



# Development of imprinted polynuclear red/electrochemically reduced graphene oxide composite for ultra-trace sensing of 6-thioguanine



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## ABSTRACT

The present work describes a novel method for the fabrication of a pencil graphite electrode modified with polynuclear red/electrochemically reduced graphene oxide (ERGO) composite, for sensing 6-thioguanine (anticancer drug), in biological and pharmaceutical samples. Herein, the neutral red monomer was electro-polymerized in the presence of 6-thioguanine (template) at  $-0.9$  V directly over ERGO nano sheets coated electrode. After the template extraction, an imprinted polymer coating on ERGO was obtained. The monomer-template (1:2) stoichiometry was optimized through density functional theory at the B3LYP/6-31 + G (d, p) level. In this work, graphene oxide (GO) sheets actually assumed exfoliated anionic texture which facilitated electro-statically deposition of cationic molecules of neutral red at neutral pH. These molecules were subsequently electro-polymerized accompanied with simultaneous electro-reduction of exfoliated GO. This resulted in highly electro-conductive polynuclear red coated ERGO nano sheets alongwith probable intercalation of the polymer into the space between the exfoliated two proximate ERGO layers. A perfect linearity in the current-concentration profile was observed for the analyte concentration in the range  $0.124$ – $78.0$  ng mL $^{-1}$ , with the detection limit  $0.02$  ng mL $^{-1}$  ( $S/N = 3$ ), in aqueous medium. The proposed sensor assured a reliable analysis of ultra-trace 6-thioguanine in real samples.

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## 1. Introduction

6-Thioguanine, (6 TG) has been a popular drug for the treatment of leukaemia in the last two decades. Apart from undesired accumulation of the 6 TG active metabolite, (6-methylmercaptapurine derivative) in liver [1], any excessive 6 TG dose may cause additional toxic side effects such as bone marrow depression and gastrointestinal complication [2]. It is reported that patients with 6 TG levels above threshold values of  $230$ – $260$  pmol/ $8 \times 10^8$  red blood cells are significantly more likely to be associated with clinical remission [3]. In the literature, high performance liquid chromatography [4,5] and mass spectrometry [6] have primarily been reported for the determination of 6 TG. However, these methods required an expensive instrumentation with typical experimental conditions and cumbersome sample preparations. Alternatively, electrochemical methods have preferably been used for the measurement of 6 TG [7–9]. However, these methods also lacked both sensitivity and selectivity. For the selective analysis of 6 TG, a

molecularly imprinted polymer (MIP)-based chemosensor, using piezoelectric micro-gravimetry, the double-layer capacity, and alternating current transducer techniques, has recently been reported [1], nevertheless, its detection sensitivity range was found to be in the higher concentration range and moreover, the measurement protocol was complicated. Therefore, a simple technique for 6 TG evaluation, dedicated with high selectivity and sensitivity, is warranted.

Electro-polymerization is a powerful tool for the development of modified electrodes, in which, a new class of organic molecules possessing unique features of ionic and electronic conductivities are used for the modification [10]. In particular, organic compounds with  $\pi$ -conjugated structures, such as redox dyes, can be employed for the construction of electrochemical sensors. This is due to the fact that the conjugated parent ring structures of the dye containing azine ring can easily be manoeuvred for electro-polymerization with the promise to develop a range of conducting polymers. For instance, neutral red (NR), a phenazine redox dye, with an amino group located on the heteroaromatic phenazine ring, makes it amenable to facilitate electro-polymerization [11]. During the electro-polymerization of NR, a radical-cation is produced by

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electro-oxidation which subsequently loses a hydrogen ion to generate a radical to initiate polymerization at a lower potential [12].

Molecular imprinting is most burgeoning technology to prepare synthetic MIPs with significant recognition ability in specific manner. Simply put, these materials are prepared by imprinting template (analyte) in the presence of functional monomer, cross-linker, and initiator in a suitable polymerizing medium (porogen). However, this technique might suffer with disadvantages of incomplete template retrieval (a necessary step to obtain the imprinted cavities in the polymer), poor binding capacity, analyte diffusion constraint, and poor ingress-egress of analyte across the binding sites mostly located in the interior core of bulk MIP. As a remedial to this, surface imprinting has successfully been adopted which involved immobilization of MIP layer on a support system, especially nanomaterials possessing high surface-to-volume ratio to enable most of binding sites situated at or approximate to the surface. Nevertheless, the problem of developing a nano thin film at the electrode surface still remains to be a challenge with application of MIP technique. Owing to the use of crosslinkers to stabilize molecular cavities in the MIP film, there could also be probable delamination of film out of the surface. In this context, the electro-polymerization technique has been found to be useful to ensure strong adherence of nano structured MIP thin film on to the conducting electrodes. This technique might have advantages for the convenient retrieval of the template molecules and creation of complementary stable cavities with everlasting memory for template rebinding, without using any crosslinker in the polymerization step.

