Original Article

FORMULATION AND CHARACTERIZATION OF SELF EMULSIFYING DRUG DELIVERY SYSTEM (SEDDS) FOR SOLUBILITY ENHANCEMENT OF POORLY WATER SOLUBLE DRUG

Ashok Kumar Rajpoot [1&2]*, Hitesh Kumar [2&3], Vaishali [1], Arvind Kumar [4], Manish Devgun [5]

[1] Moradabad Educational Trust Group of Institutions Faculty of Pharmacy, Moradabad, Uttar Pradesh, India - 244001.

[2] Faculty of Pharmaceutical Sciences, Mewar University, NH-79 Gangrar, Chittorgarh, Rajasthan, India-312901.

[3] RV Institute of Pharmacy, Moradabad Road, Bijnor, Uttar Pradesh, India-246728.

[4] SD College of Pharmacy, Muzaffarnagar, Uttar Pradesh 251001.

[5] University Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India-136119.

Email: ashokraj009@gmail.com[1]

DOI: 10.47750/pnr.2022.13.S01.247

Abstract

The current study is done for formulating and characterizing self-emulsifying drug delivery system (SEDDS). In the current research it has been shown that the SEDDS is a tedious pharmaceutical technology which has the potential for the improvement in biopharmaceutics properties like absorption, metabolism, distribution and excretion of Cyclosporin A also opens new window for developing the procedures to enhance the solubility of low water solubilised drugs. The stability in gastrointestinal drug as well as rate of absorption of drug in SEDDS can be increased by this approach. 3 types of methods were used for the formulation of Self emulsifying drug delivery system (SEDDS) containing cyclosporine: Cold homogenisation method, Hot Homogenisation method and method of injection with the use of emulsifying agent and stabilising agent.The optimisation of formulation variables were done with the use of 33 factorial design. The analysation of 3 independent formulation variables were done at the time of study which includes the concentration of surfactant (A), concentration of stabiliser (B) and lipid concentration (C). The droplet size (X) and polydispersity index (Y) were the investigated dependent variables .The complete experiment includes 27 designed points. The pattern of release of optimized formulation was almost similar to the release pattern of marketed formulation. Less nephrotoxicity and less inflammation is observed in SEDDS containing Cyclosporin A when compared with available marketed product. Hence, SEDDS containing Cyclosporin A is a system which is very important for the enhancement of solubility of drugs which have very less solubility in water.

Keywords: SEDDS, Cyclosporin, Solubilisation, Bioavailability.

INTRODUCTION

"Empirical-based" novel drug design is converted to "knowledge-based" drug design now a days. Activity of Pharmacological action can be seen with the mixing of maximum throughput screening (MTS) and Advancement in biotechnology and in combination with synthetic approaches which results in a number of various new chemical moieties (NCMs). On the other hand, design of molecules rationally cannot be treated as delivery of drug rationally because delivery of drug does not takes place of itself[1]. However, New chemical moieties (NCMs) developed in the ancient time did not reaches to the market because formulation was developed with the identification of active drug molecules but this developed formulation possess many problems like formulation possess less physicochemical properties like solubilisation and stabilisation, other problems of pharmaceutical properties such as permeabilisation and enzymatic stabilisation which results in non-reaching of about 40% of NCMs to market place [2]. Unaccepted efficacy and safety problems are the main characteristics of the new chemical moieties (NCMs) due to which they are fail at the time of preclinical trial experiments or they are thrown out from the reach of industry processes, so they are not able to reach to the shop of the chemist [3]. Systemic reach of the drug to the blood is very less, highly intra-subject and inter-subject variation and proportion of their dose is very less are the few problems which are connected to the drugs [4]. These drawbacks (Systemic reach of the drug to the blood is very less, highly intra-subject and inter-subject variation and proportion of their dose is very less) can be improved with development of many new techniques. So many techniques are given in the literature which consists of Drug can be incorporated in oils [5], Formulation of Suspensions[6], formulation of emulsions[7], Formulation of liposomes [8], cyclodextrins can be used[9], coprecipitates [10], micronization [11]; Vogt et al., 2008)[12], nanoparticles [13], permeation enhancers [14] and lipid-based vehicles[15][16][17].

MATERIALS

LGM Pharma provided Cyclosporin A as a gift sample. S.D. Fine Chem. Limited, Mumbai was used for purchasing Stearic acid and Polyoxyethylene sorbitan mono oleate (Tween 80). All other of the excipients taken for the formulation of SEDDS were pharmaceutically acceptable for administration orally.

