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FORMULATION AND EVALUATION OF CELECOXIB –LOADED NANOSIZED EMULSION AS TRANSDERMAL DRUG DELIVERY VEHICLE

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ABSTRACT

Celecoxib, a selective cyclo- oxygenase – 2 inhibitor has been recommended orally for the treatment of arthritis and osteoarthritis. Long term oral administration of celecoxib produces serious gastrointestinal side effects. Therefore the aim of the present investigation was to assess the potential of nanosized emulsion formulations for transdermal delivery of celecoxib (CXB), to reduce the side effects produced by oral administration of the drug, to increase the stability and to produce a novel controlled delivery system with better pharmaceutical and therapeutic properties. Optimized oil in water nanosized emulsion of celecoxib was prepared by ultra sonication method. The prepared nano- sized emulsion was subjected to physical characterization, Drug content analysis and stability tests. Skin permeation mechanism of celecoxib from nanosized emulsion was evaluated by *In-vitro* skin permeation studies, activation energy measurement and histopathological examination. The anti-inflammatory effects of formulations were compared with marketed formulation by carrageenan-induced paw edema in rats. The nanosized emulsions showed acceptable physical properties and exhibited slow drug release. Photomicrograph of skin sample supports the *in-vitro* skin permeation data of Celecoxib. The Results revealed that the nanosized emulsion was able to cross the skin and produce expected Anti inflammatory and Analgesic effect. These results suggested that nanosized emulsions can be used as potential vehicles for improved transdermal delivery of Celecoxib.

INTRODUCTION: Non steroidal anti-inflammatory (NSAIDs) are the most commonly used drugs to reduce pain and inflammation [1]. Celecoxib (CXB), a selective cyclo-oxygenase-2 (Cox-2) inhibitor, has been recommended orally for the treatment of arthritis and osteoarthritis. It is marketed as capsule form of 100and 200mg which possess poor solubility, stability and bioavailability. Long-term oral administration of CXB causes serious gastrointestinal side effects [2].Therefore, an improved CXB formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of body like bones, ligaments etc. One of the most promising techniques for enhancement of transdermal permeation of drugs is the micro emulsion (or) nanoemulsion technique [3, 4]. Nano emulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and co surfactant molecules having droplet size less than 100 nm [5, 6].This paper describes the potential of the nanosized emulsion system in transdermal delivery of CXB using pharmaceutically acceptable ingredients. The formulation of Celecoxib nanosized emulsion was aimed to reduce the side effects of marketed capsule dosage form, to improve the stability and to produce a controlled drug delivery system.

MATERIALS AND METHODS:

Materials: Celecoxib (CXB) was a kind gift from micro labs pharmaceuticals, Hosur (India). Chitosan and Polaxomer were gift samples from orchid chemical Healthcare, Chennai (India), Castor oil and coconut oil were procured from commercial sources. Acetic acid and acetonitrile were procured from Ranbaxy fine chemicals limited, New Delhi (India). Chloroform was procured from Reachem Laboratory Chemicals Pvt. Ltd., Chennai (India). All other chemicals used in the study were of analytical reagent grade.

Celecoxib Solubility in Single Oil (Or) Oil Mixtures: 5 ml of the selected single oil (castor oil, coconut oil, sesame oil and arachis oil) or the selected oil mixtures in 3:2 ratio (castor oil : sesame oil, castor oil: coconut oil and sesame oil: arachis oil) were taken in a 50 ml beaker. To this 25 mg of celecoxib was added slowly into the beaker with constant stirring. The procedure was repeated until traces of drug molecules remain un- dissolved at the bottom of the beaker. In some cases, the drug-oil mixtures were heated to attain a saturated solution. The experiment was repeated three times with each one of the oil or oil mixtures. The reported value was the mean of the three determinations [7, 8].

PREPARATION OF NANOSIZED EMULSION:

Table 1: COMPOSITIONS OF NANOSIZED EMULSION F-I AND F-II

INGREDIENTS	FORMULATION-I (60 ml)	FORMULATION-II (60 ml)
Celecoxib	150mg	250mg
Castor Oil	2.5ml	2.5ml
Coconut Oil	2.5ml	2.5ml
Chitosan	200mg	200mg
Polaxomer	350mg	350mg
0.05 M Acetic Acid	5ml	5ml
Double Distilled Water	50ml	50ml

Castor oil and coconut oil were taken in a beaker and heated up to 70^0C. Similarly chitosan was taken in a beaker and to this 0.05M acetic acid was added and heated up to 70^0C. Separately Polaxomer was taken in a beaker which contains specified amount of

distilled water and heated up to 70^0C. Celecoxib was added to the oil phase and this mixture was added to the chitosan solution and then the Polaxomer solution was added to the above and stirred well. The temperature was maintained at 70^0C. Then the mixture was sonicated for 3-5 min at 300^0V. The nanosized emulsion was collected immediately in cool water after sonication [9]. (Refer Table 1).

