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NEBIVOLOL HYDROCHLORIDE LOADED NANOSTRUCTURED LIPID CARRIERS AS TRANSDERMAL DELIVERY SYSTEM:-PART 2:- HYDROGEL PREPARATION, EVALUATION AND PERMEATION STUDY

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Abstract:

Nebivolol hydrochloride (NEB), is a third generation highly selective β_1-blocker, it has an antihypertensive properties. Its elimination half-life is around 10 hrs while its oral bioavailability is about 12%. The objective of the current study was to develop nanostructured lipid carriers (NLCs) for transdermal delivery of (NEB). Through the preparation, characterization and conducting of an in vitro study for (NEB) loaded (NLCs), the formulation of NEB-NLCs based hydrogel using different types of gelling agents was introduced. Moreover, the incorporation of lipid nanoparticles into carbapol 934 as hydrogel base in different formulations was described in this study. The optimized formula (350 mg Glyceryl monostearate, 150 mg oleic acid, 2% (W/W) span 80, and 2% (W/W) Cremophor EL) was tested for entrapment efficiency, particle size and loading capacity then incorporated into hydrogel for expedient transdermal application. A number of measures were implemented for the NEB-NLCs based hydrogel, the results for the optimized formula were found to be as the following: particle size 228 nm, polydispersity index 0.3, zeta potential -29mV, pH 7.05 viscosity 7210cps, spreadability 6 cm, drug content 95%, and Ex Vitro skin permeation 90.8%. The transmission electron microscopy (TEM) and the optical microscope study revealed almost spherical shaped nanoparticles. Nebivolol based hydrogel demonstrated no skin irritation and showed a prolong release for up to 24 hrs. The flux for the permeation study through rat skin was found to be (143µg/cm^2/hr). Carbapol 934 was used as a gelling agent; the obtained formula gave evidence for good spreadability, homogeneity and rheological behavior. In conclusion, the data

obtained from this study illustrated a successful development of NEB-NLCs-based hydrogel in the increase of the encapsulation efficiency of colloidal lipid carriers. The advantages of the colloidal lipid carriers of the improved performance were in terms of stability and provides a sustaining NEB transdermal effect.

Keywords: Nebivolol hydrochloride; Nanostructured lipid carriers, Hydrogel, Transdermal delivery

Introduction

In the last decade, the prefix "nano" has an increasing application to various fields of knowledge. Nanoscience, nanotechnology and nanomaterials or nanochemistry, all represent examples of few terms that occur repeatedly in scientific reports, books as well as in daily newspapers, it has been recognized for a wide range of audience even for non experts. International system (IS) of units is used to indicate a reduction factor of 10^9 times[1]. Transdermal. Nebivolol hydrochloride (NEB) is a lipophilic β_1-blocker, devoid of intrinsic sympathomimetic and membrane stabilizing activity. Clinically, (NEB) is administered as a racemic mixture of equal proportions; d-isomer ((SRRR)-nebivolol a potent cardioselective β1 adrenoceptor blocker) and L-isomer ((RSSS)-nebivolol with favorable hemodynamic profile[2,3]. These two enantiomers possess unequal potency regarding to β-receptor blocking activity and nitric oxide mediated vasodilation. Moreover, the blend of (SRRR and RSSS) has a greater antihypertensive activity than either enantiomer alone[4]. Nebivolol hydrochloride is an official drug in the British & Indian Pharmacopoeia[5]. The molecular weight for NEB is 441.9 while for the free bases are 405.4[6]. The lipid nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLCs) are stable colloidal systems with notable compensations such as drug delivery systems, i.e. biocompatibility, biodegradability, physicochemical stability, versatility and controlled drug release. [7,8] Furthermore, they are providing controlled release profiles for many substances. Aqueous dispersions of lipid nanoparticles are being investigated as drug delivery systems for different therapeutic purposes. Their most interesting characteristic is the possibility to be used topically and transdermally, while other systems have to be incorporated into commonly used dermal carriers, such as creams or hydrogels, in order to have a proper semisolid consistency[7,8].

Materials and Methods

Nebivolol (NEB) and transcutol P were purchased from ProvizerPharma, India. Oleic acid was supplied by Riedel De Haen AGHonnover, German, Cremophore EL (Polyoxy l35 Castor oil) was purchased from HiMedia Lab Pvt. Ltd, India. Span 80 was provided by Hopkin&Williams LTD, England. Poloxamer 188 (Pluronic F-68) Lutrol® and Lecithin (Phosphatidylcholine purity 72.7%) were purchased by Sigma-Aldrich, Chemie GMBH, Germany. Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Diethyl ether Carbapol 934 and Sodium alginatewere provided by BDH Chemicals Ltd., Poole, England. Glycerylmonostearate was supplied by BDH Chemicals Ltd. Poole, England.Myverol™18-04K was provided by Gattee fosse, France. Chitosan , HPMC was purchased from Fluka AG. Chem.

