Self-stratifying antimicrobial coatings

Self-Stratifying Antimicrobial Coatings

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Mustafa Barış Yağcı

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Annem ve Kerem' ime …

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Chapter 1

General Introduction

1.1. Organic Coatings

A coating is a liquid, powder, or paste-like material covering that is applied to the surface of an object, which is called substrate. Today, coatings are present almost everywhere in daily life, such as on the walls, refrigerators, cabinets, furniture, wires, CDs, houses, cars computer systems, aircrafts, papers etc. [1]. One can distinguish between inorganic and organic coatings. That comes to the mind with respect to the very first term organic coatings is *paint* taking us back 20.000 years [2,3]. Organic coatings principally consist of polymeric material, pigments, fillers and various additives. The polymer works as the binder, and constitutes a matrix in which all other constituents are solubilized or dispersed. Although coatings are distinguished as organic and inorganic, many coatings consist of inorganic pigment particles dispersed in an organic matrix [1].

There are several methods available for applying a coating layer to the surfaces, such as brushing, dip coating, flow coating, curtain coating, roller coating, spraying, electrodeposition and chemiphoretic deposition. The ultimate choice of the application method depends on many factors: whether the coating mixture is a solid, liquid or powder, whether coated films are thick or thin, water-borne or oil-based. In addition, the nature and the shape of the surface are also very important to choose the application method [4-6].

Industrial organic coatings are mostly used for protection of materials against the environmental effects (*e.g.* UV-radiation, moisture), chemicals, corrosion etc. [1,7]. In that respect, highly crosslinked coating structures render a proper protection for the substrates by possessing high resistance to solvents and mechanical stress.

Furthermore, since science and technology develop in an ever fastening rate over the years, the purpose of the surface coatings is no longer limited to protective and decorative purposes. Functional polymer coatings, such as protective, antireflective, superhydrophobic, easily cleanable, barrier, anti-fouling, antimicrobial etc. have been investigated extensively over the past decades. Therefore, functional polymer coatings, such as anticorrosive, antireflective, superhydrophobic, easily cleanable, barrier, anti-fouling, and antimicrobial etc. have been investigated extensively over the past decades [8-22].

The study described in this thesis will combine the main idea of two groups of the functional coatings: the self-segregating easily cleanable coatings (as the method) and the antimicrobial coatings (as the property).

1.2. Self-Stratifying/Segregating Coatings

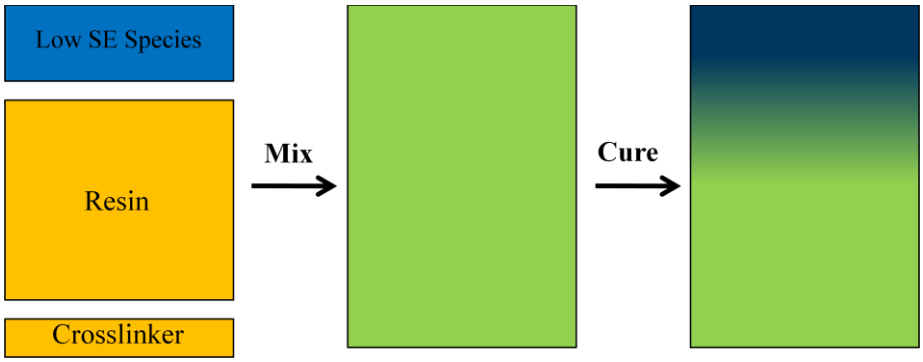
A self-stratifying coating is a coating which segregates spontaneously into two continuous interconnected layers during the process of film formation [23]. The self-stratification concept was found by Funke in 1976 [24].

Over the years, self-stratified coatings have been used regularly for preparing low surface-energy materials [10-13,25,26]. The idea behind it is to prepare easily cleanable surface coatings. Since low surface-energy materials have quite week interactions with any kind of dirt or other contaminants, they are very suitable for this purpose. Such films are often prepared by incorporation of low surface-energy moieties (fluorine, silicone, etc.) into the coating formulations [26-29]. However, the surface tension is not a bulk property but a surface property, and a high amount of (useless)

low surface-energy groups in the bulk would increase the production cost. That is why, during the past decades, the surface stratification concept has attracted a large scientific and industrial attention, as way to prepare such materials.

In self-stratifying coatings, low surface-energy groups are mainly concentrated at the air-film interface and their concentration in the bulk is low (Scheme 1.1). If there is a significant difference in surface energies of film components, an autonomous driving force makes lower surface-energy species move towards the air/film interface to minimize the surface energy. This concept has been utilized for the preparation of low surface-energy surface-segregated coatings [25].

Scheme 1.1. A scheme of self-stratification process of low surface energy (SE) species in a coating mixture [12].



In this concept, relatively small (easy to diffuse) low surface energy species with functional reactive groups are tethered to the polymeric networks. Typically, polydimethylsiloxanes and perfluoroalkyl alcohols, amines or epoxies are commonly used to prepare such coatings in polyacrylic, melamine, polyurethane and epoxy formulations.

1.3. Antimicrobial Polymers

Microbial infection constitutes one of the most serious problems in several areas in which hygiene holds a great importance (*e.g.* medical devices, drugs, health care applications, water purification systems, textile, and food packaging). Antimicrobial agents are materials which are capable of killing such pathogenic microorganisms [30]. Low molecular weight antimicrobial agents have been being widely used for the sterilization of water, as antimicrobial drugs (antibiotics), as food preservatives, and for soil sterilization [31]. However, these agents have many disadvantages, such as short-term antimicrobial ability and toxicity to the environment [32-33]. In the last two decades, novel antimicrobial polymers have offered a promise for antimicrobial applications by minimizing the environmental problems (by reducing the residual toxicity of the agents) and prolonging the lifetime of the antimicrobial agents. Also, polymeric antimicrobial agents are nonvolatile, chemically stable, do not permeate through skin and have been observed to reduce the incidences of the infection caused biomaterial implant failures [34-38]. For example, Worley and Sun have been widely used polymerized n-halamine compounds to synthesize bacteriocidal polymers [39-47]. Synthesized polymers, after activated with chlorine, have shown very promising antimicrobial activities.

The ideal antimicrobial polymer should fulfill the following major requirements: (1) biocidal to a broad spectrum of pathogenic microorganisms, (2) environmentally friendly (should not be toxic or irritating), (3) long-term (preferably permanently) active, (4) easily and inexpensively synthesized, (5) stable (should not decompose to toxic products), (6) can be regenerated upon loss of activity, and (7) not soluble in water for many applications [48].

Antimicrobial polymers are commonly obtained either by synthesizing monomeric biocide moieties and then polymerizing subsequently or copolymerizing with another monomer [49-55]. The grafting of antimicrobial agents into natural occurring or synthetic polymers is also another approach to obtain such materials in various applications. The differences in such materials will be discussed in the following chapters in more details.

Furthermore, the mechanism of action of antimicrobial systems is based on either the slow release of toxic antibacterial agents or contact killing (no release of any active agent) [56,57]. Tethering long-tailed cationic moieties (*e.g.* quaternary ammonium compounds) to polymer matrixes, as an example to the latter case, has been studied extensively by various research groups [56,58-63]. Especially, Klibanov *et al.* have been done comprehensive studies on contact killing polymeric materials (e.g. with alkylated polyethyleneimines) [57-59,64,65]. It is well-known that immobilized quaternary ammonium compounds (if they have sufficiently long hydrophobic tails) possess antibacterial properties by interacting with and disrupting bacterial cell membranes [66-68]. When the bacterial cells are in a contact with such surfaces, long hydrophobic chains presumably penetrate and disrupt the cell membranes. One major drawback of such commonly used systems is their unnecessary antimicrobial compound concentration in the bulk. Our approach is to use surface segregation of these compounds since only the ones at the top of the surface are biocidal.

Many factors have found to affect the antimicrobial efficacy of these materials, such as molecular weight of the polymer, spacer length between active site and the backbone, hydrophobic tail length attached to the active site, and the hydrophilic-hydrophobic balance of the material [69,70]. Some

reports indicate that the nature of the counterions is influential as well [49,71], but evidence to the contrary also exists [72].

1.4. Objectives and the Scope of the Thesis

The objective of this thesis is the preparation of environmentally-benign antimicrobial coatings with a surface-enrichment in antimicrobial groups via a self-stratification approach.

Both methods and products of this study have been designed according to the various requirements of an application-oriented antimicrobial surface coating: strong antibacterial activity, durable and non-toxic nature, no leaching of any active component, non-altered bulk properties.

Quaternary ammonium compounds (QACs) have been chosen as antimicrobial agents due to their highly bactericidal and non-toxic nature. Contact killing, as the mechanism of action of QACs, is a safe and environmentally friendly way to prevent microbial growth. In this mechanism, antimicrobial activity is a surface property just like the low surface-energy films. Thus, the incorporation of a low amount of low surface-energy and highly antimicrobial quaternary ammonium compounds into the polymeric network by using the self-stratification approach would give the best results in terms of good surface and non-altered bulk properties.

Chapter 2 describes the synthesis of long hydrocarbon tailed quaternary ammonium compounds. Synthesized compounds are used to prepare polyurethane coatings *via* a self-stratification approach. Analyses

and protocols to examine surface and antimicrobial properties will mainly be discussed in this chapter.

Chapter 3 introduces perfluoroalkyl chains in quaternary ammonium structures. The advantages and the effects of perfluoroalkyl chains will be studied in this chapter in detail.

Chapter 4 describes the incorporation of some commercially available ionic liquid quaternary ammonium compounds into the current polymeric network in order to prepare antimicrobial coatings. In this chapter QAC segregation at the air/film interface is not expected due to the hydrophilic nature of the ionic liquid structures.

In *Chapter 5*, a model method for preparing self-stratified surface coatings will be attempted. Unlike the previous chapters, instead of long and hydrophobic QACs, highly hydrophilic QAC structures are used in this study. The idea is that these compounds segregate at the film surface by using an “inverse” self-stratification approach. However, this model system has multiple problems and drawbacks which will be discussed in this chapter.

Finally, some conclusions and remarks will be given in *Chapter 6*.

1.5. References

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Chapter 2

Preparation of Polyurethane Coatings Based on Hydrocarbon Tailed Quaternary Ammonium Compounds*

Summary

In this chapter the preparation of polyurethane surface coatings which contain two different antimicrobial quaternary ammonium compounds via a self-stratification approach is described. Self-stratification is proven by contact angle and X-ray photoelectron spectroscopy techniques. Strong antimicrobial activity test results are obtained against S. aureus and E. coli. The contact killing mechanism of biocidal activity is confirmed by a zone of inhibition test. The effect of spacer length between the antimicrobial moiety and coating surface on antimicrobial activity is observed to enhance the biocidal property.

***** Part of the results presented in this chapter have been published in: Yagci,M.B.; Bolca, S.; Heuts, J.P.A.; Ming, W.; de With, G. *Prog. Org. Coat.*, **2011**, *72*, 305.

2.1. Introduction

Increased general desire for hygiene in everyday life as well as in the (medical) industry has prompted a strong interest in antimicrobial coatings to prevent the growth and/or accumulation of harmful bacteria. The common way to prepare bactericidal materials is to saturate them with some antibacterial agents. The activity of such systems is based on the slow release of the antibacterial agents, such as antibiotics [1,2], metal ions (*e.g.* Ag^{+}) [3-7], and biocidal cationic moieties (*e.g.* phosponium or ammonium salts) [8-12] into the surroundings over time. Although this approach has proven to be highly effective, the active biocides will be exhausted after some time. Also, leaching of toxic biocides and heavy metal ions into the surroundings is not desired from an environmental point of view [13]. Therefore, polymers/coatings exerting antimicrobial effects upon contact with bacteria without releasing toxic agents are of strong current interest [13-17]. Grafting the quaternary ammonium compounds (with a long hydrophobic tail) to the coating systems has been studied extensively by various groups [18-31]. The biocidal mechanism of such coatings is based on so called "contact killing". The essence of this mechanism is as follows: It is well known that the bacterial cell surface is usually negatively charged. The cytoplasmic membrane is semi-permeable membrane and is composed of a phospholipid bilayer with some functional proteins. The cationic hydrophilic-hydrophobic property (high lipophilicity) of long tailed cationic structures (*e.g.* QACs) provides a good surfactant character resulting in a high binding affinity for bacterial cells. This phenomenon causes damage to the integrity of the cell membrane followed by cytoplasmic membrane disruption, and finally results in cell lysis [32,33]. The step-by-step action of the cationic biocides may be considered as follows [34]: (1) adsorption onto the bacterial cell surface, (2) diffusion through the cell wall, (3) adsorption onto the cytoplasmic membrane, (4) disruption of the cytoplasmic

membrane, (5) leakage of the cytoplasmic constituents, and (6) death of the cell. This mechanism of action has been seen clearly by Kenawy and Mahmoud [35]. They synthesized copolymers of 2-chloroethylvinyl ether and different acrylates with immobilized quaternary ammonium or phosphonium salts. Then, they have successfully demonstrated the cytoplasmic membrane disruption of *S. aureus* cells by an electron microscope and a release of potassium ions from the cytoplasm, that both caused by the synthesized cationic polymers.

As explained above, the positive charge results in stronger polymer-membrane interactions by strengthening the electrostatic attraction between positively charged QAC and negatively charged bacterial cell membrane. In addition to that, the positive charge has also another very important feature. Basically, the long hydrophobic chains, themselves, have a capability of penetrating and disrupting the cell membranes of the bacterial cell (like bursting a bubble). However possessing hydrophobic long chains is not enough to exert biocidal activity. Besides from being entropically disinclined to stay erect, such chains would hydrophobically attract each other resulting in a shapeless mass instead of forming separate individual chains like needles. Thus, making such chains positively charged, like in the QAC structures, should rigidify them and their hydrophobic association should be prevented [13, 36-38].

The present study aimed at preparing such antimicrobial coatings with only their surface enriched in contact killing quaternary ammonium compounds by using a surface segregation strategy. For contact killing coating systems the antimicrobial property is a surface property, and consequently there is no need for killing groups to be present in the bulk of the coating, potentially resulting in undesired bulk properties. Our system

should allow the preparation of coatings with unaltered bulk and optimized antimicrobial surface properties.

2.2. Experimental

2.2.1. Materials

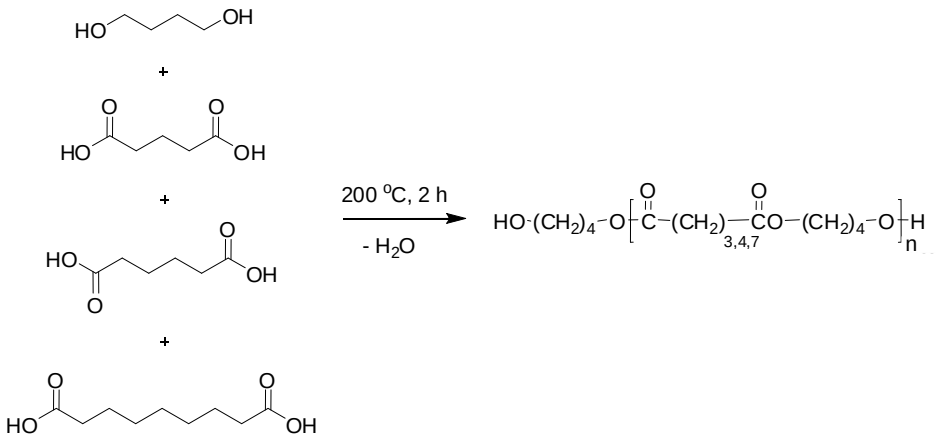
Adipic acid (>99%), glutaric acid (>99%), azelaic acid (~88%), and 1,4-butanediol (>98%) were purchased from Merck and used as received. A dialkyl tinoxide (Fascat 4101) was used as catalyst for direct esterification. N,N-dimethylundecyl amine (97%), 3-dimethylamino-1-propanol (99%), 11-bromo-1-undecanol (>99%), 1-bromoundecane (98%) were purchased (Aldrich) and used as received. A polyisocyanate crosslinker (containing mainly the isocyanurate trimer of hexamethylene diisocyanate), with the trade name Desmodur N3600, was obtained from Bayer AG. Microscopic glass slides (20 mm x 20 mm x 0.15 mm; Menzel-Glazer) were used as substrate for film preparation. Solvents were used as received without any further purification.

2.2.2. Synthesis of Solventless Liquid Oligoester

This hydroxyl di-functionalized linear oligoester (SLO) (Scheme 2.1), was synthesized by direct esterification between a mixture of diacids and 1,4-butanediol as follows [39]: adipic acid, glutaric acid, and azelaic acid (0.3 mol each), 1,4-butanediol (1.2 mol), and Fascat 4101 catalyst (0.1% of total weight) were added into 500 ml 4-neck-round bottom flask equipped with mechanical stirrer, thermometer, and Dean-Stark condenser. The flask was gradually heated to 140 ^{o}C (under continuous N_2 flow), and the temperature rose to 200 ^{o}C in 2 h. After 90% of the theoretical amount of

water product was collected in the Dean-Stark trap, the reaction was stopped. The obtained liquid product was dried under vacuum at 60 °C for 48 h to remove the trace amount of water.

Scheme 2.1. Synthetic scheme of solventless liquid oligoester (SLO).

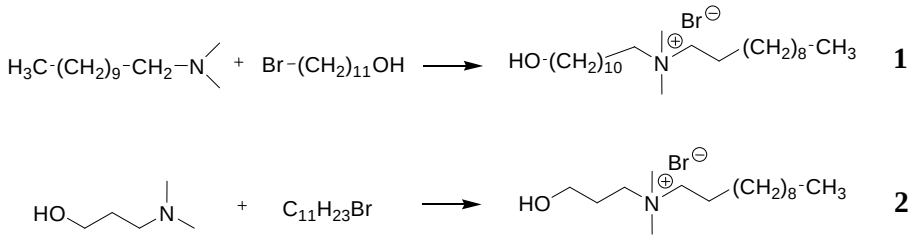


2.2.3. Synthesis of Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) with two different spacer lengths were synthesized by a one-step quaternization reaction [40]. QAC **1** (Scheme 2.2.1) was prepared by reacting 3.9 mmol N,N-dimethylundecyl amine with 4.2 mmol 11-bromo-1-undecanol. The starting materials were added to a 25 ml 2-neck round bottom flask and refluxed at 80 °C. After 30 min of stirring, the reaction mixture was cooled to room temperature and a 1:3 (v:v) propanol:methanol mixture was poured into the flask. After another 50 min of reaction at 80 °C the solvent mixture was evaporated. The product was purified by precipitating in diethyl ether twice. The obtained white solid was dried under vacuum at 60 °C for 3 h (Yield: 82%). QAC **2** (Scheme 2.2.2) was obtained from the reaction between 3-

dimethylamino-1-propanol (1.5 mmol) and 1-bromoundecane (1.3 mmol) using a similar experimental and purification protocol using 12 h reaction time instead of 50 min (Yield: 77%).

Scheme 2.2. Synthetic scheme of QAC **1** and QAC **2**.



2.2.4. Polyurethane Film Preparation

Polyurethane coatings were prepared from a mixture of solventless liquid oligoester, synthesized QACs, and the polyisocyanate crosslinker with chloroform as the solvent (60 wt% solids content) as shown in Scheme 2.3.

The molar ratio of NCO/OH was adjusted at 1.1 to ensure full conversion of OH groups. Coating mixtures were applied on glass substrates using a roller film applicator. After application, films were first kept at room temperature for 30 min to give time to the QACs to diffuse towards the surface, then cured at 50 ^{o}C for 2.5 h. After curing, the films were dried overnight at 50 ^{o}C under vacuum to remove any trace amount of solvent. In order to remove any possible unreacted QAC, samples were first intensively rinsed with deionized water, then washed in a soxhlet set-up (deionized water was used as washing liquid) for 45 min and dried under vacuum at 50 ^{o}C overnight. The QAC content is shown in Table 2.1.

Scheme 2.3. Synthetic scheme of the reactive components of a typical coating mixture.