Drug Content Analysis: 1 ml of nanosized emulsion was dissolved with 3 ml of acetonitrile in a 100ml standard volumetric flask and make up to 100 ml with chloroform. From this 2ml of sample was taken in a 10ml standard volumetric flask and chloroform was added to make up to 10 ml. The absorbance of the resulting solution was measured by UV spectrophotometer at 255 nm [9].

Droplet Size Analysis: Droplet size distribution of optimized nanosized emulsion was determined by photon correlation spectroscopy, using a zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was monitored at 25^0C at a scattering angle of 90^0. A solid-state laser diode was used as light source. The nanosized emulsion sample was suitably diluted with distilled water and filtered through 0.22 µm membrane filter to eliminate multiscattering phenomena. The diluted sample was then placed in quartz couvette and subjected to droplet size analysis [10].

Surface/Interface- Charge Measurement (Zeta Potential): Zeta potential particle mobility of nanosized emulsion samples were calculated in a Zeta Potential analyzer (Zeta plus, Brookhaven Instruments Corp., Holtsville, NY). Few ml of emulsion formulation was diluted with nano- pure deionized water and then filled in conductivity cell fitted with gold electrodes. [11]

Stability Assessment of Nanosized Emulsion: The Celecoxib nanosized emulsion was stored at 24ºC, 60% RH and 37 ºC, 75% RH in air tight glass bottle for 45days as a short term study. The samples were taken at the end of 45days [12].The samples were evaluated for physical characteristics including appearance, odor, sedimentation, re- dispersibility and drug content, *invitro* skin permeation studies.

Ex-Vivo Drug Release Studies:

Preparation of Full Thickness Rat Skin: Male Wistar rats were sacrificed with prolonged ether anesthesia and the abdominal skin of each rat was excised. Hairs on the skin of animals were removed with electrical clipper, subcutaneous tissues were surgically removed and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. The skin was then washed with distilled water, wrapped in aluminium foil and stored in a deep freezer at -20°C till further use.

Preparation of Epidermis and Stratum Corneum: The skin was treated with 1 M sodium bromide solution in distilled water for four hours. The epidermis from full thickness skin was separated using cotton swab, which was moistened with water. Epidermal sheet was cleaned by washing with distilled water and dried under vacuum and examined for cuts or holes if any. Stratum corneum (SC) samples were prepared by floating freshly prepared epidermis membrane on 0.1% trypsin solution for 12 hrs. Then SC sheets are cleaned by washing with distilled water.

Determination of Activation Energy: In vitro skin permeation study of CXB across rat skin

was carried out at, 37 °C in the methanolic PBS of pH 7.4 (30:70). The studies were performed on a modified Keshary- Chien diffusion cell with an effective diffusional area of 4.76 cm2 and 35 ml of receiver chamber capacity. In the donor compartment, 1 ml of Nano emulsion formulation was taken. Receiver compartment was composed of the vehicle (methanolic PBS pH 7.4). Samples are withdrawn at regular intervals (2, 4, 6, 8 and 12 hours) and filtered through 0.45 µm membrane filter and analyzed for percentage drug release by UV spectrophotometer at 255 nm.

Permeability coefficients were calculated at the above said temperature and activation energy of CXB was determined from Arrhenius relationship given as follows [10, 13, 14].

$$P = P_o e^{-Ea/RT}$$

Or

$$\text{Log } P = Ea/2.303\ RT + \log P_o$$

Where, Ea is the activation energy, R is gas constant (1. 987 kcal/mol), T is absolute temperature in K, P is the permeability coefficient, and Po is the Arrhenius factor.

Histopathological Examination of Skin Specimens: Abdominal skins of Wistar rats were treated with optimized celecoxib nanosized emulsion (F-II) in methanolic phosphate buffer pH 7.4. After 24 hours, the rats were sacrificed and the skin samples were taken from treated and untreated (control) areas. Each specimen was stored in 10% formalin solution in methanolic PBS pH 7.4. The specimens were cut into section vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. These samples were then observed under light microscope and compared with control sample. In each skin sample, three different sites (epidermis, dermis and subcutaneous fat layer) were scanned and evaluated for mechanism of skin permeation enhancement [10, 15].

