

Design of a Resonance Rayleigh Scattering Technique using PbS QDs-Gutathione Nanocomposite for Epinephrine drug Detection in Real samples

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Abstract

In this study, a description of spectrofluorometer method for the measure of epinephrine (EP) drug in urine and blood samples using lead sulfide (PbS) quantum dots via glutathione nanocomposite as a sensor using a resonance Rayleigh scattering (RRS) technique. The sensor was characterized using FTIR, XRD, SEM, and TEM. The scattering intensity (ΔI_{RRS}) signal was detected by a fluorescence detector at $\lambda_{(ex)} = 325$ nm. Under the optimum conditions, the calibration plot is linear in the (EP) drug concentration range of (0.050–200.0 ng L⁻¹). The standard deviations of (1.2 %), detection limit (LOD) of the method (0.015 ng L⁻¹) and quantification (LOQ) of the method (0.044 ng L⁻¹) in time 50 s, 325 nm for sensor level response PbS QDs-glutathione nanocomposite with (96 %) confidence evaluated. Moreover, a PbS QDs-glutathione nanocomposite sensor with RRS technique for the analysis of (EP) drug in urine and blood samples with high % recoveries (94.0 - 99.2 %), low % RSD values (< 3 %) was used. This method offers a reliable approach for detecting various drugs in clinical and pharmaceutical settings.

Keywords

Epinephrine drug, Glutathione, PbS quantum dots, Sensor, Resonance Rayleigh Scattering.

1. INTRODUCTION

In recent years, significant impact of the global depletion of resources has driven the transition for researchers to focus on more sustainable approaches in analytical chemistry [1, 2]. Epinephrine (adrenaline) flips the body into alert mode: it speeds up the heartbeat, boosts blood pressure, enlarges the pupils, opens the airways, and constricts blood vessels. It also helps drive alertness and focus, supports learning and memory, and helps set the sleep-wake rhythm [3, 4]. Rapid absorption and prolonged action allow one-time dosing. In nerves and body fluids it exists as a large organic cation. It has been misused in sport to speed liver glycogen breakdown and release fatty acids, increasing energy [5-7]. Therefore, developed an accurate, sensitive and rapid method for quantitative determination of

epinephrine in human fluid, and other samples with different matrices is necessary.

Recently, increasing attention has been given to spectrophotometric and fluorometric methods based on microextraction and ionic liquid techniques for the selective determination of target species (inorganic, organic, and biomolecular) in complex matrices. Accordingly, the development of simple, selective, and delicate method for determining drug samples and biomolecules was highlighted [8-10]. In this methods detection species was done with high sensitivity and excellent selectivity for the discerning and accurate of samples.

Hence, the analytically for detecting of EP concentration in various samples well as biological samples, and pharmacological research is still required. Several instrumental

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techniques have been reported for (EP) drug analysis, including liquid chromatography [11], molecularly imprinted polymer [12] voltammetry [13] and spectrophotometry [14, 15]. fluorometrically techniques is one of the most widely-used techniques for determining drugs has been reported in real samples [16, 17]. Accordingly, developing a simple, selective, and delicate method like quantum dots (QDs), and RRS technique, measurement in quantitative detection of drugs and bio-imaging was highlighted [18-21].

Quantum dots (QDs) are fascinating nanomaterials, prized for their unique properties. They exhibit wavelength-dependent emission, meaning there can change based on the light they absorb. Beyond their vibrant optical qualities, QDs are highly desirable due to their excellent water solubility, low toxicity, and superior biocompatibility. These attributes position QDs as a versatile and environmentally friendly semiconductor option, and those extremely potential to be used in the domain of sensing and imaging especially useful for precise detection of drugs in samples, primarily because of their strong fluorescent and resonance Rayleigh scattering (RRS) technique [22, 23].

Typical points are made of binary compounds II-VI, and III-V groups. However, semiconductor elements from others can be used for QDs fabrication. PbS is a highly interesting material to produce such QDs. PbS is often prepared in the form of QDs by easy, and diverse methods, from chemical routes to electro deposition [24-29]. The application of PbS QDs, widespread use comes with serious health risks. Long-term exposure can cause major problems impotence in men, miscarriage in pregnant women, and damage to multiple body systems including the brain, heart, blood, and reproductive organs. Unlike uncoated PbS QDs, the used PbS QDs-glutathione nanocomposite show minimal toxicity, making them promising candidates for use in sensors and biomedical applications [30].

Glutathione has no toxicity and is hence considered biodegradable. Besides the thiol group, glutathione possesses the surface amino and carboxyl functional groups that provide the using of glutathione as one nanoparticle [27, 28]. Due to these properties, PbS QDs-glutathione nanocomposite gained special attention in diagnostic resonance Rayleigh scattering technique and other spectrophotometric, and spectrofluorimetric methods [23, 31].

