International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

Formulation And Characterization Of Nanocarriers Bearing Lopinavir Emulsome

Brajesh Kumar[1*], Prabhat Jain[1], Naveen Gupta[1], Dharmendra Singh Rajput[1]
[1]Department of Pharmacy, Madhyanchal Professional University, Bhopal, M. P.

Abstract: *Nanocarriers, with their tunable surface characteristics and internal structures, offer enhanced delivery options for both small-molecule drugs and biologics. In recent years, research has emphasized the design of specific nanocarriers to minimize systemic exposure and reduce unintended side effects. The suggested study set out to create a carrier technology that might carry lopinavir topically without causing any side effects or toxicity. Nanocarrier emulsomes has the potential to increase drug solubility and bioavailability, decrease dosage requirements, and enhance pharmacological activity. Particle size, and by extension, PDI and zetapotential action, are positively correlated with solid core concentration and lipid content, according to the results. To optimize the impact of different surfactants at varied concentrations to identify the penetration rate or drug entrapment efficiency within the solid lipid core, the results of all dependent variables led to the selection of formulation LEMs4.*
Keywords: *Nanoparticles, Nanocarriers, Emulsomes, Lopinavir*

INTRODUCTION: Transdermal drug delivery system is topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate [1]. Transdermal drug delivery system has been in existence for a long time. In the past, the most commonly applied systems were topically applied creams and ointments for dermatological disorders. Transdermal drug delivery is the non-invasive delivery of medications from the surface of skin-the largest and most accessible organ of human body- through its layers, to the circulatory system [2].

TDDS offers many advantages over conventional injection and oral methods. Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous Input of drugs with short biological half-lives and eliminates pulsed entry into systemic circulation, which often causes undesirable side effects the common ingredients which are used for the preparation of TDDS. Several important advantages of transdermal drug delivery are limitation of hepatic first-pass metabolism, enhancement of therapeutic efficiency, and maintenance of steady plasma level of the drug [3]. Under normal circumstances, the predominant route is through the intercellular spaces. The predominant route is via intercellular spaces.

The diffusional pathlength is therefore much longer than the simple thickness of the stratum corneum (~20 mm) and has been estimated as long as 500 mm. Importantly, the intercellular spaces contain structured lipids and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before it reaches the junction between the stratum corneum and the viable epidermis [4]. The transepidermal route across the continuous stratum corneum comprises transport via intracellular and intercellular spaces. The polar molecules mainly diffuse through the polar pathway consisting of "boundwater". within the hydrated stratum corneum, whereas the non- polar molecules dissolve and diffuse through the non- aqueous lipid matrix of the stratum corneum. The transappendageal route transports substances via the sweat glands and the hair follicles with their associated sebaceous glands, but it is considered to be of minor importance because of relatively smaller area (less than 0.1% of total surface) [5].

Nanoemulsions/ Sub-micron emulsions are oil-in-water emulsions with an average droplet size ranging from 100 to 500 nm. They have very good stability and they do not undergo phase separation during storage. They have a liquid lipophilic core and are appropriate for lipophilic compound transportation. Many studies showed reduced transepidermal water loss, which means support to the barrier function of the skin. Nanoemulsion viscosity is very low, which is interesting because they can be produced as sprays [6]. Microemulsions formulations have been shown to be superior for cutaneous delivery compared to other conventional vehicles. These systems are identified as transparent mixtures of water, oil and surfactants. They are thermodynamically stable and optically isotropic.

Microemulsions are spontaneously produced in a narrow range of oil-water-surfactant composition, represented on pseudo-ternary diagram phases. They are dynamic systems with continuously fluctuating interfaces. Their good dermal and transdermal delivery properties could be attributed to their excellent solubilising properties [7]. Their high solubilising properties improve biodispen sibility and thus reduce

the efficient dose thereby increasing tolerability. Furthermore, their restructuring effect on skin and hair (due to their high lipid content) make microemulsion formulations adapt to altered skin and hair conditions [8]. Emulsomes, which are nanoparticles made of lipid, are frequently utilized as drug delivery mechanisms. Their structure can be compared to a cross between liposomes and solid lipid nanoparticles because they are made up of an oil phase enclosed by a phospholipid bilayer. A head-to-tail arrangement of two layers of phospholipids, with their hydrophilic heads facing outward and their hydrophobic tails facing inside, forms the phospholipid bilayer in emulsomes [9]. This bilayer gives the emulsomes a stable structure and works to keep the encapsulated oil phase from deteriorating. Depending on the required qualities of the final formulation, the oil phase in emulsomes can be made up of a range of different oils. Triglycerides, fatty acids, and other lipid-based substances are examples of frequently used oils.

