Division of Pharmaceutics[1], Faculty of Pharmacy, Rhodes University, Makhanda, South Africa; Department of Pharmacy[2], DDT College of Medicine Gaborone, Botswana

Preformulation characterization and identification of excipients for nevirapine loaded niosomes

B. A. Witika[1,2], R. B. Walker[1,*]

Received September 23, 2020, accepted December 1, 2020

**Corresponding author: Roderick B. Walker, Division of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Makhanda, 6140, South Africa*
r.b.walker@ru.ac.za

Pharmazie 76: 77-83 (2021) *doi: 10.1691/ph.2021.0137*

Nevirapine (NVP) is used for the management of HIV/AIDS but must be dosed frequently, exhibits unpredictable bioavailability and a side effect profile that includes hepato- and dermo-toxicity. Niosomes are a colloidal drug delivery system that may be used to overcome the low bioavailability, side effect profile and frequent dosing needed when using conventional drug delivery systems. The compatibility of NVP with sorbitan esters, polysorbate, cholesterol and dihexadecyl phosphate (DCP) was investigated using Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), Fourier Transform Infra-red Spectroscopy (FTIR) and X-ray Powder Diffraction (XRPD). Screening studies were undertaken to identify potential excipients that would produce niosomes with target critical quality attributes (CQA) *viz,* a particle size (PS) < 1000 nm, a polydispersity index (PDI) < 0.500 and an entrapment efficiency > 90%. The results revealed that sorbitan esters in combination with cholesterol and 5 µmol DCP produced niosomes with the best CQA and Zeta potential (ZP) < -30 mV which suggests good stability of the niosomes on storage. Sorbitan esters produced the smallest niosomes of < 400 nm diameter with a PDI < 0.400 and an entrapment efficiency > 78% without cholesterol. The addition of cholesterol and DCP was essential to form niosomes with target CQA.

1. Introduction

Bilayered drug delivery systems such as niosomes or liposomes and unilayered lipid carriers such as solid lipid nanoparticles and nanostructured lipid carriers offer advantages over conventional drug delivery systems (Baillie et al. 1985; Wissing et al. 2004; Désormeaux and Bergeron 2005; Tsai et al. 2012; Witika and Walker 2019) which include the possibility of sustained release profiles, optimal dosing intervals, improved side effect profiles and the potential to administer lower doses.

Niosomes are microscopic lamellar structures similar to liposomes produced using non-ionic surfactants (Azmin et al. 1985; Baillie et al. 1985; Hunter et al. 1988; Kumar et al. 2011). The amphiphiles used include sorbitan esters and/or polysorbates and/or ethoxylated alcohols and/or sucrose esters which are stabilized by addition of cholesterol and small amounts of cationic or anionic surfactants such as (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulphate (DOTAP) and diacetyl phosphate (Baillie et al. 1985; Hunter et al. 1988; Uchegbu and Vyas 1998; Biswal et al. 2008).

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with other antiretroviral (ARV) molecules for treatment of HIV to halt the progression to AIDS (Smith et al. 2001; Lange 2002; Warnke et al. 2007). NNRTI act by non-competitive binding to the non-allosteric site of the reverse transcriptase enzyme (Beach 1998). NVP is hepato- and dermatotoxic, requires frequent dosing which may result in poor adherence and viral resistance (Sulkowski et al. 2002; Lemańska et al. 2008). Niosomes may circumvent the challenges associated with NVP therapy and have been used with different degrees of success when encapsulating ARV molecules such as lamivudine (Mukka and Fatima 2016), zidovudine (Ruckmani and Sankar 2010), stavudine (Begum et al. 2014) and nevirapine (Mehta and Jindal 2015; Witika and Walker 2019).

Preformulation studies are conducted to facilitate the development of quality dosage forms by design (Tolieng et al. 2017) and are defined as the study of the physicochemical, physicomechanical and biopharmaceutical properties of an active pharmaceutical ingredient (API) and compatibility with potential excipients prior to formulation development activities. Preformulation is a fundamental step required to generate an understanding of challenges associated with an API and potential excipients including potential degradation, impact on pharmacokinetics, bioavailability, toxicity, selection of an appropriate form of the API and other formulation additives (Gopinath and Naidu 2011; Sahitya et al. 2012). Any physico-chemical or other interaction between an API and excipients to be used in a formulation may affect the physical, chemical, therapeutic, organoleptic, pharmacological and stability of a dosage form ultimately affecting the accuracy of dose or potentiating side effects with a negative impact on patient adherence (Sahitya et al. 2012).

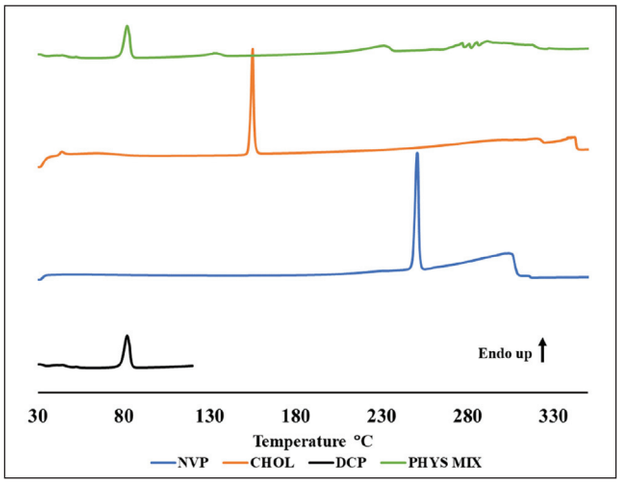

Fig. 1: DSC thermogram of NVP, CHOL, DCP and a 1:1:1 physical mixture.

