

**A hybrid molecularly imprinted polymer coated quantum dots nanocomposite  
optosensor for highly sensitive and selective determination of salbutamol in animal  
feeds and meat samples**

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### Abstract

A hybrid molecularly imprinted polymer coated quantum dots nanocomposite (MIP-coated QDs) was synthesized and applied as a fluorescence probe for the highly sensitive and selective determination of salbutamol. The hybrid MIP-coated QDs nanocomposite was synthesized via a copolymerization process in the presence of thioglycolic acid-capped CdTe QDs using salbutamol as a template, 3-aminopropyltriethoxysilane (APTES) as the functional monomer and tetraethyl orthosilicate (TEOS) as a cross-linker. The optimum molar ratio of template, monomer and cross-linker was 1:6:20. The fluorescence intensity of hybrid MIP-coated QDs was efficiently quenched after salbutamol rebinds to the recognition sites, as a result of charge transfer from QDs to salbutamol. The synthesized hybrid MIP-coated QDs nanocomposite showed a high sensitivity and good selectivity toward salbutamol. Under the optimal recognition conditions, the fluorescence intensity was quenched linearly with increasing concentration of salbutamol in the range of 0.10-25.0  $\mu\text{g L}^{-1}$  with a detection limit of 0.034  $\mu\text{g L}^{-1}$ . The developed hybrid optosensor was successfully applied towards the determination of salbutamol in animal feeds and meat samples. Satisfactory recoveries were obtained in the range of 85 to 98 % with a standard deviation of less than 8 %. Furthermore, the accuracy of the developed hybrid MIP-coated QDs nanocomposite was investigated by comparing with a conventional HPLC method with the results obtained using the two methods agreeing well with each other. The advantages of this sensing method are simplicity, rapidity, cost-effectiveness, high sensitivity and good selectivity.

**Keywords:** Quantum dots, molecularly imprinted polymer, salbutamol, optosensor

## Introduction

Salbutamol is one of the most common  $\beta$ -agonist antibiotics used in human and veterinary medicine to treat asthma, exercise-induced bronchoconstriction and chronic obstructive pulmonary disease [1]. It is also extensively misused in the livestock industry since it can promote animal growth and increase feeding efficiency by reducing fat deposition and enhancing protein accretion [2]. Thus, it is frequently added to livestock feed to improve lean meat-to-fat ratios, which can result in residues remaining in animal meat and delivery to humans along the food chain. This misuse raised serious concerns about a toxicological risk for the consumer [3]. The residues of salbutamol in edible tissues might lead to harmful effects and potential hazards towards human health such as headache, nervousness, muscular tremors, diabetes, hyperthyroidism and cardiac palpitations [4, 5]. It could also potentially lead to the evolution of antibiotic resistant pathogens. To ensure food safety and protect human health, the European Union (EU) has set strict regulations for the  $\beta$ -agonists including salbutamol, banning their use in animal feed. Therefore, it is important to develop a simple, convenient, rapid, cost-effective, sensitive and selective analytical method for the determination of salbutamol residues in animal feeds and meat samples.

Various analytical methods have been developed and used for the determination of salbutamol such as high performance liquid chromatography (HPLC) [6], liquid chromatography-mass spectrometry (LC-MS) [7, 8], gas chromatography-mass spectrometry (GC-MS) [9], electrochemical [10-12] and capillary electrophoresis [13-15]. However, these methods are time consuming, requiring expensive instrument and complex sample preparation steps. In addition, HPLC methods often require large amounts of organic solvents to be used as a mobile phase. To overcome these drawbacks, fluorescence spectroscopy is an interesting technique due to its simple measurement, cost-effectiveness and high-throughput [16]. The sensitivity and selectivity of this method is dependent on the type of fluorescence

probe used [17]. In recent years, quantum dot nanoparticles (QDs) have attracted increasingly more attention and been widely used as a sensitive fluorescence probe due to their excellent optical properties such as narrow and tunable emission spectrum, broad excitation spectrum and good photostability [18, 19]. However, the sensors developed using QDs with an unmodified surface often display a lack of selectivity [20], which means they are not suitable for the determination of trace target analytes in complex samples. Therefore, to improve the selectivity of the sensor, molecularly imprinted polymers (MIP) are an interesting family of materials that can be used in conjunction with QDs [21, 22]. MIPs can be prepared by a copolymerization method using functional monomers and cross-linkers in the presence of a template molecule which is also the target analyte [23, 24]. After polymerization, the template molecule can be removed and specific recognition sites complementary in shape, size and functional groups to the template molecule are obtained in the polymer network [25]. Not only do they provide highly specific recognition sites but MIPs are also easy to prepare, are low cost, have high chemical stability and potential application a wide range of possible target molecules [16, 26]. MIPs have been widely applied in many fields such as solid phase extraction for sample separation [27-29], a polymer coating on an optical fiber for gas sensing [30], modification of electrodes for electrochemical sensors [31] and in paper based devices [32]. It also will be a potentially powerful material to improve the selectivity of optical sensors.

In this work, hybrid MIP-coated QDs nanocomposite fluorescent probes were synthesized and applied for the first time towards the determination of salbutamol. The synthesized hybrid MIP-coated QDs nanocomposites were characterized and their sensing properties were investigated for salbutamol detection. The developed fluorescence probe was also successfully applied for the determination of salbutamol in animal feeds and meat

samples. The accuracy of this developed optosensing protocol was evaluated in spiked samples and also compared with a HPLC method.

## **Materials and methods**

### **Materials**

Tellurium powder (-200 mesh, 99.8%), cadmium chloride ( $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ), sodium borohydride ( $\text{NaBH}_4$ ), 3-aminopropyltriethoxysilane (APTES), tetraethyl orthosilicate (TEOS), thioglycolic acid (TGA) and salbutamol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, sodium hydrogen carbonate, ammonia solution, acetonitrile, methanol and ethanol were obtained from Merck (Darmstadt, Germany). Phosphoric acid and sodium hydroxide were from Labscan (Bangkok, Thailand). Deionized water was obtained from a Maxima ultrapure water system (18.2 M $\Omega$ ) (Elgastat Maxima, ELGA, UK).

