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Graphene oxide/polyethyleneglycol composite coated stir bar for sorptive extraction of fluoroquinolones from chicken muscle and liver

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ABSTRACT

Graphene oxide (GO) is an ideal adsorbent for polar and less polar compounds due to its hexagonal carbon network structure with oxygen-containing groups, while its strong hydrophilicity and water solubility limited its application in sample pretreatment techniques. Herein, GO was composited with polyethyleneglycol (PEG) or polyaniline (PAN) through intermolecular interactions to improve its stability, and the GO/PEG and GO/PAN composite coated stir bars were prepared by sol–gel technique. Compared with GO/PAN composite and polydimethylsiloxane (PDMS) coated stir bar, the prepared GO/PEG composite coated stir bar exhibited higher extraction efficiency for five fluoroquinolones (FQs). Based on it, a method of GO/PEG composite coated stir bar sorptive extraction (SBSE) combined with high-performance liquid chromatography-fluorescence detector (HPLC-FLD) was proposed. The factors influencing SBSE, such as sample pH, salt effect, stirring rate, extraction time, desorption solvent and desorption time, were optimized, and the analytical performance of the developed SBSE-HPLC-FLD method was evaluated. The limits of detection (LODs) for five FQs were in the range of 0.0045–0.0079 $\mu g\,L^{-1}$, and the enrichment factors (EFs) were in the range of 41.5–65.5-fold (theoretical enrichment factor was 100-fold). The reproducibility was also investigated at concentrations of 0.05 $\mu g\,L^{-1}$ and the relative standard deviations (RSDs, $n = 6$) were found to be in the range of 4.6–12.1%. The proposed method was successfully applied for the determination of FQs in chicken muscle and chicken liver samples.

1. Introduction

Fluoroquinolones (FQs) are a group of synthetic, highly potent antibiotics widely used in human and veterinary medicine for the treatment of respiratory diseases and enteric bacterial infections. Their antibacterial activity is based on a selective inhibition of bacterial DNA gyrase synthesis, an essential bacterial enzyme that maintains spherical twist in DNA, which prevents bacterial multiplication [1,2]. So far, the extensive use of FQs in veterinary has promoted the accumulation of their residues in foods derived from animals, which may trigger allergic reactions in some hypersensitive individuals and most importantly induce pathogens resistant to clinical drugs in humans [3]. Consequently the European Union has established maximum residues limits (MRLs) for several FQs in foodstuffs of animal origin through the Council Regulation 2377/90//EEC [4], specifically 100–300 $\mu g\,kg^{-1}$ for enrofloxacin, 300–1900 $\mu g\,kg^{-1}$ for difloxacin, and 250–1000 $\mu g\,kg^{-1}$ for flumequine in poultry. To facilitate the monitoring of FQs concentration level in poultry and foods, the development of sensitive and efficient methods for the quantification of FQs in animal derived foods is necessary.

High-performance liquid chromatography (HPLC) [5–7] and capillary electrophoresis (CE) [8,9] have been applied for FQs analysis. Among them, HPLC is the most popular analytical technique for FQs due to the merits of good resolution, high reproducibility, and easy automation. Compared with the mass spectrometry (MS) [10,11] and ultraviolet (UV) detector [12], fluorescence detection (FLD) [13–16] is more widely used due to the advantages of high sensitivity, low cost and easy combination with HPLC for FQs analysis. However, FQs in animal derived foods are at trace level, and the real sample matrix is quite complicated; therefore, a suitable sample pretreatment technique to remove sample matrix and enrich FQs before instrumental analysis is highly demanded. Liquid liquid extraction (LLE) [17–19], solid phase extraction (SPE) [20–23] are commonly used sample preparation methods for FQs analysis. While, LLE and SPE consume large amounts of organic solvent and often require complex time-consuming multi-step procedures which may cause contamination or losses of target analytes

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and deteriorate the accuracy. On the other hand, miniaturized pretreatment techniques are preferred for animal derived foods which involve limited sample amount available, and modern trends in analytical chemistry are toward the simplification, miniaturization and low-consumption of solvent/sample. Stir bar sorptive extraction (SBSE) is a novel environmentally friendly microextraction technique which was developed from solid phase microextraction (SPME) in 1999 [24]. Due to its advantages of simplicity, rapidity, sample clean-up ability and high extraction efficiency, SBSE has been successfully applied in environmental, food and biological samples for pharmaceutical analysis. However, the lack of stir bar coatings with high affinity towards polar or less polar analytes limits the wide application of SBSE. To the best of our knowledge, only a mixed coating based on poly(methacrylic acid-3-sulfopropyl ester potassium salt-divinylbenzene) monolithic material was employed for SBSE of four FQs from wastewater [7]. In this monolithic material, the extraction of target FQs was based on the cation-exchange and hydrophobic interaction.