The oil phase forms a core that can enclose hydrophobic medications or other active components as it is disseminated throughout the phospholipid bilayer. The phospholipid bilayer and oil phase are not the only components that may be present in emulsomes; additional substances including surfactants, stabilizers, and targeting ligands may also be present. These extra ingredients may aid in enhancing the emulsomes' efficacy and durability as well as their capacity to target particular tissues or cells. Emulsomes overall structure is quite adaptable and can be customized to fit the unique requirements of various drug delivery applications. It is feasible to make emulsomes that are stable, efficient, and well-tolerated by the body by carefully choosing the ingredients and perfecting the formulation [10]

Topical route of administration for therapeutic agent provides direct contact with desired site of the skin. Dosage forms via topical routes i.e. ointment, cream and gel are insufficient to provide medicament at target site usuallyin higher concentration. Novel drug delivery system like liposomes, emulsomes, niosomes, dendrimers and nanoparticles etc. helps to target the tissues through skin layer and can provide better therapy for viral infection. Emulsomes offers an effective topical drug delivery system owing to its potential of high retention flux as well as high skin retention of drug and hence enhanced anti-cancer activity and reduce skin irritation. Advantages of emulsomes are: protect medicament from harsh gastric environment, improve solubility and bioavailability of drug, reduce dose of drug, improve pharmacological activity, also provides sustained release of medicament by prolongs the release of medicament and provide target specific drug delivery. The aim of the study is to develop a carrier system for the management of viral infection topically by using lopinavir. Topical route of administration for therapeutic agent provides direct contact with desired site of the skin.

MATERIAL AND METHODS:

Determination of maximum absorption wavelength (λmax): The determination of absorption maxima was determined by UV scanning of drug solution under ultraviolet spectrophotometer between 200 to 400 nm wavelengths offer drug sample i.e. lopinavir.

Preparation of standard calibration curve of lopinavir in phosphate buffer pH 7.4 solutions: The accurately weighed required quantity 10 mg of drug sample i.e. lopinavir mixed in volumetric flask containing 10 ml of phosphate buffer pH 7.4 solvent. The solution was sonicated in bath sonicator for 20 min. The resulting solution was having concentration of 1000 µg/ml solution. Now, take 1 ml of above solution, diluted with phosphate buffer pH 7.4 solvent up to 100 ml in another volumetric flask separately and again sonicated for 20 min. The resulting solution was having concentration of 10 µg / ml solution. Take, 2.0 ml, 4.0 ml, 6.0 ml upto 10.0 ml aliquots withdrawn from above solution, diluted up to 10 ml with phosphate buffer pH 7.4 solvent in volumetric flasks. The resulting solutions containing 2 µg / ml, 4 µg / ml, 6 µg / ml, upto 10 µg / ml respectively. The absorbance of all resulting solution was calculated individually at 234 nm with phosphate buffer pH 7.4 as a blank. The absorbance was measured and standard curve was plotted between absorbance vs. concentration.

Preformulation study:

Organoleptic characteristic test: The organoleptic characteristic test of drug sample i.e. lopinavir was performed by using sensory organs. The parameters such as color, odor and taste were recorded.

Physical characteristics density: The drug (Lopinavir) powders were precisely weighed and transfer through a glass funnel into graduated cylinder and the volume was noted and bulk density was determined. The tapped density was determined using tapped density apparatus. Bulk and tapped densities of lopinavir were to be 0.718 gm / cm^3 and 0.776 gm / cm^3.

Particle size: The particle size distribution of lopinavir drug sample was investigated by using microscopic method. Two types of micrometers i.e. ocular micrometer and stage micrometer fitted in compound

microscope. The experiment was performed in triplilcate manner and average particle size (mean) was calculated.

