



Biogenic synthesis of nanomaterials

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博士論文

ナノ材料の生物学的合成

Biogenic synthesis of nanomaterials

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Chapter 1.

Introduction: Biogenic synthesis of nanomaterial

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Introduction: Biogenic synthesis of nanomaterial: An overview

Nanotechnology can be described as the manipulation of matter on an atomic and molecular scale [1]. As per the National Nanotechnology Initiative, nanotechnology can be defined as the manipulation of matter with at least one dimension in the size range from 1 to 100 nanometers (nm) or 10^{-9} of a meter. This miniaturization allows them to exhibit novel and significantly improved physical, chemical, and biological properties, phenomena, and processes owing to their size. When characteristic structural features are intermediate between isolated atoms and bulk materials in the range of about 1-100 nm, the objects often display physical attributes substantially different from those displayed by either atoms or bulk materials. The properties of material changes as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant at the nano-scale size-dependent properties are observed. These interesting and sometimes unexpected properties of nanoparticles (NPs) are therefore largely due to the large surface area of the material which governs the contribution made by the small bulk of material [2]. Important behavioral changes are caused not only by continuous modification of characteristics with diminishing size, but also by the emergence of totally new phenomena such as quantum confinement [3], a typical example of which is that the color of light emitting from semiconductor nanoparticles depends on their sizes.

The importance of atomic scale architecture can be well understood in case of another interesting nanomaterial-carbon nanotube (CNTs) where the sp^2 hybridized carbon chains result in one of the most resilient structures ever known to mankind [4]. If this folded tube is zipped along one of its axis, we obtain another 2-D structure called graphene which is better known for its transparency and excellent conducting properties [5, 6].

Therefore, we see that there is a huge difference between the properties a nanomaterial may possess depending upon its size and atomic composition. This makes nanotechnology all the

more exciting as tweaking any of these properties with an intelligent mix-n-match approach may result in novel nanocomposite with advanced properties. Therefore, nanotechnology as defined by size is naturally very wide and interdisciplinary, including fields of science as diverse as surface science, molecular biology, organic chemistry semiconductor physics, micro-fabrication, applied physics etc.[7, 8, 9] The associated research and applications are indeed diverse, ranging from improvisation of conventional device physics to totally new techniques based on molecular self-assembly and developing new materials with controlled dimensions at the atomic scale. Figure1. Shows current areas of application with regard to nanotechnology.

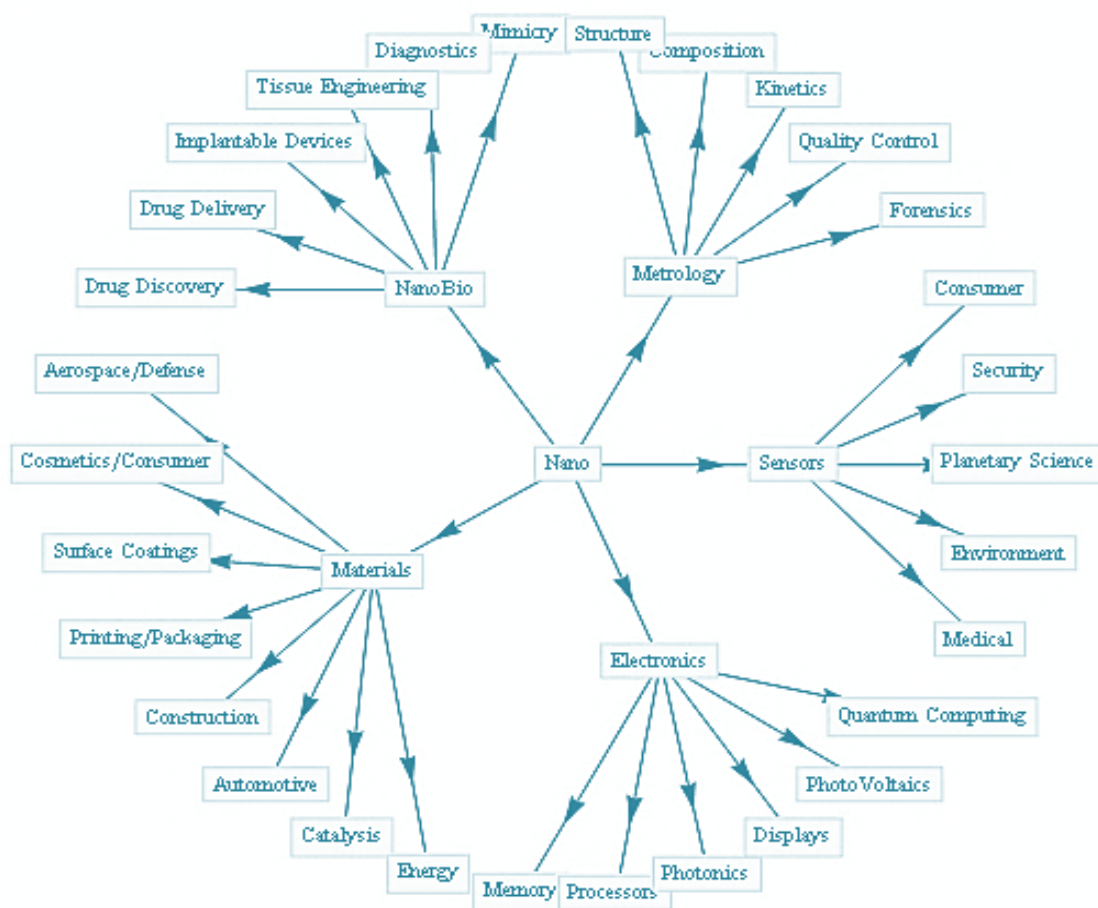


Figure 1. Showing developing application areas for nanotechnology.

(idea: <http://newton.umsl.edu/phillf/nsp08w.html>)

Nanotechnology: Historical perspective

The manipulation of material at the atomic scale to create new functional materials and properties sounds like it should be a profoundly modern concept. However, artisans from the past also controlled matter at the tiniest scales thereby altering the bulk state properties.

By modern-day standards, they were working in a branch of nanotechnology called nanocomposite. These are bulk materials in which nanoscale particles are mixed to improve the properties of the overall or composite material. There are a number of relatively famous examples of ancient artifacts which were created using nanocomposites as shown in Figure 2.



Figure 2. Showing ancient artifacts incorporating nanocomposites (a) Lycurgus cup (green when light reflected, red when light shone inside); (b) Mayan artifact showing azure pigment known as ‘Mayan blue’; (c) Damascus sword with inset showing HRTEM image confirming CNT encapsulated cementite.

For example, the Lycurgus cup is a stunning decorative Roman treasure from about AD400 made of a glass that changes color when light is shone through it [10].

The glass contains gold-silver alloyed nanoparticles distributed in a way as to make the glass look green in reflected light. However, when light passes through the cup, it reveals a brilliant red color. Similarly, corrosion resistant blue pigment known as Maya Blue, first produced in AD800, was discovered in the ancient Mayan city of Chichen Itza [11]. It is complex material containing clay with nanopores into which indigo dye was combined chemically to create an environmentally-stable pigment. Damascus steel swords originating in western India were made between AD300 and AD1700 and are known for their impressive strength, shatter resistance and exceptionally sharp cutting edge. The steel blades was later observed to be containing nanoscale wire-and-tube-like structures which certainly enhanced the material's properties owing to its source of iron mines in India which were rich in carbon and other transition impurities [12]. However, those craftsmen were highly skilled yet unaware that they are working at nanoscale. They developed these materials by trial and error and didn't know the processes going on inside the solids. These examples however are sufficient to assert that ancient alchemists knew about properties enhancement these materials may possess even though they had no confirmed scientific proof for the same.

Michael Faraday provided the first scientific statement about the optical properties of nanometer-scale metals in his classic 1857 paper. In a subsequent paper, Turner pointed out that: "It is well known that when thin leaves of gold or silver are mounted upon glass and heated to a temperature that is well below a red heat ($\sim 500^\circ\text{C}$), a remarkable change of properties takes place, whereby the continuity of the metallic film is destroyed. The result is that white light is now freely transmitted, reflection is correspondingly diminished, while the electrical resistivity is enormously increased" [13].

Nanotechnology: Evolving modern perspective

Concept of nanotechnology was first provided in 1959 by renowned physicist Richard Feynman in his talk ‘There's Plenty of Room at the Bottom’, in which he described the possibility of synthesis via direct manipulation of atoms [14]. The term "nano-technology" was first used by Norio Taniguchi in 1974 to describe semiconductor processes such as thin film deposition and ion beam milling exhibiting characteristic control on the order of a nanometer [15]. Inspired by these ideas, K. Eric Drexler an American engineer used the term "nanotechnology" in his 1986 book ‘Engines of Creation: The Coming Era of Nanotechnology’, which proposed the idea of a nanoscale "assembler" which would be able to build a copy of itself and of other items of arbitrary complexity with atomic control. Thus, Drexler’s theoretical and public work led to the emergence of nanotechnology as a field in the 1980s by developing and popularizing a conceptual framework for nanotechnology and initiating experimental advances that attracted additional large-scale attention to the prospects of attaining atomic control of matter. With advances in high-resolution instruments capable of viewing atomic scale architecture like Scanning Tunneling microscopy and Atomic Force Microscope (both developed in 1981) enabled study and manipulation of atoms at nanoscale [16, 17]. These inventions helped discovery of fullerenes in 1986 [18] followed by carbon nanotubes in 1991 [19] which started potential applications for nanoscale electronics and devices. This followed a series of nanofabrication techniques in preparation of nanocomposites and functional nanomaterials which created the modern avenue of material science (as highlighted in Table 1). However, with growing advent in application of nanomaterials, it became important that the production of these materials should be carried out in a sustainable manner.

Nature's ideas and green chemistry: Biogenic nanomaterials, scope and application of our current research

In recent years, nanoscience and nanotechnology has made considerable progress towards synthesis and characterization of new materials due to their wide-spread application in everyday life. Today, we are living in a time where nanotechnology is bound to gain its momentum as compared in the past which is highlighted in Figure 3.

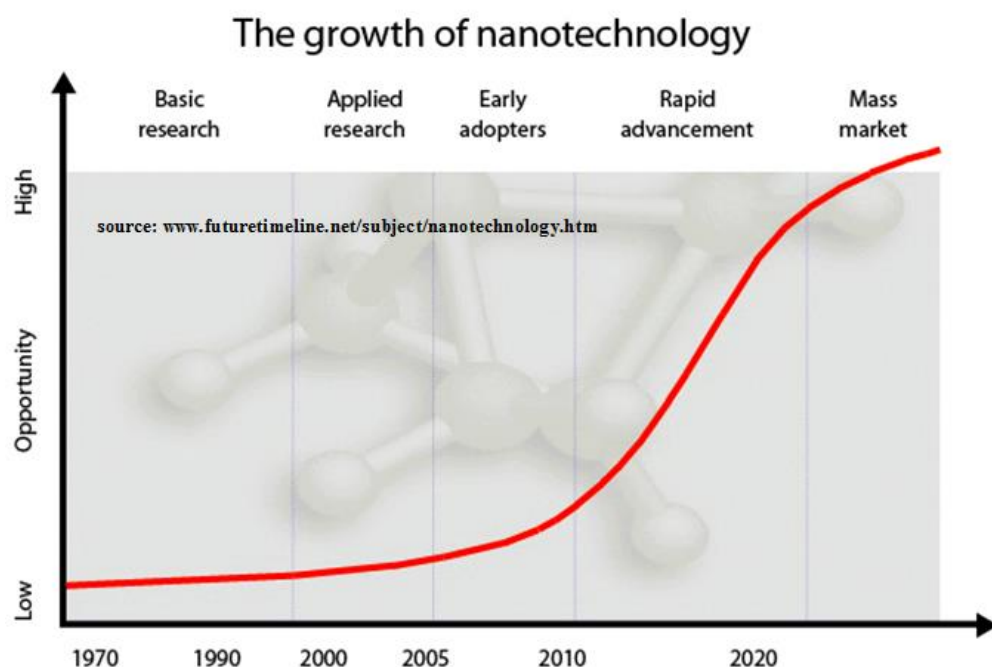


Figure 3. Showing progress of nanotechnology. Note that currently we are still in investigative stage.

Much of this success was possible due to chemical modifications or functionalization of nanomaterials including nanoparticles, carbon nanotubes (CNTs) surface and relatively new substrate- graphene. These nanomaterials have found tremendous usage in electronics, sensors, biomedical applications, catalysis, H₂ storage etc. However, synthesis and surface modification of such nanomaterials requires extensive usage of toxic chemicals/solvents, tedious process control and difficulties in scale up. To overcome these issues, several biogenic synthesis processes have been reported owing to the constant need for cost-effective eco-friendly synthesis of such nanomaterials with advanced properties.

Generally speaking, the first way is to start with a bulk material and then break it into smaller pieces using mechanical, chemical or other form of energy (top-down). An opposite approach is to synthesize the material from atomic or molecular species via chemical reactions, allowing for the precursor particles to grow in size (bottom-up). Then there are functional approaches like Biogenic fabrications, which seek to develop components of a desired functionality without regard to how they might be assembled.

Biogenic synthesis of nanomaterials revolves around the philosophy of utilizing active compounds present in our nature for synthesizing/imparting novel properties which in turn can provide a breakthrough in its application [20, 21, 22]. Biomimetic approaches are relatively new owing to constant need in sustainability and advanced material properties [23]. With the help of nature's molecules, chemical engineers have found ways to create new materials that can do everything ranging from bone/tissue regeneration, cancer treatment, efficient chemical catalysis and clean energy production [24]. This subset of green chemistry focuses on minimizing the hazard while aiming towards improved efficiency of any chemical choice. This novel ideology of utilizing bio-nano complexes has the potential to open doors for several cost-effective eco-friendly applications along with the promise to provide foundation for advanced nano-bio hybrid systems in future. This requires identification and extraction of active biomolecules which may originate from any living system or part of living system with the possibility of creating bio-nano hybrid systems [25]. Also, due to its biological origin, these nanomaterials are generally observed to be eco-friendly and non-toxic thereby proving to be excellent material for drug delivery [26] and clean energy [27].

Another advantage of biogenic materials is that the resulting product is due the virtue of one or more compounds and may not be easily replicated via chemical methods. Thus, in many ways, biogenic nanomaterial properties rather novel and are intrinsic to the biomolecules being used giving them an edge over any previously reported chemical method for the similar application. Besides the academic interest in biogenic nanomaterials, it is anticipated that the

use of natural, abundant nanomaterials, e.g., cellulose, chitin, collagen, and silk, could provide affordable functional nanomaterials in developing countries [28]. Some of these advantages of biogenic materials are summarized in Figure 4.



Figure 4. Showing advantages of biogenic nanomaterials.

It is interesting to note that some of the preliminary green synthesis of nanomaterials (biogenic study) can be traced back in early 2000s with no clear knowledge of the existing mechanism [29, 30, 31]. The next decade saw a substantial rise in research works related with the green synthesis aspect highlighted by biogenic synthesis of nanomaterials as highlighted in Figure 5. The following data was collected via ‘Google scholar’ with time limited search string consisting of “green synthesis” and nanomaterials. The following data makes it clear that green synthesis of nanomaterials is a newly evolving field where biogenic synthesis has the potential to contribute significantly.

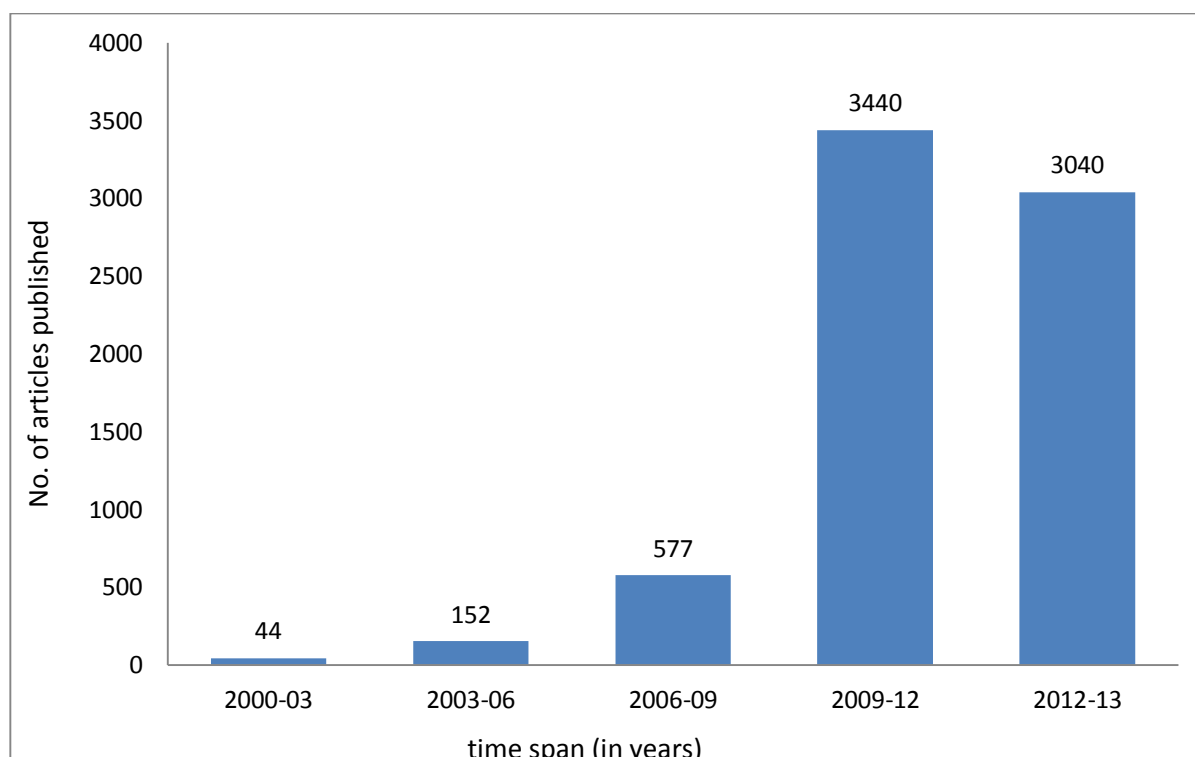


Figure 5. Showing number of scientific articles published in the area of green synthesis of nanomaterials via biogenic means.

This makes the work presented in this thesis all the more important as it contributes significantly in this newly evolving field of biogenic material synthesis as highlighted by the comparative analysis presented in Table 1. From the origin of biogenic synthesis where a ‘complex broth’ may provide the necessary functionality, we try to streamline it towards biomimetic synthesis where individual molecules can be used instead for greater control and advanced properties. With ever growing technologies being developed by incorporating nanomaterials, it becomes important that associated green chemistry of it should also keep up the pace and provide novel routes for achieving the same.

Table 1. Showing chronological development in nanoscience and nanotechnology.

Time line (in years)	Event happened	Impact of the event
1959	Feynman's famous 'There's plenty of room at the bottom' talk	Proposed synthesis via direct manipulation of atoms
1974	Dr. Norio Taniguchi coined the word 'nano-technology'	Proposed improved semiconductor process by control on the order of nanometer
1981	Scanning Tunneling Microscope invented by Binnig and Rohrer	Unprecedented visualization of individual atoms and bonds.
1985	Fullerenes discovered by Smalley et al.	Suggested potential applications of carbonaceous materials at nanoscale.
1986	Eric K. Drexler's famous 'Engines of creations: The coming era of nanotechnology' published	Stirred public awareness and government interests
1991	Dr. Sumio Iijima, often cited as inventor of carbon nanotubes publishes his observations in Nature.	Unprecedented interest in the carbon nanostructures and since fuelled intense research in nanotechnology.

1999	First working example of synthetic nanosensor by researchers at Georgia Institute of Technology.	Opened new avenues in miniaturization of sensor technology greatly benefitting biomedical technologies.
2000s	Commercialization of nanotechnology based materials like nanoparticles, carbon fillers etc.	Silver nanoparticles as effective antibacterial agents, advanced (miniaturized) semiconductor devices and nanotubes for reinforced fabric.
Early 2000-2003	Preliminary reporting on biogenic synthesis of nanomaterials published	Focus shifted on eco-friendly synthesis of nanomaterials with advanced properties.
2004	Discovery of graphene by Geim and Novoselov	Opened ground-breaking technologies in field of semiconductors and electronics
2012	Srivastava et al. published the first theory of multiple monometallic nanoparticle synthesis via single strain of bacteria	Strongly suggested the impact of extracellular peptides/polysaccharides in nanoparticle synthesis and fabrication

2013	Srivastava et al. proposed a novel Trp-mediated supramolecular interface theory for synthesis of Au@Pd nanoparticles,	First reporting of active amino acid mediated synthesis of core shell nanoparticles with improved catalytic activity.
2013	Srivastava et al. presented a novel green strategy for surface modification of CNTs and graphene via garlic phytochemicals.	Provided first ever single-step thiolation of carbonaceous materials via biogenic methods.

For example, bacteria are an exciting category of microorganisms which have an innate ability to reduce metallic ions to their respective metallic nanoparticles. They therefore function as cost-effective ‘green’ nanofactories. In fact, interactions between metals and microbes have been used for such biological applications as biomineralization, bioremediation, bioleaching and biocorrosion, and the microbial synthesis of nanoparticles has emerged as a promising field of research that bridges applied microbiology and nanotechnology.

It should be noted that although various physical and chemical methods are extensively used to produce monodisperse metallic nanoparticles, surfactants (for stability) and use of toxic chemicals (reductants) needs immediate and effective alternative. The use of toxic chemicals on the surface of nanoparticles and non-polar solvents in their synthesis limits application in the clinical field. Therefore, the development of non-toxic, clean and bio-compatible nanoparticles is a welcome sign [32]. Bacteria can produce inorganic nanoparticles either intracellularly or extracellularly with a well-defined shape and monodispersity [33, 34]. The same has been observed in class of various plant extracts [35]. However, the ability to obtain

extracellular nanoparticles thereby allowing re-usability of the stock culture clearly makes bacteria an ideal candidate for nanoparticle synthesis.

It is interesting that nanoparticles are increasingly being synthesized by bacteria even though the exact mechanism is still in its infancy. Some of the studies mentioned above have pointed out that bacterial nanoparticle synthesis depends on such factors as strain type, physical conditions like pH, time and temperature and the type and concentration of the metallic salt.

We published a novel one bacterial strain producing an array of different nanoparticles which showed for the first time that inherent reducing mechanism has the capacity to produce different types of stable nanoparticles under aqueous reaction condition [36]. Therefore, it is highly desirable to understand the underlying mechanism/responsible groups for synthesis of these nanoparticles so that they can be extracted and utilized for bulk state production. We confirmed that certain extracellular membrane bound proteins/Lipopolysaccharides may be responsible for reduction of metallic cations into its respective reduced form (nanoparticles).

Therefore, in our primary research pertaining to chapter 2, we tried to locate the origin of these bioactive compounds with an aim to isolate and study their impact in-situ. It is to be noted that in biogenic synthesis, the observed effect is generally cumulative in nature. We concluded that extracellular membrane fraction of *E. coli* K12 was responsible for the biogenic synthesis of gold nanoparticles at room temperature without pH adjustment. We concluded that certain proteins embedded in the membrane fraction causes formation and stabilization of Au NPs. In the absence of these proteins (activity loss by β -met treatment), no nanoparticle formation was observed. Further, we utilized a novel ideology proposed by Macaski et al. in 2012 where they reported biomass supported heterogeneous catalyst preparation for oxidation of benzyl alcohol [37]. We utilized our Au NP-MBF as a heterogeneous catalyst for oxidation of 4-nitrophenol. Finally, we went a step ahead and incorporated the extracted protein in silica-based column to show continuous synthesis of gold nanoparticles.

During the course of this study, we were aware that proteins which are basically chains of amino acid show catalytic activity by the virtue of certain specific amino acid folding (active site). An interesting study done by Serpell et al. (2011) drew our attention where core-shell nanoparticles were synthesized via anion coordination chemistry [38]. From our past experiences, we wanted to see if the same is possible by supramolecular interaction via tryptophan [Trp] mediated amine linkages formed the foundation for chapter 3 and chapter 4. Trp with its ability to form poly-Trp layer over gold surface provides the necessary ‘framework stability’ for producing core-shell structures. In this study, we demonstrate for the first time, biosynthesis of Au@Pd and Au@Pt bimetallic nanoparticles via tryptophan in aqueous reaction mixture at room temperature. Gold seeds were produced by incorporating tryptophan in the reaction mixture followed by successive reduction of Pd/Pt over the gold nanoparticle surface. Further, catalytic activity of Au@Pd NPs and Au@Pt NPs were tested respectively for oxidation of 4-NP and electro-oxidation of water to produce hydrogen. We provided an innovative strategy synthesis for core-shell nanoparticle synthesis and tentative application. This concludes our major research segment pertaining to biogenic nanoparticle synthesis and its tentative properties/applications.

This allowed us to shift our focus to second class of nanomaterials namely the carbonaceous materials. It is interesting to note that although quite a few numbers of studies have been reported for synthesis of metallic nanoparticles by biogenic means, not much information is available for carbonaceous substrates. An important reason for the same is that although carbon nanotubes are providing cutting edge nanocomposites, they need extremely harsh surface treatment (pre-treatment) for effective functionalization. In fact, composites are a third class of materials made of other nanomaterials that are combined with large bulky materials or other nanoparticles. Therefore, it becomes important that the first two classes of materials namely nanoparticles and carbonaceous materials can be effectively used to create biogenic nanocomposites thereby forming the basis for chapter 5 and chapter 6. Although a

few techniques were reported in 2009 dealing with direct metal deposition over carbon nanotubes (seeding), there exists no one-step thiolation technique to functionalize and attach nanoparticles over the modified CNT surface. An interesting study done by Zanella et al. caught our attention where they suggested that CNTs are not absolutely perfect structures and can be modified by mercaptothiols [39]. These defects in turn can be targeted for surface modification and subsequent treatment of nanoparticles. For us, this meant that an alternate strategy can be tested to modify these CNTs as by certain biogenic means. Garlic proved to be an ideal candidate as it happens to be a natural source of organosulfur compounds where total sulfur content ranges from 0.35 – 1% of its dry weight. In this study, we reported multiwalled CNT (MWCNT) surface modification by utilizing natural organosulfurs from *Allium sativum* (Garlic) under ambient reaction conditions with an acid-free green process and its effect on the deposition of Au NPs onto the modified MWCNT surface. We concluded that alliin/allicin organothiols can thiolize MWCNT surface thereby promoting Au NP particle deposition.

This strategy was further analyzed for surface modification of graphene as discussed in chapter 6. We further utilized pure alliin for one-step thiolation of graphene and subsequent attachment of Au NPs thereby reasserting our claim that plant phytochemicals like alliin/allicin can be effectively used to carbonaceous surface thiolation. It is interesting to note that although graphene is a relatively new substrate, there exist some biogenic strategies for its surface modification. This can be because of the fact that while CNTs are chemically inert structures requiring extreme chemical treatment, graphene on the other hand consists of several reactive oxygenated groups and essentially needs a reduction mechanism to convert from graphene oxide into reduced graphene oxide. However, we provided the first one-step thiolation strategy via partial-reduction of graphene oxide substrate.

References

- [1] E. K. Drexler (2006) Engines of Creation: The Coming Era of Nanotechnology. Doubleday, New York.
- [2] G. Schmid (2011) Nanoparticles: From theory to application. John Wiley & Sons, New Jersey.
- [3] G. D. Stucky, J. E. M Dougal, Science 1990, 247, 669.
- [4] T. Filleter, R. Bernal, S. Li, H. D. Espinosa, Adv. Mater. 2011, 23, 2855.
- [5] A. K. Geim, K. S. Novoselov, Nat. Mater. 2007, 6, 183.
- [6] J. Meyer, Nature 2007, 446, 7131.
- [7] Analysis: This is the first publicly available on-line inventory of nanotechnology-based consumer products". The Project on Emerging Nanotechnologies. 2008
- [8] J. L. West, N. J. Halas, Curr. Opi. Biotechnol. 2000, 11, 215.
- [9] C. P. Poole, F. J, Owens (2003) Introduction to Nanotechnology, John Wiley & Sons, New Jersey.
- [10] I. Freestone, N. Meeks, M. Sax, C. Higgitt, Gold Bull. 2007, 40, 4.
- [11] C. Giacomo, R. Giustetto, C. Reyes-Valerio, G. Richiardi , XXX Congresso Associazione Italiana di Cristallografia 2000, 48, 115.
- [12] M. Reibold, P. Paufler, A. A. Levin, W. Kochmann, N. Patzke. and D. C.Meyer, Nature 2006, 444, 286.
- [13] M. Faraday, Philos. Trans. R. Soc. 1857, 147, 145.
- [14] J. Mehra, Biogr. Mem. Fellows R. Soc. 2002, 48, 97.
- [15] N. Taniguchi, Process Intlernational Conference on Production Engineering Tokyo, Part II, Japan Society of Precision Engineering, 1974.
- [16] G. Binnig, H. Rohrer "Scanning tunneling microscopy", IBM Jour. Res. Dev. 1986, 30, 4.
- [17] K. M. Lang, D. A. Hite, R. W. Simmonds, R. McDermott, D. P. Pappas, J. M. Martinis, Rev. of Sci. Inst. 2004, 75, 2726.

- [18] H. W. Kroto, J. R. Heath, S. C. O'Brien, R. F. Curl, R. E. Smalley, *Nature* 1985, 318, 162.
- [19] S. Iijima, *Nature* 1991, 354, 56.
- [20] K. Liu, J. J. Zhang, F. F. Cheng, T. T. Zheng, C. Wang, J. J. Zhu *J. Mater. Chem.*, 2011, 21, 12034.
- [22] P. Fratzl, *Jour. R. Soc. Interf.* 2007, 4, 637–642
- [23] S. S. R Challa (2010) *Biomimetic and Bioinspired Nanomaterials (Volume 7)* Wiley-VCH, Weinheim.
- [24] W. Pompe, G. Rodel, H. J. Weiss, M. Mertig (2013) *Bio-Nanomaterials: Designing Materials Inspired by Nature*. John Wiley & Sons.
- [25] E. Ruiz-Hitzky, M. Darder, P. Aranda (2008) *An Introduction to Bio-nanohybrid Materials*, in *Bio-inorganic Hybrid Nanomaterials: Strategies, Syntheses, Characterization and Applications*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
- [26] Zhang et al. *Nat. Comm.*, 2013, 4, 1867
- [27] Wang et al. <http://arxiv.org/abs/1306.3015> (Biologically inspired graphene-chlorophyll phototransistors with high gain)
- [28] Alvarez et al. *Jour. Mater. Chem. A*, 2013, 1, 5469
- [29] R. R. Naik, S. J. Stringer, Gunjan Agarwal, S. E. Jones, M. O. Stone. *Nat. Mater.* 2002, 1, 169.
- [30] P. Raveendran, J. Fu, S. L. Wallen, *J. Am. Chem. Soc.* 2003, 125, 46 13940.
- [31] M. Sastry et al. *Coll. Surf. B. Biointerfaces*, 28, 2003, 313.
- [32] N. Sakthivel, K. B. Narayanan, *Adv. Coll. Int. Sci.*, 2011, 169, 59.
- [33] N. Sakthivel, K. B. Narayanan, *Adv. Coll. Int. Sci.*, 2010, 156, 1.
- [34] M. Gericke, A. Pinches, *Hydrometallurgy*, 2006, 83, 132.
- [35] A. K. Mittal, Y. Chisti, U. C. Banerjee, *Biotechnol. Adv.* 2013, 31, 246.
- [36] S. K. Srivastava, M. Constanti, *Jour. Nanopart. Res.* 2012, 14, 831.

- [37] L. E. Macaskie et al. J. R. Soc. Interface 2012, 9, 1705.
- [38] C. J. Serpell, J. Cookson, D. Ozkaya, P. D. Beer, Nat. Chem. 2011, 3, 478.
- [39] R. Zanella, E. V. Basiuk, P. Santiago, V. A. Basiuk, E. Mireles, I Puente-Lee , J. M. Saniger, Jour. Phy. Chem. B 2005, 109, 16290.

