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Detection Of Urea By Polyaniline Based Modified Sensor With Geranium Particles.

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Abstract

A novel amperometric urea biosensor has been developed for selective and quantitative recognition of urea by immobilizing urease onto polyaniline (PANI) based nanocomposite graphite paste with geranium (Ga) electrode and monitoring the amperometric response caused by the immobilized urease reaction system. Urease immobilization on electrode was investigated using aamperometric method, and factors affecting its immobilization such as concentration of urease, pH was discussed in detail. Organized materials were characterized by analytical techniques such as UV-Vis, XRD and FE-SEM analysis. The performance of the developed urea biosensor was evaluated for polyaniline and polypyrrole, obtained urea biosensor exhibited shorter response time (3 s), wider linear range, lower detection limitand good stability with about 95% of the original response signal retained after 2 month for PANI

Keywords: Amperometric; biosensor; immobilization.

1. Introduction

The determination of urea is of great interest in various fields such as the pharmaceutical and food industries, environmental protection, fertilizers, but the most important applications are in biomedical and clinical analysis. Urea is actually a waste product of protein degradation and the main nitrogenous component of urine, produced in the liver and excreted by the kidneys. Some pathological conditions, such as renal insufficiency, hyperpyrexia, hyperthyroidism, leukemia, burns, diarrheal diseases and diabetes mellitus, manifest themselves outside the range of urea concentrations (2.5-7.5) mM in blood and 10-30 g in urine collected 24 - h sampling [1,2]). Therefore, it is important to detect urea in serum or urine samples [3]. Real samples are usually diluted prior to analysis to reduce the matrix effect, so M-level detection limits are necessary [4]. Urea is commonly detected by spectrophotometric analysis [5], but alternative methods have been proposed, including sensor detection, which represents a simple and cost-effective method. Urease (Ur) has been used as a biological sensing material for the production of thermal [6–8], amperometric [9–12], conductometric [13–15], piezoelectric [16], optical [17] and potentiometric [18] urea sensors. An interesting class of urea sensors are those that use electrosynthesized polymers [19-20]. In this case, the special advantages of the sensor application of these materials are combined with different transduction mechanisms. If the polymer has the role of an immobilization matrix [21–23], incorporation of the enzyme into the electrode has been achieved either by introducing the enzyme directly into the polymerization solution or by other means such as electrostatic interactions with the components, cross-linking on the electrode. In all cases, there is limited or no control over the amount of immobilized enzyme.[23-26] Enzyme immobilization is an important aspect for the development of biosensors and bioreactors. In general, enzyme immobilization studies focus on the choice of immobilized material, immobilized methods, and immobilized enzyme properties. Many methods have been developed for enzyme immobilization, but usually one of four methods is used: physical adsorption, entrapment, copolymerization, and covalent bonding [27-28].

In this work, we describe the performance of a polyaniline-modified graphite paste electrode (GPE) for the detection of urea in laboratory samples using an amperometric technique with the addition of polyaniline (PANI), a highly conductive graphite paste-supporting polymer. A high-performance polymer-modified graphite paste electrode is demonstrated by speciation and determination of urea forms in pharmaceutical formulations, urine sample, seawater samples. The proposed amperometric method was validated using inductively coupled plasma atomic emission spectrometry (ICP-AES) [29-30].

2.Experimental part

2.1. Materials and chemicals

Urea (99%), urease purchased from Pathozyme, India. polyaniline purchased from Sigma Aldrich. Additional pure graphite powder (particle size 240 × 10^{-6}m) obtained from Loba chemie Pvt. Ltd. India, heavy oil or mineral oil (viscosity 37 ° C is 64cS) purchased from a High Quality Lab, Mumbai, India. The platinum thread is 0.2 mm wide and 6 cm long found in Jyotirling Lab, India.

Method of treating Geranium (Ga)

Geranium essential oil plant has been used to treat health conditions for centuries. There is scientific data indicating that it may be beneficial for a number of conditions, such as anxiety, depression, infection, and pain management. It's thought to have antibacterial, antioxidant, and anti-inflammatory properties. Ga use with graphite powder due to its conductive nature.