Novelty this work, development and validation of a RRS technique for the detection of (EP)

drug in human fluid, and other samples, Because, PbS QDs-glutathione nanocomposite have exciting features, such as fluorescence emission intensity (F.L), and resonance Rayleigh scattering (RRS). This approach integrates both RRS technique and spectrofluorimetric methods, capitalizing on the unique properties of PbS QDs-glutathione nanocomposite. These quantum dots offer strong fluorescence emission (F.L) and enhanced RRS intensity, making them highly effective for sensitive and selective drug detection. Their dual optical features provide a powerful platform for analytical applications in complex biological matrices.

The work presented here involved the design of the resonance Rayleigh scattering technique to determine (EP) drug using PbS QDs-glutathione nanocomposite. Moreover, to determine (EP) drug content, various influential factors, such as (pH, time, etc.), were considered. The performance of PbS QDs-glutathione nanocomposite to detect (EP) drug was investigated through a series of tests.

2. EXPERIMENTAL

This in present study was conducted: 1- Synthesis of the PbS QDs-glutathione nanocomposite. 2- Determination of the structural properties of the sensor. 3- Investigation of the sensor performance for optimization of parameters and diagnosis of (EP) drug in real samples.

2.1. Materials and methods

All chemicals of lead nitrate $\text{Pb}(\text{NO}_3)_2$ (99 %), thiourea $\text{SC}(\text{NH}_2)_2$ (99.0 %), Glutathione (99 %) were bought from Sigma-Aldrich Company (USA). Epinephrine drug (98%, from Sobhan Daru Company Iran). A spectrofluorometer (Agilent Technologies, USA) was employed to evaluate the scattering intensity (ΔI_{RRS}) excitation and emission signals. FTIR (Fourier-transform infrared spectroscopy) was conducted using an AVATAR, Thermo (USA). The X-ray diffraction patterns were taken via an X-ray diffractometer (Bruker AXS- D8 Advance), and Transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan) was performed at an accelerating voltage of 200 keV to characterize the morphology of the samples. Complementary morphological analysis was conducted using (SEM, JEOL JSM 6610LV, Japan) operated at 15 kV. Regulated by an automated pH controller (Metrohm 902 Titrando) equipped with a precision glass electrode. The chemical structure of glutathione is shown in (Fig. 1).

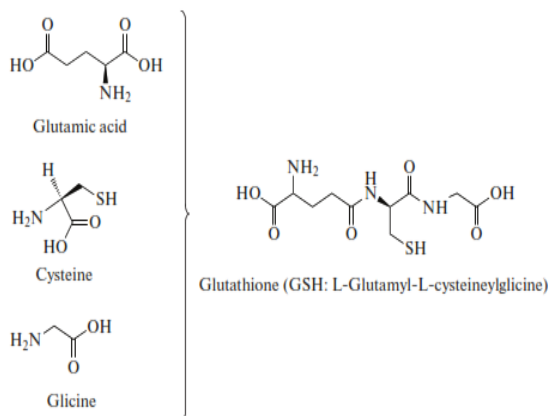


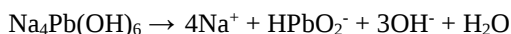
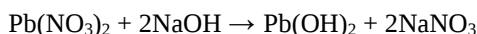
Fig. 1. The chemical structure of glutathione.

2.2. Urine and blood sample preparation

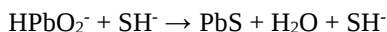
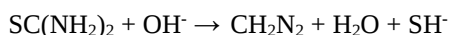
Human plasma samples were obtained from the blood transfusion organization in Omidyeh city and stored frozen at -5°C . Before use, the samples were allowed to reach room temperature and then analyzed using the described method after a ten-fold dilution. Urine samples, collected from volunteers, were stored at 3°C . Prior to analysis, these samples were also brought to room temperature and examined using the presented method after a three-fold dilution, without any purification or filtration steps [15, 24].

2.3. Synthesis of PbS QDs-glutathione nanocomposite

The synthesis of PbS QDs-glutathione nanocomposite was executed at ambient temperature by the precipitation method. First, PbS nanoparticles were synthesized by reacting 0.1 M lead nitrate with 0.45 M thiourea, and added sodium hydroxide 0.1 M in 20 mL in double distilled water [29, 33].



In the alkaline medium, the thiourea decomposes and releases S^{2-} ions, and leading to the formation of PbS nanoparticles [29].



