Surface Modified Polymers to Introduce Antibacterial Activities



Haiming Li

Wolfson College

University of Oxford

A thesis submitted for the degree of MSc by research

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Abstract

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This thesis is concerned with the chemical surface modification of several commercial industrial polymers in order to introduce antibacterial activity. Surface modification of polymers including LDPE, PE-fibre, MDPE, UHMPE and PS-XAD4 by carbene insertion reaction and diazo coupling was carried out using two diazo systems, urea diaryl diazo system and bis (dimethylamino) diaryl system. Successful surface modification on polymers was achieved while bulk properties of polymers were maintained.

ATR IR and combustion analysis were used to characterize modified polymer. Clear ATR IR absorption peaks and colour change during diazo coupling indicated successful surface modification. Surface coverage of modified polymers was calculated using nitrogen content detected by combustion analysis. Modified polymers binding with several biocidal agents including hydrogen peroxide, bleach and penicillin V were prepared and antibacterial activity was tested against *S. aureus* and *E. coli*. Modified polymers using the urea diazo system showed negative bioassay results, while modified polymer using the bis (dimethylamino) diaryl system showed excellent antibacterial activity.

Acknowledgement

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Finally my thanks would to my parents and friends for their loving consideration and support for me in this project.

Abbreviations

A^o	limiting area
Ar	aryl
ATR	attenuated total reflectance
DCM	dichloromethane
DMSO	dimethylsulphoxide
E. coli	*Escherichia coli*
Et	ethyl
EPR	electron paramagnetic resonance
ESI	electrospray ionisation
h	hours
hv	irradiation
Hz	Hertz
IR	infra-red
J	coupling constant
λ	wavelength
λ_{max}	wavelength maxima
Me	methyl
min	minutes
mp	melting point
MS	mass spectrometry
m/z	mass/charge ratio

NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
Ph	phenyl
ppm	part per million
PS	polystyrene
PS XAD	polystyrene Amberlite® XAD4
R	alkyl
S. aureus	*Staphylococcus aureus*
t	*tert*
THF	tetrahydrofuran
TLC	thin layer chromatography
UV	ultra-violet
v_{max}	absorption maxima
v/v	volume/volume
VIS	visible
w/v	weight/volume

Contents Page

1. Introduction 1

1.1 Antibacterial polymers 1

1.1.1 Background 1

1.1.2 Design and Classification of Antibacterial Polymers 3

1.1.3 Factors Affecting the Antibacterial Activity 11

1.1.4 Major Applications of Anbacterial Polymers 14

1.2 Surface Modification Techniques 18

1.3 Diaryldiazo Compounds 20

1.4 Structure and Reactivity of Carbenes 21

2. Results and Discussion 22

2.1 Urea diaryl diazo system 22

2.2 Bis (dimethylamino) diaryl system 37

3. Conclusion 48

4. Future work 49

5. Experimental 50

6. Reference 64

1. Introduction

1.1 Antibacterial Polymers

1.1.1 Background

Bacterial contamination by microorganisms has drawn lots of attention recently in many areas, such as medical devices, hospital, food packaging and storage, water purification, etc.[1] For example, Bowersock et al. showed that the infection caused by bacterial contamination has become a major problem in implant medical devices industry.[2] The traditional solution uses high doses of antibiotics to kill bacteria, but also generates high resistance to these antibiotics in the bacterial population. As a result, medical implant devices often have to be removed due to the infection, which cause serious damage to patients, hospital and medical devices industry.[1]

Antibacterial agents which could kill microorganisms are one solution to this problem. Kenawy et al. demonstrate that antibacterial agents with low molecular weight have been used for antibacterial drug and food preservation industry.[1,3] However, the residual toxicity is a unsolved problem.

Heat and ionizing radiation are traditional treatments to used kill bacteria attached on polymer materials which are used in medical devices.[4] But when these materials are exposed, contamination by microorganisms can still be found. As a result, antibacterial polymers, which show high resistance to

bacterial contamination, are considered to be an ideal solution. Recently antibacterial polymers have attracted significant interest in various areas. For example, antibacterial polymer fibres are used as bandages in hygienic applications, and antifungal polymer coatings are also widely used in swimming pool and shower walls, which reduce not only biofouling but also pathogenic microorganisms releasing.[5]

The application of antibacterial polymers has numerous advantages. For example, Worley et al.[1] showed that using these polymers could make several antibacterial agents more efficient. Therefore the loading of antibacterial agents, as well as the residual toxicity, can be reduced. In addition, Worley's research demonstrated that the lifetime of antibacterial agents has been extended by using antibacterial polymers. Polymeric antibacterial agents are also more stable, which minimize the losses caused by photolytic decomposition and transportation.[1] As previously indicated, the infection caused by bacterial contamination is the main cause of implant failure in medical devices industry. By using antibacterial polymers, this type of loss can be significantly reduced.

Since an antibacterial polymer has numerous advantages and wide potential applications, an ideal antibacterial polymer should meet some basic requirements, as shown in Table 1.1 according to Worley.[1]

Basic Requirements for Antibacterial Polymers
1. Easily synthesised and low cost
2. Long lifetime and stable in the storage
3. No decomposition or emit toxic products
4. No toxic or irritating to people
5. Can be regenerated when losing antibacterial activity
6. Biocidal to a wide range of pathogenic microorganisms
7. Not soluble in water

Table 1.1. Basic requirements of antibacterial polymers[1]

1.1.2 Design and Classification of Antibacterial Polymers

Timofeeva et al. showed that the design of antibacterial polymer needs to be complementary to the structural characteristics of outer envelope of various bacterial cells.[6] Based on their research, one important characteristic is the net negative charge, which is "provided by the lipoteichoic acid molecules of Gram-positive bacteria cell wall (CW), the lipopolysaccharides and phospholipids of Gram-negative bacteria outer membrane (OM), and the cytoplasmic membrane (CM)".[6] According to the structural characteristics of the net negative charge, a major part of antibacterial polymer design is the inclusion of cationic hydrophilic–hydrophobic macromolecular systems, the target of which is cytoplasmic membrane.[6] The aim of antibacterial polymers is to provide high surface-activity, adsorption/absorption activity and binding

ability for bacterial cells to disrupt the structure of cell membranes, resulting in the leakage of cytoplasmic contents and cell lysis.[6] Based on the reaction mechanism, antibacterial polymers are generally classified into three types, quaternary ammonium/phosphonium polymers, N-halamine-based antimicrobial polymers and sliver containing polymers, and these are discussed in more detail below.

Quaternary ammonium/ phosphonium polymers

Ikeda et al. demonstrated that quaternary ammonium/ phosphonium polymers had high antibacterial activities against *Staphylococcus aureus* and *E. coli*.[7] In their research, polymethacrylate containing pendant biguanide groups and polyvinylbenzyl ammonium chloride were synthesized (shown in **Figure 1**) and characterized by various methods, including TEM, AFM, and monitoring of the loss of bacterial cells was performed to investigate the biocidal activities of quaternary ammonium/phosphonium polymers.[6,7] According to their results, high antibacterial activities are introduced when polymers contain a cationic charge on the quaternary ammonium/phosphonium groups, which disrupted either outer membrane or cell wall, resulting in cell lysis.[6,8,9] In addition, phosphonium polymers, which were synthesized by Mahmound and Kenawy using copolymers with phosphonium salts, showed high antibacterial activities. Using these materials, the cytoplasmic membrane (CM) was disrupted by phosphonium cationic polymers and potassium ions were released. [9]

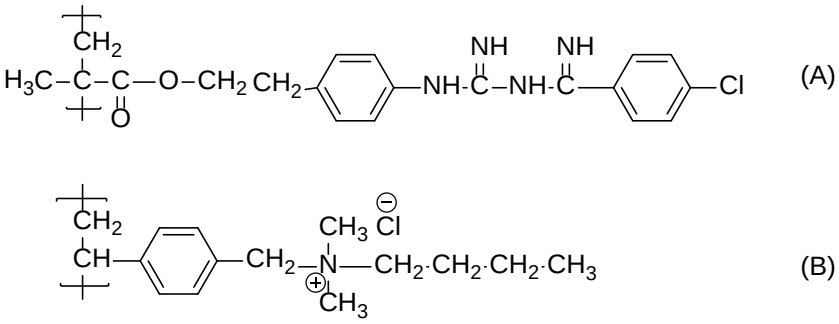
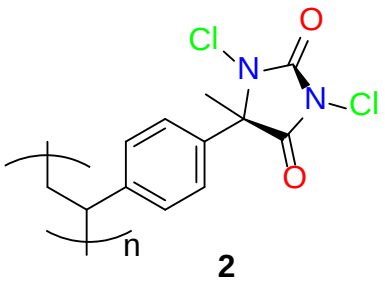


Figure 1. (**1a**) Polymethacrylate containing pendant biguanide groups. (**1b**) Polyvinylbenzyl ammonium chloride

N-Halamine-based antimicrobial polymers

N-Halamine-based antimicrobial polymers are another major type of antibacterial polymers, which are widely used in medical devices to prevent the transmission of infection after surgery. N-Halamine-based antimicrobial polymers refer to polymers which contain one or more nitrogen-halogen covalent bonds,[10] usually generated by the halogenation of imide, amide or amine groups.[10,11] The reaction mechanism of N-Halamine–based polymers is distinct from the quaternary ammonium/ phosphonium polymers. The halogen species are stabilized by nitrogen atoms and slowly released into the environment as the parent halogen (chlorine, bromine, iodine) to impart antibacterial activities.[6] Oxidative halogen species transfer through the charged microbial cell walls, resulting in cell inhibition or cell inactivation. Compared with inorganic halogens such as chlorine or bromine, organic N-Halamines are more stable, less corrosive and have less tendency to generate halogenated hydrocarbons.[12]

N-Halamine-based antimicrobial polymers have been significantly developed by Worley and Sun.[1, 5, 11] For instance, they used polystyrene as the polymer substrate and when it was appropriately modified and when treated with chlorine gave poly (l,3-dichloro-5-methyl-5-(4'-vinylphenyl)-hydantoin) which contained two nitrogen-chlorine bonds (**Figure 2**). This compound performs as a contact biocidal polymer which showed high antibacterial ability against *S. aureus* and *E. coli*.[13] They also used the same polymer but treated it with bromine. Two materials were tested at different pH, temperature and flow rate. The results showed that N-bromine polymer had higher biocidal ability and was more efficient than the N-chloramine polymer, [14] suggesting that N-bromoamines and free bromine are more biocidal than N-chloroamines and free chlorine.

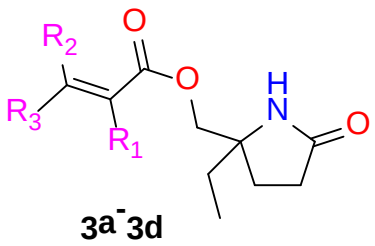


2

Figure 2. The structure of poly (l,3-dichloro-5-methyl-5-(4'-vinylphenyl)-hydantoin) **2**[13]

Eknoian and Worley have developed a new series of N-halamine poymers by grafting N-halamine monomer such as 4-(alkyl acryloxymethyl)-4-ethyl-2-oxazolidinones (**Figure 3)**, with commercial

monomers or polymers, such as polystyrene, poly vinyl chloride, poly vinyl alcohol. [15] All the resulting grafted polymer such as poly (acrylonitrile-co-3-halo-4-(alkyl acryloxymethyl)-4-ethyl-2-oxazolidinone) and poly (vinyl alcohol-co-3-halo-4-(alkyl acryloxymethyl)-4-ethyl-2-oxazolidinone), performed as efficient disinfectants to inactive bacteria based on the positive results of granular bacterial testing.[15] They also coated these N-halamine polymers on different substrates and still showed as surface active disinfectants.

