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Formulation and *in-vitro* evaluation of self microemulsifying drug delivery system (SMEDDS) of Furosemide

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Abstract

Aim of the present investigation was to develop the Self Microemulsifying Drug Delivery System (SMEDDS) of Furosemide. Furosemide is Class IV molecule according to BCS (Biopharmaceutical Classification System), having low solubility and low permeability. Prepared optimized SMEDDS of Furosemide composed of CAPTEX 500 as oil and Cremophore EL as surfactant in 20:80 ratio. Optimized SMEDDS of Furosemide showed increase in dissolution rate of Furosemide in Simulated Gastric Fluid (SGF) and 5.8 pH phosphate buffer, irrespective of pH compared the Marketed Tablet LASIX ® (Furosemide 40 mg).

Keywords: SMEDDS, Dissolution Rate, Furosemide

INTRODUCTION

Furosemide (FUR) is a potent loop diuretic, chemically designated as 4-chloro-2-(2-furylmethylamino)-5- sulfamoyl-benzoic acid. It is a white to slightly yellow, odorless, crystalline powder, practically insoluble in water (10 µg/mL), sparingly soluble in alcohol, freely soluble in dilute alkali solutions and insoluble in dilute acids [1]. The rate of absorption and the extent of bioavailability for such an insoluble hydrophobic drug are controlled by the rate of dissolution in the gastrointestinal fluids. Improvement of aqueous solubility in such a case is a valuable aim to improve therapeutic efficacy. Hence, attempts are being made to increase the rate of dissolution of such poorly water soluble hydrophobic drugs, to increase their effectiveness and simultaneously reduce their doses, variability and hence their toxic effects by using SMEDDS technology.

The terminal Half-life of Furosemide is approximately 2 hours and the mean oral bioavailability is 60% with Tmax 1.5 hr [2]. According to BCS (Biopharmaceutical Classification System) Furosemide, having low solubility and low permeability BCS Class IV [3].

A mixture of oil and surfactant (especially non-ionic) forms clear and transparent isotropic solution known as self-emulsifying system (SES) [4]. Microemulsion preconcentrate, also known as self-microemulsifying drug delivery system (SMEDDS), upon dilution with aqueous media, accompanied by gentle agitation, spontaneously forms clear isotropic solutions or microemulsions. Compared to ready-to-use microemulsions, it has improved physical stability profile upon long-term storage, and can be filled directly into soft or hard gelatin capsules for convenient oral delivery. In recent years several successful oral pharmaceutical products have been marketed as lipid systems, notably cyclosporin A (originally marketed as 'Sandimmune E' and now as the improved product 'Neoral E') and the two HIV protease inhibitors, ritonavir and saquinavir. Consequently, there is now considerable interest in the potential of lipid formulations for oral administration [5]. In SEDDS and SMEDDS, the surfactants which are used have HLB<12 and HLB>15 respectively [6].

The objective of the present investigation was to develop the Self Microemulsifying Drug Delivery System (SMEDDS) of Furosemide to increase the dissolution rate of Furosemide in Simulated Gastric Fluid (SGF) and 5.8 pH phosphate buffer, irrespective of pH compared the Marketed Tablet LASIX ® (Furosemide 40 mg).

MATERIALS AND METHODS

Furosemide obtained as a gift sample from Aventis Pharma. Ltd. Ankaleshwar, INDIA. CAPTEX 500, CAPTEX 300, CAPTEX 350, CAPMUL MCM, CAPMUL PG8 obtained as a gift sample from ABITEC CORPORATION, Ohio, USA. LABRAFILL M 2125 CS, LABRAFILL M 1944 CS, LABRAFACE CC, LAUROGLYCOL 90 obtained as a gift sample from Colorcon India, Goa (GATTEFOSSE, FRANCE). CREMOPHORE EL & CREMOPHORE RH obtained as a gift sample from BASF Ltd, INDIA. Lasix Tablet IP 40 mg (Mfg. By: Aventis Pharma. Ltd. Ankaleshwar. INDIA) purchased from local Market. All other chemicals used were of analytical reagent grade and double distilled water was used throughout the experiments.

