

ANNEXURES

ANNEXURE A

Annexure A contains the following poster:

Grobler, L. Grobler, A., Haynes, R. Steyn, F. & Wiesner, L. The effect of Pheroid® technology on the bioavailability of artemisone in primates. 1st Pan-African summer School in Nanomedicine, CSIR, Pretoria, 2012. (Poster).

Lizette Grobler^a, Anne Grobler^b, Richard Haynes^b, Faans Steyn^c and Lubbe Wiesner^d

^a Department of Pharmaceutics, Unit for Drug Research and Development, School of Pharmacy, North-West University, Potchefstroom, South Africa, ^b DST/NWU Predclinical Drug Development Platform, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa, ^c Statistical Consultation Service, North-West University, Potchefstroom, South Africa, ^d Division of Clinical Pharmacology, University of Cape Town, Medical School, Observatory, 7925, Cape Town, South Africa

Artemisinin-based combination treatments is recommended by the World Health Organization as first line treatment of uncomplicated malaria¹. Artemisinins have a low aqueous solubility that causes poor, inconsistent absorption upon oral administration, which in turn results in low bioavailability. Their short elimination half-life is also a limitation².

Phero[®] technology is an emulsion type formulation that can be manipulated in terms of structure, size, morphology and function in order to adapt to the chemical class and size of the drug. Mechanically, this need to be determined. It can entrap, transport and deliver pharmacologically active compounds and other molecules¹ in terms of artemisone, bioavailability studies have been performed for reference and Phero[®]-entrapped artemisone in a C57 BL/6 mouse model⁸. Significant differences were found between these two formulations with a greatly increased C_{max} (1550.0 ng/ml vs. 809.5 ng/ml) for artemisone-Phero[®] vesicle formulation compared to the artemisone reference formulation). The AUC_{0-24} and AUC_{0-12} also increased (2219.0 ng h/ml and 3094.0 ng h/ml vs. 458.7 ng h/ml and 604.6 ng h/ml with the Phero[®] vesicle formulation).

The purpose of this study was to progress the investigation into the enhancement of the pharmacokinetic profile of artemisone in the Pheroid® delivery system to a primate model.

Artemisone was prepared at the Hong Kong University of Science and Technology. Pro-Pheroid® was prepared by gassing an oil phase consisting of the active pharmaceutical ingredient (API), PEG 400, vitamin F ethyl ester, Cremaphor EL, *d*-D- α -tocopherol, BHA and BHT with N₂O

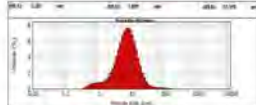


Figure 1: Particle size distribution of artemisone-entrapped artemisone incorporated into pro-Phenid®

In vivo PK

Reference (artemisonone) and test formulations (artemisonone entrapped into pro-Pheroid®) were administered to vervet monkeys ($n = 10$) as a single dose of 60 mg/kg, using capsules as dosage form. A crossover design with a one week washout period was used.

Blood samples were drawn in heparin through percutaneous venipuncture of the femoral vein pre-dose and 7 times post dose up to 10 hours. Plasma samples were prepared by centrifugation at 4 °C and immediately frozen at -196 °C and then stored at -80 °C. Parent drug concentrations and metabolite M1 in the plasma samples were determined using an LC/MS/MS assay. Calibration standards and quality control standards were prepared in blank mouse plasma. Artemisinin was used as internal standard. An Agilent 1200 series autosampler injected 2 µl onto an HPLC column with detection by API 3200 mass spectrometer.

In vitro metabolic stability

Artemisone-reference and pro-Pheoid® formulations were incubated with enzyme (0.5 mg/mL) and 0.1 M phosphate buffer (pH 7.4). The reaction was initiated by the addition of NADPH. An aliquot was taken at various time points up to 45 min. The reaction was stopped by the addition of ice cold acetonitrile. Samples were stored at 4 °C to allow protein precipitation and centrifuged at 10 000 g for 15 minutes at 4 °C. Supernatants were stored at -20 °C until analysis on a UPLC-QTOF MS/MS with a Waters Sample Manager (autosampler) and Waters Sympact HDS G1 (spectrometer).

