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PAPER

Bienzyme functionalized electro-chemically reduced transparent graphene oxide platform for triglyceride detection

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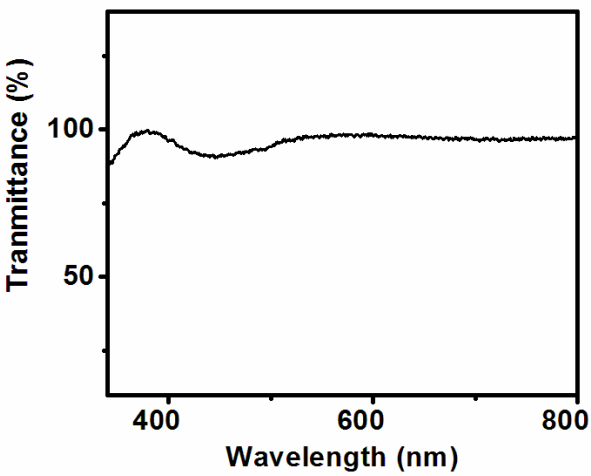

Figure S1. Transmittance of the ERGO/ITO electrode prepared by using the current technique.

Reversible nature of bioelectrode

Table-S1 demonstrates reversible nature of ERGO/ITO, TB/ERGO ITO, LIP-GDH/TB/ERGO/ ITO bioelectrodes. The difference in peak potential (cathodic and anodic) of all the electrodes is almost same, (0.25 V to 0.27 V) and I_{pa}/I_{pc} ratio remains within 0.8 to 0.9 i.e. close to 1. Interestingly, there is slight increase in ΔE for bi-enzymatic bioelectrode but Ipa/Ipc increases up to 0.9 from 0.8 (TB/ERGO/ITO) confirming reversible nature of the bi-enzymatic bio-electrode.

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Table- S1 Electrochemical results of the electrodes

Electrodes	Potential difference; ΔE (V)	Anodic cathod peak current ratio; Ipa/Ipc
ERGO/ ITO	0.25	~0.8
TB/ERGO/ ITO	0.24	~0.8
LIP-GDH/ TB/ERGO/ITO	0.27	~0.9

Development of alternative bio-chemical pathway of triglyceride bi-enzymatic system

Chemicals and Instruments:

Figure. S2 represents the steps of catalytic decomposition triglyceride in presence of LIP and GDH. LIP enzyme hydrolyses tributyrin (TG, triglyceride) to produce fatty acids along with glycerol molecules. Glycerol dehydrogenase (GDH) enzyme then catalysis the oxidation of glycerol into Dihydroxyacetone in presence of NAD+. At the same time, NAD+ converts to NADH by accepting the electrons from glycerol and later on NADH gives up electron to convert to NAD+. The redox mechanism of NADH/NAD+ at a solid electrode surface is irreversible and frequently results in electrode fouling. It is, therefore required to replace NAD+/NADH by other suitable mediator. In this paper, TB/ERGO/ITO and $[Fe(CN)_6]^{3-/4-}$ are used as mediators in place of NADH in the path of this biological cycle. TB/ERGO/ITO on the electrode surface and $[Fe(CN)_6]^{3-/4-}$in electrolyte have been investigated in place of NAD+/NADH. The proposed alternative biochemical cycle is interpreted in Figure. S2b.

