Colloids of Gold Nanoparticles Protected from Aggregation with Arabinogalactan

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Summary: We study the colloids of gold nanoparticles (GNPs) covered with water-soluble natural branched polysaccharide arabinogalactan (ARB) which is used as reducing and capping agent in the GNPs colloidal synthesis. A set of the ARB-GNP composites with gold content ranging from 3.1 to 11.9% was investigated. Transmission electron microscopy of dried colloids shows that the clustering of spherical GNPs into dimers and trimers increases with the gold content. The ARB-GNP colloids are studied by static and dynamic light scattering resonantly enhanced by the GNP surface plasmons. The GNPs clustering within the colloids is manifested by the scattered light depolarization. Spherical form of the colloids is proved by the analysis of their translational and rotational diffusion coefficients obtained via dynamic light scattering.

Keywords: clustering; colloids; depolarized dynamic light scattering; gold nanoparticles; nanobiocomposites

Introduction

Nanobiocomposites of gold nanoparticles (GNPs) with natural polymers obtained in colloidal nanoparticles synthesis have gained considerable attention in modern interdisciplinary technologies.[1–7] A large variety of nanobiocomposites has been obtained with polysaccharides serving as both reducing and stabilising agents for green synthesis of metallic nanoparticles.[8–13] GNPs can be used as biosensors and biomarkers due to the fact that the GNPs surface plasmon resonance belongs to the visible range. The surface plasmon resonance depends strongly on the size, form and clustering of GNPs.[7] The GNP clusters are even more efficient scatterers than single GNPs due to the strong enhancement of electric field between coupled GNPs.[14,15] However, colloidal synthesis of nanoparticles usually results in single nanoparticles surrounded by a shell of a capping agent, preventing the nanoparticles from aggregation. Most part of the nanoparticles clusters was obtained on substrates after the nanoparticles precipitation caused by the colloidal shell destruction.[16,17] The GNP clustering directly during the colloidal synthesis is obtained mainly by means of thiol-modified ligands.[14,18–20]

In the present article, we report the study of the colloids of GNP covered with branched water-soluble polysaccharide arabinogalactan (ARB). Solubility in water, biocompatibility and biodegradation make ARB attractive in biomedical applications.[21] The ARB-GNP composites has been shown to be possess an antimicrobial activity.[11] We found that ARB coating provides a unique possibility of producing colloids, containing GNP clusters. The ARB/GNP content regulates the GNP clusters formation. Dynamic and static light scattering were applied for the first time to study the effect of the GNPs cluster formation.

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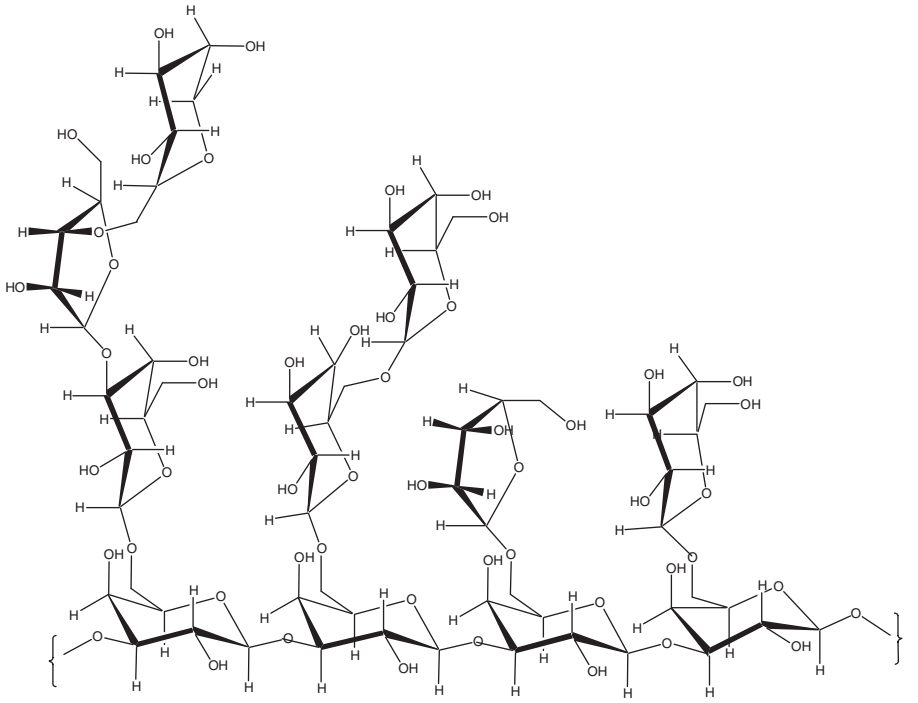
Experimental Part

