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Ultrasensitive and selective spectrofluorimetric determination of *S*-nitrosothiols by solid-phase extraction

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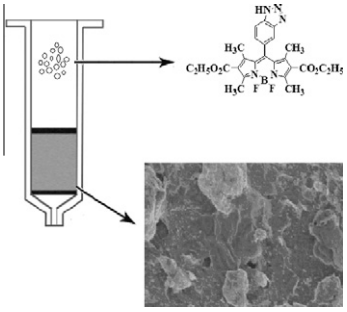
HIGHLIGHTS

- TiO_2–Gr nanocomposite was used as SPE adsorbent.
- A novel spectrofluorimetry for *S*-nitrosothiols determination was developed.
- The developed method has low detection limit and wide linear range.

GRAPHICAL ABSTRACT



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ABSTRACT

This present work describes the ultrasensitive and selective spectrofluorimetric determination of *S*-nitrosothiols by solid-phase extraction based on a novel adsorbent TiO_2–graphene nanocomposite. 1,3,5,7-Tetramethyl-2,6-dicarbethoxy-8-(3,4-diaminophenyl)-difluoroboradiaza-*s*-indacence is used as fluorescent probe for *S*-nitrosothiols label. The procedure is based on the fluorescent probe selective reaction with *S*-nitrosothiols to form highly fluorescent product, its extraction to the TiO_2–graphene-packed SPE cartridge and spectrofluorimetric determination. The experimental variables affecting the extraction procedure, such as the type of the eluent and its volume, sample pH, and sample volume, have been studied. Under the optimized extraction conditions, the method showed good linearity in the range of 0.5–100 nM. The limit of detection was 0.08 nM (signal-to-noise ratio = 3). Relative standard deviation was 2.5%. The developed method was applied to the determination of *S*-nitrosothiols in human blood samples with recoveries of 92.0–104.0%. This work revealed the great potentials of TiO_2–graphene as an excellent sorbent material in the analysis of biological samples.

Introduction

The obligatory role of nitric oxide (NO) in the regulation of cardiovascular system has been widely recognized. However, NO is known to be extremely unstable and susceptible to inactivation by heme iron, non-heme iron, superoxide anion, oxygen, and other biochemical species [1]. Indeed, it has been suggested that NO could be stabilized by covalent bonding with thiols such as glutathione, cysteine, albumin, and hemoglobin [2,3], forming *S*-nitrosocysteine (SNOCys), *S*-nitrosoglutathione (SNOGSH), *S*-nitrosoalbumin, and *S*-nitrosohemoglobin, respectively. Therefore, accurate evaluation of *S*-nitrosothiols (SNOs) in circulating blood is of great interest in assessing the in vivo regulatory state of NO/cGMP system not only by the endothelial NO, but also by NO-releasing vasodilators in clinical use such as glyceryl trinitrate [4]. Some analytical methods for the determination of RSNO have been developed. These mainly include spectrophotometry [5], chemiluminescence [6], electrometric methods [7], chromatography [8] and spectrofluorimetry [9,10], etc.

However, the extremely low concentrations in biological samples and the complexity of their matrices make determination of SNOs challenging tasks. Because of this, there is a critical demand for rapid and simple preparation techniques especially extraction

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techniques, for evaluating and monitoring SNOs from different biological samples at trace level. Solid-phase extraction (SPE) is an efficient sample pre-treatment method, routinely used for the extraction of compounds from liquid or solid matrices. SPE approach, in which adsorbents have been treated as media for the retaining of target compounds, followed by selective elution, has been broadly utilized due to the advantages of less organic solvents, no emulsion formation like in liquid–liquid extraction, and short time, etc. [11].

The choice of appropriate adsorbent is a critical factor to obtain good recovery and high enrichment factor in SPE procedure. Graphene (Gr) is a fascinating carbon nanomaterial, which posses a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice. It has a large specific surface area (theoretical value 2630 m^2/g) which suggests a high sorption capacity [12]. Furthermore, special structure makes both sides of the planar sheets of Gr are available for molecule adsorption. Actually, Gr and its composite have been reported using as adsorbent in SPE for different compounds enrichment [13–17]. However, many of the interesting and unique properties of Gr can only be realized after it is integrated into more complex assemblies [18]. Moreover, two-dimensional plane structure of Gr provides a vast platform for loading various nanoparticles, offering a new way to develop catalytic, magnetic, and optoelectronic materials [19]. It has also been reported that the integration of carbon-based materials and metal oxide usually shows synergistic effects in applications [20], so there is a reason to expect the integration of Gr and other nanoparticles has the similar effect on the SPE of SNOs. TiO_2 nanoparticles is reported having large surface area and containing much ion-exchangeable OH groups in the interlayer and surface [21], and it has gained great interests in analytical chemistry because of its high chemical stability, durability, corrosion resistance, non toxicity and cost effectiveness. Micro-scaled TiO_2 has been used as stationary phase in HPLC applications [22] as well as a solid phase extraction adsorbent for selective extraction of phosphopeptides [23]. TiO_2 were demonstrated to be excellent adsorbents of inorganic cations and anions as well as organic compound [24].

