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Development of gold nanoparticles supported membrane as an efficient material for protein detection

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Abstract

A development method for gold nanoparticles (GNPs), supported on nitrocellulose membrane by a simple contact between a GNPs solution was prepared with Tetra aurochloro acid (HAUCL4) solutions precursor. Nitrocellulose membranes are of particular interest in many bimolecular analyses, biosensors, cosmetic industries, especially for detection of specific proteins or peptides and nucleic acid sequences. These kinds of membrane currently suffer from insufficient detection sensitivity and low retention value for proteins less than 5 KDa. These deficiencies can be overcomed by constructing hierarchically structured separation layer on a porous membrane using GNPs colloidal solution. The surface modified membranes were able to effectively detect proteins less than 5 KDa than conventional membranes, since metals have high affinity towards protein. Activities of GNPs supported membrane were studied through western blot experiment and their performances were compared with the conventional membrane. The coated membrane performance increased which was proved by western blot experiment.

Key words

Biosensor, Goldnanoparticles, Nitrocellulosemembrane, Small protein, Western blot

Introduction

New opportunities are opening in biology and medicine by combining molecular properties in nature with synthetic nano scale materials (Emami *et al*., 2012). Several varieties of nano particles with biomedical relevance are available including, polymeric nano particles, metal nano particles, liposomes, micelles, quantum dots dendrimers and nano assemblies. For example, nanosized-TiO2 can cause inflammatory response in airways of rats and mice (Yu-MiJeon *et al.,* 2013). Among the approaches for exploiting nano technology in medicine, nano particles offer some unique advantages as sensing, image enhancement and delivery agents (Sahoo *et al.,* 2003).For biosensing, western blotting has become an indispensable tool and is used as a routine technique for protein analysis. However, detection of proteins/peptides below certain size (around 10 – 15kDa) by Western blot is difficult, as they may pass through the membrane during the electro-transfer step and their weak binding to the membrane may leads to their loss during numerous washes of the blotting procedure (Duchesne *et al.,* 2007). Modifications of membrane surface have been suggested recently as a method of enhancing desirable membrane properties or providing new, unique functionalities. In order to enhance these designer features, coating of membranes by metal nanoparticles has been explored (Marques *et al.,* 2008; Muniz-Miranda *et al*., 2004; Sosibo *et al*., 2012). One of the ability of metal nano particles in photo catalytic activity has been widely used in various applications such as methyl orange degradation by Sm doped BiOBr/RGO composite (Vadivel *et al.,* 2014). GNPs play an important role in biomedical research and applications due to their small dimensions and high biocompatibility (Bisker *et al*., 2012). The widespread use of GNPs as labels in

diagnostics and detection is due to a unique combination of chemical and physical properties that allow biological molecules to be detected at low concentrations. Taking advantage of the capacity of noble metal nanoparticles to bind proteins, some authors have demonstrated that such coated membranes are highly effective for the western blot detection of small molecular weight (fewer than 10 kDa) proteins and peptides. These membranes capture the proteins during the electro-transfer, and thus prevent protein loss (Duchesne *et al*., 2007).

In view of the above, the present study was carried out to develop a low cost modified Western blot protocol by using GNPs-coated Nitrocellulose membranes which allowed the detection of small protein preventing their loss during electro-transfer with a sensitivity similar to that obtained for high molecular weight proteins (above 30kDa). For this purpose, gold supported Nitrocellulose membranes were prepared and characterized, and their activity was tested by western blot experiment.

Materials and Methods

Chemical : Tetra auro chloric acid ($HAuCl_4$) and Trisodium citrate were purchased from LAB CHEMICALS, Chennai, India. Nitrocellulose membranes with pore size of 0.45µm and low molecular weight protein of insulin was purchased from Sigma-Aldrich.

Synthesis of GNPs : Plant samples were collected from local area, Chennai and was identified as *Phyllanthus amarus (*Schum by Thonn) by Siddha Research Centre, Arumbakkam, Chennai. The GNPs were synthesized by bio-chemical methods are called biological GNPs (BGNPs) (Annamalai *et al.*, 2012) and chemical GNPs (CGNPs) (Zabetakis *et al.*, 2012) respectively. Finally, the pellets were re-dispersed in distilled water and air dried to obtain the powdered form of GNPs. The GNPs solution was analyzed with Shimadzu double beam spectrophotometer for its maximum surface plasmon resonance. A 0.3 ml of solution was taken and was diluted to 3 ml with distilled water and the reading was taken in a double beam spectrophotometer at 400 to 700 nm wavelength (Naheed Ahmad*et al.,* 2012). The maximum absorbance peak obtained confirmed the presence of GNPs in the solution.