The glutathione powders were used as a base medium and 20 mL of the above solutions were prepared separately. Separately, 20 mL of each solution were prepared; 10 mL portions were then combined and magnetically stirred to ensure

homogeneity. After ~ 25 min the mixture turned gray evidence of PbS formation and stirring continued for 2 h. The resulting PbS QD-glutathione nanocomposite was collected and oven-dried at 60°C as the process shown in (Fig. 2).

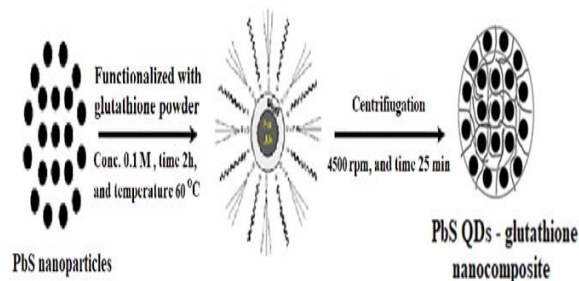


Fig. 2. Synthesis of PbS QDs-glutathione nanocomposite using lead sulfide nanoparticles and glutathione powder.

2.4. Mechanism of scattering intensity (ΔI_{RRS}) technique complex between (EP) drug and PbS QDs-glutathione nanocomposite

The PbS QD-glutathione nanocomposite shows high sensitivity toward EP. Owing to its large specific surface area, it can reversibly capture $\sim 96\%$ of EP from samples. The combination of extensive surface area and efficient electron transfer yields a strong RRS response. The proposed mechanism is illustrated in (Fig. 3).

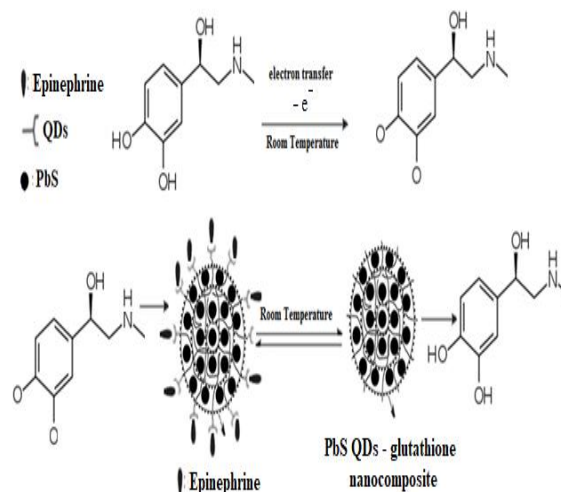


Fig. 3. Mechanism of excitation scattering intensity (ΔI_{RRS}) technique complex (EP) drug and PbS QDs-glutathione nanocomposite).

2.5. Schematic of the excitation RRS technique for (EP) drug detection using PbS QDs-glutathione nanocomposite.

Figure 4, shows the aggregation of (EP) drug occurs when PbS QDs-glutathione nanocomposite reacts with the (EP) drug. The reaction ((EP) drug and PbS QDs-glutathione nanocomposite) complex excitation spectra at a wavelength of 325 nm [32, 33]. The sensitivity of the PbS QDs functionalized glutathione, and RRS intensity was significantly enhanced owing to the high absorbing efficiency of glutathione to (EP) drug. The detection sensitivity can be significantly improved to ng L^{-1} level by monitoring of signal changes of high sensitivity RRS by PbS QDs-glutathione nanocomposite. As shown in (Fig. 4).

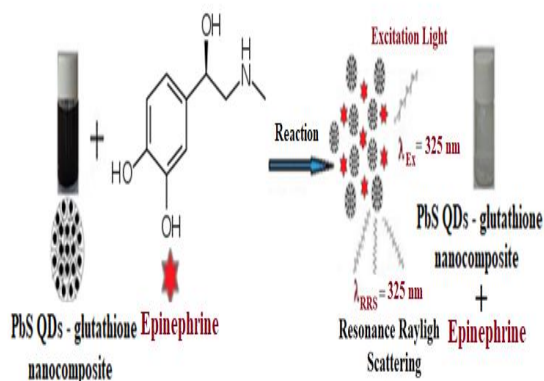


Fig. 4. Schematic of the excitation scattering intensity (ΔI_{RRS}) spectra between (EP) drug and PbS QDs-glutathione nanocomposite complex.

