SAFE AND NON-FOULING REACTIVE ANTIBACTERIAL AGENTS ON COTTON FABRICS

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Safe and Non-fouling Reactive Antibacterial Agents on Cotton Fabrics

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CERTIFICATE OF ORIGINALITY

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Abstract

The study focuses on the synthesis of reactive sulfobetaine antibacterial agents which are used in cotton fabrics to solve the problems in bacterial multi-drug resistance, bacterial contamination and washing durability. Generally, bacteria adhering on cotton fabrics surfaces form biofilms, which are difficult removed. The biofilms provide ideal shelters and allow them to metabolize safely within the environment. Therefore, it is significant and essential for researchers to develop new and effective antimicrobial agents that kill bacteria or prevent bacterial adhesion and biofilm formation on the fabric surfaces.

This thesis presented three reactive antibacterial agents and the related characterization on structure, antibacterial property, physical property, non-fouling property, washing durability and cytotoxicity.

1. Pyridine sulfobetaine

The obtained pyridine sulfobetaine structure was confirmed by ^{1}H-NMR spectra and ESI-MS. The thermostability and antibacterial activity were characterized by TGA and bacterial growth kinetics. At a lower concentration, para-pyridine sulfobetaine showed excellent antibacterial activities when acted on gram-negative *E.coli* and gram-positive *S.aureus*, with the MIC of 8.36 μmol/ml and 10.45 μmol/ml against *E.coli* and *S.aureus* respectively. When applied it to cotton fabric with a pad-dry-cure finishing,

the antibacterial rate of the modified cotton fabric could reach 99.90% or higher against both gram-negative *E.coli* and gram-positive *S.aureus*. In addition, compared to the raw cotton fabric, the modified cotton fabric exhibited slightly reduced air permeability and significantly improved hydrophilicity. The as-modified cotton fabric is more skin compatible confirmed by skin stimulation test and non-toxic. Therefore para-pyridine sulfobetaine could be a good alternative for medical fabric and other relevant applications.

2. s-triazine sulfobetaine

The s-triazine sulfobetaine was synthesized via cyanuric chloride mono-substitution reaction. Its structure was confirmed by ^{1}H-NMR and ^{13}C NMR spectra. The MIC was quantitatively determined by measuring bacterial growth kinetics in liquid media. The MIC of s-triazine sulfobetaine against *S.aureus* and *E.coli* were 2 mg/ml and more than 6 mg/ml respectively.

3. BTCA-betaine

BTCA was used as cross-linking to give cotton fabric durable washing durability. The concentration of BTCA are 5% and 8%. The air permeability, tearing strength, antibacterial activity, washing durability, non-fouling property and cytotoxicity were evaluated according to the related standard respectively. The air permeability and tearing strength of modified cotton fabric reduced. The modified cotton fabric showed

excellent antibacterial activity, washing durability and non-fouling property. Via SEM and fluorescence microscope observation, the modified cotton fabric showed no cytotoxicity on fibroblast. It indicates that the modified cotton fabric is biocompatible and safe.

List of Publications

Journals:

1. Yujuan Guo, Xingli Zhu, Yuanfeng Wang, Chuilin Lai, Kaikai Ma, Bin Fei, John H Xin*. Study of antibacterial pyridine sulfobetaine with sound safety and non-fouling property on medical cotton fabric for wound care. (Submitted to Chemical Engineering Journal)

2. Yujuan Guo, Kaikai Ma, Yuanfeng Wang, Chuilin Lai, Bin Fei, John H Xin*. Safe and durable cotton fabric modified with BTCA and amino sulfobetaine. (In preparation)

3. Shiguo Chen, Yujuan Guo, et al. Synergistic antibacterial mechanism and coating application of copper/titanium dioxide nanoparticles. Chemical engineering Journal. 2014, 256, 238-246.

4. Huawen Hu, Xiaowen Wang, Dagang Miao, Yuanfeng Wang, Chuilin Lai, Yujuan Guo, Wenyi Wang, John H. Xin and Hong Hu. A pH-mediated enhancement of the graphene carbocatalyst activity for the reduction of 4-nitrophenol. Chem. Commun., 2015, 51, 16699--16702

5. Yuanfeng Wang, Baitai Qian, Chuilin Lai, Xiaowen Wang, Kaikai Ma, Yujuan Guo, Xingli Zhu, Bin Fei, and John H. Xin*. Flexible Slippery Surface to Manipulate Droplet Coalescence and Sliding, and Its Practicability in Wind-Resistant Water Collection.

ACS Appl. Mater. Interfaces 2017, 9, 24428−24432.

6. Yuanfeng Wang, Chuilin Lai, Xiaowen Wang, Yang Liu, Huawen Hu, Yujuan Guo, Kaikai Ma, Bin Fei, and John H. Xin*. Beads-on-String Structured Nanofibers for Smart and Reversible Oil/ Water Separation with Outstanding Antifouling Property. ACS Appl. Mater. Interfaces 2016, 8, 25612−25620.

International conference:

1. J H Xin, Y J Guo. Safe and non-fouling medical cotton gauze treated by pyridine sulfobetaine. AUTEX 2017, 5.

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Symbols and Abbreviations	Notes
EPS	exopolysaccharides
QACs	quaternary ammonium compounds
PEG	Poly (ethylene glycol)
CC	Cyanuric chloride
BTCA	1,2,3,4-butane tetracarboxylic acid
NMR	Nuclear magnetic resonance
ESI-MS	Electrospray Ionization-Mass Spectrometry
MIC	minimum inhibitory concentration
MBC	minimum bactericidal concentration

CC	Cyanuric chloride
SHP	sodium hypophosphite
MTT	methyl thiazolyl tetrazolium
SEM	Scanning electron microscope
NPs	nanoparticles
AgNP	Silver nanoparticles
CuNP	Copper NP
CS	chitosan
ROS	reactive oxygen species
TiO_2	Titanium dioxide

SPR	surface plasmon resonance
ZnO NPs	Zinc oxide nanoparticles
QASs	Quaternary ammonium salts
AC	activated carbon
PHMB	Polyhexamethylene biguanides
SSPB	siloxane sulfopropylbetaine
DBTDL	dibutyltin dilaurate
1, 3-PS	1, 3 - propane sultone
THF	tetrahydrofuran
D_2O	Deuterium oxide

PBS	phosphate-buffered saline
EDX	energy dispersive X-ray
TGA	thermos-gravimetric analyzer
OD_{600}	optical density at 600 nm
CFU	colony forming units
R	antibacterial rate
MeOH	Methyl alcohol
*S*er	scores of erythema
*S*ed	scores of edema
Boc_2O	di-tert-butyl carbonate

E.coli	*Escherichia Coli*
S.aureus	*Staphylococcus aureus*
FBS	Fetal calf serum
DMEM	Dulbecco's modified eagle medium
DAPI	4', 6-diamidino-2-phenylindole
FITC	Fluorescein isothiocyanate

Chapter 1

Introduction

1.1 Research background

Microbial infection and the emergence of multi-drug resistant bacterial strains remain the most serious problems in some areas, such as medical devices, food packaging, food storage, textiles, and so on. Generally, bacteria adhering on these natural and man-made surfaces form biofilms, which are difficult to be removed [1].

Initially, the biofilm attachment to the surfaces is reversible. Thereafter the synthesis of insoluble exopolysaccharides (EPS) encase the bacteria in a three dimensional matrix [2]. With the accumulation of EPS, the bacteria colonies develop into mature biofilm. The biofilms provide ideal shelters to protect bacteria inside metabolizing safely, and persisting within the environment. Therefore, it is significant and essential for researchers to investigate some new and effective antimicrobial agents that can kill bacteria or prevent bacterial adhesion to the surfaces from biofilm formation. Recently, the researchers mainly focused the research on antibacterial agents which can be divided into three categories: organic, inorganic and natural antibacterial agents [3] and the related antimicrobial mechanisms have been explored to well guide the study of the antimicrobial agents.

For the antimicrobial agents that can kill bacteria were called bactericide, almost all the organic, inorganic and natural antimicrobial agents are bactericide. The others belong to bacteriostatic, which can resist the growth of the bacteria or resist the

adhesion to the surfaces.

The antibacterial modification of cotton fabrics need to meet many requirements. Firstly, it should be broad spectrum. The modified cotton fabrics have to nontoxic and no skin stimulation. Also washing durability against laundering is another important factors that need to be considered. The physical properties of modified cotton fabrics should not be negative.

Recently, quaternary ammonium compounds (QACs), N-halamine, triclosan and polybiguanides are the main organic antimicrobial agents [4] that are well studied and used in daily life. All of them have some disadvantages when they were used in textiles finishing. The antibacterial durability of textiles finished with these agents without reactive groups is poor, the antibacterial agents are easily to leaching from the textiles after several times washing [5]. Moreover, Triclosan has been banned in textiles and other products because of its produced toxic polychlorinated dioxins upon exposure to sunlight [6]**.** The polybiguanides have weak light tolerance, which affects the application greatly [7]. Their heat resistance is also poor.

Nowadays, comparing with organic antibacterial agents, inorganic metallic simple and their compounds such as Ag, TiO_2, Cu, Au and ZnO and so on are widely used for their excellent properties in heat resistance, chemical stability and long life [8-11]. Natural antibacterial agents are mainly chitosan and their derivatives due to their good biocompatibility and environment friendly. However, the antibacterial activity of the

chitosan is pH-sensitive and limits to acidic conditions [12]. The chitosan also shows weak adhesion to cellulose fibres and is leached gradually from the fibre surface by repeated laundering [13].

Medical cotton fabric, as a specially designed dressing for medical wound, is widely used to cover wounds. The utilization of cotton fabric has many advantages: (1) keeping the wound clean and moist, (2) powerful and effective absorption of fluid exudates, and (3) simple and low cost production process. However, cotton fabric lacks the ability to protect wound from bacterial contamination[14]. The great air permeability of cotton fabric leads to dehydration of the wound surface. As a result, cotton fabric sticks to the surface and causes the second-time mechanical injury when it is removed for replacement, which gives patients pain and trauma [15]. In the past decades, researchers paid attention to the chemical modification of the cotton fabric to solve the above drawbacks. Chitosan and silver often are used for cotton fabric for improvement. However, betaine is rarely used in cotton fabric modification. Kittinaovarat et al and Venkatrajah et al reported that carboxymethylation chitosan modification of the cotton fabric improved its antibacterial and moisture holding properties, which in turn accelerate the wound healing [14, 16]. Electrospun chitosan nanofiber was also used in cotton fabric to form composite wound dressing. In combination with the electrospun nanofibers, the antibacterial and moisture holding properties of the modified cotton fabric were improved to benefit wound healing

process [15]. Silver nanoparticles were also used in cotton fabric to provide antibacterial property and improve wound healing [17]. However, chitosan and silver cannot provide cotton fabric with non-fouling property.

At present, there are only few materials which can meet the challenges of resistance of bacteria adhesion and biofilm formation. Poly (ethylene glycol) (PEG) can achieve resistance of nonspecific protein adsorption via hydration. However, the application of PEG is limited by its oxidation damage [18-20].

Betaines represent a special family of zwitterionic materials that bear both cationic moiety like QACs and anionic functional groups like sulfo-, carboxy-, and phosphobetaines on the same repeat unit [21, 22]. They are electrically neutral and can resist the growth of the bacteria and the adhesion of the bacteria to the surface to form biofilm, the mechanism was called anti-biofouling [1]. Therefore, it has attracted researchers' great interest in studying betaines and their antibacterial mechanisms.

Pyridine has a similar chemical property to tertiary amines. The derivatives of pyridine have been well studied and used in many fields, such as gene delivery [23], anticancer drug [24], antibacterial agents [25] and so on for their good properties on non-toxicity due to the delocalization of positive charge into the pyridine ring [23].

Cyanuric chloride (CC) and its derivatives have been known for a long period for their widespread application in textiles, reactive dyes and surface active agents. CC has three chlorine atoms and they are reactive toward nucleophiles. The chlorine atoms in

cyanuric chloride can be substituted by various nucleophiles under different temperature in a stepwise manner which makes its ease of the preparation of mono-, di- and tri- substituted 1, 3, 5-triazines [26]. Generally, its mono-substitution of chlorine occurs below or at 0 ºC， di-substitution at room temperature and tri-substitution above 60 ºC.

Pyridine and triazine based antibacterial agents include reactive groups and betaine structure. The reactive groups can covalently bond to the textiles to give textiles durable antibacterial activity. The betaine can prevent the formation of biofilms on the surface of the textiles.

1,2,3,4-butane tetracarboxylic acid (BTCA) is nontoxic and used as the most efficient crosslinking agent to fix antibacterial agent on cotton fabric [27]. It can react with the cellulose hydroxyl groups and the antibacterial agent amino groups via esterification. The mechanism is believed to form an intermediate cyclic anhydride initially, then react with the cellulosic hydroxyl and amino to complete the ester linkage with sodium hypophosphite as the most effective catalyst, which can decrease the reaction temperature and energy [28]. Even the washing durability can be significantly improved [29-31].

1.2 Objectives

The aimed research is aimed to study the antibacterial activity of sulfobetaine

antibacterial agents, pyridine sulfobetaine, triazine sulfobetaine and BTCA-sulfobetaine and explore the application of sulfobetaine toward cotton fabrics. Also, the washing durability and other functional properties of the modified cotton fabrics finished with the above mentioned antibacterial agents were investigated. Betaine is a new candidate of antibacterial agent because of its biocompatible, non-fouling and non-toxic properties. Based on the above mentioned, the objectives have been proposed as follows:

1. To synthesize pyridine and triazine based sulfobetaine and characterize their structures with Nuclear Magnetic Resonance (NMR) and Electrospray Ionization-Mass Spectrometry (ESI-MS).

2. To investigate the antibacterial activity by measuring the MIC (minimum inhibitory concentration) of the synthesized agents against *E.coli* and *S.aureus* using viable count method.

3. To apply antibacterial agents on cotton fabrics and investigate appropriate finishing conditions and the properties of the modified cotton fabrics, such as antibacterial activities, washing durability, tearing strength, air permeability.

4. To explore the non-fouling property of modified cotton fabrics using Scanning electron microscope (SEM).

5. To evaluate the cytotoxicity of modified cotton fabrics to skin fibroblast using

methyl thiazolyl tetrazolium (MTT) method, SEM and fluorescence microscope.

1.3 Significance of the study

In the past years, bacterial contamination is a serious problem in cotton fabric. The antibacterial finishing of cotton fabrics need to meet many requirements. They should ideally have wide spectrum antibacterial property to various species of bacteria plus anti-microbial property such as killing fungi and yeast. Then the biocompatibility (skin stimulation and cytotoxicity) is another factor that should be considered. In addition, the washing durability should be good.

The main study on antibacterial agents focuses on inorganic antibacterial agents, i.e. Ag, Cu, ZnO, organic antibacterial agents, i.e. quaternary ammonium salts, N-halamine and Polyhexamethylene biguanides (PHMB), and natural chitosan antibacterial agents. The reactive sulfobetaine antibacterial agents have not yet been fully explored and applied. The excellent washing durability, non-fouling property and non-cytotoxicity make reactive sulfobetaine antibacterial agents to be alternative and bring great breakthroughs to antibacterial cotton fabrics.

In this study, siloxane pyridine sulfobetaine, s-triazine sulfobetaine and BTCA amino sulfobetaine were synthesized. All of them can react with cotton fabric and form chemical bond which can give cotton fabric excellent antibacterial activity, washing durability and non-fouling property. Furthermore, the modified cotton fabrics showed

no skin stimulation and non-cytotoxicity.

1.4 Methodology

In this study, the following general methodologies are listed below and the details will be described in chapter 3.

1. Structure characterization: Nuclear magnetic resonance (NMR), Electrospray Ionization-Mass Spectrometry (ESI-MS).

2. Antibacterial activity: viable count method, FZ/T 73023-2006, inhibition zone.

3. Finishing: pad-dry-cure process

4. Washing durability: AATCC 61-2A method for washing.

5. Physical property: thermos-gravimetric analyzer (TGA), Scanning electron microscope (SEM), tearing strength, water absorption, and air permeability.

6. Safe and non-fouling property: Methyl Thiazolyl Tetrazolium (MTT), SEM and fluorescence microscope.

1.5 Research framework of thesis

The thesis includes 7 chapters. The framework is listed as follows:

Chapter 1-3 Establishment of thesis framework

Chapter 1 introduction outlines the research background, objectives and significance.

Chapter 2 summarizes the current study of biofilms and antibacterial agents and their advantages and disadvantages.

Chapter 3 states the detailed research methodology in structure characterization, antibacterial activity evaluation, physical property evaluation, cotton fabric morphology observation, non-fouling property investigation and cytotoxicity test.

Chapter 4-6 Study of antibacterial agents and the properties of modified cotton fabrics

In Chapter 4, pyridine sulfobetaine was synthesized and applied in cotton fabric. The structure of pyridine sulfobetaine was characterized by ^{1}H NMR and ESI-MS. The antibacterial activity of the pure antibacterial agent and the modified cotton fabric were evaluated. The physical property, non-fouling property and cytotoxicity of modified cotton fabric were also investigated according to related standard respectively.

Chapter 5 showed the synthesis of triazine sulfobetaine with mono-substitution of chlorine. The structure was characterized by ^{1}H NMR and ^{13}C NMR.

In Chapter 6, the amino sulfobetaine was used with BTCA to treat cotton fabric. The antibacterial activity, tearing strength, air permeability, washing durability of modified cotton fabric were tested respectively. Also the non-fouling property and cytotoxicity were evaluated.

Chapter 7 Conclusions and future works

Chapter 7 summarizes the main results of resent research works and states the future objectives and directions on reactive antibacterial agents and its potential applications on cotton fabrics and other materials.

Chapter 2

Literature review

2.1 Introduction

Bacterial contamination in cotton fabric is a serious problem. When bacteria adhere to cotton fabrics, biofilms were formed and difficult to be removed. Generally, the antimicrobial agents include bactericide and bacteriostatic. Almost all the organic, inorganic and natural antimicrobial agents are bactericide. The others which can resist the growth of the bacteria or resist the adhesion to the surfaces belong to bacteriostatic. Recently, quaternary ammonium compounds (QACs), N-halamine, triclosan and polybiguanides are the main organic antimicrobial agents. Ag, TiO_2, Cu, Au, TiO_2 and ZnO are the usually used inorganic antibacterial agents. Natural antibacterial agents are mainly chitosan and their derivatives. Betaine represent a special family of zwitterionic materials that can resist the growth of the bacteria and the adhesion of the bacteria to the surface to form biofilm. Therefore, it has attracted researchers' great interest in studying betaines and their antibacterial mechanisms.

2.2 Biofilms

The biofilm was first observed by a Dutch scientist from his own teeth about 300 years ago which made him the first biofilm experimenter. He called the tiny living animals for animalcules. After that, some other scientists found that most of the bacteria do not live in free lifestyle but develop in substrates with colonial growth [32]. With the development of microscopy and molecular genetic techniques, people began to

know that natural bacterial populations exist mostly in biofilm communities. It promoted the understanding and research to bacteria. Instead of living as dispersed single cells, microorganisms accumulate to the surfaces and to form polymicrobial aggregates, interacting with each other, which we call biofilms. The biofilms provide ideal shelters to protect bacteria inside metabolizing safely, and persisting within the environment.

Biofilms are communities of microorganisms attached to surfaces with their self-produced extracellular matrix, in which the biofilm cells are embedded. Numerous pathogens survive and persist within the environment through formatting biofilm [33].

2.2.1 Biofilms formation

The formation of a microbial biofilm is a dynamic process with several steps (Fig. 2.1) [32].

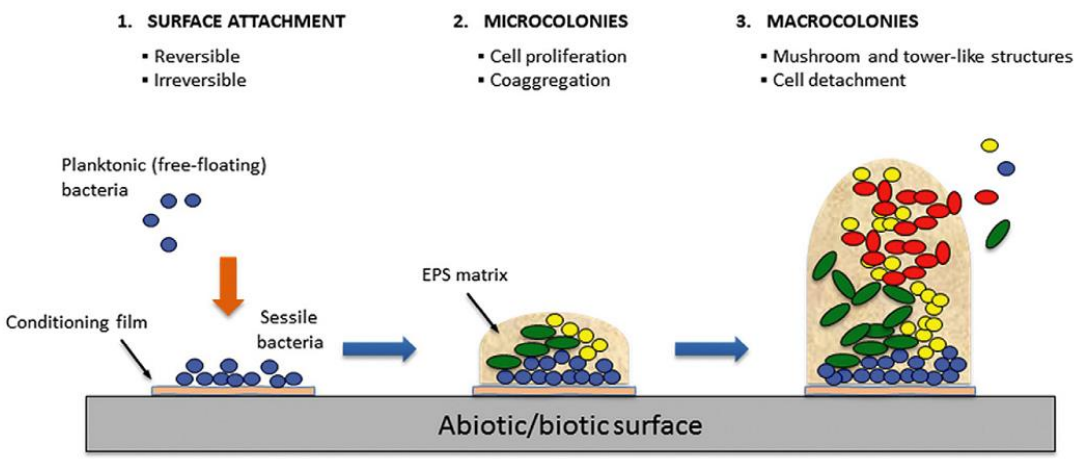


Fig 2.1. Schematic representation of the distinct steps in microbial biofilm development. The different stages in biofilm formation include initial attachment to the surface, formation of a monolayer along the surface with formation of micro-colonies, biofilm maturation with formation of a three-dimensional structure, and cell dispersion [32]. Initially, the bacterial attachment to the surfaces is reversible through the van der Waals, after that the synthesis of insoluble extracellular polymeric substances (EPS) encase the bacteria in a three dimensional matrix [2], which leads to stronger irreversible adhesion attachment. With the accumulation of EPS, the bacteria colonies develop into mature biofilm. EPS plays an important role in the biofilm formation and maintaining. The formation of biofilms depends on the environment changes and growing conditions.

The extracellular polymeric substances matrix at different dimensions was shown in Fig 2.2 [34].

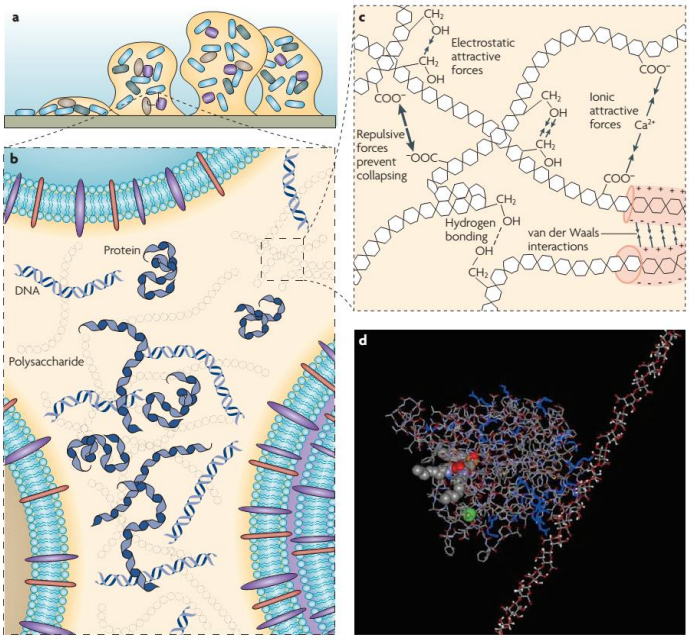


Figure 2.2. The extracellular polymeric substances matrix at different dimensions. a. A model of a bacterial biofilm attached to a solid surface. b. The major matrix components — polysaccharides, proteins and DNA. c. The classes of weak physicochemical interactions and the entanglement of biopolymers that dominate the stability of the EPS matrix. d. A molecular modelling simulation of the interaction between the exopolysaccharide alginate (right) and the extracellular enzyme lipase (left) of Pseudomonas aeruginosa in aqueous solution [34].

2.2.2 Biofilms in infectious diseases

Biofilms bring great threatening to human infections [35]. The majority of infections are due to biofilms. Actually, a large proportion of infections are related to the biofilms formatting on the surface of implanted biomedical devices, such as catheters, orthopedic, respirators and heart valves devices (Table 2.1) [32]. In fact, 95% of urinary tract infections are associated with a urinary catheter, 80% of pneumonias are associated with mechanical ventilation, and 87% of bloodstream infections are associated with intravascular devices[32, 36].

Several pathogens associated with chronic infections are linked to biofilm infections, including periodontitis, cystic fibrosis pneumonia, chronic urinary tract infections, chronic otitis media, and chronic wound infections (Table 2.2).

