received in the work[29] using the leaf extract *Acalypha indica*. Spherical SNPs were also shown by the authors of[30] using an extract of the dried leaves of the plant *Tribulus terrestris*. The sizes of the SNPs synthesized by them were in the range of (18-47) nm. The obtained SNPs retained high stability (i.e., did not aggregate with each other) even after three months of storage at 37 °C.

Along with this, SNPs received in[30] showed a pronounced antibacterial effect on a number of clinically isolated microorganisms, which have now developed resistance to many drugs. SNPs of similar shape from the leaf extract of *Mimusops elengi*, were described by Prakash with co-authors in,[31] but these silver nanoparticles had diameters in the range of 55 to 83 nm. At all, in the last ten years, leaf extracts of various plants were used to synthesize spherical SNPs;[25–64,66,73] the information about these plants, as well as the size obtained with their use of elemental silver nanoparticles are presented in Table 1 at the end of this section of the given paper. However, in some cases, when using such extracts, the researchers observed SNPs' formation having a shape different from spherical – triangular, hexagonal, cubic et al.[65-74] In most cases, the formation of spherical SNPs took place when using extracts from other parts of plant media (substrates).[75-108] So, the group of researchers[75] received spherical silver nanoparticles using *Emblica officinalis* (amla, Indian Gooseberry) fruit extract with sizes from 15 to 25 nm. Also, elemental gold nanoparticles with slightly smaller (10-20 nm) sizes. In the article,[89] spherical SNPs 25–59 nm in size were synthesized using the *Chrysanthemum indicum* flower extract. As a rule, SNPs sizes were found by means of either scanning electron microscopy (SEM) or transmission electron microscopy (TEM). An example of SEM SNPs images obtained in[94] is presented on Figure 4. A few exceptions are publications where seaweed *Ulva lactucain*,[103] *Sargassum wightii*[105] and *Sargassum vulgare*[107] were used as an accompanying agent for "green synthesis" of SNPs. SEM image of SNPs obtained in[103] is shown in Figure 5. It is significant to note that the synthesis of silver nanoparticles in these works was carried out, as a rule, in a neutral medium. The spherical SNPs size was very diverse among using plant extracts (Table 1). At the same time, however, the question of the amount effect of plant extract used in the synthesis on SNPs size is rather rarely considered—one of such works is,[47] in which *Olax scandens* leaf extract was used in the synthesis process.

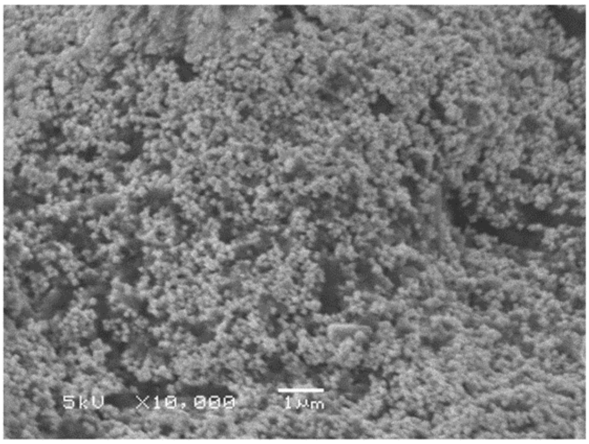


Figure 4. SEM image of SNPs obtained using aqueous peel extract of *Punica granatum*.[94]

According to the data presented in it, the average size of SNPs is 30-60 nm, however, the situation is rather more difficult: when the volume ratio of the $AgNO_3$ solutions used by them and the extract is 1:1, the size of SNPs is varied.

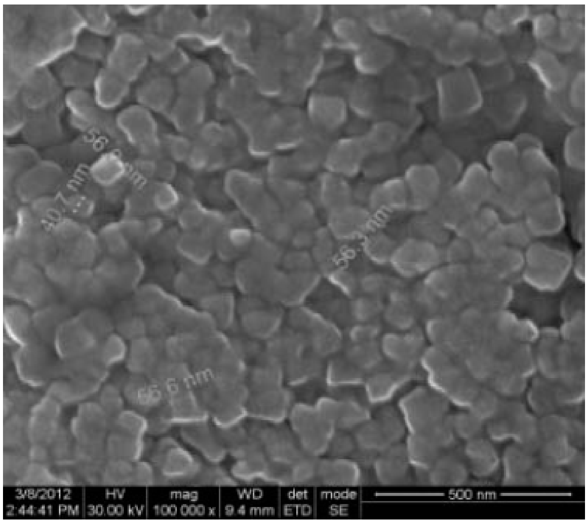


Figure 5. SEM image of SNPs obtained using aqueous extract of marine seaweed *Ulva lactucain*.[103]

in the range of 20-110 nm, at ratios of 1:2.67 and 1:3.33 – from 10 to 85 nm, with a ratio of 1:5 – from 10 to 90 nm.[47] Presumably, the same phenomenon would have also occurred with the use of other plant extracts indicated in Table 1, but this point remained out of their authors' field of view.