METHODS USED FOR THE PREPARATION OF SEDDS

3 types of methods were used for the formulation of SEDDS containing cyclosporine: Cold homogenization method, HotHomogenization method and method of injection with the use of emulsifying agent and stabilizing agent for emulsifying and stabilizing the i.e. watery and oily phases and the use of oils for the encapsulation of drug (Cyclosporin). The optimisation of method was done on the criteria of efficiency of entrapment (EE) and droplet size.

Hot homogenisation method

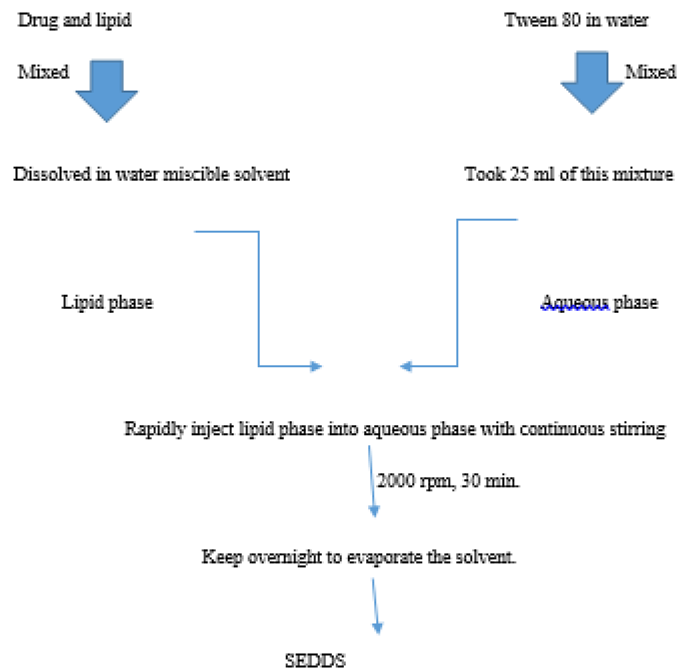
Above the m.p. of the lipids, melting dispersion usually takes place and hence it can be designated as hot homogenization of a formulation i.e. emulsion. Silver son homogeniser which is a high shear stirring device is used for dispersing the melted drug encapsulated lipid and previously heated aqueous surfactant phase at a same temperature for obtaining a coarser oil in water pre-emulsion. This pre-emulsion is used for High pressure homogenization at the temp. of the lipid above its melting point. To obtain the desired size range of nanometres and quality of the final formulation (SEDDS containing cyclosporine) is mainly influenced by the condition of pre-emulsion. High processed temperature due to lesser viscosity of the lipid matrix phase is responsible for obtaining smaller droplet size but it may lead to deterioration of drug and its carrier. Very less exposure of temperature is given for a short period of time, Hence, this method (Hot homogenization technique) can also be used for thermolabile drugs.[17]

Cold homogenisation method

The drug is dispersed or solubilised in to the melted lipid matrix and initial procedue is similar to the hot melting dispersion method. The solidification of this drug entrapped melted lipid rapidly takes place with the help of liquid nitrogen or dry ice to give assurance that moleculeis distributed uniformly in the matrix of the lipid. Ball or mortar milling is used for crushing the drug entrapped molten lipid to obtain particles of 40–105 micrometre. Increment of fragileness of lipid which results in particle communition due to low temperature. After this, cold emulsifying agent solution is used for dispersing the solid lipid matrix to yield a pre-suspension, after that homogenisation takes place at or lower than normal temperature with suitable temperature controlled and its regulation with the consideration of rise in temperature in the process of high pressure. Now, self-emulsifying drug delivery system can be formulated from lipid microparticles due to breakage because of cavitational forces.[18]

METHOD OF INJECTION

The water soluble solvents like ethanol, acetone and isopropanol were used for dissolving the solid lipid matrix to form the organic phase.[19]



OPTIMIZATION OF SEDDS

The SEDDS was formulated by one of the chosen method from 3 different methods on the criteria of smallest droplet size in nanometre range and the highest efficiency of the entrapment (EE). The method which developed droplet size which is smallest and in nanometre range and efficiency of the entrapment (EE) which was highest chosen for formulation of SEDDS.

OPTIMISATION OF FORMULATION VARIABLES

The optimisation of formulation variables were done with the use of **3^3 factorial design**. The analysation of 3 independent formulation variables were done at the time of study which includes the concentration of surfactant (A), concentration of stabiliser (B) and lipid concentration (C).

