Abdhesh et al /J. Pharm. Sci. & Res. Vol. 12(4), 2020, 524-532

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Formulation and Evaluation of Nanoemulsion Based Nanoemulgel of Aceclofenac

Abdhesh and Sanjiv Kumar Gupta

Agra Public Institute of Technology and Computer Education, Artoni, Agra – 282007, Uttar Pradesh, India.

Abstract

Aim & objective: The present work aimed to formulation and evaluation of nanoemulsion based nanoemugel of aceclofenac for improving its efficacy, stability, and permeability, bioavailability for the treatment of arthritis.

Methods: O/W Nanoemulsions were prepared using aqueous titration method oleic acid was chosen as the oil phase, Tween 20, ethanol were used as surfactant and cosurfactant respectively, on the basis of solubility studies and emulsification studies in the formulation of nanoemulsion. Pseudoternary phase diagrams were constructed to obtain the nanoemulsion region. Further optimized NE was incorporated into different concentration of Carbopol-940 to get a gel for improving convenience in superficial application of the drug. Drug loaded NE and NEG were characterized for particle size, viscosity, rheological behavior, thermodynamic stability studies.

Results: The optimized formulation was compared to conventional gel formulation and it showed higher permeation rate *in-vitro* and *ex-vivo* which justifies the nanoemulsion gel to be a promising carrier for transdermal delivery of aceclofenac

Conclusion: The optimized formulation showed higher drug release of 90.2%, compared to conventional gel that released 68.2% release in about 9 hrs. The study suggested that nanoemulsion significantly enhanced bioavailability of transdermally applied aceclofenac.

Keywords: - Solubility, Hydrophobic drug, Ternary Phase Diagram, Nanoemulsion, Nanoemulgel

INTRODUCTION

Arthritis is a word that originated from combinatorial of the Greek and Latin, "Arthron" from Greek that means Joints and "Itis" from Latin that means inflammation. Arthritis is a disease related to the joints & joints get inflammation.[1] In case of arthritis, Arthritis is starts suffering due to flawed joints, the motives behind that trigger the disease are may be possible that inadequacy of synovial fluid, cartilage damage, autoimmune infection, infections in bone joints, and infections. Arthritis is multifaceted and the few common experiences and some features are universal in arthritis: Rheumatoid arthritis, Osteo-arthritis, Ankylosing spondylitis, Gout, Juvenile arthritis, Infectious arthritis, Lupus arthritis.[2] Age is capital segment to be considering increase the risk for setting the stage of Osteo-arthritis. An intense is correlation between age and high incidence of justify Osteo-arthritis.[1] Common symptoms of joints affected to arthritis including pain in joints, swelling, inflammation, stiffness and decrease motion and movements range of joints. Symptoms can be mild, severe/moderate and can come and go.

Aceclofenac is a drug of preferential COX-2 inhibitors category. Aceclofenac has been given orally for the treatment of arthritis, rheumatoid arthritis, Osteo-arthritis, gout and ankylosing spondylitis for the relief of inflammation and pain in osteo-arthritis, it is the nonsteroidal antiinflammatory drug (NSAID) and has higher anti-inflammatory and analgesic property Aceclofenac ultra efficient inhibits the enzyme COX (cyclo-oxygenase enzyme) that is involved and responsible in the synthesis of prostaglandins (PGs), Prostacyclins (PGI) and Thromboxanes (TXA). Prostaglandins (PGs) are mediators of inflammation which causes swelling, inflammation and pain but Aceclofenac have some side-effects, ACF can causes bleeding and ulcers in gastrointestinal tract (GIT), when administered via oral route to long duration. Indications of anemia can produces because of blood loss in GIT or bleeding in GIT. Aceclofenac belongs to Biopharmaceutics Classification System (BCS) class II. It displays poor aqueous solubility (High hydrophobic solubility) and high permeability, ACF can penetrate into synovial joints of patients suffering with Osteo-arthritis and related conditions.

In prior studied Nanoemulsion is a compatible tool for transdermal drug delivery and it is defined as 'a dispersion consisting of oil, surfactant, co-surfactant and aqueous phase', nanoemulsion is a single optically isotropic and thermodynamically stable liquid vehicle with the diameter of a droplet usually in between the range of 50 and 200 nm. On account of, the low thickness of Nanoemulsions independent, Application of it in transdermal conveyance because of the cumbersome condition employments.

Recently, it have emerged as the more attractive topical drug delivery system (TDDS) and it has dual release control system, one is the nanoemulsion and second is Hydrogel. Nanoemulsions are having droplet in nano-size (10-100 nm), it rapidly penetrates and deliver active substances deeply and quickly. The use of nanoemulgels can be considered well for analgesic (NSAIDs) and anti-fungal drugs. Nanoemulgel is the addition of the nanoemulsion system incorporated into gel matrix which enhances the better skin permeation. Nanoemulgel (NEG) fills in as supply of medication and improving the arrival of medication through inward stage to external stage likewise forward. Nanoemulgel when contact to the skin, it discharges the oil beads from the nanoemulgel and these oil beads enter into the Stratum Corneum of the skin and discharge the medication at proposed site. Nanoemulgel has brilliant bond property with high solubilizing of the medication (API) in the oil stage; nanoemulgel prompts more focus inclination towards the skin that further

increment skin entrance of medication into the skin. it displays the improved properties of non-oily, spreadable, thixotropic, easily, simple to evacuate and longer time span of usability.

