Development of permanently antimicrobial coatings

by
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Wat ek is skenk U aan my, wat ek word skenk ek aan U.

(a childhood prayer)

Dedicated to my loving parents, Tommy and Ingrid

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Abstract

Water-borne coatings often contain multiple additives including pigments, dispersing agents, rheology modifiers, UV stabilizers and biocides. Due to their low molar mass and endocrine-disrupting properties, many of these additives, upon leaching from the substrate film, with time pollute water systems and become hazardous to the environment and to human health. In this study, I aimed to develop a facile method for the production of a polymeric biocide to serve as alternative to low molar mass biocides used in water-borne coatings. A secondary aim was to show that, without additional modification, the polymeric species can be used in surfactant-free *ab initio* emulsion polymerizations.

Using a two-step process, I modified a commercially available copolymer, poly(styrene-*alt*-maleic anhydride) (*SMA 1000*), with mixed amines in order to obtain latexes with inherent antimicrobial activity. In the first step, I reacted *SMA 1000* with *3-dimethylamino-1-propylamine* and aqueous ammonia to confer antimicrobial activity and water-solubility to the *SMA* copolymer. In the second step, the copolymer was incorporated into a film-forming styrene-butyl acrylate (*STY/BuA*) latex. The modified SMA was incorporated into a latex in two ways: (1) post-added to the latex, and (2) used as stabilizer in emulsion polymerization. In both cases, the latex remained stable for up to 11 months, and stability was probably due to steric stabilization of the polymer particles. Antimicrobial activity of the latex film was achieved with both methods. When the modified *SMA* was post-added, antimicrobial activity was restricted to specific areas on the eventual polymer film, and when modified *SMA* was used as stabilizer, antimicrobial activity was evenly distributed throughout the polymer film.

Fluorescence microscopy showed homogeneous distribution of antimicrobial activity upon inoculation in *Gram positive* bacteria dispersions when the modified copolymer was used as polymeric stabilizer for the synthesis of *STY/BuA* latexes. No antimicrobial activity against *Gram negative* bacteria was achieved. The homogeneous distribution of antimicrobial activity throughout the film was a result of adsorption of polymeric biocide/stabilizer to each individual latex particle. With further commercial development, high molar mass copolymers modified for antimicrobial activity may be a feasible, environmentally-friendly and healthy alternative to be used as stabilizers in emulsion polymerizations to produce water-borne coatings.

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Opsomming

Waterverf bestaan gewoonlik uit 'n verskeidenheid bestandele, onder andere: pigmente, verspreiding middels, reologie modifiseerders, UV stabiliseerders en biologies aktiewe verbindings. As gevolg van die lae molêre massa en die endokrien ontwrigtende vermoë van baie van die bestandele hou hulle 'n bedreiging in vir die omgewing in terme van waterbesoedeling en menslike gesondheid, soos hulle die film oor tyd verlaat. In hierdie studie het ek beoog om 'n eenvoudige metode vir die vervaardiging van 'n polimeries biologies aktiewe verbindings daar te stel om sodoende as 'n alternatief vir die lae molêre massa biologies aktiewe verbindings, wat tans in waterverf gebruik word, te dien. 'n Sekondêre uitkoms van die studie was om te wys dat, sonder enige adissionele omskakelings, dieselfde polimeer gebruik kan word in seep-vrye emulsie polimerisasie.

Deur gebruik te maak van 'n proses, wat uit twee stappe bestaan, het ek 'n kommersieel beskikbare kopolimeer, poly(stireen-*alt*-maleinesuuranhidried) (*SMA 1000*), met gemengde amiene reageer om 'n sintetiese lateks van stireen en butiel akrilaat (*STY/BuA*) met inherente antibakteriële aktiwiteit te verkry. In die eerste stap is *SMA 1000* met 3-dimetielamien-1-propielamien en waterige ammoniak reageer om 'n water oplosbare kopolimeer met inherente anti-bakteriële aktiwiteit te verkry. In die tweede stap is hierdie kopolimeer by 'n sintetiese lateks gevoeg op twee maniere: (1) deur dit nadat die lateks geproduseer is by te voeg, en (2) deur die kopolimeer as stabiliseerder te gebruik in die vervaardiging van die lateks. In albei gevalle is stabiele latekse verkry vir 'n tydperk van tot 11 maande. Die stabilisering was van steriese geaardheid. Albei die latekse het gevolglik anti-bakteriële eienskappe getoon. Daar was nie homogene verspreiding van die aktiwiteit in die geval waar die kopolimeer na die tyd bygevoeg is nie en het veroorsaak dat daar sekere areas van die finale film was wat geen aktiwiteit getoon het nie.

Fluoresensie mikroskopie het egter homegene verspreiding van die anti-bakteriële aktiwiteit reg deur die film getoon, na inokulasie met *Gram positiewe* bakterië suspensies wanneer die kopolimeer as polimerisasie stabiliseerder gebruik was. Geen aktiwiteit teen *Gram negatiewe* bakterië was egter verkry nie. Die homogene verspreiding was as gevolg van die feit dat die kopolimeer sterk adsorbeer op elke individuele lateks partikel wanneer dit as stabiliseerder gebruik word. Verdere ontwikkeling op 'n kommersiële basis kan daartoe lei dat polimeries biologies aktiewe verbindings as 'n lewensvatbare en omgewingsvriendelike alternatief vir heidige stabiliseerders in emulsies vir waterverf gebruik kan word.

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Table of Contents

Abstract……………………………………………………………………………...iii
Opsomming…………………………………………………………………………….iv
Acknowledgements...v
Table of Contents…………………………………………………………………....vi
List of Figures………………………………………………………………………….x
List of Tables………………………………………………………………………….xiii
List of Equations……………………………………………………………………….xiv

Chapter 1 Introduction
1.1 General Introduction………………………………………………………………......1
1.2 Objectives and thesis outline…………………………………………………………......3
References……………………………………………………………………………………......5

Chapter 2 Literature Review
2.1 Biocides………………………………………………………………………………………….6
2.1.1 Low molar mass biocides……………………………………………………………….7
2.1.2 Polymeric biocides……………………………………………………………..................8
2.1.3 Polymeric contact biocides in latex coatings………………………………………….10
2.2 Latex production via Radical Emulsion Polymerization………………………………….12
2.2.1 Radical polymerization……………………………………………………………….…12
2.2.1.1 Radical Formation and initiation…………………………………………………...12
2.2.1.2 Propagation……………………………………………………………………………….14
2.2.1.3 Termination ……………………………………………………………….....………….14
2.2.1.4 Transfer to monomer/solvent……………………………………………………......15
2.2.2 Emulsion polymerization……………………………………………………….....……16
2.2.2.1 Recipes and ingredients…………………………………………………….…….....16
2.2.2.2 Basic principles and mechanism………………………………………………….17
2.2.2.3 Particle number and growth of particles…………………………….…………….19
2.2.2.4 Zero – one and pseudo bulk conditions…………………………………………….....20
2.2.2.5 Copolymerization and composition drift…………………………………….…….21
2.2.2.6 Polymeric Surfactants……………………………………………………………………...21
2.2.2.7 Process strategies and particle morphologies……………………………….……...22
References……………………………………………………………………………………......24

Stellenbosch University http://scholar.sun.ac.za

Chapter 3: Synthesis and antimicrobial activity of styrene-*alt*-maleic anhydride

Abstract……………………………………………………………………………………27

3.1 Introduction………………………………………………………………………….27

3.2 Modification of SMA………………………………………………………………28

3.2.1 Reagents…………………………………………………………………………28

3.2.2 Modification of SMA with ammonia……………………………………………28

3.2.3 Modification of SMA with mixed amines………………………………………30

3.2.4 Optimized synthesis of modified SMA…………………………………………34

3.3 Emulsion copolymerization of styrene and butyl acrylate…………………………34

3.3.1 Reagents…………………………………………………………………………34

3.3.2 General formulation and procedure………………………………………………34

3.3.3 Addition of modified SMA to STY/BuA latex……………………………………36

3.4 Characterization and analysis…………………………………………………………36

3.4.1 Fourier Transform Infrared Spectroscopy (FT-IR)…………………………………36

3.4.2 Size Exclusion Chromatography (SEC)……………………………………………36

3.4.3 Dynamic light scattering (DLS)……………………………………………………37

3.4.4 Transmission Electron Microscopy (TEM)…………………………………………37

3.4.5 Inoculation of films in bacteria medium……………………………………………37

3.4.6 Fluorescence microscopy……………………………………………………………37

3.4.7 Differential Scanning Calorimetry (DSC)…………………………………………38

3.5 Results and discussion…………………………………………………………………38

3.5.1 Characterization of modified SMA…………………………………………………38

3.5.2 DLS, DSC and TEM analysis of STY/BuA latex…………………………………40

3.5.3 Antimicrobial activityassessment via fluorescence microscopy..................43

3.6 Conclusions and recommendations……………………………………………………44

References………………………………………………………………………………………45

Chapter 4: Modified SMA as polymeric surfactant and antimicrobial activity

Abstract…………………………………………………………………………………………46

4.1 Introduction……………………………………………………………………………………46

4.2 Emulsion polymerization with polymeric surfactant…………………………………………47

4.2.1 Reagents……………………………………………………………………………………47

4.2.2 General emulsion polymerization formulation………………………………………………47

4.2.3 Optimization of emulsion polymerization system……………………………………………48

4.2.3.1 CMC determination of polymeric surfactant………………………………………49

4.2.3.1.1 Surface Tension…………………………………………………………………50

Stellenbosch University http://scholar.sun.ac.za

4.2.3.1.2 Fluorescence Spectroscopy……………………………………………………50
4.2.3.2 Increased amount of polymeric surfactant…………………………………………50
4.2.3.3 Increasing amphiphilic character and molar mass…………………………… 51
4.3 Efficacy of polymeric surfactant……………………………………………………………52
4.3.1 Homo-polymerization of styrene with SMI-70 as surfactant and gravimetrical analysis…………………………………………………………………………………………52
4.4 Characterization and analysis……………………………………………………………53
4.4.1 Differential Scanning Calorimetry (DSC)……………………………………………53
4.4.2 Dynamic Light Scattering (DLS)...53
4.4.3 Transmission Electron Microscopy (TEM)...54
4.4.4 Inoculation of films in bacteria culture..54
4.4.5 Fluorescence microscopy…………………………………………………………54
4.5 Results and Discussion……………………………………………………………………55
4.5.1 CMC determination via surface tension and fluorescence microscopy...............55
4.5.2 DLS...57
4.5.3 DSC………………………………………………………………………………………59
4.5.4 TEM………………………………………………………………………………………60
4.5.5 Fluorescence microscopy……………………………………………………………… 61
4.5.6 Efficacy of polymeric surfactant ………………………………………………………63
4.6 Conclusion and recommendations…………………………………………………………65
References………………………………………………………………………………………67

Chapter 5: Conclusions and Recommendations………………………………………..68

Stellenbosch University http://scholar.sun.ac.za

List of figures

Chapter 2

Figure 2.1: The adsorption of various types of polymeric surfactants onto a particle surface[40]

Chapter 3

Figure 3.1: Ring opening of SMA with aqueous ammonia.

Figure 3.2: Reaction scheme for imidization of *SMA*

Figure 3.3: Reaction scheme for ring opening of imidized *SMA*

Figure 3.4: Solutions of *85%* (left) and *30%* (right) ring opened *SMA* after 5 days in water

Figure 3.5: Solutions of *15-, 30-, 45-* and *85%* ring opened SMA (Table 4) after 13 days inwater.

Figure 3.6: FT-IR spectra of *SMA* (top) and the modified (*SMI 85%*) imidized and ring-opened copolymer (bottom).

Figure 3.7: Particle size distribution from *DLS* of Sty/BuA latex with modified *SMA 85%* maleimide modification added in *5 wt%* of total solids content with $Z_{average}$ = 62.60 *nm* (*PDI* = 0.066)

Figure 3.8: *TEM* image of *Sty/BuA* latex as synthesized with *SDS* (**a**) and the same latex with the added *SMI-85* (**b**).The scale bar in the images corresponds to a length of 200 *nm*.

Figure 3.9: *DSC* thermogram for showing the glass transition temperatures for *Sty/BuA*(18.8 ^oC) latex with *85%*imidized SMA (193.2 ^oC) added in *5 wt%* of the total solids content.

Figure 3.10: Fluorescence microscopy images of three regions of pure latex film (*Sty/BuA* latex with no modified *SMA*) (a), (b), (c), stained for cell viability. Blue indicates presence of all bacterial cells (alive or dead) and red indicates presence of dead cells only.

Figure 3.11: Fluorescence microscopy images of three regions of *Sty/BuA* latex film with *5 wt% SMI85* (a), (b), (c), stained for cell viability. Colours have same meaning as in Figure 10.

Chapter 4

Figure 4.1: Uni-molecular micelle as a result of coil over globule folding of an individual chain.

Figure 4.2: Rod like, uni-molecular micelle due to self assembly of a single chain in the continuous phase, water.

Figure 4.3: *Jeffamine XTJ-507(2005 Da)* obtained from *Huntsman.*

Figure 4.4: The modified *SMA1000* copolymer with the ring opening step done with *XTJ-507(Huntsman)* instead of $NH_{3(aq)}$-solution

Figure 4.5: Plot of the surface tension measurements vs. the concentration of the polymeric surfactant in water.

Figure 4.6: Plot of the ratio of fluorescence peak intensities vs. logarithm of the concentration of polymeric surfactant for fluorescence spectroscopy with pyrene as fluorescent probe**.**

Figure 4.7: Particle Size Distribution obtained from *DLS* measurements using *SMI-85* in *1 wt%* of the emulsion formulation (*Z-avg* = 2061.0 *nm*, PDI = 0.910)

Figure 4.8: Particle Size Distribution obtained from *DLS* measurements using *SMI-85* in *2 wt%* of the emulsion formulation after 20 days

Figure 4.9: DSC thermograms for latexes obtain by post addition of *SMI-85* to *Sty/BuA* latex (a) and employing polymeric surfactants with varying degrees of functionalization; (b) *SMI-85*, (c) *SMI-55* and (d) *SMI-40*.

Figure 4.10: TEM images of latexes obtained obtained with polymeric surfactant *SMI-85* (a); *SDS* as surfactant (b);*SMI-85* added to latex produced with *SDS* as surfactant (c and d)

Figure 4.11: Particle size distribution for the latex obtained using *SMI-85* as polymeric surfactant obtained from analysis using AnalisysDocu software

Figure 4.12: Fluorescence microscopy images of *Sty/BuA* latex film with *SMI-85* as polymeric stabilzer. Images (a) and (b) shows the film stained for cell viability against *Lactobacillus sakei*. Blue indicates presence of all bacterial cells (alive or dead) and red indicates presence of dead cells only. Image (c) is an overlay of blue and red stained images.

Figure 4.13: Fluorescence microscopy images of latex film (*Sty/BuA* with *SMI-85 as polymeric stabilizer*) (a), (b) and (c) stained for cell viability against *S. aureus*. Colours have the same meaning as in *Figure 12*.

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Figure 4.14: Fluorescence microscopy images of latex film (*Sty/BuA* with *SMI-85 as polymeric stabilizer*) (a), (b) and (c) stained for cell viability against *E. coli*. Colours have the same meaning as in *Figure 12*.

Figure 4.15: Conversion versus time plot for emulsion polymerization of styrene using modified *SMA* as polymeric stabilizer.

Figure 4.16: First order kinetic plot for emulsion polymerization of styrene using modified *SMA* as polymeric stabilizer.

Figure 4.17: The particle size distribution for emulsion polymerization of styrene using modified SMA as polymeric surfactant.

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List of Tables

Chapter 3

Table 3.1: Stoichiometric amounts of $NH_{3(aq)}$ and degree of ring opening of the maleic anhydride units of the polymer chain used in the modification of *SMA*

Table 3.2: Amounts of reactants for the varying degrees of imidization

Table 3.3: Amounts of *25%* ammonia solution for ring opening of residual maleic anhydride units for each of the partially imidized SMA co-polymer entries in Table 3.2.

Table 3.4: Formulation of *Sty/BuA* emulsion polymerization with *SDS* as surfactant

Table 3.5: Qualitative assessment of solubility after5 and 13 daysof 15-*, 30-, 45-, 60-* and *85 mol%* ring opened SMA copolymers

Table 3.6: Storage stability data obtained by means of *DLS* for samples of modified *SMA* copolymers ranging from *85-* to *25%* imidization and the difference ring opened amic acid functionality, added to *Sty/BuA* latex produced with *SDS*.

Chapter 4

Table 4.1: Formulation for the synthesis of *Sty/BuA* latex with *SMI-85* as polymeric surfactant

Table 4.2: Formulation for styrene emulsion polymerization with polymeric surfactant, *SMI-70*

Table 4.3: Storage data of the evolution of Particle Size Distribution using *SMI-85* in 2 *wt%* of the emulsion formulation

Table 4.4: Latex stability over time, for the emulsion polymerization of styrene, using *SMI-70* as polymerization stabilizer.

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List of equations

Chapter 2

Equation 2.1 Initiator decomposition

Equation 2.2 Rate of initiator decomposition

Equation 2.3 Exponential decay of residual initiator concentration

Equation 2.4 Rate of initiation

Equation 2.5 Addition of first monomer species to initiator radical

Equation 2.6 Rate of initiation

Equation 2.7 Representation of polymeric radical propagation

Equation 2.8 Rate of propagation

Equation 2.9 Termination by combination

Equation 2.10 Termination by disproportionation

Equation 2.11 Rate of radical entry in emulsion polymerization

Equation 2.12 Rate of propagation in emulsion polymerization

Equation 2.13 Number of latex particles in a latex

Chapter 3

Equation 3.1 Flory-Fox equation

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Chapter 1
Introduction

1.1 General Introduction[1]

Surface coatings have been used throughout the ages for decorative purposes as well as to preserve and protect substrates surfaces. Coatings have come along way since those used by ancient civilizations in rock art found across the globe. The bulk of contemporary coatings are however still made up of three main ingredients as in ancient times. The main constituents of coatings are a) pigments, b) resins and binders and c) diluents or solvents. Coatings also contain various other additives depending on type and end application thereof. Besides different kinds of pigments, dispersing agents and rheology modifiers, they may also contain UV stabilizers, in the case of exterior coatings, as well as biocides to increase shelf life and inhibit growth and spread of bacteria. Coatings can generally be classified as decorative, protective or a combination of the two, depending on their application. We can also distinguish between water-based and solvent-based coatings according to the type of solvent or diluent used. Type and nature of the resin or binder is another way to classify coatings, where the binder may be a pure or styrene acrylic latex, polyurethane latex, epoxy resin, (un)saturated polyester resin, alkyd resin, etc. There is a wide scope for formulating decorative and protective coatings, using the array of pigments, binders and diluents available. Besides a brief explanation of non-aqueous coatings, this text will mainly focus on the make up and formulation of water-based coatings.

Water-borne coatings have become the first choice for use in nearly all applications of the coatings industry. The surge in development of water-borne coatings in areas where solvent borne coatings were previously used exclusively resulted from parallel research done on the hazards and disadvantages concerning solvent-based coatings. Water-borne coatings are mainly made up of inorganic pigments such as TiO_2, $CaCO_3$, silicates and other clays. The binders consist of film-forming polymers such as pure acrylic or styrene/acrylic synthetic latexes, containing small percentages of functional monomers to aid the ease of processing and promote adhesion to difficult substrates.
The pigments provide the necessary opacity of a coating to be able to effectively hide the substrate surface. The binders are organic film-forming polymers that are able to wet and

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bind the pigments as well as provide the primary means of adhesion to substrate surfaces. In order to achieve the necessary flow properties for application, the pigments and binder dispersion is diluted with a solvent to the desired viscosity. Solvent-borne coatings have the advantage that a wide variety of functional polymers, able to adhere to difficult substrates, can be used as long as it is readily soluble in an organic solvent. Organic solvents as diluents for coatings contribute significantly to the emission of volatile organic compounds (VOCs) having a negative impact on the environment. It is as a result of this that water-borne coatings, using water as diluent, are perceived as "green" or environmentally friendly compared to alkyd resins containing organic solvents.

