Mn-DOPPED ZnS QUANTUM DOTS AS SENSITIVE SENSOR FOR DETERMINATION OF CIPROFLOXACIN IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES

MIAN MUHAMMAD [1], BEHISHT ARA [2], FAIZ ALI [1], IZAZ AHMAD [1] AND HAFEEZ ULLAH [1]*

[1]*Department of Chemistry, University of Malakand, Khyber Pakhtunkhwa, Pakistan.*
[2]*Institute of Chemical Sciences, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.*

ABSTRACT

A simple and fast spectrofluorimetric method for determination of ciprofloxacin based on its quenching effect on the fluorescence intensity of colloidal water soluble TGA caped, Mn doped ZnS quantum dots (QDs) has been described. The QDs having characteristic fluorescence spectra with maximum excitation at 315 nm followed by an emission peak at 632 nm were characterized using Energy Dispersive X-Ray and X-ray Diffraction techniques. The effect of various parameters such as concentration of QDs, time, pH, common excipients and metal ions on the quenching phenomenon was investigated. Fluorescence quenching was found to be maximum with 10 µg mL^{-1} of QDs at pH 8 and fluorescence intensity was observed to be constant upto 45 min. The Stern-Volmer calibration plot of F^0/F as a function of ciprofloxacin concentration was found to be linear in the range of 0.5-10 µg mL^{-1} with $r^2 = 0.993$. Under optimal experimental conditions, the method was found to be interference free. The percent relative standard deviation of the proposed method, calculated against method blank, was found to be 1.10%. The limit of detection (LOD) and the limit of quantitation (LOQ) for ciprofloxacin (signal to noise ratio 3:1 for LOD and 10:1 for LOQ) were calculated to be 0.15 and 0.50 µg mL^{-1} respectively. Average percent recoveries (±SD) obtained for spiked commercial formulations; ciprol and ciproquine, and serum and urine samples were found to be in the range of 92.2±1.8% to 99.5±1.5%, 90.5±1.2% to 96.7±1.4% and 94.0±1.2% to 98.5±1.4% respectively. The data evidently proves the potential of the proposed sensor QDs in biological analysis.

Keywords: Spectrofluorimetric; Ciprofloxacin; Quenching; Quantum dots; Fluorescence intensity.

INTRODUCTION

Ciprofloxacin (Cf) is a synthetic, broad-spectrum, second-generation fluoroquinolone antibiotic that shows activity against both gram-positive and gram-negative bacteria [1] through inhibition of their DNA gyrase. It is used to treat various bacterial infections, i.e. bone and joint infections, respiratory tract infections, skin infections, several infectious diarrheas, urinary tract infections, typhoid fever, gastrointestinal and sexually transmitted diseases [2-4]. Also, the United States Food and Drug Administration has approved it to prevent or slow down anthrax after exposure [5]. There are greater chances for Cf to enter the environment via urine and wastewater due to its incomplete metabolization in the body. It has high sorption affinity onto soils and its residues have been reported in rivers, groundwater and accumulated in soils. Due to its continuous input and persistence in the environment, it affects mammalian cell replication causing adverse drug reactions and thus threatens human health. Based on the above-mentioned facts, determination of Cf residues in biological fluids (urine, blood and tissues), soil and water has been receiving more attention nowadays [4, 6].

As per United States Pharmacopeia the reference method for the quantitative determination of ciprofloxacin in Pharmaceuticals is HPLC method [2, 7]. There are several analytical methods reported in literatures for the determination of Cf in its different forms and preparations. Some of these methods are spectrophotometric [8], fluorescence [4, 9], electrochemical [10], chemiluminescence [11], capillary electrophoresis [12], chromatographic [13], and flow injection analysis (FIA) methods [3, 14]. Although some of the reported methods mentioned above have pros and cons in terms of selectivity, sensitivity, time and cost of analysis and simplicity but analytical methods always have space for improvement and extension. The associated limitations with some methods include high cost and complexity of instrumentation, unavailability of skilled technical personals and instruments (as in case of HPLC and LCMS) and longer analysis time. In many cases several sample preparation and clean-up steps are involved and some of the methods are prone to negative interferences effects.

Semiconductor nanocrystals synthesized from elements of groups II and VI or groups III and V include CdSe, CdS, CdTe, and ZnS [15]. These are usually referred to as quantum dots (QDs), and have diameters in the range of 2-10 nm. These are excellent inorganic luminophores and due to the apparent quantum confinement effect, QDs have unique optical and electrical properties such as broad excitation spectra, high luminous efficiency, narrow and symmetrical emission spectrum, controllable emission wavelength, good water dispersibility and low photobleaching sensitivity equated with the equivalent bulk materials [16]. Many reports on the use of QDs with different surface modification are available in the literature for analysis of antibiotics and other medications [4], veterinary medicines [15], compounds found in human body [16-18], neurotransmitters and radioprotectants [16, 19], metal ions [20], anions [16, 21], toxicants [22], pesticides [16, 23], antioxidants [24], explosives [16], industrial chemicals [25] and many more.

