

**XANTHINE OXIDASE
MODIFIED GLASSY
CARBON PASTE
ELECTRODES**

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Carbon is an ideal electrode substrate due to its;

- **Wide anodic potential range**
- **Low residual current**
- **Chemical inertness**
- **Low cost**
- **Fast response time**
- **Ease for fabrication in different configuration and size.**

- **Glassy carbon(GC) and carbon paste (CP) are the most widely used carbon electrodes.**
- **GC electrodes possess attractive electrochemical reactivity, good mechanical rigidity and negligible porosity.**
- **CP has the advantages of very low background current and composite nature including ease of modification and renewal.**
- **These attractive features of carbon electrodes are the reason for the considerable attention at the production of carbon-based electroanalytical sensors.**

 **Glassy carbon paste electrodes (GCPE) combines the attractive properties of composite electrodes and glassy carbon, since the preparation of this electrode includes the mixing of GC micro particles with organic pasting liquid.**

 **This new electrode material's attractive electrochemical behavior has been explored by testing this electrode in cyclic voltammetric measurements of some biologically important substances and detection of some trace metals by stripping voltammetry.**



The usage of GCPE for the biosensing of glucose has also been shown. It was observed that GCPE has better electrochemical reactivity towards the oxidation of hydrogen peroxide compared to conventional CPE.

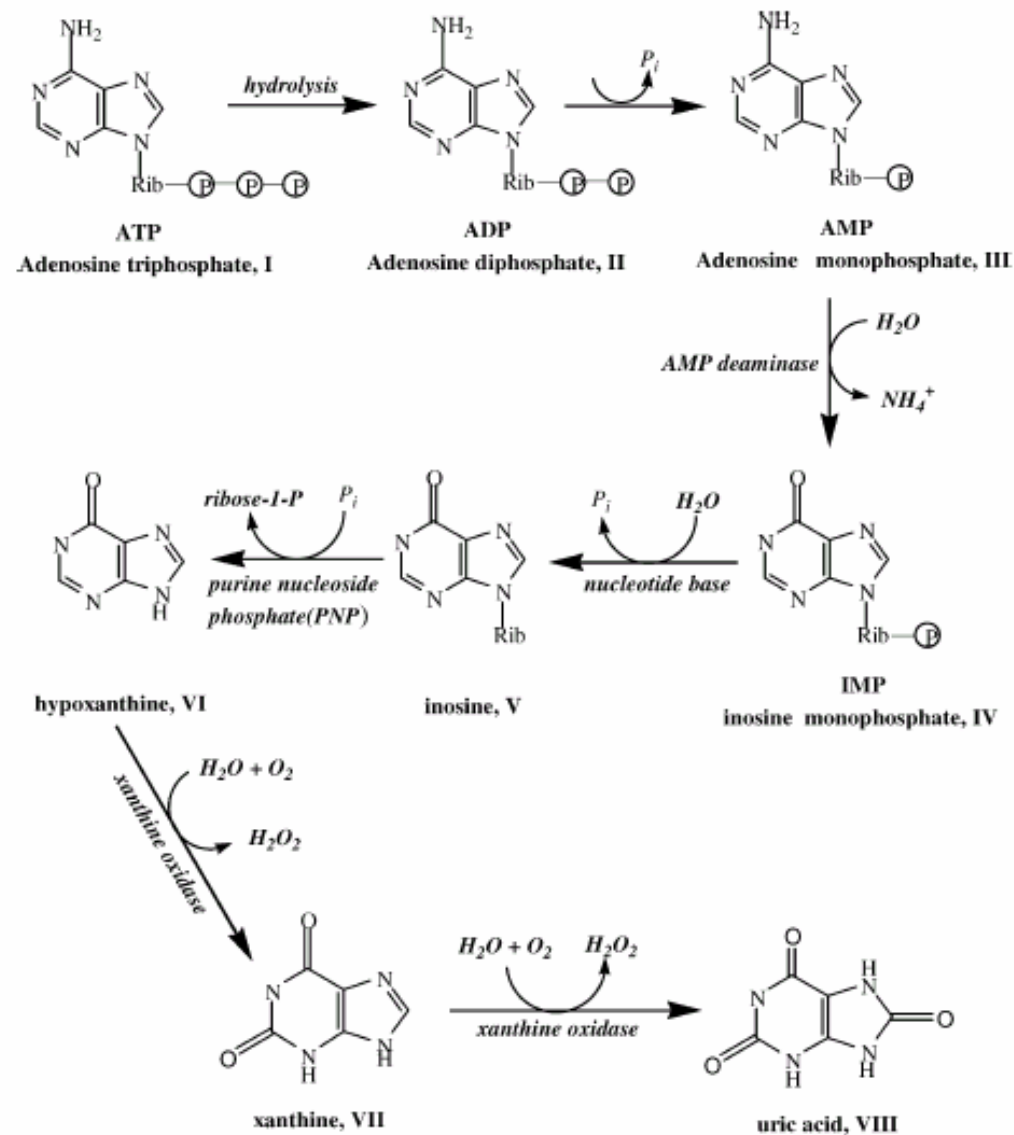


The simplicity of the preparation of GCP biosensor compared to the surface modification of GC is another attractive property of the new electrode material as a biosensor.

