Optosensor Based on Molecularly Imprinted Polymer Coated Quantum Dots Composited with Polypyrrole for Ampicillin Detection

Phannika Raksawong

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (International Program)

Prince of Songkla University

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Author	Miss Phannika Raksawong
Major Program	Chemistry (International Program)

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The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillments of the requirements for the Master of Science Degree in Chemistry (International Program)

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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 acknowledgment has been made of any assistance received.

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

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(Miss Phannika Raksawong)

Candidate

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บทคัดย่อ

วิทยานิพนธ์นี้มีจุดประสงค์เพื่อพัฒนาเซนเซอร์ทางแสงโดยประยุกต์ใช้พอลิเมอร์ลอกแบบโมเลกุลเคลือบควอนตัมดอทคอมโพสิทร่วมกับพอลิไพโรลสำหรับตรวจวิเคราะห์แอมพิซิลลิน การใช้ร่วมกันของควอนตัมดอทซึ่งมีคุณสมบัติทางแสงฟลูออเรสเซนต์ที่ดี พอลิเมอร์ลอกแบบโมเลกุลที่มีความจำเพาะเจาะจงต่อสารที่ต้องการวิเคราะห์สูง และพอลิไพโรลที่ช่วยเพิ่มประสิทธิภาพการจับสารแอมพิซิลลิน ทำให้ได้เซนเซอร์ทางแสงมีความไววิเคราะห์สูงและมีความจำเพาะเจาะจงต่อแอมพิซิลลิน และช่วยเพิ่มประสิทธิภาพการตรวจวัดแอมพิซิลลินแม้มีปริมาณน้อยได้ ภายใต้สภาวะที่เหมาะสม สัญญาณฟลูออเรสเซนต์ของตัวตรวจวัดทางแสงที่พัฒนาขึ้นลดลงเป็นเส้นตรงตามความเข้มข้นของแอมพิซิลลิน 2 ช่วง ตั้งแต่ 0.10 ถึง 25.0 ไมโครกรัมต่อลิตร และ 25.0 ถึง 100.0 ไมโครกรัมต่อลิตร มีขีดจำกัดการตรวจวัดเท่ากับ 0.05 ไมโครกรัมต่อลิตร ได้ประยุกต์ใช้วิธีที่พัฒนาขึ้นตรวจวัดแอมพิซิลลินในตัวอย่างนมและเนื้อสัตว์ โดยมีค่าร้อยละการได้กลับคืนในช่วง 81.7 ถึง 98.7 และร้อยละค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 5 อีกทั้งวิธีที่พัฒนาขึ้นให้ผลการตรวจวิเคราะห์ที่สอดคล้องกับวิธีมาตรฐานโครมาโทกราฟีของเหลวสมรรถนะสูง จุดเด่นของเซนเซอร์ทางแสงที่พัฒนาขึ้น ได้แก่ สามารถใช้งานได้ง่าย ตรวจวัดได้รวดเร็ว มีค่าความไววิเคราะห์สูง และมีความจำเพาะเจาะจงสูง วิธีที่พัฒนาขึ้นสามารถประยุกต์ใช้สำหรับตรวจวิเคราะห์สารอื่นๆ ในตัวอย่างต่างๆ ได้

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ABSTRACT

This thesis aimed to develop a nanooptosensor of molecularly imprinted polymer coated quantum dots composited with polypyrrole (PPy-QDs-MIP) for the determination of ampicillin. The integration of good fluorescence intensity of QDs, high selectivity of MIP and high affinity of PPy exhibited high sensitivity and selective recognition and facilitated the adsorption capability for trace ampicillin analysis. Under the optimal conditions, the fluorescence intensity of the developed nanocomposite probe was effectively quenched linearly with the concentration of ampicillin over two linear ranges of 0.10 to 25.0 μg L^{-1} and 25.0 to 100.0 μg L^{-1}. The limit of detection is 0.05 μg L^{-1}. The developed method was successfully applied for the determination of ampicillin in milk and meat samples and gave satisfactory recoveries between 81.7 and 98.7% and the relative standard deviations were less than 5%. The results were in good agreement with HPLC method. The advantages of developed PPy-QDs-MIP nanooptosensor were simple to operate, rapid for detection, high sensitivity and good selectivity. This developed strategy can be applied for the detection of other compounds in various samples.

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Phannika Raksawong

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this Master of Science Thesis in Chemistry (Analytical chemistry) was to develop an optosensor which composited of polypyrrole, quantum dots and molecularly imprinted polymer for the detection of ampicillin.

This developed optosensor provided a high sensitivity, good selectivity and simple to use. It can be applied for the determination of ampicillin in food samples. In addition, it can help to decrease analysis cost, analysis time and several government organizations in Thailand that can use the outcome of this work including the Ministry of Public Health, Ministry of Industry, Ministry of Environment and the Ministry of Education.

CONTENTS

CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

LIST OF ABBREVIATIONS

APTES	3-aminopropyltriethoxysilane
BET	Brunauer-Emmett-Teller
CB	Conduction band
CdS	Cadmium sulfide
CdSe	Cadmium selenide
CdTe	Cadmium telluride
EU	European Union
FLD	Fluorescence detector
FTIR	Fourier transform infrared spectroscopy
GaAs	Gallium arsenide
Ge	Germanium
GSH	Glutathione
HgS	Mercury sulfide
HPLC	High performance liquid chromatography
InAs	Indium arsenide
InP	Indium phosphide
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantification
MIP	Molecularly imprinted polymer
MPA	Mercaptopropionic acid
MRLs	Maximum residue limits
NIP	Non-imprinted polymer
PbS	Lead (II) sulfide
PbSe	Lead selenide
PPy	Polypyrrole
QDs	Quantum dots
R^2	Coefficient of determination
RSD	Relative standard deviation
SEM	Scanning electron microscopy

LIST OF ABBREVIATIONS (CONTINUED)

Si	Silicon
TEM	Transmission electron microscopy
TEOS	Tetraethyl orthosilicate
TGA	Thioglycolic acid
UV-Vis	Ultraviolet-visible
VB	Valance band
ZnS	Zinc sulfide
ZnSe	Zinc selenide
ZnTe	Zinc telluride

LIST OF PUBLICATION

Paper **Raksawong, P.**, Nurerk, P., Chullasat, K., Kanatharana, P., Bunkoed, O., A Polypyrrole doped with fluorescent CdTe quantum dots and incorporated into molecularly imprinted silica for fluorometric determination of ampicillin. ***Microchimica Acta*** 186 (2019) 338.

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1. Introduction

1.1 Background and rationale

Ampicillin is a β-lactam antibiotic which is normally used for the treatment of bacterial infection both Gram-positive and Gram-negative bacteria in humans and animals (Grant, 1980; Rafailidis *et al.*, 2007; Beltran *et al.*, 2008). It can residue in meat and milk which cause stomach cramps, diarrhea, dizziness, nausea, rash and peeling of the skin (Shrivas *et al.*, 2017). The maximum residue limits (MRLs) for ampicillin in milk and meat have been set by the European Union at 40 and 50 μg kg^{-1}, respectively. Therefore, the development of a convenient, rapid, sensitive, selective and reliable method for the detection of ampicillin in food samples is important.

Various analytical techniques have been reported for the determination of ampicillin including chromatography (Sun *et al.*, 2016), electrophoresis (Paul *et al.*, 2018) and electrochemistry (Wang *et al.*, 2017; Yu *et al.*, 2018). Although, these techniques provide accurate results, they still have some drawbacks. They are time-consuming, use large volumes of toxic organic solvent and require expensive instruments. To overcome these limitations, fluorescence spectroscopy is employed for because of simplicity, rapidity and cost-effective equipment (Liu *et al.*, 2018). However, the relatively low fluorescence intensity of ampicillin which cannot be directly detected at trace levels. The sensitivity of this method can be improved by exploiting the good optical properties of fluorescent probes. Quantum dots (QDs) are an interesting choice of fluorescent probe due to their high fluorescence intensity and good photochemical stability (Zhang *et al.*, 2011). To enhance the specificity of the analytical method, QDs can be modified with highly specific ligands or incorporated with specific materials such as molecularly imprinted polymer (MIP). MIP shows high specificity to target analytes (Carrasco *et al.*, 2016; Canfarotta *et al.*, 2018) and has good physical and chemical stability under demanding conditions of temperature, pH and pressure, using a variety of acids, bases and organic solvents (Urraca *et al.*, 2007; Sunayama *et al.*, 2016; Panjan *et al.*, 2018). The MIP is synthesized by a copolymerization reaction using ampicillin as the template. The resulting MIP interacts with a functional monomer and cross-linker to form a polymer layer (Gao *et al.*, 2007; Panahi *et al.*, 2018). After removal of template, the MIP layer contains specific binding cavities complementary in functional groups, shape and size to the target molecule (Gao

et al., 2007). MIP has been used as a chemosensor (Dabrowski *et al.*, 2018), adsorption material (Sorribes-Soriano *et al.*, 2018) and biosensor (Ekomo *et al.*, 2018). Fluorescent probes using MIP coated on QDs have engaged interest for the determination of target analytes such as amoxicillin (Chullasat *et al.*, 2018), chloramphenicol (Amjadi *et al.*, 2016) and salbutamol (Raksawong *et al.*, 2017). To enhance the adsorption ability or affinity binding of ampicillin to the fluorescent probe, the addition of polypyrrole during preparation of a nanocomposite fluorescent probe is an interesting strategy. Polypyrrole contains a π-structure which can adsorb aromatic compounds through π-π interaction (Nezhadali *et al.*, 2018). Moreover, polypyrrole has good chemical stability (Meng *et al.*, 2011).

In this thesis, a nanooptosensor was fabricated using a composite fluorescent probe of polypyrrole and quantum dots incorporated in a molecularly imprinted polymer (PPy-QDs-MIP). The nanooptosensor was characterized and the analytical performances were evaluated and compared with the results of a conventional chromatographic method (HPLC). The method was then used to detect ampicillin in food samples.

1.2 Objective

To develop a nanooptosensor of molecularly imprinted polymer coated quantum dots composited with polypyrrole for the determination of ampicillin in food samples.

1.3 Quantum dot nanoparticles

Quantum dots (QDs) are spherical particles with diameters in the range between 1 and 10 nm (Drbohlavova *et al.*, 2009). They are prepared using atoms from groups II-VI, III-V or IV-VI in the periodic table and ultimately become many different types of QDs as shown in Table 1.1 (Yu *et al.*, 2003; Sharon *et al.*, 2010; Tang *et al.*, 2013; Zhou *et al.*, 2017).

Table 1.1 Different types of quantum dot nanoparticles

Type	Quantum dots
II-VI	CdS, CdSe, CdTe, ZnS, ZnSe, ZnTe, HgS, PbS, PbSe
III-V	GaAs, InP, InAs
IV	Si, Ge

The structure of QDs consists of a core and a shell which can improve stability and dispersibility in water or buffer solution as shown in Figure 1.1.

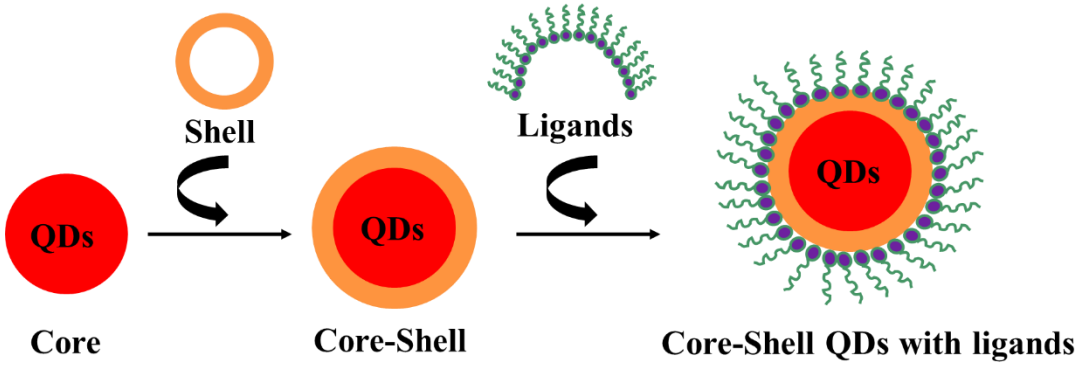

Figure 1.1 Basic structure of quantum dots (QDs)

QDs have two energy bands including valence and conduction bands. The higher energy level of valence band is occupied by electron at room temperature whereas the electrons are not occupied in the lower energy of conduction band. The electron is promoted from the valence band to conduction band by photo absorption, leading to a positively charged hole in the valence band. The different energy level is defined as band gap energy (Figure 1.2) (Reshma and Mohanan, 2019; Xu and Zheng, 2019).

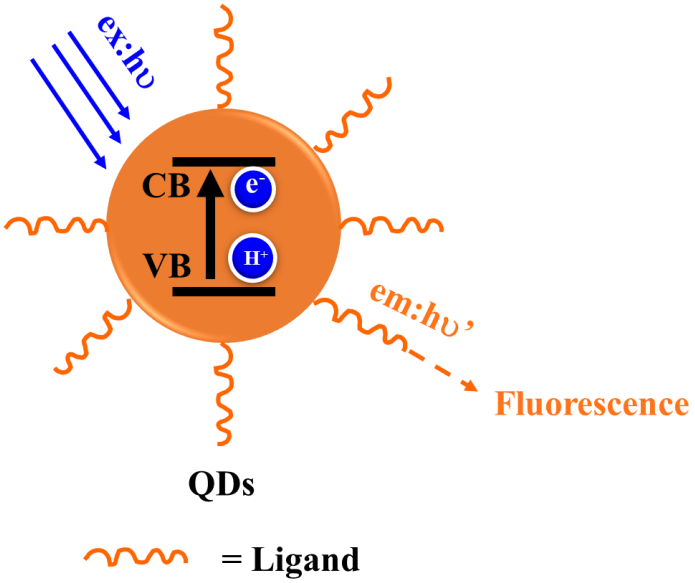

Figure 1.2 The energy band structure of QDs

The energy band gap depends on the size of QDs. The smaller size of QDs which has wider energy band gap, requires higher energy level, resulting in shorter wavelength (blue). While the larger size of QDs leads to longer wavelength (Figure 1.3) (Brus, 1984).

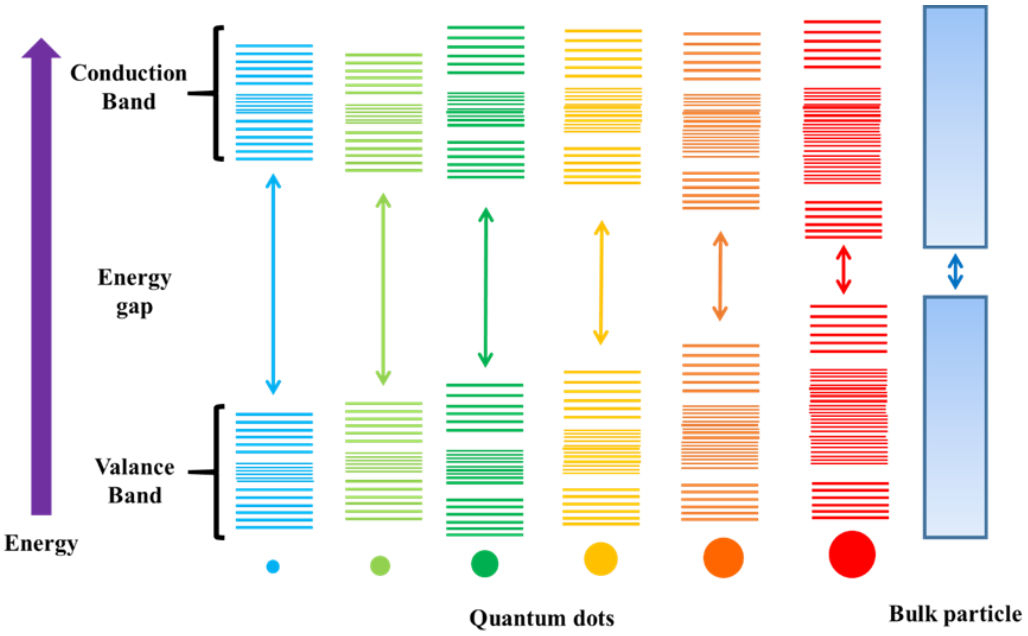

Figure 1.3 The size tunable optical properties of QDs illustrating increase in emission wavelength with increasing QDs size

QDs have attracted considerable interest due to their high fluorescence efficiency, size-dependent emission wavelengths, narrow symmetric emission and excellent photochemical stability (Bruchez *et al.*, 1998; Chan and Nie, 1998). To enhance the stability and solubility of QDs in the solution, their surfaces have been modified with capping molecules such as thioglycolic acid (TGA) (Nurerk *et al.*, 2016; Zhou *et al.*, 2017), mercaptopropionic acid (MPA) (Bunkoed and Kanatharana, 2015), cysteamine (Chen *et al.*, 2007; Ding *et al.*, 2015) and glutathione (GSH) (Adegoke *et al.*, 2012).

QDs are applied as a fluorescent probe for the highly sensitive detection of some target analytes such as perfluorooactanoic acid (Liu *et al.*, 2015), copper ion (Boonmee *et al.*, 2016), 2,4,6-trinitrotoluene (TNT) (Qian *et al.*, 2016), ceftriaxone (Samadi and Narimani, 2016). In this thesis, TGA-capped CdTe QDs were used to fabricate the fluorescence probe because they are easily synthesized under mild conditions and water soluble. The structure of TGA-capped CdTe QDs is shown in Figure 1.4.