1: Preparation of Nebivolol-loaded NLCs based hydrogel

According to the previous work[9], 100 mg NEB was incorporated into combination of different solid and liquid lipids, surfactants and co-surfactants (Table 1). Accordingly, four formulas were selected for hydrogel preparation. The optimized (NEB-NLCs) formula (F3) was selected based on the evaluation of characteristics like: particle size, entrapment efficiency, and in vitro release. Different gelling agents such as: carbopol 934, sodium alginate, chitosan, and hydroxyl propylmethyl cellulose (HPMC) were used for the conversion of NLCs dispersion into NLCs based hydrogel formulation (Table 2). Based on the compatibility with NLCs dispersion and for easier spreadability, carbopol 934 as gel forming polymer was selected (F3B). The hydrogel base was prepared by dispersing (1%w/w) carbapol 934 in distilled water, stirred for 10 mins at 1500 rpm to obtain a homogeneous gel base, and then immediately neutralized by adding drops of triethanolamine (0.5%V/V) to promote gelation. The pH of gel was adjusted to 7.4. Hydrogel was further allowed to stand overnight at a room temperature to remove any entrapped air. The hydrogel was used to disperse a freshly prepared NLCs suspension. The aqueous NLCs dispersion and hydrogel were stirred and mixed under a mechanical stirrer at a speed of 1000 rpm/15 min.

Table (1); Preparation of Different Formulas of Nebivolol - Loaded Nanostructured Lipid Carriers

Formula Code	Materials								
	GMS (mg)	OA (mg)	F68 (mg)	TCp (%)	T80 (%)	Le (%)	S80 (%)	CR (%)	Myv (%)
F1	350	150	150	5					
F2	300	200			0.8	0.4			
F3	350	150					2	2	
F4	350	150	200						0.3

Table (2); Different Nebivolol Nanostructured Lipid Carriers based Hydrogel formulations

Formula Code	Cabapol 934 (%w/w)	Chitosan (%w/v)	Sod. Alginate (mg)	HPMC (%w/v)
F3A	0.5			
F3B	1			
F3C		0.5		
F3D		1		
F3E		2		
F3F		3		
F3G			2	
F3H			3	
F3I				1
F3J				2

2: Evaluation of the NEB-NLCs based hydrogel

1. 2. Physical Examination of NEB-NLCs based hydrogel

The prepared NEB-NLCs hydrogel formulation was inspected visually for its color, appearance and consistency.

2.2. Determination of pH, Viscosity and Spreadability

The pH of the NLCs loaded hydrogel was determined using digital pHmeter (France and Hanna instruments type), standardized using standard buffer solutions (pH 4.0 and 7.0). One gram of hydrogel was dissolved in 100 mL of distilled water and stirred for 10 min then stored for 2 hrs. Results were taken in triplicate[10]. The viscosity of NLCs based hydrogel was measured using Brookfield DVII+Prodigital viscometer at 25◦C. A formulation weight of 20 gm was utilized and exposed to speed of 50 rpm. The measurements were attained in triplicate. The rheological nature of the disperse system was assayed by plotting shear stress against shear rate in a rheogram to determine if the systems are thixotropic. The readings were performed in triplicate[11].

For determination of the spreadability of hydrogel 0.5 g of gel was placed in a circle with a radius of 1cm premarked on a glass plate on top of another glass plate. A

weight of 500 gm was allowed to rest on the upper glass plate [10]. The extension in the diameter due to spreading of the hydrogels was measured with a linear scale. Experiments were done in triplicate.

3.2. Drug Content Determination

The NEB content in the hydrogel was determined by taking required quantity of the prepared gel which is equivalent to 10 mg of NEB and transferred to 100 ml of volumetric flask containing phosphate buffer (pH 7.4). Then, it was sonicated and filtered. Later, it was suitably diluted and analyzed at λ_{max} of NEB. The content of NEB was determined using UV-visible spectrophotometer at 281nm against blank [11].

4.2. Measurement of Particle Size and Polysisperisty Index of NLCs based Hydrogel

Light dynamic light scattering (LDS) was used. The aqueous NEB-NLCs was dispersed in a fixed amount of filtered distilled water (1:50) dilution of all formulations was made and placed in 1cm diameter disposable cuvette to yield a suitable scattering intensity. The mean particle size and polydispersity index PDI (The measurement of the width of size of distribution) for NEB-NLCs based hydrogel was calculated using Brookhaven Instruments Corp90 PLUS (ZetaPlus Particle Sizing, NY, Software, Version 5.34). Experiments were carried out in triplicate, and standard deviations (±SD) were calculated at a fixed scattering angle of 90°at (25°C)[12].