The nanocomposites were synthesized by reduction of gold ions of the precursor $HAuCl_4$ in alkaline solutions of arabinogalactan extracted from *Larix Sibirica*. Molecular mass of ARB was 48 000 g/mol.[22] In Scheme 1, the fragment of the ARB's structure with the backbone of *D*-galactopyranose residues linked β-(1 → 3) and short side chains, containing arabinose groups, is presented.

The reaction temperature was 90 °C, the yield was 96–99%. The nanocomposites were precipitated with ethanol and dried to a constant weight. The content of gold in the nanocomposites (ω) was varied by changing the initial concentration of $HAuCl_4$ in the reaction media. By using ARB, it is possible to obtain the colloids with $\omega < 19$ w/w%. At higher gold content, the GNPs precipitate during the synthesis.

A 20 mW helium-neon laser (COHERENT) operating at the wavelength $\lambda = 632.8$ nm was applied; the incident light intensity was reduced ten times by using a filter. The scattered light was registered in the range of scattering angles $30^\circ \leq \theta \leq 135^\circ$ by using a PHOTOCOR goniometer. A decalin index-matching bath was used. The incident light was vertically polarized. Horizontal or vertical polarization of the scattered light was attained by rotation of polarizer. In dynamic light scattering (DLS), the correlation functions were accumulated by using a 288-channel correlator (PHOTOCOR Instruments, Inc.). A software package DynaLS v2.0 (ALANGO) was used to calculate the correlation time distributions. The scattering was measured at 21 °C; the temperature was controlled within ±0.1 °. The solutions were prepared at least 10 hours before the measurements. In order to dispose of dust, the scattering cells were rinsed with benzene, vacuumized, and filled with dust-free air. The nanocomposites were dissolved in filtered water. Then the solutions were filtered again. Polyamide



Scheme 1.
Possible fragment of the ARB chain.

membrane syringe filters (CHROMAPHIL) with the pore diameter of 100 nm were used.

Microphotographs of GNPs were obtained by transmission electronic microscope Leo 906E operating at 80 kV. The samples were prepared by slow evaporation of one drop of the nanoparticles solution on a copper grid coated with formvar. The nanoparticle distributions were averaged over 100 nanoparticles for two times. The content of the GNP in the nanocomposites has been determined in Ref.[11] by an atomic-absorption method.

Table 1.
Radius of individual GNP (r), colloidal hydrodynamic radii (R^R and R^T), the depolarization factor (Δ), the radius of standard core-shell colloidal model (R_o) containing single GNP covered with a monolayer of ARB.

ω, w/w per cent	$\Delta^{1)}$	r,[2)] nm	R^R,[3)] nm	R^T,[3)] nm	R_o,[4)] Nm
0	0	–	–	3.7	–
3.1	0.06	2.7	13.7	11.7	10.1
6.7	0.10	4.3	16.0	15.0	11.7
9.3	0.17	4.8	16.5	16.5	12.2
11.9	0.24	7.0	19.9	20.1	14.3

[1)]determined by static light scattering,[2)]determined by transmission electron microscopy TEM,[3)]determined by dynamic light scattering DLS,[4)]$R_o = r(\text{GNP}) + 2R^T(\text{ARB})$.

Results

Transmission Electron Microscopy (TEM)

Figure 1 demonstrates the representative microphotographs of the ARB-GNP composites at the lowest and the highest gold contents. Figure 1 shows that spherical GNPs are formed at each gold content ω. Radius of individual GNPs was measured by TEM. Figure 1 shows that the GNPs are separated from each other at $\omega = 3.1\%$, but at higher ω some of them form small clusters (mostly dimers and trimers). The TEM photos show that the fraction of the GNP clusters increases with ω.