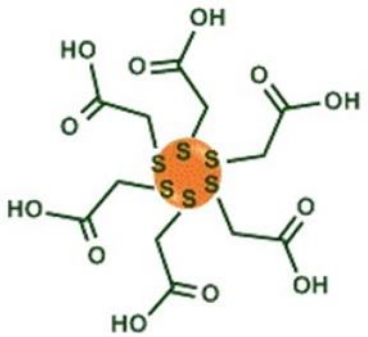

Figure 1.4 Structure of thioglycolic acid-capped CdTe QDs

1.4 Molecularly imprinted polymer (MIP)

Molecularly imprinted polymer is the attractive strategy for high binding selectivity of target analytes (Xu *et al.*, 2004; Chen *et al.*, 2016). MIP is synthesized via a copolymerization of functional monomers and cross-linkers in the presence of template molecules (target analyte). After removal of the templates, highly specific recognition sites that are complementary to the template in size, shape and functional groups are obtained (Sellergren and Allender, 2005; Lv *et al.*, 2013). The molecular

imprinting strategy is shown in Figure 1.5. MIP has many advantages such as ease of preparation, good chemical stability and low cost (Xu *et al.*, 2004; Tse Sum Bui and Haupt, 2010; Vasapollo *et al.*, 2011; Huang *et al.*, 2015).

Recently, surface of MIP has been successfully applied as recognition cavities in sensors and exhibited high selectivity for detection of trace contaminants (Shahar et al., 2017; Uzun and Turner, 2016). After surface functionalization of QDs with molecular imprinting, the molecularly imprinted polymer coated quantum dots (QDs-MIP) exhibits a high selectivity to target molecules and excellent fluorescence properties (Chantada-Vázquez et al., 2016). In this thesis, QDs embedded in MIP composite with polypyrrole (PPy-QDs-MIP) were prepared using ampicillin as a template, 3-aminopropyltriethoxysilane (APTES) as a functional monomer and tetraethoxysilane (TEOS) as a cross-linker.

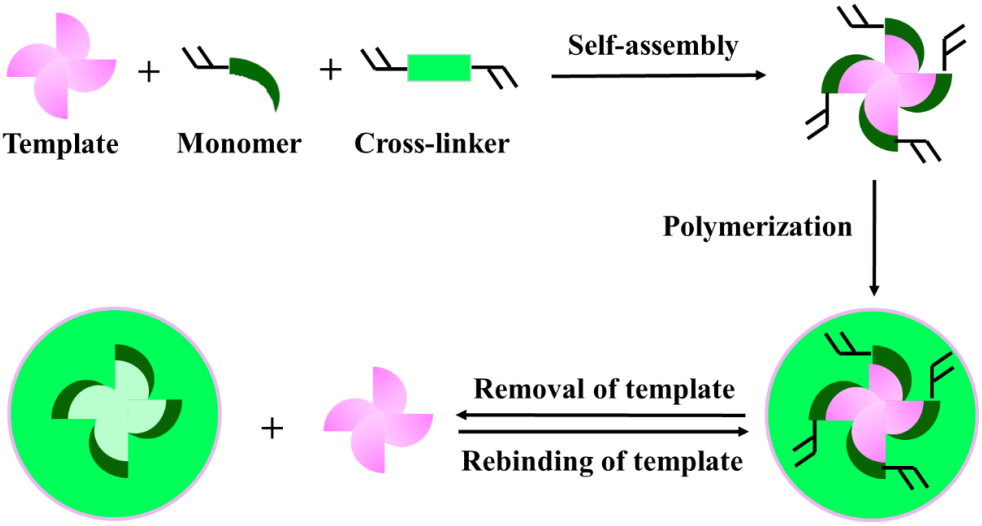

Figure 1.5 The preparation of molecularly imprinted polymer

1.5 Polypyrrole

Polypyrrole (PPy) is a conductive polymer which have been widely used in many fields due to its ease of preparation and good chemical stability (Luo *et al.*, 2010; Meng *et al.*, 2011; Tang *et al.*, 2017). It can be easily synthesized via polymerization of pyrrole monomer (Navale *et al.*, 2014). The structure of PPy contains π-conjugated structure (Figure 1.6) which can absorb aromatic compounds through π- π interaction.

The PPy nanoparticles were composited in fluorescent probe to improve the adsorption ability of ampicillin (Nezhadali *et al.*, 2018).

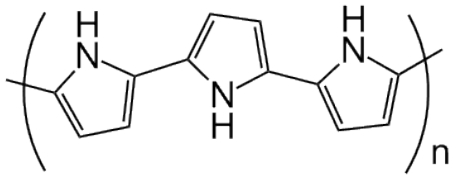

Figure 1.6 Structure of polypyrrole

2. Results and discussion

2.1 Synthesis of TGA-capped CdTe quantum dots

The TGA-capped CdTe QDs were synthesized by modification from a previous work as shown in Figure 2.1 (Ortiz *et al.*, 2016). Briefly, 5.0 mg of tellurium and 4.0 mg of sodium borohydride were mixed with 1.5 mL of deionized water in a centrifuge tube (2.0 mL) to produce a sodium hydrogen telluride (NaHTe) solution. Meanwhile, 45.0 mg of cadmium chloride and 30 μL of TGA were added to 100 mL deionized water and adjusted to pH 11.5 with sodium hydroxide (1.0 M). The mixture solution was deaerated with N_2 gas for 10 min in a three-necked flask, then heated to 90 ^oC and 1.0 mL of NaHTe was injected into the solution and continuously stirred for 15 min. Finally, the synthesized TGA-capped CdTe QDs were precipitated with 20 mL of ethanol and centrifuged at 8000 rpm for 5 min.

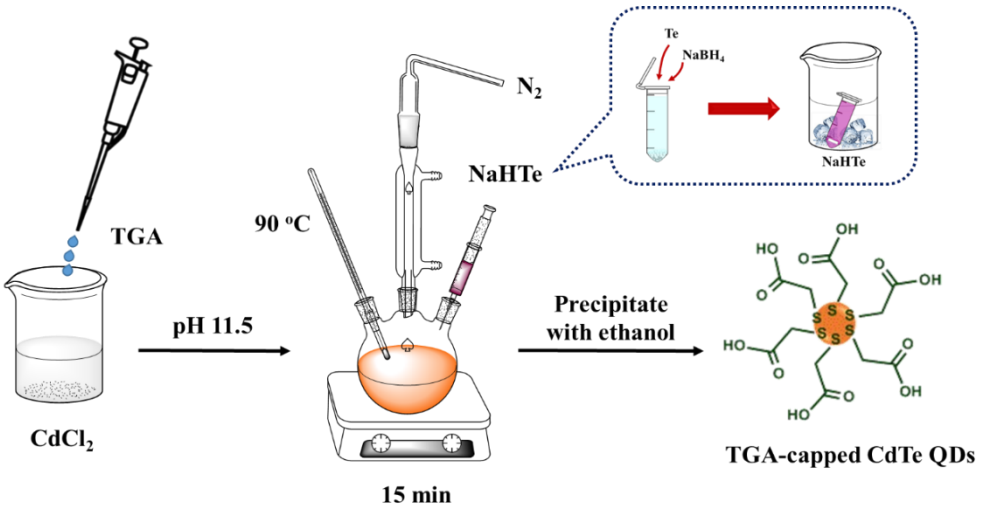

Figure 2.1 The synthesis of TGA-capped CdTe quantum dot nanoparticles

2.2 Characterization of TGA-capped CdTe quantum dots

The fluorescence spectrum of TGA-capped CdTe QDs was narrow and symmetric (Figure 2.2) and the particle size was about 2.25 nm which was calculated from the maximum absorption wavelength at 548 nm. The particle sizes of TGA-capped CdTe QDs were determined from the adsorption maximum of the UV-Vis spectrum following up equation (1) (Yu *et al.*, 2003):

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

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 TGA-capped CdTe QDs were calculated by Lambert-Beer's law; $A = \varepsilon CL$. Where, A is the absorbance of the first excitonic absorption peak, C is the concentration (mol L^{-1}) of the TGA-capped 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 TGA-capped CdTe QDs which could be obtained with formula $\varepsilon = 10043\ (D)^{2.12}$ (Yu *et al.*, 2003).

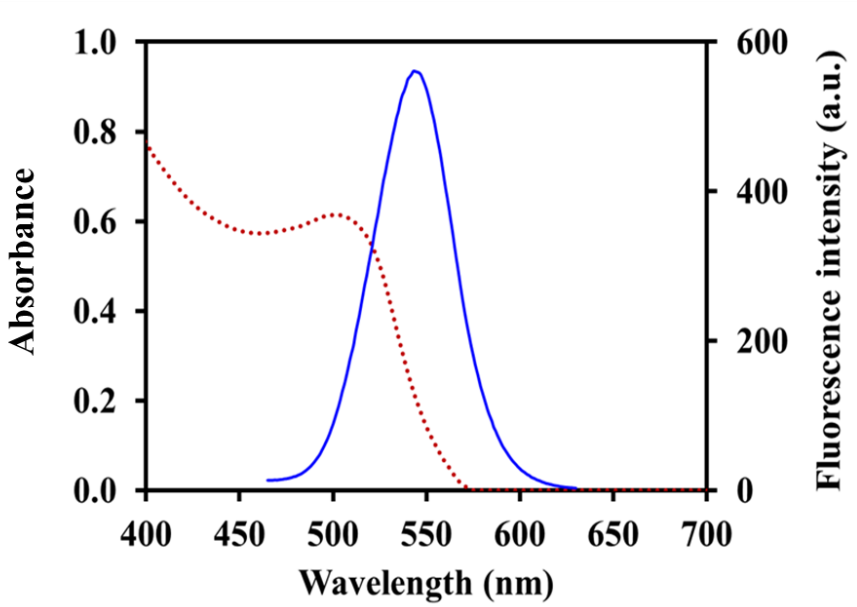

Figure 2.2 UV-Vis spectrum (dot line) and fluorescence emission spectrum (solid line) of TGA-capped CdTe QDs

2.3 Synthesis of molecularly imprinted polymer coated quantum dots composite with polypyrrole fluorescent probe

The molecularly imprinted polymer coated quantum dots composite with polypyrrole (PPy-QDs-MIP) were synthesized via a one-step copolymerization. First, 40 μL of APTES (functional monomer) was mixed with 3.0 mM of ampicillin (5.0 mL) and stirred for 1 h. Next, 10 mL of TGA-capped CdTe QDs were added to the mixture solution and continuously stirred for 1 h. Then, 120 μL of TEOS as a cross-linker, 150 μL of NH_3 (25% w/v) as a catalyst and 20 μL of polypyrrole particles were added and stirred for 6 h. Polypyrrole particles were prepared by mixing 13.0 mg of $FeCl_3$ and 400 μL of pyrrole in a 2.0 mL centrifuge tube and stirring for 1 h. APTES, template, TGA-capped CdTe QDs and PPy self-assembled via hydrogen bonding and then formed nanocomposite particles in the presence of the cross-linker (TEOS) and ammonia (catalyst) as shown in Figure 2.3. For template removal, the synthesized nanocomposites were washed three times with 10 mL of ethanol, after which no template molecules presented in the washing solution. The PPy-QDs-MIP nanoparticles were centrifuged at 7500 rpm for 10 min and dried in an oven at 60 ^{o}C. The

nanocomposite non-printed polymer (PPy-QDs-NIP) fluorescent probes were prepared under the same experimental condition without ampicillin (template molecule).

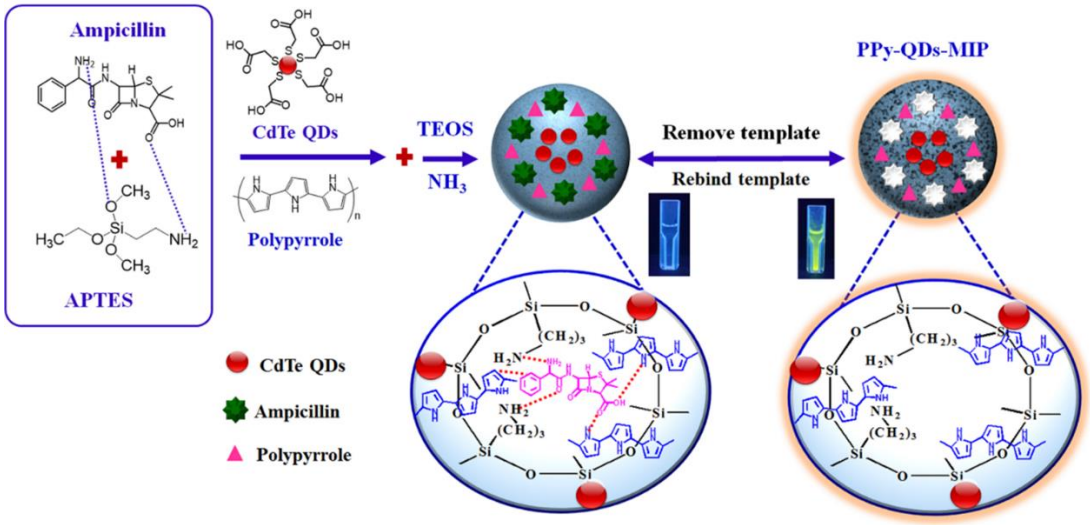

Figure 2.3 Synthesis of nanocomposite PPy-QDs -MIP fluorescent probe for ampicillin detection

The fluorescence emission intensity of PPy-QDs-MIP before removal of the template was about 60% the intensity of the emission of PPy-QDs-NIP (Figure 2.4). After removal of the template, the fluorescence emission was restored to the same intensity as PPy-QDs-NIP. The result confirmed that the PPy-QDs-MIP was successfully prepared and the template had been completely removed from the MIP layer. The method is a single-step copolymerization under soft conditions at room temperature. A photograph of nanocomposite PPy-QDs-MIP in the presence and absence of ampicillin under UV light is shown in Figure 2.5.

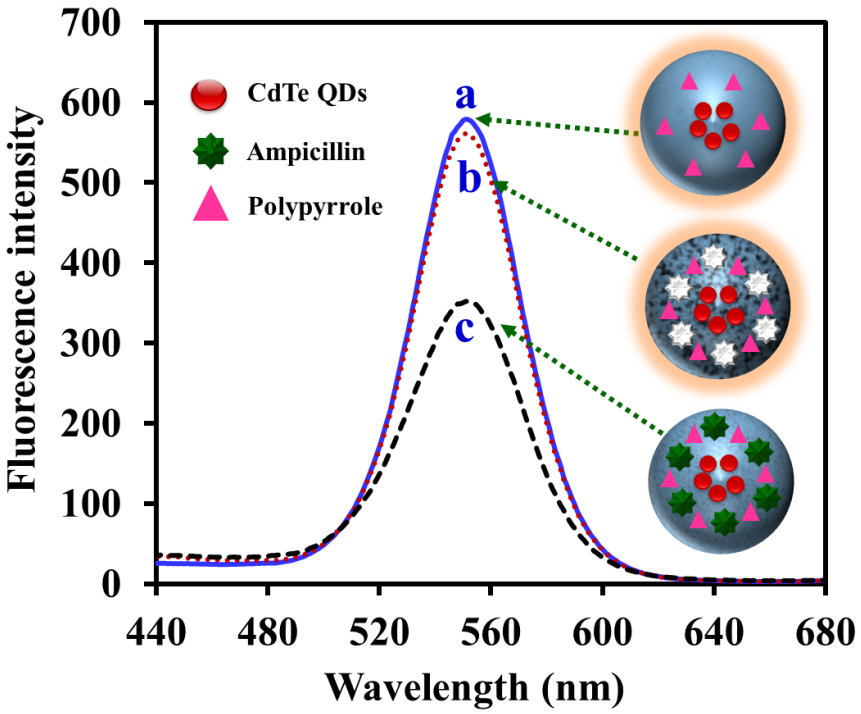

Figure 2.4 Fluorescence emission spectra of nanocomposite PPy-QDs-NIP (a), PPy-QDs-MIP without template molecule (b), PPy-QDs-MIP with template molecule (c)

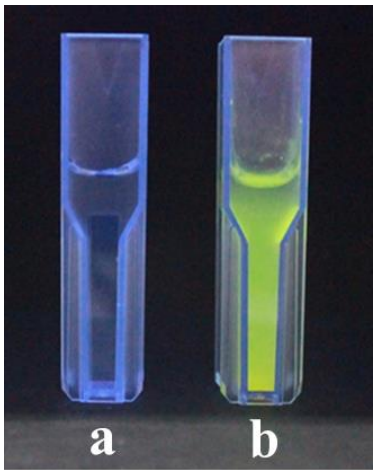

Figure 2.5 Photographs of nanocomposite PPy-QDs-MIP with (a) and without (b) ampicillin under UV light

2.4 Characterization of nanocomposite PPy-QDs-MIP fluorescent probe

The morphologies of TGA-capped CdTe QDs were investigated using TEM technique. As can be seen from Figure 2.6a, the TGA-capped CdTe QDs showed good distribution and a smooth surface. As shown in Figure 2.6b, the SEM image showed PPy-QDs-MIP of uniform spherical shape with a diameter of 200-250 nm. The TEM image of PPy-QDs-MIP indicated that TGA-capped CdTe QDs and polypyrrole particles were distributed in spherical nanocomposites and MIP was cross-linked around the QDs as shown in Figure 2.6c.