2.6. General procedure

In this procedure: first, a 10 mL volumetric flask was picked and 1 mL of (EP) drug (100.0 ng L^{-1}) was added and after 1 mL PbS QDs-glutathione nanocomposite (50.0 ng L^{-1}) into the volumetric flask in double distilled water contain. It can be seen from the figure that the RRS signal of (EP) drug and PbS QDs-glutathione nanocomposite, has been excitation/emission spectrum of sensor, the over-lapping between the scattering intensity (ΔI_{RRS}) complex ((EP) drug and PbS QDs-glutathione nanocomposite) excitation spectra at a wavelength of 325 nm [25, 34, 35]. All reaction steps were repeated by increasing the concentration (100.0 ng L^{-1}) of (EP) drug to the solution, every (50 s). In the resonance Rayleigh scattering of (EP) drug (ΔI_{RRS}) and ultimately (ΔI) $I_0 \text{ blank} - I_{\text{RRS sample}}$. There was a sharp change in the scattering intensity (ΔI_{RRS}) of the sensor in the 325 nm region at continuous increase of (EP) drug each 50 s in the solution. This indicates that complex formation resulted in the quenching of scattering intensity (ΔI_{RRS}) complex ((EP) drug and PbS QDs-glutathione nanocomposite) at the wavelength of excitation of 325 nm, as shown in (Fig. 5).

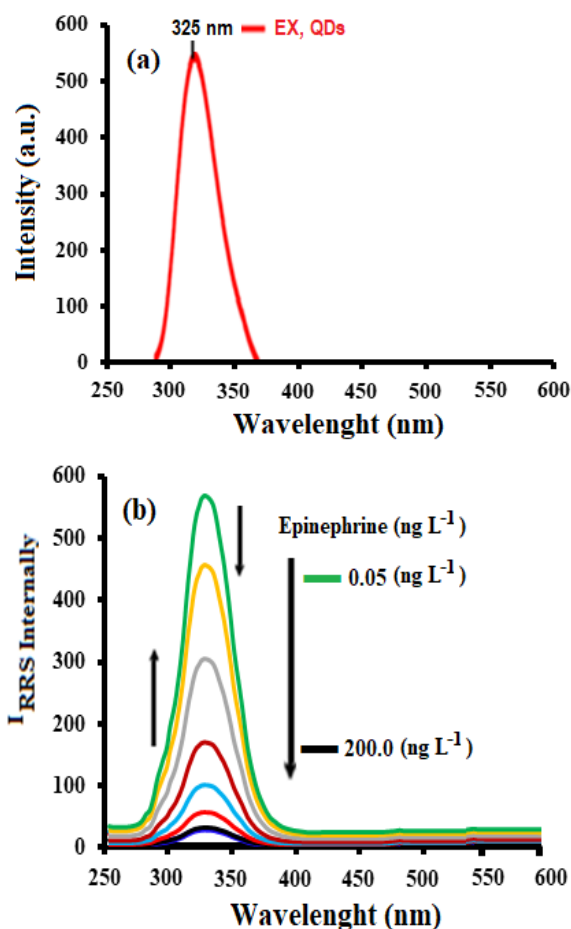


Fig. 5. (a) The scattering intensity excitation spectra of between PbS QDs-glutathione nanocomposite and (EP) drug (b) The scattering intensity (ΔI_{RRS}) technique for measurement of (EP) drug with PbS QDs-glutathione nanocomposite (pH 6.0, time 50 s in 325 nm).

3. RESULTS AND DISCUSSION

3.1. Characterization

FT-IR shows a broad O-H stretching band spanning $3350\text{--}3480 \text{ cm}^{-1}$ (max at 3442 cm^{-1}). A band at 632 cm^{-1} is assigned to the Pb-S stretch, consistent with interaction between PbS QDs and the glutathione capping thiol. The S-H stretch of neat glutathione ($\sim 2520 \text{ cm}^{-1}$) disappears in the composite, indicating thiolate binding to the QD surface. An additional band at 1444 cm^{-1} , attributed to carboxylate (COO^-) vibrations, evidences carboxyl groups on the PbS QD-glutathione nanocomposite are shown in Figure 6a [23, 30]. Different X-ray diffraction (XRD) peaks of the PbS QD-glutathione nanocomposite are shown in Figure 6b. Signals at 38.07° (111), (420), (331), (400); 44.26° (222), (311); 64.43° (220), (200); and 77.35° (422) were observed in the XRD pattern of the sensor and are attributed to Pb-S [30, 31]. The scanning electron microscopy (SEM) image of the PbS QDs-Glutathione nanocomposite

(Fig. 6c, and d) reveals a highly interconnected surface morphology, characteristic of successful structural modification. TEM images (Fig. 6e) of

the PbS QDs-glutathione nanocomposite structure, visible as black spots.

