Research article

Nanoformulated Eudragit lopinavir and preliminary release of its loaded suppositories

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ARTICLE INFO

ABSTRACT

The development of novel paediatrics formulations is critical towards achieving the UNAIDS 90-90-90 targets. According to the latest UNAIDS reports, the availability of antiretrovirals (ARVs) for children has increased significantly, from 49% in 2015 to 53% in 2017. However, this percentage is considerably lower than the 80% for pregnant women that are currently on treatment. Therefore, there is still an urgent need for an alternative child-friendly delivery system. Lopinavir (LPV) is a protease inhibitor first-line HIV treatment drugs but suffers from low aqueous solubility, bitter state, short half-life leading to a limited dissolution and variable bioavailability upon oral administration. This work focused on the fabrication and characterization of a delivery system entailing Eudragit RSPO-LPV nanoparticles loaded suppositories in two different bases to improve the bioavailability and overcome the problem encountered through oral administration emanating from poor solubility. The prepared nanoparticles by nanoprecipitation method were characterized and compounded into suppositories in fattibase and polyethylene glycol (PEG) bases using a melt fusion method. The suppositories were stored at 5 and 25 °C, and were sampled at 0, 4, 8, 12 weeks. The samples were assessed by particle size, entrapment efficiency (EE), zeta potential and polydispersity index (PDI) variations. The preliminary *in vitro* release studies were analysed by HPLC. The nanoparticles have an average particle size of 191 nm with spherical morphology, entrapment efficiency, polydispersity index and zeta potential of 79.0 ± 0.5%, 0.224, and 25.87 ± 0.41 mV respectively. The surface analysis of the nanoparticles with FTIR, SEM, PXRD and TGA indicated that the drug was truly encapsulated without any interaction. The *in vitro* release studies showed that a better release was observed in suppositories formulated with PEG than the fattibase by having higher drug concentration released. Hence, this rectal formulation might serve as an alternative for paediatric HIV treatment upon further investigation.

1. Introduction

Human immunodeficiency virus (HIV) is regarded as a major global epidemic and a serious threat to public health, especially in many developing countries (Lloyd-Sherlock et al., 2014). The recent statistics showed that 36.9 million people were living with HIV, 35.1 million adults and 1.8 million children, respectively (UNAIDS, 2018). Paediatric HIV infection in children remains a significant health issue globally (Newell et al., 2004). Although, the survival of HIV-infected children has improved with increased access of 51% receiving treatment at the end of 2017 when compared to 80% of HIV-infected pregnant women on treatments (UNAIDS, 2018). These populations of children on treatment were still not acceptable when compared to the ratio of infected children, which is high. Therefore, drastic actions must be taken into cognizance in improving paediatric HIV treatment in order to meet the millennium development goal of 90–90–90 target of eradicating HIV prevalence by 2030 (Joint United Nations Programme on HIV/AIDS (UNAIDS), 2017).

One of the foremost leading components of highly active antiretroviral therapy (HAART) applied for the treatment of HIV infections in both adults and children is LPV. LPV, as depicted in Figure 1, is a potent protease inhibitor (Maartens et al., 2014; Khan et al., 2019). It is currently being used, as a combination therapy with ritonavir (RTV) with limited oral route therapy challenges (Pham et al., 2016). This current treatment which is based on oral administrations, has been shown to
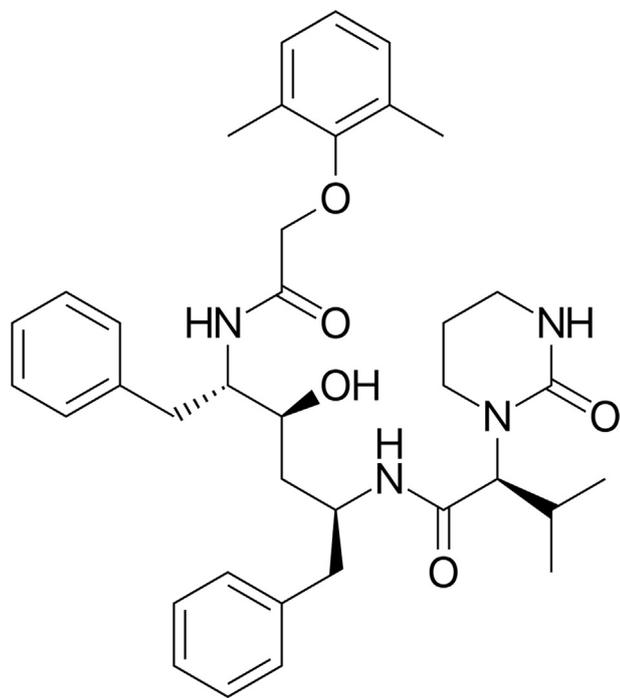
Figure 1. The chemical structure of lopinavir.

suffer from limited bioavailability when administered orally with low aqueous solubility (0.01 mg/mL) (Pham et al., 2016). In particular, most of these drugs are unbearable due to their bitter taste, high alcoholic concentration, hepatic first-pass effect and gastrointestinal side effects (Van Riet-Nales et al., 2012; Lallemant et al., 2011). Therefore, various studies have been done where nanotechnology techniques have been explored extensively to improve specific issues associated with LPV solubility, bioavailability and half-life (Abou-El-Naga et al., 2017; Joshi et al., 2016).