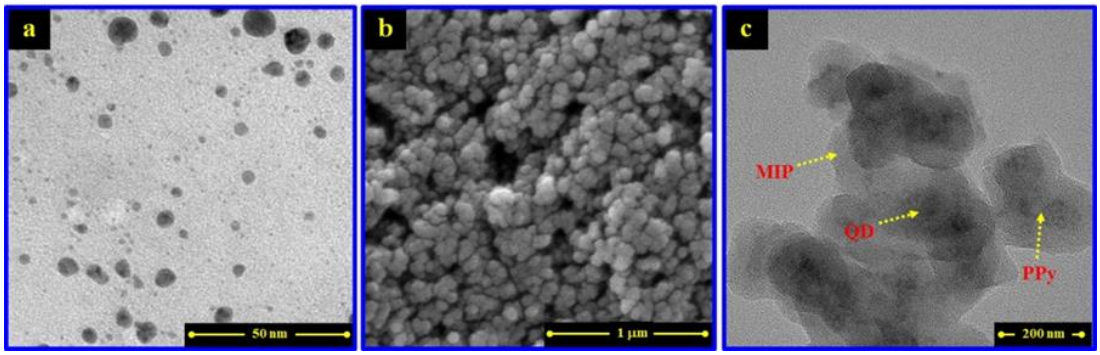

Figure 2.6 TEM image of TGA-capped CdTe QDs (a), SEM image of PPy-QDs-MIP (b) and TEM image of PPy-QDs-MIP fluorescent probe (c)

The nanocomposite PPy-QDs-MIP fluorescent probe was characterized by FTIR spectroscopy. The spectrum of TGA-capped CdTe QDs (Figure 2.7a) showed characteristic peaks at 1375 and 1566 cm^{-1}, which are due to the symmetric and asymmetric stretching of the carboxylate group (COO^-). The bands at 1229 and 3415 cm^{-1} are due to C-O and -OH stretching vibration. The FTIR spectrum of ampicillin (Figure 2.7b) showed peaks at 1690 cm^{-1} and 1774 cm^{-1} due to the C=O stretching of carboxylic and carbonyl groups. The peak at 3515 cm^{-1} is due to N-H stretching (primary amine). The peaks at 1385 and 1494 cm^{-1} are attributed to C-N stretching and N-H plane bending. The characteristic peaks of ampicillin also appeared in the FTIR spectrum of the nanocomposite PPy-QDs-MIP optosensor (Figure 2.7c). After template removal (Figure 2.7d), the absorption peaks corresponding to ampicillin disappeared. The peaks at approximately 455 and 768 cm^{-1} are due to Si-O vibration (Soledad-Rodríguez *et al.*, 2017). The bands around 1560 and 1650 cm^{-1} are due to the

C=C stretching of polypyrrole (Figure 2.7e). The results showed that the nanocomposite PPy-QDs-MIP probe was successfully synthesized for specific recognition of ampicillin.

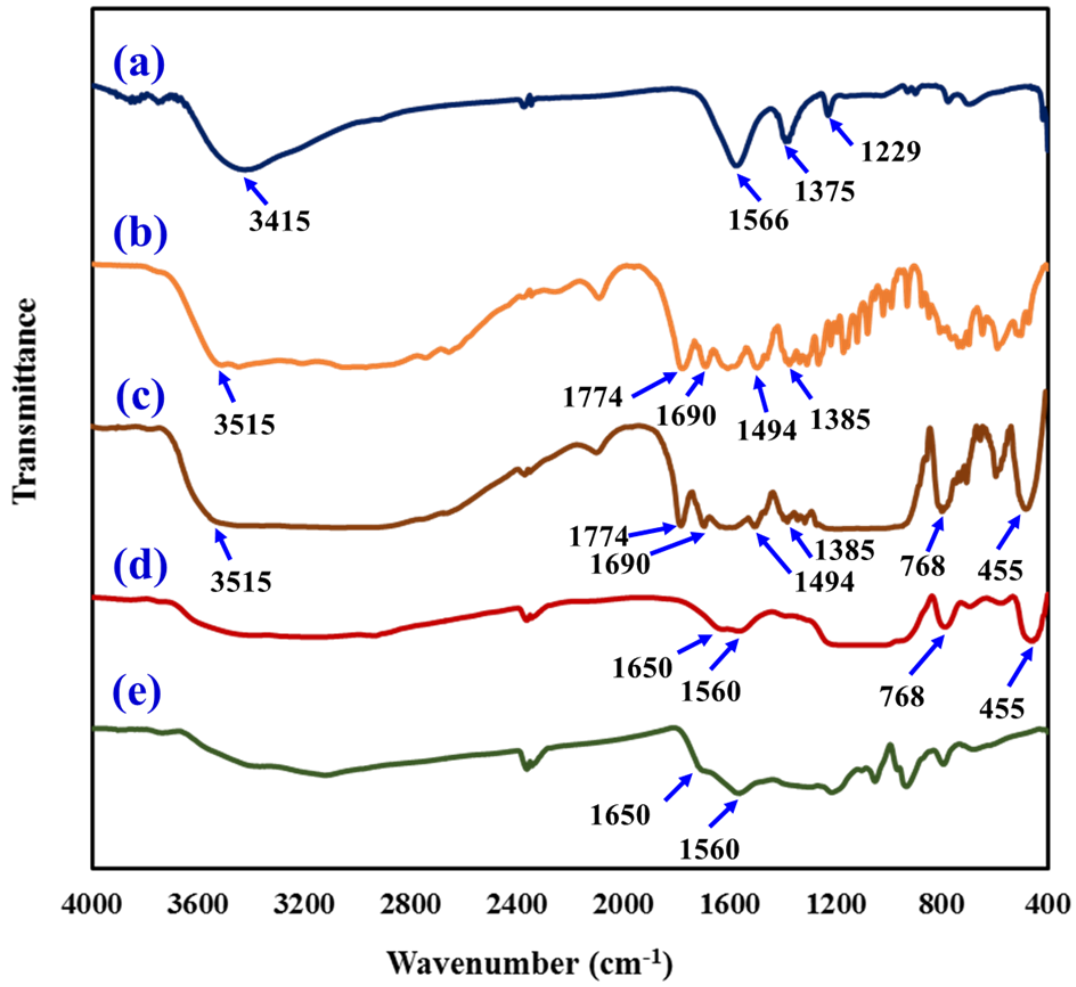

Figure 2.7 FTIR spectra of TGA-capped CdTe QDs (a), ampicillin (b), PPy-QDs-MIP before removal of ampicillin (template) (c), PPy-QDs-MIP after removal of ampicillin (template) (d) and polypyrrole (e)

The relative quantum yield (QY) was calculated according to equation (2) (Khalilzadeh *et al.*, 2009). Rhodamine 6G was used as the fluorescent standard. QYs of TGA-capped CdTe QDs, QDs-MIP and PPy-QDs-MIP were 0.90, 0.68 and 0.55, respectively.

$$QY = QY_{Std} \cdot \frac{F \cdot A_{Std} \cdot n^2}{F_{Std} \cdot A \cdot n_{Std}^2} \quad (2)$$

Where F and F_{Std} are the fluorescent areas under the fluorescent curves of the ampicillin 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 measurements of the specific surface areas of PPy-QDs-MIP and PPy-QDs-NIP were 22.2 $m^2 g^{-1}$ and 16.7 $m^2 g^{-1}$, respectively. The PPy-QDs-MIP nanooptosensor displayed greater surface area than PPy-QDs-NIP, possibly due to the presence of specific cavities of ampicillin.

2.5 Optimization of the experimental condition

During fabrication of the developed fluorescent probe, several factors can affect the sensitivity and analysis time. Consequently, effects of the ratio of template to monomer to cross-linker, the amount of polypyrrole particles, the incubation time and the pH of composite probe solution were optimized.

2.5.1 Ratio of template to monomer to cross-linker

Since it is a factor that significantly affects the number of specific binding sites and quality of MIP structure (Karaseva *et al.*, 2019), the ratios of template (T) to monomer (M) to cross-linker (C) were investigated. A molar ratio of 1: 6: 20 (T: M: C) provided the highest sensitivity as shown in Figure 2.8. In the reaction with template molecules, a small component of APTES (T: M = 1: 3) did not provide enough functional groups ($-NH_2$) to produce the necessary number of specific cavities on the MIP layer. The result was a nanoprobe of low sensitivity. On the other hand, the sensitivity was also low when the ratio of APTES to template was high (T: M = 1: 10) because the excess functional monomer led to the formation of non-imprinted

molecules within the polymer layer that inhibited binding between ampicillin and specific recognition sites. In addition, the low number of recognition sites may have been due to self-condensation of excess monomer (Amjadi and Jalili, 2017). The sensitivity was also low when using a low amount of TEOS (T: C = 1: 10). The composite PPy-QDs-MIP particles were so weak that TGA-capped CdTe QDs were easily detached during the template removal process (The Huy *et al.*, 2014). When more TEOS was used (T: C = 1: 30), the sensitivity was also low because the specific cavities in the polymer were extremely hard and rigid. When too much cross-linker is present, monomer mobility is reduced, making interaction with the template difficult. Thus, the ratio for T: M: C (ampicillin: APTES: TEOS) of 1: 6: 20 was selected for further experiment.

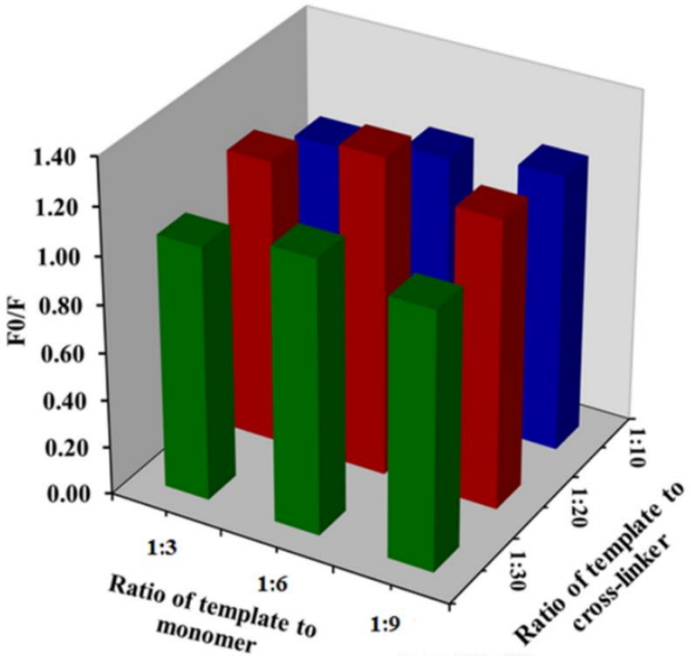

Figure 2.8 The effect of template to monomer (APTES) to cross-linker (TEOS) ratio on the fluorescence quenching of PPy-QDs-MIP fluorescent probe for ampicillin detection

2.5.2 Effect of incubation time

The incubation time of PPy-QDs-MIP with ampicillin was studied by varying the adsorption times from 1 to 30 min. The results as shown in Figure 2.9, the fluorescence quenching efficiency of PPy-QDs-MIP increased with the increments of

adsorption time up to 9 min and remained approximately constant at longer adsorption times. The adsorption time was rapid because polypyrrole enhanced the affinity between the target analyte (ampicillin) and recognition sites. Thus, the fluorescence intensity was determined after 9 min incubation of PPy-QDs-MIP and ampicillin or sample solution.

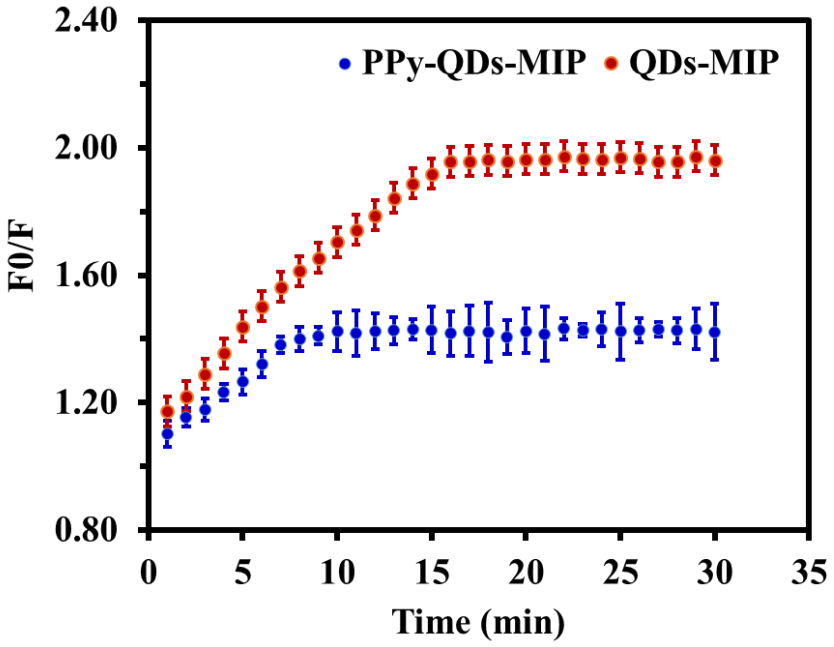

Figure 2.9 The effect of incubation time on the fluorescence quenching of QDs-MIP and PPy-QDs- MIP fluorescent probe for ampicillin detection

2.5.3 Amount of polypyrrole particles

The effect of PPy content in the PPy-QDs-MIP fluorescent probe was investigated at volumes of 20, 40, 60 and 80 μL. As can be seen from Figure 2.10, 40 μL of polypyrrole gave the highest quenching efficiency. The sensitivity decreased when the lower amount of polypyrrole was used. This result may be due to the incomplete binding between PPy-QDs-MIP and ampicillin within the incubation time of 9 min. At the higher volumes of polypyrrole, the sensitivity of the probe was also reduced, possibly because polypyrrole particles disturbed the formation of recognition sites and destroyed the polymer structure. Thus, 40 μL was selected as the appropriate amount of polypyrrole for the fabrication of the probe.

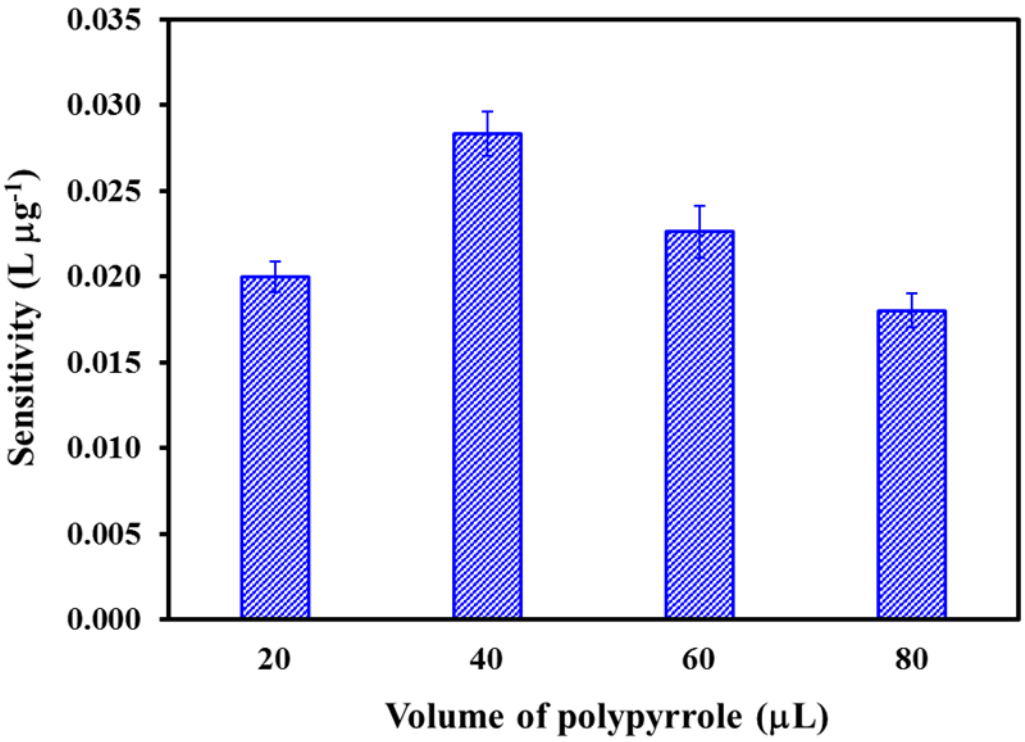

Figure 2.10 The effect of volume of polypyrrole on the fluorescence quenching of PPy- QDs-MIP fluorescent probe for ampicillin detection

2.5.4 Effect of pH

Since pH affects the binding of PPy-QDs-MIP and target molecules, the effect of pH was investigated by dispersing nanocomposite fluorescent probes in phosphate buffer at pH ranging from 5.0 to 8.0. The results as shown in Figure 2.11, buffer at pH 7.0 provided the highest sensitivity. At lower pH, the sensitivity decreased due to the protonation of the amine groups of the functional monomer in MIP particles and ampicillin (Ren and Chen, 2015). This alteration disturbed hydrogen bonding between ampicillin and specific binding sites. The sensitivity also decreased at buffer pH higher than 7.0, possibly because of deprotonation of the template molecule under the alkaline condition (Hou *et al.*, 2016). Also in alkaline solutions, silica in the MIP layer was ionized and could damage binding sites (Li et al., 2017), which could affect interaction between template molecules and the PPy-QDs-MIP probe (Figure 2.12). Therefore, a phosphate buffer at pH 7.0 was used for the preparation of the PPy-QDs-MIP solution.

