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Potentiometric urea biosensor utilizing nanobiocomposite of chitosan-iron oxide magnetic nanoparticles

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Abstract

The iron oxide (Fe_3O_4) magnetic nanoparticles have been fabricated through a simple, cheap and reproducible approach. Scanning electron microscope, x-rays powder diffraction of the fabricated nanoparticles. Furthermore, the fabrication of potentiometric urea biosensor is carried out through drop casting the initially prepared isopropanol and chitosan solution, containing Fe_3O_4 nanoparticles, on the glass fiber filter with a diameter of 2 cm and a copper wire (of thickness ~500 μm) has been utilized to extract the voltage signal from the functionalized nanoparticles. The functionalization of surface of the Fe_3O_4 nanoparticles is obtained by the electrostatically immobilization of urease onto the nanobiocomposite of the chitosan- Fe_3O_4 in order to enhance the sensitivity, specificity, stability and reusability of urea biosensor. Electrochemical detection procedure has been adopted to measure the potentiometric response over the wide logarithmic concentration range of the 0.1 mM to 80 mM. The Fe_3O_4 nanoparticles based urea biosensor depicts good sensitivity with ~42 mV per decade at room temperature. Durability of the biosensor could be considerably enhanced by applying a thin layer of the nafion. In addition, the reasonably stable output response of the biosensor has been found to be around 12 sec.

Keywords: iron oxide (Fe3O4) magnetic nanoparticles, emission characteristics, potentiometric response, urea biosensor, chitosan solution

1. Introduction:

The growing demand for clinical diagnostics in relation to kidney and liver diseases has stipulated the development of new methods for rapid and accurate measurement of urea in samples like urine, serum and blood. Normal range of urea in urine and blood is 8-20 mg/dl. An increase in urea level in blood and urine causes renal failure, urinary tract obstruction, dehydration, shock, burns and gastrointestinal bleeding. However, reduced urea level may cause hepatic failure, nephritic syndrome and cachexia (low protein and high carbohydrate diets). In comparison to conventional methods of urea detection electrochemical biosensors have been considered to provide interesting alternatives due to their simplicity, low cost and high sensitivity [1, 2].

Since biosensors allow a wide range of transduction technology so they have advantage over ordinary electrochemical sensors. Among them, the urea biosensor has widely been distributed in nature, and its analysis is of considerable interest in clinical and agricultural chemistry [3, 4]. It is known to be an important maker for evaluating uremic toxin level.

Immobilization of Ur on a suitable matrix is an important step for the fabrication of electrochemical urea sensor. In this context, hybrid nanobiocomposites possess much attraction due to synergistic effect of two components in order to obtain improved biosensing characteristics. Metal oxide nanoparticles-chitosan (CH) based hybrid composites have attracted much interest for development of a desired biosensor (5-7). Metal oxide nanoparticles such as iron oxide (Fe_3O_4) (8-10), Zinc oxide (ZnO) (11-12), cerium oxide (CeO_2) (13-14) etc. have been reported as promising matrices for the immobilization of desired biomolecules.

Among various metal oxide nanoparticles, Fe_3O_4 nanoparticles have been found to be chemically and biologically inert, they can be coated with metal catalysts, enzymes, or antibodies to increase their functionalities for biosensor applications. Moreover, immobilization of bioactive molecules onto surface charged super paramagnetic Fe_3O_4 nanoparticles is of great interest since magnetic behavior of these bio conjugates may

result in improved delivery and recovery of biomolecules for desired biosensing applications [15,16,17]. Fe_3O_4 nanoparticles have been proposed to provide a favorable micro environment for immobilization of desired enzymes directly onto electrode surface resulting in catalytic activity, electron transfer between medium and electrode or improved direct electron transfer of redox proteins [18,19,20].

Besides this existing problem of aggregation and rapid biodegradation of Fe_3O_4 nanoparticles on a given matrix containing biomolecules can perhaps be overcome by modifying these nanoparticles using CH by preparing hybrid nanobiocomposite.

