Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

In vitro properties of surface-modified solid lipid microspheres containing an antimalarial drug: halofantrine

Anthony A Attama*, Collins N Igbonekwu

Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu State, Nigeria

ARTICLE INFO

Article history:
Received 10 September 2010
Received in revised form 27 December 2010
Accepted 15 January 2011
Available online 20 April 2011

Keywords:
Solid lipid microsphere
Particle size stability
Halofantrine
Encapsulation efficiency
Release studies
In vitro evaluation

ABSTRACT

Objective: To formulate and evaluate *in vitro*, surface-modified solid lipid microspheres containing halofantrine using lipid matrix formed from goat fat and a phospholipid (P90H). **Methods:** The model drug, halofantrine in an increasing concentration of 1%, 2%, 3%, 4% and 5% w/w was incorporated into surface-modified solid lipid microspheres formulated by hot homogenization. Effect of drug concentration on the encapsulation efficiency was studied. The dispersion was evaluated using particle size, particle morphology, pH and encapsulation efficiency. The drug formulation with highest encapsulation efficiency was selected and used for the release studies and compared with the release from a commercial dosage form (Halfan® 250 mg tablet, Glaxo-Smithkline, Mayenne France) using simulated gastric fluid (SGF pH 1.2), simulated intestinal fluid (SIF pH 7.2) and phosphate buffer (pH 6.8) as biorelevant media. Results were analyzed statistically and the level of significance was taken to be $P<0.05$). **Results:** Discrete and spherical solid lipid microspheres were produced. The particle size of the dispersion was low (32.48-33.87 μm) with minimal particle growth and high encapsulation efficiencies (86.8%-91.0%) after 3 months. The pH of the microspheres dispersion changed appreciably after 3 months. *In vitro* release result obtained revealed sustained and controlled drug release from the lipid microspheres compared with the tablet dosage form. **Conclusions:** Formulation of halofantrine as solid lipid microspheres presents a better alternative to the conventional tablet formulation as the *in vitro* dissolution of the highly lipophilic halofantrine was highly improved.

1. Introduction

Pharmaceutical dosage forms are required to meet stringent standard of drug content uniformity and quality. This situation is to ensure that product consistently delivers the intended dose, thereby achieving the desired efficiency. However, formulation development is one of the most challenging aspects of pharmaceutical and biopharmaceutical innovations. It involves navigating the complexities of pharmaceutical sciences and manufacturing, while achieving regulatory compliance. The current methods of drug delivery aim to deploy medication intact to specifically targeted part of the body through a medium that can control the therapy administered by means of either a physiological or chemical trigger. To achieve these, drug delivery scientists are turning to the world of micro- and nanotechnology as particulate systems of drug delivery.

Lipid based drug delivery system such as liposomes, microemulsions, nanoemulsions, self emulsifying systems, solid lipid micro- and nanoparticle are becoming more popular because lipid materials are easily characterized and can be developed for several administration routes. The reasons for increasing interest in lipid based system include: reduced plasma profile variability, better characterization of lipidic recipient, formulation versatility, choice of different drug delivery systems and improved ability to address the key issue of technology transfer and manufacture scale up[1].

The bioavailability of orally administered lipophilic drug is low. There are usually several factors for this reason, but a particular widespread problem is poor absorption due to slow or incomplete dissolution in gastrointestinal tract. Formulation strategies to enhance solubility will ultimately increase bioavailability. Solid lipid based drug

*Corresponding author: Anthony A Attama, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.
Tel: +234(0)8036618204
E-mail: aaattama@yahoo.com
Foundation project: Funded by the International Foundation for Science (IFS), Sweden (IFS No. F/4467-1).

