Chitosan blend iron oxide nanostructure-based biosensor for healthy & malignant tissue glucose/urea detection

M W Akram1,2,*, M F Alam1,3,4,*, H N Ji1,2,*** A Mahmood1, M Z Iqbal4, M R Saleem3, N. Amin3 and A G Wu4

1 Institute of Fundamental and Frontier Sciences, University of Electronic Science and Technology, Chengdu, Sichuan province, China
2 School of Materials and Energy, University of Electronic Science and Technology of China, Chengdu 610054, China
3 Department of Physics, GC University, 38000, Faisalabad, Pakistan
4 Key Laboratory of Magnetic Materials and Devices & Division of Functional Materials and Nanodevices, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences, No. 1219 Zhongguan West Road, Ningbo, China

*E-mail: waseem.physicist@gmail.com; **E-mail: fakharphy@gmail.com;
***E-mail: hainingji@163.com (H N Ji);

Abstract. A urea trapped chitosan-Fe3O4 based nano-composite biosensor of controlled morphology is successfully fabricated by applying the aqueous chemical growth technique. Final product of nanodevice was characterized by applying various techniques. Log of the concentration of various solutions (1 to 80 mM) ranged between 0.0 to 2.5 mM for urea and glucose sample and an output signal of ~ 42 mV per decade was detected at room temperature. It disclosed the presence of chitosan composite with Fe3O4 results enhanced active surface area of sensing along with up to the mark precession, as compared to traditional biosensor for immobilization of enzymes. The current developed form of chitosan-Fe3O4 nano-composite biosensor illustrated a significant stable potentiometric response in the minimal time period. Besides, nafion/polypyrrole addition to the top surface of CH-Fe3O4 nanocomposite not only protected it from degradation but also upgraded the characteristic of sensor regarding rapid electron transfer enhancement and higher shelf life of this hybrid form of nanomaterial. The advanced form of this voltammetry implied that Ur-GLDH/CH-Fe3O4 bioelectrode is found to be very sensitive in the range of 4-120 mg/dl urea/glucose concentration and can detect as low amount as 0.4mg/dl.

1. Introduction
Initially, the glucose-based biosensors were mostly immobilized and used electrochemically to detect the glucose in blood and food products. Later on, the concept of glucose enzyme-based electrode was proposed by Clark and Lyons in 1962 [1]. In connection to this, the biosensor immobilized-glucose on
an electrode provided higher simplicity and sensitivity. However, coated magnetic nanoparticles (NPs) are becoming more prominent due to their significant role in biomedical applications, for instance, hyperthermia therapy, drug delivery, biosensors and magnetic separation [2, 3]. As an example, chitosan coated magnetic nanoparticles can provide efficient solid-state nanomaterial with versatile biosensing applications. It is reported that chitosan-coated Fe₃O₄ NPs were biocompatible and exhibited strong super-paramagnetic properties hence offered low toxicity [4]. These particles could potentially improve the delivery, and recovery of biomolecules for various biomedical applications. Both chitosan and iron oxide are low-cost material which enabled an extensive use in many practical biomedical applications [5].

Chitosan-based NPs are essential and excellent stabilizing agents, film-forming abilities, mechanical strength, biocompatibility, and non-toxicity, higher permeability towards the water, surface reactivity, and cost-effectiveness among various NPs, used in this context [6]. The amino groups of chitosan provide a hydrophilic environment and thereby enhance the biocompatibility. It is reported that the pH of chitosan-coated iron oxide nanoparticles should be above 6, which promotes biomedical compatibility [7]. Nanoparticle-based biosensors are being preferred due to their large surface to volume ratio, high absorption power, and high surface reactivity nowadays [8]. These immobilized nanoparticles can transfer the electrons between electrodes via active sites of the enzyme. A preferred method of synthesizing Fe₃O₄ NPs is the co-precipitation method because it is simple and carried out under mild conditions without the use of harmful solvents [9]. A key challenge in the synthesis is to achieve optimal size and shape of the nanoparticles by controlling the pH, temperature, ionic strength and salt properties of the pH during incorporation of the surface coating agent. NPs typically aggregate due to high surface energy and magnetization.

Currently, desired, stable and well-shaped nano-biosensor fabrication is a challenging task. Anyhow, the primary focus of this study is to develop most reliable, facile, up to the mark, novel ultra-small nanoparticles (individual/hybrid) with multiple future applications.

2. Materials and method

2.1. Reagents and preparation of solutions
Iron (II) Chloride Tetrahydrate FeCl₂.4H₂O (0.274M), Iron (III) Chloride Hexahydrate FeCl₃.6H₂O (0.213M), Iron Acetylacetonate, Oleic Acid & Oleylamine, Chitosan, and triethylamine has been purchased from Aladdin China Mainland. Indium tin oxide (ITO) coated glass plates has been attained from the Blazers, UK. All relevant chemicals used are of molecular biology grade. The substances were prepared by deionized water.

