Available online on 25.12.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Formulation, Characterization, In-vitro Evaluation and Ex-Vivo Studies of Doxepin Loaded Self Micro Emulsifying Drug Delivery system

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Article Info:

Article History:

Received 13 October 2021
Reviewed 17 December 2021
Accepted 21 December 2021
Published 25 December 2021

Cite this article as:

Tallapally M, Dunaka S, Jakku S, Janapareddi K, Formulation, Characterization, *In-vitro* Evaluation and Ex-Vivo Studies of Doxepin Loaded Self Micro Emulsifying Drug Delivery system, Journal of Drug Delivery and Therapeutics. 2021; 11(6-S):145-151

DOI: http://dx.doi.org/10.22270/jddt.v11i6-S.5150

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Abstract

The objective of the present study was to develop a novel Self Micro emulsifying drug delivery system (SMEDDS) of Doxepin to increase the oral bioavailability by escaping metabolism from the effect of the CYP450 enzyme, and evaluate its stability, drug release by In-vitro and Ex-vivo drug permeation studies. The solubility of Doxepin in oils, surfactants and co-surfactants was determined. Pseudo ternary phase diagrams were constructed for oil, Smix and water to identify the microemulsion forming region. The optimized SMEDDS formulation was composed of Oleicacid, Tween, PEG, Drug in ratios of 25:60:15:5.The SMEDDS were evaluated for self-micro emulsification time, PDI, Zeta potential, globule size, drug content. Optimized formulation (F6) was showed globule size of 37.1nm, PDI 0.21, zeta potential-22.3mv and drug content of 98.21%. *Invitro* drug release studies were carried out in 0.1N HCL for 2hrs followed by pH 6.8 phosphate buffer for 10 hrs using dialysis bag method. Optimized formulation F6 converted to solid SMEDDS by physical adsorption technique Neusilin US2 as carrier. Ex-vivo permeation studies were performed for the optimized solid formulation (F6-S) and drug suspension using normal sac method.The percentage drug release of optimized formulation was significantly high when compared to drug suspension. Through this we can conclude that bioavailability of formulation of doxepin could be enhanced significantly by formulating into solid SMEDDS or liquid SMEDDS.

Keywords: Self Micro emulsifying drug delivery system, Pseudo ternary phase diagram, Doxepin, Ex-vivo permeation.

INTRODUCTION

Doxepinis a tricyclic antidepressant. Inhibits serotonin-norepinephrine reuptake inhibitor (SNRI). Doxepin is a medication used to treat major depressive disorders and anxiety disorders. It has mild to moderate benefits for sleeping problems and it is having low oral bioavailability 29% which is due to extensive first pass metabolism. Self-micro emulsifying drug delivery system (SMEDDS) are defined as isotropic mixtures of natural or synthetic oils, surfactants, and cosolvents/cosurfactants that have a unique ability to form fine oil-in-water (o/w) microemulsions upon by dilution in aqueous media, such as GI fluids [1]. SMEDDS spread readily in the GI tract, and self-emulsification occurs due to motility of the stomach and the intestine [2]. Fine oil droplets would pass rapidly from the stomach and promote the wide distribution of the drug throughout the GI tract, thereby minimizing the irritation frequently encountered during extended contact between drug and the gut wall[3].In this study a series of formulations were prepared by varying concentrations of oils (10- 25%), surfactants (30-65%) and co surfactants (10-60%)[4, 5]. In the present study an attempt was made to improve oral bioavailability of doxepin by formulating in to self-micro emulsifying drug delivery systems [2].

MATERIALS AND METHODS

Doxepin was obtained as gift sample from Dr Reddy's Laboratory, Hyderabad. Labra sol, Transcutol-P were purchased from Gattefosse India pvt ltd. Tween 80 was purchased from Hi media, Mumbai. PEG 400, Oleic acid were from SD chemicals, Mumbai. Olive oil was from Sigma chemicals, Mumbai and methanol (HPLC grade) from Merk, Mumbaiwas used in the present study.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) of pure drug (Doxepin) was determined by using DSC instrument. The empty aluminum pan was used as reference cell. About 5-15 mg of drug was taken in thepierced DCS aluminum pan and crimped in the temperature range of 50-200°C. The heating rate was10°C/min, nitrogen served as purged gas and the system was cooled down by liquid nitrogen. Theendothermic peaks were observed[1].