MATERIALS AND METHODS

Materials

Aceclofenac was a kind gift sample from Trends Remeice, Pvt. Ltd. Other chemicals like Oleic acid, Iso-propyl myristate, Ethanol, Propylene glycol, Tween 20 (polyoxyethylene sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monqoleate), Olive oil, Castor oil and ethanol were from CDH, Mumbai, India Carbopol 934 and Carbopol940 Qualickems Fine Chem Pvt. Ltd and Transcutol P (Monoethyl ether of diethylene glycol) was from CDH, Mumbai, India. Distilled water was used throughout the study and all other reagents used were of analytical grade.

METHODS

Screening of Excipients by Solubility studies

The solvency of Aceclofenac in different oils (Liquid paraffin, Isopropyl myristate, olive oil, oleic corrosive), surfactants (Tween-80, Tween-20, and range80), and cosurfactants (propylene glycol, ethanol and PEG-400) was controlled by dissolving an abundance measure of Aceclofenac in 2 ml of the choose oils, surfactants, and cosurfactants in 5-ml limit plug vials independently. An abundance measure of Aceclofenac was added to every 5-mL-limit plug vial and blended utilizing a vortex blender. The blend vials were then kept at 37°C ± 1.0°C in the isothermal shaker (Nirmal International, Delhi) for 72 hrs to get to balance. The equilibrated tests were expelled from shaker and centrifuged up to 15 minutes at 3000 rpm. The supernatant fluid was taken out and separated through a 0.45-µm layer channel. The grouping of Aceclofenac was resolved in each oil, surfactant and co-surfactant by UV spectrophotometer at its individual λ_{max}=276nm.

PREPARATION OF NANOEMULSION

Prepare the nanoemulsions to the selected formulations from the construction of pseudo ternary phase diagram and by using sonication technique by the ultra sonicator.

PREPARATION OF NANOEMULGEL FORMULATION

Various polymers (carbopol 934, carbopol 940, HPMC 5-CPS, sodium alginate) of concentration 1%w/v were initially used for the preparation of nanoemulgels. The nanoemulgels were formulated and checked for their physical appearance.

Nanoemulsion base gels are set up by fuse of 1 g of Carbopol 940 specialist in an adequate amount of water. This gelling operator arrangement is place under dim conditions for 25 hours until finishes welling framework acquired. At that point the medication stacked nanoemulsion is gradually added to the thick arrangement of gelling specialist under attractive stirring.The pH is balanced out by the option of 0.1 ml of triethanolamine (TEA).The framed nanoemulgels are kept for 24 h to acquire a homogeneous scattering of gel.

RESULTS AND DISCUSSION

Screening of various components by Solubility Studies: - As described in table 1, the solubility of ACF was found to be highest in Oleic acid as compared to other oils. Hence, Oleic acid was selected as the oil phase. The drug was found to be more soluble in Tween®20 among surfactants and in ethanol among Co-surfactants. Oleic acid, tween 20 and ethanol were selected as oil, surfactant and co-surfactant respectively on the basis of solubility data.The concentration of Acelofenac in various excipients at 37°C was determined by UV spectrophotometer solubility results presented Table-1.

Table: - 1 Solubility studies of Aceclofenac in various excipients at 25°C

Sr. No.	Solvents	Solubility (mg/ml)
1	Oleic acid	52.01±1.77
2	Olive oil	08.25±0.45
3	Sunflower oil	05.25±1.52
4	Castor oil	08.35±0.84
5	Liquid Paraffin	04.19±1.78
6	Isopropyl myristate	17.22±0.25
7	Ethanol	84.56±1.43
8	Tween 80	40.80±1.02
9	Tween 20	51.23±2.73
10	Propylene glycol	22.40±1.05
11	Polyethylene glycol	41.92±2.35

Different combinations of oil, surfactants and co-surfactants: - Based on the results of solubility studies, following five potential different combinations(1:1 ,1:2 ,1:3,2:1 and 3:1) of oil, surfactants and co-surfactants with different Smix ratio were used for further study.

