African Journal of Pharmaceutical Research & Development

Vol. 10 No.1; pp. 001-010 (2018)

PREPARATION AND CHARACTERIZATION OF ARTEMETHER-LOADED PLGA NANOPARTICLES

Nnamani P.O [1, 2,*], Hansen S[2], Lehr C-M [2, 3]

[1]Drug Delivery and Nanomedicines Research Group, Department of Pharmaceutics, University of Nigeria

[2]Department of Drug Delivery, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Center for Infection Research, Saarland University, Saarbrücken, Germany

[3]Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbrücken, Germany

ABSTRACT

PLGA particles of antimalarial drug, artemether (ART) were produced by emulsification-diffusion method and characterized. Fresh samples, reconstituted as well as stored samples were studied for particle size, polydispersity index (PDI) and zeta potential; thermal property by differential scanning calorimetry (DSC) while morphology was done by transmission electron microscopy (TEM) in addition to interaction study done by Fourier transform infrared spectroscopy (FTIR). Encapsulation efficiency and drug loading were determined by HPLC. Result shows stable monodispersed (0.01-0.21) spherical particles (201-246 nm) with molecular dispersion of 10 mg of ART while achieving encapsulation efficiency of ~13 % and drug loading of ~17 % superior to values obtained for the batch loaded with 5 mg of ART. FTIR showed interaction of OH-group of ART with C=O of PLGA resulting in shifts and/or reduction in peak intensity. The study reveals the feasibility of ART loaded PLGA nanoparticle (~246 nm) as having high potential for skin delivery since particle size is critical for tissue penetration, *in vitro* drug release, *in vivo* performances as well as degradation behavior. In an outlook, we are currently investigating the skin permeation and follicular uptake of these particles to hope for an alternative topical and/or transdermal antimalarial regimen of ART.

KEYWORDS: Malaria; Artemether; PLGA; Emulsion diffusion; HPLC; Characterization

INTRODUCTION

Poly (lactic-co-glycolic acid, PLGA) is one of the most widely used generally-regarded as safe (GRAS) biocompatible and biodegradable polymer due to its easily metabolized end products of hydrolysis (lactic acid and glycolic acid) [1]. It is available as copolymer of different molecular weights [2]. This versatile polymer has recently gained interest due to its prevention of drug degradation, reduction of drug side effects through improved therapeutic effects, controlled, sustained and targeted delivery of so many drugs [3-7] through so many routes [8] as well as facilitating drug uptake in cells through a number of mechanisms thereby targeting specific tissues or organs of the body [9-13].

The versatility of this polymer has necessitated our investigation into its potential as a carrier for the highly lipophilic antimalarial drug, artemether (ART) which is only available as oral tablet and intramuscular injection. ART is one of the very potent short-acting schizonticidal artemisinin derivatives currently in use as first line treatment of malaria. Malaria is the world's most wide spread parasitic infectious disease with serious morbidity and mortality especially in less than 5 years and pregnant women [14]. Widespread and increased resistance to antimalarial drugs has contributed

***Corresponding author:** petra.nnamani@unn.edu.ng; +234 (0) 803 696 3979*

enormous difficulties in controlling malaria thereby posing considerable intellectual, technical and humanitarian challenges. In response to development of drug resistance to monotherapies like chloroquine (CQ) and sulfadoxine-pyrimethamine (SP), artemisinin combination therapy (ACT) was recommended by the World Health Organization (WHO) as the first line treatment of falciparum malaria in all endemic regions [15, 16], as a means of improving treatment efficacy and slowing the spread of resistance due to recrudescence.

Artemether (ART) is the methyl ether derivative of dihydroartemisinin, also called dihydroartemissinin methyl ether which has shown superior antimalarial activity over all other derivatives including arteether, artesunate, etc [17]. ART has fairly rapid absorption after oral administration with peak plasma concentrations reached about 2 h, with no dose modification in renal or hepatic impairment [18, 19]. It is therefore frequently dosed (twice daily for at least 3-5 days) with associated numerous side-effects especially nausea-vomiting tendency which makes people discontinue treatment or comply poorly. Due to the pain of injection, the parenteral route is a no go area as well. Non-compliance to complete course of treatment therefore makes room for development of resistance, which is already emerging [18]. This makes it pertinent to explore novel drug delivery systems to redesign a carrier which could better modify drug properties of this very important drug so that while its long acting partner drug is taken orally (once a day and/or all at once, as the case with some ACTs), ART could be used not as injection or oral but rubbed on the skin or impregnated as a patch to reduce side effects and improve patient acceptability. So far, ART has been successfully delivered to the systemic circulation through the skin using carefully selected nanostructured lipid carriers [18]. In this study however, another approach was sought to exploit PLGA as a polymeric carrier to deliver ART and the resultant PLGA nanoparticles (PLGA NPs) were preliminarily characterized to understand their disposition for such. An outlook from this would be to study the skin performance of the nanoparticles through the hair follicle and/or the intact skin as an alternative low-dose, more patient-friendly alternative to conventional forms of injection and/or tablets.

