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# 3-Aminopropyltrimethoxysilane and organic electron donors mediated synthesis of functional amphiphilic Gold nanoparticles and its Bioanalytical Applications

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**Precise control over functionality and nanogeometry during synthesis of gold nanoparticles (AuNPs) and dispersibility of the same in variety of solvents has been a challenging requirement for practical applications. The role of organic reducing agents i.e. 3-Glycidoxypropyltrimethoxysilane (3-GPTMS), Tetrahydrofuran hydroperoxide (THF-HPO) and Cyclohexanone has shown potentiality for meeting these requirement during the conversion of 3-aminopropyltrimethoxysialne (3-APTMS)-capped gold ions into AuNPs. The reaction product of these reducing agents with 3-APTMS during AuNPs synthesis is catalytic in nature for THF-HPO and cyclohexanone due to formation of inorganic-organic hybrid and non catalytic for 3-GPTMS. The presence and absence of such reaction products justify the difference in catalytic ability of the AuNPs as a function of organic reducing agents which profusely affects the inherent properties of AuNPs for specific applications. The use of cyclohexanone in place of 3-GPTMS or THF-HPO together with 3-APTMS outclasses the other two in imparting better stability of amphiphilic AuNPs with reduced silanol content. The as synthesized AuNPs enable the formation of nanocomposite (PBNPs-AuNPs) dispersion with Prussian blue nanoparticles (PBNPs). Further, PBNPs-AuNPs may also be converted homogeneous nanocomposite suspension with ruthenium bipyridyl solution justifying even better catalytic ability than that of HRP. The resulting nanomaterial suspension, one way, efficiently probe the Glucose oxidase catalyzed reactions based on peroxidase mimetic ability and, other way, display excellent electrocatalytic activity during the electrochemical sensing of $H_2O_2$. The peroxidase mimetic ability of nanomaterial has been found to vary as a function of 3-APTMS concentration which confirms the potential role of functional gold nanoparticles in bioanalytical applications.**

## INTRODUCTION

The requirement for practical usability of gold nanoparticles in a variety of organic and aqueous solvent has directed the use of bipolar organic reagents for synthesis of functional nanoparticles. Further, functionalization of noble metal nanoparticles with organic amine has received greater attention from many angles e.g. : (1) amine-capped nanocrystals are nearly as stable as their thiol-capped counterparts however, unlike thiols, amines are weakly bound on Au surface;[1] (2) their functional use in cancer therapy as drug carriers, photothermal agents, contrast agents and radiosensitizers;[2] (3) susceptibility for the formation of biocompatible linkage; [3, 4] and (4) organic amine acts as potential stabilizer for nanoparticles.[5, 6] These advantages of organic amine have directed us to investigate its role during the synthesis of noble metal nanoparticles.[7, 8] The use of 3-APTMS have been very well documented as potential stabilizer for noble metal nanoparticles.[9] We investigated in detail the role of 3-APTMS during the synthesis noble metal nanoparticles that required the participation of 3-APTMS compatible organic reducing agents, like 3 glycidoxypropyltrimethoxysilane (3-GPTMS) and tetrahydrofuranhydroperoxide (THF-HPO).[10, 11] During the course of AuNPs synthesis, these reducing agents interact differently with 3-APTMS resulting in the formation of organic moiety dependent reaction product that may alter the inherent properties of as synthesized AuNPs. Such product may also influence the dispersion ability of resulting AuNPs in different polar and non-polar solvents for specific application due to bipolar nature of 3-APTMS and polarity/non-polarity of organic reducing agents. Earlier finding on the use of 3-GPTMS along with 3-APTMS during nanoparticles synthesis in methanolic medium resulted AuNPs mostly dispersible in organic solvents. [12] Similarly the use of THF-HPO with 3-APTMS during such synthesis provided AuNPs mostly dispersible in aqueous medium[11]. The dependence of reducing agent on solvent required for the synthesis of AuNPs resulted into differential kinetic limitation when allowed to interact with 3-APTMS capped $Au^{3+}$ ions. Such findings revealed central role of 3-APTMS during noble metal nanoparticles synthesis and direct major contribution of organic functionalities (3-GPTMS or THF-HPO) for precise control of desired properties for practical applications. Recently, we have reported the synthesis of Prussian blue nanoparticles mediated by 3-APTMS and cyclohexanone.[13] Such findings revealed that 3-APTMS capped hexacyanoferrate ions undergo controlled conversion into polycrystalline Prussian blue with excellent electrochemical behaviour for practical applications.[13] Similar process also enable the synthesis of polycrystalline mixed metal hexacyanoferrate with nearly all combination of transition metal ions.[14] Cyclohexanone in aqueous media shows a biphasic system however, even in such condition the controlled synthesis of nanoparticles are recorded.[13-15] Cyclohexanone mediated synthesis of AuNPs has been reported by Uppal et al.[15] The biphasic system of nanoparticles resulted transition in nanogeometry of AuNPs with time suggesting the need of stabilizer and directed to examine in details the role of 3-APTMS during nanoparticles conversion.[9, 10] Further the use of cyclohexanone with 3-APTMS might prove effective in enhancing catalytic activity through the formation of organic-inorganic hybrid since the interaction of functional alkoxysilane and small organic molecule results the formation of such catalytic material. [16] The hydrophobic behaviour of cyclohexanone and micellar activity of 3-APTMS may allow the synthesis of functional AuNPs suitable for the formation nanocomposite with variety of known catalytic materials (e.g. metal hexacyanoferrate, ruthenium bipyridyl) dispersible in suitable solvent for practical bioanalytical application. Accordingly a detailed investigation on the role of 3-APTMS mediated conversion of gold cations in the presence of cyclohexanone is sought from following angles: (1) conversion of 3-APTMS capped gold ions to respective nanoparticles in the presence of cyclohexanone, (2) characterization and application of 3-APTMS and cyclohexanone mediated AuNPs as a function of functional ability and nanogeometry, (3) checking the dispersibility of as synthesized cyclohexanone mediated AuNPs at different ratio of 3-APTMS and cyclohexanone, (4) ability of AuNPs to form nanocomposite displaying peroxidase mimetic ability for probing glucose oxidase catalyzed reactions. Further the rate of 3-APTMS mediated synthesis of AuNPs may be the function of organic reducing agents accordingly, a comparison on the role of 3-GPTMS, THF-HPO and cyclohexanone on the following points are also undertaken: (a) time required during nanoparticles conversion as a function of organic moieties, (b) catalytic ability of nanoparticles based on functional ability and nanogeometry, (c) stability of as synthesized nanoparticles for practical applications, and (d) dispersibility of as synthesized nanoparticles in different solvents as a function of organic moieties. The results on these lines are reported in this communication.

## EXPERIMENTAL SECTION

### Materials

3-Aminopropyltrimethoxysilane (3-APTMS), 3-Glycidoxypropyltrimethoxysilane, Chloroauric Acid and o-dianisidine were obtained from Aldrich Chem. Co.; Cyclohexanone was obtained from S.D. Fine-Chem. Pvt. Ltd.; Toluene, Hydrogen peroxide, Ethyl acetate, Dichloromethane and Acetonitrile were obtained from Merck, India. All other chemicals employed were of analytical grade. Aqueous solutions were prepared by using double distilled-deionized water (Alga water purification system). All the experiments were performed at room temperature unless otherwise mentioned.

