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# *IN-VITRO* AND *IN-VIVO* EVALUATION OF NIOSOMES CONTAINING CELECOXIB

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**Keywords:**

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**ABSTRACT:** Niosomes as a vesicular system are well documented for delivering drugs, in a controlled manner to enhance bioavailability and get better therapeutic effect over a longer period of time. Celecoxib is an NSAID, selective COX-2 inhibitor, which is used for treatment of rheumatoid arthritis, osteoarthritis, and acute pain. The present study dealt with the preparation and characterization of celecoxib niosomes. The selected niosomes was incorporated into gel as an effective transdermal formulation of celecoxib with the aim to improve skin permeability and sustained delivery of celecoxib. Celecoxib loaded niosomes were prepared by the thin-film hydration method using different proportions of Span 60 or 40, cholesterol with or without adding stearylamine and dicetyl phosphate as a positive and negative charge-inducing agent. The results showed that neutral niosomes gave the highest encapsulation efficiency, more than 90%. All formulations were characterized using transmission electron microscopy, differential scanning calorimetry, vesicle size, and zeta potential. The vesicle size of celecoxib niosomes ranged from 132 to 826 nm. The *in-vitro* release studies revealed that most celecoxib niosomal formulations released more than 90% of its drug content within 48 h. Physical stability study conducted on the two selected celecoxib niosomal formulations gave better stability, upon storage. *In-vivo* performance of niosomal celecoxib gel was evaluated by assessing anti-inflammatory activity by carrageenan-induced rat paw edema model using digital plethysmometer. The application of niosomal gel produced significant reduction of rat paw edema compared to control and conventional celecoxib gel especially after 24 h, indicating better skin permeation and prolonged drug release.

**INTRODUCTION:** Vesicular systems are considered as novel means of delivering the drug in controlled manner to enhance bioavailability and get therapeutic effect over a longer period of time.

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Niosomes are non-ionic surfactant vesicles that are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures [1].

They are useful for delivery of both hydrophilic drugs, which are encapsulated in the interior aqueous compartment, and hydrophobic drugs which are intercalated in outer lipid layers [1]. Inflammation is defined as the local response of living tissues to injury due to any agent, leading to the local accumulation of blood cells and fluids [2].

Arthritis is the general term for a family of diseases characterized by joint inflammation, pain, and swelling that lead to permanent joint damage and a loss of flexibility and mobility. Rheumatoid arthritis is the most common inflammatory arthritis that mainly targets the synovial membrane of diarthrodial joints [3].

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID), a highly lipophilic, poorly aqueous solubility and wettability drug, however, give rise to difficulties in the design of pharmaceutical formulations and lead to variable oral and poor bioavailability between 22% and 40% by conventional capsule dosage form, its half-Life about 11 h [4]. Celecoxib, as a COX-2 specific inhibitor promoting a reduction of the inflammatory process and maintaining normal physiological levels of prostaglandin in stomach and kidneys, it appears to have a gastrointestinal safety profile superior to the traditional NSAIDs causing significantly less ulceration and displaying equivalent anti-inflammatory and analgesic efficacy, so it is used for the treatment of chronic inflammatory cases such as; rheumatoid arthritis, osteoarthritis [5].

An arthritic condition demands a controlled release drug delivery system for a prolonged period that can satisfy the goals of the treatment like the reduction of pain and inflammation and prevention of adverse effects of drugs. The oral use of celecoxib was restricted and makes it a good candidate for transdermal administration to overcome its poor bioavailability, bypassing hepatic metabolism and minimizing systemic side effects due to site-specific drug delivery.

The aim of this work is to formulate, prepare, characterize and evaluate niosomes as a drug delivery system for transdermal administration of celecoxib to improve its bioavailability and anti-inflammatory efficacy through niosomal encapsulation with objectives of decreasing the toxic effects and prolonging its action.

## MATERIALS AND METHODS:

**Materials:** Celecoxib was a kindly gift sample from European Egyptian Pharm. Ind. Company, Alexandria, Egypt. Sorbitan monostearate (Span 60), Merck Schuchardt OHG, Germany. Sorbitan monopalmitate (Span 40), Aldrich Chemistry, Sigma-Aldrich, Germany. Cholesterol from Lanolin, minimum 99% (GC), Fluka Biochemika, Germany. Dihexadecylhydrogen- Phosphate, Sigma-Aldrich Chemie GmbH, Germany. Octadecylamine (Stearylamine) (SA), Sigma-Aldrich Co., St. Louis, Mo., USA. Chloroform HPLC, Panreac Quimica SA, Barcelona, Spain.Dodeca-Tungesto Phosphoric Acid extra pure, Fine- Chem. Ltd., India. Methyl alcohol analytical reagent Fisher scientific UK Limited, UK. Cellulose membrane (molecular weight cut-off 12,000-14,000 g/mole), Sigma-Aldrich, chemie GmbH, Steinheim, Germany. Acetonitrile HPLC, Tedia, USA. Carrageenan, Sigma, St. Louis, MO, USA. Carbapol® 934 was a kindly gift sample from CID Company, Cairo, Egypt.

**Methods:**

**Spectrophotometric Assay of Celecoxib:** Celecoxib in methanol was assayed by ultraviolet spectroscopy. The decision for using a 100% methanol was based on its sensitivity, stability and preparation time [6]. A stock solution was prepared by dissolving accurately weighed 25 mg of celecoxib in 250 ml methanol to achieve a final concentration of 100 µg/ml. This stock solution was diluted ten folds obtaining a concentration of 10µg/ml to determine the $\lambda_{max}$ of celecoxib. Then, six concentrations of celecoxib in methanol were prepared for creating the calibration curve. These concentrations were 2, 4, 6, 8, 10 and 12 µg/ml, prepared by serial dilutions of the stock solution. A linear relationship was established between different concentrations of celecoxib and UV absorbance. The slope was calculated for the mean of three determinations.

**Preparation of Celecoxib Niosomes:** Niosomes were prepared by the thin-film hydration method originally reported by Baillie *et al.* [7] for niosomes preparation. Two types of nonionic surfactants were used for the preparation of celecoxib niosomes, namely; Span 60 and Span 40, Cholesterol (CHOL) with or without adding Dicetylphosphate (DCP), as a negative charge inducing agent to the niosomal system to prevent niosomes aggregation and increase the stability of niosomal dispersions. To study the effect of positively charged molecules when added to niosomal formulations, Stearylamine (SA) was

used as a positive charge inducer. All celecoxib niosomal formulations prepared were illustrated in **Table 1**.

