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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation Development and *in Vitro* Evaluation of Nano emulsion Drug Delivery of Diclofenac Sodium

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ABSTRACT

This Paper carried out to develop and evaluate nanoemulsion of diclofenac sodium by using oleic acid for the treatment of inflammation, pain and problems associate with joints, muscles and bones these include rheumatoid arthritis, osteoarthritis and gout. Design of nanoemulsion was applied for topical use of drugs, having the potential to increases of the solubility of poorly water-soluble drugs. To avoid the first pass metabolism and these formulations have the capacity to controle mild dyspepsia, heartburn, ulceration, hemorrhage and adverse effect on g.i.t by reducing the dose frequency due to longer duration of action by given formulation. So, the sustained release of drugs for longer period will improve the patient's compliances. To delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilization capacity and long shelf life. Nanoemulsion was prepared by water triturated and mechanical stirrer method using oleic acid as oil phase, tween-80 as surfactant and polyethylene glycol-400 as co-surfactant. Different oils, surfactants and co-surfactants were screened to select ideal components of nanoemulsions with good solubility and excellent skin penetration of diclofenac sodium. The solubility of diclofenac sodium was highest in oleic acid followed by olive oil, isopropyl myristate and isopropyl palmitate. Nanoemulsion formulation F-5 exhibited 93.5% higher drug content then other formulations. Among all formulations, the highest permeation flux of 95.62 µg/cm²/hour was observed in formulation F-5.

Keywords: *Nanoemulsion, diclofenac sodium, in vitro dissolution studies and drug content.*

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation [1]. Diclofenac sodium, an NSAID, has potential to treat inflammatory disorders like rheumatoid arthritis and osteoarthritis [2, 3]. Formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body, such as bones, ligaments, joints, tendons, and muscles. There has been increased interest during recent years in the use of topical vehicle enhancers and solvents to achieve these goals [4]. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and co surfactant molecules having a droplet size of less than 100 nm [5]. Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties in vitro [6-7]. Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions [10-18]. This article describes the potential of nanoemulsion systems in transdermal delivery of diclofenac sodium using pharmaceutically acceptable ingredients without using additional permeation enhancers, because excipients of nanoemulsions themselves act as permeation enhancers.

MATERIAL AND METHODS

Materials

Diclofenac sodium was gift sample from OAPL Laboratories Himachal Pradesh. Tween 80, polyethylene glycol-400, Oleic acid, cremophor RH 40 and methanol were purchased from CDH (New Delhi, India). All other chemicals were of analytical grade. Freshly distilled water was used throughout the work.

SOLUBILITY OF DICLOFENAC SODIUM

The solubility of diclofenac sodium in various oils (soya bean oil, oleic acid, ethyl oleate and isopropyl palmitate), surfactants (tween-20 and tween- 80, tween-60) and co-surfactants (polyethylene glycol-400 and cremophor RH 40) was determined by dissolving an excess amount of diclofenac sodium in 2 ml of

each of the selected oils, surfactants, and co-surfactants in 5-ml capacity stoppered vials separately. A combination of oils was also used for determination of solubility. An excess amount of diclofenac sodium was added to each 5-ml capacity stoppered vial and mixed using a vortex mixer. The mixture vials were then kept at 37 ± 1.0 C in an isothermal shaker for 72 hours to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was takenandfilteredthrougha0.45-µmmembrane filter. The concentration of diclofenac sodium was determined in each oils, surfactants, co-surfactants in presence of methanol using Spectrophotometer at their respective λ max. Formulations were dissolved in a beaker containing oil, surfactant and co-surfactant.

PREPARATION OF NANOEMULSION

On the basis of the **"water trituration method**" the oil (Oleic acid), drug (Diclofenac sodium), surfactant (Tween 80) and co-surfactant (PEG-400) were selected due to their greater solubility enhancement effect on diclofenac sodium. This mixture was mixed by mechanical stirrer at 4000 rpm, until a transparent preparation was obtained. All the mixture was stored at ambient temperature for further use [19].