MATERIALS AND METHODS

Materials

Artemether (Fig 1a) was a gift from Ipca Laboratories Limited India while PLGA (Resomer RG 50:50H; inherent viscosity 0.31 dl/g; Fig. 1b) was obtained from Boehringer Ingelheim GmbH & Co. KG, Ingelheim, Germany and polyvinyl alcohol Mowiol® 4–88 (PVA) from Kuraray Specialties Europe GmbH, Frankfurt, Germany. All other solvents and chemicals used were of the highest purity available. Deionized water (Milli Q Plus System, Millipore, Bedford, MA, USA) was used throughout the study.

Chemical compounds

Chemical compounds studied in this article
Artemether (PubChem CID: 68911); PLGA (PubChem CID: 36797); Polyvinyl alcohol (PubChem CID: 11199)

Methods

Emulsion-diffusion method

PLGA nanoparticles containing graded concentrations of ART (0, 5 and 10 mg) were prepared using an emulsion-diffusion method [20]. Some 100 mg PLGA was dissolved in ethyl acetate to which ART was also added and dissolved under magnetic stirring at 1000 rpm (Thermomixer comfort, Eppendorf, Germany) for 5 min. This was added drop wise to a 2 ml of 2.5 %w/v PVA solution under magnetic stirring at 1000 rpm for 5 min. The resultant pre-emulsion was subjected to high speed mixing at 20,000 rpm using an Ultra-Turrax T25 homogenizer (Polytron PT 2500E, Kinematica, USA) for 5 min [20]. The nanoemulsion was diluted with 15 ml volume of deionized water to destabilize the equilibrium between the two phases thereby causing the solvent to diffuse into the external phase leading to reduction of interfacial tension [21], and then in nanoparticle formation which gradually becomes poorer in solvent [21]. The system was stirred overnight at 1000 rpm.

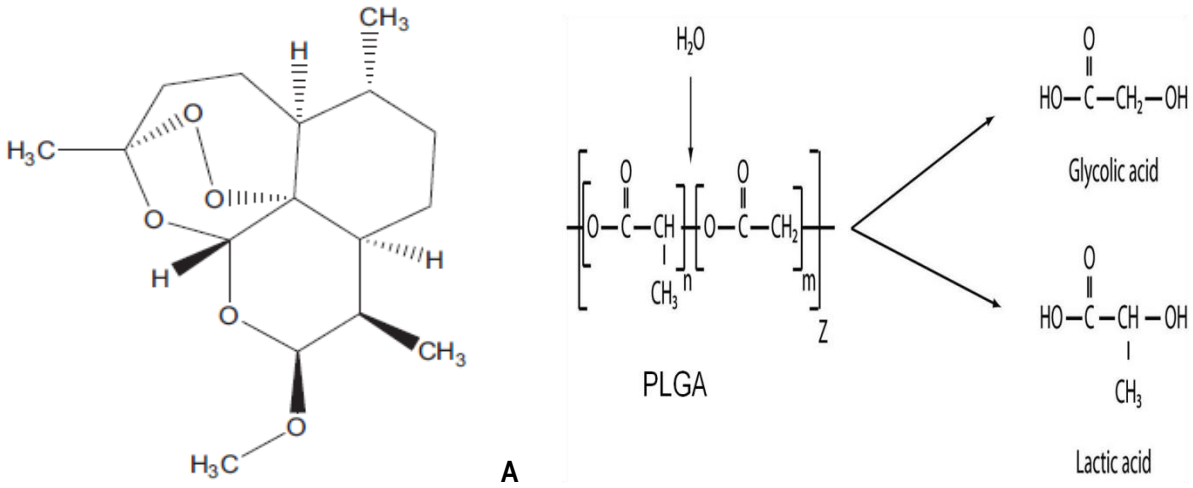


Figure 1: Figure 1: Chemical structure of (A) Artemether and (B) PLGA
(n = number of units of lactic acid; m = number of units of glycolic acid).

PLGA NPs were collected by centrifugation at 43,400×g for 15 min at room temperature, washed three times with deionized water, and frozen at -80 °C prior to lyophilization (Martin Christ, Alpha 2–4 LSC GmbH, Osterode, Germany) for 48 h. PLGA NP were stored as freeze-dried powder and re-suspended in deionized water to obtain the desired concentration prior to use. ART-PLGA NP was stored as stable suspension at 4–8 °C for a maximum of 14 days after manufacturing. Immediately before each experiment, the hydrodynamic diameter, size distribution, and zeta potential were determined using a Zetasizer Nano-ZS (Malvern, Malvern UK).

Physicochemical properties of PLGA-particles

Particle size and zeta potential measurements were conducted using a Zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). Particle size was measured by photon correlation spectroscopy (PCS) while zeta potential determinations were based on electrophoretic mobility.