OPTIMIZATION OF VARIABLES OF PROCESS

The optimization of variables of the process were done. The study of variables which are dependent of the method like revolution per minute, stirring time, the temperature at which preparation was developed and the process of filtration which affected the suspension system that resulted in the effect on the droplet size of the SEDDS. The study of individual effects of preparation and process parameters were done while developing SEDDS. The effect was determined as droplet size (nm) and particle size distribution as polydispersity index of the SEDDS so formulated.

Table 1: Response surface regression characterization of Cyclosporin A loaded SEDDS formulations showing 27 runs

Preparation Code	**Concentration of surfactant (%v/v)**	**Concentration of lipid (w/w %)**	**Concentration of stabiliser (w/v %)**
F1	2	3	2
F2	4	3	2
F3	6	3	2
F4	4	5	0.5
F5	6	5	0.5
F6	2	5	0.5
F7	6	7	1
F8	2	7	1
F9	4	5	2
F10	**6**	**3**	**1**
F11	6	5	2
F12	2	7	0.5
F13	2	3	0.5
F14	6	5	1
F15	4	7	2
F16	4	5	1
F17	6	7	2
F18	2	5	1
F19	6	7	0.5
F20	4	5	0.5
F21	2	7	2
F22	4	3	1
F23	4	7	1

F24	2	5	2
F25	4	7	0.5
F26	6	3	0.5
F27	2	3	1

Characterization of Cyclosporin A Loaded Self-Emulsifying Drug Delivery System

1. Efficiency of the Entrapment (EE) and Loading of the drug
2. Assay of drug by HPTLC.
3. Yield of SEDDS
4. Particle size distribution
5. Powder X-ray Diffraction (XRD) study
6. Zeta Potential determination
7. Transmission electron microscopy (TEM)
8. Differential scanning calorimetry (DSC)
9. In-vitro release study
10. Scanning electron microscopy (SEM)

RESULTS AND DISCUSSION

Methods Used for the Preparation of SEDDS

Three different methods were used for the preparation of Cyclosporin A loaded SEDDS like Hot homogenisation method, Cold Homogenisation method and method of injection. Efficiency of the entrapment and droplet size were the selected criteria for the optimization of method.

Table 2: Average droplet size and percent entrapment efficiency (% EE) of SEDDS by various methods (Mean ± SD, n=3)

Methods	**Average Droplet size**	**% Entrapment Efficiency**
Stearic acid as a lipid carrier		
Hot homogenisation method	156.1 + 26.8	88.93 + 6.56
Cold Homogenisation method	319.6 + 16.1	68.72 + 5.58
Injection method	788.3 + 23.5	54.23 + 7.32

Optimisation of Formulation Parameters

The method of response surface was used for the optimisation of formulation parameters. Statistically, Relationship between the various explanatory parameters and more than two response parameters are determined by the Methodology of Response surface (MRS). The three- dimensional space graphs or contour plots can be used for representing the response graphically that will help to view the shape of the response surface clearly. The analysation of 3 independent formulation variables were done at the time of study which includes the concentration of surfactant (A), concentration of stabiliser (B) and lipid concentration (C). The droplet size (X) and polydispersity index (Y) were the investigated dependent variables for the selection of desired and Optimized SEDDS formulation.

Fig. 1: The effect of Concentration of surfactant and stabilizer on average droplet size can be seen in 3D curve of response surface.

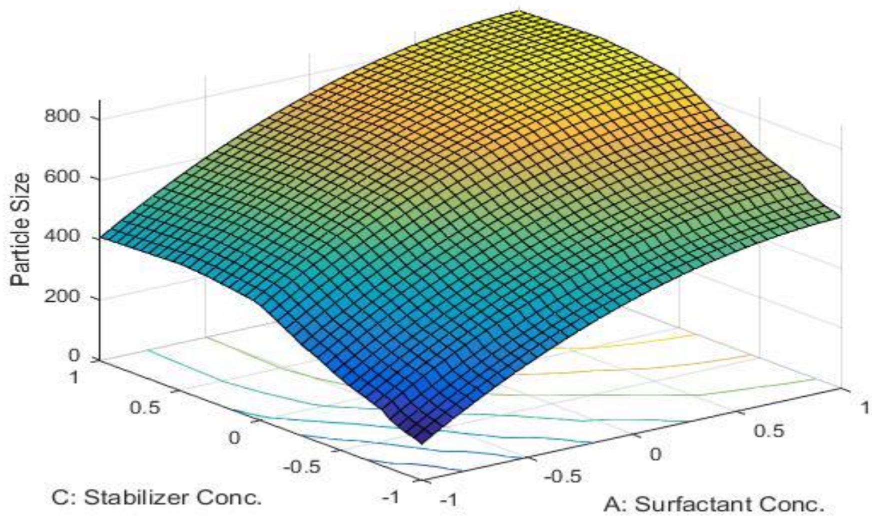


Fig. 2: The influence of Concentration of Stabiliser and Lipid on mean droplet size can be seen in 3Dcurve of response surface.