Chitosan (CH) (N-deacetylated derivative of Chitin) is alinear copolymer of glucosamine and N-acetylglucosamine units and naturally found in the exoskeleton of crustaceans, fungal cell walls and in other biological materials. Among other reported properties CH has received attention for enzyme immobilization. For biosensor applications, recent attempts have been made for improving optical and electrical properties of CH by dispersing superparamagnetic Fe_3O_4 nanoparticles. (21, 22)

Ajeet Kaushik et al. (2009) have fabricated Fe_3O_4-CH hybrid nanobiocomposite for urea sensor [23]. The nano bio composite of Fe_3O_4 nanoparticles and chitosan have recently been reported for use in the detection of glucose, urea, mycotoxin, phenolic compounds and ferritin. [16, 24, 25,26]

We report results of studies relating to immobilization of Urs on to Fe_3O_4-CH nanobiocomposite deposited on glass fiber filter substrate for fabrication of urea sensor. The reported Fe_3O_4-CH nanobiocomposite have been fabricated by using simple two electrode method.

2. Materials and Methods:

Urease (E.C.3.5.1.5 from jack Bean 100 u/mg) and urea (Acsreagnt 99.9%) have been procured from Sigma Aldrich (USA). Phosphate buffer, 10mM solution (PBS) was prepared from Na_2HPO_4 and KH_2PO_4 (Sigma Aldrich) with Sodium Chlorate in 0.1315 mM and pH was adjusted to 7.4. A stock urea solution of 100mM was prepared in PBS. The low concentration standard solution of urea was also prepared before the measurements. For making Fe_3O_4-CH nanobiocomposite, following steps have been followed. Chitosan sol gel was prepared in 1% acetic acid and 1 M hydrochloric acid solutions and kept on stirring for

24 hours. The Fe_3O_4 magnetic nanoparticles were mixed with deionized water and stirred for 1 hour. The iron oxide magnetic nanoparticles were suspended in the sol gel of chitosan for making suspension. This suspension has been dispense on a copper wire (d=500um) mounted on a glass fiber filter. The iron oxide magnetic nanoparticles based biosensor electrode was developed by drop wise dispersion of suspension, based on sol gel with suspended iron oxide magnetic particles, on copper wire. Then the Fe_3O_4-CH nanobiocomposite based biosensing electrode was immobilized with urease using simple physical adsorption method. The urease solution was prepared in PBS of pH 7.4 with activity of 2mg/ml. The potentiometric measurement in different concentrations of urea was carried out using potentiometric method. The cell assembly was consisted of the following elements; the immobilized Fe_3O_4-CH nanobiocomposite based biosensing electrode was used as working electrode and Ag/AgCl as reference electrode. A pH meter (Model 215, Denver Instrument) was used to measure the potentiometric output voltage in the experiments.

3. Results and discussion

The representative XRD pattern showing all diffraction peaks of the iron oxide magnetic nanoparticle sample as shown in Figure 1 which can be readily indexed to the cubic spinel Fe_3O_4 having the lattice parameter α =8.072Å and reveals high consistency with the JCPDS card No. 76-1802. It can be clearly seen from a XRD diffraction pattern that synthesized iron oxide nanoparticles have high purity and good crystal quality. Moreover, broadening of the diffraction peaks confirms the small particle size and high crystalline nature of the material.

Schematic and sensing measurement in Figure (2) shows the experimental setup for the potentiometric biosensing mechanism consisting of a standard Ag/AgCl reference electrode and the immobilized Fe_3O_4/Cu working electrode. The chemical equation of urea hydrolysis illustrates the release of ammonia (NH_3) and CO_2 gases as given below:

$$(NH_2)2CO + H_2O\text{……..} > 2NH_3 + CO_2$$

When urease immobilized iron oxide magnetic nano particles mounted on glass fiber filter with Cu wire was tested into urea solution, then urease enzyme present on nano surface rapidly hydrolysis the urea into ammonia and carbon dioxide. The fact for the generation of output voltage is based on the reaction of ammonia with water through acid base chemistry

resulting charged ions. The potentiometric response was carried out for different urea concentrations varying from 0.1 mM to 80 mM as shown in figure (3). As a result, highly quick output response was given by the proposed urease immobilized magnetic nanoparticles based sensor electrode for the detection of urea with sensitivity of 42 mV/decade.

The potential difference (EMF) between the urease/ Fe_3O_4-CH/Cu biosensor electrode and the reference electrode (Ag/AgCl), changes with the variation in the composition of the test electrolyte solution. According to the mechanism, the potential difference is accredited to the accumulation of ammonium ions on the surface of the urease/ Fe_3O_4-CH /Cu bioelectrode, where the potential of the reference electrode (Ag/AgCl) is illustrated to have a constant value of 222.34 mV at room temperature [27].

