Journal of Pharmaceutical Investigation (2020) 50:201–208
https://doi.org/10.1007/s40005-019-00458-8

Online ISSN 2093-6214
Print ISSN 2093-5552

ORIGINAL ARTICLE

Encapsulation and physicochemical evaluation of efavirenz in liposomes

Nnamdi Ikemefuna Okafor[1] · Christian Isalomboto Nkanga[1] · Roderick Bryan Walker[2] · Xavier Siwe Noundou[1] · Rui Werner Marçedo Krause[1]

Received: 25 March 2019 / Accepted: 3 August 2019 / Published online: 21 September 2019
© The Korean Society of Pharmaceutical Sciences and Technology 2019

Abstract

Purpose Antiretroviral therapy remains the most effective means of managing the human immune deficiency virus/acquired immune deficiency syndrome (HIV/AIDS). Application of therapeutics has been hampered by factors including poor bioavailability of most anti-retroviral compounds (ARV), side effects and an alarming emergence of drug resistant strains of the virus.
Methods Recent developments and use of drug delivery systems (DDS) has shown potential for improving the pharmacological profile of ARV. Amongst these complex DDS, liposomes have been explored for delivery of ARV. In this study, we have aimed at exploring efficient encapsulation of efavirenz (EFV), a potent ARV using different mass ratios of crude soybean lecithin and cholesterol.
Results The EFV-loaded liposomes (EFL) were prepared using thin film hydration and evaluated for particle size, zeta potential (ZP), encapsulation efficiency (EE%), morphology and drug release studies. Differential scanning calorimetry (DSC), X-ray diffraction (XRD), energy dispersity spectroscopy (EDS) and Fourier transform infrared (FTIR) spectroscopy were used for comprehensive physicochemical characterization of EFL. EFL exhibited high encapsulation (99%) in 1:1 crude lecithin to cholesterol mass ratio. The average particle size and Zeta Potential of EFL were found to be 411.10 ± 7.40 nm and $-53.5.3 \pm 0.06$ mV, respectively. EFL showed a relatively controlled EFV release behaviour that was similar to the dissolution profile of un-encapsulated EFV.
Conclusion This suggests that EFL represents a promising vehicle for effective EFV delivery while providing the advantages of a nano-scaled delivery system.

Keywords HIV/AIDS · ARV · Efavirenz · Drug delivery systems · Liposomes

Introduction

The human immunodeficiency virus and acquired immune deficiency syndrome HIV/AIDS is one of the most deadly and life threatening infectious diseases that is a challenge to public health globally (Ojewole et al. 2008). According to the United Nation over 36.9 million people globally are living with HIV/AIDS including 1.8 million children (UNAIDS 2019). An estimated 21.7 million people globally were receiving antiretroviral therapy (ART) as of 2017 (UNAIDS 2019). HIV patients are mostly subjected to intensive highly antiretroviral therapy (HAART) that involves frequent administration of multiple ARV. Unfortunately, the use of this therapy is for temporal treatment and effective management of the virus and only reduces the replication of the virus within infected cells, thereby improving the quality of life of patients (Gupta and Jain 2010). Being a lengthy drug administration regimen, HAART results in poor patient adherence due to severe adverse effects associated with ARV, which leads to therapeutic failure and development of resistance (Shah and Amiji 2006; Ramana et al. 2010). In addition, the intracellular location of HIV makes it relatively inaccessible to ARV, which in return become ineffective in controlling the viral proliferation.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40005-019-00458-8) contains supplementary material, which is available to authorized users.

✉ Rui Werner Marçedo Krause
r.krause@ru.ac.za

[1] Department of Chemistry, Faculty of Science, Rhodes University, Grahamstown 6140, Eastern Cape, South Africa

[2] Division of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Grahamstown 6140, Eastern Cape, South Africa

Another concern is the fact that the ARV exhibit poor oral bioavailability due to hepatic first pass metabolism, short half-life, which necessitates a steady administration of high doses that leads to low patient adherence (Li and Chan 1999). Since ARV remain pivotal to HAART there is an urgent need to improve the pharmacological profiles of ARs.

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) approved by the U.S. Food and Drug Administration (FDA) for treatment of HIV infection in adults and in children 3 months of age who weigh at least 7 lb 12 oz (3.5 kg). The poor solubility of efavirenz in water requires high doses required for therapeutic activity, hence 800 mg daily is recommended (Gaur et al. 2014; Velmurugan et al. 2014). This results in several adverse effects associated with efavirenz including insomnia, liver failure, severe rash, nausea, diarrhoea, confusion, depression, hallucination and serious psychiatric effects such as suicidal ideation (Carr and Cooper 2000; Ramana et al. 2014).