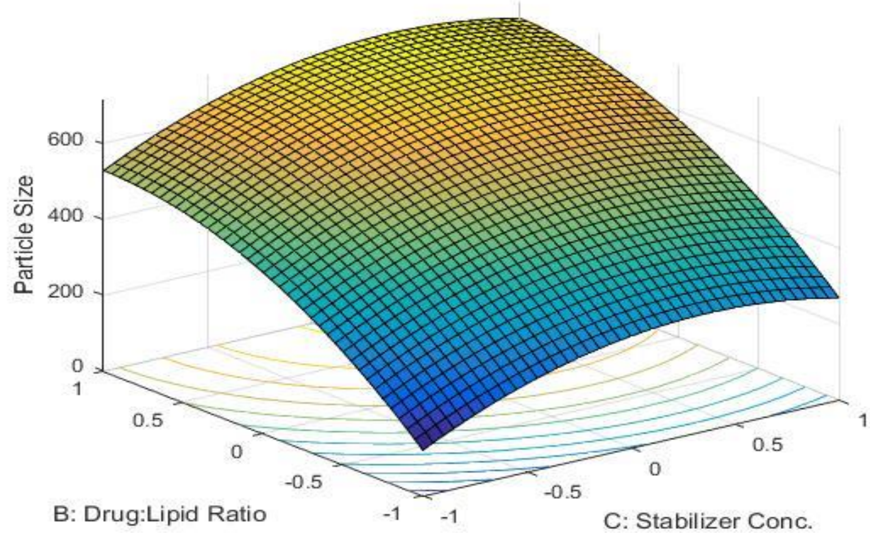


Fig. 3: The influence of Concentration of surfactant and Lipid on mean droplet size can be seen in 3Dcurve of response surface.

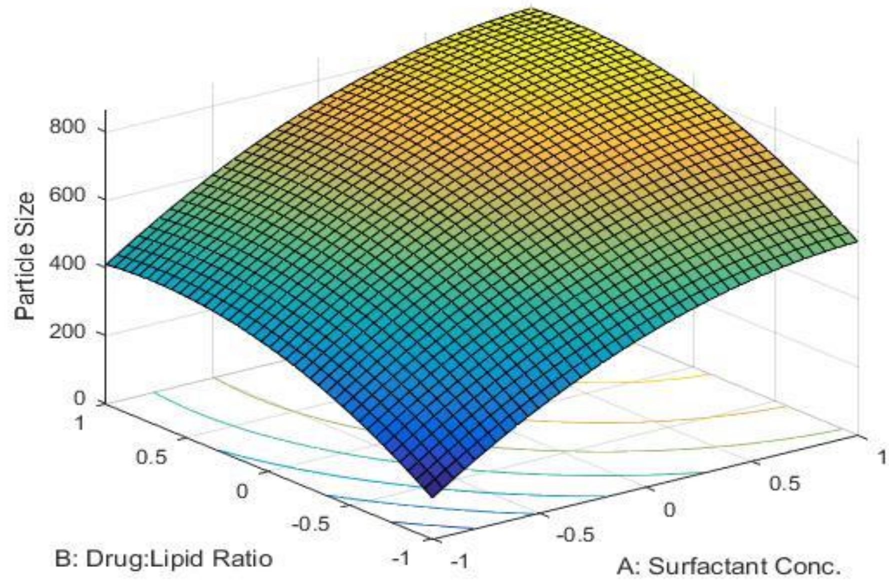


Fig. 4: The influence of Concentration of stabilizer and surfactant on droplet size distribution (PDI) can be seen in 3Dcurve of response surface.

