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PREPARATION AND CHARACTERIZATION OF SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM CONTAINING ANTI-HYPERTENSIVE DRUG''

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Abstract

The present work is to prepare and characterization of self nano emulsifying drug delivery system containing Anti-hypertensive drug. Losartan is a competitive antagonist and inverse agonist of angiotensin 2 receptor. The SNEDDS is prepared by Sonication method using a components of SPAN 60/Eudragit RS 100 as a surfactant, PVA as a Co-surfactant, Iso propyl alcohol as a solvent and DCM as a co-solvent. The prepared SNEDDS was evaluated for Fourier transform infrared spectroscopy, Surface morphology, particle size, zeta potential, drug entrapment efficiency, visual assessment, self-emulsification time, Robustness to dilution, *in-vitro* drug release and short term stability studies. The *in-vitro* drug release data of all the formulations were found to be zero order over a period of 24 h and Formulation F_7 shows good results for the drug release kinetics as controlled release. The stability studies data was found that there was no such difference in drug EE and *in-vitro* drug release.
Key words: SNEDDS, Losartan potassium, Surfactant, Co-Surfactant, Solvent, Co-solvent, Sonication method, Self nanoemulsification.

INTRODUCTION

Oral delivery route is that the most convenient route for drug administration to attain desired therapeutic effects and also the greatest degree of patient compliance, particularly for chronic condition diseases. Super molecule primarily based formulations, as well as self-nanoemulsifying formulations, is that the promising technologies for PWSD delivery and has shown to boost the oral absorption of those medicine. Self-nanoemulsifying Drug Delivery system (SNEDDS) is isotropic mixture of oil, natural or synthetic surfactants and co-surfactants that have a ability of forming fine oil in water (O/W) nano-emulsions under mild Agitation followed by aqueous media. Self-Nano emulsifying Drug Delivery System having size vary of globules is a smaller amount than 100nm underneath dispersion of water. numerous strategies for choice of parts and methodology of preparation that square measure mentioned in review of literature, For to attain the SNEDDS the category II and sophistication III medicine square measure best suited.

Components of SNEDDS and their concentrations have an effect on drop size, emulsification potency and unharness property of fashioned nanoemulsion. SNEDDS square measure ready by trial and error basis to urge smart region forming nanoemulsion. But this can be time consuming and needs a bigger range of trails.

The objective of this study was to prepare and characterize the self nanoemulsifying drug delivery system containing Anti-hypertensive drug (Losartan potassium) by sonication methodology.

MATERIALS AND METHOD

Materials:

Losartan potassium was procured from Nirupama K V, Dept. of pharmaceutics, Bharathi college of pharmacy, Mandya as a gift sample,

SPAN 60, Eudragit RS 100, Eudragit RSPO was supplied from Yarrow chemicals, Polyvinyl alcohol (PVA) was supplied from Fisher scientifics, Iso propyl Alcohol, Dichloromethane, NaOH and Potassium Dihydrogen Orthophosphate was supplied from SD Fine chemicals.

Method:

Preparation of SNEDDS by Sonication Method

Solution of surfactant SPAN 60/ Eudragit RS 100 in Iso propyl alcohol was mixed with Co-surfactant polyvinyl alcohol by using controlled flow rate syringe pump 3ml/min rate. During this mixing the aqueous phase was sonicated using a probe sonicator set at 10 KHz of energy output (Labman Pro-500) to produce oil in water type of emulsion. place this preparation for magnetic stirrer at 1000 rpm until the organic phase is to evaporate. The obtained nanoparticles were recovered by centrifugation (Remi PR 24) at 10,000 rpm for 15-20 min and washed thrice with distilled water. The washing water was removed by a further centrifugation and nanoparticles were dried.