The absorption spectra of nanoparticles were recorded using a Hitachi U-2900 Spectrophotometer. Transmission electron microscopy (TEM) studies were performed using Morgagni 268D (Fei Electron Optics) and electrochemical measurements were made with electrochemical workstation CHI 660B (CH Instrument, USA).

### 3-APTMS and Cyclohexanone mediated Synthesis of AuNPs and its nanocomposite with Prussian blue and Ruthenium bipyridyl.

In a typical procedure 1ml sol of AuNPs was prepared by adding 100µl of desired concentration of 3-APTMS to 100µl of 0.025M $HAuCl_4$.The mixture was stirred over cyclomixer for 2 minutes followed by addition of 500µl of cyclohexanone and stirred again on vertex mixer. Methanol was added to make the desired volume. The reaction mixture was then left undisturbed in dark for 1-3 hrs. Appearance of red, purple and blue colour of resulting sol indicated the formation of AuNPs. The nanocomposite of PBNPs-AuNPs was made by mixing as synthesized AuNPs and Prussian blue nanoparticles (PBNPs) made as described earlier.[13] AuNPs (100µl) and PBNPs (50µl) were mixed by stirring and incubated for 5 minutes resulting to formation of homogeneous PBNPS-AuNPs sol. Aqueous solution of Ruthenium bipyridyl (Ru(bpy) (5 mM, 5µl) was added into 15 µl of PBNPs-AuNPs sol to obtain homogeneous dispersion of PB-AuNPs-Ru(bpy) nanocomposite.

## Electrochemical measurement

AuNPs of two sizes $AuNP_1$ (blue) and $AuNP_2$ (red) are sonicated with Prussian blue (PB) suspension which was made by controlled mixing of ferrous sulphate and potassium ferricyanide following standard process. The PB-AuNPs suspension is allowed to be adsorbed thoroughly on graphite particles (1-2µ) and dried at 90°C overnight. The adsorbed nanocomposite on graphite particles was incorporated within graphite paste electrode having composition: PBNPs-AuNPs 2.5 % (w/w), graphite powder 67.5 % (w/w), Nujol oil 30 % (w/w). The well of electrode body (MF-2010 obtained from Bioanalytical Systems, West Lafayette, IN, USA) was filled with active graphite paste. The paste surface was manually smoothed on a clean paper. Electrochemical measurements were performed in a three-electrode configuration equipped with graphite paste electrode as working electrode, an Ag/AgCl reference and a platinum plate counter electrode respectively with a working volume of 3 mL. All electrochemical experiment were performed in 0.1M phosphate buffer solution (pH 7.0) containing 0.5M KCl.

### Peroxidase like catalytic Activity of AuNPs

The peroxidase like activity of as synthesized nanoparticles was determined spectrophotometrically by measuring the formation of oxidized product of o-dianisidine at 430nm ($11.3 mM^{-1} cm^{-1}$) using a Hitachi U-2900 spectrophotometer. Typically, the o-dianisidine (20mM, 10µl) oxidation activity was measured in water (2ml) in the presence of Hydrogen peroxide (10µl). AuNPs (5µl) were added to start the reaction. Experiment was also conducted replacing o-dianisidine with 3,3',5,5'-Tetramethylbenzidine (TMB).

Glucose detection was performed as follows: (a) 40 µl of 10mg $mL^{-1}$ Glucose Oxidase and 200 µl of glucose at different concentrations in 0.1M phosphate buffer (pH 7.0) were incubated at 35°C for 45min; (b) 50µl of o-dianisidine (0.5mM), 15µl of the PB-AuNPs-Ru (bpy) and 1695µl of 0.1phosphate buffer (pH 7.0) were added to the above reaction solution.; (c) the reaction mixture was incubated at 45°C for 30min followed by measurement of absorption at 430nm.

## RESULTS AND DISCUSSION

### Requirement of organic reducing agents during 3-APTMS mediated synthesis of AuNPs

The synthesis of AuNPs either in aqueous or organic systems have their own limitations specifically for the transfer of newly formed nanoparticles from a polar to a non-polar environment or vice-versa. Such requirements may involve the use of some phase transfer reagents , ionic liquid etc.[17, 18] These limitations can be eliminated by the use of suitable reagents having bipolar behaviour together acting as both reducing and stabilizing agents during the conversion of noble metal salts into respective nanoparticles. The use of organic amine has shown their potentiality as reagent and stabilizer of the nanomaterial.[9, 11, 19, 20] We have recently demonstrated that 3-APTMS although acts as a potential reagent for the conversion of noble metal cations into respective nanoparticles but required the participation of additional organic reagent during nanoparticles synthesis. Accordingly, the role of 3-GPTMS and THF-HPO for real time synthesis (*i.e* within less than 2 h) of AuNPs from 3-APTMS capped $Au^{3+}$ has been demonstrated.[10, 11] 3-APTMS alone does not enable the synthesis of AuNPs under similar condition even after 24 h (Supporting Fig.S1). The findings on these lines have shown organic moieties (3-GPTMS and THF-HPO) dependent behaviour of resulting nanoparticles with significant variation in their catalytic ability despite the use of 3-APTMS as one of reagent in both cases. This difference in catalytic behaviour of AuNPs as a function of 3-GPTMS or THF-HPO is due to the formation of organic-inorganic hybrid in the later case that enhances the catalytic ability of AuNPs. The major difference in both synthetic protocol and catalytic behaviour are found as follows: (1) 3-GPTMS allows the synthesis of AuNPs only in methanolic medium whereas the same with THF-HPO takes place in water, (2) The nanoparticles made through 3-APTMS and 3-GPTMS are mostly dispersible in organic solvent with limited dispersibility in water under specific ratio of 3-APTMS/3-GPTMS, (3) THF-HPO and 3-APTMS mediated synthesis of nanoparticles are mostly dispersible in relatively more polar solvent and not dispersible in non-polar media. The limited dispersibility of 3-GPTMS and THF-HPO mediated AuNPs led to the search for another reducing agent with dispersibility in variety of solvents. Further Uppal *et.al.*, have observed the synthesis of AuNPs only in the presence of cyclohexanone which is a biphasic system with transition in nanogeometry as a function of time[15]. Accordingly, attempt on the synthesis of AuNPs utilizing cyclohexanone and 3-APTMS in a monophasic system has been made. These findings revealed significant role of organic reagent during 3-APTMS mediated conversion of noble metal nanoparticles and required a comparative investigation. Fig.1A shows the real time synthesis of AuNPs mediated by 3-APTMS and THF-HPO whereas Fig.1B shows the similar result replacing THF-HPO by clohexanone. The results as shown in Fig.1 clearly demonstrate the real time conversion of AuNPs with significant variation in the use of 3-APTMS concentration when the reducing agents are exchanged. Cyclohexanone requires low concentration of 3-APTMS under similar conditions. It is to be noted that the synthesis of AuNPs require methanolic medium when cyclohexanone is used as reducing agent whereas THF-HPO enable the synthesis in water.