Construction of standard graph of Doxepin

The standard graph of Doxepin was constructed in different solvents such as methanol, 0.1N HCL and phosphate buffersof pH 6.8 and 7.4 by using UV- visible spectrophotometer (Lab India, 3000+).10 mg of Doxepin was weighedand dissolved in 10 ml of solvent to get

a1mg/ml (stockA). Stock - A was further diluted to obtaindesired concentrations. The absorbance of the samples was measured by UV-Visible Spectrophotometerat 297 nm and the solvent was used as blank. A standard graph was plotted by taking concentration (µg)on X-axis and absorbance (nm) onY-axis[2].

Solubilitystudies

The solubility was determined by using equilibrium solubility method. Inthis method the solubility of Doxepin in various oils, surfactants and co-surfactants was determined, 2ml of each of the selected vehicles was taken in a glass vial to which excess of Doxepin was added. The mixture was heated at 40°C in a water bath to facilitate the solubilization and mixed using vortex to facilitate uniform dispersion. Then, the mixture was agitated in a water bath shaker at 37°C for 48hrs. After reaching equilibrium, samples were collected centrifuged at 4000 rpm for 10min and the supernatant was filtered through a membrane filter (0.45 µm). The drug content in the filtratewas quantified by UV method after suitabledilution[6,7].

Pseudo-ternary phase diagram study

The pseudo-ternary phase diagrams of oil, Smix and water were plotted using CHEMIX software, version 7.00 with different ratios of surfactants and co-surfactants (1:1, 2:1, 3:1, 1:2). The weight ratio of oil to surfactant mixture (Smix) was varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. To the homogenous mixture of oil and surfactant, water was added in small increments. Following each addition, the mixture in a test tube was vortexed for 2-3min. The point at which the mixture becomes turbid was considered as the endpoint of the titration. They were allowed to settle for 30min. The mixture was examined visually for phase separation or transparency. If transparent, water was added further till it becomes turbid and the quantity of water added was noted [8, 9].

Preparation of SMEDDS

A series of the self-micro emulsifying systems were prepared with varying concentrations of oil (20-35%), and Smix (10- 60%). Oil and surfactant were added and the final mixture was vortexed until aclear solution was obtained. The mixture was stored at room temperature, and evaluated.[4, 10,11].

Droplet size analysis

Formulations were diluted 50 times 1000 times with double distilled water. The resultant microemulsion was subjected to droplet size analysis. The size of the globule was measured at 90^0angle at the temperature of 25^0 C by zeta sizer (nano zs-90 malvern) [12].

Assay of SMEDDS

SMEDDS formulation/suspension equivalent to 5 mg of Doxepin was accurately weighed and diluted to 5 mL using double distilled water to produce 1 mg/ml. From this 1 ml was taken and diluted to 10 ml with methanol and drug content was estimated by UV-Visible spectrophotometer [11].

In-vitro drug release studies (in pH 6.8 & pH 1.2)

Drug release experiments were conducted using a dialysis bag method. Initially, the dialysis tubing was soaked in the medium for 1 hr at 40^0C. The In vitro drug release test was performed in (0.1 N HCl (pH 1.2) for 2 hrs and in simulated intestinal fluid (pH 6.8) for 10 hrs. About 1 ml of SMEDDS formulation containing 5 mg of Doxepin was placed in a dialysis bag made up of amembrane having a molecular weight cut off 12000-14000 and suspended in 100 mL of the medium and the contents were mixed on a magnetic stirrer at room temperature. Samples (5ml) were collected at regular time intervals and replaced with an equal volume of fresh medium. The release profile of Doxepin suspension was also studied by the same method. All the samples were analyzedby UV -Visible Spectrophotometer at λ max 297nm after appropriate dilution of the sample and the dilution factor was noted[11].

