Colloid Polym Sci (2005) 284: 97–101
DOI 10.1007/s00396-005-1339-9

SHORT COMMUNICATION

Takeshi Sakura
Tadahito Takahashi
Kazunori Kataoka
Yukio Nagasaki

One-pot preparation of mono-dispersed and physiologically stabilized gold colloid

Received: 30 January 2005
Accepted: 14 March 2005
Published online: 22 July 2005

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00396-005-1339-9

T. Sakura · T. Takahashi
Y. Nagasaki (✉)
Pre-venture Program, Japan Science and Technology Corporation (JST),
Tokatsu Techno-Plaza,
Kashiwanoha 5-4-6,
Kashiwa 277-0882, Japan
E-mail: nagasaki@nagalabo.jp
Tel.: +81-29-8535749
Fax: +81-29-8535749

K. Kataoka
Department of Materials Engineering,
Graduate School of Engineering,
The University of Tokyo, Hongo 7-3-1,
Bunkyo-ku Tokyo 113-8656, Japan

Y. Nagasaki
Tsukuba Research Center for Interdisciplinary Materials Science,
University of Tsukuba, Tennoudai 1-1-1,
Tsukuba 305-8573, Japan

Abstract We have developed a rapid and simple method for the preparation of nearly mono-dispersed gold colloids with a fairly high concentration above 10 mM using hydroxylamine as the reducing agents, in the presence of α-methoxy-ω-mercaptoethyl-poly(ethylene glycol) (MeO-PEG-SH). It was found that a hydroxylamine acted not only as a reducing agent, but also as a nucleation agent under alkaline reaction conditions. Though the colloid concentration was fairly high, the dispersion stability was remarkably improved even in a high ionic strength in the range greater than 1 M NaCl, in which conventional citrate gold colloids immediately flocculate and precipitate. The obtained colloid was successfully redispersed in aqueous media after lyophilization. In addition, the prepared gold colloid reduced a protein adsorption significantly on its surface. Concerning these results, the obtained colloidal dispersion may be suitable for biological applications, since a regionally concentrated colloidal dispersion with dispersion stability is required for bio-labeling and bio-imaging systems.

Keywords Gold colloid · High concentration · Hydroxylamine · α-methoxy-ω-mercaptoethyl-poly(ethylene glycol) · MeO-PEG-SH · Dispersion stability

Introduction

Recently, much attention has been paid to nano-scale colloidal metal particles in many fields, such as electronics, catalysis and clinical diagnostics [1–4]. Most of such colloids are prepared by the reduction of tetrachloroauric acid using a specific reducing agent and are stably dispersed in aqueous media by ionic repulsive forces. For versatile applications of such gold colloids, however, the stabilization by the ionic

repulsive force is not fully satisfied. For example, under physiological conditions for clinical applications such as immuno-colloidal imagings and diagnostics [5, 6], the gold colloids tend to aggregate due to the compensation of the electrostatic repulsive force by the high ionic strength.

Recently, we reported a preparation method for stable gold colloids having PEG tethered chains on its surface, which improves both the non-fouling character and dispersion stability [7]. In this method, however, the size distribution of the obtained gold colloid was rather broader because of the uncontrolled reduction process due to the solid reducing agent, $NaBH_4$. Natan et al. reported that a hydroxylamine has no nucleation ability of the aurate cation but has a suitable reducing ability on the gold surface [8, 9]. During our experiments with hydroxylamine in the presence of mercapto-ended PEG, however, we found that the hydroxylamine possessed both nucleation and reduction abilities of the aurate cation. Under suitable conditions, the obtained gold colloid had a nearly mono-dispersed distribution with a high colloidal concentration. Since the prepared gold colloid possessed PEG tethered chains on the surface, it was extremely high dispersion stability even in very high salt concentration. In addition, non-fouling character was confirmed in serum solution.

There are a few reports on rapid and simple preparation methods for a highly concentrated gold colloid in an aqueous phase [10, 11], retaining both high dispersion stability and non-fouling character in biological environment. We now report a new preparation method of a mono-dispersed gold colloid having a PEG tethered chain on the surface using hydroxylamine as the reducing agent.

Experimental

The UV/vis absorption spectra were recorded using a Beckman Coulter DU530 spectro-photometer with a 1-nm resolution. The transmission electron micrograph (TEM) was obtained using a Carl Zeiss LEO 922 operating at 160 kV. The particle diameters (Table 1) were determined using Scion Image software Ver. Beta 4.02 (Scion Corporation) freely available on the web. The diameters of approximately 200 particles were used for calculations of average particle size (D_{mean}) and their size standard deviation (SD).

