

# Fabrication of Gold Nanoparticles-Deposited Glass Plate by Electroless Metal Plating Technique and Immobilization of Proteins on It

Yoshio Kobayashi, and Yuya Ishii

**Abstract**— This work performed immobilization of proteins on a glass plate, on which Au nanoparticles are deposited (Au-glass). A method for fabricating the Au-glass plate was An electroless metal plating technique that was composed of (1) adsorption of  $\text{Sn}^{2+}$  ions, (2) deposition of metallic Ag nuclei generated by reducing  $\text{Ag}^+$  ions with  $\text{Sn}^{2+}$  ions on the  $\text{Sn}^{2+}$ - adsorbed sites, and (3) deposition of Au nanoparticles by reducing  $\text{Au}^+$  ions on the Ag nuclei-deposited surface. According to visible (VIS) extinction spectroscopy, a surface plasmon resonance absorption peak was observed at 522.6 nm, which indicated that Au nanoparticles were successfully deposited on the glass plate. Protein-immobilization was performed in aqueous solution with bovine serum albumin (BSA) in the presence of the Au-glass plate. VIS extinction spectroscopy revealed that the BSA was adsorbed on the Au particle surface.

**Keywords**—Au nanoparticle, Deposition, Electroless metal plating, Protein-immobilization

## I. INTRODUCTION

**M**ETALS such as Au and Ag are being used widely in various fields. Their nanometer-sized particles have special interest because they are expected to exhibit unique properties different from those of bulk material [1,2], which is called 'size effect'. Nanoparticles of such metals show a special optical property like surface plasmon resonance (SPR) absorption [1,3,4], which depends on particle size, particle shape and surface condition.

Besides the optical property derived from the size effect, such metals have the ability to adsorb proteins on their surface through amino groups and thiol groups of proteins. This means that such metals can be used as support for immobilized enzyme. To take full advantage of surface, a decrease in their size is effective, because total surface area of metal increases with the decrease in size. Those metals face at a problem: They take cost. From a view point of effective use of metals, the metals are desired to be reused after their use.

Nanoparticles tend to aggregate, which deteriorates their

Yoshio Kobayashi is with the Department of Biomolecular Functional Engineering, College of Engineering, Ibaraki University, Hitachi, Ibaraki 316-8511 Japan (corresponding author's phone: +81-294-38-5052; e-mail: [ykoba@mx.ibaraki.ac.jp](mailto:ykoba@mx.ibaraki.ac.jp)).

Yuya Ishi is with the with the Department of Biomolecular Functional Engineering, College of Engineering, Ibaraki University, Hitachi, Ibaraki 316-8511 Japan (e-mail: [11NM502L@hcs.ibaraki.ac.jp](mailto:11NM502L@hcs.ibaraki.ac.jp)).

unique properties derived from the size effect. Immobilizing the nanoparticles on supports such as powders and plates is a candidate to prevent the aggregation.

An electroless metal plating technique can make metallic films plated on insulating support materials [5-8]. In our previous work, Au nanoparticles were successfully deposited on silica spheres by the electroless metal plating technique [9]. In another previous work of our group, the electroless metal plating technique was extended to deposition of Au nanoparticles on glass plates (Au-glass) [10]. The peak wavelength of SPR absorption of Au nanoparticles for the Au-glass plate depended on dielectric constant of the solution, which expected that the Au-glass was used as a sensor for measuring dielectric constant of solution.

In the present work, the Au-glass plate developed in our previous work [10] is examined as a support for immobilizing proteins. The present work also studies on recovery of the Au-glass plate toward reusable support of immobilized enzyme.

