MethodsX 7 (2020) 100896

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

A modified and simplified method for purification of gold nanoparticles ☆

Irem Kulu [a,b], Rui Huang [a], Bhavna Kalyanaraman [c], Vincent M. Rotello [a,*]

[a] Department of Chemistry, University of Massachusetts, 710 North Pleasant Street, Amherst, MA, 01003, United States
[b] Department of Chemistry, Faculty of Science, Gebze Technical University,41400 Gebze, Kocaeli, Turkey
[c] School of Chemical and Biotechnology, Sastra Deemed University, 613 401 Thanjavur, Tamil Nadu, India

A B S T R A C T

2 nm gold nanoparticles (AuNPs) have promising applications within drug and protein delivery, bioimaging, and biosensing. By performing ligand place-exchange reactions, AuNPs protected with alkanethiolate ligands can be functionalized to regulate their behaviors. In this reaction, a new ligand is incorporated by mixing a thiol with the AuNPs. To remove the excess new ligand as well as the displaced thiolate, dialysis has previously been the most widely used method. However, this purification method is time-consuming and fails to remove unwanted thiols completely. In this study, we describe a fast and efficient procedure to purify AuNP aqueous solution through liquid-liquid extraction using dichloromethane.

- We demonstrate a facile way to purify AuNPs after ligand place-exchange reactions through liquid-liquid extraction.
- Liquid-liquid extraction is a simple, inexpensive and efficient method for AuNP purification.
- This protocol enables us to completely purify AuNPs in a few hours and can be used as a much quicker and more scaleable valid alternative to dialysis.

A R T I C L E I N F O
Method name: Purification of gold nanoparticles from free ligand through liquid-liquid extraction
Keywords: Gold nanoparticles, Purification, Extraction
Article history: Received 21 January 2020; Accepted 7 April 2020; Available online 21 April 2020

☆ **Direct Submission or Co-Submission:** Direct Submission

* Corresponding author.
E-mail addresses: iremkulu@gtu.edu.tr (I. Kulu), ruihuang@chem.umass.edu (R. Huang), bhavnakalyanaraman@gmail.com (B. Kalyanaraman), rotello@chem.umass.edu (V.M. Rotello).

https://doi.org/10.1016/j.mex.2020.100896

Specifications table

Subject Area	Chemistry
More specific subject area	Gold nanoparticle synthesis and purification
Method name	Purification of gold nanoparticles from free ligand through liquid-liquid extraction
Name and reference of original method	Entrapment of Hydrophobic Drugs in Nanoparticle Monolayers with Efficient Release into Cancer Cells *J. Am. Chem. Soc 0.*200913141360–1361
Resource availability	https://doi.org/10.1021/ja808137c

Method

Method details

A ligand place-exchange reaction of the new thiol ligand dissolved in CH_2Cl_2: MeOH (1:1, 4 ml) with pentanethiolate-coated AuNPs (d= ~2 nm) was carried out for 3 days at rt (~25 °C). The final reaction mixture contained the desired AuNPs, the excess new ligand and replaced thiolate. The solvent was removed under reduced pressure and then washed with organic solvents (hexanes followed by ether). Next, the AuNPs were redissolved in water and then further purified by liquid-liquid extraction using CH_2Cl_2. Nuclear Magnetic Resonance (NMR) was used to check the purity.

Step 1: Monolayer Exchange Reaction

Materials

All organic solvents were purchased from Fisher and purged through nitrogen before being used. Aqueous AuNP solutions were prepared with deionized water produced by a Millipore System.

Procedure

Pentanethiolate-coated AuNPs (d= ~2 nm) were synthesized using the Brust-Schiffrin gold core synthesis method [1]. Tetraethylene glycol trimethyl ammonium (TTMA) ligands were synthesized according to the literature (~70 ligands per particle) [2]. The TTMA AuNPs were then synthesized using the Murray ligand exchange reaction following a previously reported protocol [3]. Briefly, 30 mg of pentanethiolate-coated AuNPs was dissolved in 4 mL of purged CH_2Cl_2 and the solution was allowed to stir. Further, 90 mg of TTMA ligand was dissolved in 4 mL purged CH_2Cl_2: MeOH (1:1) solution. Then the ligand solution was slowly added to pentanethiolate-coated AuNPs while stirring. The solution mixture was then purged with N_2 to remove any trace of CH_2Cl_2. The ligand exchange reaction was then carried out for 3 days under continuous stirring.

Step 2: Purification of AuNPs

After the reaction was carried out for 3 days, the solvent was evaporated without any heating. AuNP residue was washed with hexanes (4 × 10 mL) and diethyl ether (8 × 10 mL) and then the solid residue was dissolved in 10 mL MiliQ water. Next, the solution of AuNPs was further purified by liquid-liquid extraction using CH_2Cl_2.

