

Development of a multifunctional catalyst for a “relay” reaction

Anal Kr. Ganai ^{a,b}, Pravin Shinde ^a, Basab B. Dhar ^b, Sayam Sen Gupta ^{*b},

B.L.V. Prasad ^{*a}

**^aMaterials Chemistry Division, ^bChemical Engineering and Process Development
Division, National Chemical Laboratory, Dr Homi Bhabha Road, Pune 411008, India**

Electronic Supplementary Information

Experimental:

(A) Materials:

Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), β -glucosidase, 4-nitrophenyl- β -glucopyranoside, NaBH_4 , HAuCl_4 and (3-glycidyloxypropyl)trimethoxysilane were purchased from Sigma Aldrich and were used as received. All the other solvents were purchased from Merck, India and used as received.

(B) Experimental

(a) Preparation of Au Nanoparticles:

Au Nanoparticles was prepared by borohydride reduction method.¹ 500 mL of 10^{-4} M HAuCl_4 was prepared. To this solution 50 mg NaBH_4 was added slowly. The reaction mixture was left for overnight for complete reduction.

(b) Preparation of Au@mSiO₂ particles:

In a round bottom flask, 20 mL of the above prepared Au sol was taken. Cetyltrimethylammonium bromide (CTAB, 0.007 mg, 10^{-3} mol) was dissolved in that solution and stirred for half hour. NaOH (aq) (2.00 M, 100 μL) was then added to CTAB solution, followed by drop wise addition of tetraethyl orthosilicate (20 μL , 0.091 mmol). The reaction mixture was stirred for 2 hrs and then centrifuged twice with ethanol. To remove the CTAB completely, the particles were refluxed overnight in ethanol. Then the solution was centrifuged again and these particles were stored as dispersion in ethanol for further usage.

(c) Grafting of epoxide groups onto Au@mSiO₂:

The grafting of epoxide functional group was performed according to a procedure reported before.² In a typical reaction, 15 mg of Au@mSiO₂ nanoparticles were dispersed in 50 mL dry toluene. To this dispersion, (3-glycidyloxypropyl)trimethoxysilane (10 μL , 0.04 mmol) was added and the resultant reaction mixture was heated at 85 °C for 12 hrs. After completion of the reaction, the resultant epoxide grafted core shell nanoparticles were centrifuged and

washed several times with ethanol to remove the unreacted (3-glycidyloxypropyl)trimethoxysilane.

(d) Immobilization of enzyme:

Covalent enzyme anchoring was carried out following reported methods.²⁻⁴ First epoxide grafted Au@mSiO₂ nanoparticles (3 mg) were dispersed in PBS buffer (pH = 7.4, 0.1 mM) by sonication. Then these were incubated with beta-glucosidase (2 mg, 1.48 X 10⁻⁸ mol) over a night at 4 °C. The resulting hybrid nanoparticles, Au@mSiO₂@glucosidase, were separated by centrifugation, washed several times with PBS buffer to remove unreacted and absorbed enzyme. The particles was dispersed in 4 mL of PBS Buffer and used as a stock. Enzyme conjugated nanoparticles were stored at 4 °C. The amount of enzyme immobilized on Au@mSiO₂@glucosidase nanoparticles was determined using UV-Vis spectroscopy by monitoring the absorbance at 280 nm. Unfunctionalized Au@mSiO₂ nanoparticles were used as a blank for all measurements.