The detection sensitivity of an imprinted sensor is not only based on the amount of effective imprinted sites on the surface of the sensor, but also governed by the use of nanomaterials [such as iron, gold, silica nanoparticles, polyaniline nanofibers, polystyrene core colloids, carbon nanotubes (CNTs) and quantum dots, etc.] in the coated film at the electrode surface [13]. Among varieties of nanomaterials, graphene and its derivative GO have been reckoned as most promising candidate for application as supports to prepare MIP composites. Graphene nanosheet is a one-atom thick planar sheet of  $sp^2$ -bonded carbon atom, which are densely packed in honeycomb crystal lattice having larger surface area (theoretical value  $2630 \text{ m}^2 \text{ g}^{-1}$ ) than graphite (i.e.,  $\sim 10 \text{ m}^2 \text{ g}^{-1}$ ) and CNTs (i.e.,  $1315 \text{ m}^2 \text{ g}^{-1}$ ). Such unique characteristics help providing extraordinary higher conductivity (i.e.,  $550 \text{ S cm}^{-1}$ ) for fabrication of highly sensitive MIP composites [14]. In addition, the small dimension of graphene can also be beneficial to the generation of a nano thin film of MIP composite.

In this work, we present a simple technique for the development of imprinted polymer modified ERGO based electrochemical sensor. For this, GO was prepared by using the modified Hummers method which eventually assumed an exfoliated GO texture [15]. In this method, the oxidation of graphite disrupts the  $sp^2$ -hybridized carbon network of the stacked graphene sheets to give rise to defects with wider spacing between adjacent sheets as a result of oxygen functionalities on both sides of the carbon basal plane. It has been revealed that the oxidation of graphite increased the distance between the adjacent graphite sheets from 0.332 nm to 0.834 nm [16] under hydration effect with intercalation of water molecules in the exfoliated space. Exfoliated GO sheets could be negatively charged, as a result of ionization of the carboxylic acid and phenolic hydroxyl groups after dispersion in water [17]. Besides, the large electron delocalized graphene may exert a strong force to bind the aromatic monomer. Accordingly, the cationic NR, monomer could be adsorbed over and in between the space provided by exfoliated GO sheets, owing to both electrostatic as well as aromatic interactions. Subsequently, this was subjected to electro-

polymerization in the presence of template (6 TG). During electro-polymerization process of NR, GO could also get simultaneously reduced to yield ERGO restoring its  $\pi$ -network, and thereby the electrical conductivity. Notably, the electrochemical reduction to obtain ERGO is preferred to chemical reduction method using hydrazine because the handling of hydrazine is somewhat hazardous [18]. Till date, MIP-modified electrochemical sensors based on GO/reduced GO have been reported for 17 $\beta$ -estradiol, 2,4-dinitrophenol, dopamine, tetrabromobisphenol A, endocrine, 4-nitrophenol, and so on [13,19–23]. In these works, the graphene modified electrodes were fabricated mainly by applying the approaches of self-assembly, sol–gel route, and surface imprinting on the functionalized graphene supports. However, the major concern realized so far is the stability of the imprinted film, poor electrode-electrolyte contact owing to the relatively thick imprinted membranes, and above all the analyte (template) leaching during the detection process. Thus, in the present work, we have resorted for the first time to utilize exfoliated space of graphene for the firm adherence of electro-polymerized MIP nano structured film, consisted of well defined molecular cavities, for the strong and specific adsorption of the template molecules, under the effect of non-covalent and aromatic  $\pi$ – $\pi$  interactions.

## 2. Experimental

### 2.1. Reagents

Demineralized triple distilled water (conducting range  $0.06$ – $0.07 \times 10^{-6} \text{ S cm}^{-1}$ ) was used throughout in this work. Graphite powder, natural,  $\sim 325$  mesh, 99.95% was purchased from Alfa, Aesar (UK). Potassium ferricyanide,  $\text{KMnO}_4$ , and NR were purchased from Loba chemie (Mumbai, India).  $\text{H}_2\text{SO}_4$  (98%), and  $\text{H}_2\text{O}_2$  (5%) were purchased from E-Merck Ltd. (Mumbai, India). Solvent acetonitrile (ACN) was purchased from Spectrochem Pvt. Ltd. (Mumbai, India). Authentic samples of 6 TG and all its interferent(s), were obtained from Sigma–Aldrich (Steinheim, Germany) and Fluka (Steinheim, Germany). Phosphate buffer solution (pH 7.5, ionic strength 0.05 M) was used as a supporting electrolyte. The analyte stock solution was prepared by initially dissolving 6 TG (0.0125 g) in 80  $\mu\text{L}$  of 2.0 M NaOH and volume was made to 25 mL with water. All test solutions of 6 TG were prepared by diluting stock solution with water. The pH values of solutions were adjusted by addition of a few drops of either 0.1 M HCl or 0.1 M NaOH. The human blood serum and urine were obtained from the Institute of Medical Sciences, Banaras Hindu University, and stored for a week in a refrigerator at  $-4^\circ\text{C}$ , before use. These biological samples were found to be spoiled after a week. Any pretreatment (ultracentrifugation, ultra-filtration, deproteinization, etc.) of blood serum sample is not advised since it may lead to inaccuracy in trace level determination of test analyte. Tioguanina (6 TG tablet, labelled as 40 mg TG per tablet) was purchased from GlaxoSmithKline Pharmaceuticals Ltd (India). Biological samples were used after spiking with analyte. However, the tablet were first pulverized and then dissolved in water for analysis. Pencil rods (HB grade, 0.5 mm diameter, 60 mm size) were purchased from Hi Par, Camlin Ltd. (Mumbai, India).

### 2.2. Apparatus

Differential pulse adsorptive stripping voltammetry (DPASV) and cyclic voltammetry (CV) were performed on a portable potentiostat  $\mu$ -Stat 200 (Drop Sens S.L. Oviedo, Spain), which was connected via USB connection to a computer installed with the measurement software Drop View (Drop Sens). The electrochemical cell was consisted of MIP- ERGO composite modified PGE,

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