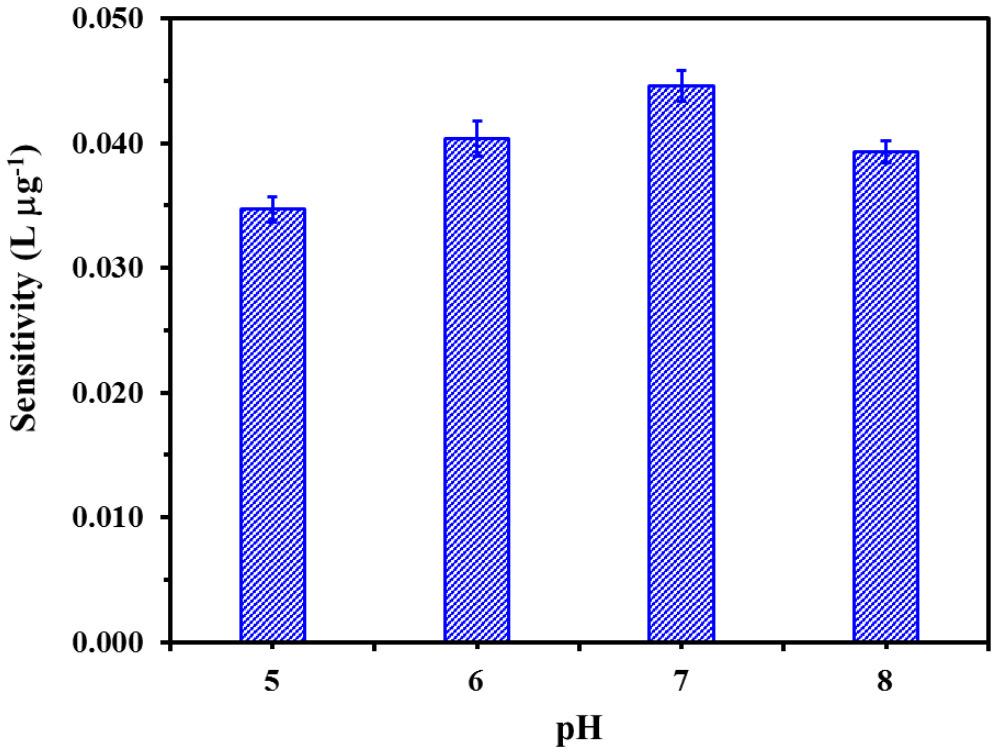

Figure 2.11 The effect of pH on the fluorescence quenching of PPy-QDs-MIP fluorescent probe for ampicillin detection

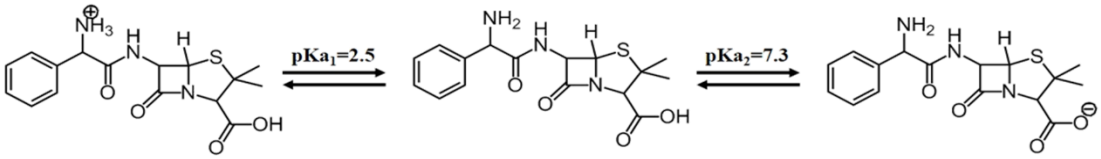

Figure 2.12 Speciation of ampicillin under different pH

2.6 Effect of different fluorescent probes for the determination of ampicillin

The quenching efficiency of the different fluorescent probes, QDs-NIP, PPy-QDs-NIP, QDs-MIP and PPy-QDs-MIP were compared. QDs-NIP exhibited the lowest quenching efficiency in the detection of ampicillin as shown in Table 2.1 and Figure 2.13. This result was due to the absence of specific recognition sites to bind with the target analyte. The PPy-QDs-MIP fluorescent probe provided the highest sensitivity because polypyrrole increased quenching efficiency during ampicillin detection by enhancing the adsorption of ampicillin on the probe via hydrogen bonding and π-π interaction. While, MIP helped to enhance specific binding.

Table 2.1 Comparison of different fluorescent probes for the determination of ampicillin

Fluorescent probes	**Sensitivity (L μg^{-1})**
QDs-NIP	0.0012 ± 0.0005
PPy-QDs -NIP	0.0014 ± 0.0010
QDs-MIP	0.0031 ± 0.0013
PPy-QDs-MIP	0.4250 ± 0.0009

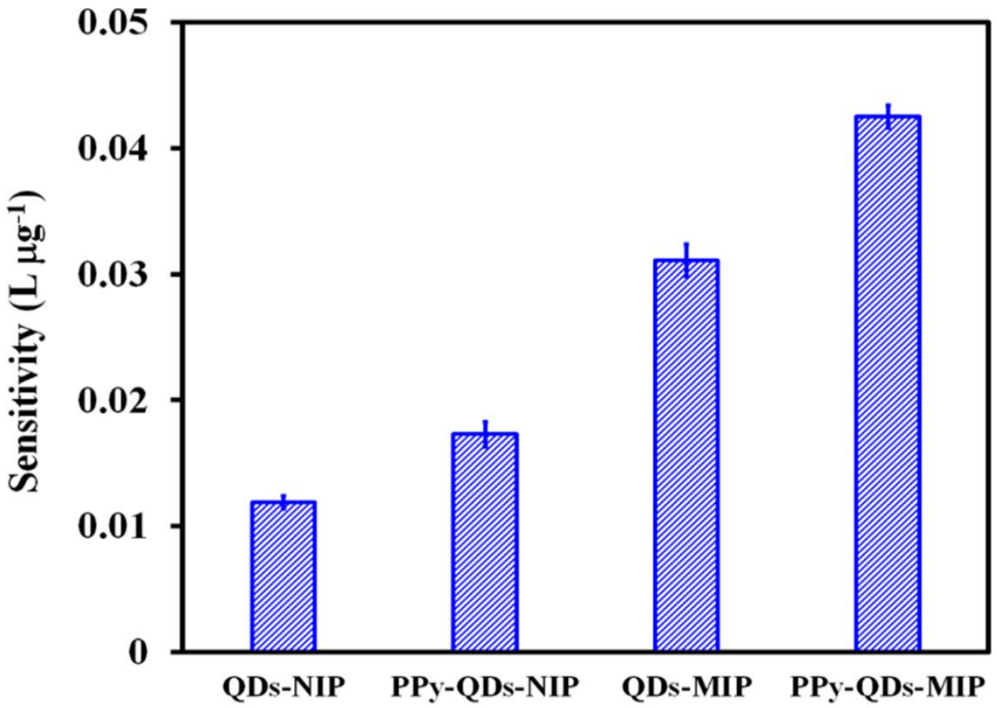

Figure 2.13 The sensitivity of various fluorescent probes for ampicillin detection

2.7 Fluorometric determination of ampicillin

To study the quenching efficiency of PPy-QDs-MIP during ampicillin detection, a series of different concentrations of ampicillin solutions were used to evaluate the quantitative analysis. The fluorescence emission of PPy-QDs-MIP fluorescent probes decreased according to the ampicillin concentration (Figure 2.14) and slightly decreased for PPy-QDs-NIP (Figure 2.15). The quenching of fluorescence in the presence of ampicillin is due to the energy transfer from QDs when ampicillin binds with amino groups of APTES on the surface of QDs. The fluorescence quenching efficiency of PPy-QDs-MIP is described by the Stern-Volmer according to equation (3) (Wang *et al.*, 2009):

$$F0/F = 1 + K_{sv}\,[C], \qquad (3)$$

where F0 and F are the fluorescence intensity without and with ampicillin, respectively, K_{sv} is the Stern-Volmer quenching constant and [C] is the ampicillin concentration.

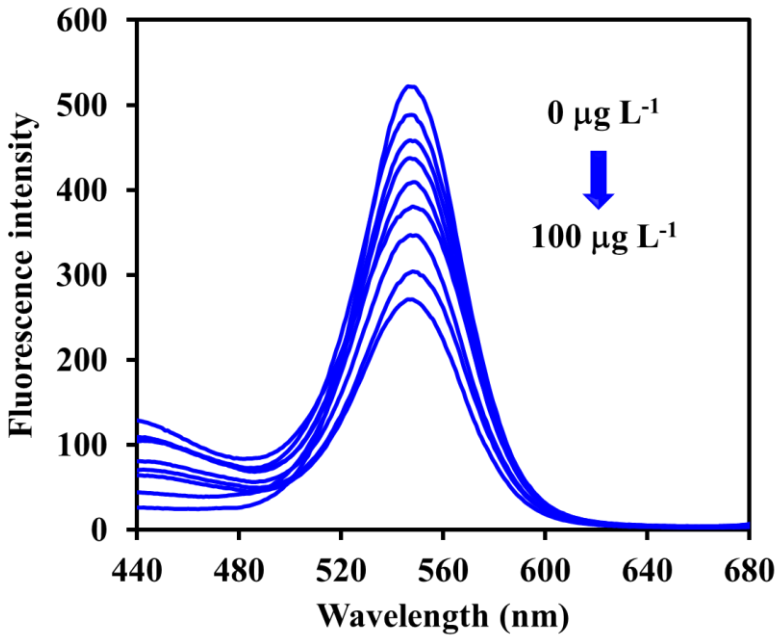

Figure 2.14 The fluorescence emission spectra of nanocomposite PPy-QDs-MIP fluorescent probe

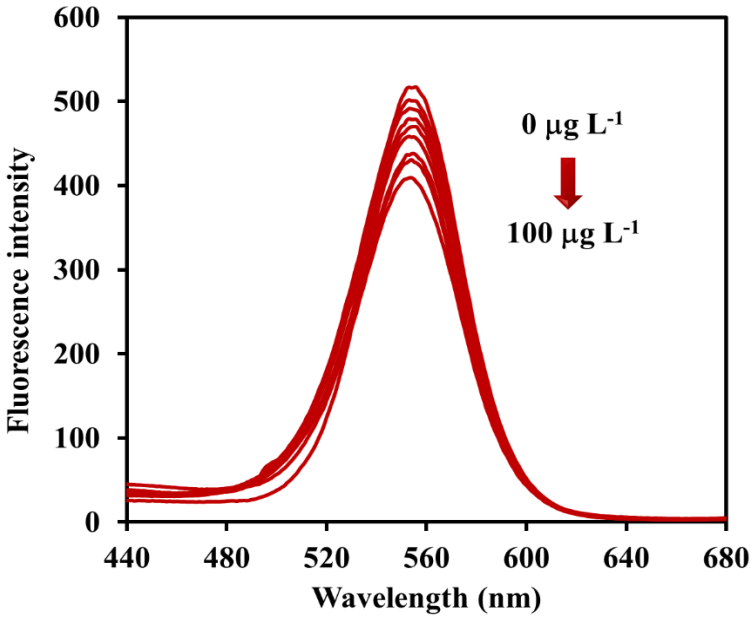

Figure 2.15 The fluorescence emission spectra of nanocomposite PPy-QDs-NIP fluorescent probe

2.8 Analytical performance of PPy-QDs-MIP fluorescent probe for the detection of ampicillin

The analytical characteristics of the PPy-QDs-MIP probe investigated under the optimum conditions were linearity, limit of detection (LOD) and limit of quantification (LOQ). As shown in Figure 2.16, the nanocomposite PPy-QDs-MIP fluorescent probe exhibited two linear ranges for ampicillin detection: 0.10 to 25.0 µg L^{-1} and 25.0 to 100.0 µg L^{-1}. The LOD and LOQ were 0.05 and 0.12 µg L^{-1}, respectively, following the IUPAC criteria; 3σ/k and 10σ/k, respectively, where σ is the standard deviation of blank measurement (n = 20) and k is the slope of the calibration curve. However, the fluorescence intensity of PPy-QDs-NIP was not significantly different at different concentrations of ampicillin. The specificity of PPy-QDs-MIP fluorescent probe was evaluated in terms of imprinting factor (IF), which was calculated using the ratio of $K_{sv,MIP}$ and $K_{sv,NIP}$ as shown in equation (4) (Hou *et al.*, 2016). In this work, the imprinting factor (IF) of ampicillin was 7.5.

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

Where IF is the imprinting factor, while $K_{SV,MIP}$ and $K_{SV,NIP}$ is the Stern-Volmer constant of MIP and NIP, respectively.

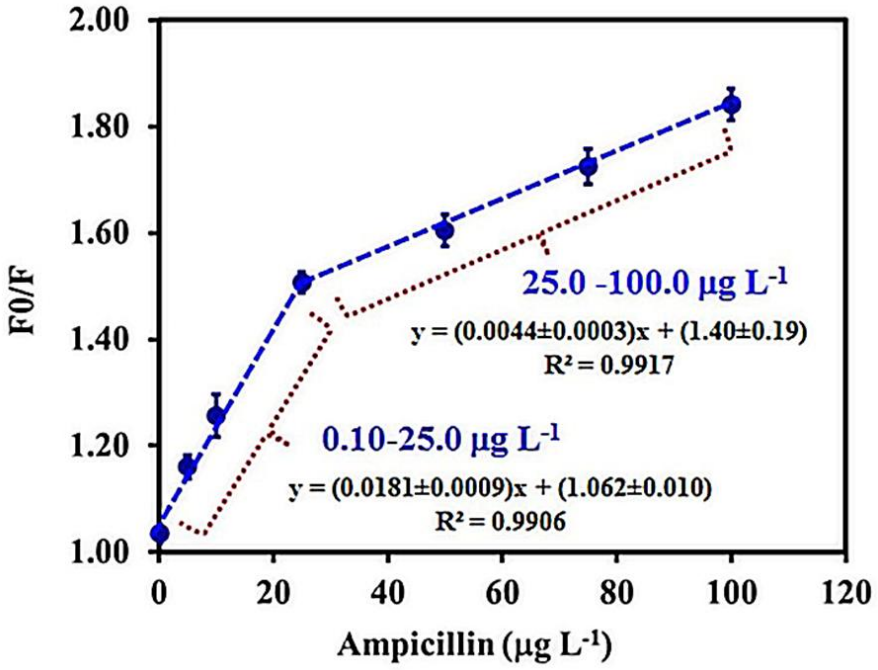

Figure 2.16 The linearity for the detection of ampicillin in the concentration range of 0.10-100.0 μg L^{-1}

2.9 Selectivity of PPy-QDs-MIP fluorescent probe for the detection of ampicillin

The selectivity of the PPy-QDs-MIP probe for the determination of ampicillin was investigated by applying the probe to detect structural analogs of ampicillin, which were amoxicillin, cephalexin, penicillin G, chloramphenicol and thiamphenicol. The PPy-QDs-MIP probe exhibited much higher sensitivity for ampicillin than for its analogs, while PPy-QDs-NIP probe exhibited similar sensitivities for ampicillin and the analog structures (Figure 2.17). The results indicated that the MIP layer contained specific recognition sites for ampicillin which were complementary in shape, size and functional groups but the PPy-QDs-NIP probe had no specific recognition sites to bind with ampicillin. The specificity of PPy-QDs-MIP was verified by a competitive binding study, which varied the ratio of ampicillin to amoxicillin ($C_{ampicillin}/C_{amoxicillin}$). As shown in Figure 2.18, the sensitivities were not significantly

different when the ratio of $C_{ampicillin}/C_{amoxicillin}$ was increased. The result confirmed that the synthesis of the PPy-QDs-MIP florescent probe produced binding sites that were highly specific to ampicillin.

