Summary and future prospects

Malaria is a curable disease that still claims countless numbers of lives each year. The development of new treatments and new antimalarials have been slow and this is further complicated by the rapid spreading scourge of resistant malaria. Even relatively new treatments are becoming ineffective at an alarming rate, for example, resistance to artemisinin derivatives has recently been discovered (WHO, 2009; Dondorp *et al.*, 2009). The time required for resistance to occur is short; therefore ways to overcome resistance need to be explored and implemented. The ways in which resistance can be overcome and controlled are as follows: combination therapies, chemosensitisers and drug delivery systems (WHO, 2009: Van Schalkwyk *et al.*, 2001; Sharma & Sharma, 1997). Other problems often occur as well. In the case of amodiaquine, which has shown great efficacy against even chloroquine resistant malaria, serious side-effects have considerably limited the use thereof considerably (Olliaro & Taylor, 2003).

Drug delivery systems have proven themselves very effective in the optimalisation of current treatments. The liposomal drug delivery system has become particularly popular as liposomes can be made for specific applications as well as being versatile. The advantages of liposomes found earlier include improved pharmacodynamics and pharmacokinetics, decreased toxicity and enhanced activity against pathogens (Drulis-Kawa *et al.*, 2006). Unfortunately other concerns may arise in the use of drug delivery systems, with problems such as instability, encapsulating efficacy and toxicity which may occur (New, 1990; Qui *et al.*, 2008). Therefore, before starting a new formulation study, certain aspects have to be addressed.

The chemical and physical properties, as well as possible toxicity of any new drug delivery system need to be investigated to ensure safety and efficacy of any new treatment. This can be done by employing accelerated stability studies and examining the different properties of the formulations, which include morphology studies, size determinations, entrapment efficacy and pH-determinations (New, 1990). Toxicity studies are often very complicated and expensive therefore, conducting *in vitro* studies, beforehand, is an effective way to quickly eliminate potentially toxic formulations (Blomme, 2008). The determination of the levels of ROS and lipid peroxidation caused by a formulation is a good measure of possible toxicity, as both these mechanisms are responsible for a wide variety of conditions and damage (Halliwell, 2007; Halliwell, 2006; Halliwell & Whiteman, 2004).

In this study, liposomes were manufactured according to the film hydration method, as well as the incorporation of amodiaquine into the formulation was achieved. The formulations were

characterised according to size, morphology and entrapment efficacy. The different formulations were subjected to accelerated stability studies to examine the effect of high stress situations on the size, pH, morphology and entrapment efficacy. Toxicity of the formulations on erythrocytes was evaluated by determining the levels of ROS and levels of lipid peroxidation caused by the different formulations.

Solubility of amodiaquine was the first subject explored to ensure sufficient concentrations of the drug to be encapsulated in the liposomes. The solubility of amodiaquine was found to be higher in more acidic environments, therefore, to keep the liposomes compatible with cells, a buffer with a pH of 6 was chosen for the liposomal formulation, as the amount of amodiaquine that could be dissolved was sufficient. Liposomes were prepared according to the film hydration method, and amodiaquine could be successfully entrapped in the aqueous interior of the liposomes.

Initial characterisation of the liposomes could now be done, with the morphological studies revealing the liposomes as spherical objects. Initial median sizes were between 0.73 and 0.87 µm for both formulations with a span varying between 17.12 and 20.14 µm. The entrapment efficacy of liposomes with incorporated amodiaquine resulted in entrapment efficacies ranging between 29 and 54%.

