Iranian Journal of Analytical Chemistry
Volume 7, Issue 2, September 2020 (47-56) Origonal Research Article

مجله ایرانی شیمی تجزیه
دوره۷، شماره ۲، مهرماه ۱۳۹۹ (۵۶-۴۷)

Determining the Amount of Metronidazole Drug in Blood and Urine Samples With the help of PbS Sensor functionalized With Gelatin as a Fluorescence- Enhanced Probe.

Shirin Bouroumand, Farzaneh Marahel*, Fereydoon Khazali
Department of Chemistry, Omidiyeh Branch, Islamic Azad University, Omidiyeh, Iran.

Received: 23 July 2020 **Accepted:** 27 August 2020
DOI: 10.30473/ijac.2021.56671.1175

Abstract
Fluorescent chemical sensors to detect drugs, by increasing fluorescence emission and absorption or by shutting down, because they are non-destructive, the ability to show decomposed concentrations, fast response, high accuracy have been considered and used. In this research, a chemical sensor was synthesized PbS functionalized with gelatin quantum dots for (MNZ) drug. The calibration curve was linear in the range of (0.1 to 10.0 µg L^{-1}). The standard deviation of (3.5%), and detection limit of the method (2.2 µg L^{-1} in time 50 sec, 285 nm) were obtained for sensor level response PbS Quantum Dot–Gelatin Nano composites sensor with (95%) confidence evaluated. The observed outcomes confirmed the suitability recovery and a very low detection limit for measuring the (MNZ) drug. The method fluorometric introduced to measure (MNZ) drug in real samples such as urine and blood was used and can be used for hospital samples. The chemical PbS Quantum Dot–Gelatin Nano composites sensor made it possible as an excellent sensor with good reproducibility.

Keywords
Metronidazole Drug; Fluorescence; Sensor; PbS with Gelatin Synthesis; Quantum Dots.

1. INTRODUCTION

Determining the amount of drug used in the biological sample is very important to follow the amount of its effect in the body system. Accordingly, different methods with high sensitivity, selectivity and efficiency, as well appropriate analysis for the determination, extraction and measurement are presented of drugs in real samples [1]. One of the biggest problems in the decomposition of biological samples is the existence of different species and their effect on the decomposition process of the drug. For this reason, many drug measurement methods are based on separation methods such as gas chromatography and high-performance liquid chromatography that are very time-consuming methods with difficult working conditions [2].

Metronidazole (1-(2-hydroxyethyl)-2 -methyl-5-nitroimidazole) is an antibiotics belonging to the nitromidazole class which is prescribed to treat parasitic and bacterial infections in human beings and is commonly effective for giardiasis, amebiasis and dracunculiasis [3]. The veterinary community may apply metronidazole to prevent and treat putrefactions. It is also used in the aqua culture industry as growth-promoting feed additive. High doses and long- term treatment with (MNZ) may be associated with the development of neutropenia, increased risk of peripheral neuropathy, ataxia and seizure [4]. For that reason, precise and reliable calculation of rare (MNZ) in biological samples is crucial for guaranteeing consumers' health. Diverse quantitative analytical method including: electrophoresis [5], voltammetry [6], nano composites electrodes-based voltammetry [7], capillary gas chromatography HPLC [8-10], HPTLC [11], liquid chromatography coupled with mass spectrometry (LC–MS) [12] and spectrophotometry [13] have been utilized for determining MNZ in different matrices. In addition, spectrophotometry [14] and flow injection [15] have been reported among the most used methods in determining (MNZ) medicaments. Attention has newly been drawn to noble metal nanoparticles-based UV–visible spectrophotometric and fluorometrically methods for selective and delicate reorganization of target species (inorganic, organic and biomolecules) in different complex matrices. Accordingly, developing a simple, selective and delicate method like fluorescence emission intensity measurement in determining (MNZ) drug was highlighted. In this method sensing (MNZ) drug was done with high sensitivity and excellent selectivity for discerning and accurate reorganization of species (1-inorganic 2-organic and 3- biomolecules) in different intricate matrices, attention has been drawn to noble metal nanoparticles-based with the help of the quenching properties of quantum dots method [16].

*Corresponding Author: Farzane.marahel.fm@gmail.com

It is only recently that attention has been directed to fluorescent sensors in tracing medicament owing to their outstanding properties like simplicity in operation, great selectivity, high-sensitivity, and real-time monitoring [17]. Numerous fluorescence probes, in the past few decades, have been announced for recognition of medicaments like organic dyes [18] and quantum dots (QDs) [19]. Those QDs, Therefore, the necessity of developing alternative and ecologically friendly materials became apparent [20,21]. Which is identical to the electronic Fermi wavelength, the nature of molecules in fluorescent sensors like 1-discrete energy level, 2-good light stability, 3-strong light luminescence, 4-biocompatibility and other exclusive physiochemical characteristics made them extremely potential in domain of sensing and imaging [22]. However, PbS nano composites can by various methods such as the application of stabilizing and reducing chemicals of glotaraldehyde, formaldehyde, polyethylene glycol, glucose, hydrate and sodium borehydride electrochemical heating, photochemical reduction prepared [23]. Gelatin as a natural, completely non-toxic and biocompatible polymer derived from collagen, is a very suitable option for coating nanoparticles of lead sulfide quantum dots. Because it can be made into fine and stable particles and at the same time it can be used as a drug transfer agent, and more importantly, its by-products are absorbable in the body, which creates significant stability in the form of cross-linking in these materials. To be destroyed or decomposed [24].

In the present article, an uncomplicated facile strategy was employed in preparing water-soluble, stable PbS with gelatin by utilizing glotaraldehyde as a stabilizer. As it is shown in (Fig.1), the existence of (MNZ) provokes the aggregation of nano clusters with improvement of fluorescence intensity. In addition, successful application of nano probes in detecting (MNZ) medicament in different real samples along with their significant efficiency and perfect recovery prove their great potentialities in practical application. In the current article, a fluorometric method was designed for determining (MNZ). The extreme sensitivity, electivity and simplicity of the proposed method led to the absolute superiority of this method over other aforementioned ones. The method was effectively applied in determining (MNZ) in blood and urine samples.

