ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

Preparation and *in Vitro* Evaluation of Solid-Lipid Nanoparticles (from Dika Wax) for Enhanced Delivery of Nevirapine in HIV/Aids Management

S. O. Abali & G. C. Onunkwo
University of Port Harcourt, Nigeria

Abstract:- The preparation and assessment of solid lipid nanoparticles (*SLNs*) of nevirapine with improved oral delivery for better management of HIV/AIDS was the aim of this research. Eight batches of *SLNs* of nevirapine were produced from Dika wax and evaluated for particle charges and distribution of the sizes of particles using Zeta sizer, surface shape with Cryo-Transmission Electron Microspcope (Cryo-TEM), chemical interaction between drug and excipients with Fourier Transform Infrared Spectroscope (FTIR). Loading capacity, encapsulation efficiency and *in vitro* drug release properties were determined. Release profiles were compared with f_2 statistic, one-way ANOVA and students'*t*-test. From the results obtained, Cryo-TEM revealed that the SLNs were round to oval in shape with smooth external surface. Zeta sizer particle sizes and distribution analysis indicated quality results for Nevirapine SLN Batches 15 and 18. The zeta potential results were: -16.83 ± 0.404 mV for Batch 1, -44.30 ± 0.624 mV for Batch 15 and -40.03 ± 2.65 mV for Batch 18. Batches 15 and 18 SLNs had loading capacities of 6.71% and 9.82% respectively and encapsulation efficiencies of 49.35% and 70.19% respectively. In vitro dissolution showed 102% release for batch 18 and 87.5% release for Batch 15 with a dissolution efficiency of 65% for Batch 15 and 83% for Batch 18 SLNs. f_2 statistic, ANOVA and students' *t*-test revealed Batch 15 SLNs are similar to Batch 18 SLN. In conclusion, Batches 15 and 18 SLNs have good properties for enhancing the delivery of nevirapine as extended release dosage forms for better management of HIV/AIDS.

Keywords:- *Solid Lipid Nanoparticle, Nevirapine, Dika Wax, HIV/AIDS.*

I. INTRODUCTION

HIV/AIDS is a significant global cause of death and has both medical and economic repercussions on society. The primary source of the virus and infected cells in individuals with HIV is the lymphoid tissue [1,2]. During the disease progression, lymphoid tissues are identified as the main location of CD4+ T cell infection; nevertheless, follicular dendritic cells (FDCs) are actually the primary origin of viral RNA in lymphoid tissue [3]. Nevirapine is a widely recognized antiretroviral medication utilized for managing HIV-1 infection and AIDS. It falls under the category of non-nucleoside reverse transcriptase inhibitors and structurally belongs to the group of compounds known as dipyridodiazepinones. [4]. Nevirapine has been categorized by the Food and Drug Administration (FDA) as a Class II medicine under the Biopharmaceutics Classification System (BCS), which means that it has low solubility but high permeability. At a neutral pH, Nevirapine's solubility in water is 1mg/ml, and it becomes highly soluble at a pH below 3. [5]. Considering this poor solubility drawback, lipid-based formulations have been extensively studied as potential approaches to improve the bioavailability of drugs that are poorly water soluble like nevirapine when administered orally. Moreover, the presence of lipids can enhance the absorption of nevirapine through various mechanisms, such as stimulating the secretion of bile and pancreatic fluids by the gall bladder and prolonging the time it stays in the stomach [6]. Solid lipid nanoparticles (*SLNs*) have shown promise as delivery systems for lipophilic and poorly water-soluble molecules like nevirapine. The exploration of locally available solid lipids like Dika wax also became very necessary in the preparation of *SLNs* in order to reduce cost of production and increase availability of raw material for drug delivery. Dika wax is an edible vegetable wax derived from the seeds of *Irvingia gabonenesis* tree, also known as bush mango tree. It has melting point of 46 °C [7, 8, 9]. Dika wax has before now been applied in the preparation of solid lipid microparticles [10,11]. So, there is need for preparation of nevirapine in the form of solid lipid nanoparticle using Dika wax and other solid lipids in order to enhance the bioavailability of nevirapine.

II. METHODS

A. Differential Scanning Colorimetry (DSC) for Nevirapine

A Mettler Toledo 821 DSC apparatus (Mettler Toledo AG, Greifensee, Switzerland) was used to obtain DSC thermograms. Indium was used to calibrate the temperature axis and the cell constant. Aluminum pans with holes punched through were used to quantify and analyze powder samples weighing four to six milligrams. The NVP samples were continuously purged of nitrogen (80 ml/min) while being heated at different rates of 10 °C/min over a temperature range of 0-300°C [10, 12].

B. Preparation of Solid Lipid Nanoparticles (SLNs) of Nevirapine

The solid lipid nanoparticles of nevirapine were prepared using the formula in table-1 below (after trial of so many other formulae for stability). The lipid matrix

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

containing either stearic with dika wax or dika wax with coconut oil and Phospholipon 90H or dika wax with Phospholipon 90H only was melted at a temperature above 70 ºC in a beaker. But, the sorbitol, sorbic acid, tween 80 and nevirapine were dissolved in water at the same temperature. The two mixtures were mixed and homogenized in a 50 ml test tube using Ultra Turax (T25) homogenizer for 15 minutes at a rotation velocity of 18,000 rpm to generate an emulsion. The emulsion was allowed to cool at room temperature. The solid lipid nanoparticles (*SLNs*) of nevirapine present in the emulsion as globules were harvested as solids by freeze drying in a lyophilizer for 12 – 15 hours. The *SLNs* of nevirapine were collected and stored at freezing temperatures [10, 11].