Characterization : Scanning Electron Microscope (SEM) technique was employed to visualize size and shape of GNPs. A Philips XL30 SEM was used. Dried suspension of synthesized GNPs was used for analysis. SEM samples of aqueous suspension of GNPs was fabricated by dropping the suspension onto clean electric plate and allowed water to completely evaporate. Energy Dispersive X-Ray Analysis was used to determine the presence of elemental gold in synthesized GNPs solution. The samples were dried at room temperature and analyzed for the composition of samples in the synthesized nano particles. To identify the possible molecules responsible for reduction of gold ions and capping of reduced GNPs synthesized by bio-chemical methods, Fourier transformed infrared radiation spectroscopy (FTIR) was performed. They were centrifuged at 10000 rpm and supernatant was discarded and pellets were washed with deionized water. The pellets were scanned on Perkin-Elmer FTIR spectrum in transmittance mode at a resolution of 2 cm^{-1} (Javad*et al.,* 2012). The Nitrocellulose membranes was immersed in 30 ml of GNPs solution for overnight incubation. The membrane attracted the nano particles, as apparent by the reduced optical properties of nano particles solution remaining after the incubation. In case of overnight immersion, the remaining solution became almost transparent. This suggest that a strong electrostatic attraction between the nano particles and Nitrocellulose membranes. After incubation, initially the white membrane acquired a black color, whose intensity increased with incubation time (Xie *et al.,* 2012). To reveal the structure of the Nitrocellulose membranes deposited with GNPs, the morphology of membrane surface and cross-section was observed by SEM analysis. The elemental composition of the GNPs deposited on the membrane was determined by EDAX. The uncoated membrane morphology was also studied for comparison studies. Western blot experiment was carried out in order to assess the membrane performance on low molecular weight protein detection.

Results and Discussion

The yellow colour of $HAuCl_4$ reaction mixture turned into purple and black colour for chemical and biological method respectively as shown in (Fig. 1 a,b). Sharon and co-workers observed that the surface plasmon resonance (SPR) bands were centred between 500 – 600 nm confirmed the formation of GNPs in the solution (Pandey *et al*., 2012). Maximum absorbance peaks were observed at 526±2.45 nm and 540± 2.95 nm and the optimum time was 20 and 60 min for bio-chemical method (Fig. 1 a,b). Fig. 2a, b shows the SEM image of synthesized GNPs of average size 17nm and 18nm by bio-chemical methods. Fig. 2 c, d depicts the EDAX spectrum of weight percentage of gold present in synthesized sample was 61.87 % and 53.85 % by bio-chemical methods. The FT-IR spectrum of BGNPs showed bands at 3411 cm^{-1}, 2151 cm^{-1}, 1649 cm^{-1} and 1652 cm^{-1}, corresponding to N-H stretching (primary and secondary amines, amides), CN nitrile groups, -C=C- aromatics, -C=O- ketone, aldehyde groups respectively (Fig. 3). The bonds or functional groups, such as -C-O- and -C=C- derived from heterocyclic compounds e.g. alkaloid or flavones, and the amide bond