Solubility studies

Screening of excipients can be done by determining the equilibrium solubility of Furosemide in different oils and surfactants. Two ml of each of selected oil, surfactant sample was added in glass vial containing excess amount of Furosemide, the drug was mixed in oil manually with glass rod for ½ h, after that the vials were kept in sonicator for 2 h. Mixture was kept in water bath for 48 hr for reaching the equilibrium. After 48 hr these vials were centrifuged at 3000 rpm for 20 min. After centrifugation the amount of dissolved drug was determined by diluting the supernatant in ethanol by UV- spectrophotometer at 272.6 nm. [7-9]

2.1.1 Solubility determination of Furosemide in different ratios of selected oil and surfactants:

Two ml of each of selected oil sample was added in glass vial containing excess amount of Furosemide, the drug was mixed in respective oil & surfactant manually with glass rod for 0.5 hr, after that the vials were kept in sonicator for 2 hr. Mixture was kept in water bath for 48 hr for reaching the equilibrium. After 48 hr these vials were centrifuged at 3000 rpm for 20 min. After centrifugation the amount of dissolved drug was determined by diluting the supernatant in ethanol by UV- spectrophotometer at 272.6 nm. [10, 11]

Combination of oil and surfactant were as follows,

1) CAPTEX 500 (Oil) + TWEEN 80 (Surfactant)
2) CAPTEX 500 (Oil) + CREMOHORE EL (Surfactant)
3) CAPTEX 500 (Oil) + TWEEN 80: CREMOPHORE EL (1:1) (Surfactant)

2.3 Ternary Phase Diagram Construction of SMEDDS:

In order to find out the concentration range of components for the existing range of microemulsions, pseudo-ternary phase diagrams were constructed using H_2O titration method. Three phase diagrams were prepared with the

1) CAPTEX 500: TWEEN 80
2) CAPTEX 500: CREMOPHORE EL and
3) CAPTEX 500: TWEEN 80: CREMOPHORE EL (1:1)

For each phase diagram, the ratios of oil: surfactant were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 w/w. The mixtures of oil and surfactant at certain weight ratios were diluted with H_2O, under moderate stirring with mechanical shaking. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions or coarse emulsions. The data obtained was subjected to **TRIDRAW 4.1** software for fabrication of ternary plot. [8, 10-14]

2.4 Formulation of Self Micro-emulsifying Drug Delivery System (SMEDDS):

Three types of Self Micro-Emulsifying System (SMES) was formulated which are as follows,

1) CAPTEX 500 (Oil) + TWEEN 80 (Surfactant)
2) CAPTEX 500 (Oil) + CREMOHORE EL (Surfactant)
3) CAPTEX 500 (Oil) + TWEEN 80: CREMOPHORE EL (1:1) (Surfactant)

The compositions of different formulation are as shown in Table 1. In all formulations CAPTEX 500 was used as oil phase and two different surfactants (i.e., TWEEN 80 & CREMEOPHORE EL) were used either in combination or alone containing Furosemide 40 mg. [8, 12, 13, 15, 16]

Table 1: Composition of Self Micro-Emulsifying Drug Delivery System (SMEDDS) of Furosemide

All Formulations containing 40 mg of Furosemide						
CAPTEX 500 (OIL)	**F C**	**CREMOPHORE EL (Surfactant)**	**F C**	**TWEEN 80 (Surfactant)**	**F C**	**CREMOPHORE EL: TWEEN 80 (1:1) (Surfactant)**
90%	C1	10%	T1	10%	CT1	10%
80%	C2	20%	T2	20%	CT2	20%
70%	C3	30%	T3	30%	CT3	30%
60%	C4	40%	T4	40%	CT4	40%
50%	C5	50%	T5	50%	CT5	50%
40%	C6	60%	T6	60%	CT6	60%
30%	C7	70%	T7	70%	CT7	70%
20%	C8	80%	T8	80%	CT8	80%
10%	C9	90%	T9	90%	CT9	90%

F.C: Formulation Code

The oil and surfactant were weighed as per its percentage in glass vial, and Furosemide (40 mg) was added in this mixture and mixed with glass rod for ½ h, and then sonicated the vial in sonicator for 2 h. The prepared SMEDDS (600 µL) was filled in hard gelatin capsule shell size '0' with the help of micropipette.

Evaluation of SMEDDS:

2.5.1 Drug Content:

Prepared SMEDDS containing Furosemide equivalent to 40 mg was added in 50 mL volumetric flask (VF) containing ethanol and mixed it well with shaking or inverting the VF for two to three times. 0.1 mL of this solution was diluted with 25 mL fresh ethanol and drug content was determined using UV-spectrophotometer at 272.6 nm.