Table 1: Formulae for Preparation of Solid Lipid Nanoparticles of Nevirapine

Batch	Composition of Ingredients (%w/w)									
	Lipid Matrix	Stearic Acid	Dika Wax	Coconut Oil	P90H	Sorbitol	Sorbic Acid	Tween 80	NVP	Water to
1	15	-	10	-	5	4	0.05	1.5	0	100
3	15	-	10	-	5	4	0.05	1.5	3	100
6	15	-	5	-	10	4	0.05	1.5	3	100
9	15	-	11.25	-	3.75	4	0.05	1.5	3	100
12	15	-	9	3	3	4	0.05	1.5	3	100
15	5	3.33	1.67	-	-	4	0.05	1.5	3	100
18	5	1.67	3.33	-	-	4	0.05	1.5	3	100
21	15	-	6	6	3	4	0.05	1.5	3	100

P90H = Phospholipon 90H

C. Evaluation of Nevirapine SLNs

➢ *Fourier Transform Infra-Red Spectral Analysis (FTIR) of Nevirapine and Nevirapine SLNs*

FTIR spectra from the Japan-made Shimadzu IR spectrophotometer (FTIR-8300) were used to assess the drug's chemical stability and compatibility with the excipient. The FTIR spectra of nevirapine and solid lipid nanoparticles of nevirapine were obtained by mixing with potassium bromide and pressing at 1 ton/unit. The 4000- 400 cm^{-1} wavenumber range of the FTIR spectra was scanned [13].

➢ *Transmission Electron Microscopy (TEM) of Nevirapine SLNs*

The TEM analysis of the nanoparticles was done to determine the particles morphology using the Cryo-TEM machine at standard operating conditions [14].

➢ *Zeta Sizer Analysis of Particle Sizes of Nevirapine SLNs*

The Zetatrac 10.6.2 Instrument was employed in measuring the sizes of the particles and polydispersity index of nevirapine *SLNs* using a nanoparticle size analyzer (Microtrac Inc., USA). Recently constituted 1 %w/v dispersions of nanoparticles in ethanol were sonicated for 2 minutes before examining the sizes of the particles and the zeta potential using a Zetatrac, to estimate the total mean diameter of nevirapine *SLNs*. An AC electric field with a high frequency was used to vibrate the charged particles. The brownian motion power spectrum, which was a part of the power spectrum arising from oscillating particles, is examined using the modulated power spectrum (MPS) method. Mean particle size and zeta potential were assessed after the preparation of a nevirapine *SLN* dispersion with the necessary obscuration [15].

➢ *In vitro Dissolution Studies of Nevirapine SLNs*

Dialysis membrane was soaked for 15hours using the freshly prepared 0.1N HCl. The *In Vitro* drug release was done using the USP paddle method with freshly prepared 0.1N HCL as the dissolution medium. A volume of 900 milliliters of the dissolution medium was utilized, and it was kept in the bath at 37±0.5°C. The revolution speed of the dissolving unit paddles was adjusted to 50 revolutions per minute, or rpm. A100mg weight of each batch of Nevirapine Solid Lipid Nanoparticles was placed in the dialysis membrane and tied with a thin thread. Each batch was tied individually to a paddle and placed in the 900 ml dissolution medium composed of 0.1N HCl and dissolution commenced immediately. A 3ml each of the samples was withdrawn using a 5ml pipette at intervals of 5minutes, 10minutes, 20minutes, 30minutes, 60minutes, 120 minutes, 180 minutes, 240minutes, 300minutes, 360minutes, 420minutes, 480minutes, 540minutes, 600 minutes, 660minutes and 720minutes. The different samples for each time were placed in previously labelled sample bottles. The dissolution was done in duplicates while maintaining sink conditions by exchanging the quantity of sample withdrawn with equivalent volume of bland dissolution medium [5].

