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Evaluation of the physical properties and stability of two lipid drug delivery systems containing mefloquine

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ABSTRACT

Stability data is used to determine the change the product has undergone over a certain time period at specific temperatures. In the present study, the physical stability characterized by size, pH and entrapment efficacy of mefloquine loaded liposomes and Pheroid™ vesicles were investigated. Size was accurately determined by flow cytometry. Entrapment efficacy, after unentrapped drug was removed was successfully determined by UV-spectrophotometry. The formulations contained 0.5% (m/v) mefloquine and results showed that mefloquine interfered with the formation of lipid bilayer of the liposomes. Liposomes increased in size from 5.22 ± 0.03 μm to 9.71 ± 1.11 μm with accelerated stability and large aggregates were observed. A notable difference in stability testing of Pheroid™ vesicles was seen with no significant increase in size. Entrapment efficacy of 68.72 ± 0.04% (5 °C), 67.45 ± 2.92% (25 °C) and 67.45 ± 2.92% (30 °C) were obtained at the different storage conditions. With these findings the mefloquine loaded Pheroid™ vesicles are stable and should be used investigated for the possible increase in efficacy and bioavailability and decrease toxicity.

1. Introduction

Due to an increase in demand for more effective and safer drugs, drug delivery systems development has increased in the last decades (Buszello and Müller, 2000; Ranade and Hollinger, 2004). These systems can transport drugs for site specific delivery leading to enhanced efficacy and bioavailability, decrease adverse reactions and improve patient compliance (Date et al., 2007; Mahato, 2007; Ranade and Hollinger, 2004). Colloidal drug carriers consist of a dispersed phase in a continuous phase and have been investigated for targeted delivery (Buszello and Müller, 2000; Date et al., 2007; Mahato, 2007).

The most extensively researched colloidal system is liposomes (Sharma and Sharma, 1997). The vesicles are composed of a lipid bilayer with an aqueous compartment (New, 1990; Sharma and Sharma, 1997) and can entrap both hydrophilic and lipophilic drugs increasing the versatility of liposomes (Date et al., 2007; New, 1990). Liposome properties vary considerably with lipid composition, size and method of preparation (Betageri et al., 1993). Liposomes differ in size from 80 nm to 100 μm and can be manipulated according to specific requirements (New, 1990; Sharma and Sharma, 1997). Site specific delivery, sustained release and reduction in toxicity are some of the advantages of this lipid drug delivery system (Betageri et al., 1993; Date et al., 2007; New, 1990). Research into the application of liposomes in malaria include artemether (Joshi et al., 2008a,b), artesunate (Gabriels and Plaizier-Vercammen, 2003), chloroquine (Qiu et al., 2008) and primaquine (Stensrud et al., 2000).

Pheroid™ technology is a patented colloidal delivery system consisting of a dispersed phase of plant and essential fatty acid in a nitrous oxide saturated continuous water phase. Pheroid™ also consists of a lipid bilayer that is dynamic and constantly changing, but has high stability. Like liposomes, Pheroid™ can entrap both hydrophilic and lipophilic drugs. Pheroid™ lipid carrier system can be manipulated in formulation, structure and size for various applications (Grobler et al., 2007). Pheroid™ has been successfully used in nasal peptide delivery (Du Plessis et al., 2010), transdermaly (Gerber et al., 2008; Grobler et al., 2007; Saunders et al., 1999) and cosmetic application (Grobler et al., 2007).

Malaria affects billions of people in 109 countries worldwide, almost half situated in Africa (WHO, 2010a,b)(WHO, 2010a,c). Malaria is responsible for 20% of deaths in children (WHO, 2010b). Anti-parasitic disease drug discovery only accounts for 1% of new drug development (Date et al., 2007). Mefloquine, a blood schizonticidal drug, is used as treatment and chemoprophylaxis of malaria (Basco, 2007; Rosenthal, 2004). Mefloquine in combination with artemisinins showed to be highly effective with good tolerability (Bouyou-Akotet et al., 2010; Congpuong et al., 2010).

Abbreviations: CLSM, Confocal laser scanning microscopy; %EE, Percentage entrapment efficacy; FSC, Forward light scattering; ICH, International Conference on Harmonisation; MQ, Mefloquine hydrochloride; PBS, Phosphate buffer saline; RH, Relative humidity; WHO, World Health Orginization.

