Novel Biocidal Formulations; the Effect of Polymer Structure on Antibacterial Activity and Aqueous Solutions Behaviour

A thesis submitted to The University of Manchester for the degree of Doctor of Philosophy (PhD) in the Faculty of Science and Engineering

2016

Fatimah Mohammed Alzahrani

School of Chemistry
The Table of Contents

Table of Contents 2
Abbreviations 8
List of Figures 9
List of Tables 17
List of Schemes 19
Abstract 20
Declaration 21
Copyright statement 22

Chapter 1. Introduction and Background

Introduction. 24
1.1 Opening statement. 24
1.2 Polyelectrolytes materials. 25
1.3 Antibacterial agents. 28
1.3.1 Cationically charged biocides. 28
1.4 Solution interactions. 29
1.4.1 Surfactants. 29
1.4.2 Mixtures of polyelectrolytes and ionic surfactant. 30
1.5 Designing antibacterial polymeric material. 31
1.5.1 Controlling polymer structures towards effective antibacterial polymers. 32
1.5.1.1 Free Radical polymerization. 32
1.5.1.2 Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization. 34
1.5.1.3 Mechanism of RAFT polymerization. 34
1.5.1.4 Chain Transfer Agents CTA (RAFT Agents). 35
1.5.2 Type of bacteria. 36
1.5.3 Mechanism of antimicrobial action. 38
1.5.4 Factors affecting antimicrobial activity. 39
1.5.4.1 Molecular weight effect. 39
1.5.4.2 Counterion effect. 40
1.5.4.3 Length of the alkyl hydrophobic chain. 41
1.5.4.4 Amphiphilic balance and charge density effect 42
1.6 Antimicrobial surfaces. 43
1.6.1 The main strategies for antibacterial surfaces design. 44
1.6.1.1 Contact kill (chemical grafting). 44
1.6.1.2 Polyelectrolyte multilayers (PEMs). 45
1.6.1.3 Adhesion resistance. 45
1.7 Tannins and tannic acid. 45
1.7.1 Antimicrobial and antiviral properties of tannins. 46
1.7.2 Tannic acid and polymer mixtures. 47
1.8 References. 47

Chapter 2: Experimental

2.1 Reagents. 55
2.2 Phase separation procedure. 56
2.2.1 Preparation of surfactant-polymer solutions. 56
Determination of critical phase separation concentration.

Bacterial strains, media and growth conditions.

Techniques.

Gel permeation chromatography (GPC).

Dynamic light scattering (DLS) and Zeta potential.

Nuclear magnetic resonance (NMR) spectroscopy.

Surface tension measurements.

Pyrene fluorescence spectroscopy.

Viscometry.

MIC determination.

Preparation of glycerol stocks of different types of bacteria.

L.B broth.

Preparation of LB agar plate.

Recovery of single colony from stored cultures.

Preparation of overnight culture.

Microtitre plate assay.

References.

Chapter 3: Synthesis and Structural Characterization of Polymers

Introduction.

Homopolymers.

Materials used to synthesize and characterize homopolymers.

Synthesis of homopolymers.

Calculation for homopolymers.

Synthesis of PMADQUAT via free radical polymerization.

Synthesis of PMADQUAT via RAFT polymerization.

Structural characterization of homopolymers.

1H NMR.

Gel permeation chromatography.

Statistical copolymers.

Materials used to synthesize and characterize statistical copolymers.

Synthesis of statistical copolymers.

Calculation for statistical copolymers.

Synthesis route of statistical copolymers.

a) Synthesis of poly(MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>).

b) Synthesis of poly(MADQUAT<sub>x</sub>-s-PEGMA<sub>y</sub>).

Characterization of statistical copolymers.

Confirmation of statistical copolymer formation by 1H NMR.

Determination of copolymer composition by 1H NMR.

Gel permeation chromatography.

Di-block polymers.

Materials used to synthesize and characterize di-block polymers.

RAFT polymerization of di-block polymers.

Calculations.

Example of di-block calculation: Poly (MADQUAT<sub>75</sub>-b-MMA<sub>25</sub>).

a) Calculation of the first block: MADQUAT<sub>75</sub>.

b) Calculation of the second block: MMA<sub>25</sub>.

c) Calculation of the initiator: VAZO-67.

Synthesis route of di-block polymers: poly(MADQUAT<sub>x</sub>-b-MMA<sub>y</sub>).
3.4.3 Characterization of di-block polymers. 80
3.4.3.1 Confirmation of di-block formation by $^1$H NMR. 80
3.4.3.2 Determination of copolymer composition by $^1$H NMR. 82
3.4.3.3 Gel permeation chromatography. 83
3.5 Core-shell quaternary nanoparticle polymers. 83
3.6 Characterization of didecyldimethylammonium chloride (DDAC). 84
3.6.1 Sample preparation for $^1$H NMR. 84
3.7 Summary and conclusion. 85
3.8 References. 86

Chapter 4. Aqueous Solutions Behavior of Polyelectrolytes

4.1 Introduction. 89
4.2 Surface tension. 90
4.3 Pyrene Fluorescence spectroscopy. 92
4.4 Interaction between surface tension and pyrene fluorescence spectroscopy. 93
4.4.1 Statistical Polymers; poly(MADQUAT$_x$-s-MMA$_y$). 94
  a/Poly(MADQUAT$_{25}$-s-MMA$_{75}$). 94
  b/Poly(MADQUAT$_{50}$-s-MMA$_{50}$). 94
  c/Poly(MADQUAT$_{75}$-s-MMA$_{25}$). 94
4.4.2 Di-block; Poly (DMCx-b-MMAy). 95
  a/Poly(MADQUAT$_{25}$-b-MMA$_{75}$). 95
  b/Poly(MADQUAT$_{50}$-b-MMA$_{50}$). 95
  c/Poly(MADQUAT$_{75}$-b-MMA$_{25}$). 95
4.4.3 Discussion. 96
4.5 DLS and Zeta potential. 97
4.6 Viscosity. 101
4.6.1 Relative viscosity. 101
4.6.2 Determination of intrinsic viscosity. 104
4.7 Summary and conclusion. 110
4.8 References. 111

Chapter 5. Phase Separations of Like Charged Polyelectrolyte and Cationic Surfactant

5.1 Introduction. 114
5.1.1 Cationic surfactants. 115
5.1.2 Phase separation in polyelectrolytes and cationic surfactant. 115
5.2 Phase separation experiments. 116
5.2.1 Materials. 116
5.2.2 Preparation of surfactant-polymers solutions. 117
5.2.3 Determination of critical phase separation concentration. 117
  a/ Phase separation behaviour of the homopolymer. 119
  b/ Phase separation behaviour of statistical copolymers. 119
  c/ Phase separation behaviour of di-block polymers. 121
5.3 Comparison of phase separation data. 122
5.4 Discussion. 123
5.5 Summary and conclusion. 124
Chapter 6. A Study of Various Classes of Antibacterial Polymers Bearing Quaternary Ammonium Salts

6.1 Introduction.
6.1.1 Polymers with quaternary ammonium salts as antibacterial agents.
6.1.2 Designing antibacterial polymeric materials.
6.1.3 Mechanism of action.
6.1.4 Chemical structure and molecular weight of polymers.
6.2 Antibacterial testing.
6.2.1 Determination of planktonic MICs of cationic polymers.
   6.2.1.1 Homopolymers.
      a) PMADQUAT.
      b) PEGMA.
   6.2.1.2 Statistical copolymers.
      a) Poly(MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>).
         1) Poly(MADQUAT<sub>25</sub>-s-MMA<sub>75</sub>).
         2) Poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>).
         3) Poly(MADQUAT<sub>75</sub>-s-MMA<sub>25</sub>).
      b) Poly(MADQUAT<sub>x</sub>-s-PEGMA<sub>y</sub>).
         1) Poly(MADQUAT<sub>50</sub>-s-PEGMA<sub>50</sub>).
         2) Poly(MADQUAT<sub>75</sub>-s-PEGMA<sub>25</sub>).
         3) Poly(MADQUAT<sub>95</sub>-s-PEGMA<sub>5</sub>).
   6.2.1.3 Cationic amphiphilic di-block polymers: Poly(MADQUAT<sub>x</sub>-b-MMA<sub>y</sub>).
      1) Poly(MADQUAT<sub>25</sub>-b-MMA<sub>75</sub>).
      2) Poly(MADQUAT<sub>50</sub>-b-MMA<sub>50</sub>).
      3) Poly(MADQUAT<sub>75</sub>-b-MMA<sub>25</sub>).
6.3 Determination of biofilm MICs of cationic polymers.
6.3.1 Homopolymers
6.3.1.1 1) PMADQUAT
6.3.1.2 2) PEGMA
6.3.2 Statistical copolymers
6.3.2.1 a) Poly(MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>).
      1) Poly(MADQUAT<sub>25</sub>-s-MMA<sub>75</sub>).
      2) Poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>).
      3) Poly(MADQUAT<sub>75</sub>-s-MMA<sub>25</sub>).
   6.3.2.2 b) Poly(MADQUAT<sub>x</sub>-s-PEGMA<sub>y</sub>).
      1) Poly(MADQUAT<sub>50</sub>-s-PEGMA<sub>50</sub>).
      2) Poly(MADQUAT<sub>75</sub>-s-PEGMA<sub>25</sub>).
      3) Poly(MADQUAT<sub>95</sub>-s-PEGMA<sub>5</sub>).
6.3.3 Cationic amphiphilic di-block polymers: poly(MADQUAT<sub>x</sub>-b-MMA<sub>y</sub>).
      1) Poly(MADQUAT<sub>25</sub>-b-MMA<sub>75</sub>).
      2) Poly(MADQUAT<sub>50</sub>-b-MMA<sub>50</sub>).
      3) Poly(MADQUAT<sub>75</sub>-b-MMA<sub>25</sub>).
6.4 Discussions.
6.4.1 MICs of polymers.
6.4.1.1 Comparing homopolymers and statistical copolymers.
6.4.1.2 Comparing amphiphilic di-block polymers and statistical copolymers.
6.5 Study of a series of core-shell nanoparticles quaternary ammonium with...
various alkyl side groups.

6.5.1 Core-shell quaternary ammonium nanoparticles.

1) ZY/24 148
2) ZY/26 149
3) ZY/27 150
4) ZY/28 150

6.5.2 Antibacterial efficacy of mixtures of core-shell quaternary ammonium nanoparticles.

1) ZY/36 151
2) ZY/37 152
3) ZY/38 152
4) ZY/39 153

6.5.3 Effects of core-shell quaternary ammonium nanoparticles on gram-negative biofilms.

1) ZY/24 154
2) ZY/26 154
3) ZY/27 155
4) ZY/28 155

6.6 Discussion.

6.6.1 Comparing core-shell quaternary ammonium nanoparticles and the mixtures.

6.7 Summary and conclusion.

6.8 References.

Chapter 7. A Study of the Antibacterial Activity of Tannic Acid and Tannic Acid/Amphiphilic Cationic Polymer Mixtures

7.1 Introduction. 165
7.2 Antibacterial activity of tannic acid. 165
7.2.1 Sample preparation. 165
7.2.2 Bacterial growth assays. 166
7.2.3 Preliminary study of tannic acid as antibacterial agent against five strains of planktonic bacteria. 166
7.2.4 MICs of tannic acid at unadjusted pH for planktonic bacteria. 168
7.2.5 MICs of tannic acid for biofilms. 169
7.3 The effect of tannic acid/PMADQUAT homopolymer mixtures on bacteria in planktonic and biofilm form. 170
7.3.1 Preparing mixtures of PMADQUAT and tannic. 170
7.3.2 MICs of TA/PMADQUAT mixtures for planktonic bacteria. 171
7.3.3 MICs of TA/PMADQUAT mixtures for bacteria in biofilms. 172
7.3.4 Comparing MICs of PMADQUAT, tannic acid and TA/PMADQUAT mixtures; 173
7.3.4.1 On planktonic. 173
7.3.4.2 On biofilms. 173
7.4 The effect of tannic acid/statistical copolymer poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>) mixtures on bacteria in planktonic and biofilm form; 173
7.4.1 On planktonic. 173
7.4.2 On biofilms. 174
7.4.3 Comparing MICs of poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>), tannic acid and TA/poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>) mixtures; 175
7.4.3.1 On planktonic. 175

6
7.4.3.2 On biofilms.

7.5 The effect of tannic acid/poly(MADQUAT$_{50}$-b-MMA$_{50}$) di-block polymer mixtures on bacteria in planktonic and biofilm form;

7.5.1 On planktonic

7.5.2 On biofilms

7.5.3 Comparing the MICs of poly(MADQUAT$_{50}$-b-MMA$_{50}$), tannic acid and TA/poly(MADQUAT$_{50}$-b-MMA$_{50}$) mixtures;

7.5.3.1 On planktonic

7.5.3.2 On biofilms

7.6 Summary and conclusion.

7.7 References.

Chapter 8. Conclusion and Further Work

8.1 Summary and conclusion.

8.1.1 Polymer synthesis, characterizations and aqueous solution behaviour.

8.1.2 The phase separation of mixtures of like charge surfactant/polymer systems.

8.1.3 Antibacterial Activity of different cationic polymers classes.

8.2 Further work.

8.3 References.

Chapter 9. Appendices

9.1 Particle size of all obtained polymers.

1/PMADQUAT.

2/p(MADQUAT$_{75}$-b-MMA$_{25}$).

3/p(MADQUAT$_{50}$-b-MMA$_{50}$).

4/p(MADQUAT$_{25}$-b-MMA$_{75}$).

5/p(MADQUAT$_{75}$-s-MMA$_{25}$).

6/p(MADQUAT$_{50}$-s-MMA$_{50}$).

7/p(MADQUAT$_{25}$-s-MMA$_{75}$).

9.2 Huggins/Kraemer plots with extrapolation to t=0 used to determine intrinsic viscosity [$\eta$].

a/ statistical copolymers.

b/ di-block polymers.

9.3 Relative viscosity, Ln relative viscosity and 1-1/Relative viscosity curves;

a/ statistical copolymers.

b/ di-block polymers

9.4 Fedros equation curves;

a/ statistical copolymers

b/ di-block polymers

9.5 A detailed report about the synthesis route of core-shell quaternary ammonium nanoparticles.

9.6 References.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDAC</td>
<td>Didecyldimethylammonium chloride</td>
</tr>
<tr>
<td>PMADQUAT</td>
<td>Poly[2-(methacryloyloxy) ethyl] trimethylammoniumchloride</td>
</tr>
<tr>
<td>PEGMA</td>
<td>Poly(ethyleneglycol) methyl ether methacrylate</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>VAZO-67</td>
<td>2,2’-azobis(2-methylbutyronitrile)</td>
</tr>
<tr>
<td>T.A</td>
<td>Tannic acid</td>
</tr>
<tr>
<td>Raft Agent(CDB)</td>
<td>2-phenyl-2-propyl benzodithioate or Cumyl dithiobenzoate</td>
</tr>
<tr>
<td>T.G</td>
<td>1-thioglycerol</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>QAS/QAC</td>
<td>Quaternary ammonium salts/Quaternary ammonium compounds.</td>
</tr>
<tr>
<td>RAFT polymerization</td>
<td>Reversible Addition-Fragmentation Chain Transfer polymerization.</td>
</tr>
<tr>
<td>wt%</td>
<td>Weight percent</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees celsius</td>
</tr>
<tr>
<td>D2O</td>
<td>Deuterium oxide. Solvent</td>
</tr>
<tr>
<td>CDCL3</td>
<td>Deuterochloroform. Solvent</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol. Solvent</td>
</tr>
<tr>
<td>Mn</td>
<td>Number average molecular mass</td>
</tr>
<tr>
<td>M_w</td>
<td>Weight average molecular mass</td>
</tr>
<tr>
<td>PDI</td>
<td>Poly dispersity index</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of Polymerization</td>
</tr>
<tr>
<td>LB</td>
<td>Lysogeny Broth or Luria-Bertani</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
</tbody>
</table>
List of Figures

**Figure 1.1.** Examples of some of functional groups that can be used to introduce cationic charges to the structure of a polymer. 25

**Figure 1.2** Schematic diagram of a surfactant molecule. 29

**Figure 1.3.** Chemical structures of alkyltrimethyl ammonium, alkylidimethylbenzyl ammonium and dialkyldimethyl ammonium, (R= alkyl group). 30

**Figure 1.4.** A comparison of conventional free radical polymerization (FRP) and Controlled Living polymerization (CRP). 33

**Figure 1.5.** Cell wall structure of gram-positive and gram-negative bacteria. 37

**Figure 1.6.** Explanation of the bactericidal mechanism against the gram-negative bacteria. 38

**Figure 1.7.** Tributyl (4-vinylbenzyl) phosphonium salt monomer. 40

**Figure 1.8.** Optimization of balance between cationic and hydrophobic group. 43

**Figure 1.9.** General principles of antimicrobial surfaces. 44

**Figure 1.10.** Schematic of layer-by-layer deposition used to assemble polyelectrolyte multilayer films on support substrates 45

**Figure 2.1.** Pyrene fluorescence with illustration of the first and third vibrational peaks. 60

**Figure 3.1.** $^1$H NMR spectrum (400 MHz, CDCl$_3$) of MADQUAT monomer. 69

**Figure 3.2.** $^1$H NMR spectrum (400 MHz, D$_2$O) of PMADQUAT homo-polymer. 69

**Figure 3.3.** $^1$H NMR spectrum (400 MHz, D$_2$O) of PMADQUAT after 30 hrs. Synthesized by RAFT in ethanol at 70-75 °C (before purification). 70

**Figure 3.4.** $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; p(MADQUAT$_{75}$-s-MMA$_{25}$). 73

**Figure 3.5.** $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; p(MADQUAT$_{50}$-s-MMA$_{50}$). 74

**Figure 3.6.** $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; p(MADQUAT$_{25}$-s-MMA$_{75}$). 74

**Figure 3.7.** $^1$H NMR spectrum (400 MHz, D$_2$O) of p(MADQUAT$_{75}$-b-MMA$_{25}$). 80

**Figure 3.8.** $^1$H NMR spectrum (400 MHz, D$_2$O) of p(MADQUAT$_{50}$-b-MMA$_{50}$). 81

**Figure 3.9.** $^1$H NMR spectrum (400 MHz, D$_2$O) of p(MADQUAT$_{25}$-b-MMA$_{75}$). 81

**Figure 3.10.** $^1$H NMR spectra (400 MHz, D$_2$O) of all di-block polymers; (p(MADQUAT$_x$-b-MMA$_y$)). 82

**Figure 3.11.** $^1$H NMR spectrum (400 MHz, D$_2$O) of DDAC. 84

**Figure 4.1.** Surface tension curves as function of the logarithm concentrations of p(MADQUAT$_x$s-MMA$_y$), T= 25 0C; solvent-water. 90

**Figure 4.2.** Surface tension curves as function of the logarithm concentrations of p(MADQUAT$_x$b-MMA$_y$), T= 25 0C; solvent-water. 90

**Figure 4.3.** Schematic representation of an image charge effect at the air/water interface. 92

**Figure 4.4.** Pyrene fluorescence curves as function of the logarithm concentrations of p(MADQUAT$_x$s-MMA$_y$), T= 25 0C; solvent-water. 93

**Figure 4.5.** Pyrene fluorescence curves as function of the logarithm concentrations of p(MADQUAT$_x$b-MMA$_y$), T= 25 0C; solvent-water. 93

**Figure 4.6.** Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the I$_3$/I$_1$ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT$_{25}$-s-MMA$_{75}$), T= 25 0C; solvent-water. 94

**Figure 4.7.** Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the I$_3$/I$_1$ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT$_{50}$-s-MMA$_{50}$), T= 25 0C; solvent-water. 94
Figure 4.8. Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the \(I_3/I_1\) ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\(_{75}\)-s-MMA\(_{25}\)), \(T=25\) °C; solvent-water.

Figure 4.9. Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the \(I_3/I_1\) ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\(_{25}\)-b-MMA\(_{75}\)), \(T=25\) °C; solvent-water.

Figure 4.10. Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the \(I_3/I_1\) ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\(_{50}\)-b-MMA\(_{50}\)), \(T=25\) °C; solvent-water.

Figure 4.11. Interaction between surface tension and pyrene fluorescence spectroscopy: variations of the \(I_3/I_1\) ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\(_{75}\)-b-MMA\(_{25}\)), \(T=25\) °C; solvent-water.

Figure 4.12. Zeta potential of pMADQUAT, p(MADQUAT\(_x\)-s-MMA\(_y\)) and p(MADQUAT\(_x\)-b-MMA\(_y\)) as a function of molar ratio of MADQUAT and polymer structures, \(T=25\) °C; solvent-water.

Figure 4.13. Changes in the Relative viscosity of block and statistical copolymers’ aqueous solutions measured at various mass concentrations (g/dL) at \(25\) °C.

Figure 4.14. Log viscosities/ (mPa.s) of the diblock polymers plotted as a function of log Concentration/ (wt.%) for aqueous solutions at \(25\) °C.

Figure 4.15. Log viscosities/ (mPa.s) of the statistical copolymers plotted as a function of log concentration (wt%) for aqueous solutions at \(25\) °C.

Figure 4.16. TEM image of micelle aggregation of p(MADQUAT\(_x\)-co-SAM\(_y\)) in water (1.26 g/L).

Figure 4.17. The reduced viscosity \(\eta_{SP}/c\) vs. c for block and statistical copolymers in water/ NaCl solutions at \(25\) °C.

Figure 4.18. Adsorption of polymer molecules (+) on the capillary walls (-).

Figure 4.19. Huggins/Kraemer plot for p(MADQUAT\(_{75}\)-s-MMA\(_{25}\)) with extrapolation to \(t=0\) used to determine intrinsic viscosity[\(\eta\)].

Figure 4.20. of \(\eta_{rel}, \eta_{rel} \text{on} \) and \(1 - 1/\eta_{rel} \) of pMADQUAT\(_{75}\)-s-MMA\(_{25}\) as function of polymer concentration.

Figure 4.21. Representation of Fuoss equation for p(MADQUAT\(_x\)-s-MMA\(_y\)) and p(MADQUAT\(_x\)-b-MMA\(_y\)) as function of polymer concentration.

Figure 4.22. Schematic presentation of the difference between amphiphilic statistical copolymers and diblock polymers aggregation.

Figure 5.1. Phase separation boundary for PMADQUAT (0.75 wt. %) / DDAC (3 wt. %) / water at \(25\) °C.

Figure 5.2. \(^1\text{H NMR of top phase and bottom phase for PMADQUAT (0.75 wt. %) / DDAC (3 wt. %) water sample, 24 hrs. since last agitation.}

Figure 5.3. \(^1\text{H NMR of top phase and bottom phase for poly(MADQUAT}_{50}-\text{s-MMA}_{50})(0.75 \text{ wt\%}) / \text{DDAC (3 wt\%)} \) water sample, 24 hrs. since last agitation.

Figure 5.4. \(^1\text{H NMR of top phase and bottom phase for poly(MADQUAT}_{50}-\text{b-MMA}_{50})(0.75 \text{ wt\%}) / \text{DDAC (5 wt\%)} \) water sample, 24 hrs. since last agitation.

Figure 5.5. Determination of critical phase separation concentration of the homopolymers PMADQUAT / DAC / Water solutions.

Figure 5.6. Phase separation diagram of statistical copolymers; poly (MADQUAT\(_x\)-s-
MMA, and DDAC. Data points illustrate the phase separation boundaries.

**Figure 5.7.** The critical phase separation concentrations as function of the molar ratio of the cationic moieties (MADQUAT) of the polymers.

**Figure 5.8.** Phase separation diagram of di-block polymers; poly (MADQUAT-MMA) and DDAC. Data points illustrate the phase separation boundaries.

**Figure 5.10.** Comparing between Phase separation diagram of homo-polymers, statistical copolymers and di-block polymers.

**Figure 5.11.** Data points illustrate the effect of the molar ratio of MADQUAT and polymers’ structures on phase separation boundaries, at constant concentration (1 wt% of all polymers).

**Figure 6.1.** The effect of homopolymer; PMADQUAT on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.2.** The effect of homopolymer; PEGMA on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.3.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.4.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.5.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.6.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.7.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.8.** The effect of statistical copolymer; poly(MADQUAT-MMA) on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
Figure 6.9. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on planktonic growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S.aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.10. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) on planktonic growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.11. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}) on planktonic growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.12. The effect of homopolymer; PMADQUAT on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.13. The effect of homopolymer PEGMA on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{K. pneumoniae} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.14. The effect of statistical copolymer; poly(MADQUAT\textsubscript{25}s-MMA\textsubscript{75}) on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.15. The effect of statistical copolymer; poly(MADQUAT\textsubscript{50}s-MMA\textsubscript{50}) on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.16. The effect of statistical copolymer; poly(MADQUAT\textsubscript{75}s-MMA\textsubscript{25}) on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S. aureus} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.17. The effect of statistical copolymer; poly(MADQUAT\textsubscript{50}s-PEGMA\textsubscript{50}) on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{K. pneumoniae} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.18. The effect of statistical copolymer; poly(MADQUAT\textsubscript{75}s-PEGMA\textsubscript{25}) on biofilm growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{K. pneumoniae} and \textit{P. aeruginose}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
Figure 6.19. The effect of statistical copolymer; poly(MADQUAT95-s-PEGMA5) on biofilm growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.20. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT25-b-MMA75) on biofilm growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, S.aureus and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.21. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT50-b-MMA50) on biofilm growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, S.aureus and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.22. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT75-b-MMA25) on biofilm growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, S.aureus and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.23. Schematic presentation shows the different between block and statistical copolymers antibacterial action.

Figure 6.24. The effect of ZY/24 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.25. The effect of ZY/26 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.26. The effect of ZY/27 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.27. The effect of ZY/28 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.28. The effect of ZY/36 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 6.29. The effect of ZY/37 polymer on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells
involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.30.** The effect of ZY/38 polymer on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.31.** The effect of ZY/39 polymer on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.32.** The effect of ZY/24 polymer on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.33.** The effect of ZY/26 polymer on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.34.** The effect of ZY/27 polymer on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 6.35.** The effect of ZY/28 polymer on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, K. pneumoniae and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 7.1.** Preliminary study of tannic acid on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, S. aureus and P. aeruginose*. The results are expressed as the mean of 8 replicate wells involving one biological replicate for each strain. Error bars indicate the standard error of the mean.

**Figure 7.2.** The effect of Tannic Acid unadjusted for pH on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, S. aureus and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 7.3.** Growing *Pseudomonas aeruginosa* bacteria after treatment with tannic acid at concentrations of 0.01, 0.1, 0.25 and 0.5 wt%.

**Figure 7.4.** The effect of Tannic Acid on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical islate, S. aureus and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Figure 7.5.** Tannic acid (0.5 wt%) in LB medium solutions 1) without PMADQUAT...
and 2) with PMADQUAT at t=0.

Figure 7.6. The effect of Tannic Acid (0.1 wt%)/PMADQUAT homopolymer mixtures on planktonic growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 7.7. The effect of Tannic Acid (0.1 wt%)/PMADQUAT on biofilm growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 7.8. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT-50-50-MMA) mixtures on biofilm growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 7.9. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT-50-50-MMA) mixtures on planktonic growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 7.10. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT-50-b-MMA) di-block polymer mixtures on planktonic growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 7.11. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT-50-b-MMA) di-block polymer mixtures on biofilm growth was assessed by microtitre assay on *E.coli K12*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

Figure 9.1. ^1^H NMR spectrum (400 MHz, D$_2$O) of poly(MADQUAT$_{75}$-b-MMA$_{25}$) showing ~95% conversion (about 48 hrs.) of each block.

Figure 9.2. ^1^H NMR spectrum (400 MHz, D$_2$O) of poly(MADQUAT$_{25}$-s-MMA$_{75}$) showing ~95% conversion after 30 hrs.

Figure 9.3. Particle size distribution of PMADQUAT at various concentrations measured by DLS.

Figure 9.4. Particle size distribution of p(MADQUAT$_{75}$-b-MMA$_{25}$) at various concentrations measured by DLS.

Figure 9.5. Particle size distribution of p(MADQUAT$_{50}$-b-MMA$_{50}$) at various concentrations measured by DLS.

Figure 9.6. Particle size distribution of p(MADQUAT$_{25}$-b-MMA$_{75}$) at various concentrations measured by DLS.

Figure 9.7. Particle size distribution of p(MADQUAT$_{75}$-s-MMA$_{25}$) at various concentrations measured by DLS.

Figure 9.8. Particle size distribution of p(MADQUAT$_{50}$-s-MMA$_{50}$) at various concentrations measured by DLS.

Figure 9.9. Particle size distribution of p(MADQUAT$_{25}$-s-MMA$_{75}$) at various concentrations measured by DLS.

Figure 9.10. Huggins/Kraemer plot for p(MADQUAT$_{50}$-s-MMA$_{50}$) with extrapolation to
t=0 used to determine intrinsic viscosity $\eta$.

Figure 9.11. Huggins/Kraemer plot for $p(MADQUAT_{25}-s\text{-}MMA_{75})$ with extrapolation to $t=0$ used to determine intrinsic viscosity $\eta$.

Figure 9.12. Huggins/Kraemer plot for $p(MADQUAT_{75}-b\text{-}MMA_{25})$ with extrapolation to $t=0$ used to determine intrinsic viscosity $\eta$.

Figure 9.13. Huggins/Kraemer plot for $p(MADQUAT_{50}-b\text{-}MMA_{50})$ with extrapolation to $t=0$ used to determine intrinsic viscosity $\eta$.

Figure 9.14. Huggins/Kraemer plot for $p(MADQUAT_{25}-b\text{-}MMA_{75})$ with extrapolation to $t=0$ used to determine intrinsic viscosity $\eta$.

Figure 9.15. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $pMADQUAT_{75}-s\text{-MMA}_{25}$ as function of polymer concentration.

Figure 9.16. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $pMADQUAT_{50}-s\text{-MMA}_{50}$ as function of polymer concentration.

Figure 9.17. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $pMADQUAT_{25}-b\text{-MMA}_{75}$ as function of polymer concentration.

Figure 9.18. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $pMADQUAT_{75}-b\text{-MMA}_{25}$ as function of polymer concentration.

Figure 9.19. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $pMADQUAT_{50}-b\text{-MMA}_{30}$ as function of polymer concentration.

Figure 9.20. Relative viscosity, Ln Relative viscosity and $1/\text{Relative Viscosity}$ of $p(MADQUAT_{25}-b\text{-MMA}_{75})$ as function of polymer concentration.

Figure 9.21. Representation of Fedros equation for three statistical copolymers with different molar ratio of MADQUAT and MMA; $p(MADQUAT_{x}-s\text{-MMA}_{y})$.

Figure 9.22. Representation of Fedros equation for three diblock polymers with different molar ratio of MADQUAT and MMA; $p(MADQUAT_{x}-b\text{-MMA}_{y})$.

Figure 9.23. The optical density of control plate of tannic acid at different concentrations without adjusting pH.

Figure 9.24. The optical density of control plate of tannic acid at different concentrations, pH 7.

Figure 9.25. Formation of precipitations at high concentrations of Tannic Acid.

Figure 9.26. Tannic Acid/L.B media mixture precipitations at t=0 and t=3hrs.

Figure 9.27. Tannic Acid (1, 0.5, 0.25, 0.1 and 0.01 wt%)/L.B media/cationic polymer mixture after 3 hrs.

Figure 9.28. Plots of (A) total particle number in the reaction, (B) $Z$-average particle diameter and PDI, (C) particle size distribution as conversion% of polyMMA-co-MATMAC.

Figure 9.29. The Mass spectra for the amino azide and quaternary ammonium azides.
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Examples of gram-positive and gram-negative bacteria.</td>
<td>38</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Names and chemical structures of monomers used.</td>
<td>55</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Names and chemical structures of RAFT agent and Free radical source used.</td>
<td>56</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Chemical structures of didecyldimethylammonium chloride (DDAC) and Tannic Acid (TA).</td>
<td>57</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Bacterial strains.</td>
<td>58</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Reagents used for LB broth and agar.</td>
<td>67</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Calculated mass of each compound used in synthesizing PMADQUAT by RAFT and free radical polymerization.</td>
<td>70</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>PMADQUAT homopolymer integral areas.</td>
<td>71</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Summary of $M_n$, $M_w$ and PDI from GPC for PMADQUAT homopolymers.</td>
<td>71</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Summary of calculated molar ratio of each polymer of the statistical polymers from $^1$H NMR.</td>
<td>75</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>Summary of $M_n$, $M_w$ and PDI from GPC for statistical copolymers.</td>
<td>76</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>Calculated $M_n$ and DP values of synthesized di-block polymers.</td>
<td>78</td>
</tr>
<tr>
<td>Table 3.7</td>
<td>Molar ratio of each block of the di-block polymers, as calculated and by $^1$H NMR.</td>
<td>83</td>
</tr>
<tr>
<td>Table 3.8</td>
<td>$M_w$, $M_n$ and PDI of di-block polymers determined by aqueous GPC.</td>
<td>84</td>
</tr>
<tr>
<td>Table 3.9</td>
<td>Side chain and particle structure of Zhou’s Core-Shell nanoparticles.</td>
<td>85</td>
</tr>
<tr>
<td>Table 3.10</td>
<td>Summary of $M_w$, $M_n$ and PDI of all synthesised polymers by aqueous GPC.</td>
<td>86</td>
</tr>
<tr>
<td>Table 3.11</td>
<td>Summary of $M_w$, $M_n$ and PDI of synthesized diblock polymers by Aqueous GPC, H NMR and Target values.</td>
<td>89</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Summary of $M_w$, $M_n$ and PDI of used polymers.</td>
<td>97</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Tabulated (cac) values. results obtained by surface tensiometry and pyrene Fluorescence.</td>
<td>99</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>particles size, PDI and zeta potential of pMADQUAT, T=25 °C in water.</td>
<td>99</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>particles size, PDI and zeta potential of di-block polymers, T=25 °C in water.</td>
<td>100</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>particles size, PDI and zeta potential of statistical copolymers, T=25 °C in water.</td>
<td>101</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Viscosity definitions.</td>
<td>104</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Different equations used to determine intrinsic viscosity.</td>
<td>108</td>
</tr>
<tr>
<td>Table 4.8</td>
<td>Intrinsic viscosity values(dL/g) obtained for both block and statistical copolymers with different ratio of MADQUAT and MMA.</td>
<td>109</td>
</tr>
<tr>
<td>Table 4.9</td>
<td>Overlap concentration, c*, for polymers investigated.</td>
<td>116</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Mw, Mn and PDI of used polymers in phase separation experiment which determined by aqueous GPC.</td>
<td>116</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>Didecyldimethylammonium Chloride (DDAC).</td>
<td>116</td>
</tr>
<tr>
<td>Table 6.1</td>
<td>Summary of $M_w$, $M_n$ and PDI of homo and statistical synthesized polymers by Aqueous GPC.</td>
<td>129</td>
</tr>
<tr>
<td>Table 6.2</td>
<td>Summary of $M_w$, $M_n$ and PDI of synthesized di-block polymers tested for antibacterial activity by Aqueous GPC, H NMR and Target values.</td>
<td>130</td>
</tr>
<tr>
<td>Table 6.3</td>
<td>MIC values of cationic polymers for planktonic bacteria.</td>
<td>144</td>
</tr>
<tr>
<td>Table 6.4</td>
<td>MIC values of cationic polymers for bacteria in biofilms.</td>
<td>145</td>
</tr>
<tr>
<td>Table 6.5</td>
<td>Side chain and particle structure of core-shell quaternary ammonium nanoparticles.</td>
<td>148</td>
</tr>
<tr>
<td>Table 6.6</td>
<td>Percentages (%) of each core-shell quaternary ammonium nanoparticles in the mixtures tested.</td>
<td>151</td>
</tr>
<tr>
<td>Table 6.7</td>
<td>MIC values of Core-shell quaternary ammonium nanoparticles for</td>
<td></td>
</tr>
</tbody>
</table>
planktonic bacterial strains.