Chapter 2.

Biogenic synthesis and
characterization of gold
nanoparticles by *Escherichia coli* K12
and its heterogeneous catalysis in
degradation of 4-nitrophenol

Chapter 2.

Biogenic synthesis and characterization of gold nanoparticles by *Escherichia coli* K12 and its heterogeneous catalysis in degradation of 4-nitrophenol

1. Introduction

Gold nanoparticle (Au NP), being the most stable mono-metallic nanoparticle, promises to be a key material and building block for newer technologies in the twenty-first century. Gold in its bulk state is regarded as a noble metal and is very unreactive because of its completely filled d-band [1]. However, at nanoscale, it is proving to be an important material for catalysis owing to its shape, size and crystal structure arrangement [2]. Due to this new set of properties, it has found wide-scale application in optics, electronics, catalysis, fabrication and biomedical utilities [3]. Generally speaking, physical methods of producing gold nanoparticles involve heating of gold at reduced pressure to generate gold vapour, while chemical synthesis requires a reducing agent (generally citrate) followed by addition of a stabilizing agent [4-7]. However, these chemical methods deliver at the cost of expensive reducing and capping agents and toxic solvents along with tedious process control. To overcome these issues, several biogenic synthesis processes have been reported owing to the constant need for cost-effective eco-friendly synthesis of Au NPs. Microbial systems have found an important role in nanoparticle production due to their natural mechanism for detoxification of metallic ions through reduction which can be achieved extracellularly or intracellularly through bioaccumulation, precipitation, biomineralization and biosorption. Ogi et al. [8] showed gold nanoparticle formation in the presence of H₂ gas pumped with *Shewanella algae* cell extract. Similarly, gold nanoparticles of different shapes and sizes were produced using bacterial and fungal strains [9-12]. However, apart from the stated advantages, biological synthesis suffers from poor mono-dispersity, random aggregation, non-uniform shapes and problems in scale-up, etc. [13]. Though, in recent times, many organisms have been reported to produce nanoparticles, scientific understanding on the mechanism and the

machinery related to its production is still in its infancy. Therefore, there is a need to improve upon this green synthesis process with an aim to understand the underlying mechanism and design a working prototype for biomimetic production of Au NPs. These nanoparticles, upon being adhered to a matrix, may serve as a better catalyst than bulk metal due to greater accessibility to surface atoms and low coordination number especially in the case of water treatment. Among several water pollutants, nitroaromatic compounds are considered as the most toxic and refractory pollutants, of which the permissible range is as low as 1 to 20 ppb. However, these are common in production of dyes, explosives and pesticides among many others; thus, their industrial production is considered as an environmental hazard [14]. Upon being released into the environment, these nitrophenols pose significant public health issues by exhibiting carcinogenic and mutagenic potential in humans [15].

Normally, it takes a long time for degradation of nitrophenols in water which poses considerable risk if it seeps into aquifers along with the groundwater. These nitrophenols tend to get accumulated in deep soil and stays indefinitely. Although, several water treatment methods are available like chemical precipitation, ion exchange adsorption, filtration and membrane systems, they are slow and non-destructive. Therefore, there is a need to remove these highly toxic compounds with efficient catalytic systems. Generally, nanoparticles are immobilized onto supporting materials like silica, zeolites, resins, alumina, microgels, latex, etc. which are inert to the reactants and provide a rigid framework to the nanoparticles. The gold-supported catalysts can then be used to carry out partial or complete oxidation of hydrocarbons, carbon monoxide, nitric oxide, etc. [16].

In a recent study, Deplanche et al. [17] showed coating of palladium followed by gold over *Escherichia coli* surface in the presence of H₂ to produce biomass-supported Au-Pd core-shell-type structures and subsequent oxidation of benzyl alcohol. Likewise, we believe that bacterial biomass is essentially carbonaceous matter which can be used to serve as a matrix

for preparing a heterogeneous catalyst with the incorporation of nanoparticles. With this aim, we utilized *E. coli* K12 strain to check its potential for producing Au^0 from AuCl_4^- . This strain has been known for its reduction activity as shown with bioremediation studies [18, 19]. Here, we report bio-reduction of gold cations at room temperature to yield well-dispersed nanoparticles (Au^0). The resulting nanoparticles were characterized by ultraviolet-visible (UV-vis) spectroscopy, atomic force microscopy (AFM), selected-area electron diffraction (SAED), transmission electron microscopy (TEM) and X-ray diffraction (XRD). Additionally, the extracellular reduction mechanism was examined by Fourier transformation-infrared spectroscopy (FT-IR), zeta potential (Z-pot) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). We observed that certain membrane-embedded proteins in the extracellular membrane fraction of the cell are responsible for reducing gold cation to stable Au^0 state. Further, these membrane-bound gold nanoparticles were utilized to produce a heterogeneous catalyst in degradation of 4-nitrophenol (4-NP). This biosynthesis study provides an excellent platform for the production of gold nanoparticles by bacterial membrane-bound proteins. The resulting membrane-bound nanoparticles can be prepared into an eco-friendly cost-effective bionanocomposite to serve as an efficient catalyst in complete degradation of 4-nitrophenol.

2. Experimental

2.1 Bacterial strain and growth conditions

E. coli K12 cells were procured from our existing strain collection and were cultured in nutrient broth (10 g L^{-1} peptone, 10 g L^{-1} meat extract, 0.5 g L^{-1} NaCl) at 27°C and 120 rpm for 24 h in screw-capped flasks. After a day of incubation, the culture was centrifuged at $10,000\times g$ for 10 min, and the resulting bacterial pellet was separated and retained. The bacterial pellet was thoroughly washed three times in sodium saline followed by washing

three times in Milli-Q water (Millipore, Tokyo, Japan) to remove any unwanted material sticking to the cells. These cells were weighed, and 0.5 g wet weight of pellet was prepared to be used later. The washed cells suspended in 10 mL of distilled water gave a solution with a cell concentration of 5.2×10^{11} cells mL⁻¹.

To determine whether or not intact cells were required for Au NP formation, *E. coli* K12 cells were cultured and harvested as in the previously described method. The cells were then disrupted by autoclaving (120°C at 15 psi for 30 min). This caused complete lysis of the bacterial cells which was later centrifuged at 15,000×*g* for 60 min to separate the membrane fraction (pellet) from the soluble (supernatant) fraction. Membrane-bound fraction (MBF) pellet was pooled together and washed thrice with Milli-Q water and re-centrifuged again at 15,000×*g* for 30 min. Finally, 2 g of MBF pellet (wet wt.) was retained to be incorporated with 10 mL of 0.01 M HAuCl₄ solution (Nacalai Tesque, Kyoto, Japan). Although pH was measured at this stage (pH 2.8), no adjustment was made. Control reactions included 0.01 M HAuCl₄ solution prepared with soluble (supernatant) fraction and uninoculated HAuCl₄ solution prepared with Milli-Q water.

2.2 Characterization of biogenic gold nanoparticles

UV-visible spectra were obtained using a Hitachi U2000A UV-vis spectrophotometer (Tokyo, Japan). The formation of Au NPs was monitored by UV-vis spectra of the reaction mixture from 210 to 800 nm.

Primary study of nanoparticle shape and size was carried out using an SPI-3800N atomic force microscope with SPA 400 soundproof housing sample holder connected to an imaging system (Seiko Instruments, Chiba, Japan). Five microliters was taken from the reaction mixture and placed on the glass grid and dried at room temperature. The images were obtained using SPIWin (3800N) ver. 3.02J (Wyandotte, MI, USA).

Morphology and grain size of these nanoparticles were analysed using a Hitachi H-7100 transmission electron microscope. Two microliters was taken from the two reaction mixtures and placed on carbon-coated copper grids and dried at room temperature. The transmission electron micrographs and the SAED patterns were recorded at an acceleration voltage of 100 kV. The images were analysed using the ImageJ 1.43M software.

FT-IR analysis was done using Jasco FT/IR-680 plus (Easton, MD, USA) coupled to a high-performance computer. The samples (100 μ L) were placed over the ATR analyser, and the resulting spectra were analysed using Spectra Manager ver. 1.06.02. Zeta potential measurements were performed using the Malvern Zetasizer Nano ZS model ZEN3600 (Malvern, UK) equipped with a standard 633-nm laser.

Confirmatory study of resulting Au NPs was done by XRD using a Rigaku RINT-TTR diffractometer (Tokyo, Japan) equipped with a parallel incident beam (Göbel mirror) and a vertical θ - θ goniometer. Samples were placed directly on a sample holder. The X-ray diffractometer was operated at 50 kV and 300 mA to generate $\text{CuK}\alpha$ radiation. The scan rate was set to 5° min^{-1} . Identification of the metallic gold was obtained from the JCPDS database.

2.3 Preparation of biomass-supported Au nanocatalyst in 4-nitrophenol degradation

The reduction of 4-NP by NaBH_4 was studied as a model reaction to probe catalytic efficiency of a biomass-supported Au catalyst for heterogeneous systems. Under experimental conditions, reduction does not proceed at all simply with the addition of NaBH_4 or biomass alone. However, in the presence of a biomass-supported Au catalyst, it proceeds to completion with formation of 4-aminophenol (4-AP). To study the reaction in a quartz cuvette, 2.77 mL of water was mixed with 30 μ L (10^{-2} M) of 4-NP solution and 200 μ L of freshly prepared NaBH_4 (10^{-1} M) was added. The Au NP reaction mixture along with the MBF was dried for 24 h at 90°C , and 5 mg of biomass-Au NP composite (size approximately

50 nm, 4.2×10^{-6} mol dm⁻³) was added to the above reaction mixture. A similar technique was used by Narayanan and Sakthivel [20] by coating fungal mycelia-coated Au NPs on glass beads. UV-vis spectra of the sample were recorded at every 2-min interval in the range of 200 to 600 nm. The rate constant of the reduction process was determined by measuring the change in absorbance of the initially observed peak at 400 nm, for the nitrophelate ion as the function of time.

3. Results and discussion

Gold nanoparticle formation was primarily observed by UV-vis spectroscopy and AFM. After a series of experimentations, we found that MBF of *E. coli* K12 strain has certain proteins which are responsible for reducing Au cations into Au NPs. A distinct pink colour was observed due to the phenomenon of surface plasmon resonance (SPR) [21] (Figure 1a) in the reaction mixture containing MBF of the bacterial cell after 24 h. No colour formation was present in the control sample consisting of soluble fraction (Figure 1b) and gold ion solution without inoculum (Figure 1c). The same is shown in the inset of Figure 1. UV-vis spectra (Figure 1) of aqueous reaction mixtures showed no increase in absorbance after 24 h, suggesting formation of stable nanoparticles in the reaction mixture. It should be noted that the SPR peak broadening and associated decreased intensity is because of the interaction between the membrane fraction and Au NPs in the reaction mixture. [22] This can be understood by the fact that when these Au NPs are in the vicinity of bacterial cells, membrane fraction or lipopolysaccharides, they tend to adhere to these substrates, thereby

reducing the peak intensity (adding scattering background) as compared to otherwise observed SPR of Au NPs alone. This also suggests that in the case of biogenic synthesis of nanoparticles, the presence and intensity of SPR should not be the sole criterion for concentration assessment.

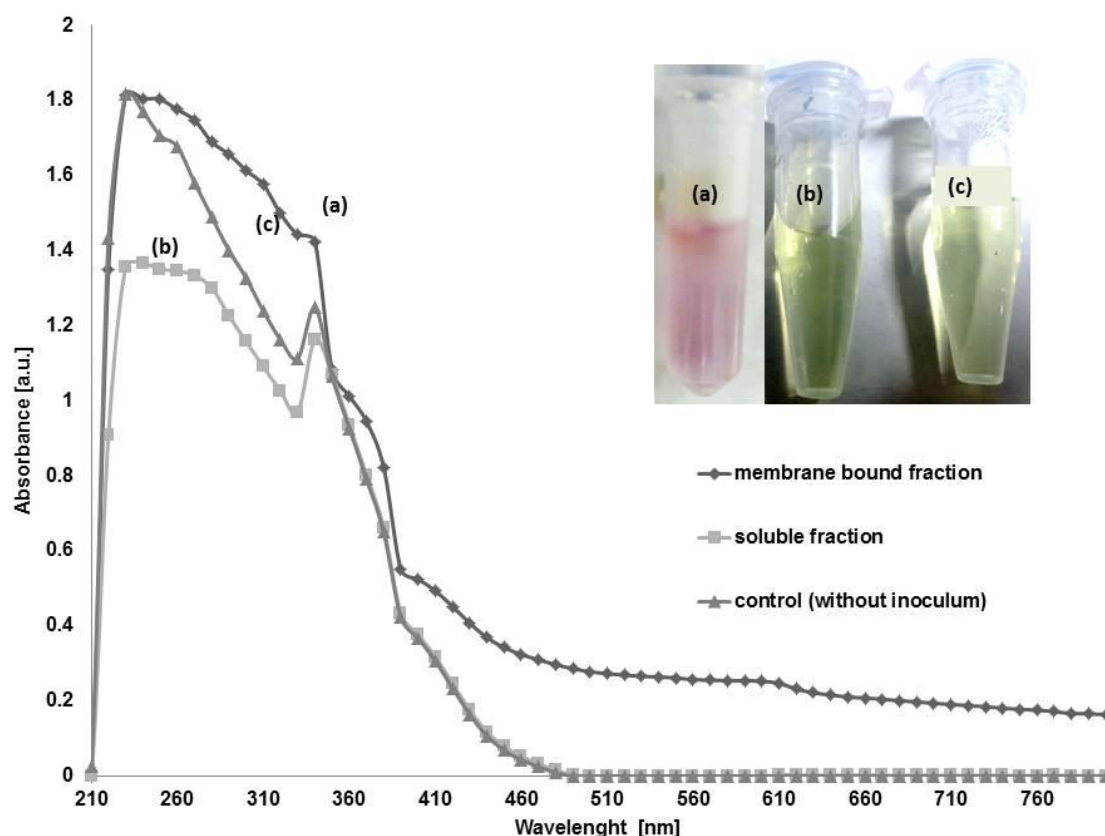


Figure 1. UV-Vis spectra observed after 24 h. (a) SPR due to Au NP produced by MBF; (b) no SPR absorbance in soluble fraction; (c) no SPR absorbance in gold ion solution without inoculum. The inset Figure corroborating the same in the above-mentioned samples, respectively.

It is important to note that no colour change was observed in control solutions consisting of cell soluble fraction and gold cation solution (without inoculum), suggesting the absence of nanoparticle formation. This was further verified when these samples were examined by AFM as shown in Figure 2.

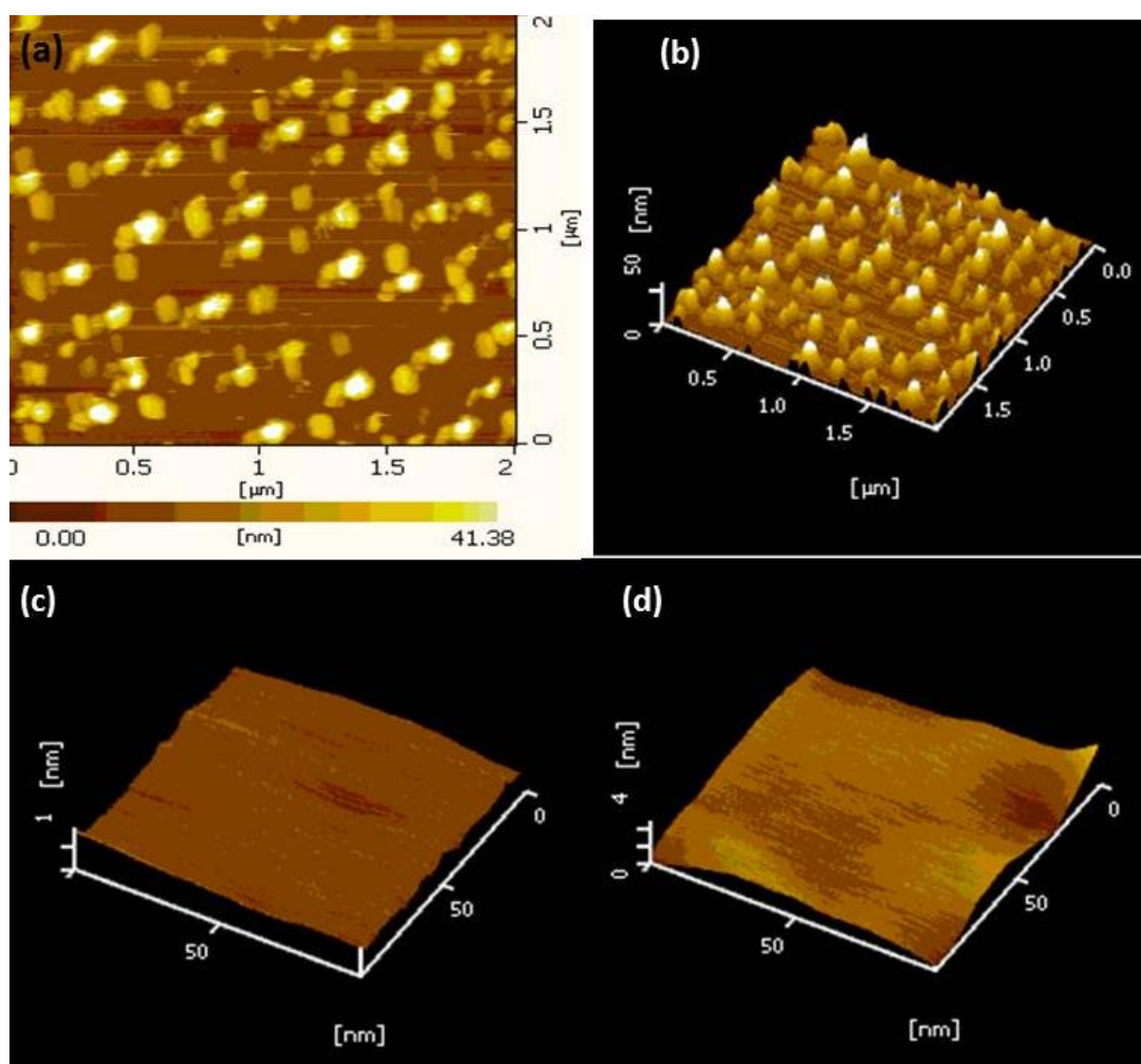


Figure 2. AFM imaging of biogenic Au nanospheres after 24 h by membrane-bound fraction of cells as (a) 2-D shape distribution; (b) 3-D size distribution; (c) 3D image of soluble cell extract (no NPs observed); (d) 3-D image of control without inoculum (no NPs observed).

The AFM probe detected discrete circular nanoparticles (Figure 2a, b) from the MBF reaction mixture, while no such formation was observed in the soluble fraction or gold cation solution without inoculum (Figure 2c, d). The 2D profile obtained by AFM suggested strong shape control (circular) with a size around 50 nm. This strong shape control indicated that apart from reducing proteins present in the MBF, certain organic groups must be acting as

stabilizing agent. To investigate the same, the membrane-bound reaction mixture was subjected to FT-IR analysis to analyse the chemical groups responsible for nanoparticle synthesis. FT-IR spectra (Figure 3a) showed distinct absorption in the region 1,800 to 1,600 cm^{-1} responsible for amide linkages in the reaction mixture.

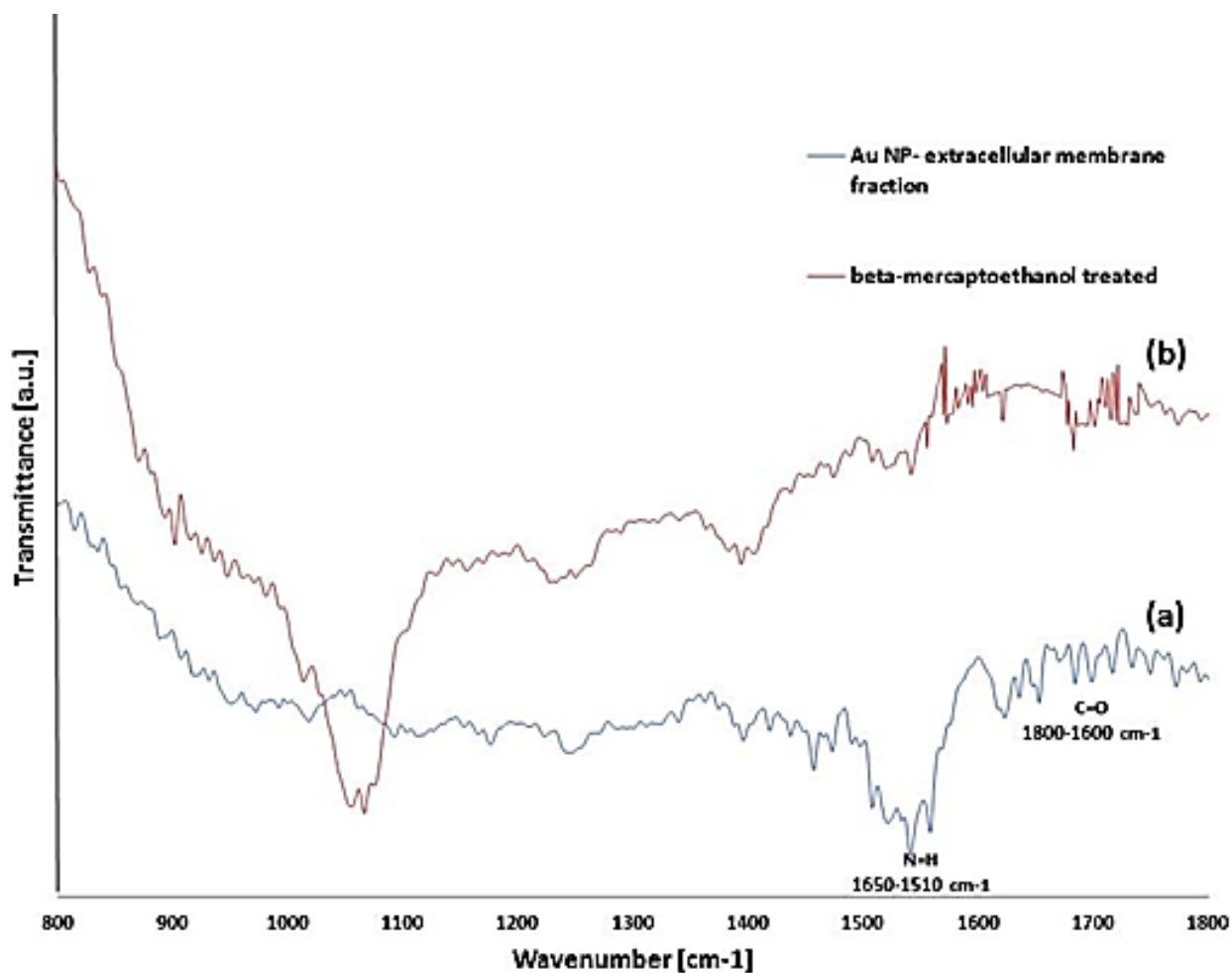


Figure 3. FT-IR spectra of reaction solution. (a) Membrane-bound fraction with Au NPs (indicated in blue); (b) membrane-bound fraction treated with β -mercaptoethanol (indicated in red).

FT-IR spectra (Figure 3a) confirmed the presence of vibration bands centred at 1,841, 1,787, 1,756, 1,725, 1,692, 1,680, 1,661, 1,650, 1,634 and 1,603 cm^{-1} . This highlights the presence of amide I (C=O) and amide II (N=H) groups present in the reaction mixture. It is likely that

the amide carbonyl group (C=O) arises from peptide coupling in proteins from the extracellular membrane fraction of the bacterial cell. This supports the fact that the secondary amide C=O stretching which forms protein/Au bioconjugates may have a role in stabilization of nanoparticles [23]. Generally, in the case of biogenic synthesis, the presence of active chemical groups like amino, sulfhydryl and carboxylic groups plays a key role in reduction of metallic ions and subsequent formation of nano/microparticles. Since amino and carboxyl groups were detected by FT-IR, it strongly suggested towards the presence of certain proteins in the reaction medium responsible for Au NP biosynthesis.

Further, aqueous stability of Au NPs were tested by zeta potential analysis. It should be noted that if active groups on biomass carry greater positive charge at low pH, it weakens the reducing power of biomass and allows AuCl_4^- ions to get closer to the reaction site [24]. This decreases the reaction rate and causes strong biosorption between Au NPs and biomass resulting in particle aggregation. Since the bacterial cell wall of *E. coli* is negatively charged, it tends to thermodynamically favour the formation of nanoparticles at low pH as observed in our case. This was confirmed by zeta potential analysis of the Au NP solution with a mean Z-pot of -24.5 ± 3.1 mV, suggesting a stable gold colloid solution. To further investigate the role of proteins in nanoparticle formation, MBF was treated with 1% β -mercaptoethanol (β -met) and heated for 30 min at 95°C. This treatment caused disruption of disulfide bonds within the multimeric chains of peptide and eventually resulted in loss of activity. In the absence of reducing activity by membrane-bound proteins, no nanoparticle formation was observed with β -met-treated MBF. This was further verified by FT-IR analysis (Figure 3b) with disappearance of most bands around the $1,600\text{ cm}^{-1}$ region. The peak observed at $1,075\text{ cm}^{-1}$ corresponds to the thiocarbonyl group due to the addition of mercaptoethanol in the reaction mixture. This suggested that certain membrane-embedded proteins may be responsible for reducing Au^{3+} to Au nanoparticles (Au^0). The membrane proteins responsible

for nanoparticle synthesis were run along with β -met-treated membrane proteins in SDS-PAGE gel (data not shown) which confirmed the presence of different sizes of protein bands in the reaction mixture, of which 25 and 73 KDa seemed to be of importance. Upon treatment of MBF with β -met, there was absence of a protein band at 73 KDa, and subsequently, no nanoparticle formation was observed, suggesting its crucial role in the reduction process. Also, a shorter peptide (25 KDa) was found to be adhered to the synthesized nanoparticles, suggesting its role in stabilization of nanoparticles. SDS-PAGE analysis showed strong evidence of membrane bound proteins responsible for Au NP production. After a series of experimentations, we excised the SDS-PAGE gel protein lanes and extracted all the proteins bands as per 'Passive Elution of Proteins from Polyacrylamide Gel Pieces' protocol. These extracted protein fractions were tested for the reduction of HAuCl_4^- into Au^0 .

Membrane bound fraction showed several protein fractions as shown in Fig 4b. This is compared to a protein ladder (Precision PlusTM Protein Standard 250 KDa) in Figure 4a. Subsequently, each of the visible protein bands was excised as mentioned above. After a completing protein separation and purification steps, these membrane protein fractions were tested for their reduction potential by their introduction in HAuCl_4 solution (0.01 M). We observed that the protein fraction of size $\sim 73\text{KDa}$ in particular, has a strong effect on reducing HAuCl_4^- into Au^0 along with a smaller protein fraction of size around 25 KDa which may be responsible for nanoparticle stabilization as highlighted in Figure 4d. This 25 KDa protein was also found to be 'wrapped' around the Au NPs as described in the manuscript. Further, when the reaction mixture was treated with β -met most of the secondary amide peaks were disappeared (FT-IR) suggesting loss of catalytic activity by these proteins as no nanoparticle formation was observed (Figure 4c).

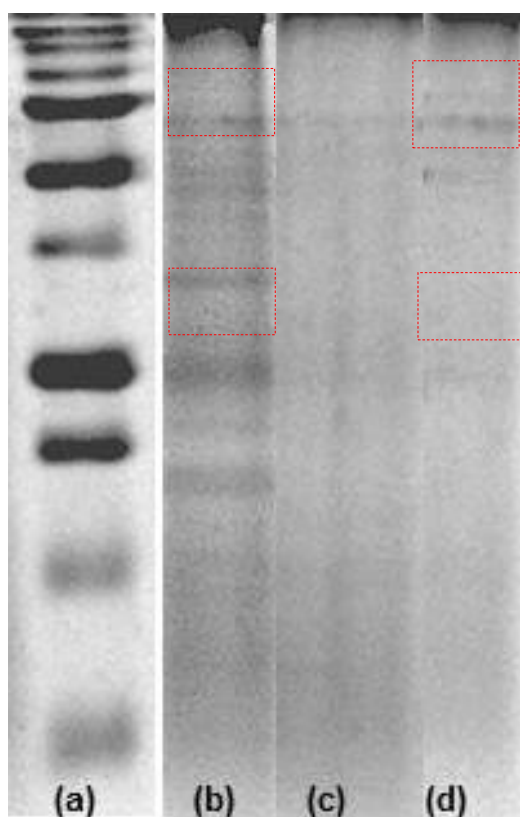


Figure 4. SDS-PAGE gel lanes showing (a) Precision plusTM protein ladder in comparison with (b) membrane bound fraction proteins, (c) membrane bound fraction treated with β -met and (d) protein fraction of size around 25KDa and 75KDa which showed strong sing for Au NP synthesis and stabilization.

This is in accordance with our recently reported study where we concluded that ionic reduction in some bacteria takes place due to certain proteins along the lipopolysaccharides/cell wall which reduces the metallic ions in its vicinity of the bacterial cell, thereby producing stable nanoparticles [25]. Subsequently, resulting nanoparticles were analysed by TEM and XRD. TEM images (Figure 5a) confirmed the presence of discrete nanoparticles in the range of approximately 50 nm. Some small nanoparticles were also visualized suggesting inherent polydispersity as generally observed in the case of biogenic synthesis. Nanoparticle size was calculated without the encasing membrane-bound proteins. It was observed that the nanoparticles obtained were highly discrete, were circular in shape and did not show aggregation with the neighbouring particles. Also, single-crystalline

structures of biogenic nanoparticles were further supported by their corresponding SAED analysis as shown in Figure 5b with characteristic {111}, {200} and {220} diffraction patterns suggesting a face-centred cube (fcc) arrangement.

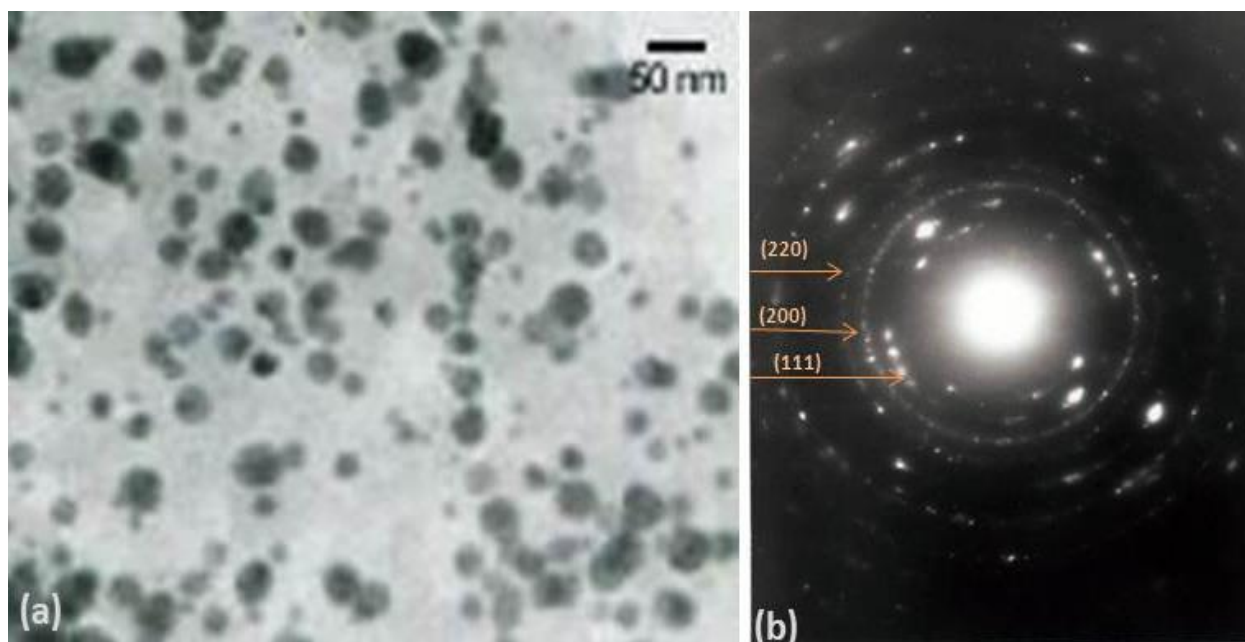


Figure 5. TEM images of biogenic Au nanoparticles after 24 h. (a) Discrete gold nanoparticles of size approximately 50 nm; (b) SAED pattern of obtained Au NPs.

