Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i03.3849

FORMULATION AND EVALUATION OF SELF-NANO EMULSIFYING SYSTEM OF ORNIDAZOLE (SNEDDS) AS ANTI-BACTERIAL AGENT

Poonam Ghorpade[1*], Vishal R. Rasve[2], Mahesh Kshirsagar[3], Snehal Lad[4], Vaishnavi Katkar[5]

[1-5]SAJVPM's, College of Pharmaceutical sciences and research center, Kada, Beed, MH-414202

***Corresponding Author: -** Poonam Ghorpade
*SAJVPM's, College of Pharmaceutical sciences and research center, Kada, Beed, MH-414202

ABSTRACT: -
The current research work study deals with the development of SNEDSS of ornidazole. The pure drug ornidazole is act as an antifungal treat on the fungal infection. SNEDDS definitions were effectively planned by developing pseudo ternary phases and assessed. To Formulate and Evaluate the self nano-emulsifying drug delivery system (SNEDDS) ofpoorly water-soluble drug Ornidazole by using different Oil, Surfactants, Co-surfactant. To improve the solubility of Ornidazole. To construct the ternary phase diagrams of SNEDDS. To formulate the liquid SNEDDS. To evaluate the physicochemical characterization of developed formulations. To perform *In-vitro* characterization of developed formulations. The development of SNEDDS drug is selected based on its solubility and bioavailability. The Ornidazole is BCS class II drug having poor solubility and high permeability hence it is good choice of drug for development of SNEDDS.[8] The short elimination half-life of ornidazole is 12-13 hrs. Then to gives reducing the dose frequency by oral administration shows better first pass effect. The bioavailability is 90 %. The preparation of SEDDS were characterized for % yield, % drug content, Zeta potential & DSC, in-vitro drug release (%CDR) for anti-protozoal activity. The results give prepared SEDDS formulation enhanced drug content. In the optimized A7 trial which release ornidazole. 18.56±1.2 % in 5 min. & remaining drug released up to 60 min. which is 98.94 ±2.45%. The SEDDS formulation were found to be stable under stability condition, which that the better drug delivery system (ODDS) & Novel drug delivery system for improved therapeutic effect of anti-protozoal anti-amoebic drug ornidazole.

Keywords: anti-protozoal, ornidazole, Self Emulsifying Drug Delivery System, anti-amoebic, In-Vitro drug release, bioavailability, Self emulsifying nano-drug delivery system.

INTRODUCTION

Oral drug delivery is the ideal route of drug administration as it is simple, most convenient, safest, noninvasive and most economical. Because of the low aqueous solubility, the oral delivery of hydrophobic drugs presents a major challenge.[1]

So we can use self-emulsifying drug delivery systems (SEDDS), which are isotropic mixtures of oils, surfactants, solvents and co-solvents/co-surfactants, for the design of formulations to improve the oral absorption of highly lipophilic drugs. When SEDDS administered orally in soft or hard gelatin capsules it forms stable oil in water (o/w) emulsionsupon aqueous dilution due to the gentle agitation of the gastrointestinal fluids.[2]

SNEDDS formulation's efficiency on oral absorption of drug depends on many formulations related

parameters, such as surfactant concentration, oil/surfactant ratio, and polarity of the emulsion, droplet size and charge, determination of the self-emulsification ability. Hence, only specific pharmaceutical excipient combinations will give efficient self-emulsifying systems. From many studies that have been carried out, concludes that there are few drug products on the pharmaceutical market formulated as SNEDDS which confirming the difficulty of formulating hydrophobic drug compounds into these formulations. The improvement in the oralbioavailability of hydrophobic drug compounds has been used for each case. The fact that almost 40% of the new chemical entities are hydrophobic in nature indicates that studies with SNEDDS will continue and more drug compounds formulated as SNEDDS will have more advantages and will be in the pharmaceutical market in the future.[2]

Presently the SNEDDS, the high-level strategy of nano emulsion was utilized to foster a measurement structure having high medication solvency and bioavailability. Due to their oneof kind thermodynamic strength, there are increments considerations for its utilization as clever medication conveyance framework. Nano emulsion is generally utilized measurement's structure accessible in market. It by and large used to upgrade the solvency and bioavailability of the ineffectively dissolvable medications.[3]

Ornidazole inhibits the growth of protozoa by interacting with the DNA of the micro-organism and inhibiting the protein synthesis, thereby leading to death of the micro-organism.

Self-emulsifying drug delivery system (SEDDS) are utilized to address low bioavailability issues of inadequately dissolvable and profoundly penetrable mixtures. A hydrophobic medication can be disintegrated in these frameworks, empowering them to be directed as a unit dose structure for per-oral organization. While Self Emulsifying drug delivery system (SEDDS) definition is delivered in the lumen of the gastrointestinal parcel, they interact with GI liquid and structure a fine emulsion (micro/nano) So called as in situ emulsification or self- emulsification which further prompts solubilization of medication that can in this way be consumed by lymphatic pathways, bypassing the hepatic first-pass impact.[9, 24.]

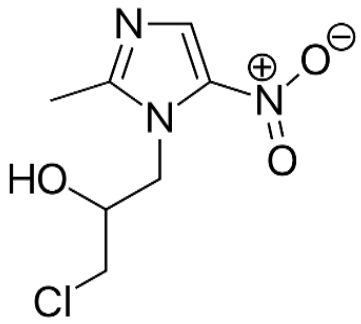
The self-Nano emulsifying drug delivery systems (SNEDDS) are nano-emulsions shaped by SEDDS. SNEDDS are likewise called as self nano emulsion pre-concentrate or as anhydrous structures frequently the nano emulsion. They are heterogeneous scatterings of two immiscible fluids (oil-in-water [O/W] or water-in-oil [W/O]) having a mean drop size in the nanometric scale (normally 20-200 nm), relies upon technique for planning. Upon weakening with water and under state of delicate or gentle unsettling like those which would beexperienced in gastrointestinal tract (GIT) by the stomach related motility of the GIT inside the body. SNEDDS are thermodynamically steady straightforward or clear definitions. Comparable to surfactants and co-surfactants, nonionized scattering of (O/W) nano emulsionshave been balanced out. The SNEDDS is one of the stable nano emulsions, giving a wide connection point to medicate parting among oil and fluid stage, this further develops drug disintegration rates and increments drug plan bioavailability.

The self-nano emulsifying drug delivery system was grown principally using medium chain fatty substance oils and non-ionic surfactant, which is fundamental for oral administration. TheSNEDDS is one of the stable nano emulsions, giving a wide connection point to tranquilize parting among oil and watery stage, this further develops drug disintegration rates and increments drug plan bioavailability. These are the favored medication conveyance framework due to their steadiness, basic oral organization practicability. The SNEDDS is one of the stable nano emulsions, giving a wide point of interaction to medicate parting among oil and watery stage, this further develops drug disintegration rates and increments drug plan bioavailability.[10 11]

MATERIALS AND METHODS: -

DRUG PROFILE:

Ornidazole: -



IUPAC name: 1-Chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol

Introduction: - Ornidazole is a nitroimidazole agent indicated in the treatment of infections such as trichomoniasis, amebiasis, and giardiasis. It is used in the treatment of susceptible protozoal infections and for the treatment of anaerobic bacterial infections.