Ga was dried under sun and impurities were separated manually. It was boiled with distilled water for 2 h to make it free from colored compounds. It was then filtered. The residual material so obtained was dried at 80 ◦ C in hot air oven for 24 h. Ga thus treated was ground till fine particles in the range of 1–10 m are obtained. The material was stored in an airtight glass vial for further use. This powdered Ga was employed as a modifier in the GPE . It is to be noted here that the composition of Ga varies from one vegetable species to another or even depending on the time at which harvest process is carried out. It is expected that its adsorptive behavior for urea would not change drastically.

2.2. Characterization

UV-Vis was recorded in the at room temperature at an average distance of 200- 800 nm using a Jena specord 210 spectrophotometer. FT-IR display was recorded on Ocean Optics HPX-2000 (Fiber coupled) spectrometer at a scale of 4000 -500 cm-1. The FE-SEM transmitted by the JEOL JSM-7500F is a very high-resolution electronic field filter (FE-SEM) equipped with a very high FE weapon and an unusual low focus cluster). All pH measurements are completed in Systronic (display pH 362 frame) pH meter. Potentiometric response characteristics were assessed with a 41/2 Digit True RMS Multimeter (MODEL 1085).

2.3 Synthesis of Graphite-PANI-geranium particles (Gr/PANI/Ga).

Combination of 70:20:5:5 graphite powder: mineral oil: PANI: Ga newly prepared pestle, this pestle is allowed to mix for 60 minutes. The glue was then filled with teflon micropipette. The platinum wire is inserted pest for electrical contact, Smooth and fresh electrode surfaces were obtained by squeezing out 0.5mm of paste from the tip, scraping off the excess and polishing it against butter paper. By using same method Gr/Ga/PANI electrode made for amperometric study.

2.4 Amperometric study.

The AgCl electrode as reference electrode, Graphite as counter electrode and Gr/Ga/PANI immobilized urease was employed as working electrode, respectively. After mounting the three electrodes in the cell, a small amount of aqueous solution was introduced into the cell. When the amperometric response became stable, urea solution (0.01 M to 0.1 M) was introduced into the cell. Timedependent change in the potential was recorded by a potentiostat.

3. Results and Discussion

3.1 UV-vis. Study

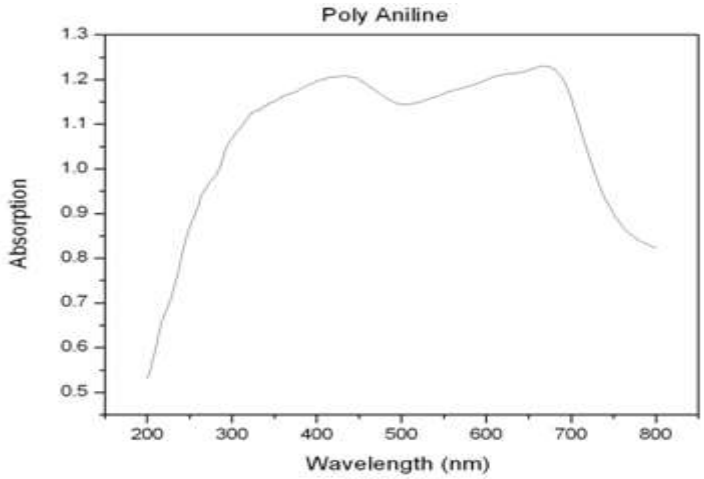

Figure 2 UV-visible spectrum of Gr/PANI/Ga

Fig 2 shows the range recorded in by UV-visible spectra. All spectra were recorded in the wavelength scope of 200-800 nm. The shoulder is showing up at 440 nm for H_2SO_4 compares to the development of ES (Emeraldine salt) stage independent of their natural supporting electrolyte. It demonstrates extremely great likeness with prior revealed work [20-21]. UV-Vis range uncovered that the response medium showed assimilation and around 250 nm and 300 nm for test which is credited to blue move for thioglycerol topped Ga particles.