delivery systems have been shown to enhance dissolution and bioavailability of lipophilic drugs[2]. Halofantrine is phenanthrene methanol derivative antimalarial agent active against asexual form of *Plasmodium falciparum*[3]. It is practically insoluble in water but freely soluble in methanol and sparingly soluble in ethanol[4]. The aim of the present study was to formulate and evaluate *in vitro* surface-modified solid lipid microspheres drug delivery system of halofantrine. This novel method of formulation of this drug has not been reported previously. However, it is believed that bioavailability of halofantrine is increase in the presence of fats. It was worthwhile to formulate lipid microspheres containing halofantrine using biocompatible lipids and assess the properties of such dosage form *in vitro*. In different studies, halofantrine was formulated as lipid emulsion or microemulsion concentrate[5-8]. Phospholipid employed in this study has several advantages in drug delivery. Advantages of phospholipids formulations not only comprise enhanced bioavailability of drugs with low aqueous solubility or low membrane penetration potential, but also improvement or alteration of uptake and release of drugs, protection of sensitive active agents from degradation in the gastrointestinal tract, reduction of gastrointestinal side effects of non-steroidal anti-inflammatory drugs and even masking of bitter taste of orally administered drugs[2].

2. Materials and methods

2.1. Materials

These following materials were used as procured from their supplier's without further purification: methanol (M&B Dagenham, England), activated charcoal, sorbitol, polysorbate 80 (Merck, Darmstadt Germany), sodium hydroxide (BDH, Poole England), monobasic potassium phosphate, hydrochloric acid (Sigma, USA), disodium hydrogen phosphate, sodium dihydrogen phosphate (Sigma-Aldrich, Germany), Halfan® 250 mg tablets (Glaxo-Smithkline, France), thiomersal (West Yorkshire, England), Phospholipon 90H (Natterman, Köln, Germany). Goat fat was obtained from a batch processed in our laboratory.

2.2. Extraction of goat fat and physicochemical characterization

Goat fat was extracted from the adipose tissue of *Capra hircus* by wet rendering achieved by subjecting the adipose tissue to moist heat by boiling with half its weight of water for 45 min. The molten fat was separated from aqueous phase after solidification. The extracted lipid was deodorized and decolorized to a white mass as earlier reported[9] and the following physicochemical properties determined using standard procedure[10]: melting point, refractive index, saponification value, unsaponifiable matter, acetyl value, iodine value and peroxide value.

2.3. Preparation of bulk lipid matrix

The bulk lipid matrix consisting of 30% w/w phospholipid in goat fat was prepared by fusion.

2.4. Preparation of unloaded and drug-loaded solid lipid microspheres

The unloaded and drug-loaded solid lipid microspheres were formulated by melt-homogenization technique[11] using Ultra-Turrax (T25 Basic, Ika Staufen, Germany) at 5 000 rpm for 10 min. In the case of drug containing microspheres, the drug was first mixed with the molten lipid matrix as it is stable in this lipid at that temperature. The quantities used are stated in Table 1.

2.5. Evaluation of the formulated solid lipid microspheres

2.5.1. Determination of pH

The pH of the different batches of the unloaded and drug-loaded solid lipid microsphere formulations (unlyophilized dispersions) were measured using a pH meter (Digital pH Meter, Labtech) at different time intervals. Triplicate determinations were done.

2.5.2. Determination of particle size and morphological characterization of solid lipid microspheres

The particle size of the surface-modified lipid

Table 1
Preparation formula for unloaded and drug loaded lipid microspheres(% w/w).

Batches of lipid microspheres	Lipid matrix	Halofantrine	Polysorbate 80	Sobitol	Thiomersal	Distilled water
U1	7.5	–	0.0	4	0.005	100
U2	7.5	–	0.3	4	0.005	100
U3	7.5	–	0.6	4	0.005	100
U4	7.5	–	1.0	4	0.005	100
U5	7.5	–	1.5	4	0.005	100
H1	7.5	1	1.5	4	0.005	100
H2	7.5	2	1.5	4	0.005	100
H3	7.5	3	1.5	4	0.005	100
H4	7.5	4	1.5	4	0.005	100
H5	7.5	5	1.5	4	0.005	100

U1 to U5 represent microspheres prepared with 0.0%, 3.0%, 3.6%, 1.0% and 1.5% w/w of polysorbate 80%, respectively; H1 to H5 represent microspheres containing 1%, 2%, 3%, 4% and 5% w/w of halofantrine, respectively.

microspheres was determined by computerized image analysis[11] on a photomicroscope (Lieca, Germany). With the aid of the software in the microscope, the projected perimeter diameters of the particles corresponding to the particle sizes of the lipid microspheres were determined and average calculated. The particle morphologies were also observed and photomicrographs taken. All these were done in time dependent manner.