Chemical nature and structure: - Ornidazole is a C-nitro compound that is 5-nitroimidazole in which the hydrogens at positions 1 and 2 are replaced by 3-chloro-2-hydroxypropyl and methyl groups, respectively. It has a heterocyclic structure consisting of a nitroimidazole-based nucleus with a 2-hydroxy3- chloro-propyl group in position 1 and a methyl group in position 2. It is synthesized from 5-nitroimidazole derivatives. Ornidazole is known chemically as 1-(2-hydroxy-3-chloropropyl)-2-methyl-5-nitroimidazole (C7H10ClN3O).

Mechanism of Action: - Ornidazole is a nitro imidazole which has broad spectrum cidal activity against Protozoa and some anaerobic bacteria. Its selective toxicity to anaerobic microbes involves 1. Drug enters the cell by diffusion, 2. Nitro group of drugs is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules. 3. DNA helix destabilization &strand breakage has been observed.

METHODOLOGY

Selection of S:mix ratio :

S:mix ratio for the preparation of formulation was selected by using different types of surfactant and co-surfactant depending upon solubility of drug in it.

Batchcode	S:mixratio	Surfactant (Tween 80) ml	Co-surfactant (PEG 400) ml
A1	1:1	1	1
A2	2:1	2	1
A3	1:2	1	2

Procedure:

Weighed accurate amount of surfactant as per above table and transpered it intest tube.
Then co-surfactant was added as per given quantity into surfactant containingtest tube.
Then mixture was vortex for 10 to 15 mins and kept for 24hrs.
The final ratio was selected on the basis of phase separation.
The batch was rejected if any phase separation was observed visually.

Preparation of SNEDDS

The procedure for preparation of batches, was perform as per given in below. The procedure simply performed by using the magnetic stirrer (Remilab FHMS-3491 and then using sonicator (Citizen Digital ultrasonic cleaner CD-4820).

Procedure

Surfactant and co-surfactant (S:mix) were accurately weighed and then vortex for 5-10 min. After that S: mix was placed in R.T. for 10 mins. Half quantity of drug was dissolve in S: mix by continuous stirring for 10 to 15 mins. Measure accurate quantity of oil and dissolve remaining quantity of drug in it. Then add oil phase into S: mix solution by continuous stirring. These formulations were stired for 20 to 30 min at R.T. After stirring, sonicate the mixture for 15 mins. An isotropic mixture was formed.

Formula for liquid SNEDDS

The formulation of ornidazole SNEDDS involves three to five components i.e. oil phase, aqueous phase and a primary surfactant and in many cases the secondary phase co-surfactant and sometimes an electrolyte. Their formulation is highly specific process involving spontaneous interaction among the constituents' molecules.

Evaluation of liquid SNEDDS

pH determination: The pH of detailing is resolved just by electric pH meter. The electric bar plunged in thearrangement and the pH was acquired.

pH Range: The reach goes from 0-14, with 7 being neutral, pH of below 7 show acidic, while a pHmore than 7 show base.

Density: The density of the final formulation checked by using density bottle. The empty bottle weight and the solution filled bottle weight is measure and final density was calculated as per given formula-

(W3-W1)/(W2-W1) *A

Whrere,

W1 – weight of empty bottle

W2 – weight of bottle with water W3 – weight of bottle with solutionA-density of water i.e., 0.9960.

Viscosity:

The rheological properties or stream properties was concentrated by utilizing consistencystudy. The methodology was performed at 20rpm. The methodology includes basically dippingthe electric bar in the arrangement and the thickness was get at advanced viscometer.

Dispersibility Test

The Dispersibility test for SNEDDS completed to survey the similarity to scatter the medication into emulsion and the size of coming about globules to sort them as SNEDDS. It was performed by utilizing USP Paddle type disintegration mechanical assembly by basically blend the SNEDDS in 500 ml of water at 37± 0.5 °C and delicately mix thearrangement 50rpm. In the wake of blending with water, the SNEDDS definition frames acombination, which was in various sort contingents on the classes given according to norms.

Table 01: Type of formulation depending upon visual observation

Sr No.	Dispersibility and Appearance	Grade	Time of Self Emulsification(min)
1	Rapidly forming Nano emulsion, having a Transparent or bluish appearance.	A	Within 30 seconds
2	Rapidly forming, slightly less transparent emulsion, having a bluish white Appearance.	B	Within 1 min
3	It is a Fine Whitish milky emulsion	C	Within 2 min
4	Dull, greyish white emulsion having slightly oily appearance that is slow to Emulsification process.	D	Within or Longer than 3 min
5	Formulation, exhibiting either less or minimal emulsification with big oil Globules existing on the surface.	E	Longer than 3min

Determination of self-emulsification time

The self- emulsification time of SNEDDS was determined by USP type II dissolution apparatus. 1 ml of formulation was added drop wise in 500ml distilled water at 37± 0.5 ℃. The gently agitation was provided by a standard stainless steel dissolution paddle which rotating at 50 rpm. The emulsification time assessed by visually.

Thermodynamic stability studies

This thermodynamic study of the formulation was performed to check the stability of the sample in different temperature and humidity conditions. In this study three tests were performed that was as below.

Heating cooling cycle

Three cycles were performed between refrigerator temperature (4 ℃) and 40 ℃ and humidity of 75± 5 with storage at each temperature of not less than 24 hours was done. That formulation, which was stable at these temperatures, was select for next centrifugation test.

Centrifugation

After heating cooling cycle, the passed formulations were centrifuge at 3500 rpm for 30 min. Those formulations that did not show any phase separation was select for the further test parameter.

Freeze thaw cycle

Three freeze-thaw cycles for the formulations between -21℃ and +25℃ performed. Those formulations, which are passed thermodynamic stress tests, were further takes place for the Dispersibility test for assessing the efficiency of self-emulsification.

Cloudy Point Measurement

Prepared SNEDDS were compared for cloud point value. Each formulation was diluted with the water in a ratio of 1:100 and it placed in a water bath with gradual increase in temperature. At the cloud point, the drop point, the drop in sample % transmittance from the zero point was measured spectrophotometrically.

Refractive Index Measurement

The optical clarity of the optimized liquid SNEDDS formulation was determined in terms of refractive index using Abbe's refractometer.

Drug content estimation

The liquid SNEDDS containing Ornidazole, equivalent to 10mg was diluted with appropriate quantity of ethanol. The sample was mixed thoroughly to dissolve the drug in ethanol by continuous stirring. Drug content in the solvent extract is filtered through 0.45 μm membrane filter. Drug content was analyzed with suitable analytical method against standard solvent solution of drug.

Zeta potential determination

Zeta-potential of SNEDDS (1 ml) diluted 100 times with distilled water was determined using the Zetasizer (nanozs) (Malvern instruments Ltd. Malvern, U.K.). The size of particle and zeta-potential calculated by using this test.

Differential Scanning Calorimetry

DSC examination of all examples completed utilizing TA 60 WS Instrument differential checking calorimeter. Tests of 2mg of the singular sub, for example, unadulterated Ornidazole. SNEDDS were recorded by setting test in the aluminum skillet and keeping in mind that a vacant container was utilized as reference warmed. Thermo gram were gotten by the DSC 60 warm analyzer program and recorded at steady diagram speed of 1 inch/min. The thermo gram, progress temperature range were

recorded. DSC decides, is any sort of compound cooperationhappened or not.

Fourier Transform-Infrared spectroscopy

FTIR spectra for the medication and the excipients of the advanced details were gotten to really look at utilitarian gathering and compatibility of medication with excipients. One drop ofupgraded plan is blended in with ATR and utilized for the investigation of FTIR range. Unadulterated medication was additionally blended in with ATR and range was acquired. Bothspectra were analyzed for potential deviations.

