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Enhancement of the solubility and bioavailability of Ursodeoxycholic acid through a self nano emulsifying drug delivery system

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Abstract
Ursodeoxycholic acid (UDCA) is a bile acid that presents high lipophilicity which is used in the treatment of cholestatic liver diseases and belongs to class II Biopharmaceutical Classification System (BCS), which exhibits low water solubility and high intestinal permeability which leads to poor oral absorption. The goal of this study was to develop a Self Nano Emulsifying Drug Delivery System (SNEDDS) of UDCA to improve its solubility and bioavailability. The excipients were selected based on the maximum solubility of UDCA in various oil, surfactant & cosurfactant and observed by UV spectrophotometer. Different combinations of oils, surfactant, and cosurfactant was optimised by 3^2 factorial design to prepare UDCA-SNEDDS formulation. Cinnamon oil, span 80, Ethanol were used as an oil, surfactant and co-surfactant respectively. The selected formulation F5 were analysed for Size, Self emulsification time, Drug content, Scanning Electron Microscopy (SEM) and *In-vitro* dissolution study. The UDCA-SNEDDS formulation exhibited Size 277.8nm and good PDI with proper Stability. The invitro drug release in phosphate buffer P^H 6.8 from selected UDCA-SNEDDS F5 was about 63% after 8 hours. These outcomes imply that UDCA-SNEDDS may serve as an effective means of enhancing UDCA's liver activity by improving the solubility and bioavailability of UDCA.

Keywords: Bioavailability, poor water solubility, self nano emulsifying drug delivery system, ursodeoxycholic acid

Introduction
Oral delivery route is the most convenient route for drug administration to achieve desired therapeutic effects and the greatest degree of patient compliance, especially for chronic condition diseases. Around fourty percent of new chemical entities developed by the pharmaceutical industry are poorly soluble or lipophilic compounds, which result poor oral bioavailability, high intra and inter subject variability and lack of dose proportionality. These drugs are classified as class II drug by the Biopharmaceutical classification system (BCS), drugs with a poor aqueous solubility and high permeability.
Different formulation approaches like micronization, solid dispersion and complexation with cyclodextrins have been utilized to resolve such problems. Indeed, in some selected cases, these approaches have been successful but offers many other disadvantages. But more recently there have been much focus on the utility of Self Nano Emulsifying Drug Delivery System (SNEDDS). SNEDDS are well known for their potential to enhance the solubility and absorption of the lipophilic drugs by increasing the surface area and decreasing the size of oil droplets that are readily digestible and incorporated into mixed micelles that can pass the intestinal lumen. SNEDDS have distinct features—which make them superior to conventional micro and nano emulsions—including log term stability, patient compliance, palatability, reduction in dose, ease of formulation, and scale-up synthesis.

Ursodeoxycholic acid
Ursodeoxycholic acid **(**UDCA**)** is a naturally occuring bile acid that is used to treat cholestatic liver disease and dissolve cholesteral gall stones. The solubility of Ursodeoxycholic acid in aqueous medium was very low (i.e) 0.0197 mg/ml in water. Absolute bioavailability of Ursodeoxycholic acid was 15.2% and biological half life is 3.5 to 5.8 days that results into poor bioavailability after oral administration.
Hence UDCA was selected as a model drug for this study. UDCA is available in various doses [150mg, 300mg] for our study half amount of available dose (75mg) was selected to limit the total formulation volume. The aim of this study was to develop a SNEDDS containing a poor water soluble drug (Ursodeoxycholic acid).

Materials and method
Ursodeoxycholic acid was a gift sample by Medreich Pharmaceuticals Pvt Ltd, Bangalore. Span 80, Cinnamon oil, Ethanol were used which are of laboratory grade and available at college.