3.2 FTIR study:

Fig. 3 shows the cellular composition of PANI-matched samples at a distance of 4000-400 cm^{-1} by the FTIR spectra. Bonds of 1463 cm^{-1} and 1597 cm^{-1} correspond to C = C which extends the vibration of benzoid and quinoid rings respectively. The height of 1259 cm^{-1} is the band of the CN expansion feature and the weak taste at 3462 cm^{-1} given the NH stretch mode. The absorption band from 1101 cm^{-1} is defined as the vibration band Nitrogen quinine (N = Q = N). In addition a band at 860 cm^{-1} can be inscribed on the CH without bending the plane of the scented ring that clearly supports the shape of the PANI. The polymer shows that the absorption bands at 2968 are due to the uneven expansion of the CH and the uniform proportions of the CH. These belts match the Aniline features, showing excellent consistency. The FTIR line results therefore confirm the formation of Polyaniline. [22-25]

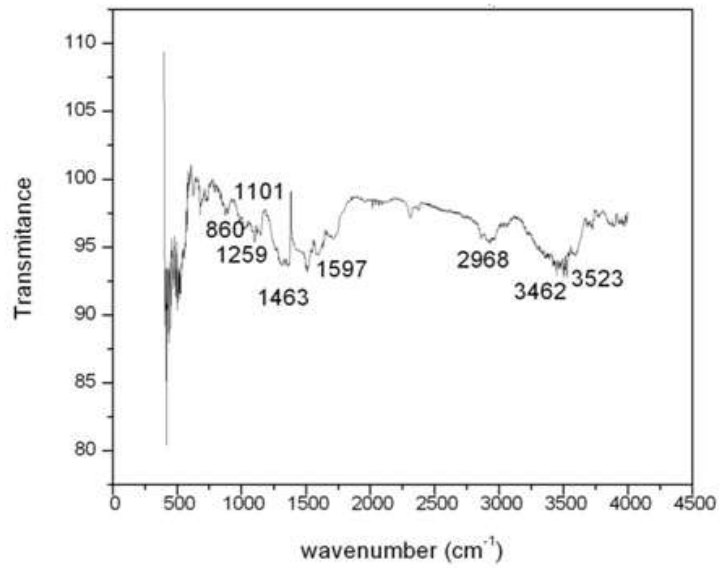

Fig.3 FTIR spectrum of Gr/PANI/Ga

3.3 SEM study

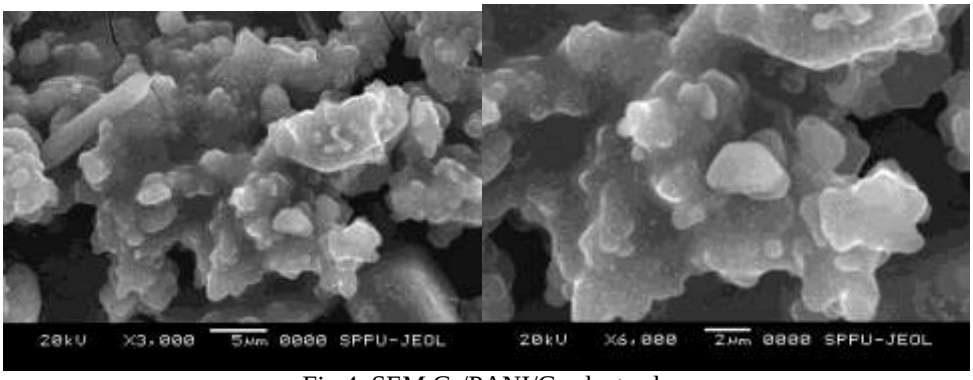

Fig.4. SEM Gr/PANI/Ga electrode

Fig.4 shows morphological structure of Gr / PANI / Ga using SEM. It exhibits a three-dimensional open composition with aniline-like texture in nature and no independent graphite particles can be observed it indicate the radiant adhesion of urea to graphite, Geraniumparticles provides mechanical strength to electrode. Porosity improved the absorbing effect of electrode to analyte. This interaction effect will stimulate higher performance of the sensor.
Furthermore, SEM images proved that the proposed electrode before immobilization serves as an excellent host-guest platform for biomolecules immobilization.

Optimization of process parameter

3.4 Effect of pH

The pH study was carried out by varying the pH in the range of 2 to 9. The pH of the testsolution was adjusted using HCl and NaOH. It also prevents the loss of the enzyme activityunder immobilization conditions [24]. Therefore enzyme sensor response depends on the workingpH of the sampling solution. The effect of pH on the behavior of the enzyme electrode wasstudied with 0.1 M phosphate buffer solution (PBS) with 0.05 M of urea sample with bothelectrode Gr / PANI and Gr / PANI / Ga. The electrochemical response is quite good at pH ranging from 5 to 8 and the most extreme occur at pH 6.5 of Gr / PANI (Fig. 5 a) and pH 7 of Gr / PANI / Ga (Figure 5 b).

