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Formulation and Evaluation of Nanoemulsion for Targeting and Systemic Delivery of Diclofenac Sodium

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Abstract **Original Research Article**

The aim of the present study was to prepare a nanoemulsion of diclofenac sodium for reduce side effects and improved oral bioavailability to treat arthritic conditions and investigate the potential of a nanoemulsion formulation for targeting and systemic delivery of diclofenac sodium. Various oil-in-water nanoemulsions were prepared by the titration emulsification method. The nanoemulsion area was identified by constructing pseudoternary phase diagrams. The prepared nanoemulsions were subjected to different thermodynamic stability tests. The nanoemulsion formulations that passed thermodynamic stability tests were characterized for viscosity, droplet size, transmission electron microscopy, size and zeta potential, and refractive index. The physical stability of nanoemulsion was studied using autoclaving, centrifugal, desorption (dilution effect) stresses and on storage. The selected formulations, F1, F2 and F3, were relatively stable during centrifugal stress, dilution stress and on storage. The cumulative percentage drug release from F1, F2 and F3 showed more release in pH 6.8 phosphate buffer than in pH 1.2 HCl. During oral bioavailability studies, the nanoemulsion showed higher serum concentrations than a suspension. The relative bioavailability of the nanoemulsion formulations F1, F2 and F3 were found to be that of F4 suspension and were statistically significant. Of all, the nanoemulsion (F3) was superior in improving bioavailability, when compared with plain emulsion (F1) and (F2). The study helps in designing the oral nanoemulsions to improve the oral bioavailability of diclofenac sodium.

Keywords: Nanoemulsion formulation, Diclofenac sodium, Oral bioavailability, Anti-inflammatory drug, DSC, SEM and TEM.

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INTRODUCTION

Diclofenac Sodium (DS) is widely used for antirheumatic, analgesic, osteoarthritis, and antipyretic activities [1]. The main challenge in systemic drug delivery via oral route is to overcome the arthritis problem. Many approaches have been used to enhance the penetration of drugs though rheumatoid arthritis [2, 3]. Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties in vitro [4, 5]. Nanoemulsions are novel drug delivery systems consisting of emulsified oil and water systems with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm and can exist as oil-in-water (o/w) or water-in-oil (w/o) form, where the core of the particle is either oil or water, respectively. Nanoemulsions are made from pharmaceutical surfactants that are generally regarded as safe (GRAS). The surfactant type and concentration in the aqueous phase are chosen to provide good stability against coalescence. Several types of oils-natural semi-synthetic and synthetic are used in the formulation of nanoemulsions. The capacity of nanoemulsions to dissolve large quantities of low soluble drugs along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation make them ideal drug delivery vectors [6]. The major advantages of nanoemulsions as drug delivery carriers include increased drug loading, enhanced drug solubility and bioavailability, and reduced patient variability, controlled drug release, and protection from enzymatic degradation [3]. Nanoemulsion droplet sizes fall typically in the range of 20-200 nm and show narrow size distribution [9]. Since, the preparation of the first nanoemulsion in the 1940s, it can be of three types such as oil-in-water (O/W), water-in-oil (W/O), and bicontinuous. The transformation between these three types can be achieved by varying the components of the emulsions. Each type of the nanoemulsions serves as a template for preparing polymer latex particles, Nanoporous polymeric solids etc [8-11].

Nanoemulsions have brought about a new direction and theological ideas to build better and more effective diagnostic and agents for different biomedical- based applications in the current biotechnology industry.

An ideal drug delivery system fulfils the objective of maximizing therapeutic effect while minimizing toxicity. With the progress in time and advances in science and technology, dosage forms have evolved from simple mixtures and pills, to highly sophisticated systems,which are known as novel drug delivery systems [7]. A lot of techniques are available for enhancing absorption of poorly water-soluble drugs, like use of lipid-based systems. Thus enhancement of aqueous solubility in such case is a valuable goal to successfully formulate them into bioavailable dosage forms. A range of novel strategies are currently being developed for efficient delivery of poorly water-soluble drugs, such as the formulation of amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes [8, 9].

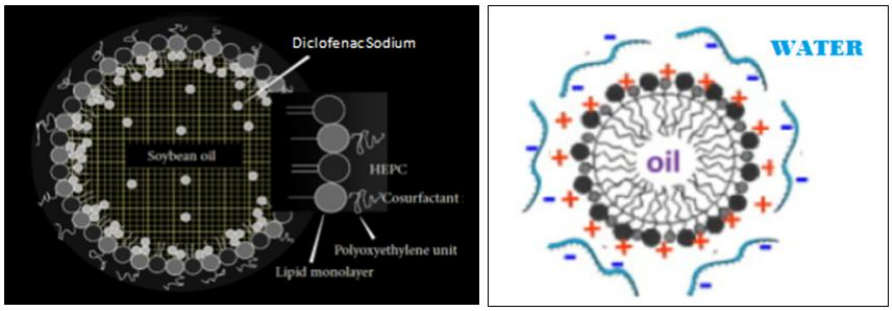

Fig-1: Diclofenac sodium-loaded nanoemulsion, figure-2: nanoemulsion- diclofenac sodium complex

Perspective drug delivery systems can be defined as mechanisms to introduce therapeutic agents into the body. Chewing leaves and roots of medical plants and inhalation of soot from the burning of medical substances are examples of drug delivery from the earliest times. However, these primitive approaches of delivering drugs lacked a very basic need in drug delivery; that is, consistency and uniformity (a required drug dose). This led to the development of different drug delivery methods in the later part of the eighteenth and early nineteenth century. Those methods included pills, syrups, capsules, tablets, elixirs, solutions, extracts, emulsions, suspension, cachets, troches, lozenges, nebulizers, and many other traditional delivery mechanisms. Many of these delivery mechanisms use the drugs derived from plant extracts [10].

Preparation of Nanoemulsion

Nanoemulsions are non-equilibrium systems of structured liquids [24-26], and so their preparation involves the input of a large amount of either energy or surfactants and in some cases a combination of both. As a result, high energy or low energy methods can be used in their formulation [25]. The high-energy method utilizes me- chanical devices to create intensely disruptive forces which break up the oil and water phases to form nanosized droplets. This can be achieved with ultrasonicators, microfluidiser and high pressure homogenisers [12-14].

Particle size here will depend on the type of instruments employed and their operating conditions like time and temperature along with sample properties and composition [15]. This method allows for a greater control of particle size and a large choice of composition, which in turn controls the stability, rheology and colour of the emulsion. Although high-energy emulsification methods yield nanoemulsions with desired properties and have industrial scalability, they may not be suitable for thermolabile drugs such as retinoids and macromolecules, including proteins, enzymes and nucleic acids.

Nanoemulsion can be prepared by a low energy emulsification method, which has been recently developed according to the phase behavior and properties of the constituents, to promote the formation of ultra-small droplets [16, 17]. These low-energy techniques include self-emulsification, phase transition and phase inversion temperature methods [18]. The low energy method is interesting because it utilizes the stored energy of the system to form small droplets. This emulsification can be brought about by changing the parameters which would affect the hydrophilic lipophilic balance (HLB) of the system like temperature, composition, etc [19, 20].

Table-1: Nanoemulsion preparation methods

Method of nanoemulsion prepration	
High energy emulsification method	Low energy emulsification method
Ultrasonification	Phase inversion method
High pressure homogenization	Solvent Displacement method
Using microfludizer	Phase Inversion Composition Method
Using high pressure homogenizer	

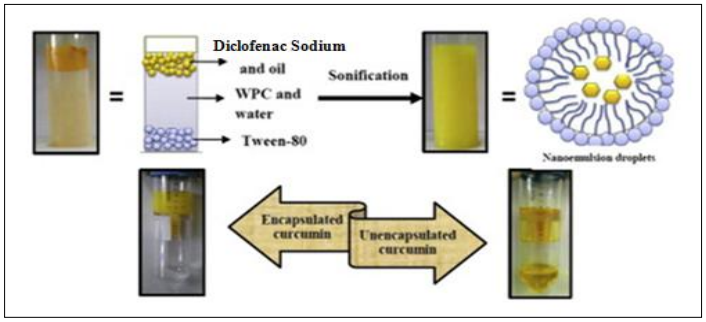

Fig-3: Prepration of nanoemulsion by ultrasonication method

Advantages of nanoemulsion

The attraction of nanoemulsions for application in personal care and cosmetics as well as in health care is due to the following advantages.

The very small droplet size causes a large reduction in the gravity force and the Brownian motion may be sufficient for overcoming gravity. This means that no creaming or sedimentation occurs on storage.

