# Optosensor and Sample Preparation Techniques for the Determination of Veterinary Drugs and Pesticides

**Kochaporn Chullasat**

**A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry**

**Prince of Songkla University**

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**Thesis Title** Optosensor and Sample Preparation Techniques for the Determination of Veterinary Drugs and Pesticides

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**Major Program** Chemistry

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Dean of Graduate School

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

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(Miss Kochaporn Chullasat)

Candidate

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.

……...……...…….Signature

(Miss Kochaporn Chullasat)

Candidate

**ชื่อวิทยานิพนธ์** เซนเซอร์ทางแสงและเทคนิคการเตรียมตัวอย่างสำหรับการวิเคราะห์ยาปฏิชีวนะที่ใช้ในสัตว์และยาฆ่าแมลง
**ผู้เขียน** นางสาวกชพร จุลสัตย์
**สาขาวิชา** เคมี
**ปีการศึกษา** ๒๕๖๑

## บทคัดย่อ

วิทยานิพนธ์นี้มีจุดประสงค์เพื่อพัฒนาเทคนิคการวิเคราะห์สำหรับตรวจวัดปริมาณยาปฏิชีวนะที่ใช้ในสัตว์และยาฆ่าแมลงที่ตกค้างปริมาณน้อยในตัวอย่างสิ่งแวดล้อมและอาหาร โดยแบ่งออกเป็น ๒ ส่วนคือ การพัฒนาเซนเซอร์ทางแสง และเทคนิคการเตรียมตัวอย่าง

ส่วนแรกเป็นการพัฒนาตัวตรวจวัดทางแสงฟลูออเรสเซนต์ที่มีความจำเพาะเจาะจงต่อสารที่ต้องการวิเคราะห์ โดยการประยุกต์ใช้ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลเพื่อตรวจวิเคราะห์อะมอกซิซิลลิน ซาบูลทามอล เซฟไตรอะโซน และเซฟาเลกซิน ตัวตรวจวัดเตรียมได้โดยอาศัยกระบวนการการเกิดโซล-เจล โดยใช้ ๓-อะมิโนโพรพิลเอทอกซีไซเรนเป็นมอนอเมอร์ เตตระเอทอกซีไซเรนเป็นสารเชื่อมขวาง และสารที่ต้องการวิเคราะห์เป็นโมเลกุลต้นแบบ หลังจากที่ล้างโมเลกุลต้นแบบออกจากชั้นพอลิเมอร์จะได้ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลที่มีช่องว่างที่จำเพาะเจาะจงต่อสารที่ต้องการวิเคราะห์ด้วยขนาด รูปร่างและหมู่ฟังก์ชัน โดยควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลที่สังเคราะห์ได้มีความจำเพาะเจาะจงเมื่อเทียบกับควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ไม่ลอกแบบโมเลกุลต่ออะมอกซิซิลลิน ๔๓.๖ เท่า ต่อซาบูลทามอล ๗.๑๔ เท่า ต่อเซฟไตรอะโซน ๓๐.๐ เท่าและต่อเซฟาเลกซิน ๒๖.๕ เท่า แสดงให้เห็นว่าพอลิเมอร์ลอกแบบโมเลกุลที่สังเคราะห์ได้มีความจำเพาะเจาะจงสูง และให้ค่าการตอบสนองเชิงเส้นในช่วงความเข้มข้น ๐.๒๐ – ๕๐.๐ ไมโครกรัมต่อลิตร และขีดจำกัดการตรวจเท่ากับ ๐.๑๔ ไมโครกรัมต่อลิตร ต่ออะมอกซิซิลลิน และ ๐.๑๐ – ๒๕.๐ ไมโครกรัมต่อลิตร และ ๐.๐๓ ไมโครกรัมต่อลิตร ต่อซาบูลทามอล และตัวตรวจวัดควบคู่ทางแสงฟลูออเรสเซนต์ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลให้ค่าสัญญาณตอบสนองในช่วงความเข้มข้น ๐.๑๐ – ๕๐.๐ ไมโครกรัมต่อลิตร ต่อเซฟไตรอะโซน และต่อเซฟาเลกซิน โดยมีขีดจำกัดการตรวจวัดเท่ากับ ๐.๐๖ และ ๑.๐๐ ไมโครกรัมต่อลิตร ต่อเซฟไตรอะโซน และต่อเซฟาเลกซิน ตามลำดับ ได้ประยุกต์ใช้ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลเพื่อตรวจวิเคราะห์อะมอกซิซิลลินในตัวอย่างนม ไข่ และน้ำผึ้ง พบว่าได้ร้อยละการได้กลับคืนอยู่ในช่วง ๘๕ ถึง ๑๐๒ และมีร้อยละค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า ๖ และไฮบริดควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลเพื่อตรวจวิเคราะห์ซาบูลทามอล ในตัวอย่างเนื้อสัตว์ และอาหารสัตว์ พบว่าได้ร้อยละการได้

กลับคืนอยู่ในช่วง ๘๕ ถึง ๙๘ และมีร้อยละค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า ๘ และตัวตรวจวัดควบคู่ทางแสงฟลูออเรสเซนต์ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลเพื่อตรวจวิเคราะห์เซฟไตรอะโซน และเซฟาเลกซินในตัวอย่างนม พบว่าได้ร้อยละการได้กลับคืนอยู่ในช่วง ๘๓ ถึง ๙๙ และร้อยละค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า ๘ นอกจากนี้เมื่อนำตัวตรวจวัดทางแสงฟลูออเรสเซนต์ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลที่พัฒนาขึ้นมาทดสอบเปรียบเทียบกับวิธีวิเคราะห์มาตรฐานโครมาโทกราฟีของเหลวสมรรถนะสูงพบว่าผลการวิเคราะห์ทั้งสองวิธีสอดคล้องกัน แสดงว่าวิธีที่พัฒนาขึ้นมีความน่าเชื่อถือและสามารถนำไปประยุกต์ใช้ในการตรวจวิเคราะห์ปริมาณอะมอกซิซิลลิน ซาบูลทามอล เซฟไตรอะโซน และเซฟาเลกซินในตัวอย่างนม ไข่ น้ำผึ้ง เนื้อสัตว์และอาหารสัตว์ได้ นอกจากนี้ตัวตรวจวัดทางแสงฟลูออเรสเซนต์ควอนตัมดอทคอมโพสิทร่วมกับพอลิเมอร์ลอกแบบโมเลกุลที่พัฒนาขึ้นมีข้อดีคือสามารถทำได้ง่าย ตรวจวัดได้รวดเร็ว ราคาถูก ให้ค่าความไววิเคราะห์สูงและมีความจำเพาะเจาะจงต่อสารที่ต้องการวิเคราะห์ที่ดี

ในส่วนที่สองเป็นการพัฒนาเทคนิคการเตรียมตัวอย่างสำหรับสกัดยาปฏิชีวนะและยาฆ่าแมลงก่อนการวิเคราะห์ด้วยเทคนิคโครมาโทกราฟี โดยประกอบด้วยสองงาน งานวิจัยแรกคือการพัฒนาตัวดูดซับของแข็งชนิดใหม่ในลักษณะครัยโอเจลคอมโพสิทระหว่างพอลิไวนิลแอลกอฮอล์และแกรฟีนออกไซด์เคลือบด้วยพอลิไพโรลสำหรับประยุกต์ใช้ในเทคนิคการสกัดด้วยตัวดูดซับของแข็งในการสกัดและเพิ่มความเข้มข้นของซัลโฟนาไมด์ในตัวอย่างน้ำ โดยการประยุกต์ใช้พอลิไพโรลและแกร-ฟีนออกไซด์เพื่อเพิ่มพื้นที่ผิวสัมผัสในการดูดซับซัลโฟนาไมด์โดยสามารถเกิดพันธะไฮโดรเจน อันตรกิริยาแบบ $\pi$-$\pi$ และไฮโดรโฟบิก พอลิไวนิลแอลกอฮอล์ครัยโอเจลซึ่งมีรูพรุนมากสามารถช่วยลดความดันต้านกลับของตัวดูดซับ ภายใต้สภาวะที่เหมาะสมวิธีที่พัฒนาขึ้นให้ช่วงความเป็นเส้นตรงตั้งแต่ ๐.๒๐ ถึง ๑๐๐ ไมโครกรัมต่อลิตร และขีดจำกัดการตรวจวัดเท่ากับ ๐.๒๐ ไมโครกรัมต่อลิตร สำหรับการตรวจวิเคราะห์ซัลฟาไดอะซีน ซัลฟาไทอะโซล และซัลฟาเมอราซีน และให้ช่วงการตอบสนองเชิงเส้นความเป็นเส้นตรงตั้งแต่ ๐.๑๐ ถึง ๑๐๐ ไมโครกรัมต่อลิตร และขีดจำกัดการตรวจวัดเท่ากับ ๐.๑๐ ไมโครกรัมต่อลิตร สำหรับการตรวจวิเคราะห์ซัลฟาเมทาซีน ซัลฟามอนอเมทอกซีน และซัลฟาไดเมทอกซีน ตัวดูดซับที่พัฒนาขึ้นมีประสิทธิภาพในการสกัดที่ดีโดยให้ค่าร้อยละการได้กลับคืนอยู่ในช่วง ๘๕.๕ ถึง ๙๙.๐ และมีค่าร้อยละเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า ๕ ตัวดูดซับที่พัฒนาขึ้นให้ค่าการเตรียมซ้ำที่ดี และสามารถใช้ซ้ำได้อย่างน้อย ๑๐ ครั้ง แสดงให้เห็นว่าตัวดูดซับที่พัฒนาขึ้นสามารถนำไปประยุกต์ใช้ในการสกัดและเพิ่มความเข้มข้นของซัลโฟนาไมด์ในตัวอย่างน้ำได้ งานวิจัยสุดท้ายคือการประยุกต์ใช้เทคนิคการสกัดด้วยตัวทำละลายที่มีฟองในหยดเดียวสำหรับการสกัดและวิเคราะห์หาปริมาณของยาฆ่าแมลงในกลุ่มคาร์บาเมตในตัวอย่างน้ำ การกักฟองอากาศไว้ภายในหยดตัวทำละลายอินทรีย์เป็นการเพิ่มพื้นที่ผิวของตัวทำละลายส่งผลทำให้สามารถเพิ่มประสิทธิภาพการสกัดได้ ภายใต้สภาวะที่เหมาะสมวิธีที่พัฒนาขึ้นให้ช่วงการตอบสนองเชิงเส้นสำหรับการวิเคราะห์คาร์

บาเมตในช่วง ๐.๐๕ ถึง ๒๐.๐ ไมโครกรัมต่อลิตร และขีดจำกัดการตรวจวัดอยู่ในช่วง ๐.๐๒ ถึง ๐.๐๔ ไมโครกรัมต่อลิตร ได้ประยุกต์ใช้วิธีพัฒนาขึ้นในการวิเคราะห์หาปริมาณยาฆ่าแมลงกลุ่มคาร์บาเมตในตัวอย่างน้ำโดยให้ค่าร้อยละการได้กลับคืนที่ดีในช่วง ๘๒ ถึง ๙๙ และค่าร้อยละเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า ๙ อีกทั้งวิธีที่พัฒนาขึ้นมีข้อดีคือสามารถทำได้ง่าย ใช้ปริมาณตัวทำละลายอินทรีย์น้อย เป็นมิตรกับสิ่งแวดล้อม และราคาถูก

โดยสรุปผลของการศึกษาแสดงให้เห็นว่าเซนเซอร์ทางแสงและเทคนิคการเตรียมตัวอย่างที่พัฒนาขึ้นสามารถประยุกต์ใช้ในการตรวจวิเคราะห์การตกค้างของยาที่ใช้ในสัตว์และยาฆ่าแมลงในตัวอย่างจริงได้อย่างมีประสิทธิภาพ สามารถทำได้ง่าย ราคาถูก รวดเร็ว มีความไววิเคราะห์สูง ความจำเพาะเจาะจงที่ดี มีความน่าเชื่อถือและถูกต้องแม่นยำ สามารถใช้เป็นวิธีทางเลือกสำหรับประยุกต์ใช้ในการวิเคราะห์ยาที่ใช้ในสัตว์ ยาฆ่าแมลง และสารอื่นๆ ในตัวอย่างชนิดต่างๆ ได้

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## Abstract

This thesis aimed to develop new analytical techniques for the determination of trace veterinary drugs and pesticides in environment and food samples. It consist of two-part including the optosensor and sample preparation techniques.

The first part focused on the development of novel fluorescence probe using molecularly imprinted polymer composited with quantum dots nanoparticles. The facile method for coating a molecularly imprinted polymer onto quantum dots (MIP-QDs) which are CdTe QDs and graphene quantum dots (GQDs) were successfully formulated and used for the first time as a highly selective and sensitive fluorescence probes for the determination of trace amoxicillin, salbutamol, ceftriaxone and cephalexin. The nanocomposite MIP-QDs fluorescence probes were prepared via a sol-gel process with 3-aminopropyl-ethoxysilane as a functional monomer, tetraethoxysilane as a cross-linker and the target analyte as a template molecule. After removal of the template molecules from the polymer layer, MIP-QDs containing cavities specific to the target analyte were obtained. The obtained cavities of MIP-QDs were specific to target analyte (amoxicillin, salbutamol, ceftriaxone and cephalexin) by size, shape and functional group. The fluorescence intensity of MIP-QDs was more strongly quenched by target analytes by comparing with the non- imprinted polymer (NIP-QDs) with high imprinting factor of 43.6 for amoxicillin, 7.14 for salbutamol, 30.0 for ceftriaxone and 26.5 for cephalexin, respectively. The synthesized nanocomposite MIP-QDs showed a high sensitivity and good selectivity toward to amoxicillin in the concentration range of 0.20 - 50.0 μg $L^{-1}$ with a low detection limit of 0.14 μg $L^{-1}$ and 0.10 - 25.0 μg $L^{-1}$ with a low detection limit of 0.03 μg $L^{-1}$ for salbutamol detection. The dual nanocomposite fluorescence probe showed a good linearity from 0.10 to 50.0 μg $L^{-1}$ with a low detection limit of 0.06 and 1.00 μg $L^{-1}$ for cephalexin and ceftriaxone,

respectively. The developed nanocomposite MIP-QDs was successfully applied toward the determination of amoxicillin in milk, eggs and honey with a good recovery of 85 – 102 % and the relative standard deviation of less than 6 % being achieved. The hybrid MIP- QDs fluorescence probe was effectively applied for the determination of salbutamol in animal feed and meat samples with a satisfactory recovery of 85 to 98 % (RSD < 8). The developed dual nanocomposite MIP-QDs fluorescence probes were applied for the detection of ceftriaxone and cephalexin in the milk sample with a satisfactory recovery of 83 - 99 % and RSD lower than 8 %. Furthermore, the accuracy of the developed nanocomposite MIP-QDs fluorescence probes were investigated by comparing with HPLC method with the results obtained using the two methods agreeing well with each other. It demonstrated that the developed method is reliable and can be applied for real sample analysis. The advantages of the developed optosensor are simplicity, rapidity, cost- effectiveness, high sensitivity and good selectivity.

The second part focused on the development of sample preparation techniques for the determination of veterinary drugs and pesticides using chromatography analysis. Two sub-project were carried out. The first one is a hybrid monolith sorbent of polypyrrole coated graphene oxide incorporated into a polyvinyl alcohol cryogel. It was successfully synthesized and used as a sorbent in solid-phase extraction technique for extraction and preconcentration of trace sulfonamides. The large surface areas with many adsorption sites of polypyrrole (PPY) and graphene oxide (GO) facilitated the high adsorption ability of sulfonamides via hydrogen bonding, π-π and hydrophobic interaction. The high porosity of the polyvinyl alcohol (PVA) cryogel helped to reduce the backpressure that occurs in a conventional packed SPE cartridge. Under the optimum conditions, the developed method provided a wide linear range from 0.20 to 100 μg $L^{-1}$ with a low detection limit of 0.20 μg $L^{-1}$ for sulfadiazine, sulfathiazole and sulfamerazine and from 0.10 to 100 μg $L^{-1}$ with a low detection of 0.10 μg $L^{-1}$ for sulfamethazine, sulfamonomethoxine and sulfadimethoxine. The developed hybrid monolith polypyrrole-coated graphene oxide embedded in the polyvinyl alcohol cryogel sorbent provided good recoveries in the range of 85.5 - 99.0 % with RSD less than 5 %. The sorbent offered a good reproducibility and can be reused at least 10 times. It was successfully applied for the extraction and preconcentration of trace amount

sulfonamides in the environmental water samples. The last project is an application of bubble-in-single drop microextraction (BI-SDME) for the extraction and analysis of eight carbamate pesticides from the environmental water sample. The increasing of the extraction solvent surface area when encapsulated air bubble in the microdroplet caused the greater extraction efficiency with still less volume of extraction solvent. Under the optimal conditions, the developed method showed a wide linearity in the range of 0.05 to 20.0 μg $L^{-1}$ with the low limit of detection in the range of 0.02 to 0.04 μg $L^{-1}$. This method was successfully applied for the determination of carbamate pesticides in the environmental water samples with good recoveries of 82 - 99 % and RSD less than 9 %. The advantages of this method is solventless, environmentally friendly, low cost and simple to operate.

In conclusion, the optosensor and sample preparation techniques were successfully developed and applied for determination of trace veterinary drugs and pesticides in real sample with a satisfactory performance. There are many advantages such as simple to prepare, cost-effective, rapid, high sensitivity and good selectivity. It provided good accuracy and precision. These developed method can be used as an alternative method for the determination of other organic compounds in a various samples.

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# Acknowledgments

The completion of this thesis would be impossible without the help of many people. I hereby express my gratitude to those who have contributed to the completion of this thesis:

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Kochaporn Chullasat

## The Relevant of the Research Work to Thailand

The purpose of this Doctor of Philosophy Thesis in Chemistry is to develop optosensor and sample preparation techniques for determination of trace veterinary drugs and pesticides residues in the environment and food-products. These developed methods provided a high efficiency, high sensitivity, good selectivity, simple to use and cost-effective. It can be used for determination of amoxicillin, salbutamol, ceftriaxone, cephalexin, sulfonamides and carbamate pesticides in real sample i.e. milk, egg, honey, meat, animal feed and the environmental water. In addition, it can be used as an alternative method for determination of veterinary drugs, pesticides and other organic compounds in various governmental agencies and private sectors in Thailand.

# Contents

# Contents (Cont.)

# List of Figures

# List of Abbreviations

| | |
|---|---|
| BI-SDME | Bubble in single drop microextraction |
| DI-SDME | Direct immersion single drop microextraction |
| EU | The European Union |
| GO | Graphene oxide |
| GQDs | Graphene quantum dots |
| HF-LMPE | Hollow fiber-based liquid phase microextraction |
| HS-SDME | Headspace single dropmicroextraction |
| LLE | Liquid-liquid extraction |
| LLME | Liquid-liquid microextraction |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MIPs | Molecularly imprinted polymers |
| MRLs | Maximum residue limits |
| MSPD | Matrix solid phase dispersion |
| MSPE | Magnetic solid phase extraction |
| PPY | Polypyrrole |
| QDs | Quantum dots |
| SBSE | Stir bar sorptive extraction |
| SDME | Single drop microextraction |
| SPE | Solid phase extraction |
| SPR | Surface plasmon resonance |

## List of Publications

This thesis is organized into two parts including a development of optosensor based on the fluorescence quenching of quantum dots composited into molecularly imprinted polymer and sample preparation techniques for trace veterinary drugs and pesticides. The following of thesis is referred by their Roman numerals in the text.

**Paper I** **Chullasat, K.**, Nurerk, P., Kanatharana, P., Davis, F., Bunkoed, O., A facile optosensing protocol based on molecularly imprinted polymer coated on CdTe quantum dots for highly sensitive and selective amoxicillin detection. ***Sensors and Actuators B: Chemical*** 254 (2018), 255-263.

**Paper II** Raksawong, P., **Chullasat, K.**, Nurerk, P., Kanatharana, P., Davis, F., 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.

**Paper III** **Chullasat, K.**, Kanatharana, P., Bunkoed, O., Nanocomposite optosensor of dual quantum dot fluorescence probes simultaneously detects cephalexin and ceftriaxone. ***Sensors and Actuators B: Chemical*** 254 (2019), 689-697.

**Paper IV** **Chullasat, K.**, Nurerk, P., Kanatharana, P., Kueseng, P., Sukchuay, T., Bunkoed, O., Hybrid monolith sorbent of polypyrrole-coated graphene oxide incorporated into a polyvinyl alcohol cryogel for extraction and enrichment of sulfonamides from water samples. ***Analytica Chimica Acta*** 961 (2017), 59-66.

**Paper V** **Chullasat, K.**, Huang, Z., Bunkoed, O., Kanatharana, P., Lee, H. K., Bubble-in-single drop microextraction for the analysis of carbamate pesticides from water samples.

*Manuscript.*

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| Title | Development of chemical sensors and sample preparation techniques for trace veterinary drugs and pesticides |

# Paper III

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# 1. Introduction

## 1.1 Background and rationale

In the recent years, the contamination of veterinary drugs and pesticides in food and environment have become a growing problem. The veterinary drugs, the antibacterial drugs are widely used for treatment and prevention infectious disease in animal and also used as a growth-promoting feed while the pesticides are extensively used for protection crop from pest (Bargańska *et al.*, 2018; Chen *et al.*, 2016). However, the overuse or misuse of veterinary drugs and pesticides in agriculture industry led to the residue and accumulate in the environment and food-producing animals through the food chain (Farré *et al.*, 2014; Marazuela and Bogialli, 2009; Masiá *et al.*, 2016). The accumulated and bioamplification of these compounds in the food chain can cause severe side-effects to human health (Peixoto *et al.*, 2016; Santos and Ramos, 2016). For example, the exposure of a veterinary drugs in food product such as β-lactam antibiotic, sulfonamides and cephalosopin antibiotics can cause hypersensitivity in humans such as headache, vomiting, stomach upset, diarrhea and nausea (Gamba *et al.*, 2009; Kılıç *et al.*, 2018; Kumar *et al.*, 2017; Raksawong *et al.*, 2017). Some carbamate and their metabolites are suspected to be carcinogen and mutagen (Saraji and Esteki, 2008; Woo, 1983) and the exposure of carbamates can cause some adverse effects i.e. vomiting, headache, diarrhea, urinary incontinence, bronchospasm, and dyspnea (Jin *et al.*, 2004; Rosman *et al.*, 2009).

To assure the human health, many countries have been set the maximum residue limits (MRLs) for veterinary drugs and pesticides. There are more than 1000 active substance indicated as pesticides and a group of veterinary drugs which have been set MRLs in food and environment (Masiá *et al.*, 2016). For instance, the European Union (EU) set the MRLs for sulfonamides of 100 μg $kg^{-1}$ in animal tissues and 4 μg $kg^{-1}$ in milk and egg, for amoxicillin of 50 μg $kg^{-1}$ in animal origin and 4 μg $kg^{-1}$ in milk, cephalexin of 200 μg $kg^{-1}$ in animal tissues and 100 μg $kg^{-1}$ in milk, for individual pesticides of 0.1 μg $L^{-1}$ and for total pesticides of 0.5 μg $L^{-1}$ in drinking water (European Commission, 1990, 1998, 2010). Therefore, the monitoring of veterinary drugs and pesticides in environment and food is an important.

Various analytical methods have been developed for the determination of veterinary drugs and pesticides such as chromatography (Karageorgou *et al.*, 2016;

Masiá *et al.*, 2016; Sun *et al.*, 2016), electrochemical methods (Balooei *et al.*, 2017; Yang and Zhao, 2015), surface plasmon resonance (Tomassetti *et al.*, 2015; Yola *et al.*, 2014), and spectrophotometry (Al-Abachi *et al.*, 2005; Shahrouei *et al.*, 2018).

For the determination of single or two target analytes, spectrofluorometry is an interesting alternative method because of it has a short analysis time, easy to use, low-cost equipment and required a small amount of sample (Lobnik *et al.*, 2012; Szulczyński and Gębicki, 2017). For the determination of analytes, it measures the enhancement of the quenching of fluorescence intensity when the chemical formation was occurred (Wolfbeis, 1985). Several organic dyes have been used as a fluorescence probe for the detection of various target analytes (Sorouraddin *et al.*, 2009). However, it still have some drawbacks i.e. broad and asymmetric band (Resch-Genger *et al.*, 2008). Instead of organic dyes, quantum dots (QDs) is an attractive fluorescence probe due to its exhibit a wide adsorption range and a narrow emission band with symmetric shape (Hezinger *et al.*, 2008; Resch-Genger *et al.*, 2008). In addition, QDs are photochemical stability, long fluorescence lifetime and good water dispersibility (Hezinger *et al.*, 2008; Wei *et al.*, 2016). There are several works used quantum dots as a fluorescence probes for determination of various analytes such as copper ion ($Cu^{2+}$) (Nurerk *et al.*, 2016), silver ion ($Ag^{+}$) (Cai *et al.*, 2014), salicylic acid (Bunkoed and Kanatharana, 2015), ciprofloxacin (Yuphintharakun *et al.*, 2018), protein (Zamora-Gálvez *et al.*, 2018) and cancer cell (Dutta Chowdhury *et al.*, 2018; Phan *et al.*, 2018).

To improve the selectivity of QDs (as a fluorescence probe), the surface of QDs can be modified or composited with some specific materials (Nurerk *et al.*, 2016). Molecularly imprinted polymers (MIPs) are an attractive material to modify on the surface of quantum dots due to its easy to prepare, inexpensive, good stability and high selectivity (Niu *et al.*, 2016; Turiel and Martín-Esteban, 2010). MIPs are affinity polymers and can be synthesized to be specific to a target analyte (template) (Garcia *et al.*, 2011). This material is highly cross-linked three-dimensional network polymers, formed by polymerization between a functional monomer, cross-linker and template molecules (target analytes) (Beltran *et al.*, 2010; Turiel and Martín-Esteban, 2010). After the polymerizations were completed, the template molecules were removed and the specific cavities were obtained (Chao *et al.*, 2016). Then the first part of thesis was focused on the development of optosensor based on the fluorescence quenching of

quantum dots composited with molecularly imprinted polymer for the determination of amoxicillin (**Paper I**), salbutamol (**Paper II**), ceftriaxone and cephalexin (**Paper III**).

For multi-analyte determination, the analytical method that widely used is chromatography which can separate analytes using analytical column (Masiá *et al.*, 2016; Villaverde *et al.*, 2016). However, the residue and contamination of target analyte at trace levels in real sample and high matrix interferences, generally requires a suitable sample preparation to preconcentrate the target analyte and clean up the sample before instrumental analysis (Li *et al.*, 2015; Santaladchaiyakit *et al.*, 2012).

Many sample preparation methods have been developed and applied for the extraction of veterinary drugs and pesticides such as solid phase extraction (SPE) (Chen *et al.*, 2015; Dmitrienko *et al.*, 2015; Tian *et al.*, 2013), matrix solid phase dispersion (MSPD) (Wang *et al.*, 2015), magnetic solid phase extraction (MSPE) (Ibarra *et al.*, 2014; Shi and Ye, 2015; Sukchuay *et al.*, 2015), stir bar sorptive extraction (SBSE) (Yu and Hu, 2012), liquid-liquid extraction (LLE) (Premarathne *et al.*, 2015), liquid-liquid microextraction (LLME) (Arroyo-Manzanares *et al.*, 2014; Song *et al.*, 2014), hollow fiber-based liquid phase microextraction (HF-LMPE) (Ramos Payan *et al.*, 2011) single drop microextraction (Amvrazi *et al.*, 2012; Pano-Farias *et al.*, 2017; Xiao *et al.*, 2006) and bubble in single drop microextraction (Guo *et al.*, 2016; Williams *et al.*, 2014). Among these sample preparation method, solid phase extraction (SPE) and bubble-in-single drop microextraction (BI-SDME) are interesting methods due to its use less amount of organic solvent, easy to use, and can be applied for high-throughput determination (Farajzadeh *et al.*, 2014; Samsidar *et al.*, 2018; Xu *et al.*, 2007).

Therefore, the aim of the second part of this thesis is to develop sample preparation method for the determination of veterinary drugs and pesticides using solid phase extraction (**Paper IV**) and bubble-in-single drop microextraction (**Paper V**).

## 1.2 Objective

The objective of this thesis is to develop an optosensor and sample preparation techniques for trace veterinary drugs and pesticides detection. To achieve this objective, two part which consists of the development of novel fluorescence probes and a sample preparation techniques were carried out as follows.

A facile optosensing was synthesized based on molecularly imprinted polymer composite with quantum dot and used as a selective and sensitive fluorescence probe for detection of amoxicillin (**Paper I**), salbutamol (**Paper II**) and dual detection of cephalexin and ceftriaxone (**Paper III**). This optosensors were successfully applied to determine the residue of veterinary drugs in food samples.

The hybrid monolith sorbent of polypyrrole coated graphene oxide incorporated into a polyvinyl alcohol cryogel was synthesized and used as a solid-phase extraction sorbent for the extraction and preconcentration of trace sulfonamides (**Paper IV**). The last work is an application of bubble-in-single drop microextraction for the extraction and analysis of eight carbamate pesticides from the environmental water samples (**Paper V**).

## 2. Optosensor

Optosensor or optical sensor is a group of chemical sensor in which electromagnetic radiation is used to generate the analytical signal in a transducer. The interaction of an analytes with a specific receptor cause the changing of radiation properties which is related to the concentration of the analytes. (Hulanicki *et al.*, 1991; Janata, 2009). Optosensor can be based on various optical principles such as absorbance, reflectance, luminescence and fluorescence. It allow the measurement not only of the intensity of light, but also other related properties such as lifetime, refractive index, diffraction, scattering and polarization (Jerónimo *et al*., 2007). The common transducers used in optosensor are UV-visible spectrophotometer (Felton *et al.*, 2018), and fluorescence spectrometer (Bunkoed and Kanatharana, 2015; Luo *et al.*, 2017).

Among various transducer, fluorescence sensor is an attractive transducer due to it inexpensive equipment, high sensitivity, short analysis time and non-destructive sample (Lakowicz, 2006; Lobnik *et al.*, 2012). The fluorescence sensor directly measures the spectral property changing (i.e. intensity of excitation spectrum, emission spectrum, or lifetime) of the fluorescence probe. In analytical application generally measure the changing in enhancement or quenching of the fluorescence intensity (Lakowicz, 2006) (Figure I).

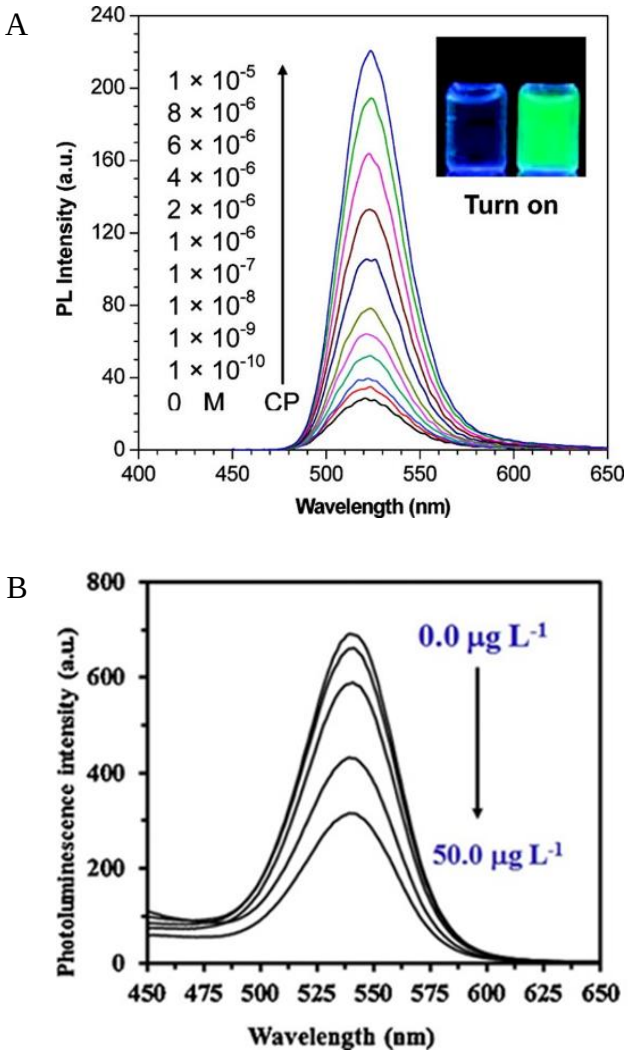



**Figure I** Spectra observation of fluorescence change. (A) The fluorescence enhancement for chlorpyrifos determination (reprinted from Zhang and coworker, 2010; copyright with permission from ACS Publication) (Zhang *et al.*, 2010) and (B) fluorescence quenching for amoxicillin determination **(Paper I)** (reprinted from Chullasat and coworker, 2017; copyright with permission from Elsevier) (Chullasat *et al.*, 2018).

Various sensors based on changing of fluorescence intensity have been reported for veterinary drugs and pesticides detection such as chlorpyrifos (Zhang *et al.*, 2010), kanamycin (Zhu *et al.*, 2018), ciprofloxacin (Yuphintharakun *et al.*, 2018), sulfamethazine (He *et al.*, 2018), chloramphenicol (Wang *et al.*, 2014),

organophosphorus pesticides (Luo *et al.*, 2018), thiacloprid (Liu *et al.*, 2018) and carbamate pesticides (Wei *et al.*, 2018).

## 3. Sample preparation method for the determination of veterinary drugs and pesticides

Sample preparation is a key step which used prior to instrumental analysis. Normally, the residues of veterinary drugs and pesticides in environment and food sample are lower than the detection limit of instrument. Herein, the sample preparation procedure is required for clean-up, elimination of the matrix interference in sample, analyte-preconcentration and sensitivity enhancement (Barchanska *et al.*, 2018; Hashemi *et al.*, 2018; Peixoto *et al.*, 2016).

Trend in sample preparation for determination of veterinary drugs and pesticides have evolved toward to reduce or eliminate of organic solvent, use a small sample size, generally strategy extract for multi-compound and able to apply for high-throughput determination (Dmitrienko *et al.*, 2014; Marazuela and Bogialli, 2009). Among various sample preparation method, solid phase extraction (SPE) and bubble-in-single drop microextraction (BI-SDME) are interesting method due to its use less amount of organic solvent, easy to use and can apply for high-throughput determination (Farajzadeh *et al.*, 2014; Samsidar *et al.*, 2018; Xu *et al.*, 2007).

