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# Amperometric detection of cholesterol by nanocomposite graphite paste electrode

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## Abstract

*A novel electrochemical cholesterol biosensor based on graphite (Gr) paste electrode modified with polyaniline (PANI) and has been developed for selective and quantitative recognition of cholesterol by immobilizing enzyme cholesterol oxidase (ChO) for monitoring the potentiometric response. Cholesterol oxidase immobilization on electrode was investigated using a amperometric method, and factors affecting its immobilization such as potential, pH was discussed in detail. The amperometric of the developed cholesterol biosensor was evaluated, and the obtained cholesterol biosensor exhibited shorter response time (3 s), wider range 0.1-10 µM and the detection limit were found to be $1x10^{-9}$M. Enzyme activity is 85% retained for 60 days.*

**Keywords:** Electrochemical, Biosensor, Immobilization.

## Introduction

There is expanded interest for growing quick and touchy strategies for the assurance of different analytes show in the entire blood. Sensors have been thought to be a standout amongst the most reasonable gadgets because of selectivity, quick reaction, and smaller than expected size, solid and re-producible outcomes. Biosensors utilizing immobilized catalysts as the sensor electrode acknowledgment component are among the broadly examined gadgets for both key investigation and applications perspective[1-4]. In this specific situation, plan and investigation of new amperometric sensor electrode for the evaluation of convergence of different analytes of pathogenic premium, for example lactate glucose and urea including cholesterol have gotten impressive consideration[5-10].

Fat esters are key com-pounds for people since it is the pieces of cowardice and brain cells and forerunners of the other bio-true blue components, for example, temper dangerous and the steroid Cholesterol and its unsaturated hormones levels[11,12]. The determination of cholesterol in blood is a quick constraint for confirmation and desire of a couple heart sicknesses, arteriosclerosis, thrombosis, and cerebral[13–15]. A pair cholesterol biosensors together with enzymatic and non-enzymatic strategies[16–20]. Regardless, these routinely have sure bothers like nonattendance of specificity as well as selectivity in light of the closeness of different encroaching responses and the function of shaky and hazardous chemicals.

Characteristic driving polymers have starting late ascended an another group of electro active materials and fascinating effect for creative work[21–24]. These are a direct result of the way it have the capacity to balance oppositely charge composite substances within their imperfect driving states and release them in their impartial securing states[25]. Polymer of pyrrole (Ppy) is seen to be very convincing among different coordinating polymers[26–28]. Its small oxidation potential engages a guiding composite material produced from watery plans that were impeccable to a huge segment of the natural parts[29]. Also, composite shows polymerization, good electrical conductivity, substance quality and capability to outline unsupported composite are included central focuses for its use towards biosensors.

There are many reports are accessible for affirmation of free cholesterol utilizing cholesterol oxidase (ChO) bound teo driving polymer framework (polypyrrole or polyaniline)[28,30]. Vidal et al[31] have beginning late engineered a cholesterol amperometric biosensor by co-entanglement of cholesterol oxidase and ferrocene mono-carboxylic damaging in a stream structure. The cholesterol biosensor was seen to be depended on affectability and selectivity of central individuals. It has been re-ported that the constructed cathode was more noticeable affectability as a result of addition in electrolytic capability for electro-blend oxidation of $H_2O_2$ which was made by enzyme immobilization method[32]. It shows the capacity to balance oppositely charged composite substances in its oxidized leading state.

Endeavors produced biosensors for aggregate cholesterol assessment. In such a specific condition chemically synthesized polymer was used for the improvement of the cholesterol sensor[33]. A optical fiber gadget has created by co-immobilizing ChO enzyme and on pre-actuated nylon film for nothing and aggregate cholesterol determination[34].

In this present work, author reported consequences for orderly reviews of the specialized improvement and streamlining of an amperometric sensor seen by immobilizing ChO in PANI and graphite (Gr) composite anode. Reaction of Gr/PANI/ChO anodes as an element of cholesterol oxidase fixation, temperature, connected potential; pH capacity time has likewise been examined.

## Materials and methods

**Materials and chemicals:** Cholesterol (99%), cholesterol oxidase was purchased from Sigma Aldrich, Graphite fine powder extra pure (particle size $240\times10^{-6}$ m) obtained from Loba chemie Pvt. Ltd. India, Paraffin liquid heavy or mineral oil (viscosity at $37^{\circ}$C is 64 cS) purchased from High purity lab, Mumbai, India.