In vivo bioavailability

Table1: Pharmacokinetic parameters of the oral artemisone reference and Pheroid® formulations

Dosage	Drug measured	AUC ₀₋₁₂ (mg h/L)	p-value	C _{max} (mg/L)	p-value	T _{max} (hours)	p-value	t _{1/2} (hours)	p-value
Artemesione		184.41 ± 134.40		47.39 ± 44.53	0.16	5.11 ± 2.52	0.14	3.17 ± 1.78	
Artemesione	Artemesione parent drug	121.83 ± 521.41	0.07	103.21 ± 2.57		3.3 ± 4.45		4.45 ± 3.47	0.61
Phloroglucinol		303.14 ± 320.23		75.73 ± 69.26	0.31	3 ± 1.25	0.5	3.52 ± 1.63	0.47
Artemesione	Metabolite M1	836.48 ± 891.44	0.45	140.76 ± 43.42		2.8 ± 1.48		3.83 ± 4.45	

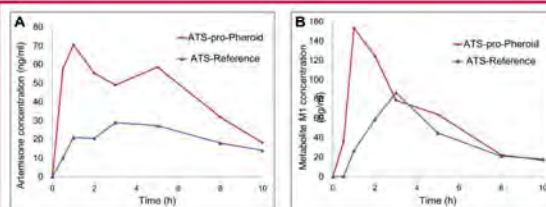


Figure 2: Mean concentration vs. time graph of the oral artemisinin reference and pro-Pheroid® formulations. A) artemisinin concentration in ng/ml and B) Metabolite M1 concentration in ng/ml.

In vitro metabolic stability

Table 2: *In vitro* intrinsic clearance (CL_{int}) of the artemisone reference and artemisone-pro-Phieroid® formulations in HLM, HIM, MLM and MIM and estimated *in vivo* hepatic clearance (CL_h) for HLM and MLM.

	In vitro CL_{int} (μ l/min/kg)				Estimated in vivo CL (ml/min/kg)	
	HLM	MLM	HIM	MIM	HLM	MLM
ATS reference	111.00	1533.33	11.00	0.00 *	18.25	34.28
ATS-pro-Pheindol®	0.06	0.00 *	0.00 *	0.00 *	0.08	0.00

* No microsomal metabolism occurred.

After log-transformation of the pharmacokinetic parameters C_{max} , AUC and V_d (and a Box-Cox transformation of $t_{1/2}$) the two formulations did not differ significantly ($p>0.05$).

A: The mean artemisone C_{max} , AUC and $t_{1/2}$ are higher with the test formulation compared with the reference formulation and the T_{max} and V_d (6.24 ± 6.65 vs. 13.22 ± 20.81 litre, $p = 0.17$) were lower.

B: The mean M1 C_{max} , AUC and $t_{1/2}$ were higher with the test formulation and the T_{max} were similar for both formulations.

The artemisone plasma levels are much lower than expected for the dosages administered. Previous studies established that a lower bioavailability for certain drugs occurs in monkeys, compared to humans, due to incomplete gastrointestinal absorption, greater first-pass metabolism, or a combination of these factors⁵.

The *in vitro* metabolism studies made it clear that very slow metabolism of the artemisone reference occurred in HIM with no metabolism in the MIM. The pro-Pheroid® formulation inhibited artemisone catabolism in all microsome preparations. The *in vitro* intrinsic clearance of the MLM is much higher than that of HLM. If extrapolated to the estimated hepatic clearance *in vivo*, the monkey CL_H is almost twice the CL_H in humans.

Also, if azamulin, a CYP3A4 inhibitor, is added to the reaction mixture, metabolism of artemisone is inhibited in the human liver microsomes (% drug remaining = 99.1%) but not in the monkey liver microsomes (% activity remaining = 2.81%). Therefore, the monkey CYP3A4 in the monkey might not be the enzyme responsible for the catabolism of artemisone or alternatively the monkey CYP3A4 is not inhibited by azamulin.

