Silver-chitosan nanobiocomposite as urea biosensor

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Silver nanoparticles (Ag NPs) were synthesized by aqueous chemical growth technique. The above mentioned synthesized materials were characterized by applying scanning electron microscope (SEM) and X-ray diffraction for confirmation of morphological analysis, compositional purity, and crystalline property and emission characteristics as well. In order to fabricate the urea biosensor (potentiometric), a solution of deionized water and chitosan was prepared having Ag NPs. The said solution was dropped on the glass fiber filter having diameter of 2 cm. A wire of copper having thickness of approximately 500 μm was used for the voltage signal to pull out from the said working nanoparticles (NPs). To improve the strength, sensitivity and the quality of the potentiometric urea biosensor, a specific functional surface of Ag NPs was attained by electrostatic restrained of an enzyme (urease) onto the chitosan-Ag (a nanobiocomposite). The potentiometric reaction was measured via electrochemical detection technique. The potentiometric urea biosensor illustrates significant sensibility at room temperature with ≈ 42 mV as per span. Furthermore, the said biosensor showed an appropriate stable response within 7 sec.

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1. Introduction

Nanomaterials have several applications in different areas such as electronics, sensing, energy conversion, biomedicine and photonics. Due to having distinctive physiochemical properties, nanomaterials have attained a great importance. Many researchers have shown that nanoparticles/nanomaterials such like silver nanoparticles [1] magnetic nanoparticles [2] and gold nanoparticles [3] and carbon nanotubes [4] have significant potential to hold biological molecules and opens up new possibilities for scientists and researchers in the different fields.

Gold nanoparticles have been used to carry the biological molecules. However, silver nanoparticles (Ag NPs) are attractive for researchers due to its catalytic properties. Moreover, silver nanoparticles (Ag NPs) have electrical and thermal conductivity very high as compared with the other metals. The said nanoparticles (Ag NPs) have antibacterial properties as well as have the ability to improve the surface enhanced optical properties [5, 6]. These materials also offer a proper environment in order to maintain the different biological activities [7] and also enable the transfer of electrons among the trapped biomolecules and the electrode surface. Hence, silver nanoparticles have been used to manufacture the electrochemical biosensors with enriched systematic performance [8, 9]. Electrochemical investigation offers several advantages such as fast detection, low cost and high sensitivity. For the proper growth of biosensors, incorporation of the biological molecules into electrodes is of much interest.

As reported above, Ag nanoparticles are fascinating due to their low cost as compare to gold nanoparticles. Most importantly, properties of Ag nanoparticles like good conductivity, excellent catalytic properties and ability to transfer the electrons among the trapped enzymes and the electrodes as well, are harvested for fabrication of electrochemical urea biosensor. Since, trapped biological molecules on different nanoparticles enhance the bioactivity and stability of enzymes. So, in the present research work, choice of Ag nanoparticles as a substrate for immobilization of urease is based on their property of effective enzyme stability and bioactivity. Moreover, effort has also been made to arrange Ag nanoparticles in order as a substrate so that their tendency of aggregation and rapid biodegradation can be overcome.

In this work, we synthesized Ag nanoparticles for urea biosensor application. The present study reveals structural and morphological characterization of the Ag nanoparticles and their use for the miniaturization of facile, repeatable and reproducible fabrication method for the electrochemical urea biosensor. The potentiometric urea sensing measurements have been performed through a simple two electrode experimental set-up. The fabrication, characterization and analytical performance of the modified biosensor based on the Ag nanoparticle to develop an electrochemical urea sensor were also described in current conducted experimental work.

2. Materials and methods

2.1 Particle synthesis

Silver nanoparticles (NPs Ag) were synthesized by the same method as adopted by Hussain et al. [10]. In addition, a weaker reductant such as citrate was used instead of strong reductant Aniline. Silver Nitrate ($AgNO_3$) was purchased from Sigma Aldrich and used as such for the growth of Ag nanoparticles (NPs). Cetyl trimethylammonium bromide (CTAB) and Citrate (Merck, 99%) were also purchased and used as such. For the preparation of stalk solutions of different reagents, deionized water as well as doubly distilled water was used.