Flow properties determination: The flow properties of drug sample were investigated for the identification of movement of drug particles through the hopper during the mixing with other excipients. Carr's index, Hausner's ratio and angle of repose were determined to characterize the flow properties of drug powder. The angle of repose (θ) was measured by fixed height method. The Carr's index (I_C) and Hausner's ratio (H_R) angle of repose of drug powders were calculating according to following equation:

Carr's Index (I_C) = $\rho_{Tapped} - \rho_{Bulk} / \rho_{Tapped}$

Hausner's ratio (H_R) = $\rho_{Tapped} / \rho_{Bulk}$

Angle of repose (θ) = $\tan^{-1}$ 2 H / D

Where H is the surface area of the free-standing height of the powder pile and D is diameter of pile that formed after powder flow from the glass funnel.

Solubility profile of drug samples: Solubility of drug lopinavir (LPV) was determined separately in various aqueous media of different pH solution. Phosphate buffer pH 6.8 and phosphate buffer pH 7.4 were mixed with Sodium thiosulphate to avoid oxidation. The excess amount of drug sample lopinavir (LPV) was mixed with 100 ml of medium with constant stirring for 6 h at room temperature, then resulting solution filtered with 0.45µm pore size whatmann filter paper. The drug solubilized in media was determined by analyzing filtered solution by UV spectrophotometer method. All procedure was done in triplicate.

Partition coefficient: The partition coefficient of lopinavir was determined to calculate the hydrophobecity/ hydrophilicity of drug sample in 100 ml of mixed solvent system. It was determined using mixture of n-octanol: phosphate buffer pH 7.4 solution, taken this mixture (100 ml) in separating funnel 100 mg of drug was added into, shaken for 24 h until equilibrium reached. After that separating funnel was kept to stand for 2h in a stand then both layer i.e. n-octanol and phosphate buffer were separated and collected individually, filtered. The quantity of drug dissolved in phosphate buffer medium was determined by UV spectroscopic method. The amount of drug in n-octanol was obtained by subtracting the quantity of drug in phosphate buffer medium from the quantity of drug taken. The partition coefficient of drug sample was calculated from following equation.

$$Log\ P\ (n-oct\ /\ pH\ 7.4)\ =\ Log\ (C\ Oct\ /\ C\ pH\ 7.4)\ equilibrium$$

The partition coefficient of Lopinavir (MT) was found 0.319**.**

Melting point: Melting point was established by capillary tube method in digital melting point apparatus. The powder of drug sample was filled in glass capillary tube with tapping pinch of pure drug sample. The filled in capillary tube is previously sealed from one end with flame. The filled capillary tube was kept stand in melting point apparatus. Temperature at which the drug powder was started to melt was noted and experiment performed in triplicate**.**

Preparation of nanocarriers emulsomes:

Preparation of solid lipid nanoparticles: Preparation of solid lipid nanoparticles via hot homogenization method was performed at a temperature above the melting point of lipid. A number of various formulations were prepared by using the solid lipid content as Solid lipids glycerol monostearate (GMS), spermaceti (SM), in different ratio were dissolved in minimum quantity of methanol in 50 ml round bottom flask (**Table 1**).

Preparation of hydration of film: Appropriate quantities of lipid, active ingredient drug lopinavir (10 mg), were weighed and mixed at a temperature 10℃ above the melting point of the lipid in a water bath. In another beaker, phosphate buffer saline pH 7.4 was heated to the same temperature along with the hydrophilic surfactant and was placed under continuous stirring. The afore mentioned lipid phase was then added drop wise to the aqueous surfactant solution and kept under stirring for a few hours at 2700 Rpm. The dispersion was then sonicated for 30-40 min and stored. A thermodynamically stable system was formed. The hydration film mixture was hydrated at temperature 37±1°C. After hydration, formulation was left for two hours and get multilamminar vesicles as emulsomes. The SLN dispersions were cooled in ice baths with gentle stirring for 5 minutes. The prepared SLN dispersions were kept in sterile screw-capped glass vials and kept at 5°C ± 3°C for later use.

Table 1: Composition of nanocarriers emulsomes

	Lipid content (X_1)		

Formulation Code	Spermaceti (SM) (mg) X_a	Glycerol monostearate (GMS) (mg) X_b	Triglycerides (Trimyristin) (TM) (mg) X_c	Amount of surfactant (X_2) (%) (Tween 20)	Addition of sonication time (X_3) (Min.)
LEMs1	150	0	150	10	10
LEMs2	0	150	150	10	10
LEMs3	150	50	100	10	10
LEMs4	50	150	100	10	10
LEMs5	150	100	50	10	10
LEMs6	50	100	150	10	10
LEMs7	100	150	50	10	10
LEMs8	100	50	150	10	10

Characterization of nanocarriers emulsomes: Optimized nanocarriers emulsomes formulation was characterized for size and size distribution, shape and surface morphology, entrapment efficiency and zeta potential.