Moreover, the selectivity of the urease/ Fe_3O_4-CH /Cu bio sensor electrode was examined by adding different interferents, e.g. uric acid, glucose and sodium Pyruvate to the urea solution. It was also observed that the size of working electrode and volume of analyte solution has no effect on the output response of the proposed urea sensor electrode. The repeatability of urease/Fe_3O_4-CH/Cu biosensor electrode was carried out in the detected range of urea concentrations for three consecutive days and it was found that the sensor electrode had given almost similar response. The sensor electrode has demonstrated a fast time response less than 12 s for the detected concentrations of urea. The shelf life of ureases immobilized iron oxide magnetic particles was found to be three weeks and it was found that sensor electrode retained activity of 90% of initial activity, which reflects that the magnetic particles provide strong binding with the urease molecules.

4. Conclusion

A potentiometric urea biosensor based on Fe_3O_4-CH nanobiocomposite by immobilization of urease enzyme has been fabricated. The x-rays powder diffraction reveals purity and crystallinity of the iron oxide nanoparticle.

The potentiometric response of ~42 mV per decade at room temperature was observed over the wide logarithmic concentration range of the 0.1 mM to 80 mM .The presented biosensor shows negligible influence to interferents which reveals that this biosensor is useful for detection of urea in samples with some interferers. Throughout the experiment, the sensor

showed good performance in sensitivity, stability, reproducibility and selectivity due to reasonable stable output response of the biosensor around 12 sec.

All these useful features can make the presented biosensor suitable for practical application in medical, food or other areas. Moreover, the presented biosensor is feasible in use for on-spot clinical diagnosis. Moreover, the fabrication method is found simple and easy and can be extended to immobilize other enzymes, and other bioactive molecules with low isoelectric points for multiple biosensor designs.

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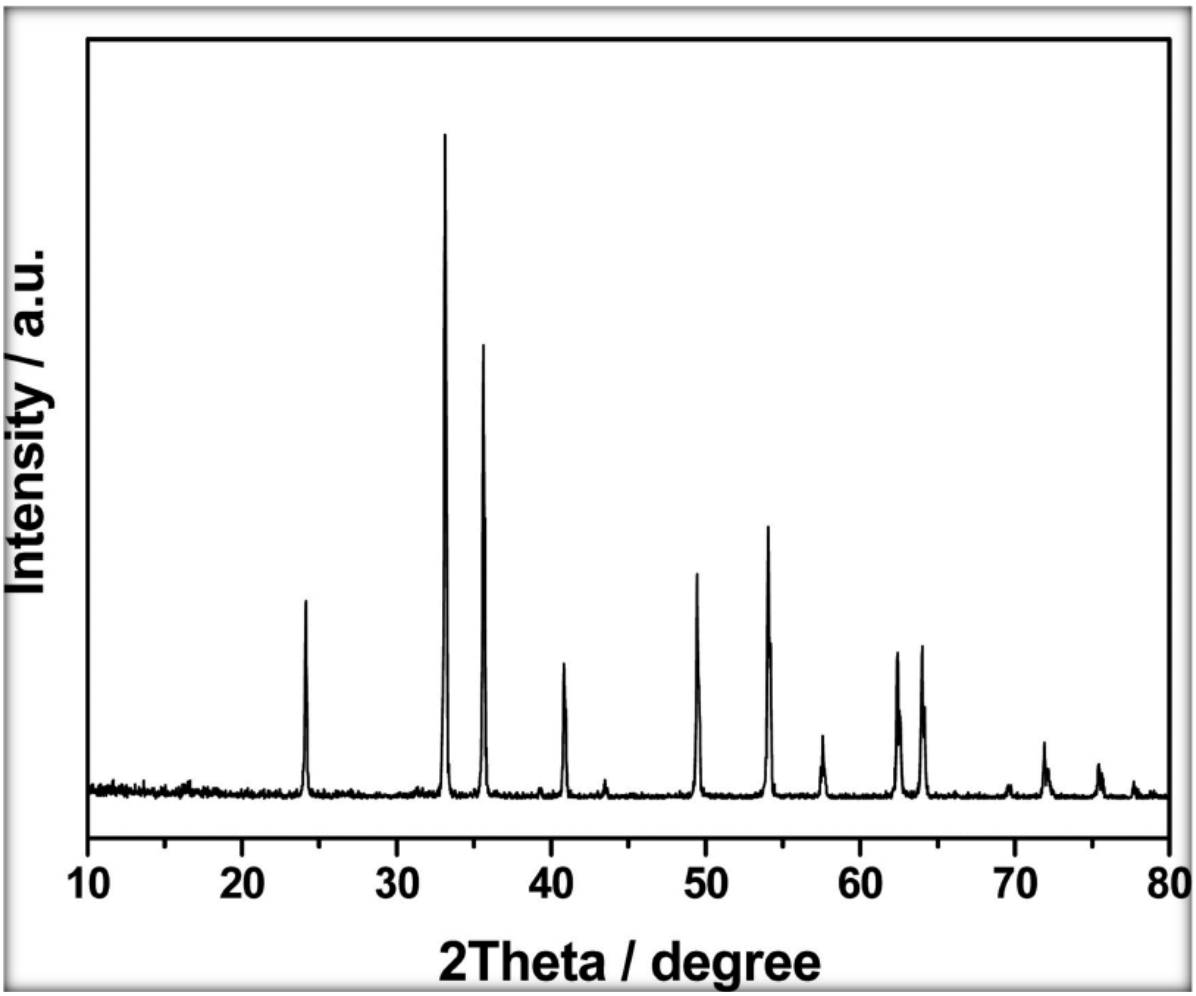