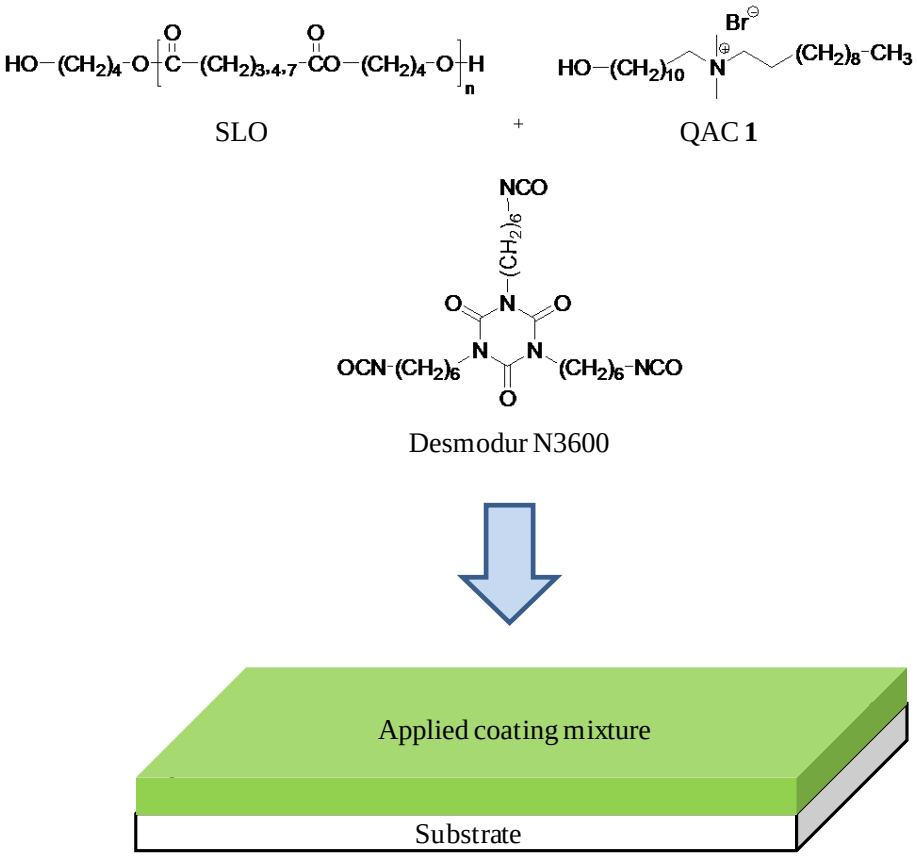


Table 2.1. The QAC content (in bulk) in the prepared coating systems.

Coating Systems	n_{NCO}/n_{OH}	QAC Content (mmol/g) (wt %)			
1	1.1	0.014 (0.6)	0.025 (1.2)	0.035 (1.6)	0.048 (2.2)
2	1.1	0.014 (0.5)	0.025 (0.9)	0.035 (1.2)	0.048 (1.6)

2.2.5. Characterization Techniques

^{1}H-NMR spectra were recorded using a Varian 400 spectrometer at 25 ^oC operating at 400.162 MHz. The sample concentration was set to be about 3 wt% in $CDCl_3$ (containing 1% TMS) as the NMR solvent. A delay time of 3 seconds was used and a total number of 16 scans were accumulated. The thickness of the synthesized coatings was measured using a Twin-Check instrument (List-Magnetic GmbH). The glass transition temperatures were determined on a Perkin Elmer Pyris 1 differential scanning calorimeter (DSC) at a heating rate of 20 ^oC/min, in a temperature range of -100 to + 100 ^oC. The T_g of the films was determined by the half C_p extrapolation method during the second heating run. Dynamic contact angle measurements were carried out using a Dataphysics OCA 30 instrument using deionized water as probe liquid. Dynamic advancing and receding angles were measured by the Sessile Drop technique while the probe liquid was added to and withdrawn from the drop, respectively. All the contact angles were determined by averaging values measured at three different points on each sample. X-ray photoelectron spectroscopy (XPS) measurements were performed using a VG-Escalab 200 spectrometer using an aluminum anode (Al Kα = 1468.3 eV) at electron take-off angles of 90^o and 30^o (between the film surface and the axis of the analyzer lens). Spectra were recorded using a VGX900 data system. All C1s peaks corresponding to hydrocarbons were calibrated at a binding energy of 284.5 eV to correct for the energy shift caused by charging. The Br/C atomic ratios were estimated from the area ratios under the corresponding XPS curves (Atomic sensitivity factors were used as follows: C 1s = 0.205, Br 3d = 0.59).

2.2.6. Antimicrobial Test Procedure

The bacterial cell grows by duplicating itself. By definition, when one cell divides to form two, one generation has occurred. The generation time is the time required for the cell population to double. Depending on the growth medium and the incubation conditions, many bacteria have generation times of 1-3 h under the optimum conditions. However, a few organisms grow much faster (*e.g. E. coli* can complete a cycle in 20 min. under laboratory conditions). Bacterial cell growth is exponential, which means that bacterial organisms grow exponentially. In such a growth, the rate of increase in cell number is slow initially but increases at an ever faster rate. Thus, in later stages this results in an explosive increase in the cell numbers [41].

Total cell count is a direct measurement for determining the number of single-celled microorganisms. In principle, the number of cells is counted by observation under a microscope. A known amount of microbial suspension is placed on a glass slide which has precisely measured small grids (to form squares of known area). The number of cells per unit area of grid can be counted under the microscope, and then the number of cells per milliliter of suspension can be calculated by multiplying this number by the number of unit grids covered by suspension. In such a microscopic counting, both living and dead cells are counted. However, in many cases like antimicrobial activity measurements, people are interested in only live cells. For this purpose the viable count method is preferred [41].

Viable count is the determination of the number of cells in the sample capable of forming colonies on an agar medium. The assumption made here is that *each viable cell can generate one colony*. In this method, a volume (0.1 ml or less) of a diluted culture is spread over an agar plate. After

an appropriate incubation time, the number of colonies formed is counted. A serial dilution (mostly 10-fold dilutions) of the culture is necessary here in order to increase the accuracy. It is important that the number of colonies forming on the agar plate is not too large (on crowded plates colonies may not form, and some colonies may fuse) and not too small (for statistical significance). To more clearly state the results of a viable count, the data are often expressed as the number of colony forming units (cfu) since a cfu may contain one or more cells [41].

2.2.6.1. Bacterial Log Reduction Test

The bacterial log reduction test method has been designed to quantitatively test the antimicrobial property of plastics over a 24 h period of contact. In most of the studies JIS Z 2801 and ISO 22196 standard test protocols or their modified versions are used to examine the antimicrobial efficacy of polymeric materials [42-44].

Bacterial log reduction tests principally consist of the following steps. The test microorganism is grown in a liquid culture medium. The suspension of test microorganism is standardized by dilution to 10^5 - 10^8 cfu/ml. Control and test surfaces are inoculated with microorganisms, in triplicate, covered with a thin, sterile film, and then are allowed to incubate in a humid environment for 24 h. After incubation, microbial concentrations are determined by the viable count method (see above) after a serial dilution as follows [45,46]:

$$\text{CFU/ml} = \text{Counts} \times (1/\text{ml suspension on plate}) \times 10^{\#\ \text{serial dilution}}$$

The effect of an antimicrobial compound is then measured by subtracting the log reduction between the control sample and the test sample:

$$\text{Log Reduction} = \log_{10} \text{Control (cfu/ml)} - \log_{10} \text{Sample (cfu/ml)}$$

In order to be called antimicrobial against a certain type of bacterium, a test sample should show at least 2 $\log_{10}$ reduction (i.e. a hundred-fold reduction).

Since this method is quantitative and results tend to be reproducible, in this study we also use a very similar protocol as follows:

Cultures of *Staphylococcus aureus* and *Escherichia coli* were grown aerobically during 24 h at 37 °C in sterile Luria-Bertani (LB) broth (normal rich growth medium: NaCl 5 g/l; yeast extract 5 g/l; trypton 10 g/l). These active growing cultures were diluted in saline (NaCl 8.5 g/l) to a concentration of 10^5 cfu/ml and, subsequently, distributed over 25-well Sterilin plates (10^5 *S. aureus* or *E. coli* per well) containing 1 cm² of coating/well, except for the control well without coating. An incubation time of 24 h at 37 ^{o}C was used without shaking the Sterilin plates. Although plates were not shaken in order to avoid wrinkling and rolling up of the sample films, full contact between bacterial solution and prepared films was ensured by adding only 1 ml of bacterial solution on 1 cm^2 samples. After 24 h of incubation, a dilution series of each treatment was made and 100 µl of 3 dilutions were plated on sterile LB agar (LB broth + agar 15 g/l). After 24 h, the number of colonies on each plate was determined to get the corresponding concentration of living bacteria.

2.2.6.2. Kirby-Bauer (Zone of Inhibition) Test

Although the Kirby-Bauer test was developed for examining the antimicrobial efficacy of small, diffusive antimicrobial molecules (*e.g.* antibiotics), this test is also commonly used as a leach-out test for polymeric antimicrobials [28,47,48]. First, a microbial suspension is spread over a sterile agar plate. Then, the antimicrobial agent is applied on the center of the agar plate. During the incubation period, the plated bacteria can grow only where there is no antimicrobial agent. In case of any leaching of the agent from the sample to the surroundings (agar in this case), bacterial growth is inhibited in a zone around the sample, the width of which is determined by the diffusion of the active compound in the surroundings (Figure 2.1).

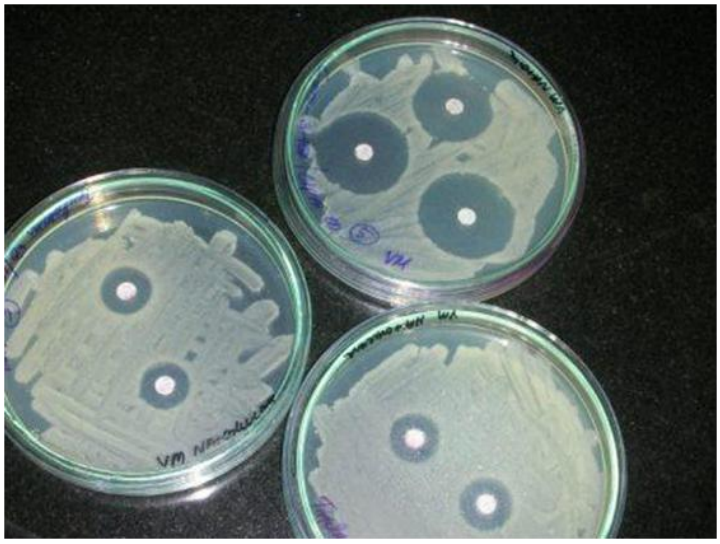


Figure 2.1. Inhibition zones of Ciprofloxacin on different bacillus species [49].

In accordance to the literature [28,47,48], we used this method to prove that the used antimicrobial groups in this study are covalently attached

to the polymer network and do not leach out from the ultimate coating to the surroundings. Any possible leaching of the active compound would result in inhibited bacterial growth around the films as seen in figure 2.1.

With this purpose, some representative samples that showed antimicrobial activity were tested for any possible leaching of active QAC species. Lawns of *S. aureus* were plated on blood agar. Films (1 cm x 1 cm) were then placed upon these lawns with their active side on the agar. The plates were placed in a 37 ^{o}C incubator for 24 h and afterwards evaluated for the presence of inhibition zones [48].

2.3. Results and Discussion

2.3.1. Synthesis and Characterization of the Solventless Liquid Oligoester

As the first step of the study, an oligoester which should later constitute the bulk structure of the ultimate films was synthesized by direct esterification between a diol and a mixture of diacids. The aim of using a mixture of diacids was to reduce the regularity of the ester backbone and ensure that the oligoester would be liquid at ambient temperature. All the chain ends were targeted to be hydroxyl in order to enable the further crosslinking of oligoester with polyisocyanate. This was achieved by controlling the degree of condensation stoichiometrically [39]. Towards the end of the reaction, the flow rate of nitrogen was increased to help the water removal and prevent transesterification, and consequently, carry the condensation to completion.

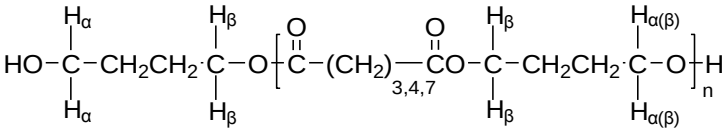


Figure 2.2. Different characteristic protons (H_α and H_β) in the synthesized solventless liquid oligoester.

The ^{1}H-NMR spectrum of the synthesized oligoester is given in Figure 2.3. The degree of condensation (d_c) was calculated from the peak area ratio of the peak at 4.1 ppm (which corresponds to the proton in the methylene adjacent to the ester group (-C$\underline{H_2}$-O-CO), H_β in Figure 2.2) and the peak at 3.7 ppm (which corresponds to the proton in the methylene adjacent to hydroxyl group (-C$\underline{H_2}$-OH), H_α in Figure 2.2) [39,50].

$$d_c = \text{area}(H_\beta)/\text{area}(H_\alpha)$$

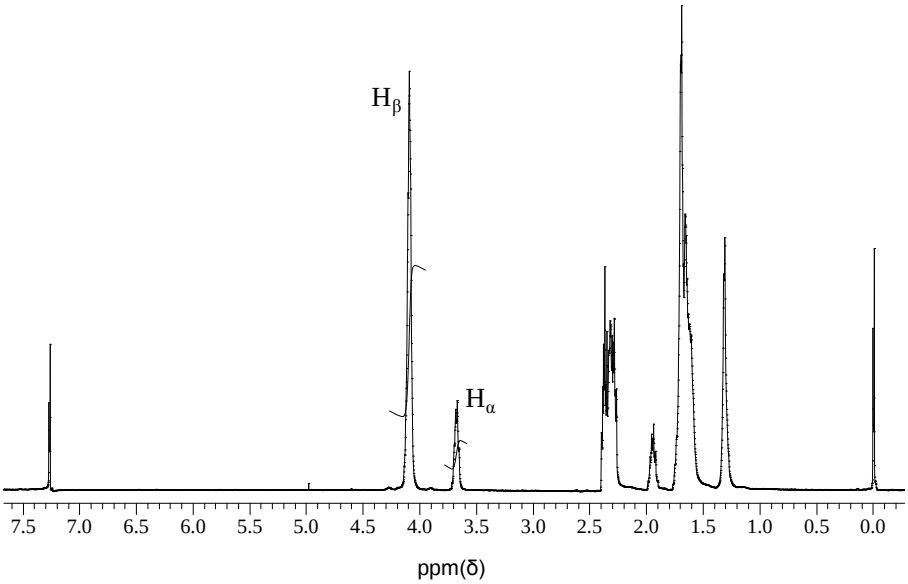


Figure 2.3. ^{1}H-NMR spectrum of solventless liquid oligoester.

By using the obtained degree of condensation, the average molecular weight (M_n) of the oligoester was calculated by the following equation

$$M_n = d_c(MW_{(diacid)} + MW_{(diol)} - 2MW_{(water)}) + MW_{(diol)}$$

where $MW_{(diacid)}$, $MW_{(diol)}$, and $MW_{(water)}$ are the molecular weights of diacids (average of the three), 1,4-butanediol, and water, respectively.

The hydroxyl number (the number of milligrams of potassium hydroxide equivalent to the hydroxyl groups in 1 g of material) of the synthesized oligoester was determined by end group titration according to a test method, TM-2432 by DSM Resins. This value is important to know prior to the film formulation in order to match the correct NCO/OH molar ratio in the ultimate film. Briefly, the hydroxyl groups in the oligoester are first acetylated with a known amount of acetic anhydride, the excess anhydride is decomposed with water, and the formed acetic acid is titrated with a methanolic potassium hydroxide solution. The number average molecular weight of the oligoester can be deduced from the hydroxyl number, considering that all chains end with a hydroxyl group. Also, the acid value (similarly, the number of milligrams of potassium hydroxide equivalent to the carboxyl groups in 1 g of material) of the oligoester was similarly checked (according to the TM-2401 test method by DSM Resins), and found to be lower than 1 mg KOH/1 g oligoester, confirming all the chain ends were hydroxyl capped. All the related values are listed in Table 2.2.

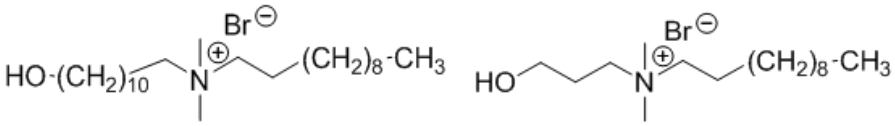
Table 2.2. Molecular weight calculations of the solventless liquid oligoester.

d_c by ^{1}H-NMR	M_n (g/mol) by ^{1}H-NMR	Hydroxyl no.	Acid no.	M_n (g/mol) by titration
4.55	1,044	108	0.76	1,038

As seen in Table 2.2, the average molecular weight of the oligoester obtained by titration (M_n = 1,038 g/mol) was found to be in a good agreement with that obtained by ^{1}H-NMR (M_n = 1,044 g/mol).

2.3.2. Synthesis and Characterization of the Hydrocarbon Tailed Quaternary Ammonium Compounds

The two quaternary ammonium compounds were synthesized *via* a direct quaternization reaction between either a dimethylalkyl amine and a haloalcohol or a dimethylamino alcohol and a haloalkane (Figure 2.4).



1 **2**

Figure 2.4. Chemical structures of QAC **1** and QAC **2**.

The completion of the reaction was checked with ^{1}H-NMR by monitoring the complete disappearance of the characteristic peak of the methyl protons of the tertiary amine at 2.2 ppm (f in Figure 2.5.A) and the generation of those of the quaternary ammonium at 3.4 ppm (f in Figure 2.5.B). A representative NMR spectrum is given in Figure 2.5.

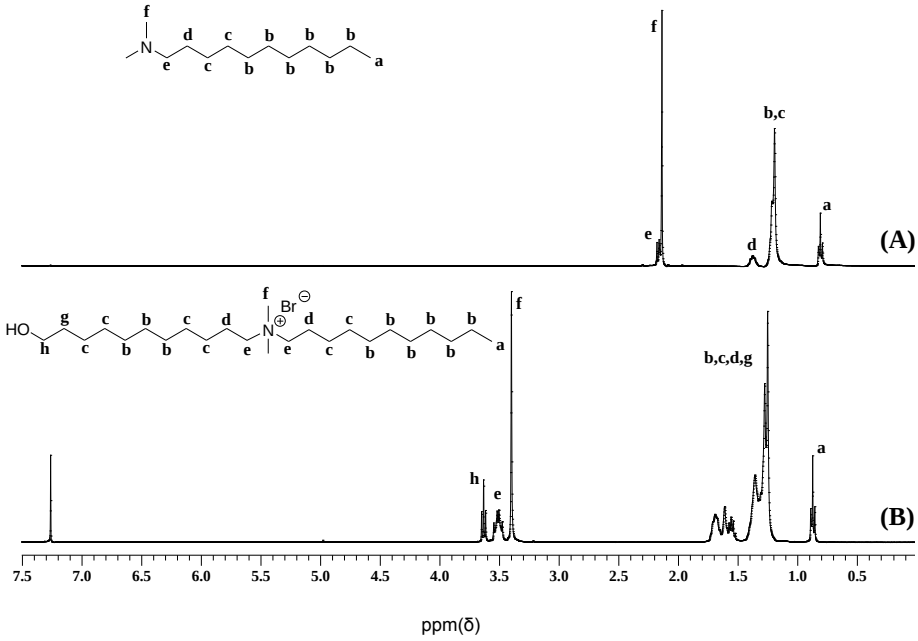


Figure 2.5. ^{1}H-NMR spectra of (**A**) N,N-dimethylundecyl amine, and (**B**) QAC **1**.

As seen in the Figure 2.5, the complete disappearance of the peak at 2.2 ppm confirms the full conversion in the quaternization reaction.

2.3.3. Preparation of Coatings

In the film recipe the molar ratio of NCO/OH moieties was adjusted to 1.1 in advance. The reason is to ensure all the OH-end-capped QAC compounds are covalently attached to the polymer backbone, thus to eliminate the possibility of leaching of free QAC compounds to the environment. For that purpose, the hydroxyl number of SLO was determined by end group titration prior to the film formation as mentioned previously. Similarly, the isocyanate content of Desmodur N3600 crosslinker was determined as mol % according to the ASTM D 2572-91 test method. In

brief, the NCO groups are first reacted with an excess of di-n-butylamine. After the reaction is complete, the excess of di-n-butylamine is determined by back titration with standard hydrochloric acid.

After curing, the thickness of the prepared films was measured with a Twin-Check instrument and found to be 40-60 μm. Prepared films showed good solvent resistance as tested by the acetone double-rub test. Coatings survived from 100 times of double-rub. The glass transition temperature T_g as obtained from DSC measurements was found to be between -42 and -44 ^oC for all films.

2.3.4. Surface Properties of the Coatings

2.3.4.1. Contact Angle Results

Once the films were cured, the QAC presence at the surface was monitored by contact angle (CA) measurement. In this study, since the quaternary ammonium compounds possess a dual-nature structure (possessing both hydrophobic and hydrophilic moieties in their structure) and a surface reorientation under aqueous environment seemed to be possible, dynamic advancing and receding contact angles were measured instead of static contact angles. The average contact angle values were obtained from three measurements from different points on each sample by Sessile Drop technique. Deionized water was used as the probe liquid. The CA data of the films containing an increasing concentration of QACs are shown in Figure 2.6.