In order to prove the concept, following analysis have been conducted. Firstly, TB/ERGO/ITO is exposed to glycerol to perform the control test of TB/ERGO/ITO electrode to check the effect of matrix (ERGO/ITO electrode) on glycerol (Figure S3a). Secondly, the bio-electrode based on only GDH/TB/ERGO/ITO is exposed to various concentrations of glycerol (Figure S3b). The control testing of the electrode as reflected in Figure S4a expresses that TB/ERGO/ITO electrode is not responding towards glycerol as significant change in peak current is not observed in the presence of glycerol. Increased value of peak potential from 0.39 V to 0.51 V is due to the presence of insulating glycerol in the medium. Figure S4b represents the CV studies of alternate bio-chemical scheme, i.e cyclic voltammogram of GDH/TB/ERGO/ITO in presence of glycerol. The CV studies of TB/ERGO/ITO and response studies of GDH/TB/ERGO/ITO bioelectrodes with various concentration of glycerol have been investigated in PBS (50mM, pH 7.8, 0.9% NaCl) containing 5mM $[Fe(CN)_6]^{3-/4-}$at scan rate of 50 mVs^{-1}. The control testing shows that peak current values of TB/ERGO/ITO in presence of glycerol remain at 0.35 mA similar to bare electrode. The anodic peak current magnitude of GDH/TB/ERGO/ITO bio-electrode is decreased from 0.35 mA [TB/ERGO/ITO] to 0.26 mA [GDH/TB/ERGO/ITO] and potential is shifted to higher value after immobilization of GDH due to the hindrance caused by

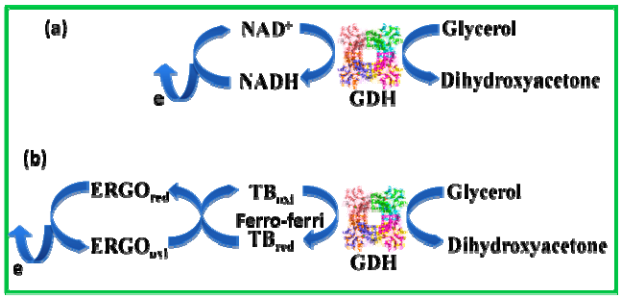

Fig. S2. (a) Conventional scheme of oxidation of glycerol in presence of GDH (b) proposed scheme of oxidation of glycerol in presence of GDH.

macromolecular structure of GDH indicating successful immobilization of GDH onto TB/ERGO/ITO surface. When the GDH/TB/ERGO/ITO bio-electrode is exposed to various concentrations of glycerol (50 mg dL^{-1} to 400 mg dL^{-1}), the peak current gradually increases with increase in concentration of glycerol which proves that GDH/TB/ERGO/ITO bio-electrode oxidises glycerol to di-hydroxy acetone in presence of TB/ERGO/ITO and $[Fe(CN)_6]^{3-/4-}$.The Figure S3 b represents the response of GDH/TB/ERGO/ITO to glycerol of only one concentration (other datas are not shown) and the raise of peak current from 0.26 mA to 0.31 mA is observed in presence of glycerol (200mg dL^{-1}) in $[Fe(CN)_6]^{3-/4-}$medium. It can be inferred that the GDH/TB/ERGO/ITO bio-electrode catalyzes oxidation of glycerol to dihydroxy acetone (Figure S3b) in presence of chemical mediators i.e. TB and ERGO (TB/ERGO/ITO) on the surface and ferro-ferri in the electrolyte without biological mediator NAD+/NADH.