All the scaffolds having QDs as the main skeleton and used for fluorescence-sensing involve change in fluorescence intensity of QDs. The nanoflurophores especially, metal chalcogenides are doped with transition metals semiconductors in which the doped ions act as luminescence centers, intensify the luminescence and shorten the life time due to the interaction of the sp electron hole of the metal chalcogenide and the 3d electrons of Mn^{2+} ion. These have excellent optical properties in comparison to their corresponding host nano-materials [15]. In the past decades, Cadmium QDs have been widely used for a variety of luminance-based purposes [26]. However, the toxic effects of cadmium QDs restricted their application in biological and environmental detection [27]. Fortunately, ZnS QDs having low toxicity, wide band gap energy (3.7 eV) [17] for various dopants and small exciton Bohr radius is the perfect host material to replace Cd [28]. Mn-doped ZnS QD causes the red-shift of the initial emission wavelength of ZnS quantum dots from 450 nm to 590 nm [29], where the redshift is triggered by the variation of transition from 4T1 to 6A1 in ZnS crystal stimulated by Mn ions [30].

In order to improve the selectivity, solubility in aqueous/biological system and stability of doped quantum dots compared to their corresponding bare ones and to diminish the QDs' crystal defect, surface ligands engineering is carried out [17]. Several capping agents including amino acids [31], cetyltrimethyl ammonium bromide, polyethylenimine, DNA sequence, thioglycolic acid, d-penicillamine, mercaptopropionic acid, N-(2-hydroxybenzyl)- cysteine, thioglycerol, peptide, etc. have been reported [17].

The aim of the present work was to develop a simple, sensitive and fast spectrofluorimetric method for determination of ciprofloxacin based on its quenching effect on the fluorescence intensity of colloidal water soluble TGA caped, Mn doped ZnS quantum dots (QDs). The QDs having characteristic fluorescence spectra were characterized using Energy Dispersive X-Ray and X-ray Diffraction techniques. The effect of various parameters such as concentration of QDs, time, pH, common excipient and metal ions on the quenching phenomenon was investigated. The method was validated using various statistical parameters and average % recovery from commercial formulations was investigated. The method under optimized conditions was found to be interference free and was successfully applied to determine ciprofloxacin in spiked serum and urine samples.

**Corresponding author email: mianchem@uom.edu.pk*

EXPERIMENTAL

Chemicals and Reagents

Analytical grade chemicals were used throughout the research work and no further purification was carried out. Required chemicals were zinc sulphate heptahydrate (Scharlau, Spain, EU), Manganese sulphate monohydrate, sodium sulphide monohydrate, thioglycolic acid, (TGA), and ethanol from Merck (Darmstadt Germany), phosphoric acid, acetic acid, boric acid, hydrochloric acid and sodium hydroxide (Sigma–Aldrich, St. Louis, USA). Standard reference of ciprofloxacin was thankfully received from Shaheen pharmaceutical Industry in Swat. Commercial formulations of ciprofloxacin such as ciproquine and ciprol were brought from local market.

Instruments

Spectroflourophotometer (RF5301 PC, Shimadzu, Japan) having 150-watt Xenon lamp as excitation source with 1.0 cm quartz cell was used throughout the experimental work for fluorescence measurements. The emission and excitation slit width was 4 nm for fluorimetric operation. Required chemicals were weighed using electrical digital balance Schimadzu ATY224 (Shimadzu Corp., Kyoto, Japan) and stirring of the reaction mixture was carried out with the help of heating magnetic stirrer (Model Hei-Tec P/N: 505-30000-00, Heidolph Germany). X-ray Diffraction (XRD) analysis of the QDs was done using X-ray diffractometer (Rigaku D/Max-II, Japan) with Cu-Kα radiation (40 kV, 30 mA). Energy Dispersive X-Ray (EDX) (JSM 5910, JEOL Japan) was employed for the elemental analysis of the QDs. Borosilicate glassware and apparatus were used for respective operation.

Preparation of TGA capped Mn dopped ZnS QDs

Colloidal water-soluble Mn doped ZnS QDs capped with TGA were synthesized by arrested precipitation method via the procedure described by Gonzalez [32] with little modification. In a glass beaker, 0.3 mL of TGA, 10 mL of $ZnSO_4.7H_2O$ (0.014 M) and 5.0 ml of $MnSO_4.H_2O$ (0.0012 M) solutions were mixed and by drop wise addition of NaOH (1.0 M), with vigorous stirring, pH of the mixture was adjusted up to 8.0 ± 0.5. During addition of NaOH solution to the mixture of TGA and Zn^{2+}, it was observed that the colour of the solution became yellow along with little whitish curds formation. The curds disappeared immediately and finally clear solution was obtained. This change was due to different dissociation levels of sulfihydryl and carboxylate groups at different pH levels, shaping Zn thio complexes with different structures. After the adjustment of pH, 10 mL of $Na_2S.9H_2O$ (0.014 M) was added drop wise with vigorous stirring. Mn dopped ZnS QDs capped with TGA obtained were separated in precipitate from by the addition of ethanol and then filtering the mixture. The residue was air dried and a portion of the QDs were subjected to characterization.