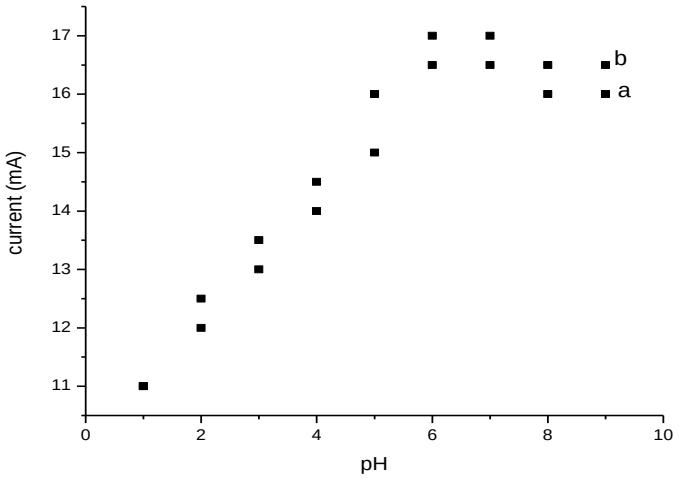

Fig.5 Effect of pH on Gr/PANI (a) and Gr/PANI/ Ga (b).

3.4 Current response

Graphite powder when treated with Geranium -particle it forms weak bond with Ag-NPs. This is Geranium- graphite nano-composite. The composite formed has electron acceptor property i.e. Lewis acid. So, we added electron donor PANI to this composite to form Graphite-Ag-NPs-PANI electron donor acceptor system where Geranium nano-particles act as a conducting wire. To apply it for urea sensing it was kept in a pot and counter electrode dipped into it. urea is strong electron donor than PANI and immobilize PANI and changes shown by ammeter [25]. Depending upon concentration of urea it shows different readings.

Figure 6 (a-b) shows the current response for various concentration of urea. Figure 6 (a) shows the amperometric detection of Gr / PANI / Ga Fig. 6 (b) indicates the reaction of Gr / PANI.. When the potential of the enzyme electrode was set at 0.6 V is as shown in Fig.6 It was found that the response current of the enzyme electrode easily reaches to steady state. The relationship between response current and urea concentration in 0.1 M phosphate buffer pH 7 is shown. It was found that, current increases with increasing urea concentration in the range of 0.1x10-6 to 1.2x10-6M. amperometric response of Gr/PANI/Ga it shows better response. In the present case, assuming that the enzyme is uniformly distributed throughout the electrode, the reaction takes place predominantly on the surface of the electrode in the lower concentration. Platinum wire help in oxidation process therefore no any secondary enzymes required for oxidation, when urea is oxides ammonia is formed and it not take part in reaction. However, the reaction on the surface of the electrode and the diffusion occurring simultaneously at higher concentrations delays the response time. With increasing concentrations of urea, the response current also increased and finally reached to steady state value. Fig.6 shows the steady-state potential dependence calibration curve for the each individual urea concentration. Fig 6, the response of Gr/PANI/Ga to urea conc. was found to be wide linear range of 1x10-6 to 7x10-6 M. This linearity range is in well conformity with that obtained in the amperometric response of sensor is proper in proportion to urea concentration for Gr/PANI/Ga electrode. It shows the Geranium nanoparticles play the significant role in sensing application