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Adverse reactions include gastrointestinal and neurological disturbances (Rosenthal, 2004). Neurotoxicity has been demonstrated *in vitro* (Dow et al., 2003), *in vivo* (Dow et al., 2006) and numerous reports of neurological symptoms in humans exist (Arguin and Steele, 2010; Rendi-Wagner et al., 2002). Mefloquine has been successfully formulated in emulsions with satisfactory stability and high antimalarial activity (Mbela et al., 1998, 1994). Stability is mainly evaluated to ensure the product will remain in a state of adequate quality throughout its shelf life and is most difficult to attain (Rhodes, 1979). Stability testing under different environmental factors provides evidence of the quality of the product needed for registration (ICH, 2006).

These lipid based colloidal drug delivery systems is similar in structure, size and drug loading ability. The present study is intended to evaluate the physical stability of mefloquine loaded Pheroid™ vesicles and liposomes under different storage condition for three months. Characteristics under investigation include the flow cytometric evaluation of size, entrapment efficacy by means of UV-spectrophotometry and pH.

2. Materials and methods

2.1. Materials

Vitamin F ethyl ester was obtained from Chemimpo (South Africa), Cremophor® RH40 from BASF (Germany) and DL-α-tocopherol from DSM (Basel, Switzerland). High grade chloroform and methanol were obtained from Rochelle Chemicals (South Africa). The following were purchased from Sigma–Aldrich® (St. Louis, MO, USA): cholesterol; L-α-phosphatidylcholine; Nile Red; and Sephadex® G50. Mefloquine hydrochloride (MQ) was purchased from Sifaviton S.p.A (Mairano, Italy). Flow Cytometry Size Calibration Kit and FluoSpheres® Fluorescent Microspheres were purchased from Molecular Probes (Invitrogen®, Breda, The Netherlands).

2.2. Preparation of lipid drug carriers

Pheroid™ vesicles were prepared according to Du Plessis et al. (2010). Briefly, the oil phase comprises of vitamin F, Cremophor® RH40 and DL-α-tocopherol was added to nitrous oxide saturated water. Loaded vesicles were prepared as described above with the addition of MQ to the DL-α-tocopherol. Liposomes were prepared by the film hydration method (Mozafari, 2005; New, 1990; Yamabe et al., 2003). The lipid phase consisting of cholesterol (1%, w/v) and L-α-phosphatidylcholine (1.5%, w/v) were dissolved in an appropriate volume of chloroform:methanol (2:1, v/v) solution. The organic solvent was slowly removed under reduced pressure obtaining a thin film of lipids on the inner wall of the flask. A lipid suspension was attained by hydration of the film in phosphate buffer solution (PBS) (pH 7.4) and swirling with glass beads until all lipids were dispersed. Particle downsizing was obtained by sonication for 5–10 min at 4 °C. Loaded liposomes were attained from adding MQ to the organic phase before evaporation.

2.3. Determination of particle size distribution

The size distribution of the particles was determined using a FACSCalibur™ (Becton and Dickson, Mountain View, CA, USA) benchtop flow cytometer equipped with a 488 nm Argon ion laser linked to CellQuest Pro software (2002, Becton and Dickson, Mountain View, CA, USA). Forward light scatter (FSC) was set to logarithmic scale and a flow rate of less than 2000 events per second was used to prevent coincidence. A total of 100,000 events were recorded. Data were processed with FCS Express V3 software (De Novo Software, CA, USA).

Size calibration beads ranging in size from 0.5–15 μm were analysed for the calibration. Histograms of FSC values were used to determine the geometric mean of each peak corresponding to a bead size (Childers et al., 1989; Sato et al., 2006; Vorauer-Uhl et al., 2000). Data were transposed to give a log-log graph and fitted with linear regression yielding an equation $y = mx + b$. XY coordinate that defines the curve as given in GraphPad Prism (GraphPad Software, Inc., CA, USA) was used to determine the size ranges. Gates representing different size ranges were set and used to determine the size distribution. A diluted sample was analysed with a FSC histogram with gates, representing different size ranges, to determine the percentage size distribution. The span of each formulation was calculated by the following equation:

$$\text{Span}\,(\mu\text{m}) = \frac{S_{95\%} - S_{5\%}}{S_{50\%}}$$

where $S_{95\%}$ represents the size where 95% of particles is smaller, $S_{5\%}$ is the size where 5% of particles is smaller and $S_{50\%}$ the median.