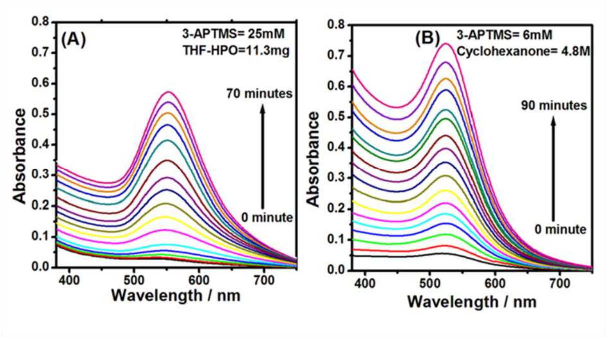


Fig.1 Real time synthesis of AuNPs mediated by 3-APTMS - THF-HPO (A) and 3-APTMS-cyclohexanone (B)

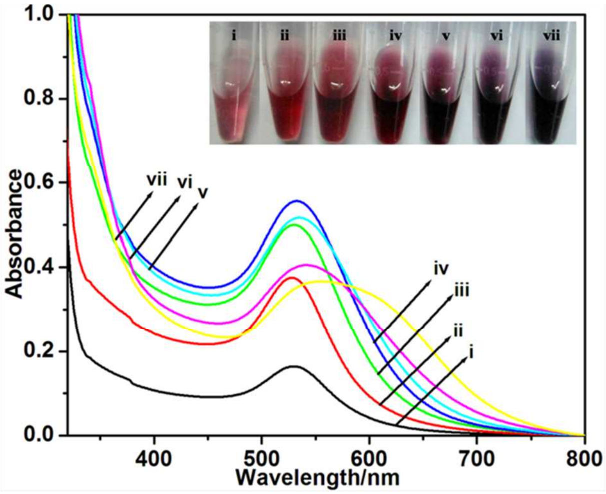
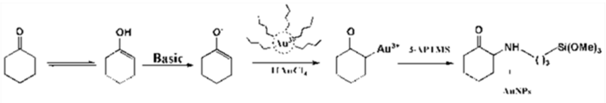


Fig.2. UV-VIS Spectra showing change in $\lambda_{max}$ of AuNPs made using constant concentration of cyclohexanone (4.8M ) and varying concentration of 3-APTMS; (i) 3mM (ii)4mM (iii)6mM (iv)8mM (v)9mM (vi)10mM (vii)12mM



Scheme 1 Proposed mechanism of 3-APTMS and cyclohexanone mediated synthesis of AuNPs

### Cyclohexanone and 3-APTMS mediated synthesis of AuNPs.

Cyclohexanone efficiently enable the synthesis of AuNPs in the presence of 3-APTMS in methanolic medium with excellent nanogeometry under specific ratio of 3-APTMS/cyclohexanone within 0.5-3 h at room temperature. The concentrations of both the reagents significantly control the synthesis of AuNPs. First, we investigated the synthesis of AuNPs under two conditions: (a) varying the concentrations of 3-APTMS while keeping the constant concentrations of cyclohexanone, (b) Keeping 3-APTMS cocentrations constant while changing the concentrations of cyclohexanone. Fig.2 shows the visual photographs along with respective absorption maxima of AuNPs synthesized at different concentrations of 3-APTMS (3mM-12mM) keeping contant concentrations of cyclohexanone (4.8M) displaying gradual variations in SPR and shows the siginifance of 3-APTMS during AuNPs synthesis. We chose two different concentrations of 3-APTMS (5mM and 10mM) to witness the effect of Cyclohexanone concentration on the AuNPs. The higher concentration of 3-APTMS (10mM) resulted in blue nanoparticle whereas lower concentration (5mM) largely gave red nanoparticle as shown in Fig.3A and Fig.3B respectively.

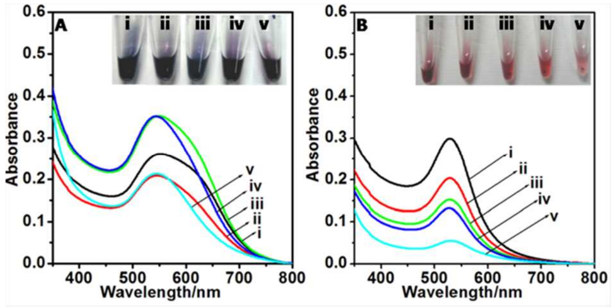


**Fig.3.** UV-VIS Spectra showing change in $\lambda_{max}$ of AuNPs made using : (A) constant concentration of 3-APTMS (10mM) and varying cyclohexanone concentration (i)1.0 M , (ii)1.5M, (iii) 2.0M , (iv) 3.0M (v)4.0M; (B) constant concentration of 3-APTMS (5mM) and varying cyclohexanone concentration; (i) 4M (ii) 5.0M (iii) 6.0M (iv) 7.0M (V) 8.0M

The morphology and nanogeometry of as synthesized AuNPs are examined by transmission electron microscopy. Fig.4 shows the TEM images of AuNPs revealing the range of average size of 3nm to 45nm corresponding to 3-APTMS concentrations of 8mM, 10mM, 12mM and 15mM respectively at 4.8M cyclohexanone. These findings show that there is a maximum and minimum limit of 3-APTMS (3mM – 15mM) and cyclohexanone (1.0M-7.0M ) concentrations beyond which the synthesis AuNPs does not occur. Such specific requirement of 3-APTMS and cyclohexanone could be possible due to micellar characteristic and the requirement of critical miceller concentration of the analytes. Below 3mM and above 15mM there was no sign of AuNPs formation at any cyclohexanone concentration. Such limitation may also be linked to specific CMC of cyclohexanone during 3-APTMS mediated conversion of nanoparticles. An optimum concentration of each component is required for AuNPs due to micellar behaviour of 3-APTMS. The proposed mechanism for the 3-APTMS and cyclohexanone mediated synthesis is shown in scheme-1: Cyclohexanone in the prevailing medium undergoes keto-enol tautomerism. Enolate ion acts as an electron donor to 3-APTMS capped $Au^{3+}$ ion, which in turn acts as a lewis acid, leading to the formation of AuNPs. It is important to compare the role of other reducing agents like 3-GPTMS and THF-HPO with cyclohexanone required for the synthesis of AuNPs. Organic moieties (3-GPTMS, THF-HPO and cyclohexanone) used for the synthesis of 3-APTMS and $Au^{3+}$ mediated AuNPs differ in following respects (i) 3-GPTMS and cyclohexanone have affinity for organic medium whereas that of THF-HPO is to aqueous medium, (ii) 3-GPTMS is a bulky group whereas cyclohexanone and THF-HPO are comparitively smaller moieties, (iii) Tendency of the reducing agents to form Organic-inorganic hybrid during AuNPs synthesis and (iv) 3-GPTMS, THF-HPO and cyclohexanone have different CMC allowing variable interaction with 3-APTMS capped gold ions during nanoparticle synthesis. The difference in properties of as synthesized AuNPs using different reducing agent is born out of these differences in their nature and discussed vide infra.