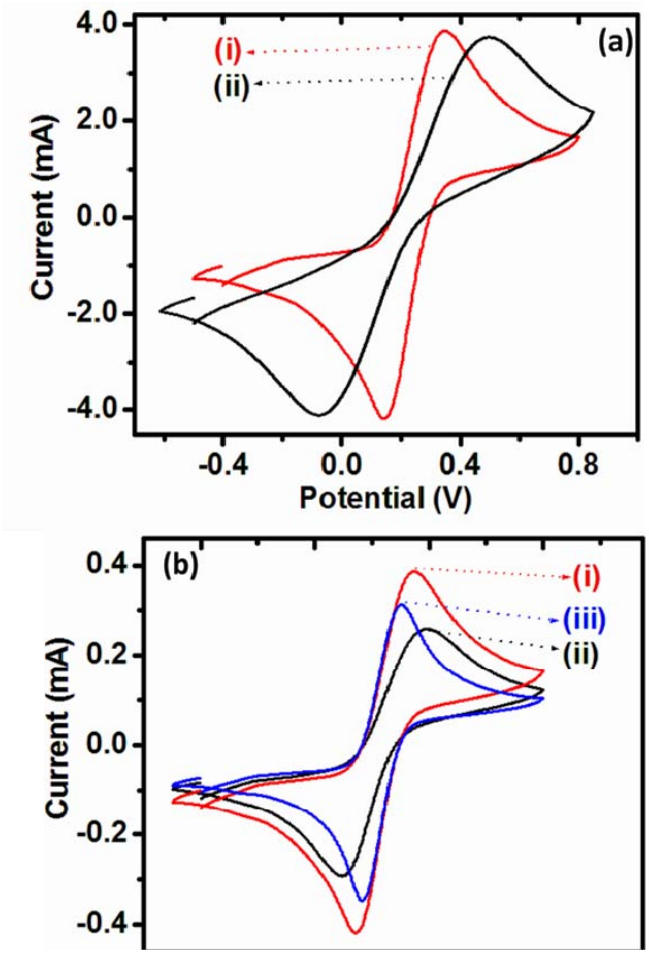

Fig. S3.(a) CV studies of (i) TB/ERGO/ITO electrode and (ii) TB/ERGO/ITO in presence of glycerol in PBS (50mM, pH 7.8, 0.9% NaCl) containing 5mM $[Fe(CN)_6]^{3-/4-}$at a scan rate of 50 mVs^{-1} (b) CV studies of (i) TB/ERGO/ITO (ii) GDH/TB/ERGO/ITO (b) (iii) GDH/TB/ERGO/ITO in presence of glycerol.

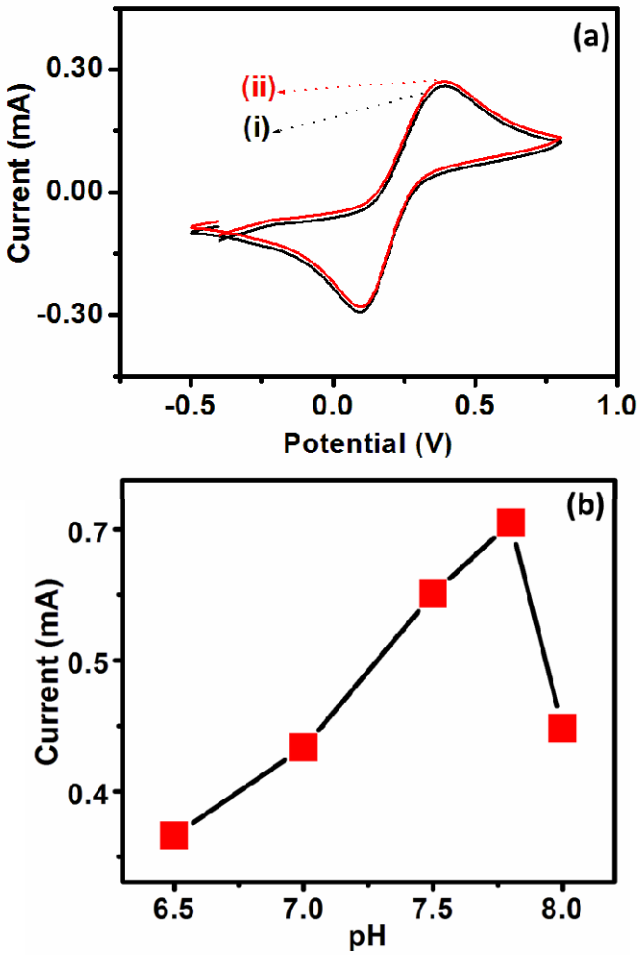

Fig. S4 (a) CV studies of LIP-GDH/TB/ERGO /ITO bio-electrode without (i) tributyrin and with (ii) 25 mg dL^{-1} of tributyrin . (b) Effect of pH on response of LIP-GDH/TB/ERGO /ITO bio-electrode

Figure S4 shows the effect of pH on the response of LIP-GDH/TB/ERGO/ITO bio-electrode in the pH ranging from 6.5 to 8. The optimum response of the GDH/TB/ERGO/ITO bio-electrode ia achieved at pH 7.8.

