

# Preparation, stability and *in vitro* evaluation of liposomes containing amodiaquine

**Jacques C. Scholtz**

**(B.Pharm)**

Dissertation submitted in fulfilment of the requirements for the degree

**MAGISTER SCIENTIAE (PHARMACEUTICS)**

at the

POTCHEFSTROOM CAMPUS OF THE NORTH-WEST UNIVERSITY

Supervisor: Dr. Lissinda Du Plessis

Co-supervisor: Prof. A.F. Kotzé

November 2010

Potchefstroom



NORTH-WEST UNIVERSITY  
YUNIBESITHI YA BOKONE-BOPHIRIMA  
NOORDWES-UNIVERSITEIT

**“Do not pray for easy lives.  
Pray to be stronger men!  
Do not pray for tasks equal  
to your powers. Pray for  
powers equal to your tasks.  
Then the doing of your  
work shall be no miracle,  
but you shall be a miracle”  
- Philip Brooks**

**“The most exciting phrase  
to hear in science, the one  
that heralds new  
discoveries, is not ‘Eureka!’  
but ‘That’s funny...’”  
- Isaac Asimov**

# Acknowledgements

I would like to start off by thanking and praising our Heavenly Father for the opportunities, the abilities and the blessings that I have received thus far in my life.

I would like to thank my parents, **Jacques** and **Sarita Scholtz** for giving me life and raising me to be the person I am today. I would also like to thank my sister, **Nadia Scholtz**. Thank you all for paving the way towards my academic success. Nothing could replace your love, support and care.

I would like to thank Dr. **Lissinda du Plessis**, my supervisor for her help, guidance and patience with me and my strange ways. Your help and guidance was paramount to the successful completion of my study.

Prof. **Awie Kotzé** my co-supervisor for always having an open door policy, and making the time to listen and help.

The **Innovation fund** for their monetary support.

**Stephnie Nieuwoudt**, thank you for walking this road with me and always being there to help and to encourage me. The blood sweat and tears shed in this time will bear fruit for us both.

Prof. **Wilna Liebenberg** for the use of her laboratories and equipment. I appreciate it very much.

Prof. **Lesley Greyvenstein** for the language editing.

I would like to thank all my friends **Christo, Jeanine, Ruan, Nicolene, Theunis, Chucky, HeLska, Cerenus, Michael, Geodelle, Jandré, Michelle** and **Lizl** for your unfailing support and friendship during the course of my study. You guys and girls mean the world to me.

To my family (especially the **Bothma's**), your support in the tough times really helped me through.

To all my colleagues at the Department of Pharmaceutics, thank you for the fun times, chatting and joking in the office. Wish you all the best and success for your future.

Special thanks go out to **Chrizaan Slabbert, Righard Lemmer** and **Herman van der Watt**. The help and guidance you each gave me in each separate field where you specialise was just amazing. Thank you for your time and help that you offered so willingly. Thank you all very much. People like you make this world a fantastic place and I can't wait to take on the world with you all by my side.

# Table of Contents

<b>Table of Contents</b>	<b>i</b>
<b>List of Figures</b>	<b>vi</b>
<b>List of Tables</b>	<b>x</b>
<b>List of Abbreviations</b>	<b>xi</b>
<b>Abstract</b>	<b>xiii</b>
<b>Uittreksel</b>	<b>xv</b>
<b>Introduction and aim of study</b>	<b>1</b>
<b>Chapter 1 ~ Malaria</b>	
1.1. Introduction	4
1.2. Malaria around the world	5
1.3. Malaria in South Africa	5
1.4. Biology of <i>Plasmodium</i>	7
1.4.1. Asexual stage	7
1.4.2. Sexual stage	9
1.5. Clinical appearance of malaria	9
1.6. Drug resistance	11
1.7. Malaria treatment: South African regimes	13
1.7.1. Treatment of uncomplicated <i>P. falciparum</i> malaria	13
1.7.2. Treatment of severe <i>P. falciparum</i> malaria	15
1.7.3. Treatment of non- <i>P. falciparum</i> infections	17
1.8. Antimalarial drugs	17
1.8.1. Classification of antimalarial compounds	17
1.9. Quinoline antimalarials	18
1.9.1. Mechanism of action	18
1.9.1.1. DNA Intercalation	19
1.9.1.2. Inhibition of haemoglobin degradation	19
1.9.1.3. Haem polymerisation theory	19
1.9.1.4. Integrated model	20

