COLORIMETRIC DETECTION OF NORFLOXACIN AND CEFTAZIDIME BY MODIFIED MATERIALS

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การตรวจวัดเชิงสีของนอร์ฟลอกซาซินและเซฟตาซิดิมด้วยวัสดุดัดแปร

น.ส.ลลิตพรรณ หงษ์ทะนี

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Field of Study	Chemistry
Thesis Advisor	Associate Professor Dr. FUANGFA UNOB
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ลลิตพรรณ หงษ์ทะนี : การตรวจวัดเชิงสีของนอร์ฟลอกซาซินและเซฟตาซิดิมด้วยวัสดุดัดแปร. (COLORIMETRIC DETECTION OF NORFLOXACIN AND CEFTAZIDIME BY MODIFIED MATERIALS) อ.ที่ปรึกษาหลัก : รศ. ดร.เฟื่องฟ้า อุ่นอบ, อ.ที่ปรึกษาร่วม : รศ. ดร.ณรงค์ ประไพรักษ์สิทธิ์

วิธีการใหม่ได้ถูกพัฒนาขึ้นสำหรับการตรวจวัดเชิงสีของนอร์ฟลอกซาซินและเซฟตาซิดิม โดยใช้เรซินพอลิเมอร์และฟองน้ำใยสำลีที่ถูกดัดแปรทางเคมีเป็นวัสดุตรวจวัด ตามลำดับ สำหรับการตรวจวัดนอร์ฟลอกซาซิน สภาวะที่เหมาะสมได้จากการดัดแปรเรซินพอลิเมอร์ด้วยคองโกเรดเข้มข้น 6.0 ไมโครโมลาร์ ที่พีเอช 6 เพื่อใช้ในการสกัดนอร์ฟลอกซาซินในตัวอย่างปริมาตร 10 มิลลิลิตร ที่พีเอช 2 ปฏิกิริยาเกิดขึ้นสมบูรณ์ภายใน 15 นาที ในส่วนของการตรวจวัดเซฟตาซิดิมได้ทำการเตรียมฟองน้ำจากสำลีที่ดัดแปรด้วยโพลีเอทีลีนอิมมีนและทำการพิสูจน์เอกลักษณ์ด้วยเทคนิคเอฟทีไออาร์ เอสอีเอ็ม และการทดสอบด้วยนินไฮดริน ในการเตรียมฟองน้ำที่ดัดแปรด้วยโพลีเอทีลีนอิมมีน ทำการดัดแปรเส้นใยสำลีโดยใช้ 3-อะมิโนโพรพิลไตรเอทอกซีไซเลนเข้มข้น 170 มิลลิโมลาร์ต่อการดัดแปรเส้นใยสำลี 1 กรัม และใช้โพลีเอทีลีนอิมมีนเข้มข้น 210 ไมโครโมลาร์ต่อการกราฟต์ฟองน้ำที่ผ่านการดัดแปรด้วย 3-อะมิโนโพรพิลไตรเอทอกซีไซเลน 0.5 กรัม ซึ่งการตรวจวัดเชิงสีของเซฟตาซิดิมอาศัยการเกิดสีย้อมเอโซบนพื้นผิวของฟองน้ำสามารถเกิดขึ้นได้โดยใช้สารละลายผสมของกรดไฮโดรคลอริกเข้มข้น 0.5 โมลาร์ โซเดียมไนไตรท์เข้มข้น 30 มิลลิโมลาร์ และกรดโครโมโทรปิกเข้มข้น 25 ไมโครโมลาร์ ภายใต้สภาวะที่เหมาะสมวิธีการนี้ให้ค่าความเป็นเส้นตรงในช่วง 0 ถึง 1.5 มิลลิกรัมต่อลิตร และ 0.5 ถึง 3.0 มิลลิกรัมต่อลิตรสำหรับการตรวจวัดนอร์ฟลอกซาซินและเซฟตาซิดิม ตามลำดับ ขีดจำกัดในการตรวจวัดของนอร์ฟลอกซาซินและเซฟตาซิดิม คือ 0.23 มิลลิกรัมต่อลิตร และ 0.06 มิลลิกรัมต่อลิตร ตามลำดับ ได้นำวิธีการนี้ไปใช้ในการตรวจวัดนอร์ฟลอกซาซินและเซฟตาซิดิมในน้ำตัวอย่างที่เติมสารมาตรฐาน ซึ่งได้ร้อยละการได้กลับคืนอยู่ในช่วง 90 – 106 และ 83 – 103 ตามลำดับ และมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่าร้อยละ 10.53

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2565 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: norfloxacin, ceftazidime, colorimetric detection, polymer resin, cotton sponge, Congo red, polyethyleneimine, chromotropic acid

Lalitphan Hongtanee : COLORIMETRIC DETECTION OF NORFLOXACIN AND CEFTAZIDIME BY MODIFIED MATERIALS. Advisor: Assoc. Prof. Dr. FUANGFA UNOB Co-advisor: Assoc. Prof. Dr. NARONG PRAPHAIRAKSIT

Novel approaches were developed for the colorimetric detection of norfloxacin and ceftazidime using chemically modified polymer resin and cotton sponge as sensing materials, respectively. For norfloxacin detection, the optimum condition was achieved by using a resin modified with 6.0 μM Congo red at pH 6 to extract norfloxacin in 10 mL of sample at pH 2. The reaction was completed within 15 minutes. Regarding ceftazidime detection, a cotton sponge functionalized with polyethyleneimine (PEI) was fabricated and characterized by FTIR, SEM, and ninhydrin test. To fabricate the PEI-sponge, 170 mM 3-aminopropyl triethoxysilane (APTES) was used to modify 1.0 g cotton fibers and 210 μM PEI for grafting on 0.5 g of APTES-sponges. The colorimetric detection of ceftazidime based on the azo dye formation on the sponge surface was achieved by using a reagent mixture containing 0.5 M HCl, 30 mM $NaNO_2$, and 25 μM chromotropic acid. Under optimized conditions, the methods provide a linear range from 0 – 1.5 mg L^{-1} and 0.5 – 3.0 mg L^{-1} for norfloxacin and ceftazidime determination, respectively. The limit of detection of norfloxacin and ceftazidime was found to be 0.23 mg L^{-1} and 0.06 mg L^{-1}, respectively. The methods were applied to detect norfloxacin and ceftazidime in spiked water samples with recoveries ranging from 90 – 106% and 83 – 103%, respectively, and % RSD lower than 10.53%.

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Student's Signature .....

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Co-advisor's Signature .....

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Lalitphan Hongtanee

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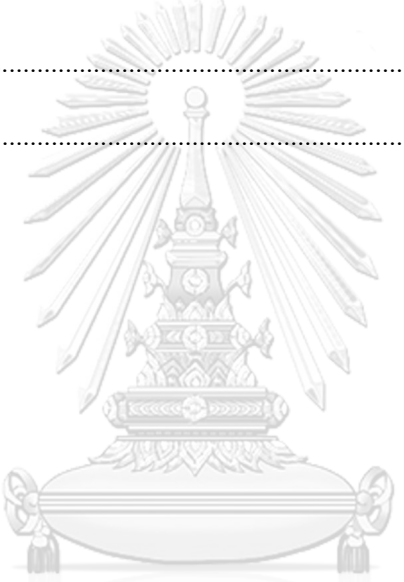

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LIST OF ABBREVIATIONS

%RSD	Relative standard deviation percentage
µM	Micro molar
AOAC	Association of Official Analytical Chemists
APTES	3-aminopropyltriethoxysilane
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
CEF	Ceftazidime
L	Liter
LOD	Limit of detection
LOQ	Limit of quantitation
M	Molar
MBA	N,N'-Methylenebisacrylamide
mg L^{-1}	Milligram per liter
min	Minute
mL	Milliliter
mM	Milli molar
NOR	Norfloxacin
PEI	Polyethyleneimine
PEI-sponge	Cotton sponge modified with polyethyleneimine
pKa	Acid dissociation constant
SD	Standard deviation
SEM	Scanning electron microscope

CHAPTER I
INTRODUCTION

1.1 Statement of problem

Antibiotics are important medicines used to treat bacterial infections in humans and animals. They are commonly used to treat various infections such as respiratory tract infections, urinary tract infections, and abdominal infections [1, 2]. These drugs are consumed orally in the form of tablet or suspension, and afterward, secreted from the body in its original form [3]. The widespread use of antibiotics, notably norfloxacin and ceftazidime, may cause antibiotic pollution in the environmental resources, such as surface water, groundwater, and drinking water [4]. This could lead the bacteria to develop a resistance to such medication, thereby causing an increase in antibiotic-resistant bacteria [5, 6]. Therefore, it is critical to track the quantity of norfloxacin and ceftazidime in environmental resources to prevent any long-term impacts on human health.

There are several analytical methods that can be applied for the determination of norfloxacin and ceftazidime, including high performance liquid chromatography (HPLC) [7, 8], electrochemistry [9, 10], spectrofluorimetry [11, 12], and UV-visible spectrophotometry [13, 14]. However, these existing methods require complicated instrumentation and skilled operators to perform such analysis. A simple colorimetric method such as digital image colorimetry for norfloxacin and ceftazidime determination thus is an attractive alternative due to their reasonable cost, ease of use, and ability for on-site detection. Several colorimetric reactions have been applied to detect norfloxacin and ceftazidime, including metal-ligand complexation [15-17], redox reaction [18, 19], charge transfer complexation [20-22], ion-pair complexation [13, 23-26], and azo dye formation [27, 28]. Among these, two colorimetric reactions, ion-pair complexation and azo dye formation, are chosen in this work due to their ease of operation. Given that the structure of norfloxacin exists as zwitterion, it can react with anionic or cationic dyes to form ion-pair complexes under controlled solution pH. Herein, we aim to detect norfloxacin through the ion-

pair complexation between norfloxacin and Congo red as shown in Figure 1.1. Under acidic condition, the protonated form of Congo red was deprotonated by a basic nitrogen atom of norfloxacin, yielding an ion-pair complex between norfloxacin and deprotonated form of Congo red. The color change from blue-purple of protonated Congo red to red-pink of its unprotonated form was observed.

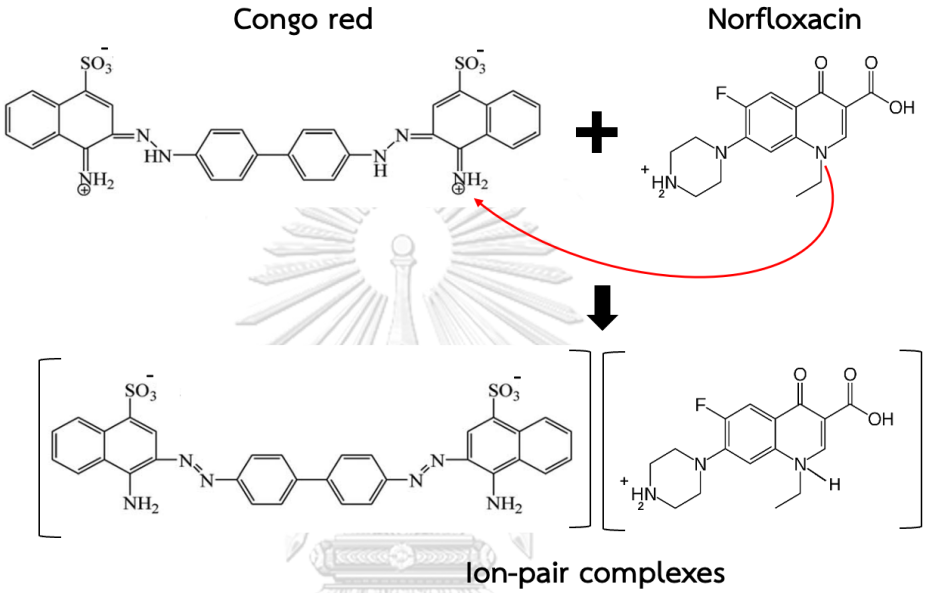


Figure 1.1 The proposed ion-pair complexation between norfloxacin and Congo red.

In the case of ceftazidime, its structure contains aromatic amine, which can be diazotized by sodium nitrite in acidic condition to form diazonium ion. The diazotized ceftazidime can further react with a coupling reagent to form an azo dye product. In this work, the diazotized ceftazidime was subsequently subjected to a coupling reaction with chromotropic acid, leading to the formation of a colored compound as depicted in Figure 1.2 [29]. Chromotropic acid has been utilized as a coupling reagent for various analytes, such as gabapentin drug [30], formaldehyde [31], , and nitrite [32].

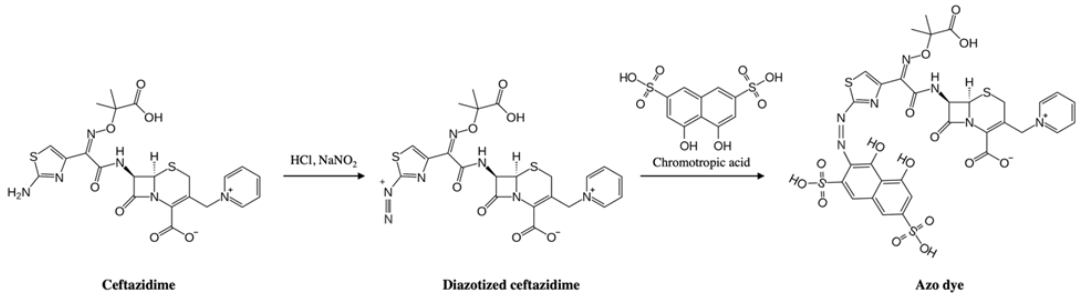


Figure 1.2 A proposed reaction for ceftazidime detection based on azo dye formation using chromotropic acid as the coupling reagent [29].

However, the colorimetric detection of norfloxacin and ceftazidime at low level still suffers from a lack of sensitivity. To improve the method sensitivity, the extraction of analyte onto a solid platform is highly recommended. In this work, we developed solid platforms for the colorimetric detection of norfloxacin and ceftazidime using chemically modified polymer resin and cotton sponge, respectively. The modified materials were used to extract the analytes through electrostatic interaction and the colorimetric reactions were performed on the material surface. For norfloxacin detection, a resin (Amberlite XAD-7) was modified with Congo red through hydrophobic interaction. The dye-modified resin was then used to extract norfloxacin and the colorimetric detection based on ion-pair complexation was conducted on the resin surface, resulting in a change of resin color. For ceftazidime detection, a cellulose sponge was fabricated from the cotton fibers modified with 3-aminopropyltriethoxysilane (APTES), followed by grafting of polyethyleneimine (PEI) on the APTES-sponge using epichlorohydrin as a crosslinker (Figure 1.3) [29]. This PEI modified cotton sponge (PEI-sponge) with positively charged surface was used to extract ceftazidime from sample solution through electrostatic interaction. After that, the extracted ceftazidime on the PEI-sponge was detected by diazotization and coupling reaction as previously proposed (Figure 1.2). The material was photographed and the photo was subjected to Image J software to obtain color intensity for quantitative analysis. These developed materials were applied to detect norfloxacin and ceftazidime in water samples.

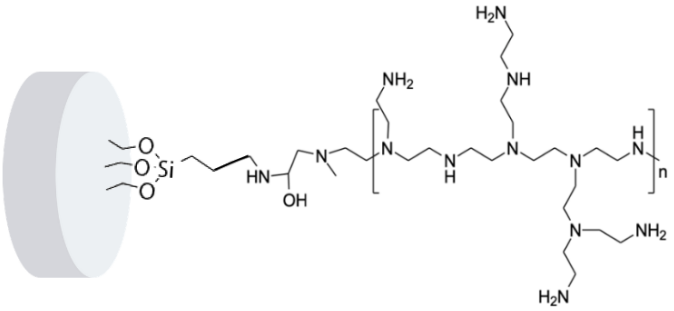


Figure 1.3 The proposed structure of PEI-sponge for ceftazidime detection [29].

1.2 Research objective

The objective of this research is to develop analytical methods to extract and detect low-level norfloxacin and ceftazidime using fabricated solid platforms and digital-image based colorimetry.

1.3 Scope of the research

The scope of this research covers the fabrication of solid platforms for extracting norfloxacin and ceftazidime and their colorimetric detection. For norfloxacin detection, Congo red as dye binding reagent was used to modify the surface of resin. Regarding the ceftazidime detection, 3-aminopropyltriethoxysilane and polyethyleneimine were employed to functionalize the cotton fibers and cotton sponge, respectively. The PEI-sponge was characterized by ATR-FTIR, ninhydrin test, SEM, and water uptake. The colorimetric detection of ceftazidime was based on the azo dye formation on the sponge surface using chromotropic acid as a coupling reagent. Parameters affecting the determination including concentration of modifying reagents, sample solution pH, sample volume, and reaction time were optimized. Finally, the proposed materials were used to extract and detect norfloxacin and ceftazidime in real water samples.

1.4 Benefits of Research

This research aimed to obtain new solid platforms for highly sensitive and selective determination of norfloxacin and ceftazidime in water samples using a simple digital image colorimetric method.

CHAPTER II
THEORY AND LITERATURE REVIEW

2.1 Antibiotics

Antibiotics are a type of medication used to treat bacterial infections. They are substances produced by microorganisms (such as bacteria, fungi, and certain molds) or synthesized in laboratories [33]. Antibiotics work by either killing bacteria (bactericidal) or inhibiting their growth (bacteriostatic) [34]. These drugs can be classified into different classes based on their chemical structure, mechanism of action, and spectrum of activity [35]. Some common classes of antibiotics base on their chemical structure include cephalosporins, fluoroquinolones, sulfonamides, and tetracyclines (Table 2.1). Each class has its own unique properties and is used to treat different types of bacterial infections [36, 37].

Antibiotics, especially fluoroquinolones and cephalosporins, have been increasingly used to treat several infectious diseases caused by bacteria including urinary tract infections [2, 38] and respiratory tract infections [1, 39]. However, the use of these antibiotics in large amount may cause antibiotic pollution in the environment through various sources, including agricultural runoff, animal waste, pharmaceutical industrial waste, and sewage [40]. One of the main pathways is through excretion by humans and animals in its original form, as well as through disposal of unused or expired antibiotics [41]. Thus, antibiotic residues have been detected in surface water, groundwater, drinking water, air, and soil, as well as in various animal species, including fish and shrimp [4, 5]. Several studies have been conducted to measure the levels of antibiotics in water sources as shown in Table 2.2. Antibiotic contamination in environmental resources can contribute to the development of antibiotic resistance in environmental bacteria, which can be transferred to human through direct contact or food and water consumption [42, 43]. Moreover, it can have toxic effects on non-target organisms, including aquatic plants and animals, as well as aquatic microorganisms [40]. Consequently, the monitoring of antibiotic residues in environmental resources is crucial for preventing long-term

effect on human health. This study focused on the determination of antibiotic residues in water samples, namely norfloxacin in fluoroquinolones, and ceftazidime in cephalosporins classes.

Table 2.1 Some common classes of antibiotics [36, 37].

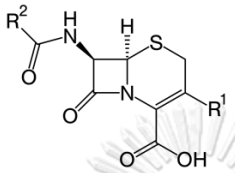
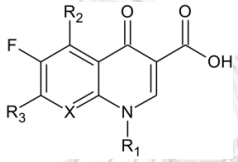
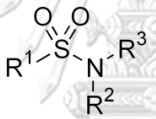
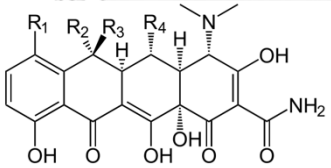
Class	Structure	Example	Mode of action
Cephalosporins		Ceftazidime, Ceftriaxone, Cefalexin	Inhibit bacteria cell wall biosynthesis
Fluoroquinolones		Norfloxacin, Ciprofloxacin, Levofloxacin	Interfere with bacteria DNA replication and transcription
Sulfonamides		Sulfanilamide, Sulfadiazine,	Prevent their growth and multiplication
Tetracyclines		Tetracycline, Oxytetracycline, Doxycycline	Inhibit the synthesis of proteins, preventing growth.

Table 2.2 The levels of antibiotic residues in various water sources.

Water sources	Antibiotics class	Levels	Ref.
Wastewater	Cephalosporins, Quinolone, Sulphonamide	0.01 – 14.5 ug L^{-1}	[44]
Tap water	Fluoroquinolone	7.9 – 679.7 ng L^{-1}	[45]
Drinking water	Cephalosporins, Quinolone, Sulfonamide, Macrolides	0.1 – 10 ng L^{-1}	[46]

2.1.1 Norfloxacin

Norfloxacin is a synthetic broad-spectrum antibiotic that belongs to the fluoroquinolone class (Figure 2.1). It is active against a wide range of aerobic gram-positive and gram-negative organisms, especially *Pseudomonas aeruginosa* [47]. Several bacterial infections were treated by norfloxacin e.g. urinary tract infections caused by *Escherichia coli* [48] and other gram-negative bacteria. Norfloxacin works by inhibiting the activity of DNA gyrase (topoisomerase II) and topoisomerase IV, which are essential enzymes involved in bacterial DNA replication, transcription, and repair [49]. By inhibiting these enzymes, norfloxacin prevents bacteria from multiplying and spreading. After oral administration, norfloxacin is quickly absorbed and excreted mainly in urine through the metabolism at the liver.

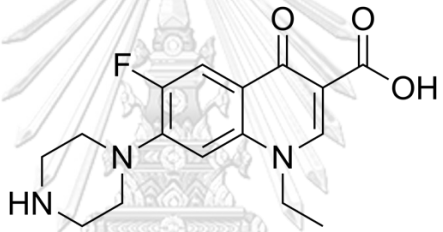


Figure 2.1 Structure of norfloxacin.

Norfloxacin is a crystalline powder that is white to pale yellow in color. It is slightly soluble in methanol and ethanol, and freely soluble in glacial acetic acid [50]. The solubility of norfloxacin in water is pH dependent as it is classified as a zwitterionic molecule [51]. The zwitterion is formed due to its carboxyl group (-COOH) and quinolone nitrogen group (-NH) in its structure. At physiological pH (6.22 – 8.51) [52], the carboxyl group becomes deprotonated and carries a negative charge, while the quinolone nitrogen group is protonated showing a positive charge, yielding the zwitterionic form. Therefore, a high solubility is achieved when pH is less than 5 or more than 10, while a low solubility is in neutral pH region [53] as shown in Figure 2.2.

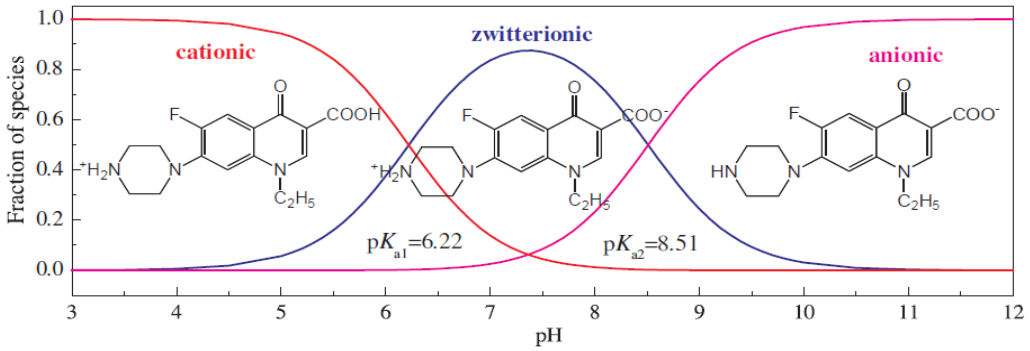


Figure 2.2 Structure of norfloxacin and the species distribution at different pH [52].