Hydroxylamine (99.999% purity, 50 wt% solution in water, Aldrich), hydrogen tetrachloroaurate(III) tetrahydrate (99.9% purity, Wako Pure Chemical Ltd.), MeO-PEG-SH (M_n 5,200, $M_w/M_n = 1.02$, NOF Co., Ltd.), normal rabbit serum (NRS) (Sigma) and bovine serum albumin (BSA) (Wako Pure Chemical Ltd) were purchased and used as received.

We first examined the influence of the reaction mixture at different pH values from 2 to 13. A 700 µl volume of the aqueous solution of MeO-PEG-SH (40.1 mg/ml) was added to a 50 µl hydroxylamine solution (0.2 mmol/ml). The pH was adjusted by sodium hydroxide or hydrochloric acid to 2.0–13.0 and then brought to 800 µl with the addition of water. To

Table 1 Preparation of gold colloid in the presence of MeO-PEG-SH reduced by hydroxylamine under alkaline conditions

Run	Hydroxylamine (mmol/ml)	MeO-PEG-SH (mg/ml)	Temperature (°C)	λ_{max}[b] (nm)	PWMH[c] (nm)	A_{max}[d]	D_{mean}[e] (nm)	SD[f] (nm)
1↓[a]	0	9.5	25					
2↓	0	38	25					
3	0	151	25					
4↓	0.2	0	25					
5	0.2	9.5	25	533	118	1.74	10.4	3.0
6	0.2	38	25	529	98	1.62	10.6	4.1
7	0.2	151	25	548	162	0.36	11.2	6.2
8↓	1	0	25					
9	1	9.5	25	521	98	1.96	9.9	3.9
10	1	38	25	528	102	1.90	14.7	6.8
11	1	151	25	546	176	1.29	13.4	5.7
12↓	2	0	25					
13	2	9.5	25	525	112	1.96	13.6	5.6
14	2	38	25	538	144	1.91	15.4	7.0
15	2	151	25	541	188	1.52	12.7	6.4
16	0.2	9.5	4	521	82	1.72	10.0	1.9
17	0.2	9.5	60	543	154	1.70	15.5	5.3

Reaction conditions (pH = 13.00–13.10)
[a]Samples marked arrows (↓) showed precipitation
[b]Plasmon absorption wavelength
[c]Straight forward as twice the difference between λ_{max} and the λ of half maximal absorbance to the red shift of the λ_{max}
[d]Absorbance at λ_{max}
[e]Mean diameter of the particle calculated from TEM photographs
[f]Standard deviation of the particle diameter

this solution, 100 μl of an aqueous solution of $HAuCl_4$ (0.1 mmol/ml) was added under vigorous agitation at room temperature.

For further investigation, the effect of the concentrations of hydroxylamine and MeO-PEG-SH concentrations as well as the reaction temperatures were systematically studied for the preparation of highly concentrated colloidal dispersion under alkaline conditions (pH = 13.00–13.10), which was chosen as a consequence of the pH effect study (Table 1).

Commercially available gold colloids prepared by the citrate reduction method [12] were purchased from Poly Science, the concentration and the size of which were 0.29 mM and 10 nm, respectively. The citrate gold colloid was concentrated and used as the controls (samples A and B). A 45.6 ml aliquot of the original citrate colloid dispersion (0.29 mM) was centrifuged at 18,000× g for 4 h and the precipitant was brought to 1.2 ml of water to prepare the 11 mM gold colloid dispersion (sample A). A 50 ml aliquot of the original citrate gold colloid was mixed with 2.56 ml of MeO-PEG-SH (4 mg/ml) and centrifuged at 18,000×g for 8 h and the precipitant was brought to 1.32 ml of water to adjust the same concentration as sample 16 (11.1 mM)(sample B). Centrifuge refining was done three times to remove any free MeO-PEG-SH and citrate ion. A 1.2 ml aliquot of sample 16 was also centrifuge-refined three times without any concentration in order to remove any free MeO-PEG-SH and hydroxylamine.

The centrifugation- and lyophilization-concentrating stability of samples 16, A and B were studied. A 200 μl aliquot of samples 16, A and B were lyophilized and 200 μl of water was then added to them.

The dispersion stability along with non-fouling protein adsorption in NRS and BSA were estimated as follows: After a 100 μl volume of NRS or BSA was added to a 100 μl volume of the centrifuge-refined samples, the gold colloid was incubated overnight at 4°C and centrifuged (18,000×g for 12 h). The UV/vis spectra of the supernatants were monitored to estimate the protein adsorption properties on these gold surfaces. Detailed protein solutions investigated in this study are summarized in Table S1.