Determination of vesicle size and size distribution: Maintaining constant size and size distribution for a prolonged period of time is an indication of stability of nanocarriers emulsomes. Electron microscopy is widely used for the assessment of surface morphology, size and size distribution of nanocarriers emulsomes. Besides the routine laboratory techniques such as gel chromatography etc. techniques based on light scattering and electron microscopy are need to be applied for statistically significant analysis of size and size distribution of the carriers. The average vesicle size and size distribution was determined by photon correlation spectroscopy using zeta sizer. The sample of dispersion was diluted to 1:9 with distilled deionized water.

Determination of Zeta potential: The zeta potential of particles is the overall charge that the particle acquires in a particular medium. The knowledge of the zeta potential of a preparation can help to predict the fate of the preparation in vivo and to assess the stability of colloidal systems. Zeta potential of nanocarriers emulsomes formulations were assessed by p-hoton correlation spectroscopy using Zetasizer nanoseries using a flow-through cell.

Determination of shape and surface morphology: Shape and surface morphology of nanocarriers emulsomes was determined by Transmission Electron Microscope (TEM) technique. The sample (10µl) was placed on the grids and allowed to stand at room temperature for 90 sec and excess of the fluid was removed by touching the edge of filter paper. All samples were examined under a Transmission Electron Microscope (Tecnai G2, Hillsboro Oregon, USA) at an acceleration voltage of 100 kV and photomicrographs were taken at 1400X.

Determination of Entrapment Efficiency: The entrapment efficiency was determined after separation of the unentrapped drug by the use of minicolumn centrifugation method (Fry et. al. 1978; New 1990). Sephadex G-50 (1.2gm) was swelled in 20 ml of 0.9% NaCl solution for 5 hours at room temperature with occational shaking. The gel was formed and it was stored at 4°C. To prepare the minicolumn, the hydrated gel was filled up to top in the barrel of 1mL disposable syringe, plugged with whatman filter pad. Then barrel placed in the centrifuge and centrifuged at 2000 rpm for 3 min to remove saline solution. Eluted volume was removed from the centrifuged tubes and exactly 0.2mL of nanocarriers suspension (undiluted) was applied drop wise on the gel bed in the center. Columns were again centrifuged at 2000 rpm for 3 min to expel and remove void volume containing nanocarriers emulsomes to the centrifuge tubes. Elute was remove and 0.25 mL of saline was applied to each column and centrifuge as previously. The amount of drug entrapped in the vesicle was then determined by disrupting the vesicle using 1 ml of 0.1%v/v triton-X 100, filtering it and the drug content was determined using UV-Vis spectroscopy at 307 nm.

The percentage efficiency was determined by following equation:

$$\text{Percentage drug entrapment efficiency} = \frac{\text{Amount of entrapped}}{\text{Total amount of drug}} \text{X100}$$

RESULTS AND DISCUSSION:

Determination of maximum absorption wavelength (λmax): The absorption maxima (λ-max) of lopinavir (10 µg / ml) in pH 7.4 phosphate buffer solution were found to be at 234 nm. The spectrum peak point graph of absorbance of drug vs. wavelength was recorded. Lopinavir was estimated in-vitro by reported UV spectrophotometric methods. The drug lopinavir was also estimated in the dissolution medium (pH 7.4 phosphate buffer).

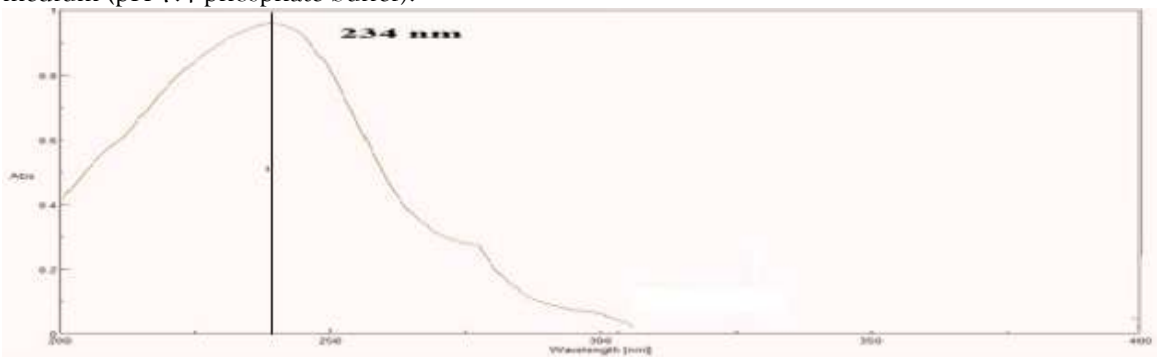

Figure 1: Absorption maxima (λ-max) of lopinavir in phosphate buffer pH 7.4 solution (10 µg/ml)