Depolarization of Scattered Light

Table 1 lists the depolarization factors $\Delta = I_{VH}/I_{VV}$, where I_{VH} and I_{VV} are the intensities of horizontally and vertically polarized components of the scattered light at $\theta \to 180^\circ$. Taking into account that GNPs are spherical, we conclude that single GNPs cannot depolarize light. The depolarization of scattered light can be produced only by non-spherical scatterers,[23] such as gold nanorods[24] or GNPs coupled in dimers and trimers.[25] Basing on the GNPs clustering observed by TEM, we assumed that the non-zero depolarization is produced by the GNP clusters. Table 1 shows that Δ increases with the gold content. This indicates that the fraction of light scattered by clusters and the mean aspect ratio of clusters increase with ω. In Ref.[22], we have modeled the GNP clusters with aspect ratio p by gold nanorods with the same aspect ratio. Comparing the $\Delta(p)$ dependence calculated for gold nanorods,[24] with Δ presented in Table 1, we have shown that

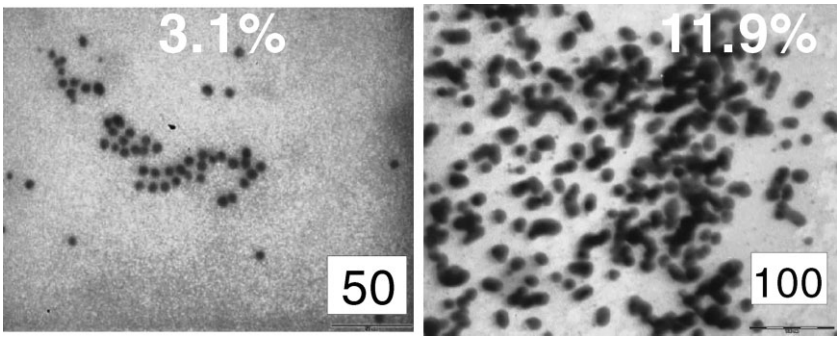


Figure 1.
TEM microphotos (the nanometer scale and the weight content of GNPs are indicated).

the observed range of Δ corresponds to $1.8 < p < 2.1$, e.g. primarily to the GNP dimers.

Dynamic Light Scattering

It is known that DLS reflects both translational (D^T) and rotational (D^R) diffusion coefficients at $\Delta \neq 0$, and the distribution of correlation times is bimodal.[23] The slow mode correlation rate $1/\tau_{slow}$ depends on the scattering vector q as:

$$1/\tau_{slow} = D^T q^2 \quad (1)$$

where $q = 4\pi n \sin(\theta/2)/\lambda$ and n is the solvent refractive index.

The fast mode correlation rate ($1/\tau_{fast}$) depends on q^2 as:

$$1/\tau_{fast} = 6D^R + D^T q^2 \quad (2)$$

Figure 2 shows the representative bimodal distributions of the colloidal correlation time. We showed that the correlation rates obey Eqs. (1) and (2). Table 1 lists the hydrodynamic radii R^T and R^R determined from D^T and D^R, respectively, under the assumption of the spherical form of the colloids.

Table 1 indicates that $R^R \approx R^T$ for all colloids. This points out the spherical form of the colloids. Table 1 shows that for each colloid, R^T is larger than the GNPs radius r. This difference can be attributed both to the contribution of the capped polymer and the increase of the core volume due to the GNPs clustering.

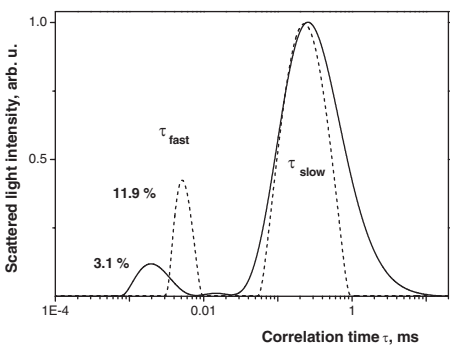
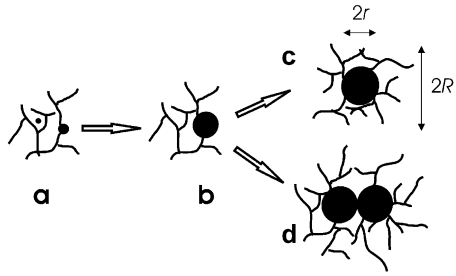


Figure 2.
DLS. Typical bimodal correlation time distributions of the colloids (scattering angle $\theta = 90^\circ$). The weight content of gold (ω) is indicated.