2.1.2 Ceftazidime

Ceftazidime is a broad-spectrum antibiotic that is commonly used to treat various bacterial infections. It belongs to the class of cephalosporin antibiotics and is considered as a third-generation cephalosporin containing 7-aminacephalosporic (7-ACA) and substituted chains as shown in Figure 2.3 [54]. Ceftazidime has more potent activity against gram-negative bacteria, including *Pseudomonas aeruginosa*, *Enterobacteriaceae*, and *Haemophilus influenzae* [55, 56]. This drug can bind with penicillin-binding proteins (PBPs) on bacterial cell walls, leading to a prevention of the linking of peptidoglycan strands [57]. This makes the bacterial cell wall weak and unable to withstand the osmotic pressure, resulting in cell lysis and death. Ceftazidime is available in several forms, including powder for injection and injectable solution. Following its administration to the body, ceftazidime is subsequently excreted in its original form without metabolism through kidney.

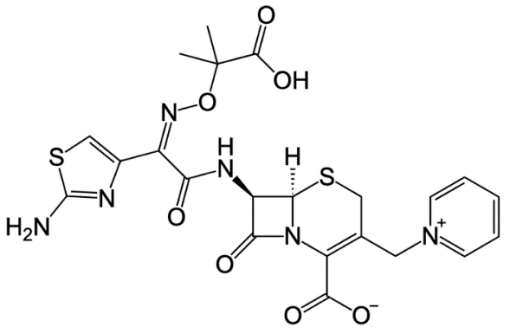


Figure 2.3 Structure of ceftazidime.

Ceftazidime has two pK_a values: pK_{a1} at 2.77 of carboxylic acid and pk_{a2} at 4.26 of aminothiazolyl [58]. At a pH below 2.7, ceftazidime exists primarily in its protonated form and as the pH increases and surpasses the pK_{a1}, carboxyl group is partly deprotonated, resulting in its partially deprotonated form. At pH between 2.77 and 4.26, ceftazidime exists as a zwitterion with an overall neutral charge, as the positive charge of aminothiazolyl group and negative charge of carboxyl group balance each other out. When the pH exceeds 4.26, ceftazidime exists in its anionic form [59].

2.2 Analytical methods for norfloxacin and ceftazidime detection

Several analytical techniques have been applied for the determination of norfloxacin and ceftazidime including spectrophotometry, electrochemistry, and chromatography. Some recent analytical methods for norfloxacin and ceftazidime determination are summarized in Table 2.3. UV-visible spectrophotometry is one of the most wildly used methods to detect antibiotics in pharmaceutical dosage forms [13, 60, 61]. However, the detection sensitivity and selectivity of this method is worse than the HPLC method. HPLC has been used to separate and quantify the drugs in various matrices, including biological fluids, such as plasma and urine [8, 62]. Although HPLC technique provides wider linear range and good selectivity [7, 63], it requires expensive and sophisticated instrumentations, proficient users, and long analysis time. Fluorescent techniques could provide better selectivity and sensitivity compared to ultraviolet (UV) techniques. There are various fluorescent probes used for norfloxacin and ceftazidime detection. Carbon dots (CDs) and quantum dots (QDs), a type of fluorescent nanomaterial, have recently received a great deal of attention. The fluorescence intensities of CDs can be modulated, either enhanced or decreased, upon exposure to norfloxacin and ceftazidime through various interactions including H-bond, charge transfer, inner filter effect (IFE), and agglomeration-induced quenching [11, 64-66]. Moreover, CDs and QDs can be functionalized with specific reagents and embedded in a molecularly imprinted polymer (MIP) to improve sensitivity and selectivity. Bunkoed and co-worker

fabricated nanoprobes by incorporating graphene quantum dots and magnetite nanoparticles into a MIP (GQDs@Fe_3O_4/MIP) for ceftazidime detection [67]. This reaction was based on fluorescence quenching due to the energy transfer when ceftazidime was bound with amino group of aminopropyltriethoxysilane (APTES) on the surface of GQDs. Amiri and co-worker fabricated CDs embedded in silica MIP (CDs-SMIP) and the fluorescence quenching mechanism was based on electron transfer due to the formation of hydrogen bond between ceftazidime and NH_2 groups of APTES [12].

Table 2.3 Summary of some analytical methods for norfloxacin and ceftazidime determination.

Analytes	Methods	Linear range	LOD	Ref.
Norfloxacin	Spectrophotometry using bromophenol blue to form ion-pair complexation with norfloxacin	5 – 150 mg L^{-1}	-	[13]
	Liquid chromatography with fluorescent detection (LC-FD) using fluorescent labelling	200-2000 µg L^{-1}	150 µg L^{-1}	[7]
	HPLC with fluorescent detection based on the derivatization of norfloxacin with 4-chloro-7-nitrobenzofurazan	10 – 500 ug L^{-1}	0.2 ug L^{-1}	[63]
	Spectrofluorometry using orange-red emitting copper nanoclusters as fluorescent nanoprobe for fluorescence quenching through the inner filter effect (IFE) and agglomeration-induced quenching	0.05 – 250 µM	17 nM	[11]
	Spectrofluorometry using magnetic nanoparticles as adsorbent and sulfur-doped carbon dots as a	0.02 – 1.25 µM	4.6 nM	[66]

Analytes	Methods	Linear range	LOD	Ref.
	fluorescent probe			
Ceftazidime	Spectrophotometric method based on azo-dye formation using 4-tert-butylphenol as a coupling reagent	1 – 10 mg L^{-1}	0.31 mg L^{-1}	[60]
	Spectrophotometric method based on azo-dye formation using 1-Naphthol and 2-Naphthol as coupling reagents	3 – 50 mg L^{-1}	0.81 mg L^{-1}	[61]
	RP-HPLC with UV-detection	0.1 -100 mg L^{-1}	0.1 mg L^{-1}	[62]
	HPLC-MS/MS	1 – 100 mg L^{-1}	1 mg L^{-1}	[8]
	Spectrofluorometry based on fluorescence quenching of PEG	0.24 – 2.4 µg L^{-1}	0.005 µg L^{-1}	[64]
	Spectrofluorometry based on the reaction with fluorescamine reagent	0.03 – 0.3 µg L^{-1}	0.003 µg L^{-1}	[65]
	Fluorescent nanosensor using graphene quantum dots and magnetite nanoparticles embedded in molecularly imprinted polymer	0.1 – 10 µg L^{-1}	0.05 µg L^{-1}	[67]
	Fluorescent nanosensor using carbon dots embedded in silica molecularly imprinted polymer	0.18 – 1.3 µg L^{-1}	0.06 µg L^{-1}	[12]

2.3 Colorimetric reactions for norfloxacin and ceftazidime determination

Colorimetric detection is a technique based on the visual observation or measurement of a color change to detect the concentration of target analytes. It is a widely used and accessible method for qualitative and quantitative analysis for various analytes due to its cost-effectiveness, ease of use, and on-site detection capability. This technique relies on the interaction between the analyte and a color-producing reagent or indicator. There are various colorimetric reactions used to detect drugs such as metal-ligand complexation [15-17], redox reaction [18, 19],

charge transfer complexation [20-22], ion-pair complexation [13, 23-26], and azo dye formation [27, 28, 30] as shown in Table 2.4.

Table 2.4 Summary of some colorimetric reagents and reactions for pharmaceutical determination.

Colorimetric reaction	**Analyte**	**Reagent**	**Method**	**Ref.**
Metal-ligand complexation	Fluoroquinolones	Fe^{3+}	Smartphone based colorimetry	[15]
	Quinolones	Fe^{3+}	Spectrofluorometry	[16]
	Ceftazidime	Fe^{3+}	Spectrophotometry	[17]
Redox reaction	Norfloxacin	3,3′,5,5′-tetramethylbenzidine (TMB)	Spectrofluorometry	[18]
	Pharmaceutical (Ceftazidime)	Carbocyanine dye	Spectrofluorometry	[19]
Charge transfer complexation	Fluoroquinolones (Norfloxacin)	Bromocresol green	Spectrophotometry	[20]
	Norfloxacin	Sudan III	Spectrophotometry	[21]
	Cephalosporins	Chloranilic acid	Spectrophotometry	[22]
Ion-pair complexation	Norfloxacin	Bromophenol blue	Spectrophotometry	[13]
	Fluoroquinolones (Norfloxacin)	Eosin Y	Sequential injection analysis using UV detector	[23]
	Norfloxacin	Sudan II, Congo red, Gentian violet	Spectrophotometry	[24]
	Cephalosporins	Methylene blue	Spectrophotometry	[25]
	Cephalosporins	Ammonium reineckate	Spectrophotometry and atomic absorption spectrometry	[26]
Azo dye	Cephalosporins	*p*-	Spectrophotometry	[27]

Colorimetric reaction	Analyte	Reagent	Method	Ref.
formation		dimethylaminobenzal dehyde (DMAB) as a coupling reagent		
	Cephalosporins	3-amino phenol (AP) as a coupling reagent	Spectrophotometry	[28]

Among them, dye binding reagents are attractive alternative reagents for colorimetric assay in response to concerns over the toxicity of metal ions and the instability of oxidizing agents. Based on norfloxacin structure as zwitterion, it could form ion-pair complexes with cationic or anionic dyes under controlled solution pH. Various reagents for dye binding, including Congo red [24], bromophenol blue [13], and Eosin Y [23], have been employed for norfloxacin determination.

Chaiyasing and coworkers used Eosin Y as a dye reagent to detect fluoroquinolone residues, including norfloxacin, ciprofloxacin, and enrofloxacin, in fish by a sequential injection analysis (SIA) method [23]. The reaction is based on the formation of ion-pair complexes between fluoroquinolone and Eosin Y in Britton-Robinson buffer at pH 2.0 (Figure 2.4), producing pink colored complexes with maximum absorption at 522, 525, and 527 nm for norfloxacin, ciprofloxacin, and enrofloxacin, respectively. The linear ranges for determination were 0.05 - 10.0, 0.10 - 10.0, and 0.05 - 10.0 mg/L for norfloxacin, ciprofloxacin and enrofloxacin, respectively with detection limit in the range of 0.013- 0.019 mg L^{-1}.

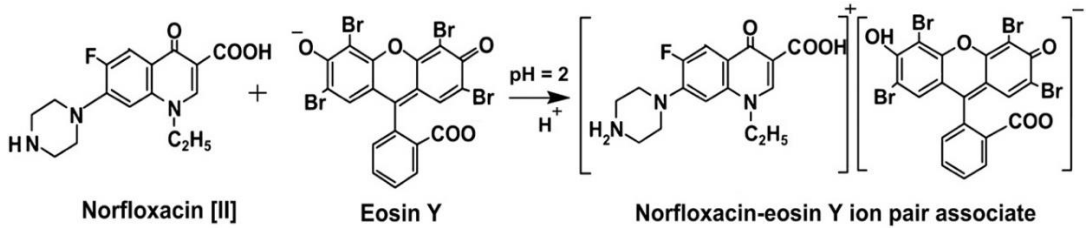


Figure 2.4 Ion-pair complexation between norfloxacin and Eosin Y [23].

Al-Tamrah and coworker determined norfloxacin using bromophenol blue as dye binding reagent [13]. At pH 4.1, a basic nitrogen atom of norfloxacin reacted with

bromophenol blue to form a yellow complex through the ion-pair complexation reaction (Figure 2.5). The colored ion-pair complexes were determined by a spectrophotometric method with the maximum absorption wavelength at 416 nm. The linear range was found in the range of 5 to 150 mg L^{-1} with relative standard deviation of less than 2%.

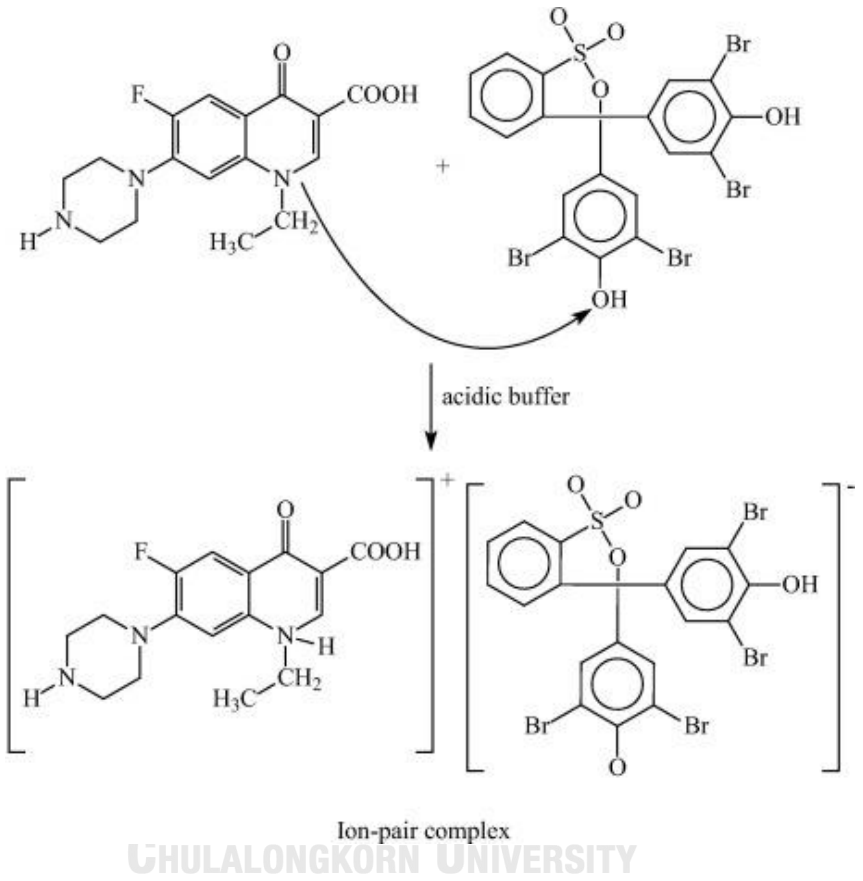


Figure 2.5 Ion-pair complexation between norfloxacin and bromophenol blue [13].

For ceftazidime detection, another popular colorimetric reaction is azo dye formation. This reaction is based on the formation of azo compounds by coupling a diazonium salt with a coupling reagent. The Griess reaction, a widely utilized colorimetric method based on azo dye formation, is commonly employed for nitrite detection [32, 68]. In this standard method in Figure 2.6, sulfanilamide (**1**) is subjected to a diazotization reaction with nitrite under acidic condition. The obtained diazonium ion (**2**) further reacts with N-(1-naphthyl) ethylenediamine (NED) (**3**) through coupling reaction to yield an azo dye product (**4**) [69].

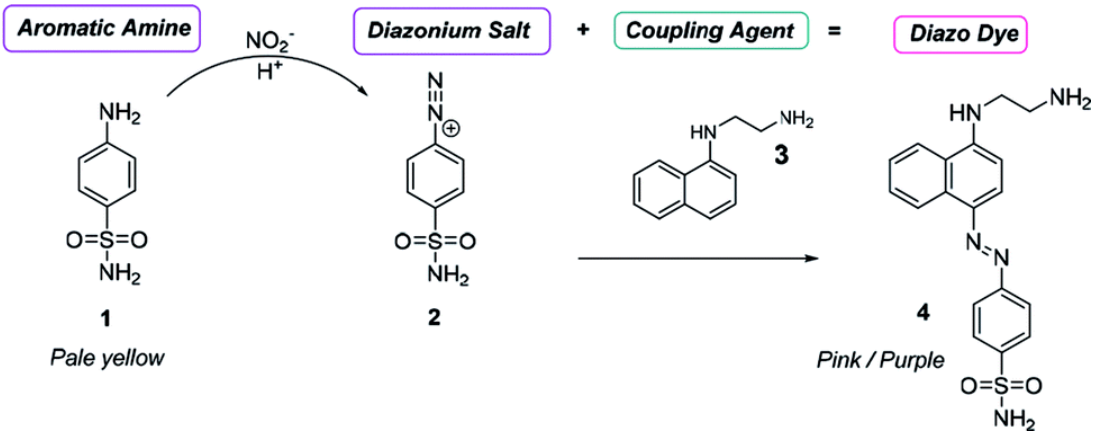


Figure 2.6 Griess reaction for the detection of nitrite in aqueous media [69].

Additionally, this reaction has been adopted for the analysis of different pharmaceutical compounds, such as antiepileptic drugs like gabapentin [51], anti-malarial drugs [56], and cephalosporin antibiotics (e.g., ceftriaxone, cefixime, ceftazidime) [26, 50], using different aromatic amines and coupling reagents. Adegbolagun and coworkers developed a spectrophotometric method for the determination of gabapentin using chromotropic acid as a coupling reagent [30]. The method relied on the diazo coupling reaction between diazotized gabapentin and chromotropic acid, forming an orange azo adduct with the maximum absorption wavelength at 470 nm (Figure 2.7). The linear range for such analysis was established in the range of 1 – 6 mg L^{-1}. For sample analysis, the method was able to produce results with good accuracy (recovery range of 97.6 – 103.1%) and precision (%RSD < 0.65%).

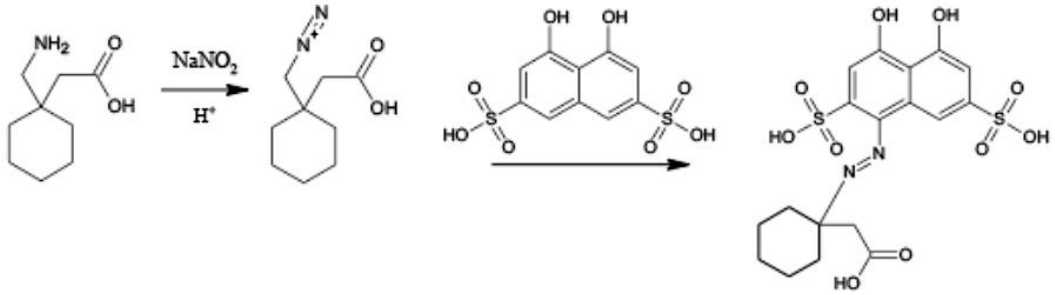


Figure 2.7 The coupling reaction between diazotized gabapentin and chromotropic acid.

Roopa and Jayanna reported the use of 3-aminophenol as a coupling reagent for spectrophotometric determination of four cephalosporins including ceftriaxone (CEFT), cefatoxime (CEFX), ceftazidime (CEZD), and cefepime (CEPM) in bulk and in pharmaceutical formulations [28]. The method was based on the formation of orange red colored product (λ_{max} = 500 nm) through the diazotization of cephalosporins in acidic medium, followed by coupling with 3-aminophenol as illustrated in Figure 2.8. The working linear ranges for cephalosporins determination were from 20 – 168 mgL^{-1} and the method could produce analytical results with acceptable precision and accuracy.

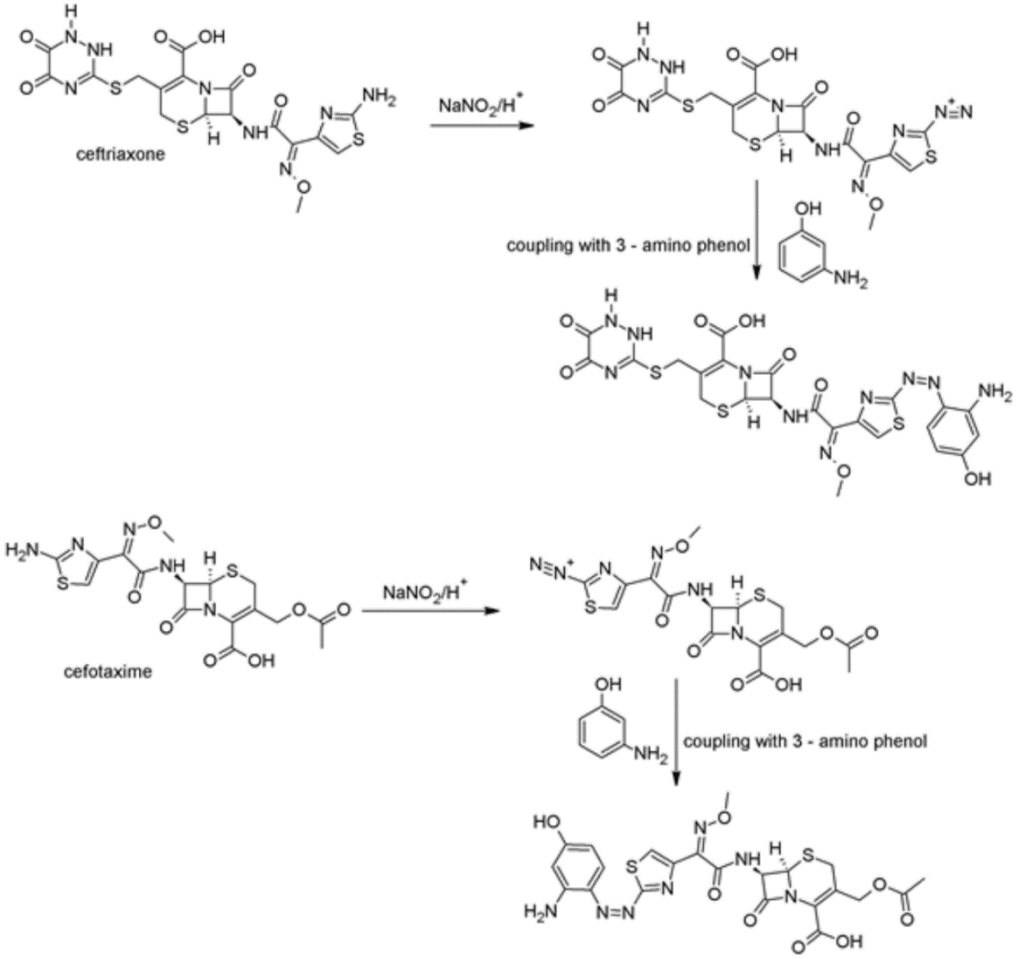


Figure 2.8 Reaction pathway of ceftriaxone and cefatoxime with 3-aminophenol [28].

The presence of an aromatic amine in the structure of ceftazidime allows the diazotization reaction and subsequent coupling reaction with a suitable reagent to

occur. In this study, chromotropic acid was selected as the coupling reagent instead of the traditional N-(1-naphthyl)ethylenediamine (NED) due to its lower toxicity [70]. Chromotropic acid has been successfully used as a chromogenic reagent for the detection of various substances, including formaldehyde [31], gabapentin drug [30], and nitrite [32].

From the previously reported colorimetric methods, they still require a UV-vis spectrophotometer and fluorometer for the analysis and professional users to perform the experiment. To solve this problem, digital image colorimetric detection is an attractive alternative due to its portability, affordability, and easy operation. Moreover, level of norfloxacin and ceftazidime residues in aquaculture is low, and hence sensitive and selective methods are desired. To improve the sensitivity and selectivity of the detection, separating analytes from sample matrix using solid phase extraction is an appealing option. In this work, different solid platforms were applied for analytes extraction. With proper surface modification, the colorimetric reactions with specific reagents were performed on the solid surface, followed by the digital image colorimetric detection.

2.4 Materials for analyte extraction

2.4.1 Polymer resin (Amberlite XAD-7)

Amberlite XAD-7 is a hydrophobic polymeric adsorbent based on a styrene-divinylbenzene copolymer matrix (Figure 2.9). It possesses a highly cross-linked structure, which contributes to its excellent thermal and chemical stability [71]. The resin exhibits a non-polar nature, making it suitable for adsorption of non-polar compounds. The major adsorption mechanism of non-polar compounds on the resin is based on the hydrophobic interaction. The adsorption capacity of Amberlite XAD-7 depends on various factors such as resin particle size, porosity, and surface area. These properties can be specifically modified to enhance the resin performance and suit its applications.