Figure 1: XRD pattern of iron oxide nanoparticles.

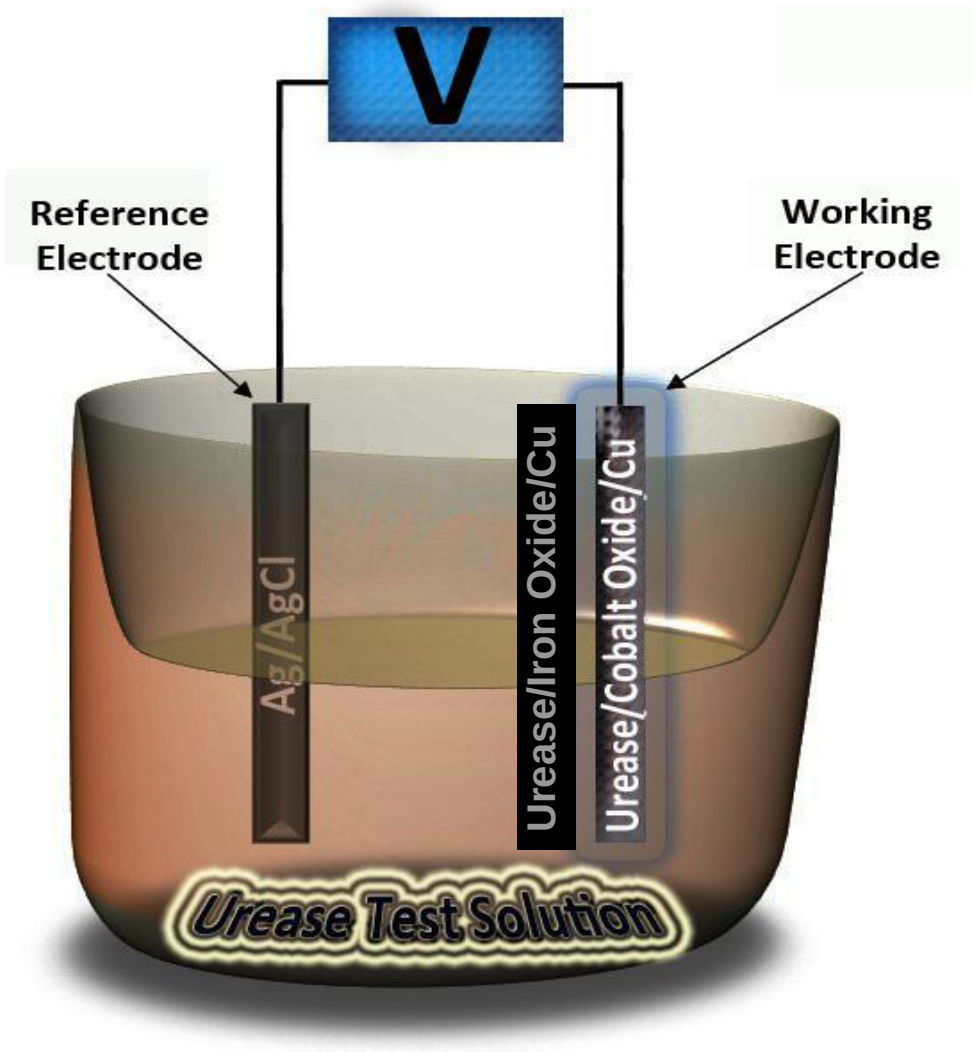

Figure 2: Experimental set-up for potentiometric urea measurements.