- The small droplet size also prevents any flocculation of the droplets.
- Weak flocculation is prevented and this enables the system to remain dispersed with no separation.
- The small droplets also prevent their coalescence, since these droplets are elastic, Surface fluctuations are prevented.
- Nanoemulsions are suitable for efficient delivery of active ingredients through the skin. The large surface area of the emulsion system allows rapid penetration of actives.
- The transparent nature of the system, their fluidity (at reasonable oil concentrations) as well as the absence of any thickeners may give them a pleasant aesthetic character and skin feel.
- Unlike microemulsions (which require a high surfactant concentration, usually in the region of 20% and higher), nanoemulsions can be prepared using reasonable surfactant concentration. For a 20% O/W nanoemulsion, a surfactant concentration in the region of 5% - 10% may be sufficient. Nanoemulsions are usually formulated with surfactants, which are approved for human consumption (GRAS), they can be taken by enteric route.
- The small size of the droplets allows them to de-posit uniformly on substrates. Wetting, spreading and penetration may be also enhanced as a result of the low surface tension of the whole system and the low interfacial tension of the O/W droplets.
- Nanoemulsions can be applied for delivery of fragrants, which may be incorporated in many personal care products. This could also be applied in perfumes, which are desirable to be formulated alcohol free.
- Nanoemulsions may be applied as a substitute for liposomes and vesicles (which are much less stable) and it is possible in some cases to build lamellar liquid crystalline phases around the nanoemulsion droplets [21, 22].

Disadvantages of nanoemulsion

Inspite of the above advantages, nanoemulsions have only attracted interest in recent years for the following reasons.

- Preparation of nanoemulsions requires in many cases special application techniques, such as the use of high pressure homogenisers as well as ultrasonics. Such equipment (such as the Microfluidiser) became available only in recent years.
- There is a perception in the personal care and cosmetic industry that nanoemulsions are expensive to pro- duce. Expensive equipment are required as well as the use of high concentrations of emulsifiers.
- Lack of understanding of the mechanism of production of submicron droplets and the role of surfactants and cosurfactants.
- Lack of demonstration of the benefits that can be obtained from using nanoemulsions when compared with the classical macroemulsion systems.
- Lack of understanding of the interfacial chemistry that is involved in production of nanoemulsions [21, 22].

MATERIALS AND METHODS

MATERIALS

Various chemicals, solvents, instruments and glassware are used during project work are listed below in Table 5.1, and Table 5.2.

Table-2: List of Chemicals

S. No.	Chemicals
1.	Diclofenac sodium
2.	Sodium CMC
3.	Tween 80
4.	Methanol
5.	Olive oil
6.	Arachis oil
7.	Castor oil
8	Liquid paraffin

Table-3: List of Equipments

S. No.	Equipments
1.	UV-spectrophotometer
2.	FTIR spectrophotometer
3.	Melting point apparatus
4.	Electronic balance
5.	Differential scanning calorimeter
6.	Magnetic stirrer
7.	Mechanical shaker
8.	Brookfield viscometer
9.	pH meter
10.	Thermometer
11.	Zeta potential and particle size analyzer

METHODS

PREFORMULATION STUDIES

Identification of Drug

Infrared spectroscopy (IR), ultra violet (UV) and melting point are used for identification and purity of drug sample. Diclofenac sodium was identified by various techniques which are following:

Organoleptic Property of the Drug

Drug (Diclofenac sodium) was Physically Characterized on the basis of colour, odour and taste. All these parameter ware recorded and compared with standard.

Identification of Drug by U.V Spectroscopy

10 mg of Diclofenac sodium was taken in volumetric flask and volume make up to 100 ml with methanol, 10 ml of above solution is diluted with methanol up to 100 ml and then it was scanned between 200 nm to 400 nm. The solution showed absorbance maximum at 274 nm in figure 6.1.

Identification of Drug by I.R. spectroscopy

The FTIR spectral analysis was carried out by pressed pellet technique. IR spectrum of any substance gives information about the group present in a specific substance. An IR spectrum of drug was taken using (KBr potassium bromide) pellets. Small quantities of drug sample were mixed with oil, and a drop was placed between KBr pellets and spread uniformly. The pellets were placed in the holder, and an infrared spectrum was taken. The range of scanning was 400-4000 cm^{-1}, Different peaks in the infrared spectrum were interpreted for presence of various group in the structure of the drug. The observed IR spectra of the drug are shown in Figure 6.2 and Figure 6.3.

Differential Scanning Calorimeter (DSC)

The sample of diclofenac sodium (about 5 mg) was loaded and sealed into DSC pan with a DSC loading puncher. The sample was scanned between 30-350°C at a heating rate of 10°C/ min, under nitrogen atmosphere (60 ml/min flow rate), using a differential scanning calorimeter, Perkin Elmer pyris 6 DSC (Massachusetts, U.S.A). An empty pan was used as a reference

Melting Point Determination

The temperature at which the solid and liquid phases are in equilibrium is called the melting point of substance. The melting point of a drug can be measured using three techniques:

- Hot stage microscopy
- Capillary melting method
- Differential scanning calorimeters thermal analysis

A melting point determination is a good first indication of purity since the presence of relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. Melting point of Diclofenac sodium was determined by capillary method using melting point apparatus.

10 mg of the drug sample was weighed accurately and placed into a capillary tube. Tube was placed in the melting point apparatus and was heated to a temperature below 5-10°C of the temperature at which powder started to melt, and temperature at which the sample started to melt was observed.

Solubility Determination

The solubility study of drug was performed in different solvents (e.g. methanol, ethanol, acetone, ethyl acetate, 0.1N HCl). A known quantity of drug was transferred in series of different solvents having volume 5ml in test tubes. Excess amount of drug was added to different solvents till the solution became saturated and these test tubes were shaken by mechanical shaker for 1 hr under constant vibration at constant temperature. After this period the solution were centrifuged. The supernatant was then analyzed by U.V. spectrophotometer (Shimadzu-1700, Japan) atλ_{max} 276 nm with appropriate dilution. Three determinations were carried out before each sample to calculate the solubility of Diclofenac sodium in different solvents.

Determination of Partition Coefficient of Drug

Partition coefficient of a drug is a measure of its hydrophilic-lipophilic balance (HLB). It can be defined as the ratio of unionized drug distributed between the organic and aqueous phase in equilibrium. Partition coefficient (solid water quotient of drug distribution) has a number of applications which are relevant to preformulation.

- Solubility both in aqueous and in mixed solvents
- Drug absorption *In-vivo*: applied to a homologous drug series for structure activity relationships
- Partition chromatography: choice of column (HPLC) and choice of mobile phase (eluent)

Partition coefficient of drug sample was determined by shake flask method. Equal volume of water (or phosphate buffer pH 6.8) and *n*-octanol were taken in glass stoppered flask and added accurately weight amount (10 mg) of Diclofenac sodium. The mixture was shaken for 24 hours at room temperature with the help of wrist action shaker. The two phases are

separated by separating funnel and the aqueous phase was analyzed spectrophotometrically at 273 nm for drug content after appropriate dilution. The drug concentration in n-octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of drug. The partition coefficient P is expressed as by the equation:

$$\textbf{Log P} = \frac{\textbf{Concentration in n-octanol}}{\textbf{Concentration in water}}$$

N-octanol is used because the properties of n-octanol are thought to resemble those of lipid bilayer membranes. It has therefore been suggested that distribution that distribution of chemicals into n-octanol simulates, to a certain extent, their ability to passively diffuse across biological membranes.

Procedure of Standard Curve Preparation

Standard Stock Solution of Diclofenac Sodium

Accurately weighed 10 mg of Diclofenac sodium and was dissolved in 100 ml of methanol, from this stock solution 10 ml was withdrawn and transferred into 100 ml volumetric flask. Volume was made with methanol in order to get standard stock solution containing 100 µg/ml.

Preparation of 0.2M Sodium Hydroxide Solution

Dissolved 8.0gm of Sodium hydroxide in distilled water and diluted the volume up to 1000 ml with distilled water.

Standard Stock Solution of Diclofenac Sodium

Accurately weighed 10 mg of Diclofenac sodium and was dissolved in 100 ml of methanol, from this stock solution 10 ml was withdrawn and transferred into 100 ml volumetric flask. Volume was made with methanol in order to get standard stock solution containing 100 µg/ml.