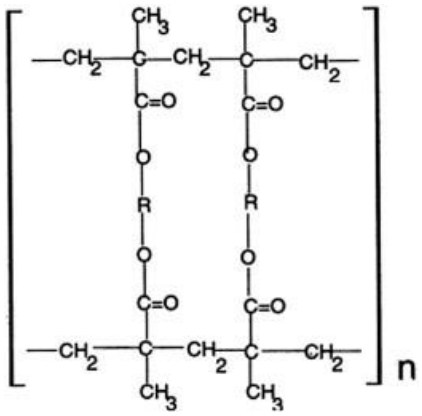


Figure 2.9 Chemical structure of Amberlite XAD-7 resin [72]

Amberlite XAD-7 has found its extensive use in various applications, including separation and purification processes. The resin is widely employed for removing organic pollutants, such as aromatic hydrocarbons [73], pesticides [74], oil [75], and pharmaceutical residues [72], from wastewater and contaminated soils. Moreover, The amberlite XAD-7 could be modified with specific reagents to enhance the removal of metal ions in environmental samples including Ce (III) and La (III) [76], Cr (VI) [77], and Ni (II) [78]. It has been reported that Congo red modified Amberlite XAD-7 resin was used for the preconcentration and determination of trace metals in fish using flame atomic absorption spectrometry [79]. In this work, the Amberlite XAD-7 resin was modified with Congo red for the extraction and colorimetric detection of norfloxacin based on ion-pair complexation reaction.

2.4.2 Cellulose sponge

Cellulose sponge or cellulose aerogels are three-dimensional nanoporous structures fabricated from cellulose. They possess a highly porous network with an interior of more than 98% air, low density, and excellent mechanical properties [80]. Furthermore, the ease of functionalization is another remarkable feature of these flexible aerogels [81, 82]. In addition, cellulose is considered a third-generation green material found abundantly in nature. These attributes make cellulose aerogels

attractive for various applications, including their use as sorbents, heat insulation, biomedical material, and sensors [83-88].

Cellulose aerogel is typically synthesized through a three-step procedure [89]. First, cellulose or its derivatives are dissolved or dispersed in a proper liquid to form a colloid. Next, a cellulose gel is formed using the sol-gel process, often referred to as gelation. The addition of cross-linking agents could drive the colloid solution to form the gel with a strong interaction [90]. Finally, the cellulose gel is freeze-dried, preserving its three-dimensional porous structure. Cellulose can be obtained from various biomass such as rice straw, wheat straw, bamboo pulp, cotton, wood, and potato tubers. Some recent applications of cellulose aerogel are summarized in Table 2.5.

Table 2.5 Summary of recent applications of cellulose aerogel in various fields.

Application	**Source of cellulose**	**Detail**	**Ref.**
Sorbent	Cotton staple	Cellulose-based aerogels were fabricated by directional freeze-drying and used as sound-absorption materials	[83]
Sorbent	α-Cellulose	Cellulose aerogel beads were functionalized with PEI to extract Cr^{6+} through electrostatic interaction, followed by a redox reaction for removing Cr^{6+}.	[84]
Sorbent	Hardwood pulp	PEI grafted cellulose nanofibril aerogel was used to adsorb Cu^{2+} from wastewater through metal-ligand complexation between Cu^{2+} and amino groups of PEI.	[85]
Heat insulation	Waste cotton fabric	Magnesium hydroxide nanoparticles was incorporated in cellulose hydrogel, followed by freeze-drying for the use as a heat insulating material.	[86]
Biomedical material	Bamboo pulp	Cellulose nanofibrils were modified with amino group, followed by grafting with PEI using	[87]

Application	Source of cellulose	Detail	Ref.
		glutaraldehyde as a crosslinker for drug delivery.	
Sensor	Rice straw	Cellulose aerogel was incorporated with halochromic hydrazonal chromophore for colorimetric detection of ammonia.	[88]

Polyethyleneimine grafted cellulose sponge

One of the prominent applications of cellulose aerogels is in adsorption and separation processes. Due to their high surface area and porosity, cellulose aerogels have been utilized as efficient adsorbents for the extraction and preconcentration of analytes from complex matrices. Furthermore, one promising approach to enhance the extraction efficiency of cellulose-based composites is by incorporating polyethyleneimine (PEI). PEI is a cationic polymer that can be used to extract anion compounds. PEI is a linear or branched polymer with repeating units of ethyleneimine monomers, which contain primary, secondary, and tertiary amine groups (Figure 2.10) [91].

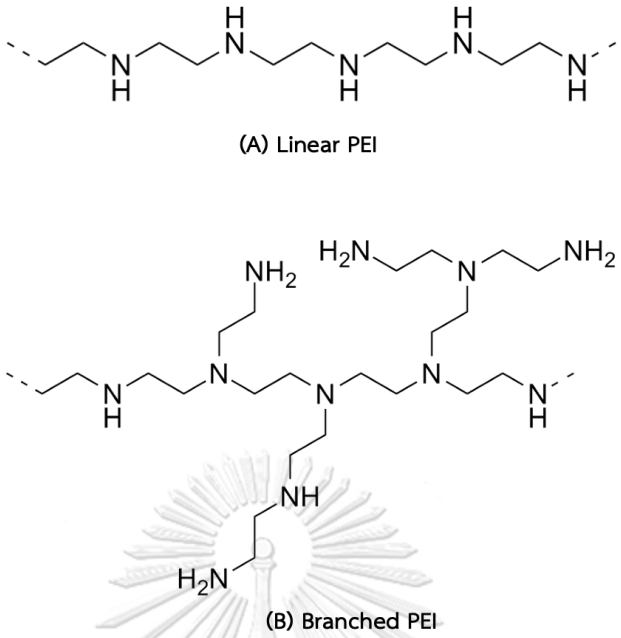


Figure 2.10 Chemical structures of (A) linear PEI and (B) branched PEI.

To chemically modify the cellulose with PEI, proper cross-linkers are required. The widely used cross-linkers are epichlorohydrin and glutaraldehyde. In the case of using epichlorohydrin as a cross-linker, it requires heating for the epoxy ring opening by amine group of PEI, resulting in the grafting of PEI on cellulose surface as shown in Figure 2.11 [92].

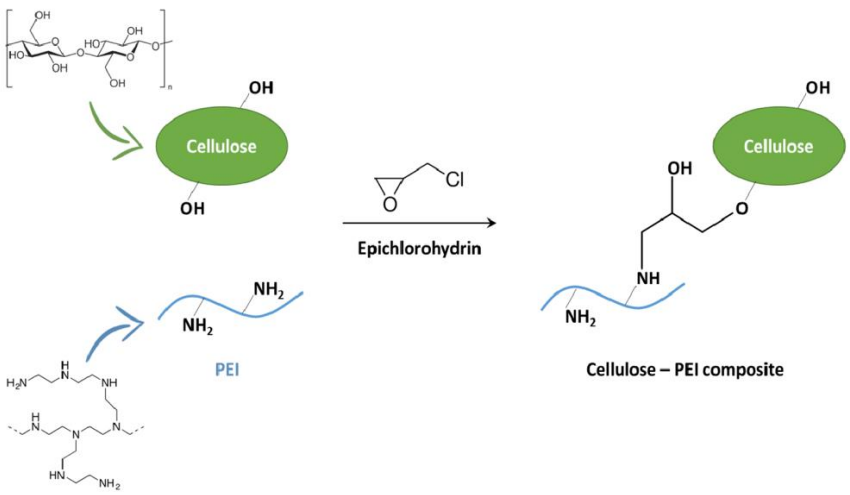


Figure 2. 11 Schematic of grafting of PEI on cellulose using epichlorohydrin as crosslinker [92].

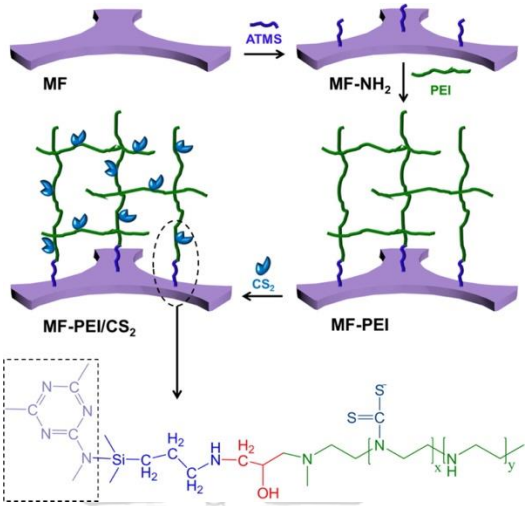


Figure 2.12 Schematic illustration for the fabrication of MF-PEI/CS_2 sponge [93].

Some previous works reported the cellulose surface modification using 3-aminopropyltriethoxysilane (APTES) followed by cross-linking with the PEI [93, 94]. Huang and co-workers [93] first coated the surface of melamine sponge with APTES to furnish more NH_2 groups, and then grafted with PEI by chemical crosslinking with epichlorohydrin (Figure 2.12). The obtained PEI modified melamine sponge (MF-PEI sponge) was esterified with CS_2 to introduce dithiocarbamate groups, resulting in MF-PEI/CS_2 sponge for the removal of copper (II) ions from aqueous solution.

Wang and Won [94] prepared amine-functionalized multiwall carbon nanotubes (MWCNTs) by grafting APTES on the surface of MWCNTs and crosslinking PEI with APTES-MWCNTs using epichlorohydrin as a crosslinker for the removal of Reactive Yellow 2 from water. The scheme of the reaction pathway of the PEI/APTES-MWCNTs preparation is shown in Figure 2.13.

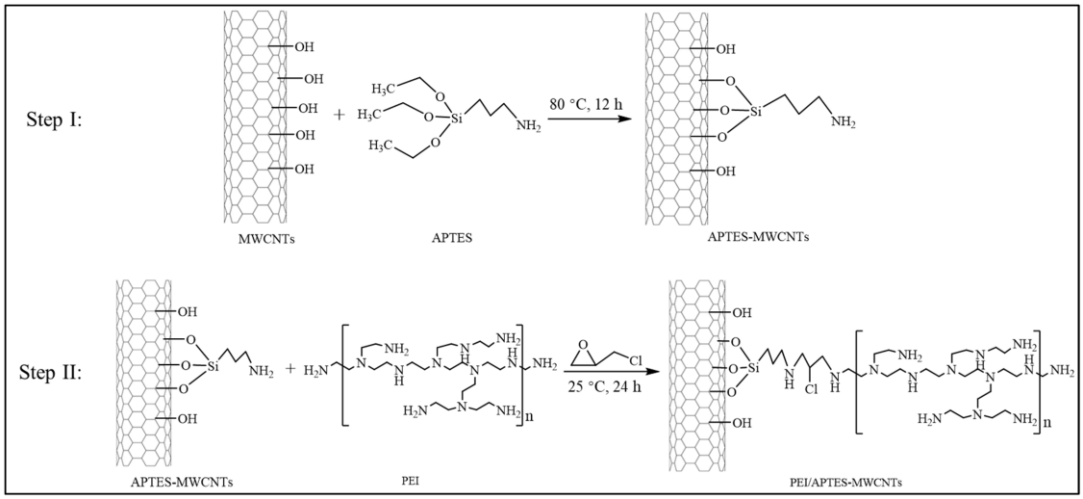


Figure 2.13 The scheme of the reaction pathway of PEI/APTES-MWCNTs preparation [94].

In this work, we fabricated cellulose sponges from cotton fibers that were modified with APTES, followed by grafting the APTES-sponge with PEI using epichlorohydrin as a crosslinker (Figure 2.14). This PEI-sponge was used for the extraction and detection of ceftazidime by colorimetric detection.

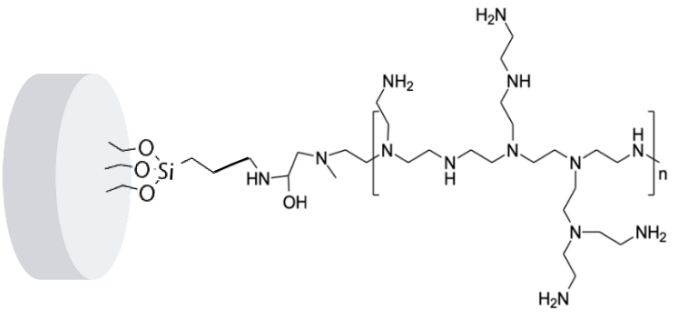


Figure 2.14 The proposed structure of PEI-sponge.

CHAPTER III

DETERMINATION OF NORFLOXACIN BY DYE-MODIFIED RESIN

3.1 Experimental

3.1.1 Materials and chemicals

Materials and chemicals used in this research are listed in Table 3.1. All chemicals were analytical reagent grade (AR) and used without further purification.

Table 3.1 List of materials and chemicals.

Chemicals	Supplier
Amberlite XAD-7 (20-60 mesh)	Sigma-Aldrich (Germany)
Norfloxacin	Sigma-Aldrich (Germany)
Congo red	Merck (Germany)
Hydrochloric acid	Merck (Germany)
Sodium hydroxide	Merck (Germany)

3.1.2 Instruments

The instruments used in this study are UV-visible spectrophotometer (Hewlett Packard 8453) and pH meter (SevenCompact^{TM} S220-Basic, Mettler Toledo, Switzerland).

3.1.3 Colorimetric reaction of norfloxacin in solution

The colorimetric reaction between norfloxacin and Congo red and the effect of solution pH were investigated by observing the absorption spectra of mixture solutions using a UV/Vis spectrophotometry. For the preliminary test, the effect of pH of solution was studied by varying the pH of solution from pH 3 – 6. The

mixture solution was prepared by adding 0.5 mL of 0.72 mM Congo red into 1 mL of 100 mg L^{-1} norfloxacin. The final volume was made up to 10 mL with 0.01 M buffer solution of desired. The absorbance intensities of the mixture solutions with and without norfloxacin were measured by a UV-Vis spectrophotometer.

The reaction between norfloxacin and Congo red at the suitable pH was further investigated using a UV-Vis spectrophotometer. Standard norfloxacin solutions of various concentrations (0 – 10 mg L^{-1}) were prepared in 0.01 M citric-citrate buffer pH 4, and to each solution, 0.5 mL of Congo red (0.72 mM) was added. The final volume of each solution was adjusted to 10 mL using Milli-Q water. The absorption spectra of norfloxacin and Congo red complex were recorded.

3.1.4 Material modification and norfloxacin detection

Amberlite XAD-7 resin (0.05 g) was suspended in 2 mL of Congo red solution containing 0.05 M NaCl under stirring at room temperature for 1 h. After the modification, the modified resin was separated using a Buchner funnel filter kit and was washed with deionized water to remove the excess reagent. For norfloxacin detection, the modified resin was immersed in a norfloxacin solution and stirred for a specified period. The material was then separated from solution and placed into a sample holder using a dropper. The resin color was observed by taking a photo of the material using a digital camera (Fuji XA-2) in a studio box and subjecting the photo to Image-J software. The color intensity was measured in red mode (I). The results are reported in term of ΔI ($\Delta I = I_{sample} - I_{blank}$) where I_{blank} and I_{sample} are the color intensity on resin obtained from the analysis of blank solution and sample solution, respectively.

The schematic diagram for the determination of norfloxacin using the Congo red modified resin is presented in Figure 3.1. The effects of pH of Congo red solution, Congo red concentration, sample volume, and reaction time were investigated.

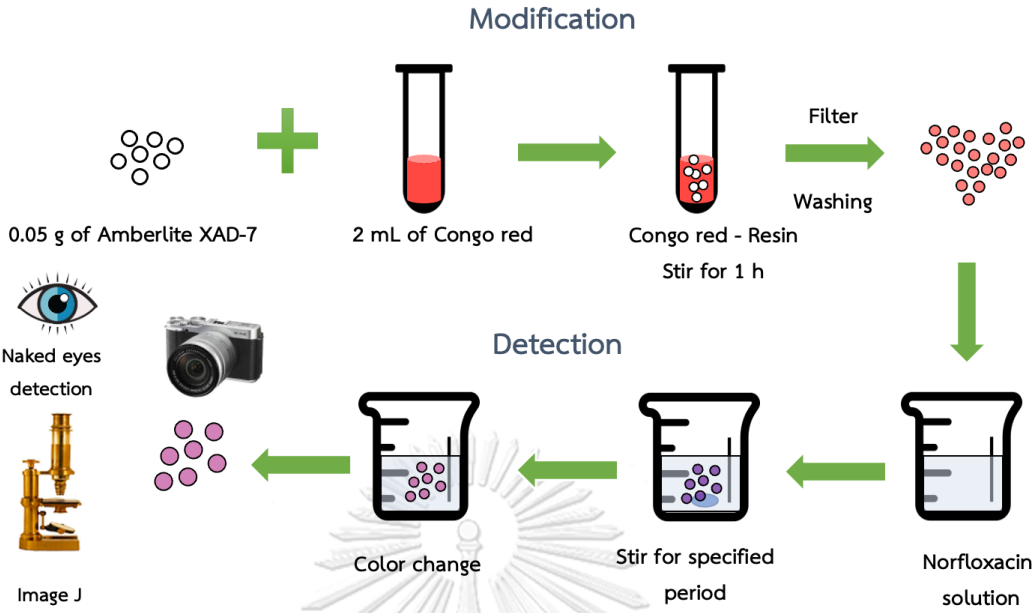


Figure 3.1 The schematic diagram for the determination of norfloxacin using the Congo red modified resin.

3.1.4.1 Effect of pH of Congo red solution for modification

The pH of Congo red solution used to modify resin (Amberlite XAD-7) was studied in the range of pH 2 – 6. Congo red solutions (4.48 μM) were prepared in 0.05 M citric-citrate buffer or 0.05 M NaCl, and the pH of each solution was adjusted using HCl and NaOH to the desired pH. After the modification process, the color of resin was detected by taking a photo of the material. Additionally, the resins modified with Congo red of different pH were used to detect norfloxacin in the concentration range of 0 to 1.5 mg L^{-1} (at pH 3).

3.1.4.2 Effect of Congo red concentration

The detection sensitivity and the analytical linear range for norfloxacin determination could be influenced by the concentration of Congo red used for resin modification. Various concentrations of Congo red solution ranging from 3.75 to 6.75 μM were prepared in the solution containing 0.05 M NaCl at pH 6. Subsequently, 2 mL of this Congo red solution was added to 0.05 g of resin, and the

mixture was stirred for 1 hour. The resulting resin exhibited an orange-red color and was then introduced into a norfloxacin solution (10 mL, pH 3) in the concentration range of 0 to 1 mg L^{-1}. After being stirred for 1 h, the modified resin was separated from the solution and placed in a sample holder for photographing.

3.1.4.3 Effect of sample volume

The effect of sample volume on the detection of norfloxacin was investigated in the range of 10 to 20 mL. The resins modified with 5.25 and 6.00 μM Congo red at pH 6 were used to detect norfloxacin (pH 3) in the concentration ranging from 0 – 0.75 mg L^{-1}. The modified resins were stirred in norfloxacin solution for 1 h before measuring the color intensity in red mode through the Image-J software.

3.1.4.4 Effect of reaction time

The reaction time for norfloxacin detection was optimized. The resin modified with 6.00 μM Congo red was immersed in 10 mL norfloxacin solution in the concentration range of 0 to 0.75 mg L^{-1} at pH 3 for the length of reaction time varying from 15 to 60 minutes. After separating modified resin from the solution, a photo of the material was taken and subjected to Image-J software for the analysis of color intensity.

3.1.5 Method performance and water sample analysis

The analytical performance for norfloxacin detection was evaluated by applying this method to detect norfloxacin real water samples. From the previous investigation, the most suitable pH for standard norfloxacin detection was pH 3. However, in real water samples, the starting color of modified resin after being immersed in water samples was red in the absence of norfloxacin. It might be because some basic species in real water samples could deprotonate Congo red. To

minimize this inference effect, the pH of standard norfloxacin solutions and sample solutions was adjusted to 2 prior to the analysis.

The calibration curve was constructed by plotting between the color intensities and the concentration of norfloxacin in the range of 0 to 1.50 mg L^{-1}. This standard curve was employed to quantify the level of norfloxacin in real water samples and evaluate the accuracy and precision of the developed method. Norfloxacin standard was spiked into the real water samples to obtain the added concentrations of 0.5 and 1.0 mg L^{-1} for the recovery test. For water sample analysis, the drinking water and tap water were filtered through Whatman® membranes with a pore size of 0.45 μm and the pH of sample solutions were adjusted to ca. 2 using HCl solution before the analysis. To prepare the resin, 0.05 g of resin was immersed in 2 mL of 6.0 μM Congo red solution (pH 6) containing 0.05 M NaCl for 1 hour. After that, the modified resin was stirred in 10 mL sample solution (pH 2) for 15 minutes. Then, the material was separated from solution and placed in a sample holder for photographing. The color intensity was measured in red mode using the Image-J program. The method was applied to detect norfloxacin in non-spiked and spiked samples and the percent recovery was calculated using equation (**1**).

$$\%\text{Recovery} = \frac{C_{spike\ sample} - C_{non-spkied\ sample}}{C_{added}} \times 100 \quad (1)$$

3.2 Results and discussion

3.2.1 Colorimetric reaction of norfloxacin in solution

Congo red is an anionic dye that is commonly used as a pH indicator. It contains $-NH_2$ and $-SO_3$ functional group with pk_a of 4.1 (Figure 3.2) [95]. In acidic solution, the amine groups of Congo red is protonated, and in strong acidic solution (pH ≤ 3), the dye molecule exists predominantly in its protonated form which is blue in color. The UV-Vis absorption spectrum of that solution showed the maximum absorption at 570 nm. On the contrary, by increasing the pH to above its pK_a (pH ≥ 5), the amine group is not protonated, and this form of Congo red exhibits a red

color with the maximum absorption at 497 nm when analyzed by a UV-Vis spectrophotomer. At the pH between 3 to 5, there is a mixture of these forms, appearing as purple color in solution. Given the structure of norfloxacin, it contains basic nitrogen atoms which can be protonated by abstracting a proton from the protonated Congo red in acidic solution (Figure 3.3). The protonated norfloxacin and deprotonated Congo red form an ion-pair complex and the red color of deprotonated Congo red appears in the solution despite acidic condition.

To follow the reaction between norfloxacin and Congo red, the Congo red solution without and with norfloxacin (10 mg L^{-1}) were analyzed by a UV/Vis spectrophotometer and their spectra were recorded. The effect of solutions pH affecting the form of Congo red and the reaction was investigated in a range of 3 to 6 (Figure 3.4).

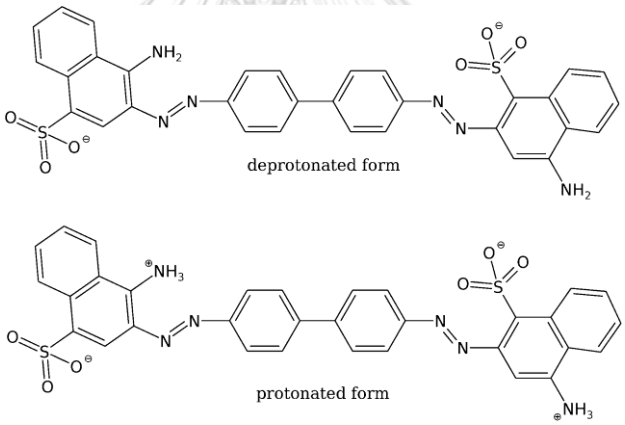


Figure 3.2 Structure of the deprotonated and protonated Congo red [95].