Procedure for spectrofluorimetric determination of ciprofloxacin

Required solutions of QDs were prepared which remained soluble and stable in distilled water at room temperature without any precipitation. In order to optimize the concentration of QDs and the pH of the medium for maximum fluorescence quenching and maximum sensitivity, different volumes of the stock solution (100 µg mL^{-1}) of QDs were taken in a series of 10 mL volumetric flasks for achieving concentration in the range of 8-12 µg mL^{-1}, added 2.0 mL of 20 µg mL^{-1} of standard Cf solution to each flask and emission fluorescence intensity (FI) of the QDs (represented as F°) and that of Cf added QDs (represented as F) was measured at 632 nm after excitation at 315 nm against reagent blank using spectroflourimeter. The relative FI (represented as F°/F) was calculated which shows the extent of fluorescence quenching.

Similarly, for investigation of the effect of pH on the quenching phenomenon, in a number of volumetric flasks (10 mL), 1.0 mL of QDs solution (100 µg mL^{-1}), 2.0 mL of Britton-Robinson buffer solution in the range of pH 2-10 and 2.0 mL of standard ciprofloxacin solution were mixed and made the volume with distilled water up to the mark and fluorescence intensity in each case was measured as mentioned above.

In order to study the fluorescence quenching as a function of concentration of Cf, in several volumetric flasks (10 mL), 10 µg mL^{-1} solutions of QDs and different volumes of Cf solution to achieve concentration in the range of 0.2-10 µg mL^{-1} were mixed. To each flask, 2.0 mL of buffer of pH 8 was added and each solution diluted up to the mark with distilled water. The blank solution of QDs was prepared without the addition of Cf and measured the fluorescence intensity.

Effect of excipients and common ions on quantification of Cf (Interference study)

Interference study was carried out by mixing 2.0 µg mL^{-1} ciprofloxacin, 2, 4 and 6 µg mL^{-1} solutions of common excipients in the ratio of 1:1, 1:2 and 1:3. Each solution was buffered using 2.0 mL of buffer of pH 8 and distilled water was added for dilution upto the final volume of 10 mL. The fluorescence measurements were done under the optimized conditions as a function of the excipient concentration and % recoveries were calculated.

Sample solution preparation

Two tablets of each commercial brand (Ciprol 500 mg, Ceproquine 250 mg) were weighed and average mass of each tablet was found to be 1.787 and 0.890 g for ciprol and ciproquine respectively. Tablets were ground into fine powder, mixed and weighed, dissolved in ethanol (20 mL), with vigorous shaking, then filtered and transferred to volumetric flasks (100 mL) and diluted with distilled water. The proposed method was extended to quantify Cf in each commercial formulation of three different concentrations (2.0, 4.0 and 6.0 µg mL^{-1}). The quantity of active ingredient of Cf was calculated using standard calibration curve and the respective linear equation.

In a number of volumetric flasks (10 mL), known amount of each commercial formulation (equivalent to 2.0 µg mL^{-1}) and 2.0, 4.0 and 6.0 µg mL^{-1} of standard sample were mixed in triplicates that formed final concentration as 4.0, 6.0 and 8.0 µg mL^{-1} of Cf with 2.0 mL of 10 µg mL^{-1} of QDs and 2.0 mL of buffer of pH 8 and dilution was done with distilled water. For quantification purposes, the successive steps of the proposed method were followed, concentration of the drug was determined and % recoveries calculated.

Urine and blood serum samples were provided by a local pathology laboratory collected from a healthy male volunteer following all ethical guidelines and stored frozen until analyzed. During analysis, triplicates of 1.0 mL of serum sample was spiked with variable concentration of Cf (2.0-6.0 µg mL^{-1}), then acetonitrile (1.2 mL) was added to remove serum protein. Subsequently, for removal of the serum protein residues, the mixture was centrifuged for 15 min at 3500 rpm and the supernatant was transferred to a 10 mL volumetric flask in each case. Then FI was measured using the procedure outlined above and recovery in each case was calculated.

Urine samples (4.0 mL each) were spiked with different concentration of Cf (2.0-6.0 µg mL^{-1}) in triplicates. To each mixture 2.0 mL of 10 µg mL^{-1} of QDs and 2.0 mL of buffer of pH 8 were added and final dilution was done with distilled water. For each solution FI was measured and concentration and the subsequent % recovery of Cf was calculated.