Accelerated stability studies were done. Morphological evaluations showed slight changes, with the addition of oil droplets being the only difference from initial formulations. The pH of both the formulations stayed relatively constant, with the liposomes containing amodiaquine starting off at a lower pH (5.94 ± 0.013 at 5 ºC, 5.86 ± 0.023 at 25 ºC and 5.91 ± 0.007 at 40 ºC) than the regular liposomes (6.01 ± 0.015 at 5 ºC, 5.85 ± 0.009 at 25 ºC and 5.88 ± 0.007 at 40 ºC). The formulations were deemed stable in terms of pH. Size determination revealed that both the liposome formulations at 5 ºC remained stable in terms of median size and span, as the median size and span did not change drastically. At the higher temperatures, median size rose in both formulations towards day 70 and 84, with the span decreasing. This indicated that the formulations were unstable at higher temperatures. Entrapment efficacy of the liposomes incorporated with amodiaquine increased as time passed. Unfortunately amodiaquine appeared to precipitate from solution over time, thus indicating that the increased reading may not be the result of increased entrapment efficacy. The *in vitro* toxicity studies displayed low levels of ROS and lipid peroxidation in both formulations, indicating that the formulations were not toxic to erythrocytes.

Finally, it can, therefore, be concluded that liposomes with incorporated amodiaquine could possibly be used as a treatment for malaria.

A few future prospects were identified during the course of this study:

- The stability of liposomes could possibly be increased with the use of freeze drying or coating the liposomes.
- Targeted liposomes may be explored such as stealth liposomes. This may also lead to increased stability of liposomes, depending on the formulation variables.
- Testing on different strains of malaria, efficacy studies as well as ROS and lipid peroxidation to see if differences in results occur on different strains of malaria.
- *In vivo* toxicity, as well as *in vivo* efficacy and bioavailability studies can be done.
- Incorporating different drug combinations into the liposomes, to align with the WHO's recommended treatment guidelines.
- The active loading of drugs into liposomes to increase the entrapment efficacy may be explored.

References

ALVING, C.R., STECK, E.A., CHAPMAN, W.L., WAITS, W.B., HENDRICKS, L.D., SWARTZ, G.M. & HANSON, W.L. 1978. Therapy of leishmaniasis: superior efficacies of liposome encapsulated drugs. *Proceedings of the National Academy of Sciences of the United States of America*, 75(6):2959-2963. Available: JSTOR Complete.

AMER, J., GOLDFARB, A. & FIBACH, E. 2004. Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. *Cytometry A*, 60A(1):73-80. Abstract in MEDLINE

AMONROSA, L.F., CORBELLINI, G. & COLUZZI, M. 2005. Lessons learned from malaria: Italy's past and sub-Saharan future. *Health and place*, 11(1):67-73. Available: ScienceDirect.

ANDERSAG, H., BREITNER, S. & HUNG, J. 1942. Quinoline compounds and process of making the same. *Chemistry abstracts*, 683-692.

BAKKER-WOUDENBERG, I.A.J.M. 2002. Long-circulating sterically stabilized liposomes as carriers of agents for treatment of infection or for imaging infectious foci. *International journal of antimicrobial agents*, 19(4):299-311. Available: ScienceDirect.

BASCO, L.K. 2007. Field application of *in vitro* assays for the sensitivity of human malaria parasites to antimalarial drugs. Geneva: World Health Organization. 191 p.

BAUM, J., GILBERGER, T., FRISCHKNECHT, F. & MEISSNER, M. 2008. Host-cell invasion by malaria parasites: insights from *Plasmodium* and *Toxoplasma*. *Trends in parasitology*, 24(12):557-563. Available: ScienceDirect.

BAYOMI, M.A., AL-ANGARY, A.A., AL-MESHAL, M.A. & AL-DARDIRI, M.M. 1998. *In vivo* evaluation of artether liposomes. *International journal of pharmaceutics*, 175(1):1-7. Available: ScienceDirect.

BD BIOSCIENCES. 2000. Introduction to flow cytometry: a learning guide. San Jose: BD Biosciences. 52 p.

BECKER, K., TILLEY, L., VENNERSTROM, J.L., ROBERTS, D., ROGERSON, S. & GINSBERG, H. 2004. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *International journal of parasitology*, 34(2):163-189. Abstract in MEDLINE.