5.2. Zeta Potential determination of NEB- NLCs based Hydrogel

Zeta potential of NLCs based hydrogel was measured using (NanoBrookZeta PLAS) Zetasizer. The NanoBrookZetaPALS determines zeta potential using Phase Analysis Light Scattering technique which is up to 1000 times more sensitive than traditional light scattering methods based on the shifted frequency spectrum. Samples were placed in disposable zeta cells

and the NLC suspensions were diluted with distilled water (1:100) of all the formulations to get a uniform dispersion prior to analysis. The conductivity of the diluted sample was measured to choose the detection model. The whole measurement was carried out at 25°C[11].

6.2. Visualization by Optical Microscope

Optical microscope (BX51M Model) offered a reflected light illumination. One drop of each prepared (NEB) dispersions was examined under the optical microscope using an (1000x) magnifying power. Particle behavior, shape and morphology were investigated.

7.2. Visualization by transmission electron microscope (TEM)

The size and morphology of the selected formula was examined using TEM (PHILIPS CM 10), with an accelerating voltage of 100 K_{VA}. The work was conducted by placing one drop of the sample on a copper grid coated with a formvar carbon film and allowed to stand at room temperature for 90 sec to form a thin film. Excess of the solution was wicked away with the aid of filter paper. The grid was allowed to thoroughly dry in air, the sample was viewed and ready for analysis and photomicrographs were taken at suitable magnification

3. In-vitro Skin Permeability of NEB-NLCs based hydrogel

1.3. Preparation of Rat Skin (Diffusion Membrane)

Albino rats (4-6 week old males) were euthanized. Then the abdominal skin was shaved lightly with an electrical clipper taking care to prevent any damage to the surface of the skin. A rectangular section of abdominal skin several centimeters in each dimension were excised from the animal using a sharp blade. The skin was lifted easily from the animal after incision was made. **The defatting procedure:** the skin was defatted by wiping with a cotton tip soaked in diethyl ether to remove the subcutaneous fat and scraping the dermal side to remove the muscle and blood vessels. The skin was wiped again with a cotton tip soaked in ether to prevent any adhering fats and kept in a phosphate buffer pH 7.4 for about 2 hrs in a water bath at constant temperature of 37^{o}C to allow water soluble UV absorbing material to leach out. The buffer was change three times during this period with fresh amounts. Then the prepared skin for diffusion study was stored in a phosphate buffer for 24hrs in the refrigerator at 2°C before use[13].

2.3. Permeation Study of NLCs based hydrogel

Franz diffusion cell Equipment-MCF10, was used in all diffusion studies. It consists of six Franz diffusion cells arranged in a water jacket with a heater to obtain a constant experimental temperature of 32 ± 1^{o}C, on magnetic stirrers at equal speed of rotation, temperature and rotation were equilibrated electronically. All Franz diffusion cells used in the experiment consisted of two compartments: Upper donor compartment and lower receptor compartment. Phosphate buffer solution pH 7.4 was used as a receptor medium with volume of 30 ml for all the release studies of NEB-NLCs to assure sink condition. Firstly, all receptor compartments were filled

with phosphate buffer solution pH 7.4 and the assembled set up left in instrument to equilibrate to experimental temperature as to get rid of air bubbles for at least half an hour. Then the rat skin membrane with surface area of 3.97 cm^2 were mounted between the two compartments of the diffusion cells in such a way that the SC layer was facing the donor compartment and the dermis facing the receptor compartment, and fastened with an O-ring. The solution in receptor compartment was agitated with a magnetic stirrer at 100 rpm[14]. After assembling the described set up, samples of 1 ml were taken periodically through the sampling port from the receptor compartment at pre determined time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 10, 15, 20 and 24 hours), and replaced with an equal volume of fresh receptor solution at temperature of 32 ± 1^oC to maintain a constant volume of the receptor phase. Samples were analyzed for NEB content using UV-visible spectrophotometer. A cumulative amount of drug diffused was calculated[15]. All results were repeater in triplicates and presented in mean values ± standard deviations.

4. Skin irritation study

The primary skin irritation test was very important to estimate the irritancy of substances that applied repeatedly to the skin of humans. Irritancy test was done using healthy male albino rat weighing approximately 200 gm. Firstly, the left dorsal surface of the rat was shaved carefully with an electrical clipper then cleaned with rectified spirit then distilled water and left for drying. The optimized NEB-NLCs hydrogel was placed on the left dorsal surface of the rat, and skin irritation from the formulation was determined by observations for 7 days for any skin sensitivity and reactions such as redness, erythema, edema, and skin rash [10].