RESULTS AND DISCUSSION

The fluorescent spectra of TGA capped Mn doped ZnS QDs was recorded by scanning the λ_{ex} and λ_{em} in the range of 250-750 nm and it was observed that QDs show excitation peak at 315 nm followed by emission peak at 632 nm. The broad band emission at longer wavelength (632 nm) was due to trap state emission between the valance and conduction band that originates from surface of QDs.

Mechanistic approach to quantification of Cf using TGA capped Mn-ZnS QDs

Nowadays, the application of QDs for the sensory system based on quenching of the FI of QDs has got extensive attention because of electron transfer (ET), fluorescence resonance energy transfer, or other interactions that occur at the surface of QDs. Recently, it has been reported in various studies that electron transfer occurs when hole or electron acceptor specie get attached at the QDs surface and thus affect the recombination of electron-hole pair and decreases the fluorescent intensity [33]. The above facts justify the capability of Cf to get adsorbed onto the surface of Mn-ZnS QDs capped by TGA, thus affecting radiative recombination mechanism which reduces the fluorescence intensity.

Characterization of QDs

Energy Dispersive X-Ray (EDX) analysis is an important tool for elemental analysis and for detection of impurity. Figures 1a and 1b shows the EDX spectra of Mn doped ZnS and TGA capped Mn doped ZnS respectively. Figure 1a shows peak for Mn, Zn and S confirming the formation of Mn doped ZnS. Figure 1b

shows the peaks for C, O, Mn, Zn and S, which confirm the synthesis of TGA capped Mn doped ZnS. All the peaks were related with the constituent elements, verifying no impurities are present.

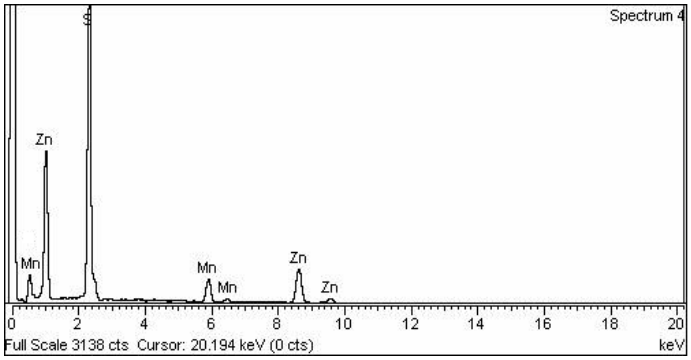

Figure 1a. EDX spectra of Mn doped ZnS QDs.

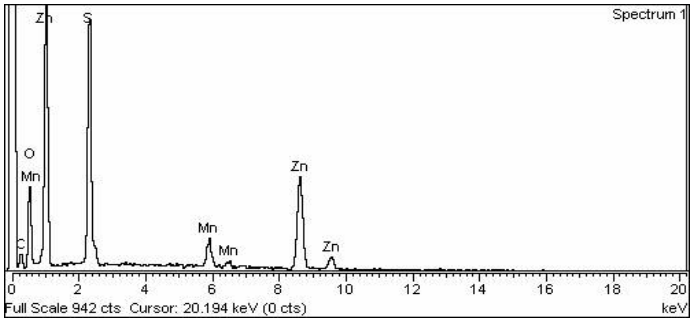

Figure 1b. EDX spectra of TGA-capped Mn doped ZnS QDs.

Figures 2 a and b show the X-ray Diffraction (XRD) pattern of TGA capped Mn-ZnS and Mn-ZnS respectively. The XRD spectrum shows that the peaks appeared at 28.6º, 48º and 55.4º indicates the crystal plane of (111), (220) and (311) respectively, which suggests the nanocrystals cubic closed packed structure of Zinc blend. The findings are in good agreement with those reported in the literature [JCPDS NO: 5-0566] [34]. From the width of the XRD peak the average grain size was estimated to be 6 nm using the Scherrer equation. Since the particle size is greater than excitonic Bohr radius of ZnS (2.5 nm), the particles are confined to a weak quantum regime [35].

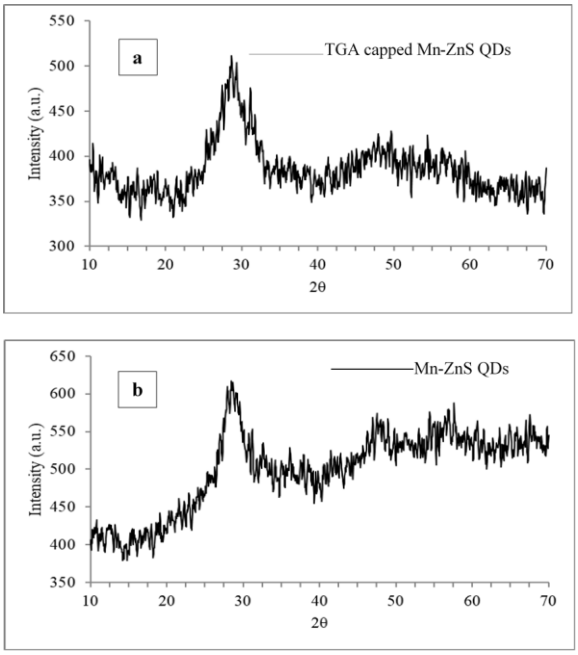

Figure 2. XRD pattern of (a) TGA capped Mn-ZnS QDs and (b) Mn-ZnS QDs.