(C) Material Characterization:

The samples were imaged using an FEI Technai F30 machine that is operated at 300 kV. The TEM samples were prepared by drop casting the sample on the carbon coated Cu grid. FT-IR spectra were recorded on a Perkin Elmer FT-IR spectrum GX instrument operated at a resolution of 4 cm⁻¹ by making KBr pellets. Pellets were prepared by mixing 3 mg of sample with 97 mg of KBr. The catalytic reaction was monitored by UV-vis spectra on a Cary 300 Conc UV-Visible spectrophotometer operated at a resolution of 2 nm. Nitrogen adsorption and desorption studies at -196 °C were carried out using an Quadrasorb SI instrument. Before the nitrogen adsorption measurements, the samples were degassed overnight under vacuum using a FloVac Degasser at 100 °C. Multipoint BET surface area was obtained from the nitrogen adsorption isotherm in the relative pressure range from 0.15 to 0.5. Solid dried samples were coated on glass slides and characterized with powder X-ray diffraction (Xpert

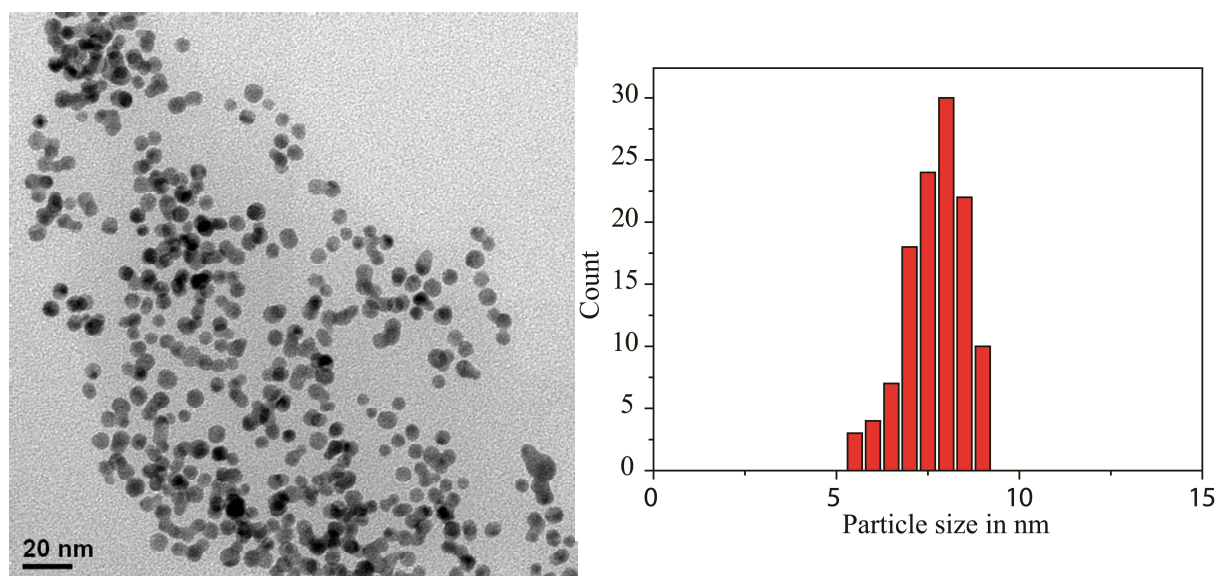
Pro, Panalytical, Cu K α) in the 2 θ range of 30–80 degree which was operated at 40 kV and 30 mA. For XPS analysis the gold coordinated functionalized silica nanoparticles were deposited on a sample holder by spreading the powder. The measurements were carried out on a VG MicroTech ESCA 3000 instrument at a pressure of better than 10⁻⁹ Torr. The general scan and Si 2p, C 1s, O 1s, and Au 4f core level spectra were recorded with unmonochromatized Mg-K α radiation (photon energy ~ 1253.6 eV) at pass energy of 50 eV and electron take off angle (angle between electron emission direction and surface plane) of 60°. The overall resolution of measurement is thus 1 eV for the XPS measurements.