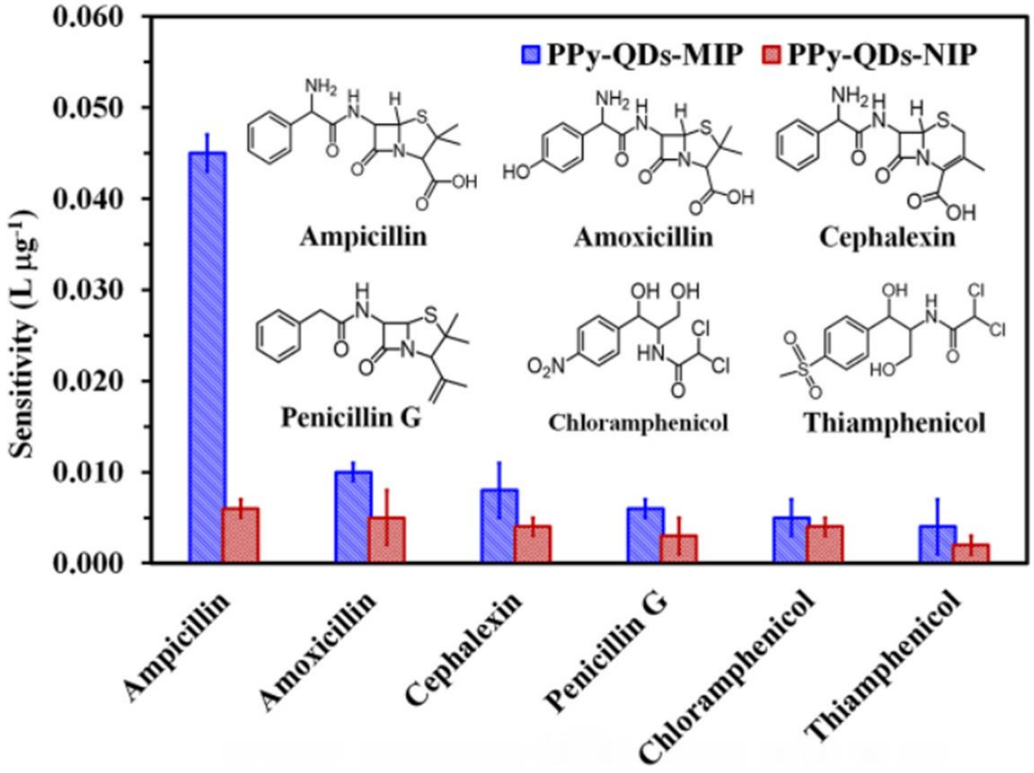

Figure 2.17 The selectivity of PPy-QDs-MIP probe and PPy-QDs-NIP probe for ampicillin detection

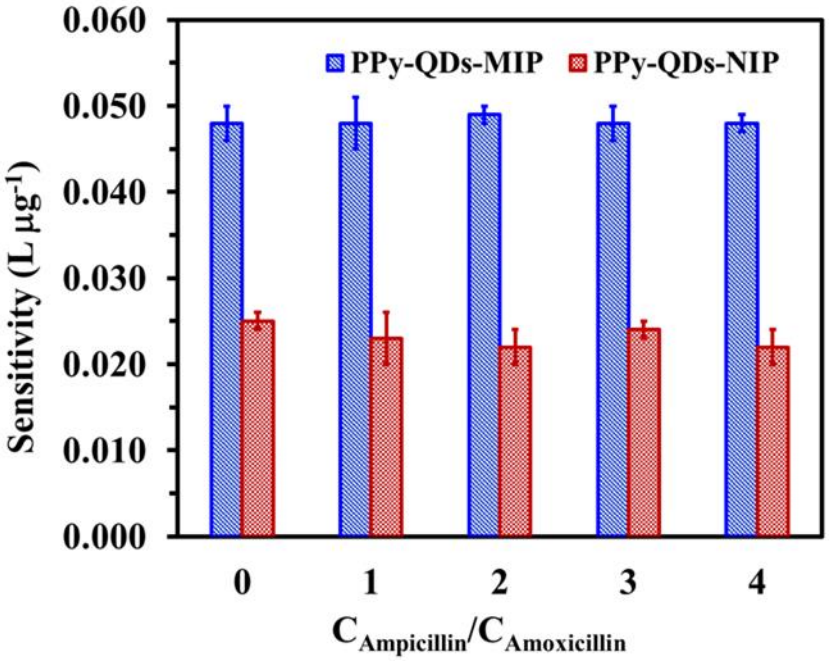

Figure 2.18 The competitive binding between ampicillin and amoxicillin

2.10 Reproducibility and stability of nanocomposite PPy-QDs-MIP fluorescent probe

The reproducibility of PPy-QDs-MIP fluorescent probe was investigated by synthesizing six different batches under the optimum conditions for the determination of ampicillin at 10 μg L^{-1}. The RSD was 3.2% which indicated that the synthesis of PPy-QDs-MIP had a good reproducibility. The stability of the fluorescent probe was also investigated and there was no significant difference in the fluorescence intensity of PPy-QDs-MIP within 360 min (response>90%), as shown in Figure 2.19. The result confirmed the good stability of the PPy-QDs-MIP fluorescent probe.

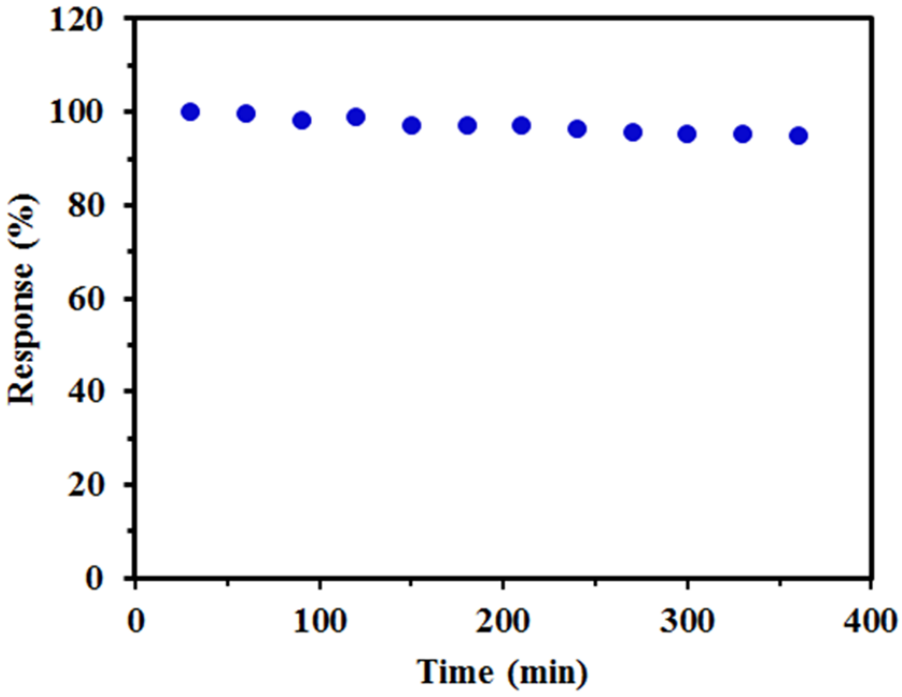

Figure 2.19 Stability of nanocomposite PPy-QDs-MIP fluorescent probe in 10 mM phosphate buffer (pH 7.0)

2.11 The analysis of ampicillin in food samples

Milk and meat samples were collected from local markets in Hat Yai, Songkhla, Thailand. The pretreatment procedure of milk samples was adapted from a previous report (Wu *et al.*, 2016). To precipitate the protein and fat, milk (5.0 mL) was added into a polypropylene tube, mixed with 15 mL of acetonitrile and centrifuged for 10 min. Then, the clear top phase was collected and evaporated to dryness at 60 ^{o}C. The extractant was re-dissolved with 5.0 mL of phosphate buffer (pH 7.0) and mixed with PPy-QDs-MIP for the analysis of ampicillin.

The pretreatment procedure of meat samples (pork and chicken) was also adapted from a previous report (Luo *et al.*, 1997). Briefly, 15 mL of phosphate buffer (10 mM) at pH 5.0 was added to 5.0 g of blended meat samples and centrifuged for 15 min. The clear top phase solution was mixed with 2.0 mL of acetic acid (5% w/v), vortexed for 3 min and centrifuged at 6500 rpm for 15 min. The supernatant was filtered through a 42 Whatman filter and diluted ten times with phosphate buffer (pH 7.0). Then, the extractant was mixed with the PPy-QDs-MIP for the determination of ampicillin.

The developed fluorescent probe was applied to detect ampicillin in milk and meat samples and the results are summarized in Table 2.2. Low amounts of ampicillin were found in milk (0.76-2.18 μg kg^{-1}) and meat (1.16-1.23 μg kg^{-1}). The detected concentrations were lower than the MRL values set by the EU: 40 μg kg^{-1} in milk and 50 μg kg^{-1} in animal tissue. The accuracy of the nanooptosensor was also investigated by the detection of ampicillin in milk and meat samples spiked at 0.5, 2.0, 4.0 and 10.0 μg kg^{-1}. Satisfactory recoveries were obtained in a range of 81.7 to 98.7% and RSDs were lower than 5%. The results of the developed nanooptosensor was compared with the HPLC techniques. The HPLC conditions for the analysis of ampicillin are shown in Table 2.3. The spiked samples were analyzed by both the nanooptosensor and HPLC. The chromatograms of ampicillin in real samples (milk) are shown in Figure 2.20. A good correlation between the PPy-QDs-MIP nanooptosensor and the HPLC method is shown in Figure 2.21. The determination coefficient (R^2) was 0.9985. These results indicate that the developed nanooptosensor can be used as an accurate analytical method to determine ampicillin in milk and meat samples.

Table 2.2 The analysis of ampicillin in milk and meat samples (n = 6)

Sample	Ampicillin (μg kg^{-1})		Recovery (%)	RSD (%)
	Added	Found		
Milk I	0.0	1.05	-	-
	0.5	1.53	96.3	4.0
	2.0	2.95	94.8	2.8
	4.0	5.00	98.7	0.8
	10.0	10.23	91.8	0.9
Milk II	0.0	0.76	-	-
	0.5	1.23	95.8	3.7
	2.0	2.69	96.6	3.2
	4.0	4.68	97.9	1.7
	10.0	10.58	98.2	1.9
Milk III	0.0	1.74	-	-
	0.5	2.17	86.3	3.8
	2.0	2.62	94.0	0.2
	4.0	5.06	83.7	1.7
	10.0	10.05	83.1	1.1
Milk IV	0.0	2.18	-	-
	0.5	2.65	93.9	3.2
	2.0	3.96	88.9	2.6
	4.0	6.06	97.0	2.4
	10.0	11.59	94.1	1.9
Pork	0.0	1.23	-	-
	0.5	1.76	87.7	2.6
	2.0	3.26	96.9	1.3
	4.0	4.27	85.0	3.7
	10.0	11.10	97.7	2.5
Chicken	0.0	1.16	-	-
	0.5	1.59	86.0	0.1
	2.0	2.79	81.7	2.4
	4.0	4.82	91.5	4.4
	10.0	10.78	96.2	0.4

Table 2.3 HPLC condition for the analysis of ampicillin

Parameters	**Conditions**
Column	VertiSep™ UPS C18 column (4.6 × 150 mm, 5 μm)
Flow rate	1.0 mL min^{-1}
Mobile phase	Acetonitrile:10 mM NaH_2PO_4 (20:80% v/v)
Detector	Diode array detector (λ = 220 nm)
Injection volume	20 μL
Column temperature	30 ^oC

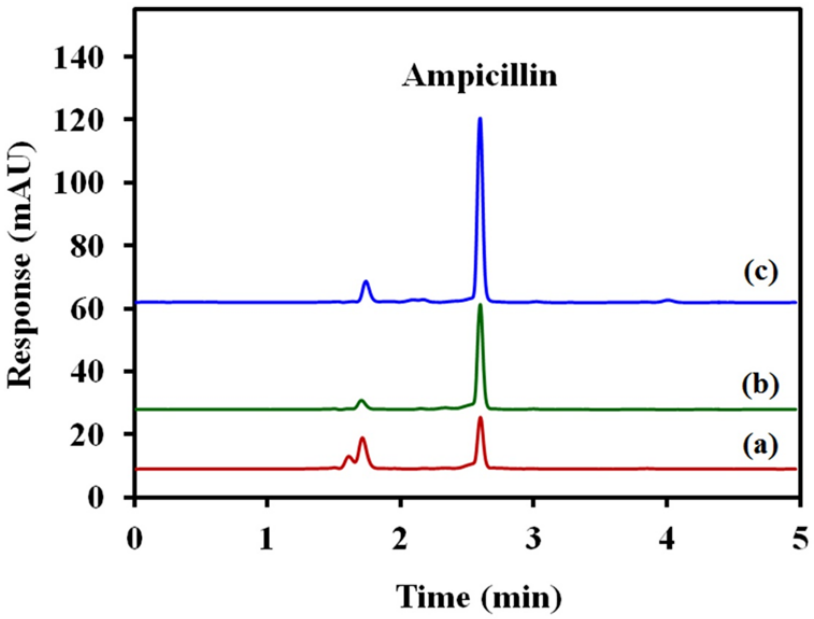

Figure 2.20 HPLC chromatograms of spiked milk sample at 2.0 (a), 4.0 (b) and 10.0 mg kg^{-1} (c)

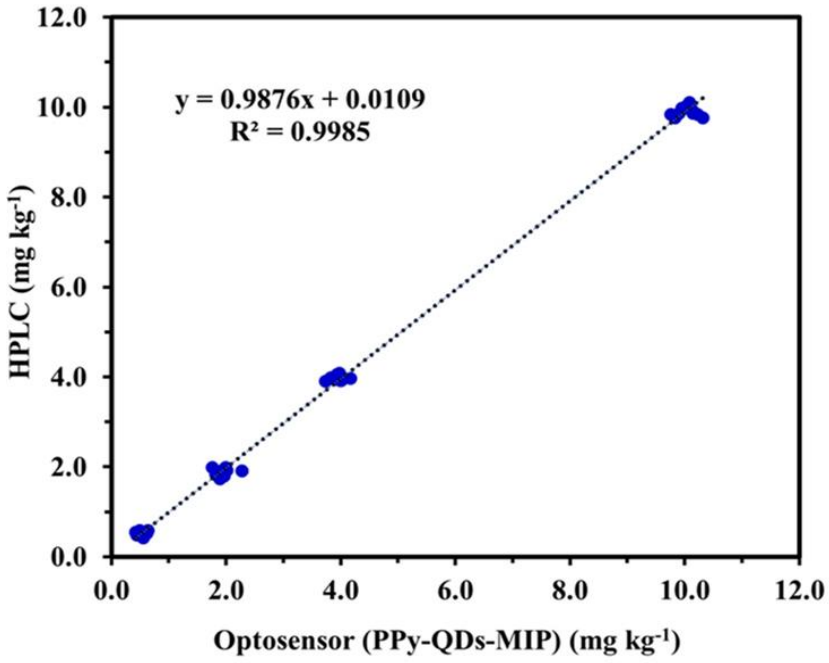

Figure 2.21 The correlation between the nanooptosensor using PPy-QDs- MIP fluorescent probe and HPLC technique for ampicillin detection in milk sample

2.12 Comparison of PPy-QDs-MIP nanooptosensor with other previous works

The analytical performance of the nanooptosensor for ampicillin detection was compared with previous methods as shown in Table 2.4. The PPy-QDs-MIP probe exhibited a wide linear range and LOD was lower than in the other works (Luo *et al.*, 1997; Khalilzadeh *et al.*, 2009; Xie *et al.*, 2012; Shrivas *et al.*, 2017; Soledad-Rodríguez *et al.*, 2017), while the recovery and precision were comparable to other methods (Khalilzadeh *et al.*, 2009; Wu *et al.*, 2016; Soledad-Rodríguez *et al.*, 2017; Wang *et al.*, 2017). The comparison confirmed that the nanooptosensor using the PPy-QDs-MIP fluorescent probe was highly sensitive for the determination of ampicillin. In addition, this work required shorter analysis time and had a cheaper analysis cost than the chromatographic techniques.

Table 2.4 Analytical performances of nanooptosensor using PPy-QDs-MIP fluorescent probes and other previous works for ampicillin detection

Analytical method	**Samples**	**Linear range ($\mu g\ L^{-1}$)**	**LOD ($\mu g\ L^{-1}$)**	**Recovery (%)**	**RSD (%)**	**References**
HPLC-FLD	Eggs	5.0-800.0	0.4	77.6-82.0	6.3-8.7	(Xie *et al.*, 2012)
HPLC-FLD	Bovine milk	0.2-9.4	0.2	87.0-91.6	1.6-3.8	(Luo *et al.*, 1997)
HPLC-UV	Milk and blood	5.0-200.0	0.05	92.1-107.6	1.4-4.6	(Wu *et al.*, 2016)
MIP/HPLC-UV	Milk	100.0-500.0	10.7	> 95.0	< 7.0	(Soledad-Rodríguez *et al.*, 2017)
Spectrophotometry	Urine	25.0-1200.0	10.0	92.5-95.0	3.8-5.7	(Shrivas *et al.*, 2017)
Electrochemical	Pharmaceutical capsule and urine	0.4-105.8	0.1	81.2-95.0	0.9-2.6	(Khalilzadeh *et al.*, 2009)
PPy-QDs-MIP spectrofluorometry	Milk and meat	0.10-25.0 25.0-100.0	0.05	81.7- 98.7	< 5.0	This work

HPLC = high performance liquid chromatography; FLD = fluorescence detector; UV = ultraviolet-visible detector

3. Concluding remarks

A nanooptosensor using PPy-QDs-MIP fluorescent probe was successfully fabricated and utilized for ampicillin detection. Integrating the good optical property of QDs, the high specificity of MIP and the high affinity of polypyrrole, the sensor exhibited high sensitivity, selective recognition and provided a rapid method for trace ampicillin analysis. The nanooptosensor detected ampicillin in milk and meat samples with good accuracy (recoveries of 81.7-98.7%). The analytical results of this nanooptosensor also agreed well with the results of a HPLC detection method. Other advantages of the nanooptosensor included a simple, rapid procedure and cost-effective equipment. The simple developed strategy can be modified for the determination of other organic compounds in various matrix interferences.