Contrary to acute infections, which are mostly the result of planktonic (free-

floating) growth, in chronic bacterial infections, the planktonic phenotype generally exists only transiently, and usually as a minor population. Since chronic infections are fundamentally different from acute infections, different strategies are necessary to treat the biofilm infections more efficiently.

Table 2.1. Biofilms of indwelling medical devices

Medical device	Principle microorganisms
Contact lens	*P.aeruginoosa*, Gram-positive cocci
Denture	*Candida* spp.
Urinary catheter	*E.coli*, *Candida* spp., *E.faecalis*, *P.mirabilis*, *K.pneumoniae*
Central venous catheter	CoNS*, *S.aureus*
Mechanical heart valve	CoNS*, *S.aureus*
Artificial hip prosthesis	CoNS*, *S.aureus*, *Enterococcus* spp.
Voice prostheses	*C.albicans*, CoNS
Endotracheal tubes	Enteric Gram-negative species

* CoNS: coagulase-negative staphylococci.

Table 2.2. Diversity of human infections involving biofilms [32]

Anatomic location	Infectious disease	Microorganisms
Eye	Ocular infections	*S. aureus*
Ear	Otitis media	Non-typeable *H. influenzae*
Mouth	Dental caries	Acidogenic Gram-positive cocci
	Endodontic infections	Gram-positive anaerobic species, *Bacteroides*, *Neisseria*, *Fusobacterium*
	Periodontitis	Gram-negative anaerobic oral bacteria
		P. aeruginosa, *B. cepacia*
Respiratory tract	CF pneumonia	*S. aureus* and CoNS
	Chronic sinusitis	Viridans streptococci
Heart	Endocarditis	Gram-positive cocci
	Musculoskeletal	

Muscle	infections	
	Osteomyelitis	Various bacterial species
Bone	Necrotizing fasciitis	Group A streptococci
Skin	Diabetic foot infection	*Corynebacterium* spp. and various obligate anaerobes
Foot		
	Bacterial prostatis	*E. coli* and other Gram-negative species
Prostate gland		*S. aureus*
	Staphylococcal toxic shock syndrome	Enteric bacteria
Vagina		
	Gallstone	*E. coli*, Enterococci, *Klebsiella*
	Urinary tract infection	
Biliary system		*E. coli*
Urinary tract		
	Persistent diarrhea	
Large intestine		

The fact that microbial biofilms represent a protected mode of growth that allows

cells to survive hostile environments presents a challenge for treating chronic biofilm infections [37, 38]. Chronic infections are thus important clinically because bacteria in biofilms resist host immune responses and antibiotic treatment and these characteristics are often cited as the reason bacteria have the ability to persist for a long time in the human body.

2.2.3 Multi-drug tolerance of biofilms

The tolerance to antibiotics depends on multiple factors. It is thus essential to understand the mechanisms that promote tolerance to antimicrobials in order to develop novel strategies to treat biofilm infections. Schematic representation of the different mechanisms involved in the multi-drug tolerance of biofilms was shown in Fig 2.3 [32].

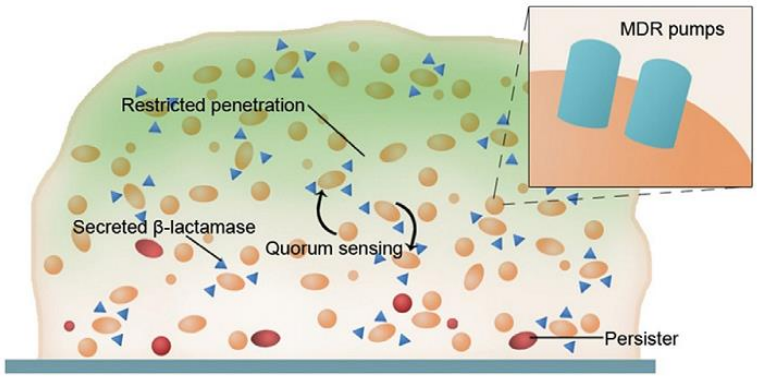


Fig 2.3. Schematic representation of the different mechanisms involved in the multi-drug tolerance of biofilms. The EPS matrix acts to restrict the penetration and diffusion of some antimicrobials. Bacteria within the biofilm can also secrete β-lactamases into their surrounding environment and/or increase the expression of multi-drug resistance

(MDR) efflux pumps. The activation of quorum-sensing systems along with different concentration gradient of nutrients, oxygen, and metabolic waste products also make important contributions to antibiotic tolerance and resistance to the host immune system. The presence of persisters that are resistant to killing by all antibiotics may also be responsible for recalcitrant biofilm infections [32].

In the natural world, about 99% of bacteria live in biofilm lifestyle. And at least 65% of bacterial infections have biofilms as an integral part of their pathogenesis [32]. The existence of biofilms brings great challenge in the treatment of biofilm infections. Hence, to develop some novel antibiofilm strategies and study the tolerance mechanisms are essential to target microbial biofilm infections.

2.3 Antimicrobial agents

2.3.1 Novel metal nanoparticles

Among inorganic antimicrobial agents, Ag, Cu, TiO_2 and ZnO have been employed extensively to fight microbial infections [39-43]. Their properties of broad-spectrum, heat resistance, chemical stability, low cost and low toxicity attracts researchers' great interest.

The inorganic antimicrobial agents' bactericidal properties are strongly influenced by their size, composition, structure and shape [44, 45]. Compared with bulk metallic

substance, the synthesis of nanoparticles (NPs) attracts a great interest for their unique chemical, electrical, optical and physical properties [10, 11]. The antimicrobial activities of nanoparticles is attributed to their small particle size and high specific surface area [46]. The larger surface area enables the nanoparticles interaction with the microorganisms and increase the concentration released from the particle surface [12], which increases the antimicrobial activity. The main antibacterial mechanisms of nanoparticles is to damage the lipids, proteins and DNA of microorganisms [47].

2.3.1.1 Silver nanoparticles (AgNP)

Silver nanoparticles have been widely used as antibacterial agents in clothing, water purification, wound dressings and so on [48]. Different sizes, morphologies and stabilities AgNP can be obtained using different methods, such as plasma sprayed technology [49], sol-gel method [50], optical interference lithography method [51], photochemical method [52] and so on.

A novel collagen-stabilized silver nanoparticles were synthesized by photochemical method at room temperature, displayed extraordinary stability. They showed bactericidal activity to *E.coli* and *B.megaterium* and non-toxic against human fibroblasts and keratinocytes [52]. AgNPs were generated on the surface of dopamine modified cotton fabrics in aqueous solution, Ag ions were reduced by dopamine to Ag nanoparticles, the fabric showed excellent durable antibacterial activity [39].

To control the release of nanoparticles or metal ions, prolong the release time and therefore enhance the washing durability, the nanoparticles have been embedded into some polymer matrices. The polymer/nanosilver composite coating combines the properties of polymers and nanosilver synergistically. The composite coating has bactericidal or adhesion resistant properties, it is also biocompatible and environmental friendly [53].

Rogheih Damerchely [54] and his colleagues combined different amounts of Ag NPs with nylon 6 fibers using melt spinning method, the obtained composite showed good antibacterial activity against gram-negative and gram-positive bacteria. The multifunctional fibers can be used in many fields, such as underwear and health socks.

Using patented deposition technology with silver nanoparticles can give wool fabrics antibacterial activity. The samples' antibacterial activity was tested against *E. coli* and with an amount of 10% of silver, the fibers showed good antibacterial efficiency even after several times washing [55].

About the antibacterial mechanisms of silver nanoparticles to bacteria, there are many theories. Several studies suggest that silver nanoparticles interact with the cell membrane and some of them also penetrate the bacterial cell wall, thereby causing the death of bacteria [56, 57]. Ag^+ ions binding to the cell membrane can also penetrate inside the bacteria. When the ions penetrate the bacterial cell, they interact with some

compounds such as DNA and protein, which induced their losing of replication and inactivation [58]. Also within the bacterial cell, they can attack its respiratory chain and inhibit cell division, leading to death of the bacterium [56, 59].

2.3.1.2 Copper NP (CuNP)

Cu is abundant in the earth, an essential trace element in most living organisms, environmentally benign, and relatively inexpensive. Therefore, Cu is a good candidate as an antibacterial agent. To date, many approaches have been used for the preparation of Cu nanoparticles, including laser ablation [60], sonochemical and electrochemical [61], chemical reduction [62] and photochemical methods [63] and so on. Rupareliaet al. reported that Ag and Cu nanoparticles have great promise as antimicrobial agents against *E. coli*, *Bacillus subtilis* and *S. aureus* [64].

Chen et al [65, 66] prepared Cu/TiO_2 and Cu/TiO_2/CS (chitosan) nanoparticles using photocatalytic reduction method. The prepared nanoparticles exhibited excellent antibacterial activities against both *E. coli* and *S. aureus* and are viable alternative candidates for future applications as high-activity antibacterial agents. The photocatalytic reduction mechanism is showed in Fig 2.4.

$$TiO_2 \xrightarrow{h\nu} TiO_2\ (h^+ + e^-) \qquad (1)$$

$$Cu^{2+} + e^- \longrightarrow Cu^+ \xrightarrow{e^-} Cu^0 \qquad (2)$$

Fig 2.4. Photocatalytic reduction mechanism

Fig 2.5 [66] showed the surface morphology of *E.coli* before and after treating with Cu nanoparticles. Cu nanoparticles damaged the bacteria cells. The antibacterial mechanisms that showed in Fig 2.6 can be concluded as follows: the Cu nanoparticles can penetrate to the cell wall and cause the disruption of cell wall/membrane, also the generation of ROS (reactive oxygen species), mostly hydroxyl radicals, H_2O_2 and singlet oxygen, can cause lipid oxidation, and lead to the lethal damage to cell, then the cytoplasm leach out and cause the final cell deformation and death.

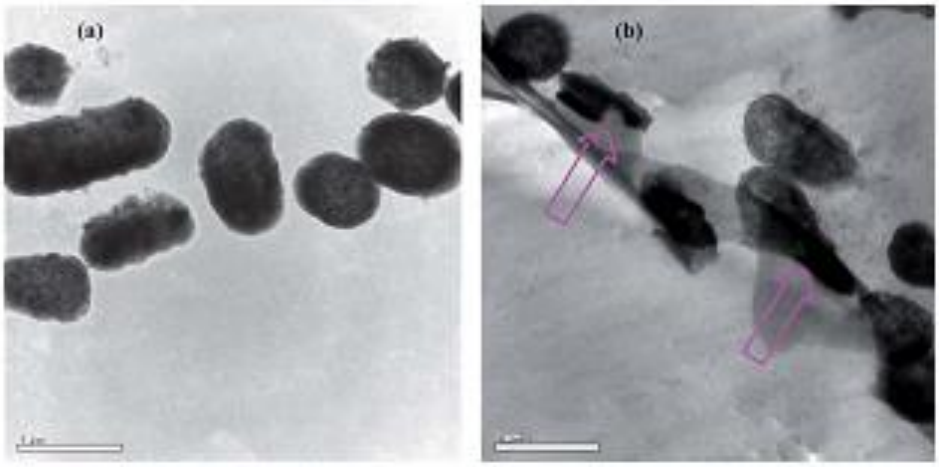


Fig 2.5. TEM images showing the surface morphology of *E.coli* before and after treating with Cu nanoparticles.

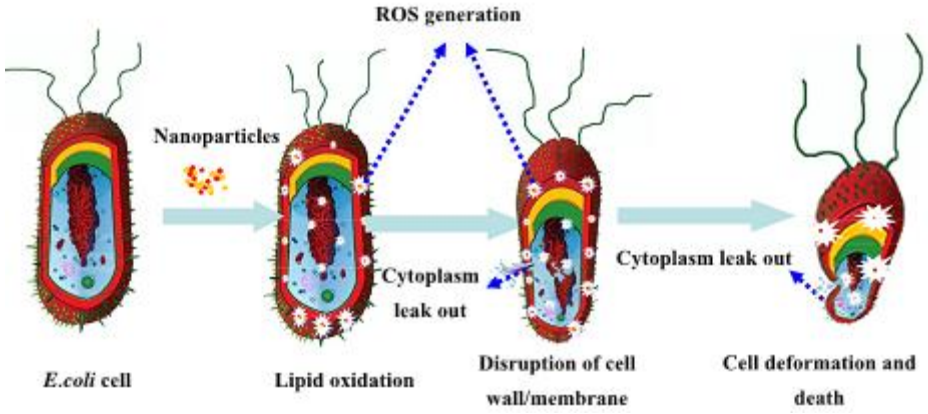


Fig 2.6. Antibacterial mechanism of Cu/TiO_2 nanoparticles

2.3.1.3 Titanium dioxide (TiO_2)

The use of metal oxide nanoparticles as antimicrobial agents attracts researchers' attention. Recently, the hybrid of metal oxide NPs (mainly TiO_2 and ZnO) and noble metals has been studied for the increase in photocatalytic efficiency [67, 68]. Noble metal NPs have been shown to increase the photoenergy conversion efficiency of semiconductors by increasing the efficiency of charge carrier separation, and extending light absorption and facilitating creation of electron-hole pairs induced by the surface plasmon resonance (SPR) effect [69, 70].

TiO_2 is one of the most popular photocatalysts. In the presence of ultraviolet light, TiO_2 in anatase form is capable of decomposing organic compounds and microorganisms on its surface [71]. Due to this ability TiO_2 has high potential in many fields of application, such as medicine, cotton fabric, architecture, and water and air

purification [41, 68]. When light (usually UV light) is absorbed, the photo-excited nanoparticle stores energy by charge separation and creating electron-hole pairs. The fate of the electron-hole pairs determines the chemical and biological reactivity of the photo-excited nanoparticle [72]. Pure semiconductors usually exhibit low photoenergy conversion efficiency probably because of their relatively low charge separation efficiency and fast recombination of charge carriers [12].

When applied TiO_2 onto cotton fabrics, it can give cotton fabrics antibacterial activity, self-cleaning and water repellence properties [73, 74]. TiO_2 is also widely used in disinfection for its photocatalytic properties. Moreover, it was reported that the addition of noble metals (e.g. Ag) to TiO_2 may enhance the overall photocatalytic efficiency and the damage to bacterial cells [75, 76].

2.3.1.4 Zinc oxide nanoparticles (ZnO NPs)

ZnO nanoparticles are nontoxic, biocompatible and have been used in many fields, such as cosmetics, drug carriers and medical materials. Under visible light, ZnO nanoparticles is broad-spectrum [77].

To enhance photocatalytic and antibacterial activity of ZnO, some noble metal nanoparticles were decorated on ZnO NPs. The hybrid of ZnO/Au antibacterial activity toward gram positive and gram negative bacteria was significantly enhanced for the enhancement of ROS generation [43], their photocatalytic activity is also enhanced

which attributed to a higher efficiency of electron transport and charge carrier separation induced by Au NPs. The enhanced ROS generation, photocatalytic and antibacterial activity of ZnO/Au hybrid nanostructures showed a distinctive Au/ZnO ratio dependence [43].

Several studies stated that ZnO produce increased levels of reactive oxygen species (ROS), mostly hydroxyl radicals, H_2O_2 and singlet oxygen, which contribute to the antibacterial activity of ZnO nanoparticles [78]. An alternative hypothesis suggested that the binding of ZnO nanoparticles to the bacterial surface is due to electrostatic forces that directly kill bacteria, ZnO nanoparticles lead to an increase in cell death, probably due to disruption of the bacterial cell wall [77, 79].

2.3.2 Organic antibacterial agents

2.3.2.1 Quaternary ammonium salts (QASs)

QASs are broad-spectrum against gram-negative and gram-positive bacteria, fungi and some viruses [12, 80]**.** It can be used in many science and technology fields [81, 82]. The length of the alkyl chain and the number of cationic ammonium groups in the molecule can affect the antibacterial activity of QASs [83].

When QASs are used in textiles, they are attached in cotton fabrics via ionic interaction between cationic QASs and anionic cotton surface [84-86]. They are easier

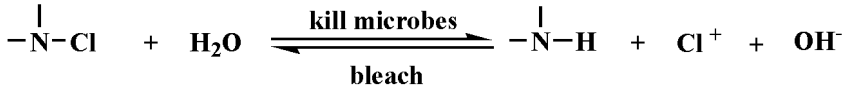
to leach from the textiles for no functional groups covalently bonding to the textiles. This in case results in the poor washing durability. Zhilong Shi and his colleagues functionalized quaternary ammonium groups to activated carbon (AC) to achieve antibacterial properties. The functionalized ACs show highly effective antibacterial activities against *E.coli* and *S.aureus*. Furthermore, the functionalized ACs can be used in repeated antibacterial applications with little loss in efficacy [87]. The commercial QAS product is known as AEM 5700 by Dow Corning. The MIC against *E.coli* and *S.aureus* is 10-100 mg/L. the main component of AEM 5700 is 3-trimethoxysilylpropyldimethyloctadecyl ammonium chloride.

The mechanism of QASs can be concluded in the following aspects. The cationic ammonium group of the QAS can attractively interact the negatively charged bacteria cell through electrostatic attraction [88]. This causes the interruption of cell membrane, affects the protein and DNA multiplication, leading to lethal death of bacteira [89]. With long hydrocarbon chain in the in the structure of the QAS, the QAS can interact the bacteria cell both through the cationic nitrogen of the ammonium group and the long hydrocarbon chain penetration, and interrupt the cell [83]. It also can bind to membranes in other cells and to other biological compounds with negative charges and hydrophobic structures and, if exposure time is sufficiently long, such binding could also cause damage to mammalian cells [90].

2.3.2.2 N-halamine

N-halamine compounds were widely applied onto cotton [91-93], nylon [94], polyester [95] and other synthetic fabrics [96, 97].

N-halamine structures mostly contain imide, amide, and amine halamine bonds possessing certain structural features. The cyclic halamines have demonstrated many advantages as biocidal materials applied on cotton fabrics, medical devices and water purification [4, 98-102], such as great stability in a wide range of temperatures and pH values, rapid inactivation of a broad spectrum of microorganisms against bacteria, yeast, viruses and fungi [102], and the unique rechargeable feature using chlorine bleach (scheme 2.1) [3, 103].



Scheme 2.1. Regenerable N-helamin

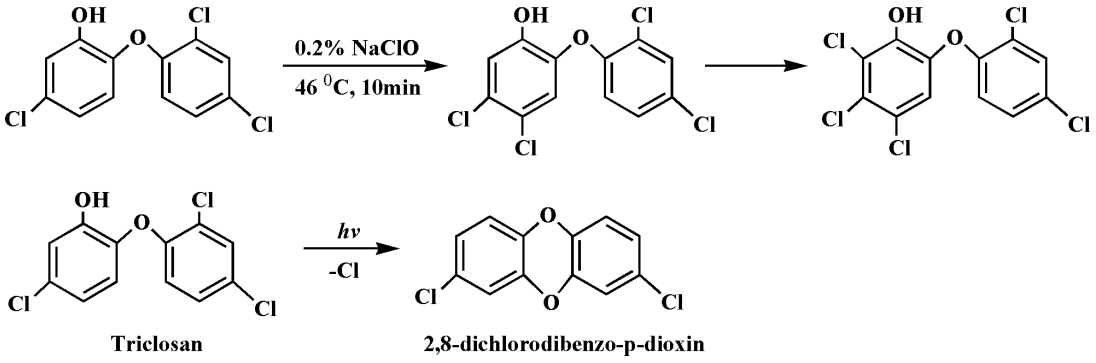
Liu et al [104] used polymeric N-halamine on cotton fabric. The modified cotton fabric showed excellent antibacterial activities against *E.coli* and *S.aureus*. The washing durability is strong. Also the modified cotton fabric showed no skin stimulation. It indicated that the modified cotton fabric can be used in healthcare applications.

The antibacterial activity is due to the oxidative ability of the N-Cl bond. However,

the remaining of chloride on the surface of cotton fabrics results in odor and discolors fabrics.

2.3.2.3 Triclosan

Triclosan ((2, 4, 4'-trichloro-2'-hydroxydiphenyl ether) is a broad-spectrum antimicrobial agent with a MIC of less than 10 ppm against many common bacterial species [105]. It inhibits microbial growth by blocking lipid bio-synthesis [6]. However, it can cause bacterial resistance. When triclosan is exposed to sunlight in the environment, it breaks down into 2,8-dichlorodibenzo-p-dioxin which is chemically related other toxic polychlorinated dioxins (scheme 2.2) [3].



Scheme 2.2. Breaking down of Triclosan

2.3.2.4 Polyhexamethylene biguanides (PHMB)

Polyhexamethylene biguanides (PHMB) (Fig 2.7), being a potent and broad spectrum bactericidal agent with low toxicity (MIC = 0.5–10 ppm, Arch technical

information) has been successfully used in the food industry, wound dressings [106], swimming pools [107] and in mouthwashes [7, 108] .

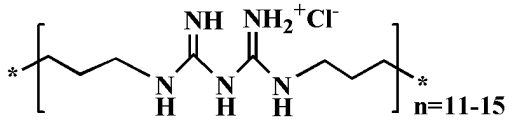


Fig 2.7. Structure of PHMB

Reputex 20, a commercially available polyhexamethylene biguanide antimicrobial agent for textile finishing, was applied to cotton textiles at various concentrations [109].

The release of this agent from the textile submerged in water was studied. The release of the commercial polyhexamethylene biguanide from a fabric at close to static conditions showed that within some minutes a concentration above the MIC of certain microorganisms such as *E. coli* is released into the liquor. A time survivor study was conducted with *E. coli* bacterium. The agent behaved as a bacteriostat at lower concentrations of application. At higher concentrations (1.6–2% o.w.f.), a biocidal or bactericidal action was noted where no viable bacteria were recovered from the textile [3].

2.3.3 Natural antibacterial agents

2.3.3.1 Chitosan

Chitosan derived from the shells of shrimps and other sea crustaceans. It is widely

used in several fields such as the food, medical, cosmetic, and textile industries.

Chitosan is a well-known biopolymer for its non-toxicity, biocompatibility and biodegradability, and possesses antibacterial activity against Gram-negative and Gram-positive bacteria [12, 13].

However, the antibacterial activity of the chitosan is pH-sensitive and limits to acidic conditions [12]. The chitosan also shows weak adhesion to cellulose fibres and is leached gradually from the fibre surface by repeated laundering [13].

Some studies focus on the composite of chitosan with metal nanocomposites, such as chitosan–silver nanocomposites. It is showed that the composite exhibits higher antibacterial activity than any component acting alone. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the chitosan–silver nanoparticles were found to be lower than those reported for other types of silver nanoparticles [110].

Chitosan is positive charged which could interact with anionic groups on the cell surface, disrupt the cell membrane resulting in increasing of permeability and the leakage of cellular. It can also interact with the DNA to affect the duplication [111]. Another mechanism stated that chitosan with trace elements or essential nutrients forms chelates, which can inhibit the activity of enzymes [112].

2.3.3.2 Capsaicin

Capsaicin is another natural antibacterial agent. When capsaicin was embedded in a silica matrix from a sol-gel process, the composite showed good antibacterial effect, and when it was coated on wool fabrics, the fabrics exhibited excellent antibacterial efficiency after laundry washes, capsaicin was still attached to the fibers after laundering [113].

2.3.3.3 Moxa oil

Moxa leaf is commonly used as a traditional medicine. It was used in cotton fabrics by microencapsulation method to be combined with gelatin-arabic gum microcapsules and gave the cotton fabric good antibacterial activity [114].

All the inorganic, organic and natural antibacterial agents have their advantages and disadvantages during their application. All the advantages and disadvantages are concluded in the following table.

Table 2.3. Advantages and disadvantages of antibacterial agents

	Advantages	Disadvantages
Metal particles	High and broad-spectrum, high temperature resistance	Poor durability, toxic and adverse influence on fabric color
Metal oxides	High and broad-spectrum, good thermo-stability	Need light, poor dispersion and stability
QACs	broad-spectrum, high antibacterial activity	easily leaching, weak tolerance to washing
N-halamine	broad-spectrum, good stability and regeneration	the residual chlorine can cause fiber damage and odors
PHMB	broad-spectrum, high safety approved by U.S. FDA and EPA	weak tolerance to light
Triclosan	broad-spectrum good durability and thermo-stability	producing toxic dioxins under light
Chitosan	broad-spectrum antibacterial activity; bio-compatible; environmentally friendly	high pH-sensitivity; poor water solubility; low antibacterial activity.