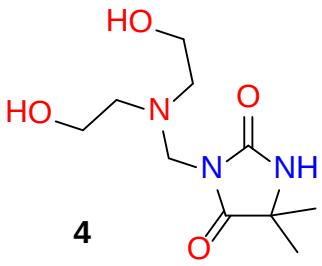


3a = R_1 = R_2 = R_3 = H
3b = R_1 = R_3 = H, R_2 = CH_3
3c = R_1 = R_2 = H, R_3 = CH_3
3d = R_1 = H, R_2 = R_3 = CH_3

Figure 3. Structures of several N-halamine monomers **3a-3d** used in the experiments[15]

Worley and his coworkers have synthesized a novel water-soluble N-halamine monomer (**Figure 4**) which can be coated and recycling used on polymer substrates. They copolymerized a waterborne acrylic polyol and an isocyanate to prepare a coating for polymer substrates, such as polyurethane.[16] The coating was chlorinated by submersion into bleach. The chlorinated N-halamine coating on polyurethane showed high inactivation of bacteria in granular bio-testing. More importantly, after the chlorine loading, as well as the

antibacterial activity, decreased, recycling was achieved by exposing the coated polyurethane to more free chlorine.[16] The lifetime of biocidal coating was measured for over six months, which suggested this polymer might find potential use in medical devices and antifouling areas.



4

Figure 4. Structure of unhalogenated N-halamine monomer **4**

Since Worley's research shows polymer N-halamine coating on polymer substrates may have long lifetime and can be recycled, both of which are significant advantages in industry, further studies were developed in this area. Sun and his co-workers have conducted similar researches about N-halamine coating on polymer substrates. They synthesized unhalogenated N-halamine polyurethane polymers using 5, 5-dimethyl hydantoin (DMH) as N-halamine precursor, and 1,6-hexamethylene diisocyanate (HDI) as a coupling agent.[17] Once the polymer was treated with bleach, covalent bounded DMH moieties were transformed into N-halamines.[17] The stable N-halamine groups provided efficient, long-term and rechargeable antibacterial abilities against *E. coli* and *S. aureus*.

Worley and Sun and their co-workers synthetized a novel cyclic-amine monomer, 1-acryloyl-2,2,5,5-tetramethylimidazolidin-4-one (ACTMIO).[18] The ACTMIO was copolymerized with commercial acrylic and vinyl monomer to generate unhalogenated N-halamine monomers.[17] After N-halamine monomer was grafted on polymeric substrates such as polypropylene and polyester, bleach was treated on polymers to generate antibacterial activities.[17] Bioassay test results showed positive, durable and recyclable antibacterial efficacies against *E. coli*. Worley suggested the reason might be some amide groups in the copolymer transferred into stable N-halamine groups.

Worley and his co-workers not only studied carbon-based N-halamine polymers but also silicon-based N-halamine polymers. N-Halamine 3-triethoxysilylpropyl-5, 5- dimethylhydantoin was synthesized and coated on polymers, such as paper, cotton and polyurethane paints.[19] After polymer surfaces were treated with bleach, biodical efficacy test was conducted to investigate their antibacterial abilities. Positive testing results showed polymers had high antimicrobial ability against *S. aureus*.

Silver containing polymers

Except for N-halamine-based antibacterial polymers, silver is also widely used in producing antimicrobial materials. For instance, Sambhy and his co-workers used silver bromide nanoparticle/polymer composites to investigate their

antimicrobial ability.[20] The bioassay test results showed that the composite had high antimicrobial activities against both Gram-negative (such as *E. coli*) and Gram-positive (such as *S. aureus*) bacteria on surfaces and in solution.[20] Radheshkumar and coworkers investigated the silver antibacterial activity of polypropylene containing elemental silver composites.[21] They found that antibiocidal species Ag^+ was released from composite in aqueous environment.[21] The released silver ions showed not only excellent antibacterial efficiency but also long releasing time.[21] Sondi and his coworkers investigated the antibiocidal mechanism of silver nanoparticles and found that silver nanoparticles had high antibacterial activity against *E. coli* by damaging cell wall and penetrating through cell membrane to kill bacteria.[22]

However, the unavoidable toxicity of silver containing antibacterial materials has been noticed and studied by scientists. For example, Jones and Hoek investigated antibacterial effects of different silver nanomaterials.[23] They tested antibacterial activity and toxicity of silver nanoparticles, stabilizer silver salts, and polymer/silver composites. All silver- containing materials showed antibacterial activities but silver particle toxicity is unavoidable. The toxicity is influenced by not only silver particle features, such as size, shape and crystallinity, but also chemical conditions, such as pH, ionic strength and composition.[21] They found that large amount of silver nanoparticles had cytotoxic and genotoxic effects in organisms including humans.[23] They

suggested that silver should only be carefully used as antibacterial materials. Atiyeh and co-workers did similar research about the properties and application of silver containing materials.[24] They found that when silver sulfadiazine was used to treat burn wounds, cells like keratinocytes and fibroblasts were killed by exposing to silver.[24,2526]

1.1.3 Factors Affecting the Antibacterial Activity

The antibacterial activity of polymers is influenced by various factors, such as molecular weight, alkylation, and nature of counterions.[1, 6, 7, 27]

Effects of molecular weight

Molecular weight of polymers has profound effects in the determination of antimicrobial activities. For instance, Cooper and co-workers found that the antimicrobial activity of quaternary ammonium poly (propyleneimine) showed a parabolic dependence on molecular weight.[27, 28] Hönig and coworker found that activity rapidly decreased with the loss of molecular weight.[29]. Ikeda and his co-workers found that molecular weight (MW) played an important role in controlling antimicrobial activity of polyactoins. The relationship of MW and activity show a bell-like shape curve, which indicated an optimal MW range to achieve best antibacterial activity.[23] Ikeda and colleagues suggested a possible theory to explain the dependence of antimicrobial activity on molecular weight. They consider the lethal process of cations biocides as

follows: “(1) adsorption onto the cell surface of bacteria, (2) diffusion through the cell wall (CW), (3) adsorption onto the cytoplasmic membrane, (4) disruption of the cytoplasmic membrane, (5) leakage of the cytoplasmic constituents, and (6) death of the cell”. [1,30] As molecular weight increases, adsorption of higher MW polycations (step 3) on bacterial cell is more likely to occur than that of monomeric cations.[1,22]

Alkylation effects

Among various structural parameters of polymer which have profound effects on antimicrobial activity, the length of the alkyl chain is the most important one. For example, Ikeda and his colleagues found that poly (alkyldimethyl(vinylbenzyl)ammonium chloride) showed best antimicrobial efficacy when alkyl radical reached the longest C_{12}.[1, 7] However, the biocidal activity of polymers does not always show a proportional relationship with longer alkyl tails. Chen and co-workers found that quaternized poly(propyleneimine) showed a parabolic dependence of biocidal activity on the chain length of quaternary ammonium groups. [1, 24] Their results showed highest activity was achieved when the chain length reached C_{10}.[1, 24] Klibanov and colleagues found that the length of alkyl tail of quaternized PVP polymers should be not over C_6 in order to avoid the rapid decrease of biocidal activity.[1,31] They suggested the decreasing activity was due to the aggregation of polymer chains with the long alkyl tails on the surface.[1, 27] Meanwhile,

although longer length of alkyl chains can enhance the adsorption/absorption ability to kill bacteria, it changes the hydrophilic-hydrophobic balance of antimicrobial polymer which is also an important role in killing bacteria.

Counterion effects

The antimicrobial activity of poly [tributyl (4- vinylbenzyl) phosphonium] salts synthesized by Kanazawa and his co-workers showed dependence on counteranion against *S. aureus*.[1,32] The biocidal activity was founded to be low in the presence of a counteranion. They suggested that the counteranion had tendency to form tight ion pair with phosphonium ion to prevent the release of free ions.[1, 28] Cooper and colleagues found that the biocidal activity of the quaternary ammonium polymers had relationship with various types of counteranion.[24] Based on their research, polymers with bromide anions showed better biocidal activity than that with chloride anions.[24] However, Panarin and his co-workers found that the antimicrobial activity of synthesized homopolymer with quaternary ammonium groups showed on relationship with types of counteranions, including chloride, bromide and iodide. The mechanism of how counteranions affect biocidal activity is not clear and further research is needed in the future.

1.1.4 Major Applications of Antibacterial Polymers

Since antibacterial polymers have been developed for decades, they are

widely used in various fields, such as water treatment, medical and health-care industry, food industry and textile products.

Water treatment

Soluble chlorine containing disinfectants are commonly used to sterilize water. However, the toxicity of residual agents causes serious and unavoidable problems.

For instance, the residue can accumulate in the food chain in the environment.[1] Meanwhile, carcinogenic chemicals, such as trihalomethane analogues, are generated when free chlorine or related compounds react with organic substances in the water.[1] A possible solution for this problem is to use insoluble disinfectants to kill microorganisms in the water by mere contact and with release of few reactive biocidal agents.[1,15,33] Antibacterial polymers are ideal for this application. For instance, Tyagi and co-workers synthesized an insoluble polymer disinfectant which is an insoluble matrix based on iodinated poly(methyl methacrylate-*co*-*N*-vinyl-2-pyrrolidone).[1, 34] Worley and co-workers have prepared several N-halamine antibacterial polymers for water treatment.[35,36,37] They found that N-halamine antibacterial polymers were ideal in this application due to their high biocidal ability, long-term stability and recharge ability.[1]

Application in medical and health-care industry

In hospitals, active agents with low molecular weight compounds, which can easily penetrate into cells and quickly excreted from human body, tend to be used.[1] Therefore, high and repeated doses are required to maintain the therapeutic effect, which results in serious problems, such as drug resistance.[1]

An ideal solution is to attach drugs such as antibacterial agents to a macromolecular biodegradable polymer matrix. This solution can not only improve therapeutic efficacy but also control the safety of delivery and drug release rate.[1] Kenawy and co-workers prepared an antibacterial polymer which contained electrospun fibers containing tetracycline hydrochloride on polymer matrix, such as poly (ethylene-*co*-vinyl acetate) and poly(lactic acid).[1, 38, 39] Their study showed a smooth release of tetracycline hydrochloride.

Except for drug delivery, antibacterial polymers are also widely as implant medical devices. Since bacterial infection during post-surgery recovery results in serious problems, antibacterial polymers are preferred to be used as medical implant devices due to their high biocidal ability, long-term stability and recharge ability.

Food industry

Microorganism contamination has always been a major problem in the food industry. The contamination not only causes shorter the shelf-life of food, as well as lowered industry profit, but also increases the risk of illness caused by ingestion of contaminated food.[1] As a result, easily processed and effective antibacterial food packaging has become a major challenge in the food industry.[1, 40] Traditional methods of preventing food from microorganism contamination include thermal processing, freezing, irradiation and adding antibacterial agents.[1] However, the traditional methods are not suitable for all food products, such as fresh meats or fish and ready-to eat products. [1,41]

Antibacterial food packaging is a possible solution. Antibacterial polymers are considered to be an ideal material for antibacterial food packaging industry. Jatomea and co-workers investigated the biocidal mechanism of antibacterial food packaging. They suggested three mechanisms as follows: “(1) reducing the growth rate and maximum growth population of bacteria, (2) extending the lag phase of microorganisms, (3) inactivating bacteria by contact”. [1, 42] Hotckiss showed that antibacterial polymer packaging could significantly prolong the shelf-time of food and reduce the growth rate of bacteria by direct contact. [1,43] Meanwhile, antibacterial polymer packaging reduces the risk of recontamination during processing and delivery of food products and they are

specifically used in self-sterilizing food products, such as fruit juices.