Preparation of standard calibration curve of lopinavir in phosphate buffer pH 7.4 solutions: The calibration curves in the various dissolution medium (pH 7.4 phosphate buffer) were prepared with drug lopinavir solutions of known concentrations. The absorbance was measured and plotted against drug concentration. The calibration curves show excellent linearity of data as evidenced by the values of correlation coefficients that were found to be greater than 0.99. The curves were found to be recti-linear in the concentration range 0 µg / ml to 10 µg / ml for the drug Lopinavir. The result of Specificity, showed all result specific in nature**.** Intra-day and Inter-day precision of the UV spectrophotometric methods was evaluated for drug lopinavir in same concentration i.e. 10 µg / ml with variability in time period of estimation. The results of inter-day and intra-day precision were also performed**.** The percent relative standard deviation was found to be less than 1 % i.e. the method was precise. The recovery of drug from pH 7.4 phosphate buffer was also estimated.

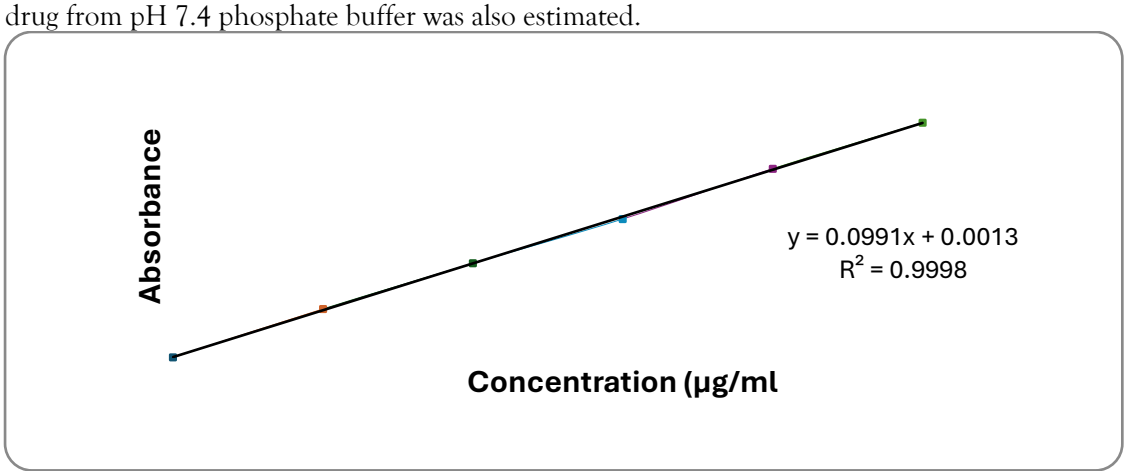

Figure 2: Standard curve of lopinavir in phosphate buffer pH 7.4 solution (234 nm)

Preformulation study:

Organoleptic characteristic test: The sensory evaluation test (organoleptic study) of drug sample i.e. Color, odor and taste etc were observed. The drug sample Lopinavir was Yellow to orange-brown in color, odourless, slightly bitter in taste and crystalline in nature.

Physical characteristics density: The drug (Lopinavir) powders were precisely determined Bulk densities and tapped density as 0.718 gm / cm^3 and 0.776 gm / cm^3 respectively.

Particle size: The particle size distribution of drug sample was found 98 µm.

Flow properties determination: The flow properties of drug sample were investigated as Carr's index (I_C) and Hausner's ratio (H_R) angle of repose of drug powders. The flow properties of drug powders were characterized. Bulk and tapped densities of lopinavir were to be 0.718 gm / cm^3 and 0.776 gm / cm^3. The result was concluded that unmilled powders have good to passable type of flow in nature. Carr's index (%), Hausner's ratio and Angle of repose θ were 9.17±0.018, 1.11±0.002 and 21.2±0.011 respectively.