2.3.4 Non-fouling materials

Non-fouling materials are investigated and applied in a wide spectrum from engineering to industry [115-120]. The most widely used non-fouling materials are PEG [121] and zwitterionic materials [122]. Their non-fouling property results from the surface hydration. The hydration in PEG is formed through hydrogen bonding between water molecules and the ether oxygen atoms while in zwitterionic materials is formed through electrostatic-induced hydrogen bonding [123-125]. The differences of hydration between PEG and zwitterionic materials sulfobetaine was showed in Fig 2.8 [126].

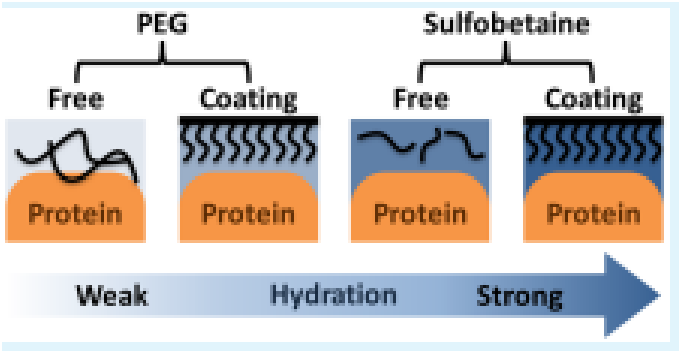


Fig 2.8. The hydration difference between PEG and sulfobetaine

Fig 2.8 showed that water was strongly bonded with the sulfobetaine molecules. The hydration is strong, whereas the hydration between proteins and free PEG solutions was weak. The strong hydration made sulfobetaine show more excellent non-fouling property than PEG. Though PEG and their derivatives are widely applied on non-fouling areas [127, 128] , the application is limited for its oxidative degradation [129, 130] .

2.3.4.1 Zwitterionic materials

Zwitterionic materials have both cationic and anionic groups in the moieties, but they are charge neutral. The unique structure makes that they can be used in many fields [131-135] . Carboxybetaine (CB) and sulfobetaine (SB) based zwitterionic materials are the most typical [136-139]. The differences between CB and SB moieties in hydration was studied in Fig 2.9 [140].

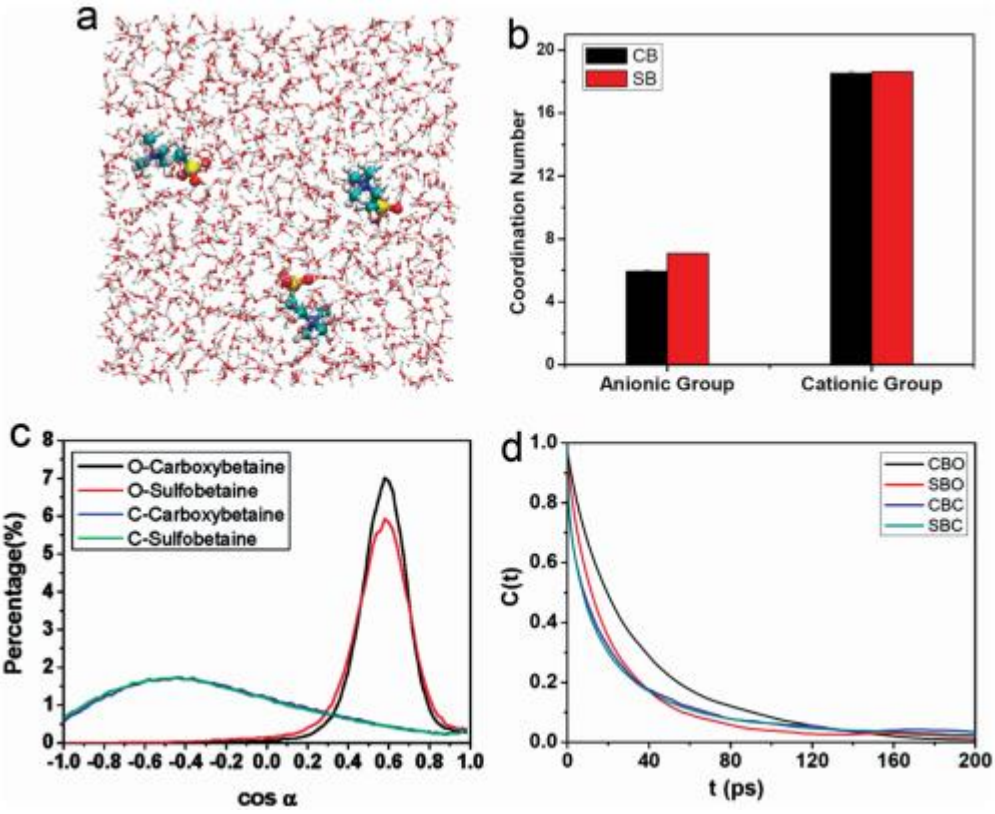


Fig 2.9. Differences between CB and SB moieties in hydration. a) The simulation configuration, b) number of water molecules in the hydration shell of the anionic and cationic groups of CB and SB moieties, c) dipole orientation distributions, and d) the residence curves of water molecules in the hydration shells of the anionic and cationic groups of CB and SB moieties. [140]

Simulation results showed that the major difference in hydration between the two zwitterionic moieties occurs around the anionic groups: SB moieties have more water molecules in the hydration shell, whereas CB moieties retain individual water molecules in the hydration shell longer.

Betaines represent a special family of zwitterionic materials that bear both cationic moiety like QACs and anionic functional groups like sulfo-, carboxy-, and phosphobetaines on the same repeat unit [21, 22]. For the antipolyrelectrolyte characteristics, electrolyte-response, bio/blood compatibility and antibiofouling properties, synthetic betaines have attracted a significant amount of interest. Previous studies mainly connect the betaine groups with the polymer backbone though ether [141], amide [142], imide [143], or other hydrolysable chemical bonds. These structures lead to the removal of the betaine groups in water by hydrolysis. In addition, previously developed betaines have no reactive groups available to form strong bonds with the substrate. These disadvantages lead to the leaching from the substrate and the decrease of antibacterial activity during the utilization process.

Chen et al [144-146] synthesized a kind of antibacterial agent siloxane sulfopropylbetaine (SSPB), which contains reactive alkoxysilane groups and has good antibacterial activity. The textile finished with the agent showed good antibacterial activity, SSPB is nonleachable, and it does not induce skin stimulation and is nontoxic to animals. What's more, the mechanical properties of the textiles finished with the SSPB, such as the breaking strength are greatly improved.

About the mechanisms of zwitterionic materials, it showed bacteriostasis, that means the reduction of bacteria adhesion [123]. Zwitterionic materials containing both positive and negative charged units and possessing the maximum polarity can bind

water molecules even more strongly and stably via electro statically induced hydration. The reduction of bacteria adhesion is due to their intrinsically strong hydration via electrostatic interactions [1].

Though the SSPB showed high antibacterial activity, it is not the best. Due to the antibacterial mechanisms, the antibacterial activity can be improved via increasing the length of alkyl chain attached to -N, which will improve the hydrophobicity of the agents; in this case, the agents will show their better antibacterial activity by breaking bacteria's survival environment to reduce the adhesion of the bacteria and inhibit bacteria's growth [147].

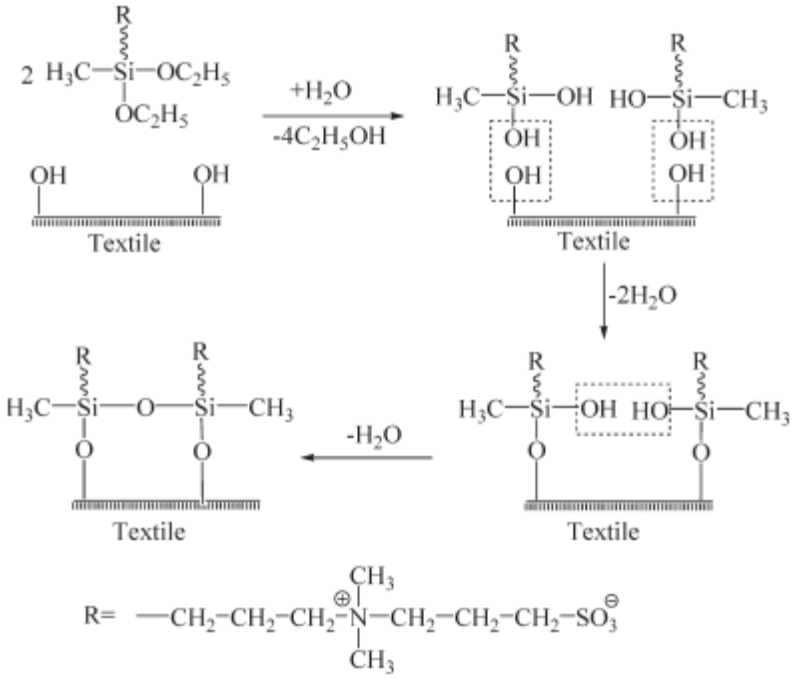


Fig 2.10. Reactive principle of cotton textile finished with SSPB

2.3.5 Pyridine

Pyridine has a similar chemical property to tertiary amines, the derivatives of pyridine have been well studied and used in many fields, such as gene delivery [23], anticancer drug [24], antibacterial agents [25] and so on for their good properties on non-toxicity due to the delocalization of positive charge into the pyridine ring [23].

2.3.6 Cyanuric chloride

Cyanuric chloride (CC) and its derivatives have been known for a long period for their widespread application in textiles, reactive dyes and surface active agents. CC have three chlorine atoms and they are reactive toward nucleophiles. The chlorine atoms in cyanuric chloride can be substituted by various nucleophiles under different temperature in a stepwise manner which makes its ease of the preparation of mono-, di- and tri- substituted 1, 3, 5-triazines [26]. Generally, its mono-substitution of chlorine occurs below or at 0 ^{o}C， di-substitution at room temperature and tri-substitution above 60 ^{o}C.

2.3.7 1, 2, 3, 4-butane tetracarboxylic acid (BTCA)

BTCA is one of the most efficient and widely used cross-linker with sodium hypophosphite (SHP) as catalyst in cotton fabric and imparts cotton fabric multi-functional properties. When cotton fabrics were finished with TiO_2 and BTCA via pad-

dry-cure method, it can give cotton fabric UV-protecting, self-cleaning, water-repellence, flame retardant and antibacterial properties [74, 148, 149]. Chitosan was attached onto cotton fabrics using BTCA to give cotton fabrics antibacterial activities against *E.coli* and *S.aureus* and washing durability [31]. Also, the crease recovery and breaking strength of treated cotton fabric changed. BTCA was used in color cotton to link dyes and cotton to overcome the wrinkling problem [150].

Chapter 3

Methodology

3.1 Introduction

The research methodology detail listed in this chapter includes four parts: experimental plan, materials section, experiment procedures, characterization and evaluation techniques. The characterization methods include nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). The antibacterial activity of antibacterial agents and the modified cotton fabric was evaluated via bacteria growth kinetics. The air permeability, water absorption, tearing strength, non-fouling property were evaluated and measured according to the related standard respectively. Also the cytotoxicity of the modified cotton fabric was investigated using MTT method, SEM and fluorescence microscope.

3.2 Experimental plan

This study aims to investigate the properties of cotton fabric modified with reactive antibacterial agents that include pyridine sulfobetaine, s-triazine sulfobetaine and BTCA-amino sulfobetaine. The research scheme is shown in the following figure 3.1.

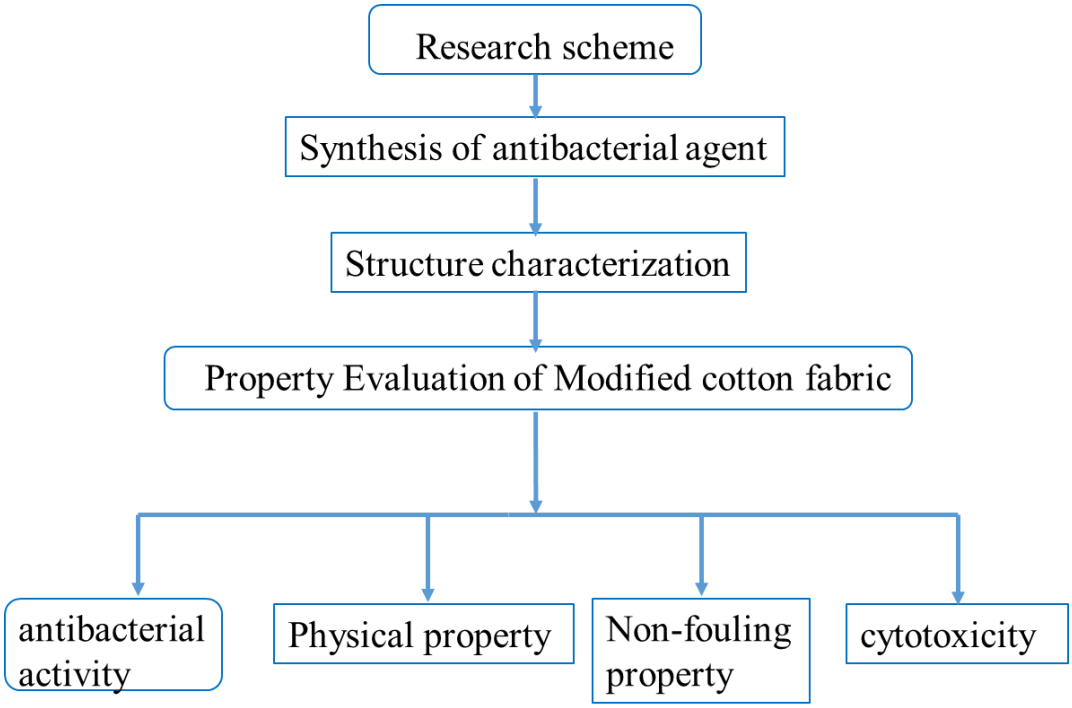


Fig 3.1. Research scheme of the whole thesis

In this study, sulfobetaine was chosen as the antibacterial agent to give cotton fabric antibacterial activity and non-fouling property. Antibacterial property is one of the necessary and important features when it was used in cotton fabric. After modification, the physical properties were evaluated compared with pristine cotton fabric. Betaine can resist bacteria adhesion and growth on the surface of cotton fabric and give cotton fabric anti-biofouling property. It is an important influence factor to avoid cotton fabric contamination. The bio compatible properties such as skin stimulation and cytotoxicity are the most important factors when it is used in medical areas. They were systematically evaluated according to the related standard respectively.

The research scheme was made according to the above descriptions. The following section will introduce the experimental materials, procedures, characterization and property investigation.

3.3 Materials

3.3.1 Materials for synthesis of pyridine sulfobetaine

Isocyanate group can easily react with hydroxyl in the catalyst of dibutyltin dilaurate (DBTDL). The tertiary amine in pyridine can react with 1, 3 - propane sultone (1, 3-PS) at room temperature to form quaternary amine cationic moiety and sulfo anionic group. With the two steps reaction, pyridine sulfobetaine was synthesized after purification in diethyl ether and dried at vacuum. It is water soluble. The structure was characterized with NMR in Deuterium oxide (D_2O) solvent.

4-(Hydroxymethyl) pyridine, 3-(Triethoxysilyl) propyl Isocyanate, dibutyltin dilaurate (DBTDL)，1, 3 - propane sultone (1, 3-PS), superdry tetrahydrofuran (THF), Deuterium oxide (D_2O), diethyl ether, were purchased from J&K Scientific Ltd., Hong Kong and used without further purification.

3.3.2 Materials for synthesis of s-triazine sulfobetaine

The three chlorine atoms in cyanuric chloride can be substituted by various nucleophiles, such as amino and hydroxyl group. In this case, cyanuric chloride can act

as cross linker to link amino sulfobetaine and cotton fabric to give cotton fabric durable antibacterial activity. When one of the chlorine atoms was substituted with amino group, the other two chlorine atoms can react with hydroxyl groups in cotton fabric. Firstly, amino sulfobetaine was synthesized. Then the sulfobetaine was linked to cyanuric chloride via amino group at 0 ^{o}C or below.

N, N-diethylethylenediamine, dichloromethane, di-tert-butyl carbonate (Boc_2O), anhydrous Na_2SO_4, 1, 3-propane sultone, Cyanuric chloride (CC), were purchased from *J&K Scientific Ltd*., Hong Kong and used without further purification.

3.3.3 Materials for BTCA-amino sulfobetaine finishing procedure

1, 2, 3, 4-butane tetracarboxylic acid (BTCA) is nontoxic and used as the most efficient crosslinking agent to fix antibacterial agent on cotton fabric. In BTCA structure, there are four carboxyl groups, which can react with amino group and hydroxyl group in the catalyst of sodium hypophosphite. The amino sulfobetaine can be linked to cotton fabric via chemical bonding to give durable antibacterial activity after laundering.

1, 2, 3, 4-butane tetracarboxylic acid (BTCA) and sodium hypophosphite were purchased from *J&K Scientific Ltd*., Hong Kong and used without further purification.

3.3.4 Bacteria and materials for antibacterial test

In this study, two common bacteria gram-negative *Escherichia Coli* (*E.coli* 8099) and gram-positive *Staphylococcus aureus* (*S.aureus* ATCC 6538) were selected to evaluate the antibacterial activity of pure antibacterial agents and modified cotton fabric.

E.coli 8099, *S.aureus* ATCC 6538, Tween-80, nutrient broth, nutrient agar, were purchased from Guangdong Huankai microbial technology co., Ltd. NaCl was purchased from *J&K Scientific Ltd.*, Hong Kong .

3.3.5 Materials for laundering

According to AATCC 61-2A standard, steel ball and 1993 AATCC standard reference detergent were used in the launderingd procedure.

3.3.6 Materials for non-fouling test

Phosphate-buffered saline (PBS) solution (pH=7.2), Glutaraldehyde solution (3%), and ethyl alcohol were purchased from *J&K Scientific Ltd.*, Hong Kong and used without further purification.

3.3.7 Materials for skin stimulation and cytotoxicity test

Skin stimulation and cell cytotoxicity are the most important factors to evaluate whether the materials are biocompatible and safe or not when they were applied to

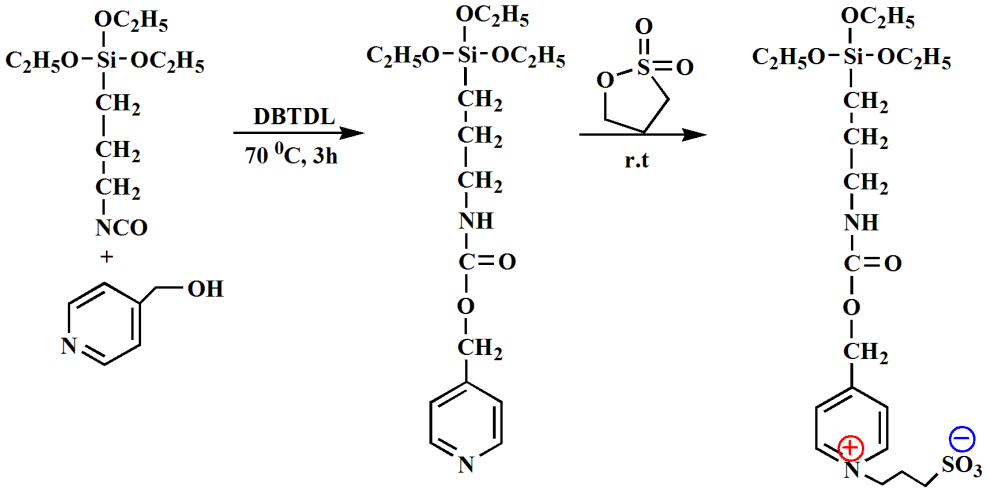
medical materials.

In this study, mature rabbits and mouse fibroblast (L929) are selected to evaluate the skin stimulation and cytotoxicity of modified cotton fabric. Fetal calf serum (FBS), Dulbecco's modified eagle medium (DMEM), Penicillin-Streptomycin, methyl thiazolyl tetrazolium (MTT), Hyclone, phosphate buffered saline (PBS), dimethylsulfoxide (DMSO), Bovine Serum Albumin (BSA), Triton, 4 % paraformaldehyde, Fluorescein isothiocyanate (FITC)-phalloidin, 4', 6-diamidino-2-phenylindole (DAPI), 6 well plate, 24 well plate and 96 well plate were purchased from Shenzhen Saidejie *Scientific Ltd*., China.

3.4 Experiment procedures

3.4.1 Synthesis of Para-Pyridine sulfobetaine

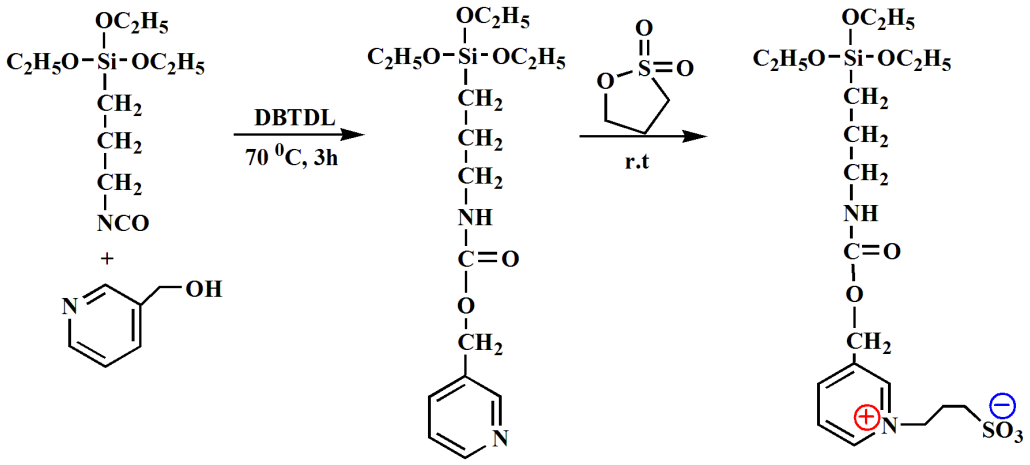
The Synthetic routine of para-pyridine betaine is presented in scheme 3.1 [151]. 4-(Hydroxymethyl) pyridine (11mmol) and 3-(Triethoxysilyl) propyl Isocyanate (10 mmol) were dissolved in 10 ml THF separately. The solution was added to a flask and then 0.1 ml DBTDL was added. The mixture was stirred at 70 °C under N_2 for 3 h with a reflux condenser. 10 mmol 1, 3-PS (dissolved in 10 ml THF) was added to the mixture at room temperature for 24 h (Scheme 3). The para-pyridine betaine precipitate was obtained after being washed with diethyl ether for several times and dried under vacuum.



Scheme 3.1. Synthetic routine of para-pyridine betaine

3.4.2 Synthesis of Meta-pyridine sulfobetaine

The Synthetic routine of meta-pyridine betaine is presented in scheme 3.2. 3-(Hydroxymethyl) pyridine (11mmol) and 3-(Triethoxysilyl) propyl Isocyanate (10 mmol) were dissolved in 10 ml THF separately, the solution was added to a flask and then 0.1 ml DBTDL was added, the mixture was stirred at 70 °C under N_2 for 3 h with a reflux condenser. 10 mmol 1, 3-PS (dissolved in 10 ml THF) was added to the mixture at room temperature for 24 h (Scheme 4). The meta-pyridine sulfobetaine precipitate was obtained after being washed with diethyl ether for several times and dried under vacuum.



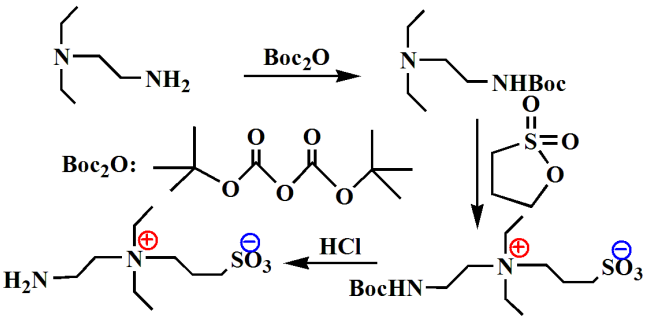
Scheme 3.2. Synthetic routine of meta-pyridine betaine

3.4.3 Synthesis of s-triazine-sulfobetaine

The protection and deprotection of amino is presented in scheme 3.3 [152]. To a solution of N, N-diethylethylenediamine in dried dichloromethane, was added dropwise to the solution of di-tert-butyl carbonate (Boc_2O) in dichloromethane slowly at 4 °C under rigorous stirring. The reaction was kept overnight at room temperature. The reaction mixture was washed with brine three times. The combined organic phase was dried by anhydrous Na_2SO_4. Removal of the solvent gives the product tert-butyl 2-(diethylamino) ethyl carbamate as a colorless oil.