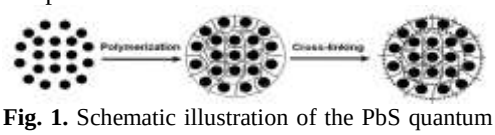

Fig. 1. Schematic illustration of the PbS quantum dots sensor functionalized with gelatin synthesis.

2. EXPERIMENTAL

2.1. Instruments

The instrument used to put all spectra and absorbance measurements on record was a Shimadzu 1601 PC UV-V spectrophotometer with a 1cm cell (Shimadzu, Japan). All the recordings were done at room temperature. X-ray photoelectron spectroscopy (XPS) measurements were performed. Fourier transform infrared (FT-IR) spectra were registered on a PerkinElmer (FT-IR spectrum BX, Germany). On a Horiba JY Fluorolog-3 molecule fluorometer (Paris, France), Time-resolved luminescence intensity decay was registered and by using a 385 nm laser light source, samples were excited. The pH of the solutions was measured using a Jenway 3510 pH-meter which was calibrated against two standard buffer solutions at pH 4.0 and 10.0. Through the instrumentality of a Hamilton syringe (10 µL), small volumes of reagent were transferred into the cell.The reference cell had a membrane with no indicator. All measurements were carried out in the absorbance mode.

2.2. Reagents and materials

Metronidazole medicament (98.0%) was purchased from India Company while all chemicals of lead nitrate ($Pb(NO_3)_2$), sodium soulfid (Na_2S) , gelatin, and glotaraldehyde were bought from Merck Company. Also, hydrochloric acid and methanol were provided from Merck Company (Merck, Darmstadt, Germany). From boric acid / acetic acid / phosphoric acid (1.0 M each), acetic acid/tri chloric acetate (1.0 M) buffers (pH 2–7) were prepared and they were tested as supporting electrolytes. By adding 0.2M sodium hydroxide, the pH > 7.0 was adjusted. stock solutions of (10.0 µg L^{-1}) of interfering medicaments were developed.

2.3. Pretreatment of real samples

In a 50 mL beaker, treatment of a 10 mL portion of a urine sample (or a spiked urine sample) in hospital (Ahvaz, Shiraz, and Boushehr) were done using 10 mL of concentrated HNO_3 (63%) and an $HClO_4$ (70%) mixture of 2:1 and then covered with a watch glass. Then on a hot plate, the treated sample of the balloon was heated (100°C 15 min/ 150°C 10 min). Next by removing the watch glass, the acid was left to evaporate to dryness at 150°C. After that by adding $HClO_4$ (3 mL) to the resulting white residue, the mixture was heated at 160°C to dryness. The whole heating process was done under a hood while necessary safety precautions were practiced. Upon adding five milliliters of 1 M H_2SO_4, the mixture was heated at 150°C for 1 min and then with the help of a 50 mL volumetric flask, desired volume made up to the mark. Seven mL of the obtained clear

solution was picked and the analysis was performed according to the explained procedure [25].

In the presence of an oxidizing agent and 10 mL of concentrated HNO_3, the exact amount of homogenized human blood sample (20 mL) in hospital (Ahvaz, Shiraz and Boushehr) were digested in a 200 mL balloon and then 2 mL $HClO_4$ 70 % was added and heated for 1 h. With the help of a what man No. 42 filter paper into a 250 mL calibrated flask and its pH was adjusted to desired value and diluted to mark with de-ionized water. In all of real and synthetic sample amount of (MNZ) drug was found by standard addition method [26].

2.4. Synthesis of PbS Quantum Dot–Gelatin nanocomposites Sensor

The synthesis of nanoparticle PbS was done in reactive solution concocted utilizing lead nitrate ($Pb(NO_3)_2$) and sodium soulfid (Na_2S) both with concentration of (0.1 M and 0.1M). The Gelatin pellets were utilized as a base medium and its concentration was adjusted to (0.1 M). The preparation of 20 mL of all the above solutions was performed separately utilizing DW (distilled water) as a solvent. Afterwards they were mixed in a beaker. Two milligrams of glotaraldyhyde (25%) as a complexing agent was added into the solution. complexing agents could help the metal ions to bond easily. The immersion of the reactive vessel with solution into 20 ml acetone at 40 ^{oC} and pressure of 5-10 mbar was performed subsequently. To measure the temperature of the bath solution in the vessel, a thermometer was used. Also the constancy of temperature was checked using a temperature sensor and dimer with temperature controller attached to the vessel. Moreover, to guarantee the homogeneity of the mixture, the solution was stirred well with the help of a magnetic stirrer. The solution was first colorless but after (30 min) changed to yellow and abruptly turned into gray color which is a good indication of chemical reactions and a good indication for the formation of PbS. The stirring of the reactive solution was continued for 2 hours. The powder was eventually collected and dried in a hot air oven at 57 ^{OC}) [27].

2.5. Procedure Fluorescent Detection measurements

The ensuing steps were followed for a typical fluorescence emission intensity method experiment: first, a 10 ml volumetric flask was picked and 1ml of MNZ (10.0µg L^{-1}) was added to it. Second, 1ml PbS Quantum Dot–Gelatin nano composites (2.5×10^{-2} $molL^{-1}$) and then 2 ml of glotaraldehyde (25%) were put into the volumetric flask. The reaction time start point was recorded. After (10 sec), the solution was mixed for another 10 sec and then by adding DW (distilled water) it was volume. The difference between the quantities of the absorption in a wavelength equal to (285 nm) in a time interval equal to (40- 60 sec), was estimated. By adding (MNZ) to the solution, it was observed that fluorescence emission intensity of the acetonitrile solution of PbS quantum dot–gelatin nanocomposites at wavelength of (335 nm) dropped. At the same time, with the help of fluorometric and UV–visible spectrum (ΔI b), the apparent spectral evolution including the formation of a well-defined isosbestic point at around (285 nm) was estimated. All reaction steps were repeated by increasing the concentration (0.2µg L^{-1}) of the (MNZ) drug every (10 sec). Moreover, all the steps were repeated for a reaction. In the fluorometric of (MNZ) drug (ΔI b) and ultimately (ΔI) I_0 blank-I sample. There was a sharp change in the fluorescence emission of the sensor in the 285 nm region, a continuous increase of (MNZ) drug at intervals of (10 sec) in solution and changes in the fluorescence emission intensity of the sensor, peak fluorescence emission during 285 nm, with an increase in fluorescence emission intensity, can be seen in (fig. 3A). UV–visible spectrum (AAb). All these steps would be repeated for a reaction without the presence of (MNZ) drug (AAb), finally (AA) AAblank-AAsample is calculated. The spectrum changes are due to the addition of (MNZ) drug in the range of (0.0µg L^{-1} at 1.2µg L^{-1}) and the formation of a complex. As can be seen, the complex ((MNZ) drug-sensor) has two absorption peaks, the first at a wavelength of 345 nm and the second at a peak Appears at a wavelength of 420 nm. (Fig.3B) [28].