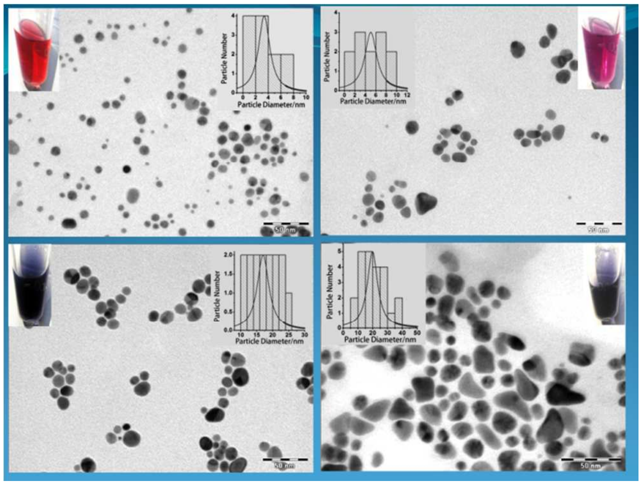


**Fig.4.** TEM images of AuNPs made at constant cyclohexanone (4.8M) and varying 3-APTMS concentrations of 8mM (red), 10mM (purple), 12mM (blue), and 15mM (violet).

## Effect of organic reducing agents on the dispersibility of AuNPs

Dispersibility of AuNPs largely depends on the medium which in turn is determined by the apparent polar or nonpolar behaviour of the organic moieties (3-GPTMS/THF-HPO/Cyclohexanone). First we discuss the Dispersibility of AuNPs made using 3-APTMS and cyclohexanone both in aqueous and organic solvents viz. Water, Methanol and Dichloromethane (DCM) based on absorption spectra and visual photographs. AuNPs are profusely dispersible in DCM and methanol at all concentration of 3-APTMS and cyclohexanone. However the dispersibility of the same in water is affected at higher concentrations of cyclohexanone. Dispersibility of AuNPs in different solvents is shown in Fig.5(A, B, C). In order to make detailed investigation on dispersibility of AuNPs in organic solvents, the absorption spectra of the same as a function of AuNPs concentrations in water, dichloromethane, Acetonitrile and ethyl acetate was recorded as shown in Fig.6 (A). The result shows a linear relationship between the absorbance and the concentration of AuNPs with significant variation in $\lambda_{max}$ as a function of the nature of solvents. The dependence of $\lambda_{max}$ on the refractive index and polarity index of these solvent is shown in Fig.6 (B) has also been recorded. These results justified the use of AuNPs both in aqueous and non-aqueous medium. THF-HPO enables the dispersibilty of AuNPs in aqueous and methanolic medium. Solvents like DCM and Toluene rejects the nanoparticle completely resulting in a two phasic mixture. Dispersibility of AuNPs made with THF-HPO in different media has been presented in supplementary information (Fig.S2). Dispersibility of AuNPs with 3-GPTMS as organic reducing moiety in different media has been reported earlier where the nanoparticles are mostly dispersible in organic phase with limited dispersibility in water.[12]. Dispersibility of as synthesized AuNPs as a function of 3-GPTMS, THF-HPO and cyclohexanone is mainly due to micellar behaviour of 3-APTMS and CMC of the reaction products generated in reaction medium.

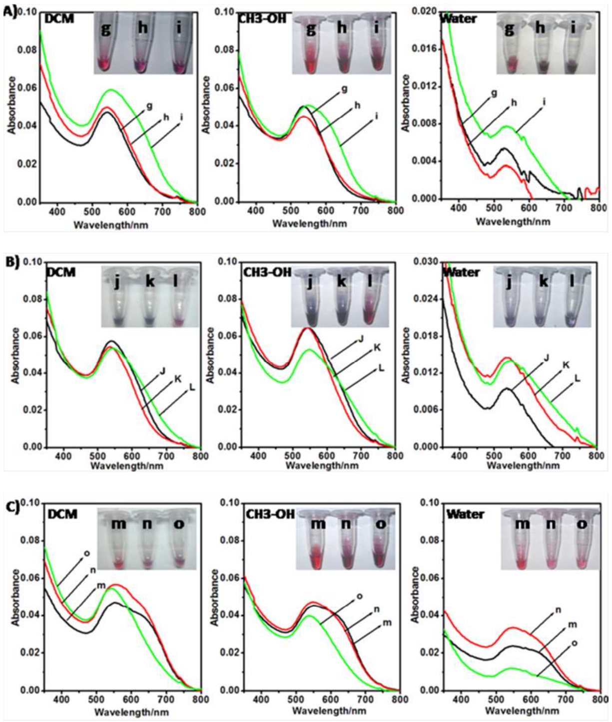


**Fig.5.** UV-VIS spectra of AuNPs and their dispersibility in DCM , Methanol and water made using constant concentration of ; (A) Cyclohexanone (4.8M) and varying concentrations of 3-APTMS: (g) 5mM, (h) 7mM, and (i) 9mM; (B) Cyclohexanone (3.8M) and varying concentrations of 3-APTMS : (j)5mM, (k)7mM, and (l)9mM; (C) 3-APTMS (10mM) and varying concentrations of Cyclohexanone: (m) 0.9M , (n) 1.8M, and (o) 3.8M. Photographs of corresponding AuNPs sol in same solvents are shown in insets to (A), (B) and (C) respectively.

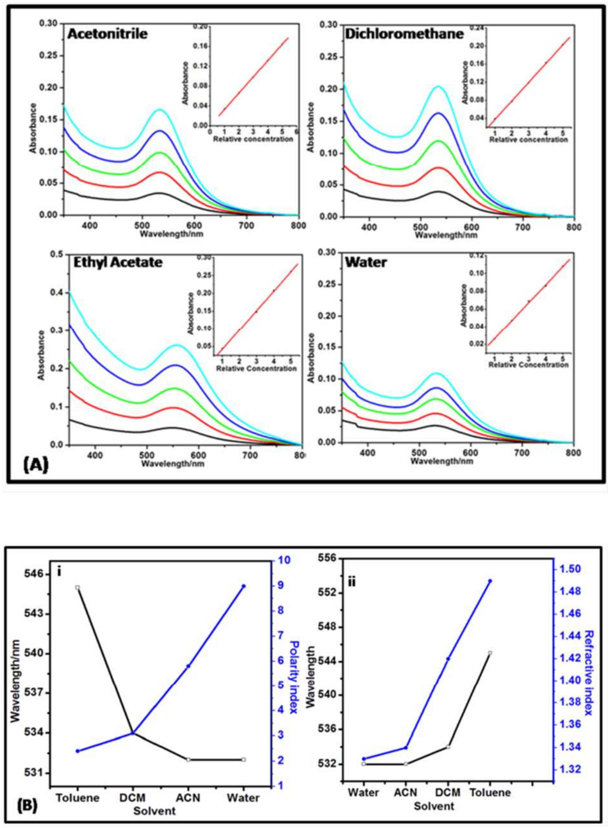


Fig.6.A) UV-VIS spectra of AuNPs in (i) Acetonitrile (ii) Dichloromethane (iii) Ethyl Acetate and (iv) water. Inset shows the dependence of absorption maxima (λmax) on relative concentration. (B) Dependence of λmax on Polarity index (i) Refractive index (ii).

