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Enhancing selectivity in spectrofluorimetric determination of tryptophan by using graphene oxide nanosheets

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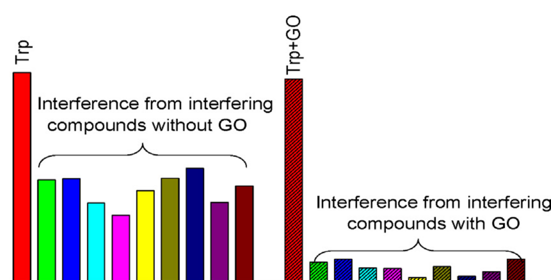
HIGHLIGHTS

- A simple and clean method for L-Trp determination was developed using GO nanosheets.
- Uptaking of L-Trp was achieved by reaction in presence of GO nanosheets.
- Selectivity of L-Trp determination was highly improved using GO nanosheets.

GRAPHICAL ABSTRACT



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ABSTRACT

Reaction of formaldehyde with amino acids followed by oxidation with hydrogen peroxide to produce a fluorophore Norharman product is well known and was used for the spectrofluorimetric determination of L-tryptophan (Trp). This study aimed to use graphene oxide (GO) to enhance the selectivity and sensitivity of Trp in presence of other amino acids and possible interfering compounds. Different parameters such as pH, temperature, incubation time, and concentrations of formaldehyde, H_2O_2 and GO were studied to optimize the condition of determination. Experimental data showed that the maximum fluorescence intensity was achieved in pH 7.0–9.0 phosphate buffer mixed with 7–10% (v/v) formaldehyde and 1–2% (v/v) H_2O_2 as oxidizing agent at 60 °C for 1 h. On the basis of calibration curve of various concentrations of Trp in the presence of 20 $\mu g\,mL^{-1}$ GO, the lower limit of detection (LOD) of Trp was determined as 0.092 nmol mL^{-1} and the lower limit of quantification (LOQ) was 0.3 nmol mL^{-1}. The selectivity of Trp in presence of other amino acids and possible interfering compounds were studied with and without GO. The data obtained after inner filter effect corrections revealed that the selectivity of Trp in presence of amino acids and other possible interfering agents was improved in the range of 76–96%, compared with that in absence of GO. The enhancement of selectivity in the presence of GO indicates that the Trp and other amino acid and possible interfering compounds were adsorbed by GO, and the selective uptaking of Trp-by the reaction with formaldehyde followed by oxidation with H_2O_2 at 60 °C with high selectivity and sensitivity was achieved successfully.

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1. Introduction

Graphene oxide (GO) is a novel single-atom-thick and two-dimensional carbon material produced by oxidation of graphite, containing oxygen functional groups, such as epoxides, phenol hydroxyls, and carboxylic groups [1–3]. The oxygenated lattice of GO not only facilitates better water solubility and stability but also allows noncovalent interaction with diols, amine functional groups, and phenyls in biomolecules through electrostatic

Abbreviations: GO, graphene oxide; Trp, L-tryptophan; Asn, L-asparagine; His, L-histidine; Phe, L-phenylalanine; Arg, L-arginine; Tyr, L-tyrosine; Lys, L-lysine; FD, formaldehyde; THCAs, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids; LOD, lower limit of detection; LOQ, limit of quantification.

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interaction, π–π stacking, and hydrogen bonding to enable recognizing of biomolecules with detectable specificity [4]. Recently, GO has attracted considerable attention due to its extraordinary electronic, optical and thermal properties in comparison to other nanomaterials. The superior characters of GO, such as large surface area, good water dispersibility and biocompatibility, facile surface modification and low manufacturing cost, make it a promising material for biotechnology and biosensing application [5,6]. GO has been shown to quench organic fluorescence molecules due to long range nanoscale energy transfer [7]. Most recent experimental results indicate that some amino acids, peptides, and proteins can be quickly adsorbed onto the surface of GO nanosheets because of electrostatic interaction, hydrophobic interaction and hydrogen bonding [8].