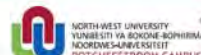
Entrapment of artemisone in the Pheroid® delivery system improves the pharmacokinetic profile of artemisone, although statistical significance could not be shown as a result of the low plasma levels in the limited number of animals used. The vervet monkey model may not be the most suitable model for evaluation of artemisone bioavailability.

The *in vitro* results suggest that artemisone is protected by the Pheroid® delivery system against microsomal metabolism.

[illegible]

We wish to acknowledge

2. The participation of the Swiss Tropical and Public Health Institute for their advice and support.



ANNEXURE B

Annexure B contains the following poster:

Grobler, L. Grobler, A., Haynes, R. & Steyn, F. The effect of Pheroid® technology on the bioavailability of artemisone in primates. 33rd Annual Conference of the Academy of Pharmaceutical Sciences of South Africa, Rhodes University, Grahamstown, 2012. (Poster).

Lizette Grobler ^a, Anne Grobler ^b, Richard Haynes ^b, Faans Steyn ^c

* Department of Pharmaceutics, Unit for Drug Research and Development, School of Pharmacy, North-West University, Potchefstroom, South Africa, ^b DST/NWU Preclinical Drug Development Platform, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa, ^c Statistical Consultation Service, North-West University, Potchefstroom, South Africa



Results

The new artemisinin derivative, artemisone, shows enhanced antimalarial activity against chloroquine-sensitive and -resistant *P. falciparum* cell lines *in vitro* and against chloroquine sensitive *P. berghei* and chloroquine resistant *Pyopli* rodent models and in *Aotus* monkey-*P. falciparum* models *in vivo* when compared to the most widely used artemisinin derivative, artesunate³.

Pheroi® technology is an emulsion type formulation that can be manipulated in terms of structure, size, morphology and function depending on the type and size of the drug molecules that needs to be delivered. It can entrap, transport and deliver pharmacologically active compounds and other molecules⁴. In terms of artemisinin, bioavailability studies have been performed for reference and Pheroi®-entrapped artemisinin in a C57 BL/6 mouse model⁵. Significant differences were found between these two formulations with a greatly increased C_{max} of 1550.0 ng/ml for artemisinin-Pheroi® vesicle formulation compared to a C_{max} of 809.5 ng/ml for the artemisinin reference formulation. The AUC_{0-24h} and AUC_{0-∞} also increased from 458.7 ng h/ml and 604.6 ng h/ml for the artemisinin reference formulation and 2219.0 ng h/ml and 3094.0 ng h/ml for the artemisinin-Pheroi® vesicle formulation.

The purpose of this research was to investigate the enhancement of the pharmacokinetic profile of artemisone when incorporated in the Pheroid® delivery system by using a primate model.

Artemisones were prepared at the Hong Kong University of Science and Technology. Pro-Pheroid® were prepared by gassing an oil phase consisting of the active pharmaceutical ingredient (API), PEG 400, vitamin F ethyl ester, Cremaphor EL, d-D-α-tocopherol, BHA and BHT with N₂O under high pressure.

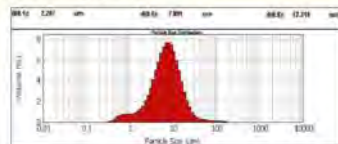


Figure 1: Particle size distribution of artemisone-entrapped artemisone incorporated into pro-Pheroid®

Reference (artemisono) and test formulations (artemisono incorporated into pro-Pheroid®) were administered to vervet monkeys ($n = 10$) as a single dose of 60 mg/kg, using capsules as dosage form. A crossover model was used with a one week washout period in-between.

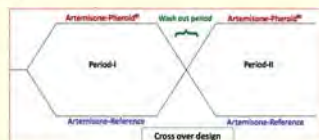


Figure 2: Primate study design

Blood samples were drawn in heparin through percutaneous venipuncture of the femoral vein at pre-dose and 0.5, 1, 2, 3, 5, 8 and 10 hr post dose time intervals. Plasma samples were prepared by centrifugation (4 °C) and immediately frozen at -196 °C and then stored at -80 °C. Parent drug concentrations and metabolite M1 in the plasma samples were determined using an LC/MS/MS method.