Standard Graph of Diclofenac Sodium

Form this standard stock solution, a series of dilution (10, 20, 30, 40, 50 µg/ml) were prepared using methanol. The absorbance of these solutions was measured spectrophotometrically against blank solution of methanol at 245 nm for Diclofenac sodium.

Preparation of pH 6.8 Phosphate Buffer (Simulated Saliva pH)

Place 50 ml of potassium dihydrogen phosphate buffer in a 200 ml volumetric flask. Add 22.4 ml of sodium hydroxide, mixed and volume was made up to 200 ml with distilled water.

Calibration Curve in 0.1N HCl

100 mg of Diclofenac sodium was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved in 100ml of 0.1N hydrochloric acid to get a solution of 1000 µg/ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with 0.1N HCl to get a solution of 100 ug/ml (Stock solution II). Further, 10 ml. of stock solution II was diluted up to 50ml with methyl orange solution (1%w/v) and extracted with chloroform (3x15 ml). Organic layers were separated and pooled. The volume of pooled organic layer was made up to 100 ml with sodium acetate solution (Stoke solution III). This stock solution III was used to prepare a series of standard Diclofenac sodium solutions as discussed below.

Procedure

From stock solution III aliquots of 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, & 2 ml were transferred to a series of 10 ml volumetric flasks. The volume was made upto 10 ml with 0.1N HCl to give 2, 4, 6, 8, 10, 12, 14, 16, 18, & 20 µg/ml of Diclofenac sodium. The absorbance of these solutions was measured at 508 nm against blank as shown in table 6.6. The standard plot obtained by the absorbance is shown in Figure 5.5 and the U.V graph is shown in Figure 6.5.

Calibration curve in Phosphate Buffer pH 6.8

Procedure

100 mg of Diclofenac sodium was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved in phosphate buffer pH 6.8 to get a solution of 1000 µg/ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with phosphate buffer pH 6.8 (Stock solution II). Further, 10 ml. of stock solution II was diluted up to 50 ml with methyl orange solution (1%w/v) and extracted with chloroform (3x15 ml). Organic layers were separated and pooled. The volume of pooled organic layer was made up to 100 ml with sodium acetate solution (Stoke solution III). This stock solution III was used to prepare a series of standard Diclofenac sodium solutions, concentration ranging from 10-20 ug/ml and the absorbance of these solutions was measured at 465 nm against blank as shown in table 6.7. The standard plot obtained by the absorbance is shown in figure 5.7 and the U.V graph is shown in Figure 6.6.

Calibration Curve in Phosphate Buffer pH 7.4:

Procedure

100 mg of Diclofenac sodium was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved in 100 ml phosphate buffer pH 7.4 to get a solution of 1000 µg/ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with phosphate buffer pH 7.4 to get a solution of 100 ug/ml (Stock solution II). Further, 10 ml. of stock solution II was diluted up to 50ml with methyl orange solution (1%w/v) and extracted with chloroform (3 × 15 ml). Organic layers were separated and pooled. The volume of pooled organic layer was made up to 100 ml with sodium acetate solution (Stoke solution III). This stock solution III was used to prepare a series of standard Diclofenac sodium solutions ranging from conc. 10-20 ug/ml and the absorbance of these solutions was

measured at 465 nm against blank as shown in Table 6.8. The standard plot obtained by the absorbance is shown in Figure 5.9 and the U.V graph is shown in figure 6.7.

FORMULATION DEVELOPMENT AND CHARACTERIZATION

Formulation of Nanoemulsion

Selection of Oil, Surfactant and Cosurfactant

Selection of excipients was done on the basis of solubility and miscibility studies. To evaluate the solubility of Diclofenac Sodium in different oils, surfactants and co-surfactants, excess amount of diclofenac sodium was added to each 2 ml of oils (Olive oil, Castor oil, Arachis oil, Liquid paraffin), surfactants (Tween 80) and co-surfactants (sodium CMC) in 5 ml stoppered vials and mixed using vortex mixer (Nirmal International, Delhi, India). The vials were then placed in an isothermal shaker at 25 ± 2ºC (Nirmal International, Delhi, India) for 72 h to reach equilibrium (shake flask method). The equilibrated samples were removed from shaker and centrifuged at 10,000 rpm for 0.25 h using a high speed centrifuge (Sigma- 3K30, Sigma Laboratory Centrifuges, Osterode am Harz, Germany). The supernatant was separated, dissolved in methanol and filtered through 0.2 µm membrane filter (Hi Media, India). The concentration of drug was determined by using UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) at 224 nm. The solubility studies were carried out in triplicate and results were reported as ±SD [23].

For miscibility studies equal amount (1:1 ratio) of selected oil was added to surfactant or co-surfactant and mixed using vortex mixer (Nirmal International, Delhi, India) for about 0.25 h and then the mixtures were allowed to stand for 24 h at room temperature and observed for any sign of turbidity, phase separation or colour change. Those mixtures which showed good miscibility with no sign of turbidity and phase separation and appeared clear were considered for the development of nanoemulsion. The oil, surfactant and co-surfactant which showed maximum solubility of Diclofenac Sodium were taken for further studies [23].

Construction of Pseudo-Ternary Phase Diagrams

In order to find out the region into which maximum amount of NE formation takes place, pseudo-ternary phase diagrams were constructed by aqueous phase titration method. Different phase diagrams were prepared from the result of solubility studies using Castor oil (oil phase), Tween 80 (surfactant), sodium CMC (co-surfactant) and distilled water (aqueous phase). Surfactant and co-surfactant (Smix) were mixed in different volume ratios (1:1, 1:2, 2:1, 3:1, 4:1 and 5:1) to obtain different pseudo-ternary phase diagrams. For each phase diagram, oil and Smix were mixed and vortexed thoroughly at different volume ratios starting from 1:9 to 9:1 in different glass vials [12, 14]. Ten different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5 (2:7), 1:3 (2:6), 3:7 (1:2.3), 1:2, 4:6 (1:1.5), were made so that maximum ratios were covered to form a clear and homogenous system. Slow titration with aqueous phase was done to these various mixtures of oil and Smix, using micropipette under continuous stirring by vortex mixing until formation of transparent oil in water (O/W) NE took place. Phase diagrams were plotted using CHEMIX School software version 4.0 (Arne Standnes, USA).The nanoemulsion region is marked on a pseudoternary phase diagram with one axis representing the aqueous phase, the second one representing oil and the third representing Smix (a mixture of surfactant and co-surfactant) at a fixed mass ratio [23].

Preparation of Diclofenac Sodium loaded NE

The Diclofenac Sodium loaded NE was prepared by titration method. In this method, predetermined amount of diclofenac sodium was dissolved in oil phase (Castor oil) using vortex mixer (Nirmal International, Delhi, India). To this mixture, fixed amount of Smix (Tween 80: Transcutol-P) was added and stirred continuously on magnetic stirrer (Remi Instrument Ltd., Mumbai, India). Then the specified amount of distilled water was added drop by drop to this mixture and stirred continuously until transparent and homogeneous NE is produced [23,24].

Physical Stability Testing of Nanoemulsions

Heating-Cooling Cycle

This test was done to see the effect of variations in temperature on the stability of nanoemulsion. In this test, diclofenac sodium loaded NE was subjected to store for three cycles between refrigerator temperatures i.e. 4ºC and 45ºC for not less than 48 h at each temperature [25].

Centrifugation Study

In this study, diclofenac sodium loaded NE was centrifuged at 5000 rpm for 30 mins to see any phase separation, creaming or cracking [25].

Freeze-Thaw Cycle

In this study, diclofenac sodium loaded NE was subjected to three freeze thaw cycles between -21ºC and +25ºC with storage at each temperature for not less than 48 h to find out the efficiency of dispersibility [25].

Characterization of Optimized NanoemulsionPercentage

Transmittance (%T)

The percentage transmittance (%T) of the prepared nanoemulsions was measured using UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) at 650 nm against distilled water as a blank [23].

Determination of Globule Size and Polydispersity Index

Globule size and polydispersity index (PDI) of NE was measured by using a Zetasizer (Nano- ZS90, Malvern Instruments, Worcestershire, UK) after suitable dilution with distilled water previously filtered with 0.45 µm membrane filters. Sample of 1 ml of NE was taken into clear polystyrene cuvettes for globule size and polydispersity index. Zetasizer is based on the principle of dynamic light scattering (DLS). In DLS, the sample is illuminated at scattering angle of 900 using helium-neon laser beam at the wavelength of 633 nm using an Avalanche photo diode detector and the intensity of the scattered light produced by the Brownian motion of the particles was analyzed that was dependent upon the size of the particles. All measurements were carried out at 25ºC [26].

