Optosensor based on multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for ciprofloxacin detection

Naphat Yuphintharakun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Chemistry

Prince of Songkla University

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Major Program	Chemistry

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Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

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Candidate

ชื่อวิทยานิพนธ์ เซนเซอร์ทางแสงโดยใช้ท่อนาโนคาร์บอนผนังหลายชั้นร่วมกับควอนตัมดอทและพอลิเมอร์ลอกแบบโมเลกุลสำหรับตรวจวัดซิโปรฟลอกซาซิน

ผู้เขียน นางสาวณภัทร ยุพินทรกุล

สาขา เคมี

ปีการศึกษา 2560

บทคัดย่อ

วิทยานิพนธ์นี้ได้พัฒนาเซนเซอร์ทางแสงโดยวัดสัญญาณฟลูออเรสเซนต์ที่ลดลงของควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลและท่อนาโนคาร์บอนผนังหลายชั้นที่ปรับปรุงหมู่ฟังก์ชั่นด้วยหมู่คาร์บอกซิลิกสำหรับตรวจวิเคราะห์ซิโปรฟลอกซาซิน ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุล ทำให้เซนเซอร์ที่พัฒนาขึ้นมีความจำเพาะเจาะจงกับซิโปรฟลอกซาซินและมีความเสถียรที่ดี อีกทั้งท่อนาโนคาร์บอนผนังหลายชั้นที่ปรับปรุงหมู่ฟังก์ชั่นด้วยหมู่คาร์บอกซิลิกสามารถเพิ่มประสิทธิภาพการจับกับสารซิโปรฟลอกซาซิน ทำให้สามารถตรวจวัดซิโปรฟลอกซาซินได้เร็วขึ้น โดยศึกษาสภาวะที่เหมาะสมเพื่อเพิ่มประสิทธิภาพในการตรวจวัด ได้แก่ พีเอชของสารละลายบัพเฟอร์ เวลาที่ใช้ในการเกิดอันตรกิริยา ปริมาณของท่อนาโนคาร์บอนผนังหลายชั้นที่ปรับปรุงหมู่ฟังก์ชั่นด้วยหมู่คาร์บอกซิลิก และอัตราส่วนโมลของแม่แบบต่อมอนอเมอร์ต่อสารเชื่อมขวางภายใต้สภาวะที่เหมาะสมวิธีที่พัฒนาขึ้นให้ช่วงความเป็นเส้นตรงตั้งแต่ 0.10 ถึง 1.0 ไมโครกรัมต่อลิตร และ 1.0 ถึง 100.0 ไมโครกรัมต่อลิตร มีขีดจำกัดในการตรวจวัด 0.066 ไมโครกรัมต่อลิตร วิธีที่พัฒนาขึ้นสามารถประยุกต์ใช้สำหรับการตรวจวัดซิโปรฟลอกซาซินในตัวอย่างเนื้อไก่และนมได้ โดยมีค่าร้อยละการได้กลับคืนอยู่ในช่วง 82.6 ถึง 98.4 เปอร์เซ็นต์ และมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 8 เปอร์เซ็นต์ อีกทั้งวิธีที่พัฒนาขึ้นให้ผลการตรวจวิเคราะห์ที่สอดคล้องกับวิธีมาตรฐาน (เทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูง) ข้อดีของเซนเซอร์ทางแสงที่พัฒนาขึ้นคือ มีความไววิเคราะห์สูง มีความจำเพาะเจาะจงสูง ใช้งานง่าย ราคาถูกและรวดเร็ว

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Abstract

This thesis aimed to develop an optosensor based on the fluorescence quenching of quantum dots composited with molecularly imprinted polymer and carboxylic functionalized multiwall carbon nanotubes (COOH@MWCNT-MIP-QDs) for the detection of ciprofloxacin. The quantum dots composited with MIP provided a good selectivity and stability. The carboxylic functionalized multiwall carbon nanotubes helped to enhance the adsorption ability which can reduce the analysis time for ciprofloxacin detection. The effect of various parameters were optimized *i.e.* pH of buffer solution, incubation time, concentration of COOH-functionalized multiwall carbon nanotubes and ratio of template to monomer to cross-linker. Under the optimum conditions, the calibration curves were linear over the concentration range of 0.10 to 1.0 µg L^{-1} and 1.0 to 100.0 µg L^{-1}. The limit of detection was 0.066 µg L^{-1}. The developed method was successfully applied for the determination of ciprofloxacin in chicken muscle and milk samples. The satisfactory recoveries were obtained in the range of 82.6 to 98.4 % and the relative standard deviations were less than 8 %. The developed method was compared with HPLC method and the results were in good agreement with HPLC. The advantages of this method including high sensitivity, good selectivity, simple to use, cost-effective and rapid.

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Finally, I would like to thank my family for their loves, encouragement and support throughout my life.

Naphat Yuphintharakun

The Relevance of the Research Work to Thailand

The purpose of this Master of Science Thesis in Chemistry (Analytical chemistry) is to develop and enhance the performance of an optosensor which composited of COOH-functionalized multiwall carbon nanotubes, quantum dots and molecularly imprinted polymer for ciprofloxacin detection.

This developed optosensor is high sensitivity, good selectivity, simple to use and can be applied for the determination of ciprofloxacin in chicken muscle and milk samples. The developed method in this thesis can help to reduce analysis cost, analysis time and several government organizations in Thailand can use the outcome of this work include the Ministry of Public Health, Ministry of Industry, Ministry of Environment and the Ministry of Education.

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List of abbreviations

APTES	3-aminopropyltriethoxysilane
CB	Conduction band
CdS	Cadmium sulfide
CdSe	Cadmium selenide
CdTe	Cadmium telluride
CIP	Ciprofloxacin
COOH	Carboxylic acid
DDS	Drug delivery systems
EU	European Union
FLD	Fluorescence detector
FT-IR	Fourier transform infrared spectroscopy
GaP	Gallium phosphide
GSH	Glutathione
HPLC	High performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantification
MIP	Molecularly imprinted polymer
MPA	Mercaptopropionic acid
MRL	Maximum residue limit
MSA	Mercaptosuccinic acid
MWCNT	Multiwall carbon nanotubes
NIP	Non-imprinted polymer
PDA	Photodiode array detector
QDs	Quantum dots
R^2	Coefficient of determination
RSD	Relative standard deviation
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TEOS	Tetraethyl orthosilicate

List of abbreviations (Continued)

TGA	Thioglycolic acid
UV-Vis	Ultraviolet-visible
VB	Valence band

List of Publication

Paper **Yuphintharakun, N.,** Nurerk, P., Chullasat, K., Kanatharana, P., Davis, F., Sooksawat, D., and Bunkoed, O. A nanocomposite optosensor containing carboxylic functionalized multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for highly selective and sensitive detection of ciprofloxacin. *Spectrochim Acta A Mol Biomol Spectrosc* 201, **2018**, 382–391.

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1. Introduction

1.1 Background and the rationale

Ciprofloxacin is a fluoroquinolone antibiotic which is extensively used for the treatment of urinary, respiratory and digestive infections in humans and livestock (Gayen and Chaplin, 2016). It is also misused in the livestock industry since treating animals with these agents can increase productivity. However this can become a serious problem, since the antibiotics can be expressed in meat and milk leading to potential toxicity or allergic hypersensitivity reactions in humans. There is also a serious issue in that this practice may lead to the generation of antibiotic resistant human pathogens. For food safety and to protect human health, the European Union (EU) has set the maximum residue limit (MRL) for ciprofloxacin at 100 μg kg^{-1} in milk, chicken and pig muscle. Thus, it is of great importance to develop a convenient, rapid and reliable method for the determination of ciprofloxacin in food samples. Several methods have been reported for the determination of ciprofloxacin such as high-performance liquid chromatography (HPLC) (Chen *et al.*, 2014; Locatelli *et al.*, 2015; Vella *et al.*, 2015) capillary electrophoresis (Moreno-González *et al.*, 2017) and electrochemical techniques (Bagheri *et al.*, 2016; Shan *et al.*, 2016). However, these techniques can be complicated, require expensive instrument and highly skilled personnel and are time consuming. To overcome these problems, spectrofluorimetry has attracted interest as an alternative method due to its simplicity, rapidity and low cost. To improve the sensitivity of the method, quantum dots (QDs) nanoparticles have been used as a sensitive fluorescence probes for the determination of traces of various target analytes such as salicylic acid (Bunkoed and Kanatharana, 2015), glucose (Yu *et al.*, 2017), H_2O_2 (Gong *et al.*, 2017), 6-mercaptopurine (Jin *et al.*, 2017), ochratoxin A (Yao *et al.*, 2017), kaempferol (Tan *et al.*, 2014) and copper (II) ion (Geng *et al.*, 2017). Compared with organic fluorescent dyes, QDs have many unique optical properties, high fluorescence intensity, tunable size-dependent photoluminescence, good photostability and narrow symmetric emission (Amin *et al.*, 2017; Zhu *et al.*, 2017). For the determination of trace target analytes in real samples with high matrix interferences normally requires highly selective methods. To improve the further selectivity of these

methods, molecularly imprinted polymers (MIPs) have received considerable attention in recent years because of their high specificity and easy preparation (Wackerlig and Lieberzeit, 2015). MIPs are normally prepared by co-polymerization process of cross-linkers with functional monomers that form complexes with template molecules (analytes) prior to polymerization. After removal of the template molecules from the polymer the specific cavities which are complementary to the template molecule by size, shape and functional group can be obtained leading to the ability to rebind template molecule with high specificity (Piletsky *et al.*, 2012). MIPs not only provide high selectivity binding material but also have high stability meaning they can be used under extreme condition such as high pressure, temperature, extreme pH and in organic solvents. Since MIPs are cost-effective and robust materials they have been widely used in many fields such as solid phase extraction (Theodoridis *et al.*, 2006), solid phase microextraction (Zhao *et al.*, 2015; Asiabi *et al.*, 2016; Terzopoulou *et al.*, 2016), chemosensor and biosensor (Ji *et al.*, 2015; Kumar Singh and Singh, 2015; Zhou *et al.*, 2017), capillary electrophoresis (Zack *et al.*, 2010), enantiomeric separations (Yong *et al.*, 2010) and drug delivery systems (DDS) (Abdollahi *et al.*, 2018). For sensor applications, the composite fluorescence probes using QDs incorporating into MIPs have been developed as highly selective fluorescence probes for the determination of some target compounds such as salbutamol (Raksawong *et al.*, 2017), patulin (Zhang *et al.*, 2017), sulfadiazine (Ding *et al.*, 2017), sulfadimidine (Zhou *et al.*, 2017), malachite green (Wu *et al.*, 2017), tetracycline (Zhang and Chen, 2016), cocaine (Chantada-Vázquez *et al.*, 2016) and amoxicillin (Chullasat *et al.*, 2018). To improve the kinetic adsorption or affinity binding of ciprofloxacin, addition of carboxylic functionalized multiwall carbon nanotubes is an interesting alternative approach since they contain π structure which can adsorb aromatic compounds via π-π interactions (Xu *et al.*, 2017). The carboxylic functionalisation of multiwall carbon nanotubes can improve their dispersibility in aqueous media and it is easy to achieve further covalent functionalisation with other materials (Barabás *et al.*, 2015).

In this work, a nanocomposite optosensor containing COOH functionalized MWCNT and CdTe quantum dots embedded into a molecularly imprinted polymer was

developed for the determination of trace ciprofloxacin. The determination of ciprofloxacin is based on the fluorescence quenching when target analyte is bound to the specific recognition sites on the developed fluorescence probes. This combined the good fluorescence properties of QDs, with the high selectivity of MIPs and high adsorption affinity of COOH@MWCNT to produce a rapid, highly sensitive optosensor and for the determination of ciprofloxacin with good selectivity. The developed optosensor was applied to determine ciprofloxacin in chicken muscle and milk samples and also compared with a HPLC method.

1.2 Objective

To develop an optosensor using a nanocomposite of the carboxylic functionalized multiwall carbon nanotubes and quantum dots embedded into a molecularly imprinted polymer for the determination of trace ciprofloxacin in food samples.

1.3 Quantum dots nanoparticles

Quantum dots (QDs) are a semiconductor nanocrystal with diameters in the range of 1-10 nanometers (10-50 atoms). It can be made from an element consist of group II-VI, III-V, or IV-VI (Murray et al., 1993), such as cadmium sulfide (CdS) (Ngamdee *et al.*, 2017), cadmium selenide (CdSe) (Sajwan *et al.*, 2017), cadmium telluride (CdTe) (Chullasat *et al.*, 2018), zinc sulfide (ZnS) (Safitri *et al.*, 2017), gallium phosphide (GaP) (Yue *et al.*, 2006), lead sulfide (PbS) (Deng *et al.*, 2012). The structure of QDs as a point in nanometer-scale of semiconductor particles that it displays behavior within the atom or the quantum molecule based on principles of quantum physics. In addition, QDs possessing unique optical, chemical and electronic properties due to quantum confinement effects (Nsibande and Forbes, 2016). QDs typically have a narrow emission spectra, high photochemical stability, and tunable size. QDs can differ in the emission color or the color of QDs to be influenced by changing the size. A smaller size leads to lower wavelengths

(blueshift) and a larger size show the redshift in the fluorescence emission peak (Figure 1.1) (Frasco and Chaniotakis, 2009).

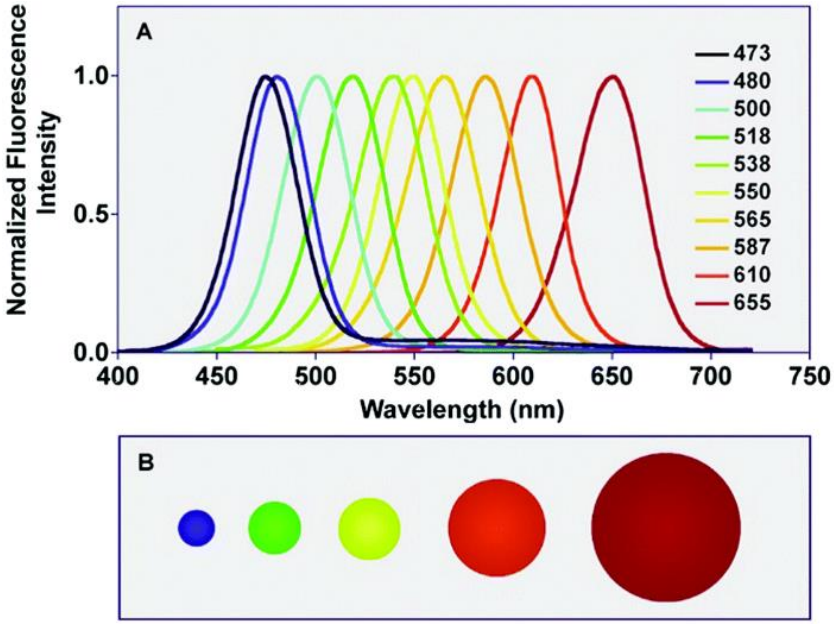

Figure 1.1 The size-dependent fluorescence emission spectra of QDs (A) and different relative particle sizes (B) (Girma *et al.*, 2017).

Structurally, QDs consist of a core, shell and capping molecule (Figure 1.2). The core of QDs is the crystalline element of group II-VI, III-V or IV-VI element. The QDs' core responsible for the fundamental optical properties include light absorption and emission. The core type can define approximately the wavelength of QDs such as cadmium selenide (CdSe) had emissive visible under visible excitation (Wang *et al*., 2017) and cadmium telluride (CdTe) had emissive visible under near-infrared excitation (Smyder and Krauss, 2011). CdSe and CdTe QDs exhibit the wavelength emission spectra range between 450 to 650 and 500 to 700 nm respectively (Mashinchian *et al*., 2014). However, the particle size is a fine adjustment of the light wavelength such as CdS core with diameter around 6 nm emits fluorescent wavelength at 525 nm, CdTe core with diameter around 2 nm emits fluorescent wavelength at 545 nm (Sai and Kong, 2011).

The shell is used to improve the optical properties of the QDs and to protect the core from surrounding medium such as ZnS in order to enhance the quantum yield. (Fontes *et al.*, 2012).

The capping molecules or stabilizer through modification of QDs surface. It can provide QDs stability and solubility in buffer solution. To maintain a high resistance to photophysical properties in aqueous media (Vasudevan *et al.*, 2015). QDs are typically stabilized by thioglycolic acid (TGA), mercaptopropionic acid (MPA), glutathione (GSH) and mercaptosuccinic acid (MSA) (Masab *et al.*, 2018).

The QDs are fluorescent nanoparticles with the size-controlled and shape-controlled absorption in the electronic states. QDs have bandgap energy that must be promotes an electron from the valence band (VB) to the conduction band (CB) leaving a hole in the VB and forming an exciton to produce light with energy equal to the bandgap energy. This effect refers to the quantum confinement. The bandgap increases as the size of the QDs decreases that the bandgap energy of QDs that vary as a size. Thus, the larger size is red-shifted to lower energy. It makes possible to tune the optical spectra by changing their size (Figure 1.3) (Freeman and Willner, 2012).

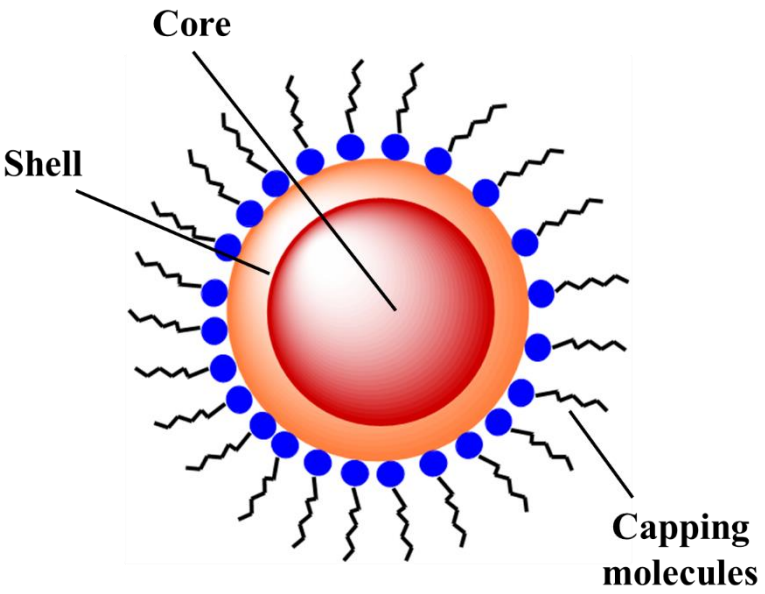

Figure 1.2 The structure of a functionalized core-shell QDs.

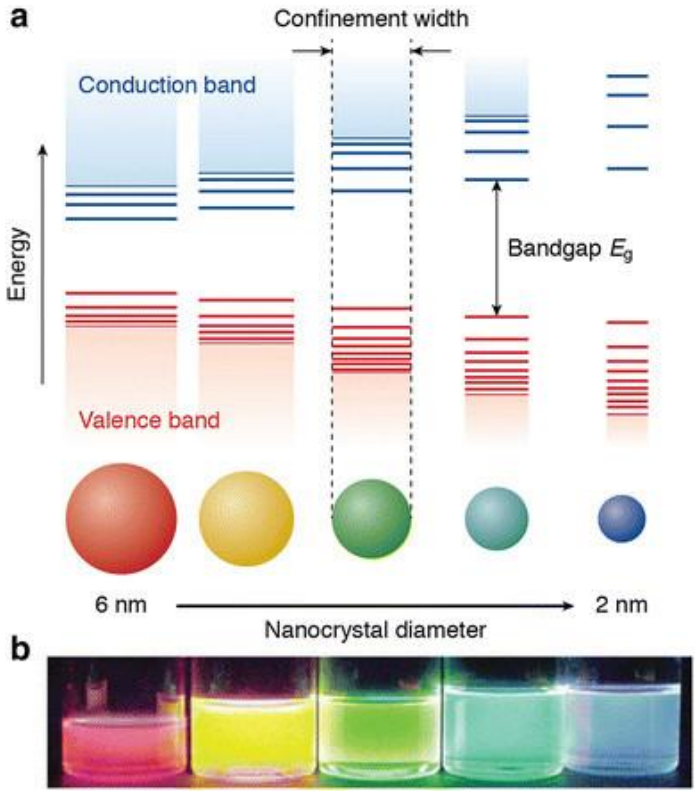

Figure 1.3 The energy band structure of the QDs (A) and the colloidal solutions of QDs with different sizes under UV light (B) (Rabouw and de Mello Donega, 2016).