2.5.3. Drug content analysis

A 5 mL volume of each of the drug-loaded solid lipid microspheres was centrifuged at 8 400 × *g* for 20 min to obtain aqueous and non-aqueous phases. A 20-fold dilution of the aqueous phase was prepared with methanol and the absorbance determined in a UV-Vis spectrophotometer (UNICO UV-2102 PC, USA) at a predetermined wavelength of 248 nm, and the encapsulation efficiency of the five drug-loaded formulations were calculated using Eqn. 1[12]. The regression equation of Beer's plot for halofantrine in methanol was A = 2.448C (r^2=0.977), where A is the absorbance of the solution and C is halofantrine concentration.

$$EE\% = \frac{\text{Actual amount}}{\text{Theoretical amount}} \times 100 \quad \text{Eqn. 1}$$

2.6. Preparation of biorelevant media for in vitro release studies

Simulated gastric fluid (SGF, pH 1.2) without pepsin, simulated intestinal fluid (SIF, pH 7.2) without pancreatin and phosphate buffer (pH 6.8) were prepared following standard procedures[13,14].

2.7. In vitro release studies

A Franz diffusion cell[15] was used for the studies. In each case, a finite volume of the optimized solid lipid microsphere formulation (H5) was placed in the donor compartment of the Franz diffusion cell separated from the receptor compartment by an artificial membrane (polycarbonate) (pore size 0.22 μm). The receptor compartment was filled with phosphate buffer pH 6.8, SGF (pH 1.2) or SIF (pH 7.2) and maintained at a temperature of (37±1) ℃ by means of a thermostatically controlled water bath, with agitation provided by a magnetic stirring bar at 50 rpm. Aliquot was removed and replaced by an equal volume of the receptor phase at different time interval up to 8 h and the samples collected analyzed for halofantrine content spectrophotometrically using a spectrophotometer (UNICO UV-2102 PC, USA). The drug content at each time point was calculated by reference to Beer's calibration in each biorelevent medium. The formulations (Halfan® 250 mg tablet and halofantrine-containing surface-modified solid lipid microspheres) were used for *in vitro* release studies[16].

2.8. Data and statistical analysis

All experiments were performed in replicates (at least, *n*= 3) for validity of statistical analysis. Results were expressed as mean ± SD. ANOVA and Student *t*-tests were performed on the data sets generated using Origin® for Windows, SPSS and SigmaPlot® 11. Differences were considered significant for *P* values < 0.05.

3. Results

3.1. Particle size analysis

Figures 1 and 2 show the particle size distribution of the unloaded and drug loaded lipid microspheres respectively. It was from Figure 1 that solid lipid microspheres with the least particle size (Batch U5) was selected for the formulation of drug loaded solid lipid microspheres.

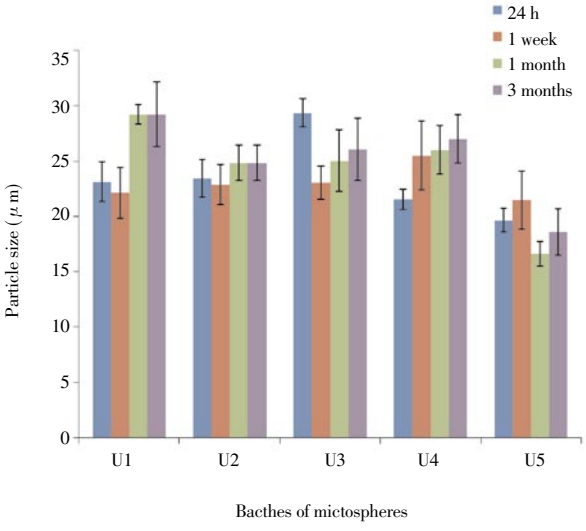

Figure 1. Time-dependent particle size analysis of unloaded solid lipid microspheres (mean ± SD).
U1 to U5 represent solid lipid microspheres prepared with 0.0%, 3.0%, 3.6%, 1.0% and 1.5% w/w of polysorbate 80, respectively.