1.10. Chloroquine	20
1.10.1. Properties	21
1.10.2. Pharmacokinetics	22
1.10.3. Side-effects	23
1.11. Amodiaquine	23
1.11.1. Properties	24
1.11.2. Pharmacokinetics	25
1.11.3. Side-effects	25
1.12. Resistance to quinolines	25
1.12.1. Cross resistance between quinolines	26
1.12.2. Overcoming resistance	27
1.12.2.1. Combination therapies	27
1.12.2.2. Chemosensitisers	27
1.12.2.3. Drug delivery systems	27
1.13. Conclusion	28

## Chapter 2 ~ Liposomes

2.1. Introduction	30
2.2. Components of liposome structure	30
2.2.1. Phospholipids	31
2.2.1.1. Phosphatidylcholine	33
2.2.2. Cholesterol	34
2.3. Classification of liposomes	34
2.3.1. Characterization of liposomes according to size and shape	34
2.3.2. Classification of liposomes according to composition	35
2.3.3. Classification of liposomes according to production method	36
2.4. Advantages of Liposomal drug delivery	38
2.4.1. Improvement of pharmacodynamics	38
2.4.2. Liposomes can be made target selective	39
2.4.3. Enhanced activity of drugs against intracellular pathogens	39
2.4.4. Enhanced activity of drugs against extracellular pathogens	40
2.5. Disadvantages of liposomes	40
2.5.1. Sterilization	41

2.5.2. Short shelf life and stability	41
2.5.3. Encapsulation efficacy	42
2.5.4. Removal from circulation by Reticulo-endothelial system (RES)	42
2.6. Interactions of liposomes with cells	43
2.6.1. Intermembrane transfer	43
2.6.2. Contact release	43
2.6.3. Adsorption	44
2.6.4. Fusion	44
2.6.5. Phagocytosis or endocytosis	44
2.7. Commercial products containing liposomes	45
2.8. Conclusion	45

### Chapter 3 ~ Physicochemical properties and cellular toxicity evaluation

3.1. Introduction	47
3.2. Stability studies	47
3.2.1. Accelerated stability studies	48
3.2.2. Size determination	48
3.2.3. Size determination – Fluorescence Activates Cell Sorter (FACS)	49
3.2.4. Entrapment efficacy	49
3.3. <i>In vitro</i> evaluation of liposome toxicity	50
3.3.1. Reactive oxidative species and lipid peroxidation	50
3.3.1.1. Damage caused by oxidative stress	51
3.3.1.2. DNA damage	52
3.3.1.3. Protein damage	53
3.3.1.4. Lipid damage (Lipid peroxidation)	53
3.3.2. Defence against oxidative stress	54
3.3.2.1. Antioxidant enzymes	54
3.3.2.2. Low molecular weight antioxidants (LMWA)	54
3.3.3. Oxidative stress in <i>P. falciparum</i>	55
3.4. Conclusion	56

### Chapter 4 ~ Experimental methods, results and discussions

4.1.	Introduction	58
4.2.	Experimental design	58
4.3.	Preparation, characterization and stability of liposomes containing amodiaquine	60
4.3.1.	Solubility study of amodiaquine (method development)	60
4.3.1.1.	Apparatus and materials	60
4.3.1.2.	Method	60
4.3.1.3.	Results and discussion	62
4.3.2.	Manufacturing of liposomes and amodiaquine entrapped liposomes	63
4.3.2.1.	Materials	64
4.3.2.2.	Method	64
4.3.3.	Morphological evaluation of liposomes and amodiaquine entrapped liposomes	64
4.3.3.1.	Materials and methods	65
4.3.3.2.	Results and discussion	65
4.3.4.	Accelerated stability testing	66
4.3.5.	Size determination	66
4.3.5.1.	Materials	66
4.3.5.2.	Method	67
4.3.5.3.	Statistical analysis	69
4.3.5.4.	Results and discussion	69
4.3.6.	Determination of pH	75
4.3.6.1.	Apparatus and method	75
4.3.6.2.	Statistical analysis	75
4.3.6.3.	Results and discussion	76
4.3.7.	Entrapment efficacy and leakage	80
4.3.7.1.	Apparatus and method	80
4.3.7.2.	Statistical analysis	81
4.3.7.3.	Results and discussion	82
4.4.	<i>In vitro</i> cultivation of <i>P. falciparum</i>	84
4.4.1.	Materials	85
4.4.2.	Cultivation	85
4.5.	Microscope evaluation and determination of parasitemia	86