SNPs from plant extracts with a shape different from spherical were noted in a relatively small number of published works. In refs.,[32,65,97] the synthesis of trigonal SNPs having the shape of triangular plates was described. Hexagonal and spherical shapes of SNPs with a size of (121±2) nm were described by Arokiyaraj et al., using water extracts of *Rheum palmatum* roots.[88] The synthesized SNPs showed very high antibacterial activity against such pathogenic microorganisms as *Staphylococcus* and *Pseudomonas*. Using a leaf extract of *Alysicarpus monilifer*, the authors of[66] could obtain monodisperse SNPs of predominantly spherical shape with a small hexagonal distortion with sizes in the range (5–45) nm and average particle size (15±2) nm. Simultaneously, along with spherical SNPs, they discovered the formation of a certain amounts of SNPs having a trigonal tabular and hexagonal tabular shape. A small amount of the mixed-phase containing hexagonal SNPs was also noted in the article.[27]

SNPs with a cubic form from plant extracts were also known in refs.[67-69] In the first of these publications, elemental silver nanoparticles were prepared using a leaf extract of *Melia azedarach*. The cubic SNPs synthesized in[67] had a size of about 80 nm (Figure 6). The authors of the article[68] reported biosynthesis of SNPs having a spherical and cubic shape, using leaf extracts from *Eucalyptus macrocarpa* and carried out at room temperature.

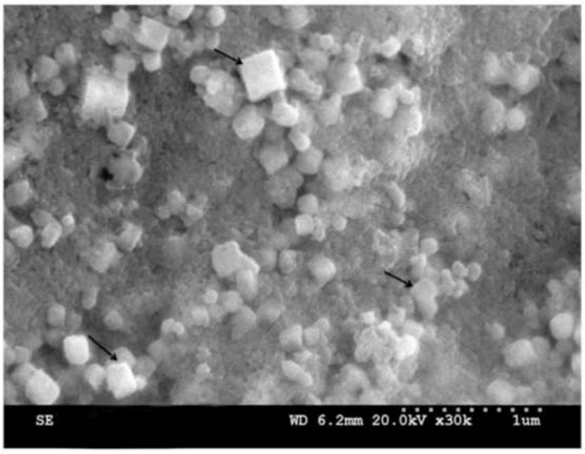


Figure 6. SEM image of SNPs obtained using aqueous leaf extract of *Melia azedarach*.[67]

During their experiment, it was also found that this extract acts both as a reducing and stabilizing agent in the process of synthesis. Herewith, according to the TEM data, the size of spherical SNPs was varied in the range (10–100) nm, while the size of cubic SNPs was in the range (10–50) nm. It should be noted in this connection that the three-dimensional FESEM image obtained several hours after the completion of the experiment showed that namely cubic but not spherical nanoparticles with sizes from 50 to 100 nm became the dominant shapes.[68] In the work,[69] three different plants were used to receive such cubic nanoparticles, namely *Cucurbita maxima*, *Moringa oleifera*, and *Acorus calamus*. In this regard, it is interesting that various parts of these plants (peels, leaves and rhizome, respectively) were used to prepare plant extracts. The sizes of silver nanoparticles obtained in[69] were varied in the range of 30–70 nm.

The authors of the work[70] observed the formation of prismatic SNPs with sizes of 22–65 nm using leaf extracts of *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* for "green synthesis." For these SNPs, a strong tendency toward coalescence was found, which is most represented in the case of *Centella asiatica* and least in the case of *Syzygium cumini* (Figure 7). Baharara et al. in the publication[92] described the synthesis of elemental silver nanoparticles, some of which had a pentagonal-tabular shape; in the absorption spectra of these SNPs, a distinct maximum was observed at 450 nm.[92] Along with this, in a number of works on biosynthesis using plant extracts, namely,[71-74, 82,95] other (mainly irregular) forms of SNPs were noted. So, in[72] by using leaf extract *Artemisia nilagirica*, silver nanoparticles shown in Figure 8 were synthesized. As can be seen, they are shapeless conglomerates and blocks resembling a pile of stones.

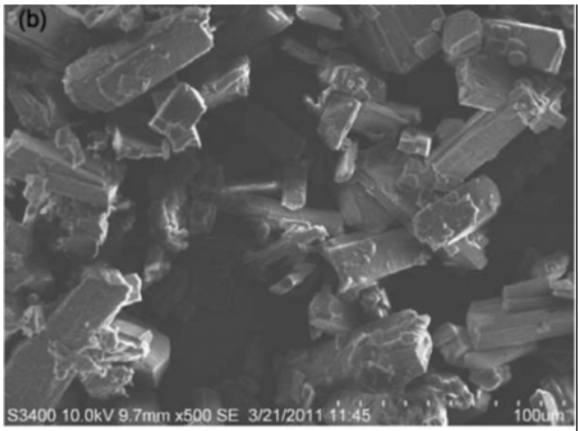


Figure 7. SEM image of SNPs obtained using aqueous leaf extract of *Syzygium cumini*.[70]

From the extract of the leaves of *Tinospora cordifolia*[73] SNPs particles were obtained with an external form that resembles a highly distorted spherical. With the use of extracts of *Leucas aspera* and leaves of *Hyptis suaveolens* particles, some of which had a distorted spherical shape, and other polygonal shapes were shown.[74]

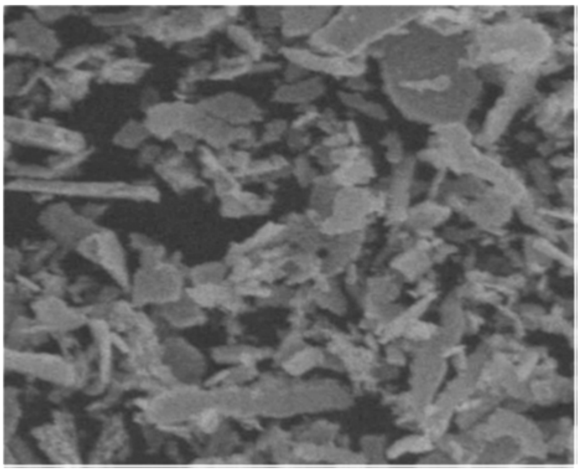


Figure 8. SEM image of SNPs obtained using aqueous leaf extract of *Artemisia nilagirica*.[72]