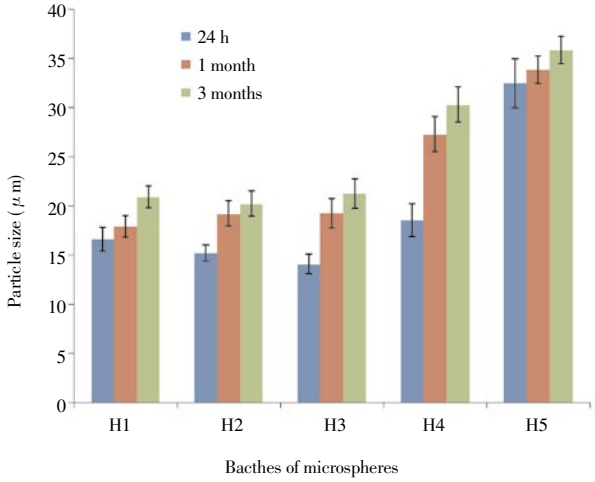

Figure 2. Time-dependent particle size analysis of halofantrine-loaded solid lipid microspheres (μm).
H1 to H5 represent solid lipid microspheres containing 1%, 2%, 3%, 4% and 5% w/w of halofantrine, respectively.

3.2. pH analysis

The pH stability profiles for unloaded solid lipid microspheres containing respectively, 0.0%, 0.3%, 0.6%, 1.0%, and 1.5% w/w of polysorbate 80 (batches U1 to U5) and the halofantrine-loaded solid lipid microspheres (batches H1 to H5) are presented in Figures 3 and 4, respectively.

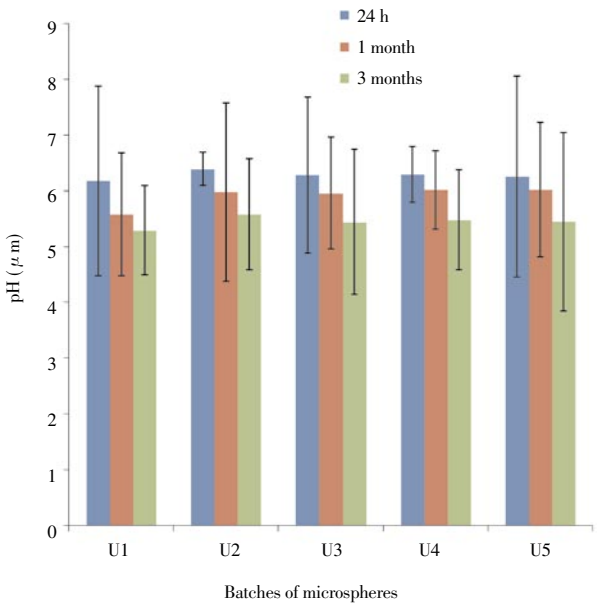

Figure 3. Time-dependent pH analysis of the unloaded solid lipid microsphere.
U1 to U5 represent microspheres prepared with 0.0%, 3.0%, 3.6%, 1.0% and 1.5% w/w of polysorbate 80, respectively.