## Effect of organic reducing agents on the Catalytic ability of AuNPs

One of the important property as predicted in scheme-1 is the formation of organic-inorganic hybrid between organic amine linked to alkoxysilane and cyclohexanone. The formation of such hybrid material enhance the catalytic property of AuNPs from those in absence of the same. An increase in 3-APTMS concentration will allow an increase in catalytic activity. Cyclohexanone mediated AuNPs synthesized at different 3-APTMS concentrations were checked for their catalytic behaviour as peroxidase mimetic that allows the catalytic oxidation of o-dianisidine in the presence of $H_2O_2$ as shown in equation-1.

$$H_2O_2+\text{o-dianisidine}_{(red.)} \xrightarrow{\text{AuNPs}} \text{o-dianisidine}_{(ox.)}+H_2O \qquad (1)$$

The catalytic behaviour of AuNPs can be examined from the absorption maxima of the oxidized product of o-dianisidine close to 430 nm. It is important here to understand the catalytic behaviour of 3-APTMS itself if any. The finding as shown in supporting information S3 justify that 3-APTMS does not enable the formation of oxidized reaction product of o-dianisidine and confirm the its non-catalytic activity. Catalytic behaviour of AuNPs is found to increase with increasing 3-APTMS concentration irrespective of its size as shown in Fig. 7(A,B,C,D) recorded in methanol and acetonitrile justifying the practical usability of nanomaterial in different solvents. In order to confirm the catalytic ability as a function of functional ability, we also examined the peroxidise mimetic behaviour of AuNPs made with increasing concentrations of cyclohexanone keeping constant concentrations of 3-APTMS yielding AuNPs of increasing nanogeometry. The results on catalytic behaviour of AuNPs recorded in methanol are shown in Fig. 7 E. The results reveal an increase in catalytic ability with an increase in nanogeometry when functional ability of the nanomaterial remains consistent.

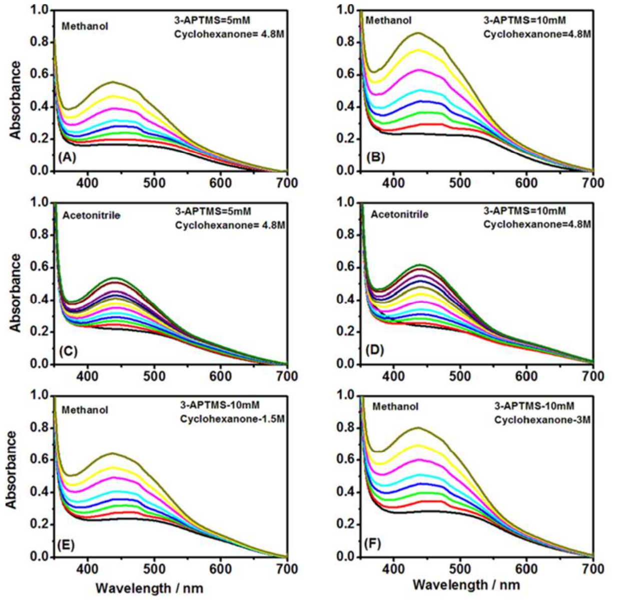


Fig.7. UV-VIS spectra of o-dianisidine-$H_2O_2$ system catalyzed by AuNPs in methanol (A, B) acetonitrile (C, D) justifying the role of functional ability; Curves E and F shows results on same line as a function of nanogeometry.

Thus catalytic behaviour of AuNPs primarily depends on functional ability of 3-APTMS rather than nanogeometry. Similar property of AuNPs made from the use of THF-HPO has been observed as reported earlier.[11] 3-GPTMS does not result in the formation of organic inorganic hybrid thus catalysis largely depend on nanogeometry irrespective of 3-APTMS concentration. Use of AuNPs as peroxidase mimetic gave a very high Km value, which means it is very weak to be used as peroxidase mimetic. For a material to work as potential substitute of peroxidase mimetic it should be stable enough at room temperature and its peroxidase mimetic ability should be equivalent or higher than that of peroxidase enzyme under feasible experimental conditions. In addition to that the resulting nanocomposite should have homogeneous dispersion in reaction medium as per requirement of homogeneous catalysis. Such requirements restrict the practical usability of much such catalytic material due to poor solubility of the same in the working solvent. Accordingly, there is need to improve mimetic ability of AuNPs by making the nanocomposite of the same having dispersibility for homogeneous catalysis. 3-APTMS and cyclohexanone mediated Prussian blue nanoparticles (PBNPs), behaves as artificial peroxidase**,** the nanocomposite of the same with as synthesized AuNPs may be perfect peroxidase replacement since the resulting PBNP-AuNPs nanocomposite is dispersible in aqueous medium. The possibility of nanocomposite formation may be further increased since the reacting systems of both PBNPs and AuNPs possesses similar reaction products resulting the formation of monophasic dispersion useful for homogeneous catalysis. The PBNP-AuNPs nanocomposite has been characterized by transmission electron microscopy. The microstructure and diffraction pattern as shown in Fig.8 confirm the formation of polycrystalline PBNPs—AuNPs nanocomposite.

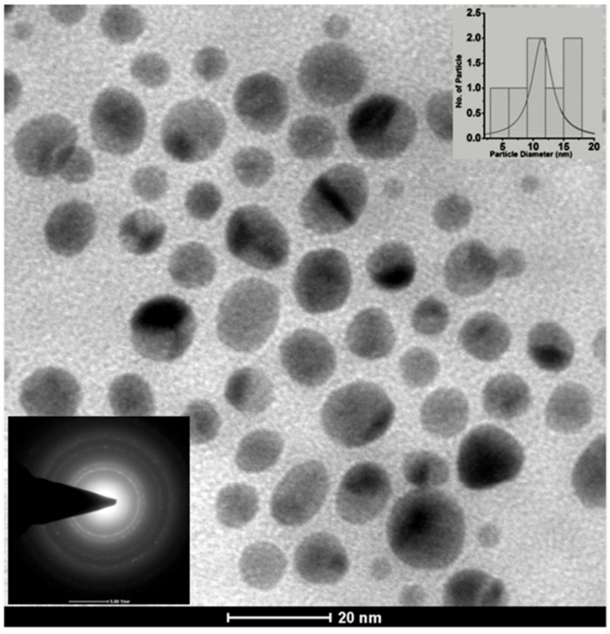
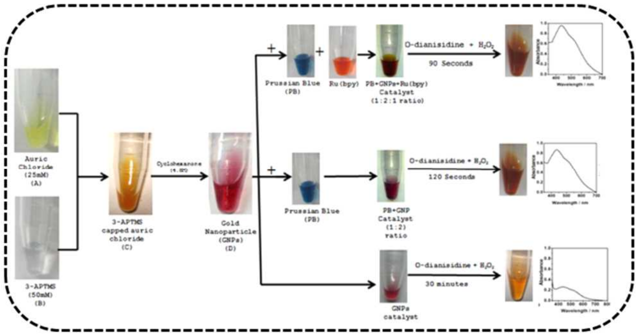


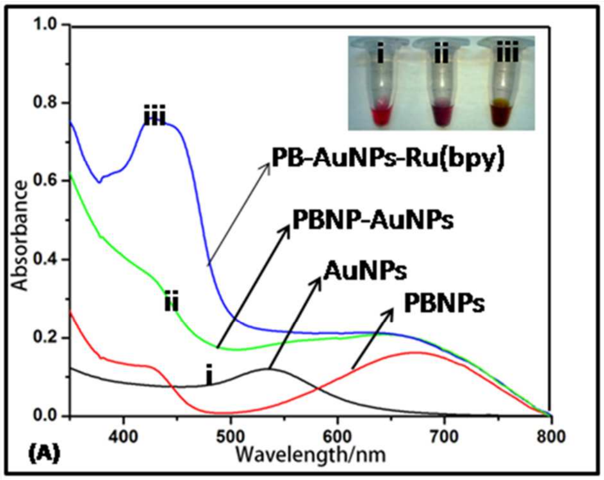
**Fig.8**.TEM image of PBNP-AuNPs nanocomposite along with respective diffraction pattern.

