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Development and Characterization of Nanoemulsion Gel for Topical Drug Delivery of Nabumetone

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ABSTRACT

The aim of the present study was to develop nanoemulsion formulation for topical delivery of Nabumetone to enhance the water solubility as well as bioavailability of the drug. O/W nanoemulsions were prepared by the spontaneous emulsification method. Pseudoternary phase diagrams were constructed to obtain the nanoemulsion region. Light liquid paraffin was chosen as the oil phase, Tween 80 and PEG400 were used as surfactant and co-surfactant respectively. F-A1 to F-A6 different formulations of Nabumetone loaded nanoemulsion papered successfully by Spontaneous emulsification method and characterized for particle size, zeta potential, thermodynamic stability study, rheology study. Further nanoemulsion was incorporated into 3%, 4% chitosan to get a gel for improving convenience in superficial application of drug. Menthol was used as penetration enhancer. E-A1 to E-A6 different formulations of Nanoemulsion Gel was prepared. The formulations were evaluated for rheological studies, spreadability, bioadhesion strength, skin irritation studies, *in-vitro* release, *ex-vivo* release studies, anti-inflammatory activity in arthritis. Anti-inflammatory activity of prepared nanoemulsion gel was compared with marketed diclofenac sodium emulgel. Study concluded that topical Nanoemulsion Gel of Nabumetone possess an effective anti-inflammatory activity in Arthritis.

INTRODUCTION

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is the main route of topical drug delivery system. Drugs are administered topically for their action at the site of application or for systemic effect. Dermatological products applied to skin are diverse in formulation and range in consistency from liquid to powder but the most popular products are semisolid preparation. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. Gels are a relatively newer class of dosage form created by entrapment of large amounts of aqueous or hydro-alcoholic liquid in a network of colloidal solid particles, which may consist of inorganic substances, such as aluminum salts or organic polymers of natural or synthetic origin. They have a higher aqueous component that permits greater dissolution of drugs, and also permit easy migration of the drug through a vehicle that is essentially a liquid, compared with the ointment or cream base. These are superior in terms of use and patient acceptability. In spite of many advantages of gels, a major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation, emulgels are prepared and used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels. Both oil-in-water and water-in-oil emulsions are extensively used for their therapeutic properties and as vehicles to deliver various drugs to the skin. Emulsions possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to penetrate the skin. In addition; the formulator can control the viscosity, appearance and degree of greasiness of cosmetic or dermatological emulsions. Oil-in-water emulsions are most useful as water washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, compatible with several excipients and water-soluble or miscible. Emulgels are emulsions, either of the oil-in-water or water-in-oil type, which are gelled by mixing with a gelling agent. They have a high patient acceptability since they possess the previously mentioned advantages of both emulsions and gels. Therefore, they have been recently used as vehicles to deliver various drugs to the skin. [1]

MATERIALS AND METHODS

Materials

Nabumetone was gifted from Triveni Chemicals Gujrat, Chitosan Purchased from Ozone International, Mumbai, Light liquid paraffin purchased from SD Fine Chemicals, Mumbai, Tween 80, PEG 400, Glacial acetic acid, Methanol, Benzalkonium Chloride, Menthol, Sodium Hydroxide purchased from Loba chemical Mumbai, Glycerin purchased from Merck Specialities Pvt. Ltd. All chemicals and solvents used in this study were of analytical reagent grade. Freshly prepared distilled water was used throughout the work.

Method

Solubility study

Solubility of Nabumetone was determined in Methanol, Phosphate buffer of pH 5.5, Phosphate buffer of pH 6.8.

Determination of solubility of Nabumetone in oils

Excess of drug in 2ml of each of selected oils in 5 ml stopped vials and mixed and kept at 37±1^{o}C in an isothermal shaker for 72 h. After 72hr sample was removed and centrifuged at 3000 rpm for 15 min dilutions of these solutions prepared in methanol from 2 to 10 ppm. The absorbance of each standard solution was determining spectrophotometrically at 272nm. The Beer's-Lambert's plot was constructed by plotting concentration Vs its corresponding absorbance. [2]

Pseudo-Ternary Phase Diagram Study

On the basis of solubility study of drug, light liquid paraffin was selected as the oil phase. Tween 80 and PEG400 were selected as surfactant and co-surfactant as per their emulsification capability for the system. Distilled water was used as an aqueous phase for the construction of phase diagram for the determination of existence zone of nanoemulsion. Pseudoternary phase diagrams were constructed using aqueous titration method. To construct pseudoternary phase diagrams the oil phase was mix with surfactant: co-surfactant used for titrations are 1:1, 1:2, 2:1.the mixture was titrated with distilled water until it turned turbid.

The volume of water used was recorded water titration was continued until a clear, Isotropic and thermodynamically stable dispersion with low viscosity was obtained. [3]

Compatibility study

a) Fourier transforms Infrared spectroscopy (FT-IR)

The IR Spectra of Nabumetone and excipients were recorded by Shimadzu S 8400 FTIR spectrophotometer. Sample was prepared by KBr disc method and examined in the transmission mode. Spectrum was measured over frequency range of 4000-400 cm^{-1}. The peaks obtained in the spectra were then compared with the corresponding functional groups in structure of Nabumetone.

b) Differential Scanning calorimetry (DSC)

DSC thermogram of Nabumetone, Nabumetone and Chitosan and formulation was recorded on TA WS Thermal analyzer (Shimadzu).The samples were hermetically sealed in aluminum pans and heated at a constant rate of 10 ^{o}C/min over temperature range of 40 to 300 ^{o}C.Inert atmosphere was maintained by purging nitrogen gas at flow rate of 50ml/min.

Preparation of Nanoemulsion of Nabumetone

Different formulations of Nanoemulsion were prepared by using the varying amount of emulsifier by spontaneous emulsification method.

The oil phases of the emulsion were prepared by dissolving PEG 400 and Tween 80 in Light liquid paraffin. The drug Nabumetone was dissolved in Methanol. This solution was mixed with the aqueous phase. Both the oily and aqueous phase were homogenized at 6000rpm for 1 hr. Oil phase was injected to the aqueous phase with continuous homogenization at 6000 rpm for 6 to 8 hrs. [4]

Table 1: Manufacturing formula for Nanoemulsion formulations

Code	% Drug	% Light liquid paraffin	% Smix Tween 80:PEG 400	% Methanol	% Water
F-A1	3%	6%	30% (1:1)	2%	59%
F-A2	3%	6%	35% (1:1)	2%	54%
F-A3	3%	6%	40% (1:1)	2%	49%
F-A4	3%	6%	30% (2:1)	2%	59%
F-A5	3%	6%	35% (2:1)	2%	54%
F-A6	3%	6%	40% (2:1)	2%	49%

Figure 1: Nanoemulsion and Conventional Emulsion of Nabumetone

Characterisation of nanoemulsion

a) Physical appearance

The prepared formulations were inspected visually for their color and appearance.

b) Particle size Measurement

Particle size of nanoemulsion was measured by Scattering light intensity scattering angle 90^0cat temperature 25^oC viscosity of dispersion medium 0.894mPa.S at count rate 2283kCps.

c) Zeta potential measurement

Zeta potential of nanoemulsion was measured at temperature 25^oC and viscosity of dispersion medium 0.895mPa.S at conductivity 0.098ms/cm and electrovoltage 3.9v.

d) Thermodynamic stability study

Thermodynamic stability of the Nanoemulsions system was determined by performing following tests.

- **Heating Cooling Cycle**

Nanoemulsion formulations were subjected to six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature not less than 48h. Stable formulations were then subjected to centrifugation test.

- **Centrifugation**

Nanoemulsion formulations were centrifuged at 3500 rpm for 30 min and those formulations which did not show any phase separation were taken for the freeze-thaw stress test.

- **Freeze-Thaw Cycle**

In this the formulation was subjected to three freeze-thaw cycles between -21°C and +25°C with storage at each temperature for not less than 48 h was done for the formulations. [4]

e) Rheology study of Nanoemulsion

The viscosity of Nanoemulsions of different formulations was measured at 10 rpm for 3 min at 25^{o}C by Brookfield type rotary viscometer with spindle 62. [4]

2. Preparation of Chitosan Gel

Chitosan gels were prepared by incorporating different concentration, 3%, 4% w/v of chitosan in 1% v/v Glacial acetic acid in double distilled water.

