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Preliminary characterization of PEGylated nanostructured lipid carriers as potential drug delivery system

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ABSTRACT: This study is a preliminary characterization of PEGylated nanostructured lipid carriers as potential drug delivery system using appropriate standard methods. DSC traces showed that PEG 4000 was the most crystalline of all the lipids and Softisan® 154 was the least crystalline due to high enthalpy (-36mW/mg) and low enthalpy (-8.9 mW/mg) respectively. It was noticeable that the endothermic peak heights of PEGylated lipid matrix became smaller compared with the original starting lipid. All demonstrated good potentials for use as nanostructured lipid carrier drug delivery system but LMA, LMB and LMC turned to be the preferred in other of better thermal activity. SEM analysis revealed evidence of coarse structure amorphous than the individual lipidic systems. By implication showed increase drug loading capacity, it follows that that PEGylated nanostructured might be employed as NLCs carriers to serve as alternative transdermal delivery systems to improve the solubility of some poorly soluble drugs.

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Pharmaceutical industries presently focused in the development of more effective delivery systems for currently available active pharmaceutical ingredients as increased treatment failure, low efficiency and resistance continue to be the key setback in the management of diseases. Many molecule with very promising activity and therapeutic potentials did not reach the clinical trial stage because of absence of effective delivery systems due to poor solubility, unacceptable plasma variation levels, low bioavailability and large biodistribution (Mehnert and Mäder,2001). There is improvement of delivery of many therapeutic molecules with innovative solutions of nanoparticle which is alternative that overcome the many challenges associated with efficaciousness and safety (De Jong and Borm, 2008). Nanoparticles possess unique features like functional surface which are significant and substantial volume –to surface ratio that allow them to adsorb easily to other compounds (Shidhaye *et al.*, 2008).The first generation of lipid based nanoparticles were originally developed as a simulation of o/w emulsion where the internal oily phase was replaced by a solid lipid matrix which was reported in early 1990s (Müller *et al.*, 2011). Due to

some limitations of nanoparticles like poor drug loading capacity, instabilities due polymorphic transitions and increase in the risk of drug expulsion from the formulation during storage, using the solid and liquid mixtures that form amorphous solid at both body and room temperature, (Müller *et al.*, 2011) developed nanostructured lipid carrier. Lipid based nanotechnology for delivery system for active pharmaceutical ingredient can be applied in delivery systems such as nanoemulsion and nanostructured lipid carrier (Hallan *et al*., 2021; Matei *et al*., 2021). Nanostructured lipid carriers have been exploited in recent years as drug delivery systems targeted to different regions using various administration routes and appear to be attractive method for the delivery of lipophilic drugs (Nnamani *et al*., 2014). Development of nanocarrier could also be made from phospholipids, lipids and polymer (Nnamani *et al*., 2021; Attama *et al*., 2016; Nnamani *et al.*, 2020). Nanostructured lipid carriers are developed also as drug delivery system through the skin because they are biocompatible, lipid in nature, non-toxic and irritant material. In addition, nanostructured lipid carrier are characterized by large surface area which enables longer contact time of the formulation with the skin to attend sustained drug delivery (Sharma et al., 2013). Transdermal drug delivery systems are dosage forms developed to deliver therapeutic agent across a patient's skin (Alkilani *et al.*, 2015). With transdermal drug delivery system undesirable side effects are brought to minimal, as the delivery system provide controlled constant administration of drug which allow continuous input of drugs with short biological half – lives and eliminate pulsed entry into the systemic circulation devoid of first pass metabolism which enhance bioavailability (Jain 2001). PEGylation, generally described as the process of both covalent and non-covalent molecular amalgamation or attachment of polyethylene glycols. It has been studied extensively with the goal of improving the pharmacokinetic behaviour of lipids and as well as drugs (Kenechukwu *et al*., (2015). PEGylated active drugs and lipids exhibit prolonged half-life, higher stability, water solubility, lower immunogenicity and antigenicity, as well as potential for specific cell targeting. Increased in production cost and in binding activity are some of the pitfalls (Chime *et al*., 2013). PEG 4000 has been used in earlier studies for the delivery of many bioactives and drugs (Obeidat *et a*l., 2010; Sundram *et al*., .2003).