Garima and co-worker prepared LPV-loaded poly lactic-co-glycolic acid (PLGA) nanoparticles (NPs) using nanoprecipitation method, and their results showed an improved bioavailability and control drug release potential without co-administration with ritonavir (Joshi et al., 2016). However, oral administration is mostly suitable for adults when considering the formulations on the market with all the shortcomings which are an indicator that children are not in cognizance consideration when they are prepared (DNDI, 2018). Thereby, arises the call for formulations that will overcome these challenges encountered using oral and intravenous administration which will be highly acceptable to the children and the caregivers through other routes. The rectal route is proposed as alternative routes. This is due to its ease of administration (Van Riet-Nales et al., 2012), which resulted in paediatric clinical studies that proposed its equivalence as a promising alternative route for the treatment of children (Sarmento and das Neves, 2012).

Few studies have been reported on the usage of the rectal delivery system especially for antiretroviral (ARV), such as zidovudine (Priya et al., 2015b), dapivirine (Das Neves et al., 2013a), stavudine,

lamivudine and nevirapine (Padmavathi et al., 2015) which demonstrated a controlled release of the ARVs. It is worth noting that no studies have been carried out on formulating LPV nanoparticles (LPV-NPs) into suppositories. Likewise, there is no LPV suppository in the market, and no information exists on their rectal availability. There is an urgency to formulate a child-friendly delivery system, with well tolerable properties as a crucial need for children living with HIV (Ham et al., 2017; Schlatter et al., 2016). This proposed formulation is to overcome the challenges of poor palatability, swallowing, regimen complexity, storage and transportation exhibited by oral formulated ARVs. In the present study, a modified nanoprecipitation method was used to prepare Eudragit RSPO-LPV nanoparticles (Nagavarma et al., 2012a; Lepeltier et al., 2014). The fabricated nanoparticles were then characterized and loaded into suppositories by fusion method (Akin-Ajani et al., 2019) and analyzed for *in vitro* released studies.

2. Materials and methods

2.1. Materials

Eudragit RSPO was donated by Evonik Rohm, GmbH, (Germany). Lopinavir with 98 % purity was purchased from DB Fine chemicals (South Africa). The Pluronic F-127 and the polyethylene glycol (PEG 1500 & 3350) were purchased from Sigma Aldrich USA. Pharmaceutical excipients, namely, Fattibase™ was purchased from Paddocks Laboratories, Minneapolis, (USA). Potassium hydrogen phosphate (KH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl) and sodium chloride (NaCl) were purchased from Sigma Aldrich (South Africa). Acetonitrile and methanol were purchased from Merck (South Africa), and hydrochloric acid was purchased from Glassworld (South Africa). All other chemicals and reagents were of analytical grade.

2.2. Preparation of lopinavir nanoparticles

Eudragit RSPO nanoparticles containing LPV were prepared by a modified nanoprecipitation method (Nagavarma et al., 2012a; Lepeltier et al., 2014). The drug and polymer were accurately weighed and dissolved in 5 mL mixture of ethanol and acetone (1:1) respectively at room temperature. The drug and polymer 25 mg and 50 mg (1:2) respectively were mixed using a magnetic stirrer for an hour (Nagavarma et al., 2012a; Lepeltier et al., 2014). Thereafter, the organic phase (drug and polymer) was added dropwise into the 1% surfactant (Pluronic F-127) by a syringe under the high stirring speed of 15000 rpm using Omni PDH (USA) homogenizer for 10 min. After evaporation of the organic solvent from the internal phase in a fume hood for 12 h at 700 rpm using IKA RT10 Power 10-position magnetic hot plate (Germany). The precipitated loaded nanoparticles were isolated by using a centrifuge at 13500 rpm for 15 min and washed three times with distilled water using Hermle Z 326 K (Germany). This is to remove the surfactant in order to obtain surfactant free nanoparticles, the supernatant from the first cycle was taken and measured the absorbance at 259 nm to calculate the encapsulation efficiency (EE). This is calculated by determining the amount of non-encapsulated LPV in the aqueous surfactant solution, against the total amount of drug added to the formulation using a standard calibration curve. The produced suspension was freeze dried for 48 h at
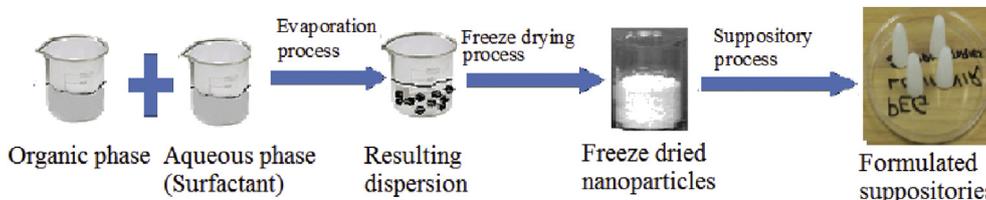

Figure 2. Diagrammatic representation of the preparation of Eudragit RSPO-LPV suppositories.