4.5.1. Materials	86
4.5.2. Methods	86
4.6. In Vitro studies (Flow cytometric determination of reactive oxygen species and lipid peroxidation)	87
4.6.1. Analysis of reactive oxygen species (ROS)	87
4.6.1.1. Materials	88
4.6.1.2. Method	88
4.6.1.3. Statistical analysis	90
4.6.1.4. Results and discussion	90
4.6.2. Analysis of Lipid peroxidation	94
4.6.2.1. Materials	94
4.6.2.2. Method	94
4.6.2.3. Statistical analysis	96
4.6.2.4. Results and discussion	96
4.7. Conclusion	99
<b>Summary and future prospects</b>	<b>100</b>
<b>References</b>	<b>103</b>
<b>Annexure A: Ethical Application</b>	<b>114</b>
<b>Annexure B: Certificate of analysis: Amodiaquine</b>	<b>115</b>
<b>Annexure C: Size data</b>	<b>116</b>
<b>Annexure D: pH data</b>	<b>132</b>
<b>Annexure E: Entrapment efficacy data</b>	<b>134</b>
<b>Annexure F: ROS data</b>	<b>137</b>
<b>Annexure G: Lipid peroxidation data</b>	<b>145</b>

# List of Figures

<b>Figure 1.1:</b> Map showing malaria risk areas in South Africa	6
<b>Figure 1.2:</b> A schematic representation of the lifecycle of <i>P. Falciparum</i>	8
<b>Figure 1.3:</b> The development of chloroquine from quinine	22
<b>Figure 2.1:</b> Illustration of the basic elements of a lipid, with the arrangement into the lipid bilayer structure	31
<b>Figure 2.2:</b> Illustration of the basic form the lipid bilayer forms in an aqueous solution. The position of the drugs formulated into liposomes is also displayed	32
<b>Figure 2.3:</b> The main classes of phospholipids that contain choline	33
<b>Figure 2.4:</b> The chemical structure of cholesterol	34
<b>Figure 2.5:</b> A simplified illustration production methods of Liposomes	36
<b>Figure 2.6:</b> A simplified illustration of the active loading of Liposomes	42
<b>Figure 3.1:</b> The formation of Reactive oxygen species	52
<b>Figure 3.2:</b> Lipid peroxidation as a cyclic process	53
<b>Figure 3.3:</b> A schematic of the two main methods used by <i>P. falciparum</i> to detoxify haem	56
<b>Figure 4.1:</b> Part one in the experimental design. The preparation, characterization and stability as laid out in the steps followed in this study	59
<b>Figure 4.2:</b> Part two in the experimental design. <i>In vitro</i> evaluations, as laid out in the steps followed in this study.	59
<b>Figure 4.3:</b> An illustration of the absorbance curves created by AQ in different pH values	61
<b>Figure 4.4:</b> The calibration curves of amodiaquine in different pH values	62
<b>Figure 4.5:</b> Micrograph pictures of liposomes in suspension, as seen under CLSM	65

- Figure 4.6:** A representative sample of the size determination study scatter plot from the FACSCalibur™ before being processed with FlowJo™. The figure portrays both the forward and side scatter as analysed from the size determination sample. 67
- Figure 4.7:** The forward scatter plot of a representative liposome size analysis turned into a histogram. The size distribution and span is calculated by adding the different sized gates here (not illustrated as the gates are unclear on such a small scale). 68
- Figure 4.8:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with a buffer of pH 6 at 5°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 69
- Figure 4.9:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with a buffer of pH 6 at 25°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 70
- Figure 4.10:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with a buffer of pH 6 at 40°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 71
- Figure 4.11:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with entrapped amodiaquine with buffer of pH 6 at 5°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 72
- Figure 4.12:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with entrapped amodiaquine with buffer of pH 6 at 25°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 73
- Figure 4.13:** Illustrates the median size (in  $\mu\text{m}$ ) and the size distribution (span in  $\mu\text{m}$ ) of liposomes manufactured with entrapped amodiaquine with buffer of pH 6 at 40°C over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 74
- Figure 4.14:** The pH for liposomes manufactured with just a pH 6 buffer in three different temperatures over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 78