Particle morphology

Microstructure of PLGA particles were observed using transmission electron microscope (JOEL 1210, JOEL Inc., Boston, MA, USA). Initially, samples were reconstituted in deionized water, diluted and deposited on film-coated copper grids and the samples were allowed an overnight drying at room temperature. The dried samples were visualized under TEM.

Entrapment efficiency (EE) and drug loading (DL)

Encapsulation efficiency (EE) of ART-loaded PLGA particles was determined by ultrafiltration using Vivaspin filter tubes (Vivaspin, Germany), with filter membrane (MWCO 10,000). Two milliliter aliquot of reconstituted sample was placed in the upper chamber and the sample recovery chamber was fitted below the membrane in the lower compartment. The unit was closed and centrifuged at 43,400×g for 15 min using a centrifuge (Model 420R Rotina Hettich, Germany). The filtrate was appropriately diluted with acetonitrile: water (90:10) and the amount of ART in the aqueous phase was estimated by a validated RP-HPLC. The EE and DL were calculated from the following equations:

$$EE\ (\%) = \frac{Real\ ART-loading}{Theoretical\ ART-loading}\ x\ 100 \ldots\ldots\ldots\ldots\ldots (1)$$

$$DL\ (\%) = \frac{Amount\ of\ encapsulated\ ART}{Total\ weight\ of\ the\ particles\ in\ the\ formulation}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

HPLC method for artemether

This was done according to a previous study [18]. Briefly, HPLC determination of ART was performed using a Dionex P680 HPLC pump (ASI-100 automated sample injector) equipped with UV/VIS detectors operating at 208 nm (210 and 214 nm). Samples were chromatographed on a stainless steel C18 reverse phase column (250 x 4.0 mm) packed with 5 µm particles (Lichrospher® 100 RP-18). Elution was conducted with a mobile phase of acetonitrile: water (90:10) at a flow rate of 40 µl

/min at 40 °C for 5 min. A calibration curve was plotted for ART in the concentration range of 5-10 µg/ml. A good linear relationship was observed between the concentration of ART and the peak area of ART with a percentage correlation coefficient of 99.7904 %. The required studies were carried out to estimate the precision and accuracy of the HPLC method.

Differential scanning calorimetry (DSC)

Thermal properties of the drug, polymer and formulations were studied using a differential scanning calorimeter (DSC Q100 TA Instrument, Germany). Briefly, at least 3-5 mg of sample was weighed in aluminum pans, heated from 25 to 150 °C at 10 °C /min under constant flushing with nitrogen (10 ml/min). The DSC parameters, such as temperature onset, maximum peak, and enthalpy, were generated after baseline corrections with empty aluminum pan as standard.

Fourier-transform infrared spectroscopy

The infrared spectra were recorded over the range 4000-400 cm^{-1} in a Perkin-Elmer (Model SPECTRUM 1000 FTIR) spectrometer. PLGA NP were mixed thoroughly with KBr and pressed into thin pellets using a pressure of 14,000 pound for 2 min. Spectra of pure drug, polymer and formulations were recorded.

Statistical analysis

Determinations were done in triplicates for validity of statistical analysis. All the data generated were expressed as mean ± standard deviation. Statistical significance was determined using student t-test, with $P<0.05$ considered to be statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the general properties of the formulations. Lyophilized PLGA particles were readily dispersible in deionized water upon reconstitution showing that trehalose was an effective cryoprotectant at the used concentration of 15 %w/v. This agreed with literature that trehalose maintains size and monodispersibility of PLGA particles after lyophilization (Fig. 2), which otherwise would have resulted in aggregation due to immense surface area of the system causing thermodynamic instability [22]. Freeze drying has proved to be a stable method of preserving unstable colloidal particles over long periods of time [21, 23]. However, the morphology of ART-PLGA NPs appeared not to have dried completely before TEM analysis. ART like many other drugs can therefore be conveniently encapsulated into PLGA particles and be stably stored as powder as can be seen from the zeta potential values (Table 1).ART encapsulation was however higher for 10 mg concentration (than 5 mg) achieving up to ~13 % EE and 17 % loading efficiency.