Textile products

Recently antibacterial polymers have drawn lots of attention in manufacturing textile products, such as sportswear and hygienic clothing. Antibacterial textile products can not only protect users from bacteria but also prevent textile products from biodeterioration. Vigo and co-workers prepared antibacterial fibre based on poly (vinyl alcohol) (PVA) for textile products. The fibre reacted with 5-nitrofurylacrolein showed antibacterial activity when nitro compound was released in the presence of moisture (**Figure 5**). [1, 44] Antibacterial polyamide fibres (known as nylon) are widely used in textile products. Buchenska showed that polyamide 6 (PA6) grafted with acrylic acid showed excellent biocidal activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. Coli*.[1,45]

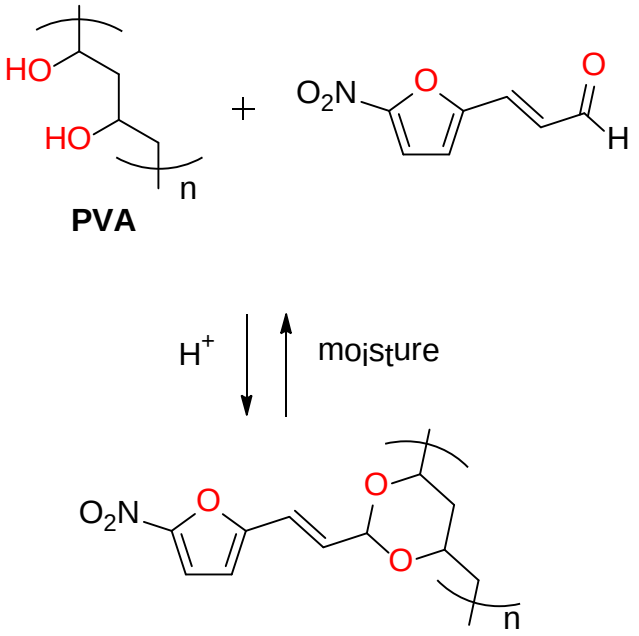


Figure 5. Synthesis of PVA based antibacterial fibre under acidic conditions with

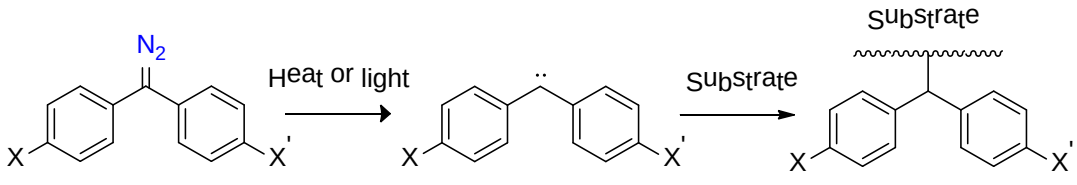
(E)-3-(5-nitrofuran-2-yl)acrylaldehyde and its reversibility by the addition of water[1]

1.2 Surface modification techniques

Polymers which function as antibacterial materials have drawn significant levels of attention because of their numerous advantages, including diversity of chemical functionality, low cost, easy handling and wide availability.[46] However, the surface modification methods to control surface characteristics to suit a specific application without changing the bulk properties of polymers are of vital importance. Traditional modification methods include mechanical treatments, chemical treatments, and surface activation techniques.[47] For example, a common mechanical treatment is abrasion to increase the roughness of surface of substrate materials, which is performed with either emery paper or water jets containing abrasion powders.[1] Traditional chemical treatments are oxidation and chlorination. Several new surface activation techniques such as atom bombardment, plasma treatment, laser treatment, electron beam are now widely used in surface modification industry.[48] For example, Huh et al. exposed poly(ethylene terephthalate) (PET) texture to oxygen plasma glow discharge to generate peroxides on the surface.[49] However, these surface modification methods are not ideal because they usually require significant infrastructure and high operating cost. For chemical treatments, because of the harsh reaction conditions, the number of polymer materials, which are suitable for these surface modification methods to achieve direct surface modification, is also limited.[2] As a result, an alternative

method, which is versatile for a wide range of polymers and still efficient under mild reaction conditions to achieve direct surface modifications, needs development.

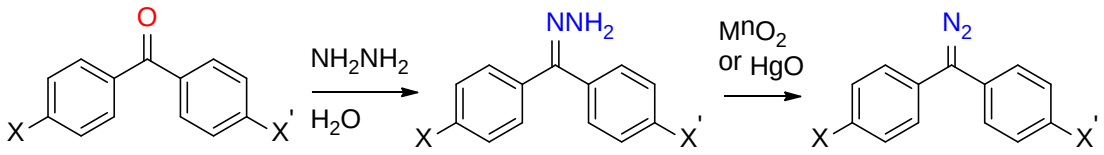
Surface modification using carbene insertion reaction would seem to be a possible chemical method for this application. Carbene insertion chemistry could introduce diverse chemical functionality on the surface of substrate materials without changing bulk properties of materials.[50] The Moloney group has done extensive research in this field, showing that surface modification can be successful achieved by the reaction between the surface of substrate materials and diaryldiazo compounds which are used as carbene procursors while the bulk properties of substrate materials are maintained. As shown in **Scheme 1.1**, the carbene is generated by exposing diaryldiazo compounds to UV light or heat, which then reacts with the surface to achieve covalent bonding.[51] Using this technique, not only a wide range of materials, such as polystyrene, ultra-high molecular weight polyethylene, silica and diamond have been reported to be successfully modified but also their colouration, biocompatibility, protein binding , protein adhesion, changes in contact angles, introduction of antibacterials activities can be enhanced. [52,53]



Scheme 1.1. Surface modification using carbene insertion reaction

1.3 Diaryldiazo Compounds

The generation of diaryldiazo compounds is of vital importance in the surface modification using carbene insertion chemistry. The general synthetic procedures of diaryldiazo compounds, which are convenient carbene precursors, are shown in **Scheme 1.2**. Benzophenone initially reacts with hydrazine monohydrate, to give the hydrazone and then oxidation is performed using manganese (IV) dioxide or mercury oxide to generate the desired diaryldiazo compounds. The key to a successful synthesis of diaryldiazo compound is the functional groups attached to the aryl-rings. Unlike carbenes, the precursor diaryldiazo compounds are less reactive and can be stored for a longer time at cold temperature in the dark. Exposure to light or high temperature causes decomposition of diaryldiazo compounds to the desired carbenes.



Scheme 1.2. Synthesis of diaryldiazo compounds

With the successful synthesis of diaryldiazo compounds, surface modification could be performed. After evaporating solvents, under mild reaction conditions (heat or light), reactive carbenes would be generated with the decomposition

of diazo compound.[4] An irreversible carbene reaction occurs on the surface of substrate materials, either by bond-insertion to X-H (X=C, O, N, S) or addition reaction at C=C bond. [2, 6, 7]

1.4 Structure and Reactivity of Carbenes

Carbenes are very reactive intermediates at room temperature due to their specific structure, in which one carbon atom possesses six valence electrons, usually in sp^2 hybridisation. Four electrons form two bonds in sp^2 orbitals while the other two are lone pair electrons.[4] According to the spin state of lone pair electrons, carbenes are divided into two states, singlet and triplet states, as shown in **Figure 6.** In solution, as previously described, the selectivity of singlet state carbenes is reacting via X-H insertion reactions where X commonly includes oxygen, nitrogen, sulphur and carbon.[54] Triplet state carbenes, on the other hand, react via radical pathways with abstraction and recombination process.[8]

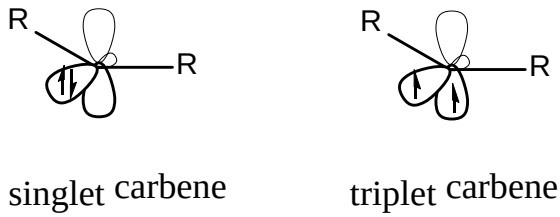


Figure 6. Two states of carbene intermediates

2. Results and Discussion

2.1 Urea diaryl diazo system

Urea diaryl diazo system has been proven to be successful in the surface modification of polymers based on the research of Moloney's group.[55] PS-XAD4 beads were used and successfully modified using urea diaryl diazo system. After modification, modified polymer was treated with hydrogen peroxide and showed high antibacterial activity in bioassay tests. In the modification, urea diaryl compound acts as a host for binding hydrogen peroxide due to hydrogen bonding formed between two molecules, which results in high stability and general releasing of hydrogen peroxide functioning as biocidal agents. PS is chosen as the polymer matrix because of its porous structure and high surface area. However, in the polymer industry, PS is not an ideal material due to the porous structure and poor mechanical properties such as tensile strength and yield strength. Polyethylene, on the other hand, is a widely used commercial polymer material in material industry as a result of its relatively high mechanical properties and low prices. But polyethylene was rarely used in surface modification because of its dense structure and low surface area. In this project, the aim is the surface modification of polyethylene as a novel polymer matrix using urea diaryl diazo system based on prior research.

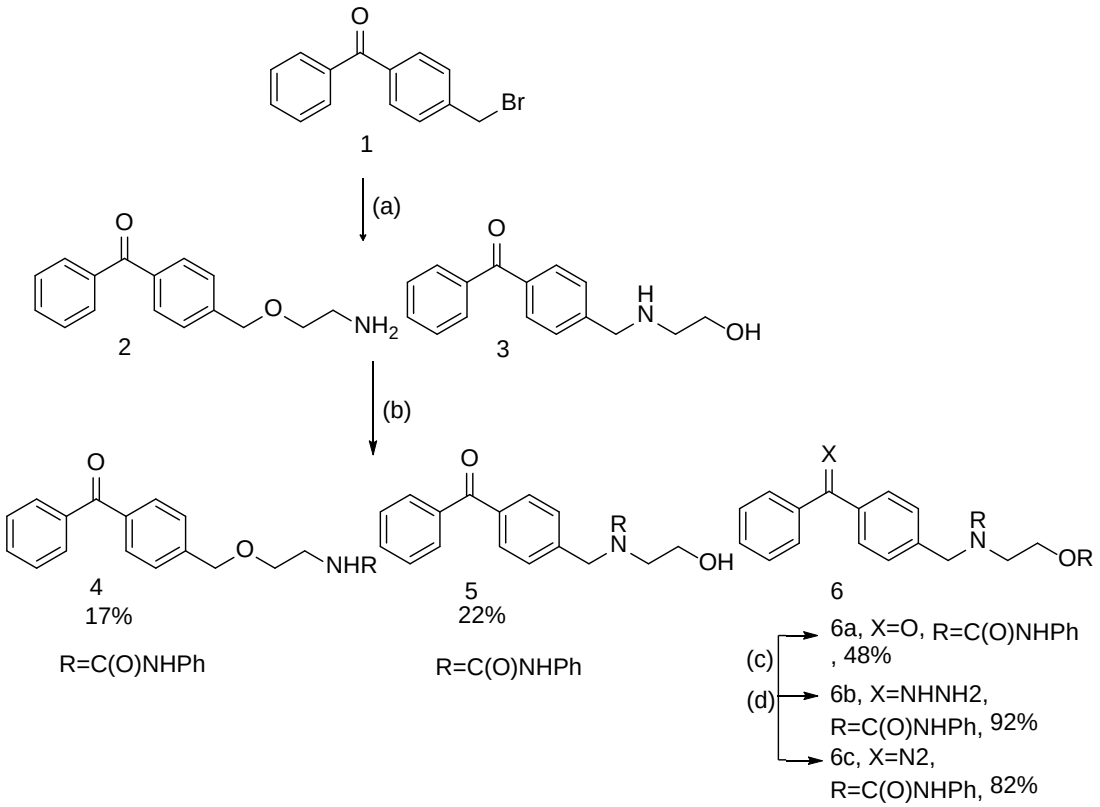
Amine compounds **2** and **3** was synthesized by the reaction of 4-bromomethylbenzophenone **1** with ethanolamine through a known procedure, as shown in **Scheme 2.1**.[55, 56] The presence of the $ArCH_2O$ system in compound **2** was confirmed by the singlet at a chemical shift of δ 4.63, resulting from the benzylic methylene group.[51] The separation and purification of compound **2** and **3** by flash column chromatography were impossible due to the similar polarities of two compounds.