The purpose of this study was to characterize, identify and select excipients that would be suitable for the manufacture of NVP loaded niosomes by undertaking well designed and appropriate preformulation studies.

2. Investigations, results and discussion

2.1. Differential Scanning Calorimetry (DSC)

The DSC thermogram depicted in Fig. 1 reveals melting endotherms at 247.53, 151.90 and 79.94 °C for NVP, cholesterol and DCP. The thermogram for the physical ternary mixture in Fig. 1 reveals the presence of all identifying peaks in their crystalline nature and revealed an endotherm peak of melting at 81.46 °C for DCP indicating that the molecule was crystalline in nature. The endotherm for NVP was broadened with a melting point at 233.27 °C the melting point of CHOL was 141.41 °C. However, The reduction in enthalpy for the endotherms can be attributed to mixing rather than any amorphism or changes in polymorphism and must be further investigated using XRPD (Kim et al. 1985). The presence of the melting endotherms provides some evidence that NVP, CHOL and DCP do not undergo or exhibit significant changes in crystallinity when combined.

2.2. Fourier Transform Infra-Red Spectroscopy (FTIR)

The IR spectra depicted in Fig. 2 show the characteristic vibrations of NVP in addition to those for a quaternary mixture of NVP and all excipients. The spectra reveal that significant chemical interactions between NVP and any of the potential excipients for this formulation are unlikely. Any shift(s) in the frequencies of vibration observed are most likely due to hydrogen bonding rather than a chemical reactions (Nie et al. 2005). The reduction of intensity of vibration for the functional groups is likely due to mixing and dilution and not due to a chemical interaction or alterations.

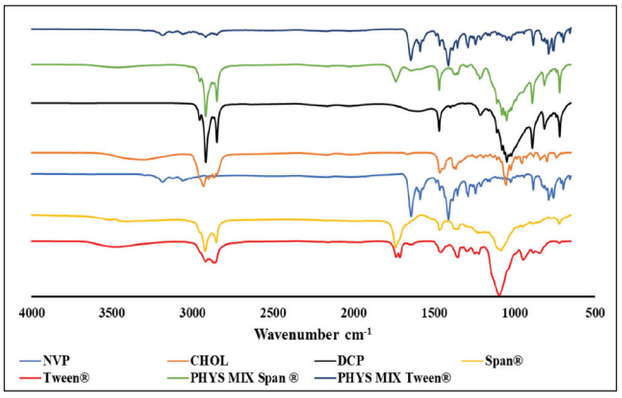

Fig. 2: FTIR of NVP, excipients and 1:1 physical mixture of NVP and non-ionic surfactant used in the manufacture of niosomes.

The characteristic vibration frequencies for all excipients and NVP are summarized in Table 1. The FTIR spectrum for NVP reveals characteristic N-H, C-N stretching of the 7-membered ring at 3295-3188 cm^{-1} and a C-O stretching vibration for a cyclic amide at 1646 cm^{-1} (Sarkar et al. 2008). In addition, the characteristic peaks for cholesterol the for the OH bond at 3305.30 cm^{-1}. The asymmetric CH of the methyl and the -C=C- are visible at 2926.52 cm^{-1} and 1668.28 cm^{-1}, respectively.

2.3. X-Ray Powder Diffraction (XRPD)

XRPD was used as to investigate whether NVP when mixed with potential excipients exhibited a loss of crystallinity. The relative intensity of the peaks in the diffractogram is a measure of the electron density around specific atoms in the molecule. The location of the highest peaks must show consistency when testing physical mixtures for crystallinity to be maintained (Chung 1974, 1975).

Table 1: Assignment of key vibration frequencies for functional groups of NVP, CHOL, DCP, Span® and Tween®

Functional group/assignment	Frequency of vibration cm^{-1}				
	NVP	CHOL	DCP	Span®	Tween®
CH_2 twisting	1153.33				
Ring Breathing and deformation	883.97				
C-H asymmetric bending	1465.11				
C-H symmetric bending	1383.01				
-C=O bending	1288.31				
CH deformation of rings	1210.17				
-C=C-C aromatic ring stretching	1585.82				
CH_2 rocking	818.18				
Aromatic C-H in plane bending	3061.37				
OH, in plane bending	1242.21				
N-H stretching of 7-membered ring	3183.67				
CH_2 of cyclopropyl ring stretching	1410.32				
C=O stretching of cyclic amide	1644.36				
O–H bond vibration of free hydroxyl		3305.30		3418.88	3382.95
Asymmetric CH stretch of methyl group		2926.52	2926.52		
-C=C- stretching		1668.28			
O-CH stretch			2955.56		
Asymmetric CH stretch of methyl group				2926.52	2955.32
C-C stretch (skeletal vibrations)				889.23	887.93

The XRD diffractograms for NVP, cholesterol and DCP are depicted in Fig. 3 and are a graphic representation of the crystallinity of the compounds under investigation.
NVP exhibits signals and peaks at angles, 2θ = 13.50°, 19.00° and 26.00°. The sharpness of the peaks is an indication of the degree of crystallinity of NVP. The XRD diffractograms for the excipients evaluated for their potential to manufacture NVP containing niosomes revealed that cholesterol and DCP exist as crystalline compounds with intensity peaks for cholesterol at angles, 2θ = 15.50°, 15.56°, 17.45° and 18.25° and at 2θ = 19.90°, 22.08°, and 23.87° for DCP