Table 6.8. MIC values of Core-shell quaternary ammonium nanoparticles for bacterial strains in biofilms.

Table 6.9. Summary of MIC values of cationic polymers for planktonic strains.

Table 6.10. Summary of MIC values of cationic polymers for biofilm strains.

Table 7.1. MIC values of tannic acid at pH 7 for various planktonic bacterial strains.

Table 7.2. MIC values of tannic acid unadjusted for pH for different strains of planktonic Bacteria.

Table 7.3. MIC values of tannic acid against different bacterial strains in biofilm.

Table 7.4. MICs of tannic acid/PMADQUAT mixtures for planktonic bacteria.

Table 7.5. MICs of tannic acid/PMADQUAT mixtures for bacteria in biofilms.

Table 7.6. Comparing MICs of PMADQUAT, tannic acid and TA/PMADQUAT mixtures for planktonic bacteria.

Table 7.7. Comparing MICs of PMADQUAT, tannic acid and TA/PMADQUAT mixtures on bacteria in biofilms.

Table 7.8. Comparing MICs of poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50}), tannic acid and TA/poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50}) mixtures on planktonic bacteria.

Table 7.9. Comparing MICs of poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50}), tannic acid and TA/poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50}) mixtures on bacteria in biofilms.

Table 7.10. Comparing MICs of poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}), tannic acid and TA/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) mixtures on planktonic bacteria.

Table 7.11. Comparing MICs of poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}), tannic acid and TA/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) mixtures on biofilm bacteria.

Table 7.12. Summary of MIC values of mixtures of TA/PMADQUAT, TA/poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50}) and TA/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}), on planktonic and biofilm bacteria.

Table 9.1 Summary of polymerization feed monomers and physical characterization, Z average diameters and polydispersion index by DLS.

Table 9.2 Composition and characterisation data for core and core-shell crosslinking polymers.

Table 9.3 The structures and Mw of the amino azide and quaternary ammonium azides.

Table 9.4 Characteristics of different type polymer particles.
List of Schemes

**Scheme 1.1.** Bearing the cationic charge in the side chain. 26
**Scheme 1.2.** Bearing the cationic charge in the backbone of the polymer. 26
**Scheme 1.3.** Free radical polymerization mechanism. 33
**Scheme 1.4.** Mechanism of reversible-addition fragmentation chain transfer (RAFT) Polymerization. 35
**Scheme 1.5.** Structural features of thiocarbonylthio RAFT agent and intermediate formed on radical addition. 36
**Scheme 3.1.** Synthesis of homopolymer PMADQUAT by free radical polymerization. 68
**Scheme 3.2.** Synthesis of poly(MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>) copolymers. 72
**Scheme 3.3.** Synthesis of poly(MADQUAT<sub>x</sub>-s-PEGMA<sub>y</sub>) copolymers. 73
**Scheme 3.4.** Synthesis of poly(MADQUAT<sub>x</sub>-b-MMA<sub>y</sub>) di-block polymers by RAFT Polymerization. 80
**Scheme 9.1.** Click chemistry. 198
**Scheme 9.2.** Strategy for attaching functional molecules (represented by the rectangular block) to particle surfaces using click chemistry. 198
**Scheme 9.3.** Emulsion MMA, MATMAC and PMA polymerisation (1), synthesis of quaternary ammonium azide with various side groups (2) and alkyne-azole cycloadition click functionalization of polymer (3). 199
**Scheme 9.4.** The procedure employed to synthesize core–shell particles composed of ploy MMA-co-MATMAC core crosslinked DEG DMA with a poly(PMA-co-PEGMA) shell. 201
The University of Manchester Faculty of Science and Engineering  
Fatimah M. Alzahrani  
A thesis submitted for the degree of Doctor of Philosophy  
Novel Biocidal Formulations; the effect of polymer structure in the antibacterial activity and aqueous solutions behaviour.  
22\textsuperscript{nd} November 2016

Abstract
New strains of bacteria have developed resistance to many of the limited number of antibiotics currently available in the market. The use of conventional antimicrobial agents is also increasingly subject to restrictions because of the environmental damage arising from the toxicity of their residues. These factors strongly justify research into ways of fighting these microorganisms physically rather than chemically, reducing the quantities of antibacterial agents used while maintaining antibacterial activity for longer.

This study is in three main parts. The first involves the synthesis of four series of quaternary ammonium compounds whose main constituent is a cationic monomer, poly [2-(methacryloyloxy) ethyl trimethylammonium chloride] (MADQUAT). Two types of cationic polymer were synthesized: a) statistical copolymers of MADQUAT and either methyl methacrylate (MMA) or poly (ethyleneglycol) methyl ether methacrylate (PEGMA) and b) amphiphilic di-block polymers. The statistical copolymers poly (MADQUAT\textsubscript{x}-s-MMA\textsubscript{y}) and poly (MADQUAT\textsubscript{x}-s-PEGMA\textsubscript{y}) were prepared by the one-pot method of a modified conventional free-radical polymerization using 2,2’-azobis (2-methyl butyronitrile) as initiator. The amphiphilic di-block polymers were synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization. The resultant products were characterized by several techniques: aqueous GPC, \textsuperscript{1}H NMR, Zeta potential and DLS. The behaviour of aqueous solutions of both types of cationic polymer was investigated by studying surface tension, pyrene fluorescence and viscosity; the results indicate that these polymers are not surface active. The behaviour and critical aggregation concentration of both di-block and statistical cationic polymers in aqueous solution were significantly affected by polymer structure and the molar ratio of MADQUAT to MMA.

The second part of the study investigates the interactions between like-charged cationic surfactant didecyl dimethyl ammonium chloride (DDAC) and polyelectrolytes: a homopolymer, poly MADQUAT, statistical copolymers poly (MADQUAT\textsubscript{x}-s-MMA\textsubscript{y}) and di-block polymers poly (MADQUAT\textsubscript{x}-b-MMA\textsubscript{y}). In each case the system separates into a lower polyelectrolyte-rich and an upper surfactant-rich phase with different phase separation boundaries, confirmed by \textsuperscript{1}H NMR.

The third part of the study measures minimum inhibitory concentrations of these polymers against both gram-positive and gram-negative bacterial strains, finding that these polymers have an interesting antibacterial activity. However, the structures of cationic polymers have an insignificant impact on antibacterial activity.
Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of The University of Manchester or any other university or any other institute of learning.

Fatimah Mohammed Alzahrani
22/11/2016
Copyright statement

i. The author of this thesis (including any appendices and/or schedules to this thesis owns certain copyright or related rights in it the “Copyright” and s/ he has given the University of Manchester (the “University”) certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made only in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be descried in this thesis may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=487), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see http://www.manchester.ac.uk/library/aboutus/regulations) and in The University’s policy on Presentation of Theses.
Chapter 1

Introduction and Background
**Introduction**

**1.1 Opening statement**

New strains of bacteria have developed resistance to many of the limited number of antibiotics currently available in the market. Health departments are alarmed by the growth of this phenomena, hence advocating a serious search to address this problem before it is too late. The problem is further compounded by the fact that new stringent regulations are adopted on the use of the conventional antimicrobial agents due to their toxic wastes in polluting the environment. These hurdles have prompted a change in the strategy pursued in fighting microorganisms by exploring fighting it physically rather than chemically via antibiotics. This has the advantage of lowering the amount of the antimicrobial agents used and at the same time prolonging its antimicrobial activity.

The aim of the project is to study the effect of polymers’ structure in antibacterial activity and aqueous solutions behaviours.

1) RAFT and Free radical polymerizations will be applied in order to synthesis a series of amphiphilic polymers bearing quaternary ammonium salts with different structures and molar ratios.

2) Then different techniques will be used to characterize the obtained polymers and their aqueous solutions, such as aqueous GPC, $^1$H NMR, Zeta potential, DLS, pyrene fluorescence, surface tension and viscosity.

3) Phase separation phenomena will be investigated in mixtures of like charge surfactant-polyelectrolyte. In more details, this part of research will study binary mixtures of cationic (Homopolymer, di-block polymers and statistical copolymers) and a cationic surfactant (DDAC). Then, the interaction of binary mixtures of cationic polymers and cationic surfactant will be studied, specifically its phase separation into two clear layers, polyelectrolytes-rich layer (bottom phase) and surfactant-rich layer (upper phase).

4) The antibacterial activity of:

1/ various classes of polymers bearing [2-(methacryloyloxy)ethyl] trimethyl ammonium chloride (MADQUAT) with a variety of structures, molecular weights, length of alkyl side groups and amphiphilic balance (MADQUAT, MMA and PEGMA).

2/ tannic acid and T.A/amphiphilic cationic polymer mixtures.

Will be studied on planktonic and biofilm growths of both gram-negative and gram-positive bacteria.
1.2 Polyelectrolytes Materials

Polyelectrolytes materials are an important class of polymers “that exhibit various interesting phenomena due to their dual character of highly charged electrolytes and macromolecular chain molecules”.\(^2\) They can be categorized into three types depending on their source; natural polymers such as DNA modified natural polymers such as cellulose and synthetic polymers such as poly(styrene sulfonic acid). These polymers can also be categorized into polyanions and polycations according to their charge.\(^2\)

Polycations, being positively charged polymer molecules are attracted to the negatively charged outer membrane of bacteria cells and if they possess a balanced amphiphilic character, they have the ability to kill bacteria cells through disrupting the outer and the cytoplasmic membrane causing lysis of the cell and eventually its death.\(^2\) A limited number of functional groups can be used to introduce cationic charges to the structure of a polymer and that is due to accessibility and/or stability. Examples of some of these groups are quaternary ammonium and phosphonium and tertiary sulfonium, all of which have been used in the synthesis and applications of polycationic antimicrobial polymers (Fig. 1.1), with the quaternary nitrogen containing structures being the most synthesized and used.

![Examples of functional groups](image)

**Figure. 1.1.** Examples of some of functional groups that can be used to introduce cationic charges to the structure of a polymer.

Polycations can be synthesized by covalently attaching the required number and identity of the quaternary ammonium group to the polymer backbone, giving a wide variety of structures and properties. Two synthetic routes can be used for preparing the cationic polyelectrolytes containing quaternary ammonium groups namely:\(^3\)

- a chain or step polymerization using appropriate monomers, or
- cationic functionalization of reactive precursor polymers
Most of the cationic quaternary polyelectrolytes are prepared by conventional or controlled free radical polymerization (CTR). CTR provide a well-defined molecular structure with a narrow molecular weight distribution, hence better control of the physiochemical properties of the synthesized polymer.\textsuperscript{4,5}

Vinyl monomers are widely used for the preparation of polyelectrolytes of polycation type. Chain polymerization of cationic vinyl monomers produces polyelectrolytes having the cationic charge in the side chain i.e. a pendant type as shown in the scheme 1.1.

\[ \text{Scheme 1.1. Bearing the cationic charge in the side chain.} \textsuperscript{3} \]

However, when step polymerization is used for the synthesis cationic polymers, the cationic charge appears as part of the polymer backbone as shown in the scheme 1.2.

\[ \text{Scheme 1.2. Bearing the cationic charge in the backbone of the polymer.} \textsuperscript{3} \]

Many cationic polyelectrolytes have been synthesized by functionalization of reactive precursor polymers. The three most common quaternization reactions used for the introduction of quaternary nitrogen in the polymer backbone are:\textsuperscript{3}

1. The quarternization of a halide containing polymer with a tertiary amine NR\textsubscript{3}

\[ \text{NR}_3 \]
Chapter 1. Introduction and background

2. The quaternization of a polymeric tertiary amine with alkyl R-X or aryl halide.

\[
\begin{align*}
\text{R} & \quad \text{N} \quad \text{R}
\end{align*}
\]

\[
\text{RX} \quad \rightarrow \quad \text{N}^+ \text{R}_3
\]

3. The quaterization of polymers containing OH-groups by suitable agents like 2,3-epoxypropyltrimethylammonium chlorides.

Commercially available poly(propylene imine) dendrimers with 32 surface primary amine groups were modified by Cooper and co-workers\(^6\) in order to introduce the quaternary ammonium function on the dendrimers. Modification was carried out in two steps. In the first step the primary amine group was reacted to produce the halogen-containing dendrimers. The quaternary ammonium–functionalized poly(propylene imine) was obtained by reacting the halogen-containing dendrimer prepared in the first step with tertiary amines. Poly (chloroethyl vinyl ether-co-methyl methacrylate) linear copolymer was obtained by free radical polymerization of chloroethyl vinyl ether and methyl methacrylate. The ammonium and phosphonium salts of the linear copolymer were then synthesized by reacting with triethylamine, triphenylphosphine and tributylphosphine.\(^7\)

Quaternary ammonium and phosphonium salts were immobilized on chloroacetylated poly(glycidyl methacrylate) homopolymer,\(^8\) as well as on a modified copolymer of glycidyl methacrylate (GMA) and 2-hydroxyethyl methacrylate.\(^9\)

Copolymer beads which have quaternary ammonium groups were prepared by Nonaka et al (1994).\(^10\) The copolymer GMA -1,4-divinylbenzene copolymer beads was reacted with hydrogen chloride to prepare the halogen containing copolymer, which was eventually treated with different amines, including triethylamine, \(N, N\)-dimethyloctylamine, \(N,N\)-dimethyldecylamine and \(N,N\)-dimethylhexadecylamine. The quaternary ammonium groups in the resin were found to have an antimicrobial activity.

Punyani et al (2006)\(^11\) reported the synthesis of iodine containing quaternary amine methacrylate copolymers. A two-step reaction was used to prepare the monomer, with the first involving the reaction of ethylene glycol dimethacrylate with piperazine, followed by a second step in which the synthesized monomer was quaternized by reacting with 1-
iodooctane. The quaternized monomer was copolymerized with 2-hydroxyethyl methacrylate by free radical polymerization. Copolymers were found to have antimicrobial properties.

Copolymers of (meth) acrylic monomers represent the largest group of nitrogen containing polycations. Copolymerization is an appropriate method by which the charge density, a key parameter for polycations can be controlled. Apart from that, copolymerization provides a method for allowing certain monomers which might be unreactive in homopolymerization to polymerize. One more advantage for the use of copolymerization is that an additional functionality can be introduced in the polymeric material.3

1.3 Antimicrobial Agents

“Materials having the ability of killing pathogenic organisms are categorized as antimicrobial agents”12. The biomedical community is worried about the contamination coming from microorganism and the resistance of some of its strains to the currently available antibiotics due to its mutation. This constitutes a major challenge in combating infections diseases.13 Antibacterial agent plays a crucial role in the preservation of different products such as food, cosmetic and medical. The most common antimicrobial agent can be classified in four broad categories14; a) the electrophilic agents contain organic biocide, for example formaldehyde and isothiazolones and inorganic ions, for example, copper, silver and mercury; b) the oxidants, for example peroxide and chlorine; c) cationic active biocide, for example, chlorhexidine and quaternary ammonium compounds (QACs)15 d) alcohols, for example, phenoxyethanol. However, the small molecules have two main disadvantages; its short-lived antimicrobial activity together with its toxic waste causing environmental pollution which limit its use.16 On the other hand, the polymeric antimicrobial agents have many advantages including its chemical stability and not penetrating the skin.17,18 Hence, the use of biocide polymers will be of great benefit towards a healthier living.

1.3.1 Cationically Charged Biocides

The discovery of the capability of charged molecules and cationic charges in annihilating colonies of bacteria when it these are attached to surfaces paved the way for employing polymer chemistry and technology to enter the field of fighting microorganism. The biocidal cationic polymer being positively charged molecule will have the ability to attract
the negatively charged bacteria cell membrane by electrostatic attraction. This action leads to a disruption of the bacteria cell membrane and eventually its death. 19

The use of polymeric antimicrobial agents offers an attractive alternative to the currently used antimicrobial agents and overcoming the drawbacks of using it mentioned earlier in regards polluting the environment and the short-lived action. In addition to that the use of antimicrobial polymeric material have the advantage of being non-volatile and chemically stable and may contribute to increasing the selectivity and efficiency of the antimicrobial agents. 7,9,20 Hence it is no wonder that the area of research in polymeric materials with antimicrobial properties is gaining considerable interest on an academic and industrial level.

1.4 Solution interactions

The interaction between biocidally active surfactant (DDAC) and 3 different types of polyelectrolytes (homo-polymer, block and statistical copolymers) will be explored in this research.

1.4.1 Surfactants

Surfactants are amphiphilic molecules that contain a hydrophilic head-group and a hydrophobic tail. 21 (Fig.1.2).

![Figure 1.2. Schematic diagram of a surfactant molecule.](image)

In QAC surfactants the quaternary ammonium nitrogen is the hydrophilic head-group while the alkyl/aryl groups form the hydrophobic tail. This amphiphilic structure makes surfactants highly surface active, this feature leads to an increase in the absorbance to the negatively charged outer membrane of bacteria cell. So, these surfactants are used as antimicrobial materials such as alkyl trimethyl ammonium chloride, dialkyl dimethyl ammonium chloride and alkylidimethylbenzyl ammonium chloride. 21 The chemical structures of alkyltrimethyl ammonium, alkylidimethylbenzyl ammonium and dialkylidimethyl ammonium, (R= alkyl group), are given in figure 1.3.
Surfactants are categorized according to the head group charge into three types; 1/non-ionic surfactants; the head groups of this type do not contain electrostatic charge. 2/ionic surfactant; the head groups of this type have a charge, a negative charge in the anionic surfactant or a positive charge in the cationic surfactant) and 3/ Zwitterionic surfactants, which contain both types of charges within the head groups.\(^{22,23}\)

1.4.2 Mixtures of polyelectrolytes and ionic surfactant

Recently studying the solutions behaviour of aqueous solution mixtures of polymers and surfactants has had a significant interest because of their widespread applications and relative complex behaviour. Many of these mixtures undergo isothermal phase separation, which have importance in different application. For example, in water purification to remove surfactant contaminations.\(^{24}\)

Kalwarczyk. E. et al\(^{24}\), have investigated four different types of mixtures; a) anionic polyelectrolytes and anionic surfactant) cationic polyelectrolytes and cationic surfactant, c) cationic polyelectrolytes and non-ionic surfactant) and e) anionic polyelectrolytes and non-ionic surfactant. They found that phase separation can be induced by two strategies; 1) by addition of like charge ionic polyelectrolytes and surfactant, 2) by addition of inorganic salt to non-ionic water-soluble polymers. Phase separation in ternary mixtures of water/surfactant/polymer is driven by different force; adding water-soluble polymers to the mixture of water and surfactant induce attractive interaction between micelles, which considered as the depletion interaction which caused by entropy changes.\(^{24}\)

Entropic depletion interaction prevents polymers from getting too close to the micelles. Geometric constants keep a certain distance between the centre mass of the polymer coil to the micelle. This distance is approximately \(2(a+R_g)\), where; \(a\)=radius of the micelle and \(R_g =\)
radius of gyration of the polymer. Polyelectrolyte molecules are hampered from entering the region between the micelles if these are close enough to each other, hence the polyelectrolyte is prevented from separating the micelles and the region between them is said to be depleted of polymer. Outside the depletion domain, the polyelectrolytes surrounding the micelles induce an osmotic pressure that brings the micelles together and eventually causing phase separation within the solution mixtures.\(^{25,26}\)

Noor (2013) et al.\(^{27}\) investigated the interaction in aqueous solutions between ionic surfactant; cationic (CTAB) and anionic (SDS) and non-ionic di-block polymers (E79B34). From this study it is found that mixing polymer with surfactant induces aggregation of surfactant onto the polymer chain. Micelle starts to form by increasing the surfactants concentrations (CAC) which falls below the critical micelle concentration (CMC) of the surfactant.

The size of polymer/surfactant mixed micelle increase due to incorporation of surfactant (SDS) and block copolymer to the mixed micelle, while in the cationic surfactant (CTAB) they found the opposite i.e. reduction in the mixed micelle size, because of solubilisation of the surfactant molecules in the core of the copolymer. Results obtained from a study conducted by Bhattacharya, P. and Chakravorti, S., were found to be in agreement to that of Noor et al (2013). Their study demonstrated the effect of mixing three types of surfactants; 1) cationic; cetyltrimethyl ammonium bromide (CTAB) 2) anionic; sodium dodecyl sulphate (SDS) and 3) non-ionic polyethylene glycol sorbitan monolaurate (Tween-20) with an amphiphilic diblock polymer; polyethylene-b-polyethylene glycol (PE-b-PEG) in copolymers’ micelle properties. They concluded that the addition of the cationic and non-ionic surfactant leads to the establishment of a new water tight screened-off region of polymer micelle, while adding anionic surfactant leads to interruption of micelle process. These results were obtained by dynamic light scattering DLS and the steady state fluorescence anisotropy.\(^{28}\)

### 1.5 Designing Antibacterial Polymeric Material

Three significant factors must be taken into account to design and improve the antimicrobial agents; understanding the outer membrane of bacteria, understanding antibacterial mechanism of cationic polymers and studying the key factors that relate chemical and physical structure of polymers and the efficacy of the antibacterial agent.\(^{29}\)
1.5.1 Controlling polymer structures towards effective antibacterial polymers

F. Palermo and K. Kurdoa reported that synthetic polymers are becoming increasingly used in various applications due to researchers’ ability to tailor their chemical and physical properties.\(^{30}\)

The major part of any antimicrobial polymer is designed based on the structural features of the outer membrane of the bacteria cell.\(^{31}\) Hence, it is essential to provide a brief description of the two types of bacteria cell wall, which will become relevant later on in the review. QASs with various structures have been developed, leading to a significant improvement in cationic biocides via the direct polymerization of monomers bearing QAS groups or covalently incorporating QAS compounds into ordinary synthetic or natural polymers.\(^{32}\) The most common characteristic in effective antimicrobial materials is the existence of an amphiphilic structure.\(^{29}\) QAS compounds are known as membrane-active agents i.e. they interact with the cytoplasmic membrane of bacteria.\(^{29,33}\)

To induce amphiphilicity in the polymers made in this research, 2-(methacryloyloxy) ethyl] trimethyl ammonium chloride (MADQUAT) was chosen to provide the hydrophilic segment, and methyl methacrylate (MMA) to provide the hydrophobicity. Hence, two routes of synthesis have been used; one-pot method employing modified conventional free-radical polymerization and reversible addition fragmentation chain transfer (RAFT) polymerization.

1.5.1.1. Free radical polymerization

Free radical polymerization (FRP) is one type of chain growth polymerization in which the polymer growth occurs by successive addition of monomer units. The monomer unit is converted into an active propagating radical by the homolytic fission of its double bond. As with any other free radical mechanism FRP proceeds via four distinct processes as shown in the scheme below (scheme 1.3).
Chapter 1. Introduction and background

Scheme 1.3. Free radical polymerization mechanism.\[^{34}\]

FRP has many benefits and is widely used method for the preparation of polymers. However, some limitation is imposed in regards to the degree of control over the polymer structure and its molecular distribution due to the rapid chain growth plus the existence of the fast irreversible termination.\[^{35}\] Apart from that FRP is not a practical method for producing polymers with complex structure or block copolymers.

Figure 1.4. A comparison of conventional Free Radical polymerization(FRP) and Controlled Living polymerization(CRP).\[^{34}\]
1.5.1.2 Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization

Reversible addition-fragmentation chain transfer (RAFT) which was discovered in 1998 by a team of researchers working at the Commonwealth Industrial Research Organization (CSIRO)\textsuperscript{36} caused a revolution in the field of controlled free radical polymerization. Controlled free radical polymerization (CRP) is the term used to describe nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), together with RAFT. The importance and significance of CRP when compared with other conventional free radical polymerization techniques is that it allows considerable control over the molecular weight of the polymeric material produced as well as its molecular weight distribution (narrow polydispersities), the ability to construct well-defined and complex macromolecular architectures. Macromolecular architectures include block and graft copolymers and star and branched polymers.\textsuperscript{37,38} Conventional free radical polymerization do not have the ability to deliver such unique polymer characteristics due to its uncontrolled nature. Controlling the molecular weight and the architecture of the polymer is of paramount importance as this defines the properties of the produced polymer and its suitability for use in a desired field.

RAFT process has been shown to be tolerant to a wide range of monomers of different functionalities, solvents including aqueous solutions, level of impurities and can be carried out over a wide range of temperatures. This makes it one of the most versatile methods of CRP.

1.5.1.3 Mechanism of RAFT Polymerization

RAFT is a type of CRP involving a conventional radical polymerization that is mediated by a RAFT agent. The most efficient of these RAFT agents are thiocarbonylthio compounds.\textsuperscript{39} A number of steps are involved in RAFT polymerization namely: initiation, pre-equilibrium, re-initiation, main equilibrium, propagation and termination.\textsuperscript{35, 40-42}
In the initiation step a polymeric radical is formed with n monomer units (P\textsubscript{n}). These are the propagating radicals, which react with the RAFT agent to form RAFT adduct radical (intermediate radical), and a dynamic equilibrium is established in which this intermediate may undergo fragmentation reaction to yield either the starting material or a polymeric RAFT agent (S=C(Z)S\textsubscript{-}P\textsubscript{n}) termed as dormant chains, and the reinitiating R free radicals. The free radical produced here from the leaving R group can attack monomers to form another polymeric radical P\textsubscript{m} that is involved in the main chain equilibrium step. Most chains are dormant at the end of RAFT polymerization which contains the dithiocarbonyl end-group, that means the polymer is living. The proportion of the dead polymer chains in this mechanism is usually less than 10 %.

### 1.5.1.4 Chain Transfer Agents CTA (RAFT Agents)

The RAFT process involves performing a polymerization in the presence of a chain transfer reagent (RAFT reagent) capable to react by reversible addition-fragmentation chain transfer as shown in the mechanism above. Careful selection of CTA is a pre-requisite for successful implementation of the RAFT polymerization process. Compounds used as CTAs have to
fulfill certain stringent requirements in order to be effective in the RAFT process. The CTA used has (i) to ensure relatively fast rates of addition and fragmentation when compared to the rate of propagation in order to consume rapidly the initial CTA and the equilibration of the dormant and active species and (ii) the produced R radical must have the ability for reinitiating the polymerization to ensure the continuity of the chain process. Thiocarbonlythio compounds, whose general structure is given below, are usually used as RAFT agents in the polymerization process.

![Scheme 1.5](image)

**Scheme 1.5.** Structural features of thiocarbonlythio RAFT agent and intermediate formed on radical addition.

The nature of Z and R substituents determine the effectiveness of the agent. Z affects the stability of the intermediate radical and promotes addition to \( C = S \) bond, while R is a good free radical leaving group having the ability of reinitiating polymerization. Different RAFT agents are chosen for the different monomers and conditions for various polymerizations. Thus dithiobenzoates and trithiocarbonates are used for controlling polymerizations of conjugated monomers such as styrenes, acrylates and methacrylates, all of which are categorized as activated monomers, while Xanthates are found to be more suitable as RAFT agents for nonconjugated monomers such as vinyl acetate and N-vinyl pyrrolidone, that are regarded as less activated monomers.

**1.5.2 Type of Bacteria**

The gram staining standard procedure is used to distinguish between the two types of bacteria's cell walls. This procedure involves using a dye, usually crystal violet (4-[4-dimethylaminophenyl]-phenyl-methyl]-N,N-dimethyl-aniline), to dye the bacteria. This is
followed by alcohol extraction. If the bacteria cell retains the dye, it is categorized as gram-positive, otherwise it would be gram-negative, see some example of bacteria strains, Table 1.1. The difference observed in the interaction of the dye with the two types of bacteria cells is due to the difference in the chemical identity or structure of the two cell walls, see figure 1.5. The gram-positive cell wall which is about 30 nm thick is positioned outside the cell membrane. The various molecules that make up the cell wall plays a crucial role in the survival of the bacteria cell. Polysaccharide-peptide complex (peptidoglycan) is the main component of the bacteria cell wall and it contains polysaccharides, polypeptides and teichoic acids. These molecules are bonded covalently to the peptidoglycan skeleton network. Teichoic acid is an anionic polymer comprised of glycerol or ribitol derivatives chains connected together via phosphodiester bridges. These molecules are responsible for both the function and integrity of the cell membrane as they can bind with specific cations to achieve that. Gram-negative bacteria has a different structure, hence the different properties. Its cell wall is 3-8 nm thick and has less of peptidoglycan and has an outer membrane outside the peptidoglycan layer. The outer membrane comprises of fatty acid residues present within the interior of lipid layer, while polysaccharides are exposed at the membrane surface.

In contrast to the gram-positive bacteria where the one lipid membrane is covered by a thick network of cross-linked peptidoglycan, the outer membrane of gram-negative bacteria has a high content of LPS. Due to the complexity of the outer membrane of gram-negative bacteria, it is more resistant to antimicrobial agents than that of outer membrane of the gram-positive bacteria. The presence of porin channels in the structure of their outer membrane hinders the penetration of antimicrobial material.

![Figure 1.5. Cell wall structure of gram-positive and gram-negative bacteria.](image)
Table 1.1 Examples of gram-positive and gram-negative bacteria

<table>
<thead>
<tr>
<th>Gram-positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

1.5.3 Mechanism of Antimicrobial Action

Various polycations have been reported to possess antimicrobial properties and it was further suggested that the mechanism of action involves the interaction with and disruption of the integrity of the cell membrane.\(^{17,49,50}\) The mechanism is still not understood on a detailed molecular level, however it is believed that the antimicrobial mechanism does not include specific receptor-mediated interactions.\(^{51}\) The following sequence of elementary steps have been suggested to be associated with mode of the lethal action of the cationic biocides(Figure1.6).\(^{52,53}\)

![Figure 1.6. Explanation of the bactericidal mechanism against the gram-negative bacteria.\(^{54}\)](image)

- Adsorption of the positively charged biocides onto the negatively charged bacterial membrane
Penetration of the bacterial cell wall
- Translocation and adsorption followed by binding to cytoplasm membrane
- Disruption of the integrity of the cytoplasm membrane and its disintegration
- Leakage of the cytoplasmic contents including electrolytes such as phosphate and potassium ions as well as the release of nucleic materials such as DNA and RNA
- Death of the cell

1.5.4 Factors Affecting Antimicrobial Activity

1.5.4.1 Molecular weight effect

Polymers in general have a large range of molecular weights and polydispersities and that could be the reason behind the observed diversity of biological function. The average molecular weight of a polymer, as well as its molecular weight distribution play an important role in defining its biological functions and in particular its toxicity.\(^ {31} \)

Polycationic biocides with pendant phosphonium salts was synthesized by Ikeda et al\(^ {52} \) and compared its antimicrobial activity with its corresponding monomers. The conclusion of their work was that the activity as antibacterial increases as the molecular weight increases.\(^ {52,55} \) The antimicrobial action was found to reach an optimum within a molecular weight between \(5 \times 10^4\) and \(1.2 \times 10^5\) Da. Below \(5 \times 10^4\) Da the antimicrobial activity was observed to increase with the increase in the molecular weight. However, when the molecular weight exceeded \(1.2 \times 10^5\), the antimicrobial activity was seen to decrease. This relationship was explained on the basis of the permeability through the bacterial cell wall. The biocidal action of poly-(triaalkylyvinylbenzlammonium chloride) against \textit{S.aureus} was investigated and was observed to increase monotonically with molecular weight up to \(7.7 \times 10^4\) Da.\(^ {47} \)

The effect of the molecular weight of poly (tributyl- 4-vinylbenzyl phosphonium chloride on its antimicrobial activity against \textit{S.aureus} was investigated by Kanazawa et al.\(^ {56} \) The antibacterial activity was found to increase with the increase of the molecular weight of the polymer from \(1.6 \times 10^4\) to \(9.4 \times 10^4\) Da.
A parabolic dependence of antimicrobial activity on the molecular weight was reported by two independent studies. However, the antimicrobial activity of copolymers of vinylmine, methyl acrylate and N-vinyl pyrrolidone with pendent quaternary ammonium groups were found not to be dependent on the molecular weight of the polymers investigated. In trying to find out an explanation for this discrepancy in the results, one has to consider the type of bacteria used in the investigations. The most commonly used *S.aureus* bacteria is a Gram-positive type, characterized by loose cell wall hence diffusion across it seems to be relatively easy for molecules with a molecular weights ranging from $5 \times 10^4$ to $9 \times 10^4$. However, this is not the case for *E.coli*, Gram-negative bacteria, where the outer membrane of cell wall constitutes an additional physical barrier for foreign molecules.

### 1.5.4.2 Counterion effect

Homopolymers of vinylamine methyl acrylate with pendent quaternary ammonium groups were synthesized with different counteranions by Panarin et al and their antibacterial activity were examined. The nature of the counteranions, namely chloride, bromide and iodide, were found to have no effect on antimicrobial activities. In contrast, investigating the dependence of the antibacterial activity of poly (tributyl-4-vinylbenzyl phosphonium chloride) with different counteranions, see figure 1.7, against *S.aureus* in silane demonstrated a strong counteranions effect on the antibacterial properties.