Therefore, the development of a carrier such as liposomes is of interest for enhancing the solubility of efavirenz to provide possible targeted and controlled delivery of ARV which may result in improved bioavailability and reduced side effects of the molecule (Mallipeddi and Rohan 2010; Ochubiojo et al. 2012).

A number of reports addressing the encapsulation of efavirenz using different polymeric nano-material such as polymeric micelles (Chiappetta et al. 2010), solid lipid nano-particles (Makwana et al. 2015), nano-emulsions (Senapati et al. 2016) have been reported which have demonstrated improved bioavailability. To the best of our knowledge, there is no report for encapsulation of efavirenz in liposomes.

Liposomes are small artificial vesicles of spherical shape prepared using phospholipids and cholesterol, and have been extensively studied since the 1970's as delivery system (Gregoriadis and Florence 1993; Sathigari et al. 2009; Dwivedi and Verma 2013) Liposomes have improved the therapeutic index of established or emerging ARV through modification of absorption and metabolism resulting in a prolonged plasma half-life and reduced toxicity (Chowdary and Naresh 2011; Chowdary and Enturi 2013). Liposomes are commonly used as vehicles for poorly soluble drugs to facilitate intracellular delivery to infected cells (Zylberberg and Matosevic 2016).

Therefore, liposomal encapsulation of efavirenz was investigated and characterization undertaken using a variety of physicochemical techniques.

Materials and methods

Materials

The soybean lecithin used was purchased from Sigma Aldrich (Oakland, USA) and cholesterol were procured from Carlotiba/Divisione chemical (Brescia, Italy). Efavirenz, mono- and dibasic sodium phosphate, orthophosphoric acid, chloroform, methanol, acetonitrile (chromatographic grade) were purchased from Merck (Johannesburg, South Africa) and were used without further purification.

Validation study

The HPLC method for quantitation of efavirenz was validated for linearity, precision (inter and intra-day) and accuracy. The chromatographic system comprised an HP1100 Agilent LC-MSD (Foster City, USA), with a quaternary solvent delivery module fitted with an in-line degasser, DAD detector, MSD 1100, Chemstation software was used for the collection and analysis of data. A Zorbax Elipse plus C18 4.6 mm i.d. × 150 mm 5 µm column was used for HPLC analysis.

The chromatographic conditions were isocratic with a mobile phase of acetonitrile, HPLC grade water and orthophosphoric acid (70:30:0.1) at a flow rate of 1.0 ml/min, an injection volume of 20 µl and UV detection set at 252 nm. A standard stock solution of efavirenz was prepared in mobile phase at a concentration of 2000 µg/ml and serial dilutions with the mobile phase performed to produce solutions in the concentration range of 8–40 µg/ml. All the samples were filtered using a polyvinylidene difluoride (PVDF) 0.45 µm Millipore filter (Peoria, USA) prior to chromatographic experiments.

Validation experiments were conducted over 4 days and samples injected daily (n = 3) except for the 20 µg/ml standard that was injected six times daily. Samples were eluted within 7 min with a retention time for efavirenz of 5.254 min. Peak areas were plotted against concentration (8–40 µg/ml) to produce a calibration curve and for determination of the linearity and range assessed by evaluation of the regression equation and correlation coefficient. The percent recovery and relative standard deviation (RSD) were calculated to establish precision and accuracy, respectively. The limits of detection (LOD) and quantitation (LOQ) of the procedure was established using the standard deviation for the lowest concentration of the calibration curve to the regression equation three times for LOD and 10 for LOQ respectively (Branch 2005; Shabir et al. 2007; Nkanga et al. 2017).

Liposome manufacture

The lipid components viz., soybean lecithin and cholesterol were tested in different quantities as described in (Table 1). The lipids were dissolved in 1 ml chloroform in a clean, moisture free 25 ml round bottom flask. The solution was dried using a rotary evaporator R-205, (Buchi, Switzerland) at 200 rpm for 5 min. The thin film layer produced

Table 1 Lipid composition of liposomes

Formulation code	Lecithin amount (mg)	Cholesterol amount (mg)
F1	100	0
F2	75	25
F3	50	50

was stored in a desiccator overnight at room temperature of 22 ± 3 °C. The film was hydrated using 8 ml phosphate buffer pH 7.4 for 1 h at 60 °C and agitated at 400 rpm. A control solution of efavirenz without lipid was treated under the same conditions to determine the encapsulation efficiency (EE). Based on EE data, only formulations with the greatest %EE was selected for further evaluations and a blank or efavirenz-free liposome was prepared using the same preparation procedure for the purposes of comparative analyses.