The completely unusual "flower-like" form of SNPs was observed by Pourjavadi and Soleyman in ref.[98] In their study, with the remarkable title "Novel silver nano-wedges for killing microorganisms," a peculiar photochemical surface biosynthesis was first applied to produce SNPs using the "Salep" (tuber extract of *Orchis mascula*). SEM image of such SNPs is shown in Figure 9. In the framework of such a synthesis, the reduction of Ag(I) to SNPs, in addition to the extract itself, was facilitated by sunlight (the UV radiation). "Salep" also served as an effective capping biomaterial, providing the formation of "flower-like" self-organizing structures in the form of unique silver "nano-wedges". In addition, there was an aggregation of such structures, which resulted in SNPs' formation with the above unusual shape. In concluding this section, we note that during the biosynthesis of Ag-NP using plant extracts, it has not yet.

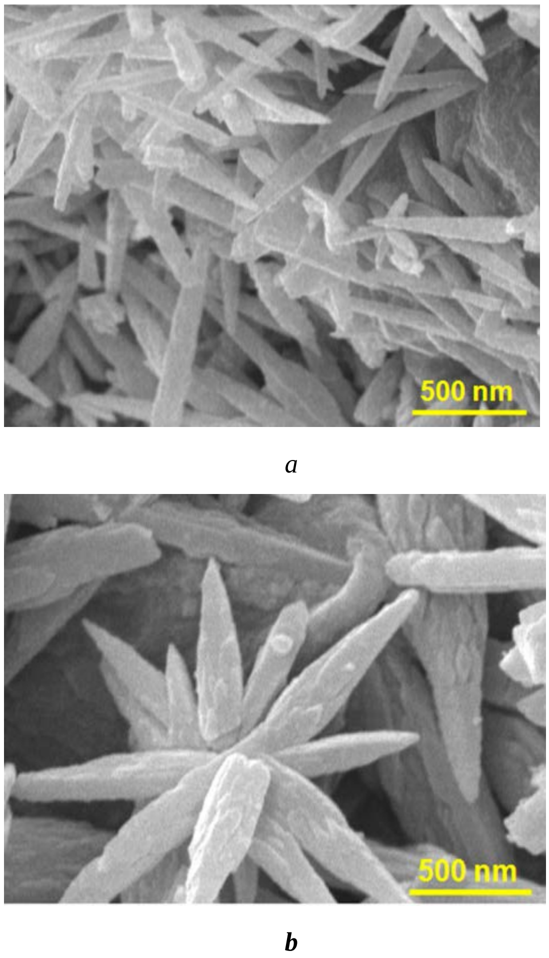


Figure 9. SEM images of nano-wedges (*a*) and "flower-like" SNPs (*b*) were obtained using aqueous tuber extract *Orchis mascula*.[98]

It has been possible to obtain such SNPs shapes as nanorods, nanowires, or nanobars, as observed during physicochemical synthesis of Ag-NP, particularly in the publications.[109–111] As can be seen from the data presented in Table 1, in most cases for SNPs synthesized using plant extracts, in the visible region of the spectrum, either a single absorption band with a maximum in the range of 400-460 nm or a "wing" band with a maximum in the UV region were detected. Accordingly, their colloidal solutions are usually colored in orange, red, or red-brown. However, any correlation between the sizes, as well as the shape of the nanoparticles and the position of this maximum in the UV-Vis absorption spectra, as can be seen from experimental data presented in Table 1, was not observed. In any case, in none of the works published to present time and, somehow or other, connected to the problem of SNPs biosynthesis, the question of such a correlation has not been discussed or even raised. Review articles[112-118] were also devoted to the discussion of recent results on SNPs biosynthesis using plant extracts, in which references to a number of other, earlier works devoted to the "green synthesis" of silver nanoparticles using extracts of various plants can be found. A possible mechanism of the plant extracts influence on the process of formation of SNPs has been considered in detail in recently published reviews.[116,118]

Table 1. Sizes and shapes of SNPs obtained by "green synthesis" using plant extracts