Thermodynamic stability studies and Visual observationsof combination I-V: - In case of combination I,S_{mix} ratio 1:1 the nanoemulsions passes the thermodynamic stability test and some formulations in visual observations lies in A category and some are in C category,small)nanoemulsion formulations were obtained as compared to combination I. In S_{mix} ratio 1:2 there was small increase in nanoemulsion formulations as compared to 1:1. As co-surfactant concentration was increased from 1:1 to 1:3, thermodynamic stability test passes amount of oil that could be solubilized increased the nanoemulsion formulations increased. Similarly, when surfactant concentration was increased from 1:1 to 2:1 less solubilisation of oil and less thermodynamic stability and visual observations lies in A category and some are in C & D category.(P=Passed), (F= Failed)

Table: - 2 Thermodynamic stability and dispersible test of different formulation selected from combination (I-V)

S_{mix} ratio (S: CoS)	Percentage w/w of different components in formulation				Observations based on the preparation thermodynamic stability studies and dispersible test				Inference
	Oil	S_{mix}		Aqueous	Heating Cooling Cycle	Centrifugation	Freeze thaw Cycle	Visual Observation	
1:1	1.0	1.0	1.0	7.0	P	P	P	Grade A	Passed
	1.2	1.2	1.2	6.4	P	P	P	Grade B	Failed
	1.5	1.5	1.5	5.5	P	F	P	Grade C	Failed
1:2	1.0	1.0	2.0	6.0	P	P	P	Grade A	Passed
	1.2	1.2	2.4	5.2	P	P	P	Grade A	Passed
	1.5	1.5	3.0	4.0	P	F	–	Grade B	Failed
	1.8	1.8	3.6	2.8	F	–	–	Grade C	Failed
1:3	1.0			6.0	P	P	P	Grade A	Passed
	1.2	0.71.2	2.23.6	5.0	P	P	P	Grade A	Passed
	1.5	0.91.8	2.75.4	4.0	P	P	P	Grade A	Passed
	1.8			2.8	F	–	–	Grade B	Failed
2:1	1.0	2.62.4	1.31.2	5.0	P	P	P	Grade A	Passed
	1.2	3.2	1.6	4.0	P	P	F	Grade C	Failed
	1.5	4.0	2.0	2.5	F	–	–	Grade D	Failed
	1.8			1.0	F	–	–	Grade D	Failed
3:1	1.0			6.0	P	P	P	Grade C	Failed
	1.2	2.25	0.75	5.2	P	P	P	Grade C	Failed
	1.5	2.70	0.90	4.0	P	F	–	Grade D	Failed
	1.8			2.8	F	–	–	Grade D	Failed

Construction of Pseudoternary phase diagram : -

Pseudo Ternary Phase Diagram of Acelofenac in Different Combinations Smix ratioTween 20:Ethanol (1:1)

This combination comprised of oil **Acelofenac**, surfactant Tween 20, co-surfactant ethanol and water. Phase diagram were constructed with varying ratio of oil and S_{mix} and S_{mix} ratios in this combination was used 1:1 1:2 ,1:3,1:4,2;1 ,3:1 and oil/S_{mix} ratio in 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2 and 1:1.The points shown in pink color represents 'A' category (Emulsion which is clear and bluish in appearance) of visual observation. The points shown in yellowish-orange color represents 'B' category (Slightly less clear emulsion and slight bluish appearance) of visual observation. The points, shown in green color represent 'C' category (Bright white emulsion, similar to milky in appearance) of visual observation. The points shown in blue color represents 'D' category (Dull grayish white emulsion with a slightly oily appearance) of visual observation. The points shown in purple color represents 'E' category (Poor or minimal emulsification with large oil droplets) of visual observation. Further increase in the oil concentration decreased the capacity of the nanoemulsion formation and further increase in the surfactant concentration with respect to co-surfactant decreased the area of the nanoemulsion. The concentration of oil that could be soluble according to the phase diagram was 08% wt/wt using S_{mix} 16%w/w and 76% water, another optimized combination .in which 10% wt/wt of oil could be soluble in the phase diagram with S_{mix} 10% and water 80% w/w. futher this study done all combination and result find below.

Pseudo Ternary Phase Diagram of Acelofenac in Different Combinations Smix ratioTween 20:Ethanol (1:2)

The concentration of oil that could be soluble according to the phase diagram was 10% wt/wt using S_{mix}20%w/w and 70% water, another optimized combination in which 12% wt/wt of oil could be soluble in the phase diagram with S_{mix}24% and water 64% w/w.

Pseudo Ternary Phase Diagram of Acelofenac in Different Combinations Smix ratioTween 20:Ethanol (1:3)

The concentration of oil that could be soluble according to the phase diagram was 12% wt/wt using S_{mix} 36%w/w and 52% water, another optimized combination in which 15% wt/wt of oil could be soluble in the phase diagram with S_{mix} 30% and water 55% w/w.