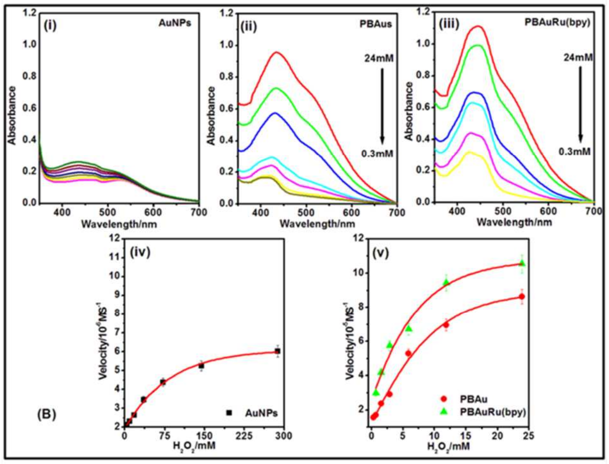
It is also noteworthy if the performance of nanocomposite could further be enhanced in the presence of other suitable material manipulating the catalytic ability for desired application which could only be possible on the processability of the PBNP-AuNPs suspension. Tris (2,2-bipyridyl) ruthenium [Ru(bpy)] is an important functional optical material which displays capping affinity with 3-APTMS and retains inherent photoactivity. [21, 22] This property of Ru(bpy) prompted us to use it to form nanodispersion with 3-APTMS functionalized nanocomposite of PBNP and AuNPs and indeed the excellent monophasic nanomaterial for homogeneou**s** catalysis was obtained. The schematic presentation of peroxidise mimetic activity is shown in scheme-2 depicting the ratio of various components participated in the formation of nanocomposite dispersion. It is to be noted from the details given in scheme-2 that when 3-APTMS (B) is added in methanolic gold salt solution (A), a dark yellow coloured product(C) is formed justifying the specific interaction of gold ions with 3-APTMS.



Scheme.2. Schematic presentation of various stages of nanocomposite formation for homogeneous catalysis.

The formation of nanocomposite dispersion of as synthesized AuNPs are examined by mixing the each component under ambient conditions. Fig.9A shows the UV-VIS spectra of AuNPs, PBNPs, PBNPs-AuNPs and PBNPs-AuNPs-Ru(bpy). The results demonstrate the formation of nanocomposite dispersion for practical applications as peroxidase mimetic. It should be noted that PBNPs-AuNPs-Ru(bpy) shows characteristic absorbance between 400-460 nm (Fig.9 A) however, there use as peroxidase mimetic at 430 nm provide valuable information on probing oxidized product of o-dianisidine. We have examined the peroxidase mimetic ability of the AuNPs, AuNPs-PBNPs and the AuNPs-PBNPs-Ru(bpy) nanocomposite as shown in Fig.9B (i) (ii) (iii) respectively for o-dianisidine-$H_2O_2$ system. Km value for AuNPs was calculated from the results shown in Fig. 9B (iv) and for that of AuNPs-PBNPs and AuNPs-PBNPs-Ru (bpy) from Fig. 9B (v). The Km value for AuNPs is found to be 30.4mM , which means it is very weak to be used as peroxidase mimic and the km for PBNPs-AuNPs comes out to be 4.6mM and shows comparable to that recorded on using HRP under practical experimental conditions.[23] Addition of Ru(bpy) to the nanocomposite further enhances the mimetic ability with a Km to the order of 2.9mM. The mimetic ability of the nanocomposite has been very effective in precise probing of glucose oxidase catalysed reaction. Based on the peroxidase like property of PB-AuNPs-Ru(bpy), we intended to detect glucose content by utilizing o-dianisidine as a chromogenic substrate analogous to that reported earlier based on the measurement of $H_2O_2$ as a function of a GOx catalyzed reaction.[24-26] The $H_2O_2$ formed as a function of the GOx catalyzed oxidation of glucose was monitored spectrophotometrically using the oxidation product of o-dianisidine to indirectly measure the concentration of glucose. A typical glucose concentration–response curve is shown in Fig.9(C) and the inset shows the corresponding calibration plot. The linear range and lowest detection limit for glucose was found to be 0.5– 3mM and 0.5mM, respectively.





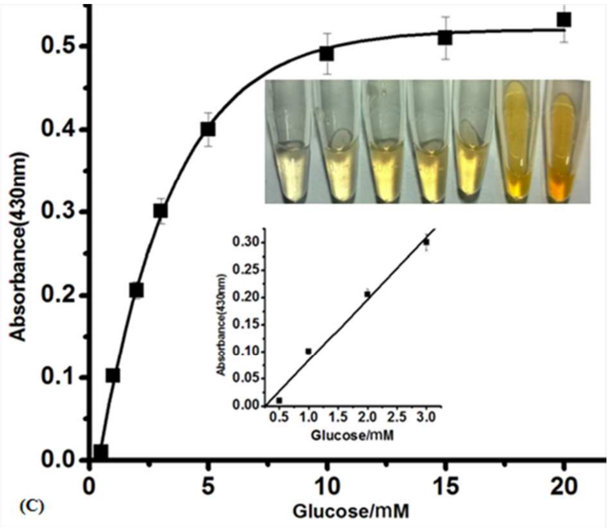


Fig.9. (A) UV-VIS spectra of AuNPs (i), PBNPs-AuNPs (ii), PBNPs-AuNPs-Ru(bpy (iii), inset to Fig.9(A) shows the visual photographs of AuNPs (i), PBNP-AuNPs (ii) and PBNP-AuNPs-Ru(bpy) (iii); [B] UV-VIS spectra of o-dianisidine-$H_2O_2$ system catalyzed by AuNPs (i), PBAuNPs (ii), and PBAuRu(bpy) (iii); Kinetic analysis of o-dianisidine-$H_2O_2$ system catalyzed by AuNPs (iv), PBNPs-AuNPs and PBNPs-AuNPs-Ru(bpy) (v); [C] Response curve for glucose detection using glucose oxidase catalyzed formation of $H_2O_2$ monitored by PB-AuNP-Ru(bpy)- o-dianisidine system; Inset to 9C shows linear calibration plot for glucose and photographs shown colour change as a function of glucose concentrations.

The findings on peroxidase mimetic activity has been recorded using o-dianisidine as substrate that shows absorption maxima at 430 nm and may introduce misleading information due to absorption maxima of Ru(bpy) falling between similar range. Accordingly we also recorded similar observation using 3,3',5,5'-Tetramethylbenzidine (TMB) as substrate replacing o-dianisidine. Similar findings as shown in Fig.10 confirm the catalytic activity of nanocomposite as peroxidase mimetic.

