Amino Coated Gold Nanorods Based Amperometric Glucose Detection

Shikha Sharma[1], Navendu Goswami[2], Nidhi Gupta[1] and Sudha Srivastava[1]*

[1]Department of Biotechnology,
[2]Department of Physics and Material Science & Engineering,
Jaypee Institute of Information Technology,
Noida 201307, INDIA

Corresponding Author Email: sudha.srivastava@jiit.ac.in

Abstract

Citrate capped gold nanoseeds of uniform size and shape were prepared and used for CTAB-assisted growth of gold nanorods. Further, shape and size dependent hydrodynamic sedimentation behavior of nanostructure is exploited for separation of gold nanorods from mixture containing particles of different shape and size. The surface functionalization of gold nanorods with amino acids allows its efficient interaction with the enzyme and makes it a promising platform for applications including medical diagnostics and sensing devices. Further, the biosensing efficiency of the glucose biosensor fabricated by immobilizing glucose oxidase (GOx) onto gold nanorods was investigated. Under optimum conditions, the fabricated biosensor showed low detection limit, excellent storage stability and high sensitivity. The biosensor has a wide linear range for the detection of glucose from 5mM to 40mM with 6 s response time. The retained activity of glucose oxidase ensures the biocompatibility of amino coated gold nanorods and shows that it can be extended to other enzymes as well for development of novel biosensors.

Keywords: *Gold nanorods, Amino acids, Glucose Biosensor, Biocompatible.*

1. Introduction

With the advent of nanotechnology biosensor research witnessed an exponential upsurge through exploitation of unique optical, electromagnetic and catalytic properties of the nanomaterials, much different from their bulk counterparts. The above unique properties are further dependent on the morphology [1,2] and size[3,4] of the nanostructures and especially interesting behavior is observed in anisotropic nanostructures. The unique optical characteristics including multiple plasmon bands [5] and light absorption in the near infrared (NIR) regions in case of one-, two- and three- dimensional anisotropic nanostructures make them suitable for various optical and optothermal applications [6-8]. Moreover, the electrochemical and mechanical properties are much more enhanced than their isotropic counterparts [9,10]. Recently we have shown that coupled chain like gold nanoparticles results in better electron transfer rates and anisotropic shape ensures better approachability of the modified electrode surface and the active sites of the biomolecules [11].

A controlled synthesis of uniform anisotropic structures is difficult to achieve [12]. Generally, a mixture of different shape nanostructures is obtained during the synthesis of

nanorods via seed mediated method[13-14]. In this method the gold seeds are prepared by reduction of metal salt with a strong reducing agent. Further, in the growth solution the weak reducing agent along with the surfactant helps in longitudinal growth of these nano seeds to form rods or wires. The aspect ratio of the synthesized rods can be controlled by varying the concentration of seeds and precursor in the growth solution.[15] The separation of different nanostructures on the basis of shape is a major challenge for researchers. Different post purification techniques including column chromatography[16], capillary electrophoresis[17], dialysis[18], etc., are generally used for separation of nanostructures. The percentage purity of product varies with the efficiency of the technique used for separation. However, the above mentioned method does not allow the separation of nanostructures on the basis of their shape.

In this paper, we have separated the nanorods from a mixture of nanostructures through a simple one step process i.e., by varying the centrifugal force. The surfactant coated gold nanorods were further functionalized with amino acid in order to enhance their biocompatibility. The fact that nanorods and nanotubes exhibit excellent conductivity was further exploited to construct a highly sensitive amperometric glucose biosensor. The nanorods modified biosensor exhibited high sensitivity, fast amperometric response time of less than 6 s, wide linear range from 5mM to 40mM, and low detection limit 5mM.

2. Experimental Section

2.1 Preparation of gold nanorods

The gold nanorods were synthesized following well-known seed mediated route described in literature [13]. First, gold nanoparticles with size less than 10 nm were prepared via citrate reduction method. After few hours, the synthesized seeds were added to growth solution containing $HAuCl_4$, ascorbic acid and CTAB. The resultant solution was kept undisturbed overnight. The separation of nanorods from the mixture containing spherical nanoparticles and nanorods was performed depending on the centrifugal speed. The mixture was centrifuged at different rpm in order to verify the effect of centrifugal speed on extent of separation. The pellet was redispersed in deionized water and titrated to a pH value between 1.0 and 7.0. Further, to the colloidal solution, a freshly prepared solution of aspargine was added to make the nanorods a better platform for enzyme immobilization.