Table 1: Formulation table of SNEDDS containing Losartan potassium

Formulation code	Losartan potassium (mg)	SPAN 60 (w/v)	Polyvinyl alcohol (PVA) (%)	Eudragit RS 100 (w/v)	Eudragit RSPO (w/v)	Isopropyl alcohol (ml)
F1	100	100	0.5	100	-	30
F2	100	150	0.5	150	-	30
F3	100	200	0.5	200	-	30
F4	100	100	0.5	-	100	30
F5	100	150	0.5	-	150	30
F6	100	200	0.5	-	200	30
F7	100	200	0.5	100	100	30

Characterization of SNEDDS

Drug - Excipient Compatibility Study

The compatibility of components and drug was evaluated by FT-IR study.

FTIR Study:

FTIR spectra of pure drugs, physical mixture of SPAN 60 and Eudragit RS 100, RSPO and drug loaded Nanoparticles were recorded on a BRUKER IR spectrophotometer and scanned in the spectral region between 4000 cm^{-1} and 600 cm^{-1}.

Surface morphology:

The surface morphology is most commonly measured by Scanning Electron microscopy. The surface morphology has been studied by using JEOL JSMT -330A Scanning electron microscopy (SEM).

Particle size:

The particle size and distribution is measured by Malvern Zeta sizer by Wet technique. The average particle sizes of the individual batch of Nanoparticles were reported.

Zeta potential:

The Zeta potential of a nanoparticle is commonly used to characterize the surface charge property of Nanoparticles. Zeta potential is measured by Malvern zeta analyzer.

Drug entrapment efficiency:

The self emulsifying Nanoparticles were separated from the aqueous medium by ultracentrifugation at 10,000 RPM for 15-20 min. Then the resulting supernatant solution was decanted and dispersed into phosphate buffer pH 7.4. Thus the procedure was repeated twice to remove the un-entrapped drug molecules completely. The amount of drug entrapped in the nanoparticle was determined as the difference between the total amount of drug used to prepare the Nanoparticles and the amount of drug present in the aqueous medium.

$$\text{Drug entrapment efficiency} = \frac{\text{amount of drug released from the lysed nanoparticle}}{\text{Amount of drug initially taken to prepare nanoparticle}} \times 100$$

Evaluation parameters of SNEDDS

Visual observation:

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

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**.**

Table 2: Grades for the visual assessment of self nano emulsifying formulation

Grade	Visibility
I	Clear or slightly bluish white in appearance within 1 min
II	Slightly less clear; bluish white in appearance <2 min
III	Milky in appearance with in 3 min
IV	Dull white which is slightly in appearance, slow to emulsify> 3 min
V	Turbid in appearance >3 min

Robustness to dilution:

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

Dissolution Rate study on Self emulsifying Nanoparticles:

In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 50 rpm. The prepared Nanoparticles were immersed in 900ml of phosphate buffer solution in a vessel, and temperature was maintained at 37±0.20°C. Required quantity 5ml of the medium was withdrawn at specific time periods and the same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were analyzed using UV spectrophotometer (SHIMADZU 1700).

Stability studies

The prepared self emulsifying Nanoparticles were packed in screw capped HDPE bottles and were stored at 40± 20 C and 75 % RH for 45 days. After storage for 45 days, the products were tested for drug entrapment efficiency and drug release study as per the ICH guidelines.

RESULTS AND DISCUSSION

Pre-formulation studies:

Analytical methods

I. Determination of λ max of Losartan potassium

The λ max of Losartan potassium was found to be 234 nm. The spectrum traced using UV spectrophotometer (UV1700, Shimadzu, Japan).