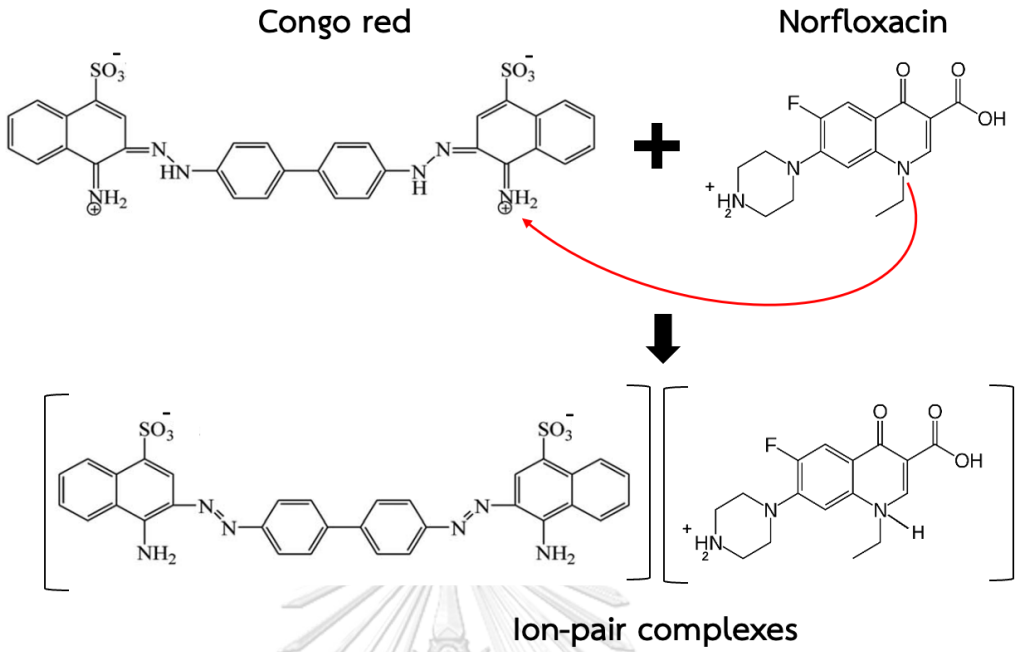


Figure 3.3 The proposed reaction between Congo red and norfloxacin through the ion-pair complexation reaction in acidic solution.

From the solution color shown in Figure 3.4, the most distinct color change between the Congo red blank and the mixture of Congo red and norfloxacin was observed at pH 4. At this pH, without norfloxacin, Congo red was in its protonated form showing blue-purple color. After mixing with norfloxacin, the solution color changed to red-purple, revealing the formation of the basic form of Congo red. Additionally, the spectrum of the mixture showed the shift of maximum adsorption from 570 nm of Congo red blank at pH 4 to 497 nm. The maximum absorption at 270 nm was attributed to norfloxacin in the mixture, as compared to the pure norfloxacin spectrum in Figure 3.5. Hence, it can be concluded that the solution color changed from blue-purple to red-pink due to the ion-pair complexation between Congo red and norfloxacin at pH 4.

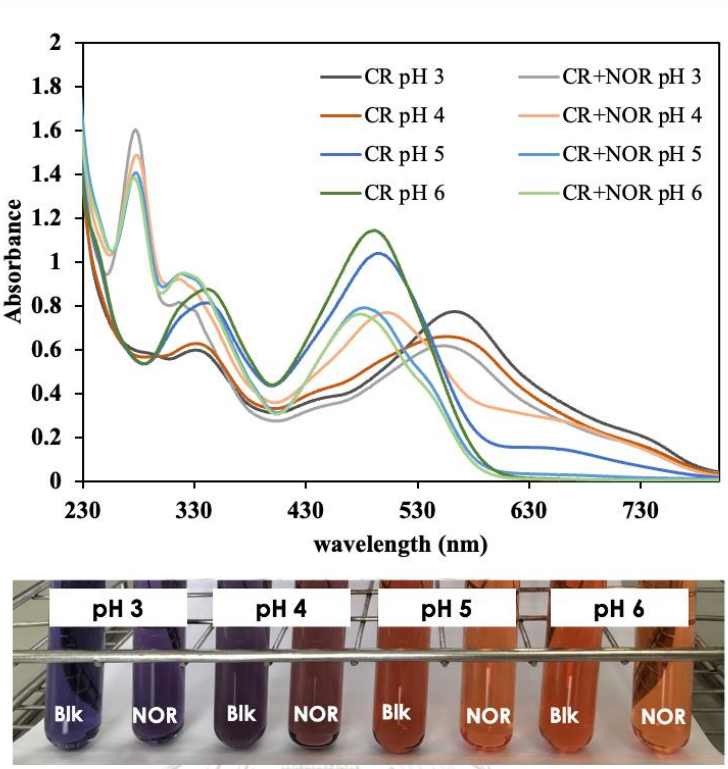


Figure 3.4 Absorption spectra and solution color of 36 μM Congo red and mixture of 10 mg L^{-1} norfloxacin and 36 μM Congo red at different pH.

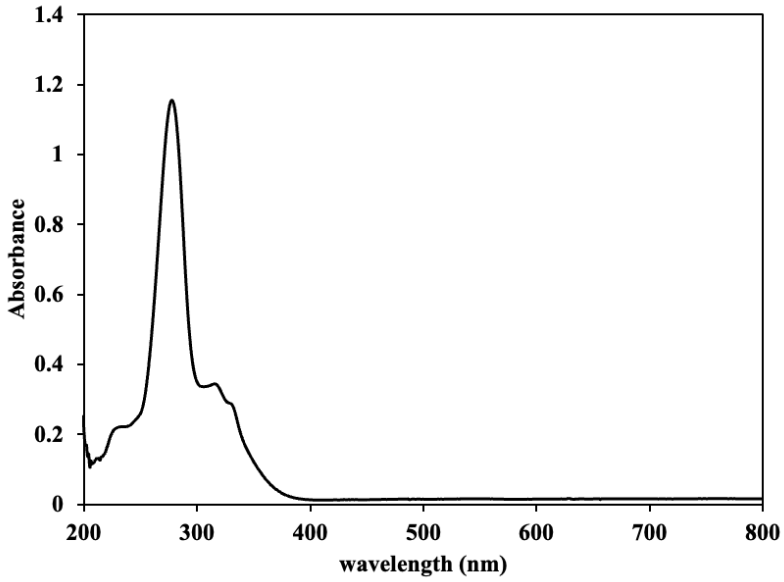


Figure 3.5 Absorption spectrum of 10 mg L^{-1} norfloxacin solution.

The color change of Congo red solution depended on the concentration of norfloxacin. As illustrated in Figure 3.6, the solution color became redder with increasing concentration of norfloxacin. The spectra also showed increased absorption at 497 nm of the deprotonated form of Congo red, proportionally to the norfloxacin concentration. Therefore, the reaction between Congo red and norfloxacin was confirmed and further applied in norfloxacin detection using Congo red modified resin.

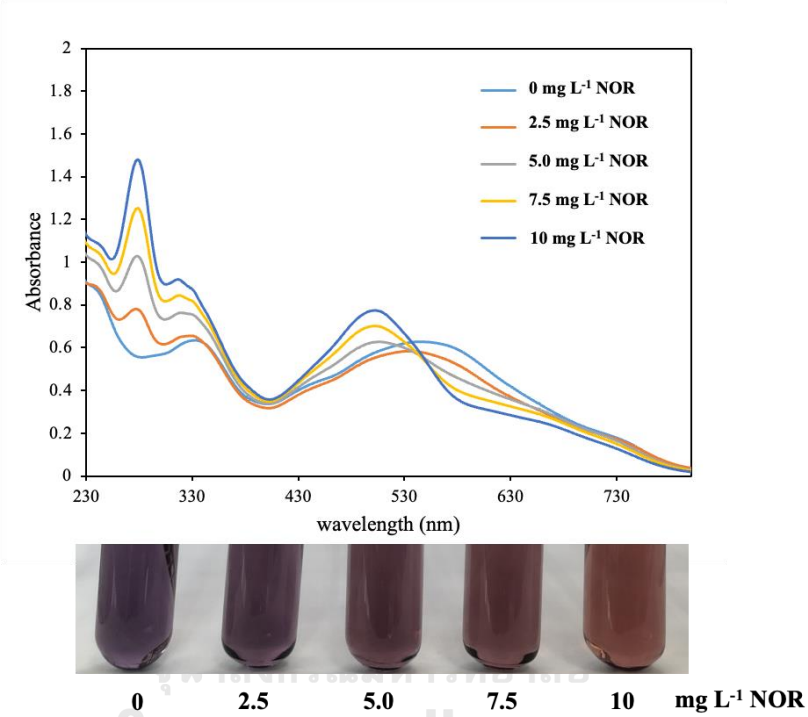


Figure 3.6 Absorption spectra and solution color of 36 μM Congo red reacted with the different concentrations of norfloxacin at pH 4.

3.2.2 Optimization of norfloxacin detection

3.2.2.1 Effect of pH of Congo red solution on resin modification

In solid platform, Congo red was used to modify resin (amberlite XAD-7) to extract and detect norfloxacin. Amberlite XAD-7 resins can adsorb non-polar compounds through hydrophobic interactions. Moreover, some previous studies have been reported that Amberlite XAD-7 is moderately hydrophilic with the pH_{pzc} of 6.2 [72]. It has been used to remove dyes and pharmaceuticals from surface

water [96, 97]. The aim of this study was to obtain a condition for the resin modification by Congo red with adequate amount for norfloxacin detection. The pH of solution plays a significant role for the dye adsorption onto the resin because it affects the form of Congo red in solution and its hydrophilicity. In this study, the resin was modified with Congo red solution of various pH values (pH 2 to pH 6). In Figure 3.7, the results show that the resins modified with Congo red at pH 2.0 and pH 3.0 had pale blue and purple color, respectively, indicating a small amount of Congo red adsorbed on the resin. Under strong acidic condition, Congo red molecule bearing the deprotonated sulfonate group ($R\text{-}SO_3^-$) and protonated amine group is highly hydrophilic, compared to the other pH. Consequently, the adsorption of Congo red on the resin was less favorable. On the other hand, the resins modified with Congo red at a higher pH (pH 4 – pH 6) showed intense red-orange color. It might be because at this pH range, amine group of Congo red is deprotonated to its neutral form, leading to the decreased hydrophilicity of Congo red. Therefore, the adsorption of Congo red on the resin was more favorable through hydrophobic interactions.

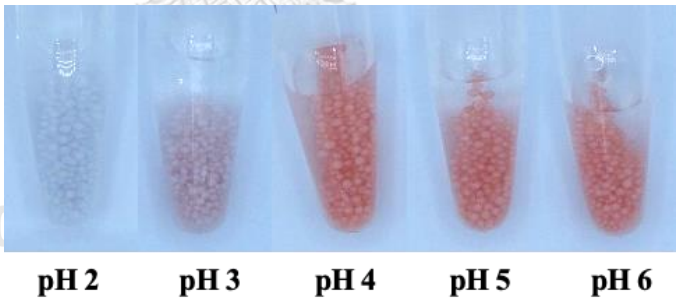


Figure 3.7 The color of resin modified with Congo red at different pH values.

In order to use the resin modified with Congo red at pH 4-6 for norfloxacin detection, the Congo red on resin surface should be in its protonated form (purple color). In this work, the pH of norfloxacin was adjusted to pH 3 to turn the Congo red on the resin surface to its protonated from. Hence, the color of resin was purple in the sample solution at the beginning and subsequently changed to red-pink in the presence of norfloxacin. Moreover, at pH 3, norfloxacin is positively

charged due to the protonation (pKa 6.22) and extracted onto the resin through electrostatic interaction with the negatively charged SO_3^- of Congo red. Then, the reaction involving the ion-pair complex formation further occurred on the resin surface. The schematic diagram of norfloxacin extraction and detection is shown in Figure 3.8.

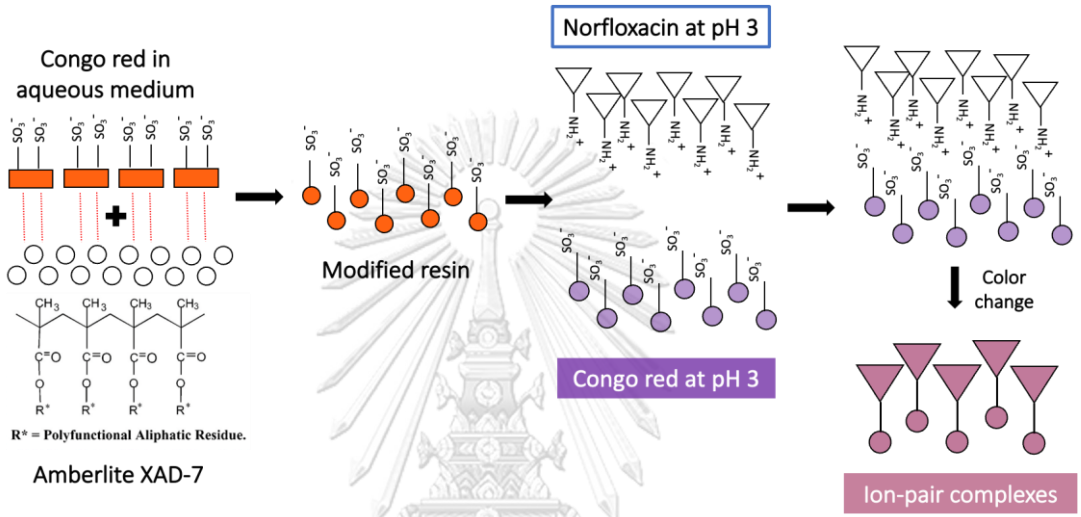


Figure 3.8 The schematic diagram of norfloxacin extraction and detection using Congo red modified resin.

To further investigate the effect, the resins modified with Congo red of various pH (pH 3 – 6) were applied to detect norfloxacin (0 – 1.5 mg L^{-1}) at pH 3. The results in Figure 3.9 showed that the color intensities of the resin modified with Congo red at pH 3 rarely changed in the presence of norfloxacin and the difference of color intensity between the sample and blank (ΔI) was too low. This is because there was a large amount of protonated Congo red on the resin and a small amount of ion-pair complex formed on the surface that could not yield a distinct color change on the resin due to the intense blue background. When the resin was modified with a higher pH (pH 4 – pH 6), there were both protonated and deprotonated forms of Congo red on the resin. When these resins were applied with the solution of pH 3, their color turned to blue purple as the blank background, making it easy on the observation of the reddish color change. The highest ΔI

intensities at a low concentration range of norfloxacin (0 – 1.0 mg L^{-1}) were achieved when the resin was modified with Congo red at pH 6. Therefore, a Congo red solution of pH 6 was chosen for resin modification in this work.

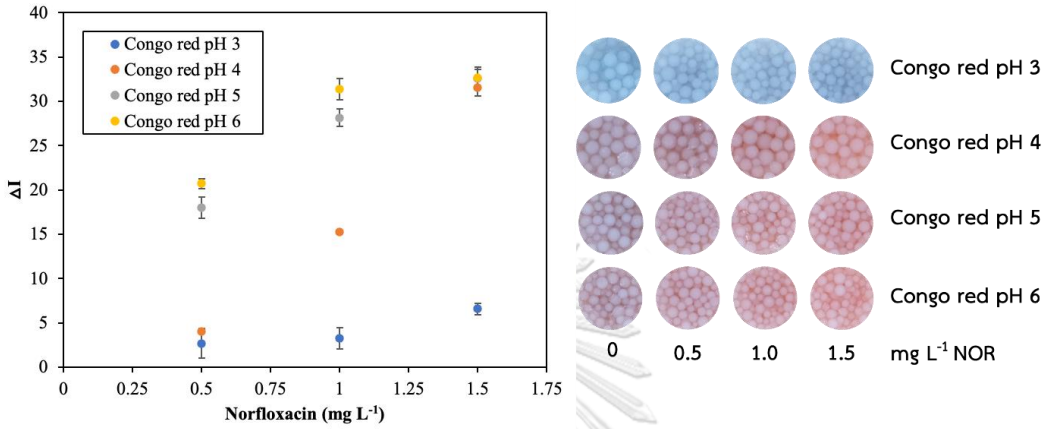


Figure 3. 9 The effect of pH of Congo red modified resin on the detection of norfloxacin at pH 3 and the photo of the obtained materials**.**

3.2.2.2 Effect of Congo red concentration

The concentration of Congo red used to modify resin could affect the color intensity on resin and analytical linear range for norfloxacin determination. The concentration of Congo red solution (pH 6) containing 0.05 M NaCl was varied from 3.75 to 6.75 μM for the determination of norfloxacin in the concentration range of 0 – 1 mg L^{-1}. In this experiment, NaCl was added into Congo red solution to increase the ionic strength and attain the salting out effect. Thus, the solubility of the dyes was decreased and hence, higher amount of dyes was adsorbed on the resins surface [98]. The results in Figure 3.10 showed the color of resin changed from purple to red in a low concentration range of norfloxacin (0 to 0.50 mg L^{-1}) when the concentration of Congo red was low (3.75 – 4.48 μM). However, it is difficult to distinguish the color of resin in a higher concentration of norfloxacin by naked eyes due to insufficient amount of Congo red on resin surface. As the concentration of Congo red increased to 5.25 μM and higher, a clear color change of resin was

observed and proportional to norfloxacin concentration. Nevertheless, at the highest concentration of Congo red (6.75 μM), the change of resin color did not much improve compared to a lower concentration (6.00 μM). It could be explained by the limited surface of resin for Congo red modification. Therefore, to avoid the reagent consumption, the concentration of Congo red of 5.25 and 6.00 μM were chosen for further investigation.

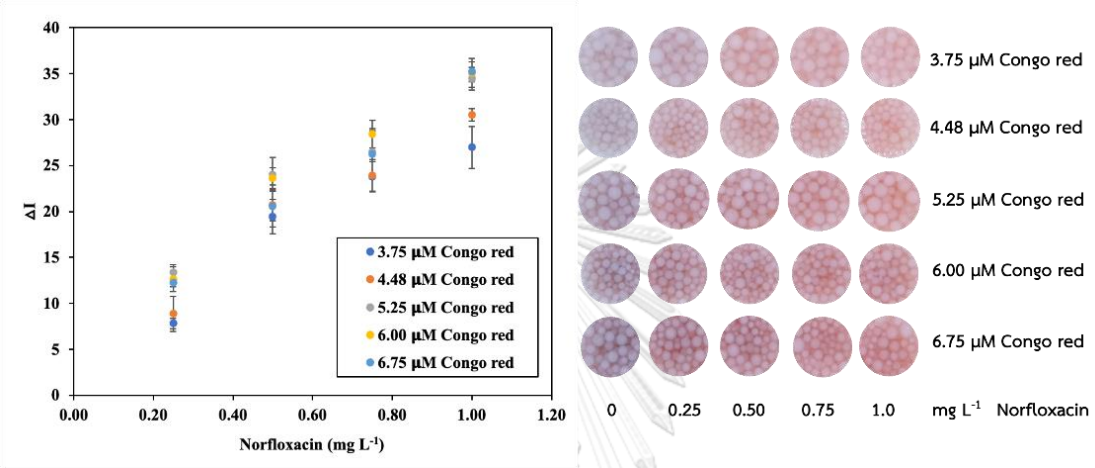


Figure 3.10 The effect of Congo red concentration on the resin color for norfloxacin detection and the photo of the obtained materials.

3.2.2.3 Effect of sample volume

To improve the sensitivity of the method, the sample volume was increased from 10 to 20 mL. The resin modified with 5.25 and 6.00 μM Congo red (pH 6) were used to detect norfloxacin in a concentration range from 0 to 0.75 mg L^{-1} at pH 3 (Figure 3.11). The results show that the color of resin modified with 5.25 μM Congo red was paler and the sensitivity decreased when the sample volume increased (Figure 3.11A), probably due to the leaching of Congo red to sample solution. This was confirmed by the paler resin color observed in the blank solution when the sample volume was increased. The leaching of dye also led to high deviation of ΔI values observed in all experiments.

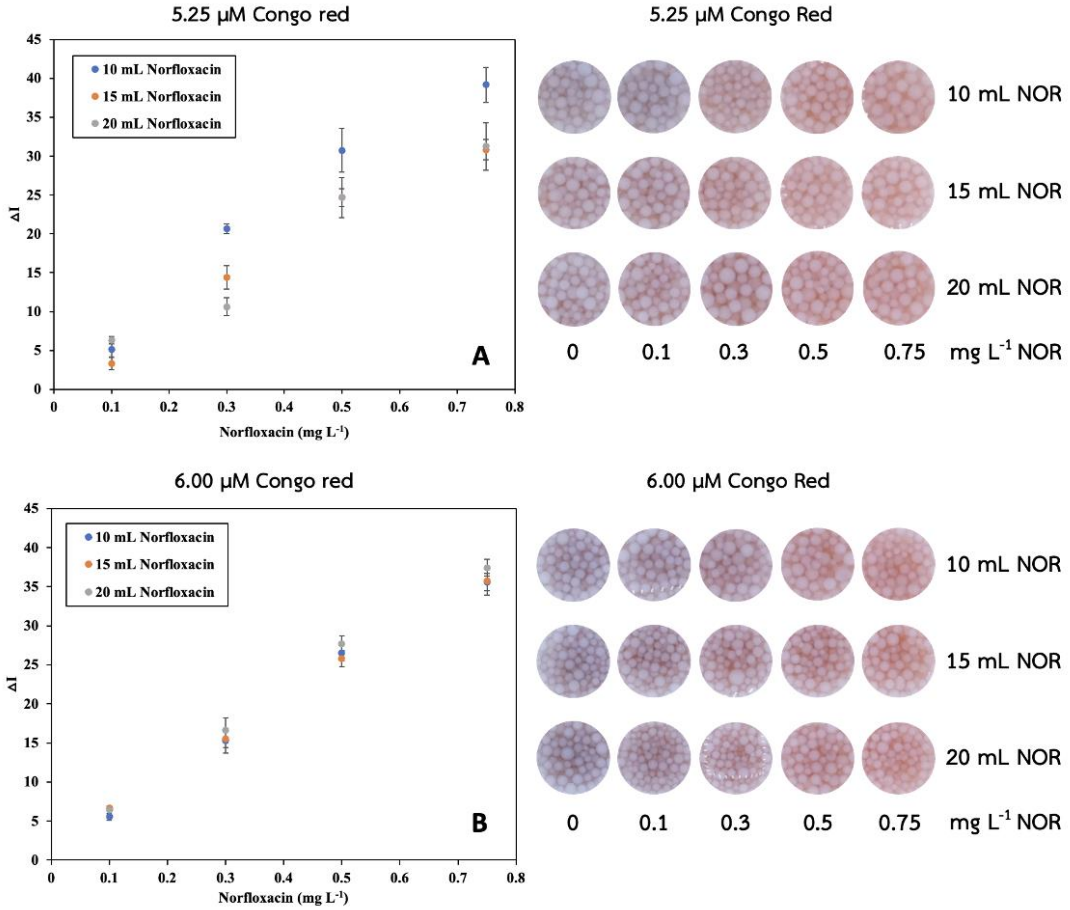


Figure 3.11 The effect of sample volume on the color intensity of the resin modified with (A) 5.25 µM Congo red and (B) 6.00 µM Congo red in the detection of norfloxacin.