BERGQVIST, Y. & DOMEIJ-NYBERG, B. 1983. Distribution of chloroquine and its metabolite desethyl-chloroquine in human blood cells and it's implications for the quantitative determination of those compounds in serum and plasma. *Journal of chromatography*, 272(1):137-148. Available: ScienceDirect.

BETAGERI, G.V., JENKINS, S.A. & PARSONS, D.L. 1993. Liposome drug delivery systems. Lancaster: Technomic Publishing Company. 135 p.

BLOMME, S. 2008. Toxicological evaluation of liposomal antimicrobials. Ontario: Heritage branch. 168 p.

SHAPIRO, T.A. & GOLDBERG, D.E. 2006. Chemotherapy of protozoal infections: Malaria. (*In* Brunton, L.L., Lazo, J.S. & Parker, K.L., *eds.* Goodman & Gilman's the pharmacological basis of therapeutics. 11th ed. http://www.accessmedicine.com/content.aspx?aID=946964.) Date of access: 27 Feb. 2010.

CENTRE FOR DISEASE CONTROL AND PREVENTION (CDC). 2010. Malaria. http://www.cdc.gov/malaria/ Date of access: 11 Mar. 2010.

CENTRE FOR DISEASE CONTROL AND PREVENTION (CDC). 2010. Malaria life cycle. http://www.cdc.gov/malaria/about/biology/index/html. Date of access: 25 Feb. 2010.

CENTRE FOR DISEASE CONTROL AND PREVENTION (CDC). 2010. Preventing malaria in travellers. http://www.cdc.gov/malaria/resources/pdf/travelers.pdf. Date of access: 12 Apr. 2010.

CHILDERS, N.K., MICHALEK, S.M., ELDRIDGE, J.H., DENYS, F.R., BERRY, A.K. & MCGHEE, J.R. 1989. Characterization of liposomes suspension by flow cytometry. *Journal of immunological methods*, 119(1):135-143. Available: ScienceDirect.

CHURCHILL, F.C., PATCHEN, L. C., CAMPBELL, C.C., SCHWARTZ, I.K., NGUYEN-DINH, P. & DICKINSON, C.M. 1985. Amodiaquine as a prodrug: importance of metabolite(s) in the antimalarial effect of amodiaquine in human. *Life sciences*, 36(1):53-62. Available: ScienceDirect.

COHEN, W.D. 1982. The cytomorphic system of anucleate non-mammalian erythrocytes. *Protoplasma*, 113(1):23-32. Available: SpringerLink.

COX, F.E.G. 2002. History of human parasitology. *Clinical biology reviews*, 15(4):595-612. Available: American Society for Microbiology.

COX-SINGH, J., DAVIS, T.M., LEE, K.S., MATUSOP, A. & RATNAM, S. 2008. *Plasmodium knowelesi* malaria in humans is widely distributed and potentially life threatning. *Clinical infective disease*, 46(2):165-171. Abstract in MEDLINE.

DAILY, J.P. 2006. Antimalarial drug therapy: the role of parasite biology and drug resistance. *Journal of clinical pharmacology*, 46(12):1487-1497. Available: Sage Premier 2010.

DATE, A.A., JOSHI, M.D. & PATRAVALE, V.B. 2007. Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. *Advanced drug delivery reviews*, 59(6):505-521. Available: ScienceDirect.

DEAMER, D.W. 2010. From "banghasomes" to liposomes: a memoir of Alec Bangham 1921-2010. *The FASEB Journal: Official publication of the Federation of American Societies for Experimental Sciences*, 24(5):1308-1310. Available: EBSCO Open Access Journals.

DEOL, P. & KHULLER, G.K. 1997. Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. *Biomedica et biophysica acta*, 1334:161-172.

DEPARTMENT of Health ***see*** SOUTH AFRICA. Department of Health. 2008.

DEPARTMENT of Health ***see*** SOUTH AFRICA. Department of Health. 2009.

DERMARDEROSIAN, A. 2006. A history of quinine. http://quinine.com/history.html. Date of access: 3 November 2009.

