Spectrophotometric analysis of phenols, which involves a hemin–graphene hybrid nanoparticles with peroxidase-like activity

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HIGHLIGHTS

- Chemical synthesis of hemin–graphene hybrid nanosheets with peroxidase – like activity.
- Determination of phenols using the different colored benzodiazepine reaction products.
- Spectroscopic analysis of phenols in different waters using multivariate calibrations.
- The application of hemin–graphene hybrid nanosheets as a peroxidase mimetic.

GRAPHICAL ABSTRACT
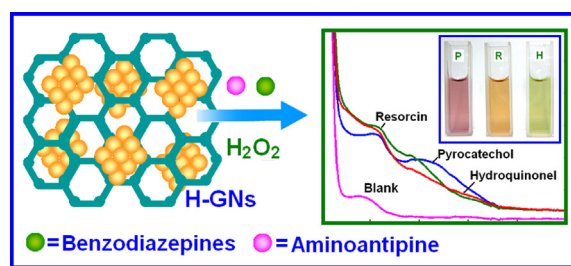
ARTICLE INFO

ABSTRACT

Phenols are well known noxious compounds, which are often found in various water sources. A novel analytical method has been researched and developed based on the properties of hemin–graphene hybrid nanosheets (H–GNs). These nanosheets were synthesized using a wet-chemical method, and they have peroxidase-like activity. Also, in the presence of H_2O_2, the nanosheets are efficient catalysts for the oxidation of the substrate, 4-aminoantipine (4-AP), and the phenols. The products of such an oxidation reaction are the colored quinone-imines (benzodiazepines). Importantly, these products enabled the differentiation of the three common phenols – pyrocatechol, resorcin and hydroquinone, with the use of a novel, spectroscopic method, which was developed for the simultaneous determination of the above three analytes. This spectroscopic method produced linear calibrations for the pyrocatechol (0.4–4.0 mg L^{-1}), resorcin (0.2–2.0 mg L^{-1}) and hydroquinone (0.8–8.0 mg L^{-1}) analytes. In addition, kinetic and spectral data, obtained from the formation of the colored benzodiazepines, were used to establish multi-variate calibrations for the prediction of the three phenol analytes found in various kinds of water; partial least squares (PLS), principal component regression (PCR) and artificial neural network (ANN) models were used and the PLS model performed best.

1. Introduction

In general, phenolic compounds are regarded as some of the most toxic substances, yet they are often found in ground and surface waters [1]. In this context, it has been well established that a very large number of phenolic compounds are released globally each year into the environment, e.g. agricultural fields and water supplies, and thus, such compounds may pose a significant threat to human health, ecosystems and homeland security [1]. Consequently, a continuing development of methodology for the analysis of these compounds is appropriate, particularly, if such methods are relatively simple, inexpensive and are aimed to analyze phenols in complex matrices. Some existing

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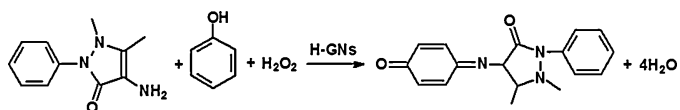
Scheme 1. Reaction of 4-aminophenazone (4-AP) with phenol; this reaction forms the basis of the method involving H–GNs; the novel method mimics and replaces the method based on the natural peroxidase catalyst.

techniques for the analysis of phenols include electrochemistry [2], gas chromatography–mass spectrometry (GC–MS) [3], chemi-luminescence [4] and kinetic spectrophotometry [5]. Also, Klibanov et al. [6] developed an enzyme-based method for water treatment with the use of horseradish peroxidase (HRP) – a catalyst, which facilitates the removal of phenols; Zhang et al. [7,8] were the first to apply HPR immobilized on graphene oxide for stabilization of phenolic compounds. However, in practice, the removal, separation or individual detection of the benzenediol isomers remains challenging because these molecules have very similar structures. Therefore, research and development of methods, which facilitate the detection or removal of phenols, remain particularly useful.

A possible approach for such research is to investigate the potential of the hemin–graphene hybrid nanosheets (H–GNs) to mimic the enzyme catalysts for phenols. The background to this approach is based on the properties and behavior of natural enzymes. Thus, these substances are efficient biological catalysts, which have been investigated and applied in medicine, chemistry, biology and agriculture; they are highly efficient and are substrate specific under mild conditions [9,10]. However, these enzymes are affected by environmental changes, such as temperature, water acidity and inhibitors; also, they are time-consuming to prepare, are costly to purify and can be difficult to store [11]. Thus, there has been an attempt to design and prepare substances, which mimic the properties of natural enzymes such as their high catalytic activity and substrate selectivity. Such work is reflected in the studies of nanoparticles [12–15], polyoxometalates [16,17], polymers [18], porphyrin [19–21], and magnetic nanocomposites [22]; the analytes included H_2O_2, glucose and thrombin. Zuo et al. [23] found that carbon nanotube/magnetic nanoparticles have an intrinsic enzyme mimetic activity similar to that of HRP; the latter is an effective substance for the treatment of phenol compounds in wastewater. Jv et al. [13] found that positively charged gold nanoparticles mimic peroxidase, and catalyze the substrate 3,3,5,5-tetramethylbenzidine in the presence of H_2O_2; these observations formed the basis of a colorimetric method with a good detection limit (LOD) of 5×10^{-7} M. Zargar et al. [24] used silver nanoparticles for the determination of resorcinol; the method was based on localized surface plasmon resonance, and was characterized by a low LOD value of 1.2×10^{-6} M.