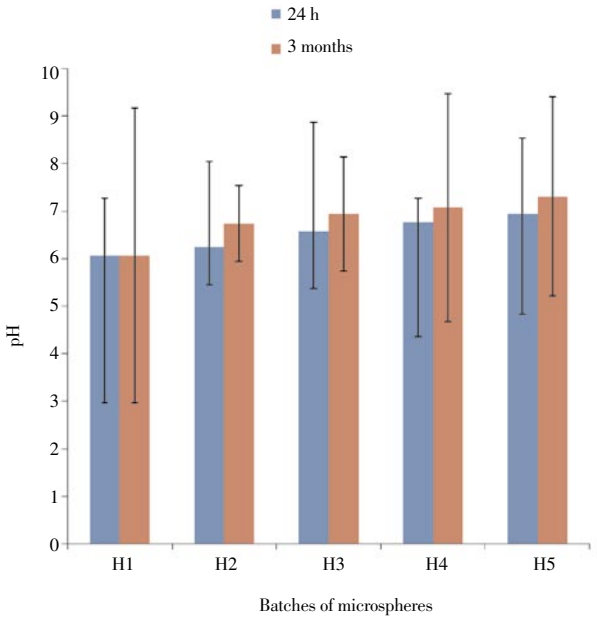

Figure 4. Time-dependent pH analysis of halofantrine-load solid lipid microspheres.
H1 to H5 represent microspheres containing 1%, 2%, 3%, 4% and 5% w/w of halofantrine, respectively.

3.3. Particle morphology

Figures 5 and 6 show the photomicrographs of the unloaded and drug loaded solid lipid microspheres respectively, after 1 month of storage at 30 ℃.

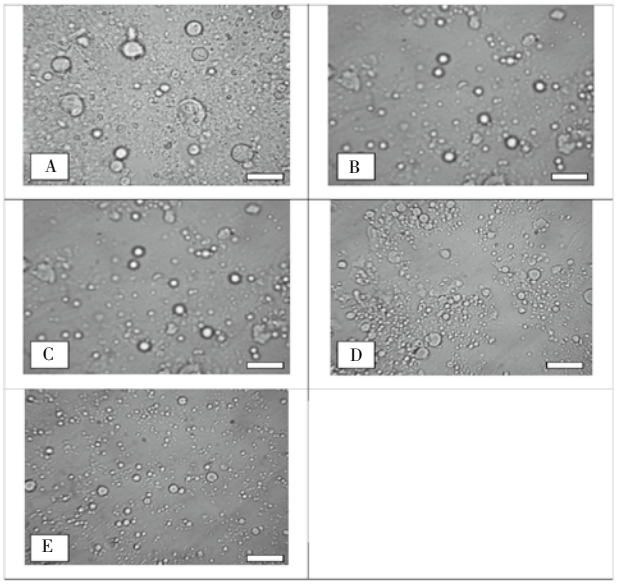

Figure 5. Photomicrographs of the lipid microspheres containing no drug after 1 month of tormulation.
A=0.5% w/w polysorbate 80; B=0.3% w/w polysorbate 80; C=0.6% w/w polysorbate 80; D=1.0% w/w polysorbate 80; and E=1.5% w/w polysorbate 80. Bar represents 50 μm.

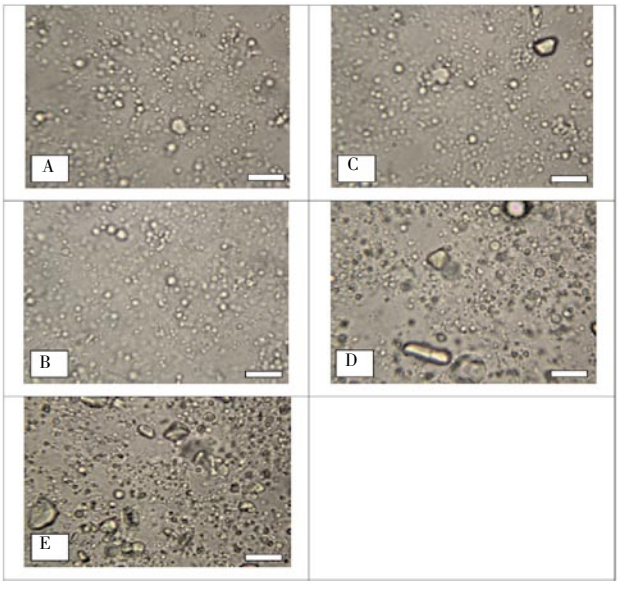

Figure 6. Photomicrographs of the drug containing lipid microspheres after 1 month of formulation.
A=1.5% w/w polysorbate (80±1)% w/w halotantrine; B=1.5% w/w polysorbate (80±2)% w/w halofamtrine; C=3% w/w halofantrine; D=1.5% w/w polysorbate (80±4)% w/w halotantrine; and E=1.5% w/w polysorbate (80±5)% w/w halotantrine. Bartepresents 50 μm.