Table 1. Eudragit RSPO-LPV nanoparticles suppositories formulation compositions.

S/N	Ingredients	F1 (%)	F2 (%)
1	LPV NPs	100	100
2	PEG 1500	25	
3	PEG 3350	75	
4	Fattibase		100

-40°C to obtain a fine powder nanoparticle using Christ Alpha plus 1–4 (Germany) freeze dryer. This method is indicated in the schematic diagram in (Figure 2).

2.3. Preparation of suppositories

The Eudragit RSPO-LPV suppositories were prepared using fattibase and PEG mixtures (1500 & 3350) as two bases. It was prepared by the fusion method using metal moulds (Akin-Ajani et al., 2019). The displacement value of each base was determined, and the amount of Eudragit RSPO-LPV required for each suppository formulation was calculated (Akin-Ajani et al., 2019). The mixture of the melted bases and the nanoparticles were thoroughly mixed to ensure homogenous mixture before pouring them into the moulds, which resulted in uniform distribution with uniform appearances. Therefore, two different types of formulation (F1 & F2) were made with four ingredients and each suppository containing an equivalent amount of the drug in each one-gram suppository as shown in Table 1.

2.4. Particle size, particle size distribution and zeta potential

The Eudragit RSPO-LPV nanoparticles were characterized in terms of mean particle size diameter, the polydispersity index (PDI) and the zeta potential using Malvern Zetasizer Nano ZS (Malvern instrument, United Kingdom) installed with DTS software by a dynamic light scattering (DLS)-based method (Salatin et al., 2017a, b). The nanoparticles formulations were sonicated in distilled water to minimize the interparticle interactions before analysing. It was analysed three times with the Nano ZS instruments to obtained average results.

2.5. Encapsulation efficiency

The encapsulation efficiency (EE) was determined by UV-vis spectrophotometer (Merck Spectroquant® Prove 300, Germany). The prepared Eudragit RSPO-LPV nanoparticles were isolated using a centrifuge at 13500 rpm for 10 min through the supernatant obtained, which was analysed. The EE of the nanoparticles was calculated using the following equation.

$$EE = \frac{Wt - W_f}{Wt}$$

W_t is the total amount of pure LPV and W_f is the total amount of free LPV in the supernatant (Zhang et al., 2019b; Matlhola et al., 2015b).

2.6. Fourier transmission infra-red spectroscopy

The drug excipients compatibility studies of the Eudragit RSPO-LPV nanoparticles, Eudragit RSPO and LPV were obtained by using an Agilent Technologies Cary 600 Series FTIR Spectrometer (USA). The samples were dispersed in dry potassium bromide (KBr). The spectra were run between the range 4000 cm^{-1} – 500 cm^{-1} (Song et al., 2008).

2.7. Scanning electron microscopy

The morphological studies of the Eudragit RSPO-LPV nanoparticles, Eudragit RSPO and LPV, were examined by scanning electron microscope (FEI Quanta 250 FEG SEM, UK) operating at 10 kV. The nanoparticles were dusted onto double-sided tape on an aluminium stub and coated with gold using a cold sputter coater prior to imaging. Coated samples were then scanned, and photomicrographs were taken (Esmaeili et al., 2015).

2.8. X-ray diffraction

Crystallinity phase identification of Eudragit RSPO-LPV nanoparticles, Eudragit RSPO and LPV were carried out on PANalyticalX'pert Pro (PANalytical, Almelo, Netherlands). The sample was placed in a glass sample holder, irradiated and measured under the following conditions: Anode, Cu; Kα1, 1.5405 Å; Kα2, 1.54443 Å; K-Beta, 1.39225 Å; Kα1/Kα2 ratio, 0.5; Generator settings, 40 mA, 45 kV; divergence slit, 0.957°, fixed; step size, 0.017° in 2θ; scan step times, 19.685 s; temperature, 25 °C. The data was analyzed using X'Pert Data Collector software version 4.0A.

2.9. Differential scanning calorimetry

Thermal characteristics of Eudragit RSPO-LPV nanoparticles, Eudragit RSPO and LPV were carried out using a differential scanning calorimeter (DSC with software star 'e' Mettler Toledo, Greifensee, Switzerland) at a heating rate of 10 °C/min over a temperature range of 30–400 °C under an inert atmosphere with a nitrogen gas flow of 35 mL/min.

2.10. Thermogravimetric

Thermogravimetric studies of Eudragit RSPO-LPV nanoparticles, Eudragit RSPO and LPV were characterized using a Mettler DTG 3+ (Mettler Toledo, Greifensee, Switzerland) instrument. Powder samples, weighing about 5–8 mg was placed in aluminium crimp cells, open or sealed (100 µl) and heated to an end temperature range of 30–400 °C, at a heating rate of 10 °C/min, with a nitrogen gas flow of 35 mL/min.

