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A simple and sensitive resonance Rayleigh scattering-energy transfer method for amino acids coupling its Ruhemann's purple and graphene oxide probe

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ABSTRACT

In pH 7.2 KH_2PO_4-NaOH buffer solution, graphene oxide (GO) has strong resonance Rayleigh scattering (RRS) effect at 400 nm, and amino acid reacted with ninhydrin to form blue-violet complex Ruhemann's purple (RP) with a absorption peak at 400 nm. RPs can strongly adsorbed on the surface of GO, and the RRS donor of GO probes coupled with the receptor of RP that reduced the RRS intensity at 400 nm due to the RRS-energy transfer (RRS-ET) from the GO to RP. With the increase of amino acid concentration, the RRS intensity quenched linearly at 400 nm due to the RRS-ET enhancing. The quenched intensity responds linearly with glutamic acid concentration in the range of 0.2–200 μmol L^{-1}, with a detection limit of 0.08 μmol L^{-1}. This simple and sensitive RRS-ET method was used to detect the content of amino acid in oral liquid, with satisfactory results.

1. Introduction

Amino acids are not only the material in protein synthesis but also some precursors of several important physiological activators, and it is an essential nutrient in the human body. The amino acid content in normal blood was consistent, such as 4–6 mg/100 mL in plasma and 6.5–9.6 mg/100 mL in haemocyte. Either too low or too high content of amino acid will be unfavorable to body health. Too low amino acid in serum content directly affects the normal body growth. The disturbance of amino acid metabolism closely correlates with hepatic encephalopathy and tumour. So the amino acid analysis is of great significance in disease diagnosis and treatment [1]. At present, main analysis methods for amino acid included spectrophotometry [2], fluorescence method [3–5], resonance Rayleigh scattering (RRS) method [6,7], surface enhanced Raman scattering (SERS) method [8,9], chemiluminescence method [10,11] etc. Spectrophotometry was simple, fast and economic, but most amino acids had without absorption on ultraviolet-visible area and the absorption peak is susceptible to pH of solution. Fluorescence method was simple and fast, but the sensitivity needed to be improved and most amino acids had without fluorescence. SERS was rapidity, sensitivity and specificity but there are some issues to be resolved such as reproducibility and the synthesis of SERS substrate [12]. Chemiluminescence method had the features of simple operation, low determination limit, wide detection range and analysis quickness, but there are some defects in the direct determination of some amino acids, expensive luminescent reagents and poor selectivity [13].

RRS method is a simple, rapid, high sensitive spectral analysis technology. As a direct liquid sampling technique, RRS method has been widely applied to the trace element analysis of trace metal ion, small molecule drugs, protein, nucleic acid and surfactant [14–20]. Nanoparticles had a strong RRS when it interacted with light. If a substance adsorbed on a nanoparticles surface and there is the overlap between the nanoparticles RRS spectra and the substance absorption spectra, the RRS energy transfer (RRS-ET) takes place, and a RRS-ET spectral method could be developed. Recently, a selective RRS-ET spectral method was establish for detection of trace tea polyphenols, formaldehyde, fluorinion, iodate and hydrogen peroxide based on the RRS-ET [21–24]. Graphene is a single-layer sheet structure composed of sp^2-hybridized carbon atoms arranged in a honeycomb lattice and has emerged as a rising star in nanomaterials science owing to its excellent physical and chemical properties [25]. But application of grapheme is limited by its

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difficulties of poor hydrophilicity and easily aggregated. Graphene oxide (GO) is a promising precursor for preparing graphene and it is of graphene and oxygen domain property, especially, it is a very stable and water-soluble nanomaterial [26]. It has been applied to biochemical analysis due to the ability of efficient adsorption, amphipathy, the ability of fluorescence quenching and the properties of Raman scattering [27–29]. Using GO as RRS probe, it has been applied to RRS detection of protein, fluorine ion, iodide and H_2O_2 [23,30,31]. However, there are no reports about RRS-ET methods for detection amino acids with GO as RRS probe. Here, a new RRS-ET analysis platform was developed to detect amino acids in amino acids oral liquid, with high sensitivity, good selectivity, high stability, and simple operation.

2. Experimental

2.1. Apparatus and reagents

A model of F-7000 fluorescence spectrophotometer (Hitachi, Japan), a model of TU-1901 double-beam UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.), a model of FEI Quanta 200 FEG scanning electron microscope (SEM) (FEI Co., Ltd., Holand), a model of nanoparticle and Zeta potential analyzer (Malvern Company, England), and a model of SK8200LH ultrasonic reactor (Shanghai Guide Ultrasonic Instruments Co. Ltd.) were used.

A pH 7.2 phosphate buffer solution (PBS): A 50 mL 0.2 mol L^{-1} KH_2PO_4 solution and 35 mL 0.2 mol L^{-1} NaOH solution were mixed and diluted to 200 mL, with a concentration of 50 mmol L^{-1} PO_4^{3-}. A 20 g L^{-1} ninhydrin solution: a 2 g ninhydrin hydrate was dissolved in 100 mL water by ultrasonic dispersion. A 1.0 mmol/L glycine (Gly), glutamic acid (Glu), lysine (Lys), aspartic acid (Asp) and phenylalanine (Phe) standard solution were prepared. GO was prepared by the Hummer procedure [30], and a 0.1000 g GO was dissolved in 100 mL water by ultrasonic dispersion to obtain a 0.1 g/L GO solution. A 173.1 μg mL^1 carbon nanoparticle (CNP) solution: 1.0 mL Hero high carbon ink (Shanghai Fine Stationery Co., Ltd) which quality after drying is 173.1 mg mL^{-1} was stepwise diluted 1000 times. All reagents were of analytical grade and the water was doubly distilled.