Pseudo Ternary Phase Diagram of Acelofenac in Different Combinations Smix ratioTween 20:Ethanol (2:1)

The concentration of oil that could be soluble according to the phase diagram was 10% wt/wt using S_{mix}40%w/w and 50% water, another optimized combination in which 10% wt/wt of oil could be soluble in the phase diagram with S_{mix} 30% and water 60% w/w. Further increase in the oil concentration decreased the capacity of the nanoemulsion formation and further increase in the surfactant concentration with respect to co-surfactant decreased the area of the nanoemulsion. The corresponding phase diagram of this region is represented in Figure1

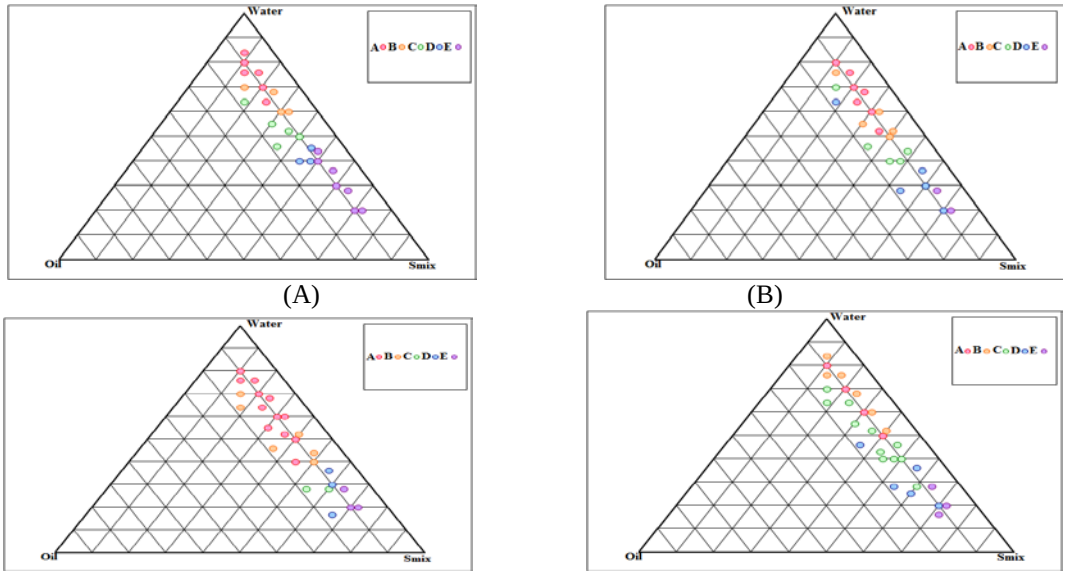

Figure 1: Pseudoternary phase diagram of formulation composed of oil (oleic acid), mixture of surfactant (Tween 20) and cosurfactant(ethanol) dispersed with water at 37∘C. For surfactant: cosurfactant ratio of (a) 1 :1, (b) 1 : 2, and (c) 1 :3 (d) 2;1

The composition of selected nanoemulsion formulations are shown in Table 3

Table -3 Compositions of Selected Nanoemulsion Formulations

Code	Oil	S_{mix}	Water	Oil/S_{mix} Ratio	S_{mix} Ratio
NE-1	10	10	80	1:1	1:1
NE-2	8	16	76	1:2	1:1
NE-3	12	36	52	1:3	1:3
NE-4	15	30	55	1:2	1:3
NE-5	10	40	50	1:4	2:1
NE-6	10	30	60	1:3	2:1
NE-7	12	24	64	1:2	1:2
NE-8	10	10	80	1:1	1:2

CHARACTERIZATION OF NANOEMULSIONS

Nanoemulsion Droplet Size Analysis

The droplet size increased with the increase in concentrationof oil in the formulations. The droplet size of formulation NE1, containing 10% oil, was (56.95 ±1.46 nm). The droplet size of formulation NE8 was(68.3 ± 5.26 nm), significantly different($P < 0.05$). All the formulations had droplets in the nano range, which is very well evident from the Table 4 . Different optimized nanoemulsions particle size analysis as shown in Figure2.

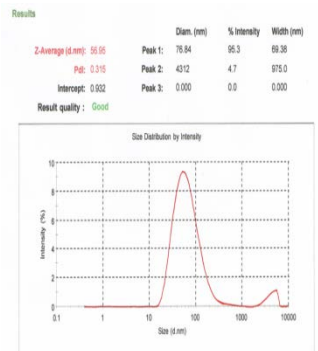

Fig - 2(A)

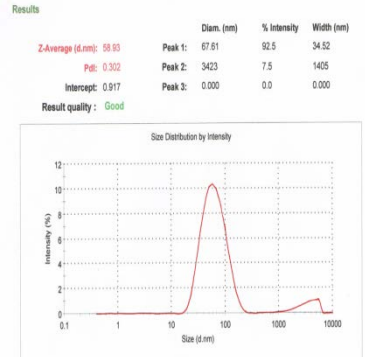

fig- 2 (B)

fig 2 (C)

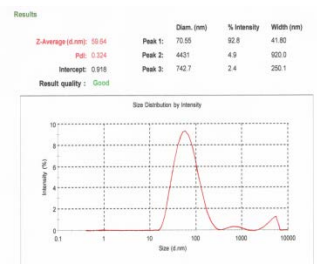

Fig - 2 (D)