### **Instrumental**

Fourier transform infrared spectra (FTIR) were recorded with a Spectrum BX FTIR spectroscope (PerkinElmer, Waltham, MA, USA). The morphology of TGA-capped CdTe QDs and hybrid MIP-coated QDs nanocomposites were observed with a JEM-2010 transmission electron microscope (TEM) (JEOL, Tokyo, Japan) and by scanning electron microscopy (JSM-5200, JEOL, Tokyo, Japan). UV spectra were recorded on an Avaspec 2048 spectrometer (Avantes, Apeldoorn, The Netherlands). Fluorescence intensity was measured using a RF-5310 spectrofluorometer (Shimadzu, Tokyo, Japan). BET surface areas of hybrid MIP-coated QDs and NIP-coated QDs were determined using a ASAP2460 (Micromeritics, USA).

### **Synthesis of TGA-capped CdTe QDs**

The synthesis of TGA-capped CdTe QDs was adapted from previous work [33, 34]. Briefly, 50 mg of tellurium powder and 38 mg of  $\text{NaBH}_4$  were dissolved in 1.0 mL deionized

water to produce a NaHTe solution. Meanwhile, 4.5 mg of CdCl<sub>2</sub> and 30.0 μL of TGA were dissolved in 100 mL of deionized water. This solution mixture was adjusted to pH 11.5 with 1.0 M NaOH, placed into a three-necked flask and deaerated by bubbling with N<sub>2</sub> for 20 min. Under vigorous stirring, 0.5 mL of NaHTe solution was rapidly injected into the mixture solution under a N<sub>2</sub> atmosphere. The solution was then refluxed for 10 min at 95°C. The resulting mixture was precipitated with ethanol and the resultant product collected by centrifugation at 5000 rpm for 10 min. Finally, the TGA-capped CdTe QDs nanoparticles were dried under vacuum and stored in a desiccator for further use.

### **Synthesis of hybrid MIP and NIP-coated CdTe QDs nanocomposite**

The MIP-coated QDs were prepared via a sol-gel copolymerization process. Salbutamol, APTES, and TEOS were used as template molecule, functional monomer and cross-linker, respectively. Briefly, 6.0 mg of salbutamol and 35 μL of APTES were dissolved in 5.0 mL of deionized water in a brown bottle and stirred at 500 rpm for 1.0 h. Then, 5.0 mL of TGA-capped CdTe QDs (10.0 μM), 110 μL of TEOS and 150 μL of 25% NH<sub>3</sub> were added and continuously stirred for 6 h. Finally, the resulting products were washed three times with 10 mL of ethanol to remove templates and unreacted substances. The hybrid MIP-coated QDs nanocomposites were collected by centrifugation at 5000 rpm for 10 min and dried at 50°C. The NIP-coated CdTe QDs nanocomposites were also prepared under the same condition without addition of template molecule (salbutamol).

### **Fluorescence measurement**

The slit width of both the excitation and emission were 10 nm. The excitation wavelength was set at 355 nm and the emission wavelengths were recorded in the range of 450-650 nm. Hybrid MIP-coated QDs (6.0 μg L<sup>-1</sup>) were dispersed in 300 μL 0.01 M carbonate buffer solution (pH 9.0) and then mixed with 100 μL of salbutamol standard

solution or sample solution. After incubation under gentle rotation for 20 min, the solution mixture was transferred into a quartz cuvette and the fluorescence intensity was recorded using a fluorescence spectrophotometer. All fluorescence measurement were carried out at room temperature (25°C) under identical conditions.

### **Sample preparation**

Animal feeds and meat samples were purchased from local markets in Songkhla province, Thailand. The extraction procedure of salbutamol in animal feeds was adapted from previous work [6]. Briefly 1.00 g of animal feed was extracted with 5.0 mL of 0.20 M phosphoric acid and methanol (1:4 v/v) using sonication for 15 min followed by centrifugation at 5000 rpm for 10 min. The supernatant was transferred into a 50 mL polypropylene centrifuge tube and 1.0 mL of HCl (0.1 M) was added to the solution to remove proteins, the mixture was then centrifuged at 5000 rpm for 5 min. The supernatant was evaporated to dryness at 60°C and the residue then dissolved in 1.0 mL of deionized before analysis by the developed hybrid MIP-coated QDs fluorescence method.

The extraction procedure of salbutamol from meat samples was adapted from previous work [35]. Briefly, 1.00 g of homogenized pork or beef samples were extracted with 2.0 mL of ethanol for 10 min using sonication and then centrifugation at 16000 rpm for 5 min. The supernatant was transferred into a 15 mL polypropylene centrifuge tube. The extraction was repeated twice and the supernatants were combined together and defatted with 2.0 mL of hexane. After being shaken for 2 min, the mixture was centrifuged at 5000 rpm for 5.0 min and the degreasing phase was removed. The ethanol phase was then evaporated to dryness at 60°C and the residue then dissolved in 1.0 mL of deionized before analysis by the developed hybrid MIP-coated QDs fluorescence method.

### **Analysis by HPLC method**

The HPLC condition for the determination of salbutamol was adopted from a previous report [6]. The determination of the salbutamol was carried out using a 1100 series HPLC system (Agilent Technologies Inc., Germany) and the data acquired using ChemStation software. The separation was performed on an Ascentis® C18 (5  $\mu\text{m}$ , 4.6 mm  $\times$  150 mm, Supelco) analytical column. The mobile phase consisted of 0.05 % acetic acid with 4.0 mM 1-pentanesulfonate sodium salt (80 %) and acetonitrile (20 %). The flow rate of mobile phase was 0.5 mL min<sup>-1</sup> and column temperature was set at 30 °C. Salbutamol was detected using an excitation and emission wavelength of 226 and 310 nm, respectively.