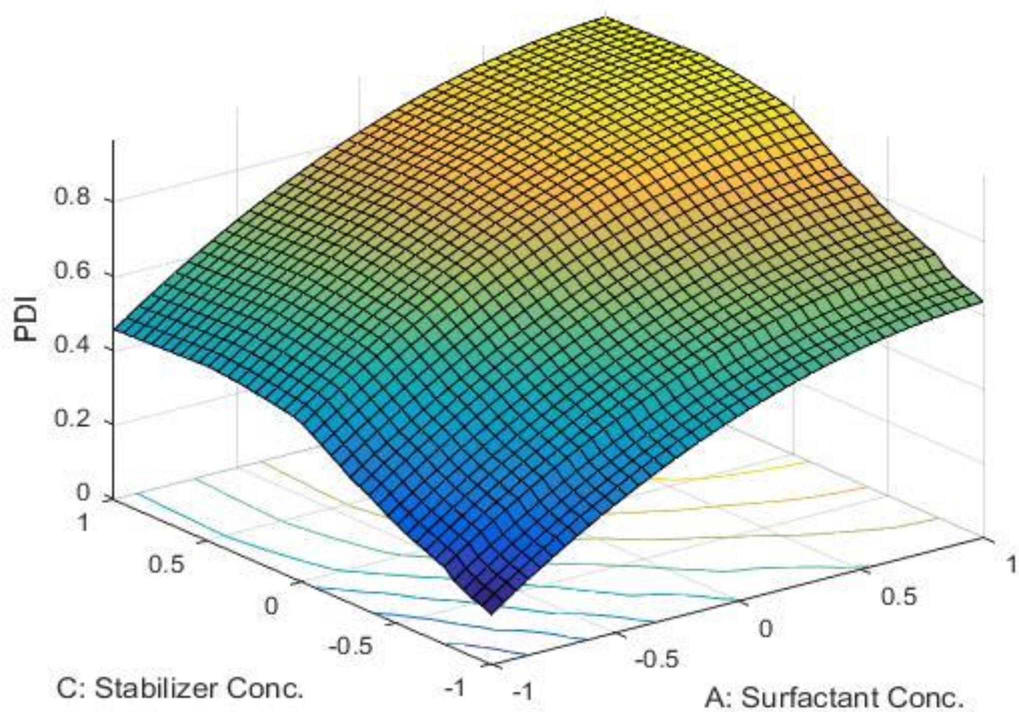


Fig. 5: The influence of Concentration of Lipid and Stabiliser on droplet size distribution (PDI) can be seen in 3D curve of response surface.

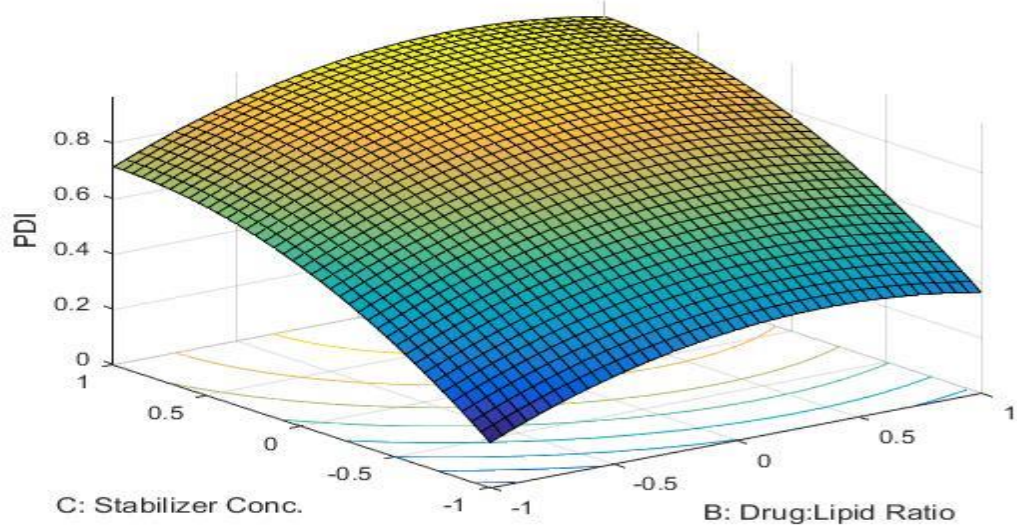
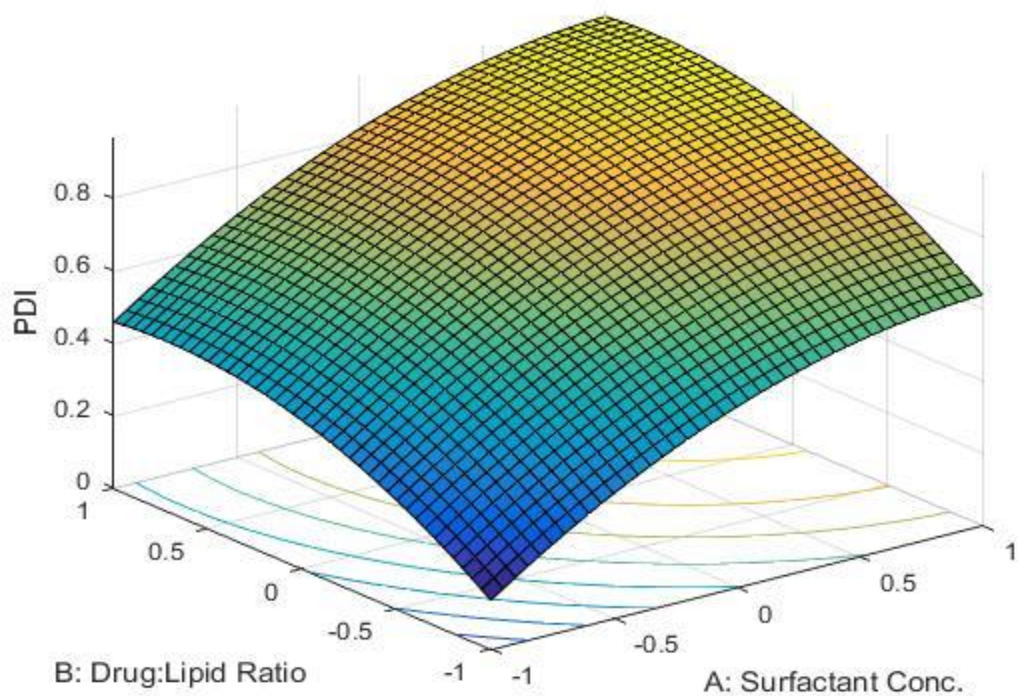