## II. EXPERIMENTAL

### A. Materials

Tin chloride (anhydrous) (97%) and trifluoroacetic acid (98%) were used for preparation of "Sn solution". For preparation of "Ag solution", used were silver nitrate (99.8%) and aqueous ammonia (28.0-30%). Oromerse Part B (Au electroless plating solution), formaldehyde solution (36.0-38%), sodium bicarbonate (99.5-100.3%), sodium sulfite (97%) and sulfuric acid (95%) were used for preparation of "Au solution". Affinity between the glass plate surface and ions of metals such as Sn, Ag, and Au was improved with polyvinylpyrrolidone (PVP, K=30). Protein used for immobilization on glass was bovine serum albumin (BSA). The supports for Au-deposition were sodium glass plates (18×18 mm, 0.12-0.17 mm thick). Oromerse Part B, BSA, sodium glass plates and the other chemicals were supplied by Technic Inc., Merk Ltd., Matsunami Glass Ind., Ltd. and Kanto Chemical Co., Inc., respectively. All chemicals were used as received. Water that was ion-exchanged and distilled with Shimadzu SWAC-500 was used in all the preparations.

### B. Preparation

Prior to Au-deposition by the electroless metal plating, the glass plates were pre-treated by in turn immersing in liquids of

acetone, water, PVP aqueous solution, and then water for improving affinity between the glass plate surface and the various metal ions.

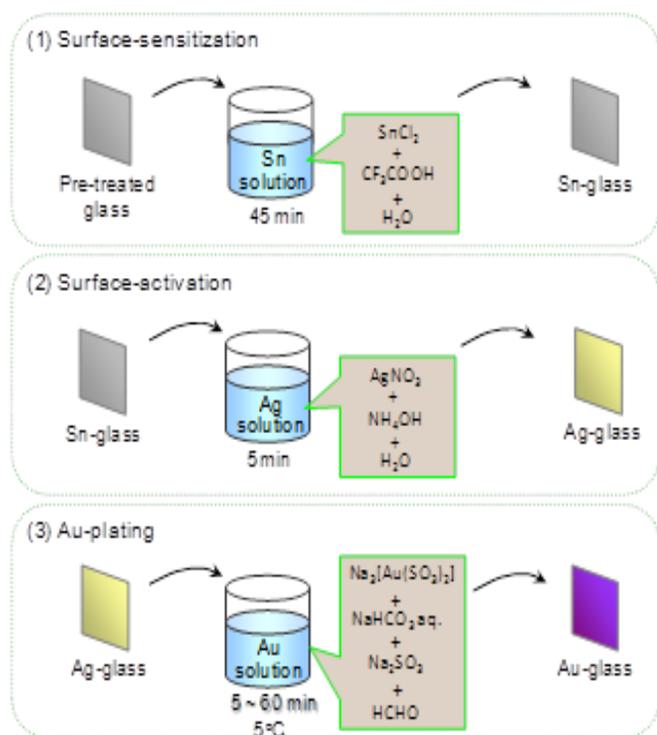


Fig. 1 Sketch of procedure for electroless Au-plating technique.

The electroless metal plating technique was employed for Au deposition on the pre-treated glass plate, according to a work performed by Menon and Martin [5]. This technique was composed of three steps, as shown in Fig. 1. The first step was surface sensitization of the glass plate or adsorption of  $\text{Sn}^{2+}$  on the plate. The glass plate was immersed into Sn solution, which was prepared by dissolving the tin chloride (0.05 g) in the water (10 mL) with addition of the trifluoroacetic acid (0.05 mL). After 45 min, the glass plate was washed with immersion in water (Sn-glass). The second step was surface activation or deposition of Ag nuclei on the plate. The Sn-glass plate was immersed into Ag solution, which was prepared by dissolving the silver nitrate (0.06 g) in the water (10 mL), adding several drops of the aqueous ammonia to form brownish fine precipitate of silver (I) oxide, and then adding aqueous ammonia further to make the solution transparent due to formation of  $\text{Ag}(\text{NH}_3)_2^+$  complex. After 5 min, the glass plate was also washed with immersion in water (Ag-glass). The third step was Au-plating or deposition of Au nanoparticles on the plate. The Ag-glass plate was immersed into Au solution, which was prepared by mixing the water (10 mL), the Oromerse Part B (0.25 mL), the formaldehyde solution (0.5 mL), the sodium bicarbonate (0.021 g), the sodium sulfite (0.16 g) and several drops of the sulfuric acid. The deposition times were 5-60 min. The obtained glass plate was washed also washed with immersion in water, and then annealed at  $500^\circ\text{C}$  in air (Au-glass).