Solid phase extraction (SPE) is the most frequently used sample preparation technique for the extraction of various analytes in environment and food (Ha *et al.*, 2016; Kılıç *et al.*, 2018). The principle of SPE is based on the partition of analytes between the liquid phase (sample solution) and the solid phase (sorbent). The analyte enrichment was obtained from the adsorption of analyte on the solid sorbent, in this step the interference can be eliminated by un-retaining on a solid sorbent (Figure II) (Hashemi *et al.*, 2018; Moldoveanu and David, 2015). Afterwards, the small amount of suitable organic solvent was used to elute the analyte and following analysis with instrument.

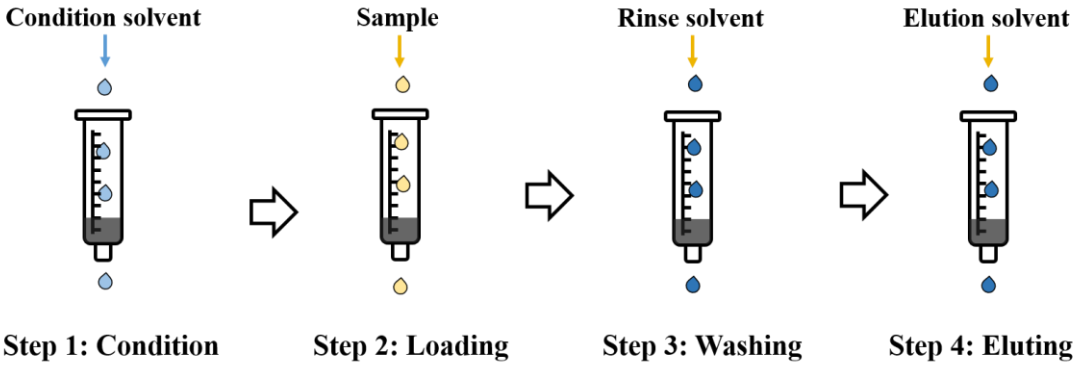



**Figure II** Solid phase extraction procedure.

For SPE technique, the appropriate of sorbent is significant factor to improve the selectivity, capacity and extraction efficiency. The sorbent not only interact specifically with the target analyte but also eliminate some interferences from the sample (Hashemi *et al.*, 2018). The commercial sorbent normally is a silica bonded with various functional group which packed in a cartridge. However, the commercial sorbent have the main drawback about the cartridge clogging when used high throughput of sample (Gong *et al.*, 2015; Vergara-Barberán *et al.*, 2016). The commercial cartridges are also expensive and cannot be reused (Bunkoed *et al.*, 2016). To overcome these problem, the high porosity materials can be applied as solid sorbent such as cryogel (Figure III) (Kueseng *et al.*, 2010; Noosang *et al.*, 2015), cellulose acetate filter (Bunkoed *et al.*, 2016).

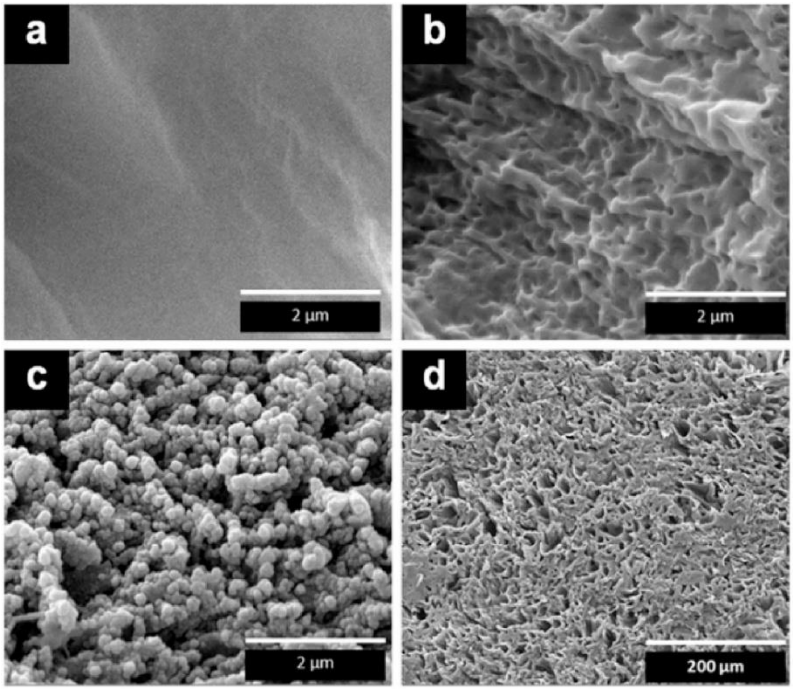



**Figure III** SEM image of high porosity (cryogel) apply as a solid sorbent for sulfonamides determination (**Paper IV**), the PVA cryogel (a), composite GO/PVA cryogel (b), hybrid monolith PPY/GO/PVA cryogel (c) and cross section of hybrid monolith PPY/GO/PVA cryogel sorbent (d). (Reprinted form Chullasat and coworkers, 2017; copyright with permission form Elsevier) (Chullasat *et al.*, 2017).

Bubble in single drop microextraction (BI-SDME) is the developed technique from single drop microextraction (SDME). SDME is a simple microextraction technique which does not require any special device or instrument. It consist of hanging drop of solvent, mostly less than 5 µL, over the headspace in equilibrium with the sample which call headspace single drop microextraction (HS-SDME) or in total immersion in the analytes containing sample solution which call direct immersion single drop microextraction (DI-SDME) (Figure IV) (Kokosa, 2015; Nerín, 2016; Xu *et al.*, 2007). The extraction procedure is performed by the partition coefficient between two phases, the liquid phase of microdroplet (extraction solvent) and the gas phase over sample solution for HS-SDME or the liquid phase of a sample solution for DI-SDME. The advantages of this technique are easy to handle and the derivatization reaction can

be carried out in the same time with the extraction step (Jeannot *et al.*, 2010; Pakade and Tewary, 2010).

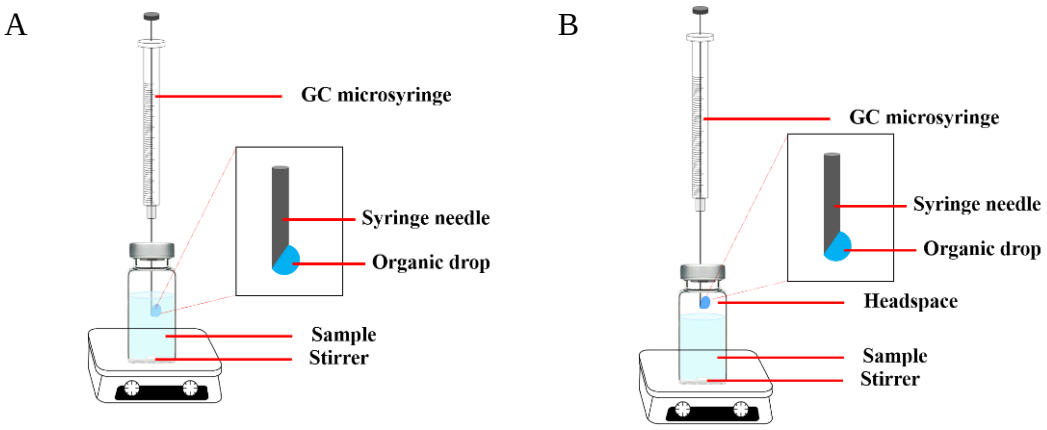



**Figure IV** Direct immersion single-drop microextraction (A) and head single-drop microextraction (B).

Bubble-in-single drop microextraction can be performed by adding the bubble in the hanging microdroplet of extraction solvent. The certain volume of air bubble introduce into the microdroplet resulting a bubble in the microdroplet which can improved the extraction efficiency due to its increase the surface area of microdroplet and occur the thin film phenomena (Figure V) (Guo *et al.*, 2016; Williams *et al.*, 2011; Xie *et al.*, 2014).

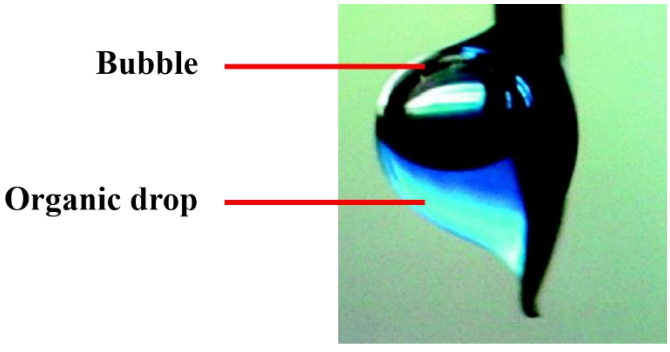



**Figure V** The photograph showing the "bubble in single drop" microextraction (BI-SDME) (reprinted from William and co-worker, 2011; copyright with permission from ACS Publications) (Williams *et al.*, 2011).

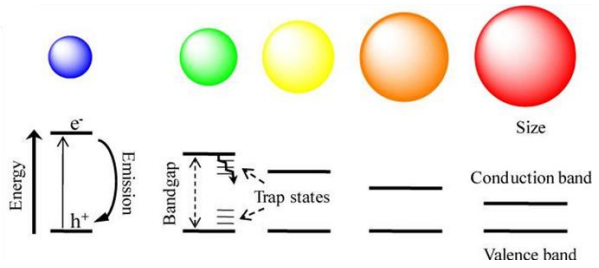
The BI-SDME technique has been developed and used for the extraction of atrazine and metolachlor (Williams *et al.*, 2014; Williams *et al.*, 2011), nitro musk (Guo *et al.*, 2016), hexestrol and diethylstilbestrol (George, 2016) and some growth hormones (George *et al.*, 2015).

## 4. Quantum dots nanoparticles

Quantum dots (QDs), colloidal semiconductor nanocrystals, are one kind of fluorescence materials with physical dimensions smaller than the Bohr exciton radius which is the distance between electron and hole pairs (Frigerio *et al.*, 2012; Vasudevan *et al.*, 2015). As a nanometer size of QDs, it provids an advantage as a high surface-to-volume ratio. Moreover, there are unique optical properties *i.e.* size-dependent, long-term photostability and photoluminescence (Reiss *et al.*, 2009; Trindade *et al.*, 2001).

QDs are small size in the range of 1 to 10 nm (10 – 50 atom), cause the energy levels are separate to the valence and conductance band. The band gap between the valence band (the lower energy level) and conductance band (the higher energy level) depends on size of quantum dots (Bera *et al.*, 2010; Drbohlavova *et al.*, 2009). A small size provides a large band gap than a larger size (Figure VIA). The larger band gap, a larger separation of the energy level of valence and conductance band, result in the shorter emission wavelengths (blue shift) while a smaller band gap results in the longer emission wavelength (red shift) (Figure VIB) (Costa-Fernández *et al.*, 2006; Frasco and Chaniotakis, 2009).

A




B

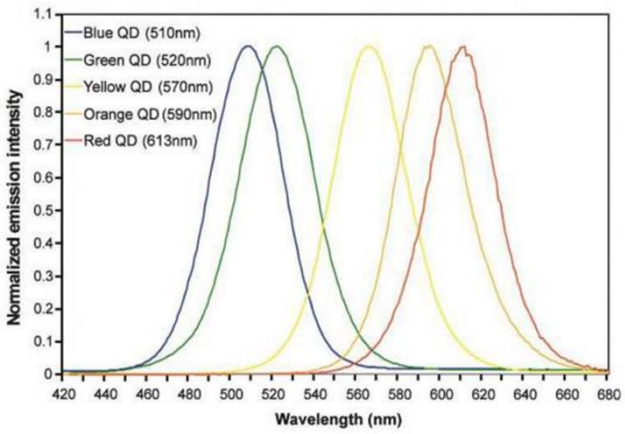



**Figure VI** The energy band gap of quantum dots (A) and size-dependent of emission spectra (B) (reprinted from Frasco and coworker, 2009 copyright with permission from MDPI Publisher) (Frasco and Chaniotakis, 2009).

Various types of QDs have been synthesized which normally consist of a couple of group II-VI, III-V or IV-VI such as CdTe (Bunkoed and Kanatharana, 2015; Wan *et al.*, 2018; Yuphintharakun *et al.*, 2018), CdSe (Boonmee *et al.*, 2016; Eftekhari-Sis *et al.*, 2018; Tang *et al.*, 2014), ZnS (Koneswaran and Narayanaswamy, 2009; Mandal *et al.*, 2012), InP (Micic *et al.*, 1997; Micic *et al.*, 1994) and PbSe (Maikov *et al.*, 2010). For the synthesis of QDs, the colloidal chemistry method is a good strategy to prepare QDs which provided high luminescence efficiency, narrow and size distribution (Frasco and Chaniotakis, 2009; Frigerio *et al.*, 2012). Apart from controlling QDs size, the colloidal chemistry method able to modify their surface with ligand which increase the solubility of QDs in the solution and prevent the agglomeration (Bera *et al.*, 2010; Vasudevan *et al.*, 2015). Several ligands have been modified on the surface of QDs such as thioglycolic acid, TGA (Nurerk *et al.*, 2016; Wang *et al.*, 2018), mercaptopropionic acid (Bunkoed and Kanatharana, 2015; Zhang

*et al.*, 2017), cysteamine (Boonmee *et al.*, 2016; Ding *et al.*, 2015), glutathione (Chang *et al.*, 2017; Peng *et al.*, 2018) and Mercaptosuccinic acid (Cai *et al.*, 2014). However, short-chain thiol with another functional group such as amino, carboxylic acid, or hydroxilic provides bright color of QDs with a flexible surface chemistry, provide a stable for years (Frigerio *et al.*, 2012).

QDs have been applied as photoluminescence probe for determination various analytes such as ions, molecules, proteins and cells (Bunkoed and Kanatharana, 2015; Montoro Bustos *et al.*, 2015; Nurerk *et al.*, 2016; Rocha *et al.*, 2016)

## 5. Graphene oxide and graphene quantum dots

Graphene oxide (GO) is a two-dimension (2D) single-atom of carbon sheet ($sp^2$ hybridized) with O functional group (hydroxyl, carboxylic, and epoxide) (Figure VII) (Perrozzi *et al.*, 2015) which have been fascinated because its high surface-to-volume ratio, high flexibility and outstanding thermal/chemical stability (Chen *et al.*, 2012; Zhang *et al.*, 2012). GO is a hydrophilic and can interact with the carboxylic, hydroxyl functional compounds via hydrogen bonding (Pan *et al.*, 2016). Furthermore, GO has strongly interacted with hydrophobic and carbon-based ring substance via π-π interaction. (Chen *et al.*, 2012; Liu *et al.*, 2012). GO was used as an adsorbent for extraction of various compounds. For example, the 3D urea-based porous organic polymer hybrid with graphene oxide (Urea-POP/GO) was used as an adsorbent for serum albumin extraction (Miri *et al.*, 2018) and composite of graphene oxide and gold nanoparticle was used as an adsorbent for mycotoxin determination (Jiang *et al.*, 2018).

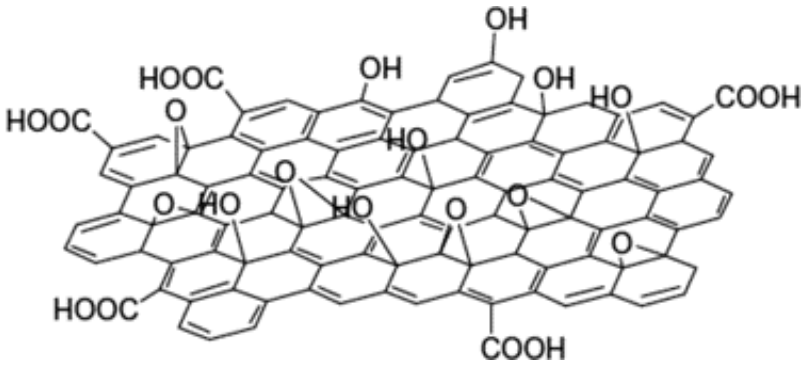



**Figure VII** GO structure

Graphene quantum dots (GQDs) are a single or few layers of graphene with a tiny size in a zero-dimension (0D) which associated with quantum confinement and edge effect (Zhang *et al.*, 2012). There are biocompatibility, intensively luminescence and well disperse in various solvents (Mehrzad-Samarin *et al.*, 2017; Zhou *et al.*, 2015). GQDs structure as shown in figure VIII, exhibited the high surface area with the carboxylic and hydroxyl moieties, thus it can be dispersed in water and interacted with several compounds (Zor *et al.*, 2015).

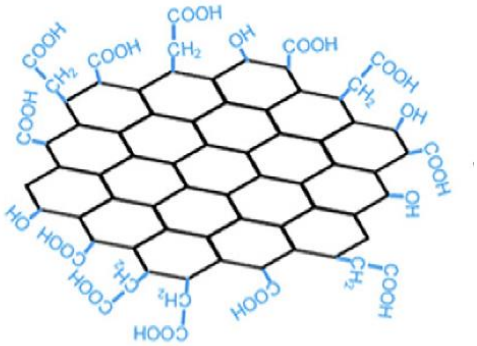


**Figure VIII** The graphene quantum dots (GQDs) structure (reprinted from Dong and coworker, 2012; copyright with permission from Elsevier) (Dong *et al.*, 2012b).

GQDs show the adsorption peak around 230 nm because of the $\pi \rightarrow \pi^*$ aromatic transition $sp^2$ domains and the wavelength between 270 and 390 nm which related with the $n \rightarrow \pi^*$ transition of C = O (Pan *et al.*, 2010; Zhu *et al.*, 2012). After the adsorption of GQDs though the graphene $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions lead to the strong photoluminescence stability of GQDs (Zhang *et al.*, 2012).

Several fluorescence sensors based on graphene quantum dots have been reported for some substances such as metronidazole (Mehrzad-Samarin *et al.*, 2017), tributyltin (Zor *et al.*, 2015), dopamine (Zhou *et al.*, 2015) and chlorine (Dong *et al.*, 2012a).

## 6. Molecularly imprinted polymers (MIPs)

The most important part for the sensor which greatly considered for determination of target analyte is recognition part. Molecularly imprinted polymers (MIPs) are attractive materials as a recognition site for specific analyte due to its easy preparation process, good stability and high selectivity (Niu *et al.*, 2016; Turiel and Martín-Esteban, 2010). MIPs are the affinity-synthesized polymer with created recognition sites able to specifically rebind to a target analytes. The preparation procedure of MIPs is showed in Figure IX, the copolymerization of the functional monomer and cross-linker in the presence of template molecules. After polymerization, the template molecule was removed and a binding site which specific with shape, size and functionalities complementary to a target analyte were obtained (Turiel and Martín-Esteban, 2010).

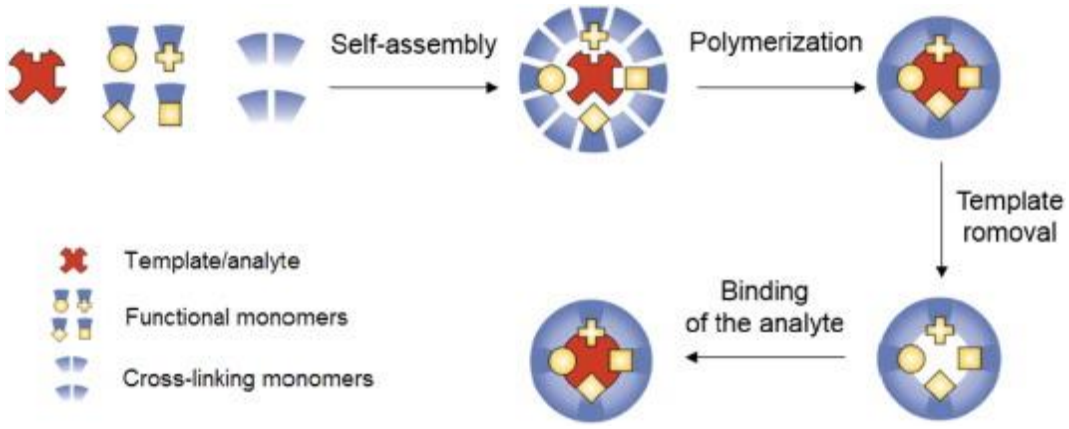



**Figure IX** The preparation of molecularly imprinted polymers (reprinted from Iskierko and coworker, 2017; copyright with permission from Elsevier) (Iskierko *et al.*, 2016).

MIPs based on fluorescence sensors have been reported for determination of veterinary drugs and pesticides. For instance, MIP composited with QDs was used

as fluorescence probe for determination of amoxicillin (Paper I) (Chullasat *et al.*, 2018) and salbutamol (Paper II) (Raksawong *et al.*, 2017), thiacloprid (Liu *et al.*, 2018), dimethyl methyl phosphonate (Zhang *et al.*, 2016) and tributyltin (Zor *et al.*, 2015). The characteristic of fluorescence changed (Figure X) when the recognition site in MIPs construction interact with the target analyte including either fluorescence brightness (enhancement or quenching), wavelength characterizing (blue or red shift), and lifetime via signal transduction mechanism (Yang *et al.*, 2018).

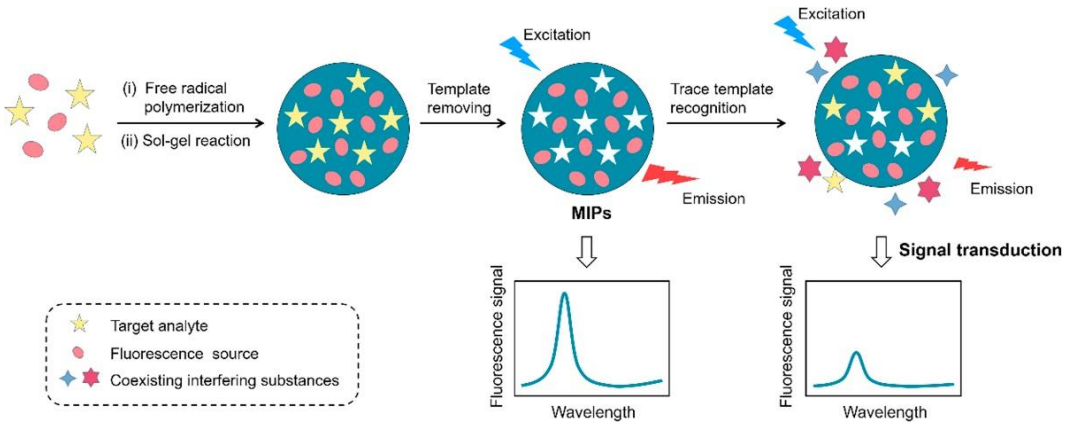



**Figure X** The optosensor based on fluorescence quenching of composite QDs and MIP probe (reprinted from Yang and coworker, 2018; copyright with permission from Elsevier) (Yang *et al.*, 2018)

## 7. Cryogel

Cryogels are an attractive supporting material due to its an extremely high porosity sponge-like material and high flexibility (Gun'ko *et al.*, 2013; Kueseng *et al.*, 2010). It can be made in any desired shape *i.e.* block, disk, tube and rod (Lozinsky *et al.*, 2003; Noosang *et al.*, 2015). Cryogels are fabricated in moderately frozen solutions of gel precursors (Hixon *et al.*, 2017; Lozinsky *et al.*, 2003). In the process (Figure XI), the mixture of polymer (precursors) solution, which was contained in the desired container and added gel-forming (cross-linker) agent, is frozen at temperature leading to gelation occurs during this period (between -5 and -20 °C). The solvent crystals act as a porogen while the solution remains in liquid micro-phase surrounding them. After frozen with sufficient time, the cryogel was formed and thawed at room temperature.

The obtained macroporous structure of cryogel results in a network in sponge-like and mechanically stable which can be applied as supporting materials (Hixon *et al.*, 2017; Lozinsky *et al.*, 2003).

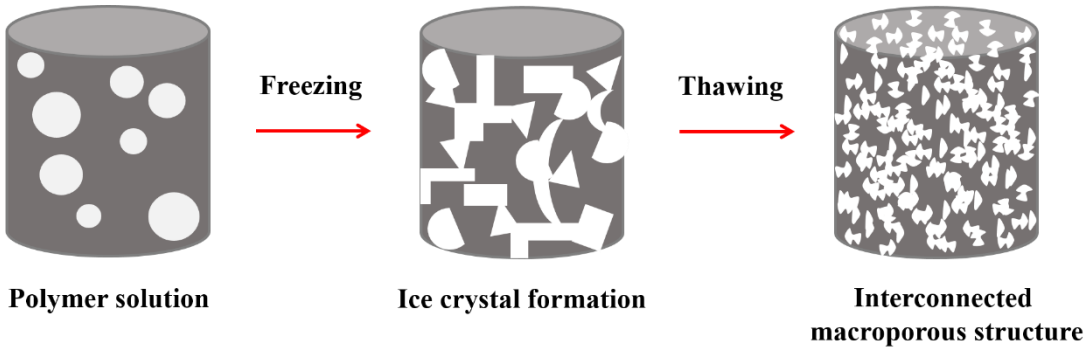



**Figure XI** Cryogel preparation stage resulting a formation of a macroporous sponge-like structure.

Cryogel material has been applied in various field such as bioreactors (Jain *et al.*, 2011), cell preparation (Aliperta *et al.*, 2017), tissue engineered scaffolds (Fassina *et al.*, 2010), and separation matrices (Kueseng *et al.*, 2010; Noosang *et al.*, 2015).

## 8. Polypyrrole

Polypyrrole (PPY) is a conducting polymer with poly-conjugation in the π-system of their backbone (Figure XII), have been attractive material due to its good stability and easy to synthesis (Sukchuay *et al.*, 2015; Wang *et al.*, 2001). PPY can be synthesized chemically or electrochemically through oxidative polymerization of pyrrole monomer. The chemical synthesis of PPY performed through the oxidation of pyrrole monomer with an oxidant such as ferric chloride ($FeCl_3$), ammonium persulfate ($(NH_4)_2S_2O_8$) or hydrogen peroxide ($H_2O_2$) (Tat'yana and Oleg, 1997).

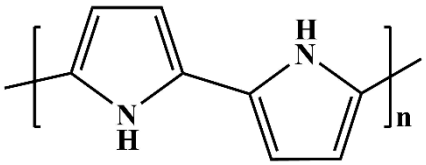



**Figure XII** Structure of polypyrrole

Since, PPY structure has π- conjugated backbone and amine functional group which can adsorb benzenoid compounds via hydrogen bonding, hydrophobic and π-π interaction (Sukchuay *et al.*, 2015; Zhao *et al.*, 2016), PPY is attractive materials for sorbent preparation to improve the extraction efficiency. Several works used and applied PPY as enhancing materials for extraction and determination of veterinary drugs and pesticides such as sulfonamide (**Paper IV**) (Chullasat *et al.*, 2017), endocrine-disrupting compounds (Bunkoed *et al.*, 2016), phthalic acid esters (Zhao *et al.*, 2016), linezolid, daptomycin and moxifloxacin (Szultka *et al.*, 2010)

## 9. Analytical performance

The method performance of the developed method must be validated. The analytical validation characteristics which should be considered including selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), sensitivity, accuracy and precision (APVMA, 2004; Magnusson and Örnemark, 2014).

### 9.1 Selectivity

Selectivity of the method refers to the point which can estimate particular analyte(s) in a complex sample without interferences from other components in the sample (APVMA, 2004). The selectivity of the analytical method can be tested with a structurally similar to target analyte which should be negative or less response (Taverniers *et al.*, 2004). In this thesis, the selectivity of the developed fluorescence sensor based on molecularly imprinted polymer composite with quantum dots (MIP-QDs) (**Paper I-III**) was investigated by comparing the intensity of MIP-QDs after interacting with the target analytes and other compounds. As shown in Figure XIII, the selectivity of hybrid MIP-coated QDs was investigated by comparing the sensitivity (in term of $K_{sv}$) of hybrid MIP-coated QDs after interacted with salbutamol and other

antibiotics. The results indicated that this fluorescence probe is high selectivity to salbutamol (Raksawong *et al.*, 2017).

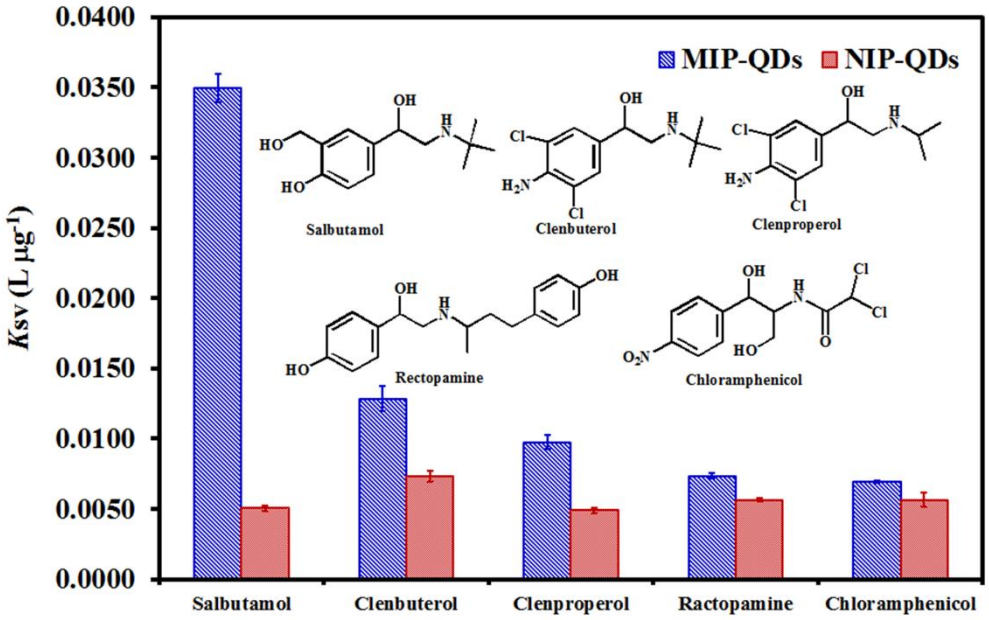



**Figure XIII** The selectivity of hybrid MIP-coated QDs for salbutamol determination (reprinted from Raksawong and coworker, 2017; copyright with permission from Springer) (Raksawong *et al.*, 2017)

### 9.2 Linearity

Linearity is the ability of the analytical method to encourage response which is directly proportional to the concentration of the target analytes in samples within the certain range (Magnusson and Örnemark, 2014). In general, the linearity is assessed by determining 3 to 6 replications of at least 4 different concentration of analytes. The linearity was plotted between the response (y-axis) and the concentration of the analyte (x-axis) and a linear regression was obtained with a coefficient of determination ($R^2$) more than 0.99 (APVMA, 2004). In this thesis, the linearity was plotted between the fluorescence intensity (F0/F) and concentration of amoxicillin (in µg $L^{-1}$ range) (**Paper I**), salbutamol (in µg $L^{-1}$ range) (**Paper II**), ceftriaxone (in µg $L^{-1}$ range) and cephalexin (in µg $L^{-1}$ range) (**Paper III**) for optosensor and the peak area and the concentration of sulfonamide (in µg $L^{-1}$ range) (**Paper IV**) and carbamate

pesticides (in µg $L^{-1}$ range) (**Paper V**), all sub-projects provided a coefficient of determination higher than 0.99.

### 9.3 Limit of detection and limit of quantification

Limit of detection (LOD) is defined as the lowest concentration or quantity of the target analyte which can be reliable detection using a given analytical method while limit of quantification is defined as the lowest concentration or quantity of the target analyte in the sample which can be measured with a reasonable statistical certainty (Magnusson and Örnemark, 2014). There are several methods for estimating LOD and LOQ, depended on the analytical technique. In this thesis, two methods were used to estimate the LOD and LOQ.

For optosensor based on the fluorescence quenching of molecularly imprinted polymer composite with quantum dots for the analysis of amoxicillin (Paper I), salbutamol (Paper II), ceftriaxone and cephalexin (Paper III), LOD and LOQ were evaluated based on the International Conference of Harmonization (ICH) as following equation: (ICH, 2005; Taverniers *et al.*, 2004).

$$LOD = 3S_{bl} / m$$

$$LOQ = 10S_{bl} / m$$

Where $S_{bl}$ is the standard deviation on the measurements of blank (n = 20) and m is the sensitivity of the method or the slope of the linearity.

For the development of sample preparation technique for determination of sulfonamides (Paper IV) and carbamate pesticides (Paper V) using the chromatography technique, LOD and LOQ were estimated using the signal-to-noise ratio (S/N) or the peak-to-peak noise around the retention of analyte (Figure XIV) as a recommendation of EURACHEM guideline (Magnusson and Örnemark, 2014).

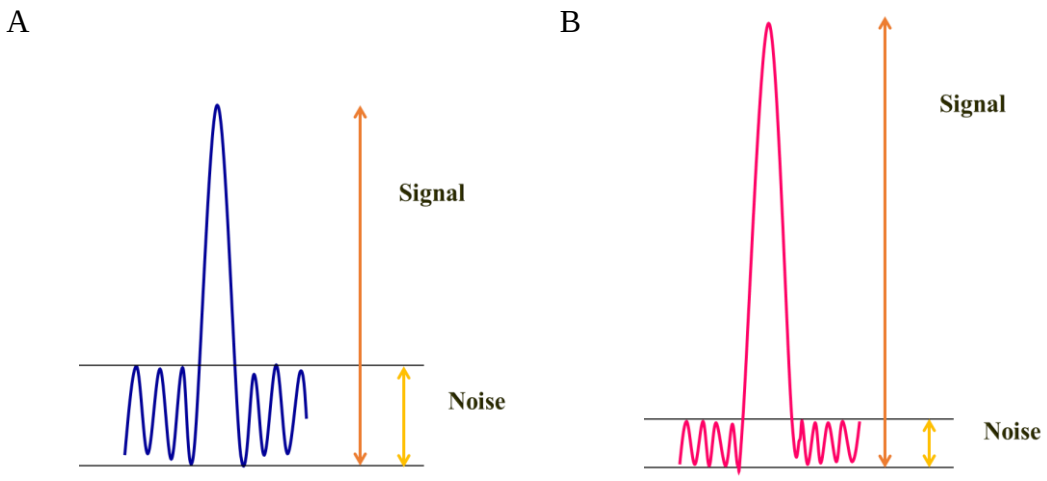



**Figure XIV** The measurement of the limit of detection (A) and limit of quantification (B) based on signal-to-noise (S/N)

### 9.4 Sensitivity

The sensitivity of a method is referred to the changing of the response of an instrumental measurement which divided by the corresponding change in the stimulus (Taverniers *et al.*, 2004). In this thesis, the sensitivity of the developed method was evaluated from the slope of the linearity.