Preparation of Doxepin suspension

Sodium carboxymethylcellulose suspension was prepared by dissolving 0.5g of Na CMC in water and volume was made up to 100 ml Accurately weighed the amount (10 mg) of Doxepin was taken in a mortar and triturated with (10 ml) 0.5 % Na CMC solution to get 1mg/ml Doxepin suspension [10,13].

Preparation of Solid SMEDDS

The optimized formulation was adsorbed onto Neusilin (Magnesium aluminium metasilicate; Fuji chemicals) to produce solid SMEDDS (S-SMEDDS). The formulation was placed in a China dish, a pre weighed quantity of Neusilin was added in small increments with continuous mixing to form a powder. The obtained formulation was filled into a capsule and preserved in an airtight container for further use [14,15].

Evaluation of Solid SMEDDS

Solid SMEDDS were evaluated for angle of repose, Bulk density, compressibility index, Hausner's ratio to determine the flow properties of solid SMEDDS [16, 17].

Assay of solid SMEDDS

S-SMEDDS formulation equivalent to 10 mg of Doxepin was diluted with 10 mL of double distilled water to produce 1 mg/ml. From this 1 mL was taken and diluted to 10 mL with methanol and drug content was estimated by UV method.

Dissolution of solid and liquid SMEDDS and Drug suspension

In-vitro drug dissolution studies were carried out in 0.1N HCL and pH 6.8phosphate buffer. Solid SMEDDS filled in capsules and liquidSMEDDS, Drug suspension equivalent to 5 mg of Doxepin was placed in 900 ml of medium and rotated at 50 rpm and maintained at 37°C± 0.5. Aliquots of samples (5 ml) were withdrawn at predetermined time intervals up to 2hrs and replaced with fresh medium. samples after filtration were analyzed for Doxepin content by UV-Visible Spectrophotometer at 297 nm [5, 18]

***Ex-vivo* absorption studies (Normal sac method)**

Ex-vivo studies were performed for liquid SMEDDS (F6) and drug suspension. By normal sac method using rat small intestine. The permeation of the formulation and drug suspension were calculated [19]. In this study, male Wister rat weighing between 200-250 g (n=2) was taken and subjected to overnight fasting. The rat was sacrificed by cervical dislocation technique and jejunum of 4 cm length was isolated, flushed with saline solution and transferred into oxygenated Krebs's Ringer solution. The one end of the segment was tied with thread and filled with the sample or control (1 ml) and the other end was tied. The segment was immersed in 100 mL of Krebs's ringer solution in a beaker and the medium was oxygenated using an aerator and agitatedonmagneticstirrerat50-rpmmaintainingatemperatureof37°C.Atregulartime intervals (15, 30, 45, 60, 75, 90, 105, 120 min) 2ml samples were withdrawn from the beaker and analyzed for drug content by UV method after filtration[4, 15,20].

RESULTS AND DISCUSSION

Solubility studies

Solubility of Doxepin in various solvents was shown in Table1, Tween 80 showed the highes solubility for doxepin, followed by PEG400 and oleic acid than other oils and surfactants.

Table1: Solubility of Doxepin in different oils, surfactants, co-surfactants

s.no	vehicles	solubility (mg/ml)
1	Oleic acid	54.56
2	Olive oil	24.82
3	Sesame oil	23.97
4	Soya bean oil	15.49
5	Sunflower oil	13.16
6	Arachis oil	6.38
7	PEG 400	65.24
8	Tween 80	56.85
9	Propylene glycol	39.86
10	Tween 20	33.41
11	Labra sol	20.41
12	Span 80	15.58
13	Glycerol	13.38