A mixture of either Span 60 or Span 40, CHOL with or without charge inducing agents was weighted according to the molar ratios investigated, then accurately weighed 20 mg of celecoxib was added to the lipid mixture. All ingredients were dissolved in 10 ml chloroform and then the organic solvent was removed by rotary evaporation under reduced pressure, on a water bath at 60 ± 2 ºC.

The deposited lipid film was then hydrated with 10 ml normal saline by rotation in a water bath, at 60 ± 2 ºC for 45 min.

**TABLE 1: COMPOSITION OF THE PREPARED CELECOXIB NIOSOMAL FORMULATIONS**

| Formula | Niosomes type | Molar ratio | | | | |
|---|---|---|---|---|---|---|
| | | Non-ionic surfactant | | CHOL | Charge inducing agent | |
| | | Span 60 | Span 40 | | DCP | SA |
| $F_1$ | Neutral | 1 | | 1 | | |
| $F_2$ | Negatively | 1 | | 1 | 0.1 | |
| $F_3$ | Positively | 1 | | 1 | | 0.1 |
| $F_4$ | Neutral | 2 | | 1 | | |
| $F_5$ | Negatively | 2 | | 1 | 0.2 | |
| $F_6$ | Positively | 2 | | 1 | | 0.2 |
| $F_7$ | Neutral | | 1 | 1 | | |
| $F_8$ | Negatively | | 1 | 1 | 0.1 | |
| $F_9$ | Positively | | 1 | 1 | | 0.1 |
| $F_{10}$ | Neutral | | 2 | 1 | | |
| $F_{11}$ | Negatively | | 2 | 1 | 0.2 | |
| $F_{12}$ | Positively | | 2 | 1 | | 0.2 |

**Estimation of Niosomes Entrapment Efficiency for Celecoxib:** The free celecoxib was separated from the niosome-intercalated celecoxib by cooling centrifugation adjusted to 5200 × g at – 4 ºC using the refrigerated centrifuge. The niosomal pellets were then washed once. The washed pellets were resuspended in 10 ml normal saline and the amount of celecoxib entrapped in niosomes was determined by lysis of 1 ml of resuspended pellets with 50 ml methanol followed by ultrasonication in the bath-type ultrasonicator for 30 min [8], then the solution was double filtered with micropore filter (pore size 0.2 µm) to discard any niosomal particles may be suspended in the solution. A clear solution was obtained which was measured spectrophotometrically at 252 nm against drug-free niosomes, prepared by the same way, as a blank.

Entrapment efficiency (EE %) = (amount of drug entrapped/ total amount of drug added) × 100.

**Characterization of Celecoxib Niosomes:**

**Transmission Electron Microscopy (TEM):** Morphological characteristics of celecoxib niosomes were studied by TEM. 1 ml from the niosomal suspensions were diluted fivefold with normal saline and mixed well prior to the examination. The stain used was 1% phosphotungstic acid. The investigated niosomes was clearly observed by the transmission electron microscope (JEOL, JEM-1230, Tokyo, Japan). Photographs were taken using suitable magnifications.

**Differential Scanning Calorimetry (DSC):** The thermal characteristics of the prepared niosomal formulations were investigated to study the possible interactions between celecoxib and vesicle ingredients using differential scanning calorimetry. These niosomal formulations were prepared, separated, washed and then the dehydrated niosomal pellets were used for this thermal analysis.

The weights used for the niosomal preparations were equivalent to 2 mg Span 60 or Span 40. A heating rate of 5 °C/min was employed over a temperature range (20 - 250) °C. The analysis took place under a nitrogen atmosphere using aluminum pan. Computer presentations of DSC thermograms were provided.

**Zeta-potential and Vesicle Size Determinations:** Niosomal zeta-potentialand vesicle size for the prepared celecoxib niosomeswere determined using dynamic light scattering (DLS) based on laser

diffraction at room temperature by Zeta Potential/ Particle Sizer NICOMP™ equipped with a 5 mW laser with a wavelength output of 632.8 nm and a scattering angle of 90.0º, which is capable of measuring vesicles in 1 nm - 5µm size range.

At investigation time, all niosomal suspensions were diluted with de-ionized water (1:100 v/v). The polydispersity index (P.I.) was determined as an indication of homogeneity.

(P.I.) = Standard deviation/ Mean droplet size

***In-vitro* release Profiles of Celecoxib Niosomes:** This study was conducted on neutral and negatively charged celecoxib niosomal formulations, namely; $F_1$, $F_2$, $F_4$, $F_5$, $F_7$, $F_8$, $F_{10}$, and $F_{11}$ celecoxib niosomes. It should be noted that positively charged celecoxib niosomes, namely; $F_3$, $F_6$, $F_9$, and $F_{12}$ were omitted from this experiment due to aggregation and their low stability previously observed and reported [9].

A one ml sample from each niosomal suspensions was transferred to a cellophane membrane, molecular weight cut off 12000 – 14000, immersed in a beaker containing 100 ml PBS (pH 5.5) containing 40% methanol, based on solubility characteristic of the drug [10], to fulfill sink condition.

The beaker was placed in a water bath shaker to maintain the temperature at 32 ± 2 ºC, with gentle agitation. At predetermined time intervals (1, 2, 3, 4, 6, 8, 24 and 48 h); 4 ml of the release medium were withdrawn for analysis and replaced with an equal volume of fresh PBS (pH 5.5) containing 40% methanol at 32 ± 2 ºC to maintain a constant volume. The absorbance of the collected samples was measured spectrophotometrically.

**Physical Stability Study of Celecoxib Niosomes:** Two niosomal formulations were selected for this study which are; negatively charged niosomes of molar ratio Span 60: CHOL: DCP (1:1:0.1) representing Span 60 formulations and neutral niosomes of molar ratio Span 40: CHOL (2:1) representing Span 40 formulations. Ten ml from each batch were sealed in 20 ml glass vials and stored at refrigerator temperature (2 – 8 ºC) for a period of 3 months. Samples from each batch were withdrawn at definite time intervals after 1, 2 and 3 months of storage and the retention amount of the entrapped drug in the vesicles was determined after separation from leached drug. Stability for the formulation was defined in terms of retaining its initial entrapment efficiency for three months duration as the following equation [13]:

Celecoxib retained in niosomes = (Amount of celecoxib entrapped after storage / Amount of celecoxib entrapped before storage) × 100

The vesicle size for each sample was analyzed and the data were compared to the freshly prepared niosomes.

