



Polyaniline based Modified Sensor with Silver Nanoparticles for Detection of Urea

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A novel polyaniline (PANI) based amperometric urea biosensor developed for selective and quantitative sensing of urea using mediator enzyme urease on graphite paste electrode modified with silver nanoparticle (Ag) (PANI/Gr/AgNO₃) and without silver nanoparticle (PANI/Gr) and measure the amperometric response caused by the immobilized urease reaction system. Urease immobilization on electrode was investigated using an amperometric method and factors like pH after its immobilization discussed. Prepared materials were characterized by analytical techniques such as ultra violet visible and SEM analysis. The performance of the developed urea biosensor was evaluated and obtained urea biosensor exhibited shorter response time (3 s), wider linear range, lower detection limit and good stability with about 90% of the original response signal retained after 2 months for silver nanoparticle.

Keywords: Amperometric, Polyaniline, Silver nanoparticles, Biosensor, Enzyme urease, Graphite paste electrode, Urea.

INTRODUCTION

Urea's commitment is of great interest in various fields such as the pharmaceutical and food industry, environmental protection, fertilizers, but the most important applications are for environmental analysis. Truth be told, urea is the result of the depletion of proteins and the basic nitrogen component, which is carried to the liver and excreted by the kidneys. A few diseases, for example, kidney failure, hyperpyrexia, hyperthyroidism, leukemia, dementia, diarrhea and diabetes are characterized by high concentrations of urea (2.5-7.5 mM) in the blood and 10-30 g in urine collected in 24 h experiments [1,2]. In this way, it is important to detect urea in serum or urine tests [3]. Real models tend to be weak before testing to reduce grid impact, so M-level recognition parameters are important [4]. Urea is often identified by spectrophotometric probes [5], but suggested selection techniques include sensory location, speaking direct financial savings strategies. Urease has been used as a natural discovery material for warmth [6-8], amperometric [9-12], conductometric [13-15], piezoelectric [16], optical [5] and potentiometric [17] urea sensors. The attractive phase of the urea nerve is mentioned by those who use electro-synthesized polymers [18]. In this case, the optimal conditions

for the use of these sensors are combined with a variety of transmission elements. While the polymer has the function of the deformity structure, fuse of the catalyst in the body is obtained by delivering proteins directly into the polymerization system or by various means, for example, electrostatic's interaction with phases, the anode connection. In all cases, there is an abnormal or uncontrolled dosage of inactive chemical doses [19].

In the current work, we report the preparation of graphite glue cathode (GPE) without AgNO₃ for separating urea from samples of a research center using an amperometric system with the expansion of polyaniline (PANI) an advanced polymer that supports graphite glue. The incomparable kill polymers transform the anode of graphite glue is shown by speciation and confirmation of urea frames in pharmaceutical systems, urine testing and seawater test. The proposed amperometric strategy was approved using combined inductively coupled plasma-atomic emission spectrometry (ICP-AES) technique.

EXPERIMENTAL

Urea (99%) and urease were purchased from Pathozyme, India, while polyaniline was purchased from Sigma-Aldrich.

Additional pure graphite powder (particle size 240×10^{-6} m) was obtained from Loba chemie Pvt. Ltd. India, whereas heavy oil or mineral oil (viscosity 64cS at 37 °C) was purchased from a High Quality Lab, Mumbai, India. The platinum thread was 0.2 mm wide and 6 cm long procured from Jyotirling Lab, India.

Characterization: UV-Vis was recorded in the air 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).

Synthesis of graphite-PANI-silver nanoparticles (Gr/PANI/Ag): Combination of 70:20:5:5 graphite powder:mineral oil:PANI:AgNO₃ was allowed to mix for 60 min to prepare paste. The glue was then filled with Teflon micro-pipette tape. The platinum thread was polished with glue, contact with electricity. Smooth and new areas of the anode were obtained by pressing 0.5 mm of glue from the tip, scraping the mass and cleaned with margarine paper.