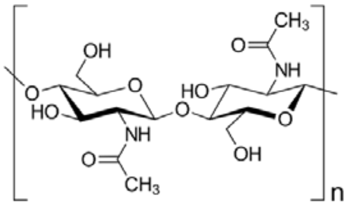
Plant	Part used for the synthesis	Shape (form)	Size of SNPs, *nm*	λ_{max}, *nm*	Ref.
Pelargonium graveolens (Geranium)	Leaves	Spherical	16-40	440	25
Aloe Vera	« ---- »	Spherical	15.2±4.2	410	26
Capsicum annuum	« ---- »	Spherical	50-70	428	27
Lippia citriodora (Lemon Verbena)	« ---- »	Spherical	15-30	430-440	28
Acalypha indica	« ---- »	Spherical	20-30	425	29
Tribulus terrestris	« ---- »	Spherical	18-47	450	30
Mimusops elengi	« ---- »	Spherical	55-83	440	31
Cinnamomum canphora	« ---- »	Spherical	55-80	440	32
Eclipta	« ---- »	Spherical	2-6	419	33
Ocimum sanctum (Tulsi)	« ---- »	Spherical	4-30	413	34
Cassia auriculata	« ---- »	Spherical	1-100	450	35
Euphorbia hirta	« ---- »	Spherical	29-31	380, 460	36
Nerium indicum	« ---- »				
Rosa chinensis	« ---- »	Spherical	25-60	No data	37
Iresine herbstii	« ---- »	Spherical	44-64	420	38
Hibiscus cannabinus	« ---- »	Spherical	9-10	446	39
Piper pedicellatum	« ---- »	Spherical	2-30	440	40
Tithonia diversifolia	« ---- »	Spherical	~25	Absent	41
Ficus panda	« ---- »	Spherical	12-36	421	42
Citrullus colocynthis	« ---- »	Spherical	13.37	No data	43
Alternanthera sessilis	« ---- »	Spherical	30-50	420	44
Podophyllum hexandrum	« ---- »	Spherical	~14	430	45
Olea europaea (Olive)	« ---- »	Spherical	20-25	441-456	46
Olax scandens	« ---- »	Spherical	30-60	410-430	47
Delonix elata	« ---- »	Spherical	35-45	432	48
Skimmia laureola	« ---- »	Spherical	No data	460	49
Butea monosperma	« ---- »	Spherical	20-80	440-475	50
Capparis decidua	« ---- »	Spherical	1.5-25	452	51
Azadirachta indica	« ---- »	Spherical	~34	436-446	52
Azadirachta indica	« ---- »	Spherical	<40	400-450	53
Capparis spinosa	« ---- »	Spherical	10-40	420	54
Ziziphus oenoplia	« ---- »	Spherical	10	436	55
Croton bonplandianum Baill.	« ---- »	Spherical	32	425	56
Rubus glaucus	« ---- »	Spherical	12-50	440-445	57
Raphanus sativus	« ---- »	Spherical	4-30	426	58
Melia azedarach	« ---- »	Spherical	34-48	482	59
Calliandra haematocephala	« ---- »	Spherical	13.5-91.3	414	60
Crocus sativus	« ---- »	Spherical	12-20	450	61
Costus afer	« ---- »	Spherical	~20	405-411	62
Datura stramonium	« ---- »	Spherical	15-20	444	63
Ficus talboti	« ---- »	Spherical	10-14	438	64
Syzygium cumini,	« ---- »	Triangular	53	420	65
Citrus sinensis	« ---- »		41		
Solanum tricobatum	« ---- »		52		
Centella asiatica	« ---- »		42		
Alysicarpus monilifer	« ---- »	Hexagonal, Spherical	5-45	422	66
Melia azedarach	« ---- »	Cubic	78	436	67
Eucalyptus macrocarpa	« ---- »	Cubic	10-50	430	68
Moringa oleifera	« ---- »	Cubic	30-70	Absent	69
Ocimum tenuiflorum	« ---- »	Prismatic	28	420	70

Solanum tricobatum	« ---- »		22.3	420	
Syzygium cumini	« ---- »		26.5	420	
Centella asiatica	« ---- »		28.4	415	
Citrus sinensis	« ---- »		65	415	
Annona squamosa	« ---- »	Irregular	~300	420	71
Artemisia nilagirica	« ---- »	Irregular	≤30	463	72
Tinospora cordifolia	« ---- »	Irregular, spherical	~30	430	73
Leucas aspera	« ---- »	Irregular	7-22	401	74
Hyptis suaveolens		Polygonal	5-25	408	
Emblica Officinalis (Amla, Indian Gooseberry)	Fruits	Spherical	15-25	400-420	75
Tribulus terrestris	« ---- »	Spherical	16-28	435	76
Ananas comosus	« ---- »	Spherical	~12	430	77
Piper longum	« ---- »	Spherical	~46	465	78
Adansonia digitata	« ---- »	Spherical	3-57	434	79
*Emblica officinali*s	« ---- »	Spherical	15-20	425	80
Cleome viscosa	« ---- »	Spherical	20-50	410-430	81
Solanum trilobatum	« ---- »	Spherical	12.5-41.9	440	82
		Polygonal	41-42	420	
Trianthema decandra	Root	Spherical	36-74	Absent	83
Citrullus colocynthis	« ---- »	Spherical	7.39	No data	43
Rheum emodi	« ---- »	Spherical	10-40	425	84
Erythrina indica lam	« ---- »	Spherical	20-118	438	85
Morinda citrifolia	« ---- »	Spherical	30-55	413	86
Potentilla fulgens	« ---- »	Spherical	10-15	410	87
Rheum palmatum	« ---- »	Hexagonal	121±2	440	88
Acorus calamus	« ---- »	Cubic	30-70	Absent	69
Chrysanthemum indicum	Flowers	Spherical	25-59	430	89
Cocos nucifera	« ---- »	Spherical	~22	420	90
Plumeria alba	« ---- »	Spherical	36.2	455	91
Syzygium cumini	« ---- »	Spherical	<40	400-450	53
Cucurbita maxima	« ---- »	Cubic	30-70	Absent	69
Achillea biebersteinii	« ---- »	Pentagonal, Spherical	10-40	450	92
Dimocarpus longan	Peel	Spherical	8-22	No data	93
Punica granatum	« ---- »	Spherical	20-40	Absent	94
Musa paradisiaca (banana)	« ---- »	Irregular	~24	433	95
Citrullus colocynthis	Callus	Spherical	31	No data	96
Dioscorea bulbifera	Tuber	Triangular, nanorod	8-20	455	97
Órchis máscula	« ---- »	"Flower-like"	<100 (width) ~500 (length)	444	98
Artemisia marschalliana	Aerial part	Spherical	5-50	430	99
Anthemis atropatana	Aerial part	Spherical	10-80	430	100
Boswellia serrata	Gum	Spherical	7.5±3.8	420	101
Citrullus colocynthis	Seeds	Spherical	16.57	No data	43
Cola nitida	Pods	Spherical	12-80	431	102
Ulva lactuca (algae)	Whole plant	Spherical	76	434	103
Cissus quadrangularis	Whole plant	Spherical	50-100	450	104
Sargassum wightii (algae)	Whole plant	Spherical	5-22	439	105
Allium sativum	Whole plant	Spherical	100-800	No data	106
Sargassum vulgare (algae)	Whole plant	Spherical	~10	No data	107
Cymodocea serrulate (seagrass)	Whole plant	Spherical	17-29	430	108