2.4. Morphological analysis

The morphology of the particles was assessed by confocal laser scanning microscopy (CLSM). Micrographs were taken with a Nikon D-Eclipse C1 confocal laser scanning microscope equipped with a DXM 1200 digital camera with real time imaging (Nikon, The Netherlands). A 60 × 1.40 ApoPlanar oil immersion objective and a medium pinhole were used. The microscope was endowed with a green krypton laser (488/515 nm) and a red helium neon laser (505/564 nm). Nile red (1 mg/ml) was added to the formulation and incubated for 15 min in the dark. The samples were placed on microscope slides, covered with a glass coverslip and analysed (Du Plessis et al., 2010; Saunders et al., 1999).

2.5. Determination of entrapment efficacy

Entrapment of MQ in Pheroid™ vesicles and liposomes were analysed by ultraviolet spectrophotometry (Shimadzu, Kyota, Japan) with a 1 cm quartz cell. For the separation of the unentrapped from the entrapped, a Sephadex® G50 was used as described by Fry et al. (1978). In short, Sephadex® in PBS was left for 24 h to swell. A 2 ml syringe plugged with a filter was filled with Sephadex® and placed in a centrifuge tube. The column was centrifuged at 2000 rpm for 5 min whereafter 200 μl sample was carefully added to the top of the column. After 5 min, PBS was added to the top of the column and centrifuged, expelling the liposomes and Pheroid™ vesicles into the tube (Fry et al., 1978; New, 1990). The column was placed in a new tube and methanol was added to the column to remove any MQ. After centrifuging the sample was filtered, diluted and analysed.

Samples were compared to a standard curve of MQ. Standard solutions with known concentrations of MQ in methanol were prepared and absorbance was measured at 283 nm. The calibration curve was determined by plotting the peak absorbance against concentrations and fitted with linear regression (Rao and Murthy, 2002). The percentage entrapment of MQ was determined as described by Maestrelli et al. (2005) using the following equation:

$$\%\ \text{Entrapment efficacy}\,(\%\,\text{EE}) = \frac{\text{initial drug load} - \text{unentrapped drug}}{\text{initial drug load}} \times 100$$

A comparative study to determine the entrapment efficacy of the Pheroid™ vesicles in relation to when the drug was added to the emulsion was evaluated. MQ, in the same concentration (0.5% m/v)

Table 1
Size and pH of Pheroid™ and liposomes at different temperature over three months. Results are presented as mean ± SEM ($n = 2$) in μm and p-values was determine by Tukey's posthoc test.

	Size (μm)				pH			
	Initial	Month 1	Month 2	Month 3	Initial	Month 1	Month 2	Month 3
P 5 °C	3.07 ± 0.01	2.68 ± 0.03 $p = 0.630$	3.04 ± 0.06 $p = 1.000$	3.11 ± 0.05 $p = 1.000$	6.54 ± 0.01	6.21 ± 0.47 $p = 0.980$	6.34 ± 0.45 $p = 0.999$	6.18 ± 0.41 $p = 0.970$
P 25 °C	3.07 ± 0.01	2.70 ± 0.02 $p = 0.985$	3.54 ± 0.55 $p = 0.952$	3.00 ± 0.03 $p = 1.000$	7.93 ± 0.01	7.09 ± 0.08 $p = 0.016$	5.70 ± 0.12 $p = 0.001$	4.98 ± 0.12 $p = 0.001$
P 30 °C	3.07 ± 0.01	2.74 ± 0.01 $p = 0.002$	2.94 ± 0.02 $p = 0.225$	3.02 ± 0.07 $p = 0.946$	6.65 ± 0.02	6.14 ± 0.01 $p = 0.001$	4.89 ± 0.03 $p = 0.001$	3.47 ± 0.03 $p = 0.001$
L 5 °C	6.46 ± 0.01	10.10 ± 0.46 $p = 0.807$	12.34 ± 3.93 $p = 0.597$	13.46 ± 3.96 $p = 0.433$	7.40 ± 0.01	7.23 ± 0.09 $p = 0.785$	7.20 ± 0.09 $p = 0.656$	7.16 ± 0.12 $p = 0.483$
L 25 °C	6.46 ± 0.01	10.76 ± 0.51 $p = 0.194$	7.86 ± 0.45 $p = 0.957$	10.27 ± 2.07 $p = 0.282$	7.36 ± 0.03	6.37 ± 0.05 $p = 0.025$	5.60 ± 0.10 $p = 0.001$	5.04 ± 0.39 $p = 0.001$
L 30 °C	6.46 ± 0.01	8.61 ± 1.03 $p = 0.830$	10.52 ± 0.99 $p = 0.293$	13.86 ± 2.50 $p = 0.027$	7.29 ± 0.02	6.00 ± 0.01 $p = 0.001$	5.15 ± 0.02 $p = 0.001$	5.07 ± 0.02 $p = 0.001$

P: Pheroid™ vesicles; L: liposomes.

as the loaded vesicles, was added to the Pheroid™ vesicles. Entrapment efficacy and size distribution was analysed on a daily basis for 14 days.