Silver Nitrate ($AgNO_3$) was used as main precursor and CTAB as a stabilizing agent. A specific quantity (0.01mol) of $AgNO_3$ and CTAB was introduced into the reaction vessel. Water was also added into reaction vessel. A colorless mixture $AgNO_3$, CTAB and water was obtained. Additionally, 0.01 mol of citrate solution was added as a reducing agent. The transparent colorless mixture, as mentioned above, was transformed into a pale yellow color. This formation of pale yellow color was indication of silver nanoparticles.

2.2 Fabrication of working electrode

Working electrode of chitosan-Ag was fabricated by making chitosan sol gel in 1 M hydrochloric acid and acetic acid (1%). The obtained solution was kept on stirring for 24 hours. On the other hand, silver nanoparticles (Ag NPs) were mixed in deionized water and kept on stirring for 1 hour. The solution containing Ag NPs was mixed with the chitosan sol gel in order to make suspension. The obtained suspension was distributed on a wire of copper (of thickness =500µm) by drop wise dispersion. This copper wire was mounted on a glass fiber filter having diameter of 2cm. Finally, the physical adsorption method was used to immobilize the biosensor electrode based on chitosan-Ag nanobiocomposite with urease. The schematic diagram of the above mentioned process is shown in Fig. 4.

2.3 Potentiometric measurements

All the chemicals like Urea (Acsreagnt 99.9%), Urease (E.C.3.5.1.5), Na_2HPO_4 and KH_2PO_4 were purchased from sigma Aldrich (USA). A 10 mM solution of phosphate buffer saline (PBS) was obtained from KH_2PO_4 and Na_2HPO_4 with sodium chlorate. The pH of the said solution was adjusted to 7.4. A stock solution (urea in PBS) of 100 mM was made. In addition, a standard solution of urea having low concentration was also prepared. On the other hand, a solution of urease in PBS (pH=7.4) was prepared having activity 2mg/ml. Furthermore, the potentiometric method was applied for measurements for different concentrations of urea solution. In the cell assembly, trapped chitosan-Ag (Ag-CH) based biosensing electrode was sued as functional electrode as well as $Ag/AgCl_2$ was used as reference electrode. A schematic diagram is shown as in Fig. 5. In these experimental steps, the potentiometric output voltage was measured by a pH meter (Denver Instrument, Model 215).

3. Results and discussion

In order to confirm the morphological and structural analysis of Ag, we applied field emission scanning electron microscopy (FE-SEM) of high resolution. Globular porous morphology of Ag nanoparticle has been observed from the FE-SEM image as shown in Fig. 1. It can also be observed that grown nanoparticles have a diameter in the range between 80 to 100 nm (figure 1). Our results shows better morphological aspects have been found as compared to previously reported work [11-13].

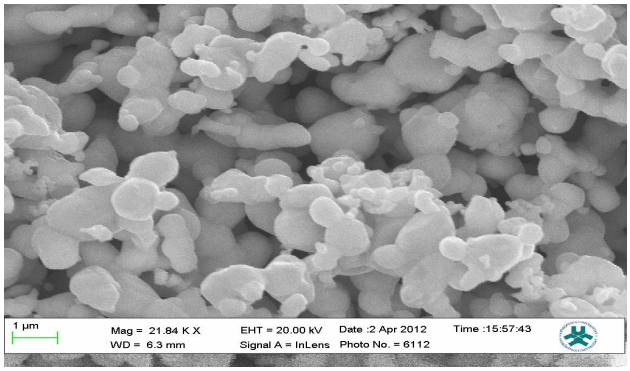

Fig. 1. SEM image of the nanocrystalline Ag.

Fig. 2 shows the x-ray diffraction (XRD) pattern of Ag nanoparticles. There are three large peaks of Ag nanoparticles and the pattern is consistent with JCPDS card No.00-002-1098 and the lattice parameter α = 4.0772. XRD pattern shows that Ag nanoparticles are highly crystalline and pure. In addition, particle size is very small as confirmed by the broadening of the peaks. Small particle size as well as the crystalline nature is a tool for microstructural investigation of nanostructures.