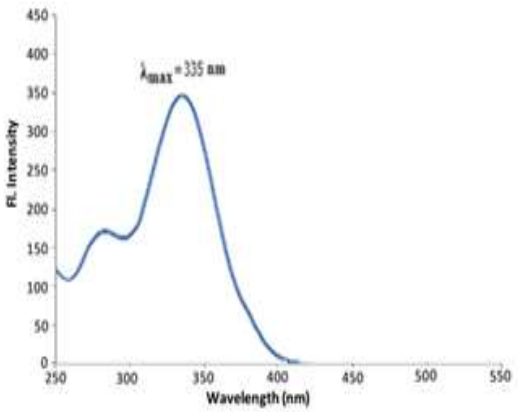

Fig. 2. The Fluorescent Detection of product PbS quantum dots–gelatin nanocomposites

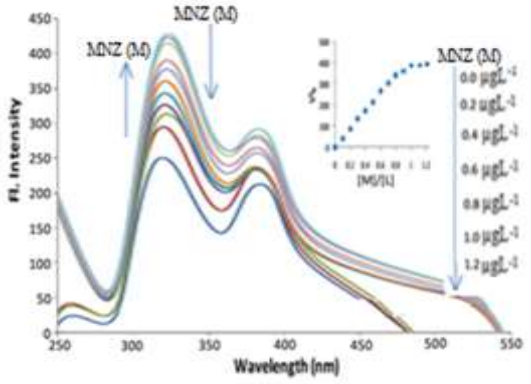

Fig. 3A. The Fluorescent Detection of product PbS quantum dots–gelatin nano composites and (MNZ) medicament (10 sec), and increasing concentration of the (MNZ) medicament solution (0.2µg L^{-1}).

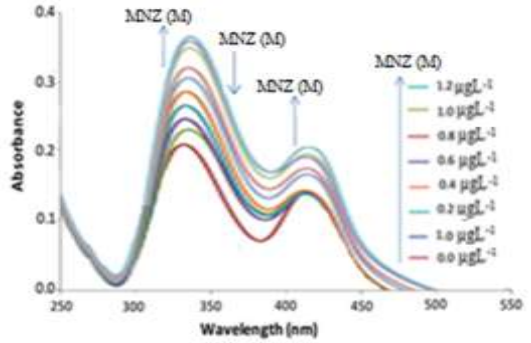

Fig. 3B. The absorption spectra of product PbS quantum dots–gelatin nano composites and (MNZ) medicament (10 sec), and increasing concentration of the (MNZ) medicament solution (0.2µg L^{-1}).

3. RESULT AND DISCUSSION

3.1. Characterization of PbS Quantum Dot–Gelatin Nano composites Synthesis

3.1.1. IR analysis

The results of IR for the samples of uncoated PbS Quantum Dots and the sample coated with gelatin can be seen in (Fig. 4a). In the figure for the uncoated sample, two very weak bands are seen in 835 cm^{-1} and 1110 cm^{-1}, which are characteristic of the presence of the Pb-S bond, and the reason for the severity of the weakness of these bands is only due to covalent bonding and very weak vibrations. Also, the indicator bands in 1350 cm^{-1} and 1380 cm^{-1} indicate the presence of nitro compounds, and the presence of a band at 3445 cm^{-1} indicates the O-H groups in the sample. The IR spectrum of the gelatin-coated specimen indicates the bands associated with the coating at 3416 cm^{-1} and 1733 cm^{-1} belonging to the N-H bond and the vibrations associated with the C=O bond in the 1633 cm^{-1} region. Pb-S covalent bond bands have also been observed, except that they have appeared in regions 985 cm^{-1} and 1110 cm^{-1} [29].

3.1.2. XRD analysis

In Fig. 4b, the XRD pattern of the PbS Quantum Dot–Gelatin nano composites is displayed. The polycrystalline nature of the synthesized nano powders is clearly discernible. All observable signals relative to (111), (200), (220), (311), (222), (400), (331), (420) and (422) planes are relevant to the pure cubic phase of PbS. Obviously the perfect PbS Quantum Dot–Gelatin nano composites, however the great intensity of signal at 25.8 (111) and 30.5 (200) confirmed that there has been a slight amount of material in amorphous state. The perfect synthesis of PbS Quantum Dot–Gelatin nano composites is obvious through looking at XRD pattern of crystal [30].

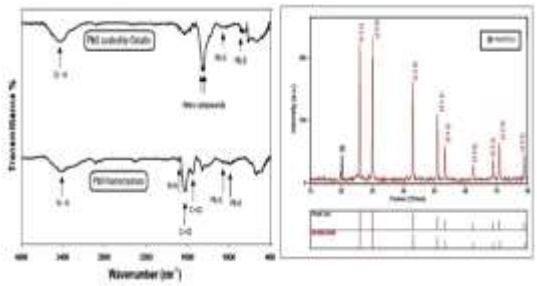

Fig. 4. The (a) FT-IR transmittance spectrum image and (b) XRD of the preparation of synthesized PbS Quantum Dot–Gelatin Nano composites.