***Ex-vivo* Permeation Studies for Celecoxib from Niosomal Carbopol Gel Base:** The *ex-vivo* permeation studies were conducted across hairless abdominal rat skin. The abdominal hair of male wistar albino rats (120 – 160 gm) was removed carefully using electric shaver. After the animals were sacrificed, the hair-free abdominal skin was excised with surgical blade and scissors. The adhering subcutaneous fat was trimmed off with care not to damage stratum corneum and the prepared skin was freshly used. The whole skin was equilibrated in phosphate-buffered saline, PBS (pH 7.4, to simulate human blood pH) for one hour before the experiment.

The study was assessed using Franz diffusion cell. The PBS (pH 7.4) containing 40% v/v methanol was used as the receptor medium in the diffusion cell. The skin was sandwiched between the receptor compartment and the donor compartment in such a way that the dermal portion was continuously bathed with the receptor fluid maintained at 37 ± 1 ºC to simulate human blood temperature.

The membrane surface area available for diffusion was 3.14 $cm^2$. The donor site was charged with the investigated formulation containing 2 mg celecoxib. The receptor compartment was constantly stirred using magnetic stirrer. Samples of the receptor fluid, about 2 ml, were withdrawn at different time intervals namely; 1, 2, 3, up to 24 hrs and replaced immediately with the same volume of fresh PBS (pH 7.4) containing 40% v/v methanol solution to maintain the sink conditions constantly. The amount of drug permeated through the skin was determined by the HPLC method.

**Carrageenan-Induced Paw Edema:** The model of carrageenan-induced paw edema was carried out in rats according to the method of Winter et *al.* [12] The right paw was marked with ink at the level of tibiotarsic articulation; basal paw volume ($V_i$) was measured by a volume displacement method using a water digital plethysmometer 7500 (Ugo Basile, Comerio, Italy) by immersing the paw till the level of tibiotarsic articulation into the container of the plethysmometer and the displacement volume (in ml) was measured by two platinum electrodes introduced beforehand into the container. Carrageenan suspension (0.1 ml of 1% w/v, in saline) was injected into the sub plantar surface of the right hind paw and the paw volume was measured again at 1, 3, 4, 6 and 24 h after carrageenan injection ($V_f$).

The increase in paw volume was calculated as the percentage of edema compared to the basal paw volume according to the formula:

$$\text{Edema (\%)} = [(V_f - V_i) / V_i] \times 100$$

While the percentage of inhibition of the severity of inflammation was calculated according to the following formula:

$$\text{\% of inhibition} = [(1 - V_t / V_c)] \times 100$$

Where $V_t$ represents mean edema volume of rats in the test groups and $V_c$ is the mean edema volume of rats in the control group.

## RESULTS AND DISCUSSION:

**Spectrophotometric Assay of Celecoxib:** The spectrum of celecoxib in methanol is shown in **Fig. 1**.

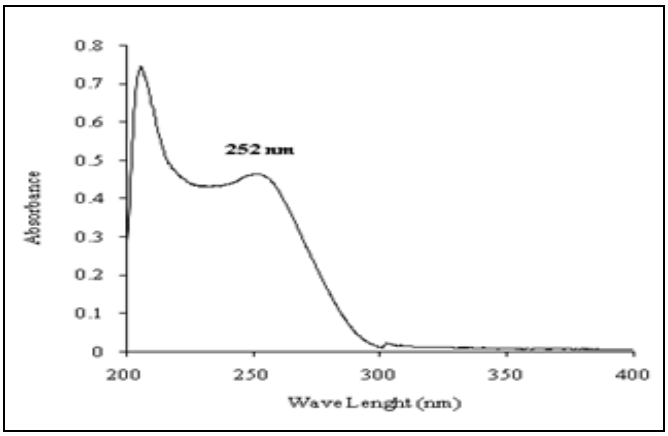



**FIG. 1: SPECTRUM (200 - 400 nm) FOR CELECOXIB IN METHANOL**

The wavelength of maximum absorbance (λmax) was found to be at 252 nm. A direct relationship was established between different concentrations of celecoxib in methanol and UV absorbance at 252nm with a linear regression ($R^2$ = 0.9993) and the slope was calculated to be (0.05199). From these results, the spectrophotometric assay of celecoxib can be used to estimate celecoxib concentrations in methanol.

**Entrapment Efficiency (EE%):** The entrapment efficiencies of all niosomal formulations are illustrated in **Table 2**, where celecoxib was successfully entrapped in all these formulations.

**TABLE 2: ENTRAPMENT EFFICIENCIES (EE %), AVERAGE ZETA-POTENTIAL, MEAN VESICLE DIAMETER AND POLYDISPERSITY INDEX OF ALL INVESTIGATED CELECOXIB NIOSOMAL FORMULATIONS**

| Formula | EE% ± S.D.* | Average Zeta-potential (mV)# | Mean Vesicle Diameter (nm) | Polydispersity index (P.I.) |
|---|---|---|---|---|
| $F_1$ | 96.79 ± 1.56 | -39.26 | 393.8 | 0.641 |
| $F_2$ | 90.74 ± 1.05 | -52.31 | 383.1 | 0.666 |
| $F_3$ | 79.60 ± 2.67 | 0.71 | 751.7 | 0.973 |
| $F_4$ | 90.79 ± 2.84 | -37.75 | 294.3 | 0.690 |
| $F_5$ | 84.45 ± 2.47 | -57.29 | 458.3 | 0.924 |
| $F_6$ | 73.66 ± 3.75 | 10.31 | 825.9 | 0.986 |
| $F_7$ | 92.83 ± 1.57 | -41.57 | 164.7 | 0.564 |
| $F_8$ | 85.71 ± 3.23 | -43.61 | 169.2 | 0.559 |
| $F_9$ | 64.43 ± 3.98 | 2.46 | 616.3 | 0.783 |
| $F_{10}$ | 90.28 ± 3.8 | -31.74 | 132.3 | 0.679 |
| $F_{11}$ | 85.66 ± 1.88 | -49.00 | 299.1 | 0.677 |
| $F_{12}$ | 77.18 ± 2.57 | 3.24 | 666.3 | 1.070 |

* Each value is a mean of at least 4 batches using 20 mg celecoxib and 100 mg total of non-ionic surfactant and lipid(s), per each batch.
# The value of zeta-potential is the average of three samples and cycles determinations of the zeta-potential.

**Effect of Niosomal Surface Charge on (EE %) of Celecoxib:** Statistical analysis of the data by one way ANOVA test ($P<0.05$) revealed a significant higher encapsulation percentage of celecoxib when comparing neutral niosomal formulation prepared either from Span 60 or 40 to their equivalent

charged ones. Addition of charge inducing agents decrease the amount of celecoxib entrapped may be due to the repulsion between the niosomal bilayers which led to increasing of the leaking space in the bilayer membranes and therefore permit the release of the encapsulated drug [13]. These findings were confirmed when comparing the two negatively charged niosomes, $F_2$ and $F_5$, where there is a significant difference in the percentage entrapment of celecoxib at ($P<0.05$). These could be explained by what was mentioned previously that increasing the amount of DCP, in case of $F_5$, led to more repulsion between the bilayers and hence increase the leakage of entrapped drug compared to $F_2$ [13].