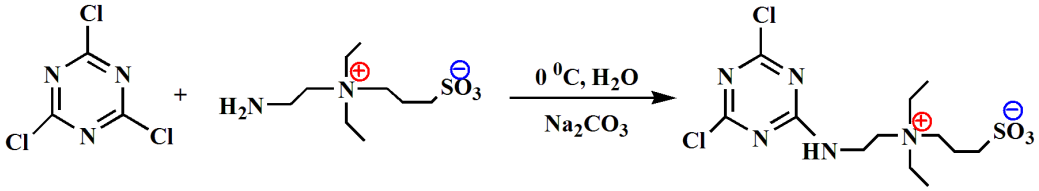
The solution of tert-butyl 2-(diethylamino) ethyl carbamate and 1, 3-propane sultone in anhydrous chloroform was heated to 60 °C under stirring overnight. The precipitate was collected and washed three times with chloroform to produce Boc-protected 2-amino-ethyl-sulfobetaine as a white solid.

Deprotection of Boc-protected 2-amino-ethyl-sulfobetaine was performed in HCl aqueous solution to give 2-amino-ethyl-sulfobetaine (Scheme 3.3).



Scheme 3.3. Amino protection and deprotection

The synthetic routine of amine mono-substitution *s*-triazine sulfobetaine (A-m-triazine-SB) is presented in scheme 3.4. Cyanuric chloride (CC) was suspended in deionized water and stirred at 0 °C for 10 minutes in three-necked round bottom flask, then the solution of 2-amino-ethyl-SB was added to the flask, the reaction was carried at 0 °C for 3 hours producing a clear solution. The pH of the solution was maintained at 3-4 using 20% of sodium carbonate solution (Scheme 3.4). The product precipitate was obtained by salt out through adding sodium sulfate.



Scheme 3.4. Synthetic routine of s-triazine-sulfobetaine

3.4.4 Cotton fabric finishing

3.4.4.1 Cotton fabric finished with pyridine sulfobetaine

Antibacterial textiles are usually produced by adding antibacterial agents to textiles through a padding process**.** The advantages of such application are high productivity and relatively low processing cost [153]. The cotton fabrics were padded twice through a treating solution containing the as-synthesized pyridine sulfobetaine antibacterial agent using a padding machine. Concentration of the solution is 8g/L. After being padded with 90% pick-up, the samples were immediately dried at 90 °C for 1.5 min, and then cured at 140 °C for 2 min. Then the modified fabrics were washed and dried in 90 ºC to remove the unreacted chemicals.

The performance of antibacterial functions depends on the amount of the agents on the fibers. This aims to study how the antibacterial agents' concentration affect the antibacterial activity. In this experiment, the cotton textiles were finished at different concentrations at the same padding pressure to find the minimum concentration that can make good antibacterial activity (Table 3.1).

Table 3.1. Finishing conditions in different concentrations

sample	Agent concentration (g/L)	Padding Pressure (kg/cm^2)	Pick up (%)	Drying	Curing
1	4	2	90.78		
2	6	2	90.53		
3	8	2	94.43	90 ºC	140 ºC
4	10	2	96.26	1.5min	2min
5	12	2	95.89		

3.4.4.2 Cotton fabric finished with BTCA-amino sulfobetaine

The fabrics were finished with 5% and 8% betaine and BTCA in the catalyst of sodium hypophosphite. Both the amino sulfobetaine and BTCA were dissolved in deionized water. The cotton fabrics were finished with pad-dry-cure process. After immersing the cotton fabrics were padded through a padder to control a 70% wet pick-up. The fabrics were dried at 80 ºC and cured at 160 ºC for 3 minutes respectively. Then the modified fabrics were washed with distilled water to remove the unreacted chemicals and dried in 90 ºC.

3.4.4.3 Laundering process (Standard: AATCC 61 - 2A)

However, many textiles finished with the above method exhibit low durability

against repeated laundering. The performance and washing durability of antibacterial functions depends on the amount of the agents on the fibers. In this experiment, to evaluate the durability of antibacterial, the samples had been washed 10, 20 and 30 times.

Sample preparation: the size of the samples required is 50 × 150 mm.

Test conditions: 49 °C, total liquor volume 150 ml, percent powder detergent of total volume 0.15, No. steel balls 50 and time 45 min.

Adjust laundering machine to maintain 49 °C. Prepare 150 ml wash liquor in 90 × 200 mm lever lock stainless steel canisters and add 0.225 g 1993 AATCC Standard Reference detergent. Then add 50 steel balls to the canister and clamp the covers. Fasten the canister on the rotor of the laundering machine and run it to preheat the canisters. After 2 min, stop the rotor and enter a sample into the solution. Run the laundering machine at 40 ± 2 rpm for 45 min for 5 times.

3.5 Characterization and property evaluation test

3.5.1 NMR

NMR is used to analyze and determine the structure of the synthesized antibacterial agents. ^{1}H NMR spectra were obtained using a Varian Unity Inova 500 MHz NMR Spectrometer at room temperature using D_2O as the solvent.

3.5.2 ESI-MS

ESI-MS is used to further analyze and determine the molecular of the obtained antibacterial agent. Pyridine sulfobetaine was tested using methanol as the solution at room temperature.

3.5.3 Thermo-gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) is used to evaluate the thermostability of antibacterial agent and determine the temperature when it is used in cotton fabric.

TGA was conducted with a thermos-gravimetric analyzer (TGA, METTLER-TOLEDO) under N_2 atmosphere with a flow capacity of 50 mL/min and a heating rate of 10 °C/min from 25 to 800 °C.

3.5.4 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) with energy dispersive X-ray detector (EDX) (JEOL JSM-6490) was used to observe the surface morphology and detect the composition of raw and modified cotton fabric. SEM was also used to observe the bacteria adhesion on a raw and modified cotton fabric surface to evaluate non-fouling mechanism. Before the SEM observation, a thin gold film was sputtered onto the samples surface.

3.5.5 Air permeability

The air permeability of raw cotton fabrics and modified cotton fabrics were tested according to the ASTM D737-04 (2012) standard method in a standard atmosphere, which is 21 ± 1 °C with 65 ± 2 % of relative humidity. The specimen is clamped over the test head opening. Start the vacuum pump to make it at 100 Pa. The test area was 5.08 cm^2. The results were recorded by averaging the values of five samples in SI units as mm/s [15, 154].

3.5.6 Water absorption

The level of water absorption is the percentage increase of the sample weight. Water absorption of cotton fabric was detected by following the ASTM D4772-09 standard method. First, the fabric samples at a weight of 1 g were dried at 45 °C for 24 h, then weighted and soaked in 100 ml distilled water for 1h at room temperature. After that, the samples were removed and hung for 30 s and weighted again. The result is a mean value of five samples [14].

3.5.7 Tearing strength

Determination of tear force using ballistic pendulum method (Elmendorf) (ISO 13937-1:2000/Cor.1:2004). This method is for the determination of tear force of textile fabrics. The measurement of the tear force is required to propagate a single-rip tear of

defined length from a cut in a fabric when a sudden force is applied.

Select the mass of the pendulum so that the measurements taken from the test specimens give results between 30% and 70% of the full scale rang of the corresponding measuring scale. Check that the apparatus is set at zero. Move the pendulum to the raised position.

Mounting of test specimens. Position the specimen in the jaws so that the long side of the test specimen is parallel to the upper edge of the jaws. Clamp the test specimen centrally and with the bottom edges of the test specimen carefully set against the bottom stop of the jaws. Using the knife, cut a slit of 20mm ± 0.5mm in the side opposite the notch, leaving a tear length of 43mm ± 0.5mm.

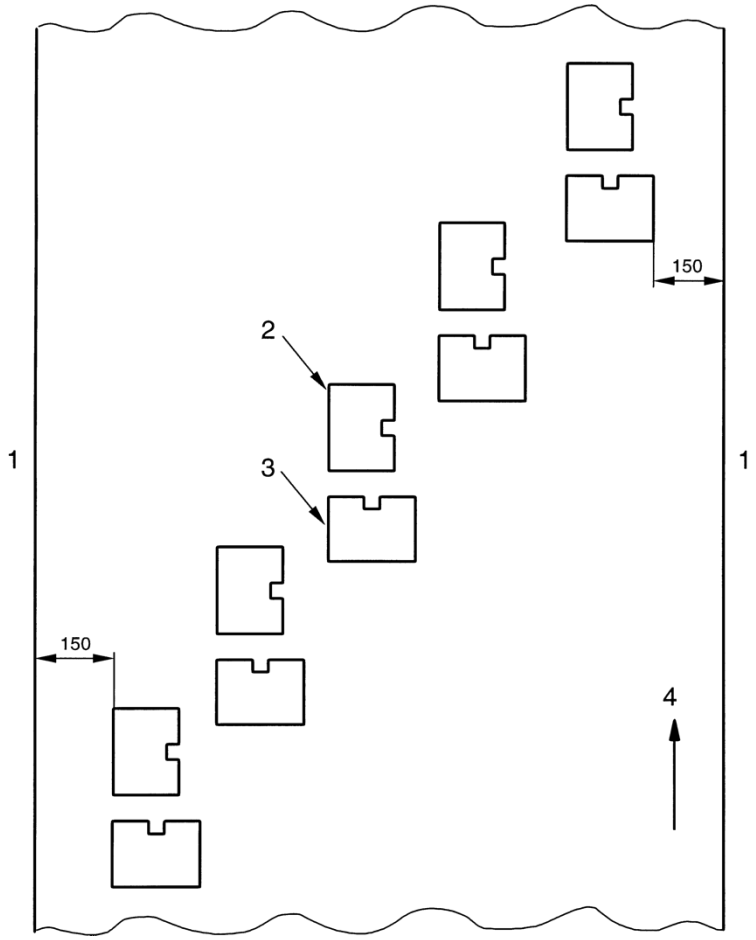


Fig 3.2. Example of pattern for cutting out test specimens from the laboratory sample. 1. Edge; 2. Specimen for tear “across warp”; 3. Specimen for tear “across weft”; 4. warp

3.5.8 Inhibition zone method

The inhibition zone was measured according to FZ/T 73023-2006 standard (China’s Textile Industry Standard). Using this method can investigate whether the cotton fabric is antibacterial or not qualitatively. The samples were prepared at a size of 1.5 cm ×1.5 cm and sterilized at 103 kPa 121 °C for 15 minutes to kill the bacteria on

the surface. The initial bacteria concentration is 1×10^6 cfu/ml ~5×10^6 cfu/ml. Nutrient agar was poured into plate until it became solid. The bacteria were inoculated onto the agar plate, and then the samples were put onto the agar surface. The plates were inverted and incubated at 37 ℃ for 24 h. The inhibition zone was measured. Each experiment was carried out in triplicate and the mean results were reported for analysis.

3.5.9 Antibacterial kinetics of pyridine sulfobetaine

In this experiment, the minimal inhibition concentration (MIC: the lowest concentration of the antibacterial agents that can completely inhibit the growth of bacteria) of pyridine sulfobetaine was quantitatively determined. To evaluate the antibacterial activities against gram-negative *E.coli* and gram-positive *S.aureus*, the effect of pyridine sulfobetaine antibacterial agent on the bacterial growth kinetics in liquid media was quantitatively studied. Bacterial growth rate was determined by measuring the optical density at 600 nm (OD_{600}) using a UV-vis spectrophotometer (UV-vis) based on the turbidity of cell suspension [155, 156]. The lower the OD_{600} is, the better antibacterial activities are carried out. For this experiment, bacteria grew to an approximate OD_{600} of 0.1, and then they were mixed with pyridine sulfobetaine antibacterial agent of various concentrations. The OD_{600} value was measured every hour until 24th hours.

3.5.10 Antibacterial property evaluation of modified cotton fabrics

The antibacterial performance of cotton fabrics finished with pyridine sulfobetaine against *E.coli* (Gram-negative) and *S.aureus* (Gram-positive) before and after washing was quantitatively evaluated by the viable colony counting method according to FZ/T 73023-2006 standard (China's textile industry standard). For these experiments, the initial bacteria concentration was approximately 0.7×10^5 CFU/ml ~ 1.5×10^5 CFU/ml (CFU: colony forming units).

The samples of both control and modified cotton fabrics were sterilized at 103 kPa for 15 minutes to kill the bacteria on the surface. Then a 0.2 ml bacteria solution was added onto the samples. At a temperature of 37 ± 1 °C, the samples were incubated for 24 h. Afterwards, the samples were washed with 0.87% NaCl solution containing Tween 80 and then 0.05ml of the washing solution was added onto different dishes containing nutrient agar. After 24 h of incubation under a temperature of 37 ± 1 °C, bacterial colonies on the agar were counted and the antibacterial rate was calculated using the equation below:

$$R\ (\%) = \frac{A-B}{A}\times100$$

Where A is the number of bacterial colonies after 24 h contact on the raw cotton fabrics (control), and B is the number of bacterial colonies after 24 h contact on the modified

cotton fabrics finished with pyridine sulfobetaine. Each experiment was carried out in triplicate and the mean results were reported for analysis.

3.5.11 Non-fouling property

Bacteria on cotton fabric grow to form biofilms and cause contamination, which significantly affects the color of fabric and bring about odor. Here, the non-fouling property of modified cotton fabric was evaluated by observing biofilm formation using SEM. Briefly, both the raw and modified cotton fabrics were incubated in bacteria suspension for 24 h and then they were taken out. Afterwards, the cotton fabrics were rinsed twice with a phosphate-buffered saline (PBS) solution (pH=7.2) to remove the nutrient broth. Glutaraldehyde solution (3%) was used to fix the bacteria onto fabric surface. Finally, the cotton fabrics were dehydrated in a series of ethanol solutions (30, 50, 70, 80, 85, 90, 95,100 % v/v) and dried in vacuum [154].

3.5.12 Skin stimulation

Skin stimulation of modified cotton fabric was evaluated according to Chinese standard GB 15979-2002 on four healthy adult New Zealand rabbits (two male and two female) at a weight of 2.2-2.3 kg. Cotton fabric samples were prepared at a size of 2.5 cm × 2.5 cm and wetted in saline. Rabbit hair with an area of 3 cm × 3 cm at two sides of spine were cut 24 hours before the experiment. Fabric samples were pasted on the left side and patched as a control on the right side. After 4 hours, all the test substances

were cleared. The skin reaction (erythema and edema) was observed and recorded after 1hour, 24hours and 48hours.

3.5.13 Test for vitro cytotoxicity

The cytotoxicity of modified cotton fabric is evaluated using MTT method, SEM and fluorescence microscope observation according to ISO 10993-5: 1999 standard [157-162].

Cell recovery: L929 cells were taken out from liquid nitrogen container and put in 37 ℃ water bath until melt. The cell suspension were transferred to culture bottle with 6-8 ml DMEM culture medium (10% FBS, 90% DMEM and 2% Penicillin-Streptomycin) and cultivated in incubator for 24 h at 37 ℃ with 5% of CO_2. Cell inoculation: using trypsin to digest the cell and make it to suspension. Then add 200 μl cell suspension to 24 well plate with 200 μl DMEM culture to make the cell concentration 2×10^4. After blending, put the plate in incubator for 24 h at 37 ℃ with 5% of CO_2.

3.5.13.1 MTT

Succinodehydrogenase in living cell mitochondria can restore MTT to insoluble blue violet crystal formazan and deposit in cell. DMSO can solve formazan in living cells. The amount of formazan is in direct proportion to cell number. To measure the

optical density at 492nm (OD492) using ELIASA can reflect the number of living cells.

The cell in 24 well plate was incubated for 1 day, 3 days, 5 days and 7 days. Discard the culture medium and wash the cell with PBS for 1 time. Add 360 µl culture medium and 40 µl MTT (5mg/ml) to the well and cultivate in incubator for 4 h at 37 °C with 5% of CO_2. Then discard the medium and add 400 µl DMSO in each well. The plate was kept in dark place for 30 min at room temperature. After that, 100 µl the above mentioned DMSO solution was added to 96 well plate and measured OD492 value using ELIASA. Each experiment was repeated for 3 times [157, 163, 164] .

3.5.13.2 SEM

The above mentioned inoculated cell was cultivated for 5 days. Then discard the medium and wash the cell for 3 times using PBS. Fix the cell using 4% paraformaldehyde overnight. After that the paraformaldehyde was discarded and washed the cell 2 times with PBS. The cell was dehydrated with 30%, 50%, 70%, 80%, 90%, and 95% ethyl alcohol for 10 min. Finally the cell was dried in vacuum and observed under SEM [165].

3.5.13.3 Fluorescence microscope

The above mentioned inoculated cell was cultivated for 3 days. Then discard the medium and wash the cell for 3 times using PBS. Fix the cell using 4%

paraformaldehyde for 2 h. After that the paraformaldehyde was discarded and washed the cell 3 times with PBS. Deal the cell with 0.1 % Triton solution for 3 min. Then discard Triton solution and washed the cell 3 times with PBS. Add 2% (wt/v) Bovine Serum Albumin (BSA) for 20 min. Finally using phalloidine to dye for 30 min and DAPI for 5 min and observe the cell under fluorescence microscope [165].

Chapter 4

Pyridine sulfobetaine

4.1 Introduction

In this chapter, we synthesized a kind of zwitterionic chemical-pyridine sulfobetaine which is nontoxic and provides non-fouling property to the treated surface. Both the para-pyridine sulfobetaine and meta-pyridine sulfobetaine were synthesized. The meta-pyridine sulfobetaine is just DMSO soluble, so it can not be used in cotton fabric finishing. All the following test was carried on para-pyridine sulfobetaine. When applied it to medical cotton fabrics, the problem of biofilm breeding can be prevented. The cotton fabrics were padded through a pyridine sulfobetaine solution with a concentration of 8.0 g/l by pad-dry-cure process at the temperature of 90 ℃ and 140 ℃ for dry and cure respectively, and the wet pickup was 90 wt. %. Thus the add-on of pyridine sulfobetaine is 7.2mg/g fabric. With a pad-dry-cure finishing, the antibacterial rate of the modified cotton fabric could reach 99.90% or higher against both gram-negative *E.coli* and gram-positive *S.aureus*. In addition, compared to the raw cotton fabric, the modified cotton fabric exhibited slightly reduced air permeability and significantly improved hydrophilicity, which could keep wound moist and facilitate wound healing. The as-modified cotton fabric is more skin compatible confirmed by skin stimulation test. Via MTT assay, SEM and fluorescence microscope observation, the modified cotton fabric showed non-cytotoxicity.

Pyridine has a similar chemical property to tertiary amines. It is easily attacked by alkylating agents [166]. The derivatives of pyridine have been well studied and used in

many fields, such as gene delivery [23, 167], anticancer drug [24, 168], antibacterial agents [25, 169] for their good properties on non-toxicity [170] due to the delocalization of positive charge into the pyridine ring[171, 172]. In this chapter, siloxane Isocyanate was chosen for the reactive property of siloxane groups with hydroxy in cotton fabrics. 4-(Hydroxymethyl) pyridine as medium can both react with Isocyanate and 1, 3 - propane sultone to introduce siloxane group and betaine to the structure.

4.2 Results and discussion

4.2.1 Structure characterization

4.2.1.1 Para-pyridine sulfobetaine

The para-pyridine sulfobetaine was synthesized according to scheme 3.1 described in chapter 3.4.1. After purification with diethyl ether for several times and dried under vacuum, white powder was obtained (showed in Fig 4.1). The yield is about 90%.

Fig 4.1 para-pyridine sulfobetaine powder

The structure was characterized by ^{1}H NMR using D_2O as the solvent. The result was showed in Fig 4.2.

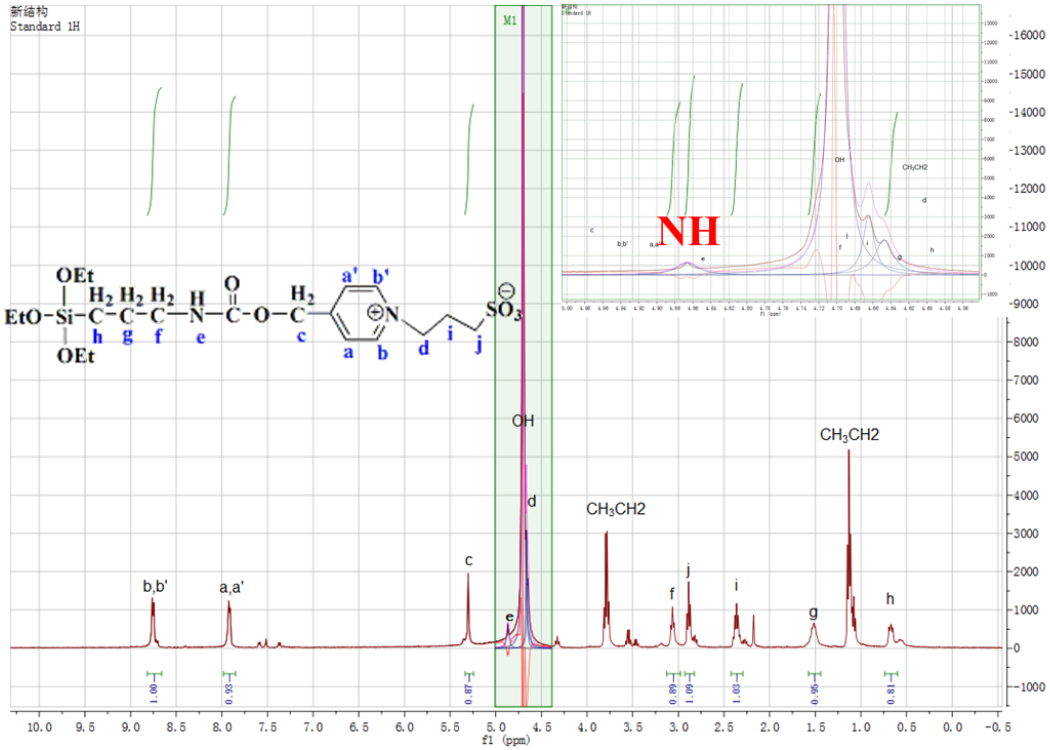


Figure 4.2. ^{1}H NMR spectrum of para-pyridine sulfobetaine

Figure 4.2 presents ^{1}H NMR spectra of siloxane 4-pyridine betaine. ^{1}H NMR (500 MHz, D_2O):δ (ppm): 0.67(h, 2H), 1.52(g, 2H), 2.36(i, 2H), 2.86(j, 2H), 3.06(f, 2H), 4.67(d, 2H), 4.87(e, 1H), 5.30(c, 2H), 7.92(a, a', 2H), 8.75(b, b', 2H). The chemical shift of δ= 4.87 is the active hydrogen of NH, which is the important hydrogen attribution indicated the successful synthesis of para-pyridine sulfobetaine. It indicates that the para-pyridine sulfobetaine was successfully synthesized.

4.2.1.2 Meta-pyridine sulfobetaine

The meta-pyridine sulfobetaine was synthesized according to scheme 3.2 described in chapter 3.4.2. After purification with diethyl ether for several times and

dried under vacuum, white powder was obtained. The structure was characterized by ^{1}H NMR using DMSO as the solvent. The result was showed in Fig 4.3.

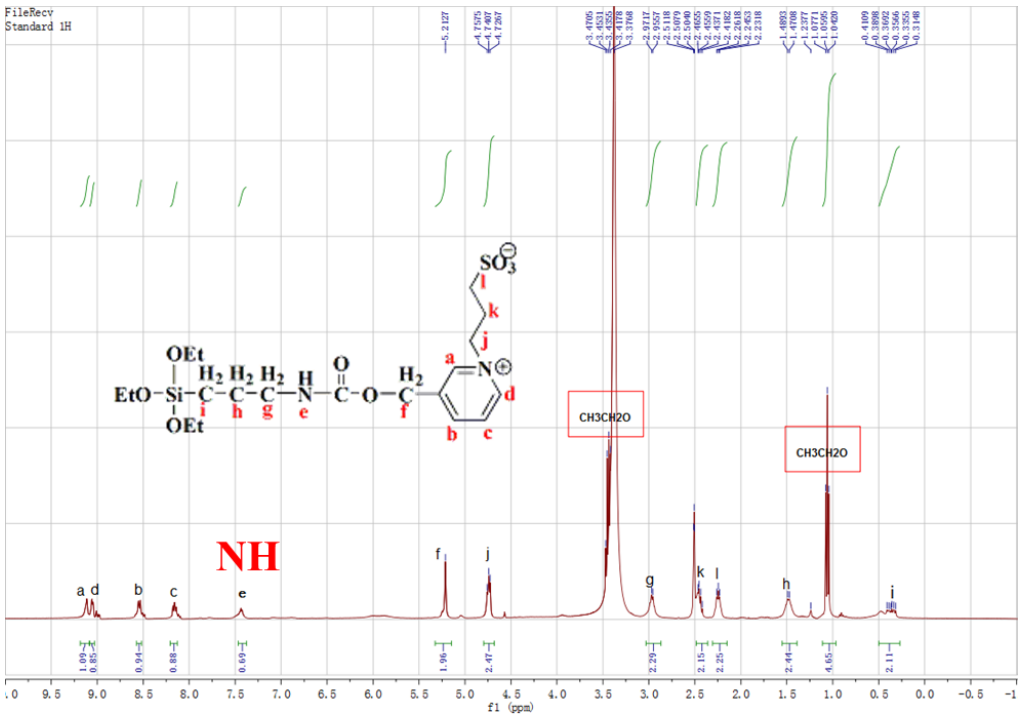


Figure 4.3. ^{1}H NMR spectra of meta-pyridine sulfobetaine

Figure 4.3 presents ^{1}H NMR spectra of meta-pyridine sulfobetaine. ^{1}H NMR (500 MHz, DMSO): δ (ppm): 0.36(i, 2H), 1.48(h, 2H), 2.25(l, 2H), 2.44(k, 2H), 2.96(g, 2H), 4.74(j, 2H), 5.21(f, 2H), 7.43(e, 1H), 8.16(c, 1H), 8.53(b, 1H), 9.05(d, 1H), 9.11(a, 1H). It indicates that the para-pyridine sulfobetaine was successfully synthesized.

