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Review Article

DESIGN & DEVELOPMENT OF MICRO EMULSION DRUG DELIVERY SYSTEM OF NISOLDIPINE FOR IMPROVEMENT OF ORAL BIOAVAILABILITY

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ABSTRACT
Nisoldipine Microemulsion (ME) is a thermodynamically stable, transparent, isotropic system which is used to enhance the bioavailability and solubility of poor water soluble drugs formulated by using only pharmaceutical excipients. Nisoldipine ME systems composed of surfactant & co surfactant that aim to decrease the interfacial tensions between water and oil facilitating their flow in the porous medium and enhance the oral bioavailability. Nisoldipine ME formulations were prepared through the water titration method by varying the ratio of oil, surfactant, co-surfactant and water, keeping the concentration of Nisoldipine constant in each case. Evaluation of ME were done for following parameters such as ph measurement, quality test-dilution test, dye test, percentage transmittance, viscosity measurement, electro conductivity measurement, droplet size & zeta potential, centrifugation test, drug content, In vitro studies and stability studies The developed nisoldipine ME contain IPM (6%), Tween 80 (30%), PEG 400 (10%), and distilled water (54%), which is transparent fluid with a globule size of 77.57 nm showed higher in vitro drug release when compared with pure drug suspension and relative bioavailability of the drug from nisoldipine ME was found to be 85.34% within 6 hrs. *In vivo* pharmacokinetic study and drug diffusion from the micro emulsions revealed that nisoldipine ME formulation can be employed to improve the tissue retention of a poorly soluble drug showing first-pass metabolism. These findings pave the way for the development of a variety of sustained drug delivery applications.

Keywords: *Nisoldipine, Micro emulsion, Bioavailability, Water Titration Method, Pharmacokinetics, Release kinetics*

INTRODUCTION
Microemulsions (ME) are optically transparent, low viscous and thermodynamically stable dispersions of oil and water. ME was stabilized by an interfacial film of a surfactant, usually in combination with a co-surfactant (Flanagan and Singh 2006). It is used as vehicles to deliver many kinds of drugs because of their thermodynamic stability, ease of preparation and good appearance (Deng *et al.*, 2015). Nisoldipine is a second generation long term acting calcium channel blocker. The vascular selectivity of nisoldipine is 10 times more than that of felodipine, isradipine, and nicardipine and 100 times more than that of amlodipine and nifedipine (Scholz, 1997). The bioavailability of nisoldipine is about 5% and cytochrome P4503A4 enzymes are supposed to play an important role in the metabolism of nisoldipine (Opie, 1997). Researchers were reported various methods, to improve the bioavailability of nisoldipine, including nanoparticles and solid solution formulation or self emulsifying drug delivery system (SEDDS) however these all are less capable to improve the oral bioavailability of nisoldipine (Fasinu *et al.*, 2011). So, in the present study we have developed nisoldipine micro emulsion in order to increase its aqueous solubility and bioavailability by using Surfactant Tween 80 and co-surfactant PEG 400, which reduces the energy required to increase the surface area (Gershanik and Benita 2000).So, that spontaneous dispersion of water or oil droplets occurs and the system is thermodynamically stable (Vitale and Katz 2003). The aim of present work is to formulate nisoldipine micro emulsion novel drug delivery system and assess its physicochemical characterization via improving the oral bioavailability. To filter out the optimal

formulation, the viscosity studies and micro emulsion droplet size tests were conducted. The spherical nature and size homogeneity of the micro emulsion droplets were revealed by transmission electron microscopy (Kelmann *et al.,* 2007).

MATERIALS AND METHODS

Materials

Gift sample of Nisoldipine was provided from IPCA Lab Pvt. Ltd. Mumbai, PEG 400 and Methanol were procured from Loba Chemical, Tween 80 and Isopropyl myristate (IPM) were procured from merck industries (India). All other chemicals and reagents used in the study were of analytical grade.