Alkyd resins consist of oil-based polyester resins diluted with a range of aliphatic and aromatic organic solvents. Long, medium and short oil alkyds are excellent protective coatings for indoor and outdoor wood finishes. The main characteristics being superior water resistance as well as oxidative crosslinking ability that provide excellent barrier properties to withstand weathering and degradation. The crosslinking ability of solvent-borne coatings is also used in protective coatings for metals and in marine environments. The mechanism of film formation after application involves evaporation of the solvent leaving behind an entangled polymer network adhered to the pigments and substrate surface. Toxic and often carcinogenic solvents inhaled by applicators coupled with long term exposure led to many health problems in this sector. Also, solvent-based paint needs to be properly disposed of as hazardous waste, but in practice this is often not the case and waste paint end up in municipal landfill sites and is a cause for growing concern. The introduction of latex-based water-borne coatings has paved the way to significantly reduce health risks involved with application of coatings. The waste water generated can also be effectively treated by flocculation of the polymers and pigments contained therein. This reduces the environmental impact by no introduction of toxic organic compounds or solvents into the environment.Binders consisting of synthetic latexes also increase sustainability given their production in emulsion polymerization systems using water as the continuous phase. Despite appearing to be a viable alternative to reduce VOC emissions and decrease the generation of hazardous waste, water-borne coatings suffer from their own unique environmental concerns. The concerns lie within the need for organic emulsifiers/surfactants to produce the synthetic binders as well as aiding the dispersion of the inorganic/organic constituents of the coating. The use of water as diluent also allows for the growth of bacteria and fungi, affecting the shelf life and stability of coatings. In order to prevent this, a multitude of biocides are available for use in coating formulations. Biocides also help to prevent unsightly biofouling and the growth and spread of

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bacteria after application. The polymer component of the coating can easily be flocculated and disposed of, but the low molar mass surfactants and biocides invariably end up in industrial and municipal waste water[2]. Effective treatment or removal of these species in municipal sewage treatment plants is often not possible. Most of these compounds are non-biodegradable or degrade relatively slowly, leading to the accumulation of these species in the environment and microorganisms. Bioaccumulation in microorganisms, plants and mammals is a cause for great concern, since surfactants such as alkyl phenol ethoxylates (*APEO*) are considered endocrine disrupting species[3]. Accumulation of biocides in the environment may also lead to the proliferation of biocide-resistant bacteria[4]. Due to the increasing importance to preserve resources and commodities such as potable water, it is important to find new and improved processes that are sustainable and do not cause irreversible environmental damage. In this study, we took these concerns under consideration and propose an alternative biocide and stabilizer to eliminate the environmental concerns with low molar mass biocides and surfactants used in water-based coatings.

1.2 Objectives and thesis outline

The present study aims to provide a platform for the production of a viable alternative to low molar mass biocides used in the coatings industry. It also investigates the probability of using the same species to act as co-stabilizer or stabilizer in the synthesis of synthetic latexes used as binders in water-based coatings. The study focuses on suitably functionalizing a commercially available copolymer containing reactive anhydride units in the polymer backbone by reaction with mixed amine compounds. The reaction of the polymer with mixed amines aims to confer both inherent antimicrobial properties as well as water-solubility to the polymer. It goes on to investigate the incorporation of the modified copolymer species to act as co-stabilizer in a synthetic latex produced via emulsion polymerization.

Chapter 2 gives a broad overview of the application and nature of low molar mass biocides-, polymeric biocides and contact biocides. It also goes on to discuss the production of synthetic latexes by means of radical polymerization in a heterogeneous emulsion polymerization system.

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Chapter 3 describes the modification of a reactive commercial copolymer, poly(styrene-*alt*-maleic anhydride) (*SMA 1000, Sartomer)*, to introduce antimicrobial activity and water-solubility by reaction with mixed amines. The modified copolymer is subsequently incorporated into a film-forming synthetic latex of which the antimicrobial properties are assessed by means of fluorescence microscopy.

Chapter 4 makes use of the modified copolymer used in the previous chapter as a polymeric stabilizer for an emulsion polymerization system. It is shown that stable latexes are obtained via this process and inherent antimicrobial activity is conferred to the latex films obtained.

Chapter 5 provides the conclusions, in which a reflection is given on the objectives of the study. It goes on to discuss possible routes for future work and recommendations for the optimization of the use of modified copolymers as biocides and stabilizers in binders for water-based coatings.

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Chapter 2
Literature review

2.1 Biocides

Biocides or antimicrobial agents are chemical species capable of killing, or inhibiting the growth of microorganisms such as bacteria, fungi and algae[1]. Compounds that show antimicrobial activity range from organic molecules with amine functionality to organo-metalic and halogenated compounds. They are widely used where microbial contamination of surfaces and products needs to be prevented. A number of consumer goods like cleaning detergents as well as sterile packaging material contain compounds with antimicrobial activity. The architectural coatings industry is another key market in which consumers desire that the aesthetic appearance of painted surfaces should last a substantial period of time. Without the use of antimicrobial agents undesired staining and deterioration of the coating may occur due to fungal or microbial growth. The growth and spread of microorganisms on a substrate surface is known as biofouling. The prevention of biofouling is even more crucial in the case of protective coatings applied to the hulls of ships. Undesired growth of algae and other microorganisms can increase drag leading to higher fuel consumption and less maneuverability. Coupled with the higher cost of fuel is the need for regular dry docking to remove the microorganisms from the hull, leading to loss of income for ship owners[2].

Inhibition of the growth and spread of bacteria has recently become an area of growing research interest given the increasing demand for interior and exterior antimicrobial coatings for hospitals and day care centers to prevent the spread of infectious bacteria[3]. In various fields from medicine to food packaging it is crucial to have a sterile environment but is currently filled with substrates that allow for the growth of bacteria[4]. Substrates can effectively be cleaned with detergents/bleach and doing this on a regular basis, still only allows for temporary bacteria free environments[5-6]. The low level residual toxicity of low molar mass biocides in detergents is a cause of growing concern in areas where it is used on a regular basis[7]. Cleaning with household detergents leads to the generation of a substantial amount of wastewater. Ineffective treatment and removal of biocides in wastewater treatment processes leads to their accumulation in the environment, specifically fresh water bodies[8-9]. If they are present in high enough concentrations it may result in the poisoning of microorganisms and ecosystems. Another major concern is evidence of bacteria becoming increasingly resistant to conventional low molar mass biocides [10-11]. Selective survival of biocide resistant bacteria means that bacteria can build up resistance

against antibiotics, having adverse effects on the effective treatment of infections, posing a risk to human health [10,12-13]. Research is underway to produce contact biocides with no residual toxicity while being relatively inert to form part of the material or coating and allow it to be inherently antimicrobial[14].

Conventional biocides are used in solutions or in polymeric matrices. In order for biocides to function, they need to leach out of the matrix they are embedded in, and come into contact with the bacteria. Once in contact with bacteria, inhibition of growth or killing is achieved by disrupting the cell wall of the organism. With the cell wall no longer intact, the cell contents leak out and cell death occurs. Antimicrobial agents of this kind are classified as contact biocides. Alternatively, biocides are taken up by bacteria cells where they disrupt essential cell functions such as metabolic processes, also leading to cell death. The way biocides are introduced and function causes the material to lose activity over time, as biocides become less concentrated. The continuous leaching of biocides is a problem since the residual toxicity of free biocides poses environmental hazards.

This problem can be partially overcome by polymeric contact biocides. Where theoretically no biocide activity is lost over time as well as having no active agent against which bacteria can build up resistance[15]. The added advantage of polymeric biocides is the possibility of reducing the environmental impact of biocides since polymers are relatively inert and can be removed from wastewater with greater ease.

2.1.1 Low molar mass biocides

Low molar mass biocides are typically made up of halogens, metal ions, organo-metalic compounds as well as quaternary ammonium salts[7,12,16] and comprise all biocides that are not polymeric in nature. They are often impregnated into a polymer matrix, and use the water absorbed by the matrix as a vehicle to leach out and kill bacteria on the material surface and nearby surroundings[17-18]. Due to intrinsic properties of the high molar mass polymeric material, the matrix stays intact and only mild swelling may occur. High level toxicity of species such as organo-tin compounds (e.g. tributyl-tin) has resulted in the banning of its use in antifouling coatings for marine applications[19-20]. The use of organo-tin compounds has been phased out over the last couple of decades, but trace amounts at toxic levels to microorganisms in harbors still remain a cause of concern. Contemporary use of silver and copper ions pertaining to the activity of the Ag^{+} and Cu^{2+} ions against a wide range of bacteria saw this type of biocides gain increased popularity[14,21]. The species act via Ag^{+} binding

strongly to any electron donor groups in biological molecules containing sulfur, oxygen or nitrogen, which are all present in biological molecules in various forms. Cell wall disruption can similarly be achieved by introduction of the highly electronegative halogens in the form of quaternary ammonium salts and other derivatives of these low molar mass biocides[22]. In the case of temporary medical or surgical implants, the short time of antimicrobial activity is not a problem. However, the impregnation and leaching from latex coatings is not ideal given the call for long term antimicrobial activity in coatings. It is possible to develop ways of slowing down the release of the active species by diffusion control through a tailored matrix or only triggering the release under specific conditions such as specific *pH*-levels. These routes are often not practically or economically feasible and ultimately do not address the low level environmental toxicity of the biocides once it has left the polymer film. A major drawback attributed to the slow degradation of free biocides in the environment.

2.1.2 Polymeric biocides

Contact biocides can be attached to the backbone of chemically stable polymeric species that have no active ingredient that react with or bind to components within the bacteria cells. Instead, along with the biocide moieties in the backbone, they provide a charged surface either positive or negative that disrupts the cell wall and leads to bacterial cell death[23].

Advantages of polymeric biocides are[24]**:**

- Chemically stable
- Non volatile
- No residual toxicity
- No loss of biocidal activity over time

Polymeric biocides find large scale application in biomedical applications such as sterile bandages, clothing and equipment[25]. Macromolecules as antimicrobial agents are nonvolatile, chemically stable and incapable of penetrating through the skin. No loss of activity and no residual toxicity are thus suffered due to volatilization, photolytic decomposition or transportation.

Exclusive use of polymeric biocides shows promise of reducing the residual toxicity of antimicrobial agents whilst increasing the time span over which they can remain active.

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Despite the possible advantages over low molar mass biocides, the feasibility of polymeric contact biocides still relies strongly on whether they meet the following criteria[24]:

- Easy and inexpensive to synthesize;
- Long-term viability at the conditions of its intended application;
- Do not decompose to- or emit any toxic products;
- Should not be toxic or be an irritant whilst being handled;
- Antimicrobial viability can be regenerated upon loss of activity;
- Activity towards a broad range of pathogenic microorganisms in short contact times

The activity of polymeric biocides is affected by various factors such as molar mass, spacer length between active site and polymer backbone and hydrophilic/hydrophobic balance. In the case of quaternary ammonium and phosphonium compounds as biocides, the nature and type of the counter ions can also play a significant role.

Iked et al. have shown that the biocidal action of poly acrylate with grafted biguanide groups against *S. aureus* is dependent on molar mass of the polymeric biocide. The optimal range for these species is between 50k and 120k Da[26-27]. The cut off for sufficient bacterial viability was 50k Da and the activity increases with an increase in molar mass up to 120k Da. Another study by Kanazwa et al. on the antimicrobial activity of poly(tributyl 4-vinylbenzene phosphonium chloride) has also shown that an increase in molar mass is accompanied by an increase in biocidal activity. They went ahead explaining it to be the result of the action of the biocide to achieve lethal action in terms of cell death and proposed the following:

Increase in molar mass leads to an increase in:

- Adsorption onto bacterial cell surface
- Diffusion through the cell wall
- Adsorption onto the cytoplasmic membrane
- Disruption of the cytoplasmic membrane
- Leakage of cytoplasmic constituents and subsequent cell death

This cycle was further pointed out to be much more effective in terms the antimicrobial activity of polymeric species viz low molar mass species as biocides[28]. The increase in activity is due to multiple active sites in a polymer chain being able to adsorb to the cell wall and causing disruption of the cell wall, which leads to cell death. Polymeric biocides with this increased biocide capabilities can be more effective against a wide range of bacteria cells.

In general, bacteria can be classified in terms of the nature and sophistication of the cell wall. *Gram positive* bacteria such as *S. aureus* generally have a loose cell wall, while *Gram negative* bacteria (*E. coli*) have an outer membrane incorporated in the cell wall that forms an additional barrier for foreign molecules. Biocidal activity against *Gram positive* bacteria is often easily achieved whereas *Gram negative* bacteria provide more of a challenge and here polymeric biocides can play a significant role. The effectiveness of biocides incorporated into polymer backbones can also be increased by the choice of counter ions in cases such as ammonium and phosphonium salts[7]. Quaternary ammonium salts with bromide anions are more effective biocides than salts where chloride anions are the counter ions for the active site[28] . In another study involving phosphonium salts it has been shown that the nature of the counterion and its ability to associate strongly with the phosphonium ion forming a tight ion-pair had a lower degree of biocidal activity[24]. The biocidal activity of phosphonium counterions which can dissociate to free ions easily, showed higher efficacy towards killing bacteria. The loss of free ions can cause the biocide to lose its activity over time but biocidal activity, upon depletion of bound chlorine ions, can be regenerated by exposing the surface to dilute bleach solution. As opposed to conventional application of bleach to remove bacteria and fungi, this provides a much milder approach and reduces residual toxicity while providing an almost permanently antimicrobial surface. The possibility to easily functionalize surfaces with biocides and be able to regenerate their activity shows great potential in our search for inherently antimicrobial coatings.

2.1.3 Polymeric contact biocides in latex coatings

Literature contains various examples of how to incorporate contact biocides for the purposes of developing water-borne coatings. The most common of which is covalently bonding active biocide species to functional groups in the backbone of polymers. Copolymerization with functional monomers to produce the polymers that make up the final latex component of the coating is an even more effective route to introduce active biocide species[29-31] . Alternatively they could be grafted onto the particles or even act a polymeric surfactant for latex production. Used as polymeric surfactant, polymeric biocides are strongly adsorbed to individual particles and upon film formation remain distributed throughout the entire film[32]. The polymeric surfactant eventually forms part of the bulk system and allows for antimicrobial activity over a longer period of time due to the absence of leaching of the active ingredients as well as "fresh" biocide moieties being exposed as degradation of the binder

film proceeds. Quaternary ammonium compounds (QACs) are ideal candidates for this, showing good activity against bacteria they also allow for easy incorporation as functional monomers or by reaction with functional groups in the polymer[32-33]. The major disadvantage of covalently bonded contact biocides is the number and distribution of the species throughout the surface. The number of biocide species bonded to and exposed on the surface is largely dependent on how many functional groups for immobilization are contained in the polymer chains of the latex. Biocides immobilized on the film surface result in these species not being effective towards killing bacteria in nearby surroundings due to their immobility. Another concern is that once the bacteria cells are dead they may still stick to and accumulate on the substrate surface[34]. Accumulation on top of and around the active sites leads to deactivation of the surface-active biocides as well as the likelyhood of unsightly biofouling, still able to take place. Ways of preventing this type of biofouling led to the notion to produce ultra-hydrophobic surfaces. The extremely hydrophobic nature of the surface causes the biological tissue of bacteria and organisms not to adhere to the surface, circumventing the need for highly effective biocides. The drawback of this technique is that although it substantially reduces the number of microbes adhering to the surface, it still does not allow for a completely microbe-free substrate surface, even in combination with leaching antimicrobial agents. Self cleaning or self polishing coatings are another way of preventing unsightly biofouling[35]. They operate by built-in continuous degradation mechanisms of the surface layer thereby exposing a “new” surface layer all the time. This however is not a good solution due to the shortened lifetime of protective coatings and chalking in decorative coatings. Chalking is generally an undesired effect associated with binder degradation and exposure of bound pigments. Chalking leads to problems with greater water absorption, increased dirt pickup and reduction or loss of abrasion resistance. Among the various strategies available to produce polymeric biocides for water-borne latex coatings there remains a lot of room for improvement of its efficiency. Stricter legislation and environmental policies, coupled with consumer demand for “greener” products, makes improving on current systems a matter of urgency.

2.2 Latex production via Radical Emulsion Polymerization

Emulsion polymerization is a process in which mono-disperse polymer particles in the nano meter range (50-350 *nm*) are easily attainable in a radical polymerization reaction. The process consists of dispersing monomers in a continuous water phase along with surface active species (surfactants) which stabilizes the monomer droplets, the growing polymer particles and the resulting latex product. It has been an industrially viable process since the early 20th century but only after the conceptual understanding provided by Harkins (1947) and the quantitative description of the mechanism by Smith & Ewart (1948)[36] has it gained considerable interest, both academically as well as industrially [36-37]. Today this technique finds wide-spread use in the production of synthetic latexes for producing commodities ranging from coatings to cosmetics and even drug delivery systems. The latex component in coatings plays the important role of binding together all its components whilst being the primary means of allowing coatings to adhere to the substrate surface. Emulsion polymerization allows for tailor-made binder properties due to a variety of development and process strategies. These strategies ensure that the production of binders with rather complicated particle morphologies can easily be achieved. This section gives a brief overview of the polymerization system, formulation and process strategies involved in the production of binders for water-based coatings.

2.2.1 Radical polymerization

Radical polymerization is a chain growth polymerization technique popular for its versatility and can be utilized in a number of polymerization processes. It allows for the production of relatively high molar mass polymer chains, out of a wide range of monomers in: bulk-, solution- and heterogeneous polymerization systems. The reaction takes place in a series of kinetic events namely: (1) radical formation, (2) initiation, (3) propagation and (4) termination. The four events mentioned above will briefly be discussed in this text and for a more in depth explanation, the reader can consult the book by Moad and Solomon on free radical polymerization[38].

2.2.1.1 Radical Formation and initiation

Radicals can be generated in a number of ways such as photo-initiation, gamma radiation, electrochemical, redox reactions and thermal decomposition. With the latter technique

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being the most conventional route for introducing and maintaining a consistent radical flux in polymerization systems. The decomposition can be represented schematically (2.1) where the initiator (I) decomposes into two radicals (R•) with a decomposition rate coefficient of k_d.

$$I \xrightarrow{k_d} 2R\bullet \qquad (2.1)$$

The decomposition rate coefficient (k_d) is highly temperature dependent and the rate of decomposition is given by the following equation.

$$R_d = \frac{d[R\bullet]}{dt} = 2k_d[I] \qquad (2.2)$$

Since the decomposition rate coefficient is dependent on temperature, the rate of decomposition increases with increasing temperature. The residual initiator concentration over time can be presented as an exponential decay plot using the equation below (2.3) derived from (2.2)

$$[I] = [I]_0 e^{-k_d t}, \qquad (2.3)$$

where $[I]_0$ is the initial concentration at $t = 0$. Not all of the generated radicals initiate the growth of polymer chains and can be destroyed or lost by combination with oxygen or solvent molecules to form stable compounds. Introduction of an efficiency factor, f, into the expression for the rate of initiation (v_i) below, accounts for this. Consequently, the rate of initiation is described according to equation 2.4.

$$v_i = 2k_d f[I] \qquad (2.4)$$

The addition of the first monomer species (M) to an initiator radical ($R\bullet$) is defined as the initiation (2.5) of a species that will propagate and become a polymer chain.

$$R\bullet + M \xrightarrow{k_i} RM\bullet \qquad (2.5)$$

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The rate of initiation is given by equation 2.6:

$$R_i = k_i[R\bullet][M] \tag{2.6}$$

Radicals will initiate a monomer species and propagation ensues in a matter of seconds, often leading to polymer chains containing up to 10^3 monomer units or more.

2.2.1.2 Propagation

Propagation is the rapid addition of monomers to an initiated chain carrying an active radical centre. In this process, the propagation rate coefficient is assumed to be independent of the chain length of polymer chains containing an active radical centre. The propagation of an initiated monomer species can be represented as:

$$\begin{aligned} &RM\bullet + M \xrightarrow{k_p} -M_2\bullet \\ &-M_2\bullet + M \xrightarrow{k_p} -M_3\bullet \\ &-M_i + M \xrightarrow{k_p} -M_{i+1} \end{aligned} \tag{2.7}$$

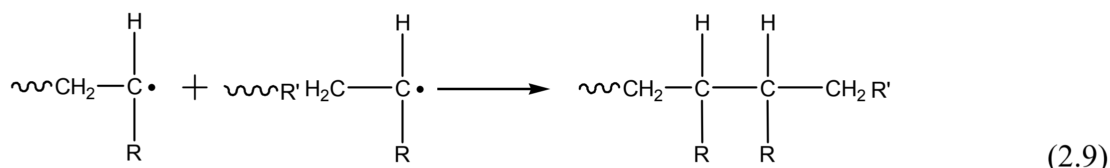
The rate of propagation is given by:

$$R_p = k_p[M\bullet][M] \tag{2.8}$$

2.2.1.3 Termination

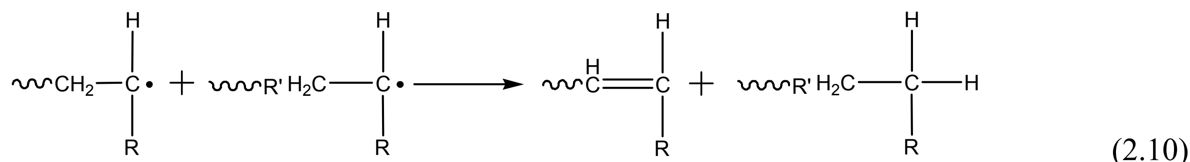
Termination can occur in one of two ways, by (a) combination and (b) disproportionation.

a) ***Combination*****:** Termination by combination occurs when two active radical centres combine to form a new bond. The radical centres of two growing chains combine to form one "dead" chain containing no active radical centre as shown in Equation 2.9.



