Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2024, **34**, 78–80

Mendeleev Communications

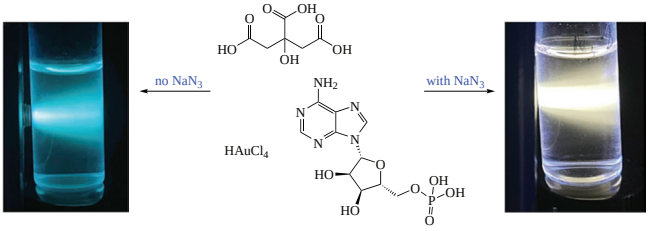
Synthesis of fluorescent gold nanoclusters in the presence of adenosine monophosphate: effect of azide ions

Evgeny A. Karpushkin,* Natalia K. Ivanova, Ekaterina A. Mesnyankina and Vladimir G. Sergeyev

Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: eukarr@gmai.com

DOI: 10.1016/j.mencom.2024.01.023

It has been demonstrated that fluorescence of gold nanoclusters formed *via* $HAuCl_4$ reduction with citrate buffered solution in the presence of adenosine monophosphate can be tuned *via* addition of NaN_3. No fluorescent products have been detected at large excess of NaN_3, whereas at intermediate concentrations of NaN_3 blue–green fluorescence has been gradually changed to yellow and weakened.



Keywords: gold, nanoparticles, nanoclusters, DNA, fluorescence.

Gold nanoclusters (AuNCs) are special objects consisting of several to several hundred metal atoms and thus closing the gap between single molecular species and conventional metal nanoparticles. Their size, typically below 2 nm, determines certain specific properties such as strong fluorescence and absence of surface plasmon resonance absorption. Owing to these optical properties as well as pronounced photostability and low toxicity, AuNCs have been applied in the fields of biological sensing, labelling, and imaging.[1] Since functional properties of AuNCs have been found highly sensitive to their size, shape, and nature of the capping agent, a variety of methods to prepare AuNCs in the presence of proteins, DNA, dendrimers, and thiolate species acting as template have been elaborated.[2]

An interesting procedure for the synthesis of AuNCs has been suggested[3] and further developed.[4] It comprises the mixing of aqueous solutions of $HAuCl_4$ and adenosine monophosphate (AMP) with citrate buffered solution and incubation of the mixture during several hours to several days (ambient temperature to 90 °C). It has been found that the presence of citrate is essential to obtain AuNCs, whereas ionizable adenine derivatives (AMP and adenosine triphosphate) have been found the most efficient in affording the fluorescent product, among other nucleotides; they act as stabilizers preventing aggregation of gold nanoclusters or their growth into larger nanoparticles. Moreover, it has been found that the procedure is sensitive to the mixture stoichiometry, the order of mixing, and even UV illumination during the synthesis. Other nucleotides could be used to produce fluorescent AuNCs as well, when ascorbic acid is used as reducing agent in a similar procedure.[5] Such nucleotide-assisted synthesis can serve as a convenient model for elucidating the mechanism of gold nanoparticles and nanoclusters formation in the presence of DNA molecules without any external reducing agent, recently reported.[6,7] Sodium azide is a well-known preservative inhibiting microbial activity,[8] it has been widely used to stabilize various solutions during storage. Since at the same time azide can act as ligand forming gold complexes,[9] it seemed important to probe its effect on the above procedure of the AuNCs synthesis.

Aiming to further explore reliability of the AMP-assisted synthesis of AuNCs and optimize its conditions, we came to a procedure† to prepare stable strongly fluorescent AuNCs avoiding UV exposure or prolonged incubation. The procedure afforded a sample with bright blue–green UV-induced fluorescence (λ_{ex} = 365 nm, $\lambda_{em,max} \approx$ 490 nm).

When the same synthesis was performed with addition of large excess of sodium azide with respect to $HAuCl_4$ (10 mM; c_{azide}/c_{Au} = 100), the resulting mixture turned yellow immediately when mixed with $HAuCl_4$ and revealed no fluorescence upon the heating. To better investigate that effect, we prepared a series of samples in the presence of low to moderate amount of sodium azide in the reaction mixture (c_{azide}/c_{Au} = 0.1–22), other synthesis conditions being the same. Selected results are presented in Figure 1.