Anti Inflammatory Studies: The anti-inflammatory and sustaining actions of the optimized formulations were evaluated by the carrageenan-induced hind paw edema method in Wistar rats. Young male Wistar rats, weighing 180–220 g, were randomly divided into 4 groups for control, F-I, F-II and marketed formulation, (F-III) each containing 6 rats. The animals were kept under standard laboratory conditions, temperature at 25 ± 1 °C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, six per cage, with free access to standard laboratory diet (Lipton Feed) and water *ad libitum.* Doses for the rats were calculated based on the mass of the animals according to the surface area ratio. The abdominal region of the rats was shaved 12 h before starting the experiments, except in the control group. Celecoxib formulations F-I, F-II, and F-III were applied on the shaved abdominal region of all animals (except in the control group) half an hour before sub planter injection of carrageenan into right paws. Paw edema was induced by injecting 0.1 mL of 1% (w\v) homogeneous suspension of carrageenan in distilled water. The paw volume was measured at 0, 1, 2, 4, 6 and 24 h after injection using a digital Plethysmometer. The amount of paw swelling was determined from time to time and expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema produced by each formulation treated group was calculated against the respective control group [8, 16].

Study of Analgesic Effect on Rat using Tail Flick Method: Weigh and number the rat. Take basal reaction time to radiant heat by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (Flicking response) is taken as the end point. Normally a rat withdraws its tail within 3-5 sec. A cut off period of 10-12 sec is observed to prevent damage to the tail .Any animal failing to withdraw its tail in 3-5 sec is rejected from the study. Take at least 3-5 basal reaction times for each rat at a gap of 5 minutes to confirm normal behavior of the animal. Celecoxib formulations F-II and F-III were applied transdermally (except in the control group) and the reaction time at 15, 30, 60 and 120 minutes were noted. As the reaction time reaches 10 sec it is considered as maximum analgesia and the tail is removed from the source of heat to avoid tissue damage. Calculate percentage increase in reaction time (index of analgesia) at each time interval [17].

RESULTS AND DISCUSSION:

Drug Solubility in Oil/Oil Mixture: Solubility of Celecoxib was tested in different oils and oil mixtures. The solubility of Celecoxib was found to be more in castor oil (200mg/ml) and least in Arachis oil (20mg/ml).Similarly the solubility of Celecoxib was found to be more in castor oil (3ml) + coconut oil (2ml) mixture (15mg/ml) and less in sesame oil (3 ml)+ Arachis oil (2 ml) mixture (10mg/ml). Based on the report castor oil and coconut oil were selected for the formulation of nanosized emulsion.

Drug Content Analysis: Formulation -I and formulation-II was tested for drug content uniformity. Formulation-I contains 150mg of drug and the drug content was found to be 146.4mg. Similarly Formulation -II contains 250mg of drug and the drug content was found to be 248.0mg. The standard deviations among the values were found to be small. This indicates that the drug is distributed almost uniformly through out in the formulations of nanosized emulsion. (Refer table 2).

TABLE 2: DRUG CONTENT ANALYSIS

BATCH CODE	ABSORBANCE	DRUG CONTENT (MG)
Formulation-I	0.062	146.4±0.125
Formulation-II	0.105	248.0±0.111

All the values are expressed as mean ± standard deviation n =3

Physical Characterization of Nanosized Emulsion: The droplet size of the nanosized emulsion was found to be 200± 26 nm. All the droplets were found in nanometer range which indicated the suitability of formulation for Transdermal drug delivery. Particles with zeta potentials more positive than +30mVand more negative than -30mV are normally considered stable. The nanosized emulsion demonstrated a greater zeta potential +40.02 ± 1.20mV (increasing positive charge), which indicates the dispersion stability.

Stability Assessment of Nanosized Emulsion: Stability testing for Celecoxib nanosized emulsion was performed. The nanosized emulsions were subjected to stability study for 45days in a stability chamber. No significant changes in physical characteristics including appearance, odor, sedimentation and re-dispersibility of emulsions were found. In control, Formulation-I and Formulation-II, no floccules were observed. This may be due to the absence of agglomeration and high surface charge measurement. In the control sample, no creaming was observed in the disperse medium under the influence of

gravitational force. But in the samples formulation-I and formulation-II, creaming was observed in the disperse medium under the influence of gravitational force. No coalescence and breaking were found in the control, Formulation-I, and Formulation-II. Short term stability studies on Celecoxib loaded nanosized formulations indicated that there are no significant changes in drug content and drug release profiles.