### 9.5 Accuracy

The accuracy of the analytical method is defined as the degree to which the determined value of the analyte in a sample relates to the true value (APVMA, 2004). The accuracy can be measured in different ways and the method should be suitable to the matrix. Recovery was evaluated to express the accuracy of the developed method. The recovery (%) was carried out by comparing the obtained response form the spiking sample and the standard solution at three concentration (at least three replicated) with follow equation (Magnusson and Örnemark, 2014).

$$\text{Recovery (\%)} = \frac{x' - x}{x_{spiked}} \times 100$$

Where x' is the mean value of the concentration or amount of the spiked sample, x is the concentration or amount of analyte in the sample and $x_{spiked}$ is the added concentration or amount of the target analyte(s).

The acceptable recovery is depending on the concentration which added to the sample, the purpose of the analysis and types of sample. In this thesis, the concentration range of the detected analytes was in μg $L^{-1}$ level which the acceptable range is 70 – 125% (Magnusson and Örnemark, 2014). The achieved recoveries of the spiked sample were in the range of 82 to 102 % which were acceptable (**Paper I – V**).

### 9.6 Precision

The precision is defined as the degree of agreement of replicate measurements under the optimum condition. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation of the measurement. The acceptable recommendation for precision at μg $L^{-1}$ level is the relative standard deviation less than 21 % (Magnusson and Örnemark, 2014). In this thesis, the determination of amoxicillin, salbutamol, ceftriaxone and cephalexin was performed five replication measurement using the synthesized fluorescence probe (MIP composite with QDs). Under the optimum conditions, the relative standard deviations were obtained less than 10 % (**Paper I-III**). The determination of carbamate pesticides (**Paper V**) using bubble-in-single drop microextraction, the precision was reported as the intra-day and inter-day precision. The intra-day precision of the developed method was investigated by analyzing spiked water sample (n = 6) at 10 μg $L^{-1}$ of carbamate pesticides within the same working day, the obtained relative standard deviations less than 7. The inter-day precision was carried out by analyzing spiked water sample (n = 6) at 10 μg $L^{-1}$ of carbamate pesticides, the obtained relative standard deviation of less than 8. The precision was acceptable with RSD lower than 15 for the spiked concentration of 10 μg $L^{-1}$.

### 9.7 Reproducibility

Reproducibility refers to the point of agreement of the results when operative conditions are different times. Reproducibility usually refers to the standard deviation (SD) or the relative standard deviation (RSD) of the same samples at different times. In this thesis, the reproducibility of the developed method was evaluated by preparing six different batches of each fluorescence probe or sorbent under the optimum conditions. The acceptable recommendation from the Association of Official Agricultural Chemists (AOAC) of reproducibility in the μg $L^{-1}$ level is the RSD less

than 16 % (Magnusson and Örnemark, 2014). For this thesis, the obtained RSD for the synthesis of fluorescence probes were less than 6% (**Paper I –III**), and the hybrid monolith PPY/GO/PVA cryogel sorbent was less than 10% (**Paper IV**).

## 10. Concluding remark

This thesis successfully developed analytical method including optosensor and sample preparation techniques. Optosensor is based on the fluorescence quenching of molecularly imprinted polymer embedded within quantum dots and sample preparation techniques are solid phase extraction and bubble-in-single drop microextraction for the analysis using chromatography technique. These methods can improve the analytical performance for the determination of trace veterinary drugs and pesticides.

In the first part, the optosensor using nanocomposite fluorescence probes which combined the excellent optical property of quantum dots nanoparticles and high selectivity of the molecularly imprinted polymer. Under optimum conditions, the developed fluorescence probes (MIPs-QDs) were applied for the determination of the veterinary drugs. It exhibited linear fluorescence quenching for amoxicillin in the concentration range of 0.20 – 50.0 µg $L^{-1}$ with a low detection limit of 0.14 µg $L^{-1}$ (**Paper I**) and 0.10 – 25.0 µg $L^{-1}$ with a low detection limit of 0.03 µg $L^{-1}$ for salbutamol (**Paper II**). The dual nanocomposite fluorescence probe showed a good linearity from 0.10 to 50.0 µg $L^{-1}$ with a low detection limit of 0.06 and 1.00 µg $L^{-1}$ for cephalexin and ceftriaxone, respectively (**Paper III**). The results of these developed optosensors were compared with a conventional method (HPLC) for veterinary drugs determination, it showed agree well with good correlation between two methods. The advantages of these methods are simple, rapid, cost-effective, high sensitivity, selective and reliable fluorescence sensing protocol. The developed methods were successfully applied to the determination of the veterinary drugs in complex sample *i.e.* milk, egg, honey, meat and animal feed samples. It showed a facile and versatile sensing preparation which can be used as an alternative procedure for the sensitive and selective recognition method of the target analyte.

For the second part, two sub-project of sample preparation techniques were developed for the determination of veterinary drugs and pesticides. The first one is a

hybrid monoliths of polypyrrole-coated graphene oxide incorporated into a PVA cryogel which was successfully prepared and applied for the extraction and enrichment of six sulfonamides from the environmental water sample (**Paper IV**). The combination of polypyrrole and graphene oxide can help to improve the extraction efficiency of trace sulfonamide due to their high specific surface area and the greater adsorption by the sites for the target analytes and the high porosity of PVA cryogel can help to prevent high backpressure that normally occurs with conventional packed SPE cartridges. The results showed that the developed method offering a high extraction efficiency, good sensitivity, accuracy and precision. The other one is a development and application of a bubble-in-single drop microextraction (BI-SDME) for determination of trace amount of eight carbamate pesticides in the environmental water sample (**Paper V**). This developed method used less amount of extraction solvent (1.0 µg $L^{-1}$) which demonstrated that it is environmentally friendly method. It showed a good linearity in the range of 0.05 – 20.0 µg $L^{-1}$ with high sensitivity, acceptable precision and accuracy. Furthermore, this developed method is easy to handle using simple and low-cost equipment and short analysis time.

The results indicated that these developed methods can be applied for the determination of trace veterinary drugs and pesticides in real sample such as milk, egg, honey, meat, animal feed and environmental water samples with a good analytical performances. In addition, these developed method can be used as an alternative method for determination of some other organic compounds in a various sample.

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# Appendices

# Paper I

**Chullasat, K.**, Nurerk, P., Kanatharana, P., Davis, F., Bunkoed, O., A facile optosensing protocol based on molecularly imprinted polymer coated on CdTe quantum dots for highly sensitive and selective amoxicillin detection. *Sensors and Actuators B: Chemical* 254 (2018). 255-263.

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# A facile optosensing protocol based on molecularly imprinted polymer coated on CdTe quantum dots for highly sensitive and selective amoxicillin detection

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ABSTRACT

A facile method for coating a molecularly imprinted polymer onto CdTe quantum dots (MIP-QDs) was successfully formulated and for the first time used as a highly selective and sensitive photoluminescence probe for the determination of trace amoxicillin. The MIP-QDs were prepared using a sol-gel process with 3-aminopropylethoxysilane as a functional monomer, tetraethoxysilane as a cross-linker and amoxicillin as a template molecule. After removal of the template molecule from the polymer layer, MIP-QDs containing cavities specific to amoxicillin were obtained. The hydrogen bonding between the amino group of 3-aminopropylethoxysilane and functional groups of amoxicillin and the specific size and shape of the cavity provided good selectivity. The photoluminescence intensity of MIP-QDs was more strongly quenched by amoxicillin compared to a non-imprinted polymer (NIP-QDs) with an imprinting factor of 43.6. Under optimum conditions, the photoluminescence intensity of MIP-QDs was decreased in response to increase amoxicillin concentration with good linearity in the range of 0.20–50.0 $\mu g\,L^{-1}$. The limit of detection and the limit of quantitation were 0.14 $\mu g\,L^{-1}$ and 0.46 $\mu g\,L^{-1}$, respectively. The developed method showed good repeatability and reproducibility with the relative standard deviation being less than 6%. This developed method was successfully applied for the determination of amoxicillin in egg, milk and honey samples with a satisfactory recovery of 85–102% being achieved.



## 1. Introduction

Amoxicillin is a synthetic antibiotic in the β-lactam class of antibiotics and is extensively used to treat infectious diseases in human and animal, being active against both Gram-positive and Gram-negative bacteria [1–3] due to its broad spectrum antibiotic activity and low cost [4]. However, the usage of antibiotics in food-producing animals can lead to the presence of residues in food and the environment [4] which can cause some side-effects such as hypersensitivity in humans [5]. To assure consumer safety, the European Union (EU) has set a maximum residue limit (MRL) of amoxicillin of 50 $\mu g\,kg^{-1}$ in animal tissues and 4.0 $\mu g\,kg^{-1}$ in milk [6]. Therefore, the monitoring of amoxicillin in food products is an important application. Various analytical methods have been developed for the determination of amoxicillin such as chromatography [4,7,8], electrochemical methods [2,3,9], surface plasmon resonance [10] and spectrophotometry [1,11–13]. Among these methods, fluorescence spectroscopy is an interesting alternative method because it has a short analysis time, is relatively simple to use, uses low cost equipment and requires small sample amounts and minimal consumption of organic solvents [12,14]. Several organic dyes have been used as photoluminescence probes to detect various target analytes [15]; however they often have drawbacks such as broad emission bands and mostly asymmetric spectra [16]. To overcome this problems, quantum dots (QDs) have attracted much attention in recent years for use as photoluminescence probes for the determination of ions, molecules, proteins and cells [17–20] due to their desirable optical properties such as size-dependent emission, narrow symmetric emission

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bands, long photoluminescence lifetime, photochemical stability and good water dispersibility [21,22]. To improve the selectively of this method the surface of QDs need to be modified with target-specific materials [18]. Molecularly imprinted polymers (MIPs) are an attractive strategy to modify the surface of quantum dots. MIPs are affinity polymers and can be synthesized to be specific to a target molecule, they are easy to prepare, inexpensive and display good stability [23]. These materials are highly cross-linked three-dimensional network polymers, formed by polymerization between a functional monomer and a cross-linking monomer whilst including template molecules (target analytes) [24,25]. After the polymerizations were complete, the template molecules were removed and specific cavities were obtained [26]. MIPs combined with QDs have been developed as a photoluminescence probe for a selective determination of some compounds i.e. histamine [27], malachite green [28], chlorpyritos [29] and α-fetoprotein [30].

In this work, molecularly imprinted polymers coated on quantum dots (MIP-QDs) were synthesized and used as a photoluminescence probe for the highly sensitive and selective determination of amoxicillin. The photoluminescence properties and morphology of the synthesized MIP-QDs were investigated and characterized. The effects of various parameters on the analytical performance were also optimized. The developed MIP-QDs were applied for the determination of amoxicillin in egg, milk and honey samples. The developed method was compared with a HPLC method and the recovery from these samples was also investigated.

## 2. Experimental

### 2.1. Chemicals and reagents

All chemicals were of analytical grade, amoxicillin, 3-aminopropyl triethoxysilane (APTES, ≥98%), tetraethyl orthosilicate (TEOS, ≥99%), tellurium powder (99.8%), thioglycolic acid (TGA) and sodium borohydride ($NaBH_4$) were purchased form Sigma-Aldrich (St. Louis, MO, USA). $CdCl_2{\cdot}2H_2O$ was purchased from Asia Pacific Specialty Chemicals Co. Ltd. (NSW, Australia). Tris (hydroxymethyl) aminomethane and ethanol (≥98%) were purchased from Merck (Frankfurt, Germany). Ammonia and sodium hydroxide were purchased from RCI Labscan (Bangkok, Thailand). Ultrapure water was from a water purification system (18.2 MΩ $cm^{-1}$) (Elgastat Maxima, ELGA, UK)

### 2.2. Instrumental

Fluorescence spectroscopy was performed using a Shimadzu RF-5301PC spectrofluorophotometer (Kyoto, Japan). UV/Vis absorption spectra were recorded using an Avaspec 2048 spectrometer (Avantes, Apeldoorn, Netherlands). The morphologies of MIP-QDs and NIP-QDs were obtained using a scanning electron microscope (JSM-5200, JEOL, Tokyo, Japan) and the FTIR spectra were recorded using KBr pellets in a FTIR spectroscopy (PerkinElmer, Waltham, MA, USA). TEM images were obtained from a TECNAI T20 $G^2$ transmission electron microscope (FEI, The Netherlands). BET surface areas of MIP-QDs and NIP-QDs were determined using Quantachrome Autosorb 1 system (Quantachrome Instruments, USA).

### 2.3. Synthesis of TGA- capped CdTe QDs

TGA-capped CdTe quantum dots were synthesized using a method modified from previous work [17,18]. Firstly, 50 mg of tellurium powder and 40 mg of $NaBH_4$ were dissolved in 1.0 mL of deionized water and stirred under a nitrogen atmosphere to prepare a NaHTe solution. Also, 0.05 g of $CdCl_2$ and 25 μL TGA were dissolved in 100 mL of deionized water and adjusted to pH 11.5 with 1.0 M NaOH. Then, this solution mixture was placed into a three-necked flask and deaerated by bubbling with nitrogen gas for 15 min. Under vigorous stirring, 500 μL of NaHTe solution was rapidly injected into the solution mixture under a nitrogen atmosphere. Then the solution was refluxed for 10 min at 90 °C. After being cooled to room temperature, the resulting product was precipitated with ethanol to remove excess reagents and centrifuged at 3500 RCF for 10 min. The obtained TGA-capped CdTe QDs were dried in an oven at 40 °C for 1 h and kept in a desiccator for further use.

### 2.4. Synthesis of molecularly imprinted polymer coated CdTe quantum dots (MIP-QDs)

The MIP-coated QDs were synthesized via a sol-gel copolymerization process. Briefly, 6.6 mg of amoxicillin (template) was dissolved in 5.0 mL of deionized water and mixed with 30 μL APTES (functional monomer) in a brown bottle and stirred at room temperature (25 °C) for 1 h. Then, 15 mL of TGA-capped CdTe QDs ($7.5 \times 10^{-5}$ M), 135 μL of TEOS (cross-linker) and 150 μL of 25% ammonia solution were added and continuously stirred for 6 h. The resultant products were collected by centrifugation at 3500 RCF for 10 min and washed three times with 10 mL of ethanol to remove template molecules, no amoxicillin (template) could be detected in the washing solvent (ethanol) by UV spectroscopy. Finally, the MIP-QDs were dried in an oven at 40 °C for 1 h. The non-imprinted polymer coated QDs (NIP-QDs) were also prepared through the same procedure but without addition of the template molecule.

### 2.5. Photoluminescence measurements

Photoluminescence measurements were performed using a spectra band pass of the excitation and emission of 10 nm, an excitation wavelength of 355 nm and recording the emission in the range of 400–700 nm. MIP or NIP-coated QDs (6.0 μg $L^{-1}$) were dispersed in 10 mM of Tris-HCl buffer solution (pH 8.0). The measurements were obtained by mixing of 150 μL of MIP-QDs or NIP-QDs solution with 50 μL of amoxicillin solution or sample solution. After incubation under gentle rotation for 30 min, the mixture solution was transferred into a quartz cuvette and the photoluminescence intensity was recorded using a fluorescence spectrophotometer. All measurements were performed at room temperature (25 °C) for convenient analysis.

### 2.6. Sample preparation of food samples

All samples were purchased from the local market in Hat Yai, Songkhla, Thailand. Milk samples were pretreated according to the previous report [31]. Briefly, 30 mL of raw milk was transferred to a 50 mL polypropylene centrifuge tube and centrifuged at 1260 RCF for 15 min to precipitate fat. Then 10 mL of acetonitrile was added into the defatted milk to deproteinize it. Subsequently, the mixture was vortexed and centrifuged at 2240 RCF for 15 min. Then, the supernatant was collected and evaporated to dryness at 40 °C. The extract was redissolved with 2.0 mL of phosphate buffer and analysed by the developed method. For honey samples, the sample preparation was modified from previous work [3]. Briefly, 10 mL of honey was transferred to a 50 mL polypropylene centrifuge tube and diluted with 10 mL of distilled water. The mixture was then vortexed for 1 min followed by centrifuging at 2240 RCF for 20 min and the supernatant was evaporated at 60 °C. Then, 2.0 mL of phosphate buffer was added and vigorously vortexed for 10 s before analysis. The preparation of egg sample was modified from previous work [4], whole egg white was homogenized and 5.0 g of homogenized egg was transferred into a 50 mL polypropylene centrifuge tube and 10 mL of acetonitrile was added. The mixture was extracted by ultrasonification for 15 min and then centrifugation at 2240

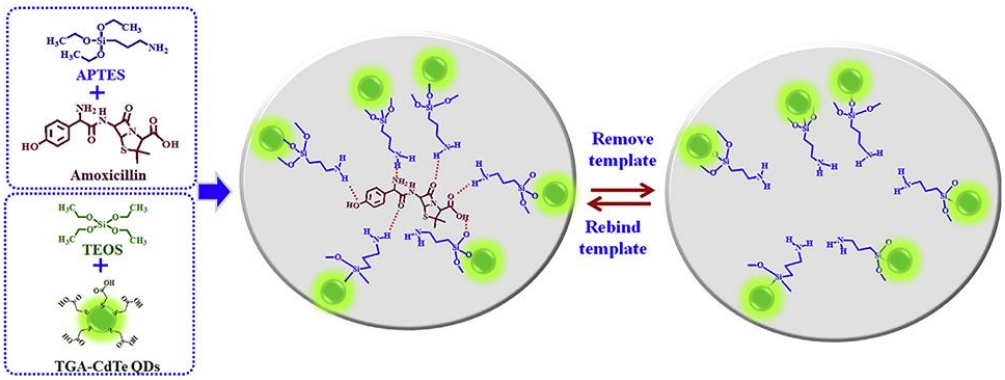


**Fig. 1.** The synthesis process of MIP-QDs for the recognition of amoxicillin.

RCF for 10 min, the supernatant was transferred to another 50 mL polypropylene centrifuge tube. Then 1.0 mL of 4.0 M ammonium acetate buffer (pH 6.74) was added and mixed by vortexing. 20 mL of dichloromethane was added into the mixture and vortexed for 1 min and centrifuged at 2240 RCF for 10 min; the supernatant was collected and evaporated at 40 °C. The extractant was redissolved with 2.0 mL of phosphate buffer and filtered through a 0.22 μm membrane filter before analysis.

## 3. Results and discussion

### 3.1. The synthesis of MIP coated on TGA-capped CdTe quantum dots (MIP-QDs)

The MIP-QDs were synthesized as shown in Fig. 1. TGA-capped CdTe QDs has a carboxylic group which can interact with TEOS and APTES to facilitate formation of a sol-gel MIP coated onto the QDs. The strong non-covalent interaction between APTES and amoxicillin occurred during the molecular imprinting process. The amino group of APTES can interact with functional groups of amoxicillin such as the carboxylic group, amino group and hydroxyl group though hydrogen bonding. The synthesized MIP-QDs and NIP-QDs have a symmetric emission at 545 nm when excited at 355 nm.

The photoluminescence intensity of MIP-coated QDs before the removal of templates molecules was relatively weak (Fig. 2a). However, the photoluminescence intensity of MIP-QDs was restored dramatically after removal of template molecules (Fig. 2b). The photoluminescence intensity was restored to almost the same value as obtained with NIP-QDs (Fig. 2c). These results indicated that the template molecules were almost completely removed from the recognition cavities in the MIP-QDs. In addition, the photoluminescence signal was sharp which indicated that the size of MIP-QDs were homogeneous. Fig. 2d and e showed the photoluminescence photographs of MIP-QDs without and with amoxicillin.

### 3.2. Characterizations of MIP-QDs

The photoluminescence spectrum and absorption spectrum of TGA-capped CdTe QDs are shown in Fig. S1. The TGA-capped CdTe QDs showed a narrow and symmetric photoluminescence spectrum. The maximum absorption wavelength of the TGA-capped CdTe QDs at 500 nm was used to calculate the particle size of QDs as described in previous work [18], the average particle size was approximately 2.5 nm. The TEM images of the TGA-capped CdTe QDs and MIP-coated CdTe QDs are shown in Fig. 3A and B. The TEM

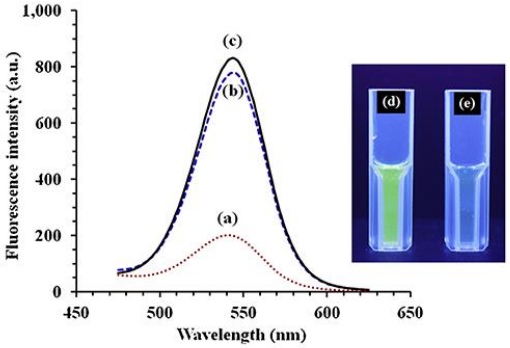


**Fig. 2.** Photoluminescence spectra of MIP-CdTe QDs before removal of template molecules (a) and after removal of template molecules (b), NIP-CdTe QDs (c). The photoluminescence photographs of MIP-CdTe QDs (d) and MIP-CdTe QDs + amoxicillin (e).

image exhibits the uniform size and distribution of TGA-capped CdTe and their particle size agreed with those calculated.

The morphology of MIP-QDs was investigated by SEM. As shown in Fig. 3C, they have a uniform spherical shape and narrow size distribution with diameters in the range of 180–200 nm. It is clear that the particles diameter increased significantly after coating with MIP compared with the original TGA-capped CdTe QDs. This indicated that the MIP-QDs have a large surface area with effective imprinting sites to bind the template molecule.

The FT-IR spectrum of TGA capped CdTe QDs (Fig. 4a) showed characteristic peaks at 1375 and 1572 $cm^{-1}$ which corresponded to the C=O stretching and deformation vibration of carboxylic group. The absorption peaks at 3400 and 1225 $cm^{-1}$ were attributed to the O—H stretching and C—O stretching. The FT-IR spectrum of amoxicillin (Fig. 4b) exhibited an absorption band at 3463 $cm^{-1}$ corresponding to the N—H stretching of primary amine. The absorption band at 1777 $cm^{-1}$ and 1687 $cm^{-1}$ corresponded to the C=O stretching of carbonyl and carboxylic group, respectively. The absorption peak at 1590 $cm^{-1}$ was due to the C—C stretching of the thiazole ring. The FT-IR spectrum of MIP-QDs before removal of the template (amoxicillin) is shown in Fig. 4c. The absorption peak at 1065 $cm^{-1}$ was ascribed to Si—O—Si asymmetric stretching. The Si—O vibrations bands were shown at 460 and 784 $cm^{-1}$. After removing the template the absorption peaks at 1777, 1687 and

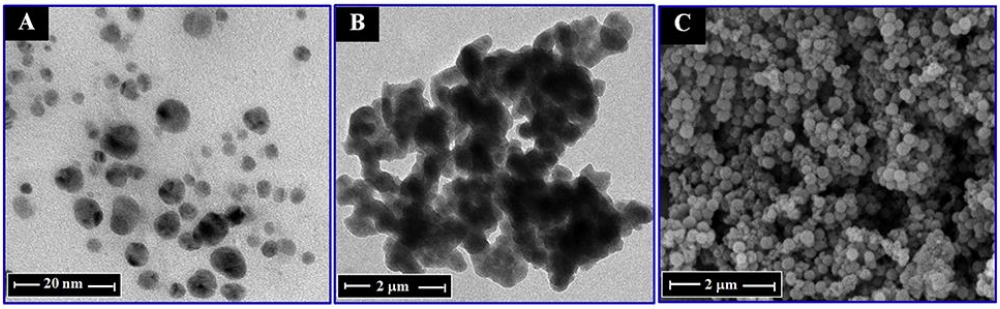


**Fig. 3.** TEM images of TGA-capped CdTe QDs (A), MIP-QDs (B) and SEM image of MIP-QDs (C).

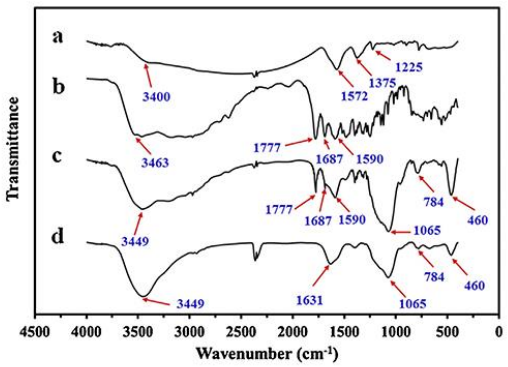


**Fig. 4.** FTIR spectra of (a) TGA capped CdTe QDs, (b) amoxicillin, (c) MIP-QDs before removal of the template and (d) MIP-QDs after removal of the template.

1590 $cm^{-1}$ which related to amoxicillin were absent (Fig. 4d). The results indicated that the molecularly imprinted polymer was successfully synthesized and coated on the CdTe-QDs to form MIP-QDs for selective recognition for amoxicillin.

The photoluminescence quantum yields of the TGA-capped CdTe QDs and MIP-coated QDs were 0.67 and 0.30, respectively. The BET surface area of MIP-QDs and NIP-QDs were $12.00 \pm 0.18\ m^2\ g^{-1}$ and $11.50 \pm 0.15\ m^2\ g^{-1}$, respectively. The MIP-QDs showed higher surface area than NIP-QDs, this possibly results from the imprinted cavity of the template (amoxicillin).

### 3.3. Optimization of the determination conditions

To obtain the highest sensitivity and shortest analysis time, the ratio of amoxicillin solution to MIP-QDs solution, molar ratio of template to monomer, incubation time and pH value were investigated and optimized. The sensitivity was obtained by the determination of amoxicillin in the concentration range of 0.20–50.0 $\mu g\ L^{-1}$. All optimization parameters were performed at room temperature (25 °C) for convenient analysis.

#### 3.3.1. The ratio of amoxicillin solution to MIP-QDs solution

A number of different ratios of amoxicillin solution to MIP-QDs solution (80 $\mu g\ L^{-1}$) were investigated for the determination of amoxicillin *i.e.* 1:1, 1:2, 1:3 and 1:4. The results are shown in Fig. 5A, the sensitivity increased when the ratio of MIP-QDs increased up to ratio of 1:3 and after that there was no further increase in sensitivity at higher ratio. Therefore, the ratio of amoxicillin solution to MIP-QDs solution of 1:3 was chosen for further studies.

#### 3.3.2. Effect of incubation time

To ensure the completion of binding between amoxicillin and the recognition site of MIP-QDs, the effect of incubation time between amoxicillin and MIP-QDs was investigated from 0 to 60 min. As shown in Fig. 5B, the photoluminescence intensity change (F0/F) of MIP-QDs increased up to an incubation time of 30 min and above that remains almost constant. Therefore, an incubation time of 30 min was selected for further experiments. Although, the incubation time between MIP-QDs and amoxicillin was 30 min, however, the measurement of the photoluminescence intensity requires only 2 min. This indicated that the developed method is capable of a high throughput of approximately 30 samples per 1 h.

#### 3.3.3. Ratio of template to monomer

To obtain the highest the sensitivity of MIP-QDs for the determination of amoxicillin, the effect of molar ratio of template to monomer was investigated and the results are shown in Fig. 5C. The highest sensitivity was obtained at the molar ratio of 1:8. The molar ratio of template to monomer of 1:6 provided lower sensitivity due to its low number of recognition sites for the target analytes. Also, the sensitivity was also decreased at the molar ratio of 1:10, possibly due to the site forming excess monomer which might inhibit the binding of recognition sites and the target analytes, resulting in the decrease in sensitivity.

#### 3.3.4. Effect of pH

MIP-QDs are sensitive to their surrounding environment and the pH value had a significant effect on the photoluminescence intensity of MIP-QDs [32]. Extremes of pH value both high and low will affect the binding efficiency of MIP-QDs and target analytes [33]. Therefore, the influence of the pH of buffer solution (Tris-HCl) used to prepare MIP-QDs solution in the range of 6.0–10.0 were investigated. The results are showed in Fig. 5D, the pH value had a significant influence on the photoluminescence quenching of MIP-QDs with the highest sensitivity being obtained at pH 8.0. The sensitivity decreased at lower pH due to hydrogen ion in the solution affecting the hydrogen bonding between MIP-QDs and amoxicillin [34] as well as causing the protonation of the amine groups of amoxicillin. At a pH higher than 8.0, the sensitivity was also decreased, possibly because of degradation or ionisation of the template molecule [34,35]. The $pK_a$ values of amoxicillin are 2.4, 7.4 and 9.6 [36]. In addition, the silica shell can be ionized under highly alkaline condition which can cause damage to the structure of the

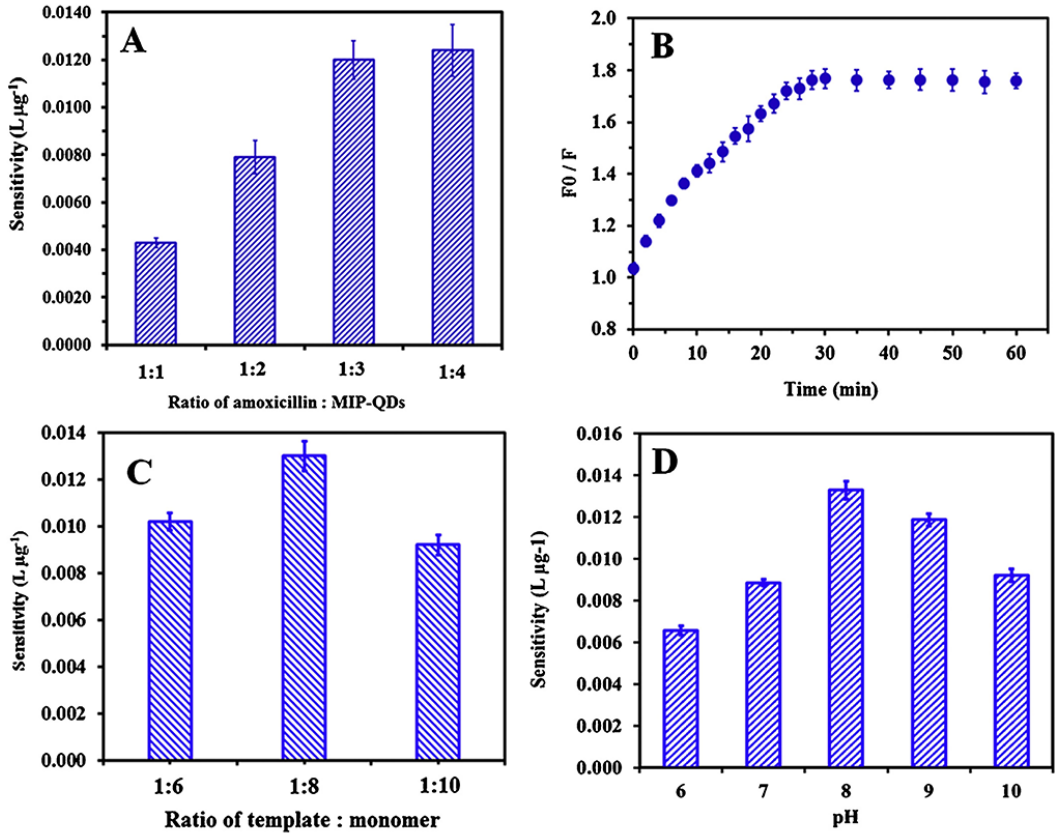


**Fig. 5.** (A) The effect of ratio of amoxicillin solution to MIP-QDs solution, (B) incubation time, (C) molar ratio of template to monomer and (D) pH value on the photoluminescence quenching of MIP-QDs for the determination of amoxicillin.

binding site. As a result, a Tris-HCl buffer solution pH of 8.0 was selected as an optimum value and used for the further experiment.

### 3.4. Comparison between MIP-QDs and NIP-QDs for the determination of amoxicillin

To investigate the recognition ability of the MIP-QDs compared to NIP-QDs, the photoluminescence intensity of MIP-QDs and NIP-QDs with different concentration of amoxicillin were investigated. As shown in Fig. 6A, the photoluminescence intensity of MIP-QDs clearly decreased with increasing concentration of amoxicillin whereas the photoluminescence intensity of NIP-QDs does not significantly change (Fig. 6B). It can be seen in Fig. 6C; the photoluminescence intensity is proportionally quenched much more for MIP-QDs than for NIP-QDs. The higher quenching efficiency or better sensitivity of MIP-QDs results from their specific binding affinity towards amoxicillin due to the cavities in MIP-QDs. These results indicated the synthesized MIP-QDs could be used as a facile and effective method to detect amoxicillin.

The photoluminescence quenching of the procedure could be described by the Stern-Volmer equation [27–30]:

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

where $F_0$ and F are the photoluminescence intensities of MIP-QDs in the absence and presence of a given concentration of amoxicillin respectively. $K_{sv}$ is the Stern-Volmer constant and [C] is the concentration of quencher (amoxicillin). The equation was used to quantify quenching constant of the MIPs and NIPs. The imprinting factor (IF), which is the ratio of $K_{sv}$ of the MIP-QDs and NIP-QDs (IF = $K_{sv}$,MIP/$K_{sv}$,NIP) was used to evaluate the selectivity of sensing materials. Under optimum conditions, the imprinting factor was calculated to be 43.6 which indicates the specific cavities of MIP are capable of selective adsorption of amoxicillin leading as demonstrated by greatly increased photoluminescence quenching efficiency.