2.2 Characterization of nanorods

The aspect ratio estimation of the synthesized gold nanorods was done using transmission electron microscope (TEM). Morgagini 268 transmission electron microscope with magnification of 8900 x. The optical properties of nanorods (AuNRs) were further assessed by recording UV–VIS absorbance spectra using a cuvette free spectrophotometer ND 1000 UV-VIS Spectrophotometer from Nanodrop technologies operated at 12 V.

2.3 Response Time Measurement

The disposable gold electrode was supplied by Drop Sens.The electrodes were screen printed on to a ceramic substrate and electric contacts were made of silver. The electrochemical cell consists of gold as working and counter electrode whereas reference electrode is of silver. Cleaning of working electrode was done using ethanol and distilled water. 5 µl of APT (5mM)

was deposited on to the working electrode and was incubated for 3 hrs. The electrode was rinsed with double distilled water to remove unbound APT. 5 µl of the cross linking agent (10% (v/v) aqueous solution of glutaraldehyde) was added drop wise and allowed to dry. Amino functionalized gold nanorods were immobilized onto the electrode surface. Unbound gold nanorods were removed by washing the electrodes 2-3 times with double distilled water. Finally, 20U of *glucose oxidase* was immobilized on electrode surface coated with functionalized gold nanorods. The following day, the electrode was rinsed with water, dried and stored in PBS (pH 7.4) at 4°C for further use.

A screen printed electrode containing three electrodes (where working electrode modified with gold nanorods was used as platform for *glucose oxidase* immobilization, a gold electrode act as a counter electrode, and silver electrode is used as a reference electrode). A micro ammeter was connected in series with the working electrode to measure the current. Water and PBS acted as negative control which showed no current. When the current trends were stable different glucose concentrations were prepared & the current response in each was observed by applying a constant voltage of 0.4V. Response time was calculated as the time required to sense 95% change in concentration.

3. Results and Discussion

Gold nanostructures prepared using the above protocols were separated on the basis of shape using centrifugation. The morphological analysis of the solution separated at different centrifugal speeds is done using TEM. Figure. 1a represents the TEM micrograph of solution centrifuged at 9000 rpm showing a mixture of rods of different aspect ratios and spherical nanoparticles. Spherical nanoparticles of gold showed an average diameter of 15 nm, whereas the average length and diameter of synthesized gold nanorods was 224 nm, and 20 nm, respectively. The sample was further characterized using UV-vis spectroscopy. A broad absorption peak from 520 nm to 750 nm indicating a high polydispersity in size of nanorods and nanoparticles is observed (see absorption spectra of Figure 1a). These particles and rods were separated based on their hydrodynamic velocity by centrifuging the solution containing mixture of nanostructures (nanorods and nanoparticles) at 6000 rpm for 20 min. Rods being heavier in mass would have larger terminal settling velocity and hence settle down faster along the walls of the centrifugation tubes while comparatively lighter spherical particles tend to take longer time and settle at the bottom of the centrifugation tube. The red colored pellet of nanoparticles at the bottom of the tube when characterized using UV-vis spectroscopy shows sharp single absorption peak at 526 nm whereas the purplish black pellet at the wall of centrifuge tube shows two distinct peaks at 534 and 675 nm (see absorption spectra of Figure 1b), confirming the anisotropic character of the synthesized nanorods. The morphological analysis of the pellets was further done using TEM which confirmed the separation of two different shape nanostructures using centrifugation (Fig 1(b-c)). The nanorods of different aspect ratios were further separated by resuspending the wall pellet in 1.5 ml of water followed by differential centrifugation from 5000 rpm to 9000 rpm.