3.1.3. Surface morphology

After employing SEM micrograph, the morphological properties and particle size distribution of the PbS Quantum Dot–Gelatin Nano composites are exhibited in the Fig.5, graph. The spheroid nature of the particles was evident. The particle size distribution varied since they could agglomerate but the average particle size was estimated to be in the range of 37- 44 nm which was very close to what was determined by XRD analysis [31].

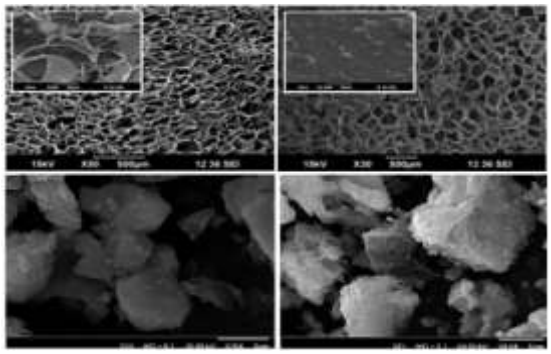

Fig. 5. The (SEM) image of synthesized PbS Quantum Dot–Gelatin Nano composites.

3.2. Optimization of Sensing Conditions

It is interesting that the fluorescence intensity of the as-prepared PbS quantum dot–gelatin nano

composites was significantly enhanced in the presence of (MNZ) drugs. To obtain a highly sensitive response for the detection of (MNZ) drugs, the optimization of pH values, PbS quantum dot–gelatin nano composites, and incubation time was carried out systematically.

In this section, the best type of buffer and its volume for maximum absorption (MNZ) drug with PbS quantum dot–gelatin nano composites sensor are investigated. To this step, the procedure is as follows: In 10 ml balloons, separately 1 ml of (MNZ) drug (10.0 µg L^{-1}) and a volume of each type of acetic acid / boric acid / phosphoric acid buffer and then 1 ml of 1 ml PbS quantum dot–gelatin nano composites (2.5×10^{-2}mol L^{-1}), 2 ml glotaraldehyde (25%), to the solution inside the balloon and after (50 sec), the adsorption reaction of the solutions by the device read a fluorescence. The results are shown in (Fig. 6 A). Based on the results, 1 ml of acetic acid buffer shows the highest percentage for the determination of (MNZ) drug, so acetic acid / tri chloric acetate buffer (1.0 M) to adjust the pH solution as the optimal buffer.

After measuring the fluorescence intensity of the solution, a thorough investigation was carried out on the fluctuating pH values in the range of 2-9 for the MNZ-PbS Quantum Dot–Gelatin nano composites complex at 285 nm. As evident in Fig. 6B, fluorescence intensity increased rapidly on changing the pH from 1.0 to 4.0, while it decreased at pH values higher than 4.0. This phenomenon might be because of the weak complexion at lower pH values (pH < 4.0). On the other hand, the reduced response of the proposed PbS Quantum Dot–Gelatin nano composites sensor for the determining (MNZ) at pH > 4.0 could be due to a possible formation of the hydroxide of MNZ drug in solution. Thus, pH 4.0 was selected as a favorable pH for all subsequent experiments [32,33]. Accordingly, pH 4.0 was chosen as the perfect pH value for detecting (MNZ). Concurrently, (1 ml MNZ 10.0 µgL^{-1}) solution, 2 ml glotaraldehyde (25%) and 1ml PbS Quantum Dot–Gelatin nano composites (0.5×10^{-3} to 4.0×10^{-2} mol L^{-1}) were mixed in a volumetric flask 10 ml using DW (distilled water) to find out about the impact of PbS Quantum Dot–Gelatin nano composites on the reaction rate. Again fluorescence intensity of solution was assessed. The previously mentioned operation has been replicated for blank solution (the solution in the absence of (MNZ)). The findings are exhibited in Fig. 6C. Consequently (2.5×10^{-2} mol L^{-1}) based on those findings was determined as the perfect concentration. Also, the impact of reaction time on the absorbance spectrum was investigated. Based on Fig. 6D, it has become apparent that the fluorescence intensity enhanced expeditiously and reached its peak at around (50 sec). After 50 sec, a relative stability was spotted in the fluorescence intensity. Thus (50 sec) was determined as the perfect reaction time in this experiment.

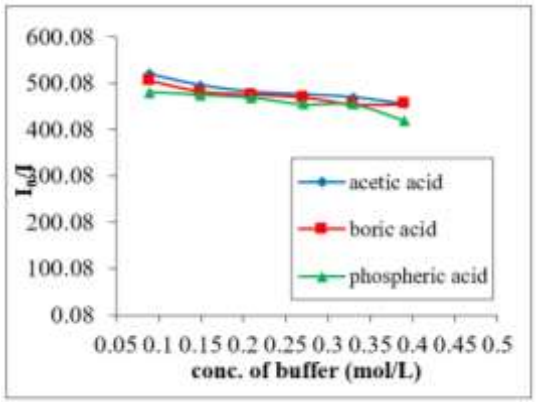

Fig. 6A. The efficacy of buffer concentration on the reaction rate. (aqueous sample volume, 10 mL: PbS Quantum Dot–Gelatin Nano composites, 2.5×10^{-2} M, MNZ = 10.0 µgL^{-1}, time 60 sec, 285 nm).

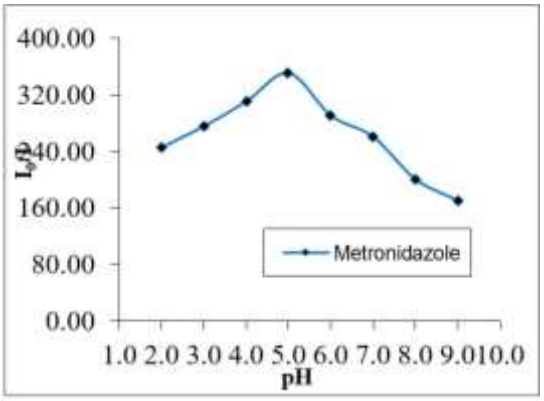

Fig. 6B. The impact of pH on the reaction rate. (aqueous sample volume, 10 mL: PbS Quantum Dot–Gelatin Nano composites, 2.5×10^{-2} M, MNZ = 10.0 µgL^{-1}, time 60 sec, 285 nm).