Zeta Potential Measurement

The sample of 1ml was taken into disposable folded capillary cell and zeta potential was determined using zeta potential measuring instrument (ZS90, Malvern Instruments, and Worcestershire, UK). In case of zeta potential, electric field of -120 to 120V applies. Due to which particles move with a velocity related to their zeta potential. This velocity is measured using a He–Ne laser at the wavelength of 633 nm [26].

Transmission Electron Microscopy

The surface morphology of nanoemulsion was done using Transmission electron microscope (TEM) (CM 200, Philips Briarcliff Manor, NY, USA) to determine the shape of the dispersed phase. A drop of diluted NE was applied to a 300 mesh carbon coated copper grid and left for 1 min. Then the grid was kept inverted and stained with 1% phosphotungstic acid (PTA). Then the sample was allowed to dry and observed in TEM [26].

In-vitro Release Study

Dialysis Membrane

The dialysis membrane (capacity was 60 ml/ft, average flat width was 2.5 mm, diameter was 16 mm) used in this study to check the in-vitro release performance. Prior to the in-vitro release study, the proper treatment was done according to the directions written on the package. Glycerin was removed by washing in running water for 3-4 h. Sulphur compounds were removed by treating it with 0.3% w/v sodium sulphide solution at 80 ± 0.5ºC for 1 min and then washed with hot water at 60 ± 0.5ºC for 2 min.

After this treatment 0.2% v/v sulphuric acid was used to acidify the membrane and then rinsed with hot water to remove acid. Finally the membrane was immersed in simulated cerebrospinal fluid (CSF) (pH 7.4, 37 ± 2ºC) so that the pores remain saturated with that medium.

In-vitro Release by Dialysis Membrane

In-vitro release tests of the formulations and drug solution were performed using dialysis membrane (MWCO =12,000-14,000 Da, Hi Media, Mumbai, India) in simulated cerebrospinal fluid (CSF) (pH 7.4, 37 ± 2ºC). The dialysis bag was pretreated by soaking it in the simulated CSF for 24 h prior to the release study. The apparatus was set at 100 rpm and was maintained at 37±2ºC. In order to perform these tests, 2 ml of the formulation and drug solution (containing equivalent to 0.474 mg of drug) were placed in separate dialysis bag and dipped in 100 ml simulated CSF (containing 25% w/v methanol to maintain sink condition) maintained over magnetic stirrer (Remi Instrument Ltd., Mumbai, India). Three milliliters of samples was withdrawn at regular time intervals of 0, 0.5, 1, 2, 3, 4, 8, 10 and 24 h and the same amount of fresh simulated CSF was replaced every time to maintain sink condition. The samples were then analyzed in triplicate at 264 nm by UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) after suitable dilution. Cumulative amount of Diclofenac Sodium released was calculated by using the given equation [24, 26].

DRUG RELEASE KINETICS

The drug release kinetics were studied by various kinetic models such as Korsmeyer-peppas, Higuchi plot, first order plot and zero order plot. To study the release kinetics, data obtained from in vitro drug release studies were plotted in various kinetic models: zero order as cumulative amount of drug released *vs.* time, first order as log cumulative percentage of drug remaining *vs.* time, and Higuchi's model as cumulative percentage of drug released *vs.* square root of time.

The best fit model was confirmed by the value of correlation coefficient near to 1. The data was presented for the most appropriate model.

Zero Order: Graph was plotted between cumulative amounts of drug released *vs.* time

$$C = K0\ t \qquad \text{Eqn (1)}$$

Where, K0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration Vs time would yield a straight line with a slope equal to K0 and intercept the origin of the axes.

First Order: Graph was plotted between log cumulative percentages of drug remaining *vs.* time

$$\text{Log } C = \text{Log } C0 - kt/2.303 \qquad \text{Eqn (2)}$$

Where, C_0 is the initial concentration of drug, k is the first order constant, and t is the time.

Higuchi's Model: Graph was plotted between cumulative percentages of drug released *vs.* square root of time.

$$Q = Kt1/2 \qquad \text{Eqn (3)}$$

Where, K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time.

Korsmeyer-Peppas: The dissolution data was also fitted to the well-known Korsmeyer Peppas equation (as log cumulative percentage of drug released Vs log time), which is often used to describe the drug release behavior from polymeric systems and the exponent n was calculated through the slope of the straight line.

$$Mt /M\infty = Ktn \text{ or } \log Mt /M\infty = \log K + n \log t \qquad \text{Eqn (4)}$$

Where, Mt /M∞ is the fractional solute release, Mt is the amount of drug released at time t, M∞ is the amount of drug release after infinite time, t is the release time, K is a kinetic release rate constant characteristic of the drug/polymer system, and n is the diffusional exponent that characterizes the mechanism of drug release.

If the exponent n = 0.45, then the drug release mechanism is Fickian diffusion, and if 0.45 < n < 0.89, then it is non-Fickian or anomalous diffusion. An exponent value of 0.89 is indicative of Case-II Transport or typical zero-order release [26].

Interpretation of Diffusion Release Mechanisms

Table-4: Value of 'n' with corresponding drug release mechanism

Release Exponent 'n'	Mechanism of drug transport
< 0.5	Fickian transport
0.5< n < 1.0	Non – Fickian Transport
1.0	Case II transport
> 1.0	Super case II transport

Storage Stability

Three formulations of the optimized NE were prepared. These formulations were kept at a temperature of 40 ± 2ºC and 75 ± 5% RH for three months. Samples were withdrawn after specified time intervals (0, 30, 60 and 90 days) and examined visually for any physical change in the formulation. Globule size, zeta potential and % transmittance were determined at the end of 0, 30, 60 and 90 days.

Statistical Analysis

The results of the present study are reported as mean ± standard deviation. Comparison between the two groups was done by using Student's t-test. Differences were considered significant at **p<0.05.

RESULT AND DISCUSSION

Pre-formulation studies

Diclofenac sodium was procured from Yarrow Chem, Mumbai. It was identified and characterized as per the identification test given in the Indian Pharmacopoeia (2010) and United State Pharmacopoeia.

Identification of the Drug

Organoleptic Property

- Colour: - White or Crystalline powder
- Odor: - Odourless
- Appearance: - White
- Moisture sensitivity: - slightly hygroscopic

Identification of Drug by U.V Spectroscopy

Diclofenac sodium was scanned between 230 nm to 360 nm. The solution showed absorbance maximum at 276 nm. (Spectra of Diclofenac sodium in Methonal show below in Figure 4).

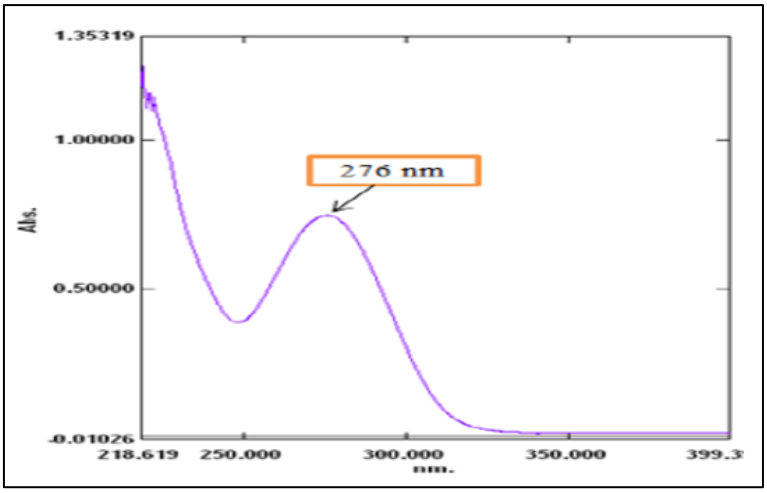

Fig-4: UV Scan of Diclofenac sodium in Methanol (276 nm)

FTIR Spectroscopy

The IR spectrum of the obtained sample was done acc. to the procedure mention in section 5.2.1.4 and complied with the IR spectrum of reference standard of Diclofenac sodium. IR spectra of sample drug show similar characteristic peaks. Figure 6.2 shows IR spectra analysis of standard drug Diclofenac sodium and Figure 6.3 shows the IR spectra of sample drug and the interpretation is shown in table 5**.**