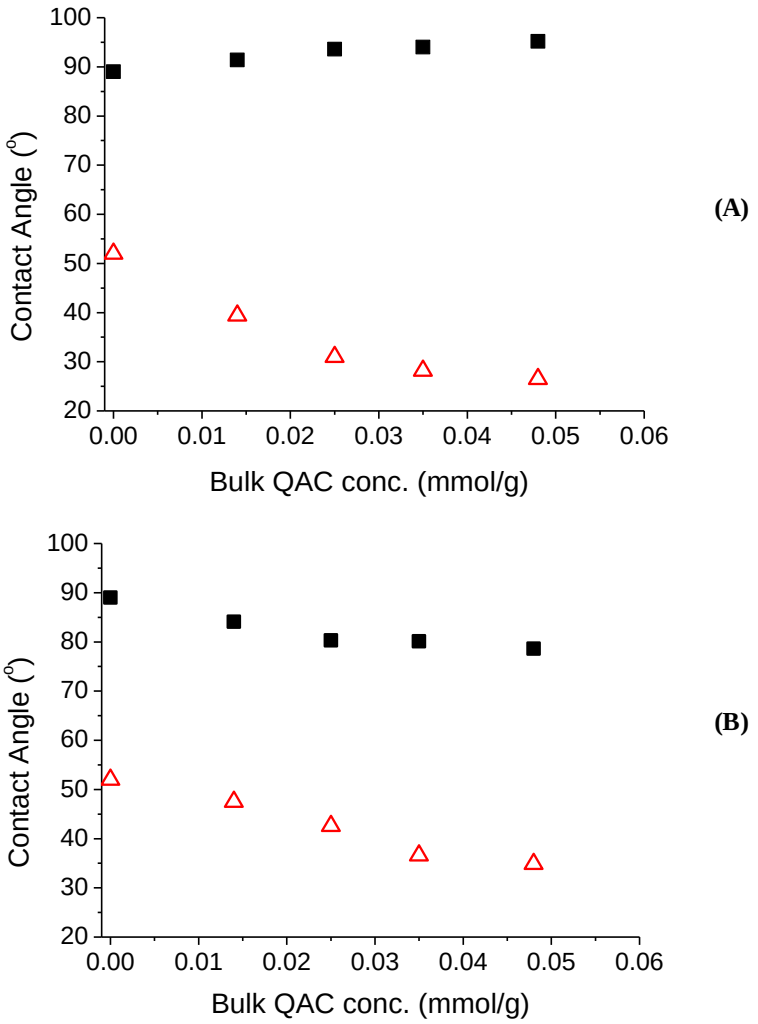


Figure 2.6. Advancing and receding water contact angles of the systems (**A**) **1** and (**B**) **2**: (■) θ_{adv}; (△) θ_{rec}.

As can be seen from Figure 2.6, coatings with QACs **1** and **2** showed only a small effect on the advancing contact angle θ_{adv} for water with increasing QAC concentration. This behavior is likely due to the combined effects from the hydrophobic tail and the hydrophilic quaternary ammonium moiety. The hydrophobic groups tend to increase θ_{adv}, whereas the

hydrophilic QACs tend to decrease it. Thus, these two effects may cancel each other largely as likely happened in our system. As Tingey *et al*. [51] have demonstrated extensively, when such a dual-natured surface is exposed to water, surface reorientation takes place in order to reduce the interfacial free energy. More hydrophilic moieties tent to be present at the polymer surface to replace or reduce regions of more apolar character. Since the hydrophilic phase acts as channels in which the water can recede (it holds back the draining motion of the contact line causing low receding angles) the receding contact angle θ_{rec} is more sensitive towards the hydrophilic moieties. Thus, the obvious decrease in θ_{rec} in both systems in our study is a clear indication of QAC presence at the air-film interface. However, contact angle measurements give information about the surface and are not enough to comment on surface segregation. Thus, QAC segregation towards the film surface was examined with X-ray Photoelectron Spectroscopy.

2.3.4.2. X-ray Photoelectron Spectroscopy Results

X-ray photoelectron spectroscopy was used to determine the relative QAC concentration at different depths from the film surface (Figures 2.7-2.11). The data obtained were compared with the calculated bulk ratio using the applied film composition with the assumption of no QAC segregation. Any QAC enrichment at the top of the surface would be proven if the obtained data were higher than the calculated bulk values. For this purpose a specific element for the QAC (the Br counterion in this case) over total carbon ratio was measured.

Figure 2.7 shows the wide XPS spectra of the coatings with and without the presence of QAC. A peak intensity correction was made by keeping the carbon peak intensity constant since the total C concentration

does not change significantly by the addition of QAC even at the highest concentration used (less than 1.5 mol%). In this way it is much easier to have an idea about the relative concentration changes of a specific atom in different samples, qualitatively (Figure 2.8 and 2.10).

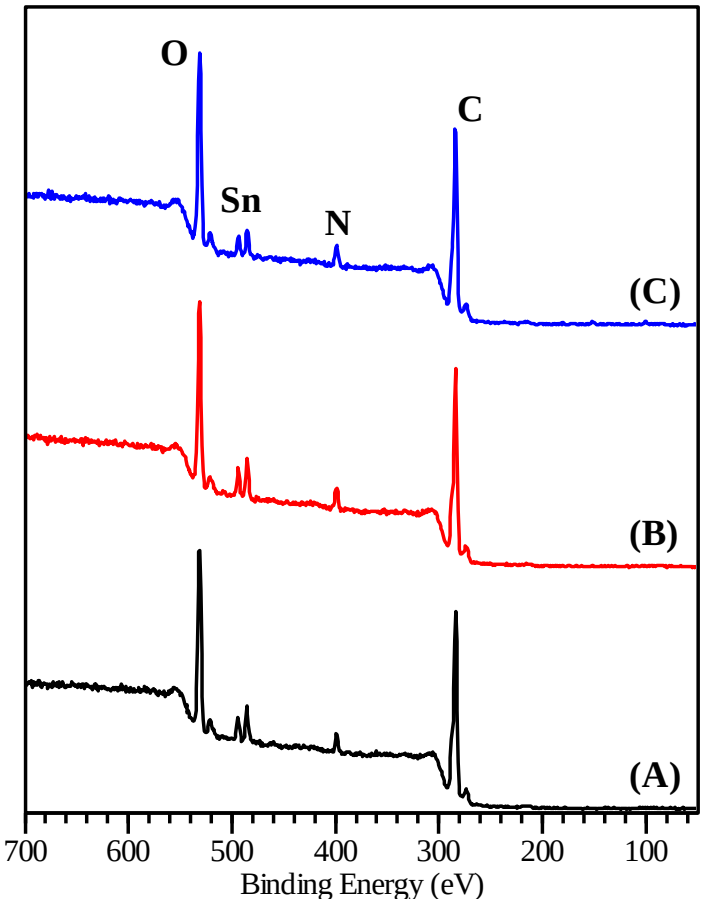


Figure 2.7. XPS wide spectra of (**A**) the coating without any QAC, (**B**) the coating that contains 0.035 mmol/g QAC **1**, (**C**) the coating that contains 0.035 mmol/g QAC **2**. The spectra were recorded at take-off angle of 90°, corresponding to the probe depth of ~10 nm.

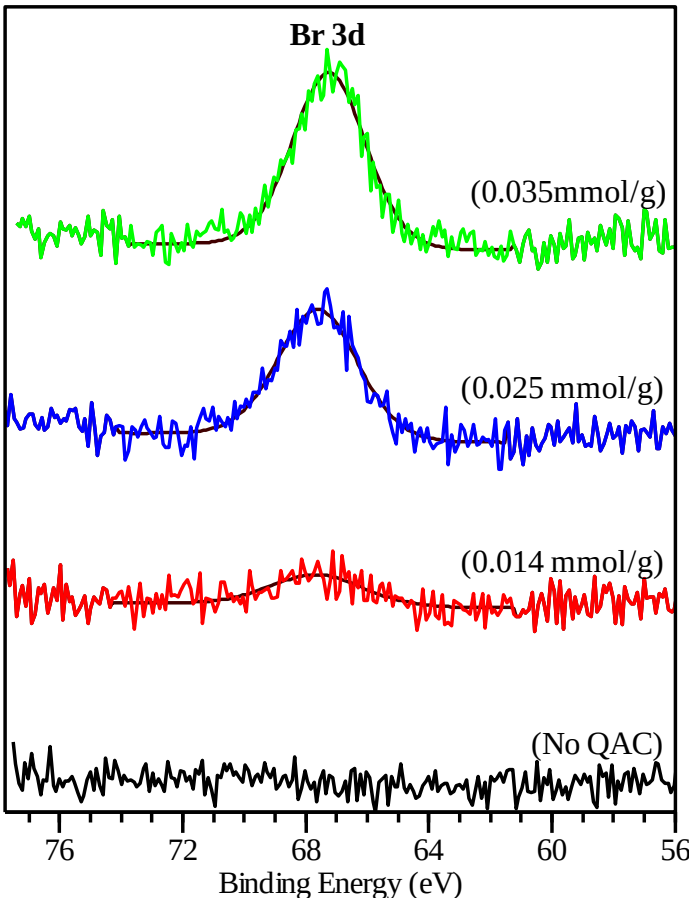


Figure 2.8. XPS Br 3d signals in the top 10 nm layer of the surface of samples of System **1**.

Figure 2.8 shows the Br signals of the coating surfaces containing QAC **1** which were recorded by XPS. A clear increase in Br (and therefore QAC) concentration is observed with increasing the QAC concentration (as expected) and was found to be in a good agreement with the receding water contact angle values in Figure 2.6.

In order to quantify the QAC concentrations on the sample surface, Br and C regions were monitored and plotted as Br/total C. Since the contributions of the other atoms from quaternary ammonium compounds like

N and O to the whole coatings mixture were very small (max. 1.4 mol% for N and 0.3 mol% for O), detection of the concentration change in N or O was not possible.

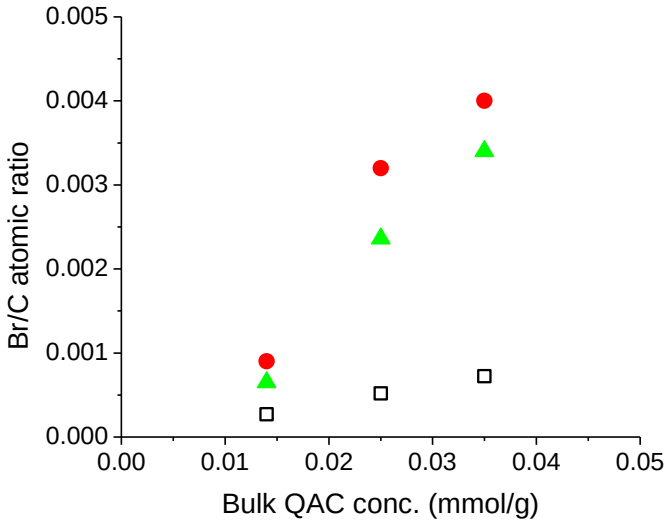


Figure 2.9. Br/C ratio in the top 5 and 10 nm of films from System **1**: (□) Bulk average; (▲) 10 nm; (●) 5 nm.

Thus, in Figure 2.9, Br/C atomic ratios in the top 10 and 5 nm of the surfaces are plotted. Also, the Br/C ratios from the QAC concentrations in coating recipe were calculated and shown in the graph. According to the measurements, relative Br concentrations in the top 10 nm were found to be much higher (3 to 5 fold) than the calculated bulk values suggesting hydrophobic quaternary ammonium compound enrichment at the surface. In addition to this, it was observed that the Br/C ratios in the top 5 nm were even higher than those in 10 nm proving a further QAC enrichment towards shallower probe depts. These observations clearly suggest antimicrobial compound segregation towards the surface of the film.

For the atomic ratio calculations we did not make any peak intensity correction, and all the calculations were made independently.

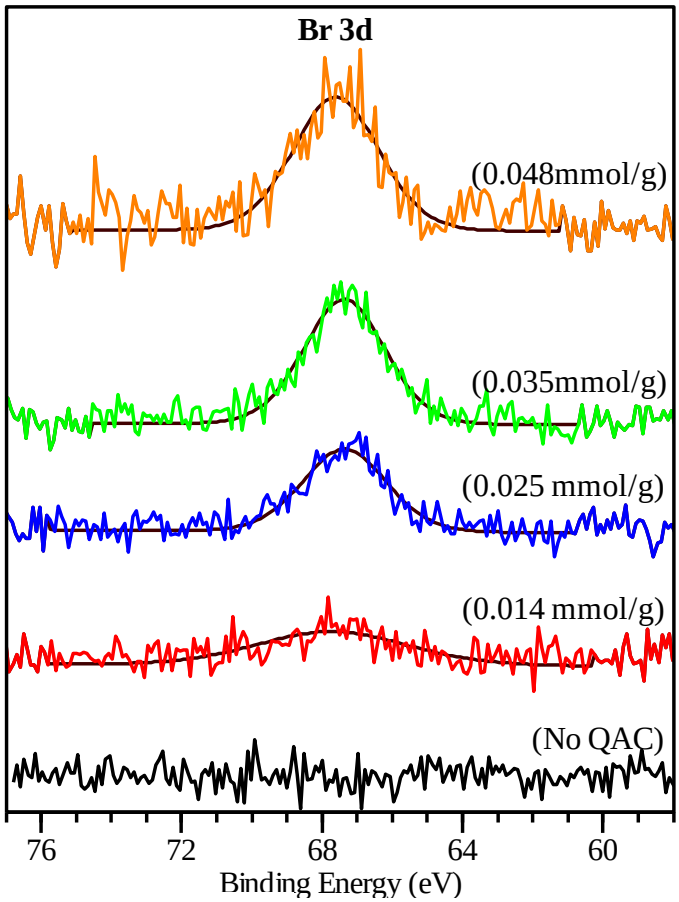


Figure 2.10. XPS Br 3d signals in the top 10 nm of the surface of samples from System **2**.

For system **2** similar calculations were made. Br/C rations in the top 10 nm of the prepared films can be seen in Figure 2.11.

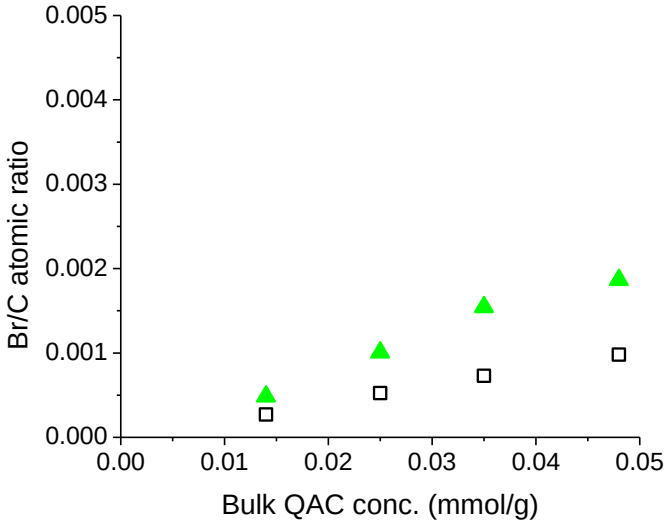


Figure 2.11. Br/C ratio in the top 10 nm of films from System **2**: (□) Bulk average; (▲) 10 nm.

Similar to system **1**, QAC segregation was observed in system 2 as well. This time, measured Br concentrations were found to be ~2 fold higher than the bulk averages. When compared to system **1**, surface segregation of QACs was found to be less effective in system **2**. The reason for this is that QAC **2** has a shorter hydrophobic spacer than QAC **1**, resulting in a more hydrophilic nature, and therefore it is less diffusive towards the air/film interface than QAC **1**.

2.3.5. Antimicrobial Activity of the Coatings

One of the conventional measures of bactericidal/bacteriostatic activity is monitoring the reduction of the number of viable bacterial cells ($\log_{10}$ scale) as colony forming units (cfu) in a certain time (this process follows first-order kinetics). The test procedure followed is similar to the standard antimicrobial susceptibility test protocols like ISO 22196 and JIS Z

2801. This method basically involves the immersion of test and control samples in bacterial suspensions with a known concentration of bacteria (10^5 to 10^8 cfu/ml) for a specified amount of time, and then estimating the number of viable microorganisms using a specified microbiological technique. During incubation, each viable organism divides many times, forming a single colony. Based on the number of cfu and the dilution levels, the number of viable organisms after the sample immersion is estimated [43]. For this purpose, the resulting films were tested against Gram-positive *S. aureus* and Gram-negative *E. coli* bacterial species for 24 h of incubation (Table 2.3).

Table 2.3. Bacterial $\log_{10}$ reduction after 24 h of incubation of 10^5 bacteria with 1 cm^2 of films.

Bulk QAC conc.	*Staphylococcus aureus*		*Escherichia coli*	
(mmol/g)	System **1**	System **2**	System **1**	System **2**
0[a]	0.5	0.5	0.8	0.8
0.014	3	1	1	1
0.025	5	2	2	1
0.035	5	3	5	1
0.048	5	2	5	3

[a] The control film was a polyurethane film with no quaternary ammonium compound.

In this study, an antimicrobial activity was defined as a decrease of at least 3 $\log_{10}$ units of a bacterial suspension (10^5 bacteria/ml) within 24 h. The standard antimicrobial susceptibility test protocols like ISO 22196 and JIS Z 2801 use a 2 $\log_{10}$ reduction and this criterion yields similar, though less discriminative, results.

The longer-spacered-QAC containing system **1** showed strong antimicrobial activity against *S. aureus* at every bulk QAC concentration (from 0.014 mmol QAC/g (0.6 wt%) to 0.048 mmol/g (2.2 wt%)). In addition, a 5 $\log_{10}$ killing efficiency (which means that not even 1 single colony was detected at any dilution level) was observed at all the concentrations higher than 0.014 mmol/g. Against *E. coli*, system **1** was found to be slightly less active and the desired activity was observed at 0.035 mmol QAC/g (1.6 wt%) concentration.

In contrast, the shorter-spacered-QAC containing system **2** was found to be less effective when compared to system **1** in accordance with the literature [52-54]. A minimally desired 3 $\log_{10}$ reduction was obtained with higher QAC concentrations for this system (0.035 mmol/g against *S. aureus* and 0.048 mmol/g against *E. coli*). Moreover, 5 $\log_{10}$ reduction was not obtained at any applied QAC concentration for system **2**. Similar to the longer spacered counterparts, films containing QAC **2** were slightly more active against Gram-positive *S. aureus* than Gram-negative *E. coli*., which has a more complex cell membrane.

2.3.5.1. Zone of Inhibition Test

Free quaternary ammonium compounds are known to be quite effective against bacteria. Any small amount of unreacted QAC in the system might deceive the antimicrobial test results. The zone-of-inhibition test is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms. Thus, in order to prove that the antimicrobial mechanism of our system is contact killing and not the slow release of biocidal compounds, a representative film sample from the

more active system **1** was exposed to a zone-of-inhibition test for 24 h of incubation [48].

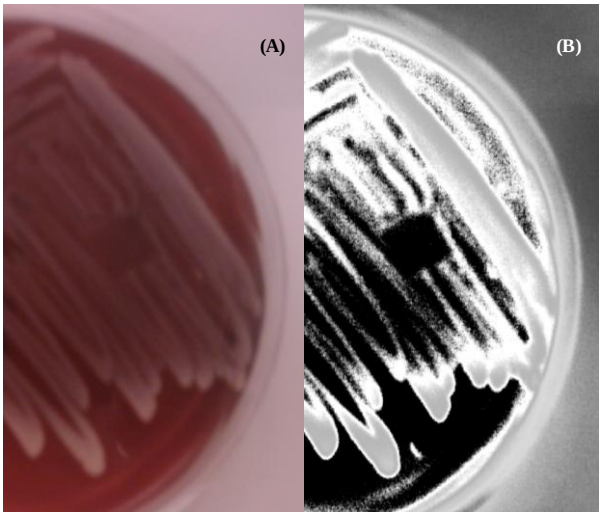


Figure 2.12. Picture of a zone-of-inhibition test result of 0.048 mmol/g QAC **1** containing sample, (Fig. **B** is the Adobe Photoshop modified version of the real picture (Fig. **A**) to help the reader to see the contrast easily).

As can be seen in Figure 2.12 the tested sample did not show any inhibition zone, indicating no evidence of any biocidal QAC leaching from the coating. This suggests that the antimicrobial effect of our films occurs only *via* surface contact. In addition to the zone-of-inhibition test, the water, which was used to extract the films for 45 min in a soxhlet set-up, was tested against the same bacterial species and did not show any biocidal activity within the detection limits suggesting that all quaternary ammonium compounds were covalently bonded to the polymer network.