Table 1: Formulation Design.

FORMULATIONCODE	DICLOFENAC SODIUM (mg)	OLEIC ACID	TWEEN-80	PEG-400	DISTILLD WATER	FINAL VOLUME
F-1	50	2	1.5	1.5	25	30
F-2	50	2	4.5	4.5	19	30
F-3	50	2	3.5	1.5	23	30
F-4	50	2	5.5	2.5	20	30
F-5	50	2	9.5	4.5	14	30

EVALUATION AND CHARACTERIZATION OF NANOEMULSION FORMULATION

Optical Transparency

Optical transparency of the formulation was determined by inspecting the sample in clear and transparent container under the presence of good light against reflection into the eyes, and viewed against black white illuminated background.

Determination of pH

pH was measured using a pH meter of a glass electrode. pH fundamentally represents the value of hydrogen ion activity in solutions/sample.

Viscosity Measurements

Viscosity is a measure of the resistance of a fluid which is being deformed by either sheer stress or tensile stress. In everyday terms (and for fluids only), viscosity is "thickness" or "internal friction". Viscosity describes a fluids internal resistance to flow and was determined by the Brookfield viscometer.

Droplet Size Measurements

Droplet size analysis was done by Dynamic Light Scattering (DLS). Dynamic light scattering (sometimes referred to as photon correlation spectroscopy or quasielastic light scattering) is a technique for measuring thesize of particles typically in the submicron region. 1 gm of each formulation were diluted with 250 ml of phosphate buffer pH 7.4. The volumetric flasks were inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulations, particle size was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due tothe brownian motion of the droplets as function of time using a Zetasizer Nano series (Malvern Instrument, DTS.Ver.4.10 serial no.MAL 500999). Light scattering was monitored at 25 ºC [5].

Zeta Potential Determination

Zeta potential for nanoemulsion was determined using zetasizer has 3000 (Malvern instrument ltd., UK). 1 gm of each formulations were diluted with MilliQ water up to 50 ml. Zeta potential were measured by using Malvern Zetasizer. Equipped with a 4.0 mW He-Ne red laser (633nm)[19]

Drug Content Studies

1 ml of naoemulsion formulation was transferred into a beaker containing 10 ml methanol. The content of the beaker were stirred for 30 minutes and then kept for 24 hours. After 24 hours the content of beaker were transferred into centrifuged tube and centrifuged at the 3000 rpm for 10 minutes. Supernatant was separated and filtered then 0.1 ml of the supernatant was diluted appropriately with phosphate buffer saline (PBS) pH 7.4 and assayed spectrophotometrically for drug content.

In-Vitro Drug Diffusion Studies

Preparation of Goat skin

Franz diffusion cell is used to obtain the drug release profile of the nanoemulsion formulation in the Selected formulations were further studied for skin permeation using goat ear skin, obtained from the slaughter house after sacrificing the animal within 1 hour. The average thickness of the goat skin

was0.28±0.06 mm and then the hair was removed from the upper portion of skin surface using an animal hair clipper, and, subsequently, full thickness of the skin was harvested. The fatty layer, adhering to the dermis side, was removed by surgical scalpel. Finally, these excised skins were thoroughly rinsed with distilled water and packed in aluminum foils. The skin sample were stored at - 20□C and used within a week.