- Figure 4.15:** The pH for AQ entrapped liposomes in three different temperatures over a period of 84 days. Results are shown as mean  $\pm$  SEM (n=3). 80
- Figure 4.16:** The calibration curve used to determine the concentration of AQ in a pH of 6. (n=3)  $r^2 = 0.9832$ .  $y = 0.0357x - 0.005$ . 81
- Figure 4.17:** The entrapment efficacy of amodiaquine in the liposome formulation in 5°C. Results are shown as mean  $\pm$  SEM (n=3). 82
- Figure 4.18:** The entrapment efficacy of amodiaquine in the liposome formulation in 25°C. Results are shown as mean  $\pm$  SEM (n=3). 83
- Figure 4.19:** The entrapment efficacy of amodiaquine in the liposome formulation in 40°C. Results are shown as mean  $\pm$  SEM (n=3). 83
- Figure 4.20:** A representative sample of a scatter plot as gotten from the FACSCalibur™ before being processed with FlowJo™. The figure portrays both the forward and side scatter when a ROS analysis was done on erythrocytes. 88
- Figure 4.21:** The fluorescence histogram of a representative erythrocyte sample, illustrating fluorescent species (Stained) and non-fluorescent species (Unstained) after processing with FlowJo™. 89
- Figure 4.22:** The amount of intracellular ROS detected in erythrocytes (RBC) and *P. falciparum* infected erythrocytes (iRBC). The numbers on the x-axis denote the concentration of liposomes added to the RBS solution before incubation. Results are shown as mean  $\pm$  SEM (n=2) and a factor of the unstained cells. 91
- Figure 4.23:** The amount of intracellular ROS detected in erythrocytes (RBC) and *P. falciparum* infected erythrocytes (iRBC). The numbers on the x-axis denote the concentration of liposomes with entrapped amodiaquine then diluted with liposomes with no entrapped drug, which are added to the RBS solution before incubation. Results are shown as mean  $\pm$  SEM (n=2) and a factor of the unstained cells. 93
- Figure 4.24:** A representative sample of a scatter from the FACSCalibur™ before being processed with FlowJo™. The figure portrays both the forward and side scatter when a lipid peroxidation analysis was done. 94

- Figure 4.25:** The fluorescence (FL 1) histogram of a representative erythrocyte sample, fluorescent species (2) and non-fluorescent species (1) after processing with FlowJo™. 95
- Figure 4.26:** The amount of lipid peroxidation detected in erythrocytes (RBC) and *P. falciparum* infected erythrocytes (iRBC). The numbers on the x-axis denote the concentration of liposomes added to the RBS solution before incubation. Results are shown as mean  $\pm$  SEM (n=2), a factor of the unstained cells and are also inverted (1/x). 97
- Figure 4.27:** The amount of lipid peroxidation detected in different solutions of erythrocytes (RBC) and *Plasmodium falciparum* infected erythrocytes (iRBC). The numbers on the x-axis denote the concentration of liposomes with entrapped amodiaquine then diluted with liposomes with no entrapped drug, which were added to the RBS solution before incubation. Results are shown as mean  $\pm$  SEM (n=2), a factor of the unstained cells and are also inverted (1/x). 98

# List of Tables

<b>Table 1.1:</b> Introduction dates of specific antimalarial drugs and time taken for the appearance of resistance	12
<b>Table 1.2:</b> Treatment regime for uncomplicated <i>P. falciparum</i> malaria	14
<b>Table 1.3:</b> Treatment regime for severe <i>P. falciparum</i> malaria	16
<b>Table 1.4:</b> The physical properties of chloroquine	21
<b>Table 1.5:</b> The physical properties of amodiaquine	24
<b>Table 4.1:</b> The results from the solubility study of amodiaquine in a wide pH range	63
<b>Table 4.2:</b> The pH of the liposomes with buffer (pH 6). Results represented as mean $\pm$ SEM (n=3)	77
<b>Table 4.3:</b> The pH in the liposomes with AQ entrapped. Results represented as mean $\pm$ SEM (n=3)	79
<b>Table 4.4:</b> Amounts of the reagents used to make the cultivation medium	85