Ex-Vivo Drug Release Studies: The Celecoxib release from nanosized emulsion formulation-I, formulation-II and marketed formulation-III was studied in phosphate buffer pH 7.4. The drug release from the Formulation-I, Formulation-II and marketed formulation-III varied with respect to time. (Refer Table 3).

TABLE 3: COMPARATIVE EX-VIVO DRUG RELEASE DATAS OF FORMULATION-I, FORMULATION-II AND MARKETED FORMULATION-III

Time in hours	Percentage Drug release (F-I)	Percentage Drug release (F-II)	Percentage drug release Marketed formulation (F-III)
0	0	0	0
2	24.48	25.51	33.67
4	28.33	34.99	51.67
6	32.78	41.75	61.46
8	36.10	50.38	70.18
10	41.57	57.79	79.05
12	46.28	68.67	89.88

The drug Release from formulation-I, formulation-II, and Marketed formulation-III after 12 hours was found to be 46.28%, 68.67%, and 89.88% respectively. Drug release was found to be more in marketed formulation. Drug release from Formulation-II was more than Formulation-I (Refer Fig. 1).

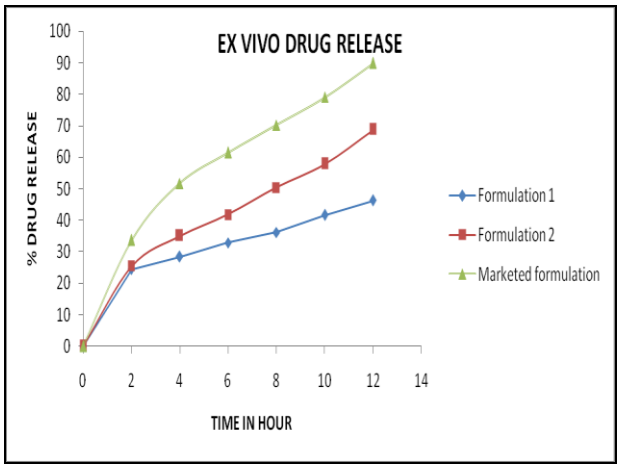

Fig. 1: COMPARATIVE EX-VIVO DRUG RELEASE PROFILES OF FORMULATION-I, FORMULATION-II AND MARKETED FORMULATION-III

These results reveal that the drug release from nanosized emulsion was slower than the marketed product. So the drug release from the nanosized emulsion formulation can be sustained for more than 12 hours.

Activation Energy: Comparison of activation energies of two tested nanosized emulsion formulations with marketed formulation indicates that the nanosized emulsions had higher activation energy than marketed formulation (Refer fig. 2). This means that the emulsions are better penetrated through the skin than marketed formulation.

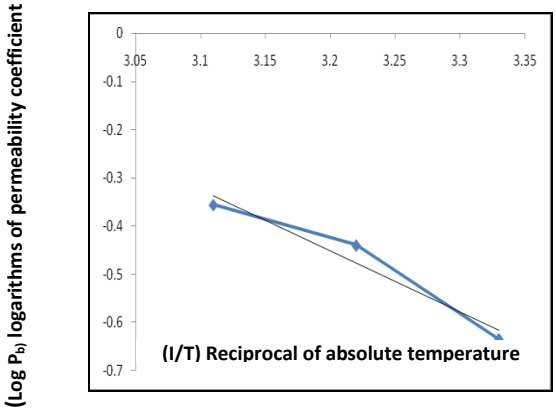

FIG. 2 ARRHENIUS PLOT OF FORMULATION-II PERMEATION ACROSS RAT SKIN

Histopathological Results: The photomicrographs of control (untreated skin) showed normal skin with well defined epidermal and dermal layers. Keratin layer was well formed and lied just adjacent to the top most layer of the epidermis. Dermis was devoid of any inflammatory cells. Skin appendages were within normal limits (fig. 3).