Methodology
Pre-formulation studies
a. Melting point
A capillary tube was sealed with a Bunsen burner and it was filled with the drug ursodeoxycholic acid through the open end. The drug – filled capillary tube was placed in the melting point apparatus and temperature at which the drug started to melt was noted.

b. Solubility studies
1g of the drug was dissolved in 1ml, 10ml, 30 ml and 100ml of various solvents depending upon its solubility. The resulting solubility was compared with the solubility limits specified in the Indian pharmacopeia.

c. Determination of lambda max
10 mg of drug was dissolved in 10 ml of ethanol to prepare stock solution – A of concentration 1mg/ml. 1ml of stock-A was further diluted to 100 ml to get stock-B of concentration 20 mcg/ml.This solution was scanned from 200-400 nm in a UV spectrophotometer to determine the lambda max.

d. Standard Curve

100mg of drug was dissolved in 100ml of ethanol to get a stock solution –A of concentration 100mcg/ml. 1ml of stock –A was diluted to 10 ml to give a stock – BA serial dilutions of the stock-B were done to get solutions of concentrations 10, 20, 30, 40, 50, 60, 70,80 mcg/ml. These solutions were analyzed in the UV spectrophotometer at the 216 nm. A calibration curve was plotted with concentration on x-axis and the absorbance on the y-axis, the correlation coefficient was also calculated.

e. Screening of Components

Solubility studies: The solubility of Ursodeoxycholic acid in various oils, surfactants and co- surfactants was done by the vial shake method. An excess of drug was added to the vial containing 5ml of the oil/surfactant and was sealed with an aluminium foil. The sealed vial was heated at 40°C and then centrifuged at 15,000 rpm for 10 mins. The insoluble drug was removed by filtering it and the resulting solution was analyzed in the UV spectrophotometer.

f. Emulsification study

1. **Surfactant**: 3 ml of the surfactant was mixed with 3 ml of the selected oil, heated to 50°C and diluted to 50 ml with water. The ease of emulsification was observed by the number of flask inversions required for the formation of an emulsion. They were also observed visually for any signs of phase separation or turbidity.
2. **Co-surfactant**: 1 ml of co-surfactant, 2 ml of surfactant and 3 ml of the selected oil were taken and heated to 50°C and 3 ml of this mixture was diluted to 50 ml. This was visually assessed.

Preparation of SNEDDS

The drug was weighted to 0.75mg and was mixed with the specified amount of oil. To this the specified amount of the surfactant and co surfactant were added. It was stirred in magnetic stirrer for about 1 hour in which 4ml of water is added drop by drop, after which it was stored in room temperature.

Table 1: Composition of Ursodeoxycholic acid SNEDDS formulation

Formulation code	Drug (mg)	Oil (cinnamon oil) (ml)	Surfactant span-80(ml)	Co-surfactant ethanol(ml)
FT1	0.75	1	10	4
FT2	0.75	2	10	4
FT3	0.75	3	10	4
FT4	0.75	1	12	4
FT5	0.75	2	12	4
FT6	0.75	3	12	4
FT7	0.75	1	14	4
FT8	0.75	2	14	4
FT9	0.75	3	14	4

Evaluation parameters of liquid SNEDDS

a. Visual observation

The formulation were diluted and made to stand for 24 hours at 37° C. They were observed for phase separation and turbidity.

Grades for the visual assessment of self nano emulsifying formulation

Grade	Visibility
I	Clear or slightly bluish white in appearance within I Minute
II	Slightly less clear; bluish white in appearance < 2 minutes
III	Milky in appearance within 3 minutes
IV	Dull white which is slightly in appearance, slow to emulsify > 3 minutes
V	Turbid in appearance >3 minutes

b. Self-emulsification time

1ml of formulations was added to 100 ml of distilled water at 37° C being agitated at 100 rpm. The time required for the formation of a milky emulsion was noted**.**

c. Robustness to dilution

The formulations were diluted to 10 ml, 50 ml, and 100 ml and were observed over a period of 24 hours for phase separation or signs of precipitation.

d. Droplet size and Zeta potential

1ml of formulation was diluted to 100 ml with distilled water and sonicated for 15 minutes. The resulting nano-emulsion was checked for droplet size and zeta potential in a particle size analyzer (Malvern zetasizer). The average droplet size and zeta potential was determined.

e. Drug content

1ml of formulations were taken and diluted sufficiently, these solutions were then analyzed in the UV spectrophotometer. The drug concentration present was extrapolated from the standard graph. The drug content was calculated using the below formula.