***In-vitro* release**

This test was performed by using USP type 2 dissolution paddle type apparatus. Percent drug dissolved at different time intervals calculated using Beer Lambert's Equation. The procedure for this test given in below flow chart.

Stability

Stability test was carried out as per ICH guidelines. Stability tests are a lot less complex and required less much of the time for coarse scattering,where drops sizes and stage changes should be followed. To conquer the issue of metastable arrangement which are not thermodynamically steady and gets some margin to separate, thermodynamic solidness test is suggested.

Table 02: Stability study specification as per ICH guidelines

Study condition specification	Time periods
40°C ± 2°C/ 75% RH ± 5% RH	30 days
40°C ± 2°C/ 75% RH ± 5% RH	60 days
40°C ± 2°C/ 75% RH ± 5% RH	90 days

RESULT AND DISCUSSION

Preformulation Study of Ornidazole

Physical Characteristic of Ornidazole

Table 03: Physical Characteristic of Ornidazole

Sr no.	Test	Observation	Inference
1	Color	White or yellowish Powder	Complies with IP
2	Odor	Characteristic	Complies with IP
3	Surface Nature	Amorphous Powder	Complies with IP

The physical characteristics of Ornidazole complies with IP

Melting Point

Table 04: Melting point of Ornidazole

Sr no.	Standard M.P (^{o}C)	Observed M.P. (^{o}C)	Mean M.P (^{o}C)
1	85-98	86	89
2		88	
3		93	

Melting point was found 89^{o}C While as per standard literature it is reported to be 85-98^{o}C. So, it can be concluded that Ornidazole was identified and indicating purity of sample.

Analytical methodology: -

UV spectrophotometrically analysis

Determination of λ max and calibration curve Ornidazole in Ethanol

Determination of Ornidazole in ethanol:

The Ornidazole drug exhibited an absorption maximum at 314 nm. A linear relationship between the λ max and the concentration of Ornidazole was established over the examined concentration range (2-10µg/ml). Linear regression data are given in table 05 and 06.

Analytical calibration curve of Ornidazole in ethanol

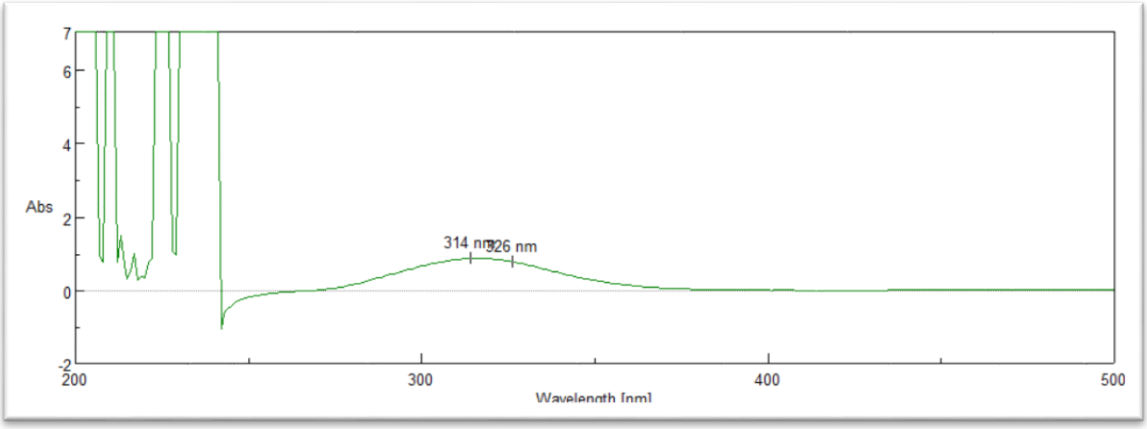


Table 05: Absorbance and conc. data of Ornidazole in ethanol

Sr.no.	Conc. (µg/ml)	Absorbance
1	2	0.1789
2	4	0.2925
3	6	0.3796
4	8	0.4732
5	10	0.7333

Table 06: Calibration data of Ornidazole in ethanol

Sr no.	λ max(nm)	Solvent	Conc. Range (µg/ml)	Regression Equation	Regression Coefficient (R^2)
1	314	Ethanol	2-10	Y=1.7857x+2.8571	0.9766

The calibration curve of Ornidazole in ethanol was found to be linear in the range of2-10 µg/ml and coefficient of regression was found 0.9766.

Solubility study

Results of solubility study of Ornidazole in different oil, surfactant and co-surfactant are given in table. From the results of solubility study of Ornidazole in different oil,surfactants, co surfactants it was found that Ornidazole was more soluble in Mentha oil (50.40 mg/ml) as oil, Tween 80 (31.50 mg/ml) as surfactant and PEG-200 (56.23 mg/ml) as a Co-surfactant than other vehicles. Hence Mentha oil as oil, Tween 80 is as surfactant and PEG- 200 as a Co-surfactant Ornidazole in various oil, surfactant, and co-surfactant in shown in figure 01, 02, 03 and table 07, 08, 09 .

Selection of Oil, Surfactant and Co-surfactant

Table 07: Solubility of Ornidazole in different oil

Sr no.	Oils	Solubility (mg/ml)
1	Peppermint oil	21.99
2	**Mentha oil**	**50.40**
3	Eucalyptus oil	19.04
4	Oleic acid	22.85

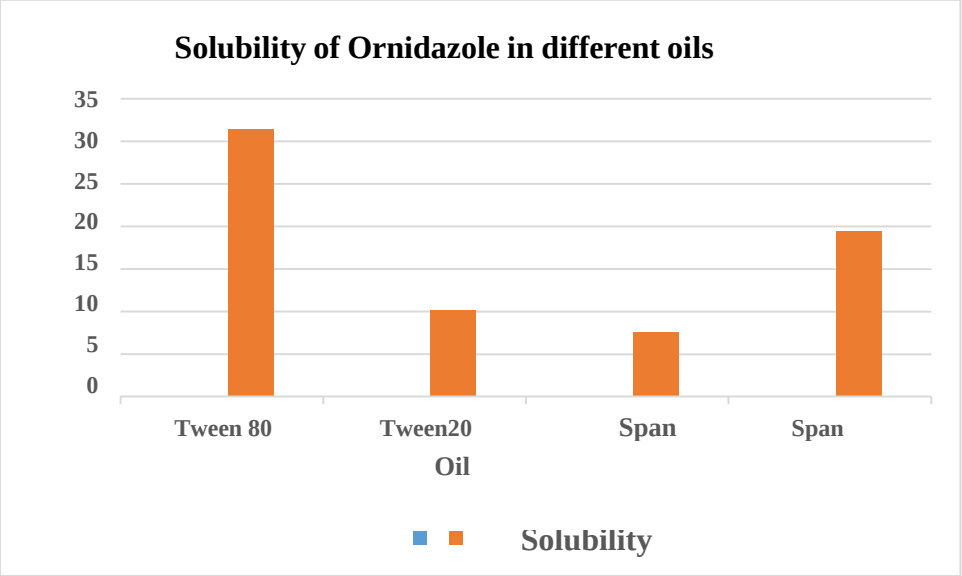


Figure 01: Solubility of Ornidazole in different oil

Table 08: Solubility of Ornidazole in different Surfactant

Sr no.	Surfactant	Solubility (mg/ml)
1	**Tween 80**	**31.50**
2	Tween 20	10.20
3	Span 80	07.57
4	Span 20	19.51