2.4. Conclusions

Two long-hydrocarbon-tailed quaternary ammonium compounds were successfully synthesized. Incorporation of the synthesized QACs into the polyurethane network is presented. Water contact angle and X-ray photoelectron spectroscopy measurements proved QAC segregation on the surface. Covalent attachment of the QACs to the polymer network was concluded since no inhibition zone was detected and the extraction solutions of the films did not show any antimicrobial activity.

According to the antimicrobial activity tests, system **1** with a longer spacer group between N^+ and the film surface is much more active than its shorter counterpart system 2.

Since there was no observation of unreacted QAC leaching out from the polymer matrix, it is concluded that contact killing is the only mechanism of action for these films.

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Chapter 3

Preparation of Polyurethane Coatings Based on Fluorinated Quaternary Ammonium Compounds*

Summary

In this chapter perfluoroalkyl QACs are chemically incorporated in the polymer matrix. Enhanced surface segregation, enhanced surface hydrophobicity, and ease of following QAC concentration at the surface by XPS (by following the chemically bonded fluorine signal instead of the counterion) are targeted. Two different film preparation approaches (one-pot and pre-reaction) are presented in this chapter. Surface segregation occurs in both approaches but less effectively in pre-reaction approach. Longer perfluoroalkyl tail is found to enhance surface segregation and antimicrobial activity.

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3.1. Introduction

The bacteriocidal properties of quaternary ammonium compounds were first discovered during the late 1800s, among the carbonium dyes, such as auramin, methyl violet, and malachite green [1]. These compounds were found to be very effective against Gram-positive organisms. Jacobs and Heidelberger further studied their antibacterial activity of substituted hexamethylene tetraammonium salts against other types of organisms [2-5]. In 1928, Hartman and Kagi demonstrated the bacteriocidal activity of the QAC salts of acylated alkylene diamines [6]. However, it was not until 1935 that the full potential of QACs was recognized by the chemical community, when Domagk synthesized a long-chain benzalkonium chloride QAC [7]. The further characterization of its antibacterial activities proved that QACs were effective against a wider variety of bacterial organisms. Later, in the 20th century, researchers became more interested in the synthesis of water-soluble QACs for potential applications as surfactants [8,9] anti-electrostatic agents [10], anti-corrosive agents[11], disinfectants[12], and phase-transfer catalysts [13]. These newly developed quaternary ammonium salts showed antibacterial activity against not only Gram-positive and Gram-negative bacteria, but also pathogens such as fungi and protozoa [14]. These discoveries opened a huge variety of application areas to QACs (in both monomeric and polymeric forms) in common household products, and especially for general environmental sanitation in hospitals and food production facilities [15-29].

Fluorine attachment to QAC structures have already been studied by different purposes, such as micelle formation of surfactants [30], phase transfer catalysis [31], corrosion protection of low-carbon steels [32], and as solvent for the synthesis of super critical carbon dioxide [33]. Recently, Wynne *et al.* studied the preparation of low-surface energy antimicrobial

polymers [34-37]. They prepared polyurethane films having random soft blocks of quaternized alkylammonium oxetanes as the antimicrobial species. To implement the low surface energy concept, these researchers did not use fluorinated QACs, however they incorporated some fluorinated alkyl chains into the polyurethane network as side chains.

In this study, we have utilized both ideas: the fluorinated QAC and fluorinated low surface energy films. During the past few years, our group has successfully prepared low-surface energy polymer films with perfluorinated chains [38-44]. In what follows, the synthesis of two novel fluorinated quaternary ammonium compounds and their incorporation of the polyurethane network will be discussed. The effect of these compounds on film surface and antimicrobial properties will be demonstrated in detail. Also, in addition to our typical film preparation method, a so called pre-reaction, approach will be used to prepare new coatings. The aim of this is binding the QACs covalently to the crosslinker before preparing the ultimate film, as an additional control of having no free QAC in the final cross-linked films.

3.2. Experimental

3.2.1. Materials

The synthesis of solventless liquid oligoester was explained in the previous chapter. For QAC synthesis, 3-dimethylamino-1-propanol (99%) was purchased (Aldrich) and used as received. 1H,1H,2H,2H-perfluorodecyl iodide (97%) and 1H,1H,2H,2H-perfluorohexyl iodide (98%) were purchased from Fluorochem and used as received. A similar polyisocyanate crosslinker to Desmodur N3600, with the trade name Tolonate HDT-LV, was obtained from Rhodia. Aluminum panels (152 x 76 x 0.6 mm; Q-Panel

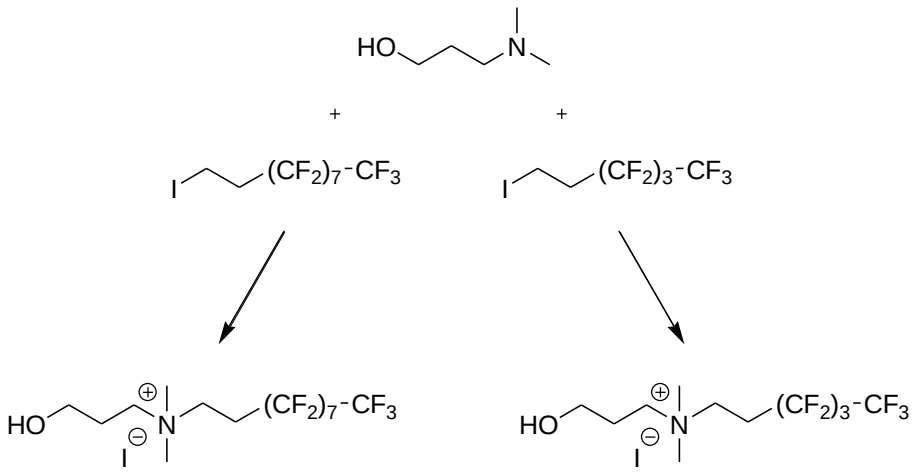
Co) were used as substrates. Besides deionized water, anhydrous hexadecane (Aldrich) was also used as probe liquid for contact angle measurements. Solvents were used as received without any further purification.

3.2.2. Synthesis of Solventless Liquid Oligoester

The solventless liquid oligoester was synthesized using the same procedure as described in *Chapter 2*. The number average molecular weight of the SLO was determined to be 1087 g/mol by end group titration and 1123 g/mol by ^{1}H-NMR.

3.2.3. Synthesis of Quaternary Ammonium Compounds

Scheme 3.1. Synthetic scheme of QAC **3** and QAC **4**.



3 **4**

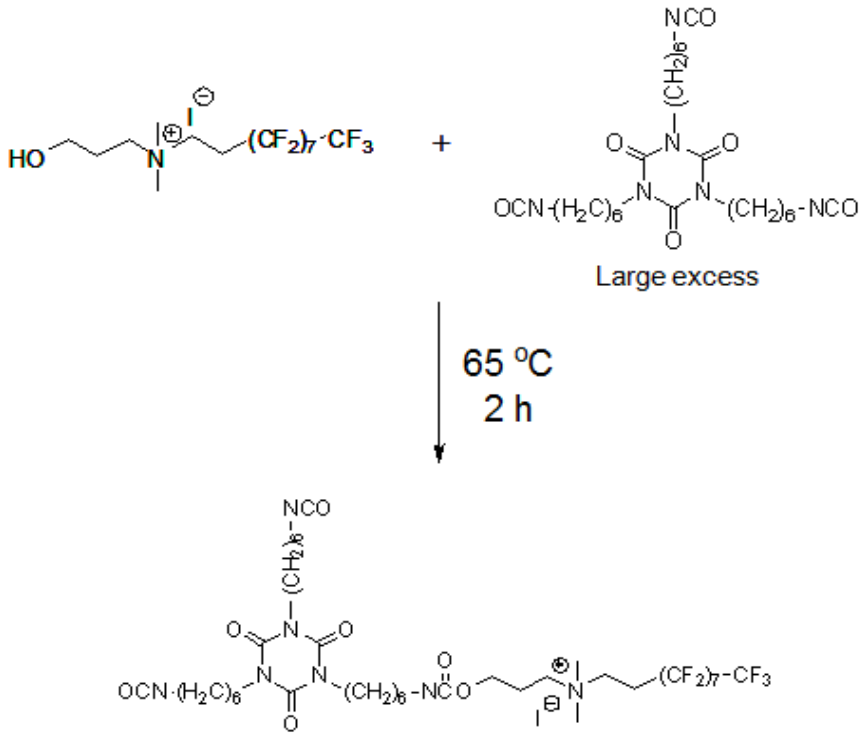
Perfluorinated quaternary ammonium compounds **3** and **4** (Scheme 3.1), with fluorinated tails of two different lengths, were prepared by reacting 3-dimethylamino-1-propanol with 1H,1H,2H,2H-perfluorodecyl iodide and 1H,1H,2H,2H-perfluorohexyl iodide, respectively. The alcohol and 1.1 eq. alkyl halides were added to a 25 ml 2-neck round bottom flask and heated to 80 ^{o}C under N_2 flow. After 90 min of reaction time, the obtained products were precipitated in diethyl ether and dried under vacuum at room temperature for 48 h (Yields: 56% and 38%, respectively).

3.2.4. Synthesis of Partially Fluorinated Isocyanate Crosslinker

4.474 g (8.17 mmol) Tolonate HDT-LV and 15 ml dry methylene iodide were put in a 50 ml 3-neck round bottom flask. 0.3386 g (0.5 mmol) QAC **3** was dissolved in 10 ml dry methylene iodide and then added dropwise to the reaction flask. The reaction was carried out for 2 h at 65 ^{o}C under Ar atmosphere. After the reaction (Scheme 3.2), the solvent was evaporated using a rotary evaporator at room temperature.

The pre-reaction approach was based on the idea of binding the QAC moiety covalently to the crosslinker before preparing the ultimate film, to ensure there is no free QAC in the final cross-linked films. To this end, 1 equivalent fluorinated QAC was reacted with 49 equivalents of triisocyanate crosslinker under Ar atmosphere for 2 h at 65 ^{o}C. The obtained product was later used to prepare a new set of antimicrobial films.

Scheme 3.2. Synthetic scheme of partially fluorinated isocyanate crosslinker (System **5**).



3.2.5. Polyurethane Film Preparation

Polyurethane coatings using the one-pot approach followed the same protocol as their hydrocarbon-tailed counterparts, which was explained in *Chapter 2*. Prepared mixtures were applied on aluminum substrates using a Doctor Blade film applicator. After application, films were cured at 80 °C for 1.5 h. Using the pre-reaction approach, the partially fluorinated crosslinker **5** was mixed with SLO and additional Tolonate crosslinker (in order to vary the final QAC concentration), then applied and cured as described above.

The molar ratio of NCO/OH was adjusted at 0.9 this time to ensure full conversion since the QAC compounds were attached to the system in advance. After curing, the films were dried overnight at 50 ^{o}C under vacuum to remove any trace amounts of solvent. In order to remove any possible unreacted QAC, samples were intensively washed with acetone in a soxhlet set-up for 45 min and dried under vacuum at 50 ^{o}C for 12 h. The QAC content in all three systems is shown in Table 3.1.

Table 3.1. The QAC content (in bulk) in the prepared coating systems.

Coating Systems	n_{NCO}/n_{OH}	QAC Content (mmol/g) (wt %)								
3	1.1	0.007 (0.5)	0.014 (0.9)			0.025 (1.7)	0.035 (2.4)	0.048 (3.2)		
4	1.1	0.007 (0.3)	0.014 (0.6)			0.025 (1.2)	0.035 (1.7)	0.048 (2.3)	0.075 (3.6)	0.100 (4.9)
5 *	0.9		0.013 (0.7)	0.018 (0.9)	0.022 (1.2)					

* System **5** is the prereaction product of QAC **3** and not another R_f-QAC.

3.2.6. Characterization Techniques

^{1}H and ^{19}F NMR spectra were recorded on a Varian 400 spectrometer at 25 ^{o}C operating at 400.162 MHz for ^{1}H and 376.487 MHz for ^{19}F, respectively. The sample concentration was set to be about 3 wt% in $CDCl_3$ (containing 1% TMS) as the NMR solvent. For ^{1}H-NMR, a delay time of 3 s was used and a total number of 16 scans were accumulated, whereas a delay time of 4 s and 128 scans were used for ^{19}F-NMR. Attenuated total-reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was performed on a Bio-Rad Excalibur FTS3000MX infrared spectrometer with an ATR diamond unit (Golden Gate). Spectra of the surfaces were recorded in the range of 4000-650 cm^{-1} with a resolution of 4 cm^{-1}. A full spectrum was taken every 30 s. One background spectrum (averaged from 100 scans co-added) was collected just before starting the measurements. The signal assigned to the CH_2 stretching peak at 2926 cm^{-1} was used as internal standard. The thickness of the synthesized coatings was measured with a Twin-Check instrument (List-Magnetic GmbH). The glass transition temperatures were determined on a Perkin Elmer Pyris 1 differential scanning calorimeter (DSC) at a heating rate of 20 ^{o}C/min, in a temperature range of -100 to + 100 ^{o}C. The T_g of the films was determined by the half C_p extrapolation method during the second heating run. Dynamic contact angle measurements were carried out using a Dataphysics OCA 30 instrument using deionized water and hexadecane as probe liquids. X-ray photoelectron spectroscopy (XPS) measurements were performed using a VG-Escalab 200 spectrometer using an aluminum anode (Al Kα = 1468.3 eV) at electron take-off angles of 90^{o} and 30^{o} (between the film surface and the axis of the analyzer lens). Spectra were recorded using a VGX900 data system. All C1s peaks corresponding to hydrocarbons were calibrated at a binding energy of 284.5 eV to correct for the energy shift caused by charging. The F/C and I/C atomic ratios were estimated from the area ratios

under the corresponding XPS curves (Atomic sensitivity factors were used as follows: C 1s = 0.205, F 1s = 1.0, I $3d_{5/2}$ = 4.4). Atomic Force Microscopy (AFM) measurements were performed with Solver P47H (NT-MDT) in non-contact mode. An NSG 11S cantilever was chosen, and the typical resonance frequency was 140 kHz.

3.2.7. Antimicrobial Test Procedure

Bacterial test reduction tests and zone of inhibition tests were carried out according to the test procedures described in *Chapter 2*. In the bacterial reduction test, besides 24 h of bacterial incubation, prepared films were also tested for additionally 2 h of incubation time.

3.3. Results and Discussion

3.3.1. Synthesis and Characterization of the Perfluorinated Quaternary Ammonium Compounds

The two perfluoroalkyl tail containing quaternary ammonium compounds were synthesized *via* a condensation reaction of 3-dimethylamino-1-propanol with fluorinated alkyl iodide chains. The completion of the reaction was again checked with ^{1}H-NMR by monitoring the disappearance of the methyl protons peak of the tertiary amine and the generation of those of the quaternary ammonium at 2.8 ppm. A representative NMR spectrum is given in Figure 3.1.

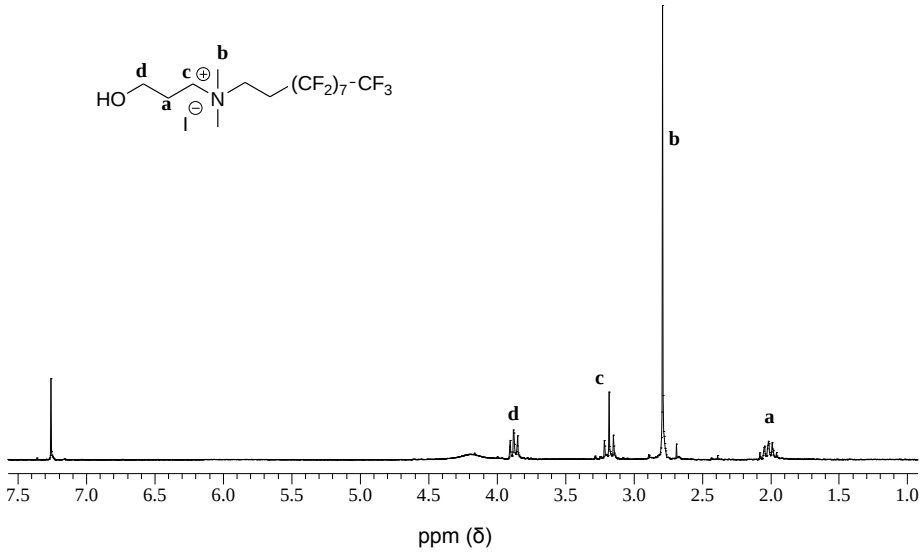


Figure 3.1. ^{1}H-NMR spectrum of QAC **3**.

The four novel compounds so far were designed to stimulate the autonomous self-stratification behavior at the surface by decreasing the surface tension. Both QAC **1** and **2** have the same long, hydrophobic hydrocarbon tail (11 C), but the spacer length between the quaternary ammonium and the backbone differs (C_{11} for **1** and C_3 for **2**). By incorporation of the perfluoroalkyl chains (QACs **3** and **4**) to the system now an enhanced segregation at the film surface (due to the highly hydrophobic nature of the perfluorinated tails) and enhanced surface hydrophobicity were targeted. Incorporation of fluorinated chains would also facilitate the determination of the surface QAC concentration by XPS (by following the chemically bonded fluorine signal in addition to that of the counterion). The ^{19}F-NMR spectrum of QAC **3** is shown in Figure 3.2.

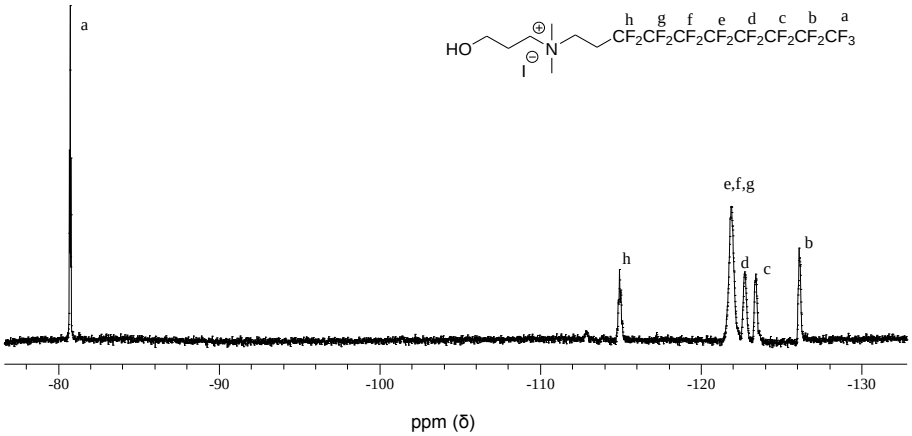


Figure 3.2. ^{19}F-NMR spectrum of QAC **3**.

3.3.2. Thermal Curing and Characterization of the Coatings

In order to monitor the covalent incorporation of the synthesized QACs into the polymer matrix, a representative film formation reaction was monitored by real-time ATR-FTIR spectroscopy prior to the film preparation (Figure 3.3).

It was observed that the peaks corresponding to the –OH group (3534 cm^{-1}) and –NCO group (2267 cm^{-1}) decrease steadily during the reaction as expected. New peaks emerged at 3387 cm^{-1} and at 1525 cm^{-1} as a result of formed urethane linkages. The CH_2 stretching peak (2935 cm^{-1}) remained the same throughout the reaction and was taken as the internal standard.

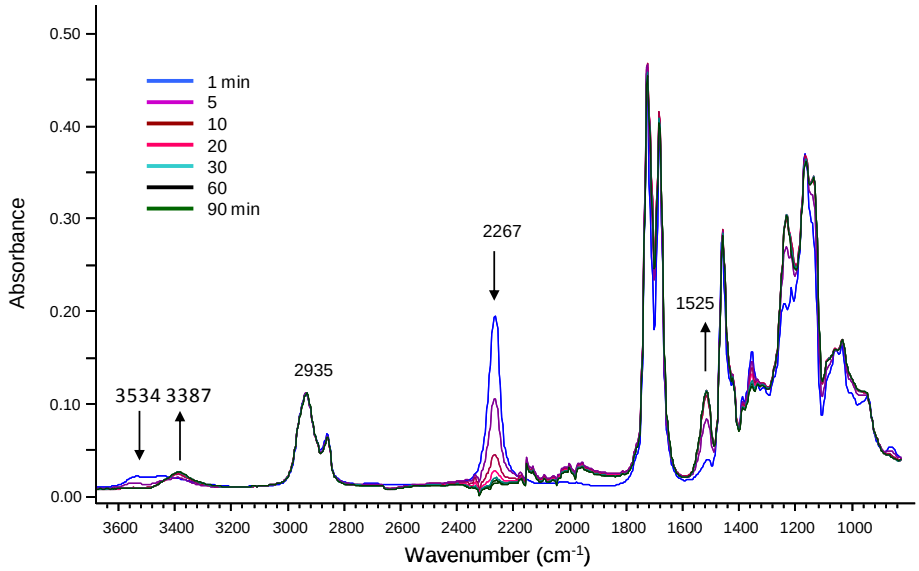


Figure 3.3. Real-time ATR-FTIR spectra for the thermal curing of a polyurethane system (0.048 mmol/g QAC **3** containing) at 80 ^{o}C.

