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Carbon dots as a fluorescent probe for label-free detection of physiological potassium level in human serum and red blood cells

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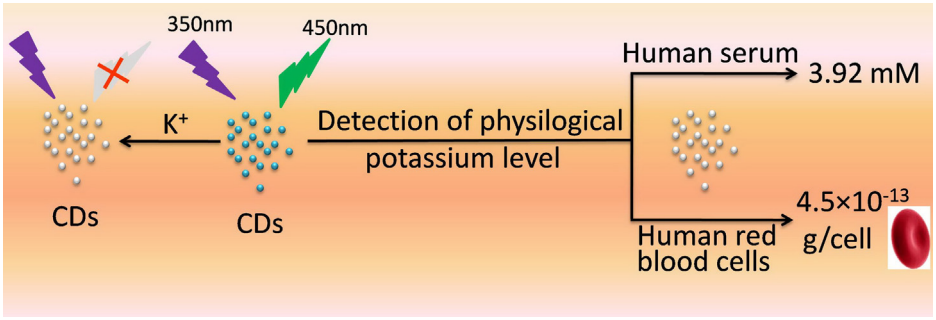
HIGHLIGHTS

- A unique photoluminescence carbon dots with larger size were prepared by microwave-assisted method.
- A highly sensitive CD-based fluorescence probe for label-free detection of K^+ was established.
- Physiological potassium levels of human serum and red blood cells may be directly and rapidly detected.

GRAPHICAL ABSTRACT



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ABSTRACT

A unique photoluminescence carbon dots (CDs) with larger size were prepared by microwave-assisted method. Complex functional groups on the surface of the CDs facilitate the nanoparticles to form affinity with some metal ions. Taking advantage of the effective fluorescence quenching effect of K^+, a highly sensitive CD-based fluorescence analytical system for label-free detection of K^+ with limit of detection (LOD) 1.0×10^{-12} M was established. The concentrations of potassium ion in biological samples such as human serum are usually found at millimolar levels or even higher. The proposed method begins with a substantial dilution of the sample to place the K^+ concentration in the dynamic range for quantification, which covers 3 orders of magnitude. This offers some advantages: the detection of K^+ only needs very small quantities of biological samples, and the dilution of samples such as serum may effectively eliminate the potential interferences that often originate from the background matrix. The determined potassium levels were satisfactory and closely comparable with the results given by the hospital, indicating that this fluorescent probe is applicable to detection of physiological potassium level with high accuracy. Compared with other relative biosensors requiring modified design, bio-molecular modification or/and sophisticated instruments, this CD-based sensor is very simple, cost-effective and easy detection, suggesting great potential applications for successively monitoring physiological potassium level and the change in biological system.

1. Introduction

Carbon-based nonmaterials have attracted much attention due to their unique properties. Owing to the robust chemical inertness, size dependent photoluminescence, high photostability against photo bleaching and blinking, low cytotoxicity and excellent

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biocompatibility, carbon dots (CDs) have been successfully utilized in bio-imaging, sensors, photocatalysis and optoelectronic devices [1–6]. As new class of fluorescent materials, CDs and graphene quantum dots (GQDs) have been extensively studied as sensing probes for monitoring many materials such as thrombin, glucose, Hg^{2+}, Cu^{2+} and Fe^{3+} [7–12]. Recently, CDs have been actively pursued and applied in biosensing [13], which greatly facilitate the sensing application of CDs and provoke further efforts to develop their bio-analytical applications.

Potassium ions play key roles in biological systems, such as nerve transmission, maintenance of muscular strength and extracellular osmolarity, enzyme activation, regulation of blood pressure [14–18]. Abnormal K^+ concentration is a symptom of several diseases, including kidney diseases, alcoholism, anorexia, bulimia, heart disease, diabetes and cancer [19]. Thus, selective and sensitive K^+ sensors are important for the detection of physiological potassium level to facilitate diagnosis and treatment. A variety of analytical methods for K^+ determination have been developed, including atomic absorption spectrophotometry [20,21], electrochemical method [22,23], spectrophotometry [24], atomic emission spectrometry [25], ion chromatography [26,27] and so on. Most of them, however, require complicated instrumentation and involve cumbersome laboratory procedures, which limit the scope of their practical application. Some of them also suffer from disadvantages such as relatively high detection limits and narrow detection ranges. Among the biologically important cations, accurate analysis of K^+ is more difficult because there are many conditions involving variations in the electrolyte content of the body fluids. One of the difficulties in monitoring K^+ under physiological conditions comes from the coexistence of Na^+. It is therefore of great significance to explore a rapid analytical assay for detection of K^+ in biological systems. Therefore, a simple and sensitive alternative without using complex instruments is highly desired Scheme 1.