II. Standard plot of Losartan potassium

The standard plot was established for Losartan potassium in Phosphate buffer pH 7.4. Absorbance measured at 234 nm and a graph of concentration versus absorbance was plotted

Table 3: Standard Calibration curve of Losartan in Phosphate buffer 7.4

Sl. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.152
3	4	0.323
4	6	0.467
5	8	0.639
6	10	0.752

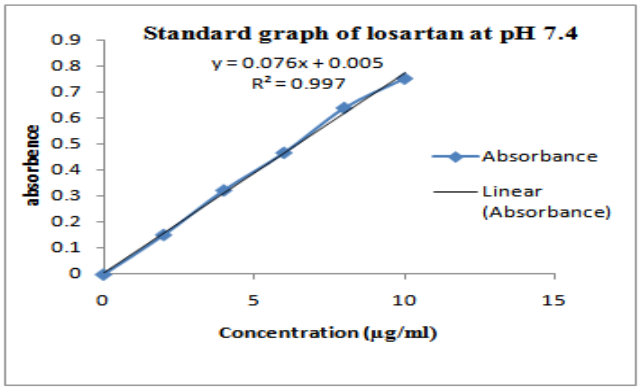

Figure 1: Calibration curve of Losartan in phosphate buffer pH 7.4

Solubility analysis of drug (Losartan)

Practically completely soluble in Dichloromethane, Isopropyl alcohol, Ethanol, Methanol and Water.

Characterization of SNEDDS

Compatibility studies:

From the FTIR spectra of the pure drug and the combination of drug with the surfactant and co-surfactant was observed that all the characteristics peaks of Losartan is present in the combined spectra as well thus indicating the compatibility of the drug with the components . The individual FTIR spectra of the pure drug Losartan and physical mixture of SPAN 60, Eudragit RS100 and Eudragit RSPO as well as combination spectra of the drug and components (formulation F_7) are shown in Figure No. 13, 14 and 15. It was found that the drug was compatible with polymer in physical mixture.

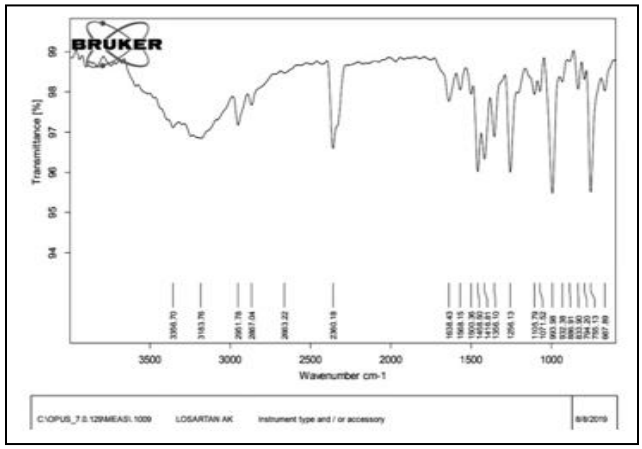

Figure 2: IR spectra of Pure Losartan potassium Drug

The FTIR spectrum of the Losartan pure drug was found to be similar to standard spectrum of Losartan as in IP. The spectrum of Losartan showed the following functional groups at their frequencies.

Characteristic group	**cm^{-1}**
O-H stretching	3356
N-H stretching	3183
C-H stretching	2951
C=C stretching	1638
C=N stretching	1568
C-class stretching	755

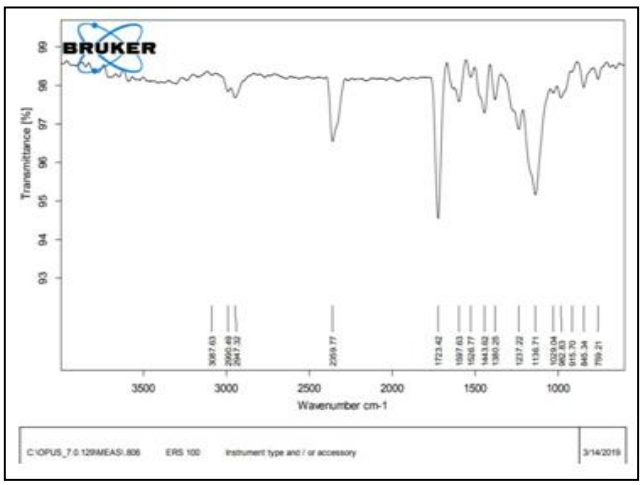

Figure 3: IR spectra of physical mixture of SPAN 60, Eudragit RS 100, RSPO and PVA**.**