As can be seen in Figure 3.3, the –OH peak disappeared after 10 min of reaction which proves a 100% conversion of the added QACs. It can also be seen that the peak at 2267 cm^{-1} did not disappear completely, indicating that the crosslinker was in small excess. However, to be able to discard any possible trace amount of unreacted compound or any possible impurity, cured films were further subjected to soxhlet extraction using acetone.

The final ~50 µm thick ultimate films showed good solvent resistance as tested by the acetone double-rub test. Coatings survived from 100 times of double-rub. The glass transition temperature T_g was measured to be to be around -40 ^{o}C for all films.

3.3.3. Surface Properties of the Coatings

3.3.3.1. Contact Angle Results

Quaternary ammonium compound enrichment at the film surface was first examined by dynamic contact angle (CA) measurements using deionized water and hexadecane as probe liquids. The CA data of the films containing an increasing concentration of QACs are shown in Figures 3.4 - 3.6.

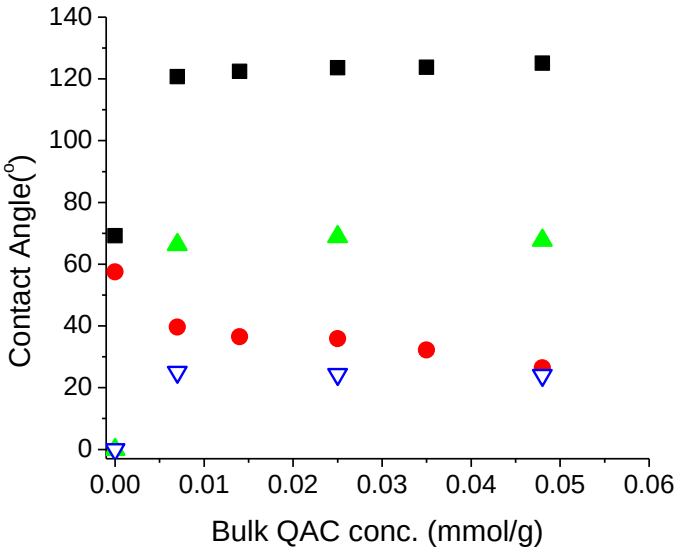


Figure 3.4. Advancing and receding contact angles of the system **3** with two different probe liquids: (■) θ_{adv} (H_2O); (●) θ_{rec} (H_2O); (▲) θ_{adv} ($C_{16}H_{34}$); (▽) θ_{rec} ($C_{16}H_{34}$).

According to Figure 3.4 in contrast to the other systems (also hydrocarbon ones), the incorporation of QAC **3** (with a long fluorinated tail) into the polyurethane films resulted in a large difference in both advancing and receding contact angles of water when compared to the system without QAC. Also, advancing water contact angle reached plateau values at 0.014

mmol/g (0.9 wt%) bulk QAC concentration, suggesting that the surface of the film is basically saturated with QAC **3** at this concentration and further increase of the QAC content will not enhance the surface QAC enrichment. In contrast, system **4** showed a very similar trend to systems **1** and **2** (Figure 3.5). This suggests that system **3** has a high fluorine content on the surface, whereas system **4** does not.

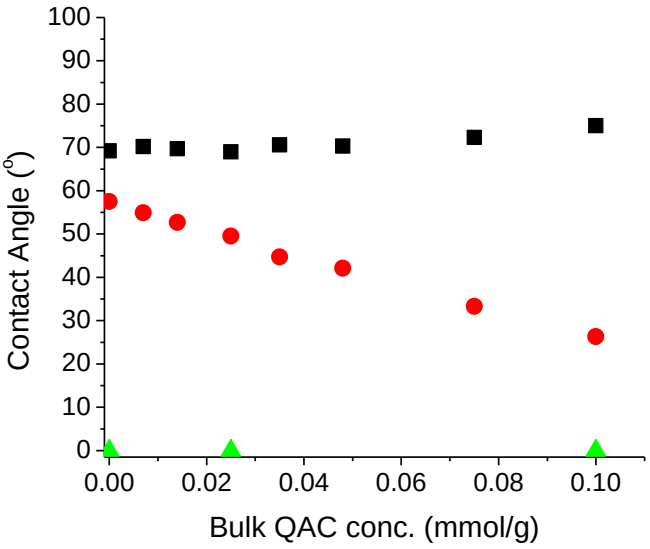


Figure 3.5. Advancing and receding contact angles of the system **4** with two different probe liquids: (■) θ_{adv} (H_2O); (●) θ_{rec} (H_2O); (▲) θ_{adv} ($C_{16}H_{34}$).

When the probe liquid was switched to the lower surface energy hexadecane (Figures 3.4 and 3.5), the control film, which does not contain any QAC, showed a contact angle close to 0^o. Due to the higher solvent affinity, system **3** showed much lower advancing and receding contact angles than it showed against water. System **4**, like the control film, showed a contact angle close to 0^o and this trend was consistent with water contact angle values.

Incorporation of the QAC at an earlier stage of the synthesis (i.e., the pre-reaction approach) results in films that display much higher advancing water contact angles than those without any QAC (Figure 3.6). This result suggests that the QACs are still mobile enough to move towards the surface. However, for this system the advancing contact angle values were found to be about 15^{o} lower than their one-pot counterparts produced with QAC **3**. This behavior is mainly due to the lower mobility of the fluorinated tails of the QAC **3**. Since hydrophobic QAC groups were covalently bonded to the polyisocyanate crosslinker at the time of film formation, diffusion towards the surface was less effective, resulting in a lower QAC concentration at the surface of the film.

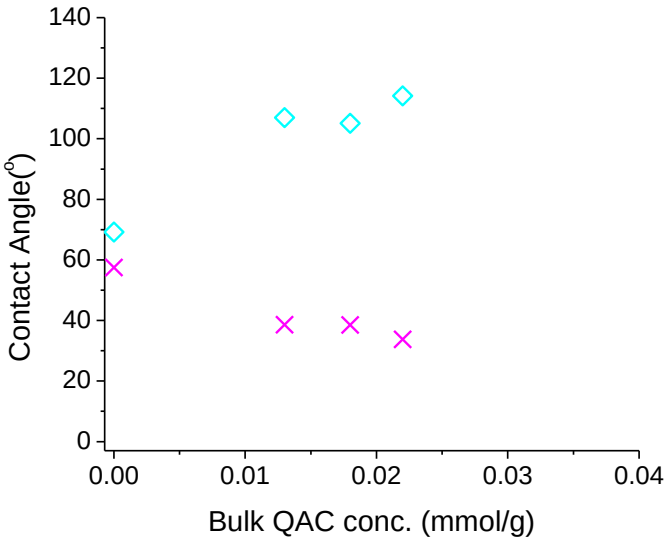


Figure 3.6. Advancing and receding water contact angles of the system **5** (pre-reaction approach): (◇) θ_{adv}; (×) θ_{rec}.

3.3.3.2. X-ray Photoelectron Spectroscopy Results

After examining the QAC enrichment at the films' surface by contact angle measurements, relative QAC concentrations at the surface were investigated by X-ray photoelectron spectroscopy (Figures 3.7 - 3.13).

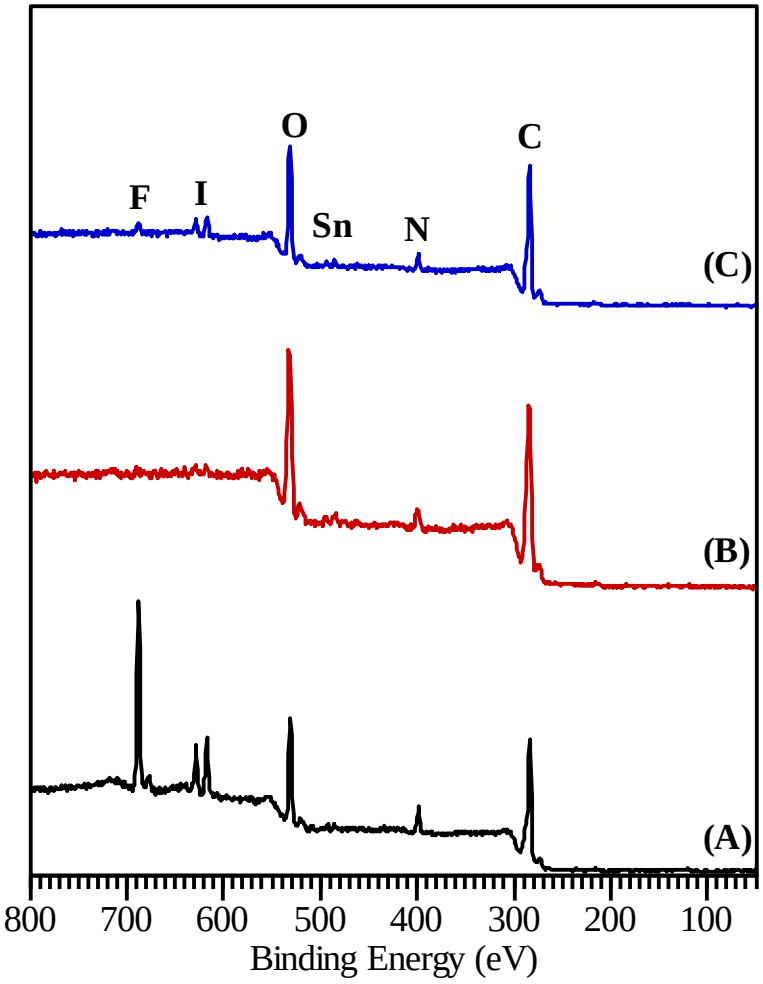


Figure 3.7. XPS wide spectra of (**A**) the coating that contains 0.048 mmol/g QAC **3**, (**B**) the coating that contains 0.048 mmol/g QAC **4**, (**C**) the coating that contains 0.100 mmol/g QAC **4**. The spectra were recorded at take-off angle of 30°, corresponding to a probe depth of ~5 nm.

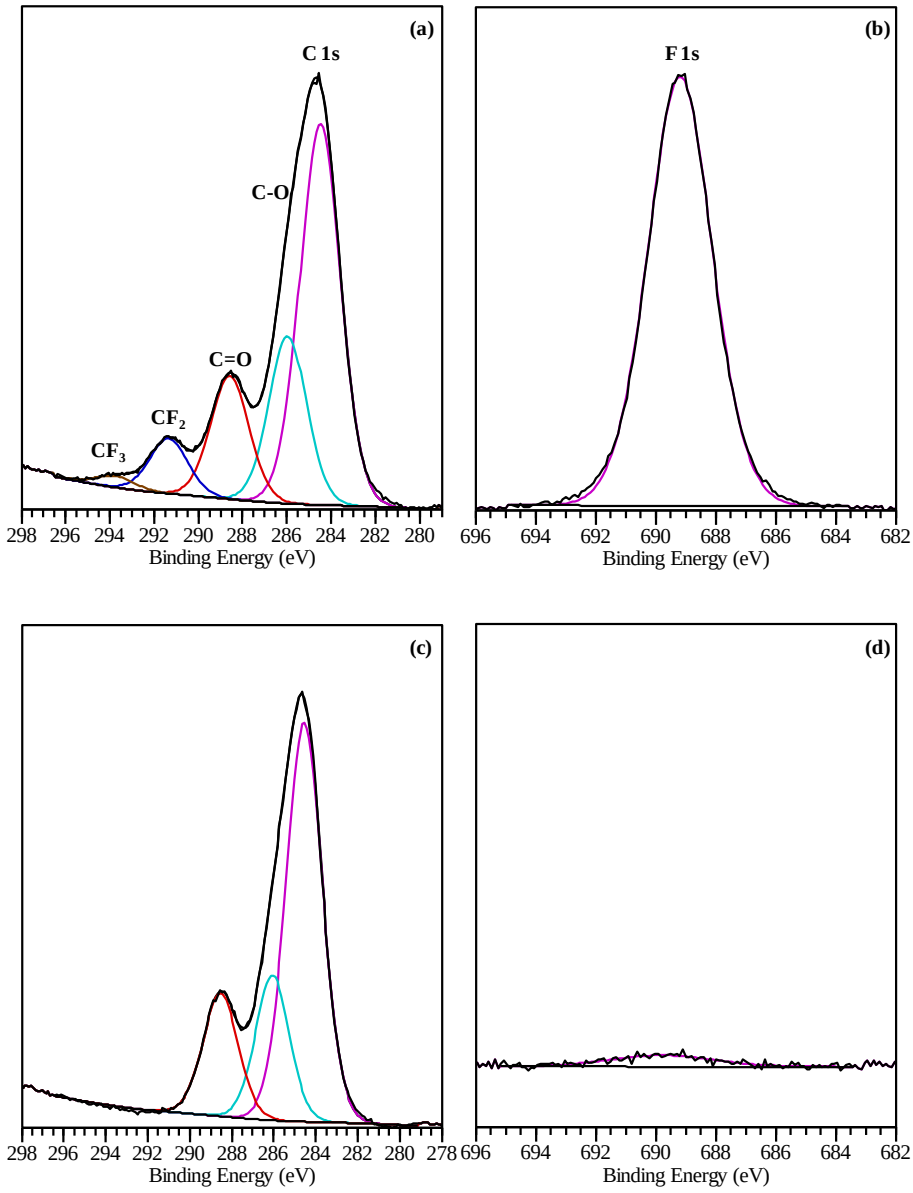


Figure 3.8. XPS C 1s and F 1s signals in top 5 nm for 0.048 mmol/g QAC containing systems **3** (**a**,**b**) and **4** (**c**,**d**).

In *Chapter 2*, monitoring the counterion (Br/C) was the only option for XPS measurements. However, since covalently bonded fluorine is also a specific element for QACs **3** and **4**, this time, the F/C ratio was also monitored for such systems in addition to the counterion concentration (I/C) (Figure 3.9). Also, in this way, by having both covalently bonded F and counterion I concentrations, the reliability of monitoring counterion concentration for systems **1** and **2** would be double checked.

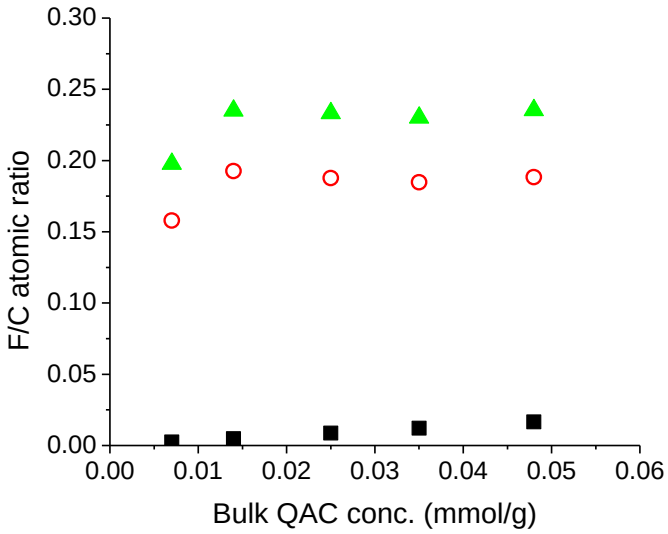


Figure 3.9. F/C ratio in the top 5 and 10 nm of films containing QAC **3**: (■) Bulk average; (○) 10 nm; (▲) 5 nm.

According to the F/C atomic ratio of system **3** (Figure 3.9), measured values were found to be up to 50 fold higher than bulk averages. Also, the XPS results in the top 5 nm were observed to be about 25% higher than those in the top 10 nm which further confirms the QAC segregation at the film surface. Thus, similar to the systems **1** and **2**, the F/C ratio values for system **3** were found to be consistent with QAC segregation at the film surface. With a good agreement with contact angle measurements, F/C ratio reached plateau values at the QAC **3** bulk concentration of 0.014 mmol/g.

This indicated that the coating surface can be saturated with fluorinated species at a certain level of fluorine content. Further increase in fluorinated QAC concentration does not influence the surface properties of system 3 coatings.

The situation is very different for QAC **4**. As can be seen in Figure 3.10, no fluorine enrichment at the surface for QAC **4** is observed, suggesting that segregation did not occur (can be seen also in Figure 3.8 c and d). This unexpected trend was reproducible in repeated experiments. Certainly, there is no driving force for the QAC **4** to surface segregate, likely due to that the respective effects of R_f (C_4F_9) and N^+ cancelled each other in their surface energy.

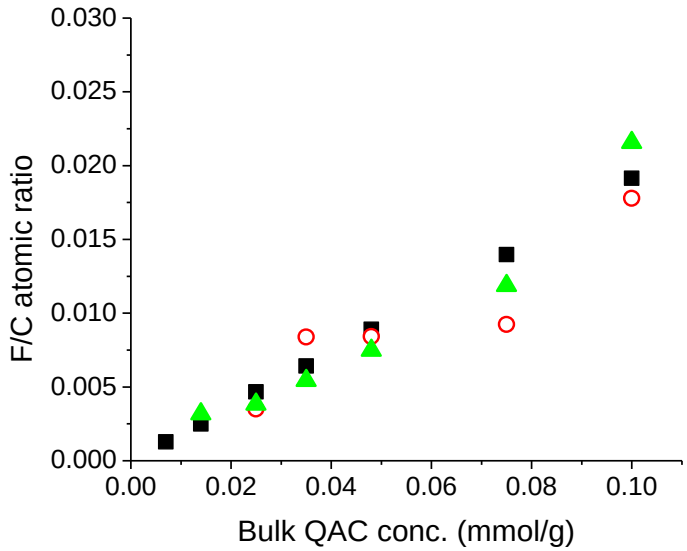


Figure 3.10. F/C ratio in the top 5 and 10 nm of films containing QAC **4**: (■) Bulk average; (○) 10 nm; (▲) 5 nm.

In order to make a better comparison between the results obtained for QACs **1** and **2** on the one hand and QAC **3** on the other, the I/C ratios for

QACs **3** and **4** were also measured (Figures 3.11 and 3.12). For QAC **3** a large increase in the surface I/C ratios was seen as compared to the bulk values, which is consistent with the F/C results in Figure 3.9. The I/C results for QAC **4**, however, showed a different trend than the F/C results. Whereas there did not seem to be any surface enrichment in terms of F/C, the I/C results do suggest this. This result casts some doubt as to whether following the counterion concentration is the most adequate parameter for proving surface segregation (also for systems **1** and **2**). However, that was the only possible way for us to monitor systems **1** and **2**.

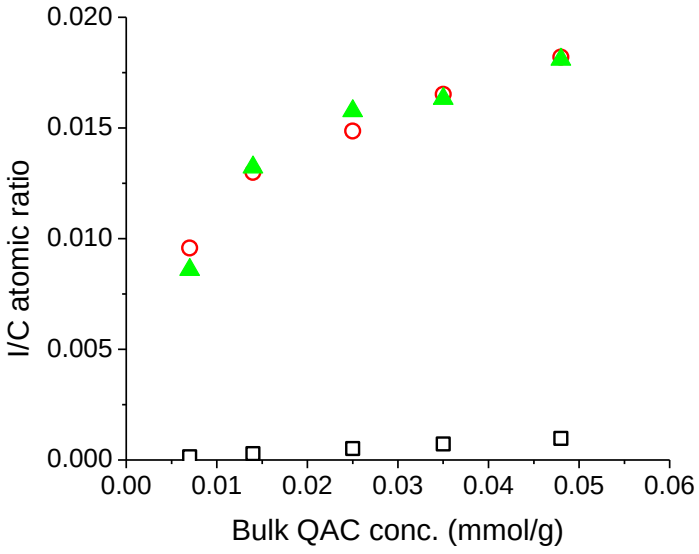


Figure 3.11. I/C ratio in the top 5 and 10 nm of films containing QAC **3**: (□) Bulk average; (○) 10 nm; (▲) 5 nm.

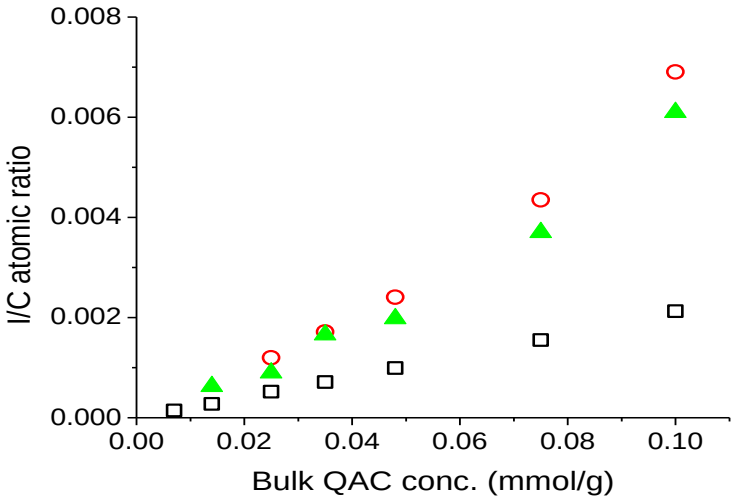


Figure 3.12. I/C ratio in the top 5 and 10 nm of films containing QAC **4**: (□) Bulk average; (○) 10 nm; (▲) 5 nm.

