Original Research

Formulation and Evaluation of Nanoemulsion for Bioavailability Enhancement of Metaxalone

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ABSTRACT

Introduction: Sustained or controlled release of drug, genetic material and biologically active ingredient are achieved through nanoemulsion which is the novel drug delivery system. Nanoemulsion is an anisotropically clear and kinetically or thermodynamically stable liquid solution, consisting of oil, surfactant and an aqueous phase, generally droplet diameter of 10-500 nm.

Aims: The aim of the present research was formulation and evaluation of nanoemulsion for bioavailability enhancement of metaxalone drug having poor water solubility.

Methods: Nanoemulsion was formulated by high shear homogenization technique using sesame oil, tween 80 and PEG 400 as a surfactant and characterized by particle size determination, polydispersity index, entrapment efficiency and zeta potential. The formulations were prepared by mixing the appropriate amount of surfactant and co-surfactant and oil. The formulation was mixed until complete dispersion occurs at 400-500 C temperature with 100 rpm. An appropriate quantity of the drug was added and the final mixture was mixed by vortexing until a transparent solution was obtained. Formulations were homogenised using a high-speed homogenizer.

Results: The result confirmed that all the droplet size was less than 500 nm in size and there is no aggregation between the droplets and other parameters are suitable for enhancing the bioavailability of metaxalone drug. The optimized formulation contains droplet size 94.03nm and zeta potential -47.2 mV. *In-vitro* dissolution study of nanoemulsion showed 90.35 release of drug within 45 minutes.

Conclusion: It was concluded that nanoemulsion of metaxalone using high shear homogenization technique gave improved drug release and could potentially improve the bioavailability of the drug.

Key Words: Nanoemulsion, Metaxalone, Zeta potential, High shear homogenization, Ternary phase diagram

INTRODUCTION

The development of a new drug entity is a highly expensive and time-consuming process. Different methods have been attempted for the improvement of the safety efficacy ratio of old drugs such as individualizing drug therapy, dose titration, and therapeutic drug monitoring. Delivering drug at a controlled rate, slow delivery, targeted delivery are other very attractive methods and have been pursued vigorously. There are the different route of administration of drug but the most commonly preferred route for drug delivery is the oral administration because of its convenience, simplicity, and patient acceptance.[1]Approximately 40-70% of new chemical entities has low aqueous solubility and are not well absorbed after oral administration, ultimately leading to poor bioavailability which is a major challenge to the modern drug delivery system. Metaxalone is a muscle relaxant and in the brain, it blocks nerve impulses. Metaxalone is used in the treatment of skeletal muscle conditions like injury or pain.[2]

Nanoemulsion consists of the aqueous phase, oil, and surfactant. Through very small size droplets of the nanoemulsions, better drug absorption and targeting were achieved.[3] The conventional emulsion system is not only improved by the nanoemulsion but also opens new opportunities for other drugs to be designed accurately with the correct dosing, better bioavailability leading to minimum side effects. The interfacial film of surfactant and co-surfactant molecules stabilized the nanoemulsions having oil and water.

To correlate the different transdermal drug release from a variety of delivery systems various studies has been performed. It was observed in studies that the drug release from different formulations like solid lipid nanoparticles, nanoemulsion, and polymeric nanosuspension, are proved to be proficient transdermal delivery vehicle.[4]

Techniques used to enhance solubility

The various techniques generally employed in order to improve the solubility of poorly water soluble drugs are shown in Figure 1.
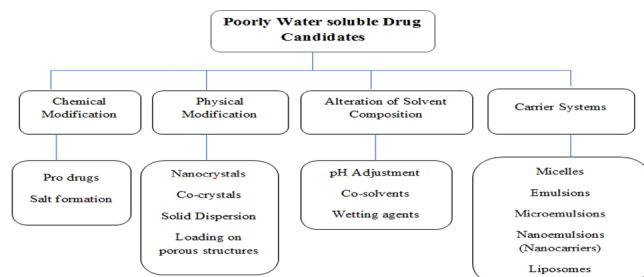

Figure 1: Solubility enhancement techniques.

Particle size reduction has been a much smarter approach that can be applied to the nonspecific formulation for several years. Micronization of drug leads to an increase in surface area which proportionally increases dissolution and absorption.[5] In the case of a very low solubility compound, micronization fails to improve solubility, therefore further steps to reduce the particle size to submicron range (nanometer range) have been developed to increase the dissolution rate and saturation solubility, subsequently improving the bioavailability[5]. Nanoemulsion is also known as sub-micron emulsions, mini-emulsions, ultrafine emulsions are thermodynamically unstable transparent dispersions of oil and water stabilized by surfactant molecules having a droplet size of less than 100 nm.