Meta-pyridine betaine was tested in MeOH at room temperature using ESI-MS. The spectrum (Fig 4.4) shows prominent cluster peaks at *m/z* 479.1875 attributed to {pyridine· H^{+}} (Calcd *m/z* 479.1878) (Fig 4.5) and at *m/z* 501.1697 attributed to

{pyridine· Na^+} (Calcd *m/z* 501.1697) (Fig 4.6). The observed peaks well match their calculated ones.

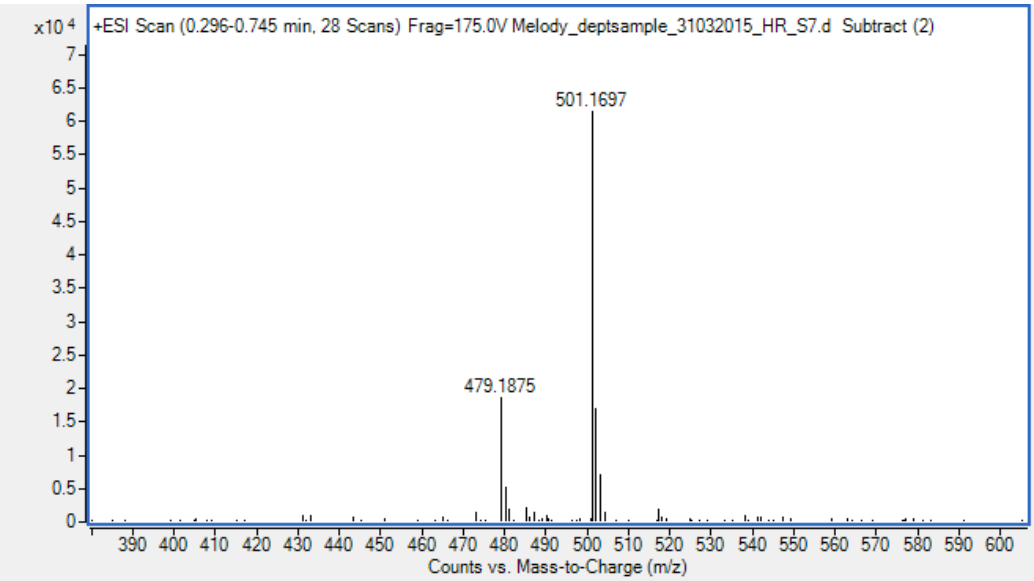


Figure 4.4. ESI mass spectra of siloxane meta-pyridine betaine in MeOH at room temperature

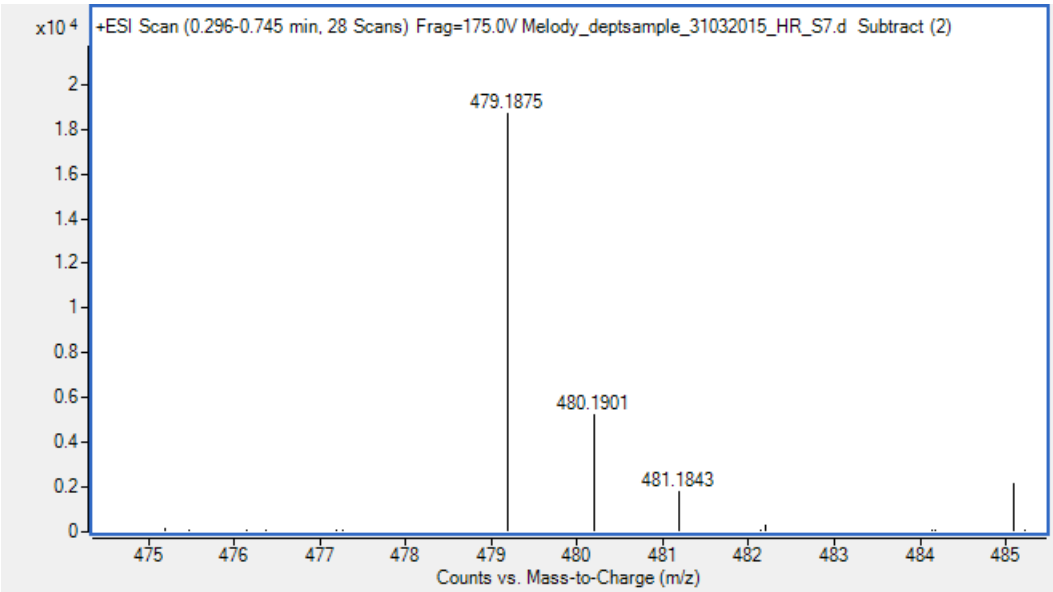


Figure 4.5. ESI mass spectra of siloxane meta-pyridine betaine in {pyridine· H^+}

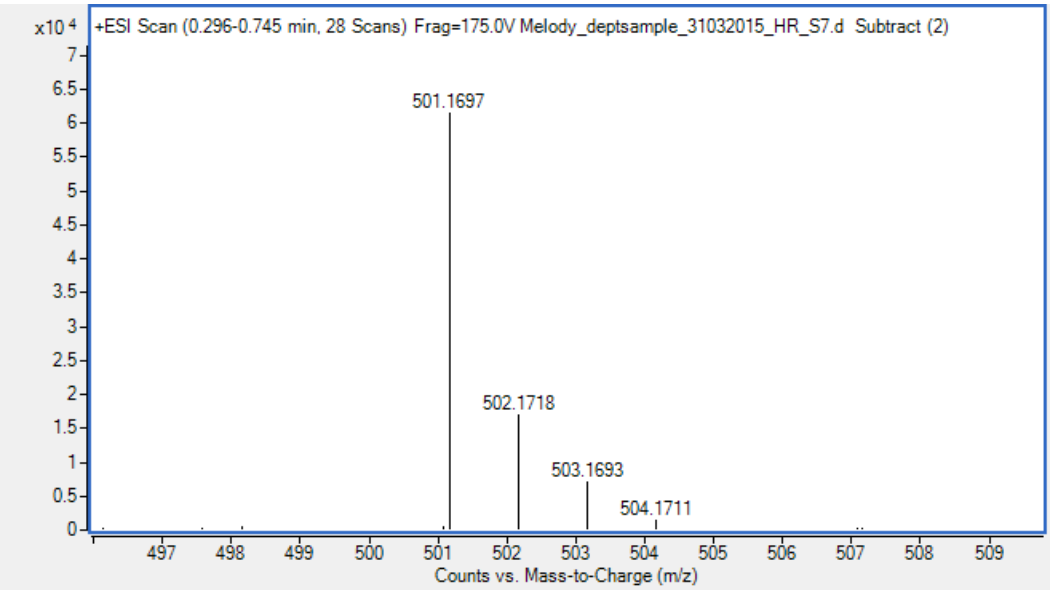


Figure 4.6. ESI mass spectra of siloxane meta-pyridine sulfobetaine in {pyridine· Na^+}

4.2.2 Thermal stability

Thermal stability of para-pyridine sulfobetaine was investigated by thermogravimetric analysis under N_2 atmosphere with a flow capacity of 50 mL/min and a heating rate of 10 °C/min from 25 °C to 800 °C. TG curve (as seen in the Supporting Information in Fig 4.7) shows that the start decompose temperature is 220 °C, and the start decompose temperature for 96% residual weight is 290 °C. The maximum rate of weight loss occurs at 398 °C. It indicates that para-pyridine sulfobetaine has good thermal stability, which can be used in a high temperature environment.

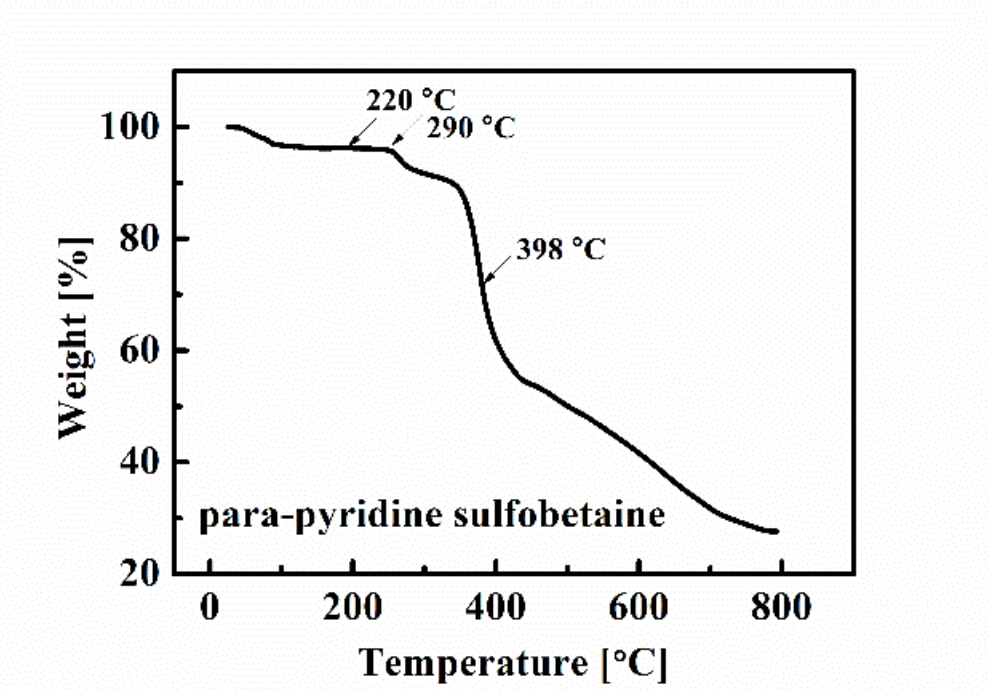


Fig 4.7. TGA analysis of para-pyridine sulfobetaine

4.2.3 Surface morphology of modified cotton fabric

SEM with EDX was used to investigate the surface morphologies of raw cotton fabric and cotton fabric finished with para-pyridine sulfobetaine. SEM images gave us direct observation of coating on fabric surface and the distribution of the coating. Fig 4.8 shows the SEM images of cotton fibers in a raw cotton fabric and a modified cotton fabric. A nonuniform coating bonded to the surface of modified cotton fabric was observed, whereas no coating was observed on the surface of raw cotton fabric. It indicates that pyridine sulfobetaine was successfully attached to the surface of the cotton fabric. Fig 4.9 shows the surface composition of a modified cotton fabric. It contains C, O, N, S, Si elements, and N, S and Si are from the pyridine sulfobetaine.

This again confirms that pyridine sulfobetaine was bound successfully to the modified cotton fabric.

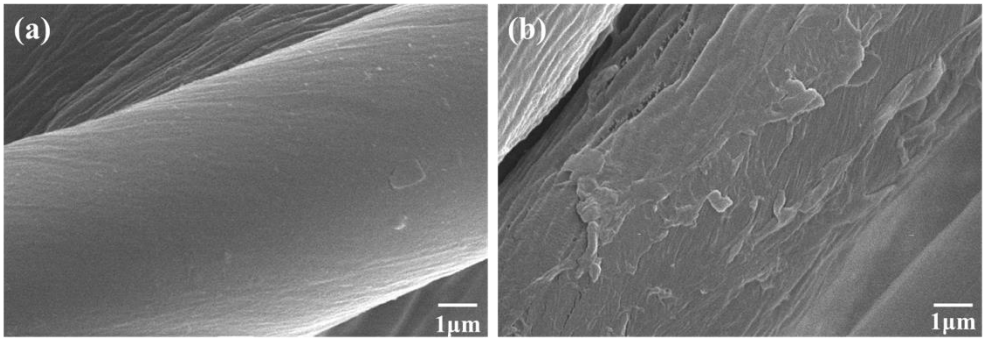


Fig 4.8. SEM images of cotton fiber in cotton fabrics before (a) and after finishing process (b) with para-pyridine sulfobetaine.

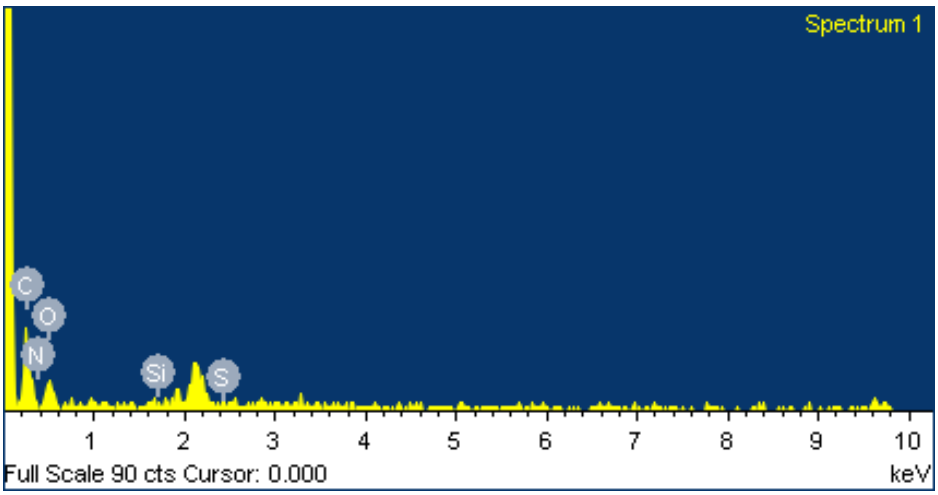
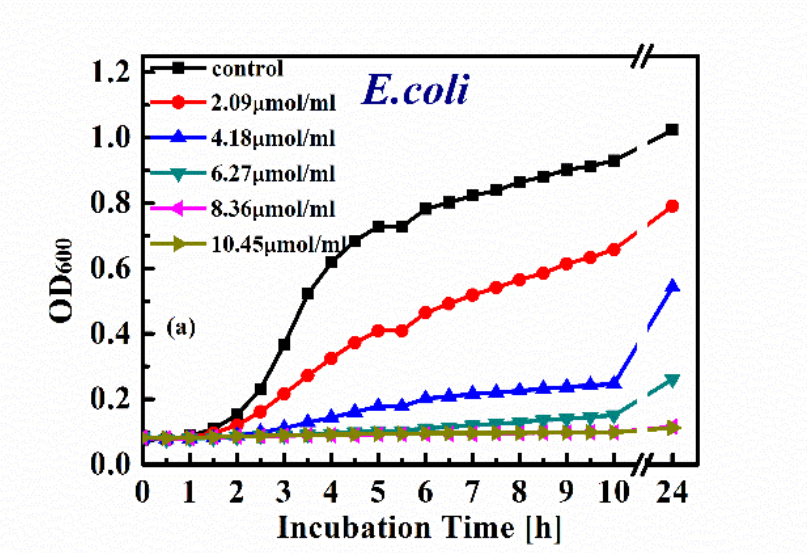


Fig 4.9. EDX result of the modified cotton fabric with para-pyridine sulfobetaine.

4.2.4 Antibacterial kinetics of para-pyridine sulfobetaine

The Effect of pyridine sulfobetaine on bacterial growth was investigated in liquid

media with different concentrations of para-pyridine sulfobetiane based on the turbidity of cell suspension. Bacterial growth was determined by measuring the optical density of cell suspension at 600 nm with different pyridine sulfobetaine concentrations. Fig 4.10 shows the OD_{600} curves of *E.coli* and *S.aureus*. As the concentration of pyridine sulfobetaine increases, bacterial growth slows down. It indicated that the concentration of 2.09 μmol/ml retarded the bacterial growth of *E.coli* and *S.aureus*. At the concentration of 8.36 μmol/ml, the growth of *E.coli* could be completely inhibited. At the concentration of 10.45 μmol/ml, the growth of *S.aureus* approached to be completely inhibited. Therefore, the MIC of pyridine sulfobetaine is between 6.27 and 8.36 μmol/ml against *E.coli* and approached 10.45 μmol/ml against *S.aureus*, respectively. It showed that pyridine sulfobetaine has excellent antibacterial activities at a lower concentration than the reported antibacterial agent SSPB with a MIC of 70 μmol/ml against *E.coli* and *S.aureus*, respectively [144].



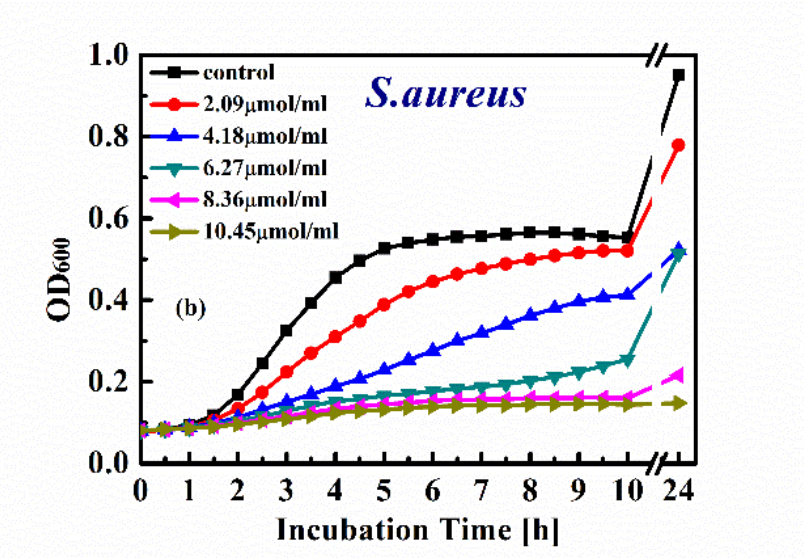


Fig 4.10. Optical density curves indicating bacterial growth kinetics, (a) *E.coli*, (b) *S.aureus*, in solutions of different concentrations of para-pyridine sulfobetaine.

4.2.5 Antibacterial property of modified cotton fabric

Antibacterial activities of modified cotton fabrics finished with para-pyridine sulfobetaine were evaluated by antibacterial rate according to the standard FZ/T 73023-2006. Fig 4.11 shows the antibacterial activity of modified cotton fabric finished with pyridine sulfobetaine against *E.coli* and *S.aureus*. It is found that the count of both bacteria colony decreased sharply after 24 hours contact. The antibacterial rate reached 99.99% against *E.coli* and 99.90% against *S.aureus* respectively. This implies that the modified cotton fabric has excellent antibacterial activity against both gram-negative *E.coli* and gram-positive *S.aureus*.

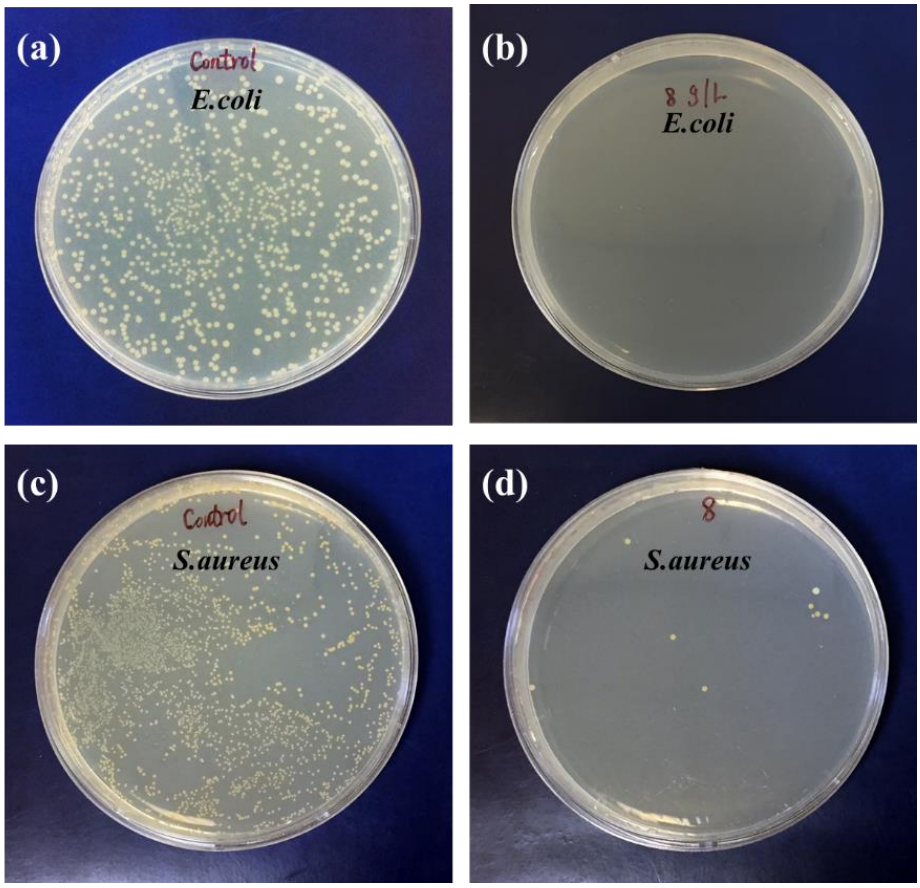


Fig 4.11. Antibacterial activity of cotton fabric. Colony count after 24 hours contact. (a) *E.coli*, raw cotton fabric, (b) *E.coli*, modified cotton fabric with para-pyridine sulfobetaine, (c) *S.aureus*, raw cotton fabric, (d) *S.aureus*, modified cotton fabric with para-pyridine sulfobetaine.

4.2.6 Antibacterial activity of cotton textiles-before washing

In this experiment, the antibacterial activity of cotton textiles finished with different concentrations against *E.coli* were evaluated using the above method. Table 4.1 showed the results of antibacterial rate. At a concentration of 8 g/L, the antibacterial

rate can reach to 96.88% against to *E.coli*, which indicates that the cotton textiles finished with 8 g/L antibacterial agent can have excellent antibacterial activity. The concentration is lower than 10 g/L of siloxane sulfobetaine, which showed that the cotton textiles finished with pyridine sulfobetaine at a lower concentration can obtain excellent antibacterial activity.

Table 4.1. Antibacterial rate against *E.coli* in different concentrations

sample	Agent concentration (g/L)	Padding Pressure (kg/cm^2)	Antibacterial rate (R) (%)
1	4	2	0
2	6	2	36.27
3	8	2	96.88
4	10	2	96.94
5	12	2	97.93

4.2.7 Antibacterial activity of cotton textiles-after washing

Table 4.2 showed antibacterial rate of cotton textiles finished with pyridine sulfobetaine after 20 times washing. It is found that after 20 times washing, antibacterial rate decreased a lot, just 24.29%, 26.71% and 55.00%, which showed poor antibacterial durability of pyridine sulfobetaine after repeated laundering. Though the durability of pyridine sulfobetaine is not ideal, it can be used in one-off medical fabric and bandage

to resist the attachment of bacteria, avoiding wound infection.

Table 4.2. Antibacterial rate against *E.coli* after 20 times washing

sample	Washing times	Agent concentration (g/L)	Antibacterial rate (R) %
1	20	8	24.29
2	20	10	26.71
3	20	12	55.00

4.2.8 Non-fouling property of modified cotton fabrics

Betaine is theoretically electroneutral due to the anionic and cationic groups in the same structure, which should resist the formation of bacterial biofilm on the surface of cotton fabric. As shown in Fig 4.12, bacteria formed biofilm on surface of raw cotton fabric (Fig 4.12 a, c). However, there was no biofilm formation on the surface of modified cotton fabric (Fig 4.12 b, d). This indicates that betaine strongly resisted bacterial adhesion and biofilm formation on modified cotton fabric surface. The modified cotton fabric showed excellent non-fouling property. Therefore, it is expected that the pyridine sulfobetaine treated cotton fabric is good for wound healing due to its non-fouling property.