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

III. RESULTS

➢ *Preliminary Spectral Analysis of Nevirapine and PEG 4000*

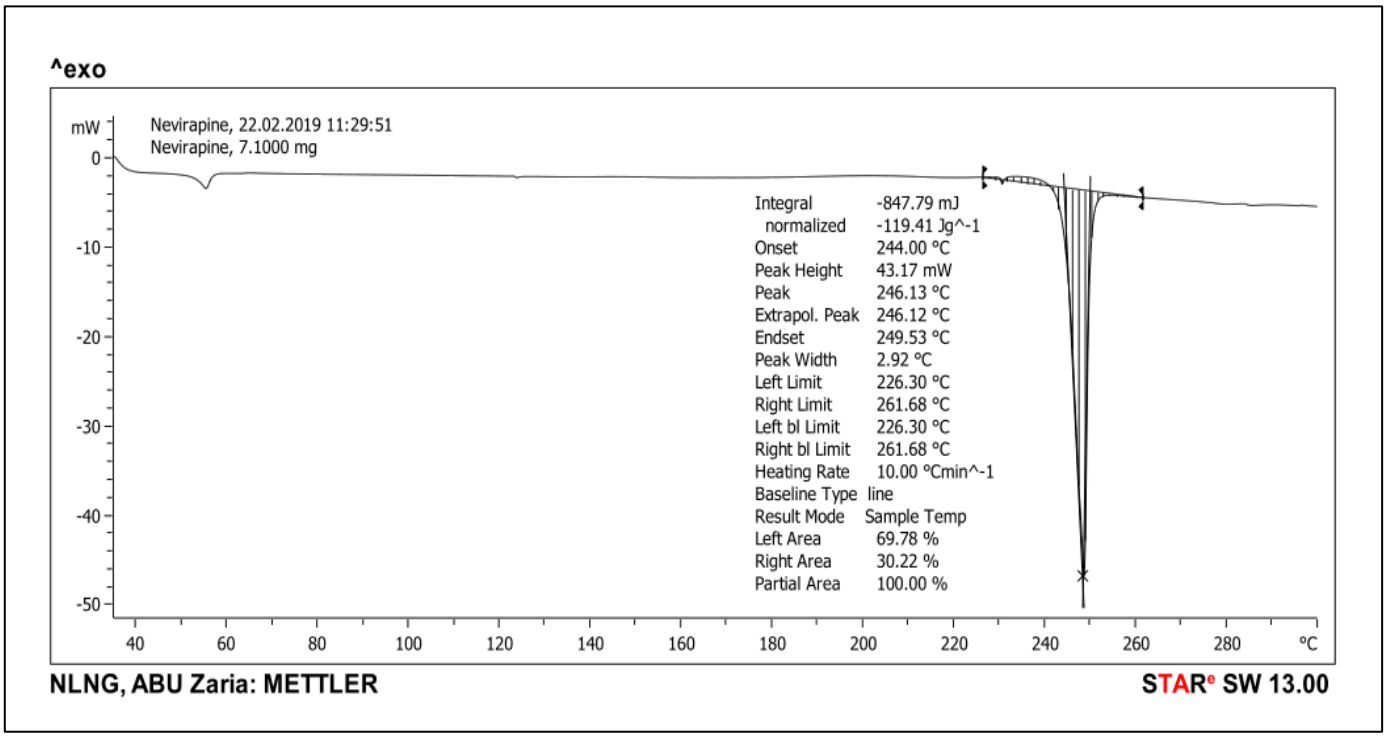

Fig 1: DSC Thermogram of Nevirapine

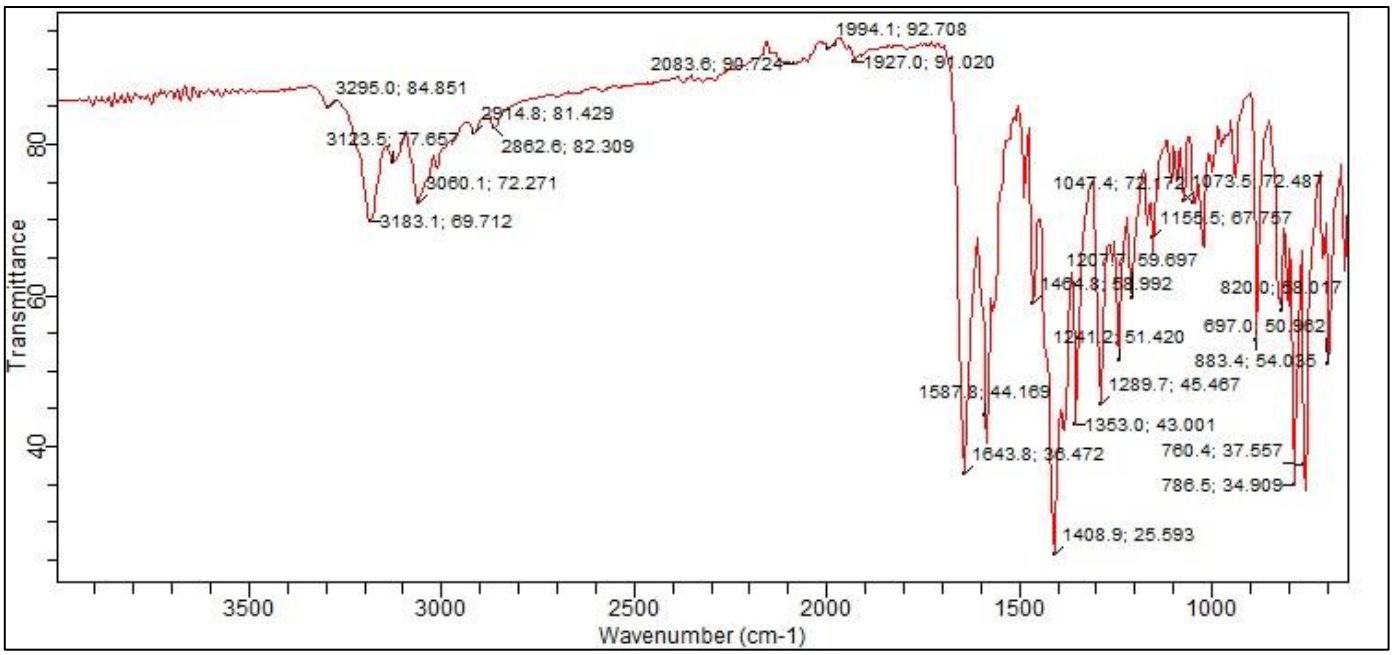

Fig 2: FTIR Spectrum of Nevirapine

The DSC thermogram of our nevirapine pure sample in figure 1 above revealed a single sharp melting endotherm at 246.13°C, which means that the drug is not a hydrate and it is also not a solvate. But the FTIR spectrum of nevirapine as a single agent in figure 2 below showed that nevirapine has a sharp peak at wavenumber of 1643 cm^{-1} suggesting the presence of carbonyl (C=O) stretching vibration. There were also strong sharp peaks wavenumbers of 3060 cm^{-1} which could be due to C-H stretching vibration while the peak at 3183 cm^{-1} may be associated with O-H or N-H stretching vibration. All these peaks at different wavenumbers which have been associated with nevirapine is line with other evaluations of nevirapine in the literature [16,17].