Finally, confirmation of gold nanoparticles was done via XRD which confirmed the presence of synthesized gold (Figure 6). Bragg's reflections observed in the diffraction pattern could be indexed on the basis of fcc-type crystal arrangement. The strong diffraction peak at 38.21° is ascribed to the {111} facet of the fcc-metal gold structure. The other two peaks can be attributed to {200} and {220} facets at 44.19° and 64.45° , respectively. It is important to note that the ratio of intensity between {200} and {111} peaks is lower than the standard value (0.47 versus 0.53). Also, the ratio between {220} and {111} peaks is lower than the standard value (0.32 versus 0.33). These observations indicate that gold nanoplates (and not nanospheres, although both will exhibit circular plane) were formed in majority by the reduction of Au(III) by membrane-bound fraction of *E. coli* K12 and are dominated by {111}

facets. Further, most of the {111} planes parallel to the surface of the supporting substrate were sampled.

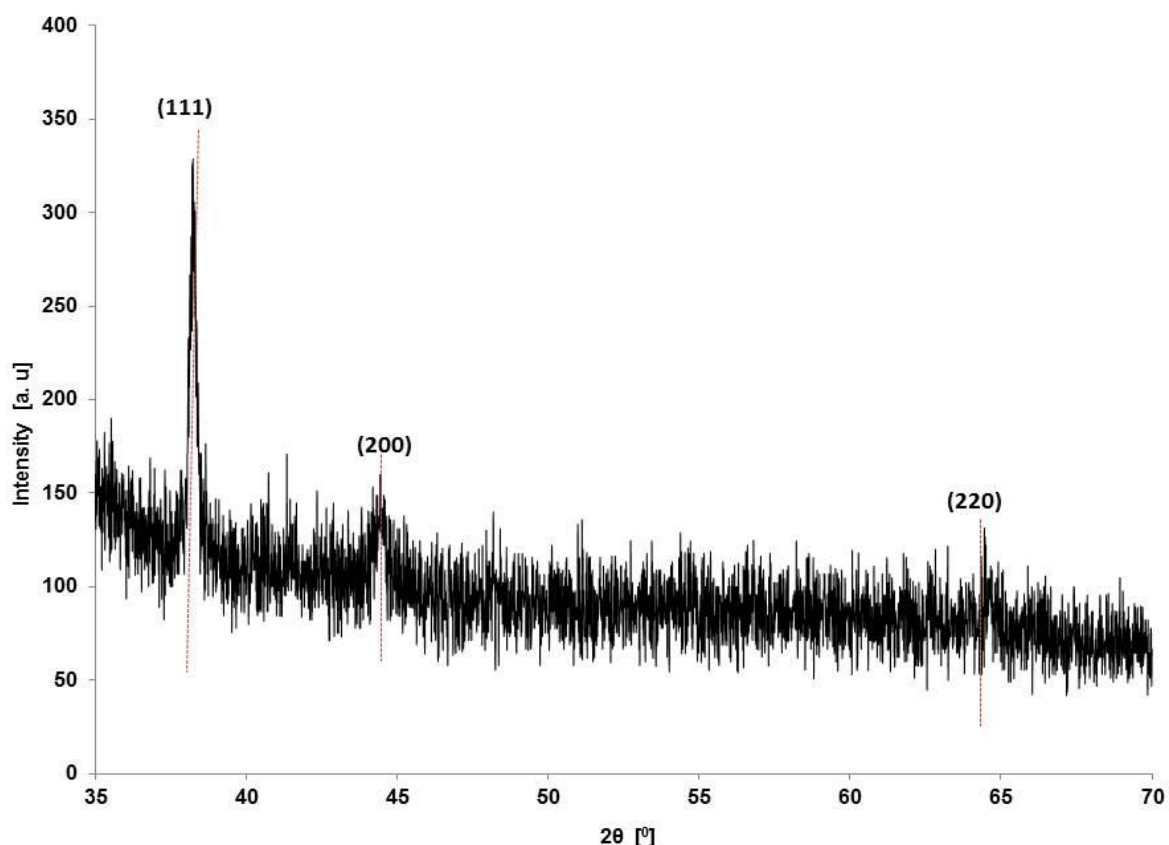


Figure 6. XRD spectra of Au⁰ as obtained by membrane-bound fraction of *E. coli* K12 cells.

Since, microbial mediated nanoparticle synthesis has several advantages over chemical methods and needs to compete for a continuous bulk-scale production process. Therefore, we tried producing biogenic Au NPs by constructing a ‘continuous reactor’ prototype where reduction is achieved by feeding gold cation solution from one end and obtaining Au nanoparticles from the other end of the reactor column. Au NP synthesis column was prepared by extracting MBF as described previously and packing it with silica in a microcolumn (Fig. 7a) in ratio 1:10 (w/w). It was expected that membrane bound proteins will cause reduction of gold ions yielding nanoparticle solution from the other end of the column. In this experiment, 10 mL of 0.01M HAuCl₄ solution was passed through a column

consisting of membrane bound fraction (MBF) of *E. coli* K12 and silica packed tightly in a column. Likewise, control reaction consisted of silica packed column only and gold cationic solution without any inoculum. It was observed that the reaction mixture showed visible color change after 30 minutes and the solution collected from column indicated presence of Au NP due to color change from pale yellow to light pink (Fig. 7b) due to SPR while no such result was observed in control reactions.

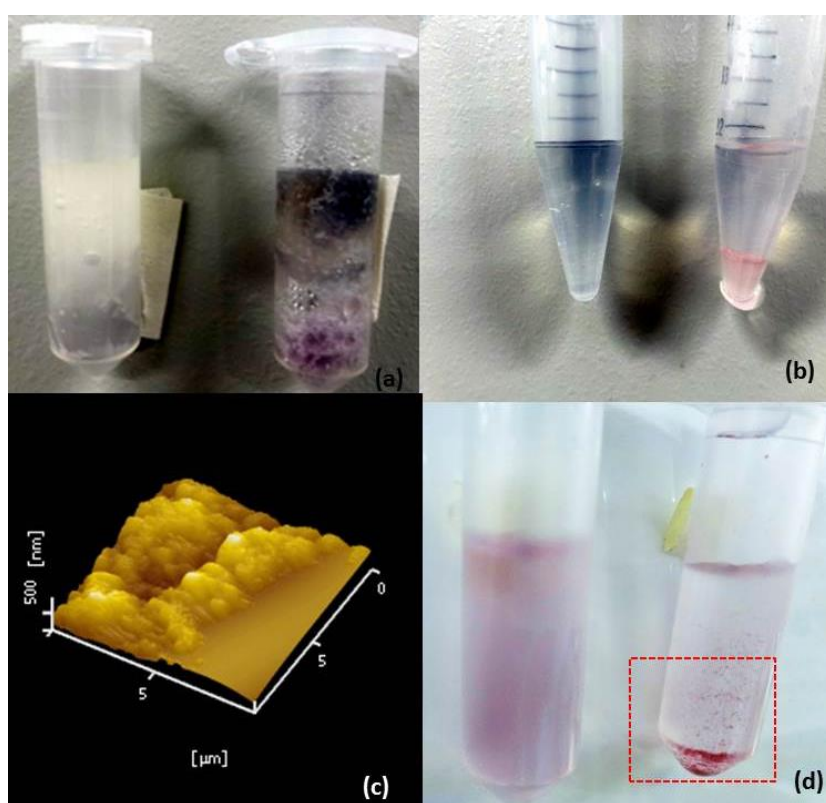


Figure 7. Incorporation of MBF of *E. coli* K12 with silica in a micro-column: (L to R) (a) silica column without MBF after passage of gold cation solution, silica column column with MBF after passage of gold cation solution; (b) visible color change in the cation solution passed through MBF bound column due to SPR by formation of Au NPs (c) AFM visualisation of Au NP produced by MBF-silica column d particle aggregation (indicated region) in MBF-silica column as compared to MBF reaction mixture.

As expected, resulting nanoparticles showed certain degree of aggregation (Fig. 7d) as observed visually since there was no incorporation of stabilizing agent to stabilize resulting nanoparticles. This was confirmed by zeta-potential analysis where mean Z-pot came out to be -0.47 mV suggesting particles are instable in solution. AFM analysis (Fig. 7c) showed greater aggregation of nanoparticles in the range of 250 - 500 nm by our crude 'biogenic-nanoparticle column reactor'. Particle size can be greatly controlled by incorporating capping agents like SDS and glycerol in collection beaker. We are currently examining column properties based on its regeneration potential, nanoparticle yield and means to obtain monodisperse NPs.

Finally, Catalytic activity of Au-MBF biocatalyst in 4-nitrophenol degradation was tested. Aqueous 4-NP shows maximum UV-vis absorbance at 317 nm [26]. When NaBH₄ (pH > 12) was added to reduce 4-NP, an intense yellow colour appeared due to formation of 4-nitrophenolate ion red-shifting the absorption peak to 400 nm [27]. The reaction does not proceed, and the peak remained for several days in the absence of Au catalyst [28, 29]. Also, no peak change was observed in the control reaction consisting of MBF only without Au NPs. Normally, -NO₂-containing aromatic compounds are inert to the reduction via NaBH₄. However, with the addition of MBF-Au NP biocatalyst, the colour faded to a colourless solution (as shown in Figure 8a) and the peak at 400 nm decreases with the appearance of the peak at 290 nm corresponding to the formation of 4-AP [30]. Au NPs present in the biocomposite helped in the transfer of electron from BH₄⁻ ions to the nitro group of 4-NP and reducing it to 4-AP, which was qualitatively monitored by UV-vis spectroscopy as shown in Figure 8b. Since the concentration of bionanocomposite catalysing the reaction was very low, measurement of the absorption spectra of 4-NP and the reduction product 4-AP was not disturbed by the light scattering due to the catalyst carrier particles in the reaction mixture. As the concentration of NaBH₄ used was much higher than that of 4-NP, it is assumed that the concentration of BH₄⁻ remains constant during the reaction, and in this context, the order of

reaction can be considered as a pseudo-first-order reaction [31]. We found good linear correlation of $\ln(A)$ and time, and the kinetic reaction rate constant under the given set of reaction conditions was estimated to be $1.24 \times 10^{-2} \text{ min}^{-1}$. However, it should be noted that the reduction rate of 4-NP can be influenced by the concentration of catalyst, size of catalyst, concentration of reactants and temperature [32]. Here, we observed that the biomass-supported catalyst proved to be a sturdy substitute for catalyst matrix as biogenic nanoparticles tend to adhere/adsorb to the biomaterial matrix because of certain active chemical groups, which in turn may impart additional stability to the biocatalyst framework.

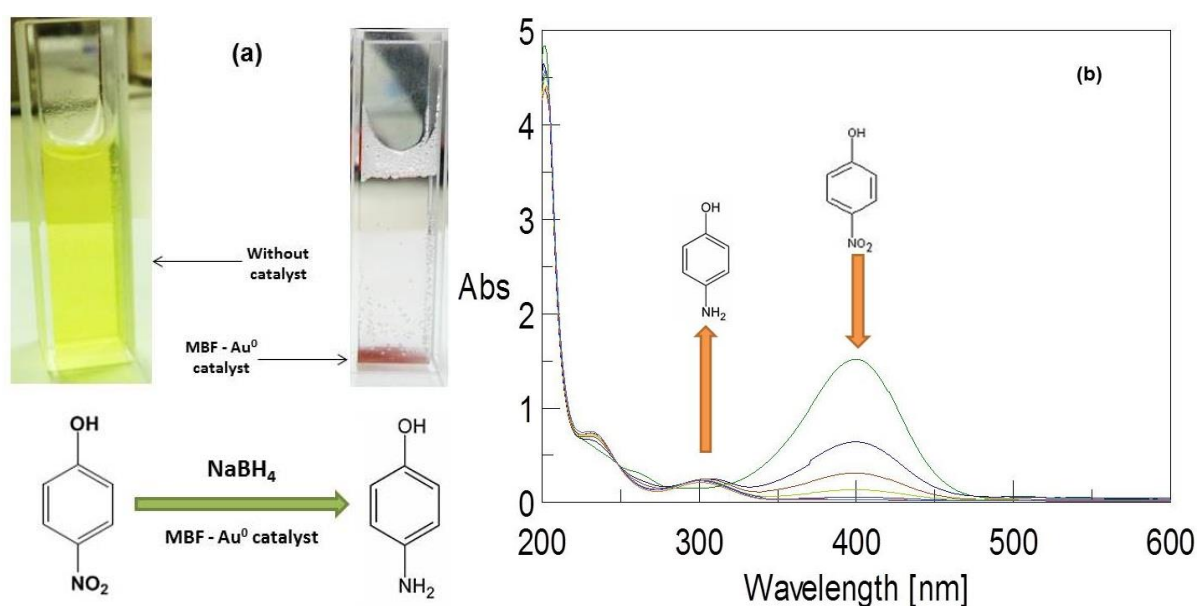


Figure 8. Degradation of 4-nitrophenol and UV-vis absorption spectra. (a) Schematic representation of degradation of 4-nitrophenol from pale yellow into colourless solution in the presence of MBF-Au⁰ heterogeneous catalyst; (b) UV-vis absorption spectra during the reduction of 4-nitrophenolate ion by Au NPs bound to MBF over a time period of 10 min.

Further, the biomass alone in the absence of Au NPs was inert to the reaction. This ‘green catalyst’ will greatly reduce the cost incurred in bioremediation with an added advantage of being a totally eco-friendly synthesis process. Although there may be a few drawbacks like polydispersity of nanoparticles which may affect the quality of nanobiocatalyst, nonetheless

considering the economic viability and facile green synthesis, this study helps in better understanding of bacteria-mediated nanoparticle synthesis and associated development of biocatalysts for the reduction of nitroaromatic pollutants.

4. Conclusion

Extracellular membrane fraction of *E. coli* K12 was found to be responsible for the biogenic synthesis of gold nanoparticles at room temperature without pH adjustment. Gold nanoplates were obtained in majority in the size range of around 50 nm. We concluded that certain proteins embedded in the membrane fraction cause formation and stabilization of Au NPs. In the absence of these proteins (activity loss by β -met treatment), no nanoparticle formation was observed. This was further supported by the MBF incorporated silica column showing continuous Au NP production. Since biogenic nanoparticles are stabilized 'naturally' in the presence of active biomass, their efficacy in the preparation of heterogeneous catalyst was also examined. We provided an innovative approach to utilize biogenic gold nanoparticles adsorbed over the cell membrane fraction to be used as a heterogeneous catalyst for catalysing complete degradation of 4-NP. A distinct advantage of this study lies in the fact that the facile green synthesis process can be seamlessly aligned with the preparation of nanobiocatalyst which may find numerous applications in catalysis, bioremediation studies, etc. This research has the potential to promote membrane fractions (proteins) for continuous synthesis of different types of NPs (see Additional file 1) and subsequent development of associated bionanocomposite resulting in improved material synthesis and application by biogenic systems.

References

- [1] G. C. Bond, D. T. Thompson, *Catal. Rev. Sci. Eng.* 1995, 41, 319.
- [2] R. Narayanan, M. A. El-Sayed, *J. Phys. Chem. B* 2005, 109, 12663.
- [3] M. C. Daniel, D. Astruc, *Chem. Rev.* 2004, 104, 293.
- [4] C. J. Murphy, T. K. Sau, A. M. Gole, C. J. G. Orendorff, J. L. Gou, S. E. Hunyadi, T. Li, *J. Phys. Chem. B* 2005, 109, 13857.
- [5] M. P. Pileni, *Nat. Mater.* 2003, 2, 145.
- [6] M. Brust, C. J. Kiely, *Coll. Surf. A* 2002, 202, 175.
- [7] R. Genc, G. Clergeaud, M. Ortiz, C. K. O'Sullivan, *Langmuir* 2011, 27, 10894.
- [8] T. Ogi, N. Saitoh, T. Nomura, Y. Konishi, *J. Nanopart. Res.* 2010, 12, 2531.
- [9] B. Nair, T. Pradeep, *Cryst. Growth Des.* 2002, 2, 293.
- [10] M. Gericke, A. Pinches *Gold Bull.* 2006, 39, 22.
- [11] S. K. Das, A. R. Das, A. K. Guha, *Langmuir* 2009, 25, 8192.
- [12] K. N. Thakkar, S. S. Mhatre, R. Y. Parikh, *Nanomed-Nanotechnol.* 2010, 6, 257.
- [13] K. B. Narayanan, N. Sakthivel, *Adv. Colloid Interface. Sci.* 2010, 156, 1.
- [14] G. Booth (2007) *Nitro Compounds, Aromatic* in *Ullmann's Encyclopedia of Industrial Chemistry*. New York: Wiley.
- [15] R. P. Pohanish (2011) *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens*. Elsevier, Amsterdam.
- [16] M. Haruta, *ChemInform.* 1997, 28, 153.
- [17] K. Deplanche, M. L. Merroun, M. Casadesus, D. T. Tran, I. P. Mikheenko, J. A. Bennett, J. Zhu, I. P. Jones, G. A. Attard, J. Wood, S. Selenska-Pobell, L. E. Macaskie, *J. R. Soc. Interface* 2012, 9, 1705.
- [18] M. Pazirandeh, B. M. Wells, R. L. Ryan, *Appl. Environ. Microbiol.* 1998, 64, 4068.

- [19] D. F. Ackerley, Y. Barak , S. V. Lynch, J. Curtin, A. Matin, J. Bacteriol. 2006, 188, 3371.
- [20] K. B. Narayanan, N. Sakthivel, J. Hazard. Mater. 2011, 189, 519.
- [21] S. Link, M. A. El-Sayed, Int. Rev. Phys. Chem. 2000, 19, 409.
- [22] S. Basu, S. Panigrahi, S. Praharaj , S. K. Ghosh, S. Pande, S. Jana, T. Pal, New J. Chem. 2006, 30,1333.
- [23] A. Gole, C. Dash, V. Ramachandran, A. B. Mandale, S. R. Sainkar, A. B. Mandale, M. Rao, M. Sastry, Langmuir 2001, 17, 1674.
- [24] S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang, N. Gu, Mater. Lett. 2007, 61, 3984.
- [25] S. K. Srivastava, M. Constanti, J. Nanopart. Res. 2012, 14, 831.
- [26] N. Pradhan, A. Pal, T. Pal, Langmuir 2001, 17, 1800.
- [27] S. K. Ghosh, M. Mandal, S. Kundu, S. Nath , T. Pal, Appl. Catala-Gen. 2004, 268, 61.
- [28] K. Hayakawa, T. Yoshimura, K. Esumi, Langmuir 2003, 19, 5517.
- [29] Y. Lu, Y. Mei, M. Ballauff , J. Phys. Chem. B 2006, 110,3930.
- [30] N. Pradhan, A. Pal, T. Pal, Coll. Surf. A 2002, 196, 247.
- [31] R. W. J. Scott, O. M. Wilson, R. M. Crooks, J. Phys. Chem. B 2005, 109,692.
- [32] S. Panigrahi, S. Basu, S. Praharaj , S. Pande, S. Jana, A. Pal, S. K. Ghosh, T. Pal J. Phys. Chem. C 2007, 111, 4596.

Chapter 3.

Green synthesis of Au, Pd
and Au@Pd core-shell
nanoparticles via
tryptophan induced
supramolecular interface.

Chapter 3.

Green synthesis of Au, Pd and Au@Pd core-shell nanoparticles via tryptophan induced supramolecular interface.

1. Introduction

Bimetallic nanoparticles have gained importance because of their composition dependent optical, catalytic and magnetic properties which are different as compared to their monometallic counterparts [1]. Au@Pd bimetallic nanoparticles with core-shell morphology in particular have proved to be an excellent catalyst for a variety of reactions including oxidation reactions which are of importance in field of fuel cells [2], semiconductors [3], drug delivery [4] and so on. Au@Pd bimetallic nanoparticles with core-shell morphology in particular have proved to be excellent catalysts for a variety of reactions including oxidation reactions which are of importance in field of fuel cells, semiconductors, drug delivery and so on. These nanoparticles upon being adhered to a matrix, may serves as a better catalyst than bulk metal due to greater accessibility to surface atoms and low coordination number especially in case of water treatment. Among several water pollutants, nitroaromatic compounds are considered as the most toxic and refractory pollutants whose permissible range is as low as 1 to 20 ppb. However, these are common in production of dyes, explosives, pesticides among many others and thus their industrial production is considered as an environmental hazard. Upon being released into the environment, these nitrophenols pose significant public health issues by exhibiting carcinogenic and mutagenic potential in humans. Thus, there is a need to develop cost-effective methods for removing nitroaromatic pollutants from water by utilizing these catalytic nanoparticles.

A number of strategic routes have been proposed for synthesis of core-shell structures including Toshima's sacrificial hydrogen method [5], surface-specific reductants [6], galvanic displacements [7], controlled simultaneous reduction [8] and exploitation of core/shell preferences through annealing [9]. However, most of these methods require stringent process control, expensive instruments and usage of several toxic solvents etc. Therefore, there is an urgent need to produce these valuable bimetallic structures in a more sustainable and eco-friendly manner.

Biogenic synthesis of nanomaterials has recently gained a lot of importance by utilizing naturally occurring compounds as the basis for new material synthesis. We have demonstrated a range of biogenic materials in form of monometallic nanoparticle synthesis [10] to preparation of heterogeneous catalyst via bacteria [11] covering an array of nanoparticles including Au, Ag, Pd, Fe, Rh, Ni, Ru, Pt, Co, and Li. These bio-inspired materials have advanced chemical properties with inherent potential to support a variety of novel technologies. Recently, Huang et al. [12] reported Au@Pd core-shell nanoparticles via plant tannin as reductant and stabilizer. Although, biogenic nanoparticle synthesis is still in its infancy, these studies among others clearly demonstrated that multiple complex reactions and subsequent stabilization especially in case of bimetallic nanoparticles [13, 14, 15] can be achieved by biological entities.

However, it should be noted that while alloyed nanoparticles are relatively easy to produce, discreet stable core-shell structures via biogenic means still remains a challenge. This can be understood because one of the key characteristics of biogenic synthesis is the 'cumulative effect' of active reaction mixture generally consisting of large complex molecules/compounds which may be difficult to isolate/purify. This remains a major drawback in most the biogenic processes limiting their use in large scale production. Therefore, there is a need to target smaller active chemical groups present in the 'biogenic broth' in order to assist biogenic studies evolve towards biomimetic technologies.

Amino compounds including amino acids promises to be among the potential biosynthesis agent which have been tested as effective reducing agents for the preparation of monometallic nanoparticles [16, 17]. These amino acids in aqueous environment exhibit their characteristic zwitter ion structure which has the potential to impart additional supramolecular functionality and stability for bimetallic nanoparticles. This amino group mediated supramolecular functionality is important in synthesis and stabilization of core-shell structures as demonstrated by Serpell et al.[18] by imparting anion distribution via imidazole-amide linkages. Tryptophan is one such interesting amino acid which has unique chemical properties of reducing and stabilizing metallic nanoparticles. Several studies showed that tryptophan plays an important role in reduction of monometallic nanoparticles (Au) via oxidation of amino acid and forms poly-Trp which caps and stabilizes the resulting nanoparticles [19, 20].

In this study, we demonstrate for the first time, biosynthesis of Au@Pd bimetallic nanoparticles via tryptophan in aqueous reaction mixture at room temperature. Gold seeds were produced by incorporating tryptophan in the reaction mixture followed by successive reduction of Pd over the gold nanoparticle surface. To confirm the nature of nanoparticles and responsible chemical groups, various characterization techniques including UV-Vis spectroscopy, transmission electron microscopy (TEM), aberration-corrected high angle annular dark field scanning transmission electron microscopy (AC-HAADF-STEM), energy dispersive spectrometry (STEM-EDS), Zeta-potential analysis, X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) were employed. These analyses suggested formation of Au@Pd core-shell type bimetallic nanoparticles in the aqueous reaction mixture due to anion coordination functionality imparted by poly-Trp layer around the seed Au nanoparticles. The use of Trp as reducing and stabilizing agent to obtain bimetallic nanoparticles with core-shell morphology promises facile, cost-effective, one-pot green synthesis of multimetallic nanoparticles in future.

2. Experimental

2.1 Synthesis of Au, Pd and Au@Pd nanoparticles

Chloroauric acid (HAuCl_4) and Palladium chloride (PdCl_2) were obtained from Aldrich (Tokyo, Japan) and were used directly without pre-treatment. L-Tryptophan was obtained from Nacalai tesque (Kyoto, Japan).

Ultrapure water (resistivity $\sim 18.2 \text{ M}\Omega\cdot\text{cm}$) was used as a solvent throughout the experiments. Well-mixed gold cation stock solution at 0.5 mM (10 mL) and Palladium cation stock solution (0.5 mM, 10mL) was prepared. To prepare Pd (II) ionic solution, pre-weighed PdCl_2 was introduced in 5mL of 5mM HCl followed by volume make-up by addition of ultrapure water. Trp aqueous stock solution was prepared at a concentration of 0.5 mM (20 mL). Cation solutions consisting of Au:Pd was used in the volumetric ratio of 1:1.

Gold nanoparticle solution was prepared by spontaneous reduction of 10 mL gold cation solution in presence of 2 mL of Trp solution with vigorous stirring for 2 hours followed by intermittent sonication for 30 seconds at every 15 min. Au NPs prepared at this stage served as a seed (core) for deposition of Pd (shell) along the surface of Au NPs. It should be noted that the size of core-shell structures can be controlled by controlling the size of Au NPs at seeding stage. For Au@Pd bimetallic nanoparticles, palladium precursor solution (5 mL) was introduced with gold NP seed solution (5mL) along with 1 mL of Trp solution. The resulting mixture was stirred for 2 hours followed by intermittent sonication for 10 seconds at every 30 minutes. The Pd NP solution was prepared in the same way as mentioned above for Au NP seed synthesis. Therefore, three distinct types of nanoparticles were synthesized namely Au NPs, Pd NPs and Au@Pd NPs.

2.2 Characterization of Au, Pd and Au@Pd nanoparticles

UV-visible spectra were obtained using a JASCO V670 UV-vis Spectrophotometer.

Nanoparticle formation was monitored from 400-800 nm at 2000nm/min scan rate. This was done as the focus of this study was in the visible region (LSPR) which provided vital details about Au@Pd bimetallic nanoparticle formation. Morphology and grain size analysis of these nanoparticles (Au, Pd, Au@Pd NPs) was carried out using a JEOL JEM-2010 transmission electron microscope (TEM) operating at an acceleration voltage of 200 kV. One micro-litre was taken from the reaction mixtures and placed on carbon coated copper grids and dried at room temperature. The images were analysed using ImageJ 1.43M software. Individual Au@Pd nanoparticles were further analysed by aberration-corrected STEM-HAADF microscopy (JEOL 2100F, 200 kV, ~0.1 nm point resolution) to observe the precise structure of resulting core-shell nanoparticles. Observed nanoparticles were also analysed by JED-2300T STEM-EDS for obtaining composition architecture.

X-ray diffraction (XRD) patterns were obtained using a Rigaku RINT-TTR diffractometer equipped with a parallel beam collimation and a vertical θ - θ goniometer. Samples were placed directly on sample holder. The X-Ray diffractometer was operated at 50 kV and 300 mA to generate $\text{CuK}\alpha$ radiation. The scan rate was set to 5° min^{-1} .

Zeta-potential measurements were performed using a Malvern Zetasizer Nano ZS model ZEN3600 (Worcestershire, UK) equipped with a standard 633 nm laser.

Surface analysis for Au@Pd bimetallic NPs was also carried out by X-ray photoelectron spectroscopy (XPS) obtained from JEOL JPS9010 MC photoelectron spectrometer operating at 10 kV and 30 mA to generate $(\text{Al})\text{K}\alpha$ radiation. The results obtained were analysed by SpecSurf ver. 1.7.3.9 software.

2.3 Preparation of nanoparticle based catalyst in degradation of 4-Nitrophenol

The reduction of 4-NP by NaBH_4 was studied as a model reaction to probe catalytic efficiency of Au, Pd and Au@Pd catalyst for heterogeneous systems. Under the experimental condition, reduction does not proceed at all simply with addition of NaBH_4 or biomass alone. But in presence of catalyst coated glass beads, it proceeds to completion with formation of 4-Aminophenol (4-AP). To study the reaction in a quartz cuvette, 2.77 mL water was mixed with 30 μL (10^{-2} M) 4-NP solution and 200 μL of freshly prepared NaBH_4 (10^{-1} M) was added. Catalyst coated glass beads were prepared by modified protocol of Shen et al. (Langmuir 2012, 28, 7519). Porous glass beads were prepared by autoclaving the glass beads (2 mg) in water (100 mL) at 121 °C for 2 hours. Afterward, the reactor was cooled to room temperature through natural cooling. Eventually, the porous glass beads were and washed several times with deionized water before further use. A total of 20 mg of these glass beads was added to Au, Pd and Au@Pd nanoparticle solution (5 mL) stated previously. The nanoparticle loading of around 2×10^{-3} g/L was introduced into the reaction cuvette. The mixtures were stirred for 12 h at 60° C to evaporate the solvent (water). These nanoparticle coated glass beads were then dried at 90°C for 24 hours. UV-Vis spectra of the 4-NP sample were recorded at every 2 min interval in the range of 200-600 nm. The percentage degradation of 4-NP was determined by measuring the change in absorbance of the initially observed peak at 400 nm, for the nitrophenolate ion as the function of time.

3. Results and discussion

Of the two general approaches for synthesizing bimetallic nanoparticles, successive reduction approach normally gives better control over the shape and atomic ratio as compared to simultaneous approach especially with respect to core-shell structure. For the same reason, we utilized successive reduction approach to yield Au@Pd bimetallic NPs with core-shell structures. The synthesis strategy used in this work has three steps as hypothesized in Figure 1.

Firstly, Au core nanoparticles were formed and got stabilized via poly-Trp layer due to indole group H-bonded supramolecular functionality [21, 22]. Next, the shell metal (Pd II) was introduced in the aqueous reaction mixture in its cationic state which got associated with the core ligands (via Trp).