**Synthesis of enzyme based biosensor Gr/PANI/ChO (working electrode):** Organization of 65:25:5 graphite: mineral oil: PANI this pestle allowed to homogenize for 60 minutes. The glue was then filled in a plastic syringe micropipette tip. A platinum wire was analyzed through the glue, to offer an electrical contact. Delicate and new anode surfaces were gotten by crushing out 0.5 mm of glue from the tip, scratching off the overabundance and cleaning it against spread paper.

ChO immobilization on the composite surface done by dropping of the PBS (pH7) containing the compound on the anode surface, which additionally dried at a controlled temperature. The working cathode was set up by dropping 7.0 µL of refined cholesterol oxidase arrangement onto the surface of the Gr/PANI/ChO terminal and brooding it at 20°C for 24 h. The terminal was washed with refined water, dried and put away at 4°C before utilize. This constitutes a genuine commitment from the composite surface to the proficiency of the biosensor without the cross-connecting operators to make clinging to the dynamic destinations of catalysts, in this way hindering their action. The undefined material attached to the PANI is related to the ChO.

**Arrangement of compound arrangements:** The enzyme ChO, about 4.0 U/ml was arranged instantly before use in frosty potassium phosphate support (400 mM) at constant pH.

## Results and discussion

**SEM study:** In Figure-1 are the morphological components of Gr/PANI/ChO anodes utilizing SEM. It unmistakably demonstrates permeable morphology of cathode surface. This nature supportive for effectively immobilization of chemicals for the biosensor application It indicates more uniform in nature and no isolated graphite particles could be watched, which shows the astounding adherence of PANI to graphite, Moreover, homogeneous covering of the catalyst demonstrated that the proposed anode before immobilization fills in as a great host-visitor stage for biomolecules immobilization.

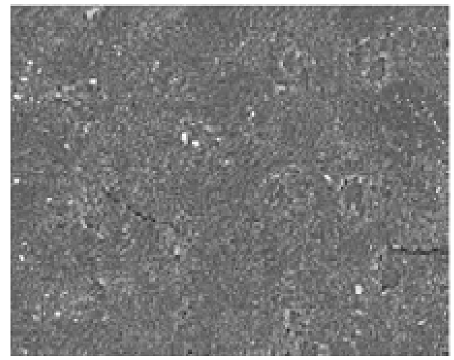

**Figure-1:** SEM image of Gr/PANI/ChO at 20 µM.

**Determination of potential response of Gr/PANi/ChO electrodes:** For impact of working potential observed during amperometric reaction of Gr/PANI/ChO terminal for 4 mM cholesterol is appeared in Figure-2. The electro-chemical effect was recorded by applying diverse inclination potential to the compound cathodes in phosphate cradle 400mM and ChO (4 mM). For Gr/PANI/ChO anodes, the reaction current expanded in gradually, after 0.5V which might be because of the likelihood of meddling species getting corroded at higher potential. These outcomes likewise supported 0.5 V as working potential. Keeping these outcomes in examination a capability of 0.5 V as picked as the working potential for further reviews.

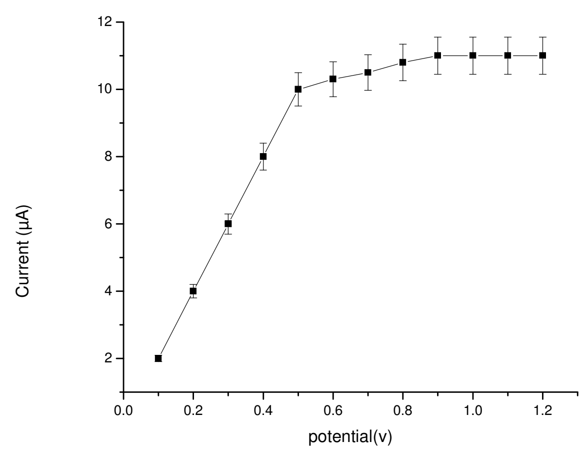


**Figure-2:** Effect of potential on Gr/PANI/ChO electrode.

**Effect of pH:** The pH study was completed by changing the pH in the scope of 1 to 10. The pH of the test arrangement was balanced utilizing $H_2SO_4$ and NaOH. It likewise maintains a strategic distance from the loss of the chemical movement. Hence catalyst sensor reaction relies on upon the functioning pH of the examining arrangement. The impact of pH on the conduct of the protein cathode was studied with 0.1 M Phosphate cushion arrangement with 5 mM cholesterol. The unfaltering state streams at 0.5 V as a component of pH qualities is appeared in Figure-3 the electrochemical reaction is very great at pH extending from 5 to 8 and the most extreme current happened at pH 7.