### 3.5. Selectivity of MIP-QDs for the determination of amoxicillin

The selectivity of the developed MIP-QDs was investigated by comparing the photoluminescence intensity of MIP-QDs after interaction with the target analyte (amoxicillin) and other antibiotics. The potential interferent antibiotics used were ampicillin (AMP), cephalexin (CEP), penicillin G (PEG), chloramphenicol (CRP) and thiamphenicol (TAP).The photoluminescence intensity of MIP-QDs showed a high response to amoxicillin and a much lower signal for other antibiotics. The Stern-Volmer constant ($K_{sv}$) values

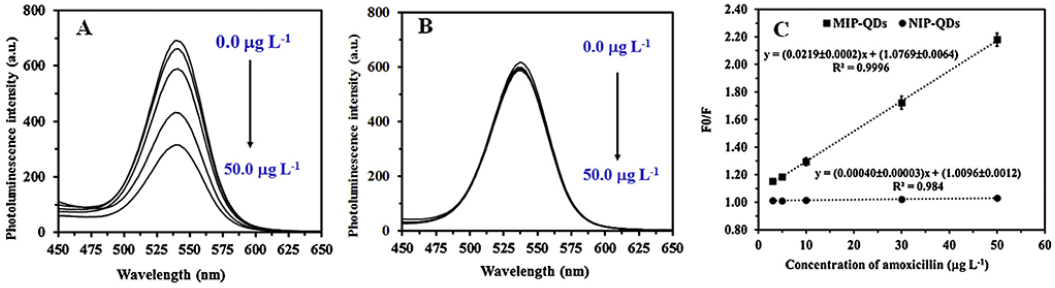


**Fig. 6.** Photoluminescence spectra of MIP-QDs (A), NIP-QDs (B) and the calibration curve (C) in the presence of amoxicillin in the concentration range of 0.0–50.0 μg $L^{-1}$.

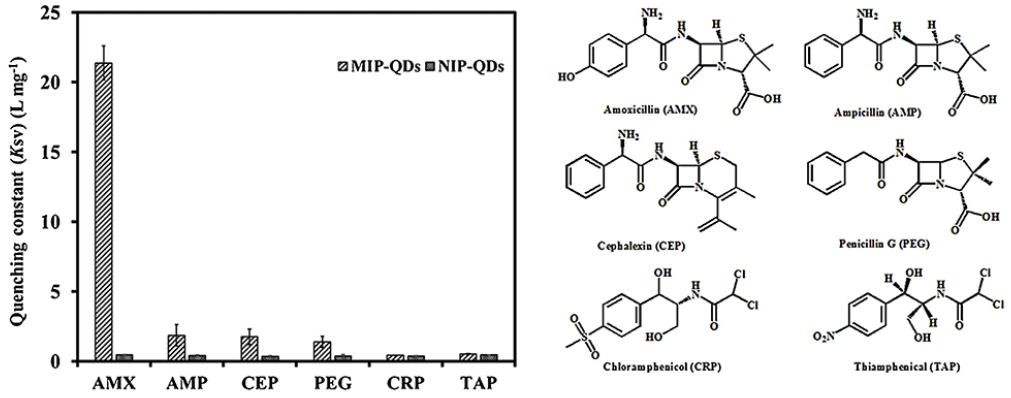


**Fig. 7.** Selectivity of MIP-QDs, Stern-Volmer constant for photoluminescence quenching of MIP-QDs for amoxicillin and other antibiotics (results are mean of three replicates).

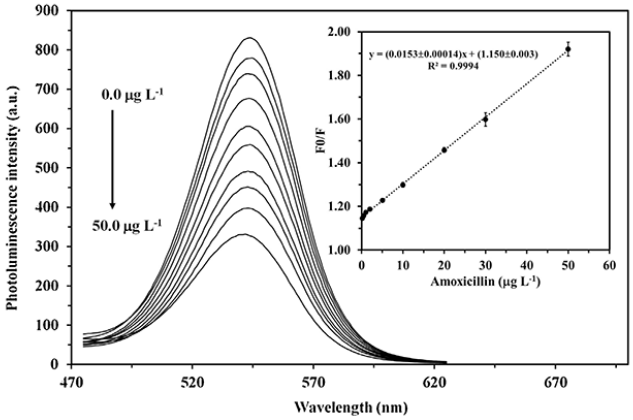


**Fig. 8.** Photoluminescence spectra of MIP-QDs at different concentration of amoxicillin and (inset) calibration curve, (results are mean of three replicates).

of amoxicillin interaction with MIP-QDs were higher than other antibiotics as shown in Fig. 7. These results indicated the MIP-QDs have a good selectivity for amoxicillin detection. This can be explained by the synthesis process of MIP-QDs producing many specific recognition sites with respect to the amoxicillin on the surface of MIP-QDs, the analyte therefore could be bound strongly to the MIP-QDs leading to quenching of the photoluminescence intensity.

### 3.6. Stability of MIP-QDs

The stability of the synthesized MIP-QDs in a Tris-HCl buffer solution (pH 8.0) was investigated at room temperature (25 °C) by the repeated measurement of the photoluminescence intensity over time. As shown in Fig. S2, the photoluminescence intensity decreased less than 1.0% after 12 h. The stability of the solid powder of MIP-QDs was also investigated by keeping it in a desiccator

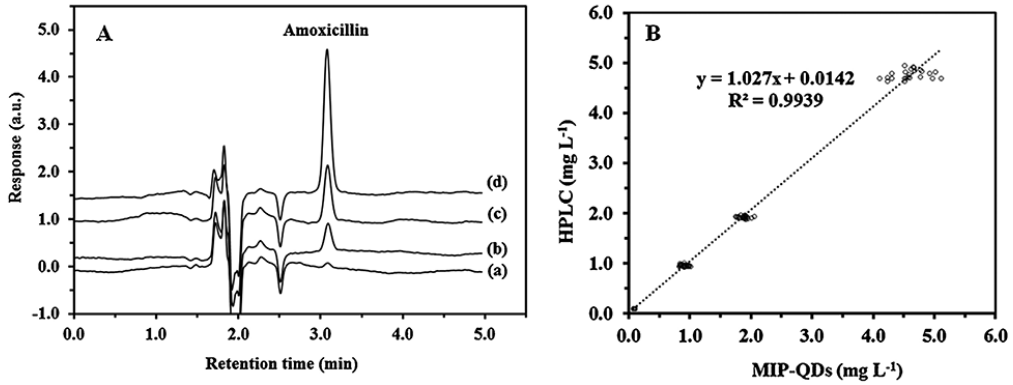


**Fig. 9.** (A) HPLC chromatogram of spiked egg sample at different concentration of amoxicillin; (a) 0.10 mg $L^{-1}$, (b) 1.0 mg $L^{-1}$, (c) 2.0 mg $L^{-1}$ and (d) 5.0 mg $L^{-1}$, (B) Correlation between MIP-QDs and HPLC methods for the determination of amoxicillin in food samples.

at 25 °C and the photoluminescence intensity showed no significant changes after 3 months (Fig. S3). These results indicated that MIP-QDs had good stability since coating the CdTe QDs with an MIP outside layer helps to improve the QDs photo stability.

### 3.7. Analytical performance of MIP-QDs for the determination of amoxicillin

The analytical performance of the developed method was evaluated including linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility. The MIP-QDs exhibited linear photoluminescence quenching for amoxicillin detection in the concentration range from 0.20 to 50.0 μg $L^{-1}$ with a coefficient of determination ($R^2$) of 0.9994 as shown in Fig. 8. The LOD and LOQ were 0.14 μg $L^{-1}$ and 0.46 μg $L^{-1}$, respectively, based on IUPAC criteria, 3σ/k and 10σ/k, respectively., where σ is the standard deviation of the blank measurement (n = 20) and k is the slope of the calibration curve.

Five replicate measurements of amoxicillin (50 μg $L^{-1}$) were performed to evaluate the precision of the MIP-QDs measurement. Under the optimum conditions, the relative standard deviation was 2.7%, indicating good measurement repeatability.

The reproducibility of synthesized MIP-QDs was investigated by preparing six different batches of MIP-QDs under identical conditions. The synthesized MIP-QDs were used to determine amoxicillin at a concentration of 50 μg $L^{-1}$ under optimum condition. The relative standard deviation (RSD) was 5.0%, indicating good reproducibility of the MIP-QDs preparation process.

### 3.8. Application of MIP-QDs for the determination of amoxicillin in food samples

The developed method using MIP-QDs as photoluminescence probes was applied to determine amoxicillin in a range of food samples i.e. egg, milk and honey. The concentration of amoxicillin was found in milk sample as supplied at a concentration of 0.50 μg $L^{-1}$. The accuracy of the developed method was also investigated by spiking the food samples with amoxicillin at four different concentrations (1.0, 10, 20, 50 μg $L^{-1}$). The sample preparation procedures were described in Section 2.5. The amoxicillin recovery values of spiked samples were obtained in the range of 85.3–102.0% with a relative standard deviation less than 6% (Table 1). The result showed that MIP-QDs can be used in the determination of amoxicillin in food samples with good accuracy and precision.

**Table 1**
Application of MIP-QDs and analytical results for the determination of amoxicillin in food samples ($n = 3$).

| Samples | Amoxicillin (μg $L^{-1}$) | | Recovery (%) | RSD |
|---|---|---|---|---|
| | A mount added | Amount found | | |
| Egg 1 | 0.0 | n.d. | – | – |
| | 1.0 | 0.86 | 86.0 | 1.3 |
| | 10.0 | 8.79 | 87.9 | 3.6 |
| | 20.0 | 18.79 | 94.0 | 3.1 |
| | 50.0 | 45.81 | 91.6 | 1.3 |
| Egg 2 | 0.0 | n.d. | – | – |
| | 1.0 | 0.85 | 85.3 | 3.3 |
| | 10.0 | 8.63 | 86.3 | 4.6 |
| | 20.0 | 18.81 | 94.1 | 3.5 |
| | 50.0 | 46.75 | 93.5 | 2.0 |
| Milk 1 | 0.0 | n.d. | – | – |
| | 1.0 | 1.02 | 102.0 | 2.7 |
| | 10.0 | 9.75 | 97.4 | 2.2 |
| | 20.0 | 19.53 | 97.7 | 4.6 |
| | 50.0 | 46.12 | 92.2 | 1.8 |
| Milk 2 | 0.0 | 0.50 | – | 2.0 |
| | 1.0 | 1.48 | 97.8 | 5.7 |
| | 10.0 | 9.97 | 94.7 | 3.9 |
| | 20.0 | 19.62 | 95.6 | 2.4 |
| | 50.0 | 47.0 | 93.0 | 2.3 |
| Honey 1 | 0.0 | n.d. | – | – |
| | 1.0 | 0.86 | 86.0 | 1.7 |
| | 10.0 | 8.91 | 89.1 | 3.7 |
| | 20.0 | 18.41 | 92.1 | 2.2 |
| | 50.0 | 43.57 | 87.1 | 3.6 |
| Honey 2 | 0.0 | n.d. | – | – |
| | 1.0 | 0.99 | 98.5 | 3.1 |
| | 10.0 | 8.72 | 87.2 | 3.5 |
| | 20.0 | 18.12 | 90.6 | 4.2 |
| | 50.0 | 48.43 | 96.9 | 4.8 |

n.d. = not detectable.

The developed MIP-QDs method was compared with a HPLC method. The three different sample types (egg, milk, honey) were spiked with the four different concentrations of amoxicillin. The extracted sample solutions were analysed using both MIP-QDs and HPLC method. The typical HPLC chromatogram of spiked egg samples is shown in Fig. 9A. The correlation between both methods was very good as shown in Fig. 9B; the coefficient of determination ($R^2$) was 0.9939. The result indicated that the developed method agrees

**Table 2**
Comparison of the developed MIP-QDs method with other methods for the determination of amoxicillin.

| Detection technique | Samples | Linear range ($\mu g\,L^{-1}$) | LOD ($\mu g\,L^{-1}$) | Recovery (%) | RSD (%) | References |
|---|---|---|---|---|---|---|
| MIP grown on MWCNTs/electrochemical | Milk and Honey | 36.5–2190.0 | 32.5 | 88–96 | 1.8–3.8 | [3] |
| SPME/HPLC-UV | Plasma | 1000–50,000 | 1210 | – | 5.9 | [37] |
| HFCU/HPLC/UV | Human Plasma | 100–20,000 | 25 | 83.1–86.8 | 3.1 | [38] |
| SPE/HPLC-UV | Milk and river water | 2–500 | 1.5 | 93–103 | 11 | [39] |
| Polyglutamic acid modified glassy carbon electrode/electrochemical | Human urine | 730.8–9135.0 | 336.2 | 96.2–106.7 | 1.4–2.5 | [40] |
| SPE/spectrophotometric | Pharmaceutical formulations, human urine | 5–1000 | 3.0 | 96.2–102.1 | 0.7–1.3 | [41] |
| FIA/spectrophotometric | Pharmaceutical preparation samples | 2000–40,000 | 637 | 97–102 | 0.4–1.5 | [13] |
| Colorimetric | Capsules and oral suspension | 110–1644 | 54.8 | – | 2.1 | [42] |
| MIP-QDs | Egg, Milk, Honey | 0.2–50 | 0.14 | 85–102 | 1.–5.7 | This work |

SPME = Solid phase microextraction; SPE = Solid phase extraction; HFCU = Hollow fiber centrifugal ultrafiltration; MIP = Molecularly imprinted polymer; MWCNTs = Multiwalled carbon nanotubes; FIA = Flow injection analysis.

well with the HPLC method and can be used for the determination of trace amoxicillin in food samples.

### 3.9. Comparison of the developed MIP-QDs method with other methods

The analytical performances of the developed MIP-QDs was compared with other works for the determination of amoxicillin and summarized in Table 2. The developed method provided a wide linear range and the limit of detection was much lower than other methods [3,13,37–42], while the recovery and precision of this method were comparable to or better than other methods. Moreover, when comparing this method with chromatographic methods [37–39], the photoluminescence measurement is simpler, faster, cost-effective and did not require any organic solvents such as normally used in the HPLC mobile phase. In addition, the selectivity of this work was improved with the use of MIPs and sensitivity was improved with quantum dots. Therefore, the developed MIP-QDs method can be used as an effective method for a simple, rapid, cost-effective, sensitive and selective determination of amoxicillin in food samples.

## 4. Conclusion

The facile synthesis of a molecularly imprinted polymer coated onto CdTe quantum dots was successfully achieved and the resultant composite used as a highly sensitive and selective photoluminescence probe for the determination of amoxicillin. The detection is based on the photoluminescence quenching of the MIP-QDs after the binding of amoxicillin into the specific cavities of MIP-QDs. The MIP-QDs photoluminescence probe combined the advantages of the high sensitivity of QDs and good selectivity of the MIP, demonstrating high sensitivity allowing detection of amoxicillin at trace levels and good selectivity to amoxicillin based on shape, size and functional group interactions. The developed MIP coated CdTe QDs was successfully applied to the determination of amoxicillin in various complex sample matrices *i.e.* eggs, milk and honey. The advantages of this method include simplicity, rapidity, high sensitivity, good selectivity and cost efficiency.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2017.07.062.

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# Paper II

Raksawong, P., **Chullasat, K.**, Nurerk, P., Kanatharana, P., Davis, F., 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(20) (2017). 4697-4707.

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PAPER IN FOREFRONT

# A hybrid molecularly imprinted polymer coated quantum dot 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 (MIP)-coated quantum dot (QD) nanocomposite was synthesized and applied as a fluorescence probe for the highly sensitive and selective determination of salbutamol. The hybrid MIP-coated QD nanocomposite was synthesized via a copolymerization process in the presence of thioglycolic acid capped CdTe QDs with salbutamol as a template, 3-aminopropyltriethoxysilane as the functional monomer, and tetraethyl orthosilicate as a cross-linker. The optimum molar ratio of template, monomer, and cross-linker was 1:6:20. The fluorescence intensity of the hybrid MIP-coated QDs was efficiently quenched after salbutamol rebound to the recognition sites, as a result of charge transfer from QDs to salbutamol. The synthesized hybrid MIP-coated QD nanocomposite showed 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 from 0.10 to 25.0 μg $L^{-1}$, with a detection limit of 0.034 μg $L^{-1}$. The hybrid optosensor developed was successfully applied in the determination of salbutamol in animal feeds and meat samples. Satisfactory recoveries were obtained in the range from 85% to 98%, with a standard deviation of less than 8%. Furthermore, the accuracy of the hybrid MIP-coated QD nanocomposite was investigated by comparison with a conventional high-performance liquid chromatography method, with the results obtained with 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 commonest β-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 toward human health, such as headache, nervousness, muscular tremors, diabetes, hyperthyroidism, and cardiac palpitations [4, 5]. They could also potentially lead to the evolution of antibiotic resistant pathogens. To ensure food safety and protect human health, the European Union has issued strict regulations for β-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

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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 [7, 8], gas chromatography–mass spectrometry ([9], electrochemical methods [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 because of its simple measurement, cost-effectiveness, and high throughput [16]. The sensitivity and selectivity of this method are dependent on the type of fluorescence probe used [17]. In recent years, quantum dot (QD) nanoparticles have attracted increasingly more attention and have been widely used as a sensitive fluorescence probe because of their excellent optical properties, such as narrow and tunable emission spectrum, broad excitation spectrum, and good photostability [18, 19]. However, the sensors developed with 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 (MIPs) 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 that 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, MIPs are also easy to prepare, have low cost, have high chemical stability, and have potential application for a wide range of possible target molecules [16, 26]. MIPs have been widely applied in many fields, such as in solid-phase extraction for sample separation [27–29], as a polymer coating on an optical fiber for gas sensing [30], as modification of electrodes for electrochemical sensors [31], and in paper-based devices [32]. They are also a potentially powerful material to improve the selectivity of optical sensors.

In this work, hybrid MIP-coated QD nanocomposite fluorescent probes were synthesized and applied for the first time for the determination of salbutamol. The synthesized hybrid MIP-coated QD nanocomposites were characterized and their sensing properties were investigated for salbutamol detection. The fluorescence probe developed was also successfully applied for the determination of salbutamol in animal feeds and meat samples. The accuracy of the optosensing protocol was evaluated in spiked samples and was also compared with that of an HPLC method.

## Materials and methods

### Materials

Tellurium powder (-200 mesh, 99.8%), cadmium chloride ($CdCl_2 \cdot 2H_2O$), sodium borohydride ($NaBH_4$), 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 an Elgastat Maxima ultrapure water system (18.2 MΩ cm) (ELGA LabWater, High Wycombe, UK).

### Instrumental

Fourier transform IR spectra (FTIR) were recorded with a Spectrum BX FTIR spectroscope (PerkinElmer, Waltham, MA, USA). The morphologies of TGA-capped CdTe QD and hybrid MIP-coated QD nanocomposites were observed with a JEM-2010 transmission electron microscope (JEOL, Tokyo, Japan) and a JSM-5200 scanning electron microscope (JEOL, Tokyo, Japan). UV spectra were recorded with an Avaspec 2048 spectrometer (Avantes, Apeldoorn, Netherlands). Fluorescence intensity was measured with an RF-5310 spectrofluorometer (Shimadzu, Tokyo, Japan). Brunauer–Emmett–Teller surface areas of hybrid MIP-coated QDs and nonimprinted polymer (NIP)-coated QDs were determined with an ASAP 2460 surface area and porosity analyzer (Micromeritics, Norcross, GA, 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 $NaBH_4$ were dissolved in 1.0 mL of deionized water to produce a NaHTe solution. Meanwhile, 4.5 mg of $CdCl_2$ and 30.0 μL of TGA were dissolved in 100 mL of deionized water. The pH of this solution mixture was adjusted to 11.5 with 1.0 M NaOH, placed in a three-necked flask, and deaerated by bubbling with $N_2$ for 20 min. Under vigorous stirring, 0.5 mL of NaHTe solution was rapidly injected into the mixture solution under a $N_2$ atmosphere. The solution was then refluxed for 10 min at 95 °C. The resulting mixture was precipitated with ethanol, and the resultant product was collected by centrifugation at 5000 rpm for 10 min. Finally, the TGA-capped CdTe QD nanoparticles were dried under a vacuum and stored in a desiccator for further use.

### Synthesis of hybrid MIP and NIP-coated CdTe QD nanocomposite

The MIP-coated QDs were prepared via a sol–gel copolymerization process. Salbutamol, APTES, and TEOS were used as the 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 the mixture was stirred at 500 rpm for 1 h. Then, 5.0 mL of TGA-capped CdTe QDs (10.0 μM), 110 μL of TEOS, and 150 μL of 25% $NH_3$ were added and the mixture was 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 QD nanocomposites were collected by centrifugation at 5000 rpm for 10 min and dried at 50 °C. The NIP-coated CdTe QD nanocomposites were also prepared under the same conditions without addition of the template molecule (salbutamol).

### Fluorescence measurement

The widths of the excitation and emission slits were both 10 nm. The excitation wavelength was set at 355 nm, and emission was recorded in the range from 450 to 650 nm. Hybrid MIP-coated QDs (6.0 μg $L^{-1}$) were dispersed in 300 μL of 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 with a fluorescence spectrophotometer. All fluorescence measurements were performed 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 procedure for extraction 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) by 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 was then dissolved in 1.0 mL of deionized before analysis by the hybrid MIP-coated QD fluorescence method developed.

The procedure for extraction of salbutamol from meat samples was adapted from previous work [35]. Briefly, 1.00 g of homogenized pork or beef samples was extracted with 2.0 mL of ethanol for 10 min by sonication and then centrifugation at 16,000 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 and defatted with 2.0 mL of hexane. After being shaken for 2 min, the mixture was centrifuged at 5000 rpm for 5 min and the degreasing phase was removed. The ethanol phase was then evaporated to dryness at 60 °C, and the residue was then dissolved in 1.0 mL of deionized before analysis by the hybrid MIP-coated QD fluorescence method developed.

### Analysis by the HPLC method

The HPLC conditions for the determination of salbutamol were adopted from a previous report [6]. The determination of salbutamol was performed with a 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany), and the data were acquired with ChemStation. The separation was performed on an Ascentis® $C_{18}$ (5 μm, 4.6 mm × 150 mm) analytical column (Supelco). 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 the mobile phase was 0.5 mL $min^{-1}$, and the column temperature was set at 30 °C. Salbutamol was detected with an excitation wavelength of 226 nm and an emission wavelength of 310 nm.

## Results and discussion

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

Hybrid MIP-coated QD nanocomposites were prepared via copolymerization as shown in Fig. 1. The copolymerization occurred in the presence of TGA-capped CdTe QDs, salbutamol as the template molecule, APTES as the functional monomer, and TEOS as the 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 $–NH_2$ binding sites. Then the amino groups further interacted 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 conditions but without addition of the template molecule. Figure 2 shows the fluorescence intensities of NIP-coated QDs (Fig. 2, curve a) and MIP-coated QDs after (Fig. 2, curve b) and before (Fig. 2, curve c) removal of the template. The fluorescence intensities of MIP-coated QDs before removal of template were about 20% of the fluorescence intensities of NIP-coated QDs. The fluorescence intensity of

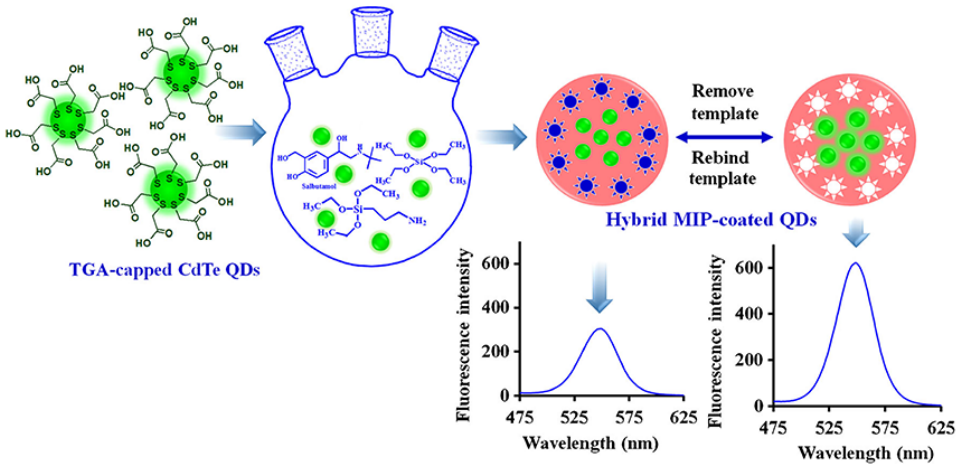


**Fig. 1** Synthesis of hybrid molecularly imprinted polymer (*MIP*)-coated quantum dot (*QD*) nanocomposite for salbutamol detection. *TGA* thioglycolic acid

MIP-coated QDs was significantly increased after removal of the template molecules. This indicates that the MIP was successfully synthesized and the template molecule was removed from the MIP-coated QD nanocomposite particles. The advantage of this method is the one-step polymerization process under mild conditions that can be performed at room temperature (26 ± 2 °C).

The absorption and fluorescence spectra of TGA-capped CdTe QDs are shown in Fig. S1; the maximum emission was 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 QD nanocomposites was also investigated by scanning electron microscopy. As shown in Fig. 3, images a and b, they have a uniform spherical shape, and their diameters range from 220 to 300 nm. The particle diameter increased significantly after coating with the MIP compared with the original TGA-capped CdTe QDs. These results indicate that the hybrid MIP-coated QDs have a large surface area with effective imprinting sites to bind the template molecule.

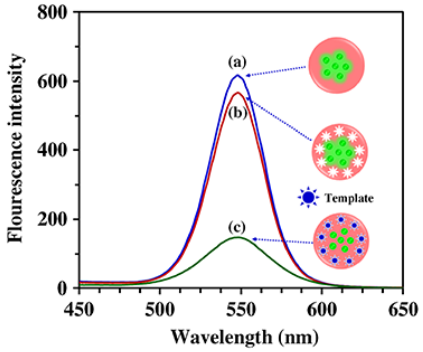


**Fig. 2** Fluorescence spectra of nonimprinted polymer (NIP)-coated QDs (*a*), MIP-coated QDs after removal of the template (*b*) and MIP-coated QDs before removal of the template (*c*)

The transmission electron microscope images of MIP-coated CdTe QDs demonstrate the QDs are small dots distributed within the polymer matrix of the MIP (Fig. 3, image c).

The FTIR spectrum of TGA-capped CdTe QDs (Fig. 4, spectrum a) has characteristic peaks at 1376 and 1585 $cm^{-1}$, which corresponded to the C = O symmetric and asymmetric stretching of the carboxylic group. The absorption peaks at 3450 and 1225 $cm^{-1}$ were attributed to O–H stretching and C–O stretching. The FTIR spectrum of salbutamol (Fig. 4, spectrum b) has an absorption band at 1500 $cm^{-1}$ corresponding to O–H bending [36]. The absorption peak at 1100 $cm^{-1}$ is due to C–O stretching. The absorption peaks at 3240 and 3400 $cm^{-1}$ are due to N–H and O–H stretching. The absorption peak at 1616 $cm^{-1}$ is due to aromatic stretching. The FTIR spectrum of hybrid MIP-coated QD nanocomposite before removal of the template (salbutamol) is shown in Fig. 4, spectrum c. The absorption peak at 1063 $cm^{-1}$ was ascribed to Si–O–Si asymmetric stretching. The Si–O vibration band is at 460 $cm^{-1}$. After removal of the template, the absorption peaks at 1100, 1500, 1616, and 3240 $cm^{-1}$, which are related to salbutamol, were absent (Fig. 4, spectrum d). The broad absorption band at 3409 $cm^{-1}$ and the absorption peak at 1600 $cm^{-1}$ result from the N–H stretching vibration of the aminopropyl group. The results indicate that the MIP was successfully synthesized and that the CdTe QDs were coated with it to form hybrid MIP-coated QDs for selective recognition of salbutamol.

The Brunauer–Emmett–Teller surface area of hybrid MIP-coated QDs and NIP-coated QDs were 52.77 and 44.75 $m^2\ g^{-1}$ respectively. The hybrid MIP-coated QDs had a slightly greater surface area than the NIP-coated QDs; this could result from the imprinted cavity of the template.

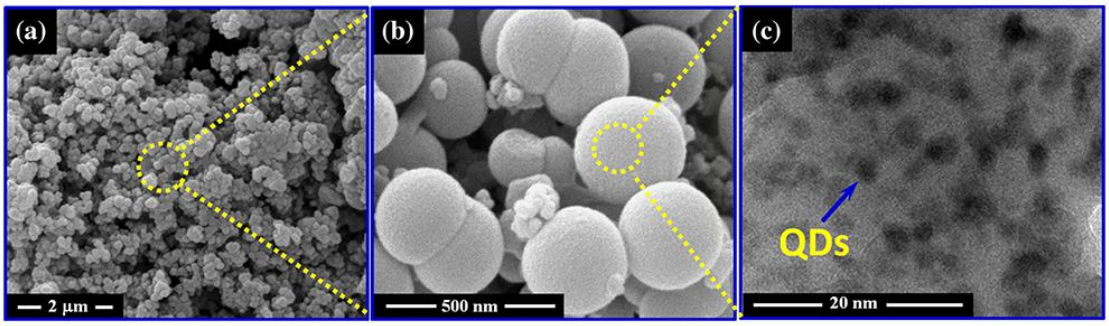


**Fig. 3** Scanning electron microscope images of hybrid MIP-coated QD nanocomposites at × 20,000 magnification (*a*) and × 80,000 magnification (*b*) and transmission electron microscope images of hybrid MIP-coated QD nanocomposites (*c*)

## 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, molar ratio of template to monomer, and molar ratio of template to cross-linker). Therefore, these parameters were investigated and optimized to obtain the highest sensitivity and shortest analysis time.

### *Effect of incubation time*

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.

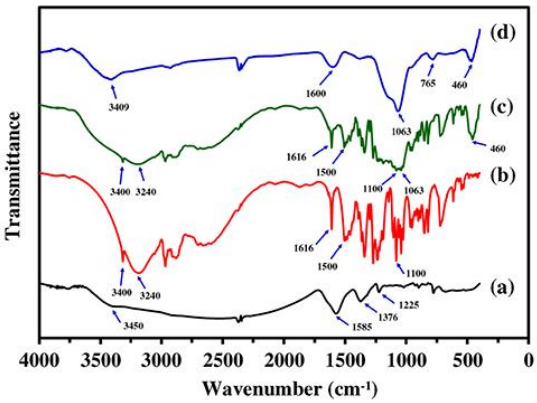


**Fig. 4** Fourier transform IR spectra of TGA-capped CdTe QDs (*a*), salbutamol (*b*), hybrid MIP-coated QDs before removal of the template (*c*) and hybrid MIP-coated QDs after removal of the template (*d*)

### *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 from 7.0 to 10.0 on the sensitivity was investigated. As shown in Fig. 5b, the highest sensitivity was obtained at pH 9.0. The sensitivity decreased under acidic conditions because of 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 the polymer and salbutamol. The sensitivity also decreased at pH greater 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 conditions, which can damage the structure of the binding sites and potentially electrostatic repulsion between the silica and ionized substrate molecules. Therefore, the determination was performed with hybrid MIP-coated QDs in buffer solution at pH 9.0.

### *Ratio of template to monomer*

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

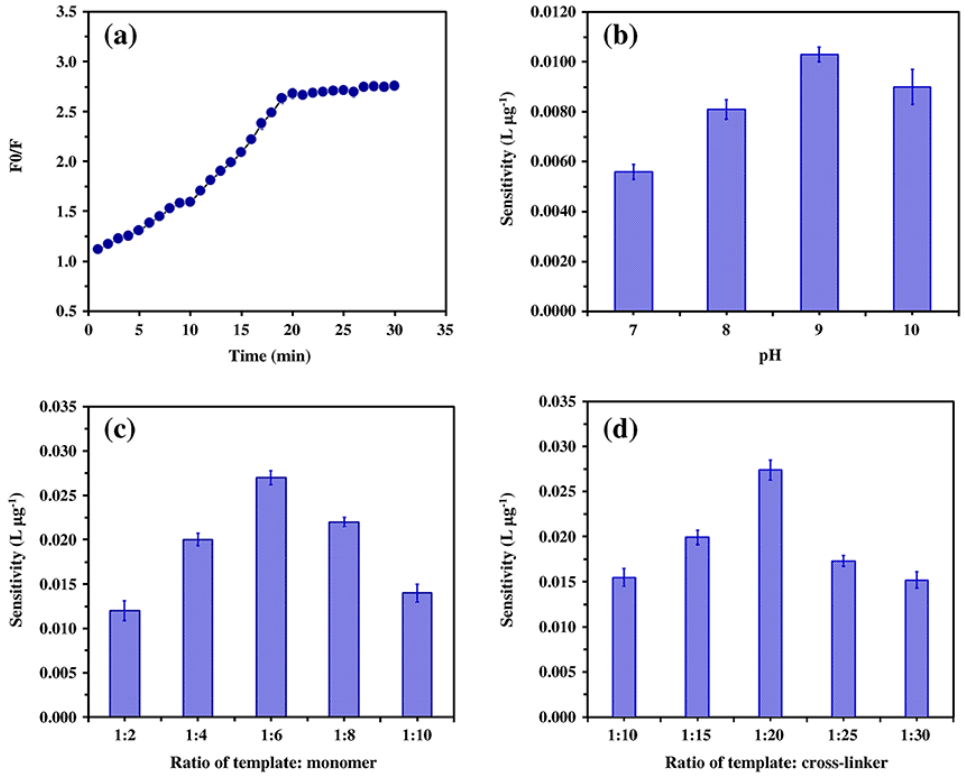


**Fig. 5** Influence of incubation time (**a**), pH (**b**), molar ratio of template to monomer (**c**), and molar ratio of template to cross-linker (**d**) on the fluorescence quenching of hybrid MIP-coated QDs for the determination of salbutamol

*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 a ratio of 1:20, and the sensitivity decreased with further increases in concentration of the cross-linker (Fig. 5d). Low sensitivity was obtained at low concentration of the cross-linker because of the MIP structure being physically weaker and less rigid. This means that the formation of specific recognition sites is less effective and also that the CdTe QDs were easily disconnected from the polymer during the template removal process. However, too high a concentration of the cross-linker also resulted in low sensitivity because of excessive cross-linking potentially blocking the diffusion and motion of the 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. Figure 6a shows the fluorescence spectra of hybrid MIP-coated QDs with different concentrations of salbutamol. The fluorescence intensity was quenched gradually with 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 greater than that of NIP-coated QDs (Fig. 6c). The quenching efficiency of hybrid MIP-coated QDs with regard 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

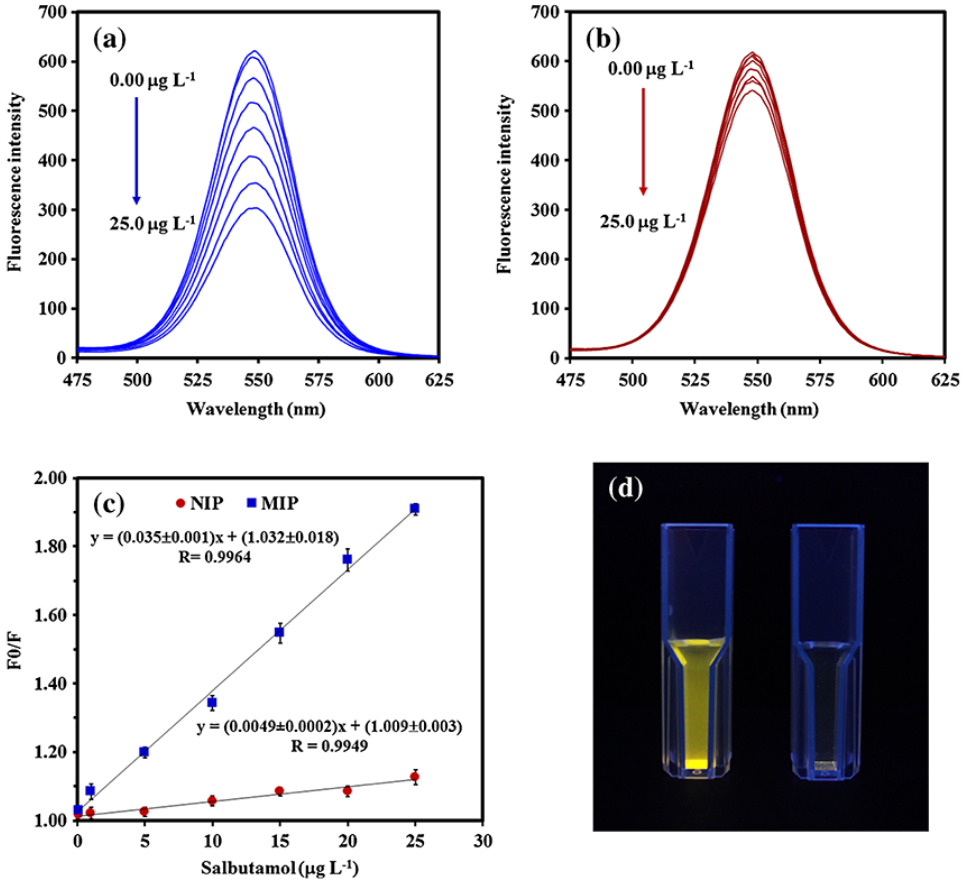


**Fig. 6** Fluorescence spectra of hybrid MIP-coated QDs (**a**) and NIP-coated QDs (**b**), calibration curve of hybrid MIP-coated QDs and NIP-coated QDs (**c**), and photographs of cuvettes containing solutions of hybrid MIP-coated QDs without (*left*) and with (*right*) salbutamol under UV light (**d**)

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 with regard 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 the QDs to salbutamol, thereby leading to fluorescence quenching of hybrid MIP-coated QDs. Photographs showing fluorescence of hybrid MIP-coated QDs with and without salbutamol are shown in Fig. 6d. Although 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 for salbutamol

The selectivity of hybrid MIP-coated QD nanocomposite was evaluated by our determining $K_{SV}$ of other compounds structurally related to salbutamol: namely, clenbuterol, clenproperol, ractopamine, and chloramphenicol. The results are shown in Fig. 7; $K_{SV}$ of salbutamol was much higher than that of these structural analogues. The imprinting factor, which is the ratio of $K_{SV}$ of the hybrid MIP-coated QDs and NIP-coated QDs ($K_{SV,MIP}/K_{SV,NIP}$) was used to evaluate the selectivity of the sensing materials. Under optimum conditions, the imprinting factors for 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 that match the shape, size, and functional groups of the template molecule (salbutamol).