Compound **4**, **5** and **6a** were synthesized by direction reaction of this mixture with phenylisocyanate and separation by flash column chromatography. Phenylisocyanate were used instead of hydrogen isocyanate because of the high stability of phenylisocyanate. Based on the MSDS provide by supplier company, the boiling point of phenylisocyanate is 158°C while the boiling point of hydrogen isocyanate is 38.5°C. phenylisocyanate is more stable because of its conjugation structure. Moloney and co-workers reported in their early work only the formation of compound **6a**.[57] This suggested that the reason of formation of **5** and **6a** might be the insufficient phenylisocyanate used in the reaction. In the reaction, the equivalence of phenylisocyanate used was 1.4. However, at least 2.0 equivalence of phenylisocyanate was needed to achieve the formation of **6a** from **3**. As a result, only the formation of 6a could be achieved using higher equivalence of phenylisocyanate. Compound **6a** was mainly investigated because the research of Moloney's group suggested that

compound **6a** gave better modification and higher surface loading on polymers.[51]

Compound **6a** was distinguished by IR spectrum (ketone absorption at 1722 cm^{-1} and urea absorption at 1654 cm^{-1}) and expected NMR spectroscopic data.[51] Hydrazone **6b** was synthesized by the reaction of compound **6a** with large numbers of equivalents of hydrazine monohydrate. Then hydrazone **6b** was converted to diazo compound **6c** by oxidation using manganese dioxide. Because diazo compound was highly reactive at room temperature and unstable in $CDCl_3$, clear NMR spectroscopic data of **6c** was impossible to get to confirm the chemical structure. However, IR absorption peaks at 1712 cm^{-1} (diazo) and 1651 cm^{-1} (urea) indicated the successful conversion from hydrazone **6b** to diazo compound **6c** had occurred. No decomposition of **6c** was observed when it was stored in the freezer at -4°C for weeks.[51]

Scheme 2.1. Introduction of urea function groups.

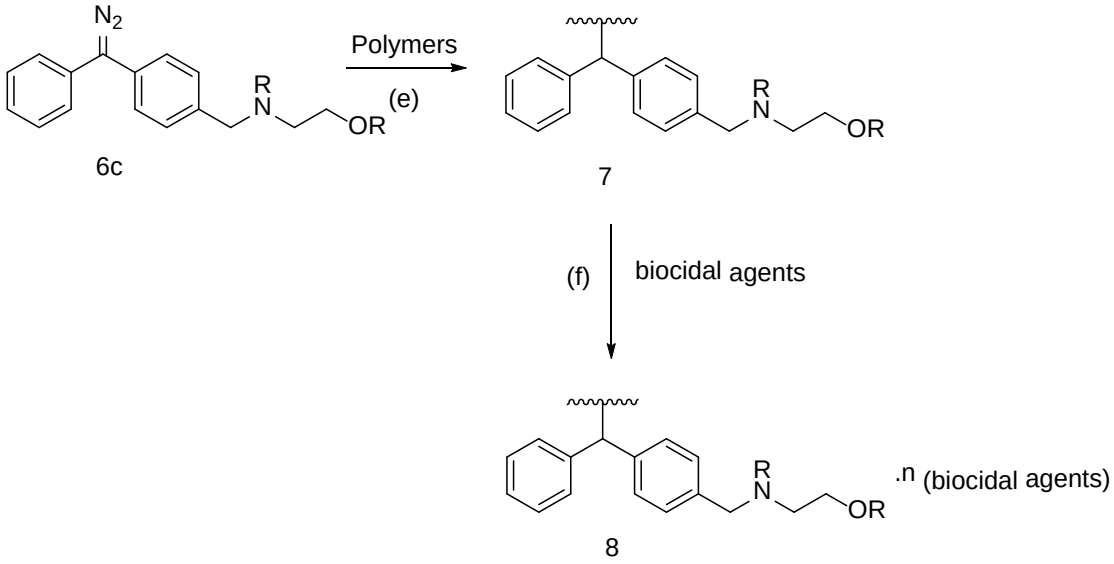


Reagents and conditions: (a) NaH, ethanolamine, 4-bromomethyl benzophenone, dry THF, stir for 18h; (b) triethylamine, phenyl isocyanate, dry DCM, stir for 16h; (c) hydrazine monohydrate (15 equivalences), methanol, reflux 16h; (d) MnO_2, $NaSO_4$, sat. KOH in ethanol, THF, stir for 16h in the dark

Diazo compound **6c** was used to modify the surface of several polymers (LDPE, MDPE, UHMPE and PE fibre,), as shown in **Scheme 2.2**. Absorption of **6c** onto the surface of polymer by removal of organic solvents under vacuum gave material which showed an even purple colouration over the surface. This materials with 6c absorbed on the polymer was heated at 110°C for 15

minutes, to give modified polymer **7**. Importantly decolouration was observed during the process, consistent with the collapse of the diazo compound.

Scheme 2.2 Coupling of diazo compound on polymers and binding with biocidal agents



Reagents and conditions: (e) heat at 110 °C for 15min and wash by water; (f) polymer submerged in biocidal agent solution for 18h in the freezer

ATR IR and Combustion Analysis

The modified polymer was characterized by ATR IR and combustion analysis.. The surface modification on polymer surface was confirmed to be successful because two C=O peaks at 1710 cm^{-1} (C=O stretching, urethane) and 1650 cm^{-1} (C=O stretching, urea) were observed.

The nitrogen content of polymer **7** was calculated by combustion analysis, as shown in **Table 2.1** and **Table 2.2**. All blank polymers showed no nitrogen

content in combustion analysis. Based on Table 2.2, the calculated surface coverage values of LDPE, MDPE and UHMPE powders are over 100%, which indicates the calculated results are unreliable. The reason for LDPE and MDPE powders is that nitrogen content on the surface of both materials is too low that it is beyond the detect limit of equipment. In addition, the calculated value of PE-fibre provides the upper-limit of surface coverage. Moloney and co-workers used same compound to modifify PS-XAD4 beads and the surface coverage of modified PS was 4.2%.[55] Compared to this value, modified polyethylene-based polymers seem to have higher surface coverage.

For the measurement of surface area, MDPE and UHMPE are expected to have higher values than LDPE because the size of MDPE used is about 100μm and the size of UHMPE is about 35-50μm while that of LDPE is about 1000μm. However, based on the BET analysis, the surface area of three materials is about the same. The reason might be that the surface area measurement of micro-sized polymer powders using BET is difficult due to the high sensitivity of equipment and the error of measured value might be high. Based on the measured surface area value of UHMPE, the calculated particle size that measured value implies (assuming spherical particles) is 2.52μm, which is much lower than the value (35-50μm) given by supplied company. The difference between two values indicates that the error of surface area measurement of micro-sized polymer powders using BET might be high. The

calculation are shown as follow,

$$\mathrm{S(Surface\ area)} = 2.5\ m^2/\mathrm{g}\ ,\ \mathrm{d\ (Density)} = 0.945\ (\mathrm{g}/cm^3)$$

$$m_0 = d \times V_0 = d \times \frac{4}{3}\pi r^3 = 3.96 \times 10^6 r^3 \mathrm{g}$$

$$\mathrm{N(number\ of\ particles)} = \frac{1\ \mathrm{g}}{3.96 \times 10^6 r^3\ \mathrm{g}} = 2.5 \times 10^{-7} r^{-3}$$

$$S_0\text{(surface area of one particle)} = 4\pi r^2 = 12.56 r^2\ (m^2)$$

$$\mathrm{S} = S_0 \times N = 2.5 \times 10^{-7} r^{-3} \times 12.56 r^2 = 2.5$$

$$\mathrm{r} = \frac{2.5 \times 10^{-7} \times 12.56}{2.5} = 1.26 \times 10^{-6} m = 1.26\ (\mu\mathrm{m})$$

$$\mathrm{Particle\ size\ of\ UHMPE} = 2\mathrm{r} = 2.52\ (\mu\mathrm{m})$$

Polymer	C/%	H/%	N/%
LDPE	85.44	13.84	<0.1±0.01
MDPE	84.64	14.04	<0.1±0.01
UHMPE	85.57	14.02	0.21±0.02
PE-fibre	82.72	13.81	0.13±0.01

Table 2.1 Combustion analysis of modified polymers using urea diaryl diazo system (%=% per gram) (Error in the %N is about 10%)

	%N per gram	Number of carbene compound on surface modification per gram	Surface area of Polymer using BET m^2/g	Number of carbene compound on surface modification per cm^2	%surface coverage
LDPE	<0.10± 0.01	1.43E+19± 1.43E+18	2.0	7.17 E+14± 7.17 E+13	<728±73
MDPE	<0.10± 0.01	1.43E+19± 1.43E+18	1.9	7.55E+14± 7.55E+13	<766±77
UHMPE	0.21± 0.02	3.01E+19± 2.87E+18	2.5	1.20E+15± 1.15E+14	<1223± 116
PE-fibre	0.13± 0.01	1.86E+19± 1.43E+18	42.0	4.44E+13± 3.41E+12	45±4

Table 2.2. Surface coverage of modified polymers using urea diaryl diazo system

Surface area measurement using BET

Surface area of polymers was measured using BET (Brunauer, Emmett and Teller) surface measurement instrument by Dr Ashley Shepherd. All the calculating procedures were conducted by equipment. Only the principles of calculation are introduced in this section. In BET theory, the surface area of a

powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface.[59] Physical absorption is due to van der waals forces between gas molecules and surface of test powders. The principle of calculation of surface area is treating experimental data to BET adsorption isotherm equation, which are shown below.

$$\frac{p}{v(p_0 - p)} = \frac{1}{v_m c} + \frac{c-1}{v_m c}\frac{p}{p_0}$$

p = partial vapour pressure of adsorbate gas in equilibrium with the surface at 77.4 K (b.p. of liquid nitrogen), in pascals,

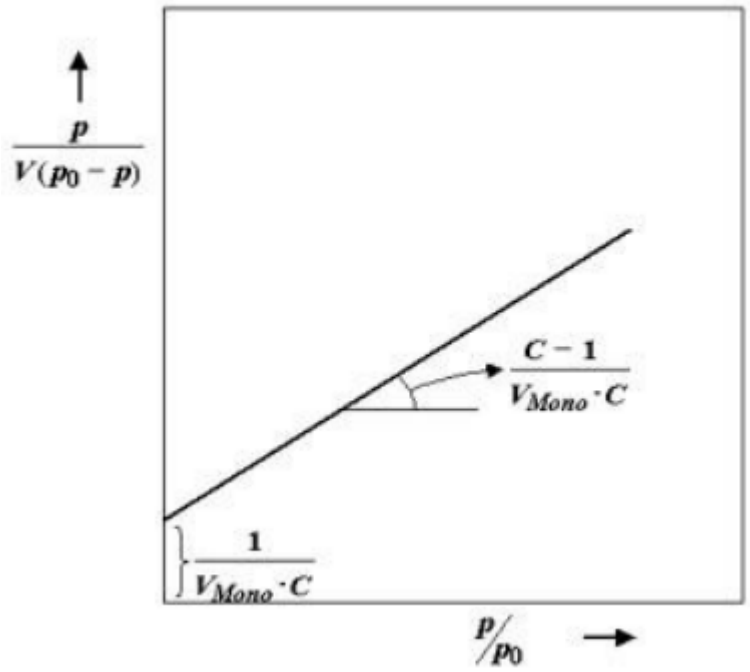
p_o = saturated pressure of adsorbate gas, in pascals,

V_a = volume of gas adsorbed at standard temperature and pressure (STP) [273.15 K and atmospheric pressure (1.013×10^5 Pa)], in millilitres,

V_m = volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in millilitres,

C = dimensionless constant that is related to the enthalpy of adsorption of the adsorbate gas on the powder sample.