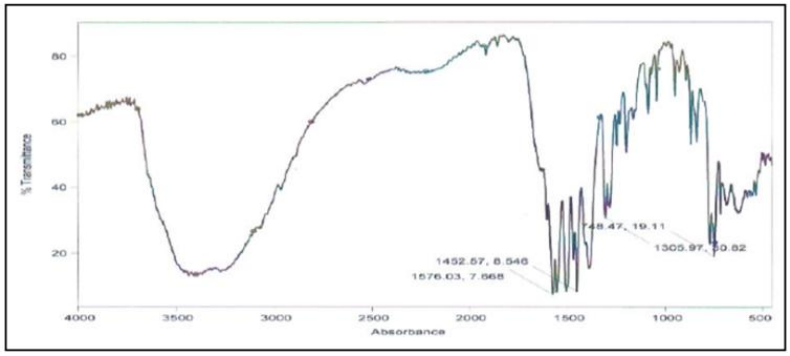

Fig-5: IR Spectra Analysis of Standard Diclofenac sodium (I.P. 1996)

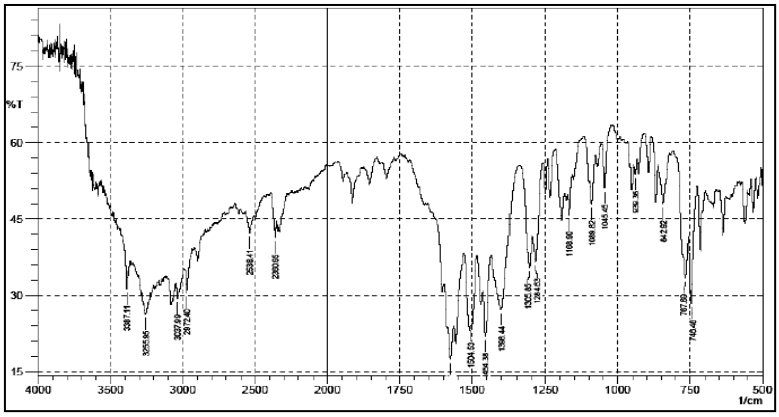

Fig-6: IR Spectra Analysis of Diclofenac sodium (Sample)

Table-5: Interpretation of Diclofenac sodium

S. no.	Reported peaks (cm^{-1}) in standard drug	Observed peak (cm^{-1}) of sample drug	Inference
1	785-540 (s)	766.87 (s)	C-Cl stretching
2	1300-1000 (s)	1197.19 (m)	C-N (amines) stretching
3	1350-1000 (m-s)	1273.95 (s)	C-O stretching
4	3150-3050 (s)	3250.92 (s)	C-H (aromatic) stretching
5	3500-3100 (m)	3252.92 (m)	N-H stretching

The peaks of sample drug are very close to the peaks of standard drug so it indicates the sample of Diclofenac sodium is authentic.

Differential Scanning Calorimeter (DSC)

The DSC thermogram of pure RHC is shown in figure 5.1. DSC thermogram showed a sharp endothermic peak at 161.92°C that was in agreement with the reported value 160-165°C. Thus it could be concluded that the sample of Diclofenac sodium was authentic and pure.

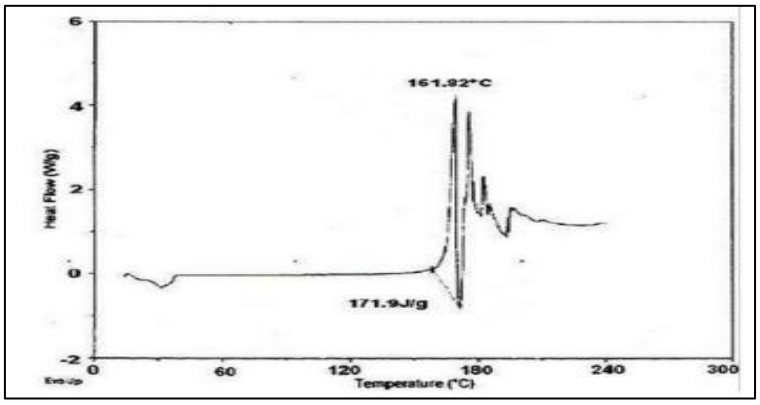

Fig-5: DSC thermogram of Diclofenac sodium

Determination of Melting Point

Melting point range of the drug having from 283-285 ℃ and Melting point of the drug was found to be 283.6 ºC. So the drug was found to be suitable for the formulation.

Solubility Study of the Drug

Qualitative

It was found that Diclofenac sodium was soluble in most of the organic solvent and insoluble in water as shown in table 6.

Table-6: Solubility Study of Drug

S. No.	Solvent	Interference
1	Water	Soluble
2	Ethanol	Insoluble
3	Methanol	Soluble
4	Chloroform	Insoluble
5	Acetone	Insoluble

Quantitative

The results of Quantitative solubility of the drug are given below in the following table 7.

Table-7: Solubility Study of Drug

S. No.	Solvent	Interference
1	Water	1.36 mg of drug was present in 1 ml of distilled water
2	Ethanol	0.024 mg of drug was present in 1ml of ethanol
3	Methanol	3.312 mg of drug was present in 1 ml of Methanol
4	7.4 pH Buffer	5.198 mg of drug was present in 1 ml of 7.4 pH buffer

Partition Coefficient of Diclofenac sodium

Partition coefficient of the drug was determined by the procedure mention under the section 5.2.1.7 and shown in table 6.4. The value of log P was found out to be 1.28. The standard value of log P for the drug is 1.40.

Table-8 Partition coefficient of Diclofenac sodium

Water: n- octanol (ml)	Conc. of drug in water (µg/ml)	Conc. of drug in n-octanol (µg/ml)	Log P
1:1	5.52	12.8	1.28

Preparation of Calibration Curve of Diclofenac sodium

Calibration curve of Diclofenac sodium were prepared as per the procedure mentioned.

Table-9: Standard Curve of Diclofenac sodium in Methanol

S. no	Concentration (µg/ml)	Absorbance
1.	0	0.000
2.	1	0.014
3.	2	0.025
4.	3	0.047
5.	4	0.055
6.	5	0.068
7.	6	0.081
8.	7	0.093
9.	8	0.105
10.	9	0.123
11.	10	0.131

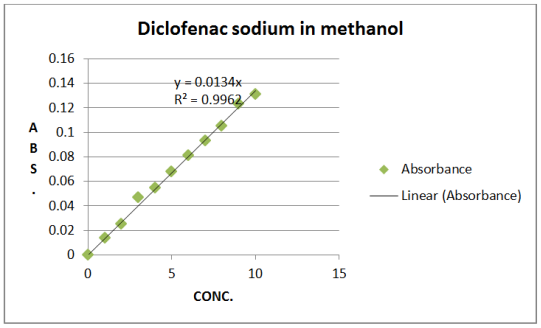

Fig-7: Calibration Curve of Diclofenac sodium in methanol at λ_{max} 276 nm

Table-10: Standard Curve Data of Diclofenac sodium in 0.1N HCl

S.No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.018
3	2	0.029
4	3	0.044
5	4	0.059
6	5	0.066
7	6	0.081
8	7	0.095
9	8	0.109
10	9	0.117
11	10	0.131

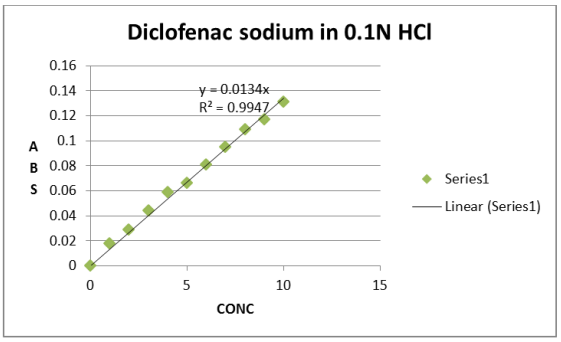

Fig-7: Calibration curve of Diclofenac sodium in 0.1N HCl at λ_{max} 276 nm

Table-11: Standard Curve Data of Diclofenac sodium in PBS pH 6.8

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.018
3	2	0.037
4	3	0.056
5	4	0.074
6	5	0.092
7	6	0.108
8	7	0.127
9	8	0.149
10	9	0.168
11	10	0.189

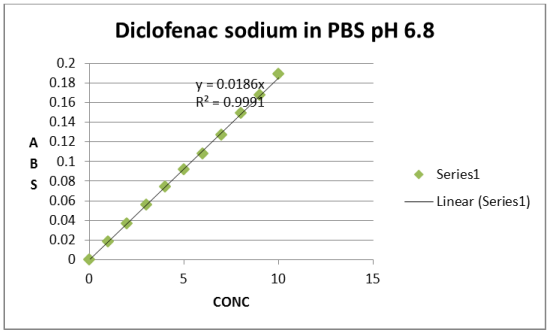

Fig-8: Calibration curve of Diclofenac sodium in PBS pH 6.8 at λ_{max} 276 nm

Table-12: Standard Curve Data of Diclofenac sodium in PBS pH 7.4

S. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	1	0.016
3	2	0.031
4	3	0.055
5	4	0.072
6	5	0.093
7	6	0.111
8	7	0.129
9	8	0.151
10	9	0.162
11	10	0.191