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Publication

A polypyrrole doped with fluorescent CdTe quantum dots and incorporated into molecularly imprinted silica for fluorometric determination of ampicillin

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ORIGINAL PAPER

A polypyrrole doped with fluorescent CdTe quantum dots and incorporated into molecularly imprinted silica for fluorometric determination of ampicillin

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Abstract
A fluorometric method is described for the detection of ampicillin. A polypyrrole containing fluorescent CdTe quantum dots was incorporated into a silica-based molecularly imprinted polymer. The composite MIP displays good fluorescence (with excitation/emission maxima at 355/548 nm), and high selectivity and affinity for ampicillin due to the use of polypyrrole. Ampicillin is found to quench the fluorescence of composite much more strongly than the emission of a non-imprinted polymer. The imprinting factor of 7.5 implies that the nanocomposite probe contains specific binding sites. The MIP probe has two linear response ranges, one from 0.10 to 25 μg L^{-1} of ampicillin, and one from 25 to 100 μg L^{-1}. The limit of detection is 0.05 μg L^{-1}. The method was applied to the determination of ampicillin in (spiked) milk and meat samples and gave recoveries between 81.7 and 98.7%. The results agreed well with HPLC techniques.

Keywords Nanoprobe · Fluorescence · Quenching · Optical · Composite · CdTe · Emission · Template · Monomer · Cross-linker

Introduction

Frequent use of ampicillin leaves residues in meat and milk which can cause stomach cramps, diarrhea, dizziness, nausea, rash and peeling of the skin when consumed by humans [1]. The maximum residue limits (MRLs) for ampicillin in milk and meat have been set by the European Union at 40 and 50 μg kg^{-1}, respectively. Therefore, the development of a convenient, rapid, sensitive, selective and reliable method for the detection of ampicillin in food samples is important.

Various analytical techniques have been reported for the determination of ampicillin including chromatography [2], electrophoresis [3] and electrochemistry [4, 5]. Although, these techniques provided accurate results, they still had some drawbacks. They used large volumes of toxic organic solvent, were time consuming and required expensive instruments. To overcome these limitations, fluorescence spectroscopy was employed for its technical simplicity, high sensitivity, rapidity and cost-effective equipment [6]. However, the relatively low fluorescence intensity of ampicillin which cannot be detected at trace levels. The sensitivity of this method can be improved by exploiting the good optical properties of fluorescence probes. Quantum dots (QDs) were an interesting choice of fluorescence probe due to their high fluorescence intensity and good photochemical stability [7]. To enhance the specificity of the analytical method, QDs can be modified with highly specific ligands or incorporated with specific materials such as molecularly imprinted polymers (MIP). MIP showed high specificity to target analytes [8, 9] and had good physical and chemical stability under demanding conditions of temperature, pH and pressure, using a variety of acids, bases and organic solvents [10–12]. The MIP was synthesized by a co-polymerization reaction using ampicillin as the template. The resulting MIP interacts with a functional monomer and cross linker to form a polymer layer [13]. After removal of template, the MIP layer contained specific binding cavities complementary in functional groups, shape and size to the target molecule [14]. MIPs have been used as a chemosensor [15], adsorption material [16] and biosensor [17]. Fluorescence probes using MIPs coated on QDs have engaged interest for the

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determination of target analytes such as amoxicillin [18], chloramphenicol [19] and salbutamol [20]. To enhance the adsorption ability or affinity binding of ampicillin to the fluorescence probe, the addition of polypyrrole during preparation of a nanocomposite fluorescence probe is an interesting strategy. Polypyrrole contains a π structure which can adsorb aromatic compounds through π-π interactions [21]. Moreover, polypyrrole had good chemical stability [22].

In this work, a nanooptosensor was fabricated using a composite fluorescence probe of polypyrrole and quantum dots incorporated in a molecularly imprinted polymer (PPy-QDs-MIP). The method was then used to detect and analyze ampicillin in food samples. The nanooptosensor was characterized and the analytical performances were evaluated and compared with the results of a conventional chromatographic method.

Experimental

Chemicals and materials

Ampicillin trihydrate and tetraethoxyl orthosilicate (TEOS) were from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan, https://www.tcichemicals.com). Thioglycolic acid (TGA), sodium borohydride, cadmium chloride ($CdCl_2.2H_2O$), tellurium (99.8%), pyrrole and 3-aminopropyltriethoxysilane (APTES) were from Sigma-Aldrich (MO, USA, https://www.sigmaaldrich.com). Iron (III) chloride was purchased from VWR Prolabo Chemical (Leuven, Belgium, https://www.be.vwr.com). Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) and disodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$) were from Univar Chemical (Washington, USA, https://www.univar.com). Sodium hydroxide, ethanol (≥98.0%) was from RCI Labscan (Bangkok, Thailand, http://www.rcilabscan.com).

Instrumental

A RF-5301PC spectrofluorophotometer (Shimadzu, Japan, https://www.shimadzu.com) was used for fluorescence measurement. UV-Vis absorption was measured with an Avaspec 2048 spectrometer (Avantes, Netherlands, https://www.avantes.com). Fourier transform infrared (FTIR) spectroscopy was performed using a BX FTIR spectroscope (PerkinElmer, USA, http://www.perkinelmer.com). Transmission electron microscope (JEM-2010) and scanning electron microscope (JSM-5200) (JEOL, Japan, https://www.jeol.co.jp) were used for morphological investigation. The Brunauer-Emmett-Teller (BET) surface areas of PPy-QDs-MIP and PPy-QDs-NIP fluorescence probes were investigated using the ASAP 2460 system, (Micromeritics, USA, https://www.micromeritics.com).

Synthesis of CdTe quantum dots nanoparticles

The CdTe QDs were synthesized by modifying a previous work [23]. Briefly, 5.0 mg of tellurium and 4.0 mg of sodium borohydride were mixed in 1.50 mL of deionized water in a centrifuge tube (2.0 mL) to produce a sodium hydrogen telluride (NaHTe) solution. 45.0 mg of cadmium chloride and 30 μL of TGA were added to 100 mL deionized water and adjusted to pH 11.5 with sodium hydroxide (1.0 M). The mixture solution was deaerated with N_2 gas for 10 min in a three-necked flask, then heated to 90 °C and 1.0 mL of NaHTe was injected into the solution and continuously stirred for 15 min. Finally, the synthesized CdTe QDs were precipitated with 20 mL of ethanol and centrifuged at 8000 rpm for 5 min.

Synthesis of PPy-QD-MIP and PPy-QD-NIP fluorescent probes

The PPy-QDs-MIP were synthesized via a one-step copolymerization. First, 40 μL of APTES (functional monomer) were mixed with 3.0 mM of ampicillin (5.0 mL) and stirred for 1 h. Then, 10 mL of CdTe QDs were added to the mixture and continuously stirred for 1 h. Then, 120 μL of TEOS as a cross-linker, 150 μL of NH_3 (25% *w/v*) as a catalyst and 20 μL of polypyrrole particles were added and stirred for 6 h. Polypyrrole particles were prepared by mixing 13.0 mg of $FeCl_3$ and 400 μL of pyrrole in a 2.0 mL centrifuge tube and stirring for 1 h. For template removal, the synthesized nanocomposites were washed three times with 10 mL of ethanol, after which no template molecules presented in the washing solution. The PPy-QDs-MIP were centrifuged at 7500 rpm for 10 min and dried in an oven at 60 °C. The PPy-QDs-NIP fluorescence probes were prepared under the same experimental condition without ampicillin (template molecule).

Fluorescence quenching studies

Fluorescence intensity was measured by setting the slit widths at 10 nm for the excitation and emission. The wavelength for excitation was set at 355 nm and fluorescence emission was recorded from 400 to 700 nm. PPy-QDs-MIP or PPy-QDs-NIP fluorescence probe solutions (6.0 μg L^{-1}) were prepared by dispersion in phosphate buffer (10 mM) at pH 7.0. The 300 μL of PPy-QDs-MIP or PPy-QDs-NIP solution were mixed with ampicillin solution or sample (100 μL). Then, the mixture was incubated under gentle rotation for the appropriate time. The measurement of fluorescence emission was carried out with and without ampicillin and recorded as F and F0, respectively.

Sample preparation of milk and meat

Milk and meat samples were collected from local markets in Hat Yai, Songkhla, Thailand. The pretreatment procedure of milk samples was adapted from a previous report [24]. To precipitate the proteins and fats, milk (5.0 mL) was added into a polypropylene tube, mixed with 15 mL of acetonitrile and centrifuged for 10 min. Then, the clear top phase was collected and evaporated to dryness at 60 °C. The extractant was re-dissolved with 5.0 mL of phosphate buffer (pH 7.0) and mixed with PPy-QDs-MIP for the analysis of ampicillin.

The pretreatment procedure of meat samples (pork and chicken) was also adapted from a previous report [25]. Briefly, 15 mL of phosphate buffer (10 mM) at pH 5.0 was added to 5.0 g of blended meat samples and centrifuged for 15 min. The clear top phase solution was mixed with 2.0 mL of acetic acid (5% *w/v*), vortexed for 3 min and centrifuged at 6500 rpm for 15 min. The supernatant was filtered through a 42 Whatman filter and diluted ten times with phosphate buffer (pH 7.0). Then, the extractant was mixed with the PPy-QDs-MIP for the determination of ampicillin.

Results and discussion

Choice of materials

In this work, nanocomposite PPy-QDs-MIP fluorescence probe was developed for the detection of trace ampicillin. QDs was chosen as sensing material due to their high fluorescence intensity and good photochemical stability. MIP was used to improve the selectivity of composite probe. While, polypyrrole can help to improve the adsorption ability which can interact with ampicillin via π-interaction and hydrogen bonding.

Characterization of CdTe quantum dots (QDs) and nanocomposite PPy-QD-MIP fluorescent probe

The fluorescence spectrum of CdTe QDs was narrow and symmetric (Fig. S1) and the particle size was about 2.25 nm which was calculated from the maximum absorption wavelength at 505 nm [26].

The nanocomposite PPy-QDs-MIP fluorescence probe was synthesized via a co-polymerization process using ampicillin as the template, APTES as a monomer and TEOS as a cross-linker. APTES, template, TGA-CdTe QDs and PPy self-assembled via hydrogen bonding and then formed nanocomposite particles in the presence of the cross-linker (TEOS) and ammonia (catalyst) (Fig. 1). The fluorescence emission intensity of PPy-QDs-MIP before removal of the template was about 60% the intensity of the emission of PPy-QDs-NIP (Fig. S2). After removal of the template, the fluorescence emission was restored to the same intensity as PPy-QDs-NIP. The result confirmed that the PPy-QDs-MIP was successfully prepared and the template had been completely removed from the MIP layer. The method is a single-step copolymerization under soft conditions at room temperature (25 ± 2 °C).

The morphologies of CdTe QDs were investigated using TEM (Fig. 2a). The QDs showed good distribution and a smooth surface. The SEM (Fig. 2b) and TEM (Fig. 2c) images shows PPy-QDs-MIP of uniform spherical shape with a diameter of 200–250 nm.

The nanocomposite PPy-QDs-MIP fluorescence probe was characterized by FTIR spectroscopy. The spectrum of TGA-CdTe QDs (Fig. S3a) shows characteristic peaks at 1375 and 1566 cm^{-1}, which are due to the symmetric and asymmetric stretching of the carboxylate group (COO^-). The bands at 1229 and 3415 cm^{-1} are due to C-O and -OH stretching vibration. The FTIR spectrum of ampicillin (Fig. S3b) shows peaks at 1690 cm^{-1} and 1774 cm^{-1} due to the C=O stretching of carboxylic and carbonyl groups. The peak at 3515 cm^{-1} is due to N-H stretching (primary amine). The peaks at 1385 and 1494 cm^{-1} were attributed to C-N stretching and N-H plane bending. The characteristic peaks of ampicillin also appeared in the FTIR spectrum of the nanocomposite PPy-QDs-MIP optosensor (Fig. S3c). After removal of template (Fig. S3d), the absorption peaks corresponding to ampicillin disappeared. The peaks at approximately 455 and 768 cm^{-1} are due to Si-O vibration [27]. The bands around 1560 and 1650 cm^{-1} are due to the C=C stretching of polypyrrole (Fig. S3e). The results showed that the nanocomposite PPy-QDs-MIP probe was successfully synthesized for specific recognition of ampicillin.

The quantum yield (QY) was calculated according to a previous work [28]. Rhodamine 6G was used as the fluorescence standard. QYs of CdTe QDs, QDs-MIP and PPy-QDs-MIP were 0.90, 0.68 and 0.55, respectively. The BET measurements of the specific surface areas of PPy-QDs-MIP and PPy-QDs-NIP were 22.2 $m^2 g^{-1}$ and 16.7 $m^2 g^{-1}$, respectively. The PPy-QDs-MIP nanooptosensor displayed greater surface area than PPy-QDs-NIP, possibly due to the presence of specific cavities of ampicillin.

Optimization of method

During the fabrication of the fluorescent probe, several factors can affect the sensitivity and analysis time of the probe. The following parameters were optimized: (a) ratio of template to monomer and cross-linker; (b) incubation time; (c) amount of PPy particles, (d) pH of composite probe solution; Respective data and Figure are given in the Electronic Supporting Material. The following experimental conditions were found to give the best results: (Fig. S4a) best ratio of template to monomer to cross-linker: 1: 6: 20; (Fig. S4b) optimal

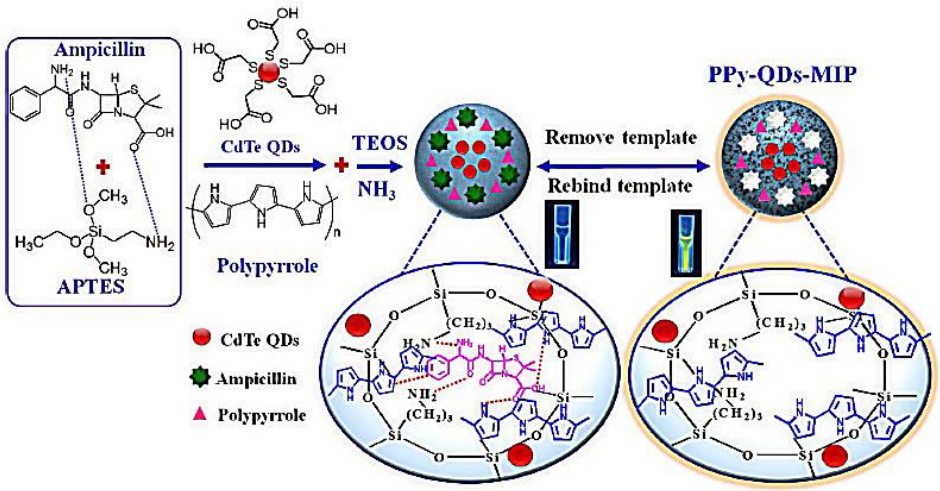

Fig. 1 Synthesis of nanocomposites PPy-QDs-MIP fluorescent probe for ampicillin detection

incubation time: 9 min; (Fig. S4c) amount of PPy particles: 40 μL and (Fig. S4d) pH of composite probe solution: pH 7.

Effect of different fluorescent probes for determination of ampicillin

The quenching efficiency of the different fluorescence probes, QDs-NIP, PPy-QDs-NIP, QDs-MIP and PPy-QDs-MIP, were compared. QDs-NIP exhibited the lowest quenching efficiency in the detection of ampicillin (Fig. 3). This result was due to the absence of specific recognition sites to bind with the target analyte. The PPy-QDs-MIP fluorescence probe provided the highest sensitivity because polypyrrole increased quenching efficiency during ampicillin detection by enhancing the adsorption of ampicillin on the probe via hydrogen bonding and π-interaction. MIP helped to enhance specific binding.

Fluorometric determination of ampicillin

To study the quenching efficiency of PPy-QDs-MIP during ampicillin detection, a series of different concentrations of ampicillin solutions were used to evaluate the quantitative analysis. The fluorescence emission of PPy-QDs-MIP fluorescence probes decreased according to the ampicillin concentration (Fig. 4a) and slightly decreased for PPy-QDs-NIP (Fig. 4b). The quenching of fluorescence in the presence of ampicillin is due to the energy transfer from QDs when ampicillin binds with amino groups of APTES on the surface of QDs. The fluorescence quenching efficiency of PPy-QDs-MIP is described by the Stern-Volmer equation [29]:

$$F0/F = 1 + K_{sv}\,[C],$$

where F_0 and F are the fluorescence intensity without and with ampicillin, respectively, K_{sv} is the Stern-

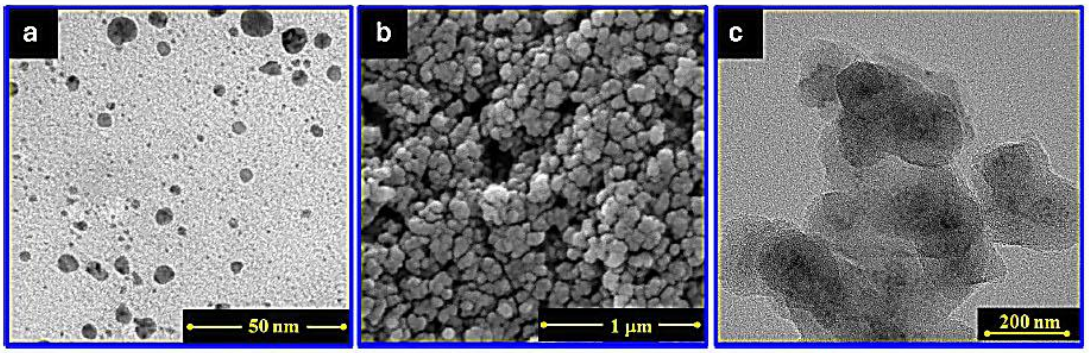

Fig. 2 TEM images of TGA-CdTe QDs (a), SEM image of PPy-QDs-MIP (b) and TEM images of PPy-QDs-MIP fluorescence probe (c)

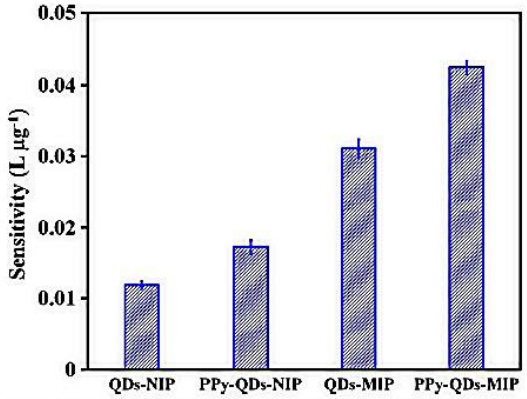

Fig. 3 The sensitivity of various fluorescence probes for the detection of ampicillin with excitation/emission maxima at 355/548 nm

Volmer quenching constant and [C] is the ampicillin concentration.