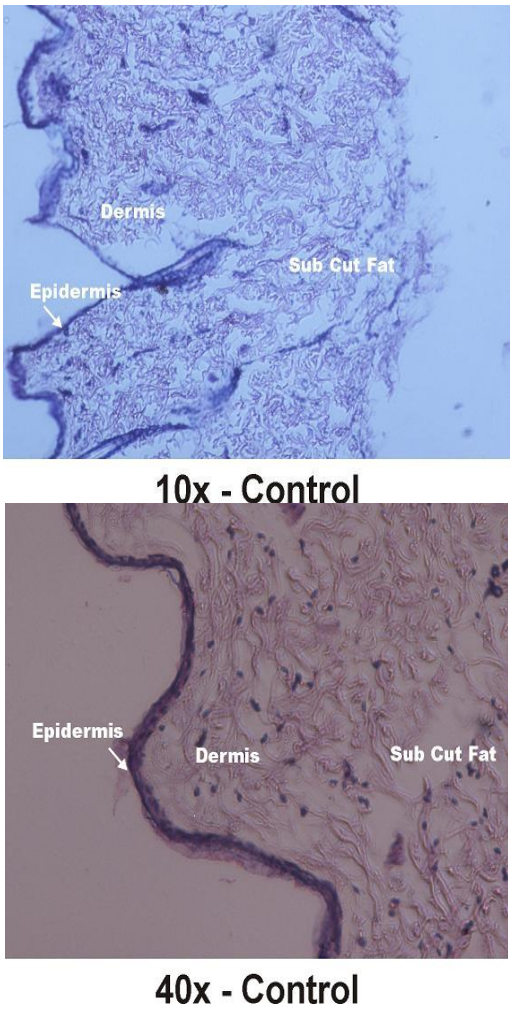

FIG. 3: PHOTOMICROGRAPHS OF SKIN SAMPLE FROM CONTROL GROUP ANIMAL SHOWING NORMAL EPIDERMIS, DERMIS AND SUBCUTANEOUS TISSUES AT LOW POWER VIEW (10X) AND HIGH POWER VIEW(40X)

When the skin was treated with nano sized emulsion formulation (F-II) for 24h, significant changes were observed in the skin morphology. Low power photomicrograph of skin sample showed epidermis with a prominent keratin layer, a normal dermis and subcutaneous tissues. High power photomicrograph of skin sample showed a thickened and reduplicated stratum corneum with up to 8 distinct layers. The epidermis showed increase in its cellular layer to 4-6 cells. Dermis does not show any edema or inflammatory cell infiltration. The disruption of lipid bilayers was clearly evident as distinct voids and empty spaces were visible in the epidermis region (Refer Fig. 4). These observations support the in vitro skin permeation data of C X B.

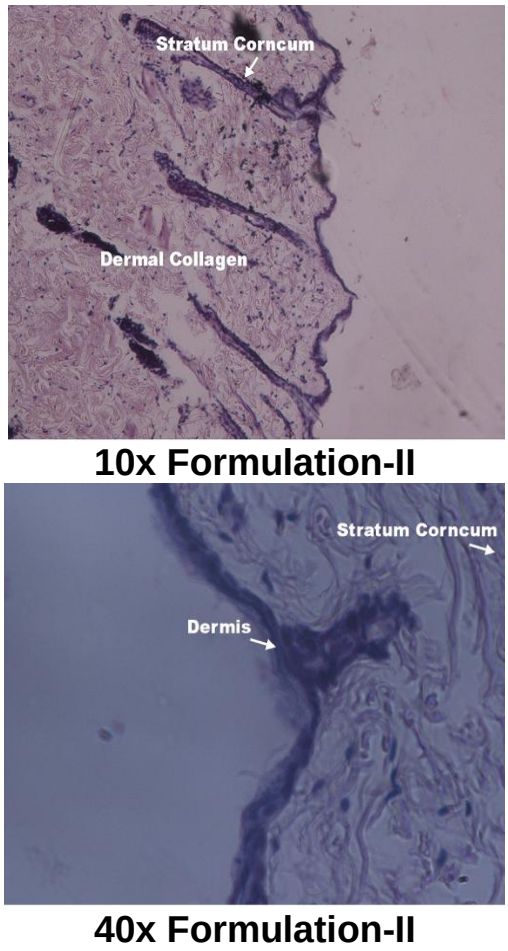

FIG. 4: PHOTOMICROGRAPHS OF SKIN SAMPLE FROM NANOSIZED EMULSION TREATED ANIMAL AT LOW POWER VIEW (10X) AND HIGH POWER VIEW (40X)

Anti-Inflammatory Studies: Anti inflammatory activity of Celecoxib loaded nanosized emulsion was compared with a standard Celecoxib marketed formulation by carrageenan induced paw edema method. Celecoxib nanosized formulations F-I and F-II produced significant Anti inflammatory effect by reducing carrageenan induced paw edema. Formulation-II showed better anti inflammatory activity than Formulation -I (Refer Table 4).

