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Fluorescence chemodosimeter for dopamine based on the inner filter effect of the *in situ* generation of silver nanoparticles and fluorescent dye

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ABSTRACT

A new strategy for the sensitive and selective detection of dopamine (DA) was proposed. The chemodosimeter design was based on the measurement of the fluorescent quenching of fluorescein dye caused by the *in situ* generation of silver nanoparticles (AgNPs). The AgNPs can be simply generated by a reaction between DA and Ag^+ in the presence of polymethacrylic acid (PMAA). In addition, the generated AgNPs possess the maximum surface plasmon resonance (SPR) at 440 nm and an increase in the SPR intensity with an increasing DA concentration. Basically, fluorescein dye can emit the fluorescent intensity maximum at 513 nm with excitation at 487 nm. Thus, fluorescent quenching was achieved due to an inner filter effect from the overlap between the excitation spectrum of the fluorescein dye and the SPR spectrum of the generated AgNPs. The degree of fluorescent quenching linearly depends on the number of generated AgNPs that can be directly related to the concentration of DA. The proposed chemodosimeter can be used to detect DA in a working linear concentration range of 1.0–5.0 μM at a detection limit of 10.6 nM. This chemodosimeter was successfully applied to determine DA in a real urine sample and a dopamine injection formulation with satisfactory results.

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1. Introduction

Dopamine (DA) is a naturally occurring catecholamine compound in the human body [1]. This compound is an important neurotransmitter for various functions in mammalian central nervous systems, such as body movement or even when sleeping [2,3]. The changes in the DA level in certain situations can be related to physical and psychological disorders [4]. DA is an important indicator compound for the identification of schizophrenia and Parkinson's disease [5,6]. Therefore, a diagnosis of these diseases requires highly accurate, rapid and sensitive measurements of DA concentration. Thus, substantial efforts have been taken to quantify DA by several approaches.

There have been various studies regarding the development of detection strategies for the sensitive monitoring of DA, such as high-performance liquid chromatography [7], capillary electrophoresis [8], electrochemistry [9], colorimetric assay [10], and enzymatic methods [11]. However, most of these techniques do not meet the increasing requirements for developing a simple, fast, facile, low-cost, sensitive, and selective method. Fluorescent methods have attracted much attention

[12–17] due to their high sensitivity, fast analysis speed, convenient signal transduction with low background interference, easy operation, and quick response. Therefore, the further development of high sensitivity and selectivity chemodosimeters for DA based on a fluorescence method is of interest.

The *in situ* generation of AgNPs is a facile process that is useful for various purposes, such as chemodosimeter fabrication (direct or indirect detection) [18–20], and these particles can serve as catalysts [21,22] and reductants [23]. This strategy needs a suitable reducing agent, such as ascorbic acid [24], tannic acid [25], 4-mercaptophenylboronic acid [26], or carbon dots [27], to reduce Ag^+ to Ag^0 in the presence of an appropriate stabilizer or a template. In addition, the *in situ* generation of AgNPs by DA has been reported [28–30]. From the literature, the AgNPs have been easily generated by the appropriate reducing agents in a one-pot reaction. The resulting nanoparticle characteristics, such as particle shape and size, are basically strongly dependent on the type of reducing agent, template, reaction temperature and concentration of the Ag^+ source, which can be exhibited by an SPR spectrum.

The inner filter effect (IFE) is one popular strategy for the fabrication of a fluorescence chemodosimeter. Its popularity is because the IFE can provide advantages over other approaches, namely, it does not need covalent links between the absorber and the fluorophore, which provide a comparatively simple and facile approach for the detection

of target analytes [31]. Thus, this approach can be used to freely design many types of new fluorescent chemodosimeters. The basic IFE principle is caused by the shielding between the excitation wavelengths of the fluorophore with the absorption spectrum of the absorber. There have been reports regarding many types of fluorophore and absorber couples for the fabrication of the fluorescent chemodosimeter-based IFE phenomena [32]. As the conventional absorber usually has a problem with its extinction coefficient, which can also restrict the sensitivity of the IFE, metal nanoparticles, such as AgNPs, are therefore the one efficient candidate that can be used as the absorber during an IFE process. This efficiency is because AgNPs possess an extremely large extinction coefficient that originates from its surface plasmon resonance [33,34]. Moreover, the SPR characteristic of the AgNPs strongly depends on their sizes and shapes [35–37]. This attribute signifies that the SPR spectrum of the AgNPs can be easily tuned to overlap with the excitation spectra of the chosen fluorophores. However, there is no report regarding the *in situ* generation of AgNPs coupled with a fluorescein dye for the detection of DA.