Furthermore, the H–GNs systems can be synthesized relatively simply with the use of wet-chemistry, which relies on the π–π stacking interactions between the various components of the system. Such systems, in the presence of hydrogen peroxide and 4-aminoantipine (4-AP; which acts as a hydrogen donor), can react with phenols to form quinone-imine (Scheme 1).

Graphene has a single layer of sp^2-bonded carbon atoms, as well as high thermal stability, good mechanical strength and relatively high electrical conductivity [25–27]. Graphene oxide (GO), is the common parent material for graphene; it facilitates the formation of the graphene material with a large specific surface area, which has the potential to stabilize molecules and promote the catalytic activity and stability of such supported molecular systems as, e.g. hemin, HRP and Fe_3O_4NPs; cation–π interactions or π–π stacking are commonly invoked to account for the molecular stabilization,

which, however, still allows for the preservation of the intrinsic properties of the graphene [28].

Hemin, which is the active center of hemin-proteins, including cytochromes, myoglobins and hemoglobins, plays an important role in the catalysis of a variety of oxidation reactions similar to that of the peroxidase enzyme [21]. However, direct application of hemin as an oxidation catalyst leads to passivation because hemin is insoluble in water and produces molecular aggregation of the inactive dimers. A possible method to solve this problem is to modify synthetically the porphyrin structure to improve the catalytic activity or stability [29]; silica, zeolites, nanoparticles and graphehe are commonly used as the high surface area materials to improve the properties of the porphyrin. Recently, the deposition of porphyrin derivatives on the graphene oxide sheets was successfully realized with the aid of π–π interactions at the porphyrin–graphene interface [30,31]. Guo et al. [32] reported that H–GNs had intrinsic peroxidase-like activity, and could selectively detect the single-nucleotide polymorphism. Xue et al. [33] also found that graphene – supported hemin acted as a highly active biomimetic catalyst in the oxidation of pyrogallol. However, in general, literature suggests that the catalytic ability of H–GN conjugates has not been adequately explored.

The spectrophotometric technique has been widely used for quantitative single or simultaneous analysis of many different analytes from, for example, industrial, food, environmental and pharmaceutical sources [34–36]. For simultaneous analyses where significantly overlapping spectral bands often occur, chemometrics methods of data analysis have been applied successfully to resolve the overlapping profiles [5,37,38]. Thus, common multivariate calibration methods included principal component regression (PCR), partial least squares (PLS) and artificial neural networks (ANN); recently, such methods have been applied for multi-component kinetic modeling of data [5,38].

Thus, the aims of this work were: (1) to synthesize the H–GNs material with the use of a wet-chemistry method and to demonstrate that this material can facilitate the individual colorimetric analysis of the commonly met with important phenols – pyrocatechol, resorcin and hydroquinone, which are often found in various water samples; (2) to extend this methodology for the simultaneous analysis of the same three phenols but using their colored benzodiazepines, with the use of different chemometrics calibration models; (3) to compare the analytical performance of these calibrations, particularly with the analytical results obtained with the use of a standard HPLC method.

2. Experimental

2.1. Chemicals and materials

Graphite powder (gray material, 99%, w/w) was purchased from Sigma–Aldrich Co., Shanghai, China. Ammonia solution (28%, w/w), hemin (98%, w/w), 4-aminoantipine (98%, w/w), H_2O_2 (30%, w/w), and hydrazine solution (85%, w/w) were prepared according to standard methods [39] and all reagents were obtained from the Aladdin Industrial Corporation Co., Shanghai, China. Pyrocatechol, resorcin and hydroquinone (all >98% pure, w/w) were obtained from the Shanghai Chemical Plant, China, and their 20.0 mg L^{-1} aqueous solutions were prepared by standard methods [39]. Tris–HCl buffer (0.05 M, pH8.5), was prepared by mixing 10 mL 0.2 M 2-amino-2-(hydroxymethyl)-1,3-propanediol with 8 mL 0.2 M HCl, and diluted to 50 mL with water. All of the above chemicals were Analytical Grade reagents. Double distilled water was used throughout in this work.