In the experiment, different values of p/p_o are set by BET surface measurement equipment. A value of Va is measured at each of not less than 3 values of P/Po. Then BET value 1/(Va *(Po/P-1)) is plotted against P/P_o according to the equation above. This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient, r, of the linear regression is not less than 0.9975; that is, r^2 is not less than 0.995. From the resulting linear plot, the slope, which is equal to $(C - 1)/V_mC$, and the intercept, which is equal to $1/V_mC$, are evaluated by linear regression analysis. From these values, V_m is calculated as 1/(*slope + intercept*), while C is calculated as (*slope/intercept*) + 1.



From the value of V_m so determined, for single point measurement, the specific surface area, S, in $m^2 \cdot g^{-1}$, is calculated by the equation:

$$S = \frac{V_m N_a}{m \times 22400}$$

N = Avogadro constant (6.022×10^{23} mol^{-1}),

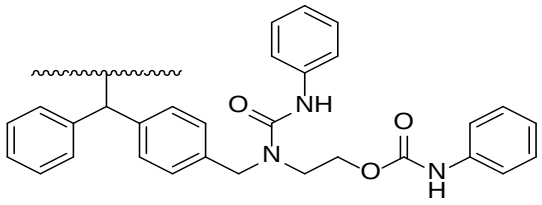
a = effective cross-sectional area of one adsorbate molecule, in square metres (0.162 nm^2 for nitrogen and 0.195 nm^2 for krypton),

m = mass of test powder, in grams,

22400 = volume occupied by 1 mole of the adsorbate gas at STP allowing for minor departures from the ideal, in millilitres.

The calculation of surface coverage of surface modification:

There is an example of the calculation of combustion analysis data. Urea compound **6c** modified PS-XAD4 is used as example. (Data from Moloney's research[55])



Surface loading: %N=0.21, as measured by combustion analysis

Moles of N per gram of polymer

$=(\%N/100)/M_r(N) = (0.21/100)/14=1.5\times10^{-4}$

Moles of carbene compound on surface modification per gram of polymer

=moles of N per gram of polymer/atoms of N in surface modification=$1.5\times10^{-4}/3=5\times10^{-5}$

Number of molecules of carbene compound on surface modification per gram of polymer

= Moles of carbene compound on surface modification per gram of polymer × Av = $(5\times10^{-5})\times(6.02214\times10^{23})=3.01\times10^{19}$

Number of molecules of carbene compound on surface modification per cm^2 (according to literature, surface area of PS-XAD4 is 725 m^2/g)

$=(3.01\times10^{19})/(725\times100\times100)=4.15\times10^{12}$

Surface coverage:

Minimum projection area of carbene compound on modified polymer (A_0) is 101.54 $Å^2$. (http://www.chemicalize.org/)

Maximum number of carbene molecules possible per cm^2

$=1/ A_0=1/(1.015\times10^{-14})=9.85\times10^{13}$

%Surface coverage = (actual number of molecules per cm^2)/ (maximum number of carbene molecules possible per cm^2)$\times100=(4.15\times10^{12})/(9.85\times10^{13})\times100= 4.2\%$

In the calculation, the minimum projection area was used to estimate the maximum number of carbene molecules possible per cm^2, which was used to calculate %surface coverage. The reason of using minimum projection area was shown in **Figure 2.1**. Based on the 3D model of carbene compound on modified surface, the ideal modification was that every carbene compound molecule was inserted to polymer matrix as the structure shown in **Figure 2.1**. However, due to rotation of polymer chains and space barrier, not all carbene compounds could ideally insert into polymer matrix. Therefore the minimum projection area could only be use to estimate maximum of carbene molecules possible per cm^2 and upper limit of surface coverage. The minimum projection 3D model was shown in **Figure 2.2**.

Figure 2.1 3D model of inserted carbene compound

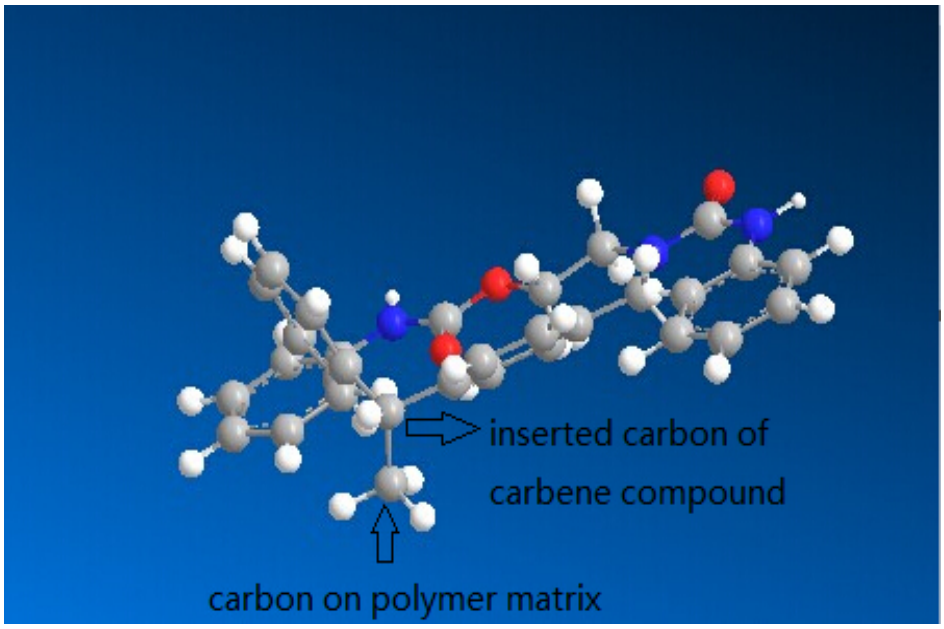
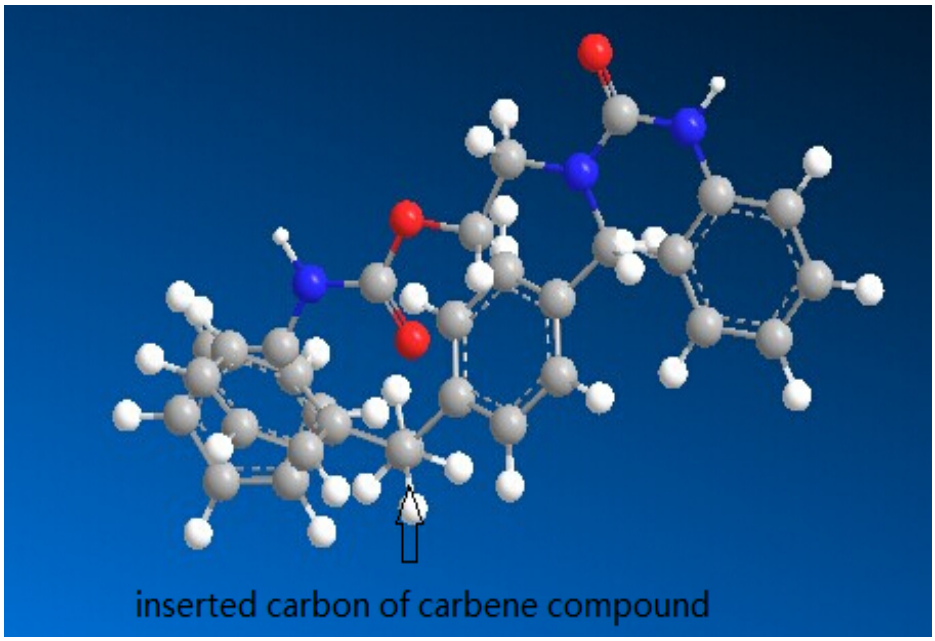


Figure 2.2 The minimum projection 3D model of carbene compound



Hole-plate bioassay test

Hydrogen peroxide was chosen as biocidal agent to investigate antibacterial properties of modified polymers. Moloney and his co-workers used same urea diaryl diazo system to modify PS –XAD4 beads and then treated with hydrogen peroxide. [55] They found that urea function group could form H-bonding with hydrogen peroxide. Hydrogen atom on hydrogen peroxide showed positive charge due to strong electronegativity of oxygen atom in hydrogen peroxide. Lone pair of electrons on nitrogen atom of urea function group attacked positive hydrogen and generated H-bonding between hydrogen peroxide and urea group, which resulted in relatively high stability and general releasing of hydrogen peroxide on the surface of modified polymer. After characterization by ATR IR and combustion analysis, modified polymers **7** were also treated

hydrogen peroxide to investigate their antibacterial activity.[51]

Modified polymers were submerged in 10% hydrogen peroxide solution for 18 hours in the freezer and then washed with water three times. Hole-plate bioassay tests were conducted to investigate the biocidal activity against *S. aureus* and *E. coli*. However, all hole-plate bioassay tests showed negative results, as shown in **Table 2.3**. Since all modified polymers had different surface coverage (PE-fibre appears to have high surface coverage), the failure in bioassay test caused by low surface loading on polymers was unlikely. We suggested the negative bioassay results might be caused by the low activity of urea diazo compound **6c** as a result of insufficient bounding ability of biocidal agent (hydrogen peroxide).

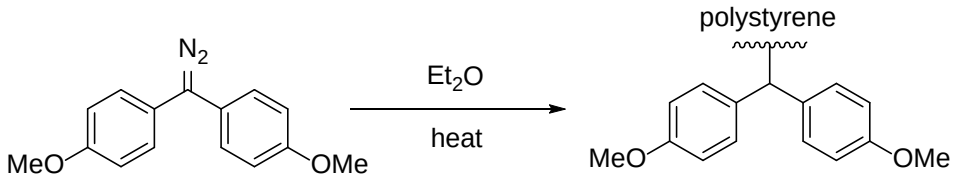
Zone of inhibition/ mm	LDPE			FIBRE			MDPE			UHMPE		
	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2
E. coli	0	0	0	0	0	0	0	0	0	0	0	0
S.aureus	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.3. Bioassay test results of **6c** modified polymers (B=Blank polymer, M=Modified polymer) (unit of zone of inhibition=mm)

2.2 Bis (dimethylamino) diaryl system

Since surface modification using urea diazo compound **6c** failed in binding biocidal agents due to its low reactivity, a more highly reactive diazo system was required. Moloney and co-worked prepared methoxy-activated carbene system (**Scheme 2.3**) to successfully modify polystyrene.[58] However, it was suspected that an even more reactive system would be needed to modify low surface energy materials, such as PE and PP.[44, 55]

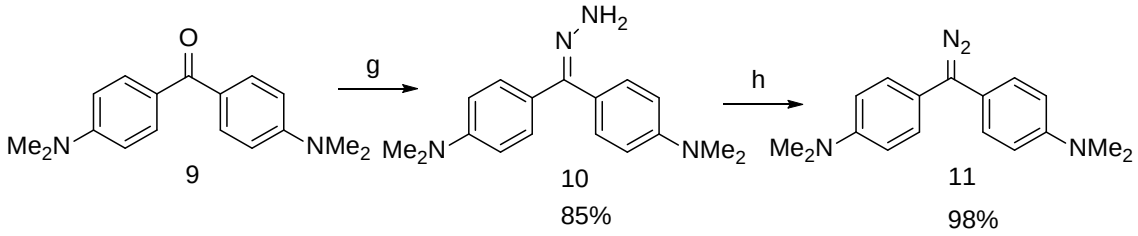
Scheme 2.3. Methoxy-activated carbene system