This work focused on the CdTe QDs stabilized by thioglycolic acid (TGA) as a capping agent due to it is easily synthesized under mild conditions and water-soluble. The structure of TGA-capped CdTe QDs as showed in Figure 1.4.

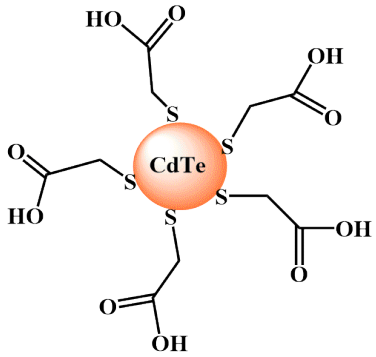

Figure 1.4 The thioglycolic acid-capped CdTe QDs.

1.4 Molecularly imprinted polymers

The molecularly imprinted polymers (MIPs) is a technique to create the molecular recognition site for specific with the template, which is called the lock and key model. This technique involves of a functional monomer, cross−linker and molecular template by the co-polymerization process. The functional monomers are responsible for the binding interactions in the imprinted binding sites. The function of the cross-linker in the polymer network is to arrange the monomer into specific sites and directions around the template molecules and thereby maintain the binding site structure (specific cavities). After template removal, the polymer contain specific cavities like template molecules in size, shape and functional group (Alexander *et al.*, 2006). The molecular imprinting process as described in Figure 1.5.

Molecularly imprinted polymers is becoming increasingly popular due to its stability, ease of preparation and low cost. It can be used to prepare highly selective fluorescence probe for the detection of target analytes. MIPs were composited with QDs and used as sensitive and selective optosensor. In this work, MIP composited with quantum dots nanoparticles and COOH@MWCNT are synthesized using 3-aminopropyl triethoxysilane (APTES) as functional monomer, tetraethoxysilane (TEOS) as cross-linker and ciprofloxacin (CIP) as template molecule. The functional monomer provides the amino groups on the surface to attract template molecules by hydrogen bonding.

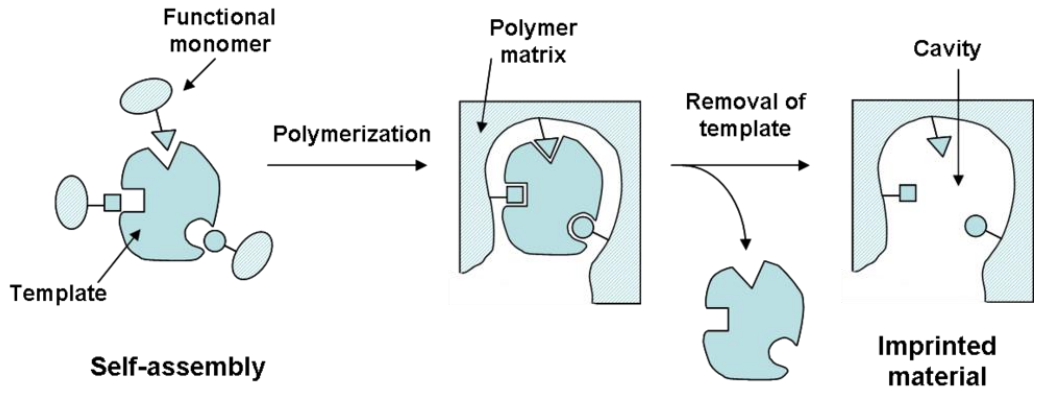

Figure 1.5 The molecular imprinting process (Sarafraz-Yazdi and Razavi, 2015).

1.5 Carboxylic functionalized multiwall carbon nanotubes (COOH@MWCNT)

Carboxylic functionalized multiwall carbon nanotubes (COOH@MWCNT) have a large surface areas, good chemical stability, good electrical conductivities and contain π structure (Arıer and Uysal, 2017). The combination of MIP-QDs with COOH@MWCNT as the composite materials to improve the kinetic adsorption or affinity binding of ciprofloxacin which can adsorb aromatic compounds via π-π interactions. The carboxylic acid functionalisation of multiwall carbon nanotubes can improve their dispersibility in aqueous media (Xu *et al.*, 2017) (Figure 1.6).

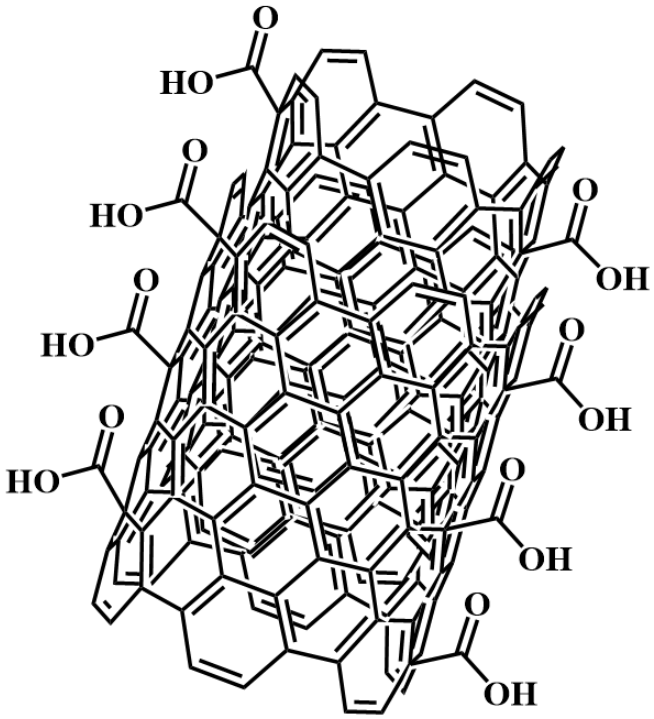

Figure 1.6 The carboxylic functionalized multiwall carbon nanotubes (COOH@MWCNT).

2. Results and discussion

2.1 Synthesis of thioglycolic acid-capped CdTe quantum dots

Thioglycolic acid-capped CdTe QDs were synthesized according to previous work with some modification as shown in Figure 2.1 (Bunkoed and Kanatharana, 2015). Briefly, a NaHTe solution was prepared by dissolved 0.050 g of Te powder and 0.045 g of $NaBH_4$ in 2.0 mL of deionised water. Separately, 0.046 g of $CdCl_2 \cdot H_2O$ and 30 µL of thioglycolic acid were mixed with 100 mL of deionised water in a beaker to form the cadmium precursor. The mixture was adjusted to pH 11.50 with 1.0 M NaOH and then transferred to a three-necked flask followed by bubbling with nitrogen gas for 10 min. The solution was refluxed under nitrogen atmosphere until the temperature was 90 °C and then 0.50 mL of the NaHTe aqueous solution was injected into the solution under vigorous stirring and continually refluxed for 10 min and then cooled to room temperature (27±2 °C). The thioglycolic acid-capped CdTe QDs were precipitated with ethanol and centrifuged at 5000 rpm for 10 min to eliminate the excess reagents. The TGA-capped CdTe QDs were dried in an oven at 50 °C for 4 h and stored in a desiccator at room temperature (25 °C) until used.

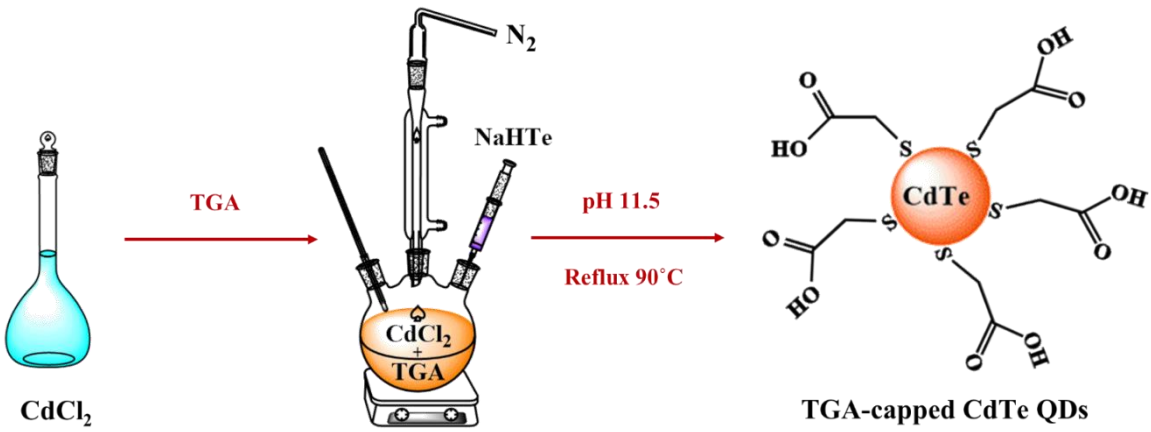

Figure 2.1 The synthesis of thioglycolic acid-capped CdTe QDs.

2.2 Characterization of TGA capped-CdTe QDs

Fluorescence spectrum and UV-Vis spectrum of TGA-capped CdTe QDs are shown in Figure 2.2. TGA-capped CdTe QDs showed a narrow and symmetric fluorescence spectrum with the maximum emission wavelength being 540 nm. The calculated particle size of CdTe QDs was 2.10 nm using the method described in previous work) (Yu *et al.*, 2003). The particle sizes of CdTe QDs were determined from the absorption maximum of the UV-vis spectra according to equation (1) (Yu *et al.*, 2003):

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0094)\lambda - 194.84 \qquad (1)$$

Where D (nm) is the size of the CdTe QDs, and λ (nm) is the wavelength of the first excitonic absorption peak. The concentrations of the CdTe QDs were calculated by Lambert-Beer's law; A = εCL. Where, A is the absorbance of the first excitonic absorption peak, C is the concentration (mol/L) of the CdTe QDs, L is the path length (cm) of the radiation beam used for recording the absorption spectrum, and ε is the extinction coefficient per mole of CdTe QDs which could be obtained with formula $\varepsilon = 10043\ (D)^{2.12}$.

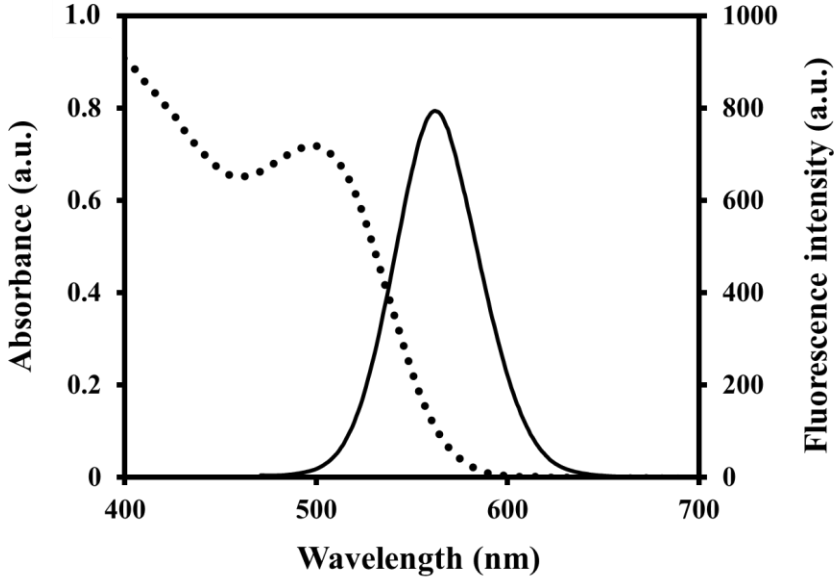

Figure 2.2 UV-Vis spectrum (dot line) and fluorescence emission spectrum (solid line) of TGA-capped CdTe QDs.

2.3 Synthesis of nanocomposite the carboxylic functionalized multiwall carbon nanotubes based on quantum dots coated molecularly imprinted polymer (COOH@MWCNT-MIP-QDs)

The carboxylic functionalized multiwall carbon nanotubes and QDs were incorporated into MIP (COOH@MWCNT-MIP-QDs) via a sol-gel copolymerization process. Briefly, 8.3 mg of ciprofloxacin (template) was dissolved in 10 mL of deionised water and then 0.005 g of COOH@MWCNT was added to the solution. Then 47.8 µL of APTES (functional monomer) and 5.0 mL of CdTe QDs were sequentially added in the mixture solution and stirred for 1 h. Then, 112 µL of TEOS (cross-linker) and 150 µL of ammonia solution (25 wt %) were added to the solution which was then continuously stirred for 5 h. After polymerization, the nanocomposite COOH@MWCNT-MIP-QDs were obtained and the template removed by washing with three portions of 10 mL of ethanol, the washing process of template was investigated by measuring the washings solution absorption at 260 nm. The nanocomposite COOH@MWCNT-MIP-OQs were collected by centrifugation at 5000 rpm for 15 min and dried at 50ºC for 4 h. The nanocomposite non-imprinted polymer (COOH@MWCNT-NIP-QDs) was also synthesized under the identical conditions but without the addition of ciprofloxacin (template).

The nanocomposite COOH@MWCNT-MIP-QDs optosensor were synthesized via copolymerization process in the presence of COOH@MWCNT, TGA-capped CdTe QDs, APTES as functional monomer, TEOS as cross-linker, ciprofloxacin as template and NH_3 as a catalyst. As shown in Figure 2.3, a carboxylic group of TGA-capped CdTe QDs and COOH@MWCNT can interact with amino groups ($-NH_2$) of APTES to facilitate formation of sol-gel layer via hydrogen bonding. While, non-covalent interaction between APTES and ciprofloxacin (template) occurred during the molecularly imprinting process, for example the amino group can interact with the carboxylic group of ciprofloxacin through hydrogen bonding. COOH@MWCNT can interact with ciprofloxacin via π-π interaction and also hydrogen bonding.

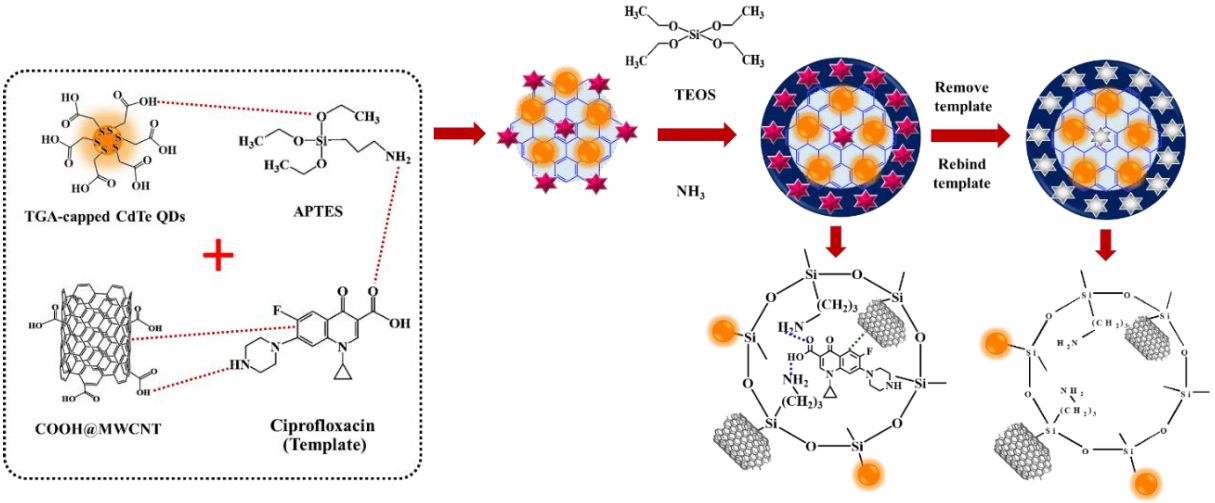

Figure 2.3 The synthesis of nanocomposite COOH@MWCNT-MIP-QDs optosensors for the specific recognition of ciprofloxacin.

The synthesized nanocomposite COOH@MWCNT-MIP-QDs optosensor showed a high symmetric emission at 544 nm. Figure 2.4 shows the fluorescence spectra of COOH@MWCNT-NIP-QDs (spectrum a), COOH@MWCNT-MIP-QDs after washing during which template was removed (spectrum b) and COOH@MWCNT-MIP-QDs before removal of template (spectrum c). Prior to the removal of the template, the fluorescence intensity of COOH@MWCNT-MIP-QDs was relatively low about 50 % of that the NIP, while after removal of template molecule its fluorescence intensity was restored to almost the same level as found for NIP-QDs (97.0 %). This result confirms that the template was completely removed from the MIP layer. This facile synthesis method can be performed under mind condition at room temperature (27 °C). The photographs of nanocomposite COOH@MWCNT-MIP-QDs in the presence (Figure 2.4d) and absence (Figure 2.4e) of ciprofloxacin under UV light.

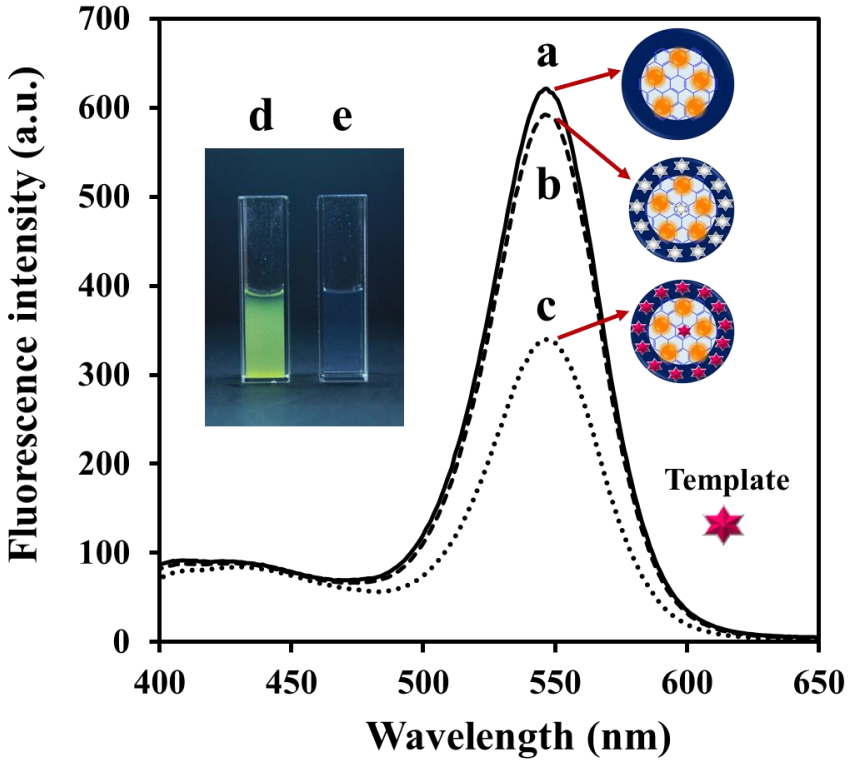

Figure 2.4 Fluorescence spectra of nanocomposite COOH@MWCNT-NIP-QDs (a), COOH@MWCNT-MIP-QDs after removal of template molecule (b), COOH@MWCNT-MIP-QDs before removal of template molecule (c), photographs of COOH@MWCNT-MIP-QDs in Tris-HCl buffer solution (d) and COOH@MWCNT-MIP-QDs + 1.0 mg L^{-1} of ciprofloxacin (e) under UV light.

2.4 Characterization of nanocomposite COOH@MWCNT-MIP-QDs optosensor

TEM images of TGA-capped CdTe QDs and nanocomposite MIP-QDs are shown in Figure 2.5A and 2.5B. The QDs nanoparticles were distributed within the MIP layer of the nanocomposite fluorescence probe. The results of TEM image confirm that QDs were embedded into the molecularly imprinted polymer matrix. The morphological structures of nanocomposite COOH@MWCNT-MIP-QDs were also investigated by the SEM technique. As can be seen from Figure 2.5C, they exhibit a spherical morphology and

a rough surface which indicated that specific recognition sites remained in the nanocomposite fluorescence probe.