# List of Abbreviations

**AQ:** Amodiaquine

**CDC:** Centre for Disease Control

**CL:** Conventional liposomes

**DCFH-DA:** 2',7'-dichlorofluorescein diacetate

**DOH:** Department of Health (South Africa)

**EDL:** Essential Drug List

**FACS:** Fluorescence Activated Cell Sorter

**FP:** Ferriprotoporphyrin IX

**Fluorescein-DHPE:** N-(fluorescein-5-thiocarbonyl)-1,2-diheade-canoyl-sn-glycero-3-phosphoethanolamine

**FSC:** Forward scatter

**Hb:** Haemoglobin

**iRBC:** *Plasmodium* infected Red blood cells

**LCL:** Long Circulating Liposomes

**LMWA:** Low molecular weight antioxidants

**LUV:** Large Unilamellar Vesicles

**MLV:** Multilamellar Vesicles

**NIAID:** National Institute of Allergy and Infectious diseases

**NOS:** Reactive nitrogen species

**OLV:** Oligolamellar Vesicles

**PBS:** Phosphate buffer solution

**PC:** Phosphatidyl choline

**RBC:** Red blood cells or erythrocytes

**ROS:** Reactive oxidative species

**RPMI:** Roswell Park Memorial Institute (refers to the buffer)

**SSC:** Side scatter

**SUV:** Small Unilamellar Vesicles

**T<sub>c</sub>:** Phase transition temperature

**UV:** Ultra violet

**WHO:** World Health Organization

# Abstract

**Title:** Preparation, stability and *in vitro* evaluation of liposomes containing amodiaquine.

**Keywords:** Malaria, liposomes, amodiaquine, Plasmodium falciparum, stability, toxicity, entrapment efficacy, size determination, reactive oxygen species, lipid peroxidation.

Malaria is a curable disease that claims nearly one million lives each year. Problems with the treatment of malaria arise as resistance spreads and new treatment options are becoming less effective. The need for new treatments are of the utmost importance. Liposomes combined with antimalarials are a new avenue for research as liposomes can increase the efficacy of drugs against pathogens, as well as decreasing toxicity. Amodiaquine is a drug with known toxicity issues, but has proven to be effective and is, therefore, a prime candidate to be incorporated into the liposomal drug delivery system.

The aim of this study was to prepare, characterize and evaluate the toxicity of the liposomes with incorporated amodiaquine. The solubility of amodiaquine was determined and liposomes formulated with, and without, amodiaquine entrapped. Accelerated stability studies (at 5 °C, 25 °C with relative humidity of 60% and 40 °C with a relative humidity of 40%) were conducted during which the size, pH, morphology and the entrapment efficacy was determined. The toxicity was determined *in vitro* by analysing the levels of reactive oxidative species and lipid peroxidation caused by the formulations to erythrocytes infected with *P. falciparum* as well as uninfected erythrocytes with flow cytometry.

The solubility study of amodiaquine in different pH buffers showed that amodiaquine was more soluble at lower pH values. Solubility in solution with pH 4.5 was  $36.3359 \pm 0.7904$  mg/ml when compared to the solubility at pH 6.8, which was  $15.6052 \pm 1.1126$  mg/ml. A buffer with a pH of 6 was used to ensure adequate solubility and acceptable compatibility with cells. Liposomes with incorporated amodiaquine were formulated with entrapment efficacies starting at  $29.038 \pm 2.599\%$  and increasing to  $51.914 \pm 1.683\%$ . The accelerated stability studies showed the median sizes and span values remained constant for both liposome and amodiaquine incorporated liposomes at 5 °C. The higher temperatures, i.e. 25 °C and 40 °C, displayed increases in the median size, and decreases in the span for both formulations. The conclusion can, therefore, be made that both liposome and amodiaquine incorporated liposomes are stable at lower temperatures. The entrapment efficacy increased from initial values to nearly 100% during the course of the stability study. This was attributed to amodiaquine precipitating from the solution. The pH values of the liposomes and amodiaquine incorporated liposomes remained

constant for each formulation; though the amodiaquine incorporated liposomes had a lower starting pH, the formulations are both thought to be stable in terms of the pH.

Toxicity studies revealed low levels of reactive oxygen species as well as low levels of lipid peroxidation for both liposome and amodiaquine incorporated liposomes, on both erythrocyte and *Plasmodium* infected erythrocytes. From the toxicity studies it can be concluded that liposomes and amodiaquine incorporated liposomes are not toxic to erythrocytes and infected erythrocytes.