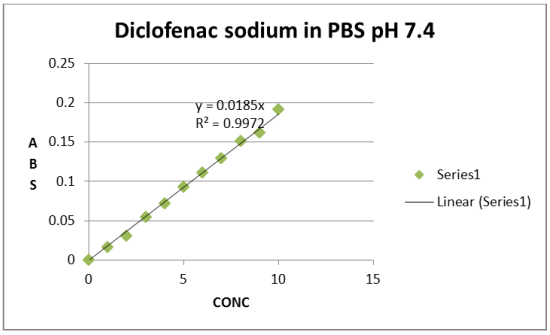

Fig-9: Calibration curve of Diclofenac sodium in PBS pH 6.8 at λ_{max} 276 nm

Formulation of nanoemulsion

Selection of oil, surfactant and cosurfactant

Nanoemulsion required oil, surfactant and co-surfactant for the formulation. The most important criteria for fabricating NE are the selection of most suitable components. A good solubility of drug in different components namely in oils, surfactants and co-surfactants remains the pre-requisite criteria. The aim of this study was to develop an intranasal drug delivery system where the dose volume should comparatively very low. Therefore, solubility of drug in oils is more important as the ability of NE is to maintain the desired dose in solubilized form.

The high solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in the solubilized form. The solubility of Diclofenac sodium was determined in oils, surfactants and co-surfactants. The results are shown in table 5.24. Among selected oils, Diclofenac Sodium had highest solubility in Castor oil (1.65 ± 0.07 mg/ml) followed by olive oil (0.31 ± 0.03 mg/ml) which might be due to the fact that Castor oil is itself an emulsifier also which has both lipophilic and hydrophilic group. Therefore Castor oil was selected as the oil phase. Among surfactants, diclofenac sodium showed highest solubility in Tween 80 (44 ± 2.1 mg/ml).

Therefore Tween 80 was selected as the surfactants. Tween 80 belongs to the class of non-ionic surfactant and is widely used since it is less toxic compared to ionic surfactant and is less affected by pH and ionic strength. For o/w emulsion, surfactant should have HLB > 10. Tween 80 has hydrophilic lipophilic balance (HLB) value is 15. Among co-surfactants, sodium CMC showed highest solubility of 49 ± 2.5 mg/ml. Therefore Transcutol-P was selected as the co-surfactant. Sodium CMC having HLB value of 4.2 has an ability to form transparent and stable NE.

Co-surfactant intercalates between surfactant molecules which decreases the interactions between polar head group at the interfacial layer, increases flexibility of interfacial film around nanoemulsion droplets and also increases the fluidity of the interfacial film by penetrating into the surfactant monolayer. Figure 5.9 represents solubility data for diclofenac sodium indifferent oils, surfactants and co-surfactants. All chemicals used were non-irritant and nonsensitizing to the skin, pharmaceutically acceptable and fall under GRAS (generally regarded as safe) category.

Table-13: Solubility of Diclofenac sodium in different oils, surfactants and co-surfactants

S. no.	oils, surfactants and co-surfactants	Solubility
1	Olive oil	0.31 ± 0.03
2	Arachis oil	0.74 ± 0.08
3	Castor oil	1.65 ± 0.07
4	Liquid paraffin	0.23 ± 0.02
5	Tween 80	44 ± 2.1 mg/ml
6	Sodium CMC	49 ± 2.5 mg/ml

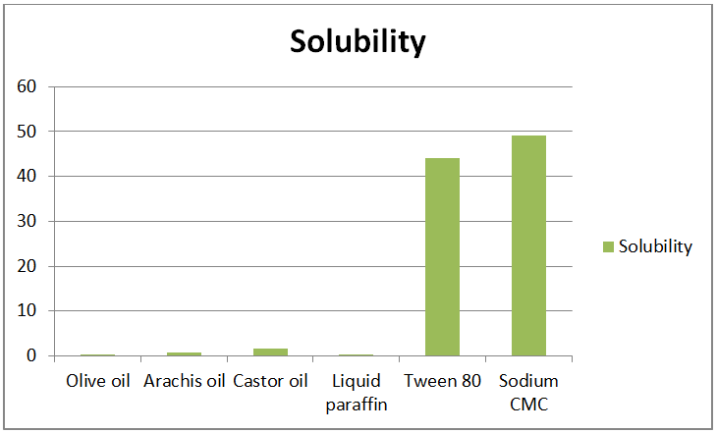

Fig-10: Solubility of Diclofenac sodium in different oils, surfactants and co-surfactants

CONCLUSION

Solubility study of Diclofenac sodium was done in different oils, surfactants and co-surfactants. On the basis of solubility study, Castor oil was selected as oil phase, Tween 80 as surfactant and sodium CMC as co-surfactant.

Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed separately for each Smix ratio and the results are presented in figure 5.9. Ternary plots were constructed using Castor oil as oil phase, Tween 80 as surfactant, sodium CMC as co-surfactant and distilled water as aqueous phase. Six phase diagrams of Smix ratios of 1:1, 1:2, 2:1, 3:1, 4:1 and 5:1 with shaded

region showing formation of NE system are presented in figure 5.10. From the figure, it was observed that Smix ratio of 4:1 showed maximum NE region when compared to 1:1, 1:2, 2:1, 3:1 and 5:1.

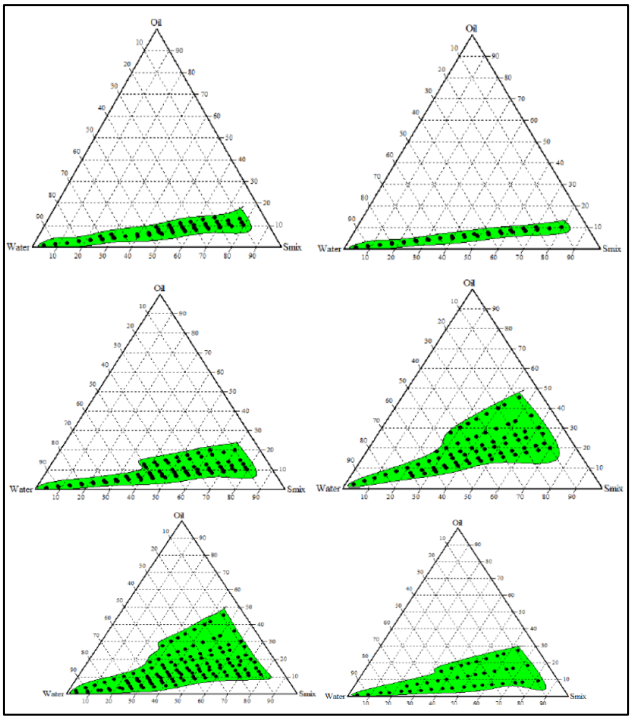

Fig-10: Pseudo-ternary phase diagrams system containing the following components: Castor oil as oil, Tween 80 as surfactant, sodium CMC as cosurfactant. Dotted area shows O/W nanoemulsion region in different ratio of surfactant to cosurfactant in 1:1, 1:2, 2:1, 3:1, 4:1 and 5:1

Table-14: Percentage nanoemulsion region obtained for different ratios of Tween 80: sodium CMC

Ratios of Tween 80 : sodium CMC	Percentage nanoemulsion region ± SD (n=3)
1:1	18.27 ± 0.45
1:2	11.64 ± 0.64
2:1	22.82 ± 0.57
3:1	41.16 ± 1.02
4:1	47.21 ± 0.89
5:1	27.11 ± 0.59

CONCLUSION

Pseudo-ternary phase diagrams were constructed by aqueous phase titration method. S_{mix} ratio of 4:1 was selected for NE as it showed maximum nanoemulsion region which was determined by the cut and weigh method.

Physical Stability testing of nanoemulsions

Nanoemulsions are considered to be kinetically stable systems which are produced at a particular concentration of oil, surfactant and water, with no sign of phase separation, creaming or cracking. Optimized Diclofenac sodium loaded nanoemulsion was subjected to different stress/thermodynamic stability tests like heating-cooling cycle, centrifugation study and freeze-thaw cycle. It was observed that there was no sign of instability such as precipitation, phase separation, creaming, cracking and coalescence during these stress/thermodynamic stability tests.