3.4. Encapsulation efficiency (EE%)

The encapsulation efficiencies of the solid lipid microspheres are presented in Figure 7.

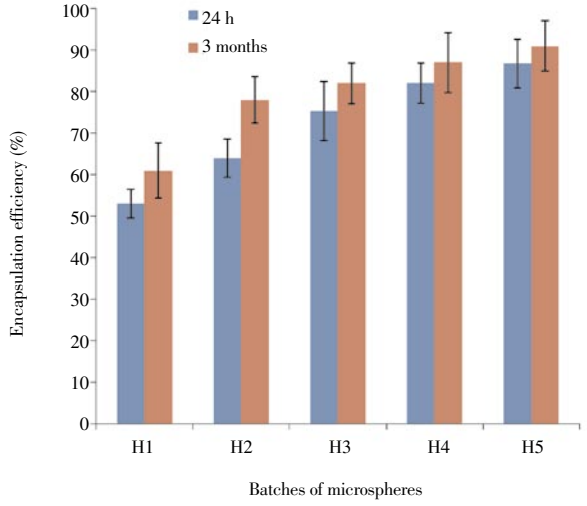

Figure 7. Encapsulation efficiency (EE%).

3.5. In vitro drug release

Figure 8 shows *in vitro* release of halofantrine for both the formulated lipid microspheres (Batch H5) and that of the dispersed Halfan® tablet in SGF, SIF and PB (phosphate buffer).

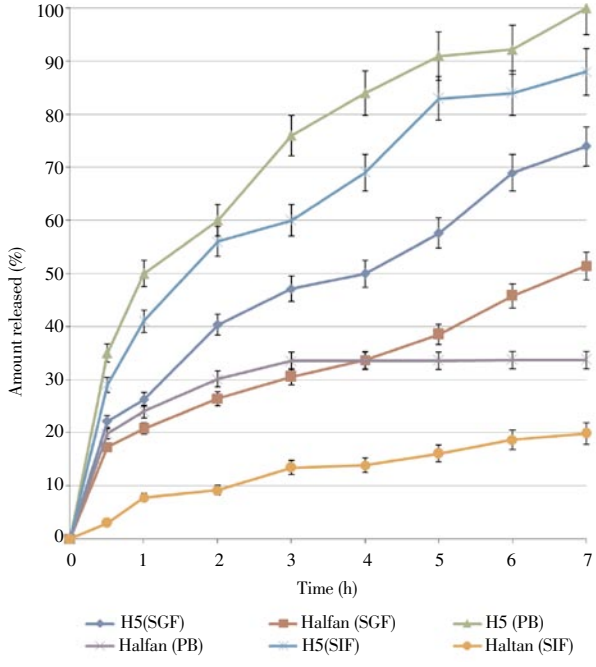

Figure 8. Release profile of halofantrine from the formulation and the commercial tablet in different biorelevant media.

4. Discussion

Particle size analysis shows that increase in concentration of polysorbate 80 resulted in decrease in particle size at constant homogenization condition. This is consistent with earlier findings[17]. On storage, there were minor increases in the particle size after 3 months indicating high particle size stability, although, the particle sizes were low after formulation. The particle sizes were between (18.6±2.1) μm to (29.3±2.9) μm for the unloaded solid lipid microspheres after 3 months and (35.9±1.4) μm to (20.9±1.1) μm for the drug loaded lipid microspheres. It was also observed that there was increase in particle size on drug loading, which also increased as the drug content increased. This indicated that the drug was located mostly in the solid core of the microsphere since it is highly lipophilic. Particle size, particle size distribution and stability are a major issue considered by formulation scientists when formulating dispersed systems[18,19].