(2.9)

b) ***Disproportionation***: The abstraction of a hydrogen, by an active radical centre of one growing chain, at the chain end of another results in disproportionation. This leads to two dead chains, one with a saturated chain end and the other with an unsaturated chain end. The unsaturated chain end is still susceptible towards radical reactions, and may for example copolymerize with a propagating chain or be reinitiated by a newly formed primary radical. Termination by disproportionation is shown in Equation 2.10.



(2.10)

The rate at which either of these two events occurs depends on the rate of diffusion of the polymer chains and subsequently that of the active radical centres. As the polymerization reaction reaches high conversions and the reactor contents become more viscous, the polymer chains have a slower diffusion rate. A lower diffusion rate of long polymer chains at higher conversion decreases the chances of termination of active radical centres. The decrease in the probability of termination occurring, may lead to an increased polymerization rate towards the end of the reaction, known as the Trommsdorf-Nourish auto-acceleration effect.

2.2.1.4 Transfer to monomer/solvent

During polymerization, transfer reactions may occur. Radical transfer to monomer, solvent or polymer does not necessarily affect the amount or concentration of radicals in the system but significantly influences the molar masses obtainable. In some instances it is required to add a chain transfer agent in order to limit the polymer chains to a certain degree of polymerization or molar mass.

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2.2.2 Emulsion polymerization[39]

2.2.2.1 Recipes and ingredients

The following ingredients are dispersed in a continuous water phase in a typical *ab initio* emulsion formulation or recipe:

a) Monomers

The monomers used in emulsion polymerization often have partial or limited solubility in water. Common monomers for emulsion systems include stryrene, acrylates, methacrylates and acrylic acids. Functional monomers are frequently employed in small quantities to increase the stabilization of latex products. In addition, monomers containing amines, epoxy or hydroxyl groups are employed to achieve crosslinking reactivity or confer ability to adhere to difficult substrates in applications such as adhesives and binders for water based coatings.

b) Surfactants

Surfactants take on the role of emulsifier or stabilizer within an emulsion polymerization system and the subsequent latex. A surfactant is a surface active compound containing both hydrophilic and hydrophobic segments. Different classes of surfactants, classified according to the hydrophilic group exist namely, anionic, cationic, non-ionic and pH dependent amphoteric surfactants[40]. All of which fulfill the role of stabilizer of monomer droplets and the formation of micelles to stabilize the growing polymer particles leading up to the stable latex. Surfactants also come in the form of polymeric stabilizers where the stabilization is of a more steric nature as is the case with partially hydrolysed polyvinyl acetate.

c) ***Initiators***

Water as well as oil soluble initiators can be employed in emulsion systems. However, examples of single initiator systems based on water-soluble potassium-, sodium- and ammonium persulphate salts are most commonly found in literature. The radicals are formed in the water phase where they subsequently initiate polymerization to form surface active *z-mers* (surface active oligomers) that migrate from the continuous water phase into micelles where they further propagate to form polymer chains. Besides the pH sensitivity of persulphate initiators they remain the most straightforward and trusted means of initiation for emulsion polymerizations.

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d) ***Buffer reagents***

The counter ions involved in ionic stabilization are provided by buffer reagents or *pH* adjusters. The stabilization effect of ionic surfactants often relies on an electric double layer formed among particles and this gives rise to synthetic latexes having a certain surface charge and zeta potential. The zeta potential of an emulsion is useful in predicting the long term stability of an emulsion.

e) ***Chain transfer agents***

Chain transfer agents, most commonly mercaptans, are employed to reduce the molar mass of polymers in radical polymerization reactions ensuring a suitable or desired molar mass is achieved. Emulsion polymerization often leads to molar masses that are too high for a given application and various types of chain transfer agents are used to avoid this. It is vital to employ these species in emulsion polymerization systems to control gelation where crosslinking may occur.

2.2.2.2 Basic principles and mechanism

With the inception of emulsion polymerization it was proposed that each radical generated in the aqueous phase will enter a monomer droplet and continue to propagate within it [39]. The droplet nucleation mechanism was assumed to be the dominant nucleation mechanism and that each droplet, upon nucleation continues to grow into a polymer particle. It is only true for mini-emulsion polymerization, a variation on emulsion polymerization, and not for emulsion polymerizations with added surfactants. The following section discusses why micellar nucleation, in the presence of surfactants, is the dominant form of nucleation.

Micellar Nucleation: Upon initiation, a monomer will rapidly add more monomer units until a short chain surface active species, *z-mer*, is formed. The short chain *z-mer* species are no longer soluble in water and have one of the following possible fates. It will either; a) propagate further b) reach its solubility limit and precipitate; c) undergo aqueous phase termination; d) enter into a monomer droplet or existing particle and e) enter a monomer swollen micelle. The latter being the most likely due to the number concentration and surface area of micelles exceeding that of monomer droplets by some orders of magnitude. The rate of radical entry is given by:

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$$k_e = 4\pi DNr \tag{2.11}$$

Where D represents the diffusion coefficient, N represents the number concentration of monomer droplets or micelles and r is the radius micelles, droplets or particles. Micellar nucleation strongly governs the rate of polymerization and conversion during the initial stages of the polymerization due to the fact that surface active *z-mers* preferentially enter and propagate within micelles.

Homogeneous nucleation: In the absence of a surfactant or after total depletion of surfactant species due to adsorption to growing polymer particles, homogeneous nucleation becomes possible. The fate of newly formed *z-mers,* not able to enter into any swollen micelles or growing particles, are a) entry into a monomer droplet, b) aqueous termination or c) further propagation. Radical species that continue to propagate in the continuous phase will reach a critical degree of polymerization. At this stage the polymer chain, being hydrophobic, will fold onto it self in order to decrease its surface tension while the charged radical chain ends provide some stability. Due to the hydrophobic nature of this species they will subsequently be swollen with monomer. The process of entry and propagation of a radical species into a monomer swollen polymer coil is known as homogeneous nucleation.

Upon initiation and nucleation emulsion polymerization proceeds in three distinct phases and can be classified as *Intervals I, II* and *III*:

Interval I: This stage is associated with the presence of large monomer droplets and a vast number of small micelles dispersed throughout the continuous phase in the reactor. Upon initiator decomposition and initiation in the continuous phase, surface-active radical species enter into the micelles and continue to polymerize. A monomer diffusion gradient from the continuous phase develops causing depletion of monomer droplets and monomer swollen growing particles. During this stage of the polymerization, the number and size of growing particles increase, resulting in an increase in the rate of polymerization. This stage is thus associated with the nucleation of micelles and the rapid growth of particles leading to relatively fast monomer conversion.

***Interval II*:** During this stage of the polymerization, no new particles are formed and all of the micelles have disappeared. The micelles are depleted by preferential adsorption of the

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surfactant molecules onto the surface of the growing polymer particles that increase in size during the first stage of the polymerization. Some of the surfactant molecules, previously part of micelles may also, aid stabilization of the monomer droplets that are still present but much smaller in size compared to Interval I. The main characteristic of this phase in the reaction is that the number of particles in the system remains constant, while the particle size is increasing. Monomer concentration within the growing particle remains constant. Also, the rate of polymerization throughout Interval II remains fairly constant due to no new particles being formed and the monomer concentration in swollen particles remaining constant.

***Interval III*:** This stage in the reaction marks the disappearance of monomer droplets. All of the remaining monomer in the system is present in the monomer swollen particles. No transfer of monomer through the continuous phase occurs and the remaining monomer within the particles is consumed, leading to a decrease in monomer concentration as well as rate of polymerization. However, as higher conversions are obtained the rate of termination decreases due to increase in viscosity leading to slower diffusion rates. A low rate of diffusion of radical chain ends often leads to an auto acceleration effect causing a spike in the rate of polymerization towards the very end of this stage and the reaction.

2.2.2.3 Particle number and growth of particles

After initiation, particles continue to grow throughout *Intervals I, II* and *III*. The rate of propagation equation for radical polymerization is modified for emulsion polymerization to take into account the fact that propagation occurs mainly within latex particles (2.12).

$$R_p = k_p[M]_p \bar{n} \frac{N_p}{N_{AV} V_s} \tag{2.12}$$

The number of latex particles remains constant after *Interval I*, since no new particles are initiated after this stage of the polymerization. It is possible to calculate the number of latex particles for a given system using Equation 2.13,

$$N_p = \frac{m_{Monomer}}{\frac{4}{3}\pi r^3 d_p} \tag{2.13}$$

In Equation 2.13, N_p is the number of particles; r denotes the unswollen particle radius and d_p the density of the polymer. Determination of the number and size of particles is important because the size and number of particles will influence the workable viscosity and flow properties of a latex.

2.2.2.4 Zero – one and pseudo bulk conditions

In emulsion polymerization, radicals are isolated and confined within the growing particles. Rates of propagation and termination are by implication very different for emulsion polymerization as is the case for bulk or solution polymerization. Kinetics in emulsion polymerization is governed by the so called "zero-one" conditions of emulsion polymerization. Building on the earlier theory of Smith and Ewart, a growing particle in emulsion polymerization contains either 1 or 0 radicals or radical chain ends. When a radical enters a particle already containing one, termination occurs instantaneously leading to a "dead" or "switched off" particle. Once a radical enters a particle formerly not containing any radical it leads to a so-called "switched on" particle in which the radical continues to propagate in the monomer-rich environment. A steady state arises in which the average number of radicals in a particle, $\bar{n}$, is less or equal to $\frac{1}{2}$. Meaning that on average half the particles are switched on and half of them switched off and monomer conversion continues only in the ones containing a radical or radical chain end. This is only true for moderate conversion, since at higher conversions termination reactions are diffusion controlled and more than one radical may be able to propagate within a particle.
Under zero-one conditions, the isolation (compartmentalization) of radicals is significant but this is not the case under pseudo-bulk considerations. As a consequence, the rate of termination is higher in the case of a pseudo-bulk system compared to zero-one conditions. Radical species are able to exit the particle and re-enter a different one due to its partial solubility and ability to be transferred across the continuous water phase. This leads to a system where $\bar{n}$ is sufficiently large and no significant isolation of radicals can be considered. Under pseudo-bulk conditions there is a high flux of radicals and rapid entry and exit of radicals in and out of particles occurs resulting in particles containing more than one radical at any given time. Each particle can thus be considered as a small reactor in which the kinetics is that of a conventional bulk polymerization system.

2.2.2.5 Copolymerization and composition drift

The composition of polymer chains in emulsion copolymerization is subject to a) reactivity ratios of the monomers and b) the ratio of the monomers in the growing particles or loci of polymerization. The monomer ratio within the particles is further subject to the partitioning of monomers from droplet reservoirs across the continuous phase into the particles. The monomer ratio within system may differ from the ratio within particles if there is a significant difference in water solubility of monomers. Copolymer composition drift will occur to a large extent if the more reactive monomer is the less water soluble of the two. The opposite is true if the more reactive monomer is also more soluble in water. To obtain a homogeneous copolymer composition in heterogeneous polymerization systems remains a challenge but a number of processing strategies have been developed to overcome these challenges. These processing strategies and their effect on copolymer composition and particle morphology are discussed in Section 2.2.2.7.

2.2.2.6 Polymeric Surfactants[40]

It is possible to stabilize oil-in-water emulsions using surface-active species that are polymeric in nature. These types of surface-active polymers have come to be known as polymeric surfactants. They are widely used in a similar fashion as their low molar mass counterparts in the stabilization of latexes in a variety of commodity products ranging from coatings to pharmaceuticals. The simplest types of polymeric surfactants are homopolymers made up of ethylene oxide or N-vinyl pyrrolidone. These polymers are readily soluble in water and as a result have little affinity to adsorb onto the O/W interface. They may however adsorb significantly to the solid/liquid interface in synthetic latexes. Homopolymers are however not the most effective emulsifiers and polymeric surfactants are predominantly made up of block or graft copolymers. Block copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) as polymeric surfactants are widely used and are commercially available under trade names such as Pluronic (BASF, Germany) and Synperonic PE (ICI, UK). They act under the assumption that the PPO section is strongly adsorbed at the hydrophobic oil or particle surface with the PEO segments dangling in the aqueous phase, subsequently obtaining steric stabilization of the dispersed phase. The adsorption and conformation of polymeric surfactants onto the

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interface is commonly described as a series of loops, trains and tails, illustrated in Figure 2.1.

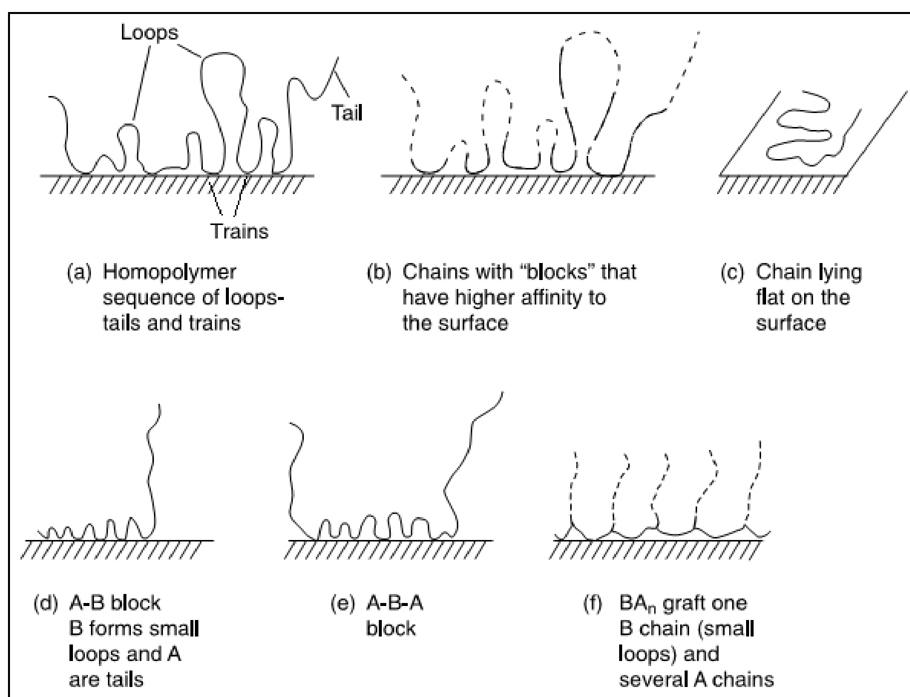

Figure 2.1: The adsorption of various types of polymeric surfactants onto a particle surface[40]

In order to understand how stabilization by polymeric surfactant is achieved it is crucial to have a sense of the nature of adsorption and conformation of these species in the heterogeneous system. It is however often difficult to establish since not only polymer/solvent interactions are at play, but also the polymer/surface and surface/solvent interactions. Based on existing models polymeric surfactants irreversibly adsorb to the particle surface and stabilization is achieved sterically by means of the trains, loops and tails presented in Figure 2.1, after adsorption.

For a more in depth explanation of how these interactions are modeled the reader can refer to a review on polymeric surfactants in disperse systems by Tadros [41].

2.2.2.7 Process strategies and particle morphologies

The mode of operation of emulsion polymerization has a significant effect on the topology of the polymer chains as well as particle morphologies obtained. By operating the polymerization in a semi-batch fashion (addition of monomers at a constant rate) it is possible to obtain a narrow chemical composition distribution and reduce the heterogeneity

often observed for batch emulsion polymerizations. Two modes of semi-batch or constant addition emulsion polymerization exist: a) Constant addition under flooded conditions and b) constant addition under starved conditions. Under flooded conditions the monomer feed is added at a rate much higher than the rate of polymerization of the individual monomers in the system. Starved conditions on the other hand involve feeding the monomers at a slower rate than the overall rate of polymerization. Feeding the monomers in this fashion allows for a steady state to develop where high monomer conversion is maintained and the monomer ratio within the growing polymer particles is the same as that of the feed composition. This implies that the polymerization of monomers is equivalent to their rate of addition and copolymers are obtained with a chemical composition identical to the ratio of the monomers in the feed. By changing the feed composition as well as feed rate, various chemical compositions and core shell particles can be produced via emulsion polymerization. Core-shell refers to the inside of a particle having a largely different composition to that of the polymer chains making up the shell of the particle. Core-shell particles are often produced with various niche applications in mind and to confer specific properties the latex particles not attainable by mere copolymerization of different monomers. Examples are particles with a core made up of a high glass transition polymer and a shell with a low glass transition polymer. This type of particle will have the ability to coalesce and film form easily at room temperature but still have superior mechanical strength and durability due to the hard core. It is also possible to produce hollow particles where the core can be dissolved in a suitable solvent given the fact that the shell feed contained a difunctional crosslinking monomer to maintain its structural integrity. The encapsulation of inorganic particles to produce composite latexes is also beneficial in order to ensure the optimal distribution of inorganic material throughout a latex and subsequent latex film.

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Chapter 3

Synthesis and antimicrobial activity of modified styrene-alt-maleic anhydride

Abstract

A water insoluble alternating copolymer, *poly(styrene-alt-maleic anhydride) (SMA)*, is modified to yield a polymeric species that is both water-soluble and contains inherent antimicrobial activity. The modified polymeric species is then incorporated into synthetic latexes and their antimicrobial activity is tested.

3.1 Introduction

Biocides are widely used in the coatings industry to prevent or inhibit bacterial growth in storage containers as well as after application on substrate surfaces[1]. The current use of low molar mass biocides is a source of major concern because biocides leach out of the final products or substrate films and result in residual toxicity in freshwater systems including rivers and dams as well as ground water sources [2-3]. Theoretically, this problem could be circumvented by suitably functionalizing or modifying a polymeric species to produce a macromolecular biocide[4].A polymeric biocide integrated into the film would be unable to leach from the final substrate film. Wastewater treatment would also be much simpler because macromolecular species can easily be removed from wastewater by means of a suitable flocculent[5]. Most polymers, such as those produced by free radical polymerization of alpha olefins, are fairly inert and do not allow for any further modification. However, copolymerization of a stable monomer with one that allows for further modification produces polymeric species that can easily be functionalized, post-polymerization.

For example, the copolymer *SMA*, contains maleic anhydride units that are highly reactive and susceptible to nucleophilic addition reactions by various amine compounds[6]. *SMA* is a strongly alternating copolymer and, depending on the excess of *STY* relative to *MAnh* in the monomer mixture, a block of *STY* of varying chain length can be incorporated towards the end of the reaction by using controlled living radical polymerization[7]. *SMA* undergoes self-emulsification after incorporation of a suitable nucleophile via the maleic anhydride units in the polymer chain to introduce specific functional groups into the polymer chain[8].Various tertiary amine and quaternary ammonium compounds have shown a great degree of antimicrobial activity[9]. After modification it is potentially possible to incorporate amine-functionalized *SMA* into synthetic latexes, which would open possibilities to use the modified *SMA* as a polymeric

biocide in latexes. In this study we aim to incorporate such modified species into an existing latex in order to establish: (1) whether or not the latex will remain stable over time and (2) the effect of the addition of functionalized copolymer on the properties of the synthetic latex.

Stabilization due to the modified copolymer should be achieved either sterically or electrostatically in order to act as a co-stabilizer along with the surfactant. For both methods, the stability of the latex pre- and post-addition of the modified copolymer was verified by means of dynamic light scattering (*DLS*) to measure any changes in particle size and distribution. The film formation and properties such as miscibility of the various copolymers in the latex were determined by Transmission Electron Microscopy (*TEM*) along with thermal analysis by Differential Scanning Calorimetery (*DSC*). Fluorescence microscopy was used to verify the extent of bacterial growth on the films relative to a control of a film without any biocide incorporated into the latex.

3.2 Modification of *SMA*

The modification of *SMA* described in this section was derived from literature, where similar modifications led to the manufacturing of nano-fibrous antimicrobial materials[10].

3.2.1 Reagents

SMA1000 (*Sartomer; alternating co-polymer 1:1 Styrene to maleic anhydride,* M_n =2351 *g/mol,* *Đ*=1.99), *3-dimethylamino-1-propylamine* (*Fluka; 98%*), acetone (*Sasol; 98.5%*) and *ethyl acetate* (*chemically pure, KIMIX*) were used as received. The water used as continuous phase was distilled de-ionized water (*DDI*).

3.2.2 Modification of SMA with ammonia

In order for the hydrophobic *SMA* copolymer to be water-soluble it was necessary for it to undergo modification to increase its hydrophilic nature. Despite the amount of the polar maleic anhydride contained in the copolymer it was water-insoluble, even at low molar masses as was the case with *SMA 1000* used in this study. Water-solubility could be attained by ring-opening the maleic anhydride units and converting them to amic acids by reaction with aqueous ammonia. The reaction by which the ring-opening occurred was by nucleophilic addition of aqueous ammonium (Fig. 3.1).