## Results and discussion

### The synthesis and characterization of hybrid MIP-coated QDs nanocomposite

Hybrid MIP-coated QDs nanocomposites were prepared via copolymerization process as shown in **Fig. 1**. The copolymerization occurred in the presence of TGA-capped CdTe QDs, salbutamol as template molecule, APTES as functional monomer and TEOS as cross-linker. The silica nanospheres were fabricated via the hydrolysis and condensation reaction of TEOS and APTES. The resulting APTES coating on the surface of CdTe QDs provided –NH<sub>2</sub> binding sites. Then the amino groups further interact with salbutamol via hydrogen bonding and then the specific recognition sites were formed around the template molecule in the nanocomposites. NIP-coated QDs were also prepared under the same experimental condition but without addition of template molecule. **Fig. 2** showed the fluorescence intensities of NIP-coated QDs (**Fig. 2a**) and MIP-coated QDs after (**Fig. 2b**) and before removal of template (**Fig. 2c**). The fluorescence intensities of MIP-coated QDs before removal of template were about 20 % of the NIP-coated QDs. The fluorescence intensity of MIP-coated QDs was significantly increased after removal of the template molecules. This result indicated that the MIP was successfully synthesized and template molecule was removed from the MIP-coated QDs nanocomposite particles. The advantages of this method

is the one-step polymerization process under mild conditions which can be carried out at room temperature ( $26 \pm 2^\circ\text{C}$ ).

The absorption and fluorescence spectra of TGA-capped CdTe QDs are shown in **Fig. S1**, the maximum emission appeared at 545 nm. The particle size was 2.35 nm, calculated from the maximum absorption peak according to previous work [33].

The morphology of hybrid MIP-coated QDs nanocomposites were also investigated by the SEM technique. As shown in **Fig. 3a** and **3b**, they have a uniform spherical shape and their diameters are in the range of 220 - 300 nm. The particles diameter increased significantly after coating with the MIP compared with original TGA-capped CdTe QDs. These results indicated that the hybrid MIP-coated QDs have a large surface area with effective imprinting sites to bind the template molecule.

The TEM images of MIP-coated CdTe QDs demonstrated the QDs are small dots distributed within the polymer matrix of the MIP (**Fig.3c**).

The FT-IR spectrum of TGA-capped CdTe QDs (**Fig. 4a**) showed a characteristic peak at 1376 and 1585  $\text{cm}^{-1}$  which corresponded to the C=O symmetric and asymmetric stretching of carboxylic group. The absorption peaks at 3450 and 1225  $\text{cm}^{-1}$  were attributed to the O-H stretching and C-O stretching. The FT-IR spectrum of salbutamol (**Fig. 4b**) exhibited an absorption band at 1500  $\text{cm}^{-1}$  corresponding to the O-H bending [36]. The absorption peak at 1100  $\text{cm}^{-1}$  was due to the C-O stretching. The absorption peak at 3240 and 3400  $\text{cm}^{-1}$  were due to N-H and O-H stretching. The absorption peak at 1616  $\text{cm}^{-1}$  was due to aromatic stretching. The FT-IR spectrum of hybrid MIP-coated QDs nanocomposite before removal of the template (salbutamol) is shown in **Fig. 4c**. The absorption peak at 1063  $\text{cm}^{-1}$  was ascribed to Si-O-Si asymmetric stretching. The Si-O vibrations band was shown at 460  $\text{cm}^{-1}$ . After removal of the template the absorption peaks at 1100, 1500, 1616 and 3240  $\text{cm}^{-1}$  which related to salbutamol were absent (**Fig. 4d**). The broad absorption band at 3409  $\text{cm}^{-1}$

and the absorption peak at  $1600\text{ cm}^{-1}$  indicate the N-H stretching vibration of the aminopropyl group. The results indicated that the MIP was successfully synthesized and coated on the CdTe QDs to form hybrid MIP-coated QDs for selective recognition of salbutamol.

The BET surface area of hybrid MIP-coated QDs and NIP-coated QDs were  $52.77\text{ m}^2\text{ g}^{-1}$  and  $44.75\text{ m}^2\text{ g}^{-1}$ , respectively. The hybrid MIP-coated QDs showed slightly higher surface area than NIP-coated QDs, this could result from the imprinted cavity of the template.

### **Optimization of recognition and the determination conditions**

Several factors could potentially influence the recognition ability of hybrid MIP-coated QDs for the determination of salbutamol i.e., incubation time, pH value, molar ratio of template to monomer and cross-linker. Therefore, these parameters were investigated and optimized to obtain the highest sensitivity and shortest analysis time.

#### **Effect of incubation time**

In order to obtain the highest sensitivity with the shortest analysis time, the binding performance of salbutamol with hybrid MIP-coated QDs was investigated. A certain amount of salbutamol was mixed with hybrid MIP-coated QDs and then the fluorescence intensities were recorded at different incubation times. As shown in **Fig. 5a**, the fluorescence intensity ( $F_0/F$ ) increases with increased incubation time up to 20 min and then remains almost constant. Therefore, 20 min was selected for further experiments.

#### **Effect of pH**

Hybrid MIP-coated QDs are sensitive to chemical changes in their surrounding environment and pH has a significant effect on the sensitivity of the analytical method. Therefore, the influence of pH in the range of 7.0-10.0 on the sensitivity was investigated. As shown in **Fig. 5b**, the highest sensitivity was obtained at pH 9.0. The sensitivity was decreased under acidic condition due to the hydrogen bonding between hybrid MIP-coated

QDs and salbutamol being decreased by hydrogen ion in the solution, possibly due to the protonation of the amine groups present in both polymer and salbutamol. The sensitivity was also decreased at pH value higher than 9.0, possibly due to the template molecules being deprotonated under such alkaline conditions. In addition, the silica shell will be ionized under highly alkaline condition which can cause damage to the structure of the binding sites and potentially electrostatic repulsion between the silica and ionized substrate molecules. Therefore, the determination was carried out using hybrid MIP-coated QDs in buffer solution at pH 9.0.

### **Ratio of template to monomer**

It was reported that the molar ratio of template to functional monomer was an important factor for the formation of specific recognition sites. In order to obtain the highest quality of hybrid MIP-coated QDs for detection of salbutamol, the effect of molar ratio of template to monomer was evaluated and optimized. As shown in **Fig. 5c**, the highest sensitivity was obtained at the molar ratio of 1:6. As shown in **Fig. S2**, low molar ratio (1:2) led to the formation of small particles of hybrid MIP-coated QDs which provided less recognition sites for target analyte. The sensitivity was also decreased at a high molar ratio of template to monomer (1:8) due to the excess monomer forming non-imprinted regions within the polymer layer, which reduced, perhaps by blocking, the binding between recognition sites and target analyte. Therefore, a molar ratio of template to monomer of 1:6 was used for further experiments.