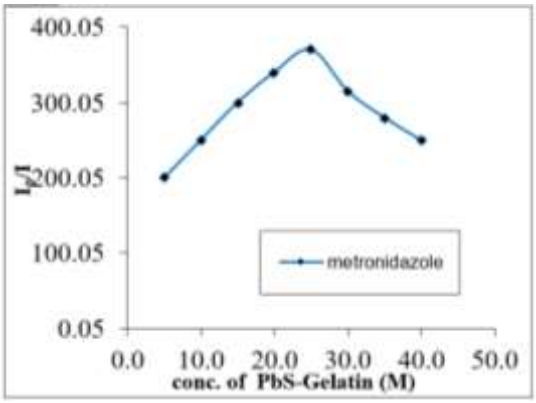

Fig. 6C. The impact of PbS Quantum Dot–Gelatin Nano composites on the reaction rate. (aqueous sample volume, 10 mL: pH =4, MNZ = 10.0 µgL^{-1}, time 50 sec, 285 nm).

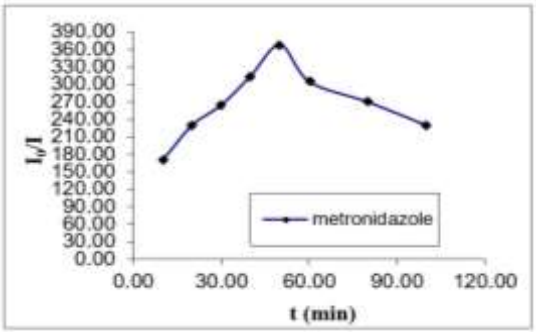

Fig. 6D. The impact of time on the reaction rate. (aqueous sample volume, 10 mL: PbS Quantum Dot–Gelatin Nano composites, 2.5×10^{-2} M, MNZ =10.0 μgL^{-1}, pH 4, 285 nm).

3.3. Response time

As is known, the response time (t95 %) of a sensor, the time required for the response of the sensor towards a certain concentration of the measured ion to reach (95%) of its final value (steady state). Controlling the response time of the membrane is attainable via checking the needed time for the analytic to disperse from the volume of the solution to the PbS Quantum Dot–Gelatin Nano composites interface and to connect with the (MNZ) medicament solution. Through registering the change in absorbance intensity (at from pH=4 in 285 nm) to (MNZ) medicament solution of (10.0 μgL^{-1}), the response time of the present membrane was examined. As evident in (Fig.7), it is revealed that the PbS Quantum Dot–Gelatin Nano composites reached (95%) of the final signal at (5 sec at 120 sec) contingent upon the time, is one of the predominant parameters that must be determined experimentally, the time-dependent response [34].

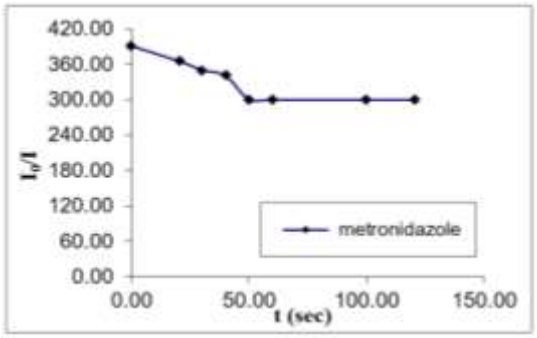

Fig. 7. as a function of time, typical response curve of the PbS Quantum Dot–Gelatin Nano composites was observed to reach 95% of the final signal at (5 – 120 sec) at 285 nm was subjected to (10.0 μgL^{-1} MNZ).

3.4. Analytical specifications and Calibration graph and reproducibility

After optimizing the factors affecting the measurement of (MNZ), the grading curve was plotted under optimized conditions (fig.8). As shown the adsorption intensity in the range of (MNZ) medicament (0.01-10.0 $\mu g\ L^{-1}$), is linearly related to the concentration of (MNZ) drug, and this error follows the equation y = 0.0279x + 0.0154, where is the concentration (MNZ) drug x (μgL^{-1}), is equal to 0.9977 in terms of molar and correlation (R^2).

Also, for 6 replicates, measurement of (MNZ) drug (10.0 $\mu g\ L^{-1}$), solution with optimized conditions, the relative standard deviation (R.S.D) for the response of PbS Quantum Dot–Gelatin nanocomposites towards a (10.0 $\mu g\ L^{-1}$) of (MNZ) medicament was (3.5%) and reproducibility of the response of different PbS Quantum Dot–Gelatin nanocomposites was also studied. The determination of (10.0 $\mu g\ L^{-1}$) (MNZ) drug. The relative standard deviation for the response of PbS Quantum Dot–Gelatin nanocomposites was (2.2%) (fig.8) [35].

3.5. Optimum values of parameters

The optimum values of parameters are demonstrated in Table.1. The method can be used as an alternative method for (MNZ) medicament measurement owing to advantages like excellent selectivity and sensitivity, low cost, simplicity, low detection limit and no need in utilizing organic harmful solvent.

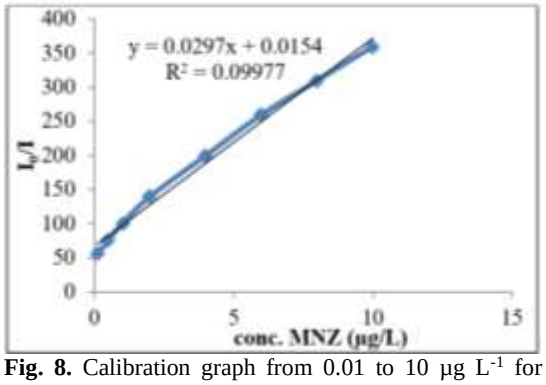

Fig. 8. Calibration graph from 0.01 to 10 $\mu g\ L^{-1}$ for metronidazole medicament

Table 1. Investigation of method repeatability at conditions.