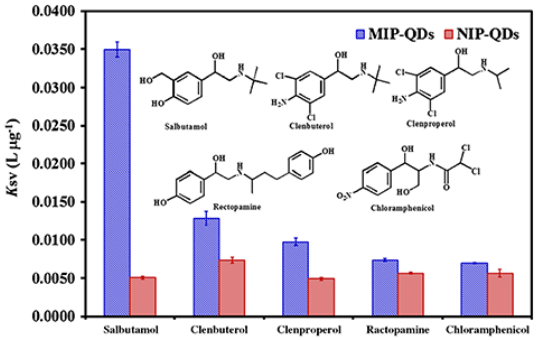


**Fig. 7** Selectivity of hybrid MIP-coated QDs and NIP-coated QDs for salbutamol, clenbuterol, clenproperol, ractopamine, and chloramphenicol

The adsorption ability of the NIP-coated QDs was also investigated. $K_{SV}$ of salbutamol was similar to that of 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 performance of the method developed was evaluated, including linearity, limit of detection, and limit of quantification. The hybrid MIP-coated QDs exhibited linear fluorescence quenching ($F_0/F$) for salbutamol detection in the concentration range from 0.10 to 25.0 μg $L^{-1}$, with a coefficient of determination ($R^2$) of 0.9966. The limit of detection was 0.034 μg $L^{-1}$and the limit of quantification was 0.11 μg $L^{-1}$, 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 the preparation of the hybrid MIP-coated QDs was investigated by preparation of six different batches of MIP-coated QDs under identical experimental conditions. The relative standard deviation of the six different batches was 6%, which indicated that the preparation of the hybrid MIP-coated QDs has good reproducibility.

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

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

The optosensing method based on hybrid MIP-coated QD nanocomposite was applied to detect salbutamol in three different types of animal feeds (porcine, poultry, and bovine) and in pork and beef meat samples (the results are shown in Table 1). Salbutamol was detected in porcine feed at 9.8 μg $kg^{-1}$, and no salbutamol was detected in pork or beef samples. The accuracy of this method was also investigated by addition of standard solution to 1.00 g of homogenized sample to obtain final concentrations of 2.0, 5.0, 10.0, and 20.0 μg $kg^{-1}$. These spiked samples were vortexed for 15 s and allowed to stand at room temperature for 1 h to ensure that the analyte was incorporated into the sample matrix. The spiked samples were then extracted and analyzed by the method developed. 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 indicate that the hybrid MIP-coated QD nanocomposite method was reliable and can be used as a high-throughput method for the determination of salbutamol in complex samples.

The method was also compared with the HPLC method. The samples were spiked with four different concentrations of salbutamol and extracted as described in "Sample preparation." The extracted sample solutions were analyzed by both the hybrid MIP-coated QD method and the HPLC

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

| Sample | Concentration of salbutamol (μg $kg^{-1}$) | | 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 | ND | - | - |
| | 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 | ND | - | - |
| | 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 | ND | - | - |
| | 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 | ND | - | - |
| | 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 |

*ND* not detectable, *RSD* relative standard deviation

**Table 2** Comparison of optosensing based on the hybrid molecularly imprinted polymer (*MIP*)-coated quantum dot (*QD*) method with other methods for the determination of salbutamol

| Analytical method | Samples | Linear range (μg $L^{-1}$) | LOD (μg $L^{-1}$) | Recovery (%) | RSD (%) | Reference |
|---|---|---|---|---|---|---|
| Ultraperformance liquid chromatography–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 immobilized on eggshell membrane as a fluorescence probe] | Urine | 5-100 | 3.5 | 85-102 | 3.2 | [39] |
| Immunochromatography | 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 | 500-10,000 | 200 | 85-91 | 3.0-3.1 | [42] |
| Capillary electrophoresis | Urine | 2000-30,000 | 500 | 98-101 | 1.5-3.8 | [43] |
| Capillary electrophoresis | Swine feed | 2000-100,000 | 1070 | 100-104 | 1.0-3.0 | [15] |
| Electrochemical method | Salbutamol sulfate injections | 12-47,800 | 12.0 | 95-103 | 1.5-4.6 | [44] |
| Hybrid MIP-coated QD nanocomposite | Animal feeds and meat | 0.10-25.0 | 0.034 | 85-98 | 0.4-7.7 | This work |

*LOD* limit of detection

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 indicates that the results from the hybrid MIP-coated QD method agreed well with the results from 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 QD method with other methods for the determination of salbutamol

Several methods have been reported for the determination of salbutamol in various samples. The analytical performance of the fluorescence sensor based on the hybrid MIP-coated QD optosensing protocol was compared with other methods described in previous work (Table 2). The hybrid MIP-coated QD optosensors provided a wide linear range and lower detection limit than reported in other work, whereas the recovery and standard deviation of this method were comparable with those in previous work. These results demonstrate that the hybrid MIP-coated QDs are highly sensitive and can be used for the determination of trace salbutamol in complex samples. Moreover, this method is simple, rapid, and cost-effective and demonstrates good selectivity.

## Conclusions

A hybrid MIP-coated QD nanocomposite was developed and used as an optosensor for the detection of salbutamol based on electron-transfer-induced fluorescence quenching of QDs. The 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 of target analytes.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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# Paper III

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# Nanocomposite optosensor of dual quantum dot fluorescence probes for simultaneous detection of cephalexin and ceftriaxone

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ABSTRACT

An optosensor based on the fluorescence quenching of two different nanocomposite fluorescence probes was developed for simultaneous detection of cephalexin and ceftriaxone. The nanocomposite fluorescence probes were synthesized by incorporating graphene quantum dots (GQDs) and cadmium telluride quantum dots (CdTe QDs) in a molecularly imprinted polymer (MIP). The MIP-GQDs and MIP-CdTe QDs exhibited high emission fluorescence intensities at 440 and 575 nm, respectively. The mesoporous structure of the MIP layer provided recognition sites specific to the template analytes. At the optimum condition, the fluorescence intensities of the probes were quenched linearly as the concentrations of cephalexin and ceftriaxone increased from 0.10 to 50.0 μg $L^{-1}$. The very low detection limits were 0.06 and 0.10 μg $L^{-1}$ for cephalexin and ceftriaxone, respectively. The developed dual nanocomposite fluorescence probes were used for the detection of cephalexin and ceftriaxone in milk samples. The recoveries of cephalexin and ceftriaxone in four spiked milk samples ranged from 83.0 to 98.7% with RSD lower than 8%. This developed nanocomposite optosensor was highly sensitive and selective for trace detection of cephalexin and ceftriaxone in milk samples and produced results that were in good agreement with the results of HPLC analysis of the same samples.

## 1. Introduction

Cephalexin and ceftriaxone are cephalosporin antibiotics which are normally used to treat a variety of infections in livestock. However, the misuse of these drugs leaves residues in animal products that can cause stomach upset, diarrhea, loss of appetite, nausea and vomiting in humans who eat the animal products. Therefore, the monitoring of these antibiotic residues in food is of some importance. Various methods have been reported for the detection of cephalexin and ceftriaxone. These methods have included electrochemistry [1], liquid chromatography [2–6], micellar electrokinetic chromatography [7], fluorescent polarization immunoassay [8,9], and spectrophotometry [10]. Although liquid chromatography is the most widely used of these analytical methods, it requires expensive instruments, a long analysis time and uses large volumes of toxic organic solvent for the mobile phase. To overcome these limitations, spectrofluorimetry is an interesting technique due to its simplicity, rapidity and low cost. However, for trace analysis in complex metrics samples, the sensitivity and selectivity of the analytical method need to be improved. To enhance the sensitivity of spectrofluorimetry, quantum dot and carbon dot fluorescence probes show promise due to their good optical properties and chemical stability. An optosensor using quantum dots and carbon dots was a sensitive method for the monitoring of compounds such as bleomycin [11], $Co^{2+}$ [12], kanamycin [13], $Fe^{3+}$ [14] and dopamine [15]. To increase the selectivity of an optosensor, the use of composite fluorescence probes of quantum dots and carbon dots with a molecularly imprinted polymer (MIP) has attracted much attention. The MIP is normally synthesized via sol-gel copolymerization in the presence of template molecules (target analyte), a functional monomer and a cross-linker. After removal of the template, the obtained specific recognition sites have the complementary shape, size and function to rebind with the target analyte (template). In addition, MIP has exhibited good chemical stability and ease of synthesis. MIPs have been used in solid phase extraction sorbents [16], composite magnetic adsorbents [17,18], the removal of toxic compounds or purification [19] and electrode modification for electrochemical sensing [20,21]. MIP composited QD fluorescence probes were developed for sensitive and selective detection of organic compounds, including salbutamol [22], amoxicillin [23], ciprofloxacin [24], malachite green [25], melamine [26] and sulfadimidine [27]. However, these methods can detect only a single target analyte.

In this work, dual nanocomposite fluorescence probes were

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developed and used for the simultaneous detection of cephalexin and ceftriaxone. The developed dual optosensor consists of composite graphene quantum dots (GQDs) and cadmium telluride quantum dots (CdTe QDs) incorporated into an MIP. The MIP-GQDs and MIP-CdTe QDs exhibited a strong fluorescence intensity at different emission wavelengths, which can enable simultaneous detection of cephalexin and ceftriaxone. The developed dual nanocomposite optosensor was applied to determine cephalexin and ceftriaxone in milk samples. The performance of the developed nanocomposite optosensor was also compared with the performance of an HPLC detection method.

## 2. Experimental

### 2.1. Chemicals and materials

Citric acid, 3-aminopropyl triethoxysilane (APTES, ≥98%), sodium borohydride ($NaBH_4$), cadmium chloride ($CdCl_2$) and tellurium powder (99.8%) were purchased from Sigma-Aldrich (MO, USA). Cephalexin, ceftriaxone, thioglycolic acid (TGA) and tetraethyl orthosilicate (TEOS, ≥99%) were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Sodium hydroxide, methanol, acetonitrile, ethanol and ammonia were purchased from RCI Labscan (Bangkok, Thailand). Dialysis membrane was from Spectrum Laboratories, Inc. (Rancho Dominguez, CA, USA).

### 2.2. Instrumental

Fluorescence intensity was measured with an RF-5310PC spectrofluorophotometer from Shimadzu (Tokyo, Japan) and UV–vis spectra were produced with an Avaspec 2048 spectrometer (Apeldoom, Netherlands). FT-IR spectra were recorded on a BX FTIR spectroscope from PerkinElmer (MA, USA). The morphologies of GQDs, MIP-GQDs, TGA-CdTe QDs and MIP-CdTe QDs were observed using a JSM-5200 scanning electron microscope from JEOL (Tokyo, Japan) and a Philip TECNAI 20 transmission electron microscope (Eindhoven, Netherlands).

### 2.3. Synthesis of GQDs and MIP-GQDs fluorescence probe

GQDs were synthesized by direct pyrolysis of citric acid following the method of a previous work with minor modification [28]. Briefly, citric acid (2.0 g) was heated at 200 °C using a heating mantle until the liquid became pale yellow and added dropwise into 100 mL of 0.25 M sodium hydroxide solution and stirred for 30 min. The obtained solution was dialyzed in a dialysis bag (12 kDa cut off) for 24 h. The synthesized GQD nanoparticles in solution were stored at 4 °C until use.

The MIP-GQDs were synthesized as described in a previous work but with some alteration [29]. First, 5 mmol of APTES were added into 20 mL of GQDs in solution under vigorous stirring and heated at 40 °C for 30 min. The GQDs were coated with APTES by precipitation with isooctane twice and dispersed in ethanol. To synthesize MIP-GQDs, 0.10 g of cephalexin was mixed with 10.0 mL of APTES-coated GQDs in solution and stirred for 30 min. Then, 2.0 mL of APTES, 1.0 mL of TEOS and 20 mL of $NH_3$ were added into the solution and stirred for 30 min. Finally, the template (cephalexin) was removed by washing with a mixture of acetic acid and methanol (1:9). The NIP-GQDs were prepared under the same condition as MIP-GQDs but without adding the template (cephalexin).

### 2.4. Synthesis of thioglycolic acid-capped CdTe QDs and MIP-CdTe QD fluorescence probes

The procedure for the synthesis of TGA-capped CdTe QDs was adapted from reported procedures [30,31]. First, a NaHTe solution was prepared by dissolving 75.0 mg of tellurium and 57.0 mg of $NaBH_4$ in deionized water (1.50 mL) and stirring for 1 h. Meanwhile, 4.50 mg of $CdCl_2$ and 30 μL of thioglycolic acid were dissolved in deionized water (100 mL) and adjusted to pH 11.5 with 1.0 M NaOH. This mixture solution was transferred into a three-necked flask and deaerated by $N_2$ gas for 10 min. Subsequently, 0.5 mL of the NaHTe solution was injected into the mixture solution under $N_2$ atmosphere with vigorous stirring. The solution was then heated under nitrogen atmosphere at 90 °C for 15 min. The TGA-capped CdTe QD nanoparticles were precipitated with ethanol and dried in a vacuum at 50 °C for 6 h.

The nanocomposite MIP-CdTe QDs were prepared by following a reported method with minor modification [23]. Briefly, 15.0 mg of ceftriaxone (template) and 50 μL of APTES (monomer) were added into a brown bottle containing 5.0 mL of deionized water and the mixture was stirred at 600 rpm for 1 h. Subsequently, 5.0 mL of the prepared TGA-capped CdTe QDs (10.0 μM), 120 μL of TEOS (cross-linker) and 150 μL of $NH_3$ (25% w/v) were added and stirred for 4 h. After polymerization, the template molecules were removed by washing with ethanol and the hybrid nanocomposite MIP-CdTe QDs were separated by centrifugation at 5000 rpm for 15 min and dried at 50 °C for 4 h. The nanocomposite non-imprinted polymer CdTe QDs (NIP-CdTe QDs) were synthesized under the same condition without the addition of template (ceftriaxone).

### 2.5. Fluorescence measurement procedure

The MIP-GQDs and MIP-CdTe QDs were dispersed in citrate-phosphate buffer solution. To measure fluorescence intensities, 150 μL of fluorescence probes (MIP-GQDs and MIP-CdTe QDs) were mixed with 50 μL of standard (ceftriaxone and cephalexin) or sample solution. Using an excitation wavelength of 355 nm, emissions were recorded from 400 to 700 nm with a slit width of 10 nm. All analyses were performed at 25 °C (room temperature).

### 2.6. Sample pretreatment of milk samples

Milk samples were obtained from local markets in Songkhla province, Thailand. The samples were pretreated using a modified method from a previous report [4]. In brief, milk samples of 10.0 mL were defatted by centrifugation at 5000 rpm for 10 min. Then, 10 mL of acetonitrile was added into the defatted milk to remove proteins. The mixture was centrifuged at 5000 rpm for 20 min and the supernatant was collected and evaporated to dryness at 50 °C. The residue was redissolved with 10.0 mL of deionized water and analyzed with the developed optosensor.

## 3. Results and discussion

### 3.1. The synthesis and characterization of GQD, CdTe QD, MIP-GQD and MIP-CdTe QD fluorescence probes

The prepared GQDs and CdTe QDs produced symmetrical and narrow UV–vis and fluorescence spectra at the maximum emission wavelengths of 440 nm and 575 nm, respectively (**Fig. S1 and Fig. S2**).

The nanocomposite MIP-GQDs were prepared by sol-gel polymerization. The functional monomer (APTES) interacted with the synthesized GQDs through hydrogen bonding and polymerization occurred after the addition of the cross-linker (TEOS), template (Cephalexin) and catalyst ($NH_3$). After polymerization was complete, the template molecules were removed and the obtained fabricated nanocomposite MIP-GQD fluorescence probes contained specific recognition sites on their particles.

The nanocomposite MIP-CdTe QDs were prepared via sol-gel copolymerization. A template molecule (ceftriaxone) and a functional monomer (APTES) were self-assembled through hydrogen bonding and coated the surface of as-prepared TGA-capped CdTe QDs. After adding a cross-linker (TEOS) and $NH_3$, the CdTe QDs were entrapped in the MIP layer to form the nanocomposite MIP-CdTe QD fluorescence probes.

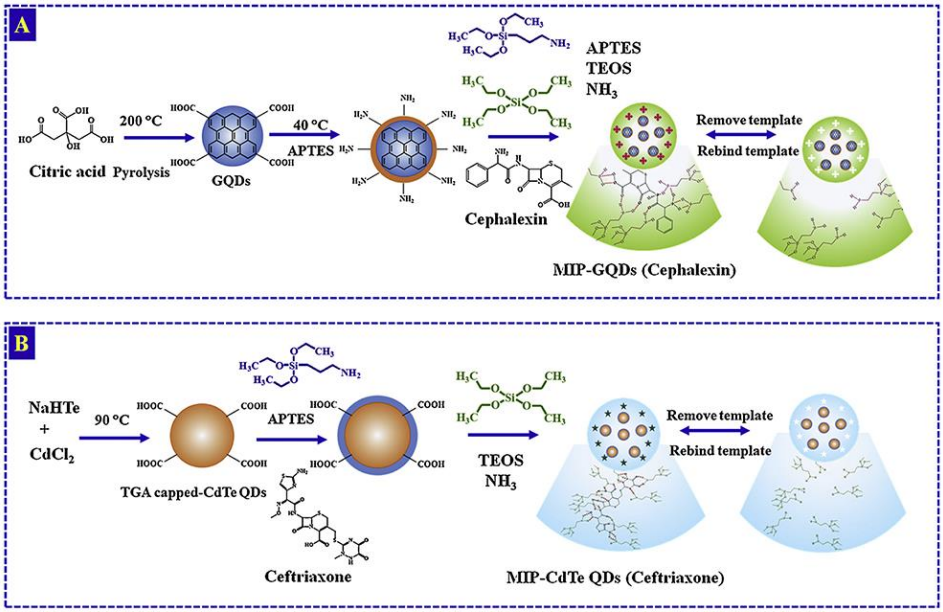


**Fig. 1.** The synthesis procedure of MIP-GQDs (A) and MIP-CdTe QDs (B).

The synthesis processes of MIP-GQDs and MIP-CdTe QDs are represented in Fig. 1.

Before removal of the template, the fluorescence intensity of the MIP-GQDs was about 35.0% that of the NIP-GQDs and the fluorescence intensity of the MIP-CdTe QDs about 22.5% that of the NIP-CdTe QDs (Fig. 2a). After removal of the templates (Fig. 2b), the fluorescence intensities were almost the same as the NIP-GQDs and the NIP-CdTe QDs (Fig. 2c), indicating successful removal of the templates from the composite fluorescence probes. In addition, the removal of the templates were also investigated by the analysis of both target analytes in washing solvent (methanol) using HPLC technique. As shown in Fig. S3, no signal of both ceftriaxone and cephalexin in the washing solvent which can be confirmed that the template molecules were completely removed from MIP layer. These nanocomposite fluorescence probes simultaneously detected the target analytes, cephalexin and ceftriaxone, which could rebind to the generated specific recognition sites.

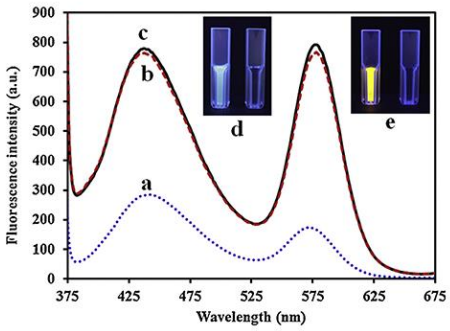


**Fig. 2.** Fluorescence spectra of a mixture of MIP-GQDs and MIP-CdTe QDs (a) before and (b) after removal of template molecules and (c) of a mixture of NIP-GQDs and NIP-CdTe QDs, (d) photographs of MIP-GQDs under UV light (right) with and (left) without template and (e) photographs of MIP-CdTe QDs (right) with and (left) without template.

Photographs of the MIP-GQDs and MIP-CdTe QDs under UV light with and without templates are shown in Fig. 2**d** and e, respectively.

The morphologies of GQDs and CdTe QDs were investigated by transmission electron microscopy (TEM). From the TEM images, the average particle size of GQDs (Fig. 3A) and CdTe QDs (Fig. 3B) were 5.0 ± 1.1 nm and 4.8 ± 1.3 nm, respectively. The surface morphologies of the MIP-GQD and MIP-CdTe QD fluorescence probes were investigated using SEM. The SEM images of both MIP-GQDs (Fig. 3C) and MIP-CdTe QDs (Fig. 3D) revealed uniform spherical nanoparticles with a rough surface and the average particle sizes were 305 ± 10 nm and 302 ± 15 nm, respectively. The rough surfaces of the MIP-GQDs and MIP-CdTe QDs indicated the presence of a large number of specific imprinted cavities on the particles.

The sysnthesized nanocomposite MIP-GQDs and MIP-CdTe QDs were also investigated by FT-IR spectroscopy. The FT-IR spectrum of the GQDs Fig. 4**(a)** showed a broad band at 3368 $cm^{-1}$ that corresponded to −OH stretching, and a peak at 1725 $cm^{-1}$ due to C=O stretching. The characteristic peaks at 2951 and 1350 $cm^{-1}$ are attributed to C−H stretching vibration. The peak at about 1508 $cm^{-1}$ is due to skeletal vibration of aromatic rings [28]. Fig. 4**(b)** shows the charecteristic peaks of MIP-GQDs after removal of the template. The peaks at 1067 and 465 $cm^{-1}$ are attributed to Si−O−Si asymmetric vibration and Si−O symmetric bending, respectively. The FT-IR spectrum of MIP-GQDs before removal of the cephalexin template (Fig. 4**(c)**) shows peaks at 1760 and 3275 $cm^{-1}$, which were due to C=O and N−H stretching vibration and appear at the same wavelengths as peaks in the FT-IR spectrum of cephalexine (Fig. 4**(d)**). The charecteristic peaks of the TGA-capped CdTe QDs can be seen in Fig. 4**(e)** present a broad peak at about 3450 $cm^{-1}$ and a peak at 1222 $cm^{-1}$ which were respectively attributed to O−H and C−O stretching of carboxylic groups; the strong absorption peaks at 1376 and 1509 $cm^{-1}$ are the symmetric and asymmetric stretching of the carboxylate group.

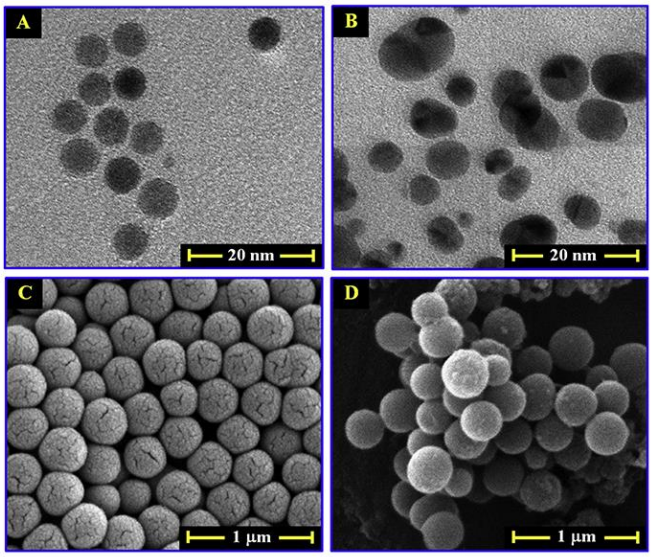


**Fig. 3.** TEM images of GQDs (A) and TGA-capped CdTe QDs (B) and SEM images of MIP-GQDs (C) and MIP-CdTe QDs (D).

Fig. 4(**f**) shows the charecteristic peaks of MIP-CdTe QDs after removal of the template. The broad and strong absorption peak at 1067 is due to Si—O—Si asymmetric vibration and the characteristic peak at 465 $cm^{-1}$ is assigned to Si-O symmetric bending. The FT-IR spectrum of MIP-CdTe QDs before removal of the ceftriaxone template (Fig. 4(**g**)) shows strong absorption peaks at 1742 and 1592 $cm^{-1}$, due to C=O and C=N stretching vibration, respectively. Absorption peaks are present at the same wavelenghts in the FT-IR spectrum of ceftriaxone (Fig. 4(**h**)). These results indicated that nanocomposite MIP-GQDs and MIP-CdTe QDs were successfully synthesized to contain specific imprinted recognition sites to enable re-binding of cephalexin and ceftriaxone.

### 3.2. Optimization of experimental conditions

#### 3.2.1. Effect of pH

To investigate the influence of pH on the sensitivity of the developed probes, the MIP-GQDs and MIP-CdTe QDs were dispersed in citrate-phosphate buffer solutions in a pH range between 5.0 and 6.5. The highest sensitivity of both MIP-GQDs and MIP-CdTe QDs was obtained at pH 5.5 (Fig. 5A). The sensitivity decreased at higher pH because of surface defects caused by ionization of the silica layer. The sensitivity also decreased at pH lower than 5.5 because the $-NH_2$ group of the functional monomer (APTES) in the specific cavities underwent protonation, which reduced hydrogen bonding between the target analytes and the functional monomer [32]. From these results, pH 5.5 was chosen as the optimum value for the simultaneous detection of cephalexin and ceftriaxone.

#### 3.2.2. Effect of incubation time

The effect of incubation time was investigated to determine the time dependency of the adsorption of the target analytes at the surface of the MIP-GQDs and MIP-CdTe QDs. The fluorescence probes were dispersed

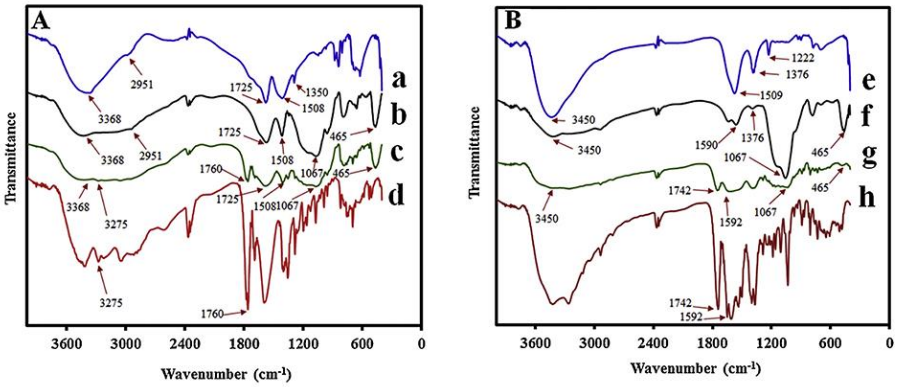


**Fig. 4.** (A) FT-IR spectra of GQDs (a), MIP-GQDs after template removal (b), MIP-GQDs before template removal (c) and cephalexin (d). (B) FT-IR spectra of CdTe QDs (e), MIP-CdTe QDs after template removal (f), MIP-CdTe QDs before template removal (g) and ceftriaxone (h).

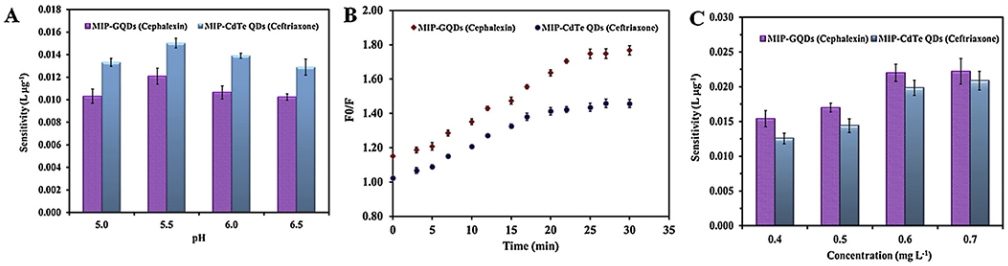


**Fig. 5.** Effect of pH (A), incubation time (B) and concentration of MIP-GQDs and MIP-CdTe QDs (C) on the fluorescence quenching of nanocomposite fluorescence probes for the simultaneous detection of cephalexin and ceftriaxone.

in citrate-phosphate buffer solution at pH 5.5, mixed with the target analytes and the fluorescence intensity was recorded at room temperature (25 ± 1 °C) from 0 to 30 min. The results showed that fluorescence quenching increased as the incubation time increased from 0 to 25 min and then it remained almost constant during further incubation (Fig. 5B). Therefore, 25 min was selected as the optimum incubation time for the simultaneous detection of cephalexin and ceftriaxone.

### 3.2.3. Effect of concentration of MIP-GQDs and MIP-CdTe QDs

To investigate the effect of the concentration of the probes on the system's sensitivity in detecting cephalexin and ceftriaxone, the concentration of MIP-GQDs and MIP-CdTe QDs was varied between 0.4 and 0.7 mg $L^{-1}$ at room temperature (25 ± 1 °C). The sensitivity increased when the concentration of both fluorescence probes was increased from 0.4 to 0.6 mg $L^{-1}$ and it remained almost constant at the higher concentration (0.7 mg $L^{-1}$) (Fig. 5C). Therefore, 0.6 mg $L^{-1}$ was selected as the optimum concentration of MIP-GQD and MIP-CdTe QD fluorescence probes for future experiments.

### 3.2.4. Effect of mole ratio of template to monomer to cross-linker

The thickness and particle size of MIP-GQDs and MIP-CdTe QDs depend on the amounts of the polymerization precursors in the synthesis. Therefore, the mole ratio of template to monomer to cross-linker had to be optimized. The highest sensitivity of the MIP-GQDs was obtained from a synthesis at the mole ratio of 1:30:15 (Fig. 6A) and at 1:8:15 for MIP-CdTe QDs (Fig. 6B). The sensitivity decreased at the low amount of monomer maybe due to low generation of functional groups on the surface of the probes, which leads to fewer recognition sites. The sensitivity also decreased at the higher amount of monomer because excess monomer can cause self-condensation during synthesis, which reduces the formation of recognition sites [33].

The amount of cross-linker (TEOS) used in the synthesis also affected the sensitivity of the MIP-GQDs and MIP-CdTe QD towards cephalexin and ceftriaxone. When the probes were synthesized with a low amount of cross-linker, sensitivity decreased due to the instability of the MIP formation. The sensitivity was also impaired by the larger amount of cross-linker because excess cross-linker blocked the binding between the functional monomers and the template molecules [34].

### 3.3. Quenching mechanism

The fluorescence quenching mechanism of the MIP-GQDs and MIP-CdTe QDs after interaction with the target analytes was previously described in the literature. Hydrogen bonding occurred between cephalexin or ceftriaxone and the amino groups (-$NH_2$) of the functional monomer (APTES) on the surface of nanocomposite fluorescence probes. This led to fluorescence quenching due to electron transfer from conduction bands of the QDs to the lowest unoccupied molecular orbital of the target analyte (cephalexin or ceftriaxone) [26]. In addition, there was no overlap between the absorption spectra of target analytes (cephalexin and ceftriaxone) and fluorescence emission spectra of fluorescence probes (MIP-GQDs or MIP-CdTe QDs) (**Fig. S4A and B**). These results indicated that energy transfer was not a possible quenching mechanism [35,36].