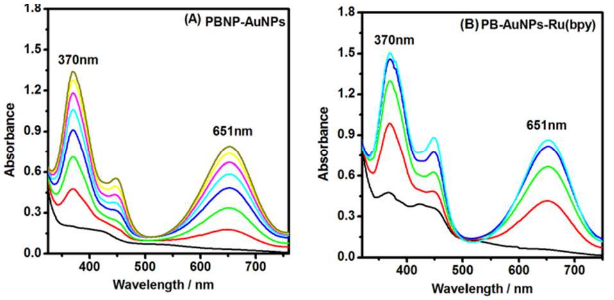


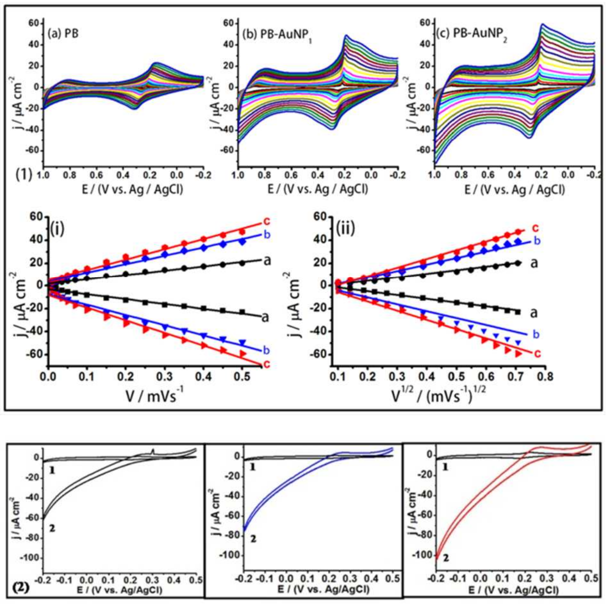
**Fig.10.** VIS spectra of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) -$H_2O_2$ system catalyzed PBAuRu(bpy).

**Effect of organic reducing agents on the stability of AuNPs**

A comparative study on the stability of AuNPs made from the use of 3-APTMS capped gold ions in the presence of 3-GPTMS, THF-HPO and cyclohexanone has been made. The results on the stability of AuNPs made with cyclohexanone are shown in Supporting Fig.S4 Justifying that as synthesized AuNPs is stable for >6 months. Although 3-GPTMS mediated AuNPs remained stable for a long time (> 90 days) but suffered the problem of hydrolysis due to Si-O-Si linkage, which has been reduced several folds when 3-GPTMS is replaced by cyclohexanone. THF-HPO is least stable out these three.

**Electrocatalytic ability of AuNPs and its nanocomposite.**

The results on peroxidase mimetic ability of AuNPs based on UV-VIS spectroscopy directed us to evaluate the electrocatalytic ability of the same for wider practical application of the nonmaterial. Since AuNPs are not potential electroactive species required for precise evaluation of electrocatalytic performances, it is convenient to make nanocomposite with known potential redox material for such investigation and the choice of Prussian blue (PB) again seems to be reasonable. Since the evaluation of electrocatalytic ability for practical application is based on the dynamics of oxidation and reduction of PB-AuNPs and subsequent measurement of electron exchange during electrochemical sensing, the role of 3-APTMS present in nanocomposite may play crucial role as 3-APTMS serve as potential electron donors in polar medium (scheme-1) during AuNPs synthesis. The results shown in Fig.9B (II) showed the application of PBNPs made from 3-APTMS and cyclohexanone as reported earlier,[13] the similar nanocomposite for electroanalytical application may introduce severe problem during efficient electron transfer due to large concentrations of 3-APTMS and may require detailed investigation. Accordingly, at first instant, we chose to use conventional Prussian blue (PB) made from potassium hexacyanoferrate and ferrous sulphate to make nanocomposite with as synthesized gold nanoparticles of two different size $AuNP_1$ (blue) and $AuNP_2$ (red) in order of increasing nanogeometry. The as prepared PB-AuNPs are adsorbed on graphite particles and incorporated into graphite paste electrode for understanding heterogeneous electrocatalysis based on probing the electrochemical behaviour of surface confined redox species. Fig.11[1]( a, b, and c) shows the voltammograms of these modified electrode for PB (a), PB-$AuNP_1$ (b) and PB-$AuNP_2$ (c) at different scan rate in 0.1M phosphate buffer (pH 7.0) containing 0.5M KCl. The results as shown in Fig.11[1] ( a, b, and c) shows the redox behaviour of Prussian blue as a function of AuNPs nanogeometry and reveals faster electron exchange on increasing the nanogeometry of AuNPs as evaluated from the plots (i) and (ii) shown in insets to Fig.11(1) of respective voltammograms. Since PB is used as artificial peroxidase, the finding on the electrocatalytic reduction of $H_2O_2$ at the surface of the modified electrodes has also been investigated. Fig.11[2] (a, b, and c) shows the voltammograms of these modified electrodes in absence (1) and the presence (2) of 2 mM $H_2O_2$ in 0.1 M phosphate buffer pH 7.0 at the scan rate of 10 mV/s for PB (a), PB-$AuNP_1$ (b) and PB-$AuNP_2$ (c) respectively. The results as shown in Fig.11 [2] (a, b and c) clearly reveals subsequent enhancement in electrocatalytic property as a function of AuNPs nanogeometry. Finally we studied amperometric analysis of $H_2O_2$ at constant operating potential of 0.05V vs., Ag/AgCl in 0.1 M phosphate buffer pH 7.0 as shown in Fig.11[3] for PB (a), PB-$AuNP_1$ (b) and PB-$AuNP_2$ (c). The electrocatalytic responses (Fig.11 [3]) again confirm the role of AuNPs nanogeometry on electrocatalysis and justify an increase of the same on increasing nanogeometry.



