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Amperometric Detection of Urea by Polyaniline and Polypyrrole Based Nanocomposite Graphite Paste Electrode: A Comparative Study

B Pawase[1], K.S. Paithankar[2] V.K. Gade[3]
[1,2,]Department of Physics Research Center, Ahmednagar College Ahmednagar(M.S.) India
[3]Department of Physics, Shri Anand College, Pathardi (M.S.) India

Abstract :A novel amperometric urea biosensor has been developed for selective and quantitative recognition of urea by immobilizing urease onto polyaniline and polypyrrole based nanocomposite graphite paste electrode and monitoring the amperometric response caused by the immobilized urease reaction system. Urease immobilization on electrode was investigated using an ampere metric 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 limit and good stability with about 95% of the original response signal retained after 2 month fopolyaniline.
Keywords: Amperometric; biosensor; immobilization.

I. INTRODUCTION

Urea determination is of great interest in different fields as pharmaceutical and food industry, environmental protection, fertilizers, but the most important applications are in biomedical and clinical analysis. In fact, urea is a waste product of protein degradation and the main nitrogen component of urine, produced in the liver and eliminated by the kidneys. Some pathologies such as renal insufficiency, hyperpyrexia, hyperthyroidism, leukemia, burns, diarrheal diseases and diabetes mellitus are reflected by out-of-range urea concentrations (2.5–7.5) mM in the blood and 10–30 g in urine collected by a 24-h sampling [1,2]). Therefore, it is important to detect urea in serum or urine samples [3]. Real samples are typically diluted before analysis to reduce matrix influence, so detection limits at M level are necessary [4].Urea is routinely detected by spectro photo metric analysis [5], but alternative methods have been proposed including sensor detection, which represents a simple and cost-effective method. Urease (Ur) has been utilized as the biological sensing material for the fabrication of thermal [6–8], ampere metric [9–12], conduct metric [13–15], piezoelectric [16], optical [17] and potentio metric [18] urea sensors. An interesting class of urea sensors is represented by those employing electrosyn the sized polymers [19-20]. In this case, the peculiar advantages of sensor application of these materials are combined with different transduction mechanisms. When the polymer has the role of immobilization matrix [21–23], incorporation of enzyme in the electrode has been obtained either by introducing enzyme directly in the polymerization solution or by other means such as electrostatic interactions with components, cross-linking onto electrode. In all cases, there is scarce or no control of the amount of immobilized enzyme.[23-26] Enzyme immobilization is an important aspect for the development of biosensors and bioreactors. In general, the immobilization studies of enzyme focus on the immobilized material selection, immobilized methods and immobilized characters of enzyme. Many methods have been developed for the enzyme immobilization but usually one of four methods is used: physical adsorption, entrapment, co-polymerization and covalent at attachment [27-28]. In the present work, we report the performance of a graphite paste electrode (GPE) modified by polyaniline and polypyrrole, for detecting of urea in laboratory samples using amperometric technique with addition of polyaniline (PANI) and polypyrrole (Ppy) a good conducting polymer supporting to graphite paste. The superior performance polymers modified graphite paste electrode is demonstrated by the speciation and determination of urea forms in pharmaceutical formulations, urine sample, sea water samples. The proposed ampere metric method has been validated by using inductively coupled plasma-atomic emission spectrometry (ICP-AES) [29-30].

II. EXPERIMENTAL

A. Materials and chemicals

Urea (99%), urease was purchased from Pathozyme, India. polyaniline and polypyrrole purchased from Sigma Aldrich. Graphite fine powder extra pure (particle size 240×10^{-6} m) obtained from Lobachemie Pvt. Ltd. India, Paraffin liquid heavy or mineral oil (viscosity at 37°C is 64 cS) purchased from High purity lab, Mumbai, India. Platinum wire has 0.2 mm diameter and 6 cm length obtained from Jyotirling Lab, India.

B. . Characterization

X-ray powder diffraction (XRD) patterns have been recorded on a model D8 Bruker AXS with monochromatic Cu radiation (40 kV and 30 mA), over the 2θ collection range of 20–80°. UV–Visible spectra (UV–Vis) were recorded in air at room temperature in the wavelength range of 200–800 nm using a Jena specord 210 spectrophotometer. FT-IR spectra were recorded on a Ocean optics HPX-2000 (Fiber coupled) spectrometer in the range of 4000-500 cm^{-1}. FE-SEM carried by JEOL JSM-7500F is an ultra-high resolution field emission scanning electron microscope (FE-SEM) equipped with a high brightness conical FE gun and a low aberration conical objective lens). All pH measurements were carried out on a Systronic (model μ pH system 362) pH meter. Potentiometric response characteristics were studied with a $4^{1/2}$ Digit True RMS Multimeter (MODEL 1085).