The fluorescence quenching of MIP-GQDs and MIP-CdTe QDs can be quantified by the Stern-Volmer equation as follows:

$$F0/F = 1 + Ksv[Q],$$

where F and F0 are the fluorescence intensity in the presence and absence of a quencher, respectively; and K*sv* and [Q] are the quenching

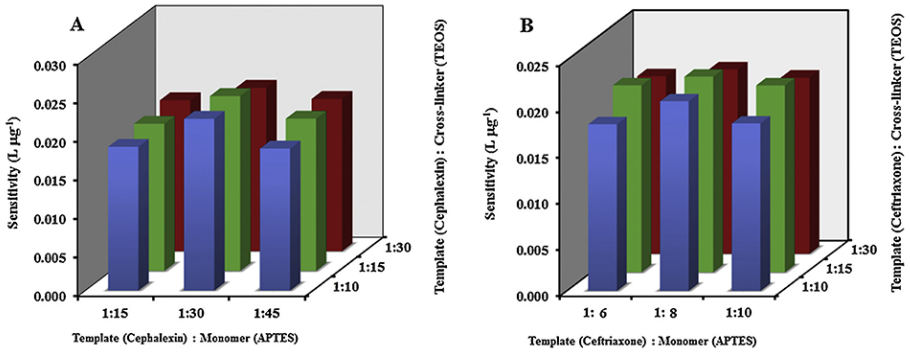


**Fig. 6.** The influences of mole ratio of template to monomer to cross-linker in MIP-GQDs (A) and MIP-CdTe QDs (B). The experimental conditions were MIP-GQDs and MIP-CdTe QDs dispersed in citrate-phosphate buffer solution at pH 5.5, incubation time of 25 min and concentration of MIP-GQDs and MIP-CdTe QDs was 0.6 mg $L^{-1}$.

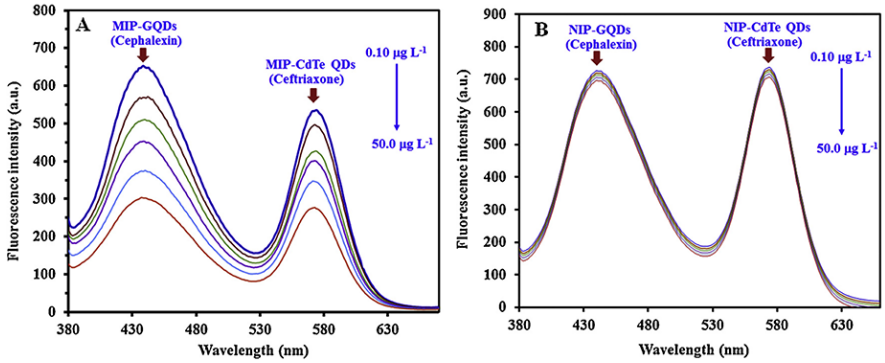


**Fig. 7.** Fluorescence spectra of nanocomposites MIP-GQDs and MIP-CdTe QDs (A) and NIP-GQDs and NIP-CdTe QDs (B) in the presence of different concentrations of cephalexin and ceftriaxone. The experimental conditions were MIP-GQDs and MIP-CdTe QDs dispersed in citrate-phosphate buffer solution at pH 5.5, incubation time of 25 min and concentration of MIP-GQDs and MIP-CdTe QDs was 0.6 mg $L^{-1}$.

constant and the concentration of the quencher, respectively. The imprinting factor (IF) can be calculated from the ration of $Ksv_{,MIP}/Ksv_{,NIP}$. The IF of the MIP-GQDs and MIP-CdTe QDs for simultaneous detection of cephalexin and ceftriaxone were 26.5 and 30.0, respectively. The results indicated that the developed nanocomposite fluorescence probes had a high specificity to the target analytes.

### 3.4. Analytical performance of MIP-GQDs and MIP-CdTe QDs for simultaneous determination of cephalexin and ceftriaxone

The analytical performance of the developed nanocomposite fluorescence probes was investigated under the optimum experimental conditions. The fluorescence spectra of nanocomposite MIP-GQDs mixed with MIP-CdTe QDs and NIP-GQDs mixed with NIP-CdTe QDs in the presence of different concentrations of cephalexin and ceftriaxone are shown in Fig. 7**A and B**, respectively. The developed MIP-GQDs and MIP-CdTe QDs showed a good linearity from 0.10 to 50.0 µg $L^{-1}$ (Fig. 8). The coefficient of determination ($R^2$) of the MIP-GQDs and MIP-CdTe QDs were 0.9980 and 0.9958, respectively. The LODs ($3SD_{blank}$/slope) of cephalexin and ceftriaxone were 0.06 and 0.10 µg $L^{-1}$, respectively. The LOQs ($10SD_{blank}$/slope) of cephalexin and ceftriaxone were 0.20and 0.32 µg $L^{-1}$, respectively. These very low LODs and LOQs indicated that the developed optosensor was highly sensitive in the simultaneous detection of trace cephalexin and ceftriaxone.

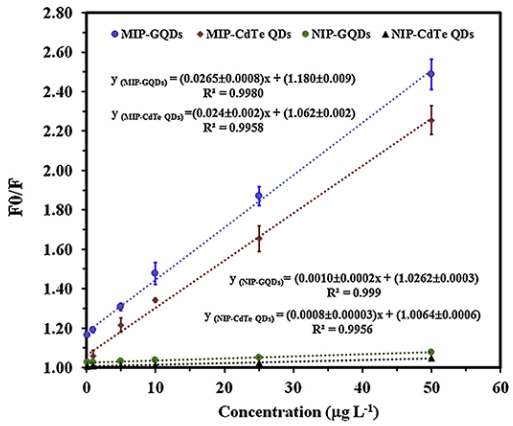


**Fig. 8.** The linearity of the simultaneous detection of cephalexin and ceftriaxone using dual nanocomposite MIP-GQDs and MIP-CdTe QDs.

### 3.5. Selectivity of nanocomposite MIP-GQDs and MIP-CdTe QDs in simultaneous detection of cephalexin and ceftriaxone

To determine the selectivity of the developed MIP-GQDs and MIP-CdTe QDs, the sensitivity of the dual optosensor towards the target analytes was compared with its sensitivity in the detection of the structural analogs cefixime, ceftazidime and cefadroxil. The level of sensitivity was obtained by measuring the response of the optosensor to the target analytes and their analogs in concentrations ranging from 1.0 to 25.0 µg $L^{-1}$. The developed optosensor showed a higher sensitivity towards cephalexin and ceftriaxone than it did towards the structural analogs (Fig. 9). By synthesizing the fluorescence probes with a molecularly imprinted polymer, large numbers of recognition binding sites specific to the target analytes were produced on the surface of the MIP-GQDs and MIP-CdTe QDs. Therefore, the target analytes could bind rapidly and strongly to the surface of the fluorescence probes, leading to fluorescence quenching. The structural analogs were not molecularly complementary to the recognition cavities and could not, therefore, effectively quench the fluorescence intensity of the probes.

### 3.6. Stability and reproducibility

The synthesized fluorescence probes demonstrated a good stability under the optimum experimental conditions. In citrate-phosphate buffer at pH 5.5, the fluorescence intensity of both probes was measured every 30 min and did not significantly change during a 5 h period **(Fig. S5)**. The good stability of these nanocomposite optosensors could be attributed to the protective effect of the MIP on the surface of the quantum dots.

The reproducibility of the synthesis of the MIP-GQDs and MIP-CdTe QDs was evaluated by preparing six different batches of each fluorescence probe under the conditions described in Sections 2.3 and 2.4. The RSDs for the synthesis of MIP-GQDs and MIP-CdTe QDs were 1.6 and 1.9%, respectively **(Fig. S6)**. The results showed that the synthesis of both probes had a good reproducibility.

### 3.7. Analysis of cephalexin and ceftriaxone in milk samples

The developed optosensor based on dual fluorescence probes was applied for simultaneous detection of cephalexin and ceftriaxone in milk samples. Before analysis with the developed optosensor, milk samples were pretreated as described in Section 2.6. Low

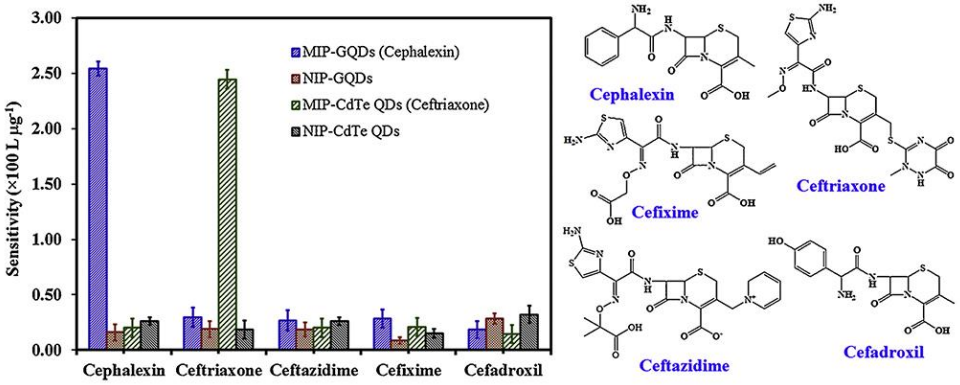


**Fig. 9.** The selectivity of the nanocomposite MIP-GQDs and MIP-CdTe QDs fluorescence probes for the simultaneous detection of cephalexin and ceftriaxone. Experimental condition were incubation time of 25 min and buffer solution pH 5.5.

**Table 1**
The determination of cephalexin and ceftriaxone in milk samples using dual fluorescence probes of MIP-GQDs and MIP-CdTe QDs ($n$ = 3).

| Samples | Added (μg $L^{-1}$) | Cephalexin | | | Ceftriaxone | | |
|---|---|---|---|---|---|---|---|
| | | Found (μg $L^{-1}$) | Recovery (%) | RSD (%) | Found (μg $L^{-1}$) | Recovery (%) | RSD (%) |
| Milk 1 | 0.0 | n.d. | – | – | n.d. | – | – |
| | 5.0 | 4.76 | 95.2 | 4.1 | 4.58 | 91.6 | 6.2 |
| | 10.0 | 8.60 | 86.0 | 6.3 | 9.48 | 94.8 | 4.2 |
| | 25.0 | 23.72 | 94.9 | 3.8 | 24.23 | 96.0 | 3.3 |
| | 50.0 | 47.76 | 95.5 | 3.7 | 48.71 | 94.6 | 3.0 |
| Milk 2 | 0.0 | n.d. | – | – | n.d. | – | – |
| | 5.0 | 4.61 | 92.2 | 6.9 | 4.81 | 96.1 | 2.7 |
| | 10.0 | 8.87 | 88.7 | 5.1 | 9.45 | 94.5 | 4.3 |
| | 25.0 | 24.45 | 97.8 | 2.0 | 21.86 | 87.4 | 3.9 |
| | 50.0 | 47.20 | 94.4 | 4.9 | 49.36 | 98.7 | 0.7 |
| Milk 3 | 0.0 | 0.79 | – | 4.0 | n.d. | – | – |
| | 5.0 | 5.10 | 86.2 | 5.7 | 4.23 | 84.5 | 7.9 |
| | 10.0 | 9.3 | 85.3 | 3.8 | 8.3 | 83.0 | 2.1 |
| | 25.0 | 24.3 | 94.1 | 1.8 | 22.41 | 89.6 | 4.4 |
| | 50.0 | 47.9 | 94.3 | 5.7 | 48.19 | 96.4 | 2.4 |
| Milk 4 | 0.0 | n.d. | – | – | n.d. | – | – |
| | 5.0 | 4.76 | 95.2 | 5.8 | 4.67 | 93.4 | 1.2 |
| | 10.0 | 9.80 | 98.1 | 1.7 | 9.08 | 90.8 | 7.5 |
| | 20.0 | 23.81 | 95.2 | 5.0 | 22.60 | 90.4 | 3.1 |
| | 50.0 | 46.40 | 92.8 | 6.5 | 48.81 | 97.6 | 1.6 |

n.d. = not detectable.

concentrations of cephalexin were detected in the milk samples (Table 1). The accuracy in terms of recovery was also evaluated by spiking standard solutions of cephalexin and ceftriaxone in milk samples at four different concentrations (5.0 10.0 25.0 and 50.0 μg $L^{-1}$). Satisfactory recoveries were obtained ranging from 83.0 to 98.7% with an RSD less than 8%. These results demonstrated that the nanocomposite optosensor was reliable for the determination of cephalexin and ceftriaxone.

In addition, the output of the developed optosensor was compared with the results of HPLC analysis. The HPLC chromatogram of a spiked milk sample is shown in Fig. 10**A**. A very good correlation between the two optosensors and the HPLC method was obtained with a coefficient of determination ($R^2$) better than 0.99 (Fig. 10**B and C**).

### 3.8. Comparison of developed optosensor with other methods

The performance of the developed optosensor was compared with analytical methods reported in other works (Table 2). The present method exhibited a wide linear range with LODs much lower than LODs in the other reported works of trace analysis. The accuracy in terms of recoveries was better than some methods and similar to others. The repeatability of the synthesis was satisfactory. In addition, this developed method is simple to operate, gives rapid measurements and the equipment costs much less than equipment for HPLC techniques.

## 4. Conclusion

Dual nanocomposite fluorescence probes, based on GQDs and CdTe QDs encapsulated in a molecularly imprinted polymer, were developed for the simultaneous detection of cephalexin and ceftriaxone. Integrating the high specificity of a molecularly imprinted polymer and the strong fluorescence properties of GQDs and CdTe QDs produced a highly sensitive and selective optosensor of fluorescence probes which can simultaneously detect cephalexin and ceftriaxone at trace levels. This versatile dual nanocomposite optosensor can be used as an alternative, simple, rapid and reliable method for the analysis of cephalexin and ceftriaxone in milk samples. In addition, this simple approach can be used to fabricate nanocomposite fluorescence probes for

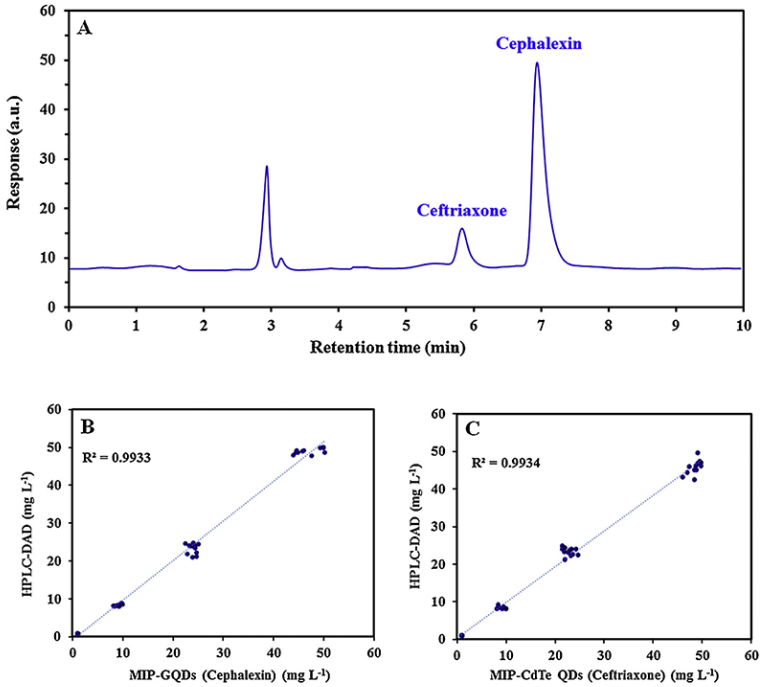


**Fig. 10.** Typical HPLC chromatogram of spiked milk sample with 10.0 μg $L^{-1}$ of cephalexin and ceftriaxone (A). Correlation between an HPLC method for detection of cephalexin and ceftriaxone and an optosensor using MIP-GQDs fluorescence probe (B), and MIP-CdTe QDs fluorescence probe (C).

**Table 2**
Comparison of developed optosensor with other methods for the detection of cephalexin and ceftriaxone.

| Analytical method | Analytes | Linear range (μg $L^{-1}$) | Limits of detection (μg $L^{-1}$) | Recovery (%) | RSD (%) | References |
|---|---|---|---|---|---|---|
| Differential pulse voltammetry | Cephalexin | 174-243,313 | 35 | 92-96 | 1–7 | [37] |
| Spectrofluorimetry | Ceftriaxone | 1,664–55,457 | 555 | | < 1 | [38] |
| UHPLC-MS/MS | Ceftriaxone | – | 64 | 71- 115 | < 23 | [39] |
| Voltammetry | Ceftriaxone | 6-5,546 | 5 | 90-101 | 3 | [40] |
| Chemiluminescence | Ceftriaxone | 11-4,436 | 2.7 | 97- 106 | – | [41] |
| Spectrofluorimetry | Ceftriaxone | 400-20,000 | 1.3 | 97- 100 | 5 | [42] |
| Spectrofluorimetry | Ceftriaxone | 200-20,000 | 19.4 | 94- 100 | 3 | [43] |
| HPLC-DAD | Ceftriaxone | 200-54,000 | 180 | 99- 100 | < 2 | [44] |
| HPLC-DAD | Cephalexin | 15-240 | 3.0 | 87-99 | < 7 | [45] |
| Fluorescence polarization immunoassay | Cephalexin | 4.7-83.5 | 1.8 | 88- 115 | 3–14 | [9] |
| HPLC-DAD | Cephalexin | 2-20 | 4.0 | 79- 83 | 2–4 | [46] |
| Spectrofluorimetry | Cephalexin | 1,181-34,739 | 288 | 96- 101 | < 5 | [47] |
| HPLC-DAD | Cephalexin | – | 11.6 | 93-106 | < 13 | [5] |
| Spectrofluorimetry | Cephalexin | 0.10-50.0 | 0.06 | 83-99 | < 8 | This work |
| MIP-GQDs and MIP-CdTe QDs | Ceftriaxone | | 0.10 | | | |

simultaneous monitoring of other organic compounds and other real-life samples.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2018.11.003.

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# Paper IV

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# Hybrid monolith sorbent of polypyrrole-coated graphene oxide incorporated into a polyvinyl alcohol cryogel for extraction and enrichment of sulfonamides from water samples

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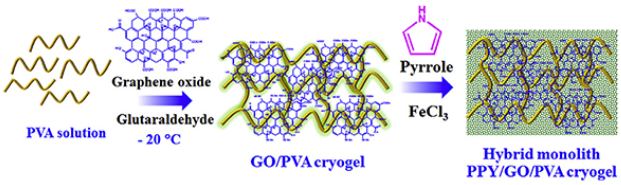
## HIGHLIGHTS

- A hybrid monolith polypyrrole-coated graphene oxide incorporated into polyvinyl alcohol cryogel sorbent was developed.
- Its high surface area facilitated the high adsorption capability of the sulfonamides.
- The hybrid monolith PPY/GO/PVA cryogel showed a high extraction efficiency for sulfonamides (85.5–99.0%).

## GRAPHICAL ABSTRACT

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## ABSTRACT

A hybrid monolith sorbent of polypyrrole-coated graphene oxide embedded in polyvinyl alcohol cryogel was prepared and used as an effective solid phase extraction sorbent for the determination of trace sulfonamides. The large surface areas with many adsorption sites of polypyrrole and graphene oxide facilitated the high adsorption of sulfonamides via hydrogen bonding, π-π and hydrophobic interactions. The high porosity of the polyvinyl alcohol cryogel helped to reduce the back pressure that occurs in a conventional packed solid phase extraction cartridge. The effecting parameters on the extraction efficiency including the type of sorbent, the polymerization time, desorption conditions, the sample pH, the sample volume, the sample flow rate, and ionic strength were investigated and optimized. Under the optimum conditions, the developed method provided a wide linear range from 0.20 to 100.0 μg $L^{-1}$ for sulfadiazine, sulfathiazole and sulfamerazine; and from 0.10 to 100 μg $L^{-1}$ for sulfamethazine, sulfamonomethoxine and sulfadimethoxine. The limits of detection were 0.20 μg $L^{-1}$ for sulfadiazine, sulfathiazole and sulfamerazine; and 0.10 μg $L^{-1}$ for sulfamethazine, sulfamonomethoxine and sulfadimethoxine. The developed hybrid monolith polypyrrole-coated graphene oxide embedded in the polyvinyl alcohol cryogel sorbent provided good recoveries in the range of 85.5–99.0% with RSDs of less than 5.0%. The sorbent offered a good reproducibility, was robust and can be reused at least 10 times. It

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was successfully applied for the extraction and enrichment of sulfonamides from normal and supplemented water samples.



## 1. Introduction

Sulfonamides are synthetic antibiotics of low cost and high efficiency that are extensively used to treat diseases, prevent infections and promote growth [1]. However, they are poorly absorbed by the organism and their extensive use leads to their wide release into environmental water, mostly in excreted urine and feces [2,3]. Water-borne sulfonamides can enter the food chain and cause serious allergic reactions in humans. Therefore, it is important to develop a simple, sensitive and reliable analytical method for the determination of sulfonamides. High performance liquid chromatography (HPLC) is the most widely used method due to its high sensitivity, selectivity and good precision [4,5]. However, at trace levels in real samples, sulfonamides are usually present together with high matrix interferences, therefore, an instrumental analysis generally requires a suitable sample treatment to preconcentrate the target analytes and remove the matrix interferences [6].

Many sample preparation methods have been developed and applied for the extraction of sulfonamides such as matrix solid phase dispersion (MSPD) [5], magnetic solid phase extraction (MSPE) [7–9], liquid-liquid extraction (LLE) [10], liquid-liquid microextraction (LLME) [11,12], stir bar sorptive extraction (SBSE) [13], hollow fiber-based liquid phase microextraction (HF-LPME) [14], and solid phase extraction (SPE) [3,15,16]. Among these methods, SPE is one of the most widely used for the extraction and preconcentration of target analytes at trace level in aqueous solution due to its high preconcentration efficiency [17]. However, the main drawback of conventional packed SPE cartridges is the cartridge clogging that occurs when large sample volumes are loaded through the sorbent [18,19]. The cartridges are also expensive and cannot be reused [20]. This problem of cartridge clogging can be addressed using porous materials. Cryogel is a good choice of porous material for the preparation of solid phase extraction sorbent due to its extremely high porosity [21,22]. Poly(vinyl alcohol) (PVA) is now widely used to prepare cryogel material due to its non-toxicity, biodegradability and low cost [23,24]. However, PVA cryogels has a low surface area and low adsorption capacity for target analytes, therefore, the entrapment of adsorbent particles within the cryogels and high affinity coatings can increase their specific surface area and obtain a higher extraction efficiency and better selectivity. Recently, multiwall carbon nanotube, functionalized with sulfonate group (MWCNTs-$SO_3^-$) incorporated in PVA cryogel has been developed and used as an SPE sorbent to separate β-agonists from animal feeds [21] and molecularly imprinted polymer (MIP) composite cryogel was used to separate propranolol from complex sample [22]. These sorbents provided a high extraction efficiency and effectively reduce matrix interferences. Their elastic and highly porous interconnected structure provide a low back pressure [25].

Graphene oxide (GO) can help to enhance the adsorption of target analytes due to its large surface area [26]. It is a derivate of graphene and contains hydroxyl, epoxide and carboxyl groups on its surface that can adsorb sulfonamides via hydrogen bonding [9,27,28]. In addition, the existence of π-conjugated structure also endows GO with a strong affinity for benzenoid structures [29]. Another material that can improve the adsorption of sulfonamides from aqueous samples is a conducting polymer such as polypyrrole, which adsorbs benzenoid compounds via hydrogen bonding, π–π and hydrophobic interactions [30].

This work has focused on a synthesis of polypyrrole-coated GO that was incorporated into a PVA cryogel as a newly-designed hybrid monolith sorbent for the extraction and preconcentration of trace sulfonamides. Combining the high adsorption properties of the polypyrrole and graphene oxide, it not only improves the extraction efficiency and enrichment of sulfonamides but also reduces matrix interferences. This composite monolith porous materials facilitates to minimize any back pressure in a test cartridge. Due to their toxicity, sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SME), sulfamethazine (SMT), sulfamonomethoxine (SMM), and sulfadimethoxine (SDM) were selected as test compounds to investigate the performance of the developed method.

## 2. Experimental

### 2.1. Chemicals and reagents

Poly (vinyl alcohol) (PVA) (MW 72,000 g $mol^{-1}$, ≥98% hydrolyzed) and acetic acid were from Merck (Darmstadt, Germany). Acetonitrile, methanol, pyrrole, glutaraldehyde, iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), graphene oxide powder, 15–20 sheets, 4–10% edge-oxidized, sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SME), sulfamethazine (SMT), sulfamonomethoxine (SMM), sulfadimethoxine (SDM), were purchased from Sigma–Aldrich (Steinheim, Germany). Deionized water was from a Maxima ultrapure system (ELGA, Buckinghamshire, England). The GF/F Glass Microfiber filter was from Whatman International Ltd (Maidstone, English). The HLB cartridge was from Waters (Milford, USA).

### 2.2. Instrumentals

Chromatographic separation and determination of the sulfonamides was performed on the 1100 series (Agilent Technologies Inc., Germany) and the data were acquired using ChemStation software. The separation was conducted on a VertiSep™pHendure C18 analytical column, 5 μm, 150 mm × 4.6 mm i.d. (Vertical chromatography Co., Ltd., Bangkok, Thailand). The mobile phase consisted of (A) water and (B) acetonitrile. A gradient elution was conducted by changing the composition of the mobile phase as follows; 0–3 min, 16% B; 3–6 min, 16–35% B; 6–8 min, 35–40% B; 8–10 min, 40–45% B; 10–12 min, 45% B and 12–15 min, 45-16% B. The flow rate of the mobile phase was 1.0 mL $min^{-1}$. The injection volume and column temperature were 20 μL and 30 °C, respectively. All the target sulfonamides were detected at 270 nm. The FTIR spectra were determined by FTIR spectroscopy (PerkinElmer, Waltham, MA, USA). The morphology of the developed sorbent was studied by scanning electron microscopy (JSM-5200, JEOL, Tokyo, Japan). The surface area of the developed sorbent were determined from nitrogen adsorption and desorption isotherms using Quantachrome Autosorb 1 system (Quantachome Instruments, USA).

### 2.3. Preparation of hybrid monolith PPY/GO/PVA cryogel sorbent

The preparation method for the polypyrrole-coated graphene oxide entrapped in polyvinyl alcohol cryogel sorbent (PPY/GO/PVA cryogel) is shown in Fig. 1. PVA solution (3.0% w/v) was prepared by dissolving PVA powder in deionized water at 90 °C and stirring for 10 min to obtain a homogeneous solution. Then, the PVA solution was cooled at room temperature (27 ± 2 °C) and the pH was adjusted to 1.0 with 5.0 M HCl [31,32]. Subsequently, the graphene oxide powder was added into the PVA solution (0.050% w/v) and dispersed by ultrasonication for 10 min and 1.0 mL of the mixed solution was then poured into a polypropylene tube, 20.0 μL of glutaraldehyde (crosslinking agent) was added and the solution vortexed for 10 s to obtain a homogeneous solution. The mixed solution was then kept in the freezer at −20 °C for 12 h. The frozen composited GO/PVA cryogel was removed from the tube and thawed at room temperature (27 ± 2 °C). Then it was washed with deionized water until neutral pH was obtained.

For the polypyrrole coating on the composite GO/PVA cryogel was produced by placing the GO/PVA cryogel into 2-propanol and stirring at 500 rpm for 10 min. Then the residual 2-propanol was removed. The GO/PVA cryogel was transferred to the pyrrole solution, incubated for 30 min to saturate the cryogel with pyrrole monomer and then the residual pyrrole solution was removed. The polymerization of the polypyrrole was adopted from previous report [33], 0.60 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 2-propanol (20 mL) and added to a rotator tube containing the pyrrole saturated GO/PVA cryogel. The polymerization was performed on a rotator mixer at room temperature (27 ± 2 °C) for 2.0 h. The resulting hybrid monolith polypyrrole-coated GO/PVA cryogel sorbent was washed first with 10.0 mL of 2-propanol, then with methanol and finally with deionized water. Subsequently, the sorbent was packed in polypropylene cartridges (5.0 mL) and used as a solid phase extraction sorbent for the extraction of sulfonamides from solutions.

### 2.4. Solid phase extraction procedure

The developed hybrid monolith PPY/GO/PVA cryogel sorbent was packed into a 5.0 mL polypropylene tube between two membrane filters. The membrane filter was used to protect the sorbent from some matrix interferences in real samples and then the cartridge was connected with an SPE vacuum manifold. Before sample loading, the sorbent was conditioned with 2.0 mL of methanol and then with deionized water. The certain volumes of the samples were loaded through the sorbent at certain flow rates and then washed with 2.0 mL of deionized water. The retained sulfonamides were eluted from the PPY/GO/PVA cryogel sorbent with a known volume of methanol (eluent) at a flow rate of 1.0 mL $min^{-1}$. The collected eluent was then evaporated to dryness at 60 °C and the dry residue was redissolved with 1.0 mL of mobile phase. Finally, the resulting solution was filtered through a 0.22 μm disposable PTFE syringe filter and 20.0 μL was injected into the HPLC system for analysis.

### 2.5. Water samples

Tap water samples were collected from the laboratory, river water samples were collected from two rivers in Songkhla province, two samples of livestock wastewater were collected from Songkhla province and lake water samples were collected from Songkhla Lake, Songkhla province, Thailand. All samples were filtered through 0.45 μm nylon membrane filters and stored at 4 °C until analysis.

## 3. Results and discussions

### 3.1. Characterization of hybrid monolith PPY/GO/PVA cryogel sorbent

The morphologies of the PVA cryogel, composite GO/PVA cryogel and hybrid monolith PPY/GO/PVA cryogel were investigated

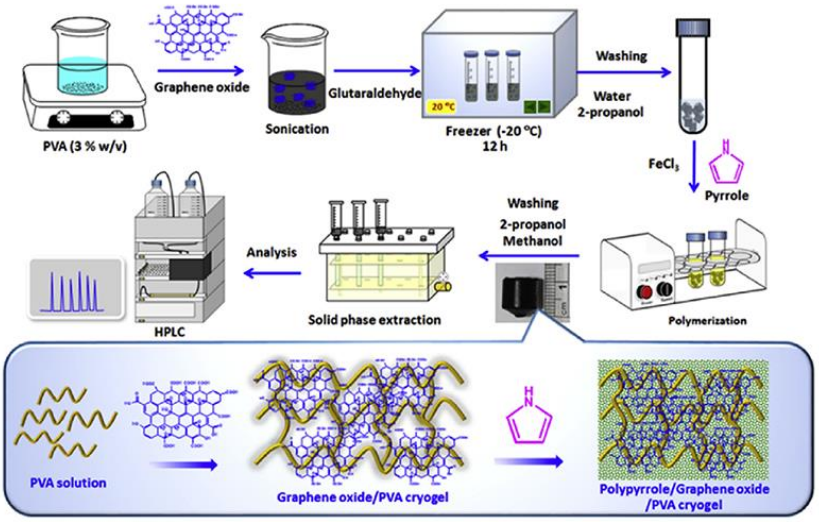


**Fig. 1.** Schematic diagram of the preparation of hybrid monolith PPY/GO/PVA cryogel sorbent.

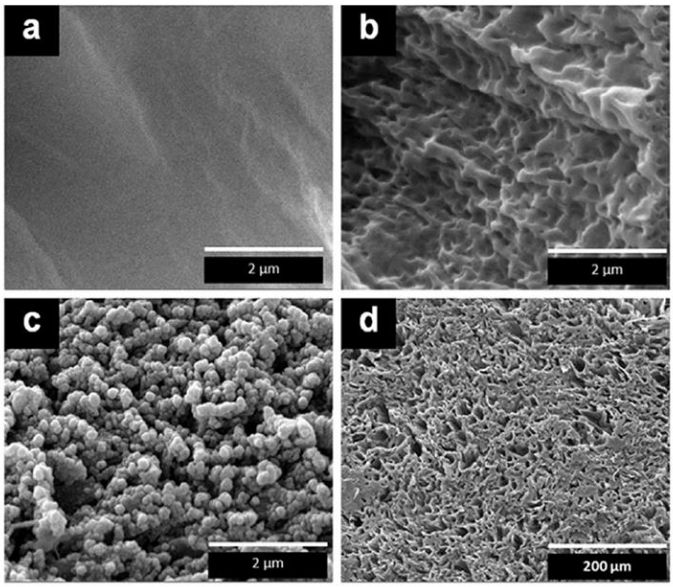


**Fig. 2.** SEM images of the PVA cryogel (a), composite GO/PVA cryogel (b), hybrid monolith PPY/GO/PVA cryogel (c) and cross section of hybrid monolith PPY/GO/PVA cryogel sorbent (d).

by SEM technique and are illustrated in Fig. 2. The SEM images show that the cryogel sorbent had a smooth surface (Fig. 2a). The SEM image of the composite GO/PVA cryogel (Fig. 2b) showed a rough surface which indicates that the GO was well distributed and entrapped in the PVA cryogel. Fig. 2c shows the SEM image of a hybrid monolith PPY/GO/PVA cryogel displaying the polypyrrole layer's typical "cauliflower" morphology on the surface of the GO/PVA cryogel. The nanostructure of the polypyrrole helped to increase the surface area for adsorption of the target analytes. The cross-sectional SEM image (Fig. 2d) shows that the hybrid monolith PPY/GO/PVA cryogel sorbent had the high porosity. The pore structure benefitted from its increasing mass transfer and also by decreasing the back pressure during sample loading.

FTIR spectra of the GO, PVA cryogel, GO/PVA cryogel and monolith hybrid PPY/GO/PVA cryogel are shown in Fig. S1. Graphene oxide showed a characteristic peak at 1624 $cm^{-1}$ and 1401 $cm^{-1}$ were corresponded to the C=O stretching and deformation vibration from carboxyl. The absorption band at 3420 $cm^{-1}$ and 1262 $cm^{-1}$ were attributed to the O–H stretching and C–O stretching vibrations from-COOH group. The FTIR spectrum of PVA exhibited the absorption band at 3322 $cm^{-1}$ and 2946 $cm^{-1}$ corresponding to the hydroxyl groups and the $-CH_2-$ asymmetric stretching. The absorption band at 1432 $cm^{-1}$ were attributed to the O–H and C–H bending. The peak at 1096 $cm^{-1}$ was attributed to C–O group. The absorption peak at 1633 and 1570 $cm^{-1}$ were attributed to the absorption peaks of pyrrole ring. The peak at 923 $cm^{-1}$ was attributed to C–H wagging. It indicates that polypyrrole was successfully coated onto the surface of composited GO/PVA cryogel sorbent.