Amperometric study: AgCl cathode as a reference anode, graphite as a counter terminal and Gr/PANI/AgNO₃ with a weak urease used as a working anode, each. After three terminals were placed in the cell, a small amount of water was brought into the cell. At the time when the amperometric reaction was damaged, urea (0.01-0.1 M) concentrations were introduced into the cell. The subsequent change in power was recorded by the potentiostat.

RESULTS AND DISCUSSION

UV-vis study: The spectrum was recorded in the wavelength range of 300-800 nm (Fig. 1). The shoulder is showing up at 491 nm for H₂SO₄ compares to the development of Emeraldine salt stage independent of their natural supporting electrolyte. It demonstrates similar resemblance with previous work [19,20]. The UV-Vis range revealed that the response medium showed absorption around 250 nm and 300 nm which is attributed to blue move for thioglycerol capped silver nanoparticles.

FTIR study: For the PANI-matched samples, Fig. 2 presents the cellular composition measured at 4000-400 cm^{-1} through FTIR. The bands appearing at 1597 and 1463 cm^{-1} corresponded to the C=C vibrations of quinoid and benzoid rings, respectively. The bands at 1259 and 3462 cm^{-1} correspond to CN expansion and NH stretch modes, respectively. The absorption band observed at 1101 cm^{-1} is related to the nitrogen quinine (N=Q=N) vibration. The band observed at 860 cm^{-1} without bending the plane of the scented ring, which supports the PANI shape, was ascribed to CH. For the polymer, absorption bands appeared at 2968 cm^{-1} due to the uniform proportions and uneven expansion of CH. These bands coincide with the features of aniline. Therefore, the FTIR results confirmed polyaniline formation [21-24].