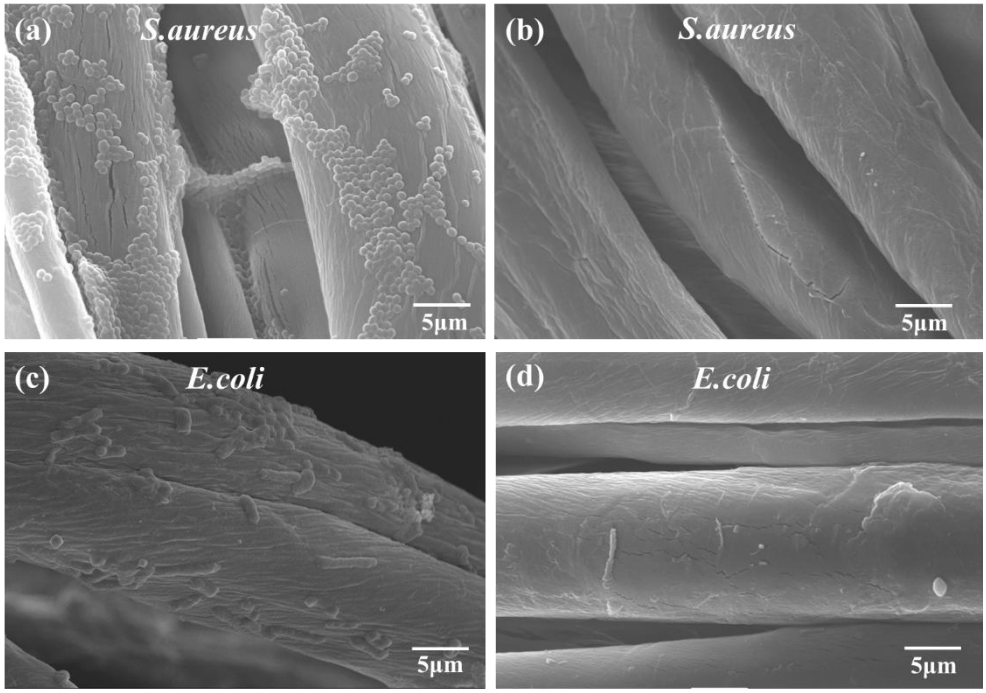


Fig 4.12. Non-fouling property. SEM images. a, c) raw cotton fabric, b, d) modified cotton fabric. The bacteria used are *S.aureus* in a, b) and *E.coli* in c, d).

4.2.9 Physical properties of cotton fabric

Air permeability and hydrophilicity are two key physical impact factors on the wound healing of medical fabric. The air permeability and hydrophilicity of both raw cotton fabric and modified cotton fabric were investigated according to the ASTM D737-04 (2012) standard and the ASTM D4772-09 standard respectively. As shown in Fig 4.13 (a), after finishing, the air permeability of the modified cotton fabric decreased a little more than raw cotton fabric. The decrease of air permeability may be due to the cross-linking between para-pyridine sulfobetaine and cotton fabric. Also the shrinking of cotton fabric decreased the number of pore. This is beneficial to medical fabric,

because lower air permeability could decrease dehydration of the wound surface and reduce the second injury when it is replaced. Otherwise, the fabric usually sticks to the wound surface and results in patients' second pain and trauma. Fig 4.13 (b) shows that water absorption of the modified cotton fabric increased significantly after finishing. It indicates that water holding capability of the modified cotton fabric is enhanced, which is significant to wound healing.

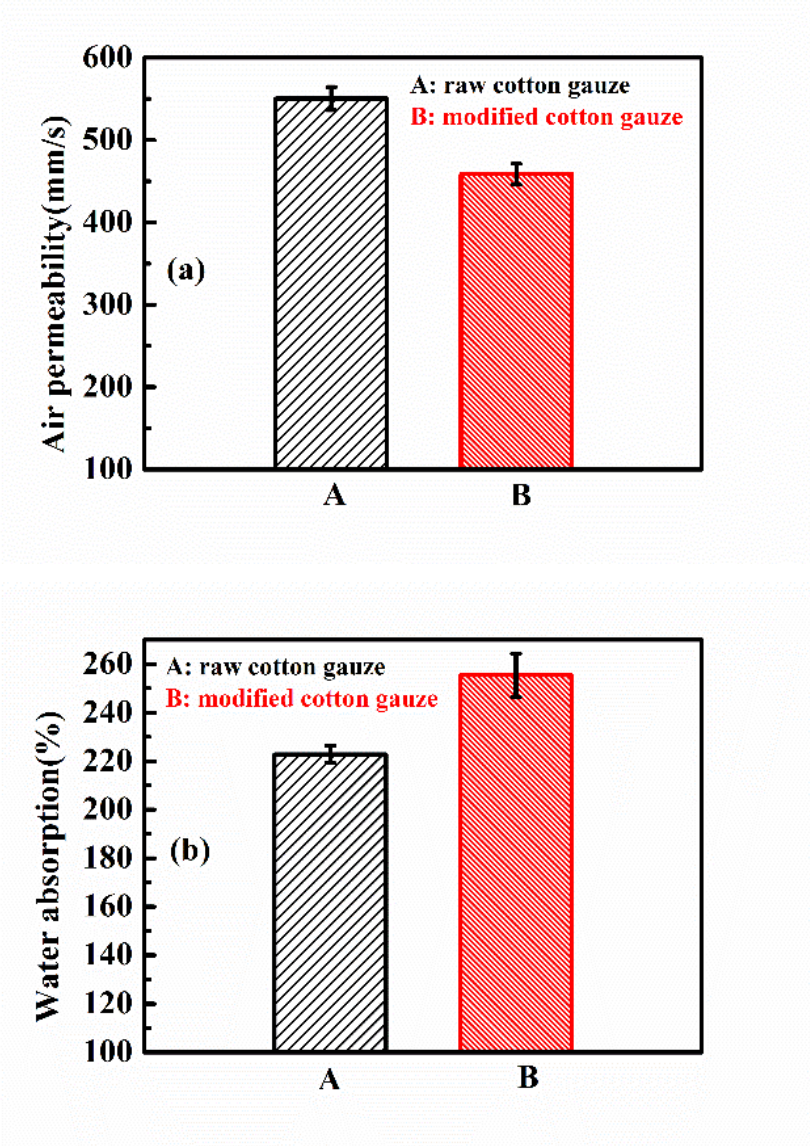


Fig 4.13. Air permeability (a) and water absorption (b) of cotton fabrics.

4.2.10 Skin stimulation

It is important to consider the safety of cotton fabric when it is used on human body. The safety of the modified cotton fabric is evaluated by skin stimulation test on rabbit. The test was conducted in SGS Guangzhou Branch. The results are summarized in Table 4.3. It shows that the scores of erythema (*S*er) and edema (*S*ed) are 0 after 1h, 24h and 48h contact. This means that the pyridine sulfobetaine antibacterial agent will not cause any skin stimulation. Thus, the antibacterial agent is skin compatible and safe for medical fabric application.

Table 4.3 Skin stimulation results of finished cotton fabric

sex		1h				24h				48h			
	W	Sample		control		sample		control		sample		control	
	(kg)	*S*er	*S*ed	*S*er	*S*ed	*S*er	*S*ed	*S*er	*S*ed	*S*er	*S*ed	*S*er	*S*ed
male	2.2	0	0	0	0	0	0	0	0	0	0	0	0
male	2.2	0	0	0	0	0	0	0	0	0	0	0	0
female	2.3	0	0	0	0	0	0	0	0	0	0	0	0
female	2.3	0	0	0	0	0	0	0	0	0	0	0	0
Average *S*		0		0		0		0		0		0	

W: weight of the rabbits; *Ser*: scores of erythema; *Sed*: scores of edema.

4.2.11 Cytotoxicity

4.2.11.1 MTT

The vitro cytotoxicity of modified cotton fabrics were quantitatively evaluated by measuring MTT assay using direct contact according to ISO 10993-5: 1999 standard. To measure the optical density at 492nm (OD492) using ELIASA can reflect the number of living cells. The results was shown in Fig 4.14.

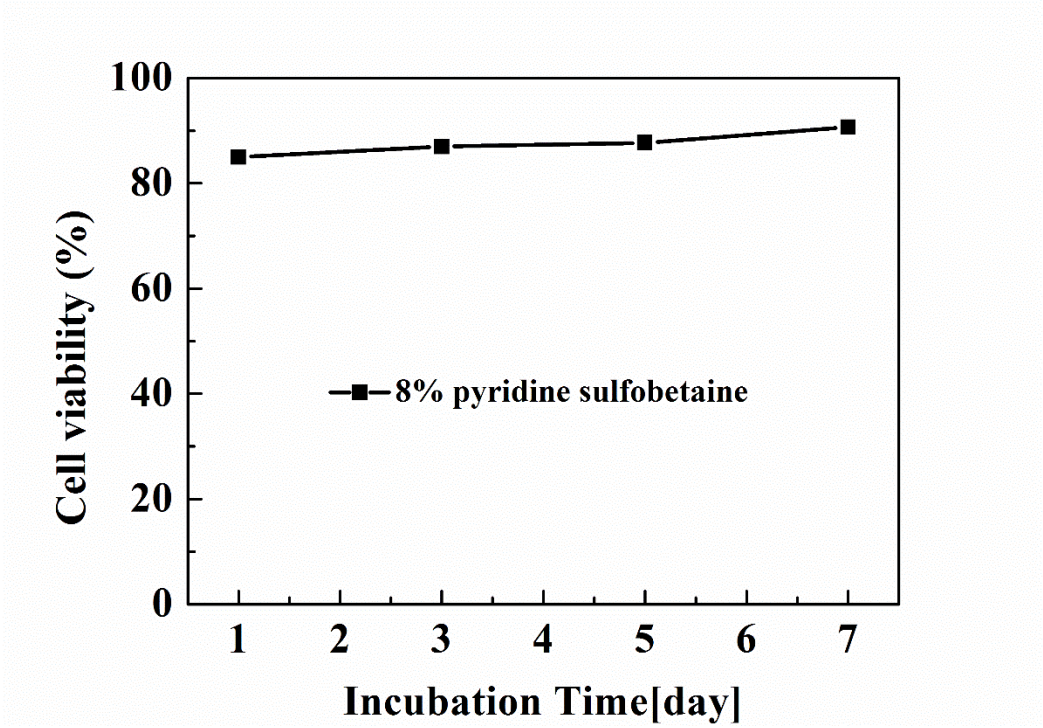


Fig 4.14. Vitro cell cytotoxicity results of modified cotton fabrics

Fig 4.14 showed that the cell viability is above 80% with a 1day, 3days, 5 days

and 7days incubation. It indicates that the modified cotton fabrics with pyridine sulfobetaine is non-cytotoxicity. The modified cotton fabrics can be applied onto medical cotton fabrics and other related fields.

4.2.11.2 Fluorescence microscope and SEM observation

The cytotoxicity of modified cotton fabrics is also qualitatively evaluated via fluorescence microscope and SEM by observing the morphology of cells. The results were shown in Fig 4.15 and Fig 4.16.

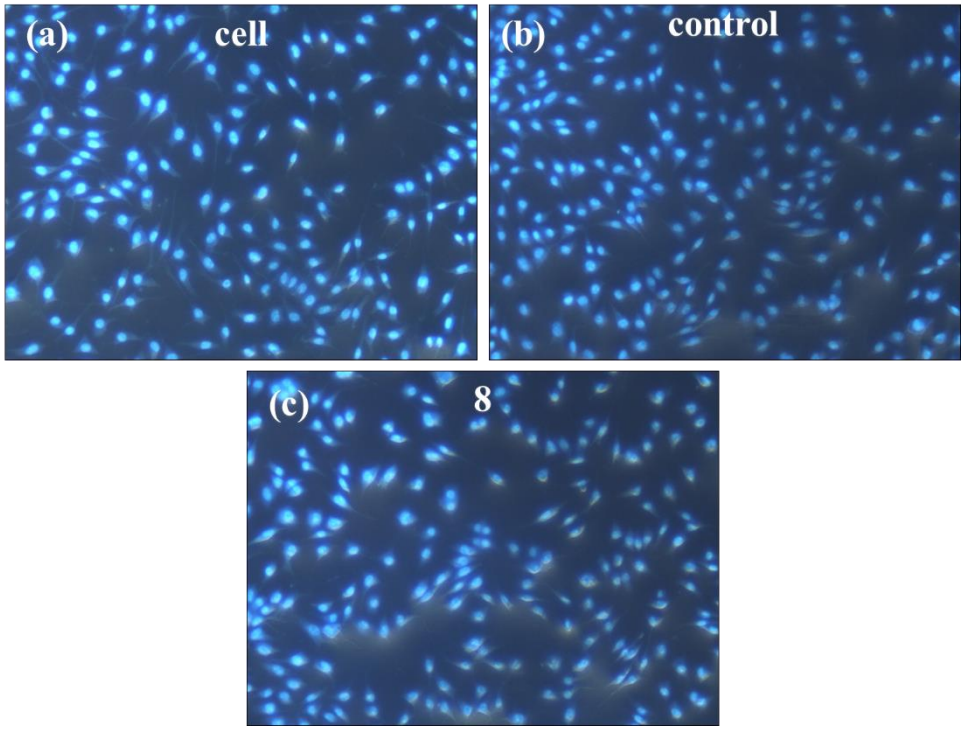


Fig 4.15. Fluorescence microscope images. (a) cells; (b) cells incubated with raw cotton fabrics; (c) cells incubated with modified cotton fabrics

The cells were inoculated with raw and modified cotton fabrics for 3 days. Then the cells were dyed with FITC and DAPI. The cells were observed under fluorescence microscope. Fig 4.15 showed the morphology of cells (Fig 4.15, a), cells incubated with raw cotton fabric (Fig 4.15, b) and cells incubated with modified cotton fabric (Fig 4.15, c). We can see that compared with the morphology of cells, the morphology of cells incubated with raw and modified cotton fabrics did not changed, with no cell breaking. The morphology is integrated. It indicates that the modified cotton fabrics are non-cytotoxicity.

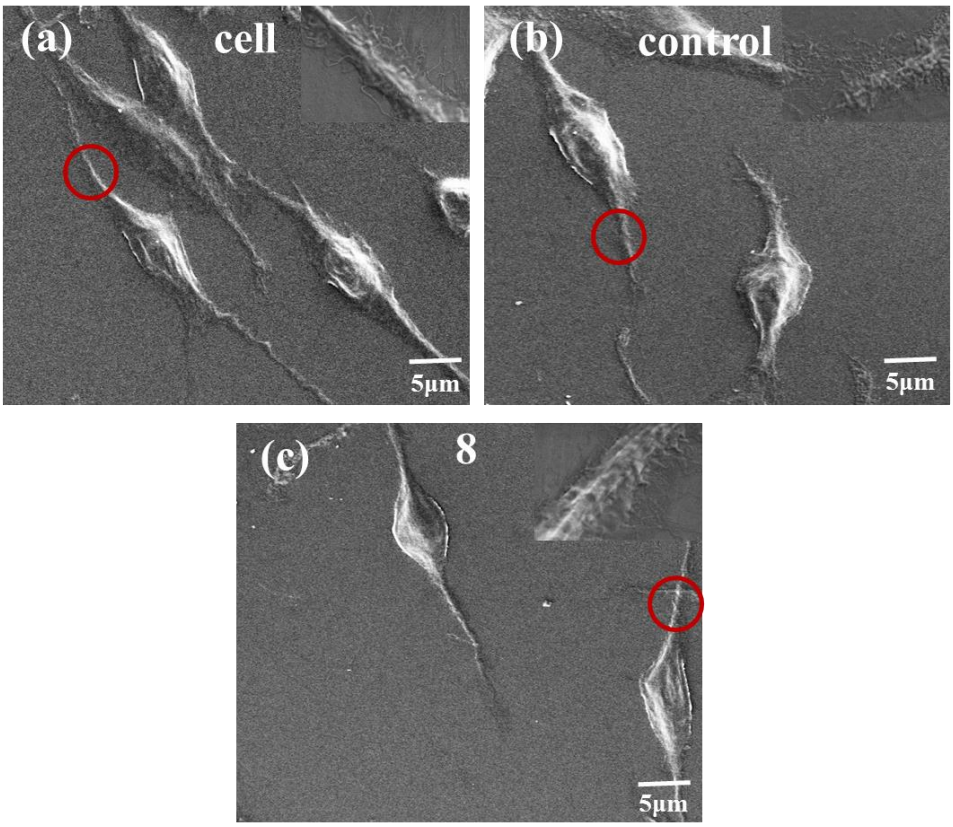


Fig 4.16. SEM images of cells. (a) cells; (b) cells incubated with raw cotton fabrics; (c)

cells incubated with modified cotton fabrics

After 5 days incubation, the cells was fixed and dehydrated. Then the cells were observed under SEM. Fig 4.16 showed the cells morphology of cells (Fig 4.16, a), cells incubated with raw cotton fabric (Fig 4.16, b) and cells incubated with modified cotton fabric (Fig 4.16, c). The small images on the top right corner showed the amplification of the labeled part with red circle. It showed that the cells' morphology is integrated without breaking. And the flagellums did not atrophy. It further indicates that the modified cotton fabrics are non-cytotoxicity to cells.

4.3 Conclusions

In this study, pyridine sulfobetaine has been successfully synthesized. At a lower concentration, para-pyridine sulfobetaine showed excellent antibacterial activities when acted on gram-negative *E.coli* and gram-positive *S.aureus*, with the MIC of 8.36 μmol/ml and 10.45 μmol/ml against *E.coli* and *S.aureus* respectively. The antibacterial rate of as-modified cotton fabric reaches 99.90% or higher against *E.coli* and *S.aureus* within 24 hours contact time. The modified cotton fabric showed efficient non-fouling antibacterial function which inhibited bacteria adhesion and biofilm formation on modified cotton fabric surfaces. In addition, the slight reduction of air permeability and great improvement of water absorption can help wound healing due to keeping moist. Furthermore, the modified cotton fabric is biocompatible with no skin stimulation and

non-cytotoxicity. Therefore pyridine sulfobetaine could be a good alternative for medical fabric and other relevant applications.

Chapter 5

s-triazine sulfobetaine

5.1 Introduction

In this chapter, s-triazine sulfobetaine was synthesized via mono-substitution nucleophilic reaction. The intermediate product Boc-protected 2-amino-ethyl-sulfobetaine structure was characterized by ^{1}H NMR and ^{13}C NMR. The structure of s-triazine was also characterized by NMR, but the result is not right, which is due to the unsuccessful purification. The MIC of s-triazine sulfobetaine against to *S.aureus* and *E.coli* were 2 mg/ml and more than 6 mg/ml respectively.

Cyanuric chloride (CC) and its derivatives have been known for a long period for their widespread application in textiles and reactive dyes [173, 174]. The chlorine atoms in cyanuric chloride can be substituted by amino group below or at 0 °C with sodium carbonate adjusting the pH value.

5.2 Results and discussion

5.2.1 Structure characterization

Boc-protected 2- amino-ethyl-sulfobetaine was synthesized according to scheme 3.3 described in chapter 3.4.3. The structure was characterized by ^{1}H NMR and ^{13}C NMR using D_2O as the solvent. The result was showed in Fig 5.1, Fig 5.2 and Fig 5.3.

Fig 5.1 presents ^{1}H NMR spectrum of Boc-protected 2- amino-ethyl-sulfobetaine. ^{1}H NMR (500 MHz, D_2O): δ (ppm): 1.29(a, 3H), 1.47(f, 3H), 2.57(h, 2H), 3.24(g, 2H),

3.37(d, e, 2H), 3.47(c, i, 2H), 7.20(b, 1H). The chemical shift of δ= 7.20 is the active hydrogen of NH, which is the important hydrogen attribution indicated the successful synthesis of Boc-protected 2- amino-ethyl-sulfobetaine in this experiment.

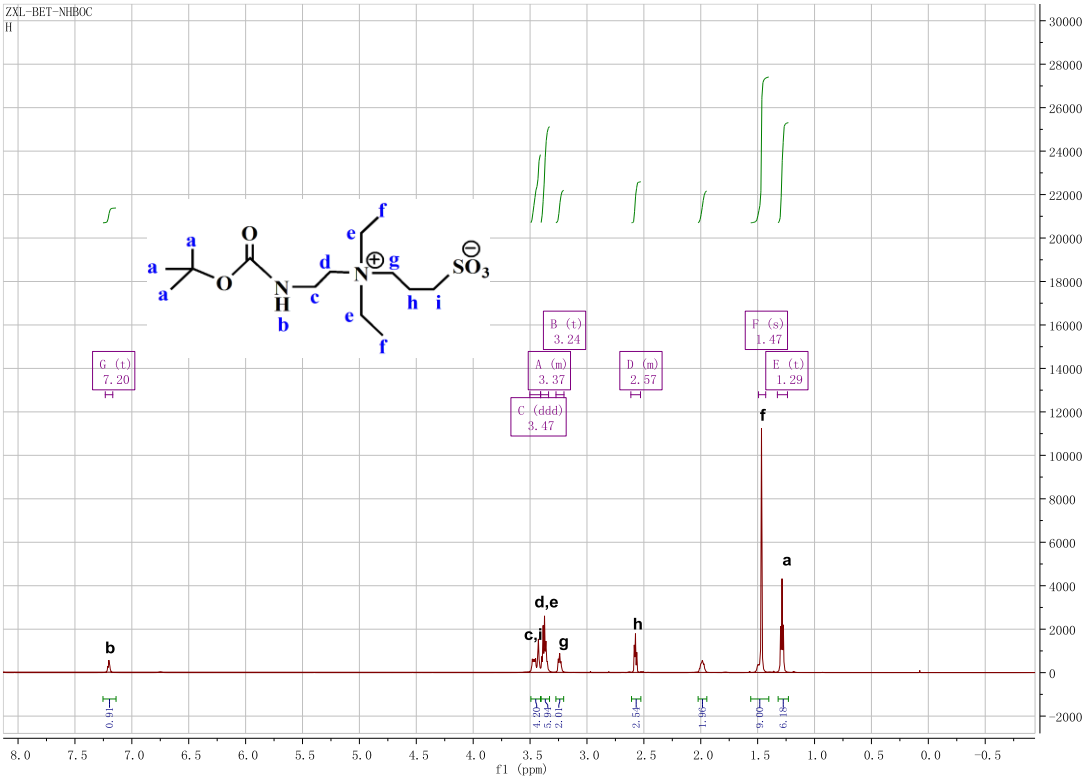


Figure 5.1. ^{1}H NMR spectrum of Boc-protected 2- amino-ethyl-sulfobetaine

Fig 5.2 presents ^{13}C NMR spectrum of Boc-protected 2- amino-ethyl-sulfobetaine. ^{13}C NMR (500 MHz, D_2O): δ (ppm): 28.59 (a, CH_3), 79 (b, C), 156.13 (c, C), 33.77 (d, CH_2), 56.39 (e, CH_2), 53.38 (f, CH_2), 7.45 (g, CH_3), 55.04 (h, CH_2), 18.42 (i, CH_2), 47.72 (j, CH_3). The chemical shift of δ = 55.04, δ = 18.42 and δ = 47.72 indicated the successful synthesis of Boc-protected 2- amino-ethyl-sulfobetaine in this experiment.

Fig 5.1 and 5.2 indicates that the the Boc-protected 2- amino-ethyl-sulfobetaine

was successfully synthesized.