Determination of encapsulation efficiency

The yellow liposomal suspension was transferred to a 50 ml centrifuge tube. The mixture was centrifuged at 4000 rpm using MSE Mistral 1000 (London, UK) centrifuge for 5 min to remove non-encapsulated efavirenz. The supernatant was decanted and homogenized by ultrasonic agitation using the water bath sonicator (Digital Cleaner, Spellbound 909, London, UK) at 60 °C for 1 h. The homogenized liposomes were freeze dried using the Apolo scientific Lyo Lab-300 (Johannesburg, South Africa) and stored at 4 °C in a desiccator until further analysis. The isolated pellets (viz., non-encapsulated efavirenz particles) were dissolved in 30 ml of the mobile phase of acetonitrile, and the resultant solution was subjected to HPLC analysis of efavirenz. For the assessment of encapsulation efficiency (EE), a positive control dispersion of efavirenz (without lipids) was produced following the liposome's preparation procedure "except addition of lipids". The resultant control sample was used for theoretical estimation of the total amount of efavirenz used in the formulation (Nkanga and Krause 2018). The validated HPLC method was used for determination of efavirenz concentration in each sample using efavirenz standard calibration curve. Prior to HPLC analysis, samples were diluted 1/100 using mobile phase and filtered through PVDF 0.45 µm Millipore filters. The encapsulation efficiency (%EE) was calculated using the following equation (Panwar et al. 2010; Costa et al. 2014).

$$\%EE = \frac{\text{Efavirenz total amount} - \text{Efavirenz pellet amount}}{\text{Efavirenz total amount}} \times 100 \quad (1)$$

Particle size analysis

Freeze-dried liposomes were dispersed in HPLC grade water at room temperature (22 ± 3 °C) for particle size, size distribution and Zeta Potential elucidation using dynamic light scattering (Nano Malvern Instrument, Worcestershire, UK) at a scattering angle of 173°.

Morphology of liposomes

The morphology of liposomes was determined using transmission electron microscopy (Carl Zeiss Libra120 kV, Oberkochen, Germany). The aqueous samples used for particle size and Zeta Potential were spotted onto a copper grid and left at room temperature overnight to dry prior to microscopic experiments.

Differential scanning calorimetry (DSC)

The thermal behaviour of pure efavirenz, efavirenz loaded liposomes and empty liposomes was investigated using a Perkin Elmer Model 6000 DSC (Waltham, USA). The DSC thermograms were recorded over the temperature range 50 to 175 °C at a heat flow rate of 10 °C/min under a nitrogen atmosphere set at a flow rate of 20 ml/min.

Fourier transform infrared (FTIR)

The FTIR spectra of pure efavirenz, efavirenz loaded liposomes and empty liposomes were collected using a Perkin Elmer Model 100 Spectrum FT-IR Spectrometer (Shelton, USA) at 8 scans over the wavenumber range 4000–650 cm^{-1}.

X-ray diffraction (XRD)

X-ray diffraction analysis of pure efavirenz, efavirenz loaded liposomes and empty liposomes was conducted to evaluate the crystalline nature of materials using a Bruker D8 Discover XRD (Berlin, Germany). The freeze-dried samples were placed into a sample holder, and the pattern generated and analysed in the 2-Θ range 10° to 80°. A cu-kα Discover equipped with a lynx Eye detector and a slit width of 1.1 mm and 6.0 mm was used for collection of all XRD spectra.

Energy dispersive spectroscopy (EDS)

The elemental surface composition of pure efavirenz, efavirenz loaded liposomes and empty liposomes were evaluated using a TESCAN thermionic emission SEM (VEGA3, Eching, Germany) connected to an INCA penta FET.

EFV in vitro release

Prior to evaluating EFV release, freeze-dried efavirenz loaded liposomes that had demonstrated the best %EE was evaluated for efavirenz content. A five 5 ml aliquot of the liposome dispersion used for DLS experiments was placed into a 25 ml A grade volumetric flask containing 10 ml methanol in order to disrupt the liposome structure and ensure dissolution of efavirenz. This solution was then made up to volume with HPLC grade water. The volumetric flask was then sonicated using a Digital Ultrasonic Cleaner, Spellbound 909 sonicator at 60 °C for 30 min and analysed using the validated HPLC method.

In vitro release was investigated using dialysis method described by (Dixit et al. 2010). A 3 ml aliquot of the liposome suspension equivalent to 6 mg efavirenz was transferred into a membrane weight cut off (MWCO) of 12,000–14,000D dialysis bag (DM70, Himedia) of pore size 2.4 nm placed into 150 ml phosphate buffer saline (PBS) pH 7.4 at 37 °C and stirred at 100 rpm. Samples (5 ml) were removed from the bulk solution at 0.5, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h analysis and replaced with an equivalent volume of fresh PBS. An aqueous solution of efavirenz raw material was also tested using the same conditions in order to evaluate EFV diffusion in comparison to release from liposomes. All experiments were conducted in triplicate.