Results and Discussion:

1. Preparation of NEB-NLCs based Hydrogel

Based on the particle size, the entrapment efficiency and the in Vitro release profiles of NEB based hydrogel (F 1, 2, 3 and 4) which showed optimum physicochemical properties, were selected for the formulation of the hydrogel for transdermal NEB delivery by incorporation into (1%W/W) carbapol 934. However, (F3B) was chosen as the selected formula as it exhibited the best release profile, viscosity, spreadability, pH, and % drug content.

2. Evaluation of NEB-NLCs based hydrogel

1.2. Visual Inspection

The visual inspection of NEB-NLCs based hydrogel was found to be off-white in color homogenous and showed smooth texture. Hydrogel loaded with NEB-NLCs (F43B) was stored in a tight closed glass container, protected from light. The physical properties of the selected formulas after two months of storage was examined, no changes in the original off-white color or unpleasant odor were observed, which indicates no probability of microorganism growth and hence, the physical stability of the selected formula.

2.2. pH, Viscosity and Spredability Determination

The pH of the prepared NLCs loaded hydrogel was between 6.9±0.02 to 7.05±0.05 that lies in normal range of skin pH (4.5-7) as shown in (Table 3). Viscosity of the prepared hydrogel is mainly dependent on the gelling agent used. In the current study, when carbapol 934 (1%W/W) being under hydration condition it can form a physically bounded structure that is crucial for providing the proper mechanical strength to the hydrogel. Accordingly there is noticeable difference in viscosity values of the control hydrogel and NLCs based hydrogel which can be justified as the participation of nanostructured lipid carriers on the viscosity of the formulation. Spredability is a crucial factor to uniform and ease the application of topical and transdermal preparations from the patient`s compliance point of view. The application of the formulation to the inflamed skin is more comfortable if the base spreads easily, enhancing maximum slip and drag[16].Spreadability was found to be 6±1 cm for the optimized NEB-NLCs hydrogel formula (F3B) which indicates excellent spreadability as the large diameter signifies better spreadability.

3.2. Drug Content Evaluation in the Prepared Hydrogel

Drug content of the optimized NEB-NLCs based hydrogel(F3B) was found to be 95.0%±0.6 and gave better drug loading capacity of formulation as shown in (Table 3).

Table (3); pH, Viscosity, Spreadability and % Drug Content Evaluation of the different NEB-NLCs Based Hydrogels

Formula Code	pH	Viscosity (cp)(50rpm)	Spredability (cm)	%Drug Content
Control	6.02±0.02	3521±3.2	2.9±1.5	80.3±0.6
F1B	6.10±0.03	7622±1.4	3.5±1.0	87.1±0.1
F2B	6.78±0.11	7834±2.5	4.9±0.5	86.0±0.5
F3B	7.05±0.05	7210±1.2	6.0±1.0	95.0±0.6
F4B	7.20±0.12	7243±2.3	5.2±1.5	94.1±0.7

4.2. Particle Size, Polydispersity Index and Zeta potential evaluation of NEB-NLCs based Hydrogel

The average particle size, Polydispersity index and zeta potential of NEB-NLCs based hydrogel (F3B) was found to be 228nm, 0.3, -29.0 respectively. Zeta potential value of the freshly prepared hydrogel (F3B) is illustrated in figure (1). Meanwhile, figures (2 A, B, C and D) for the NEB-NLCs based hydrogel (F3) under TEM. TEM micrographs showed nearly rounded nanoparticle surrounded by a homogeneous shading with a rather uniform distribution and no prominent sign of aggregate remained in the hydrogel. However, there was no drug detected in the blank NLCs (figure 3), whose particle size was smaller than the NEB-NLCs. Such results were confirmed by optical microscope images (Figure 4) which suggests that the optimized formula (F3) resembled the drug-enriched core model (i.e. the drug occupies the core of the

particles), (NEB) was dispersed well due to its miscibility in the lipid matrix. In such a model, the core is surrounded by a practically drug-free lipid shell and homogenous hydrophilic polymer[17,18].

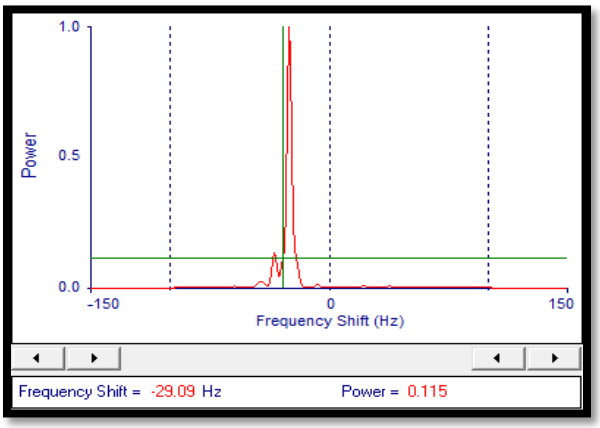

Figure (1); Zeta Potential of the freshly prepared (NEB-NLCs) based hydrogel (F3B)