![Tributyl (4-vinylbenzyl) phosphonium salt monomer.](image)

The antibacterial activity was found to increase in the order of chloride > tetrafluoride> perchlorate> hexafluorophosphate, which is in accordance with the solubility products of the polymers. Previously it was reported that antibacterial activity vanishes when polycations are cross-linked or insolubilized.
1.5.4.3 Length of the alkyl hydrophobic chain

The effect of the length of the alkyl hydrophobic chain on the antimicrobial activity of a cationic biocide has been investigated by many researchers with the aim of determining the most potent biocides. Thus Gilbert et al\textsuperscript{61} investigated the antibacterial activity of a number of alkyl trimethylammonium bromides against \textit{S. aureus}, \textit{Saccharomyces cerevisiae}, and \textit{Pseudomonas aeruginosa}. A parabolic relationship between the antibacterial activity and the n-alkyl chain length was found. Highest antibacterial activity was found for C\textsubscript{10} and C\textsubscript{12}. Chen et al\textsuperscript{6} reported a parabolic relationship between the antimicrobial activity of quaternized poly(propylene imine) dendrimers and the length of the hydrophobic chain, with dendrimer biocides having C\textsubscript{10} hydrophobes showing the highest potency. The presence of the parabolic relationship between the antimicrobial activity and the alkyl chain length has been attributed (1) the existence of two binding sites on the surface, whose affinities to the short and long alkyl substituents are different or (2) different aggregational behaviour for long and short hydrophobes.\textsuperscript{6}

In a separate study Ikeda et al\textsuperscript{62} investigated poly(trialkylvinyl-benzylammonium chloride) concluded that the antibacterial activity was the highest for the longest chain C\textsubscript{12} that was studied. Nonaka et al\textsuperscript{63,64} investigated the antimicrobial activity of methacryloyl-ethyl trialkyl phosphonium chlorides/N-isopropylacrylamide copolymers. The antimicrobial activity of the copolymers against \textit{E. coli} was found to increase with increasing the alkyl chain length attached to the phosphonium groups in the copolymer. Nakagawa et al\textsuperscript{65} investigated the effect of the alkyl chain length of an immobilized quaternary ammonium salts onto glass beads on the antimicrobial activity of the polymer against \textit{E. coli}. Glass beads having C\textsubscript{2}-C\textsubscript{4} alkyl chains exhibited lower activity, while those containing C\textsubscript{8}-C\textsubscript{18} demonstrated higher antimicrobial activity. Glass beads having C\textsubscript{10} alkyl chains proved to be the most potent.

According to Klibanov\textsuperscript{66} the length of alkyl tails for alkylated quaternized PVP polymers should not be excessive in order to avoid a massive decrease in the biocidal efficacy of the immobilized polymer. This was seen to be mainly due to the stronger hydrophobic interactions and aggregation of polymer molecules that takes place with large alkyl spacers on the surface.\textsuperscript{13,67} In contrast to the previous findings Panarin and co-workers\textsuperscript{58} reported an absence of relationship between the length of the alkyl chain and the antimicrobial activity.
of cationic quaternary copolymers based on methyl acrylate, vinylamine and N-vinyl pyrrolidone having pendant quaternary ammonium groups. However, the authors found an increase of several orders of magnitude in activity of the monomers as the alkyl chain length was increased from $C_1$ to $C_{16}$.

Variation in the length of the alkyl chains brings with it changes in certain characteristic features and parameters of the polymer under investigation. According to Timofeeva et al (2011), changing the length of the alkyl chain might boost the adsorption/absorption ability of the polymer as well as its lipophilicity. This might lead to varying the amphiphilic balance of a polymer hence varying the efficacy of the biocidal polymer against different microbes in a different manner.

### 1.5.4.4 Amphiphilic balance and charge density effect

Many studies have reported the ability of charged molecules in solution to kill bacteria. It has been further realized that positively charged groups attached to polymers in cationic polymer materials including quaternary ammonium or phosphonium or sulfonium are capable of killing microorganisms. Apart from the chemical identity of the charged the spatial arrangement of positive charges within the amphiphilic polymers, density and number are key parameters in determining the antimicrobial activity of the biocidal polymer. Kugler et al (2005) reported that a minimum charge density is required where bacterial death occurs rapidly when its adsorbed on the substrates functionalized with cationic quaternary ammonium groups. It was further reported that the threshold is dependent on the type of bacteria investigated. A difference by a factor of ten was observed between *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus epidermidis* (Gram-positive bacteria). It is well known that the balance between the cationic charge and hydrophobicity, sometimes referred to as ‘cionic-hydrophobic’ or ‘hydrophilic-lipophilic balance’ or amphiphilic balance, play an important role in the antimicrobial activity of the biocidal polymer. One has to realize that highly cationic polymeric materials while being able to attach to the bacterial cell which is negatively charged efficiently due to the strong electrostatic attraction, their ability to insert into the hydrophobic core of the membranes is limited and that in turn restricts their activity. Biocidal polymeric materials with excessive hydrophobicity are toxic to both human and bacterial cells since its hydrophobic nature enhances binding and translocation or penetration into human cell membranes even in the absence of electrostatic attraction. However when an optimum ratio
Chapter 1. Introduction and background

is reached between the cationic and hydrophobic residues in the antimicrobial polymeric material, the biocidal polymer can selectively kill the microorganism without causing any harm to the human cells (Fig. 1.8).\textsuperscript{73,74}

Optimization of the amphiphilic balance in the synthetic polymers have been achieved by employing three different methods (Palmero and Kuroda 2010):\textsuperscript{54} (1) modifying the hydrophobic-cationic side chains in random copolymer, (2) varying the hydrophobic moieties in random copolymers or amphiphilic homopolymers, and (3) reduction of the hydrophobicity of polymer disinfectants by conjugation with neutral, hydrophilic electrical groups like polyethylene glycol (PEG).

![Amphiphilic Balance](image)

**Figure 1.8.** Optimization of balance between cationic and hydrophobic group.\textsuperscript{54}

1.6 Antimicrobial Surfaces

Bacteria growing on surfaces often form biofilms, the formation of these biofilms consist of two main steps; adhesion of bacterial cells to substrate surface followed by an initial reversible interaction between the bacteria membrane and the substrate. This is followed by a second step that involves specific and nonspecific interaction between adhesion proteins expressed on the bacteria surface structures and the binding molecules on the substrate surface.\textsuperscript{75}

Biofilms cause a lot of serious infections because these biofilms have complex adhesion mechanisms that change with bacteria types, and the genetic mutation that offers protection against environmental stresses.\textsuperscript{76} Furthermore, these biofilms are very efficient at colonizing medical devices (e.g., catheters, implants, etc.) causing failure of implants.\textsuperscript{77}

All above reasons lead to urgent demand to improve antimicrobial surfaces and define the essential challenges connected with controlling the adhesion of bacteria to surfaces and
identification of key material features that can be employed to inhibit or promote bacterial adhesion and biofilm formation.

**Figure 1.9.** General principles of antimicrobial surfaces.

### 1.6.1 The Main Strategies for Antibacterial Surfaces Design

Different techniques have been used to create antimicrobial surfaces by attaching polymers to surfaces such as Contact Kill (Chemical grafting techniques), and Polyelectrolyte multilayers (PEMs) and Adhesion resistance which will be explained in more details, see figure 1.9 which shows the general principles of antimicrobial surfaces.

#### 1.6.1.1 Contact Kill (Chemical grafting)

These types of antimicrobial surfaces are permanently modified via attaching the biocide material to the substrate by elaborate techniques (covalent attaching). For instance, Polyethylenimine (PEI) was attaches to amino-glass via 1,4-dibromobutane, followed by methylation and alkylation with long chain alkylhalides. The drawbacks of this elaborate techniques; they can be applied only in the laboratory and very expensive to create.

The main advantage of using this strategy is that the biocide is never release to the environment so it can be considered as environmentally friendly. Park et al have found an alternative method which helps overcome the disadvantage of multistep method where they hydrophobically anchor the biocide to the surface by a single-step painting-like coating procedures, i.e., Slides of polyethylene or Glass were immersed into organic solutions of optimally hydrophobic N-alkyl-polyethylenimine with varying the molecular weight and the hydrophobicity of the alkyl moiety. This was followed by solvent evaporation. The produced polycation-coated slides were found to have the ability to kill on contact bacterial
cells.\textsuperscript{80}

1.6.1.2 Polyelectrolyte Multilayers (PEMs)

The possibility of employing layer-by-layer assembly of polyelectrolyte multi-layers as antimicrobial coating was suggested by Lichter et al.\textsuperscript{76} The authors indicated that the potential of using the PEM assembly lies in the fact that it can accommodate a wide range of polyelectrolytes that encompass silver, QACs, peptides and chitosan, hence paving the way for a wide range of applications.\textsuperscript{81,82,83,84} PEMs layer-by-layer or coating method has been developed to include coating of a substrate several times, with a cationic polyelectrolyte first followed by anionic polyelectrolyte coating (see figure 1.10). These polyelectrolyte layers function as biocides by slowly releasing the active component due to changes in temperature or pH.\textsuperscript{76}

![Figure 1.10. Schematic of layer-by-layer deposition used to assemble polyelectrolyte multilayer films on support substrates.\textsuperscript{76}]

1.6.1.3 Adhesion Resistance

The main aim of design these surfaces is to avoid the bacterial adhesion on the material surfaces by preventing the early stages of biofilm formation. For example, poly(ethylene glycol, PEG) grafted into surfaces leads to form a highly hydrated layers that have a great effect in prevention E.Coli cells’ adsorption.\textsuperscript{85}

1.7 Tannins and Tannic acid

Plant extracts, employed in processing animal skin into leather was called tannins since ancient times.\textsuperscript{86} It was not till 1962 that their chemical nature was revealed as polyphenolic compounds.\textsuperscript{87} Vegetable tannins, which are a group of phenolic compounds commonly referred to as tannic acid. Some of these tannins are water soluble having molecular weights ranging from 500 to nearly 3000, while others with molecular weights ranging from 3000 to
over 30000 are insoluble ones. Tannins can form complexes with carbohydrates and proteins.88

Two types of tannins are known, namely hydrolysable and condensed tannins. Hydrolysable tannins are polyesters formed from an organic acid and sugar moiety mostly glucose can undergo hydrolytic cleavage of their ester bond into its sugar and acid moiety when treated with diluted acid. Based on the acid produced when these compounds are hydrolysed they are called gallotannins if the acid produced is gallic acid they are called gallotannins, and ellagitannins if ellagic acid is formed.

1.7.1 Antimicrobial and antiviral properties of tannins

Food poisoning caused by naturally occurring pathogens constitutes a major health problem associated with drainage of financial resources. Antimicrobial agents can be employed to prevent the growth of pathogens in food. Tannins present in plant extracts were found to exhibit bactericidal effects on many types of bacteria including *Streptococcus mutans, Strep. sobrinus*, *Strep. Salivarius* and *Actinomyces viscosi*.89 Inhibition of the growth of several food-borne bacteria by some plant extracts has been demonstrated by Chung et. al.90 (1990) also.

Many mechanisms have been suggested through which tannins operate to affect bacterial growth including: dispossession of the substrates necessary for microbial growth, inhibition of the extracellular microbial enzymes or interfering with microbial metabolism through oxidative phosphorylation.91 Deprivation of bacteria from essential metal ions present in its environment and required for its growth by complexation with tannins, is also one of the envisaged mechanisms by which tannins could affect the microbial growth.92, 93

Many tannins especially of the condensed type like proanthocyanidins present in berries and grapes have bacteriostatic effects due to their anti-adhesion properties. It has been demonstrated that consumption of cranberry products prevents the adhesion of *Escherichia coli* strains to the uroepithelium94,95, hence interfering with this rather essential initial step for the propagation and progress of the infection process.96 Howell et. al.97 investigated the anti-adhesion activity of proanthocyanidins present in different juices including cranberry, grapes and apple. The authors concluded that only cranberry juice with A-type linkages in proanthocyanidins is the one associated with anti-adhesion activity. Antiviral activity has been also reported for many of the tannins, both hydrolysable as well as the condensed
ones. Radiolabelled studies demonstrated that the antiviral effect for both types of tannins is due to the inhibition of virus absorption.\textsuperscript{98} Several hydrolysable tannins were found to have an anti-human immunodeficiency virus activity (anti-HIV). It is believed that inhibition of the virus absorptions is due to the binding of the tannins to the viral envelope inhibiting viral adherence and penetration of the plasma membrane.\textsuperscript{99}

### 1.7.2 Tannic acid and polymer mixtures

The presence of multifunctional polar groups in natural polyphenolic compounds together with their amphiphatic nature allow them to form complexes with a wide variety of macromolecules via both intra- and intermolecular H-bonding interactions beside hydrophobic and cation-\(\pi\) interactions.\textsuperscript{92} Generation of novel colloidal structures has been attempted by exploiting the interactions of methylcellulose and with the green tea polyphenol epigallocatechin gallate for encapsulation applications.\textsuperscript{100, 101} Soluble complexes are formed through the interactions of polyphenols and macromolecules, which through self-associating can grow to gradually into large particles leading eventually to its sedimentation.\textsuperscript{100} Modification of polymer functionalities including interfacial adsorption and bulk gelling through the interactions between the polyphenols and polymers is of great interest and importance for polymer and material scientists.

Mixtures of polymers with tannic acid have been reported in few studies. It mixes with polyethylene glycol (PEG),\textsuperscript{102} polyvinyl pyrrolidone(PVP),\textsuperscript{103} methylcellulose\textsuperscript{104} and a block copolymer ; poly(ethylene oxide)-block-poly(2-hydroxylethyl methacrylate) (PEO-b-PHEMA).\textsuperscript{105} The study of cationic polymers and Tannic Acid mixtures was firstly conducted by Shutava, T. et. al.(2005), where they used tannins as negatively charged structural blocks for LbL assembly in alternation with two different cationic polymers; strong poly (dimethyl diallylamide) (PDDA) and weak poly(allylamine) (PAH) to design polyelectrolyte microcapsule with adjustable drug loading-release profile.\textsuperscript{106}

### 1.8 References


31. L. Timofeeva and N. Kleshcheva, *Applied Microbiology and Biotechnology*, 2011, **89**.
50. Y. Endo, T. Tani and M. Kodama, *Applied and Environmental Microbiology*, 1987, **53**.
52. T. Ikeda, H. Yamaguchi and S. Tazuke, *Antimicrobial Agents and Chemotherapy*, 1984, **26**.
55. A. Kanazawa, T. Ikeda and T. Endo, *Journal of Polymer Science Part a-Polymer Chemistry*, 1993, **31**.
56. A. Kanazawa, T. Ikeda and T. Endo, *Journal of Polymer Science Part a-Polymer Chemistry*, 1993, **31**.
Chapter 1. Introduction and background


Chapter 2
Experimental
Chapter 2: Experimental

This chapter provides a detailed overview of the reagents, experimental techniques, and general procedures employed during the course of this research.

2.1 Reagents

Monomers: [2-(methacryloyloxy) ethyl] trimethylammoniumchloride (MADQUAT), 80 wt% in H₂O; poly(ethylene glycol) methyl ether methacrylate, Mn = 360 g·mol⁻¹ (PEGMA); methyl methacrylate (MMA) containing ≤30 ppm MEHQ as inhibitor, 99%, all from Sigma-Aldrich. The structures of these reagents are shown in Tables 2.1, 2.2 and 2.3.

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Chemical name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical structure" /></td>
<td>[2-(methacryloyloxy) ethyl] trimethyl ammonium chloride</td>
<td>MADQUAT</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical structure" /></td>
<td>poly(ethylene glycol) methyl ether methacrylate</td>
<td>PEGMA</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical structure" /></td>
<td>methyl methacrylate</td>
<td>MMA</td>
</tr>
</tbody>
</table>

Table 2.1. Names and chemical structures of monomers used

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Chemical name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4" alt="Chemical structure" /></td>
<td>2,2'-azobis(2-methyl butyronitrile)</td>
<td>VAZO-67</td>
</tr>
<tr>
<td><img src="image5" alt="Chemical structure" /></td>
<td>Cumyl dithiobenzoate, or 2-phenyl-2-propyl benzodithioate</td>
<td>RAFT agent (CDB)</td>
</tr>
</tbody>
</table>

Table 2.2. Names and chemical structures of RAFT agent and Free radicle source used.
**Reagents:** Free radical initiator: 2,2'-azobis(2-methylbutyronitrile), VAZO-67 (DuPont). RAFT agent: 2-phenyl-2-propyl benzodithioate (99% (HPLC), 731269, Sigma-Aldrich). 1-thioglycerol (HSCH$_2$CH(OH)CH$_2$OH, TG, 97%, Sigma Aldrich). Solvents: ethanol $\geq$ 99.8%, n-Hexane 99%, deuteron chloroform (CDCL$_3$), deuterium oxide (D$_2$O), all of standard laboratory reagent grade from Sigma-Aldrich. Surfactant: didecyl(dimethyl)ammonium chloride (DDAC) (Trade name: Lonza Bardac 2240), obtained from Lonza as a 40 wt% solution in water. Tannic acid (TA) (Source: Chinese natural gall nuts, Sigma Aldrich). All chemicals were used as received without further purification. Table 2.2 shows the chemical structures of DDAC and TA.

**Table 2.3.** Chemical structures of didecyl(dimethyl)ammonium chloride (DDAC) and tannic acid (TA)

<table>
<thead>
<tr>
<th>Didecyl(dimethyl)ammonium chloride</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H$_2$C</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>R=C$<em>{10}$H$</em>{21}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tannic acid (gallotannin/tannin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{76}$H$</em>{52}$O$_{46}$</td>
</tr>
</tbody>
</table>

**2.2 Phase separation procedures**

**2.2.1 Preparation of surfactant-polymer solutions**

Different stock solutions were prepared of both polymers (0.25, 0.5, 1, 1.5 and 3 wt%) and DDAC (7, 10, 15, 20 and 25 wt%). Millipore filtered water was used for all solutions. Fresh
solutions were used for all experiments in 10 ml polypropylene tubes. Samples were prepared at desired concentrations from the appropriate stock solutions, then they were stirred for 40-60 minutes with a magnetic stirrer. Phase separation was determined after 24-48 hours. The cationic surfactant DDAC was mixed with three types of cationic polymers: homo-polymer, statistical copolymers and di-block polymers (with different molar ratios of MADQUAT cationic moiety). Phase separation was induced depending on the concentration of both cationic polymers and cationic surfactant, and on polymer properties.

2.2.2 Determination of critical phase separation concentration

A series of 6 ml solutions were prepared as follows. A constant concentration (0.13, 0.25, 0.5, 0.75 and 1 wt%) of polyelectrolyte was mixed with different concentrations of surfactant at room temperature. The solutions were stirred for about 1 hour with a magnetic stirrer. Phase separation was determined after 24-48 hrs. The lowest concentration of surfactant that induced phase separation was determined as the critical phase separation concentration.

\(^1\)H NMR was conducted on both upper and lower phases to confirm that the upper phase contained the surfactant and that the lower phase contained the polymer.

2.3 Bacterial strains, media and growth conditions

Two types of bacterial strains were used in this study: gram-negative and gram-positive. They were supplied by the Central Manchester Foundation Trust (Clinical Sciences Building 2, Manchester, UK) and their references are listed in Table 2.4. Bacterial glycerol stocks were prepared in 80% glycerol (Fisher Scientific Ltd.) then frozen at -80 °C. Stock bacterial strains were cultured every 2-3 weeks on Luria-Bertani (LB) agar plates, and then incubated at 37 °C around 18 hours. The working culture plates were stored in a fridge at 4 °C. Overnight cultures were prepared by inoculating 10 mL LB broth (50 mL Falcon tube) with 5 colonies of bacteria from the working culture plates, then incubating at 37 °C for 16-18 hours with shaking at 200 rpm.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli, XLI-BLUE, a K-12 phage</td>
<td>Laboratory strain</td>
</tr>
<tr>
<td>Escherichia coli, U125544</td>
<td>Clinical isolate from urinary tract infection</td>
</tr>
<tr>
<td>Klebsiella pneumonia, Clinical isolate 1</td>
<td>Clinical isolate from urinary tract infection</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa, Clinical isolate 1</td>
<td>Clinical isolate from urinary tract infection</td>
</tr>
<tr>
<td>Staphylococcus aureus, ATCC 6538</td>
<td>Laboratory strain</td>
</tr>
</tbody>
</table>
All reagents used for preparing L.B broth and L.B agar are listed in Table 2.5.

**Table 2.5.** Reagents used for L.B broth and agar.

<table>
<thead>
<tr>
<th>L.B broth</th>
<th>10 g tryptone¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 g yeast extract¹</td>
</tr>
<tr>
<td></td>
<td>10 g NaCl²</td>
</tr>
<tr>
<td></td>
<td>made up to 1 L with distilled water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L.B agar</th>
<th>10 g tryptone¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 g yeast extract¹</td>
</tr>
<tr>
<td></td>
<td>10 g NaCl²</td>
</tr>
<tr>
<td></td>
<td>15 g agar³</td>
</tr>
<tr>
<td></td>
<td>made up to 1 L with distilled water</td>
</tr>
</tbody>
</table>

Material suppliers: ¹ Becton, Dickenson Ltd.; ² Fisher Scientific Ltd.; ³ Melford Laboratories Ltd.

### 2.4 Techniques

#### 2.4.1 Gel permeation chromatography (GPC)

The aqueous GPC technique was followed, using a Hi column (TSK-GEL 5000 and 6000) and an ERC 7515A refractive index detector, at a flow rate of 0.5 ml/min at 38-40 °C. Chromatograms were calibrated using polyethylene glycol standards. The samples were made up in 0.1 M citric acid (containing 0.02 % sodium azide) at 0.2 % w/v, with a drop of ethylene glycol added as a flow time marker. Samples were left overnight, then passed through a 0.45 µm filter prior to injection.

#### 2.4.2 Dynamic light scattering (DLS) and Zeta potential ¹,²

DLS is one of the techniques most commonly used to determine particle size and zeta potential. The light from a laser shone onto a solution undergoes Doppler shift because the particles are in Brownian motion. When the light hits the moving particles, they re-radiate it at different frequencies, the difference being related to the size of the particles. The autocorrelation function enables the determination of the diffusion coefficient. Once this has been determined, the Einstein-Stokes equation (Equation 2.1) can be used to determine the hydrodynamic radius of a particle.

$$R_H = \frac{kT}{6\pi\eta D}$$  \hspace{1cm} (2.1)

Where $R_H =$ hydrodynamic radius, $k =$ Boltzmann constant, $T =$ absolute temperature, $\eta =$ viscosity of solution, $D =$ diffusion coefficient.
Particle size and zeta potential were measured at 25 °C by dynamic light scattering, using a Zetasizer-Nano (Malvern Instruments) comprising a He-Ne laser, 632.8 nm, and a 173° back-scattering detector. Samples were placed in a disposable folded capillary cell (DTS1070), and the result is an average of at least three measurements, each consisting of 12-15 repetitions, and the average result was calculated as the final hydrodynamic diameters (D_h) and ζ-potential. using the CONTIN program to deconvolute the size distribution and zeta-potential. Polymers solutions with different concentrations were prepared in DI. water and filtered by using a 0.45 μm disposable filter.

2.4.3 Nuclear magnetic resonance (NMR) spectroscopy

A Bruker 400 MHz ¹H NMR spectrometer was used. Samples were dissolved in either CDCl₃ or D₂O and filtered by using a 0.45 μm disposable filter then transfer into the NMR tube.

2.4.4 Surface tension measurements

Using a White Electrical and Industrial Company Ltd. OS Torsion Balance, a 4 cm circumference platinum ring was submerged into a small dish containing the polymer solution under investigation. The ring was then slowly lifted from the polymer solution. The point at which the ring detached from the surface of the polymer solution into air was read and noted as the surface tension of the polymer solution. This was repeated until three consecutive concordant results were obtained. Precision was ± 0.5 mN/m.

2.4.5 Pyrene fluorescence spectroscopy

Fluorescence spectroscopy is a useful technique that has been used in the investigation of the physiochemical properties of polymers through analysing both the intensity and the features of the radiation emitted from it in the form of fluorescence.

When a molecule absorbs light energy from the UV-visible region characterized by a wavelength range of 200-900 nm, it gets to an exited state that is associated with a transition to a higher electronic state. However, the excited state does not last and the molecule comes back to its ground state emitting the energy it absorbed. The emitted light energy will be of specific wavelength. Usually some of the absorbed energy will be lost within the molecule to vibrational energy states or heat, hence, the emitted light energy will have a longer
wavelength than the one which caused the transition to the excited state. One of the very common and successful fluorescence probe is Pyrene molecule. This is because the relative intensities of the five bands in the vibrational fine structure of its fluorescence spectra is sensitive to both polarity and the chemical nature of its environment.\textsuperscript{3,4} Emission characteristics of Pyrene were observed to change on moving from a hydrophilic to a hydrophobic environment. The change in the fluorescent emission spectrum of pyrene is associated with a change in its conformational structure that affects its molecular electronic structure. The ratio of relative intensities of I (373 nm) and III (383 nm) peaks ($I_3/I_1$), of pyrene emission spectrum is shown in Figure 2.1.

In the absence of micelles, the fluorescence emission spectra of a dilute aqueous solution of amphiphilic polymer to which pyrene has been added, will be similar to that shown in fig. 2.1. As the concentration of the of the amphiphilic polymer solution increases the critical micelle concentration (CMC) is reached at one point above which micelles formation commences. Since the core of the micelle is hydrophobic, pyrene would reside in the core as it is an organic compound composed of hydrogen and carbon only. This results in a noticeable change in the $I_3/I_1$ peak ratio. This behaviour can be employed in determining the CMC of an amphiphilic polymer, when the variation in the $I_3/I_1$ ratio is plotted as a function of polymer concentration.

![Pyrene fluorescence with illustration of the first and third vibrational peaks.](image)

**Figure 2.1.** Pyrene fluorescence with illustration of the first and third vibrational peaks.

Pyrene solution preparation: 5 mg of pyrene was firstly dissolved in 10 mL of methanol. Then, solution was shaken and left for several hours to ensure it is totally dissolved. Next step, was the 10 times dilution of the prepared solution (pipette 1 mL into 10 mL volumetric
flasks), then 20 µL of pyrene solution was added to 2 ml of aqueous solution of each polymer at different concentrations and left over night. Pyrene/polymer solution was transferred into a quartz glass cell. Cary Eclipse fluorescence spectrophotometer (Varian) was used to measure the fluorescence emission. Pyrene was excited at 335 nm using excitation and emission slits of 2.5 and 2.5 nm respectively, and emissions were recorded at 373 and 382-385 nm, corresponding to the first (I₁) and third (I₃) vibrational peaks respectively.

2.4.6 Viscometry
The viscosity of the obtained polymers was measured using an ASTM Ubblelohde capillary viscometer (size 1C, nominal constant = 0.03, PSL ref. 1643/05). The viscometer was placed in a beaker filled with water to control temperature around 25 ºC ±1 via a thermometer and hotplate. An average of three measurements were taken for each sample. The flow time of 20 ml of deionized water was first measured as t₀ in a clean viscometer. Then, the flow time of polymers solutions with known concentration were recorded as t. The stock solutions of these samples were prepared by mass/volume. The value of relative viscosity was calculated by dividing the flow time of solution into the flow time of the solvent directly.

2.5 MIC determination
To determine the MIC value of each inhibitor, different preparations were made. MIC is defined as the minimum concentration of polymer solution that inhibits bacterial growth after overnight incubation.⁶

2.5.1 Preparation of glycerol stocks of different types of bacteria
Into 2 ml sterile screw top tubes were added 1 ml of 80% sterile glycerol solution and 1 ml of overnight inoculum (a 10 ml culture of bacteria grown for 16-19 hours at 37 ºC in L.B broth) to prepare 1:1 bacteria glycerol stocks, which were subsequently frozen at -80 ºC.

2.5.2 L.B broth
The L.B broth used in this study was prepared by dissolving 10 g tryptone, 5 g yeast extract and 5 g NaCl in 1L of deionised (DI) water using a magnetic stirrer. The solution obtained was poured into 5 x 200 ml bottles, which were then autoclaved at 212 ºC for 50 minutes.
2.5.3 Preparation of L.B agar plates

To prepare the L.B agar plates, 1.25 g of NaCl, 2.25 g tryptone, 1.25 g of yeast extract and 3.75 g agar were dissolved in 250 mL of DI water using a magnetic stirrer, the solution was autoclaved for 50 minutes at 212 °C, then the solution was divided into 12 Petri dishes, which were left upside down with lids on at room temperature overnight to help dry the plates prior to them being stored in a refrigerator.

2.5.4 Recovery of single colonies from stored cultures

Using a flamed wire loop, a small amount of glycerol stock was streaked onto each agar plate, which was then incubated upside down at 37 °C for 12-24 hours until single colonies developed.

2.5.5 Preparation of overnight culture

Five colonies of certain bacteria were picked from streaked plates and replaced into 10 ml L.B broth. The wire loop used for inoculation was flamed before it touched the colonies and after the inoculation. The cultures were grown overnight at 37 °C with vigorous shaking.

2.5.6 Microtitre plate assay

In a modified version of the assay described by Deighton,\textsuperscript{7} 1/100 dilutions of overnight cultures of the bacteria were prepared in LB broth and 200 µL of the dilution of each strain was transferred into the wells of a flat-bottomed untreated polystyrene 96-well microtitre plate (Greiner Bio-one Ltd., UK, ref. code 655161). Eight replicate wells were used for each concentration of inhibitor, 8 wells were each inoculated with 200 µL inoculum alone as positive growth control and 200 µL of LB broth with no organism were transferred into a further 8 wells to act as a negative control. All experiments with microtitre plates were performed with a multichannel pipette. The microtitre plates were incubated for 8 hours at 37 °C.

The excess media and any planktonic cells were removed and each well was washed with 200 µL sterile phosphate buffered saline (PBS) (Sigma-Aldrich), then the washed plates were left to dry overnight at room temperature. Next, each well was stained with 150 µL of 0.4 % w/v ammonium crystal violet for 10 minutes, then washed with running tap water until the excess stain was removed and the running water appeared colourless. The plates were left to dry overnight at room temperature and the ammonium crystal violet was
solubilised using 200 µL 100 % ethanol (analytical reagent grade, ethanol absolute, Fisher Scientific UK Ltd.). The biofilm density was determined by measuring the optical density of each well using a spectrophotometer plate reader (Synergy HT/Bio-TEK).

2.6 References

Chapter 3
Synthesis and Structural Characterization of Polymers
Chapter 3: Synthesis and Structural Characterization of Polymers

This chapter describes the synthesis and characterization of the polymers used in this study.

3.1 Introduction

The term ‘amphiphilic’ denotes “molecules which have affinity for two different types of environments, i.e. hydrophilic and lipophilic”. Amphiphilic antimicrobial polymers have a strong potential as effective antimicrobials that have low tendency to develop resistance, are less toxic to human cells and have long-lasting antibacterial activity. Polymeric materials bearing quaternary ammonium or phosphonium salts (QAS, QPS) have been used in various antimicrobial-relevant applications. QASs are the antimicrobial agents most commonly found in US households (in 57.8% of products) according to a survey of 500 disinfectant agents registered with the Environmental Protection Agency. QASs with various structures have been developed, leading to a significant improvement in cationic biocides via the direct polymerization of monomers bearing QAS groups or covalently incorporating QAS compounds into ordinary synthetic or natural polymers.

To induce amphiphilicity in the polymers made in this research, 2-(methacryloyloxy) ethyl]trimethyl ammonium chloride (MADQUAT) was chosen to provide the hydrophilic QAS, and methyl methacrylate (MMA) to provide the hydrophobicity. Inducing a hydrophobic group in cationic polymers leads to a decrease in toxicity and an increase in antibacterial activities. MADQUAT is an excellent monomer that can be used in synthesizing homopolymers or copolymers with various monomers which can be used in different applications such as antimicrobial materials and drug delivery. Chowdhury P. and coworkers prepared quaternized polymer/ nanosilica composite having core-shell structures by synthesizing MADQUAT via one step polymerization onto the surface of silicon dioxide nanopowder (SDNP). Then they tested against Salmonella typhi. A good result as antibacterial of PMADQUAT/SDNP composite was observed at concentration of 400 µg/mL where the inhibition zone was found to be 10 mm.

Amphiphilic cationic polymers containing MADQUAT, MMA and PEGMA were synthesized at various molar ratios with different polymeric structures. The statistical copolymers were synthesized by the one-pot method employing modified conventional free-radical polymerization, while amphiphilic di-block polymers were synthesized by reversible addition fragmentation chain transfer (RAFT). Homo-polymer was synthesized by both routes.
These polymers were characterized by proton NMR for % conversion and final composition, and aqueous gel permeation chromatography (GPC) to determine molecular weight and molecular weight distribution.

3.2 Homopolymers

3.2.1 Materials used to synthesize and characterize homopolymers

Monomers: 2-(methacryloyloxy) ethyl] trimethylammoniumchloride (MADQUAT) (80 wt% in H₂O, Sigma-Aldrich) and poly(ethyleneglycol) methyl ether methacrylate, Mn = 360 g.mol⁻¹ (PEGMA). Free radical initiator: 2,2’-azobis(2-methylbutyronitrile) (VAZO-67) (DuPont). RAFT agent: 2-phenyl-2-propyl benzodithioate (cumyl dithiobenzoate; CDB) (99% (HPLC), 731269, Sigma-Aldrich). Solvents: ethanol ≥ 99.8%, n-hexane 99%, deuterium chloroform (CDCl₃), deuterium oxide (D₂O) (all of standard laboratory reagent grade, Sigma Aldrich).

3.2.2 Synthesis of homopolymers

PMADQUAT was synthesized by conventional free radical polymerization and also by RAFT polymerization.

3.2.2.1 Calculation for homopolymers

The homopolymer PMADQUAT was prepared by two routes: free radical polymerization at 70-75 °C, using VAZO-67 as initiator, and RAFT polymerization in the presence CDB as a RAFT chain transfer agent and VAZO-67 as initiator at 70-75 °C. Details of synthesis are provided in this chapter. PMADQUAT calculations are detailed in Table 3.1.
### Table 3.1. Calculated mass of each compound used in synthesizing PMADQUAT by RAFT and free radical polymerization.