2.11. Stability studies

Stability studies of prepared Eudragit RSPO-LPV nanoparticles were assessed according to International Conference on Harmonization (ICH) Q1A (R2) guidelines (ICH Harmonized Tripartite Guideline, 2003). Briefly, Eudragit RSPO-LPV nanoparticles were stored in sealed glass vials at 25 °C/60 ± 5% relative humidity in the stability chamber (Remi, Mumbai, India). Control samples were stored at 5 °C in a refrigerator. This was carried out on the basis of particle size, entrapment efficiency (EE), zeta potential and polydispersity index (PDI) variations for three months. For products, adequate shelf-life, dosage forms and active ingredients must be stable chemically and physically for extended periods.

2.12. In vitro drug release

The in vitro drug release of Eudragit RSPO-LPV suppositories were carried out by USP rotating basket dissolution apparatus (Ramadan, 2013a). Each suppository was placed in the basket and was lowered to a height 5 mm from the bottom of the vessel containing 100 mL, citric acid/phosphate buffer (50 mM) solution pH 7.4 at a temperature range 37 °C and rotation of 75 rpm. At the predetermined time interval of, 0, 5, 10, 15, 30, 45, 60 and 90 min, 3 mL aliquot was withdrawn and analysed by HPLC for the concentration of drug released. The dissolution medium was replaced by an equal volume of fresh buffer dissolution media after each withdrawal to maintain the total volume. The drug release data were normalized by converting drug concentration in solution to a percentage of the cumulative drug release. An HPLC system Agilent 1200 series; Agilent Technologies Inc., Santa Clara, CA (USA) composes of quaternary pump, degasser, autosampler, Phenomenex C18 RP column (5 ìm packing, 4.6 × 150 mm), Phenomenex C18 RP guard column, and
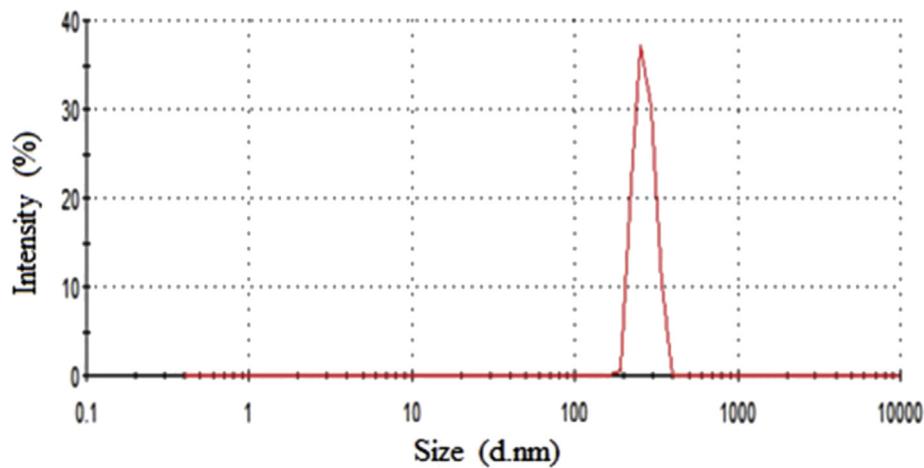
Figure 3. Particle size distribution by intensity as a function of particle size for Eudragit RSPO-nanoparticles.
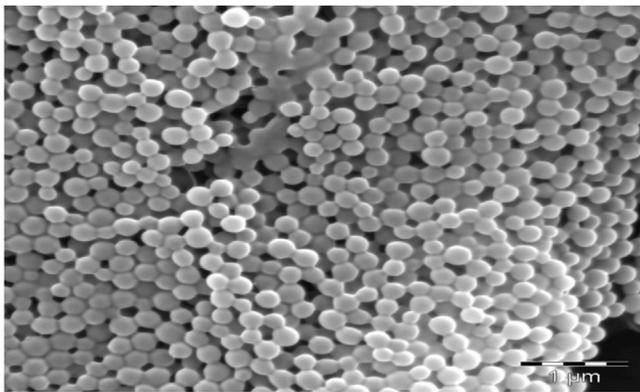

Figure 4. SEM micrograph of Eudragit RSPO-LPV nanoparticles.

diode array detector was employed. The mobile phase was composed of a mixture of 0.1 M methanol and phosphate buffer in the ratio of 85:15. The method was developed and stabilized for 1 h with the mobile flow rate maintained at 1.0 mL/min, with baseline monitoring prior to actual analysis. The column temperature was maintained at 25 °C, and the detection was performed at 259 nm.

3. Results and discussion

3.1. Particle size, particle size distribution and zeta potential

Eudragit RSPO-LPV exhibited a uniform size distribution and the average particle diameters of approximately 190.9 ± 0.21 nm and polydispersity index 0.224 (Figure 3), which was found to be almost similar to the value of drug-loaded nanoparticles of 180.6 ± 2.32 nm obtained through LPV-loaded polylactic-co-glycolic acid (PLGA) nano-particles (NPs) (Joshi et al., 2016). Eudragit RSPO being a positively charged polymer imparts cationic nature with values ranging from 25.6 to 26.0 mV, similar to the value obtained in RHT-Eudragit nanoparticles (Salatin et al., 2017a, b). It is a well-established fact that a zeta potential with greater absolute value is an indicator of higher and better stability of the colloidal systems (Hans and Lowman, 2002; Wang and Keller, 2009). Therefore, symbolising a stable formulation developed.