TABLE 4: ANTI INFLAMATORY EFFECTS OF F-I, F-II AND MARKETED FORMULATION (F-III)

FORMULATIONS	1ST HOUR	2ND HOUR	4TH HOUR	6TH HOUR	24TH HOUR
Control	1.53±0.06	1.66±0.05	1.77±0.03	1.82±0.02	1.93±0.03
Formulation -I	1.23±0.08[c]	1.13±0.10[c]	1.03±0.11[c]	0.87±0.09[c]	0.72±0.07[b,c]
Formulation-II	1.21±0.03[c]	1.13±0.04[c]	0.96±0.04[c,]	0.85±0.04[c,]	0.67±0.03[a]
Marketed formulation – III	1.06±0.11[a]	0.94±0.06[a]	0.87±0.04[b]	0.78±0.05[b,c]	0.49±0.01[a]

Values are mean ±SD, n=6; Paw volume was taken for the percentage inhibition calculations.
[c]P<0.05, [b]P<0.01, [a]P<0.001, with respect to control

The slow anti-inflammatory activity of nanosized emulsion compared to marketed formulation may be due to the controlled release of drug from the Celecoxib loaded nanosized emulsion.

Analgesic Activity of Celecoxib: The analgesic effect of Celecoxib nanosized emulsion (F-II) was compared with a standard marketed product (F-III) on rat using tail flick method. Celecoxib nanosized emulsion (F-II) produced significant analgesic effect on rats. (Refer fig. 5).

Study of Analgesic Effect on Rat using Tail Flick Method:

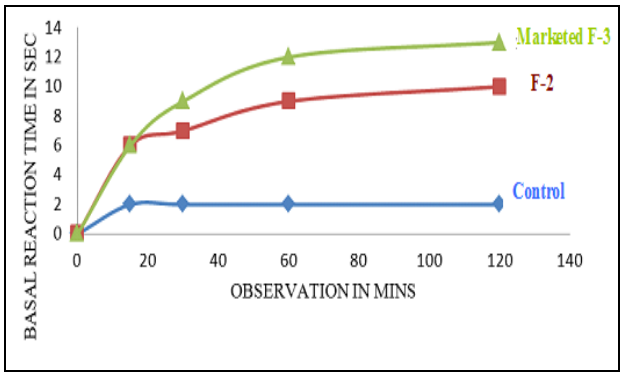

Fig. 5: ANALGESIC EFFECT OF FORMULATION-II AND MARKETED PRODUCT

However the Analgesic effect of nanosized emulsion (F-II) was not superior as compared to Celecoxib marketed formulation. This could be due to controlled release of drug from nanosized emulsion.

CONCLUSION: Celecoxib nanosized emulsion using castor oil and coconut oil along with Polaxomer and Chitosan polymer can be formulated by ultra sonication method. The drug content analysis concluded that the drug was uniformly distributed throughout the formulation. The droplet size of the nanosized emulsion was found to be in nm range which

indicates the suitability of formulation for transdermal drug delivery. The nanosized emulsion demonstrated a greater zeta potential (increasing positive charge) which indicates the dispersion stability. The nanosized emulsion was found to be stable for 45 days at 24^0 C and 37^0 C. *Ex vivo* drug release data reveals that the drug release was slower than the marketed product. Photomicrograph of skin sample supports the *in vitro* skin permeation data of celecoxib. *In vivo* studies showed that the drug loaded nanosized emulsion was able to cross the skin and produce expected anti inflammatory and analgesic effect. These results suggested that the nanosized emulsions can be successfully used to improve the stability and provide a controlled release of drug. The serious gastrointestinal side effects produced by oral administration of celecoxib can be minimized by nanosized emulsions. Thus the nanosized emulsions can be used as potential vehicles for improved transdermal delivery of celecoxib.

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ABBREVIATIONS: CXB = Celecoxib, NSAIDS = Non Steroidal anti inflammatory Drugs, nm = nano meter, RH = Relative humidity, SC = Stratum corneum PBS = Phosphate Buffer Solution.

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