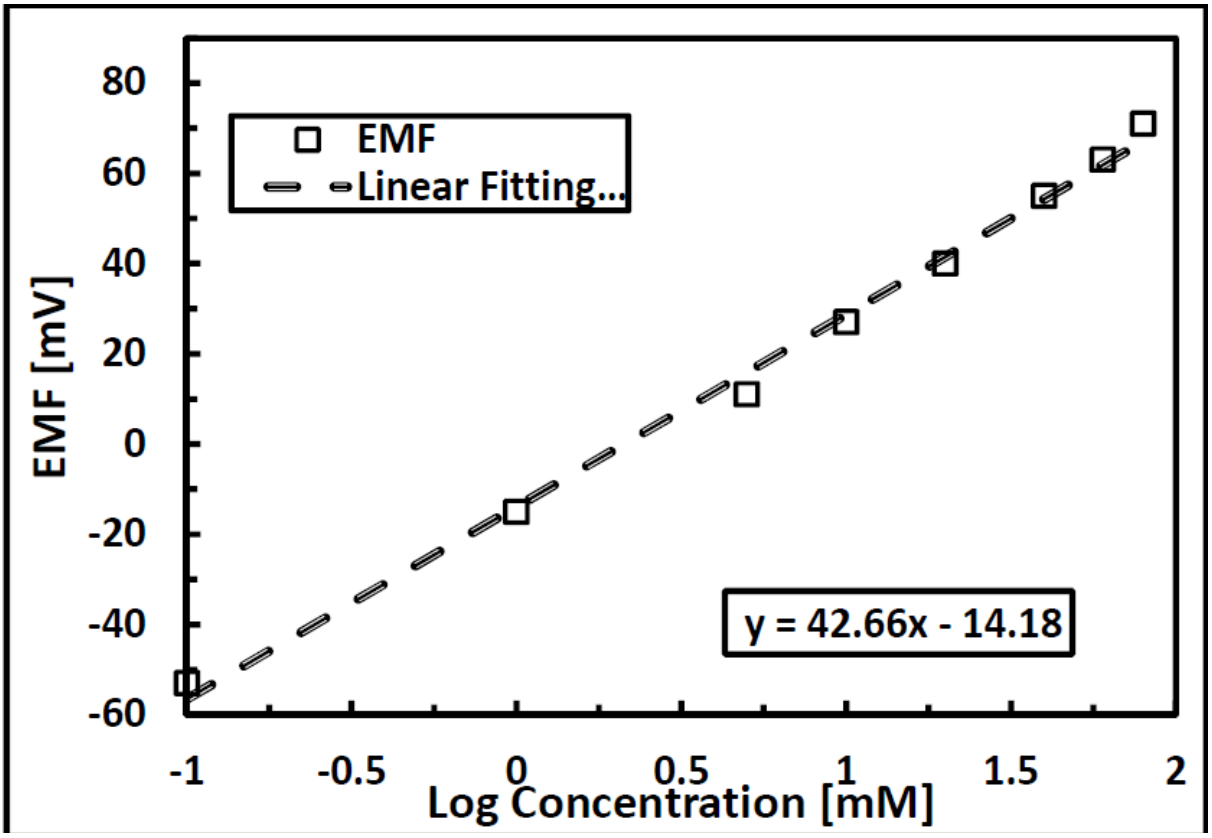

Figure3: Sensitivity response curve measured for logarithmic concentration range from 0.1 mM to 80 mM.