Softisan® 154 is a brand name for hydrogenated palm oil. It is one of the six Softisan® types, all of which are hard fats based on blends of natural triglycerides (Sundram 2003). Others variants include Softisan® 100, 133, 134, 138 and 142. Softisan® hard-fats are readily soluble in diethyl ether, toluene and acetone, almost insoluble in methylene chloride, 96 % ethanol and water and miscible with other fats and oils. DSC is an informative technique that reveals the changes that occur in a material with change in temperature (Özdemir *et al*., (2019). DSC generally are used to obtain information such as the melting and crystallization behavior of the sample under investigation such as crystal ordering of solid lipid and internal polymorphism and FTIR is used to investigate indication of interaction between the lipid matrices as well as other ingredients used in the preparation of NLC formulations(Özdemir 2019). Hence, the objective of this study was to formulate nanostructured lipid carriers as a potential transdermal drug delivery system.

MATERIALS AND METHODS

Materials: Transcutol®HP gotten from Gattefosse SAS (saint priest Cedex, France), Phospolipon® 90H, (Phospholipid GmbH, Köln, Germany), Softisan® 154 pellets ((Gattefossé, Saint Priest Cedex, France), Polyethylene glycol 4000 (Ph. Eur. Carl Roth GmbH +Co. KG Karlsruhe, Germany)

Preparation of lipid matrices for Nanostructured lipid carrier (NLCs) formulation: The lipid matrices were prepared in paraffin oil bath by fusion (Friedrich *et al*., 2003) using a hot plate (Ika RCT basic, Ika.Staufen , Germany). It was established from the previous studies (Chime et al.,2013; Kenechukwu *et al*., 2015; Attama *et al*., 2015; Ugwu *et al*.,2018) that 7: 3 is one of the optimal ratios for the combination of hard fats(e.g Softisan® 154, beewax) and phospholipids (Phospholipon®90H- P90H).A combination of Softisan® 154 and Phospholipon® 90H was chosen in admixture with liquid lipid (Transcutol®) and polyethyelen glycol (PEG) 4000 based on this in preparing structured lipid matrices for NCLs formulation. Five batches of lipid matrices with different ratios (1:1:3, 2:1:3, 2:2:3, 3:1:1 & 3:1:2) were formulated as shown in the table 1. By continuous blending, the optimum ratio of Softisan® 154, Phospholipon® 90H and polyethyelen glycol (PEG) 4000 was determined. 6g of the Phospholipid (Phospholipon® 90H) was briefly weighed and placed in a beaker, 14g of the Softisan® 154 (hard fat) then was also weighed and melted together with the Phospholipon® 90H over a temperature- regulated oil bath at 90^0C (containing liquid paraffin) placed on hot-plate magnetic stirrer assembly, stirred and allowed to solidify. (Phospholipon® 90H) was weighed and placed in a beaker. Then 14 g of the hard fat (Softisan® 154) was also weighed and melted together with the phospholipid over a temperature-regulated oil bath (containing liquid paraffin) placed on hot-plate magnetic stirrer assembly, stirred and

allowed to solidify. Based on Table 1, appropriate quantities of Softisan® 154 and Phospholipon® 90H were melted in a water bath and different quantities of Transcutol® and PEG 4000 were gradually added to the mixtures and stirred continuously.

Table 1: Formulation composition of lipid matrices for NLCs formulation

S/N	Ratio of components	Softisan®154 &P90H (7:3) % w/w	Transcutol® % w/w	PEG4000 % w/w
A	1:1:3	2	2	6
B	2:1:3	3.3	1.6	5
C	2:2:3	2.9	2.9	4.3
D	3:1:1	6	2	2
E	3:1:2	5	1.6	3.3

Characterization of the lipid matrices: Differential scanning calorimetry (DSC): The melting transitions and changes in heat capacity of all the lipid matrices were determined using a calorimeter (Netzsch DSC 204 F1, Germany). About 3mg to 5 mg of each lipid matrix was weighed into an aluminum pan, hermetically sealed and the thermal behaviour determined in the range of 20 – 250 °C at a heating rate of 5 °C/min. The temperature was held at 80 °C for 10 min and thereafter, cooled at the rate of 5 to 10 °C/min. Baselines were determined using an empty pan, and all the thermograms were baseline-corrected. Lipid matrices with desirable thermal properties were selected and further analyzed.