### **Ratio of template to cross-linker**

The effect of cross-linker concentration was also investigated at different molar ratios of template to cross-linker from 1:10 to 1:30. The sensitivity increased with increasing concentration of cross-linker up to the ratio of 1:20 and the sensitivity was decreased with further increases in concentration of cross-linker (**Fig. 5d**). A low sensitivity was obtained at

low concentration of cross-linker due to the MIP structure being physically weaker and less rigid. This means that the formation of specific recognition sites is less effective and also the CdTe QDs were easily disconnected from the polymer during the template removal process. However, too high a concentration of cross-linker also provided low sensitivity due to excessive cross-linking potentially blocking the diffusion and motion of functional monomer (APTES), interfering with its binding with template molecules and leading to a low concentration of binding sites for target analytes within the MIP layer. Therefore, a molar ratio 1:20 was chosen for subsequent experiments.

### **Recognition ability and quenching efficiency of hybrid MIP-coated QDs and NIP-coated QDs for the determination of salbutamol**

The recognition ability of hybrid MIP-coated QDs versus NIP-coated QDs was investigated. **Fig. 6a** shows the fluorescence spectra of hybrid MIP-coated QDs with different concentrations of salbutamol. Their fluorescence intensity was quenched gradually with the increasing concentration of salbutamol. However, the fluorescence intensity of NIP-coated QDs shows only a small decrease at the same concentration of salbutamol (**Fig. 6b**). It can be clearly seen that the fluorescence quenching of hybrid MIP-coated QDs was much higher than that of NIP-coated QDs (**Fig. 6c**). The quenching efficiency of hybrid MIP-coated QDs to salbutamol was investigated according to the Stern-Volmer equation.

$$F_0/F = 1 + K_{sv}[C]$$

Where  $F_0$  and  $F$  are the fluorescence intensity of hybrid MIP-coated QDs in the absence and present of salbutamol, respectively,  $[C]$  is the concentration of salbutamol (quencher) and  $K_{sv}$  is the quenching constant of the quencher. The quenching efficiencies of hybrid MIP-coated QDs to salbutamol were much higher than those of NIP-coated QDs. This is because of the presence of specific recognition sites for salbutamol in the hybrid MIP-coated QDs. When

salbutamol molecules bind with the functional groups in the recognition site via hydrogen bonding and other interactions, this results in electron transfer from QDs to salbutamol, thereby leading to fluorescence quenching of hybrid MIP-coated QDs. The photographs showing fluorescence of hybrid MIP-coated QDs with and without salbutamol are shown in **Fig. 6d**. While, no recognition sites were formed on the surface of NIP-coated QDs, salbutamol can be physically adsorbed on the surface of NIP-coated QDs via hydrogen bonding between salbutamol and  $-NH_2$  groups located on the surface of NIP-coated QDs.

#### **Selectivity of hybrid MIP-coated QDs to salbutamol**

The selectivity of hybrid MIP-coated QDs nanocomposite was evaluated by determining the  $K_{sv}$  of others compounds structurally related to salbutamol namely clenbuterol, clenproperol, ractopamine and chloramphenicol. The results are shown in **Fig. 7**; the  $K_{sv}$  of salbutamol was much higher than these structural analogues. The imprinting factor (IF), which is the ratio of  $K_{sv}$  of the hybrid MIP-coated QDs and NIP-coated QDs ( $IF = K_{sv,MIP}/K_{sv,NIP}$ ) was used to evaluate the selectivity of sensing materials. Under optimum conditions. The imprinting factor of salbutamol, clenbuterol, clenproperol, ractopamine and chloramphenicol were 7.14, 1.75, 1.99, 1.30 and 1.22, respectively. It appears the hybrid MIP-coated QDs have many specific imprinted cavities which match the shape, size and functional groups of the template molecule (salbutamol).

The adsorption ability of NIP-coated QDs was also investigated, the  $K_{sv}$  of salbutamol was similar to the other structural analogues which confirmed there were no specific recognition sites in the NIP-coated QDs

#### **Analytical performance of hybrid MIP-coated QDs for the determination of salbutamol**

Under the optimal conditions, the analytical performances of the developed method was evaluated including linearity, limit of detection (LOD) and limit of quantification (LOQ). The hybrid MIP-coated QDs exhibited linear fluorescence quenching ( $F_0/F$ ) for salbutamol

detection in the concentration range of 0.10-25.0  $\mu\text{g L}^{-1}$  with a coefficient of determination ( $R^2$ ) of 0.9966. The LOD and LOQ were 0.034 and 0.11  $\mu\text{g L}^{-1}$ , based on three times and ten times the standard deviation of the blank signal divided by the slope of the calibration curve, respectively.

### **Reproducibility and stability**

The reproducibility of hybrid MIP-coated QDs preparation was investigated by preparing six different batches of MIP-coated QDs under identical experimental condition. The relative standard deviation of six different batches was 6 %, which indicated that the preparation of hybrid MIP-coated QDs demonstrates good reproducibility.

The stability of hybrid MIP-coated QDs in 0.010 M carbonate buffer solution (pH 9.0) over the time was also investigated. As shown in **Fig. S3**, the fluorescence intensity of hybrid MIP-coated QDs showed no significant changes within 300 min. The stability of the solid powder of hybrid MIP-coated QDs was also investigated by keeping it in a desiccator at 25°C and it was found that the fluorescence intensity showed no significant changes after 5 months (**Fig. S4**). These results indicated that the hybrid MIP-coated QDs optosensing probe has good stability.