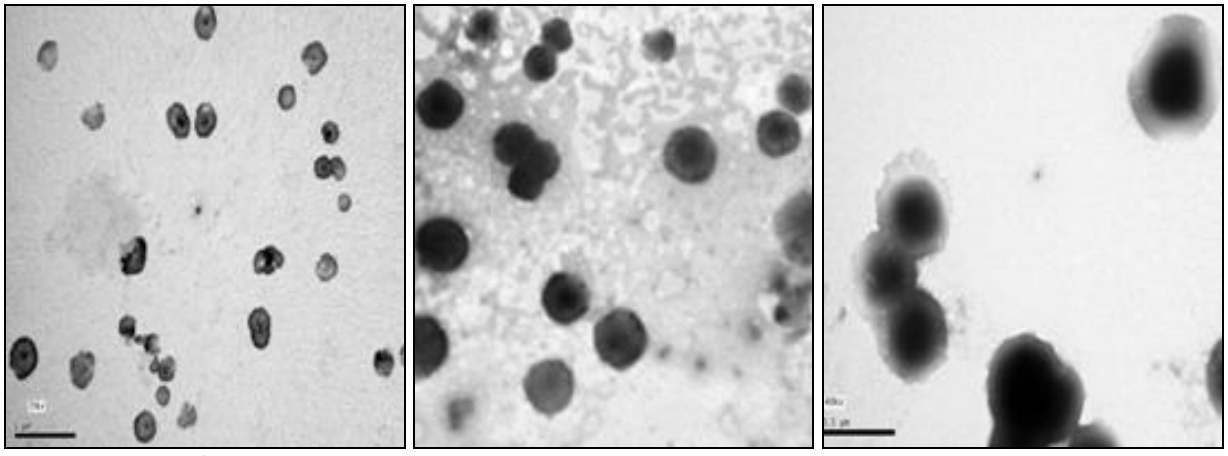
In case of positively charged niosomes $F_3$, $F_6$, $F_9$ and $F_{12}$, a significant decreasing in the mean of celecoxib entrapped is noticed when compared to neutral and negatively charged niosomes. This could be explained by the aggregations occurred and observed during the preparation led to the formation of sticky coarse particles in the niosomal suspension [9].

**Effect of Molar Ratio on (EE %) of Celecoxib:** The results reveal that neutral, negatively and positively charged celecoxib niosomes, $F_1$, $F_2$, $F_3$, $F_7$ and $F_8$ of the molar ratio Span/CHOL (1:1) and Span/CHOL/CIA (1:1:0.1), possessed higher entrapment efficiencies when compared to their alternative molar ratio neutral celecoxib niosomes at the molar ratio Span /CHOL (2:1),$F_4$ and $F_{10}$, negatively and positively charged celecoxib niosomes at the molar ratio Span/CHOL/CIA (2:1:0.2), $F_5$, $F_6$ and $F_{11}$. This could be explained by increasing the CHOL content resulted in an improvement of the total amount of drug encapsulated due to an increase in the lipophilic behavior and crystallinity of the surfactant bilayer of niosomes [14]. Accordingly, it could be concluded that celecoxib, which is a highly hydrophobic drug, intercalated in these lipophilic portions.

**Transmission Electron Microscopy (TEM):** Considering the electron micrographs, the vesicles exist in disperse and aggregate collections. Also, they reveal well stained niosomal vesicles, where the outer lipophilic domains and the inner hydrophilic domains are well-identified **Fig. 2**.

**Fig. 2A** and **2B** demonstrate the electron micrographs of neutral celecoxib niosomes $F_1$ and negatively charged celecoxib niosomes of the molar ratio, Span $F_{11}$, reveal well-identified vesicles which are present in a nearly perfect sphere-like shape having a large internal aqueous space and a smooth vesicle surface.

**Fig. 2C** demonstrates the electron micrograph of positively charged celecoxib niosomes $F_6$ which show heavily dyed niosomal vesicles, with irregular borders and without definite shape or structure. This could be explained by the aggregation formed as a result of the presence of positive charge, induced by stearylamine [9].



**A. $F_1$** **B. $F_{11}$** **C. F6**

**FIG. 2: TEM OF NEUTRAL CELECOXIB NIOSOMES ($F_1$), NEGATIVELY CHARGED CELECOXIB NIOSOMES ($F_{11}$) AND POSITIVELY CHARGED CELECOXIB NIOSOMES ($F_6$)**

**Differential Scanning Calorimetery (DSC):** The thermograms reveal sharp melting endothermic peaks for the niosomal individual components indicating their crystallinity. A definite

endothermic peak representing the gel-liquid transition temperature of Span 60 or 40 employed for the preparation of the niosomal formulations. Decreasing in the value of Span's transition temperatures as well as a distinct peak broadening of all niosomal formulations, compared to Span in its pure form, where the inclusion of CHOL in niosomal preparation, didn't shift the transition temperatures of Spans to higher temperatures [15]. Also, DSC thermograms show disappearance of the characteristic endothermal peak of celecoxib. This may suggest that there is a significant interaction of celecoxib with the niosomal bilayer components and can account for the enhanced entrapment of celecoxib into these formulations [16].

All these findings indicate that there were significant-good interactions of all niosomal components forming the bilayers of niosomes and enhanced entrapment of celecoxib into these formulations.