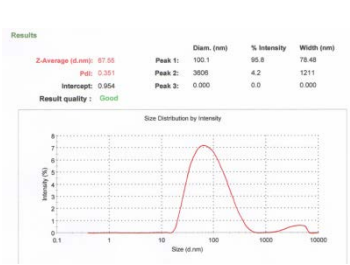

fig - 2 (E)

fig- 2(F)

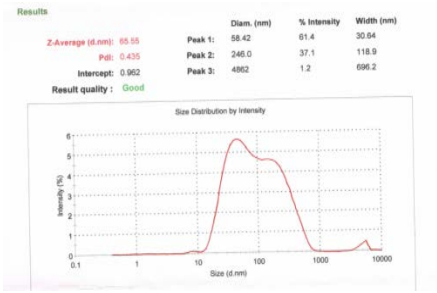

Fig - 2 (G)

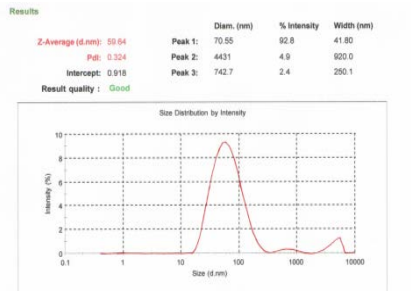

fig- 2(H)

Fig-2 Particle Size Analysis of AcelofenacNanoemulsion Formulation Oil:S_{mix} (1:1), S_{mix} (1:1), Oil:S_{mix} (1:2), S_{mix} (1:1),Oil:S_{mix} (1:3), S_{mix} (1:3, Oil:S_{mix} (1:2), S_{mix} (1:3) Oil:S_{mix} (1:4), S_{mix} (2:1) ,Oil:S_{mix} (1:3), S_{mix} (2:1) andOil: S_{mix} (1:2), S_{mix} (1:2)

Table-4 Drolet Size, Polydispersity values, Zeta Potential and Conductivity of the Nanoemulsion Formulations (N=3)

Code	Droplet Size±SD(nm)	Polydispersity	Zp (mv)	Conductivity
NE-1	56.95±1.46	0.315	-41.9	0.0655
NE-2	58.93±1.5	0.302	-37.6	0.0606
NE-3	57.31±1.48	0.247	-36.5	0.0674
NE-4	58.11±1.53	0.370	-35.3	0.117
NE-5	53.58±1.57	0.380	-34.8	0.0658
NE-6	65.55±1.62	0.435	-31.9	0.0803
NE-7	67.55±1.65	0.351	-31.1	0.0854
NE-8	65.96±1.72	0.456	-30.6	0.0885

Viscosity Determination

The viscosities of the selected formulations were determined. The viscosity of formulation NE1 (26.28 ± 1.22cP) was lower than that of any other formulation, and this difference was significant (P <0.05). The viscosity of formulation NE 8 was highest (60.52 ± 2.91cP), but it was observed that the viscosity of the nanoemulsion formulations generally were low.The values are given in Table5.

pH determination

The pH value determination monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. The nanoemulsions had stable pH values between (5.22- 5.52) for almost all formulations tested.The values are given in Table 5

Refractive Index

The mean values of the refractive index of drug-loaded formulations NE1 (1.432±0.004) and NE8 (1.423±0.004) the refractive index values of blank, NE1 (1.439±0.004) andNE8 (1.432±0.004) were compared it was found that there were no significant differences between the values.The values are given in Table 5

Transmission Electron Microscope

In the TEM positive image, the nanoemulsion appeared dark and the surroundings were bright. Some droplet sizes were measured, as TEM is capable of point-to-point resolution. These sizes were in agreement with the droplet size distribution measured using photon correlation spectroscopy. The microscopic observations of nanoemulsion showed that it had a spherical shape particles ranging < 100nm.As shown in Figure3

Table-5 Viscosity, pH and Refractive Index of selected Nanoemulsions

Code	Viscosity mean±SD(cP)	pH	Refractive Index
NE-1	26.28±1.22	5.52	1.43
NE-2	28.52±1.68	5.48	
NE-3	34.46±1.82	5.36	
NE-4	38.22±1.70	5.32	
NE-5	44.12±1.70	5.40	
NE-6	46.34±1.86	5.38	
NE-7	52.62±1.94	5.28	
NE-8	60.52±2.91	5.22	