In the present work, GCPE was modified with xanthine oxidase that is very important for the purine metabolism in humans.

The development of a sensor for xanthine and hypoxanthine is of medical and biological importance.

Metabolic pathway for purine nucleotides and deoxy nucleotides





Various methods including spectrophotometry and chromatography were utilized for the detection of these compounds. However, these methods are costly and require pretreatment of the food samples prior to the measuring step.



Proposed system is based on the chronoamperometric monitoring of the current that occurs due to the oxidation of the hydrogen peroxide which liberates during the enzymatic reaction. The characterization of the system was performed and then the biosensor was applied for xanthine and hypoxanthine detection in the plasma samples.

MATERIALS AND METHOD

Chronoamperometric experiments were carried out with the PALM SENS electrochemical measurement system from PALM Instruments B.V., (Netherlands).





The electrodes were inserted into the cell through its Teflon cover. Ag/AgCl were used as counter and reference electrode, respectively.

- **XO, 0.06 units/mg solid**
 - **Glassy carbon micro particles (0.4-12 μm diameters)**
 - **Mineral oil**
 - **Phosphate buffer (0.05 M, pH 7.0) was served as supporting electrolyte.**
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- **Plasma samples were obtained from a local hospital and denatured by adding 0.5 M HClO_4 . The obtained plasma sample was diluted 100 times and standard addition method was used for analytical assays.**

ELECTRODE PREPARATION



- **XO based glassy carbon paste electrode was prepared at different portions (63:10:27, 66:7:27, 70:3:27 72:1:27 (w/w) % GC microparticles/XO/mineral oil) by hand-mixing of glassy carbon particles with enzyme and mineral oil.**

- **A portion of the resulting paste was then packed firmly into the electrode cavity (3.0 mm diameter and 5 mm depth) of a PTFE sieve. Electrical contact was established via a copper wire.**
- **The paste surface was smoothed on a weighing paper. The surface of the resulting paste electrodes were smoothed and rinsed carefully with double distilled water.**

- **Chronoamperometric measurements were carried out in 0.05 M phosphate buffer (pH 7.0) medium under the operating potential of +0.9 V.**
- **The duration of each analysis was 100 second and the transient current decayed to a steady state value after 50 sec. in the presence of supporting electrolyte.**

- **The current changes were registered by a potentiostat. After completion of the measurement, the electrode was rinsed with distilled water and allowed to equilibrate before another measurement.**

RESULTS

Effect of enzyme amount

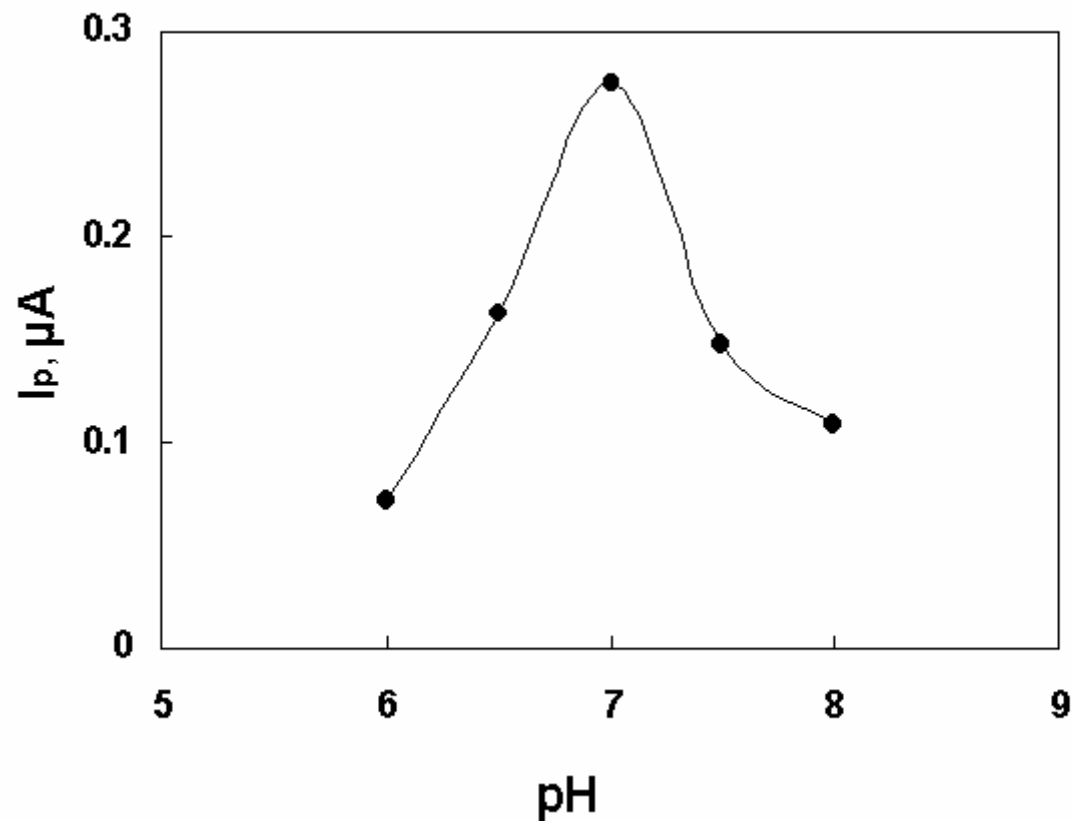
Table 1. Response characteristics of the systems for xanthine determination