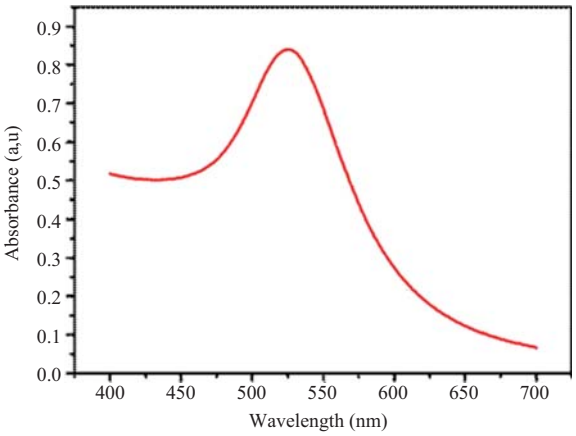

Fig. 1a : UV spectra of BGNPs

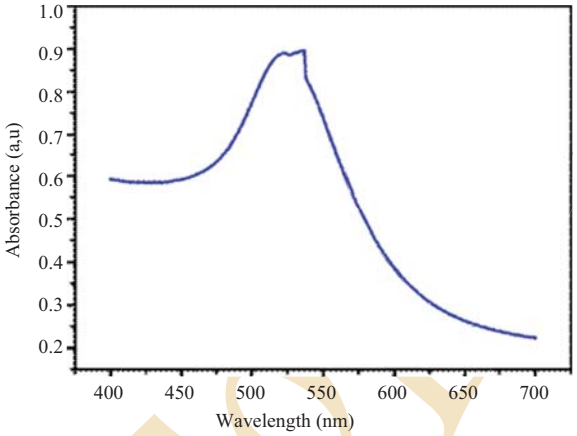

Fig. 1b : UV spectrometry of CGNP's

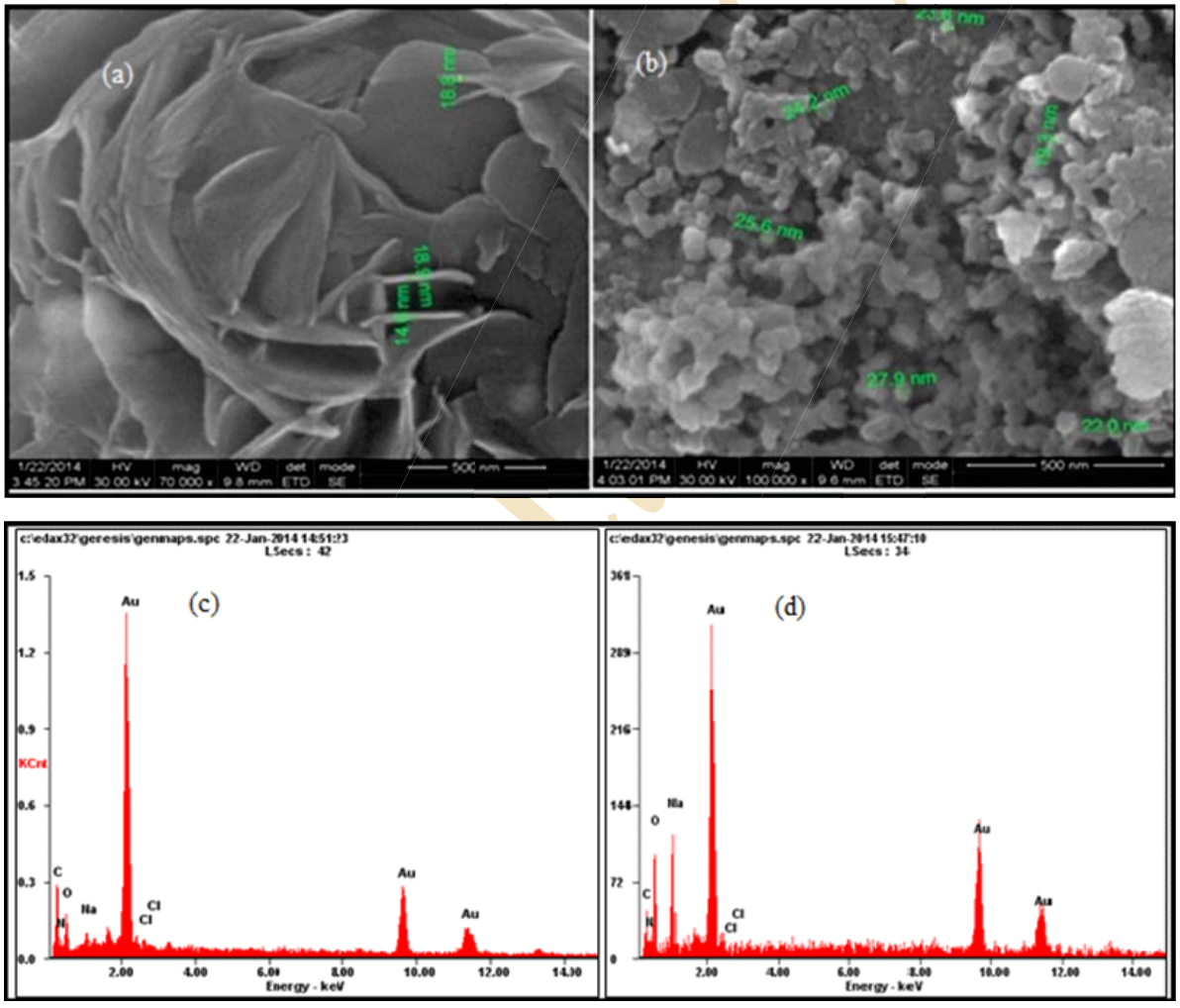

Fig. 2 : SEM image for a) BGNPs b) CGNPs c) EDAX spectrum for BGNPs d) is EDAX spectrum for CGNPs