Tryptophan (Trp) is an essential amino acid needed for normal growth in infants and for nitrogen balance in adults. Trp cannot be synthesized in the human body and thus must be obtained from food or supplements. Trp serves as a precursor for many neurotransmitters and neurochemicals, including serotonin and melatonin. Melatonin is known to help improve sleep, and serotonin is needed to improve mood and mental health. Trp supplements have been used for some time as antidepressants, sleep aids and weight-loss aids. Many analytical methods have been established for its determination with a variety of sample matrices [9,10], such as spectrophotometric [11], spectrofluorimetric [12,13], high performance liquid chromatography (HPLC) [14,15], capillary electrophoresis [16], and electrochemical methods including traditional and modified electrodes [9,10,17–19]. A simpler, more sensitive and low-cost determination of Trp is of great significance to human health. Furthermore, as Trp coexists with different amino acids in body fluids, its selective determination is also very important in pharmaceutical preparations, body fluids and biological samples [10]. One of the most important spectrofluorimetric method to determine Trp is the formation of fluorophore Norharman product by reaction with formaldehyde followed by oxidation with H_2O_2 [13,20]. In the present study, we aim to improve the selectivity and sensitivity of Trp determination using GO nanosheets as a chemical sensor in Norharman reaction. In this method, Trp and other amino acids or possible interfering compounds are first adsorbed on the GO surface by non-covalent interactions, and then Trp is selectively uptaken by the chemical reaction with formaldehyde followed by the oxidation with H_2O_2, recovering the fluorescence of fluorophore of Norharman product. In the presence of GO, other amino acids or interfering compounds like ascorbic, tartaric and uric acid do not participate in the Norharman reaction.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were of analytical reagent grade unless otherwise stated. Deionized water used in the experiments was obtained from a Modulab 2020 Water purification system (Continental Water System Corp. San Antonio, USA) with resistivity more than 18 MΩ cm and pH 5.6 at 20.0 ± 0.5 °C. To prepare 2×10^{-5} M of stock solution of L-tryptophan (Trp), 2.04 mg Trp was dissolved in 500 mL volumetric flask using deionized water and the diluted solutions of Trp up to 5×10^{-8} M were prepared in 0.1 M phosphate buffer of pH 7.5–8.0. Amino acids and other interfering compounds were prepared in 1×10^{-5} M in phosphate buffer before using directly. L-tryptophan (Trp), L-asparagine (Asn), L-histidine (His), L-phenylalanine (Phe), L-arginine (Arg), L-tyrosine (Tyr), L-lysine (Lys), L-ascorbic acid, tartaric acid and uric acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Formaldehyde solution (37%) and hydrogen peroxide (30%) were obtained from MP Biomedicals (Solon, OH, USA). 25% Formaldehyde solution (FD) was prepared by mixing 37% formaldehyde with phosphate buffer of pH 7.5–8.0 in 2:1 (v/v) ratio. Single layer GO was purchased from ACS Material LLC (Medford, MA, USA).

2.2. Apparatus

All spectrofluorimetric measurements were carried out at 20.0 ± 0.1 °C with Fluorolog-3 spectrofluorometer (Horiba Scientific, Edison, NJ, USA). The slit widths for excitation and emission were both set at 5 nm. A bench-top sonicator of Model 1510 was used to disperse graphene oxide in water (Branson, Danbury, CT, USA). A bench-top M545 pH meter was applied to adjust pH (Pinnacle, Corning, NY, USA). The oven used in this study was an autoblot micro-hybridization oven (Bellco Glass Inc., Vineland, NJ, USA).

2.3. Methods

2.3.1. General procedure

To a 5 mL clean and sterilized vial, the prepared solutions were added in the following order: 1.0 mL of 2×10^{-6} M of Trp solution, 1.0 mL of 100 μg mL^{-1} graphene oxide (GO), shacking well for 3 min, then 1.8 mL of FD (25%) and 1.0 mL phosphate buffer solution. After mixing, the solution was stored for 2 h at room temperature, and then 200 μL of H_2O_2 was added. The mixture was shacked well every 5 min and incubated in an oven at 60 °C for 1 h. The above method was used for the subsequent measurements of emission spectra under effects of temperature, incubation time and the effect of reagent concentrations of formaldehyde and H_2O_2. After cooling the above solution, the spectrofluorimetric measurements were monitored at excitation wavelength 290 nm and emission wavelength 450 nm ($\lambda_{ex}/\lambda_{em}$ = 290/450 nm). The data obtained were the average of 5 measurements.

2.3.2. Effect of pH

Measurements of the pH dependence of 2.0 mL of 2×10^{-6} M Trp and formaldehyde reaction were performed over a wide range of pH 2–10. The pH was adjusted using dilute sodium hydroxide and/or hydrochloric acid. The fluorescence intensity fluorophore Norharman product was measured of different pHs at $\lambda_{ex}/\lambda_{em}$ = 290/450 nm. Each point was repeated for 3 measurements.

2.3.3. Effect of temperature and incubation time

The effect of temperature on the fluorescence intensity of the reaction of 1.0 mL of 2×10^{-6} M Trp solution with FD at 40, 50 and 60 °C were plotted against the incubation time until stable fluorescence intensity at 450 nm was reached. The data presented in the curve were the average of 3 measurements.

2.3.4. Effect of chemical reagents

2.3.4.1. Formaldehyde concentration. Various amounts of formaldehyde (FD) concentrations ranged from 2 to 10% of the total volume of 5 mL mixed with 1.0 mL of 2×10^{-6} M Trp solution as in general method and the fluorescence intensity at ($\lambda_{ex}/\lambda_{em}$ = 290/450 nm) against the FD concentrations was plotted.

2.3.4.2. Hydrogen peroxide concentration. Hydrogen peroxide concentrations ranged from 0.1 to 10.0% of the total volume 5 mL were mixed thoroughly with 1.0 mL of 2×10^{-6} M Trp solution as in previous general procedure. The fluorescence intensity at ($\lambda_{ex}/\lambda_{em}$ = 290/450 nm) against the H_2O_2 concentrations was plotted.

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