***In Vitro* skin Permeation Study**

In- vitro permeation study of drug from F-1, F-2, F-3, F-4 and F-5 diclofenac Sodium nanoemulsion formulation was carried out using *Goat skin.* The average thickness of the skin was 0.28±0.06 mm Skins were allowed to hydrate for 1 hour before being mounted on the open ended diffusion with the stratum corneum facing the donor compartment and the dermal side faced the receiver compartment. The receptor compartment was consist of 400ml of phosphate buffer (pH 7.4) in 500ml beaker and its temperature was maintained at 37 ±0.5°C and stirred at 300rpm throughout the experiment. About 1ml diclofenac Sodium nano emulsion was placed in *Goat skin* tied to the one end of open – ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer. Sample were taken from receptor medium at 0, 0.50, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8, 9, 10, 11, 12, 23, 24hrs and replaced immediately with an equal volume of fresh phosphate buffer equilibrated at 37 ±0.5°C. All the samples were analyzed for diclofenac Sodium content at 276nm by UV spectrophotometer. Cumulative amount of drug permeation was calculated from standard calibration curve.

Permeation Study

Apparatus: Open ended diffusion cylinder
Speed: 300rpm
P^H: 7.4
Time: 0.50-24hrs
Temperature: 37□C
λ_{max}: 276nm

RESULT AND DISCUSSION

After the preparation and evaluation of diclofenac sodium nanoemulsion the present investigation showed that its appearance, PH, viscosity, mechanical stress, particle size, in vitro drug release and drug content was found out –Stability of the prepared nanoemulsion formulations was assessed using accelerated temperature study. The drug content, pH, viscosity of the best formulation F-5were subjected to stability studies at 400C/75% RH up to 3 months.

F-5 Sample showed excellent results in these studies. Drug degradation was found to be in the range (93.1, 89.5%, and 87.5%) after three months. Viscosity values after three months compared to the initial viscosity were in the range 0-1cps, while pH changed 6-5 units only. In all cases, F-5 showed the small changes in these parameters. Overall results from the stability studies indicated that the nanoemulsions were chemically stable for three months.

Table 2: Appearance of Formulations

FORMULATIONS	*APPEARANCE*
F-1	Milky
F-2	Milky
F-3	Clear
F-4	Milky
F-5	Clear

Table 3: Comparative pH values of Formulations

FORMULATIONS	*pH*
F-1	5.42±0.02
F-2	5.75±0.03
F-3	4.35±0.06
F-4	5.81±0.03
F-5	6.42±0.02

Table 4: Comparative Viscosity values of Formulations

FORMULATIONS	*VISCOSITY(cps)*
F-1	51.8
F-2	76.8
F-3	91.5
F-4	92.4
F-5	118.2

Table 5: Comparative study of mechanical stress in Formulations

CENTRIFUGATION TIME (min)	*%PHASE SEPARATION*				
	F-1	F-2	F-3	F-4	F-5
10	-	-	-	-	2
30	4	-	-	-	6
60	8	2	-	12	10

Table 6: Comparative in vitro Skin permeation rate of diclofenac sodium nanoemulsions

TIME IN HOURS	*CUMULATIVE % DRUG PERMEATED (µg/cm²)*				
	F-1	F-2	F-3	F-4	F-5
0	0	0	0	0	0
0.50	0.97	1.28	6.00	5.72	9.19
1	2.27	2.91	12.66	11.47	14.22
2	4.52	5.47	18.70	17.22	19.66
4	7.37	9.26	24.36	22.98	25.41
6	10.84	13.08	30.05	28.74	31.44
8	14.31	17.17	35.15	34.49	37.85
10	17.78	21.89	40.49	39.91	44.94
12	21.61	26.93	46.18	44.66	53.64
14	26.07	26.93	52.46	49.69	62.67
16	30.82	32.77	59.06	54.10	72.89
18	36.18	38.15	65.98	59.45	77.30
20	41.83	47.28	73.21	65.42	82.02
22	47.81	57.91	76.71	72.02	82.86
24	54.09	79.29	80.55	78.92	95.62

Table 7: Model fitting of the in vitro Permeation data of various diclofenac sodium nanoemulsion

Formulations	**r² Value**				
	Zero order	**First order**	**Higuchi**	**Korsmeyer poppas**	**Diffusion exponent**
F-1	0.987	0.952	0.952	0.979	0.06
F-2	0.919	0.976	0.987	0.966	0.1
F-3	0.985	0.976	0.996	0.895	0.8
F-4	0.985	0.976	0.996	0.875	0.7
F-5	0.993	0.658	0.963	0.738	0.07