Preformulation study

Melting point:

The melting point of Nisoldipine was determined using the open capillary method. The drug sample was filled into a capillary and placed in a melting point apparatus The tube was heated and the temperature at which the drug melted was noted (Wong, 1993).

Solubility determination:

Solubility of the Nisoldipine was determined in different solvents like methanol, chloroform, hexane, acetone, water, oils such as Oleic acid, Isopropyl myristate, castor oil, surfactants such as tween 80,tween 20,span 80 & co surfactants such as polyethylene glycol 400, polyethylene glycol 200 (Islam, 2012). Drug was added in excess to different oils, surfactants & co surfactants and stirred for 24 hrs on magnetic stirrer. After stirring samples was centrifuged at 3000 rpm for 3-5 min and the drug in the supernatant was analyzed at λ max 237 nm after proper dilution with Methanol (Bhandari *et al.*,2017).

Determination of λ max of drug by UV spectrometer:

100mg of Nisoldipine was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in an adequate amount of methanol and the volume was made up to 100 ml with methanol so as to obtain a stock solution of 1000 µg/ml. A dilution of 20 µg/ml concentration was made from the above stock solution with the methanol and the resulting solution was scanned on a double-beam UV-visible spectrophotometer (Shimadzu 1800) between wavelength ranges of 200 nm to 400 nm.

Calibration curve of nisoldipine in methanol:

A standard curve was prepared in the concentration range of 5-25 µg/ml. For the preparation of calibration curve, stock solution was prepared by dissolving 50 mg of accurately weighed Nisoldipine in 50 ml of methanol. Further 10ml of this solution was pippeted into 100 ml of volumetric and diluted to 100 ml with methanol. From this 5,10,15,20 and 25 ml pipetted into a series of 10 ml volumetric and volume was made up to 10 ml with methanol to get 5,10,15,20 and 25 µg/ml of Nisoldipine respectively. The optical density values of resulting solutions were measured at 237 nm in UV spectrophotometer.

Calibration curve of nisoldipine in 0.1 N HCL:

50 mg of Nisoldipine was accurately weighed and dissolved in 50ml of 0.1 N HCL containing 1% SLS into a volumetric flask (1000 mg/ml) respectively. 10 ml of this solution was taken and made up to 100 ml with 0.1 N HCL, which gives 100 mg/ml concentrations (stock solution). From this stock solution, concentration of 5, 10, 15, 20,25mg/ml in 0.1 N HCL solutions were prepared. The absorbance of the diluted solution was measured at 237 nm respectively and a standard plot was drawn using the data obtained. The correlation coefficient was calculated by linear regression analysis.

Drug excipient interaction study:

Infrared spectrophotometry is a useful analytical technique utilized to check the chemical interaction b/w the drug and other excipients used in the formulation. It determined by ABB FT-IR series model-MB 3000.The sample were prepared by the potassium bromide (KBr) disc method. The KBr discs were prepared by compressing the powder and scanning range was kept from 4000-400 cm^{-1}. The drug was mixed with the excipients (IPM, Tween 80, PEG 400) individually and packed in amber color vials and kept at 40ºC for 1month.the IR spectrum of the pure drug was compared with that of the physical mixture to check for any possible drug excipient interaction (Van Eerdenbrugh and Taylor 2013).

Partition coefficient:

Isopropyl myristate oil was used for the investigation of the oil-water partition coefficient of Nisoldipine. A 30mg of the drug was dissolved in 20ml of oil and 20ml of distil water was added and shaken for 30min and kept aside for overnight at room temperature to achieve equilibrium. The aqueous layer was separated by a separating funnel, centrifuged to remove any entrapped oil globules or particles and the supernatant liquid was separated. From the supernatant 1ml was taken and made up to 100ml with distil water. Further 1ml of this solution was pippeted into 10 ml of volumetric flask and diluted to 10 ml with distil water and analyzed at λmax 237 nm. Then oil/water partition coefficient was calculated using the formula k = Cwater/Coil.