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

➢ *In Vitro Dissolution Studies of Nevirapine Solid Lipid Nanoparticles*

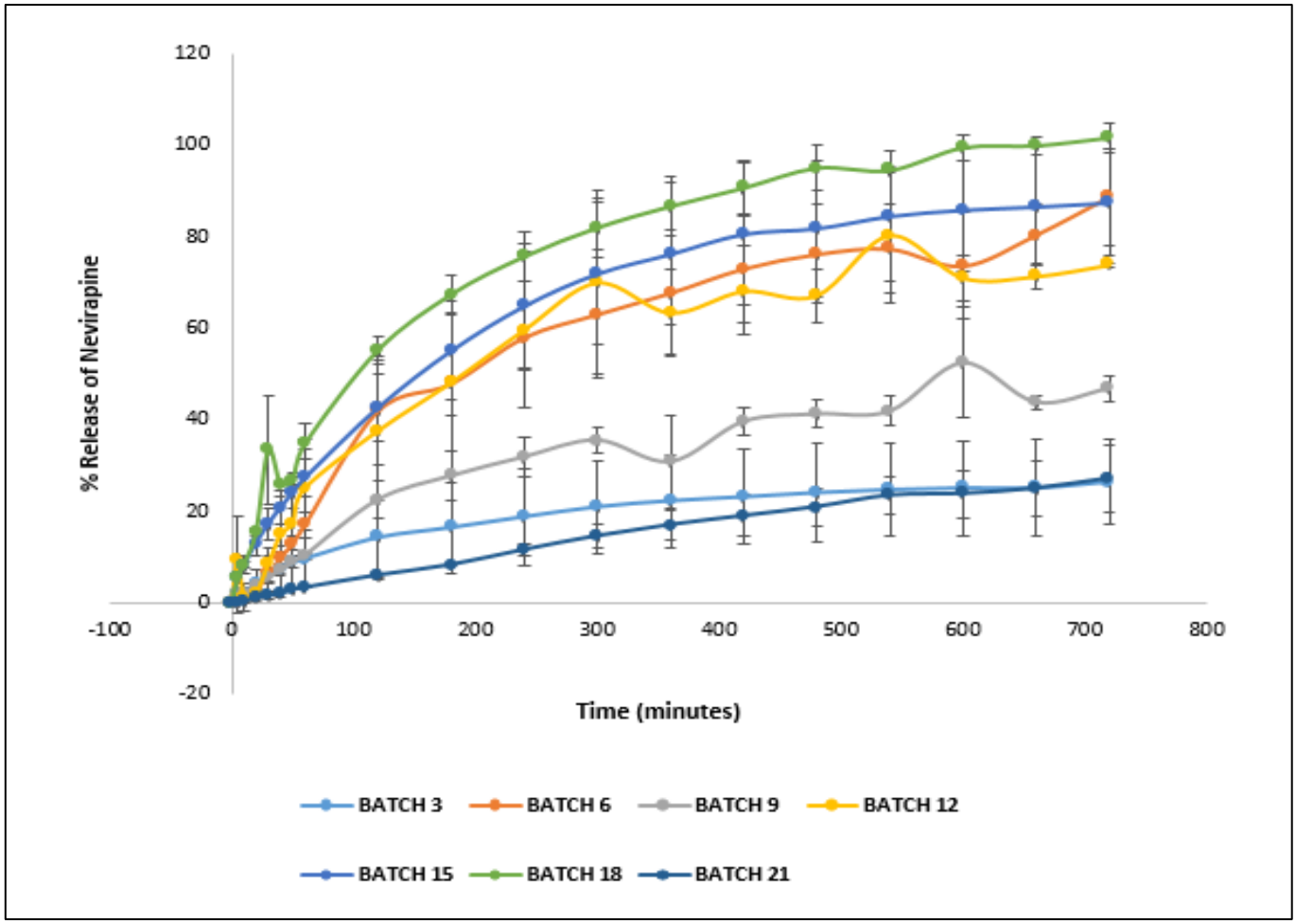

Fig 3: In Vitro Release of Nevirapine from Batches 3,6,9,12,15,18 and 21 *SLNs*

Table 2: Comparing Dissolution Profiles using Similarity factor (f_2)

Batches	**21**	**18**	**15**	**12**	**9**	**6**	**3**
21	**N/A**	13	18	22	40	21	66
18	13	**N/A**	50	36	21	34	15
15	18	50	**N/A**	51	27	55	19
12	22	36	51	**N/A**	33	60	24
9	40	21	27	33	**N/A**	31	45
6	21	34	55	60	31	**N/A**	22
3	66	15	19	24	45	22	**N/A**

Table 3: Comparing dissolution profiles using difference factor (f_1)

Batches	**21**	**18**	**15**	**12**	**9**	**6**	**3**
21	**N/A**	81	78	73	54	74	26
18	81	**N/A**	15	28	59	27	74
15	78	15	**N/A**	28	52	15	70
12	73	28	28	**N/A**	43	-1	64
9	54	59	52	43	**N/A**	-76	38
6	74	27	15	-1	-76	**N/A**	65
3	26	74	70	64	38	65	**N/A**

The *in vitro* drug release profiles was plotted as percentage of drug release verses time. From the result of the *in vitro* dissolution studies of the *SLNs*, batch 18 *SLNs* had the highest percentage release of 101% after 12 hours (720 minutes) followed by batches 6, 15 and 12 with percentage release of 88.5%, 87.5% and 73.9% respectively as shown in figure 3 above. But, batches 3, 9 and 21 had the least release (<50%) of 26.5%, 46.8% and 27.1% respectively. It

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

suggested that if the dissolution study is extended to 24 hours, the SLN batches that had poor release may have improved drug release (this could be for further studies). Recall that batches 15 and 18 that had one of the best release profiles contain only 5% lipid matrices consisting of stearic acid and dika wax but batch 6 and 12 had 15% lipid matrix made of dika wax + P90H (at ratio 1:2) and dika wax + coconut oil + P90H (at ratio 3:1:1) respectively. It is not yet well understood why these batches of *SLNs* had better release studies but we are speculating that it could be due to lower concentrations of dika wax (below 10%) and coconut oil (≤3%) [17,18].

When the dissolution profiles of the *SLNs* were compared using f_2 - similarity factor, batch 18 was exclusively similar to batch 15 and also, batch 21 was exclusively similar to batch 3. There is usually similarity when $f_2 \geq 50$. But batches 15, 12 and 6 had inter-batch similarity. But, when comparing the dissolution profiles of the SLNs using the difference factor (f_1), only batches 18 and 15 and then, batches 15 and 6 were similar respectively. In this case, there is similarity, when $f_1 \leq 15$. Dissolution profile similarity studies above implies that the SLN batches whose dissolution profiles are similar could be used as a substitute for one another or interchanged during therapy. [13,19].

The dissolution efficiency of the three selected batches showed that batch 18 had the highest dissolution efficiency of 77.6% while batches 6, 12 and 15 had a dissolution efficiency of 59.8%, 57.8% and 66.7% respectively. Since some of the dissolution profiles showed similarity, a population standard deviation (SD) of dissolution efficiency between the similar profiles was conducted and it was discovered that batches 15 and 18 had SD of 5.47%, batches 6, 12 and 15 had SD of 3.80% while batches 3 and 21 had SD of 2.17% as shown in Table 5. This further confirms their similarity since the deviations are still within the prescribed ±10% deviation from average content [13]. The similar dissolution profiles were also compared with T-test: Two- Sample with Equal Variances and ANOVA: Single Factor as shown in Table 5.