Calibrations standards and quality control standards were prepared in blank primate plasma. Artemisinin was used as the internal standard. An Agilent 1200 series autosampler injected 2 μ l onto HPLC column with detection by API 3200 mass spectrometer.

Table 1: Pharmacokinetic parameters of the oral artemisinin reference and Pheroid® formulations

Dosage	Drug measured	AUC ₀₋₂₄ (ng·h/ml)	C _{max} (ng/ml)	T _{max} (hours)	t _{1/2} (hours)
Artemisone	Artemisone parent drug	184.41 ± 134.40	47.39 ± 44.53	5.11 ± 2.52	3.17 ± 1.68
Artemesone-Phenol ^d		421.83 ± 521.42	103.21 ± 51.57	3.3 ± 2.44	4.45 ± 3.37
Artemisone	Metabolite M1	203.14 ± 320.23	75.73 ± 49.32	3 ± 2.45	3.52 ± 1.03
Artemesone-Phenol ^d		835.48 ± 891.44	140.78 ± 83.46	2.8 ± 1.48	6.35 ± 4.45

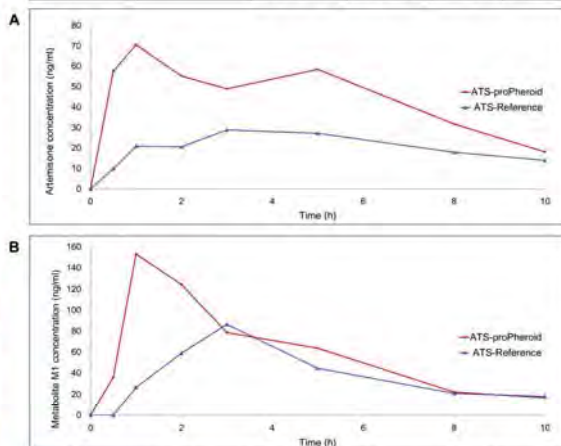


Figure 3: Mean concentration vs. time graph of the oral artemisone reference and pro-Pheroid® formulations
A) artemisone concentration in ng/ml and B) Metabolite M1 concentration in ng/ml

After log-transformation of the pharmacokinetic parameters C_{max} , AUC and V_d (and a Box-Cox transformation of $t_{1/2}$) the two formulations did not differ significantly ($p>0.05$).

After log-transformation of the pharmacokinetic parameters C_{max} , AUC and V_d (and a Box-Cox transformation of $t_{1/2}$) the two formulations did not differ significantly ($p>0.05$).

A: The mean artemisinin C_{max} (103.21 $\pm$ 151.57 vs. 47.39 $\pm$ 44.53 ng/ml, $p = 0.16$), AUC (421.83 $\pm$ 521.42 vs 184.41 $\pm$ 134.40 ng h/ml, $p = 0.07$) and $t_{1/2}$ (4.45 $\pm$ 3.47 vs. 3.17 $\pm$ 1.68 hours, $p = 0.61$) seems to be higher with the test formulation compared with the reference formulation and the T_{max} (3.3 $\pm$ 2.44 vs 5.11 $\pm$ 2.52 hours, $p = 0.14$) and V_d (6.24 $\pm$ 6.65 vs. 13.22 $\pm$ 20.81 litre, $p = 0.17$) were lower.

B: The mean M1 C_{max} (140.78 ± 243.42 vs. 75.73 ± 80.26 ng/ml, $p = 0.31$), AUC (635.48 ± 891.44 vs. 303.14 ± 320.23 ng h/ml, $p = 0.45$) and $t_{1/2}$ (6.35 ± 4.45 vs. 3.52 ± 1.03 hours, $p = 0.47$) seems to be higher with the test formulation and the T_{max} (2.8 ± 1.48 vs. 3.1 ± 1.25 hours, $p = 0.50$) were similar for both formulations.

The artemisone plasma levels are much lower than expected for the dosages administered. Previous studies established that a lower bioavailability for certain drugs occurs in monkeys, compared to humans, due to incomplete gastrointestinal absorption, greater first-pass metabolism, or a combination of these factors⁵.