Prepared 1% of Glacial acetic acid by dissolving 1ml of Glacial acetic acid in Double distilled water. 5 gm Menthol was dissolved in 1% Glacial acetic acid. A weighted amount of chitosan was taken and dispersed over 1% Glacial acetic acid for 2 h still all the chitosan is soaked and homogenized for 2h at 6000 rpm. After homogenization chitosan gel was subjected to two cycles of sonication for 15 min to expel out the entrapped air bubbles from the prepared gel. Similarly, other gel formulations were prepared. pH was adjusted 6 to 6.5 by 0.2 M NaOH. [5, 6]

Table 2: Manufacturing formula for Chitosan Gel Formulation

Code	Chitosan	Menthol	1% Glacial acetic acid	0.2M NaOH
GF1	3 %	5%	Q.S	Q.S
GF2	4%	5%	Q.S	Q.S

Characterization of gel

a) pH Determination

pH determination of prepared formulations was done by using digital pH meter. The procedure was carried out by taking gel in 250 ml beaker immersing pH meter into the formulation and readings of pH meter were recorded. Same process was repeated two more times with the same formulation. Similar procedure was used for the determination of the pH of all the prepared formulation thrice.

b) Rheology study of Gel

The viscosity of Chitosan gel of different formulations was measured at 10 rpm for 3 min at 25^{o}C by Brookfield type rotary viscometer with spindle 64. [7]

3. Preparation of Nanoemulsion Gel

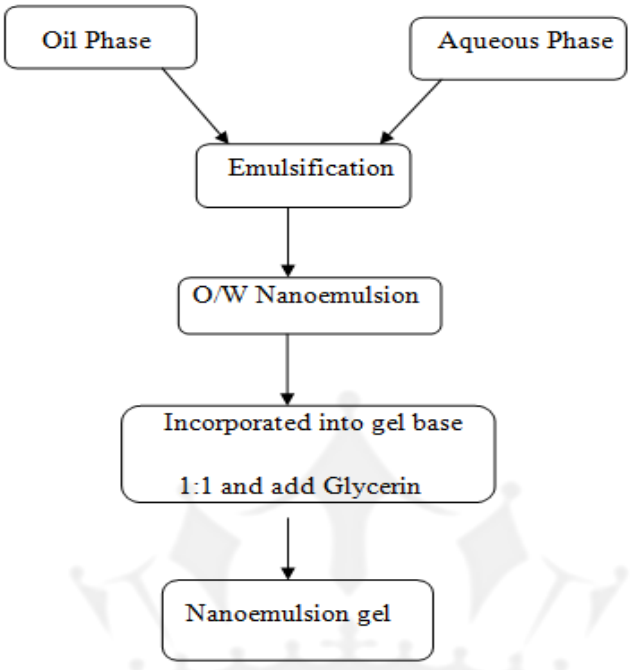


Figure 2: Flow chart of Preparation of Nanoemulsion Gel

Manufacturing formula for Nanoemulsion Gel

Table 3: Manufacturing formula for Nanoemulsion Gel

Ingredient	Quantity (%w/w)					
	E-A1	**E-A2**	**E-A3**	**E-A4**	**E-A5**	**E-A6**
Nabumetone	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
Light liquid paraffin	3%	3%	3%	3%	3%	3%
Smix	15%	17.5%	20%	15%	17.5%	20%
Methanol	1%	1%	1%	1%	1%	1%
Chitosan	2%	2%	2%	1.5%	1.5%	1.5%
Menthol	2.5%	2.5%	2.5%	2.5%	2.5%	2.5%
Glacial acetic acid	1%	1%	1%	1%	1%	1%
Benzalkonium chloride	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%
Glycerin	3%	3%	3%	3%	3%	3%
Water(Q.S)	100%	100%	100%	100%	100%	100%
0.2M NaOH	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Characterization of Nanoemulsion Gel

a) Physical appearance

The prepared Nanoemulsion gel formulations were inspected visually for their color, homogeneity, consistency, grittiness and phase separation.

b) pH Determination

pH determination of prepared formulations was done by using digital pH meter. The procedure was carried out by taking Nanoemulsion Gel in 250 ml beaker immersing pH meter into the formulation and readings of pH meter were recorded. Same process was repeated two more times with the same formulation. Similar procedure was used for the determination of the pH of all the prepared formulation thrice.

c) Rheology study of Nanoemulsion Gel

The viscosity of Nanoemulsion Gel of different formulations was measured at 10 rpm for 3 min at 25^{o}C by Brookfield type rotary viscometer with spindle 63.

d) Spreadability

Spreadability is determined by apparatus suggested by Mutimer et al (1956) which is suitably modified in the laboratory and used for the study. It consists of a wooden block, which is provided by a pulley at one end. By this method, spreadability is measured on the basis of 'Slip' and 'Drag' characteristics of emulgels. A ground glass slide is fixed on this block. An excess of Nanoemulsion Gel (about 2 gm) under study is placed on this ground slide. The Nanoemulsion gel was sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the Nanoemulsion gel between the slides. Excess of the Nanoemulsion Gel was scrapped off from the edges. The top plate was subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. Spreadability was calculated by using the formula.

$$S = M.L/T$$

Where, S = Spreadability,

M = Weight tied to upper slide,

L = Length of glass slides

T = Time taken to separate the slides completely from each other.

e) Extrudability

The extrudability test was carried out using hardness tester. A 5 gm of Nanoemulsion Gel was filled into the aluminum collapsible tubes. The plunged is subjected to hold the tube properly. The 1gm/cm^2 applied for the 30 sec. Then measured the quantity of Nanoemulsion gel extruded from the tube repeat procedure for three times.

f) Swelling index

To determine the swelling index of prepared Nanoemulsion gel 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml 0.1 N

NaOH. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows.

$$\text{Swelling Index (SW) \%} = [(Wt - Wo) / Wo] \times 100$$

Where, (SW) % = Equilibrium percent swelling.

Wt = Weight of swollen emulgel after time t.

Wo = Original weight of emulgel at zero time.

g) *Ex–vivo* bioadhesive strength measurement of Nanoemulsion-Gel

The modified method is used for the measurement of bioadhesive strength. The fresh skin of rat was cut into pieces and washed with 0.1 N NaOH. Two pieces of skin were tied to the two glass slide separately from that one glass slide was fixed on the wooden piece and another piece is tied with the balance on right-hand side. The right and left pans were balanced by adding extra weight to the left-hand pan. 1 gm of topical emulgel was placed between these two slides containing hairless skin pieces, and extra weight from the left pan was removed to sandwich the two pieces of skin and some pressure was applied to remove the presence of air. The balance was kept in this position for 5 minutes. Weight added slowly at 200 mg/ min to the left-hand pan until the patch detached from the skin surface. The weight (gram force) required to detach the emulgel from the skin surface gave the measure of bioadhesive strength. The bioadhesive strength was calculated by using following formula.

$$\text{Bioadhesive Strength} = \text{Weight required (in gms)} / \text{Area (cm}^2\text{)}$$

h) Drug content Determination

1.33gm of Nanoemulsion gel was taken dissolved using 100ml of methanol and sonicated for the period of 15 min filtered it by whatman filter paper. Further dilutions were made by using methanol prepared concentration within Beer's range. The absorbance was measured at 272 nm by UV-Visible spectrophotometer and drug content was determined.

i) *In-vitro* release studies

The *in-vitro* drug release studies were carried out using a Franz diffusion cell. The formulation was applied the surface of egg membrane which was placed between donor and receptor compartment of the Franz diffusion cell. Phosphate buffer pH 5.5 was used as a dissolution media. The temperature of the cell was maintained at 37^{o}C by circulating water

jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. Sample (5 ml) was withdrawn at suitable time intervals and dilute up to 10ml with same solvent and replaced with equal amounts of fresh dissolution media. Samples were analyzed spectrophotometrically at 271 nm and the cumulative % drug release was calculated.

Figure 3: *In-vitro* drug release by Franz diffusion cell

j) *Ex-vivo* drug release study

The *ex-vivo* drug release study of selected formulations was carried out in a Franz diffusion cell, Using rat skin. A section of skin was cut and placed in the space between the donor and receptor compartment of the FD cell, keeping the dorsal side upward. Phosphate buffer pH 5.5 was used as dissolution media. The temperature of the cell was maintained constant at 32^{o}C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. Sample (5 ml) was withdrawn at suitable time intervals and dilutes up to 10ml with same solvent and replaced with equal amounts of fresh dissolution media. Samples were analyzed spectrophotometrically at 271 nm and the cumulative % drug release was calculated.

k) Skin irritation test (patch test)

A set of 2 rats was used in the study. The Nanoemulsion gel was applied on the properly shaven skin of rat. Undesirable skin changes, i.e., change in color, change in skin morphology were checked after 24 h.

l) Accelerated Stability studies of Nanoemulsion Gel

Stability studies were performed according to ICH guidelines. The prepared nanoemulsion gel was packed in aluminum collapsible tubes (5 g) and subjected to stability studies. The formulations were stored in hot air oven at 37 ± 2°C, 45 ± 2°C, and 60 ± 2°C for a period of 3 months. The samples were analyzed for drug content every month for 3 months by UV-Visible spectrophotometer. Stability study was carried out by measuring the change in pH of gel at regular interval of time. [8-11]

Animal study of Nanoemulsion Gel

The anti-inflammatory activity of prepared nanoemulsion gel was studied by using Wistar rats with weight 100-150 gm. Total numbers of animals used in these experiments were 8 and marketed Diclofenac emulgel was used as the standard control. [12, 13]

Complete Freund's adjuvant-induced Arthritic model for Nabumetone Nanoemulsion Gel study

Table 4: Protocol of Animal study

Group No. (N=2)	Treatment with dose/day	Observation parameters
1	Vehicle control (distilled water)	On 8th day 1) Inflammatory assessment On 16th day 1)Inflammatory assessment
2	Negative control (plain arthritis) CFA 0.1 ml by right hind paw of the rats on 1st day	
3	Nabumetone Nanoemulsion gel treated group CFA on 1st day by right hind paw and from 8th to 16th day 100mg/kg B.W. Formulation on the paw topically	
4	Diclofenac Emulgel treated group CFA on 1st day by right hind paw from 8th to 16th day 100mg/kg B.W. Diclofenac emulgel on the paw topically.	