The BET specific surface area of different sorbents were also determined by $N_2$ adsorption-desorption isotherms. The surface area of the PVA cryogel, GO/PVA cryogel, PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cyogel equal to 16.57, 18.51, 24.34 and 34.52 $m^2g^{-1}$, respectively. This results indicated that polypyrrole coated graphene oxide incorporated into PVA cryogel can improve the surface area and extraction efficiency of the target analytes.

### 3.2. Optimization of solid phase extraction

The highest extraction efficiency, the lowest solvent consumption and the shortest sample preparation time were then carefully investigated. The parameters that may affect the extraction efficiency of the sulfonamides using the hybrid monolith PPY/GO/PVA cryogel sorbents included the type of sorbent, the polymerization time, concentration of graphene oxide, the desorption conditions, the sample pH, the sample flow rate, and the sample volume and ionic strength were optimized. The recovery was used to evaluate the extraction efficiency of the developed method. All optimization experiments were performed in triplicate.

#### 3.2.1. Effect of the type of sorbent

The extraction efficiency of sulfonamides using the PVA cryogel, composite GO/PVA cryogel, composite PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cryogel sorbent were first investigated. The results indicated that the extraction efficiency of the hybrid monolith PPY/GO/PVA cryogel sorbent was higher than those obtained using the PVA, GO/PVA and PPY/PVA sorbent (Fig. 3). The adsorption capacity of the PVA cryogel, composite GO/PVA cryogel, composite PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cryogel sorbent for sulfadiazine equal to 0.42, 1.11, 3.72 and 6.36 mg $g^{-1}$, respectively. The improvement of the extraction efficiency and adsorption capacity of the hybrid monolith PPY/GO/PVA cryogel sorbent for the extraction of sulfonamide were due to the combination of GO and PPY which increase the surface area and multiply the adsorption sites. Sulfonamides can be adsorbed via hydrogen bonding, hydrophobic and π-π interaction. Therefore, hybrid monolith PPY/GO/PVA cryogel was chosen as SPE sorbent for the extraction and enrichment of sulfonamides.

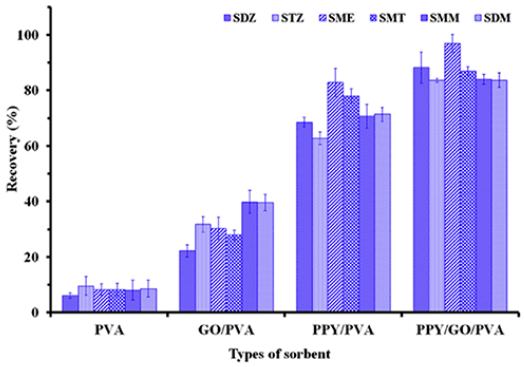


**Fig. 3.** Comparison of the recovery of the PVA, GO/PVA, PPY/PVA and PPY/GO/PVA sorbent for extraction of sulfonamides.

### 3.2.2. *Effect of polymerization time and concentration of graphene oxide*

The polymerization time is one of the important parameters that affects the thickness of the polypyrrole layer and further affects the extraction capacity of the sorbent. Therefore, the effect of the polymerization time was investigated and the results are shown in Fig. S2, the extraction efficiency increased when the polymerization time increase from 1.0 to 2.0 h probably due to the increasing amount of the polypyrrole particles. However, the extraction efficiency decreased when the polymerization time was longer than 2.0 h. The reason may be that the polypyrrole layer was too dense (Fig. S3), resulting in a reduced surface area and adsorption sites. The surface area of hybrid monolith PPY/GO/PVA cryogel sorbent at the polymerization time of 1.0, 2.0, 4.0 and 6.0 h were 30.50, 34.52, 30.40 and 23.48 $m^2g^{-1}$ respectively. Thus, the polymerization time of 2.0 h was enough to achieve a satisfactory recovery and was selected for subsequent experiments.

The concentration of graphene oxide was investigated in the concentration range of 0.010–0.10% w/v. The extraction efficiencies of sulfonamides increased as the concentration of graphene oxide increased from 0.010 to 0.050 %w/w, and it remained almost constant with any further increase of graphene oxide concentration (Fig. S4). Thus, the optimal concentration of graphene oxide of hybrid monolith PPY/GO/PVA cryogel sorbent was 0.050 %w/v.

### 3.2.3. *Desorption condition*

After the extraction was completed, the adsorbed sulfonamides were eluted from the sorbent. To obtain the highest eluting efficiency, several solvents with different polarities were investigated *i.e.* acetonitrile, methanol, ethyl acetate and dichloromethane with the polarity index of 5.8, 5.1, 4.3 and 3.1, respectively. Since sulfonamides are polar compounds, their elution with slightly more polar solvents is better than with less polar solvents (Fig. 4a). In this work, methanol gave the highest recovery, therefore, it was selected as the desorption solvent in subsequent experiments. The effect of the desorption solvent volume was also investigated. The result indicated that 2.0 mL of methanol was sufficient for the desorption of all target sulfonamides (Fig. 4b).

### 3.2.4. *Effect of sample pH*

The sample pH is an important factor affecting chemical species of target analytes and also the effective surface charge of the sorbent, which can affect the extraction efficiency of target analytes. Sulfonamides are amphoteric compounds that can have cationic, neutral or anionic forms. Therefore, the pH of the sample solution needs to be investigated and optimized. In this work, the sample pH was adjusted with HCl or NaOH in the range of 3.0–11.0. As shown in Fig. 4c, there was no significant difference in the recovery of sulfonamides between pH 3.0 and pH 9.0, in which range sulfonamides are in a neutral form, and the recovery decreased at a pH higher than 9.0 due to their having an anionic form in that pH range [34,35]; carboxyl acid group of graphene oxide could also be deprotonated under the same condition [36]. Therefore, the presence of repulsive interaction between the sulfonamides and the sorbent resulted in decreasing extraction efficiency. These results indicated that the sulfonamides adsorbed on the surface polypyrrole and graphene oxide through hydrogen bonding, π-π and hydrophobic interaction. Considering that the pH of the real samples was lower than 9.0, therefore no need to adjust the sample pH.

### 3.2.5. *Effect of sample volume*

Although high enrichment factors were obtained with increasing sample volume, the enrichment was limited by the number of available adsorption sites of the sorbent. In this work, influence of sample volume on the extraction efficiency of sulfonamides was investigated in the range of 5.0–40.0 mL. As shown in Fig. 4d, the recovery was reduced when the sample volume was greater than 20.0 mL. Considering the enrichment factor and extraction efficiency, 20.0 mL was chosen as an optimum sample volume for the next experiment.

### 3.2.6. *Effect of sample flow rate*

For the SPE procedure, a fast sample flow rate would not allow the analytes to be adsorbed completely onto the SPE sorbents, while a slow sample flow rate would make the extraction time too long. Therefore, the sample flow rate needs to be optimized to obtain the fastest flow rate that provides the highest extraction efficiency. The influence of the sample flow rate on the recovery of sulfonamides was investigated in the range of 0.5–5.0 mL $min^{-1}$. As shown in Fig. S5, the extraction efficiency decreased when the sample flow rate was faster than 2.0 mL $min^{-1}$. Therefore, the 2.0 mL $min^{-1}$ was selected as the optimum sample flow rate.

### 3.2.7. *Effect of salt concentration*

The content of salt may affect the partitioning of the analytes between sorbent and sample solution, which can enhance or decrease the extraction efficiency. Therefore, the influence of salt addition on the extraction efficiency of sulfonamides was investigated by the addition of NaCl in spiked deionized water in the range of 0.0–15.0% w/v. The results are shown in Fig. S6. The salt content of up to 2.0% w/v did not have a significant effect on the extraction efficiency of sulfonamides and the recovery decreased when the concentration of salt was higher than 2.0% w/v. This may due to the increased viscosity of the sample solution and the consequent reduction in the mass transfer of analytes to the sorbent and diminished adsorption ability of the developed sorbent. Therefore, further experiments were performed without salt addition.

## 3.3. *Analytical performance*

To evaluate the analytical performance of the developed method for the extraction and determination of sulfonamides. Under the optimum conditions, the linearity, limit of detection (LOD) and limit of quantification (LOQ) were investigated and the results are summarized in Table 1. Good linearity was obtained in the range of 0.20–100.0 μg $L^{-1}$ for SDZ, STZ, and SME; and in the range of 0.10–100.0 μg $L^{-1}$ for SMT, SMM and SDM with a coefficients of determination ($R^2$) greater than 0.99 and the relative standard deviations (RSD) less than 8.0%. The LOD and LOQ based on the

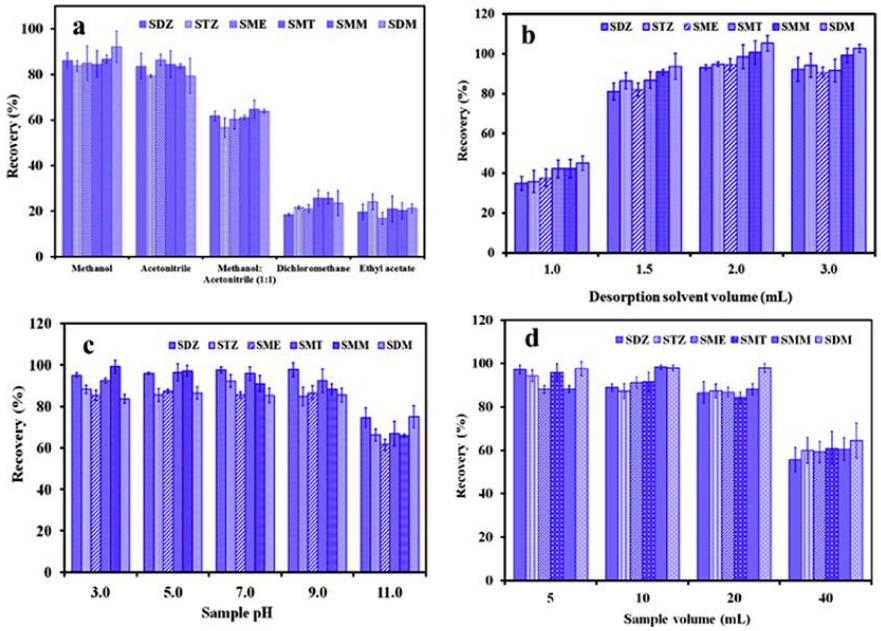


**Fig. 4.** Effect of desorption solvent (a), desorption solvent volume (b), sample pH (c) and sample volume (d) on the recovery of sulfonamides using the hybrid monolith PPY/GO/PVA cryogel sorbent.

signal-to-noise ratios of 3 and 10 were 0.20 and 0.80 μg $L^{-1}$ for the SDZ, STZ, and SME; and 0.10 and 0.40 μg $L^{-1}$ for the SMT, SMM and SDM. It can be concluded that the developed method can be used for the determination of trace sulfonamides in real samples.

### 3.4. Real samples analysis

The developed hybrid monolith PPY/GO/PVA cryogel sorbent was applied for the extraction of sulfonamides from different water samples including tap water, river water, livestock wastewater and lake water. The results are shown in Table S1. SMM was detected in livestock wastewater at a concentration of lower than the LOQ and SMT was also detected in livestock wastewater in the concentration range of lower than LOQ to 0.77 ± 0.03 μg $L^{-1}$. To evaluate the accuracy of the developed method, the water samples were spiked with sulfonamides at three concentrations (1.00, 5.00 and 20.00 μg $L^{-1}$) and then extracted and analyzed under the optimum conditions. The results are shown in Table S2. The recoveries of all target sulfonamides were in the range of 85.5–99.0% with relative standard deviations less than 5%. The satisfactory recoveries were within the acceptable range as recommended by the AOAC (70–120%) [37]. The chromatograms of spiked wastewater samples

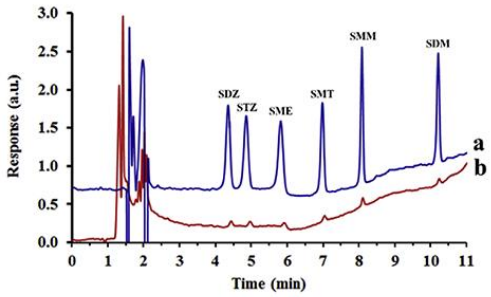


**Fig. 5.** Typical chromatograms of spiked wastewater sample (1.0 μg $L^{-1}$) with (a) and without (b) extraction using the hybrid monolith PPY/GO/PVA cryogel sorbent.

**Table 1**
Analytical performance for the determination of sulfonamides using the hybrid monolith PPY/GO/PVA cryogel sorbent.

| Sulfonamides | Linear range (μg $L^{-1}$) | Regression line equation | $R^2$ | LOD[a] (μg $L^{-1}$) | LOQ[b] (μg $L^{-1}$) | RSD (%) |
|---|---|---|---|---|---|---|
| SDZ | 0.20–100 | y = (0.082 ± 0.003)x + (3.10 ± 2.29) | 0.9926 | 0.20 | 0.80 | 0.7–6.1 |
| STZ | 0.20–100 | y = (0.081 ± 0.003)x + (2.77 ± 2.15) | 0.9932 | 0.20 | 0.80 | 0.8–5.5 |
| SME | 0.20–100 | y = (0.084 ± 0.003)x + (3.32 ± 2.51) | 0.9938 | 0.20 | 0.80 | 1.2–7.1 |
| SMT | 0.10–100 | y = (0.073 ± 0.003)x + (2.06 ± 2.12) | 0.9921 | 0.10 | 0.40 | 1.6–5.6 |
| SMM | 0.10–100 | y = (0.080 ± 0.003)x + (2.79 ± 2.39) | 0.9917 | 0.10 | 0.40 | 2.0–6.3 |
| SDM | 0.10–100 | y = (0.074 ± 0.003)x - (2.37 ± 2.15) | 0.9920 | 0.10 | 0.40 | 2.0–5.7 |

[a] LOD is the lowest concentration of an analyte that can be detected, but not necessarily quantified.
[b] LOQ is the lowest concentration of an analyte that can be determined with acceptable precision and accuracy.

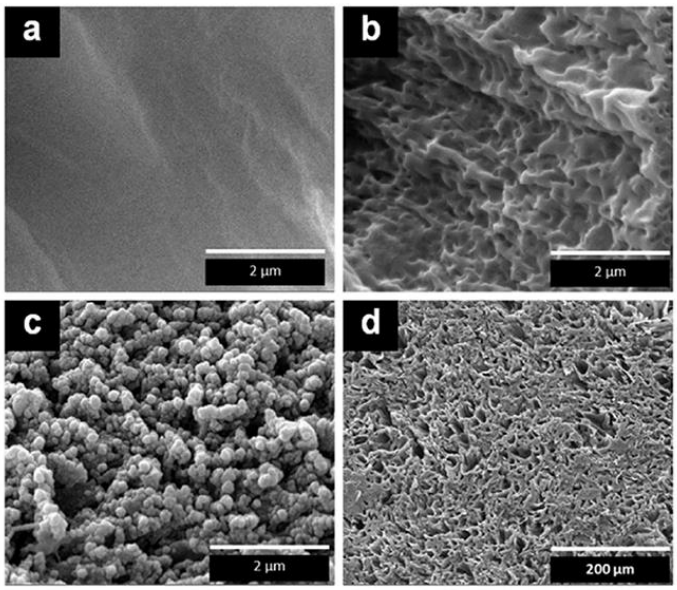


**Fig. 2.** SEM images of the PVA cryogel (a), composite GO/PVA cryogel (b), hybrid monolith PPY/GO/PVA cryogel (c) and cross section of hybrid monolith PPY/GO/PVA cryogel sorbent (d).

by SEM technique and are illustrated in Fig. 2. The SEM images show that the cryogel sorbent had a smooth surface (Fig. 2a). The SEM image of the composite GO/PVA cryogel (Fig. 2b) showed a rough surface which indicates that the GO was well distributed and entrapped in the PVA cryogel. Fig. 2c shows the SEM image of a hybrid monolith PPY/GO/PVA cryogel displaying the polypyrrole layer's typical "cauliflower" morphology on the surface of the GO/PVA cryogel. The nanostructure of the polypyrrole helped to increase the surface area for adsorption of the target analytes. The cross-sectional SEM image (Fig. 2d) shows that the hybrid monolith PPY/GO/PVA cryogel sorbent had the high porosity. The pore structure benefitted from its increasing mass transfer and also by decreasing the back pressure during sample loading.

FTIR spectra of the GO, PVA cryogel, GO/PVA cryogel and monolith hybrid PPY/GO/PVA cryogel are shown in Fig. S1. Graphene oxide showed a characteristic peak at 1624 $cm^{-1}$ and 1401 $cm^{-1}$ were corresponded to the C=O stretching and deformation vibration from carboxyl. The absorption band at 3420 $cm^{-1}$ and 1262 $cm^{-1}$ were attributed to the O–H stretching and C–O stretching vibrations from-COOH group. The FTIR spectrum of PVA exhibited the absorption band at 3322 $cm^{-1}$ and 2946 $cm^{-1}$ corresponding to the hydroxyl groups and the $-CH_2-$ asymmetric stretching. The absorption band at 1432 $cm^{-1}$ were attributed to the O–H and C–H bending. The peak at 1096 $cm^{-1}$ was attributed to C–O group. The absorption peak at 1633 and 1570 $cm^{-1}$ were attributed to the absorption peaks of pyrrole ring. The peak at 923 $cm^{-1}$ was attributed to C–H wagging. It indicates that polypyrrole was successfully coated onto the surface of composited GO/PVA cryogel sorbent.

The BET specific surface area of different sorbents were also determined by $N_2$ adsorption-desorption isotherms. The surface area of the PVA cryogel, GO/PVA cryogel, PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cyogel equal to 16.57, 18.51, 24.34 and 34.52 $m^2g^{-1}$, respectively. This results indicated that polypyrrole coated graphene oxide incorporated into PVA cryogel can improve the surface area and extraction efficiency of the target analytes.

### 3.2. Optimization of solid phase extraction

The highest extraction efficiency, the lowest solvent consumption and the shortest sample preparation time were then carefully investigated. The parameters that may affect the extraction efficiency of the sulfonamides using the hybrid monolith PPY/GO/PVA cryogel sorbents included the type of sorbent, the polymerization time, concentration of graphene oxide, the desorption conditions, the sample pH, the sample flow rate, and the sample volume and ionic strength were optimized. The recovery was used to evaluate the extraction efficiency of the developed method. All optimization experiments were performed in triplicate.

#### 3.2.1. Effect of the type of sorbent

The extraction efficiency of sulfonamides using the PVA cryogel, composite GO/PVA cryogel, composite PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cryogel sorbent were first investigated. The results indicated that the extraction efficiency of the hybrid monolith PPY/GO/PVA cryogel sorbent was higher than those obtained using the PVA, GO/PVA and PPY/PVA sorbent (Fig. 3). The adsorption capacity of the PVA cryogel, composite GO/PVA cryogel, composite PPY/PVA cryogel and hybrid monolith PPY/GO/PVA cryogel sorbent for sulfadiazine equal to 0.42, 1.11, 3.72 and 6.36 mg $g^{-1}$, respectively. The improvement of the extraction efficiency and adsorption capacity of the hybrid monolith PPY/GO/PVA cryogel sorbent for the extraction of sulfonamide were due to the combination of GO and PPY which increase the surface area and multiply the adsorption sites. Sulfonamides can be adsorbed via hydrogen bonding, hydrophobic and π-π interaction. Therefore, hybrid monolith PPY/GO/PVA cryogel was chosen as SPE sorbent for the extraction and enrichment of sulfonamides.

**Table 2**
Comparison of the developed method with other methods for the determination of sulfonamides.

| Method | Adsorbent | Sample | LOD (μg $L^{-1}$) | Recovery (%) | Reference |
|---|---|---|---|---|---|
| HPLC-UV | $CoFe_2O_4$–graphene | Milk | 1.59 | 62–104 | [6] |
| HPLC-DAD | Magnetite–embedded with silica functionalized with phenyl chains | Milk | 7.0–14.0 | 81.9–115.0 | [8] |
| HPLC-UV | Microwave–assisted liquid–liquid microextraction | Water | 0.33–0.85 | 75.1–115.8 | [11] |
| HPLC-DAD | Molecularly imprinted polymers | Water | 0.2–3.0 | 70–120 | [34] |
| HPLC-DAD | Micro–solid phase extraction (μ-SPE) using $TiO_2$ nanotube arrays | Water | 0.27–0.60 | 82.8–101.7 | [38] |
| HPLC-UV | Polypropylene membrane protected micro–solid phase extraction (MP–μ–SPE) | Milk | 0.38–0.62 | 71.3–83.8 | [39] |
| HPLC-UV | Magnetic molecularly imprinted polymers (MMIPs) | Water | 0.76–1.2 | 61.2–94.1 | [40] |
| HPLC-DAD | Hybrid monolith PPY/GO/PVA cryogel | Water | 0.1–0.2 | 85.5–99.0 | This work |

with and without extraction using hybrid monolith PPY/GO/PVA cryogel sorbent are shown in Fig. 5. The results indicated that extraction using the developed method can improve the method detection limit and can be used to detect trace sulfonamides.

### 3.5. Reproducibility and reusability

The reproducibility of the hybrid monolith PPY/GO/PVA cryogel sorbent was investigated in terms of lot-to-lot reproducibility by the preparation of six different lots under the same conditions. These sorbents were then applied to extract sulfonamides under the optimum conditions. The relative standard deviations of the average recovery from the six different lots were lower than 10% (Fig. S7). This result indicated a good reproducibility of the sorbent preparation.

The reusability of the hybrid monolith PPY/GO/PVA cryogel sorbent was also investigated by spiking sulfonamides in wastewater samples. After the first use, the sorbent was washed with 2.0 mL of methanol and then the carryover effects were investigated. No target analytes peaks were detected, which indicated that the developed sorbent could be reused. As shown in Fig. S8, the hybrid monolith PPY/GO/PVA cryogel sorbent could be reused at least 10 times without loss of the extraction efficiency (>80%). These results also indicated that the coating of polypyrrole on the surface of the graphene oxide incorporated in the PVA cryogel has a good stability. After 10 cycles, the recovery of sulfonamides was decreased may be due to the losing of polypyrrole particles from the sorbent (Fig. S9). On the other hand, traditional particle-packed SPE sorbent cartridges cannot be reused due to the difficulty of completely removing adsorbed interferences.

### 3.6. Comparison between the hybrid monolith PPY/GO/PVA cryogel sorbent and a traditional packed SPE cartridge

The extraction efficiency of the hybrid monolith PPY/GO/PVA cryogel sorbent was compared to a traditional packed SPE cartridge, HLB sorbent. The recoveries for the hybrid monolith PPY/GO/PVA cryogel sorbent of SDZ, STZ, SME, SMT, SMM and SDM were 88.1 ± 5.0, 86.6 ± 3.8, 96 ± 3.2, 87.0 ± 1.5, 85.6 ± 3.5, 87.6 ± 2.6%, respectively. For the traditionally packed SPE cartridge, the recoveries were 72.6 ± 4.0, 72.0 ± 4.9, 80.3 ± 3.3, 76.0 ± 5.6, 79.7 ± 6.0, and 71.0 ± 4.4%, respectively. The better recoveries were obtained using the hybrid monolith PPY/GO/PVA cryogel sorbent due to its larger surface area and high adsorption sites.

### 3.7. Comparison with other extraction methods

The analytical performance of the developed method for the determination of sulfonamides based on the hybrid monolith PPY/GO/PVA cryogel sorbent was compared with other reported methods (Table 2). The LODs of the developed method were lower than those of the previous methods [6,8,11,34,38–40]. The extraction efficiency of the developed method provided results better than [6,11,34,39,40] or comparable with the other methods [8,38]. These results have demonstrated that the developed method is highly sensitive and accurate for the determination of trace sulfonamides in water samples. In addition, the hybrid monolith PPY/GO/PVA cryogel sorbent can be reused at least 10 times which also helps to reduce the analysis costs.

## 4. Conclusions

In this work, a hybrid monoliths of polypyrrole-coated graphene oxide incorporated into a PVA cryogel were successfully prepared and applied for the extraction and enrichment of six sulfonamides from different water samples. The combination of polypyrrole and graphene oxide helped to improve the extraction efficiency of the sulfonamides due to their high specific surface area and the greater adsorption by the sites for the target analytes. The high porosity of the PVA cryogel helped to prevent the high back pressure that normally occurs with conventional packed SPE cartridges. The results showed that the developed method offers good sensitivity, accuracy and precision. The satisfactory recoveries from different water samples indicated that the developed method can be used as an efficient extraction and preconcentration method for the determination of trace sulfonamides in water samples.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2017.01.052.

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# Paper V

**Chullasat, K.**, Huang, Z., Bunkoed, O., Kanatharana, P., Lee, H. K., Bubble-in-single drop microextraction for the analysis of carbamate pesticides from water samples.

(Manuscript)

# Bubble-in-single drop microextraction for the analysis of carbamate pesticides from water samples

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**Abstract**

Solvent less bubble-in-single drop microextraction (BI-SDME) coupled with gas chromatography-mass spectrometry was developed and used for the extraction of carbamate pesticides from environmental water samples. The desirable condition affecting the BI-SDME performance including type of extraction solvent and its volume, volume of air bubble, extraction time, stirring rate, ionic strength and sample pH were optimized. In BI-SDME, the increasing of surface area of extraction solvent when encapsulated air bubble in droplet causing the greater extraction efficiency. Under the optimal condition, the developed method showed good linearity in the range of 0.05 to 20.0 μg $L^{-1}$ with the coefficients of determination ($R^2$) higher than 0.99. The limit of detections and limit of quantifications were obtained in the concentration range of 0.02 - 0.04 μg $L^{-1}$ and 0.05 - 0.13 μg $L^{-1}$, respectively. This method was successfully applied for the determination of carbamate pesticides in water samples with good recoveries were achieved from 81.7% to 99.0% and the relative standard deviation less than 9%. This developed method was demonstrated to enhance the enrichment factor for the determination of trace carbamate pesticides in water sample. The advantages of this method are solvent less, environmental friendly, low cost and simple to operate.

Keywords: Bubble-in-drop, Microextraction, Carbamate, Pesticides

## 1. Introduction

Carbamates are a group of pesticide that have been extensively used as insecticide or herbicide to improve agriculture products in many countries [1, 2]. The extensively used of carbamates leading to the residue in environment and agriculture products. Although carbamates are a broad biological activity with relatively short persistence lifetime under natural condition comparing organochlorine and organophosphate, however, some carbamates and their metabolites are suspected to be carcinogens and mutagen [3, 4]. The structure of carbamates are derived from carbamic acid which act as inhibitors of acetylcholinesterase activity (AChE) and prevent acetylcholine to build up [5, 6]. The exposure of carbamates can cause some adverse effects in human such as headaches, vomiting, diarrhea, urinary incontinence, bronchospasm, and dyspnea [7, 8]. The European Union directive on drinking water quality established a maximum permissible concentration for individual pesticides of 0.1 μg $L^{-1}$ and 0.5 μg $L^{-1}$ for total pesticides [9]. Therefore, the monitoring of carbamates is an important.

Several method have been reported for the determination of carbamates such as high performance liquid chromatography [10-12] and gas chromatography with a various detector [13-15]. However, the residue of carbamates in environment are at trace level and presented of various matrix interferences, the sample pretreatment and preconcentration steps are normally required prior to instrumental analysis. Several sample preparation methods have been developed for the extraction of carbamate pesticides such as stir bar sorptive extraction [12], hollow fiber liquid-phase microextraction [16], liquid-phase microextraction [13], dispersive liquid–liquid microextraction (DLLME) [17, 18] and single-drop microextraction (SDME) [3, 19]. Among of these method, SDME has attracted much attention due to it provided high extraction efficiency using small amount of extraction solvent and also simply operated with a common laboratory equipment, inexpensive and able to use a small amount of sample and compatibility with GC [20-22]. SDME has been developed in two modes depending on the analyte and sample properties including direct immersion (DI-SDME) and headspace (HS-SDME). Carbamates are moderated polar, low vapor pressure and low water solubility [23, 24], appropriated analysis with DI-SDME. A microdroplet (normally less than 3 μL) of water-immiscible solvent hanging on the needle tip of

syringe is directly immersed in the sample solution, and the extraction efficiency is generally achieved by the stirring. The target analyte can passive diffuse into the extraction solvent. After extraction, the microdroplet is withdrawn back into the microsyringe and directly injected to GC or HPLC [25, 26]. However, the sensitivity of method need to be improved for trace residue analysis. In recent year, the increasing surface area of microdroplet has been developed by introducing the known volume of air into the microdroplet resulting a bubble in the droplet which can improve the efficiencies [27, 28]. In addition, the increasing surface area, the occurring thin film phenomena also improved the extraction efficiency [29, 30]. This method called "Bubble-in-single drop" microextraction (BI-SDME).

In the present study, we proposed BI-SDME for the determination of eight carbamate pesticides consist of propham, chlorpropham, promecarb, carbofuran, aminocarb, pirimicarb, carbaryl and methiocarb. The extraction parameters affecting the extraction efficiency such as extraction solvent, volume of extraction solvent and bubble, extraction time, stirring rate, ionic strength, and sample pH were optimized. The performances of this method were evaluated and applied to detect the residue of carbamates in environmental water sample.

## 2. Materials and methods

### 2.1 Chemicals and reagents

Propham, chlorpropham, promecarb, carbofuran, aminocarb, pirimicarb, carbaryl and methiocarb were purchased from Chem Service (West Chester, PA). All of organic solvents were high performance liquid chromatography grade. Trichloromethane and cyclohexane were purchased from Tedia Company (Fairfield, OH, USA). Toluene and butyl acetate were purchased from Fisher (Loughborough, UK) and iso-octane was obtained from Merck (Darmstadt, Germany). Sodium chloride was supplied by Goodrich Chemical Enterprise (Singapore). Ultrapure water was produced on a ELGA Purelab Option-Q (High Wycombe, UK).

The individual stock solutions of each standard carbamates were prepared by dissolving in methanol at 1000 mg $L^{-1}$ and stored at 4 °C. Working standard solution were prepared daily by mixing all of stock standard solution in methanol.

**2.2 Instrumental**

Gas chromatographic analysis was carried out using a Shimadzu QP2010 GC-MS system (Kyoto, Japan) and a DB-5MS fused silica capillary column (30 m × 0.25 mm. i.d., 0.25 μm film thickness) (J&W scientific, Folsom, CA). High purity (99.999%) of helium gas was used as the carrier gas at constant flow rate of 1.0 mL $min^{-1}$. The injection (1.0 μL) were carried out in the splitless mode; after 1 min, a split ratio of 1: 10 was maintained throughout the run. The injection temperature was kept at 270 °C. The oven programming included an initial temperature of 60 °C (held for 3 min), the temperature increased by 10 °C $min^{-1}$ to 140 °C, then ramped by 8 °C $min^{-1}$ to 200 °C, following ramped by 15 °C $min^{-1}$ to 230 °C and held for 2 min. The total time for one GC run was 22.50 min. The MS system was operated in the electron impact ionization mode, and the interface temperature was set at 280 °C. A mass range of *m/z* 50 – 350 was scanned to confirm the retention time of the analytes and selective ion monitoring (SIM) mode was performed with the molecular ion and one to two characteristic fragment ions of each compound as follow: *m/z* 41, 43, 93 for propham; *m/z* 43, 127, 213 for chlorpropham; *m/z* 135, 150, 91 for promecarb; *m/z* 164, 149, 57 for carbofuran; *m/z* 151, 150, 136 for aminocarb; *m/z* 166, 72, 238 for pirimicarb; *m/z* 144, 115, 116 for carbaryl and *m/z* 168, 153, 109 for methiocarb.

**2.3 Bubble in drop microextraction procedure**

A 10 μL Hamilton GC microsyringe with bevel tip (Reno, Nevada) was used to perform the BI-SDME experiments for the extraction of carbamates from water sample. Before extraction, the microsyringe was washed three times with methanol and the extraction solvent. Firstly, a specific volume of air was drew into the microsyringe, following the known volume of organic solvent; the plunger is depressed. The microsyringe held with a stand and clamps then insert through the septum of the sample vial and immerse into the sample solution. The bevel tip of the extraction needle protruded to a depth of 1.0 cm below the surface of the sample solution (Fig. 1a). Then, these contents were introduced into the aqueous solution by gentle depression of the plunger, resulting the air to form a bubble contained within the microdroplet (Fig. 1b). The sample solution was agitated with magnetic stirrer for extraction. After extraction,

the organic solvent was retracted into the syringe and directly injected into the GC-MS (Fig. 1c).

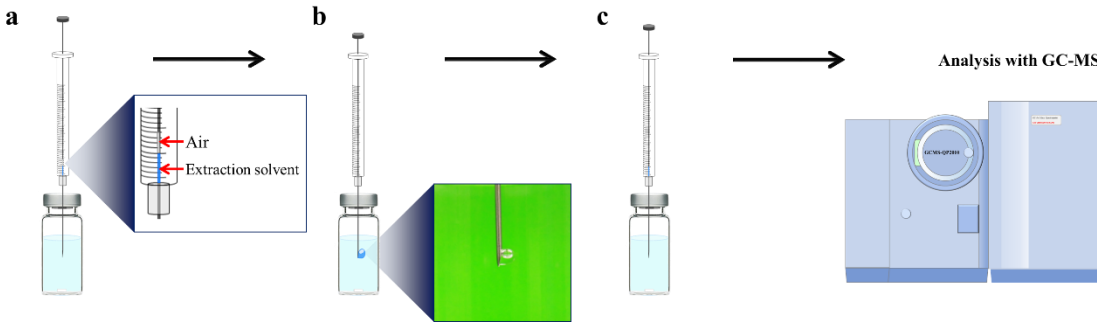


Fig. 1. Schematic of bubble-in-single drop microextraction (BI-SDME): (a) the obtained air following with extraction solvent in the syringe was moved to the sample solution, (b) the bubble-in-drop microdroplet was hold on the bevel tip for extraction step and (c) the extracted carbamates in extraction solvent was withdrawn by depressing of the plunger.