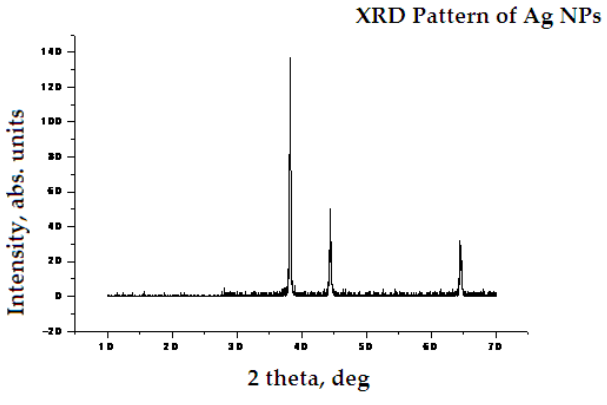

Fig. 2. XRD pattern of Ag nanoparticles.

Uniform layer of Ag NPs on a substrate by using chitosan was also investigated by SEM. The SEM image shown in Fig. 3 revealed that the Ag NPs are present in the uniform layer over the substrate. This could be due to the use of chitosan to overcome the existing problem of nanoparticles like aggregation and biodegradation when attached on substrate. The schematic image of fabrication of working electrode based on Ag is shown in Fig. 4. Working electrode was made by dispensing Ag-CH-urease nanobiocomposite on a wire of copper having thickness of 500µm.

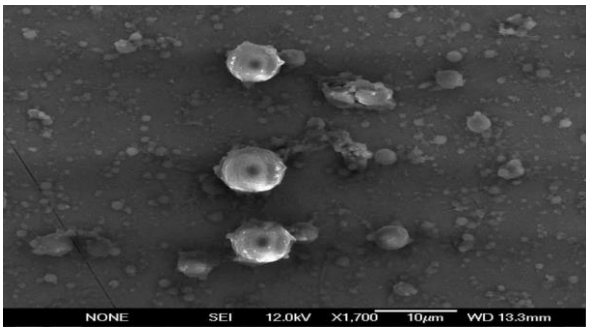

Fig. 3. SEM of Ag Nanoparticles.

Zhang et al. [14] reported the technique for fabrication of working electrode based on Fe_3O_4 [14]. That reported method is lengthy and time consuming and requires more chemicals for fabrication. Alternative technique was applied for the fabrication of working electrode of metal nanoparticle Ag which is illustrated in Fig. 4. This technique is easy, simple and less time consuming. The measurements were made by the potentiometric biosensing as reported above.

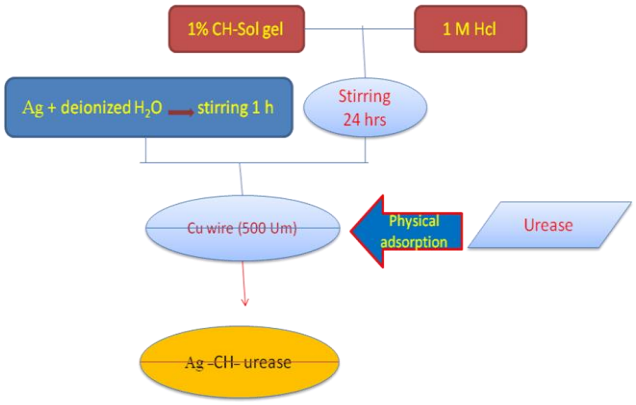

Fig. 4. Schematic diagram of the process of preparation of working electrode (CH-Ag).

The whole mechanism involved two electrodes, one electrode made of CH-Ag as an immobilized functional electrode and the second one as reference electrode consisted with Ag/AgCl. Ammonia (NH_3) and carbon dioxide (CO_2) released from the chemical reaction which is shown by the following equation.

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$$

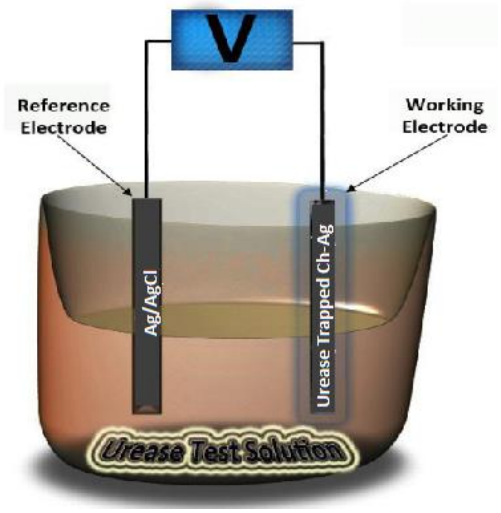

Fig. 5. Experimental set-up for potentiometric urea measurements.