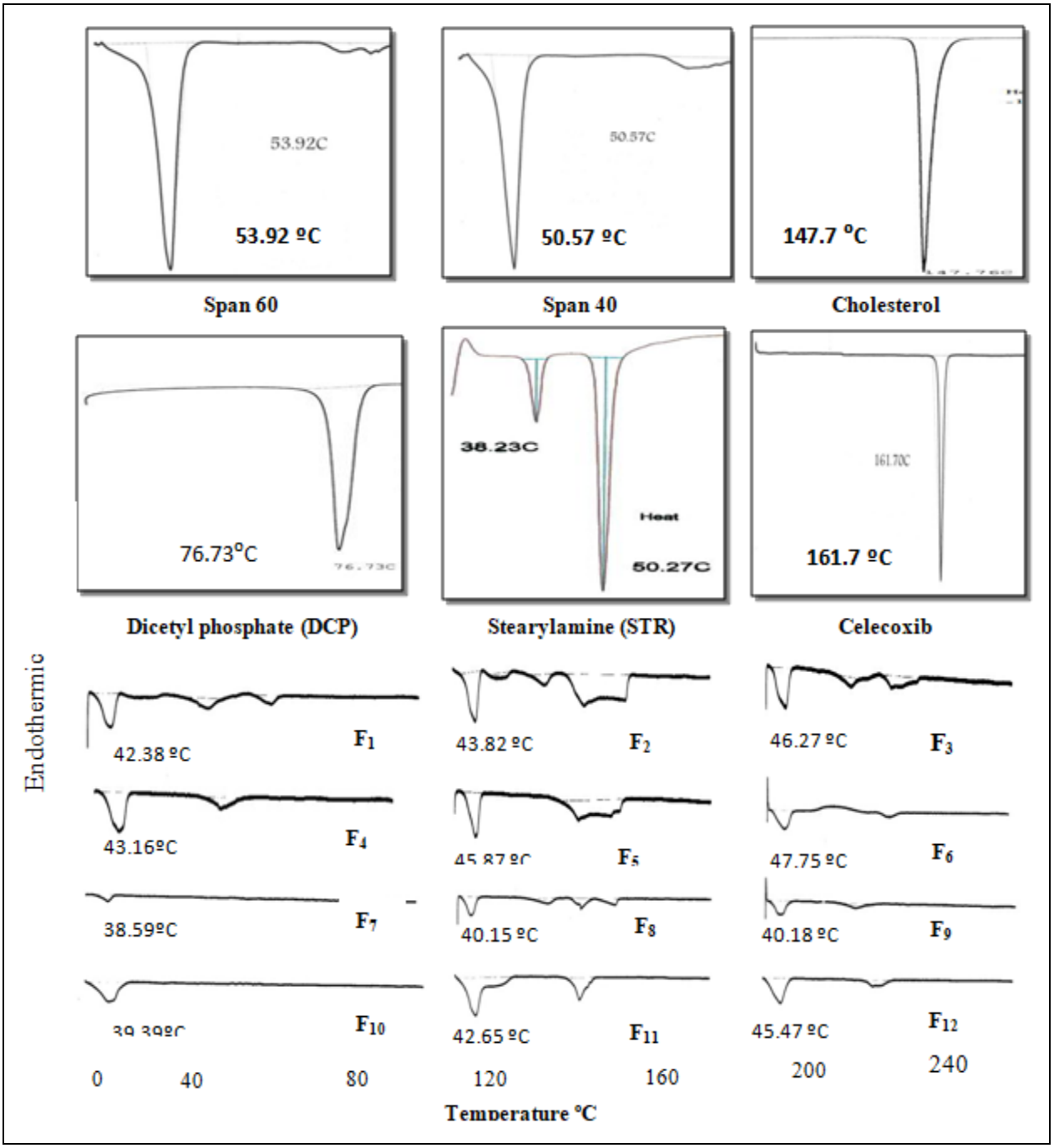



**FIG. 3: DSC THERMOGRAM OF NIOSOMAL PURE COMPONENTS AND CELECOXIB NIOSOMAL FORMULATIONS, $F_1 – F_{12}$**

**Zeta Potential Determination: Table 2** reveals that the neutral celecoxib niosomes for both the investigated Spans, *viz.* 60 and 40, and the following two molar ratios Span/CHOL (1:1 and 2:1), give negative zeta potential values more than (-30 mV) indicating stable systems [17]. While negatively charged niosomes prepared using Span 60 and 40/CHOL/DCP (1:1:0.1 and 2:1:0.2) give higher negative values of zeta potential, due to addition of negatively charged lipids (DCP), which indicates more stability of these colloidal systems compared to neutral niosomes. On the contrary, niosomes prepared using positive charge inducing agents (stearylamine) neutralize the negative charge carried by the system gives low zeta potential values resulting in aggregation of the particles which indicates low stability of the system.

**Vesicle Size Determination: Table 2** showed the results of vesicle size determinations, it could be concluded that neutral celecoxib niosomes, ($F_1$ and $F_7$), of molar ratio Span/CHOL (1:1), exhibited markedly larger vesicle sizes compared to the other alternative molar ratio, Span /CHOL (2:1) neutral celecoxib niosomes, ($F_4$ and $F_{10}$). These results are in agreement with previous studies showing that an increasing in the percentage of CHOL caused the size of vesicles to increase, where CHOL would be more likely to increase the number of bilayers since it has little effect on the charge at the bilayer surface and interlayer separation [18]. Also, the increase in the ratio of the negative charge inducer, DCP, or positive charge inducer, SA, in the niosomal formulations, $F_5$, $F_6$, $F_{11,}$ and $F_{12}$, increased the vesicle size compared to other niosomal formulations, $F_2$, $F_3$, $F_{8,}$ and $F_9$, correspondingly. This increase in vesicle size could be explained by the higher charge inducing agents' content, in addition to lower cholesterol content. Also, the inclusion of charged molecules in the composition may increase the separation between bilayers of vesicles due to repulsion between similar charges, thus increasing the size of the internal aqueous compartment resulting in the formation of vesicles larger in size [19].

***In-vitro* Release Studies of Celecoxib Niosomes:** Furthermore *in-vitro* release studies are often performed to niosomal formulations to predict some indications of their *in-vivo* performance. **Fig. 4** demonstrate the release pattern of the investigated niosomal formulations. Celecoxib released from niosomes occurred in two phases, a controlled release phase that lasted for 8 h, characterized by a relatively fast drug release rate, more than 50% of the entrapped celecoxib could be released, followed by a steady phase with a reduced and slow-release rate that was maintained for 48 hours, and showed more than 90% drug released.

This biphasic release pattern is in accordance with previously reported studies and seems to be a general characteristic of bilayer vesicles [20]. It could be explained by the following sequences of events; the initial moderate phase may be due to the diffusion of free unentrapped celecoxib and desorption of the drug from the surface of niosomal vesicles. The sustained slower release phase is related to the diffusion of celecoxib gradually through the bilayers into the medium [21].