The analytical characteristics of the PPy-QDs-MIP probes investigated under the optimum conditions were linearity, limit of detection (LOD) and limit of quantification (LOQ). The nanocomposite PPy-QDs-MIP fluorescence probe exhibited two linear ranges for ampicillin detection: 0.10 to 25 μg L^{-1} and 25 to 100 μg L^{-1} (Fig. 4c). The LOD and LOQ were 0.05 and 0.12 μg L^{-1}, respectively, following the IUPAC criteria. The fluorescence intensity of PPy-QDs-NIP, however, was not significantly different at different concentrations of ampicillin. The specificity of PPy-QDs-MIP fluorescence probes were evaluated in terms of imprinting factor (IF), which was calculated using the ratio of $K_{sv,MIP}$ and $K_{sv,NIP}$. In this work, the imprinting factor (IF) of ampicillin was 7.5. A photograph of nanocomposite PPy-QDs-MIP in the presence and absence of ampicillin is shown in Fig. 4d.

Selectivity

The selectivity of the PPy-QDs-MIP for the determination of ampicillin was investigated by applying the probe to detect structural analogs of ampicillin, which were amoxicillin, cephalexin, penicillin G, chloramphenicol and thiamphenicol. Figure 5a indicates that the PPy-QDs-MIP probe exhibited a much higher sensitivity for ampicillin than for its analogs, while PPy-QDs-NIP exhibited similar sensitivities for ampicillin and the analog structures. The results indicate that the MIP layer contained specific recognition sites for ampicillin which were complementary in shape, size and functional groups but the PPy-QDs-NIP probe had no specific recognition sites to bind with ampicillin. The specificity of PPy-QDs-MIP was verified by a competitive binding study, which varied the ratio of amoxicillin to ampicillin ($C_{amoxicillin}/C_{ampicillin}$). The sensitivities were not significantly different when the ratio of $C_{amoxicillin}/C_{ampicillin}$ was increased (Fig. 5b). The result confirmed that the synthesis of the PPy-QDs-MIP florescence probe produced binding sites that were highly specific to ampicillin.

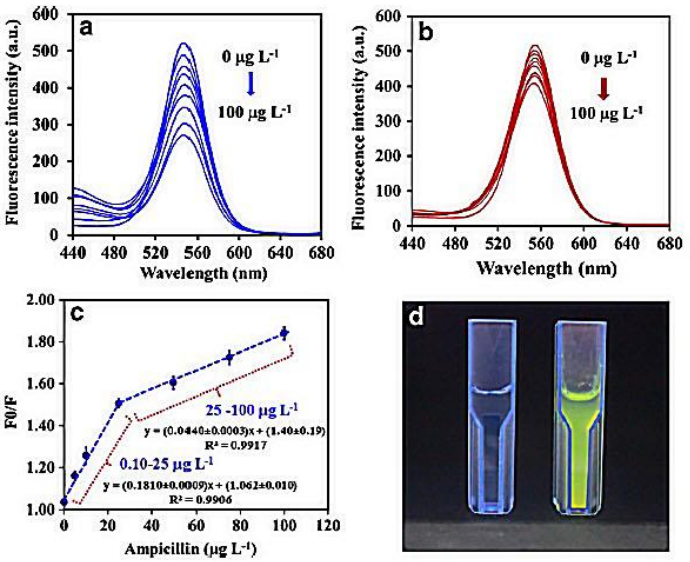

Fig. 4 The fluorescence emission spectra of nanocomposite PPy-QDs-MIP fluorescence probe (a), PPy-QDs-NIP fluorescence probe (b), linearity for the detection of ampicillin (with excitation/emission maxima at 355/548 nm) (c) and photograph of nanocomposite PPy-QDs-MIP with (left) and without (right) ampicillin (d)

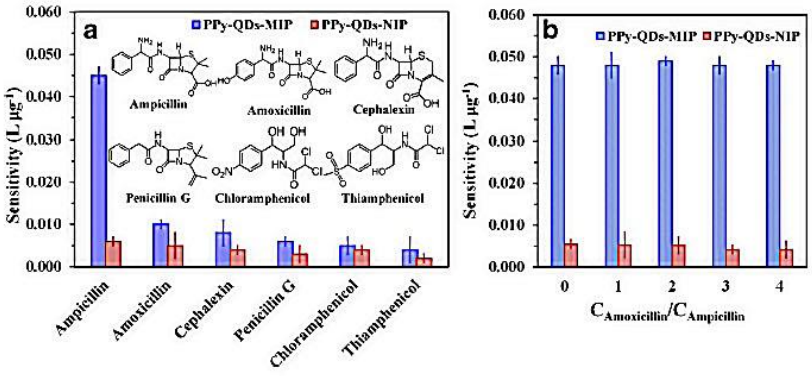

Fig. 5 Selectivity of PPy-QDs-MIP and PPy-QDs-NIP for ampicillin detection (a) and competitive binding between amoxicillin and ampicillin (b)

Reproducibility and stability

The reproducibility of PPy-QDs-MIP fluorescence probe was investigated by synthesising six different batches under the optimum conditions for the determination of ampicillin at 10 μg L^{-1}. The RSD was 3.2% which indicated that the synthesis of PPy-QDs-MIP had a good reproducibility. The stability of the fluorescence probe was also investigated and there was no significant difference in the fluorescence intensity of PPy-QDs-MIP within 360 min (response>90%), as shown in Fig. S5. The result confirmed the good stability of the PPy-QDs-MIP fluorescence probe.

Analysis of food samples

The PPy-QDs-MIP fluorescence probe was applied to detect ampicillin in milk and meat samples and the results are summarized in Table S1. Low amounts of ampicillin were found in milk (0.76–2.18 μg kg^{-1}) and meat (1.16–1.23 μg kg^{-1}). The detected concentrations were lower than the MRL values set by the EU: 40 μg kg^{-1} in milk and 50 μg kg^{-1} in animal tissue. The accuracy of the nanooptosensor was also investigated by the detection of ampicillin in milk and meat samples spiked at 0.5, 2.0, 4.0 and 10.0 μg kg^{-1}. Satisfactory recoveries were obtained in a range of 81.7 to 98.7% and RSDs were lower than 5%. The results of the nanooptosensor was compared with the HPLC techniques. The spiked samples were analyzed by both the nanooptosensor and HPLC. The chromatograms of ampicillin in real samples (milk) is shown in Fig. S6a. A good correlation between the PPy-QDs-MIP nanooptosensor and the HPLC method is shown in Fig. S6b. The determination coefficient (R^2) was 0.998. These results indicate that the nanooptosensor can be used as an accurate analytical method to determine ampicillin in milk and meat samples.

Comparison of the PPy-QD-MIP nanooptosensor with previous works

The analytical performance of the nanooptosensor for ampicillin detection was compared with previous methods (Table 1). The PPy-QDs-MIP probe exhibited a wide linear range and LOD was lower than in other works [1, 30–33], while the recovery and precision were comparable to other methods [4, 24, 32, 33]. The comparison confirmed that the nanooptosensor using the PPy-QDs-MIP fluorescence probe is highly sensitive for the determination of ampicillin. In addition, this work required shorter analysis time and had a cheaper analysis cost than chromatographic techniques.

Table 1 Analytical performances of nanooptosensor using PPy-QDs-MIP fluorescence probes and other previous works for ampicillin detection

Analytical method	Samples	Linear range (μg L^{-1})	LOD (μg L^{-1})	Recovery (%)	RSD (%)	References
HPLC-FLD	Eggs	5.0–800	0.4	77.6–82.0	6.3–8.7	[30]
HPLC-FLD	Bovine milk	0.2–9.4	0.2	91.6–87.0	1.6–3.8	[31]
HPLC-UV	Milk and blood	5.0–200	0.05	92.1–107.6	1.4–4.6	[24]
MIP sorbent/HPLC-UV	Milk	100–500	10.7	>95.0	< 7.0	[32]
Spectrophotometry	Urine	25–1200	10.0	92.5–95.0	3.8–5.7	[1]
Electrochemical	Milk	0.00035–1.75	0.00013	95.5–105.5	5.1	[4]
Electrochemical	Pharmaceutical capsule and urine	0.4–106	0.1	81.2–95.0	0.9–2.6	[33]
PPy-QDs-MIP Spectrofluorimetry	Milk and meat	0.10–25 25–100	0.05	81.7–98.7	< 5.0	This work

Conclusion

A nano-optosensor using PPy-QDs-MIP fluorescence probe was successfully fabricated and utilized for ampicillin detection. Integrating the good optical property of QDs, the high specificity of MIPs and the high affinity of polypyrrole, the method exhibited high sensitivity and selective recognition and provided a rapid method for trace ampicillin analysis. The nanooptosensor detected ampicillin in milk and meat samples with good accuracy (recoveries of 81.7–98.7%). The analytical results of this nanooptosensor also agreed well with the results of an HPLC detection method. Other advantages of the nanooptosensor included a rapid procedure and cost-effective equipment. The strategy can be modified for the determination of other organic compounds in various matrix interferences.

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Compliance with ethical standards The authors declare that they have no competing interests.

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Electronic Supporting Material
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A nanooptosensor of polypyrrole and quantum dots incorporated in molecularly imprinted polymer for trace ampicillin detection

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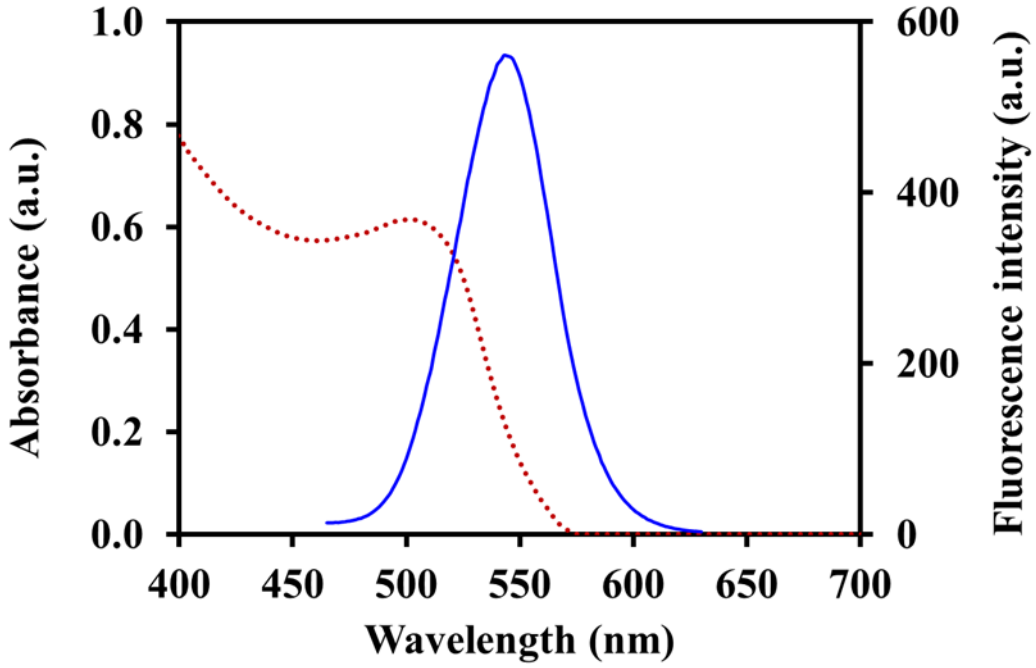

Fig. S1 UV-Vis spectrum (dot line) and fluorescence emission spectrum (solid line) of TGA-capped CdTe QDs.

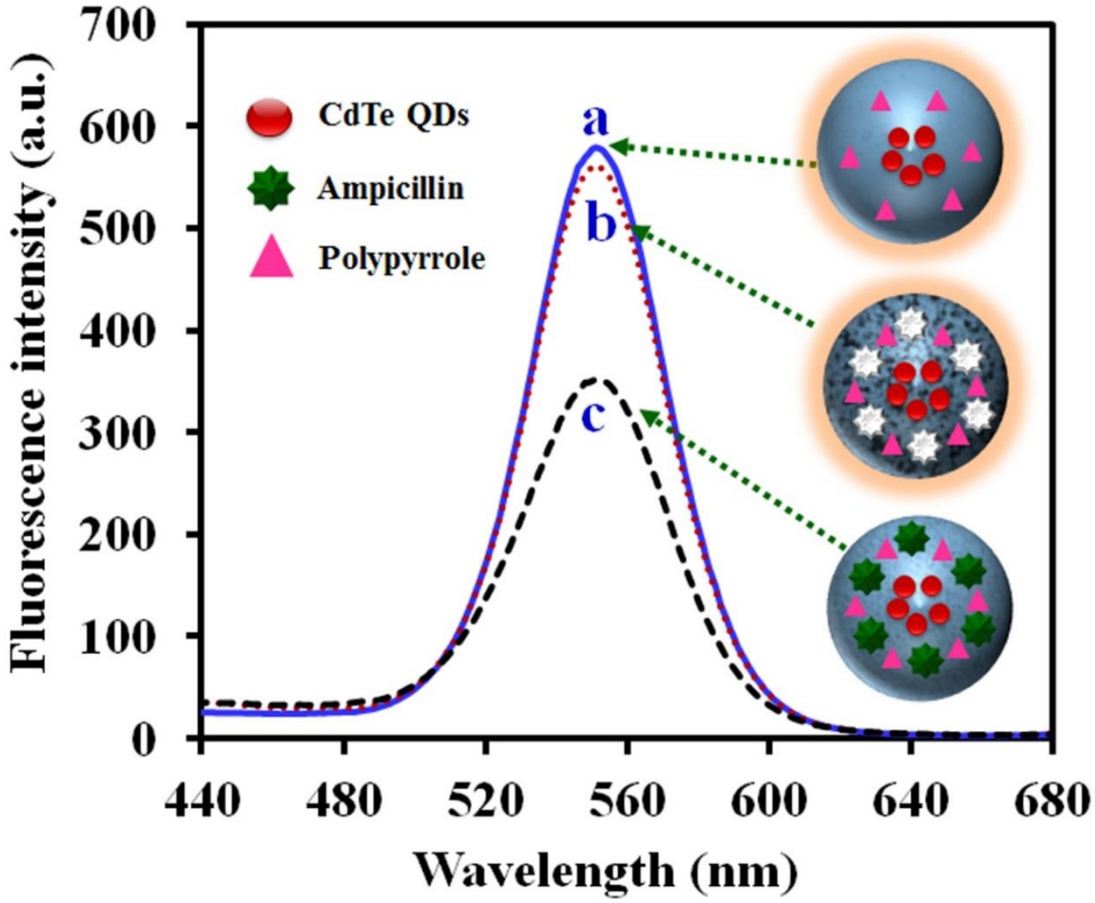

Fig. S2 Fluorescence emission spectra of nanocomposites PPy-QDs-NIP (a), PPy-QDs-MIP without template molecule (b) and PPy-QDs-MIP with template molecule (c).