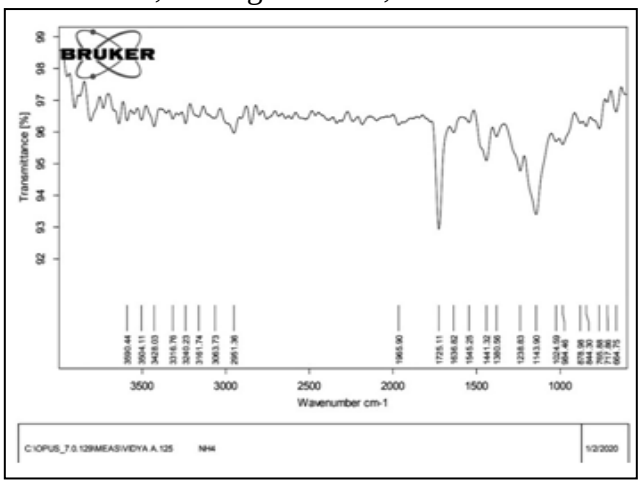

Figure 4: IR spectra of formulation LP7

Report: IR spectra of SNEDDS LP6 shows peaks at:-

- Peaks at 3428.03 shows O-H stretching
- Peaks at 3161.74 shows N-H stretching
- Peaks at 2951.36 shows C-H stretching
- Peaks at 1636.82 shows C=C stretching
- Peaks at 1545.25 shows C=N stretching
- Peaks at 765.88 shows C- Class stretching

Scanning electron microscopy (SEM)

Determination of surface morphology and shape was done by ZEISS EVO. US Scanning Electron Microscope. The SNEDDS formulations shows the spherical shape and smooth in surface area.