2.2. Preparation of iron oxide nanoparticles
Combine all above chemicals into a suitable capacity of the beaker. For homogenous perform sonication of 3-4 minutes and allow it for 15 minutes of vigorously magnetic stirring. Now the solution is ready for the next step. For the further pursuit of the desired nanomaterial, place an updated form of a chemical solution in hydrothermal reactor Teflon and the internal space of autoclave around Teflon filled with 10 ml ethanol and 2.5 ml of deionized water. Proceed the reaction for approximately 04 hours at 300°C till the few nanometers of iron oxide nanoparticles were developed. On the next day put off the cover and wait for the oven cool down to room temperature. After thorough examination, the dark brown/blackish precipitate can be visualized via naked eye. Wash this prepared form of iron nanoparticles with ethanol thrice gently with the help of centrifuge having 11500 rpm for 10 min at room temperature. Next, add 97% of n-hexane C₆H₁₄ for perfect dispersion and a suitable amount of cyclohexane for non-polar chain/mixing mode. Finally, store it at room temperature the nanomaterial should be dark brown/black color.

2.3. Preparation of hybrid form of chitosan with iron NPs (CH-Fe₃O₄ NPs)
Take 0.5% chitosan (CH) solution and dissolve CH (50 mg) in 100 ml of acetate buffer (0.05 M; pH 4.4). The defined amount of Fe₃O₄ nanoparticles was spread in the CH solution at 25 °C by continuous stirring to get a viscid solution of CH with homogeneously dispersed Fe₃O₄ nanoparticles. The resultant form of CH-Fe₃O₄ nano-composite was dried 60°C for 5 h and characterized.

3. Results and Discussion

3.1. Characterization

To confirm the morphological and structural analysis of iron nanoparticles (Fe₃O₄) and CH-doped iron nanoparticles (CH-doped iron NPs), the scanning electron microscopy (SEM) was employed for the structural analysis. A globular aggregated form of nanoparticles morphology was observed, the particle sizes of Fe₃O₄ and CH-Fe₃O₄ composite were investigated to be from 5 nm to 30 nm (indicated by marking some nanoparticles).

Figure 1 shows the scanning electron microscope (SEM) analysis of Fe₃O₄ NPs based working electrodes. A series of experiments and various synthesis processes were performed for the perfect form of fabricated biosensing working electrodes within a range of 5nm to 30 nm.

![Figure 1. Scanning Electron Microscopy (SEM) Analysis of Iron Oxide Nanoparticles](image)

3.2. XRD Analysis

Calculation: Structural parameters such as lattice constant "a", unit cell volume and crystallite size "D" were calculated from the XRD data using equations (1-3) described below.

\[ a = \frac{\lambda}{2\sin\theta} = \sqrt{h^2 + k^2 + l^2} \quad \ldots \ldots (1) \]
\[ V_{cell} = a^3 \quad \ldots \ldots (2) \]
\[ D = \frac{k\lambda}{\beta_{hkl}\cos\theta} \quad \ldots \ldots (3) \]

Here \( h, k, \) and \( l \) are the Miller indices, \( V \) is the unit cell volume, \( Z \) represents eight molecules per unit cell of the spinel structure, “\( N_A \)” is the Avg.’s number and “\( M \)” is the molecular weight of the sample. Here “\( k \)” is the shape factor, \( \lambda \) is the wavelength of the X-rays, \( \theta \) is the diffraction angle, and \( \beta_{hkl} \) represents the full-width half maxima.

Analysis: figure 2 shows the XRD pattern of Fe₃O₄ ferrite synthesized by chemical method. A bi-phase structure was observed. The observed patterns have prominent diffraction peaks with these reflection planes (220), (311), (222), (400), (440) and (511), which is indexed by computer software Jade 5 [10]. The lattice constant “\( a \)” was measured by the equation. Corresponding to the heat treatment temperature, the crystallite size was 94 nm. Crystal sizes of less than 50 nm have been described in this study, and it has been reported that crystalline sizes below 50 nm can be used to provide a suitable signal to noise ratio in high-density recording media received [11]. Many researchers have previously reported similar results for polycrystalline spinel ferrites synthesized by several methods [12-13].
Table 1. Lattice constant (a), crystallite size (nm), unit cell volume of Fe$_3$O$_4$

| Crystallite size (nm) | Lattice constant (Å) | Volume (Å$^3$) |
|-----------------------|----------------------|----------------|
| 94                    | 8.4146               | 595.799        |

3.3. Sensitivity measurements

As a result of hydrolysis unease enzyme presented onto the surface of Iron Oxide NPs along with polypyrrole composite mounted through a glass substrate via Ti and Au thin film deposition. The potentiometric measurement was conceded out at different values of pH ranging from 3 to 11 as depicted by figure 3. Physiological investigation response for calibration of stability and performance Fe$_3$O$_4$ NPs based biosensor were inspected using a pH range of 3 to 11, but the neutral pH, which was 7, was selected as the best pH range for stability, performance, and sensitivity parameter. Besides, the selectivity of the fabricated nano-biosensing electrode or were tested by the accumulation of various interfaces, e.g., glucose and uric acid to test the solution containing urea.
Figure 3. Sensitivity response of biosensor based on Fe$_3$O$_4$ at pH values range of pH 3-11.