Protein-immobilization was performed for the Au-glass plate.

The Au-glass plate was immersed in 0.12 g/L BSA solution at room temperature for 4 h. The immersed Au-glass plate was washed by immersing it in water.

### C. Characterization

The Au-glass plates were characterized by visible (VIS) extinction spectroscopy and X-ray diffractometry (XRD). VIS extinction spectra were measured in air with a Shimadzu UV-3101PC spectrophotometer. XRD measurements were carried out with a Rigaku RAD-B X-ray diffractometer at 50 kV and 150 mA with  $\text{CuK}_{\alpha 1}$  radiation.

## III. RESULTS AND DISCUSSION

### A. Morphology of Au-glass plate

Figs. 2 (a) and (b) show photographs of the Au-glass plate prior to annealing. The plate for the deposition time of 5 min had a purple tone, which appeared to be derived from SPR absorption of Au nanoparticles. This observation implied that Au nanoparticles were deposited on the glass plate with the present method. An increase in the deposition time to 60 min made the purple tone increased. This indicated that the amount of Au deposited on the glass plate increased with increasing the deposition time. Fig. 3 shows XRD patterns of the plates. No dominant peaks appeared in the sodium glass plate, except for a broad peak at ca. 25 degree that was due to amorphous. In contrast, for the Au-plate glass, peaks were detected at 38.3 and 45.0 degree. They were attributed to (111) and (200) planes of cubic gold (JCPDS card No. 04-0784). Accordingly, the metallic gold was successfully produced with the present method. Fig. 2 (c) shows a photograph of the Au-glass plate after annealing. The plate also had a purple tone. This observation implied that Au nanoparticles were still present on the glass plate even after the annealing. The color of Au-glass plate became slightly reddish after the annealing. This indicated that morphology of the Au nanoparticles was varied with the annealing.

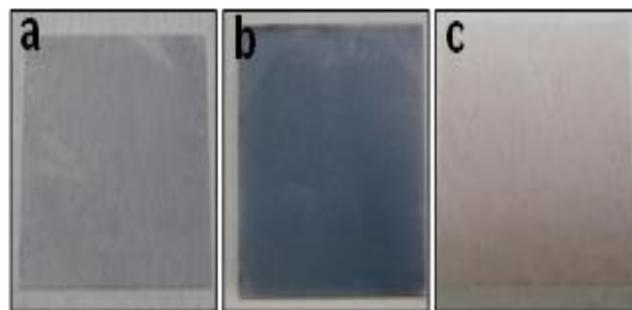


Fig. 2 Photographs of Au-glass plates. Images (a) and (b) show Au-glass plates prior to annealing, in which the deposition times were (a) 5 and (b) 60 min. Image (c) shows the sample (a) after annealing.

Fig. 3 (a) shows a VIS extinction spectrum of the Au-glass plate. The annealed Au-glass plate was used for the measurement. A peak that was clearly observed at 522.6 nm was attributed to the SPR absorption [1], which supported the

deposition of Au nanoparticles on the glass plate.