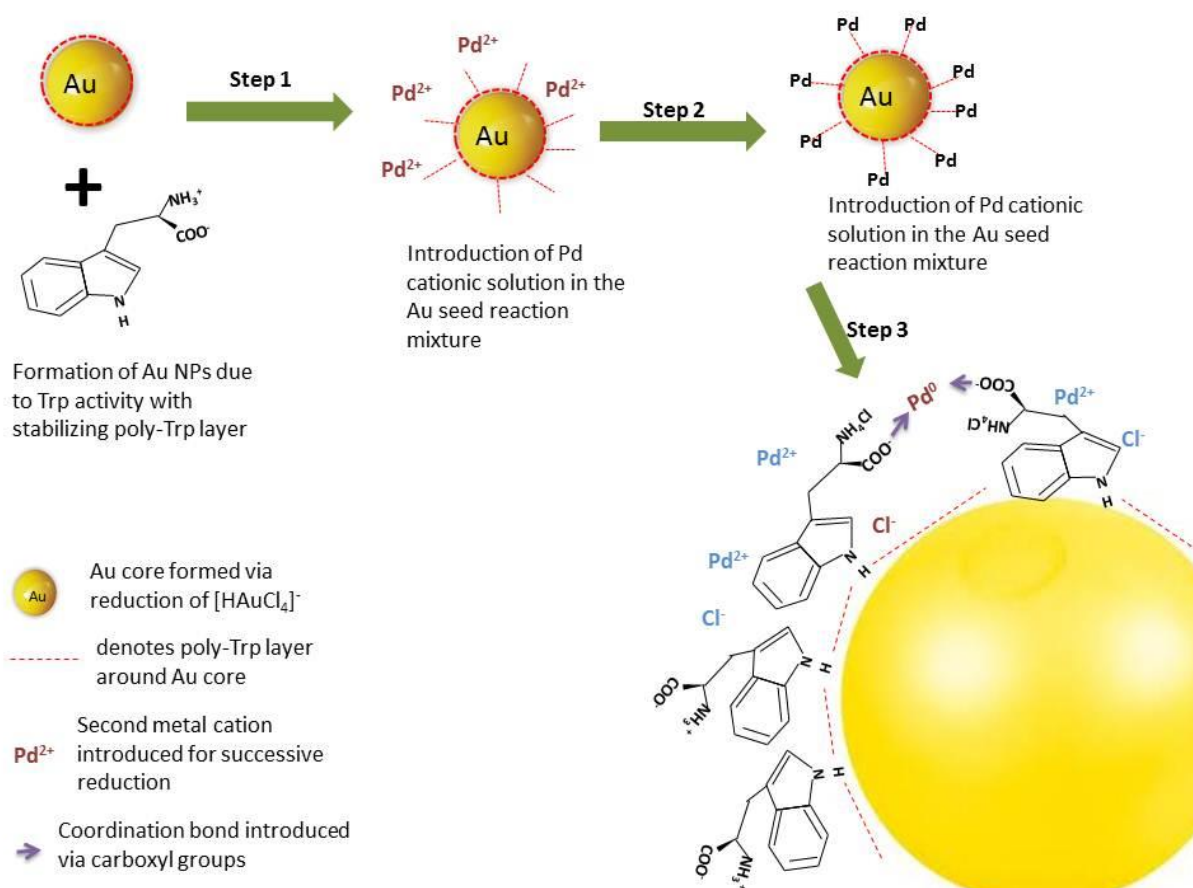


Figure 1. Schematic representation of anion coordination synthetic strategy for Au@Pd nanoparticles.

This results in sequestering of Pd (II) onto the surface of the Au core via active carboxyl groups. Finally, the surface bound Pd (II) gets reduced over the Au core, yielding core-shell structures. Figure 2 show UV-vis absorbance spectra of Trp synthesized Au@Pd nanoparticles and reaction mixtures of monometallic Au and Pd nanoparticles. The color change between the three reaction mixtures was visually distinguishable after the completion of reaction. Gold nanoparticles can be seen with distinct pink color, while Au@Pd NP solution turned black-brown followed by the orange-brown color of Pd NP solution (inset Figure 2).

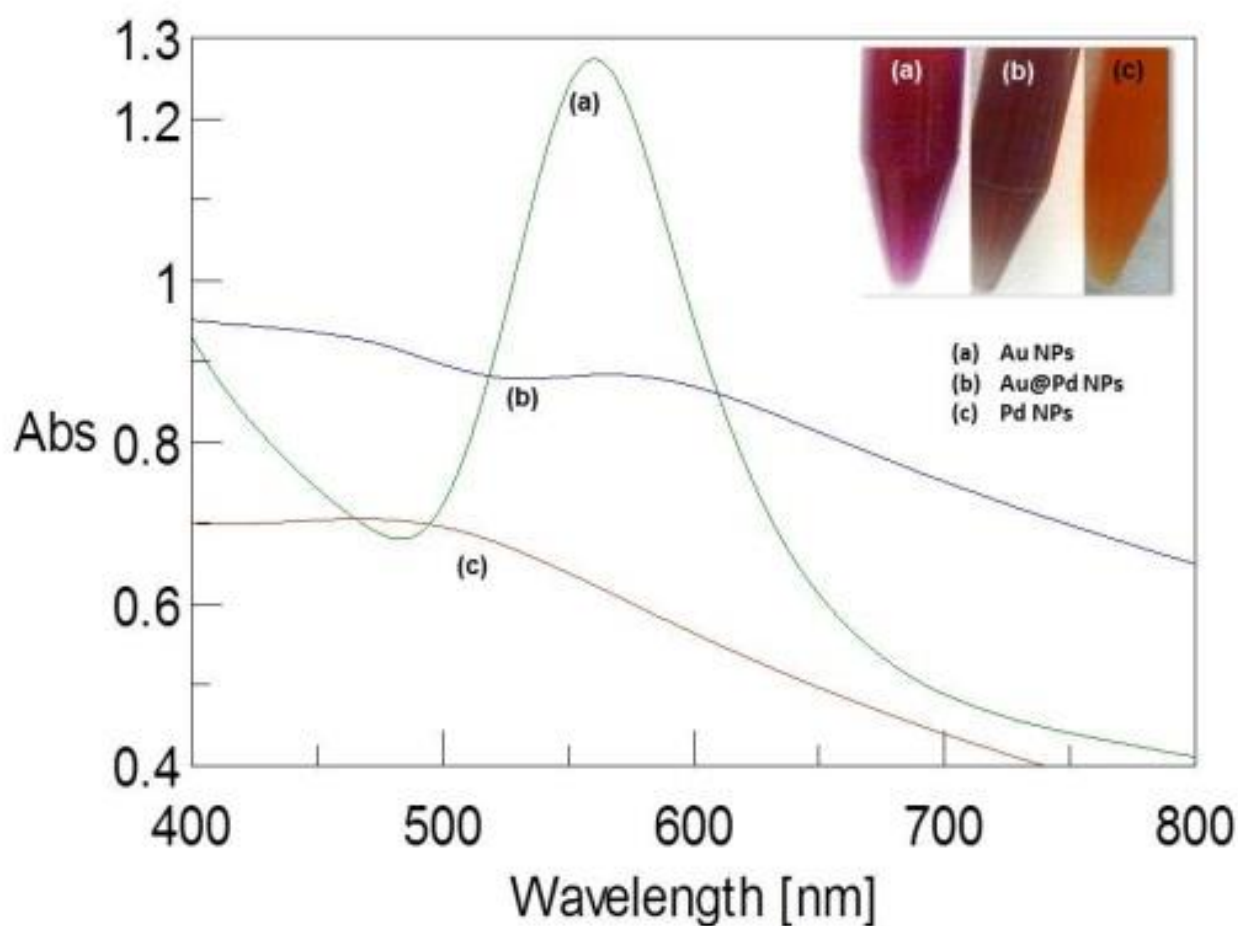


Figure 2. Visible spectra of Au, Pd and Au@Pd nanoparticles. Inset image depicting observed color in each of the above mentioned reaction mixture.

These results also suggest that the bimetallic nanoparticle (Au@Pd) mixtures present in the reaction solution are not phase separated when compared with monometallic NPs. Au NPs showed characteristic localized surface plasmon resonance (LSPR) peak centered at 550 nm. The LSPR peak was drastically reduced in case of Au@Pd bimetallic NPs and completely absent in case of Pd NP reaction mixture after the completion of reaction. This spectral behaviour reasonably indicates towards the core-shell structure. In such closely associated structures (like core-shell), the surface electron density in the Au core increases at the expense of electrons from the outer Pd shell. This happens because Pd has a higher electron chemical potential (5.32 eV vs. 5.00 eV). Thus, a thicker Pd shell coating on the Au core results in the gradual damping of the plasmon of Au core [23]. To further confirm the interaction between Au NP surface and subsequent Pd deposition, UV-vis spectroscopy was carried out (400-800 nm) to observe the coating of Pd over Au core as a function of time as shown in Figure 3.

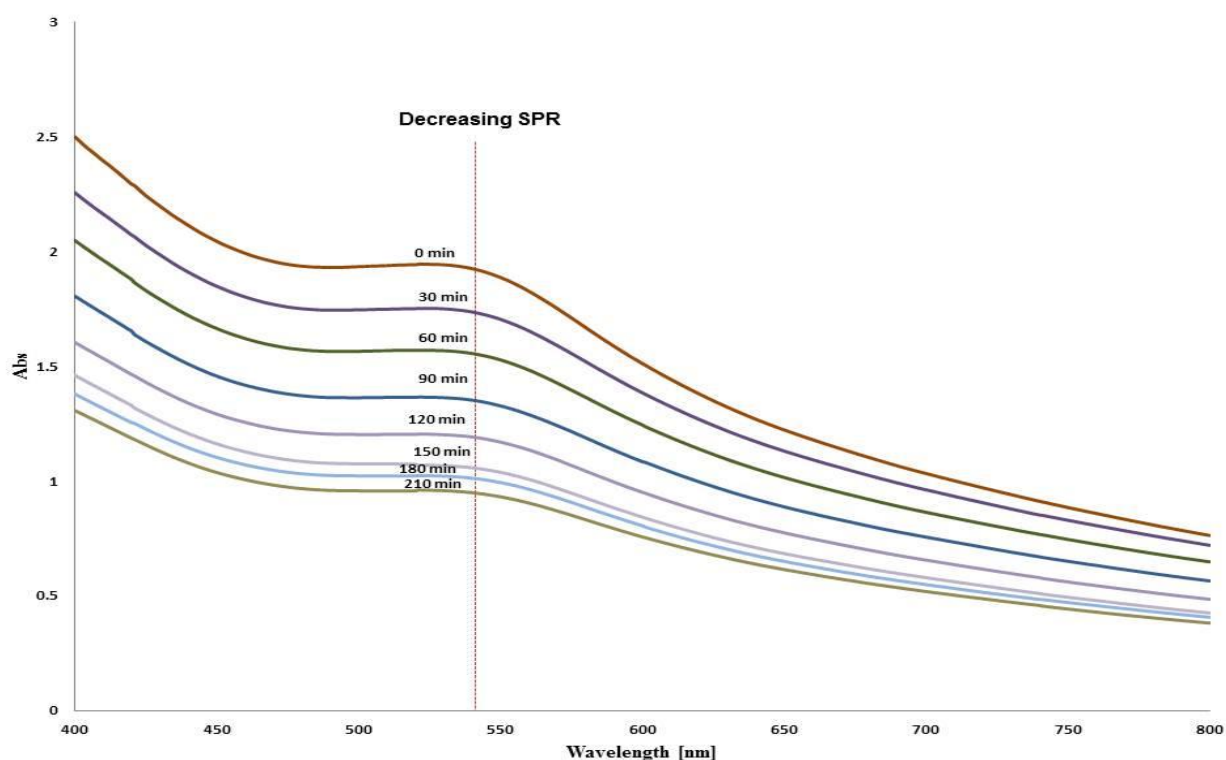


Figure 3. Visible spectra showing formation of Pd layer over the synthesized Au core. Each line denotes an interval of 30 min.

Each successive spectrum was taken at a 30 minute interval up to three hours. This also confirmed that the reaction (deposition of Pd over Au) nearly reached to completion after 3 hours. The gradual decrease in LSPR can be easily observed at around 550 nm as the reaction proceeds to completion. This also suggests that the Pd shell thickness can be controlled by varying reaction time while keeping the other parameters constant. Overall, UV-vis analysis strongly suggested towards the chemical interaction between the Au nanoseed surface and subsequent coating deposition of palladium. To confirm and characterize the formation of Au@Pd bimetallic nanoparticles with core shell morphology in the solution, we carried out TEM followed by high resolution STEM and EDS. TEM imaging (Figure 4) confirmed truncated octahedral type Au NPs of size around 50 nm in the reaction mixture as shown in Figure 4a.

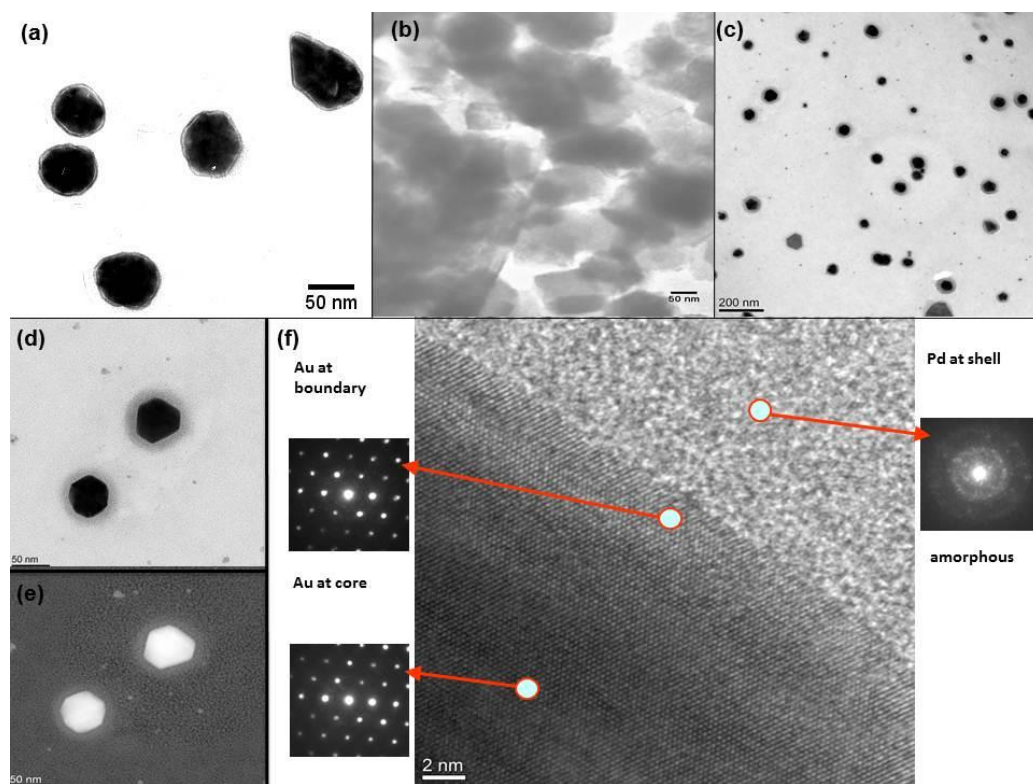


Figure 4. Showing TEM images of (a) Au NPs, (b) Pd NPs, (c) Au@Pd NP population, (d) individual Au@Pd NPs (highlighted in fig 4c), (e) AC-STEM-HAADF imaging of individual Au@Pd NPs, (f) STEM imaging of individual Au@Pd NP boundary layer.

Observed Au NPs were highly dispersed over the sample grid as Trp has already been proved to be an efficient reducing and capping agent for Au NPs. However, unlike Au NPs, Pd NPs lacked shape control and showed greater degree of aggregation as shown in Figure 4b. TEM analysis of Au@Pd bimetallic nanoparticles population showed well dispersed Au@Pd bimetallic nanoparticles. Fig 4e shows individual Au@Pd NPs (as highlighted in fig 4 c) where the dense Au core can be clearly seen surrounded by the Pd layer. We observed Au@Pd NPs in the range of ~60 nm where Au core size was about ~50 nm and Pd shell thickness in the range of 5-10 nm.

Aberration-corrected high-angle annular dark-field scanning TEM (AC-HAADF-STEM) was used to confirm core-shell morphology of the observed Au@Pd NPs. HAADF-STEM imaging (fig 4 e) displayed a brighter gold core due to its heavier atomic weight while palladium shell appeared comparatively less bright due to its low atomic weight. Highly discreet Au@Pd boundary layer was also observed (fig 4f) where the Au core in the center showed characteristic Au (111) diffraction pattern. Also, in the absence of any distinct diffraction pattern, Pd shell was rather amorphous in nature. Thus, Au@Pd NPs observed in this study largely comprises of crystalline Au core with largely amorphous Pd shell. This was further confirmed with subsequent XRD analysis of Au, Pd and Au@Pd NPs as shown in Figure 5.

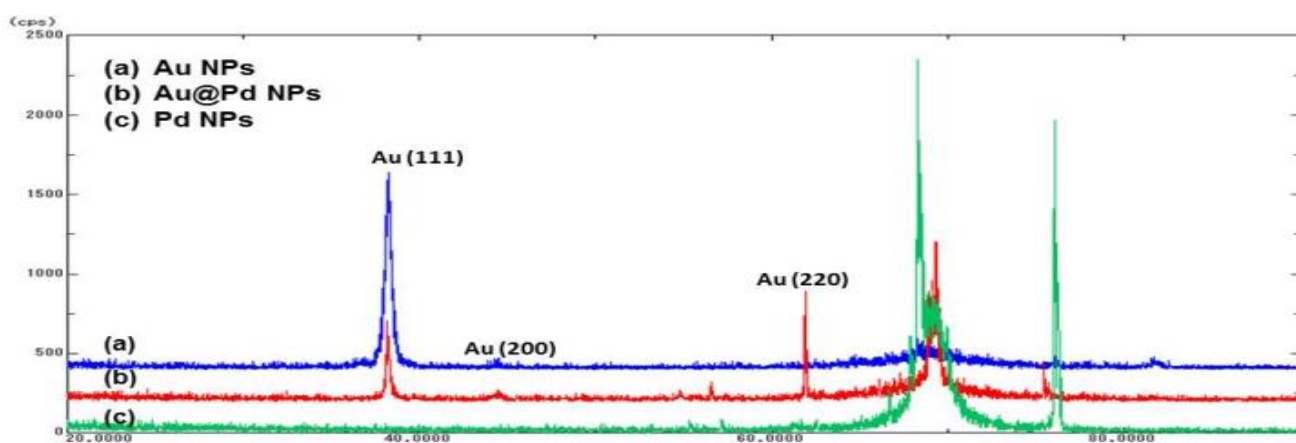


Figure 5. Showing XRD pattern of (a) Au NPs, (b) Au@Pd NPs, (c) Pd NPs.

As observed from XRD, the truncated octahedron Au NP seeds (Figure 5 a) showed a very strong Bragg's diffraction at (111) at 38.05° followed by a very weak diffraction at 44.57° which can be assigned to the (200) crystal plane. In case of Au@Pd NPs (Figure 5b), a distinct peak was observed at 68.19° suggesting Au (220) crystal re-arrangement normally observed in core-shell structures. Finally, no credible peaks can be assigned to Pd, in the case of Pd NPs (Figure 5c) and Au@Pd NPs confirming that the majority of reduced palladium was amorphous in nature. This can be understood as the observed reductive reaction under the supramolecular effect of Trp is rather quick when Pd (II) ions are reduced to Pd atoms. Hence, the resulting Pd atoms are produced rapidly at room temperature where Pd atoms stick onto particles without relaxation/surface tension to balance sites thereby producing a disordered structure i.e. the amorphous state of palladium [24]. STEM-EDS elemental mapping was used to verify the element distribution over the observed Au@Pd NPs (fig 6).

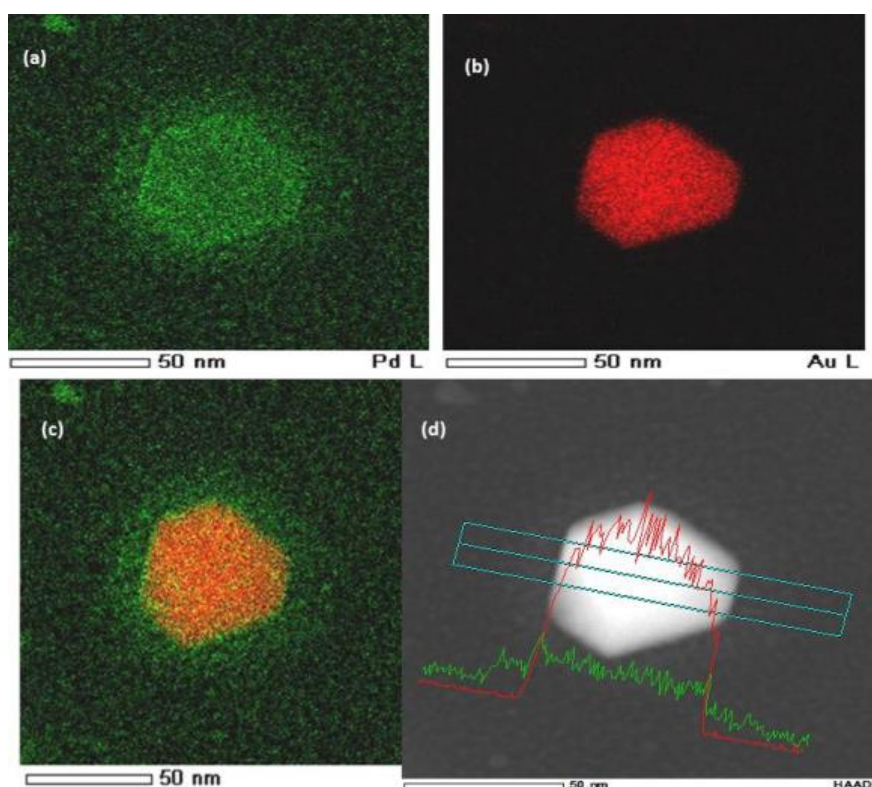


Figure 6. STEM-EDX elemental mapping of (a) Pd, (b) Au, (c) Au@Pd NP (overlay), (d) line profile of resulting Au@Pd NP.

Colored elemental mapping images showed that Au atoms (red, fig 6b) and Pd atoms (green, fig 6a) were discreetly distributed with dense Au core at the centre and Pd at the boundary giving rise to characteristic core-shell structure (fig 6c). EDS elemental line scanning (fig. 6d) on single particles confirmed that the Au@Pd NPs (~60 nm) were composed of distinct Au rich centre and Pd rich boundary responsible for typical core-shell (not alloyed) morphology. This analysis reveals the presence of both Au and Pd in the same nanoparticle. As the concentration of Au is higher in the NP under observation, the signal of Au is more intense than that of the Pd. However, careful observation also reveals that the signal of Pd is slightly broader than that of the Au. This indicates the existence of Pd as shell and Au as core. It must be noted that the interaction profile between Au and Pd largely depends on the particle shape and order of the two metallic species. Similar HAADF-STEM results for Au@Pd NPs were also reported by Han et al. [25].

These Au@Pd nanoparticles exhibiting core-shell morphology were further subjected to XPS analysis (fig 7) to confirm the metallic nature of Au and Pd along with the responsible chemical groups present in the reaction mixture. Metallic shell of Pd was confirmed (fig 7b) by characteristic Pd 3d 5/2 and Pd 3d 3/2 peaks at 340.18 eV and 334.73 eV respectively. Au core was denoted at 89.7 eV and 84.3 eV as observed in fig 7d.

Since Trp was the only active chemical agent used in this reaction, interesting observation was made when C1s and N1s (fig 7 a, c) XPS spectra of Au@Pd were observed (amino and carboxyl ends).

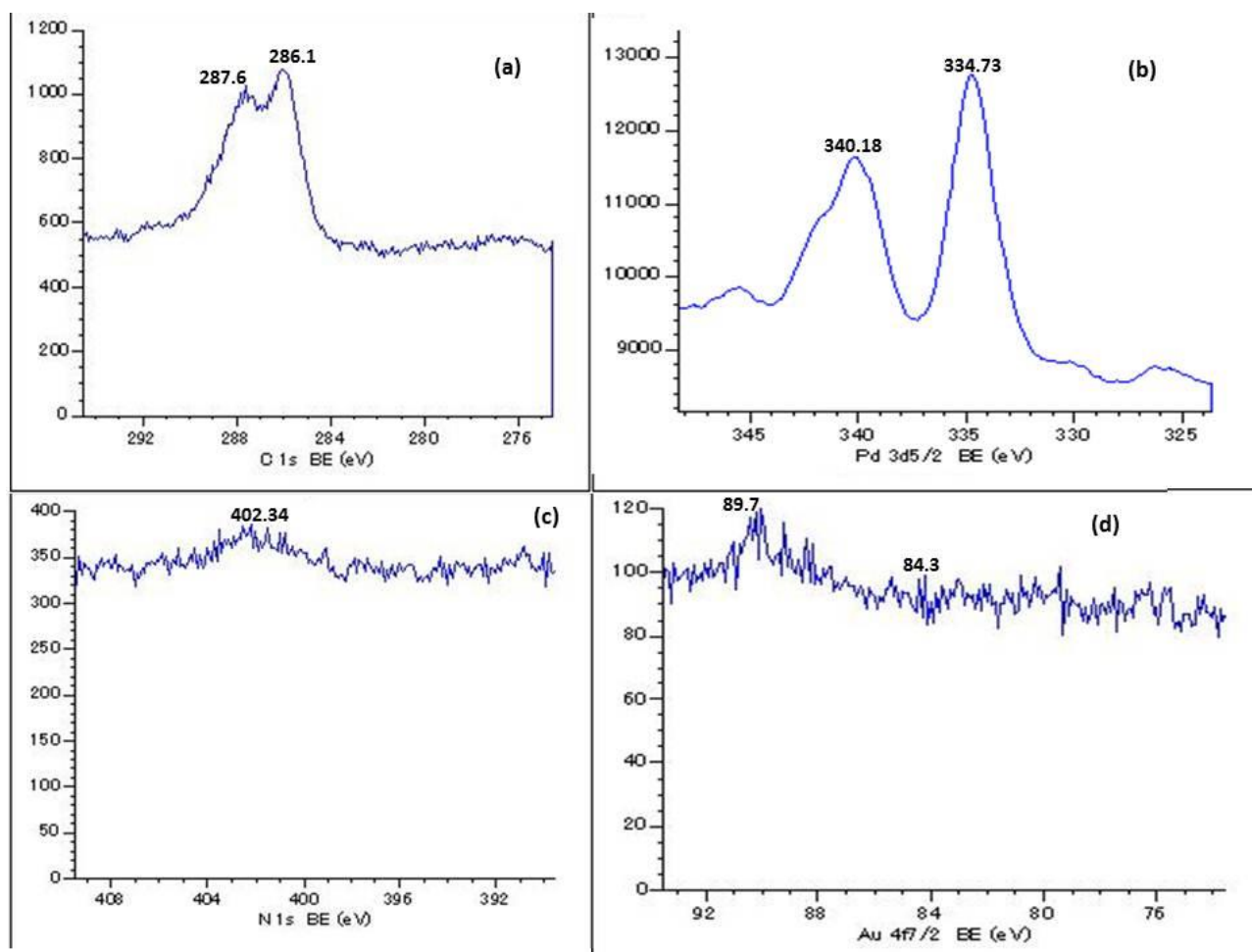


Figure 7. XPS spectra of Au@Pd NPs exhibiting following regions as (a) C 1s (b) Pd 3d (c) N 1s and (d) Au 4f.

C1s spectra doublet showed the presence of carboxyl group at 286.05 eV along with probable presence of amine carboxyl (NH-C=O) peak at 287.6 eV. Further, N1s spectra showed remarkable presence of NH₄Cl (402.3 eV) in the reaction mixture.

Based on the reduction potential of Au (III) and Pd (II) (AuCl₄⁻/Au, +1.002 vs. SHE (standard hydrogen electrode) and Pd²⁺ (PdCl₂²⁻/Pd, +0.915 V vs. SHE), we can easily expect that the Au (III) will be preferentially reduced over the Pd (II) under our experimental reaction conditions [26].

In another study by Lee et al. [27] synthesis of Au@Pd core-shell nanooctahedrons by utilizing CTAC as both reducing and stabilizing agent was reported. Similarly, in our reaction mixture, Trp plays a key role in reduction and subsequent stabilization of core-shell structure. The presence of indole functional group together with amine and carboxyl group is the important structural characteristic of the aromatic amino acid residue tryptophan. Studies have shown that the salt-bridge structure involving the COOH group appears to be energetically more favourable than the two comparably interacting indole and NH_2 group [28]. This leaves the indole group free to stabilize the resulting Au NP via indole polymerization (poly-Trp) obtained by the hydrogen linking between the N-group of interacting indole groups and followed by the deposition of palladium over the Au NP surface in the presence of NH_3^+ and COO^- zwitterion state.

Z-pot analysis of all three NP reaction mixtures was carried out to understand the relative stability of resulting nanoparticles in aqueous solution. Au NPs synthesized via Trp showed high stability in aqueous reaction mixture with average Z-pot as +18.8 eV clearly due to the presence indole polymerized surface granting extra stability to Au NPs along with exposed amino groups (NH_3^+) amino group. Therefore, in order to achieve Pd deposition over the Au surface, chemical interactions involving free NH_3^+ of amino group with PdCl_4^- over the indole polymerized surface should result in decreased Z-potential (as observed by XPS by formation of NH_4Cl). The resulting Au@Pd NPs demonstrated mean zeta potential as -2.26 eV suggesting that the resulting bimetallic nanoparticles with core-shell morphology nanoparticles are somewhat stable in the aqueous reaction mixture. This decrease in positive charge can be attributed to quenching of free amino groups present along the indole group stabilized Au NP surface. Pd NPs showed a mean Z-pot as -0.07 eV suggesting greater particle aggregations in the reaction mixture as observed earlier by TEM.

We believe that the temporal separation of the formation of Au octahedron from the formation of Pd layer owing to the presence of capping properties of Trp and associated

coordination supramolecular chemistry is the key to the formation of core-shell structure. The new structural arrangement in Au@Pd NPs confirmed the core-shell nature of the resulting nanoparticles. The novel green process promises Trp as a potential agent for synthesis and stabilization of bimetallic nanoparticles with core-shell morphology (like Au@Pd NPs). Finally, catalytic efficiency of the synthesized Au, Pd and Au@Pd NPs were tested by the degradation of 4-nitrophenol. Finally, catalytic activity of Au, Pd and Au@Pd NPs were tested by oxidation of 4-Nitrophenol. Both Au and Pd NPs have found tremendous catalytic usage as described previously but not Au@Pd bimetallic NPs in degradation of nitroaromatic pollutants. Aqueous 4-NP shows maximum UV-Vis absorbance at 317 nm. When NaBH₄ (pH>12) was added to reduce 4-NP, an intense yellow color appeared due to formation of 4-nitrophenolate ion red-shifting the absorption peak to 400 nm. The reaction does not proceed and the peak remained for several days in absence of catalyst. Normally, -NO₂ containing aromatic compounds are inert to the reduction of NaBH₄. But, with the addition of glass bead coated nanoparticles, the color faded to a colourless solution and the peak at 400 nm decreases with the extent of reaction time. Au, Pd and Au@Pd NPs coated over the glass beads helped in transfer of electron from BH₄⁻ ions to nitro group of 4-NP and reducing it to 4-AP, which was qualitatively monitored by UV-Vis spectroscopy as shown in Figure 6b. Since, the concentration of catalyst coated glass beads catalysing the reaction was very low, measurement of the absorption spectra of 4-NP and its subsequent reduction into 4-AP, was not disturbed by the light scattering due to the catalyst carrier particles in the reaction mixture. We compared the degradation potential (Figure 8) of Au, Pd and Au@Pd nanoparticles coated glass beads under same reaction condition to give us an idea about their efficacy in heterogeneous catalysis.