From the plot in Figure 1(*a*) it is to be seen that the increase in the azide content led to gradual weakening of the main emission maximum at 490 nm; at the same time, the long-wave shoulder at ~600 nm appeared and grew, which corresponded to the change in the fluorescence color from blue–green to yellow [Figure 1(*b*)]. The shape of the absorption spectra [Figure 1(*c*)] was not changed at c_{azide}/c_{Au} = 0–2.2; intensity of the shoulder at $\lambda \approx$ 305 nm (assigned to AMP-coordinated AuNCs) passed through a minimum at c_{azide}/c_{Au} = 0.86. At the highest content of sodium azide (c_{azide}/c_{Au} = 22), the long-wave shoulder at the AMP absorption band became a distinct maximum and exhibited a slight red shift to $\lambda \approx$ 315 nm. The values of quantum yield of fluorescence (estimated in comparison with quinine sulfate reference, QY 54%[10]) shown in Figure 1(*d*) evidenced that

† The sample was prepared as follows. An aqueous solution containing 4 mM of citric acid, 16 mM of sodium citrate, and 1 mM of AMP (pH 5.9) was kept at room temperature during 10 min; after that, $HAuCl_4$ was added to concentration of 0.1 mM and the mixture was heated at 90 °C during 3 h. The electronic absorption and fluorescence spectra were recorded using a Vernier Ocean Optics modular system equipped with DH-2000 light source (absorption) or 365-nm diode (fluorescence) and Maya Pro detector. The measurements were performed at room temperature in 10-mm quartz cells.

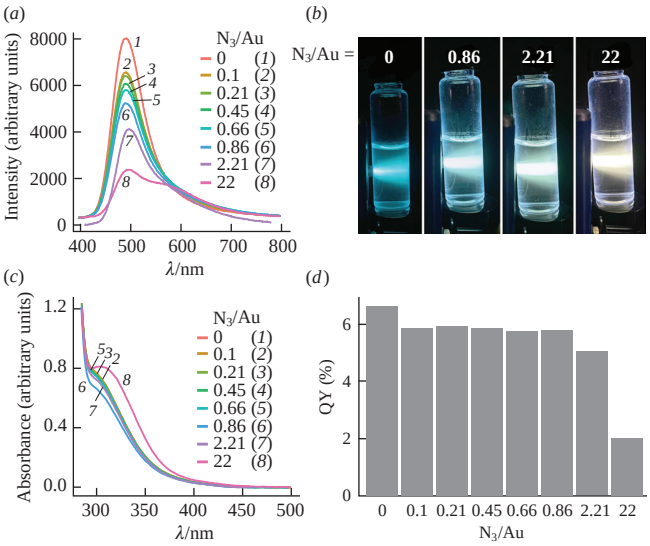


Figure 1 (*a*) Emission spectra (λ_{ex}= 365 nm), (*b*) view of fluorescence (λ_{ex} = 365 nm), (*c*) electronic absorption spectra, and (*d*) quantum yield of fluorescence of the AuNCs prepared at different excess of sodium azide with respect to $HAuCl_4$.

addition of a small amount of sodium azide led to certain decrease in the fluorescence efficiency from 6.6 to 5.9%, which remained constant in the c_{azide}/c_{Au} range of 0.1–0.86. Further increase in the sodium azide concentration led to reduction of the emission quantum yield to 2% at c_{azide}/c_{Au} = 22. Thus, the addition of less than 1 mol of sodium azide per a mol of $HAuCl_4$ resulted in slight decrease in the sample fluorescence intensity, its quantum yield remaining nearly constant. When the amount of NaN_3 was increased to c_{azide}/c_{Au} = 2.2 or 22, *i.e.* when the amount of azide ions was comparable to that of AMP, the species exhibiting yellow fluorescence instead of blue–green one were formed, and the overall quantum yield of fluorescence was decreased. At even greater excess of sodium azide (hundredfold with respect to $HAuCl_4$ and tenfold with respect to AMP), the formation of fluorescent AuNCs was completely suppressed.