2.5.2 Phase separation study:

Each SMEDDS (0.05 mL) was added to glass test tube containing 5 mL of 0.1 N HCl and Distilled water. After inverting the test tube for 3-4 times, each mixture was stored for a period of 2 hr and phase separation was observed visually. [11, 13]

2.5.3 Viscosity determination of SMEDDS:

Twenty gram of each of formulation was weighed and transferred to beaker and the viscosity of formulation was determined with the help of Brookfield Viscometer DV-E model, spindle no 6, at 10 rpm for 5 min**. [7]**

2.5.4 Droplet Size Determination of SMEDDS:

Droplet size distribution of resultant emulsion was determined by using a multiple scattering angle detector (**Beckman Coulter N4 Plus Model, Italy),** The scattering intensity data were obtained at an angle of 90^0 and analyzed by a digital correlator (photon correlation spectroscopy (PCS)) to calculate the droplet size. Analysis was carried out using double distilled water (DW**). [12-18]**

2.5.5 *In-Vitro* Dissolution Study of SMEDDS:

Dissolution study was carried out using USP Type II apparatus (paddle method) at 50 rpm, and at 37± 0.5^0C. Formulations with droplet size below 100 nm preferably selected for Dissolution study in simulated gastric fluid (SGF) 1.2 pH & 5.8 pH

Phosphate Buffer:

Prepared SMEDDS capsule was placed in 900 ml of dissolution medium (SGF) and 5.8 pH Phosphate Buffer after every 5 min interval 10 mL of aliquot was withdrawn and filtered through Whatman Filter paper (40 no.) and same was replaced with fresh dissolution media to maintain the sink condition. Study was carried out for 60 min. Amount of drug dissolved was determined using UV- spectrophotometer at 274.6 nm. Same procedure was applied for marketed tablets (M) Lasix® 40 mg. [8-11, 13]

2.5.6: *In-Vitro* Diffusion Study:

Diffusion study was carried out in 7.4 pH saline phosphate buffer. Formulation C8, T8 and CT8 containing 40 mg of drug was filled individually in 7 cm hollow activated dialysis membrane bag with one end was tied with thread. This formulation was diluted 10 times with SGF (1.2 pH) in the bag for formation of microemulsion and the other end of bag was also tied with thread. The bag was held in place with the aid of stand in beaker containing 200 mL of 7.4 pH saline phosphate buffer. The medium was stirred at 50 rpm with magnetic bead at 37^0C ± 0.5^0C. After each one hour sample was withdrawn and diluted with same medium.

Same volume of fresh medium was transferred to beaker for maintaining the sink condition. Amount of drug diffused was determined using UV-spectrophotometer at 276.8 nm. **[7, 8, 10]**

2.5.7 Statistical treatment to diffusion data:

All the statistical calculation were performed by using Graph Pad Instat Demo (DATA SET 1. ISD). Data are expressed as mean ± S.D. Data were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnett test. P value less than 0.05 was considered statistically significant. C8 was considered as control.

RESULTS AND DISCUSSION

3.1 Solubility Study:

Furosemide showed highest solubility in CAPTEX 500 (Oil), TWEEN 80 (Surfactant) and CREMOPHORE EL (Surfactant) than other oils and surfactants (Figure 1&2). Hence these exicipients were selected to formulate the SMEDDS of Furosemide.