Parameter	Optimum Value for metronidazole drug
metronidazole drug (M)	(10.0 $\mu g\ L^{-1}$)
PbS Quantum Dot–Gelatin Nano composites (M)	(2.5×10^{-2} M)
pH	4.0
Equilibration time (sec)	(50 sec)
Linear range (LDR)	(0.1 - 10.0 $\mu g\ L^{-1}$)
Detection limit (LOD)	(2.2 $\mu g\ L^{-1}$)
Relative Standard Deviations (RSD)	(3.5%)
Accuracy and precision	High
Advantages	High repeatability, sensitivity, selectivity, wide linear range and no need to organic solvent
Disadvantages	Do not preconcentrate

3.6. Interference Studies

After establishing the measurement method, to evaluate the selectivity of the prepared PbS Quantum Dot–Gelatin Nano composites sensor for determining the (MNZ) drug the effect of various substances on the determination of (MNZ) drug (10.0 μg L^{-1}) for method respectively was tested under optimum conditions. Several representative potential interferences such as inorganic cautions, anions, molecular species and drugs were investigated individually for their effect on (MNZ) drug recovery. Tolerance Limits were defined by the concentration of interferences which caused on <5% error in the determination of (MNZ) drug [36]. The obtained mean recoveries and standard devastation ranged between 99.0% - 96.0% and ± 1.0 - 2.0 respectively are shown in (Table. 2).

The results showed that most of the other medications studied did not have much effect on the measurement of (MNZ) drug and among them, compounds with a more similar structure or with more functional groups are more disturbing, which It may be related to their hydrogen interactions or the molecule of the (MNZ) drug and thus reduce the measurement of the (MNZ) drug in the analytic sample. As exhibited in (Table.2), the tolerance limit was determined as the max concentration of the interfering substance which resulted in an error less than (±5%) for determination of (MNZ) drug. The So selectivity of the recommended method was proven.

3.7. Application of the real sample

In order to evaluate the efficiency of the proposed sensor for determining (MNZ) drug in real samples, this PbS Quantum Dot–Gelatin Nano composites sensor was used to measure (MNZ) medication in urine and blood human samples according to the instructions mentioned for (MNZ) medication experiment 3, replicates measuring section [37]. The obtained percentage percentiles in (Table.3), indicate that the prepared sensor has a very good performance for determining the drug (MNZ) medication in urine and blood human samples. Therefore, the determining of (MNZ) medication in samples was confirmed utilizing standard addition method. The level of the (MNZ) medicament was estimated to be below the detection limit of related element. Based on the outcomes of replicating analyses for each sample, it was shown that the medication retrievals were mainly quantitative with a low RSD. The potentiality of the recommended method for the determination of trace quantities of these elements in distinct samples was proven.

Table 2. Impacts of the matrix medicaments on the retrieving of the examined (MNZ) drug (N=6).

Foreign species	Tolerance limit (ng/mL)
Amoxicillin, Ampicilline, Acetominophene, Cortisone, Cyclosporine	1000
Tramadol, Metadone	750
NH_4^+, Mg^{2+}, F^-, K^+, Cu^{2+}, Fe^{3+}, Ca^{2+}, Cl^-, I^-	500
Naratriptan, Rizatriptan, Sumatriptan, and Zolmitriptan	100
Vitamin B1, Vitamin B12, Vitamin B6	50

Table. 3: Recovery of trace (MNZ) from urine and blood sample after employing presented procedure (n=3).

Samples	Added (μg mL^{-1})	Founded (μg mL^{-1})	RSD %	Recovery %
Urine hospital Boushehr	0/00 0/15	0/74 1/26	1/1 1/2	---- 99
Blood hospital Boushehr	0/00 0/15	0/42 1/00	1/1 1/4	---- 103
Urine hospital Shiraz	0/00 0/15	0/29 0/80	3/7 2/8	---- 102
Blood hospital Shiraz	0/00 0/15	1/49 1/97	3/8 3/0	---- 98
Urine hospital Ahvaz	0/00 0/15	0/44 0/95	2/3 1/8	---- 102/2
Blood hospital Ahvaz	0/00 0/15	0/67 1/44	2/5 2/1	---- 101/7

4. CONCLUSION

The investigation in this article focused on measuring the amount of trace (MNZ) medicament utilizing PbS quantum dots–gelatin nanocomposites sensor, in the company of by utilizing glotaraldehyde as a stabilizer sensor. A successful analytical method for measuring (MNZ) medicament was prosperously developed via utilizing a sensitized fluorescence emission with the help of PbS quantum dots–gelatin nanocomposites. The method can be used as an alternative method for (MNZ) medicament measurement owing to advantages like excellent selectivity and sensitivity, low cost, simplicity, low detection limit and no need in utilizing organic harmful solvent or extraction.

The reaction was evaluated by measuring the absorption rate of (MNZ) drug, the optimum

conditions. For determination (MNZ) drug in solution we used a prepared from PbS quantum dots–gelatin nanocomposite sensor and fluorometric method. The calibration curve was linear in the range of (0.01 to 10.0 μg L^{-1}). The standard deviation of (3.5%), and detection limit of the method (2.2 μg L^{-1} in time 50 sec, 285 nm) were obtained for sensor level response PbS quantum dots–gelatin nanocomposites with (95%) confidence evaluated. The lowest determining error (MNZ) drug could be obtained in a short time, which strongly confirms the greater contribution for the deletion of (MNZ) drug by PbS quantum dots–gelatin nanocomposites sensor.

ACKNOWLEDGEMENTS

The authors would like to acknowledge that the research was partially supported by the Islamic Azad University, Branch of Omidiyeh Iran.

REFERENCES

[1] H. Shemer, Y.K. Kunukcu and K.G. Linden, Degradation of the pharmaceutical metronidazole via UV, Fenton and photo-Fenton processes. *Chemosphere*. 63 (2006) 269.

[2] S. Benitez–Martinez, A.I. Lopez-Lorente and M. Valcarcel, Multilayer grapheme-gold nanoparticle hybrid substrate for the sers determination of metronidazole. *Micro. Chem. J*. 121 (2015) 6.