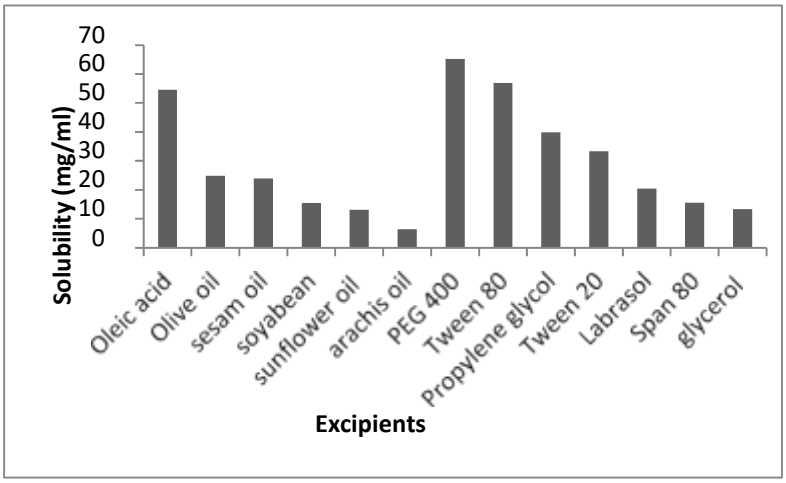

Figure1: solubility of doxepin in different oils, surfactants & co-surfactants

Pseudo ternary phase diagrams

Pseudo ternary phase diagrams were plotted using CHEMIX software version 7.00. Phase diagrams were constructed in the presence of Doxepin to obtain the optimum concentrations of oil, surfactant, and co surfactant. SMEDDS form fine oil–water emulsions with only gentle agitation up on its introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. Surfactants form a layer of interface and reduced the interfacial energy as well as providing a mechanical barrier to coalescence. The visual test is measured the apparent spontaneity of emulsion formation. The series of SMEDDS were prepared and their self-emulsifying properties were observed by adding water visually. Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying region and to optimize the concentration of oil. Smix at 3:1 ratio of tween 80, PEG 400 yielded maximum micro emulsion region. Hence 3:1 ratio of tween 80, PEG 400 as Smix were selected for the SMEDDS preparation and formulation optimization. Tween 80 is a biocompatible emulsifier. PEG 400 is a stabilizer and co surfactant which imparts flexibility to the surface of the globule.