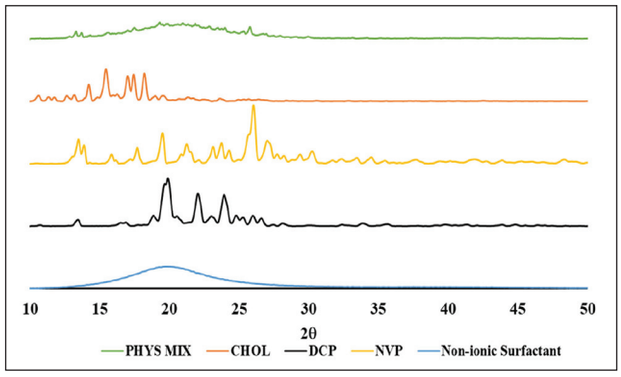

Fig. 3: XPRD diffractogram of NVP, a physical mixture and potential excipients used for the manufacture of NVP containing niosomes.

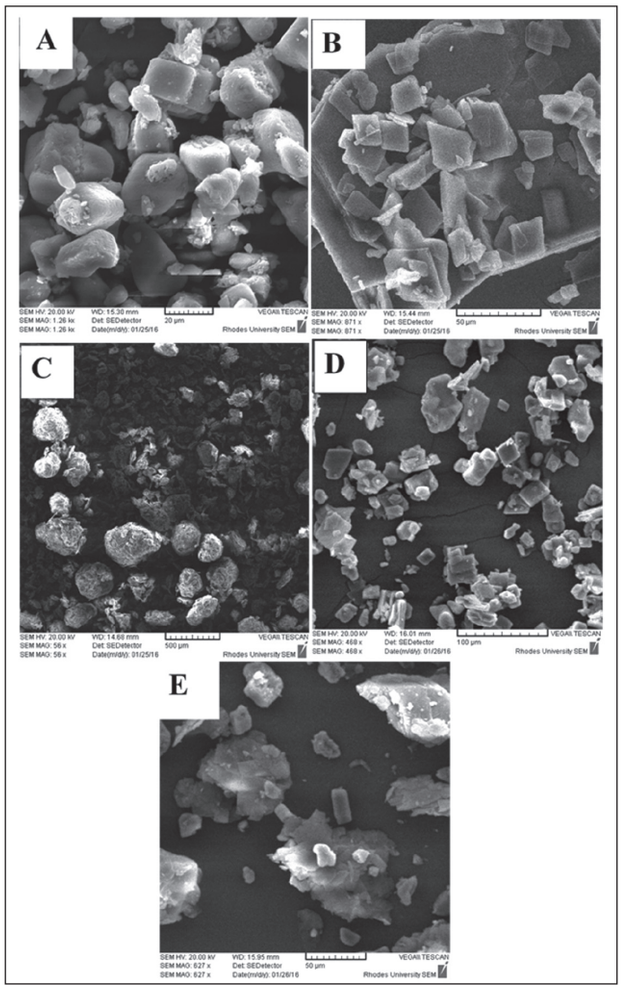

Fig. 4: Scanning electron micrograph of NVP and solid excipients used for the manufacture of NVP containing niosomes.

Sorbitan esters and polysorbates exist as amorphous liquids with the exception of sorbitan monooleate that exists as an amorphous solid at 22 °C. A typical diffractogram of an amorphous compound, which is a solid state form associated with the non-ionic surfactants, is depicted in Fig. 3. Unlike FTIR spectra in which the characteristic molecular differences are evident, XRPD diffractograms for all sorbitan esters and polysorbates tested will be identical with an amorphous halo (Griffin 1949) evident in the diffractogram.
The diffractogram in Fig. 3 depicts the result for an equivalent weight quaternary mixture of NVP, DCP, cholesterol and polysorbate 80 and is a typical representation of a diffractogram of all quaternary mixtures tested. The diffractogram for the quaternary mixture exhibits the characteristic peaks for NVP, DCP and cholesterol suggesting that they maintain their crystallinity when included in a physical mixture.

2.4. Scanning Electron Microscopy (SEM)

The scanning electron micrographs of NVP crystals (A), cholesterol (B) and DCP (C) are depicted in Fig. 4. NVP exists in sub-rounded/irregularly shaped particles that occur in aggregates of medium sphericity, similar to what has been previously reported (Sarkar et al. 2008). Unlike NVP, cholesterol occurs in well-defined particles of regular shape. The cholesterol particles were approximately 50 µm in size and these findings are in agreement with the previously reported shape of cholesterol (Uskoković and Matijević 2007). In contrast, the particles of DCP are almost spherical and occur as independent units with an average diameter of approximately 200 µm.
Figure also depicts the micrographs of binary mixtures of NVP and cholesterol (D) or DCP (E) are also depicted in Fig. 4. The cholesterol particles are clearly identifiable as slightly bigger and regularly shaped than the NVP particles which are identifiable as irregularly shaped small particles and it is evident that no change in the crystal structure of the molecules is visible. The micrograph (E) reveals large DCP particles and numerous small particles of NVP with unchanged surface morphology or physical interaction which suggest the absence of any physical interaction between NVP and DCP.

2.5. Screening studies

2.5.1. Surfactants, cholesterol and DCP

In general, niosomes manufactured using surfactants with a high HLB value exhibit larger PS than those manufactured with compounds of low HLB value, as they tend to attract water into the niosomes. Furthermore, the addition of CHOL to the niosome formulation compositions results i.n the formation of carriers largerthan those without CHOL as it is sequestered in spaces between the surfactant molecules, resulting in the formation of larger sized vesicles.