Pre-reaction approach:

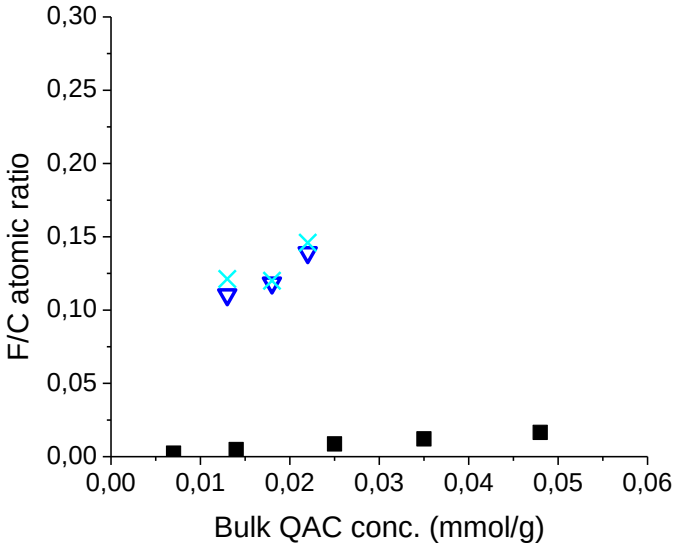


Figure 3.13. F/C ratio in the top 5 and 10 nm of system **5** (pre-reaction approach): (■) Bulk average; (▽) 10 nm; (×) 5 nm.

In Figure 3.13, F/C ratios for the pre-reacted version of system **3** (system **5**) are shown. It can be seen that XPS measurements for the system **5** films still result in a much higher F/C ratio than its bulk value, consistent with the contact angle measurements. This shows that, although the fluorinated tails were largely hindered, some diffusion towards the surface took place. However, we did not observe much difference in two different probe depths. This supports the idea that surface segregation was not as efficient as in system **3**.

3.3.3.3. Atomic Force Microscopy Results

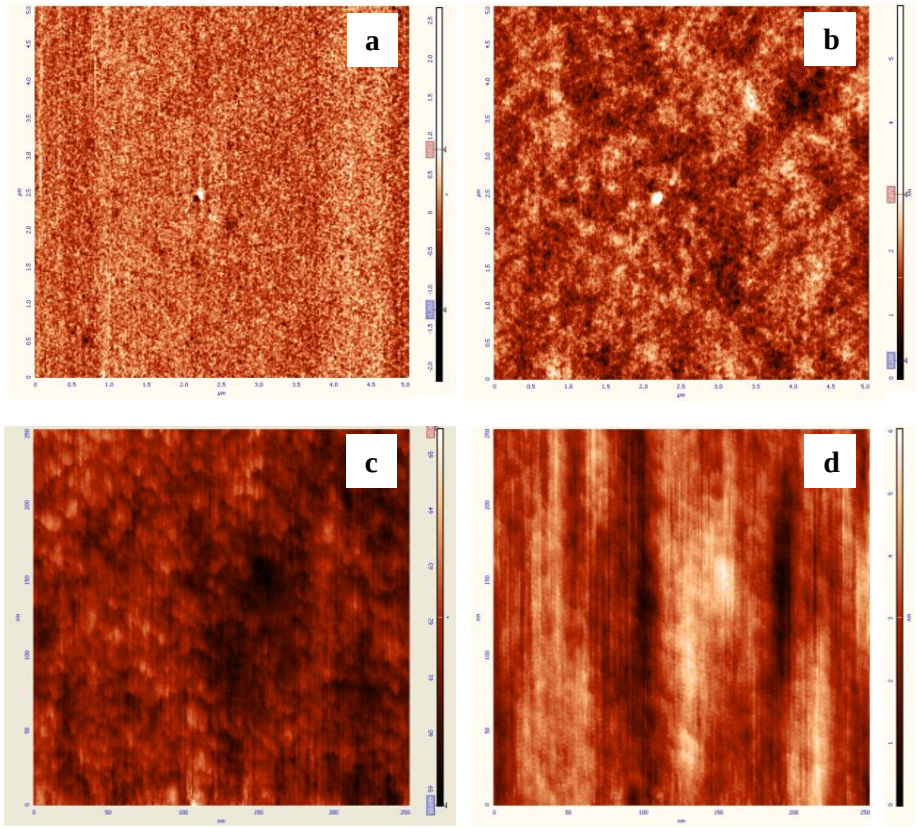


Figure 3.14. AFM images (5 µm x 5 µm and 250 nm x 250 nm) of the surface of 0.048 mmol/g QAC **3** containing film: (**a.c**) phase images, (**b,d**) topography images.

The AFM phase image shown in Figure 3.14 a clearly shows that there is no phase contrast on the film which contains QAC **3** at the highest concentration. This indicates that the distribution of the fluorinated QAC **3** is homogenous over the film surface.

As a result of this observation, any surface inhomogeneity in the other systems (which are less hydrophobic systems) is not expected.

3.3.4. Antimicrobial Activity of the Coatings

The bacterial log reduction tests were carried out as explained in the previous chapter in detail. Prepared films were tested against Gram-positive *S. aureus* and Gram-negative *E. coli* bacterial species for 24 h of incubation (Table 3.2).

Table 3.2. Bacterial $\log_{10}$ reduction after 24 h of incubation of 10^5 bacteria with 1 cm^2 of films.

Bulk QAC conc.	*Staphylococcus aureus*		*Escherichia coli*	
(mmol/g)	System **3**	System **4**	System **3**	System **4**
0[a]	0.5	0.5	0.8	0.8
0.007	5	3	5	3
0.014	5	2	5	5
0.025	5	5	5	3
0.035	5	5	5	5
0.048	5	4	5	5
0.075		5		5
0.100		5		5

[a] The control film was a polyurethane film with no quaternary ammonium compound.

As can be seen above, all of the R_f-QAC-containing films showed strong antimicrobial activities against both types of bacteria. System **3** was more effective with a 5 $\log_{10}$ reduction of both bacterial species observed at all concentrations applied. Due to its hydrophobic long perfluoroalkyl chains, QAC **3** was more efficiently diffusing R_f-QAC towards the film surface and, as a result, the surface concentration of QAC **3** was higher than QAC **4**.

Also, in order to estimate the antimicrobial efficiency of the films in a shorter period of time, similar tests to some chosen samples were made after 2 h of incubation instead of 24 h (Table 3.3).

Table 3.3. Bacterial $\log_{10}$ reduction after 2 h of incubation of 10^5 bacteria with 1 cm^2 of films.

Bulk QAC conc.	*Staphylococcus aureus*		*Escherichia coli*	
(mmol/g)	System **3**	System **4**	System **3**	System **4**
0[a]	0	0	0	0
0.025	1		0	
0.035	1	1	1	0
0.048	2	1	1	0
0.075		2		1
0.100		2		1

[a] The control film was a polyurethane film with no quaternary ammonium compound.

As seen in the table 3.3, tested films showed some minor activities when compared to the control film. However, 2 h of action time was not enough for any of them films to reach the desired 3 $\log_{10}$ bactericidal results.

Pre-reaction approach:

Table 3.4. Bacterial $\log_{10}$ reduction after 24 h of incubation of 10^5 bacteria with 1 cm^2 of films synthesized using the pre-reaction (QAC **5**).

QAC conc. (mmol/g)	*Staphylococcus aureus*	*Escherichia coli*
0[a]	0.5	0.8
0.013	5	5
0.018	5	5
0.022	5	5

[a] The control film was a polyurethane film with no quaternary ammonium compound.

Like their one-pot counterparts (system **3**), films from the pre-reaction approach also showed a 5 $\log_{10}$ reduction against both Gram-positive and Gram-negative bacteria (Table 3.4).

Table 3.5. Bacterial $\log_{10}$ reduction after 2 h of incubation of 10^5 bacteria with 1 cm^2 of films synthesized using the pre-reaction (QAC **5**).

QAC conc. (mmol/g)	*Staphylococcus aureus*	*Escherichia coli*
0[a]	0	0
0.013	0	0
0.018	0	0
0.022	0	1

[a] The control film was a polyurethane film with no quaternary ammonium compound.

However, 2 h of action time was not enough for them to show any activity against both Gram-positive and Gram-negative bacterial species. Longer contact time is needed for these films (Table 3.5).

3.3.4.1. Zone of Inhibition Test

In order to further prove that the perfluorinated QACs in one-pot approach were covalently bonded to the network and there is not free QAC leaching out from the films during the bacterial log reduction test protocol, a representative coating (2 cm x 2 cm) from system **3** (with the highest QAC concentration used) was exposed to a zone of inhibition test for 24 h of incubation.

Figure 3.15. Picture of a zone-of-inhibition test result of 0.048 mmol/g QAC **3** containing sample, (Fig. **B** is the Adobe Photoshop modified version of the real picture (Fig. **A**) to help the reader to see the contrast easily).

In Figure 3.15 it is clearly seen that any inhibition zone around the tested sample was not observed. This proves that for the prepared coatings microbial action was contact-killing instead of releasing antimicrobial agents to the surroundings. Moreover, we again did not observe any antimicrobial activity in the soxhlet extraction liquids for these films like in systems **1** and **2**.

3.4. Conclusions

The synthesis of the two novel perfluoroalkyl-tailed-quaternary ammonium compounds is presented. The synthesized compounds were incorporated into the polyurethane matrix in one-pot and pre-reaction approaches.

For the one-pot approach, the longer perfluorinated-tailed-QAC (QAC **3**) system was more efficient in terms of surface segregation. The addition of 0.9 wt% (0.014 mmol/g) QAC **3** was enough to reach the saturation concentration at the top surface. Surface segregation also occurred in films using the pre-reaction approach but less effectively due to the hindered mobility of the hydrophobic QAC.

The prepared films showed strong antimicrobial activities against both Gram-positive and Gram-negative type of bacteria even at very low concentrations (*e.g.* 0.5 wt%, 0.007 mmol/g for system **3**). As no inhibition zone was observed and the extraction solutions of the films did not show any antimicrobial activity, it is proven that the QACs are covalently bonded to the polymer network and that contact killing is the mechanism for these films. Hence, we conclude that the one-pot approach and not the pre-reaction approach is the optimal approach for preparing these coatings since QAC segregation is limited in the pre-reaction approach.

3.5. References

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Chapter 4

Preparation of Polyurethane Coatings Based on Ionic Liquid Quaternary Ammonium Compounds*

Summary

In this chapter commercially available ionic liquids in the form of quaternary ammonium compounds are incorporated in the coatings recipe. The surface and the antimicrobial properties of the prepared films are discussed. Of all the ionic liquid structures, the one with the longest tail and spacer is found to be the most effective against the tested bacteria species. Leaching of any active ionic liquid structure is not observed. Since these compounds are very hydrophilic, the self-stratification approach is not within the scope of this chapter.

* Part of the results presented in this chapter have been published in: Yagci,M.B.; Bolca, S.; Heuts, J.P.A.; Ming, W.; de With, G. *Prog. Org. Coat.*, **2011**, *72*, 343.

4.1. Introduction

Ionic liquids (ILs) are generally defined as salts that exist in the liquid phase below 100 ^{o}C. These salts comprise both organic cations (*e.g.* imidazolium, ammonium, pyridinium, phosphonium, piperidinium, morpholinium, sulfonium, and pyrylium) and various anions. Common ionic liquid anions are hexafluorophosphate (PF_6^-), tetrafluoroborate (BF_4^-), chloride (Cl^-), nitrate (NO_3^-) and bromide (Br^-) [1-5]. Anions of inorganic origin have been also reported in some studies [6]. The positive charges in IL structures are localized on the nitrogen, phosphorus, sulfur, or oxygen atom.

The first IL was prepared by Paul Walden in 1914 by the protonation of ethylamine with nitric acid [7-9]. Due to their unique characteristics, such as being non-volatile, non-flammable, and having generally low melting points, high thermal stability, and low viscosity, ionic liquids have started to receive considerable interest from the chemical industry by the end of 1900s. Today, ionic liquids have become a benign replacement of industrial chemicals/solvents. They are widely used in organic chemistry [10-22], polymer synthesis [23-27], separation, and extraction processes [28-31], biocatalysis [32,33], battery systems [34,35], electrochemical deposition and electrochemistry[36,37], and in several systems as lubricants [38,39].

In addition to their environmental advantages as green solvents, lately, their structural similarities to quaternary ammonium compounds have made ionic liquids attractive for pharmaceutical research. Their possible antimicrobial character has been investigated by different groups, recently [40-47]. Especially, Pernak *et al.* have designed novel antibacterial ionic liquid compounds to use as liquids in embalming and tissue preservation

[48-58]. Their extensive studies on bacteriocidal property of these compounds ended up with very promising results.

Quite recently, McNally *et al.* [59] designed and synthesized novel dual functional ionic liquids, which act as a plasticizer for poly (vinyl chloride) and exhibit antimicrobial activity. However, the prepared polymer was found to be non-effective against a wide range of Gram-negative type of test species. Also, it was observed that IL quaternary ammonium compounds were leaching out from the system since the IL compounds were not tethered to the polymer network covalently (they were incorporated into the network by simple blending).

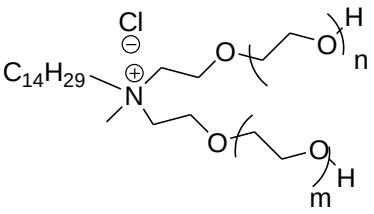
In the present chapter, we will discuss the preparation of surface coatings with a permanent antimicrobial property by covalent incorporation of some N^+ containing ionic liquid compounds to a polyurethane network. An IL-based antimicrobial surface coating is reported for the first time in the literature.

4.2. Experimental

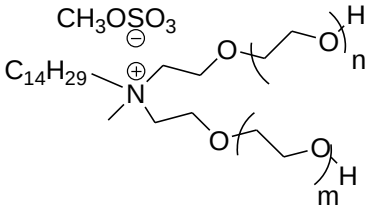
4.2.1. Materials

The usual procedure which was explained before was used to synthesize the solventless liquid oligoester. The triisocyanate crosslinker (Tolonate HDT-LV) was obtained from Rhodia and its NCO content was checked by the titration method as specified in ASTM D2572-91 before application. Ionic liquids (Tego IL-K5, IL-K5MS, and IL-T16ES) (Degussa Goldschmidt GmbH) (Figure 4.1) were dried under vacuum before being used. Aluminum panels (152 x 76 x 0.6 mm) were purchased from Q-Panel Co. Di-n-butyldilauryltin was purchased (ABCR GmbH) and used as

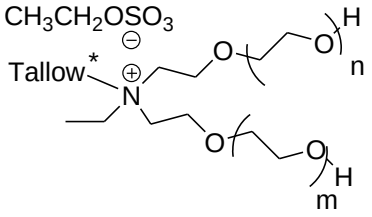
catalyst in the film preparation. The solvent N-methyl pyrrolidone (99.5%) (Biosolve b.v.) was used as received.



Tego IL K5 (n+m=13)



Tego IL K5MS (n+m=3)



Tego IL T16ES (n+m=14)
* Tallow: C16 - C18 hydrocarbon chain

Figure 4.1. Ionic liquid quaternary ammonium compound (IL-QAC) structures.

4.2.2. Polyurethane Film Preparation

Polyurethane coatings were prepared from a mixture of SLO, IL-QACs and polyisocyanate crosslinker with NMP as solvent (50 wt% solid content). 0.5 wt% di-n-butyldilauryltin catalyst was added to the coating mixture. The molar ratio of NCO/OH was adjusted at 1.1 to ensure full conversion of the OH groups. From film to film, only the type of IL-QAC and the ratio of $OH_{SLO}/OH_{IL\text{-}QAC}$ were varied while maintaining $NCO/OH_{total}=1.1$. Coating mixtures were applied on aluminum substrates

using a square film applicator, after which they were cured at 80 ^{o}C for 30 min. After curing, the films were dried for 12 h at 50 ^{o}C under vacuum to remove trace amounts of solvent. In order to remove any possible unreacted QAC, samples were intensively washed first with deionized water, then with acetone, and subsequently dried in vacuum oven at 50 ^{o}C for 12 h. The QAC contents of the prepared films are shown in Table 4.1.

Table 4.1. The QAC content of the prepared coatings.

Coating Systems	**n_{NCO}/n_{OH}**	**QAC Content (mol %) (wt %)**				
K5	1.1	6 (6)	14 (16)	29 (33)	43 (51)	58 (69)
K5MS	1.1	6 (4)	14 (11)	29 (23)	43 (39)	58 (58)
T16ES	1.1	6 (9)	14 (22)	29 (41)	43 (60)	58 (76)

4.2.3. Characterization Techniques

Attenuated total-reflection Fourier-transform infrared (ATR-FTIR) spectroscopy was performed on a Bio-Rad Excalibur FTS3000MX infrared spectrometer with an ATR diamond unit (Golden Gate). Spectra of the surfaces were recorded in the range of 4000-650 cm^{-1} with a resolution of 4 cm^{-1}. A full spectrum was taken every 30 s. One background spectrum (averaged from 100 scans co-added) was collected just before starting the measurements. The signal assigned to the CH_2 stretching at 2926 cm^{-1} was used as internal standard. The glass transition temperatures (T_g) were determined on a Perkin Elmer Pyris 1 differential scanning calorimeter

(DSC) using a heating rate of 20 ^{o}C/min, between a temperature range of -80 to +40 ^{o}C. The T_g of the films was determined by the half C_p extrapolation method during the second heating run. The surface tension of the ionic liquids was measured by the Du Nouy ring method with a Kruss Tensiometer. Dynamic contact angle measurements were carried out with a Dataphysics OCA 30 instrument, using deionized water as probe liquid. Dynamic advancing and receding angles were measured by Sessile Drop technique while the probe liquid was added to and withdrawn from the drop, respectively. All the contact angles were determined by averaging values measured at least at three different points on each sample.

4.3. Results and Discussion

4.3.1. Preparation and Characterization of the Coatings

In order to investigate the ionic liquid incorporation the polyurethane film formation reaction was monitored by real-time ATR-FTIR spectrometry. For this test the molar ratio of NCO/OH was switched from 1.1 to 0.9 to be able to measure the NCO conversion by monitoring the complete disappearance of the NCO peak.

As can be seen in Figure 4.2, taking the CH_2 stretching peak (2926 cm^{-1}) as the internal standard, the peak corresponding to the –NCO group (2269 cm^{-1}) decreased steadily during the reaction until complete disappearance. This observation proved a full conversion of –NCO groups of the crosslinker after 10 min. of reaction.

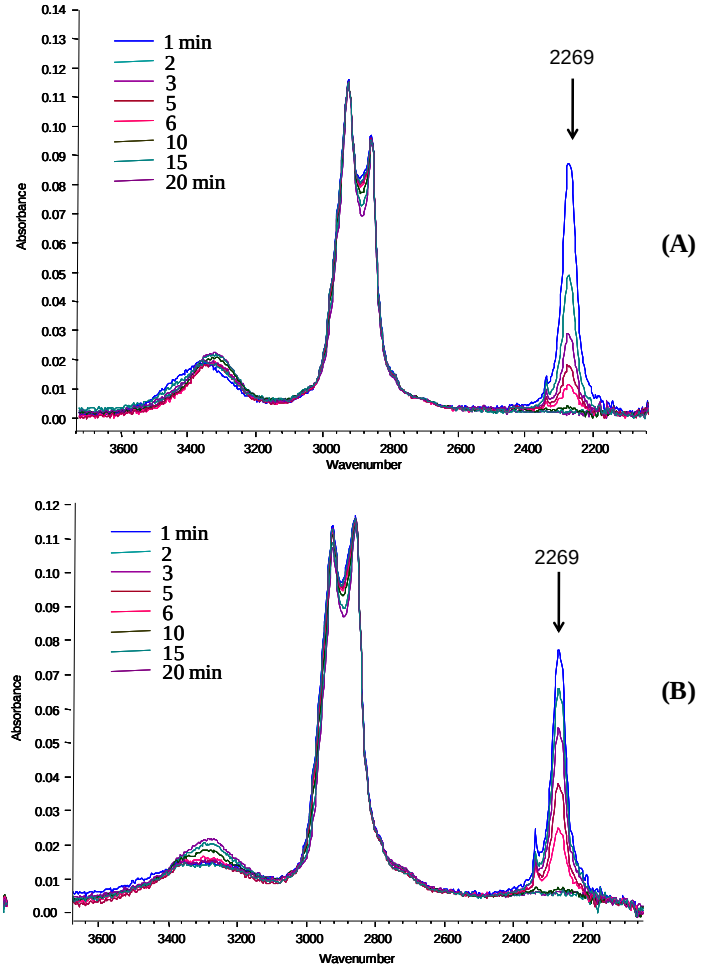


Figure 4.2. Real-time ATR-FTIR spectra for the thermal curing of: (**A**) Tego IL-K5 and (**B**) Tego IL-K5MS by triisocyanate crosslinker.