Characterization of the particles was followed by activity analysis of the free enzyme in aqueous solution (as shown in figure 2(a)) and after immobilization onto gold nanorods (see Figure 2(b)). Activity was assayed by studying the rate of formation of Quinoneimine dye at 37°C. It was observed that after immobilization onto gold nanorods there was a three-fold enhancement

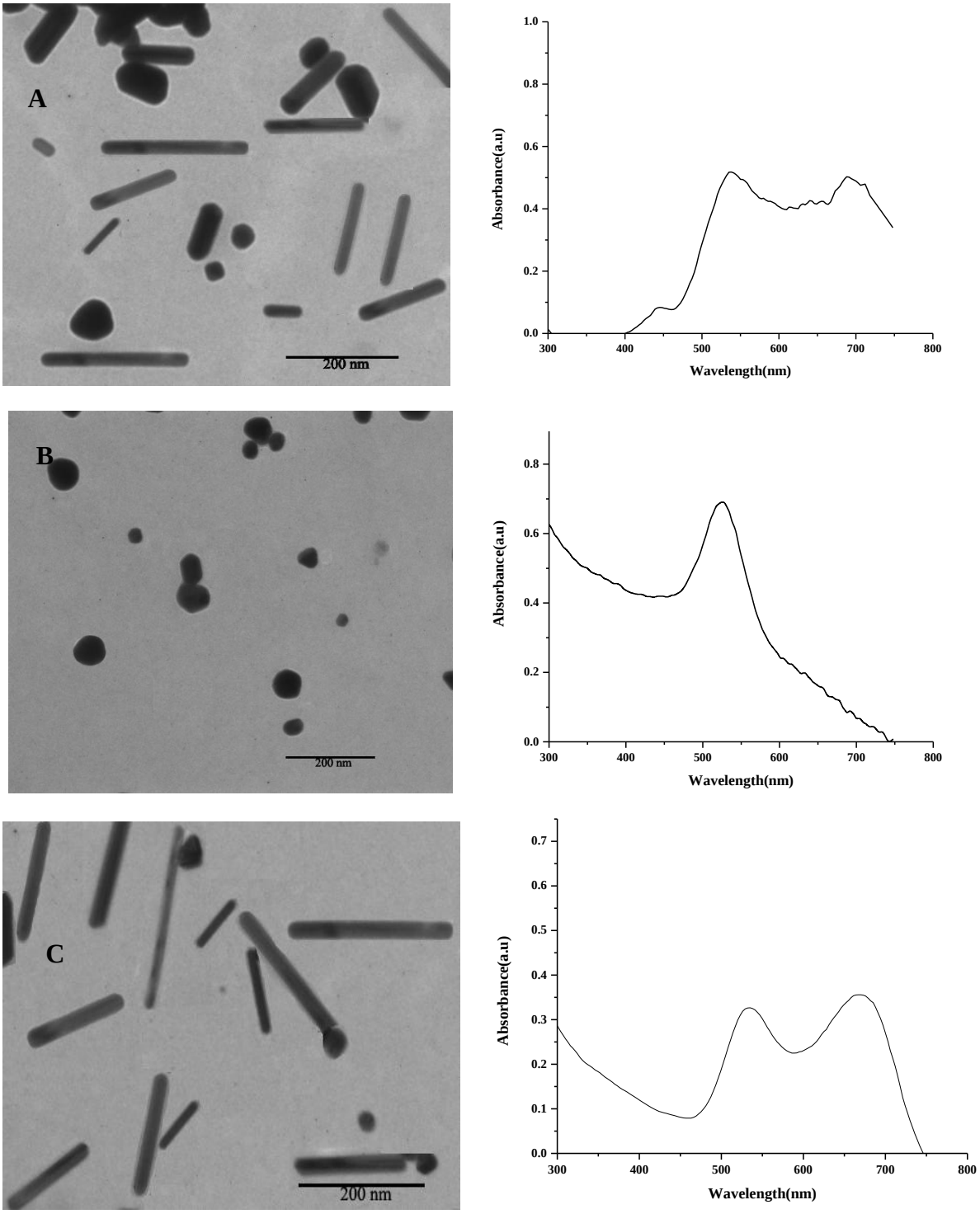

Fig 1 TEM micrographs of A) mixture of rods and NPs B) NPs after separation C) Rods after separation and their corresponding UV-Visible absorption spectra

in the specific activity of the enzyme as compared to the free enzyme Table 1. This indicates that the immobilization onto nanorods is enhancing the enzyme activity significantly. This also