Solubility profile: Solubility of drug was determined separately in various aqueous media of different pH solution as various dissolution medium phosphate buffer pH 6.8 and phosphate buffer pH 7.4 showed at Table 6.3. The solubility of Lopinavir at Water, 0.1 N HCl, Phosphate buffer pH 4.5, Phosphate buffer

pH 6.8 and Phosphate buffer pH 7.4 were 0.0114 (mg / ml), 0.086 (mg / ml), 0.721 (mg / ml), 1.022 (mg / ml) and 1.161 (mg / ml) respectively**.**

Partition coefficient: The partition coefficient of drug was determined to calculate the hydrophobicity/ hydrophilicity. The partition coefficient of lopinavir was found 0.319**.**

Melting point: Melting point of drug sample was noted and experiment performed in triplicate is 185 ± 0.115°C**.**

Optimization of nanocarriers emulsomes: The prepared various formulations from LEMs1 to LEMs8 according to 3^2 factorial design. The content of solid lipid core triglycerides (Trimyristin; TM) were coated with single layer of lipid content and double layer of combination of two layers of Solid lipids glycerol monostearate (GMS), spermaceti (SM). The various combinations of glycerol monostearate: spermaceti: trimysterin optimize with constant concentration of surfactant as penetration enhancer of drug. The penetration enhancer affects the insertation of drug lopinavir amount inside the solid lipoid core (TM) through the single or double layer of lipid layers. The sonication time affect the multiilaminar vesicles to unilaminar vesicles and the process also useful for overcome the problem of agglomeration of vesicles. The result of all dependent variables was evaluated and among all the formulations LEMs4 having glycerol monostearate:spermaceti:trimysterin (0.5:1.5:1.0) was selected best formulation. The prepared nanocarriers emulsomes lipid layers ratio varying from 0.5 to 1.5 in with solid lipid triglycerides (Trimysterin) provides a hydrophobic core. The result concluded that as the concentration of solid core varies and the amount of lipid content increase the particle size increase, thus increase the PDI and zetapotential action. The variations of result of all parameters also showed between the optimization of single or double layer. The optimization of sonication time was improving the particle size of LEMs1 and LEMs2, because of single layer of emulsomes and showed smaller size than the other formulations. Thus, the result of particle size distribution, PDI **(Figure 3)** and zetapotential action **(Figure 4)** of LEMs1 and LEMs2 was equivalent to the formulations LEMs4, because of amount of solid lipid core (TM) with addition of more amounts of PC and less amount of GMS. The change of vesicles size and drug entrapment efficiency also correlated with each other, due to thickness of wall of lipid layers. The formulation LEMs4 has more than 80% drug entrapment with addition of tween 80 in 10% concentrations. All the result of dependent variables concluded that the formulation LEMs4 was selected for the optimization of effect of various surfactants in different concentration to identify the penetration rate or drug entrapment efficiency inside the solid lipid core. The optimization of sonication time also evaluated for identification of effect vesicular size and shape.

Table 2: Various parameters of nanocarriers emulsomes

Formulation Code	Particle size (nm)	Layers	Zeta potential (mV)	PDI	Drug Entrapment (%)
LEMs1	127.03±1.01	Single	-21.11±1.01	0.213±0.02	76.53±1.3
LEMs2	125.02±1.02	Single	-21.12±1.02	0.216±0.03	79.78±1.1
LEMs3	130.01±0.02	Double	-23.11±1.02	0.223±0.17	71.86±1.1
LEMs4	129.11±1.05	Double	-23.91±1.03	0.224±0.04	81.37±0.8
LEMs5	131.11±1.01	Double	-21.11±1.02	0.221±0.02	68.32±1.2
LEMs6	129.11±1.11	Double	-22.05±1.01	0.224±0.02	76.53±0.9
LEMs7	133.12±1.02	Double	-21.82±1.02	0.227±0.03	63.05±0.8
LEMs8	131.21±1.12	Double	-23.11±1.02	0.226±0.02	65.12±1.2