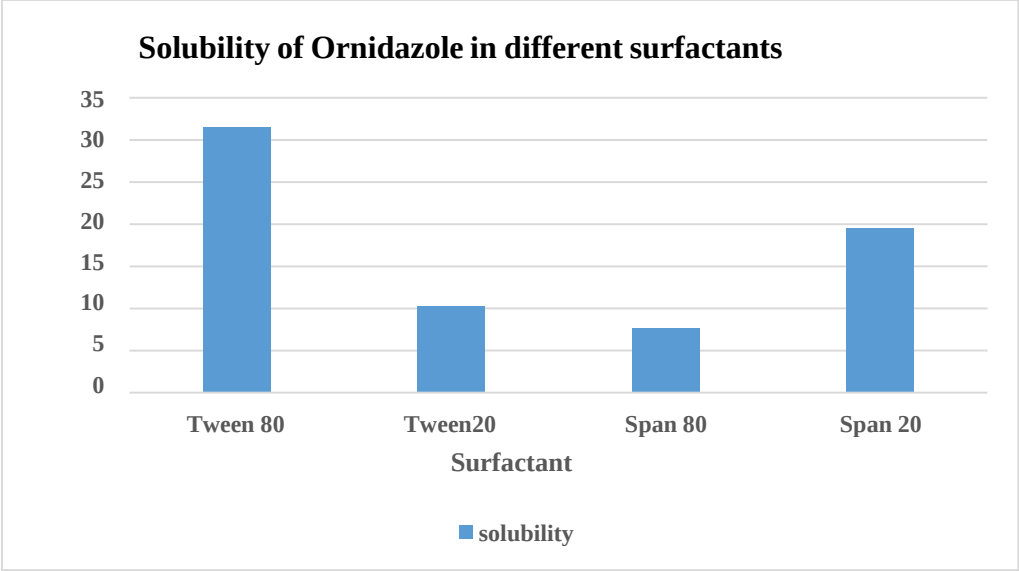


Figure 02: Solubility of Ornidazole in different Surfactant

Table 09: Solubility of Ornidazole in different Co-Surfactant

Sr no.	Co-Surfactant	Solubility (mg/ml)
1	PEG 400	18.90
2	**PEG 200**	**56.23**
3	Propylene glycol	11.87

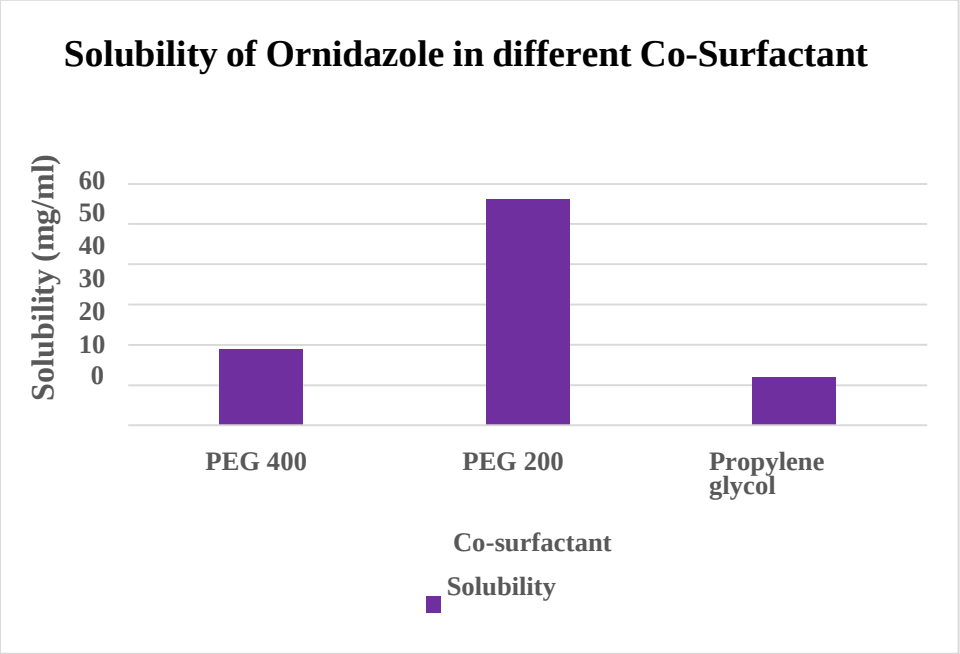


Figure 03: Solubility of Ornidazole in different Co-Surfactant.

Pseudo ternary phase diagram:

The Study on phase behavior using water Titration Method. Phase diagram were constructed using Mentha oil as an oil phase, Tween 80 and PEG 200 asS: mix and water by using CHEMIX software. Obtained phase diagram was given below. Phase diagram by using ratio 1:1 Phase diagram was draw by using CHEMIX software. The 3 points were considered for diagram i.e., oil, S: mix and water. The obtained area of nano emulsion region is represented by colored portion.

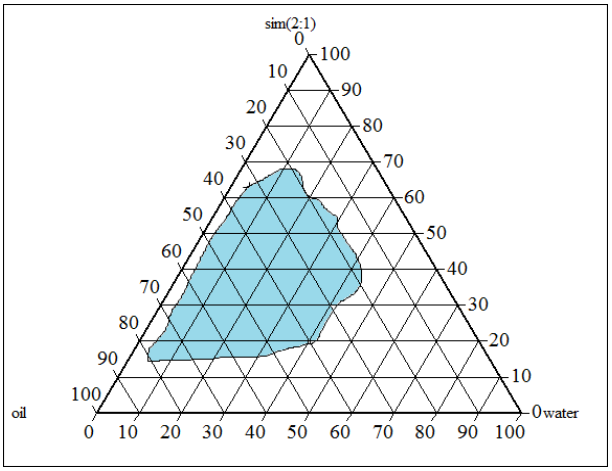


Figure 04: Pseudo ternary phase diagram of oil, S:mix and water with ratio of 1:1

- **Phase diagram by using ratio 2:1**

Phase diagram was draw by using oil: Smix ratio 2:1. The obtained area of nanoemulsion region is represented by coloured portion.

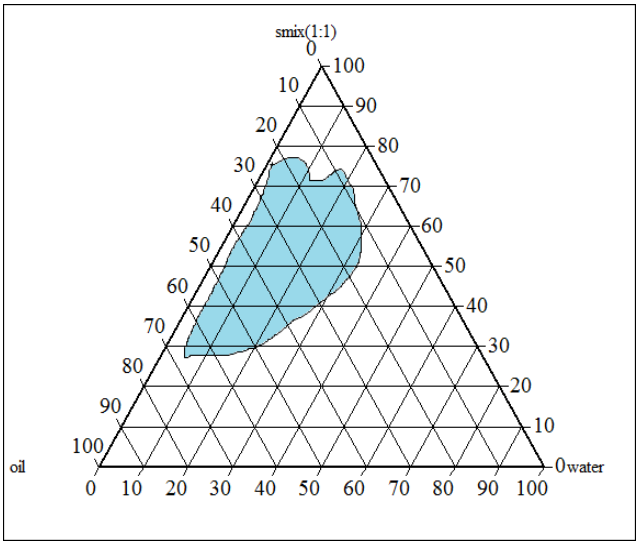


Figure 05: Pseudo ternary phase diagram of oil, S:mix and water with ratio of 2:1

Results of preliminary trials

Selection of S:mix ratio :

The different trials was performed for the selection of S:mix ratio. In the process surfactant and co-surfactant was used as per there solubility trials. Batch A1 shows good result as they doesnot show any phase separation and formed clear, transparent solution as compared to other two below in table 10

Table 10: Result of trials for selection of S:mix ratio

Batchcode	S:mixratio	Surfactant (Tween 80)ml	Co-surfactant (PEG 400) ml	Observation	Result
A1	1:1	1	1	Stable	Pass
A2	2:1	2	1	Thick solution	Fail
A3	1:2	1	2	Thick solution	Fail

- **Phase diagram by using ratio 2:1**

Phase diagram was draw by using oil: Smix ratio 2:1. The obtained area of nanoemulsion regionis represented by coloured portion.