The pH stability profiles show that the pH of the unloaded solid lipid microspheres significantly changed ($P<0.05$) after storage. The pH profile of the drug-loaded solid lipid microspheres of the different batches showed the pH values were fairly constant between 24 h and 1 month presenting values of 6.1±1.2 to 6.9±1.6 and 6.1±3.1 to 7.3 ±2.1 respectively. The pH values were affected by drug loading as there was a significant difference ($P<0.05$) between the pH values of the unloaded and drug loaded solid lipid microspheres. One has to be aware that since this formulation is particulate in nature and is dispersed in aqueous medium, particle surface pH in some systems may be 2 to 3 units different from the pH of the bulk because of double layer forces of attraction/adsorption of other ions in the continuous phase[20].

The photomicrographs of the solid lipid microspheres revealed that they are crystalline in nature, spherical and uniform with a smooth surface. The particles appeared to be aggregate in nature without evidence of any collapsed particles. It should be noted that the micrograph presents the particles in two dimensions and particles viewed edge-on may not appear spherical.

The encapsulation efficiencies of the solid lipid microspheres indicated very high EE%. It can be inferred that the encapsulation efficiency increased with the increasing concentration of drug loaded into the lipid microspheres and also with duration of time. The significant increase ($P<0.05$) in the EE% with time indicates that after preparation, the lipid microspheres remained fluid. Analysis of the drug content within 24 h would be erroneous since complete crystallization had not occurred. Migration of drug from the aqueous phase into the lipid microspheres continued until complete crystallization had reached because the drug is highly lipophilic. The percentage of drug entrapped is also dependent upon the physicochemical properties of the drug, the nature of the lipid components selected, the solvent and the manner in which the components were combined.

In the *in vitro* release studies of halofantrine for both the formulated lipid microspheres (Batch H5) and that of the dispersed Halfan® tablet in SGF, SIF and PB (phosphate buffer), it was observed that halofantrine release was greater from the lipid microspheres than from

the tablet formulation (crushed) at the end of the release period. This was as a result of solubility enhancement by the phospholipid-containing solid lipid microsphere. Phospholipid formulations not only enhance bioavailability of drugs with low aqueous solubility or low membrane penetration potential, but also improve or alter uptake and release of drugs[2,21,22]. The release profile revealed that the formulations tested in different biorelevant media had different maximum release. The novel formulation containing 5% w/w halofantrine released maximally after 7 h in phosphate buffer (pH 6.8), while the commercial dosage form could only achieve about 30% maximum release in same media after 7 h. There was also a controlled release of halofantrine from the novel lipid microsphere formulation compared with the commercial dosage form. There was no burst release from the novel lipid microsphere dosage form. Release followed this order: PB > SIF > SGF. The decreased release rate of halofantrine from the tablet may be attributed to the granular nature of Halfan tablet and it has to dissolve before releasing the drug and also the solubility of the drug in the release media. Release studies were conducted in different biorelevant media to ascertain the possible behaviour of the formulation as it transits the entire gastrointestinal system since the formulation is intended for oral administration.

The solid lipid microspheres containing halofantrine exhibited an excellent release profile compared with the commercial table dosage form (Halfan®). This was as a result of the increase in solubility of the lipophilic drug. It is envisaged that there would be controlled release of halofantrine *in vivo* from this novel dosage form and this will ultimately enhance boavailability and a better therapeutic effect will be obtained. It is recommended that further research into use of a lipid matrix and phospholipid in the formulation of halofantrine be embarked upon.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research work was funded by the International Foundation for Science (IFS), Sweden (IFS No. F/4467-1). Dr. A. A. Attama is highly grateful to the Foundation and the Organization.