On the other hand, for the resin modified with 6.00 µM Congo red, an increase of sample volume from 10 to 20 mL did not yield a significant change of color intensity as shown in Figure 3.11B. It was likely that there was sufficient content of Congo red left on the resin for the reaction with norfloxacin. Moreover, the detection sensitivity (6.00 µM Congo red) did not improve when increasing the sample volume due to the limitation of resin capacity. Therefore, to minimize sample consumption while maintaining a good detection sensitivity, 10 mL of sample volume and 6.0 µM Congo red were selected for further studies.

3.2.2.4 Effect of reaction time

The effect of reaction time between Congo red on the resin and norfloxacin in the solution on the color development on the resin was investigated in the period of 15 – 60 minutes (Figure 3.12). The modified resin was immersed in 10 mL norfloxacin solution (0 – 0.75 mg L^{-1}) at pH 3 and the color of resin was observed at different reaction times. The results showed that the color was fully developed on the modified resin within 15 minutes at room temperature. A longer reaction time neither yielded significant increase in ΔI nor improved the detection sensitivity, indicating that the reaction between Congo red and norfloxacin was completed under this experimental set-up. To shorten the analysis, the reaction time of 15 minutes was selected.

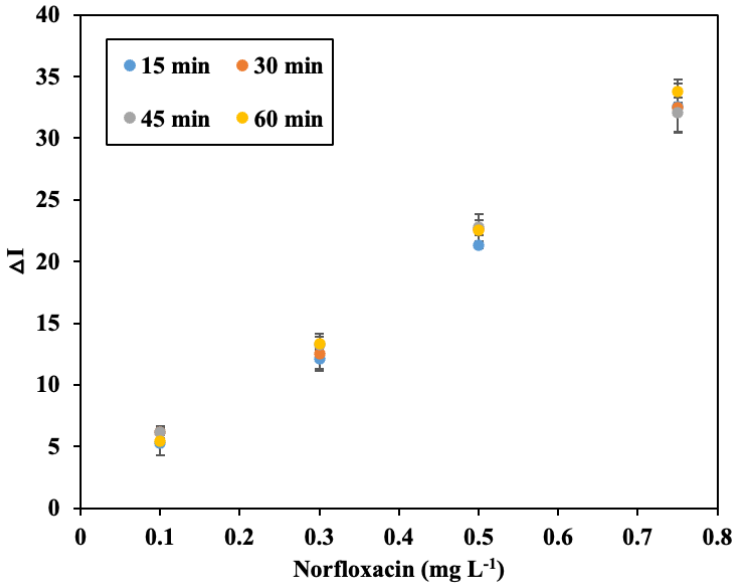


Figure 3.12 The effect of reaction time on the color intensity for the detection of norfloxacin.

3.2.3 Method performance and water sample analysis

3.2.3.1 Calibration curve and linearity

The optimal conditions for the standard norfloxacin detection were used as follows. The resin was modified with a 6.00 μM Congo red solution and

used to extract and detect norfloxacin in 10 mL solution of pH 3 for a 15-minute period. An external calibration curve was obtained by plotting the ΔI value against the concentration of norfloxacin as shown in Figure 3.13. This graph illustrates a linear relationship in the range of 0 – 0.75 mg L^{-1} norfloxacin with a linear regression equation of y = 42.788x + 0.1345 and correlation (R^2) of 0.9976. Moreover, the resin color changed from purple to pink as shown below.

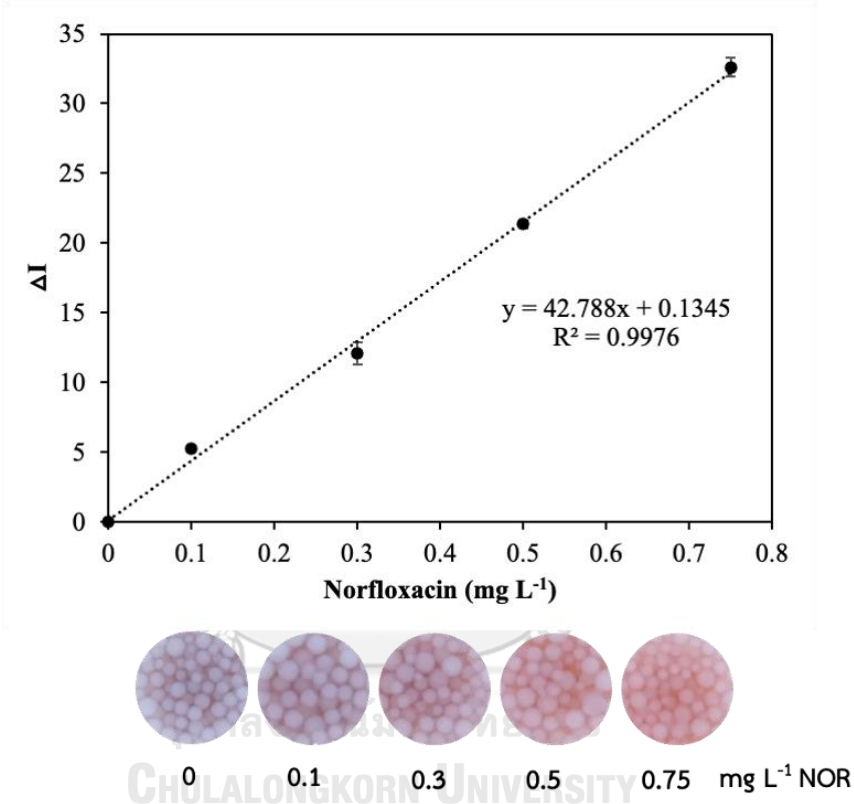


Figure 3.13 Calibration curve for the determination of standard norfloxacin using Congo red modified resin and photos of the obtained materials.

To apply the method for water sample analysis, a preliminary test was conducted to observe the effect of sample matrix. A water sample was collected from Chao Phraya River (Thailand) and norfloxacin was spiked into the water sample to obtain the final concentration of 0.2 and 0.4 mg L^{-1}. The experiment was performed under optimized conditions. However, the unsatisfied results were observed as shown in Figure 3.14. The color of materials changed to red in the

analysis of both non-spiked and spiked samples, seemingly due to the deprotonation of Congo red by other basic species in the sample. The most important compounds in water that attribute to alkalinity include the carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions. As shown in Figure 3.15, the presence of $NaHCO_3$ in water can change the color of resin to red orange without norfloxacin.

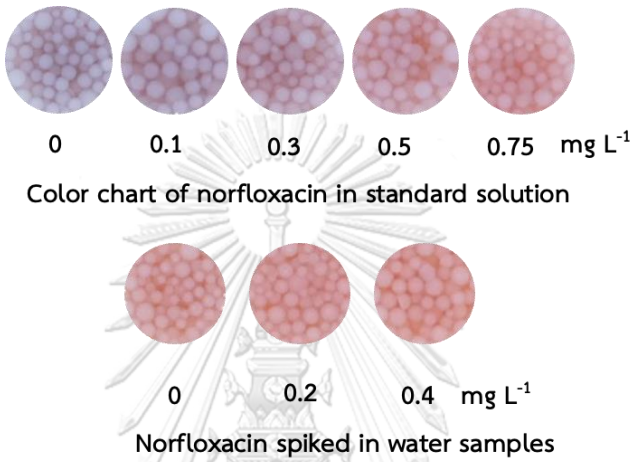


Figure 3.14 Color chart for norfloxacin detection and the color of materials obtained from the analysis of water sample (Chao Phraya River) and spiked samples.

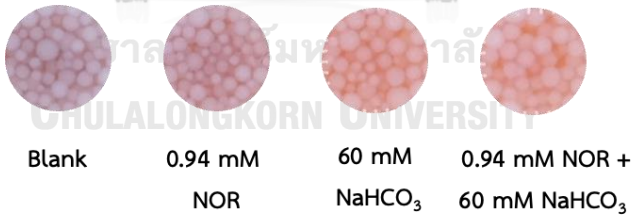


Figure 3.15 The resin color in the presence of $NaHCO_3$.

To minimize the effect of basic species, the pH of norfloxacin solution or sample solution was decreased to pH 2. The effect of matrix was investigated by comparing the color obtained from the standard norfloxacin solutions and drinking water samples spiked with norfloxacin. The results showed that there was no significant difference in color intensities using paired *t*-test at 95% confidence

level as shown in Figure 3.16. Therefore, this condition was further employed to construct the external calibration curve.

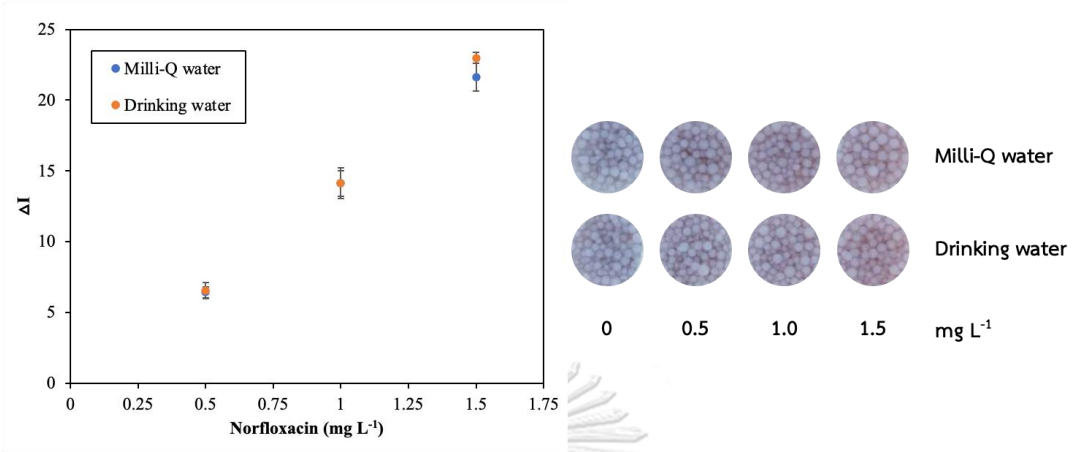


Figure 3.16 The effect of matrix on the detection of norfloxacin at pH 2 using Congo red modified resin.

Under the new condition, an external standard curve was constructed by plotting between the ΔI value and the concentration of norfloxacin (Figure 3.17). The linear relationship was obtained in the concentration range from 0 – 1.5 mg L^{-1} with a linear regression equation of y = 14.499x – 0.4824 and correlation (R^2) of 0.9972. Moreover, the change of materials color from blue-purple to pink-purple could be observed by naked eyes, as shown in the color chart.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of response (S_y) and the slope of calibration curve (S) following the formula: LOD = 3.3(S_y/S) and LOQ = 10(S_y/S). The LOD and LOQ of this proposed method was found to be 0.23 and 0.70 mg L^{-1}, respectively.

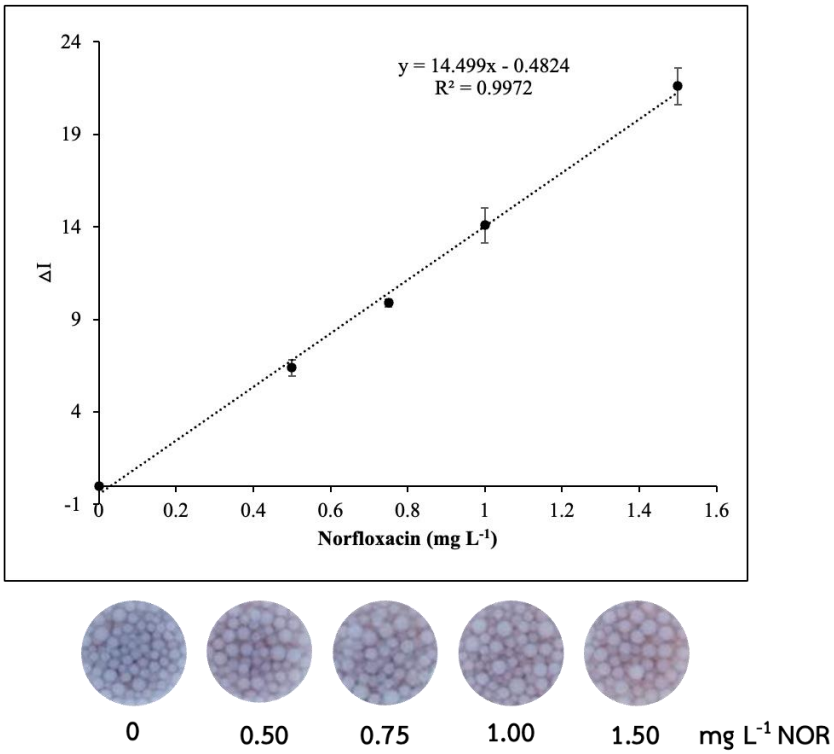


Figure 3. 17 External calibration curve and the color chart for the detection of norfloxacin using Congo red modified resin.

3.2.3.2 Accuracy and precision in water sample analysis

The proposed method was applied to detect norfloxacin in water samples. Two different norfloxacin levels (0.5 and 1.0 mg L^{-1}) were spiked in water samples including drinking water and tap water. The standard solutions of norfloxacin prepared in Milli-Q water were used to construct the external standard calibration curve in the concentration range of 0 to 1.50 mg L^{-1}. The recovery of norfloxacin in spiked water samples using the developed method was obtained in the range of 90 – 106% and the relative standard deviation (%RSD) ranged from 7.50 – 8.96% (Table 3.2). According to the AOAC international guidelines for the determination of analytes at a concentration level of 1 mg L^{-1}, acceptable accuracy and precision are defined by a recovery ranging from 80 to 110% and the %RSD lower than 16% [99]. The recovery obtained from the detection of norfloxacin in both drinking water and tap water were in the acceptable range. However, the recovery of norfloxacin in tap

water spiked with 0.5 mg L^{-1} level was slightly high. This may be attributed to the colored species in water samples that might yield a pale positive background color which strongly affected the detection of norfloxacin at low level. Hence, a sample pretreatment is highly recommended prior to the analysis to minimize the effect of interfering species. Conclusively, the Congo red modified resin could be applied to detect norfloxacin in real water samples with satisfied accuracy and precision using only a digital camera.

Table 3.2 The determination of norfloxacin in water samples using the developed method.

Sample	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD (%)
Drinking water	0	n.d.	-	-
	0.5	0.48±0.04	97	8.08
	1.0	1.01±0.08	101	7.51
Tap water	0	n.d.	-	-
	0.5	0.53±0.05	106	8.96
	1.0	0.90±0.07	90	7.50

Results presented as mean ± SD (n = 3)

n.d. = not detectable

CHAPTER IV

Determination of ceftazidime based on azo dye formation on PEI-sponge

4.1 Experimental

4.1.1 Materials and chemicals

Cotton fibers were purchased from a local store in Thailand. Acrylic slides as substrates were obtained from Tan Soon Huat products co., LTD., Bangkok, Thailand. Chemicals used for the detection of ceftazidime are listed in Table 4.1. All chemicals were of analytical reagent grade (AR) and used without further purification. All chemical solutions were prepared in deionized water (DI) or Mill-Q water, if not specified.

Table 4.1 List of chemicals.

Chemicals	Supplier
3-aminopropyltriethoxysilane (APTES), average MW ~221.37	Tokyo Chemical Industry Co. Ltd.
Polyethyleneimine (PEI), average MW ~60,000	Alfa Aeser (Massachusetts, USA)
Ceftazidime	Tokyo Chemical Industry Co. Ltd.
Hydrochloric acid	Merck (Germany)
Sodium nitrite	Daejung (Siheung-si, South Korea)
Chromotropic acid	Merck (Germany)
Epichlorohydrin	Merck (Germany)
Sodium hydroxide	Merck (Germany)

Chemicals	Supplier
Ethanol	RCI Labscan (Bangkok, Thailand)
Urea	Merck (Germany)
N,N'-Methylenebisacrylamide (MBA)	Sigma-Aldrich (Germany)

4.1.2 Instruments

The instruments used in this study are listed in Table 4.2.

Table 4.2 List of apparatus.

Apparatus	Company, model
UV-visible spectrophotometer	Hewlett Packard 8453
Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)	Nicolet 6700
pH meter	SevenCompact™ S220-Basic, (Mettler Toledo, Switzerland)
Scanning electron microscope	JEOL/IT-100 (Japan)
Freeze-dryer	Labconco™ (USA)
High performance liquid chromatograph (HPLC)	1290 Infinity II Agilent Technologies Inc., (Germany)

4.1.3 Colorimetric reactions of ceftazidime in solution

The formation of azo dye product was investigated using a UV-Vis spectrophotometer. The azo compound was obtained through the coupling reaction between the diazotized ceftazidime and chromotropic acid. The diazotization of ceftazidime was performed by mixing 0.5 mL of 1000 mg L^{-1} ceftazidime with 0.1 mL of 5 M HCl and 0.25 mL of 50 mM sodium nitrite, and the mixture volume was made

up to 1 mL with Milli-Q water. The reaction mixture was stirred for 30 min in an ice bath **(**at 0 **–** 5 °C**).** After that, 0**.**2 mL of 0.5 mM chromotropic acid and 0.8 mL of Milli-Q water were added into the mixture and continuously stirred for 15 min. The product was incubated at ambient temperature before analysis by a UV-Vis spectrophotometer. The solution color was stable for up to an hour. The schematic of colorimetric detection of ceftazidime in solution is depicted in Figure 4.1.

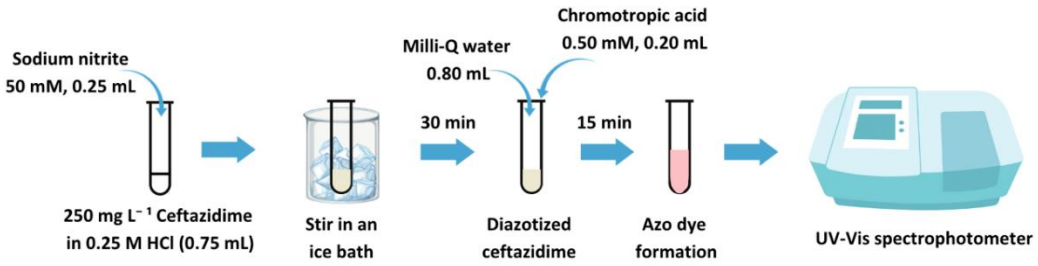


Figure 4.1 Schematic of colorimetric detection of ceftazidime in solution**.**

4.1.4 Fabrication of polyethyleneimine modified cotton sponge

Cotton fibers were initially functionalized with 3-aminopropyltriethoxysilane (APTES). The obtained APTES-cotton fibers were further used to produce cotton sponge, prior to the surface grafting with polyethyleneimine (PEI) through crosslink reaction using epichlorohydrin as a crosslinker. The preparation of APTES-modified cotton fibers and the cotton sponge were modified from the method proposed by Huang et al. [93] and Lou et al. [83], respectively.

Cotton fibers (1.00 g) were initially soaked in ethanol (100 mL) containing a specific amount of 3-aminopropyl triethoxysilane (APTES) and stirred at 55°C for 5 h. After that, the obtained APTES-cotton fibers were washed with ethanol and dried at room temperature. To fabricate the cotton sponge, the APTES-cotton fibers were dispersed in a pre-cooled alkali solution containing 7 wt% NaOH and 13 wt% urea and stirred in an ice bath for 1 h to obtain a viscous transparent cellulose solution. Subsequently, 0.6 % w/v N,N'-methylene bisacrylamide (MBA) as a cross-linker was added into the mixture and stirred for 1 h. To obtain the cellulose hydrogel, 1.5 g of

the mixture was transferred to a small plastic bottle and solidified at room temperature for 12 h. The gel was further soaked in deionized water to remove excessive chemicals until pH of the soaking water was neutral. The gels were pre-frozen in a freezer before freeze-drying process. Finally, APTES-sponges were obtained and kept in a desiccator for further surface modification (Figure 4.2).

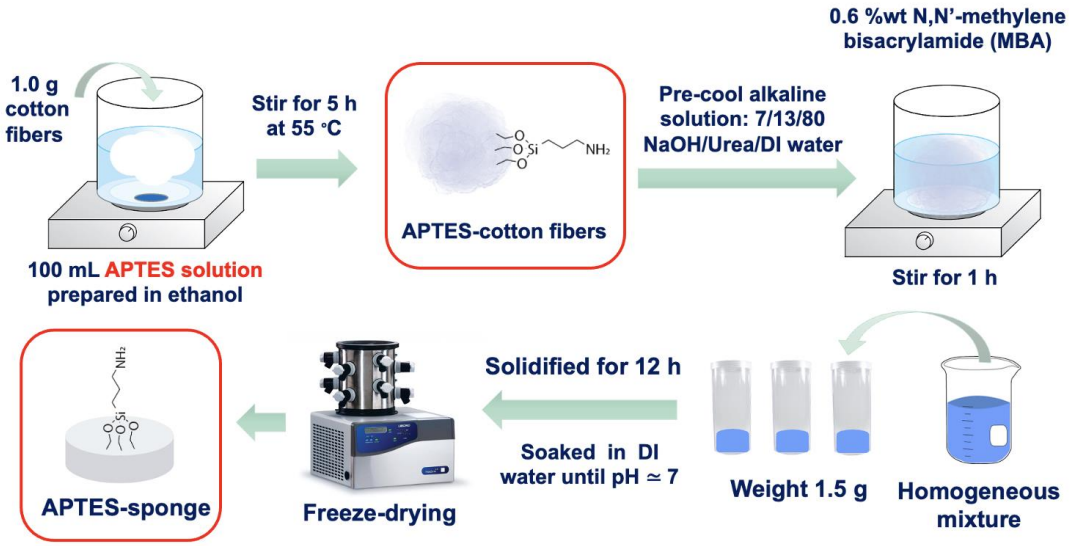


Figure 4.2 Schematic of the fabrication of APTES-sponges.

For the surface grafting with PEI (Figure 4.3), the APTES-sponges (0.50 g) were immersed in a solution of 1:3 (v/v) ethanol/water (60 mL) containing 0.125 M sodium hydroxide and heated to 55℃ under continuous stirring. Epichlorohydrin (1.5 mL) was subsequently added into the mixture. After 30 minutes ofmixing, a specific amount of PEI was introduced into the mixture and further stirred for 3 hours at 55℃. The resulting PEI-sponges were then washed with ethanol and dried overnight at ambient temperature.

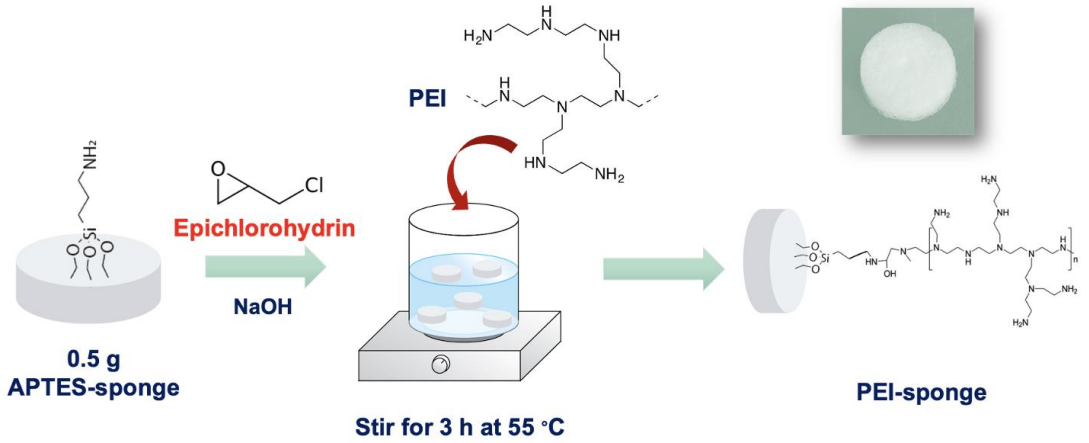


Figure 4.3 The grafting of PEI on APTES-sponges.