SNPs OBTAINING WITH USING VARIOUS ANIMAL PRODUCTS

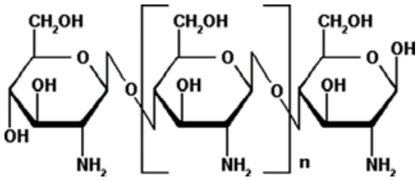
The literature contains a number of data showing the possibility of using for the synthesis of SNPs various products of animal origin, in various natural compounds having high molecular weight. Owing to the large size of their molecules, the molecular mass of which (M) is tens and hundreds of thousands of carbon units (c.u., Daltons), their role in this process is reduced mainly to function (3), i.e., substrates that hinder the aggregation of already formed nanoparticles [although in principle their participation in the functions referred to in paragraph **3** (1-2) is not excluded]; in that way, these nanoparticles are immobilized in masses of these substrates. As it may, we note immediately that the number of publications devoted to the synthesis of SNPs using such substrates is much smaller than the number of publications using various plants, if only because their choice is much more limited.

The most commonly used animal object for SNPs synthesis is chitin (poly-N-acetyl-D-glucoso-2-amine) **4**.I



4.I

and chitosan **4**.II, macromolecules of which consist of randomly linked β-(1-4)-D-glucosamine units and N-acetyl-D-glucosamine, obtained only from chitin



4.II

Chitin is known to be the main component of the arthropod exoskeleton; chitosan is usually obtained artificially, but in nature, it is found in the cell walls of the cells of fungi of the *Zygomycota* department (in combination with chitin) and crustacean shells. Chitosan is characterized by so-called mucoadhesive properties (ability to adhere to various mucous membranes),[119] which seems to be very important for creating drugs that enter the body through the mucous membranes.[120-122] In this connection, it seems appropriate to obtain chitosan-immobilized SNPs, which could be used as effective antibacterial agents. Now, however, only fragmentary information is available.[123-138] The influence of chitosan molecular weight on SNPs dimensional characteristics when they were formed in situ as a result of the reduction of $AgNO_3$ precursor in a solution of this biopolymer was studied by Apryatina et al.[123,124] It is very important that the authors of the given works were able to regulate the size of silver nanoparticles formed in the range from 8 to 12 nm by changing the molecular weight of chitosan (which also acts a stabilizer of silver nanoparticles occurring during biosynthesis). The effect of the chitosan (M) molecular mass on SNPs' spectral characteristics is also interesting: for example, at M = 40.000 c.u. the absorption maximum in the visible spectrum ($\lambda_{max.}$) is at 424 nm, at M = 127.000 c.u., at 412 nm, at M = 165.000 c.u., at 400 nm, at M = 240.000 c.u., at 383 nm. Herewith, SNPs formed in chitosan solutions with a higher molecular mass and having a size of 8 nm, exhibit much more pronounced bactericidal activity than SNPs with a size of 12 nm,[123,124] in a recently published paper[125] Uryupina with co-authors obtained pseudospherical SNPs with an average size of 65 nm using chitosan.

In refs.[126-130] another derivative of chitin, namely O-carboxymethyl chitin, was tested as a substrate, and the use of γ-radiation from the ^{60}Co isotope contributed to the restoration of the $AgNO_3$ precursor. As a result of the studies, new radiation-induced bactericidal metal-polymer nanosystems containing the above biopolymer and elemental silver nanoparticles were created. Besides, by varying the dose of γ-radiation, as well as the degree of filling of the biopolymer macromolecules with Ag^+ ions, the authors of works[126,127] were able to create macromolecular systems with silver nanoparticles 1–5 nm in size and, most importantly, to control these sizes during the experiment. The SNPs obtained in these studies, however, had an irregular shape. Researches in the field of chitosan-immobilized SNPs undoubtedly require their continuation. The possibility of using carboxymethyl chitosan[128,133.135,137] and 3,4-dihydroxyphenyl acetic-conjugated oligochitosan[139] for the synthesis of SNPs as well as nanoparticles of other noble metals – gold and platinum[133] was also noted. In such synthesis, SNPs having spherical or irregular forms are formed; the sample of such nanoparticles is shown in Figure 10.

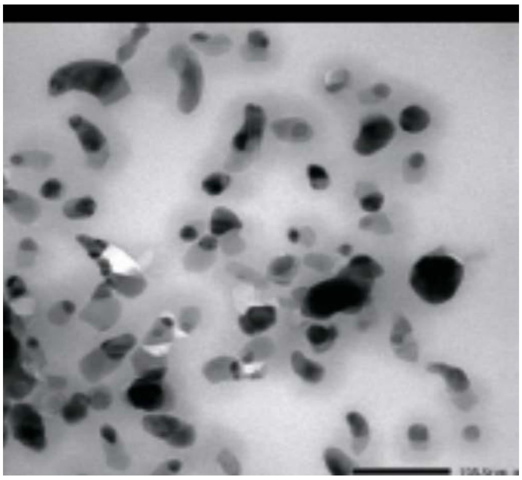
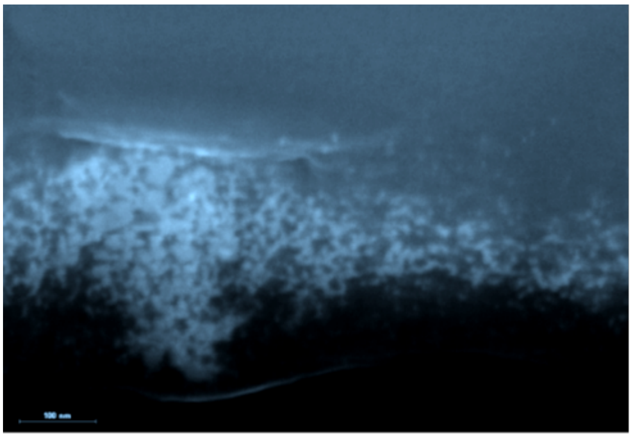