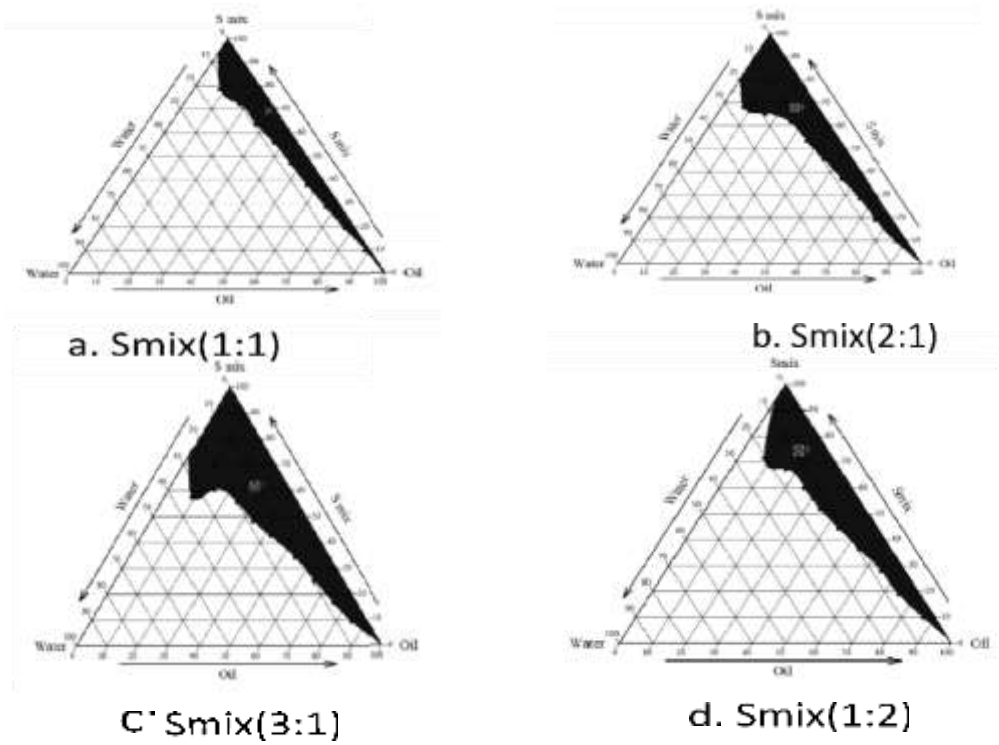

Figure 2: Pseudo ternary phase diagram

Table2: Composition of SMEDDS formulations

Formulation code	Oleic acid mg	Tween 80 mg	PEG400 mg	Visual observation (1:1000)	Droplet size (nm) (1:50)
F1	20	60	20	Transparent	45.78
F2	20	50	30	Transparent	78.91
F3	20	40	40	Transparent	97.42
F4	20	30	50	Transparent	191.6
F5	20	20	60	Turbid	274.7
F6	25	60	15	Transparent	36.17
F7	25	50	25	Transparent	52.52
F8	25	40	35	Transparent	121.72
F9	25	30	45	Transparent	138.31
F10	25	20	55	Turbid	229.7
F11	30	60	10	Transparent	70.76
F12	30	50	20	Transparent	92.6
F13	30	40	30	Transparent	174.6
F14	30	30	40	Transparent	181.48
F15	30	20	50	Turbid	266.8
F16	35	55	10	Transparent	89.02
F17	35	45	20	Transparent	167.9
F18	35	35	30	Transparent	167.8
F19	35	25	40	Turbid	209.12
F20	35	15	50	Turbid	320.4

*All the formulations contain 5mg of the drug

In visual observation study (1:1000), Among the formulations 15 were transparent and 5 turbid upon dilution The formulations become turbid due to larger globule size(>200nm) dilution. Transparency indicated the formation of SMEDDS. The size analysis was conducted at 1:50 dilution to delineate the region of SMEDDS formation. SMEDDS which yielded very low size (36.17) were further diluted to 1000 times to know the stability of SMEDDS. Accordingly, F1, F6, F7, F11 formulations produced a globule size of 45.78, 36.17, 52.52, 70.76 nm respectively selected for further studies.

Droplet size analysis and determination of zeta potential

The experiment was conducted as described in methodology wherein the dilution was 1:50 in water. The droplet size, polydispersity index and zeta potential were shown in table 3.

Table 3: Droplet size, polydispersity index (PDI), emulsification time and drug content of SMEDDS

Formulation code	Droplet size (nm)	PDI ± SD	Zetapotential(mV)	Emulsification time (Sec)	Drug content (%)
F1	45.76±1.8	0.255±0.02	-18.3±0.2	49	96.52
F6	37.10±2.1	0.210±0.06	-23.2±0.6	42	98.21
F7	52.94±1.07	0.274±0.07	-13.3±2.6	54	95.10
F11	71.52±0.4	0.296±0.06	-15.1±1.08	58	92.85

* PDI value <0.3 signifies good homogeneity of dispersed globules

***In-vitro* release studies**

In vitro release studies were conducted in 0.1N HCl, for 2hrs followed by 10 hrs in 6.8 pH phosphate buffer. The drug suspension showed relatively lowest cumulative release i.e., (44.65%) when compared with formulations (F1, F6, F7, F11) and F6 resulted in the highest cumulative release i.e. (83.26%) when compared with other formulations.