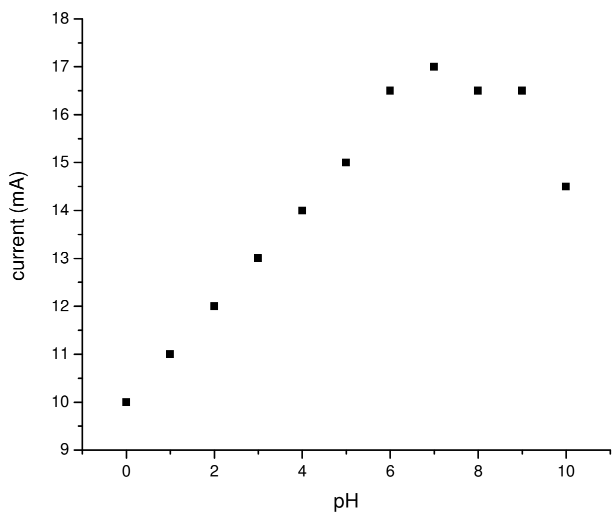


**Figure-3:** (a) Effect of pH on Gr/PANI/ChO at potential 0.5V.

**Current-time response of Gr/PANI/ChO electrodes:** The current–time relationship when the ability of the compound terminal was set at 0.5 V is as showed up in Figure-4 It was discovered that the response current of the concoction anode adequately reaches to persevering state. The association between response current and cholesterol center in 0.1 M phosphate bolster at pH 7, it was discovered that, present augmentations with extending cholesterol obsession in the range $0.1x10^{-8}$ to $10x10^{-8}$ M. In the present case, expecting that the protein is reliably appropriated all through the cathode, the reaction happens overwhelmingly on the surface of the anode in the lower center. In any case, the reaction on the surface of the anode and the scattering happening in the meantime at the reaction time. With expanding centralizations of cholesterol, the reaction current additionally expanded lastly come to unfaltering state esteem.

Figure demonstrates the relentless state potential reliance adjustment bend for the every individual cholesterol focus in the scope of 1x10-8 to 10x10-8M. The reaction of Gr/PANI/ChO to cholesterol was observed to be wide straight scope of 1x10-8 to 10x10-8M and as far as possible was observed to be 1x10-8 M. Linearity range is well concurrence with that acquired in the amperometric reaction of sensor is fitting in extent to cholesterol fixation.

**Storage stability:** Long time dependability is a standout amongst the most noteworthy elements required for the palatable use of a biosensor as appeared in Figure-5. To assess the capacity solidness, the sensor was tried for 10 weeks of capacity in 0.1 M phosphate support pH 7 at 25°C. There is a slight diminishing in affectability of the sensor of around 12% from the underlying quality, uncovering decent protection of the bioactivity than other work[10].