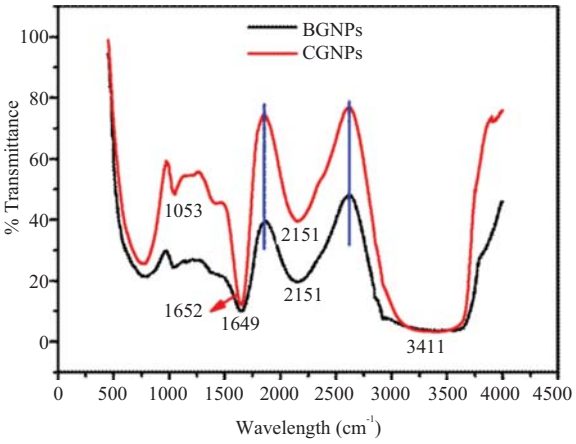

Fig. 3 : UV spectrometry of CGNP's

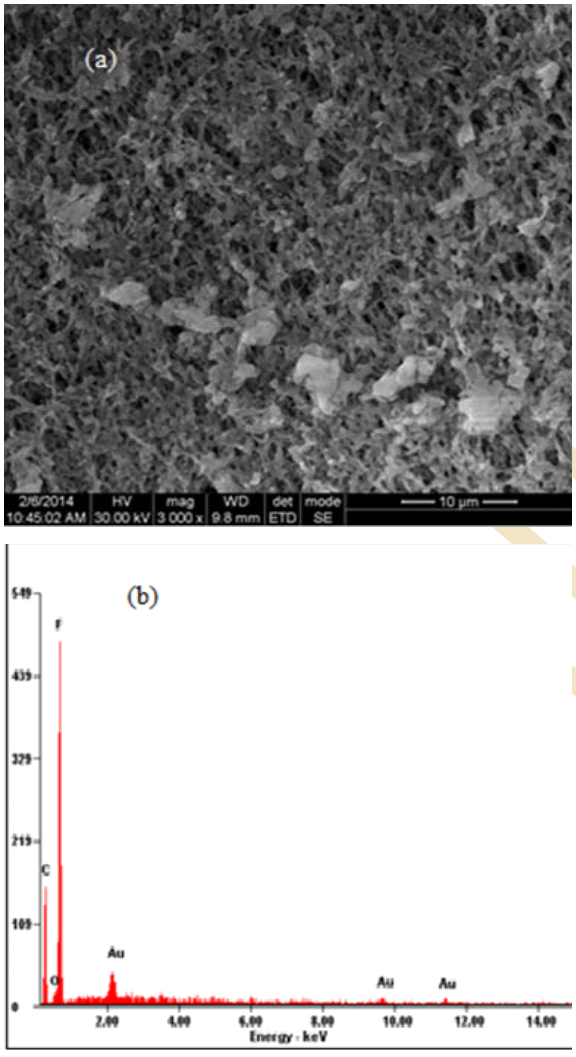

Fig. 4 : UV spectrometry of CGNP's

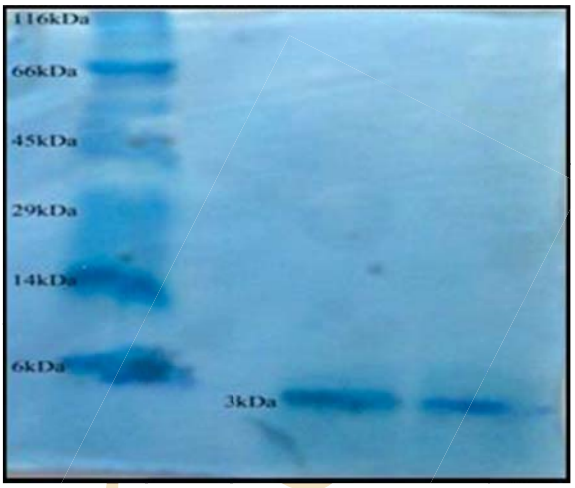

Fig. 5 : SDS PAGEgel (15%): Broad range marker and Insulin (3 kDa)