2.6. Stability testing

Three month stability testing of the lipid emulsions was conducted as described by the ICH (International Conference on Harmonisation) (ICH, 2006). Storage conditions were 5 °C ± 3 °C (further referred to as only as 5 °C) and for accelerated stability 25 °C ± 2 °C/60% RH ± 5% RH (RH: relative humidity) (referred to as 25 °C) and 30 °C ± 2 °C/65% RH ± 5% RH (referred to as 30 °C). Analysis included pH, size, entrapment efficacy and morphology.

2.7. Statistical analysis

The statistical difference between the initial values and those determined over time were analysed by one-way ANOVA using Statistica 9 (StatSoft, San Diegao, California, USA). Tukey's post hoc test was performed to determine the significant differences in the data. Values obtained during the three month stability were compared to the initial values. A p-value less than 0.05 was termed significant and less than 0.005 highly significant.

3. Results

3.1. Size determination

Size determination of lipid drug delivery systems are usually conducted with dynamic light scattering (Sintov and Botner, 2006; Vicentini et al., 2010) but large sample volumes are needed and is based on the equivalent sphere principle (Gaumet et al., 2008). Flow cytometry can measure and analyse physical characterizations, like size of a single particle as it passes through a beam of light (BD Biosciences, 2002). Flow cytometry technology is a reliable and accurate quantitative tool for evaluation of particles (Shapiro, 2003). Comparision between the abe mentioned methods resulted in FACS analysis being an accurate and easy method with small sample volumes (Slabbert et al., 2010). FSC analysis of calibration beads gave distinctive peaks. The geometric mean of each peak was plotted against the size of the beads. The graph was fitted with linear regression to give an equation of $y = 1.607x + 0.4496$ and a regression of $R^2 = 0.9978$.

3.2. Characterization of formulations

Pheroid™ vesicles showed only a small change in particle size at all three temperatures over three months as noted in Table 1. A decrease in size to around 2.7 μm is observed for 5 °C, 25 °C and 30 °C at month one increasing to 3.11 ± 0.05 μm, 3.00 ± 0.03 μm and 3.02 ± 0.07 μm respectively. This was not statistically significant. Size distribution of Pheroid™ vesicles showed a bimodal distribution (data not shown) with no significant change. Nile Red accumulated in the Pheroid™ vesicle yielding micrographs of clearly distinguishable spherical structures (Fig. 1). No structural change was noted during the stability period. Pheroid™ vesicles at 5 °C showed a stable pH value with only a slight decrease in pH. A significant decrease in pH was observed at 25 °C and 30 °C ($p = 0.001$). No correlations between any of the characteristics were evident.

Liposomes, without MQ showed a initial particle size of 6.46 ± 0.01 μm as noted in Table 1. Over the three month stability testing at 5 °C and 30 °C an increase in size is observed. A significant difference in size is seen at month 3 of the liposome formulation stored at 30 °C ($p = 0.027$). Micrographs of the lipid bilayer of the liposomes are clearly visible when stained with Nile Red (Fig. 1). No difference in structure of the liposomes was noted on the micrographs over three months, but an increase in size was observed. Liposomes kept at 5 °C during the stability testing showed a slight decrease in pH from 7.40 ± 0.01 to 7.16 ± 0.12. However, at 25 °C and 30 °C a highly significant decrease in pH is observed ($p \leq 0.01$) at all three months (Table 1). A correlation between time and pH at 25 °C and 30 °C were seen, yielding a correlation of $r = -0.9684$ and $r = -0.9396$ respectively (results not shown). The pH had a significant influence ($p < 0.0001$) on size at 30 °C with a correlation of $r = -0.8060$. No other correlations were noted.