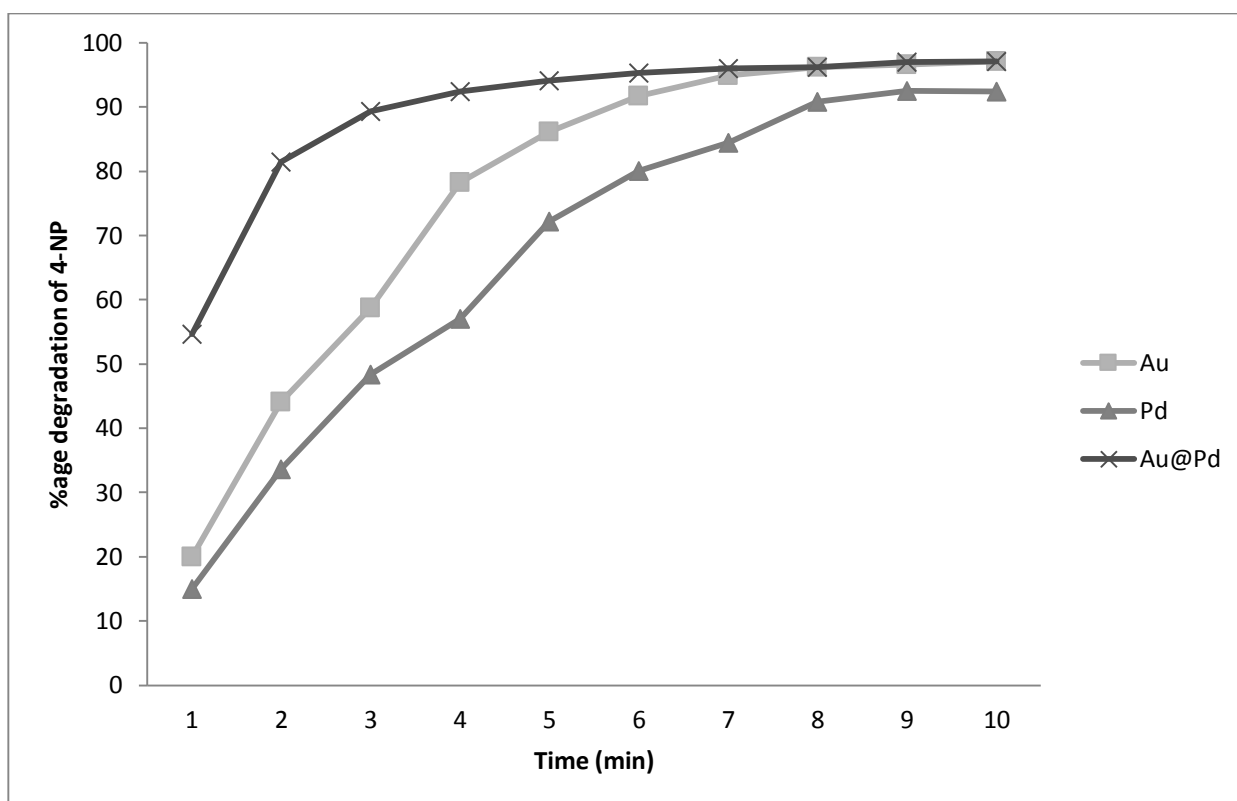


Figure 8. Showing percentage degradation of 4-Nitrophenol by Au, Pd and Au@Pd nanoparticle coated glass beads.

Au@Pd bimetallic nanoparticles proved to be a very efficient catalyst in degradation of 4-NP as compared to Au and Pd NPs coated over glass beads. In the first sixty seconds of the reaction, Au@Pd coated glass beads degraded about 55% of 4-NP as compared to 20% and 15 % by Au and Pd NPs respectively. At the end of 5 minutes, Au@Pd NPs degraded 94% of 4-NP as compared to 86% and 72% by Au and Pd NPs respectively. However, at the end of reaction at 10 minutes, all the three coated NPs showed similar results with Au and Au@Pd as 97% while Pd as 92%. This intense catalytic activity of Au@Pd bimetallic nanoparticles exceeded than that of individual metal NPs (Au and Pd). In fact this increased activity by bimetallic system arises due the synergistic effect between Au and Pd which is well-recognized phenomenon in liquid phase oxidation. It is interesting to note that while Pd NPs alone were amorphous in nature, they were rather poor in oxidation of 4-NP. However, in

case of Au@Pd NPs the interfacial layer containing crystalline Au and Pd crystals greatly improved the degradation potential. This preliminary study clearly demonstrates effective catalytic activity in all the three types of nanoparticles (Au, Pd, and Au@Pd NPs) synthesized via Trp in aqueous environment.

Finally, it should be noted that the reduction rate of 4-NP can be influenced by the concentration of catalyst, size of catalyst, concentration of reactants and temperature.

Although there may be a few drawbacks like polydispersity of nanoparticles which may affect the catalytic potential, nonetheless considering the economic viability and facile green synthesis, this study helps in better understanding of bimetallic nanoparticle synthesis and its associated application for the reduction of nitroaromatic pollutants.

4. Conclusion

In summary, we presented simple one-pot green synthesis of Au NPs, Pd NPs and Au@Pd bimetallic NPs with core-shell structure. Since the focus of this study was towards the Au@Pd bimetallic NPs, we observed Au octahedron forming the core (~50 nm) with Pd layer (~5 nm) forming the shell. This Pd layer deposition was found to be time dependent keeping other reaction parameters constant. The resulting NPs were characterized by TEM, XRD and Z-pot analysis. Further, Au@Pd NPs were analysed by AC-HAADF-STEM, STEM-EDS and XPS to confirm and analyse the resulting core-shell morphology. Although, a lot of research has been put into the study of bimetallic nanoparticle (with emphasis to core-shell structure), our study demonstrate for the first time, synthesis of stable Au@Pd bimetallic nanoparticles (core-shell type) by successive reduction approach in presence of an amino acid (Trp) at room temperature. The resulting nanoparticles will find numerous applications in study of physiochemical properties of heterostructured nanomaterial's and several other catalytic applications. Further, tryptophan will not induce any biological toxicity as compared to other chemical counterparts and nanoparticles synthesized by this technique may find several important uses in biological systems and diagnostics due to its inherent UV fluorescence. An interdisciplinary research will aid in better understanding of the underlying mechanism and process improvement.

References

- [1] N. Toshima, T. Yonezawa, *New J. Chem.* 1998, 22, 1179.
- [2] W. Zhou, J.Y. Lee, *Electrochem. Comm.* 2007, 9, 1725.
- [3] K.J. Major, C. De, S. O. Obare, *Plasmonics* 2009, 4, 61.
- [4] K. S. Soppimath, D. C. W. Tan, Y. Y. Yang, *Adv. Mater.* 2005, 17, 318.
- [5] N. Toshima, Y. Shiraishi, A. Shiotsuki, D. Ikenaga, Y. Wang, *Eur. Phys. J. D.* 2001, 16, 209.
- [6] K. R. Brown, M. J. Natan, *Langmuir* 1998, 14, 726.
- [7] W. R. Lee, M. G. Kim, J. R. Choi, J. I. Park, S. J. Ko, S. J. Oh, J. Cheon, *J. Am. Chem. Soc.* 2005, 127, 16090.
- [8] K. Mallik, M. Mandal, N. Pradhan, T. Pal, *Nano Lett.* 2001, 1, 319.
- [9] K. J. Mayrhofer, V. Juhart, K. Hartl, M. Hanzlik, M. Arenz, *Angew. Chem. Int. Ed.* 2009, 48, 3529.
- [10] S. K. Srivastava, M. Constanti J. *Nanopart. Res.* 2012, 14, 831.
- [11] S. K. Srivastava, R. Yamada, C. Ogino, A. Kondo, *Nanoscale Res. Lett.* 2013, 8, 70.
- [12] X. Huang, H. Wu, S. Pu, W. Zhang, X. Liao and B. Shi. *Green Chem.*, 2011, 13, 950.
- [13] G. Zhan, J. Huang, M. Du, I. A. Rauf, Y. Ma, Q. Li, *Mater Lett.* 2011, 65, 2989.
- [14] P. S. Schabes-Retchkiman,; G. Canizal, R. Herrera-Becerra, C. Zorrilla, H. B. Liu, J. A. Ascencio, *Opt. Mater.* 2006, 29, 95.
- [15] S. Senapati, A. Ahmad, M. I. Khan, M. Sastry, R. Kumar, *Small* 2005, 1, 517.
- [16] X. Liu, J. Zhang, X. Guo, S. Wu, S. Wang, *Nanoscale* 2010, 2, 1178.
- [17] R. R. Naik, S. J. Stringer, G. Agarwal, S. E. Jones, M. O. Stone, *Nat. mater.* 2002, 1, 169.
- [18] C. J. Serpell, J. Cookson, D. Ozkaya, P. D. Beer, *Nat. Chem.* 2011, 3, 478.
- [19] P.R. Selvakannan, S. Mandal, S. Phadtare, A. Gole, R. Pasricha, S. D. Adyanthaya, J. *Colloid Interf. Sci.* 2004, 269, 97–102.

- [20] M. Iosin, P. Baldeck, S. Astilean, J. Nanopart. Res. 2010, 12, 2843.
- [21] J. D. Newman G. J. Blanchard, Langmuir 2006 20, 5882.
- [22] S. Si, T.K. Mandal, Chem.-Eur. J. 2007,13, 3160–3168
- [23] J. W. Hu, Y. Zhang, J. F. Li, Z. Liu, B. Ren, S. G. Sun, Z. Q. Tian, T. Lian, Chem. Phys. Lett. 2005, 408, 354.
- [24] W. Lu, B. Wang, K. Wang, X. Wang, J.G. Hou, Langmuir, 2003, 19, 5887.
- [25] J. W. Hong, D. Kim, Y. W. Lee, M. Kim, S. W. Kang, S. W. Han, Angew. Chem. Int. Ed. 2011, 50, 8876.
- [26] K. Y. Lee, M. Kim, Y. W. Lee, J. J. Lee, S. W. Han, Chem. Phys. Lett. 2007, 440, 249.
- [27] Y. W. Lee, M. Kim, Z. H. Kim, S. W. Han, J. Am. Chem. Soc. 2009, 131, 17036.
- [28] P. Joshi, V. Shewale, R. Pandey, V. Shanker, S. Hussain, S. P. Karna, J. Phys. Chem. C 2011, 115, 22818.

Chapter 4.

Tryptophan mediated green synthesis of Au@Pt nanoparticles and its application in hydrogen evolution reaction by electro-oxidation of water.

Tryptophan mediated green synthesis of Au@Pt nanoparticles and its application for the hydrogen evolution reaction by electro-oxidation of water.

1. Introduction

Bimetallic nanoparticles (NPs) have gained importance because of their composition dependent optical, catalytic and magnetic properties which are different as compared to their monometallic counterparts [1, 2]. Au@Pt core-shell structures in particular have gained a lot of importance in catalysis and fuel cells due to their unique hybrid structure [3, 4]. The importance of core-shell morphology in case of Au@Pt NPs can be understood by the fact that Au-Pt alloy NPs are not ideal candidates since the co-deposition of process of Au and Pt is difficult to achieve in order to obtain monodisperse NPs with high surface area [5]. Therefore, several techniques have been reported to synthesize these core-shell structures mainly based on successive reduction approach and step by step reduction approach. However, most of these techniques require excessive usage of solvents, expensive instrumentation and tedious process control [6, 7, 8]. Therefore, there is a need to produce these valuable core-shell structures in a more facile and eco-friendly manner. Biogenic synthesis of nanoparticles has the potential to deliver the same with their inherent “green synthesis” mechanism while centered on the philosophy of utilizing active bio-compounds present in the nature for imparting novel material properties [9, 10, 11]. This becomes more important if we consider their application in biological systems (low toxicity) and clean energy (eco-friendly production). Previously, we reported a novel approach for synthesizing Au@Pd core-shell nanoparticles via tryptophan (Trp) in aqueous environment [12] thereby confirming that the zwitterion structure of amino acids (like Trp) has potential to impart additional supramolecular functionality and stability for synthesis of core-shell nanoparticles. Here, we extend the usage of our protocol by reporting room

temperature synthesis of uniform sized Au@Pt NPs and its application in electro-oxidation of water to produce hydrogen.

Platinum being one of the rarest elements in the Earth's crust also happens to be responsible for next generation of fuel cells. However, high costs associated with higher Pt loading remains indispensable for achieving feasible fuel cell technology has been a big challenge [13]. Adzic et al. reported the first Pt based core-shell catalyst for fuel cell reaction by underpotential deposition of Cu and subsequent displacement with Pt [14]. Although, electrochemical approach is not feasible for mass production of these catalytically active nanoparticles. This makes our current study all the more important as it not only extends the scope of core-shell structures which are capable of being synthesized by Trp-mediated approach but also provides a lucrative opportunity for facile bulk production of these Au@Pt NPs in green energy production. Au@Pt core-shell nanoparticles of size around ~50 nm were produced by utilization of tryptophan in aqueous reaction mixture at room temperature. Gold seeds (Au NPs) were produced by incorporating tryptophan in the reaction mixture followed by successive reduction of Pt over the Au NP surface resulting in core-shell morphology. Resulting nanoparticles were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), aberration-corrected Scanning TEM-HAADF microscopy, EDX, X-ray diffraction (XRD) and cyclic voltammetry (CV). These analyses showed formation of bimetallic Au@Pt core-shell type nanoparticles in the aqueous reaction mixture. Subsequently, resulting Au@Pt nanoparticles were incorporated over glassy carbon electrode to access its efficiency in electro-oxidation of water.

2. Experimental

2.1 Synthesis of Au, Pt and Au@Pt nanoparticles

Chloroauric acid (HAuCl_4) and Potassium tetrachloroplatinate (K_2PtCl_4) were obtained from Scharlau Chemie (Spain) and were used without any pre-treatment. L-Tryptophan was obtained from Sigma (Spain). Ultrapure water (resistivity $\sim 18.2 \text{ M}\Omega\cdot\text{cm}$) was used as a solvent throughout the experiments. Well-mixed gold cation stock solution at 1 mM (10 mL) and platinum cation stock solution (1 mM, 10mL) was prepared. Trp aqueous stock solution was prepared at a concentration of 4 mM (20 mL). Cation solutions consisting of Au:Pt was used in the volumetric ratio of 1:1.

Gold nanoparticle solution was prepared by spontaneous reduction of 10 mL gold cation solution in presence of 2 mL of Trp solution with vigorous stirring for 2 hours followed by intermittent sonication for 30 seconds at every 15 min. It should be noted that the size of core-shell structures can be controlled by controlling the size of Au NPs at seeding stage. For Au@Pt core-shell NPs, platinum precursor solution (5 mL) was introduced with gold NP seed solution (5mL) along with 1 mL of Trp solution. The resulting mixture was stirred for 2 hours followed by intermittent sonication for 10 seconds at every 30 minutes. The Pt NP solution was prepared in the same way as mentioned above for Au NP seed synthesis.

Therefore, three distinct types of nanoparticles were synthesized namely Au NPs, Pt NPs and Au@Pt NPs.

2.2 Characterization of Au, Pt and Au@Pt nanoparticles

UV-visible spectra were obtained using a CARY 100 Bio UV-Vis Spectrophotometer and were analyzed using Varian's Cary Win UV Scan ver. 3.00 (182) software. Nanoparticle formation was monitored from 200-800 nm at 2000nm/min scan rate. The focus of this study was in the visible region of Au NPs around 540 nm (LSPR) to monitor the progress of the reaction.

Morphology and grain size analysis of resulting Au@Pt NPs was carried out using a JEOL 1011 transmission electron microscope operated at 80 keV with an ultrahigh-resolution pole

piece providing a point resolution of 2 Å. Individual Au@Pt nanoparticles were further analyzed by aberration-corrected STEM-HAADF microscopy (JEOL 2100F, 200 kV, ~0.1 nm point resolution) to observe the precise structure of resulting core-shell nanoparticles. One micro-liter was taken from the reaction mixtures and placed on carbon coated copper grids and dried at room temperature. The images were analyzed using ImageJ 1.43M software. Observed nanoparticles were also analyzed by JED-2300T STEM-EDS for obtaining composition architecture.

X-ray diffraction (XRD) patterns were obtained using a Siemens D5000 diffractometer equipped with Bragg–Brentano parafocusing geometry and a vertical θ - θ goniometer. A drop of the sample (100 μ L) was deposited on to a low background Si (510) sample holder. The X-Ray diffractometer was operated at 40 kV to generate CuK α radiation. The data were collected with an angular step of 0.05° at 12 s per step and sample rotation.

2.3 Electrochemical characterization/measurements

All electrochemical measurements were performed with a potentiostat/galvanostat PGSTAT 12 Autolab controlled with the General Purpose Electrochemical System (GPES). A three-electrode setup was used with glassy carbon (GC) as working electrode ($\phi = 0.126$ cm²), a platinum wire as counter electrode and Ag/AgCl as pseudo-reference electrode. Before the measurements, the electrochemical cell was purged with N₂ gas for 10 minutes.

The characterization of the core-shell NP morphology was realized out by diluting the different NP pellets in 20 μ l of 0.5 M H₂SO₄. This solution was electrochemically characterized by cyclic voltammetry between -0.2 V and 1.6 V at a scan rate of 0.1 V/s.

Au, Pt, and Au@Pt mixture solutions were mixed and centrifuged twice with 2 ml Milli-Q water removing the supernatant in order to eliminate the excess of Trp.

25 μ l of each NP reaction sample was loaded on the surface of GC electrode by drop casting under hot-air flow till dried completely. The electrocatalytic properties of Au@Pt NPs

deposited on the surface of the GC electrode were examined by the determination of hydrogen evolution reaction (HER) in basic medium [15, 16]. The NP-modified GC electrodes were characterized by cyclic voltammetry between 0 V and -2 V at a scan rate of 0.1 V/s applying 25 consecutive cycles. 90 mM of Tris-buffered saline solution (pH 8.0) was used as conductive medium and Au, Pt and GC without particles were used as controls and in each case.

3. Results and discussion

UV-vis absorbance spectra (Figure 1) of Trp synthesized Au@Pt nanoparticles were compared with the Au NP formation spectra asserting formation of Pt shell around Au core.

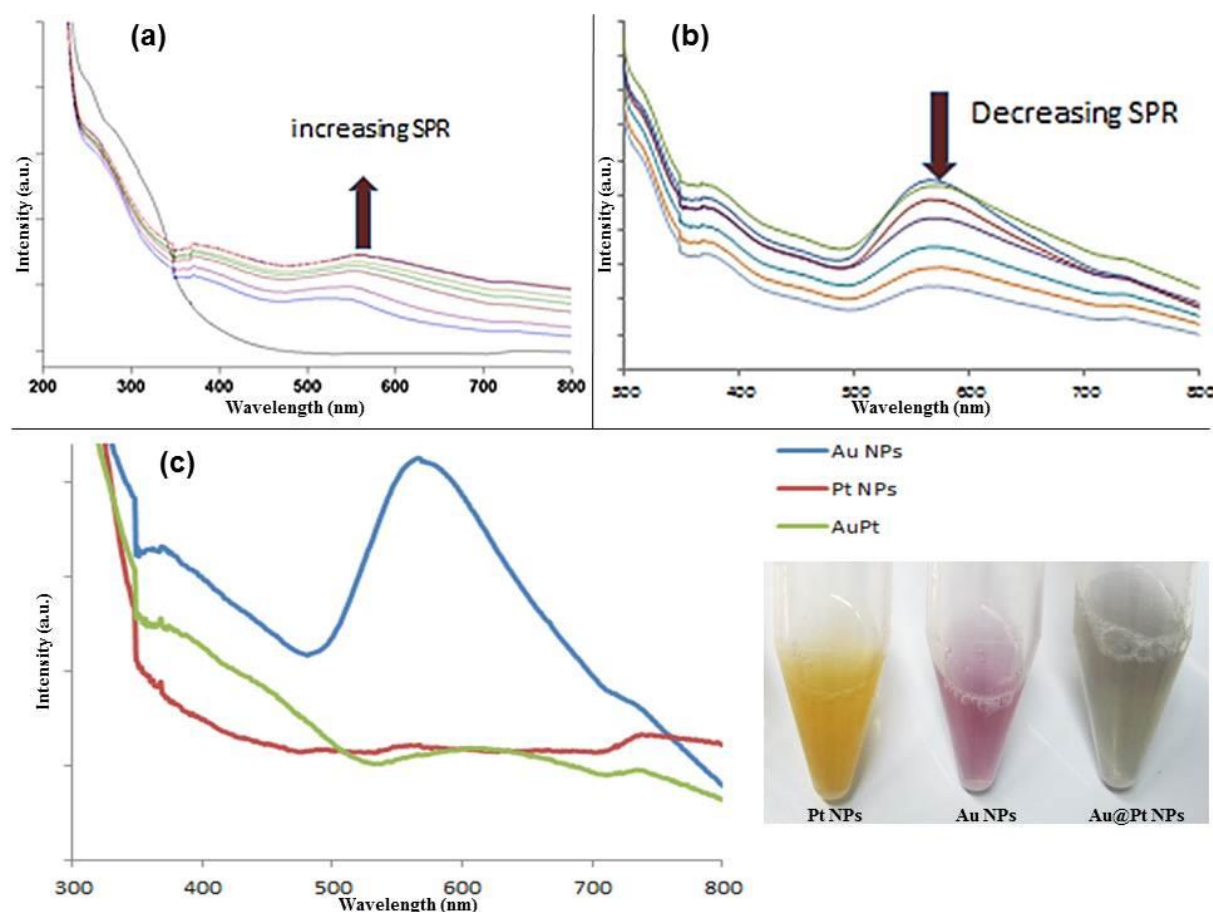


Figure 1. Showing growth characteristics in terms of SPR in (a) formation of Au NPs; (b) deposition of Pt shell over Au NPs; (c) Difference in SPR after the completion of reaction (inset: showing visible color difference between all three nanoparticle reaction mixtures).

Au NP spectra showed increasing Localized Surface Plasmon Resonance (LSPR) centered at around 540 nm over a time period of every 15 minutes as showing in Figure 1a. The gradual increase in LSPR was stabilized over a time period of 120 minutes suggesting completion of Au NP formation. These Au NPs were then utilized for the formation of Pt shell formation which resulted in marked decrease in absorbance at 540 nm over a period of time as highlighted in Figure 1b.

This also suggests that the Pt shell thickness can be controlled by manipulating the reaction time while keeping the other parameters constant. Figure 2 compares absorbance spectra of Au, Pt and Au@Pt NPs over UV- Vis region. The LSPR peak was drastically reduced in case of Au@Pt NPs and completely absent in case of Pt NP reaction mixture after the completion of reaction. This spectral behavior reasonably indicates towards the core-shell structure formation as the surface electron density in the Au core increases at the expense of electrons from the outer Pt shell [17]. This happens because Pt has a higher electron chemical potential (5.65 eV vs. 5.00 eV). Thus, a thicker Pt shell coating on the Au core results in the gradual damping of the plasmon of Au core. It is interesting to note that while Au NPs showed characteristic pink color due to SPR, at the end of the reaction (120 min) the visible pink color of Au NPs seeds was absent with characteristic black color dispersion clearly marking the formation of thin Pt shell over the Au core (Figure 1 c). Likewise, no SPR was observed in case of Pt NPs. This visible color difference between Au, Pt and Au@Pt NPs is also highlighted in the inset of Figure 1c.

To confirm and characterize the formation of Au@Pt NPs in the aqueous solution, we carried out TEM followed by high resolution STEM and EDS. TEM imaging confirmed Au@Pt core-shell nanoparticles of size around ~50 nm as shown in Figure 2a.

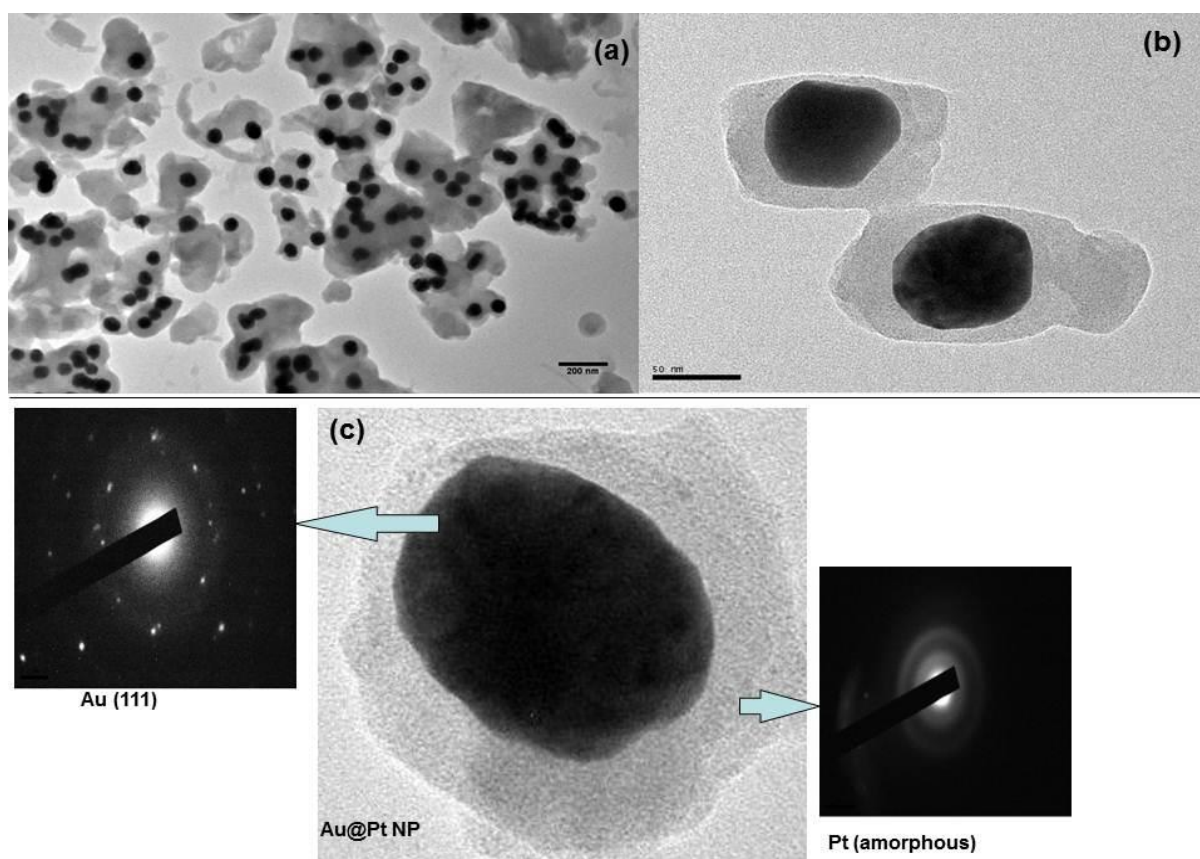


Figure 2. TEM images showing (a) Au@Pt NP particles; (b) discrete Au@Pt NP particles with core size ~ 50 nm and shell ~ 5 nm; (c) HAADF-STEM image of resulting Au@Pt NP showing crystalline Au (111) core and largely amorphous Pt shell.

We observed Au core of size around ~ 45 nm and Pt shell of ~ 5 nm thickness as shown in Figure 2b. Aberration-corrected high-angle annular dark-field scanning TEM (AC-HAADF-STEM) was used to confirm core-shell morphology of the observed Au@Pt NPs. Highly discrete Au@Pt boundary layer was also observed (fig 2c) where the Au core in the center showed characteristic Au (111) diffraction pattern. Also, in the absence of any distinct diffraction pattern, Pt shell was rather amorphous in nature. Thus, Au@Pt NPs observed in this study largely comprises of crystalline Au core with largely amorphous Pt shell. This was further confirmed with subsequent XRD analysis of Au, Pt and Au@Pt NPs.

As observed from XRD, Au NPs (Figure 3 a) showed a very strong Bragg's diffraction at (111) at 38.2° followed by (200) at 44.35° .

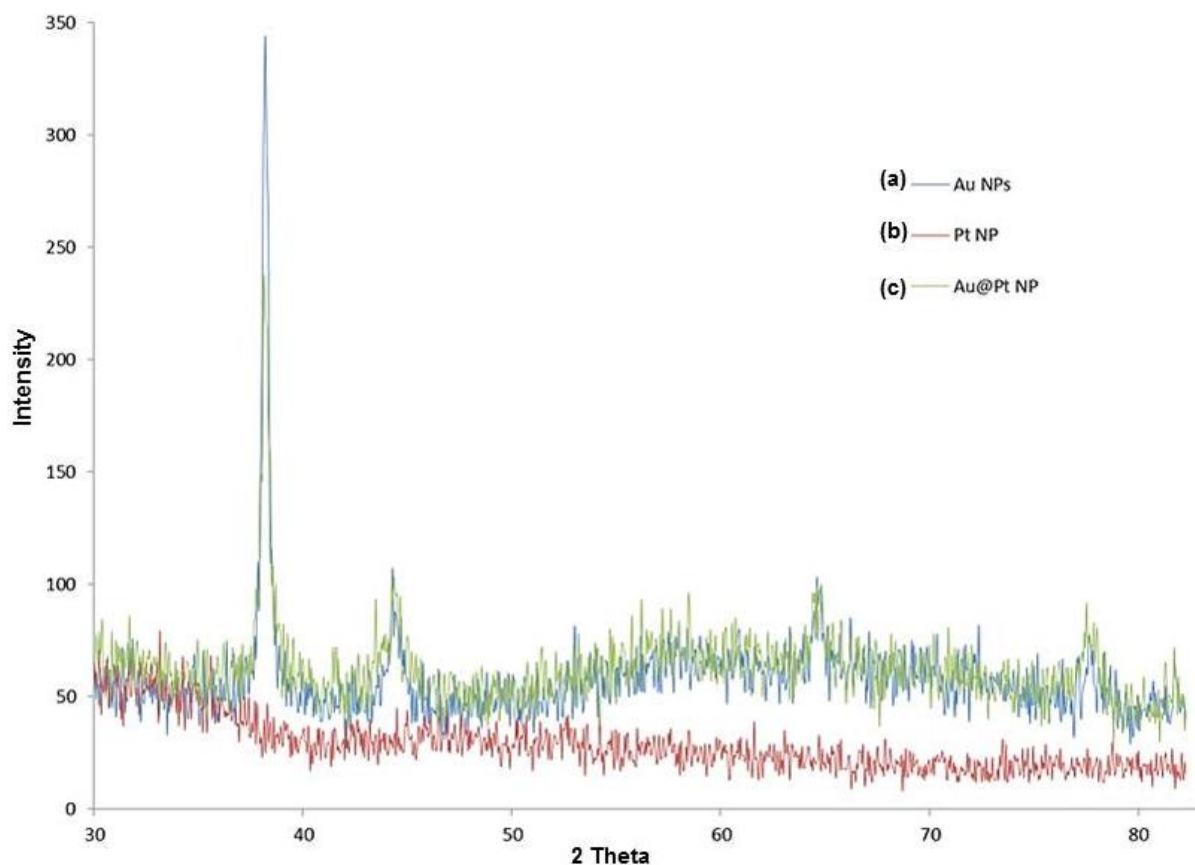


Figure 3. XRD patterns of (a) Au NPs; (b) Pt NPs; (c) Au@Pt NPs.

In case of Au@Pt NPs (Figure 3b), a distinct peak broadening was observed for Au (111) at 38.15° and Au (200) at 43.5° which is normally observed in core-shell structures. Finally, no credible peaks can be assigned to Pt (Figure 3c); in the case of Pt NPs and Au@Pt NPs confirming that the majority of reduced platinum was amorphous in nature. It should be noted that core-shell structures are capable of generating new electrocatalytic properties via interaction between shell (Pt) and underlying core metal (Au) as well as geometrical structures of Pt such as monolayer, island and intermixing phase. Thin amorphous platinum films are highly desirable end-products sought after for electrode preparation and electro-oxidation reactions. Further, Pt based bimetallic systems for electrochemical reactions have been proven to show that qualitative changes in the surface properties takes place during the

deposition of a catalytically active component and amorphous Pt shell may offer higher catalytic activity (synergistic effect) and selectivity for specific reaction [18].

HAADF-STEM imaging (Figure 4a) displayed a brighter gold core due to its heavier atomic weight while platinum shell appeared comparatively less bright due to its low atomic weight.