C. Synthesis of Graphite-PANI (Gr/PANI) and Graphite-Ppy (Gr/Ppy) nanocomposite electrode

Composition of 70:25:5 graphite powder: mineral oil: PANI pestle freshly prepared, this pestle allowed to homogenize for one hour. The paste was then filled in a teflon micropipette tip. A platinum wire was dissected through the paste, to provide an 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/PPy electrode made for amperometric study.

D. Urease immobilization on electrode

Urease immobilization on the composite surface carried out by dropping of the PBS (pH7) containing the enzyme on the electrode surface, which further dried at a controlled temperature. The working electrode was prepared by dropping 2mg of purified urease solution onto the surface of the Gr/PANI and Gr/PPy electrode. The electrodes were rinsed with distilled water, dried.This constitutes a real contribution from the composite surface to the efficiency of the biosensor without the cross-linking agents to make bonding to the active sites of enzymes, thus inhibiting their activity.

E. Amperometric determination.

The AgCl electrode as reference electrode, Graphite as counter electrode and Gr/PANI, Gr/PPy with 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. Time-dependent change in the potential was recorded by a potentiostat.

III. RESULTS AND DISCUSSION

A. UV-vis. Study

Fig.1(a-b) shown the optical absorption spectrum of synthesized Gr/PANI (a) and Gr/PPy (b) electrode. The spectrum recorded in directly without any solution on it. All spectra were recorded in the wavelength range of 300-800 nm. The shoulder is appearing at 491 nm corresponds to the formation of ES (Emeraldine salt) phase irrespective of their organic supporting electrolyte. Peak at 600-650 shows PANI has good adhesion to graphite powder and wavelength 550-600 shows the indication of PPy. It shows very good resemblance with earlier reported work [7-8]. Fig.2 (b).shown peakis just shifted at 499 nm.

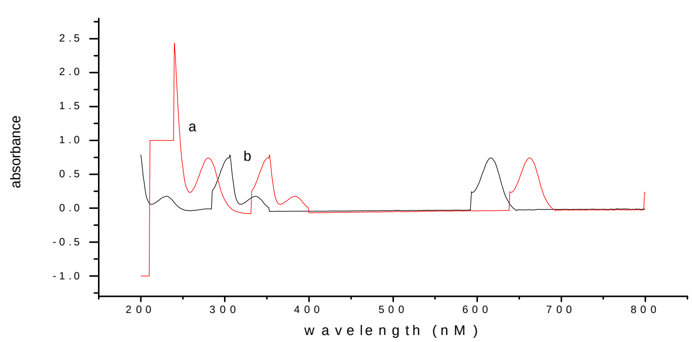

Fig. 1 (a-b) UV–Visible study of (a) Gr/PANI (b) Gr/PPy

B. SEM study

Fig.2compares the morphological features of (a) Gr/PANI, (b)Gr/PPy electrodes using SEM. [Fig. 2(a)]. Both images are uniform in nature and no separated graphite particles could be observed, which demonstrates the excellent adherence of urea to graphite [Fig. 2(b)]. Hence, it is expected that surface will contribute to the adsorption of analyte. This synergistic effect will lead to a better performance of sensor electrode.