Drug content=concentration X dilution factor X correction factor X volume of formulation.

Morphological studies

a. Scanning electron microscopy

The morphology and size of the prepared SNEDDS was observed by SEM. Samples were fixed on a brass stub using double sided adhesive tape and were made electrically conductive by coating with a thin layer of gold and SEM images were recorded at 15 KeV accelerating voltage.

b. *In-vitro* drug release

The *In-vitro* Drug release profile of selected Ursodeoxycholic acid SNEDDS formulation F5 were carried by using Dialysis Bag Diffusion Technique. In Dialysis Bag, the tested formulation was placed in a glass beaker containing acceptor medium as phosphate buffer pH 6.8 and incubated at 37 degree Celsius in a Magnetic stirrer. The *In-vitro* Drug release was determined.

Results and discussion

Preformulation study

1. **Melting point**
   The melting point was found to be 272˚C which confirms the identification of the drug.

2. **Solubility study**
   Solubility of ursodeoxycholic acid in different solvents are

- Water – insoluble.
- Ethanol – soluble.
- Dimethyl sulphoxide (DMSO)– soluble.
- Dimethyl formamide –soluble.

3. Infrared studies

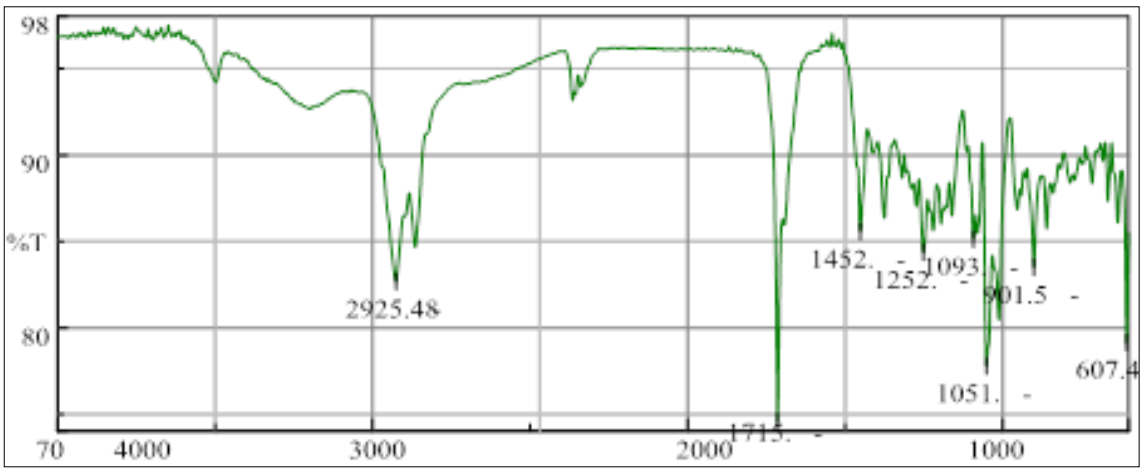


Fig 1: FTIR data of Ursodeoxycholic acid

Table 2: FTIR data of Ursodeoxycholic acid

S.no	Wave no (cm^{-1})	Assignment
1	2925.4	Acid Stretching
2	1715	C=O Stretching
3	1051.9	C-O Stretching