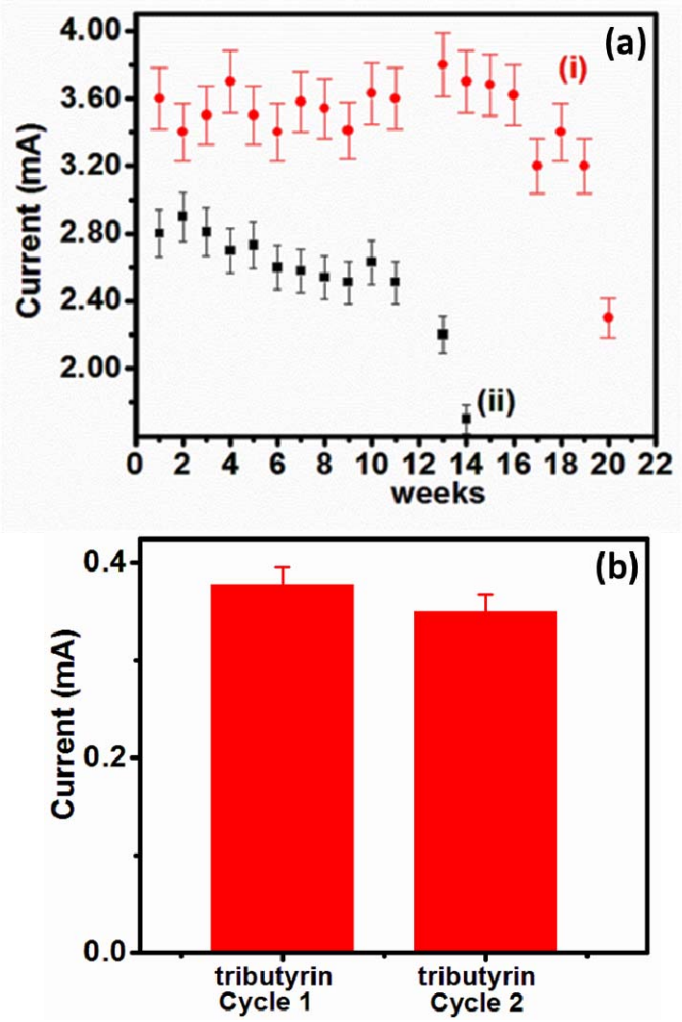

Fig. S5 (a) . Shelf-life study of electrodes in PBS (50mM, pH 7.8, 0.9% NaCl) containing 5mM $[Fe(CN)_6]^{3-/4-}$ at of at a scan rate of 50 mVs^{-1}(i) LIP-GDH/TB/ERGO/ITO bioelectrode and (ii) ERGO/ITO electrode. (b) Effect on response of LIP-GDH/TB/ERGO/ITO bioelectrode by applying consecutive cycle of CV.

Table-S2. Characteristics of the proposed TG electrode along with the other bio electrodes reported from literature.

Electrode material	Detection limit (mM)	Linear range (mM)	Response time (sec)	Ref.
Iridium nanoparticles/ carbon	NA	0 - 10	NA	6
Polyvinyl alchol/ Pt	0.21	0.56 - 2.25	2	12
Polyvinyl chloride/ Pt	0.11	0.56 - 2.25	30	13
Polyaniline/SWCNTS/ ITO	0.56	0.5 - 4.5	12	15
MWCNT-Cerium oxide nanoparticles/ GC	0.005	0.01 - 1.13	25	28
Cerium oxide film/ ITO	0.37	0.5 - 5.6	20	31
Zinc oxide nanoparticles/ Pt	0.22	0.5 - 7.3	6	32
Collagen membrane	0.11	0.11 - 5.6	900	33
AV membrane/ Pt	0.005	0.002 - 2	-	34
Cellulose acetate/ Pt	0.2	0.2 - 3.5	40	35
LIP-ERGO/ITO	NA	0.5-3.3	12	18
LIP-GDH/TB/ERGO/ITO	0.18	0.5-4.51	12	Present