Table 8: Release kinetics of F-5

Time in hrs	**Log Time**	**√Time**	**% Cumulative drug release**	**Log Cumu. %drub release**	**Log %Cumu. of drug remained**
0.50	- 0.3010	0.71	9.19	0.96	0.044
1.00	0.0000	1.00	14.22	1.15	1.01
2.00	0.3010	1.41	19.66	1.2935	1.09
4.00	0.6021	2.00	25.41	1.40	0.14
6.00	0.7782	2.45	31.44	1.4974	1.18
8.00	0.9031	2.83	37.85	1.57	1.19
10.00	1.0000	3.16	44.94	1.65	0.20
12.00	1.0792	3.46	53.64	1.72	1.19
14.00	1.1461	3.74	62.67	1.79	1.17
16.00	1.2041	4.00	72.89	1.86	1.13
18.00	1.2553	4.24	77.30	1.88	1.11
20.00	1.3010	4.47	82.02	1.91	1.09
22.00	1.3424	4.69	87.86	1.94	1.06
24.00	1.3802	4.90	95.62	1.98	1.02

Figure No. 1 F-5 Zero order plot

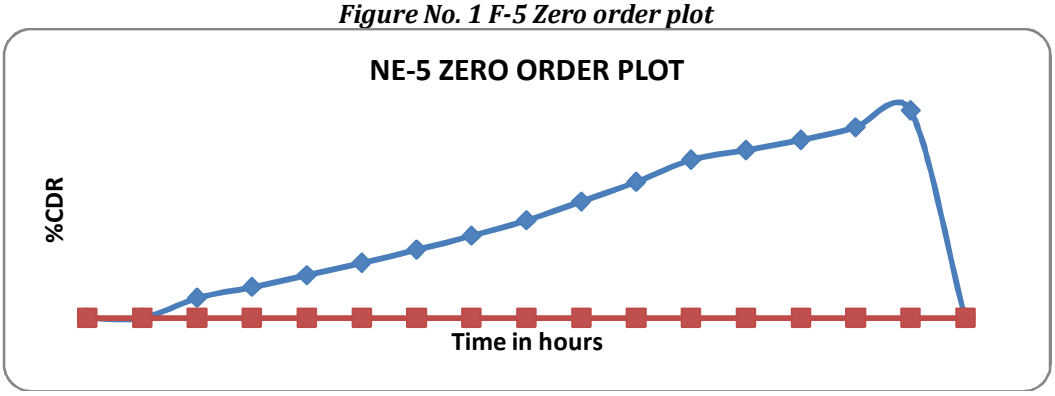

Figure No. 2 F-5 First order plot

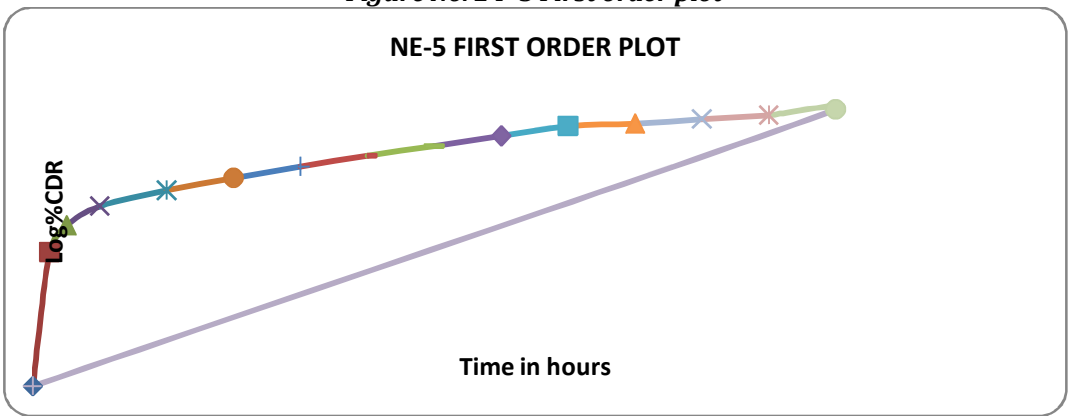

Figure No. 3 F-5 Higuchi plot

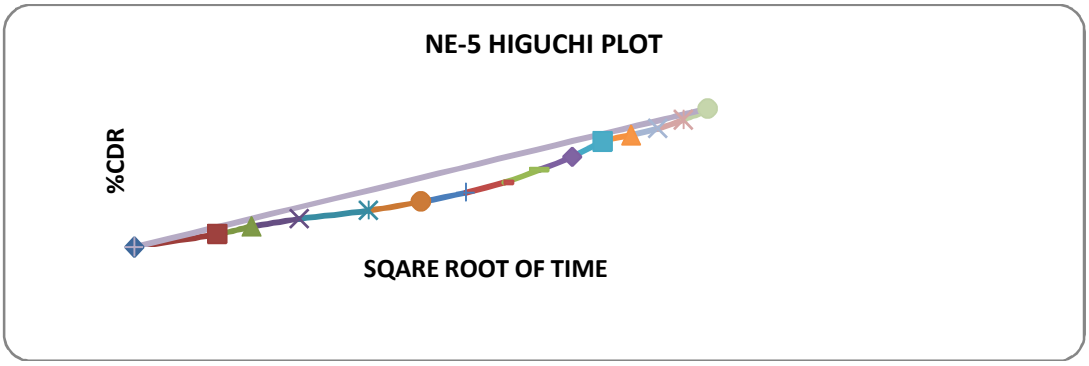

Figure No. 4 F-5 Korsmeyer Pappas plot

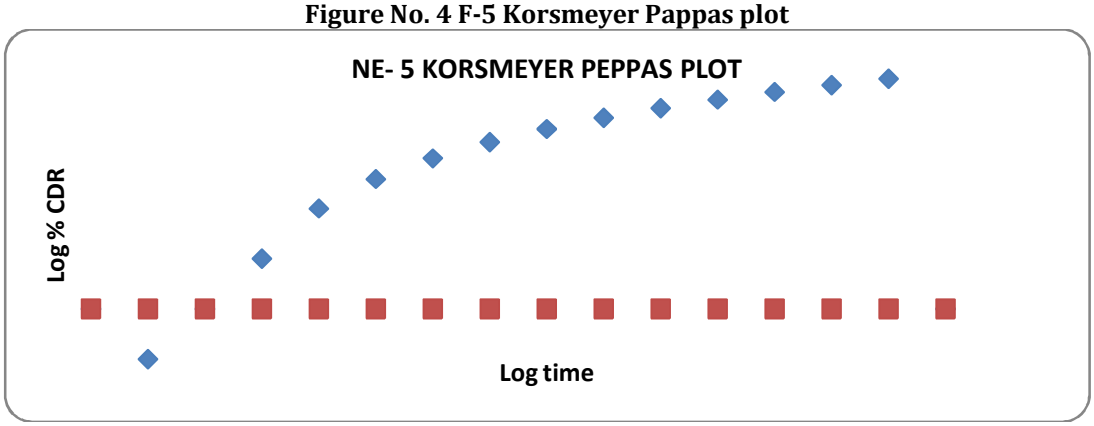

Table 9: Stability Study of Nano emulsion (F-5)

S. No.	Formulation F-5	Before storage	Storage at 40□c		
			1st	2nd	3rd
1	Drug content (%)	93.5	93.1	89.5	87.5
2	PH	6.42±0.02	6.42±0.02	6.42±0.02	6.42±0.02
3	Viscosity(cps)	92.4	92.4	92.4	92.4

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