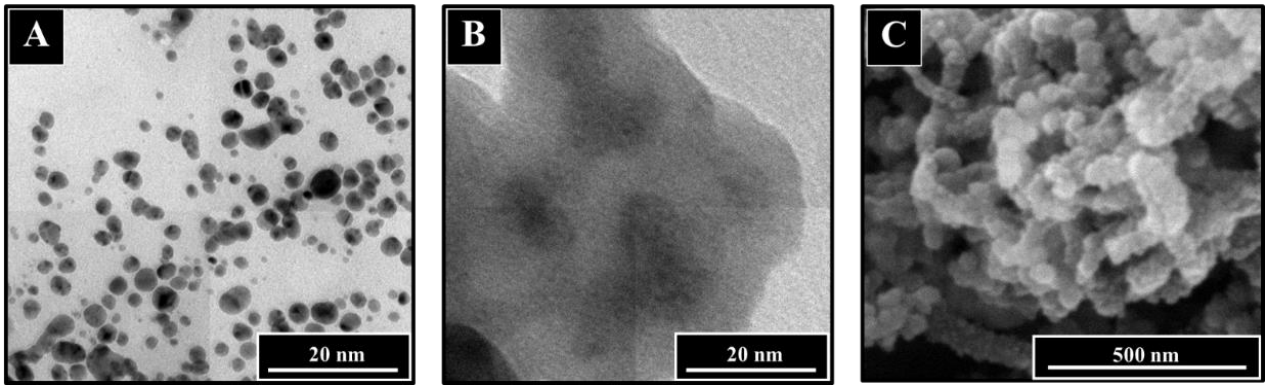

Figure 2.5 TEM images of (A) TGA-capped CdTe QDs, (B) nanocomposite MIP-QDs and (C) SEM image of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe.

FT-IR spectroscopy was performed to investigate the functional group of nanocomposite optosensor. The characteristic peak of TGA-capped CdTe QDs as shown in Fig. Figure 2.6a, the absorption peak at 1375 and 1582 cm^{-1} due to the C=O symmetric and asymmetric stretching of the carboxylate. The bands at 1224 and 3448 were the stretching vibration of C-O and O-H, respectively. FT-IR spectrum of ciprofloxacin (Figure 2.6b) showed characteristic peaks at 1050 cm^{-1} corresponding to C-F stretching. The peaks at 1410 cm^{-1} and 1620 cm^{-1} corresponded to C=O stretching and N-H bending of the quinolone ring, respectively. The absorption peak at 2900 cm^{-1} was due to C-H stretching of cyclopropyl group. Figure 2.6c shows the FT-IR spectrum of hybrid nanocomposite COOH@MWCNT-MIP-QDs optosensor before removal of template (ciprofloxacin). The absorption peak at 1060 cm^{-1} was due to Si-O-Si asymmetric stretching. The peaks around at about 460 and 760 cm^{-1} are assigned to the Si-O vibration band. The broad absorption band about at 3440 cm^{-1} corresponding to N-H stretching vibration of aminopropyl group. After removal of template the absorption peaks which related to

ciprofloxacin were absent (Figure 2.6d). The band around 1628 cm^{-1} was due to the C=C stretching of the carbon nanotubes backbone (Figure 2.6e). These results indicated that a hybrid nanocomposite COOH@MWCNT-MIP-QDs was successfully synthesized for selective recognition for ciprofloxacin.

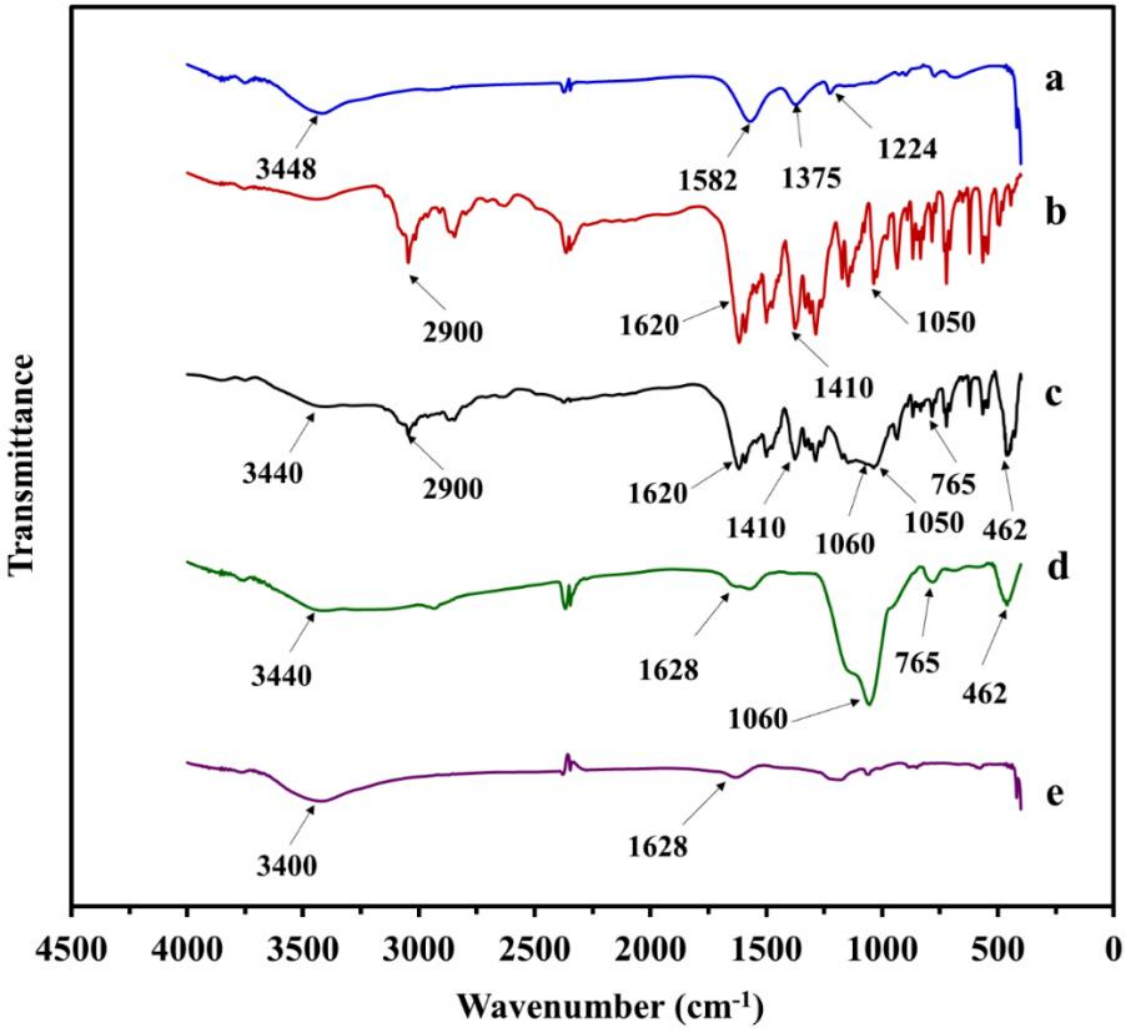

Figure 2.6 FT-IR spectra of (a) TGA-capped CdTe QDs, (b) ciprofloxacin, (c) COOH@MWCNT-MIP-QDs with template molecule (ciprofloxacin), (d) COOH@MWCNT-MIP-QDs without template molecule (ciprofloxacin) and (e) COOH@MWCNT.

The fluorescence quantum yield of CdTe QDs, MIP-QDs and COOH@MWCNT-MIP-QDs were 0.89 and 0.62 and 0.48 respectively, using Rhodamine 6G as a reference. The fluorescence quantum yields (Φ) were determined according to equation (2) (Masilela and Nyokong, 2011):

$$\Phi_F = \Phi_{F(Std)} \bullet \frac{F \cdot A_{Std} \cdot n^2}{F_{Std} \cdot A \cdot n_{Std}^2} \quad (2)$$

Where F and F_{Std} are the fluorescence areas under the fluorescence curves of the ciprofloxacin in the sample and the reference, respectively. A and A_{Std} are the absorbance of the sample and the reference, and n and n_{std} are the refraction index of solvents used for the sample and reference, respectively. The BET surface areas of COOH@MWCNT-NIP-QDs and COOH@MWCNT-MIP-QDs were 46.10 and 50.25 m^2g^{-1}, respectively. The nanocomposite COOH@MWCNT-MIP-QDs optosensor showed higher surface area than NIP-QDs, possibly because of the imprinted cavity of the template molecule.

2.5 Optimization of the analysis system

Several parameters influencing the fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs optosensors for the determination of ciprofloxacin *i.e.* incubation time, pH, amount of COOH@MWCNT, ratio of template to cross-linker and ratio of template to monomer were optimized. The highest quenching efficiency (sensitivity) and the shortest analysis time were considered to be the optimum values.

2.5.1 Effect of the incubation time

To investigate the binding performances of nanocomposite COOH@MWCNT-MIP-QDs and MIP-QDs with ciprofloxacin, the adsorption time was studied. As can be seen from Figure 2.7, the fluorescence intensity of COOH@MWCNT-MIP-QDs and MIP-QDs showed significant increases up to 15 min and 22 min, respectively. Above these times, the fluorescence intensity remained almost constant with the rise of adsorption time.

The equilibrium binding of COOH@MWCNT-MIP-QDs was faster than MIP-QDs by about 7 min which indicated that COOH@MWCNT can help to improve mass-transfer speed between the ciprofloxacin and recognition sites. Thus, nanocomposite COOH@MWCNT-MIP-QDs was used as a rapid fluorescence probe for ciprofloxacin detection and an equilibrium time of 15 min was sufficient to obtain complete ciprofloxacin adsorption.

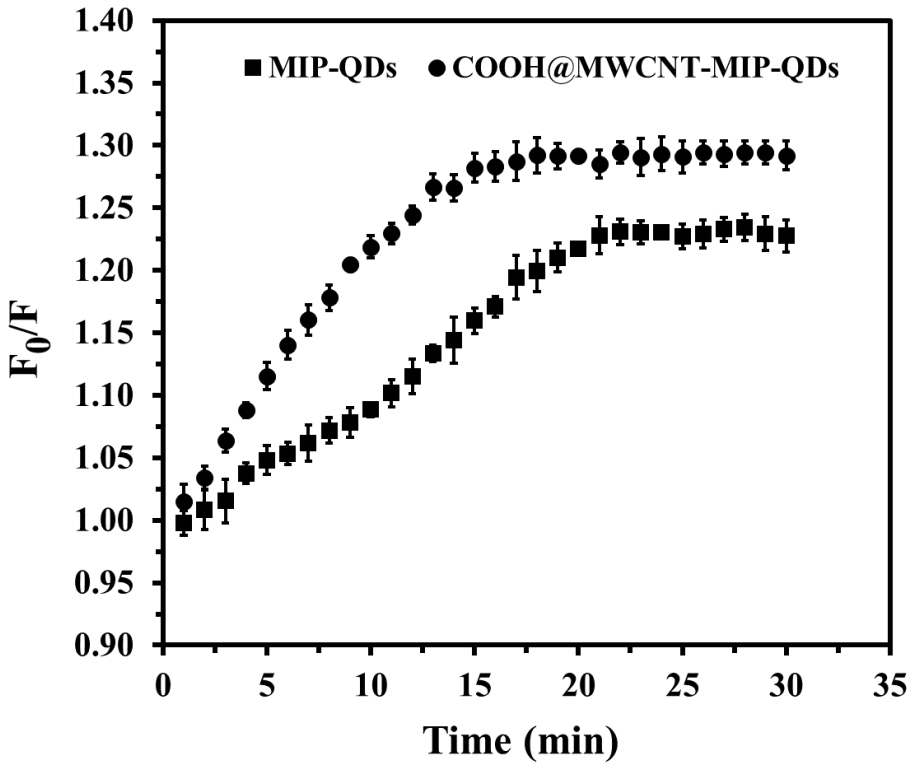

Figure 2.7 The effect of incubation time on the fluorescence quenching of COOH@MWCNT-MIP-QDs and MIP-QDs for the determination of ciprofloxacin (*n*=3).

2.5.2 Effect of pH

It was reported that the pH value had a significant effect on the fluorescence quenching of QDs due to their sensitivity to chemicals in the surrounding environment such as acids, bases, metal ions and organic molecules (Ren and Chen, 2015; Geng *et al.*, 2017). In this work, the effect of pH in the range of 6.0 to 9.0 was investigated for the determination of ciprofloxacin. The results as shown in Figure 2.8, the highest fluorescence quenching was obtained at a pH of 7.0. Since, the template molecule and MIP are bound through hydrogen bonding, the binding efficiency was decreased by hydrogen ion under acidic medium (pH<7) which causes a decrease in the interaction between template molecule and binding site. The fluorescence quenching was also decreased at pH higher than 7.0 due to the degradation or ionization of the template molecule under the alkaline condition. Moreover, the silica layer was unstable and will ionise under highly alkaline solution which can cause damage to the binding site of nanocomposite COOH@MWCNT-MIP-QDs probe thereby affecting the interaction between template and optosensing probe (Figure 2.9) (Li *et al.*, 2017). Therefore, a Tris-HCl buffer solution at pH 7.0 was chosen as optimum value for binding media and used for the further experiments.

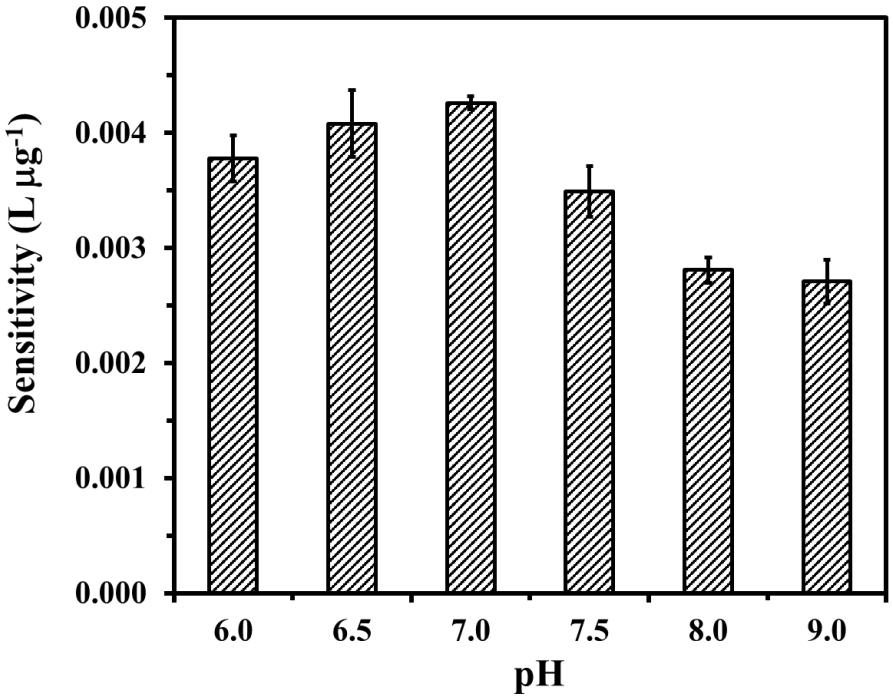

Figure 2.8 The effect of pH value on the fluorescence quenching of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe for ciprofloxacin detection.

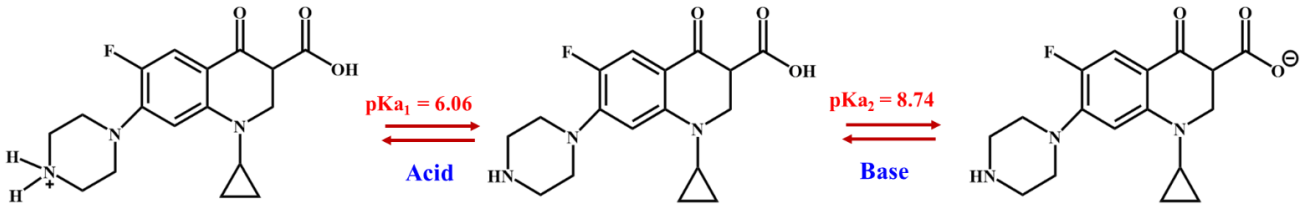

Figure 2.9 Speciation of ciprofloxacin under different pH conditions.

2.5.3 Amount of carboxylic functionalized multiwall carbon nanotubes (COOH@MWCNT)

The effect of amount of COOH@MWCNT in nanocomposite fluorescence probe was also optimized to obtain the high sensitivity for the determination of ciprofloxacin. The results as shown in Figure 2.10, the highest sensitivity was obtained an amount of COOH@MWCNT of 0.0005 % w/v. At lower amount of COOH@MWCNT, the composites showed lower sensitivity, possibly the adsorption was not complete with an incubation time of 15 min. However, the sensitivity was also decreased at higher amount of COOH@MWCNT, this could be possibly due the COOH@MWCNT disrupting the polymer structure, leading to the decrease of the number of binding sites in the MIP layer. Therefore, 0.0005 % w/v of COOH@MWCNT was selected for further experiment.

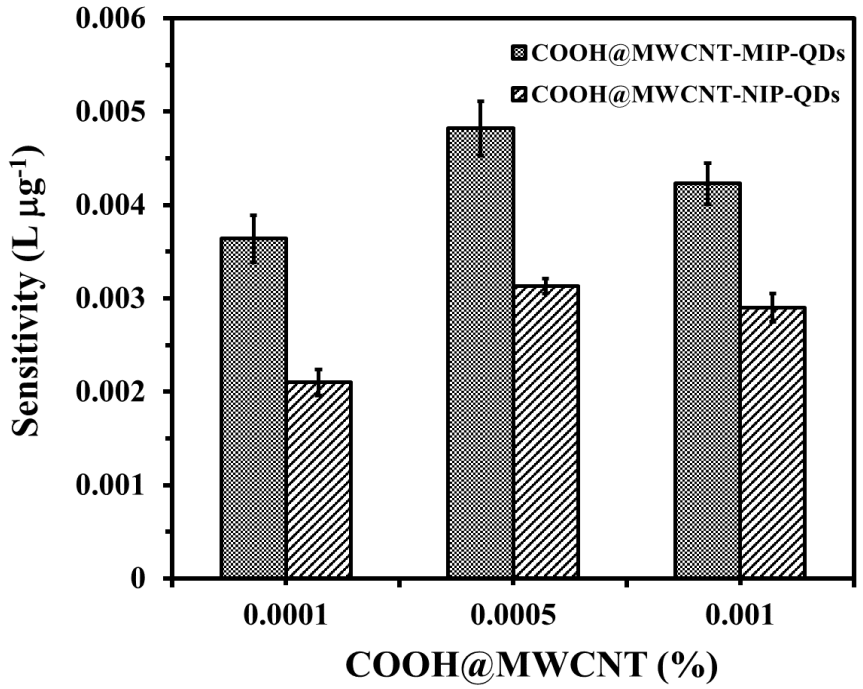

Figure 2.10 The effect of amount of COOH@MWCNT on the fluorescence quenching of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe for ciprofloxacin detection.

2.5.4 Ratio of template to cross-linker

TEOS is normally used as cross-linker to prepare MIP and it effect to the recognition ability of the MIP (Wu *et al.*, 2017; Zhou *et al.*, 2017). Thus, the effect of molar ratio of template to cross-linker was investigated to obtain the highest sensitivity for the determination of ciprofloxacin. As shown in Figure 2.11, the molar ratio of template to cross-linker of 1:20 provided the highest sensitivity. The sensitivity was decreased at lower amount of cross-linker (1:10) due to lower levels of crosslinking movement leading to the MIP-QDs structure being physically weaker and allowing an increase of molecular movement causing the formation of recognition sites to be less effective. The sensitivity was also decreased at higher amount of cross-linker (1:30 and 1:40) because of large amount of cross-linker results in a highly rigid polymer, providing highly rigid recognition sites. Also, excessive cross-linking can block the movement of functional monomer which reduces the interaction between target analyte and functional monomer (Xu *et al.*, 2013). Therefore, the molar ratio of template to cross-linker of 1:20 was chosen for further experiment.