The T-test and ANOVA which were done at a significance level of $p < 0.05$ showed that the dissolution profiles compared are similar because the dissolution profile groups compared had p-value > 0.05 for both statistical determinations [20].

Table 4: AUC and Dissolution Efficiency (DE) of the *in Vitro* Dissolution Profiles

Batch	AUC (mg.hr/ml)	DE (%)
3	14,247.02	19.78752
6	43,026.57	59.75912
9	24,001.71	33.33571
12	41,639.42	57.83253
15	48,004.26	66.67259
18	55,878.95	77.60965
21	11,127.12	15.45433

Table 5: Statistical Comparison of the Similar Dissolution Profiles

Batch	T-test *p*-value	ANOVA *p*-value		STD of DE (%)	
15 & 18	0.4506	0.4506		5.4700	
3 & 21	0.2211	0.2211		2.1700	
6, 12 & 15	Not Applicable	0.7162		3.8000	

N/B: Significance level is represented with p-value = 0.05, STD=Standard Deviation, DE=Dissolution Efficiency

Table 6: Zeta Sizer Analysis of Nevirapine SLNs

Batch	Peak1±SDnm (%Vol)	Peak2±SDnm (%Vol)	Peak3±SDnm (%Vol)	PDI	Result Quality
3	838.8±111 (91.49)	55.11±7.413(8.60)	-	0.732	Poor
6	782.0±259.6 (100)	-	-	0.646	Poor
9	412.8±55.11 (65.8)	219.2±28.77(34.2)	-	0.820	Poor
12	1349±394.2 (92.0)	5159±798.7(7.3)	296.8±64.02(0.7)	0.477	Poor
15	10.29±2.76 (34.1)	40.18±24.3 (62.7)	559±140.2(2.4)	0.277	Good
18	40.18±24.19 (65.0)	10.33±2.787(31.7)	558.8±140.4 (2.4)	0.271	Good
21	307.8±60.01(47.9)	121.0±16.60(33.3)	47.75±6.495(18.8)	0.762	Poor

SDnm=Standard Deviation in nanometer, PDI=Polydispersity Index, %Vol= % Volume

Table 6 above is a summary of the particle size determination and analysis of the different *SLNs* batches using the zeta sizer apparatus. Though all the SLN batches had particles with sizes that are within the nano-range, yet their polydispersity index (PDI) which is usually above 0.3 shows that their result quality is low except SLN batches 15 and 18 had good result quality with PDI of 0.277 and 0.271 respectively. M. Danaei et al. (2018) found that a PDI of 0.3 or lower is considered acceptable and indicates a uniform population of phospholipid vesicles in drug delivery applications with lipid-based carriers like liposome and nanoliposome formulations [21].