<table>
<thead>
<tr>
<th></th>
<th>RAFT agent</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Via RAFT polymerization</strong></td>
<td>RAFT agent</td>
<td>100 mg, 0.37 mmol</td>
</tr>
<tr>
<td></td>
<td>MADQUAT (80 wt% in water)</td>
<td></td>
</tr>
<tr>
<td>RAFT agent</td>
<td>0.00037(mol) × 207.7 (g mol⁻¹) ×100 = 7.636 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.636 ÷ 0.8 = 9.545 g</td>
<td></td>
</tr>
<tr>
<td>[RAFT]:[VAZO-67] = 1:4¹⁶</td>
<td>3.677×10⁻⁴×192 = 0.018 g</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>20 mL</td>
<td></td>
</tr>
<tr>
<td><strong>Via free radical polymerization</strong></td>
<td>MADQUAT (80 wt% in water)</td>
<td>33.0 (g) × 0.8 = 26.4 g</td>
</tr>
<tr>
<td></td>
<td>VAZO-67 (1% of total monomer concentration)</td>
<td>0.127 mol × 0.01 × 192.26 (g mol⁻¹) = 0.244 g</td>
</tr>
<tr>
<td>Ethanol</td>
<td>60 mL</td>
<td></td>
</tr>
</tbody>
</table>

#### 3.2.2.2 Synthesis of PMADQUAT via free radical polymerization

The linear homopolymer was synthesized as shown in Scheme 3.1, with molar ratio of the initiator (1% over the total monomer concentration). A mixture of MADQUAT monomer (26.4 g) and ethanol (60 mL) was prepared in a two-necked round-bottomed glass flask and heated to 70-75 °C with continuous stirring to ensure good mixing under inert atmosphere by purging with nitrogen. Polymerization was started by adding to the mixture 0.244 g of initiator VAZO-67 dissolved in 1-3 mL of ethanol. The mixture was then left stirring overnight (around 22-24 hrs.). The polymerization was monitored by ¹H NMR and when the desired conversion of around 95% was reached, the reaction mixture was quenched by cooling the flask in an external ice-bath. The viscous liquid that was obtained was dropped into a glass vessel containing 50 mL cold n-hexane, with continuous stirring. Two phases were obtained: a liquid upper layer of unreacted monomer dissolved in n-hexane, which was discarded, and a transparent viscous liquid bottom layer of polymer, which was poured onto a plate and left overnight at room temperature for the ethanol solvent to evaporate. On the next day, the polymer was placed in a vacuum oven at 50 °C to ensure the complete evaporation of any residual solvent overnight. The polymer obtained (PMADQUAT) was a white solid and the yield was about 85%. It was confirmed to be free of solvent and residual monomer by ¹H-NMR.
Chapter 3: Synthesis and Structural Characterization of Polymers

3.2.2.3 Synthesis of PMADQUAT via RAFT polymerization

PMADQUAT homopolymer was synthesized via RAFT as follows: a solution of CDB, (100 mg), MADQUAT (9.545 g), VAZO-67 (0.018 g) and ethanol (20 mL) was prepared in a two-necked round-bottomed flask equipped with a condenser and stirrer bar. The solution was degassed by nitrogen purge for 30 min at room temperature and then heated to 70-75 °C. Polymerization was monitored by $^1$H NMR and when the desired conversion of around 99% was reached, the reaction mixture was quenched by cooling the flask in an external ice-bath. The viscous liquid obtained was dropped into a glass vessel containing 30 mL cold n-hexane with continuous stirring. Two phases were obtained: a liquid upper layer of unreacted monomer dissolved in n-hexane, which was discarded, and a pinkish viscous bottom layer, which was collected and poured onto a plate in order to remove the solvent. On the next day, the polymer was placed in a vacuum oven at 50 °C overnight. A yield of about 80% of pinkish solid polymer was obtained and was confirmed to be free of solvent and residual monomer by $^1$H NMR.

3.2.3 Structural characterization of homopolymers

3.2.3.1 $^1$H NMR

$^1$H NMR spectra were used to determine polymer structures and the conversion of monomers to polymers. In the olefinic region, $\equiv$CH of the monomers gave proton resonances at 5.5-6 ppm (Figure 3.1), then as monomer dwindled and polymer became abundant, these resonances started disappearing and new proton resonances appeared around 1 ppm, as shown in Figures 3.2 and 3.3. The new resonances corresponded to $\equiv$CH of the polymer formed. These observations provide clear evidence of conversion to polymer.
Figure 3.1. $^1$H NMR spectrum (400 MHz, CDCl$_3$) of MADQUAT monomer.

Figure 3.2. $^1$H NMR spectrum (400 MHz, D$_2$O) of PMADQUAT homopolymer.
Figure 3.3. $^1$H NMR spectrum (400 MHz, D$_2$O) of PMADQUAT after 30 hrs. synthesized by RAFT in ethanol at 70-75 °C (before purification).

The integrals from Figure 3.3 are tabulated below:

<table>
<thead>
<tr>
<th>Integral</th>
<th>Normalized integral area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>384</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

From the NMR spectrum (Figure 3.3) and Table 3.2, conversion of MADQUAT was calculated as follows:

\[
\frac{\text{Integral 1}}{\text{Integral 1} + (2 \times \text{Integral 2})} = \frac{384}{384 + (2 \times 1)} = \frac{384}{386} = 99.5% 
\]

The method used in the above calculation of the conversion of PMADQUAT was repeated for the other polymers.

3.2.3.2 Gel permeation chromatography

Aqueous GPC was used to determine number average molecular mass ($M_n$), weight average molecular mass ($M_w$) and polydispersity index (PDI) of the synthesized polymers (Table 3.3).
Table 3.3. Summary of \( M_n \), \( M_w \) and PDI from GPC for PMADQUAT homopolymers.

<table>
<thead>
<tr>
<th>Synthesis method</th>
<th>( M_n ) (g mol(^{-1}))</th>
<th>( M_w ) (g mol(^{-1}))</th>
<th>( M_w/M_n ) (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free radical polymerization</td>
<td>68,000</td>
<td>541,000</td>
<td>7.9</td>
</tr>
<tr>
<td>RAFT polymerization</td>
<td>9,000</td>
<td>15,000</td>
<td>1.6</td>
</tr>
</tbody>
</table>

3.3 Statistical copolymers

3.3.1 Materials used to synthesize and characterize statistical copolymers

Monomers: 2-(methacryloyloxy) ethyl] trimethylammoniumchloride (MADQUAT), (80 wt% in H\(_2\)O) and methyl methacrylate (MMA, containing \( \leq 30 \) ppm MEHQ as inhibitor, 99%), poly(ethylene glycol) methyl ether methacrylate, \( M_n = 360 \) (g mol\(^{-1}\)) (PEGMA), all from Sigma-Aldrich. Free radical initiator: VAZO-67 (DuPont). 1-thioglycerol (TG, 97%, Sigma Aldrich). Solvents: ethanol \( \geq 99.8\% \), n-Hexane 99%, CDCL\(_3\), D\(_2\)O, all of standard laboratory reagent grade from Sigma Aldrich.

3.3.2 Synthesis of statistical copolymers

3.3.2.1 Calculation for statistical copolymers

The calculation for the synthesis of poly(MADQUAT\(_{50}\)-s-MMA\(_{50}\)) which follows is given as an example which applies to all of the statistical polymers synthesized in this work:

First monomer: MADQUAT\(_{50}\) = 0.05 mol

So, 0.05 mol \( \times 207.7 \) g mol\(^{-1}\) = 10.385 g

Second monomer: MMA\(_{50}\) = 0.05 mol

So, 0.05 mol \( \times 100.12 \) g mol\(^{-1}\) = 5.006 g

VAZO-67: 1% of total monomer concentration

So, \((0.05 \text{ mol} + 0.05 \text{ mol}) \times 0.01 \times 192.26 \text{ g mol}^{-1} = 0.192 \text{ g}\)

TG: 0.5% of total monomer concentration

So, \((0.05 \text{ mol} + 0.05 \text{ mol}) \times 0.005 \times 108.16 \text{ g mol}^{-1} = 0.054 \text{ g}\)

3.3.2.2 Synthesis route of statistical copolymers

Two series of copolymers with different mole ratios, poly(MADQUAT\(_x\)-s-MMA\(_y\)) and poly(MADQUAT\(_x\)-s-PEGMA\(_y\)), were synthesized by conventional one-pot free radical polymerization in the presence of a free radical agent (VAZO-67) in ethanol as solvent. Details of synthesis routes are given below for one example of each series.
a) Synthesis of poly(MADQUAT$_{x}$-s-MMA$_{y}$)

To synthesize poly(MADQUAT$_{75}$-s-MMA$_{25}$), a solution of MADQUAT monomer (7.79 g), MMA monomer (1.25 g), TG (0.027 g) and 30 mL of ethanol was prepared. The solution was degassed before adding VAZO-67 initiator (0.095 g) dissolved in ethanol (3 mL). The mixture was left stirring vigorously for 24 hours at 70-75 °C under nitrogen gas. The polymer obtained was purified using cold n-hexane and recovered by rotatory evaporation at 40 °C. As the mole ratio of MMA to MADQUAT was increased the polymer became whiter and the yield increased. Thus, the yields were 80%, 86% and 90% for poly(MADQUAT$_{75}$-s-MMA$_{25}$), poly(MADQUAT$_{50}$-s-MMA$_{50}$) and poly(MADQUAT$_{25}$-s-MMA$_{75}$) respectively. The polymers obtained were confirmed to be free of solvent and residual monomer by $^1$H NMR.

b) Synthesis of poly(MADQUAT$_{x}$-s-PEGMA$_{y}$)

A solution of MADQUAT monomer (6.08 g), PEGMA monomer (5.73 g) and ethanol (20 mL) was prepared in a two-necked round-bottomed glass flask and heated to 70 °C with continuous stirring to ensure good mixing under inert atmosphere by purging with nitrogen. To the solution, VAZO-67, 1% (0.075 g) dissolved in methanol (3 mL) was added. The mixture was left stirring overnight (around 22-24 hrs.) under inert atmosphere at 70-75 °C. The viscous liquid that was obtained was dropped into a glass vessel containing 50 mL cold n-hexane, with continuous stirring. Two layers were obtained: an upper liquid layer comprising unreacted monomer dissolved in n-hexane, which was discarded, and a bottom layer of transparent viscous liquid which contained the polymer. This was collected and poured onto a plate in order to remove the solvent. On the next day, the polymer was placed in a vacuum oven at 50 °C overnight. A yield of about 77% solid yellowish polymer was obtained and was confirmed by $^1$H NMR to be free of solvents and residual monomer.
Scheme 3.3. Synthesis of poly ((MADQUATx-s-PEGMAy) copolymers.

3.3.3 Characterization of statistical copolymers

3.3.3.1 Confirmation of statistical copolymer formation by $^1$H NMR

Figures 3.4, 3.5 and 3.6 show the $^1$H NMR spectra of the three poly(MADQUAT$_x$-s-MMA$_y$) copolymers, where peak 3 (3.59 ppm) is attributed to $\text{–OCH}_3$ in methyl methacrylate and peak 4 (~3.2 ppm) to $\text{–N}^+\text{(CH}_3\text{)}_3$ in MADQUAT, these results confirm the formation of the statistical copolymers.

Figure 3.4. $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; poly(MADQUAT$_{75}$-s-MMA$_{25}$).
Figure 3.5. $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; poly(MADQUAT$_{50}$-s-MMA$_{50}$).

Figure 3.6. $^1$H NMR spectrum (400 MHz, D$_2$O) of the statistical copolymer; poly(MADQUAT$_{25}$-s-MMA$_{75}$).
3.3.3.2 Determination of copolymer composition by $^1$H NMR

The ratios of MADQUAT to MMA in poly(MADQUAT-s-MMA) statistical copolymers were estimated from $^1$H NMR, by comparing –N(CH$_3$)$_3$ (from the MADQUAT moiety) to –OCH$_3$ (from the MMA moiety), using equation 3.1:

$$\% \text{ block } x = \frac{\alpha_x}{m_x} \times 100\%$$

(3.1)

where $\alpha_x$ is the area of the $^1$H NMR peak of –N(CH$_3$)$_3$; $m_x$ is the number of protons of –N(CH$_3$)$_3$; $\alpha_y$ is the area of the $^1$H NMR peak of –OCH$_3$; $m_y$ is the number of protons of –OCH$_3$. Substituting into equation 3.1:

$$\% \text{ MADQUAT} = \frac{0.32}{\frac{0.32}{9} + \frac{0.04}{3}} \times 100\% = \frac{0.036}{0.049} \times 100\% = 73$$

$$\% \text{ MMA} = \frac{0.04}{\frac{0.32}{9} + \frac{0.04}{3}} \times 100\% = \frac{0.013}{0.049} \times 100\% = 27$$

The above calculation was applied to other polymers and the resulting monomer ratios are listed in Table 3.4:

<table>
<thead>
<tr>
<th>Polymer name, indicating calculated monomer ratio</th>
<th>Monomer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target</td>
</tr>
<tr>
<td></td>
<td>MADQUAT</td>
</tr>
<tr>
<td>poly (MADQUAT$<em>{75}$-s-MMA$</em>{25}$)</td>
<td>75</td>
</tr>
<tr>
<td>poly (MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</td>
<td>50</td>
</tr>
<tr>
<td>poly (MADQUAT$<em>{25}$-s-MMA$</em>{75}$)</td>
<td>25</td>
</tr>
</tbody>
</table>

The reactivity ratios of both monomers can be estimated from proton NMR, where the targeted molar ratio (degree of polymerization) is almost equal to the estimated values from $^1$H NMR (see table, 3.4). That means the reactivity ratio is almost 1:1 leading to statistical copolymers.

3.3.3.3 Gel permeation chromatography

The aqueous GPC technique was used to determine the molecular weights of the statistical copolymers obtained (Table 3.5).
Table 3.5. Summary of \( M_n \), \( M_w \) and \( PDI \) from GPC for statistical copolymers.

<table>
<thead>
<tr>
<th>Polymer name</th>
<th>( M_n ) (g mol(^{-1}))</th>
<th>( M_w ) (g mol(^{-1}))</th>
<th>( M_w/M_n ) (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(MADQUAT(<em>{95})s-PEGMA(</em>{5}))</td>
<td>16,000</td>
<td>329,000</td>
<td>20.8</td>
</tr>
<tr>
<td>poly(MADQUAT(<em>{75})s-PEGMA(</em>{25}))</td>
<td>8,000</td>
<td>148,000</td>
<td>17.6</td>
</tr>
<tr>
<td>poly(MADQUAT(<em>{75})s-MMA(</em>{25}))</td>
<td>11,000</td>
<td>35,000</td>
<td>3</td>
</tr>
<tr>
<td>poly(MADQUAT(<em>{50})s-MMA(</em>{50}))</td>
<td>8,000</td>
<td>24,000</td>
<td>2.9</td>
</tr>
<tr>
<td>poly(MADQUAT(<em>{25})s-MMA(</em>{75}))</td>
<td>3000</td>
<td>7000</td>
<td>2</td>
</tr>
</tbody>
</table>

3.4 Di-block polymers

Amphiphilic di-block polymers bearing MADQUAT and MMA were synthesized by RAFT polymerization in the presence of CDB as a RAFT chain transfer agent. The aim was to prepare amphiphilic di-block poly(MADQUAT\(_{x}\)-b-MMA\(_{y}\)) polymers of controlled structure and molecular weight.\(^{18,19}\) Their molecular weights were obtained by aqueous GPC calibrated with polyethylene glycol standards. The polymerization kinetics were monitored by \(^1\)H NMR spectroscopy, comparing the signals attributed to the monomers and corresponding monomeric units in poly(MADQUAT\(_{x}\)-b-MMA\(_{y}\)). RAFT polymerization of MADQUAT and MMA in the ratio [RAFT]:[VAZO-67] = 1:4 demonstrated their “living” character, enabling the synthesis of poly(MADQUAT-b-MMA) with molecular weight \( M_n \) in the range 4000-2600 (GPC), 18,000-12,000 (calculated), at various molar ratios of the monomers and low polydispersity (\( M_w/M_n = 1.2\text{--}1.3 \)).

3.4.1 Materials used to synthesize and characterize di-block polymers

Monomers: 2-(methacryloyloxy) ethyl] trimethylammoniumchloride (MADQUAT), (80 wt% in H\(_2\)O) and MMA (containing \( \leq 30 \) ppm MEHQ as inhibitor, 99%), both from Sigma-Aldrich. RAFT agent: CDB (99% (HPLC), 731269, Sigma-Aldrich). Free radical initiator: VAZO-67 (DuPont). Solvents: ethanol \( \geq 99.8\% \), n-Hexane 99%, CDCL\(_3\), D\(_2\)O, all of standard laboratory reagent grade from Sigma Aldrich.

3.4.2 RAFT polymerization of di-block polymers

3.4.2.1 Calculations

In RAFT polymerisation a specific mole ratio can be targeted and the following calculation was used to synthesize di-block polymers with a certain mole ratio of each block.

\[
\text{Mass of RAFT agent} = m_r \tag{3.2}
\]

\[
\text{Mole of RAFT} = n_r = \frac{m_r}{M_w \text{ of RAFT}} \tag{3.3}
\]

\[
\text{Mass of monomer} = m_m = n_r \times M_w \text{ of monomer} \times \text{targeted mole ratio of the block} \tag{3.4}
\]
Example of di-block calculation: Poly (MADQUAT\textsubscript{75}-b-MMA\textsubscript{25})

RAFT controls the reaction; 100 mg of RAFT agent (M\textsubscript{w} = 272 g mol\textsuperscript{-1}) was used, so the number of moles of RAFT needed was

\[ \frac{100 \text{ mg}}{272 \text{ g mol}^{-1}} = 3.677 \times 10^{-4} \text{ mol} \]

a) Calculation of the first block: MADQUAT\textsubscript{75}

The targeted mole ratio of the first block is 75, so the mass of MADQUAT required is

\[ 3.677 \times 10^{-4} \times 207.7 \times 75 = 5.727 \text{ g} \]

Dividing by the concentration of monomer (80%) gives

\[ 5.727 \div 0.8 = 7.159 \text{ g} \]

b) Calculation of the second block: MMA\textsubscript{25}

The targeted mole ratio of the second block is 25, so the mass of MMA required is

\[ 3.677 \times 10^{-4} \times 100.12 \times 25 = 0.920 \text{ g} \]

c) Calculation of the initiator: VAZO-67

The ratio of RAFT agent to initiator is 1:4, so the mass of initiator required is

\[ \frac{3.677 \times 10^{-4}}{4} \times 192 = 0.018 \text{ g} \]

Calculation of molecular weight of each block

Molecular weights of reagents used were:
MADQUAT=207.70 (g mol\textsuperscript{-1})
MMA=100.12 (g mol\textsuperscript{-1})
RAFT=272.00 (g mol\textsuperscript{-1})

The molecular weight of each block was calculated as follows:

Calculate the number of moles of RAFT agent (n\textsubscript{r})

\[ n_r = \text{grams of RAFT} \div M_w \text{ of RAFT} \]

Calculate the number of moles of monomer (n\textsubscript{m})

\[ n_m = \text{grams of monomer} \div M_w \text{ of the monomer} \]

Calculate the degree of polymerization for each block (DP)

\[ DP = (\text{mole of monomer} \div \text{mole of RAFT}) \times \text{conversion} \]

Finally, calculate the M\textsubscript{n} of each block

\[ M_n = DP \times M_w \text{ (monomer)} \]

The results of these calculations are listed in Table 3.6.
Table 3.6. Target $M_n$ and DP values of synthesized di-block polymers.

<table>
<thead>
<tr>
<th>Calculated molar ratio</th>
<th>Polymer</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{75}$-b-MMA$</em>{25}$)</td>
<td>Poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</td>
<td>Poly(MADQUAT$<em>{25}$-b-MMA$</em>{75}$)</td>
</tr>
<tr>
<td>[RAFT] (mol)</td>
<td>0.00037</td>
<td>0.00037</td>
<td>0.00037</td>
</tr>
<tr>
<td>[MADQUAT] (mol)</td>
<td>0.0276</td>
<td>0.0185</td>
<td>0.0093</td>
</tr>
<tr>
<td>DP of MADQUAT block</td>
<td>75</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>$M_n$ of MADQUAT block (g mol$^{-1}$)</td>
<td>15,600</td>
<td>10,000</td>
<td>5,200</td>
</tr>
<tr>
<td>[MMA] (mol)</td>
<td>0.00919</td>
<td>0.0186</td>
<td>0.0276</td>
</tr>
<tr>
<td>DP of MMA block</td>
<td>25</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>$M_n$ of MMA block (g mol$^{-1}$)</td>
<td>2,500</td>
<td>5,000</td>
<td>7,600</td>
</tr>
<tr>
<td>Combined $M_n$ (g mol$^{-1}$)</td>
<td>18,100</td>
<td>15,000</td>
<td>12,800</td>
</tr>
</tbody>
</table>

$M_n$ can also be calculated in a different way, using the calculated number of repeating units (n)$_{17}$ as follows, see Figures 3.7, 3.8 and 3.9:

$$M_n \text{ of poly (MADQUAT}_x\text{-b- MMA}_y) =$$

$$n_{MADQUAT} \left( \frac{a_{MADQUAT} \times m_{MADQUAT} \times n_{CDB}}{a_{CDB} \times m_{CDB}} + 119 \right)$$

Where, $a_{MADQUAT}$ is the area (intensity) of MADQUAT from proton NMR; $n_{MADQUAT}$ is the number of repeating units of MADQUAT; $m_{MADQUAT}$ is the number of proton of MADQUAT (9 protons); $a_{CDB}$ is the area (intensity) of RAFT (CDB) from proton NMR; $n_{CDB}$ is the number of repeating units of CDB (1); $m_{CDB}$ is the number of protons of the dithiobenzoate end group (2 protons).
For poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}),
\[ n_{\text{MADQUAT}} = \frac{a_{\text{MADQUAT}} \times m_{\text{CDB}} \times n_{\text{CDB}}}{a_{\text{CDB}} \times m_{\text{MADQUAT}}} = \frac{313 \times 2 \times 1}{1.58 \times 9} = 44 \]
\[ n_{\text{MMA}} = \frac{a_{\text{MMA}} \times m_{\text{CDB}} \times n_{\text{CDB}}}{a_{\text{CDB}} \times m_{\text{MMA}}} = \frac{34.8 \times 2 \times 1}{1.58 \times 3} = 15 \]
Therefore, \( M_n = 153 + (207.7 \times 44) + (100.12 \times 15) + 119 = 153 + 9139 + 1502 + 119 = 10,900 \text{ g mol}\textsuperscript{-1} \).

Similarly, for poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}),
\( M_n = 153 + (207.7 \times 37.6) + (100.12 \times 33) + 119 = 153 + 7622 + 3303 + 119 = 11,200 \text{ g mol}\textsuperscript{-1} \);
for poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}),
\( M_n = 153 + (207.7 \times 2.23) + (100.12 \times 15.5) + 119 = 153 + 463 + 1552 + 119 = 2,300 \text{ g mol}\textsuperscript{-1} \);
and for PMADQUAT,
\( M_n = 153 + (207.7 \times 84) + 119 = 17,700 \text{ g mol}\textsuperscript{-1} \).

### 3.4.2.2 Synthesis route of di-block polymers: poly(MADQUAT\textsubscript{x}-b-MMA\textsubscript{y})

Di-block polymers were synthesized by the RAFT process as shown in Scheme 3.4. Details are given for poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}); the same route was followed for the other di-block polymers in this series. To synthesise PMADQUAT, a solution of CDB (100 mg), MADQUAT (7.159 g), VAZO-67 (0.018 g) and ethanol (15 mL) was prepared in a two-necked round-bottomed flask equipped with a condenser and stirrer bar. The solution was degassed by nitrogen purge for 30 min at room temperature, then heated to 70-75 °C. Polymerization was monitored by \(^1\text{H} \text{NMR}\) and when the desired conversion of around 95% was reached, the reaction mixture was quenched by cooling in an external ice-bath. To the solution were added the second monomer (MMA, 0.92 g), ethanol (5 mL) and VAZO-67 (0.018 g) were added. The mixture was reheated to 70-75 °C and left stirring. The copolymerization was also monitored by \(^1\text{H} \text{NMR}\) and the reaction was stopped when approximately 95% of MMA monomer had been consumed about 48 hours (see figure 9.1 in appendices). It was found that the mole ratio of MMA to MADQUAT affected the colour of the copolymer, i.e. increasing this ratio gave a stronger shade of pink. The yields were 94%, 88% and 68% for poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}), poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) and poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) respectively; some polymer was lost during the purification process, which affected the final yield. The polymers obtained were confirmed to be free of solvent and residual monomer by \(^1\text{H} \text{NMR}\).
Scheme 3.4. Synthesis of poly(MADQUAT$_x$-b-MMA$_y$) di-block polymers by RAFT polymerization.

3.4.3 Characterization of di-block polymers

3.4.3.1 Confirmation of di-block formation by $^1$H NMR

Figures 3.7, 3.8 and 3.9 show $^1$H NMR spectra of the three poly(MADQUAT$_x$-b-MMA$_y$) di-block polymers synthesized by RAFT as described above.

Figure 3.7. $^1$H NMR spectrum (400 MHz, D$_2$O) of poly(MADQUAT$_{75}$-b-MMA$_{25}$).
In the $^1$H NMR spectra, the signals of aromatic protons of CDB at 7.1-7.3 ppm for S=C(Z)S- can be clearly observed.\cite{20,21} The signals at 0.89-1.05 ppm can be attributed to the protons of methyl groups in the main polymer chain, while the signals of olefinic protons in
the residual monomers appear in the range of 5.67-5.06 ppm. These observations can be used as a clear evidence of conversion to polymer.

The significant signals at 3.19 and 3.75 ppm are those of protons in the $-\text{N}$(CH$_3$)$_3$ group in MADQUAT and the $-\text{OCH}_3$ group in MMA respectively. Both signals can be used to calculate the composition of the di-block polymers. From Figure 3.10, it can be seen that the intensity of the peak at 3.6 ppm, corresponding to the resonance of the $-\text{OCH}_3$ protons, increases as the molar ratio of MMA increases, i.e. at ratios of 25, 50 and 75, the intensity values were 0.03, 0.06 and 0.11 respectively. The peak at 3.6 ppm also starts to overlap with the peak at 3.75 ppm, corresponding to $-\text{CH}_2-$ in CH$_2-$N(CH$_3$)$_3$. This provides proof of di-block formation at different molar ratios of MADQUAT and MMA.

![Figure 3.10. $^1$H NMR spectra (400 MHz, D$_2$O) of all di-block polymers; poly(MADQUAT$_x$-b-MMA$_y$).](image)

### 3.4.3.2 Determination of copolymer composition by $^1$H NMR

The monomer ratios of MADQUAT to MMA in di-block poly(MADQUAT-b-MMA) polymers were estimated from $^1$H NMR, by comparing $-\text{N}$(CH$_3$)$_3$ (from the MADQUAT block) to $-\text{OCH}_3$ (from the MMA block), using equation 3.1:

\[
\% \text{ MADQUAT(block)} = \frac{0.3}{0.3 + 0.03} \times 100\% = \frac{0.3}{0.34} \times 100\% = 75
\]

\[
\% \text{ MMA(block)} = \frac{0.03}{0.3 + 0.03} \times 100\% = \frac{0.03}{0.34} \times 100\% = 25
\]

The above calculation was applied to all three polymers and their blocks; results are listed in Table 3.7.
Table 3.7. Molar ratio of each block of the di-block polymers, as calculated and by $^1$H NMR

<table>
<thead>
<tr>
<th>Polymer name (calculated molar ratio)</th>
<th>Molar ratio by $^1$H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMADQUAT</td>
</tr>
<tr>
<td>poly(MADQUAT$<em>{75}$-b-MMA$</em>{25}$)</td>
<td>75</td>
</tr>
<tr>
<td>poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</td>
<td>52</td>
</tr>
<tr>
<td>poly(MADQUAT$<em>{25}$-b-MMA$</em>{75}$)</td>
<td>36</td>
</tr>
</tbody>
</table>

3.4.3.3 Gel permeation chromatography

Aqueous GPC was applied to all synthesized di-block polymers, allowing $M_w$, $M_n$ and PDI values to be determined, as displayed in Table 3.4.

Table 3.8. $M_w$, $M_n$ and PDI of di-block polymers determined by aqueous GPC

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$M_w$ (g mol$^{-1}$)</th>
<th>$M_n$ (g mol$^{-1}$)</th>
<th>$M_w/M_n$ (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^{st}$ block: PMADQUAT$_{75}$</td>
<td>11,400</td>
<td>4,200</td>
<td>2.7</td>
</tr>
<tr>
<td>poly(MADQUAT$<em>{75}$-b-MMA$</em>{25}$)</td>
<td>5,300</td>
<td>4,100</td>
<td>1.3</td>
</tr>
<tr>
<td>1$^{st}$ block: PMADQUAT$_{50}$</td>
<td>6,200</td>
<td>4,700</td>
<td>1.3</td>
</tr>
<tr>
<td>poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</td>
<td>4,800</td>
<td>3,400</td>
<td>1.3</td>
</tr>
<tr>
<td>1$^{st}$ block: PMADQUAT$_{25}$</td>
<td>3,600</td>
<td>2,200</td>
<td>1.2</td>
</tr>
<tr>
<td>poly (MADQUAT$<em>{25}$-b-MMA$</em>{75}$)</td>
<td>3,600</td>
<td>2,200</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The aqueous GPC data in Table 3.8 show that as the molar ratio of the MMA block increased, the molecular weight of the polymers decreased considerably. This result can be explained by the increasing molar ratio of the hydrophobic MMA moiety causing the polymer chains to shrink in water, leading to smaller particles. The principle of GPC separation is known to rely on the hydrodynamic volume$^{16,17}$ (size) of the polymer being tested, so this shrinking behaviour of the polymers significantly effects the molecular weight estimated by GPC. The values of PDI given in Table 3.8, as estimated from aqueous GPC analysis, indicate that all three di-block polymers had very low polydispersity.

$^1$H NMR spectroscopy is a fairly accurate method of determining molecular weight from the intensity of the significant peaks of the di-block polymers, i.e. those corresponding to –N(CH$_3$)$_3$, –OCH$_3$ and the end group.

3.5 Core-shell quaternary nanoparticle polymers

A series of core-shell quaternary nanoparticles with various alkyl side groups, synthesized by emulsion polymerization, were provided by Dr. Zhou Yang. These polymeric
nanoparticles have a particle size around 300-550 nm measured by DLS. The aim of studying these nanoparticles was to determine the relation between increasing the alkylation of their side groups and their efficiency as antimicrobial materials. More information and details about the synthesis and the characterizations of these polymers are provided in chapter 9; Appendix.

Table 3.9. Side chain and particle structure of Core-shell quaternary nanoparticles.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Side group</th>
<th>Particle structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY-24</td>
<td>Alkyne particle</td>
<td><img src="image" alt="Alkyne particle" /></td>
</tr>
<tr>
<td>ZY-26</td>
<td>C₄H₉</td>
<td><img src="image" alt="Particle structure" /></td>
</tr>
<tr>
<td>ZY-27</td>
<td>C₈H₁₇</td>
<td><img src="image" alt="Particle structure" /></td>
</tr>
<tr>
<td>ZY-28</td>
<td>C₁₂H₂₅</td>
<td><img src="image" alt="Particle structure" /></td>
</tr>
</tbody>
</table>

3.6 Characterization of didecyldimethylammonium chloride (DDAC)

3.6.1 Sample preparation for ¹H NMR

To prepare samples for ¹H NMR (figure 3.11), 3 mL of DDAC (40 wt%) was placed in a vacuum oven for 48 hrs at 80 °C, then 0.1 mL of the dried material was rehydrated by D₂O.

![1H NMR spectrum](image)

**Figure 3.11.** ¹H NMR spectrum (400 MHz, D₂O) of DDAC.
3.7 Summary and conclusion

Three amphiphilic di-block polymers and three statistical copolymers were synthesized by RAFT and free radical polymerization respectively, with various molecular weights, molar ratios of hydrophilic to hydrophobic moieties and structures. Proton NMR was used to investigate their number-average molecular weight, conversion and polymer composition, while Aqueous Gel Permeation Chromatography (GPC) was applied on all synthesized polymers, which helps to determination of weight average molecular mass ($M_w$), number average molecular mass ($M_n$) and polydispersity index (PDI), $M_w$. the values of $M_n$ and PDI of all obtained polymers are listed in table 3.10. However, it was found that the molecular weight of block and statistical copolymers decreased as the molar ratio of MMA increased. Molecular weight was estimated more accurately by means of $^1$H NMR for di-block polymers.

Table 3.10. Summary of $M_w$, $M_n$ and PDI of homo and statistical synthesized polymers by Aqueous GPC.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>Polymers</th>
<th>$M_w$ (g.mol$^{-1}$)</th>
<th>$M_n$ (g.mol$^{-1}$)</th>
<th>PDI</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo-polymer</td>
<td>PMADQUAT (via Free radical polymerization)</td>
<td>541,000</td>
<td>68,000</td>
<td>7.9</td>
<td><img src="image1" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>PMADQUAT (via RAFT polymerization)</td>
<td>15,000</td>
<td>9,000</td>
<td>1.6</td>
<td><img src="image2" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td>Poly(MADQUAT$_{95}$-s-PEGMA$_5$)</td>
<td>329,000</td>
<td>16,000</td>
<td>20.8</td>
<td><img src="image3" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{75}$-s-PEGMA$</em>{25}$)</td>
<td>148,000</td>
<td>8,000</td>
<td>17.6</td>
<td><img src="image4" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{75}$-s-MMA$</em>{25}$)</td>
<td>35,000</td>
<td>11,000</td>
<td>3</td>
<td><img src="image5" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</td>
<td>24,000</td>
<td>8,000</td>
<td>2.9</td>
<td><img src="image6" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{25}$-s-MMA$</em>{75}$)</td>
<td>7,0000</td>
<td>3000</td>
<td>2</td>
<td><img src="image7" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</td>
<td>4,800</td>
<td>3400</td>
<td>1.3</td>
<td><img src="image8" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT$<em>{25}$-s-MMA$</em>{75}$)</td>
<td>3,200</td>
<td>2600</td>
<td>1.2</td>
<td><img src="image9" alt="Chemical structure" /></td>
</tr>
</tbody>
</table>
Table 3.11. Summary of $M_w$, $M_n$ and PDI of synthesized diblock polymers by Aqueous GPC, H NMR and Target values.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>Polymers</th>
<th>Mw (g.mol$^{-1}$)</th>
<th>PDI</th>
<th>Mn (g.mol$^{-1}$)</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-block polymers</td>
<td>1) Poly(MADQUAT$<em>{75}$-s-MMA$</em>{25}$)</td>
<td>5,300</td>
<td>1.3</td>
<td>4,100</td>
<td>18,100</td>
</tr>
<tr>
<td></td>
<td>2) Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</td>
<td>4,800</td>
<td>1.3</td>
<td>3,400</td>
<td>15,100</td>
</tr>
<tr>
<td></td>
<td>3) Poly(MADQUAT$<em>{25}$-s-MMA$</em>{75}$)</td>
<td>3,200</td>
<td>1.2</td>
<td>2,600</td>
<td>12,800</td>
</tr>
</tbody>
</table>

3.8 References


Chapter 4
Aqueous Solutions Behavior of Polyelectrolytes
Chapter 4 Aqueous Solutions Behavior of Polyelectrolytes

4.1 Introduction

This chapter aims to study the aqueous solutions’ behaviour of both block and statistical copolymers. It is well known that the critical aggregation concentration (cac) is one of the most essential physical parameter for amphiphilic polymers. Many techniques can be applied to study the aqueous solution behavior and to estimate the value of (cac) such as surface tension, pyrene fluorescence, DLS; dynamic light scattering, zeta potential and viscosity which have been used in the present study. The (cac) is generally lower in amphiphilic block copolymers than in small molecules that can be explained by the hydrophobic segment in amphiphilic copolymers is longer than those found in conventional surfactants.¹

In the previous chapter, all the details about the synthesized polymers; synthesis routes and polymer characteristics were provided. For clarity, table 4.1 illustrates the key characteristics of the studied polymers.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>M_w (g.mol⁻¹)</th>
<th>M_n (g.mol⁻¹)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical polymers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT_{75-s-MMA_{25}})</td>
<td>35,000</td>
<td>11,000</td>
<td>3</td>
</tr>
<tr>
<td>Poly(MADQUAT_{50-s-MMA_{50}})</td>
<td>24,000</td>
<td>8,000</td>
<td>2.9</td>
</tr>
<tr>
<td>Poly(MADQUAT_{25-s-MMA_{75}})</td>
<td>7,000</td>
<td>3,000</td>
<td>2</td>
</tr>
<tr>
<td>Di-block polymers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M_w (g.mol⁻¹)</td>
<td>PDI</td>
<td>Mn (g.mol⁻¹)</td>
</tr>
<tr>
<td></td>
<td>GPC</td>
<td>Target</td>
<td>¹H NMR</td>
</tr>
<tr>
<td>Poly(MADQUAT_{75-b-MMA_{25}})</td>
<td>5,300</td>
<td>1.3</td>
<td>4,100</td>
</tr>
<tr>
<td>Poly(MADQUAT_{50-b-MMA_{50}})</td>
<td>4,800</td>
<td>1.3</td>
<td>3,400</td>
</tr>
<tr>
<td>Poly(MADQUAT_{25-b-MMA_{75}})</td>
<td>3,200</td>
<td>1.2</td>
<td>2,600</td>
</tr>
</tbody>
</table>

¹
4.2 Surface tension

Surface tension and pyrene fluorescence measurements are employed to measure the critical aggregation/micelle concentration.

