



# Electro-deposited poly-luminol molecularly imprinted polymer coating on carboxyl graphene for stir bar sorptive extraction of estrogens in milk



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## ABSTRACT

Electrochemical polymerization of luminol molecularly imprinted polymer on carboxyl graphene (MIP/CG) was developed as stir bar sorptive extraction (SBSE) coating for selective pre-concentration and specific recognition of bisphenol A (BPA), hexoestrol and diethylstilbestrol in milk samples. Luminol was employed as monomer and BPA as the template to prepare MIP under 0–0.6 V electro-polymerization. Carboxyl graphene was modified on pencil lead as the substrate to increase extraction capacity. The preparation and extraction conditions affecting the extraction efficiency were optimized. Under the optimized conditions, a good linearity of three estrogens was obtained in the range of 4–1000 ng mL<sup>-1</sup>. The average recoveries at the three spiked levels of the three estrogens ranged from 83.4% to 96.3% with the relative standard deviations (RSD) ≤ 7.1%. The limits of detection were in the range of 0.36–1.09 ng mL<sup>-1</sup>. The developed method with low cost, high selectivity and good reproducibility can be potentially applied for determining trace estrogens in complex food samples.

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## 1. Introduction

Estrogens, especially the exogenous estrogens, are associated with endocrine disruption, which have become a public concerned issue. Some studies indicated that these estrogens or their metabolites play a role in human carcinogenesis, such as in prostate, breast, and ovarian cancers [1–4]. Furthermore, these exogenous estrogens (EEs), such as exogenous hexoestrol (HEX), diethylstilbestrol (DES) and bisphenol A (BPA), show toxic and carcinogenic activity even at low levels [5–8]. It is urgent and significant to develop a simple, sensitive and selective analytical method to monitor EE residues in food samples.

First of all, it is important to develop a simple sample-preparation technique for the extraction and enrichment of estrogens from complex sample matrices. Stir bar sorptive extraction (SBSE) is one of the most popular methods with many

advantages, such as high sensitivity and extraction capacity, good reproducibility. The coating is the key of SBSE which significantly affects the extraction performance. Recently, lots of home-made coatings were developed to improve the performance of SBSE for the detection of estrogens, such as polydimethylsiloxane/β-cyclodextrin [9], polydimethylsiloxane/metal-organic frameworks [10], and ethylene glycol-silicone [11]. However, most coatings have no selectivity towards targets. They can also extract undesired organic pollutants that maybe lead to matrix interference for quantification. Based on this reason, it is important to improve the specificity of the coating.

Molecularly imprinted polymers (MIP), a kind of man-made porous materials, is a good candidate because of its outstanding advantages of specific selectivity, good chemical stability, easy and cost-effective preparation. It could selectively recognize a single or a family of related molecules based on the complementary of structure and chemical bonds between targets and MIP cavities [12–16]. However, the use of MIP by traditional imprinting methods still faces challenges, such as low binding capacity, poor site accessibility and slow binding kinetics because of the deeply embedded recognition sites in highly cross-linked polymer network [17]. Thus,

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the adsorbent based on a thin surface molecularly imprinted polymer coating modified on some substrate with high specific area can partially solve the problem.

The preparation procedure of traditional MIP, such as block polymerization or sol gel method, is always tedious and laborious, and it is difficult to control the thickness and surface evenness of MIP film [17,18]. Recently, electrochemical polymerization of MIP has become a popular and new synthesis mode due to the characteristics of simplicity, convenience and easy to control [19]. Except for above advantages, the preparation process could be accomplished within a short polymerization time at constant potential. Moreover, the electrode potential was the driving force of initiator and polymerization reaction without additional cross-linking and initiator agent. Meanwhile, the polymerization reaction could be completed at room temperature and the preparation process only need several hours. [20–22] Moreover, the thickness of film can be simply and effectively adjusted by electro-polymerization current and time.

Luminol, as a light-emitting material, was always used for optical detection [23–25]. There are one  $-\text{NH}_2$ , two  $-\text{NH}-$ , and two  $-\text{C}=\text{O}$  in luminol, which could form many hydrogen bonds with  $-\text{OH}$  in estrogens. Moreover, luminol can self-polymerize under a certain condition [25,26]. There have been many reports about preparing the poly-luminol film in electrodes for detection cholesterol molecular [27,28]. During the self-polymerization of luminol, some template estrogen molecules were embedded in the poly-luminol layer. After the removal of these embedded estrogen molecules, the estrogen imprinted sites are created. Herein, the electro-polymerization method was employed to prepare the poly-luminol MIP coating for capturing the targets.

Carboxyl graphene (CG) with good conductivity and a large specific surface has been widely employed for enlarging the extraction area for coating, which was also used as the substrate to increase the extraction capacity. Moreover, CG with carboxyl functional groups is negatively charged, which could generate a strong electrostatic interaction with positively charged poly-luminol. Meanwhile, pencil lead was adopted to fix the MIP coating instead of the poor conductivity of the typical matrix silica fiber [29] or stainless steel wire [30] hindered electropolymerization process. While, pencil lead had a large active electrode surface area, thus it is suitable for modification or electrochemical polymerization with the advantage of chemical inertness, low cost, low background current, wide potential window and ease of modification [31].