Fig. 6: The influence of Concentration of Lipid and surfactant on droplet size distribution (PDI) can be seen in 3D curve of response surface.



The size of droplet and distribution of droplet size (pdi) was predicted as 155.4 nm and 0.167, respectively by the software. The size of droplet and distribution of droplet size (pdi) was experimentally found as 156.9 nm and 0.176 and when these values are compared with predetermined readings predicted with matlab software was very near to the previously predicted results. In the current research, predetermined readings predicted with matlab software was very near to the previously predicted results indicated that optimization achieved was reliable.

Table 3: Selected Parameters with their optimised values

Variables	Parameters	Optimised values
Formulation	**Concentration of Surfactant**	6 % V/V
	Concentration of Lipid	**3**% W/W
	Concentration of stabiliser	**1** % W/V
Process	**RPM**	2000 rpm
	Time	60 min.
	Temperature	25 °C
	Filtration	With filtration

EVALUATION OF CYCLOSPORIN A LOADED SELF- EMULSIFYING DRUG DELIVERY SYSTEM

Drug loading and Entrapment Efficiency (EE)

Table 4: % EE, drug loading, % yield Cyclosporin A loaded Self-Emulsifying Drug Delivery System.

Preparation	Entrapment Efficiency (%EE)	Drug loading (% DL)	Yield (%y)
Cyclosporin A loaded SEDDS	88.5 + 2.19	20.50 + 0.43	94.20 + 2.3

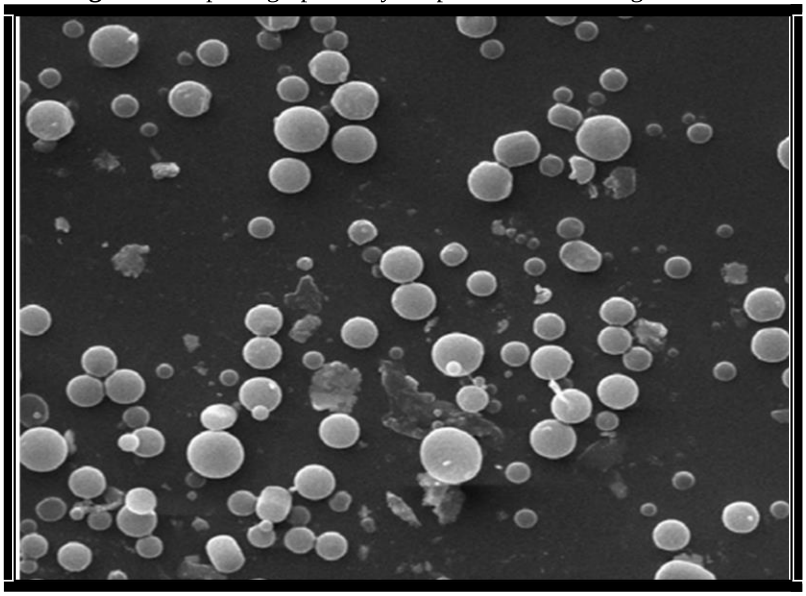
Particle size distribution

Table 5 : Zeta potential andsize of droplet of Cyclosporin A loaded SEDDS (n=3).