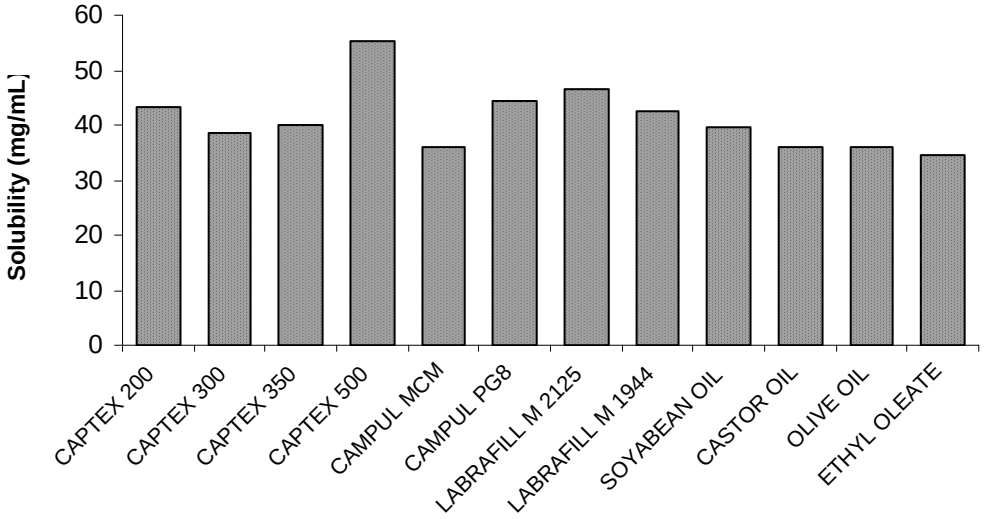

Figure. 1: Solubility of Furosemide in different oils

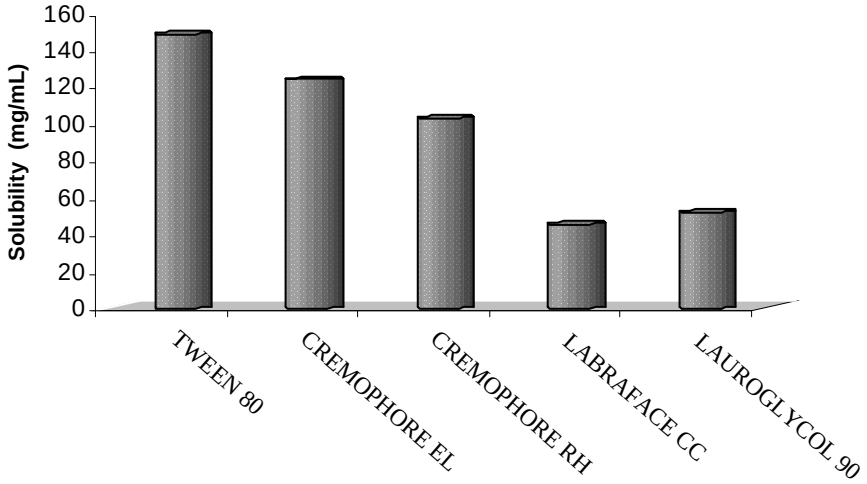

Figure. 2: Solubility of Furosemide in different surfactants

Solubility of Furosemide also varied in the different combination of selected oil and surfactants. TWEEN 80: CREMOPHORE EL (1:1) was taken instead of considering single surfactant. Combined use of surfactant showed good results compared to single (Table 2). From results it was observed that solubility of Furosemide increases with increasing the concentration of surfactant in all the three cases but in 1:1 combination of TWEEN 80 & CREMOPHORE EL solubility was more as compared to single surfactant combinations.

Table 2: Solubility of Furosemide in different ratios of selected oil and surfactant

S. N.	Ratio of Oil:Surfactant	Solubility (mg/ml)* Mean ± S.D.		
		CAPTEX 500 + CREMOPHORE EL	CAPTEX 500 + TWEEN 80	CAPTEX 500 + CREMOPHORE EL: TWEEN 80 (1:1)
1	9:1	61.305 ± 0.279	59.274 ± 0.207	63.268 ± 1.195
2	8:2	64.439 ± 0.221	65.196 ± 0.289	68.502 ± 0.585
3	7:3	68.296 ± 0.389	68.020 ± 0.469	75.527 ± 0.425
4	6:4	72.944 ± 0.145	71.705 ± 1.365	78.316 ± 0.591
5	5:5	79.177 ± 0.166	74.563 ± 0.524	91.402 ± 0.847
6	4:6	82.896 ± 0.123	81.002 ± 1.409	97.807 ± 1.019
7	3:7	90.885 ± 0.114	89.741± 0.720	103.557 ± 0.488
8	2:8	98.495 ± 0.070	95.771 ± 0.470	107.345 ± 0.496
9	1:9	111.58 ± 0.671	120.84 ± 0.599	124.218 ± 0.431

** (n=3)*

For the development of SMEDDS, CAPTEX 500 was used as oil phase in all formulations only surfactant used were different i.e. TWEEN 80, CREMOPHORE EL and TWEEN 80: CREMOPHORE EL (1:1)

3.2 Ternary Phase Diagram Construction of SMEDDS

From ternary phase diagram it was observed that there was formation of almost same microemulsion region in all three types of SMEDDS without any significant difference, this might be due to similar HLB values of both TWEEN 80 and CREMOPHORE EL. Hence it was not possible to found out the best SMEDDS, which gives more microemulsion region, from ternary phase diagram construction (Figure 3, 4, 5).

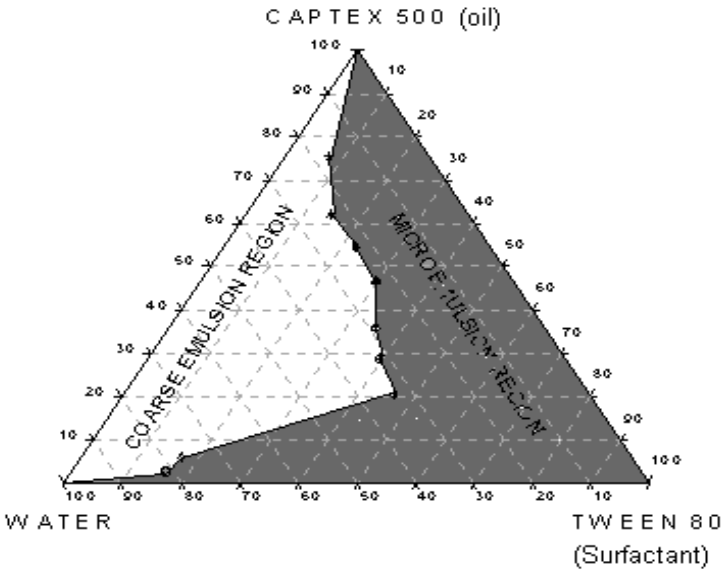

Figure. 3: Ternary phase diagram of CAPTEX 500 (Oil) and TWEEN 80 (Surfactant)