RESULT AND DISCUSSION

Solubility study

Solubility of Nabumetone in different solvents are shown in Table 5. Drug showed more solubility in Methanol than phosphate buffer of pH 5.5 and phosphate buffer of pH 6.8.

Table 5: Solubility of Nabumetone in different solvent

Sr. No.	Solvent	Solubility mg/ml
1	Methanol	34.3
2	Phosphate buffer of p^H 5.5	26.1
3	Phosphate buffer of p^H 6.8	20.7

Determination of solubility of Nabumetone in oils

Solubility of Nabumetone in different oils. Light liquid paraffin Olive oil Castor oil are shown in Table 6.

Table 6: Solubility of Nabumetone in different oils.

Sr. No.	Solvent	Solubility mg/ml
1	Light liquid paraffin	28.9
2	Olive oil	5.1
3	Castor oil	2.5

Pseudo-Ternary Phase Diagram Study

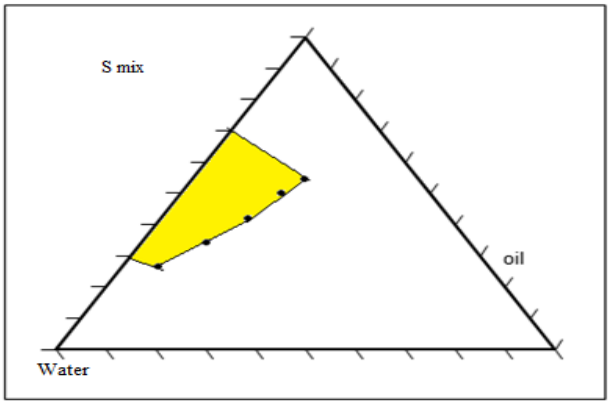


Figure 4: Pseudo-ternary phase diagram of 1:1Smix Ratio

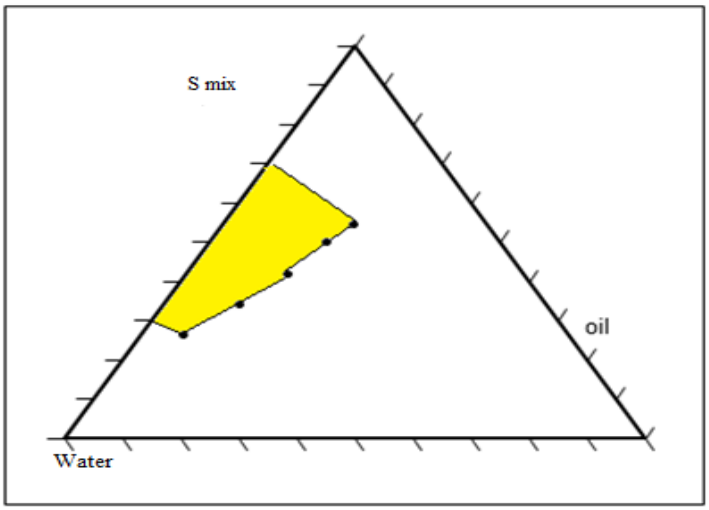


Figure 5: Pseudo-ternary phase diagram of 1:2 Smix Ratio

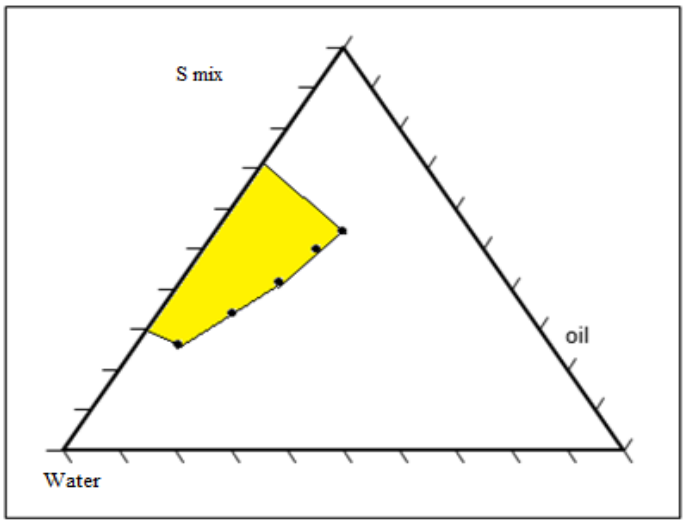


Figure 6: Pseudo-ternary phase diagram of 2:1Smix Ratio

A ternary phase diagram explains the selection of the formulations from the phase diagrams to avoid metastable formulations having minimum surfactant concentration, in the least possible time. Ternary phase diagrams were constructed by varying Tween 80: PEG-400 ratios as 1:1, 1:2, and 2:1.The shaded areas of phase diagrams show the nanoemulsion regions, whereas the non-shaded area displays the emulsion region. Thus, the ternary phase system of Tween 80: PEG-400, (1:1, 2:1) that exhibited maximum area for nanoemulsion formation was selected for the optimization of nanoemulsion batches. It was clearly evident that an increase in the concentration of Tween 80 resulted in a decrease in globule size but 1:2 ratio of Tween 80: PEG-400 does not show the long time stability so these rejected.

Compatibility study

a) Fourier transforms Infrared spectroscopy (FT-IR)

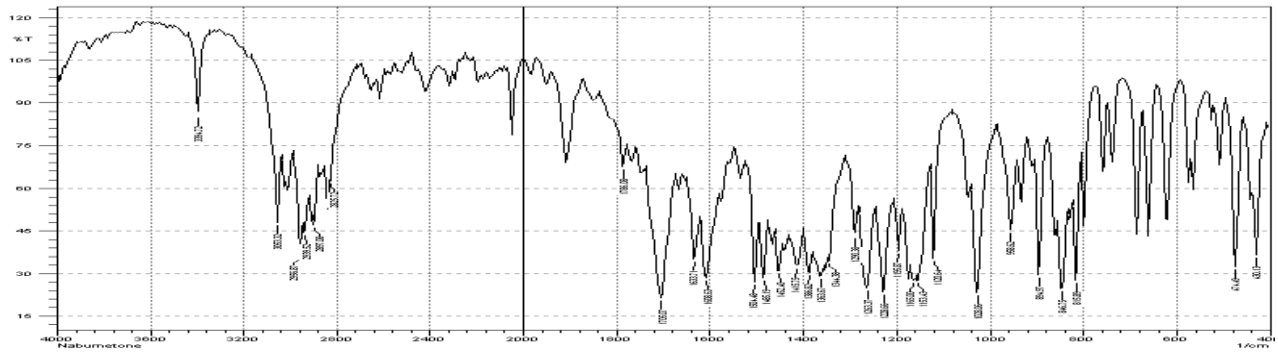


Figure 7: Fourier transforms Infrared spectrum of Nabumetone

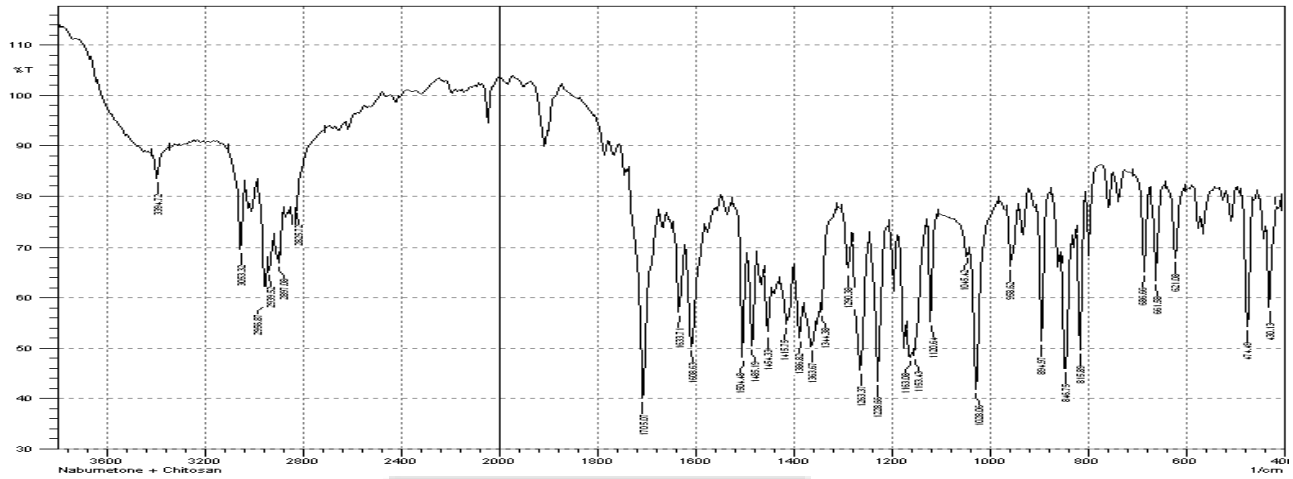


Figure 8: Fourier transforms Infrared spectrum of Nabumetone and Chitosan.