After the curing conditions were optimized using the ATR-FTIR data, the polyurethane films were cast and cured at 80 ^{o}C for 30 min. The ratio of the number of moles of OH from oligoester over the number of moles OH from IL-QAC was set at 2/0, 1.8/0.2, 1.5/0.5, 1/1, 0.5/1.5, and 0/2.

The thickness of the prepared films was measured to be about 30 µm by a Twin-Check instrument. The glass transition temperature T_g as obtained

from DSC measurements was found to be around -35 ^{o}C for all IL-QAC containing films and -42 ^{o}C for the film which does not contain any QAC. This limited difference will not affect significantly the mechanical behavior of the coating.

In addition, the water-swellability of the films was studied, as due to the presence of hydrophilic ionic liquid moieties the polyurethane system might be more susceptible to water-uptake. All the prepared films were exposed to water (dropped onto the film surface) for 1 h in order to let the films swell. Thereafter, the films were dried at 50 ^{o}C for 3 h. It was observed that all three systems were slightly swollen under the water droplets and recovered after drying. Only the IL-K5 containing films showed delamination after drying. Delamination was observed in all IL-K5 concentrations.

4.3.2. Surface Properties of the Coatings

Once the polyurethane films were cured, water contact angle measurements were performed on the film surfaces. Contact angle data of prepared films containing IL-QACs with increasing concentrations are shown in Figure 4.3.

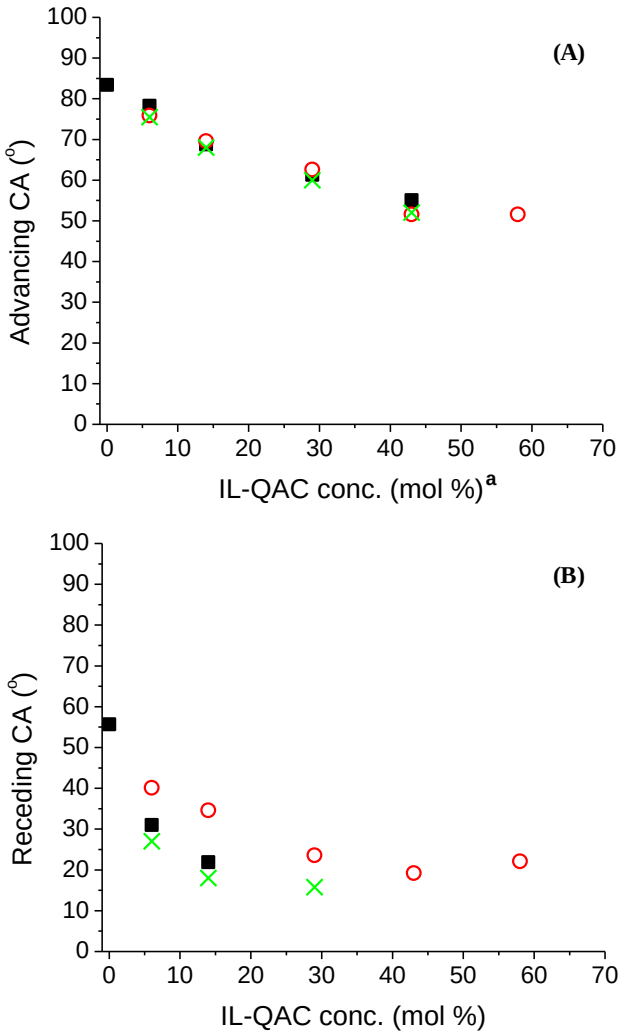


Figure 4.3. Water contact angle measurements on the surfaces of the prepared films which contains: (■) Tego IL-K5; (○) Tego IL-K5MS; (×) Tego IL-T16ES.

[a] Mol fraction of ionic liquid with respect to all of the monomers. (Missing data points are due to the impossibility of measuring the CA since the corresponding samples swell using water as the probe liquid during the measurement).

These measurements showed a decrease in both advancing (θ_{adv}) and receding (θ_{rec}) contact angles to all systems. As explained in *Chapter 2* in detail, in an aqueous environment, the hydrophobic segments (long hydrocarbon tails of QACs in our case) tend to increase the contact angle whereas the hydrophilic segments (PEO chains) tend to decrease it. In the current IL-QAC systems it seems that the hydrophobic tails reorient in order to decrease the surface tension of the coating; the hydrophilic nature of the relatively long PEO chains overcompensates this resulting in a decrease in measured contact angles. The receding contact angle of the IL-K5MS system was observed to be higher than for the other systems, probably due to its much shorter PEO spacer.

Also, it can be seen that all three IL-QACs showed similar advancing contact angle profiles although they have different tail and spacer lengths. In order to understand this behaviour, the surface tension of the three ionic liquids was measured (Table 4.2).

Table 4.2. Surface tension of the ionic liquids at 20 ^{o}C.

Ionic Liquid	γ_L (mN/m)
Tego IL-K5	40.5
Tego IL-K5MS	37.0
Tego IL-T16ES	39.0

As seen in Table 4.2, even though all three IL-QACs show structural differences, their surface tensions are quite similar. Considering these similar surface active properties in the ionic liquids, it seems likely that they contribute similarly to the surface properties of the prepared films.

4.3.3. Antimicrobial Activity of the Coatings

Prior to the preparation and curing of the films, the antimicrobial activity of the commercially available IL-QACs was examined against the same bacterial species and it was observed that all three IL-QACs showed at least 4 $\log_{10}$ reduction against both *S. aureus* and *E. coli* even at very low concentrations.

After having established that the ionic liquids showed antimicrobial activity, the antimicrobial activity of the IL-containing films was examined similarly after 24 h of incubation of the bacteria (Table 4.3).

Table 4.3. Bacterial $\log_{10}$ reduction after 24 h of incubation of 10^5 bacteria with 1 cm^2 of prepared films.

IL-QAC conc. (mol%)	OH_{SLO}/ $OH_{IL\text{-}QAC}$	*Staphylococcus aureus*			*Escherichia coli*		
		K5	K5MS	T16ES	K5	K5MS	T16ES
0[a]	2.0/0.0	0.5	0.5	0.5	0.8	0.8	0.8
6	1.8/0.2	3	2	5	0	2	4
14	1.5/0.5	3	2	5	3	4	5
29	1.0/1.0	3	2	5	2	4	5
43	0.5/1.5	5	2	5	4	5	5
58	0.0/2.0	5	2		5	5	

[a] The control film was a polyurethane film with no quaternary ammonium compound.

Accordingly, all three types of polyurethane films showed strong antimicrobial activity against *E. coli*. Initially long hydrocarbon-tailed IL-

QACs (C14 and C16) have been chosen in view of the previously made studies on the effect of the chain length of quaternary ammonium compounds with respect to antibacterial activity [38,39]. The longest tailed (C16) IL-T16ES system showed the strongest effect (5 $\log_{10}$ reduction, 100%) at almost all IL-QAC concentrations. The more hydrophobic IL-K5MS was slightly more effective than IL-K5 in disrupting the complex cell wall of *E. coli*. Also, in terms of activity against Gram-positive *S. aureus*, the IL-T16ES containing system showed the best results with 5 $\log_{10}$ reductions at almost all concentrations tested. In contrast, IL-K5MS system, the one with the shortest spacer chain did not reach the minimally desired 3 $\log_{10}$ reduction at any concentration against this bacterial species.

Table 4.4. Bacterial $\log_{10}$ reduction after 2 h of incubation of 10^5 bacteria with 1 cm^2 of prepared films.

IL-QAC conc. (mol%)	OH_{SLO} / $OH_{IL\text{-}QAC}$	*Staphylococcus aureus*			*Escherichia coli*		
		K5	K5MS	T16ES	K5	K5MS	T16ES
0[a]	2.0/0.0	0	0	0	0	0	0
6	1.8/0.2	0	0	1	0	0	0
14	1.5/0.5	0	1	1	0	1	1
29	1.0/1.0	0	1	1	1	1	2
43	0.5/1.5	1	2	1	1	1	1
58	0.0/2.0		1		1	1	

[a] The control film was a polyurethane film with no quaternary ammonium compound.

Also, like in systems **3** and **4**, bacterial log reduction tests after 2 h of incubation instead of 24 h were carried out for these films (Table 4.4).

As seen in the Table 4.4, 2 h of action time was not enough for any of the prepared films to reach the desired bactericidal results. The studied coatings seemed to need longer contact times with bacteria to be able to reach higher efficiency.

4.3.3.1. Zone of Inhibition Test

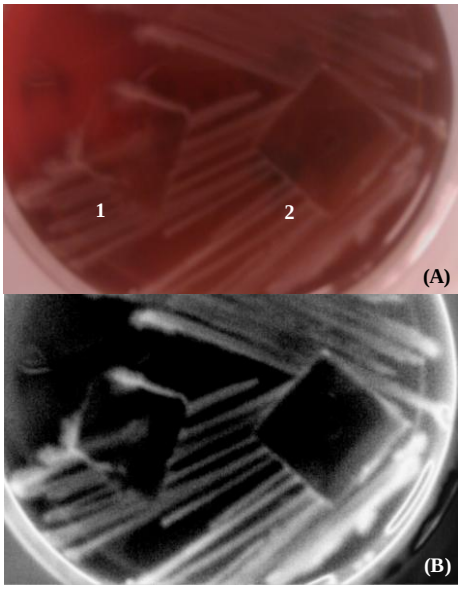


Figure 4.4. Picture of a zone of inhibition test result of (**1**) 43 mol% IL-K5 and (**2**) 29 mol% IL-T16ES containing film samples against *S. aureus* (Fig. **B** is the Adobe Photoshop modified version of the real picture (Fig. **A**) to help the reader to see the contrast easily).

According to Figure 4.4 no inhibition zones were observed upon incubation of *S. aureus* after 24 h with the films, indicating that IL-QACs did not leach from the coating into the surroundings. This suggests that the antimicrobial effect of the prepared films occurs indeed only *via* surface

contact like their other counterparts, which were discussed in previous chapters, and not by free IL-QACs for this approach.

4.4. Conclusions

In this study we have demonstrated for the first time the covalent incorporation of functional ionic liquid structures to a polymeric coating system.

According to the study, both commercial Tego IL-K5 and IL-T16ES containing polyurethane films showed promising antimicrobial effects (with certain QAC concentration) against both Gram-positive and Gram-negative bacteria, whereas IL-K5MS with the shortest spacer length resulted in preparing films that are found to be effective only against the Gram-negative *E. coli*. Tego IL-T16ES with the longest tail and spacer lengths was found to be the best in terms of antimicrobial activity of all three ionic liquid compounds. As no inhibition zones were observed, we conclude that contact killing rather than leaching of the biocide compound is mechanism of action for these films. Water contact angle measurements showed that increasing concentrations of IL-QACs in the system results in more hydrophilic surfaces. This increased water sensitivity of the coating may be limiting for some industrial applications because of water swelling although delamination behavior was observed only in IL-K5 containing films. In these cases, increasing the crosslinking density by addition of, *e.g.* 2-ethyl-2-(hydroxymethyl)-1,3-propanediol (TMP), might help to decrease the hydrophilicity.

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Chapter 5

Preparation of Polyurethane Coatings *via* an *"Inverse" Self-Stratification* Approach

Summary

In this chapter, unlike the previous chapters, short hydrophilic quaternary ammonium compounds are included in the polyurethane network. In contrast with the previous chapters, QAC enrichment at the film surface now is targeted via an "inverse" self-stratification of the QACs and not via an autonomous diffusion. This approach is based on attracting the hydrophilic, mobile, small QACs towards the film surface assisted by an external hydrophilic layer. It is described that, migration of small hydrophilic structures towards the film surface is possible by using an external layer; however, this method is not suitable to prepare antimicrobial coatings.

5.1. Introduction

So far, we have extensively investigated the self-stratification of antimicrobial quaternary ammonium compounds in a coating system *via* an autonomous segregation mechanism [1]. This method benefits from the differences in the surface energy of the components of the system, which results in the migration of low energy compounds towards the film/air interface. QACs with long hydrophobic tail were shown to migrate to this interface in polyurethane films in the previous chapters. This is a result of the hydrophobicity of the QAC structure. When this tail is significantly short, the balance between hydrophobic tails and hydrophilic N^+ shifts towards the hydrophilic side and then such mechanism is not probable anymore. In this chapter, we propose a novel method to prepare surface segregated polyurethane coatings with short tail QACs *via* an inverse self-stratification approach. In this approach a sacrificial hydrophilic layer is used to facilitate the migration of the short tailed QAC salts towards the hydrophilic layer providing the inverse self-stratification. There are no examples in the literature of the inverse self-stratification method as proposed here. Therefore, we have developed this method in parallel to the traditional stratification method used in the previous chapters.

In this new method, reactive low MW additives containing quaternary ammonium groups were incorporated into a thermal curing system to afford ammonium-surface-rich films. To test the validity of the proposed method, one synthesized and one commercial, relatively small QAC salts were initially chosen. The QACs were added to a mixture of an appropriate oligomer/crosslinker mixture (*e.g.* epoxy/amine or polyol/isocyanate mixtures). After that, the prepared mixture was thermally cured with the surface covered by a sacrificial hydrophilic layer (This hydrophilic layer can be a thin film based on non-reactive hydrophilic

polymers, such as poly(ethylene oxide) and poly(vinyl pyrrolidone), or a NaCl plate). During the thermal curing, quaternary ammonium groups were expected to migrate preferentially to the interface of the hydrophilic layer/crosslinked polymer film in order to minimize the interfacial energy. When the curing completed, the hydrophilic layer was removed by simply washing with water to give a crosslinked coating with quaternary ammonium groups at the surface. We expected such a surface enrichment of QACs in a coating system to be facilitated by the faster diffusion of small molecules.

5.2. Experimental

5.2.1. Materials

Adipic acid (>99%), glutaric acid (>99%), and 1,4-butanediol (>98%) were purchased from Merck and used as received. Fascat 4101 tin based catalyst was used the esterification reaction. Desmodur N3600 crosslinker was obtained from Bayer AG. Choline chloride (99%), 2-dimethylamino ethanol (>98%), and 1-bromobutane (99%) were purchased from Aldrich and used as received. The chemicals which were used as hydrophilic external layers, anhydrous NaCl, KBr, and poly(vinyl pyrrolidone) (M_n~40.000 g/mol) (Aldrich), were used as purchased. Microscopic glass slides (26 mm x 26 mm x 1 mm (as substrate for both film and hydrophilic layer preparation)) were obtained from Menzel-Glazer. Solvents were used as received without any further purification.

5.2.2. Synthesis of Solventless Liquid Oligoester

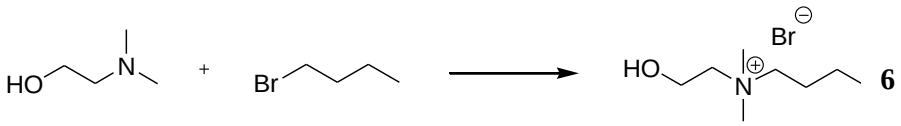
The solventless liquid oligoester was synthesized by an esterification reaction of 1,4-butanediol with eq. molar mixture of adipic and glutaric acids

at 200 ^{o}C. The details of the experimental procedure have been described in *Chapter 2*. After synthesis, the number average molecular weight was determined to be 801 g/mol by –OH and –COOH end group titrations.

5.2.3. Synthesis of Hydrophilic Quaternary Ammonium Compound

A protocol very similar to that used to synthesize QACs **1** and **2** was also used to synthesize the small size quaternary ammonium compound (Scheme 5.1). To this end, 9.5 mmol 2-dimethylamino ethanol was reacted with 10.5 mmol 1-bromobutane in a 1:3 (v:v) propanol:methanol solvent mixture at 80 ^{o}C for 90 min under N_2 atmosphere. The obtained product was purified by precipitating in cyclohexane and dried under vacuum at 60 ^{o}C for overnight (Yield: 83%).

Scheme 5.1. Synthetic scheme of the synthesized QAC **6**.



6

The structure of the synthesized compound was confirmed by ^{1}H-NMR (Figure 5.1).

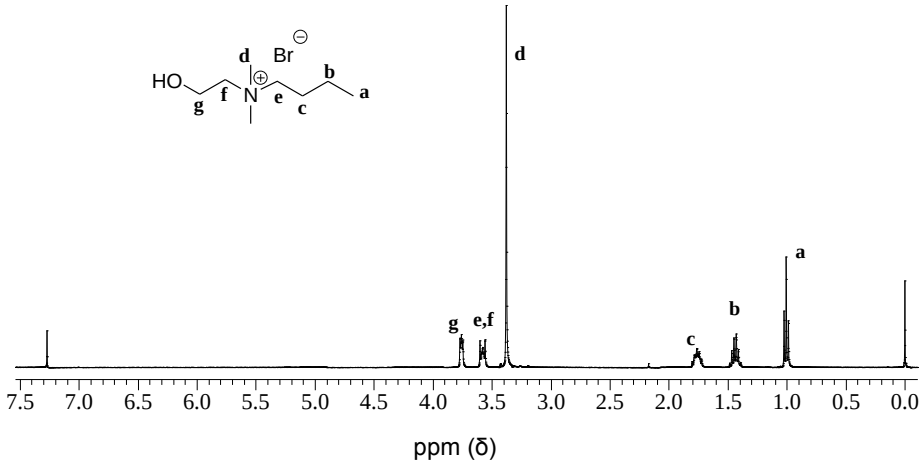


Figure 5.1. ^{1}H-NMR spectrum of QAC **6**.

5.2.4 Preparation of Hydrophilic External Layers

With the purpose of attracting hydrophilic quaternary ammonium compounds to segregate at the film surface, sacrificial hydrophilic external layers were prepared using two different methods:

a) 0.25 g NaCl or KBr salts were pressed under 500 Pa pressure for 15 min to form hydrophilic discs (15 mm in diameter). Used salts were dried under vacuum prior to be pressed and prepared discs were kept in a desiccator until being used.

b) Microscopic glass slides were coated by water soluble poly(vinyl pyrrolidone) (PVP). PVP was cast from a 30 wt% solution in ethanol by using a 60 μm spiral film applicator. Ethanol was evaporated in a vacuum oven for overnight at room temperature.

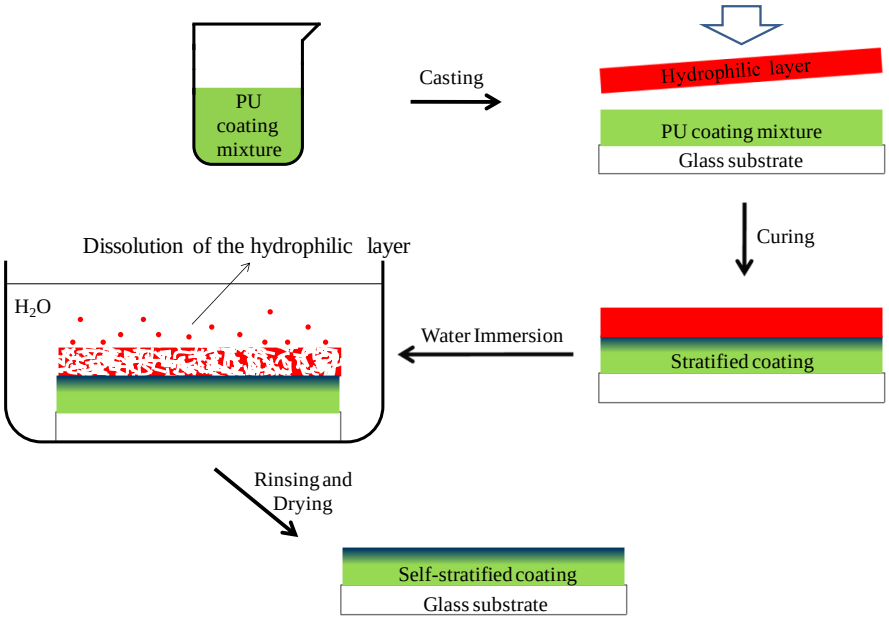
5.2.5. Polyurethane Film Preparation

The coating mixtures were prepared from one of the two quaternary ammonium compounds, oligoester diol, N3600 triisocyanate crosslinker, and either ethylene glycol or DMSO as solvent. The molar ratio of NCO/OH was adjusted at 1.1 as usual.