Droplets or small particles, with a size range of 5 nm-200 nm present in the dispersed phase have very low oil/water interfacial tension. Nanoemulsions are transparent due to the droplet size, which is less than 25% of the wavelength of visible light. Many times co-surfactant or co-solvent is used in addition to the surfactant, the oil phase, and the water phase. [6]

Methods of preparation of nanoemulsions

The schematic representation of the method of preparation of nanoemulsion is depicted below in Figure 2.

High energy approaches

Based on selected composition, i.e. surfactant compound, functional compound, and on the quantity of energy supplied, the nanoemulsions are formed by high energy methods.
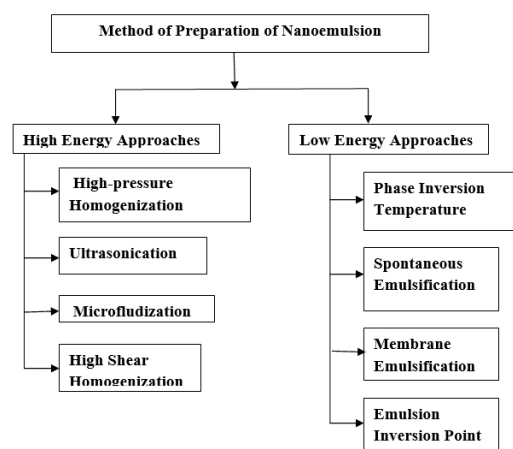

Figure 2: Methods of preparation of nanoemulsion.

1. **High-pressure homogenization:** To produce nanoemulsions of extremely low particle size (up to 1 nm) piston homogenizer / high-pressure homogenizer is used. Several forces like intense turbulence, hydraulic shear and cavitation, act together during the process to yield nanoemulsions with extremely small droplet size. Until the nanoemulsion with desired polydispersity index and droplet size is achieved the resultant product can be resubjected to high-pressure homogenization

   Application of high energy is needed for the production of small droplets (submicron). To enhance the efficiency of emulsification several procedures may be applied when producing nanoemulsions. At a high volume fraction of the disperse phase and diluted afterwards the emulsion is preferably prepared. However, during emulsification coalescence may be formed due to very high phase volume ratios, hence to create a smaller reduction in effective surface tension and mostly vanishing recoalescence more surfactant could be added.[7] Surfactant mixtures showing the high decrease in surface tension as compared to the individual components may also be utilized. Smaller droplets are formed if the surfactant is dissolved in the disperse phase rather than the continuous phase.[5,6]

2. Ultrasonication: Sonication mechanism used to reduce the droplet size of nanoemulsion or microemulsion. By this method, only small batches of nanoemulsions can be prepared and it is not applicable for large batches. For the preparation of nanoemulsion, the ultrasonication method is used by using a probe sonicator. Sonication time is the factor affecting ultrasonication.

3. Microfluidization: It is a patented mixing technology that involves a high-pressure positive displacement pump with a pressure of 500-20,000 psi. The phases pass through micro-channels because of this pressure. The massive amount of shear developed as the coarse

emulsion of two phases is mixed and proceed, and made to pass through micro-channels.

Unit the desired droplet size is obtained, the liquids are then passed through an interaction chamber microfluidizer.

4. High shear homogenizer: Silverson flow mixers in which rotors and stators have different configurations to achieve more efficient emulsification. At high rotor speed, a high rarefaction is created inside the disintegrating head, and the emulsion components are sucked in the rotor-stator unit. Under the action of centrifugal force, the emulsion is thrown away to peripheral areas, and intense dispersion occurs in the gap between the rotor and the inner wall of the stator. Then, the emulsion passes through the outer orifice of the stator at high speed and exits the apparatus.[7,8]

MATERIALS AND METHODS

Metaxalone was generously gifted by Sun Pharmaceutical Pvt. Ltd. Mumbai India. Tween 80, PEG 400 and sesame oil were procured from SD fine chemicals and all other chemicals used were of analytical grade.