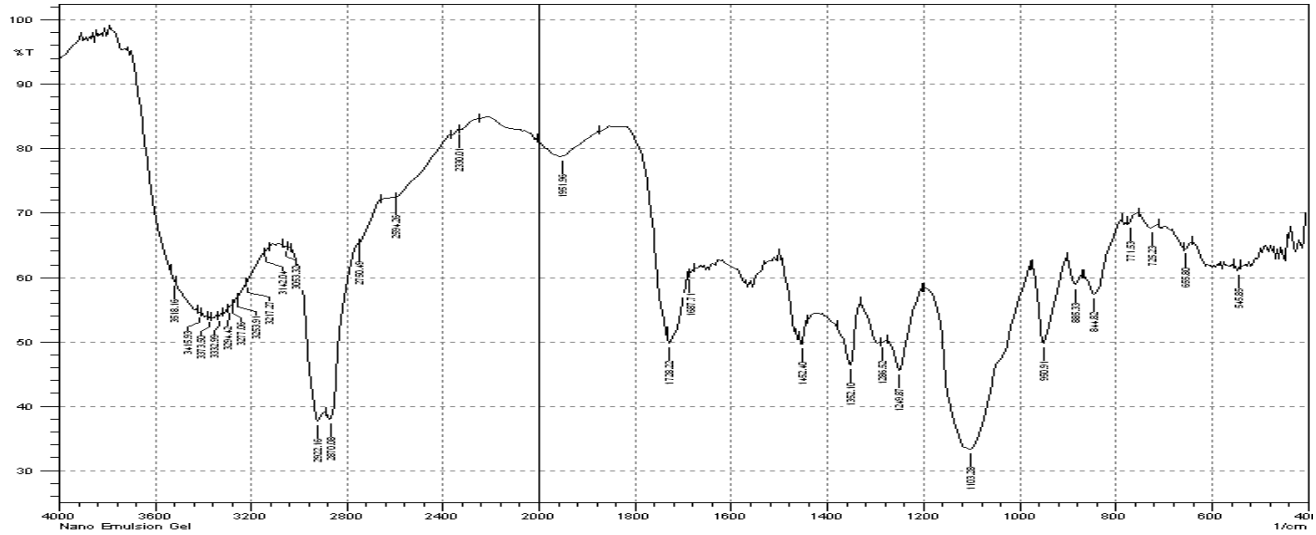


Figure 9: Fourier transforms Infrared spectrum of Nabumetone and Mixture of Excipients

Interpretation of FTIR spectra

Table 7: Interpretation of FTIR spectra

Functional groups	Actual IR Range	Observed IR Range		
		Nabumetone	Nabumetone+Chitosan	Nabumetone+ Mixture of Excipients
C-H Stretching	2970-2850	2939.52-2897.08	2897.08	2922.16-2870.63
C=O Carbonyl group	1750-1705	1786.08-1705.07	1705.07	1728.22
Ether O-CH_3	2850–2815	2825.7	2825.7	2824.5
C=C-C Aromatic ring stretching	1680-1620	1633.7-1608.63	1633.7-1608.63	1667.7

The functional groups determined were similar between standard ranges as shown in above there is no change in absorption frequency .Therefore we conclude that there is no interaction between API and Excipients.

b) Differential Scanning Colourimetry

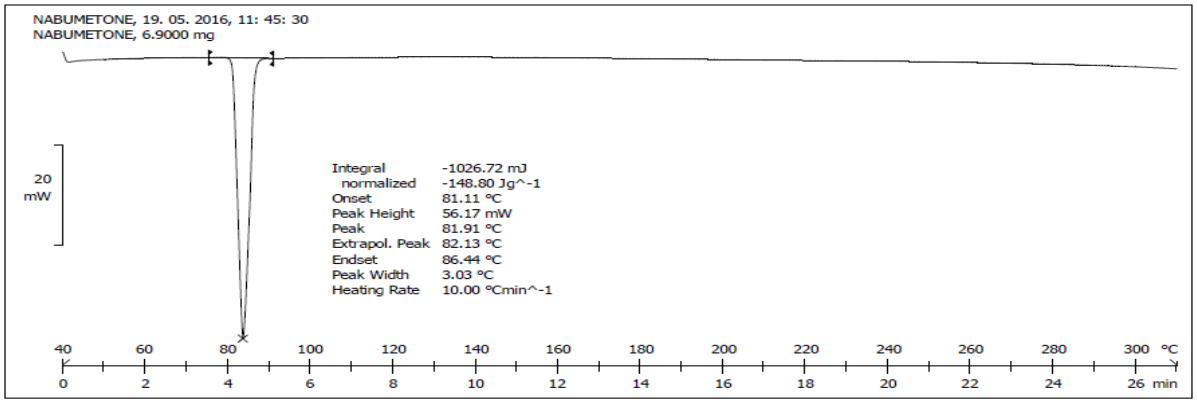


Figure 10: DSC Spectra of Nabumetone

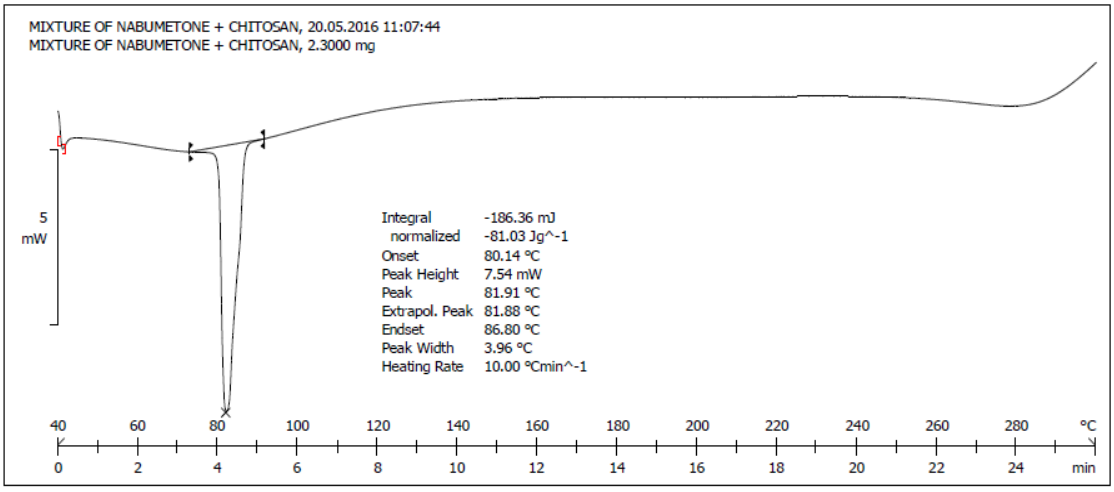


Figure 11: DSC Spectra of Nabumetone and Chitosan

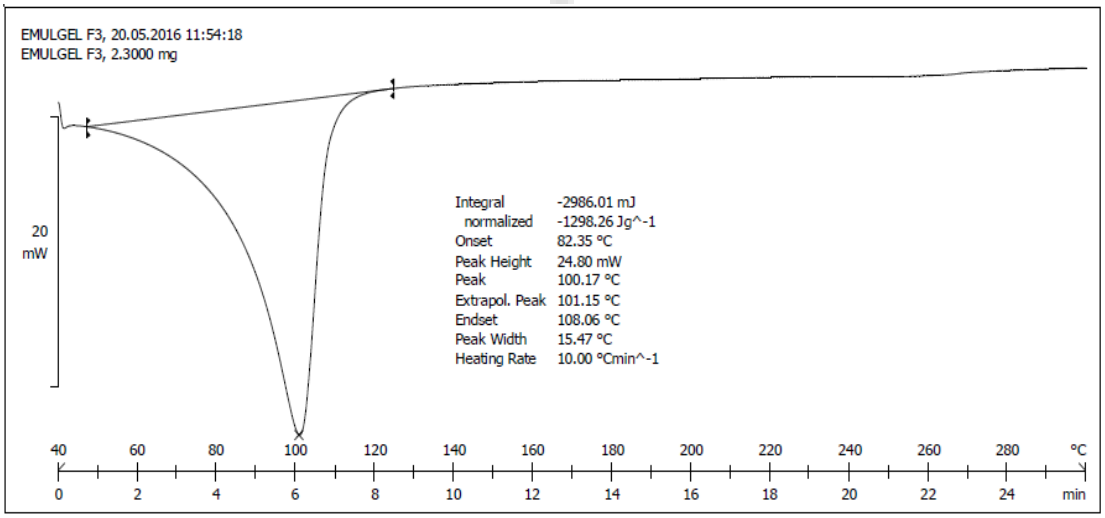


Figure 12: DSC Graph of Nabumetone and mixture of Excipients

Differential Scanning Colorimetry is a thermoanalytical technique used for analyzing thermal transitions involving thermal energy with great sensitivity. From the DSC analysis drug alone elicited a peak at 81.91^{o}C very close to the reported value of Nabumetone melting point. It was found that Nabumetone with physical mixture of Chitosan at 81.91^{o}C reflected characteristic feature of Nabumetone. These two peaks are close to each other and also another peak with drug close to each other .Thus, it was indicated that there was no physical interaction between Nabumetone and Excipients.