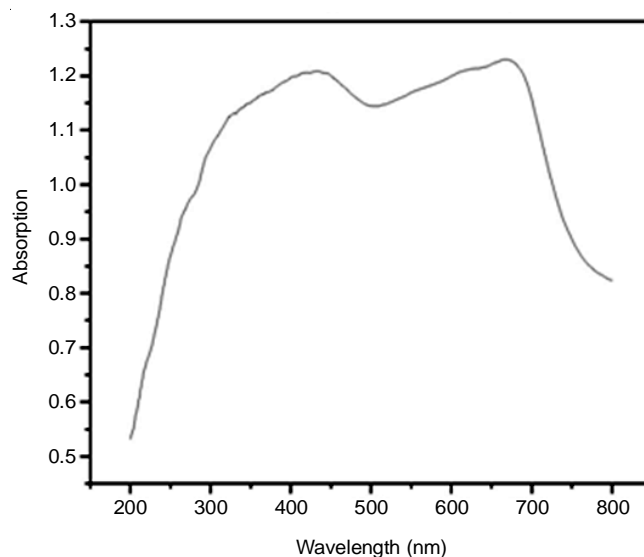


Fig. 1. UV-visible spectrum of Gr/PANI/AgNO₃

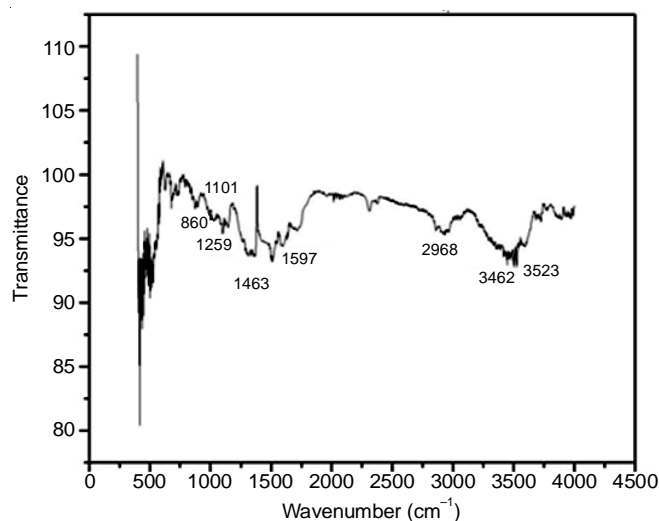
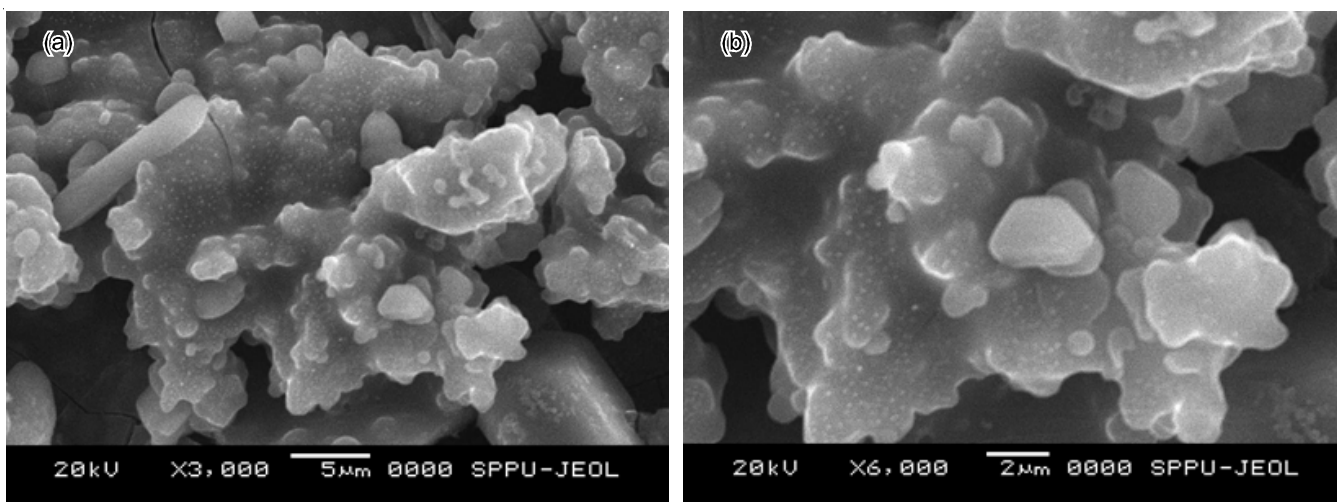
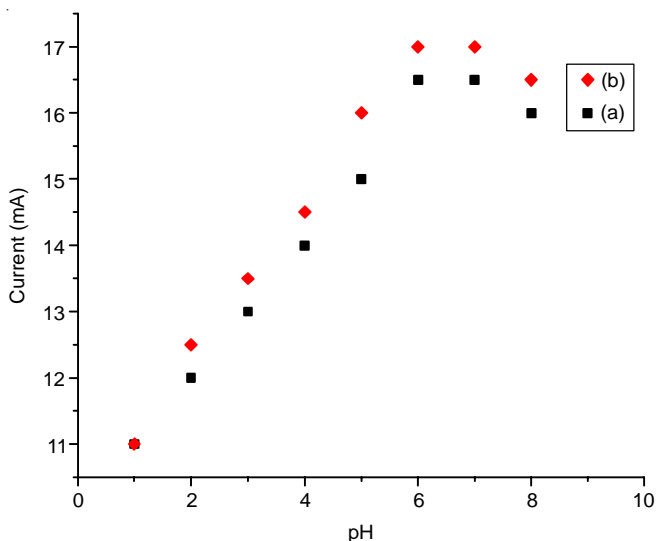


Fig. 2. FTIR spectrum of Gr/PANI/AgNO₃

SEM study: The morphology of Gr/PANI/AgNO₃ electrode was analyzed using SEM. The Gr/PANI/AgNO₃ shows a 3D open structure having an aniline-like texture without any independent graphite particles (Fig. 3). This result revealed the adhesion of graphite to urea. Silver nanoparticles provided high terminal performance and mechanical quality. For anode sensors, this interaction effect can stimulate high performance.