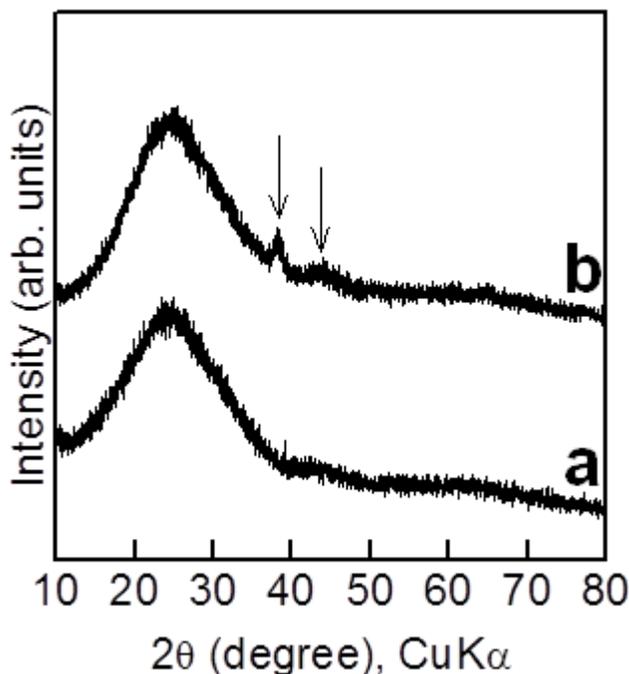


Fig. 3 XRD patterns of (a) glass plate and (b) Au-glass plate. The sample (b) was the same as the sample (b) in Fig. 2. Arrows show peaks attributed to cubic gold.

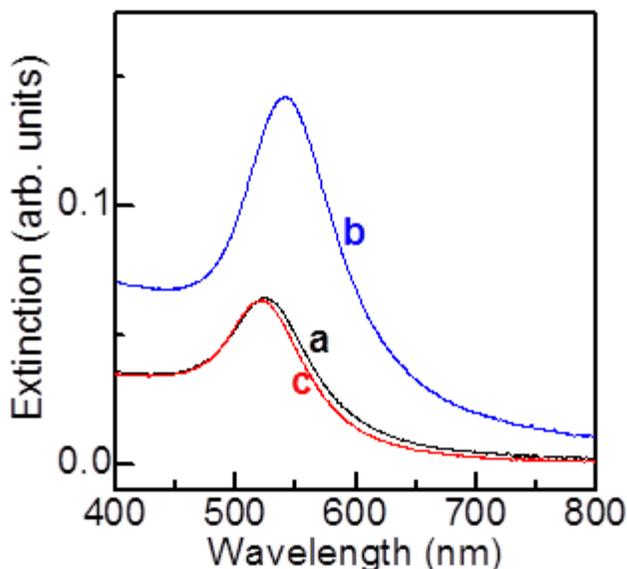


Fig. 4 VIS extinction spectra of various Au-glass plates. Sample (a): Au-glass plate, sample (b): sample (a) after BSA-immobilization, and sample (c): sample (b) after annealing.

#### B. Protein-immobilization

Fig. 3 (b) shows a VIS extinction spectrum of the Au-glass plate after the protein-immobilization. Intensity of the SPR peak increased with the protein-immobilization. Adsorption of BSA on Au nanoparticle surface probably increased apparent Au particle size. This increase in size strengthened light scattering from the particles, which provided the increase in extinction intensity. The SPR peak position shifted to 541.9 nm. The SPR absorption band in the VIS range is very sensitive to particle

size, particle shape and conditions of particle surface [1,3,4]. Adsorption of BSA was considered to vary the condition of Au surface in the present work. Accordingly, both tendency for the SPR peak intensity and position indicated successful protein-immobilization.

Fig. 3 (c) shows a VIS extinction spectrum of the BSA-immobilized Au-glass plate. The plate was annealed at 500°C in air after the protein-immobilization. The shape of spectrum was very similar to the Au-glass plate prior to the protein-immobilization, which was shown in Fig. 3 (a). This indicated that the BSA burnt on contact with air, and then the BSA-immobilized Au-glass plate was restored to its former state with no damage to Au particles by the annealing.