With this in mind, bis (dimethylamino) diaryl system which has found successful application in modifying carbon nanotubes was examined.[44] Before this experiment, using Michler's ketone to modified polymers for antibacterial application are rarely studied because the diazo compound resulting from Michler's ketone has high reactivity due to two electronic-rich amine groups and results in fast decomposition at room temperature. However, in this novel system, Michler's ketone (bis(4-(dimethylamino)phenyl)methanone) **9** was chosen as a novel starting material because of its high reactivity compared with urea diaryl diazo system. Michler's ketone **9** was treated with large numbers of equivalents of hydrazine monohydrate for conversion into

hydrazone **10**, which was then oxidized into dark green coloured diazo compound **11** (**scheme 2.4**). The diazo compound was highly reactive and must be kept at sub-ambient temperature and used as soon as possible after synthesis.[44]

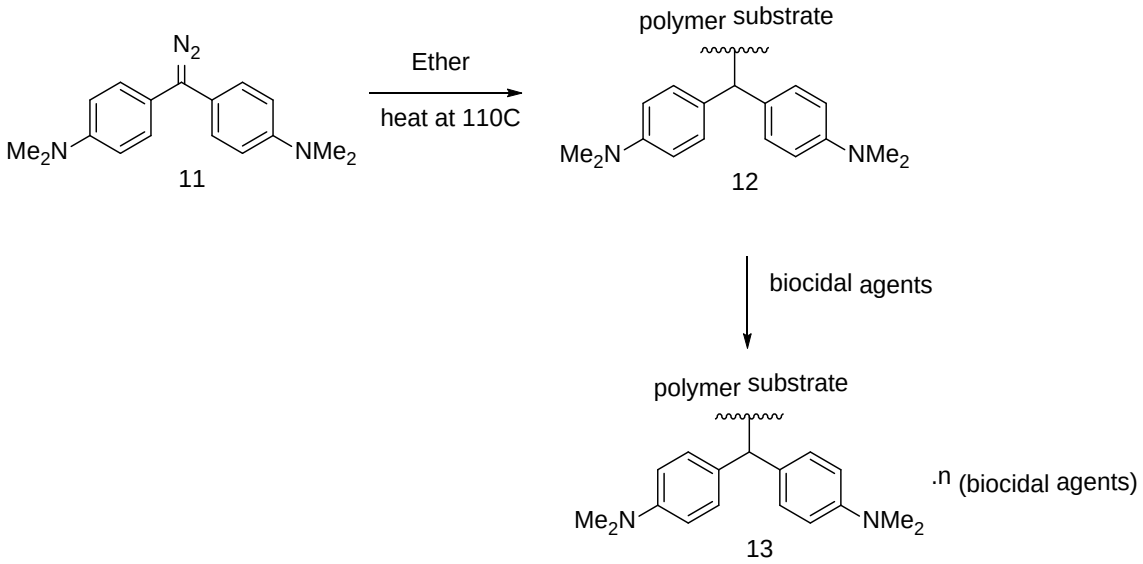
Scheme 2.4. Bis(dimethylamino)diaryl system



Reagents and conditions: (g) NH_2-NH_2.H_2O, ethanol, reflux 72h at 80°C; (h) HgO, Na_2SO_4, sat. KOH in ethanol, ethanol, stir for 16h in the dark

The coupling of diazo compound **11** onto the polymer surface was attempted, as shown in **Scheme 2.5**. Diazo compound **11** was dissolved in ether and absorption on polymer was achieved by fast evaporation of solvent in vacuum. Then the polymer was heat at 110°C for 15 minutes and washed with water and acetone. During the process, the loss of diazo colouration was observed, again consistent with decomposition of the diazo compound to the carbene. The colour of modified polymer **12** usually showed as yellow or light yellow. The modified polymer **12** was submerged into different antibacterial agents for 18 hours in the freezer to be prepared for hole-plate bioassay test against *S.aureus* and *E.Coli*.

Scheme 2.5. Modification of polymers using bis(dimethylamino)diaryl system



ATR IR and Combustion Analysis

Similar to urea diazo modified polymers, ATR IR and combustion analysis were used to characterize modified polymer **12**. Successful surface modification was confirmed by colour change from white to yellow and the observation of C-N stretching peak at 1380-1390 cm^{-1}. Nitrogen content was examined by combustion analysis, the data of which is shown in **Table 2.4**. The calculation procedures were similar to that of urea diazo compound. The minimum projection area of new modified polymer (A_0) is now estimate to be 39.22 $Å^2$. As shown in **Table 2.4**, the calculated surface coverage values of LDPE, MDPE and UHMPE were again over 100% and unreliable because the surface loading was beyond detecting limitation. The surface areas of LDPE, MDPE and UHMPE are about $2m^2/g$ and a small error in detecting surface area can cause huge difference in calculation of surface coverage. The

surface coverage value of PE-fibre and PS, however, seems to be reasonable. The much higher nitrogen content of PS-XAD4 was due to the porous structure, which results in a much larger surface area.

	%N per gram	Number of carbene compound on surface modification per gram	Surface area of Polymer using BET m^2/g	Number of carbene compound on surface modification per cm^2	%surface coverage
LDPE	<0.1±0.01	2.15E+19± 2.15E+18	2.0	1.08E+15± 1.08E+14	<422±42
MDPE	<0.1±0.01	2.15E+19± 2.15E+18	1.9	1.13E+15± 1.13E+14	<444±44
UHMPE	0.17±0.02	3.66E+19± 4.30E+18	2.5	1.46E+15± 1.72E+14	574±67
PE-fibre	0.22±0.02	4.73E+19± 4.30E+18	42.0	1.12E+14± 1.02E+13	44±4
PS-XAD 4	0.55±0.06	1.18E+20± 1.29E+19	725.0	1.63E+13± 1.78E+12	6±1

Table 2.4. The calculation of surface coverage of modified polymer using bis(dimethylamino)diaryl system

Hole-plate bioassay test

The antibacterial activity of modified polymers **12** binding with three antibacterial agents, hydrogen peroxide, bleach and penicillin V were tested using hole-plate bioassay tests.

Hydrogen peroxide and penicillin V were chosen because we believed H-bonding were generated between antibacterial molecules and modified polymer surface. Spencer and his co-workers found out that amine could form H-bonding (OH···N) with organic compound containing OH group.[60] Positive charged hydrogen atom were attacked by lone pair of electrons on nitrogen atom and resulted in H-bonding. Subramanian and co-workers found H-bonding of penicillin V formed with similar mechanism. [61] Bleach was chosen because of the formation of N-Halamine. Gang Sun and Worley found that reversible formation of N-Halamine occurred with bleach and kill bacteria by releasing free chlorine.[62]



The bioassay results of hydrogen peroxide are shown in **Table 2.5**. Based on **Table 2.5**, the antibacterial activity was observed in the modified LDPE, PE-fibre and PS-XAD4 binding hydrogen peroxide while the blank polymers and blank polymer binding with H_2O_2 showed negative results. The positive

bioassay results indicated that compared with urea diazo system, the binding ability of modified polymer using bis(dimethylamino)diaryl system was significantly enhanced. However, modified MDPE and UHMPE still showed negative bioassay results. One reason might be that the highly crystallized structure of MDPE and UHMPE prevented the carbene insertion reaction, which resulted in lower binding ability of biocidal agents. Compared with LDPE, MDPE and UHMPE have much higher crystallinity, which results in a major proportion of crystalline lamella and very small proportion of amorphous polymer chain, as shown in **Figure 7**. The strong bonding force between polymer chains folded into crystalline lamella makes carbene insertion reaction more difficult to occur.

Zone of inhibition/ mm	LDPE			FIBRE			MDPE			UHMPE			PS-XAD4		
	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2
E.Coli	0	0	21±1	0	0	32±1	0	0	0	0	0	0	0	0	41.5±1.5
S.aureus	0	0	21.5±0.5	0	0	33±0	0	0	0	0	0	0	0	0	42.5±2.5

Table 2.5 Bioassay results of modified polymer 12 binding with hydrogen peroxide (B=Blank polymer, M=Modified polymer), average value of duplicate (unit of zone of inhibition=mm)

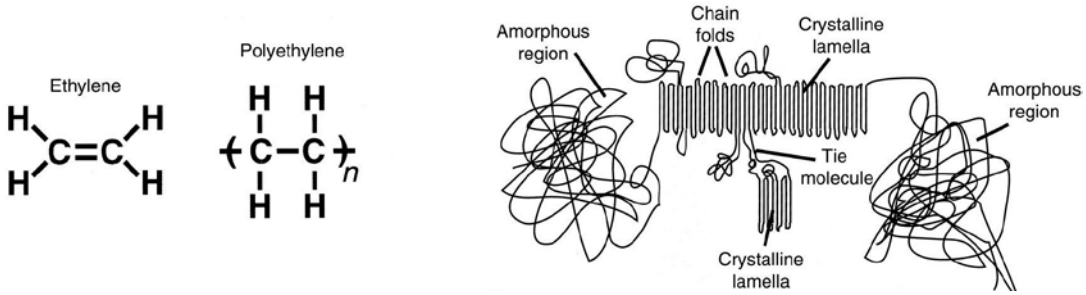


Figure 7. Crystallized structure of polyethylene

Since modified LDPE, PE-fibre and PS-XAD4 binding with hydrogen peroxide showed biocidal activity, the loading of hydrogen peroxide was quantified by iodometric titration (Table 2.6). Unfortunately the hydrogen peroxide loading of modified LDPE and PE-fibre was so low (less than 0.0001 mmol/g) that it was incalculable. For modified LDPE and PE-fibre, after one drop of 0.01M sodium thiosulfate, the dark solution immediately turned to be colourless. However, this indicated a successful modification using bis (dimethylamino) diaryl system. Compared with hydrogen peroxide loading levels (0.08-0.8 mmol/g) on polymers investigated by Moloney and Leonard, [58] the modified LDPE and PE-fibre with extremely low loading of hydrogen peroxide still showed good bioassay results, which indicated that the bis(dimethylamino)diaryl system was a very reactive system and that the binding ability of modified polymer using this system was significantly enhanced.

Polymer	LDPE	PE-fibre	PS-XAD4
H_2O_2 loading mmol/g	<0.0001	<0.0001	0.62

Table 2.6. Hydrogen peroxide loading of modified polymer using bis(dimethylamino)diaryl system

Since modified MDPE and UHMPE failed to achieve biocidal activity, only modified LDPE, PE-fibre and PS-XAD4 binding with bleach and penicillin V were investigated in a further bioassay test. The bioassay results are shown in **Table 2.7** and **Table 2.8**. Modified polymer binding with bleach showed similar results as polymer binding with hydrogen peroxide. Only modified polymer + bleach showed antibacterial activity, while blank polymer and blank polymer + bleach showed negative bioassay results, indicating no biocidal activity. Free hydrogen peroxide and bleach are relatively unstable at room temperature, and light and high temperature can accelerate their decomposition. During the washing procedure, fast decomposition of free hydrogen peroxide and bleach which are weakly absorbed on blank polymer occurs while biocidal agents binding with modified polymers by hydrogen-bonding are more stable and this decomposition is avoided. However, the bioassay results of modified polymer binding with penicillin V differ from polymer binding other two biocidal agents. Except for modified polymer binding with penicillin V, blank PS-XAD4 + penicillin V also showed antibacterial activity in the bioassay test. Penicillin V is a relatively stable compound at room temperature. Even though weakly

binding penicillin V on the surface of blank LDPE and PE is washed away, some penicillin V was left in polystyrene due to it porous structure. During bioassay test, the remaining penicillin V in blank PS-XAD4 showed some biocidal activity.