Characterization of Nanoemulsion

a) Physical appearance

Formulation was examined for appearance which shows transparent formulation. They do not show any turbidity.

b) Particle size Measurement

The of Particle size of Nanoemulsion is shown in Figure 13 and Table 8.

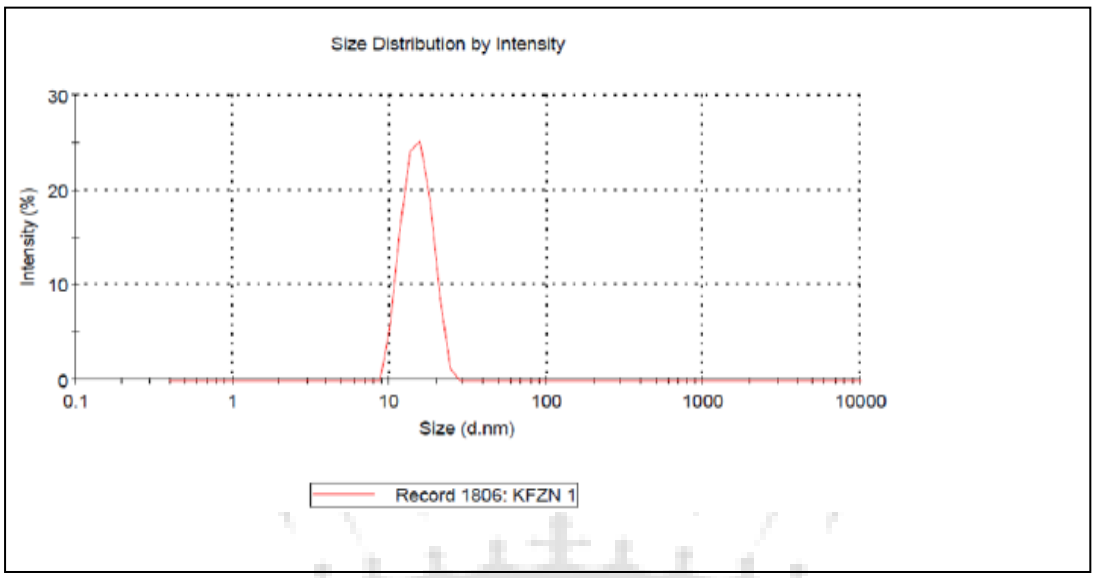


Figure 13: Particle size distribution

Table 8: Particle size of Nanoemulsion Formulations

Sr. No.	Formulation code	Particle size(nm)
1	F-A1	99.43
2	F-A2	90.41
3	F-A3	87.43
4	F-A4	78.45
5	F-A5	48.42
6	F-A6	52.43

It was concluded that peak was shown at the particle size 131.8 nm and the graph depict that it has a homogeneous distribution of particles. Thus the result showed that the particle size of

formed nanoemulsion was in the required range, therefore, a transparent nanoemulsion formulated successfully**.**

c) Zeta potential measurement

The Zeta potential of nanoemulsion is shown in Table 9 and Figure 14.

Table 9: Particle size of Nanoemulsion

Peak no	Zeta potential	Electrophoretic mobility
1	-0.1Mv	-0.000001 cm^2/Vs
2	-Mv	-cm^2/Vs
3	-mV	- cm^2/Vs

Zeta potential (mean) = -0.1mV, Electrophoretic mobility (mean) =-0.000001 CM^2/VS.

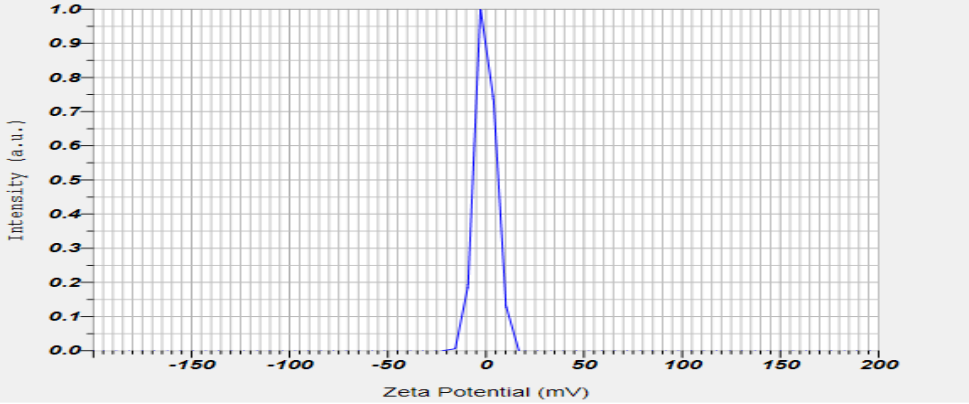


Figure 14: Zeta potential graph

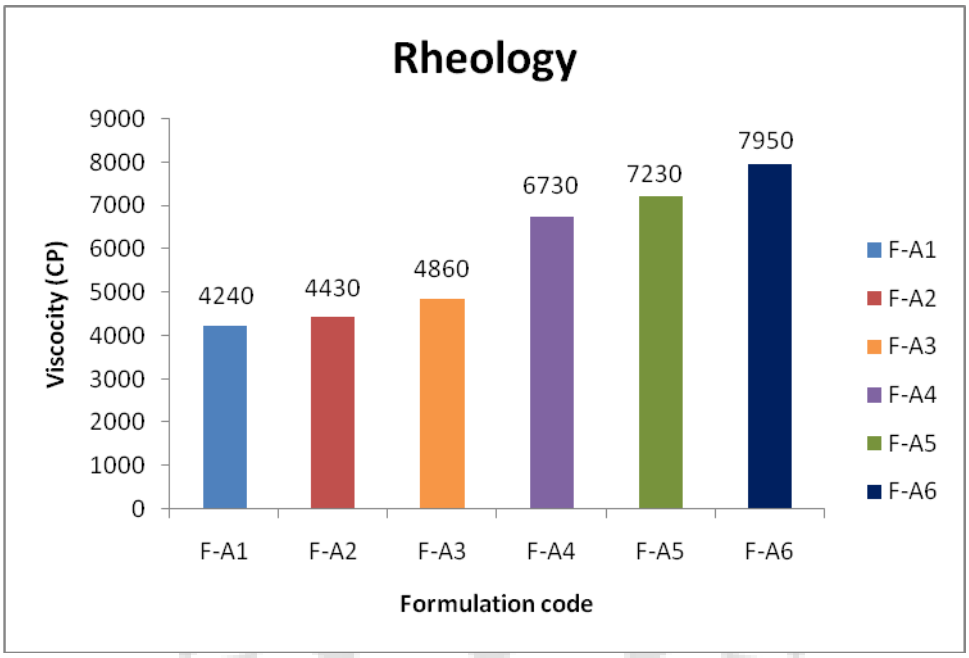
d) Thermodynamic stability study

Table 10: Thermodynamic stability study

Sr. No.	Formulation code	Heating cooling cycle	Centrifugation cycle	Freeze Thaw cycle	Inference
1	F-A1	×	√	×	Failed
2	F-A2	√	√	√	Passed
3	F-A3	√	√	√	Passed
4	F-A4	√	√	√	Passed
5	F-A5	√	√	√	Passed
6	F-A6	√	√	√	Passed

From the above study, it was concluded that formulation F-A2, F-A4, F-A5 and F-A6 are thermodynamically stable and formulation F-A1and F-A3 are thermodynamically unstable.

e) Rheology study



Graph 1: Rheology study of Nanoemulsion

The formulation F-A6 shows the high viscosity and formulation F-A1 shows low viscosity. The viscosity of nanoemulsion depends on the nature and concentration of emulsifying agents.

Characterization of Gel

a) P^H Determination

Table No.11: - P^H of gel

Sr.no	Formulation code	pH
1	GF1	6.45
2	GF2	6.47

The pH of gel in between 6 to 6.5 which lies in between normal pH range of skin which does not produce any skin irritation.

b) Rheology study of Gel

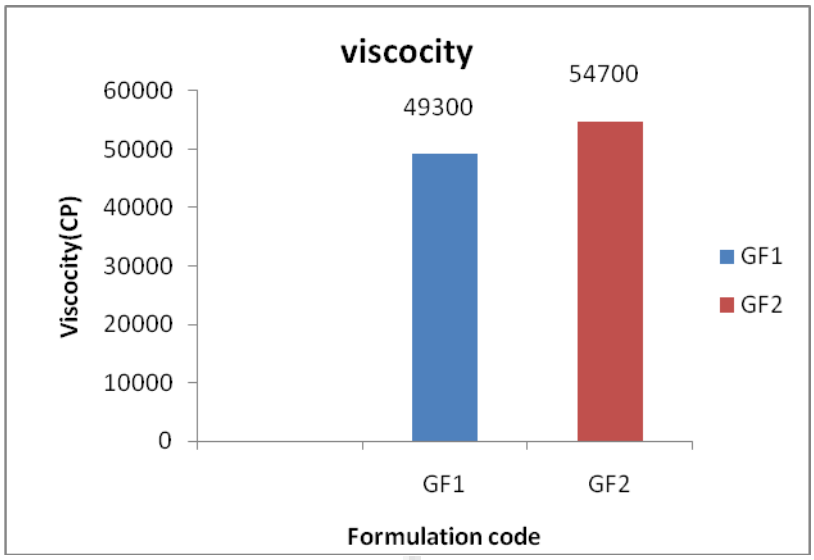


Figure 15: Rheology study of Gel Formulations

The gel formulation GF2 showed high viscosity than GF1. Viscosity of Gel depends on the concentration of Chitosan in 1% glacial acetic acid.