Pheroid™ vesicles loaded with MQ at accelerated stability testing showed a slight decrease in size with a small increase at 5 °C. It is evident that the size at all the temperatures is relatively stable as seen in Fig. 2. A highly significant difference, $p = 0.001$, is observed at week 1 30 °C. The spherical structure of Pheroid™ vesicles were small and uniform. No variation was observed in structure or size on the micrographs (Fig. 1). A significant decrease in pH ($p < 0.002$) is observed during the stability testing as seen in Table 2. Entrapment efficacy was calculated by a standard curve by plotting the peak absorbance of known concentration yielding a equation of $y = 0.01493x - 0.1006$ and $r^2 = 0.9975$. Initial entrapment efficacy of standard 0.5% (m/v) MQ formulation were 58.7 ± 0.05% and illustrating an overall increase in entrapment efficacy at month three for all the temperatures (Fig. 2). A dynamic system of decrease and increase of entrapment efficacy is observed at all three temperatures but no significant differences are seen. No correlation between the different characteristics was noted.

The liposomal formulation loaded with MQ increased in the percentage particle in the large aggregate region over time. Size

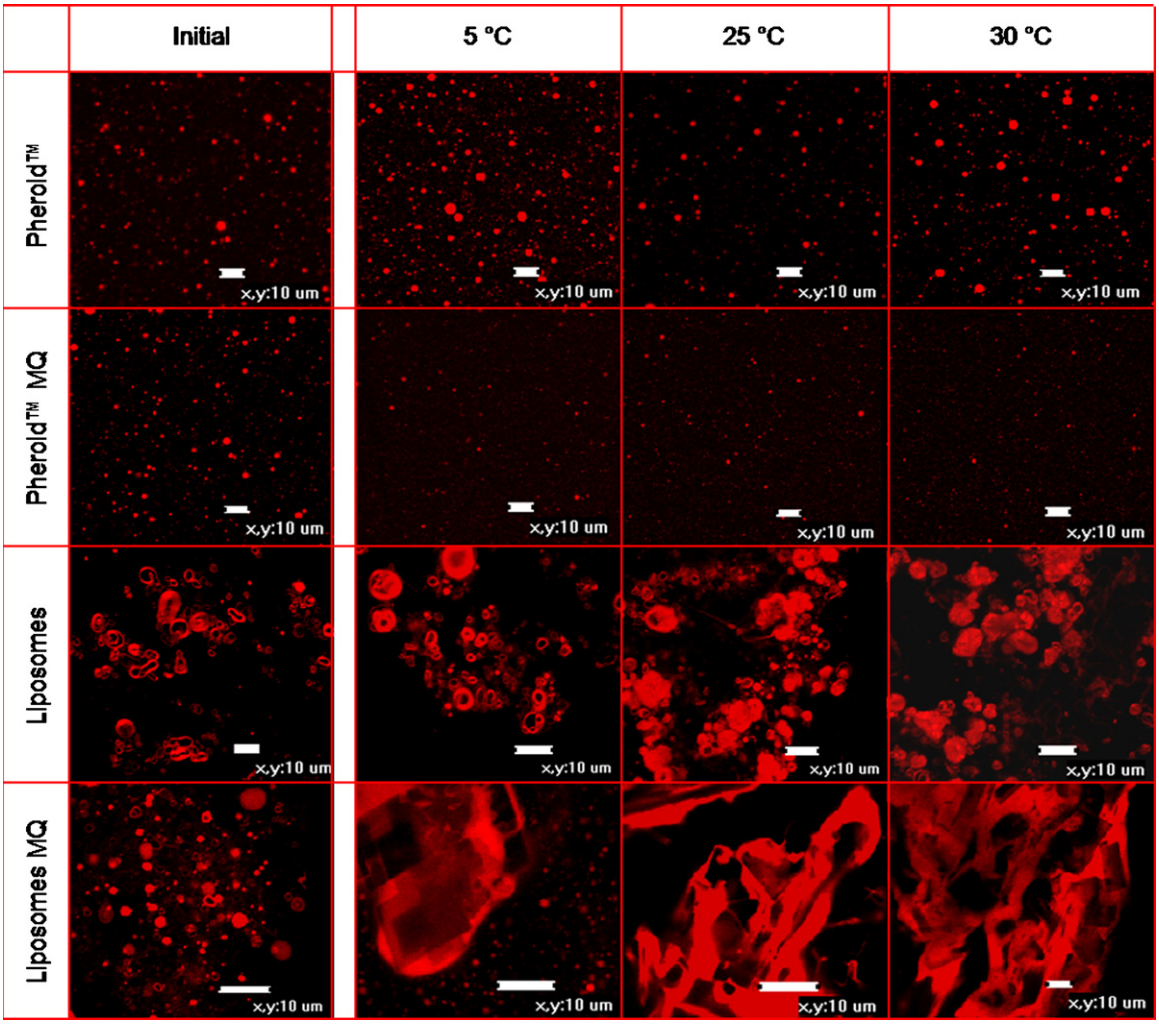

Fig. 1. Micrographs of Pheroid™ and liposomes, with and without MQ stained with Nile Red. The initial formulations are compared with micrographs at three months at the different temperatures. Spherical structures of Pheroid™ are clearly visible in red in the images. Bilayers of the liposomes without MQ are red surrounding a black aqueous volume with only a slight change in size at month three. Unilaminer liposomes loaded with MQ can be seen with the initial formulation, but only lipid structures with crystals after stability testing with small oil drops visible at 5 °C.

increased over time at all the temperatures. Initial micrographs showed distinctly formed liposomes together with oil droplets. At month three, large oil aggregates are seen with an increase in temperature, with no visible formed liposomes (Fig. 1). As seen in Table 2, liposomes kept at 5 °C showed no significant change in pH. Accelerated stability testing showed a significant decrease in pH at month two