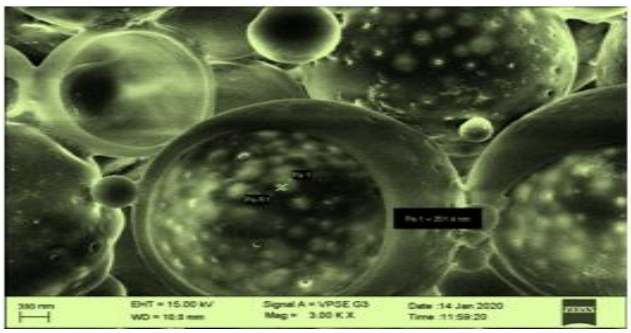

Figure 5: SEM images of LP5

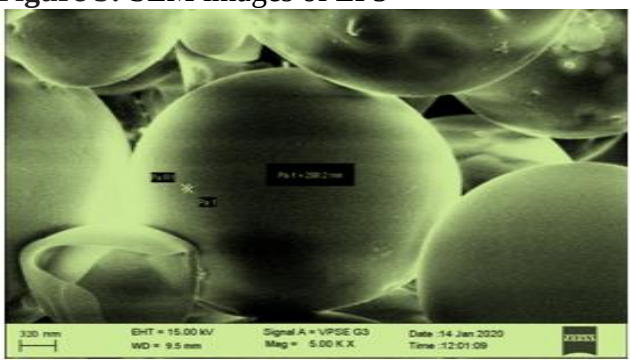

Figure 6: SEM images of LP7

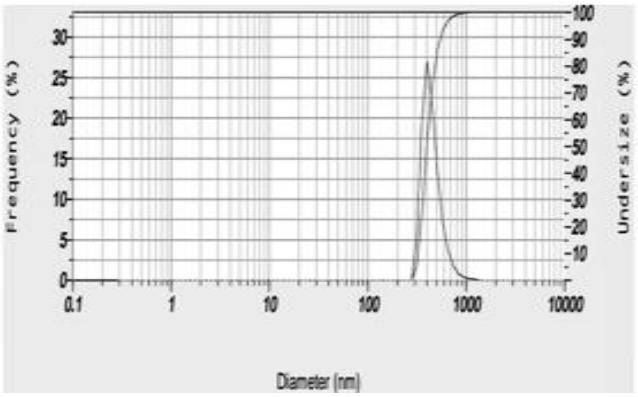

Figure 7: Particle Size analysis by size distribution by intensity

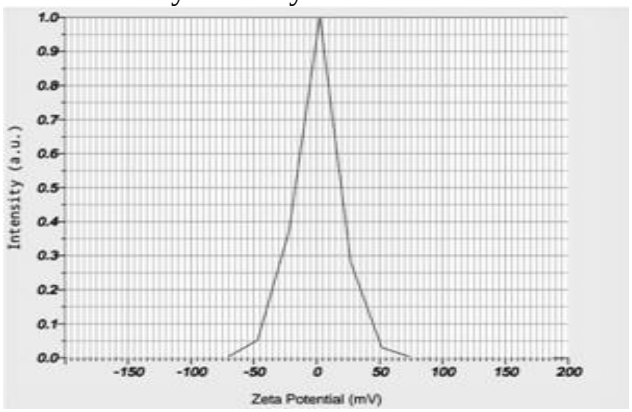

Figure 8: particle size analysis by Zeta potential distribution

Table 4: Particle size and drug entrapment efficiency analysis of SNEDDS.

Sl No.	Formulation code	Particle size (nm)	Drug EE (%)
01	F1	520	75
02	F2	460	77.7
03	F3	412	84.72
04	F4	447	85.12
05	F5	380	91.66
06	F6	480	83.51
07	F7	320	92.54

Visual assessment:

In nano-emulsion formulation only F2, F4, F5, F6 and F7 were clear. The rest of the formulations were showed precipitation.

Table 5: Visual assessment of F1-F7

Sl. No.	Formulation code	Visibility grade	Precipitation
1	F1	IV	YES
2	F2	IV	NO
3	F3	III	YES
4	F4	III	NO
5	F5	III	NO
6	F6	III	NO
7	F7	III	NO

Self emulsification time

1 ml of formulation was a added to 100ml of distilled water at 37^0C being agitated at 100 rpm. The time required for the formulation of a milky emulsion was noted for F2, F4, F5, F6 and F7 were 58 sec, 53 sec, 51 sec, 48 sec and 49 sec.

Robustness to dilution

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

In-vitro drug release study

Table 6: *In-vitro* drug release study of SNEDDS formulations F1 to F7

Time (hr)	% Drug Release						
	Formulation code						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
0	0	0	0	0	0	0	0
0.5	3.81±0.011	4.15±0.016	3.21±0.018	4.12±0.014	5.42±0.035	3.55±0.013	4.16±0.014
1	6.45±0.019	8.49±0.019	8.63±0.023	12.45±0.016	10.16±0.029	10.64±0.021	9.51±0.034
1.5	13.45±0.028	15.46±0.021	12.45±0.016	16.86±0.023	15.42±0.038	15.49±0.025	14.45±0.026
2	20.47±0.031	21.74±0.034	18.74±0.015	24.32±0.026	26.14±0.017	25.41±0.014	23.48±0.021
3	28.64±0.032	25.86±0.029	35.13±0.006	37.21±0.034	35.46±0.015	36.47±0.024	38.42±0.013
4	35.21±0.052	32.35±0.016	45.23±0.035	44.23±0.021	45.96±0.014	41.56±0.014	40.16±0.026
5	45.87±0.061	39.56±0.015	54.31±0.062	48.26±0.013	51.48±0.029	49.56±0.031	46.28±0.038
6	51.26±0.072	48.18±0.022	59.41±0.014	56.28±0.014	61.87±0.026	54.21±0.024	55.89±0.034
7	58.65±0.021	59.51±0.028	63.85±0.019	65.62±0.025	68.12±0.034	65.17±0.029	68.14±0.011
8	65.23±0.025	68.32±0.032	68.21±0.031	69.65±0.024	70.85±0.031	72.32±0.019	71.63±0.027
9	68.98±0.063	71.98±0.016	73.25±0.032	70.14±0.031	75.65±0.028	75.64±0.041	76.58±0.018
10	71.05±0.019	73.04±0.009	75.31±0.023	81.46±0.027	76.48±0.027	83.24±0.017	85.41±0.026
11	73.46±0.023	76.48±0.018	81.45±0.021	83.47±0.019	82.18±0.027	89.45±0.018	90.14±0.039
12	75.28±0.061	77.58±0.027	83.47±0.026	85.84±0.023	83.94±0.09	92.61±0.039	92.68±0.026
18	77.45±0.054	78.49±0.026	85.61±0.013	86.06±0.012	87.23±0.028	93.48±0.024	94.27±0.013
24	78.58±0.021	79.68±0.032	87.41±0.036	86.94±0.023	88.83±0.031	94.31±0.014	95.68±0.028