Fourier transform infra-red (FTIR) Spectroscopic analysis: The compatibility between the pure drug and lipid matrix was studied using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan). The spectra were recorded in the wavelength region of 4000 to 400 cm^{-1} with threshold of 1.303, sensitivity of 50 and resolution of 2 cm^{-1}. In each case, the sample was dispersed in KBr and then compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained. This procedure was followed to obtain spectra for batches of NLCs. Infrared spectra of Softisan® 154, PEG 4000 and five batches of lipid matrices were obtained using transmitance mode. About 2 %w/w of each sample with respect to the potassium bromide (KBr) disc was mixed with dry KBr (FT-IR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wave number region of 400 - 4000 cm^{-1} using FT-IR spectrophotometer (Model 500, Buck Scientific, USA) coupled to a computer with Omnic analysis software. The characteristic peaks of infra-red transmission spectra were recorded.

Scanning Electron microscopy (SEM): The morphological characteristics of selected lipid matrices were determined by a scanning electron microscope (JEOL-JSM-6 360, Japan) at different magnifications. Initially, samples were coated with gold using ion sputter at an accelerating voltage of 20 kV and then visualized under the scanning electron microscope.

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC) measurement: The DSC analyses of pure samples of individual lipids were done to gain insight of thermal behavior of the various lipids. It is very necessary to confirm the stability of lipidic or polymeric systems and to establish the possibility of modifying the properties of them (Pranshu, T, 2011; Ryan et al.,2008). The DSC thermograms (table 2) showed that all the lipids are crystalline in nature exhibiting sharp melting endothermic peak at 130.30°C , 61.30°C ,64.20°C ,120.30°C for Transcutol® , softisan® 154, PEG4000 and P90H ® with enthalpies ranges from -26.76 mW/mg , -8.9 mW/mg , -36.34 mW/mg , -24.56 mW/mg respectively. The DSC thermogram of PEG4000 (table 2) showed endothermic peak of 64.20°C with an enthalpy of -36.34 mW /mg indicating its crystalline nature while thermograms of high PEGylated lipid matrix showed different melting peaks and thermal properties as illustrated figures 3 -5 and table 2.

Table 2: Thermal properties of the lipid matrices for NLCs preparation

Sample	Melting point(°C)	Enthalpy (w/g)	Melting Temperature range (°C)	Area (J/g)
Transcutol®	130.30	-26.76	130-150	372.0
Softisan®154	61.30	-8.9	37-75	71.91
PEG4000	64.20	-36.34	50-75	67.20
P90H	120.30	-24.56	120-180	134.3
LMA (1:1:3)	87.00	-0.35	80-119	24.34
LMB (2:1:3)	62.56	-3.20	25-78	51.55
LMC (2:2:3)	60.72	-4.20	25-75	71.59
LMD (3:1:1)	62.60	-2.5	25-78	61.18
LME (3:1:2)	64.54	-2.10	25-75	65.20

There was a further modification in the crystal properties of the lipid upon addition of high ratio of PEG4000 compared to individual lipids. High PEGylated lipid matrix , LMA (1:1:3) ,LMB(2:1:3) and LMC (2:2:3) lipid matrices based on softisan®

154 and P90H ® (mixture 7: 3) : Transcutol® : PEG400 ratio gave melting point of 87.00°C [LMA (1:1:3)], 62.56°C [LMB(2:1:3)] and 60.72°C [LMC (2:2:3)] with corresponding enthalpies of -0.35 mW/mg [LMA (1:1:3)], -3.20 mW/mg [LMB(2:1:3)] and -4.20 mW/mg [LMC (2:2:3] respectively .It was particularly noticeable that endothermic peak heights became smaller compared with the starting lipids alone. This may be contributed to the heterogenous composition of the matrices and points at presence of disorder in the matrices which is vital for increased drug loading. In addition (as shown in table 2) ,there were inconsistence and variation in melting temperature range and melting points on the high ratio PEGylated lipid matrices although enthalpies were relatively lower.