In this study, a novel electrochemical preparation method of MIP was developed as the stir bar coating for the analysis of BPA, DES and HEX in milk. BPA was employed as the common template for extraction of above three estrogens according to the literature [32]. The poly-luminol MIP was prepared by electro-polymerization. CG, as the support of MIP, can largely increase the extraction area of the imprinting layer. The preparation process was simple and easy to control. The characterization, adsorption isotherms/kinetics and competitive adsorption of the new SBSE coating coupled with HPLC were investigated and the application in real milk samples was also explored and validated.

## 2. Experimental

### 2.1. Chemical reagents and materials

Bisphenol A (BPA), hexoestrol (HEX), diethylstilbestrol (DES), poly (dimethyldiallylammonium chloride) solution (PDDA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Graphene oxide was purchased from Fung Ltd. Nano (Nanjing, China). The HPLC-grade acetonitrile was obtained from CNW Technologies (Dusseldorf, Germany). 0.08% of ammonia hydroxide was prepared

with pure water (Wahaha foods Co.Ltd. Hangzhou, China) and HPLC-grade concentrated ammonia hydroxide (Aladdin, Shanghai, China). All the other reagents used in the experiment were analytical reagent grade. The mixed and individual stock solutions of three estrogens (BPA, HEX and DES) were prepared at a concentration of  $1000 \text{ mg L}^{-1}$  in methanol, and stored at  $4^\circ\text{C}$  in darkness for the further use. All the sample solutions used for HPLC analysis were filtered through a  $0.22 \mu\text{m}$  nylon membrane before injection.

The common commercial SBSE fibers with  $85 \mu\text{m}$  polydimethylsiloxane (PDMS) or polyacrylate (PA) coating were used for comparison study, which were obtained from Supelco (Bellefonte, PA, USA).

### 2.2. Instrumentation

Microscopy was performed using a SU-70 scanning electron microscope (SEM) (Hitachi, Tokyo, Japan). Electro-polymerization was performed on a CHI 620D Electrochemical Workstation (Shanghai, China). The chromatographic analysis was performed with a LC-20AT HPLC (Shimadzu, Kyoto, Japan) with a SPD-M20A detector. The separation column was an Agilent ZORBAX Eclipse XDB-C18 column ( $5 \mu\text{m}$ ,  $250 \times 4.6 \text{ mm}$ , USA).

### 2.3. Fabrication of SBSE fiber

#### 2.3.1. Preparation of carboxyl graphene (CG)

According to the previous literature [33], sodium borohydride, as the reducing agent, was added into graphene oxide suspension to get the partially reduced graphene oxide at  $80^\circ\text{C}$ , followed by washing with deionized water. Subsequently, reduced graphene oxide was diluted to give a concentration of about  $2 \text{ mg mL}^{-1}$ , and then sonicated for 1 h to give a clear solution. NaOH (1.2 g) and chloroacetic acid ( $\text{Cl}-\text{CH}_2-\text{COOH}$ ) (1.0 g) were added to the reduced graphene oxide suspension and sonicated for 2 h to convert the  $-\text{OH}$  groups to  $-\text{COOH}$  via conjugation of acetic acid. The resulting carboxyl graphene (CG) solution was neutralized, and purified by repeated rinsing and filtration.

#### 2.3.2. Modification of CG on the surface of pencil lead

The pencil lead was immersed in phosphate buffered saline (PBS, pH 6.0) containing 3% PDDA and sonicated for 30 min. At the same time, 24 mg of CG was added in 3 mL of dimethylformamide (DMF) and sonicated. Then the pencil lead modified with PDDA was removed from the PBS and put into above CG solution. After balanced for 24 h, it was taken out and dried at room temperature for further use.

#### 2.3.3. Preparation of the MIP-coated fiber

The preparation process of MIP was based on the electrochemical polymerization luminol within a certain voltage range. Polymerizations were performed by cyclic voltammetry from 0 to 0.6 V for 300 cycles at a scan rate of  $100 \text{ mV s}^{-1}$  in 0.1 M phosphate/0.1 M KCl solution (pH 6.0) containing 1 mM of BPA (the template) and 3 mM luminol. After reaction, the pencil lead was transferred into the mixture of methanol/acetic acid (9:1, v/v) stirring for 30 min. This procedure was repeated 4 times to remove the templates completely. The result showed that no BPA was detected after 4 times elution. Thus, the microextraction fiber (MIP/CG) was prepared. For comparison, the molecularly non-imprinted polymers (NIP) were prepared in an identical manner except for the absence of BPA. The preparation of the MIP coating was shown in Fig. 1.

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