Graphene oxide (GO), a precursor to graphene (G) after reduction, consists of a hexagonal carbon network bearing hydroxyl and epoxide functional groups on its "basal" plane, whereas the edges are mostly decorated by epoxy, hydroxyl and carboxyl groups [25,26]. The ultra high surface area, π–π electrostatic stacking property and rich oxygen-containing functional groups of GO offer abundant binding sites for polar and less polar analytes by hydrogen bonding and π–π interactions. Therefore, it has been realized that GO is an ideal adsorbent for polar and less polar analytes in sample pretreatment techniques. However, the direct use of GO as adsorbents for interest analytes often suffers from its irreversible aggregation and water solubility. The aggregation may reduce the sorption capacity of the adsorbent, and water solubility of GO may aggravate the adsorbent loss. To avoid the above-mentioned problems associated with GO, Liu et al. [27] developed new SPE adsorbents by covalently binding G and GO sheets to silica. The extraction performance of G and GO supported on silica as SPE adsorbents were compared towards various analytes ranging from small molecules of pollutants to biomolecules such as proteins and peptides. G with electron-rich and hydrophobic properties is considered to be a non-polar adsorbent. In contrast, GO with much more polar moieties, such as hydroxy, expoxy and carboxy groups, is suitable for the extraction of polar analytes. Han et al. [28] and Xu et al. [29] prepared Fe_3O_4/GO nanocomposites as SPE adsorbent and GO bonded fused-silica as SPME coating, respectively. The prepared GO based adsorbents/coatings were applied for the analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental water by π–π stacking interaction and hydrophobic interaction. Tabani et al. [30] combined GO based SPE and electro membrane extraction (EME) for the preconcentration of chlorophenoxy acid herbicides in environmental samples. Due to the good adsorption property of GO towards polar compounds of chlorophenoxy acid herbicides and the combination of SPE and EME, the proposed method presented good extraction performance for target analytes, with enrichment factors (EFs) of 1950–2000 and limits of detection (LODs) of 0.3–0.5 $\mu g\,L^{-1}$.

The irreversible aggregation and water solubility of GO also hampers the application of GO as SBSE coating, deteriorating the preparation reproducibility and extraction performance of GO based stir bar coating, and no GO-based coating stir bar has been prepared for real samples analysis so far. The development of GO/polymer composites is a feasible strategy to improve the extraction performance and durability of GO based stir bar coating. By solution co-blending method or in-situ polymerization, GO with oxygen-containing functional groups can be reacted with polymers by intermolecular interactions. The GO/polymer composites, such as graphene oxide/polyethyleneglycol (GO/PEG) composite, graphene oxide/polyvinyl alcohol (GO/PVA) composite obtained by solution co-blending method [31,32] and graphene oxide/polyaniline (GO/PAN) composite, graphene oxide/polypyrrole (GO/PPY) composite obtained by in-situ polymerization [33–36], have been applied in energy, catalysis, conductive plastics, etc.

In this work, GO/PEG and GO/PAN composite were synthesized by solution co-blending method and in-situ polymerization, respectively, and the GO/PEG and GO/PAN composite coated stir bars were prepared by sol–gel technique. Both GO-based composite coated stir bars were tested for the extraction of five FQs (pefloxacin, norfloxacin, ciprofloxacin, enrofloxacin, lomefloxacin) and the GO/PEG composite coated stir bar showed better extraction efficiency for target five FQs. Based on this fact, a new method of GO/PEG composite coated SBSE-HPLC-FLD was developed for the analysis of five FQs in chicken muscle and liver. The operation conditions affecting the extraction of five target FQs by GO/PEG composite coated stir bar were optimized and the analytical performance of the developed method of SBSE-HPLC-FLD was evaluated. The developed method was applied to the analysis of five FQs in chicken muscle and chicken liver samples for validation.

2. Experimental

2.1. Reagents and standards

Five fluoroquinolones (FQs) (pefloxacin, PEF; norfloxacin, NOR; ciprofloxacin. CIP; enrofloxacin, ENR; lomefloxacin, LOM) were all analytical grade and obtained from Aladdin Reagent Database Inc. (Shanghai, China). The structure, pK_a and log P of target FQs (data source from Ref. [37]) are all listed in Table S1. Each standard solution of target analytes (500 $mg\,L^{-1}$) was prepared in methanol. Working standard solutions were prepared by diluting the mixed standard solution with high purity water to the required concentration. All standard stock solutions were kept in refrigerator at 4 °C away from light.

Hydroxyl-terminated polydimethylsiloxane (OH-PDMS), γ-(2,3-epoxypropoxy) propytrimethoxysilane (KH-560), tetraethoxysilane (TEOS), and poly(methylhydrosiloxane) (PMHs) were all obtained from Chemical Plant of Wuhan University (Wuhan, China). Trifluoroacetic acid (TFA, 95%) was obtained from Beijing Chemical Works (Beijing, China). Graphite powder (325 mesh, 99.9995%) was obtained from Alfa Aesar (MA, USA). Polyethylene glycol (PEG 10000), methanol (CH_3OH), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), aniline ($C_6H_5NH_2$) and potassium persulfate ($K_2S_2O_8$) were all analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). High purity deionized water purified by the Milli-Q water purification system (18.2 MΩ cm, Millipore, Molsheim, France) was used throughout the whole experimental process. The capillary glass bars (1.0 mm I.D., 0.10 mm wall thickness) were purchased from Apparatus Factory of West China University of Medical Sciences (Sichuan, China). The commercial PDMS coated stir bar (Twister) of 2 cm long and 0.5 mm thick was purchased from Gerstel (Müllheim an der Ruhr, Germany).

2.2. Instrumentation

The chromatographic separation of target FQs was processed by an Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degassing device, a quaternary pump, a 100 μL sample loop, an ultraviolet detector and a fluorescence detector (FLD). A RP-18 column (250 mm × 4.6 mm, 5 μm, Merck KGaA, Germany) was used for the separation of target FQs, and a mixture of CH_3OH and 10 $mmol\,L^{-1}$ $NaH_2PO_4{\cdot}2H_2O$ at a volume