DONDORP, A.M., NOSTEN, F., YI, P., DAS, D., PHYO, A.P., TARNING, J., LWIN, K.M., ARIEY, F., HANPITHAKPONG, W., LEE, S.J., RINGWALD, P., SILAMUT, K., IMWONG, M., CHOTIVANICH, K., LIM, P., HERDMAN, T., YEUNG, S., SINGHASIVANON, P., DAY, N.P.J., LINDEGARDH, N., SOCHEAT, D. & WHITE, N.J. 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *The New England journal of medicine*, 361(5):455-467.

DOURMASHKIN, R.R. & ROSSE, W.F. 1966. Morphological changes in the membranes of red blood cells undergoing hemolysis. *The American journal of medicine*, 41(5):699-710. Available: ScienceDirect.

DRULIS-KAWA, Z. & DOROTKIEWICZ-JACH, A. 2010. Liposomes as delivery systems for antibiotics. *International journal of pharmaceutics*, 387(1-2):187-198. Available: ScienceDirect.

DRULIS-KAWA, Z., GUBERNATOR, J., DOROTKIEWICZ-JACH, A., DOROSZKIEWICZ, W. & KOZUBEK, A. 2006. *In vitro* microbial activity of liposomal meropenem against *Pseudomonal aeruginosa* strains. *International journal of pharmaceutics*, 315(1-2):59-66. Available: ScienceDirect.

DU PLESSIS, L., LAUBSCHER, P., JOOSTE, J., DU PLESSIS, J., FRANKEN, A., VAN AARDE, N. & ELOFF, F. 2010. Flow cytometric analasis of the oxidative status in human peripheral blood mononuclear cells of workers exposed to welding fumes. *Journal of occupational and environmental hygiene*, 7(6):367-374. Available: E-Journals from EBSCO.

FOLEY, M. & TILLEY, L. 1997. Quinoline antimalarials: mechanisms of action and resistance. *International journal of parasitology*, 27(2):231-240. Available: ScienceDirect.

FOLEY, M. & TILLEY, L. 1998. Quinoline antimalarials: mechanism of action and resistance and prospects for new agents. *Pharmacological therapy*, 79(1):55-87. Available: ScienceDirect.

FRANCIS, S.E., SULLIVAN, D.J. & GOLDBERG, D.E. 1997. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annual reviews: microbiology*, 51:97-123. Available: Annual Reviews.

GINSBERG, H., WARD, S.A. & BRAY, P.G. 1999. An integrated model of chloroquine action. *Parasitology today*, 15(9):357-360. Available: ScienceDirect.

GREGORIADIS, G. 1995. Engineering liposomes for drug delivery: progress and problems. *Trends in biotechnology*, 13(12):527-537. Available: ScienceDirect.

HALLIWELL, B. 2006. Reactive species and antioxidants: redox biology is a fundamental theme of aerobic life. *Plant physiology*, 141(2):312-322. Available: EBSCO Open Access Journals.

HALLIWELL, B. 2007. Biochemistry of oxidative stress. *Biochemical society transactions*, 35(5):1147-1150. Available: EBSCO Open Access Journals.

HALLIWELL, B. & CROSS, C.E. 1994. Oxygen derived species: their relation to human disease and environmental stress. *Environmental health perspectives*, 102(supplement 10):5-12. Available: Academic Search Premier.

HALLIWELL, B. & WHITEMAN, M. 2004. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *British journal of pharmacology*, 142(2):231-255. Available: Academic Search Premier.

HAWLEY, S.R., BRAY, P.G., PARK, B.K. & WARD, S.A. 1996. Amodiaquine accumulation in *Plasmodium falciparum* as a possible explanation for its superior antimalarial activity over chloroquine. *Molecular and biochemical parasitology*, 80(1):16-25. Available: ScienceDirect.

HILLERY, A.M. 1997. Supramolecular lipid drug delivery systems: from laboratory to clinic. A review of the recently introduced commercial liposomal and lipid-based formulations of amphotericin B. *Advanced drug delivery review*, 24(2-3):345-363. Available: ScienceDirect.