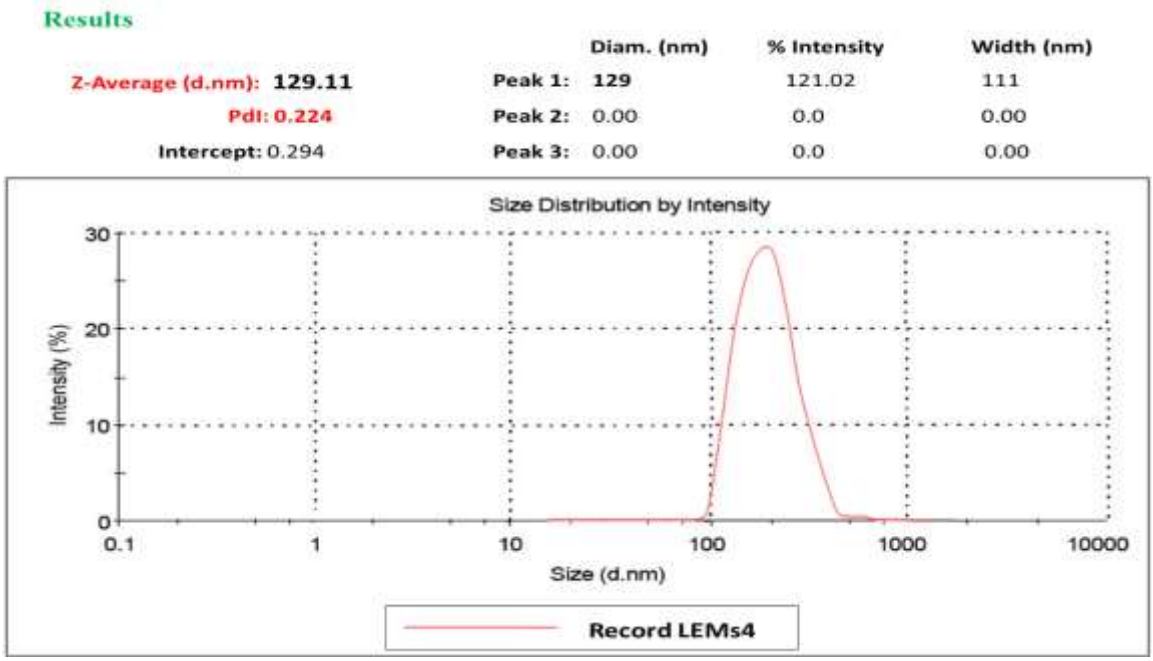

Figure 3: Particle size distribution & Polydispersity Index (PDI) of nanocarriers emulsomes (LEMs4)

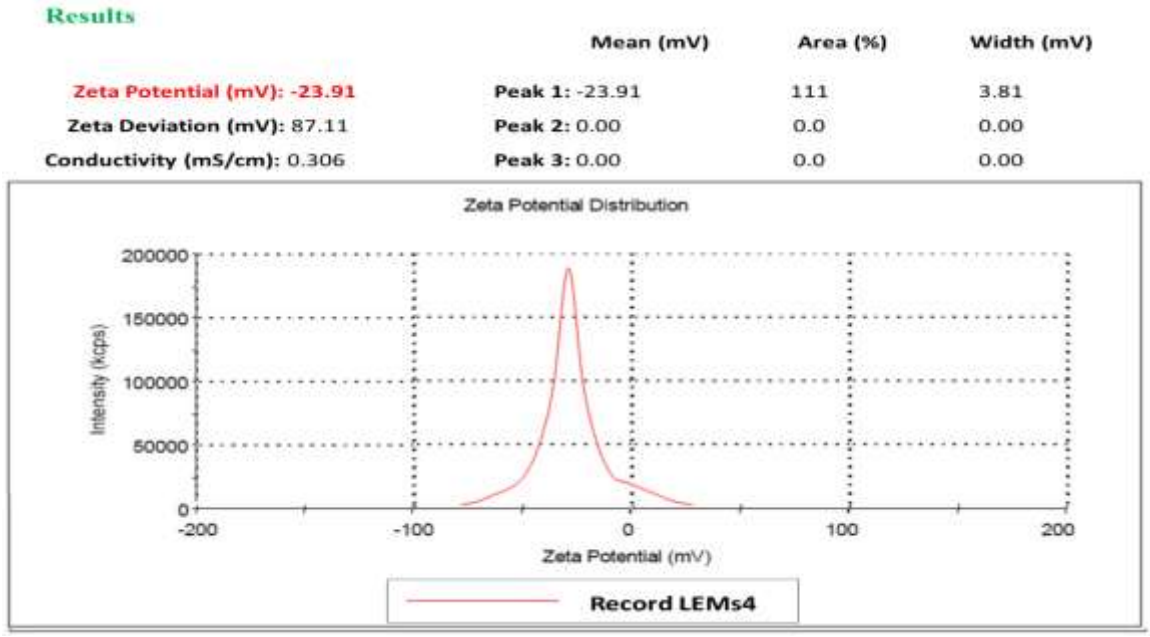

Figure 4: Zeta potential (mV) of nanocarriers emulsomes (LEMs4)