Loss on drying:

The weighing bottle was dried for 30 minutes in oven then it was allow cooling. The bottle was accurately weighed with cover. Then cover was removed and 100mg of sample was placed in to the bottle and weight. Then sample was heated at 105°C for 3 hour. Then the bottle was removed and it was placed in the desiccators. Then the material was allowed to reach room temperature and weigh and calculate.

Preparation of Nisoldipine microemulsion

ME formulations were prepared by the water titration method by varying the ratio of oil, surfactant, co-surfactant and water, keeping the concentration of nisoldipine constant in each case (Airaksinen *et al.*, 2003). Preparation of micro emulsion on the basis of the solubility studies, isopropyl myristate was selected as the oil phase. Tween 80 and PEG 400 were selected as surfactant and co surfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and co surfactant (Smix) was mixed at different mass ratios (1:1, 2:1, and 3:1, 4:1). Predetermined quantities of the drug were dissolved in the oil. Sonication was performed in bathsonicated for 5 minutes to dissolve drug. Surfactant and co-surfactant were added and mixed gently for 1hrs with the help of a homogenizer (Lab stirrer REMI) at 1000 rpm at room temperature. The mixture was then finally titrated with distilled water until a stable and transparent ME was obtained which resulted in the formulation of a transparent and homogenous micro emulsion. Different batches of micro emulsion with or without drug were prepared and select final preparation based on the transparency and physical observation. The compositions of nisoldipine micro emulsion were shown in Table 1.

Table 1: Composition of Microemulsion of Nisoldipine

S. No.	Oil:Smix Ratio	Oil: mix In ml	1:1(smix) 50:50	2:1(smix) 100:50	3:1(smix) 150:50	4:1(smix) 200:50
Dilution with water until micro emulsion remains						
1	1:9	1ml:9ml	30	40	65	80
2	2:8	2ml:8ml	20	45	60	85
3	3:7	3ml:7ml	3	3	7	12.4
4	4:6	4ml:6ml	2	2	6	6.2
5	5:5	5ml:5ml	1	1	2	3.2
6	6:4	6ml:4ml	1	1.14	2.85	3.08
7	7:3	7ml:3ml	0.7	1	1.4	2.84
8	8:2	8ml:2ml	0.6	0.10	1.3	1.56
9	9:1	9ml:1ml	0.4	0.8	1.12	1.52

Characterization (Butani *et al.*, 2014***)***

pH measurement:

pH of the micro emulsion was measured using digital pH meter. The electrode was rinsed with deionized water and blot was dried with a soft, clean paper .Then the electrode was dipped into the test solution. Then pH was recorded when the reading was stable after insertion of the electrode into the solution.

Qualitative test:

Dilution and Dye solubility tests were performed for determination of type of ME.

Dilution test:
The prepared drug loaded micro emulsion was diluted with water and it was found to be optically clear.
Dye solubility test:
The water soluble dye amaranth was added in micro emulsion and it was found uniformly distributed throughout the micro emulsion without any lump formation.
Centrifugation test:
This test was used to specify the stability of the micro emulsion whether it was monophasic or biphasic. In this, the samples were centrifuged at 3000 rpm for 30 minutes and then were examined for whether the system was monophasic or biphasic.
Conductivity measurement:
The electro conductivity of the resultant system was measured by an electroconductometer (chemiline conductometer). For the conductivity measurements, the tested Microemulsion was prepared with a 0.01N aqueous solution of sodium chloride instead of distilled water.
Viscosity measurement:
The rheological property of the micro emulsion was evaluated by a Brookfield LVDV 111 + CP viscometer at 30ºC using a CPE 42 spindle at 5 rpm.
Percent transmittance:
Transparency of both optimized ME formulation and its diluted forms (10 and 100 times with distilled water) was determined by measuring percentage transmittance through ultraviolet (UV) spectrophotometer (UV-1800 shimadzu). Percentage transmittance of samples was measured at 650 nm with purified water, taken as blank.
Globule size & zeta potential determination:
The globule size & zeta potential were measured with Malvern zetasizer (Malvern Instruments Ltd)
Drug content:
ME containing 10 mg of drug was transferred to a 25 ml volumetric flask and the volume was made up with methanol. The drug was allowed to dissolve in the solvent for 30 min than the solution was filtered and 1ml was taken in 100ml of volumetric flask and diluted up to mark with methanol. From this, 1ml pipetted into a 10 ml volumetric flask and volume was made up to 10 ml with methanol. The resultant solution was analyzed spectroscopically at 237nm.