FTIR Data of Drug + Excipient

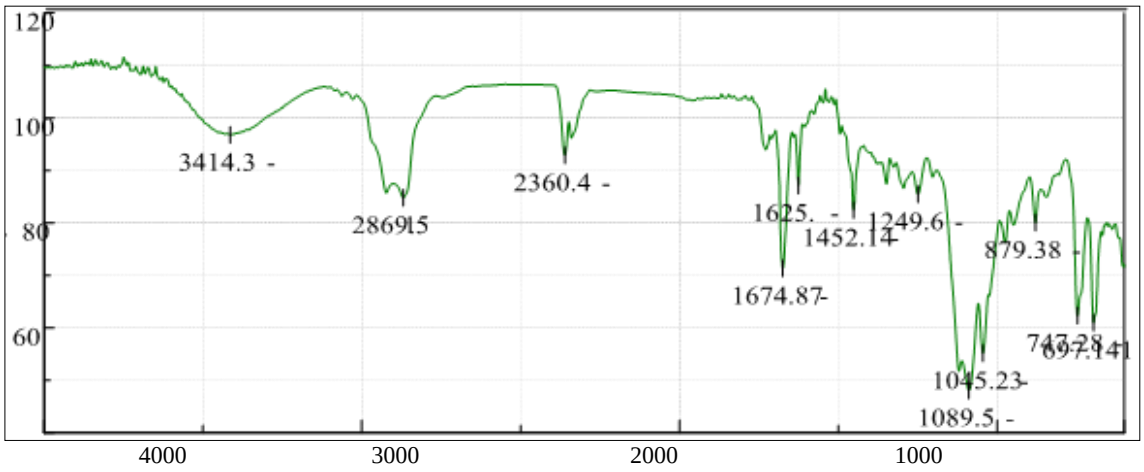


Fig 2: FTIR data of drug excipient

Table 3: FTIR data of drug excipient

S. no	Wave no (cm^{-1})	Assignment
1	2869(drug)	Acid stretching
2	1625(cinnamon oil)	C=c stretching
3	1089(span -80)	C-O alkane bending
4	3414(ethanol)	OH stretching

The FTIR study conclude that there is no major interaction occurred between drug and excipient.

Lambda max

The diluted stock which was scanned for maximum wavelength the peak at 216 nm this was selected and was used for further studies.

Standard graph

The linearity was obtained between 10-80mcg/ml of ursodeoxycholic acid and the regression value (r^2) was found to be 0.99113.

Table 4: Calibration curve of Ursodeoxycholic acid

Concentration(mcg/ml)	Absorbance at 216 nm
10	0.076
20	0.296
30	0.386

40	0.493
50	0.626
60	0.748
70	0.915
80	1.064

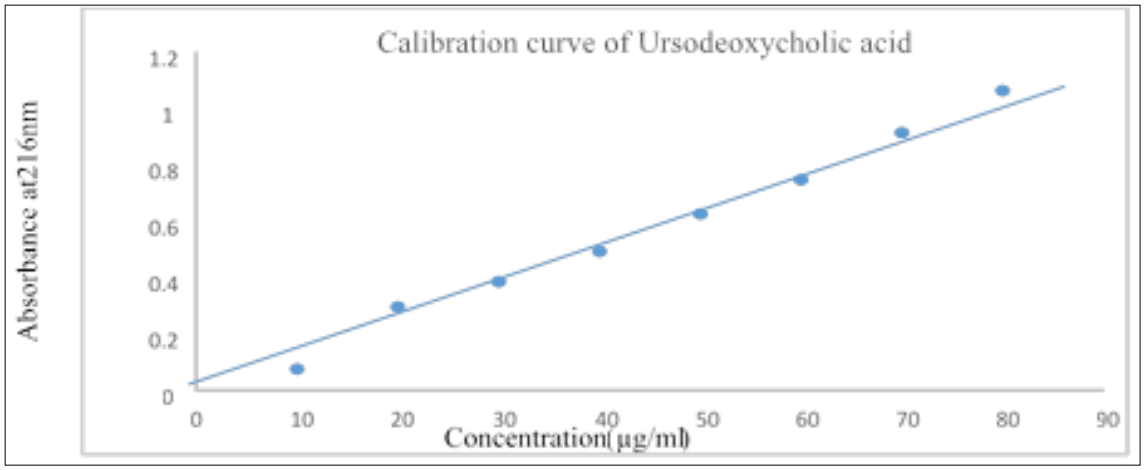


Fig 3: Calibration curve of Ursodoxycholic acid

Solubility Studies in Oil

Vehicle Solubility

Olive oil - 7.21
Cotton seed oil - 8.36
Cinnamon oil - 25
Corn oil - 4.89

Screening of Surfactant

300 ml of the surfactant was mixed with 300 ml of the selected oil, heated to 50°C and dilute to 50 ml with water. The ease of emulsification was observed by the number of flask inversions required for the formation of an emulsion.