INVITROGEN. 2003. Flow cytometry size calibration kit. http://www.invitrogen.com Date of access: 15 Jul. 2010.

JEONG, S.H., PARK, J.H. & PARK, K. 2007. Formulation issues around lipid-based oral and parenteral delivery systems. (*In*: Wasan, K.M, *ed*. Role of lipid excipients in modifying oral and parenteral drug delivery, 1st ed. Hoboken: John Wiley & Sons Inc. 32-47 p.).

KIM, H., GIAS, E.L.M. & JONES, M.N. 1999. The adsorption of cationic liposomes to *Staphylococcus aureus* biofilms. *Physiochemical and engineering aspects*, 149(1-3):561-570. Available: ScienceDirect.

KIRBY, C., CLARKE, J. & GREGORIADES, G. 1980. Effect of cholesterol content on small unilamellar liposomes on stability *in vivo* and *in vitro*. *Biochemical journal*, 186(2):591-598. Abstract in MEDLINE.

KOHEN, R. & NYSKA, A. 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic pathology*, 30(6):520-630. Available: Academic Search Premier.

KOUZNETSOV, V.V. & GOMES-BARRIO, A. 2009. Recent developments in the design and synthesis of hybrid molecules based on amodiaquine and their antiplasmodial evaluation. *European journal of medicinal chemistry*, 44(8):3091-3113. Available: ScienceDirect.

LABANA, S., PANDEY, R., SHARMA, S. & KHULLER, G.K. 2002. Chemotherapeutic activity against murine tuberculosis of once weekly administered drugs (isoniazid and rifampicin) encapsulated in liposomes. *International journal of antimimicrobial agents*, 20(4):301-304. Available: ScienceDirect.

LANKFORD, J.S. 1913. The lessons of Canal zone sanitation. *The popular science monthly*: 294-299.

LASIC, D.D. 1998. Novel applications of liposomes. *Trends in Biotechnology*, 16(7):307-321. Available: Elsevier.

MAESTRELLI, F., GONZALES-RODRIGUEZ, M.L., RABASCO, A.M. & MURA, P. 2005. Preparation and characterisation of liposomes encapsulating keptofen-cyclodexterin complexes for transdermal drug delivery. *International journal of pharmacy*, 298(1):55-67. Abstract in Medline.

MARTIN, A. 1993. Physical pharmacy: physical chemical principals in the pharmaceutical sciences. Baltimore: Lippincott Williams & Wilkins. 622 p.

MATTHEWS, B.R. 1999. Regulatory aspects of stability testing in Europe. *Drug development and industrial pharmacy*, 25(7):831-856. Available: E-Journals from EBSCO.

MAULIK, G., ASSIS, A.I., SAVVIDES, P. & MAKRIGIORGOS, G.M. 1998. Fluoresceinted phosphoethanolamine for flow-cytometric measurement of lipid peroxidation. *Free radical biology & medicine*, 25(6):645-653. Available: ScienceDirect.

MERCK RESEARCH LABORATORIES. 2008. Malaria. (*In*: Beers, M.H., Porter, S.R., Jones, T.V., Kaplan, J.L. & Berkwitz, M., *eds*. The Merck manual of diagnosis and therapy. 18th ed. New Jersey: Merck Co. 1381-1664 p.).

MITA, T., TANABE, K. & KITA, K. 2009. Spread and evolution of *Plasmodium falciparum* drug resistance. *Parasitology international*, 10(1016):9. Available: ScienceDirect.

MÜLLER, R.H., BENITA, S. & BÖHM, B., *eds*. 1998. Emulsions and nanosuspensions for the formulation of poorly soluble drugs. Stuttgart: MedPharm Scientific Publishers. 396 p.