2.2. Procedure

Into a 5 mL marked tube, a 90 μL pH 7.2 PBS, a 70 μL 20 g L^{-1} ninhydrin solution, a certain amount of amino acid standard solution were added and it was heated 15 min in 85°C water bath and cooled with tap-water. Then a 400 μL 0.1 g L^{-1} GO was added and diluted to 1.5 mL with water. The RRS spectrum was recorded, using synchronous scanning technique (λ_{ex}-λ_{em}=$\Delta\lambda$=0), volt of 450 V, both excited slit and emission slit of 5.0 nm, and emission filter of 1% T attenuator. The RRS intensity at 400 nm (I_{400nm}) and a blank value without amino acids ($I_{400nm})_b$ were measured, and the $\Delta I=(I_{400nm})_b$-I_{400nm} for amino acids were calculated.

3. Results and discussion

3.1. Principle

GO has a strong RRS peak at about 400 nm, it can be used as probe. In the presence of pH 7.2 PBS and 85 °C water bath heating, ninhydrin reacted with amino acids to form blue-violet complex Ruhemann's purple (RP) that reduced the RRS peak due to the RRS-energy transfer (RRS-ET) from the GO as donors to the RP as receptors. With the increase of glutamate concentration, the formed RP increased, and the RRS intensity quenched linearly at 400 nm due to the RRS-ET enhancing (Fig. 1). So, a simple rapid and sensitive GO RRS-energy transfer analytical platform was established to detect trace amino acids.

3.2. RRS spectra

GO is very easy to dissolve in water because it contain rech hydroxyl and carboxyl groups, and is one of most stabile in known nanosol such as graphene, carbon nanotube, carbon nanoparticle, C_{60}, nanosilver and nanogold. It exhibited four RRS peaks at 320 nm, 375 nm, 430 nm and 490 nm, and the peak at 375 nm is strongest. Thus, GO was selected as RRS probe. There is no significant change in the RRS spectra of GO when the pH 7.2 PBS and ninhydrin was added in the system. In 85 °C water bath heating, ninhydrin reacted with common amino acids (Gly, Glu, Lys, Asp, Phe) to form RP with weak RRS spectra. When GO coexist with RP, the RRS intensity quenched with the increase of amino acid and there is a peak valley at 400 nm (Fig. 2, Figs. S1-S4). Meanwhile, the RRS spectra of Gly-ninhydrin-carbon nanoparticles (CNP) were studied with a poor stability (Fig. S5). So, the GO system was selected for detection amino acids.

3.3. Absorption spectra

The absorption of ninhydrin is weak and without absorption peak in the range of 350–700 nm (Fig. 3a). Gly reacted with ninhydrin to form RP which had two equal absorption peaks at 400 nm and 565 nm. The absorption peak value responds linearly with glutamate concentration (Fig. 3b-i) due to formation of RP. The absorption value of GO is weak in pH 7.6 PBS and ninhydrin solution. When GO coexist with RP, there are also two absorption peaks at 400 nm and 565 nm (Figs. S6-S10). For the Gly-ninhydrin-CNP system, two absorption peaks were also observed at 400 nm and 565 nm (Fig. S11). However the stability is not good.

The relationship between the absorption peak and the RRS valley, that is, the RRS-ET principle, was considered. The RP exhibited two absorption peaks at 400 nm and 565 nm, is of strong intermolecular forces, and can adsorb on the surface of one dimensional nanomaterial GO that formed the RRS acceptor and RRS donor. RP can max accepted the RRS energy at 400 nm and 565 nm from the donor of GO. Thus, two RRS valleys would be observed at 400 nm and 565 nm (Fig. 4). The RRS valley at 400 nm was recorded, because the RRS intensity of GO is weak when the wavelength is more than 500 nm. Thus, the valley wavelength at 400 nm was selected for detection of trace amino acid.

3.4. Scanning electron microscopy (SEM) and laser scattering

The SEM of GO showed in Fig. 5a that exhibited nano-sheets in size of 0.1–4 μm, and the energy spectral peak appeared at 0.25 keV ascribing to carbon element of GO. Upon addition of Gly and ninhydrin respectively, the size and shape hold constant because GO was very stable and can not be decomposed by Gly or ninhydrin that may absorb on the surface by means of intermolecular forces (Fig. 5b, c). In fact, both RRS spectra showed that the two systems exhibited equal RRS signal. When both Glu and ninhydrin are coexisted in the system, the particle size and shape of GO are not changed (Fig. 5d), but the RRS signal decreased greatly at 400 nm. So, we can conclude that the phenomenon of RRS-ET occurred in the acceptor RP and donor GO. In short, the RRS intensity reduction is not caused by the particle size and shape change, but because of the RRS-ET, which is consistent with the results of SEM. The size distribution of laser scattering graph (Fig. 6a) showed that there are two peaks at 70–700 nm and 2000–7000 nm for the GO-ninhydrin system. Upon addition of Gly, there are also two peaks at 60–600 nm and 3500–7000 nm. This is also indicated that the particle size of GO are not changed greatly after adding Gly.