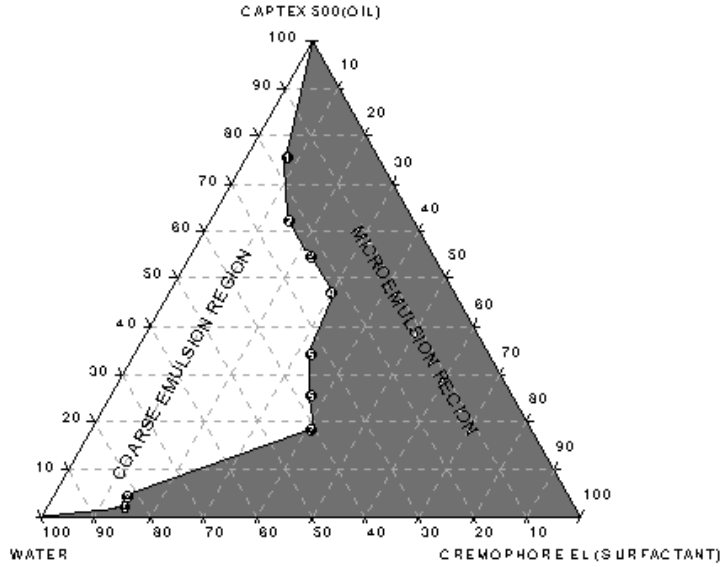

Figure. 4: Ternary phase diagram of CAPTEX 500 (Oil) and CREMOPHORE EL (Surfactant).

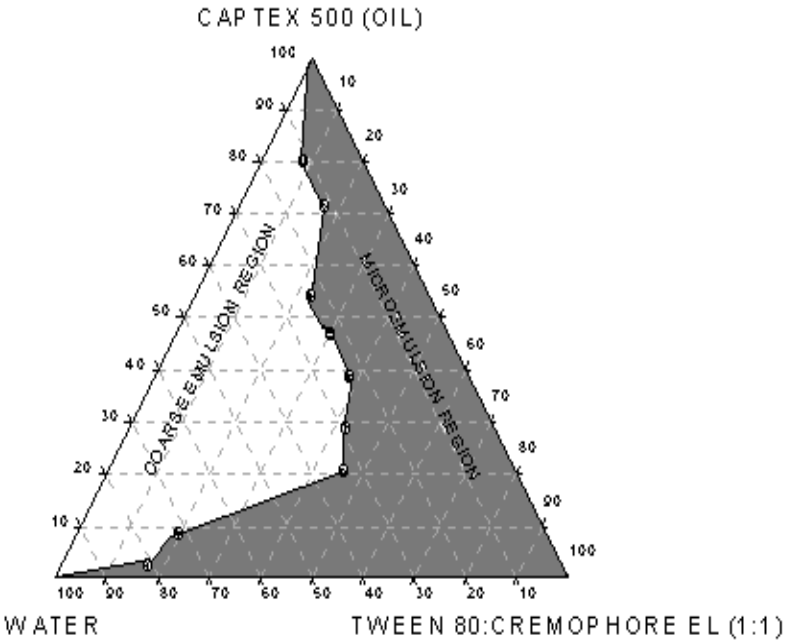

Figure. 5: Ternary phase diagram of CAPTEX 500 (Oil) and 1:1 Ratio of TWEEN 80 & CREMOPHORE EL (Surfactant)

3.3 Drug Content:

Formulation C1, C2, T1, T2 and CT1 showed low drug content because of low surfactant concentration which is unable to solubilize the 40 mg dose of Furosemide. Hence these formulations were eliminated from further evaluation procedures. (Table 3).