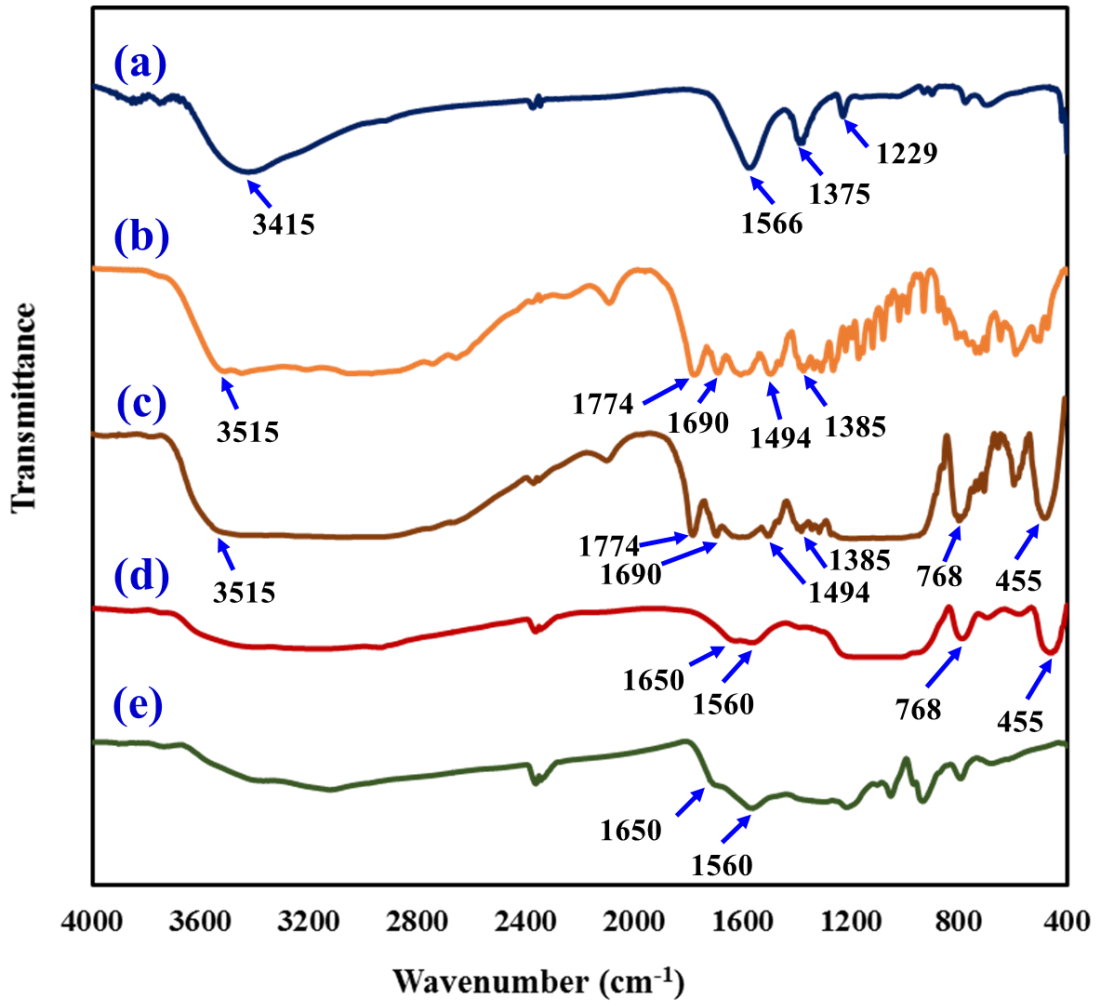

Fig. S3 FTIR spectra of (a) TGA-CdTe QDs, (b) ampicillin, (c) PPy-QDs-MIP before removal of ampicillin (template), (d) PPy-QDs-MIP after removal of the ampicillin (template) and (e) polypyrrole.

Ratio of template to monomer to cross-linker

Since it is a factor that significantly affects the number of specific binding sites and quality of MIP structure [1], the ratios of template (T) to monomer (M) to cross-linker (C) were investigated. A molar ratio of 1:6:20 (T: M: C) provided the highest sensitivity (**Fig. S4a**). In the reaction with template molecules, a small component of APTES (T: M=1: 3) did not provide enough functional groups (-NH_2) to produce the necessary number of specific cavities on the MIP layer. The result was a nanoprobe of low sensitivity. On the other hand, the sensitivity was also low when the ratio of APTES to template was high (T: M=1: 10) because the excess functional monomer led to the formation of non-imprinted molecules within the polymer layer that inhibited binding between ampicillin and specific recognition sites. In addition, the low number of recognition sites may have been due to self-condensation of excess monomer [2]. The sensitivity was also low when using a low amount of TEOS (T: C= 1: 10). The composite MIP-QD particles were so weak that QDs were easily detached during the template removal process [3]. When more TEOS was used (T: C=1: 30), the sensitivity was also low because the specific cavities in the polymer were extremely hard and rigid. When too much cross-linker is present, monomer mobility is reduced, making interaction with the template difficult. Thus, the ratio for T: M: C (ampicillin: APTES: TEOS) of 1: 6: 20 was selected for further experiment.

Effect of incubation time

The incubation time of PPy-QDs-MIP with ampicillin was studied by varying the adsorption times from 1 to 30 min. The fluorescence quenching efficiency of PPy-QDs-MIP increased with increments of adsorption time up to 9 min and remained approximately constant at longer adsorption times (**Fig. S4b**). The adsorption time was

rapid because polypyrrole enhanced the affinity between the target analyte (ampicillin) and recognition sites. Thus, the fluorescence intensity was determined after 9 min incubation of PPy-QDs-MIP and ampicillin solution or sample.

Amount of polypyrrole particles

The effect of PPy content in the PPy-QDs-MIP fluorescence probe was investigated at volumes of 20, 40, 60, and 80 μL. A volume of 40 μL of polypyrrole gave the highest quenching efficiency (**Fig. S4c**). The sensitivity decreased when the lower amount of polypyrrole was used. This result may be due to incomplete binding between PPy-QDs-MIP and ampicillin within the incubation time of 9 min. At the higher volumes of polypyrrole, the probe's sensitivity was also reduced, possibly because polypyrrole particles disturbed the formation of recognition sites and destroyed the polymer structure. Thus, 40 μL was selected as the appropriate amount of polypyrrole for the fabrication of the probe.

Effect of pH

Since pH affects the binding of PPy-QDs-MIP and target molecules, we investigated the effect of pH by dispersing nanocomposite fluorescence probes in phosphate buffer at pH ranging from 5.0 to 8.0. Buffer at pH 7.0 provided the highest sensitivity (**Fig. S4d**). At lower pH, sensitivity decreased due the protonation of the amine groups of the functional monomer in MIP particles and ampicillin [4]. This alteration disturbed hydrogen bonding between ampicillin and specific binding sites. The sensitivity also decreased at buffer pH higher than 7.0, possibly because of deprotonation of the template molecule under the alkaline condition [5]. Also in alkaline solutions, silica in the MIP layer was ionized and could damage binding sites, which could affect interaction between template molecules and the PPy-QDs-MIP

probe. Therefore, a phosphate buffer at pH 7.0 was used for the preparation of the PPy-QDs-MIPs solution.

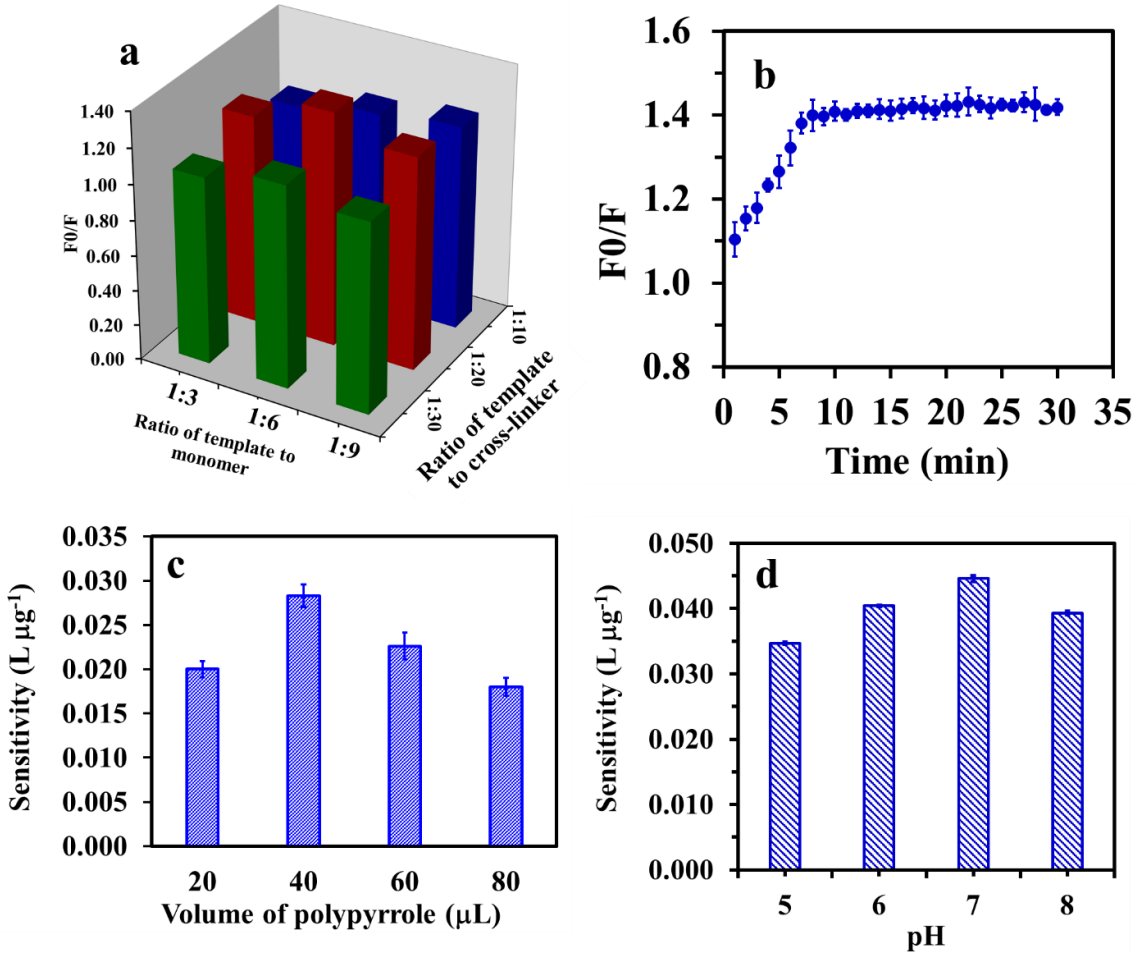

Fig. S4 Effect of template to monomer (APTES) to cross-linker (TEOS) ratio (a), incubation time (b), volume of polypyrrole (c) and pH of PPy-QDs-MIP solution (d) on the fluorescence quenching of PPy-QDs-MIP fluorescence probe for ampicillin detection.

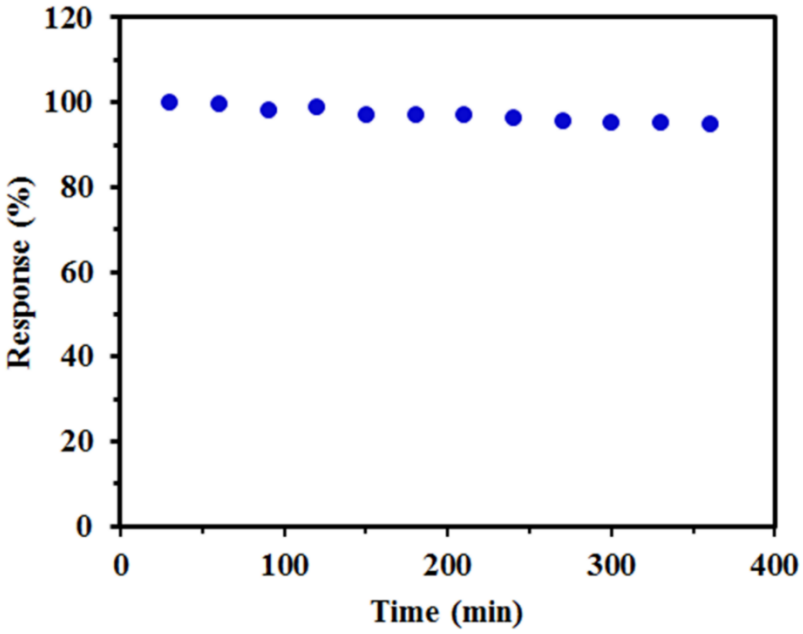

Fig. S5 Stability of nanocomposite PPy-QDs-MIP fluorescence probe in 10 mM phosphate buffer (pH 7.0).

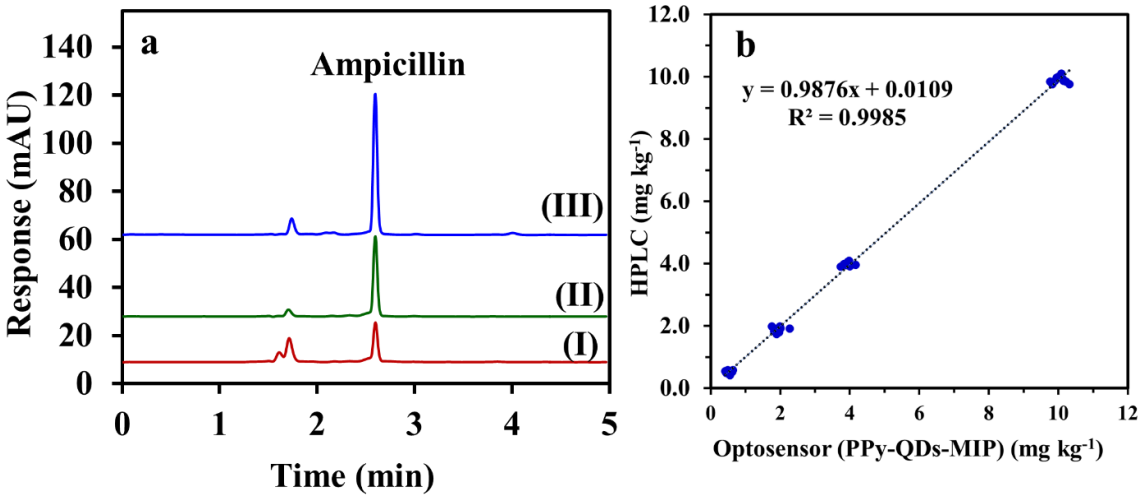

Fig. S6 HPLC chromatograms of spiked milk sample at 2.0 (I), 4.0 (II) and 10.0 mg kg^{-1} (III) (a), and the correlation between the developed nanooptosensor using PPy-QDs-MIP fluorescence probe and HPLC technique for ampicillin detection in food samples (b).

HPLC analysis

HPLC analysis was performed on the 1100 series HPLC apparatus (Agilent Technologies Inc., Germany). The analytical column was a VertiSep^{TM} pHendure C18 column (4.6×150 mm, 5μm) and the column temperature was controlled at 30 °C with an injection volume of 20 μL. The mobile phase was acetonitrile (20% v/v) and 10 mM NaH_2PO_4 (80% v/v) with a flow rate of 1.0 mL min^{-1}. The detection of ampicillin was 220 nm.

Table S1. The analysis of ampicillin in milk and meat samples (n = 6)

Sample	Ampicillin (μg kg^{-1})		Recovery (%)	RSD (%)
	Added	Found		
Milk I	0.0	1.05	-	-
	0.5	1.53	96.3	4.0
	2.0	2.95	94.8	2.8
	4.0	5.00	98.7	0.8
	10.0	10.23	91.8	0.9
Milk II	0.0	0.76	-	-
	0.5	1.23	95.8	3.7
	2.0	2.69	96.6	3.2
	4.0	4.68	97.9	1.7
	10.0	10.58	98.2	1.9
Milk III	0.0	1.74	-	-
	0.5	2.17	86.3	3.8
	2.0	2.62	94.0	0.2
	4.0	5.06	83.7	1.7
	10.0	10.05	83.1	1.1
Milk IV	0.0	2.18	-	-
	0.5	2.65	93.9	3.2
	2.0	3.96	88.9	2.6
	4.0	6.06	97.0	2.4
	10.0	11.59	94.1	1.9
Pork	0.0	1.23	-	-
	0.5	1.76	87.7	2.6
	2.0	3.26	96.9	1.3
	4.0	4.27	85.0	3.7
	10.0	11.10	97.7	2.5
Chicken	0.0	1.16	-	-
	0.5	1.59	86.0	0.1
	2.0	2.79	81.7	2.4
	4.0	4.82	91.5	4.4
	10.0	10.78	96.2	0.4

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List of poster presentations and publication

Poster presentations

Raksawong, P., Chullasat, K., Nurerk, P., Kanatharana, P., Davis, F., and Bunkoed, O. "Optosensor based on molecularly imprinted polymer coated CdTe quantum dots for salbutamol detection" The 12th Conference on Science and Technology for Youths, 3th – 4th June 2017 at Bangkok International Trade & Exhibition Centre (BITEC), Bangkok, Thailand

Raksawong, P., Kanatharana, P., and Bunkoed, O. "Optosensor based on molecularly imprinted polymer coated quantum dots nanoparticles for the determination of ampicillin" Pure and Applied Chemistry International Conference (PACCON 2018), 7th – 9th February, 2018, The 60th Anniversary of His Majesty's the King Accession to the Throne International Convention Center (ICC Hat Yai) , Songkhla, Thailand.

Raksawong, P., Chullasat, K., Nurerk, P., Kanatharana, P., and Bunkoed, O., "A hybrid molecularly imprinted polymer coated CdTe quantum dots as an optosensing for highly sensitive and selective detection of salbutamol" PERCH-CIC X: Contributing Expertise for THAILAND 4.0, 4^{th} – 7^{th} July, 2018, Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, Thailand.

Publication

Raksawong, P., Chullasat, K., Nurerk, P., Kanatharana, P., Davis, F., and Bunkoed, O. "A hybrid molecularly imprinted polymer coated quantum dot nanocomposite optosensor for highly sensitive and selective determination of salbutamol in animal feeds and meat samples". *Analytical and Bioanalytical Chemistry* 409, **2017**, 4697-4707.

Raksawong, P., Nurerk, P., Chullasat, K., Kanatharana, P., and Bunkoed, O. "A polypyrrole doped with fluorescent CdTe quantum dots and incorporated into molecularly imprinted silica for fluorometric determination of ampicillin". *Microchimica Acta* 186, **2019**, 338-345.