Results and discussion

Validation studies

The calibration curve (shown in Supplementary Information Fig. 1) reveals a linear correlation between concentration and corresponding peak area with an $R^2 = 0.9998$. The LOD and LOQ were 0.017 µg/ml and 0.038 µg/ml respectively. The interday and intraday precision data are listed in (Table 2) expressed as a relative standard deviation (% RSD) arising from the efavirenz samples. The % RSD for intraday and interday precision were < 2% and 5% respectively. The HPLC method therefore possesses the necessary repeatability and intermediate precision (Geetha et al. 2012; Paithankar 2013) for use in development studies. The percent recoveries arranged between 92.31 and 103.27% indicating the accuracy of the method as the recoveries are within the range of 90–10% (Geetha et al. 2012).

Table 2 Relative standard deviation and recovery data of validation

Concentration (µg/ml)	Intraday precision (%RSD)	Interday precision (%RSD)	Recovery (%RSD)
10	0.77	2.26	103.27
15	0.48	1.14	98.55
20	0.26	0.73	92.42
25	0.16	0.52	101.34
30	0.82	1.76	96.34
45	0.14	0.16	99.28

Encapsulation efficiency (EE)

The encapsulation efficiency (EE) increased as the amount of cholesterol incorporated into the liposomes increased. The formulation containing 50 mg each of lecithin and cholesterol exhibited the greatest EE. The increase in EE may be attributed to the effect of incorporation of cholesterol (Pattni et al. 2015) suggesting that efavirenz is embedded within the lipid bilayers as it is hydrophobic. Cholesterol increases the hydrophobicity of the liposomal membranes and creates additional voids as the hydrophobic segment content is increased facilitates inclusion of additional efavirenz molecules into the lipid bilayers (Ramana et al. 2014).

Particle size, PDI and zeta potential

The particle size, PDI and zeta potential values are summarised in Table 3. The plots of size distribution by intensity are presented in Supplementary Information Fig. 2. The mean particle size looks promising for delivery of ARV as the liposomes are sufficiently small to permeate from the leaky vasculature and target infected blood cells (Limasale et al. 2015). All formulations exhibited PDI < 3.5, revealing that homogenization resulted in uniform particle sizes (Danaei et al. 2018). The encapsulation of EFV in liposomes influenced the particle size as did increases in the amount of cholesterol used. This suggests high embedment potential of solubilized EFV within the lipid bilayers, thereby increasing the particle size of the liposomes whereas the particle size of empty liposomes were much smaller than those containing EFV. The Zeta Potential were negative indicating a negatively charged surface on the liposomes that was > − 40 mV. This suggested that the formulations would be stable and aggregation of the liposomes was unlikely. The inclusion and encapsulation of EFV had some impact on the Zeta Potential of the liposomes and no correlation with use of increased amounts of cholesterol.

Table 3 Mean particle size, polydispersity index, Zeta Potential and encapsulation efficiency of EFV loaded liposomes

Formulations	Average size ± SD (nm)	PDI ± SD	ZP ± SD (mV)	EE ± SD (%)
F1	172.72 ± 2.11	0.301 ± 0.02	− 49.77 ± 1.75	20.13 ± 2.225
F2	332.32 ± 17.04	0.337 ± 0.12	-58.82 ± 1.21	56.82 ± 1.654
F3	411.10 ± 7.40	0.319 ± 0.06	−53.53 ± 0.06	98.86 ± 0.010
Empty liposome	70.29 ± 0.53	0.272 ± 1.84	-41.82 ± 1.86	NA

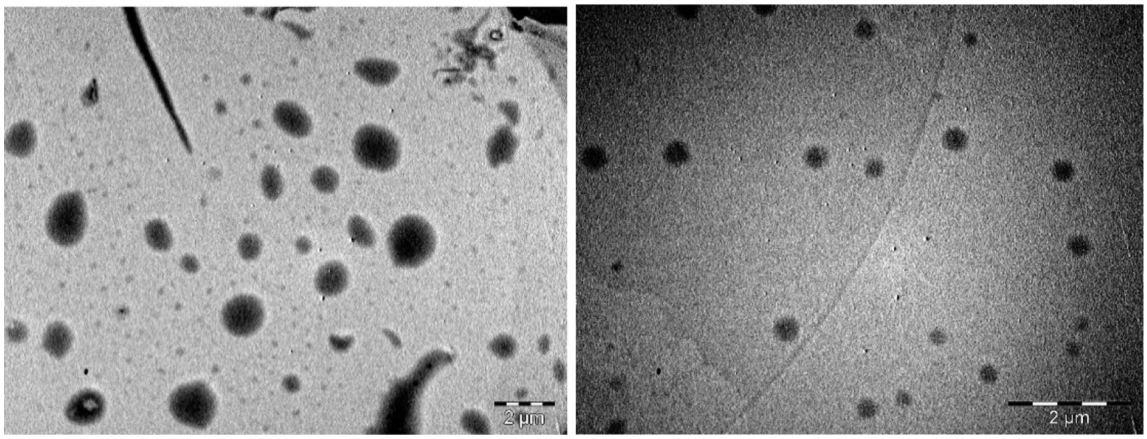

Fig. 1 TEM images of EFV loaded liposomes

Morphology

TEM analyses (Fig. 1) revealed the presence of nanosized particles spherical in shape. The nanoparticles were individually and homogenously dispersed suggesting the presence of liposomal vesicles with high surface charges as revealed with Zeta Potential measurements resulting in dispersion due to particle repulsion.