Bleach	LDPE			FIBRE			PS-XAD4		
Zone of inhibition/mm	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach
E.Coli	0	0	17±1	0	0	24.5+±1.5	0	0	36±1
S.aureus	0	0	17.5±0.5	0	0	26.5+±0.5	0	0	34.5±0.5

Table 2.7. Bioassay results of binding bleach (B=Blank polymer, M=Modified polymer), average value of duplicate (unit of zone of inhibition=mm)

Penicillin V	LDPE			FIBRE			PS-XAD4		
Zone of inhibition/mm	B	B+ PV	M+ PV	B	B+ PV	M+ PV	B	B+ PV	M+PV
E.Coli	0	0	15.5±0.5	0	0	15±0	0	32±2	41±1
S.aureus	0	0	26±2	0	0	24±4	0	44.5±1.5	50±0

Table 2.8. Bioassay results of binding Penicillin V (B=Blank polymer, M=Modified polymer, PV=Penicillin V), average value of duplicate (unit of zone of inhibition=mm)

Reproducibility and pH sensing

For modifying polymers using **11**, prevention of the decomposition during the evaporation of solvent was the major challenge, which was important for the uniformity of modified products. As **11** was a highly reactive diazo compound, different solvents including THF, ethanol and diethyl ether were investigated to optimise the solvent evaporation step. When THF and ethanol were used, decomposition of **11** was observed by obvious colour change from dark green to bright red, which caused non-uniform colour of modified polymers. However, ether showed little decomposition and modification of three polymers showed high consistency and uniformity, as shown in **Table 2.9**.

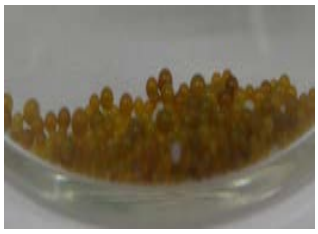
Solvent	PS-XAD4	LDPE	Fibre
THF			
Ethanol			
Ether			

Table 2.9. Uniformity of modified polymers using different solvents

Modified PS-XAD4 was submerged in different buffer solutions to investigate its pH sensitivity. Instant and obvious colour change (see **Table 2.10)** showed that modified polymers were very sensitive to pH (colour change from dark green to light yellow at pH 3 to 10) and indicated a potential application as pH sensing.

pH	3	6	8	10
THF as solvent				
Ethanol as solvent				
Ether as solvent				

Figure 2.10. Colour change of modified PS-XAD4 in different pH buffer solutions

(pH=3, 50ml of 0.1M potassium hydrogen phthalate+22.3ml of 0.1M HCl

pH=6, 50ml of 0.1M potassium dihydrogen phosphate+5.6ml of 0.1M NaOH

pH=8, 50ml of 0.025M borax+20.5ml of 0.1M HCl

pH=10, 50ml of 0.025M borax+18.3ml of 0.1M NaOH)

3. Conclusion

In this project, we investigated two diazo systems to modify polymers to examine their structure and capacity to allow the introduction of antibacterial activity.

Initial investigation showed that successful surface modification on polymer **8** was achieved using a urea-substituted diazo system by clear ATR IR results. However, modified polymers **8** bounded with different antibacterial agents did not show any antibacterial activity. A possible reason of low loading on polymer surface was proved to be unlikely as several modified polymers with different surface areas and surface loading showed all negative bioassay results. Another possible reason was that the low activity of urea diazo compound resulted in insufficient bounding ability of biocidal agent. To investigate this possible reason, a more active system which could result in higher bounding ability was needed.

A relatively more reactive bis(dimethylamino)diaryl system was used to enhance the bounding ability of biocidal agents. Successful surface modification on polymer **12** using diazo compound **11** was observed by C-N stretching from ATR IR and the polymer colour change from white to yellow. However, modified MDPE and UHMPE still showed negative bioassay test because their high crystal lamella structure prevented the carbene insertion

reaction, resulting in low binding ability of biocidal agents. Modified LDPE, PE-fibre and PS-XAD4 binding with hydrogen peroxide, bleach and penicillin V showed excellent antibacterial activity, which indicated the binding ability was significantly enhanced by bis(dimethylamino)diaryl system. Blank PS-XAD4 with penicillin V showed biocidal activity because some penicillin V remained after the wash procedure due to the porous structure of PS-XAD4. During evaporation in the solvent modification process, ether was considered to be a good solvent as little decomposition of diazo was observed and quick evaporation was achieved. Modified PS-XAD4 using bis(dimethylamino)diaryl system showed high pH sensitivity, which indicated a possible application of modified polymers.

4. Future work

The hole-plate bioassay test used in the project is not very accurate and an alternative but more accurate bioassay test system should be investigated. Moreover, quantification of the release of biocidal agents would be desirable. In addition, other antibacterial agents, such as chlorohexidine, sliver products or quaternary ammonium compounds, should be examined on these modified polymers using the bis(dimethylamino)diaryl system. Finally, more substrate materials, such as graphene which has ultra-high surface area, should be investigated.

5. Experimental

5.1 General

All reagents, solvents and polymers were purchased from commercial companies (Goodfellow Ltd and Sigma Aldrich). All reactions were carried out in dried round bottom flask using standard solvents. Reactions which needed temperature below room temperature were achived using cooling baths, ice/water (0 °C) and acetone/CO_2 (s) (-78 °C).

Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets, pre-coated with Kieselgel 60 F_{254}, the thickness of which was 0.2 mm. Chemical compound spots were visualised under UV fluorescence (λ_{max}=254 nm). Flash column chromatography was performed using silica gel 60, 0.040-0.063 mm.

Characterization of compounds was done using following equipment and settings. ^{1}H, ^{13}C, COSY and HSQC spectra were recorded on either Bruker DPX200 (200 MHz) or AVN400 (400 MHz) spectrometers. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peaks. Coupling constants (*J*) are in hertz (Hz) and the following abbreviations are: s (singlet), d (doublet), dd (doublet doublet), t (triplet), q (quartet) and m (multiplet). Low resolution mass spectra (m/z) were obtained on a Fisons Platform spectrometer using electrospray ionisation (ESI).

Surface characterizaiton was carried out using Infrared (IR) spectra, which were recorded using a Bruker Tensor 27 FT-IR spectrometer. Absorption maxima (ν_{max}) were reported in wavenumbers (cm^{-1}) and selected peaks were reported.

Surface analysis of modified polymers was obtained using ATR-IR, performed on a BIO-Rad FTS-6000 with diamond stage accessories.

Characterization of materials was performed whith following instruments. Melting points were recorded using a Stuart Scientific SMP1 melting apparatus in open capillaries. Combustion analysis of all materials was provided by MEDAC Ltd.

5.2 Synthesis

General Method (A): Functionalisation of polymer beads / powder / fibre with diazonium compounds

To a solution of diazonium compounds in diethyl ether (10 ml) was added the required amount polymer and the solvent was carefully removed in vacuum in the dark. After that, the coated polymer was heated to 110 ^{o}C for 15 minutes and cooled to room temperature. The colour change of the polymers showed a successful surface modification. Then the modified polymer was washed using

acetone and water until the washing solvent was clean and colourless. The polymers were dried for 30 min and stored in the fridge.

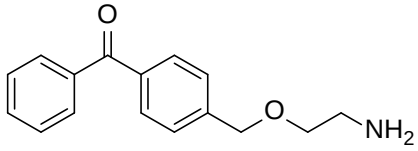
General Method (B): Binding of biocidal agents to functionalised polymers

Biocidal agents were purchased from Sigma Aldrich. 150 mg of each functionalised polymer and blank polymer (control experiment) were put into a vial and then was added 0.5 ml MeOH and 4.5 ml of the biocidal agents (such as H_2O_2, Bleach or Penicillin V, the concentration of which were shown in **Table 5.1.1**). The mixture was stored in the fridge in the dark for 18h. After that, the modified polymers were collected by filtration and washed with water (3 × 5 ml).

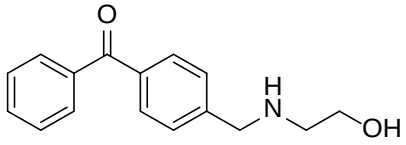
Biocidal agents	Concentration
Hydrogen peroxide	18 wt.% in H_2O
Bleach	6% in H_2O
Penicillin V	0.005 mol/L

Table 5.1.1 Concentration of biocidal agents used in bioassay test (Hydrogen peroxide and Bleach are supplied by Sigma Aldrich Company)

(4-((2-Aminoethoxy)methyl)phenyl)(phenyl)methanone **2**

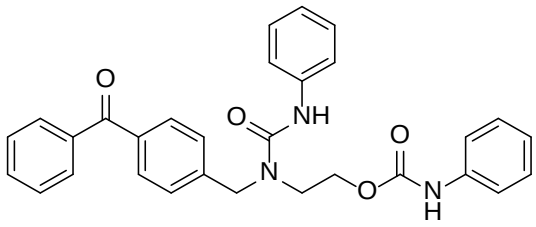


(4-(((2-Hydroxyethyl)amino)methyl)phenyl)(phenyl)methanone **3**



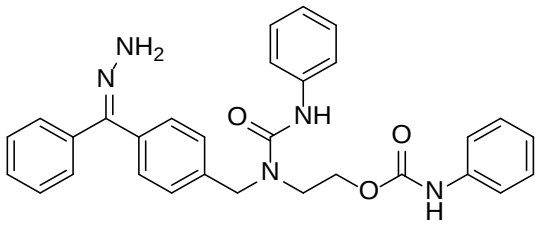
To a solution of sodium hydride (0.44 g, 60% dispersion in mineral oil, 11 mmol) in dry THF (10 ml) was added a solution of dry ethanolamine (0.87 g, 14.3 mmol) in dry THF (10 ml) and the mixture was stirred for 15 min. A solution of 4-bromomethyl benzophenone (3 g, 11 mmol) in dry THF (10 ml) was cooled to -78 ^{o}C. The mixture was warmed to room temperature and stirred for 18h and then concentrated in vacuum. The residue was partitioned using chloroform and water, and the organic layer was collected and concentrated in vacuum. δ_H ($CDCl_3$, 400 MHz) 2.83 (m, 2H, C**H$_2$**NH_2 and C**H$_2$**N), 3.70 (m, 2H, C**H$_2$**OH and C**H$_2$**O), 3.91 (s, 2H, ArC**H$_2$**N), 4.63 (s, 2H, ArC**H$_2$**O), 7.4-7.8 (M, 9H, Ar**H**); δ_C ($CDCl_3$, 400 MHz) 51.1 (Ar**C**H_2N), 53.6 (**C**H_2N), 55.6 (**C**H_2O), 64.7 (**C**H_2O), 77.5 (Ar**C**H_2O), 127.6, 128.3, 128.7, 130.4, 130.8, 132.8, 135.6, 136.8, 138.1, 145.3, 196.9; ν_{max}/cm^{-1} 1656, 1577, 1365, 1176; m/z (ESI $^{+}$) 256.2 ($[M+H]^{+}$);