Selection of oils, surfactants and co-surfactants for nanoemulsion formation

Selection of suitable oil is done based on the solubility of the drug by the shake flask method. An excess quantity of the drug was added to 10 ml of various oils in 50 ml stopper bottles.[8] The bottles were shaken at 35 °Cfor 48 hrs at 75 rpm. Then contents of each bottle were centrifuged at 3000 rpm for 15 min. The supernatant was diluted suitably with chloroform and quantification of the drug was done by UV-Spectroscopic method at 272 nm. From the literature, it was found that tween 80 was selected as surfactant because it is non-toxic and non-irritant. Solubility of the drug in tween 80 was also checked. From the literature, it was also found that most of the time PEG- 400 was used as a co-surfactant.[9]

Method of formulation of metaxalone nanoemulsion

The formulations were prepared by incorporation of metaxalone in an oil solution. Tween 80 and PEG 400 were added to the distilled water respectively and a water solution was prepared. Oil solution was added to water solution at 1000 rpm at 40°-50° C temperature. The final mixture was mixed by vortexing until a transparent solution was obtained. The formulation was homogenized using a high-speed homogenizer and finally metaxalone nanoemulsion was characterized.[9]

Optimization and preparation of nanoemulsion

Various formulations with varying concentration of surfactant, co-surfactant and oil were prepared as shown in Table 1and they were evaluated for the effect of the varying concentration on the particle size diameter, polydispersity index, zeta potential, encapsulation efficiency, pH in order to optimize the formulation. For this purpose Box-Behnken design was applied (Table 1).

Table 1: Nanoemulsion formulation for pseudo-ternary phase diagram

Batch No.	X_1 (conc. of oil) (ml)	X_2 (conc. of Smix) (ml)	X_3 Pressure (bar)
N-1	5	10	1300
N-2	20	10	1300
N-3	5	25	1300
N-4	20	25	1300
N-5	5	17.5	600
N-6	20	17.5	600
N-7	5	17.5	2000
N-8	20	17.5	1300
N-9	12.5	10	600
N-10	12.5	25	600
N-11	12.5	10	2000
N-12	12.5	25	2000
N-13	12.5	17.5	1300
N-14	12.5	17.5	1300
N-15	7.5	17.5	1300
N-16	12.5	17.5	1300
N-17	12.5	17.5	1300
N-18	12.5	17.5	1300

Solubility study

The solubility studies performed to detect the oil that solubilises the maximum amount of the drug. The solubility of the drug was determined in various oils by adding an excess amount of drug to 1 ml of selected oils (sesame oil, soya oil, castor oil) in stopper vials. The vials were kept at 25 ± 0.5°C in a Wrist action shaker for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000rpm for 15 min. The supernatant was taken and filtered through a 0.45µ membrane filter and the concentration of the drug was determined in the oils after dilution using a UV-Visible spectrophotometer at 272 nm. The result of the solubility study is given in Table 3.

Ternary phase diagram

The pseudo ternary phase diagram of oil, water, surfactant, and co-surfactant was constructed using the water titration method to determine the concentration and possibility for the preparation of metaxalone nanoemulsion. Surfactant

and cosurfactant are blended at a fixed ratio. The mixture of surfactant and cosurfactant (S_{mix}) was then mixed with the oil phase in a different ratio. Water is added dropwise with constant stirring with a magnetic stirrer until a homogeneous solution is obtained. Each system was visually observed for transparency. However, no heat is applied during the preparation. The endpoint of the titration was identified when the solution became turbid.[10] The quantity of the aqueous phase required to make the mixture turbid was noted. The schematic diagram of the pseudo-ternary phase diagram for the formulation is shown in Figure 4. The schematic diagram of the pseudo-ternary phase diagram shows the cosurfactant (S_{mix}) ratio is 5:2.

Evaluation of optimized formulation

Droplet size and polydispersity index
The droplet size of nanoemulsion was determined by dynamic light scattering with Malvern particle size distribution (Zetasizer ver. 6.20) based on laser light scattering phenomenon, which analyzes the fluctuations in light scattering. Light scattering was monitored at 25ºC at a 90º angle. Properly diluted samples of nanoemulsions were used for droplet size analysis. Average droplet size and polydispersity index were determined. Droplet size was expressed as nm and polydispersity index determines size distribution. The higher the polydispersity index the wider is the droplet size distribution.[10]

Zeta potential analysis
Nanoemulsion formulation was diluted with distilled water 50 times and 100 times. By gentle agitation, the resultant samples were prepared using a magnetic stirrer in 5 min using dynamic light scattering technique by Malvern zeta sizer (NANO ZS) zeta potential and globule size distribution (PSD) of the final nanoemulsion were examined.[11]

Encapsulation efficiency
By measuring the concentration of free drug (un-entrapped) in aqueous medium entrapment efficiency (EE) was examined. As it affects the release behaviour of drug molecule it is of prime importance. After separation of the entrapped drug from the nanoemulsion formulation, the amount of drug encapsulated per unit weight of nanoparticles was calculated:[12]

$$\text{Entrapment efficiency} = \frac{\text{Weight of total drug in formulation} - \text{Weight of drug in aqueous phase} \times 100}{\text{Weight of total drug in formulation}}$$

pH
Another important parameter of nanoemulsion is pH. The pH of the final preparation is decided by the excipients used in the formulation. Digital pH metal was utilized to measure the pH of formulations.