References

[1] Stuchlík M, Žák S. Lipid based vehicle for oral drug delivery. *Biomed Papers* 2001; **145**: 17-26.

[2] Fricker G, Kromp T, Wendel A, Blume A, Zirkel J, Rebmann H, et al. Phospholipids and lipid-based formulations in oral drug delivery. *Pharm Res* 2010; **27**: 1469-1486.

[3] Katzung BG. *Basic and clinical pharmacology*. 8th edition. McGraw Hill: New York; 2000, p. 874-875.

[4] Council of Europe. *European pharmacopeia*. 5th edition. Council of Europe: Strasbourg; 2005, p. 1790-1791.

[5] Caliph SM, Charman WN, Porter CJH. Effect of short-, medium-, and long-chain fatty acid based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and non-cannulated rats. *J Pharm Sci* 2000; **89**: 1073-1084.

[6] Holm R, Mullertz A, Pedersen GP, Kristensen HG. Comparison of the lymphatic transport of halofantrine administered in dispersed systems containing three different unsaturated fatty acids. *Pharm Res* 2001; **18**: 1299-1304.

[7] Khoo SM, Shackleford DM, Porter CJ, Edwards GA, Charman WN. Intestinal lymphatic transport of halofantrine occurs after oral administration of a unit-dose lipid-based formulation to fasted dogs. *Pharm Res* 2003; **20**: 1460-1465.

[8] Trevaskis NL, Porter CJH, Charman WN. The lymph lipid precursor pool is a key determinant of intestinal lymphatic drug transport. *J Pharmacol Exp Ther* 2006; **316**: 881-891.

[9] Attama AA, Nkemnele MO. *In vitro* evaluation of drug release from self micro-emulsifying drug delivery systems using a novel biodegradable homolipid from *Capra hircus*. *Int J Pharm* 2005; **304**: 4-10.

[10] *British pharmacopoeia*. London: Her Majesty's Stationery Office; 2007.

[11] Attama AA, Okafor CE, Builders PF, Okorie O. Formulation and *in vitro* evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium. *Drug Deliv* 2009; **16**: 448-457.

[12] Morkhade DM, Joshi SB. Evaluation of gum dammar as a novel microencapsulating material for ibuprofen and diltiazem hydrochloride. *Indian J Pharm Sci* 2007; **69**: 263-268.

[13] *European pharmacopoeia*. 7th edition. Strasbourg: Council of Europe; 2007.

[14] *United States pharmacopoeia*. Rockville: United States Pharmacopoeial Convention; 2007.

[15] Franz TJ. Percutaneous absorption-on the relevance of *in vitro* data. *J Invest Dermatol* 1975; **64**: 190-195.

[16] Shin SC, Choi CN, Choi HK. Permeation studies release from the poloxamer gel. *Dev Ind Pharm* 1999; **25**: 273-278.

[17] Attama AA, Müller-Goymann CC. Investigation of surface-modified solid lipid nanocontainers formulated with a heterolipid-templated homolipid. *Int J Pharm* 2007; **334**: 179-189.

[18] Attama AA, Schicke BC, Paepenmüller T, Müller-Goymann CC. Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: characterization. *Eur J Pharm Biopharm* 2007; **67**: 48-57.

[19] Kaur IP, Garg A, Singla AK, Aggarwal D. Vesicular systems in ocular drug delivery: an overview. *Int J Pharm* 2004; **269**: 1-14.

[20] Lasic DD, Templeton NS. Liposomes in gene therapy. *Adv Drug Deliv Rev* 1996; **20**: 221-266.

[21] Attama AA, Reichl S, Müller-Goymann CC. Sustained release and permeation of timolol from surface modified solid lipid nanoparticles through bio-engineered human cornea. *Curr Eye Res* 2009; **34**(8): 698-705.

[22] Attama AA, Reichl S, Müller-Goymann CC. Diclofenac sodium delivery to the eye: *in vitro* evaluation of novel solid lipid nanoparticle formulation using human cornea construct. *Int J Pharm* 2008; **355**: 307-313.