2.5.1.1. Sorbitan monolaurate (Span® 20)

When Span® 20 was included in the composition, niosomes of approximately 120 to 300 nm in diameter with an entrapment efficiency ranging between 79-84 % were formed. An increase in EE and diameter was observed as surfactant was increased. The PDI of niosomes manufactured without cholesterol or DCP was 0.100 to 0.300 indicating that the niosomes produced were of uniform size.

2.5.1.2. Sorbitan monostearate (Span® 60)

The use of Span® 60 yielded niosomes of 80 to 250 nm in diameter which are smaller than those prepared using sorbitan monolaurate what can be attributed to the HLB value of the surfactant. Surfactants with high HLB values interact with hydration fluids and swell to yield particles with large vesicles (Sezgin-Bayindir and Yuksel 2012). Furthermore, the surface free energy of systems decreases with an increase in hydrophobicity of surfactants, resulting in the formation of smaller particles (Yoshioka et al. 1994). The EE of Span® 60 niosomes without cholesterol was 80-84 %, which was

attributed to the lower HLB value of Span® 60 when compared to Span® 20. For NVP which is lipophilic , entrapment in likely to be more efficient when surfactants of low HLB are used (Hunter et al. 1988; Kumar et al. 2011; Sezgin-Bayindir and Yuksel 2012). The PDI of niosomes manufactured using Span® 60 was between 0.100 and 0.300 suggesting uniform size.

2.5.1.3. Sorbitan monooleate (Span® 80)

The use of Span® 80 in the composition yielded niosomes that ranged between 80 and 250 nm in size. The HLB values of Span® 80 and Span® 60 are similar and consequently the PS and EE were expected to be similar. In addition, the PDI of the niosomes manufactured was between 0.100 and 0.300 suggesting uniformity of size distribution with an EE of 80-84 % when CHOL was excluded.

2.5.1.4. Polysorbate 20 (Tween® 20)

Niosomes manufactured using polysorbate 20 were much larger than those manufactured using the sorbitan esters due, in part, to the high HLB value of this polysorbate surfactant. In addition the hydrophilicity of polysorbate surfactants has a negative impact on the EE of hydrophobic NVP (Hunter et al. 1988; Kumar et al. 2011; Sezgin-Bayindir and Yuksel 2012). The EE for polysorbate 20 niosomes ranged between 58 and 64%, which was due to the high HLB value of 16 for this excipient. The PS ranged between 100-500 nm with an associated PDI of 0.400-1.000 suggesting highly variable particles size which was in part due to the presence of NVP external to the niosomes and which may have contributed to the formation of large niosomes.

Table 2: Effect of cholesterol on the PS of NVP niosomes

Surfactant	Surfactant concentration µmol	Cholesterol content µmol	
		0	50
Sorbitan monolaurate	50	136.2 ± 48 nm	904 ± 42 nm
	90	144.3 ± 35 nm	910 ± 37 nm
	120	144.5 63 nm	908 ± 32 nm
Sorbitan monostearate	50	119.5 ± 80 nm	882 ± 82 nm
	90	121.6 ± 47 nm	894 ± 64 nm
	120	127.6 ± 39 nm	892 ± 80 nm
Sorbitan monooleate	50	112.2 ± 75 nm	870 ± 94 nm
	90	120.4 ± 50 nm	881 ± 45 nm
	120	129.5 ± 63 nm	892 ± 53 nm
Polysorbate 20	50	332.2 ± 37 nm	1153 ± 108 nm
	90	337.4 ± 61 nm	1204 ± 205 nm
	120	341.6 ± 57 nm	1279 ± 163 nm
Polysorbate 60	50	536.0 ± 38 nm	1223 ± 148 nm
	90	540.2 ± 40 nm	1296 ± 123 nm
	120	544.6 ± 39 nm	1303 ± 149 nm
Polysorbate 80	50	548.0 ± 50 nm	1293 ± 208 nm
	90	557.3 ± 46 nm	1330 ± 196 nm
	120	561.3 ± 43 nm	1320 ± 207 nm

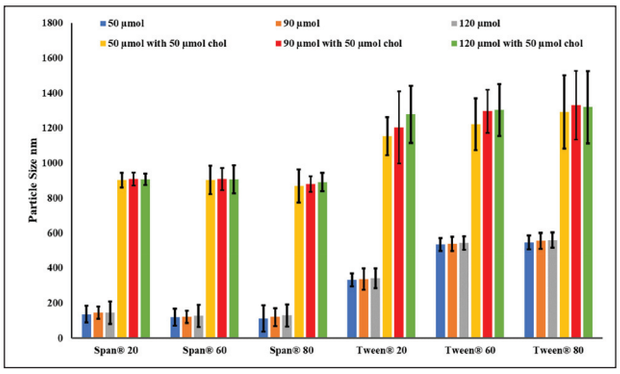

Fig. 5: Effect of CHOL on the PS of NVP niosomes.

2.5.1.5. Polysorbate 60 (Tween® 60)

Niosomes manufactured using polysorbate 60 were of dimensions similar to those formed using polysorbate 20. The HLB value of polysorbate 60 of 14.9 suggests that it is hydrophilic and therefore low entrapment of NVP was expected. The niosomes manufactured using Tween® 60 had an EE of 63-66% with an associated PDI of 0.400-1.000, indicating that the suspension was polydisperse.