Fourier transform infrared spectroscopy (FTIR)
FTIR spectra of prepared nanoemulsion were recorded on Shimadzu FTIR 8400 spectrophotometer.[13] Sample was placed in the sample holder. FTIR spectra of the drug were scanned at 4000-1000 cm^{-1}.

Differential scanning calorimetry (DSC)
The nanoemulsion was hermetically sealed in aluminium pans and heated at a constant rate of 10°C/min over a temperature range of 40°C-300°C. The inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.[11-14]

Transmission electron microscopy (TEM)
The surface morphology of nanoemulsion was studied with the help of a field emission transmission electron microscope (Joel FE-TEM, model JEM-2100F) capable of point to point resolution. A drop of nanoemulsion was deposited on a copper grid and observed after drying.[14]

In-vitro drug release study
Comparative *in vitro* drug release study was carried out between optimized formulation (N-9) and marketed formulation. The batch N-9 nanoemulsion equivalent to 4 mg of drug was placed in dialysis membrane (specification: Av. Flat width- 24.26 mm, Av. Diameter- 14.3 mm, Capacity- 1.61 ml/ft, Himedia, LA387, Dialysis Membrane-50) and diluted with 3 ml of respective dissolution medium which then tied from both sides with the thread. This dialysis membrane was then immersed in a beaker containing a 900 ml dissolution medium. Aliquots of dissolution fluid (10 ml) were withdrawn at specified time intervals (Indian Pharmacopoeia, 2014). The fresh buffer was added to the beaker at the same time, to keep the volume constant. The sample withdrawn was estimated by ultraviolet spectrophotometer.[15] Table 2 provides the USP pharmacopoeia conditions for in vitro drug release test.

Table 2: USP Pharmacopoeia condition

Parameter	Condition
Apparatus	USP Type 2 (Lab India DS 8000)
Dissolution Medium	Phosphate buffer pH 7.8
Temperature	37°C(± 0.5)
Rpm	75
Volume of Sample	10 ml
Volume of Buffer	900 ml
Amount of nanoemulsion	Solution Equivalent to one dose (4 mg)

RESULTS AND DISCUSSION

Solubility study

Solubility of given drug was checked in various oils like sesame oil, soya oil, and castor oil. Solubility of drug in tween 80 and PEG 400 were also checked. Sesame oil was selected as oil phase due to high solubility of drug as compared to other oils (Table 3).

Table 3: Solubility of metaxalone in different components

Components	Solubility (mg/ml)
Sesame oil	55.20
Soya oil	28.970
Castor oil	27.600
Tween 80	32.30
PEG 400	21.94

Ternary phase diagram

Surfactants: co surfactants (5:2) show maximum nanoemulsion area. So this ratio was selected for further formulation.

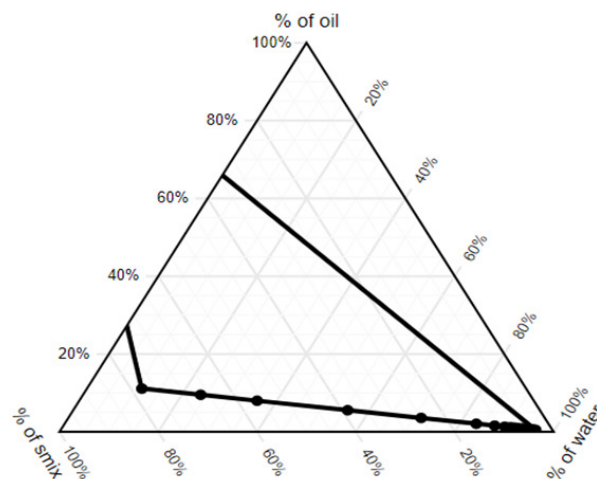Phase diagram and existence readings for nanoemulsion are shown in Figure 3.