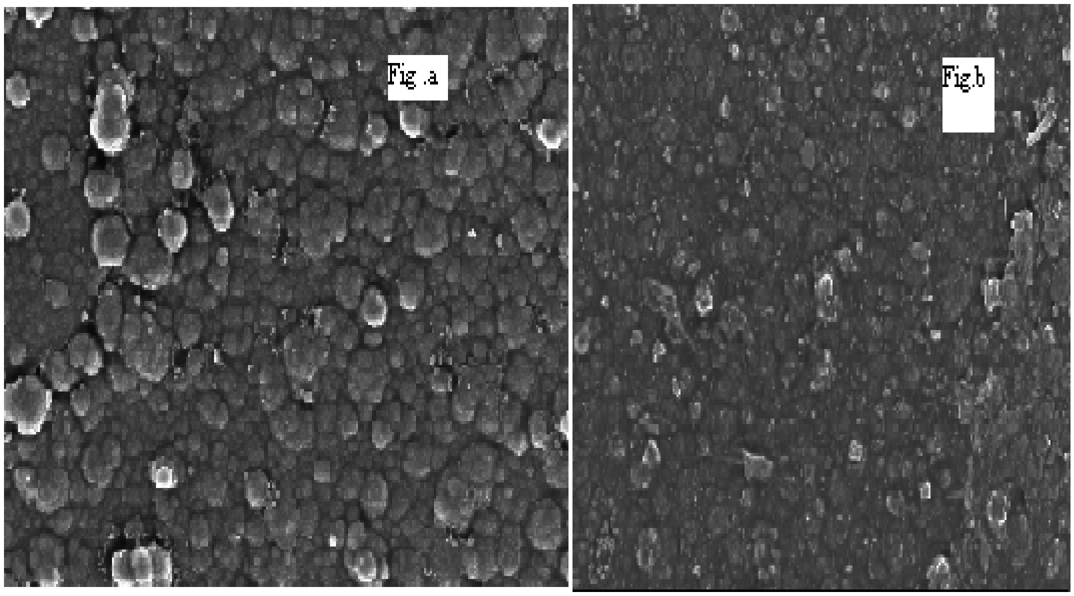

Fig. 2(a-b) SEM of (a) Gr/PANI (Mag.25KX) and (b) Gr/PPy (Mag.25KX)

C. XRD study

Fig. 3 shows the XRD pattern of Gr/PANI. It is clearly indicates that the intensity of observed peaks are better developed on the composites prepared using di and tri basic acid solutions compared with the monobasic acid. The profile of the characteristic peak of PANI at ≈25° Thus the fraction of crystalline phase found to be increased as increasing the voltages. Figure shows graphite powder exhibited the characteristic cubic (FCC) diffraction peaks at 26.1°.The XRD pattern of purified graphite nanosheets gives a distinguishable graphite peak at 26.1°.

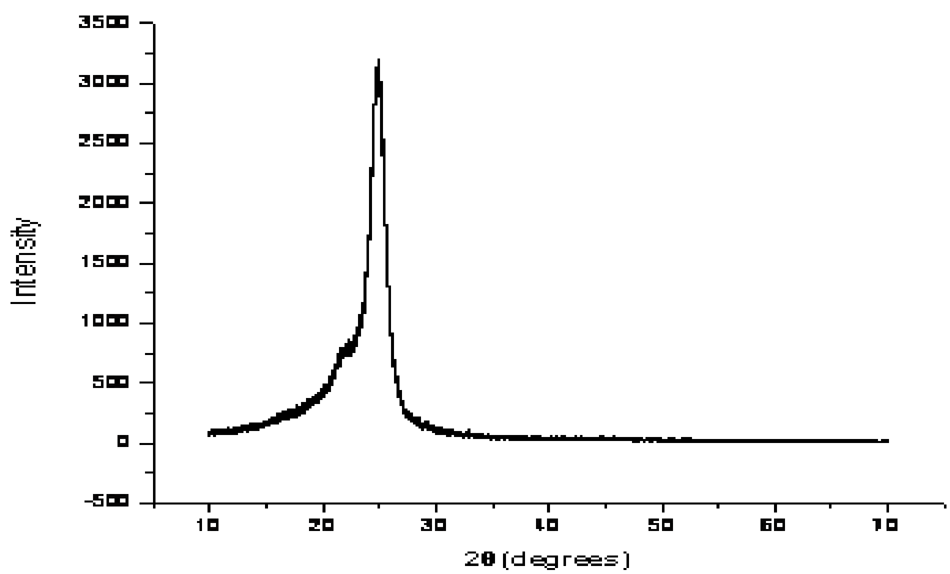

Fig. 3 XRD study of Gr/PANI

D. *Effect of concentration of pH*

The pH study was carried out by varying the pH in the range of 2 to 9. The pH of the test solution was adjusted using HCl and NaOH. It also prevents the loss of the enzyme activity under immobilization conditions [31].Therefore enzyme sensor response depends on the working pH of the sampling solution. The effect of pH on the behavior of the enzyme electrode was studied with 0.1 M phosphate buffer solution (PBS) with 0.05 M of urea sample with both electrode Gr/PANI and Gr/Ppy. The electrochemical response is quite good at pH ranging from 5 to 8 and the maximum current occurred at pH 6.5 for Gr/PANI (Fig.4.a) and pH 7 for Gr/Ppy (Fig.4.b). pH study shows the conductivity of PANI is better in acidic medium than PPy.