Optimization of the conditions for maximum fluorescence Quenching of QDs

The concentration of QDs was varied in the range of 8-14 µg mL^{-1} at a fixed concentration of Cf (4.0 µg mL^{-1}) and as shown in figure 3, it was found that maximum quenching occurs with 10 µg mL^{-1} of QDs. This concentration of QDs was selected optimum and was kept constant during further studies.

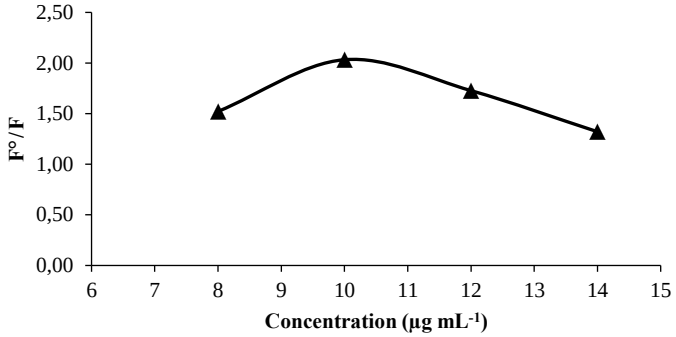

Figure 3. Quenching effect as a function of concentration of QDs.

The effect of pH on quenching was studied by using Briton Robinson buffer in the range of pH 2-10 and maximum quenching was observed at pH 8. This observation can be attributed to the fact that at lower pH, the TGA would get detached from the QDs surface and form thiol by protonation which decreases the fluorescence intensity. The results are shown in figure 4.

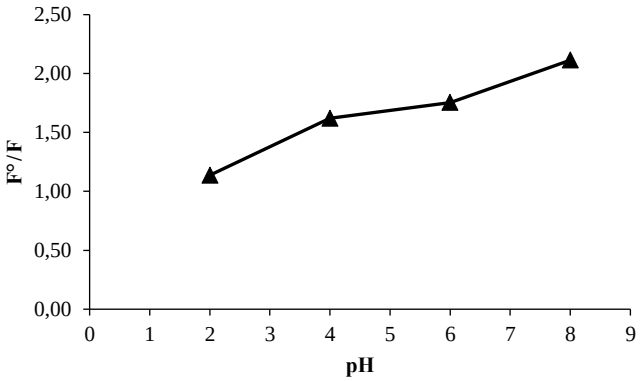

Figure 4. Effect of pH on extent of quenching of QDs by Cf.

Effect of concentration of Cf on extent of quenching of FI of QDs

The relative FI of QDs was investigated as a function of concentration of Cf in the range of (0.5-10 µg mL^{-1}) at pH 8 and it was found to increase linearly with excellent correlation coefficient (r^2=0.993). The results are shown in figure 5. The curve and the linear equation shown in figure 5 was used for calculation of concentration of Cf in subsequent studies.

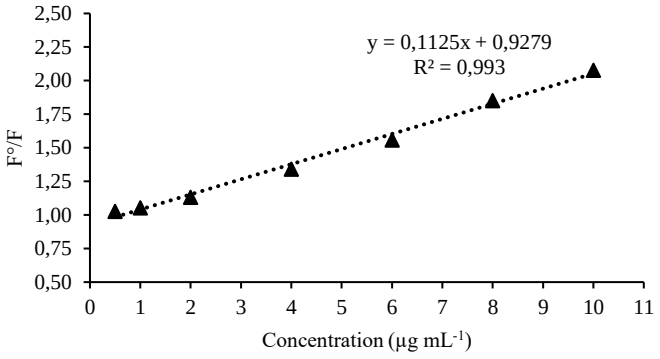

Figure 5. Effect of concentration of Cf on extent of quenching of FI of QDs.

Effect of common excipients and interference metal ions

Excipients which are normally used in the manufacturing of Cf tablets include sorbitol, glucose, lactose, sucrose, magnesium stearate and starch. These can possibly interfere with the determination following the proposed method and thus the selectivity of the method become doubtful. Likewise, metal and compound ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, HCO^{3-} and CO_3^{2-} may also interfere in the analysis. These possible interfering substances were then added sequentially to assay the samples at concentrations 1-3-fold higher than the concentration of Cf (2.0 µg mL^{-1}). The results are given in figure 6, which show that the method is interference free in both cases i.e. in case of common excipients as well as in case of common ions.