EFV in vitro release

The results of in vitro release studies conducted in PBS pH 7.4 are presented in Fig. 2. Over 72 h of experiment, EFV loaded liposomes exhibited approximately 63% EFV whereas the dissolution profile of raw EFV exhibited 79% release using the dialysis bag method. The two dissolution profiles tend to describe a biphasic pattern of release in which a sudden increase in percentage release over the first 10 h followed by slower or sustained release behaviour over the remainder of the time. However, the release data showed no significant difference between the efavirenz-loaded liposomes and free efavirenz. This behaviour could be due to the structural specificity of efavirenz as a hydrophobic compound and has been previously observed by other authors who investigated efavirenz release from microemulsion (Hemal et al. 2015). Since there is no other report discussing efavirenz release from liposomes, and there is

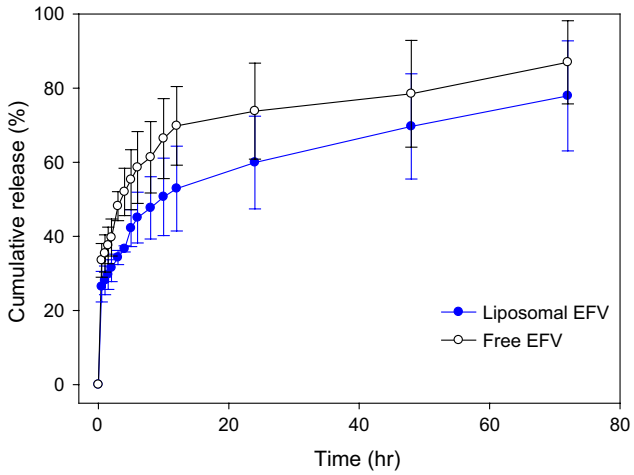

Fig. 2 EFV release from liposomes and raw material

no other study describing similar drug diffusion profiles between a free hydrophobic drug and its liposomal formulations, our findings encourage future biological evaluation of liposomes containing efavirenz. A comparative assessment of antiretroviral efficacy between free and drugs loaded liposomes would provide insights into the rate of drug diffusion in the two cases, which will allow to confirm the present observation.

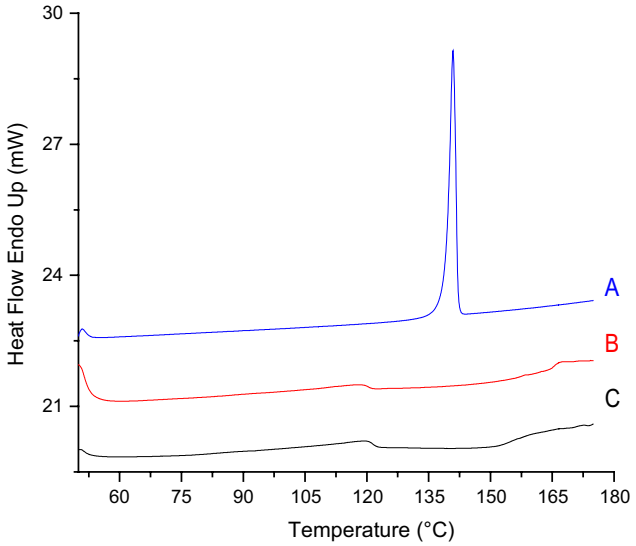

Fig. 3 DSC thermograms of A pure EFV, B EFV loaded liposomes, C empty liposomes

Differential scanning calorimetry (DSC)

DSC studies were performed to investigate the physical state of EFV encapsulated in the liposomes during continuous heating. DSC data of the pure EFV, EFV loaded liposomes and empty liposomes is depicted in Fig. 3. The presence of a sharp endothermic peak at approximately 138 °C for raw material indicates its crystalline nature. The absence of an endothermic peak in the DSC for EFV loaded liposomes suggests all components were in an amorphous state since the melting peaks of empty liposomes were not evident.