Enzyme Amount (mg)	Linear Range (μ M)	Equations	R^2
0.1	0.2-0.5	$y = 0.11x + 0.007$	0.997
0.3	0.5-10	$y = 0.04x + 0.016$	0.993
0.6	2.5-15.0	$y = 0.03x + 0.017$	0.992
0.9	5-15.0	$y = 0.01x + 0.005$	0.989

0.3 mg was chosen as optimum amount considering linear range and facility in electrode preparation.

pH Effect

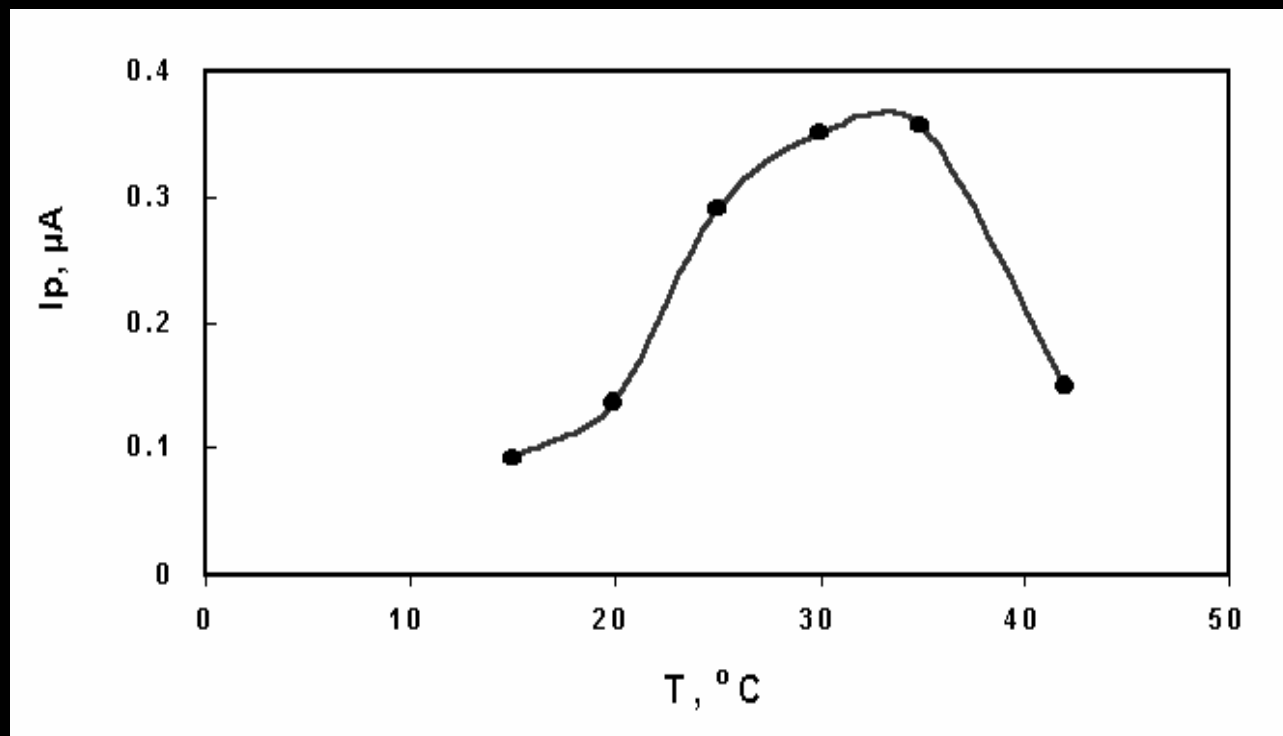
Fig. 1. Optimum pH of biosensor (pH 6.0-8.0; phosphate buffer; 50 mM, T; 25°C).