Details of the mechanism of the discovered effect of sodium azide on the AuNCs synthesis are yet to be elucidated. For the azide-free $HAuCl_4$–AMP–citrate system, it has been suggested[3] that AMP is coordinated at gold atoms to form the AMP_nAu_n species (n = 2, 3, or 4), citrate acts as reducing agent to convert Au^{III} into Au^{I} and/or Au^0, whereas charged phosphate groups of AMP prevent aggregation of the AuNCs into larger non-fluorescent nanoparticles or aggregates, due to electrostatic repulsion. Binding of AMP with gold(III) and gold(I) ions is possible *via* the N^1, N^3, N^7, and NH_2 nitrogen atoms of AMP.[11,12] On the other hand, it is known that azide anions can substitute chloride and hydroxide ligands in $[AuCl_m(OH)_{4-m}]^-$ complexes with the formation of azidoaurate species. Their gross formula, $NaAu_{1.43}N_{9.05}$, evidenced partial reduction of gold(III) into gold(I) during the synthesis.[13] Moreover, it was noted that keeping of the resulting product in an aqueous solution during several days gives a blue-gold colloid. The latter transformation can be related to the formation of polynuclear complexes with the azide ions acting as bridging ligands, which is well-known for azide complexes with heavy metal ions.[9]

Hence, we suggested that azide ions competed with AMP for the initial binding with gold(III) ions and thus altered the pathway of the fluorescent AuNCs formation. Our control experiments confirmed that suggestion. We performed a series of syntheses as described above,[‡] but eliminating certain components from the synthesis. Absorption spectra of the mixtures are shown in Figure 2(*a*). When NaN_3 was the only reactant in the mixture with $HAuCl_4$, a very strong absorption band at ~325 nm marked the formation of the yellow-colored azidoaurate complexes. The complex formed from AMP and $HAuCl_4$ revealed no strong visible-range absorption bands, yellow color of the mixture being due to long-wave tail of the UV-range absorption band. When a mixture of AMP and NaN_3 interacted with $HAuCl_4$, the absorption of the azidoaurate complexes appeared as a shoulder at ~315 nm, due to the presence of the competing AMP ligand. Citrate buffered solution converted $HAuCl_4$ into dark-brown insoluble products (likely, aggregated gold particles) with virtually no absorbing species detected in the solution. The interaction of $AuCl_4$ with the mixture of citrate buffered solution and NaN_3 also gave insoluble product, marking insufficient stabilization of the reduced gold species in the absence of AMP. The presence of AMP and the citrate buffered solution led to AuNCs, assigned to a shoulder at ~310 nm in the absorption spectrum, as discussed above.

The emission spectra in Figure 2(*b*) show that soluble fluorescent product could be obtained only using a combination of AMP (stabilizer) and citrate buffered solution (reducing agent). Other samples either contained insoluble gold species (citrate, citrate + NaN_3) or exhibited negligibly weak fluorescence (NaN_3, AMP, NaN_3 + AMP). It is interesting to notice that the product obtained under the action of the mixture of NaN_3 and AMP revealed considerable yellow fluorescence upon about 2 weeks storage [Figure 2(*c*)]. Hence, azide acted as a weak reducing agent, and fluorescent AuNCs could be obtained even in the absence of citrate.

The yellow-emitting AuNCs prepared in this study in the presence of sodium azide retained sensitive fluorescence quenching in the presence of 1–10 μM of mercury(II) ions reported for other types of AuNCs (see, for example, refs. 14, 15). At the same time, intensity of fluorescence of the blue–green-emitting AuNCs prepared in the absence of azide remained nearly constant in the presence of much higher concentration of