**Figure 4.1.** Surface tension curves as function of the logarithm concentrations of 
P(MADQUAT$_x$-s-MMA$_y$), T= 25 °C; solvent-water.

**Figure 4.2.** Surface tension curves as function of the logarithm concentrations of 
P(MADQUAT$_x$-b-MMA$_y$), T= 25 °C; solvent-water.

Figures 4.1 and 4.2 depicts the variation in the surface tension of the polymers studied; di-block polymers and statistical copolymers as a function of polymers’ concentration in aqueous media. The figures reveal that there is a plateau region at low concentrations where the surface tension shows nearly no change with concentration after which a decline is observed after a specific concentration which varies from one polymer to the other.
The cac values determined from surface tension curves are 0.01 wt% for both p(MADQUAT\textsubscript{25}-s/b-MMA\textsubscript{75}), 0.1 wt% for both p(MADQUAT\textsubscript{50}-s/b-MMA\textsubscript{50}) and 0.25 wt% for both p(MADQUAT\textsubscript{75}-s/b-MMA\textsubscript{25}). These results demonstrate that as the ratio of MADQUAT (positive charge moiety) increases the (cac) values obtained increases. The aggregation behavior for both types of polymers will get easier when MMA (hydrophobic) segments increase. p(MADQUAT\textsubscript{x}-s/b-MMA\textsubscript{y}) polymers dissolved in water which is a good solvent for MADQUAT (the hydrophilic segment), but not for MMA (the hydrophobic segment) and that leads to aggregation formation in which MMA forms the core while MADQUAT forms the corona. This conformation minimizes the interaction of MMA with water and the existence of the hydrophilic segment helps to stabilize the formed micelles. Further study is recommended into this concentration regime to reveal more information about the conformational property of the polymer aggregates and chains. Techniques such as TEM and AFM will be of benefit in this regard.

Inspection of the graphs (figure 4.1 and 4.2) reveals that in the dilute region, reduction in the surface tension of water in the aqueous solution commences when micelles start to form and not before that. Above the critical aggregation concentration, a decline in the surface tension of water is observed. The expected behaviour was a reduction in the surface tension by addition of polymers molecules which expected to adsorb at the air/water interface. That means simply that these polymers are non-surface active. This is demonstrated by the values of the surface tension that are too close to that of water in the dilute regime. Many authors reported on the non-surface activity of certain polymers.\cite{2-4} Thus Ghosh et. al.\cite{4} confirmed the ‘non-surface activity’ for cationic amphiphilic di-brock copolymers. These authors confirmed the formation of micelle and investigated its behaviour by various techniques including surface tension, light scattering and fluorescence probe. This phenomena was also reported by Matsuoka et al and Iddon et. al.\cite{5,6} The ionic nature of these polymers which have quaternary ammonium -\(\text{N}^+\)(CH\textsubscript{3})\textsubscript{3} and counter ions Cl\textsuperscript{-}, could be the main factor that creates this behaviour. However, other factor besides the ionic nature need to be mentioned. The formation of polymers micelles in the absence of surface activity can be explained by the image charge effect theory, i.e. this theory suggests a creation of a similar charge and valency at the symmetric position with the interface.\cite{5,7} This normally occurs into system that involves two media with different dielectric constants. In this study the interface involves water and air (dielectric constant \(\varepsilon\textsubscript{w} = 72\), dielectric constant \(\varepsilon\textsubscript{a}=1\))\cite{5}, respectively. And the
charged polymer chains should be localized close to the air/water interface for polymer adsorption to take place. Assuming that $Q$ is the charge located in the medium having higher dielectric constant, the image charge $Q'$ can be expressed by

$$Q' = -\left(\frac{\varepsilon_a - \varepsilon_w}{\varepsilon_a + \varepsilon_w}\right) Q \tag{1}$$

Substituting the dielectric constants of water and air in equation (1), yields $Q' = 0.957Q$.  

![Figure 4.3. Schematic representation of an image charge effect at the air/water interface.](image)

The production of an image charge of the MADQUAT chains which have the same sign and valency near the air/water interface, creates a strong electrostatic repulsion from the interface for cationic polymers chains (see Fig. 4.3) which prevent polymer molecules to adsorb at the air/water interface. Furthermore, the electrostatic repulsion forces polymer molecules to go into the water bulk phase where it starts to form micelles aggregation, because of the existence of hydrophobic (MMA) segments in the polymer chain. That is clearly seen from figs. 4.1 & 4.2, as the ratio of MADQUAT increases the curve show lower surface tension values close to water’s surface tension. This explains the mechanism by which a polymer can form micelle in the absence of adsorption at the interface. Then, at higher concentration polymers become surface active at which they start to adsorb at the air/water interface leading to a clear reduction on the surface tension of water.

**4.3 Pyrene Fluorescence Spectroscopy**

The value of $I_3/I_1$ relay on the environments of pyrene a significant change in the peak intensity ratio $I_3/I_1$ indicates the formation of the critical aggregation concentration (cac).
Figure 4.4. Pyrene fluorescence curves as function of the logarithm concentrations of p(MADQUAT$_x$-s-MMA$_y$), T= 25°C; solvent-water.

Figure 4.5. Pyrene fluorescence curves as function of the logarithm concentrations of p(MADQUAT$_x$-b-MMA$_y$), T= 25°C; solvent-water.

4.4 Interaction between surface tension and pyrene fluorescence spectroscopy

Fluorescence probe is a useful technique that can be used with surface tension to investigate cac of amphiphilic copolymers in aqueous solutions.

Figures 4.6 – 4.11, summarize the results obtained in this section for all the polymers studied. Each of these figures is a plot of $I_3/I_1$ and surface tension as a function of the concentration of each of the polymers used.
4.4.1 Statistical Polymers; poly(MADQUAT₂₅-s-MMA₇₅)

a) Poly(MADQUAT₂₅-s-MMA₇₅)

Figure 4.6. Variation of the I₃/I₁ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT₂₅-s-MMA₇₅), T= 25 °C; solvent-water.

b) Poly(MADQUAT₅₀-s-MMA₅₀)

Figure 4.7. Variations of the I₃/I₁ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT₅₀-s-MMA₅₀), T= 25 °C; solvent-water.

c) Poly(MADQUAT₇₅-s-MMA₂₅)

Figure 4.8. Variation of the I₃/I₁ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT₇₅-s-MMA₂₅), T= 25 °C; solvent-water.
4.4.2 Di-block; Poly (DMCx-b-MMAy)

a) Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75})

Figure 4.9. Variation of the $I_3/I_1$ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}), $T= 25^\circ$C; solvent-water.

b) Poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50})

Figure 4.10. Variation of the $I_3/I_1$ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}), $T= 25^\circ$C; solvent-water.

c) Poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25})

Figure 4.11. Variation of the $I_3/I_1$ ratio and surface tension as a function of polymer concentration for; poly (MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}), $T= 25^\circ$C; solvent-water.
4.4.3 Discussion

Figures 4.4 - 4.5, show that the variation in the intensity ratio $I_3/I_1$ is significantly different when di-block polymers are compared to that of statistical copolymers. Thus, while a slight increase in the values of $I_3/I_1$ are observed for the statistical polymers in the concentration range of 1.01 to 1.09, $I_3/I_1$ values increases significantly for the di-block polymers as the concentration increase from 0.99 to 1.4. The difference in both polymers fluorescence behavior it could be due to the difference in polymer aggregation, di-block polymers form micelles while statistical copolymers could just form a loose aggregation. As the mole ratio of MMA increases (more hydrophobic environment) a higher value of the intensity ratio $I_3/I_1$ was obtained.

The results obtained here as shown by Figs (4.6 – 4.11), display that at low concentrations (0.0001-0.01 wt%), the pyrene fluorescent is weak as indicated by the slight change in the values of $I_3/I_1$ ratio as pyrene is hydrophobic molecules and insoluble in water. In the dilute regime where the polymer concentrations is low, the interactions between MMA, the hydrophobic moiety in the amphiphilic polymer chains are expected to be weak (no micelle formed at these concentrations), so the hydrophobic core is not big enough to catch the hydrophobic pyrene molecule to solubilise it. In this concentration regime also the surface tension values as indicated previously, show non-surface active behaviour at low concentration. A sharp increase in $I_3/I_1$ ratio is observed at which the critical concentration (cmc) value is reached, and this is significant and more prominent for all the di-block polymers investigated. Also, the surface tension curves show the same significant change at nearly the same concentration for the polymers under investigation. At this concentration aggregation and micelles start to occur in the bulk solution. Pyrene, is hydrophobic molecules which will resides in the MMA core of the formed micelles, hence the large increase in the ratio of $I_3/I_1$. The $I_3/I_1$ curves in most of the cases are nearly a mirror image of that of the surface tension curves.

Sun, G. et al., examined the surface tension and pyrene fluorescence for the amphiphilic cationic random copolymers that contain MADQUAT, p(MADQUAT$_x$-co-SMA$_y$) which also synthesized by conventional free-radical polymerization. They have found that the critical aggregation concentration (cac) values mainly effected by the hydrophobicity of the
amphiphilic copolymers, as the SMA(hydrophobic) molar ratio increase the lower (cac) obtained which agreed with this study results.9

**Table.4.2.** Tabulated (cac) values. Results obtained by Surface tensiometry and Pyrene fluorescence.

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>p(MADQUAT&lt;sub&gt;x&lt;/sub&gt;-b-MMA&lt;sub&gt;y&lt;/sub&gt;)</th>
<th>p(MADQUAT&lt;sub&gt;x&lt;/sub&gt;-s-MMA&lt;sub&gt;y&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface tension</td>
<td>0.25 0.1 0.01</td>
<td>0.25 0.1 0.01</td>
</tr>
<tr>
<td>Pyrene fluorescence</td>
<td>0.1 0.01 0.01</td>
<td>0.1 0.25 0.1</td>
</tr>
</tbody>
</table>

**4.5 DLS and Zeta potential**

Dynamic light scattering was used to determine the hydrodynamic diameters ($D_h$) and $\zeta$-potential. DLS measurements were performed at 25 °C.

Figure 4.10 below shows the variation in Zeta potential as a function of the molar ratio of MADQUAT in the polymers investigated.

![Zeta potential graph](image)

**Figure 4.12.** Zeta potential of pMADQUAT, p(MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>) and p(MADQUAT<sub>x</sub>-b-MMA<sub>y</sub>) as a function of molar ratio of MADQUAT and polymer structures, T= 25 °C; solvent-water.

Figure 4.12 above, shows that at at higher MADQUAT molar ratio and for constant loading of 1 wt%, the Zeta potential increases. This is due to an increase in the cationic moiety $\text{–N}^+(\text{CH}_3)_3$ and Cl$^-$ ions. In addition the particel diameter as determined by DLS decreases as MMA molar ratio increases. This is because of two reasons:

1. The reduced surface charge and the lower electrostatic repulsion of polymer chains results in a more compact polymer configuration.
2. Water is nonsolvent for MMA that leads to aggregation formation into MMA core and MADQUAT corona to minimize the interaction of MMA with water. Tables 4.3-4.5 summarize the results obtained in this section for the polymers investigated. Polymers that have a high concentrations and high ratio of the positive moiety; pMADQUAT, p(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}) and p(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25}) show higher Zeta potential values which means more stable particles. It is known that molecules having Zeta potential values higher than +30 mV are stable particles.\textsuperscript{10,11} In contrast, as the concentration and ratio of MMA increases obviously the Zeta potential and size decrease. Di-bock polymers have less charge density than statistical copolymers because they have lower particles size as a result of micelle formations which have a particle size range from 27 to 200 nm while statistical copolymers range from 150 to 500 nm (see tables; 4.4 and 4.5). From tables 4.3, 4.4 and 4.5 it can be seen that a low concentration a large particle size is observed, i.e. the expected size for a single extended polymer molecule must be around 25 nm that could be a results of impurities or dust existence.
**Table 4.3.** Particle size, PDI and Zeta potential of pMADQUAT, T=25 °C in water.

<table>
<thead>
<tr>
<th>Homo-polymer</th>
<th>Sample</th>
<th>Z-average(d.nm)</th>
<th>PDI</th>
<th>Zeta potential(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/pMADQUAT</td>
<td>0.001</td>
<td>291.5</td>
<td>0.444</td>
<td>28.0 ± 22.8</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>151.4</td>
<td>0.695</td>
<td>37.0 ± 22.3</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>213.9</td>
<td>0.599</td>
<td>36.9 ± 13.4</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>133.8</td>
<td>0.554</td>
<td>45.1 ± 17.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>178.9</td>
<td>0.391</td>
<td>56.6 ± 5.02</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>126.1</td>
<td>0.499</td>
<td>60.3 ± 7.60</td>
</tr>
</tbody>
</table>

**Table 4.4.** Particle size, PDI and Zeta potential of di-block polymers, T=25 °C in water.

<table>
<thead>
<tr>
<th>Di-block polymers</th>
<th>Sample</th>
<th>Z-average(d.nm)</th>
<th>PDI</th>
<th>Zeta potential(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/poly(MADQUAT&lt;sub&gt;75&lt;/sub&gt;-b-MMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>0.001</td>
<td>178.2</td>
<td>0.584</td>
<td>9.33 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>199.3</td>
<td>0.432</td>
<td>17.3 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>218.7</td>
<td>0.285</td>
<td>37.3 ± 9.10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>180.2</td>
<td>0.293</td>
<td>31.2 ± 15.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>173.3</td>
<td>0.280</td>
<td>46.3 ± 12.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>140.1</td>
<td>0.267</td>
<td>50.3 ± 12.3</td>
</tr>
<tr>
<td>2) poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-b-MMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>0.001</td>
<td>169.4</td>
<td>1.000</td>
<td>9.31 ± 3.32</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>112.7</td>
<td>0.806</td>
<td>8.84 ± 4.61</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>67.77</td>
<td>1.000</td>
<td>19.7 ± 7.10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>81.30</td>
<td>1.000</td>
<td>39.7 ± 17.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>71.05</td>
<td>0.355</td>
<td>43.4 ± 13.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>27.85</td>
<td>0.820</td>
<td>35.8 ± 13.8</td>
</tr>
<tr>
<td>2) poly(MADQUAT&lt;sub&gt;75&lt;/sub&gt;-b-MMA&lt;sub&gt;75&lt;/sub&gt;)</td>
<td>0.001</td>
<td>151.4</td>
<td>0.420</td>
<td>3.97 ± 1.23</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>92.77</td>
<td>0.914</td>
<td>14.7 ± 3.14</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>59.30</td>
<td>0.985</td>
<td>20.69 ± 13.4</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>36.96</td>
<td>0.755</td>
<td>29.2 ± 12.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>43.13</td>
<td>0.966</td>
<td>30.0 ± 11.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30.26</td>
<td>0.791</td>
<td>31.9 ± 13.2</td>
</tr>
</tbody>
</table>
### Table 4.5. Particle size, PDI and Zeta potential of statistical copolymers, T=25 °C in water.

<table>
<thead>
<tr>
<th>Statistical polymers</th>
<th>Sample</th>
<th>Z-average (d.nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/poly(MADQUAT_{75-s-MMA}_{25})</td>
<td>0.001</td>
<td>260.1</td>
<td>0.704</td>
<td>40.9 ±15.3</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>168.6</td>
<td>1.000</td>
<td>41.4 ±13.9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>141.1</td>
<td>1.000</td>
<td>50.8 ±7.47</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>200.1</td>
<td>1.000</td>
<td>54.8 ±6.78</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>247.2</td>
<td>0.625</td>
<td>52.9 ±7.76</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>478.2</td>
<td>0.737</td>
<td>51.2 ±9.88</td>
</tr>
<tr>
<td>2) poly(MADQUAT_{50-s-MMA}_{50})</td>
<td>0.001</td>
<td>135.8</td>
<td>0.910</td>
<td>41.9 ±6.15</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>241.5</td>
<td>0.632</td>
<td>36.5 ±12.6</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>229.8</td>
<td>0.620</td>
<td>55.9 ±5.07</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>142.0</td>
<td>0.523</td>
<td>50.9 ±18.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>262.9</td>
<td>0.558</td>
<td>51.1 ±15.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>283.2</td>
<td>0.658</td>
<td>35.4 ±14.5</td>
</tr>
<tr>
<td></td>
<td>310.1</td>
<td>0.599</td>
<td>46.1 ±12.4</td>
<td></td>
</tr>
<tr>
<td>2) poly(MADQUAT_{25-s-MMA}_{75})</td>
<td>0.001</td>
<td>236.6</td>
<td>0.521</td>
<td>15.4 ±11.1</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>286.4</td>
<td>0.447</td>
<td>39.2 ±12.9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>376.7</td>
<td>1.000</td>
<td>32.8 ±13.1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>206.6</td>
<td>0.652</td>
<td>40.7 ±15.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>276.1</td>
<td>1.000</td>
<td>47.1 ±35.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>312.0</td>
<td>0.701</td>
<td>42.9 ±16.2</td>
</tr>
</tbody>
</table>
4.6 Viscosity

The viscosities of di-block polymers and statistical polymers’ solutions with 1 M of NaCl were measured using Ubbelohde capillary viscometer at T = 25 °C ± 1. Viscosity of polymers is affected by many factors including: the chemical structure, the size and the charge density as well as by the solvent properties such as, the ionic strength and the solvent polarity. Definitions of the different viscosities are provided in table 4.5.

Table 4.6. Viscosity definitions.\textsuperscript{15}

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative viscosity</td>
<td>$\eta_{rel} = \frac{\eta}{\eta_0} = \frac{t}{t_0}$</td>
</tr>
<tr>
<td>Specific viscosity</td>
<td>$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \frac{t - t_0}{t_0}$ = $\eta_{rel}$ - 1</td>
</tr>
<tr>
<td>Reduced viscosity</td>
<td>$\eta_{red} = \frac{\eta_{sp}}{c} = \frac{\eta_{rel} - 1}{c}$</td>
</tr>
<tr>
<td>Inherent viscosity</td>
<td>$\eta_{inh} = \frac{ln \eta_{rel}}{c}$</td>
</tr>
<tr>
<td>Intrinsic viscosity</td>
<td>$[\eta] = \left(\frac{\eta_{sp}}{c}\right)<em>{c=0} = \left(\eta</em>{inh}\right)_{c=0}$</td>
</tr>
</tbody>
</table>

4.6.1 Relative Viscosity

Viscosity of a polymer is dependent on its concentration and particle size. This is to be expected since the disruption of solvents' flow lines by the solute molecules leads to an increase in the fluid viscosity. Figures 4.13, illustrates the difference of the relative viscosity of various polymers’ aqueous solutions as a function of concentrations (g/dL).

General trend for studied polymers, the viscosity of a polymer aqueous solution increases gently with an increase in concentration. A dramatic increase in viscosity is observed when the concentration exceeds the critical aggregation concentration value.
Figure 4.13. Changes in the Relative viscosity of block and statistical copolymers’ aqueous solutions measured at various mass concentrations (g/dL) at 25°C with 1M NaCl.

In the dilute regime, the polymer chains are freely expanding and far apart from each other, hence less the interactions between polymer chains. While, in the high concentration the viscosity increases because of an increase in the interactions between polymer-polymer and polymer-solvent molecules. Further inspection of fig. 4.13 reveals large differences between the di-block polymers and the statistical copolymers viscosities. This is due to the difference in the molecular weight and conformation of the polymers, see table 4.1. Larger molecules lead to higher resistance to the flow than smaller molecules.

Log dynamic viscosity/Pa.s vs log c , for all polymers were plotted (Figs. 4.14 & 4.15) aiming to further investigation of the concentration regimes which helps to separate the dilute from the semi-dilute un-entangled and semi-dilute entangled regimes. Looking closely at the curves in figure 4.14, and 4.15 one can distinguish between three concentration regimes. First regime, the dilute regime (lower concentration) where the polymer chains are separated from each other and their interaction is kept to a minimum. In this concentration regime the viscosities are low for all the polymers investigated. The second regime starts as we pass a concentration of 0.1 (g/dL) for statistical polymers and 0.25 (g/dL) for di-block polymers (c^* concentration), where the increase in viscosity becomes more significant This concentration regime is considered to be the overlap
concentration regime. In this regime, the transition from the dilute to the semi-dilute regime occurs, at which the polymer chains overlapped and start to form aggregations.

![Diagram showing concentration regimes](image)

**Figure 4.14.** Log viscosities/(mPa.s) of the diblock polymers plotted as a function of log concentration/(g/dL) for aqueous solutions at 25°C.

The third concentration regime starts at around 1 g/dL for di-block polymers and 0.5 g/dL for statistical polymers, where a significant increase in the viscosity of the polymer solution is observed which could be regarded as the critical aggregation concentration (c**). In this concentration regime, polymer chains form aggregations as a result of the reduction in the 'free' volume which restricts the movement of polymers' chains.\(^{16}\) For different M\(_W\) grades of PMMA, Gupta et. al.\(^{16}\) plotted the curves of zero shear rate viscosity(Pa.s) as function of concentration, in order to do further investigation of the concentration regimes; the
dilute, the semi-dilute unentangled and semi-dilute entangled regimes. The different viscosity behaviour between statistical and di-block polymers proves the difference in polymers' conformations; statistical copolymers which show higher molecular weight, particles size and viscosity tend to form loose aggregations while di-block polymers tend to form micelle. Sun, G. et al.\textsuperscript{9} investigated self-assembly of a series of amphiphilic random copolymers p(MADQUATx-co-SAMy) in aqueous solution that have been synthesised via free radical polymerization using TEM. They demonstrated that these polymers aggregate into a core of SAM and corona of MADQUAT to stabilize micelles in water (see figure 4.16).

![Figure 4.16. TEM image of micelle aggregation of p(MADQUATx-co-SAMy) in water (1.26 g/L).\textsuperscript{9}](image)

### 4.6.2 Determination of Intrinsic Viscosity

The intrinsic viscosity is defined as the capability of the polymer to enhance the viscosity of the solution.\textsuperscript{17} The commonly used procedure for calculating the value of intrinsic viscosity is via dual extrapolation of $\eta_{rel}$ and $\ln \eta_{rel}$ to zero concentration (see figure 4.1).

#### Table 4.7. Different equations used to determine intrinsic viscosity, $[\eta]$.  

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huggins\textsuperscript{18}</td>
<td>$\frac{\eta_p}{c} = [\eta] + k' [\eta]^2 c$</td>
</tr>
<tr>
<td>Kraemer\textsuperscript{18}</td>
<td>$\frac{\ln \eta_{rel}}{c} = [\eta] + k'' [\eta]^2 c$</td>
</tr>
<tr>
<td>Tanglerpaibul and Rao\textsuperscript{19}</td>
<td>$1/\eta_{rel} = 1 + [\eta]c$; $2/\eta_{rel} = e \eta c$</td>
</tr>
<tr>
<td>Fuoss\textsuperscript{20, 21}</td>
<td>$\eta_{sp}/c = [\eta]/(1+BC^{1/2})$</td>
</tr>
<tr>
<td>Fedors\textsuperscript{21, 22}</td>
<td>$1/[2(\eta_{rel}^{1/2} - 1)] = 1/[\eta]C - 1[\eta]C_m$</td>
</tr>
</tbody>
</table>

Different methods have been used to calculate the intrinsic velocity (table 4.7), aiming to
find an estimate for the cac of polyelectrolytes in aqueous media. A solvent of 1M NaCl is used to prepare polymers solutions to determine the value of intrinsic viscosity of polyelectrolyte solutions as it can be performed only in aqueous solutions containing a high electrolyte concentration in order to supress the electrostatic forces within the cationic polymers leading to neutral polymers solutions behaviour.\textsuperscript{23}

\textbf{Figure 4.17.} The reduced viscosity $\eta_{sp}/c$ vs. c for block and statistical copolymers in water/1M NaCl solutions at 25 °C.

Figure 4.17 presents the variation of the reduced viscosity ($\eta_{sp}/c$) values as function of polymers’ concentration (from 0.01 to 6 g/dL) in aqueous solution of 1 M NaCl. At low concentrations (0.01,0.1,0.25,0.5 g/dL) all tested polymers had the typical polyelectrolytes behaviour even with the existence of salts. Similar results have been reported by Yang, H. et al.\textsuperscript{24}. The figure shows that in general and for all the solutions investigated the viscosity of solutions with higher molecular weight and higher molar ratio of MADQUAT were higher than those with higher MMA molar ratio within the concentration range studied. That can be explained by the following: in the dilution regime as the distance between quaternary ammonium c ions $-N^+(CH_3)_3$ and counter ions Cl$^-$ generated by ionization is relatively large, restriction on counter ions by the quaternary ammonium ions is kept at minimum. Simultaneously, an increase in the charge density of polymeric ions and counter ions and
electrostatic repulsion between polymeric ions in polymer chain which yields to its extension of polymer chains causing a rise of reduced viscosity.\textsuperscript{25,26}

Furthermore, the wall-effect\textsuperscript{24,27-29} which is based on the theory of polymer adsorption cannot be ignored. According to this theory, the increase of relative viscosity at low concentration is caused by polymer molecules adsorption on the surface of capillary. This adsorption causes a considerable reduction in the effective diameter of viscometer capillary. The presence of an adsorbed film of a polymer solution on the capillary wall that used for measuring viscosity was demonstrated by AFM study conducted by Zhong et al.\textsuperscript{27} The adsorption of positively charged polymer into the negatively charged capillary surface is possible (fig.4.18).

**Figure 4.18.** Adsorption of polymer molecules (+) on the capillary walls (-).

However, the wall-effect is not accepted as an explanation for this behaviour, the formed film must be thick enough to be able to affect the results plus the wall-effect must exist significantly at higher concentrations as the polymers molecules concentrations are higher, so the formed film is thicker. Hence that behaviour at lower concentration can be explained by measurements errors or the existence of some impurities.

In the concentration range (1-4 g/dL) all polymers curves show natural polymers behaviour. The values of reduced viscosity increase with increasing polymers’ concentrations. Above (5 g/dL) a drastic increase in the reduced viscosity is observed and that could be due to critical aggregation behaviour, especially for p(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}}) which contains a high molar ratio of the hydrophilic segment. Hence, to determine the intrinsic viscosity of polymers the Huggins-Kraemer plots in concentrations range (1 to 4 g/dL) were plotted. Figure 4.19 shows the Huggins-Kraemer plots for p(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}}).
Figure 4.19. Huggins/Kraemer plot for p(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25}) with extrapolation to $t=0$ used to determine intrinsic viscosity $[\eta]$.

The Huggins/Kraemer plots of all the polymers investigated are provided in the appendices (figures 9.10-9.14). Table 4.6 below summarizes the intrinsic viscosities obtained in this study for the polymers under investigation.

Higiro J. et al. (2005)\textsuperscript{19} and others \textsuperscript{18,30} reported a method for the determination of the intrinsic viscosity based on slopes of the plots of $\eta_{rel}$, $\ln(\eta_{rel})$ and $1 - 1/\eta_{rel}$ vs. polymer concentration which yielded plot relationships with reasonably large linear regression coefficients (Figure 4.20).

Figure 4.20. Plots of $\eta_{rel}$, $\ln(\eta_{rel})$ on and $1 - 1/\eta_{rel}$ of pMADQUAT\textsubscript{75}-s-MMA\textsubscript{25}) as function of polymer concentration.

The other plots of $\eta_{rel}$, $\ln(\eta_{rel})$ and $1 - 1/\eta_{rel}$ function of polymer concentration are provided in the appendices (figures 9.15-9.20).

The values obtained by using these models are different (see table 4.7). However, they showed similar trends, the values of intrinsic viscosity estimated by using $\eta_{rel}$ vs. $c$ plot were larger than the values that estimated via $\ln(\eta_{rel})$ and $1 - 1/\eta_{rel}$ vs. $c$ plots. Dragan. S
and Ghimici L.\textsuperscript{21} obtained similar polyelectrolytes behaviour, hence they applied Fuoss\textsuperscript{20} and Fedors\textsuperscript{22} equations to linearized the reduced viscosity curves. Polyelectrolytes curves plotted by the Fuoss equation (figure 4.21) shows straight lines for all studied polymers over a wide range of concentration. While, the reduced viscosity curve does not linearize when using Fedors equation. (see figure 9.21 and 9.22 in appendices).

![Figure 4.21. Representation of Fuoss equation for p(MADQUAT\textsubscript{x}-s-MMA\textsubscript{y}) and p(MADQUAT\textsubscript{x}-b-MMA\textsubscript{y}) as function of polymer concentration.]

Table 4.7, summarizes the values of the intrinsic viscosities obtained for the polymers investigated, estimated from the plots mentioned earlier.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Model</th>
<th>Huggins $\eta_{sp}/c$</th>
<th>Kraemer $\ln \eta_{ret}/c$</th>
<th>Tanglerpaibul and Rao $\ln \eta_{ret}$</th>
<th>$1-1/\eta_{ret}$</th>
<th>Fuoss $c/\eta_{sp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>p(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25})</td>
<td>0.544</td>
<td>0.528</td>
<td>1.04</td>
<td>0.34</td>
<td>0.14</td>
<td>1.57</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50})</td>
<td>0.286</td>
<td>0.264</td>
<td>0.22</td>
<td>0.13</td>
<td>0.08</td>
<td>4.29</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{25}-s-MMA\textsubscript{75})</td>
<td>0.259</td>
<td>0.234</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>5.55</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25})</td>
<td>0.189</td>
<td>0.258</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>4.66</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50})</td>
<td>0.163</td>
<td>0.149</td>
<td>0.07</td>
<td>0.05</td>
<td>0.04</td>
<td>4.33</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75})</td>
<td>0.199</td>
<td>0.179</td>
<td>0.11</td>
<td>0.08</td>
<td>0.06</td>
<td>4.29</td>
</tr>
</tbody>
</table>

It can be seen from the data presented in table 4.7 that with the exception of Fuoss method, an increase in MADQUAT ratio in the statistical copolymers and di-block polymers always
yields higher values of intrinsic viscosity. The higher charge density causes more unfolded and expanded macromolecular conformations. Similar trend was reported for the copolymers of MADQUAT with 2-hydroxyethylacrylate.\textsuperscript{21} Fuoss equation results show the opposite, as the concentration of MADQUAT increases the intrinsic viscosity decreases. To conclude, intrinsic viscosity $[\eta]$ estimated from the Huggins method is the most reliable and reasonable value compared with other used methods.

The concentration range used in this study may have encompassed the overlap concentration, at which the departure from the dilute to the semi-dilute region occurs. The value of $c^*$, “which is equal to the inverse of the intrinsic viscosity is defined as the overlap concentration” and is estimated by applying $c^* = 1/[\eta]$ by Flory for flexible Gaussian coil.\textsuperscript{21,31}

<table>
<thead>
<tr>
<th>Polymers</th>
<th>$c^* = 1/[\eta]$ (g/dL)</th>
<th>Huggins</th>
<th>Kraemer</th>
<th>Tanglerpaibul and Rao</th>
<th>Fuoss</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S.T</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{75} -s-MMA\textsubscript{25})</td>
<td>1.8</td>
<td>1.9</td>
<td>1</td>
<td>2.9</td>
<td>7.1</td>
<td>0.64</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{50} -s-MMA\textsubscript{50})</td>
<td>3.5</td>
<td>3.8</td>
<td>4.5</td>
<td>7.7</td>
<td>12.5</td>
<td>0.23</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{25} -s-MMA\textsubscript{75})</td>
<td>3.9</td>
<td>4.3</td>
<td>25</td>
<td>33.3</td>
<td>50</td>
<td>0.18</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{75} -b-MMA\textsubscript{25})</td>
<td>5.3</td>
<td>3.9</td>
<td>12.5</td>
<td>16.7</td>
<td>25</td>
<td>0.21</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{50} -b-MMA\textsubscript{50})</td>
<td>6.1</td>
<td>6.7</td>
<td>14.3</td>
<td>20</td>
<td>25</td>
<td>0.23</td>
</tr>
<tr>
<td>p(MADQUAT\textsubscript{25} -b-MMA\textsubscript{75})</td>
<td>5</td>
<td>5.6</td>
<td>9</td>
<td>12.5</td>
<td>16.7</td>
<td>0.23</td>
</tr>
</tbody>
</table>

The values of $[\eta]$ that are estimated via Fuoss equation results in a closer value of $c^*$ to that experimentally estimated from log v vs. log c, surface tension and pyrene fluorescence.