They were also observed visually for any signs of phase separation or turbidity. Based on the observation the surfactant has selected for formulation.

Evaluation

Visual assessment

Formulations FT-5 showed no phase separation or turbidity. Formulations with concentrations of oil below 30% and surfactant above 70 showed SNEDDS that have good clarity and No phase separation.

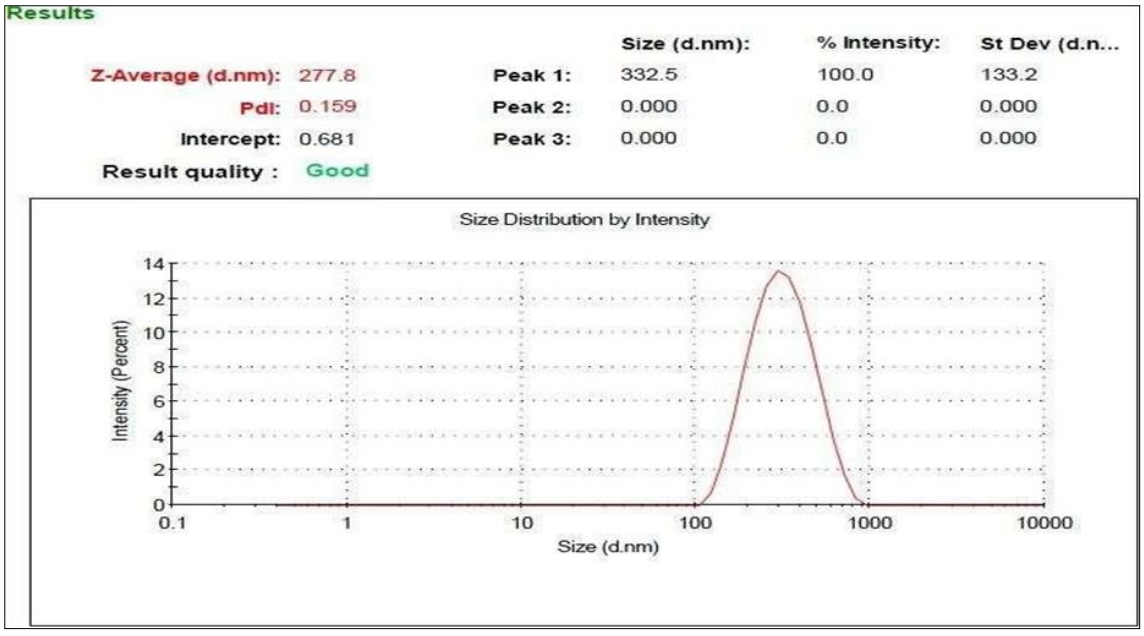
In nano-emulsion formulations only FT-5 was clear. The rest of the formulations showed precipitation.

According to the visual assessment of FT-5 was Grade III in which milky appearance occurs within 3 minutes.

Self emulsification time

The time required for the formation of a milky emulsion was noted for F5 is 2mins.