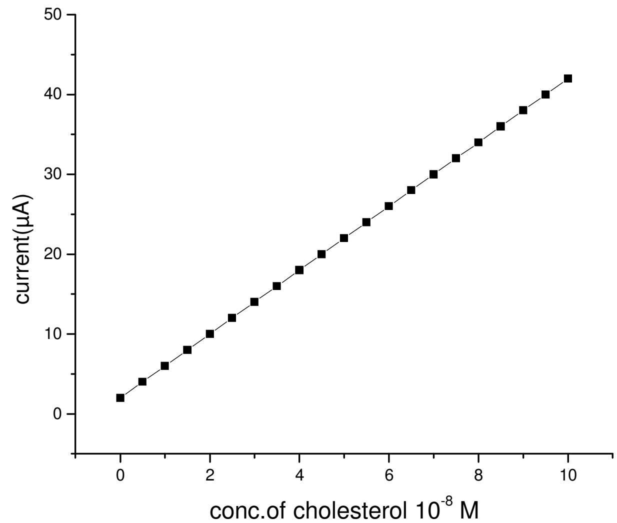


**Figure-4:** Amperometric response of Gr/PANI/ChO at potential 0.5V electrode at pH 7.

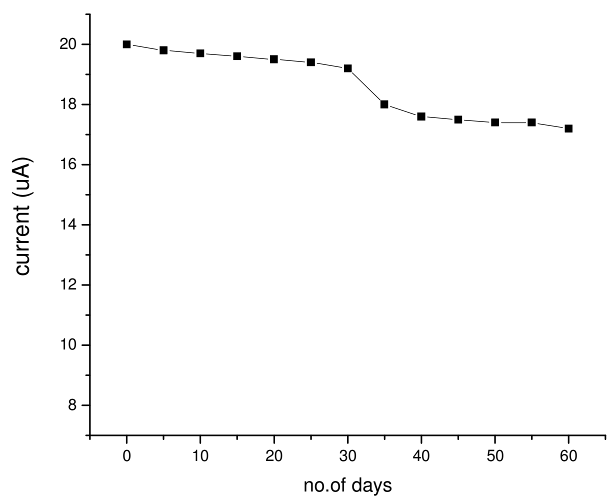


**Figure-5:** Stability of the Gr/PANI/ChO electrode storage for 60 days.

## Conclusion

A novel electrochemical cholesterol biosensor based on graphite (Gr) paste electrode modified with polyaniline (PANI) and has been developed successfully. The amperometric of the developed cholesterol biosensor was evaluated, and the obtained cholesterol biosensor exhibited shorter response time (3 s), with wider range 0.1-10 μM and the detection limit were found to be

$1x10^{-9}$M. Enzyme activity is 85% retained for 60 days. The Gr/PANI/ChO electrode shows good reproducibility and stability than conventional biosensor.

## References

**1.** Songa E.A. and Okonkwo J.O. Recent approaches to improving selectivity and sensitivity of enzyme-based biosensors for organophosphorous pesticides: A review. *Talanta*, 155, 289-304.

**2.** Stoytcheva M. and Zlatev R. (2011). Organophosphorus pesticides analysis. a Stoytcheva (Ed.), Pesticides in the Modern World - Trends in Pesticides Analysis ,InTech, Croatia, 143-164.

**3.** Stoytcheva Margarita, Gochev Velizar and Velkova Zdravka (2016). Electrochemical biosensors for direct determination of organophosphorous pesticides: a review. *Curr. Anal. Chem*., 12, 37-42.

**4.** Sassolas Audrey, Prieto-Simón Beatriz and Marty Jean-Louis (2012). Biosensors for pesticide detection: new trends. *American Journal of Analytical Chemistry*, 3, 210-232.

**5.** Cagnini Andrea, Palchetti Ilaria, Lionti Ilaria, Mascini Marco and Turner Anthony P.F. (1995). Disposable ruthenized screen-printed biosensors for pesticides monitoring. *Sensor Actuat. B-Chem*., 24, 85-89.

**6.** Solna R., Sapelnikova S., Skladal P., Winther-Nielsen M., Carlsson C., Emmenus J. and Ruzgas T. (2005). Multienzyme electrochemical array sensor for determination of phenols and pesticides. *Talanta*, 65(2), 349-357.

**7.** Ju H. and Kandlimalla V.B. (2008). Biosensors for Pesticides in Electrochemical Sensors, Biosensors and their biomedical applications. *Academic Press*. Inc., 31-56.

**8.** Wu H.Z., Lee Y.C., Lin T.K., Shih H.C., Chang F.L., Lin H.P.P. (2009). Development of an potentiometric micro-biodetector for pesticide monitoring and detection. *J. Taiwan Inst. Chem. Eng*., 40(2), 113-122.

**9.** Parham H. and Rahbar N. (2010). Square wave voltammetric determination of methyl parathion using $ZrO_2$-nanoparticles modified carbon paste electrode. *J. Hazard. Mater*, 177, 1077-1084.

**10.** Shulga O. and Kirchhoff J. (2007). An acetylcholinesterase enzyme electrode stabilized by an electrodeposited gold nanoparticle layer. *Electrochem. Commun.*, 9(5), 935-940.

**11.** Zhang L., Zhang A., Du D., Lin Y. (2012). Biosensor based on Prussian blue nanocubes/reduced graphene oxide nanocomposite for detection of organophosphorus pesticides. *Nanoscale*, 4, 4674-4679.

**12.** Gerard M., Chaubey A. and Malhotra B.D. (2002). Application of conducting polymers to biosensors. *Biosens. Bioelectron*., 17, 345-359.

**13.** Kamyabi M.A., Hajari N., Turner A.P.F. and Tiwari A. (2013). A high-performance paraxon biosensor using covalently immobilized paraxon oxidase on a poly(2,6-diaminopyridine)/carbon nanotube electrode. *Talanta*, 116, 801-808.

**14.** Aguilar R., Dávila M.M., Elizalde M.P., Mattusch M. and Wennrich R. (2004). Capability of a carbon-polyvinylchloride composite electrode for the detection of dopamine, ascorbic acid and uric acid. *Electrochim. Acta*, 49, 851-859.

**15.** Cano M., Ávila J.L., Mayén M., Mena M.L., Pingarrón J. and Rodrígez-Amaro R. (2008). A new, third generation, PVC/TTF–TCNQ composite amperometric biosensor for glucose determination. *J. Electroanal. Chem*., 615, 69-74.