Table 1: Particle characterization

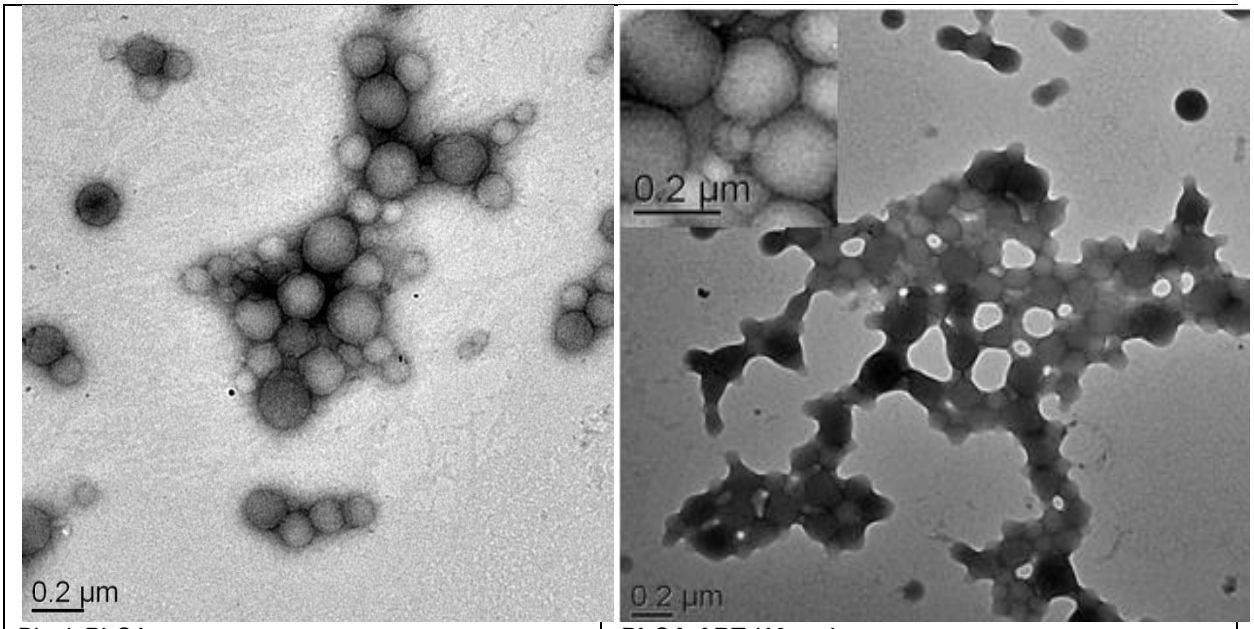
Parameters	Z-Av ± SD (nm)	PDI ± SD	ZP ± SD (mV)	Encapsulation efficiency (%)	Drug loading (%)
Blank PLGA	205.6 ± 13.20	0.025 ± 0.001	-24 ± 0.213	-	-
PLGA-ART (10mg)	249 ± 17.98	0.305 ± 0.025	-16.5 ± 0.232	12.55	16.74
PLGA-ART (5mg)	218.4 ± 9.87	0.044 ± 0.054	-13.6 ± 0.095	3.85	1.54
Redispersed particles after lyophilization					
Blank PLGA	206.6 ± 5.87	0.01 ± 0.001	-13.6 ± 0.017	-	-
PLGA-ART (10mg)	246.8 ± 10.25	0.213 ± 0.073	-15.6 ± 0.015	12.55	16.67
PLGA-ART (5mg)	202.4 ± 9.93	0.015 ± 0.012	-13.0 ± 0.019	3.92	1.54
Storage stability study					
Blank PLGA	205.7 ± 8.23	0.033 ± 0.004	-13.1 ± 0.016	-	-
PLGA-ART (10mg)	246.2 ± 8.37	0.210 ± 0.027	-12.9 ± 0.034	12.53	16.69
PLGA-ART (5mg)	201.4 ± 4.32	0.021 ± 0.009	-13.2 ± 0.013	3.61	1.49

Legend: Z-Av means particle size; PDI means polydispersity index; ZP means zeta potential

Particle size is a critical value for determining drug release and degradation behavior as well as efficiency of the system for tissue penetration or even intracellular uptake, *in vitro* drug release and *in vivo* performances [21-26]. Size determines the destination of nanoparticles in the body. However, Mahe et al. showed that particles of a size of approx. 200 nm applied to the skin were taken up by the Langerhans cells (LCs) located around the hair follicles [22]. This observation is consistent

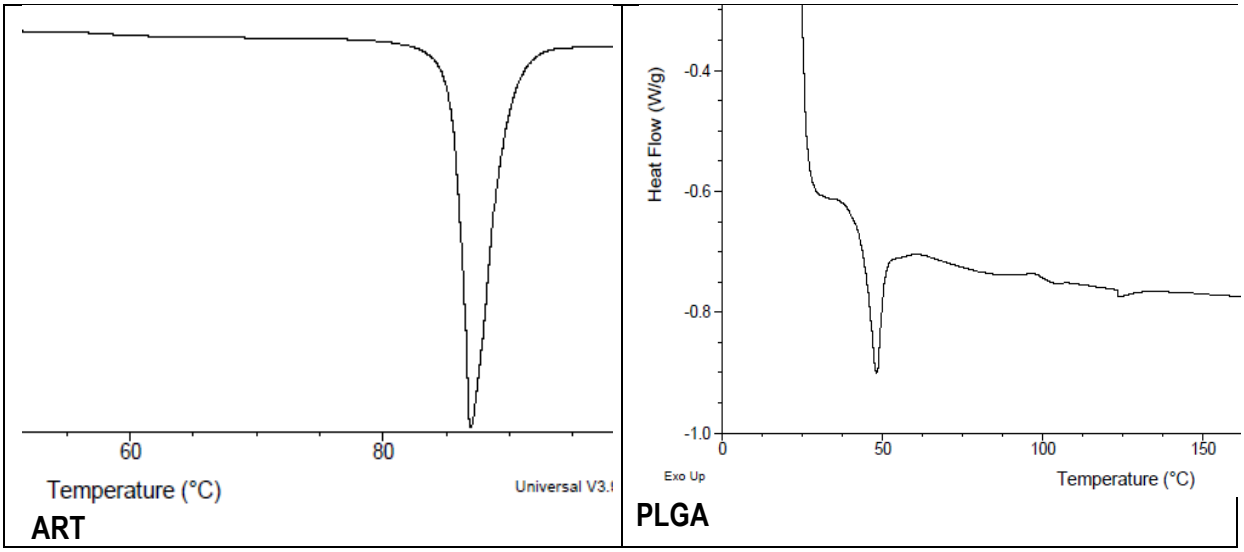
with a large body of research worldwide that nanoparticles below 250 nm size are feasible for skin delivery [18]. Since the particles in this preliminary study were ~ 246 nm, it suggests that the ART-loaded PLGA nanoparticles could be feasible for skin studies.