Characterization of Nanoemulsion Gel

a) Physical appearance

Table 12: Physical appearance of Nanoemulsion gel

Sr. No.	Formulation code	Colour and appearance	Phase separation	Grittiness	Homogeneity
1	E-A1	transparent white	None	None	Homogeneous
2	E-A2	transparent white	None	None	Homogeneous
3	E-A3	transparent white	None	None	Homogeneous
4	E-A4	transparent white	None	None	Homogeneous
5	E-A5	transparent white	None	None	Homogeneous
6	E-A6	transparent white	None	None	Homogeneous

Nanoemulsion-gel was found to be transparent white with viscous smooth and homogeneous texture.

b) pH Determination

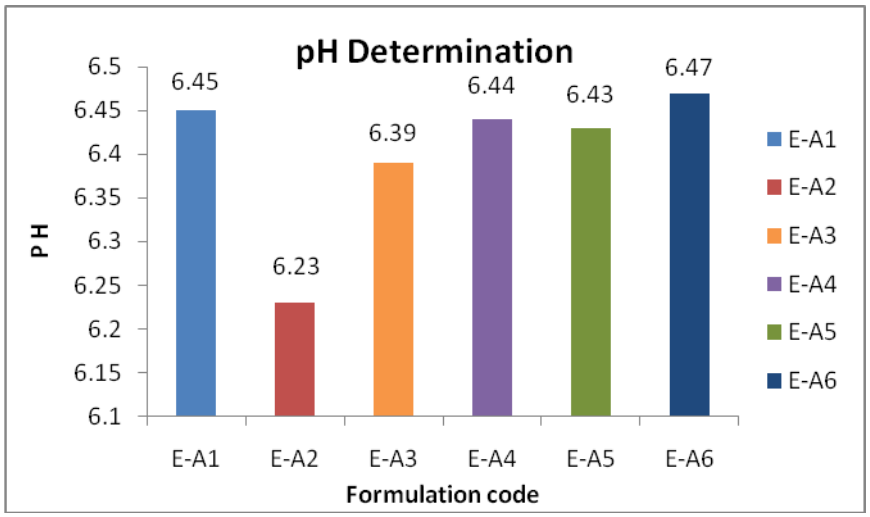


Figure 16: pH of Nanoemulsion Gel Formulations

The pH of Nanoemulsion gel was found to be in between 6 to 6.5 which lies in between normal pH range of skin hence which may not produce any skin irritation.

c) Rheology study of Nanoemulsion Gel

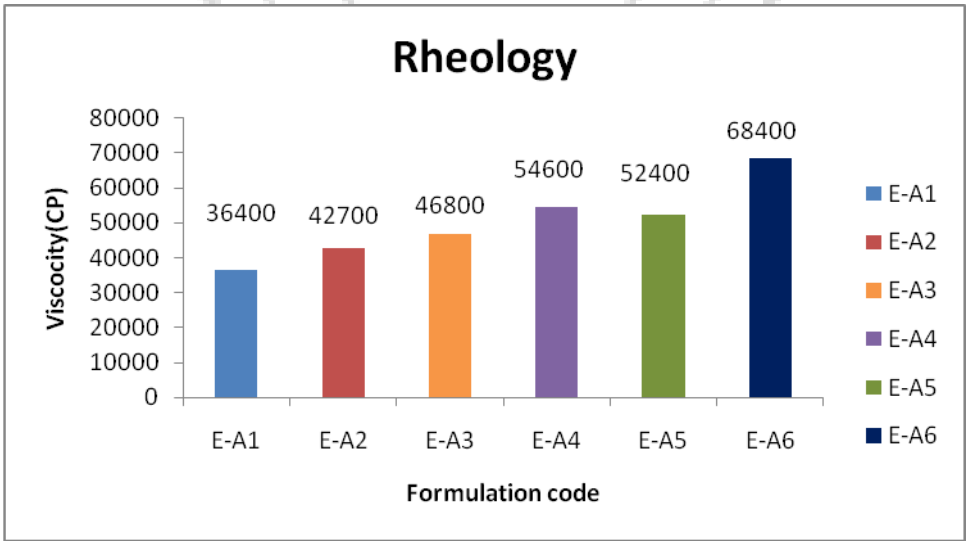


Figure 17: Rheology study of Nanoemulsion Gel Formulations

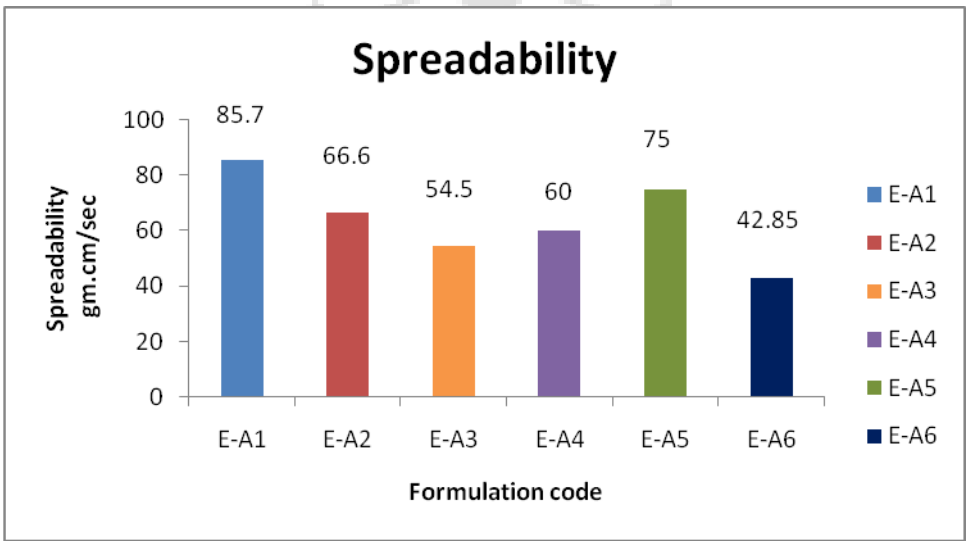
The formulation E-A5 showed high viscosity and formulation E-A1 showed low viscosity. The viscosity of Nanoemulsion gel depends upon the concentration and nature of gelling and emulsifying agents.

d) Spreadability

Table 13: Spreadability of Nanoemulsion Gel

Sr. no	Formulation code	M(gm)	L(cm)	T(sec)	Spreadability gm.cm/sec
1	E-A1	80	7.5	7	85.7
2	E-A2	80	7.5	9	66.6
3	E-A3	80	7.5	11	54.5
4	E-A4	80	7.5	10	60
5	E-A5	80	7.5	8	75
6	E-A6	80	7.5	14	42.85

The formulation E-A1 and E-A6 showed high Spreadability. Shorter interval showed the better spreadability.



Graph No.5:-Spreadability of Nanoemulsion Gel Formulations

e) Extrudability

Extrudability of Nanoemulsion Gel formulation has been checked as shown bellow.

Table No.14:-Extrudability of Nanoemulsion

Sr .no	Formulation code	Wt.extruded from tube
1	E-A1	0.67
2	E-A2	0.62
3	E-A3	0.70
4	E-A4	0.63
5	E-A5	0.72
6	E-A6	0.63

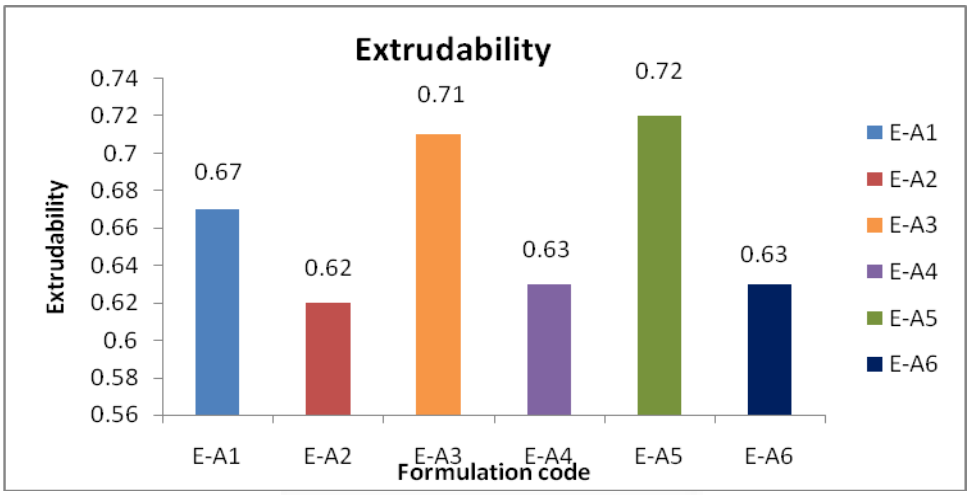


Figure 18: Extrudability of Nanoemulsion Gel Formulations

f) Swelling index

Swelling index of Nanoemulsion gel formulation is shown in Figure 19.