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

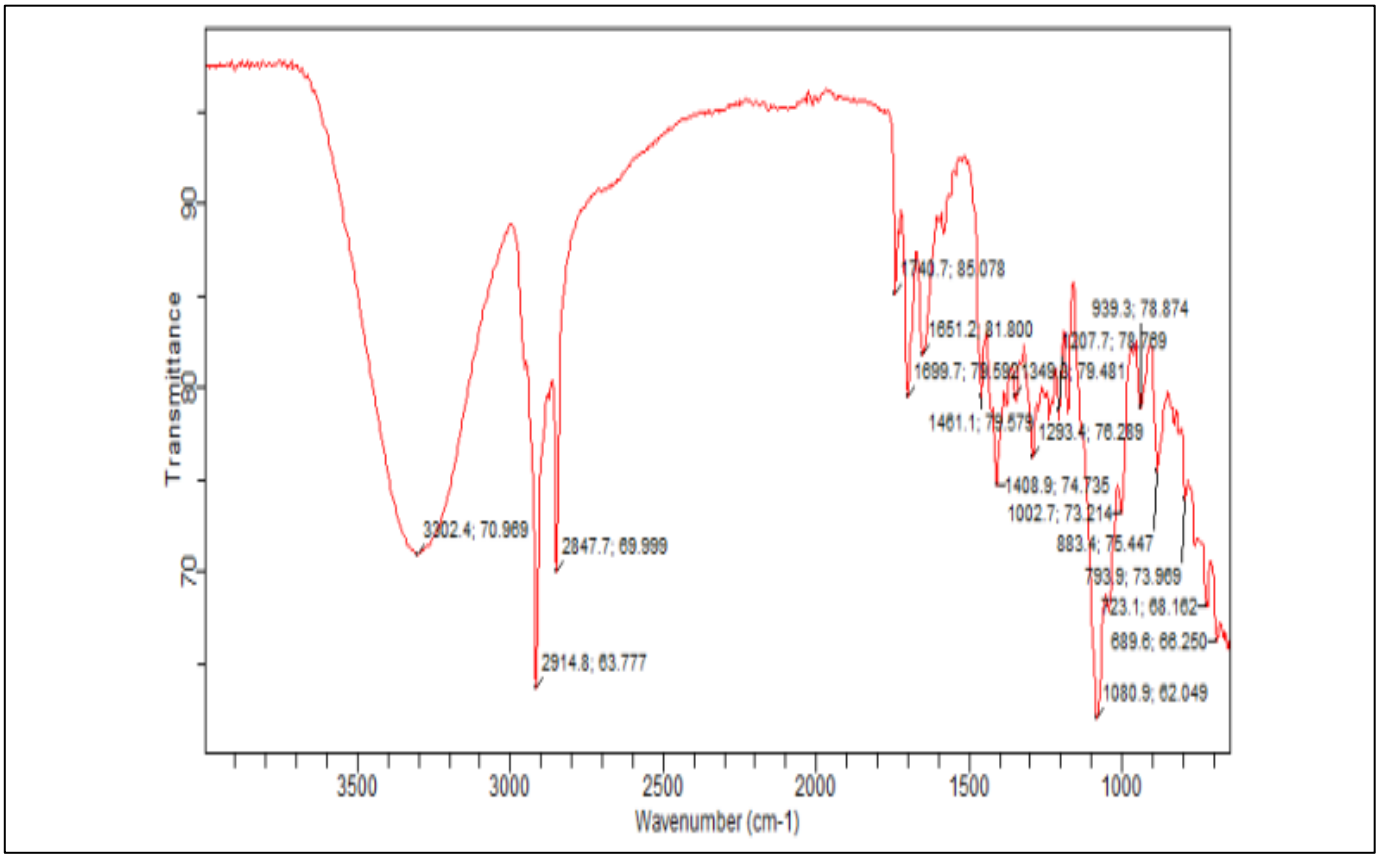

Fig 4: FTIR Spectrum of Batch 15 SLN of Nevirapine

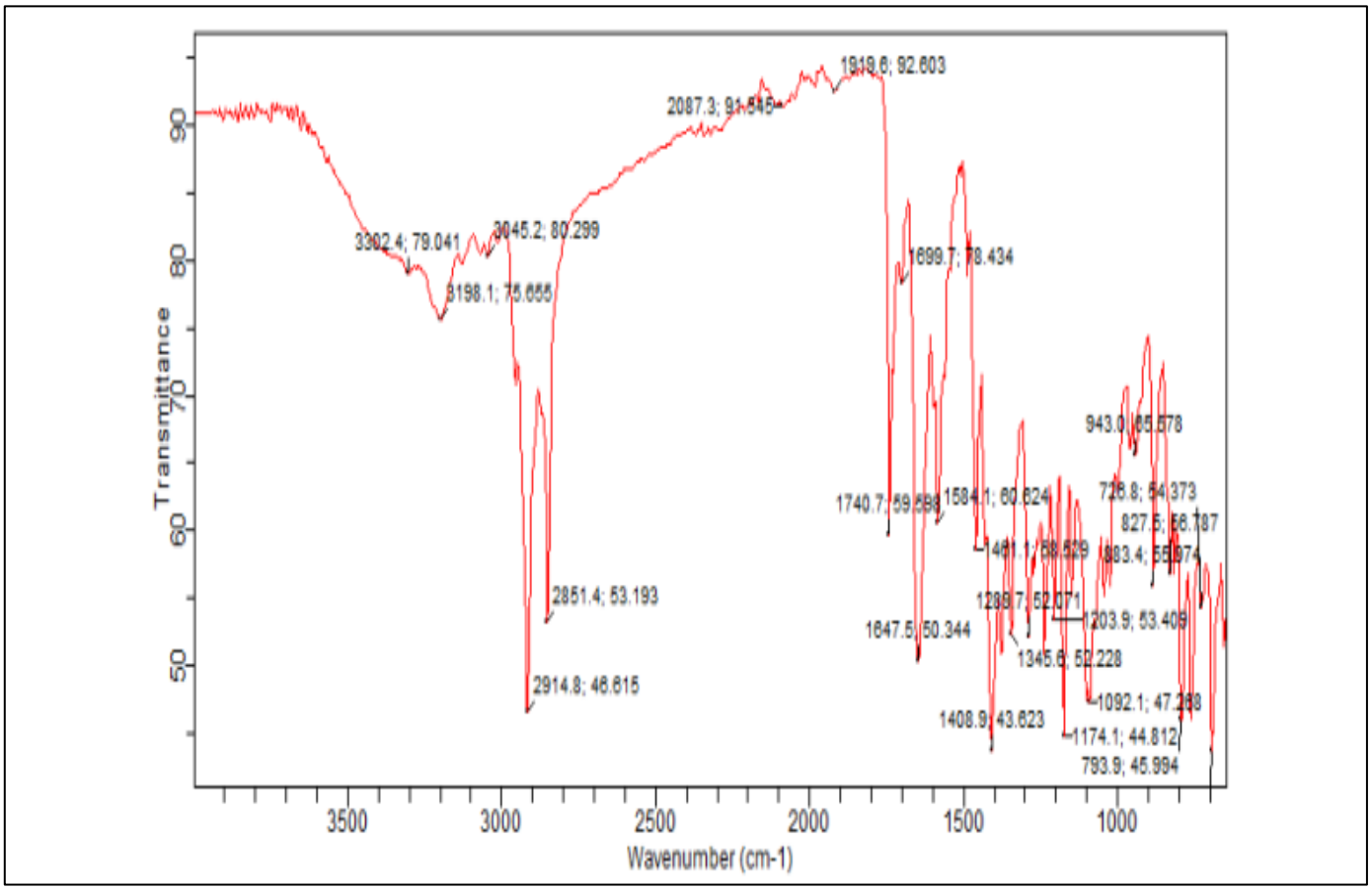

Fig 5: FTIR SPectrum of Batch 18 SLNs of Nevirapine

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

Batch 15 **SLNs** had a peak at a wavenumber of 1651.2cm^{-1} with transmittance intensity of 81.8% and another peak at 3302.4 cm^{-1} with transmittance intensity of 70.97% while batch 18 **SLNs** had peaks at wavenumbers 1647 cm^{-1} and 3302.4 cm^{-1} with transmittance intensities 50.34% and 79.04% respectively. The peaks at the wavenumbers of 1651.2cm^{-1} and 1647cm^{-1} strongly suggest the presence of carbonyl (C=O) stretching vibration which usually occur within the range 1700 - 1600 cm^{-1}. The C=O stretching vibration is the most characteristic FTIR peak of nevirapine. The other peak at 3302.4 cm^{-1} correspond to the stretching vibrations of O-H bond. The peak at 3198.1cm^{-1} indicates the presence of a seven membered ring which normally occur at the wave range 3295-3188cm^{-1} [22].