4.1.4.1 Effect of cotton fiber content

The effect of cotton fiber content was investigated by varying the amount of neat cotton fiber (1, 2, 3, and 4 wt%) in an alkali solution consisting of 7 wt% NaOH and 13 wt% urea. This cellulose mixture was stirred in an ice bath for 1 hour before adding 0.6 g of MBA. The mixture was further stirred for an hour, transferred into small plastic bottles with 1.5 g each, and left to solidify under ambient conditions. Finally, the cellulose hydrogels were frozen and subsequently subjected to freeze-drying.

4.1.4.2 Effect of APTES concentration

The effect of APTES amount used to modify the cotton fibers (1.0 g) was investigated by varying its concentration in the range of 85 to 340 mM in 100 mL ethanol. The obtained APTES-cotton fibers were used to fabricate APTES-sponges and further grafted with PEI (155 µM). The efficiency of the resulting PEI-sponges in ceftazidime detection was compared. The PEI-sponge was applied to extract ceftazidime (0.1 to 5.0 mg L^{-1}) by immersing a PEI-sponge into 80 mL ceftazidime at pH 4 for 15 min. For ceftazidime detection, 150 µL of reagent mixture containing 1.0 M HCl, 30 mM sodium nitrite, and 25 µM chromotropic acid was dropped onto the sponge and the material color was observed after 15 min of incubation time.

4.1.4.3 Effect of PEI concentration

The effect of PEI amount used to graft on APTES-sponge was investigated by modifying 0.5 g of APTES-sponge with PEI in a concentration range of 155 to 280 μM. The APTES-sponges prepared by using 170 mM APTES were soaked in 60 mL of 1:3 v/v Milli-Q water and ethanol containing 0.125 M NaOH and 1.5 mL of epichlorohydrin and heated to 55°C for 3 hours. PEI was subsequently added into the mixture and stirred for another 3 hours at 55°C. The efficiency of the obtained PEI-sponges in ceftazidime detection was evaluated and compared. The PEI-sponges were used to determine ceftazidime in the concentration range from 0.1 to 5.0 mg L^{-1} (80 mL) using a regent mixture comprising 1.0 M HCl, 30 mM $NaNO_2$, and 25 μM chromotropic acid with extraction time and detection time of 15 min.

4.1.5 Materials characterization

4.1.5.1 Attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR)

The success of APTES and PEI functionalization on the cellulose sponge was confirmed using ATR-FTIR to identify the functional groups on the material surface. Three materials including the unmodified sponge, APTES-modified sponge, and PEI-modified sponge were analyzed and the OMNIC PROGRAM was used to measure the absorbance of each functional group deposited on the sponge surface.

4.1.5.2 Ninhydrin test

To confirm the presence of amine groups functionalized on the modified materials, the ninhydrin test was performed. The test was conducted by immersing a piece of the test material (neat cotton fiber, APTES modified cotton fiber, neat cotton sponge, APTES-sponge, or PEI-sponge) in 2.0 mL of water. Then, 0.5 mL of 2 %w/v ninhydrin solution prepared in acetone was dropped into the mixture and heated to 80°C in a water bath. After 5 min, the material color was observed.

4.1.5.3 Scanning electron microscope *(SEM)*

The morphology of the unmodified sponge, APTES-sponge, and PEI-sponge were examined using a scanning electron microscope (SEM) with an accelerating voltage of 15 kV. Prior to imaging, all materials were sputter-coated with a thin layer of gold.

4.1.5.4 Water uptake

To determine the water uptake capacity, the dried cellulose cotton sponges were weighted to obtain the dry weight (w_0). The material is then immersed in water for 0 - 120 min. The material was weighed at a specific time (t) to obtain the weight after water absorption at time t (w_t) at room temperature. The water uptake ratio is calculated following eq. (**2**).

$$\text{Water uptake ratio} = \frac{w_t - w_0}{w_0} \quad (2)$$

4.1.6 Optimization of ceftazidime detection

Ceftazidime was extracted from the solution by submerging a piece of PEI-sponge (50±4 mg) into a ceftazidime solution and stirring for a specific duration. The sponge was subsequently separated from the solution and deposited onto an acrylic slide. A reagent mixture (150 µL) consisting of hydrochloric acid, sodium nitrite, and chromotropic acid was dropped onto the surface of the sponge (Figure 4.4). To complete the chemical reaction, the sponge was left at room temperature for a specific period. The material color was observed by taking a picture using a digital camera (Fuji XA-2) in a cube light box (36 cm x 23 cm x 24 cm) with controlled brightness and distance between the subject and the camera (10 cm). The photographs were analyzed using Image-J software to measure the color intensities in RGB mode (Red-Green-Blue), which were subsequently converted to CIE *L*a*b** (https://colorizer.org/). The CIE color space is a mathematical model that represents

all visible colors by using three coordinates: *L** for lightness, *a** for red/green, and *b** for yellow/blue. The change of material color was observed by comparing the color coordinates of material observed in the analysis of blank solution and the standard or sample solution and presented as color differences (ΔE) calculated by using eq. (**3**).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (3)$$

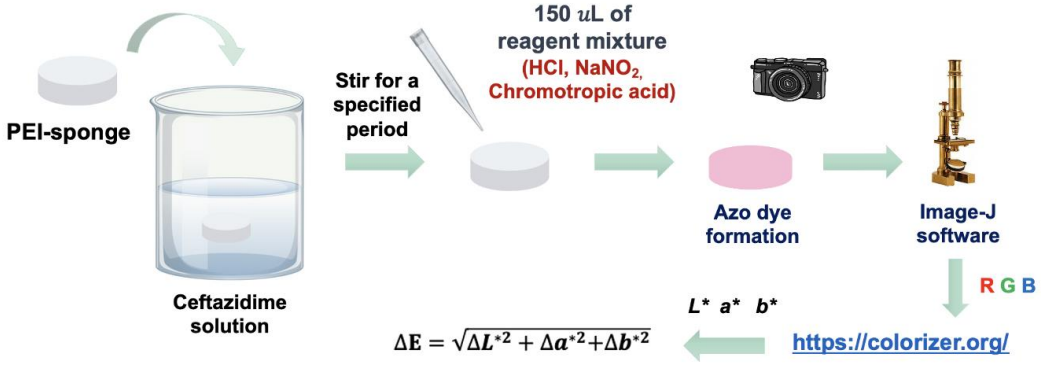


Figure 4.4 The extraction and detection of ceftazidime on the PEI-sponge.

4.1.6.1 Effect of sample volume

The impact of sample volume on the extraction of ceftazidime onto PEI-sponge was investigated. Different ceftazidime standard solutions (0.1, 1.0, 2.0, and 3.0 mg L^{-1}) were prepared with various volumes including 50, 100, 150, and 200 mL at pH 4. After the extraction step using a piece of PEI-sponge (*ca.* 50 mg) for each solution, the extracted ceftazidime on PEI-sponge surface was detected with a reagent mixture containing 0.5 M HCl, 30 mM sodium nitrite, and 25 µM chromotropic acid.

4.1.6.2 Effect of extraction time

The effect of extraction time was studied by immersing a PEI-sponge into a ceftazidime solution (0.1 to 3.0 mg L^{-1}, 150 mL, pH 4) in a period of 15, 30, and 45 min under continuous stirring. For ceftazidime detection, 150 µL of a reagent solution containing 0.5 M HCl, 30 mM sodium nitrite, and 25 µM chromotropic acid was dropped onto the material, followed by the photography process.

4.1.6.3 Effect of solution pH

The influence of pH of ceftazidime solution was examined within the range of 2.0 to 8.0. To conduct this study, a PEI-sponge was immersed in a solution (150 mL) containing 0.5, 1.5, or 3.0 mg L^{-1} ceftazidime for 30 min. The reactions on the obtained PEI-sponges were performed and detected within 15 min using a prepared reagent (0.5 M HCl, 30 mM sodium nitrite, and 25 µM chromotropic acid).

4.1.6.4 Effect of reagent concentration

The levels of hydrochloric acid, sodium nitrite, and chromotropic acid were optimized by using reagent mixtures containing different concentrations of each component to detect ceftazidime in the concentration range of 0.5 to 3.0 mg L^{-1} (150 mL, pH 4). First, the concentration of hydrochloric acid was varied as 0.25, 0.50, and 1.0 M in the reagent mixture containing 30 mM nitrite and 25 µM chromotropic acid. The effect of sodium nitrite was further studied in the range of 10 to 50 mM, while maintaining the concentration of hydrochloric acid and chromotropic acid at 0.50 M and 25 µM, respectively. The impact of chromotropic acid was eventually evaluated by varying its concentration from 10 to 50 µM in a mixture solution containing 0.50 M hydrochloric acid and 30 mM sodium nitrite.

4.1.6.5 Effect of reaction time

The effect of reaction time for the diazotization and coupling reactions was investigated and optimized for the determination of 0.5 to 3.0 mg L^{-1} ceftazidime. After dropping the reagent mixtures containing 0.50 M hydrochloric acid, 30 mM nitrite, and 25 µM chromotropic acid onto PEI-sponges, the materials were incubated at room temperature for a period varied from 15 to 60 minutes.

4.1.7 Selectivity

The impact of potential interfering species including norfloxacin (NOR), oxytetracycline (OTC), amoxicillin (AMOX), penicillin (PEN), roxithromycin (ROX), erythromycin (ERY), and gabapentin (GPN) on the detection of ceftazidime was evaluated. Binary solutions containing 3.0 µM ceftazidime (1.64 mg L^{-1}) and each interfering drug at concentration of 15 µM (OTC, ROX, and ERY) or 30 µM (NOR, AMOX, PEN, and GPN) were analyzed under the optimum conditions. The ΔE values of the PEI-sponge color observed from the analysis of pure ceftazidime solution was compared to those obtained from the analysis of binary solutions.

4.1.8 Real sample analysis and method validation

Real water samples including drinking water, tab water, and water from shrimp farming were collected and filtered through Whatman® membranes with a pore size of 0.45 µm. Prior to the analysis, the pH of the water samples was adjusted to approximately 4.0 using NaOH or HCl solutions and the samples were preserved at 4 °C in a refrigerator. To conduct the recovery test, a ceftazidime standard solution was spiked into the water samples to achieve a final concentration of either 0.75 or 1.75 mg L^{-1}. Both the non-spiked and spiked water samples were determined using the proposed PEI-sponge method under optimal conditions. The sponges modified with 170 mM APTES and 210 µM PEI were used to extract ceftazidime from sample solutions (150 mL, pH 4). After 30 min of extraction process, the extracted ceftazidime on a PEI-sponge was detected by dropping 150 µL reagent mixture containing 0.5 M hydrochloric acid, 30 mM sodium nitrite, and 25 µM chromotropic

acid on the material surface. To measure the developed color intensity, the photos of sponges were taken within 30 minutes. The external standard calibration curve was constructed by observing ΔE intensities of sponges used in the analysis of ceftazidime standard solutions and it was applied to determine ceftazidime level in real water samples.

For method validation, the amount of ceftazidime in both spiked and non-spiked samples was determined by HPLC technique. Prior to the analysis, the Zorbax eclipse XDB C18 (5 mm, 0.46 cm x 15 cm) column was washed with acetonitrile (100% v/v) and equilibrated with the mobile phase (acetonitrile:water) for an hour. The separation was achieved with a mixture of acetonitrile and water 12:88 (v/v) in the presence of 0.1% v/v acetic acid in isocratic mode with a flow rate of 0.8 mL/min, while maintaining the column temperature at 29°C. The detection was performed at a wavelength of 260 nm. The results observed from the HPLC method were compared to those obtained from the PEI-sponge method.

4.2 Results and discussion

4.2.1 Colorimetric reactions of ceftazidime in solution

To detect ceftazidime, the aromatic amine of ceftazidime is first reacted with nitrite in acid. The resulting diazotized ceftazidime undergoes the coupling reaction with chromotropic acid to produce an azo dye product. The reactions were investigated by analyzing the solution of ceftazidime, chromotropic acid, diazotized ceftazidime, and azo compound using a UV-Vis spectrophotometer (Figure 4.5).

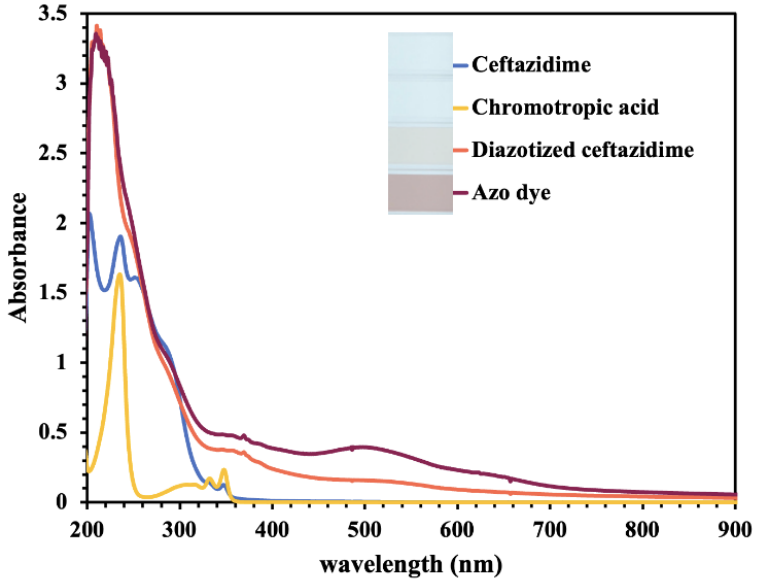


Figure 4.5 Absorption spectra of ceftazidime (125 mg L^{-1}), chromotropic acid (25 μM), diazotized ceftazidime, and the azo dye product prepared in 0.125 M HCl. (inset: photo of solutions)

Ceftazidime and chromotropic acid displayed absorption bands from 220 to 260 nm and 200 to 380 nm, respectively, with the maximum absorption at 246 nm and 235 nm. After the diazotization, a new absorption band was observed in the range of 330 – 700 nm and the solution color turned to yellow orange. The subsequent reaction with chromotropic acid generated a red-pink product in solution, and a new absorption band appeared in the range of 445 – 700 nm, with the maximum absorption at 500 nm. This newly obtained spectrum was significantly different from that of the starting compounds, indicating the formation of a new product. These results confirmed the reactions of ceftazidime and its applicability for colorimetric detection of ceftazidime in the PEI-sponge method.

4.2.2 Fabrication of polyethyleneimine modified cotton sponge

4.2.2.1 Effect of cotton fiber content

To produce a highly porous cotton sponge, the quantity of cotton fibers used in the fabrication process was carefully considered and optimized. Cotton

sponges were fabricated using various amounts of neat cotton fiber (1, 2, 3, 4 wt%) as shown in Figure 4.6. When 1 wt% cotton fiber was used, the cellulose gelation took a long time (ca. 48 h), and the obtained sponge had low mechanical strength as it collapsed during the further modification process. In contrast, the solution containing 4 wt% cotton was highly viscous and not homogeneous. As a result, the sponges obtained from the same batch had different densities, due to different amounts of cellulose in each sponge. The uniform cellulose sponges with porous structure were achieved by using 2 – 3 wt% cotton fiber.

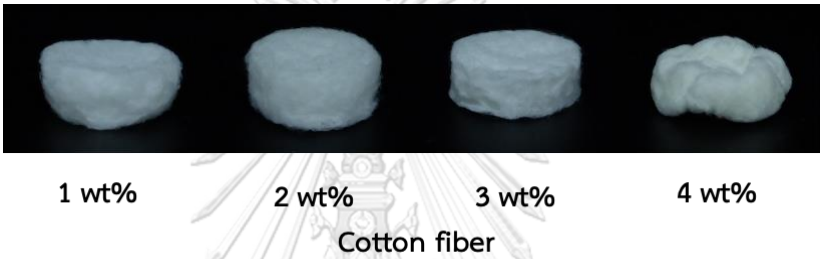


Figure 4.6 Photo of materials fabricated with different amount of cotton fiber.

To compare the water absorption ability which is related to the sponge porosity, the water uptake ratio of sponges prepared from 2% and 3%wt cotton fiber was investigated by immersing the sponges in water in the period of 0 – 120 min. The results in Figure 4.7 showed the water uptake capacities of 21.6 ± 2.09 g/g and 13.7 ± 0.24 g/g for the sponges prepared from 2 %wt and 3 %wt cotton fiber, respectively. The results indicated that the sponges produced from 2 %wt cotton fiber showed higher water absorption ability, and hence higher porosity that was suitable to extract analytes from large sample volume. This composition was employed to produce APTES-sponges.

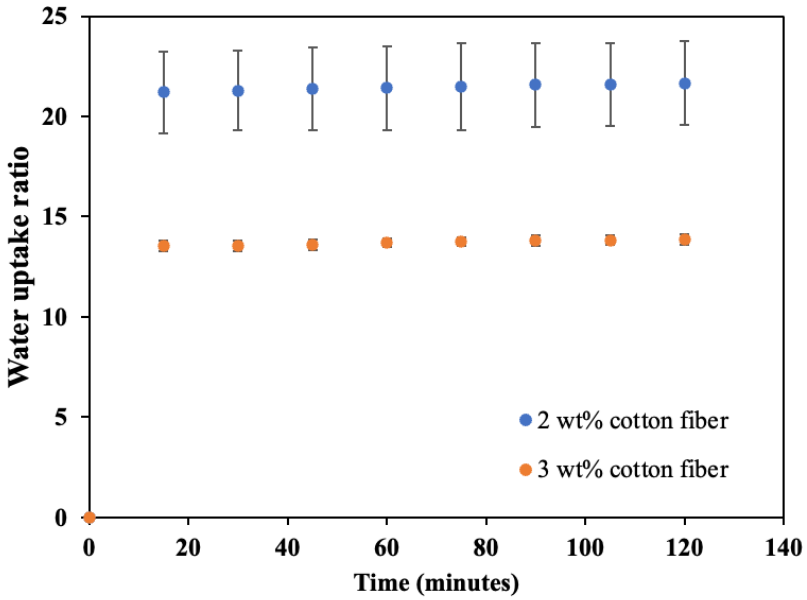


Figure 4.7 Water uptake of the cellulose sponges fabricated from 2 %wt and 3 %wt cotton fiber observed at 0-120 minutes.

4.2.2.2 Effect of APTES concentration

The concentration of APTES on the cotton fibers plays a crucial role in the content of PEI grafted on the cotton sponge, and consequently the sensitivity of ceftazidime detection. To investigate this effect, cotton fibers were modified using varied concentrations of APTES from 85 to 340 mM, while maintaining a fixed concentration of PEI (155 μM). The resulting PEI-sponges were employed to detect ceftazidime in a concentration range from 0.1 – 5.0 mg L^{-1} (Figure 4.8). The use of PEI-sponge prepared from the lowest APTES concentration (85 mM) resulted in a poor sensitivity, especially for the detection of high-level ceftazidime, likely due to a low number of PEI binding sites for ceftazidime. The ΔE values increased with increasing concentration of APTES from 85 mM to 127.5 and 170 mM due to a rising number of active sites. The highest color intensity change (ΔE) was achieved with the PEI-sponge fabricated by using 170 mM APTES-modified cotton fibers. However, APTES concentrations exceeding this value (212.5 – 340 mM) were not recommended, as the detection sensitivity deteriorated. This was attributed to the self-condensation of

excessive APTES in solution, resulting in a lower APTES content available for cotton fiber modification. Therefore, the optimal APTES concentration for cotton fiber modification under the set-up in this study was 170 mM.

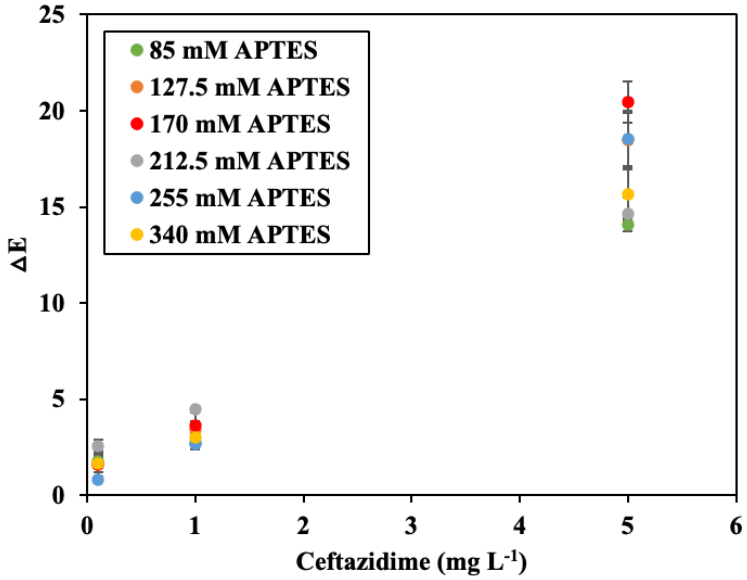


Figure 4. 8 Effect of APTES concentration used in PEI-sponge fabrication on the detection of ceftazidime.

4.2.2.3 Effect of PEI concentration

The concentration of PEI for the sponge modification was further optimized. APTES-sponges (0.5 g) were modified with three concentrations of PEI including 155, 210, and 280 μM. From preliminary results, it was found that lower concentrations of PEI did not yield a distinct color of the azo dye product on the sponge surface. This could be attributed to the inadequate amount of amine functional groups on the cotton sponge surface, which resulted in less ceftazidime extraction. By using the above-mentioned PEI concentrations, all prepared materials provided similar detection sensitivity as their color intensities were not significantly different when used in the detection of 0.1 – 5.0 mg L^{-1} ceftazidime (Figure 4.9). This suggests that the amounts of PEI used were sufficient for the reaction with APTES on the sponge surface. To ensure the complete coverage of APTES-sponge surface with

PEI, 210 µM of PEI was selected for the modification of 0.5 g of APTES-sponge under the set-up in this study.

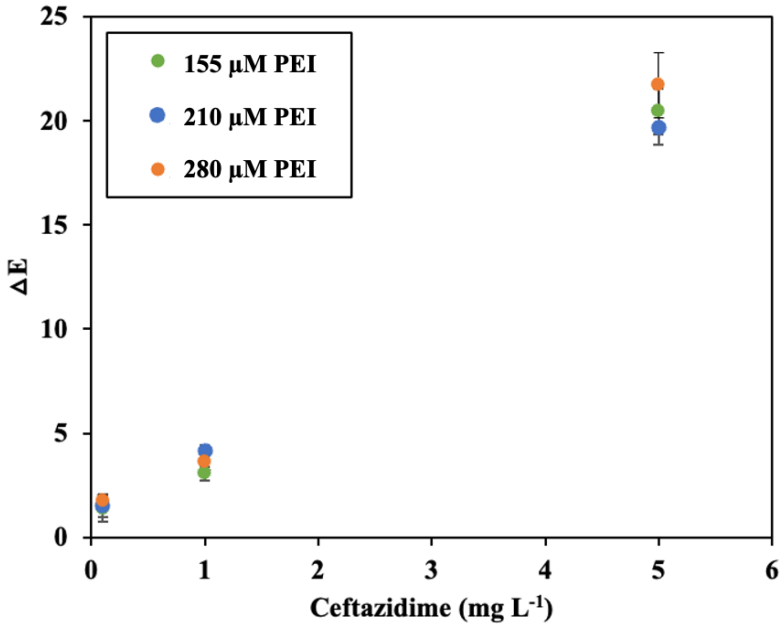


Figure 4.9 Effect of PEI concentration used in PEI-sponge fabrication on the detection of ceftazidime.