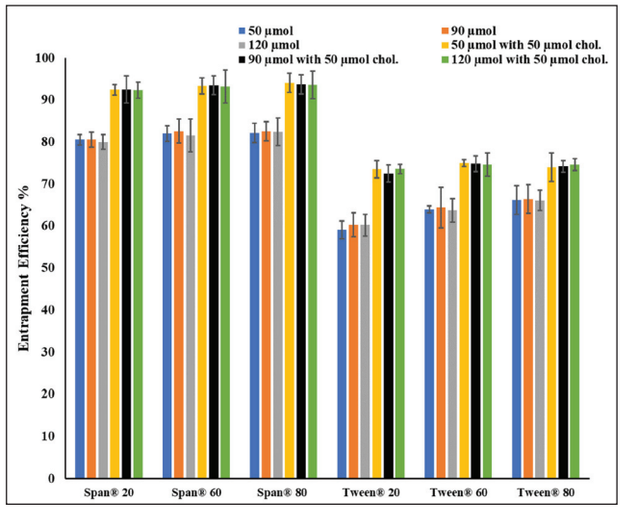

Fig. 6: Effect of CHOL on the EE of NVP in niosomes

Table 3: Effect of CHOL on the EE of NVP in niosomes

Surfactant	Surfactant concentration µmol	Cholesterol content µmol	
		0	50
Span ® 20	50	80.5	92.4
	90	80.6	92.5
	120	80.0	92.3
Span ® 60	50	82.0	93.3
	90	82.6	93.5
	120	81.5	93.2
Span ® 80	50	82.2	94.1
	90	82.5	93,7
	120	82.4	93.6
Tween® 20	50	59.1	73.5
	90	60.3	72.5
	120	60.2	73.6
Tween® 60	50	64.0	75.0
	90	64.4	74.8
	120	63.7	74.6
Tween® 80	50	66.2	74.0
	90	66.4	74.2
	120	66.1	74.6

2.5.1.6. *Polysorbate 80 (Tween® 80)*

Niosomes manufactured using polysorbate 80 were 100–500 nm in dimension with an associated PDI of 0.4-1.000 and an EE of 63-69 %. These results were almost identical to those observed for polysorbate 60. This can, in part, be explained by the HLB of 15.9 which is similar to that of polysorbate 60.

2.5.1.7. *Cholesterol*

The inclusion of CHOL had the most significant impact on the PS and EE of the niosomes. The effect of CHOL on the PS of niosomes is summarized in Table 2 and graphically depicted in Fig. 5. The PS of niosomes is affected by factors such as the inclusion or absence of CHOL in formulation compositions and in general, the size of niosomes increases with the inclusion of CHOL (Baillie et al. 1985; Yoshioka 1994; Kumar et al. 2011). The increased hydrophobicity of the bilayer by CHOL results in the NVP being sequestered in the hydrophobic bilayer. This consequentially also increases the PS.
The EE of niosomes is known to increase with the addition of CHOL (Uchegbu and Duncan 1997; Uchegbu and Vyas 1998; Ruckmani and Sankar 2010) and as expected the use of CHOL resulted in an increase in the EE of NVP or all formulations tested. The PS and EE for NVP in batches in which different surfactants without cholesterol and DCP were used are reported in Table 3 and depicted in Fig. 6 to illustrate the impact of CHOL content on EE. There is an increase in EE observed with increases in molar concentration of surfactant. This is more in cases involving surfactants with lower HLB values. The increased hydrophobicity results in a higher EE. The addition of CHOL allows for an increased amount of NVP to be sequestered in the hydrophobic bilayer and subsequent increase in EE.

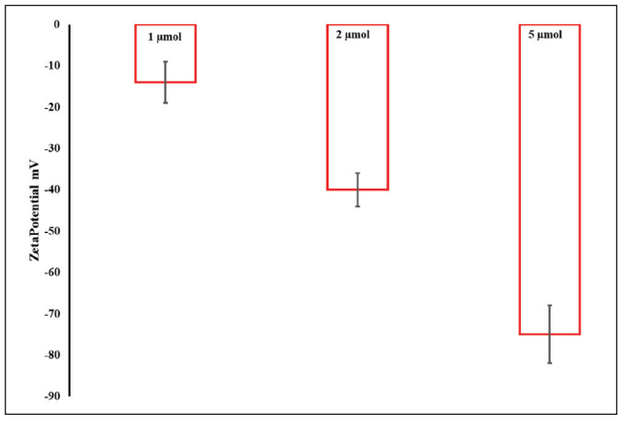

Fig. 7: Effect of DCP on the Zeta potential of NVP noisome dispersions.

2.5.1.8. *Dihexadecyl phosphate (DCP)*

The inclusion of DCP in noisome formulations also had an impact on the PS of the niosomes as it has a propensity to attract the hydration medium into the architecture of the noisome (Sezgin-Bayindir and Yuksel, 2012). The presence of DCP did not appear to have a significant effect on the PS of the niosomes. The ZP of the niosomes was affected by the inclusion of dihexadecyl phosphate which is a known charge inducer (Baillie *et al.* 1985; Yoshioka, Sternberg and Florence, 1994; Torchilin, 2000; Mokhtar *et al.* 2008). Different amounts of DCP were included and the ZP monitored so as to identify a composition level that would ensure stable NVP niosome dispersions could be produced and the impact of different molar ratios of DCP on the ZP is depicted in Fig. 7.