In this study, we propose a new IFE-based approach using *in situ*-generated AgNPs that was templated by PMAA as an IFE absorber and fluorescein dye as an IFE fluorophore for the detection of DA. The detection principle is based on the *in situ* generation of AgNPs by a reaction between DA and Ag^+ in the presence of PMAA. With a high extinction coefficient, the generated AgNPs were expected to be an efficient absorber to tune the emission of the fluorophore in the IFE-based fluorescent assays. The number for the AgNPs generated can cause the fluorescent quenching of the fluorescein dye by the IFE phenomenon. Thus, the degree of fluorescent quenching can be directly related to the concentration of DA. The parameters that are likely effect to the detection sensitivity were studied and optimized. In addition, the selectivity of the chemodosimeter was studied over other related compounds and possible interference species in a real situation. The feasibility of the proposed chemodosimeter in a real application was also demonstrated with a urine sample and dopamine injection.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade and used without further purification. Silver nitrate and fluorescein were obtained from BDH, England. Poly(methacrylic acid) sodium salt and dopamine hydrochloride were purchased from Sigma-Aldrich, Germany. Sodium hydroxide was obtained from Carlo Erba Reagents, Italy. Glacial acetic acid was purchased from QRec, Thailand. Ultrapure water (18.2 MΩ cm) was obtained from a Millipore water purification system.

2.2. Instrumentation

The absorbance and fluorescence spectra were recorded using an Agilent HP 8453 UV–Vis spectrophotometer and an RF-6000 spectrofluorometer (Shimadzu), respectively. The slit width used for both excitation and emission was 5 nm wide. The solution pH was measured *via* a UB-10 Ultra Basic pH meter (Denver Instrument Company). Transmission electron microscopy (TEM) images of the generated AgNPs were recorded using a Tecnai G^2-20 (FEI, Netherlands) under the accelerating voltage of 200 kV.

2.3. Fluorescence Detection of DA and a Selectivity Study

The appropriate volume of 10 mM $AgNO_3$ solution mixed with 0.15% of PMAA solution was added into a 10 mL volumetric flask that contained 30 μL of 1 mM DA solution and 5 mL of DI water. The mixture solution was then incubated at room temperature for 20 min before

adding 20 μL of 0.1 mM fluorescein dye. The mixture was then diluted to 10.00 mL with 0.1 M acetic-acetate buffer at pH 5.5. The fluorescence spectra of the mixture solution were then recorded by using an excitation wavelength (λ_{ex}) of 440 nm. For the selectivity study, the individual solution of possible interference species (1 mM), including urea, ascorbic acid, glucose, epinephrine, norepinephrine, benzylamine, melamine, 1,4-phenylenediamine, alanine, glycine, histidine, lysine, D-phenylalanine, tryptophan, tyramine, tyrosine, K^+, Na^+, and Ca^{2+}, was used instead of a DA solution and followed the same procedure.

2.4. Determination of DA in Real Samples

The feasibility of the proposed chemodosimeter applied to a real sample was demonstrated by using urine samples. Human urine samples were collected from healthy volunteers and used without storage. The urine samples were centrifuged at 5000 rpm, and the supernatants were separated. The urine supernatant (100 μL) was added to a 10 mL volumetric flask that contained 250 μL of 10 mM $AgNO_3$ solution mixed with 0.15% PMAA solution. The mixture solution was then incubated at room temperature for 20 min before adding 20 μL of 0.1 mM fluorescein dye. Before recording the fluorescence spectra, the mixture solution was diluted to 10 mL by 0.1 M acetic-acetate buffer at pH 5.5. For the commercial dopamine injection sample (for intravenous infusion), the sample was first diluted by a factor of 200 with deionized water following the same procedure, and the determination of DA in the urine samples was performed (but using 20 μL of the diluted sample instead of the urine sample). The concentrations of DA in the samples were calculated by using the standard calibration curve in buffer media. The accuracy of the proposed chemodosimeter was evaluated by spiking two different concentrations of DA (2 and 4 μM) into the samples under the optimized condition. Furthermore, the concentration of DA in the samples and spiked samples was also determined by high-performance liquid chromatography (HPLC).

3. Results and Discussion

3.1. Chemodosimeter Design

The chemodosimeter design was based on the reducing property of DA, which is strong enough to reduce Ag^+ to Ag^0. However, to avoid the formation of bulk Ag^0 and to achieve the silver nanoparticles, a template was critically needed in the reaction medium. Thus, polymethacrylic acid (PMAA) was chosen as the template or stabilizing agent to control the aggregation of the generated Ag^0 particles to become silver nanoparticles (AgNPs) [38]. The surface plasmon resonance (SPR) is a well-known optical property of the AgNPs that can reduce the incident intensity of the light source in the spectrophotometer and can be recorded as the absorption spectrum. If the SPR band of the generated AgNPs overlaps with the excitation spectrum of the appropriate fluorescent dye, then the fluorescent emission of that dye will decrease due the decreasing excitation intensity. Thus, the degree of fluorescent quenching can be directly related to the concentration of DA. From this chemodosimeter design, a highly selective chemodosimeter can be expected due to the specific reaction between DA and Ag^+ (in the presence of PMAA as a template), which can provide a specific SPR spectral region superimposed on the excitation spectrum of the fluorescein dye. In addition, a highly sensitive chemodosimeter can be expected from fluorescent measurements compared to the direct detection of its SPR signal.

3.2. Chemodosimeter Response

To study the generation of AgNPs from the reaction between DA and Ag^+ (in the presence of PMAA), the SPR spectrum of the generated AgNPs was investigated by spectrophotometry, and the results are