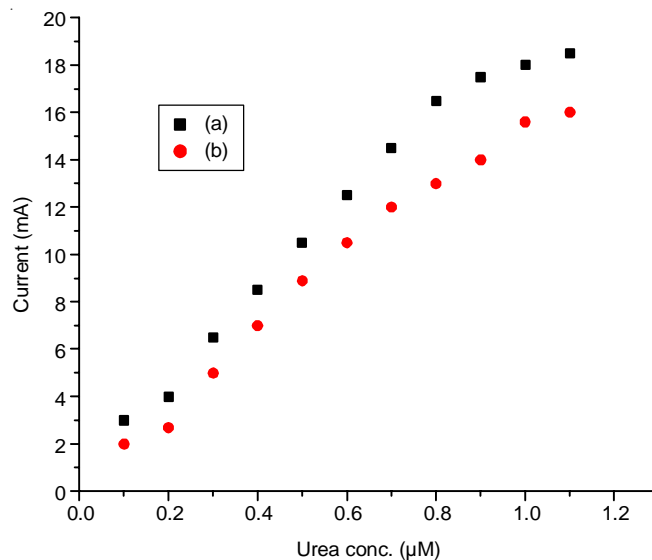
Optimization of process parameter

Effect of pH: For the investigated systems, the pH was determined using NaOH and HCl. It maintained the integrated action loss under the inactivity conditions [23]. Thus, the agent is dependent on test system's pH performance. For both the Gr/PANI/AgNO₃ and Gr/PANI cathodes, the pH effect on terminal behaviour was studied through 0.05 M urea with the phosphate cradle (PBS) arrangement. The electrochemical reaction was fast at the pH of 5-8. The most extreme reaction occurred at the pH of 6.5 and 7.0 for Gr/PANI (Fig. 4a) and Gr/PANI/AgNO₃ (Fig. 4b), respectively.

Fig. 3. SEM Gr/PANI/AgNO₃ electrodeFig. 4. Effect of pH on Gr/PANI (a) and Gr/PANI/AgNO₃ (b)

Current response: A graphite powder forms feeble bonds with the silver nanoparticles after treatment with them. The resulting complexes are Ag-graphite nanocomposites. These composites are electron acceptors means act as Lewis acids and are corrosive in nature. Therefore to this composite, polyaniline (an electron benefactor) was added to construct graphite-Ag-NPs-PANI electron contributor acceptors, in which Ag NPs serve as the leading wire. This framework was stored in a pot, and the counter terminal was immersed into it, for its application in urea detection. Urea is an electron donor compared with immobilized PANI and PANI. Changes appeared were studied with an ammeter [24].

Fig. 5(a-b) presents the current reaction for various urea classifications. The amperometric Gr/PANI/AgNO₃ and Gr/PANI reaction is shown in Fig. 5a and 5b, respectively. When the catalyst cathode power was 0.6 V, the chemical terminal current reaction attains a constant state. A relation between the urea concentration and current reaction at 0.1 M phosphate support and the pH of 7 was found. The current and urea correction increases were obtained at 0.1×10^{-6} to 1.2×10^{-6} M. The

Fig. 5. Current-concentration curve (a) Gr/PANI/AgNO₃ (b) Gr/PANI at 0.5 V

Gr/PANI/AgNO₃ amperometric reaction was better than the Gr/PANI reaction terminal. The chemical was expected to disperse consistently at anode, and at a low point, the response was assumed to occur likely without a cathode. The platinum wire, for oxidation, did not provide an optional compound required. Urea is a fragrant salt oxide and does not involve in this reaction. To adjust the response time, simultaneous distribution and external anode response are used at high resolution. With the increment in urea centralisations, the current response becomes magnified, thereby reaching a state.

Fig. 6(a-b) illustrates state-adjusted state-reliance inconsistency for all the urea concentrations. In urea, the Gr/PANI/AgNO₃ reaction achieved 1×10^{-6} to 7×10^{-6} M range (Fig. 6a) while Gr/PANI attained 2×10^{-6} to 4×10^{-6} M range (Fig. 6b). This transmission of linearity is the same as that for the amperometric sensor response, which can be allowed by the urea fixation level of the Gr/PANI/AgNO₃ anode. Silver nanoparticles play a critical role in finding the current.