derived from leaf extract are responsible for the reduction reaction. CGNPs showed band at 2151 cm^{-1} and 1053 cm^{-1} (Fig. 3). The spectrum showed the presence of -C=C-, N-H and -C=O- which are responsible for the capping of GNPs in the chemical method. These molecules act as surface coating molecules which keep away from the internal agglomeration of the particles. Consequently, the nano particles were stabilized in nanosolutions. As a result of visual observation, the white membrane acquired black colour whose intensity increased by incubation. Modification of immobilin-PVDF membrane using GNPs by simple incubation method has been demonstrated earlier (Emami *et al*., 2012; Duchesne *et al.,* 2007;Xie *et al.,* 2011). The uncoated and GNPs coated Nitrocellulose membranes was characterized by SEM analysis and morphological characteristics were studied. The SEM image clearly showed deposition of GNPs on the coated membrane (Fig.4a) and EDAX data also proved that 3.2 weight% of GNPs was deposited on the membrane (Fig.4b). Imaged the NC membrane before and after incubation with GNPs by SEM (Xie et al.,2011). The edax data clearly showed that 3.2 wt % of GNPs were only deposited on membrane. From this it is clear that trace amount of GNPs only used for membrane modification provides a better improvement of detection sensitivity of low molecular protein compared to uncoated membrane. Therefore, not much cost is needed for membrane modification using GNPs.

The GNPs covered the Nitrocellulose membranes with a homogenous layer of nanoparticles. The performance of uncoated and coated membrane were studied by western blot experiment using low molecular weight protein of Insulin (3 kDa). The SDS-PAGE analysis showed the band with respect

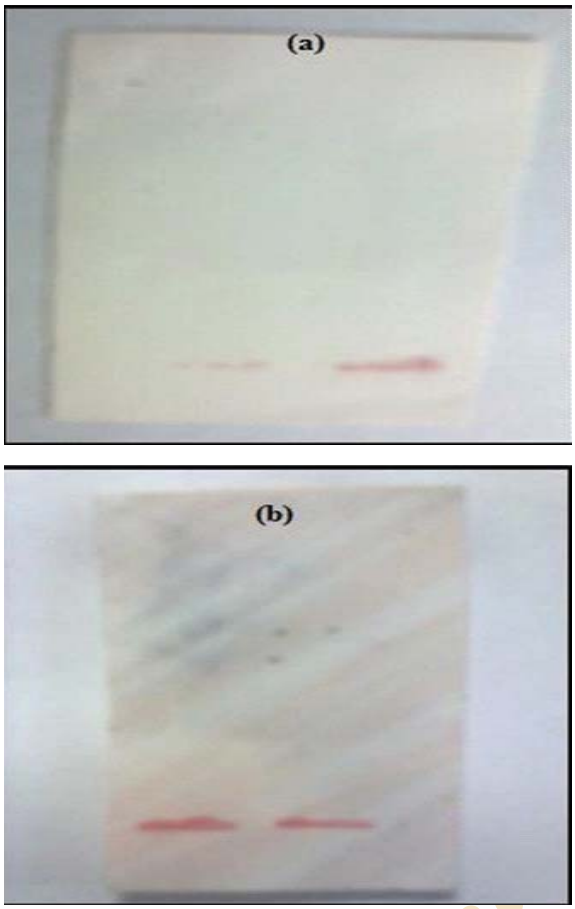

Fig. 6 : Western blot analysis of Insulin (3 kDa) a) Uncoated Nitrocellulose Membrane b) Coated Nitrocellulose Membrane

to broad range marker which confirmed that molecular weight of Insulin was 3kDa as shown in Fig. 5. During Western blotting, the functional groups of peptides bound to GNPs and they were kept in the membrane. The presence of the GNPs prevented diffusion of the peptides from the membrane after applying the voltage. The thiol (Cys) and amino groups (Arg, Lys, His, Asn, Gln) of the proteins and peptides have high affinity in binding to some metals such as gold and silver (Duchesne *et al.*, 2007). These metals were used previously in staining proteins (Emami *et al*., 2012). During electro-blotting, the proteins were loaded onto the SDS-PAGE gel (15%), which is commonly used to separate proteins in the mass range of 3 to 100kDa. The uncoated nitrocellulose membranes showed low detection sensitivity for low molecular weight protein as shown in Fig. 6a. The peptide was transferred to the gold-coated membrane by the Western blot technique (Emami *et al*., 2012). During Western blotting, the functional groups of peptides bound to the gold nanoparticles were kept in the membrane and they were not diffused from the membrane during the washing procedures. Insulin with a molecular weight of 3kDa kept in coated membrane Fig. 6b showed that detection sensitivity of low molecular weight protein was improved than uncoated membrane. The metal nano particles embedded in the membrane show other highly desirable properties such as the ability to capture low molecular weight proteins. (Xie *et al*., 2011).

The present study demonstrated that nitrocellulose membranes can be modified by simple incubation with a gold colloidal solution. These membranes were able to remove the gold nanoparticles from the gold colloid solution. The nanoparticles coated membrane play an important role in fluroscence-based bioassays and other biochemical procedures.

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