2.6. *Conclusions*

The identification, characterization and selection of excipients for the manufacture of a novel noisome technology for the delivery of NVP was successfully undertaken.
Preliminary compatibility testing revealed that NVP is compatible with polysorbate and sorbitan esters which was successfully evaluated using DSC, SEM, FTIR and XRD. The data suggest that NVP, cholesterol and DCP retained their crystallinity and did not exhibit any significant physical or chemical interaction.
The results of screening studies revealed that while NVP is readily assimilated into niosomes with all excipients tested, with or without CHOL and/or DCP, the use of sorbitan esters produced niosomes with better Critical Quality Attributes (CQA) *viz,* PS, PDI and EE when compared to the polysorbate surfactants possibly due to the lower HLB value associated with the surfactants.
The study also revealed that to maximise the EE of NVP, it is necessary to incorporate CHOL into the formulation as its use results in better sequestration of hydrophobic drugs (Khan et al. 2016).
The inclusion of DCP did not have a significant influence on the PS or EE however, incorporation of DCP into the formulation improved the stability of niosome suspensions as observed by a corresponding reduction in the ZP.

Table 4: Summary of batches manufactured

Batch No.	Surfactant	Surfactant µmol	Cholesterol µmol
F1	Sorbitan monolaurate	50	0
F2	Sorbitan monolaurate	90	0
F3	Sorbitan monolaurate	120	0
F4	Sorbitan monostearate	50	0
F5	Sorbitan monostearate	90	0
F6	Sorbitan monostearate	120	0
F7	Sorbitan monooleate	50	0
F8	Sorbitan monooleate	90	0
F9	Sorbitan monooleate	120	0
F10	Polysorbate 20	50	0
F11	Polysorbate 20	90	0
F12	Polysorbate 20	120	0
F13	Polysorbate 60	50	0
F14	Polysorbate 60	90	0
F15	Polysorbate 60	120	0
F16	Polysorbate 80	50	0
F17	Polysorbate 80	90	0
F18	Polysorbate 80	120	0
F19	Sorbitan monolaurate	50	50
F20	Sorbitan monolaurate	90	50
F21	Sorbitan monolaurate	120	50
F22	Sorbitan monostearate	50	50
F23	Sorbitan monostearate	90	50
F24	Sorbitan monostearate	120	50
F25	Sorbitan monooleate	50	50
F26	Sorbitan monooleate	90	50
F27	Sorbitan monooleate	120	50
F28	Polysorbate 20	50	50
F29	Polysorbate 20	90	50
F30	Polysorbate 20	120	50
F31	Polysorbate 60	50	50
F32	Polysorbate 60	90	50
F33	Polysorbate 60	120	50
F34	Polysorbate 80	50	50
F35	Polysorbate 80	90	50
F36	Polysorbate 80	120	50

The manufacture of NVP containing niosomes was successfully achieved and the CQA pertinent to appropriate performance were monitored in order to characterize the niosomes. Based on this data sorbitan esters were considered suitable for the production of an optimized NVP containing niosome with inclusion of CHOL and DCP likely to improve the performance of the optimised formulation.

3. Experimental

3.1. Materials

Nevirapine was donated by Aspen Pharmacare® (Port Elizabeth, South Africa). Tween® 20, 60 and 80 and Span® 20, 60 and 80 were donated by Aspen® Pharmacare (Port Elizabeth, South Africa). Cholesterol (CHOL) and dihexadecyl phosphate (DCP) were purchased from Sigma Aldrich® (Johannesburg, South Africa). Methanol UV cut off 215 nm (MeOH) and acetonitrile 200 far UV Romil-SpS™ Super Purity Solvent (ACN) (HPLC grade for UV) were purchased from Romil® Ltd (Caimbridge, United Kingdom). Chloroform AnalaR NORMAPUR was purchased from VWR Prolabo® Chemicals (Pennsylvania, United States of America). HPLC-grade water was obtained from a Milli-RO® 15 water purification system (Millipore®, Bedford, Massachutsetts, United States of America).All other reagents were at least of analytical reagent grade and used without further purification.

3.2. Methods

3.2.1. Differential Scanning Calorimetry (DSC)

Approximately 4 mg NVP, CHOL, DCP and 1:1:1 ternary physical mixture of NVP and the excipients was placed into separate aluminium pans, sealed and placing directly onto the hot stage of a Model DSC – 6000 PerkinElmer Differential Scanning Calorimeter (Massachusetts, USA) and the thermogram data generated was analysed using Pyris™ Manager Software (Massachusetts, USA). The temperature of the DSC was monitored using a central processor and a controlled heating rate of 10 °C/min was used for analysis over the temperature range 30-350 °C. All DSC analyses were conducted in triplicate (n=3) under a nitrogen atmosphere purged at a flow rate of 20 ml.min^{-1}.

3.2.2. Fourier Transform Infra-Red (FTIR) Spectroscopy

The IR absorption spectra of NVP and quaternary mixtures of NVP and excipients were generated using a Spectrum 100 FTIR ATR Spectrophotometer (Beaconsfield, United Kingdom). A small amount of the sample powder was placed onto a diamond crystal, compressed at 100 N and the spectrum generated over the wavenumber range 4000-650 cm^{-1}.

3.2.3. X-Ray Powder Diffraction (XRPD)

XRD diffraction patterns were collected with a Bruker D8 Discover (Massachusetts, USA) diffractometer equipped with a proportional counter using Cu-K_{α} radiation of 1.5405 Å, a nickel filter at 30 kV and a general current of 40 mA. XRPD data for samples on a silicon wafer slide, were collected in the range 2? = 10 ° to 50 ° at a scanning rate of 1.5 ° min^{-1} with a filter time constant of 0.38 s per step and a slit width of 6.0 mm. The diffraction data were evaluated using evaluation curve fitting (Eva) software and baseline correction was performed for each diffraction pattern, by subtracting a spline function fitted to the curved background.