➢ *Cryo-TEM Surface Morphology Results of the SLNs*

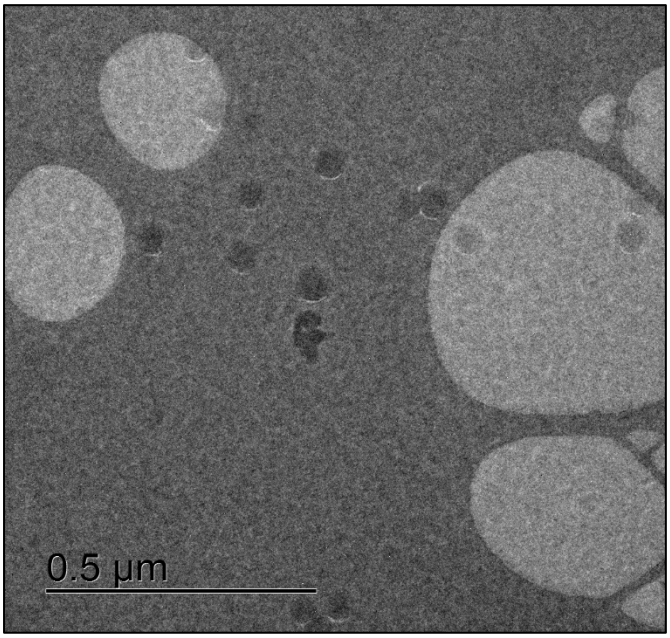

Fig 6: Cryo-TEM Result for Particle Morphology of Batch 1 SLNs

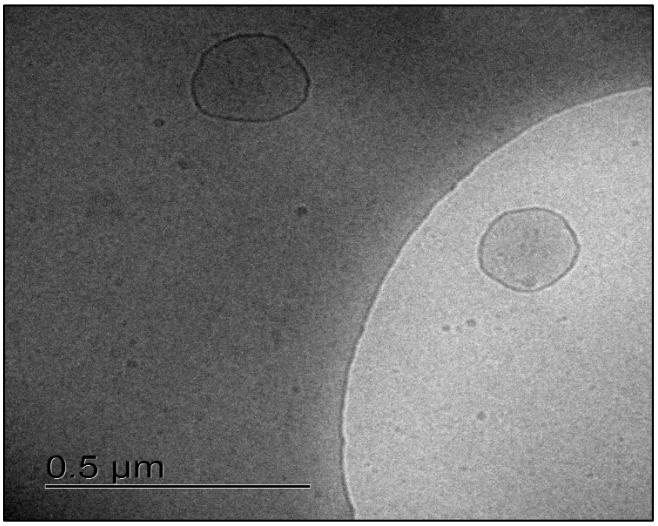

Fig 7: Cryo-TEM Results for Particle Morphology of Batch-15 SLNs

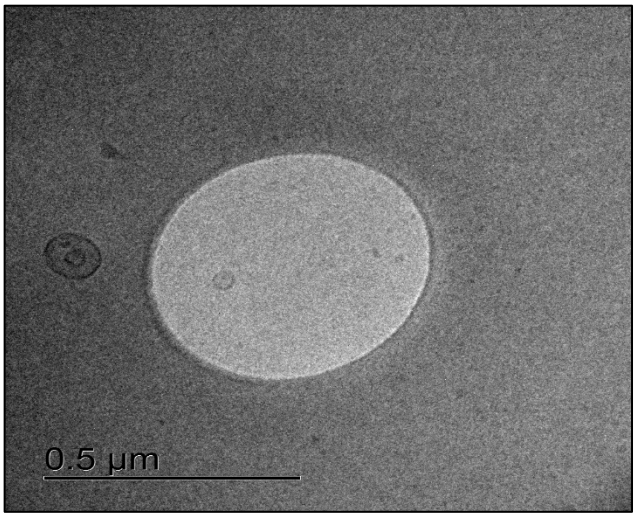

Fig 8: Cryo-TEM Results for Particle Morphology of Batch-18 SLNs

The Cryo-TEM results particle morphology for batches 1, 15, and 18 shows that the solid lipid nanoparticles are round to oval in shape which is typical of particles generated from emulsion globules; recall that the particles were generated from a lyophilization process of emulsions made from solid edible wax – dika wax [8]. It is also worth noting that the surfaces of the particles are smooth and so, the *SLNs* will not form coarse globules when re-constituted into emulsion for therapeutic administration.

➢ *Encapsulation Efficiency, Loading Capacity and Zeta Potential Results of the SLNs*

Batches 15 and 18 SLNs had loading capacities of 6.71% and 9.82% respectively and encapsulation efficiencies of 49.35% and 70.19% respectively. The zeta potential of batches 1, 15 and 18 solid lipid nanoparticles were determined with a zeta sizer and results showed that these SLN batches had zeta potentials of -16.83±0.404mV, -44.30±0.624mV and -40.03±2.65mV respectively. Expert advice revealed that distribution data was good and result met quality criteria. It's crucial to remember that the interpretation of zeta potential values for solid lipid nanoparticles may vary depending on the specific application or desired characteristics of the *SLNs*. Nanoparticles with zeta potentials exceeding +30 mV or falling below -30 mV are generally considered highly cationic or highly anionic, respectively. Conversely, particles with zeta potentials ranging from -10 to +10 mV are typically regarded as neutrally charged. More specifically, a zeta potential range between -30 mV to +30 mV is considered acceptable for colloidal systems such as SLNs. Values closer to zero may be linked to reduced stability and increased propensity for particle aggregation or coagulation. But, higher absolute values (e.g., above ±30 mV) suggest greater stability due to electrostatic repulsion between particles. Zeta potential can affect a nanoparticle's ability to penetrate membranes due to the negative charge of most cellular membranes. Cationic particles often show increased toxicity associated with cell wall degradation. Therefore, *SLN* batches 15 and 18 and expected to be highly stable due to higher zeta potential and less toxicity due to their anionic nature [15,23].

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

IV. CONCLUSION

Batches 15 and 18 SLNs have good potential for enhancing the delivery of nevirapine as extended-release dosage forms for better management of HIV/AIDS. The aforementioned batch formulae could also serve as leads for pilot scale-up and commercial production of nevirapine solid lipid nanoparticles to be presented in hard gelatin capsules or as lipid granules for reconstitution into emulsion before administration.