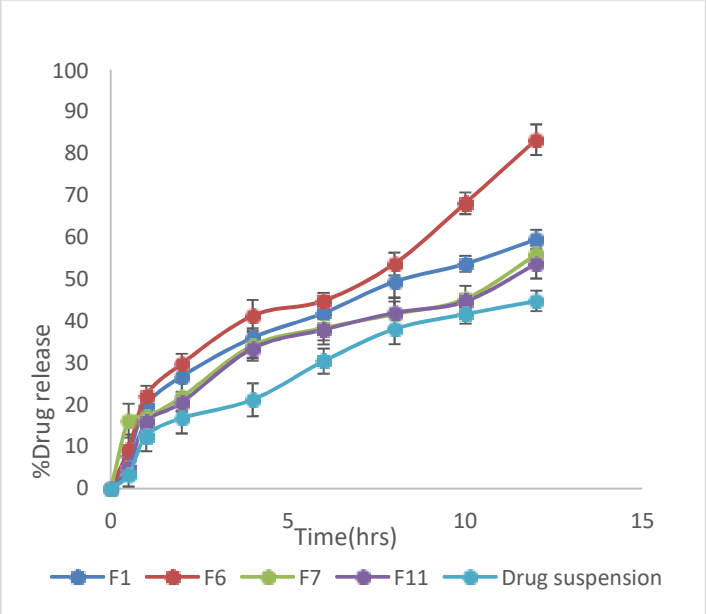

Figure3: Cumulative percent drug release from SMEDDS and drug suspension in 0.1NHCl and pH 6.8 phosphate buffer.

In vitro drug release profile of (F6) SMEDDS (98.81%) and(F6) S-SMEDDS (95.04%) and drug suspension (54.77%). When compared to liquid SMEDDS 4% low release in solid SMEDDS was observed.

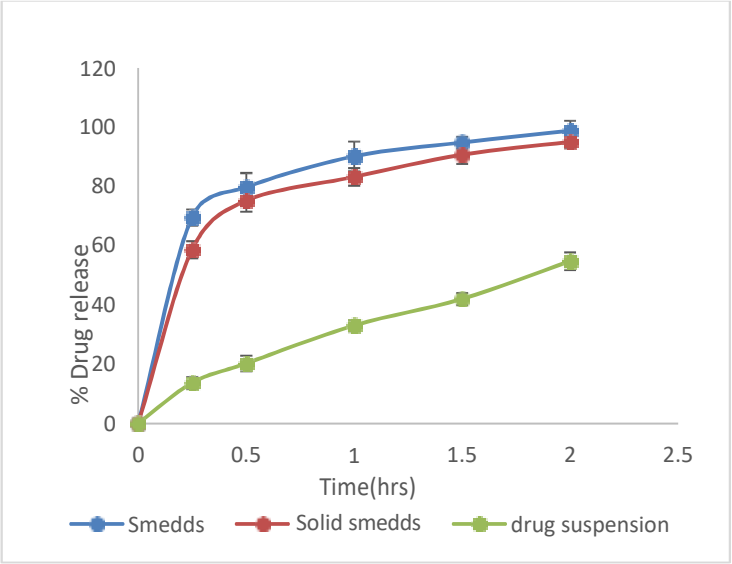

Figure4: Cumulative percent drug release from (F6) liquid SMEDDS, (F6) solid SMEDDS and drug suspension in 0.1N HCl

In vitro drug release of (F6) SMEDDS (97.25%) and (F6) S-SMEDDS (93.86%) and drug suspension (57.22%). When compared to liquid SMEDDS 4% low release in solid SMEDDS was observed.

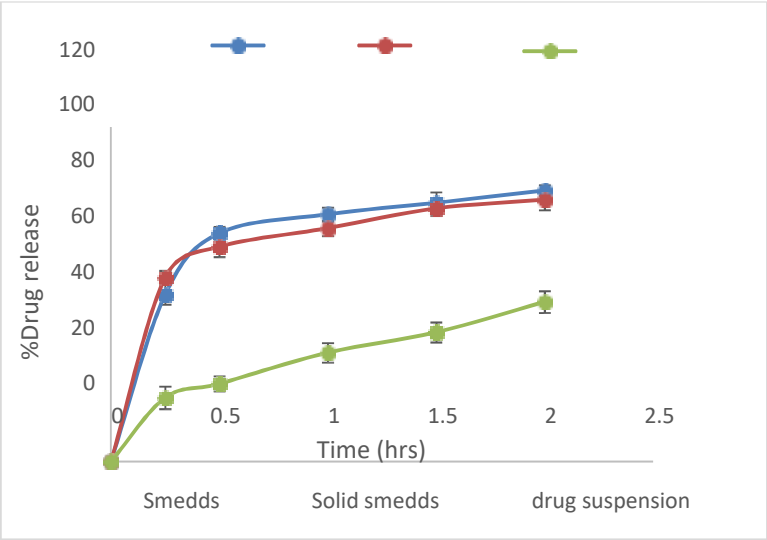

Figure5: Cumulative percent drug release from (F6) liquid SMEDDS, (F6) solid SMEDDS and Drug suspension in pH 6.8 Phosphate buffer