***In-vitro* study**

In-vitro study was carried out using cellophane membrane. The cellophane membrane was activated in distilled water for 4 hours than 2% sodium bicarbonate and 1m mol EDTA for 2 hours. Nisoldipine ME and plain drug suspension (each equivalent to 5 mg of Nisoldipine) were placed in the donor compartment. The receptor compartment was filled with dialysis medium (500ml of 0.1 N HCL).

RESULTS AND DISCUSSION

The λ_{max} of the nisoldipine was found to be 237 nm on UV spectrophotometer, which complies with the official literature The linearity range was selected 5-25 µg/ml and r2 was found to be in methanol and 0.1 N HCl 0.9937 and 0.9959 respectively Table 2, 3 & Fig. 1, 2.

Table 2. Standard Plot Data of Nisoldipine in Methanol

S No.	Concentration (µg/ml)	Absorbance (A)
1	5	0.33
2	10	0.48
3	15	0.59
4	20	0.72
5	25	0.81

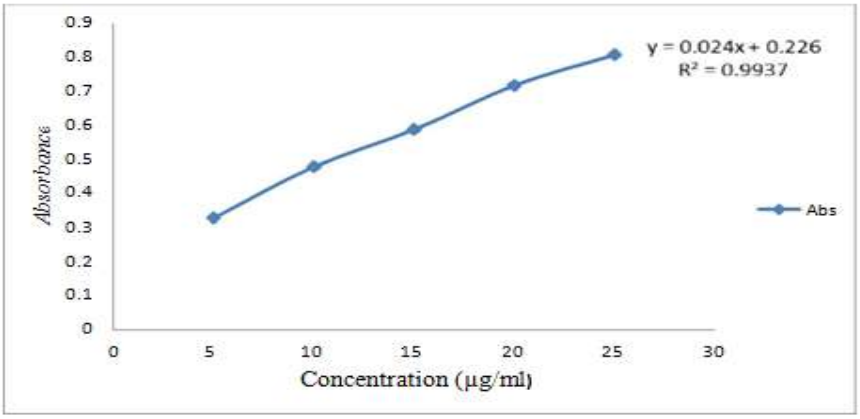

Figure 1. Calibration Curve of Nisoldipine in Methanol

Table 3. Standard Plot Data of Nisoldipine in 0.1 N HCL

S No.	Concentration (µg/ml)	Absorbance (A)
1	5	0.28
2	10	0.46
3	15	0.66
4	20	0.81
5	25	0.99