Different Drug release Kinetics Models:

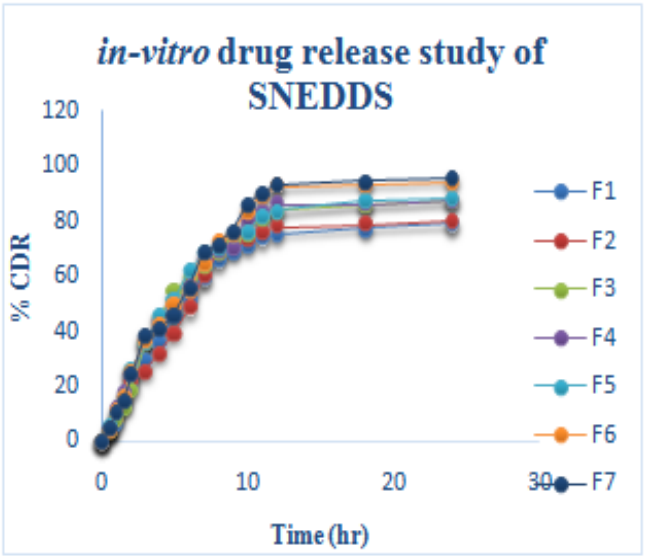

Figure 9: Graph of *in-vitro* drug release of kinetics for formulations F_1 to F_7