NAKRUMAH, L.J., RIEGELHAUPT, P.M., MOURA, P., JOHNSON, D.J., PATEL, J., HAYTON, K., FERDIG, M.T., WELLEMS, T.E., AKABAS, M.H. & FIDOCK, D.A. 2009. Probing the multifactorial basis of *Plasmodium falciparum* quinine resistance: evidence for strain-specific contribution of the sodium-proton exchanger PfNHE. *Molecular and biochemical parasitology*, 165(2):122-131. Available: ScienceDirect.

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES. 2007. Understanding malaria. http://www.niaid.nih.gov/topics/malaria. Date of access: 12 Nov. 2009.

NEW, R.R.C. 1 990. Liposomes: a practical approach. Oxford: Oxford University Press. 301 p.

NOGUEIRA, F., DIEZ, A., RADFAR, A., PEREZ-BENAVENTE, S., DO ROSARIO, V.E., PUYET, A. & BAUTISTA, J.M. 2010. Early transcriptional response to chloroquine of the *Plasmodium falciparum* antioxidant defence in sensitive and resistant clones. *Acta Tropica*, 114(2):109-115. Available: ScienceDirect.

OLLIARO, P. & MUSSANO, P. 2009. Amodiaquine for the treatment of malaria (review). Geneva: John Wiley & Sons, Ltd. 70 p.

OMRI, A. & RAVAOARINORO, M. 1996. Preparation and the effects of amikacin, netilmicin and tobramycin in free and liposomal formulations on gram-negative and gram positive bacteria. *International journal of antimicrobial agents*, 7(1):9-14. Available: ScienceDirect.

PAPAGIANNAROS, A., BORIES, C., DEMETZOS, C. & LOISEAU, P.M. 2005. Antileishmanial and trypanocidal activities of new miltefosine liposomal formulations. *Biomedicine and pharmacotherapy*, 59(10):545-550. Available: ScienceDirect.

PEARSON, R.D. 2009. Malaria extraintestinal protozoa (Merck manual online). http://www.merck.com/mmpe/sec14/ch186/ch186g.html Date of access: 18 Feb. 2010.

PEYMAN, G.A., CHARLES, H.C., LIU, K.R., KHOOBEHI, B. & NIESMAN, M. 1988. Intravitreal liposome-encapsulated drugs: a preliminary human report. *International ophthalmology*, 12(3):175-182. Available: SpringerLink.

PINTO-ALPHANDARY, H., ANDREMONT, A. & COUVREUR, P. 2000. Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications. *International journal of antimicrobial agents*, 13(3):155-168. Available: ScienceDirect.

PUGH, J. 2002. Kinetics and product stability. (*In*: Aulton, M.E., *ed*. *Pharmaceutics: the science of dosage form design*, 2nd ed. Edinburgh: Churchill Livingstone. 679 p.

QUI, L., JING, N. & JIN, Y. 2008. Preparation and *in vitro* evaluation of liposomal chloroquine diphosphate loaded transmembrane pH-gradient method. *Internationl journal of pharmaceutics*, 361(1-2):56-63. Available: ScienceDirect.

RINGWALD, P., MECHE, F.S., BICKII, J. & BASCO, L.K. 1999. *In vitro* culture and drug sensitivity assay of *Plasmodium falciparum* with nonserum substitute and acute-phase sera. *Journal of clinical microbiology*, 37(3):700-705. Available: EBSCO Open Access Journals.

ROBERTS, D.R., MALGULN, S. & MOUCHET, J. 2000. DDT House spraying and re-emerging malaria. *The lancet*, 356:330-332. Available: ScienceDirect.

ROBINSON, A.M., BANNISTER, M., CREETH, J.E. & JONES, M.N. 2001. The interaction of liposomes with mixed bacterial biofilm and their uses in the delivery of bactericide. *Colloids and surfaces A: Physiochemical and engineering aspects*, 186(1):43-53. Available: ScienceDirect.

ROERDINK, F.H., DAEMON, T., BAKKER-WOUDENBERG, I.A.J.M., STORM, G., CROMMELIN, D.J.A. & SCHERPHOF, G.L. 1987. Therapeutic utility of liposomes. (*In*: Johnson, P. & Lloyd-Jones, J.G., *eds*. *Drug delivery systems: fundamentals and techniques*, 1st ed. Chichester: Ellis Horwood Ltd. 282 p.).