The overlap concentration ($c^*$) is lower in statistical copolymers which have higher molecular weight compared with di-block polymers. Polymers with low molecular weight chains have a less occupied hydrodynamic volume which also confirmed by DLS, hence to induce overlap a higher concentration is required.\textsuperscript{16}
4.7 Summary and Conclusion

There was a variation in the surface tension of the polymers studied; namely, di-block polymers and statistical copolymers as a function as function of polymers’ concentration in aqueous media. The figures 4.1 and 4.2 reveal that there is a plateau region at low concentrations where the surface tension shows nearly no change with concentration after which a decline is observed after a specific concentration which varies from one polymer to the other. The cac values determined from surface tension curves are 0.01 for both p(MADQUAT_{25}-s/b-MMA_{75}) , 0.1 for both p(MADQUAT_{50}-s/b-MMA_{50}) and 0.25 for both p(MADQUAT_{75}-s/b-MMA_{25}). These results demonstrate that as the ratio of MADQUAT (positive charge moiety) increases the cac values obtained increases.

The intensity ratio $I_3/I_1$ is significantly different when di-block polymers are compared to that of statistical copolymers. Thus, while a slight increase in the values of $I_3/I_1$ are observed for the statistical polymers in the concentration range of 1.01 to 1.09, $I_3/I_1$ values increases significantly for the di-block polymers as the concentration increase from 0.99 to 1.4. The difference in both polymers fluorescence behavior it could be due to the difference in polymer aggregation, di-block polymers form micelles while statistical copolymers could just form a loose aggregation. As the mole ratio of MMA increases (more hydrophobic environment) a higher value of the intensity ratio $I_3/I_1$ was obtained.

At higher MADQUAT molar ratio and for constant loading of 1 wt%, the Zeta potential increases. This is due to an increase in the cationic moiety $–N^+(CH_3)$ and Cl$^-$ ions leading to larger particle size which measured bt DLS.

To determine intrinsic viscosity different equations were used; Huggins, Kraemer, Tanglerpaibul & Rao, Fuoss and Fedors. The values of $[\eta]$ that estimated via Fuoss equation results in a closer value of c* to the value of c* that experimentally estimated by log v vs. log c, surface tension and pyrene fluorescence.

As conclusion polymers structures, molecular weight, molar ratio of MADQUAT and MMA and synthesis route have a great effect on polyelectrolytes solution behaviour in aqueous solutions.
Figure 4.22. Schematic presentation of the difference between amphiphilic statistical copolymers and diblock polymers aggregation.32

4.8 References

Chapter 5
Phase Separations of Like Charged Polyelectrolyte and Cationic Surfactant
5.1 Introduction

The interaction between biocidally active surfactant (DDAC) and 3 different types of polyelectrolytes have been explored. At high concentrations a phase separation of the mixtures is induced producing two phases; the top phase is surfactant rich and the bottom phase is polyelectrolyte rich and this was confirmed by $^1$H NMR.

Different phase separation boundaries are observed; di-block polymers induce phase separation at higher concentrations of DDAC and this could be explained by the difference in polymers conformations and properties.

This study aims to study phase separation phenomena that occurs in an aqueous mixture of DDAC; didecyldimethyl ammonium chloride which is considered as quaternary ammonium surfactant and three different polymers listed below:

1/ Homo-polymer; PMADQUAT

2/ Series of statistical copolymers; poly(MADQUAT$_x$-s-MMA$_y$)

3/Series di-block polymers; poly(MADQUAT$_x$-b-MMA$_y$)

in order to explore the factors that affect the phase separation in these mixtures.
5.1.1 Cationic Surfactants

Surfactants can be defined “amphiphilic molecules that contain a hydrophilic head group and a hydrophobic tail”. DDAC is a cationic surfactant that will be used in this study; it contains quaternary ammonium with a corresponding counter ion (chloride) as part of the head group.1,2

5.1.2 Phase Separation in Polyelectrolytes and Cationic Surfactant

At higher concentrations phase separation is obtained by addition of polyelectrolytes with cationic surfactant. As a result, the solutions separate into two layers; polyelectrolytes-rich layer (lower) and surfactant-rich layer (upper).

Phase separations of like charged polyelectrolyte and cationic surfactant has been reported.3-6 For example; Kalwarczyk. E. et al3, have investigated four different types of mixtures; a) anionic polyelectrolytes and anionic surfactant) b) cationic polyelectrolytes and cationic surfactant, c) cationic polyelectrolytes and non-ionic surfactant) and e) anionic polyelectrolytes and non-ionic surfactant. They found that phase separation can be induced by two strategies; 1) by addition of like charge ionic polyelectrolytes and surfactant, 2) by addition of inorganic salt to non-ionic water-soluble polymers. Phase separation in ternary mixtures of water /surfactant /polymer is driven by different force; adding water-soluble polymers to the mixture of water and surfactant induce attractive interaction between micelles, which considered is as the depletion interaction which is caused by entropy changes.3

Phase separation exists in mixtures of like charged quaternary ammonium polyelectrolytes/surfactant/water and it depends on both concentrations of polymers and surfactant and in this research the effect of polymers structures and molar ratio of cationic moiety will be investigated which is the first part on investigating the effect of these factors in phase separation behaviour.
5.2 Phase Separation Experiments

5.2.1 Materials

The used polymers in this study were synthesised and characterized as discussed in chapter 3. See table 5.1. The surfactant didecyldimethyl ammonium (DDAC), was obtained from Lonza (Tradename-Lonza Bardac 2240), as a 40 wt.% solution in water. Sodium chloride (NaCl) (Fisher Scientific, analytical reagent grade). All chemicals were used as received without any further purification.

Table 5.1. Mw, Mn and PDI of used polymers in phase separation experiment which determined by Aqueous GPC.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>$M_n$ (g.mol$^{-1}$)</th>
<th>$M_n$ (g.mol$^{-1}$)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo-polymers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMADQUAT (RAFT)</td>
<td>15,000</td>
<td>9,000</td>
<td>1.6</td>
</tr>
<tr>
<td>Statistical polymers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{75}$-s-MMA$</em>{25}$)</td>
<td>35,000</td>
<td>11,000</td>
<td>3</td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</td>
<td>24,000</td>
<td>8,000</td>
<td>2.9</td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{25}$-s-MMA$</em>{75}$)</td>
<td>7,000</td>
<td>3,000</td>
<td>2</td>
</tr>
<tr>
<td>Di-block polymers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$M_n$ (g.mol$^{-1}$)</td>
<td>PDI</td>
<td>Mn (g.mol$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GPC</td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{75}$-b-MMA$</em>{25}$)</td>
<td>5,300</td>
<td>1.3</td>
<td>4,100</td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</td>
<td>4,800</td>
<td>1.3</td>
<td>3,400</td>
</tr>
<tr>
<td>Poly(MADQUAT$<em>{25}$-b-MMA$</em>{75}$)</td>
<td>3,200</td>
<td>1.2</td>
<td>2,600</td>
</tr>
</tbody>
</table>

Table 5.2. Didecyldimethylammonium Chloride (DDAC)

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didecyldimethylammonium Chloride (DDAC)</td>
<td>$\text{R} = \text{C}<em>{10}\text{H}</em>{21}$</td>
</tr>
</tbody>
</table>
5.2.2 Preparation of surfactant-polymers solutions

Different stock solutions were prepared for both polymers (0.25, 0.5, 1, 1.5 and 3 wt%) and DDAC (7, 10, 15, 20 and 25 wt%). Millipore filtered water was used for all solutions. Fresh solutions were always used for all experiments in 10 ml polypropylene tubes. Samples were prepared at desired concentrations from the appropriate stock solutions. Then, they were stirred for 40-60 mins. with a magnetic stirrer. Phase separation was determined after 24-48 hrs.

By mixing cationic surfactant DDAC and three types of cationic polymers’ structures; homo-polymer, statistical copolymers and di-block polymers (with different molar ratios of MADQUAT cationic moiety), phase separation was induced depending on the concentration of both cationic polymers and cationic surfactant and polymers’ properties.

5.2.3 Determination of critical phase separation concentration

A series of (6 or 5) ml solutions were prepared as following: at constant concentration (0.13, 0.25, 0.5, 0.75 or 1) of polyelectrolyte was mixed with different concentration of the surfactant. The solutions were stirred for 40-60 mins. with a magnetic stirrer. Phase separation was determined after 24-48 hrs. The lowest concentration of surfactant that induced phase separations was determined as the critical phases separation concentration, see figure 5.1.

![Surfactant Rich Phase](image)

![Polyelectrolyte Rich Phase](image)

\[ t=24 \text{ hrs.} \]

**Figure 5.1.** Phase separation boundary for PMADQUAT (0.75 wt %) / DDAC (3 wt. %) / water at 25 °C.

Proton \(^1\)H NMR was used to investigate the extent and nature of the phase separation process. In all cases, the top phase was found to be surfactant rich while the bottom phase
was found to be polyelectrolytes rich. This is shown for PMADQUAT (0.75 wt%) / DDAC (3 wt%) / water at 25 °C in figure 5.1.

**Figure 5.2.** $^1$H NMR of top phase and bottom phase for PMADQUAT (0.75 wt %) / DDAC (3 wt %) water sample, 24 hrs. since last agitation.

**Figure 5.3.** $^1$H NMR of top phase and bottom phase for poly(MADQUAT$_{50}$-b-MMA$_{50}$) (0.75 wt%) / DDAC (3 wt%) water sample, 24 hrs. since last agitation.

**Figure 5.4.** $^1$H NMR of top phase and bottom phase for poly(MADQUAT$_{50}$-b-MMA$_{50}$) (0.75 wt%) / DDAC (5 wt%) water sample, 24 hrs. since last agitation.
a/ Phase Separation behaviour of the Homo-polymers; PMADQUAT

![Graph showing phase separation concentration behavior](image)

**Figure 5.5.** Determination of critical phase separation concentration of the homo-polymers PMADQUAT / DDAC / Water solutions.

As the polymer concentration increases less concentration of DDAC is required to induce phase separation. That could be explained by considering that a critical ionic strength must be reached in each mixture to affect the separation. At higher concentration of the cationic polymer PMADQUAT there are more ions in the mixture (\(N^+(CH_3)_3\) and Cl") from the polymer so less ions are needed from the surfactant to reach the critical ionic strength to induce the phase separation.

b/ Phase Separation behaviour of statistical copolymers

Similar procedure was followed with statistical copolymers to determine the critical phase separation concentration and figure 5.6 shows all homo-polymer and statistical copolymers polymers together.
Chapter 5. Phase Separations of Like Charged Polyelectrolyte and Cationic Surfactants

Figure 5.6. Phase separation diagram of the statistical copolymers; poly (MADQUAT<sub>x</sub>-s-MMA<sub>y</sub>) and DDAC. Data points illustrate the phase separation boundaries.

Figure 5.7. The critical phase separation concentrations as function of the molar ratio of the cationic moieties (MADQUAT) of the homo-polymer and statistical polymers.

The data shows that as the hydrophilic (MADQUAT) molar ratio increases the concentration of polyelectrolyte that is needed to introduce the phase separation decreases. From the phase diagram, it could be said that the molar ratio of MADQUAT (cationic moiety) has a great effect in the phase boundary.

That could be explained as following: for homo-polymer, PMADQUAT the number of counter-ion (Cl<sup>-</sup>) and the positive charges are higher (one counter ion for every two carbon
atoms). But when the molar ratio of MMA increases there will be less counter-ion plus less charge density as there will be one counter-ion for every 4 carbon atoms for poly (MADQUAT_{50-s-MMA_{50}}) and every 8 carbon atoms for poly (MADQUAT_{25-s-MMA_{75}}).

c/ Phase Separation behaviour of Block Polymers

Three block polymers were synthesized by using reversible addition fragmentation chain transfer process (RAFT) as explained in chapter 3.

Similar steps were followed to study phase separation in these classes of cationic polymers. In block polymers the phase separation experiments were conducted using three concentrations of polyelectrolytes (0.25, 0.5 and 1 wt %).

Figure 5.8. Phase separation diagram of di-block polymers; poly (MADQUAT_{x-b-MMA_{y}}) and DDAC. Data points illustrate the phase separation boundaries.
5.3 Comparison of phase separation data

Figure 5.9. Comparing between Phase separation diagram of homo-polymers PMADQUAT, three statistical copolymers and di-block polymers.

Figure 5.10. Data points illustrate the effect of the molar ratio of MADQUAT and polymers’ structures on phase separation boundaries, at constant concentration (1 wt% of all polymers).

From fig. 5.10, it is clear that block polymers separate at higher concentration of DDAC. We can see that the block polymer, poly(MADQUAT<sub>50</sub>-b-MMA<sub>50</sub>) separates at similar
concentration as that of statistical copolymer, poly(MADQUAT$_{25}$-s-MMA$_{75}$). That could be explained by the different polymers solution behavior (polymers conformation) which have been confirmed in the previous chapter. As well as statistical copolymers have higher zeta potential values than diblock polymers. Hence, less DDAC is needed for statistical polymers to increase the ionic strength to reach the critical ionic strength that is needed to induce phase separation. Use of techniques such as SEM and TEM are needed to help to understand more about polymers’ conformations.

5.4 Discussion

To explore the effect of polymers’ structures, molar ratio of the cationic segment(MADQUAT) and concentrations phase separation was studied in a range DDAC/polymers mixtures in aqueous media. The critical phase separation concentration of these mixtures of homo-polymer, statistical copolymers and di-block polymer with DDAC were determined.

The phase separation behaviour can be caused by the electrolyte driving force where the head-group (quaternary ammonium) of surfactant (DDAC) is decreasing in size because of surrounding ionic atmosphere leading to condensation of the counter ion. Mixtures separated into two layers, top-layer, where the DDAC form immiscible oil which is not soluble in water and due to its lower density it creams into the top layer. In this layer there will be absence or reduction in the polymers molecules, hence the surfactant molecules become soluble within this layer. Is not possible to know if phase separation induced just by reducing the ionic atmosphere around the head-group of the surfactant or by forming the actual intimate ion pair. An intimate ion pair is when quaternary ammonium group the charged segment is in a direct contact with its counter ion leading to neutralize the ionic charge, hence further investigation is needed on this aspect to clarify the mechanism. We believe that the reason why surfactant/homo-polymer need lower DDAC concentration (lower ionic strength) than statistical copolymers which also need lower than di-block polymers to induce phase separation is due to the polymer different conformations, size and zeta potential values. Furthermore, phase separation behaviour can be induced by the effect of entropic depletion interactions. Which is result from the changes in the conformational entropy of the polyelectrolytes chains that limit the chains from being too close to micelle. Hence, when the space between two micelles is close enough to prevent the polymer chain from splitting them the area between the micelles is said to be depletion zone. An osmotic
pressure will be induced by the polyelectrolyte which is located outside the depletion zone between micelles leading to push micelles together and induce phase separation behaviour.\textsuperscript{2,3,6} A number of studies of similar charged colloid/polymer mixtures and low salinity have shown that the depletion interaction is occurred at much lower concentrations that needed in the neutral systems.\textsuperscript{7-10}

5.5 Summary and conclusion

In this work the phase separation has been studied for three types of mixtures of like charge surfactant/polymer systems, i.e. cationic surfactants(DDAC) and three cationic polymers; (i) homo-polymer, (ii) statistical copolymer (iii) and di-block polymers. As a solvent, pure water was used. The phase separation can be induced by addition of cationic polyelectrolyte having a charge of the same sign as that of surfactant. In each case the system separates into bottom polyelectrolyte-rich and upper surfactant-rich phase with different phase separation boundaries and that was confirmed by \textsuperscript{1}H NMR.

It was concluded that phase separation behaviour is affected by polymers’ properties such as zeta potential, molecular weight size and the concentrations of both cationic polymer and surfactant.

5.6 References

Chapter 5. Phase Separations of Like Charged Polyelectrolyte and Cationic Surfactants


Chapter 6

A Study of Various Classes of Antibacterial Polymers Bearing Quaternary Ammonium Salts
Chapter 6. A Study of Various Classes of Antibacterial Polymers Bearing Quaternary Ammonium Salts

6.1 Introduction

This chapter reports a study of the antibacterial activity of various classes of polymer bearing [2-(methacyloyloxy) ethyl] trimethyl ammonium chloride (MADQUAT) with different chemical structures. Most of them were synthesised as described in Chapter 3 and one series of core-shell nanoparticles with various alkyl side groups was provided by Dr. Zhuo Yang.

Homopolymers: PMADQUAT; PEGMA
A series of statistical copolymers: poly(MADQUAT\(_x\)-s-PEGMA\(_y\));
A series of statistical copolymers: poly(MADQUAT\(_x\)-s-MMA\(_y\));
A series of di-block polymers: poly(MADQUAT\(_x\)-b-MMA\(_y\));
And a series of core-shell quaternary nanoparticles with various alkyl side groups.

These polymers were synthesized with a variety of structures, molecular weights, length of alkyl side groups and amphiphilic balance (MADQUAT, MMA and PEGMA), aiming to discover those with the properties best able to improve the antibacterial activity of the amphiphilic polymers.

6.1.1 Polymers with quaternary ammonium salts as antibacterial agents

Polymers with quaternary ammonium salts have been tested and used in various antimicrobial-relevant applications,\(^1\) due to their wide spectrum of activity against bacteria, yeasts, viruses and fungi.\(^2\) In addition, polymeric antibacterial materials overcome the drawbacks of conventional antibacterial agents, as they are more effective, have less residual toxicity,\(^3,4\) have longer-lasting antibacterial activity\(^5\) and are much more tolerant towards the development of microbial resistance.\(^6,7\)

Quaternary ammonium compounds (QACs) are polyatomic materials that have a positively charged nitrogen atom covalently bonded to four alkyl groups (NR\(_4^+\)). The covalent bonding of the carbons to the nitrogen group means that they do not dissociate in acid-base reactions, and therefore offer the QAC a stable permanent positive ionic charge independent of the pH of the solution in which they may be.\(^8\)
6.1.2 Designing antibacterial polymeric materials

Three main factors must be taken into account when designing improved antimicrobial agents: (1) the outer cytoplasmic membrane of bacteria, which is crucial to their survival, (2) the antibacterial mechanism of cationic polymers and (3) the relation of the chemical and physical structure of polymers to their efficacy as antibacterial agents. 6

Palermo et al. report that synthetic polymers are becoming increasingly used in various applications due to researchers’ ability to tune their chemical and physical properties.9 The new synthesized polymers used in this study have optimal amphiphilicity, which comes from positively charged nitrogen (MADQUAT) and the hydrophobic moiety (MMA) which considered an untoxic monomer.10 This feature permits these polymers to interact strongly with bacterial membranes as a first step in killing the bacteria.6,11 Other important factors are molecular weight,11-13 charge density,14-16 counter-ions17-19 and the length of the alkyl hydrophilic chain.20,21

6.1.3 Mechanism of action

Various polycations have been reported to possess antimicrobial properties and it was further suggested that the mechanism of action involves interaction with and disruption of the integrity of the cell membrane.22,23 The mechanism is still not understood on a detailed molecular level, but it is believed that the antimicrobial action does not involve specific receptor-mediated interactions.24 The following sequence of elementary steps has been suggested to be associated with the mode of lethal action of cationic biocides:12,25

- Adsorption of the cationic polymers onto the negatively charged bacterial outer membrane, i.e. adhesion to anionic proteins of the membrane: the lipopolysaccharides and the negatively charged phosphate head of the phospholipid bilayer of the outer membrane
- Penetration of the bacterial cell wall, causing diffusion of the cytoplasmic membrane;20,26-28
- Translocation and adsorption, followed by binding to the cytoplasmic membrane; Disruption of the integrity of the cytoplasmic membrane and its disintegration,
- Leakage of the cytoplasmic contents, including electrolytes such as potassium ions and phosphate, as well as the release of nucleic materials such as DNA and RNA,27
- Death of the cell.
At levels of minimum inhibitory concentration (MIC), there are specific consequences to this mode of action: the biocide is integrated into the membrane, altering the phase-transition temperature. The bacterial membrane is normally fluid at physiological temperatures, but the interaction of QACs causes it to develop a liquid crystalline structure, resulting in the loss of physiological and osmoregulatory functions.\(^{29}\)

### 6.1.4 Chemical structure and molecular weight of polymers

In order to investigate the relationship between molecular weight (Mw) and antibacterial activity, the Mw of each cationic polymer used in this study was determined by aqueous gel permeation chromatography (GPC). The results are summarized in Table 6.1 and Table 6.2.

**Table 6.1.** Summary of M\(_w\), M\(_n\) and PDI of homo and statistical synthesized polymers by Aqueous GPC.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>Polymers</th>
<th>M(_w) (g.mol(^{-1}))</th>
<th>M(_n) (g.mol(^{-1}))</th>
<th>PDI</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo-polymer</td>
<td>PMADQUAT (via Free radical polymerization)</td>
<td>541,000</td>
<td>68,000</td>
<td>7.9</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>PMADQUAT (via RAFT polymerization)</td>
<td>15,000</td>
<td>9,000</td>
<td>1.6</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td>Poly(MADQUAT(_{95})-s-PEGMA(_5))</td>
<td>329,000</td>
<td>16,000</td>
<td>20.8</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{75})-s-PEGMA(</em>{25}))</td>
<td>148,000</td>
<td>8,000</td>
<td>17.6</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{75})-s-MMA(</em>{25}))</td>
<td>35,000</td>
<td>11,000</td>
<td>3</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{50})-s-MMA(</em>{50}))</td>
<td>24,000</td>
<td>8,000</td>
<td>2.9</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{25})-s-MMA(</em>{75}))</td>
<td>7,0000</td>
<td>3000</td>
<td>2</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{50})-s-MMA(</em>{50}))</td>
<td>4,800</td>
<td>3400</td>
<td>1.3</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
<tr>
<td></td>
<td>Poly(MADQUAT(<em>{25})-s-MMA(</em>{75}))</td>
<td>3,200</td>
<td>2600</td>
<td>1.2</td>
<td><img src="image" alt="Chemical structure" /></td>
</tr>
</tbody>
</table>
Table 6.2. Summary of $M_w$, $M_n$ and PDI of synthesized di-block polymers tested for antibacterial activity by Aqueous GPC, H NMR and Target values.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>Polymers</th>
<th>$M_w$ (g.mol$^{-1}$)</th>
<th>PDI</th>
<th>$M_n$ (g.mol$^{-1}$)</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-block polymers</td>
<td>1) Poly(MADQUAT$<em>{75}$s-$\text{MMA}</em>{25}$)</td>
<td>5,300</td>
<td>1.3</td>
<td>4,100</td>
<td>18,100</td>
</tr>
<tr>
<td></td>
<td>2) Poly(MADQUAT$<em>{50}$s-$\text{MMA}</em>{50}$)</td>
<td>4,800</td>
<td>1.3</td>
<td>3,400</td>
<td>15,100</td>
</tr>
<tr>
<td></td>
<td>3) Poly(MADQUAT$<em>{25}$s-$\text{MMA}</em>{75}$)</td>
<td>3,200</td>
<td>1.2</td>
<td>2,600</td>
<td>12,800</td>
</tr>
</tbody>
</table>

6.2 Antibacterial testing

The polymers obtained in this study were tested against both gram-negative and gram-positive bacterial strains in order to determine MIC values. The MIC is defined as the minimum concentration of polymer solution that inhibits bacterial growth after overnight incubation. Bacteria form biofilm that can be defined as “an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material”.

6.2.1 Determination of planktonic MICs of cationic polymers

Figures 6.1 to 6.11 show the effects of cationic polymers with different structures and mole ratios of MADQUAT, all with the pH adjusted to 7, on the planktonic form of four types of gram-negative bacteria; E. coli K 12, E. coli CI 2, Klebsiella pneumoniae and Pseudomonas aeruginosa and one type of gram-positive bacteria: Staphylococcus aureus. Planktonic growth was assessed by microtitre assay. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.2.1.1 Homopolymers

Figures 6.1-6.2 show the effect of Homo-polymers on the planktonic of three types of gram-negative bacteria and and one type of gram-positive bacteria.

**a) PMADQUAT**

The effect of Homo-polymer; PMADQUAT on the planktonic growth.

![Graph](Figure 6.1).

Figure 6.1. The effect of homopolymer; PMADQUAT on planktonic growth was assessed by microtitre assay on *E.coli K12*, *E.coli clinical isolate*, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**b) PEGMA**

The effect of Homo-polymer; PEGMA on the planktonic growth.

![Graph](Figure 6.2).

Figure 6.2. The effect of homopolymer; PEGMA on planktonic growth was assessed by microtitre assay on *E.coli K12*, *E.coli clinical isolate*, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.2.1.2 Statistical copolymers
Figures 6.3-6.8 show the effect of statistical copolymers on the planktonic of three types of gram-negative bacteria and and one type of gram-positive bacteria.

a) Poly(MADQUAT<sub>x-s</sub>-MMA<sub>y</sub>)
The effect of series of the statistical copolymer; Poly(MADQUAT<sub>x-s</sub>-MMA<sub>y</sub>) on the planktonic growth, (see figures 6.3-6.5).

1) Poly(MADQUAT<sub>25-s</sub>-MMA<sub>75</sub>)
The effect of Poly(MADQUAT<sub>25-s</sub>-MMA<sub>75</sub>) on the planktonic growth.

![Figure 6.3.](image)

Figure 6.3. The effect of statistical copolymer; poly(MADQUAT<sub>25-s</sub>-MMA<sub>75</sub>) on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, S.aureus and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) Poly(MADQUAT<sub>50-s</sub>-MMA<sub>50</sub>)
The effect of Poly(MADQUAT<sub>50-s</sub>-MMA<sub>50</sub>) on the planktonic growth.

![Figure 6.4.](image)

Figure 6.4. The effect of statistical copolymer; poly(MADQUAT<sub>50-s</sub>-MMA<sub>50</sub>) on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, S.aureus and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
3) Poly(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}})

The effect of Poly(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}}) on the planktonic growth.

**Figure 6.5.** The effect of statistical copolymer; poly(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}}) on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
b) Poly(MADQUAT<sub>x</sub>-<sub>s</sub>-PEGMA<sub>y</sub>)

The effect of series of the statistical copolymer; Poly(MADQUAT<sub>x</sub>-<sub>s</sub>-PEGMA<sub>y</sub>) on the planktonic growth, (see figures 6.6-6.8).

1) Poly(MADQUAT<sub>50</sub>-<sub>s</sub>-PEGMA<sub>50</sub>)

The effect of Poly(MADQUAT<sub>50</sub>-<sub>s</sub>-PEGMA<sub>50</sub>) on the planktonic growth.

![Figure 6.6](image1)

*Figure 6.6. The effect of statistical copolymer; poly(MADQUAT<sub>50</sub>-<sub>s</sub>-PEGMA<sub>50</sub>) on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.*

2) Poly(MADQUAT<sub>75</sub>-<sub>s</sub>-PEGMA<sub>25</sub>)

The effect of Poly(MADQUAT<sub>75</sub>-<sub>s</sub>-PEGMA<sub>25</sub>) on the planktonic growth.

![Figure 6.7](image2)

*Figure 6.7. The effect of statistical copolymer; poly(MADQUAT<sub>75</sub>-<sub>s</sub>-PEGMA<sub>25</sub>) on planktonic growth was assessed by microtitre assay on E.coli K12, E.coli clinical isolate, K. pneumoniae and P. aeruginose. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.*
**Chapter 6. A Study of Various Classes of Antibacterial Polymers Bearing Quaternary Ammonium Salts**

3) **Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5})**

The effect of Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5}) on the planktonic growth.

![Graph showing the effect of Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5}) on planktonic growth.]

**Figure 6.8.** The effect of statistical copolymer; poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5}) on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**6.2.1.3 Cationic amphiphilic di-block polymers: Poly(MADQUATx-b-MMAy)**

Figures 6.9-6.11 show the effect of di-block polymers on the planktonic of three types of gram-negative bacteria and one type of gram-positive bacteria.

1) **Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75})**

The effect of Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on the planktonic growth.

![Graph showing the effect of Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on planktonic growth.]

**Figure 6.9.** The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
2) Poly(MADQUAT\textsubscript{50-b-MMA\textsubscript{50}})

The effect of Poly(MADQUAT\textsubscript{50-b-MMA\textsubscript{50}}) on the planktonic growth.

![Figure 6.10](image)

Figure 6.10. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{50-b-MMA\textsubscript{50}}) on planktonic growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S.aureus} and \textit{P. aeruginosa}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

3) Poly(MADQUAT\textsubscript{75-b-MMA\textsubscript{25}})

The effect of Poly(MADQUAT\textsubscript{75-b-MMA\textsubscript{25}}) on the planktonic growth.

![Figure 6.11](image)

Figure 6.11. The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{75-b-MMA\textsubscript{25}}) on planktonic growth was assessed by microtitre assay on \textit{E.coli K12}, \textit{E.coli clinical isolate}, \textit{S.aureus} and \textit{P. aeruginosa}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.3 Determination of biofilm MICs of cationic polymers

Figures 6.12 to 6.22 show the effects of cationic polymers with different structures and mole ratios of MADQUAT, all with the pH adjusted to 7, on biofilms of four types of gram-negative bacteria; *E. coli K 12*, *E. coli CI*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and one type of gram-positive bacteria: *Staphylococcus aureus*. Biofilm growth was assessed by microtitre assay. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

6.3.1 Homopolymers

Figures 6.12-6.13 show the effect of Homo-polymers on the biofilms growth of three types of gram-negative bacteria and and one type of gram-positive bacteria.

1) PMADQUAT

The effect of PMADQUAT on the biofilm growth.

*Figure 6.12.* The effect of homopolymer; PMADQUAT on biofilm growth was assessed by microtitre assay on *E.coli K12*, *E.coli clinical isolate*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
2) PEGMA

The effect of PEGMA on the biofilm growth.

![Figure 6.13](image_url)

**Figure 6.13.** The effect of homopolymer PEGMA on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

### 6.3.2. Statistical copolymers

Figures 6.14-6.19 show the effect of the statistical copolymers on the biofilms growth of three types of gram-negative bacteria and one type of gram-positive bacteria.

**a) Poly(MADQUATx-s-MMAy)**

The effect of series of the statistical copolymer, Poly(MADQUAT$_{x}$-s-MMA$_{y}$) on the biofilms growth, (see figures 6.14-6.16).

**1) Poly(MADQUAT$_{25}$-s-MMA$_{75}$)**

The effect of Poly(MADQUAT$_{25}$-s-MMA$_{75}$) on the biofilms growth.
Chapter 6. A Study of Various Classes of Antibacterial Polymers Bearing Quaternary Ammonium Salts

Figure 6.14. The effect of statistical copolymer; poly(MADQUAT<sub>25</sub>-s-MMA<sub>75</sub>) on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) Poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>)

The effect of Poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>) on the biofilms growth.

Figure 6.15. The effect of statistical copolymer; poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>) on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
3) Poly(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25})

The effect of Poly(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25}) on the biofilms growth.

![Figure 6.16](image)

Figure 6.16. The effect of statistical copolymer; poly(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25}) on biofilm growth was assessed by microtitre assay on *E.coli* K12, *E.coli* clinical isolate, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

b) Poly(MADQUAT\textsubscript{x}-s-PEGMA\textsubscript{y})

The effect of series of the statistical copolymer; Poly(MADQUAT\textsubscript{x}-s-MMA\textsubscript{y}) on the biofilm growth, (see figures 6.17-6.19).

1) Poly(MADQUAT\textsubscript{50}-s-PEGMA\textsubscript{50})

The effect of Poly(MADQUAT\textsubscript{50}-s-PEGMA\textsubscript{50}) on the biofilm growth.
Figure 6.17. The effect of statistical copolymer; poly(MADQUAT\textsubscript{50}-s-PEGMA\textsubscript{50}) on biofilm growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) Poly(MADQUAT\textsubscript{75}-s-PEGMA\textsubscript{25})

The effect of Poly(MADQUAT\textsubscript{75}-s-PEGMA\textsubscript{25}) on the biofilm growth.

Figure 6.18. The effect of statistical copolymer; poly(MADQUAT\textsubscript{75}-s-PEGMA\textsubscript{25}) on biofilm growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

3) Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5})

The effect of Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5}) on the biofilm growth.

Figure 6.19. The effect of statistical copolymer; poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5}) on biofilm growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.3.3 Cationic amphiphilic di-block polymers: poly(MADQUATx-b-MMAy)

Figures 6.20-6.22 show the effect of di-block polymers on the planktonic of three types of gram-negative bacteria and and one type of gram-positive bacteria.

1) Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75})

The effect of Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on the biofilm growth.

![Graph showing the effect of Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on biofilm growth.](image)

**Figure 6.20.** The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75}) on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) Poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50})

The effect of Poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) on the biofilm growth.

![Graph showing the effect of Poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) on biofilm growth.](image)

**Figure 6.21.** The effect of cationic amphiphilic di-block polymer; poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
3) Poly(MADQUAT$_{75}$-b-MMA$_{25}$)

The effect of Poly(MADQUAT$_{75}$-b-MMA$_{25}$) on the biofilm growth.

![Figure 6.22](image_url)

**Figure 6.22.** The effect of cationic amphiphilic di-block polymer; poly(MADQUAT$_{75}$-b-MMA$_{25}$) on biofilm growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
### 6.4 Discussions

#### 6.4.1 MICs of polymers

Tables 6.3 and 6.4 summarise the MIC values of all tested polymers for bacteria in planktonic and biofilm forms respectively.

**Table 6.3.** MIC values of cationic polymers for planktonic bacteria.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>MIC (wt%)</th>
<th>Bacterial strains (planktonic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em>&lt;br&gt;K12</td>
</tr>
<tr>
<td>Homopolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEGMA</td>
<td>Not effective as antibacterial (enhances bacteria growth)</td>
<td></td>
</tr>
<tr>
<td>PMADQUAT</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Di-block Polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;25-b-MMA&lt;sub&gt;75&lt;/sub&gt;)</td>
<td>0.001</td>
<td>1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50-b-MMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75-b-MMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;25-s-MMA&lt;sub&gt;75&lt;/sub&gt;)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50-s-MMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75-s-MMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50-s-PEGMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>0.1</td>
<td>Reduced to 60%</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75-s-PEGMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;95-s-PEGMA&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>0.1</td>
<td>Reduced to 6%</td>
</tr>
<tr>
<td>Polymer class</td>
<td>MIC (wt%)</td>
<td>E. coli K12</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Homo-polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEGMA\textsubscript{100}</td>
<td>Not effective in killing bacteria (enhances biofilm growth)</td>
<td></td>
</tr>
<tr>
<td>PMADQUAT\textsubscript{20}</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td>Di-block polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{25}-b-MMA\textsubscript{75})</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50})</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25})</td>
<td>0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{25}-s-MMA\textsubscript{75})</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50}-s-MMA\textsubscript{50})</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75}-s-MMA\textsubscript{25})</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50}-s-PEGMA\textsubscript{50})</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75}-s-PEGMA\textsubscript{25})</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{95}-s-PEGMA\textsubscript{5})</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
6.4.1.1 Comparing homopolymers and statistical copolymers

Tables 6.3 and 6.4 show that PEGMA was not an effective antibacterial homopolymer, while adding MADQUAT to statistical copolymers in different molar ratios improved its antibacterial activity. Homopolymer PMADQUAT killed all types of planktonic bacteria tested at around 0.25 wt% and reduced the formation of biofilm significantly at 0.1 wt%. As to statistical copolymers of MADQUAT and MMA, it can be seen that the optimal molar ratio is 50:50. Poly(MADQUAT\textsubscript{50-s-MMA\textsubscript{50}}) has the lowest MIC values on all tested bacterial strains. A possible explanation is that could be the optimal amphiphilic balance for these polymers, which plays a crucial role in the antimicrobial activity of the biocidal polymer\textsuperscript{11,14,32} i.e. cationic moiety of polymer(MADQUAT) is able to bind to the negatively charged bacterial cell surfaces efficiently due to the strong electrostatic attraction while the existence of MMA helps to penetrate the outer membrane. Thus, statistical copolymers of MADQUAT and MMA have better MIC values than homopolymer; PMADQUAT. All statistical polymers of the poly(MADQUAT\textsubscript{x-s-MMA\textsubscript{y}}) type were found to inhibit biofilm growth of both \textit{E. coli} K12 and \textit{E. coli} Cl 2 in the range 0.001-0.1 wt%, of \textit{S. aureus} from 0.001 to 0.5 wt% and of \textit{P. aeruginosa} from 0.01 to 0.25 wt%. Therefore, these polymers were effective as antibacterial agents in inhibiting the growth of all of the bacterial strains tested.