Procedure

1. The final reaction mixture (10 mL AuNP solution) was transferred into a separating funnel and then equal volume of dichloromethane was added.
2. The organic layer was extracted from the aqueous layer phase.
3. This washing was repeated 5 times to ensure complete removal of impurities.
4. The aqueous layer was then purged with nitrogen to remove any trace of CH_2Cl_2.
5. The purified AuNP solution was then filtered using a 0.22 µm syringe filter.

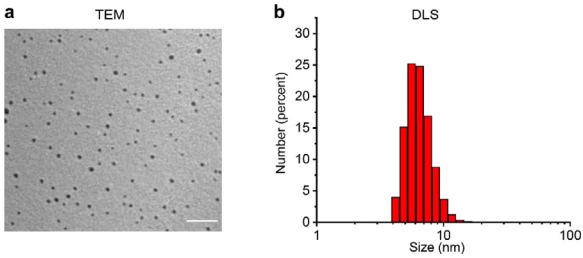

Fig. 1. TEM images (a) and DLS histogram graphs (b) of the TTMA AuNP after liquid-liquid extraction. Scale bar represents 20 nm. The diameter of TTMA AuNP was analyzed by ImageJ.

Step 3: Characterization of Gold nanoparticles

Hydrodynamic diameter of the AuNP was measured by dynamic light scattering (DLS) in DI water using a Malvern Zetasizer Nano ZS instrument. The measurement angle was 173° (backscatter). Data were analyzed by the "multiple narrow modes" (high resolution) based on non-negative-least-squares (NNLS). Dynamic light scattering data showed the formation of ~10 nm AuNPs with no aggregation observed after liquid-liquid extraction.

The particle structure was also confirmed by using transmission electron microscopy (TEM). A TEM sample of AuNP was prepared by placing one drop of the desired solution (1 μM) on to a 300-mesh Cu grid-coated with carbon film. These samples were analyzed and photographed using JEOL CX-100 electron microscopy with an acceleration voltage of 200 kV (Fig. 1).

Step 4: Structure Characterization of Gold nanoparticles with ^1H NMR Spectroscopy

^1H NMR spectroscopy was used to verify the success of the ligand place-exchange reaction and the purity of the materials (Fig. 2). 0.5 μL NMR samples (10 μM) were prepared by replacing H_2O with D_2O through centrifugation (5 times) using 10 K Amicon© ultra-centrifugal filter tube. ^1H NMR spectra in D_2O showed substantial broadening of the proton signals and no free ligands were observed.

Additional information

Background

Gold nanoparticles (AuNPs) have been widely used in bionanotechnology due to their facile synthesis, ease of functionalization, high biocompatibility, and inherent non-toxicity. Monolayer-protected AuNPs are mostly synthesized via the Brust–Schiffrin two-phase synthesis method [1]. This methodology can control the size of the AuNPs between 1.5 to 5 nm by varying reaction conditions including gold-to-thiol ratio, reduction rate, and reaction temperature. Due to the synergic effect of the thiol-gold interactions and van der Waals attractions between neighboring ligands, these alkanethiolate-protected AuNPs possess higher stability compared to most other AuNPs [4,5]. The ligand structure can be tuned through ligand place-exchange reactions as described by Murray et.al [6], which allows for the tailoring of chemical properties such as solubility, chemical reactivity, surface chemistry, cell surface interactions and binding affinity [7-9].

Ligand exchange between the 2-nm AuNP and functionalized thiols can be achieved by combining gold nanoparticles with an excess of thiol (approximately 1:3 ratio) in an appropriate solvent. Organic-soluble exchange products are prepared in a monophasic system (typically using CH_2Cl_2: MeOH as the solvent mixture) with a variety of alkyl-thiols [7]. It is particularly crucial to obtain high-purity AuNPs samples for delivery, imaging, and sensing applications as minor impurities are toxic and may affect AuNP function. It is also known that gold nanoparticles in the presence of excess thiol ligand (as with