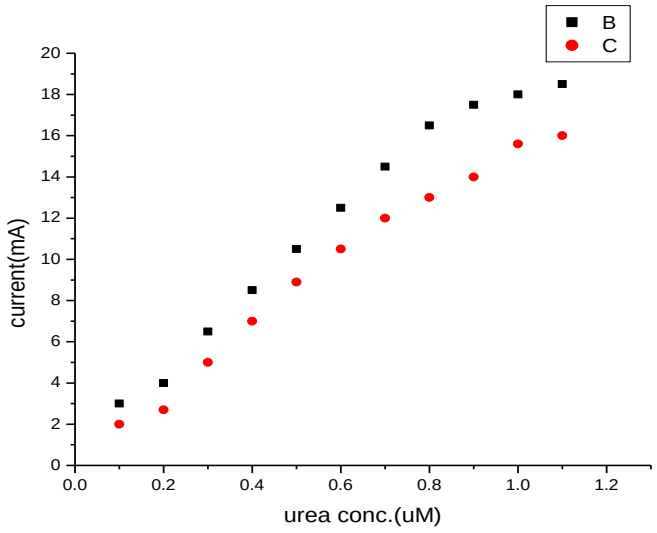

Fig. 6 (a-b) Current–concentration curve a) Gr/PANI/Ga (b) Gr/PANI at 0.5 V

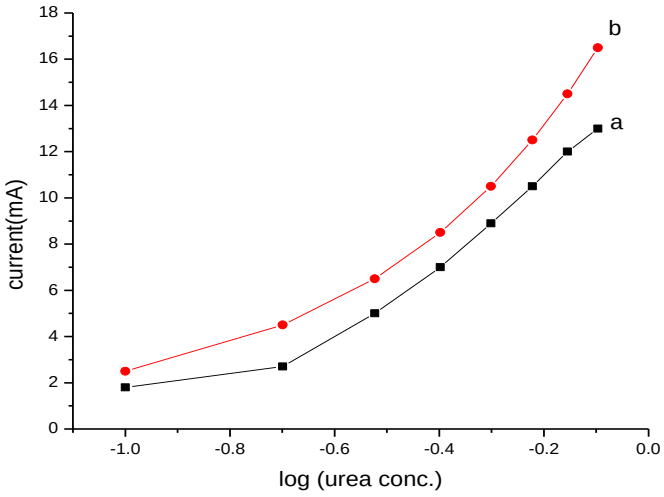

Fig. 7(a-b) Steady-state potential dependence calibration curve of biosensor (a) Gr/PANI/Ga (b) Gr/PANI at 0.5 V

Stability

Long-term stability is one of the most important factors in the efficient use of biosensor as shown in Fig. 6 To test storage stability, both sensors were tested for the last 2 months at 0.1 M phosphate buffer pH 7 at 25 ° C. There is a slight decrease in sensitivity (Gr / PANI / Ga) of about 15% from the original value, which indicates excellent ioactivity retention by sensor (Gr / PANI).

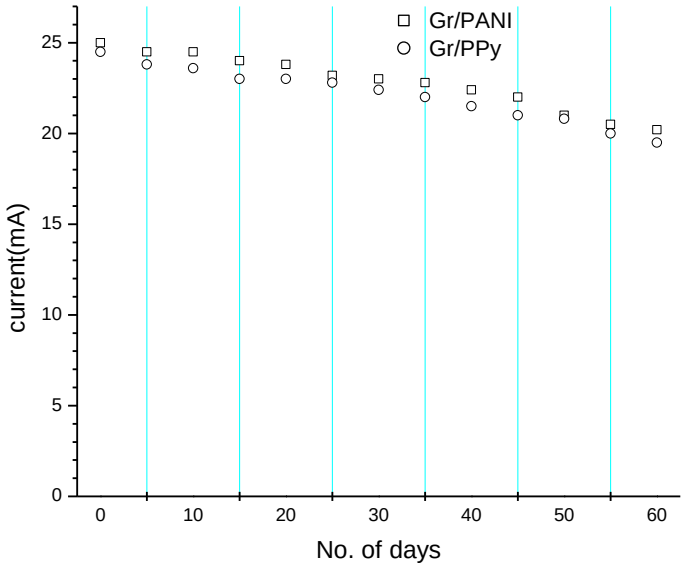

Fig.6 Stability of the a) Gr/PANI/Ga (b) Gr/PANI electrode on storage in 0.1 M PBS (pH 7) for 60 days.

Conclusion

A Gr/PANI/Ga electrode has been developed and success-fully employed for the urea determination laboratory sample. A detection limit of 0.1 × 10−6 M for urea was achieved with the use of the Gr/POA. The present work shows that, Ga is better combination with PANI and graphite powder, it shows better current response as supporting conducting polymer. Also gives the better storage stability for two months, it save the cost of enzyme. This method gives benefits such advantages as high sensitivity, low detection limit, easy handling, resistance against surface fouling, and low cost. Consequently, this method is recommended for the analyses of phosphate, antimony, glucose, creatinine in clinical as well as quality control laboratories.

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