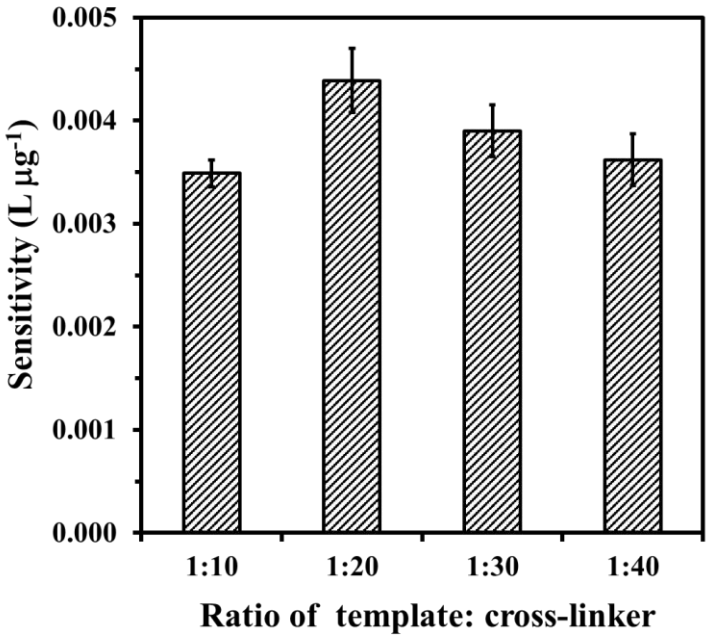

Figure 2.11 The effect of molar ratio of template to cross-linker on the fluorescence quenching of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe for ciprofloxacin detection.

2.5.5 Ratio of template to monomer

The nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe was synthesized using APTES as functional monomer and it was reported that the molar ratio of the template to monomer is an important factors on the specific recognition sites of MIP layer (Feng *et al.*, 2015). To obtain the highest sensitivity for the determination of ciprofloxacin the molar ratio of template to monomer was investigated. As shown in Figure 2.12, the highest sensitivity was obtained when the molar ratio of template to functional monomer (APTES) was 1:8. The lower amount of functional monomer (1:4 and 1:6) would produce a low number of recognition site ($–NH_2$ group) to interact with target analyte via hydrogen bonding. Also, the sensitivity was decreased at a higher amount of function monomer (1:10) due to the excess functional monomer forming non-imprinted layers

within the polymer which might inhibit the binding between target analyte and recognition sites. Thus, the molar ratio of template to monomer of 1:8 was selected for subsequent experiment.

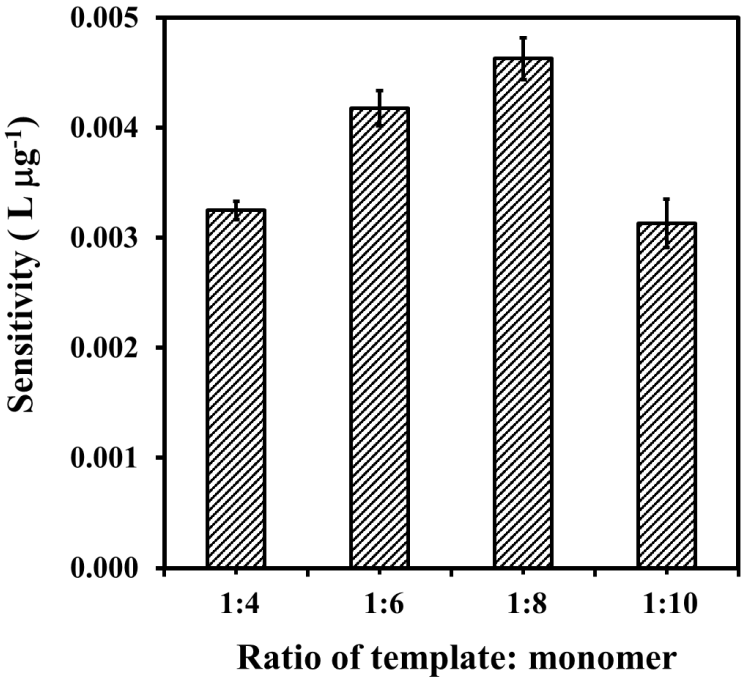

Figure 2.12 The effect of molar ratio of template to monomer on the fluorescence quenching of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe for ciprofloxacin detection.

2.6 Comparison of different fluorescence probes

The sensitivity of different fluorescence probes were investigated for the determination of ciprofloxacin including NIP-QDs, COOH@MWCNT-NIP-QDs, MIP-QDs and COOH@MWCNT-MIP-QDs. As shown in Table 2.1 and Figure 2.13. NIP-QDs showed the lowest sensitivity for ciprofloxacin detection due to they have no specific imprinted cavities for ciprofloxacin, the functional monomers were randomly orientated in the particles leading to low adsorption ability. The sensitivity was increased when incorporated COOH@MWCNT in NIP-QDs, this is because ciprofloxacin could adsorb on

the surface of COOH@MWCNT via π-π interaction leading to an increase in the quenching efficiency for ciprofloxacin detection. The nanocomposite MIP-QDs showed higher sensitivity than both NIP-QDs and COOH@MWCNT-NIP-QDs due to many specific binding sites being present in the fluorescence probes which can selectively interact with template molecule. The highest sensitivity was obtained for a nanocomposite COOH@MWCNT-MIP-QDs due to the integration of high affinity of COOH@MWCNT with ciprofloxacin and specific recognition cavities of MIP. These results confirm that the combination of COOH@MWCNT, MIP and QDs could improve the sensitivity, specificity and adsorption speed.

Table 2.1 Comparison of different fluorescence probes for the determination of ciprofloxacin

Fluorescence probes	**Sensitivity (L μg^{-1})**
NIP-QDs	0.001450 ± 0.000092
COOH@MWCNT-NIP-QDs	0.003130 ± 0.000080
MIP-QDs	0.00430 ± 0.00016
COOH@MWCNT-MIP-QDs	0.00482 ± 0.00029

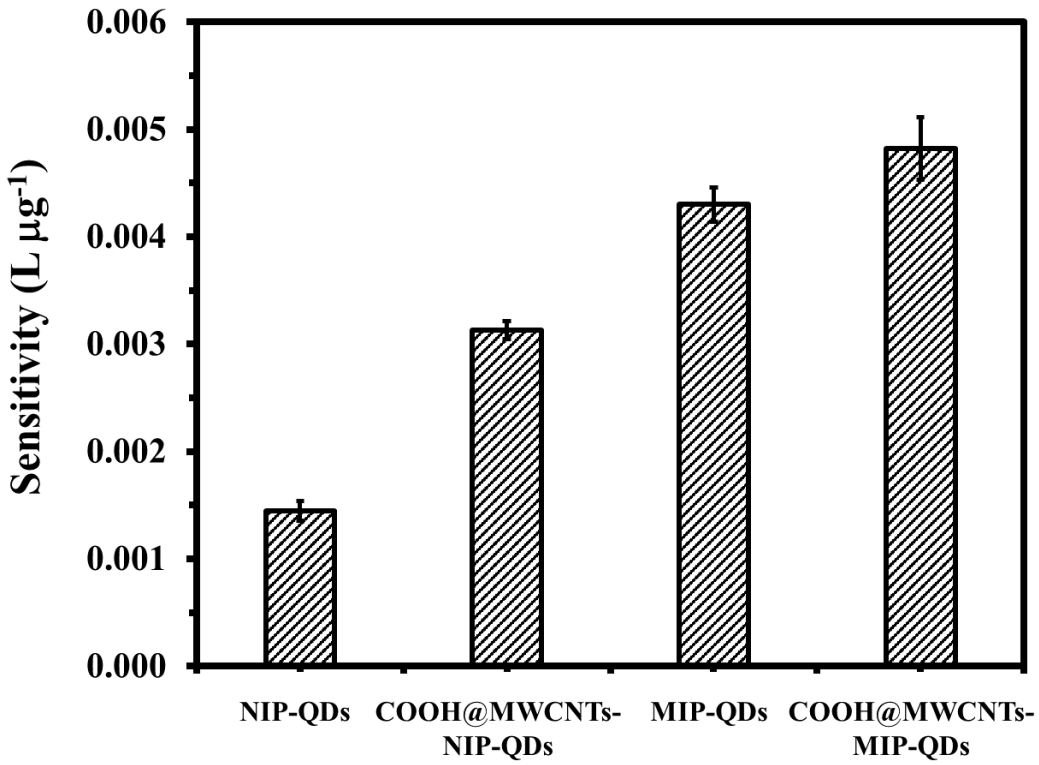

Figure 2.13 The sensitivity of different fluorescence probe for ciprofloxacin detection with incubation time was 15 min.

2.7 Fluorescence quenching mechanism

The fluorescence quenching mechanism of nanocomposite COOH@MWCNT-MIP-QDs by ciprofloxacin was described. In the presence of ciprofloxacin, hydrogen bonding could occur between ciprofloxacin and the amino groups of functional monomer on the surface of QDs. This led to the possibility that the electrons of the conduction bands of the QDs could transfer to the lowest unoccupied molecular orbital of ciprofloxacin, which would lead to the fluorescence quenching (The Huy *et al.*, 2014). Thus, the fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs is due to an electron transfer mechanism from QDs to ciprofloxacin. In addition, energy transfer was not considered to be a possible mechanism due to there being no spectral overlap between

the absorption spectrum of ciprofloxacin and the emission spectrum of COOH@MWCNT-MIP-QDs (Zhang and Chen, 2016; Lu *et al.*, 2017) (Figure 2.14).

The fluorescence quenching of the system could be described by the Stern-Volmer equation (3):

$$F_0/F = 1 + K_{sv}[C] \quad (3)$$

Where F_0 and F are the fluorescence intensity of nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe in the absence and presence of ciprofloxacin (quencher), respectively. *K*sv is the quenching constant of the quencher and [C] is the concentration of quencher (ciprofloxacin). The ratio of $K_{SV,MIP}$ to $K_{SV,NIP}$ was defined as the imprinting factor (IF).

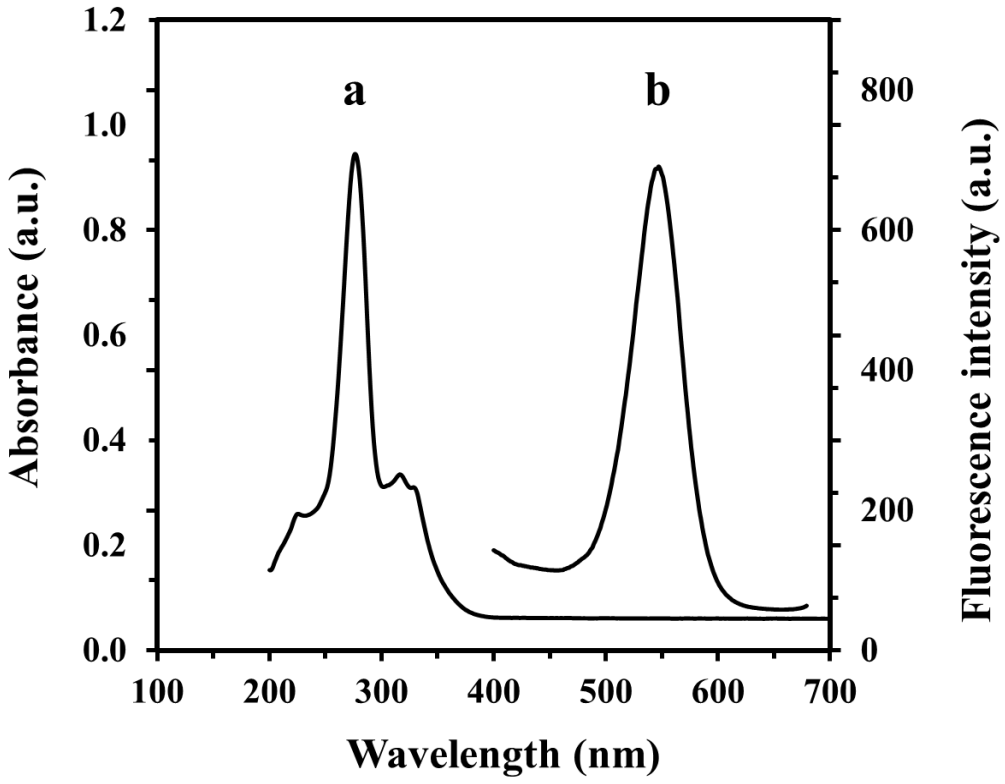

Figure 2.14 Absorption spectrum of ciprofloxacin (a) and emission spectrum of the nanocomposite COOH@MWCNT-MIP-QDs (b).

2.8 Selectivity of nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe for the determination of ciprofloxacin

The fluorescence quenchingof nanocomposite COOH@MWCNT-MIP-QDs toward other ciprofloxacin structural analogs (danofloxacin, difloxacin, enrofloxacin, norfloxacin, sarafloxacin) were investigated to study its selectivity due to hydrogen bonds can form between the structural analogs and the functional monomer in imprinting site. As shown in Figure 2.15 and 2.16, the nanocomposite COOH@MWCNT-MIP-QDs had higher fluorescence quenching than other compounds. This is because during the synthesis process of nanocomposite COOH@MWCNT-MIP-QDs, many specific imprinting sites which act as a memory of the size, shape and functional groups of ciprofloxacin were generated. However, the nanocomposite COOH@MWCNT-NIP-QDs showed low fluorescence quenching to ciprofloxacin and other compounds due to no recognition sites existing in the NIP layer and the molecules were adsorbped on the surface of NIP only through non-specific binding. The competitive binding experiment was also performed by fixing the concentration of ciprofloxacin and increasing the concentration of danofloxacin. As shown in Figure 2.17, the sensitivity were not significantly changed by the increase of the ratio of $C_{Danofloxacin}/C_{Ciprofloxacin}$which indicating that the recognition sites created by ciprofloxacin in the polymer are specific to the template molecule.

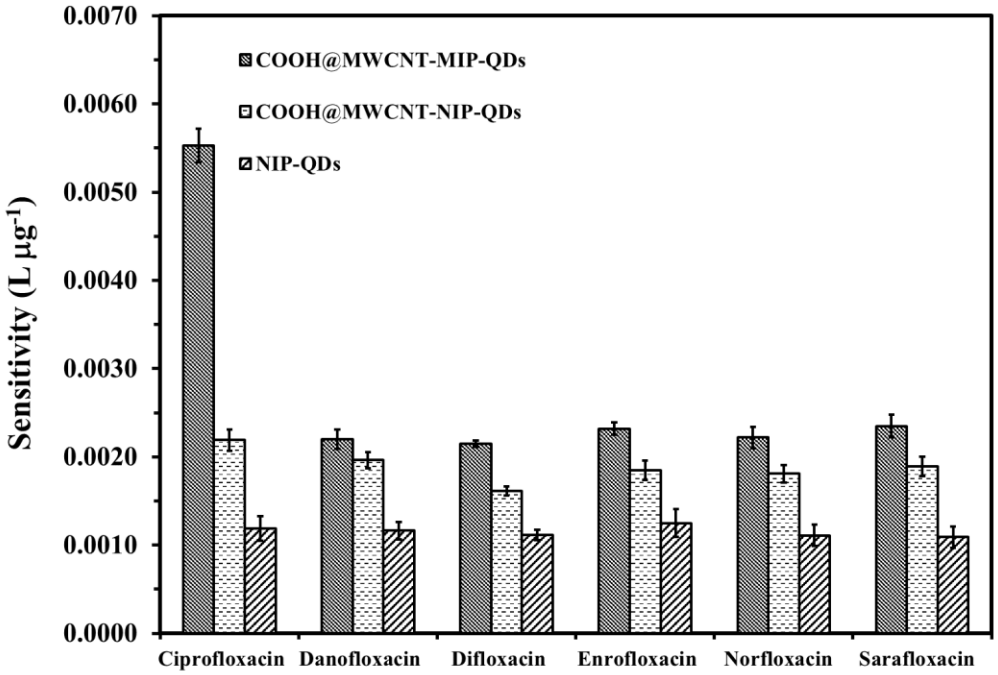

Figure 2.15 The selectivity of the nanocomposite COOH@MWCNT-MIP-QDs, COOH@MWCNT-NIP-QDs and NIP-QDs for ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, norfloxacin and sarafloxacin.

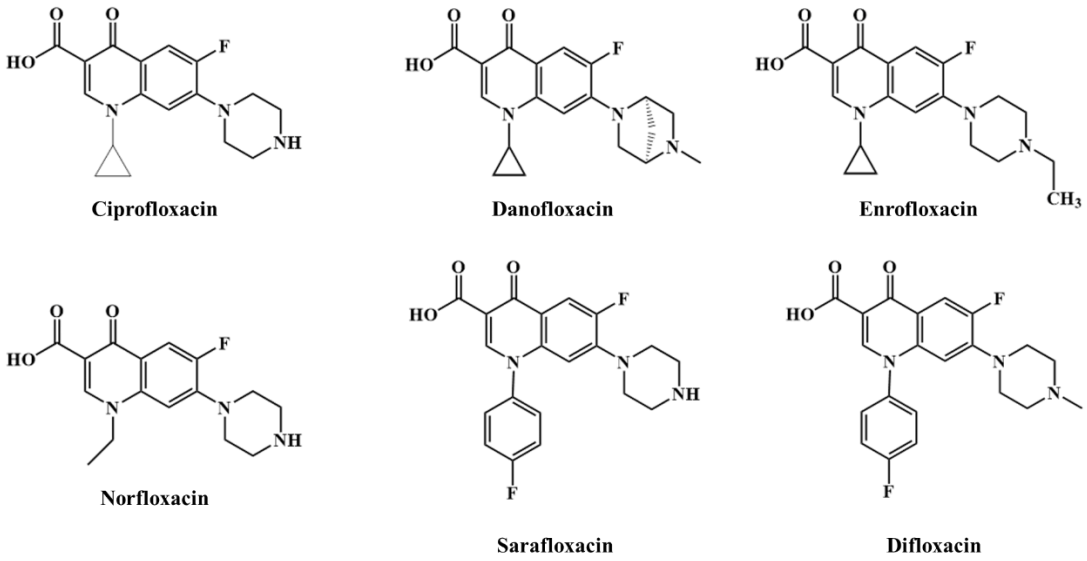

Figure 2.16 The structures of ciprofloxacin, danofloxacin, enrofloxacin, difloxacin, norfloxacin and sarafloxacin.

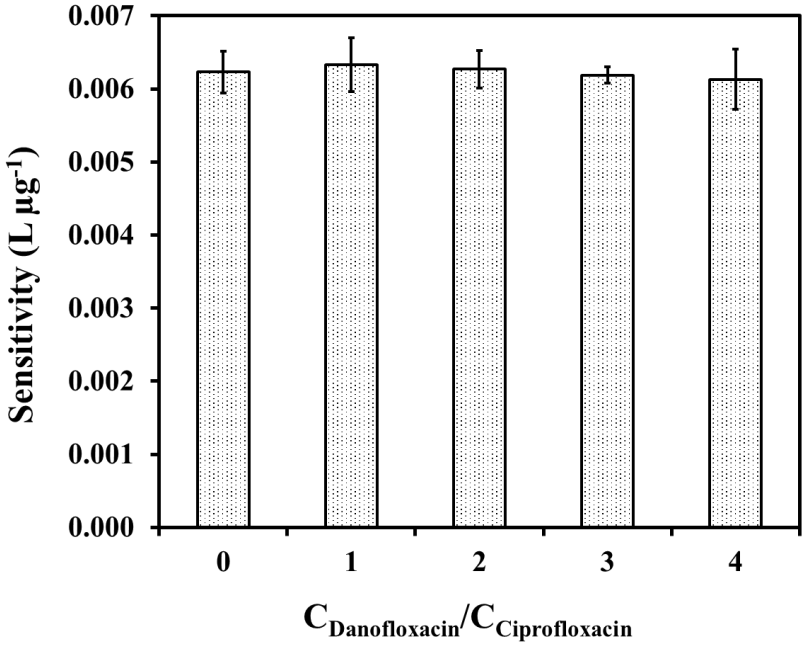

Figure 2.17 The effect of competitive analog danofloxacin on the binding of ciprofloxacin to the nanocomposite COOH@MWCNT-MIP-QDs.