Table 3: Drug content of SMEDDS

FC	% Drug Content* Mean ± S.D	FC	% Drug Content* Mean ± S.D	FC	% Drug Content* Mean ± S.D
C1	92.45 ± 0.752	T1	93.25 ± 0.452	CT1	90.08 ± 0.124
C2	92.35 ± 0.824	T2	94.17 ± 0.324	CT2	96.12 ± 0.486
C3	97.84 ± 0.369	T3	96.75 ± 0.275	CT3	98.23 ± 0.210
C4	98.82 ± 0.345	T4	98.55 ± 0.814	CT4	99.41 ± 0.284
C5	99.17 ± 0.215	T5	99.13 ± 1.022	CT5	100.83 ± 1.021
C6	99.24 ± 0.365	T6	101.24 ± 0.464	CT6	102.45 ± 0.312
C7	98.31 ± 0.425	T7	100.89 ± 0.278	CT7	100.67 ± 0.259
C8	100.02 ± 0.236	T8	99.92 ± 0.371	CT8	100.22 ± 0.646
C9	99.34 ± 0.102	T9	99.84 ± 0.543	CT9	99.98 ± 0.728

*(n=3), **F C:** Formulation Code

3.4 Phase separation study:

Phase separation study showed that all formulations subjected for this study were stable in 0.1N HCl & Distilled Water. No signs of phase separation within 2 hr, which implies formation of stable emulsion (Table 4). Hence all formulations were subjected to further evaluation.

Table 4: Phase separation results of SEDDS in 0.1 N HCl and distilled water

S. N.	FC	Phase separation		S. N.	FC	Phase separation		S. N.	FC	Phase separation	
		0.1 N HCL	D.W.			0.1 N HCL	D.W.			0.1N HCL	D.W.
1	C3	No	No	8	T3	No	No	15	CT2	No	No
2	C4	No	No	9	T4	No	No	16	CT3	No	No
3	C5	No	No	10	T5	No	No	17	CT4	No	No
4	C6	No	No	11	T6	No	No	18	CT5	No	No
5	C7	No	No	12	T7	No	No	19	CT6	No	No
6	C8	No	No	13	T8	No	No	20	CT7	No	No
7	C9	No	No	14	T9	No	No	21	CT8	No	No
--	--	--	--	--	--	--	--	22	CT9	No	No

No: Not Observed, D.W.: Distilled Water; FC: Formulation Code

3.5 Viscosity determination of SMEDDS:

From viscosity determination it was observed that as the concentration of surfactant increased viscosity of formulation also get increased. The sequence of viscosity of prepared SMEDDS batches is as follow CT > C > T (Table 5).

Table 5: Viscosity of prepared SMEDDS formulation

Formulation Code	Viscosity (mPas)	Formulation Code	Viscosity (mPas)	Formulation Code	Viscosity (mPas)
C3	2471	T3	2042	CT2	2547
C4	2598	T4	2245	CT3	2789
C5	2704	T5	2512	CT4	3014
C6	3174	T6	2874	CT5	3487
C7	3745	T7	3412	CT6	4074
C8	4351	T8	3997	CT7	4888
C9	4782	T9	4423	CT8	5096
--	--	--	- -	CT9	5274

(mPas: mili pascal)

3.6 Droplet Size Determination of SMEDDS:

From droplet size analysis it was observed that droplet size of SMEDDS of C, T, CT formulations were decreased with respect to increased concentration of surfactant. The lowest droplet size was of C8 i.e. 23.8 nm (**Table 6).**

3.7 *In-Vitro* Dissolution Study of SMEDDS:

In vitro dissolution indicates that the release of Furosemide from SMEDDS varied according to the type and ratio of the oil and surfactants. The release of Furosemide from SMEDDS become faster and increased with increase in concentration of surfactant in formulation.

Table 6. Droplet Size distribution of SMEDDS

FC	Droplet Size (nm)	FC	Droplet Size (nm)	FC	Droplet Size (nm)
C3	160.2	T3	163.5	CT2	168.3
C4	152.9	T4	155.8	CT3	160.4
C5	143.8	T5	147.4	CT4	154.6
C6	108.1	T6	110.2	CT5	145.1
C7	140.3	T7	151.6	CT6	109.7
C8	23.8	T8	29.8	CT7	148.3
C9	23.9	T9	27.9	CT8	28.3
-----	-----	-----	-----	CT9	28.5

***FC**: Formulation Code*

3.7.1 Dissolution study of SMEDDS in SGF (1.2 pH)

When *in vitro* dissolution study of marketed tablet of Furosemide (40 mg) was compared with SMEDDS of Furosemide (40 mg) in SGF & 5.8 pH phosphate buffer, marketed tablet showed only 42.2 % drug release in 60 min and complete release respectively. While all the SMEDDS formulation showed complete drug release within 60 min or less (Figure 6.). Out all SMEDDS formulation, SMEDDS containing CREMOPHORE EL showed fastest release compared to others. Faster release of drug from SMEDDS was because of small droplet size of resultant microemulsion and solubilized form of drug in lipid and surfactant mixture. This confirms the solubility of drug get increased several times which may results in improvement in oral bioavailability and as the drug present in solubilized form and in the center of lipid core in microemulsion droplet, the gastric irritating potential of drug may get reduced. Dissolution rate of marketed tablet in 5.8-pH phosphate buffer is significantly high as compared to in SGF because of high solubility of Furosemide toward alkaline pH.