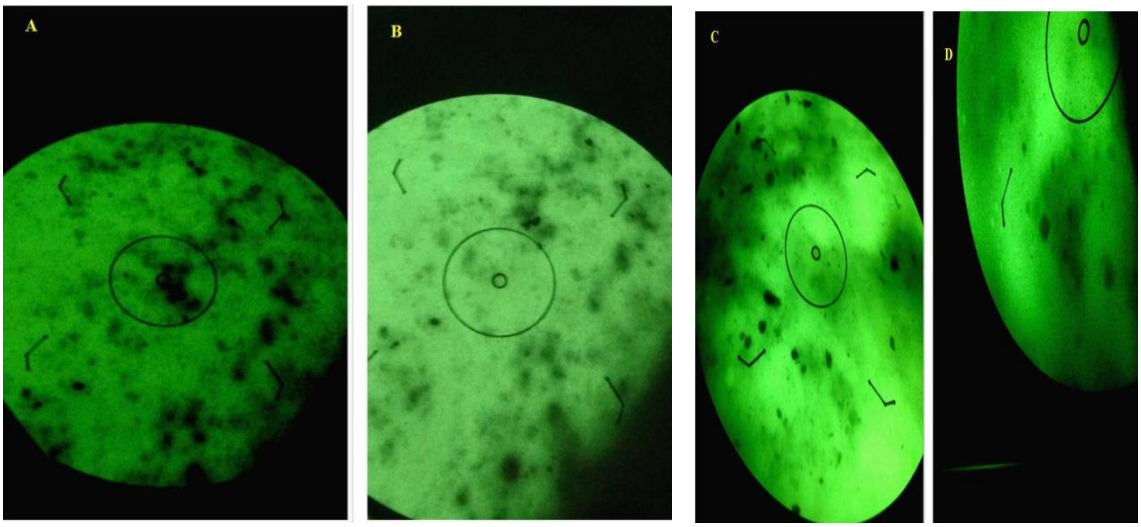

Figure (2); TEM Micrographs of A, B, C and D of NEB-NLCs, and D/Blank NEB-NLCS

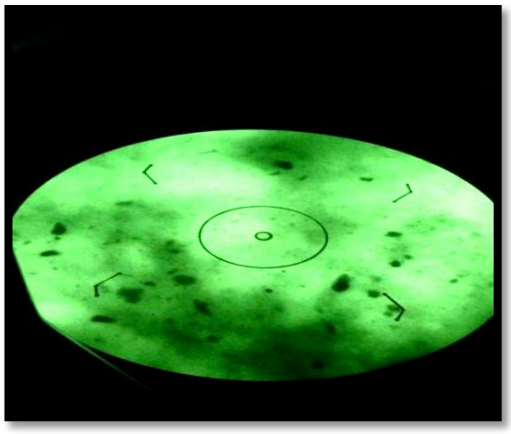

Figure (3); TEM image of NEB-NLCs based hydrogel (F3B)

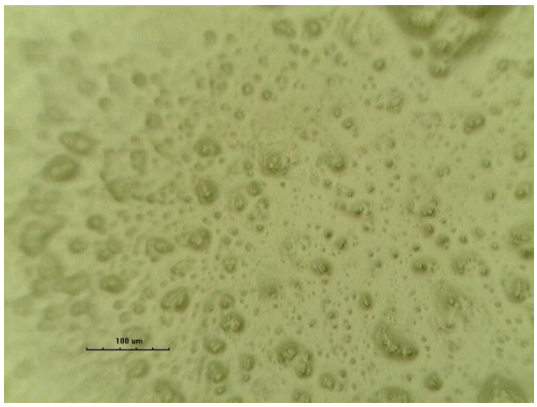

Figure (4); NEB-NLCs based hydrogel (F3B) under optical microscope

3. Skin Permeation Study of Prepared NEB-NLCs based hydrogel

Drug permeating across the skin in the vitro status represents the amount available for skin absorption for some anti hypertensive drugs such as carvidolol, propranolol and labetolol[19]. Hence, drug flux may be an indicator of the drug absorption to the targeted skin tissue. The kinetics of drug permeation which is helpful to explain the mechanisms of drug absorption via the skin. Release of drug from the vehicle must occur before the permeation of the drug into the skin. In vitro permeation study was performed to compare the permeation ability of NEB to the various NLC gel formulations (NLC-F1, NLC-F2, NLC-F3, and NLC-F4) using Franz diffusion cells. The in vitro permeation profile is portrayed in figure (5). Cumulative permeation profile of the different formulations revealed that the permeation of NEB from the NLCs hydrogel formulation (NLC-F3) is significantly high ($p<0.05$) as compared to the permeation of NEB from hydrogel formulations (NLC-F1, NLC-F2, and NLC-F4) which confirms the selection of (NLC-F3) to be the best formula. It was found that 90.8% of NEB permeated within 24 hrs of the study

as depicted in (Figure 5). The diffusion parameters such as lag time, permeation coefficient and (NEB) flux of the optimized formula were: 0.5 hr, 141 (cm/hr) × 10^{-3} and 143 (µg/cm^2.hr), respectively.