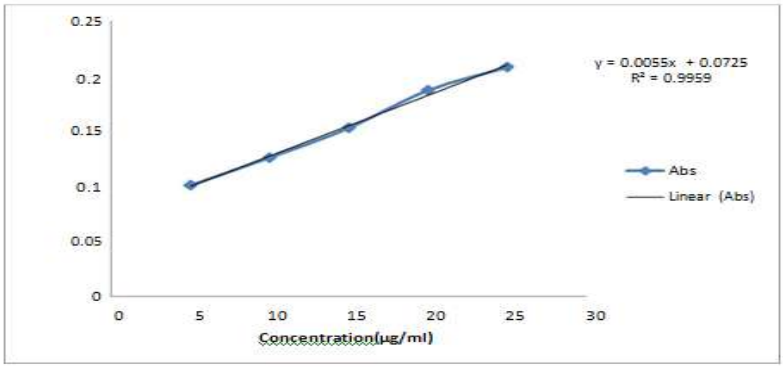

Figure 2. Calibration Curve of Nisoldipine in 0.1 N HCL

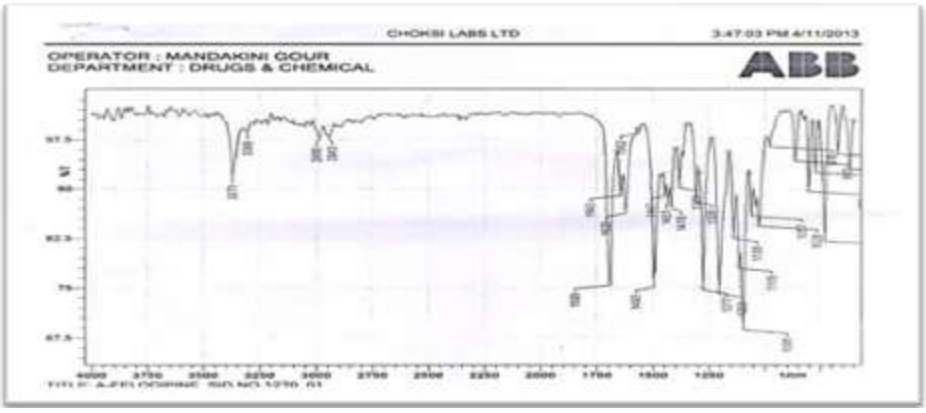

Figure 3. FTIR Spectrum of Nisoldipine

IR spectrum of any compound given information about the functional group present in particular compound. IR spectrum of nisoldipine and with all excipient was taken using KBr pellet method. Various peaks in IR spectrum were interpreted for presence of different group in the structure of drug. The spectra of FTIR (Fig.3 to 6) indicate that the sample used was nisoldipine and no interference with drug and excipient.