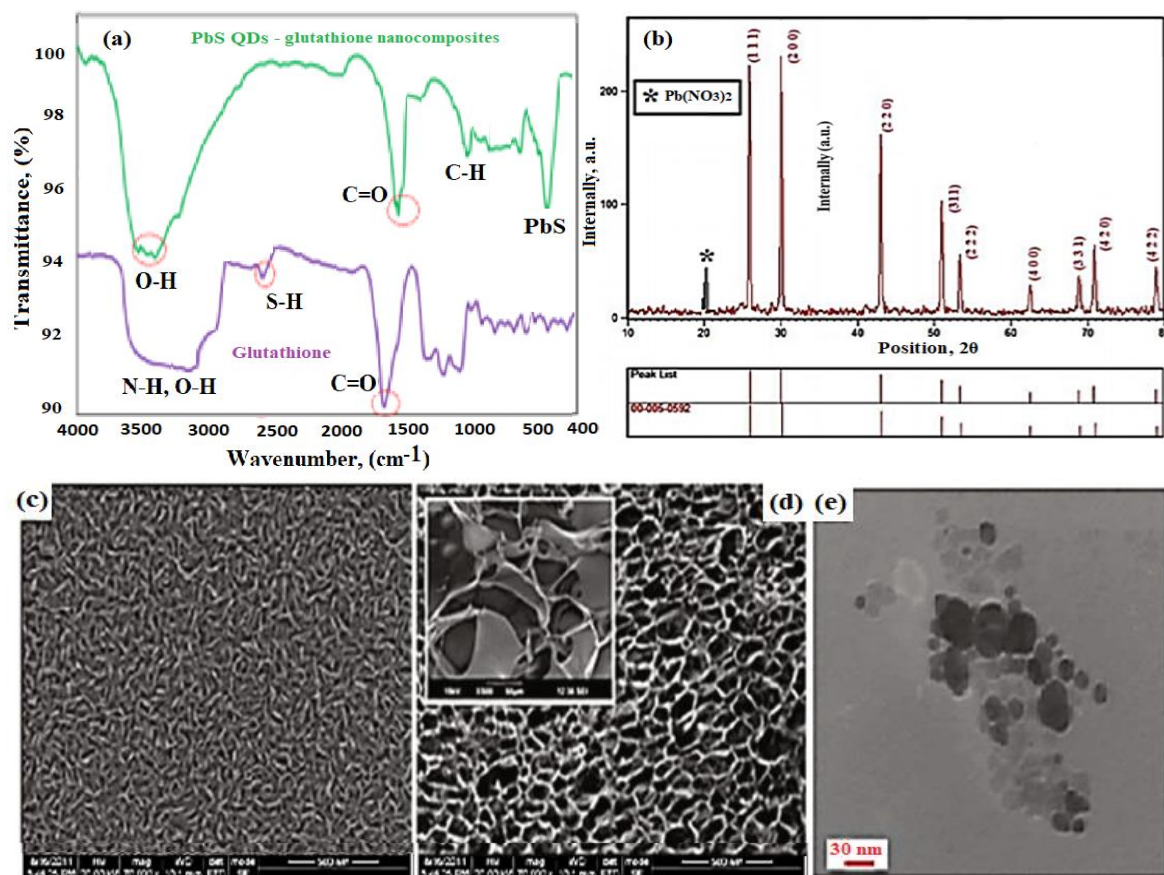


Fig. 6. (a) FT-IR spectrum (b) XRD patterns (c, d) SEM micrograph (e) TEM micrograph of surface PbS QDs-glutathione nanocomposite.

3.2. Optimization of Sensing Conditions

To obtain a highly sensitive response for the detection of (EP) drug with RRS technique, the optimization of various influential factors, such as pH, PbS QDs-glutathione nanocomposite, and reaction time.

3.2.1. Impact of pH

After measuring the scattering intensity (ΔI_{RRS}) of the solution, a thorough investigation was carried out on the influence of pH value in the range of 2.0–7.0 for the (EP) drug – PbS QDs-glutathione nanocomposite complex at 325nm. The response maximized at pH 6.0, consistent with improved nanocomposite stability and, in turn, maximal (EP) drug detection. This pH could be helpful for the stability of PbS QDs-glutathione nanocomposite, and provision of maximum detection for (EP) drug, as shown in (Fig. 7a) [20, 36].

3.2.2. Impact of PbS QDs-glutathione nanocomposite

We assessed how the PbS QD-glutathione nanocomposite influences the stability of the

resonance Rayleigh scattering signal. As shown in Fig. 7b, ΔI_{RRS} was recorded across 5–100 ng L⁻¹ (nanocomposite). The response rose to a stable maximum at 50 ng L⁻¹ after all reagents were added, so 50 ng L⁻¹ was selected as the working concentration for subsequent experiments [31, 32].