Figure 3: Pseudo-ternary phase diagram of nanoemulsion formulation.

Evaluation of optimized formulation

Results of droplet size, polydispersity index, zeta potential, encapsulation efficiency and pH are given in Table 4.

Table 4: Formulation of nanoemulsion applying Box-Behnken design

Batch No.	Droplet Size Diameter (nm)	Polydispersity index	Zeta Potential (mV)	Encapsulation Efficiency (%)	pH
N-1	125	0.659	-59.3	68.99	7.20
N-2	267.5	1.000	-7.91	50.98	6.45
N-3	152.3	0.849	-19	54.78	6.94
N-4	239.3	0.934	-7.83	54.58	7.52
N-5	144.4	0.804	-11.4	64.58	6.20
N-6	288.7	0.690	-13.4	50.90	6.40
N-7	220.5	1.000	-11.2	62.59	7.13
N-8	271.2	0.808	-57.7	67.52	6.35
N-9	94.03	0.888	-47.2	78.99	7.11
N-10	176.9	0.398	-19.3	67.89	7.05
N-11	108.9	0.558	-5.04	77.54	6.95
N-12	132	0.824	-7.49	69.70	6.11
N-13	189.8	0.560	-7.83	65.90	6.05
N-14	324.5	1.000	-7.49	57.92	6.95
N-15	378.8	0.408	-7.91	55.32	6.06
N-16	411.5	0.392	-6.06	44.98	7.51

Fourier transform infrared spectral spectroscopy

The FT-IR spectra showed characteristic C=O, C-H, N-O, N-H stretching bands at 1087.10 cm^{-1}, 1460.45 cm^{-1}, 1524.75 cm^{-1}, 3441.30 cm^{-1} respectively shown in Table 5 and Figure 4. The ortho-distribution after 1000 cm^{-1} was also observed. FT-IR spectra of the drug were matched with reference spectra.[12,13] The presence of all characteristic peaks confirmed the metaxalone nanoemulsion peak (Table 5 and Figure 4).

Table 5: Comparison of FTIR spectra of the observed peak of metaxalone and optimized batch N-9

Functional groups	Peak observed in Metaxalone API (cm⁻¹)	Peak observed in nanoemulsion batch N-9 (cm⁻¹)
C-O stretching	1078.94 cm⁻¹	1087.10 cm⁻¹
C-H bending	1452.36 cm⁻¹	1460.45 cm⁻¹
N-O stretching	1541.16 cm⁻¹	1524.75 cm⁻¹
N-H stretching	3451.45 cm⁻¹	3441.30 cm⁻¹
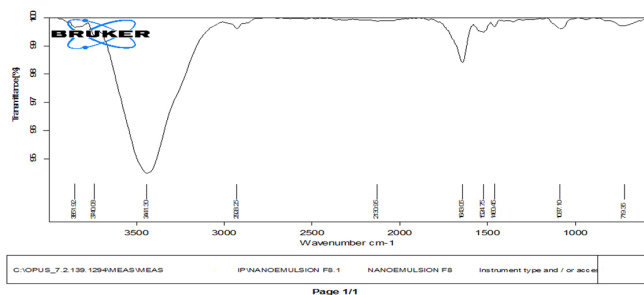
Figure 4: FTIR spectra of optimized batch N-9 of metaxalone nanoemulsion.

Differential scanning calorimetry

DSC thermogram shown in **Figure 5** indicates no interaction between metaxalone and excipients during nanoemulsion formulation. DSC study of nanoemulsion revealed that the incorporation of the drug into the nanoemulsion suggesting a molecular dispersion of the drug inside the system.[14,15] There is no difference between the peak of blank nanoemulsion and drug-loaded nanoemulsion.
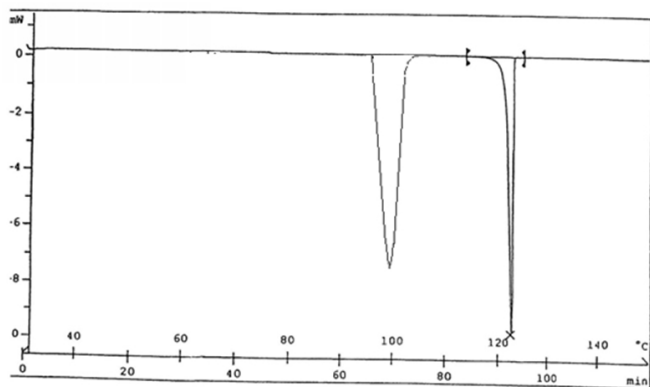

Figure 5: Differential scanning calorimetry thermogram of optimized batch N-9.