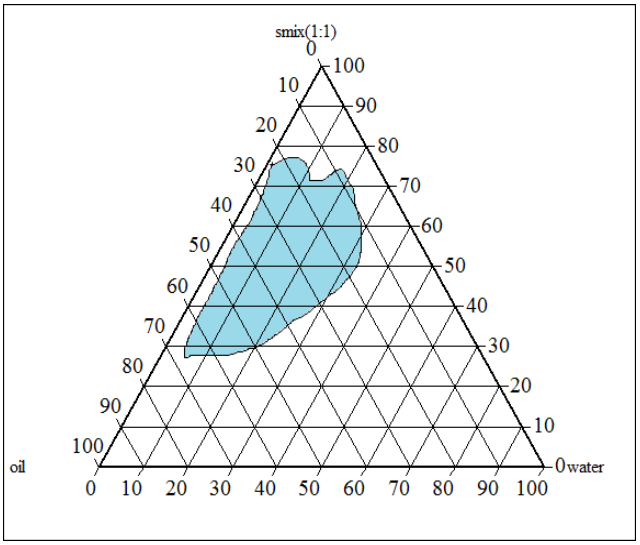


Figure 06: Pseudo ternary phase diagram of oil, S: mix and water with ratio of 2:1.

Results of preliminary trials

Result of Selection of S:mix ratio

The different trials were performed for the selection of S:mix ratio. In the process surfactant and co-surfactant was used as per there solubility trials. Batch A1 shows good result as they doesnot show any phase separation and formed clear, transparent solution as compared to other two below in table 11.

Table 11: Result of trials for selection of S:mix ratio

Batchcode	S:mixratio	Observation	Result
A1	1:1	Stable	Pass
A2	2:1	Thick solution	Fail
A3	1:2	Thick solution	Fail

Result of selection of Oil: Smix ratio

The different trials were performed for the selection of Oil: Smix ratio. The result was given below in table no 12. Batch A5, A7, A8, A9 shows stable nano emulsion.

Table 12: Result of trials for selection of Oil: Smix ratio

Batch code	Observation	Result
A1	Phase separation	Fail
A2	Phase separation	Fail
A3	Phase separation	Fail
A4	Viscous	Fail
A5	Stable	Pass
A6	Phase separation	Fail
A7	Stable	Pass
A8	Stable	Pass
A9	Stable	Pass

In the above observations, some trials show good result which does not show any phase separation. Some trials were fail because they shows phase separation and becomes viscous after kept for 24 hrs.

Result of preliminary trials with drug:

The Oil: Smix ratio was selected as per above trials. Then the drug was added in formulationtrials. The result obtained is given in table below. In the formulation, 500 mg of Ornidazole drugwas added as final concentration. Batch A5 and A7 shows viscous nano emulsion. Batch A8 and A9 shows stable nano-emulsion.

Evaluation of SNEDDS

pH determination

The formulations show basic pH which means drug shows ADME in small intestine i.e., basic nature.

Density & Viscosity determination

The viscosity of the SNEDDS formulation was obtained using Brookefield viscometer using spindle no. 61 at Room Temp and resultant obtained Nanoemulsion is in table Fomulations shows viscocity ans density in range for nano-emulsion.

Table 13: Result of viscosity of SNEDDS formulations

Batch code	pH	Density	Viscosity at rpm (cps)
A5	7.83	0.467	1.49
A7	7.30	0.7131	1.25
A8	8.37	0.579	1.68
A9	8.48	0.608	1.61

Dispersibility test

The liquid SNEDDS was evaluated for dispersibility test as given in experimental work andstudies the various formulations shows the following results in table 14.

Self-emulsification Time-Emulsification time

FormulationA5 and A7 requires SE time less than min hence it is nanoemulsion

Table 14: Result of Dispersibility and self-emulsification time of formulations

Batch code	Dispersibility/ Appearance	Grade	Time inSE(Min)	Inference
A5	**Slightly less transparent**	**B**	**Within 49**	**Pass**
A7	**Bluish white**	**A**	**Within 18**	**Pass**
A8	Milky white	C	Within 1.44	Fail
A9	Milky white	C	Within 1.18	Fail

Thermodynamic stability study:

The screening of a thermodynamic stability studies the A5, A7, A8, A9 formulations, which are observed for three evaluation parameter these, are freezes throw cycle, heating cooling cycle, and the centrifugation are given in table below. The formulation remained stable at above three parameters there was not any change occurred in formulation shows in table 15.

Table 15: Result of Screening of formulations based on thermodynamic stability Study.

Batch code	Observation based on thermodynamic stability studies		
	Freeze thawcycle	Heating/Coolingcycle	Centrifugation
A5	√	√	√
A7	√	√	√
A8	√	√	√
A9	×	×	×

% Transmittance and Clouding point determination

% Transmittance of the Ornidazole SNEDDS was gives idea about the formulation clarity and also the phase separation was easily notice by clarity and transparency of formulation. The transmittance of emulsion should be greater than 99%, that formulation have clear and transparent nature. Formulation A5 and A7 shows % transmittance >99%.

Table 16: Result of % Transmittance and Clouding point of formulation

Batch code	% Transmittance	Clouding point (^{0}c)	Refractive index
A5	99.17	74	1.509
A7	99.87	70	1.506
A8	96	80	1.527
A9	95.12	78	1.532

Refractive index

Refractive index of all formulations shown in following table. Formulations shows refractive index in range for nano emulsion.

Drug Content of SNEDDS

The drug content shows the drug uniformly distributed in the formulation. The percent drug content of all the SNEDDS formulation were found be in the range of 85.16±0.342% to 99.62±0.147%. The drugs content of the A7 formulation was found 99.62±0.147% while other formulation of drug content found less than the 97% so it was concluded that the A7 formulation have more drug content as compare to others.

Table 17: Result of Drug content of all the formulations

Batch code	% Drug content	Zeta potential (Mv)
A5	96.72±0.098	-37.4
A7	99.62±0.147	-32.3
A8	88.24±0.075	-40.6
A9	85.16±0.342	-38.5

Zeta potential determination

Zeta potential shows the stability of formulation and also particle size of the formulation. SNEDDS reports negative zeta potential value mV as shown in Fig. The surfactant (Tween80) and co surfactant (PEG-200) used in this study are non-ionic which do not contribute any charge to the Nanoemulsion particle. The reported stable SNEDDS using same excipients. This indicates that negative charge particle does not affect stability of the nano emulsion. A dividing line in between stable and unstable aqueous dispersions are generally taken place at either +30 or -30mV. Particles with the zeta potentials more than +30mV are normally considered stable. Particles with zeta potentials more than -30mV are normally considered stable.