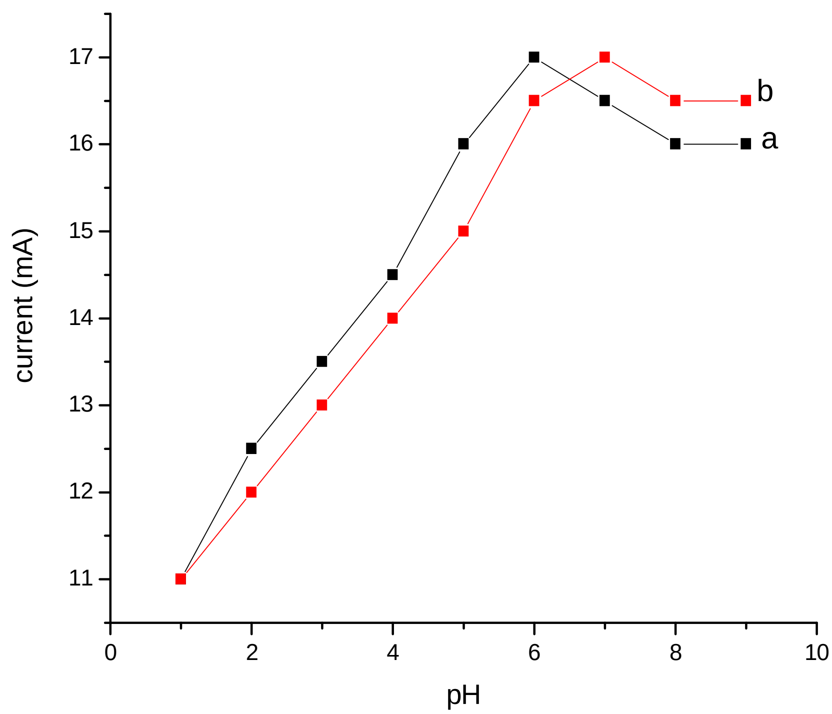

Fig.4 Effect of pH on Gr/PANI (a) and Gr/Ppy (b).

E. *Current response*

Fig.5 (a-b) shows the current response for various concentration of urea. Fig.5 (a) shows amperometric response for Gr/PANI and Fig.5(b) shows response for Gr/PPy When the potential of the enzyme electrode was set at 0.6 V is as shown in Fig.4 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 $0.1x10^{-9}$ to $1.1x10^{-9}$M. amperometric response of Gr/PANI (a) it shows better response than Gr/PPy (b) electrode . 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(a-b) shows the steady-state potential dependence calibration curve for the each individual urea concentration. Fig 6(a) The response of Gr/PANI to urea was found to be wide linear range of $1x10^{-9}$ to $7x10^{-9}$ M and for Fig 6(b) Gr/PPy it become very short $2x10^{-9}$ to $4x10^{-9}$ M. This linearity range is in well conformity with that obtained in the amperometric response of sensor is proper in proportion to urea concentration.