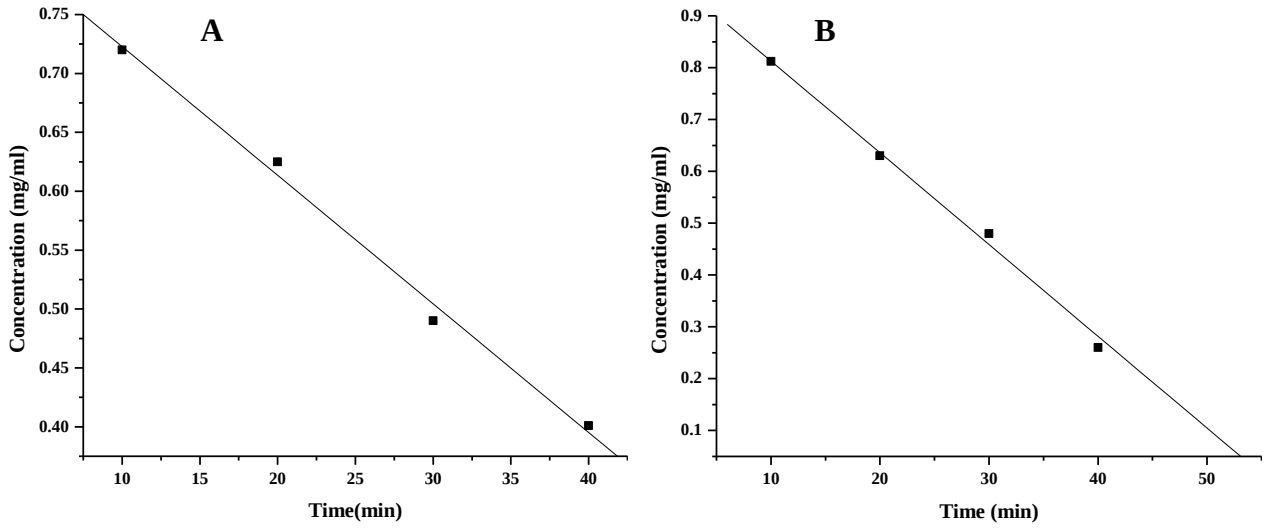

Fig2 Variation of concentration as a function of time for activity studies of a) free enzyme b) enzyme immobilized onto gold nanorods.

implies that functionalizing surfactant coated nanorods with amino acids makes them more biocompatible and thus ensuring no reduction in activity of the enzyme. Henceforth, the construction of amperometric biosensor and current measurements were carried out using screen printed gold electrode (DRP 220). A micro ammeter was connected in series to measure the amount of current produced. The current was recorded as a function of applied voltage and a linearly increasing I-V characteristic was observed. However, it has been reported that there is strong interference from ascorbic acid and uric acid at high voltages, therefore a constant voltage of 0.4 V was applied. Figure 3 shows current produced varies linearly with increasing glucose concentrations. Water and phosphate buffer were taken as negative control that gave no current response. The response time of the above biosensor, measured as the time taken to sense 95% of concentration change. The measured response time was 6 secs.

Table 1. Comparison of specific activity of free and immobilized enzyme at 37°C

S.No	Activity Analysis for	Specific Activity(mmoles/min/mg)
1	Free enzyme GOx	2.75
2	GOx immobilized onto Gold Nanorods	6.8

Comparison of the Biosensor

The performance of the fabricated GOx-AuNRs biosensor was compared with that of commercial glucometer. Choice of commercial glucometer was done on the basis of enzyme used i.e., one using glucose *oxidase* as the biomolecule for sensing the amount of glucose in the blood sample. The correlation of glucose sensing using fabricated GOx-AuNRs biosensor and that of commercial glucometer is shown in Fig. 4. A high correlative coefficient of .98 was confirms good performance characteristics of the fabricated biosensor.