***Ex-vivo* permeation studies by Normal sac method (n=2)**

The experiment was conducted for the optimized formulation (F6-Solid) and drug suspension using phosphate-buffered saline PH 7.4.

Table4: Drug permeation in phosphate-buffered saline (n=2)

Time (min)	Drug permeation (%)	
	Drug suspension	F6-Solid
15	7.43±0.90	10.42±0.90
30	17.41±0.60	20.3±0.91
45	24.30±1.20	27.86±1.20
60	32.96±0.90	38.26±0.90
75	41.52±1.20	57.34±1.21
90	49.80±0.24	71.94±0.57
105	56.4±0.90	81.89±0.38
120	67.18±1.20	94.04±0.60

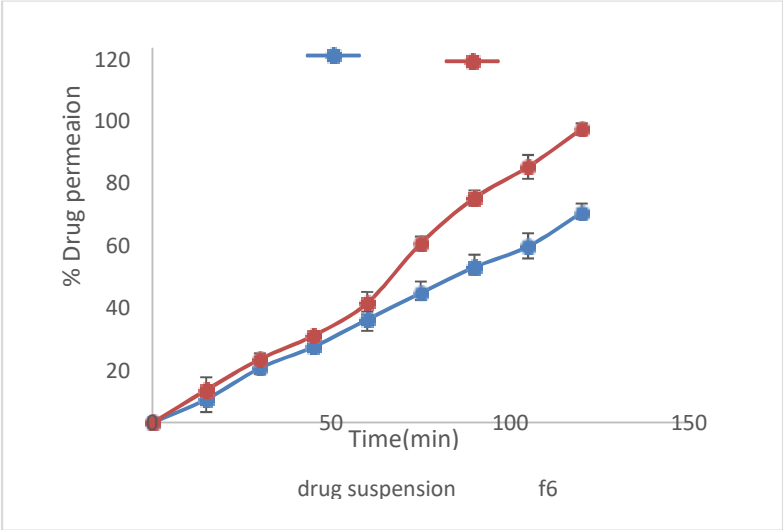

Figure 6: Drug permeation through rat ileum (normal sac)

The *Ex-vivo* release profiles were shown in (figure.6). The drug release was significantly higher (94.04%) for optimized formulation than the drug suspension (67.18%).

CONCLUSION

The formulation of SMEDDS loaded with Doxepin was developed. The formulation was stable and exhibiedglobulesizeof37.10nm, PDI 0.210, zeta potential -22.3 mV. In vitro dissolution studies revealed that release of Doxepin from solid SMEDDS (95.04%) was significantly high than the drug suspension (54.77%). Through this we can conclude that bioavailability of Doxepin could be enhanced significantly by formulating into SMEDDS.

ACKNOWLEDGEMENTS

First author is highly thankful to the Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India for providing facilities for doing research.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

REFERENCES

1 Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. European journal of pharmaceutical sciences: official journal of the European Federation for Pharmaceutical Sciences. 2006; 29(3-4):278-87. https://doi.org/10.1016/j.ejps.2006.04.016

2 Khoo S-M, Humberston AJ, Porter CJH, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. International journal of pharmaceutics. 1998; 167(1):155-64. https://doi.org/10.1016/S0378-5173(98)00054-4

3 Tang J-l, Sun J, He Z-G. Self-Emulsifying Drug Delivery Systems: Strategy for Improving Oral Delivery of Poorly Soluble Drugs. Current Drug Therapy. 2007; 2(1):85-93. https://doi.org/10.2174/157488507779422400