In this study, it has been proven that release rate of NEB is close to the flux value which indicates that NEB can easily penetrate into the stratum corneum. This suggests that partitioning into the skin and subsequent penetration are predominant steps in transdermal delivery of NEB. The mechanism for this finding is not clear, however different hypotheses have been placed forth. These include enhancement of solubility and the large surface area due to the nanoparticulate size[19]. Generally, intact particles are unable to permeate the stratum corneum. Lipid nanoparticles can deliver substances by interactions of the lipids used to construct the particles, and skin surface lipids[20]. Since NEB shows high lipophilicity thus might have a greater affinity for the SC. Nebivolol might be transported with lipid. A partitioning of NEB-NLC (F3) into the stratum corneum would lead to high accumulation of the drug. A rise in the concentration gradient leads to an elevation in the diffusion pressure of the drug into the skin. An Increased adhesiveness to skin surfaces is a general feature of nano particles. Lipid nanoparticles adhering to the skin form an adhesive film which leads to occluding the skin surface[18], thus increasing the amount of drug reaching the site of action. This effect should be meaningful for NEB since the therapeutic response to NEB can be increased by occlusion with a polyethylene film. Due to the adhesion effect, hydration of the stratum corneum was raised by reducing keratinocyte packing, and widening of the intercellular bilayers helping in facilitating drug penetration into deeper strata[19]. Generally, decreasing particle size results in increasing the adhesion effect due to disruption of the permeability barrier in the skin.

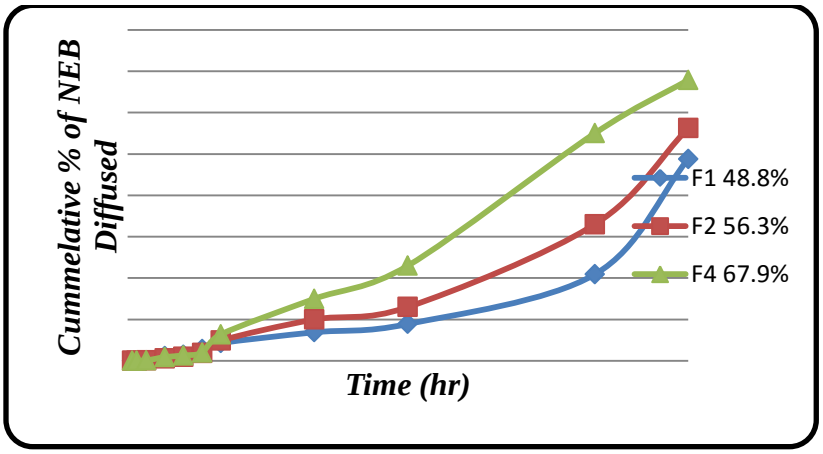

Figure (5); Cumulative % of NEB-NLCs loaded hydrogel (F1, 2, and 4 B) diffused through rat skin at 32◦C.

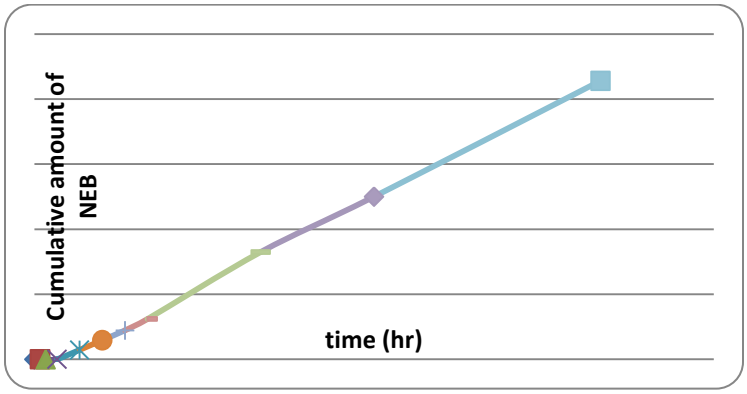

Figure (6); Cumulative % of NEB-NLCs (F3B) loaded hydrogel diffused through rat skin at 32◦C