Fig. 3 shows the DSC traces whereas Table 2 shows thermal properties of bulk materials and formulations. There was complete absence of drug peak (m.pt 86.96 °C) in all ART-loaded PLGA thermograms. Table 2 shows that it could be seen that ART-loaded PLGA NP had the least enthalpies compared to blank formulation as well as single entities of PLGA, trehalose and ART. This confirms low crystallinity of NPs, agreeing with earlier observations that emulsion-



Blank PLGA | **PLGA-ART (10 mg)**

Figure 2: Morphology of formulation by TEM photomicrographs



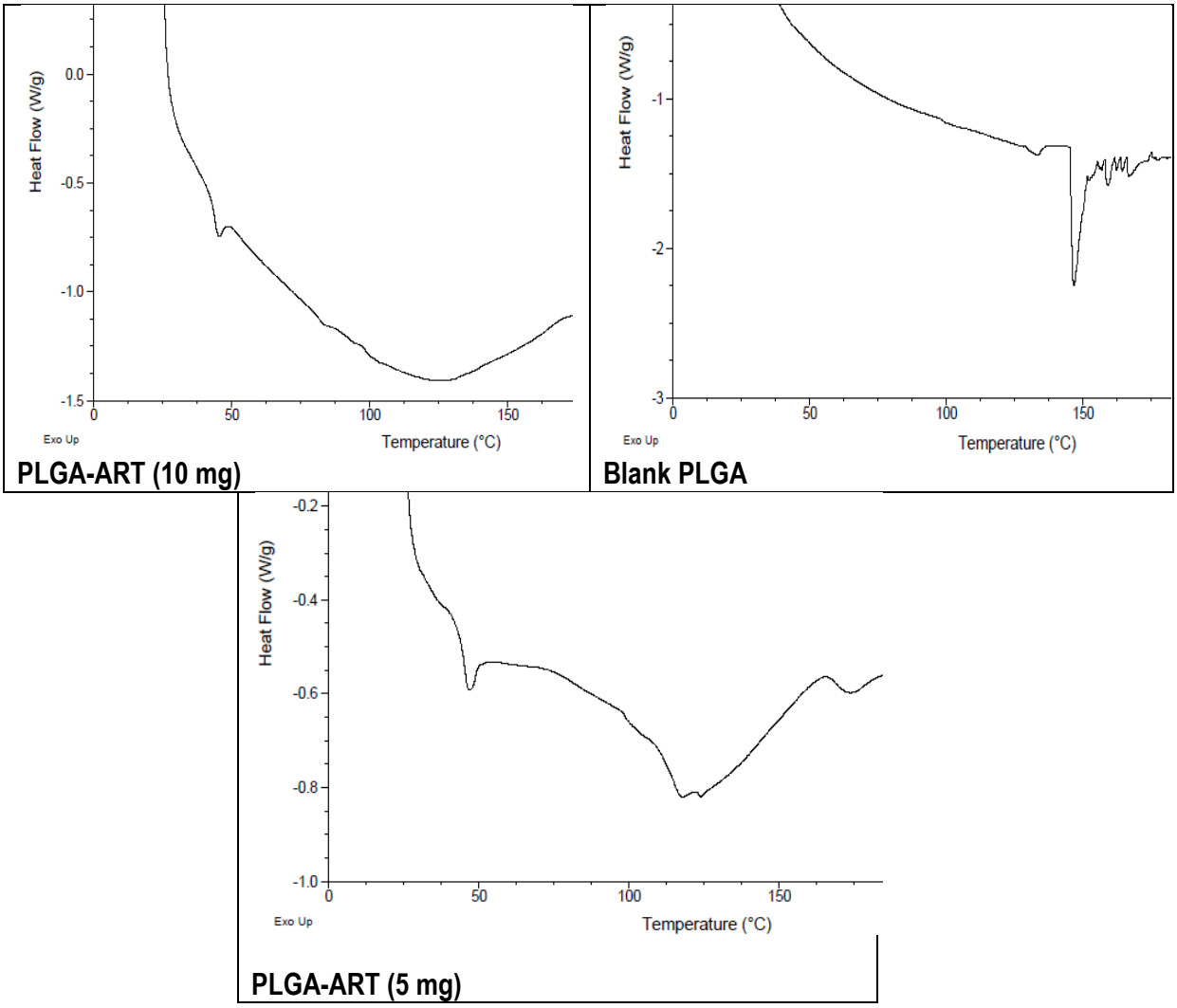


Figure 3: DSC thermograms

Table 2: Thermal properties of formulations

Formulations	Onset temp (°C)	Peak temp. (°C)	Enthalpy (J/g)	Onset temp. (°C)	Peak temp(°C)	Enthalpy (J/g)
Artemether	85.74	86.96	87.51			
Trehalose	115.58	112.55	3.800			
PLGA	46.28	48.11	1.254			
Blank PLGA	-	146.80	1.746			
ART-PLGA (10 mg)	44.02	45.15	0.3009	121.46	126.21	0.2040
ART-PLGA (5 mg)	45.34	46.62	0.5569	114.91	117.55	0.5411

diffusion method offered higher drug entrapment efficiency for poorly water soluble drug (lipophilic) than hydrophilic drugs [24].

To demonstrate that it was possible to incorporate ART into PLGA NPs, IR spectra of free-ART, ART-PLGA, PLGA and blank PLGA were obtained and presented in Fig. 4. Fourier transform infrared spectroscopy (FTIR) is an important chemical analysis method that reveals interactions at the particle surface. Spectra of ART showed peaks for OH, C–H stretching, (CH_2, CH_3) asym, -C-O