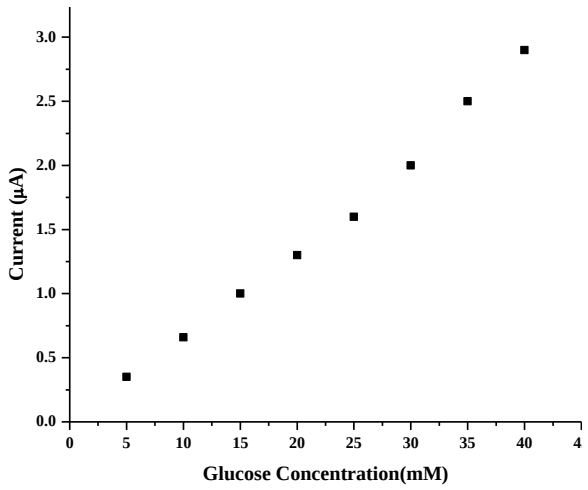

Fig. 3. Current response as a function of glucose concentration.

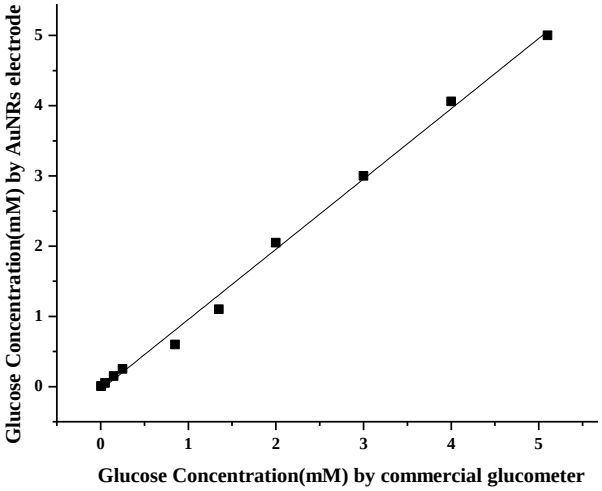

Fig. 4. Correlation glucose concentration values as measured by GOx-AuNRs electrode and commercial glucometer.

Interference: A number of possible interfering species alters the correct measurements of glucose levels. The signal alterations due to most interfering electroactive species, such as ascorbic acid and uric acid were investigated in the present study. The level of endogenous ascorbic and uric acid is about 0.125 and 0.33 mM, respectively has been reported in blood samples. At this level, no fall in reponse current signal was observed. Thus, confirming the glucose biosensor exhibited negligible response to such electroactive interfering species.

4. Conclusions

Present paper reports a simple process of centrifugation in conjuction with amino acids to yields biocompatible nanorods that can further be utilized for highly sensitive biosensor fabrication. Effective sedimentation velocity under the applied centrifugal force is the governing principle for separation of pure gold nanorods (AuNRs) from a mixture of particles and rods of different sizes and shapes. Further, decorating the AuNRs with amino acid enhances their biocompatibility and results in less denaturation of immobilized biomolecule and hence more suitable for a sensitive biosensor fabrication.

Acknowledgments

This project was funded by All India Council for Technical Education (AICTE) (File No.: 8023/BOR/RID/RPS-68/2008-09). Sophisticated and Analytical Instruments Facility, All- India Institute of Medical Sciences, New Delhi is gratefully acknowledged for TEM work. S. Sharma is thankful to JIIT University for providing research fellowship. We would like to acknowledge laboratory technicians Mr. Narendra Kumar Yadav and Mr. Subhash Jha (Biotechnology laboratory) for the much required help extended by them throughout the work.

References

[1] Xianjun Du, Dengsong Zhang, Liyi Shi, Ruihua Gao, and Jianping Zhang, "Morphology Dependence of Catalytic Properties of Ni/CeO_2Nanostructures for Carbon Dioxide Reforming of Methane" J. Phys. Chem. C, 116, 2012, pp 10009–10016.

[2] Li Z, Pan W, Zhang D, Zhan J. "Morphology-dependent gas-sensing properties of ZnO nanostructures for chlorophenol." Chem Asian J., 5, 2010, pp 1854-1859.