The measured potential difference values (EMF values) the from the working electrode of biosensor were found influence free from a volume of the test solution and the area of the dipped tip of the working electrode in the test solution. A series of experiments were performed for precise measurements and fabricated working electrode of good reproducibility and repeatability. As sensitivity response of nano-based biosensor was shown in graph depicted EMF (mV) vs. log of concentration (range 1 to 80 mM) 0.00 to 2.5 mM. As the described graph show that EMF concentration rises as increasing of solution (glucose and urea concentration). Fe$_3$O$_4$ NPS based biosensor shows the linear curve trend between EMF and sample concentration as shown in figure 4. It is evident from the figure as long as the biological sample became concentrated due to stroma’s or cancerous tissue EMF (mV) polarity signal shows an increasing trend too, similar data were already published in many articles [14].

Figure 4. Sensitivity response curve of Fe$_3$O$_4$ NPs at pH=7.
3.4. Capsulation of chitosan coating in iron oxide NPS layer

Take a suitable amount of pluronic acid and iron oxide nanoparticles allow for homogeneity till 30 minutes and incorporate dropwise form of Ccitosan solution and allow rotating by using magnetic stirrer until 4 hours at room temperature for reaction process after reaction completion washed by using suitable quantity of ethanol thrice and stored final product after filtration.

4. Conclusion

A urease-trapped CH-Fe₃O₄ nano-biocomposite-based urea biosensor of desired morphology has been successfully fabricated by applying the aqueous chemical growth technique and characterized by various methods. Log of the concentration range of multiple solutions (1mM to 80 mM) 0.00 to 2.5 mM for urea and glucose and an output signal (≈42 mV per decade) was detected at room temperature.

The current developed form of CH-Fe₃O₄ nanocomposite biosensor illustrates significant stable potentiometric response in very minimal time overall performance like reproducibility, selectivity, stability, and sensitivity of the biosensor is very up to the mark and was excellent due to a versatile and unique characteristic magnetic based biosensor valuable for medical application and other related areas like to decide non-hygienic form of food. Additionally, CH-Fe₃O₄ based sensor is also achievable for clinical diagnosis as well. However, the said biosensor showed slight influence once inspected through the addition of a variety of interferers such as glucose and uric acid of healthy and abnormal tissues/cells, which reflects that the sensor is helpful for urea detection along with such interferers.

Acknowledgments

This work was supported by the National Natural Science Foundation of China 51302030, 51272038, 61474015, 61474014, and the National Program on Key Basic Research Project (973 Program) 2013CB933301, 2018YFA0306100.

References

[1] Yang L, Ren X, Tang F and Zhang L 2009. Biosensors and Bioelectronics, 25(4), 889-5.
[2] Sophie L., Delphine F; Marc P; Alain R; Caroline R; Luce V E and Robert N M 2008 Chemical Reviews 108 (6), 2064-110.
[3] Harivardhan Reddy L; José L A; Julien N and Patrick C 2012 Chemical Reviews 112 (11), 5818-78.
[4] Nehra P, Chauhan R P, Garg N and Verma K 2018. British Journal of Biomedical Science, 75(1), 13-8
[5] Miao Y and Tan S N 2000. Analyst, 125(9), 1591-4
[6] Aziz T, Masum S M, Qadir M R, et al. 2016 Int Res J Pure Appl Chem. 11(1):1–9.
[7] Kaushik A, Solanki P R, Ansari A A, Ahmad S and Malhotra B D 2008. Electrochemistry Communications, 10(9), 1364-8.
[8] Zhang F 2015. Photon Upconversion Nanomaterials Springer Berlin Heidelberg 255-84
[9] Arakha M, Pal S, Samantarrai D, Panigrahi T K, Mallick B C, Pramanik K and Jha S 2015. Scientific Reports, 5, 14813.
[10] Hankare P P, Jadhav S D, Sankpal U B, Chavan S S, Waghmare K J and Chougule B K 2009. J. Alloys and Compounds, 475(1-2), 926-9.
[11] Ashiq M N, Ehsan M F, Iqbal M J and Gul I H 2011. J. Alloys and Compounds, 509(16), 5119-5126.
[12] Panda R N, Shih J C and Chin T S 2003. J. Magnetism and Magnetic Materials, 257(1), 79-86.
[13] Tahar L B, Artus M, Ammar S, Smiri L S, Herbst F, Vaulay M J and Fievet F 2008. J. Magnetism and Magnetic Materials, 320(23), 3242-50.
[14] Zhu C Z, Yang G H, Li H, Du D and Lin Y H 2014. Analytical Chemistry 87 (1) 230-49.