Scheme 2.
Colloidal synthesis of the ARB-GNP composites: the synthesis starts within each molecule (a, b), several ARB macromolecules sharing one GNP in the final colloid (c) the GNPs clustering (d).

Let us calculate the radius R_0 of the simplest colloid containing single GNP in the core covered with a monolayer of ARB (Scheme 2c). The shell thickness of this simplest colloid was approximated by the hydrodynamic diameter of the virgin ARB, 7.4 nm. Table 1 demonstrates that R_0 is consistent with the experimental R^T only at the lowest gold content ($\omega = 3.1\%$). Therefore, the colloid formed at $\omega = 3.1\%$ corresponds to Scheme 2c. This is in accord with the TEM image of this nanocomposite (Figure 1) showing single GNPs separated from each other. Table 1 shows that difference between R^T and R_0 increases with the gold content. Considering that the GNP dimers are more effective scatterers than single GNPs, we conclude that DLS probes mainly the larger colloids containing the GNP clusters. The observed increase of (R^T-R_0) with the gold content indicates the increasing fraction of the colloids containing the GNP clusters (like in Scheme 2d).

Discussion

It is important to realize at least qualitatively, what are the mechanisms of the GNP cluster formation. In our case, Au(III) ions are reduced to a zero-valent state within the branched macromolecules of ARB. The nuclei of gold nanocrystals can be formed

either inside the voids of the branched ARB or at the macromolecular periphery (Scheme 2a). Obviously, the nuclei which are formed inside the branched macromolecule have a limited capability of growth. The nuclei formed at the macromolecular periphery have enough space to grow further. We can imagine that the further growth of the outer GNPs nuclei results in their poor coverage by the branches of ARB. This can provoke the GNPs clustering (Scheme 3d). To our opinion, the clustering of the GNPs is promoted by the formation of hydrogen bonds between neighboring ARB molecules of the shell. The hydrogen-bonded self-assembly is an intrinsic property of polysaccharides, it was observed also in water solutions of the virgin ARB by DLS.[26] To a higher extent, the ability of ARB to keep together the metal nanoparticles in one colloidal aggregate is realized in the composites of ARB with nanoparticles of palladium. In particular, the colloids of ARB with the nanoparticles of Pd ($r = 4.9$ nm, $\omega = 4.1\%$)[12] exhibited $R_h = 66$ nm.[26] This radius is much higher than those of the ARN-GNP colloids of similar r and ω. Unlike the ARB-GNP colloids, the ARB-NP(Pd) colloids did not depolarize the scattered light,[26] thus indicating the spherically symmetric distribution of the Pd nanoparticles within the colloidal aggregate.

Conclusion

Due to the enhanced scattering from the GNPs clusters, dynamic and static light scattering is particularly sensitive for the GNPs clusters characterization. Reduction of gold ions in the alkaline solutions of ARB resulted in formation of stable GNP colloids in which the GNPs size and their clustering were controlled by the ARB/GNP content. The size of the colloid with the lowest gold content corresponded to the simple core-shell model of single GNP surrounded by a monolayer of ARB macromolecules. The fraction of the colloids with the GNP dimers and trimers in the core increased with the gold content, as indicated by the increase of the depolarization factor of light scattered from the colloids and by the increase of the colloidal radius with respect to that of the colloids with single nanoparticle inside. The mean form of the colloids was spherical according to the equality of hydrodynamic radii obtained from rotational and translational colloidal diffusion coefficients measured by DLS.