Table: 15: Physical Stability testing of nanoemulsions

FORMULATION	FT	CS	HCC	INFERENCE
NE	√	√	√	Passed

Characterization of optimized nanoemulsion

Percentage transmittance (%T)

The percentage transmittance (%T) of the prepared nanoemulsions was measured using UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) at 650 nm against distilled water as a blank.% Transmittance of optimized formulation was calculated to be 98.13 ±2.21%.

Determination of globule size and polydispersity index

Globule size and polydispersity index (PDI) of NE was measured by using a Zetasizer (Nano- ZS90, Malvern Instruments, Worcestershire, UK) after suitable dilution with distilled water previously filtered with 0.45 µm membrane filters. Mean globule size and PDI of optimized formulation was 35.75 ± 0.21 nm and 0.247 ± 0.04 respectively.

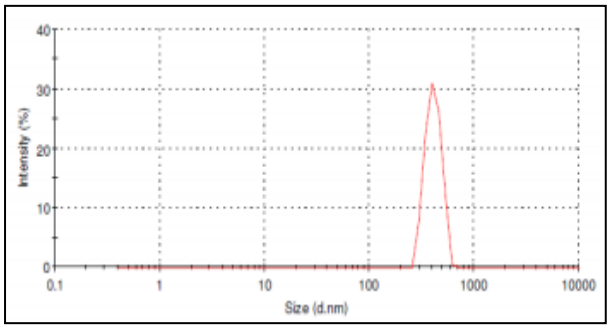

Fig-11: Globule size distribution of optimized nanoemulsion formulation

Zeta potential measurement

The sample of 1ml was taken into disposable folded capillary cell and zeta potential was determined using zeta potential measuring instrument (ZS90, Malvern Instruments, and Worcestershire, UK). In case of zeta potential, electric field of -120 to 120V applies. Zeta potential of optimized formulation was -35.2 ± 0.67 mV.

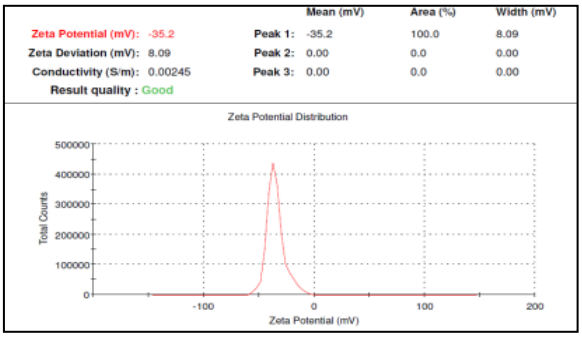

Fig-11: Zeta potential of optimized nanoemulsion formulation

Transmission Electron Microscopy

TEM study of optimized NE was done to find out more information about the morphology and mean diameter of the globules of the nanoemulsion system. TEM has indicated that most of the oil globules were of uniform shape (spherical) and in the nanometer range (size range 33–40 nm) (Figure 5.15).The sizes of globules were in the further agreement with the results obtained using dynamic light scattering (DLS).

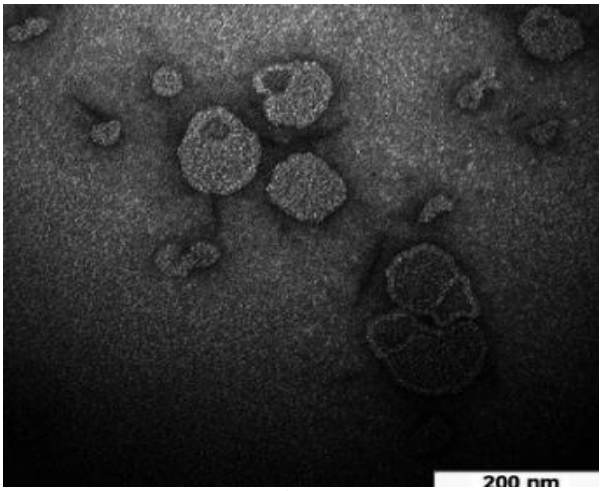

Fig-12: TEM image of optimized nanoemulsion

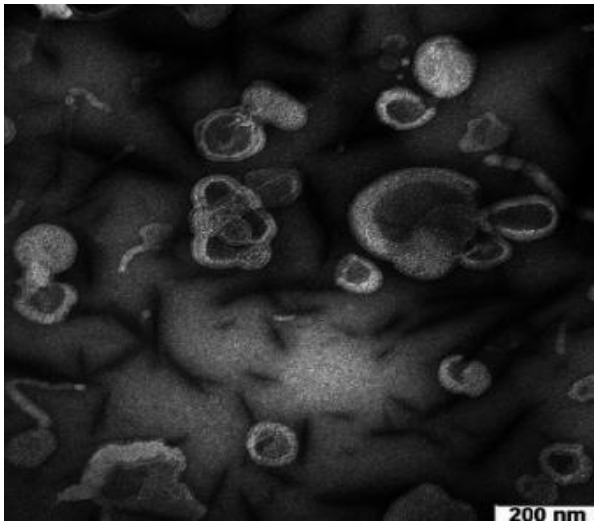

Fig-13: TEM image Diclofenac sodium

Table-16: Characterization of Diclofenac sodium loaded nano emulsions

Code	Particle size (nm)	Polydispersity	Zeta potential (mv)	Drug content (percent)
F1	128.50±3.8	0.24±0.05	-0.08±0.01	97.67±6.5
F2	72.60±1.7	0.13±0.01	-7.69±0.83	97.04±4.3
F3	27.80±0.72	0.12±0.01	-17.20±1.05	100.2±1.4
F4	121.90±4.2	0.17±0.01	-27±0.65	78.6±5.8
F5	65.29±1.9	0.28±0.04	-22.83±1.69	74.89±4.7
F6	49.42±0.8	0.21±0.01	-21.37±0.77	79.74±3.9
F7	85.2±2.7	0.09±0.01	-17.76±0.7	97.4±5.1
F8	69.30±1.62	0.12±0.01	-15.38±1.06	101.67±3.0
F9	48.31±1.73	0.273±0.04	-14.57±0.92	99.68±7.2
F10	155.6±11.4	0.281±0.06	-19.88±0.24	91.3±2.68

Data represents Mean ± SD, n=3

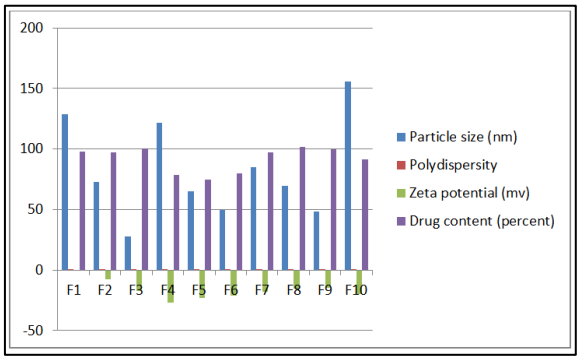

Fig-14: Characterization of Diclofenac sodium loaded nano emulsions

In-vitro release study

The percentage of cumulative drug release studies of RHC from NE and drug solution were performed in simulated CSF pH 7.4. The results of the in vitro drug release studies are presented in Figure 5.16. The percentage of cumulative release of RHC from NE was 88.90 ± 4.2 over a period of 24 h whereas almost all drugs was released from drug solution after 4 h. From the figure it was observed that NE showed initially burst release (which might be due to presence of nanodroplets near the surface of the NE) then followed by sustained release which might be due to the fact that the release of RHC from the oily core at oil-water interface was hindered by the aqueous medium (acts as a barrier for drug transport) and dialysis bag (acts as a physical barrier to the release of drug as only free drug can pass through nanosized pores). The data so obtained from in-vitro drug release studies (for the optimized formulation) were fitted to various release kinetic models such as zero order, first order, Higuchi model and Peppas model to understand the mechanism of drug release from the nanoemulsions and the results suggested that release of drug from NE follow Higuchi model as indicated by the highest value of coefficient of correlation (R2=0.961). This could be explained as the dialysis membrane acted as barrier or controlling membrane therefore diffusion process become closed to reservoir system than zero-order (concentration independent) or first-order (concentration gradient) diffusion. Hence Higuchi model was selected as best fit model.