stretching, (C-O-C) asym, and (=C-O bending) of carboxylic acid at wave numbers of 32934-2932, 2871-2843, 1430, 1276-1249, 1150-1060 and 994-779 cm^{-1} respectively. PLGA exhibited characteristic strong and narrow absorption peaks at 2978-2939 cm^{-1} (OH group), 1747 cm^{-1} (C=O stretching), 1470-1400 cm^{-1} (CH_2, CH_3) asym, 1379-1263 cm^{-1} (COOH), 1150-1060 cm^{-1} (C-O-C) asym and 943-725 cm^{-1} (=C-O bending) of carboxylic acid. Absence, shifting or

broadening of characteristic peaks in infrared spectrum of the NPs would indicate changes in the drug characteristics, amorphous form or possibilities of drug-polymer interactions. The OH group of ART therefore interacted with –OH and C═O groups of PLGA and the peak got shifted to higher wave number at 3484-3380 cm^{-1} as well as lower wave number at 1737 cm^{-1} respectively. A new broad peak was therefore observed in the ART-PLGA spectrum which could be attributed to the functional group ≡(C-H) stretching. However, interaction of proton donors (ART) with carbonyl oxygen (from PLGA) via hydrogen bonding is known to decrease the frequency of the C═O mode due to weakening of C═O bond [27]. This reduction in peak at 1737 cm^{-1} (from 1747 cm^{-1}) wave number confirms hydrogen bonding as much as the fact that simultaneous growth of C═O band of PLGA and O-H group of ART shifted to higher wave number and C═O group of ART-PLGA NP shifted to lower wave number provides strong evidence of hydrogen bonded ART with PLGA [28].