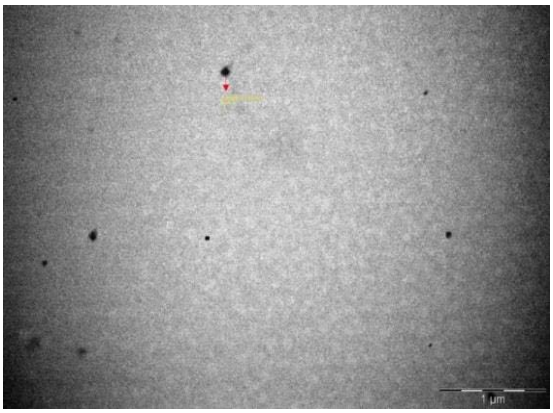

Fig: 3 Transmission Electron Microscope

The TEM of nanoemulsion appeared dark and the surroundings was bright (Figure 3), The droplet size measured using TEM, provides point-to-point resolution. The droplet size is in coordination with the Zetasizer measurement. The picture represents the formation of discrete, spherical & smooth surfaced droplets of NE clearly. The size of these oil globules were ranged from 58.3nm to 117.63nm

In vitro skin permeation studies: - *In vitro* skin permeation studies were performed to compare the release of drug from eight different nanoemulsion formulations (NE1-NE8), all having the same quantity of **Aceclofenac.** I*n vitro* skin permeation was highest in formulation NE3

Formulations of nanoemulgel from selected nanoemulsion: - The very low viscosity often exhibited by nanoemulsion is inappropriate for topical use. Therefore, the nanoemulsion-based gel was formulated by incorporating appropriate gelling agent.

Viscosity measurement: - The viscosities of prepared nanoemulgel (0.5%w/v, 1%w/v and 1.5%w/v) formulations were determined by using Brookfield Viscometer using spindle # 61 at 37±0.5°C. The spindle speed was set at 60 rpm and a single run was performed at a temperature of 37±0.5°C. The wait time for the operation was 2 mins. Each sample was performed in triplicate and the standard deviation was calculated.

Spreadability studies: - 0.5 g test formulation was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 50 g was allowed to rest on the upper glass plate for 5 min. the increase in the diameter due to spreadability of the formulation was noted

Swelling index: - To determine this parameter 1.0 gm of formulated nanoemulgel is taken onto the porous aluminum foil and which is then placed in the 10 ml solution of the 0.1 N NaOH solutions. The sample is removed with time to time and weight of sample is noted till no further change in the weight:

Swelling index (SW) % = {[Wt-Wo] / Wo } x 100

Where,

SW % = Percentage swelling,

Wt = Weight of the swollen nanoemulgel (NEG) at time t.

Wo = Original weight of the nanoemulgel (NEG)

Drug content: - 1 ml of nanoemulgel was diluted to 20 ml of methanol and sonicated. The volume was maintained up to 100 ml using phosphate buffer (pH7.4). The UV-VIS spectrophotometer (SHIMADZU-1880) was used to measure aceclofenac content after several dilutions of nanoemulgel at 276nm.

Table-6 In vitro skin permeation studies

Time(hr)	NE 1	NE 2	NE3	NE 4	NE 5	NE 6	NE 7	NE 8
0.5	8.57	8.57	9.85	6.00	5.14	4.72	4.28	3.43
1	23.24	29.24	34.09	28.09	15.99	12.09	25.33	27.81
2	44.14	52.09	59.62	45.72	32.28	23.24	39.67	50.14
4	64.95	75.00	78.14	68.81	44.19	38.05	56.57	66.90
6	75.57	84.85	86.47	78.53	52.33	54.66	67.19	69.53
8	86.24	94.14	96.19	83.00	65.62	70.14	78.81	78.57

On the basis of results NE3 Nanoemulsion selected for formulation of nanoemugel. due to low polydespersibility , optimum valve of conductivity , pH, viscosity etc. and highest vitro skin permeation **.**

Table 7: Physical characterization of nanoemulgels NEG1(0.5%w/v), NEG2(1%w/v) and NEG3(1.5%w/v)

Sr. No.	Property	NEG1 (0.5%w/v) Carbopol 940	NEG2 (1%w/v) Carbopol 940	NEG3 (1.5%w/v) Carbopol 940
1	Colour	Colorless	Colorless	Colorless
2	Appearance	Transparent	Transparent	Transparent
3	Odor	Odorless	Odorless	Odorless
4	After feel	Emollient	Emollient	Emollient
5	Type of Smear	Non-greasy	Non-greasy	Non-greasy
6	Removal	Easy	Easy	Easy
7	Phase Separation	Yes	No	No
8	Consistency	+	++	+++

Table-8 pH, Viscosity, Spreadability studies and percentages drug content of prepared Nanoemulgels

Sr. No.	Formulation Code	pH ± SD (n=3)	Viscosity (cP)±SD (n=3)	Spreadability (gcm/sec) ± SD (n=3)	%Drug content ± SD (n=3)
1	**NEG1** (0.5%w/v)	5.826± 0.519	2351 ± 9.84	118.32± 0.21	90.50± 0.012
2	**NEG2** (1%w/v)	5.516± 0.518	2319 ± 0.06	122.55± 0.39	95.70± 0.019
3	**NEG3** (1.5%w/v)	5.721± 0.707	2440 ± 4.58	120.71± 0.11	97.70 ±0.013

In vitro Drug release studies: - The diffusion study of the nano emulgel formulations was performed in Franz diffusion cell. The dialysis layer was mounted between the benefactor and receptor compartments nano emulgel definition (2% gel) was applied consistently on the dialysis film and the compartment braced together. The receptor compartment was loaded up with phosphate buffer pH. 7.4 and the hydrodynamics in the receptor compartment was kept up by blending with an attractive dab. 1ml of tests was pulled back at foreordained time interims and an equivalent volume of cushion was supplanted.The samples were analyzed by the UV-Visible spectrophotometer at 276 nm to determine the concentration. The experiment was repeated thrice. Same procedure was repeated for all the gels.