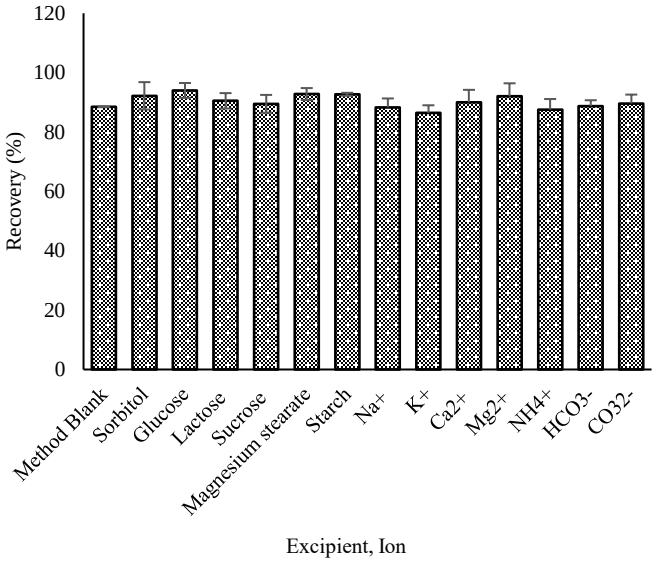

Figure 6. Effect of excipients and common ions on the % recovery of Cf.

Application of the proposed method for quantification of Cf in drug formulations and biological samples

To prove its utility, the proposed method was applied to measure the concentrations of Cf in serum and urine samples and also in commercial formulations at different spiking levels. Percent recovery of Cf from two different formulations i.e. ciprol 500 mg and ciproquine 250 mg was investigated. The quantity of active ingredient was calculated using standard calibration curve. The results as given in table 1, indicate excellent average percent recovery (±SD) in the range of 92.2±1.8 to 99.5±1.5 µg mL^{-1}.

Table 1. Recovery studies for Cf formulations in the form of tablets.

Drug Formulation	Amount of active ingredient of Cf (µg mL^{-1})			Average % Recovery±SD
	Contained in drug solution	Added	found	
Ciprol 500 mg	2	2	3.77	94.2±3.0
		4	5.82	97.0±2.7
		6	7.38	92.2±1.8
Ciproquine 250 mg		2	3.98	99.5±1.5
		4	5.86	97.6±1.4
		6	7.66	95.7±1.2

The proposed method was also applied to determine average percent recovery (±SD) of Cf from spiked human serum and urine samples. The developed method shows good applicability in term of higher percent recoveries (90.5±1.2 to 96.7±1.4 µg mL^{-1} for blood serum and 94.0±1.2 to 98.5±1.4 µg mL^{-1} for urine samples) as shown by the results given in table 2.

Table 2. Extraction recovery for Cf from spiked serum and urine samples.

Sample	Number of replicates	Amount of active ingredient of Cf (µg mL^{-1})		Average % Recovery±SD
		Added	found	
Serum	3	2	1.81	90.5±1.2
	3	4	3.87	96.7±1.4
	3	6	5.48	91.3±1.4
Urine	3	2	1.97	98.5±1.4
	3	4	3.76	94.0±1.2
	3	6	5.74	95.6±0.5

Analytical and statistical parameters

By employing the proposed method for determination of Cf under optimum conditions, replicate analysis of the lowest detectable concentration of Cf (0.5 µg mL^{-1}) and linearity studies were performed and analytical parameters for validation of the method were calculated as given in table 3. Over a concentrations range of 0.50–10 µg mL^{-1}, the calibration curve for Cf showed an excellent linearity with the equation y = 0.1125x +0.9279 and r^2 = 0.993. The standard deviation of the proposed method calculated against method blank was found to be 0.0056 µg mL^{-1}.

The limit of detection (LOD) and the limit of quantitation (LOQ) for Cf (signal to noise ratio 3:1 for LOD and 10:1 for LOQ) were calculated to be 0.15 and 0.50 µg mL^{-1} respectively. The average percentage recovery of Cf at varied spiking level (2, 4 and 6 µg mL^{-1}, *n*=3) shows the accuracy of the proposed method and was found to be in the range of 92.2±1.8 to 99.5±1.5 µg mL^{-1} for commercial formulations, 90.5±1.2 to 96.7±1.4 µg mL^{-1} for serum and 94.0±1.2 to 98.5±1.4 µg mL^{-1} for urine samples.

Table 3. Analytical and statistical parameters for the proposed method.

Parameter	Value
λex (nm)	315
λem (nm)	632
Linear range (µg mL^{-1})	0.50-10
Linear Equation	y = 0.1125x +0.9279
Correlation coefficient (r^2)	0.993
Standard deviation (µg mL^{-1})	0.0056
Relative standard deviation (%)	1.10
Limit of detection (µg mL^{-1})	0.15
Limit of quantitation (µg mL^{-1})	0.50

Comparison of the proposed method with reported analytical methods

The proposed method was compared with previously published methods for quantification of ciprofloxacin (Table 4). The linearity of the proposed method is better than spectrophotometric methods and comparable to other fluorimetric methods. The proposed method is less precise and accurate as compared to a reported method [1] which involve the use of CdS QDs but the environment friendly nature of ZnS QDs as compared to CdS QDs outweigh this deficiency.