#### IV. CONCLUSION

The Au-glass plates were fabricated by extending the electroless metal plating technique. The BSA-immobilization on the Au-glass plate was successfully performed by simply immersing it into the BSA aqueous solution. The VIS extinction spectrum of the BSA-immobilized Au-glass plate after the annealing was similar to that of the Au-glass plate prior to the BSA-immobilization. Accordingly, it was found that the BSA-immobilized Au-glass plate recovered its former state prior to the BSA-immobilization with no damage to Au particles by the annealing. This result on recovery implied that the Au-glass plate produced in the present work could be reused. Awaited are further studies on optimization of protein-immobilization conditions, a precise mechanism of protein-immobilization, and immobilized enzyme.

#### REFERENCES

- [1] S. Basu, S. K. Ghosh, S. Kundu, S. Panigrahi, S. Praharaj, S. Pande, S. Jana, and T. Pal, "Biomolecule induced nanoparticle aggregation: Effect of particle size on interparticle coupling," *J. Colloid Interface Sci.*, vol. 313, pp. 724–734, Sept. 2007.  
<http://dx.doi.org/10.1016/j.jcis.2007.04.069>
- [2] L. Wang, W. Ma, L. Xu, W. Chen, Y. Zhu, C. Xu, and N. A. Kotov, "Nanoparticle-based environmental sensors," *Mater. Sci. Eng. R*, vol. 70, pp. 265–274, Nov. 2010.  
<http://dx.doi.org/10.1016/j.mser.2010.06.012>
- [3] T. Som and B. Karmakar, "Plasmon tuning of nano-Au dichroic devitrified antimony glass nanocomposites by refractive index control," *Chem. Phys. Lett.*, vol. 479, pp. 100–104, Sept. 2009.  
<http://dx.doi.org/10.1016/j.cplett.2009.07.109>
- [4] A. Chhatre, P. Solasa, S. Sakle, R. Thakkar, and A. Mehra, "Color and surface plasmon effects in nanoparticle systems: Case of silver nanoparticles prepared by microemulsion route," *Colloid Surf. A*, vol. 404, pp. 83–92, June 2012.  
<http://dx.doi.org/10.1016/j.colsurfa.2012.04.016>
- [5] V. P. Menon and C. R. Martin, "Fabrication and evaluation of nanoelectrode ensembles," *Anal. Chem.*, vol. 67, pp. 1920–1928, July 1995.  
<http://dx.doi.org/10.1021/ac00109a003>
- [6] Q. Zhou, H. Chen, and Y. Wang, "Region-selective electroless gold plating on polycarbonate sheets by UV-patterning in combination with silver activating," *Electrochim. Acta.*, vol. 55, pp. 2542–2549, Feb. 2010.  
<http://dx.doi.org/10.1016/j.electacta.2009.12.024>
- [7] J. Hu, W. Li, J. Chen, X. Zhang, and X. Zhao, "Novel plating solution for electroless deposition of gold film onto glass surface," *Surf. Coat Technol.*, vol. 202, pp. 2922–2926, March 2008.  
<http://dx.doi.org/10.1016/j.surfcoat.2007.10.026>

- [8] K. Miyoshi, S. Fujikawa, and T. Kunitake, "Fabrication of nanoline arrays of noble metals by electroless plating and selective etching process," *Colloid Surf. A*, vol. 321, pp. 238–243, May 2008.  
<http://dx.doi.org/10.1016/j.colsurfa.2007.11.041>
- [9] Y. Kobayashi, Y. Tadaki, D. Nagao, and M. Konno, "Deposition of gold nanoparticles on silica spheres by electroless metal plating technique," *J. Colloid Interface Sci.*, vol. 283, pp. 601–604, March 2005.  
<http://dx.doi.org/10.1016/j.jcis.2004.09.002>
- [10] Y. Kobayashi and Y. Ishii, "Electroless deposited gold nanoparticles on glass plates as sensors for measuring the dielectric constant of solutions," *J. Nanoparticles*, vol. 2013, Article ID 841582, March 2013.