It was concluded that liposomes incorporating amodiaquine could possibly be used as a treatment option for malaria.

# Uittreksel

**Titel:** Die vervaardiging, stabiliteit en *in vitro* evaluering van amodiakien bevattende liposome

**Sleutelwoorde:** Malaria, liposome, amodiakien, *P. falciparum*, stabiliteit, toksisiteit, inkorporerings effektiwiteit, groottebepaling, reaktiewe suurstof spesies, lipied peroksidase.

Malaria is 'n geneesbare toestand wat meer as 'n miljoen lewens elke jaar eis. Probleme met die behandeling van malaria duik op as gevolg van verspreidende weerstandbiedendheid van die parasiete teen huidige behandelings. Daarom is dit uiters belangrik om nuwe behandelings te ontwikkel. Die kombinasie van liposome met antimalaria middels is 'n nuwe veld wat ondersoek kan word, omdat liposome die effektiwiteit teen verskeie patogene kan verbeter, sowel as om toksisiteit te verlaag. Probleme wat met amodiakien toksisiteit ondervind word, is welbekend, maar die middel beskik oor hoë effektiwiteit. Daarom is amodiakien 'n geskikte middel om in 'n afleweringssisteem ingesluit te word.

Die doel van die studie was om liposome en liposome met geïnkorporeerde amodiakien te vervaardig, te karakteriseer en die toksisiteit daarvan te evalueer. Die oplosbaarheid van amodiakien is bepaal en liposome berei, met en sonder die geneesmiddel daarin geïnkorporeer. Versnelde stabiliteitsstudies (in 5 °C, 25 °C met 'n relatiewe humiditeit van 60% en 40 °C met 'n relatiewe humiditeit van 40%) was gedoen, waartydens die grootte, pH, morfologie en inkorporerings effektiwiteit bepaal is. Daarna is die toksisiteit *in vitro* bepaal deur die vlakke van reaktiewe suurstof spesies en vlakke van lipied peroksidase, wat veroorsaak is deur verskillende formulerings op *Plasmodium* geïnfekteerde rooibloedselle en on-geïnfekteerde rooibloedselle, deur middel van vloeisitometrie.

Die oplosbaarheid van amodiakien in verskillende pH buffers is bepaal. Die oplosbaarheid studies het getoon dat amodiakien meer oplosbaar is by laer pH waardes. Oplosbaarheid by pH 4.5 was  $36.3359 \pm 0.7904$  mg/ml, in vergelyking met  $15.6052 \pm 1.1126$  mg/ml by pH 6.8. 'n Buffer met 'n pH van 6 is dus gebruik, om te verseker dat die amodiakien voldoende sal oplos, sowel as om verenigbaarheid met die selkulture te verseker. Liposome met amodiakien geïnkorporeer, kon dus vervaardig word, met aanvanklike geneesmiddel inkorporering wat begin by  $29.038 \pm 2.599\%$  en styg tot  $51.914 \pm 1.683\%$ . Versnelde stabiliteitsstudies het getoon dat grootte, sowel as die deeltjie verspreiding relatief konstant gebly het vir beide die liposome en amodiakien geïnkorporeerde liposome by 5 °C. Die hoër temperature, dit wil sê 25 °C en 40 °C, het 'n verhoging in die grootte en 'n afname in deeltjie verspreiding getoon. Hieruit kan afgelei word dat beide formulerings stabiel is by laer temperature. Die inkorporeringseffektiwiteit van die geneesmiddel het gestyg van die aanvanklike waardes tot byna 100% by al die

temperature gedurende die stabiliteitsondersoek. Dit kan toegeskryf word aan die presipitasie van amodiakien uit die oplossing. Die pH waardes van beide formulerings het konstant gebly, alhoewel die amodiakien liposome oor 'n laer aanvanklike pH geskik het. Beide formulerings is stabiel geag in terme van pH.

Toksisiteit studies het lae vlakke reaktiewe suurstof spesies, sowel as lae vlakke lipied peroksidase vir beide liposoom en amodiakien geïnkorporeerde liposoomformulerings op rooibloedselle en *Plasmodium* geïnfekteerde rooibloedselle getoon. Vanuit die toksisiteitbepaling kan afgelei word dat liposome en liposome met amodiakien geïnkorporeer, nie toksies vir rooibloedselle is nie.