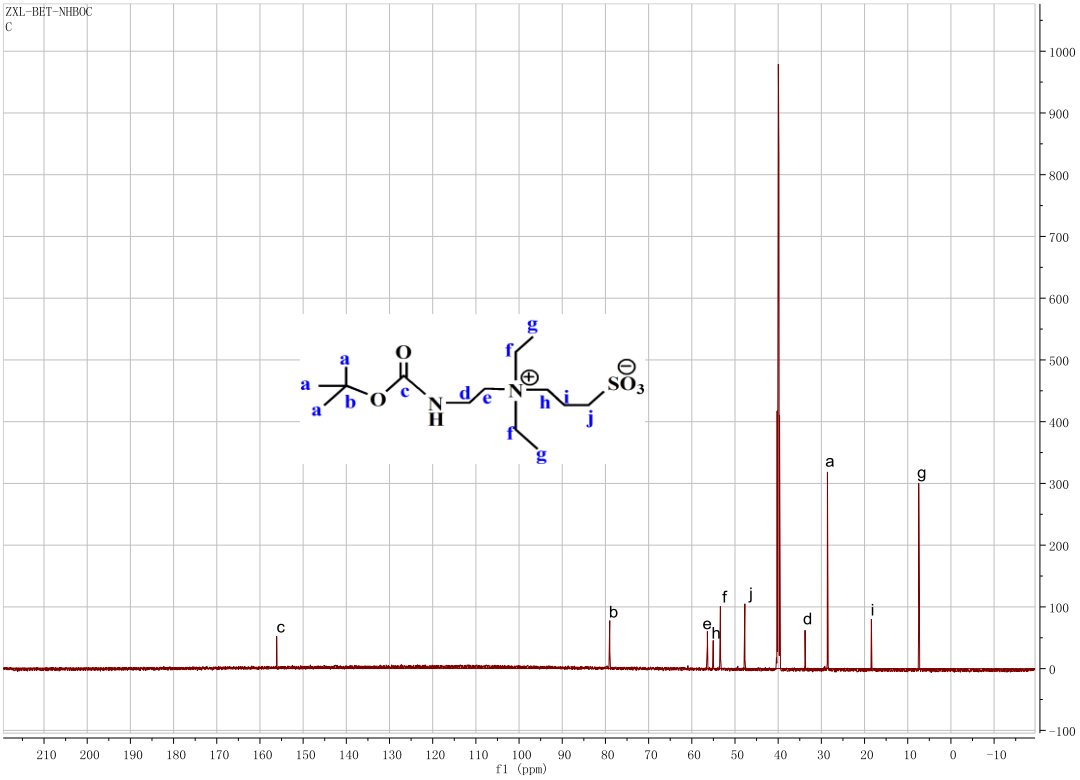


Fig 5.2. ^{13}C NMR spectrum of Boc-protected 2-amino-ethyl-sulfobetaine

Fig 5.3 presents ^{1}H NMR spectrum of s-triazine. But the result can not show the right structure of s-triazine. The reason is due to the final product is viscous, so it can not be successfully purified. But from the synthesis process, we can conclude that 2-amino-ethyl-sulfobetaine reacted with cyanuric chloride. Initially, cyanuric chloride is not soluble in water. With the process of reaction, the solution is clear. And the final product is obtained salt out through adding sodium sulfate. In that case, we need to explore other method to purify the product.

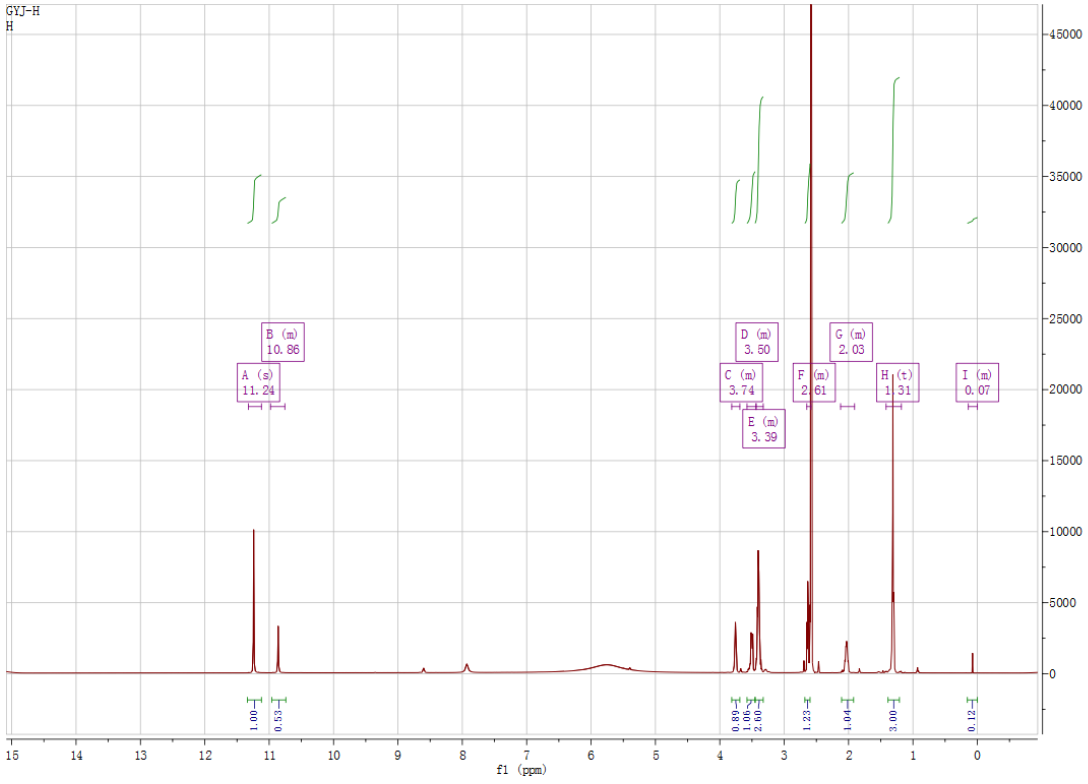


Figure 5.3. ^{1}H NMR spectrum of s-triazine sulfobetaine

5.2.2 Antibacterial activity of Amine mono-substitution s-triazine sulfobetaine

The antibacterial activity of s-triazine sulfobetaine against to *E.coli* and *S.aureus* were evaluated quantitatively by measuring the effects on the bacterial growth kinetics in liquid media based on the turbidity of the cell suspension. Fig 5.4 shows the antibacterial activities of s-triazine sulfobetaine. The OD_{600} curves in Fig 5.4 demonstrates that the antibacterial activities of s-triazine sulfobetaine increase as the concentration increases. As the concentration of s-triazine sulfobetaine increases, bacterial growth slows down. Additionally, it was found in Fig 5.4 that the s-triazine

sulfobetaine was able to slow the growth of *E.coli* and *S.aureus* at 0.5 mg/ml.

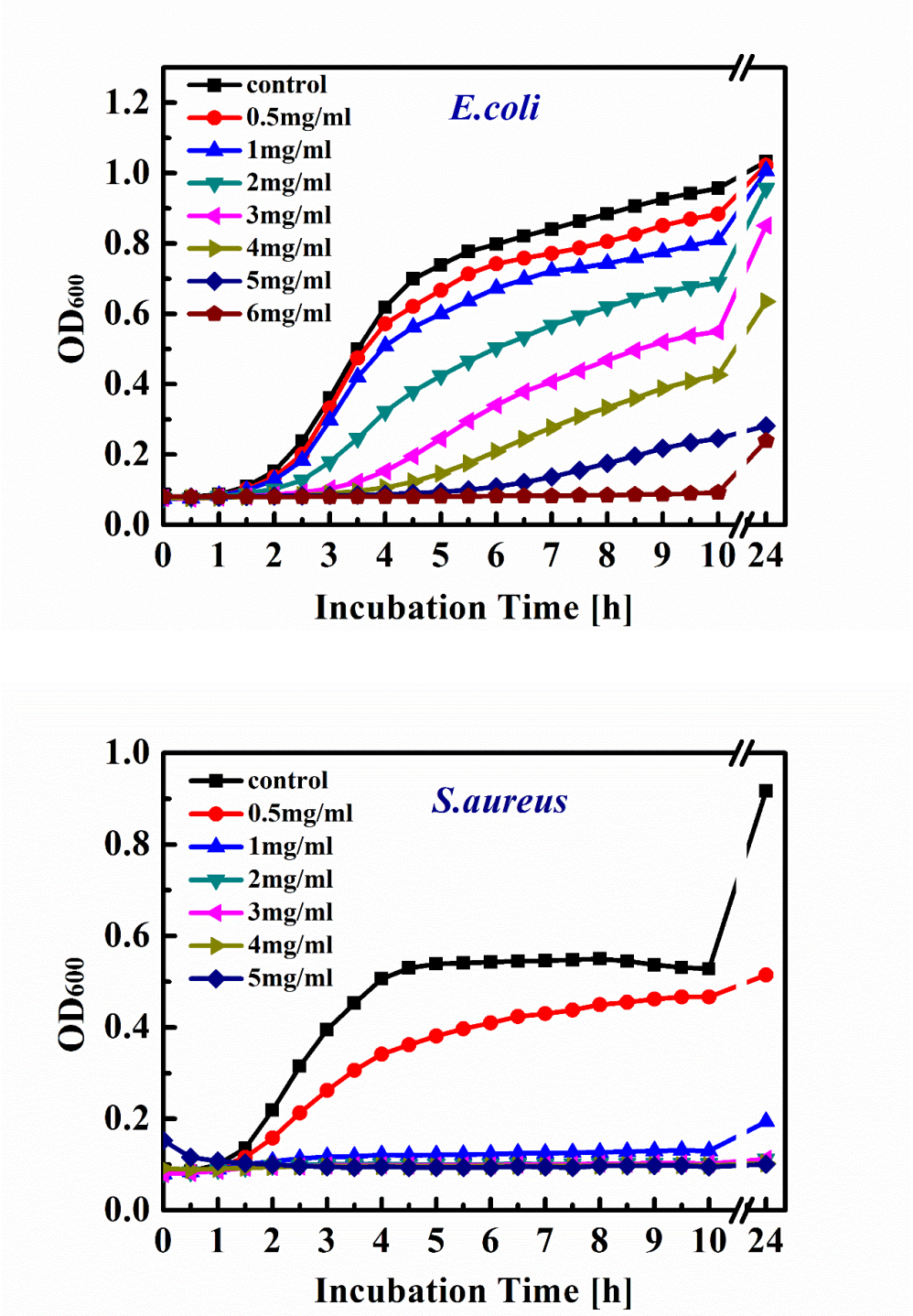


Fig 5.4. Optical density curves indicating bacterial growth, *E.coli* and *S.aureus*, *s*-

triazine sulfobetaine.

The s-triazine sulfobetaine completely inhibited the growth of *S.aureus* at 2 mg/ml, i.e., the MIC of s-triazine sulfobetaine against *S.aureus* was 2 mg/ml, which is lower than the para-pyridine sulfobetaine. That indicates s-triazine sulfobetaine has better antibacterial activity than para-pyridine sulfobetaine against *S.aureus*. At a concentration of 6 mg/ml, the growth of *E.coli* cannot be completely inhibited. It indicates that the MIC of s-triazine sulfobetaine against *E.coli* was more than 6 mg/ml, which is higher than para-pyridine sulfobetaine. That indicates s-triazine sulfobetaine has poorer antibacterial activity than para- pyridine sulfobetaine against *E.coli*.

5.3 Conclusions

In this study, s-triazine sulfobetaine was synthesized. The intermediate product Boc-protected 2-amino-ethyl-sulfobetaine was successfully synthesized which was indicated by ^{1}H NMR and ^{13}C NMR spectrum. The antibacterial activity was measured via bacteria growth kinetics. The MIC is 2 mg/ml against gram-positive *S.aureus* and more than 6 mg/ml against gram-negative *E.coli*. s-triazine sulfobetaine has better antibacterial activity than para-pyridine sulfobetaine against *S.aureus*, whereas poorer than para-pyridine sulfobetaine against *E.coli*.

However, the productivity of end product s-triazine sulfobetaine is low. It cannot be successfully purified. The structure is not successfully characterized by NMR. Then

the synthesis process is complex, which is not ideal for industry production. Though s-triazine sulfobetaine showed excellent antibacterial activity against gram-positive *S.aureus*, the antibacterial activity against gram-negative *E.coli* is poor.

Chapter 6

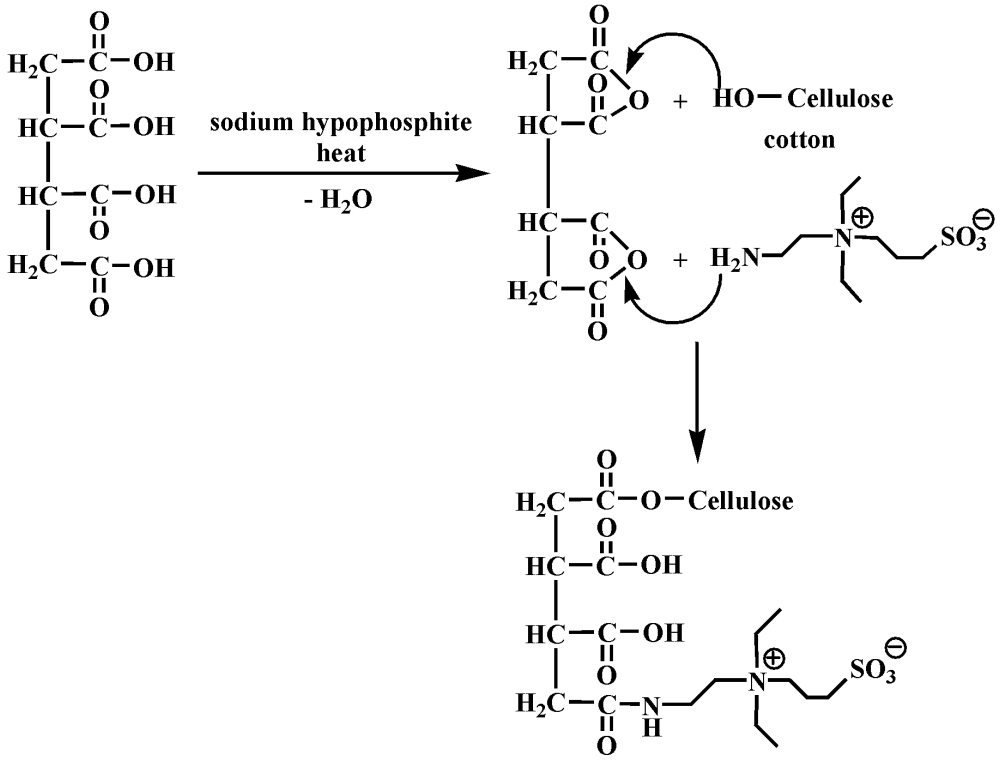
BTCA-Amino betaine

6.1 Introduction

In this chapter, the above mentioned amino sulfobetaine was applied with BTCA on cotton fabrics at a same concentration of 5% and 8%. The air permeability of modified cotton fabrics decreased little, but the tearing strength of modified cotton fabrics decreased a lot, especially at a concentration of 8%. The antibacterial activity of modified cotton fabric was measured using inhibition zone method, viable cell counting method. The modified cotton fabrics showed excellent antibacterial activity both at concentration of 5% and 8%. After 10 and 30 times washing, the antibacterial activity can reach above 90%. It indicates the modified cotton fabric have good washing durability. Also it showed good non-fouling property via SEM observation. The non-cytotoxicity of modified cotton fabric was confirmed via MTT method, SEM and fluorescence microscope observation.

1, 2, 3, 4-butane tetracarboxylic acid (BTCA) is nontoxic and usually applied on cotton fabrics to fix antibacterial agents onto cotton fabrics [175, 176]. It can react with the cellulose hydroxyl groups and the amino group in antibacterial agent. The mechanism is believed to form an intermediate cyclic anhydride initially, then react with the cellulosic hydroxyl and amino to complete the ester linkage with sodium hypophosphite as the most effective catalyst, which can decrease the reaction temperature and energy [28]. The washing durability can be significantly improved [177].

BTCA as bridge can link cotton fabric and amino sulfobetaine via chemical bonding. The scheme is showed in scheme 6.1 [178].



Scheme 6.1. The mechanism of BTCA as cross-linker

6.2 Results and discussion

The fabrics were finished with 5% and 8% betaine and BTCA with pad-dry-cure process and 70% wet pick-up. The fabrics were dried at 80 ºC and cured at 160 ºC for 3 minutes.

6.2.1 Air permeability

When the antibacterial agent is applied to cotton fabrics, air permeability is one of the most important factors that need to be evaluated. The air permeability of raw cotton fabrics and modified cotton fabrics were tested according to ASTM D737-04 (2012) standard method at 100 Pa with a test area of 5.08 cm^2. Each experiment was repeated 5 times and got an average value. The results were shown in Fig 6.1.

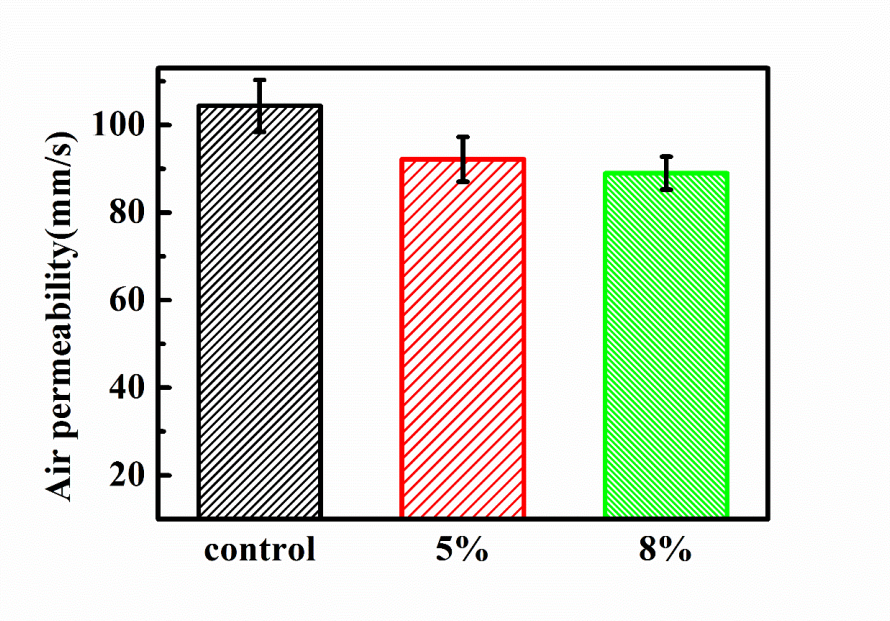


Fig 6.1. Air permeability results of cotton fabrics before (control) and after finishing (5% and 8%)

Fig 6.1 showed that the air permeability of modified cotton fabrics finished with 5% and 8% BTCA-amino betaine decreased a little. It is about 14% and 19%. What's more, with the increasing of finishing concentration, the air permeability decreased. It

maybe attributes to the coating resulted from the cross-linking of BTCA and amino sulfobetaine that decreases the rate of air through.

6.2.2 Tearing strength

The tearing strength of pristine and modified cotton fabrics both in warp and weft directions were tested using ballistic pendulum method (Elmendorf) according to ISO 13937-1:2000/Cor.1:2004 standard. The tearing strength results were shown in Fig 6.2.

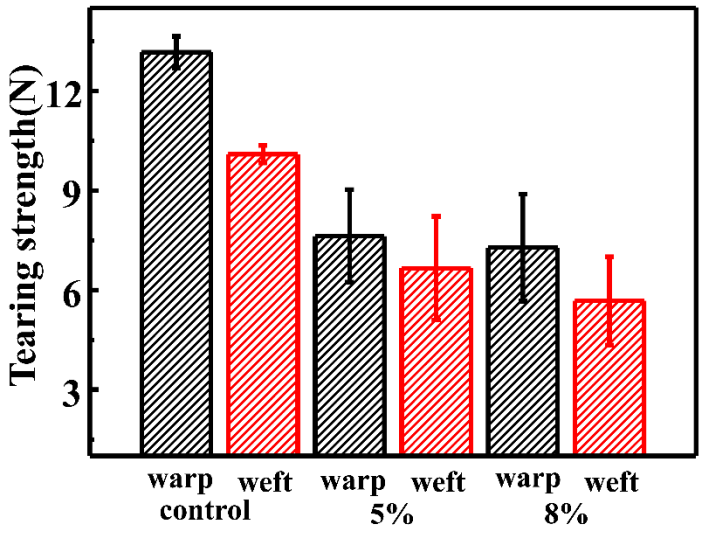


Fig 6.2. Tearing strength results of cotton fabrics before (control) and after finishing (5% and 8%) in warp and weft directions

Fig 6.2 showed that the tearing strength of modified cotton fabrics finished with 5% and 8% BTCA-amino betaine decreased a lot both in in warp and weft directions. With the increasing BTCA concentration, tearing strength decreased. It attributes to

the addition of BTCA that breaks the fiber of cotton fabrics.

6.2.3 Antibacterial activity

6.2.3.1 Inhibition zone method

The antibacterial activity of modified cotton fabrics was initially qualitatively investigated with inhibition zone method according to FZ/T 73023-2006 standard (China's Textile Industry Standard). The test bacteria are *E.coli* and *S.aureus*. The results are shown in Fig 6.3.

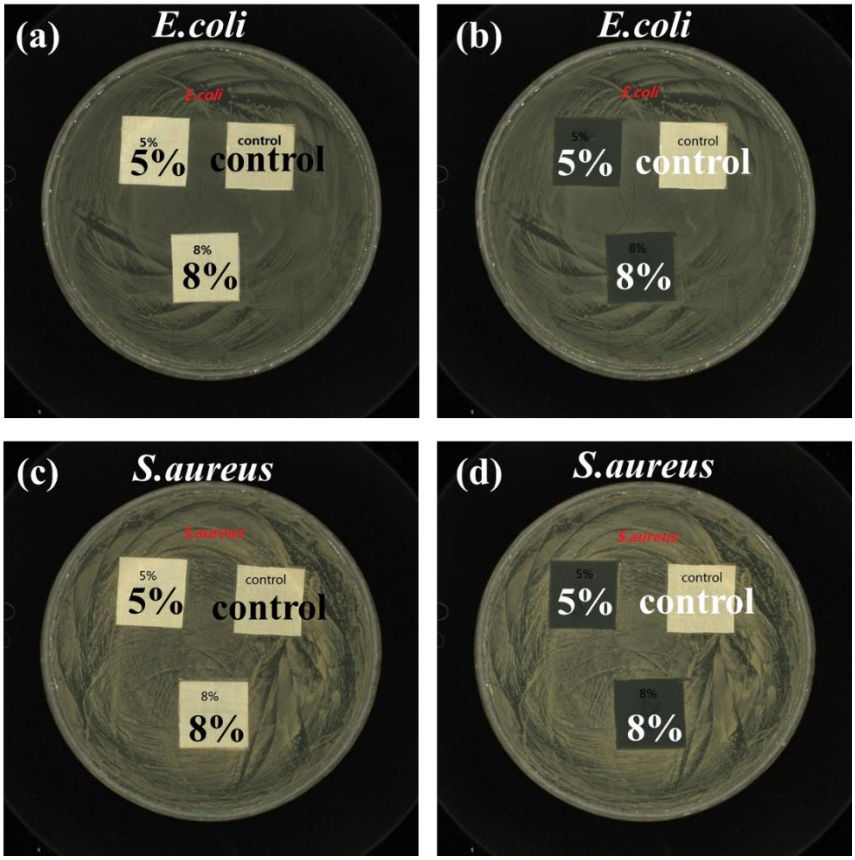


Fig 6.3. Images of inhibition zone. (a) (b), *E.coli*, (c) (d), *S.aureus*

Fig 6.3 showed the antibacterial activity of modified cotton fabrics. (a) and (c) showed no inhibition zone. It indicates that the betaine is non-leachable when it was coated on cotton fabrics. When the fabric was removed, there is no bacteria growth under the fabric. It showed that the modified fabric can resist bacteria growth. This indicates that the modified cotton fabrics have excellent antibacterial activity.

6.2.3.2 Viable cell counting method

The antibacterial activity of modified cotton fabrics with betaine and BTCA were evaluated according to FZ/T 73023-2006 standard (China's Textile Industry Standard). The results were showed in Fig 6.4.

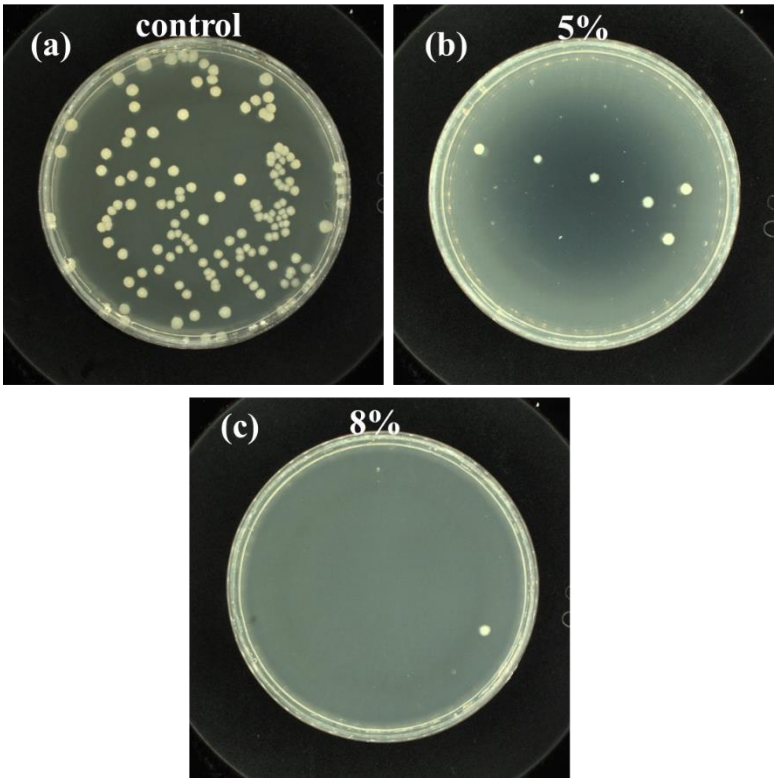


Fig 6.4. Colony count of 24 hours contact, *E.coli*, (a) raw cotton fabric, (b) 5% betaine and BTCA, (c) 8% betaine and BTCA

It is found that the count of bacteria colony decreased sharply after 24 hours contact. The antibacterial rate reached 99.96% and 99.98% at a concentration of 5% and 8% against *E.coli*, respectively. This implies that the modified cotton fabric has excellent antibacterial activity against gram-negative *E.coli*.