[3] Hrushikesh M. Joshi,Yen Po Lin, Mohammed Aslam, P. V. Prasad, Elise A. Schultz-Sikma, Robert Edelman, Thomas Meade, and Vinayak P. Dravid "Effects of shape and size of cobalt ferrite nanostructures on their MRI contrast and thermal activation" J Phys Chem C Nanomater Interfaces., 113, 2009, pp 17761–17767.

[4] Duc V. Dinh and J. H. Yang, S. M. Kang, S. W. Kim, D. H. Yoon, "Size-dependent Field-emission Properties from Triangular-shaped GaN Nanostructures" Journal of the Korean Physical Society, 55, 2009, pp. 202-206.

[5] K. A. Willets and R. P. Van Duyne, "Localized surface plasmon resonance spectroscopy and sensing," Annual Review of Physical Chemistry, 58, 2007, pp. 267–297.

[6] Y.Y. Yu, S.S. Chang, C.L. Lee, and C. R. C. Wang, "Gold nanorods: electrochemical synthesis and optical properties," Journal of Physical Chemistry B, 101, 1997, pp. 6661–6664.

[7] S. Link and M. A. El-Sayed, "Spectral Properties and Relaxation Dynamics of Surface Plasmon Electronic Oscillations in Gold and Silver Nanodots and Nanorods," Journal of Physical Chemistry B, 103, 1999, pp. 8410–8426.

[8] C. J. Murphy, T. K. Sau, A. M. Gole et al., "Anisotropic metal nanoparticles: synthesis, assembly, and optical applications," Journal of Physical Chemistry B, 109, 2005, pp. 13857–13870.

[9] Yeong Hwan Ko, Myung Sub Kim, and Jae Su Yu "Structural and optical properties of ZnO nanorods by electrochemical growth using multi-walled carbon nanotube-composed seed layers" Nanoscale Res Lett., 7, 2012, pp. 13-15.

[10] Wen-Jay Lee, Jee-Gong Chang, Shin-Pon Ju, Meng-Hsiung Weng and Chia-Hung Lee "Structure-dependent mechanical properties of ultrathin zinc oxide nanowires" Nanoscale Research Letters, 6, 2011, pp. 352-360.

[11] Shikha Sharma, Nidhi Gupta, Sudha Srivastava, "Modulating electron transfer properties of gold nanoparticles for efficient Biosensing" Biosensors and Bioelectronics, 37, 2012, pp. 30–37.

[12] Panikkanvalappil R. Sajanlal, Theruvakkattil S. Sreeprasad, Akshaya K. Samal and Thalappil Pradeep, "Anisotropic nanomaterials: structure, growth, assembly, and functions" Nano Reviews, 2, 2011, 5883 pp. 1-62.

[13] Babak Nikoobakht and Mostafa A. El-Sayed, "Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method" Chem. Mater., 15, 2003, pp. 1957–1962.

[14] Anand Gole and Catherine J. Murphy, "Seed-Mediated Synthesis of Gold Nanorods: Role of the Size and Nature of the Seed" Chem. Mater., 16, 2004, pp. 3633–3640.

[15] Hao Ming Chen, Ru-Shi Liu, and Din Ping Tsai, "A Versatile Route to the Controlled Synthesis of Gold Nanostructures" Crystal Growth & Design., 9, 2009, pp. 2079–2087.

[16] Siswoyo, Lee Wah Lim,, Toyohide Takeuchi, "Separation of gold nanoparticles with a monolithic silica capillary column in liquid chromatography", Anal Sci. 28, 2012, pp. 107-113.

[17] Sławomir Oszwałdowski, Katarzyna Zawistowska-Gibuła, and Kenneth P. Roberts, "Capillary electrophoretic separation of nanoparticles", Anal Bioanal Chem., 399, 2011, pp. 2831–2842.

[18] Hironori Yamada, Chihiro Urata, Yuko Aoyama, Shimon Osada, Yusuke Yamauchi, and Kazuyuki Kuroda, "Preparation of Colloidal Mesoporous Silica Nanoparticles with Different Diameters and Their Unique Degradation Behavior in Static Aqueous Systems" Chem. Mater., 24, 2012, pp. 1462–1471.