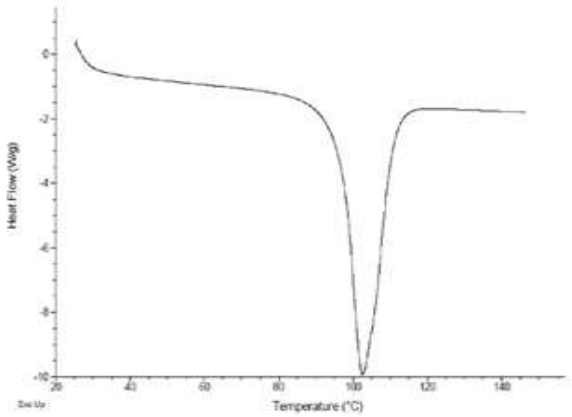

Fig.1: DSC thermogram of Transcutol®

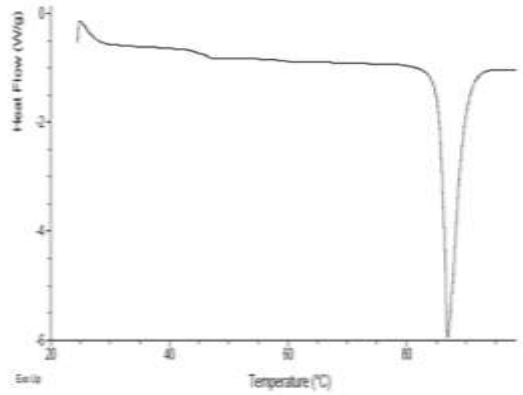

Fig.2: DSC thermogram of Softisan

LM A (1:1:3) Batch contain softisan® 154 and P90H ® (mixture 7: 3), Transcutol® and PEG400 ratio of 1:1:3, LM B (2:1:3) Batch contain softisan® and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 2:1:3, LM C (2:2:3) Batch contain softisan and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 2:2:3, LM D ((3:1:1) Batch contain softisan 154 and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 3:1:1 , LM E (3:1:2) Batch contain softisan and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 3:1:2. It was observed that mixtures of lipids modify the entire properties of the individual lipids.