and three. Initial entrapment efficacy of 63% was measured (Fig. 2). An initial increase in unentrapped MQ is observed whereafter a drastic decrease in the amount of free MQ is seen. Correlation between pH and entrapment efficacy showed a significant difference at 25 °C and 30 °C with $p = 0.001$ and $p = 0.002$ respectively, however, a correlation of only $r = -0.7944$ and $r = -0.7520$ is observed (results not shown).

Table 2
The pH of mefloquine loaded formulation over three months at different temperatures. Results are presented as mean ± SEM ($n = 2$) and p-values was determine by Tukey's post hoc test.

	Initial	Month 1	Month 2	Month 3
P 5 °C	4.56 ± 0.01	3.50 ± 0.01	3.12 ± 0.04	3.01 ± 0.02
P 25 °C	4.36 ± 0.02	2.88 ± 0.04	2.56 ± 0.02	2.50 ± 0.01
P 30 °C	4.13 ± 0.01	2.75 ± 0.01	2.51 ± 0.01	2.57 ± 0.01
L 5 °C	7.16 ± 0.03	7.20 ± 0.02*	7.22 ± 0.02*	7.17 ± 0.01*
L 25 °C	7.03 ± 0.03	6.34 ± 0.51*	4.47 ± 0.31	4.19 ± 0.19
L 30 °C	6.98 ± 0.01	5.07 ± 0.14	4.46 ± 0.08	4.33 ± 0.13

Ph: Pheroid™ vesicle; L: liposomes.
* No significant difference, p-value > 0.200.

3.3. A 14 day entrapment efficacy study of Pheroid™ vesicle formulations

The entrapment efficacy and size of two different Pheroid™ vesicle formulations were evaluated over 14 days at 5 °C to determine the possibility to entrap a drug both during or after manufacturing. The formulations used were loaded with MQ during manufacturing (PF1) and added to Pheroid™ vesicles after manufacturing (PF2). As seen in Fig. 3, PF1 had a decrease in entrapment efficacy initially, whereafter a steady increase was seen with only a 1% difference between day 0 and 14. PF2 had a 2% increase in entrapment efficacy after 2 weeks. At day 14, the entrapment efficacy of PF1 was 58.30 ± 0.20% and PF2 60.41 ± 5.59%. No statistically significant differences were seen. The size of PF1 showed an increase to 3 μm and is relatively stable up to day 14. A highly significant difference ($p \leq 0.0012$) was noted for the difference in size for PF1 with exception to day 10 in relation to day 1. An initial increase and decrease in size is observed for PF2 and levelling out from day 10 yielding a final span of 2.75 ± 0.02 μm. A significant difference in size at day 4 and 7 yielded $p = 0.0003$ and $p = 0.0196$ respectively.