4- Rheological Study

The rheological property of semisolid drug carriers is a very important physical parameter for its cutaneous application[17]. The aim of the current investigation is the rheological behavior of hydrogel when nanostructured lipid carriers are entrapped into their network. Therefore, rheological behavior of the NEB-NLCs based hydrogel was evaluated. According to this, analysis has been performed for NEB-NLCs based hydrogel formula (F43) and blank gel base as shown in figure (7 A, B and C) respectively, the obtained results were recorded after two months of hydrogel storage at 4 ºC,25 ºC and 40ºC. According to the results, the shear stress was not proportional to the shear rates in NEB-NLCs based hydrogel systems. The unique concavity of the rheogram toward the shear rate axis indicates that all developed formulations exhibited pseudoplastic flow. Such pseudoplasticity results from a colloidal network structure that aligns itself in the direction of shear, thereby decreasing as the shear rate increases the viscosity as well as will be increased[19].During all the rhological work, the temperature has been maintained at 25±0.1ºC using a thermostated water bath in order to avoid obtaining false positive results in the test for thixotropy. Figure (7) indicates that all systems show thixotropy, which in turn can be defined as an isothermal and comparatively slow recovery on standing of a material, of a consistency lost through shearing. Such result is in agreement with Sanap and Mohanta study in which, complex systems such as NLCs-loaded hydrogels exhibit a loose network connects together the sample, thixotropy proceeds from structural breakdown and re-aggregation[20].

5-Determination of Irritancy Test

Materials used in skin pharmaceuticals and cosmetics can be primary irritants, which induce irritation after a single contact or accumulative irritants that produces a fatigue reaction produced only after the application on successive days [21]. To ensure the transdermal preparations are innocuous, it is important to study the irritation effect of NEB-loaded hydrogel. The NEB-NLCs based hydrogel (F3B) was subjected to the irritancy test previously mentioned. After 7 days of application on the dorsal shaved skin of albino rat, there was visual redness developed on the skin (figure 8 and 9). In this present preliminary safety test, findings suggest

skin tolerance to the NEB-loaded hydrogel. In summary, the selected formula is considered as a nonirritant

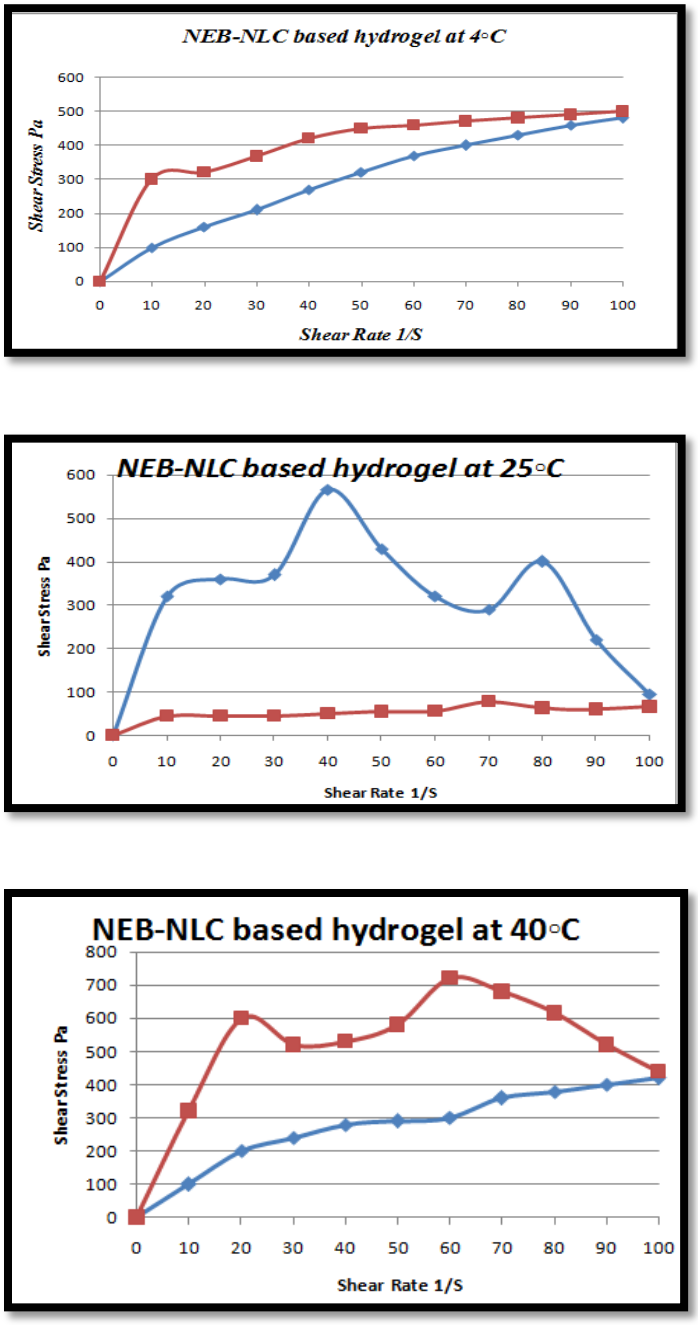

Figure (7); A,B, and C Represent Shear Rate (1/S) Vs Shear Stress (Pa) of NEB-NLCs based hydrogel after two months of storage at different temperatures (4, 25, 40◦C) respectively.