4.2.3 Materials characterization

4.2.3.1 Attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR)

The cellulose cotton fibers were modified with APTES to decorate the surface with amino groups. These amine groups on APTES-sponge further reacted with -CH_2Cl of epichlorohydrin via dehydrochlorination to introduce epoxy functional groups on their surface. The following reaction involved the amine group of PEI attacking the epoxide ring, causing the ring opening reaction at high temperature. This reaction results in the grafting of PEI onto the cotton sponge surface.

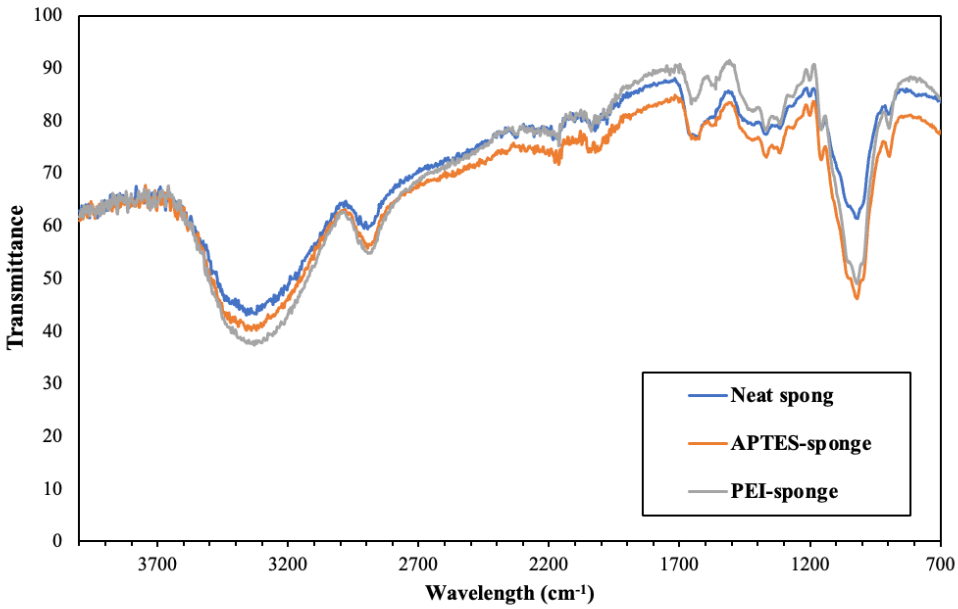


Figure 4.10 FTIR spectra of neat sponge, APTES-sponge, and PEI-sponge.

ATR-FTIR was employed to observe the functional groups on the neat cotton sponge, APTES-sponge, and PEI grafted sponge. The results from FTIR spectrum of all materials (Figure 4.10) showed the characteristic bands of cellulose including O-H stretching of hydroxyl group at 3330 cm^{-1}, C-H stretching of glucose unit at 2885 cm^{-1} and 1365 cm^{-1}, and C-O stretching of secondary alcohols and ethers functions existing in the cellulose chain backbone at 1016 cm^{-1} [100]. Moreover, the absorption peaks at 1645 cm^{-1} and 1560 cm^{-1} corresponding to -C=O stretching vibration absorption and -NH flexural vibration absorption of MBA crosslinker were also observed [101]. Compared to the neat sponge, the spectra of APTES-sponge and PEI-sponge showed stronger absorption at 3330 cm^{-1} of N-H stretching and 1365 cm^{-1} of C-N stretching corresponding to primary amine in APTES and PEI molecule [93]. These results suggested a successful functionalization of APTES and PEI on the cellulose sponge.

4.2.3.2 Ninhydrin test

The ninhydrin test was used to confirm the coverage of material surface with amine groups of APTES and PEI. The reaction is based on the formation of a complex between ninhydrin and amine group, yielding a purple or blue color product. The results in Figure 4.11 showed a yellow and brown color of reagent on the surface of neat cotton fiber and non-modified sponge, respectively. On the other hand, a purple to blue surface was obtained in the test of APTES modified cotton fiber, APTES-sponge, and PEI-sponge. These observations confirmed the successful modification of cotton fiber and sponges with APTES and PEI, which contain a high content of -NH and $-NH_2$.

Figure 4.11 Photo of materials after performing ninhydrin test.

4.2.3.3 Scanning electron microscope *(SEM)*

SEM was used to observe the surface morphologies of the neat cotton sponge, APTES-sponge, and PEI-sponge as shown in Figure 4.12. The images from the SEM revealed the porous structure of the sponges where the pores were formed by the voids between adjacent cellulose networks. The pore structure was uniformly dispersed due to the cross-linking and the freeze-drying processes [102]. The surface of the unmodified cotton appeared rough and it became smoother after the modification, because of the coverage by the modified species (APTES or PEI).

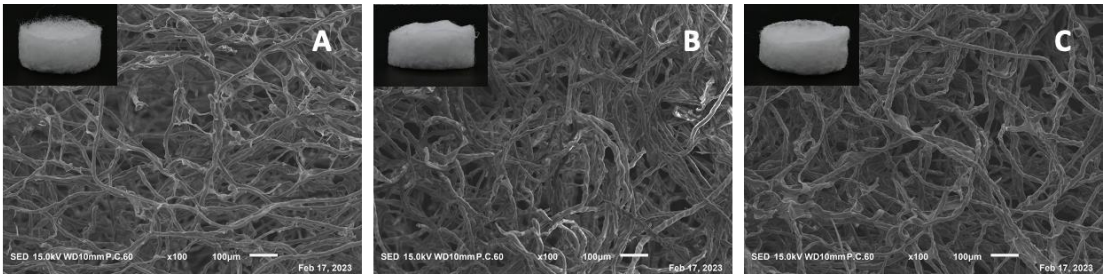


Figure 4.12 SEM images of the surface of (A) neat cotton sponge, (B) APTES-sponge, and (C) PEI-sponge (inset: photo of cotton sponges).

4.2.3.4 Water uptake

To demonstrate the ability of materials to absorb water, the water uptake experiments were conducted for the neat cotton sponge and PEI-sponge. Both materials were immersed in water for 120 min. The sponges could absorb water rapidly and their water-holding capacities reached the maximum level within 15 min and did not change afterward (Figure 4.13). Owing to their porous structure and excellent water stability, the neat cotton sponge and PEI-sponge exhibited high water uptake ratio of 21.6 ± 2.09 g/g and 18.71 ± 1.67 g/g, respectively. The water uptake ratios of the unmodified and PEI-modified sponges did not differ significantly at 95% confidence interval, revealing that the surface modification did not affect this property. The sponge can absorb a large volume of water compared to its initial weight and showed good stability in water due to its crosslinked structure. These properties are beneficial for its use as a material to extract target analytes in water sample.

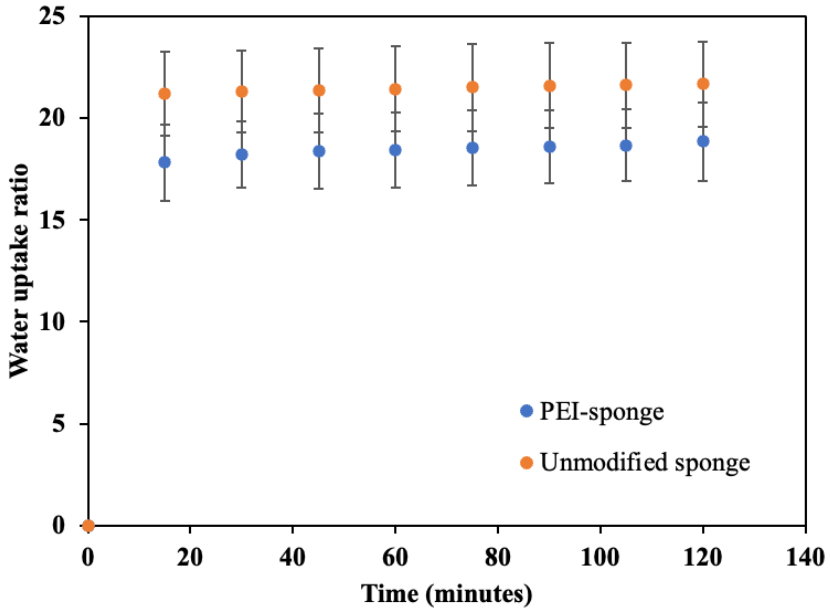


Figure 4.13 Water uptake of the unmodified sponge and PEI-sponge observed at 0-120 minutes.

4.2.4 Optimization of ceftazidime detection

4.2.4.1 Effect of sample volume

Due to a low level of ceftazidime possibly found in water samples, a highly sensitive method is required for its detection. To enhance the sensitivity of the method, the effect of sample volume was studied. An increase of sample volume would lead to a corresponding increase in the amount of ceftazidime extracted on the PEI-sponge. A PEI-sponge (ca. 50 mg) was used to extract ceftazidime (0.1 to 3.0 mg L^{-1}) in sample of different volumes ranging from 50 to 200 mL. As shown in Figure 4.14, the results indicated a significant increase in signal with an increase in sample volume from 50 to 150 mL, apparently due to the higher amount of ceftazidime extracted on the PEI-sponge. However, using a high sample volume of 200 mL resulted in a remarkable reduction of color intensity and low precision. This could be explained that the sponges of compact size were not disperse thoroughly in such a large volume. Thus, 150 mL of ceftazidime solution was chosen as an optimal sample volume used in the further experiments.

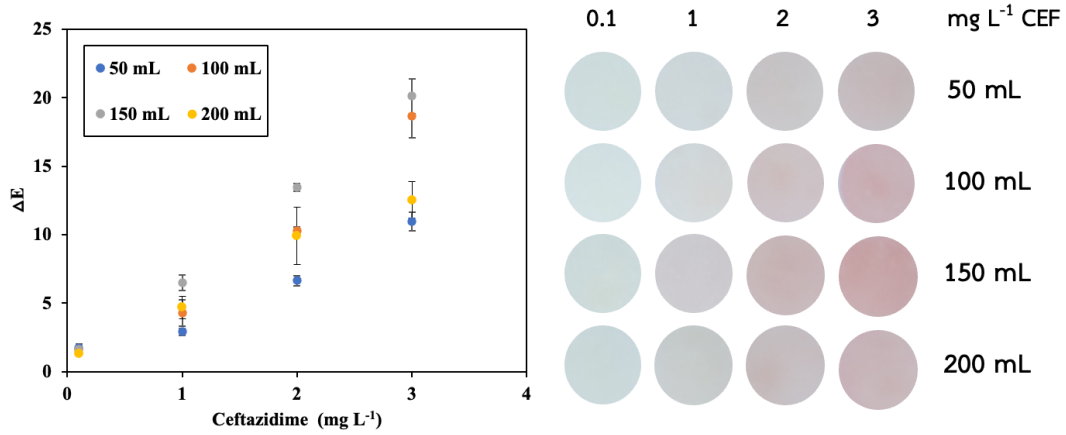


Figure 4.14 Effect of sample volume on the detection of ceftazidime using the obtained PEI-sponges.

4.2.4.2 Effect of extraction time

The impact of extraction time on the detection of ceftazidime was investigated to achieve the most efficient extraction. This study was performed by incubating a PEI-sponge in a ceftazidime solution (0.1 to 3.0 mg L^{-1}) for 15, 30 and 45 minutes (Figure 4.15).

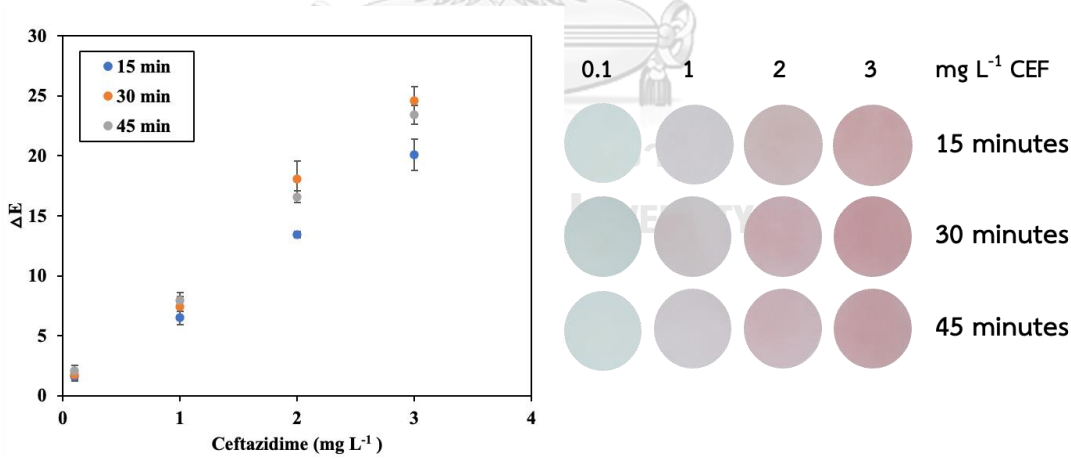


Figure 4.15 Effect of extraction time on the detection of ceftazidime using the obtained PEI-sponges and the photos of the materials.

The results illustrated that the delta E signal was strongly enhanced by increasing the extraction time from 15 to 30 minutes. By increasing the extraction time, a higher amount of ceftazidime could be extracted onto PEI-sponge,

resulting in higher content of azo dye produced on the sponge surface. Nonetheless, extending the extraction time to 45 minutes did not improve the detection sensitivity. To avoid lengthy analysis time, a 30-minute extraction time was selected for the further experiments.

4.2.4.3 Effect of solution pH

The pH of solution has a significant effect on the extraction as it affects the protonation or deprotonation of ceftazidime and PEI on the sponge surface. Ceftazidime exists in its cationic, anionic, and zwitterionic forms depending on the solution pH. The pKa of ceftazidime is approximately 2.77 for the carboxyl group and 4.26 for the aminothiazolyl group [58]. At pH below 2.77, the carboxyl group will be protonated and the amino group will be positively charged. By increasing the solution pH, ceftazidime exists as either neutral zwitterions (pH 2.77 – 4.26) or negatively charged species (pH > 4.26)[103]. In this work, PEI-sponges were used to extract ceftazidime through the electrostatic interaction between the negatively charged ceftazidime and the positively charged PEI. Considering the pk_a of the amine groups of PEI (ca. 9.5), the degree of PEI protonation increases as the solution pH decreases below 9.5 [104].

To achieve a suitable pH for quantitative extraction of ceftazidime onto the PEI-sponge surface, pH of the solution was varied in the range of 2-8. As shown in Figure 4.16, high ΔE intensities were obtained when the solution pH was adjusted to pH 4 and 6. In this pH range, ceftazidime would rather exist in its zwitterionic form and its anionic part could react well with the cationic PEI polymer, leading to higher amount of ceftazidime extracted, compared to what was observed in more acidic or basic solution. The detection sensitivity deteriorated when the pH was set to pH 2 owing to the electrostatic repulsion between the cationic ceftazidime and protonated PEI. In basic solution (pH 8), less degree of protonation of amine group on the PEI surface led to less electrostatic interaction between ceftazidime and PEI, resulting in a lower detection sensitivity. Therefore, pH 4 is the optimal pH for the extraction and detection of ceftazidime due to the strongest

electrostatic interaction between the deprotonated form of ceftazidime and protonated PEI on the sponge surface.

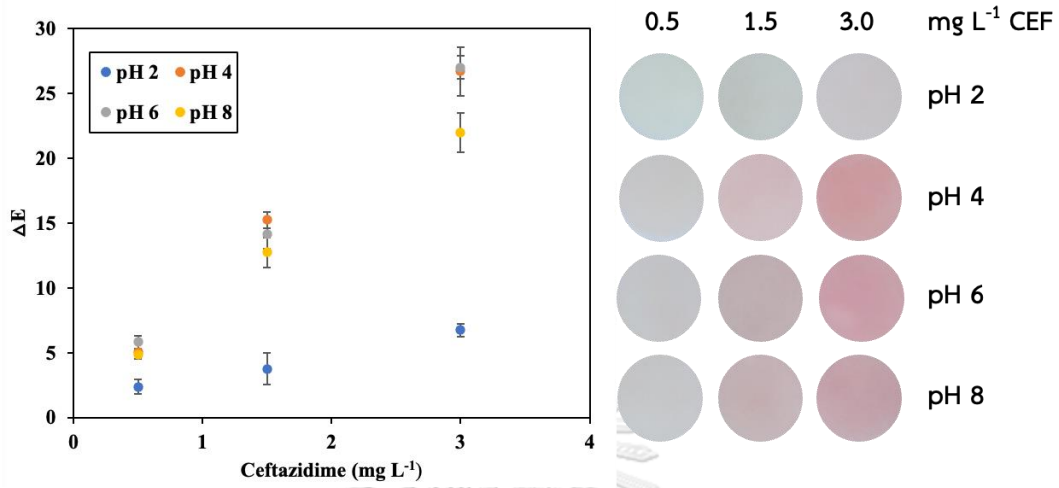


Figure 4.16 Effect of solution pH on the detection of ceftazidime using the obtained PEI-sponges and the photos of the materials.

4.2.4.4 Effect of reagent concentration

The colorimetric detection of ceftazidime relied on the formation of an azo dye of ceftazidime using chromotropic acid as coupling reagent. The aromatic amine ($-NH_2$) of ceftazidime was initially reacted with nitrite ions in the presence of hydrochloric acid to form diazonium ions. The obtained diazotized ceftazidime further underwent the coupling reaction with chromotropic acid to produce a pink or red-violet azo dye. The intensity of the color product is directly proportional to the amount of ceftazidime present in the sample. The optimizations of hydrochloric acid, sodium nitrite, and chromotropic acid concentrations were conducted for the detection of 0.5 to 3.0 mg L^{-1} ceftazidime.

4.2.4.4.1 Effect of hydrochloric acid concentration

Acid plays an important role in generating diazonium ions. To ensure that the amount of hydrochloric acid is adequate for the reaction, different concentrations of hydrochloric acid ranging from 0.25 to 1.0 M were studied while keeping the concentrations of sodium nitrite (30 mM) and chromotropic acid (25 μM)

constant. From Figure 4.17, a low concentration of hydrochloric acid (0.25 M) resulted in low color intensities on the sponges due to the insufficient nitrous acid for the diazotization reaction. Higher concentrations of hydrochloric acid (0.50 and 1.0 M) showed significant increases in color intensities, indicating the enhanced detection sensitivity. However, extremely high concentrations of HCl could lead to the rapid production of nitrous acid, which may decompose into NO and NO_2 gas before reacting with the aromatic amine [105]. Therefore, the optimal concentration of hydrochloric acid of 0.50 M was selected for further experiments.

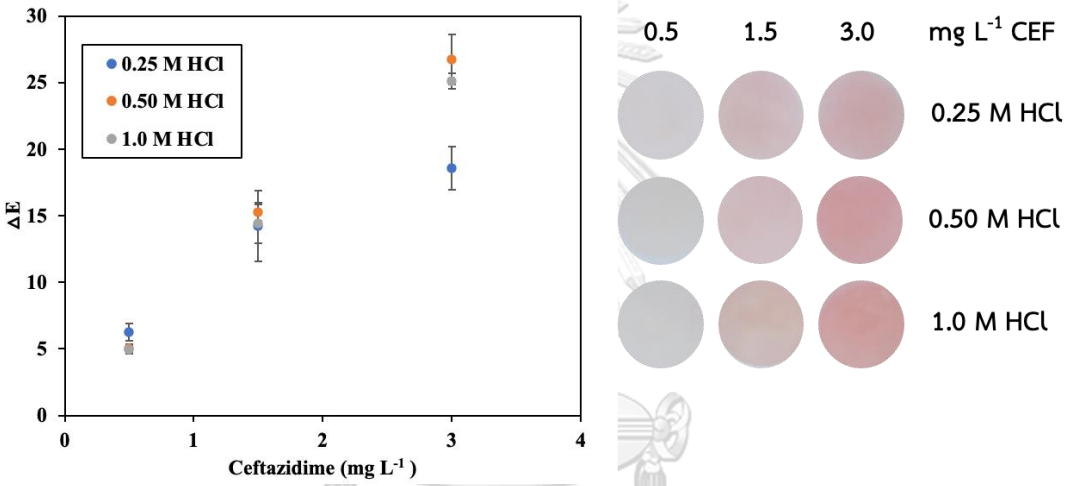


Figure 4.17 Effect of hydrochloric acid concentration on the detection of ceftazidime using the PEI-sponges and the photos of the materials.

4.2.4.4.2 Effect of sodium nitrite concentration

Nitrite concentration would affect the formation of diazonium ions and hence the detection of ceftazidime. To achieve a large amount of azo dye and improve the detection sensitivity, the concentrations of sodium nitrite were optimized in the range of 10 to 50 mM in a mixture solution containing 0.50 M hydrochloric acid and 25 μM chromotropic acid. The significant impact of sodium nitrite was observed in the detection of high-level ceftazidime (3.0 mg L^{-1}) as shown in Figure 4.18. At 10 mM sodium nitrite, the detection sensitivity was low due to an insufficient diazonium ions generated from a small amount of sodium nitrite. A

remarkable increase in the color intensity and detection sensitivity was achieved when the concentration of nitrite reached 30 mM. However, using an even higher concentration of nitrite (50 mM) did not result in any further improvement in color intensity, indicating that the nitrous acid content was already sufficient for the diazotization reaction. Therefore, a concentration of 30 mM sodium nitrite was chosen for ceftazidime detection in this concentration range based on environmental considerations.

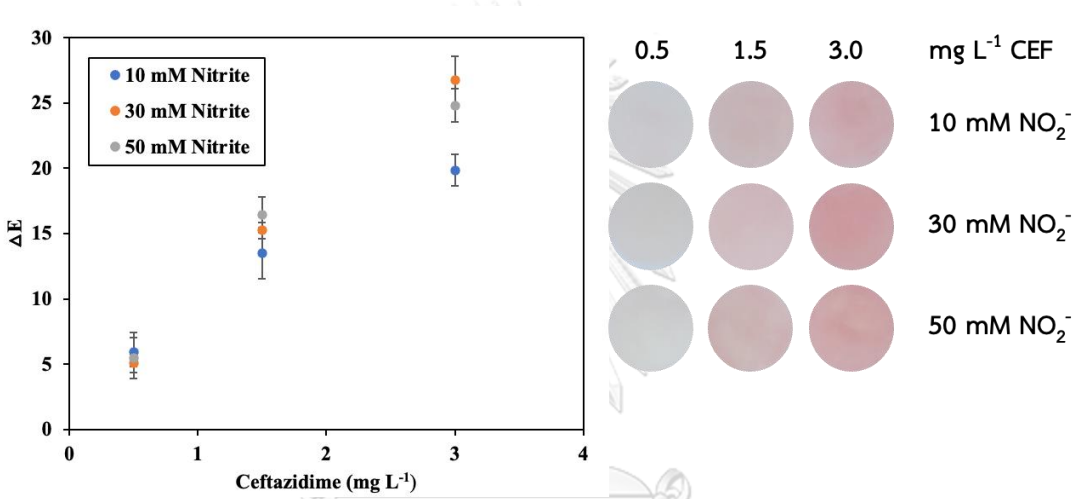


Figure 4.18 Effect of sodium nitrite concentration on the detection of ceftazidime using the PEI-sponges and the photos of the materials.

