Sensors & Actuators: B. Chemical 271 (2018) 358–366

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A smartphone-based ratiometric resonance light scattering device for field analysis of Pb^{2+} in river water samples and immunoassay of alpha fetoprotein using PbS nanoparticles as signal tag

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ARTICLE INFO

Keywords:
Smartphone-based
Ratiometric
Resonance light scattering
Field analysis
Immunoassay

ABSTRACT

Herein, we developed a smartphone-based ratiometric resonance light scattering (RLS) device for portable field analysis and immunoassay. The RLS signal was detected by the camera in a smartphone controlled by a user-edited APP software. Except the smartphone, the total cost of the detection system itself is about 60 $, including a laser pointer and battery, a twin cuvette, a collective lens, and outside box. By using the RLS brightness ratio between the sample and reference channels in the twin cuvette as the signal indicator, the baseline drifts due to the variations in incident light intensity, ambient temperature, and shooting parameters of camera, can be eliminated efficiently. A new brightness indictor, $(R - R_0)/(R_{max} - R)$, was proposed to linearize the calibration curve. Where R and R_0 are the rightness ratio in sample and blank, R_{max} is the maximum averaged brightness ratio, respectively. The applicability of the portable RLS device was demonstrated by the field analysis for Pb^{2+} in environmental water samples, using $[CTA]_2PbI_4$] (CAT = cetyltrimethyl ammonium) as the luminophore. The limit of detection for Pb^{2+} is 0.7 μg L^{-1}. By using PbS nanoparticles conjugated second antibody as the signal tag, the RLS method was applied in immunoassay to determine alpha fetoprotein with the limit of detection of 0.1 pg mL^{-1}. This smartphone-based ratiometric RLS device offers the advantages of good portability, low cost, high sensitivity and reliability.

1. Introduction

Resonance light scattering (RLS), as a kind of elastic scattering, is produced when the wavelength of incident beam is close to the molecular absorption peak of particles. The RLS technique has been attracted considerable attention in analysis because of its remarkable advantages, such as rapidness, high sensitivity, and convenience in performance [1]. Since the first application in 1993 reported by Pasternack [2], RLS based assays have been applied to detect metal ions [3,4], hydrogen sulfide [5], melamine [6], kanamycin [7], L-cysteine [8], microRNA [9], single nucleotide polymorphism [10], hepatitis A [11], thrombin [12], and so on, showing good practicability in environmental and biological analysis. Usually, the RLS signal is measured by a laboratory spectrofluorimeter. The relative expensive and bulky equipment limits the practicability of RLS in the area of portable field analysis and point-of-care (POC) chemical and biosensors.

With the continuous developments in the electronics and information technologies, smartphones are equipped with numerous components that can be employed for various measurements and detections. Recently, considerable efforts are been directing toward the utilizations of smartphone as the sensing devices in various analytical methods [13–15]. Typical examples include optical detectors [16–18], and data acquisition and interface [19–21]. In addition, the smartphone-based devices have successfully demonstrated the feasibility in portable field analysis [22–24] and immunoassay [25–29]. Compared to traditional laboratory analytical instruments, the smartphone-based devices are much more portable and cheaper, and have an inherent capacity in data storaging, processing and sharing. But to our best knowledge, a smartphone-based ratiometric RLS device has not been reported.

Herein, we reported a smartphone-based ratiometric RLS device for portable field analysis and immunoassay. By using the brightness ratio (R) between the RLS images of the sample and reference channels in a twin cuvette as signal, the influence from the variation in the intensity of the incident light, ambient temperature, and the exposure time and

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https://doi.org/10.1016/j.snb.2018.05.103
Received 23 September 2017; Received in revised form 5 May 2018; Accepted 18 May 2018
Available online 23 May 2018
0925-4005/

photosensitivity (ISO) of the camera on RLS detection is eliminated effectively. The portable smartphone-based RLS device was applied in field analysis for Pb^{2+} in environmental water samples. Lead is one of the major environmental pollutants, accompanying by severe human health risks including muscle paralysis, memory loss, anemia, cardiovascular dysfunction and mental troubles [30,31]. The World Health Organization establishes the recommended limit in drinking water is 10 μg L^{-1} for lead [32]. In the RLS method for Pb^{2+} detection, the ion-association of $[CTA]_2[PbI_4]$ (CTA = cetyltrimethyl ammonium) was utilized as the luminophore. In the immunoanalysis, PbS nanoparticles (PbS NPs) conjugated second antibody was used as the signal tag. The concentration of target antigen was quantified using the RLS signal from $[CTA]_2[PbI_4]$, which is dependent to the amount of PbS tag in the sandwich-type immunocomplex. Alpha-fetoprotein (AFP) is chosen as the model antigen, because it is a widely used tumor marker for the diagnosis of patients with germ cell tumors and hepatocellular carcinoma [33]. The smartphone-based ratiometric RLS device was demonstrated the practicability in field analysis and POC chemical and biosensors.

2. Experimental

2.1. Reagents and instruments

All other reagents and solvents were of analytical grade and used directly. Cetyltrimethyl ammoniun bromide (CTAB), potassium iodide, ascorbic acid (AA), lead nitrate, bovine serum albumin (BSA), *N*-(3-dimethylamino-propyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), glutaraldehyde (GA), and *N*-hydroxysuccinimide (NHS) were purchased from Sigma–Aldrich (Shanghai, China). Alpha fetoprotein ELISA kits were purchased from Biocell Biotechnology Co. Ltd. (Zhengzhou, China). Ultrapure water was obtained through ion-exchanger and quartz distiller. Prior to use, all solutions were filtered through 0.22 μm nylon syringe filters.