ACKNOWLEDGEMENT

We are grateful to the Academic Staff Union of Universities (ASUU), Nigeria for the support grant she gave us to aid us do the bench of this research . Thank you to Lipoid, Germany for the gift of Phospholipon 90H through Dr. C. C. Mbah of the University of Nigeria, Nsukka.

REFERENCES

[1]. Swaminathan S, Ramachandran G, Agibothu Kupparam HK, Mahalingam V, Soundararajan L, Perumal Kannabiran B, et al. Factors influencing plasma nevirapine levels: a study in HIV-infected children on generic antiretroviral treatment in India. J Antimicrob Chemother. 2011 Jun; 66(6):1354–9.

[2]. Kou H, Du X, Li Y, Xie J, Qiu Z, Ye M, et al. Comparison of nevirapine plasma concentrations between lead-in and steady-state periods in Chinese HIV-infected patients. PLoS One. 2013; 8(1):e52950.

[3]. Frontiers | Follicular Dendritic Cells of Lymph Nodes as Human Immunodeficiency Virus/Simian Immunodeficiency Virus Reservoirs and Insights on Cervical Lymph Node | Immunology [Internet]. [cited 2022 Mar 16]. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2018.00805/full

[4]. Pottabathini V, Gugulothu V, Kaliyaperumal M, Battu S. Identification, Isolation and Characterization of Unknown Acid Degradation Product of Nevirapine. American Journal of Analytical Chemistry. 2016 Aug 26; 7(9):663–78.

[5]. Raju1 A, Reddy2 AJ, Satheesh1 J, Jithan1 AV. Preparation and characterisation of nevirapine oral nanosuspensions. Indian Journal of Pharmaceutical Sciences. 2014; 76(1):62.

[6]. Poovi G, Damodharan N. Lipid nanoparticles: A challenging approach for oral delivery of BCS Class-II drugs. Future Journal of Pharmaceutical Sciences. 2018 Dec 1; 4(2):191–205.

[7]. Ross SM. African Mango (IGOB131): A Proprietary Seed Extract of Irvingia gabonensis is Found to Be Effective in Reducing Body Weight and Improving Metabolic Parameters in Overweight Humans. Holistic Nursing Practice. 2011 Jul; 25(4):215–7.

[8]. Njoku OU, Ugwuanyi JO. Nutritional and Toxicological Properties of Dika Fat (Irvingia gabonensis). Journal of Herbs, Spices & Medicinal Plants. 1997 Jul 2; 4(4):53–8.

[9]. Irvingia. In: Wikipedia [Internet]. 2023 [cited 2023 Jul 26]. Available from: https://en.wikipedia.org/w/index.php?title=Irvingia&oldid=1134643705

[10]. E.E. Chinaeke et al. Formulation of Novel Artesunate-Loaded Solid Lipid Microparticles (SLMs) Based on Dika Wax Matrices: In Vitro and in Vivo Evaluation. Journal of Drug Delivery Science and Technology. 2014; 24(1):69-77. Downloaded on 13th March, 2024 from: https://www.sciencedirect.com/science/article/abs/pii/S177322471450010X.

[11]. Chime Salome Amarachi et al. Diclofenac potassium–loaded dika fat solid lipid microparticles: In vitro and in vivo characterisation. Journal of Pharmacy Research. 2013; 1(3):227-234. Downloaded on 12th March, 2024 from: https://www.researchgate.net/publication/271954615_Diclofenac_potassium-loaded_dika_fat_solid_lipid_microparticles_In_vitro_and_in_vivo_characterisation.

[12]. Sarkar M, Perumal OP, Panchagnula R. Solid-state characterization of nevirapine. Indian Journal of Pharmaceutical Sciences. 2008; 70(5):619.

[13]. Abali S. O., Ifeoma C. Ekenna, Ochen F. Scribd. Formulation and Characterization of Extended-Release Nevirapine Solid Dispersions. International Journal of Innovative Science and Research Technology. 2022; 7(3):588-594.

[14]. Harris J.R. (2015). Transmission electron microscopy in molecular structural biology: A historical survey. Arch Biochem Biophys, 581:3-18. doi:10.1016/j.abb.2014.11.011.

[15]. Clogston JD, Patri AK. Zeta potential measurement. Methods Mol Biol. 2011; 697:63–70.

[16]. Sarkar M, Perumal OP, Panchagnula R. Solid-state characterization of nevirapine. Indian Journal of Pharmaceutical Sciences. 2008; 70(5):619.

[17]. Jr S, Strattmann RR, Alburquerque MM, Araújo A, Matos J, Neto PJ. In vitro evaluation of dissolution profiles and thermal properties of some commercial formulations of nevirapine tablets. Acta Farmaceutica Bonaerense. 2006; 1(25):76–82.

[18]. Asrade B, Tessema E, Tarekegn A. In vitro comparative quality evaluation of different brands of carbamazepine tablets commercially available in Dessie town, Northeast Ethiopia. BMC Pharmacology and Toxicology. 2023 May 25; 24(1):35.

[19]. Shah VP, Tsong Y, Sathe P, Williams RL. Dissolution Profile Comparison Using Similarity Factor, f2. Dissolution Technol. 1999; 6(3):15–15.

[20]. Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. Application of Student's t-test, Analysis of Variance, and Covariance. Ann Card Anaesth. 2019; 22(4):407–11.

[21]. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics. 2018 May 18; 10(2):57.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24APR1028

[22]. H S, M B, Tl D, Z H, As L, Ic S, et al. FTIR, Dissolution and Anti-viral Activity of Nevirapine Co-crystals. Pharm Anal Acta [Internet]. 2017 [cited 2021 Apr 26];08(09). Available from: https://www.omicsonline.org/open-access/ftir-dissolution-and-antiviral-activity-of-nevirapine-cocrystals-2153-2435-1000561.php?aid=94016

[23]. Zeta Potential - An Introduction | Malvern Panalytical [Internet]. [cited 2024 Jan 8]. Available from: https://www.malvernpanalytical.com/en/products/measurement-type/zeta-potential