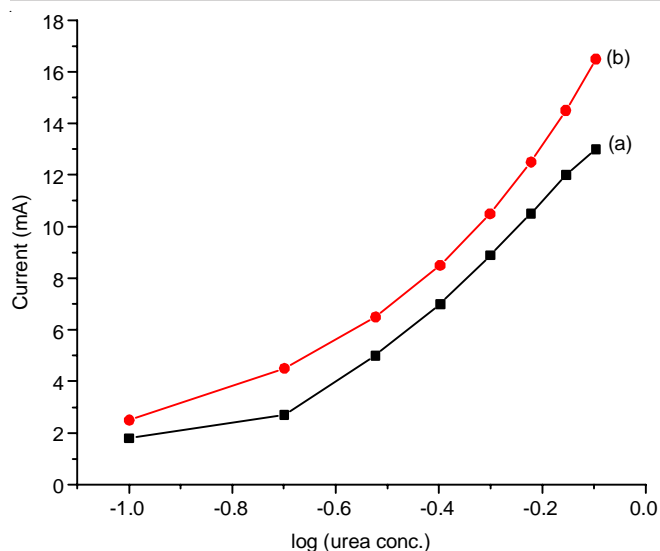


Fig. 6. Steady-state potential dependence calibration curve of biosensor (a) Gr/PANI/AgNO₃ (b) Gr/PANI at 0.5 V

Stability: For the efficient use of biosensors, long-term stability an important factor. To analyze the storage stability, both the sensors were kept in 0.1 M phosphate buffer at the pH of 7 and 25 °C for 2 months. The sensitivity for Gr/PANI/AgNO₃ decreased by approximately 15%, indicating outstanding bioactivity retention of the sensor (Gr/PANI) (Fig. 7).

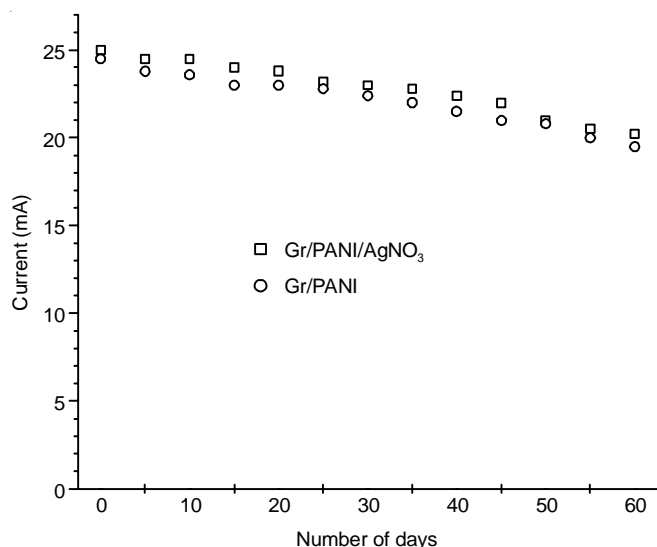


Fig. 7. Stability of the Gr/PANI/AgNO₃, Gr/PANI electrode on storage in 0.1 M PBS (pH 7) for 60 days

Conclusion

The Gr/PANI/AgNO₃ electrode was developed and applied successfully for the urea determination. A detection limit of 0.1×10^{-6} M of urea was achieved with Gr/PANI. According to the literature, the combination of AgNO₃ with the graphite powder and PANI, which as support for running polymers, provides a higher current response, works better than the Gr/PANI electrode. This electrode presents excellent storage durability of two months, thereby saving enzyme expenses. This approach presents advantages, including low

acquisition limit, high sensitivity, low cost, easy handling, and resistance to land fraud. Consequently, this method can be recommended for analyses of antimony, phosphate, creatinine and glucose for quality control.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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