Results and discussions

When tetrachloroauric acid was mixed with hydroxylamine in the presence of MeO-PEG-SH under the acidic to neutral conditions, the color of the mixture gradually changed from red to orange and then an orange-colored precipitate was observed at the bottom of the vessel, which is in good agreement with the previous reports by Natan et al [8, 9]. On the contrary, the color of the solution immediately changed from bluish black to ruby red under alkaline conditions (pH = 12–13) within a few seconds, indicating the aurate ions being reduced. The plasmonic absorption of the obtained mixtures were clearly observed at $\lambda_{max} = 526$ nm (pH 12.14) and 523 nm (pH 12.96). The colloidal particles obtained in the highest pH region (pH 12.96) showed the narrowest size distribution ($D_{mean} = 15.1$ nm, SD = 4.9 nm). On the basis of these results, it is clear that hydroxylamine acts as a suitable reducing agent to prepare mono-dispersed colloidal gold particles, which is in sharp contrast to the result of Natan et al. The detailed results of the influence of the preparation conditions, such as the concentration of hydroxylamine and MeO-PEG-SH, as well as the reaction temperatures are summarized in Table 1. As can be seen in the table, it is confirmed that the low temperature conditions (4°C) gave the narrowest size distributions (sample 16).

Figure 1 shows a UV/vis spectrum along with a TEM image of sample 16. It was confirmed that spherical gold particles with a sharp plasmon peak were observed.

It is interesting to note that the reduction of the aurate cation took place without amine (runs 1, 2 and 3 in Table 1). In this case, however, a large precipitate with a metallic gold color was obtained. This is considered to be a result of the reduction of aurate cation by oxidizing the thiols to disulfides [13]. When the reaction was carried out using hydroxylamine in the absence of

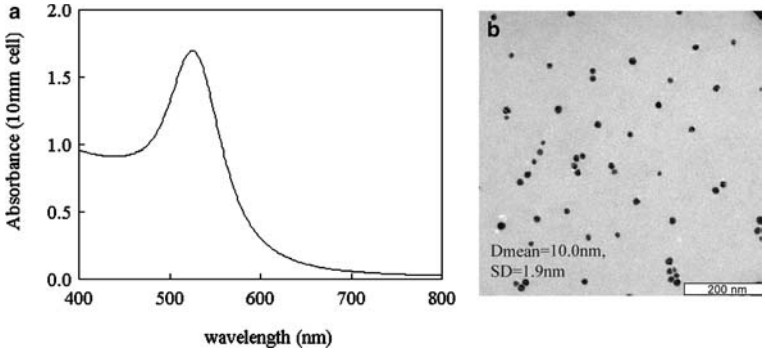

Fig. 1 **a** Absorption spectrum of sample 16 diluted 20-fold with water, **b** TEM photograph of sample 16 using a Carl Zeiss LEO 922 operating at 160 kV. Centrifuge refining was proceeded by Hitachi-koki himac CS 150GX before taking the TEM

MeO-PEG-SH, no stable colloid was obtained. Actually, the obtained colloid was flocculated immediately after the preparation. By using hydroxylamine in the presence of MeO-PEG-SH under the alkaline conditions, a gold colloid having diameters of 9–16 nm was obtained, retaining a low polydispersion factor.

In order to evaluate the stability of the prepared highly concentrated colloid, the centrifugation—redispersion purification was carried out. The gold colloid prepared by hydroxylamine in the presence of PEG-SH showed no spectral change before and after the centrifuge treatments, which is sharp contrast to the commercially available gold colloid. For example, the citrate reduced gold colloid changed its UV/vis spectra significantly after the centrifugation treatments [14]. It is reported previously that PEG chains formed on the gold colloid surface as a tethered fashion improved the dispersion stability in aqueous media [7, 15]. From these obtained data, it is concluded that the PEG tethered chains surface was constructed on the formed gold colloid during the reducing process. Detailed results are described in supporting information (Figures S0, S1).

It is well known that once the citrate gold colloid was lyophilized, complete dispersion is no longer accomplished. A large black lump was always observed even with a continuous stirring. In the case of sample 16, however, the lyophilization did not cause any cluster formation. After the lyophilization, sample 16 could be fully re-dispersed just after the addition of water. Actually, the UV/vis spectra did not change at all before and after the lyophilization. Detailed results are described in supporting information (Figure S2).