### **Application of hybrid MIP-coated QDs for the determination of salbutamol in animal feeds and meat samples**

The developed optosensing method based on hybrid MIP-coated QDs nanocomposite was applied to detect salbutamol in three different types of animal feeds (porcine, poultry and bovine) and well as pork and beef meat samples. The results are shown in **Table 1**, salbutamol was detected in porcine feed at 9.8  $\mu\text{g kg}^{-1}$  and no salbutamol was detected in pork or beef samples. The accuracy of this method was also investigated by spiking standard solution into 1.00 g of homogenized sample to obtain a final concentration of 2.0, 5.0, 10.0

and  $20.0 \mu\text{g kg}^{-1}$ . These spiked samples were vortexed for 15 s and allowed to stand at room temperature for 1.0 to ensure that the analyte was incorporated into the sample matrix. The spiked samples were then extracted and analyzed by the developed method. The recoveries for all samples were in the range from 85.1 to 98.0% with the relative standard deviation being lower than 8 %. These results indicated that the developed hybrid MIP-coated QDs nanocomposite was reliable and can be used as a high throughput method for the determination of the salbutamol in complex samples.

The developed method was also compared with the HPLC method, the samples were spiked with four different concentrations of salbutamol and extracted as described in Section 2.6. The extracted sample solutions were analyzed by both hybrid MIP-coated QDs and HPLC method. A typical HPLC chromatogram of salbutamol in real samples (porcine feed) is shown in **Fig. S5**. The correlation between both methods was good (**Fig. S6**), the coefficient of determination ( $R^2$ ) was 0.9931. This result indicated that the developed hybrid MIP-coated QDs method agreed well with the HPLC method, meaning it can be used as a fast, simple and cost-effective method for the determination of trace salbutamol in animal feeds and food samples.

### **Comparison of the hybrid MIP-coated QDs method with other methods for the determination of salbutamol**

Several methods have been reported for the determination of salbutamol in various samples, the analytical performances of the developed fluorescence sensor based on hybrid MIP-coated QDs optosensing protocol was compared with others described in previous work (**Table 2**). The developed hybrid MIP-coated QDs optosensors provided a wide linear range and lower detection limit than reported in other work, while the recovery and standard deviation of this method was comparable with previous work. These results demonstrated that the hybrid MIP-coated QDs are highly sensitive and can be used for the determination of

trace salbutamol in complex samples. Moreover, this developed method is simple, rapid, and cost-effective and demonstrates good selectivity.

## **Conclusions**

A hybrid MIP-coated QDs nanocomposite was developed and used as an optosensing method for the detection of salbutamol based on an electron transfer induced fluorescence quenching of QDs. The developed hybrid MIP-coated QDs combined the strong fluorescence property of QDs and the high selectivity of MIP, leading to a highly sensitive and selective optosensor for trace determination of salbutamol in complex samples. This simple, rapid, cost-effective, highly sensitive, selective and reliable optosensing protocol was successfully applied to determine salbutamol in animal feeds and meat samples. This facile and versatile sensor preparation can be used as an alternative procedure for the sensitive and selective recognition method of target analytes.

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## **Conflict of interest**

The authors declare that they have no conflict of interest.

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**Table 1.** The determination and the recoveries of salbutamol in real samples (n=5).

Sample	Concentration of salbutamol ( $\mu\text{g kg}^{-1}$ )		Recovery (%)	RSD (%)
	Added	Found		
Porcine feed	0.0	9.80	-	3.0
	2.0	11.65	90.6	5.2
	5.0	14.47	92.5	7.7
	10.0	19.64	98.0	3.5
	20.0	29.40	97.8	0.4
Poultry feed	0.0	n.d	-	-
	2.0	1.88	94.3	3.1
	5.0	4.83	96.7	2.0
	10.0	9.03	90.3	1.1
	20.0	19.55	97.7	1.6
Bovine feed	0.0	n.d	-	-
	2.0	1.73	86.9	1.2
	5.0	4.25	85.1	3.5
	10.0	9.52	95.2	3.1
	20.0	19.58	97.9	2.2
Pork	0.0	n.d	-	-
	2.0	2.09	88.7	2.7
	5.0	4.59	85.4	3.5
	10.0	9.07	87.6	3.3
	20.0	18.64	91.6	5.8
Beef	0.0	n.d	-	-
	2.0	1.77	85.4	0.6
	5.0	4.66	93.2	3.2
	10.0	9.52	95.2	4.5
	20.0	19.33	96.6	1.6

**Table 2.** Comparison of the developed optosensing based on hybrid MIP-coated QDs method with other works for the determination of salbutamol

<b>Analytical methods</b>	<b>Samples</b>	<b>Linear range (<math>\mu\text{g L}^{-1}</math>)</b>	<b>LODs (<math>\mu\text{g L}^{-1}</math>)</b>	<b>Recovery (%)</b>	<b>RSD (%)</b>	<b>References</b>
Ultra-performance liquid chromatography (UPLC)–quadrupole-time-of-flight mass spectrometry	Pig feeds and chicken feeds	2-200	2.0	84-101	3.1-4.8	[37]
Flow injection chemiluminescence	Pork and pork liver	0.5-100	0.15	89-120	1.5-9.0	[38]
Fluorescence sensor (R-phycoerythrin (R-PE) immobilized on eggshell membrane as a fluorescence probe)	Urine	5-100	3.5	85-102	3.2	[39]
Immuno chromatographic	Swine urine	0.1-50	0.04	90-115	4.0-7.8	[40]
Flow-injection chemiluminescence	Pharmaceutical formulations	20-100	5.0	99-100	1.5-2.0	[41]
Pressurized capillary electrochromatography	Urine	500-10000	200	85-91	3.0-3.1	[42]
Capillary electrophoresis	Urine	2,000-30000	500	98-101	1.5-3.8	[43]
Capillary electrophoresis	Swine feed	2,000-100000	1070	100-104	1.0-3.0	[15]
Electrochemical	Salbutamol sulfate injections	12-47800	12.0	95-103	1.5-4.6	[44]
Hybrid MIP-coated QDs nanocomposite	Animal feeds and meat	0.10-25.0	0.034	85-98	0.4-7.7	This work

## Figure Captions

**Fig. 1** Schematic illustration for the synthesis of hybrid MIP-coated QDs nanocomposite for salbutamol detection

**Fig. 2** The fluorescence spectra of NIP-coated QDs (a), MIP-coated QDs after removal of the template (b) and before removal of the template (c)

**Fig. 3** SEM images of hybrid MIP-coated QDs nanocomposites at 20000 magnification (a) and 80000 magnification (b) and TEM images of hybrid MIP-coated QDs nanocomposites (c).