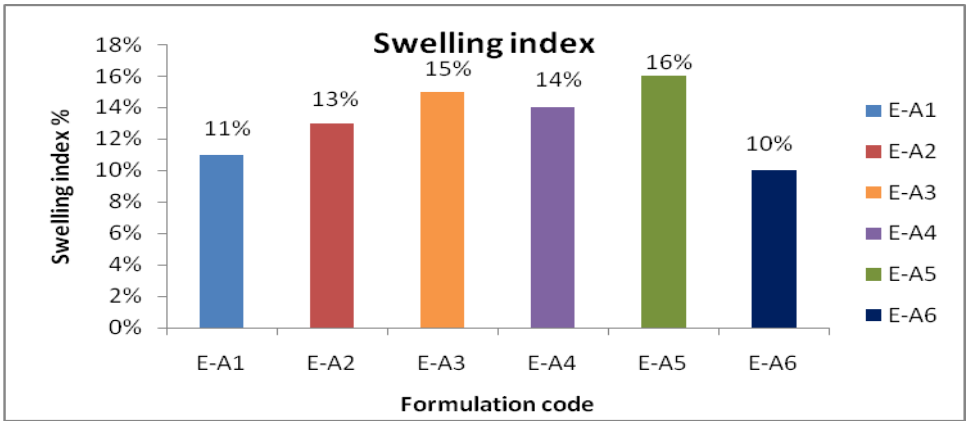


Figure 19: Swelling Index of Nanoemulsion Gel Formulations

g) *Ex-vivo* Bioadhesive strength measurement of Nanoemulsion Gel

Ex-vivo bioadhesive strength measurement of Nanoemulsion Gel formulation are shown in Figure 20.

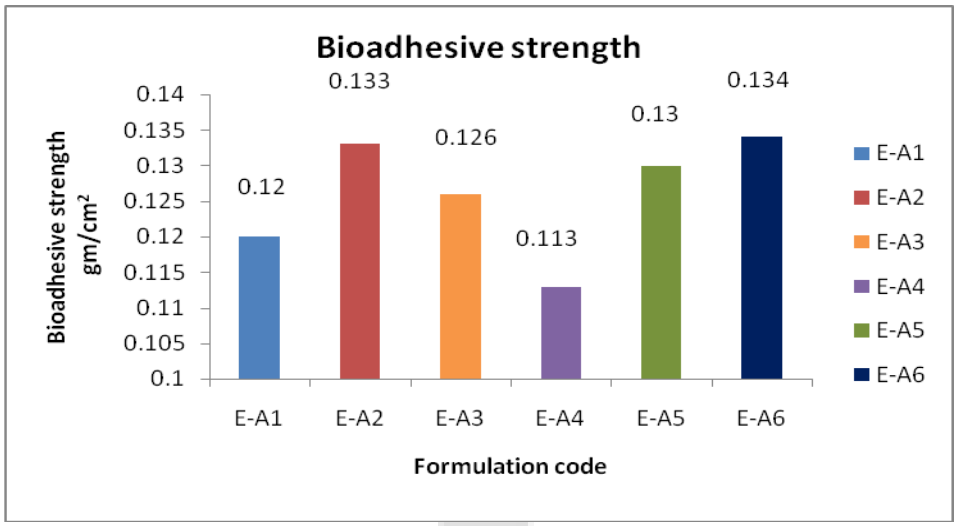


Figure 20: *Ex-vivo* Bioadhesive strength of Nanoemulsion Gel Formulations.

h) Drug content Determination

Table 15: Drug content

Sr. No.	Formulation code	Drug content
1	E-A1	87%
2	E-A2	92.03%
3	E-A3	88.89%
4	E-A4	91.7%
5	E-A5	93.55%
6	E-A6	92.55%

Drug content of Nanoemulsion gel was found in the range of 87.03% to 93.55%. The higher drug content was found in E-A5 i.e.93.55% and lower drug content was found in F1 i.e.87%.

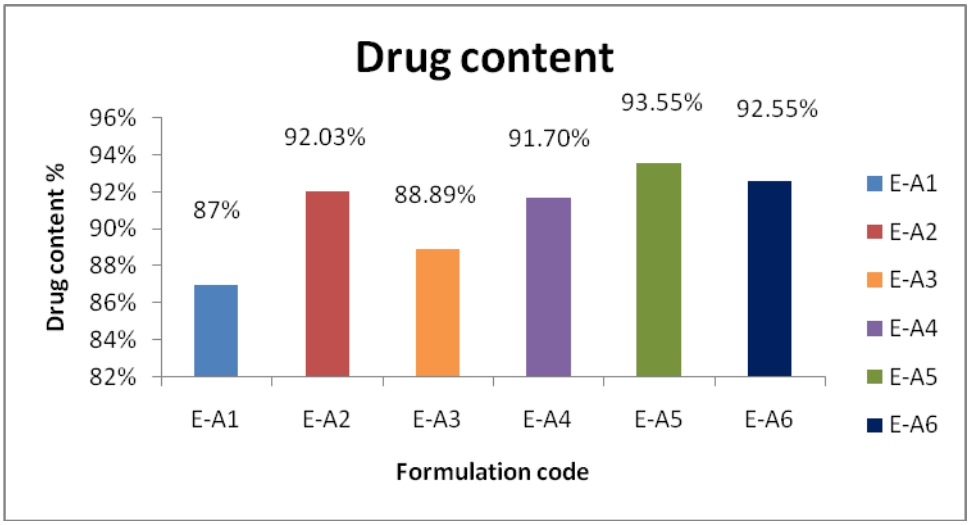


Figure 21: Drug content of Nanoemulsion Gel Formulations

i) *In-vitro* release studies

Table 16: *In-vitro* release of Nanoemulsion Gel Formulations

Time(min)	E-A1	E-A2	E-A3	E-A4	E-A5	E-A6	Conventional Emulgel
0	0	0	0	0	0	0	0
5	1.441	2.447	1.981	1.305	5.663	2.712	0.45
10	3.091	4.851	3.528	3.652	7.043	4.224	1.181
15	5.82	8.086	6.547	7.754	11.434	10.708	1.881
20	7.792	13.61	10.836	12.133	16.545	15.503	2.963
25	9.173	16.806	14.685	15.115	19.862	16.098	3.329
30	16.771	25.628	20.986	23.616	21.607	26.522	5.855
60	25.98	30.805	26.752	27.539	31.733	30.175	7.849
90	30.884	36.619	34.178	35.115	35.94	39.436	10.816
120	35.923	42.595	39.755	40.845	42.694	45.872	13.578
150	41.816	49.582	46.377	47.524	49.694	53.396	15.465
180	45.647	54.124	50.516	51.932	57.846	58.287	22.394
210	51.071	60.556	56.519	58.068	63.145	65.214	25.885
240	55.775	66.133	61.725	63.416	70.156	71.221	35.885
270	62.583	74.206	69.259	71.156	86.573	79.914	42.162
300	68.829	81.375	75.95	78.03	94.937	87.634	44.784

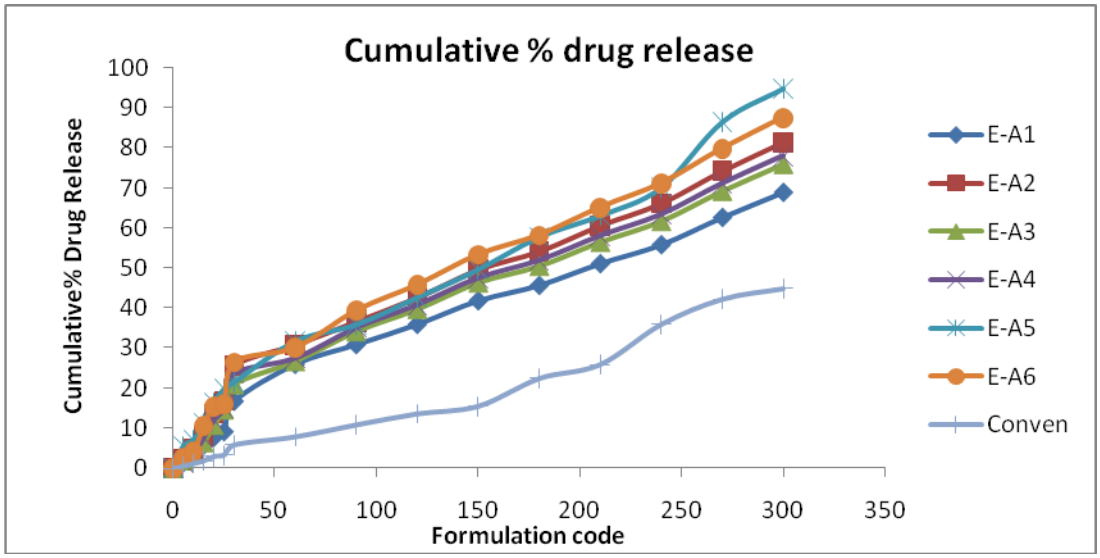


Figure 22: *In-vitro* release study

The *in-vitro* release of Nabumetone from the Nanoemulsion gel was varied in amount according to concentration of emulsifying agents used on formulations. The release of drug in following ascending order Conventional emulsion gel < E-A1 < E-A3 < E-A4 < E-A2 < E-A6 < E-A5. Where amount of % release 44.785% < 68.829% < 75.95% < 78.03% < 81.375% < 87.634% < 94.937%. From the study it was concluded that Nanoemulsion gel showed better drug release than conventional Emulsion Gel of Nabumetone within 300 min.