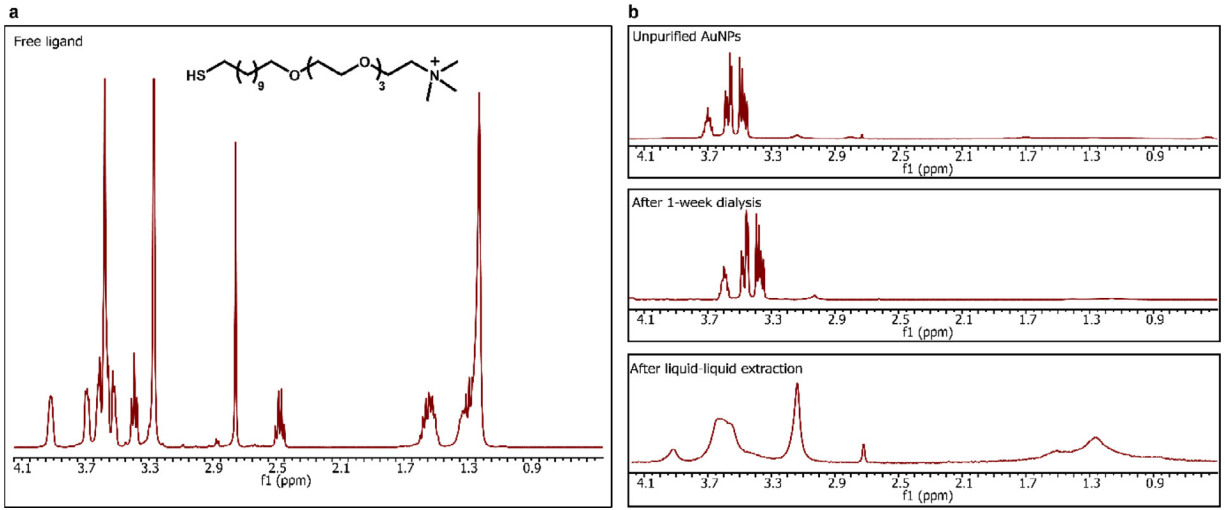

Fig. 2. 400 MHz ^{1}H NMR spectra of (a) free ligand in $CDCl_3$. (b) Unpurified AuNPs- AuNPs purified through 1-week dialysis and liquid-liquid extraction (in D_2O).

ligand exchange) will rapidly decompose [7,10]. Dialysis is often the most commonly used method for removing impurities and small molecular weight impurities. This method involves the mass transfer between two miscible liquid phases (the donor and acceptor solutions) separated by a membrane through which some chemical species are likely to pass. Miscibility between the donor and acceptor solutions is inherent to dialysis and distinguishes it from e.g., liquid–liquid extraction [11]. However, dialysis is time-consuming and sometimes fails to remove unwanted thiols completely. In this study, we optimized the method to allow the removal of unwanted thiolates from reaction byproducts using liquid-liquid extraction.

Declaration of Competing Interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgment

This research was supported by the NIH (DK121351 and EB02264)
I.K. acknowledges TÜBİTAK for the fellowship 1059B191800458.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2020.100896.

References

[1] M. Brust, M. Walker, D. Bethell, D.J. Schiffrin, R. Whyman, Synthesis of thiol-derivatised gold nanoparticles in a two-phase Liquid–Liquid system, J. Chem. Soc. Chem. Commun. (7) (1994) 801–802.
[2] C.K. Kim, P. Ghosh, C. Pagliuca, Z.-J. Zhu, S. Menichetti, V.M. Rotello, Entrapment of Hydrophobic Drugs in Nanoparticle Monolayers with Efficient Release into Cancer Cells, J. Am. Chem. Soc. 131 (4) (2009) 1360–1361.
[3] B. Yan, Z.-J. Zhu, O.R. Miranda, A. Chompoosor, V.M. Rotello, R.W. Vachet, Laser desorption/ionization mass spectrometry analysis of monolayer-protected gold nanoparticles, Anal. Bioanal. Chem. 396 (3) (2010) 1025–1035.
[4] Y.-C. Yeh, B. Creran, V.M. Rotello, Gold nanoparticles, preparation, properties, and applications in bionanotechnology, Nanoscale 4 (6) (2012) 1871–1880.
[5] M.J. Hostetler, J.E. Wingate, C.-J. Zhong, J.E. Harris, R.W. Vachet, M.R. Clark, J.D. Londono, S.J. Green, J.J. Stokes, G.D. Wignall, G.L. Glish, M.D. Porter, N.D. Evans, R.W. Murray, Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: core and monolayer properties as a function of core size, Langmuir 14 (1) (1998) 17–30.
[6] M.J. Hostetler, A.C. Templeton, R.W. Murray, Dynamics of place-exchange reactions on monolayer-protected gold cluster molecules, Langmuir 15 (11) (1999) 3782–3789.
[7] G.H. Woehrle, L.O. Brown, J.E. Hutchison, Thiol-functionalized, 1.5-nm gold nanoparticles through ligand exchange reactions: scope and mechanism of ligand exchange, J. Am. Chem. Soc. 127 (7) (2005) 2172–2183.
[8] A. Verma, F. Stellacci, Effect of surface properties on nanoparticle–cell interactions, Small 6 (1) (2010) 12–21.
[9] B. Duncan, C. Kim, V.M. Rotello, Gold nanoparticle platforms as drug and biomacromolecule delivery systems, J. Controlled Release 148 (1) (2010) 122–127.
[10] S.F. Sweeney, G.H. Woehrle, J.E. Hutchison, Rapid purification and size separation of gold nanoparticles via diafiltration, J. Am. Chem. Soc. 128 (10) (2006) 3190–3197.
[11] D.D. Cunningham, Fluidics and sample handling in clinical chemical analysis, Anal. Chim. Acta 429 (1) (2001) 1–18.