Droplet size & Zeta potential



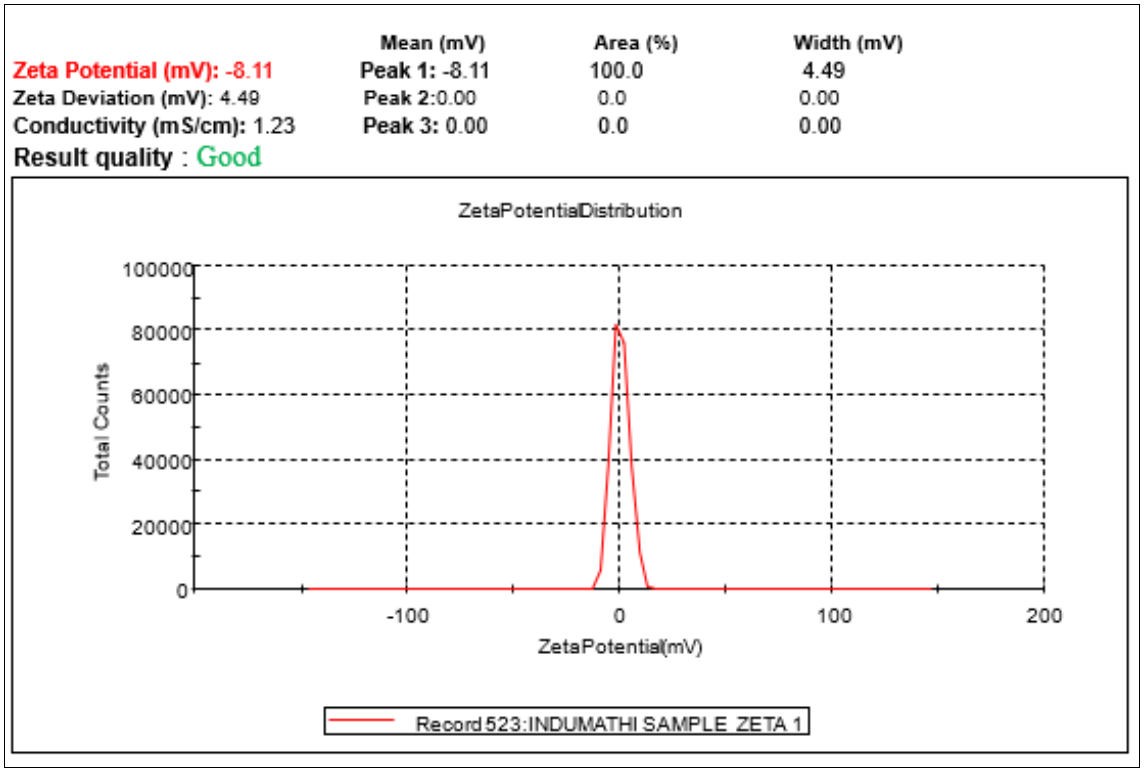


Fig 4: Droplet size & Zeta potential of FT5

Formulation of Ursodeoxycholic acid liquid SNEDDS

The formulation of Ursodeoxycholic acid L-SNEDDS was prepared by the mixture of oil, surfactant & co-surfactant.

Fig 5: Formulation of UDCA L-SNEDDS

Drug Content

The drug content we prepared Ursodeoxycholic acid L-SNEDDS was found to be 0.63 mg.

Robustness to Dilution

The formulations were diluted in various ratios to assess the performance of the L-SNEDDS in the body. The diluted L-SNEDDS showed no precipitation or phase separation indicating the stability of the nano emulsions.

***In-vitro* Drug Release**

The Ursodeoxycholic acid SNEDDS formulation FT5 provide a better drug release. The drug release profile was given by a graphical representation.

***In vitro* release of formulation FT5**

Table 5: *in vitro* drug release

Time in hrs	Cumulative % drug release
0	0
0.6	8.2
0.8	10.6
1	13.2
2	25.7
3	32
4	40.8
5	47.3
6	52.8
7	58.9
8	63.2

Summary & Conclusion

Oral route is the most convenient route of administration but it faces the problem of low oral bioavailability. Self nano emulsifying therapeutic system (SNEDDS) can be used to overcome the problems faced while using low aqueous soluble drugs. These systems form emulsion *in situ* with have good stability. This study aimed at investigating the increase in the bioavailability by administering a BCS class II drug, in a SNEDDS form and was compared to the conventional ursodeoxycholic acid tablets. It can be

concluded from the experimental study carried out that the formulation of a poorly water soluble drug, ursodeoxycholic acid into Self Nanoemulsifying Drug Delivery System yields a formulation with nano size range & good zeta potential.

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