X-ray diffraction

The XRD data for pure efavirenz, efavirenz loaded liposome and empty liposomes are depicted in Fig. 4. From these data, it appears that empty liposomes are amorphous in nature whereas both EFV loaded liposomes and EFV raw material exhibited some sharp peaks that indicate the presence of crystalline material. Despite the persistence of some crystalline peaks due to EFV in liposomes with many peaks due to EFV absent. The data suggests that changes in the physical state of EFV occurs and is corroborated by DSC data which suggests the loss of crystallinity following encapsulation in liposomes. The loss of crystallinity due to liposomal encapsulation has been reported as evidence of molecular interactions within the lipid bilayers in which the cargo molecule is trapped (Nkanga et al. 2018).

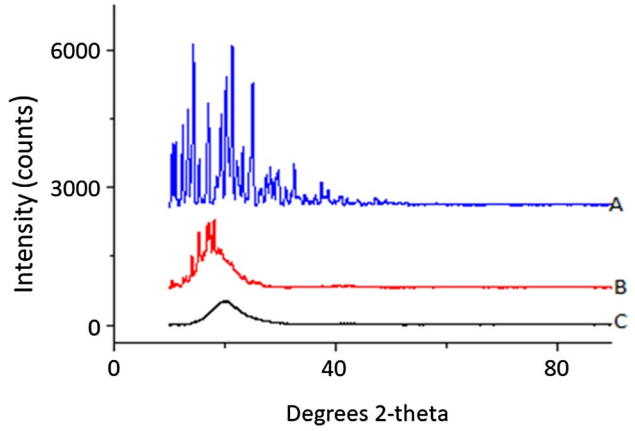

Fig. 4 XRD patterns of A pure EFV, B EFV loaded liposomes, C empty liposomes

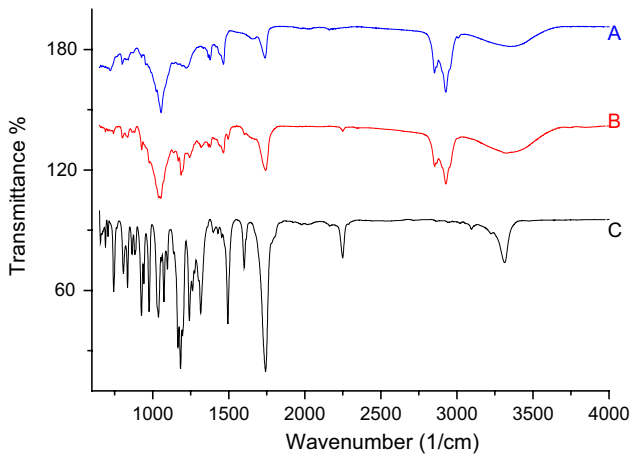

Fig. 5 FTIR spectra of A empty liposome, B EFV loaded liposome, C pure EFV

Fourier transform infrared (FTIR)

FTIR spectra of pure EFV, EFV loaded liposome and empty liposome are presented in Fig. 5. The FTIR spectrum of efavirenz raw material exhibited characteristic peaks at 689 and 652 cm^{-1} (–CF Stretch), 1096, 1057, 1074 cm^{-1} (C–O–C Stretch), 1492 cm^{-1} (C≡C) of benzene ring stretching vibration, 1742 cm^{-1} (C=O Stretching vibration) and 3314 cm^{-1} (–NH Stretch vibration) (Gaur et al. 2014). The spectrum of EFV loaded liposomes exhibited some of characteristics peaks of EFV in the same spectral regions, in addition to peaks due to the lipid components that appeared at 2911–2931 cm^{-1} and 1455–1468 cm^{-1} for the alkane C–H stretches, at 1248 cm^{-1} for the ester sigma bonds and at 1103–1110 cm^{-1} for the ether region (Senapati et al. 2016). In combination with the FT-IR spectrum of empty liposomes, it appears that there are no major EFV-lipid

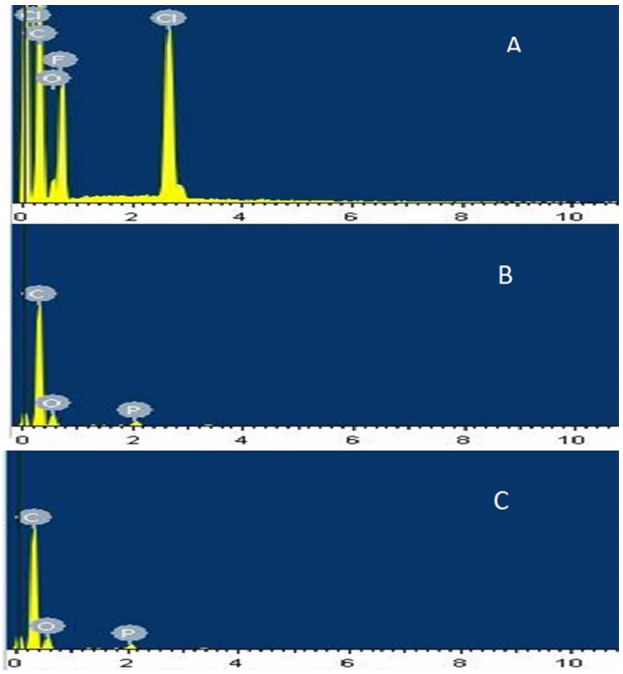

Fig. 6 EDS spectra of A pure EFV, B EFV loaded liposome, C empty liposome

interaction in loaded liposomes, suggesting that encapsulation did not affect the chemical structure of EFV (Gaur et al. 2014).