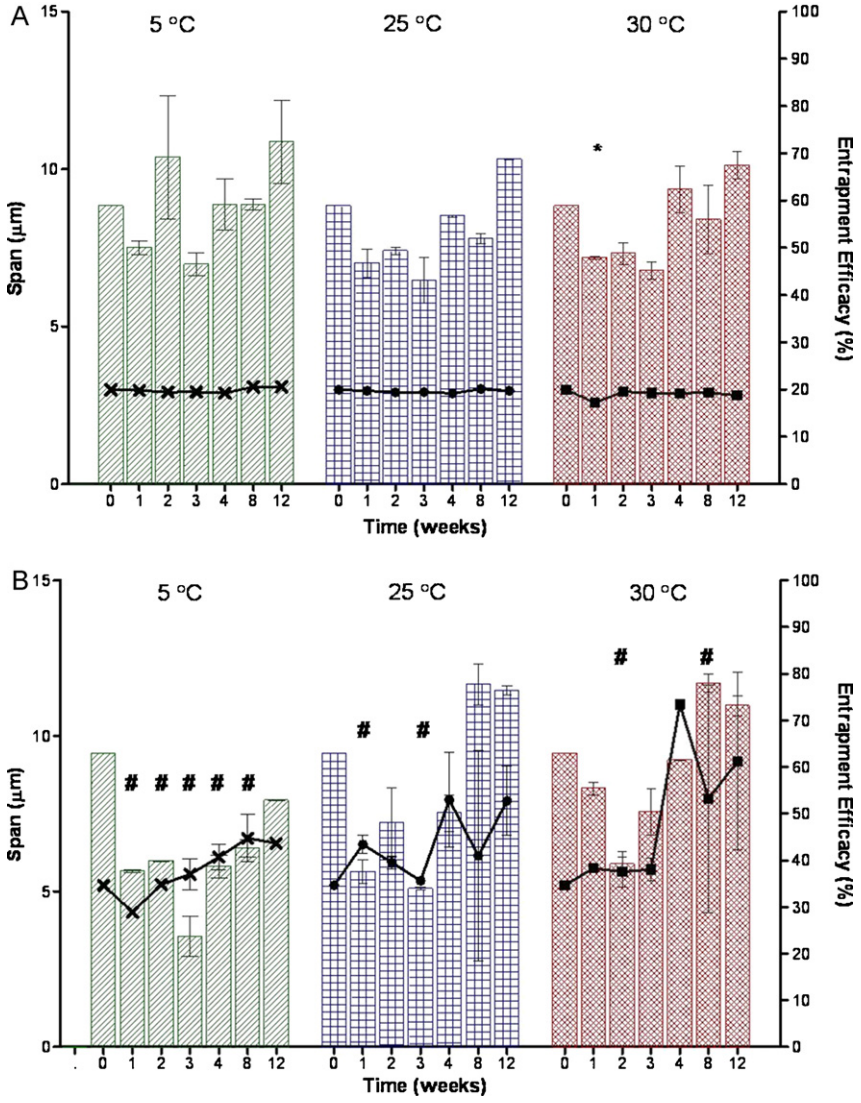

Fig. 2. Entrapment efficacy and span of Pheroid™ vesicles (A) and liposomes (B) loaded with mefloquine. Bars represent entrapment efficacy and lines span. Results are shown as mean ± SEM ($n = 2$). *Highly significant difference in size. # Highly significant difference in entrapment efficacy.

4. Discussion

The aim of the study was to determine the stability of liposomes and Pheroid™ vesicles by determination of pH, particle size and entrapment efficacy over a three month period. A limiting problem of liposomes is the physical stability including aggregation of vesicles to form larger particles (Sharma and Sharma, 1997). Results of this study showed an increase in size of liposomes at different temperatures with significant changes at 30 °C. The liposomes remained structurally stable as seen with CLSM. Liposomes loaded with MQ were initially smaller, but when stained with Nile Red, oil droplets were visible. A decrease in the amount of characteristic lipid bilayer of the liposome is seen with the inclusion of MQ in the formulation. MQ has a high affinity to bind to phospholipids (Chevli and Fitch, 1982) and can be responsible for the formation of large aggregates. Pheroid™ vesicles differed notably from liposomes. No noteworthy difference in size or structure was observed when MQ was added to the formulation. Pheroid™ vesicles stabilized to around 3 µm in diameter around day 10 with no significant difference over three months at various temperature and humidity. The slight increase and decrease of Pheroid™ vesicles could be due to the dynamic system of formation and disbanding of cesicles leading to formation of new vesicles (Grobler et al., 2007).