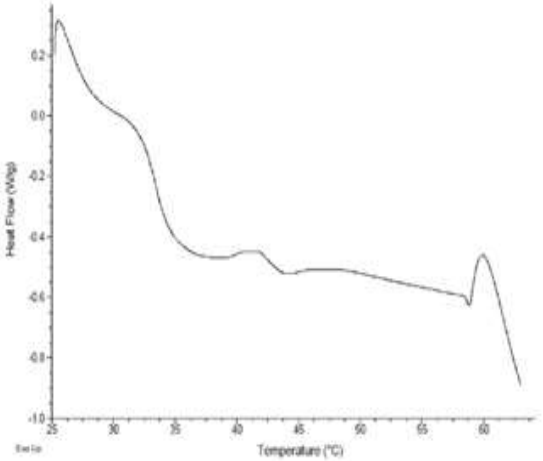

Fig.3: DSC thermogram of Lipid matrix A

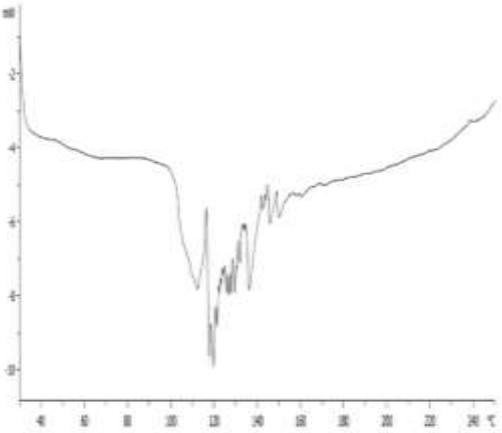

Fig.4: DSC thermogram of lipid matrix B

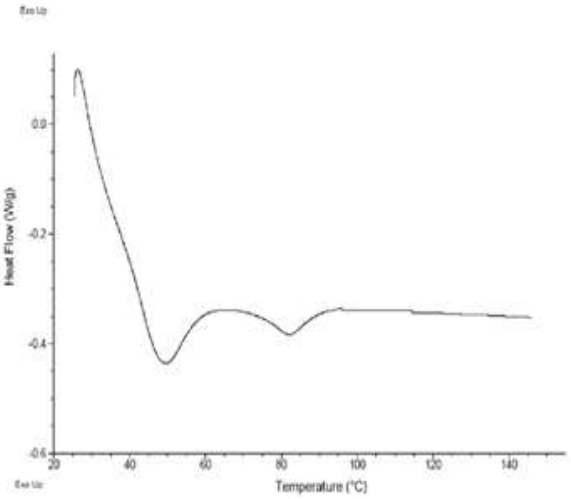

Fig. 5: DSC thermogram of lipid matrix C

These fatty acids may have interacted in such a manner as to partly disorder the original crystal arrangement of individual lipids since they have varied contents that vary widely in degree of saturation and chain length. Our observation shows that structuring of Softisan® and P90H (mixture 7: 3) with Transcutol® produced matrices with lower melting endotherms as well as enthalpies. They achieved larger distortions in the crystal arrangement of their individual matrices thereby creating numerous spaces for possible drug localization and of course will highly increase drug pay load. Reduction in enthalpy generally suggests less crystallinity of the lipid matrices (Attama *et al*., 2015). This is perhaps due to the presence of the unsaturated phospholipid molecules of admixture of Softisan® 154 and P90H ® in the ordered structures of the matrices that caused a broadening and a shift of the solid lipid- to-lipid crystal transition peak towards lower temperatures as well as PEGylation. We, therefore, infer that the lipid matrices were successfully prepared by fusion method which is simple, reproducible, scalable and cheap. The optimized processing parameters have shown that lipid structuring modifies the properties of the individual, lipids and may increase or decrease crystallinity, all batches demonstrated good potentials for use as nanostructured lipid carrier (NLCs) drug delivery system but LMA, B and C turned to the best.

Fourier transform infra-red spectrophotometer (FTIR): FT-IR is a non - destructive analysis technique used for identifying any interaction that may occur between the materials during nanoparticle formulation. Fourier transform infra-red spectrophotometer was used to ascertain the possible interactions between the constituents of the lipid matrix (Softisan® and P90H ® ,Transcutol® and PEG400) formulation as shown in table 3. By implication, the interaction of PEG4000 was more intense with batch A, B and C matrices.

Table 3: FT-IR Spectrum properties of the lipid matrices

Sample	Characteristics bands
Transcutol®	2714cm^{-1}and 2747cm^{-1}(O=C-H stretching two bands)
Softisan® 154 &P90H	2914cm^{-1}and 2847cm^{-1}(O=C-H stretching two bands)
PEG4000	2881cm^{-1} (-C-H stretching), 1468cm^{-1} (-C=H deformation)
LM A (1:1:3)	2938cm^{-1} and 2855cm^{-1}(O=C-H stretching two bands)
LM B (2:1:3)	2914cm^{-1}and 2847cm^{-1}(O=C-H stretching two bands)
LM C (2:2:3)	2918cm^{-1} ,2855cm^{-1}(O=C-H stretching two bands)
LM D (3:1:1)	28814cm^{-1}and 2739cm^{-1}(O=C-H stretching two bands)
LME (3:1 :2)	2998cm^{-1} and 2955cm^{-1}(O=C-H stretching two bands)

LM A (1:1:3) Batch contain softisan® 154 and P90H ® (mixture 7: 3), Transcutol® and PEG400 ratio of 1:1:3, LM B (2:1:3) Batch contain softisan® and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 2:1:3, LM C (2:2:3) Batch contain softisan and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 2:2:3, LM D ((3:1:1) Batch contain softisan 154 and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 3:1:1 , LM E (3:1:2) Batch contain softisan and P90H (mixture 7: 3), Transcutol® and PEG4000 ratio of 3:1:2.

SEM Analysis: SEM analysis revealed detailed microstructure of the various batches of lipid matrices showed evidence of large microstructure (coarser structure) compared to lamellar structure (discrete structure) as seen in individual lipid matrices. This implies that, they are more amorphous than the former.

*Conclusion***:** It was observed that mixtures of lipids modify the entire properties of the individual lipids, of which may have resulted in disorder of the original crystal arrangement of individual lipids. The result indicated that all batches demonstrated good potentials for use as nanostructured lipid carrier (NLCs) drug delivery system. By implication, they can be employed as NLCs carriers to transdermally deliver poorly water-soluble drug like artemether and other drugs belonging to biopharmaceutical classification scheme (BCS) classes II.

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REFERENCES

Alkilani, A; Mccrudden, MTC; Donnelly, RF (2015). *Transdermal Drug Delivery*: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the stratum corneum. 438–470.