(D) Enzymatic activity measurements:

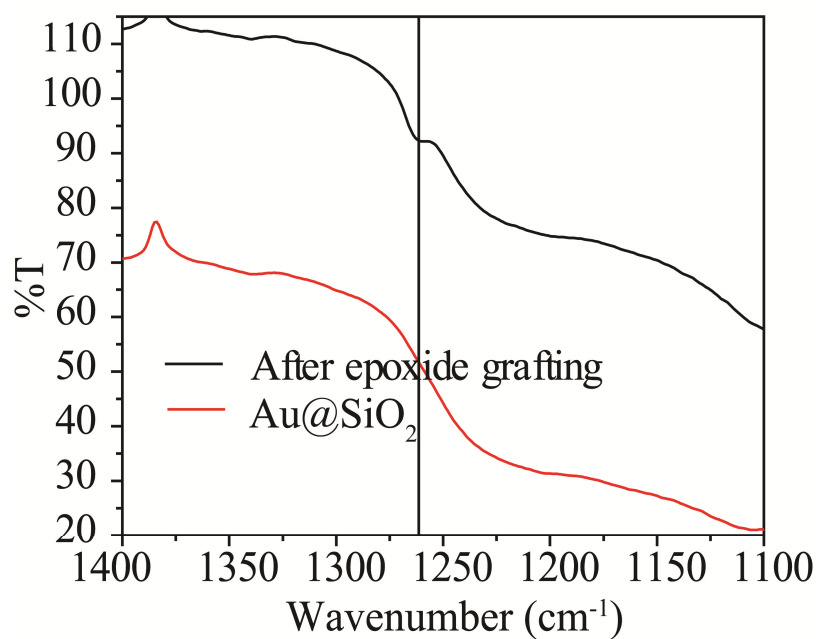
The kinetics were monitored in scanning kinetic mode of the spectrophotometer (Varion-300) using 1 mL quartz cuvette of 1.00 cm path length in a thermostatted (25.0 $\pm$ 0.5 °C) cell housing at pH 7.4. The first step was formation of *p*-nitrophenol from the hydrolysis of 4-nitrophenyl- β -glucopyranoside and second step was reduction of *p*-nitrophenol. Both the reactions were monitored at 402 nm, which corresponds to λ_{max} of *p*-nitrophenol. In a 1 mL cuvette, 200 μ L of the above mentioned stock solution of catalyst was taken and mixed with 300 μ L of Millipore water to make a total volume of 500 μ L. To this solution 0.006 mL of 0.01 M 4-nitrophenyl- β -glucopyranoside was added and the spectra was recorded with time. After the completion of the 1st reaction, NaBH₄ was added for the reduction of *p*-nitro phenol and change of O.D. was studied with time.

(E) References:

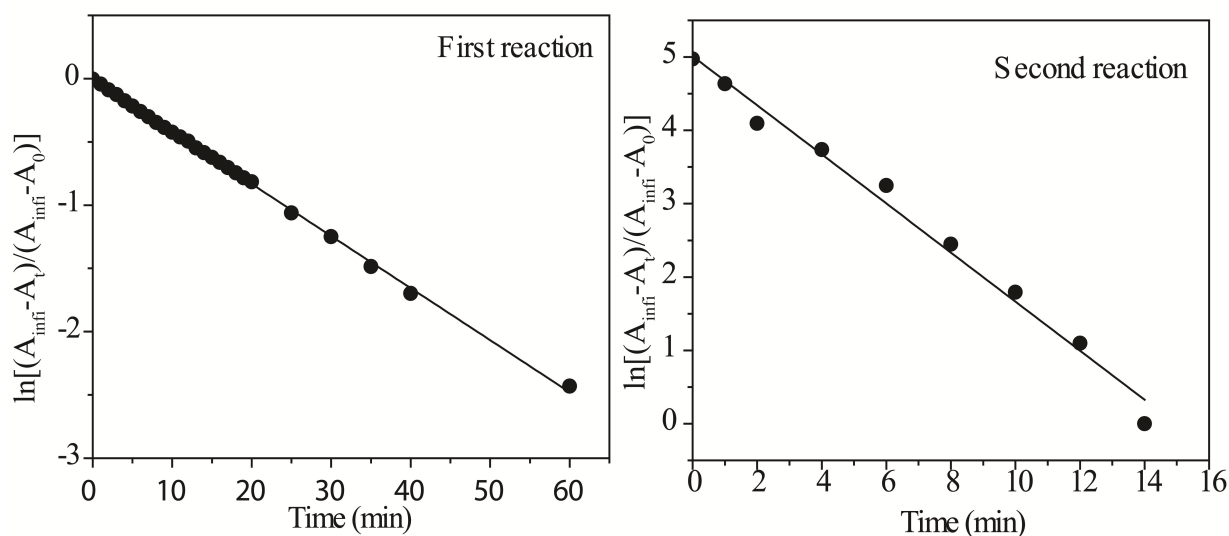
1. A. Gole, C. Dash, V. Ramakrishnan, S. R. Sainkar, A. B. Mandale, M. Rao and M. Sastry, *Langmuir*, 2001, **17**, 1674.
2. N. Brun, A. Babeau Garcia, H. Deleuze, M. F. Achard, C. Sanchez, F. Durand, V. Oestreich and R. Backov, *Chem.Mater.*, 2010, **22**, 4555.
3. R.K.Singh, Y.-W. Zhang, N.-P.-T. Nguyen, M. Jeya and J.-K. Lee, *Appl. Microbiol.Biotechnol.*, 2011, **89**, 337.
4. T. Georgelin, V. Maurice, B. Malezieux, J.-M. Siaugue and V. Cabuil, *J. Nanopart. Res.*, 2010, **12**, 675.



