β-Cyclodextrin functionalized graphene based non-enzymatic electrochemical detection of cholesterol

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Abstract

Cholesterol and its esters are membrane constituents widely found in biological systems which serve a unique purpose of modulating membrane fluidity, elasticity, and permeability making the cell walls rigid and strong. In the human serum, 80% of cholesterol exists in the ester form with normal level of 200mg/dL. The higher levels of cholesterol lead to life-threatening coronary heart diseases, cerebral thrombosis and atherosclerosis. Therefore, cholesterol level in the serum is one of the most important parameters in diagnostics and prevention of heart diseases. The electrochemical approach for biosensing has gained momentum over various analytical methodologies due to their high sensitivity and fast response time. A lot research has been performed around the electrochemical biosensing of cholesterol by the enzymatic reaction of cholesterol with cholesterol oxidase, where the concentration of either H_2O_2 generated or oxygen consumed during the enzymatic reaction is being monitored. Detection selectivity in most of these methods relies on the use of cholesterol selective enzymes which are expensive and prone to denaturation. The optical sensors are highly appreciable as an alternative for simple and cost effective methods, whereas an electrochemical non-enzymatic sensing process has an ample scope for better sensitivity.

β-cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of 7-β (1–4) glucopyranose units, with internal cavity lined with C(3)H and C(5)H hydrogen and ether-like oxygen providing a hydrophobic environment. This internal cavity of β-CD allows hydrophobic cholesterol molecules to be soluble in aqueous solution, that's why β-CDs have a high affinity for sterols as compared to other lipids in vitro.

Herein, we have presented a non-enzymatic electrochemical approach for cholesterol sensing using Graphene-β-Cyclodextrin (Grp-β-CD) hybrid system as the sensing matrix. Grp-β-CD solution was synthesized in situ where graphene oxide (GO) sheets were treated with β-CD in presence of ammonia and Sodium hydroxide. β-CD is presumed to get covalently attached over GO sheets during the reaction forming GO-β-CD followed by its reduced using hydrazine forming Grp-β-CD. Methylene Blue (MB), a redox indicator, when added into the Grp-β-CD solution, forms a host–guest complex with β-CD. When cholesterol solution was added in the solution, it replaced the MB molecule from the cavity due to its higher affinity towards β-CD, offering better detection sensitivity range via selective host–guest interaction. Graphene sheet network helps in rapid transfer of the electrochemical signal. As MB is a well known redox probe and hence can be easily detected using Differential Pulse Voltammetery (DPV) technique. To the best of our knowledge this is for the first time, a completely non-enzymatic sensing with such a high sensitivity and low detection limit is being reported, using an electrochemical DPV metric method with the targeted analyte, cholesterol. Salient features of the present study include graphene's increased solubility after β-CD functionalization, due to the covalent interactions occurring between the hydrophilic surfaces of the two and using DPV technique, where cholesterol molecule is replacing MB molecule and forming the inclusion complex within the hydrophobic core of Grp-β-CD. The detection limit of cholesterol was achieved as low as 1 mM. Also, it detects cholesterol efficiently in the micromolar concentration range with outstanding selectivity over the common interfering species.

References

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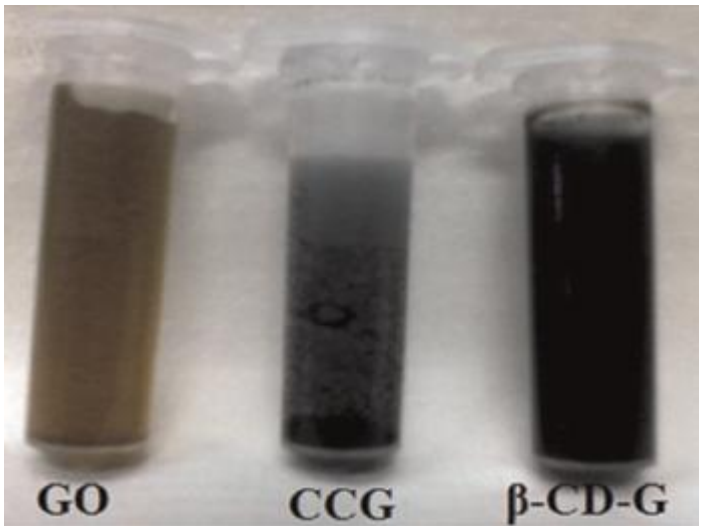
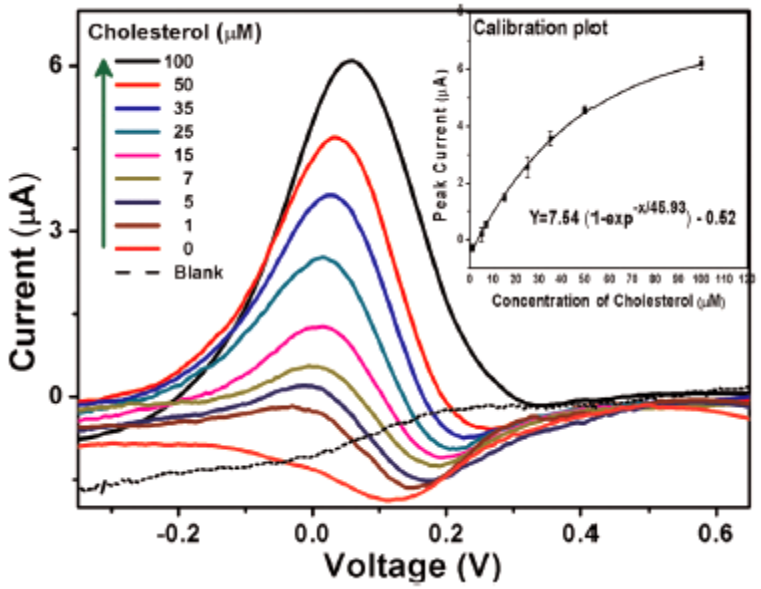
Figures:


GO
CCG
β-CD-G


Cholesterol (μM)
100
50
35
25
15
7
5
1
0
Blank
Current (μA)
Voltage (V)
Calibration plot
Peak Current (μA)
Concentration of Cholesterol (μM)
Y=7.54 (1-exp-x/45.93) - 0.52