6.4.1.2 Comparing amphiphilic di-block polymers and statistical copolymers

The best polymers at inhibiting \textit{E. coli} K12 were poly(MADQUAT\textsubscript{25-b-MMA\textsubscript{75}}) and poly(MADQUAT\textsubscript{50-s-MMA\textsubscript{50}}), both effective at 0.001 wt%. All block polymers inhibited \textit{S. aureus} at 0.01 wt%, while the statistical copolymers did so at the higher concentration range of 0.01-0.25 wt%. Polymers of both types have interesting antibacterial effects on the strains tested and polymer structure was found to have no major effect in improving this activity, only two studies which could be found for investigating the difference between statistical copolymer and block polymers on antibacterial activity which have similar finding.\textsuperscript{33,34} The most important feature of these polymers thus appears to be the presence of MADQUAT, providing a cationic moiety.

Furthermore, the diblock polymers and statistical have different antibacterial action; diblock polymers approved by Y. Oda, et al.\textsuperscript{34} caused dye leakage from lipid vesicles of \textit{E. coli} -type lipids, but not mammalian lipids. While, it was found in random copolymer system
both types of vesicles were disrupted. That is because of different polymers conformations, from chapter 4 results which confirmed that statistical copolymers form a loose aggregate with particle size range from 150 to 500 nm and they have higher molecular weight (see table 6.1) while di-block polymers particle size from 27 to 200 nm. MICs values for most polymers tested were below their observed cac, hence these results suggest that formation of micelle or aggregate is not require to exhibit antibacterial activity.

Figure 6.23 Schematic presentation shows the different between block and statistical copolymers antibacterial action.35
6.5 Study of a series of core-shell nanoparticles quaternary ammonium with various alkyl side groups

Variation in the length of alkyl chains brings with it changes in certain characteristic features and parameters of the polymers under investigation. According to Timofeeva et al. (2011), changing the length of the alkyl chain may boost the adsorption/absorption ability of the polymer, as well as its lipophilicity. This may in turn change the hydrophilic-hydrophobic balance of the polymer, which will also change its biocidal efficacy against different microbes. Table 6.5 lists the side groups of the four core-shell nanoparticles tested and illustrates their structures. Figures 6.24 to 6.28 show their biocidal efficacy against four planktonic gram-negative bacterial strains.

**Table 6.5.** Side chain and particle structure of core-shell quaternary ammonium nanoparticles.

<table>
<thead>
<tr>
<th>Polymer name</th>
<th>Side group</th>
<th>Particle structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY-24</td>
<td>Alkyne particle</td>
<td><img src="image" alt="Particle Structure" /></td>
</tr>
<tr>
<td>ZY-26</td>
<td>C₄H₉</td>
<td><img src="image" alt="Particle Structure" /></td>
</tr>
<tr>
<td>ZY-27</td>
<td>C₈H₁₇</td>
<td><img src="image" alt="Particle Structure" /></td>
</tr>
<tr>
<td>ZY-28</td>
<td>C₁₂H₂₅</td>
<td><img src="image" alt="Particle Structure" /></td>
</tr>
</tbody>
</table>

### 6.5.1 Core-shell quaternary ammonium nanoparticles

Figures 6.24 to 6.28 show the biocidal efficacy of Core-shell quaternary ammonium nanoparticles against four planktonic gram-negative bacterial strains.
1) **ZY/24**

The effect of ZY/24 polymer on planktonic growth.

![Figure 6.24.](image)

**Figure 6.24.** The effect of ZY/24 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12, E. coli clinical isolate, K. pneumoniae and P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) **ZY/26**

The effect of ZY/26 polymer on planktonic growth.

![Figure 6.25.](image)

**Figure 6.25.** The effect of ZY/26 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12, E. coli clinical isolate, K. pneumoniae and P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
3) ZY/27

The effect of ZY/27 polymer on planktonic growth.

Figure 6.26. The effect of ZY/27 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12, E. coli clinical isolate, K. pneumoniae and P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

4) ZY/28

The effect of ZY/28 polymer on planktonic growth.

Figure 6.27. The effect of ZY/28 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12, E. coli clinical isolate, K. pneumoniae and P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.5.2 Antibacterial efficacy of mixtures of core-shell quaternary ammonium nanoparticles

Antibacterial testing revealed that each polymer had a different antibacterial activity against the various planktonic strains. For example, Figure 6.25 shows that ZY/26 was strongly active against *E. coli* K12 and *E. coli* CI 2, resulting in a dramatic decrease in bacterial growth to 5% and 1%. Similarly, Figure 6.26 shows the good antibacterial activity of ZY/27 against all strains tested at 0.07 on *E. coli* K12 and *E. coli* CI 2 and 0.26 wt% on *K. Pneumoniae* and *P. Aaeruginosas*. The core-shell quaternary nanoparticles were then mixed as shown in Table 6.6, to determine whether these mixtures had better antibacterial properties.

Table 6.6. Percentages (%) of each core-shell quaternary ammonium nanoparticles in the mixtures tested.

<table>
<thead>
<tr>
<th>ZY mixtures</th>
<th>ZY/26 polymer</th>
<th>ZY/27 polymer</th>
<th>ZY/28 polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY/36</td>
<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>ZY/37</td>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>ZY/38</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>ZY/39</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1) ZY/36

The effect of ZY/36 polymer on planktonic growth.

![Figure 6.28.](image)

**Figure 6.28.** The effect of ZY/36 polymer on planktonic growth was assessed by microtitre assay on *E. coli* K12, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
2) ZY/37

The effect of ZY/37 polymer on planktonic growth.

![Graph showing the effect of ZY/37 polymer on planktonic growth](image)

**Figure 6.29.** The effect of ZY/37 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

3) ZY/38

The effect of ZY/38 polymer on planktonic growth.

![Graph showing the effect of ZY/38 polymer on planktonic growth](image)

**Figure 6.30.** The effect of ZY/38 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli* clinical isolate, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
4) **ZY/39**

The effect of ZY/39 polymer on planktonic growth.

![Graph showing the effect of ZY/39 polymer on planktonic growth.](image)

**Figure 6.31.** The effect of ZY/39 polymer on planktonic growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *K. pneumoniae*, and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

The results of the mixing experiment show that ZY/36 performed better than the other mixtures in inhibiting growth of on three types of bacteria, with similar MIC values of 0.07 wt%, while *P. aeruginosa* at 0.26%.
6.5.3 Effects of core-shell quaternary ammonium nanoparticles on gram-negative biofilms

The four core-shell quaternary ammonium polymers ZY/24, 26, 27 and 28 were also tested on biofilms of four gram-negative bacterial strains. The results are illustrated in Figures 6.32 to 6.35.

1) ZY/24

The effect of ZY/24 polymer on biofilm growth.

![Figure 6.32.](image)

**Figure 6.32.** The effect of ZY/24 polymer on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

2) ZY/26

The effect of ZY/26 polymer on biofilm growth.

![Figure 6.33.](image)

**Figure 6.33.** The effect of ZY/26 polymer on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
3) **ZY/27**

The effect of ZY/27 polymer on biofilm growth.

![Graph](image)

**Figure 6.34.** The effect of ZY/27 polymer on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

4) **ZY/28**

The effect of ZY/28 polymer on biofilm growth.

![Graph](image)

**Figure 6.35.** The effect of ZY/28 polymer on biofilm growth was assessed by microtitre assay on *E. coli K12*, *E. coli clinical isolate*, *K. pneumoniae* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
6.6 Discussion

Tables 6.7 and 6.8 summarise the MIC values of all polymers tested on bacterial strains in planktonic and biofilm form.

**Table 6.7.** MIC values of Core-shell quaternary ammonium nanoparticles for planktonic bacterial strains.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>MIC (wt%)</th>
<th>E. coli K12</th>
<th>E. coli Cl 2</th>
<th>K. Pneumoniae</th>
<th>P. Aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nanoparticle polymers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/24 polymer</td>
<td>0.7</td>
<td>0.7</td>
<td>0.26</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ZY/26 polymer</td>
<td>0.7</td>
<td>At 0.07</td>
<td>Reduced to 1%</td>
<td>Reduced to 3%</td>
<td>Reduced to 7%</td>
</tr>
<tr>
<td></td>
<td>at 0.007</td>
<td>reduced to 5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/27 polymer</td>
<td>0.07</td>
<td>0.07</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>ZY/28 polymer</td>
<td>0.26</td>
<td>Reduced to 4%</td>
<td>Reduced to 7%</td>
<td>Reduced to 7%</td>
<td></td>
</tr>
<tr>
<td><strong>ZY Mixtures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/36 polymer</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>ZY/37 polymer</td>
<td>0.007</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>ZY/38 polymer</td>
<td>0.26</td>
<td>Reduced to 7%</td>
<td>Reduced to 15%</td>
<td>Reduced to 6%</td>
<td></td>
</tr>
<tr>
<td>ZY/39 polymer</td>
<td>0.07</td>
<td>0.26</td>
<td>0.7</td>
<td>Reduced to 6%</td>
<td></td>
</tr>
</tbody>
</table>
6.6.1. Comparing Core-shell quaternary ammonium nanoparticles and the mixtures

ZY/27 and ZY/28 had higher antibacterial activity than the other ZY polymers against *E. coli* K12, perhaps because their longer alkyl side groups (C₈H₁₇ and C₁₂H₂₅) helped them to penetrate the outer bacterial membranes. Thus, the optimal length of the alkyl chain appears to be 8-12 carbon atoms for ZY polymers. Abel et al. made a similar observation, that long alkyl side groups produced a broad antibacterial activity.³⁶ Many other studies have found that a long alkyl side chain, i.e. at least eight carbon atoms, improves antibacterial activity.¹

ZY/37 (a 50:50 mixture of ZY/27 and ZY/28) had a better MIC value of 0.007 wt% for *E. coli* K12. And ZY/36 inhibits all tested bacterial strains around 0.07wt%. In general, all ZY polymers inhibited the growth of the biofilm of all bacterial strains tested, in the range of 0.07-0.26 wt%.

### 6.7 Summary and conclusion

Tables 6.9 and 6.10 summarise the full results reported above, listing MIC values of all polymers and blends tested on planktonic and biofilm strains respectively.
### Table 6.9. Summary of MIC values of cationic polymers for planktonic strains.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>MIC (Minimum inhibitory concentration)/ (µg/mL)</th>
<th>Bacterial strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli K12</td>
</tr>
<tr>
<td>Homopolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEGMA</td>
<td>Not effective as antibacterial (enhances bacterial growth)</td>
<td></td>
</tr>
<tr>
<td>PMADQUAT</td>
<td>2500</td>
<td>2500</td>
</tr>
<tr>
<td>Di-block polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{25-b-MMA\textsubscript{75}})</td>
<td>10</td>
<td>10000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50-b-MMA\textsubscript{50}})</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75-b-MMA\textsubscript{25}})</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{25-s-MMA\textsubscript{75}})</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50-s-MMA\textsubscript{50}})</td>
<td>10</td>
<td>1000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75-s-MMA\textsubscript{25}})</td>
<td>5000</td>
<td>10000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{50-s-PEGMA\textsubscript{50}})</td>
<td>1000</td>
<td>Reduced to 60%</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{75-s-PEGMA\textsubscript{25}})</td>
<td>100</td>
<td>5000</td>
</tr>
<tr>
<td>Poly(MADQUAT\textsubscript{95-s-PEGMA\textsubscript{5}})</td>
<td>1000</td>
<td>Reduced to 6%</td>
</tr>
<tr>
<td>Nanoparticle polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/24 polymer</td>
<td>7000</td>
<td>7000</td>
</tr>
<tr>
<td>ZY/26 polymer</td>
<td>7000</td>
<td>At 70 reduced to 5%</td>
</tr>
<tr>
<td>ZY/27 polymer</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>ZY/28 polymer</td>
<td>2600</td>
<td>Reduced to 4%</td>
</tr>
<tr>
<td>ZY Mixtures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/36 polymer</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>ZY/37 polymer</td>
<td>70</td>
<td>2600</td>
</tr>
<tr>
<td>ZY/38 polymer</td>
<td>2600</td>
<td>Reduced to 7%</td>
</tr>
<tr>
<td>ZY/39 polymer</td>
<td>700</td>
<td>2600</td>
</tr>
</tbody>
</table>
### Table 6.10. Summary of MIC values of cationic polymers for biofilm strains.

<table>
<thead>
<tr>
<th>Polymer class</th>
<th>MIC (µg/mL)</th>
<th>Bacterial strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli K12</td>
</tr>
<tr>
<td>Homopolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEGMA&lt;sub&gt;100&lt;/sub&gt;</td>
<td>Not effective in killing bacteria (it enhances bacteria biofilm growth)</td>
<td></td>
</tr>
<tr>
<td>PMADQUAT&lt;sub&gt;20&lt;/sub&gt;</td>
<td>10000</td>
<td>10</td>
</tr>
<tr>
<td>Di-block polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;25&lt;/sub&gt;-b-MMA&lt;sub&gt;75&lt;/sub&gt;)</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-b-MMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75&lt;/sub&gt;-b-MMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Statistical copolymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;25&lt;/sub&gt;-s-MMA&lt;sub&gt;75&lt;/sub&gt;)</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-s-MMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75&lt;/sub&gt;-s-MMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-s-PEGMA&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>5000</td>
<td>10000</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;75&lt;/sub&gt;-s-PEGMA&lt;sub&gt;25&lt;/sub&gt;)</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>Poly(MADQUAT&lt;sub&gt;95&lt;/sub&gt;-s-PEGMA&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Nanoparticle polymers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY/24 polymer</td>
<td>70</td>
<td>700</td>
</tr>
<tr>
<td>ZY/26 polymer</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>ZY/27 polymer</td>
<td>700</td>
<td>700</td>
</tr>
<tr>
<td>ZY/28 polymer</td>
<td>700 (at 70 µg/mL reduced to 11%)</td>
<td>700</td>
</tr>
</tbody>
</table>
Different cationic polymers have been tested in inhibiting the growth of both gram-positive and gram-negative bacterial strains. All polymers tested except PEGMA were able to inhibit growth in planktonic bacteria and biofilms. The proposed explanation is that these polymers contain a cationic moiety which becomes electrostatically attached to the outer membrane of the bacteria, while the presence of a hydrophobic moiety (MMA) leads to an increase in antibacterial activities\(^{37}\), i.e. helps to incorporate the polymer into lipid membranes, hence the hydrophilic-hydrophobic structure of polymers induces disruption of the bacterial membranes, leading to breakdown of the transmembrane potential, leakage of cytoplasmic contents and finally the death of the bacterial cell\(^{38}\). Both statistical copolymers and diblock polymers containing MADQUAT showed interesting activity as antibacterial agents. Unexpectedly, there was not such a strong effect of polymer structure as chemical structure on antibacterial activity. A similar study was conducted by Y. Oda, et al.\(^{35}\) which resulted in similar conclusions. However, they proved that the block copolymers have much less haemolytic (i.e. destruction of red blood cells) when they compared to the random copolymers. Hence, they confirmed that the structures of polymers has a crucial role in the antibacterial activity, which causes a different conformation of polymers which leads to have unalike interaction with the outer membrane of bacteria strains. Core-shell quaternary nanoparticles with longer alkyl side groups (C\(_8\)H\(_{17}\) and C\(_{12}\)H\(_{25}\)) had the best antibacterial activity against four classes of bacteria, i.e. *K. Pneumoniae*, *P. Aeruginosa*, *E. coli* K12 and *E. coli* CI 2. The polymer ZY/27 (side group C\(_8\)H\(_{16}\)) was found to have a minimum inhibitory concentration 700 \(\mu g/ml\) or greatly less 700 \(\mu g/ml\) for the bacteria respectively, see figure 6.25. The ZY/36 (Mixture with side groups C\(_4\)H\(_9\), C\(_8\)H\(_{16}\) and C\(_{12}\)H\(_{25}\),33:33:33) was found to have a MIC 700 \(\mu g/ml\) or greatly less 700 \(\mu g/ml\) for different bacteria respectively. As well as, The ZY/37 (Mixture with side groups C\(_8\)H\(_{16}\) and C\(_{12}\)H\(_{25}\),50:50) was found to have a MIC 70 \(\mu g/ml\) on *E.coli* K12. So far, the link between the polymer structure and antibacterial activity has been studied by using different polymers’ structures bearing MADQUAT. So, this study highlights the potential of amphiphilic polymer with different structures and properties as a new design antibacterial polymers that could help to improve the antibacterial activity plus understanding the affect of polymers’ structures on the antibacterial mechanism.
6.8 References


Chapter 7

A Study of the Antibacterial Activity of Tannic Acid and Tannic Acid/Amphiphilic Cationic Polymer Mixtures
Chapter 7. A Study of the Antibacterial Activity of Tannic Acid and Tannic Acid/Amphiphilic Cationic Polymer Mixtures

7.1 Introduction
The tannins are polyphenolic compounds commonly found in plants including cranberry, grape and green tea, and occurring in two forms: hydrolysable and condensed. The commonest hydrolysable tannin is tannic acid (TA), otherwise known as gallotannin or tannin, an anionic organic compound whose molecular weight (1772.57) is relatively high.\textsuperscript{1,2}

A number of applications of tannic acid are widely reported in the literature, including as an antimicrobial material against various types of bacteria, e.g. gram-negative \textit{Pseudomonas aeruginosa}\textsuperscript{3, 4} and gram-positive \textit{Staphylococcus aureus}.\textsuperscript{4, 5} Siddiqui and others used TA with other phenolic compounds, ellagic acid and epigallocatechin, in novel biological strategies to reduce membrane biofouling in waste water and seawater treatment, reporting that these compounds can interrupt biofilm formation. TA had the lowest optical concentration (100 mg L\textsuperscript{-1}) for biofilm control of \textit{P. aeruginosa}.\textsuperscript{3} It has also been used in anti-cancer drug delivery systems\textsuperscript{6} and eco-friendly agents to prepare metal nanoparticles.\textsuperscript{4,7}

The aim of this chapter is to investigate the antibacterial activity of tannic acid and TA/amphiphilic cationic polymer mixtures on strains of both gram-negative (\textit{E. coli} K12, \textit{E. Coli} CI 2, \textit{Klebsiella pneumonia} and \textit{P. aeruginosa}) and gram-positive (\textit{S. aureus}) bacteria.

7.2 Antibacterial activity of tannic acid

A preliminary study tested the antibacterial effects of tannic acid against strains of \textit{E. coli} K12, \textit{E. coli} CI2, \textit{Klebsiella pneumonia}, \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus} planktonic bacteria and in preventing these strains forming biofilms.

7.2.1 Sample preparation

Tannic acid was purchased from Sigma-Aldrich and was used without further purification. A stock solution (3 wt\%) of TA in water was prepared and other desired concentrations were made via dilution. The pH of each TA solution was adjusted to 7. It is important to note that at higher concentrations (from 0.1 to 1 wt\%), TA solutions begin to change colour (to a darker brown) and to form a precipitate, because at higher pH (above 6) tannic acid will hydrolyse in water; carbohydrates (i.e. glucose and polyhydric alcohol) occupy the
central core position in the TA structure, with hydroxyl groups attached to one or more phenolics (i.e. gallic acid, ellagic acid) which are partially hydrolysed into glucose and gallic acid moieties at extremes of pH.\textsuperscript{2,7,8}

### 7.2.2 Bacterial growth assays

A 1/100 dilution of an overnight culture of each bacterial strain was prepared in LB medium, then 200 \( \mu \text{L} \) of each dilution was aliquoted into wells of a flat-bottomed untreated polystyrene 96-well microtitre plate (Greiner Bio-one Ltd., UK, ref. code 655161). Sixteen replicate wells were used for each concentration of inhibitor (0.0001, 0.001, 0.01, 0.1, 0.25, 0.5 and 1 wt%): 8 wells were each inoculated with 100 \( \mu \text{L} \) inoculum alone as positive growth control and 100 \( \mu \text{L} \) of LB medium without any organism was aliquoted into a further 8 wells to act as negative control. All experiments with the microtitre plate were performed with a multichannel pipette. The microtitre plates were incubated overnight at 37 °C, then the optical density (OD 595) of each bacterial cell was quantified via a microplate reader.

### 7.2.3 Preliminary study of tannic acid as antibacterial agent against five strains of planktonic bacteria

Preliminary study of tannic acid on planktonic growth on different types of bacteria strains.

![Figure 7.1.](image-url)

**Figure 7.1.** Preliminary study of tannic acid on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical isolate, S. aureus and P. aeruginose*. The results are expressed as the mean of 8 replicate wells involving one biological replicate for each strain. Error bars indicate the standard error of the mean.
Table 7.1. MIC values of tannic acid at pH 7 for various planktonic bacterial strains.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC of tannic acid (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.001</td>
</tr>
<tr>
<td><em>E. coli</em> CI2</td>
<td>1 (at 0.01 wt% reduced to 17%)</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>At 0.01 reduced to 13%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.01</td>
</tr>
</tbody>
</table>

The results of a preliminary study of tannic acid shown in Figure 7.1 and Table 7.1 reveal interesting antibacterial activity against all bacterial strains tested, especially *E. coli* K12, *P. aeruginosa* and *S. aureus*. These results have two possible explanations: 1) Tannic acid solutions at pH 7 and at low concentration (less than 0.1 wt%) are unaffected by adjusting the pH, while at higher concentrations (0.1 wt% and above) they may break down and form a precipitate. Interference in optical density between such precipitates and bacterial cells may lead to errors and it can be seen clearly in Figure 7.1 that MIC values increased above 0.1 wt%. 2) The optical density of TA solutions are high (see Figure 9.23 in appendix) and when this value is abstracted from the total optical density of tannic acid and bacterial cells, this could reduce the value of the optical density of bacterial cells after treatment.

Therefore, to confirm the antibacterial effectiveness of TA, the experiment was repeated without adjusting the pH, thus avoiding precipitation at higher concentrations, and using only those three strains having low MIC values.
7.2.4 MICs of tannic acid at unadjusted pH for planktonic bacteria

The effect of Tannic Acid on planktonic growth.

![Graph showing MIC values of tannic acid at unadjusted pH for planktonic bacteria](image)

**Figure 7.2.** The effect of Tannic Acid unadjusted for pH on planktonic growth was assessed by microtitre assay on *E.coli K12, E.coli clinical isolate, S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Table 7.2.** MIC values of tannic acid unadjusted for pH for different strains of planktonic bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC of tannic acid (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli K12</em></td>
<td>1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>At 0.1 wt% reduced to 23%</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1 (at 0.1 reduced to 31%)</td>
</tr>
</tbody>
</table>

When TA was unadjusted for pH the MIC values were higher than with pH adjusted to 7, as Figure 7.2 and Table 7.2 show. However, a similar behaviour was observed at values above 0.1 wt%, where MIC began to increase. As noted above, this may be a result of interaction between the OD of TA solutions at higher concentrations and the OD of the bacterial cells. A further experiment was performed on *P. aeruginosa* only, as it showed stronger bacterial growth than the other strains, to clarify the cause of the increase in MIC at higher concentrations. After treatment with TA at different concentrations, *P. aeruginosa* was streaked onto LB agar plates, which were then incubated for 8 hours at 37 °C.
Figure 7.3. Growing *Pseudomonas aeruginosa* bacteria after treatment with tannic acid at concentrations of 0.01, 0.1, 0.25 and 0.5 wt%.

Figure 7.3 shows that treatment with TA reduced bacterial growth significantly at 0.25 wt% and that there was no growth at 0.5 wt%, which helps to explain the increase in MIC values at higher concentrations, which must be because of the high OD of TA solutions.

### 7.2.5 MICs of tannic acid for biofilms

The effect of Tannic Acid on biofilm growth, see figure 7.4.

![Graph of MICs for biofilms](image)

**Figure 7.4.** The effect of Tannic Acid on biofilm growth was assessed by microtitre assay on *E.coli K12, E.coli clinical isolate, S. aureus* and *P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
MICs of TA were higher for bacteria in biofilms. This may be a result of TA precipitation at higher concentrations, which will form a film.

Table 7.3. MIC values of tannic acid against different bacterial strains in biofilm.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC of tannic acid (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.5</td>
</tr>
</tbody>
</table>

7.3 The effect of tannic acid/PMADQUAT homopolymer mixtures on bacteria in planktonic and biofilm form

Having established the antibacterial effects of tannic acid alone, the next step was to study the use of mixtures of TA and cationic polymers against three bacterial strains (*E. coli* K12, *P. aeruginosa* and *S. aureus*) in both planktonic and biofilm forms.

7.3.1 Preparing mixtures of PMADQUAT and tannic acid

Figure 7.5 shows that the presence of PMADQUAT homopolymer allowed clearer and more stable solutions to be produced at high concentrations.

![Image](image.png)

Figure 7.5. Tannic acid (0.5 wt%) in LB medium solutions 1) without PMADQUAT and 2) with PMADQUAT at t=0.

To determine the optimal concentration of TA to be mixed with different concentrations of homopolymer solutions, 0.001, 0.01, 0.1, 0.25, 0.5 and 1 wt% concentrations of TA were mixed with LB medium and 0.01 wt% PMADQUAT (see figure 9.27 in the appendix). The following example describes the preparation of one such concentration: to prepare 0.5 wt%
of PMADQUAT with 0.1 wt% of TA, 5 mL of 2 wt% polymer was mixed with 2 mL of 0.4 wt% TA, then 100 µL of this mixture was mixed with 100 µL of inoculum in each well.

### 7.3.2 MICs of TA/PMADQUAT mixtures for planktonic bacteria

A constant concentration (0.1 wt%) of TA was mixed with different concentrations of PMADQUAT. Their effects on three planktonic bacterial strains are shown in Figure 7.6 and Table 7.4.

![Figure 7.6](image)

**Figure 7.6.** The effect of Tannic Acid (0.1 wt%)/PMADQUAT homopolymer mixtures on planktonic growth was assessed by microtitre assay on E.coli K12, S. aureus and P. aeruginosa. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC of tannic acid/PMADQUAT mixtures (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.01(at 0.0001 reduction into 3%)</td>
</tr>
</tbody>
</table>

**Table 7.4.** MICs of tannic acid/PMADQUAT mixtures for planktonic bacteria

171
7.3.3 MICs of TA/PMADQUAT mixtures for bacteria in biofilms

Figure 7.7 and Table 7.5 show the effect of Tannic Acid/PMADQUAT on biofilm growth.

![Graph showing the effect of Tannic Acid (0.1 wt%)/PMADQUAT on biofilm growth.](image)

**Figure 7.7.** The effect of Tannic Acid (0.1 wt%)/PMADQUAT on biofilm growth was assessed by microtitre assay on *E. coli K12*, *S. aureus* and *P. aeruginosa*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

**Table 7.5.** MICs of tannic acid/PMADQUAT mixtures for bacteria in biofilms.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MICs of tannic acid/PMADQUAT mixtures (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli K12</em></td>
<td>0.01</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.01</td>
</tr>
</tbody>
</table>
7.3.4 Comparing MICs of PMADQUAT, Tannic Acid and TA/PMADQUAT mixtures

Comparing the MICs of pure Tannic Acid, pure homo-polymer, PMADQUAT and the mixture of TA/PMADQUAT on both planktonic and biofilm growths.

7.3.4.1 On planktonic

Table 7.6 summarize all obtained MICs on planktonic growth.

**Table 7.6.** Comparing MICs of PMADQUAT, tannic acid and TA/PMADQUAT mixtures for planktonic bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>PMADQUAT</th>
<th>Tannic acid</th>
<th>TA/PMADQUAT mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.25</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Reduced to 2%</td>
<td>At 0.1 wt% reduced to 23%</td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. aureus.</em></td>
<td>0.25</td>
<td>1 (at 0.1 reduced to 31%)</td>
<td>0.01 (at 0.0001 reduced to 3%)</td>
</tr>
</tbody>
</table>

7.3.4.2 On biofilms

Table 7.7 summarize all obtained MICs on biofilm growth.

**Table 7.7.** Comparing MICs of PMADQUAT, tannic acid and TA/PMADQUAT mixtures for bacteria in biofilms.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>PMADQUAT</th>
<th>Tannic acid</th>
<th>Tannic acid/PMADQUAT mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>1</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td><em>P. Aeruginosa</em></td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>S. Aureus.</em></td>
<td>Reduced to 2%</td>
<td>0.5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Tables 7.6 and 7.7 show that the mixtures of tannic acid and PMADQUAT homopolymer gave better results on all three bacterial strains tested than either PMADQUAT or TA alone.

7.4 The effect of tannic acid/statistical copolymer poly(MADQUAT$_{50}$-s-MMA$_{50}$) mixtures on bacteria; planktonic and biofilm form.

7.4.1 On planktonic

The effect of tannic acid/statistical copolymer poly(MADQUAT$_{50}$-s-MMA$_{50}$) mixtures on bacteria planktonic growth, (Figures 7.8).
Figure 7.8. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT\textsubscript{50}$-\text{s}$-MMA\textsubscript{50}) mixtures on biofilm growth was assessed by microtitre assay on \textit{E. coli K12}, \textit{S. aureus} and \textit{P. aeruginosa}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

7.4.2 On biofilms

The effect of tannic acid/statistical copolymer poly(MADQUAT\textsubscript{50}$-\text{s}$-MMA\textsubscript{50}) mixtures on bacteria biofilm growth, (Figures 7.9).

Figure 7.9. The effect of Tannic Acid (0.1 wt%)/poly(MADQUAT\textsubscript{50}$-\text{s}$-MMA\textsubscript{50}) mixtures on biofilm growth was assessed by microtitre assay on \textit{E. coli K12}, \textit{S. aureus} and \textit{P. aeruginosa}. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
Chapter 7. A Study of the Antibacterial Activity of Tannic Acid and Tannic Acid/Amphipilic Cationic Polymer Mixtures.

7.4.3 Comparing MICs of poly(MADQUAT$_{50}$-s-MMA$_{50}$), tannic acid and TA/poly(MADQUAT$_{50}$-s-MMA$_{50}$) mixtures

Comparing the MICs of pure Tannic Acid, pure statistical copolymer, poly(MADQUAT$_{50}$-s-MMA$_{50}$) and the mixture of TA/poly(MADQUAT$_{50}$-s-MMA$_{50}$) on both planktonic and biofilm growths.

7.4.3.1 On planktonic

Table 7.8 summarize all obtained MICs on planktonic growth.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</th>
<th>Tannic acid</th>
<th>TA/poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$) mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.001</td>
<td>1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
<td>0.1 reduced to 23%</td>
<td>Reduced to 3%</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.01</td>
<td>1 (at 0.1 reduced to 31%)</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

7.4.3.2 On biofilm

Table 7.9 summarize all obtained MICs on planktonic growth.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$)</th>
<th>Tannic acid</th>
<th>TA/poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$) mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.1</td>
<td>1</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
<td>1</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Reduced to 2%</td>
<td>0.5</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Tables 7.8 and 7.9 above show that mixtures of tannic acid and poly(MADQUAT$_{50}$-s-MMA$_{50}$) gave better results than either poly(MADQUAT$_{50}$-s-MMA$_{50}$) or TA alone against gram-positive *S. aureus* bacteria in both planktonic and biofilm forms.
7.5 The effect of tannic acid/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) di-block polymer mixtures on bacteria in planktonic and biofilm form

7.5.1 On planktonic

The effect of tannic acid/di-block polymer poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) mixtures on bacteria planktonic growth, (Figures 7.10).

![Figure 7.10](Image)

**Figure 7.10.** The effect of Tannic Acid (0.1 wt\%)/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) di-block polymer mixtures on planktonic growth was assessed by microtitre assay on *E.coli K12, S. aureus and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.

7.5.2 On biofilms

The effect of tannic acid/di-block polymer poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) mixtures on bacteria biofilm growth, (Figures 7.11).

![Figure 7.11](Image)

**Figure 7.11.** The effect of Tannic Acid (0.1 wt\%)/poly(MADQUAT\textsubscript{50}-b-MMA\textsubscript{50}) mixtures on biofilm growth was assessed by microtitre assay on *E.coli K12, S. aureus and P. aeruginose*. The results are expressed as the mean of 24 replicate wells involving three biological replicates for each strain. Error bars indicate the standard error of the mean.
7.5.3 Comparing the MICs of poly(MADQUAT$_{50}$-b-MMA$_{50}$), tannic acid and TA/poly(MADQUAT$_{50}$-b-MMA$_{50}$) mixtures

Comparing the MICs of pure Tannic Acid, pure di-block polymer, poly(MADQUAT$_{50}$-b-MMA$_{50}$) and the mixture of TA/ poly(MADQUAT$_{50}$-b-MMA$_{50}$) on both planktonic and biofilm growths.

7.5.3.1 On planktonic

Table 7.10 summarize all obtained MICs on planktonic growth.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</th>
<th>Tannic acid</th>
<th>TA/poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$) mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.1</td>
<td>1</td>
<td>0.1 (at 0.0001 reduced to 19%)</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
<td>At 0.1 wt% reduced to 23%</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.01</td>
<td>1 (at 0.1 reduced to 31%)</td>
<td>0.1 (at 0.0001 reduced to 4 %)</td>
<td></td>
</tr>
</tbody>
</table>

7.5.3.2 On biofilms

Table 7.11 summarize all obtained MICs on planktonic growth.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Poly(MADQUAT$<em>{50}$-b-MMA$</em>{50}$)</th>
<th>Tannic acid</th>
<th>TA/poly(MADQUAT$<em>{50}$-s-MMA$</em>{50}$) mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K12</td>
<td>0.0001</td>
<td>1</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.1</td>
<td>1</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.0001</td>
<td>0.5</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Tables 7.10 and 7.11 show that the mixtures of tannic acid and poly(MADQUAT$_{50}$-b-MMA$_{50}$) gave good results, intermediate between those for di-block polymer and TA alone.