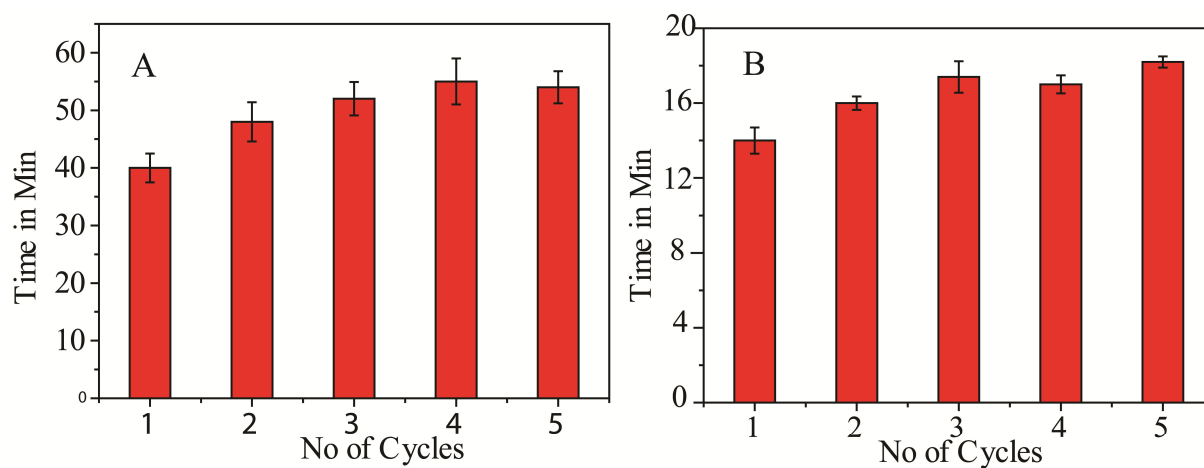
ESI Figure 1: TEM and particle size distribution of bare Au Nanoparticles.



ESI Figure 2: In the zoomed IR spectra the peak appears at 1260 cm⁻¹ after the epoxy grafting also indicates successful incorporation of epoxide in Au@mSiO₂



ESI Figure 3: Plot of $\ln[(A_{\infty} - A_t)/(A_{\infty} - A_0)]$ versus time using β -glucosidase immobilized Au@mSiO₂.



ESI Figure 4: (A) Recyclability for 5 cycles for the 1st reaction. (B) Recyclability for 5 cycles for the 2nd reaction.