Table. 9: *In-vitro* Drug release study of gel.

% Drug Release			
Time (Hrs)	**NEG -1**	**NEG - 2**	**NEG – 3**
1	14.7±0.25	12.6±2.11	10.1±1.56
2	21.4±1.65	16.8±0.38	15.5±1.22
3	29.2±1.47	23.2±0.41	22.1±2.33
4	36.2±2.45	31.3±1.22	32.0±0.25
5	45.2±2.66	41.4±2.31	43.8±0.14
6	60.8±1.48	57.1±0.29	62.2±0.52
7	66.6±0.36	63.4±0.41	76.7±1.24
8	72.7±2.35	71.2±2.41	84.6±0.36
9	79.2±2.98	86.7±1.69	90.2±0.41

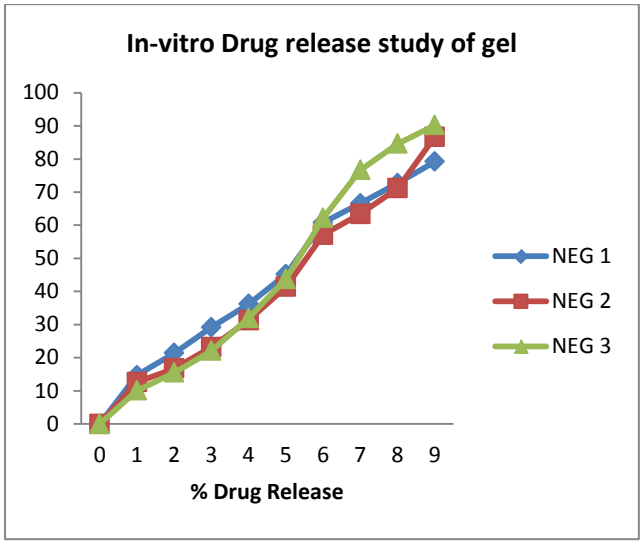

Figure No: - 4 In-vitro drug release study of nanoemulgels

Drug release comparison of nanoemulsion gel with conventional gel

In-vitro drug release of nanoemulsion gel containing carbopol-940(1%) was compared with conventional ACFgel containing carbopol-940(1%). Results were represented in Table-10.

Table – 10 Drug release comparison of nanoemulsion gel with conventional gel

% Drug Release		
Time (Hrs)	**Nanoemulgel**	**Conventional gel**
1	10.1±1.56	6.1±0.21
2	15.5±1.22	11.9±1.22
3	22.1±2.33	17.9±2.30
4	32.0±0.25	24.2±0.40
5	43.8±0.14	29.1±1.01
6	62.2±0.52	36.8±1.42
7	76.7±1.24	41.2±0.54
8	84.6±0.36	53.1±0.21
9	90.4±0.41	60.2±2.36

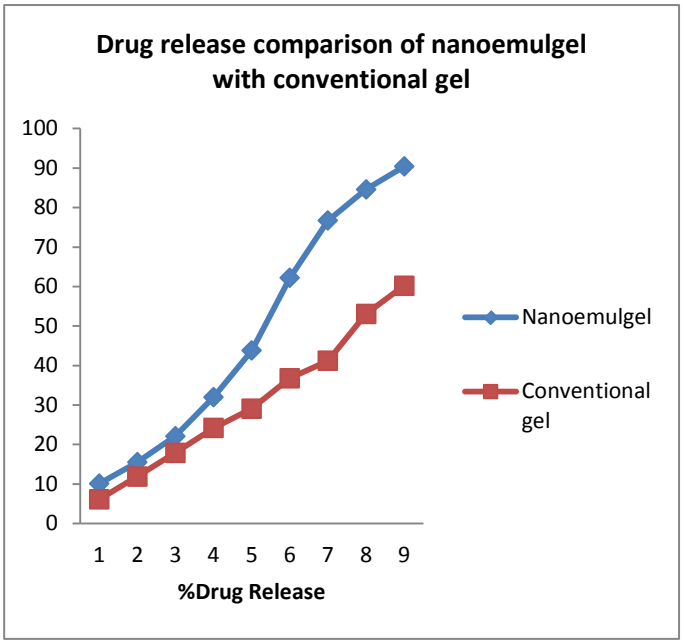

Fig -5 Drug release comparison of nanoemulsion gel with conventional gel

Stability studies at different temperature conditions: - Temperature stress thinks about were directed by putting away the detailing at various temperature conditions. Every detailing was put away in fixed glass holders in cooler (4°C), at surrounding temperature (25°C) and at quickened temperature (40°C) for 90 days. After 1, 30, 60 and 90 days, the plans were assessed for any physical change, (for example, clearness, stage partition, precipitation of medication, shading change), sedate substance and pH.