7.6 Summary and conclusion

Table 7.12 summarises all MIC values of TA/PMADQUAT, TA/poly(MADQUAT$_{50}$-s-MMA$_{50}$) and TA/poly(MADQUAT$_{50}$-b-MMA$_{50}$) mixtures obtained for planktonic and biofilm bacteria.
### Table 7.12. Summary of MIC values of mixtures of TA/PMADQUAT, TA/poly(MADQUAT<sub>50</sub>-s-MMA<sub>50</sub>) and TA/poly(MADQUAT<sub>50</sub>-b-MMA<sub>50</sub>), for planktonic and biofilm bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Planktonic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA</td>
<td>TA/PMADQUAT mixture</td>
<td>TA/poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-s-MMA&lt;sub&gt;50&lt;/sub&gt;) mixture</td>
<td>TA/poly(MADQUAT&lt;sub&gt;50&lt;/sub&gt;-b-MMA&lt;sub&gt;50&lt;/sub&gt;) mixture</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>1</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1 (at 0.0001 reduced to 19%)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0.1 reduced to 23%</td>
<td>0.1</td>
<td>Reduced to 3%</td>
<td>0.25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1 (at 0.1 reduced to 31%)</td>
<td>0.01 (at 0.0001 reduced to 3%)</td>
<td>0.0001</td>
<td>0.1 (at 0.0001 reduced to 4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (wt%)</th>
<th>Biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K12</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Tannic acid showed antibacterial activity against all bacterial strains tested. The existence of an ester linkage between gallic acid and glucose is an important factor in the antimicrobial potential of tannic acid. In more detail, it inhibited *E. coli* K12 and *S. aureus* growth at 1 wt% and it reduced *P. aeruginosa* growth to 23%. Mixing TA with cationic polymers with different structures (homopolymer, statistical copolymer and di-block polymer) increased its antibacterial activity and the mixtures had better stability and clarity than the pure TA solution. In inhibiting the growth of planktonic *E. coli* K12 bacteria, the TA/homopolymer and TA/di-block polymer mixtures were the best (0.1 wt%), while TA/di-block polymer reduced growth to 19% at a low concentration (0.0001 wt%). Conversely, the TA/statistical polymer mixture was best at inhibiting growth of the gram-positive *S. aureus* bacteria (0.0001 wt%).

The fact that the lowest MIC was for *S. aureus*, a gram-positive bacterium, may be explained by the ability of tannic acid to bind directly to the peptidoglycan layer in the bacterial outer membranes, a commonly accepted antimicrobial mechanism for polyphenols. To conclude, mixing TA with cationic polymers improved its activity against both planktonic and biofilm bacteria, especially *S. aureus*.
Chapter 7. A Study of the Antibacterial Activity of Tannic Acid and Tannic Acid/
Amphiphilic Cationic Polymer Mixtures.

7.7 References

Chapter 8
Conclusion and Further Work
Conclusion and Further Work

This chapter aims to summarise the results obtained from each chapter and presents some ideas for future work that could constitute a starting point for further research and investigations.

8.1 Summary and conclusion

8.1.1 Polymer synthesis, characterizations and aqueous solution behaviour

Different classes of amphiphilic cationic polymers including di-block polymers, statistical polymers and homo-polymers have been synthesized by RAFT polymerization or free radical polymerization, with various molecular weights, molar ratios of hydrophilic to hydrophobic moieties and structures aiming at discovering which polymers’ properties will show a better antibacterial activity. Proton NMR was used to investigate their number-average molecular weight, conversion and polymer composition, while aqueous gel permeation chromatography (GPC) was applied on all synthesised polymers, to determine the $(\text{M}_w)$, $(\text{M}_n)$ and (PDI). It was found that the molecular weight of block copolymers decreased as the molar ratio of MMA increased. Molecular weight also was estimated more accurately by means of $^1$H NMR. Core-Shell nanoparticles were provided by Dr. Zhuo Yang, a detailed report about these nanoparticles synthesis route and characterizations has been inserted in chapter 9 appendices.

Different techniques have been applied to investigate solution behaviour including micelle or aggregation formation and determination of cac of two series of polymers; p(MADQUAT$_x$-s-MMA$_y$) and p(MADQUAT$_x$-b-MMA$_y$). In the dilute region there was no drop in the surface tension values and before micelles’ formation in the aqueous solution, meaning that the polymer is non-surface active. As the ratio of MADQUAT increased the polymers showed higher surface tension values closer to that of the water. The intensity ratio $I_3/I_1$ showed a significant difference between di-block polymers and statistical copolymers. A slight increase in the values of $I_3/I_1$ range between 1.01 to 1.09 was observed for the statistical polymers in contrast to the di-block polymers where values of $I_3/I_1$ increased significantly as the polymer concentrations increase (from 0.99 to 1.4). Higher values for the intensity ratio $I_3/I_1$ were recorded as the mole ratio of MMA increased (more hydrophobic environment). At higher MADQUAT molar ratio with constant concentration (1 wt%), the Zeta potential increased due to an increase in the cationic moiety –$\text{N}^+(\text{CH}_3)_3$ ions. Additionally, the hydrodynamic particle diameter determined by DLS decreased, as
Chapter 8. Conclusion and Further Works

MMA molar ratio increased. To determine intrinsic viscosity different equations were used; Huggins, Kraemer, Tanglerpaibul & Rao, Fuoss and Fedors. The values of \([\eta]\) that was estimated via Fuoss equation results in a closer value of \(c^*\) to the value of \(c^*\) that was experimentally estimated from \(\log v \text{ vs. } \log c\) graphs, surface tension and pyrene fluorescence. It was concluded that polymers' structures, molar ratio of MADQUAT and MMA and synthesis route have great effect on polyelectrolytes solution behaviour in aqueous solutions.

8.1.2 Phase separation of mixtures of like charge surfactant/polymer systems

The phase separation was studied using three types of mixtures of like charge surfactant/polymer systems, including cationic surfactants (DDAC) and three cationic polymers; (i) homo-polymer, (ii) statistical copolymer (iii) and di-block polymers. Pure water was used as solvent. The phase separation was induced in by addition of cationic polyelectrolyte with like charge of of surfactant. In each case at high concentrations the system separates into a lower polyelectrolyte-rich and an upper surfactant-rich phase with different phase separation boundaries, confirmed by \(^1\)H NMR.

The results show that block polymers separate at higher concentration of DDAC compared to statistical polymers. That was explained by the different polymers solution behavior (polymers conformation).

8.1.3 Antibacterial Activity of different cationic polymers classes

Different cationic polymers have been tested in inhibiting the growth of both gram-positive and gram-negative bacterial strains. All polymers tested except PEGMA were able to inhibit growth in planktonic bacteria and biofilms. Both statistical copolymers and di-block polymers containing MADQUAT showed interesting activity as antibacterial agents. The best polymers at inhibiting \(E.\ coli\) K12 were poly(MADQUAT\(_{25}\)-b-MMA\(_{75}\)) and poly(MADQUAT\(_{50}\)-s-MMA\(_{50}\)), both effective at 10 \(\mu\)g/ml. All block polymers inhibited \(S.\ aureus\) at 100 \(\mu\)g/ml, while the statistical copolymers did so at the higher concentration rang of 100 - 2500 \(\mu\)g/ml.

Tannic acid showed antibacterial activity against all bacterial strains tested. In more detail, it inhibited \(E.\ coli\) K12 and \(S.\ aureus\) growth at 10000 \(\mu\)g/ml and it reduced \(P.\ aeruginosa\) growth to 23%. Mixing TA with cationic polymers with different structures (homopolymer, statistical copolymer and di-block polymer) increased its antibacterial activity and the
mixtures had better stability and clarity than the pure TA solution. In inhibiting the growth of planktonic *E. coli* K12 bacteria, the TA/homopolymer and TA/di-block polymer mixtures were the best (1000 µg/ml), while TA/di-block polymer reduced growth to 19% at a low concentration (1 µg/ml). Conversely, the TA/statistical polymer mixture was best at inhibiting growth of the gram-positive *S. aureus* bacteria (1 µg/ml). Core-shell quaternary nanoparticles with longer alkyl side groups (C₈H₁₇ and C₁₂H₂₅) had the best antibacterial activity against four classes of bacteria, i.e. *K. Pneumoniae, P.Aaeruginosa, E. coli* K12 and *E. coli* CI 2. The polymer ZY 27 (side group C₈H₁₆) was found to have the lowest inhibitory concentration 700 µg/ml or greatly less 700 µg/ml for the bacteria respectively. It is useful to compare these polymers with a commercially available biocide, poly(hexamethylenebiguanidehydrochloride) (PHMB), which has excellent antibacterial activity. Against *E. coli* K12, *E. coli* CI 2, *K. pneumoniae* and *P. aeruginosa*, for example, PHMB has MIC values of 10, 60, 200 and 100 mg/ml respectively. These are lower than the values for the polymers tested in this study. However, PHMB is reported to be toxic and subject to regulatory restrictions, so this research seeks to identify alternative antimicrobial agents which have a safer human toxicological profile.¹,²

8.2 Further Work

It is recommended to expand the techniques that might shed more light into the structure and nature of the aggregations and films of these polymers at different concentrations used. Techniques such as differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) and TEM or SEM can be used in this regard.

Interesting recent publication³ has investigated the effect of the hydrophobic modification of hydroxyethyl cellulose (HCE) and its amphiphilic derivatives (hmHEC) the intrinsic viscosity of the polymers formed. Extension of the work carried out in this thesis might consider investigating such effect.

Furthermore, study the antibacterial activity of the polymers in this study using different techniques, such as the spot-on-lawn method.⁴

Preparation of ZY-polymers with 1) longer alkyl side groups (more than 12 carbons) and study their antibacterial activity; 2) the mixtures of ZY polymers worth further study and looking at the possibility of including all on one particle; 3) resynthesizing di-block polymers with higher molecular weight and study their antibacterial activity.
It would be useful to study the diversity of interactions between tannins, amphiphilic cationic polymers and water, where all of the following types of interaction may occur: hydrogen bonding, ionic bonding and hydrophobic bonding.

The mixtures of polymers could be used in different applications, for example: Tannins could be used as negatively charged structural blocks for layer-by-layer assembly in alternation with cationic polymers in order to obtain antibacterial surfaces.\(^5\)

### 8.3 References

Chapter 9 Appendices
Chapter 9. Appendices

Figure 9.1. $^1$H NMR spectrum (400 MHz, D$_2$O) of poly(MADQUAT$_{75}$-b-MMA$_{25}$) showing ~95% conversion (about 48 hrs.) of each block.

Figure 9.2. $^1$H NMR spectrum (400 MHz, D$_2$O) of poly(MADQUAT$_{25}$-s-MMA$_{75}$) showing ~95% conversion after 30 hrs.
9.1 Particle size of all obtained polymers

1/PMADQUAT

![Figure 9.3](image1.png)

**Figure 9.3.** Particle size distribution of PMADQUAT at various concentrations measured by DLS.

2/p(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25})

![Figure 9.4](image2.png)

**Figure 9.4.** Particle size distribution of p(MADQUAT\textsubscript{75}-b-MMA\textsubscript{25}) at various concentrations measured by DLS.
Chapter 9. Appendices

3/p(MADQUAT$_{50}$-b-MMA$_{50}$)

![Graph 1](image1)

**Figure 9.5.** Particle size distribution of p(MADQUAT$_{50}$-b-MMA$_{50}$) at various concentrations measured by DLS.

4/p(MADQUAT$_{25}$-b-MMA$_{75}$)

![Graph 2](image2)

**Figure 9.6.** Particle size distribution of p(MADQUAT$_{25}$-b-MMA$_{75}$) at various concentrations measured by DLS.
5. \( p(\text{MADQUAT}_{75}-s-\text{MMA}_{25}) \)

Figure 9.7. Particle size distribution of \( p(\text{MADQUAT}_{75}-s-\text{MMA}_{25}) \) at various concentrations measured by DLS.

6. \( p(\text{MADQUAT}_{50}-s-\text{MMA}_{50}) \)

Figure 9.8. Particle size distribution of \( p(\text{MADQUAT}_{50}-s-\text{MMA}_{50}) \) at various concentrations measured by DLS.
7/p(MADQUAT\textsubscript{25}s-MMA\textsubscript{75})

**Figure 9.9.** Particle size distribution of p(MADQUAT\textsubscript{25}b-MMA\textsubscript{75}) at various concentrations measured by DLS.
Chapter 9. Appendices

9.2 Huggins/Kraemer plots with extrapolation to $t=0$ used to determine intrinsic viscosity $[\eta]$. 

a/statistical copolymers

**Figure 9.10.** Huggins/Kraemer plot for p(MADQUAT$_{50}$-s-MMA$_{50}$) with extrapolation to $t=0$ used to determine intrinsic viscosity $[\eta]$. 

**Figure 9.11.** Huggins/Kraemer plot for p(MADQUAT$_{25}$-s-MMA$_{75}$) with extrapolation to $t=0$ used to determine intrinsic viscosity $[\eta]$. 

\[
y = -0.0201x + 0.2857 \\
R^2 = 0.52558 
\]

\[
y = -0.0298x + 0.2641 \\
R^2 = 0.77877 
\]
Chapter 9. Appendices

b/ di-block polymers

**Figure 9.12.** Huggins/Kraemer plot for p(MADQUAT\(_{75}\)-b-MMA\(_{25}\)) with extrapolation to \(t=0\) used to determine intrinsic viscosity\([\eta]\).

**Figure 9.13.** Huggins/Kraemer plot for p(MADQUAT\(_{50}\)-b-MMA\(_{50}\)) with extrapolation to \(t=0\) used to determine intrinsic viscosity\([\eta]\).

**Figure 9.14.** Huggins/Kraemer plot for p(MADQUAT\(_{25}\)-b-MMA\(_{75}\)) with extrapolation to \(t=0\) used to determine intrinsic viscosity\([\eta]\).
9.3 Relative viscosity, Ln relative viscosity and 1-1/Relative Viscosity curves

a/ Statistical copolymers

Figure 9.15. Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of pMADQUAT_{75-s-MMA_{25}}) as function of polymer concentration.

Figure 4.16. Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of pMADQUAT_{50-s-MMA_{50}) as function of polymer concentration.

Figure 9.17. Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of pMADQUAT_{25-s-MMA_{75}) as function of polymer concentration.
b/di-block polymers

**Figure 9.18.** Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of pMADQUAT\textsubscript{25-b-MMA\textsubscript{25}} as function of polymer concentration.

**Figure 9.19.** Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of pMADQUAT\textsubscript{50-b-MMA\textsubscript{50}} as function of polymer concentration.

**Figure 9.20.** Relative viscosity, Ln Relative viscosity and 1-1/Relative Viscosity of p(MADQUAT\textsubscript{25-b-MMA\textsubscript{75}}) as function of polymer concentration.

\begin{align*}
y &= 0.0774x + 1.1713 \\
R^2 &= 0.98536 \\
y &= 0.0571x + 0.163 \\
R^2 &= 0.98833 \\
y &= 0.0424x + 0.1537 \\
R^2 &= 0.98695 \\
y &= 0.1107x + 1.1569 \\
R^2 &= 0.99357 \\
y &= 0.0781x + 0.1553 \\
R^2 &= 0.99586 \\
y &= 0.0556x + 0.1497 \\
R^2 &= 0.9924 \\
y &= 0.0721x + 1.1995 \\
R^2 &= 0.95618 \\
y &= 0.0529x + 0.1851 \\
R^2 &= 0.95185 \\
y &= 0.039x + 0.1711 \\
R^2 &= 0.94446 \\
y &= 0.0781x + 0.1553 \\
R^2 &= 0.9924 \\
y &= 0.0556x + 0.1497 \\
R^2 &= 0.9924
\end{align*}
9.4 Fedros equation curves

a/ statistical copolymers

Figure 9.21. Representation of Fedros equation for three statistical copolymers with different molar ratio of MADQUAT and MMA; p(MADQUAT\(_x\)-s-MMA\(_y\)).

b/ di-block polymers

Figure 9.22. Representation of Fedros equation for three diblock polymers with different molar ratio of MADQUAT and MMA; p(MADQUAT\(_x\)-b-MMA\(_y\)).
Figure 9.23. The optical Density of Control Plate of Tannic Acid at different concentrations without adjusting pH.

Figure 9.24. The Optical Density of Control Plate of Tannic Acid at different concentrations, pH 7.
Figure 9.25. Formation of precipitations at high concentrations of Tannic Acid.

Figure 9.26. Tannic Acid/L.B media mixture precipitations at t=0 and t=3hrs.

Figure 9.27. Tannic Acid (1,0.5,0.25,0.1 and 0.01 wt%)/L.B media/cationic polymer mixture after 3 hrs.
9.5 A detailed report about the synthesis route of Core-shell Nanoparticles

This report was provided by Dr. Zhuo Yang.

Introduction

Click chemistry\(^1\) was first fully described by K. Barry Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together, Scheme 9.1. A desirable Click chemistry reaction would:

- give very high chemical yields
- generate only inoffensive by-products
- be physiologically stable
- exhibit a large thermodynamic driving force (\(> 84 \text{ kJ/mol}\)).
- have simple reaction conditions
- use readily available starting materials and reagents
- use no solvent or use a solvent that is benign or easily removed (preferably water)
- provide simple product isolation by non-chromatographic method (crystallisation or distillation).

\[
\text{R-N}_3 + \text{R'} \rightarrow \text{Cu(I) (cat)} \rightarrow \text{R} + \text{N}_3\text{N-N-N-R'}
\]

Scheme 9.1. Click chemistry

Click chemistry in polymer colloids\(^2\) was studied by P. A. Lovell (Chem. Comm., 2009, (17), 2305-2307) and described that click coupling can be performed successfully at the surface of polymer nano/submicron-size particles dispersed in aqueous media, Scheme 9.2, thereby defining a generic strategy for attachment of a wide variety of functional molecules and providing a powerful, versatile route for the synthesis of surface functional particles.

\[
\text{Cu(I), room temperature}
\]

Scheme 9.2. Strategy for attaching functional molecules (represented by the rectangular block) to particle surfaces using click chemistry.

The rapid emergence of antibiotic-resistant bacteria along with increasing difficulty in biofilm treatment has caused an immediate need for the development of new classes of antimicrobial therapeutics. Grafting polymers onto and/or from surfaces antimicrobial
activity can be imparted onto a surface through the grafting of functionalized polymers, for example those terminated with quaternary ammonium functional groups. Quaternary ammonium ion-containing polymers have been proven to effectively kill cells and spores through their interactions with cell membranes. A wealth of nitrogenous amino azides can be quaternized to be biologically active. Alkyne-azide cycloaddition click functionalization of polymer colloids and various amine side groups can be subsequently polymerized. Thus antimicrobial surfaces can be prepared via click chemistry.

We have developed a functionalised antimicrobial polymer colloids prepared by emulsion Methyl methacrylate (MMA), [2-(Methacryloyloxy)ethyl]trimethyl-ammonium chloride (MATMAC) and Propargyl methacrylate (PMA) polymerisation (surfactant free) to produce alkyne particles (1), synthesis of quaternary ammonium azide with various side groups (2) and alkyne-azide cycloaddition click functionalization of polymer and they have varied side-group functionality, including quaternary ammonium with amine side chains ranging from 1 to 12 carbons long (3), shown in Scheme 3.

Scheme 9.3. Emulsion MMA, MATMAC and PMA polymerisation (1), synthesis of quaternary ammonium azide with various side groups (2) and alkyne-azide cycloaddition click functionalization of polymer (3).

The alkyl chain length refers to on the length of the quaternary ammonium. The length of this chain has been investigated to see if it affects the antimicrobial activity of the polymer.
Experimental Section

Materials

Methyl methacrylate (MMA), [2-(Methacryloyloxy)ethyl]trimethyl-ammonium chloride 80 wt% solution in water (MATMAC), Propargyl methacrylate (PMA), 2,2’-Azobis(2-methylpropionamidine) dihydrochloride (AIBA), Sodium azide, 3-dimethylamino-1-propylchloride hydrochloride, 1-Bromobutane, 1-Bromododecane, Copper(II) sulphate pentahydrate, 1-Bromooctane, Copper(I) bromide, (+)-Sodium L-ascorbate, N,N,N’,N’’,N’’’-Pentamethyl diethylenetriamine (PMDTA), N,N-Dimethyl formamide, poly-(ethylene glycol) methacrylate (PEGMA) and Di(ethylene glycol) dimethacrylate (DEGDAM) were purchased from Sigma Aldrich. All materials were used as received.

Characterization of particles (Dynamic light Scantling DLS)

A Zetasizer Nano-ZS (Malvern, UK) with Folded Capillary cell (Malvern DTS 1061) was used to measure the hydrodynamic diameter, polydistribution index(PDI), Zeta potential and electrophoretic mobility of the polymer particles.

Synthesis of polymer colloids

All reactants (MMA, MATMAC, or PMA and deionised water), except for the initiator solution, were charged in a three neck flask, with mechanical stirrer. The mixture was bubbled with nitrogen for 30 mins and then slow surface nitrogen flow was maintained. The circulator temperature was set for 80 °C. The AIBA initiator solution was added to the reactor once the internal temperature reached 80 °C. The reactor mixture was then maintained at this temperature for 2 hrs. High level of coagulated material was forming quite early in the reaction.

General synthesis of amino azides

Organic azides can be EXPLOSIVE! A guide to safe handling and storage of organic azides can be found in ‘Click Chemistry: Diverse chemical function from a few good reactions’ by Kolb et al. The shorthand notation for each side group used in this article is provided in parentheses after the specific chemical name. Amino azides were synthesized using the protocol presented by Carboni et al.

A representative example, 3-dimethylamino-1-propylchloride hydrochloride (10 g, 63 mmol) and sodium azide (8.22 g, 126 mmol) were dissolved in water (1 mL mmol⁻¹) and heated at 75 °C for 15 h. The reaction mixture was cooled in an ice bath and NaOH (4 g) was added. The solution phase separated and the organic phase was removed. The aqueous phase was extracted with diethyl ether twice. The organic layers were combined, dried with MgSO₄, and concentrated down to an oil of 3-Azido-N,N-dimethylpropan-1-amine (dimethylpropanamine) (5.70 g, 70.8% yield).
Synthesis of quaternary ammonium azides

In a typical experiment, 3-azido-N,N-dimethylpropan-1-amine (0.5 g, 3.9 mmol) was dissolved in methanol (5 ml) and added to the haloalkane (bromododecane 0.88 g, 3.54 g) dissolved in methanol (5 ml). The reaction mixture was refluxed for 20 h and then cooled to room temperature. The methanol and any unreacted 3-azido N,N-dimethylpropan-1-amine was removed under high vacuum.

Synthesis of core-shell alkyne particles

Synthesis of the two-layer particles was completed by using one of the core to provide seed particles emulsion polymerisations involving sequential metered addition of the PMA, PEGMA 360 and DEGDMA containing monomer mixtures. Scheme 4 presents the procedure employed to synthesize core–shell particles composed of poly MMA-co-MATMAC core and crosslinking DEGDMA with a poly(PMA-co-PEGMA 360) shell. The reactants for core (MMA, MATMAC and deionised water), except for the initiator solution, were charged in a three neck flask, with mechanical stirrer. The mixture was bubbled with nitrogen for 30 mins and then a slow surface nitrogen flow was maintained. The circulator temperature was set for 80 °C. The AIBA initiator solution was added to the reactor once the internal temperature reached 80 °C. The reactor mixture was then maintained at this temperature for 50 min. The reactants for shell (PMA, PEGMA and DEGDMA or AIBA, DI water) were added by syringe pump at 1 ml/min rate. The reactor mixture was then maintained 80 °C for further 2 hrs. High level of coagulated material was forming quite early in the reaction.

Scheme 9.4 The procedure employed to synthesize core–shell particles composed of poly MMA-co-MATMAC core crosslinked DEGDMA with a poly(PMA-co-PEGMA 360) shell.
General synthesis of alkyne-azide cycloaddition click functionalization of polymer colloids

The equivalents used for click reaction of azide with polymer colloid with alkyne surface groups are given in Table 1. The polymer colloids was first adjusted to pH 8 by the addition of a small amount of dilute aqueous NaOH and was then added to a small vial followed by the sodium ascorbate, CuSO₄ solution and finally the DMF was then added to aid solubility of the azide and the vial suspended in a temperature controlled oil bath at 35 ºC for 4 days.

Results and Discussion

Emulsion polymerisation to produce alkyne particles and physical characterization

Effect of monomer Ratio

In order to investigate the influence of MATMAC concentration on final particle characteristics, a series of copolymerizations of fixed MMA with MATMAC mol ratio from 2.5 to 10, the samples ZY 02, 22, 23 and 24 are shown in Table 2. The Z averages particle diameter increase from 164 to 353 nm. The influence of PMA concentration on final particle characteristics, a series of copolymerizations of fixed MMA and MATMAC with PMA varied monomer feed ratios from 0 to 10 which are shown in Table 2 samples ZY 02B, 04 and 03. The Z averages rapid increase from 164 nm until agglomeration occurred. MATMAC and PMA were conducted at varied monomer feed ratios were investigated which are shown in table 2 samples ZY 04 (MATMAC/PMA mol ratio 2.5/5=0.5, final particle size 498nm, PDI 0.2) ZY 05 (5/5 = 1, 234nm, 0.09) and ZY 24 (10/10 = 1, 353nm, 0.2). This revealed that uniform spherical particles were obtained when MATMAC/PMA mol ratio=0.5-1 and MMA 100 mol present. High concentration alkyne particles were investigated samples, such as ZY 40 (MATMAC/PMA mol ratio 1/25 without MMA, final particle size 378nm, PDI 0.2) and ZY32 (core-shell cross-linking), see Table 2.
Table 9.1 Summary of polymerization feed monomers and physical characterization, Z average diameters and polydispersion index by DLS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mol ratio</th>
<th>Z average d (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMA</td>
<td>MATMAC</td>
<td>PMA</td>
</tr>
<tr>
<td>ZY02</td>
<td>100</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>ZY 22</td>
<td>100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>ZY 23</td>
<td>100</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>ZY 04</td>
<td>100</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>ZY 05</td>
<td>100</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ZY 24</td>
<td>100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ZY 32</td>
<td>100</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Core-shell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZY 02 B</td>
<td>100</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>ZY 04</td>
<td>100</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>ZY 03</td>
<td>100</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>ZY 40</td>
<td>0</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>
Kinetics of binary copolymerization

For copolymerization of MMA with MATMAC (ZY02), particle size and PDI increased while number of particles decreased as conversion increased at 0-25%. When over 25% conversion, particle size, PDI and number of particles tended to be stable. Figure 1 (A) (B) and (C) show the curves of particle size, PDI and number of particle changes as conversion% of copolymerization.

Figure 9.28. Plots of (A) total particle number in the reaction, (B) Z-average particle diameter and PDI, (C) particle size distribution as conversion% of polyMMA-co-MATMAC.
**Characterization for core and core-shell particles**

The analysis core particle by DLS gave a Z-average particle diameter of 400 nm and Zeta potential 65mv. For the core-shell particle gave 520nm and 54 mv, no doubt due to the contribution from the shell polymerisation, see Table 3.

**Table 9.2** Composition and characterisation data for core and core-shell crosslinking polymers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mol ratio</th>
<th>PEGDMA/DEGDMA</th>
<th>MATMAC</th>
<th>PMA</th>
<th>Z average (nm)</th>
<th>PDI</th>
<th>Zeta potential mv</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZY32 Core</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>400</td>
<td>0.154</td>
<td>65</td>
</tr>
<tr>
<td>ZY32 Core-shell</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td></td>
<td>520</td>
<td>0.216</td>
<td>54</td>
</tr>
</tbody>
</table>

**Synthesis of amino azide and quaternary ammonium azides with various side groups**

The compounds structures were confirmed using Mass spectra. Representative M⁺ or s M⁺1 spectra of amino azide and quaternary ammonium azides are shown in Table 4 and Figure

**Table 9.3** The structures and Mw of the amino azide and quaternary ammonium azides

<table>
<thead>
<tr>
<th>Azides</th>
<th>M_w</th>
<th>Mol Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>128</td>
<td>C₅H₁₂N₄</td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>C₉H₂₁N₄</td>
</tr>
<tr>
<td></td>
<td>241</td>
<td>C₁₃H₂₉N₄</td>
</tr>
<tr>
<td></td>
<td>297</td>
<td>C₁₇H₃₇N₄</td>
</tr>
</tbody>
</table>
Figure 9.29. The Mass spectra for the amino azide and quaternary ammonium azides.
Alkyne-azide cycloaddition click functionalization of polymer Characteristics

Alkyne-azide cycloaddition click functionalization of polymer colloids with various amine side groups. The hydrodynamic diameter, zeta potential values and electrophoretic mobility of the different types of particles (see Table 1) are presented in Table 5.

Table 9.4. Characteristics of different type polymer particles

<table>
<thead>
<tr>
<th>Type 1: MMA/MATMAC/PMA 100/5/5, alkyne/azide 1/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample</td>
</tr>
<tr>
<td>ZY 05 Alkyne Particles</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ZY 16 Side Group C₄H₉</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ZY 15 Side Group C₈H₁₇</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ZY 18 Side Group C₁₂H₂₅</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
## Chapter 9. Appendices

Type 2: MMA/MATMAC/PMA 100/5/5, alkyne/azide 1/1.1

<table>
<thead>
<tr>
<th>sample</th>
<th>Temperature °C</th>
<th>Z average (Nm)</th>
<th>P.D.I</th>
<th>Zeta potential (mV)</th>
<th>Mobility (mcm/Vs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYang 17 Alkyne particles</td>
<td>20</td>
<td>253</td>
<td>0.191</td>
<td>69.7</td>
<td>4.791</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>278</td>
<td>0.186</td>
<td>63.3</td>
<td>5.432</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>304</td>
<td>0.088</td>
<td>59.7</td>
<td>6.382</td>
</tr>
<tr>
<td>ZYang 19 Side Group C₄H₉</td>
<td>20</td>
<td>286</td>
<td>0.198</td>
<td>35.2</td>
<td>2.497</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>310</td>
<td>0.150</td>
<td>32.9</td>
<td>2.824</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>340</td>
<td>0.046</td>
<td>31.0</td>
<td>3.317</td>
</tr>
<tr>
<td>ZYang 20 Side Group C₈H₁₇</td>
<td>20</td>
<td>275</td>
<td>0.163</td>
<td>39.1</td>
<td>2.775</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>301</td>
<td>0.144</td>
<td>36.3</td>
<td>3.133</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>320</td>
<td>0.135</td>
<td>34.6</td>
<td>3.698</td>
</tr>
<tr>
<td>ZYang 21 Side Group C₁₂H₂₅</td>
<td>20</td>
<td>239</td>
<td>0.203</td>
<td>39.2</td>
<td>2.784</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>258</td>
<td>0.201</td>
<td>36.5</td>
<td>3.135</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>278</td>
<td>0.193</td>
<td>34.5</td>
<td>3.683</td>
</tr>
</tbody>
</table>
Type 3: MMA/MATMAC/PMA 100/10/10 alkyne/azide 1/1.1 (polymers used in this study)

<table>
<thead>
<tr>
<th>sample</th>
<th>Temperature (°C)</th>
<th>Z average (nm)</th>
<th>P.D.I</th>
<th>Zeta potential (mV)</th>
<th>Mobility (mcm/Vs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyne particles</td>
<td>20</td>
<td>353</td>
<td>0.233</td>
<td>61.6</td>
<td>4.368</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>381</td>
<td>0.211</td>
<td>58.0</td>
<td>4.974</td>
</tr>
<tr>
<td>ZY 26</td>
<td>20</td>
<td>259</td>
<td>0.252</td>
<td>25.1</td>
<td>1.783</td>
</tr>
<tr>
<td>Side Group C₄H₉</td>
<td>30</td>
<td>283</td>
<td>0.270</td>
<td>23.8</td>
<td>2.046</td>
</tr>
<tr>
<td>ZY 27</td>
<td>20</td>
<td>258</td>
<td>0.258</td>
<td>28.6</td>
<td>2.031</td>
</tr>
<tr>
<td>Side Group C₈H₁₇</td>
<td>30</td>
<td>279</td>
<td>0.233</td>
<td>26.2</td>
<td>2.250</td>
</tr>
<tr>
<td>ZY 28</td>
<td>20</td>
<td>245</td>
<td>0.256</td>
<td>27.8</td>
<td>1.974</td>
</tr>
<tr>
<td>Side Group C₁₂H₂₅</td>
<td>30</td>
<td>257</td>
<td>0.220</td>
<td>25.9</td>
<td>2.224</td>
</tr>
</tbody>
</table>

Type 4 Core -shell polymers

<table>
<thead>
<tr>
<th>sample</th>
<th>Temperature (°C)</th>
<th>Z average (nm)</th>
<th>P.D.I</th>
<th>Zeta potential (mV)</th>
<th>Mobility (mcm/Vs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYang 32 Core</td>
<td>20</td>
<td>261</td>
<td>0.154</td>
<td>59.2</td>
<td>4.199</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>290</td>
<td>0.152</td>
<td>55.7</td>
<td>4.780</td>
</tr>
<tr>
<td>ZYang 32 Core-shell Alkyne</td>
<td>20</td>
<td>313</td>
<td>0.115</td>
<td>70.5</td>
<td>5.001</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>341</td>
<td>0.086</td>
<td>69.5</td>
<td>5.966</td>
</tr>
<tr>
<td>ZYang 34</td>
<td>20</td>
<td>459</td>
<td>0.237</td>
<td>28.0</td>
<td>1.986</td>
</tr>
</tbody>
</table>
A large change in the charge on the surface of the particles was observed after click functionalisation with the different quaternary ammonium azides, as shown in zeta potential measurements presented in Table 5. The cationically stabilised latex with alkyne groups exhibits a high positive zeta potential value of about +60 mV. After the click reactions the positive charge on the surface has been reduced, yielding values of between +25-30 mV. This suggests that the click reaction has been successful in positioning the alkyl groups present on the quaternary ammonium groupings near to the surface of the particles, shielding the pre-existing charge on the surface of the particles. This result shows the potential of this chemistry as a means of tuning the hydrophobicity and charge on the surface of nanoparticles.
9.6 References