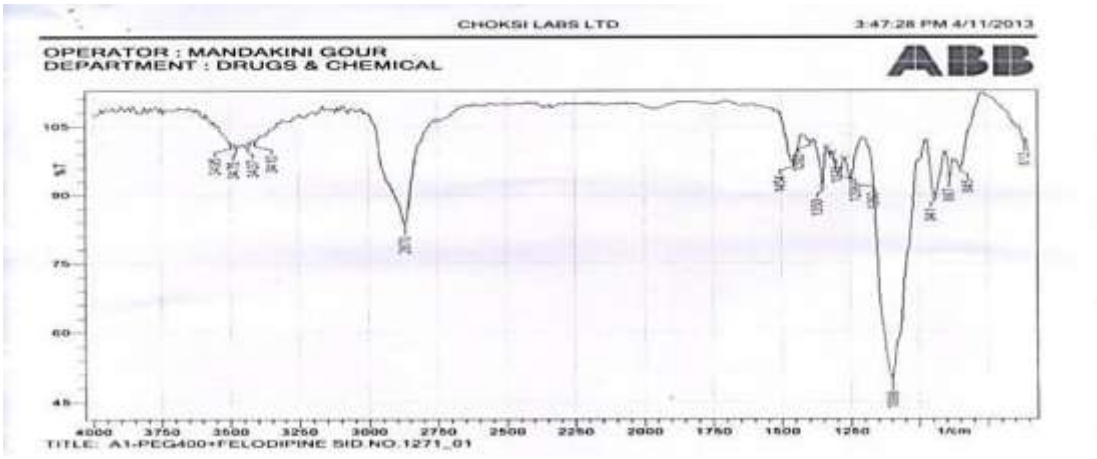

Figure 4. FTIR Spectrum of Nisoldipine + PEG 400

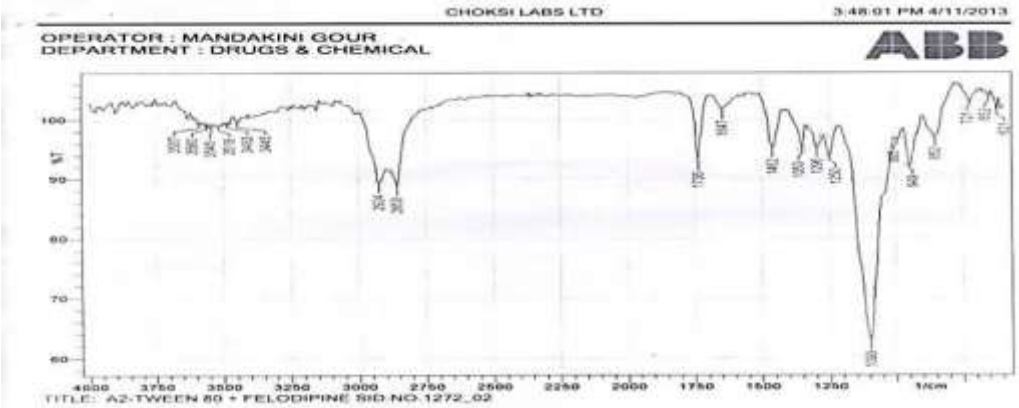

Figure 5. FTIR Spectrum of Nisoldipine + Tween 80

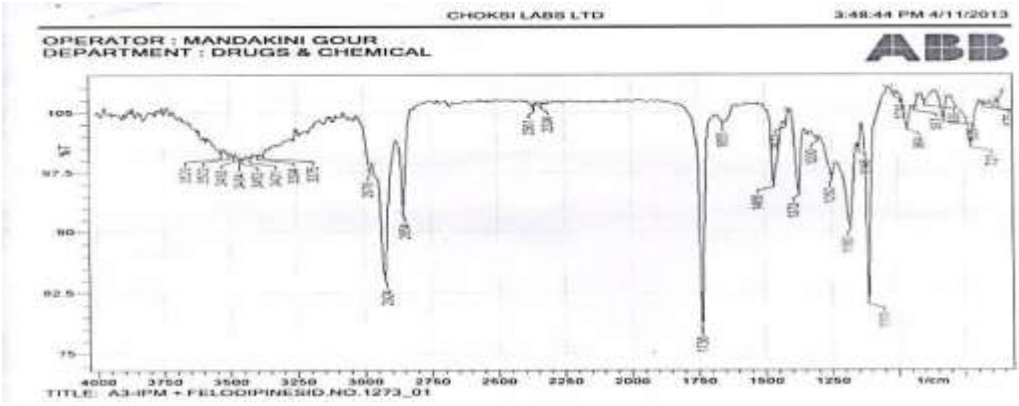

Figure 6. FTIR Spectrum of Nisoldipine + IPM

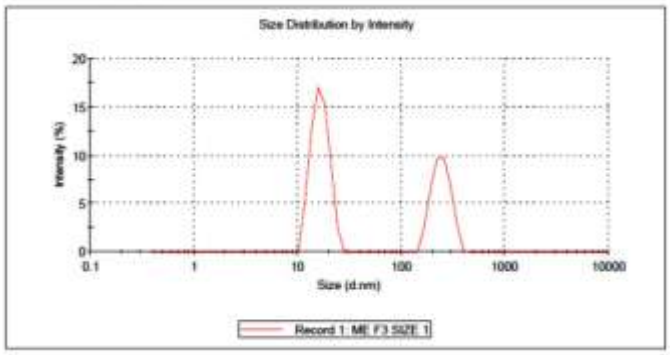

Figure 7. Globule Size of Formulation (F3)

From the dilution test and dye solubility test the prepared micro emulsion was found to be o/w type. The micro emulsion was found to be optically monophasic even after centrifuging at 3000 rpm for 30 minutes. Various tests pH measurement, Percentage transmittance, Viscosity measurement, Electro conductivity measurement, Droplet size & zeta potential, Centrifugation test, Drug content were performed. Globule size of the micro emulsions was found to be 77.57 nm (Fig. 7). Zeta potential was found to be negatively charged (-6.20) to the system (Malvern Instruments Ltd). Hence the formulations will not cause any problem due to electrostatic interaction between the micro emulsions (Fig 8).

