Electrochemical determination of diethylstilbestrol by using a magnetic nanoparticle/graphene composite film electrode

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Abstract. The magnetic nanoparticle-graphene modified magnetic carbon electrode (MCE) was fabricated and the electrochemical effect of diethylstilbestrol (DES) on this electrode was studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The factors that affected the performance of the sensor were also optimized. The experimental results exhibited good electrocatalytic activity and a fast response for the electrochemical oxidation of DES. The linear range for the determination of diethylstilbestrol was 1 to 100 µM and the detection limits were 1.06 x 10⁻⁷ M based on S/N = 3.

1. Introduction
Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen substance and its molecular structure contains two hydroxyl groups in the phenol-ring. Since the pharmacological and therapeutic effects of DES are the same as those of natural estrogen, it has been widely used in treatment of estrogen-deficiency disorders, and livestock production to promote growth [1]. However, in contrast to natural estrogen, DES is more stable and remains in the body longer [2-3]. Recently, it has been found that DES is one of the strongest potential carcinogens [4]. Therefore, the use of growth-promoting drugs for fattening livestock has been banned in many countries [5]. Based on this fact, there is an urgent need for a fast and efficient method for the determination of residues in animal tissues as well as DES in milk samples to prevent illegal usage.

Several methods have been used for determining DES [6-8]. Until now, most of the analytical methods towards this determination have required expensive instruments, trained personnel, and are very time-consuming. This added to the cost and complexity of the assay. Consequently, the method provides a convenient, simple, rapid, and economic means of detecting these food contaminants. DES has the property of electrochemical activity because of the hydroxyl groups in its molecules. Therefore, electrochemical techniques, which are often simple and less expensive, have been used to determine DES [9-13]. However, a major disadvantage of the method is its low sensitivity due to the slow electron transfer at unmodified electrodes. Interest has been focused on the development of chemically
modified electrodes for DES detection. Interestingly, so far, a magnetic nanoparticle-graphene composite has not been developed for the electrocatalytic detection of these food contaminants. In this work, a new disposable electrochemical sensor for the fast determination of DES is fabricated based on the use of a magnetic carbon electrode (MCE) modified with magnetic nanoparticles and graphene composites.

2. Materials and methods
All electrochemical experiments were carried out with a Potentiostat (Metrohm Autolab PGSTAT302N, Ecochemie, Netherlands) in a three-electrochemical cell form using a magnetic carbon electrode (MCE) as a working electrode, a platinum wire as a counter electrode, and Ag/AgCl saturated KCl as the reference electrode.

The fabrication of Fe3O4-Gr composites was described elsewhere [14-15]. Briefly, 20 mg graphene oxide in 60 mL water was ultrasonicated for 3 h, and then 100 mg EDC and 80 mg NHS were added together in the solution of graphene. The mixture was stirred for 30 min and ultrasonicated for another 30 min to form a homogenous suspension. Next, 20 mg NH2-Fe3O4 NPs were added into the suspension solution and the mixture was subjected to ultrasonication for 30 min. The reaction was carried out at 80 °C for 1 h under stirring. The Fe3O4-Gr composites were obtained by magnetic separation and washed with water several times. The obtained Fe3O4-Gr composites (1 mg/2.5 mL) were re-dispersed in DI water before further use. Finally, 5 µl of composite solution was dropped onto the MCE surface.

3. Results and discussion

3.1 Electrochemical characterization of Fe3O4-Gr composite-modified MCE
The electrochemical properties of the composite were evaluated through cyclic voltammetry (CV). The cyclic voltammograms of the Fe3O4-Gr composite-coated MCE in phosphate buffer solution at pH 7.0 at various scan rates can be seen in Figure 1. The anodic and cathodic peak currents are linear with potential scan rates ranging from 25 to 400 mV/s (inset in Figure 1). The results confirmed that composites have good electrochemical reversibility. Both the anodic peak current and the cathodic peak current were proportional to the scan rate, again demonstrating that the redox process of the Fe3O4-Gr composite on to the MCE was a surface-confined process.

![Figure 1](image-url)  
**Figure 1.** CVs of the Fe3O4-Gr/MCE in 0.1 M PBS at pH 7.0 containing 0.3 mM DES at different scan rates from inner to outer: 25, 50, 75, 100, 150, 200, 250, 300, 350 and 400 mV/s. Inset: The plot of the peak current vs. scan rates.
3.2 Optimization of DES monitoring

The accumulation time was determined in order to find the optimal operational condition for DES measuring. Thus, a plot of differential pulse voltammetric current versus accumulation time was made as shown in Figure 2. The current changed with a decrease in accumulation time from 5 to 210 seconds. Hence, the accumulation time of 5 seconds was selected as the optimized monitoring accumulation time, due to its good sensitivity.

![Figure 2](image1.png)

**Figure 2.** Effect of accumulation time on DPV peak current in 0.1 M PBS at pH 7.0 containing 0.3 mM DES.

The influence of pH on the sensor response is shown in Figure 3. With an increase of buffer pH, the CV response of the sensor shows no significant change until pH 8.0. A further increase of buffer pH led to a decrease in the response. Due to pH 7.0 being the normal pH in human and animal biological fluids and also the sensitivity of this sensor, pH 7.0 was chosen for use as the supporting electrolyte in all subsequent analytical experiments.

![Figure 3](image2.png)

**Figure 3.** Effect of pH on CV peak current in 0.1 M PBS containing 0.3 mM DES at a scan rate of 50 mV/s.
3.3 Current response to DES
The current response of Fe₃O₄-Gr composite on to the MCE was investigated under the optimal experimental conditions as shown in Figure 4.

![Figure 4](image-url)

**Figure 4** Calibration curve between the peak current and DES concentration from 1 to 500 μM in 0.1 M PBS at pH 7.0.

Upon addition of DES, the DPV response increased, indicating the fast response and high sensitivity of the electrode to DES. The calibration curve of the sensor is shown as an inset in Figure 4. The response to DES was linear in the range of 1 to 100 μM, and the sensitivity of this biosensor corresponding to the linear range was 42.2 μA/mM. The regression equation was \( Y = 0.0422x - 0.0152 \) with a correction coefficient of 0.9983, and the detection limit was 0.106 μM when the signal to noise ratio was 3.

4. Conclusions
This work demonstrates a new disposable sensor for DES based on Fe₃O₄-Gr composite modified on the magnetic carbon electrode. The electrochemical measurement showed good electrocatalytic activity and sensitivity, along with a wide range and low detection limit of the sensor to DES. This sensor fabrication could be employed for other applications of several analytes in food contaminant analysis.

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