Energy dispersive spectroscopy (EDS)

The elemental surface composition of pure EFV, EFV loaded liposomes and empty liposomes are depicted in Fig. 6. The elemental composition of EFV raw material was C, O, Cl and F. However, none of these elements were found on the surface of EFV loaded liposomes, which exhibited a similar composition with empty liposomes viz., C, O and P suggesting that no EFV was located on the surface of liposomes due to its hydrophobic nature which result in it being sequestered into the lipid bilayers of the liposome (Nkanga and Krause 2018).

Conclusion

The encapsulation of efavirenz, a hydrophobic non-nucleoside transcriptase inhibitor in crude soybean lecithin liposomes was successfully achieved. An encapsulation efficiency of 98.8% was achieved revealing the solubilizing effect of crude soybean that can solve the challenge of poor solubility of efavirenz. Furthermore, efavirenz loaded liposomes exhibited nanoparticulate characteristics, with a particle size of 411 nm and a Zeta Potential of − 54 mV, which looks promising for the targeted delivery of EFV to the infected lymphocytes. In vitro release studies revealed the potential for sustained release of EFV and for specific site delivery. The potential benefit of the liposomal efavirenz includes the possibility for surface modification of the resultant nano-system (efavirenz-loaded liposomes), for site targeting. This will minimize side effects of efavirenz as a result of poor solubility and wide spread delivery to non-infected cells. At this stage, antiviral efficacy evaluation of this mono-drug formulation could not be considered due to the alarming issue of drug resistance, of which antiretroviral drugs (ARVDs) are commonly used in combination therapies. However, our labs are currently investigating the possibility of adding one or more ARVD to the formulation under development. The multi-drug loaded liposomes will be prepared and subjected to animal studies for anti-HIV efficacy assessment since these formulations will meet the requirement for multidrug therapy that is currently recommended for HIV management. Since, to our knowledge, no evidence of the liposomal encapsulation of EFV has been reported, the insights from the present study will be valuable as a set of preliminary data. This will encourage further investigations of the encapsulation and delivery of EFV like antiretrovirals for enhanced solubility, site targeting and prolonged release using liposomes. We are currently working on surface modification of liposomes in order to delay EFV release (coating liposomes with self-adhesive polymers, like chitosan and alginate) and extend liposomes blood circulation time (by grafting polyethylene glycol).

Acknowledgements The authors wish to acknowledge the National Research Foundation South Africa through the DST innovation, Rhodes University, and Sundra Cleaning Company for their financial assistance.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

Statement of Human and animal rights This article does not contain any studies with human and animal subjects performed by any of the authors.

References

Branch SK (2005) Guidelines from the international conference on harmonisation (ICH). J Pharm Biomed Anal 38:798–805

Carr A, Cooper DA (2000) Adverse effects of antiretroviral therapy. The Lancet 356:1423–1430

Chiappetta DA, Hotcht C, Taira C, Sosnik A (2010) Efavirenz—loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significant higher oral bioavailability. Nanomedicine 5:11–23

Chowdary KPR, Enturi V (2013) Preclinical pharmacokinetic evaluation of efavirenz solid dispersions in two new modified starches. J Appl Pharm Sci 3:89–92

Chowdary KPR, Naresh A (2011) Formulation development of efavirenz tablets employing β cyclodextrin-PVP K30-SLS: a factorial study. J Appl Pharm Sci 1:130–134

Costa AP, Xu X, Burgess DJ (2014) Freeze-Anneal-Thaw cycling of unilamellar liposomes: effect on encapsulation efficiency. Pharm Res 31:97–103

Danaei M, Dehghankhold M, Ataei S, Davarani FH, Javanmard R, Dokhani A, Mozafari RM (2018) Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics 10:1–17

Dixit AR, Rajput SJ, Patel S (2010) Preparation and bioavailability assessment of SMEDDS containing valsartan. Am Assoc Pharm Sci 11:314–321

Dwivedi C, Verma S (2013) Review on preparation and characterization of liposomes with application. J Sci Innov Res Rev 2:2320–4818

Gaur PK, Mishra S, Bajpai M, Mishra A (2014) Enhanced oral bioavailability of efavirenz by solid lipid nanoparticles: in vitro drug release and pharmacokinetics studies. Biomed Res Int 9:1–9