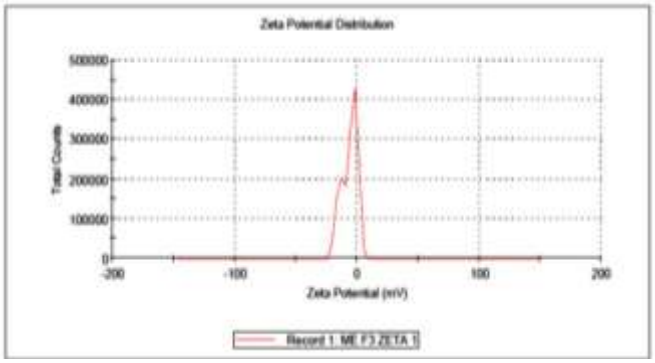

Figure 8. Zeta Potential Formulation (F3)

Drug release from optimized ME & pure drug suspension, were found to 85.34%, 55.1%. ME showed higher drug release as compared to the PDS, which may be due to the solubility-enhancing component of the surfactant and co-surfactant (Table 4 & Fig. 9).

Table 4. In Vitro Drug Release Study

S. No.	Time (min)	%Cumulative drug release (ME F3)	%Cumulative drug release (PDS)
1	15	10.12	8.1
2	30	15.22	9.0
3	60	21.89	10.2
4	90	28.98	14.7
5	120	35.87	18.6
6	150	49.1	22.5
7	180	55.65	26.0
8	210	61.22	32.2
9	240	68.91	38.2
10	270	71.2	42.8
11	300	78.2	48.5
12	330	84.16	50.2
13	360	85.34	55.1

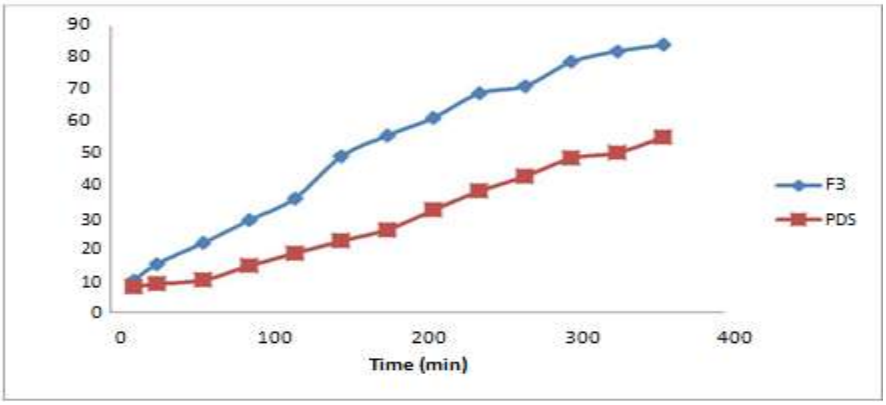

Figure 9. In Vitro Drug Release Study of F3 Formulation & PDS

CONCLUSION

Nisoldipine is used as an antihypertensive and antianginal drug, belongs to BCS class drug (low solubility and high permeability).It undergoes extensive first pass metabolism with a bioavailability of only about 15%.The major drawback in the therapeutic application and efficacy of nisoldipine as oral dosage form is its low aqueous solubility .Hence this work was planned to improve dissolution characteristics of the drug by increasing its release and solubility through micro emulsion technique. The Present study was undertaken with an aim to formulate and evaluate micro emulsion system of nisoldipine using water titration method with the addition of surfactant and co-surfactant agents. Preformulation study was carried out initially and results directed for the further course of formulation based on the Preformulation studies different batches with surfactant and co-surfactant agents addition were prepared using selected excipients. Hence, it can be concluded that the ME formulation can be employed to improve the bioavailability of a poorly soluble drug showing first-pass metabolism. However, further studies in higher animals and humans need to be performed before this formulation can be commercially exploited.

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