### 2.4 Sample preparation

The water samples including tap water, river water and waste water were collected from residential area in Singapore. The water samples were filtered through a 0.45 μm nylon membrane filter (Millipore Corporation, Billerica) to remove all particulate materials, which may cause unstable microdroplet.

## 3. Results and discussion

To achieve the best extraction efficiency, the extraction parameters including the types of extraction solvent, the volume of extraction solvent, volume of bubble in drop, extraction time, stirring rate, ionic strength and sample pH were optimized. Three replicate tests of the water samples (10 μg $L^{-1}$ for each carbamate pesticide) were used to perform the extraction efficiency of the method. The extraction efficiency of the developed method was evaluated in term of enrichment factors (EFs) which can be calculated by the ratio between the concentration of analyte in organic solvent phase ($C_{a,\,final}$) and the initial concentration of analyte in the water sample ($C_{s,\,initial}$) according to the following equation.

$$EFs = C_{a,final} / C_{s,initial}$$

The initial experiment was performed using 1.00 μL of extraction solvent, 0.40 μL of bubble, extraction time 15 min and stirring rate was 100 rpm.

### 3.1 Type of extraction solvent

The principle for selection of the extraction solvent is based on "like dissolve like". The extraction solvent using in BI-SDME technique should be low solubility in water, less toxicity, low vapor pressure and provide good drop stability [31, 32]. Various types of organic solvent including trichloromethane, iso-octane, toluene, butyl acetate and cyclohexane were investigated. The highest enrichment factors of propham, chlorpropham and pirimicarb were obtained when using toluene as the extraction solvent while the butyl acetate exhibited the highest enrichment factor for promecarb, carbofuran, aminocarb, carbaryl and methiocarb (Fig. 2). Therefore, the combination of toluene and butyl acetate were investigated. Fig. 3. indicated that a mixture of toluene and butyl acetate in the ratio of 3: 2 (v/v) exhibited a good extraction efficiency. Therefore, the mixture of toluene and butyl acetate (3: 2 v/v) was chosen for subsequent experiment.

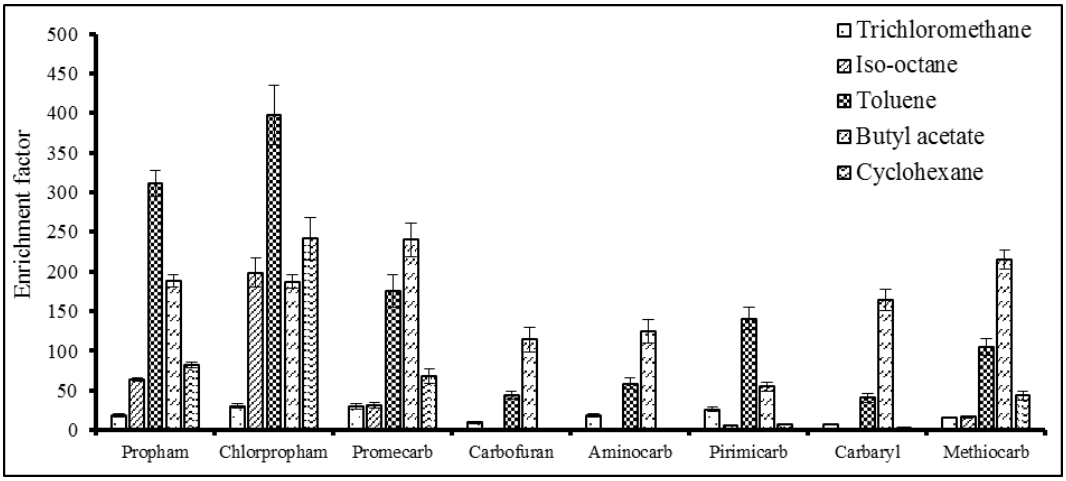


Fig. 2. Effect of extraction solvent on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

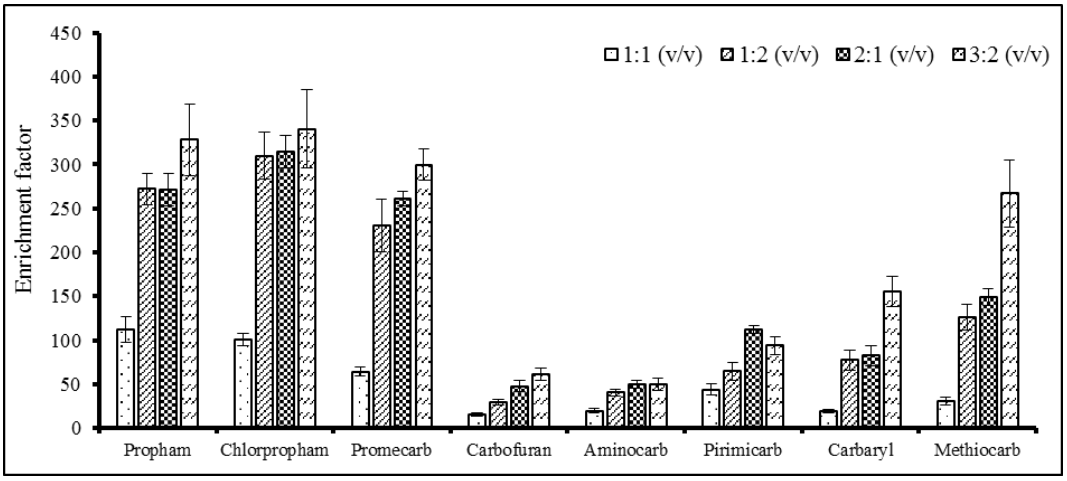


Fig. 3. Effect of a mixture of toluene and butyl acetate on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

### 3.2 The volume of extraction solvent and bubble

Mostly, the use of large volume of extraction solvent provide the high response for single drop microextraction technique [32]. However, the large extraction droplet caused buoyancy under stirring. In diffusion process, the larger volume of droplet results the longer time to reach the analyte equilibrium in extraction solvent. Then, the effect of volume of extraction solvent was investigated in the range of 0.50 – 2.00 μL. Fig. 4. showed that the extraction efficiency increased with increasing of volume of extraction solvent. However, the large volume of droplet was become unstable and buoyancy which are less reliable (the relative standard deviation increased), 1.00 μL of extraction solvent was chosen. The bubble volume within 1.00 μL of extraction solvent was also investigated. The addition of bubble to improve the extraction efficiency; however, the large bubble becomes unstable with excessive buoyancy. 0.40 μL of bubble in 1.00 μL of extraction solvent provided a good extraction efficiency with the stable droplet (Fig. 5.). Then 0.40 μL of bubble was selected for subsequent experiment.

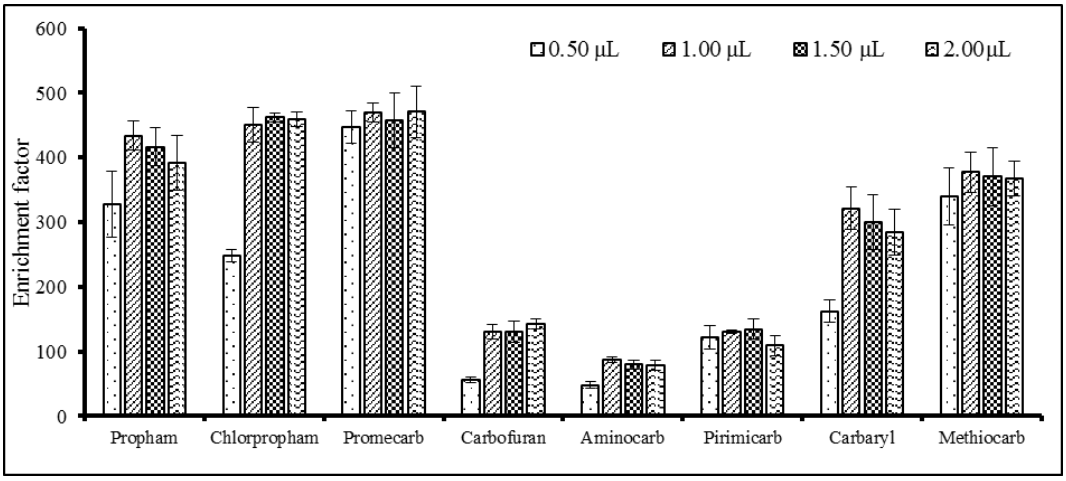


Fig. 4. Effect of extraction solvent volume on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

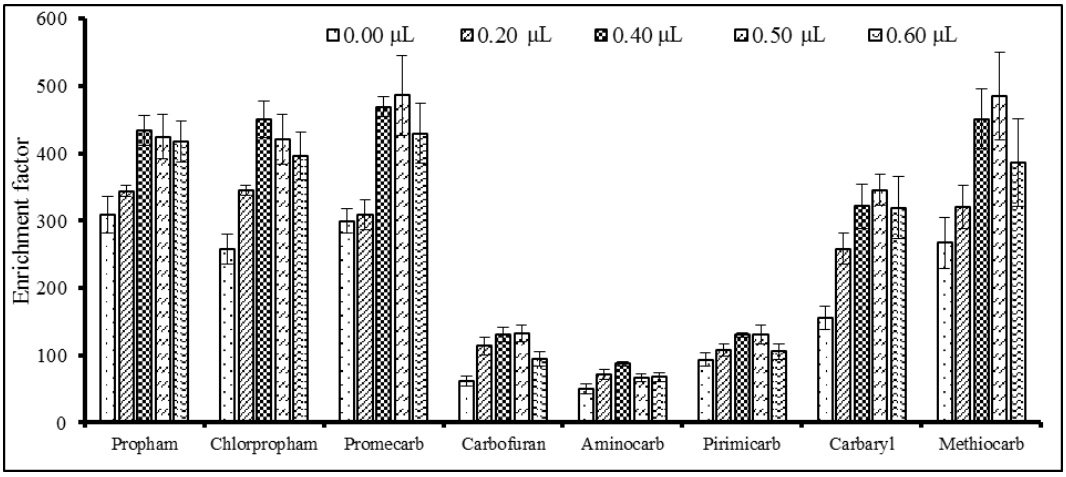


Fig. 5. Effect of bubble volume on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

### 3.3 The effect of extraction time and stirring rate

The effect of extraction time on the extraction efficiency of carbamates was investigated and the results are showed in Fig. 6. The extraction efficiency increased with increasing the extraction time from 10 min to 30 min. The extraction efficiency decreased when the extraction time longer than 30 min due to the droplet tend to dissolve, unstable and easily dislodged. Therefore, 30 min of extraction time was chosen for next experiment. The stirring of sample solution facilitates the diffusion of

the analyte into droplet, however, the faster stirring can cause buoyancy droplet and led to dislodged droplet [20, 33]. The evaluating of the stirring rate was studied at 100, 200 and 300 rpm (Fig. 7). The extraction efficiency increased with increasing the stirring rate from 100 to 200 rpm due to the increasing of mass transfer of the analytes from the sample solution into the droplet. The extraction efficiency decreased at the stirring rate higher than 200 rpm due to unstable of bubble in droplet led to dislodge. Thus, 200 rpm of the stirring rate was used for subsequent experiment.

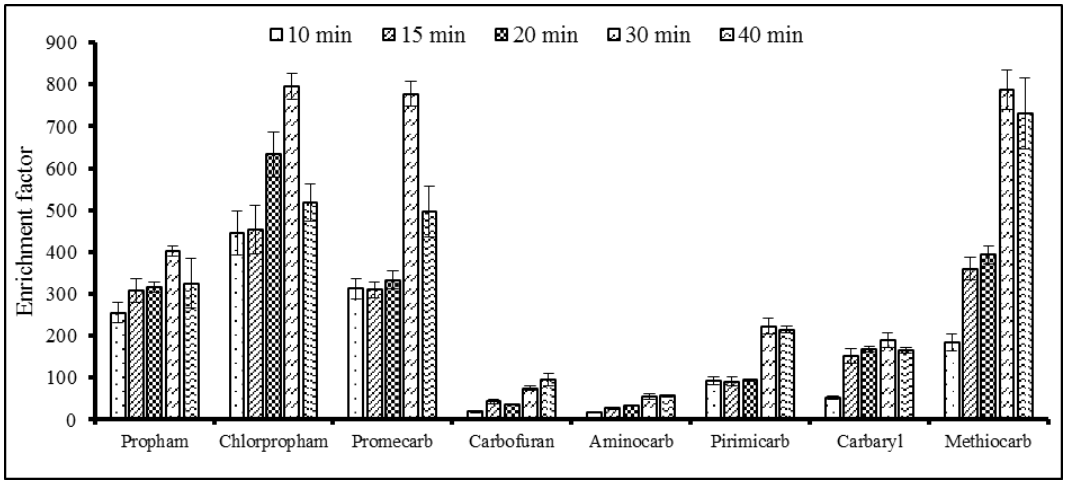


Fig. 6. Effect of extraction time on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

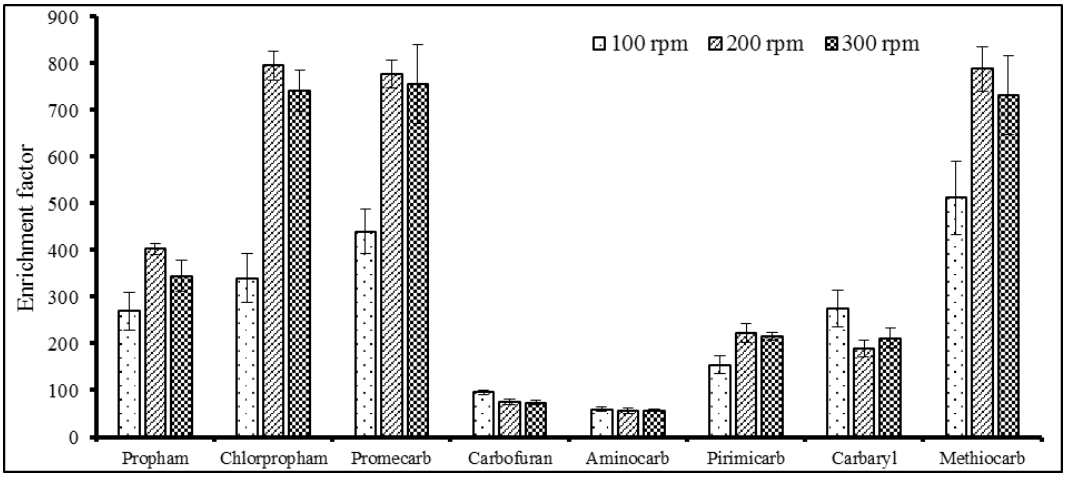


Fig. 7. Effect of stirring rate on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

### 3.4 The effect of ionic strength

The effect of ionic strength on extraction efficiency depend on the solubility of analyte in water [32]. The effect of ionic strength was evaluated by addition of different amount of NaCl in sample solution (0 - 30% w/v). The addition of salt into the sample solution generally enhance the ionic strength of the water sample. The increasing of ionic strength leading to low solubility of less polar analytes in the sample solution, it enhances their partitioning into the extraction solvent [2, 34]. As shown in Fig. 8, the extraction efficiency of carbamates increased when the concentration of salt increased up to 20% w/v. Base on the organic extraction solvent/water distribution constant ($K_{ow}$) of analyte, carbofuran, aminocarb, pirimicarb and carbaryl are dramatically enhanced the extraction efficiency, while propham, chlorpropham, promecarb and methiocarb are slightly increased with the increasing of ionic strength. The extraction efficiency of carbamates decreased at the concentration of NaCl higher than 20% w/v, it probably the occurrence of electrostatic interaction between the molecule of carbamates and salt ions in solution which may block carbamates move into the extraction solvent [35, 36]. Therefore, 20% w/v of NaCl was added in the sample for subsequent experiment.

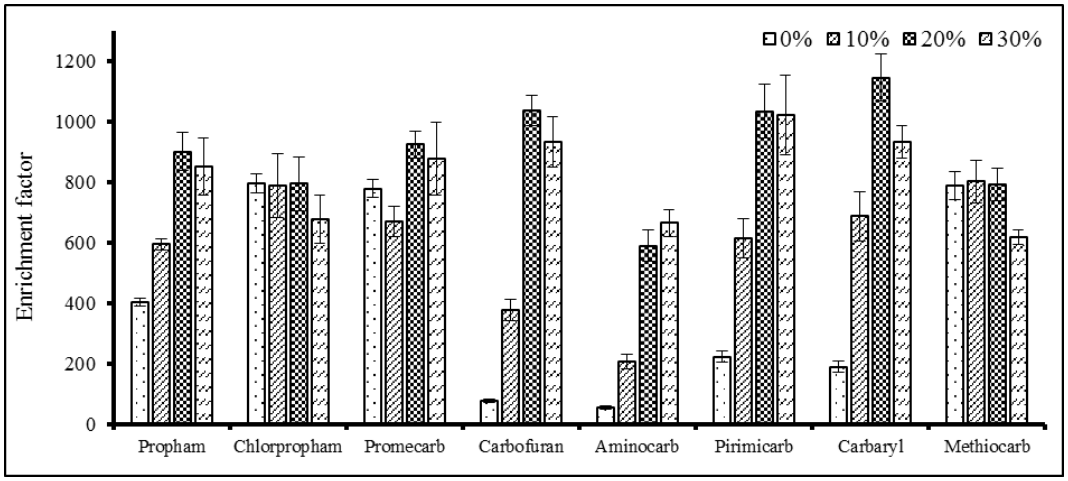


Fig. 8. Effect of ionic strength on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

### 3.5 Effect of sample pH

The change of sample pH effect to the properties of target analytes such as existence form, water solubility and extractability. Adjustment of pH intend to encourage the analyte to transfer into the organic solvent phase. The sample pH was evaluated from 3 to 9 by adjusting with 0.1 M HCl or 0.1 M NaOH. A good enrichment factor was achieved at sample pH 6 (Fig. 9) due to carbamates were in the neutral form [19]. At pH above 6 the molecule of carbamate pesticides may degraded [3, 15, 37], while at pH below 6 the amine group of aminocarb and pirimicarb may be attributed to the protonation [38]. However, the pH value of water sample is near the pH 6. Therefore, sample pH adjustment is not required.

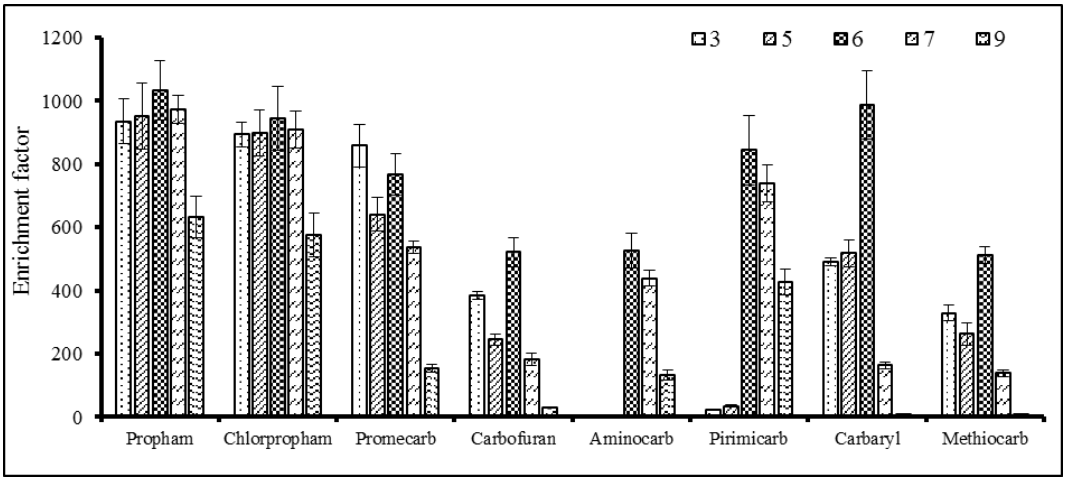


Fig. 9. Effect of sample pH on the extraction efficiency of carbamate pesticides using bubble-in-single drop microextraction

### 3.6 Comparison with traditional direct immerse single-drop microextraction (DI-SDME)

The encapsulation of bubble in microdroplet led to enlarge the surface area and thin film phenomena of the extraction solvent which helped to improve the extraction efficiency [27, 29, 30]. The developed BI-SDME method (using 1.00 μL of extraction solvent containing 0.40 μL of air bubble inside) was compared to traditional DI-SDME with different volume of extraction solvent (1.00 and 1.40 μL). As shown in Fig. 10, the developed BI-SDME method showed much higher extraction efficiency than DI-

SDME both using 1.00 and 1.40 μL of extraction solvent. This result demonstrated the increasing of surface area by encapsulation of bubble in extraction solvent droplet was encouragement factor to enhance the extraction efficiency.

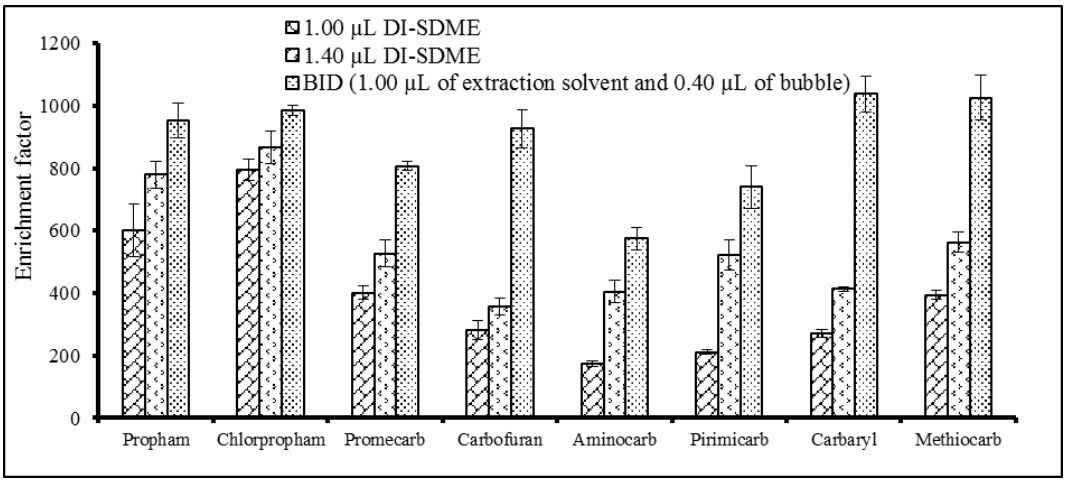


Fig. 10. Comparison of the developed BI-SDME and traditional DI-SDME

### 3.7 Analytical performance

The analytical performance of BI-SDME was evaluated by quantitative analysis of the spiked eight carbamate pesticides in water sample under the optimal condition. The linearity of this method were in the range of 0.05 – 20.0 μg $L^{-1}$ with coefficient of determination ($R^2$) were higher than 0.99 and the relative standard deviations lower than 15 (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratio of 3 and 10, respectively. The LOD and LOQ were in the range of 0.02 to 0.04 μg $L^{-1}$ and 0.05 to 0.13 μg $L^{-1}$, respectively. The results indicated that this method can be used for the determination of trace carbamate pesticides. Compared to other methods (Table 2), the developed method showed a lower detection limit with a good recovery using the small amount of solvent (1.00 μL). The intra-day and inter-day precision was behaved in order to evaluate the precision of the method. The intra-day precision of the method was investigated by analyzing six spiked water sample solution 10 μg $L^{-1}$ of carbamates within the same working day, the relative standard deviations less than 7. The inter-day precision of the method was carried out by analyzing six water sample spiked at 10 μg $L^{-1}$ of carbamates, the relative

Table 1. Analytical performances of bubble-in-drop single drop microextraction for determination of carbamates.

| Carbamates | Linear range (µg $L^{-1}$) | Regression line equation | $R^2$ | LOD (µg $L^{-1}$) | LOQ (µg $L^{-1}$) | RSD (%) |
|---|---|---|---|---|---|---|
| Propham | 0.05 – 20.0 | y = (6938 ± 9062)x + (1792 ± 7860) | 0.9946 | 0.02 | 0.07 | 5-13 |
| Chlorpropham | 0.05 – 20.0 | y = (85820 ± 3665)x + (25693 ± 31777) | 0.9910 | 0.04 | 0.13 | 2-10 |
| Promecarb | 0.05 – 20.0 | y = (35869 ± 1247)x + (15699 ± 11402) | 0.9952 | 0.03 | 0.09 | 1-12 |
| Carbofuran | 0.05 – 20.0 | y = (11327 ± 218)x + (5868 ± 1992) | 0.9985 | 0.02 | 0.05 | 2-10 |
| Aminocarb | 0.05 – 20.0 | y = (12248 ± 505)x + (9332 ± 4357) | 0.9915 | 0.02 | 0.05 | 1-9 |
| Pirimicarb | 0.05 – 20.0 | y = (77618 ± 2950)x + (1965 ± 25581) | 0.9928 | 0.02 | 0.07 | 3-11 |
| Carbaryl | 0.05 – 20.0 | y = (19352 ± 844)x + (16400 ± 7322) | 0.9906 | 0.02 | 0.05 | 2-14 |
| Methiocarb | 0.05 – 20.0 | y = (17521 ± 854)x + (4404 ± 7808) | 0.9906 | 0.03 | 0.08 | 5-15 |

Table 2. Comparison of the developed method with other methods for the determination of carbamate.

| Method | Separation method | Linear range (mg $L^{-1}$) | LOD (µg $L^{-1}$) | Sample | Recovery | Reference |
|---|---|---|---|---|---|---|
| HPLC-UV | LLE-LTP | 0.033 – 500 | 5.00 – 10.00 | Water | 89.6 – 97.5 | (Goulart et al. 2010) |
| HPLC-ESI/MS | SPME | 0.0005 – 0.1 | 0.5 - 9 | Soil | - | (Möder et al. 1999) |
| UHPLC-MS/MS | QuEChERS | 0.002 – 0.08 | 2 | Aromatic herb | 72 - 81 | (Nantia et al. 2017) |
| GC/ MS | LPME | 0.0002 – 1 | 0.05 – 0.01 | Water | 81 - 125 | (Lee and Lee 2011) |
| HPLC-DAD | DDLE | 0.001 – 2 | 0.32–0.51 | fruit juice and vegetable | 69–91 | (Farajzadeh et al. 2016) |
| HPLC-DAD | SDME | 0.002 – 1 | 0.63 | Water | 79 -120 | (Wang et al. 2012) |
| GC-MS | SDME | 0.00005 – 0.2 | 0.02 – 0.5 | Water | 89.4 – 97.4 | (Chen et al. 2009) |
| GC-MS | BID-SDME | 0.00005 – 0.02 | 0.02 – 0.04 | Water | 81.7 – 99.0 | This work |

LLE-LTP: Liquid-liquid extraction with low temperature partitioning; SPME: Solid phase microextraction; LPME: Liquid phase microextraction; DLME: Dispersive liquid microextraction; SDME: Single drop microextraction; BID SDME: Bubble-in-drop single drop microextraction

standard deviation less than 8. The precision were acceptable with RSD lower than 15 for spiked concentration of 10 μg $L^{-1}$ [39]. These results indicated that this method has a good precision for the determination of carbamates.

### 3.8 Analysis of water samples

The developed BI-SDME method was applied to detect carbamates in water sample including tap water, river water and drain water. The results are showed in Table 3, no carbamates were detected in tap water, river water and drain water. The accuracy of this method was evaluated by spiking standard carbamates in water sample at two concentrations (1.0 μg $L^{-1}$ and 10.0 μg $L^{-1}$) and then extracted and analyzed under optimum conditions. The recoveries of carbamate pesticides were in the range of 81.7% to 99.0% with the relative standard deviation less than 9%. The satisfactory recoveries were obtained within the acceptable range as recommended by the AOAC (70 - 120%) [39]. The typical chromatogram of water sample and spiked water sample after extraction with BI-SDME method are showed in Fig 11.

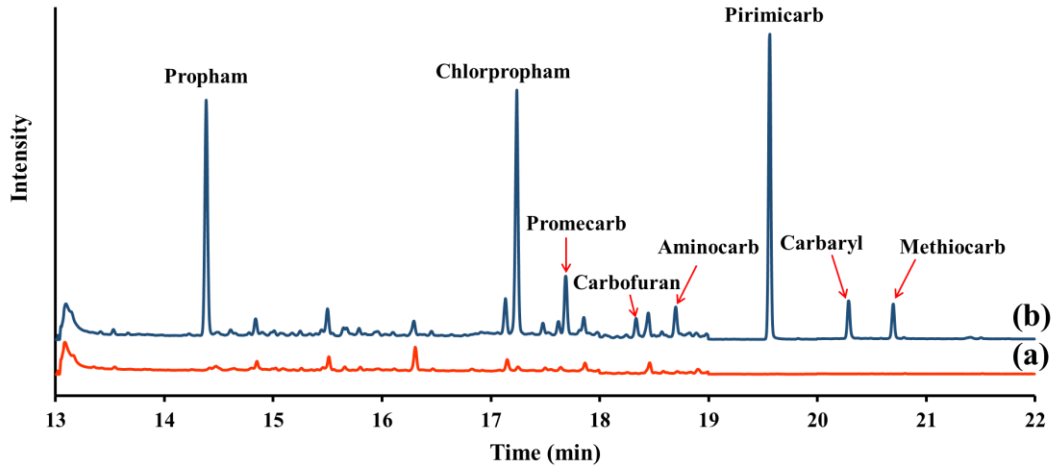


Fig. 11. Chromatogram of water sample (a) and spiked water sample (b) after extraction with BI-SDME method

## 4. Conclusion

A bubble-in-single drop microextraction (BI-SDME) was developed and applied to determine trace of carbamate pesticides in water samples. In extraction step used less amount (1.0 μL) of extraction solvent (toluene and butyl acetate) which

Table 3. The determination and the recovery of carbamate pesticides in real sample analysis.

| Analyte | Spiked ($\mu g\ mL^{-1}$) | Tap water | | River water | | Drain water | |
|---|---|---|---|---|---|---|---|
| | | Found ($\mu g\ L^{-1}$) | Recovery | Found ($\mu g\ L^{-1}$) | Recovery | Found ($\mu g\ L^{-1}$) | Recovery |
| Propham | 0.00 | ND[a] | - | ND | - | ND | - |
| | 1.00 | 0.86 | 86.2 ± 3.2 | 0.90 | 90.0 ± 7.2 | 0.92 | 92.0 ± 2.1 |
| | 10.0 | 9.40 | 94.0± 3.2 | 9.90 | 99.0 ± 1.4 | 9.23 | 92.3 ± 3.1 |
| Chlorpropham | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.95 | 95.2 ± 3.3 | 0.86 | 86.3 ± 5.4 | 0.83 | 83.5 ± 3.0 |
| | 10.0 | 8.96 | 89.6 ± 7.1 | 9.73 | 97.3 ± 2.5 | 8.85 | 88.5 ± 6.8 |
| Promecarb | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.99 | 98.6 ± 0.9 | 0.85 | 85.2 ± 6.7 | 0.93 | 93.0 ± 2.5 |
| | 10.0 | 8.47 | 84.7 ± 3.6 | 9.52 | 95.2 ± 3.7 | 8.96 | 89.6 ± 7.7 |
| Carbofuran | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.83 | 82.9 ± 2.2 | 0.83 | 83.2 ± 1.6 | 0.84 | 84.4 ± 4.1 |
| | 10.0 | 8.80 | 88.0 ± 5.8 | 8.49 | 84.9 ± 5.3 | 8.17 | 81.7 ± 1.2 |
| Aminocarb | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.87 | 86.7 ± 4.9 | 0.85 | 85.2 ± 3.7 | 0.87 | 86.7 ± 2.3 |
| | 10.0 | 9.09 | 90.9 ± 2.7 | 9.68 | 96.8 ± 2.1 | 9.72 | 97.2 ± 3.3 |
| Pirimicarb | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.94 | 94.2 ± 0.9 | 0.94 | 94.0 ± 2.2 | 0.95 | 94.7 ± 5.2 |
| | 10.0 | 8.62 | 86.2 ± 5.2 | 9.71 | 97.1 ± 1.9 | 8.53 | 85.3 ± 0.5 |
| Carbaryl | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.84 | 83.5 ± 3.1 | 0.82 | 82.3 ± 2.0 | 0.82 | 82.4 ± 4.6 |
| | 10.0 | 8.83 | 88.3 ± 4.9 | 8.92 | 89.2 ± 2.0 | 8.32 | 83.2 ± 2.9 |
| Methiocarb | 0.00 | ND | - | ND | - | ND | - |
| | 1.00 | 0.83 | 82.5 ± 0.8 | 0.86 | 86.3 ± 5.8 | 0.87 | 86.6 ± 3.8 |
| | 10.0 | 8.82 | 88.2 ± 5.3 | 9.02 | 90.2 ± 1.7 | 9.16 | 91.6 ± 6.9 |

[a]ND: Not detect

indicated this method is environmental friendly. The sufficiency of analytical performance for carbamates determination in water sample were obtained with high enrichment factor of 952, 985, 807, 926, 574, 740, 1038 and 1025 for propham, chlorpropham, promecarb, carbofuran, aminocarb, pirimicarb, carbaryl and methiocarb, respectively. The method was demonstrated good linearity in the range of 0.05 to 20.0 μg $L^{-1}$ with high sensitivity, acceptable precision and accuracy. The developed method was successfully applied for the determination of carbamate pesticide in different water samples including tap water, river water and drain water and desirable recoveries were obtained in the range of 81.7% to 99.0% with good precision (RSD in the range of 0.6 – 8.6%). Furthermore, this developed method is easy to handle using a simple equipment, short analysis time and inexpensive using less amount of solvent and low-cost equipment.

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**List of Publication**

Chullasat, K., Kanatharana, P., Bunkoed, O., Nanocomposite optosensor of dual quantum dot fluorescence probes simultaneously detects cephalexin and ceftriaxone. ***Sensors and Actuators B: Chemical*** 254 (2019), 689-697.

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**Poster Presentations**

Chullasat, K., Nurerk, P., Kanatharana, P., Kueseng, P., Sukchuay, T., Bunkoed, O., A new sorbent of polypyrrole coated composited graphene oxide/Polyvinyl alcohol cryogel for extraction of sulfonamides in water sample, The 13th Asian Conference on Analytical Sciences (ASIANALYSIS XIII), 8-11 December 2016, The Empress International Convention Center, Chiang Mai, Thailand.

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