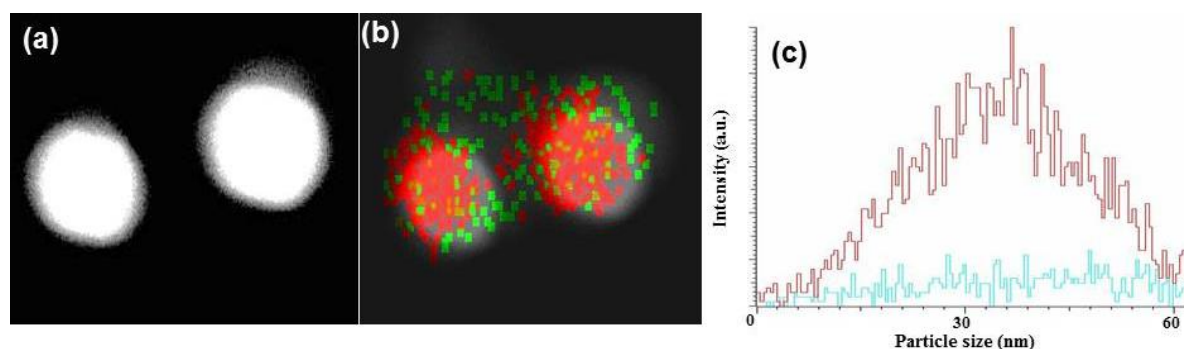


Figure 4. Showing Au@Pt NPs as observed by (a) AC-HAADF-STEM image; (b) STEM-EDS elemental mapping; (c) line scan EDS.

Colored elemental mapping images showed that Au atoms (red) and Pt atoms (green) were discretely distributed with dense Au core at the center and Pt at the boundary giving rise to characteristic core-shell structure (fig 4b). EDS elemental line scanning (fig. 4c) on single particles confirmed that the Au@Pt NPs (~50 nm) were composed of distinct Au rich center and Pt rich boundary responsible for typical core-shell (not alloyed) morphology. Similar core-shell results were also reported by Huang et al. for Au@Pd NPs [19]. Based on the reduction potential of Au (III) and Pt (II) ($\text{AuCl}_4^-/\text{Au}$, +1.002 vs. SHE (standard hydrogen electrode) and Pt^{2+} ($\text{PtCl}_4^{2-}/\text{Pt}$, +0.73 V vs. SHE), we can easily expect that the Au (III) will be preferentially reduced over the Pd (II) under our experimental reaction conditions [20]. In another study by Lee et al. synthesis of Au@Pd core-shell nanooctahedrons by utilizing CTAC as both reducing and stabilizing agent was reported [21]. Similarly, in our reaction mixture, Trp plays a key role in reduction and subsequent stabilization of core-shell structure.

Finally, prior to electrocatalytic assay, it was important to ascertain that no organic impurities or exposed Au surface (in case of Au@Pt NPs) is present which may alter the electrochemical measurements. The presence of gold can be electrochemically detected by its characteristic peaks when the metal is oxidized or reduced in the boundaries of the electrode. The voltammograms for this experiment (Fig 5.) show no peaks for the Au@Pt NP neither the negative controls (Pt, Pt (N), GC) whereas the Au NP solution yielded the representative peaks for this species.

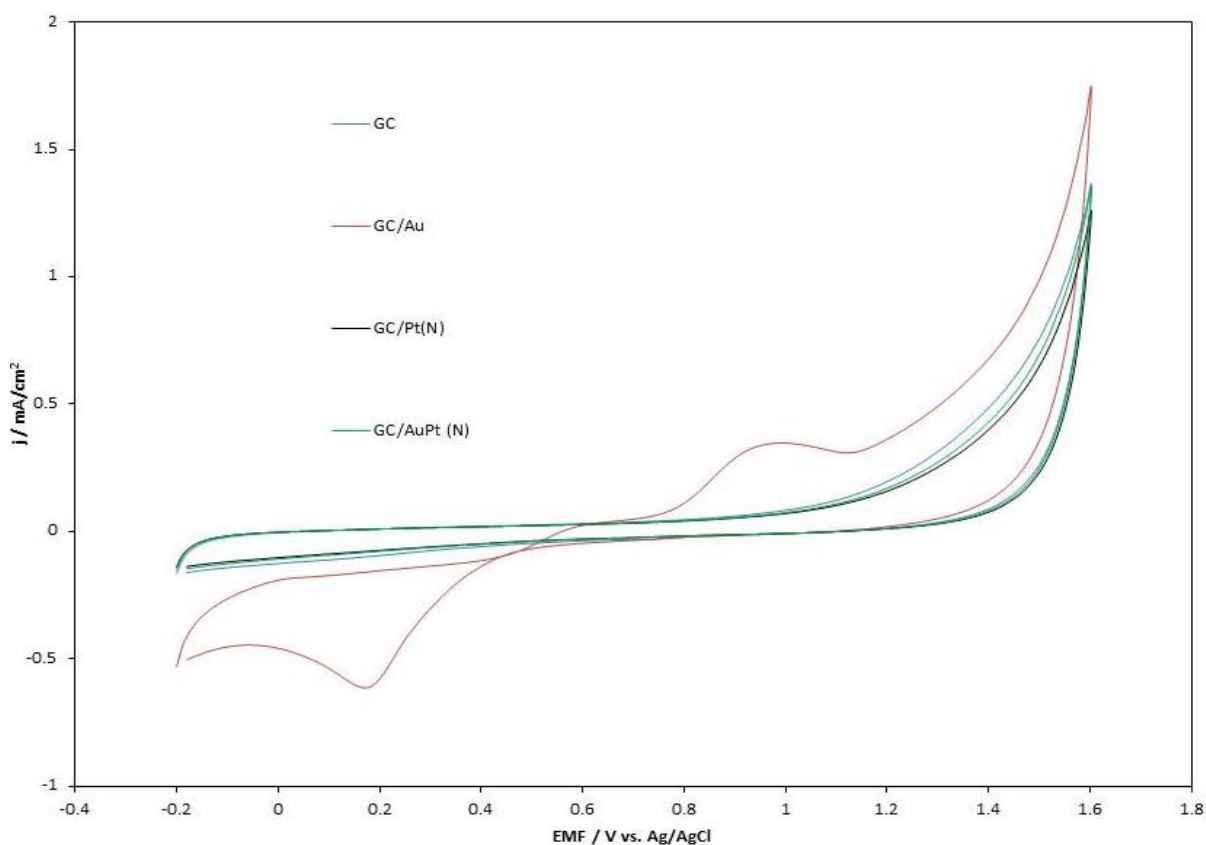


Figure 5. Acid CV characterization of exposed Au core/organic impurities.

This confirms that in case of Au@Pt NPs, there is no exposed gold surface which may rapidly oxidize upon electro-oxidation assay. Further, no organic impurity arising from Trp adherence on the outer surface was detected in any of our nanoparticle mixtures.

The catalytic efficiency of Au, Pt and Au@Pt NPs synthesized via Trp in aqueous solution was tested for electrochemical oxidation of water (hydrolysis) via experimental determination

of potential range where the hydrogen evolution reaction (HER) occurs. Three different control electrodes of gold, platinum and nitrogen-doped platinum nanoparticles respectively were also evaluated to compare the real efficiency of core-shell Au@Pt nanoparticles. Cyclic voltammetry (Figure 6) revealed different HERs for different NPs and control, yielding different evolutions of current density (Figure 7).

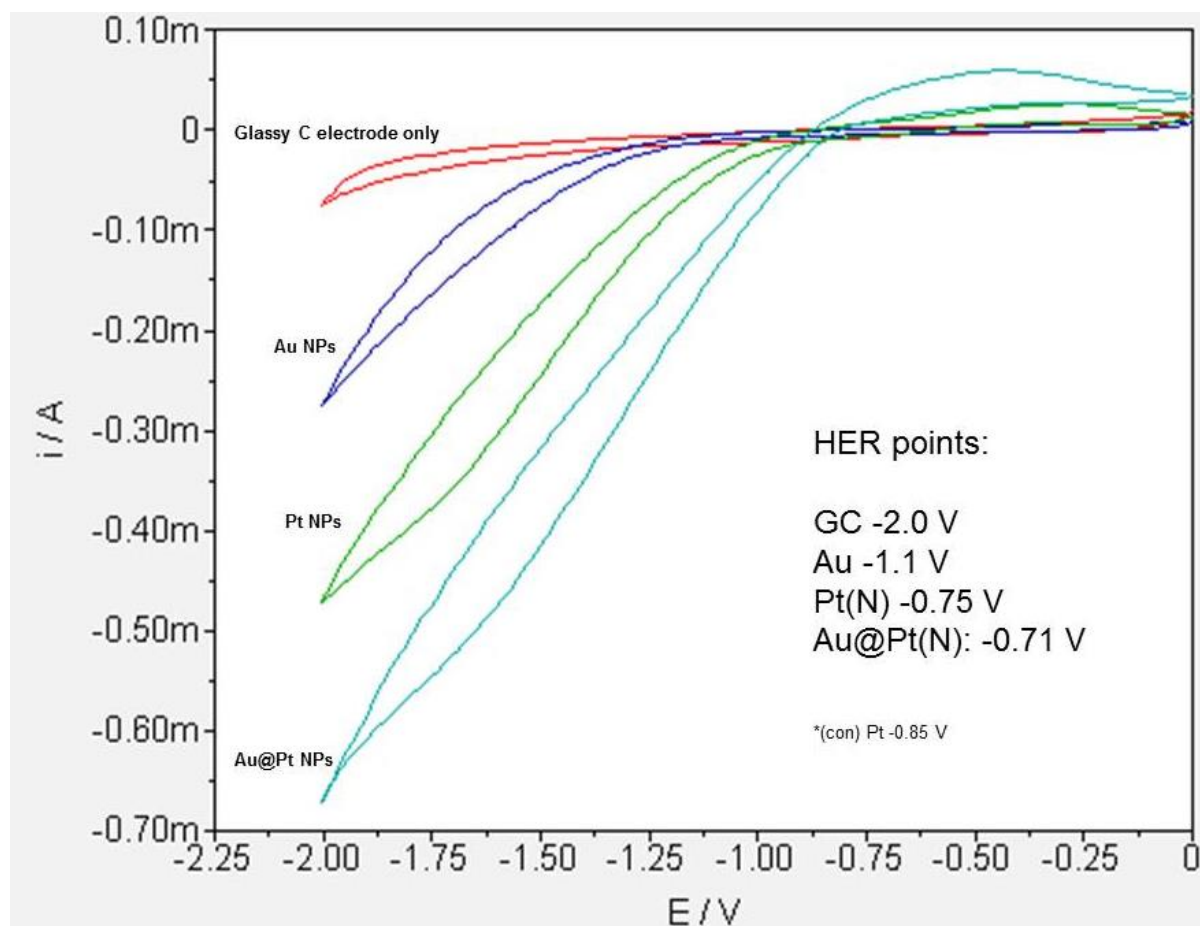


Figure 6. Electrochemical hydrogen evolution reaction.

While Au NPs showed electro-oxidation of water at an applied EMF of -1.1V, Pt NPs were at -0.85 V. The best performance both in terms of both current density and HER were attributed for the Au@Pt coated GC electrode as -0.71 V. Although the highest current densities have always been observed at -2.0 V (GC control), the efficiency under these conditions tends to decrease because the reactive species compete for the active sites of the electrode and this slows down the HER.

The evolution of the current density (i.e. the slope of current) is represented in the Fig X to clarify the HER point:

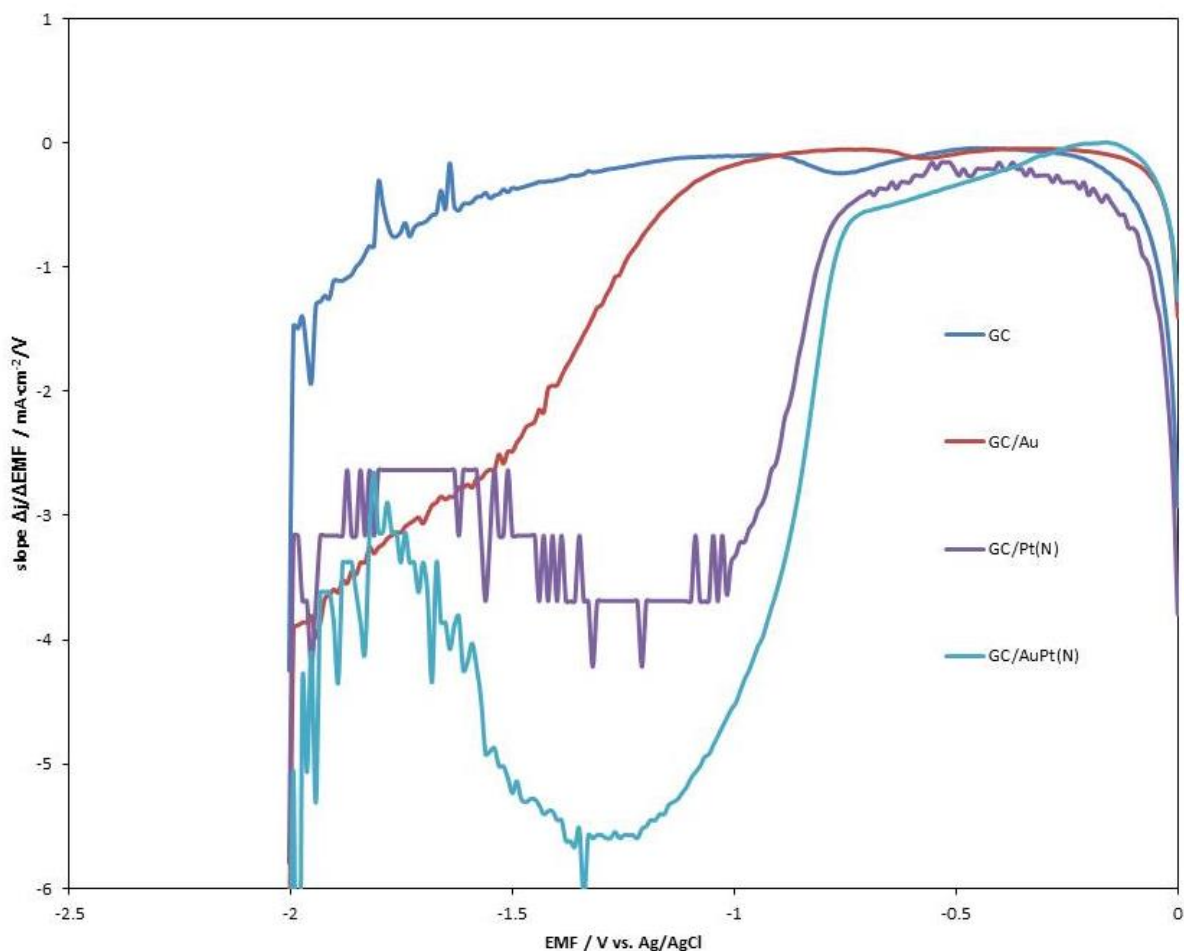


Figure 7. Evolution of the Slope for the current density.

The better HER of Au@Pt compared with pure Pt catalyst can be explained by the presence of gold which oxidizes the adsorbed species on the active sites of platinum nanoparticles and therefore can improve the catalytic activity of Au@Pt NP.

4. Conclusion

We presented a facile one-pot green synthesis of Au@Pt bimetallic NPs with core-shell structure. Since the focus of this study was towards achieving higher Pt loading, we observed Au octahedron forming the core (~50 nm) with Pt layer (~5 nm) forming the shell. We found out that the thickness of Pt layer to be time dependent keeping the other reaction parameters constant. Resulting Au@Pt NPs were characterized by TEM, XRD, AC-HAADF-STEM, STEM-EDS and EDX to confirm and analyse the resulting composition of core-shell morphology. Although, a lot of research has been put into the study of bimetallic nanoparticle (with emphasis to core-shell structure), our study demonstrate for the first time, synthesis of stable Au@Pt bimetallic nanoparticles (core-shell type) by successive reduction approach in presence of an amino acid (Trp) at room temperature. Finally, electro-oxidation potential of these nanoparticles was tested by depositing them over GC electrode for performing HER via electrolysis of water. We observed that Au@Pt NPs showed the highest HER potential with low applied potential making them an ideal candidate for fabrication electrodes for electro-oxidation reactions. We strongly believe that an interdisciplinary research will aid in better understanding of the underlying mechanism these unique crystalline(Au)@amorphous (Pt) NP may possess and will further aid in process improvement for bulk production.

References

- [1] N. Toshima, T. Yonezawa, *New J. Chem.* 1998, 22, 1179.
- [2] R. W. J. Scott, O. M. Wilson, S. K. Oh, E. A. Kenik, R. M. Crooks, *J. Am. Chem. Soc.* 2004, 126, 15583.
- [3] J. Luo, P. N. Njoki, Y. Lin, D. Mott, L. Wang, C. J. Zhong, *Langmuir* 2006, 22, 2892.
- [4] W. Tang, S. Jayaraman, T. F. Jaramillo, G. D. Stucky, E. W. McFarland, *Jour. of Phys. Chem. C* 2009, 113, 5014.
- [5] J. Luo, P. N. Njoki, Y. Lin, D. Mott, L. Wang, C. J. Zhong, *Langmuir* 2006, 22, 2892.
- [6] D. F. Yancey, E. V. Carino, R. M. Crooks, *J. Am. Chem. Soc.* 2010, 132, 10988.
- [7] H. A. Esfahani, L. Wang, Y. Yamauchi, *Chem. Commun.*, 2010, 46, 3684.
- [8] K. J. Mayrhofer, V. Juhart, K. Hartl, M. Hanzlik, M. Arenz, *Angew. Chem. Int. Ed.* 2009, 48, 3529.
- [9] S. K. Srivastava, R. Yamada, C. Ogino, A. Kondo, *Nanoscale Res. Lett.* 2013, 8, 70.
- [10] S.K. Srivastava, R. Yamada, C. Ogino, A. Kondo, *Carbon* 2013, 56, 309.
- [11] B. Wickleinab, G. S. Alvarez, *J. Mater. Chem. A* 2013, 1, 5469.
- [12] S. K. Srivastava, M. Constanti *J. Nanopart. Res.* 2012, 14, 831.
- [13] D. J. Chen, A. M. H. Duffy, I. S. Park, D. O. Atienza, C. Susut, S. G. Sun, Y. J. Tong, *J. Phys. Chem. C* 2011, 115, 8735.
- [14] J. Zhang, F. H. B. Lima, M. H. Shao, K. Sasaki, J. X. Wang, J. Hanson, R. R. Adzic, *J. Phys. Chem. B* 2005, 109, 22701.
- [15] S. A. Grigoriev, P. Millet, V. N Fateev, *Jour. Pow. source* 2008, 177, 281.
- [16] E. Ndzebet, O. Savadogo, *Int. J. Hydrogen Energy* 1995, 20, 635.
- [17] J. W. Hu, Y. Zhang, H. V. Li, Z. Liu, B. Ren, S. G. Sun, Z. Q. Tian, T. Lian, *Chem. Phy. Lett.* 2005, 408, 354.
- [18] Y. Hayakawa, A. Kawashima, H. Habazaki, K. Asami, K. Hashimoto, *Jour. of App. Electrochem.* 1992, 22, 1017.

- [19] X. Huang, H. Wu, S. Pu, W. Zhang, X. Liao, B. Shi, *Green Chem.* 2011,13, 950.
- [20] K. Y. Lee, M. Kim, Y. W. Lee, J. J. Lee and S. W. Han, *Chem. Phys. Lett.* 2007, 440, 249.
- [21] Y. W. Lee, M. Kim, Z. H. Kim and S. W. Han, *J. Am. Chem. Soc.* 2009, 131, 17036.

Chapter 5.

Sidewall modification of multiwalled carbon nanotubes by *Allivum sativum* (garlic) and its effect on the deposition of gold nanoparticles

Chapter 5.

Sidewall modification of multiwalled carbon nanotubes by *Allivum sativum* (garlic) and its effect on the deposition of gold nanoparticles

1. Introduction

In recent years, nanoscience and nanotechnology has made considerable progress towards synthesis and characterization of new materials due to their wide-spread application in everyday life. Much of this success was possible due to chemical modifications or functionalization of carbon nanotube (CNT) surface [1]. This combination of chemically modified nanotubes decorated with nanoparticles has found tremendous usage in electronics, sensors, biomedical applications, catalysis, H₂ storage etc. [2] Gold nanoparticles (Au NPs) in particular, happens to be a key material and building block for newer technologies owing to their unique set of properties at nanoscale [3]. Several methods for functionalizing CNTs followed by Au NP deposition on the modified nanotube surface were proposed recently. Satishkumar et al. [4] showed that CNTs upon harsh acid treatment allowed tagging of metal nanoparticles (including Au NPs) on the modified nanotube surface. Also, Jiang et al. [5] showed selective attachment of Au NPs on nitrogen-doped multi-walled carbon nanotubes (MWCNTs) with H₂SO₄ – HNO₃ chemical treatment. Similarly, surface activation by carboxylic acid [6] and thioamide-carboxyl [7] surface has been reported for immobilization of metal NPs. The general idea which prevailed was that pristine CNTs are inert structures and harsh pre-treatment is needed to create ‘openings’ on the CNT surface for attaching other chemical groups. However, Ellis et al. [8] demonstrated weak hydrophobic anchoring of Au NPs by utilizing thiol capping on acetone (-OH group) activated CNT surface. Similarly, other techniques were also reported [9, 10, 11] for Au NP attachment on CNT surface. However, most of these techniques were without any chemical modification of nanotube

surface thereby limiting the binding strength and capacity of Au NPs over the nanotube surface. Since, CNTs are not ideal structures and contain defect sites and impurities [12], this makes them ideal site for addition reactions [13]. These defect sites can be exploited to tag metal nanoparticles onto the CNT surface without any harsh treatment as demonstrated by Zanella et al. [14] to tag gold nanoparticles via thiol linkages [15, 16, 17]. These sulfur linkages when attached to the defect sites, open new possibilities for attaching other chemical groups (including nanoparticles) at the vacancy defect sites. Although, considerable efforts have been made in pre-treatment of CNTs as discussed previously, most of these techniques require excessive usage of toxic solvents, acid pre-treatment and rigorous process control at high temperatures.

Thus, there is a need to functionalize CNTs in a more benign and eco-friendly manner. With this aim, we hereby provide a novel biogenic method for CNT surface thiolation and subsequent attachment of Au NPs by utilizing organosulfur plant extract. Garlic is one such natural source of organosulfur compounds [18] where total sulfur content ranges from 0.35 – 1% of its dry weight [19]. Here, we report CNT surface modification by utilizing natural organosulfurs from *Allium sativum* (Garlic) and its effect on the deposition of Au NPs onto the modified CNT surface under ambient reaction conditions with an acid-free green process. This biogenic study has the potential to evolve into biomimetic study with advanced interdisciplinary research to provide innovative surface modification strategies for carbonaceous materials in future.

2. Experimental

2.1 CNT surface modification

Prepared MWCNTs from chemical vapor deposition (CVD) process (Aldrich, USA; 95% purity, 6 – 9 nm; 5 μ m) were used. CNT surface modification was carried out by freshly chopped garlic (100 g) cloves, which were crushed and extracted with 20 mL aqueous solution of water:ethanol (95:5 v/v). This extract was centrifuged at $3500 \times g$ to remove any suspended solids and the supernatant was retained. It is important to note that 80% of organosulfurs in garlic cloves convert to allicin (diallyl thiosulfinate) by enzymatic activity of allinase (naturally present in garlic) which converts alliin (3-(2-Propenylsulfinyl)alanine) upon being cut in the presence of water in a period of ten-fifteen seconds [20]. At elevated temperatures, alliin rapidly converts to diallyl trisulfide (DATS) and diallyl disulfite [21]. Since, CNT sidewall functionalization is affected with the curvature of carbon nanotubes [22], we preferred small diameter MWCNTs for thermal activation in plastic vials by solvent-free process [23] of baking reactant mixture at elevated temperature. MWCNTs (10 mg) were placed together with organosulfur extract (2 mL, 80 mg dry weight) and heated at 110°C for 4 hours in sealed vials. This reaction mixture was sonicated for 15 seconds at every hour to homogenize the reaction solution. Excess of organosulfur extract was removed from the reaction mixture by centrifugation at $3500 \times g$ for 2 min. Finally, organosulfur modified multi-walled nanotubes (to be referred as OS-MWCNT) was obtained.

2.2 Decoration of gold nanoparticles on CNTs

Gold nanoparticles were prepared by the reaction of HAuCl₄ (Sigma) and citric acid (Aldrich). In a typical experiment, 5 mg of OS-MWCNT was dispersed in 1-butanol (5 mL) and sonicated for 10 min. Then, 0.016 g of HAuCl₄ and 0.017g citric acid, both dissolved separately in 5 mL of 1-butanol were stirred for 30 min to produce Au NPs [24] before being added dropwise into the dispersion of OS-MWCNTs. Final volume was set to 10 mL.

This dispersion was vigorously stirred at room temperature for 3 h with intermittent sonication of 15 seconds at every 30 min. After this procedure, solid phase was separated by centrifugation ($3500 \times g$ for 10 min) and washed six times with 1-butanol to remove any non-linking Au NP from the CNT surface. Finally, we obtained our desired product of OS-MWCNTs decorated with Au NPs (Au-OS-MWCNT) which were dried at 60 °C and stored away from light in a vacuum desiccator. As a control test, same procedure of Au NP deposition was performed with pristine MWCNTs.

2.3 Characterization of Au-CNT nanocomposite

Preliminary analysis of Au NP deposition over the modified CNT surface was done by NanoDrop 3300 Fluorospectrometer operated at 400 nm – 600 nm with relative fluorescence units (RFU) at 540 nm. MWCNTs – with and without Au NP attachment dispersed in 1-butanol were transferred to carbon coated grid for transmission electron microscopy (TEM) measurements by Hitachi H-7100 transmission electron microscope and Jeol JEM 1011 electron microscope operated at 100 kV and 200 kV respectively. Images obtained were analysed using ImageJ 1.43M software. Transmission mode Fourier transform infrared (FT-IR) spectroscopy was carried out using Jasco FTIR-680 plus coupled to a high performance computer. The results reported here were obtained from 200 scans at a 4 cm^{-1} resolution and were verified five times with different nanocomposite samples prepared and stored under identical conditions. Energy dispersive X-ray (EDX) analysis was done to confirm sulfur linkages present over the nanotube surface using JEOL JSM6400 operating at an accelerating voltage of 15 kV and working distance of 15 mm; resulting spectra were analysed using Spectra Manager ver. 1.06.02. Raman spectra was obtained by using the 514.5 nm line of an Ar^+ laser (100mW) analyzed by a JOBIN-YVON monochromator Model U-1000 for 10 cycles each of 16 seconds. Confirmatory analysis for sidewall addition of thiol onto CNT surface was done by X-ray photoelectron spectroscopy (XPS) obtained from JEOL JPS9010

MC photoelectron spectrometer operating at 10 kV and 30 mA. The results obtained were analyzed by SpecSurf ver. 1.7.3.9 software.

3. Results and Discussion

Figure 1a shows fluorospectra of OS-MWCNT decorated with Au NPs and spectra of Au NPs alone (Figure 1b).

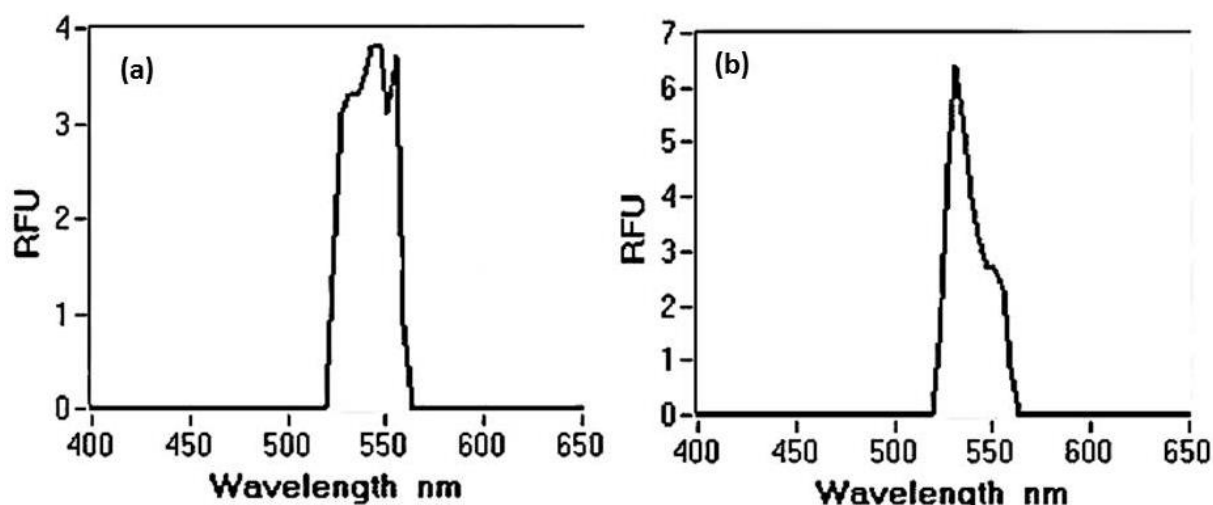


Figure 1. (a) SPR observed in Au-OS-MWCNT; (b) SPR observed in Au NP solution.

Both samples showed distinct surface plasmon resonance peak (SPR) at 540 nm when laser excitation was made in the white light region. This characteristic absorbance at 520-540 nm suggest towards the presence of gold nanoparticles linked over the modified CNT surface. The SPR peak remained after repeated washing and vigorous sonication. This preliminary result indicated that Au NPs may be attached onto the modified nanotube surface. Also, retention of optical absorption spectra suggested that there is no adverse influence on the nanoparticles. We noted that Au NPs do not attach to pristine MWCNT (control) when observed after same amount of washing as stated above. Likewise, TEM, EDX and XPS analysis also confirmed the same that no Au NP attachment was done over the pristine MWCNTs under the same set reaction conditions.

TEM images of Au-OS-MWCNTs showed gold nanoparticles on the surface of organosulfur modified MWCNTs, as is evidenced in Figure 2.

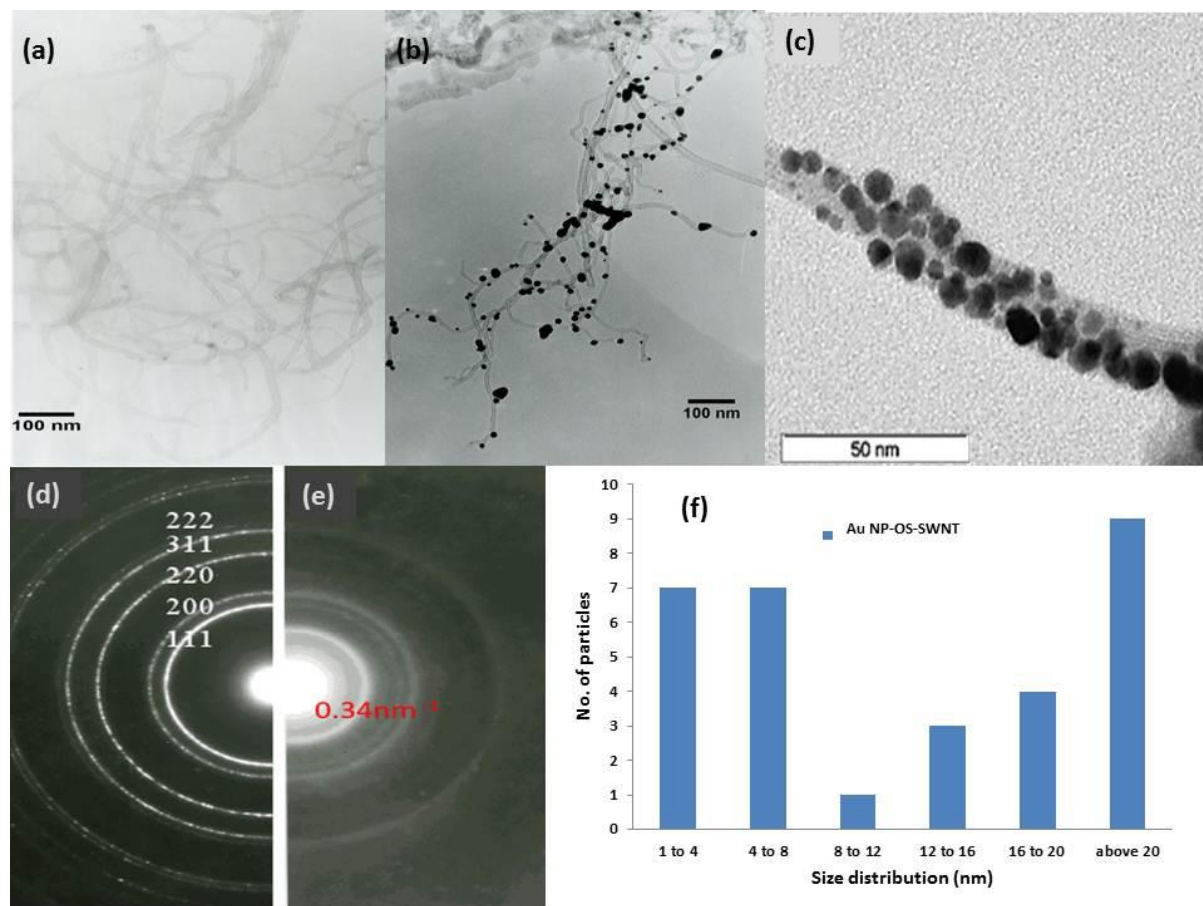


Figure 2. (a) TEM image of unmodified CNT; (b) TEM image of Au-OS-MWCNT; (c) High resolution image of Au-OS-MWCNT; (d) XRD pattern obtained from Au NP; (e) XRD pattern obtained from Au-OS-MWCNT; (f) Au NP particle size distribution on OS-MWCNT surface.