**Fig. 4** FT-IR spectra of TGA-capped CdTe QDs (a), salbutamol (b), hybrid MIP-coated QDs before removal of template (c) and hybrid MIP-coated QDs after removal of template (d)

**Fig. 5** Influence of incubation time (a), pH value (b), molar ratio of template to monomer (c) and molar ratio of template to cross-linker (d) on the fluorescence quenching of hybrid MIP-coated QDs for the determination of salbutamol.

**Fig. 6** Fluorescence emission spectra of hybrid MIP-coated QDs (a), NIP-coated QDs (b) and calibration curve of hybrid MIP-coated QDs and NIP-coated QDs (c) and photographs of cuvettes containing solutions of hybrid MIP-coated QDs without (left) and with (right) salbutamol under UV light (d).

**Fig. 7** Selectivity of hybrid MIP-coated QDs and NIP-coated QDs for salbutamol, clenbuterol, clenproperol, ractopamine and chloramphenicol

Fig. 1

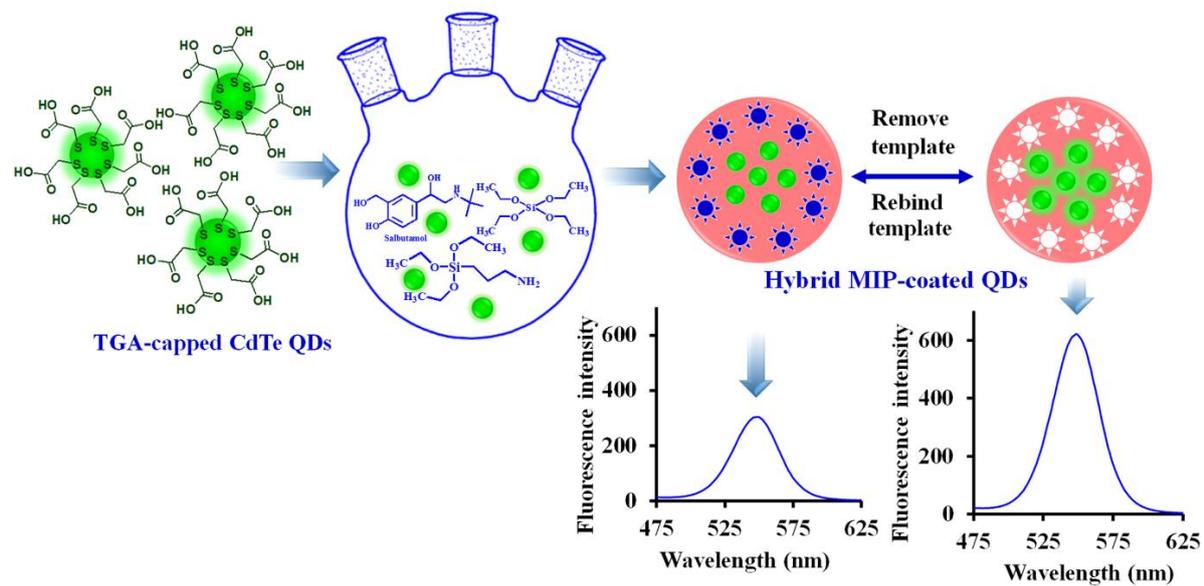


Fig. 2

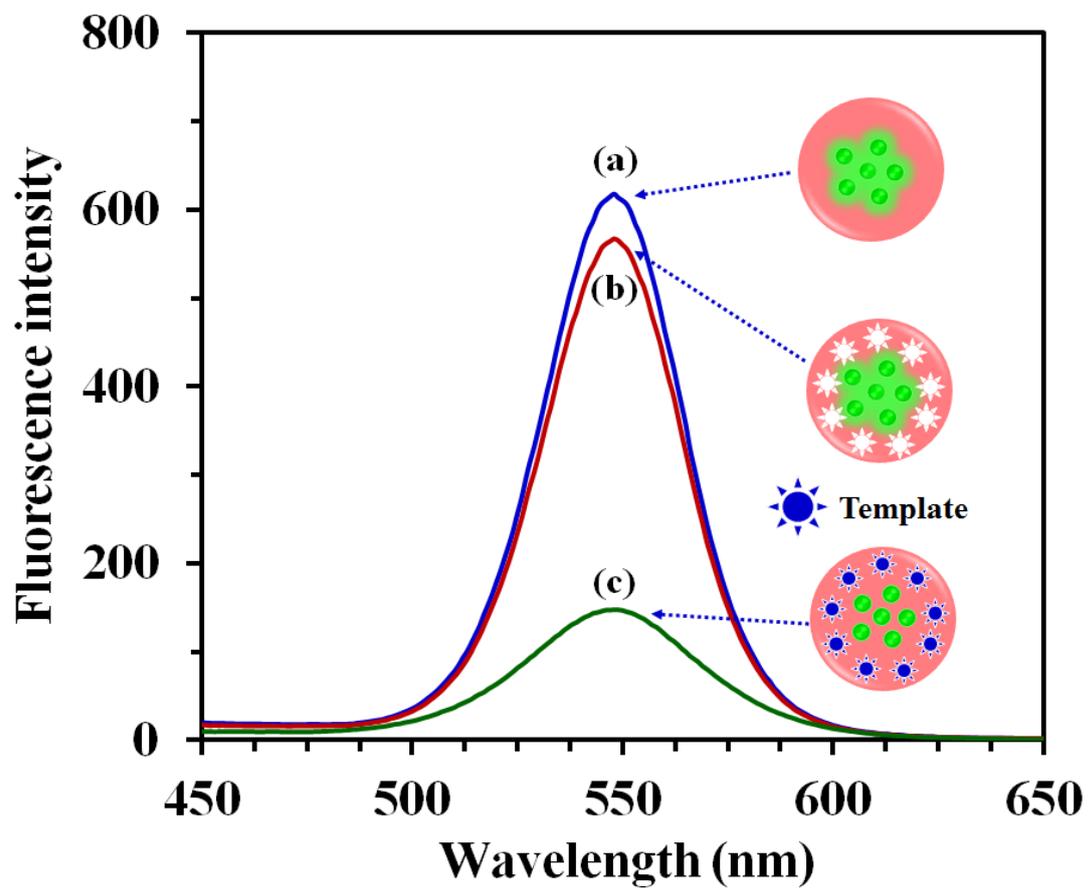


Fig. 3

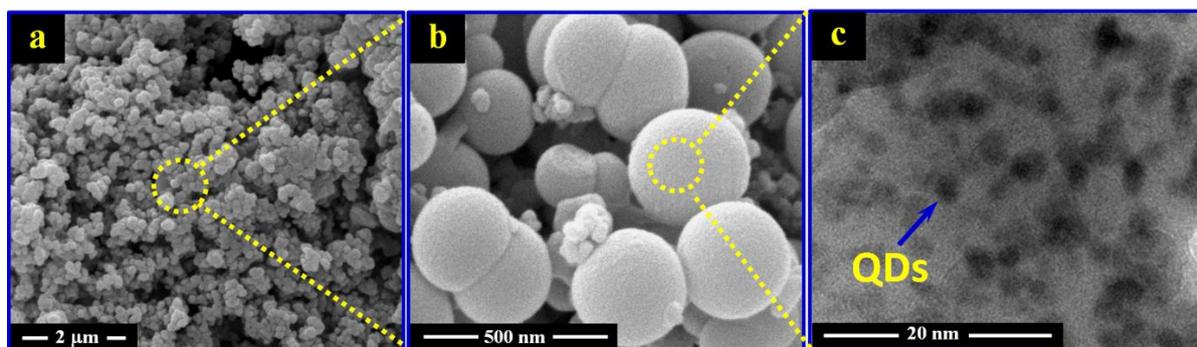


Fig. 4

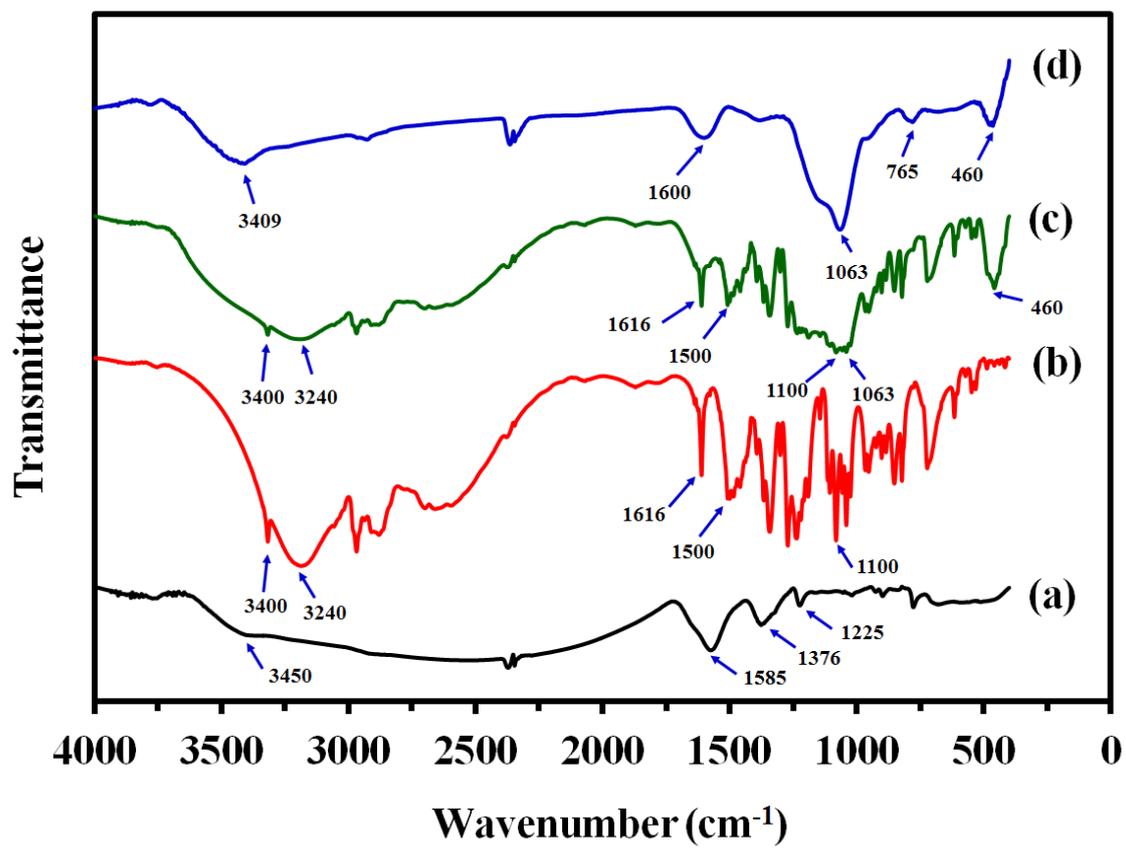


Fig. 5

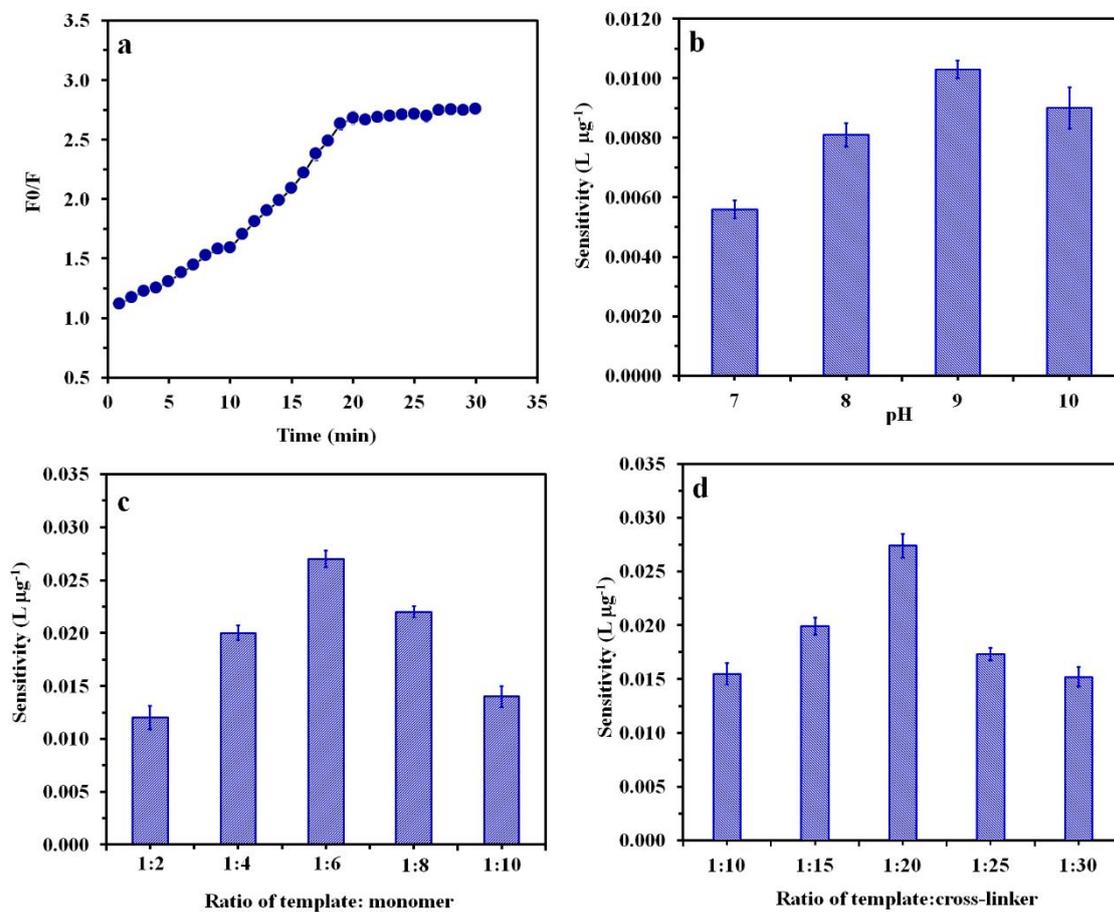


Fig. 6

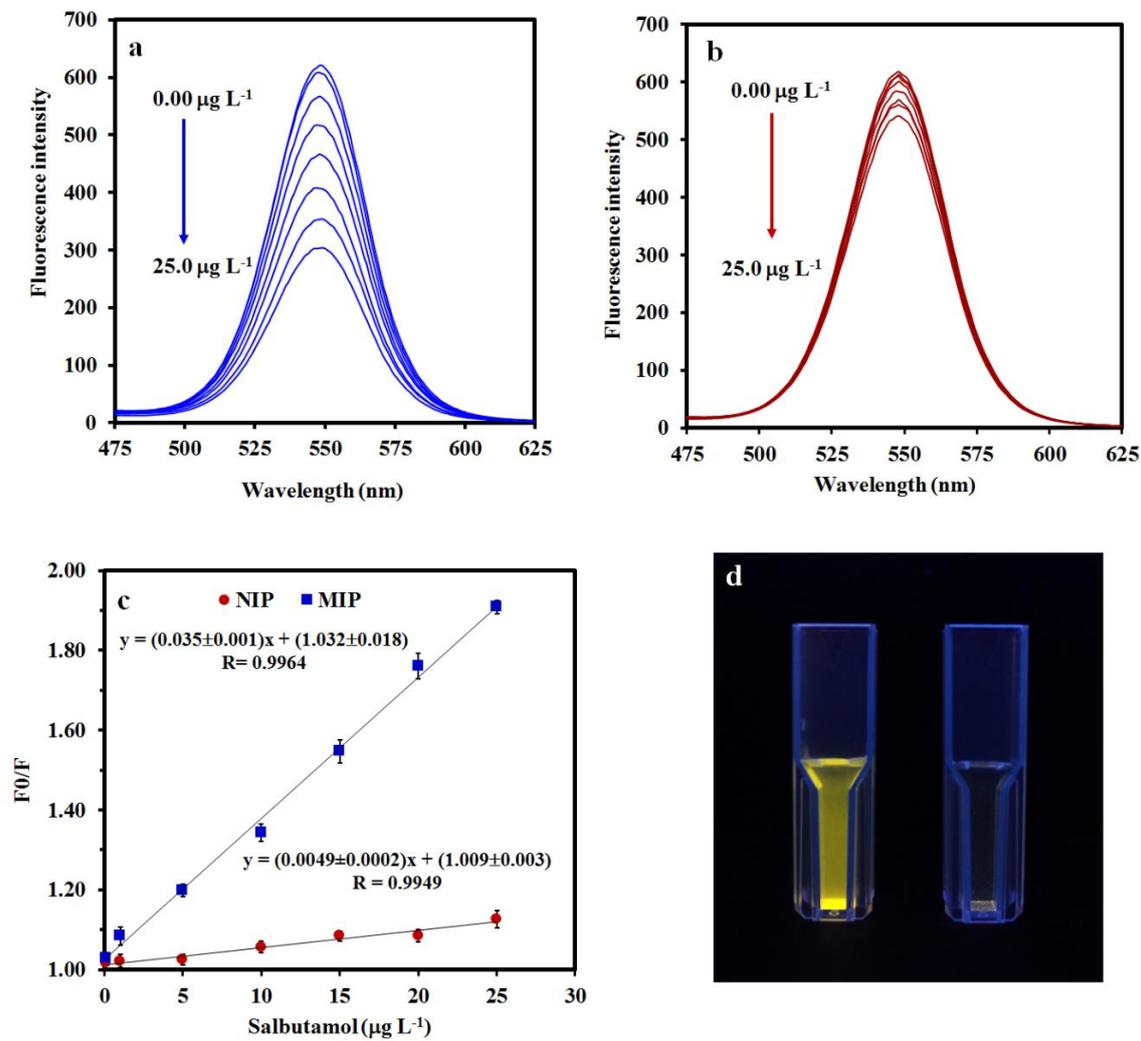


Fig. 7