Geetha G, Naga KGR, Kumar BV, Raja MG (2012) Analytical method validation: an updated review. Int J Adv Pharm Biol Chem 1:64–71

Gregoriadis G, Florence AT (1993) Liposomes in drug delivery; clinical, diagnostic and opthalmic potential. Drugs 45(I):15–28

Gupta U, Jain NK (2010) Non-polymeric nano-carriers in HIV/AIDS drug delivery and targeting. Adv Drug Deliv Rev 62(4–5):478–490

Hemal T, Patel P, Jani P (2015) Preparation and study of efavirenz microemulsion drug delivery system for enhancement of bioavailability. Eur J Pharm Med Res 5:1156–1174

Li X, Chan WK (1999) Transport, metabolism and elimination mechanisms of anti-HIV agents. Adv Drug Deliv Rev 39:81–103

Limasale YDP, Tezcaner A, Özen C, Keskin D, Banerjee S (2015) Epidermal growth factor receptor-targeted immunoliposomes for delivery of celecoxib to cancer cells. Int J Pharm 479:364–373

Makwana V, Jain R, Patel K, Nivsarkar M, Joshi A (2015) Solid lipid nanoparticles (SLN) of Efavirenz as lymph targeting drug delivery system: elucidation of mechanism of uptake using chylomicron flow blocking approach. Int J Pharm 495:439–446

Mallipeddi R, Rohan LC (2010) Progress in antiretroviral drug delivery using nanotechnology. Int J Nanomed 5:533–547

Nkanga CI, Krause RWM (2018) Conjugation of isoniazid to a zinc phthalocyanine via hydrazone linkage for pH-dependent liposomal controlled release. Appl Nanosci 8:1313–1323

Nkanga CI, Krause RWM, Noundou XS, Walker RB (2017) Preparation and characterization of isoniazid-loaded crude soybean lecithin liposomes. Int J Pharm 526:466–473

Nkanga CI, Walker RB, Krause RWM (2018) pH—dependent release of isoniazid from isonocotic acid (4-hydroxyl-benzylidene)-hydrazide loaded liposomes. J Drug Deliv Sci Technol 45:264–271

Ochubiojo ME, Chinwude I, Ibanga E, Ifianyi S (2012) Nanotechnology in drug delivery. In: Demir SA (ed) Recent advances in novel drug carrier systems, 3rd edn. IntechOpen, London, pp 70–106

Ojewole E, Mackraj I, Naidoo P, Govender T (2008) Exploring the use of novel drug delivery systems for antiretroviral drugs. Eur J Pharm Biopharm 70:697–710

Paithankar HV (2013) Hplc method validation for pharmaceuticals: a review. Int J Univ Pharm BioSci 2:229–240

Panwar P, Pandey B, Lakhera PC, Singh KP (2010) Study of albendazole-encapsulated nanosize liposomes. Int J Nanomed 5:101–108

Pattni BS, Chupin VV, Torchilin VP (2015) New developments in liposomal drug delivery. Chem Rev 115:10938–10966

Ramana LN, Sethuraman S, Ranga U, Krishnan UM (2010) Development of a liposomal nanodelivery system for nevirapine. J Biomed Sci 17:1–9

Ramana LN, Anand AR, Sethuraman S, Krishnan UM (2014) Targeting strategies for delivery of anti-HIV drugs. J Control Release 192:271–283

Sathigari S, Chadha G, Lee YHP, Wright N, Parsons DL, Rangari VK, Babu RJ (2009) Physicochemical characterization of efavirenz–cyclodextrin inclusion complexes. AAPS PharmSciTech 10:81–87

Senapati PC, Sahoo SK, Sahu AN (2016) Mixed surfactant based (SNEDDS) self-nanoemulsifying drug delivery system presenting efavirenz for enhancement of oral bioavailability. Biomed Pharmacother 80:42–51

Shabir GA, John Lough W, Arain SA, Bradshaw TK (2007) Evaluation and application of best practice in analytical method validation. J Liq Chromatogr Relat Technol 30:311–333

Shah LK, Amiji MM (2006) Intracellular delivery of saquinavir in biodegradable polymeric nanoparticles for HIV/AIDS. Pharm Res 23:2638–2645

The Joint United Nation Programme on HIV/AIDS (UNAIDS) (2019) Fact Sheet - Global Aids Update

Velmurugan S, Ali MA, Kumar P (2014) Microparticulate drug carriers: a promising approach for the delivery of anti HIV drugs. Int J Pharm Pharm Sci 6:31–39

Zylberberg C, Matosevic S (2016) Pharmaceutical liposomal drug delivery: a review of new delivery systems and a look at the regulatory landscape. Drug Deliv 23:3319–3329

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.