4 Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, et al. Development of self- micro emulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. International journal of pharmaceutics.2004; 274(1-2):65-73. https://doi.org/10.1016/j.ijpharm.2003.12.028

5 Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. International journal of pharmaceutics. 1994; 106(1):15- 23. https://doi.org/10.1016/0378-5173(94)90271-2

6 Devani M, Ashford M, Craig DQ. The emulsification and solubilization properties of polyglycolides oils in self-emulsifying formulations. The Journal of pharmacy and pharmacology. 2004; 56(3):307-16. https://doi.org/10.1211/0022357022872

7 Rang MJ, Miller CA. Spontaneous Emulsification of Oils Containing Hydrocarbon, Nonionic Surfactant, and Oleyl Alcohol. Journal of colloid and interface science. 1999; 209(1):179-92. https://doi.org/10.1006/jcis.1998.5865

8 Khan B, Bakhsh S, Khan H, Mahmood T, Rasul A. Basics of Self Micro Emulsifying Drug Delivery System. Journal of Pharmacy and Alternative Medicine. 2012; 1.

9 Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. International journal of pharmaceutics. 2001; 212(2):233-46. https://doi.org/10.1016/S0378-5173(00)00614-1

10 Boltro L, Cocaine N, De Curto D, Dobetti L, Esposito P. Enhancement and modification of etoposide release from crospovidone particles loaded with oil-surfactant blends. Pharm Dev Technol. 1997; 2(4):373-81. https://doi.org/10.3109/10837459709022636

11 Porter CJ, Charman WN. In vitro assessment of oral lipid-based formulations. Advanced drug delivery reviews. 2001;50 Suppl1: S127-47. https://doi.org/10.1016/S0169-409X(01)00182-X

12 Gershan T, Benita S. Positively charged self-emulsifying oil formulation for improving oral bioavailability of progesterone. Pharm Dev Technol. 1996; 1(2):147-57. https://doi.org/10.3109/10837459609029889

13 Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Advanced drug delivery reviews. 2008; 60(6):625-37. https://doi.org/10.1016/j.addr.2007.10.010

14 Shukla JB, Jani GK, Omi AW. FORMULATION AND EVALUATION OF ORAL SELF MICROEMULSIFYING DRUG DELIVERY SYSTEM OF CANDESARTAN CILEXETIL. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(5):238- 43.

15 Seo A, Holm P, Kristensen HG, Schaefer T. The preparation of agglomerates containing solid dispersions of diazepam by melt agglomeration in a high shear mixer. International journal of pharmaceutics. 2003; 259(1-2):161-71. https://doi.org/10.1016/S0378-5173(03)00228-X

16 Agarwal V, Siddiqui A, Ali H, Nazzal S. Dissolution and powder flow characterization of solid self-emulsified drug delivery system (SEDDS). International journal of pharmaceutics. 2009; 366(1-2):44-52. https://doi.org/10.1016/j.ijpharm.2008.08.046

17 Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. International journal of pharmaceutics. 2004; 278(1):119-31. https://doi.org/10.1016/j.ijpharm.2004.03.001

18 Cui S, Zhao C, Chen D, He Z. Self-micro emulsifying drug delivery systems (SMEDDS) for improving in vitro dissolution and oral absorption of Pueraria lobata isoflavone. Drug development and industrial pharmacy. 2005; 31(4-5):349-56. https://doi.org/10.1081/DDC-54309

19 Chitneni M, Peh KK, Darwis D, Abdulkarim M, Abdullah GZ, Qureshi MJ. Intestinal permeability studies of sulpiride incorporated into self-micro emulsifying drug delivery system (SMEDDS). Pakistan journal of pharmaceutical sciences. 2011; 24(2):113-21.

20 Yu LX, Amidon GL. A compartmental absorption and transit model for estimating oral drug absorption. International journal of pharmaceutics. 1999; 186(2):119-25. https://doi.org/10.1016/S0378-5173(99)00147-7

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