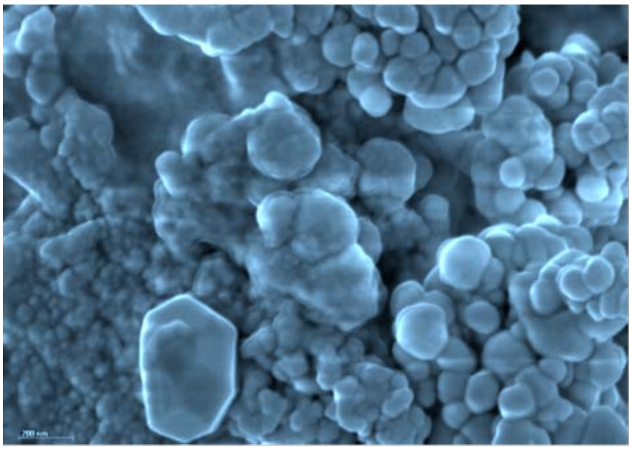
Figure 10. TEM of SNPs having irregular form obtained in ref.[139]

Another example of such a substrate is gelatin, which is the main component of various food (meat) jellies. As known from the publications,[140-148] this natural compound is a polydisperse mixture of low molecular (molecular weight M = 50.000–70.000 c.u.) and high molecular (M = 200.000–300.000 c.u.) polypeptides. The dimensional structure, which is now well studied,[144,145,147] and in this structure, there are many nanoscale size cavities, which can serve as a kind of molecular nanoreactors. The reduction reaction Ag(I)→Ag occurs, namely in such cavities; in this case, some water-insoluble silver compound (AgCl, AgBr, $Ag_4[Fe(CN)_6]$ et al.) immobilized in a gelatin mass acts as a silver-containing precursor. The reducing agent is an organic or inorganic substance with strongly pronounced electron-donor properties. One of the most suitable for this purpose is tin dichloride $SnCl_2$, which was used to obtain gelatin-immobilized SNPs in refs.[149-151] The reduction according to the Ag(I)→Ag scheme occurred in a strongly alkaline (pH~12) medium in the presence of reagents capable of forming fairly strong and water-soluble complexes with Ag(I) [thiocyanate anion SCN^-, thiosulfate anion $S_2O_3^{2-}$, ammonia NH_3, ethylenediamine, monoethanolamine, etc.). Due to this fact, not AgCl, AgBr, or $Ag_4[Fe(CN)_6]$, but Ag(I) complexes with the inorganic and organic compounds named above, were actually reduced. The pseudospherical SNPs size obtained in this variant of biosynthesis is in the range from 10 to 40 nm; this was first experimentally established in ref.[149] An example of an SEM image of such SNPs in a gelatin-immobilized matrix is shown in Figure 11, a.

It should be noted in this connection that when these nanoparticles are isolated from the gelatin matrix (for example, by the action of proteolytic enzymes, as described in ref.[152] their aggregation naturally occurs (Figure 11, b); nevertheless, a significant part of these particles retains its former nano size. Details of the producing SNPs using such a specific method were presented in publications.[150,151] The idea that in the specific conditions of chemical processes in the gelatin matrix, as well as due to the above-mentioned specific structure of the gelatin itself, nanoparticles of a wide variety of chemical compounds that can be formed in it, was expressed in a number of earlier works, particularly in reviews.[143-147]



a



b

Figure 11. SEM of pseudo-spherical SNPs obtained as a result process of “re-precipitating” of silver contained in gelatin matrix (*a*) and SNPs isolated from this matrix (*b*).[151]

In this regard, it is worth noting the work,[138] in which the combination (chitosan + gelatin) was used for SNPs synthesis; spherical nanoparticles with a size in the range of 3.7-10.5 nm and λ_{max} = 408-412 nm, were obtained here (Figure 12). Gelatin is not the only polypeptide substrate that can be used for this purpose; the various albumins are known for the synthesis of SNPs.[153–155]

For example, the chicken egg protein was used for this purpose,[153] as a result, spherical SNPs with an average size of ~ 20 nm (Figure 13) and a maximum in the visible spectral region at 425 nm was obtained.