Figure 2b shows TEM micrograph of OS-MWCNT decorated with gold nanoparticles of size 5-15 nm. A micrograph of pristine MWCNT (control) is shown in Figure 2a for comparison. Gold nanoparticles (without being deposited onto the modified nanotube surface) exhibited face-centered cubic (fcc) structure as indicated by {111} and {200} facet diffraction pattern in Figure 2d. Figure 2c shows adherence of several Au NPs along the sidewall of OS-MWCNT. Au NPs attached on the OS-MWCNT surface showed similar fcc-type diffraction

pattern associated with Au NPs alone along with the interlayer spacing of graphite sheets (0.34 nm) as shown in Figure 2e. The close proximity of Au NPs to OS-MWCNT despite constant washing suggests certain level of adherence between them. Average Au NP particle size distribution on OS-MWCNT is depicted in Figure 2f where variation in the Au NP size happened due to the presence of other organic moieties in garlic extract. This is in accordance with Zhong's [25] finding that the position of nano-gold surface plasmon band depends on particles size, distance between the Au NPs and interaction of Au NPs with molecules on the surface. Red shift (SPR @ 520-540 nm) indicates that either the interparticle spacing is short, or the particle aggregation is bigger. In our cases, if CNTs are successfully modified with organothiol groups on the sidewall of MWCNTs, gold nanoparticles should be closely packed along with certain level of particle aggregation due to other organic moieties present in the garlic extract. This was verified when gold nanoparticle solution was subjected to garlic extract under similar reaction conditions which resulted in particle aggregation. To understand more about active functional groups present over the modified nanotube surface, we carried out transmission mode FT-IR measurements.

FT-IR spectrum of unmodified MWCNT is shown in Figure 3a.

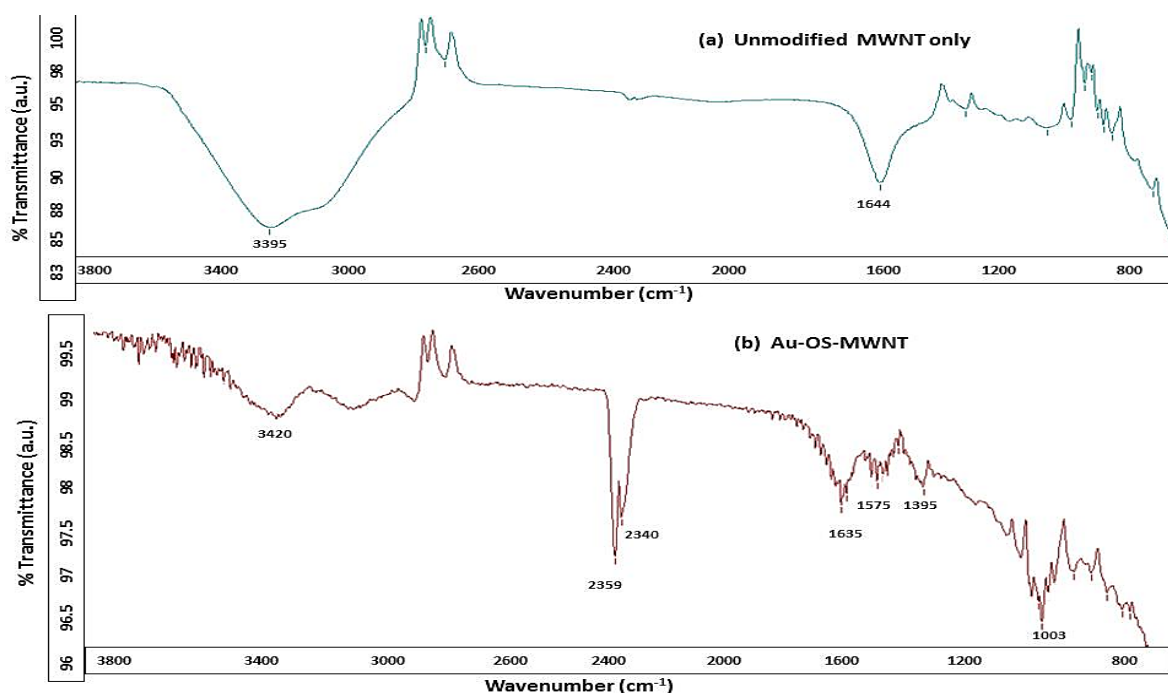


Figure 3. (a) FT-IR spectra of unmodified MWCNT; (b) FT-IR spectra of Au-OS-MWCNT.

OS-MWCNT decorated with Au NPs (Figure 3b) showed signature bands at 1635 cm^{-1} , 1575 cm^{-1} and 1395 cm^{-1} associated with the stretching of nanotube backbone. Also, evidence of utilizing openings/defects in graphite wall of MWCNT was denoted by characteristic absorbance of $\text{C}\equiv\text{C}$ at $2200 - 2400\text{ cm}^{-1}$ region. Similar result was obtained by Zhang et. al [26] with nitrile group addition on the sidewall of CNT. It is important to note that the band obtained at 1003 cm^{-1} confirms the presence of thiocarbonyl center whose presence was detected by absorption in $1250 - 1000\text{ cm}^{-1}$ region. Since, absorption occurs in the same region as C–O stretching, considerable interactions can occur within these vibrations within a single molecule. EDX study (Figure 4) further confirmed the presence of sulfur in functionalized MWCNT.

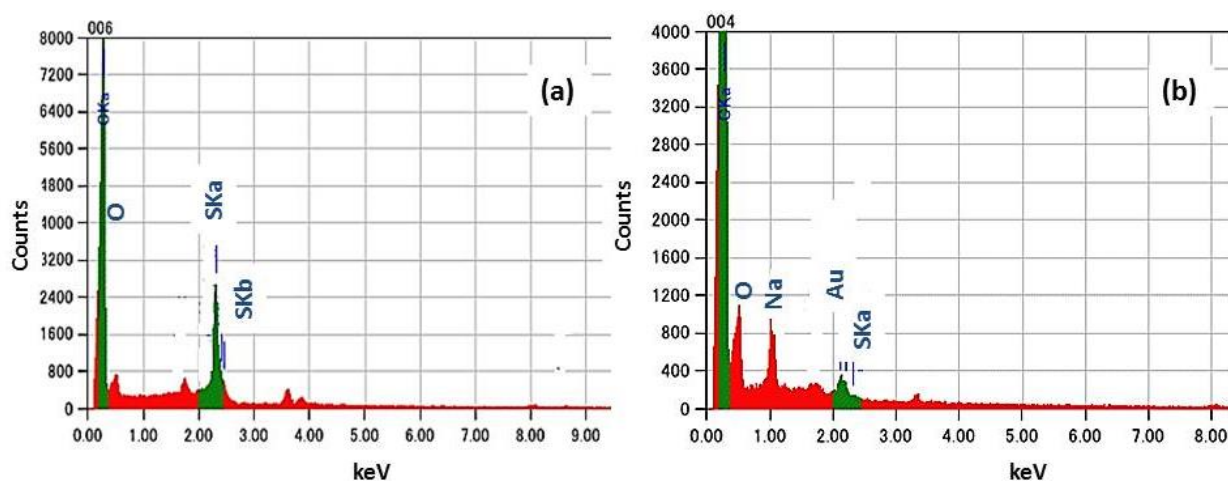


Figure 4. (a) EDX data of OS-MWCNT (no Au NP deposition; (b) EDX data of Au-OS-MWCNT.

Figure 4a shows EDX spectra of OS-MWCNT (without Au NP attachment) and Figure 4b shows Au-OS-MWCNT. Gold loading of around 0.78 atomic wt% was obtained in Au-OS-MWCNT while no such result was obtained from control sample. We also observed the characteristic 2:1 stoichiometric ratio between sulfur and gold at some reaction sites over the Au-OS-MWCNT suggesting formation of dithiols as active biolinker. However, this ratio varied from reaction site being observed over the Au-OS-MWCNT surface. We found that OS-MWCNT can have sulfur content in the range of 0.4-1 atomic wt%. Upon pyrolysis, the garlic extract confirmed the presence of around 0.17% of sulfur left in the reaction mixture. Further, the initial sulfur concentration in the organosulfur extract was found to be around 1.8%.

Surface modification was confirmed by Raman spectroscopy as shown in Figure 5.

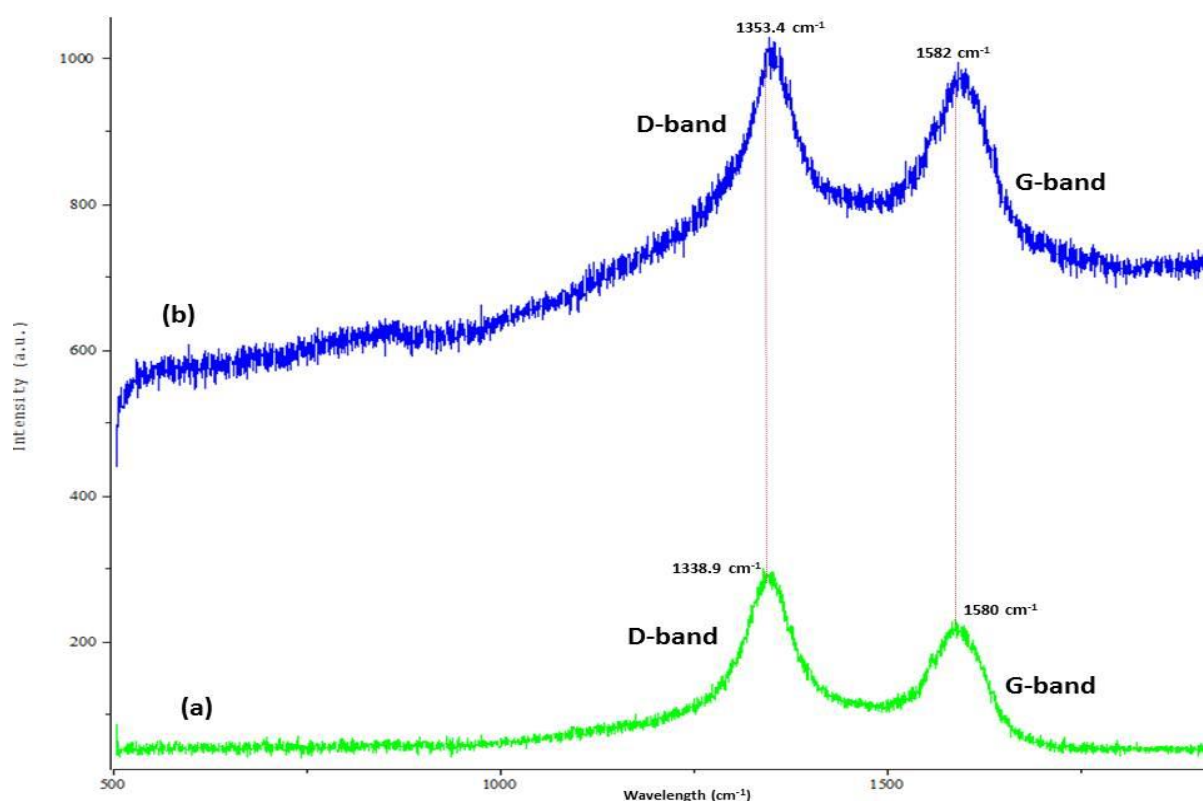


Figure 5. Raman spectra of (a) pristine MWCNT and (b) organosulfur modified MWCNT.

Figure 5a shows Raman spectra of pristine MWCNTs while Figure 5b shows that of OS-MWCNTs. Pristine MWCNTs showed G and D bands at 1580 cm^{-1} and 1338.9 cm^{-1} respectively. These G and D bands got shifted to 1582 cm^{-1} and 1353.4 cm^{-1} respectively in case of OS-MWCNTs. The higher upshift in D-band region towards the higher wavelength and lower shift in G-band is commonly observed in case of dopants containing oxygen atoms in their structure. It is known that oxygen can easily get attached to the defect sites in nanotube network leading to greater modification in defect band [27]. Further, the G/D intensity ratio of pristine MWCNTs (1.18) as compared to OS-MWCNTs (1.16) suggests decrease in relative sample purity due to CNT surface modification. These chemical modification results in the upshift of G and D bands which are related to direct electron charge transfer process from the nanotube to the acceptor molecule and are generally time-

dependent [28]. Thus, Raman spectra confirmed the surface modification took place while FT-IR and EDX suggested towards the presence of sulfur (organothiols) in the reaction mixture. Confirmatory proof of organosulfur modified nanotube surface with facilitating Au NP attachment was obtained by XPS. The presence of oxygen (and in turn, oxidation of MWCNT surface) was further highlighted in full scale spectrum of Au-OS-MWCNTs as depicted in Figure 6.

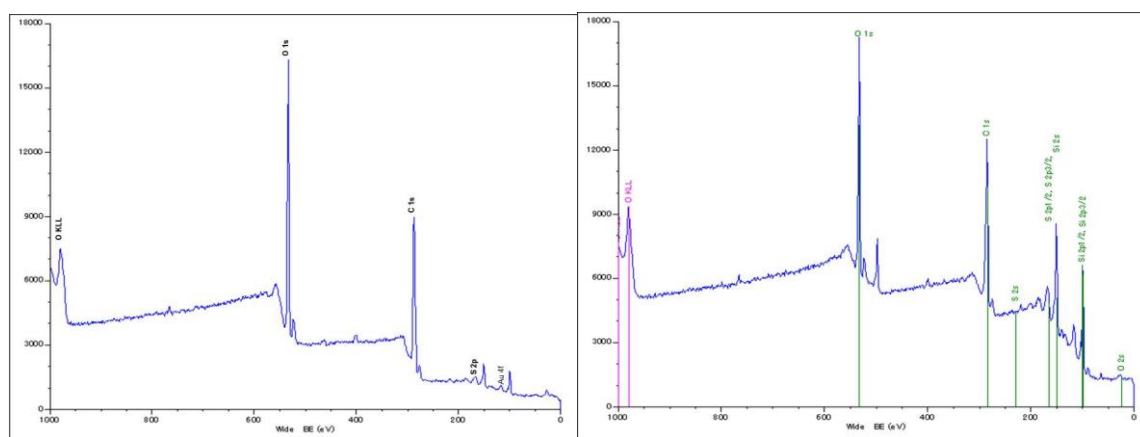


Figure 6. (L to R) Full-scale XPS spectra of Au-OS-MWCNTs and OS-MWCNTs

A further, full spectrum of OS-MWCNT (without Au NP attachment) is shown in Figure 6. The peak at position 116.3 eV is of the Aluminium sample grid and shouldn't be confused for Au (which was further confirmed as no Au NPs were visible by TEM nor any peak signal received for Au 4f region in case of OS-MWCNTs). Several peaks were observed in case of XPS spectra of OS-MWCNTs (fig. 6b) when compared with Au-OS-MWCNTs (fig 6a.) indicating towards the presence of intermediates/impurities on MWCNT surface. However, cleaning of nanotubes with butanol (as described in the manuscript) resulted in removal of these intermediates/impurities.

These results confirm that the organosulfurs present over the modified nanotube surface (thiocarbonyl and thiol linkages) are responsible for Au NP attachment.

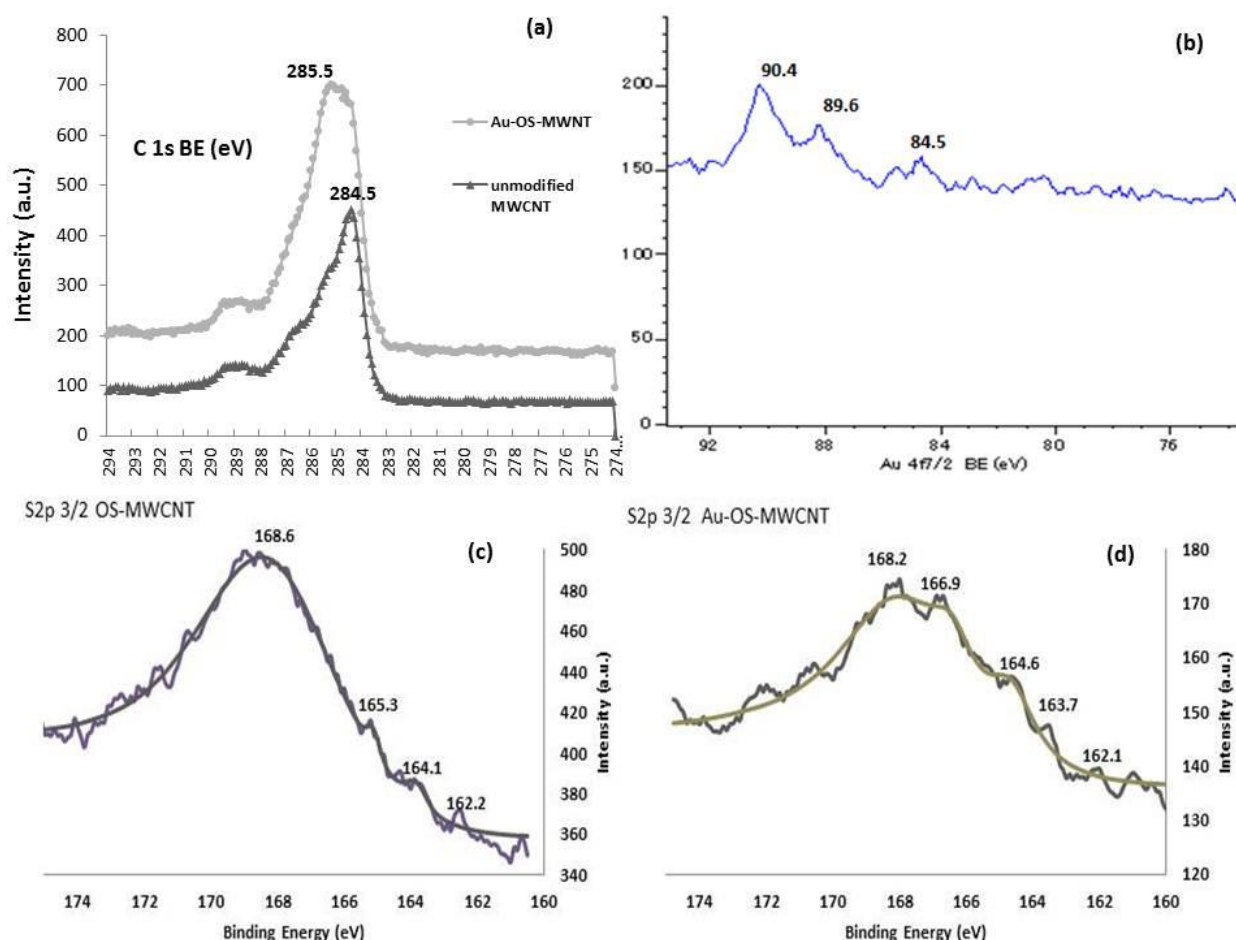


Figure 7. XPS spectra (a) C1s spectra of modified and unmodified MWCNTs; (b) Au 4f spectra of Au NPs attached onto Au-OS-MWCNTs; (c) S2p spectra of OS-MWCNT; (d) S2p spectra of Au-OS-MWCNTs

In present case (fig 7a), unmodified MWCNT C1 component of C 1s spectrum was represented at 284.5 eV [29]. This when compared to Au-OS-MWCNT composite showed an increase of C 1s spectral peak width upon functionalization, from 1.4 to 1.9 eV. This is expected when electron delocalization becomes limited [30, 31] as a result of interactions between thiols and CNT sidewall. Broadening is also observed due to overlapping of CNT and organosulfur C 1s spectral envelopes, which causes an increased intensity in binding

energy region around 286.5 eV [32] (C-SH) as observed in our case. Both contributions are manifested as increases in peak width and asymmetry. OS-MWCNTs after treatment with Au NP solution exhibits a new peak at Au4f region (Figure 7b) together with C1s peak at 285.5 eV and S2p peak between the regions 162 eV-164 eV responsible for thiol interactions. In Figure 7b, component at 84.5 eV (88.2 for the 4f5/2 component) is assigned to Au⁰. In particular, the positions of the Au4f7/2 signal assigned to the Au⁰ peak present a binding energy sensitively higher respect to the 84 eV value of bulk gold (88 eV for the 4f5/2 component). This is an indication of the presence of very small gold nanoclusters in the samples due to the presence of metallic nuclei that acts as centers for the successive grow of the gold nanoparticles. This can also result from the coalescence of Au NPs when deposited over the OS-MWCNTs as observed in this study. Further, decrease in relative peak intensities between the two S2p spectra (fig. 7 c, d) can be clearly observed after Au NP attachment which further supports that S-moieties are responsible for Au NP attachment. Characteristic sulfur-oxygen interaction at around 168 eV region was also observed which is common in case of natural organosulfurs. This confirms that the attachment of Au-NPs to modified CNT surface can be achieved via thiol and thiocarbonyl linkages. Similar results were reported by Nakamura et al. [33] for carbon surface modification via elemental sulfur and subsequent interaction with Au NPs.

Finally, based upon our experimental observation, we were able to hypothesize a free-radical mechanism (Figure 8) which may be responsible for CNT surface modification in presence of alliin/allicin as major organosulfur groups in garlic. The thiolated CNTs obtained as end-product can then be used for Au NPs deposition over the modified nanotube surface.

4. Conclusion

We have shown ‘one-pot’ sidewall attachment of Au NPs to MWCNTs treated with garlic plant extract (organosulfurs) by a facile eco-friendly process. As per our knowledge, this is the first study to effectively utilize plant phytochemicals for sidewall modification of CNTs under moderate temperature conditions. Such bio-inspired materials may facilitate novel synthesis and application routes in a more sustainable manner. Finally, an interdisciplinary research will aid in better understanding of underlying mechanism and properties this nanocomposite may possess.

References

- [1] D. Tasis, N. Tagmatarchis, A. Bianco, M. Prato, *Chem. Rev.* 2006, 106, 1105.
- [2] H. Dai, *Acc. Chem. Res.* 2002, 35, 1035.
- [3] M. A. El-Sayed, S. Eustis, *Chem. Soc. Rev.* 2006, 35, 209.
- [4] B. C. Satishkumar, E. M. Vogl, A. Govindaraj, C. N. R. Rao, *J. Phys. D Appl. Phys.* 1996, 29, 31273.
- [5] K. Jiang, A. Eitan, L. S. Schadler, P. M. Ajayan, R. W. Siegel, *Nano Lett.* 2003, 3, 275.
- [6] A. Eitan, K. Jiang, D. Dukes, R. Andrews, L. S. Schadler, *Chem. Mater.* 2003, 15, 3198.
- [7] R. Parthasarathy, X. Lin, H. M. Jaeger, *Phys. Rev. Lett.* 2001, 87, 1868071.
- [8] A. V. Ellis, K. Vijayamohan, R. Goswami, N. Chakrapani, L. S. Ramanathan, P. M. Ajayan, G. Ramanath, *Nano Lett.* 2003, 3, 279.
- [9] R. Zhang, M. Hummelgard, H. Olin, *Mater. Sci. Eng. B* 2009, 158, 48.
- [10] Y. Shia, R. Yangb, P. K. Yueta, *Carbon* 2009, 47, 1146.
- [11] D. Gingery, P. Bühlmann, *Carbon* 2009, 46, 1966.
- [12] H. Hu, P. Bhowmik, B. Zhao, M. A. Hamon, M. E. Itkis, R. C. Haddon, *Chem. Phys. Lett.* 2001, 345, 25.
- [13] J. Zhao, H. Park H, J. Han, J. P. Lu, *J. Phys. Chem. B* 2004, 108, 4227.
- [14] R. Zanella, E. V. Basiuk, P. Santiago, V. A. Basiuk, E. Mireles, I. Puente-Lee, J. M. Saniger, *J. Phys. Chem. B* 2005, 109, 16290.
- [15] S. Banerjee, S. S. Wong, *Nano Lett.* 2002, 2, 195.
- [16] L. Zhao, L. Gao, *J. Mater. Chem.* 2004, 14, 1001.
- [17] S. Harpovic, Y. Liu, K. B. Male, J. H. T. Luong, *Anal. Chem.* 2004, 76, 1083.
- [18] H. Amagase, B. L. Petesch, H. Matsuura, S. Kasuga, Y. Itakura, *J. Nutr.* 2001, 131, 955S.
- [19] G. M. Volk, *D. Stern, Hortscience* 2009, 44, 12238.
- [20] L. D. Lawson, B. D. Hughes, *Planta. Med.* 1992, 58, 345.
- [21] A. Kamel, M. Saleh (2000) *Studies in Natural Products Chemistry*, Elsevier, Amsterdam.

- [22] J. L. Bahr, J. M. Tour J. Mater. Chem. 2002, 12, 1952.
- [23] V. A. Basiuk, K. Kobayashi, T. Kaneko, Y. Negishi, E. V. Basiuk, J. M. Saniger-Blesa Nano. Lett. 2002, 2, 789.
- [24] J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, A. Plech, J. Phys. Chem. B 2006, 110, 15700.
- [25] Z. Zhong, S. Patskovskyy, P. Bouvrette, J. H. T. Luong, A. Gedanken. J. Phys. Chem. B 2004, 108, 4046.
- [26] M. Zhang, L. Su, L. Mao, Carbon 2006, 44, 276.
- [27] R. Graupner, J. Abraham, A. Vencelova, T. Seyller, F. Hennrich, M. M. Kappes et al. Phys. Chem. Chem. Phys. 2003, 5, 5472.
- [28] A. G. Souza Filho, V. Meunier, M. Terrones, B. G. Sumpter, E. B. Barros, F. Villalpando-Paez et al. Nano Lett. 2007, 7, 2383.
- [29] Y. Xing, L. Li, C. C. Chusuei, R. V. Hull, Langmuir 2005, 21, 4185.
- [30] D. Q. Yang, E. Sacher, Langmuir 2006, 22, 860.
- [31] D. Q. Yang, E. Sacher, Surf. Sci. 2002, 504, 125.
- [32] D. Q. Yang, B. Hennequin, E. Sacher, Chem. Mater. 2006, 18, 5033.
- [33] T. Nakamura, T. Ohana, Y. Hagiwara, T. Tsubota, Phys. Chem. Chem. Phys. 2009, 11, 730.

Chapter 6.

Green synthesis of thiolated graphene nanosheets by Alliin (garlic) and its effect on the deposition of gold nanoparticles

Green synthesis of thiolated graphene nanosheets by Alliin (garlic) and its effect on the deposition of gold nanoparticles

1. Introduction

Graphene has attracted tremendous attention and vast amount of research for various applications within a decade [1]. This relatively new two-dimensional nanomaterial comprising of sp^2 -bonded carbon atoms promises to be the next generation building block for composite materials [2]. Attachment of nanoparticles on graphene has offered greater opportunities in application of graphene oxide (GO) and graphene nanosheets (GNS) [3, 4, 5]. Several chemical methods for surface modification of graphene [6] have been reported including exfoliation by chemicals/strong acids [7], sheet formation by carbon vapour deposition (CVD) [8] and thermal reduction of GO under high temperatures conditions [9]. Also, non-covalent functionalization techniques exploit π - π stacking of graphene sheets via peptides [10], surfactants [11] and direct reduction of metal salts [12] in presence of GO. However, direct in-situ reduction of metallic salts over GO sheets has resulted in poor control over the particle size and morphology thereby affecting metal-GO composite properties in general [13]. Therefore, there is an urgent need for providing eco-friendly alternative for graphene functionalization without using toxic chemicals, excessive solvents, expensive instruments and tedious process control. Here, we report a unique green functionalization technique for GO surface modification via alliin (3-(2-Propenylsulfinyl) alanine) under ambient temperature conditions in aqueous solution and its effect gold nanoparticle attachment. In our previous research, we reported that organosulfur compounds in garlic particularly alliin-allycin are responsible for carbon nanotube surface thiolation and subsequent attachment of Au NPs [14]. In this study, we extended the application of alliin [15,

16, 17] found in garlic cloves (*Allivum Sativum*) in preparation of thiolated-GO from GO and subsequent attachment of Au NPs. Gold was selected as an ideal recipient for S-linkages attachment [18] (like thiol) along with several potential applications in catalysis, optics and nanobiotechnology [19].

2. Experimental

2.1 Surface modification of graphene

Alliin and Graphene Oxide (GO) were purchased from Sigma Aldrich (Japan). 4 mg of alliin was dissolved in 2 mL of H₂O:EtOH (95:5 v/v) in plastic vials and sonicated till dissolved completely. To this alliin solution, 5 mg GO were introduced and sonicated for 30 minutes. The GO-alliin mixture was then heated at 100°C for 3 hours in sealed vials. Care should be taken to seal the plastic vials properly as subsequent heating may result in accidental spillage.

This reaction mixture was sonicated for 15 seconds at every hour to homogenize the reaction solution. Excess of alliin solution was removed from the reaction mixture by centrifugation at 3500 × g for 5 minutes. Finally, the obtained organosulfur modified GNS (to be referred as OS-GNS) was dried at 60 °C and stored for further use.

At this stage, a notable color shift between GO and OS-GNS can be observed by the color change from brownish tint to black.

2.2 Synthesis and attachment of gold nanoparticles over modified GNS

Gold nanoparticles were prepared by the reaction of HAuCl₄ and citric acid. In a typical experiment, 0.016 g of HAuCl₄ and 0.019g citric acid, both dissolved separately in 5 mL of milliQ H₂O were stirred together for 60 minutes to produce Au NPs [20]. These Au NPs were then allowed to stand overnight before being added drop-wise into the dispersion of OS-GNS to make sure that no Au NP attachment happens due to seeding over the graphene surface [21]. 2mL of Au NP solution was added to 2 mg of OS-GNS in a clean plastic vial.

This dispersion was vigorously stirred at room temperature for 1 h to complete Au NP surface attachment. After this procedure, solid phase was separated by centrifugation (3500 × g for 10 min) and washed six times with milliQ water to remove any non-linking Au NPs over the OS-GNS surface. Finally, we obtained our desired product of OS-GNS decorated with Au NPs (Au-OS-GNS) which was dried at 60 °C and stored away from light in a

vacuum desiccator. As a control test, same procedure of Au NP deposition was performed with pristine GO (referred as GO+Au NPs).

2.3 Characterization studies

Preliminary analysis of Au NP deposition over the organosulfur modified GO surface was done by NanoDrop 3300 Fluorospectrometer operated at 400 nm – 600 nm with relative fluorescence units (RFU) at 540 nm.

GO – with and without Au NP attachment dispersed in H₂O were transferred to carbon coated grid for transmission electron microscopy (TEM) measurements by Joel JEM 1011 electron microscope operated at 200 kV r. Images obtained were analysed using ImageJ 1.43M software.