3.2. Encapsulation efficiency

The entrapment efficiency of 79.0 ± 0.5% was produced, which is similar to the results obtained in other LPV nanoparticles researches carried out (Patel et al., 2016; Ravi et al., 2014). It is a significant parameter in clinical applications and predominantly depends on the ability of the compound to dissolve in the matrix material or polymer
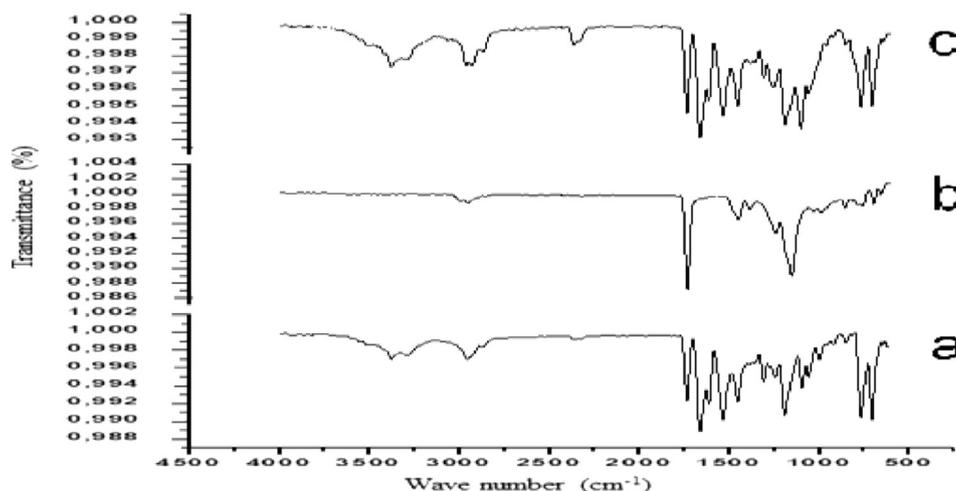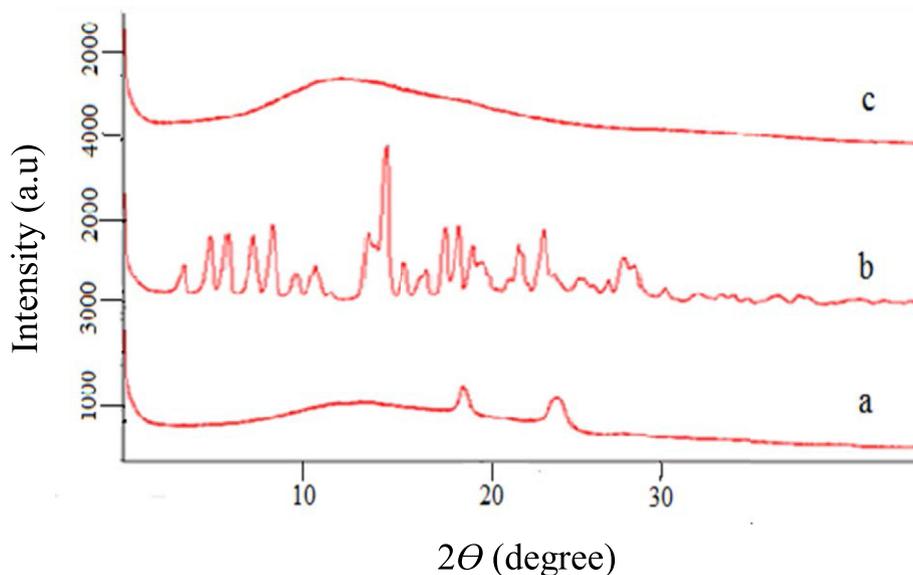

Figure 5. FTIR spectrum of (a) Eudragit RSPO-LPV nanoparticles, (b) LPV, (c) Eudragit RSPO.

Figure 6. XRD results where the y-axis represents counts per second and x-axis is theta degree; (a) Eudragit RSPO-LPV nanoparticles, (b) LPV, (c) Eudragit RSPO.

(dissolution or solid dispersal) (Kumari et al., 2010a). The entrapment efficiency obtained, which is probably due to the lipophilic nature of the drug results in the high interaction between the drug and the polymer (Varshney and Tanwar, 2010b; Mandal, 2010).

3.3. Scanning electron microscopy

Scanning electron microscopy studies in Figure 4, revealed that Eudragit RSPO-LPV nanoparticles were spherical when compared with the morphology of LPV with irregular crystals indicative that the drug was entrapped within the carrier (Ravi et al., 2015). The size distribution has average particles mean diameter of 190.9 ± 0.21 nm.