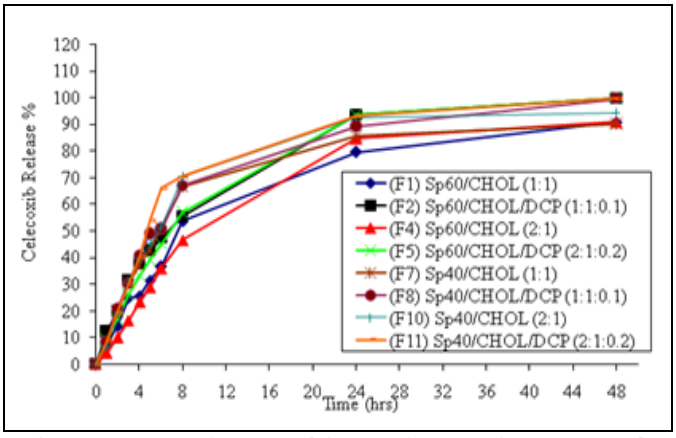



**FIG. 4: *IN-VITRO* RELEASE PROFILE OF NEUTRAL AND NEGATIVELY CHARGED CELECOXIB NIOSOMES AT 32 ± 2 ºC**

**Effect of Cholesterol Content on the Release Rates: Fig. 4** and **Table 3** reveal that $F_1$, $F_2$, $F_7$ and $F_8$, exhibited lower release rate constants namely; 14.59, 15.29, 13.64 and 14.96 $mgh^{-1/2}$ respectively, compared to their corresponding niosomal formulations $F_4$, $F_5$, $F_{10}$ and $F_{11}$, namely; 15.84, 16.08, 14.69 and 15.02 $mgh^{-1/2}$. The preceding results reveal that the increase of cholesterol content, which reaches 50 mole %, in the molar ratios Span/CHOL (1:1) and Span/CHOL/DCP (1:1:0.1) compared to 33 mole % in the molar ratios Span/CHOL (2:1) and Span/CHOL/DCP (2:1:0.2), markedly reduced the efflux of celecoxib from niosomal formulations. Cholesterol play the main role in stabilizing the

bilayers, preventing leakiness and retarding permeation of solutes enclosed in the vesicles and produced an optimum hydrophobicity which decreases the formation of the transient hydrophilic holes, by decreasing fluidity, responsible for drug release through the bilayers [22].

**Effect of Surface Charge on Release Rate:** The release pattern of negatively charged celecoxib niosomes, $F_2$, $F_5$, $F_8$, and $F_{11}$, showed higher release rates compared to their corresponding neutral celecoxib niosomes, $F_1$, $F_4$, $F_7$ and $F_{10}$. This could be explained by the inverse relation between the entrapment efficiency and the drug release, *i.e.* entrapment efficiency is a measure of the vesicle ability to retain the drug; thus the more the drug is retained in the vesicle, the slower the release profile will be. Another main reason that could be proposed for the higher release rate achieved by negatively charged celecoxib niosomes compared to neutral ones is the inclusion of DCP. The presence of DCP in the formulation led to increase the electrostatic repulsion force between intra niosomal bilayers and this facilitate the permeation of entrapped celecoxib into the release medium [13].

**Kinetics Studies of the Release Profiles for Celecoxib Niosomes: Table 3** tabulated mathematical models which are commonly used to predict the release mechanisms and compare the release profiles. The correlation coefficients ($R^2$) values reveal that the release of celecoxib from niosomal formulations, $F_1$, $F_2$, $F_4$, $F_5$, $F_7$, $F_8$, $F_{10}$, and $F_{11}$, is best-fitted to diffusion-controlled mechanism (Higuchi's model), where diffusion out of the vesicles showed the highest ($R^2$) values. The results also point to sustained release characteristics with a Higuchi pattern of drug release, where niosomes act as a reservoir system for continuous delivery of drug. These controlled-release patterns of entrapped drug reflect the high stability of these niosomal formulations.

**TABLE 3: RELEASE KINETICS OF CELECOXIB NIOSOMAL FORMULATIONS IN METHANOL/PHOSPHATE BUFFERED SALINE (PH 5.5) AT 32 ± 2ºC**

| Formula | $R^2$ value | | | | | Release Rate constant* ($mgh^{-1/2}$) |
|---|---|---|---|---|---|---|
| | Zero Order | First Order | Second Order | Third Order | Higuchi Diffusion model | |
| $F_1$ | 0.818 | 0.559 | 0.263 | 0.130 | 0.935 | 14.59 |
| $F_2$ | 0.801 | 0.571 | 0.306 | 0.164 | 0.928 | 15.29 |
| $F_4$ | 0.817 | 0.527 | 0.206 | 0.1008 | 0.936 | 15.84 |
| $F_5$ | 0.797 | 0.550 | 0.27 | 0.137 | 0.926 | 16.08 |
| $F_7$ | 0.681 | 0.436 | 0.207 | 0.109 | 0.841 | 13.64 |
| $F_8$ | 0.732 | 0.448 | 0.197 | 0.102 | 0.88 | 14.96 |
| $F_{10}$ | 0.668 | 0.398 | 0.162 | 0.086 | 0.832 | 14.69 |
| $F_{11}$ | 0.647 | 0.406 | 0.197 | 0.107 | 0.811 | 15.02 |

* Release rate (K) of Higuchi model

**Physical Stability Study of Celecoxib Niosomes:** The visual appearance observations for the two selected formulations, $F_2$ and $F_{10}$, celecoxib niosomes showed good physical stability with partial, without coarse particles, sedimentation, no layer separation, and no color change. This could be explained by the fact that the presence of surface charge is an important parameter influencing niosomal behavior; *i.e.* high zeta-potential of these systems led to electrostatic repulsion between vesicles which reduced the rate of aggregation and fusion of niosomes during storage.

**Table 4** represented the percentages of drug retained in niosomes. After 30, 60 and 90 days, the percentages of celecoxib retained in the niosomal formulations were 95.9%, 95.2%, and 93.6%, respectively in case of $F_2$ and 96.2%, 96.1% and 91.6%, in case of $F_{10}$ from their initial drug entrapped. Statistical analysis of the data by one way ANOVA test ($P<0.05$) revealed no significant differences in comparison between fresh (initial) time and after 30, 60 and 90 days. It is obvious from results that there was a direct relationship between the percentage of the drug leached out of the vesicles and aging. The investigated niosomes exhibited sufficiently stable behaviors under refrigerators storage and the advantages of the bilayer membrane were retained. These may be attributed to the fact of the presence of cholesterol in the bilayers which led to increasing membrane-stabilizing ability and dramatically reduced the leakage of encapsulated drug.

Also, **Table 4** demonstrated the vesicle size changes occurred on celecoxib niosomes formulations, $F_2$ and $F_{10}$, after 30, 60 and 90 days storage compared to their size at the initial time of the study. The results showed an increase in the vesicle sizes of the stored niosomal formulations, especially after 90 days. In case of $F_2$, the vesicle size increases after 30 days and 60 days, about 11% to 13% increase in vesicle diameters respectively, compared to the vesicle size of freshly prepared formulation. While there were markedly increase in the vesicle size after 90 days reached more than 31% from the initial value. The same results were observed in the other formulation $F_{10}$, where the vesicle sizes increased about 13%, 18% and reached 23% after 30, 60 and 90 days, respectively. These changes in vesicle size after storage might be due to slight fusion and aggregation of the niosomes after storage.