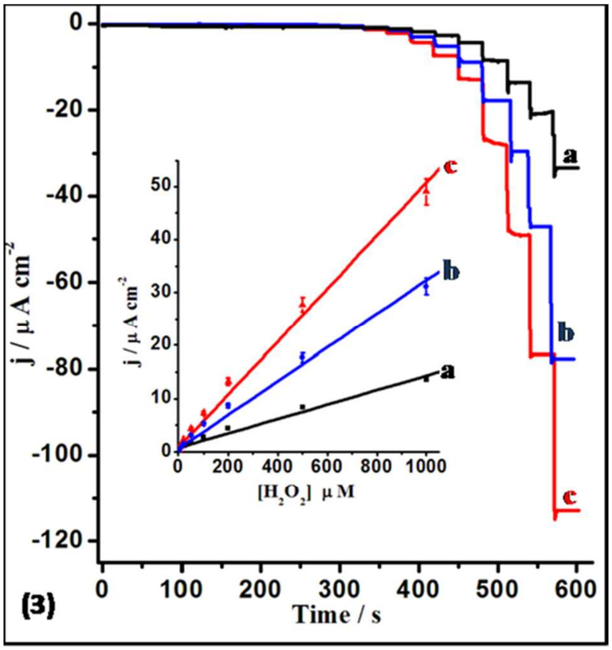


Fig.11. [1] Cyclic voltammograms of PB (a), PB-AuNP$_1$(b) and PB-AuNP$_2$ (c) in 0.1 M phosphate buffer pH 7.0 containing 0.5 M KCl at the scan rate (Vs$^{-1}$), 0.01, 0.02, 0.035, 0.050, 0.070, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50; insets to Fig.11(1) show the plot of anodic and cathodic current density vs scan rate (i) and square root of scan rate(ii) respectively; [2] Cyclic voltammograms of PB, PB-AuNP$_1$ and PB-AuNP$_2$ in absence (1) and the presence (2) of 2 mM $H_2O_2$ in 0.1 M phosphate buffer pH 7.0 containing 0.5M KCl at the scan rate of,0.01Vs$^{-1}$; [3] Amperometric response of $H_2O_2$ analysis at 0.05V vs Ag/AgCl in 0.1 M phosphate buffer pH 7.0 containing 0.5M KCl, inset to Fig.11[3] shows the linear range of calibration curves for PB(a), PB-AuNP$_1$ (b) and PB-AuNP$_2$(c).

## Conclusions

To summarize, we present a comparitive study on the role of organic reducing agents (3-GPTMS, THF-HPO and cyclohexanone) during 3-APTMS mediated controlled synthesis of AuNPs. As synthesized AuNPs differ from each other in properties like dispersibility, catalysis , stability etc. thus making them available for use in various applications. In a bid to get 3-APTMS mediated AuNPs having better stability and dispersibility than THF-HPO and 3-GPTMS we used cyclohexanone that ensures the formation of organic-inorganic hybrid and facilitates the peroxidase mimetic ability of AuNPs. AuNPs can be made dispersible to different solvents (organic or aqueous) by adjusting the constituents (3-APTMS/ Cyclohexanone) ratio. Thus, the use of different reducing agents having compatibility with different solvents results in AuNPs with significant variation in properties from each other presenting option to select AuNPs for specefic applications either in aqueous or organic media. These nanoparticles display functional ability to form monophasic nanocomposite for homogeneous catalysis with prussian blue nanoparticles and Ruthenium bipyridyl for specific application as peroxidase mimetic. In addition to that the nanoparticles also enable the formation of nanocomposite with prussian blue useful as heterogeneous redox catalyst displaying excellent electrochemical properties as a function of nanogeometry. Both mimetic and electrocatalytic ability could be explored for probing glucose oxidase catalyzed reactions justifying potential viability in biomedical applications.

## ASSOCIATED CONTENTS

### Supporting Information

3-APTMS alone does not enable the synthesis of AuNPs under similar condition even after 24 hours (Supporting Figure S1). The dispersibility of Gold nanoparticles made with 3-APTMS and THF-HPO in methanol, acetonitrile, dichloromethane (DCM) and toluene is presented in Figure S2 which clearly indicates better dispersibility in methanolic medium and undergoes red shift in acetonitrile and result biphasic system in DCM and toluene. 3-APTMS is not catalytic in nature as presented in Figure S3. The results on the stability of AuNPs made with cyclohexanone is shown in Supporting Figure S4.

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### Authors Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

## ABBREVIATIONS

3-Aminopropyltrimethoxysilane (3-APTMS), 3-glycidoxy propyltrimethoxysilane (3-GPTMS), Tetrahydrofuran hydroperoxide (THF-HPO), dichloromethane (DCM), Prussian blue nanoparticles (PBNPs), Gold Nanoparticles (AuNPs), Ruthenium bipyridyl (Ru(bpy).

## References

1. D. V. Leff, L. Brandt and J. R. Heath, *Langmuir*, 1996, **12**, 4723-4730.
2. S. Jain, D. G. Hirst and J. M. O'Sullivan, *The British Journal of Radiology*, 2012, **85**, 101-113.
3. C. Xu, K. Xu, H. Gu, X. Zhong, Z. Guo, R. Zheng, X. Zhang and B. Xu, *Journal of the American Chemical Society*, 2004, **126**, 3392-3393.
4. T. Mirzabekov, H. Kontos, M. Farzan, W. Marasco and J. Sodroski, *Nature biotechnology*, 2000, **18**, 649-654.
5. Y. Jv, B. Li and R. Cao, *Chem. Commun.*, 2010, **46**, 8017-8019.
6. S. K. Bhargava, J. M. Booth, S. Agrawal, P. Coloe and G. Kar, *Langmuir*, 2005, **21**, 5949-5956.
7. M. Aslam, L. Fu, M. Su, K. Vijayamohanan and V. P. Dravid, *J. Mater. Chem.*, 2004, **14**, 1795-1797.
8. J. Newman and G. Blanchard, *Langmuir*, 2006, **22**, 5882-5887.
9. K. Saha, S. S. Agasti, C. Kim, X. Li and V. M. Rotello, *Chemical Reviews*, 2012, **112**, 2739-2779.
10. P. C. Pandey and D. S. Chauhan, *Analyst*, 2012, **137**, 376-385.
11. P. C. Pandey and G. Pandey, *Journal of Materials Chemistry B*, 2014, **2**, 3383-3390.
12. P. C. Pandey, A. K. Pandey and G. Pandey, *Journal of Nanoscience and Nanotechnology*, 2014, **14**, 6606-6613.

13. P. C. Pandey and A. K. Pandey, *Electrochim Acta*, 2013, **87**, 1-8.
14. P. C. Pandey and A. K. Pandey, *Analyst*, 2013, **138**, 2295-2301.
15. M. A. Uppal, A. Kafizas, M. B. Ewing and I. P. Parkin, *Journal of Materials Chemistry A*, 2013, **1**, 7351-7359.
16. A. Wight and M. Davis, *Chemical reviews*, 2002, **102**, 3589-3614.
17. W. Wang, S. Efrima and O. Regev, *Langmuir*, 1998, **14**, 602-610.
18. N. Lala, S. P. Lalbegi, S. Adyanthaya and M. Sastry, *Langmuir*, 2001, **17**, 3766-3768.
19. A. C. Manikas, F. Causa, R. Della Moglie and P. A. Netti, *ACS applied materials & interfaces*, 2013, **5**, 7915-7922.
20. E. J. Guidelli, A. P. Ramos, M. E. D. Zaniquelli, P. Nicolucci and O. Baffa, *ACS applied materials & interfaces*, 2012, **4**, 5844-5851.
21. L. Qian and X. R. Yang, *Advanced Functional Materials*, 2007, **17**, 1353-1358.
22. M. R. Gill and J. A. Thomas, *Chemical Society Reviews*, 2012, **41**, 3179-3192.
23. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang and S. Perrett, *Nature nanotechnology*, 2007, **2**, 577-583.
24. W. Chen, J. Chen, Y.-B. Feng, L. Hong, Q.-Y. Chen, L.-F. Wu, X.-H. Lin and X.-H. Xia, *Analyst*, 2012, **137**, 1706-1712.
25. H. Wei and E. Wang, *Analytical Chemistry*, 2008, **80**, 2250-2254.
26. M. R. Guascito, D. Chirizzi, C. Malitesta and E. Mazzotta, *Analyst*, 2011, **136**, 164-173.