Entrapment of artemisone in the Pheroid® delivery system improves the pharmacokinetic profile of artemisone, although statistical significance could not be shown as a result of the low plasma levels in the limited number of animals used. The vervet monkey model may not be the most suitable model for evaluation of artemisone bioavailability.

[illegible]

ANNEXURE C

Annexure C contains the following poster:

Grobler, A.F, Steyn , J.D., Wiesner, L., Haynes, R., Meeding, L., Gibhard, L. & Kotze, A. Comparative pharmacokinetic analysis of novel antimalarial artemisinin derivatives after oral administration using Pheroid™ technology. 3rd Pharmaceutical Sciences Fair and Exhibition, Prague, Czech Republic. 2011(Poster).

Comparative pharmacokinetic analysis of novel antimalarial artemisinin derivatives after oral administration using Pheroid™ technology

Grobler, Anne ^a; Steyn, Dewald ^a; Meeding, Lizette ^a; Gibbard, Liezl ^a; Scholtz, L-M ^a; Wiesner, Lubbe ^b; Haynes, Richard ^{c, a}

^a North-West University, Pharmaceuticals, Potchefstroom, South Africa; ^b University of Cape Town, Clinical pharmacology, Cape Town, South Africa; ^c The Hong Kong University of Science and Technology, Department of Chemistry, Kowloon, Hong Kong

Introduction

Artemisinin have a short half life, which attributes to high recrudescence rates, and therefore limits their effectiveness¹. The new artemisinin derivative, artesunate, shows enhanced antimalarial activity against chloroquine-sensitive and -resistant *P. falciparum* cell lines *in vivo* and against chloroquine sensitive *P. berghei* and chloroquine resistant *P. yoelii* rodent models and in *Anura monoy-R. falciparum* models *in vivo*² when compared to the most widely used artemisinin derivative, artesunate.³ In Phase II trials no neurotoxicity was observed, it was well tolerated in humans and had a curative effect at doses levels of approximately half those of artesunate⁴.

Chloroquine forms part of the quinolines. During the 1930's the first quinoline anti-malarial drugs were alkaloids extracted from the cinchona tree. The biggest discovery was in 1940 when the synthetic 4-aminoquinoline chloroquine was developed. Drug resistance in malaria is a vital public health concern. Resistance of *P. falciparum* to chloroquine was first observed in 1957 and during the 1980's it spread across Africa⁵. The rapid spread of resistance to antimalarial drugs over the past few decades has led to focus on research and development of novel anti-malarial compounds, and to improve the existing drug regimens so that it can be used more effectively. The latter can be achieved by reformulation of the existing compounds into more effective dosage forms by incorporation of Pheroid™ technology which is a patented drug delivery system that has the ability to protect, transport and deliver pharmacologically active compounds. Pharmaceutical models were constructed for artesunate and chloroquine in Pheroid™ vesicle formulations, respectively.

Aim

To evaluate the effect of Pheroid™ technology on bioavailability in rodent versus non-human primate models.

Methods

Materials

Artesunate was a generous gift by the Hong Kong University of Science and Technology. Chloroquine was obtained from BD Fine Chemicals (South Africa). Pheroid™ delivery system formulations was supplied by L-M Scholtz of the North-West University.

Antimalarial drug classes

Artesunate, a 10-alkylaminoartemisinin, forms part of a class of antimalarials called the artemisinins. The quinoline anti-malarial drugs consist of quinines, 8-Aminoquinolines, 4-Aminoquinolines and Quinolines. Chloroquine forms part of the 4-Aminoquinolines.

Reference formulations

The required amount of artesunate was dissolved in analytical grade DMSO & sterile water was added to a final ratio of 1:9 v/v and allowed to sonicate for 15 minutes. The required dosage of chloroquine was dissolved in sterile water.