The results of stability studies are shown in table depicting nanoemulgels remained clear even after a period of three months at temperatures 25 ± 2°C, 40 ± 0.1°C, and 4± 0.2°C. All the formulations were found to be consistent with respect to their pH values, drug content, phase separation,and transparency during the stability study

Table-11 Stability studies at different temperature conditions

Time (h)	Temp.	% Drug content	transparency	pH	Phase separation
1 day	4ºC	97.85±0.02	+	5.73 ± 0.02	-
	25ºC	97.75±0.02	+	5.72 ± 0.13	-
	40ºC	97.64±0.02	+	5.61 ± 0.22	-
1 month	4ºC	97.72±0.02	+	5.74 ± 0.04	-
	25ºC	97.59±0.12	+	5.72 ± 0.21	-
	40ºC	97.61±0.21	+	5.73 ± 0.14	-
2 month	4ºC	97.70±0.04	+	5.74 ± 0.18	-
	25ºC	97.51±0.07	+	5.74 ± 0.16	-
	40ºC	97.41±0.21	+	5.70 ± 0.22	-
3 month	4ºC	97.62±0.14	+	5.73 ± 0.16	-
	25ºC	97.41±0.15	+	5.71 ± 0.11	-
	40ºC	97.16±0.21	+	5.73 ± 0.13	-

DISCUSSION

Nanoemulgel was proposed as carrier for transdermal delivery of Aceclofenac due to its high solubilizing ability and permeation enhancing properties . Apart from this its topical route has the potential to bypass the problems associated with chronic oral delivery of Aceclofenac.

Nanoemulgel system is drug loaded multicomponent system containing oil, surfactant cosurfactant mixture, aqueous phase, and gel base.The nanoemulsions were prepared by figuring out the concentration range of components . All eight nanoemulsions (NE1–NE8) formed were optimized for morphological structure, droplet size, viscosity, and conductivity. The TEM image revealed spherical structure, and all formulations were in nanosize range (10–100 nm) with low PDI value indicating uniformity of droplet size in formulation. The high conductivity values confirmed the O/W structure of nanoemulsions. on the basis Among the different drug-loaded formulations evaluated, formulation NE3, which exhibited satisfactory polydispersity index, viscosity, refractive index, percentage transmittance and maximum *in vitro* release of NE3 (96%), was chosen as the optimized formulation for further studies. NE3 nanoemulsion formulation was changed to gel by simply adding three different concentrations of carbopol 940 i.e. 0.5%, 1% and 1.5% to produce formulations NEG1 0.5%w/v, NEG2 1%w/v and NEG3 1.5%w/v. The nanoemulgel formulations NEG1 0.5%w/v, NEG2 1%w/v and NEG3 1.5%w/v were characterized for various parameters like physical evaluation, drug content and content uniformity, viscosity, homogeneity and grittiness, pH, spreadability and *in vitro* skin permeation. From the results of *in vitro* skin permeation of various nanoemulgel formulations NEG1 0.5%w/v, NEG2 1%w/v and NEG3 1.5%w/v it was concluded that as the concentration of gel increased from 0.5% to 1.5%, its rate of permeation through the skin was increased. The percent cumulative amount of drug permeated from nanoemulgel formulations NEG1 0.5%w/v, NEG2 1%w/v and NEG3 1.5%w/v was found to be 79.2,86.7 and 90.2 respectively as compared to conventional gel (60%).

The formulated nanoemulgel system was found to possess good permeation potential without incorporation of any chemical enhancers which are habitually irritants . Hence,the novelty of this system lies here, as the components(oil, surfactant, and especially cosurfactant) of nanoemulgel themselves acted as permeation enhancers.The stability studies were carried out at room temperature and refrigerator temperature, indicating that the formulationis stable and no change in drug content and pH was observed.Thus the nanoemulgel formulation could be beneficial in improving bioavailability and permeation of Aceclofenac for transdermal delivery for treatment of arthritis .

CONCLUSION

In conclusion, topical nanoemulgel of Aceclofenac was developed to a satisfactory level in terms of release of drug from it, optimum globule size, minimum polydispersity index (Pdi), optimum viscosity, lower surfactant concentration, higher penetration, and better skin retention. The present study endorsed topical nanoemulgel of Aceclofenac to be a promising choice over conventional topical formulations for the treatment of rheumatoid arthritis.

Acknowledgement:
The authors would like to acknowledge the guidance, suggestions and support from teachers, seniors and staff members involved in performing the research work.

Conflict of intrest: Nil

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