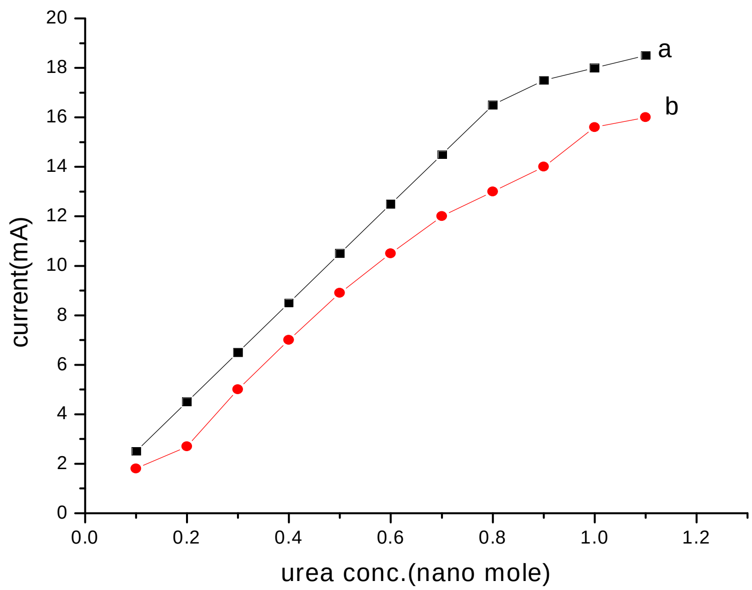

Fig. 5 (a-b) Current–concentration curve a) Gr/PANI (b) Gr/PPy at 0.6 V

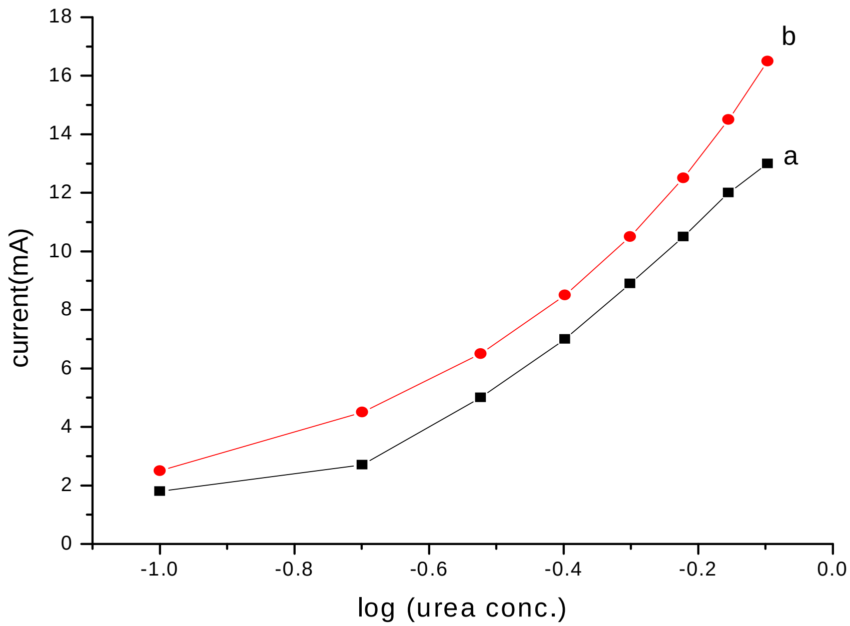

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Fig. 6(a-b) Steady-state potential dependence calibration curve of biosensor a) Gr/PANI (b) Gr/PPy

F. *Storage stability*

Long term stability is one of the most important features required for the satisfactory application of a biosensor as shown in Fig.6 In order to evaluate the storage stability, the both sensor was tested for 2 month of storage in 0.1 M phosphate buffer pH 7 at 25°C. There is a slight decrease in sensitivity of the sensor (Gr/PANI) of about 15% from the initial value, revealing a very good preservation of the bioactivity than sensor (Gr/PPy).

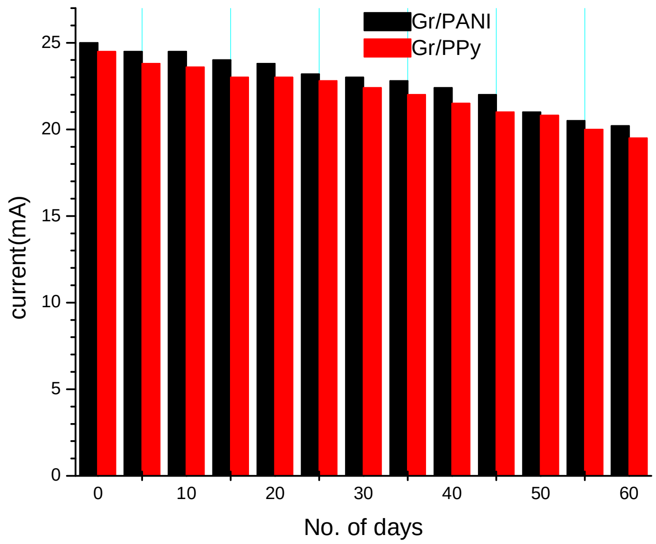

Fig.6 Stability of the a) Gr/PANI (b) Gr/PPy electrode on storage in 0.1 M PBS (pH 7) for 60 days.

IV. CONCLUSION

A Gr/PANI and Gr/PPyelectrode has been developed and success-fully employed for the urea determination laboratory sample. A detection limit of 0.1×10^{-9} M for urea was achieved with the use of the Gr/PANI. The present work shows that, PANI is better combination than PPy with graphite powder, it shows better current response as supporting conducting polymer. Gr/PANI electrode 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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