pH 7.0 was chosen as optimum value

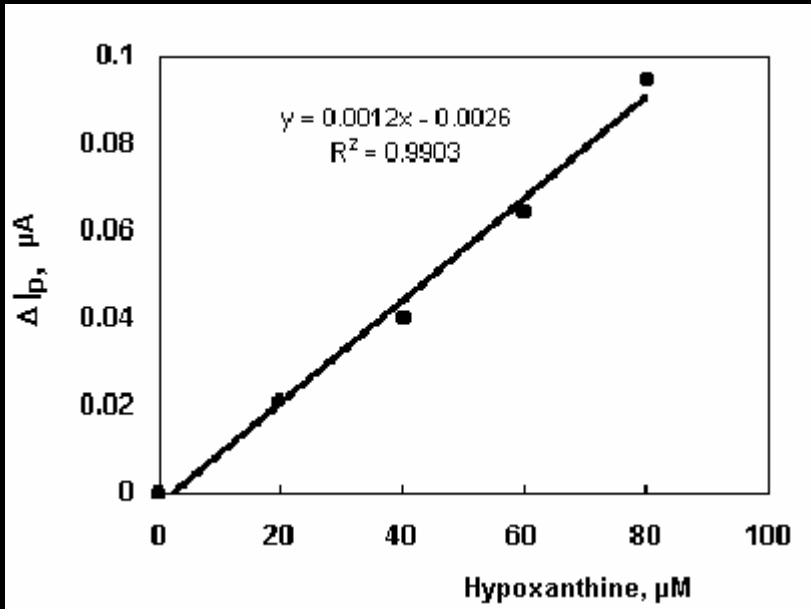
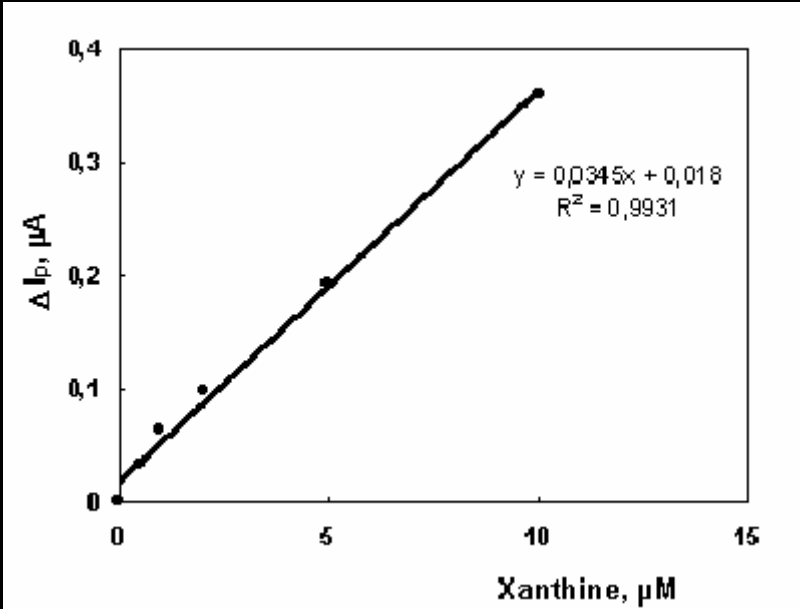
Temperature Effect

Fig. 2. Optimum temperature of biosensor (pH 7.0; phosphate buffer, 50 mM).



30-35 °C was found as optimum value

Linear range



A linearity for the biosensor was obtained in concentration range between 5.0×10^{-7} - 4.0×10^{-5} M xanthine and 2.0×10^{-5} - 8.0×10^{-5} M hypoxanthine, respectively.

Accuracy

The reproducibility of the biosensor was tested for 5.0×10^{-7} M ($n=5$) xanthine concentration and the S.D and cv were calculated as $\pm 1.8 \times 10^{-8}$ M and 4.1%, respectively.

LOD for X and HX; 1.0×10^{-7} , 5.3×10^{-6} M

Sample Application

Plasma samples which included HX (40 μM) and X (2.0 μM) were used,

HX and X amounts in samples were calculated from calibration curve as 40.23 ± 2.15 and 2.14 ± 0.11 μM , respectively.

Results are expressed as means \pm SD (n=4). The analysis indicated the absence of HX in plasma samples.

The recoveries of the spiked samples were close to 100%. Since the obtained signals from spiked samples were very close to the standard solutions, it is obvious that the nature of sample does not interfere the measurement.