3.4. Fourier transform infrared spectroscopy

Infrared spectroscopy spectra, as shown in Figure 5, was performed to identify the compatibility of LPV with Eudragit RSPO. The FTIR spectra of LPV shows major characteristic absorption peaks at 3373.02 cm^{-1} (O–H stretching, 2953.11 cm^{-1} (C–H$_2$ stretching) and 1658.62 cm^{-1}(amide bond C=O stretching) and 1531.68 cm^{-1} (urea C=O stretching) and 1352.68 cm^{-1} (C–N) which are in agreement with the

reported values from preparation and evaluation of metastable solid-state forms of LPV (Lemmer and Liebenberg, 2013). All physical characteristics absorption peaks of LPV were retained in Eudragit RSPO-LPV nanoparticles without a major shift in the structure of Eudragit polymer as a result of ionic electrostatic interaction between the drug and the polymer during the formation of nanoparticles.
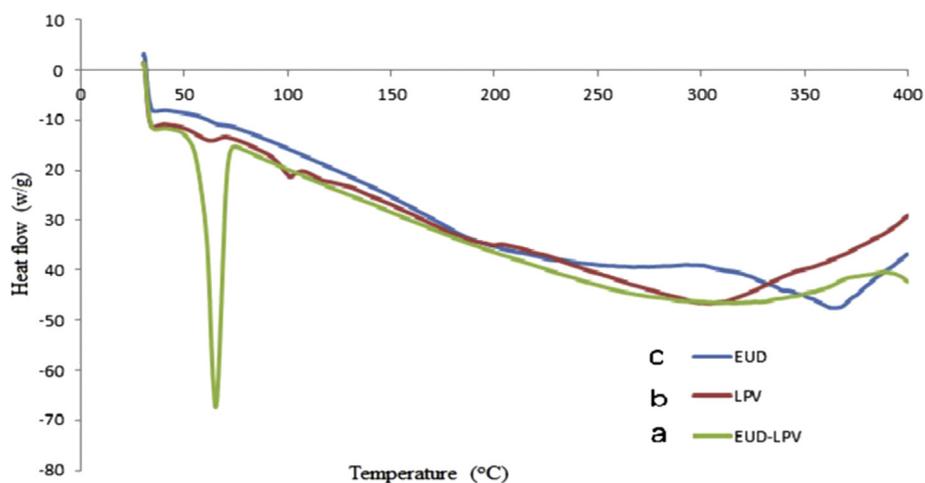
The red shift in the peak of the drug carbonyl from 1658.62 cm^{-1} to 1731.84 cm^{-1} may be attributed to the hydrogen bonding interaction between LPV O–H group and the Eudragit C=O groups in the Eudragit RSPO-LPV nanoparticles, which confirmed the compatibility of LPV with Eudragit polymers. These peaks confirmed that there was no interaction between the drug and other components.

3.5. X-ray diffraction

X-ray diffractogram pattern of the LPV, Eudragit RSPO, Eudragit RSPO-LPV nanoparticles is shown in Figure 6. LPV has shown several diffraction crystal peaks at 2θ 7 to 29.5^{o}, indicating crystallinity (Khan et al., 2019) while the Eudragit RSPO-LPV nanoparticles showed a broad, amorphous peak intensifying that there were molecular miscibility and interaction between the components. This confirmed that the drug was
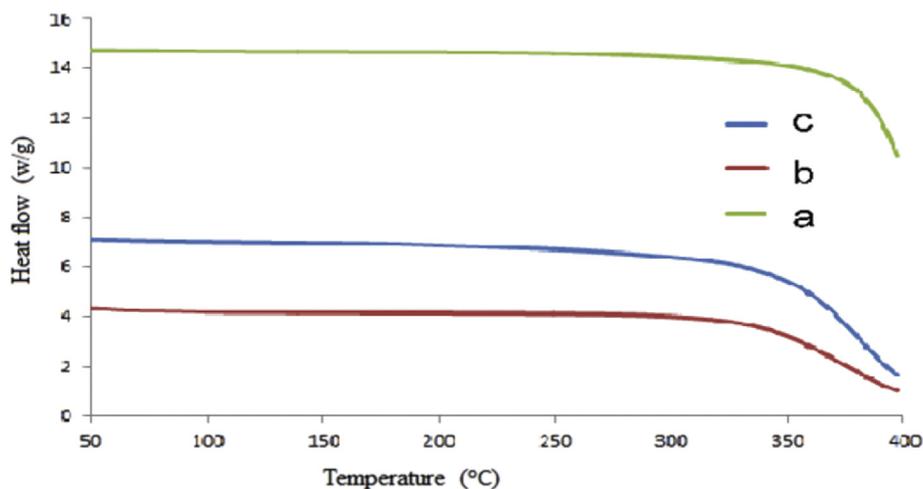
Figure 7. TGA curves where the y-axis represents heat flow (w/g) and the x-axis is the temperature (^{o}C); (a) Eudragit RSPO-LPV nanoparticles, (b) LPV, (c) Eudragit RSPO.

Figure 8. DSC thermograms where y-axis represents heat flow (w/g) and x-axis is the temperature (°C); (a) Eudragit RSPO-LPV nanoparticles, (b) LPV, (c) Eudragit RSPO.

Table 2a. Stability results of the Eudragit RSPO- LPV NPs suppositories at 5 °C.