Preparation	Droplet size (nanometer)	Polydispersity index(PDI)	Zeta potential (Millivolt)
Cyclosporin A loaded SEDDS	156.9 + 6.2	0.176 + 0.05	-36.3 + 1.2

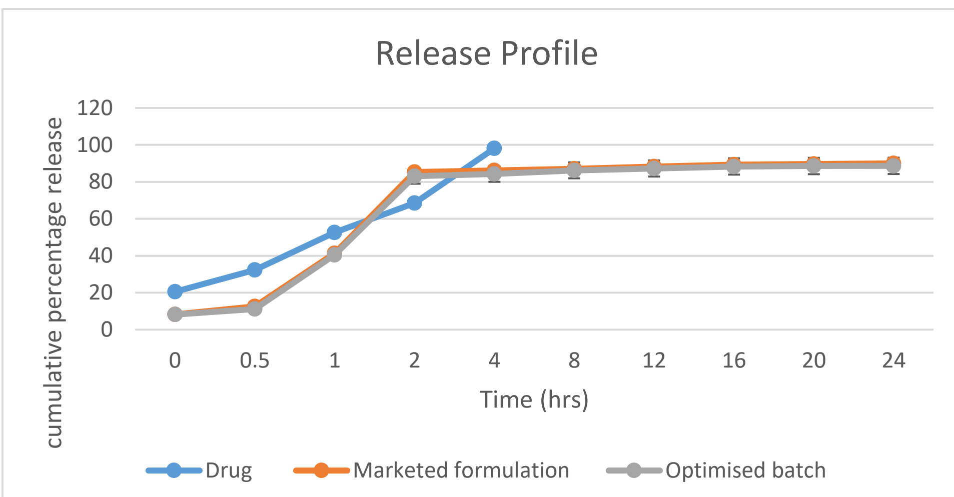
SCANNING ELECTRON MICROSCOPY (SEM)

Fig. 7: SEM photograph of Cyclosporin A Containing SEDDS.



STUDY OF IN-VITRO RELEASE PROFILE

Fig. 8: In vitro dissolution study of pure drug, SEDDS and marketed formulation in PBS buffer, pH 6.8.



The release profile of Cyclosporin A, Cyclosporin A loaded SEDDS and available marketed product was used for the study of in-vitro release within the twenty four hours of formulations preparation. In the dialysis tube of diffusion membrane, 1 millilitre of sample was taken and then ten millilitre of PBS buffer (pH 6.8) with emulsifying agent (tween 80) in a vial was taken and now place sealed tube in it. Orbital shaker was used for shaking the dispersion at body temperature and 60 stroking per min. In-vitro release profile studies were done for the comparison of the release of Cyclosporin A from Cyclosporin A loaded SEDDS exhibited controlled release properties. In case of pure Cyclosporin A drug, initial burst release (52.63 % in 1 hr) was observed and 98.12 % drug was released in 4 hours only which shows fast release of drug followed by burst release. Marketed formulation of Cyclosporin A shows a burst release of 41.23 in 1 hour but subsequent slow release of 89.28% in 24 hours. The release study of Cyclosporin A loaded SEDDS shows a release of 40.52% in 1 hour considered as burst release then it showed slowly release of 88.65% in 24 hours. The pattern of release of optimized formulation was almost similar to the release pattern of marketed formulation.

STUDY OF STABILITY

The Cyclosporin A loaded SEDDS did not shown any drastic increase in the droplet size after storing for six months at 5°C (**Table 6**). The efficiency of the entrapment (% EE) of Cyclosporin A loaded SEDDS was decreased by about 4 - 5 % and there was a slight increment in droplet size. At the temperature of 25°C, the zeta potential (mV) of Cyclosporin A loaded SEDDS was increased from- 41.6 to - 38.23 in 6 months. The crystal transition of fatty glyceride would be responsible for these alterations, which was due to the storage and temperature conditions.

Table 6: Effect of storage conditions on entrapment efficiency, droplet size and zeta potential of Cyclosporin A loaded SEDDS.