Table 4. Comparison of Cf determination by several analytical methods.

Method	Linearity range (μg mL^{-1})	RSD (%)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	Ref.
Present work	0.5-10	1.10	0.15	0.50	-
MPA-CdS QDs/Fluorescence probe	0.13-150	2.90	0.04	-	[5]
Fluorimetry	0.4-100	4.00	0.76	-	[36]
HPLC	0.06-1.5	-	0.02	-	[37]
HPLC-UV	0.05-6.0	1.80	0.15	0.05	[38]
ITP–CZE	0.1-0.15	0.60	0.05	0.10	[39]
Square-wave stripping voltammetry	0.01–0.75	6.96	0.01	0.08	[5]
Differential pulse adsorptive voltammetry	0.03–6.6	-	16.00	-	[40]
Amperometry	13.0–331	-	1.98	-	[41]
Cyclic voltammetry	0.033–331	-	0.01	0.08	[42]
Potentiometry/MIP	16.4–331	-	3.30	16.50	[43]
Potentiometry/MIP		-	3.30	-	[44]
Potentiometry/Nano-composite based electro sensor	0.3–3310	-	0.30	-	[45]
Spectrophotometric	1.0-15.0	0.79	0.17	0.52	[11]
Charge transfer/Spectrophotometric	7.5- 42.3	1.00	-	-	[46]
Reverse FIA Spectrophotometry	0.5-16	≤1.73	0.20	0.69	[3]
SPE/Spectrophotometric	0.05-0.3	1.11	0.04	0.12	[47]

ITP–CZE: Capillary isotachophoresis–capillary zone electrophoresis.
MIP: Molecularly imprinted polymers.

CONCLUSIONS

The present study describes development and validation of a simple and fast spectrofluorimetric method for determination of ciprofloxacin and is based on fluorescence quenching of the water soluble TGA capped Mn-ZnS QDs by the subject analyte. The QDs having characteristic fluorescence spectra with maximum excitation at 315 nm followed by an emission peak at 632 nm were prepared by arrested precipitation method and characterized by EDX and XRD. The effect of various factors such as pH, time and concentration of QDs on the quenching of QDs by Cf was investigated. It was found that maximum quenching occurs at 10 ppm of QDs at pH 8. The Stern-Volmer calibration plot was found to be linear in the range of 0.5-10 μg mL^{-1} with $r^2 = 0.993$. The percent relative standard deviation, LOD and LOQ of the proposed method were found to be 1.10%, 0.15 and 0.50 μg mL^{-1} respectively. High % recovery (90.5±1.2% to 98.5±1.4%) was obtained for Cf in commercial formulations and biological samples. The method was found to be free of interferences when applied in the presence of common excipients like sorbitol, starch and glucose. The starting materials that were used for QDs preparation are easily available in the laboratory. In terms of accuracy and precision, the proposed method is comparable to many of the reported methods. The data evidently proves the potential of the proposed sensor QDs in biological analysis.