3.2.3. Impact of addition and standing time

Fig. 7c, showed the impact time of the scattering intensity (ΔI_{RRS}) spectra for detecting (EP) drugs. Under the optimum condition, the effect of standing time on the stability of RRS intensity was studied. The results showed that the scattering intensity (ΔI_{RRS}) reached a maximum at 50 s after all reagents were added, and it remained stable for over 90 s without any significant change. Therefore, this system exhibits good stability and a standing time of 50 s was selected for further works [23, 34].

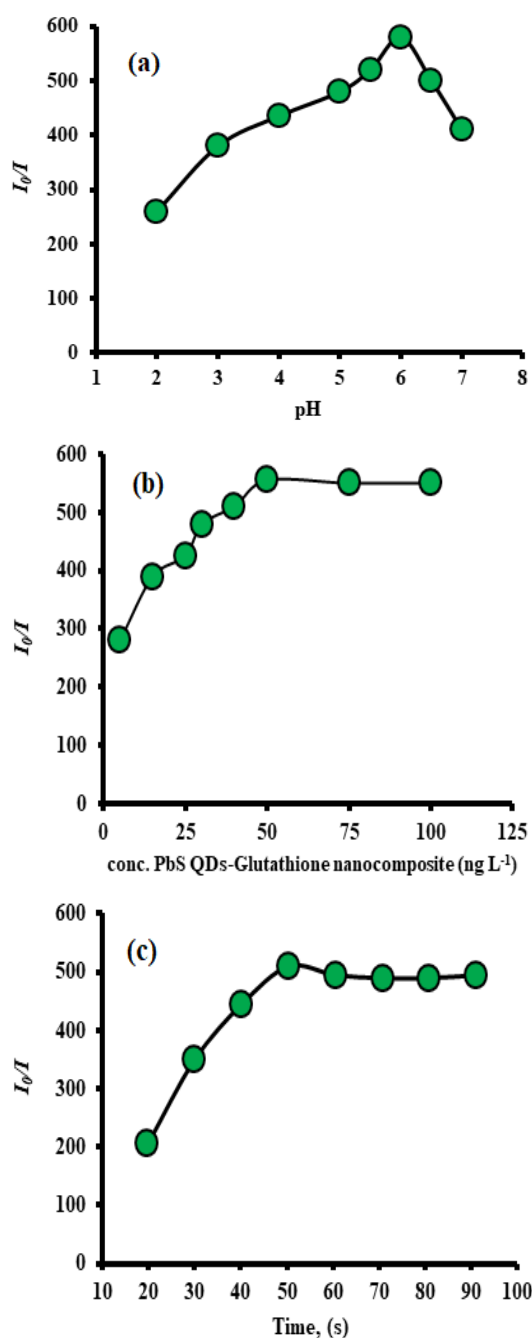


Fig. 7. (a) Impact of pH (b) Impact of PbS QDs-glutathione nanocomposite (c) Impact of time on the RRS technique (EP) drug = 100.0 ng L^{-1} in phosphate buffer solution).

3.3. Analytical application

The analytical performance of PbS QDs-glutathione nanocomposite for the determination of (EP) drug was examined. First, the calibration curve was constructed for (EP) drug concentrations from 0.050 to 200.0 ng L^{-1} using the RRS technique by spectrofluorometer method. The results are shown in Figure 8 [21, 32]. Furthermore, the correlation coefficient of 0.998 was observed for

the concentration range of 0.050 to 200.0 ng L^{-1} . The detection limit LOD is as low as 0.015 ng L^{-1} , according to $3S_b/m$, where S_b denotes the blank signal standard deviation, and m is the slope of the linear relationship, respectively. Also, for ten measurements (100.0 ng L^{-1}) of (EP) drug solution with optimized conditions, good repeatability with relative standard deviation ($\pm 1.2\%$), has been obtained. The optimum values of parameters are listed in Table. 1 [20, 34].