Pheroid™ formulations

The oil-phase of the Pheroid™ vesicle formulations were prepared by heating and mixing vitamin E ethyl ester (50.2g), Compritol® EL (27.6 g) and D-α-tocopherol (1.0 g). PEG 400 was then added (5.0 g) together with BHA (0.2 g) and mixed thoroughly. Purified water was saturated water with H₂O under high pressure. The required amount of artesunate was added to 1.0 ml of the prepared oil-phase and mixed by vortexing and placed in an ultrasonic bath for 15 minutes. Nitrous oxide saturated water was then added to the oil-phase to achieve an oil to water ratio of 1:5 v/v. The mixture was homogenised with a Heidolph Claw 600 homogeniser at 8000 rpm for 1 minute. For the chloroquine-Pheroid™ formulation, chloroquine was added after manufacturing of the Pheroid™ vesicle with overnight mixing to ensure complete entrapment of chloroquine.

Experimental approach

Reference and test formulations were administered by oral gavage to both the rodent and non-human primate groups. Venous samples were used as non-human primates. Blood was collected at the time intervals indicated in Table 1. Plasma was prepared by centrifugation (4 °C), immediately frozen at -100 °C and then stored at -80 °C.

Instrumentation

Calibration standards and quality control standards were prepared in rodent & primate plasma at full blood and stored at -80 °C.

Artemisinin: Artesunate was the internal standard. A Phenomenex Gemini-NX (50 x 2.0 mm, 5 µm) analytical column and mobile phase of methanol and ammonium acetate (90:10, v/v) was used. Flow rate was 0.5 ml/min for 3 minutes.

Chloroquine: Anodiquine was the internal standard. A Phenomenex Luna, PFP (50 x 2.0 mm, 5 µm) analytical column and mobile phase of acetonitrile and 0.1% formic acid (90:10, v/v) was used at a constant flow rate of 0.5 ml/min for 2 minutes.

Agilent 1100 series autosampler injected 2 µl. HPLC column with detection by API 3000 mass spectrometer.

Table 1: Summary of study design

Antimalarial	Species	Sample	Dosage	Time intervals for collection of blood
Artemisinin	Human (CSP full dose)	Plasma	1 mg/kg; 3 mg/kg & 10 mg/kg	0, 0.083, 0.25, 0.5 & 1 hours
	Human (CSP full dose)	Plasma	50 mg/kg	0, 0.083, 0.25, 0.5, 1 & 2 hours
	Primate (Chloroquine analogue)	Plasma	4 mg/kg & 20 mg/kg	0, 0.15, 0.5, 0.8, 0.8, 1.2, 1.5, 2, 2.5, 3, 3.5, 4, 8 & 12 hours
Chloroquine	Human (CSP full dose)	Whole blood	20 mg/kg	0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 & 24 hours
	Primate (Chloroquine analogue)	Whole blood	20 mg/kg	0, 0.15, 0.5, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 14, 16, 18, 20, 24, 36 & 48 hours



Figure 1: Primate study design

Results

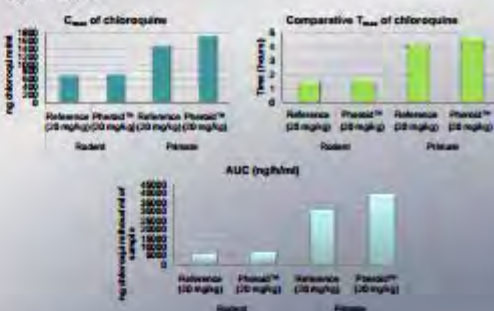
(a) Artesunate

Bioavailability was determined and a number of the parameters were investigated. The main parameters investigated during the artesunate study is reflected in Table 2.

Table 2: Bioavailability parameters for artesunate				
Artesunate		C _{max} (ng/ml)	T _{max} (hour)	AUC (ng/h/ml)
Rodent	Reference (1 mg/kg)	676.4	0.083	184.84
	Pheroid™ (1 mg/kg)	379.4	0.150	184.35
	Reference (3 mg/kg)	818.8	0.150	167
	Pheroid™ (3 mg/kg)	754.2	0.115	307
	Reference (10 mg/kg)	1510	0.183	1007.53
	Pheroid™ (10 mg/kg)	1387	0.173	665.66
Primate	Reference (50 mg/kg)	884.5	0.145	604.9
	Pheroid™ (50 mg/kg)	1580	0.33	3204
	Reference (8 mg/kg)	8.86	1.78	3.81
	Pheroid™ (8 mg/kg)	5.38	1.78	5.81

^aResults shown as average.