Parameters/weeks	0	4	8	12
Particle size (nm)	190.9	194	196	197
EE (%)	79.0	78	76	75
Zeta potential (mv)	26.0	25.6	25	25
PDI	0.224	0.203	0.215	0.241

molecularly encapsulated in the polymer, and converted from the crystalline form into the amorphous peaks on encapsulating (Khan et al., 2019). Hence, it is assumed that the poor solubility of LPV must be addressed by preparing amorphous forms of the drug and it is confirmed that the high internal energy and specific volume of the amorphous state have already been reported to enhance dissolution, solubility, and bioavailability (Hancock and Parks, 2000).

3.6. Thermogravimetric

The thermal stability analysis in Figure 7 indicated that Eudragit RSPO-LPV nanoparticles showed better stability as compared to the polymer and the drug. The weight loss occurred between 314 and 300°C for LPV, 321 and 400 °C for Eudragit RSPO while it is between 349 and 400 °C for the Eudragit RSPO-LPV nanoparticles. Any increment in the moisture contents of a polymer will result in melting temperature reduction (Cao and Bhoyro, 2001). The maximum weight loss occurs the temperature range of 314–400 °C and the results showed that the Eudragit RSPO-LPV nanoparticles were thermally stable (Lemmer and Liebenberg, 2013).

3.7. Differential scanning calorimetry

Compatibility studies to investigate the crystallinity in Figure 8 shows endothermic peaks at 66.3°C and 64.5°C for Eudragit RSPO and Eudragit RSPO-LPV nanoparticles, respectively. LPV has two peaks at 56.7°C and 96.8°C, which suggest its crystallinity nature (Khan et al.,

2019). The product crystallinity depends on temperature and melting point; any reduction in these leads to a decrease in crystallinity and amorphous formation, resulting in increased solubility. There were no significant differences in the individual endotherm and respective endotherm observed in the Eudragit RSPO-LPV loaded in suppositories, which indicates that there was no interaction between excipient and the drug.

3.8. Stability studies

Stability studies for Eudragit RSPO-LPV nanoparticles were done based on particle size, EE, zeta potential and PDI variations for three months. The results in Table 2a and Table 2b and Figure 9 showed that there was no significant ($p < 0.05$) change in assessed parameters when samples are stored at 5 °C. Similarly, Eudragit RSPO-LPV nanoparticles stored at 25 °C showed no significant change in particle size and zeta potential. However, in these samples, statistically significant ($p < 0.05$) reduction in EE was observed. The EE of Eudragit RSPO-LPV nanoparticles at the end of 3 months was 73.4% of the initial formulation. Hence, storage under refrigerated condition is recommended for Eudragit RSPO-LPV nanoparticles.

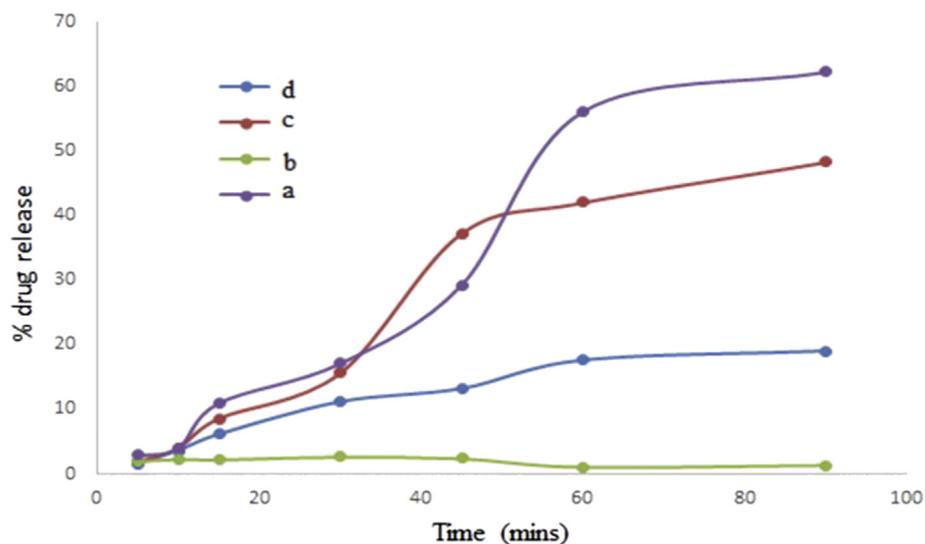### 3.9. In-vitro drug release studies

The release mechanism of the suppositories depends on its rate of dissolution (Figure 10). The *in vitro* drug release was performed to investigate the suppository's ability to release the drug Eudragit RSPO-

Table 2b. Stability results of Eudragit RSPO- LPV NPs suppositories at 25 °C.