[3] J. Muller, P. Schildknecht and N. Muller, Metabolism of nitro drugs metronidazole and nitazoxanide in Giardia lamblia: characterization of a novel nitroreductase. *J. Antimicrob. Chemoth*. 68 (2013) 1781.

[4] J. Han, L. Zhang, S. Yang, J. Wang, A highly sensitive metronidazole sensor based on a Pt nanospheres/polyfurfural film modified electrode. *J. Environ. Contam. Tox.* 92 (2014) 196.

[5] W. Jin, W. Li, Q. Xu, Q. Dong, Quantitative assay of metronidazole by capillary zone electrophoresis with amperometric detection at a gold microelectrode. *J. Electrophoresis*. 21(7) (2000) 1409-1414.

[6] M. Yang, M. Guo, Y. Feng, Y. Lei, Y. Cao, D. Zhu, Y. Yu and L. Ding, Sensitive Voltammetric Detection of Metronidazole Based on Three - Dimensional Graphene - Like Carbon Architecture / Polythionine Modified Glassy Carbon Electrode. 165 (2018) 687.

[7] I. Peng, C. Hou and X. Hu, Determination of metronidazole in pharmaceutical dosage forms based on reduction at graphene and ionic liquid composite film modified electrode. 169 (2012) 81.

[8] S. Ashour and N. Kattan, Simultaneous determination of miconazole nitrate and metronidazole in different pharmaceutical dosage forms by gas chromatography and flame ionization detector (GC-FID). *Int. J. Bio. Sci*. 6 (2010) 13.

[9] M. Silva, S. Schramm, E. Kano, E. Koono, V. Porta and C. Serra, Development and validation of a HPLC-MS-MS method for quantification of metronidazole in human plasma. *J. Chromatogr*. 47(9) (2009) 781-784.

[10] N.W. Ali, M. Gamal and M. Abdelkawy, Chromatographic methods for simultaneous determination of diiodohydroxyquinoline and metronidazole in their binary mixture. *J. Pharm. Sci*. 26 (2013) 865.

[11] S.N. Makhija and P.R. Vavia, Stability indicating HPTLC method for the simultaneous determination of pseudoephedrine and cetirizine in pharmaceutical formulations. *J. Pharm. Biomed. Anal*. 25 (2001) 663.

[12] H. Liu, F. Li, R. Yang, L. Wang and Y. Ma, Determination of common antibiotics and metronidazole in cosmetics by ultraperformance liquid chromatography tandem mass spectrometry. *J. Chinese. Chromatography*. 27 (2009) 50.

[13] S.B. Wankhede, K.A. Lad and S.S. Chitlange, Development and validation of UV-spectrophotometric methods for simultaneous estimation of cetirizine hydrochloride and phenylephrine hydrochloride in tablets. *Int. J. Pharm. Sci. Drug. Res*. 4 (2012) 222.

[14] N. Samadi and S. Narimani, Sensitive and Selective Determination of Metronidazole Using Highly Luminescent Pepper Carbon Dots. *J. Chem. Biolog. Phys. Sci*. 6 (2016) 387.

[15] E. Roy, S.K. Maity, S. Patra and P.K. Sharma, A metronidazole-probe sensor based on imprinted biocompatible nanofilm for rapid and sensitive detection of anaerobic protozoan. Adv. 4 (2014) 32881.

[16] J. Das and M. Dhua, UV-Spectrophotometric Assay Method Development and Validation of Metronidazole in Bulk and Tablet Formulation. *J. Pharma. Sci. Tech*. 3 (2014) 106.

[17] Y. Dai, K. Yao, J. Fu, K. Xue, L. Yang and K. Xu, A novel 2-(hydroxymethyl) quinolin-8-ol-based selective and sensitive fluorescence probe for Cd^{2+} ion in water and living cells. *Sensor. Actuators B.* 251 (2017) 877.

[18] W.B. Huang, W. Gu, H.X. Huang, J.B. Wang, W.X. Shen, Y.Y. Lv and J. Shen, A

porphyrin-based fluorescent probe for optical detection of toxic Cd^{2+} ion in aqueous solution and living cells. *Dyes Pigments*. 143 (2017) 427.

[19] N.B. Brahim, N.B.H. Mohamed, M. Echabaane, M. Haouari, R.B. Chaabane, M. Negrerie and H.B. Ouada, Thioglycerol-functionalized CdSe quantum dots detecting cadmium ions. *Sens. Actuators* B. 220 (2015) 1346.

[20] A. Hatamie, F. Marahel and A. Sharifat, Green synthesis of graphitic carbon nitride nanosheet (G-C_3N_4) and using it as a label-free fluorosensor for detection of metronidazole via quenching of the fluorescence, *Talanta*. 176 (2018) 518.

[21] A. Tadesse, D. RamaDevi, M. Hagos, G.R. Battu and K. Basavaiah, Facile Green Synthesis of Fluorescent Carbon Quantum Dots from Citrus Lemon Juice for Live Cell Imaging. *Asian. J. Nanoscience and Materials*. 1(2018) 36-46.

[22] C. Knoblauch, M. Griep and C. Friedrich, Recent advances in the field of bionanotechnology: An insight into optoelectric bacteriorhodopsin, quantum dots, and noble metal nanoclusters. *Sensors*. 14 (2014) 19731.

[23] M. Mirsalari, S. Elhami, Colorimetric detection of insulin in human Serum using GO/AuNPs/TX100 nanocomposite. *J. Spectrochim. Acta. A. Mol. Biomol. Spectrosc*. 240 (2020) 118617.

[24] X. Yang, M. Liu, Y. Yin, F. Tang, H. Xu, X. Liao, Green, Hydrothermal Synthesis of Fluorescent Carbon Nanodots from Gardenia, Enabling the Detection of Metronidazole in Pharmaceuticals and Rabbit Plasma. *Sensors*. 18(4) (2018) 964.

[25] C.M. Kaye, M.J. Sankey and L.A. Thomas, A rapid and specific semi-micro method involving high-pressure liquid chromatography for the assay of metronidazole in plasma, saliva, serum, urine and whole blood, *J. Clin. Pharmacology*, 9 (1980) 528.