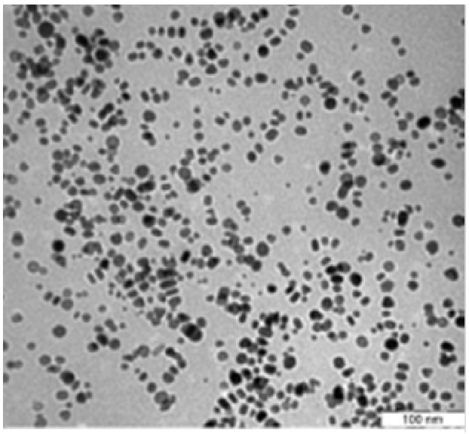


Figure 12. TEM of SNPs having spherical form obtained in[138]

The same substrate was used in[154] and in[155] – bovine serum albumin. In the work,[156] casein was used for this purpose. Some publications concerning protein- and peptide-directed syntheses of inorganic materials, and, in particular, of elemental noble metal nanoparticles having various sizes and morphologies, can be found in review.[157]

a

b

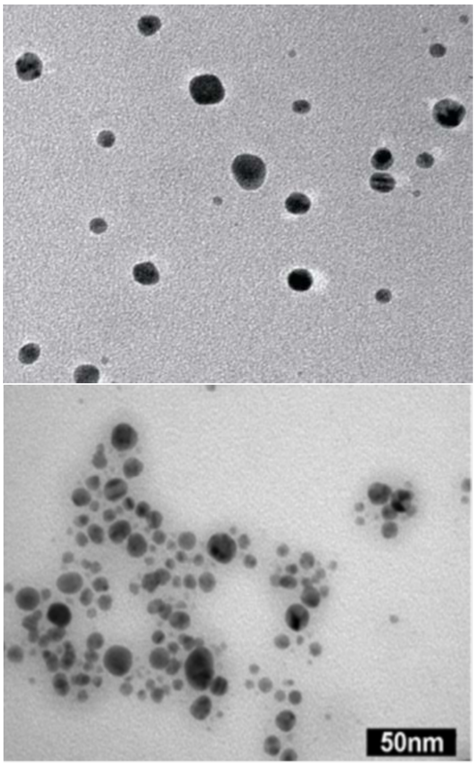


Figure 13. TEM images of SNPS obtained egg white-mediated green synthesis in ref.[153] (a) and in ref.[154] (b)

BIOAPPLICATIONS OF SNPs

Actually, SNPs have been known in anthropogenic activities since very ancient times (although nobody had any idea about their existence). For example, the detailed study of one of the late Roman Empire cultural masterpieces, namely the Lycurgus Cup (IV century ad), was shown that the glass inserts in its bronze frame owe their specific coloring (red in reflected light and gray-green in transmitted) to the presence of nanoparticles, that are 70 % elemental silver.[158]

Currently, SNPs obtained by various methods are used in very diverse fields of science and technology. Thus, an important area of application of SNPs is catalysis, which can be implemented in two versions – with the influence on the reaction system of electromagnetic radiation (photocatalysis) and without it. For example, in refs.[159,160] it was shown that SNPs on a SiO_2 matrix exhibit catalytic properties in redox reactions involving benzene, carbon monoxide, some dyes, and, possibly, many other chemical compounds. In particular, benzene under these conditions is almost completely oxidized to phenol even when the matrix's SNPs content is about 1 mass. %. Reactions between sodium borohydride and dyes such as methylene blue and eosin, in the presence of SNPs in the reaction system, proceed at a very high rate, whereas in their absence, such reactions practically do not take place. Besides, the SiO_2 substrate actually serves only to prevent the aggregation of SNPs in a colloidal solution.[160] An important feature of SNPs is that they allow to realize as photocatalysis for creation of resonant surface plasmons from light in the visible range, as well as to enhance the fluorescence intensity.[161-166] Owing to their stability and oxidation stability, elemental silver nanoparticles are widely used, for example, in electronics and photonics,[167] as a biosensor,[168] in bio catalysis,[169] for protein coagulation[170] and for drug delivery.[171] A layer of silver nanoparticles is covered with cutlery, door handles and even a keyboard and mouse for computers; they are used to create new coatings and cosmetics, in filters of air conditioning systems, in pools, showers and other places. The method of isotropic printing for the manufacture of silver microelectrodes is described, in which samples of electronic components with a minimum width of about 2 μm were received by applying a concentrated paint consisting of silver nanoparticles on semiconductor, plastic and glass substrates.[172] The foregoing, however, relates mainly to SNPs that were produced by chemical and physicochemical methods. And although SNPs obtained using the "green synthesis" could also find their application in the above areas of science and technology, their modern practical application is related to their production. As in this case, biosynthesis of SNPs, as a rule, was carried out in laboratories of biological and/or biochemical section with the participation of experts in the field of biochemistry and biotechnology; attention of researchers was focused on the application of SNPs in biology, first of all in medicine and pharmacology.

Currently, significant factual material related to bioapplications of SNPs has already exists.[173-176] First of all, it is the possibility of antimicrobial activity of SNPs; according to the data presented in the work,[6] their antimicrobial effect is more expressed than that of penicillin, biomycin and other antibiotics, due to the inhibitory effect on antibiotic-resistant strains of bacteria. According to these works' data, the effect of killing bacteria with preparations containing elemental silver nanoparticles is 1.500 times higher than phenol at the same concentration and 3.5 times higher than mercury(II) dichloride $HgCl_2$ (with much less toxicity). SNPs have an antimicrobial effect on many pathogenic microorganisms, such as *Staphylococcus aureus*, *Streptococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli*: from bacteriostatic (ability to inhibit microbial reproduction) to bactericidal (ability to destroy microbes).[6] Currently published works describe the use of biosynthesized SNPs as antibacterial agents against a wide variety of microorganisms. So, in a number of works cited above devoted to the "green synthesis" of SNPs with the participation of plant extracts, a high efficiency of silver nanoparticles against pathogenic microorganisms genus *Bacillus,*[53,54,58,73,81,83,95] *Staphylococcus,*[34,46,49,54,58,63,66,70,73,76,81,83,87,5,98,101] *Pseudomonas,*[49,55,62,70,73,76,83,87,101] *Klebsiella,*[49,55,62,70,73,81,87,97] *Escherichia,*[34,46,49,53,54,55,58,60,62,63,70,81,87,95,98] *Salmonella,*[54,55] *Enterococcus*[83] and *Serratia,*[58] was noted. The introduction of SNPs causes structural and morphological changes in cells that can lead to bacterial death. When silver nanoparticles come into contact with bacteria, they adhere to the cell wall and cell membrane, prevent replication and contribute to cell death.[177]