CONCLUSION:

Lopinavir (LPV), an antiviral drug, is a drug of choice for viral infection. Nanocarrier emulsomes is a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. Nanocarrier emulsome is an advance nanocarrier technology for poorly aqueous soluble drugs. It possesses both emulsion and liposomes feature nanocarrier emulsomes offers an effective topical drug delivery system owing to its potential of high retention flux as well as high skin retention of drug and hence enhanced anti-cancer activity and reduce skin irritation. The aim of the proposed work was to develop a carrier system for the management of viral infection topically preventing the toxic and other undesired effect of medicament. The advantages of nanocarrier emulsomal gel able to improve solubility and bioavailability of drug, reduce dose of drug with improved pharmacological activity. The result concluded that as the concentration of solid core varies and the amount of lipid content increase the particle size increase, thus increase the PDI and zetapotential action. All the result of dependent variables concluded that the formulation LEMS4 was selected for the optimization of effect of various surfactants in different concentration to identify the penetration rate or drug entrapment efficiency inside the solid lipid core.

REFERENCES:

1. Albery WJ, Hadgraft J. Percutaneous absorption: in vivo experiments. J Pharm Pharmacol 1979; 31:140–147.
2. Nemanic MK, Elias PM. In situ precipitation: a novel cyotchemical technique for visualization of permeability pathways in mammalian stratum corneum. J HistochemCytochem 1980; 28:573–578.
3. Bodde HE, Brink I Van den, Koerten HK, Dehaan FHN. Visualization of in vitro percutaneous penetration of mercuric chloride transport through intercellular space versus cellular uptake through desmosomes. J Control Release 1991; 15: 227–236.
4. Heisig M, Lieckfeldt R, Wittum G, Mazurkevich G, Lee G. Nonsteady-state descriptions of drug permeation through stratum corneum. 1. The biphasic brick and mortar model. Pharm Res 1996; 13:421–426.
5. Potts RO, Francoeur ML. The influence of stratum corneum morphology on water permeability. J Invest Dermatol 1991; 96:495–499.
6. Wang Y, Allen LV, Li C, Tu Y. Iontophoresis of hydrocortisone across hairless mouse skin: investigation of skin alteration. J Pharm Sci 1993; 82:1140-1144.
7. Turner NG, Kalia YN, Guy RH. The effect of current on skin barrier function in vivo: recovery kinetics post iontophoresis. Pharm Res 1997; 14:1252-1255.
8. Banga AK, Bose S, Ghosh TK. Iontophoresis and electroporation: comparisons and contrasts. Int J Pharm 1999; 179:1-19.
9. Guy RH, Kalia YN, Delgado-Charro MB, Merino V, López A, Marro D. Iontophoresis: electrorepulsion and electroosmosis. J Control Rel 2000: 64:129-132.
10. Schwarz C, Mehnert W, Lucks JS, Muller RH (1994) Solid lipid nanoparticles (SLNs) for controlled drug delivery: I. production, characterization and sterilization. J Control Releas 30:83–96
11. Kaur S, Nautyal U, Singh R, Singh S, Devi A. Nanostructure lipid carrier (NLC): the new generation of lipid nanoparticles. Asian Pac J Health Sci. 2015;2(2):76–93. doi: 10.21276/apjhs.2015.2.2.14.
12. Kost J, Katz N, Shapiro D, Herrmann T, Kellog S, Warner N, Custer L. Ultrasound skin permeation pre-treatment to accelerate the onset of topical anaesthesia. Proc Inter SympBioact Mater 2003.
13. Jacques SL, McAuliffe DJ, Blank IH, Parrish JA. Controlled removal of human stratum corneum by pulsed laser to enhance percutaneous transport. Patent 1988; Serial Number US 4775: 361.
14. Müller RH, Benita S, Böhm B, Emulsions and nanosuspensions for the formulation of poorly soluble drugs. Med Pharm Ed Stuttgart 1998.
15. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002; 54 (1): 131-155.