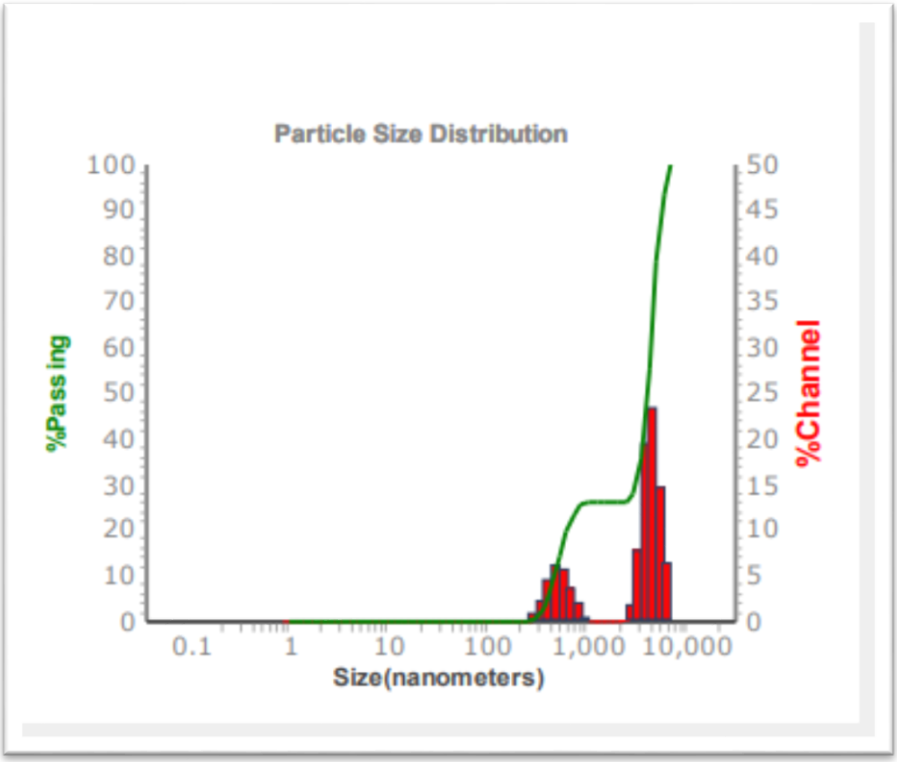


Figure 07: Result of Zeta potential of optimized batch A7

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermo analytical technique used foranalyzing thermal transitions involving thermal energy with great sensitivity.

The DSC thermogram of pure Ornidazole showed the endothermic peak at 228.77^{0}c indicated the melting point the adsorption to solid carrier formulation (A7). There was no sharp change in melting point of drug. Thus, there was no significant interaction between thedrug, surfactant and polymer.

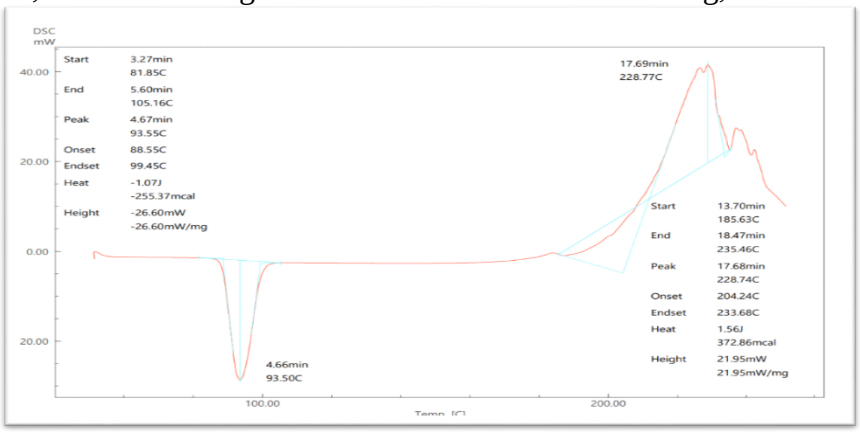


Figure 08: DSC Spectra of Ornidazole

The *In-vitro* dissolution study

The *In-vitro* dissolution test was performed by using USP- type-II dissolution test apparatusin 900 ml of Phosphate buffer pH 6.8 solutions at 37 ± 2^{0}c with the 50-rpm rotating speed. Samples of 1 ml are withdrawn at regular time interval of 5, 10, 15, 30, 45 and 60 mins and filtered by using 0.45 µm filters. An equal volume of respective dissolution medium was added to maintain the volume constant.

Drug content from the sample was analyzed using UV-spectrophotometer at 318 nm. All the measurements are done in triplicate from the three independent samples.

Table 18: *In- vitro* dissolution study of SNEDDS of formulations

% Cumulative drug release					
Time (min)	**A5**	**A7**	**A8**	**A9**	**API**
0	0	**0**	0	0	0
5	12.3	**18.56**	16.25	13.25	9.56
10	22.88	**23.65**	23.65	21.56	13.52
15	30.25	**39.45**	39.85	38.74	19.65
20	45.25	**42.87**	36.23	42.56	23.54
25	58.62	**57.89**	44.89	52.65	27.56
30	62.58	**62.11**	51.23	59.68	31.47
35	69.36	**70.12**	69.89	65.32	38.99
40	75.56	**84.45**	78.45	72.85	41.87
45	83.45	**89.56**	81.25	79.35	46.12
50	93.56	**95.15**	88.96	87.36	50.1
60	98.64	**98.94**	92.74	93.98	58.65