**TABLE 4: EFFECT OF STORAGE AT (2 – 8 ºC) ON MEAN DRUG RETAINED AND MEAN VESICLE SIZE OFTHE SELECTED TWO CELECOXIB NIOSOMES FORMULATIONS, $F_2$ AND $F_{10}$**

| Time (Days) | $F_2$ | | $F_{10}$ | |
|---|---|---|---|---|
| | Drug retained* (% ± S.D.) | Mean vesicle size (nm) | Drug retained* (% ± S.D.) | Mean vesicle size (nm) |
| Initial | 100 ± 0.00 | 1125 | 100 ± 0.23 | 1021 |
| 30 | 95.9 ± 1.06 | 1253 | 96.2 ± 2.45 | 1161 |
| 60 | 95.2 ± 3.34 | 1278 | 96.1 ± 2.84 | 1205 |
| 90 | 93.6 ± 4.82 | 1477 | 91.6 ± 6.02 | 1248 |

*Mean of 3 batches

***Ex-vivo* Permeation Studies for Celecoxib from Niosomal Carbopol Gel Base:** Niosomal formulations incorporated in gel, $F_2$ and $F_{10}$, enhanced celecoxib skin permeation, showing high cumulative amount of celecoxib permeated with relatively slow permeation rate compared to free celecoxib gel. **Fig. 5** revealed that $F_2$ celecoxib niosomal gel, exhibited the highest cumulative percentage permeation of the drug, reached 75%, with permeation rate (0.306 mg/hr) followed by $F_{10}$ celecoxib niosomal gel, with 60.55% after 24 h. Celecoxib gel, free from niosomes, showed a relatively slow permeation rate (0.182 mg/hr) and lower cumulative percentage permeation of the drug through the skin reached, only 47%, after 24 h.

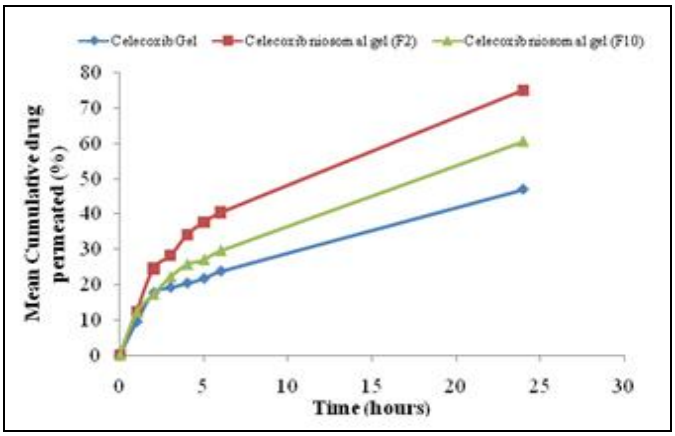



**FIG. 5: PERMEATION PROFILE OF CELECOXIB FROM CARBOPOL GEL, (F2) NIOSOMAL GEL AND (F10) DRUG-NIOSOMAL GEL THROUGH RAT SKIN**

Both non-ionic surfactants and phospholipids in niosomes can act as penetration enhancers, which are useful for increasing the permeation of many drugs. One of the most probable reasons for niosomes to enhance the permeability of drugs is structure modification of stratum corneum. It has been reported that the intercellular lipid barrier in stratum corneum would be dramatically changed to be more loose and permeable by treatment with liposomes and niosomes [24].

**Evaluation of Biological Activity of Celecoxib Niosomal Gel by Carrageenan-Induced Paw Edema:** The *in-vivo* performance of selected celecoxib niosomal formulations was assessed by measuring the reduction of edema induced by carrageenan in rat paw and comparing their anti-inflammatory activity with celecoxib Carbopol gel. Data presented in **Fig. 6** showed that transdermal application of celecoxib-Carbopol gel exhibited rapid onset of action resulted in a significant decrement in the paw volume by 48%, 49% and 26% at 3, 4, 6 h, respectively as compared to the placebo group (control), however its effect was diminished at 24 h to reach 3% edema reduction as compared to the control groups.

Regarding the effect of celecoxib niosomal gel formulations, data represented in **Fig. 6** showed that transdermal application of celecoxib present in

the selective two niosomal gel formulations; $F_2$ and $F_{10}$, resulted in a significant decrease in the paw volume, which was not rapid but the action was maintained for prolonged period of time, by 27%, 35% 47% and 37% at 3, 4, 6 and 24 h, respectively for $F_2$ gel as compared to the placebo control group. While the paw volume was decreased by 33%, 34%, 42% and 39% at 3, 4, 6 and 24 h, respectively for $F_{10}$ gel.

Statistical analysis of the data by one way ANOVA test ($P<0.05$) revealed a significant difference between the control group and the treated groups at 3, 4 and 6 h. After 24 h there was a significant difference at ($P<0.05$) between the control group and the 2 groups treated transdermally with celecoxib niosomal gel only while there was no significant difference between the control group and the group treated with celecoxib-Cabopol gel.

In conclusion, the results of the present study revealed that the anti-inflammatory efficacy of the celecoxib niosomal gel groups exhibited better sustained anti-inflammatory effect as compared to the celecoxib-Carbopol group. These results are in accordance with what was reported previously, about the depot characteristics of niosomal gel formulations which offered easy drug release from niosomes in a sustained manner for a longer period of time, reached at least 24 h, for edema reduction

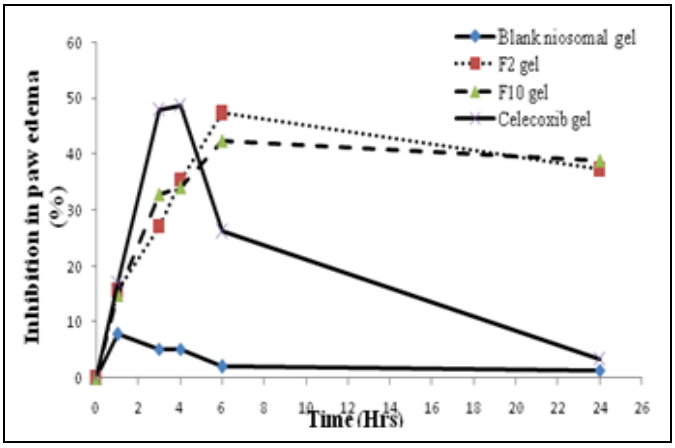



**FIG. 6: INHIBITION OF PAW EDEMA (%) CAUSED BY APPLICATION OF DIFFERENT FORMULATIONS; BLANK NIOSOMAL GEL, CELECOXIB NIOSOMAL GEL AND CELECOXIB-CARBOPOL GEL USING CARRAGEENAN-INDUCED PAW EDEMA**.