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[1] G. C. Bond, C. Louis, D. T. Thompson, in: "*Catalysis by Gold*", Imperial College Press, London **2006**, P. 367.
[2] R. K. DeLong, Ch. M. Reynolds, Y. Malcolm, A. Schaeffer, T. Severs, A. Wanekaya, *Nanotechnology, Science and Applications* **2010**, *3*, 53.
[3] I. García, M. Marradi, S. Penadés, *Nanomedicine* **2010**, *5*, 777.
[4] R. A. Rippel, A. M. Seifalian, *J. Nanosci. Nanotechnol.* **2011**, *11*, 3740.
[5] N. G. Khlebtsov, L. A. Dykman, *Chem. Soc. Rev.* **2011**, *40*, 1647.
[6] J. Polte, T. T. Ahner, F. Delissen, S. Sokolov, F. Emmerling, A. F. Thunemann, R. K. , *J. Am. Chem. Soc.* **2010**, *132*, 1296.
[7] V. H. Grassian, *J. Phys. Chem. C* **2008**, *112*, 18303.
[8] Y. Park, Y. N. Hong, A. Weyers, Y. S. Kim, R. J. Linhardt, *IET Nanobiotechnol.*, **2011**, *5*, 69.
[9] B. A. Trofimov, B. G. Sukhov, G. P. Aleksandrova, S. A. Medvedeva, L. A. Grishchenko, A. G. Mal'kina, L. P. Feoktisova, A. N. Sapozhnikov, V. I. Dubrovina, E. F. Martynovich, V. V. Tirskii, A. L. Semenov, *Doklady Chemistry* **2003**, *393*, 287.
[10] B. G. Sukhov, G. P. Aleksandrova, L. A. Grishchenko, L. P. Feoktistova, A. N. Sapozhnikov, O. A. Proidakova, A. V. T'kov, S. A. Medvedeva, B. A. Trofimov, *J. Struct. Chem.* **2007**, *48*, 922.
[11] G. P. Aleksandrova, L. A. Grishchenko, T. V. Fadeeva, B. G. Sukhov, B. A. Trofimov, *Nanotechnics* **2010**, *23*, 34.
[12] B. A. Trofimov, B. G. Sukhov, V. V. Nosyreva, A. G. Mal'kina, G. P. Aleksandrova, L. A. Grishchenko, *Doklady Chemistry* **2007**, *417*, 261.

[13] M. V. Lesnichaya, G. P. Aleksandrova, L. P. Feoktistova, A. N. Sapozhnikov, B. G. Sukhov, B. A. Trofimov, *Doklady Chemistry* **2011**, *440*, 282.
[14] D. S. Sebba, A. A. Lazarides, *J. Phys. Chem. C* **2008**, *112*, 18331.
[15] S. C. Yang, H. Kobori, C. L. He, M. H. Lin, H. Y. Chen, C. Li, M. Kanehara, T. Terenishi, S. Gwo, *Nano Lett.* **2010**, *10*, 632.
[16] Z. Y. Tang, N. A. Kotov, M. Giersig, *Science* **2002**, *297*, 237.
[17] M. Roca, N. H. Pandya, S. Nath, A. J. Haes, *Langmuir* **2010**, *26*.
[18] X. Li, W. Xu, J. Zhang, H. Jia, B. Yang, B. Zhao, B. Li, Y. Ozaki, *Langmuir* **2004**, *20*, 1298.
[19] K.-H. Su, Q.-H. Wei, X. Zhang, J. J. Mock, D. R. Smith, S. Schultz, *Nano Letters* **2003**, *3*, 1087.
[20] S. S. Kinge, M. Crego-Calama, D. N. Reinhoudt, *Langmuir*, **2007**, *23*, 8772.
[21] R. Falk, A. J. Domb, I. Polacheck, *Antimicrob. Agents Chemother.* **1999**, *43*, 1975.
[22] E. R. Gasilova, G. P. Aleksandrova, *J. Phys. Chem. C* **2011**, *115*, 24627.
[23] B. J. Berne, R. Pecora, in: "*Dynamic Light Scattering with Applications to Chemistry, Biology, and Physics*", J. Wiley & Sons, New York **1976**, p. 114.
[24] B. N. Khlebtsov, V. A. Khanadeev, N. G. Khlebtsov, *J. Phys. Chem. C* **2008**, *112*, 12760.
[25] M. Hoffmann, C. S. Wagner, L. Harnau, A. Wittemann, *ACSNano* **2009**, *3*, 3326.
[26] E. R. Gasilova, A. A. Toropova, S. V. Bushin, A. K. Khripunov, L. A. Grischenko, G. P. Aleksandrova, *J. Phys. Chem. B* **2010**, *114*, 4204.