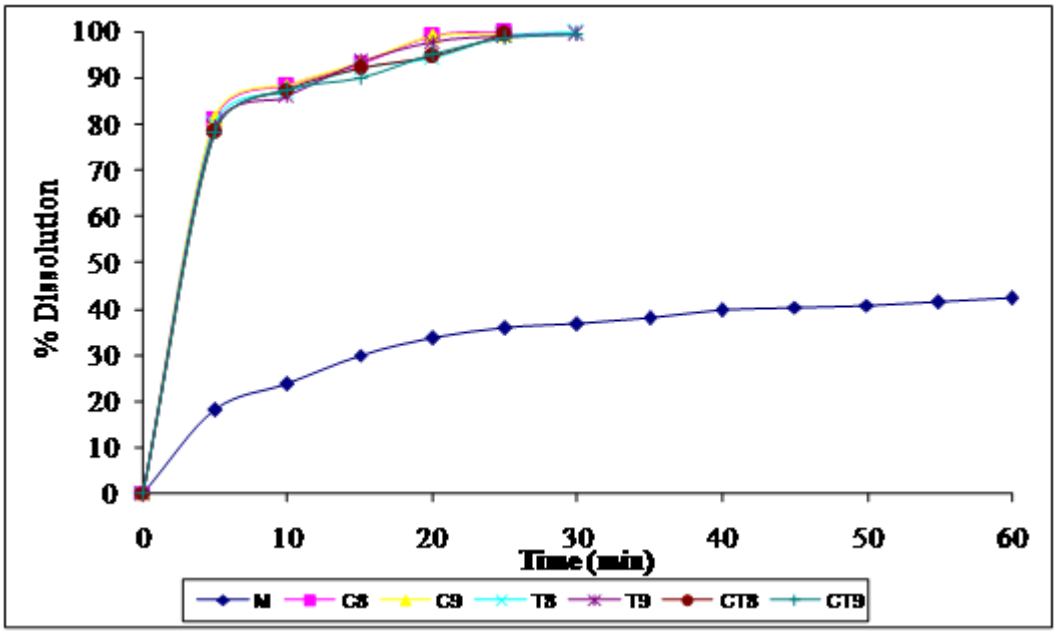

Figure 6: *In-vitro* release profiles of formulation C8,C9,T8,T9,CT8,CT9 compared with Marketed tablet in 1.2 pH SGF

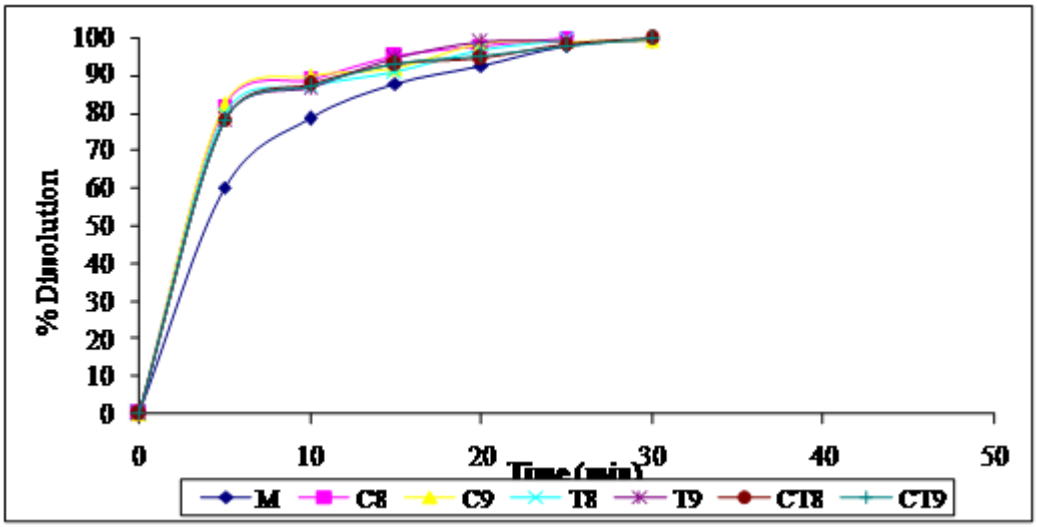

Figure 7: *In-vitro* release profiles of formulation C8,C9,T8,T9,CT8,CT9 compared with Marketed tablet in 5.8 pH Phosphate Buffer

3.8 *In-Vitro* Diffusion Study

In the diffusion study of C8, T8 and CT8, it was observed that C8 formulation showed fastest diffusion of drug i.e. 94.38 % ± 1.023 in 8 h ($P < 0.01$). (Figure. 8). The reason is droplet size of C8 batch i.e. 23.8 nm, which is the smallest droplet size amongst the all formulations. The rate of release of drug from SMEDDS depends on the droplet size of microemulsion, if the droplet size is small the rate of release of drug is fast and vice-versa. Hence it was decided to consider C8 batch as optimized batch.