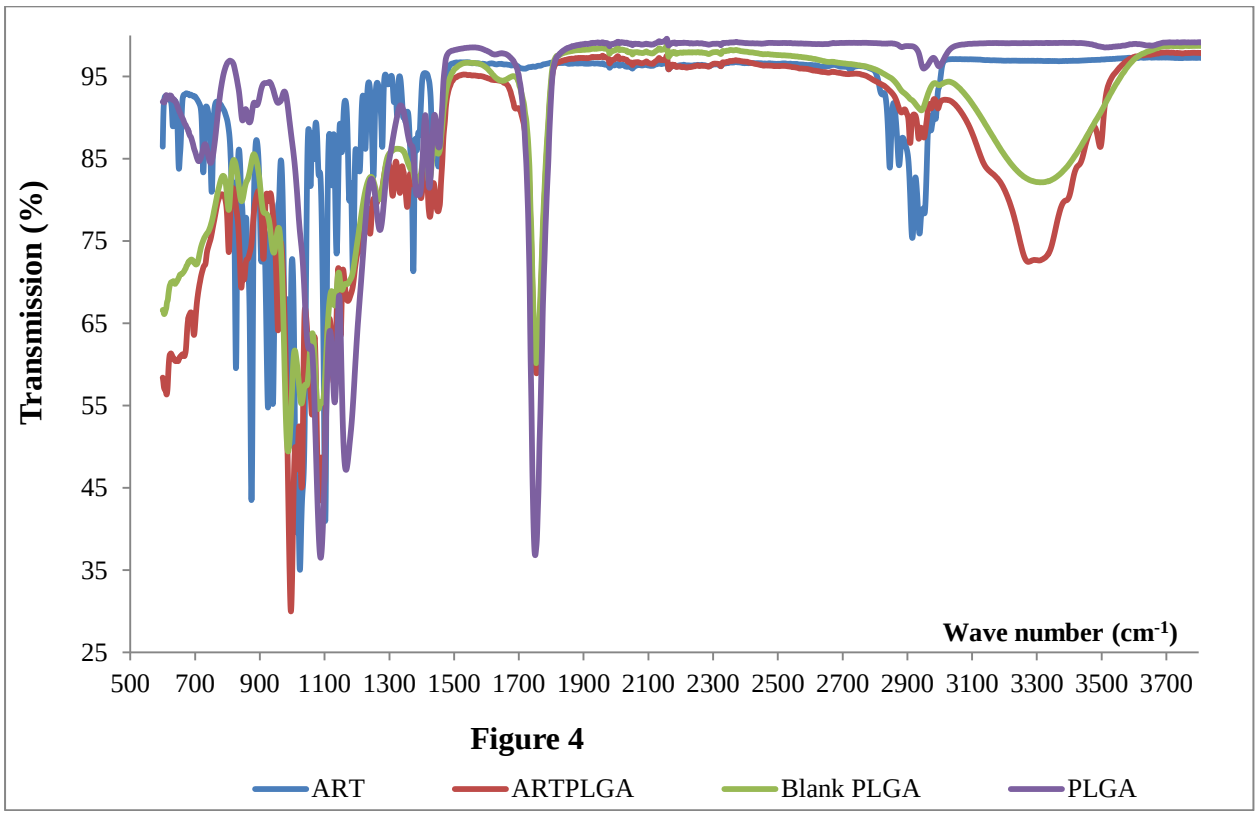


Figure 4: FTIR of formulations

CONCLUSION

PLGA has demonstrated convincing delivery properties for ART by forming less crystalline, spherically monodispersed and stable nanoparticles with high encapsulation efficiency and loading capacity. Trehalose has proven an excellent cryoprotectant for the system to reduce aggregation of the colloidal particles thereby making them stable for a long period of time. Since particle size is critical for drug release (*in vitro* and *in vivo*), tissue penetration and uptake as well as biodegradation, it would be pertinent to say that the ART-loaded PLGA NPs with sizes less than 250 nm as obtained in this preliminary study could possibly be up taken by the Langerhans cells (LCs) located around the hair follicles; hence the thrust of an outlook study which could prove this concept.