The stability of the PEGylated gold colloids was further investigated under both a high salt concentration and in the presence of serum.

When saline was added to the citrate gold colloid (sample A), a black precipitate was immediately observed (Fig. 2a). On the contrary, sample 16 showed no precipitation even under very high salt concentrations. It was confirmed that the steric repulsion of the PEG tethered chains on the colloid surface improved the dispersion stability even under the very high salt concentration conditions.

Colloid particles ranging from 1 nm to 1 μm are thermodynamically driven to flocculate due to the attractive van der Waals forces that work in the range of approximately 5 nm from the surface [16–20]. The Debye length in a 150 mM aqueous solution of a 1:1 electrolyte is ca. 0.8 nm. Thus, in physiological fluids with the above electrolyte concentration, the Debye length is often inadequate to prevent the particles from approaching close enough to where attractive van der Waals becomes the dominant interaction. Electrostatically stabilized particles coagulate under the higher electrolytes conditions (at critical coagulation concentration known as a Schultz–Hardy rule). These results proved that PEG stabilization is far less dependent of the ionic strength of the media than the electrostatic stabilization (such as the citrate colloid) and that PEG is suitable for providing a strong stabilization in physiological fluids.

Interactions of the prepared gold colloid with protein solutions were carried out to evaluate the biocompatibility. When the commercially available gold colloid was added to the NRS solution, a significant change in the UV/vis spectra was observed (Figure S4). Figure 3a shows a change in the strength of plasmon peak as a function of the NRS concentration. When a small amount of NRS was added to sample A, a significant increase in the plasmon absorption was observed. It is well known that the surface plasmon spectrum of the gold colloid is influenced by the dielectric constant of the surface environment [21]. A strong serum protein adsorption should be one of the reasons for the change in the plasmon absorption of the citrate colloid [22]. The similar tendency was observed in the case of sample B though the spectral change was not as strong as that of sample A, indicating that the serum protein interacted with the surface of sample B to some extent. Contrary to these two control samples, sample 16 showed almost no change in the plasmon absorption regardless of the serum concentration. This tendency was confirmed by

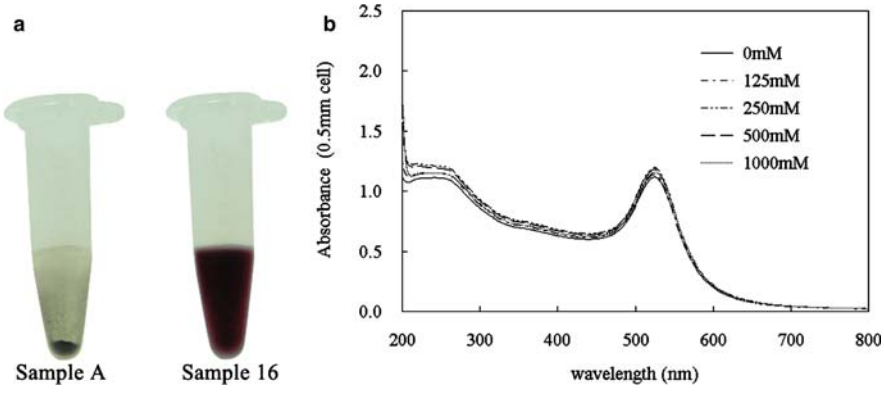

Fig. 2 **a** A photograph of gold colloid samples (A and 16) after the centrifugation in 1 M of NaCl solution (Runs 6 and 23 in Table S1), **b** UV-vis spectra of the mixture of centrifuge-refined sample 16 at the various concentrations of NaCl solution (Runs 2–6 in Table S1)