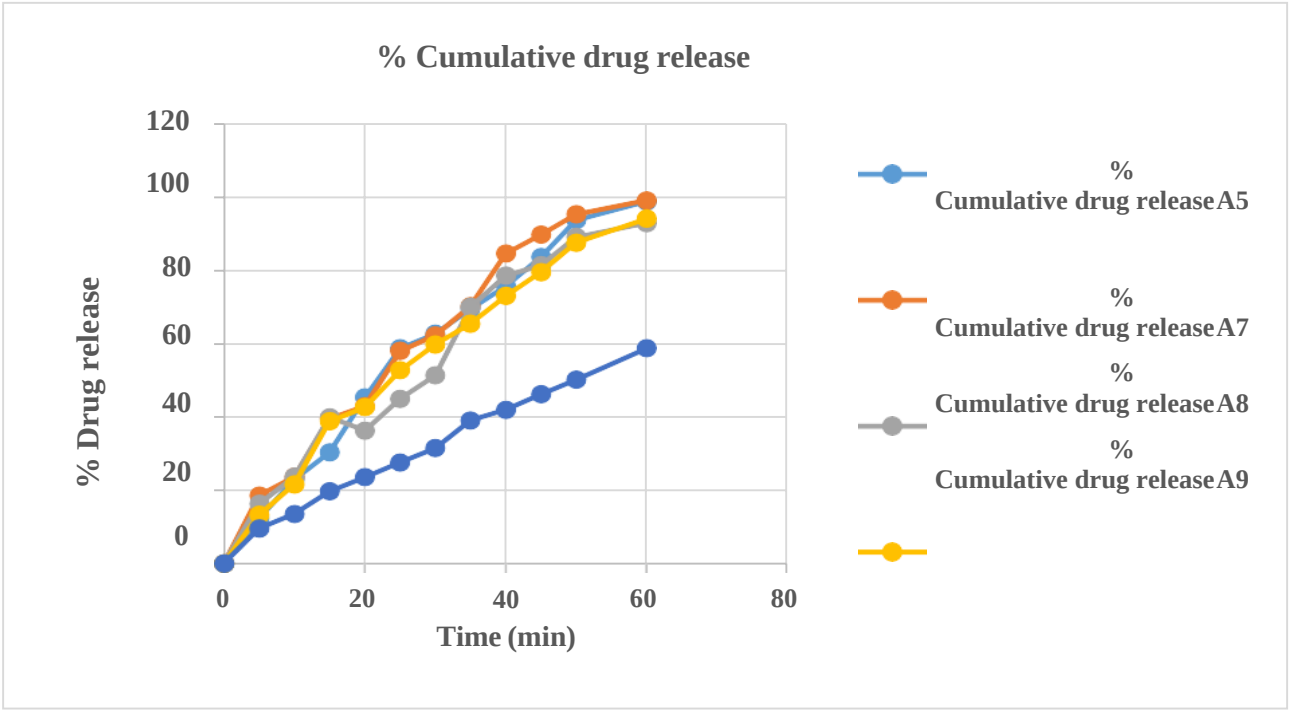


Figure 09: Graphical representation of percent drug released from the formulation

Stability study

Stability test was carried out as per ICH guidelines. From this stability study, it was found that the evaluated formulation (A7) Showed there was no influence of variety of environment factors such as temperature, humidity and light, and during storage conditions or shelf life of drug.

Table 19: Stability study data of optimized batch

	Observations		
Physical parameter	30^{th} day	60^{th} day	90^{th} day
% Drug content	98.52	98.78	99.65

CONCLUSION

The present study explores the possibilities of loading a wide variety of hydrophobic drugs and plant actives as their scale up is as well as economical too. In this research work, self nanoemulsion was prepared which contain Ornidazole as active drug and other suitable excipients which delivers through oral route**.** It is present in liquid form that is thermodynamically stable.

SNEDDS are promising approach for the formulation of drugs of BCS Class II with poor aqueous solubility. Based on present research work with preliminary trials and by evaluations the following conclusion can be made.

- Ornidazole drug is compatible with excipients such as Mentha oil, Tween 80 and PEG 200.
- The various trials were performed further to select proper ratios of excipients for preparation of self nanoemulsion. Liquid SNEDDS convert into solid by using Adsorption to solid carrier techniques successfully. Phase titration method was use for the preparation of formulation with selected excipients. Various trials conducted for preparing stable self nanoemulsion with no phase separation. By performing various trials final formula was optimize that are thermodynamically stable and having no phase separation.
- The percent drug content of all liquid SNEDDS formulation was found to be in range of 83.24 % to 99.62 %. It showed that drug was uniformly distributed in the formulated SNEDDS.
- Zeta potential of the optimized formulation (F2) was found -17.3mV which indicates high surface negative charge on the particle which in turn indicates higher stability because of the anticipated surface repulsion between similar charged particles, hence inhibiting aggregation of the colloidal

particles.

The four batches were prepared for reproducibility determination using optimized formula. All the test parameters were performed for selected four batches.

FUNDING SOURCES
This study was not funded by any of the grant.

CONFLICT OF INTEREST
The authors declare no conflicts of interest to publish this manuscript.

REFERENCES:
1. Dong Shik Kim, Jung Hyun Cho, Self- microemulsifying drug delivery systems (SMEDDS) for improved oral delivery and photostability of methotrexate, Int Journal of Nanomedicine,2019; 14: 4949–4960.
2. Gursoy R N, Benita S, Self- emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs, Biomed Pharmacother,2004 Apr;58(3):173-82.
3. Manjit Jaiswal, Rupesh Dudhe, and P. K. Sharma, Nanoemulsion: an advanced mode of drug delivery system, Biotech journal,2015 Apr; 5(2): 123–127.
4. Marlyn Laksitorini, Vivitri D. Prasasty, Paul K. Kiptoo, and Teruna J. Siahaan, Pathways and Progress in Improving Drug Delivery through the Intestinal Mucosa and Blood-Brain Barriers, HHS Public Access Ther Deliv. 2014 October; 5(10): 1143–1163.
5. Orme M, Drug Absorption in The Gut, Br. J. Anaetth,1984: 56- 59.
6. Bahman Homayun, Xueting Lin and Dr. Hyo-Jick Choi, challenges and recent progress in oral drug delivery systems for Biopharmaceuticals, Pharmaceutics 2019, 11(3)*:*129.
7. Savjani KT, Gajjar AK, Savjani JK, Drug solubility: Importance and Enhancement Techniques, ISRN Pharm. 2012; 2(12):45-56.
8. Mark G. Papich and Marilyn N. Martinez, Applying Biopharmaceutical Classification System (BCS) criteria to predict oral absorption of drugs in dogs: Challenges and pitfalls, AAPS J. 2015 Jul; 17(4): 948–964.
9. Kshitija Khedekar and Swati Mittal**,** Self Emulsifying Drug Delivery System: A Review, Ijpsr (2013); Vol.4, Issue 12:4494-4507.
10. Abhijit A Date , Neha Desai, Rahul Dixit, Mangal Nagarsenker, Self Emulsifying Drug Delivery System: formulation insights,applications and advances, Nanomedicine (Lond), 2010 Dec;5(10):1595-616.
11. Aristote B. Buya, Ana Beloqui, Patrick B. Memvanga and Veronique Préat, Self-Nano-Emulsifying Drug-Delivery Systems:From the Development to the Current Applications and Challenges in Oral Drug Delivery, Pharmaceutics 2020; Vol 12:1194.
12. Mrunali R. Patel and Shivam D. Thakore, Nanoemulsion in drug delivery, In journal Applications of Nanocomposite Materials in Drug Delivery, 2018:1-2.
13. Thakur Gurjeet Singh and Mansi Chitkara, Nanobiomaterials, In journal Fabrication and Self-Assembly of Nanobiomaterials, 2016:5-6.
14. Stefano Buzzaccaro, Antonio Tripodi, Roberto Rusconi1, Daniele Vigolo and Roberto Piazza, Kinetics of sedimentation in colloidal suspensions, J. Phys.: Condens. Matter (2008); 20: 494219- 494228.
15. Gaurang Patel, Ambikanandan Misra, Oral Delivery of Proteins and Peptides,In journal Challenges in Delivery of Therapeutic Genomics and Proteomics, 2011; Vol 1:191- 193.
16. Aristote B. Buya, Ana Beloqui, Patrick B. Memvanga and Veronique Preat, Self-Nano-Emulsifying Drug-Delivery Systems: From the Development to the Current Applications and Challenges in Oral Drug Delivery, Pharmaceutics. 2020 Dec; 12(12): 1194.
17. Zahari Vinarov, Mohammad Abdallah, Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review, European Journal of Pharmaceutical