ACKNOWLEDGEMENT

This work was supported by the TWAS-DFG fellowship (Ref. 3240263814). Dr. Petra greatly appreciates the mentorship of Prof. Dr. Claus-Michael Lehr and his team on this visit. We thank Dr. Chiara De Rossi and Mr. Peter Meiers for assistance with TEM and HPLC analysis respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. A. Kumari, S.K. Yadav, S.C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, Colloids Surf. B Biointerfaces 75 (2010) 1–18.
2. A. Prokop, J.M. Davidson, Nanovehicular intracellular delivery systems, J. Pharm. Sci. 97 (2008) 3518–3590.
3. Y. Kawashima, H. Yamamoto, H. Takeuchi, Y. Kuno, Mucoadhesive DL-lactide/glycolide copolymer nanospheres coated with chitosan to improve oral delivery of elcatonin, Pharm. Dev. Technol. 5 (2000) 77–85.
4. J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, Adv. Drug Deliv. Rev. 24 (2003) 329–347.
5. M.N. Ravi Kumar, U. Bakowsky, C.M. Lehr, Preparation and characterization of cationic PLGA nanospheres as DNA carriers, Biomaterials 25 (2004) 1771–1777.
6. J.K. Vasir, V. Labhasetwar, Biodegradable nanoparticles for cytosolic delivery of therapeutics, Adv. Drug Deliv. Rev. 10 (2007) 718–728.
7. O. Jeon, H.W. Lim, M. Lee, S.J. Song, B.S. Kim, Poly (L-lactide-co-glycolide) nanospheres conjugated with a nuclear localization signal for delivery of plasmid DNA, J. Drug Target 15 (2007) 190–198.
8. A.S. Raber, A. Mittal, J. Schäfer, U. Bakowsky, J. Reichrath, T. Vogt, U.F. Schaefer, S. Hansen, C.M. Lehr. Quantification of nanoparticle uptake into hair follicles in pig ear and human forearm, J. Control. Rel. 179 (2014) 25–32.
9. N. Nafee, S. Taetz, M. Schneider, U.F. Schaefer, C.M. Lehr, Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides, Nanomedicine 3 (2007) 173–183.
10. H. Yamamoto, Y. Kuno, S. Sugimoto, H. Takeuchi, Y. Kawashima, Surface modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions, J. Control Release 102 (2005) 373–381.
11. R. Yang, W.S. Shim, F.D. Cui, G. Cheng, X. Han, Q.R. Jin, D.D. Kim, S.J. Chung, C.K. Shim, Enhanced electrostatic interaction between chitosan-modified PLGA nanoparticle and tumor, Int. J. Pharm. 371 (2009) 142–147.
12. F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A.L. Breton, V. Préat, PLGA-based nanoparticles: An overview of biomedical applications, J. Control. Rel. 161 (2012) 505–522.
13. K. Tahara, T. Saka, H. Yamamoto, H. Takeuchi, N. Hirashima, Y. Kawashima, Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells, Int. J. Pharm. 382 (2009) 198–204.
14. WHO: World Malaria Report 2014. World Health Organisation (2014). http://www.who.int/malaria/publications/world_malaria_report_2014/en/
15. S.I. Hay, E.A. Okiro, P.W. Gething, A.P. Patil, A.J. Tatem, C.A. Guerra, R.W. Snow, Estimating the global clinical burden of *Plasmodium falciparum* malaria in 2007, PLoS Med 7 (2010) e1000290.
16. P.O Nnamani, G. Scoles, S. Kröl, Preliminary characterization of N-trimethylchitosan as a Nanocarrier for Malaria Vaccine, J. Vector Borne Dis. 48(4) (2011) 224-230.
17. O. Egunsola, K.A. Oshikoya, Comparative safety of artemether-lumefantrine and other artemisinin-based combinations in children: a systematic review, Malar. J. 12 (2013) 385.
18. P.O. Nnamani, S. Hansen, M. Windbergs, C.M. Lehr, Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application, Int. J. Pharm. 477 (2014) 208–217.
19. P. Deepa, N.P. Aditya, R.S.R Murthy, Development of artemether and lumefantrine co-loaded nanostructured lipid carriers: physicochemical characterization and *in vivo* antimalarial activity, Drug Deliv, Early Online (2014) 1–7. DOI: 10.3109/10717544.2014.905883
20. A. Mittal, A.S. Raber, U.F. Schaefer, S. Weissmann, T. Ebensen, K. Schulze, C.A. Guzmán, C.M. Lehr, S. Hansen, Non-invasive delivery of nanoparticles to hair follicles: a perspective for transcutaneous immunization, Vaccine 31 (2013) 3442-3451.
21. *A. Mittal,* K. *Schulze,* T. *Ebensen,* S.

Weissmann, S. *Hansen,* C.A. *Guzmán,*C.M. *Lehr, Inverse micellar sugar glass (IMSG) nanoparticles for transfollicular vaccination,* J. Control Rel. 206 (2015) 140-52.

22. B. Mahe, A. Vogt, C. Liard, D. Duffy, V. Abadie, O. Bonduelle, A. Boissonnas, W. Sterry, B. Verrier, U. Blume-Peytavi, B. Combadiere, Nanoparticle-based targeting of vaccine compounds to skin antigen-presenting cells by hair follicles and their transport in mice, J. Invest. Dermatol. 129 (2009) 1156-1164.
23. C.S. Campbell, L.R. Contreras-Rojas, M.B. Delgado-Charro, R.H. Guy, Objective assessment of nanoparticle disposition in mammalian skin after topical exposure, J. Control Rel. 162 (2012) 201-207.
24. I. Bala, S. Hariharan, M.N.V Ravi Kumar, PLGA Nanoparticles in Drug Delivery: The State of the Art, Critical Rev. Thera. Drug Carrier Sys. 21(5) (2004) 387–422.
25. M.O. Oyewumi, A. Kumar, Z. Cui, Nano-microparticles as immune adjuvants: correlating particle sizes and the resultant immune responses, Expert Rev. Vaccines 9 (2010) 1095-1107.
26. A. Mittal, K. Schulze, T. Ebensen, S. Weissmann, S. Hansen, C.M. Lehr, C.A. Guzman, Efficient nanoparticle-mediated needle-free transcutaneous vaccination via hair follicles requires adjuvantation, Nanomedicine: nanotechnology, biology, and medicine, 11 (1) (2015) 147-54
27. S. Bhende, N. Jadhav, Moringa Coagulant as a Stabilizer for Amorphous Solids: Part I, AAPS PharmSciTech (2012) DOI: 10.1208/s12249-012-9755-x.
28. S. Nadaf, P.O. Nnamani, N. Jadhav, Evaluation of *Prosopis africana* Seed Gum as an Extended Release Polymer for Tablet Formulation, AAPS PharmSciTech. (2014) DOI: 10.1208/s12249-014-0256-y (Online ISSN: 1530-9932).

GRAPHICAL ABSTRACT

PLGA particles of antimalarial drug, artemether (ART) were produced by emulsification-diffusion method and shown to be amorphous with 246 nm size suitable for skin study.