ROSENTHAL, P.J. 2004. Antiprotozoal drugs. (*In*: Katzung, B.G., *ed*. *Basic and clinical pharmacology*, 9th ed. Boston: McGraw-Hill. 864-875 p.).

ROSSITER, D. 2008. South African medicines formulary. 8th ed. Cape Town: Health and Medical Publishing Group. 612 p.

SALEM, I.I. & DÜZGÜNES, N. 2003. Efficacies of cyclodextrin-complexed and liposome encapsulated clarithromycin against Mycobacterium avium complex infection in human macrophages. *International journal of pharmaceutics*, 250(2):403-414. Available: ScienceDirect.

SARKAR, M., VARSHNEY, R., CHOPRA, M., SEKHRI, T., ADHIKARI, J.S. & DWARAKANATH, B.S. 2005. Flow cytometric analysis of reactive oxygen species in peripheral blood mononuclear cells of patientes with thyroid dysfunction. *Cytometry B*, 70B(1):20-23. Abstract in MEDLINE.

SCHUSTER, F.L. 2002. Cultivation of *Plasmodium spp*. *Clinical microbiology reviews*, 15(3):355-364. Available: American Society of Microbiology.

SHARMA, A. & SHARMA, U.S. 1997. Liposomes in drug delivery: progress and limitations. *International journal of pharmaceutics*, 154(2):123-140. Available: ScienceDirect.

SOLOMAN, V.R. & LEE, H. 2009. Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. *European journal of pharmacology*, 625(1-3):220-233. Available: ScienceDirect.

SOUTH AFRICA. Department of health. 2008. Guidelines for the treatment of malaria in South Africa. Pretoria: Department of health. 36 p.

SOUTH AFRICA. Department of Health. 2009. Guidelines for the prevention of malaria in South Africa. Pretoria: Department of Health. 44 p.

SPITELLER, G. 1996. Enzymatic lipid peroxidation: a consequence of cell injury? *Free radiacal biology & medicine*, 21(7):1009-1009. Available: ScienceDirect.

STAHL, W. & SIES, H. 2002. Introduction: reactive oxygen species. http://www.uniklinik-duesseldorf.de/img/ejbfile/ROS.pdf?id=48. Date of access: 12 Oct. 2010.

STORM, G. & CROMMELIN, D.J.A. 1998. Liposomes: *quo vadis*? *Plasma sources science and technology*, 1(1):19-31.

SULLIVAN, D.J. JR, MATILE, H., RIDLEY, R.G. & GOLDBERG, D.E. 1998. A common mechanism for blockade of heme polymerization by antimalarial quinolines. *The journal of biological chemistry*, 273(47):31103-31107.

SWEETMAN, S.C., *ed*. 2009. Martindale: the complete drug reference. 36th ed. London: Pharmaceutical Press. 3694 p.

TORCHILIN, V.P. 2007. Lipid-based parenteral drug delivery systems: biological implications. (*In*: Wasan, K.M., *ed*. Role of lipid excipients in modifying oral and parenteral drug delivery, 1st ed. Hoboken: John Wiley & Sons Inc. 205 p.).

TRAGER, W. & JENSEN, J.B. 1976. Human malaria parasites in continuous culture. *Science*, 193(4254):673-675. Abstract in MEDLINE.

TREN, R. & BATE, R. 2004. South Africa's war against malaria: lessons for the developing world. *CATO Institute: policy analysis*, 25 Mar. 19 p. Available: CATO Institute.

TRIPATHI, R.P., MISHRA, R.C., DWIVEDI, N., TEWARI, N. & VERMA, S.S. 2005. Current status of malaria control. *Current medicinal chemistry*, 12(22):2643-2659. Available: Academic Search Premier.