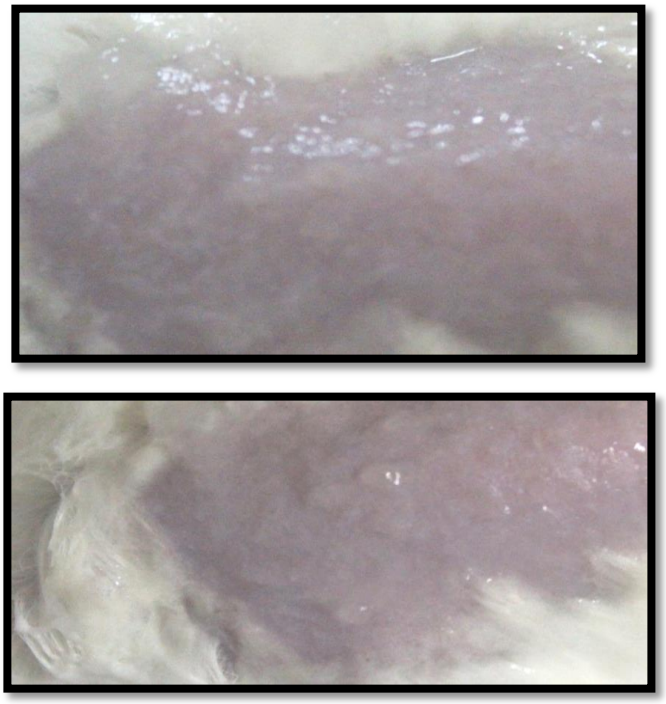

Figure (8); A and B of the applied NLCs based hydrogel of NEB on the dorsal area of rat at different magnification power

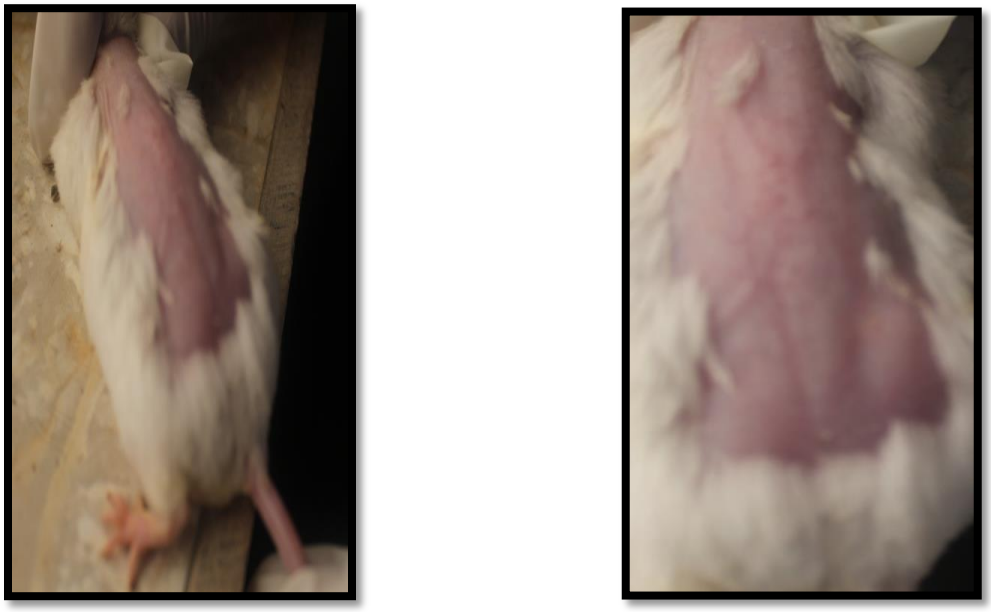

Figure (9); After 7 days of NEB-NLCs based hydrogel application

Conclusion

Nebivolol hydrochloride as a model drug was used for the preparation of nanostructured lipid carriers.The NEB-loaded NLCs could be fabricated and successfully incorporated into hydrogel for transdermal application. The Ex -Vitro skin permeation data indicated that NEB-NLCs bearing hydrogel provided sustained release of NEB. The results reflected the potential of NLC as a carrier for transdermal administration of NEB and that would demonstrate greater drug deposition into the skin. The NEB-NLCs system considered as a promising alternative drug carriers for transdermal pharmaceutics. The data presented indicated the successful development of NEB-NLCs-based hydrogel in increasing the encapsulation efficiency of colloidal lipid carriers. The advantage for the colloidal lipid carriers is in the improved performance in terms of stability and providing a sustained NEB transdermal effect.

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