Storage conditions (degree celsius)	**Storage (in months)**	**Droplet Size (nanometre)**	**Zeta Potential (millivolt)**	**Efficiency of the entrapment (% EE)**
STEARIC ACID as a lipid				
5	0 day	155.41	-39.6	91.4
	2 months	156.32	-39.8	91.2
	4 months	154.23	-38.5	89.65
	6 months	157.83	-38.4	89.35
25	0 day	156.72	-41.23	90.23
	2 months	157.89	-40.56	89.56
	4 months	156.23	-39.56	88.32
	6 months	157.21	-38.23	88.11

CONCLUSION

In the current research it has been shown that the SEDDS is a technology which have the potential for the improvement in biopharmaceutics properties. released effect of Cyclosporin A SEDDS prepared The optimized formulation of Cyclosporin A-SEDDS was prepared by Hot Homogenisation technique by using Stearic acid as a lipid at a concentration of 3%, Polysorbate 80 as an emulsifying agent at a concentration of 6%, and polyvinyl alcohol (PVA) as a stabilizing agent at a concentration of 1% in distilled water as an aqueous medium. The sustained drug release rate is because of the entrapment of drug in Cyclosporin A SEDDS which results in sustained using Stearic acid as a lipid and showing very good results in terms of droplet size (156.9 nanometre), polydispersity index (0.176), entrapment efficiency (88.5%) and sustained release pattern (88.65 % in 24 h) showed in *in vitro* dissolution study.

REFERENCES

1. Davis SS, Illum L, 1998. Drug Delivery Systems for Challenging Molecules. Int. J. Pharm, 176, 1-8.
2. Prentis RA, Lis Y, Walker SR, 1998. Pharmaceutical innovation by the 7 unowned pharmaceutical companies (1964-1985). Br. J. Clin. Pharmacy, 25, 387-396.
3. Kennedy T, 1997. Managing the drug discovery/development interface. Drug discovery today, 2(10), 436-444.
4. Kommuru TR, Gurley B, Khan MA, et al, 2001. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int. J. Pharm, 212, 233-46.
5. Burcham DL, Maurin MB, Hausner EA, et al, 1997. Improved oral bioavailability of the hypocholesterolemic DMP 565 in dogs following oral dosing in oil and glycol solutions. Biopharm. Drug Dispos, 18, 737-742.
6. Serajuddin ATM., Sheen PC, Mufson D, et al, 1988. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water soluble drug from solid dispersion. J. Pharm. Sci,77, 414-417.
7. Myers RA, Stella VJ, 1992. Factors affecting the lymphatic transport of penclomedine (NSC-338720), a lipophilic cytotoxic drug: Comparison to DDT and hexachlorobenzene. Int. J. Pharm. 80, 511-62.
8. Schwendener RA, Schott H, 1996. Lipophilic 1-âd - arabino-furanosyl cytosine derivatives in liposomal formulations for oral and parenteral antileukemic therapy in the murine L1210 leukemia model. J. Cancer Res. Clin. Oncol,122, 723-726.
9. Patil, P, Patil V, Paradkar, 2007. Formulation of a self-emulsifying system for oral delivery of simvastatin: In vitro and in vivoevaluation. Acta Pharm, 57, 111-122.
10. Nazzal S, Guven N, Reddy IK, et al, 2002. Preparation and characterization of Coenzyme Q10 - Eudragit® solid dispersion. Drug Dev. Ind. Pharm, 28(1), 49-57.
11. McInnes GT, Asbury MJ, Ramsay LE, et al, 1982. Effect of micronization on the bioavailability and pharmacologic activity of spironolactone. J. Clin. Pharmacol, 22, 410-417.
12. Voge M, Klaus K and Jennifer B.D., 2008. Dissolution enhancement of fenofibrate by micronization, cogrinding and spray-drying: Comparison with commercial preparations. Eur. J. Pharm. Biopharm, 68 (2), 283-288.
13. Shabouri MH., 2002. Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. Int. J. Pharm, 249(1-2), 101-108.
14. Aungst BJ, 2000. Intestinal permeation enhancers. J. Pharm. Sci.89(4), 429-442.
15. Chakrabarti S, Belpaire FM, 1978. Bioavailability of phenytoin in lipid containing dosage forms in rats. J. Pharm. Pharmacol, 30, 330-331.
16. Trull, AK, Tan KKC, Lan T, et al, 1995. Absorption of cyclosporine from conventional and new microemulsion oral formulations in liver transplant recipients with external biliary diversion. Br. J. clin. Pharmac, 39, 627-631.
17. Song C, Liu S,2005. A new healthy sunscreen system for human: Solid lipidnanoparticles as carrier for 3, 4, 5-trimethoxybenzoylchitin and the improvementby adding Vitamin E. Int J Biol Macromolecules, 36, 116-119.
18. Muller RH, Mader K and Gohla S,2000. "Solid lipid nanoparticles (SLN) for controlled drug delivery– A review of the state of the art", Eur. J. Pharm. Biopharm. 50, 161–177.
19. Schubert MA, Muller-Goymann CC, 2003. Solvent injection as a new approach formanufacturing lipid nanoparticles-evaluation of the method and process parameters. Eur J Pharm Biopharm , 55, 125-131.