Liposome drug loading of either lipophilic or hydrophilic drugs can be passively entrapped during manufacturing or actively after manufacturing (Chonn and Cullis, 1995). Addition of MQ (0.5% m/v), a highly lipophilic drug (Karbwang and White, 1990), to the liposome formulation yielded an initial entrapment efficacy of 62.93% whereafter leakage of the drug out of the lipid bilayer structure is observed. A decrease in the amount of free drug as determined by UV-spectrophotometry is observed over time. This can possibly be explained by the high affinity of MQ to phosphatidyl choline (Chevli and Fitch, 1982). Pheroid™ vesicles can successfully be loaded with MQ during or after manufacturing. No significant difference in the amount of drug entrapped was seen over three months. An overall increase in the amount of entrapped drug is observed at month three. The pH of MQ loaded Pheroid™ vesicles was lower than that of normal Pheroid™ vesicles, but both showed a decrease over time. Using of a buffer solution instead of water during manufacturing lead to more favourable and stable pH values (data not shown).

Liposomes are used as model membrane system and consist of artificial components that are foreign to the body (Betageri et al., 1993; New, 1990). MQ interfered with liposome formulation, structure and stability resulting in an unstable formulation. Antimalarial drugs formulated in liposomes showed high stability for

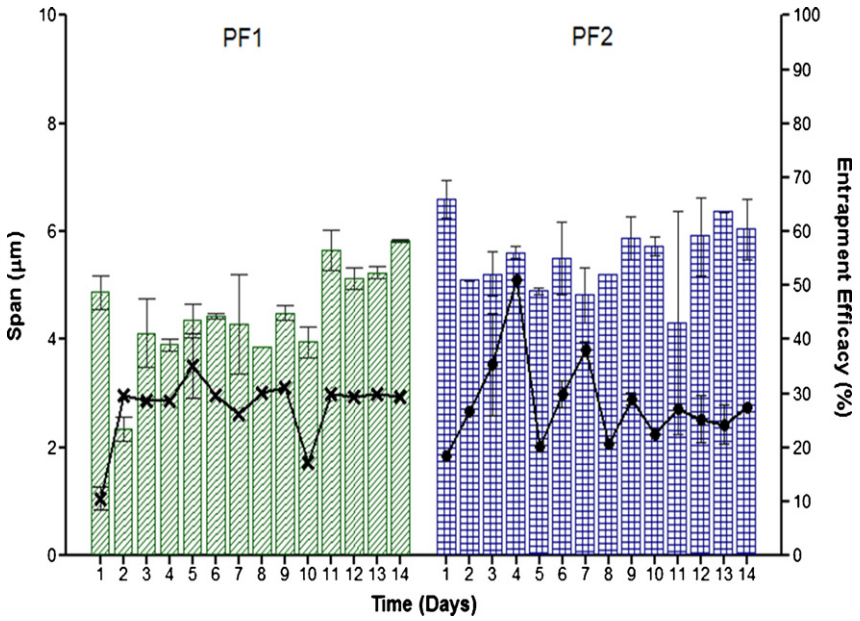

Fig. 3. Entrapment efficacy and span of Pheroid™ vesicles loaded with mefloquine. Bars represent entrapment efficacy and lines span. Results are shown as mean ± SEM ($n = 2$). PF1 - loaded with MQ during manufacturing and PF2–MQ added to Pheroid after manufacturing.

artemether (Bayoni et al., 1998), but for chloroquine, an increase in size and decrease in pH was observed over three months (Qiu et al., 2008). Pheroid™ vesicles, a lipid drug delivery system differs from liposomes. This emulsion type formulation consists of natural fatty acids (Grobler et al., 2007) and yielded stable micron size structures. The high entrapment efficacy and ability to entrap MQ during both and after manufacturing increases the versatility of the lipid drug delivery system. The pH had no notable influence on the size or entrapment efficacy of Pheroid™ vesicles.

5. Conclusion

MQ was successfully entrapped in Pheroid™ vesicles both during and after manufacturing and proved to be stable during three month stability testing. Results of size determination at the different storage conditions for both Pheroid™ vesicle formulations was around 3 μm. MQ interfered with the size and structural integrity of the liposomes with low stability compared to Pheroid™ vesicles. The liposome formulations were double the initial size of Pheroid™ vesicles increasing over time. Liposomes were unstable compared to Pheroid, but long term stability of liposomes can possibly be obtained by lyophilisation (Sharma and Sharma, 1997). Methods used to characterize the formulation during stability test showed to be accurate and sensitive. Pheroid™ vesicles were stable in size, structure and entrapment efficacy and can possibly be used to increase efficacy and bioavailability and decrease toxicity of MQ.

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