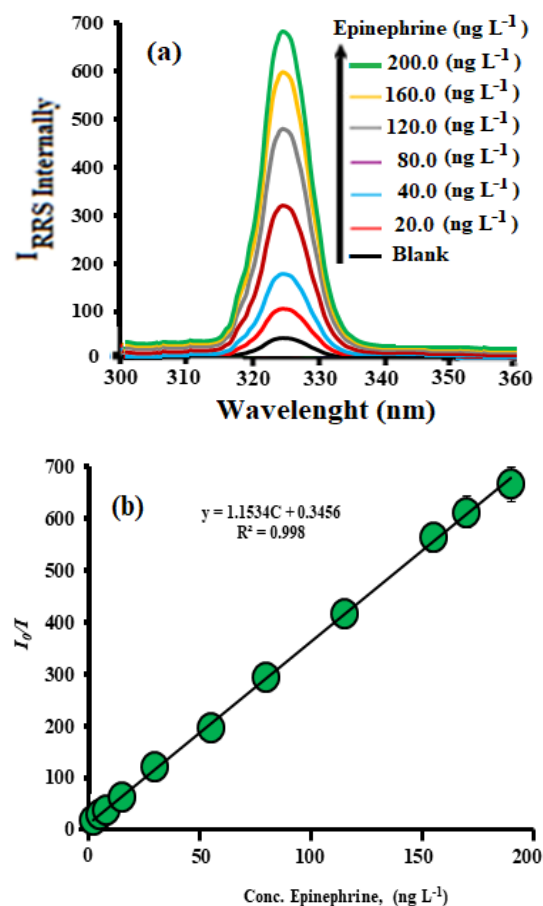


Fig. 8. (a) Scattering intensity (ΔI_{RRS}) spectra for measurement of (EP) drug with PbS QDs-glutathione nanocomposite (b) Calibration curve of (EP) drug (pH 6.0, time 50 s in 325 nm).

3.4. Interference study

The study was performed in the presence interfering species concentration that caused the change in the analyte signal to be more than 5% of the initial value, in a solutions contained of 100.0 ng L^{-1} (EP) drug in pH 6.0, 50 ng L^{-1} concentration PbS QDs-glutathione nanocomposite, time 50 s in 325 nm. The results are shown in Table. 2 [24, 35].

Table 1. Analytical figures of merit for RRS technique of (EP) drug detection.

Parameters	Optimum Value for (EP) drug
PbS QDs-glutathione nanocomposite	(50.0 ng L ⁻¹)
pH	6.0
λ_{ex}	325 nm
Time	(50 s)
Linear range	(0.050 – 200.0 ng L ⁻¹)
Limit of detection (LOD)	(0.015 ng L ⁻¹)
Limit of quantification (LOQ)	(0.044 ng L ⁻¹)
Relative standard deviations	($\pm$ 1.2 %)

Table 2. Impacts of the other analytes concentration on the determination of (EP) drug (n=3).

Interfering species	Tolerance limit (ng L ⁻¹)
Metronidazole, Sulfacetamide, Naproxen	500
Zn ²⁺ , Cd ²⁺ , Ag ⁺ , Fe ²⁺ , Al ³⁺	250
Na ⁺ , K ⁺ , Ca ²⁺	500
Cl ⁻ , I ⁻ , NO ₃ ⁻ , SO ₄ ²⁻	500

3.5. Application of the sensor prepared in the real sample

In order to evaluate the performance of the proposed novel technique for determining (EP) drug in complex solutions, this sensor was tested to measure (EP) drug level in urine and human serum samples according to the agenda mentioned in the (EP) drug measurement section. The procedure was developed to determine (EP) drug in in urine and human serum samples with 3 replicates were spiked at Δ levels (0.0, 25.0, 50.0, 75.0, and 100.0 ng L⁻¹). To evaluate the accuracy of this work, the results obtained by the proposed method were compared with HPLC method (Table. ۳). The recovery percentages obtained (94.0 to 99.2 %) indicate that the prepared sensor has excellent performance for determining (EP) drug in complex solution samples [15, 23, 36]. By comparing the results of (EP) drug measurements with the proposed method and evaluating them with t-Test, it

was found that there is no significant difference, all t_{exp} values were below t_{crit} ($t = 2.78$), the result is shown in (Table. 3). So, these results characterized the applying PbS QDs-glutathione nanocomposite sensor appropriate for the determination of (EP) drug in urine and human serum samples [37, 38].

3.6. Comparison of different methods for detection of (EP) drug

A comparison of the proposed method with the other previously reported methods demonstrates the feasibility of the RRS technique and its reliability for the analysis of (EP) drugs. Obviously, measurement of the (EP) drug by PbS QDs-glutathione nanocomposite sensor, shows the best limit of detection (LOQ), and standard deviation (RSD) for the detect of (EP) drug in comparison with other methods as shown in (Table. 4).

Table 3. Detection of trace (EP) drug from urine and blood samples after employing presented procedure (n=3).

Samples	Added (ng L ⁻¹)	Founded by RRS technique (ng L ⁻¹)	HPLC method (ng L ⁻¹)	t-Test	Recovery %
Blood samples	0.0	0.22	0.23	1.37	----
	25.0	24.55 $\pm$ 1.8			97.4
	50.0	48.96 $\pm$ 1.7			97.6
	75.0	73.68 $\pm$ 1.5			98.2
	100.0	98.5 $\pm$ 1.6			99.2
Urine samples	0.0	0.19	0.21	1.75	----
	25.0	24.4 $\pm$ 1.9			96.8
	50.0	47.2 $\pm$ 1.7			94.0
	75.0	71.9 $\pm$ 1.4			95.6
	100.0	97.1 $\pm$ 1.6			97.0

Mean value $\pm$ standard deviation (n = 3).