3.2.4. Scanning Electron Microscopy (SEM)

Particle size and shape were evaluated using a Vega® Scanning Electron Microscope (Tuscan, Czechia). Approximately 0.5 mg NVP and each of the solid excipients used during formulation development studies was separately dusted onto a graphite plate and sputter coated with gold for 20 min under vacuum. The samples were visualised using SEM at an accelerated voltage of 20 kV. Binary mixtures of NVP and excipients were also studied to determine whether physical interactions between the crystals and changes to the crystal structure had occurred.

3.2.5. Screening studies and characterisation of niosomes

Niosomes were manufactured using thin-layer hydration (Azmin et al. 1985; Baillie et al. 1985; Uchegbu et al. 1995) with approximately 40 mg of NVP and the pre-determined amount of surfactant being accurately weighed using a Mettler Toledo AG 135 (Port Elizabeth, South Africa) electronic balance and placed into a clear glass round-bottomed Glassco NS 24/29 100 ml Borosilicate 3.3 flask (Port Elizabeth, South Africa). The surfactant was evaluated at three different concentrations *viz.*, 50, 90 and 120 µmol. NVP and surfactant were dissolved without agitation in 10 ml of a 9:1 chloroform: methanol solution which was evaporated under vacuum using a Buchi™ R-215 rotary evaporator (Flawil, Switzerland) at 70 °C. The resultant film was then hydrated with phosphate buffered saline (PBS) pH 7.4 whilst rotating for 45 min at 70 °C without vacuum. The niosome suspension produced was annealed at 4 °C overnight in a Fuchsware® refrigerator (Johannesburg, South Africa). All niosomes were produced using the composition as summarized in Table 5 and characterised within 24 h of manufacture in terms of entrapment efficiency (EE), polydispersity index (PDI) and particle size (PS).

3.2.5.1. Characterisation of niosomes

3.2.5.1.1. Particle size analysis

The mean PS and PDI of the niosomes was determined using a Model Nano-ZS Zetasizer (Worcestershire, United Kingdom) with the instrument set in photon correlation spectroscopy (PCS) mode. Approximately 30 µl of an aqueous dispersion of the niosomes was diluted with 10 ml of HPLC grade water, prior to analysis. The dilute sample was then placed into a 10 x 10 x 45 mm polystyrene cell and all measurements were performed in replicate (n=5) at 25 °C with a scattering angle of 90 °. Analysis of PCS data was undertaken using Mie theory with real and imaginary refractive indices set at 1.456 and 0.01, respectively.

3.2.5.1.2. Entrapment efficiency

Entrapment efficiency (EE) of the niosomes was established by centrifuging 1 ml of an aqueous noisome suspension with an Eppendorf 3154-C centrifuge (Hamburg, Germany) at 14000 x g for 1 h. The supernatant was decanted and the resultant pellet washed using Milli-Q water after which it was centrifuged again at 14000 x g for a further 30 min, to ensure all free and unentrapped NVP was removed from the pellet. The supernatant was removed and analysed using a validated reversed-phase HPLC method (Witika 2017). The amount of NVP that had been entrapped was verified by disrupting the niosome pellet in the centrifuge tube with n-propanol and sonicating for 1 h using a Branson 8510 sonicator (Port Elizabeth, South Africa). The resultant solution was analysed using a validated reversed-phase HPLC method (Witika 2017).

3.2.5.1.3. Zeta Potential (ZP)

The ZP of the niosomes in dispersion was measured with a Model Nano-ZS Zetasizer (Worcestershire, United Kingdom) with the equipment set in the Laser Doppler Anemometry (LDA) mode (n=5). The samples were prepared for analysis as described in § 3.2.5.1.1 but placed into folded polystyrene capillary cells for the measurement.

Conflict of interest: none declared.

References

Azmin MN, Florence AT, Handjani-Vila RM, Stuart JF, Vanlerberghe G, Whittaker JS (1985) The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice. J Pharm Pharmacol 37: 237–242.

Baillie AJ, Florence AT, Hume LR, Muirhead GT, Rogerson A (1985) The preparation and properties of niosomes--non-ionic surfactant vesicles. J Pharm Pharmacol 37: 863-868.

Beach JW (1998) Chemotherapeutic agents for human immunodeficiency virus infection: Mechanism of action, pharmacokinetics, metabolism, and adverse reactions. Clin Ther 20: 2–25.

Begum MY, Dasari S, Sudhakar M, Lakshmi BVS, Manga K (2014) Development and evaluation of co-encapsulated stavudine and lamivudine niosomes for the controlled delivery. Der Pharmacia Sinica 5: 1–10.

Biswal S, Murthy BP, Sahu J, Sahho P, Amir J (2008) Vesicles of non-ionic surfactants (niosomes) and drug delivery potential. Int J Pharm Sci Nanotechnol 1: 1–8.

Chung FH (1974) Quantitative interpretation of X-ray diffraction patterns of mixtures. II. Adiabatic principle of X-ray diffraction analysis of mixtures. J Appl Crystallogr 7: 526–531.

Chung FH (1975) Quantitative interpretation of X-ray diffraction patterns of mixtures. III. Simultaneous determination of a set of reference intensities. J Appl Crytallogr 8: 17–19.