In order to assist inverse self-stratification, two approaches were developed. We named them as the sandwich, and the laminar method.

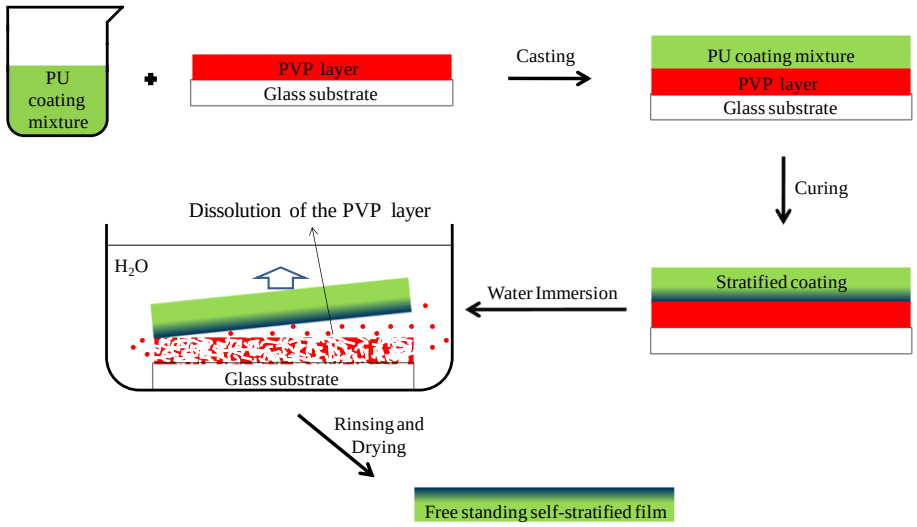
a) The Sandwich Method: In this method, first the coating mixtures were applied on the substrates with a roller film applicator. Subsequently, water soluble hydrophilic layers (NaCl or KBr pellets, or PVP films) were put on the top of the coatings prior to film curing. In order to prevent bubbling from the evaporation of the solvent, a reactive solvent (ethylene glycol), which would later incorporate into the film structure, was used in this method. Films were cured at 50 ^{o}C for 2.5 h. After curing, the ultimate systems were immersed for 4 h in deionized water in order to dissolve hydrophilic layers. After being rinsed intensively with deionized water, the films were dried in vacuum oven at room temperature for 2 days. A schematic representation of the method is shown in Scheme 5.2.

Scheme 5.2. Schematic scheme of the sandwich method.



b) <u>The Laminar Method:</u> In this method (Scheme 5.3), the coating mixtures were directly applied on hydrophilic PVP layers cast on glass substrates. After the film curing which was described above, the whole system was immersed in deionized water. After the dissolution of PVP layers, the obtained free standing films were first rinsed with water and then were dried at room temperature under vacuum. Only the PVP layers were used in this method since KBr and NaCl pellets did not give successful results.

Scheme 5.3. Schematic scheme of the laminar method.



5.2.6. Characterization Techniques

^{1}H-NMR spectra were recorded with a Varian 400 spectrometer at 25 ^{o}C operating at 400.162 MHz. Deuterated chloroform (containing 1% TMS) as the NMR solvent. Dynamic contact angle measurements were carried out with Dataphysics OCA 30 instrument using deionized water as probe liquid. The Sessile Drop technique was used for measuring dynamic advancing and receding contact angles. X-ray photoelectron spectroscopy (XPS) measurements were performed using a VG-Escalab 200 spectrometer using an aluminum anode (Al Kα = 1468.3 eV) at electron take-off angle of 90^{o} (between the film surface and the axis of the analyzer lens). The C1s peaks were calibrated at a binding energy of 284.5 eV to correct for the energy shift caused by charging. The Cl/C atomic ratios were estimated from the area ratios under the corresponding XPS curves.

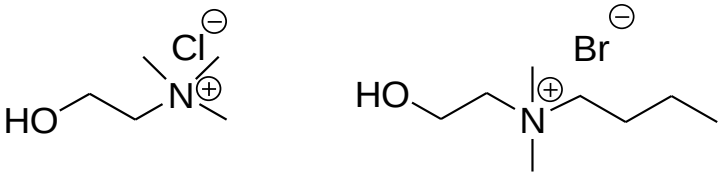
5.2.7. Antimicrobial Test Procedure

Both prepared surface coatings and the pure quaternary ammonium compounds were exposed to bacterial log reduction tests against *Staphylococcus aureus* and *Escherichia coli* type bacteria for 24 h of incubation. The test procedure is described in detail in the previous chapters.

5.3. Results and Discussion

5.3.1. Preparation of Coatings

In this part of the study, instead of using long-tailed very hydrophobic quaternary ammonium compounds, much smaller and hydrophilic QACs were chosen (Figure 5.2). Initially, a commercially available choline chloride salt was utilized in the film formation in order to test the newly proposed "inverse" self-stratification strategy.



a **b**

Figure 5.2. Used quaternary ammonium compound structures: (**a**) Choline chloride, (**b**) QAC **6**.

The ultimate films were prepared to contain 1, 2, and 5 wt% of final choline chloride as the bulk concentrations. According to the idea of inverse self-stratification, an external hydrophilic layer should be used to attract such

hydrophilic compounds. The sandwich method was initially used to attach these layers to the system. This method consists of the full coverage of the coating mixture with a solid top layer. When a non-reactive solvent is used this solid layer might hinder the evaporation of the solvent during curing. Thus, such an inhomogeneous evaporation of the solvent can result in the formation of bubbles and create rough, inhomogeneous active surface. In order to prevent this, a reactive ethylene glycol solvent, which would chemically incorporate into the film structure during curing rather then evaporating, was used in this method. However, even after using a reactive solvent, the ultimate film surfaces were extremely rough and fluffy after the removal of external hydrophilic layers. Beside the solvent evaporation, being covered by a heavy layer might be responsible for this inhomogeneity on the surfaces.

Another way to avoid surface damages due to the factors explained above was found to turn the whole system upside down (laminar method). In this method, the coating mixture was applied directly on the hydrophilic layer without covering the top with any solid layer. This time the possible QAC segregation was supposed to be on the bottom side of the films. The best result of this method was obtained when poly(vinyl pyrrolidone) (PVP) was used as the layer (Prepared NaCl and KBr pellets resulted in a very rough film surfaces). After the film formation, the sacrificial PVP layer was dissolved in distilled water to give a free standing film. Obtained films were found to be much smoother than the ones that obtained from sandwich method. The only drawback here was handling the free standing films. These films were very soft, and got wrinkled or rolled as soon as they are removed from the rinsing water bath. Yet, we still managed to investigate the surface characteristics and antimicrobial properties.

5.3.2. Surface Properties of the Coatings

5.3.2.1. Contact Angle Results

Dynamic water contact angles of the choline chloride containing films by Dataphysics OCA 30 instrument from both the PVP and the air sides. The results are presented in Figure 5.3.

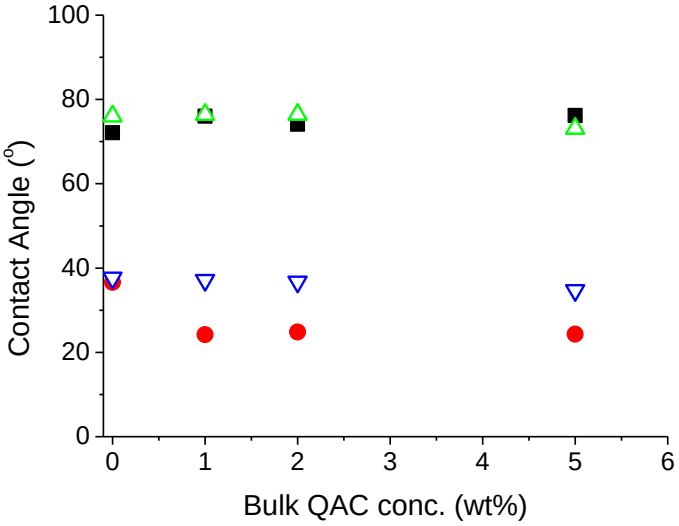


Figure 5.3. Advancing and receding water contact angles of the choline chloride system: (■) θ_{adv} (PVP side); (●) θ_{rec} (PVP side); (△) θ_{adv} (air side); (▽) θ_{rec} (air side).

Since the obtained films were free standing, it was possible to check contact angles at both the air and the PVP sides. As shown in Figure 5.3 the advancing contact angle both at the air and at the PVP side did not change considerably with increasing QAC concentration. The receding contact angles at the PVP side were observed to be about 15^{o} lower than those at the air side. This difference in receding contact angle measurements suggests a hydrophilic QAC enrichment at the PVP side.

5.3.2.2. X-ray Photoelectron Spectroscopy Results

Similarly, in order to prove "inverse" self-stratification, both the air and the PVP sides of the prepared films were monitored with X-ray photoelectron spectroscope (Figure 5.4). For choline chloride, the only indicator was the chlorine counterion. Cl/C atomic ratios at both sides of the films are shown in Figure 5.5.

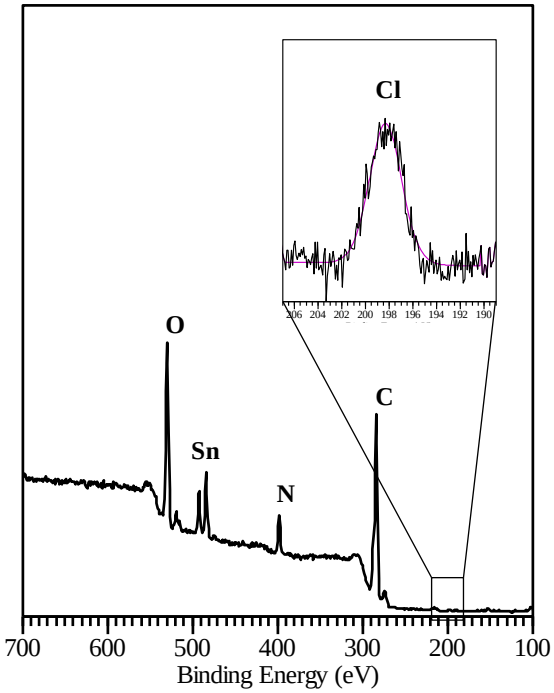


Figure 5.4. XPS wide spectrum of 2 wt% choline chloride containing film, (Spectrum was taken from the PVP side of the film at take-off angle of 90^{o}).

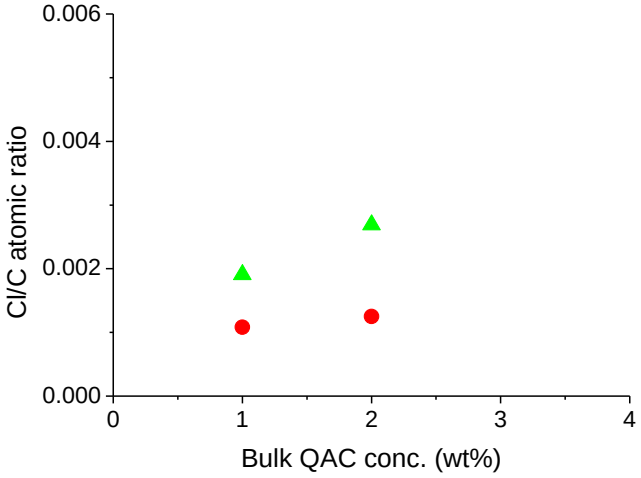


Figure 5.5. Cl/C ratio in the top 10 nm of films containing choline chloride: (●) air side (▲) PVP side.

It was observed that the Cl/C ratios at PVP side were much higher than those at the air side (Figure 5.5) proving that hydrophilic PVP layer was successful to attract hydrophilic choline chloride QACs. However, one needs to be cautious in over-interpreting such data, because the counterion is not covalently bonded to the polymer network. As discussed in *Chapter 3*, the counterion concentration may be an overestimation of the actual QAC concentration at the surface.

5.3.3. Antimicrobial Activity of the Coatings

Prepared film samples, after their surface properties were investigated, were exposed to bacterial log reduction test. Such tests were carried out against both Gram-positive *S. aureus* and Gram-negative *E. coli* bacterial species.

Table 5.1. Bacterial $\log_{10}$ reduction after 24 h of incubation of 10^5 bacteria with 1 cm^2 of choline chloride containing films.

Bulk QAC conc. (wt%)	*Staphylococcus aureus*	*Escherichia coli*
1	0	0
2	0	1
5	0	1

The bactericidal activity results of the test samples after 24 h of incubation time is presented in Table 5.1. As seen above none of the test samples showed a promising activity against either bacterial species. Considering that the surface concentration of choline chloride was not sufficient for desired microbial values, the pure choline chloride at much higher concentration was exposed to the similar tests. The pure compound at 1.0 mg/cell concentration showed a 2 $\log_{10}$ reduction against both Gram-positive and Gram-negative test species. Similarly, 1.0 mg/cell of pure QAC **6** compound showed a 2 $\log_{10}$ reduction against *E. coli* and a 4 $\log_{10}$ reduction against *S. aureus*. These results mean that, with such small QAC salts, one needs to go very high loadings to be able to reach the desired antimicrobial activity results. Addition of QACs to the coating mixture with a much higher concentration would be possible, but then the importance of the surface segregation of QAC at such a high loading would possibly be diminished.

5.4. Conclusions

In this chapter, an attempt to prepare surfaces enriched in short tailed QACs is described. Unlike the previous chapters, short and

hydrophilic quaternary ammonium compounds were chosen to be used instead of hydrophobic ones. The stratification mechanism was assisted by an external sacrificial hydrophilic layer attracting the hydrophilic QACs. Several different external layers (*e.g.* NaCl, KBr, and PVP) were employed and the best film surface was obtained by using a PVP layer. Contact angle and XPS measurements indicate a more hydrophilic and ion rich surface, suggesting the migration of the hydrophilic QACs to the polymer/hydrophilic layer interface. However, there are still several problems: handling of the films was very difficult and the films obtained suffer from surface roughness and surface inhomogeneity. Also, in application point of view, using a "sacrificial" external layer would increase the production cost of the final material.

In addition, the films containing commercial choline chloride QAC did not show any antibacterial property. This result is not completely unexpected. We know from the literature that hydrophobic tails at a certain length are essential for the polymeric systems with immobile quaternary ammonium compounds to exert antimicrobial activity [2-4]. However, films with long tailed hydrophobic QACs can be prepared with the autonomous self stratification method as we have shown in the previous chapters. Here, such small compounds were used in order to prove the principle and test the feasibility of the proposed "inverse self-stratification" method.

In conclusion, due to these multiple problems, this approach was not found to be useful for making antimicrobial films based on quaternary ammonium compounds, but found to be useful for getting hydrophilic groups to surface.

5.5. References

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Chapter 6

Conclusions

The motivation of this study was to prepare environmentally friendly and durable polymeric antimicrobial surface coatings with long-term activity for many different uses in daily life. The idea of self-stratification of quaternary ammonium compounds originated from the need to enhance the antimicrobial property of the coatings by saturating the surface with the active compounds without losing the bulk properties. To this end, the initial proposal had a two-legged approach: a) the usage of hydrophilic QACs in an "*inverse*" self-stratification strategy and b) the usage of hydrophobic QACs in an "*autonomous*" self-stratification strategy. Initially, very promising preliminary results were obtained from the autonomous self-stratification strategy, but numerous problems were faced with the inverse self-stratification strategy using hydrophilic QACs, rendering the latter strategy inappropriate for the ultimate goal of this study. Thus, we focused on the autonomous segregation of the quaternary ammonium compounds as the main approach. Prepared relatively low surface energy films with hydrocarbon tailed QACs were found to be antimicrobial and QAC segregation was shown by the contact angle and XPS measurements. However, monitoring ionically bonded counterion concentration by XPS carried the doubts about the certainty of the measurements.

The approach was further expanded to fluorinated QACs in order to enhance the self-stratification behavior because of an even lower surface energy originating from the perfluoro alkyl chains. In this way, the surface concentration of the active moieties as well as the antimicrobial activity was improved drastically by using a C_8F_{17} perfluorinated chain. An additional benefit of using fluorinated QACs was the ability to monitor the covalently attached fluorocarbons improving the reliability of the XPS measurements.

A final novel approach followed in this thesis is the development of antimicrobial polyurethane coatings based on ionic liquid QACs (IL-QACs)

using the self-stratification method. Since the selected IL-QACs are water soluble, self-stratification was not achieved, but the coatings showed excellent antimicrobial properties at high loadings of IL-QAC. A drawback of the approach is the fact, because of the hydrophilic nature of the IL-QACS, that the resultant coatings were water-sensitive, which may limit their possible applications. One may need to further improve the crosslinking density according to the type of the application.

As a final remark, the self-stratification approach followed in this thesis using hydroxyl-functional QACs in a standard polycondensation recipe can be easily adopted by the coatings industry. It should also be easily implementable using other polymerization techniques, for example in radical polymerization using quaternary ammonium ion containing (meth)acrylates and styrenics. Future studies in these directions would expand the use of the self-stratification concept in various coatings and applications.

Summary

Today, antimicrobial polymers/coatings are widely used in various areas, such as biomedical devices, pharmaceuticals, hospital buildings, textiles, food processing, and contact lenses, where sanitation is needed. Such wide application facilities have made antimicrobial materials very attractive for both academic and industrial researchers. Many methods have been developed to produce such materials with different properties. The aim of this PhD study was developing a novel method to prepare environmentally benign, surface active, long lasting antimicrobial surface coatings. This method was also targeted to be a simple one, so that it can be manufactured on an industrial scale and can be applied in our daily life.

In *Chapter 2* and *3*, we developed low surface energy polyurethane films by tethering antimicrobial quaternary ammonium compounds to the polymer network covalently. These compounds were designed and synthesized to diffuse towards the film/air interface during the film curing. In this way, a higher antimicrobial moiety concentration on the film surface was targeted. This property was gained to the QACs by attaching either long hydrocarbon (QACs **1** and **2**) or perfuorinated (QACs **3** and **4**) hydrophobic tails. QAC enrichment at the film surface was confirmed by dynamic contact angle measurements and X-ray photoelectron spectroscopy techniques. Overall, all of the QAC-containing films showed strong antimicrobial activities against the tested bacterial species. Of all systems tested, number **3** was the most effective with a 5 $\log_{10}$ reduction of both bacterial species observed at all concentrations applied. With the long perfluoroalkyl tail, QAC **3** was found to be the most efficiently diffusing QAC towards the film surface. As a result, the number of QAC molecules per cm^2 was much higher for system **3** than for the other systems. This suggests that the most

hydrophobic nature of QAC **3** resulted in the highest antimicrobial effect. The shorter spaced QAC-containing system **2** was the least effective of all four systems. We obtained minimally desired 3 $\log_{10}$ reduction only with higher QAC concentrations for this system. Also, we found that system **1** was slightly more active against the much simpler Gram-positive *S. aureus* bacteria than the complex Gram-negative *E. coli*.

In *Chapter 4*, we investigated a possible antimicrobial activity of some commercially available ionic liquids. These liquids were never used for this purpose before. The covalent incorporation of these liquids to our polyurethane network ended up with very successful antibacterial results. Hence, we successfully reported an ionic liquid based antimicrobial surface coating for the first time in the literature.

Moreover, in environmental point of view, leaching of the active QAC compounds from the films to the exterior was investigated by monitoring bacterial inhibition zone around the film samples and the antimicrobial tests of the extraction solutions of the films. None of the prepared films showed any trace of leaching out confirming all the quaternary ammonium compounds were covalently bonded to the polyurethane backbone.

Finally, we tried another method to prepare targeted antimicrobial coatings in *Chapter 5*. Instead of using self-diffusion ability of hydrophobic quaternary ammonium compounds, we utilized hydrophilic QAC structures to enrich at the film surface by forcing them to diffuse with an external attractive layer. However, we found this method impractical after facing numerous problems.

Curriculum Vitae

M. Barış Yağcı was born in Istanbul, Turkey on 08.08.1980. He studied chemistry in Marmara University, Turkey and got his B.Sc. degree in 2002. He continued his education in Boğaziçi University, Turkey and received his M.Sc. degree in polymer chemistry in 2005. His M.Sc. thesis focused on synthesis and photopolymerization of novel acrylic-based functional crosslinkers for dental applications. In the meantime, he worked as a short-term intern at the University of Southern Mississippi, USA in 1999 and 2004. In October 2005, he started his Ph.D. project in the Department of Materials and Interface Chemistry at the Eindhoven University of Technology under the supervision of Prof. dr. G. de With. The most important results of his Ph.D. research are described in this thesis.

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Barış