As a result of hydrolysis, urease enzyme presented onto the surface of CH-Ag nanobiocomposite mounted through a copper wire on glass fibre filter, get converted into NH_3 and CO_2 once tested in a solution containing urea. Reaction of ammonia with water is a key factor for the liberation of output signal by the generation of charged ions as a result of acid base interaction. The potentiometric measurements were conceded out at different values of pH ranging from 3 to 11 as shown in Fig. 6.

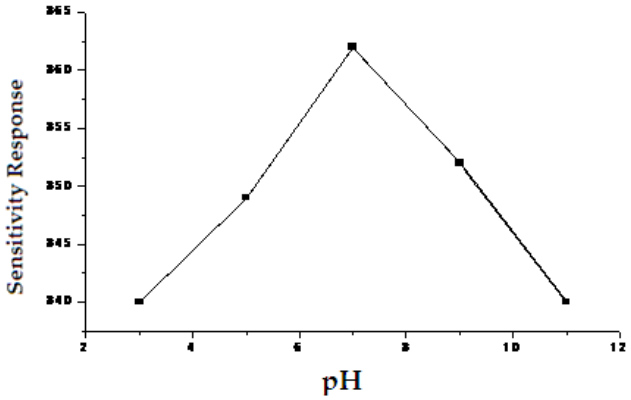

Fig. 6. Sensitivity response of the biosensor based on Ag measured at the pH values range of 3 to 11.

The physiological investigations related to the stability and performance of the biosensor in the acidic, basic and at neutral pH environments has been tested for the urea test solutions. The pH range has been selected from 3 to 11 and the maximum sensitivity value from the presented biosensor based on Ag nanobiocomposite has

been achieved at around physiological pH value of 7. The results are shown in Fig. 6.

Moreover, the selectivity of the presented biosensing electrode is inspected through the addition of variety of interferers such as glucose and uric acid to the test solution containing urea. The measured potential difference (EMF) values from working electrode are found influence free from the volume of urea test solution utilized for the experiments and area of the working electrode dipped for the detection of urea molecules. A series of consecutive experiments have been performed over the period of couple of weeks and the fabricated biosensing electrode shows good reproducibility and repeatability.

Urease trapped/immobilized CH-Ag nanobiocomposite based working electrode showed very high output response for urea detection. As a result, a sensitivity of 42mV/decade was recorded. The potential difference (EMF) among the working biosensing electrode (Ag-CH) and the reference electrode (Ag/AgCl) depends upon the composition of the urea test solution. As the composition is varied, the potential difference gets changed. The potential difference is also associated with the accretion of ammonium ions on the surface of the working electrode (CH-Ag) while that of the reference electrode have a constant value (=222.34) at room temperature [15]. Additionally, it was noted that the working electrode (CH-Ag) size and the volume of test solution have no effects on the output potential of biosensor. In addition, data shows the electrochemical response (EMF) vs. the logarithmic concentration of urea going from 1 mM to 80 mM (0.00 mM to 2.5 mM range depicted) as shown in the Fig. 7. It is good agreement of experimental data with simulated results. Graph shows that electrochemical (EMF) response gets rising by increasing the sample concentration incensement. The curve shows the linear overview. The work of same template was done by some researchers [16]. The efficiency of the proposed sensor is excellent and quick which can be calibrate within ~ 7 sec (having whole concentration range with 90% of the steady state voltage (EMF) achieved).