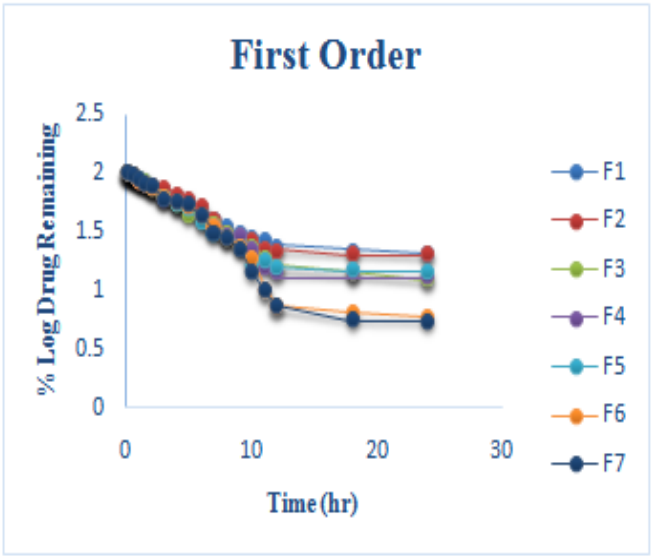

Figure 10: Graph of First order SNEDDS formulation from F_1 to F_7

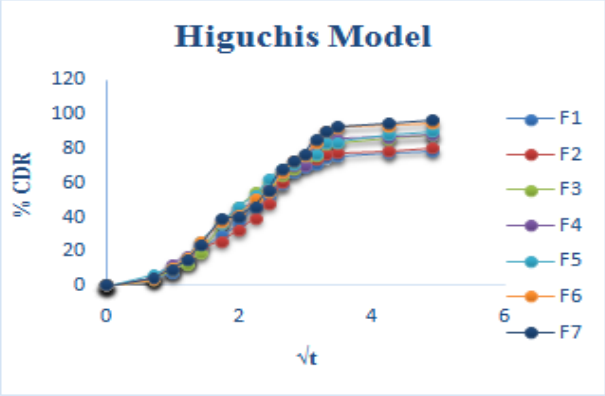

Figure 11: Graph of Higuchi's model for *in-vitro* drug release of formulation F_1 to F_7

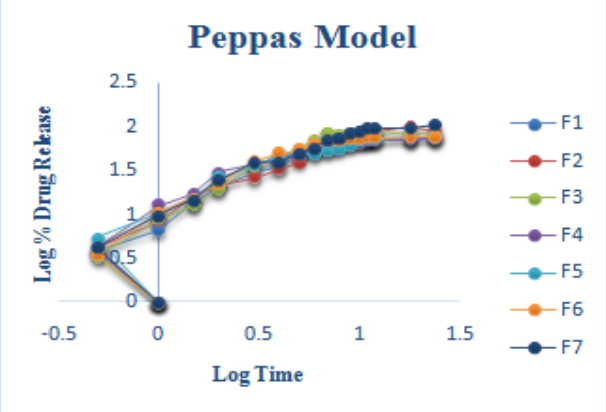

Figure 12: Graph of Peppa's model *in-vitro* drug release of formulation F_1 to F_7 for *in-vitro* drug release of formulation F_1 to F_7

Table 7: Regression co-efficient (R^2) values and 'n' values of SNEDDS according to different kinetic models

Formulation code	Zero order		First order		Higuchi	Peppas	
	R^2	n	R^2	n	R^2	R^2	n
F_1	0.851	5.166	0.921	0.102	0.947	0.956	0.911
F_2	0.857	5.322	0.904	0.109	0.938	0.967	0.868
F_3	0.842	5.631	0.946	0.128	0.947	0.947	0.947
F_4	0.850	5.441	0.934	0.125	0.957	0.948	0.830
F_5	0.857	5.698	0.944	0.137	0.955	0.946	0.885
F_6	0.871	6.078	0.948	0.177	0.959	0.966	0.847
F_7	0.851	5.696	0.924	0.137	0.9509	0.946	0.886

Stability Study Report:

The prepared self emulsifying nanoparticles were tested for drug entrapment efficiency and drug release study as per the methods described earlier. The results are given in Table.

Table 8: Drug entrapment efficiency

Formulation code	Drug entrapment efficiency (%)	
	Before stability	After stability
F7	92.54	91.82

Table 9: Percentage of drug release

Formulation code	% drug release	
	Before stability	After stability
F7	95.68	94.91

Conclusion

It can be concluded from the experimental study carried out that the formulation of a SNEDDS containing anti-hypertensive drug yields a formulation with spherical and smooth surface, nano size range & good percentage entrapment efficiency .

The particle size analysis indicated that the particles were in the size range of 320 nm to 520 nm, and showed good flow properties. The Nanoparticles were smooth, as shown by the scanning electron microscopic studies. *In-vitro* drug release showed that release from the SNEDDS gets successfully retarded for over 24h. The formulations were found to be stable in Short term stability studies. Pharmacokinetic studies indicate that the *in-vitro* drug release of the formulations fitted Peppas model and the mechanism follows non- Fickian drug release. By considering the results obtained from *in-vitro* and Stability studies, it can be suggested that there is further scope for the *in-vivo* and the Pharmacokinetic Study. Here we have selected F7 has an optimized formulation which shown good morphological features, drug entrapment efficiency and controlled drug release.

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