6.2.3.3 Washing durability

When the antibacterial agent is applied to cotton fabrics, the washing durability after many times laundering need to be evaluated to determine whether the modified cotton fabrics exhibit durable antibacterial activity. The laundering is according to AATCC 61-2A standard. The antibacterial activity after laundering was also evaluated according to FZ/T 73023-2006 standard (China's Textile Industry Standard). The antibacterial results against *E.coli* after 10 and 30 times laundering are shown in Fig 6.5.

Fig 6.5 showed that after 10 and 30 times laundering, the antibacterial rate of modified cotton fabrics with 5% and 8% BTCA and sulfobetaine can reach above 90%. In addition, after 10 times laundering, the antibacterial rate can reach 99% or above. It indicates that the modified cotton fabrics exhibit good washing durability after times laundering.

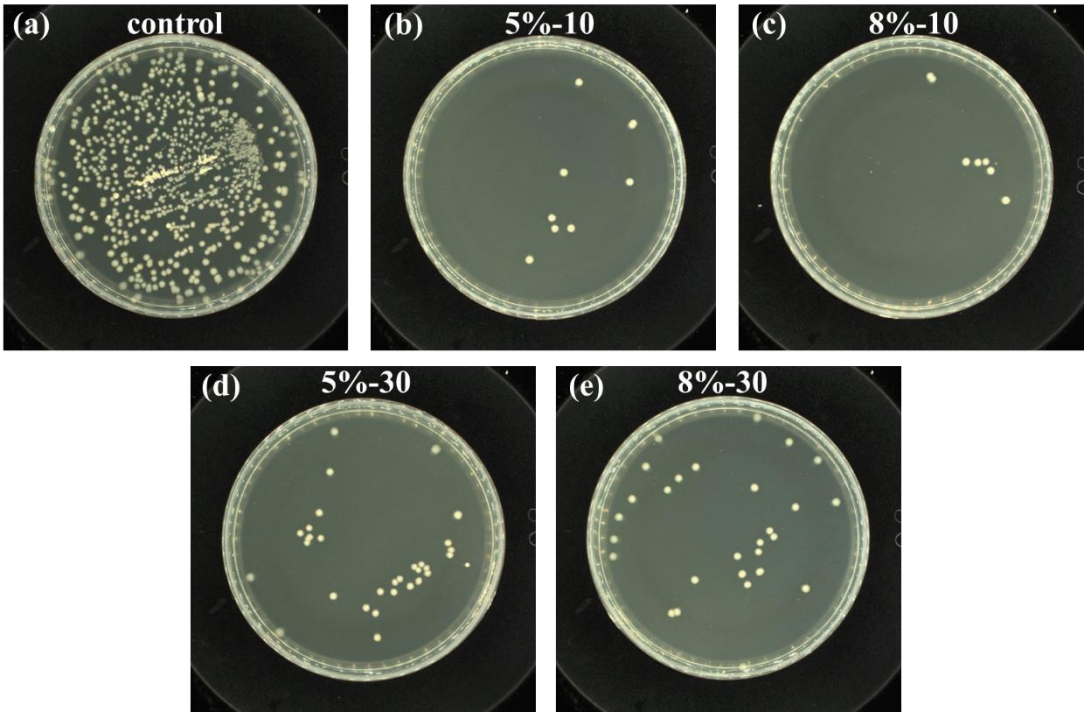


Fig 6.5. Colony count of 24 hours contact, *E.coli*, (a) raw cotton fabric, (b) 5% betaine and BTCA, 10 times washing (c) 8% betaine and BTCA, 10 times washing (d) 5% betaine and BTCA, 30 times washing (e) 8% betaine and BTCA, 30 times washing

6.2.4 Non-fouling property

Antibacterial agent resisting biofilm formation on surface of cotton fabrics is known as non-fouling property. It can avoid fabric contamination and odor. The non-fouling property of modified cotton fabric was evaluated by observing biofilm formation on cotton fabric surface using SEM. The results are shown in Fig 6.6.

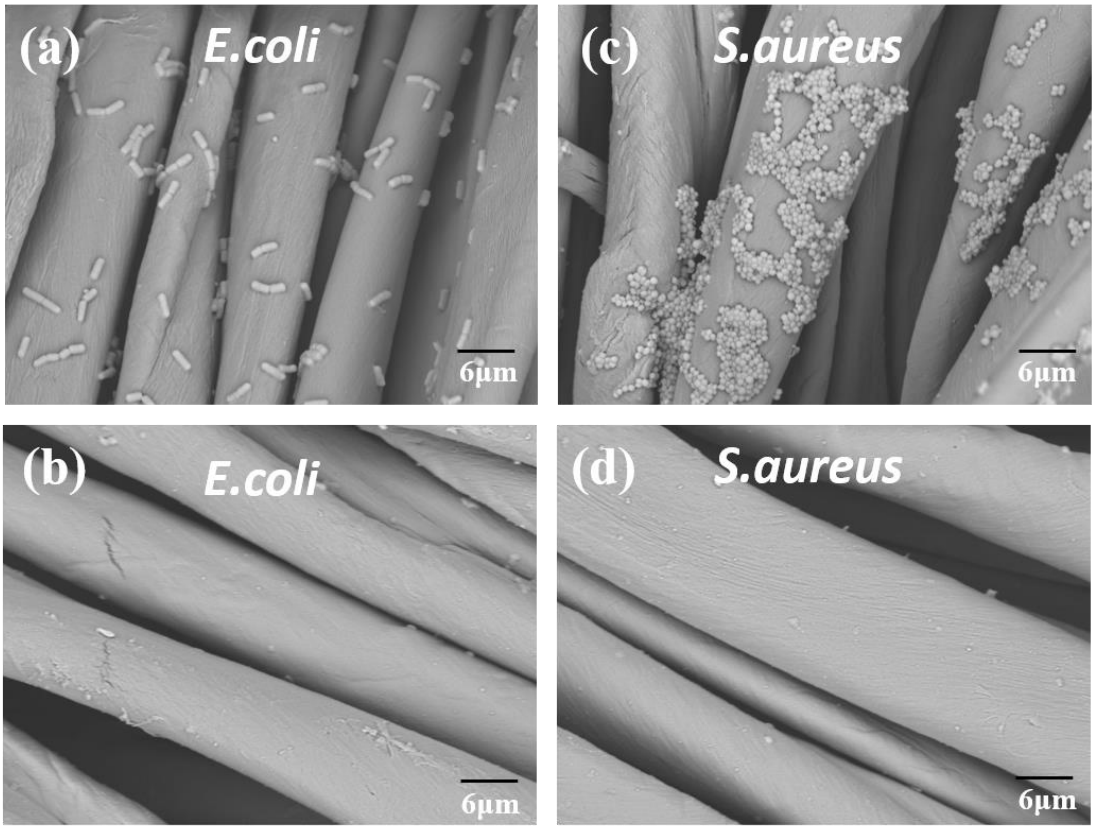


Fig 6.6. Non-fouling property. SEM images. a, c) raw cotton fabric, b, d) modified cotton fabric. The bacteria used are *E.coli* in a, b) and *S.aureus* in c, d).

As shown in Fig 6.6, bacteria formed biofilm on surface of raw cotton fabric (Fig 6.6 a, c). However, there was no biofilm formation on the surface of modified cotton fabric (Fig 6.6 b, d). This indicates that betaine strongly resisted bacterial adhesion and biofilm formation on modified cotton fabric surface. The modified cotton fabric showed excellent non-fouling property. Therefore, it is expected that the amino sulfobetaine treated cotton fabric is good for wound healing due to its non-fouling property.

6.2.5 Cytotoxicity

6.2.5.1 MTT

The vitro cytotoxicity of modified cotton fabrics were quantitatively evaluated by measuring MTT assay using direct contact according to ISO 10993-5: 1999 standard. To measure the optical density at 492nm (OD492) using ELIASA can reflect the number of living cells. The results was shown in Fig 6.7.

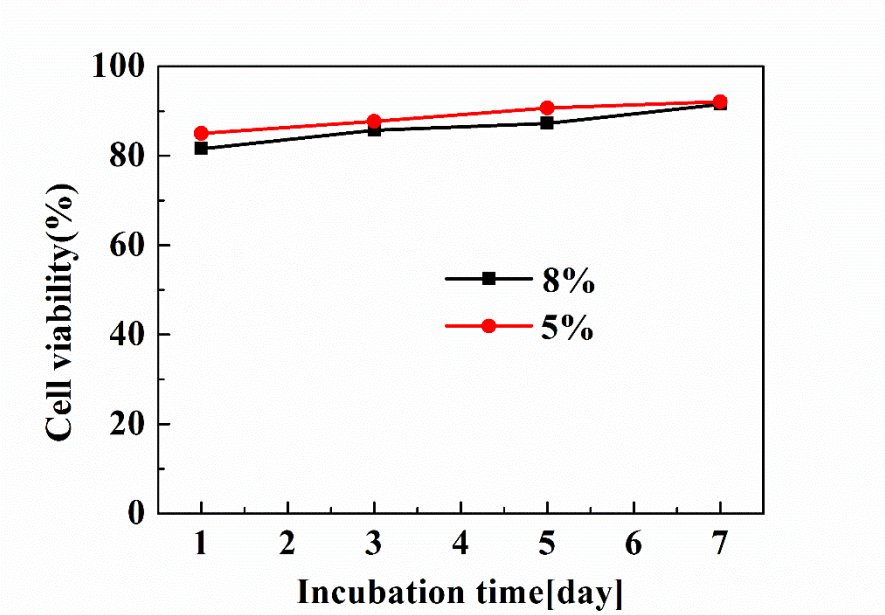


Fig 6.7. Vitro cell cytotoxicity results of modified cotton fabrics

Fig 6.7 showed that the cell viability is above 80% with a 1day, 3days, 5 days and 7days incubation. It indicates that the modified cotton fabrics with BTCA-amino sulfobetaine is non-cytotoxicity. The modified cotton fabrics can be applied onto

medical cotton fabrics and other related fields.

6.2.5.2 Fluorescence microscope and SEM observation

The cytotoxicity of modified cotton fabrics is also qualitatively evaluated via fluorescence microscope and SEM by observing the morphology of cells. The results were shown in Fig 6.8 and Fig 6.9.

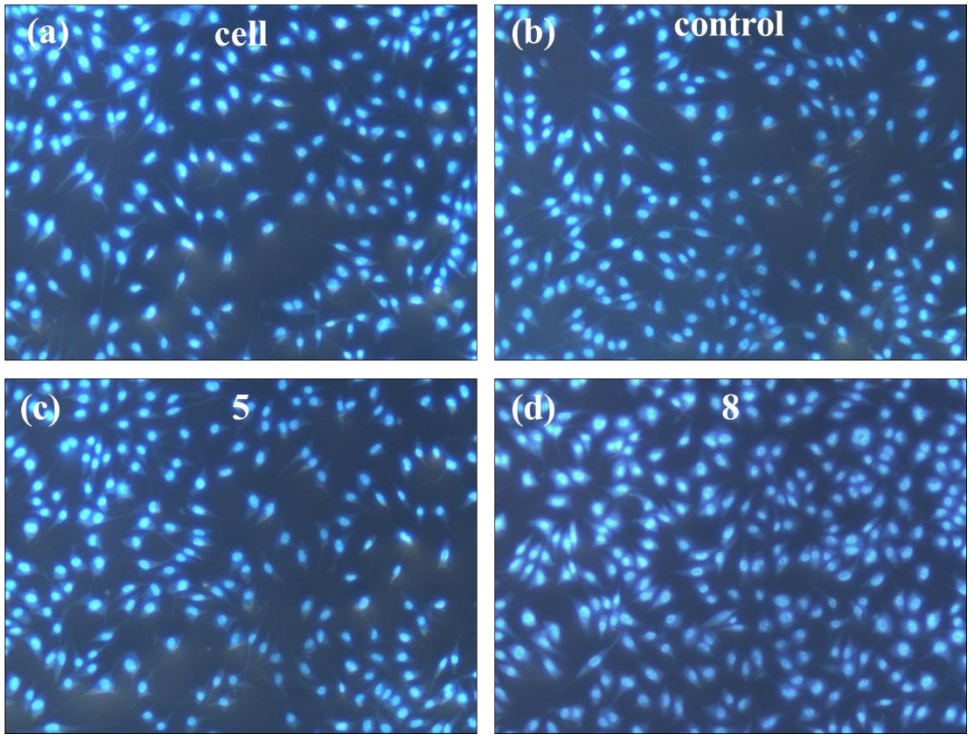


Fig 6.8. Fluorescence microscope images of cell morphology. (a) cells; (b) cells incubated with raw cotton fabrics; (c) and (d) cells incubated with modified cotton fabrics

The cells were inoculated with raw and modified cotton fabrics for 3 days. Then

the cells were dyed with FITC and DAPI. The cells were observed under fluorescence microscope. Fig 6.8 showed the morphology of cells (Fig 6.8, a), cells incubated with raw cotton fabric (Fig 6.8, b) and cells incubated with modified cotton fabric (Fig 6.8, c,d). We can see that compared with the morphology of cells, the morphology of cells incubated with raw and modified cotton fabrics did not changed, with no cell breaking. The morphology is integrated. It indicates that the modified cotton fabrics are non-cytotoxicity.

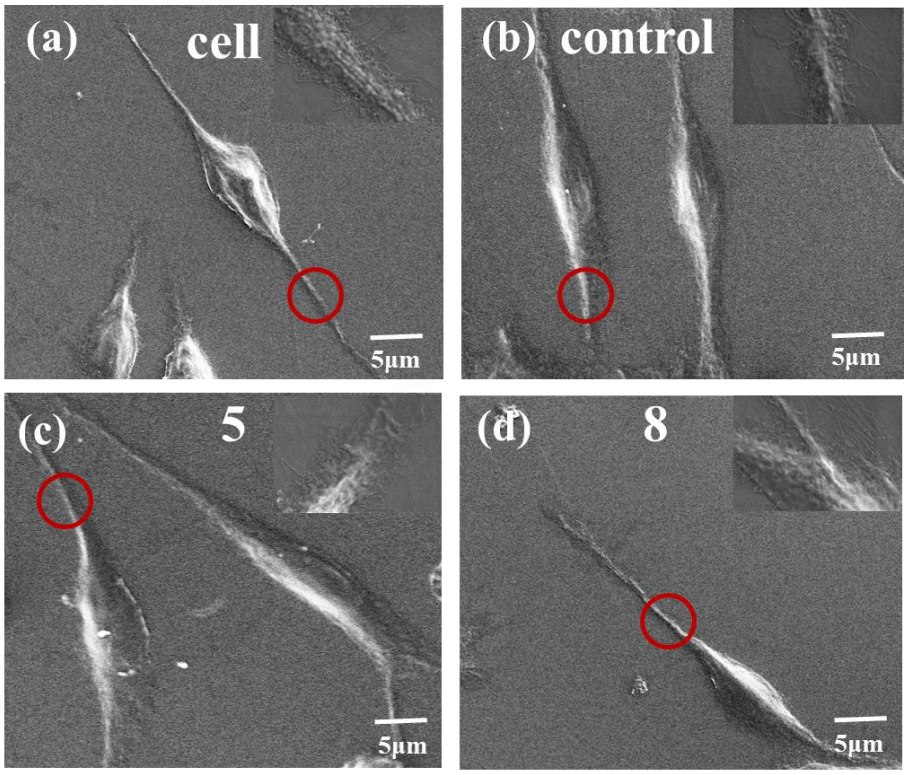


Fig 6.9 SEM images of cell morphology. (a) cells; (b) cells incubated with raw cotton fabrics; (c) and (d) cells incubated with modified cotton fabrics

After 5 days incubation, the cells was fixed and dehydrated. Then the cells were

observed under SEM. Fig 6.9 showed the cells morphology of cells (Fig 6.9, a), cells incubated with raw cotton fabric (Fig 6.9, b) and cells incubated with modified cotton fabric (Fig 6.9, c,d). The small images on the top right corner showed the amplification of the labeled part with red circle. It showed that the cells' morphology is integrated without breaking. And the flagellums did not atrophy. It further indicates that the modified cotton fabrics are non-cytotoxicity to cells.

6.3 Conclusions

In this chapter, amino sulfobetaine is attached to cotton fabrics via the cross-linking of BTCA. The air permeability and tearing strength of the modified cotton fabric decreased with the increasing of BTCA concentration. The antibacterial activity was evaluated using inhibition zone method and viable cell counting method. It showed that the modified cotton fabrics exhibit excellent antibacterial activity. Also after 30 times laundering, the antibacterial rate can reach above 90%, which indicates good washing durability. The modified cotton fabric showed good non-fouling property which can resist biofilm formation on surface. Via MTT method, SEM and fluorescence observation, the modified cotton fabric showed non-cytotoxicity. It is good candidate that can be used in medical materials.

Chapter 7

Conclusions and suggestions for future works

7.1 Conclusions

7.1.1 Pyridine sulfobetaine

In this study, pyridine sulfobetaine has been successfully synthesized. At a lower concentration, para-pyridine sulfobetaine showed excellent antibacterial activities when acted on gram-negative *E.coli* and gram-positive *S.aureus*, with the MIC of 8.36 μmol/ml and 10.45 μmol/ml against *E.coli* and *S.aureus* respectively. The antibacterial rate of as-modified cotton fabric reaches 99.90% or higher against *E.coli* and *S.aureus* within 24 hours contact time. The modified cotton fabric showed efficient non-fouling antibacterial function which inhibited bacteria adhesion and biofilm formation on modified cotton fabric surfaces. In addition, the slight reduction of air permeability and great improvement of water absorption can help wound healing due to keeping moist. Furthermore, the modified cotton fabric is biocompatible with no skin stimulation and non-cytotoxicity. Therefore pyridine sulfobetaine could be a good alternative for medical fabric and other relevant applications.

7.1.2 Triazine sulfobetaine

s-triazine sulfobetaine was synthesized. The antibacterial activity was measured via bacteria growth kinetics. The MIC is 2 mg/ml against gram-positive *S.aureus* and more than 6 mg/ml against gram-negative *E.coli*.

7.1.3 BTCA-amino sulfobetaine

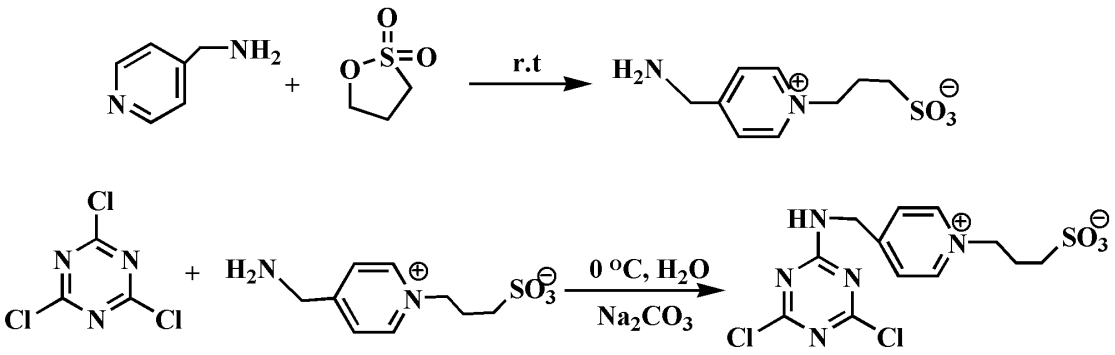
Amino sulfobetaine is attached to cotton fabrics via the cross-linking of BTCA. The air permeability and tearing strength of the modified cotton fabric decreased with the increasing of BTCA concentration. The antibacterial activity was evaluated using inhibition zone method and viable cell counting method. It showed that the modified cotton fabrics exhibit excellent antibacterial activity. Also after 30 times laundering, the antibacterial rate can reach above 90%, which indicates good washing durability. The modified cotton fabric showed good non-fouling property which can resist biofilm formation on surface. Via MTT method, SEM and fluorescence observation, the modified cotton fabric showed non-cytotoxicity. It is good candidate that can be used in medical materials.

7.2 Future work suggestions

This study developed three antibacterial agents and all of them exhibited advantages and disadvantages. The further research mainly focuses on the improvement of disadvantages and investigation of further and wide applications.

For the pyridine sulfobetaine, it exhibited good antibacterial activity, non-fouling property, non-cytotoxicity and no skin stimulation. However, its washing durability is not ideal. On the one hand, we can apply it onto one-off medical cotton fabric, which can solve the antibacterial contamination problem without durability requirement.

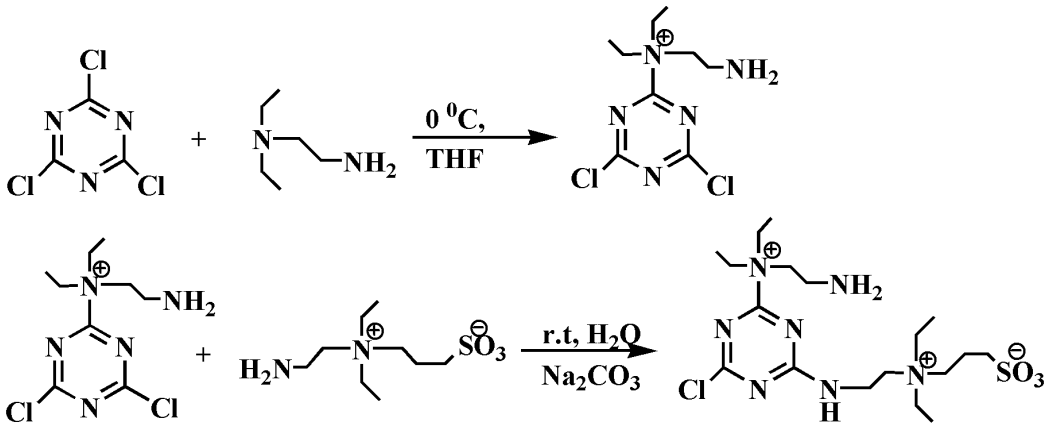
What's more, the modified cotton fabrics exhibited good air permeability and water absorption ability, which are significant to wound care. On the other hand, we need to investigate the washing durability. Though the siloxane group can react with the hydroxy groups of cotton fabrics, the chemical bond between Si-O-C is not strong which leads to the non-ideal washing durability. So we can combine pyridine sulfobetaine with cyanuric chloride. The chloride atoms can react with the hydroxy groups of cotton fabrics to form C-O-C chemical bond, which can give cotton fabrics durable washing ability. The scheme is shown in Scheme 7.1.



Scheme 7.1 Synthetic routine of triazine-pyridine sulfobetaine

For the s-triazine sulfobetaine, it can give modified cotton fabric durable washing ability. But the productivity is low. The next step is to optimize synthesis technology. Then it can be used in cotton fabrics. And we can investigate the properties of the modified cotton fabrics, such as antibacterial activity, air permeability, tearing strength, non-fouling property and skin stimulation. What's more, the chloride atoms in cyanuric chloride can be di-substituted by sulfobetaine and quaternary ammonium salts, which

can improve the antibacterial activity. The scheme is shown in scheme 7.2.



Scheme 7.2 Synthetic routine of triazine-QAC-sulfobetaine

For the BTCA-amino sulfobetaine, it exhibited good antibacterial activity and washing durability after 30 times washing. Vitro cytotoxicity test confirmed that the modified cotton fabrics are non-cytotoxicity. But the tearing strength of modified cotton fabrics decreased a lot. The next step need to modify the finishing technology to improve the tearing strength.

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