Uit die resultate kan die afleiding gemaak word dat liposome waarin amodiakien geïnkorporeer is, 'n moontlike behandelings opsie vir malaria kan wees.

# Introduction and aim of study

Worldwide, more than 1 million people die as a result of malaria. This serious disease affects the lives of more than 1.62 billion people that live in areas where malaria is endemic (WHO, 2009; CDC, 2010; Daily, 2006). Unfortunately, malaria is most wide-spread and out of control in developing countries that do not have sufficient infrastructure to handle a health crisis on such a large scale (WHO, 2009). This problem is further aggravated by the fact that malaria resistance is becoming an ever increasing and wide spread problem. This leads to inadequate treatment and treatment failures (Wongsrichanalai *et al.*, 2002). Even newly introduced treatments are not safe from the threat of treatment failure, as even the newly introduced artemisinin treatment alternatives have shown the first stage of treatment failures due to resistance (Dondorp *et al.*, 2009). Therefore, it is important to develop new treatment options and review treatment regimes.

For many years chloroquine has been the staple of malaria treatment, but chloroquine has come under fire as resistance started spreading and is now almost a global occurrence (Foley & Tilley, 1997). An alternative to chloroquine is amodiaquine, as cross-resistance to both amodiaquine and chloroquine is rare, and amodiaquine has increased efficacy even when chloroquine resistant malaria was tested (Foley & Tilley, 1997; Hawley *et al.*, 1996; Winstanley *et al.*, 1990). Amodiaquine may be an answer to many problems, but amodiaquine has an unfortunate stigma attached to it as certain severe side-effects, encountered in the 1980's, removed amodiaquine from wide-spread and prophylactic use. In 1996 the WHO reintroduced amodiaquine to the essential drug list, as extensive research showed that amodiaquine related serious side-effects are rare (Olliaro & Taylor, 2003). Unfortunately not much research has been done on amodiaquine as the use thereof has been limited (Winstanley *et al.*, 1990).

Problems in malaria treatment, such as resistant parasites and toxicity can in a large part be decreased and controlled if a drug delivery system is employed. A lipid based drug delivery system known as liposomes has proven itself to be useful in both these respects, as liposomes have in past studies, improved pharmacokinetics and bio-distribution, decreased toxicity and increased efficacy against a wide range of pathogens (Sharma & Sharma, 1997; Drulis-Kawa *et al.*, 2006). Unfortunately, as with most things in life, liposomes as a drug delivery system is not without its faults, and this needs to be closely examined as many different aspects, especially the physicochemical aspects of formulations need to be tested and examined before starting *in vivo* tests (New, 1990). It has been shown that drugs, including arthemether, chloroquine, primaquine and a whole host of others have been successfully incorporated into liposomes. The

formulations showed an increase in bioavailability, possibly overcoming resistance and often a decrease in toxicity (Qui *et al.*, 2008; Sharma & Sharma, 1997; Bayomi *et al.*, 1998).

The aims of this study were the preparation, characterisation and *in vitro* evaluation of liposomes containing amodiaquine. Therefore, in this study, a combination of amodiaquine and liposomes was prepared and tested to determine if a combination was possible and viable to formulate. Preliminary studies were done to determine if a possible combination is safe for use.

Therefore, the specific objectives of this study were:

1. Manufacturing liposomes according to the thin film hydration method.
2. Characterisation of liposomes according to morphology, size, pH and entrapment efficacy.
3. Manufacturing liposomes and incorporating amodiaquine.
4. Characterising amodiaquine entrapped liposomes according to size and entrapment efficacy.
5. Determining the stability of said formulations under high stress situations, such as accelerated stability testing.
6. To evaluate the possible toxicity of liposomes and liposomes incorporated with amodiaquine.

Chapters 1 to 3 consist of a literature study covering malaria, liposomes as a drug delivery system and the determination of the physicochemical properties of the formulations as well as the toxicity determinations. Chapter 4 consists of the experimental design, methods followed, the results and the discussions of said experiments. This study is unique as this author was unaware of any studies using a combination of liposomes and amodiaquine. This study will help determine if amodiaquine combined with liposomes is possible, viable and safe. If this is deemed to be the case, further studies may optimise this system, test its efficacy against different *Plasmodium* strains and may even move it to wide spread production and use.