Désormeaux A, Bergeron MG (2005) Lymphoid tissue targeting of anti-HIV drugs using liposomes. In: Methods in Enzymology 391, Elsevier pp. 330–351.

Gopinath R, Naidu RAS (2011) Pharmaceutical preformulation studies – current review. Int J Pharm Biol Arch 2: 1391–1400.

Griffin WC (1949) Classification of surface-active agents by" HLB". J Soc Cosmet Chem 1: 311–326.

Hunter CA, Dolan TF, Coombs GH, Baillie AJ (1988) Vesicular systems (niosomes and liposomes) for delivery of sodium stibogluconate in experimental murine visceral leishmaniasis. J Pharm Pharmacol 40: 161–165.

Khan MI, Madni A, Peltonen L (2016) Development and in-vitro characterization of sorbitan monolaurate and poloxamer 184 based niosomes for oral delivery of diacerein. Eur J Pharm Sci 95: 88–95.

Kim KH. Frank MJ, Henderson NL (1985) Application of differential scanning calorimetry to the study of solid drug dispersions. J Pharm Sci 74: 283–289.

Kumar A. Lal PJ, Amit J, Vishvabhan S (2011) Review on niosomes as novel drug delivery system. Int Res J Pharm 2: 61–65.

Kumar KK, Sasikanth K, Sabareesh M, Dorababu N (2011) Formulation and evaluation of diacerein cream. Asian J Pharm Clin Res 4: 93–98.

Lange JMA (2002) Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomised, double-blind, placebo-controlled trial', Lancet 359: 1178–1186.

Lemańska M, Jankowska M, Cielniak I, Witor A, Szetela B, Leszczyszyn-Pynka M, Wnuk A, Wiercińska-Drapało A, Olczak A, Małolepsza E, Jabłonowska E, Barałkiewicz G, Skwara P, Mian MM, Mikuła T (2008) The role of nevirapine in the antiretroviral therapy. HIV AIDS Rev 7: 17–21.

Mehta SK, Jindal N (2015) Tyloxapol niosomes as prospective drug delivery module for antiretroviral drug nevirapine. AAPS PharmSciTech US 16: 67–75.

Mokhtar M, Sammour OA, Hammad MA, Megrab NA (2008) Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int J Pharm 361: 104–111.
Mukka N, Fatima S (2016) Formulation and in-vitro evaluation of lamivudine niosomes. Indo Am J Pharm 2: 42–46.
Nie B, Stutzman J, Xie A (2005) A vibrational spectral maker for probing the hydrogen-bonding status of protonated Asp and Glu residues. Biophys J 88: 2833–2847.
Ruckmani K, Sankar V (2010) Formulation and optimization of zidovudine niosomes. AAPS PharmSciTech 11: 1119–1127.
Sahitya G, Krishnamoorthy B, Muthukumaran M (2012) Importance of preformulation studies in designing formulations for sustained release dosage forms. Int J Pharm Technol 4: 2311–2331.
Sarkar M, Perumal OP, Panchagnula R (2008) Solid-state characterization of nevirapine. Indian J Pharm Sci 70: 619–630.
Sezgin-Bayindir Z, Yuksel N (2012) Investigation of formulation variables and excipient interaction on the production of niosomes. AAPS PharmSciTech 13: 826–835.
Smith PF, DiCenzo R, Morse GD (2001) Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin Pharmacokinet 40: 893–905.
Sulkowski MS, Thomas DL, Mehta SH, Chaisson RE, Moore RD (2002) Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: role of hepatitis C and B infections. Hepatology 35: 182–189.
Tolieng V, Prasirtsak B, Sidhipol J, Tongchul N (2017) Identification and lactic acid production of bacteria isolated from soils and tree barks. Malaysian J Microbiol 13: 100–108.
Torchilin VP (2000) Drug targeting. Eur J Pharm Sci 11 Suppl 2: S81–S91.
Tsai MJ, Wu PC, Huang YB, Chang JS, Lin CL, Tsai YH, Fang JY (2012) Baicalein loaded in tocol nanostructured lipid carriers (tocol NLCs) for enhanced stability and brain targeting. Int J Pharm 423: 461–470.
Uchegbu IF, Double JA, Turton JA, Florence AT (1995) Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span 60) niosomes in the mouse. Pharm Res 12: 1019–1024.
Uchegbu IF, Duncan R (1997) Niosomes containing N-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin (PK1): Effect of method of preparation and choice of surfactant on niosome characteristics and a preliminary study of body distribution. Int J Pharm 155: 7–17.
Uchegbu IF, Vyas SP (1998) Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int J Pharm 172: 33–70.
Uskoković V, Matijević E (2007) Uniform particles of pure and silica-coated cholesterol. J Colloid Interface Sci 315: 500–511.
Warnke D, Barreto J, Temesgen Z (2007) Therapeutic review: Antiretroviral drugs. J Clin Pharmacol 47: 1570–1579.
Wissing SA, Kayser O, Müller RH (2004) Solid lipid nanoparticles for parenteral drug delivery. Adv Drug Deliv Rev 56: 1257–1272.
Witika BA (2017) The development, manufacture and characterisation of niosomes intended to deliver nevirapine to the brain. Master of Science thesis, Rhodes University.
Witika BA, Walker RB (2019) Development, manufacture and characterization of niosomes for the delivery for nevirapine. Pharmazie 74: 91–96.
Yoshioka T, Sternberg B, Florence AT (1994) Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 105: 1–6.