REFERENCES

1. R. Kharat, S. Jadhav, S. Pawar, A. Tamboli, *PharmaTutor* **3**, 51 (2015).
2. N.S. Turkie, H.Q. Munshid, *J. Pharm. Sci Res.* **11**, 1563 (2019).
3. S. Palamy, W. Ruengsitagoon, *Spectrochim. Acta A.* **190**, 129 (2018).
4. T. Madrakian, S. Maleki, A. Afkhami, *Sensor. Actuat. B-Chem.* **243**, 14 (2017).
5. xxA.-N. Kawde, M.A. Aziz, N. Odewunmi, N. Hassan, A. AlSharaa, *Arab. J. Sci. Eng.* **39**, 131 (2014).
6. J. Wang, J. Dai, M. Meng, Z. Song, J. Pan, Y. Yan, C. Li, *J. App. Polym. Sci.* **131**, (2014).
7. xxUSP38NF33, US. Pharmacopeia & National Formulary, 2016, 3162–3270.
8. A.El-Kosasy, O. Abdel-Aziz, N. Magdy, N. El Zahar, *Spectrochim. Acta A* **157**, 26 (2016).
9. M.E. El-Kommos, G.A. Saleh, S.M. El-Gizawi, M.A. Abou-Elwafa, *Talanta* **60**, 1033 (2003).
10. M.P. Deepak, G.P. Mamatha, *Anal. Bioanal. Electrochem.* **7**(5), 523 (2015).
11. Y.-D. Liang, J.-F. Song, X.-F. Yang, *Anal. Chim. Acta* **510**, 21 (2004).
12. X. Xu, L. Liu, Z. Jia, Y. Shu, *Food Chem.* **176**, 219 (2015).
13. S.N. Razzaq, M. Ashfaq, I. Mariam, I.U. Khan, S.S. Razzaq, G. Mustafa, *J. Chilean Chem. Soc.* **62**, 3572 (2017).
14. I.M. Shakir, R.F. Hassan, *Iraqi J. Sci.* **57**, 522 (2016).
15. S. Sadeghi, M. Jahani, F. Belador, *Spectrochim. Acta A* **159**, 83 (2016).
16. H. Wang, C. Chen, Y. Liu, Y. Wu, Y. Yuan, Q. Zhou, *Talanta* **198**, 242 (2019).
17. K. Abha, J. Nebu, J.A. Devi, R. Aparna, R. Anjana, A. Aswathy, S. George, *Sensor. Actuat. B-Chem.* **282**, 300 (2019).
18. S. Kulchat, W. Boonta, A. Todee, P. Sianglam, W. Ngeontae, *Spectrochim. Acta A* **196**, 7 (2018).
19. Z. Wang, Y. Zhang, B. Zhang, X. Lu, *Talanta* **190**, 1 (2018).
20. M. Hua, S. Yang, J. Ma, W. He, L. Kuang, D. Hua, *Talanta* **190**, 278 (2018).
21. B.-H. Zhang, F.-Y. Wu, Y.-M. Wu, X.-S. Zhan, *J. Fluoresc.* **20**, 243 (2010).
22. H. Bhardwaj, C. Singh, M. kumar Pandey, G. Sumana, *Sensor. Actuat. B-Chem.* **231**, 624 (2016).
23. R. Ban, J.-j. Zhu, J. Zhang, *Microchim. Acta* **181**, 1591 (2014).
24. X. Tan, S. Liu, Y. Shen, Y. He, J. Yang, *Spectrochim. Acta A* **133**, 66 (2014).
25. Y. Gong, H. Wu, Z. Fan, *Anal. Lett.* **46**, 2454 (2013).
26. W.C. Chan, S. Nie, *Science* **281**, 2016 (1998).
27. N. Pradhan, D. Goorskey, J. Thessing, X. Peng, *J. Am. Chem. Soc.* **127**, 17586 (2005).
28. H. Labiadh, B. Sellami, A. Khazri, W. Saidani, S. Khemais, *Optic. Mater.* **64**, 179 (2017).
29. J. Suyver, S. Wuister, J. Kelly, A. Meijerink, *Nano Lett.* **1**, 429 (2001).
30. A. Bol, A. Meijerink, *Phy. Rev. B* **58**, R15997 (1998).
31. P. Wu, X.-P. Yan, *Chem. Soc. Rev.* **42**, 5489 (2013).
32. E. Sotelo-Gonzalez, M.T. Fernandez-Argüelles, J.M. Costa-Fernandez, A. Sanz-Medel, *Anal. Chim. Acta* **712**, 120 (2012).

33. M. Cao, M. Liu, C. Cao, Y. Xia, L. Bao, Y. Jin, S. Yang, C. Zhu, *Spectrochim. Acta A* **75**, 1043 (2010).
34. N. S. Nirmala Jothi, G. J. Amish, R. J. Vijay, *Mater. Chem. Phy.* **138**, 186 (2013).
35. Y. Motlan, G. Zhu, K. D. Tomsia, K. McBean, M. R. Phillips, E. M. Goldys, *Optic. Mater.* **29**, 1579 (2007).
36. S. Shtykov, T. Smirnova, Y.G. Bylinkin, N. Kalashnikova, D. Zhemerichkin, *J. Anal. Chem.* **62**, 136 (2007).
37. W. Gau, H. Ploschke, K. Schmidt, B. Weber, *J. liquid Chromatogr.* **8**, 485 (1985).
38. M.T. Maya, N.J. Gonçalves, N.B. Silva, J.A. Morais, *J. Chromatogr. B* **755**, 305 (2001).
39. M. Hernández, C. Aguilar, F. Borrull, M. Calull, *J. Chromatogr. B* **772**, 163 (2002).
40. H. Yi, C. Li, *Russian J. Electrochem.* **43**, 1377 (2007).
41. L. Fotouhi, M. Alahyari, *Colloid. Surf. B* **81**, 110 (2010).
42. A.A. Ensafi, A.R. Allafchian, R. Mohammadzadeh, *Anal. Sci.* **28**, 705 (2012).
43. A.H. Kamel, W.H. Mahmoud, M.S. Mostafa, *Anal. Meth.* **3**, 957 (2011).
44. H.M. Oliveira, F.T. Moreira, M.G.F. Sales, *Electrochim. Acta* **56**, 2017 (2011).
45. F. Faridbod, T. Poursaberi, P. Norouzi, M.R. Ganjali, *Int. J. Electrochem. Sci.* **7**, 3693 (2012).
46. S. Mostafa, M. El-Sadek, E.A. Alla, *J. Pharmacol. Biomed. Anal.* **27**, 133 (2002).
47. M.I. Pascual-Reguera, G.P. Parras, A.M. Díaz, *Microchem. J.* **77**, 79 (2004).