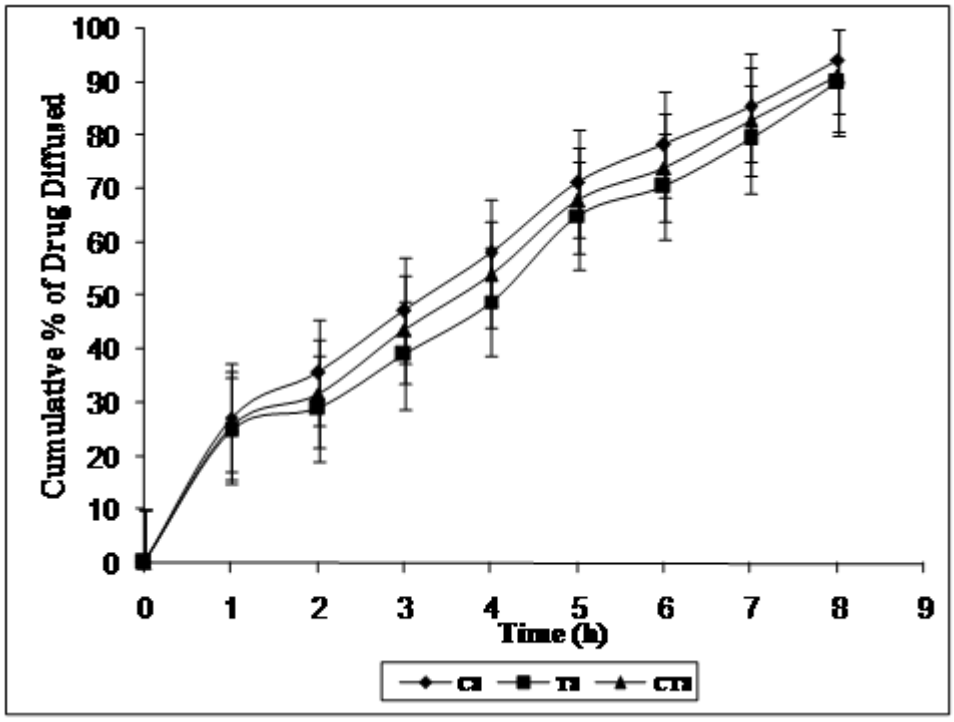

Figure 8: *In-vitro* diffusion study profiles of formulation C8, T8 and CT8

3.9 Kinetic treatment

Optimized batch C8 was treated with different kinetic equations to interpret the order of release of Furosemide and the coefficient of determination (r^2) was determined. Results indicated that the release rate of Furosemide from C8 best fitted Higuchi release pattern (r^2= 0.927 (SGF) and 0.901 (5.8 pH PB)) followed by Zero order (r^2= 0.780 (SGF) and 0.737 (5.8 pH PB)) and then first order (r^2= 0.685 (SGF) and 0.642 (5.8 pH PB)) (Table 7)

As per Korsemeyer Peppas equation, the value of n is less than 0.5 (i.e. 0.137 in SGF and 0.115 in 5.8 pH phosphate buffer) (Table 7), which indicates that the release of Furosemide from formulation C8 takes place by the mechanism of diffusion.

Table 7: Kinetic treatment to data of formulation C8

Equation	In SGF		In 5.8 pH phosphate buffer	
	Variable		Variable	
	r^2	n	r^2	n
Zero order	0.7802	--	0.7370	--
First order	0.6853	--	0.6425	--
Higuchi's (Square root of time)	0.9278	--	0.9010	--
Korsemeyer and Peppas	--	0.1376	--	0.1156

CONCLUSION

The present study revealed that SMEDDS formulation of Furosemide could be developed using CAPTEX 500 as oil and CREMOPHORE EL as a surfactant in 20:80 ratio. From dissolution study it was concluded that SMEDDS form of Furosemide showed complete and faster dissolution profile compared to marketed formulation of Furosemide i.e., LASIX® 40 mg tablet. pH independent dissolution profile of SMEDDS compared to LASIX® 40 mg tablet may definitely improve the oral bioavailability of Furosemide with reduced dose and variability.

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