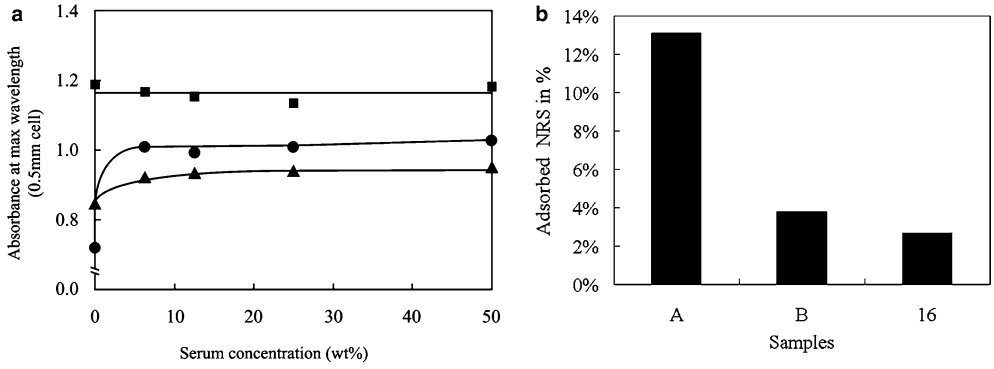

Fig. 3 **a** The change in A_{max} of gold colloid samples A (*filled circle*), B (*filled triangle*) and centrifuge-refined 16 (*filled square*) as a function of the concentrations of normal rabbit serum (NRS) (A_{max}: absorbance at λ_{max}). **b** The amount of adsorbed proteins from NRS solution (6.25 wt%) on gold surface. (The conditions are described in Runs 7, 13 and 16 in Table S1)

the quantification of the adsorbed proteins as shown in Fig. 3b. On the concentrated gold colloid surface without PEG chains (sample A), a significant protein adsorption was observed. Even in the case of commercial citrate gold colloid mixed with PEG-SH (sample B), certain amount of proteins were adsorbed on the surface. On the contrary, serum protein adsorption was fairly suppressed on the sample 16. Though sample B should possess the PEG tethered chain on the surface, the non-fouling character was not the same as in sample 16. We have already reported that the PEG chain density and length significantly influences the protein adsorption tendency [23]. Since the PEG chain length on sample B is the same as that on sample 16, the PEG chain density may be the reason for the difference in the protein adsorptions. In the case of sample 16, the PEG tethered chains form on the surface during the particle propagation process, which may be suitable for a high density brush than that after the modification of the preformed colloid by PEG-SH.

Conclusions

In conclusion, we have developed a method to prepare a highly concentrated mono-dispersed colloid using hydroxylamine as the reducing agent in the presence of MeO-PEG-SH. Lowering the molar ratio of hydroxylamine/Au and MeO-PEG-SH/Au and the reaction temperature produced a narrow size distribution. The concentration of the PEGylated gold colloid solution was 11 mM, which is fairly higher than that obtained using the conventional colloid preparation conditions. By using this method, a gold colloid of fairly high concentration was prepared while retaining its high dispersion stability. The obtained gold colloid showed a remarkable decrease in protein adsorption even when compared to the PEGylated colloid prepared by the commercially available gold colloid and PEG-SH. Such a new PEGylated gold particle is promising as one of the bio-related tools in nanobioscience.

References

1. Musick MD, Keating DC, Keefe MH, Natan MJ (1997) Chem Mater 9:1499
2. Toshima N, Wang Y (1994) Adv Mater 6:245
3. Abraham GE, Himmel PB (1997) J Nutr Environ Med 7/4:295
4. Huang MF, Kuo YC, Huang CC, Chang HT (2004) Anal Chem 76:192
5. Sokolov K, Follen M, Aaron J, Pavlova I, Malpica A, Lotan R, Richards-Kortum R (2003) Cancer Res 63:1999
6. Paciotti G, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin R, Tamarkin L (2004) Drug Deliv 11:169
7. Otsuka H, Akiyama Y, Nagasaki Y, and Kataoka K (2001) J Am Chem Soc 123:8226
8. Brown KR, Walter D, Natan MJ (2000) Chem Mater 12:306
9. Brown KR, Natan MJ, (1998) Langmuir 14:726
10. Kobayashi T, Kamo H (2000) Chem Eng 53:909
11. Kobayashi T, Ishibashi H, Kamo H, Japanese patent no. 3594803
12. Turkevich J, Stevenson PC, Hiller J (1951) Trans Faraday Soc 11:55
13. Negishi Y, Tsukuda T (2003) J Am Chem Soc 125:4046
14. Quinten M, Kreibig U (1986) Surf Sci 172:557
15. Wuelfing WP, Gross SM, Miles DT, Murray RW (1998) Am Chem Soc 120:12696
16. Israelachvili J (Eds) (1996) Intermolecular and surface forces, chap 12. Asakura
17. Leckband D, Sheth SR, Halperin A (1999) J Biomater Sci Polym Ed 10:1125
18. Napper DH (1983) Chem Technol Water Soluble Polym 233
19. Overbeek Th G (1977) Colloid Interface Sci 58:390
20. Patel PD, Russel WB (1989) J Colloid Interface Sci 131:192
21. Templeton AC, Pietron JJ, Murray RW, Mulvaney P (2000) J Phys Chem B 104:564
22. Nath N, Chilkoti A (2002) Anal Chem 74:504
23. Otsuka H, Nagasaki Y, Kataoka K, Langmuir (in press)