2.9 Analytical performance of nanocomposite COOH@MWCNT-MIP-QDs for the determination of ciprofloxacin

Under the optimal conditions, the analytical performances of the developed nanocomposite COOH@MWCNT-MIP-QDs optosensor were investigated including linearity, limit of detection and limit of quantification. As shown in Figure 2.18A, two linear relationships of calibration curve were found in the range of 0.1-1.0 μg L^{-1}; F_0/F = (0.159±0.008) + (1.038±0.005) **(**Figure 2.18B**)** and 1.0-100.0 μg L^{-1}; F_0/F = (0.0060±0.0002) + (1.206±0.009) **(**Figure 2.18C**)**. The fact that two linear calibration curves were obtained might be due to the inhomogeneity of the specific imprinting cavities on the surface of the MIP. The imprinting factors were 17.67 and 4.28, respectively. The imprinting factor (IF) was calculated according to the following equation (4) (Ren and Chen, 2015):

$$IF = \frac{K_{sv,MIP}}{K_{sv,NIP}} \quad (4)$$

Where IF is the imprinting factor, while $K_{SV,MIP}$ and $K_{SV,NIP}$ is the Stern-Volmer constant of MIP and NIP, respectively. The fluorescence spectra of nanocomposite COOH@MWCNT-MIP-QDs after mixing with various concentrations of ciprofloxacin were shown in Figure 2.19, the fluorescence intensities were significantly quenched by ciprofloxacin. The limit of detection (LOD) and limit of quantification (LOQ) were calculated following the IUPAC criteria; $3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of blank signal (n=20) and S is the slope of the calibration curve. The LOD and LOQ were 0.066 μg L^{-1} and 0.22 μg L^{-1}, respectively.

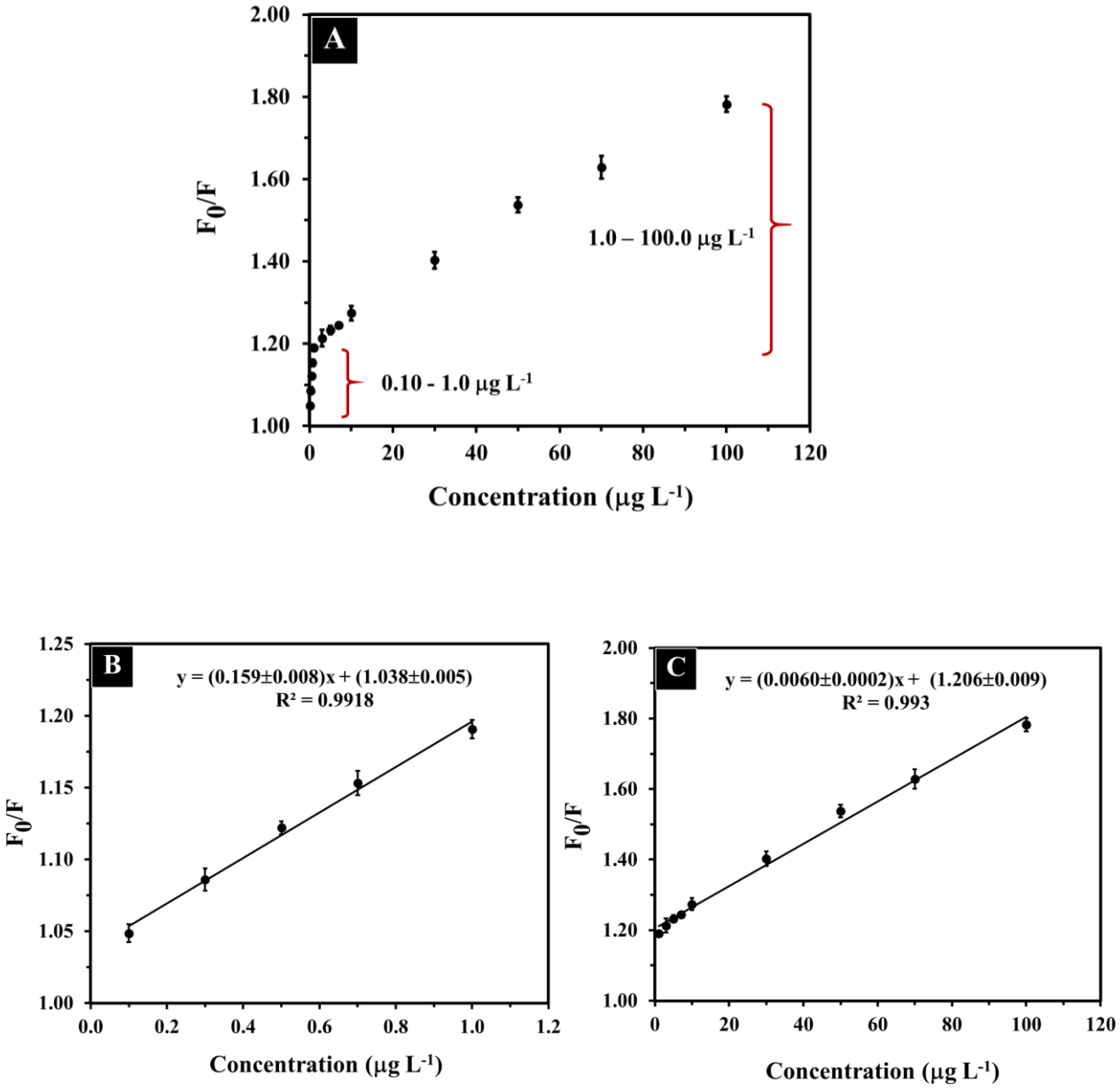

Figure 2.18 The calibration curve in the presence of ciprofloxacin in the concentration range of 0.1-100.0 μg L^{-1} (A), 0.1-1.0 μg L^{-1} (B) and 1.0-100.0 μg L^{-1}(C).

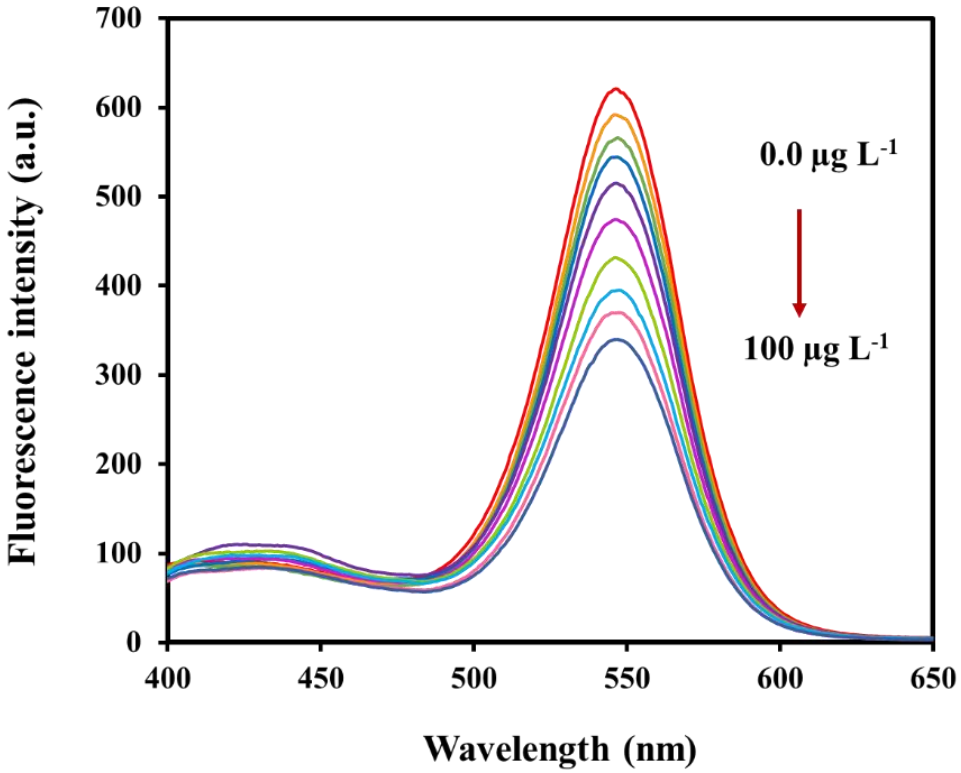

Figure 2.19 Fluorescence spectra of the nanocomposite COOH@MWCNT-MIP-QDs in the presence of ciprofloxacin.

2.10 Reproducibility and stability of COOH@MWCNT-MIP-QDs

The reproducibility of the nanocomposite COOH@MWCNT-MIP-QDs optosensors were investigated by preparing six different batches of nanocomposite COOH@MWCNT-MIP-QDs under the optimum condition at different times. The developed optosensors were used to determine ciprofloxacin in the concentration range of 1.0-50.0 µg L^{-1} and the sensitivity was used to evaluate the reproducibility. The relative standard deviation (RSD) of the six optosensing systems was 1.5 %, indicating good batch-to-batch reproducibility.

The stability of nanocomposite COOH@MWCNT-MIP-QDs and CdTe QDs were also studied under the optimized conditions by the repeated measurement of the

fluorescence intensity every 30 min at room temperature (25 °C). As shown in Figure 2.20, the fluorescence intensity of nanocomposite COOH@MWCNT-MIP-QDs did not significantly change within 300 min, while the fluorescence intensity of CdTe QDs was decreased after 90 min. It could be explained that the MIP layer helps to protect the photostability of QDs.

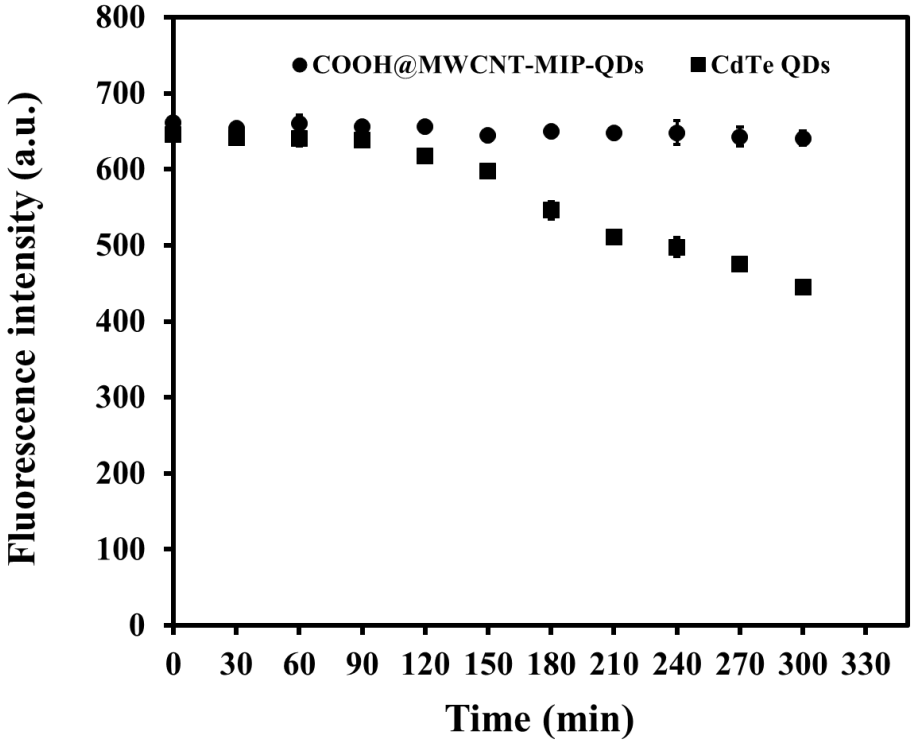

Figure 2.20 The fluorescence stability of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probes and CdTe QDs in 0.01 M Tris-HCl buffer solution (pH 7.0).

2.11 Application of nanocomposite COOH@MWCNT-MIP-QDs optosensors for the determination of ciprofloxacin in food sample

The chicken muscle and milk samples were purchased from local markets in Hat Yai city, Songkhla, Thailand. The extraction procedures of ciprofloxacin in chicken muscle was adapted from a previous report (Yorke and Froc, 2000). Briefly, 300 μL of Tris buffer

solution (pH 7.0) was added to 0.50 g of homogenized chicken muscle and vortexed for 1 min. Then, 1.0mL of acetonitrile was added into the mixture which was vortexed for 1 min and then centrifuge for 10 min. The supernatant was transferred into a 15 mL centrifuge tube and 300 μL of hexane was added and vortexed for 1 min. The extract was centrifuged at 6000 rpm and the degreasing phase was removed. The acetonitrile phase was then evaporated to dryness at 50 °C and the residue was redissolved with 10 mL deionised water and filtered through a 0.22 μm syringe filter before analysis with the developed method. The extraction procedure of ciprofloxacin from milk was adapted from previous work (Jin *et al.*, 2016). Briefly, 10 mL of the milk was transferred into a 50 mL centrifuge tube and 10 mL of acetonitrile was added followed by vortexing for 5 min. The sample was then centrifuged at 6000 rpm for 30 min. The supernatant was evaporated to dryness at 50 °C and the dried extract was then dissolved in 10 mL deionised water before mixing with the developed fluorescence probe for analysis.

The developed nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe was applied to determine ciprofloxacin in chicken muscle and milk (Table 2.2). Low concentrations of ciprofloxacin was found in chicken muscle (0.19 μg kg^{-1}) and milk (0.22-0.35 μg kg^{-1}) which were lower than the MRL value set by European Union, 100 μg kg^{-1} for chicken muscle and milk. The accuracy of the developed optosensor was also investigated by spiking standard ciprofloxacin into real samples at different concentrations. Satisfactory recoveries were obtained in the range of 82.6-98.4 % with the relative standard deviation being lower than 8 %.

The developed optosensor was also compared with a HPLC method. The HPLC condition for the analysis of ciprofloxacin as shown in Table 2.3. The spiked samples were extracted and analyzed by both the developed optosensing system and HPLC methods. Figure 2.21A shows HPLC chromatograms of spiked samples at different concentration of ciprofloxacin. The correlation between the developed optosensors and HPLC is shown in Figure 2.21B. The coefficient of determination and slope reach 1.0 and indicated that the developed optosensors agreed with the HPLC methods. Therefore, this developed method

can be used as a simple, rapid and sensitive method for the determination of trace ciprofloxacin in milk and chicken muscle.

Table 2.2 The determination of ciprofloxacin in chicken muscle and milk (*n*=5).

Sample	Added (μg kg^{-1})	Found (μg kg^{-1})	Recovery (%)	RSD (%)
Chicken muscle I	0.0	0.19	-	-
	1.0	1.16±0.02	96.2	2.4
	5.0	4.45±0.21	85.1	4.9
	10.0	9.54±0.42	93.4	4.5
	50.0	48.35±1.34	96.3	2.8
Chicken muscle II	0.0	0.19	-	-
	1.0	1.08±0.02	89.0	3.1
	5.0	4.80±0.34	92.3	7.4
	10.0	9.69±0.25	95.0	2.7
	50.0	48.02±1.31	95.7	2.7
Chicken muscle III	0.0	n.d.	-	-
	1.0	0.95±0.04	95.4	3.8
	5.0	4.82±0.13	96.5	2.7
	10.0	9.80±0.18	98.0	1.9
	50.0	49.03±0.98	98.1	2.0
Milk I	0.0	n.d.	-	-
	1.0	0.85±0.05	85.4	5.4
	5.0	4.52±0.20	90.3	4.4
	10.0	9.44±0.22	94.4	2.3
	50.0	49.20±0.46	98.4	0.9
Milk II	0.0	0.35	-	-
	1.0	1.18±0.01	82.6	1.2
	5.0	4.87±0.18	90.6	4.0
	10.0	9.88±0.20	95.2	2.0
	50.0	48.69±0.64	96.7	1.3
Milk III	0.0	0.22	-	-
	1.0	1.12±0.03	90.6	2.9
	5.0	4.58±0.24	87.3	5.4
	10.0	9.58±0.31	93.6	3.3
	50.0	48.20±0.83	96.0	1.7

n.d. = not detectable

Table 2.3 HPLC conditions for the analysis of ciprofloxacin.

Parameters	**Conditions**
Column	VertiSep™ UPS C18 column (4.6 × 150 mm, 5 μm)
Flow rate	0.90 mL min^{-1}
Mobile phase	Acetonitrile:25 mM H_3PO_4 (18:82 % v/v)
Detector	Fluorescence detector (λ_{ex} = 272 nm and λ_{em} = 448 nm)
Injection volume	20 μL

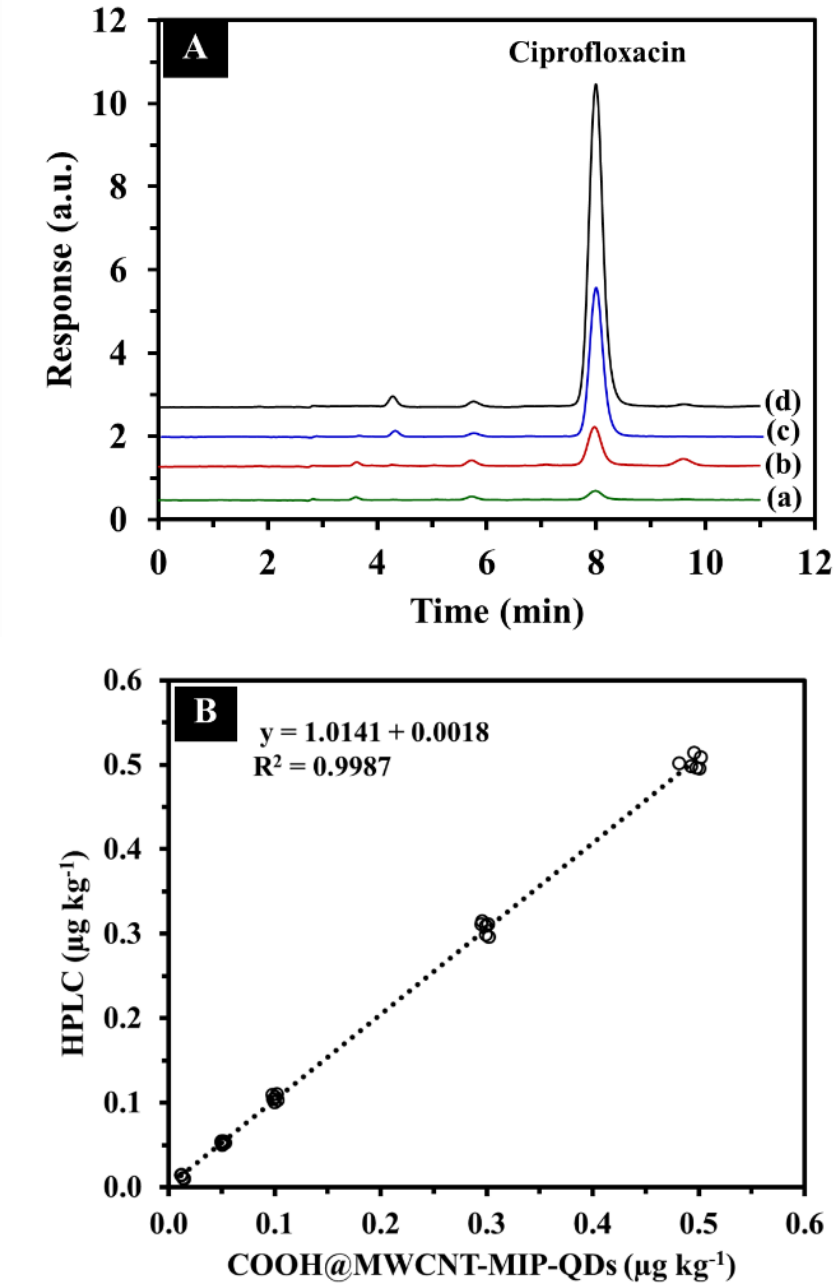

Figure 2.21 HPLC chromatograms of spiked milk samples at different concentration of ciprofloxacin; (a) 10 µg kg^{-1}, (b) 50 µg kg^{-1}, (c) 300 µg kg^{-1} and (d) 500 µg kg^{-1}(A). Correlation between nanocomposite COOH@MWCNT-MIP-QDs optosensor and the HPLC method for the determination of ciprofloxacin in chicken muscle and milk samples (B).

2.12 Comparison of the developed hybrid nanocomposite COOH@MWCNT-MIP-QDs optosensor with other methods

The analytical performance of the developed optosensor for the determination of ciprofloxacin was compared with other previous works. As summarized in Table 2.4, the developed method provided a wide linear range and the detection limits are much lower than other work which demonstrates that the nanocomposite COOH@MWCNT-MIP-QDs are highly sensitive and selective for the determination of ciprofloxacin. The recovery (82.6-98.4 %) and precision (<8 %) of this method was comparable to other methods. This developed optosensor is simple, rapid and cost effective when compared to chromatographic techniques which required expensive instrumentation and used large amount of organic solvents as mobile phase. In addition, the selectivity of this sensor was improved with the using of MIP, without requiring complicated separation processes like chromatographic methods.

Table 2.4 Comparison of the developed optosensor with other methods for the determination of ciprofloxacin.