Transmission electron microscopic (TEM)

Transmission electron microscopic images of nanoemulsion and overlay of metaxalone, blank nanoemulsion and metaxalone loaded nanoemulsion are given in Figure 6 and Figures 7 respectively. It can be concluded that the input polymor-

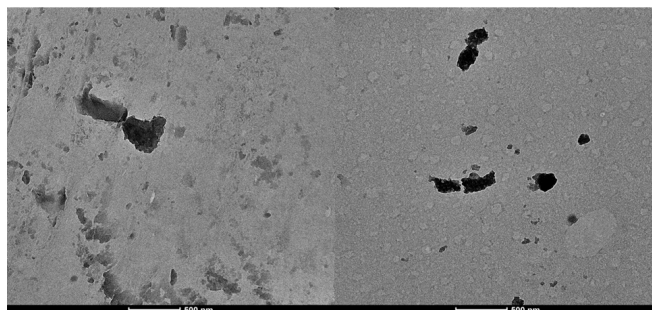phic form of metaxalone remains after nanoemulsion formulation as well and there is no polymorphic conversion.

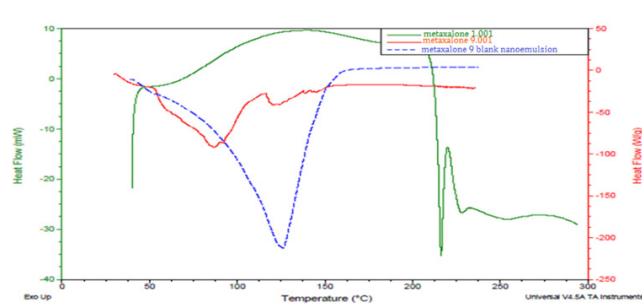**Figure 6:** Transmission electron microscopic images of nanoemulsion.

Figure 7: Overlay of metaxalone, blank nanoemulsion and metaxalone loaded nanoemulsion.

In-vitro drug release study

A comparative *in vitro* drug release study was carried out between optimized formulation and conventional formulation. The results showed that the release of the drug from the optimized batch was more than the marketed product (Table 6, Figure 8).

Table 6: Optimized formulation v/s conventional formulation

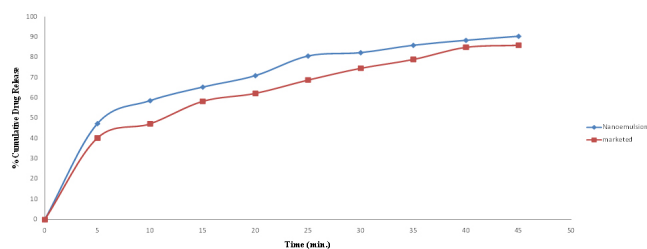| Time (Min.)	% CDR (N-9)	% CDR (Marketed Product)
5	47.32	40.25
10	58.63	47.23
15	65.32	58.32
20	70.96	62.23
25	80.63	68.8
30	82.28	74.59
35	85.92	78.96
40	88.36	84.94
45	90.35	85.95

Figure 8: *In vitro* drug release study of optimized and conventional formulations.

CONCLUSION

Nanoemulsions of metaxalone were prepared in 18 batches. The prepared batches showed droplet size in the range of 94.03 - 411.5 nm, zeta potential -5.04 to -59.3mV, encapsulation efficiency 44.98- 78.99% and pH 6.06 -7.52. Batch N-9 was found to be an optimized batch and was utilized for further characterization. The optimized batch showed 90.35% drug released in 45 minutes in comparison with the marketed product. FTIR analysis of nanoemulsion showed that there was no significant shifting of functional peaks and no overlapping of characteristics peaks and no appearance of new peaks were observed on comparison of obtained spectra with reference spectra. DSC study of nanoemulsion revealed that the incorporation of the drug into the nanoemulsion suggesting a molecular dispersion of the drug inside the system. There is no difference between the peak of blank nanoemulsion and drug-loaded nanoemulsion. TEM analysis shows that the droplet of nanoemulsion was approximately spherical with uniform distribution with some deviation. The result confirmed that all the droplet size was less than 500 nm in size and there is no aggregation between the droplets. It was concluded that nanoemulsion of metaxalone can be prepared using high shear homogenization technique with improved drug release characteristics.

ACKNOWLEDGEMENT

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