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Table 4. Comparisons of determination (EP) drug by RRS technique with other technique and methods.

Sensing platform / Method	LOQ (ng L ⁻¹)	(LDR) (ng L ⁻¹)	(RSD) (%)	Ref.
Carbon paste electrode (CPE)	0.023	0.025-23.0	1.1	[4]
Liquid chromatography (HPLC)	30.0	30-100.0	1.1	[11]
Molecularly imprinted polymer chemiluminescence (CL)	0.3	050-10.0	2.0	[12]
Voltammetry (DPV)	0.16	0.20-80.0	1.3	[13]
Spectrophotometric (UV-Vis)	8.0	20.0-200.0	2.17	[14]
Spectrophotometric (UV-Vis)	2.3	10.0-100.0	3.7	[15]
Fluorescence (F.L)	0.14-2.10	0.20-120.0	1.2	[17]
Resonance Rayleigh Scattering (RRS)	0.044	0.050-200.0	1.2	Present study

LOQ: quantification, RSD: standard deviation.

4. CONCLUSION

In this work, describes a RRS technique with spectrofluorometer method for measuring (EP) drug in real samples using PbS QDs-glutathione nanocomposite sensor. Exceptionally sensitive response in detecting (EP) drug with this sensor and time 50 s in width of the excitation scattering intensity (ΔI_{RRS}) spectra in the range (0.050 to 200.0 ng L⁻¹). The standard deviation method (1.2 %) and quantification (LOQ) of the method (0.044 ng L⁻¹) in time 50 s, 325 nm evaluated. Detection limit (LOD) values demonstrate the efficacy of the methods for the direct quantification of (EP) drug in human fluid and hospital samples, achieving high precision with a mean recovery of 96.8 % (EP) drug. Thus, PbS QDs-glutathione nanocomposite can be used as a facile, rapid and selective probe for the sensing of (EP) drug in pharmaceutical and biological samples. Further, this method offers a reliable approach for detecting various drugs in clinical and pharmaceutical settings.

Note

The real (blood and urine) samples were obtained from the hospital with the individual's consent, without listing their names, and the measurements were carried out.

Declaration of interest

No potential conflict of interest was reported by the author(s).

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طراحی تکنیک پراکندگی رزونانس ریلی با استفاده از نقاط کوانتومی سولفید سرب با پوشش گلوپاتیون برای تشخیص داروی اپی نفرین در نمونه‌های حقیقی

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چکیده

در این مطالعه، طراحی یک تکنیک پراکندگی رزونانس ریلی (RRS) با استفاده از نقاط کوانتومی سولفید سرب با پوشش گلوپاتیون به عنوان حسگر تشخیص داروی اپی نفرین در نمونه‌های واقعی اختصاص داده شد. تکنیک‌های مختلفی از جمله SEM, XRD, FTIR و TEM برای مشخص کردن سنسور استفاده شد. سیگنال شدت پراکندگی (ΔI_{RRS}) توسط یک آشکارساز فلورسانس در طول موج ۳۲۵ نانومتر شناسایی شد. تحت شرایط بهینه، نمودار کالیبراسیون در محدوده غلظت داروی اپی نفرین (۰/۰۵ تا ۲۰۰/۰ نانوگرم در لیتر) خطی است. انحراف استاندارد نسبی ($\pm 1/2$ درصد)، و حد تشخیص (LOD) روش ۰/۰۱۵ نانوگرم در لیتر، و مقدار کمی سازی (LOQ) روش ۰/۰۴۴ نانوگرم در لیتر در زمان ۵۰ ثانیه و طول موج ۳۲۵ نانومتر برای پاسخ سطح سولفید سرب با پوشش گلوپاتیون با (۹۶/۰ درصد) اطمینان ارزیابی شد. علاوه بر این، از یک حسگر نقاط کوانتومی سولفید سرب با پوشش گلوپاتیون با تکنیک پراکندگی رزونانس ریلی برای آنالیز داروی اپی نفرین در نمونه‌های ادرار و خون با درصد بازیابی بالا (۹۴/۰ تا ۹۹/۲ درصد) و درصد RSD پایین ($< 3/0$ درصد) استفاده شد. این روش یک رویکرد قابل اعتماد برای تشخیص داروهای مختلف در محیط‌های بالینی و دارویی ارائه می‌دهد.

کلید واژه‌ها

اپی نفرین، گلوپاتیون، نقاط کوانتومی سولفید سرب، حسگر، پراکندگی رزونانس ریلی.