Analytical technique	Samples	Linear range (μg L^{-1})	LOD (μg L^{-1})	Recovery (%)	RSD (%)	References
HPLC-FLD	Human plasma	20-4,000	10.0	73.0-95.0	3.0-17.0	(Muchohi *et al.*, 2011)
HPLC–UV	Human plasma	50-8,000	10.0	90.0-96.0	<4.0	(Vella *et al.*, 2015)
HPLC–PDA	Sputum samples	50-2,000	17.0	>80.0	<15.0	(Locatelli *et al.*, 2015)
HPLC-FLD	Surface water	200-2,000	100	102.5-122.2	9.2	(Prutthiwan asan *et al.*, 2016)
Electrochemical	Pharmaceutical samples and biological fluids	1.6-281.6	0.56	98.0-103.0	3.0	(Bagheri *et al.*, 2016)
Fluorescent siderophorepyoverdine	Pharmaceutical tablet	-	2,362	98.6	1.3	(Pawar *et al.*, 2016)
Electrochemical	Wastewater	3,313-26,507	16.6	98.2-107.0	< 5.0	(Garrido *et al.*, 2017)
Electrochemical	Urine samples	33-3,313	7.3	99.1-109.6	1.0-1.4	(Shan *et al.*, 2016)
Electrochemical	Urine samples	0.15 - 2.11	0.05	97.0-102.0	2.4	(Radičová *et al.*, 2017)
Electrochemical	Physiological Fluids	3,313-3,310,000	2618	98.7-104.5	0.7-0.9	(Abdel-Haleem *et al.*, 2017)
MIP based micromechanical cantilever sensor	-	497-50,000	265	94.0	1.4	(Okan *et al.*, 2017)
COOH@MWCNT-MIP-QDs optosensor	Milk and chicken	0.1-1.0 1.0-100	0.066	82.6-98.4	< 8.0	This work

HPLC= high performance liquid chromatography; FLD= fluorescence detector; UV=Ultraviolet-Visible detector; PAD= photodiode array detector; MIP = molecularly imprinted polymer; QDs = quantum dots; COOH@MWCNT = carboxylic functionalized multiwall carbon nanotubes

3 Concluding remarks

A nanocomposite COOH@MWCNT-MIP-QDs optosensor was developed for the determination of ciprofloxacin on the basis of electron transfer induced fluorescence quenching. The developed optosensor integrated the high specificity of MIP, excellent fluorescence property of QDs and high affinity of COOH@MWCNT to ciprofloxacin, demonstrating a highly selective, sensitive and rapid method for the determination of trace ciprofloxacin. This rapid, convenient and cost-effective hybrid nanocomposite optosensor was successfully applied to determine ciprofloxacin in milk and chicken muscle with a satisfactory recovery and also demonstrated excellent agreement with HPLC. This facile and versatile process for the optosensor fabrication provides an alternative method for the specific recognition of the others organic compounds.

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Publication

A nanocomposite optosensor containing carboxylic functionalized multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for highly selective and sensitive detection of ciprofloxacin

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A nanocomposite optosensor containing carboxylic functionalized multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for highly selective and sensitive detection of ciprofloxacin

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ABSTRACT

A nanocomposite optosensor consisting of carboxylic acid functionalized multiwall carbon nanotubes and CdTe quantum dots embedded inside a molecularly imprinted polymer (COOH@MWCNT-MIP-QDs) was developed for trace ciprofloxacin detection. The COOH@MWCNT-MIP-QDs were synthesized through a facile sol-gel process using ciprofloxacin as a template molecule, 3-aminopropylethoxysilane as a functional monomer and tetraethoxysilane as a cross-linker at a molar ratio of 1:8:20. The synthesized nanocomposite optosensor had high sensitivity, excellent specificity and high binding affinity to ciprofloxacin. Under optimal conditions, the fluorescence intensity of the optosensor decreased in a linear fashion with the concentration of ciprofloxacin and two linear dynamic ranges were obtained, 0.10–1.0 μg L^{-1} and 1.0–100.0 μg L^{-1} with a very low limit of detection of 0.066 μg L^{-1}. The imprinting factors of the two linear range were 17.67 and 4.28, respectively. The developed nanocomposite fluorescence probe was applied towards the determination of ciprofloxacin levels in chicken muscle and milk samples with satisfactory recoveries being obtained in the range of 82.6 to 98.4%. The results were also in good agreement with a HPLC method which indicates that the optosensor can be used as a sensitive, selective and rapid method to detect ciprofloxacin in chicken and milk samples.

1. Introduction

Ciprofloxacin is a fluoroquinolone antibiotic which is widely used for the treatment of respiratory and digestive infections in humans and livestock [1]. This drug also misused in the livestock industry since treating animals with these agents can increase productivity. However, this can become a serious problem, since the antibiotics can be expressed in meat and milk leading to potential toxicity [2] or allergic hypersensitivity reactions in humans. There is also a further serious issue that this practice may lead to the generation of antibiotic resistant human pathogens. Therefore, the European Union has set the maximum residue limit (MRL) for ciprofloxacin at 100 μg kg^{-1} in milk, chicken and pig muscle [3]. Thus, it is necessary to develop a convenient, rapid and cost-effective method for the monitoring of ciprofloxacin in food samples. Several analytical techniques have been reported for ciprofloxacin detection such as high performance liquid chromatography [4–6], capillary electrophoresis [7] and electrochemical techniques [8–11]. However, these techniques can be complicated, may require expensive instrumentation and highly skilled personnel. To overcome these drawbacks, spectrofluorimetry can be considered as an alternative method due to its simplicity, rapidity and cost effectiveness. The sensitivity of this method can be improved using high sensitive fluorescence probes such as quantum dot nanoparticles (QDs). QDs have been used for the determination of various target analytes at trace levels typical analytes include salicylic acid [12], glucose [13], H_2O_2 [14], 6-mercaptopurine [15], ochratoxin A [16], kaempferol [17] and copper (II) ion [18]. In addition, QDs have many unique optical properties such as tunable size-dependent photoluminescence, good photostability and narrow symmetric emissions [19,20]. The determination of trace target analytes in real samples with high matrix interferences normally requires highly selective methods. To further improve the selectivity of these methods, molecularly imprinted polymers (MIPs) have received considerable study due to their high specificity and facile preparation. MIPs are

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normally prepared by a *co*-polymerization process of cross-linker moieties with functional monomers that form complexes with analytes (template molecule) prior to polymerization. After the template molecules were eluted from the polymer, specific recognition sites which are complementary in size, shape and functional groups to the template can be obtained, leading to the ability to rebind template molecules with high specificity. MIPs not only provide highly selective binding material but also have high stability meaning they can be used under extreme condition such as extreme pH, high temperature and in organic solvents. Since MIPs are cost-effective and robust materials, they have been extensively used in many fields such as an adsorbent material [21], solid phase microextraction [22–24] and chemosensors and biosensors [25–27]. For sensor applications, the composite fluorescence probes using QDs incorporated into MIPs have been developed as highly selective fluorescence probes for the determination of some target compounds such as salbutamol [28], patulin [29], sulfadiazine [30], sulfadimidine [31], malachite green [32], tetracycline [33], cocaine [34] and amoxicillin [35]. To improve the kinetic adsorption or affinity binding of ciprofloxacin, addition of carboxylic acid functionalized multiwall carbon nanotubes is an interesting alternative approach since they contain an extended π structure which can adsorb aromatic compounds via π-π interactions [36]. The carboxylic acid functionalisation of multiwall carbon nanotubes can improve their dispersibility in aqueous media and it is easy to achieve further covalent functionalisation with other materials [37].

In this work, nanocomposite optosensor COOH functionalized MWCNTs and CdTe quantum dots embedded in a MIPs were synthesized for trace ciprofloxacin detection. The determination of ciprofloxacin is based on the fluorescence quenching when target analyte is bound to the binding sites on the developed fluorescence probes. This integrated the desirable optical properties of the quantum dots with the high specificity of MIPs and high adsorption affinity of COOH@MWCNT to produce a rapid, highly sensitive optosensor for the determination of ciprofloxacin with good selectivity. The developed optosensor was applied to determine ciprofloxacin in chicken muscle and milk and the accuracy of the method was investigated by comparing with a HPLC technique.

2. Experimental

2.1. Chemicals and Reagents

Tellurium powder (99.8%), sodium borohydride ($NaBH_4$), thioglycolic acid (TGA), 3-aminopropyltriethoxysilane (APTES), cadmium chloride ($CdCl_2 \cdot H_2O$) and tetraethylorthosilicate (TEOS) were from Sigma-Aldrich (St. Louis, MO, USA). Ciprofloxacin was supplied by Tokyo Chemical Industry (Tokyo, Japan), Tris (hydroxymethyl) aminomethane and methanol were obtained from Merck (Darmstadt, Germany), 25% $NH_3 \cdot H_2O$ was from QReC (New Zealand). MWCNTs were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China), Rhodamine 6G was purchased from Tokyo Chemical Industry (Tokyo, Japan). Deionised water (18.2 MΩ cm) was produced by an Elgastat Maxima water system (ELGA, High Wycombe, UK).

2.2. Apparatus

Fluorescence spectra were recorded using a RF-5301 spectrofluorometer (Shimadzu, Tokyo, Japan). An Avaspec 2048 spectrometer (Apeldoorn, The Netherlands) was used to record ultraviolet-visible absorption spectra and Fourier transform infrared (FT-IR) spectra were obtained using a Spectrum BX FTIR spectroscope (PerkinElmer, Waltham, MA, USA) on solid samples dispersed within KBr discs. Scanning electron microscope (SEM) images were obtained using a JSM-5200 microscope (JEOL, Tokyo, Japan). Transmission electron micrograph (TEM) images were obtained using a JEM-2010 microscope (JEOL, Tokyo, Japan). The surface areas were measured with an ASAP 2460 surface area and porosity analyzer (Micromeritics, Norcross, USA).

2.3. Synthesis of Thioglycolic Acid-capped CdTe Quantum Dots

The synthesis of TGA-capped CdTe QDs was performed as previously reported with some modification [38]. Briefly, 0.050 g of Te powder and 0.045 g of $NaBH_4$ were dissolved in 2.0 mL of deionised water to prepare a NaHTe solution. Separately, 0.046 g of $CdCl_2 \cdot H_2O$ and 30 μL of TGA were mixed with 100 mL of deionised water and adjusted to pH 11.50 using 1.0 M NaOH. The solution was heated under nitrogen atmosphere until the temperature was 90 °C and then 0.50 mL of the NaHTe aqueous solution was injected into the solution and the mixture refluxed for 10 min. The TGA-capped CdTe QDs were precipitated with ethanol and centrifuged at 6000 rpm for 15 min to eliminate the excess reagents. The TGA-capped CdTe QDs were dried in an oven at 50 °C for 4 h and kept in a desiccator at room temperature (25 °C) until used.

2.4. Synthesis of Nanocomposite COOH@MWCNT-MIP-QDs Fluorescence Probe

The carboxylic functionalized multiwall carbon nanotubes and QDs were incorporated into MIP via a sol-gel copolymerization process. Briefly, 8.3 mg of ciprofloxacin (template) was dissolved in 10 mL of deionised water and 0.0050 g of COOH@MWCNT was added to the solution. Then, 48.0 μL of APTES and 5.0 mL of CdTe QDs were sequentially added in the mixture solution and stirred for 1 h. Subsequently, 110 μL of TEOS and 150 μL of ammonia solution (25 wt%) were added to the solution and continuously stirred for 5 h. After polymerization, the nanocomposite COOH@MWCNT-MIP-QDs were obtained and the template removed by washing with three portions of 10 mL of ethanol, the process of template removal was investigated by measuring the washing solutions absorption at 260 nm. The nanocomposite COOH@MWCNTs-MIP-QDs were collected by centrifugation at 5000 rpm for 15 min and dried at 50 °C for 4 h. The nanocomposite non-imprinted polymer (COOH@MWCNTs-NIP-QDs) was also prepared using identical conditions except without the addition of template (ciprofloxacin).

2.5. Fluorescence Measurement

The nanocomposite COOH@MWCNT-MIP-QDs were dispersed in 0.010 M Tris-HCl buffer solution (pH 7.0) and 150 μL was mixed with 50 μL of ciprofloxacin standard solution under rotation for 15 min. The mixture was transferred into a quartz cell and its fluorescence intensity measured by setting the excitation wavelength at 272 nm and recording in the emission range of 400–700 nm. The slit widths of the excitation and emission were both 10 nm. The measurement was performed at room temperature (25 °C) for convenience of analysis.

2.6. Sample Preparation

The chicken muscle and milk samples were collected from local markets in Hat Yai city, Thailand. Extraction of ciprofloxacin from chicken muscle was carried out as described in a previous report [39]. Briefly, 300 μL of Tris buffer solution (pH 7.0) was added to 0.50 g of homogenized chicken muscle and vortexed for 1 min. Acetonitrile (1.0 mL) was added into the mixture which was vortexed for another 1 min and then sonicated for 10 min. The supernatant was separated and mixed with 300 μL of hexane into a 15 mL centrifuge tube and vortexed for 1 min. The extract was centrifuged at 6000 rpm to remove the degreasing phase. The acetonitrile phase was then evaporated to dryness at 50 °C and redissolved with 10 mL deionised water and filtered through a 0.22 μm syringe filter before analysis with the developed method.

The extraction procedure of ciprofloxacin from milk was adapted from previous work [40]. Briefly, 10.0 mL of the milk sample and

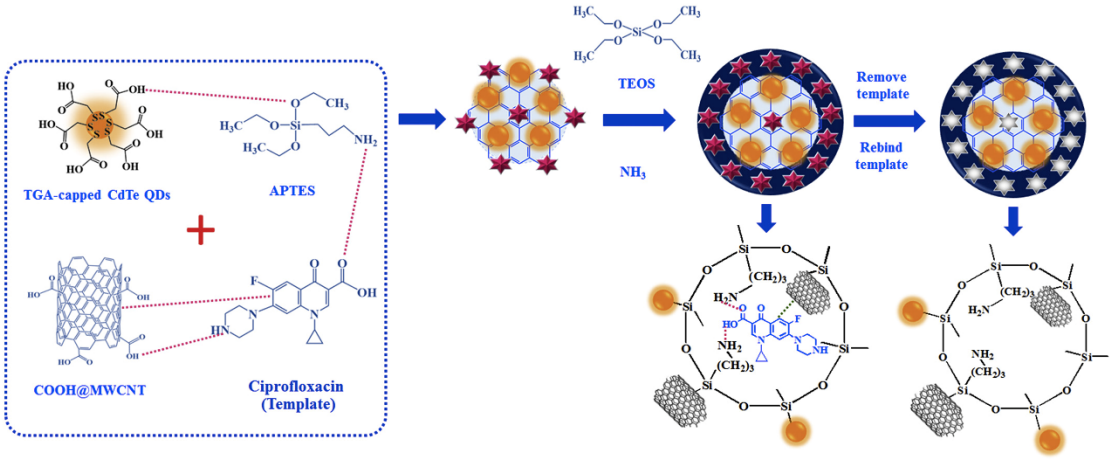

Fig. 1. The synthesis of nanocomposite COOH@MWCNT-MIP-QDs optosensors for the specific recognition of ciprofloxacin.

10 mL of acetonitrile were added into a 50 mL centrifuge tube followed by vortexing for 5 min and centrifuged at 6000 rpm for 30 min. Then, the supernatant was separated and evaporated to dryness at 50 °C, the dried extract was redissolved in 10.0 mL deionised water before mixing with the developed fluorescence probe for analysis.

2.7. Analysis by HPLC Method

HPLC analyses were carried out using an Agilent 1100 series HPLC system (Germany). The analytical column was a VertiSep™ UPS C18 column (4.6 × 150 mm, 5 μm). Acetonitrile (18%) and 25 mM H_3PO_4 (82%) was used as the mobile phase at a flow rate of 0.9 mL min^{-1}. The sample volume was 20 μL. The excitation (λ_{ex}) and emission (λ_{em}) wavelength were 272 and 448 nm, respectively.

3. Results and Discussion

3.1. The Synthesis of Nanocomposite COOH@MWCNT-MIP-QDs Optosensors for Ciprofloxacin Detection

The nanocomposite COOH@MWCNT-MIP-QDs optosensor were prepared via copolymerization process incorporating COOH@MWCNT, TGA-capped CdTe QDs, APTES (functional monomer), TEOS (cross-linker), ciprofloxacin (template) and NH_3 (catalyst). The carboxylic groups of TGA-capped CdTe QDs and COOH@MWCNT can interact with amino groups (-NH_2) of APTES to facilitate incorporation of the CdTe QDs into the sol-gel via hydrogen bonding. Also, non-covalent interaction between APTES and ciprofloxacin (template) occurred during the molecularly imprinting process, for example the amino group can interact with the carboxylic group of ciprofloxacin through hydrogen bonding. COOH@MWCNT can interact with ciprofloxacin via π-π interaction and also hydrogen bonding (Fig. 1).

The synthesized nanocomposite COOH@MWCNT-MIP-QDs optosensors showed a highly symmetric emission at 544 nm. Fig. 2 displays the fluorescence spectra of COOH@MWCNT-NIP-QDs (spectrum a), COOH@MWCNT-MIP-QDs after washing during which the template was removed (spectrum b) and COOH@MWCNT-MIP-QDs prior to removal of template molecules (spectrum c). Before template removal the fluorescence intensity of COOH@MWCNT-MIP-QDs was relatively low, about 50% of that of the NIP, while after removal of template molecule its fluorescence intensity was restored to almost the same level as found for NIP-QDs (97.0%). This result confirms that the ciprofloxacin (template) was completely removed from the MIP layer. This facile synthesis method can be performed under mild conditions at room temperature (27 °C).

3.2. Characterization of Nanocomposite COOH@MWCNT-MIP-QDs Optosensor

Fluorescence and UV–Vis spectrum of CdTe QDs are shown in Fig. S1. TGA-capped CdTe QDs showed a narrow and symmetric fluorescence spectrum with the maximum emission wavelength being 540 nm. The calculated particle size of the TGA-capped CdTe QDs was 2.10 nm using the method described in previous work [12].

Fig. 3A and B shows the TEM images of TGA-capped CdTe QDs and nanocomposite MIP-QDs. The QDs nanoparticles were distributed within the MIP layer of the nanocomposite fluorescence probe. The results of TEM imaging confirm that QDs were embedded into the molecularly imprinted polymer matrix. The morphological structures of nanocomposite COOH@MWCNT-MIP-QDs were also investigated by the SEM technique (Fig. 3C).They exhibit a rough surface which indicated that specific recognition sites presented in the nanocomposite fluorescence probe.

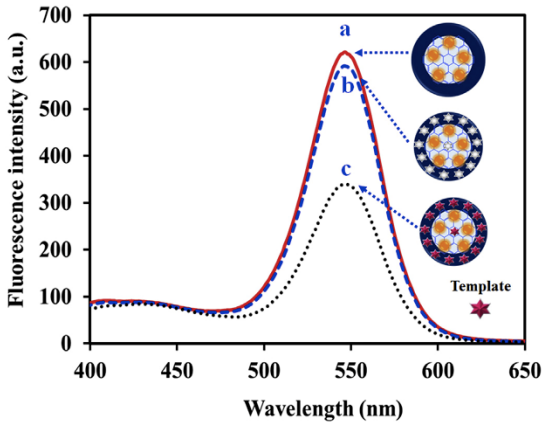

Fig. 2. Fluorescence spectra of nanocomposite COOH@MWCNT-NIP-QDs (a), COOH@MWCNT-MIP-QDs after removal of template molecule (b) and COOH@MWCNT-MIP-QDs before removal of template molecule (c).

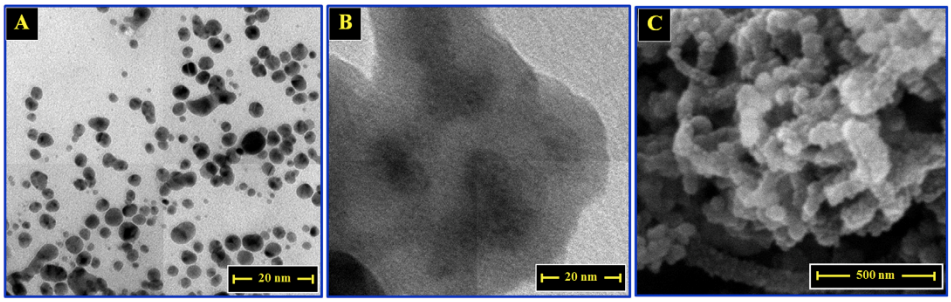

Fig. 3. TEM images of (A) TGA-capped CdTe QDs, (B) nanocomposite MIP-QDs and (C) SEM image of the nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe.