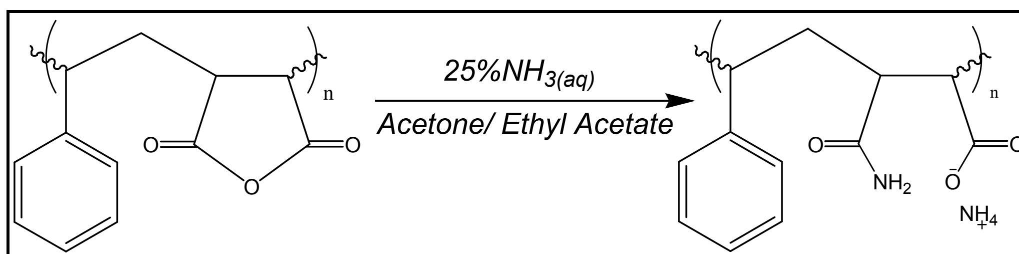

Figure 3.1: Ring opening of SMA with aqueous ammonia.

Upon modification, the ring-opened form of the maleic anhydride units contained two functional groups, a carboxyl moiety stabilized by an ammonium counter ion on the one side, and an amide moiety on the other. Carboxylic acids and amides are soluble in water and ring-opening some or all of the maleic anhydride units will increase the water-solubility of the *SMA* polymer. In order to select the optimum degree of ring-opening for sufficient solubility in water, a range of different degrees of modification were compared. The copolymerization of styrene and maleic anhydride has a strongly alternating tendency since the reactivity ratios of styrene and maleic anhydride are close to zero. Consequently, the polymer obtained from a monomer mixture containing an equimolar amount of each comonomer is an alternating copolymer. Based on the alternating character of the polymer it is straightforward to calculate the amount of ammonia necessary for partial ammonolysis of the anhydride residues. The necessary extent of reaction was determined by qualitatively assessing copolymer solubility in water, post-modification. In the event that polymeric species were only partially soluble in water, the *pH* dependence of the solubility of species of this nature was taken into account. The solubility was thus verified at slightly higher pH (ca. 8-9), which was measured with laboratory pH strips.

Procedure:

SMA 1000 (*1g*) was added to20*mLacetone* in a round-bottom flask, equipped with a magnetic follower, and dissolved under magnetic stirring. Stoichiometric amounts (Table 3.1) of*25%* aqueous solution of ammonia (*25%* $NH_{3(aq)}$) was added drop wise. As the nucleophilic addition of the amine and the subsequent ring-opening to form the amic acid occurred, the polymer precipitated out of solution. The reaction mixture was allowed to react for one hour and the *acetone* was removed by means of a rotary evaporator (*rotavap*). The polymer was then dried overnight in a petri dish with the fume hood switched on. The dissolution in water (100*mgSMA*; 5*mL*H_2O) at pH 8-9 was done by adjusting the pH with addition of $NaHCO_3$ and verifying the solubility of the species. The pH was measured with no other means than common laboratory pH strips.

Table 3.1: Stoichiometric amounts of $NH_{3(aq)}$ and degree of ring opening of the maleic anhydride units of the polymer chain used in the modification of *SMA*

%Ring opening	SMA (g)	$NH_{3(aq)}$ (g)
15%	1	0.052
30%	1	0.104
45%	1	0.156
60%	1	0.208
85%	1	0.294

3.2.3 Modification of *SMA* with mixed amines.

The modification of *SMA* with mixed amines took place in two stages. Firstly, partial imidization of the maleic anhydride units in the copolymer was achieved by ring opening the desired amount of maleic anhydride units with a functional amine and then ring closing at elevated temperature (Fig. 3.2).The imidization reaction was done by nucleophilic addition to the desired fraction of maleic anhydride units with the appropriate amine compound (*3-dimethylamino-1-propylamine*) and subsequent ring closure under vacuum at elevated temperature ($T > 100\,^{\circ}C$). The ring closure of the open rings led to the evolution of water and this was removed by doing the modification at temperatures slightly higher than $100\,^{\circ}C$ under high vacuum in a vacuum oven. The imidized copolymer was essentially water-insoluble in the *pH* range of 8-9 after pH adjustment using $NaHCO_3$ and its solubility in the solvent used for the ring-opening reaction was qualitatively assessed. After ring closure the polymer samples were again soluble in the solvent in which the ring opening reaction was carried out. Secondly, rings were opened again via ammonolysis of the residual maleic anhydride units contained in the polymer chain (Fig. 3.3). The water-insoluble copolymer was reacted with ammonia solution to yield a partially imidized water-soluble copolymer in the desired pH range (pH 8-9).

Procedure:

Part A (Figure 3.2)

2 *g SMA* was dissolved in acetone (30 *mL*) under mechanical stirring in a 50 *mL* round bottom flask. The *3-dimethylamino-1-propylamine*was added dropwise and, as in section 3.2.2, the ring-opened polymer precipitated out of solution. The amount of *3-dimethylamino-1-*

propylamine added was varied to produce five degrees of imidization: 85-, 70-, 55-, 40- and 25% (Table 3.2). The reaction mixture was stirred for an additional half hour at room temperature. The acetone was removed by means of rotary evaporation and the white precipitate formed in the reaction was placed in a vacuum oven for *20 h* at *110°C*. This was done to convert the modified maleic anhydride units from amic acid to maleimide[8]. Fourier transform infrared spectroscopy (FT-IR) was used to verify if the fully ring closed maleimide functionality was obtained after heating the polymer samples under vacuum.

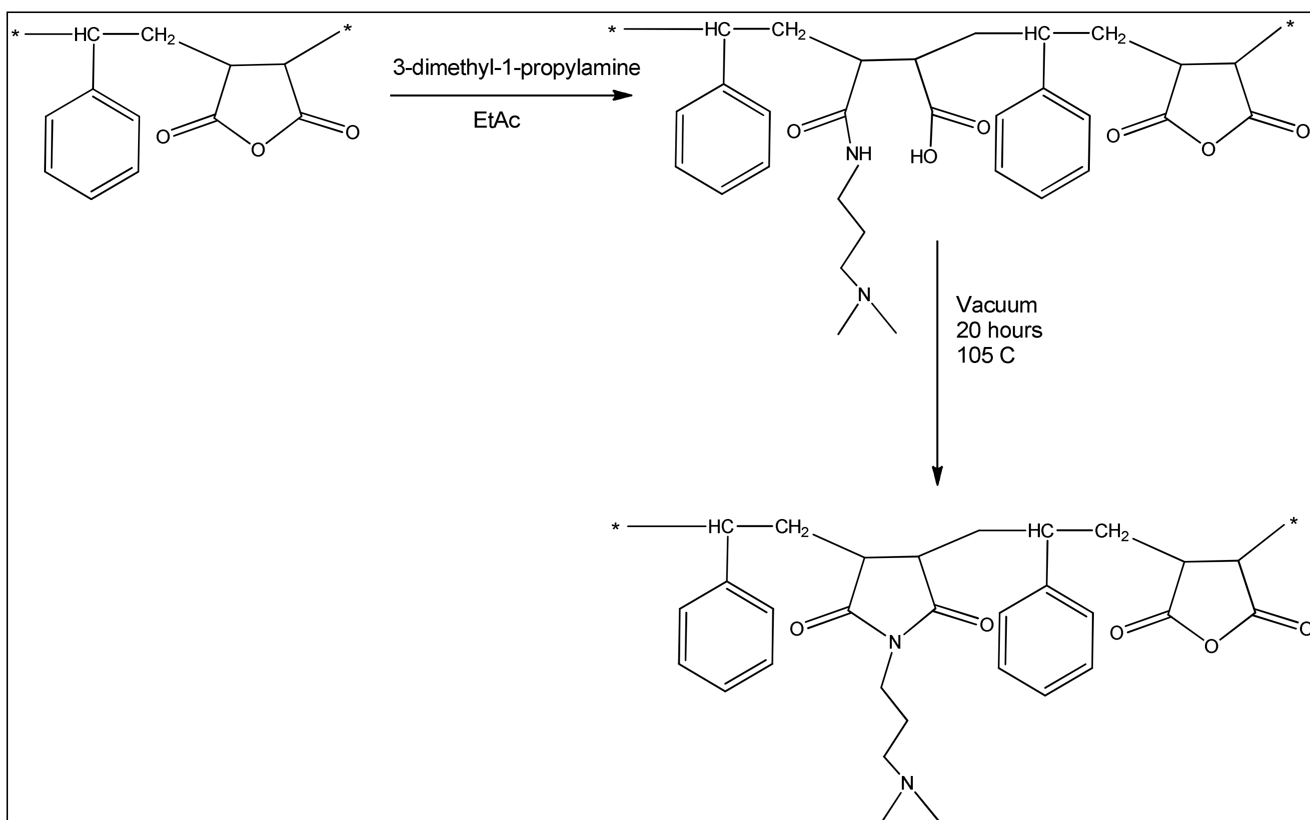

Figure 3.2: Reaction scheme for imidization of *SMA*

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Table 3.2: Amounts of reactants for the varying degrees of imidization

% Imidization	SMA (g)	$mass_{tertiary\ amine}$ (g)
85%	2	0.885
70%	2	0.729
55%	2	0.572
40%	2	0.416
25%	2	0.260

Part B (Figure 3.3)

Each of the modified copolymers with various degrees of N,N-dimethylaminopropyl maleimide functionality obtained in Part A was added to acetone (50 *mL*).To this solution, a stoichiometric amount of *25%* $NH_{3(aq)}$ was added drop wise and again a white precipitate formed. The amount of ammonia solution was varied in accordance with the percentage of ring opening – 15-, 30-, 45-, 60- and75% (Table 3.3). The solution was transferred into a petri dish and left in a fume hood to allow for solvent evaporation and then dried under high vacuum in a desiccator. A white/yellow shiny powder was recovered and its solubility in water was verified at *pH 8-9* by adjusting the pH through addition of $NaHCO_3$.The water-solubility indicated that the residual maleic anhydride units were converted to yield the ring opened amic acid, resulting in water-solubility of the polymer chains.

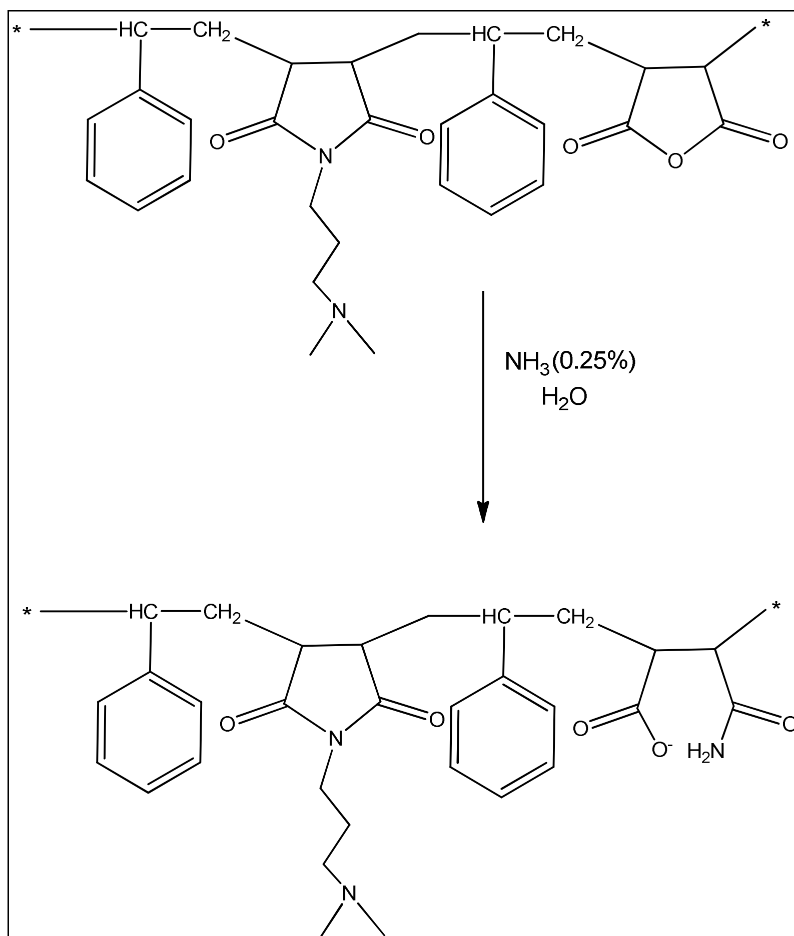

Figure 3.3: Reaction scheme for ring opening of imidized *SMA*

Table 3.3: Amounts of *25%* ammonia solution for ring opening of residual maleic anhydride unitsfor each of the partially imidized SMA co-polymer entries in *Table 2.*

% Ring opening	**mass$_{NH3}$ (g)**	**mass (25% $NH_{3(aq)}$)**
15%	**0.026**	**0.104**
30%	**0.052**	**0.208**
45%	**0.078**	**0.312**
60%	**0.104**	**0.416**
75%	**0.130**	**0.520**

3.2.4 Optimized synthesis of modified SMA

In order to make sure that no residual ammoniaor solvent were still contained in the sample, the final step of the reaction was also carried out in water instead of *acetone / ethyl acetate*. This was done to ensure that none of these compounds would interfere with the antimicrobial activity studies. The water-insoluble, imidized, polymer was added to water and the ammonia solutionwas added dropwise under magnetic stirring.The polymer could subsequently be isolated by freeze drying the solution. The imidization reaction was also done in *ethyl acetate* instead of *acetone* to avoid possible Schiff base formation that could potentially lead to a lower extent of modification of the maleic anhydride units in the copolymer chain.

3.3 Emulsion copolymerization of *styrene and butyl acrylate*

3.3.1 Reagents

Styrene (*STY*) and *butyl acrylate* (*BuA*) monomer were obtained from Freeworld Coatings Research Centre at Stellenbosch and purified by distillation under reduced pressure after removal of inhibitors by washing with $KOH_{(aq)}$ and $NaOH_{(aq)}$ respectively and drying over $MgSO_4$-anhydrous. The water-soluble initiator, *KPS (potassium persulphate)* [Sigma-Aldrich,99%], was used along with *SDS (sodium dodecyl sulphate)* [Saarchem 90%] as surfactant. Water was used as the continuous phase for the emulsion system (*Milli-Q* deionised water).

3.3.2 General formulation and procedure

Synthetic latex of *styrene* and *butyl acrylate* was produced by *ab initio* emulsion polymerization using *SDS* as surfactant. The polymerization of a copolymer of *STY/BuA* with a theoretical T_g of 18 ℃ (calculated with *Fox equation (*Equation. 3.1*),* where T_g is the copolymer glass transition temperature; $T_{g1\&2}$ and $w_{1\&2}$ denote the glass transition temperature and weight fractions of the respective monomers *1* and *2*) was synthesized by means of emulsion polymerization employing *SDS* as surfactant and *KPS* as water-soluble initiator (Table 3.4).

$$\frac{1}{T_g} = \frac{w_1}{T_{g1}} + \frac{w_2}{T_{g2}}$$

Equation 3.1: Fox equation to predict the glass transition temperature for copolymers

The polymerization was done at *70°C* at an agitation speed of 213*rpm* by means of an overhead stirrer equipped with a Teflon impeller. Degassing prior to polymerization was done with argon for *20 min* and an additional *5 min* after the initiator was added. Reaction was left to proceed for 4.5 h to ensure maximum monomer conversion was achieved.

Table 3.4: Formulation of *Sty/BuA* emulsion polymerization with *SDS* as surfactant

Component	Compound	%mass	mass (g)
Continuous phase	H_2O	76.8	120.0
Monomer	*Butyl Acrylate*	9.40	14.70
	Styrene	12.6	19.80
Initiator	*KPS*	0.12	0.188
Buffer Reagent	$NaHCO_3$	0.12	0.188
Surfactant	*SDS*	1.20	1.880

Emulsion system: ***pH 8-9***

Shear rate: **213 rpm**

Theoretical Solids %: **23%**

Procedure:

Monomer, surfactant and water were added to the *250mL* glass reactor and placed under mechanical stirring. The reactor was submersed in an oil bath set at *70°C* and the reaction mixture was degassed with argon for *20 min*. The initiator was dissolved in a vial with*5 g* water and added to the reaction mixture by means of a syringe through a rubber septum. The reaction mixture was then degassed for an additional *5 min*. After completion of the reaction, mechanical stirring was stopped and the reactor was removed from the oil bath. The reaction mixture was allowed to cool to room temperature and the solids content was determined gravimetrically. The experimental solids content was *ca. 20%*after polymerization was left to react for 4.5 h.

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3.3.3 Addition of modified *SMA* to *STY/BuA latex*

The modified *SMA* samples obtained in *Section 3.2.3*were added to the *STY/BuA* latex (*10g*) in an amount equal to *5wt% (0.1g)* of the total experimental solids content (i.e. *1wt%* of the latex). The stability of the latex was verified by means of *DLS* and *TEM* to investigate whether or not the expected co-stabilizing effect of the modified polymer was achieved and no aggregation or coagulation of species occurred.

3.4 Characterization and analysis

3.4.1 Fourier Transform Infrared Spectroscopy *(FT-IR)*

FT-IR was used to confirm the successful functionalization of the *SMA* copolymer. The polymer samples were compressed into translucent discs after mixing with *KBr*. The spectra were recorded using a *Nexus FT-IR* spectrophotometer and presented as an average of *32* scans, running the instrument in transmission mode under $N_{2(g)}$ purging, with a resolution of $4.0cm^{-1}$. The data were subsequently analysed employing *Omnic Software* and *Origin 6.2*.

3.4.2 Size Exclusion Chromatography (*SEC)*

For *SEC*, the instrument included a *Waters 1515* isocratic pump, a *Waters* inline degasser AF, a *Waters 717* plus auto sampler with a 100 µL sample loop, a *Waters 2487* dual wavelength absorbance *UV* detector, a *Waters 2414* refractive index detector at *30.0°C*. *SEC* measurements were performed on a set of two *PLgel* columns (*Polymer Laboratories*) *5µm Mixed-C (300×7.5 mm)* connected in series along with a *PLgel* guard column (*50×7.5 mm*). *THF* (stabilized by *0.125% BHT*) was used as mobile phase at a flow-rate of *1.00 mL/min*. Sample concentrations were *1.0 g/L* and injection volumes were *100 µL*. The columns were calibrated with polystyrene standards from Polymer Laboratories (*Church Stretton, Shropshire, UK*). Data processing was performed by *Breeze* version *3.30 SPA (Waters)* software.

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3.4.3 Dynamic light scattering (*DLS*)

DLS was done at the Freeworld Coatings Research Centre, Stellenbosch, on a Malvern Instruments ZetaSizer Nano-S equipped with a He-Ne laser operating at a wavelength of *633.0 nm* and the scattered light was detected at an angle of 90°. The instrument was externally calibrated with *200nm poly(styrene)* spherical particles. The samples were diluted until slightly translucent and the measurements were taken as the average of *3* measurements of *15* acquisitions each. The stability of the latex samples were also tested after addition of $CaCl_{2(aq)}$ solution of up to *20 wt%* $CaCl_2$.

3.4.4 Transmission Electron Microscopy (*TEM*)

The particle morphology of the polymer latex was investigated by means of *TEM* analysis using a *LEO 912 Omega TEM (Zeiss,Oberkochen,Germany)* at *120kV*. The latex (*1μL*) was diluted with *DDI* water (*1mL*) so as to obtain a translucent solution and *3μL* of the solution was transferred onto a piece of parafilm. Staining agent (*2% aqueous uranyl acetate*) was added to the solution and subsequently transferred to a carbon-coated 200 mesh copper grid. Excess sample was removed by means of blotting with a piece of filter paper and left to dry at room temperature before the imaging was done.

3.4.5 Inoculation of films in bacteria medium

Films of 75*μm* were cast on a microscope slide, employing the *STY/BuA*latex with the *85% SMI* (*Styrene maleimide* copolymer) added in 5%wt of the total solids content and dried in a sample drying oven @ 60°C. The slides were then placed in a petri dish along with the bacteria *Lactobacillus sakei* (*Gram positive*),in a suitable growth medium solution. The growth medium used was de Man, Ragosa and Sharp (MRS) broth obtained from BioLab Diagnostics (Midrand, South Africa). This was then inoculated at *37°C* for *24hrs*. The slides were removed and stained with fluorescent dyes and imaged via fluorescence microscopy as described below.