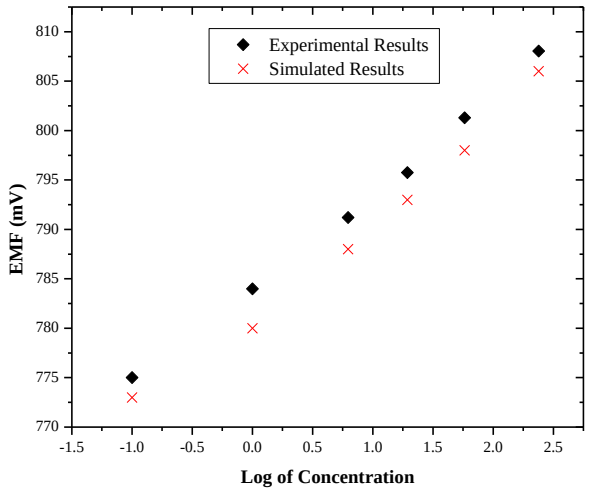

Fig. 7. Sensitivity response curve of Ag non-magnetic nanoparticles.

All the experimental steps relating urease trapped CH-Ag urea sensor electrode were repeated within the selected range of urea concentration for a couple of days. It was noticed that a very fast time (less than 10 sec) output response for the detected concentrations was given by the urea sensor. Moreover, it was found that the shelf life of urease trapped Ag nanobiocomposite lasts up to three weeks and 90 % of the initial activity of sensing electrode retained which is the reflection of strong binding of the said particles towards the urease enzyme.

4. Conclusion

A urease trapped CH-Ag nanobiocomposite based urea biosensor has been fabricated by applying aqueous chemical growth technique. Scanning electron microscope, X-ray diffraction (XRD) and Raman Spectroscopic characterization reveals globular porous morphology, purity, and crystallinity and emission characteristics of Ag nanoparticles. The concentration range of urea test solution was varied from 1 mM to 80 mM and an output signal (≈42 mV/decade) was detected at room temperature. The presented biosensor showed significant stable potentiometric response around 7 s and the overall performance like reproducibility, selectivity, stability and sensitivity of the biosensor was very good. The above mentioned features can make the said CH-Ag based sensor useful for use in medical and other areas like food. Additionally, CH-Ag based sensor is also achievable for clinical diagnosis as well. However, the said biosensor showed slight influence once inspected through the addition of variety of interferers such as glucose and uric acid which reflects that the sensor is helpful for urea detection along with such interferes. In addition to this, the said fabrication method for the preparation of immobilized urease enzyme is useful and simple and can be used for different biosensors growth.

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References

[1] J. Chin, Chem. Soc. **56**, 703(2009).
[2] W. Shi, Z. Ma, Biosens. Bioelectron. **26**, 1098 (2010).
[3] X. L. Luo, J. J. Xu, Q. Zhang, G. J. Yang, H. Y. Chen, Biosens. Bioelectron. **21**, 190 (2005).
[4] A. Kaushik et al Biosens. Bioelectron. **24**, 676 (2008).
[5] X. Kang, Z. Mai, X. Zou, P. Cai, J. J. Mo, Nanosci. Nanotechnol. **7**, 1618 (2007).
[6] Y. G. Sun, Y. N. Xia, Science **298**, 2176 (2002).
[7] M. X. Kan, X. J. Wanga, H. M. Zhang, Chin Chem. Lett. **22**, 458 (2011).
[8] C. Ren, Y. Song, Z. Li, G. Zhu, Anal. Bioanal. Chem.

381, 1179 (2005).
[9] Z. P. Chen, et al Biosens. Bioelectron. **23**, 485 (2007).
[10] J. L. Hussain, S. Kumar, A. A. Hashmi, Z. Khan, Adv. Mat. Lett. **2**(3), 188 (2011).
[11] X. Q. Liu, S. W. Tao, Y. S. Shen, Sens. Actuators B. Chem. **40**, 161 (1997).
[12] L. C. Hsu, Y. Y. Li, C. G. Lo, C. W. Huang, G. Chern, J. Phys. D: Appl. Phys **41**, 185003(2008).
[13] L. X. Yang, Y. Liang, H. Ceng, L. Y. Kong, W. Jiang, Bull. Mater. Sci. **31**, 919(2008).
[14] Z. Yhang, X. Wang, X. Yang, Analyst, **136**, 4960 (2011).
[15] W. Stumm, J. J. Morgan, Aquatic Chemistry 480 (1981).
[16] A. Tiwari, S. K. Shukla, eXPRESS Polymer Letters **9**, 553 (2009).

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