FT-IR spectroscopy was performed to investigate the functional groups of the nanocomposite optosensor. Fig. 4a shows the characteristic peaks of TGA-capped CdTe QDs, the absorption peaks at 1375 and 1582 cm^{-1} are due to the symmetric and asymmetric stretching of the carboxylate group. Bands at 1224 and 3448 cm^{-1} are the stretching vibration of C—O and O—H, respectively. FT-IR spectrum of ciprofloxacin (Fig. 4b) showed characteristic peaks at 1050 cm^{-1} due to C—F stretching and peaks at 1410 cm^{-1} and 1620 cm^{-1} due to C═O stretching and N—H bending of the quinolone ring [41], respectively. The absorption peak at 2900 cm^{-1} was due to C—H stretching. Fig. 4c shows the FT-IR spectrum of hybrid nanocomposite COOH@MWCNT-MIP-QDs optosensor before removal of template (ciprofloxacin). The characteristic peak at 1060 cm^{-1} corresponded to Si-O-Si asymmetric stretching. Absorption peaks at around 460 and 760 cm^{-1} are assigned to the Si—O vibration band and the broad absorption band at 3440 cm^{-1} corresponds to N—H stretching of aminopropyl group. After removal of template the characteristic peaks which related to ciprofloxacin disappear (Fig. 4d). The band around 1628 $cm^{-1}$was due to the C═C stretching of the carbon nanotube backbones (Fig. 4e). These results indicated that a hybrid nanocomposite COOH@MWCNT-MIP-QDs was successfully synthesized for selective recognition for ciprofloxacin.

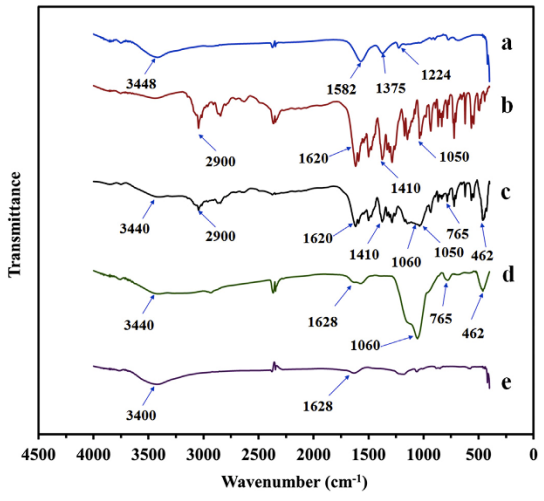

Fig. 4. FT-IR spectra of (a) TGA-capped CdTe QDs, (b) ciprofloxacin, (c) COOH@MWCNT-MIP-QDs with template molecule (ciprofloxacin), (d) COOH@MWCNT-MIP-QDs without template molecule (ciprofloxacin) and (e) COOH@MWCNT.

The fluorescence quantum yield of CdTe QDs, MIP-QDs and COOH@MWCNT-MIP-QDs were 0.89 and 0.62 and 0.48 respectively, using Rhodamine 6G as a reference [42]. The BET surface areas of COOH@MWCNT-NIP-QDs and COOH@MWCNT-MIP-QDs were 15.75 and 23.46 $m^2\ g^{-1}$ respectively. The nanocomposite COOH@MWCNT-MIP-QDs optosensor showed higher surface area than NIP-QDs, possibly because of the imprinted sites of the template.

3.3. Optimization of the Analysis System

Several parameters influencing the fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs optosensors for ciprofloxacin detection i.e. incubation time, pH, amount of COOH@MWCNT as well as the ratios of template to cross-linker and template to monomer were optimized. The highest quenching efficiency (sensitivity) and the shortest analysis time were considered to be the optimum values.

3.3.1. Effect of the Adsorption Time

To investigate the binding performances of nanocomposite COOH@MWCNT-MIP-QDs and MIP-QDs with ciprofloxacin, the effects of adsorption time were investigated. The fluorescence intensity of COOH@MWCNT-MIP-QDs and MIP-QDs showed significant increases up to adsorption times of 15 min and 22 min, respectively (Fig. S2). With longer exposures, the fluorescence intensity remained almost constant with further increases in adsorption time. The equilibrium binding of COOH@MWCNT-MIP-QDs was faster than MIP-QDs by about 7 min which indicated that COOH@MWCNT can help to improve mass-transfer speed between the ciprofloxacin and its recognition sites. Thus, nanocomposite COOH@MWCNT-MIP-QDs was used as a rapid fluorescence probe for ciprofloxacin detection.

3.3.2. Effect of pH

The pH level had significant effects on fluorescence quenching of QDs due to their sensitivity to the surrounding environment such as acids, bases, metal ions and organic molecules [18,43]. In this work, the effect of pH between 6.0 and 9.0 on the determination of ciprofloxacin was investigated. The results are shown in Fig. 5A; with highest fluorescence quenching being obtained at a pH of 7.0. Since, the template molecules and MIPs are bound through hydrogen bonding, the binding efficiency was decreased by hydrogen ion under acidic medium (pH < 7) which causes a decrease in the interaction between template molecule and binding site. The fluorescence quenching was also decreased at pH higher than 7.0 due to the degradation or ionization of the template molecule under alkaline conditions. Moreover, the silica layer was unstable and will ionize in an alkaline solution which can cause damage to the binding site of nanocomposite COOH@MWCNT-MIP-QDs probe thereby affecting the interaction between template and optosensing probe [44]. Therefore, a Tris-HCl buffer solution (pH 7.0) was chosen as an optimum binding media and used for further experiments.

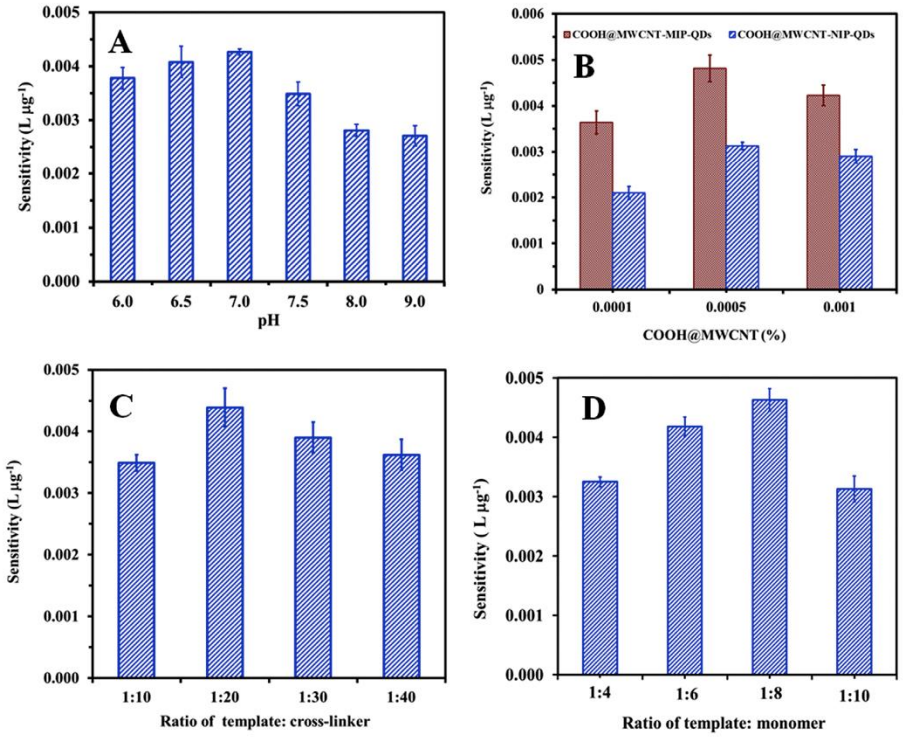

Fig. 5. (A) Effect of pH, (B) amount of COOH@MWCNTs, (C) molar ratio of template to cross-linker and (D) molar ratio of template to monomer on the fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs fluorescence probes for ciprofloxacin detection ($n = 3$).

3.3.3. Amount of Carboxylic Functionalized Multiwall Carbon Nanotubes (COOH@MWCNTs)

The effect of the amount of COOH@MWCNT in nanocomposite fluorescence probe was also optimized to obtain the high sensitivity for the determination of ciprofloxacin. The highest sensitivity was obtained using an amount of COOH@MWCNT of 0.0005% w/v (Fig. 5B). At lower levels of COOH@MWCNT, the composites showed lower sensitivity, possibly the adsorption was not complete with an incubation time of 15 min. However, the sensitivity was also decreased at higher levels of COOH@MWCNT; this could be possibly due to the MWCNTs disrupting the polymer structure, leading to the decrease of the number of recognition sites in the MIP layer. Therefore, 0.0005% w/v of COOH@MWCNT was selected for further experiment.

3.3.4. Ratio of Template to Cross-Linker

TEOS is normally used as cross-linker to prepare MIPs and it can affect the recognition ability of the MIPs [31,45]. Thus, the effect of molar ratio of template to cross-linker was investigated to obtain the highest sensitivity for the determination of ciprofloxacin. As shown in Fig. 5C, a 1:20 template to cross-linker molar ratio provided the highest sensitivity. The sensitivity was decreased at lower amount of cross-linker (1:10) due to lower levels of crosslinking leading to the MIP-QDs structure being physically weaker and allowing an increase of molecular movement causing the formation of recognition sites to be less effective. The sensitivity was also decreased at higher amount of cross-linker (1:30 and 1:40) because a large amount of cross-linker results in a highly rigid polymer, providing highly rigid recognition sites. Also, excessive cross-linking can block the movement of functional monomer which reduces the target analyte and functional monomer interaction [46]. Therefore, a molar ratio of ciprofloxacin to TEOS of 1:20 was chosen for further experiment.

3.3.5. Ratio of Template to Monomer

The nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe was synthesized using APTES as functional monomer and it is an important factors on the specific recognition sites of MIP layer [47]. In this work, the molar ratio of template to monomer was investigated with the highest sensitivity being obtained for a 1:8 template to functional monomer (APTES) molar ratio (Fig. 5D). The lower amount of functional monomer (1:4 and 1:6) would produce a low number of recognition site ($-NH_2$ group) to interact with target analyte via hydrogen bonding. Also, the sensitivity was decreased at a higher amount of functional monomer (1:10) due to the excess functional monomer forming non-imprinted layers within the polymer which might inhibit the binding between target analyte and recognition sites. Thus, the molar ratio of ciprofloxacin to APTES of 1:8 was selected for subsequent experiment.

3.4. Comparison of Different Fluorescence Probes

The sensitivity of different fluorescence probes were investigated for the determination of ciprofloxacin including NIP-QDs, COOH@MWCNT-NIP-QDs, MIP-QDs and COOH@MWCNT-MIP-QDs. As shown in Fig. 6. NIP-QDs showed the lowest sensitivity for ciprofloxacin detection due to the fact that they have no specific imprinted cavities for ciprofloxacin, the functional monomers were randomly orientated in the particles leading to low adsorption ability. The sensitivity was increased on incorporation of COOH@MWCNT in NIP-QDs, this is because ciprofloxacin could adsorb on the surface of COOH@MWCNT via π-π interactions leading to an increase in the quenching efficiency for ciprofloxacin

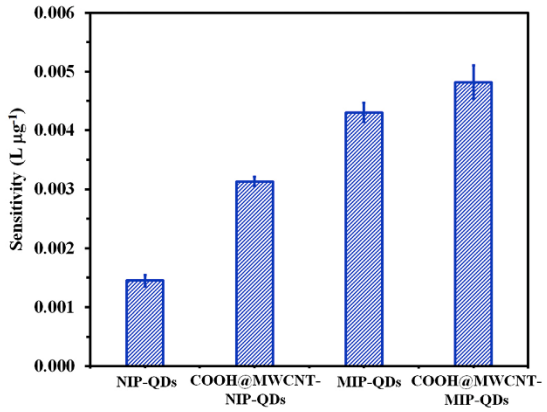

Fig. 6. The sensitivity of different fluorescence probe for ciprofloxacin detection with an incubation time of 15 min ($n = 3$).

detection. The nanocomposite MIP-QDs showed higher sensitivity than both NIP-QDs and COOH@MWCNT-NIP-QDs due to many specific binding sites being present in the fluorescence probes which can selectively interact with template molecule. The highest sensitivity was obtained for a nanocomposite COOH@MWCNT-MIP-QDs due to the integration of high affinity of COOH@MWCNT with ciprofloxacin and specific recognition cavities of MIP. These results confirm that the combination of COOH@MWCNT, MIP and QDs could improve the sensitivity, specificity and adsorption speed.

3.5. Fluorescence Quenching Mechanism

The fluorescence quenching mechanism of nanocomposite COOH@MWCNT-MIP-QDs by ciprofloxacin was described. Hydrogen bonding could occur between ciprofloxacin and the NH_2 groups of functional monomer on the surface of fluorescence probe. This led to the possibility that the electrons of the QDs conduction bands could be transferred into the lowest unoccupied molecular orbital of ciprofloxacin leading to the fluorescence quenching [48]. Thus, the fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs is due to an electron transfer mechanism from QDs to ciprofloxacin. In addition, energy transfer was not considered to be a possible mechanism due to there being no spectral overlap between the COOH@MWCNT-MIP-QDs emission spectrum and the ciprofloxacin absorption spectrum [33,42] (Fig. S3).

The fluorescence quenching of the system was described using the Stern-Volmer equation:

$$F0/F = 1 + Ksv[C]$$

where F and F0 are the fluorescence intensity of nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe in the presence and absence of quencher (ciprofloxacin), respectively. *Ksv* the quenching constant and [C] the quencher concentration. The imprinting factor (IF) was calculated from the ratio of $K_{SV,MIP}$ to $K_{SV,NIP}$.

3.6. Selectivity of Nanocomposite COOH@MWCNT-MIP-QDs for the Determination of Ciprofloxacin

The fluorescence quenching of nanocomposite COOH@MWCNT-MIP-QDs towards other ciprofloxacin structural analogs (danofloxacin, difloxacin, enrofloxacin, norfloxacin, sarafloxacin) were investigated to study its selectivity since hydrogen bonds can form between the structural analogs and the functional groups located in the imprinting sites. As shown in Fig. 7, the nanocomposite COOH@MWCNT-MIP-QDs had higher fluorescence quenching than other nanocomposites. This is because during the synthesis process of nanocomposite COOH@MWCNT-MIP-QDs, many specific imprinting sites which act as a memory of the shape, size and functional groups of ciprofloxacin were generated. However, the nanocomposite COOH@MWCNT-NIP-QDs showed low fluorescence quenching to ciprofloxacin and other compounds due to no recognition sites existing in the NIP layer and the molecules were adsorbed on the surface of NIP only through non-specific binding. A competitive binding experiment was also undertaken as shown in Fig. 7 (inset).The sensitivities were not significantly changed by increasing of the ratio of $C_{Danofloxacin}/C_{Ciprofloxacin}$ which indicated that the recognition sites are specific to the template molecule (ciprofloxacin).

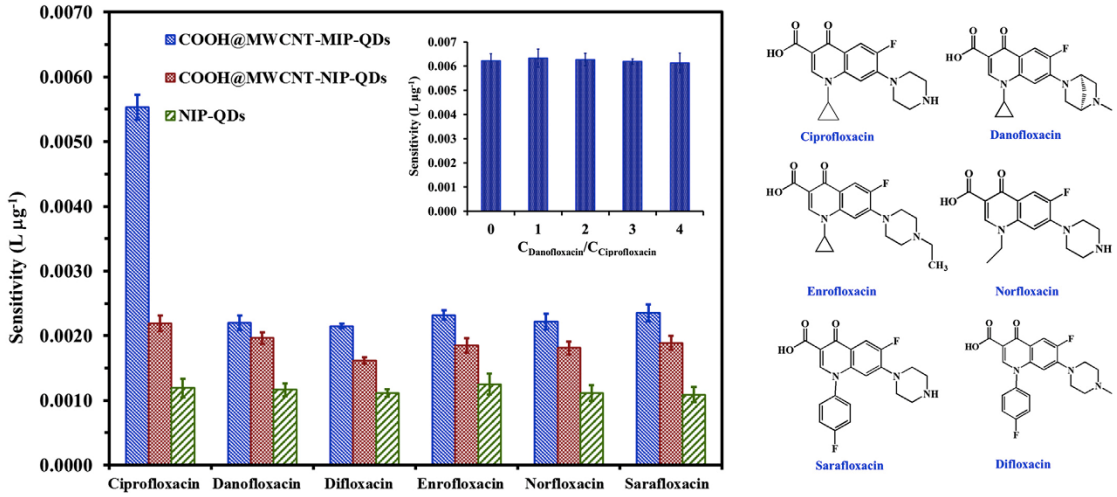

Fig. 7. The selectivity of nanocomposite COOH@MWCNT-MIP-QDs, COOH@MWCNT-NIP-QDs and NIP-QDs to ciprofloxacin and analogous molecules; (inset) effect of a competitive analog danofloxacin on the binding of ciprofloxacin to the nanocomposite COOH@MWCNT-MIP-QDs. For all results, $n = 3$.

3.7. Analytical Performance of Nanocomposite COOH@MWCNT-MIP-QDs for the Determination of Ciprofloxacin

The analytical performances of the developed nanocomposite COOH@MWCNT-MIP-QDs optosensor were investigated including linearity, limit of detection (LOD) and limit of quantification (LOQ). As shown in Fig. 8A, two linear relationships of calibration curve were found with ranges of 0.10–1.0 μg L^{-1}; F0/F = (0.159 ± 0.008) + (1.038 ± 0.005) (Fig. 8B) and 1.0–100.0 μg L^{-1}; F0/F = (0.0060 ± 0.0002) + (1.206 ± 0.009) (Fig. 8C). The fact that two linear calibration curves were obtained might be due to the inhomogeneity of the specific imprinting cavities on the surface of the MIP. The imprinting factors were 17.67 and 4.28, respectively. The fluorescence spectra of nanocomposite COOH@MWCNT-MIP-QDs after mixing with various concentrations of ciprofloxacin were shown in Fig. 8D, the fluorescence intensities were significantly quenched by ciprofloxacin. The insets are the photographs of nanocomposite COOH@MWCNT-MIP-QDs in the presence (right) and absence (left) of ciprofloxacin under UV light. The LOD and LOQ calculated following the IUPAC criteria were 0.066 μg L^{-1} and 0.22 μg L^{-1}, respectively.

3.8. Reproducibility and Stability of COOH@MWCNT-MIP-QDs

The reproducibility of the nanocomposite COOH@MWCNT-MIP-QDs optosensors were investigated by preparing six different batches of nanocomposite COOH@MWCNT-MIP-QDs under the optimal conditions at different times. The developed optosensors were used to determine ciprofloxacin in the concentration range of 1.0–50.0 μg L^{-1} and the sensitivity was used to evaluate the reproducibility. The relative standard deviation (RSD) of sensitivities of the six optosensing systems was 1.5%, indicating good batch-to-batch reproducibility.

The stability of nanocomposite COOH@MWCNT-MIP-QDs and CdTe QDs were also investigated by the repeated measurement of fluorescence intensity at room temperature (25 °C). The fluorescence intensity of nanocomposite COOH@MWCNT-MIP-QDs did not significantly change within 300 min, while the CdTe QDs fluorescence intensity was decreased after 90 min (Fig. S4). It could be deduced that the presence of a protective MIP layer helps to enhance the photostability of QDs.