3.4.6 Fluorescence microscopy

After inoculation in bacteria solution, the latex films were treated with *Hoechst 33342* and *propidium iodide*. Samples were observed on an *Olympus Cell*$^{\wedge R}$ system attached to an *IX-81*

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inverted fluorescence microscope equipped with an*F-view-II* cooled *CCD* camera (*Soft Imaging Systems*). Using a *Xenon-Arc* burner (*Olympus Biosystems GMBH*) as light source, images were excited with the *360 nm* and *572 nm* excitation filter. Emission was collected using a UBG triple- bandpass emission filter cube. An *Olympus Plan Apo N 60x/1.4 Oil objective* and the *Cell^R* imaging software have been used. Images were processed and background-subtracted using the *Cell^R* software

The fluorescence dyes used in the experiment were chosen carefully in order to distinguish between dead and living bacteria. The blue stain (*Hoechst 33342*) is able to permeate through the cell wall to the nucleus of living as well as dead cells. The red stain (*propidium iodide*) however is only capable of entering bacteria cells once the cell wall is no longer intact. Thus if a bacteria is coloured red it implies that the cell wall is no longer intact and that the bacterial cell is dead. The imaging was done in three different regions of each film to establish and compare if the same effects were visible throughout the entire film.

3.4.7 Differential Scanning Calorimetry (*DSC*)

Differential Scanning Calorimetry (*DSC*) was done using a *Q 100 V9.9* DSC instrument. The heating and cooling rates were done at *10°C/min*. TA Instruments Advantage software Release 4.2.1 was used to analyse thermograms obtained.

3.5 Results and discussion

3.5.1 Characterization of modified *SMA*

Ring opening of the maleic anhydride units in the copolymer was done to varying degrees namely, *15-, 30-, 45-, 60-* and *85 mol%*. It was expected that there will be an increase in water solubility across the range from the least amount of ring opening to the highest degree of ring opening. The object was to determine the minimum degree of ring opening to achieve water solubility. Qualitative assessment of the solubility of the *SMA* samples with no imidization and after only ammonolysis was done, showed that the copolymer containing a theoretically targeted amount of *85%* amic acid was almost completely dissolved after 5 days (Table 3.4) in water at *pH 7*. Whereas the remaining samples with a lower degree of ring opening were only partially soluble at the same conditions and *pH* but almost completely dissolved after 13 days (Table 3.5). We expected solubility to increase with degree of modification, and indeed

the greater the percentage of amic acid, the greater the degree of solubility (Table 3.5). The dissolution process of the modified copolymers was a slow process, with three of five samples more soluble at 13 days than at 5 days. Since the solubility of these species is *pH* dependent it was assessed that all of the polymer samples were readily soluble after raising the *pH* slightly to between *8* and *9*. The success of the imididization step was also qualitatively assessed by the solubility of the product in *ethyl acetate*, prior to ammonolysis. It was found that the ring-closed maleimide functionalized product was obtained given the solubility of the product, after heat treatment in a vacuum oven, in the same solvent (*ethyl acetate)* from which the ring opened polymer was isolated after precipitation.

Furthermore, the *FT-IR* spectra obtained for the polymer samples clearly showed the shift of the *maleic anhydride* peak (1780 cm^{-1}) to (1695 cm^{-1}) corresponding to the absorbance band of a *maleimide* functional group (Figure 3.6). All of the modified *SMA* samples also contain a broad band at (3433 cm^{-1}) which is normally associated with the absorbance bands for $–OH$ and $–NH_2$.

Table 3.5: Qualitative assessment of solubility after5 and 13 days of 15-*, 30-, 45-, 60-* and *85 mol%* ring opened SMA copolymers

%Modification	Day 5	Day 13
15% Amic Acid	Insoluble	Insoluble
30% Amic Acid	Not completely dissolved	Almost completely dissolved
45% Amic Acid	Not completely dissolved	Almost completely dissolved
60% Amic Acid	*Polymer wasn't recovered*	
85% Amic Acid	Almost completely dissolved	Almost completely dissolved

Solubility: 100mg dissolved in 5mL H_20, pH 7.

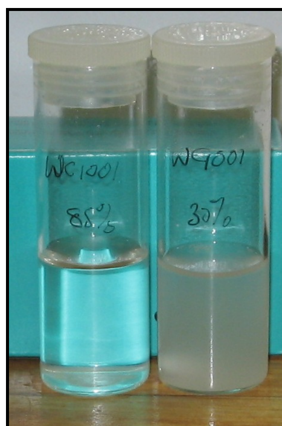

Figure 3.4: Solutions of *85%* (left) and *30%* (right) ring opened *SMA* after 5 days in water.

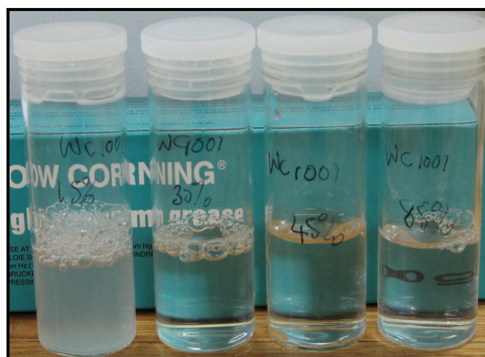

Figure 3.5: Solutions of *15-, 30-, 45-* and *85%* ring opened SMA (Table 4) after 13 days inwater.

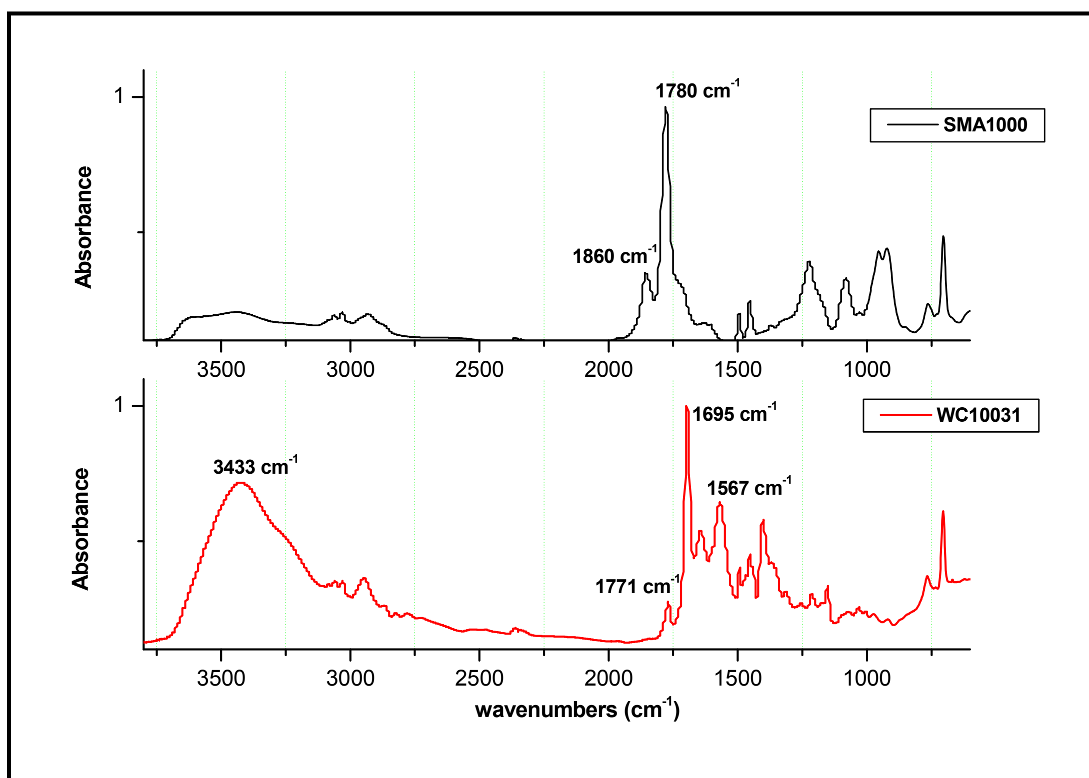

Figure 3.6: FT-IR spectra of *SMA* (top) and the modified (*SMI 85%*) imidized and ring-opened copolymer (bottom)[11].

3.5.2 *DLS, DSC* and *TEM* analysis of *STY/BuA* latex

The *STY/BuA* latex as well as the latex with the various modified *SMA* samples added proved to be visibly stable over time and this was confirmed by means of *DLS* and *TEM* analysis. The particle size and distribution results were the same for the latex synthesized with *SDS* as stabilizer and for those with the modified *SMA* copolymer added after polymerization. The modified *SMA* copolymer was modified to varying degrees as indicated in the table. The PSD as well as the average particle sizes were in good agreement with what we would expect from a conventional *ab initio* emulsion polymerization system (*50-350nm*) (Fig. 3.7). There was a

slight drop in particle size over time for the sample with the *15%* ring opening and *85%* imidization copolymer added to the latex (Table 3.6). The particle size evolution over time for the remaining latex samples, with varying degrees of modified *SMA* copolymers added, could however not be accessed since no initial values were taken for these.

Table 3.6: Storage stability data obtained by means of *DLS* for samples of modified *SMA* copolymers ranging from *85*- to *25%* imidization and the difference ring opened amic acid functionality, added to *STY/BuA* latex produced with *SDS*.

Sample	Days Stable	Z-avg (*nm*)	PDI
85% Imidized	**1**	**62.6**	**0.066**
85% Imidized	**178**	**61.9**	**0.056**
70% Imidized	**178**	**58.2**	**0.051**
55% Imidized	**178**	**59.8**	**0.053**
40% Imidized	**178**	**58.3**	**0.034**
25% Imidized	**178**	**58.9**	**0.038**

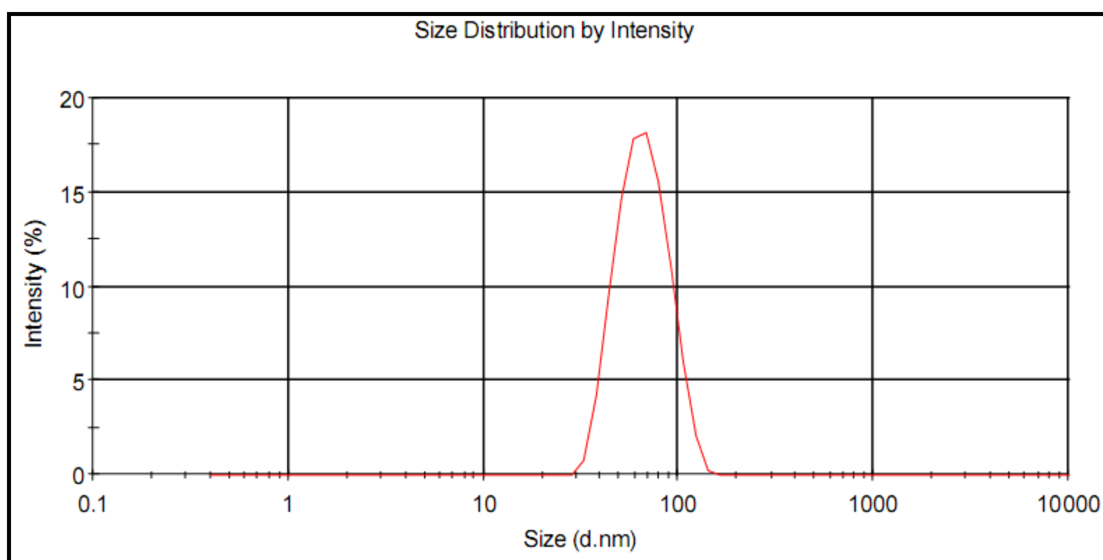

Figure 3.7: Particle size distribution from *DLS* of Sty/BuA latex with modified *SMA 85%* maleimide modification added in *5 wt%* of total solids content with $Z_{average}$ = 62.60 *nm* (*Đ* = 0.066)

The particle sizes obtained by measuring the particle diameters on the *TEM* images were in good agreement with the results obtained with *DLS* for the *STY/BuA* latex (*DLS=58 nm*; *TEM =62nm)* and for the *STY/BuA* latex with added *SMI-85* (*DLS= 62 nm; TEM=68 nm*) (Fig. 3.8).

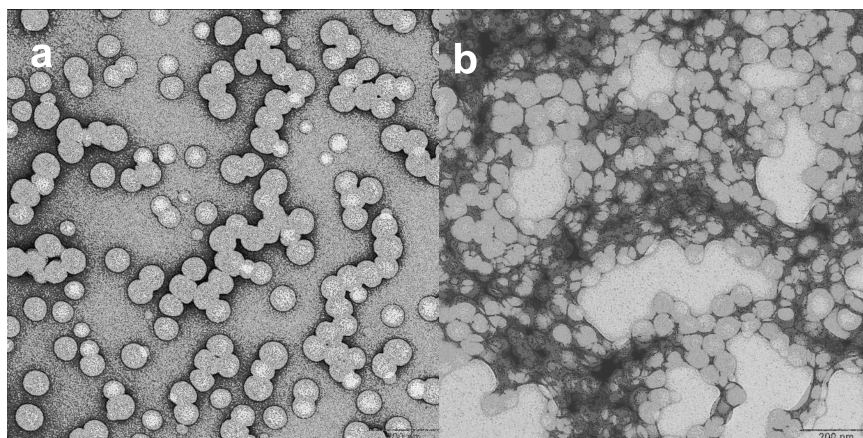

Figure 3.8: *TEM* image of *STY/BuA* latex as synthesized with *SDS* (**a**) and the same latex with the added *SMI-85* (**b**).The scale bar in the images corresponds to a length of 200 *nm*.

TEM images show that rather uniformly distributed latex particles were obtained using *SDS* as surfactant (Figure 3.8 (a)). The dark regions in the film could be attributed to the staining agent, used to improve the contrast of the images, becoming trapped in between particles, or it could be due to *SMI* trapped in between some particles which was able to absorb more of the staining agent than the particles themselves. A small degree of phase separation is visible in the *TEM* images and is confirmed by the *DSC* results obtained for the latexes. This could help to explain the appearance of localized bacterial growth inhibition apparent in the fluorescence microscopy images in the following section (3.5.3). From the *D*SC thermogram, two glass transitions (T_g) are clearly visible (Fig. 3.9), one T_g at 18.8 ^oC for the *STY/BuA* and the other at 193.2 ^oC, for the modified *SMA* copolymer with *85%* imidization and *15%* amic acid. This confirms that there is no miscibility between the added *SMI* and the *STY/BuA* polymer latex.

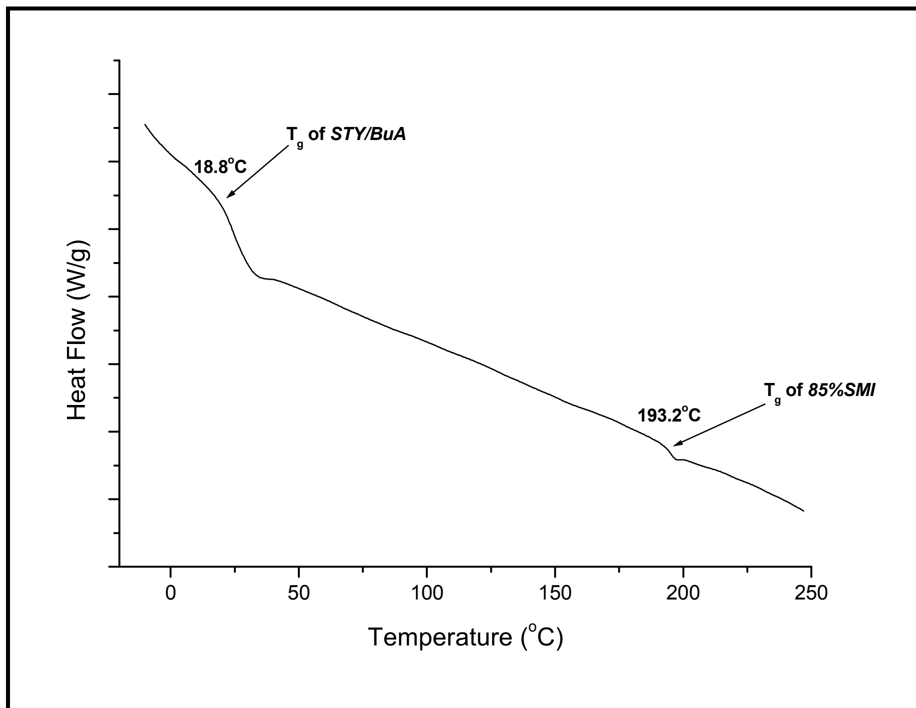

Figure 3.9: *DSC* thermogram for showing the glass transition temperatures for *Sty/BuA*(18.8 ^oC) latex with *85%*imidized SMA (193.2 ^oC) added in *5 wt%* of the total solids content.

3.5.3 Antimicrobial activityassessment via fluorescence microscopy

There was no substantial inhibition of bacterial growth in the case of the pure *STY/BuA* latex as evidenced by the majority of blue-coloured cells (Figure 3.10). The isolated red spots indicating dead cells can be attributed to natural cell death.

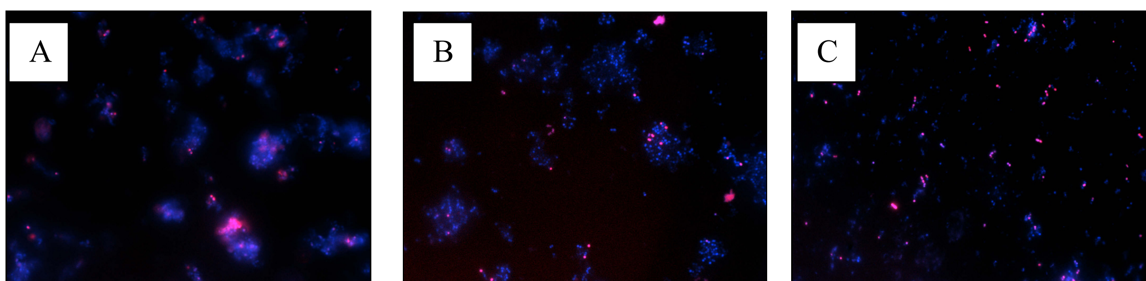

Figure 3.10: Fluorescence microscopy images of three regions of pure latex film (*STY/BuA* latex with no modified *SMA*) (a), (b), (c), stained for cell viability. Blue indicates presence of all bacterial cells (alive or dead) and red indicates presence of dead cells only.

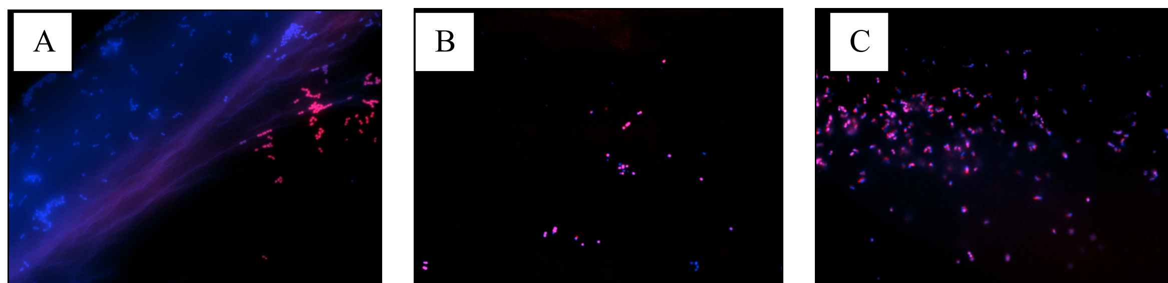

Figure 3.11: Fluorescence microscopy images of three regions of *Sty/BuA* latex film with *5 wt% SMI85*(a), (b), (c), stained for cell viability. Colours have same meaning as in Figure 10.

In the latex containing modified SMA, large scale cell death has occurred as evidenced by the large number of red-coloured cells (Figure 3.11). From the images it is clear that there are some areas where the cell viability appears to be very good (blue coloured section, Figure 3.11 (a)). The most likely explanation is phase separation between the *STY/BuA* and the *SMI-85* copolymers. This results in regions of the film that would in essence have no activity against the bacteria.

3.6 Conclusions and recommendations

Poly(styrene-*alt*-maleic anhydride) (*SMA1000*), was successfully modified. The modified polymer was successfully incorporated in a latex and subsequent latex film. The modified latex film significantly inhibited bacterial growth, as compared with a control. The inhibition, however, was not uniform throughout the film, probably due to phase separation between the *Sty/BuA* particles and added modified *SMA* copolymer which occurred during film formation. This problem might be overcome by using the biocide as a polymeric surfactant to stabilize the emulsion polymerization. This will result in the biocide associating with or forming part of each polymer particle, leading to homogeneous distribution throughout the matrix during film formation. Also, given the quite low amount of modified copolymer incorporated into the latex, the activity could potentially be increased substantially by including the copolymer in a higher concentration in the latex. Employing the modified *SMA* copolymer as polymeric surfactant instead of co-stabilizer is discussed in *Chapter 4*.