Table-17: Comparative cumulative % drug release between drug solution and NE

Time (h)	Cumulative % drug release from Diclofenac sodium solution (± SD)	Cumulative % drug release from Diclofenac sodium -NE (± SD)
0.5	35.64± 5.1	22.22 ± 4.1
1	44.89± 5.7	28.43 ± 4.8
2	64.36± 4.3	34.01 ± 5.1
3	71.18 ± 4.6	41.10 ± 4.2
4	99.18± 4.8	46.32 ± 4.3
6	-	55.76± 4.9
8	-	69.62 ± 3.5
10	-	74.21± 5.8
24	-	89.90 ± 4.2

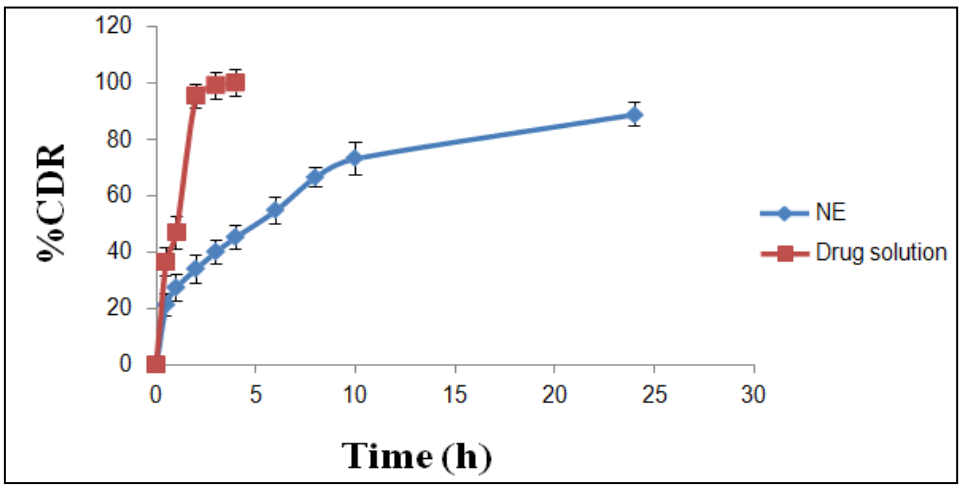

Fig-15: Comparative cumulative % drug release between drug solution and NE

Mechanism of drug release from Diclofenac sodium loaded NE

The analysis of kinetics of release of the optimized nanoemulsion was undertaken to find out the release mechanism of developed formulation.

Table-18: Kinetic analysis of Diclofenac sodium loaded NE (n=3)

Time (min)	Square root of time	Log time	% Cumulative release	Fraction drug release	Log % drug released	% Drug remaining	Log % drug Remaining
30	5.477	1.477	21.2	0.213	1.326	78.8	1.896
60	7.745	1.778	27.4	0.275	1.437	72.6	1.86
120	10.954	2.079	34	0.35	1.531	66	1.819
180	13.416	2.255	40.1	0.411	1.603	59.9	1.777
240	15.491	2.38	45.3	0.463	1.656	54.7	1.737
360	18.973	2.556	54.76	0.517	1.738	45.24	1.655
480	21.908	2.681	66.6	0.656	1.823	33.4	1.523
600	24.494	2.778	73.21	0.742	1.864	26.79	1.427
1440	37.947	3.158	88.9	0.899	1.948	11.1	1.045

Table-19: Co-efficient of correlation for optimized NE

Zero Order		First Order		Higuchi Model		Korsmeyer-peppas	
R^2	K_0	R^2	K_1	R^2	K_H	R^2	K
0.759	0.000	0.960	-0.000	0.961	0.023	0.946	0.611

Storage stability

Stability studies were conducted for all nanoemulsion compositions (F3, F4, F8, F9 and F10) that possess narrow globule size, high drug content and best drug release characteristics by storing them at 4°C for 60 days. The particle size and zeta potential were determined after 30 days and 60 days and the results were reported in Table 4 and 5. After storage at 4°C no significant change in the zeta size and zeta potential were observed in optimized formulations. After centrifugation at 5000 rpm for 5 hrs and at 10000 rpm for 30 min, no phase separation or creaming was found upon visual observation. Nanoemulsions that are stable in centrifugation, heating and cooling cycles were subjected to freezing and thawing. At the end of 3 cycles, the particle size was slightly increased, however no significant difference in zeta potential was observed.

Table-20: Effect of storage at 4°C on particle size

Code/day	F3	F4	F8	F9	F10
0 day	25.80±0.72	49.42±0.8	68.30±1.62	48.31±1.73	46.96±2.18
30th day	23.125±0.05	48.24±0.97	71.12±0.61	48.84±1.45	48.82±1.5
60th day	26.7±0.86	48.65±0.28	72.48±0.64	47.92±2.36	49.73±0.85

Data represents Mean ± SD, n=3

Table-21: Effect of storage at 4°C on zeta potential

Code/day	F3	F4	F8	F9	F10
1st day	-17.20±1.05	-21.37±0.77	-15.38±1.06	-12.57±0.92	-19.5±1.36
30th day	-17.85±0.8	-17.65±0.14	-14.32±0.59	-15.87±1.7	-17.48±1.4
60th day	-18.68±0.69	-16.44±0.17	-16.52±0.17	-15.64±0.8	-18.29±1.6

Data represents Mean ± SD n=3

CONCLUSION

The aim of the present study was to develop nanoemulsion of Diclofenac sodium that could deliver the drug through oral route to avoid first pass metabolism and to avoid the distribution to non-targeted site.

The summary of the results is given below.

From the physical properties and identification tests of the drug sample, it was concluded that the drug sample diclofenac sodium was authentic, pure and confirming to the standards.

To accomplish this research plan first a UV method for the estimation of diclofenac sodium in methanol was validated.

The highest solubility of diclofenac sodium was achieved with Castor oil. The solubility in Castor oil was 1.73 ± 2.64 mg/ml, so it was selected as an oil phase for making nanoemulsion. Nanoemulsions were formulated by titration method. The phase behavior of different surfactant, co-surfactant and their combinations was determined by constructing ternary phase diagrams. Percentage nanoemulsion region obtained for different groups were determined. The 4: 1

ratio of Tween 80: sodium CMC yielded broad regions of nanoemulsion, so this combination was selected for further studies.

The variation in the globule size, PDI and zeta potential was predicted by employing response surface methodology as the responses were the function of the emulsion composition. It was observed that with increase in concentration of Smix upto a certain level, globule size of nanoemulsions was decreased, after that further increase in concentration of Smix leads to increase in globule size which showed that emulsifier plays a vital role in the formation of emulsion. It was also observed that with increase in concentration of Smix upto a certain level, PDI of nanoemulsions was decreased, after that further increase in concentration of Smix leads to increase in PDI. It was found that when concentration of oil was increased, zeta potential of nanoemulsions was also increased. From the study, it was observed that the variation in Smix and water concentration did not show any influence on zeta potential.

From the physical stability testing, it was observed that the optimized RHC loaded NE was stable and there was no sign of instability such as precipitation, phase separation, creaming, cracking and coalescence during these tests. From the TEM studies, it was found that most of the oil globules were of uniform shape (spherical) and in the nanometer range (size range 33–40 nm) and in agreement with the results obtained using dynamic light scattering (DLS).

The percentage cumulative drug release from optimized NE was 88.90 ± 4.2 over a period of 24 h whereas almost all drugs was released from Diclofenac sodium solution after 4 h. From the study it was observed that NE showed initially burst release which might be due to presence of nanodroplets near the surface of the NE then followed by sustained release which might be due to the fact that the release of drug from the oily core at oil-water interface was hindered by the aqueous medium (acts as a barrier for drug transport) and dialysis bag (acts as a physical barrier to the release of drug as only free drug can pass through nanosized pores). From the studies of mechanism of drug release, it was suggested that release of drug from NE follow Higuchi model as indicated by the highest value of coefficient of correlation (R2 =0.961). This could be explained as the dialysis membrane acted as barrier or controlling membrane therefore diffusion process become closed to reservoir system than zero-order (concentration independent) or first-order (concentration gradient) diffusion. Hence Higuchi model was selected as best fit model.

For storage stability study, the optimized NEs were kept at a temperature of 40 ± 2ºC and 75 ± 5% RH for two months. After storage at 4°C no significant change in the zeta size and zeta potential were observed in optimized formulations. After centrifugation at 5000 rpm for 5 hrs and at 10000 rpm for 30 min, no phase separation or creaming was found upon visual observation. Nanoemulsions that are stable in centrifugation, heating and cooling cycles were subjected to freezing and thawing. At the end of 3 cycles, the particle size was slightly increased, however no significant difference in zeta potential was observed.

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