Kinetic model fitting

The best model fitted for formula E-A5 which showed 94.937 was Korsmeyer peppas with Slope n was found to be 0.7504 and k=1.3851 with R=0.9830.

j) *Ex-vivo* drug release study

Table 17: *Ex-vivo* drug release of Nanoemulsion Gel Formulations

Time (min)	E-A1	E-A2	E-A3	E-A4	E-A5	E-A6	Conventional Emulgel
0	0	0	0	0	0	0	0
5	3.094	3.399	2.99	3.656	4.09	2.836	1.006
10	4.354	4.783	3.552	5.145	5.756	6.399	1.852
15	7.257	7.972	8.587	8.576	9.594	9.896	2.177
20	10.42	11.447	11.894	12.314	13.775	13.921	3.329
25	15.082	16.569	16.789	17.824	19.939	19.703	4.281
30	22.102	24.287	23.109	26.121	29.22	27.908	5.664
60	27.646	30.372	29.906	32.672	36.559	35.283	6.741
90	32.864	36.104	35.361	38.839	43.477	41.735	7.15
120	38.226	41.995	40.968	45.177	50.536	47.403	10.8
150	44.497	48.884	47.525	52.587	58.827	55.179	14.591
180	48.575	53.362	50.787	57.404	64.215	60.233	20.394
210	54.345	59.703	56.822	64.226	71.846	67.392	26.876
240	59.35	65.202	62.055	70.141	78.965	73.598	35.246
270	66.596	73.16	69.63	78.703	88.041	82.582	43.751
300	73.028	80.228	76.357	86.306	96.546	90.56	49.598

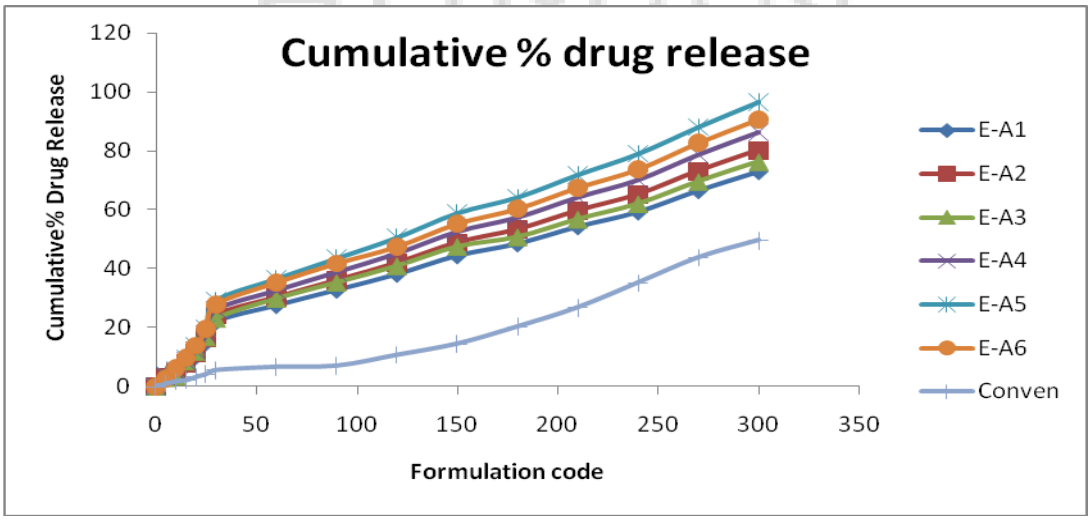


Figure 23: *Ex-vivo* drug release study

The *Ex-vivo* release of Nabumetone from the Nanoemulsion Gel was varied in amount according to concentration of emulsifying agents used in formulations. The release of drug in following ascending order Conventional emulsion gel < E-A1< E-A3 < E-A2 < E-A4 < E-A6 < E-A5. Where amount of % release 49.598% < 73.028% < 76.357 < 80.228% < 86.306 < 90.56% < 96.546%. From the study, it was concluded that Nanoemulsion gel showed better drug release than conventional Emulsion Gel of Nabumetone within 300 min.

Kinetic model fitting

The best model fitted for formula E-A5 which showed 96.546% was Korsmeyer peppas with Slope n was found to be 0.7504 and k=1.4086 with R=0.9830.

k) Skin irritation test (patch test)

There is no irritation, swelling, redness was observed after 24h by applying Nanoemulsion gel to the rat skin. Hence formulation was found to be safe for application on the skin.

m) Accelerated stability studies of Nanoemulsion Gel

Table 18: Stability study at 37^{o}C ± 2

Formulation code	Month 1		Month 2		Month 3	
	Drug content	pH	Drug content	pH	Drug content	pH
E-A1	87%	6.45	87%	6.45	86.93%	6.41
E-A2	92.03%	6.23	92.03%	6.23	91.01%	6.22
E-A3	88.89%	6.39	88.89%	6.39	88.70%	6.25
E-A4	91.7%	6.44	91.6%	6.44	91%	6.40
E-A5	93.55%	6.43	93.55%	6.43	93.55%	6.41
E-A6	92.55%	6.47	92.55%	6.47	92.30%	6.47

Table 19: Stability study at 45°C ± 2

Formulation code	Month 1		Month 2		Month 3	
	Drug content	pH	Drug content	pH	Drug content	pH
E-A1	87 %	6.45	86.90%	6.30	85.32%	6.2
E-A2	92.03%	6.23	91.10%	6.20	90.30%	6.1
E-A3	88.89%	6.39	87.30%	6.29	86.04%	6.25
E-A4	91.7%	6.44	90.40%	6.41	89.13%	6.35
E-A5	93.55%	6.43	92.35%	6.39	91.04%	6.31
E-A6	92.55%	6.47	91.20%	6.46	90.25%	6.43

Table 20: Stability study at 60°C ± 2

Formulation code	Month 1		Month 2		Month 3	
	Drug content	pH	Drug content	pH	Drug content	pH
E-A1	86.04%	6.3	85%	6.3	84%	6.2
E-A2	91.03%	6.2	90.34%	6.2	89.45%	6.1
E-A3	87.23%	6.2	86.08%	6.1	85.52%	6
E-A4	90.07%	6.3	89.74%	6.3	88.48%	6.2
E-A5	92. 34%	6.1	91.5%	6.1	90.08%	6.1
E-A6	91.45%	6.4	90.35%	6.4	89.45%	6.32

The stability study was carried out there is no significance changes in pH and drug content in formulation at 37°C ± 2**°C**.There was small changes in pH and drug content in the formulation at 45°C ± 2°C .There was significant changes in pH and drug content in formulation at 60°C ± 2°C .

Animal study of Nanoemulsion Gel

Table 22: Inflammation assessment

Sr. No.	Group	Paw edema(ml)
1	Vehicle control	1.195±0.07
2	Negative control	2.220±0.07##
3	Nabumetone Nanoemulsion Gel formulation	1.915±0.02*
4	Diclofenac Emulgel as standard	1.095±0.02**

N=6 Values are expressed as Mean±Sem comparison were made as follows ## P < 0.01 when compared with vehicle control* P < 0.05, **P < 0.01 when compared with negative control (values are expressed by one-way ANOVA)

CONCLUSION

In the coming years, topical drug delivery will be used extensively to impart better patient compliance. Since Nanoemulsion gel is helpful in enhancing spreadability, adhesion, viscosity and extrusion, this novel drug delivery become popular. Moreover, they will become a solution for loading hydrophobic drugs in water soluble gel bases for the long term stability. Similarly, in the study, topical Nanoemulsion gel of Nabumetone was formulated and subjected to physicochemical studies i.e. rheological studies, spreading coefficient studies and bioadhesive strength, *in-vitro* release studies and *ex-vivo* release studies through rat skin. *In-vitro* release of the tests formulations were performed to determine drug release from Nanoemulsion gel. From the *in-vitro* studies, formulation E-A5 showed maximum release of 94.937 % in 300 min. *Ex-vivo* drug release was also performed in which formulation E-A5 and E-A6 showed best release of 96.546% and 90.56% in 300 min. CFA-induced Arthritis and anti-inflammatory activity studied using Plethysmometer The formulations were comparable with marketed Diclofenac Emulgel. So Nabumetone Nanoemulsion gel can be used as an anti-inflammatory agent in Arthritis pain as topical drug delivery.

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