The schematic illustration of the smartphone-based ratiometric RLS device is showed in Fig. 1. A laser pointer (450 nm, 1 mW) was used as the incident light. A portable source was employed in the situation of field analysis. The light beam was perpendicular to the window of the cuvette. A glass cuvette and its frame were mounted in a black box with the dimensions of 120 × 80 × 60 mm. In the ratiometric RLS measurement, the twin cuvette with sample and reference channels was used. A smartphone (Honor 7, China) was employed to capture the RLS image by its built-in camera. The camera was about 10 mm apart from the outside wall of the cuvette. A macro lens was mounted in the light path to get a clearer RLS image. To maintain a constant focus for the RLS measurements, the auto-setting function of the camera was turned off. The shooting parameters of camera for capture images were set manually by the user-edited APP software. The brightness ratio, $R = \overline{B}_1/\overline{B}_0$, was calculated by the user-edited APP software. Where $\overline{B}_0$ and $\overline{B}_1$ are the averaged RLS image brightness (in blue component) in reference and sample channels, respectively. The user-edited APP software can be applied to the smartphones in Android system. The reliability of this APP software was confirmed in comparison with the brightness and brightness ratio data of the same images calculated by using the software of MATLAB 14.0 in PC. The RLS spectra were obtained by simultaneously scanning the excitation and emission monochromators ($\lambda_{ex} = \lambda_{em}$) in an F-4500 spectrofluorometer (Hitachi, Japan) with a 10 mm length normal fluorescent cuvette.

Fig. 1. Schematic illustration of the double-channel smartphone-based RLS detection device for portable analysis. (1) smartphone, (2) macro lens, (3) laser pointer, (4) twin cuvette and frame; (5) black box.

2.2. Field analysis of Pb^{2+} in real water samples

The field analysis of Pb^{2+} in water samples was performed at three locations in Xiaoqing River (Jinan, China). The position and surroundings of the sampling sites as well as the ambient temperature can be recorded by the smartphone. Prior to the detection, the water sample was filtered through a 0.22 μm nylon syringe filters. Then 2.50 mL of the filtered water sample was added to 5 mL plastic centrifuge tube, mixed sequentially with 1.00 mL formic acid-sodium hydroxide buffer (pH 3.8), 500 μL 20 mM AA, 500 μL 0.2 M KI and 500 μL 50 μM CTAB. The references were prepared by the same procedure by using Pb^{2+} standard solution. Finally, the RLS brightness ratio was measured by the portable device at the field. To reach the better ratiometric efficiency, a balanced calibration method was employed. First, the concentration of Pb^{2+} in the sample was estimated according to the RLS brightness ratio using the stored calibration curve and used as the initial value (C_{x0}). Then three references with finial Pb^{2+} concentrations of $C_1 \approx 0.9C_{x0}$, $C_2 \approx C_{x0}$ and $C_3 \approx 1.1C_{x0}$ were prepared. The brightness of the RLS image in the reference was adjusted in the range of 150 ± 20 by the photosensitivity (ISO) of the camera. According to the brightness ratios in the three references, (C_1, $\overline{R}_1$), (C_2, $\overline{R}_2$) and (C_3, $\overline{R}_3$), the concentration of Pb^{2+} in water sample was calculated, which is corresponded to the point of (C_x, $\overline{R}_2 = 1$) by using a regressed algorithm.

2.3. Synthesis of PbS NPs and Ab_2–PbS conjugates

The PbS NPs were synthesized via an in situ source-template-interface reaction reported in ref. [34] with slight modification. Briefly, 3 mmol ethylenediamine was dissolved in 20 mL ultrapure water, 0.234 g CS_2, and 100 μL thioglycolic acid were added. Then 10 mL 1 mM $PbNO_3$ were added dropwise in 20 min under stir. The mixture was heated at 50 °C for 30 min to decompose the residual CS_2. The resulting precipitates were collected by centrifugation and washed with ethanol and ultrapure water several times. The product was re-dispersed in ultrapure water again and stored at 4 °C for the further use. As shown in Fig.S1 in Electronic Supporting Information (ESI), the resultant PbS NPs have the diameter in the range of 25–35 nm.

The Ab_2–PbS conjugates were prepared according to the method in ref. [35] with slight modification. Firstly, 100 μL of 20 mg mL^{-1} newly prepared EDC and NHS solutions were mildly mingled with 1 mL of PbS NPs suspension (0.2 mg mL^{-1}) for 30 min at room temperature. The supernatant was removed by centrifugation. Then 1 mL of Ab_2 solution (200 μg mL^{-1}) was added and incubated for 12 h under shaking at 4 °C. After washing with phosphate buffer solution (PBS, pH 7.4, 0.081 M Na_2HPO_4 + 0.019 M NaH_2PO_4 + 0.1 M NaCl) several times, the resultant Ab_2-PbS NPs conjugates were acquired by centrifugation, redispersed to 1 mL PBS and stored at 4 °C for the further use.

2.4. Immunoassay procedure

Scheme 1 illustrates the immunoassay protocol used in this work. Firstly, the aminated surface in microtiter plate was activated with 2.5% GA (in pH 7.4 phosphate buffer) for 2 h and washed with ultrapure water. Then 50 μL of the primary antibody (Ab_1) solutions of anti-