(b) Chloroquine



Conclusion

(a) Artesunate

In rodents treated with 50mg/kg Pheroid™ formulation, a therapeutic improvement in the C_{max}, T_{max} and delay in T_{max} was observed. The relative bioavailability of artesunate was RA = 1.2 (reference) and RA = 4.57 (Pheroid™) (p < 0.0001). The absolute bioavailability was calculated as F = 0.10 (reference) and F = 0.48 (Pheroid™) (p < 0.0001).

In primates, the artesunate plasma levels are much lower than expected for the dosage administered. In contrast to the rodents, Pheroid™
 - Disposition of these parameters shown above: C_{max}, T_{max} and AUC. However, levels were as low as to not always be detectable and the study needs to be repeated with a higher dosage;
 - The bioavailability parameters investigated did not correlate to the predicted levels according to dosage. Previous studies established that a lower absolute bioavailability for certain drugs occurs in monkeys, compared to humans. Incomplete gastrointestinal absorption, greater first-pass metabolism, or a combination of the two factors may attribute to the findings. Cytochrome P450 may also play a role.

(b) Chloroquine

The entrapment of chloroquine in the Pheroid™ drug delivery system resulted in:

- As 12.2% increase in the AUC in the rodents and 2.1% in the non-human primates;
- As increased C_{max} with 5% in the rodents and 17 % in the non-human primates when compared to the Reference formulation;
- No statistical significant difference in T_{max} between the Pheroid™ formulation and the Reference formulation was observed.

Pheroid™ technology impacts differently on the bioavailability of each of the two pharmaceutical classes of antimalarials investigated.

References

1. WHO/UNAIDS, C.T. 2010. *World malaria situation, 2010*. Geneva: World Health Organization.
2. WHO/UNAIDS, C.T. 2010. *World malaria situation, 2010*. Geneva: World Health Organization.
3. WHO/UNAIDS, C.T. 2010. *World malaria situation, 2010*. Geneva: World Health Organization.
4. WHO/UNAIDS, C.T. 2010. *World malaria situation, 2010*. Geneva: World Health Organization.
5. WHO/UNAIDS, C.T. 2010. *World malaria situation, 2010*. Geneva: World Health Organization.



ANNEXURE D

Annexure D contains the Ethics application for cultivation of *Plasmodium falciparum* parasites in human blood (NWU-0008-08-S5) and for evaluation of bioavailability and efficacy of Pheroid[®]-entrapped anti-infective agents in non-human primates (NWU-00027-10-A5)



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Research Ethics Committee
North-West University
Potchefstroom Campus
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15 April 2008

Dear Ms Botha

FINAL RESPONSE: ETHICS APPLICATION: NWU-0008-08-S5

The abovementioned application has reference.

We have received satisfactory answers to all the questions posed by the Ethics panel and has therefore found the ethical aspects to be in order.

**PROF. J. DU PLESSIS
DIRECTOR**



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ETHICS APPROVAL OF PROJECT

2010-07-15

This is to certify that the next project was approved by the NWU Ethics Committee.

Project title: *Investigation into the comparative bioavailability and efficacy of Pheroid™-entrapped anti-inteactive agents in non-human primates*

Project leader: Dr. AF Grobler

Ethics number: **NWU-00027-10-A5**

Expiry date: 2015/07/11

The Ethics Committee would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the Ethics Committee for any further enquiries or requests for assistance.

The formal Ethics approval certificate will be sent to you as soon as possible.

Yours sincerely

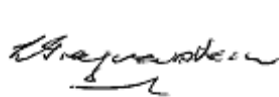
Me. Marietjie Halgryn
NWU Ethics Secretariate

ANNEXURE E

Annexure E contains the proof of language editing.

ENGLISH LANGUAGE EDITING CERTIFICATION

This is to certify that the English language editing of this thesis by Ms L Grobler was done by Prof. L.A. Greyvenstein.



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