4.2.4.4.3 Effect of chromotropic acid concentration

Chromotropic acid reacts with the diazotized ceftazidime in coupling reaction and has an impact on the production of azo dye. Therefore, the optimal concentration of chromotropic acid for efficient azo dye formation on a cotton sponge was determined in the range of 10 to 50 μM while keeping the concentration of hydrochloric acid and sodium nitrite constant at 0.50 M and 30 mM, respectively (Figure 4.19). By using 10 μM chromotropic acid, the ΔE signals of ceftazidime were low, likely because of insufficient coupling reagent to drive the coupling reaction. The detection sensitivity and ΔE values were significantly enhanced with increasing concentration of chromotropic acid for up to 25 μM.

However, the results obtained from using 50 μM chromotropic acid did not significantly differ from those obtained with 25 μM chromotropic acid. Moreover, using a very high concentration of chromotropic acid resulted in an intense yellow background color of unreacted chromotropic acid, affecting the ΔE values and the detection sensitivity. Thus, the optimal reagent concentrations for the detection of ceftazidime in this range were 25 μM chromotropic acid, 0.5 M hydrochloric acid, and 30 mM sodium nitrite.

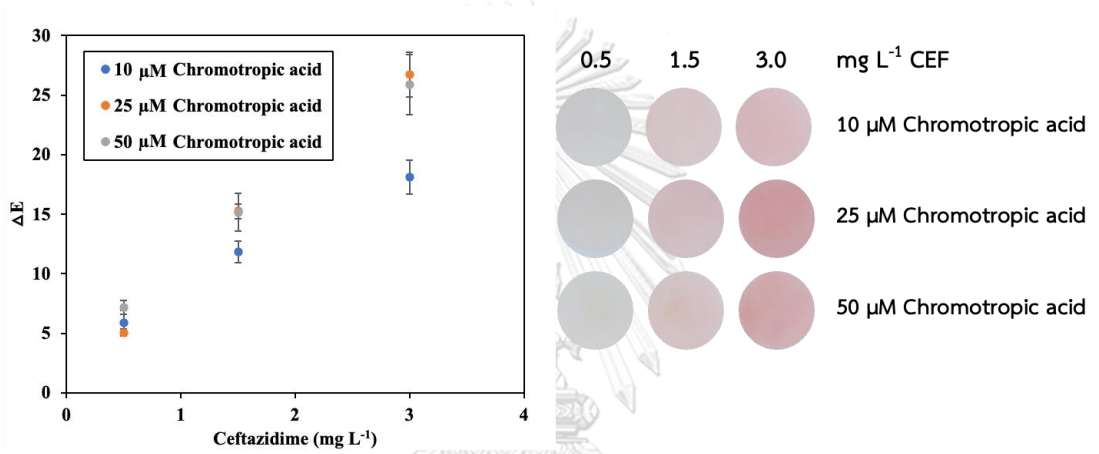


Figure 4. 19 Effect of chromotropic acid concentration on the detection of ceftazidime using the PEI-sponges and the photos of the materials.

4.2.4.5 Effect of reaction time

The completeness of diazotization and coupling reactions could be affected by the reaction time given prior to taking the material photo. After adding the reagent mixture onto the PEI-sponge, the incubation time was varied from 15 to 60 minutes (Figure 4.20). The results showed that the reaction was completed within 15 minutes when the lowest concentration of ceftazidime (0.5 mg L^{-1}) was analyzed. This was largely because the reaction was favorable as there were excessive amount of reagents to react with small amount of ceftazidime on the sponge surface. However, a longer reaction time was required for the detection of ceftazidime at higher concentrations as observed by a gradual increase of the ΔE signal up to 30 minutes. Moreover, it was found that extending the reaction time to

45 or 60 minutes did not provide any significant benefits, and in fact, the solvent evaporated during the prolonged process, leading to a paler material color and decreased sensitivity. Therefore, to attain the best reaction performance and minimize detection duration, a reaction time of 30 minutes was suggested in this study.

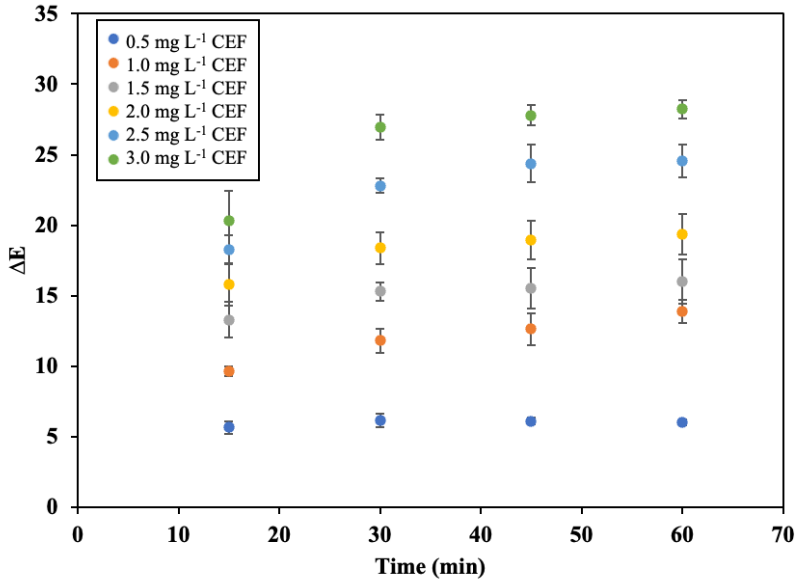


Figure 4.20 Effect of reaction time on the detection of ceftazidime using the PEI-sponges.

4.2.5 Selectivity

The effect of potential interfering species was investigated by binary mixing pure ceftazidime (3 μM) with other drugs (15 or 30 μM) including norfloxacin (NOR), oxytetracycline (OTC), amoxicillin (AMOX), penicillin (PEN), roxithromycin (ROX), erythromycin (ERY), and gabapentin (GPN) (Figure 4.21). The color intensities obtained from the pure ceftazidime solution were compared to those from the binary solutions. The results in Figure 4.22 illustrated that there was no significant difference between the binary solutions and the pure ceftazidime solution in terms of ΔE color intensity at 95% confidence interval, suggesting a superb specificity of the method for ceftazidime detection. The excellent selectivity of this approach was presumably achieved by two combined mechanisms: extraction and detection. In the extraction

step, the pH of solution was adjusted to pH 4 and only interfering drugs presented in their negatively charged forms including amoxicillin, penicillin, oxytetracycline, and ceftazidime could be extracted onto the PEI-sponge surface through electrostatic interaction. Moreover, there are three drugs including roxithromycin, erythromycin, and oxytetracycline that showed higher interfering effects than the others at its high concentration. As roxithromycin and erythromycin contain a large lactone ring with several substituents, these structures might be extracted onto the PEI-sponge surface through physisorption (e.g. π-π interaction) and compete with ceftazidime on the extraction on the surface [106]. In case of oxytetracycline, this drug has its own yellow color that could obscure the product color (pink), resulting in different ΔE color intensity. In the detection step based on azo dye formation, only drugs containing primary amine in their structure, such as amoxicillin, gabapentin, and ceftazidime, could undergo the diazotization reaction and further coupling reaction. However, the aromatic amine in the ceftazidime structure seemingly exhibited higher reactivity compared to others. Therefore, only ceftazidime extracted on PEI-sponge could obviously produce azo compound without interfering effect from other antibiotics despite their presence at 5 or 10 times higher.

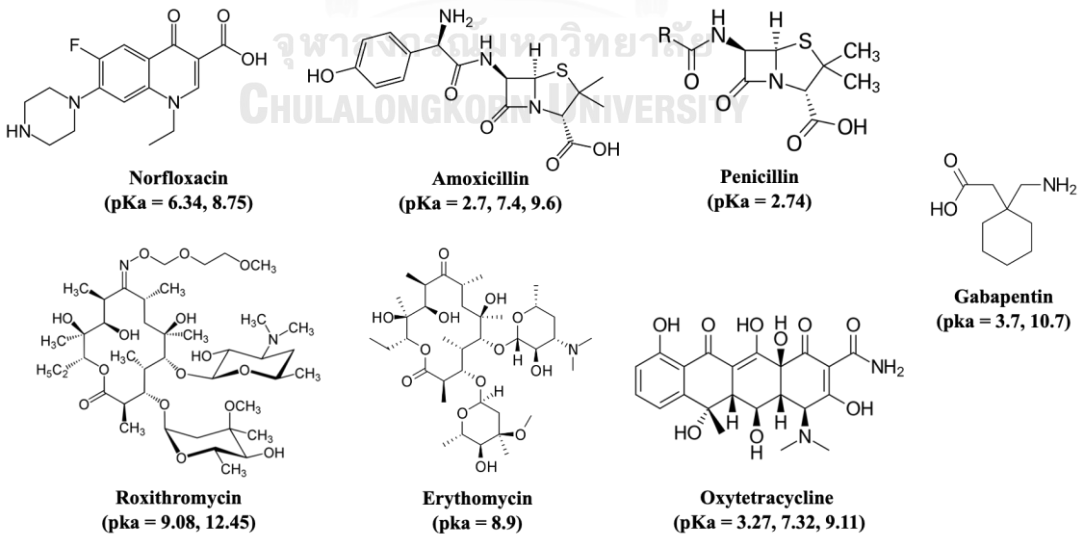


Figure 4.21 Structure of studied drugs.

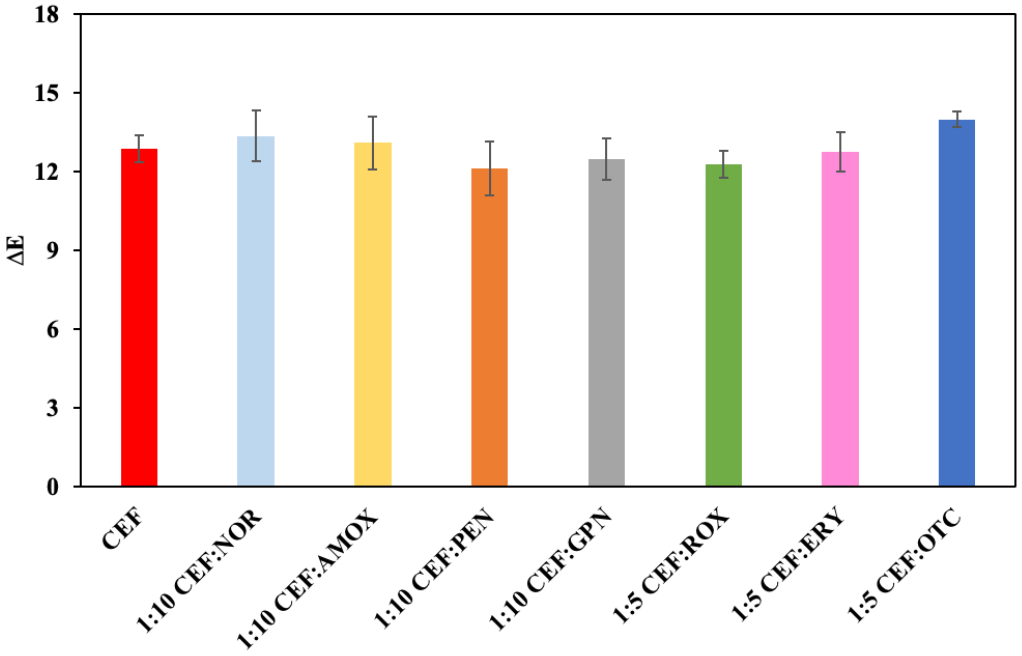


Figure 4.22 The analytical results of the modified PEI-sponges in the detection of ceftazidime (CEF) in single solution compared to binary mixtures with norfloxacin (NOR), amoxicillin (AMOX), penicillin (PEN), gabapentin (GPN), roxithromycin (ROX), erythromycin (ERY), and oxytetracycline (OTC).

4.2.6 Method validation and water sample analysis

The analytical performance of the proposed method including the linear range, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision was evaluated. The detection of ceftazidime was performed under the optimum condition described as follows. A piece of cotton sponge modified with 170 mM APTES and 210 µM PEI was immersed in 150 mL of ceftazidime solution (pH 4) for 30 min before detecting with 150 µL of a reagent mixture containing 0.5 M hydrochloric acid, 30 mM sodium nitrite, and 25 µM chromotropic acid with the reaction time of 30 min. Moreover, this method was applied to detect ceftazidime in water samples and compared with the HPLC method.

4.2.6.1 Calibration curve and linearity

Under the optimum conditions, the external calibration curve was plotted between the concentrations of ceftazidime and color intensities in terms of ΔE. The linear relationship was found in the range of 0.5 to 3.0 mg L^{-1} with the linear regression equation of y = 7.4868x + 2.2502 and correlation (R^2 value) of 0.9951. The material color changed from pale yellow to pink when the concentration of ceftazidime solution increased, and it could be detected by naked eyes as shown in Figure 4.23. The relative standard deviation (RSD) of the calibration slope was within the range of 3.13% when repeated inter-day (n=7), indicating great repeatability of the method.

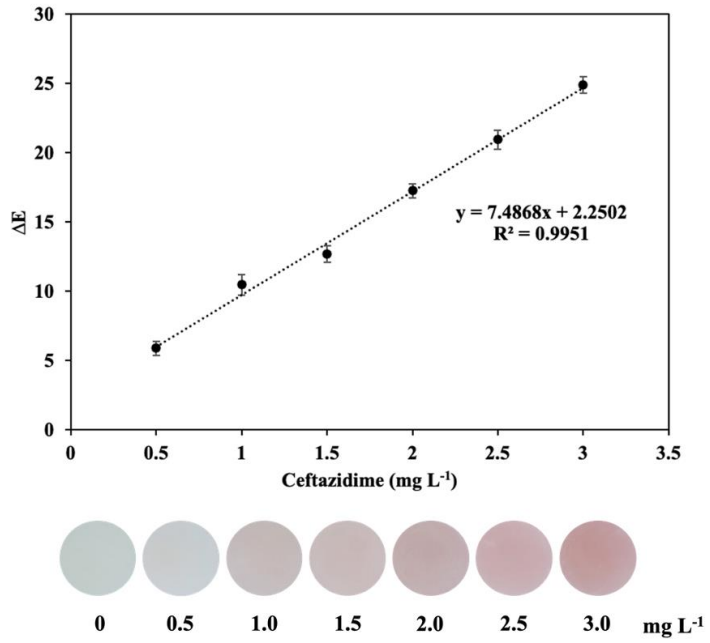


Figure 4.23 Calibration curve for ceftazidime determination by PEI-sponge and the photos of the materials.

4.2.6.2 Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) are two important parameters that are used to evaluate the sensitivity of the method. The LOD represents the lowest concentration of an analyte that can be reliably detected by the method as the signal-to-noise ratio of 3:1, while the LOQ represents

the lowest concentration that can be quantified as the signal-to-noise ratio of 10:1. The LOD and LOQ of this method were calculated to be 0.06 and 0.40 mg L^{-1}, respectively.

4.2.6.3 Accuracy and precision in water sample analysis

To evaluate the accuracy and precision of the developed method, the spike method was applied to quantify the concentration of ceftazidime in real water samples including drinking water, tap water, and shrimp farming water. Ceftazidime was spiked into water samples to obtain the final concentration of 0.75 and 1.75 mg L^{-1}. The concentrations of ceftazidime from our proposed method calculated from external standard calibration curve were compared to those obtained by the standard HPLC method. The results were reported in terms of percent recovery and %RSD for accuracy and precision, respectively. In Table 4.3, the concentrations of ceftazidime in various water samples detected by the proposed platform were not significantly different from those determined by HPLC at 95% confidence interval. The recovery of ceftazidime from spiked sample analyzed by the PEI-sponge method was found in the range of 83 – 103% and 90 – 101% for the HPLC method. The relative standard deviation (RSD) ranging from 0.67 - 4.76% and 0.52 - 7.36% were obtained by the PEI-sponge and HPLC methods, respectively. According to the AOAC international guidelines, the accuracy and precision are considered acceptable if the recovery is in the range of 80% to 110% and the relative standard deviation (%RSD) is less than 16% for the determination of an analyte at a concentration of 1 mg L^{-1} **[99]**. These outcomes demonstrated that the PEI-sponge method could be used to detect ceftazidime in water samples with a fair accuracy and precision, in compliance with the AOAC international criteria. However, the color matrix from natural water samples may affect the detection of ceftazidime by the PEI-sponge method due to its colored background. Therefore, the sample pretreatment was strongly suggested to reduce the extraction of color species before the analysis.

Table 4.3 The determination of ceftazidime in water samples using developed method.

Sample	Added	PEI-sponge method			HPLC method		
	(mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD (%)	Found (mg L^{-1})	Recovery (%)	RSD (%)
Drinking	-	n.d.	-	-	n.d.	-	-
water	0.75	0.73±0.03	97	4.76	0.73±0.02	97	3.01
	1.75	1.80±0.07	103	3.78	1.76±0.06	101	3.54
Tap water	-	n.d.	-	-	n.d.	-	-
	0.75	0.77±0.01	102	0.67	0.72±0.02	96	3.27
	1.75	1.53±0.07	88	4.33	1.63±0.01	93	0.52
Shrimp	-	n.d.	-	-	n.d.	-	-
farming	0.75	0.77±0.02	102	2.07	0.74±0.03	99	4.04
water	1.75	1.45±0.04	83	2.83	1.58±0.12	90	7.36

Results presented as mean ± SD (n = 3)

n.d. = not detectable (LOD = 0.03 mg L^{-1} in HPLC method, 0.06 mg L^{-1} in PEI-sponge method)

4.2.7 Comparison of method performance

The analytical performance of our developed method and other previously reported methods for ceftazidime detection in various samples are compared in Table 4.4. Our platform had a greater detection sensitivity than that of the spectrophotometry [60, 61] and HPLC/UV [8, 62] methods. HPLC had a good detection selectivity and a wider linear range to detect ceftazidime in biological fluids. However, they require sophisticated equipments and professional users. Moreover, spectrofluorometry [64, 65] was another sensitive and selective technique to detection of ceftazidime. To improve the sensitivity and selectivity in these spectrofluorometric methods, molecular imprinted polymers were used. AFor

instance, quantum dots embedded molecularly imprinted polymers (QDs-MIP) were prepared and employed as adsorbent for ceftazidime extraction prior to the detection [12, 67]. In our method, we extracted ceftazidime from a sample solution at specific pH on the PEI-sponge and detected ceftazidime with a specific azo dye formation reaction. With this approach, a good detection sensitivity and selectivity were achieved. In addition, the PEI-sponge could also be used with a high-volume sample, resulting in highly sensitive detection. Hence, colorimetric method developed herein show a great potential in the detection of trace ceftazidime in water samples using only a digital camera.

Table 4.4 Comparison of the presented method with other reported methods for ceftazidime detection.

Method	Linear range (mg L^{-1})	LOD (mg L^{-1})	Reaction and materials	Sample	Ref.
Spectrophotometry	1 - 10	0.314	Azo-dye formation using 4-tert-butylphenol as coupling reagent	Pure form and pharmaceutical dosage	[60]
Spectrophotometry	3 – 50	0.8102	Azo-dye formation using 1-Naphthol and 2-Naphthol as coupling reagent	Pharmaceutical formulations	[61]
Spectrofluorometry	0.24 - 2.4	0.0047	Fluorescence quenching of poly (ethylene glycol) (PEG) 2000-capped carbon quantum dots (CQDs)	Active pharmaceutical ingredient (API)	[64]
Spectrofluorometry	0.03 – 0.3	0.003	Reaction with fluorescamine reagent	Pharmaceuticals and human plasma	[65]
HPLC	0.1 – 100	0.1	RP-HPLC and UV-detection	Frog plasma sample	[62]
HPLC-MS/MS	1 – 100 0.4 – 16	1[a] 0.4[a]	HPLC-MS/MS	Human plasma Cerebrospinal fluid	[8]

Method	Linear range (mg L^{-1})	LOD (mg L^{-1})	Reaction and materials	Sample	Ref.
Fluorescent nanosensor	0.1 – 10[b]	0.05[b]	Graphene quantum dots and magnetite nanoparticles embedded in molecularly imprinted polymer	Milk	[67]
Fluorescent nanosensor	0.18 – 1.3[b]	0.06[b]	Carbon dots embedded in silica molecularly imprinted polymer	Urine sample	[12]
Digital-image colorimetry	0.5 - 3.0	0.06	Azo-dye formation on PEI-sponge using chromotropic acid as coupling reagent	Water samples	This work

[a] LOQ = Limit of quantification. [b] μg L^{-1}

CHAPTER V
CONCLUSION

5.1 Conclusion

The solid platforms modified with specific reagents for digital image colorimetric determination of norfloxacin and ceftazidime were successfully developed. For norfloxacin determination, Congo red as chromogenic reagent was modified on a resin (amberlite XAD-7) and reacted with norfloxacin on the material surface through ion-pair complexation. The optimum conditions for this method are shown in Table 5.1. This approach allowed the detection of norfloxacin in a concentration range of 0 – 1.5 mg L^{-1} with a limit of detection of 0.23 mg L^{-1}. Overall, the platform developed herein is simple, quick, and equipment-free. It shows a potential in the detection of norfloxacin in real water samples with acceptable accuracy and precision.

Table 5.1 The optimum conditions for norfloxacin determination.

Parameters	Optimum conditions
pH of Congo red	pH 6
Congo red concentration	6.00 μM
Sample volume	10 mL
Reaction time	15 minutes
pH of detection	pH 2

For ceftazidime detection, a cellulose sponge derived from APTES-cotton fiber was successfully fabricated and functionalized with PEI for the extraction of ceftazidime. The characterization of PEI-sponge by ninhydrin test and ATR-FTIR revealed the presence of amine group on its surface. The morphology obtained from SEM image of the sponge illustrated the porous structure and its water uptake ratio was high. These properties make the sponge a suitable material for the extraction of ceftazidime from large sample volume in a batch manner with ease of solid

separation from the sample. The colorimetric detection of ceftazidime was achieved through diazotization and coupling reaction, resulting in azo dye color on the material. The optimum conditions for the method are shown in Table 5.2. The color intensities of material were dependent linearly on the ceftazidime concentration and hence a calibration plot could be made for quantitative purpose in the range of 0.5 – 3.0 mg L^{-1} with a limit of detection of 0.06 mg L^{-1}. This method provides a good selectivity in ceftazidime detection over the other potential interfering drugs including norfloxacin (NOR), oxytetracycline (OTC), amoxicillin (AMOX), penicillin (PEN), roxithromycin (ROX), erythromycin (ERY), and gabapentin (GPN). The selectivity of ceftazidime determination was achieved by two steps: extraction and detection. Under the optimized condition, the proposed PEI-sponge method could be applied for the quantification of ceftazidime in water samples with acceptable accuracy and precision. This method was demonstrated as a promising alternative for low level ceftazidime determination without requiring analytical instruments.

Table 5.2 The optimum conditions for ceftazidime determination.

Parameters	Optimum conditions
Cotton fiber content	2 %wt
APTES concentration	170 mM
PEI concentration	210 μM
Sample volume	150 mL
pH of Solution	pH 4
Reagent concentrations	0.5 M HCl, 30 mM $NaNO_2$, 25 μM chromotropic acid
Reaction time	30 minutes

5.2 Suggestion for future work

Suggestion for future work is to study the shelf life of the modified materials. For norfloxacin detection, the sensitivity of this method should be improved for accurately quantifying low-level norfloxacin in real samples. To solve this restriction,

the sample pretreatment and pre-concentration step should be performed before the analysis. Moreover, the concept of PEI modification could be applied to other cellulose-based materials for ceftazidime detection in various sample types.

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