2-(1-(4-Benzoylbenzyl)-3-phenylureido)ethyl phenylcarbamate **6a**



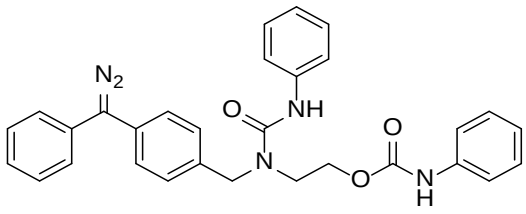
To the mixture of **2** and **3** (3.0 g, 11 mmol) was added dry DCM (20 ml) and stirred for 15 min. To the mixture was added triethylamine (2.22 g, 22 mmol) and stirred for 15 min. A solution of phenyl isocyanate (1.83 g, 15.4 mmol) in dry DCM (30 ml) was added and the mixture was stirred for 16h. Then the mixture was quenched with water and the organic layer was collected and washed sequentially with water, 2 M HCl, and water. The organic layer was dried over Na_2SO_4 and purified by flash chromatography on silica gel via elution with 2:1 petrol (40:60): ethyl acetate to yield **6** (0.92 g, 48%) as a white solid. m. p. 158 ^oC; δ_H ($CDCl_3$, 400 MHz) 3.60 (t, 2H, J=6.8, NHC**H**$_2$), 4.17 (t, 2H, J=6.8, OC**H**$_2$), 4.66 (s, 2H, NC**H**$_2$Ar), 6.95-7.01 (m, 2H, Ar**H**), 7.22-7.26 (m, 4H, Ar**H**), 7.41-7.54 (m, 8H, Ar**H**), 7.69-7.72 (m, 5H, Ar**H**), 8.11 (br. s, 1H, NCON**H**Ar); δ_c ($CDCl_3$, 400 MHz) 46.2 (N**C**H$_2$), 51.1 (Ar**C**H$_2$N), 62.5 (O**C**H$_2$CH$_2$N), 119.7, 122.4 (Ar**C**H), 127.3, 128.4, 128.9, 129.2, 130.7, 132.6 (Ar**C**H), 142.6, 151, 157; v_{max}/cm^{-1} 3339, 3058, 1722, 1654; m/z (ESI $^+$) 494.1 ([M+H]$^+$);

2-(1-(4-(Hydrazono(phenyl)methyl)benzyl)-3-phenylureido)ethyl phenylcarbamate **6b**



To a solution of **6a** (1.43 g, 2.9 mmol) in methanol (20 ml) was added hydrazine monohydrate (2.18 g, 43.5 mmol). The mixture was heated to gentle reflux for 16 h, cooled, and concentrated in vacuum. The residue was partitioned using water, and the organic layer was collected, washed with ethyl acetate, and dried over $MgSO_4$. Concentration in vacuum yielded hydrazone **6b** (1.35 g, 92%) as a white thick oil. δ_H ($CDCl_3$, 400 MHz) 3.60-3.70 (m, 4H, NHC**H**$_2$C**H**$_2$O), 4.71 (s, 2H, OC**H**$_2$Ar), 5.83 (s,2H,NN**H**$_2$), 7.01-7.52 (m, 19H, Ar**H**); δ_c ($CDCl_3$, 400 MHz) 46.2 (N**C**H$_2$), 51.1 (Ar**C**H$_2$N), 62.5 (O**C**H$_2$CH$_2$N), 119.7, 122.4 (Ar**C**H), 127.1, 128.7, 128.7, 129.2, 129.4, 130.7, 143.1, 155, 160; m/z (ESI $^+$) 508.3 ([M+H]$^+$);

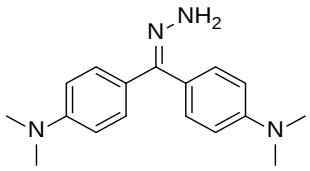
2-(1-(4-(Diazo(phenyl)methyl)benzyl)-3-phenylureido)ethyl phenylcarbamate **6c**



To a solution of **6b** (1.35 g, 2.65 mmol) in DCM (15 ml) was added a mixture of

manganese dioxide (0.6 g, 6.89 mmol), sodium sulfate (0.57 g, 3.98 mmol), and sat. KOH in ethanol (1 ml). The mixture was stirred for 16h in the dark and filtered through a pad of Celite. The filtrate was concentrated and vacuum in the dark to yield diazo compound **6c** as pink solid (1.1 g, 81.9%). The compound is reactive under light in room temperature that characterizing using NMR and Mass spectral were not possible as it decomposes during the characterization.v_{max}/cm^{-1} 2038 ($Ar_2C=N^+=N^-$),1712, 1651.

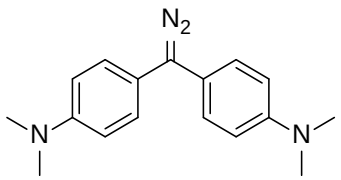
4,4'-(Hydrazonomethylene)bis(N,N-dimethylaniline) **10**



To a solution of Michler's ketone (3 g, 11 mmol) in EtOH (10 ml) was added hydrazine monohydrate (9.5 ml, 165 mmol). The mixture was heated at flux for 72h at 80 °C. Then 5 more equivalence hydrazine monohydrate (2.7 ml, 56 mmol) were added to the mixture and refluxed for another 18h. After cooling, the mixture was put into ice bath for crystallizing for 1h. The crystallized solid was filtered and washed with 2-propanol (5 ml) and dried in vacuum to yield **10** (2.6 g, 9.3 mmol, 85%) as a beige solid. m.p. 151-152 ^oC; δ_H (d_6-DMSO, 200 MHz) 3.03 (s, 12H, 4xNC**H**$_3$), 6.76 (d, 4H, *J* = 8.8, 3-, 3'-, 5-, 5'-**H**), 6.86 (d, 2H, *J* = 8.8, 3'-, 5'-**H**), 7.60 (d, 4H, *J* = 8.8, 2-, 2'-, 6-, 6'-**H**); δ_C (d_6-DMSO, 200 MHz) 39.99 (4x N**C**H$_3$), 111.1 (3-, 3'-, 5-, 5'-**C**), 113.2 (**C**), 125.7 (1-**C**), 128.7 (1'-**C**),

131.9 (2-, 2'-, 6-, 6'-**C**), 153.0 ($Ar_2C{=}NNH_2$); ν_{max}/cm^{-1} 1626, 1545, 1350, 1180; m/z (ESI $^+$) 283.2 ($[M+H]^+$);

4,4'-(Diazomethylene)bis(N,N-dimethylaniline) **11**



To a solution of **10** (0.77 g, 2.7 mmol) in EtOH (10 ml) was added yellow mercury oxide (1.24 g, 5.7 mmol), sodium sulphate (0.97 g, 6.83 mmol) and sat. potassium hydroxide in ethanol (3 ml). The mixture was stirred in the dark for 18h and filtered through a pad of celite. The filtrate was concentrated in vacuum to yield **11** (0.75 g, 98%) as a dark green solid which was storage in the freezer to avoid decomposition. The compound is so reactive under light in room temperature that characterizing using NMR and Mass spectral were not possible as it decomposes during the characterization. ν_{max}/cm^{-1} 2020 ($Ar_2C{=}N^+{=}N^-$),1606, 1519, 1356

5.3 Combustion analysis

Urea diazo compound modified polymer:

Material	Combustion analysis	mmol of N/ gram polymer	mmol of carbene compound on surface modification/ gram polymer	Molecules of carbene compound on surface modification /gram polymer
LDPE	%C: 85.44 %H: 13.84 %N: 0.15	0.11±0.01	0.04±0.01	$2.2\times10^{19}\pm2.9\times10^{18}$
MDPE	%C: 84.64 %H: 14.04 %N: <0.1	<0.07±0.01	<0.02±0.00	$<1.4\times10^{19}\pm2.8\times10^{18}$
UHMPE	%C: 85.57 %H: 14.02 %N: 0.21	0.15±0.01	0.05±0.00	$3.0\times10^{19}\pm2.9\times10^{18}$
Fibre	%C: 82.72 %H: 13.81 %N: <0.1	<0.07±0.01	<0.02±0.00	$<1.4\times10^{19}\pm2.8\times10^{18}$

Table 7. Combustion analysis of urea diazo compound modified polymer

Michler's Ketone modified polymer:

Material	Combustion analysis	mmol of N/ gram polymer	mmol of carbene compound on surface modification/ gram polymer	Molecules of carbene compound on surface modification /gram polymer
LDPE	%C: 84.07 %H: 15.46 %N: 0.33	0.236±0.01	0.118±0.01	$7.1\times10^{19}\pm4.3\times10^{18}$
Fibre	%C: 53.42 %H: 8.33 %N: 0.37	0.264±0.01	0.132±0.01	$8.0\times10^{19}\pm4.3\times10^{18}$
PS-XAD4	%C: 84.67 %H: 7.99 %N: 1.10	0.786±0.02	0.393±0.01	$2.4\times10^{20}\pm4.8\times10^{18}$

Table 8. Combustion analysis of Michler's ketone modified polymer

5.4 Hole-plate bioassay tests

Biocidal activity of modified polymers was obtained by hole-plate antibacterial bioassay test. 15 mg of each modified polymers was submerged in 1 ml 1:1

DMSO:H_2O solution and transferred into wells on *E.Coli* and *S.aureus* plates. The plates were put into the oven at 37 ^oC for 18h. Zone of inhibition was measured and recorded.

Biocidal agent: H_2O_2

First batch:

Zone of inhibition/mm	LDPE			FIBRE			MDPE			UHMPE			PS-XAD4		
	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2
E.Coli	0	0	20	0	0	31	0	0	0	0	0	0	0	0	40
S.aureus	0	0	22	0	0	33	0	0	0	0	0	0	0	0	42

Table 9. Bioassay tests of Michler's ketone modified polymer (first batch) (unit of zone of inhibition=mm)

Second Batch:

Zone of inhibition/mm	LDPE			FIBRE			MDPE			UHMPE			PS-XAD4		
	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2	B	B+ H_2O_2	M+ H_2O_2
E.Coli	0	0	22	0	0	33	0	0	0	0	0	0	0	0	43
S.aureus	0	0	21	0	0	33	0	0	0	0	0	0	0	0	45

Table 10. Bioassay tests of Michler's ketone modified polymer (second batch) (unit of zone of inhibition=mm)

Biocidal agent: Bleach

First batch

Zone of inhibition/mm	LDPE			FIBRE			PS-XAD4		
	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach
E.Coli	0	0	16	0	0	23	0	0	35
S.aureus	0	0	17	0	0	26	0	0	35

Table 11. Bioassay tests of Michler's ketone modified polymer (first batch) (unit of zone of inhibition=mm)

Second batch

Zone of inhibition/mm	LDPE			FIBRE			PS-XAD4		
	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach	B	B+ bleach	M+ bleach
E.Coli	0	0	18	0	0	26	0	0	37
S.aureus	0	0	18	0	0	27	0	0	34

Table 12. Bioassay tests of Michler's ketone modified polymer (second batch) (unit of zone of inhibition=mm)

Biocidal agent: Penicillin V

First batch

Zone of inhibition/mm	LDPE			FIBRE			PS-XAD4		
	B	B+ PV	M+ PV	B	B+ PV	M+ PV	B	B+ PV	M+PV
E.Coli	0	0	15	0	0	15	0	34	40
S.aureus	0	0	28	0	0	28	0	46	50

Table 13. Bioassay tests of Michler's ketone modified polymer (first batch) (unit of zone of inhibition=mm)

Second batch:

Zone of inhibition/mm	LDPE			FIBRE			PS-XAD4		
	B	B+ PV	M+ PV	B	B+ PV	M+ PV	B	B+ PV	M+PV
E.Coli	0	0	16	0	0	15	0	30	42
S.aureus	0	0	24	0	0	20	0	43	50

Table 14. Bioassay tests of Michler's ketone modified polymer (second batch) (unit of zone of inhibition=mm)

5.5 pH sensing

High reactive diazo compound **11** was used to modify three polymers, LDPE, fibre and PS-XAD4. Although using different solvent in sample processing showed different consistency, all modified polymers showed high pH sensing in different pH buffer solutions.

pH	3	6	8	10
THF as solvent				
Ethanol as solvent				
Ether as solvent				

Figure 4. pH sensing of **11** modified PS-XAD4 using different solvents

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