Sciences,July 2021; Volume 162:10581.
18. Michael Grimm, Mirko Koziolek, Jens-Peter Kühn and Werner Weitschies, Interindividual and intraindividual variability of fasted state gastric fluid volume and gastric emptying of water, European Journal of Pharmaceutical Sciences, 2018; Volume 127: 309-317.
19. Barbara M. Davit, Isadore Kanfer, BCS Biowaivers: Similarities and Differences Among EMA, FDA, and WHO Requirements, AAPS J. 2016 May; 18(3): 612–618.
20. B. Basanta Kumar Reddy, and A. Karunakar, Biopharmaceutics Classification System: A Regulatory Approach, Dissolution Technologies, 2011:31-37.
21. Ashish Gadhave, Determination of Hydrophilic-Lipophilic Balance Value, IJSR, 2014; Volume 3 Issue 4: 573-575.
22. Solubility, Wikipedia: The free encyclopedia.
23. Venkat Ratnam Devadasu,Rakesh K. Tekade, Physicochemical, Pharmaceutical, and Biological Considerations in GIT Absorption of Drugs, In journal of Dosage Form Design Considerations, 2018: 149-178.
24. Prakash Khadka , Jieun Ro, Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability, Asian Journal of Pharmaceutical Sciences December 2014; Volume 9, Issue 6:304-316.
25. Abhijit A Date; Neha Desai; Rahul Dixit; Mangal Nagarsenker, Self-nanoemulsifying Drug Delivery Systems: Formulation Insights, Applications and Advances, Nanomedicine. 2010; 5(10):11-15.
26. Ahmad Mustafa Masoud Eid, The Effect of Surfactant Blends on the Production of Self-Emulsifying System, IJPFR, April-June 2012; 2(2):21-31.
27. Maarit J. Rein, Mathieu Renouf, Bioavailability of bioactive food compounds: a challenging journey to bioefficacy, Br J Clin Pharmacol. 2013 Mar; 75(3): 588–602.
28. Vivek P. Chavda, Dhaval Shah, Self-emulsifying delivery systems: one step ahead in improving solubility of poorly soluble drugs, In journal Nanostructures for Cancer Therapy, 2017: 653-718.
29. Milica Markovic, Shimon Ben-Shabat, Lipids and Lipid-Processing Pathways in Drug Delivery and Therapeutics, Int J Mol Sci. 2020 May; 21(9): 3248.
30. 29) Samuel L. Jones and Anthony T. Blikslager, Disorders of the Gastrointestinal System, Equine Internal Medicine. 2004: 769–949.
31. Kazi Mohsin, Design of Lipid-Based Formulations for Oral Administration of Poorly Water-Soluble Drug Fenofibrate: Effects of Digestion, AAPS PharmSciTech. 2012 Jun; 13(2): 637–646.
32. Shweta Gupta, Rajesh Kesarla, and Abdelwahab Omri, Formulation Strategies to Improve the Bioavailability of Poorly Absorbed Drugs with Special Emphasis on Self-Emulsifying Systems, ISRN Pharm. (2013); 2013: 848043.
33. Priya Thakare, Vrushali Mogal, Pankaj Borase, Jaydeep Dusane and Sanjay Kshirsagar, Areview on self-emulsified drug delivery system, Pharmaceutical and Biological Evaluations April 2016; Vol. 3, Issue (2): 140-153.
34. Eleni Kontogiannidou, Thomas Meikopoulos, In Vitro Evaluation of Self-Nano- Emulsifying Drug Delivery Systems (SNEDDS) Containing Room Temperature Ionic Liquids(RTILs) for the Oral Delivery of Amphotericin B, Pharmaceutics. 2020 Aug; 12(8): 699.
35. Surya Kovvasu, Self-emulsifying Drug Delivery Systems and their Marketed Products: A Review, Asian Journal of Pharmaceutis,Apr-Jun 2019; 13(2): 73.
36. Y. Nakama, Surfactants, In journal of Cosmetic Science and Technology, 2017: 231-244.
37. Jean-Louis SALAGER, Book of surfactant types and uses: 1-48.
38. Marc Hirsch, Original article on Lowering surface tension – Surfactants in coating materials, February 25, 2021:1-6.
39. Ali Nasr, Effect of oils, surfactants and cosurfactants on phase behavior and physicochemical properties of self-nanoemulsifying drug delivery system (Snedds) for irbesartan and olmesartan, Int J App Pharm, 2006; Vol 8, Issue (1): 13-24.

40. Reza Fattahi, Babak Ghanbarzadeh, The effect of Macro and Nano-emulsions of cinnamon essential oil on the properties of edible active films, Food Sci Nutr. 2020 Dec; 8(12): 6568–6579.
41. Theories of Microemulsion Formation, By Pharma Tips: 2011.
42. Kaustav B, Book on Importance of Surface Energy in Nanoemulsion, 2019: 1-432.
43. Jeand Baloch, Muhammad Farhan Sohail,Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation, Medicina (Kaunas), 2019 May; 55(5): 210.
44. Kareem Rahn-Chique, German Urbina-Villalba, Article on Correct Use of the Lifshitz-Slyosov-Wagner Expression for the Calculation of the Average Radius of an Oil-In-Water (o/w) Emulsion Subject to Coalescence: 2015: 324.
45. Applications of SNEDDS, Wikipedia.
46. Mohammed Al-Swairi, Evaluation of Self-Nanoemulsifying Drug Delivery Systems(SNEDDS) for Poorly Water-Soluble Talinolol: Preparation, *in vitro* and *in vivo* Assessment, Front. Pharmacol, 2019; (10) 459: 1-15.
47. Jaydeep Patel, Garala Kevin, Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery, Int J Pharm Investig. 2011 Apr-Jun; 1(2):112–118.
48. Ornidazole Pharmacology, Medicine India.
49. Mentha oil, Wikipedia.
50. Manoj Mishra, Pharmaceutical preformulation studies in formulation and development of new dosage form: A review, International Journal of Pharma Research & Review, Oct 2016; 5(10):12-20.
51. General tests, processes and apparatus, JP XVI, 25-312.
52. Phuong Tran, Jeong-Sook Park, Recent trends of self- emulsifying drug delivery system for enhancing the oral bioavailability of poorly water- soluble drugs, Journal of pharmaceutical Investigation; (2021),51: 439-463.
53. Darna Bhikshapathi, Formulation and characterization of pioglitazone HCl self emulsifying drug delivery system, Scholars Research Library Der Pharmacia Lettre, 2013, 5 (2):292-30.
54. Ashok Meena, Formulation development of an albendazole self-emulsifying drug delivery system (SEDDS) with enhanced systemic exposure,Acta Pharm,(2012);62: 563–580.
55. Ping Gao, Development of a supersaturable SEDDS (S-SEDDS) formulation of paclitaxel with improved oral bioavailability, J Pharm Sci, 2003 Dec;92(12):2386-98.
56. Prabagar Balakrishnan, Enhanced oral bioavailability of dexibuprofen by a novel solid Self-emulsifying drug delivery system (SEDDS), European Journal of Pharmaceutics and Biopharmaceutics, Aug 2009;Volume 72, Issue 3: 539-545.
57. Nitin Pandurang Gurav, Solubility Enhancement of Satranidazole Using Self Emulsified Drug Delivery Systems, Indian Journal of Pharmaceutical Education and Research, Jul-Sep, 2016; Vol 50, Issue 3 (Suppl.): 68-75.
58. Zwanden Sule Yahaya, Preliminary investigation on coconut oil-based self-emulsifying formulation for the delivery of Metronidazole, Nigerian Journal of Pharmaceutical and Applied Science Research, January 2020; 9(1):18-24.
59. Venkatesh Miryala, Mallesh Kurakula, self- nano emulsifying drug delivery system (snedds) for oral delivery of atorvastatin formulation and bioavailability studies, Journal of Drug Delivery & Therapeutics; 2013, 3(3):131-142.
60. Sailaja Gunnam, Formulation and Evaluation of Self-emulsifying Drug Delivery System of Bosentan, Asian Journal of Pharmaceutics Apr -Jun 2018;12 (2):822- 833.