Transmission mode Fourier transform infrared (FT-IR) spectroscopy was carried out for using Jasco FTIR-680 plus coupled to a high performance computer. The results reported here were obtained from 200 scans at a 4 cm⁻¹ resolution. Confirmatory analysis for sidewall addition of S-linkages/thiol onto CNT surface was done by X-ray photoelectron spectroscopy (XPS) obtained from JEOL JPS9010 MC photoelectron spectrometer operating at 10 kV and 30 mA. The results obtained were analyzed by SpecSurf ver. 1.7.3.9 software.

3. Results and discussion

Preliminary analysis of Au NP deposition over the modified GNS surface was carried out by observing characteristic localized surface plasmon resonance peak (L-SPR) at 540 nm. GO – with and without Au NP attachment dispersed in H₂O:EtOH were also analysed by transmission electron microscopy (TEM) measurement. Finally, confirmatory analysis for surface modification and subsequent organosulfur addition onto GNS surface was done by X-ray photoelectron spectroscopy (XPS). Both Au NPs and Au-OS-GNS samples showed distinct localized SPR peak at around 540 nm when laser excitation was made in the white light region as shown in Figure 1.

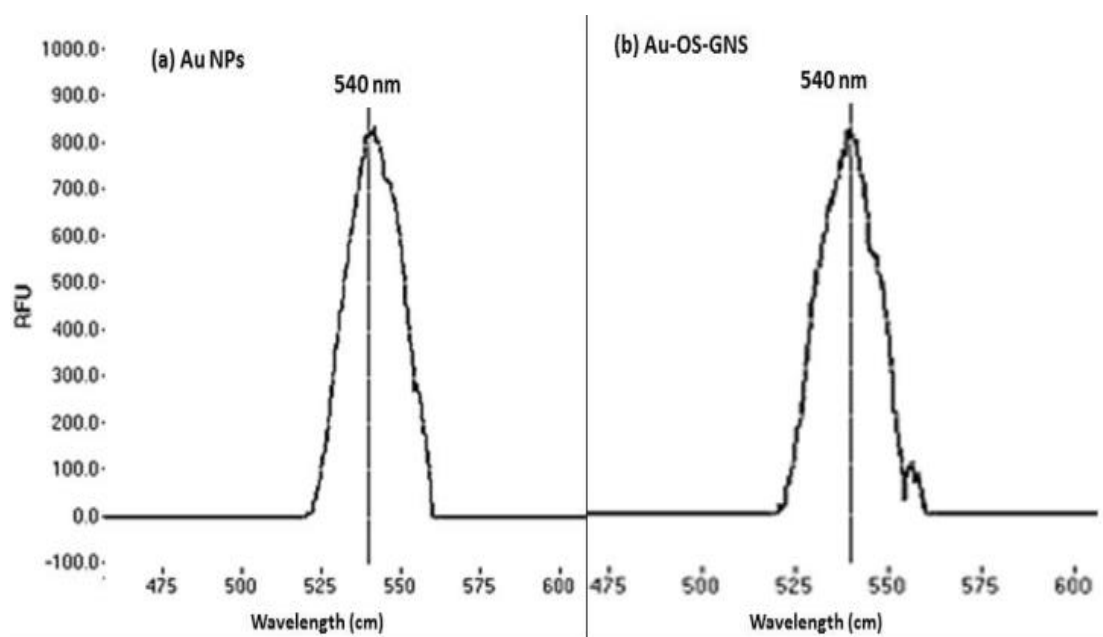


Figure 1. L-SPR observation in (a) Au NPs; (b) Au-OS-GNS

This characteristic absorbance at 520-540 nm suggest towards the presence of gold nanoparticles linked over the GNS surface. The L-SPR peak remained after vigorous sonication and repeated washing. No L-SPR peak was otherwise observed in control sample consisting of GO only and OS-GNS sample.

This preliminary result indicated that Au NPs are attached onto the GNS surface resulting in formation of Au-OS-GNS. Also, retention of optical absorption spectra suggested that there is no adverse influence on nanoparticles. TEM image of Au-OS-GNS was compared with control as shown in Figure 2.

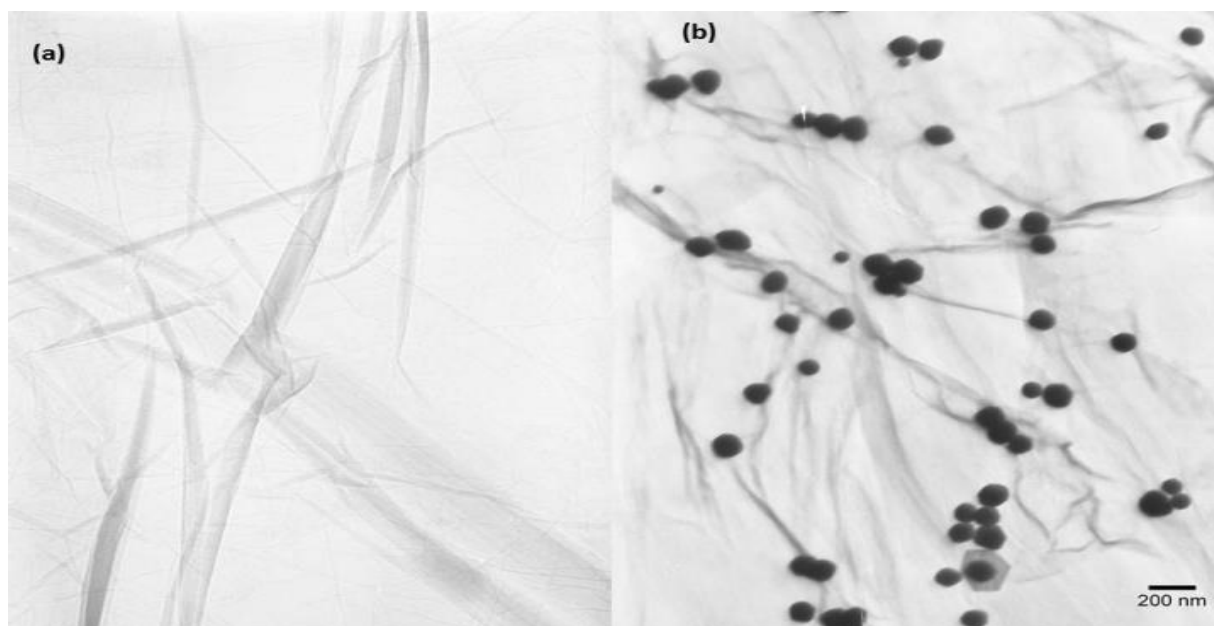


Figure 2. TEM images of (a) GO (control); (b) Au-OS-GNS.

Figure 2b (Au-OS-GNS) clearly showed Au NPs attached on to the alliin modified GNS sheets while no such attachment was observed in control sample. Several Au NPs in the size range of 60-80 nm were found to be adhered to alliin treated GO sheets (OS-GNS). Further, GO, OS-GNS and Au-OS-GNS were analysed by FTIR (Fourier Transform Infrared spectroscopy) as shown in Figure 3. FTIR spectra was analysed for different density of oxygen functionalities present at the surface of GO, OS-GNS, and Au-OS-GNS. The absorption band corresponding to the C=O carbonyl and carboxyl stretching was represented at 1706 cm^{-1} and 1251 cm^{-1} respectively [22, 23]. The C-O (epoxy) and C-O (alcoxy) stretching at 1251 cm^{-1} and 1057 cm^{-1} respectively [24]. Note that the entire spectrum showed a C=C peak centred around $1550\text{-}1650\text{ cm}^{-1}$ corresponding to remaining sp^2 character of the C-linkages. It is evident that oxygen functionalities decreased drastically in case of OS-GNS

and Au-OS-GNS. Both the reaction samples showed decrease in C=O functionalities in particular, suggesting decreased oxygen double bond character in GNS post-alliin treatment. XPS analysis was done to further study the surface of each graphene substrate (GO, OS-GO and OS-GNS-Au) and confirms chemical groups responsible for Au NP attachment. Although, the presented C1s spectra (Figure 4a) and O1s (Figure 4b) can be further divided into smaller components to precisely allocate the different bonding regions, for the ease of comparison and data interpretation, we intended it be unaltered. In present case (fig 6a), pristine GO sheets exhibit C1 component of C 1s spectrum at 288.4 eV and 286.2 corresponding to O=C-OH/C=O and C-O groups respectively [25].

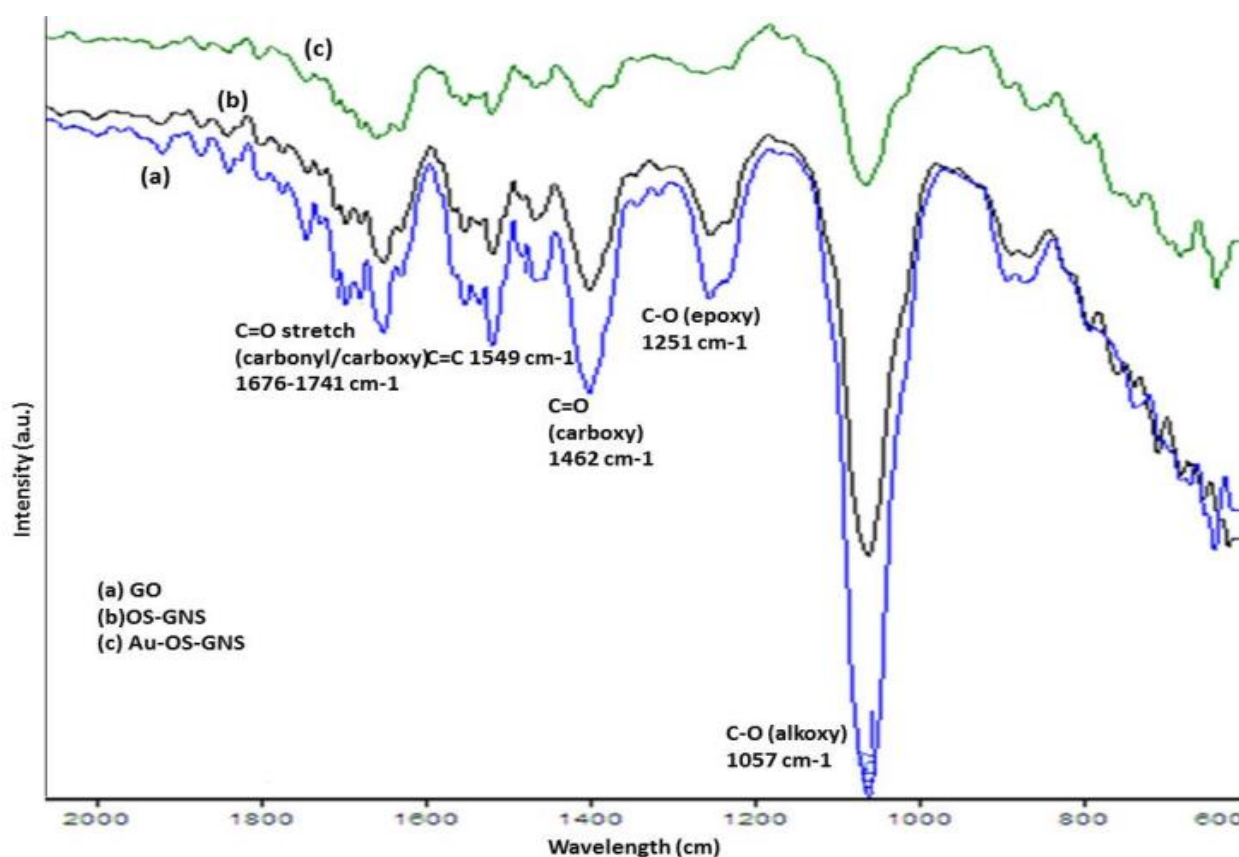


Figure 3. FT-IR spectra of (a) GO; (b) OS-GNS; (c) Au-OS-GNS.

This C1s spectrum of GO sheets when compared with OS-GNS and Au-OS-GNS clearly confirmed major peak shift from carbon-oxygen bond majority to carbon-carbon bond majority (Binding energy of C1s spectrum shifting from higher energy to lower energy). The same was highlighted by FT-IR analysis.

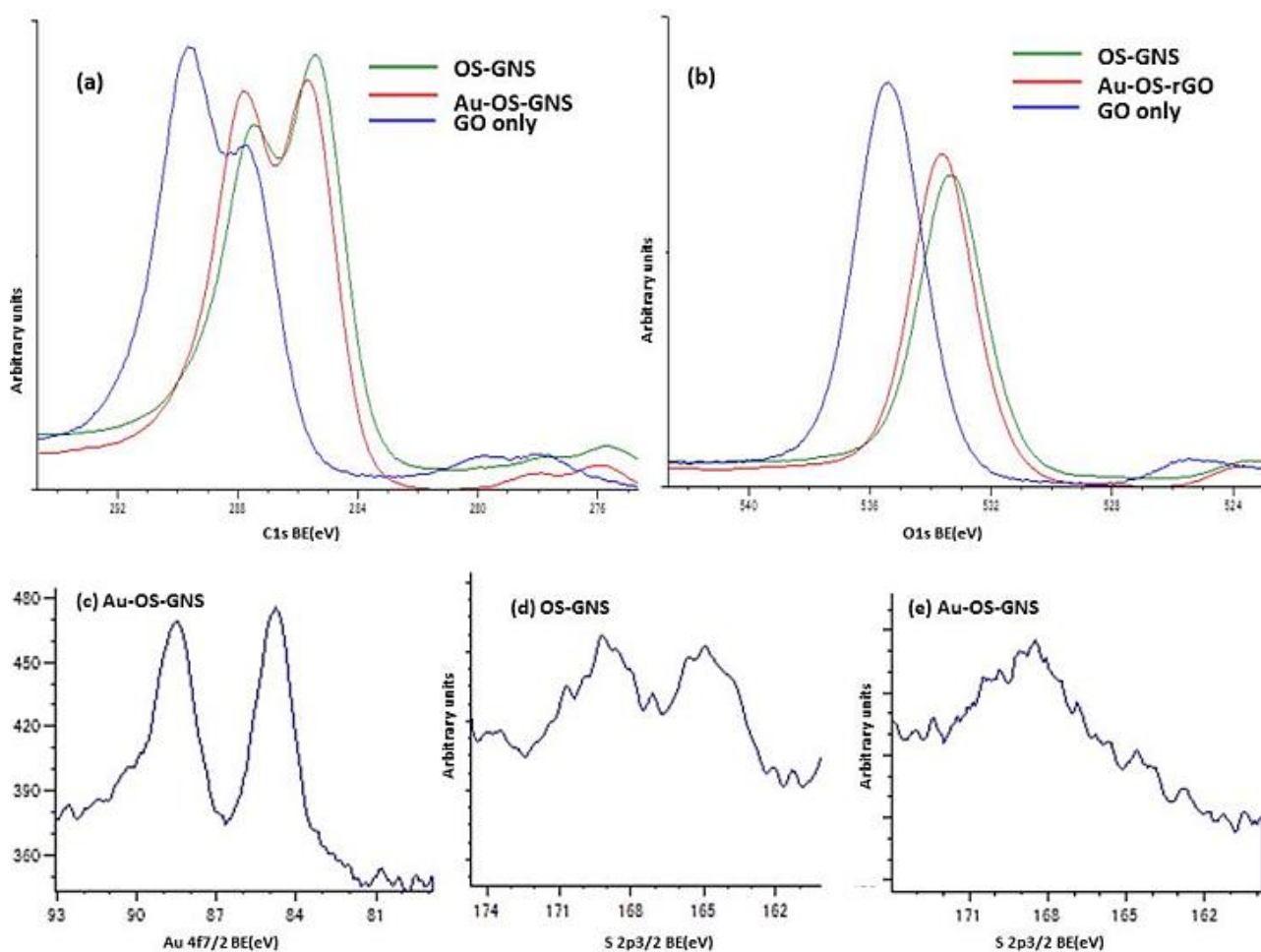


Figure 4. XPS spectra of (a) C1s region; (b) O1s region; (c) Au4f region in Au-OS-GNS; (d) S2p region in OS-GNS; S2p region in Au-OS-GNS.

The O=C-OH peak region arising due to carboxylate carbon was completely absent in OS-GNS and Au-OS-GNS with appearance of prominent C-C/C=C at lower energy regions. C1s spectra of OS-GNS showed two major peaks at 285.8 and 283.5 corresponding to singlet C-bound oxygen moieties like C-OH and C-C/C=C respectively [26]. Similarly, Au-OS-GNS (Figure 4c) showed two major peak at 286.3 eV and 283.1 eV corresponding to singlet C-O and C-C/C=C bonds. Both reaction mixture (OS-GNS and Au-OS-GNS) showed absence of peaks at higher binding energy region for C1s spectrum suggesting that -C=O and -O=C-OH group are absent once GO is treated with alliin. This decrease in oxygen bounded groups in GO and its subsequent reduction to GNS was also confirmed when O1s spectrum of reaction

and control mixtures were compared. Figure 4b shows comparison of O1s spectrum of GO with OS-GNS and Au-OS-GNS. A distinct peak shift and decrease in intensity was observed when GO (con) at 535.4 eV was compared with GNS and Au-OS-GNS at 533.6 and 533.3 respectively. The relative decrease in O1s peak intensity and peak shift towards lower BE values clearly suggest that GO has been successfully undergone partial reduction with formation of graphene sheets (GNS).

It should be noted that the essence of reduction is transformation of sp^3 -C to sp^2 -C. Also, presence of single bonded oxygen groups over the GO surface reduces the Van der Waal's forces between the C-layers and improving stability and facilitating easy exfoliation of GO into monolayers for subsequent chemical reduction.

To further confirm Au NP particle attachment, Au 4f and S2p bands were observed in OS-GNS and Au-OS-GNS. OS-GNS after treatment with Au NP solution (Au-OS-GNS) showed distinct presence of Au NPs linked to resulting Au-OS-GNS nanocomposite surface as evidenced in Figure 4c. In Figure 4c, XPS peaks at 84.7 eV and 88.5 eV corresponds to Au NPs present over OS-GNS sheets in metallic state. Also, change in peaks were observed in the S2p region in case of OS-GNS and Au-OS-GNS which were responsible for Au NP attachment as shown in Figure 4d, 4e. It is interesting to note that in case of OS-GNS, S2p component clearly showed two peaks at 169.2 eV and 164.9 eV. Characteristic oxygen-sulphur interactions are generally observed at higher energy levels (~ 168 eV) at around 168 eV region in case of natural organosulfurs. The lower binding energy at around 164 eV region corresponds to characteristic thiocarbonyl/sulfhydryl interactions. The S2p component of OS-GNS when compared with Au-OS-GNS showed absence of free thiocarbonyl linkages over OS-GNS at around 164 eV [27] and peak shifting of organothiol groups (168.4 eV) due to S-linked interactions with Au NP. This clearly demonstrates that resulting Au-OS-GNS was being formed by attachment of Au NPs via virtue of these organothiol interactions imparted over OS-GNS surface by alliin treatment.

Similar results were reported by Nakamura et. al. [28] for carbon surface modification via elemental sulphur and subsequent interaction with Au NPs. Based upon our initial observations, a free-radical mechanism comprising of ($\text{H}_3\text{C-O-S}^\bullet$) and ($\text{HC}^\bullet=\text{S}$) resulting from thermal decomposition of alliin into methyl methane thiosulfinates [29] may be responsible for this reaction. The thiolated GNS obtained as end-product can then be used for Au NPs deposition over the modified OS-GNS surface. This preliminary study highlights the chemical modification of graphene oxide by a natural phytochemical and adds valuable information to the ever growing community of biogenic/biomimetic materials. It is interesting to note that very recently a few research works have been reported suggesting use of natural products [30, 31, 32] in surface modification of graphene but no study till now claimed one-step thiolation or subsequent nanoparticle attachment.

4. Conclusion

In summary, we have demonstrated one-step thiolation of graphene and subsequent preparation of Au-graphene nanocomposite by a green process via garlic plant extract (alliin). As per our knowledge, this is the first study to effectively utilize plant phytochemicals for rapid thiolation of graphene oxide under moderate temperature conditions. Finally, an interdisciplinary research will aid in better understanding of underlying mechanism this bio-inspired material may possess and offers a promising sustainable alternative for Au-graphene nanocomposite production.

References

- [1] K. Novoselov, A. Geim, S. Morozov, D. Jiang, Y. Zhang, S. Dubonos, I. Grigorieva and A. Firsov, *Science* 2004, 306, 666.
- [2] Y. J. Gan, L. T. Sun and F. Banhart, *Small* 2008, 4, 587.
- [3] W. Hong, H. Bai, Y. Xu, Z. Yao, Z. Gu and G. Shi, *J. Phys. Chem. C* 2010, 114, 1822.
- [4] Y. Zhu, S. Murali, W. Cai, X. Li, J. W. Suk, J. R. Potts, R. S. Ruoff, *Adv. Mater.* 2010, 22, 3906.
- [5] S. Stankovich, D. A. Dikin, G. H. B. Dommett, K. M. Kohlhaas, E. J. Zimney, E. A. Stach, R. D. Piner, S. T. Nguyen and R. S. Ruoff, *Nature* 2006, 442, 282.
- [6] C. K. Chua, M. Pumera, *Chem. Soc. Rev.* 2013, 42, 3222.
- [7] T. Szabo, O. Berkesi, P. Forgo, K. Josepovits, Y. Sanakis, D. Petridis, I. Dekany, *Chem. Mater.* 2006, 18, 2740.
- [8] X. Li, W. Cai, J. An, S. Kim, J. Nah, D. Yang, R. Piner, A. Velamakanni, I. Jung, E. Tutuc, S. K. Banerjee, L. Colombo, R. S. Ruoff, *Science* 2009, 324, 1312.
- [9] H. C. Schniepp, J. L. Li, M. J. McAllister, H. Sai, M. Herrera-Alonso, D. H. Adamson, R. K. Prud'homme, R. Car, D. A. Saville, I. A. Aksay, *J. Phys. Chem. B* 2006, 110, 8535.
- [10] J. Liu, S. Fu, B. Yuan, Y. Li and Z. Deng, *J. Am. Chem. Soc.* 2010, 132, 7279.
- [11] S. Stankovich, R.D. Piner, X. Chen, N. Wu, S.T. Nguyen, R.S. Ruoff, *J. Mater. Chem.* 2006, 16, 155.
- [12] X. Zhou, X. Huang, X. Qi, S. Wu, C. Xue, F. Y. C. Boey, Q. Yan, P. Chen and Hua Zhang, *J. Phys. Chem. C* 2009, 113, 10842.
- [13] B. S. Kong, J. Geng and H. T. Jung, *Chem. Comm.* 2009, 16, 2174.
- [14] S. K. Srivastava, R. Yamada, C. Ogino, A. Kondo, *Carbon* 2013, 56, 309.
- [15] H. Amagase, B. L. Petesch, H. Matsuura, S. Kasuga, Y. Itakura, *J. Nutr.* 2001, 131, 955S.
- [16] G. M. Volk, *D. Stern, Hortscience* 2009, 44, 12238.

- [17] L. D. Lawson, B. D. Hughes, *Planta. Med.* 1992, 58,345.
- [18] H. Häkkinen, *Nat. Chem.* 2012, 4, 443.
- [19] C. M. Shen, C. Hui, T. Z. Yang, C. W. Xiao, J. F. Tian, L. H. Bao, S.T. Chen, H. Ding, H. J. Gao, *Chem. Mater.* 2008, 20, 6939.
- [20] J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, A. Plech, *J. Phys. Chem. B* 2006, 110, 15700.
- [21] G. Goncalves, P. A. A. P. Marques, C. M. Granadeiro, H. I. S. Nogueira, M. K. Singh and J. Grácio, *Chem. Mater.* 2009, 21, 4796.
- [22] S. Stankovich, R.D. Piner, S.T. Nguyen, R.S. Ruoff, *Carbon* 2006, 44, 3342.
- [23] J. I. Perdes, S. Villar-Rodil, A. Martinez-Alonso, J. M. D. Tascon, *Langmuir* 2008, 24, 10560.
- [24] A. B. Bourlinos, D. Gournis, D. Petridis, T. Szabo, A. Szeri, I. Dekany, *Langmuir* 2003, 19, 6050.
- [25] Y. Xu, H. Bai, G. Lu, C. Li, G. Shi, *J. Am. Chem. Soc.*, 2008, 130, 5856.
- [26] X. R. Zeng and T. M. Ko, *J. Polym. Sci. B: Polym. Phys.* 1997, 35, 1993.
- [27] A. Chen, J. Qi, Q. Zhao, Y. Li, G. Zhang, F. Zhang, X. Fan, *RSC Adv.* 2013, 3, 8973.
- [28] T. Nakamura, T. Ohana, Y. Hagiwara, T. Tsubota, *Phys. Chem. Chem. Phys.* 2009, 11, 730.
- [29] J W Kim, K H Kyung, *J. Food Sci.* 2003, 68, 1766.
- [30] T. Kuila, S. Bose, P. Khanra, A. K. Mishra, N. H. Kim, J. H. Lee, *Carbon* 2012, 50, 914.
- [31] J. Zhang, H. Yang, G. Shen, P. Cheng, J. Zhang, S. Guo, *Chem. Comm.* 2010, 46, 1112.
- [32] C. Zhu, S. Guo, Y. Fang, S. Dong, *ACS Nano* 2010, 4, 2429

Summary

In last two decades, nanotechnology made considerable progress and has found applications in everyday life. Biogenic nanomaterials although just a decade old, promise to be a key building block in ‘green nanomaterials’. In this thesis, we discussed in details how nanoparticles, carbonaceous materials and their combined nanocomposites can be prepared by novel green approaches and have the potential to contribute significantly in catalysis, clean energy, sensors, biomedical, composites etc. The primary focus of our research was to provide alternative biogenic routes for synthesis/functionalization of nanomaterials. For better understanding of the underlying properties, we also provided a secondary study highlighting prospective usage of such materials.

In Chapter 2, 3 and 4 of this thesis, we provided an in-depth discussion about monometallic and bimetallic nanoparticles and their application via biogenic means. Each of these research works has a novel aspect associated with it which provides a key-solution in the field of green nanocomposites. In chapter 2, we showed bacterial mediated synthesis of Au NPs and also identified the membrane bound protein fraction responsible for the same. In absence of these proteins (activity loss), no nanoparticle formation was observed. We went a step further and tried utilizing the well laid concept of carbon-support heterogeneous catalysts. We ‘baked’ bacterial membrane fraction along with Au NPs to make an interesting heterogeneous catalyst with excellent oxidation capability for 4-nitrophenol degradation. It is interesting to note that the associated published article for this research achieved the “highly accessed” status with over 2000+ downloads soon after its publication thereby suggesting the interest level generated by this work. Contrary to monometallic NPs which are being synthesized by a vast array of biogenic means, we observed that bimetallic nanoparticles are still left untouched. Upon probing further, we found out

that even though bimetallic particles can be produced via biogenic methods, achieving the same with core-shell morphology is a challenge left unaddressed. Our past experiences and batch runs suggested that amino acids like tryptophan has the potential to form and stabilize Au core by the virtue of its H-bonded poly-Trp layer. Upon investigating further, we found out that it is possible to carry out successive reduction over the pre-synthesized Au core with other metals like Pd and Pt thereby generating core-shell morphology. This was highlighted in chapter 3 and chapter 4 where we showed preliminary reporting of core-shell nanoparticle synthesis via supramolecular Trp interphase. In chapter 3, we synthesized Au@Pd NPs with the same concept and discussed in details about the surface composition and proposed mechanism. We also checked the oxidation potential of these core shell nanoparticles over silica beads for degradation of 4-NP. This study gave us confidence to tackle on of the key challenges of our current times- improved electrodes for clean energy. In chapter 4, we tried addressing two key-points simultaneously for electro-oxidation reactions involving Pt metal. Firstly, we provided a green fabrication technique thereby making a seamless technology where production, assemble and application all justifies sustainability. Second, we were able to achieve high Pt loading over Au core in a very facile manner. Finally, the applied voltage needed and associated HER with Au@Pt NPs happens to be among the best we have seen so far in literature.

Soon after this, our focus shifted to another vital component of nanomaterials- carbonaceous materials. It is interesting to note that there exist almost a handful of biogenic techniques for surface modification of carbonaceous materials like CNTs and graphene. Therefore, we dedicated the next phase of our research for developing nanocomposites including carbon-NP framework. We developed a first ever surface functionalization technique for MWCNTs by utilizing garlic clove extract as covered

in chapter 5. It is important to note that this also happens to be the first ever technique across all domains of surface modifications which facilitated one-step thiolation and subsequent Au NP attachment over the modified MWCNTs. After a while, we were able to successfully isolate and study two major components aiding in this thiolation strategy namely alliin and allicin. These two natural organothiols of garlic showed interesting chemical properties and we made us believe that a similar thiolation strategy can be worked out for graphene. Again, till date, there exists no green thiolation strategy for surface modification of graphene. In chapter 6, we utilized graphene oxide and converted it to thiolated graphene nanosheets. This was followed by subsequent attachment of Au NPs and also resulted in decreased double bond character of oxygen moieties.

Therefore, we actually worked across the length and breadth of biogenic nanomaterials by working on substrates as different as monometallic nanoparticles, bimetallic nanoparticles, CNTs, graphene and their associated composites. Further, we developed various biogenic strategies ranging from application of microbes, amino acids and plant extracts. The work presented in this thesis fully justifies our pre-set goals and contributes significantly in this ever-dominant area of biogenic materials.

Publication List

1. Srivastava, S.K., Yamada, R., Ogino, C., Kondo, A. "Green synthesis of thiolated graphene nanosheets by Alliin (garlic) and its effect on the deposition of gold nanoparticles." RSC Adv. (accepted 24 Oct 2013; DOI: 10.1039/C3RA45353G).
2. Srivastava, S.K., Hasegawa, T., Yamada, R., Ogino, C., Mizuhata, M., Kondo, A. "Green synthesis of Au, Pd and Au@Pd core-shell nanoparticles via tryptophan induced supramolecular interface." RSC Adv. (2013), 3, 18367.
3. Srivastava, S.K., Yamada, R., Ogino, C., Kondo, A. "Sidewall modification of multiwalled carbon nanotubes by Allivum sativum (garlic) and its effect on the deposition of gold nanoparticles" Carbon (2013), 56, 309.
4. Srivastava, S.K., Yamada, R., Ogino, C., Kondo, A. "Biogenic synthesis and characterization of gold nanoparticles by Escherichia coli K12 and its heterogeneous catalysis in degradation of 4-nitrophenol" Nanoscale Res. Lett. (2013), 8, 70.
5. To-be submitted

Related Research works

1. “A synergistic composition for nucleic acid staining by natural dye from *Rubia Cordifolia* derivative” by Srivastava, S.K. et al. at the Indian Institute of Technology, Kanpur, India. (Application No.1363/DEL/2010 A)
2. Srivastava, S.K., Joshi, N., “Biosurfactant production and characterization” published by Lambert Publishing House, Germany. (ISBN 978-3-8383-6022-5)
3. Srivastava, S.K., Constanti, M. “Room temperature biogenic synthesis of multiple nanoparticles (Ag, Pd, Fe, Rh, Ni, Ru, Pt, Co, and Li) by *Pseudomonas aeruginosa* SM1” *Jour. of Nanopart. Res.* (2012), 14, 831.
4. Shukla, D., Vankar, P.S., Srivastava, S.K. et al. “Bioremediation of hexavalent chromium by a cyanobacterial mat” *App. Water Sci.* (2012), 2, 245.
5. Srivastava, J., Srivastava, S.K., Vankar, P.S. "Correlation of antioxidant activity and phytochemical profile in native plants" *Nutr. & Food Sci.* (2012), 42, 71.

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~तेरी जात-ए-पाक से ताल्लुक गहरा है मेरा, मैं ठहरा गरीब और तू गरीब नवाज़~

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