Meanwhile, the so-called electronic effects are observed for SNPs with an average size of 10 nm or less. Their bactericidal activity increases sharply compared to that for SNPs with large dimensions.[178] In quantitative ratio, this effect is different for each specific type of cell, since, on the one hand, the composition of their cell membranes varies widely, on the other hand, with a decrease in the size of SNPs, their reactivity increases due to an increase in their surface area and reduce their volume. According to work[98] the discovered silver nano-wedges, due to their unique pointed shape (Figure 9), act on any microorganisms like real "daggers", tearing their bodies apart. Therefore, such SNPs with a similar form may be promising candidates for a wide range of biomedical applications, and especially in the manufacture of antibacterial drugs. The high fungicidal activity of biosynthesized SNPs on some microscopic fungi[98] was shown.

An important fact is that clearly expressed anticancer activity of silver nanoparticles was identified.[43,45,50,53,79,81,92,93,100] In ref.,[179] SNPs were proposed for cancer diagnosis and drug standards. In the publication,[180] chemotherapeutic anticancer drugs were developed with photo-soluble linkers that "attached" them to a substrate on the surface of SNPs. The principle of action of such drugs is reduced to destruction under the influence of UV radiation, resulting in its active form, which has a destructive effect on cancer cells.[180] Earlier, an alternative approach, in which the anticancer drug "attached" directly to the functionalized surface of SNPs, was proposed.[181] The advantages of cancer treatment methods described in[180,181] are that, on the one hand, the drug is transported into the patient's body without the use of any toxic compounds. On the other hand, it is selectively released precisely in the affected organ. SNPs can also help overcome multidrug resistance, which often prevents the delivery of the right drug to the affected organ.[182]

SNPs seem to be characterized by a highly synergistic bactericidal action in combination with such well-known antibiotics as penicillin, ampicillin, erythromycin, clindamycin and vancomycin; such a phenomenon has been observed, for example, against bacteria of the genus *Staphylococcus* and *Escherichia.*[183] It should be noted that preparations based on elemental nanoparticles are widely used in bone transplants to treat burns, because SNPs associated with the implant provides better antimicrobial activity and contributes to a significant reduction in the number of scars arising in the healing process of the affected tissue. Owing to antimicrobial activity, elemental silver nanoparticles also find a certain application in the food industry and food technologies, described in refs.[184,185]

Another possibility of application SNPs is their use as part of larvicidal compositions. It was proposed to use biosynthesized SNPs against malaria mosquitoes of the genus *Anopheles*,[72,89,186,188-191] and pathogens of malaria – *Plasmodium falciparum*,[192] mosquitoes-carriers of yellow fever – the genus *Aedes* and *Culex*.[71-73,186,187,189-191,193] (Interestingly, graphene was used in refs.[189,192] as one of the components of such preparations). However, the number of works devoted to the larvicidal (and insecticidal) activity of biosynthesized SNPs is still relatively small compared to that for the works about the antibacterial activity of these NP.

Nevertheless, it should be noted that SNPs are still toxic to the human body. Because SNPs dissolves to form Ag+ ions, which are known to have toxic effects,[194] some studies have been conducted to determine whether SNPs toxicity results from the release of silver ions or is associated with the nanoparticles themselves.[195,196] The results of these studies indicate that elemental silver nanoparticles can indeed cause allergies. However, these results do not exclude the possibility that SNPs' toxicity is no less associated with the formation of silver ions in cells because according to ref.,[197] SNPs and Ag+ ions have almost the same cytotoxicity. The authors of the work[198] came to the same conclusion: the combination of SNPs and Ag^+ is responsible for the toxic effect of silver nanoparticles; besides, a toxic effect on cells was shown for the SNPs regardless of free silver ions. On the other hand, the toxicity of SNPs in human cells is due to oxidative stress and inflammation caused by the formation of reactive oxygen species stimulated by either SNPs, Ag+ ions, or both.[199] According to the authors of,[200] the introduction of SNPs into tissue cells leads to free radicals, which have a potential health risk.

CONCLUSION

Thus, the perspectives of the biosynthesis of SNPs look very impressive. The list of biological substrates that have so far been used in published works is exceptionally long, and in this review paper, it is impossible even to quote all these publications. However, now the development and improvement of these methods using those discussed biological substrates, the control of the size, shape and degree of dispersion of biosynthesized SNPs cannot yet be considered to be adequately provided in the experiment. That is why, for the implementation of the controlled biosynthesis of SNPs with predetermined target parameters, undoubtedly, some new principles and methodological approaches should be elaborated. And for this, a thorough knowledge of the mechanism about the process of nanoparticle biosynthesis is necessary in general and SNPs in particular, the specifics of which in most cases remained unexplored. These bio-syntheses require information about the effect on the process of concentration-time and temperature parameters used in the experiment. In most published works, they are scattered and insufficient to make complete conclusions. A very important task is also to increase the target product's yield (i.e., synthesized SNPs). Finally, it is very important to improve existing and develop new methods of isolating nanoparticles from the parent systems they were formed (which may be necessary to produce commercially available products containing these nanoparticles).

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