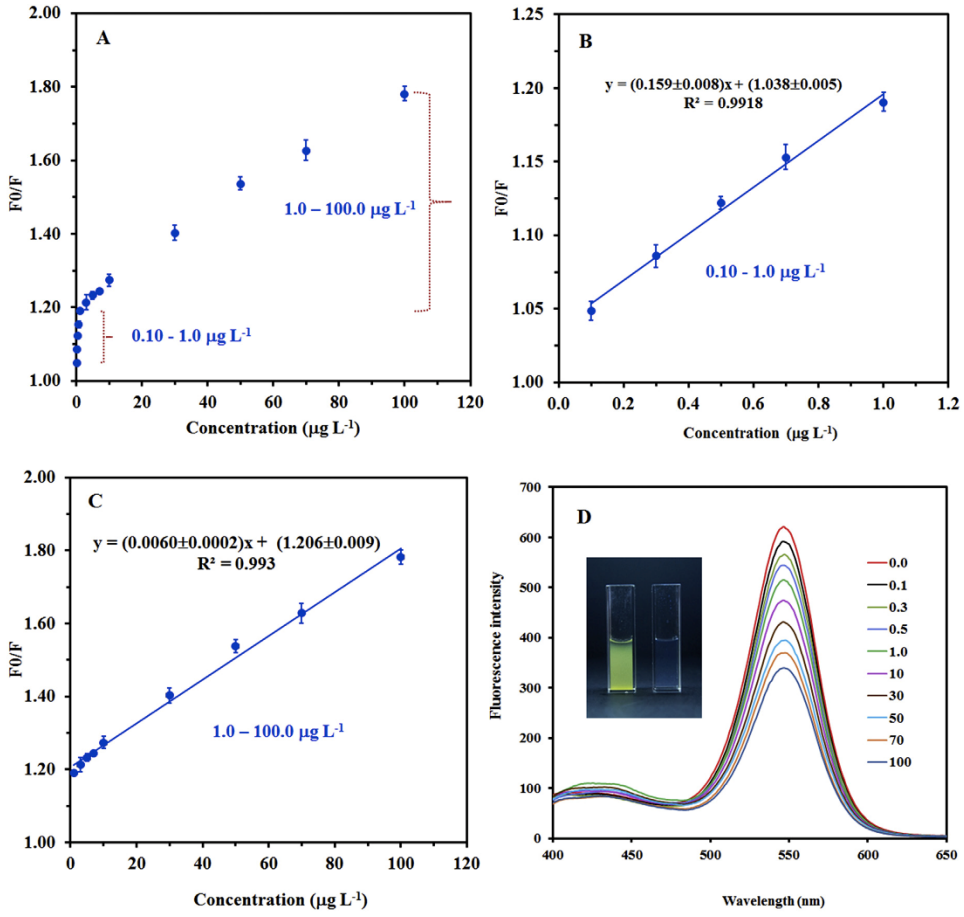

Fig. 8. (A)The linearity of ciprofloxacin in the concentration range of 0.10–100.0 μg L^{-1}, (B) 0.10–1.0 μg L^{-1}, (C) 1.0–100 μg L^{-1} ($n = 3$) and (D) fluorescence spectra of nanocomposite COOH@MWCNT-MIP-QDs with increasing concentration of ciprofloxacin; (inset) photographs of COOH@MWCNT-MIP-QDs in Tris-HCl buffer solution (left) and COOH@MWCNT-MIP-QDs + 1.0 mg L^{-1} of ciprofloxacin (right) under UV light.

Table 1
The determination of ciprofloxacin in chicken muscle and milk ($n = 5$).

Sample	Added (μg kg^{-1})	Found (μg kg^{-1})	Recovery (%)	RSD (%)
Chicken muscle I	0.0	0.19	–	–
	1.0	1.16 ± 0.02	96.2	2.4
	5.0	4.45 ± 0.21	85.1	4.9
	10.0	9.54 ± 0.42	93.4	4.5
	50.0	48.35 ± 1.34	96.3	2.8
Chicken muscle II	0.0	0.19	–	–
	1.0	1.08 ± 0.02	89.0	3.1
	5.0	4.80 ± 0.34	92.3	7.4
	10.0	9.69 ± 0.25	95.0	2.7
	50.0	48.02 ± 1.31	95.7	2.7
Chicken muscle III	0.0	n.d.	–	–
	1.0	0.95 ± 0.04	95.4	3.8
	5.0	4.82 ± 0.13	96.5	2.7
	10.0	9.80 ± 0.18	98.0	1.9
	50.0	49.03 ± 0.98	98.1	2.0
Milk I	0.0	n.d.	–	–
	1.0	0.85 ± 0.05	85.4	5.4
	5.0	4.52 ± 0.20	90.3	4.4
	10.0	9.44 ± 0.22	94.4	2.3
	50.0	49.20 ± 0.46	98.4	0.9
Milk II	0.0	0.35	–	–
	1.0	1.18 ± 0.01	82.6	1.2
	5.0	4.87 ± 0.18	90.6	4.0
	10.0	9.88 ± 0.20	95.2	2.0
	50.0	48.69 ± 0.64	96.7	1.3
Milk III	0.0	0.22	–	–
	1.0	1.12 ± 0.03	90.6	2.9
	5.0	4.58 ± 0.24	87.3	5.4
	10.0	9.58 ± 0.31	93.6	3.3
	50.0	48.20 ± 0.83	96.0	1.7

n.d. = not detectable.

3.9. Application of Nanocomposite COOH@MWCNT-MIP-QDs Optosensors for the Determination of Ciprofloxacin in Food Sample

The developed nanocomposite COOH@MWCNT-MIP-QDs fluorescence probe was applied to determine ciprofloxacin in chicken muscle and milk (Table 1). Low concentrations of ciprofloxacin were found in chicken muscle (0.19 μg kg^{-1}) and milk (0.22–0.35 μg kg^{-1}) which were lower than the MRL value set by European Union (100 μg kg^{-1}) for chicken muscle and milk. The accuracy of the developed optosensor was also investigated by spiking standard ciprofloxacin into real samples at different concentrations. Satisfactory recoveries between 82.6 and 98.4% were obtained with the relative standard deviation being lower than 8%.

The spiked samples could also be extracted and analyzed using HPLC methods. Fig. 9A shows HPLC chromatograms of spiked samples at different concentration of ciprofloxacin. The correlation between the developed optosensors and HPLC was very good with the coefficient of determination being 0.9987 (Fig. 9B). The results indicated that the developed nanocomposite COOH@MWCNT-MIP-QDs fluorescence probes can be used as a simple, rapid and sensitive method to detect trace ciprofloxacin in milk and chicken muscle.

3.10. Comparison of the Developed Hybrid Nanocomposite COOH@MWCNT-MIP-QDs Optosensor with Other Methods

The analytical performance of the developed optosensor for the determination of ciprofloxacin was compared to other works. As summarized in Table 2, the developed protocol provided a wide linear range and the detection limits are much lower than other work which demonstrates that the nanocomposite COOH@MWCNT-MIP-QDs are highly sensitive and selective for ciprofloxacin detection. The recovery (82.6–98.4%) and precision (<8%) of this method was comparable to other methods. This developed optosensor is simple, faster and lower cost than chromatographic techniques which require expensive instrumentation and potentially use large amounts of organic solvents as mobile phase. In addition, the selectivity of this sensor was improved with the use of MIPs, without requiring complicated separation processes crucial for many other methods.

4. Conclusion

A nanocomposite COOH@MWCNT-MIP-QDs optosensor was developed for the determination of ciprofloxacin based on the electron transfer induced fluorescence quenching. The developed optosensor combined the high specificity of MIPs with the excellent optical properties of QDs with the high affinity of COOH@MWCNT to ciprofloxacin, thereby demonstrating a highly selective, sensitive and rapid method for the determination of trace ciprofloxacin. This rapid, convenient and cost-effective hybrid nanocomposite optosensor was successfully applied to determine ciprofloxacin in milk and chicken muscle with a satisfactory recovery and also demonstrated excellent agreement with HPLC. This facile and versatile process for optosensor fabrication provides potentially alternative methods for the specific recognition of many other organic compounds.

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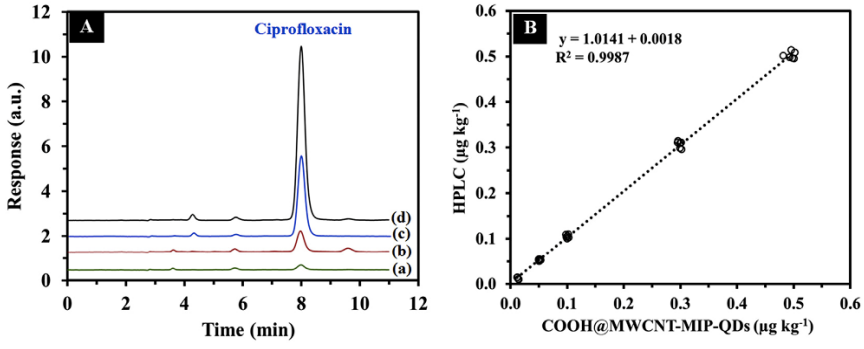

Fig. 9. (A) HPLC chromatograms of spiked milk samples at different concentration of ciprofloxacin; (a) 10 μg kg^{-1}, (b) 50 μg kg^{-1} (c) 300 μg kg^{-1} and (d) 500 μg kg^{-1}. (B) Correlation between the nanocomposite COOH@MWCNT-MIP-QDs optosensor system and the HPLC method for the determination of ciprofloxacin in milk.

Table 2
Comparison of the developed optosensor with other methods for the determination of ciprofloxacin.

Analytical technique	Samples	Linear range (μg L^{-1})	LOD (μg L^{-1})	Recovery (%)	RSD (%)	References
HPLC-FLD	Human plasma	20–4000	10.0	73.0–95.0	3.0–17.0	[49]
HPLC–UV	Human plasma	50–8000	10.0	90.0–96.0	<4.0	[5]
HPLC–PDA	Sputum samples	50–2000	17.0	>80.0	<15.0	[4]
HPLC-FLD	Surface water	200–2000	100	102.5–122.2	9.2	[50]
Electrochemical	Pharmaceutical samples and biological fluids	1.6–281.6	0.56	98.0–103.0	3.0	[9]
Fluorescent siderophorepyoverdine	Pharmaceutical tablet	–	2362	98.6	1.3	[51]
Electrochemical	Wastewater	3313–26,507	16.6	98.2–107.0	< 5.0	[52]
Electrochemical	Urine samples	33–3313	7.3	99.1–109.6	1.0–1.4	[8]
Electrochemical	Urine samples	0.15–2.11	0.05	97.0–102.0	2.4	[53]
Electrochemical	Physiological fluids	3313–3,310,000	2618	98.7–104.5	0.7–0.9	[54]
MIP based micromechanical cantilever sensor	–	497–50,000	265	94.0	1.4	[55]
COOH@MWCNT-MIP-QDs optosensor	Milk and chicken	0.1–1.0 1.0–100	0.066	82.6–98.4	<8.0	This work

HPLC = high performance liquid chromatography; FLD = fluorescence detector; UV=Ultraviolet-Visible detector; PAD = photodiode array detector; MIP = molecularly imprinted polymer; QDs = quantum dots; COOH@MWCNT = carboxylic functionalized multiwall carbon nanotubes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2018.05.034.

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Supporting Information

A nanocomposite optosensor containing carboxylic functionalized multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for highly sensitive and selective determination of ciprofloxacin

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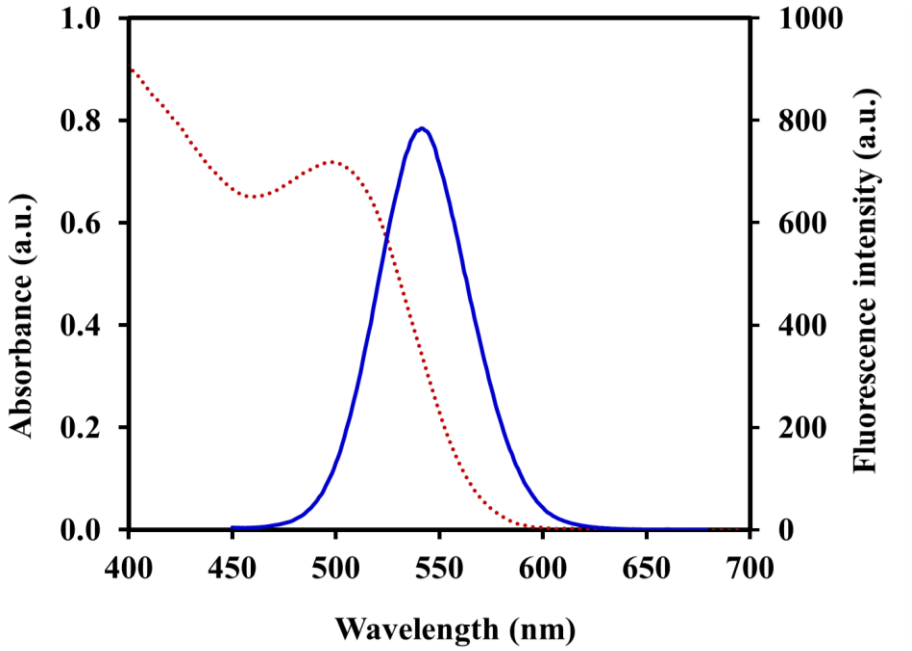

Fig. S1 UV-Vis spectrum (dot line) and fluorescence emission spectrum (solid line) of TGA-capped CdTe QDs.

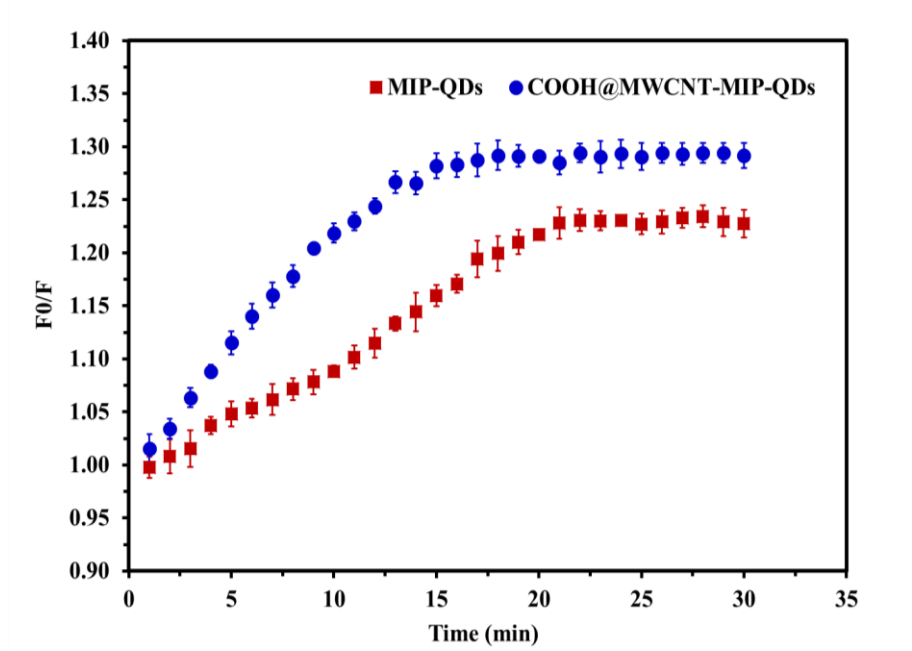

Fig. S2 Effect of incubation time on the fluorescence quenching of COOH@MWCNT-MIP-QDs and MIP-QDs for the determination of ciprofloxacin (*n*=3).

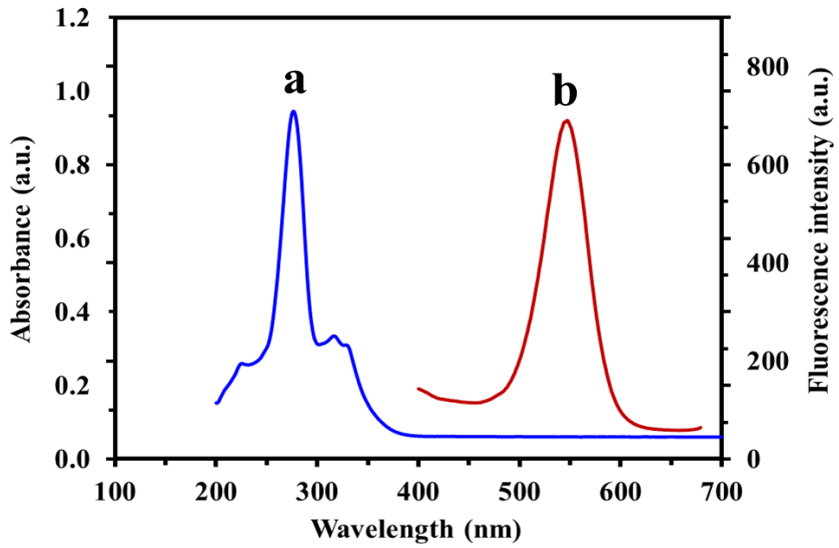

Fig. S3 Absorption spectrum of ciprofloxacin (a) and emission spectrum of nanocomposite COOH@MWCNT-MIP-QDs (b).

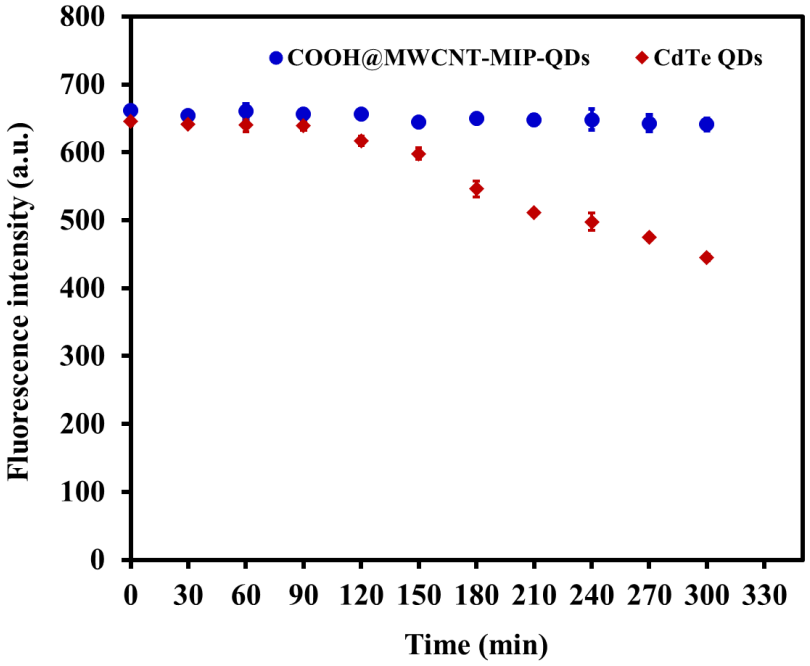

Fig. S4 The fluorescence stability of nanocomposite COOH@MWCNT-MIP-QDs fluorescence probes and CdTe QDs in 0.01 M Tris-HCl buffer solution (pH 7.0) (*n*=3)**.**

VITAE

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Educational attainment

Degree	Name of institution	Year of graduation
Bachelor of Science (Chemistry)	Prince of Songkla University	2016

Scholarship awards during enrolment

Faculty of Science and Graduate School, Prince of Songkla University (SCI600559S), the Faculty of Science Research Fund, Prince of Songkla University, for research assistantship (Contract No. 1-2559-02-009), the Thailand Research Fund, Office of the Higher Education Commission and Center of Excellence for Innovation in Chemistry (PERCH-CIC).

List of poster presentations and publication

Poster presentations

Yuphintharakun, N., Kanatharana, P., and Bunkoed, O. "Chemical sensor based on the fluorescence quenching of CdTe quantum dots for the determination of lead ions" Pure and Applied Chemistry International Conference (PACCON 2017), 2nd - 3rd February, 2017, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand.

Yuphintharakun, N., Kanatharana, P., and Bunkoed, O. "Chemical sensor for lead (Pb^{2+}) detection based on the fluorescence quenching of CdTe quantum dots" Research and Innovation for Social Stability, Prosperity and Sustainability, 8th – 9th May, 2018, Thaksin University, Songkhla, Thailand.

Yuphintharakun, N., Nurerk, P., Chullasat, K., Kanatharana, P., Davis, F., Sooksawat, D., and Bunkoed, O. “An optosensor based on molecularly imprinted polymer composited with COOH-functionalized multiwall carbon nanotube and quantum dots for highly sensitive and selective ciprofloxacin detection” PERCH-CIC Congress X, 4^{th} – 7^{th} July, 2018, Pattaya, Chonburi, Thailand.

Publication

Yuphintharakun, N., Nurerk, P., Chullasat, K., Kanatharana, P., Davis, F., Sooksawat, D., and Bunkoed, O. “A nanocomposite optosensor containing carboxylic functionalized multiwall carbon nanotubes and quantum dots incorporated into a molecularly imprinted polymer for highly selective and sensitive detection of ciprofloxacin. *Spectrochim Acta A Mol Biomol Spectrosc* 201, **2018**, 382–391.