[26] A. Shokrollahi, H.E. Haghighi, E. Niknam and K. Niknam, Application of cloud point preconcentration and flame atomic absorption spectrometry for the determination of cadmium and zinc ions in urine, blood serum and water samples. *J. Quim. Nova. Sao. Paul*. 36 (2013) 273.

[27] C. Coester, K. Langer, H. Brisen and J. Kruter, Gelatin nanoparticles by two step desolvation-a new preparation method, surface modifications and cell uptake, *J. Micro. encapsul*. 17 (2000) 187.

[28] N. Samadi and S. Narimani, Citrate Capped CdS Quantum Dots as Fluorescence Sensor for Simple and Selective Determination of Metronidazole. *Sensor. Lett. J.* 14 (2016) 530.

[29] Y. Zhao, J. Zou and W. Shi, In situ synthesis and characterization of lead sulfide nanocrystallites in the modified hyperbranched polyester by gamma-ray irradiation, *J. Mater. Sci. Eng.* 121 (2005) 20.

[30] S. Lee, S. Cho and J. Cheon, Anisotropic shape control of colloidal inorganic nanocrystals, Adv .*Mater*. 15 (2003) 441.

[31] T.S. Shyju, S. Anandhi, R. Sivakumar and R. Gopalakrishnan, Studies on Lead Sulfide (PbS) Semiconducting Thin Films Deposited from Nanoparticles and Its NLO Application. *Int. J. Nano. Sci*. 13 (2014) 1450001.

[32] M. Zohreh, S.M. Ghoreishi, M. Behpour and M. Mohammadhassan, Applied electrochemical biosensor based on covalently self-assembled monolayer at gold surface for determination of epinephrine in the presence of ascorbic acid. *Arab. J. Chem*. 10 (2017) 657–664.

[33] R. Adel, S. Ebrahim, A. Shokry, M. Soliman and Marwa Khalil, Nanocomposite of CuInS/ZnS and Nitrogen-Doped Graphene Quantum Dots for Cholesterol Sensing, *ACS Omega*. 6 (2021) 2167-2176.

[34] T.D. Thanh, J. Balamurugan, N.T. Tuan, H. Jeong, S.H. Lee, N.H. Kim and J.H. Lee, Enhanced electrocatalytic performance of an ultrafine AuPt nanoalloy framework embedded in graphene towards Epinephrine sensing. *J. Biosens. Bioelectron*. 89 (2016) 750.

[35] S.S. Liang, L. Qi, R.L. Zhang, M. Jin and Z.Q. Zhang, Ratiometric fluorescence biosensor based on CdTe quantum and carbon dots for double strand DNA detection. *Sens. Actuators.* 244 (2017) 585.

[36] P. Dutta, D. Saikia, N.C. Adhikary and N.S. Sarma, Macromolecular Systems with MSA-Capped CdTe and CdTe/ZnS Core/Shell Quantum Dots as Superselective and Ultrasensitive Optical Sensors for Picric Acid Explosive. *ACS Appl. Mater. Interfaces*. J. 7 (2015) 24778.

[37] S. Baluta, K. Malecha, A. Swist and J. Cabaj, Fluorescence Sensing Platforms for Epinephrine Detection Based on Low Temperature Cofired Ceramics. *Sensors*. 20 (2020) 1429.

تعیین مقدار داروی مترونیدازول در نمونه‌های خون و ادرار با کمک حسگرنانوذرات نقاط کوانتومی سولفید سرب پوشش داده‌شده با ژلاتین بعنوان یک پروب افزایش‌یافته با فلوئوسانس عامل‌دار شده

شیرین برومند، فرزانه مراحل*، فریدون خضعلی
گروه شیمی، دانشگاه آزاد اسلامی، واحد امیدیه، امیدیه، ایران
تاریخ دریافت: ۲ مرداد ۱۳۹۹ تاریخ پذیرش: ٦ شهریور ۱۳۹۹

چکیده
حسگرهای شیمیایی فلوئورسانس‌کننده برای شناسایی داروها، از طریق افزایش نشر فلوئورسانس و جذب و یا از طریق عمل خاموش کردن، به دلیل غیر مخرب بودن آن‌ها، توانایی نشان دادن غلظت‌های مورد تجزیه، پاسخ سریع، دقت و صحت بالا بسیار مورد توجه و استفاده قرار گرفته‌اند. در این تحقیق، حسگر شیمیایی با روشی آسان و کم هزینه از نانوذرات کوانتومی سولفید سرب با پوششی از ژلاتین در حضور گلوتارالدئید برای تعیین میزان داروی مترونیدازول سنتز گردید. منحنی کالیبراسیون در محدوده (۰,۱ تا ۱۰,۰ میکروگرم در لیتر) خطی بود. انحراف استاندارد روش (۳,۵٪) و حد تشخیص روش (۲,۲ میکروگرم در لیتر، در مدت زمان ۵۰ ثانیه، در طول موج ۲۸۵ نانومتر) برای داروی مترونیدازول محاسبه گردید. و ارزیابی زمان پاسخ دهی دارو در طول موج ۲۸۵ نانو متر در حضور حسگر شیمیایی نانوذرات سولفید سرب پوشش داده‌شده با ژلاتین در مدت زمان ۵۰ ثانیه با در صد اطمینان ۹۵ درصد حاصل گردید. نتایج مشاهده شده بهبود مناسب بودن و حد تشخیص بسیار کم برای اندازه گیری دارو (MNZ) را تأیید کرد. از روش فلوئورسنجی برای اندازه‌گیری دارو (MNZ) در نمونه‌های حقیقی مانند ادرار و خون استفاده شد و می‌تواند برای دیگر نمونه داروها و همچنین نمونه‌های بیمارستانی مورد استفاده قرار گیرد. حسگر شیمیایی نانو ذرات کوانتومی سولفید سرب با پوششی از ژلاتین این امکان را به عنوان یک سنسور عالی با قابلیت تکرارپذیری خوب فراهم کرده است.

واژه‌های کلیدی
داروی مترونیدازول، فلوئورسانس، حسگر سنتزی سولفید سرب با پوشش ژلاتین، اتصالات کوانتومی عرضی.