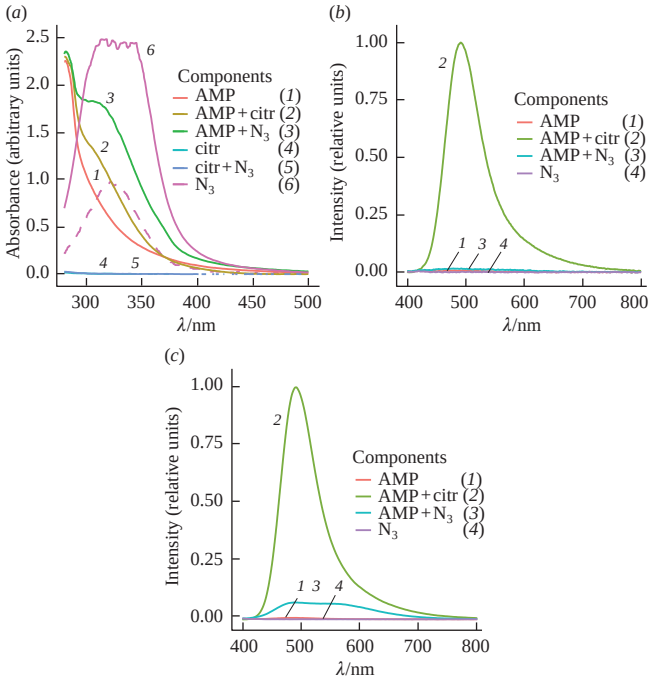


Figure 2 (*a*) Absorption spectra of the mixtures immediately after the synthesis (the dashed line corresponds to the spectrum of $HAuCl_4$ + NaN_3 mixture diluted with water); emission spectra (λ_{ex} = 365 nm) of the mixtures (*b*) immediately after synthesis and (*c*) upon 2 weeks storage. The mixtures contained $HAuCl_4$ and the components listed in the legend.

[‡] The components concentrations were as follows: 0.2 mM of $HAuCl_4$, 20 mM of citric acid, 20 mM of sodium citrate, 4 mM of AMP, and 1 mM of NaN_3.

NaN_3 (1000 μM) introduced upon the synthesis. Hence, the observed effect of azide ions was only pronounced at the stage of the AuNCs preparation. Thus, we found that introduction of small amount of sodium azide during formation of fluorescent gold nanoclusters in the presence of AMP allowed tuning of their optical properties.

This study was financially supported by the Russian Foundation for Basic Research (project no. 20-33-90296; NI and VS) and by Russian Science Foundation (project no. 21-73-20144; EK and VS).

References

1 X. Qu, Y. Li, L. Li, Y. Wang, J. Liang and J. Liang, *J. Nanomater.*, 2015, Article ID 784097.
2 H. Cui, Z.-S. Shao, Z. Song, Y.-B. Wang and H.-S. Wang, *J. Mater. Chem. C*, 2020, **8**, 14312.
3 A. Lopez and J. Liu, *J. Phys. Chem. C*, 2013, **117**, 3653.
4 J. Liu, H.-W. Li, W.-X. Wang and Y. Wu, *J. Mater. Chem. B*, 2017, **5**, 3550.
5 F. Nie, L. Ga and J. Ai, *ACS Omega*, 2019, **4**, 7643.
6 N. K. Ivanova, E. A. Karpushkin, L. I. Lopatina and V. G. Sergeyev, *Mendeleev Commun.*, 2023, **33**, 346.
7 E. A. Karpushkin, Y. D. Aleksandrov, A. V. Gibalova, A. M. Abakumov, L. I. Lopatina and V. G. Sergeyev, *Colloid. Polym. Sci.*, 2019, **297**, 433.
8 H. C. Lichstein and M. H. Saule, *J. Bacteriol.*, 1944, **47**, 239.
9 W. P. Fehlhammer and W. Beck, *Z. Anorg. Allg. Chem.*, 2013, **639**, 1053.
10 A. M. Brouwer, *Pure Appl. Chem.*, 2011, **83**, 2213.
11 J. Kundu, O. Neumann, B. G. Janesko, D. Zhang, S. Lal, A. Barhoumi, G. E. Scuseria and N. J. Halas, *J. Phys. Chem. C*, 2009, **113**, 14390.
12 D. W. Gibson, M. Beer and R. J. Barrnett, *Biochemistry*, 1971, **10**, 3669.
13 G. T. Rogers, *J. Inorg. Nucl. Chem.*, 1958, **5**, 339.
14 M. Yang, J. Yao, Y. Liu and Y. Duan, *J. Mater. Res.*, 2014, **29**, 2416.
15 J.-H. Lin, S.-J. Chen, J.-E. Lee, W.-Y. Chu, C.-J. Yu, C.-C. Chang and C.-F. Chen, *Chem. Eng. J.*, 2022, **430**, 133070.

Received: 7th September 2023; Com. 23/7244