**16.** Guzman-Vázquez de Prada A., Peña N., Parrado C., Reviejo A.J. and Pingarrón J.M. (2004). Amperometric multidetection with composite enzyme electrodes. *Talanta*, 62, 896-903.

**17.** Barsan Madalina M. and Brett Christopher M.A. (2009). A new modified conducting carbon composite electrode as sensor for ascorbate and biosensor for glucose. *Bioelectrochemistry*, 76, 135-140.

**18.** Ghica M.E. and Brett C.M.A. (2006). Development of novel glucose and pyruvate biosensors at poly (neutral red) modified carbon film electrodes. Application to natural samples. *Electroanalysis*, 18(8), 748-756.

**19.** Wang X., Watanabe H. and Uchiyama S. (2008). Amperometric l-ascorbic acid biosensors equipped with enzyme micelle membrane. *Talanta*, 74(5), 1681-1685.

**20.** Bandgar D. K. , Khuspe G.D., Pawar R.C., Lee C.S. and Patil V.B. (2014). Facile and novel route for preparation of nanostructured polyaniline (PANi) thin films. *Applied Nanoscience*, 4(1), 27-36.

**21.** Shukla S.K., Bharadvaj Anand, Tiwari Ashutosh, Parashar G.K., Dubey G.C. (2010). Synthesis and characterization of highly crystalline polyanilineelectrode promising for humid sensor. *Adv. Mat. Letts*., 1(2), 129-134.

**22.** Kumar Rajesh, Chauhan R.P., Kumar R. and Chakarvarti S.K. (2010). Synthesis of conducting polymers and their characterization. *Indian Journal of Pure & Applied Physics*, 48, 524-526.

**23.** Vivekanandan J., Ponnusamy V., Mahudeswaran A. and Vijayanand P.S. (2011). Synthesis, characterization and conductivity study of polyaniline prepared by chemical oxidative and electrochemical methods. *Archives of Applied Science Research*, 3(6), 147-153.

**24.** Tiwari A. and Singh V. (2007). Synthesis and characterization of electrical conductingchitosan-graft-polyaniline. *EXPRESS Polymer Letters*, 1(5), 308-317.

**25.** Prasad Bhim Bali, Kumar Deepak, Madhuri Rashmi and Tiwari Mahavir Prasad (2011). Ascorbic acid imprinted polymer-modified graphite electrode: A diagnostic sensor for hypovitaminosis C at ultratrace ascorbic acid level. *Sensors and Actuators B.,* 160, 418-427.

**26.** Alegret S., Céspedes F., Martínez-Fàbregas E., Martorell D. and Morales A. (2011). Carbon-polymer biocomposites for amperometric sensing. *Biosens. Bioelectron*., 11(1-2), 35-44.

**27.** Ramesan M.T. (2014). Synthesis, characterization, and properties of new conducting polyaniline/copper sulfide nanocomposites. *Polymer Engineering & Science,* 54(2), 438-445.

**28.** Ahmed Shakeel, Parveen Ameena, Dashpande Raghunandan and Roy Aashis (2013). Synthesis, characterization, and DC conductivity of polyaniline-lead oxide composites. *Chemical Papers*, 67(3), 350-356.

**29.** Kotal Moumita , Thakur Awalendra K. and Bhowmick Anil K. (2013). Polyaniline–Carbon Nanofiber Composite by a Chemical Grafting Approach and Its Supercapacitor Application. *ACS Appl. Mater. Interfaces*, 5 (17), (2013) 8374–8386.

**30.** Du D., Huang X., Cai J. and Zhang A. (2007). Comparison of pesticide sensitivity by electrochemical test based on acetylcholinesterase biosensor. *Biosens. Bioelectron*., 23(4), 285-289.

**31.** Heras J.Y., Giacobone A.F.F. and Battaglini F. (2007). Ascorbateamperometric determination using conducting copolymers from aniline and N-(3-propane sulfonic acid) aniline. *Talanta*, 71(4), 1684-1689.

**32.** Fabiano S., Tran-Minch C., Piro B., Dang L.A., Pharm M.C. and Vittori O. (2002). Poly 3,4-ethylenedioxythiophene as an entrapment support for amperometric enzyme sensor. *Mat. Sci. Eng.*, 21(1-2), 61-67.

**33.** Bright H.J. and Appleby M. (1969). The pH dependence of the individual steps in the glucose oxidase reaction *Journal of Biological Chemistry,* 244(13), 3625-3634.

**34.** Weibel H.K. and Bright H.J. (1971). The pH dependence of individual step in the Glucose Oxidase reaction. *J. Biol. Chem*, 246, 2734.