In this study, we prepared unique photoluminescence CDs with larger size using facile and green one-step microwave-assisted method. The potassium ion can effectively quench the fluorescence of the unmodified CDs, and a selective CDs-based fluorescence analytical system for label-free detection of K^+ was therefore established. The unique CDs may be used as fluorescent probe to sensitively detect physiological potassium level in human serum without any pretreatment. The results were almost in agreement with those obtained by the currently used ion-selective electrode method in clinical diagnosis. Furthermore, the physiological potassium level of human red blood cells (RBC) was determined by this proposed method.

2. Experimental

2.1. Chemicals

D-Glucose, potassium chloride, aluminum sulfate, silver nitrate, zinc nitrate, calcium nitrate, ferric chloride, manganese sulfate, copper nitrate, cobalt sulfate, nickel sulfate, sodium chloride, cadmium nitrate, ammonia chloride, plumbous nitrate, magnesium chloride, mercuric chloride were purchased from Sinopharm Chmical Reagent Co., Ltd. (Shanghai, China). All of the chemicals, unless mentioned otherwise, were of analytical reagent grade and used as received. The aqueous solutions were prepared in doubly distilled water.

2.2. Synthesis of CDs

The CDs were synthesized by a microwave-assisted method as bellow: 5 g glucose was dissolved in 75 mL of doubly distilled water with sonication to form solution. Then the solution was heated in a domestic microwave for 20 min. The obtained product was centrifuged ($16{,}000 \times g$, 30 min). After that, the resultant supernate containing luminescent CDs was dialyzed with a dialysis tube (molecular weight 1000) against doubly distilled water for 24 h, and finally the product was obtained.

2.3. Procedures

2.3.1. Fluorescent sensing of K^+

3.00 mL of CDs was placed in a quartz cell of 10 mm path length. Then, a certain amount of K^+ was added to the cell at room temperature and the corresponding fluorescence spectrum was recorded after 15 min. For comparison, different metal ions such as Mg^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Na^+, Ag^+, Fe^{3+}, Cd^{2+}, Al^{3+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Hg^{2+} were added to 3.00 mL of CDs with the final concentration of 10^{-4} M, and incubated for 15 min at room temperature. The fluorescence spectra were recorded under excitation at 360 nm.

2.3.2. Detection of potassium ion in human serum and red blood cell (RBC) samples

A series of standard potassium solution (1.0×10^{-8}, 5.0×10^{-8}, 1.0×10^{-7}, 5.0×10^{-7}, 1.0×10^{-6}, 5.0×10^{-6} M) were chosen to establish calibration curve for the subsequently quantitative detection of K^+. 30 μL of different concentrations of potassium solution was added to 3 mL of CDs solution, and the corresponding fluorescent spectra were recorded.

All the serum samples were obtained from the hospital. The serum samples were stored at 4 °C until use while the whole vein blood samples (2 mL each) were separately put into heparinized containers, diluted with 5 mL of phosphate buffer saline (PBS) containing 0.9% NaCl, and centrifuged at 2500 rpm for 5 min. After washing three times with PBS, the cells were re-suspended in PBS. The size distributions of the RBCs was measured on the Sysmex XE 5000 (Sysmex Corporation, Kobe, Japan), a fully automated blood cell counter on which the RBCs were detected at the center of the aperture employing a Sheath Flow DC detection method. In the process of the experiments, the serum was first diluted 10,000 times, then, 30 μL of diluted human serum sample without any pretreatment was added in 3 mL of CDs PBS buffer solution, and the fluorescent spectra were recorded subsequently.

2.4. Experimental instrumentation

Transmission electron microscopy (TEM) was performed on a Tecnai G2 20 (USA) operating at 100 kV. For TEM sample preparation, 1–2 μL of the CDs solution was placed on a carbon coated copper grid and allowed to dry at room temperature. UV–vis absorption spectra of the CDs were measured using Lambda 35 (PerkinElmer, USA) and the fluorescence intensity spectra were recorded by a RF-5301PC (Shimadzu, Japan) fluorescence spectrometer.

3. Results and discussion

To evaluate the optical properties of CDs, the UV–vis absorption spectrum and fluorescence spectra were investigated. Fig. 1 shows the UV–vis absorption and fluorescence spectra of the water-soluble CDs. It can be seen that there are two absorbance bands centered at around 223, 282 nm in the UV–vis absorption spectrum. Similar to the previous reports [28,29], the peak at 223 nm should be ascribed to the π–π^* transitions of the aromatic C=C sp^2 domains which cannot produce observed fluorescence signal. However, another absorption peak centered at 282 nm arises from the trapping of excited state energy of the surface states, which results in strong fluorescence [30]. When excited at 350 nm, the CDs exhibited strong PL emission centered at 450 nm.