Attama, AA; Kenechukwu, FC ; Onuigbo, EB ; Nnamani,PO, Obitte, NC; Jahn,H ; Finke ; Pretor, S; Muller -Goymann ,C (2016). Solid lipid nanoparticles encapsulating a fluorescent marker (coumarin 6) and anti-malarials – artemether and lumefantrine: evaluation of cellular uptake and anti-malarial activity. *Eur J Nanomed* 8(3).

Attama, AA; Umeyor, CE C(2015). The use of solid lipid nanoparticles for sustained drug release. *Ther Deliv.* 6(6), 669–684.

Chime, SA; Attama, AA; Kenechukwu, FC; Umeyor,

EC; Onunkwo, GC; Builders,PF(2013). Formulation, in vitro and in vivo characterisation of diclofenac potassium sustained release tablets based on solidified reverse micellar solution (SRMS). *Br J Pharm Res.* 3(1), 90 – 107.

De Jong, WH ; Borm, PJA(2008). Drug delivery and nanoparticles: Applications and hazards..*Int.J. Nanomed*. 3, 133–149.

Hallan, SS; Sguizzato, M; Esposito, E; Cortsi, R (2021). Challenges in the physical characterization of Lipid Nanoparticles. *Pharmaceutics.*13:549.

Friedrich, I ; Muller -Goymann ,C (2003). Characterization of solidified reverse micellar solutions (SRMS) and production development of SRMS-based nanosuspensions. *Eur J Pharm Biopharm.* 56, 111 – 19.

Kenechukwu, FC; Momoh, MA; Nnamani, PO; Attama, AA (2015). Solid lipid micro-dispersions (SLMs) based on PEGylated solidified reverse micellar solutions (SRMS): a novel carrier system for gentamicin. *Drug Deliv*, 1–13; DOI: 10.3109/10717544.2014.900152.

Mehnert, W ; Mäder, K (2001). Solid lipid nanoparticles: Production, characterization and applications. *Adv. Drug Deliv. Rev.* 47, 165–196.

Muller, RH ; Shegokar, R ; Keck, CM (2011). 20 Years of Lipid Nanoparticles (SLN & NLC): Present State of Development & Industrial Applications. *Curr. Drug Discov. Technol.* 8, 207–227 .

Jain ,NK, (2001) . Advances in controlled and novel drug delivery, 1stEd.,CBS Publishers and distributors, New Delhi .2001; pp 108-110. 108–110.

Nnamani, PO ; Hansen, S; Windbergs, M; Lehr, CM (2014). Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application. *Int. J. Pharm*. 477 (2014) 208–217. doi: 10.1016/j.ijpharm.2014.10.004.

Nnamani, PO; Ugwu, AA. *et al.*,(2021). Formulation and evaluation of transdermal nanogel for delivery of artemether. *Drug Deliv. Transl. Res.* 11, 1655–1674.

Nnamani, PO ; Echezona, AC; Metu, OC; Onoja, US; Ogbonna, JDN; Akpa, PA; Onunkwo, GC; Nzekwe, IT; Attama, AA (2020). Formulation and characterization of artemrther-loaded sodium alginage microcapsules. *trop .J Pharm Res* 7, 1341–1349 .

Özdemir, S; Çelik, B; Üner, M,(2019). Properties and therapeutic potential of solid lipid nanoparticles and nanostructured lipid carriers as promising colloidal drug delivery systems. *Materials for Biomedical Engineering* 451–499 , doi:10.1016/B978-0-12-816913-1.00015-5. 451–499 (2019).

Pranshu, T, (2011). Mucoadhesive drug delivery: mechanism and methods of evaluation. *Int J Pharm bBo sci* 2, 14–19.

Ryan, SM; Mantovan, G; Wang, X; Haddleton, DM, (2008). Advances in PEGylation of important biotech molecules: delivery aspects. *Experts Opin Drug Deliv*. 5: 1 – 13. 1–13

Sharma, RK; Sharma, N; Rana, SS (2013). Solid lipid nanoparticles as a carrier of metformin for transdermal delivery. *Int J Drug Deliv* 5, 137–45.

Singh,IM; Morris, A (2011). Performance of transdermal therapeutic systems: effects of biological factors. *Int J Pharm Investig.* 1, 4–9.

Ugwu,AA; Nnamani,PO; Ibezim, EC; Reginald-Opara,JN; Attama,AA,(2018).Preliminary Characterization of modified nanostructured lipid carrier as potential drug delivery system. *Afr.J Pharm Res Dev*;volume 10(2):085-090.