**CONCLUSION:** In this study, celecoxib niosomes formulations were prepared, characterized and evaluated. All the formulations showed good entrapment efficiencies ranged from 64.43 to 96.79%, in which the neutral niosomes exhibited the highest percentages of entrapment followed by negatively charged celecoxib niosomes, while the positively charged celecoxib niosomes formulations showed the least entrapment efficiencies. Zeta-potential determinations revealed a good stability indicator for neutral and negatively charged niosomes while positively charged niosomes gives low zeta potential values and result in aggregation of the particles indicating low stability of the system. Vesicle size determination using dynamic light scattering (DLS) depicted that the size of celecoxib niosomes ranged from 132 to 826 nm. Drug released occurred in two phases, a controlled release phase that lasted for 8 h, characterized by a relatively fast drug release rate, more than 50% of the entrapped celecoxib could be released, followed by a steady phase with a reduced and slow-release rate that was maintained for 48 hours, and showed more than 90 % drug released. The results also point to sustained release characteristics with a Higuchi pattern of drug release. Physical stability study was conducted on two celecoxib niosomal formulations, $F_2$ and $F_{10}$, on their suspension formulation, showed good stability characteristics, upon storage.

*In-vivo* results of this study revealed that the anti-inflammatory efficacy of the celecoxib niosomal gel showed a better sustained anti-inflammatory effect as compared to the celecoxib-Carbopol gel. These results confirmed the depot characteristics of niosomal gel formulations which offered easy drug release from niosomes in a sustained manner for a longer period of time, reached at least 24 h, for edema reduction.

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**CONFLICT OF INTEREST:** Nil

**REFERENCES:**

1. Gannu PK and Rajeshwarrao P: Nonionic surfactant vesicular systems for effective drug delivery an overview. Acta Pharmacol. Sin 2011; 1(4): 208-19.

2. Ryan GB and Majno G: Acute inflammation. A review. Am J Pathol 1977; 86(1): 183-76.
3. Firestein GS:Evolving concepts of rheumatoid arthritis. Nature 2003; 423: 356-61.
4. Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B, Maziasz TJ, Cook CS and Karim A: Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. J Pharmacol Exp Ther 2001; 297(2): 638-45.
5. Hinz B and Brune K: Cyclooxygenase-2--10 years later. J Pharmacol Exp Ther 2002; 300(2): 367-75.
6. Primo FT and Froehlich PE: Celecoxib Identification Methods. Acta Farm Bonaerense 2005; 24(3): 421-25.
7. Baillie AJ, Florence AT, Hume LR, Muirhead GT and Rogerson A: The preparation and properties of niosomes--non-ionic surfactant vesicles. J Pharm Pharmacol 1985; 37(12): 863-68.
8. El-Ridy MS, Abdelbary A, Essam T, El-Salam RM and Kassem AA: Niosomes as a potential drug delivery system for increasing the efficacy and safety of nystatin. Drug Dev Ind Pharm 2011; 37(12): 1491-08.
9. Juliano RL and Stamp D: Pharmacokinetics of liposome-encapsulated anti-tumor drugs. Studies with vinblastine, actinomycin D, cytosine arabinoside, and daunomycin. Biochem Pharmacol 1978; 27(1): 21-27.
10. Seedher N and Bhatia S: Solubility enhancement of Cox-2 inhibitors using various solvent systems. AAPS Pharm Sci Tech 2003; 4(3): E33 1-9.
11. El-Nabarawi MA, Bendas ER, El Rehem RT and Abary MY: Transdermal drug delivery of paroxetine through lipid-vesicular formulation to augment its bioavailability. Int J Pharm 2013; 443(1-2): 307-17.
12. Winter CA, Risley EA and Nuss GW: Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 544-47.
13. Ruckmani K and Sankar V:Formulation and optimization of Zidovudine niosomes. AAPS Pharm Sci Tech 2010; 11(3): 1119-27.
14. Confalonieri EO, Soraci AL, Becaluba M, Denzoin L, Rodriguez E, Riccio B and Tapia O: The disposition of free and niosomally encapsulated Rac-flurbiprofen in dairy bovines. J Vet Pharmacol Ther 2010; 33(1): 9-14.
15. Albertini G, Bossi G, Dubini B, Ponzi Bossi MG and Rustichelli F: Structural modifications in the liquid crystalline $L_\alpha$ phase of distearoyl-choline liposomes induced by variable cholesterol concentration. Mol Cryst Liq Cryst 1990; 179: 383-04.
16. Nasr M, Mansour S, Mortada ND and Elshamy AA: Vesicular aceclofenac systems: a comparative study between liposomes and niosomes. J Microencapsul 2008; 25(7): 499-12.
17. Hanaor DAH, Michelazzi M, Leonelli C and Sorrell CC: The effects of carboxylic acids on the aqueous dispersion and electrophoretic deposition of $ZrO_2$. J Eur Ceram Soc 2012; 32(1): 235-44.
18. Varshosaz J, Pardakhty A, Hajhashemi VI and Najafabadi AR: Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. Drug Deliv 2003; 10(4): 251-62.
19. Balakrishnan P, Shanmugam S, Lee WS, Lee WM, Kim JO, Oh DH, Kim DD, Kim JS, Yoo BK, Choi HG, Woo JS and Yong CS: Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery. Int J Pharm 2009; 377(1-2): 1-8.
20. Khazaeli P, Pardakhty A and Shoorabi H: Caffeine-loaded niosomes: characterization and *in-vitro* release studies. Drug Deliv 2007; 14(7): 447-52.
21. Azeem A, Anwer MK and Talegaonkar S: Niosomes in sustained and targeted drug delivery: some recent advances. J Drug Target 2009; 17(9): 671-89.
22. Cocera M, Lopez O, Coderch L, Parra JL and De la Maza A: Permeability investigations of phospholipids liposomes by adding cholesterol. Colloids Surf A: Physicochem Eng Asp 2003; 221: 9-17.
23. Barry BW: Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001; 14(2): 101-14.
24. Kaur K, Jain S, Sapra B and Tiwary AK: Niosomal gel for site-specific sustained delivery of anti-arthritic drug: *in-vitro-in vivo* evaluation. Curr Drug Deliv 2007; 4(4): 276-82.


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