Parameters/weeks	0	1	8	12
Particle size (nm)	190.9	198	201	203
EE (%)	79.0	75	66	58
Zeta potential (mV)	26.0	25.3	25	25
PDI	0.224	0.212	0.220	0.246
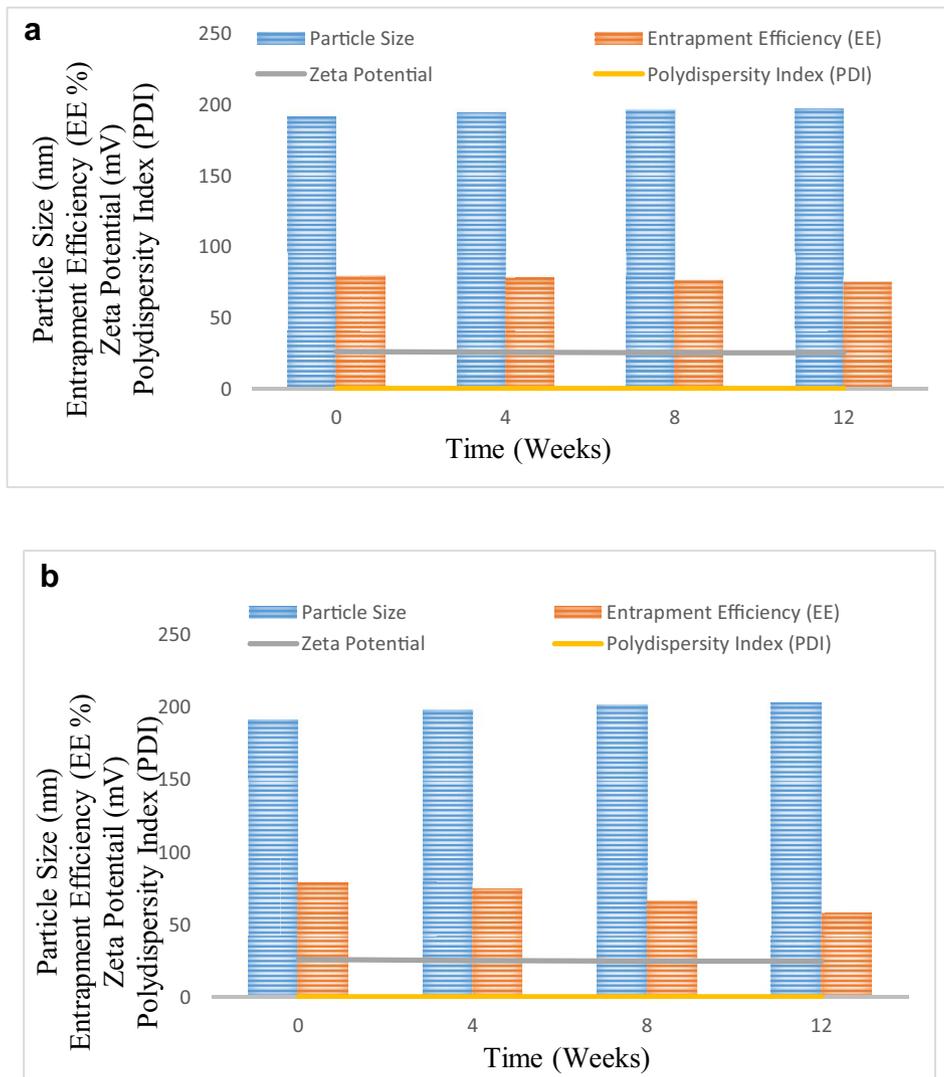
Figure 9. a. Stability results of Eudragit RSPO- LPV NPs in terms of mean particle size, entrapment efficiency (EE), zeta potential and polydispersity index (PDI) stored at 5 °C for 12 weeks. b. Stability results of the Eudragit RSPO- LPV NPs in terms of mean particle size, entrapment efficiency (EE), zeta potential and polydispersity index (PDI) stored at 25 °C and 60% ± 5% RH for 12 weeks.

Figure 10. The release studies profile where the y-axis represents percentage drug release and the x-axis is time (minutes); (a) Fattibase suppositories (b) LPV, (c) Eudragit RSPO-LPV nanoparticles, (d) PEG suppositories.

LPV nanoparticles in two different bases. The results show suppositories prepared with a PEG mixture having a prominent higher release of almost 72 % increase between 30-90 min. The Eudragit RSPO-LPV nanoparticles show 68 % release, and the pure drug had a release of 41 % with the fattibase release declining. The release was as a result of the difference in the two bases, one was oleaginous, and the other was water soluble. Meanwhile, the drug is hydrophobic which resulted in a higher release in the water soluble base, but the oil base withheld the release since drugs with high affinity for the base will not be quickly released into the rectal fluid for absorption. It could also be attributed to the rapid softening and solubilizing properties of the hydrophilic base. This confirmed that hydrophobic drug would exhibit a higher affinity for the lipophilic base while the fattibase would entrap the drug and hinder the migration of the drug out into the rectum for absorption (Abass et al., 2012b). In addition, the drug released depend on the diffusion rate of the drug from the matrix of the Eudragit RSPO polymer reported by Vandenberg et al., 2000).

4. Conclusion

Eudragit RSPO-LPV nanoparticles were successfully prepared with nanoprecipitation and loaded into suppositories by the fusion method. The results obtained when characterised indicated that the drug was indeed encapsulated and could be a potential carrier for controlled drug delivery. This result is supported by the preliminary *in vitro* release study of suppositories using two different bases. Suppositories formulated in PEG bases gave better release properties than those in the fattibase, an indication of a good released which can be investigated further as a better alternative route of drug administration for children paediatric HIV treatment.

Declarations

Author contribution statement

L.M. Katata-Seru, B.M. Ojo, O. Okubanjo, R. Soremekun and O.S. Aremu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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