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Chapter 4

Modified SMA as polymeric surfactant and antimicrobial activity

Abstract

This chapter describes the use of *SMI-85* as a polymeric surfactant for emulsion polymerization. *SMI-85* is a copolymer made up of *85%* maleimide functionality and *15%* amic acid functionality, derived from reacting mixed amines with an alternating copolymer of *SMA 1000*. When used as polymeric surfactant, the functionalized copolymer (*SMI-85,* unless otherwise stated) is more homogeneously distributed in a latex film, providing antimicrobial activity throughout the entire surface. As a result, homogeneous antimicrobial activity is achieved. We use kinetic studies to investigate the efficacy of the polymeric biocide as a surfactant in comparison with literature results for regular low molar mass surfactants in a styrene emulsion polymerization. We attempt to determine the critical micelle concentration of the proposed polymeric surfactant. Finally, we discuss the most likely mechanism for achieving stable latexes. We conclude that the locus of polymerization is provided by monomer swollen uni-molecular micelles.

4.1 Introduction

Primary dispersions or synthetic latexes are widely used in the formulation of water-borne coatings[1]. These latexes are produced through heterogeneous emulsion polymerization reactions utilizing a surface active species (surfactant) and a water-soluble initiator to polymerize dispersions of monomeric species (e.g. acrylics, epoxies, urethanes) in a continuous water phase[2]. Surfactants such as alkyl phenol ethoxylate (*APEO)* are endocrine disrupters which mimic hormones and steroids. Due to their low molar mass, they often leach out of the final film and eventually become environmental contaminants of water sources, with adverse effects on animal and human health[3-4]. Replacing traditional surfactants with higher molar mass alternatives may reduce the risk of leaching and environmental pollution.

Modified copolymers of *SMA* can be successfully dispersed/dissolved in water to obtain stable dispersions and allow their incorporation into latex binders[5-6]. Additionally, the ability of *SMA* to undergo a range of modifications, enables the development of latex binders with a variety of properties, ranging from adhesion to antifungal properties. In Chapter 3, we showed that *SMA* modified with amine compounds post-added to a synthetic latex, confers antimicrobial

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activity to the system without disrupting latex stability[5-6]. In Chapter 4, we expand on these earlier findings by testing whether modified *SMA* can be used as a polymeric surfactant in emulsion polymerization.

In this study, we show that a functionalized copolymer based on *SMA* is capable of stabilizing an emulsion polymerization reaction and bestowing inherent antimicrobial properties to the resulting latex. We first performed an emulsion polymerization using modified *SMA* as a polymerization stabilizer. We then optimized the polymerization system and characterized the resulting latex products. Finally, we discuss the implications of our findings for the development of latex binders in water-borne coatings.

4.2 Emulsion polymerization with polymeric surfactant

This section describes the synthesis of stable emulsion latexes employing the modified *SMA* copolymer as a polymeric surfactant. It also describes attempts in determining the *CMC* of the proposed polymeric surfactant as well as an additional modification to that done in *Chapter 3*.

4.2.1 Reagents

Styrene (STY) and *butyl acrylate (BuA)* monomer were obtained from Freeworld Coatings Research Centre at Stellenbosch. The monomers were washed with $KOH_{(aq)}$ and $NaOH_{(aq)}$ respectfully, to remove the inhibitors and subsequently distilled under reduced pressure after drying over $MgSO_4$-anhydrous. The water soluble initiator, *KPS (potassium persulphate)* [*Sigma-Aldrich, 99%*], was used along with modified *SMA* (*SMI-85*) as surfactant. Water was used as the continuous phase for the emulsion system (*Milli-Q* deionised water). *Sodium carbonate* and *sodium bicarbonate* were used as buffer reagents in the polymerization system. *Jeffamine XTJ-507 (2005 Da,* Huntsman), a low molar mass *poly(ethylene glycol)* polymer with one amine functionalized chain end, was used as received.

4.2.2 General emulsion polymerization formulation

The same procedure was followed as in *Chapter 3* that was used for the synthesis of the latex employing *SDS* as surfactant. The same weight percentages of modified *SMA* were used in order to compare the results obtained for the antimicrobial viability study in *Chapter 3*. The emulsion polymerization was formulated with *SMI-85* making up *ca. 1 wt%* of the final latex. This amount

was assumed to be well above the *CMC*. The efforts to determine the CMC will be discussed later in the chapter, for the proposed polymeric surfactant.

Table 4.1: Formulation for the synthesis of *Sty/BuA* latex with *SMI-85* as polymeric surfactant

Component	Compound	%mass	mass (g)
Continuous phase	H_2O	77.0	100.0
Monomer	*Butyl Acrylate*	9.20	12.00
	Styrene	12.3	16.00
Initiator	*KPS*	0.10	0.134
Buffer reagent	$NaHCO_3$	0.10	0.134
Surfactant	*SMI-85*	1.10	1.430

The polymerization was done at $70\ ^oC$ in a 250 *mL* reactor with four baffles. An agitation speed of *284 rpm,* by means of an overhead stirrer equipped with a single blade Teflon impeller, was used. Prior to polymerization, the reaction mixture was degassed with argon for *20 minutes* and an additional *5 minutes* after the initiator, *KPS* dissolved in 5 g H_2O, was added.

4.2.3 Optimization of emulsion polymerization system

Attempts to determine the *CMC* of the polymeric surfactant were performed in order to determine the minimum amount of polymeric surfactant necessary for stabilization of the polymerization reaction. An additional modification with a polymeric amine (*Jeffamine*) was also done in an attempt to obtain a copolymer species that would form true micellar conformations in the continuous water phase.

Figure 4.1 and 4.2 shows the possible conformations for modified SMA as opposed to the conventional micelles normally formed by low molar mass emulsion polymerization stabilizers such as *SDS*.

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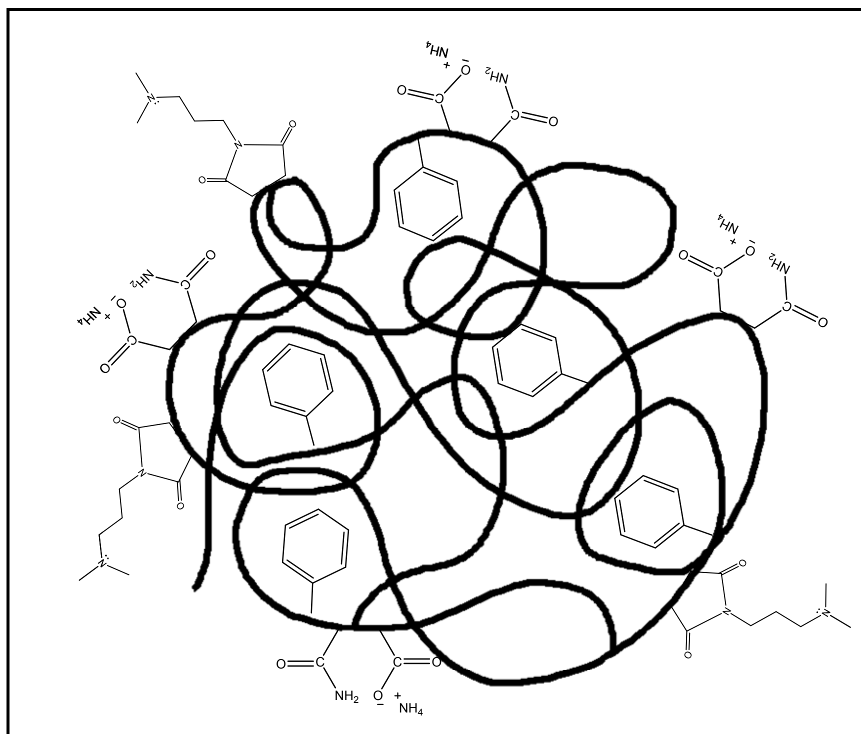

Figure 4.1: Uni-molecular micelle as a result of coil over globule folding of an individual chain.

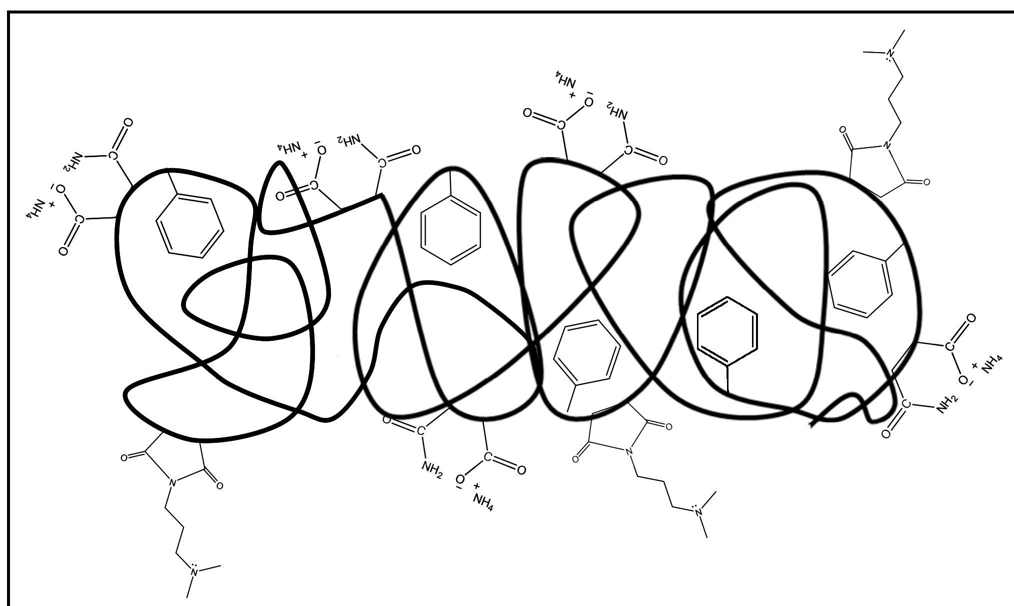

Figure 4.2: Rod like, uni-molecular micelle due to self assembly of a single chain in the continuous phase, water.

4.2.3.1 *CMC* determination of polymeric surfactant

The *CMC* determination was done in two ways: Surface tension measurements as well as Fluorescence Spectroscopy using pyrene as a fluorescent probe.

4.2.3.1.1 Surface Tension

Surface tension measurements were done on a *KRÜSS Tensiometer K11/MK3*. A stock solution of *1 mg/mL* of modified copolymer in a $Na_2CO_3/NaHCO_3$ buffer of *pH 9.16* was made up and subsequently diluted. The measurements were done using a platinum *Du Nouy* surface tension ring. First, the ring was burned using a butane flame and fitted into the instrument. *50 mL* of sample was placed in the sample chamber and the instrument set to record an average of *5* measurements.

4.2.3.1.2 Fluorescence Spectroscopy

Fluorescence spectroscopy was done on a *Perkin Elmer LS50B Luminescence Spectrometer* using the water insoluble fluorescent probe, pyrene, and an acquisition range of 300 – 360 nm and standard cuvette with a path length of 1 *cm*. A solution of *pyrene/acetone* (1.8×10^{-4} *mg/mL*) was added to a concentration range of *SMI-85* in water. The solution vials were covered in tin foil and left stirring overnight to allow for the evaporation of acetone. The results were analysed and expressed as the ratio between the intensity at *338 nm* over *333 nm* and plotted against the logarithm of the concentration range.

4.2.3.2 Increased amount of polymeric surfactant

Since the *CMC* determination was inconclusive an additional emulsion polymerization was done using double the amount of polymeric surfactant. Literature on polymeric surfactants indicate that in general the *CMC* values for these species are much lower then in the case of low molar mass species and using it above *SDS' CMC* in principle means that you are using it above the *CMC* for the *SMI*. Since the main concern is the coagulation occurring during emulsion polymerization, doubling the amount of surfactant could lead to more micellar nucleation since the number and surface area of micelles would theoretically increase by increasing the concentration or weight percentage of polymeric surfactant in the formulation.

The same procedure was used to do the emulsion polymerization as in section *4.2.1* but with double the amount of polymeric surfactant (*SMI-85*). Now instead of *1 wt%* surfactant *2 wt%* (*ca. 10 wt%* of the total theoretical solids content) was used in the formulation. A

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stirring rate of *294 rpm* was used and the polymerization was left to react for *4 hours* after degassing for *20 minutes* with argon. *DLS* analysis was done on the latex and the results will be discussed later in this chapter.

4.2.3.3 Increasing amphiphilic character and molar mass

In order to increase the length of the amphiphilic sections of the modified copolymer the following *Jeffamine* was incorporated in a similar fashion to the way the *3-amino-1-propyldimethylamine* was introduced in *Chapter 3.* This was done in the hope of aiding the modified copolymer to form conventional micellar conformations in water, with the hydrophobic portions towards the centre of the micelles and the hydrophilic sections pointing outwards into the water.

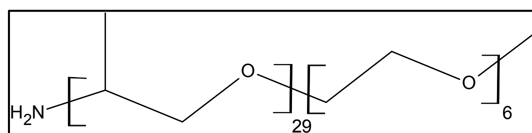

Figure 4.3: *Jeffamine XTJ-507(2005 Da)* obtained from *Huntsman.*

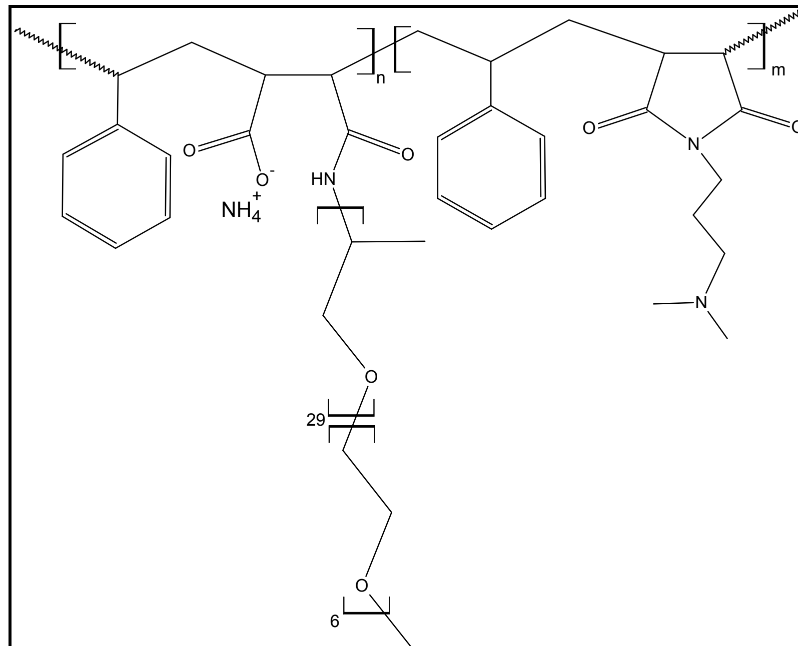

Figure 4.4: The modified *SMA1000* copolymer with the ring opening step done with *XTJ-507(Huntsman)* instead of $NH_{3(aq)}$-solution

Procedure

SMA1000 (*2 g*) was dissolved in *EtAc* under magnetic stirring in a round bottom flask and *3-aminopropyldimethylamine* (*8.67 mmol; 0.884 g*) was added dropwise, the precipitated polymer was isolated and heated overnight in a vacuum oven at *110* ^oC. The imidized polymer was added to water along with the *Jeffamine* (*1.53 mmol; 3.06 g*). It was left to react until the entire amount of polymer was dissolved and the solution was subsequently freeze dried to recover the polymer.

After freeze drying *ca. 5.7 g* polymer was recovered. The polymer sample was then used as a polymeric surfactant employing the same formulation used in section *4.2.1*, but no stable latex was obtained and full coagulation occurred during polymerization.

4.3 Efficacy of polymeric surfactant

We established if the proposed polymeric surfactant provides sufficient control of an emulsion polymerization system, compared to literature results for a styrene emulsion polymerization employing a regular low molar mass surfactant. The conversion versus time was measured, and a first order kinetics plot of a styrene emulsion polymerization was constructed.

4.3.1 Homo-polymerization of styrene with *SMI-70* as surfactant and gravimetrical analysis

The polymerization was carried out in a similar fashion as the one using *SMI-85* as polymeric surfactant, with the only difference in the formulation being the exclusion of *BuA* monomer.

Table 4.2: Formulation for styrene emulsion polymerization with polymeric surfactant, *SMI-70*

Component	Compound	%mass	mass (g)
Continuous phase	H_2O	76.0	100.0
Monomer	*Styrene*	21.0	28.00
Initiator	*KPS*	0.65	0.862
Buffer reagent	$NaHCO_3$	0.57	0.756
	Na_2CO_3	0.08	0.106
Surfactant	*SMI-70*	2.15	2.860

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The styrene monomer, modified copolymer (*SMI*) and water, buffered at *pH 9.16* with a *Na_2CO_3/$NaHCO_3$ buffer,* was added to a *250 mL* glass reactor and placed under mechanical stirring at 284 *rpm*. The reactor was submersed in an oil bath set at $70\ ^oC$ and the reaction mixture was degassed with argon for *20 minutes*. The initiator (*KPS*) was dissolved in a vial with 5 g H_2O and added to the reaction mixture by means of a syringe through a rubber septum. Degassing was done for an additional *5 minutes*. Sampling was done every *5 minutes* for *1 hour* and every *15 minutes* thereafter (*ca. 2 hours*). The solids contents of all the samples were determined gravimetrically. After completion of the reaction, mechanical stirring was stopped and the reactor was removed from the oil bath. Reaction mixture was allowed to cool down to room temperature and the solids content of the latex was determined gravimetrically.

4.4 Characterization and analysis

4.4.1 Differential Scanning Calorimetry (*DSC)*

DSC was performed using a *Q 100 V9.9 DSC* instrument. The heating and cooling rates were done at $10\ ^oC/min$. TA Instruments Advantage software Release *4.2.1* was used to analyse thermograms obtained.

4.4.2 Dynamic Light Scattering *(DLS)*

DLS was carried out at the Freeworld Coatings Research Centre, Stellenbosch, on a Malvern Instruments ZetaSizer Nano-S equipped with a He-Ne laser operating at a wavelength of *633.0 nm* and the scattered light was detected at an angle of 90°. The instrument was externally calibrated with *200 nm poly(styrene)* spherical particles. The samples were diluted until slightly translucent and the measurements were taken as the average of *3* measurements of *15* acquisitions each. The stability of the latex samples was also tested after addition of $CaCl_{2(aq)}$ solution.

4.4.3 Transmission Electron Microscopy (*TEM)*

The particle morphology of the polymer latex was investigated by means of transmission electron microscopy (*TEM*) analysis using a LEO 912 Omega TEM (Zeiss, Oberkochen, Germany) at *120 kV*. The latex (*1 μL*) was diluted with DDI water (*1 mL*) so as to obtain a

translucent solution and *3 μL* of the solution was transferred onto a piece of parafilm. Staining agent (*2% aqueous uranyl acetate*) was added to the solution and subsequently transferred to a carbon coated *200* mesh copper grid. Excess sample was removed by means of blotting with a piece of filter paper and left to dry at room temperature before the imaging was done.

4.4.4 Inoculation of films in bacteria culture

Films of 75 *μm* (wet film thickness) were cast on a microscope slide, employing the latex prepared with *SMI-85* as polymeric surfactant and dried in a sample drying oven at *60 ^{o}C*. The slides were then placed in a petri dish along with the bacteria *Lactobacillus sakei* (*Gram positive*), *S .aureus (Gram Positive)* and *E.Coli (Gram Negative)*, in a suitable growth medium solution. The growth media used for the respective bacteria were, de Man, Ragosa and Sharp (MRS) broth, brain heart infusion (BHI) broth and lysogeny broth (LB) obtained from BioLab Diagnostics (Midrand, South Africa). This was then inoculated at 37 ^{o}C for 24 hours. The slides were removed, and stained with the fluorescent dyes as indicated in section 4.4.5, and imaged via fluorescence microscopy.

4.4.5 Fluorescence microscopy

Bacteria were treated with *Hoechst 33342* and *propidium idodide*. Samples were observed on an Olympus Cell^^{R} system attached to an *IX-81* inverted fluorescence microscope equipped with a F-view-II cooled *CCD* camera (Soft Imaging Systems). Using a Xenon-Arc burner (Olympus Biosystems GMBH) as light source, images were excited with the *360 nm* and *572 nm* excitation filter. Emission was collected using a UBG triple- bandpass emission filter cube. An Olympus Plan Apo N 60x/1.4 Oil objective and the Cell^^{R} imaging software have been used. Images were processed and background-subtracted using the Cell^^{R} software

The fluorescence dyes used in the experiment were chosen carefully in order to distinguish between dead and living bacteria. The blue stain (*Hoechst 33342*) is able to permeate through the cell wall to the centre of living as well as dead cells. The red stain (*propidium iodide*) however is only capable of entering the bacteria cells once the cell wall is no longer intact. Thus if a bacteria is coloured red it implies that the cell wall is no longer intact and that the bacteria cell in question is dead.

4.5 Results and Discussion

4.5.1 *CMC* determination via surface tension and fluorescence microscopy

Figure 4.5 shows that the surface tension dramatically decreases from *75 mN/m* (surface tension of pure water), to *ca. 40 mN/m*. There is however no recovery of the surface tension and no critical concentration from which micelle formation could be deduced in this concentration range by means of surface tension measurements.