TSURUO, T., IIDA, H., TSUKAGOSHI, S. & SAKURAI, Y. 1981. Overcoming of viscristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of viscristine and visblastine by verapamil. *Cancer Research*, 41:1967-1972. Available: EBSCO Open Access Journals.

TUTEJA, R. 2007. Malaria: an overview. *The Federation of European Biochemical Societies journal*, 274(18):4670-4679. Abstract in MEDLINE.

UNITED STATES PHARMACOPEIA. 2010. USP monographs: amodiaquine hydrochloride. http://www.uspnf.com/uspnf/pub/index?usp=33&nf=28&s=1&official on=October1,2009 Date of access: 28 Jan. 2010.

VAN ETTEN, E.W.M., VAN VIANEN, W., TIJHUIS, R.H.G., STORM, G. & BAKKER-WOUDENBERG, I.A.J.M. 1995. Sterically stabilized amphotericin B-liposomes: toxicity and

biodistribution in mice. *Journal of controlled release*, 37(1-2):123-129. Available: ScienceDirect.

VAN RENSWOUDE, J. & HOEKSTRA, D. 1981. Cell-induced leakage of liposomal contents. *Biochemistry*, 20(3):540-546.Available: American Chemical Society Web Editions (ACS).

VAN SCHALKWYK, D.A. & EGAN, T.J. 2006. Quinoline resistance reversing agents for the malaria parasite *Plasmodium falciparum*. *Drug resistance updates*, 9(4-5):211-226. Available: ScienceDirect.

VAN SCHALKWYK, D.A., WALDEN, J.C. & SMITH, P.J. 2001. Reversal of chloroquine resistance in *Plasmodium falciparum* using combinations of chemosensitizers. *Antimicrobial agents and chemotherapy*, 45(11):3171-3174. Available: EBSCO Open Access Journals.

VORAUER-UHL, K., WAGNER, A., BORTH, N. & KATINGER, H. 2000. Determination of liposome size and distribution by flow cytometry. *Cytometry*, 39(2):166-171. Abstract in MEDLINE.

WALGATE, R. 2001. WHO Bulletin library.. <http://www.whqlibdoc.who.int/bulletin/2001/issue2/79(2).pdf. Date of access: 3 Jul. 2010

WANG, H. & JOSEPH, J.A. 1999. Quantifying cellular oxidative stress by dichlorofluorecein assay, using microplate reader. *Free radical biology & medicine*, 27(5-6):612-616. Available: ScienceDirect.

WARD, S.A. & BRAY, P.G. 2001. Is reversal of chloroquine resistance ready for the clinic? *The lancet*, 357(9260):904. Available: ScienceDirect.

WARHURST, D.C. 1995. Haemozoin and the mode of action of blood schizontocides: more controversy. *Parasitology today*, 11(6):204-205. Available: ScienceDirect.

WINSTANLEY, P.A., COLEMAN, J.W., MAGGS, J.L, BRECKENBRIDGE, A.M. & PARK, B.K. 1990. The toxicity of amodiaquine and its principal metabolites towards mononuclear leucocytes and granulocyte/monocyte colony forming unit. *British journal of clinical pharmacology*, 29(4):479-485. Available: Academic Search Premier.

WISER, M.F. 2003. Malaria lifecycle. http://www.tulane.edu/~wiser/malaria/ Date of access: 3 Nov. 2009.

WONGSRICHANALAI, C., PICKARD, A.L., WERNSDORFER, W.H. & MESHNICK, S.R. 2002. Epidemiology of drug-resistant malaria. *The lancet: infectious diseases*, 2(4):209-218. Abstract in MEDLINE

WOODWARD, R.B. & DOERING, W.E. 1945. The total synthesis of quinine. *Journal of the American Chemical Society*, 67(5):860-874. Available: American Chemical Society Web Editions (ACS).

WHO (WORLD HEALTH ORGANIZATION). 2009. Guidelines for the treatment of malaria. Geneva: World Health Organization. 194 p.

WHO (WORLD HEALTH ORGANIZATION). 2009. World malaria report. Geneva: World Health Organization. 66 p.