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 °C, 5.86 ± 0.023 at 25 °C and 5.91 ± 0.007 at 40 °C) than the regular liposomes (6.01 ± 0.015 at 5 °C, 5.85 ± 0.009 at 25 °C and 5.88 ± 0.007 at 40 °C). The formulations were deemed stable in terms of pH. Size determination revealed that both the liposome formulations at 5 °C 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.


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