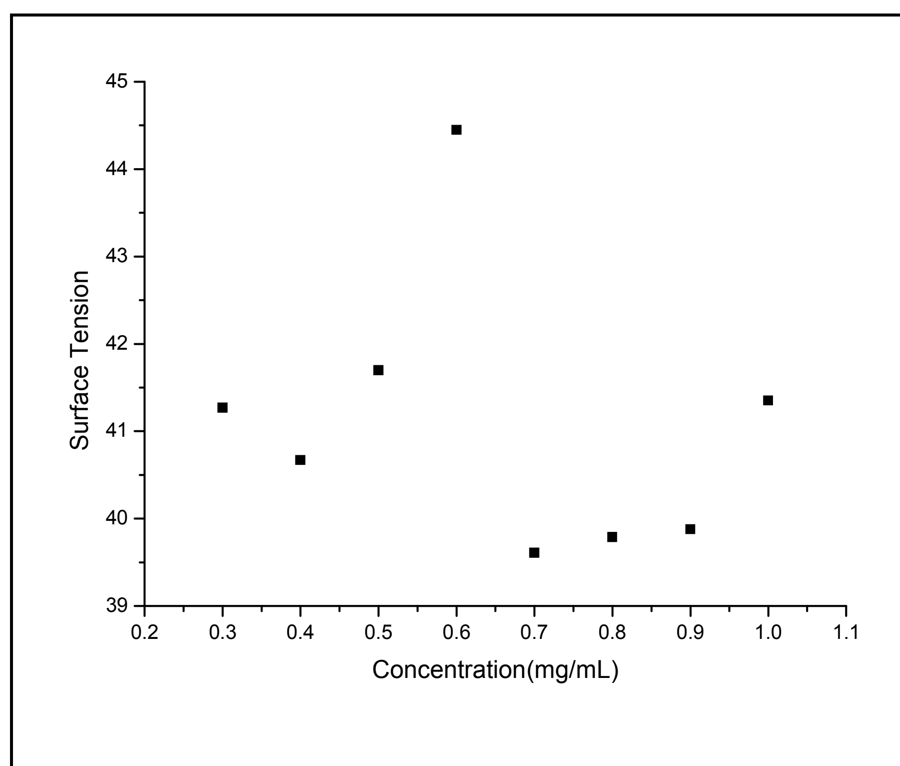

Figure 4.5: Plot of the surface tension measurements vs. the concentration of the polymeric surfactant in water.

Figure 4.6 shows the ratio of the pyrene fluorescence peak intensities at *338 nm* and *333 nm* vs. the logarithm of the concentration range of *SMI-85* in water. The graph does not show any significant trend as compared to literature results for *CMC* determinations with pyrene as fluorescent probe.

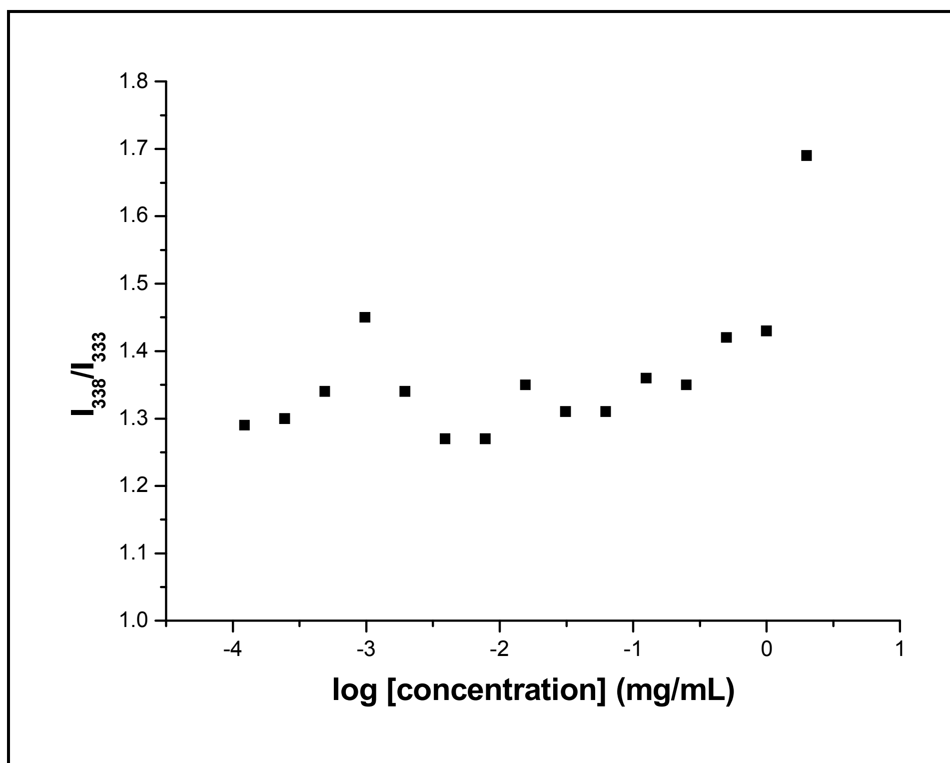

Figure 4.6: Plot of the ratio of fluorescence peak intensities vs. logarithm of the concentration of polymeric surfactant for fluorescence spectroscopy with pyrene as fluorescent probe[7].

Using the polymeric surfactant in 1 *wt%* of the emulsion formulation (Section 4.2.2) led to a great deal of coagulation or grit formation as well as a bimodal particle size distribution. The large amount of coagulation due to a possible high degree of homogeneous nucleation occurring in the emulsion system gave rise to the argument that there was a lack of control due to not enough surfactant used. In order to ensure that micellar nucleation occurs predominantly, one needs to make sure that the number and surface area of the micelles is much larger than that of the monomer droplets. If the monomer droplet surface area is larger, droplet nucleation becomes more likely. Thus the amount of surfactant to be used was increased. With an increase in amount of polymeric stabilizer used, there was a marked increase in the control and monodisperse particles with a narrow size distribution were obtained.

Coagulation of particles and insufficient control during and after polymerization can be attributed to uni-molecular micelle formation in the system. Under this hypothesis a relatively large fraction of the unimolecular micelles get initiated, leading to a too large surface area for the amount of polymeric surfactant. This then results in coalescence of growing particles until the surface area is reduced to a situation that is in line with the amount of polymeric surfactant, resulting in the formation of a stable particle. Insufficient exchange of the polymeric stabilizer among growing particles thus leads to no stabilization of those formed by other nucleation

mechanisms. Latex particles as a result of droplet nucleation are thus not stabilized by adsorption of free polymeric stabilizer, leading to large scale coagulation.The likelyhood of uni-molecular micelles of *SMI-85* being formed and providing the locus of polymerization is also substantiated by the fact that no *CMC* could effectively be determined for these species.

4.5.2 *DLS*

The latex based on the formulation used in the emulsion system employing *SDS* as surfactant resulted in a large degree of coagulation evident from the *DLS* results shown below. The results show a bimodal distribution with some of the particle sizes around *200 nm* and the others larger than 1 μm. Hence there is not enough stabilization of the system from the polymeric surfactant so as to ensure that micellar nucleation is the dominant form of nucleation and that micelles form the sole locus of polymerization.

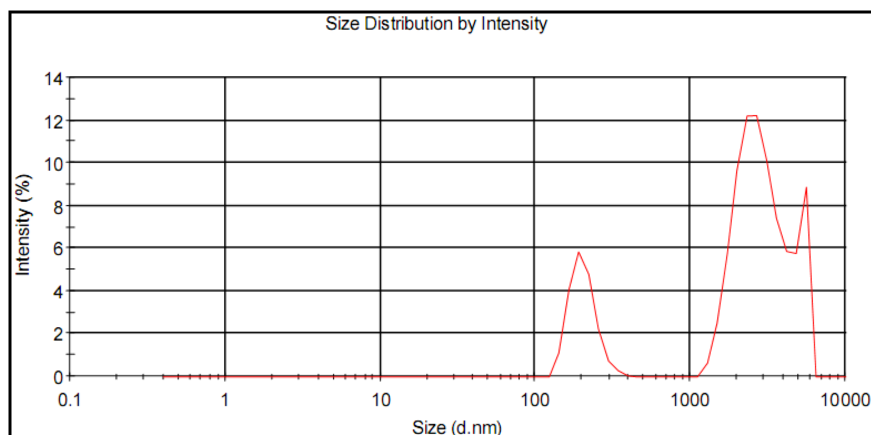

Figure 4.7: Particle Size Distribution obtained from *DLS* measurements using *SMI-85* in *1 wt%* of the emulsion formulation (*Z-avg* = 2061.0 *nm*, Đ = 0.910)

By doubling the amount of polymeric surfactant used, much better control was achieved giving a narrow particle size distribution and particle sizes in accordance with conventional emulsion polymerization systems. The latex's initial solids content was close to the theoretical solids percentage but over a period of 6 days after the latex was synthesized, visible precipitation of some of the particles occurred. Roughly *30%* of the solids content is lost during this process. This implies that some coagulation or settling of existing particles not stabilized by any ionic or steric mechanisms occurs during the first couple of days. The remaining latex is however extremely stable over time with no pronounced change in *PSD* or average particle size, evident from the storage data of up to 11 months.

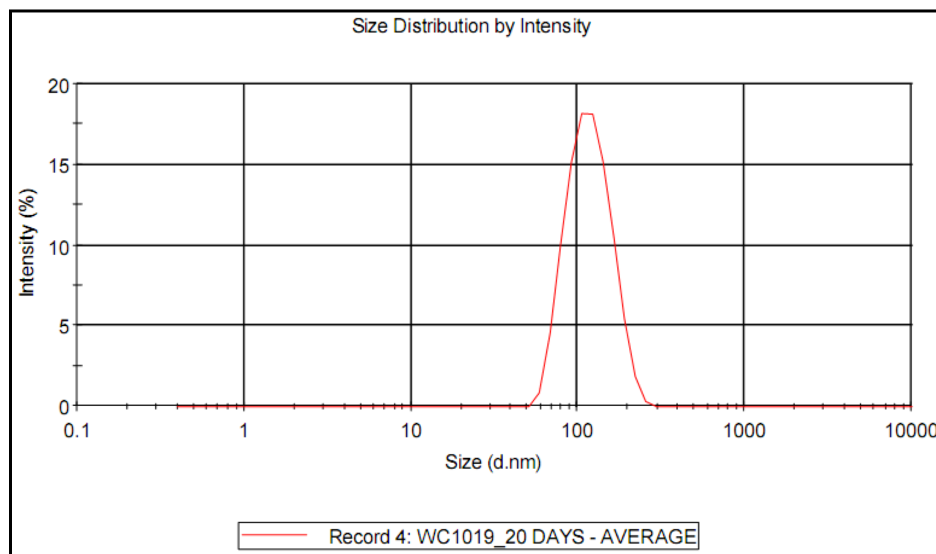

Figure 4.8: Particle Size Distribution obtained from *DLS* measurements using *SMI-85* in *2 wt%* of the emulsion formulation after 20 days

Table 4.3: Storage data of the evolution of Particle Size Distribution using *SMI-85* in 2 *wt%* of the emulsion formulation

Sample	Days Stable	*Z-avg (nm)*	PDI
	1	248.9	0.420
Sty/BuA* with *SMI-85	30	110.0	0.043
as polymeric	63	110.5	0.051
surfactant	178	107.0	0.046
	339	108.0	0.025

The zeta potential for the latex sample was *ca.* -10 *mV* and the latex stabilization was subsequently expected to be a combination of steric as well as ionic stabilization. To verify this hypothesis, the destabilization of the latex was attempted by the addition of an excess of ions. However, the latex remained stable even up to concentrations as high as *20 wt%* $CaCl_{2(aq)}$-solution. The latex stability at relatively high electrolyte content suggests that the stabilization of the latex particles is predominantly achieved by means of steric stabilization.

4.5.3 *DSC*

Miscible polymer blends exhibit only one intermediate glass transition temperature, which is located towards the T_g of the polymer making up the bulk of the polymer sample. In the

thermograms obtained, the glass transition appearing at *193 ^{o}C* is no longer visible. This indicates that a miscible polymer blend was obtained using the modified *SMA* copolymer as a polymeric surfactant. A likely explanation for the miscibility would be that the modified *SMA* copolymer is grafted onto the polymer particles during polymerization. Further evidence that a miscible blend was obtained is the shift in the glass transition corresponding to the T_g for the *Sty/BuA* latex from *18°C* to *23°C*, *25°C* and *34°C* respectively. Since the *Sty/BuA* copolymer makes up the bulk of the sample the final T_g for the blend is closer to that of the *Sty/BuA* copolymer but shifted slightly to the higher temperature and T_g of the modified *SMA* copolymer.

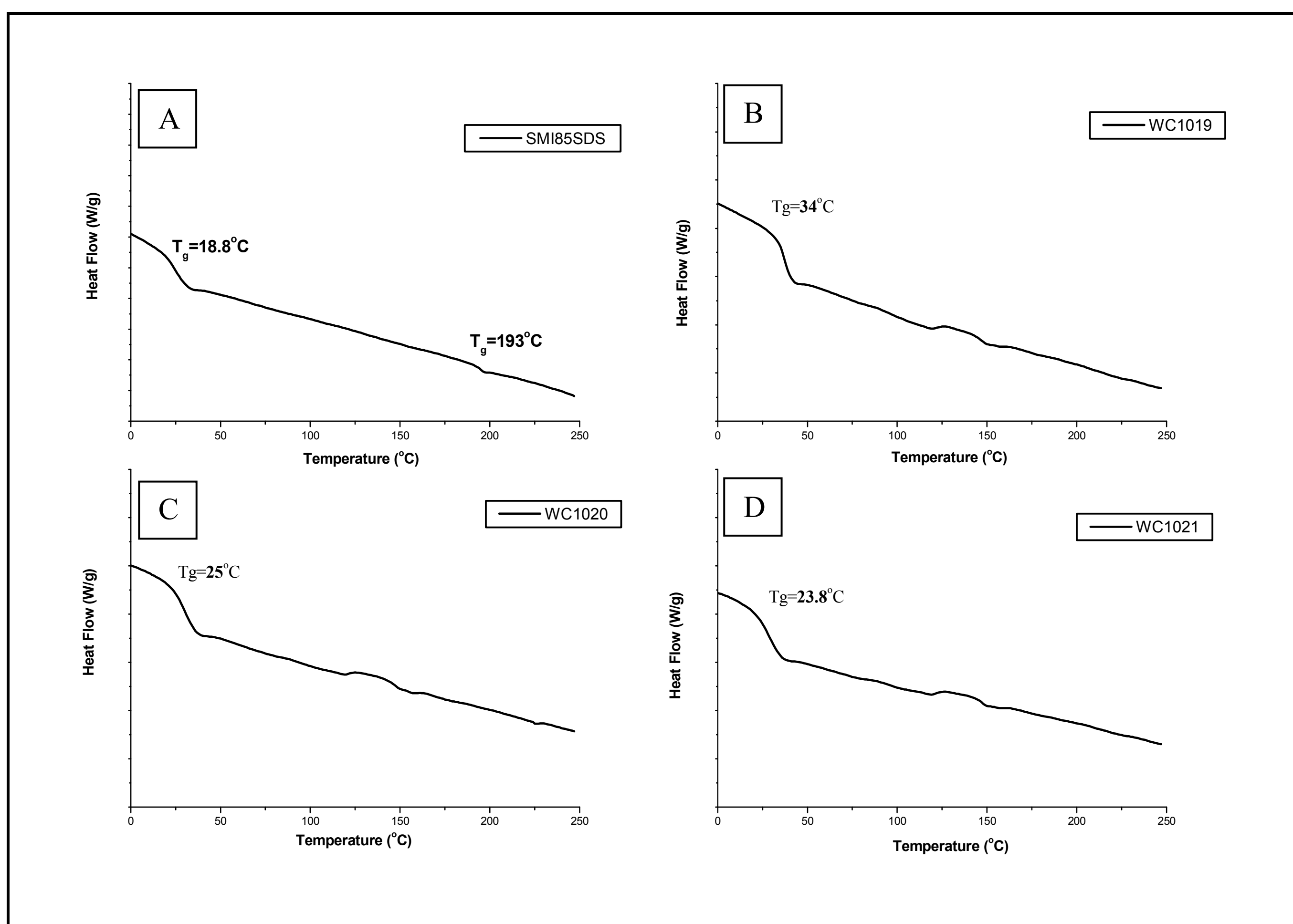

Figure 4.9: DSC thermograms for latexes obtain by post addition of *SMI-85* to *Sty/BuA* latex (a) and employing polymeric surfactants with varying degrees of functionalization; (b) *SMI-85*, (c) *SMI-55* and (d) *SMI-40*.

In addition to the disappearance of the T_g corresponding to the modified *SMA* and the shift to an intermediate T_g for the polymer blend obtained, there is another transition (T = 120 ^{o}C - 150 ^{o}C) visible in the thermograms. The transition occurred in the same place regardless of the degree of modification of the *SMI* used as polymerization stabilizer. Compared to the

glass transition this event is very small and it is not easy to attribute it to any significant thermal event. No further investigation to establish the source and nature of the event was conducted since it had no effect on the outcome of this study.

4.5.4 TEM

The *TEM* images obtained for the latex produced on the basis of the polymeric surfactant shows very well defined particles that are fairly homogeneously distributed. This is further confirmation or evidence that stabilization by the polymeric surfactant is achieved by a combination of steric and electrostatic effects. The *DLS* results indicate that micellar nucleation is most likely the dominant nucleation mechanism. This in turn means that the polymeric surfactant creates an electronegative particle surface and along with the positively charged ammonium and buffer salt ions creates a stabilizing electric double layer resulting in a zeta potential of *ca.* -10 *mV*.

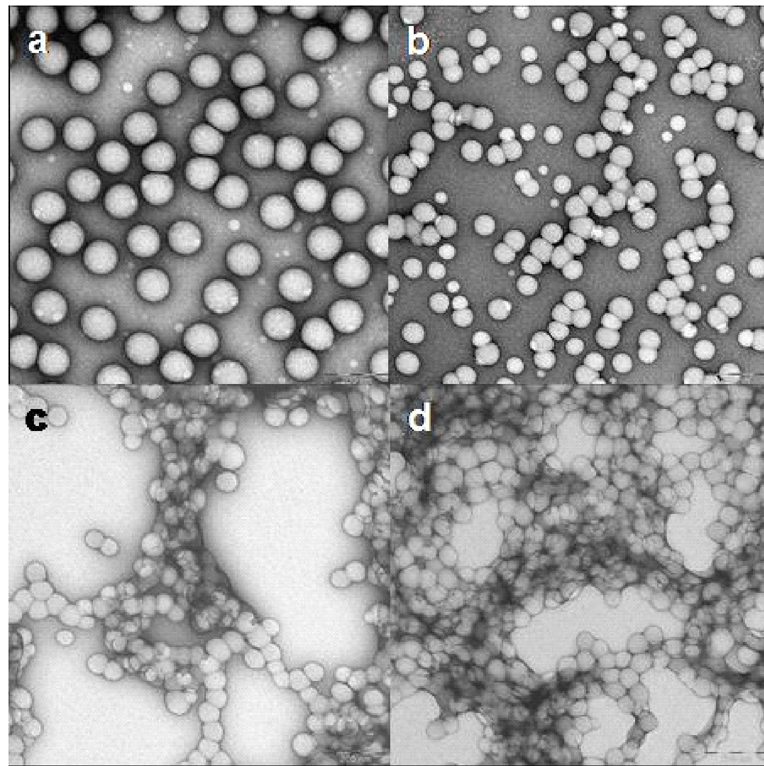

Figure 4.10: TEM images of latexes obtained obtained with polymeric surfactant *SMI-85* (a); *SDS* as surfactant (b);*SMI-85* added to latex produced with *SDS* as surfactant (c and d)

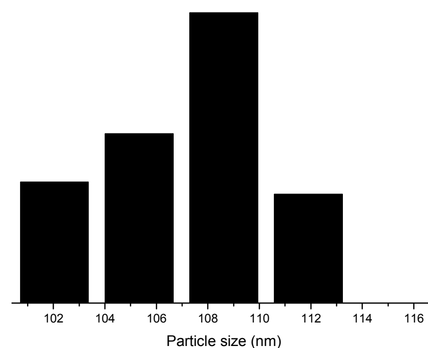

Figure 4.11: Particle size distribution for the latex obtained using *SMI-85* as polymeric surfactant obtained from analysis using AnalisysDocu software.

4.5.5 Fluorescence microscopy

The latex with *2 wt%,* modified *SMA* copolymer appears to have activity against Gram positive bacteria evident from the fluorescence microscope images of the films inoculated in growth medium along with *Lactobacillus sakei* and *S.aureus*. The isolated regions of activity are also eliminated by the use of the modified *SMA* copolymer as polymeric surfactant since there is large scale cell death throughout the entire film. This is apparent from *TEM* images (Figure 4.10 (a)) where the particles appear to be homogeneously distributed and fluorescence images (Figure 4.12) that show activity throughout the entire film.