Spectrofluorimetric method of determination has several advantages including low detection limit, sensitivity, selectivity, low cost and less time consuming. Difluoroboradiaza-*s*-indacenes (BODIPY) has advantages of high extinction coefficients, high fluorescence quantum efficiency and stability to light [9]. It has been reported that Hg^{2+} can effectively displace NO from GSNO, and then NO reacts with *o*-phenylenediamine to form fluorescent derivative [9]. 1,3,5,7-tetramethyl-2,6-dicarbethoxy-8-(3,4-diaminophenyl)-difluoroboradiaza-*s*-indacence (TMDCDABO) is an excellent fluorescent probe [25]. Its self-fluorescence is very weak, and the quantum efficiencies can increase more than 100 times after reaction with NO. In this work, a novel method was developed for the ultrasensitive and selective determination of SNOs in biological samples using fluorescent probe 1,3,5,7-tetramethyl-2,6-dicarbethoxy-8-(3,4-diaminophenyl)-difluoroboradiaza-*s*-indacence (TMDCDABO) derivatization followed by SPE and spectrofluorimetric detection. TiO_2–Gr was used as SPE adsorbents. Several key influence parameters were investigated in detail for good SPE efficiency. The method was demonstrated to be applicable for the analysis of SNOs in human blood samples.

Experimental

Apparatus

All fluorescence measurements were performed on a Cary Eclipse Fluorescence Spectrophotometer (Varian, USA) with 1-cm path length quartz cells. Instrument excitation and emission slits both were adjusted to 5 nm. The pH value of solution was measured using a pHS-3C meter (Shanghai Leici Equipment Factory, China). Scanning electron microscopy (SEM) images were obtained on a Hitachi S-4800 scanning electron microscope. The SPE cartridges (1 mL) were obtained from Agilent (Agilent, Palo Alto, CA, USA).

Reagents

1,3,5,7-Tetramethyl-2,6-dicarbethoxy-8-(3,4-diaminophenyl)-difluoroboradiaza-*s*-indacence (TMDCDABO) was synthesized according to the references [25,26]. The stock solution of 1×10^{-3} mol L^{-1} TMDCDABO was prepared in methanol (MeOH). Glutathione (GSH) (reduced form, free acid 98–100%) was obtained from Shanghai Chemicals (Shanghai, China). Phosphate buffered saline (PBS) consisted of 10.0 g L^{-1} NaCl, 0.25 g L^{-1} KCl, 1.44 g L^{-1} Na_2HPO_4, 0.25 g L^{-1} KH_2PO_4 and adjusted to pH 7.0 [25]. Unless otherwise specified, all reagents were of analytical reagent grade used without further purification and all solutions were prepared from double-distilled water.

Preparation of SNOs

SNOs were prepared fresh for each experiment by incubating 100 mM thiol (GSH) with 100 mM sodium nitrite in the presence of 250 mM HCl and 0.1 mM EDTA for 30 min. The solutions rapidly turned red upon exposure to water, forming the corresponding SNOs. The stock solutions were stored on ice. The final concentrations were determined using the reported extinction coefficients at 334–338 nm for SNOGSH (ε_{334nm} = 780 mol^{-1} cm^{-1}) [27].

Preparation of TiO_2–Gr nanocomposite

Graphene oxide (GO) was prepared from graphite powder by the modified Hummers method [28]. Graphite was added in a mixture containing 12 mL concentrated H_2SO_4, 2.5 g $K_2S_2O_8$ and 2.5 g P_2O_5. The solution was heated to 80 °C and kept stirring for 5 h. The mixture was diluted with deionized water (500 mL). The product was obtained by filtering using 0.2 μm Nylon film and dried naturally. The product was re-oxidized by Hummers and Offeman method to produce the graphite oxide. Exfoliation was carried out by sonicating 0.1 mg mL^{-1} graphite oxide dispersion for 1 h. TiO_2–Gr nanocomposite was prepared according to the reference [29]. In short, 0.2 mL of $Ti(O^iPr)_4$ was added to the GO suspension (1 mg mL^{-1}) and ultrasonicated for 1 h. The mixture was then transferred to a 25-mL Teflon-sealed autoclave and kept at 130 °C for 12 h. The product was isolated by filtration and rinsed thoroughly with water and ethanol, respectively. The product was then dried in vacuum. The TiO_2–Gr nanocomposite was obtained in the form of black powder.

Derivatization procedures

Hg^{2+} can effectively displace NO from GSNO, and then NO reacts with TMDCDABO to form TMDCDABO derivative. The derivatization procedures were according to the reference [10]. 2 mL 2.0×10^{-5} mol L^{-1} TMDCDABO was transferred to a 10 mL volumetric flask. Then 2 mL PBS, 0.05 mL 5×10^{-4} mol L^{-1} SNOGSH and 1 mL of 1×10^{-3} mol L^{-1} Hg^{2+} solution were added. The mixture was diluted to the mark with water and kept at 30 °C for 15 min. The chemical structure of TMDCDABO and its reaction with SNOGSH are shown in Fig. 1.