Lactobacillus sakei

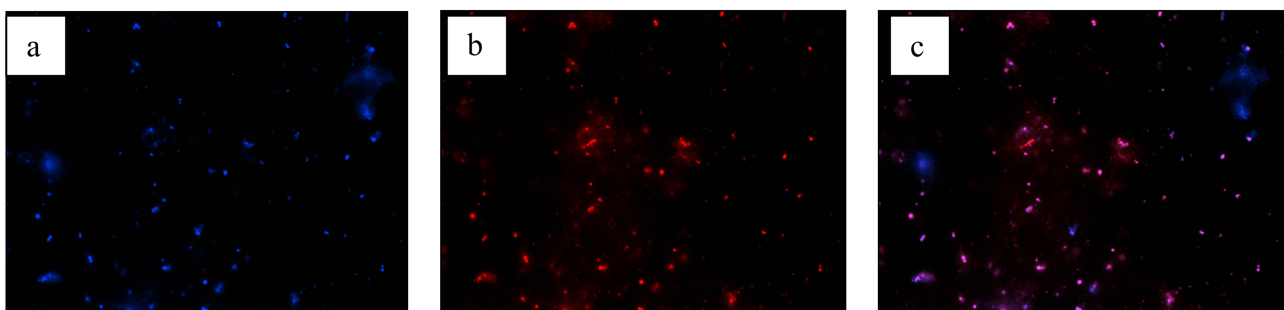

Figure 4.12: Fluorescence microscopy images of *STY/BuA* latex film with *SMI-85* as polymeric stabilzer. Images (a) and (b) shows the film stained for cell viability against *Lactobacillus sakei*. Blue indicates presence of all bacterial cells (alive or dead) and red indicates presence of dead cells only. Image (c) is an overlay of blue and red stained images.

The image on the left shows all the bacteria that form part of the biofilm which formed on the latex film. The image in the middle shows how many of those bacteria cells are dead since the blue dye can enter into both alive and dead cells, but the red dye can only permeate into the interior of the cell once the cell is dead. The image on the right is an overlay of the two images on the left indicating that the majority of the cells are in fact dead and that the contact biocide has antimicrobial activity against the bacteria.

S. aureus

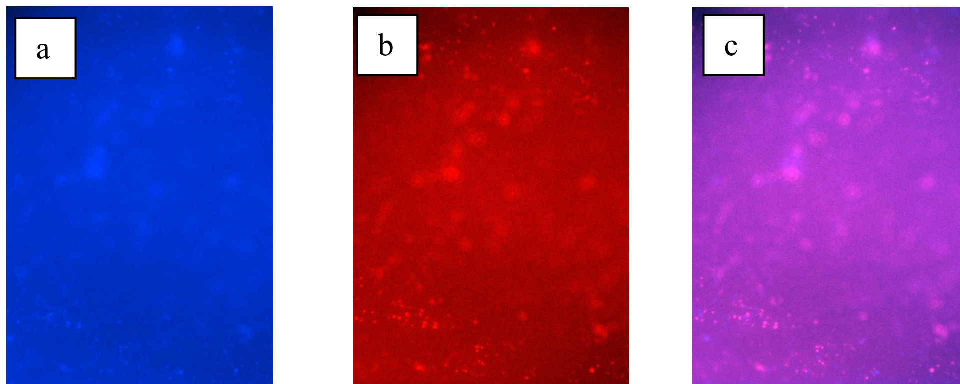

Figure 4.13: Fluorescence microscopy images of latex film (*STY/BuA* with *SMI-85 as polymeric stabilizer*) (a), (b) and (c) stained for cell viability against *S. aureus*. Colours have the same meaning as in *Figure 4.12*.

In the case of activity studies against *S.aureus* the same applies as for the previous fluorescence microscope images, where blue indicates alive as well as dead bacteria and red shows only those bacteria cells that are dead. The image in purple is again an overlay of the red and blue fluorescent stains indicating that the bulk of the bacteria cells are no longer alive. In comparison to the images for *Lactobacillus sakei*, the images show a more pronounced fluorescence throughout the film. The individual bacteria stained with agents are however still clearly visible as dots in the image. Background subtraction was done for the images in Figure 4.13 (*S. Aureus*) in a similar fashion to that of Figure 4.12 (*Lactobacillus sakei*). The more pronounced effect in Figure 4.13 can be attributed to the fluorescence of bacteria in different focal plains or auto fluorescence of the substrate film which is often observed and is largely sample and film region specific. The same latex was used for this study as in the case of the *Lactobacillus sakei* and it appears that the latex retains its activity over time since the activity against *S.aureus* was done more than six months after the latex was synthesized.

E. coli

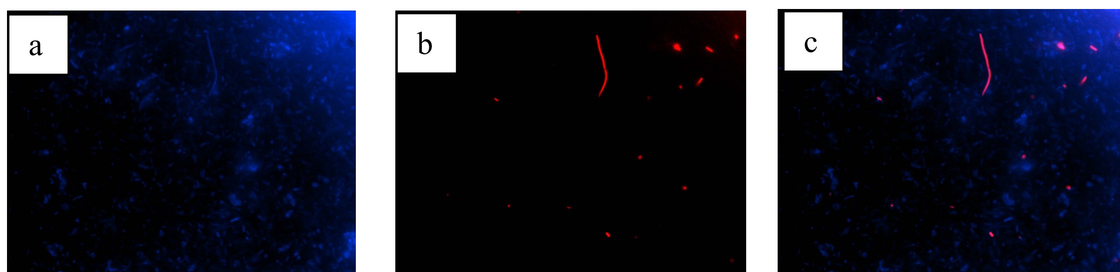

Figure 4.14: Fluorescence microscopy images of latex film (*STY/BuA* with *SMI-85 as polymeric stabilizer*) (a), (b) and (c) stained for cell viability against *E. coli*. Colours have the same meaning as in *Figure 4.12*.

The same latex used in viability studies for both Gram positive bacteria does not show any activity or viability against the Gram negative bacteria, *E. coli.* In the image there are some dead cells but in comparison as seen in the overlay of dead and alive cells (Figure 4.14 (c)) the majority of cells are still alive. The amount of dead cells is considered to be the result of natural cell death that occurred and not due to any biocidal activity from the modified *SMA* incorporated into the latex film.

4.5.6 Efficacy of polymeric surfactant

The gravimetrically calculated conversion versus time results for the homopolymerization of styrene shows a typical trend for an ab initio emulsion polymerization employing a conventional low molar mass species. In the absence of any other means of stabilization, besides the modified *SMA* copolymer proved that it is a viable alternative for low molar mass surfactants as stabilizer for emulsion polymerization. The stabilization effect is attributed to the formation of uni-molecular micelles that swell to equilibrium with monomer and upon initiation provide the locus of polymerization. The graph (Figure 4.15) shows the three distinct phases expected for a conventional ab initio emulsion polymerization. There is a distinct period in the polymerization where there is steady growth in the over all conversion and a relatively high conversion is reached in a relatively short period of time, compared to bulk or solution polymerization. The conversion then reaches a plateau signalling the disappearance of the monomer droplet reservoirs and the slight increase in conversion after 60 minutes can only be the result of the polymerization of residual monomer trapped within the polymer particles that formed in Stage I and continued to grow in Stage II of the polymerization.

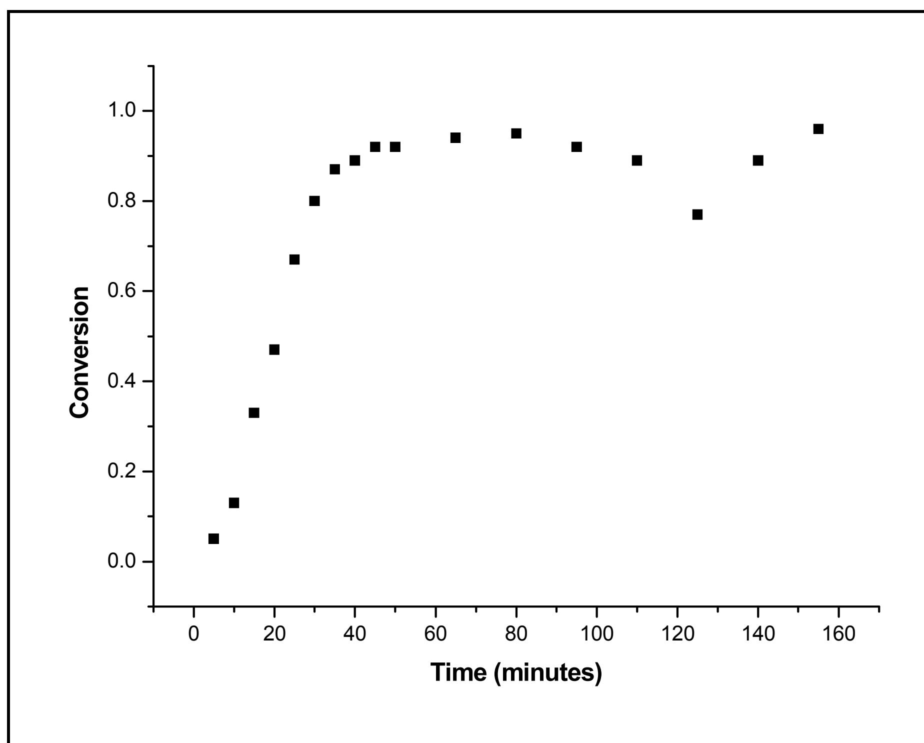

Figure 4.15: Conversion versus time plot for emulsion polymerization of styrene using modified *SMA* as polymeric stabilizer.

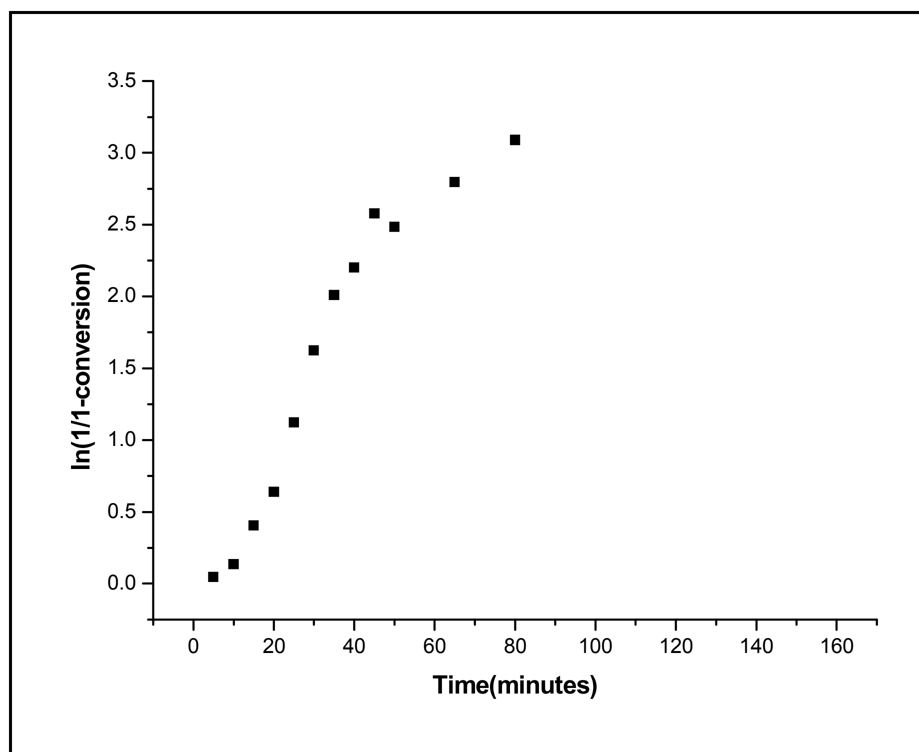

Figure 4.16: First order kinetic plot for emulsion polymerization of styrene using modified *SMA* as polymeric stabilizer.

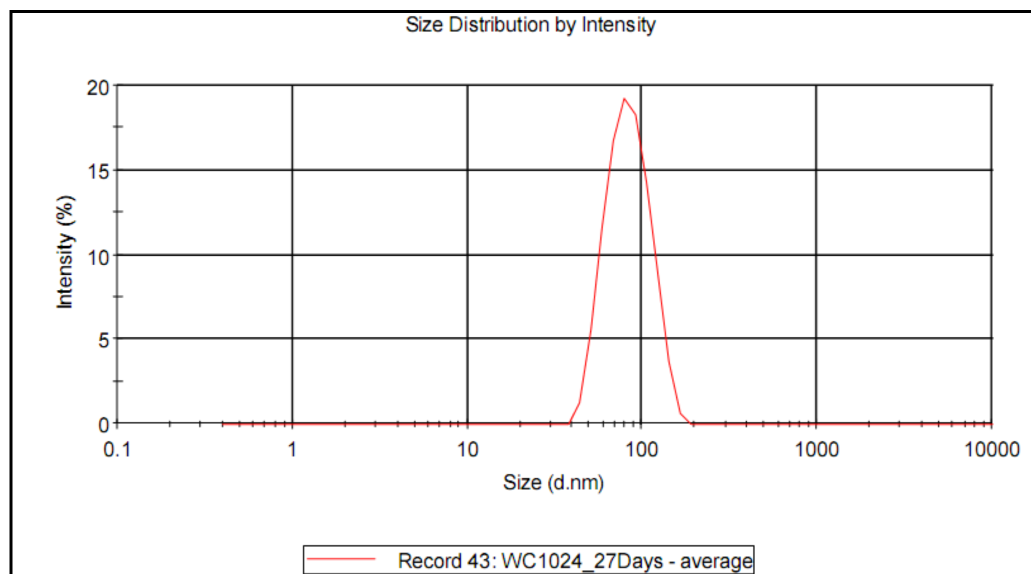

Figure 4.17: The particle size distribution for emulsion polymerization of styrene using modified *SMA* as polymeric surfactant.

Table 4.4: Latex stability over time, for the emulsion polymerization of styrene, using *SMI-70* as polymerization stabilizer.

Days Stable	***Z-avg* (*nm*)**	**PDI**
6	78.8	0.059
25	79.4	0.066
115	78.5	0.063

There is no evidence of conventional micelle formation when using modified *SMA* as polymeric surfactant in an *ab initio* emulsion formulation. The trends observed during the typical stages of the polymerization do however mirror that of a conventional *ab initio* emulsion polymerization, employing a low molar mass surfactant.

4.6 Conclusion and recommendations

According to the DLS results, little to no stabilization effect is evident when using the modified SMA polymeric surfactant in 1 *wt%* of the formulation. However particle sizes in agreement with conventional emulsion systems with low molar mass surfactants as well as fairly narrow PSDs are observed when using the polymeric surfactant in double (2 *wt%*) weight percentage of the formulation.

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The great loss in solids content can be attributed to some coagulation that occurred after and during polymerization. Optimization to minimize the amount of coagulation forms part of future studies. One way of optimizing the formulation will be to experimentally verify the optimal amount of SMI needed for complete stabilization. Future work can also focus on using the coagulum and precipitated polymer particles as seed latex for the manufacture of micron sized particles with inherent biocidal activity. These large particles with still an extremely large surface area can in turn be used in a packed column to act as contact biocide for the removal of bacteria in water, in water purification systems.

From the gravimetrical analysis of conversion versus time for the homopolymerization of styrene employing the polymeric stabilizer, the kinetics follows the expected trend for emulsion polymerization systems and is in excellent agreement with the trends in literature when using low molar mass surfactants.

The occurrence of isolated regions of activity for where modified *SMA* copolymer was added to an existing *STY/BuA* latex film was overcome by employing the modified *SMA* as polymeric surfactant, evident from the *TEM* and fluorescence microscopy images. In both imaging techniques, the modified SMA polymer appears homogeneously distributed throughout the sample and the latex films appear to have homogeneous antimicrobial activity throughout the entire film.

The modified *SMA* copolymer employed as contact biocide in the latex film appears to only have activity against *Gram positive* bacteria (*Lactobacillus sakei* and *S. aureus*) but not against *Gram negative* bacteria (*E. coli*). Additional functionalization or the quarternization of the tertiary amine in the current study could potentially provide the necessary activity to act as a contact biocide against *E.coli* and other *Gram negative* bacteria.

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Chapter 5
Conclusions and Recommendations

The present study set out to verify whether it is possible to suitably functionalize a commercially available copolymer containing reactive functional groups to confer inherent antimicrobial activity to the polymer species. It also aimed to substantiate whether the proposed polymer species would act as a co-stabilizer in synthetic latexes or dispersing agent in emulsion polymerization systems, with retention of its inherent antimicrobial activity.

Chapter 3 ultimately concludes that antimicrobial activity against *Gram positive* bacteria (*Lactobacillus sakei*) was achieved by modification of a low molar mass poly(styrene-*alt*-maleic anhydride) (SMA *1000*) with mixed amine compounds and that the functionalized copolymer can be incorporated into synthetic latexes without compromising latex stability.
*SMA 1000*was suitably functionalized by a series of reactions with mixed amines to obtain a water-soluble copolymer with inherent antimicrobial activity. Qualitative solubility assessments of *SMA 1000* modified to varying degrees with aqueous ammonia and *3-dimethylamino-1-propylamine* show that a minimum theoretical modification of 15% ammonolysis is needed to achieve complete dissolution at pH 8-9. It was shown that incorporation of the styrene/maleimide copolymer into *STY/BuA* synthetic latexes led to a stable dispersion without visible coagulation. It can be concluded that addition of the copolymer had no affect on latex stability. Fluorescence imaging of *STY/BuA* latex films, with a fixed amount of *SMI* copolymer added showed that large scale cell death occurred during inoculation of the filmsin bacteria growth solutions. Relative to a control of the same *STY/BuA* latex films containing none of the *SMI* copolymer, it is concluded that the *SMI* copolymer confers antimicrobial activity to synthetic latexes and subsequent films. The antimicrobial activity was corroborated via fluorescence images on a blank experiment (pure *STY/BuA* latex film inoculated in a similar fashion) that showed no sign of cell death.

Evidence of the successful modification of *SMA 1000* was obtained by means of FT-IR spectroscopy but future work can focus on quantitatively assessing the extent of the modification to determine the minimal degree of modification to achieve water solubility. This will then ensure an optimal amount of antimicrobial functionality to be incorporated into the polymer backbone. Post addition of the modified copolymer led to isolated areas of antimicrobial activity in the eventual polymer film.

Chapter 4 concludes that *SMI* copolymers are capable of stabilizing a *STY/BuA* emulsion copolymerization conferring antimicrobial activity to the latex. This was evident from the fluorescence images obtained after inoculation of the films in *Gram positive* bacteria solutions of *S. aureas* and *Lactobacillus sakei*. The films, more specifically the *SMI* copolymer, showed no antimicrobial activity against the *Gram negative* bacteria, *E. coli*. Despite not having activity against Gram negative bacteria, using SMI as stabilizer for the production of synthetic latexes overcame the problem of non-homogeneous distribution of antimicrobial activity. The non-homogeneous distribution observed in Chapter 3 is concluded to be a result of the process of post addition of the SMI copolymer. Incompatibility between the two copolymers leads to phase separation within the final latex film, resulting in isolated regions of antimicrobial activity. Based on the fact that activity is observed throughout the entire film surface we conclude that using the *SMI* copolymer as stabilizer ensures that *SMI* is adsorbed in sufficient amounts to each individual particle. This results in homogeneous distribution of *SMI* in the film upon film formation. This notion was also substantiated by the TEM images taken of the latex. These images show particles in the nano meter range with a narrow particle size distribution. In the absence of any added stabilizer, this can only be the result of stabilization of each particle by a particular amount of *SMI* copolymer.

Although stable dispersions with the desired particle size and distribution were obtained, future work should focus on optimizing the amount of stabilizer used. An optimized amount of stabilizer would potentially eliminate the large scale grit formation during polymerization and subsequent loss of total solids content, observed in Chapter 4. It would also be interesting to see if control over particle size can be achieved and what amounts of solids content, at workable viscosities, can be obtained by this system. Once the emulsion process is optimized, the latex product can be used in a coating formulation to ascertain whether it leads to long term antimicrobial activity of the coating.It will also be worth while to look into the possibility of an additional reaction to convert the tertiary amines incorporated in this study to quaternary ammonium functional groups. Quaternary ammonium functional groups incorporated via this route may well lead to activity against *Gram negative* bacteria as well as to antifungal properties.

In the light of the absolute necessity for scientists to come up with (1) viable alternatives for biocides with low level residual toxicity and (2) polymerization stabilizers that are not endocrine disrupting, this study provides a valid platform on which future work can be based. The results, discussions and conclusions of this study indicate that modification of SMA copolymers with mixed

amines provides a facile way of producing an emulsion polymerization stabilizer/polymeric surfactant that confers inherent antimicrobial activity to the latex produced.