



IN VIVO STUDY OF SELF-EMULSIFYING DRUG DELIVERY SYSTEM (SEDDES)

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Abstract

The main aim of the present study was “In vivo study of self-emulsifying drug delivery system (SEDDES)”. *Ex-vivo* drug release study of optimized formulation was performed using male albino rat skin. The rat epidermis was mounted onto a Franz diffusion cell in such a way that the dermis side was in contact with receptor solution. Freshly excised abdominal skin was used for *ex-vivo* studies, whose hair had been previously removed. The permeation profiles were constructed by plotting the cumulative amount of amlodipine permeated per unit rat skin area ($\mu\text{g}/\text{cm}^2$) versus time. The anti-inflammatory activity of prepared ibuprofen SEDDES was evaluated by the *carrageenan*-induced rat hind paw edema method.

KEYWORD: - In vivo, anti-inflammatory activity, permeation activity.

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1. INTRODUCTION

Self-emulsifying drug delivery systems (SEDDES) have gained exposure for their ability to increase solubility and bioavailability of poorly soluble drugs, SEDDES are isotropic mixtures of oils and surfactants, sometimes containing cosolvents, and can be used for the design of formulations in order to improve the oral absorption of highly lipophilic compounds.^{1,2} About 40% of the drug candidates identified via combinatorial screening programs are poorly water soluble. The aqueous solubility of poorly water-soluble drugs is usually less than 100 $\mu\text{g}/\text{ml}$. Especially poorly soluble, highly permeable active pharmaceutical ingredients (BCS Class II drugs) represent a technological challenge, as their poor bioavailability is solely caused by poor water solubility resulting in low drug absorption. Different techniques have been reported in the literature to achieve better drug dissolution rates.^{3,4,5} To improve dissolution/solubility and oral bioavailability of a formulation one of the approaches is self-

dispersing lipid formulations (SDLF's), SDLFs, surfactant dispersions, solid lipid nanoparticles, liposomes, emulsions and oils are various lipid-based formulations. There are two types of SDLFs which includes Self Emulsifying Drug Delivery Systems (SEDDES) and Self-Micro Emulsifying Drug Delivery self-emulsification depends on include various related Systems (SMEDDS).^{6,7,8}

Formulation of SEDDES

SEDDES are composed of oil, hydrophilic surfactant, and a co-solvent. The process of self-emulsification is only specific to certain combinations of pharmaceutical excipients. It depends on the type of oil and surfactant pair, their ratios, the surfactant concentration and the temperature at which self-emulsification occurs. The primary step during formulation of a SEDDES is the identification of these specific combinations of excipients and construct a phase diagram which shows various concentrations of excipients that possess self-emulsification.^{9,10,11} Mutual miscibility of

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these excipients is also important for producing a stable liquid formulation. Long chain triglycerides (LCT) are usually immiscible with hydrophilic surfactants and co-solvents. Polar oils such as mixed glycerides show an affinity towards hydrophilic surfactants and thus are miscible with the surfactant and aids in self-dispersion of the formulation. The diversity of the chemical nature of lipids used may lead to immiscibility on long-term storage, so it is essential to perform physical stability tests on the formulation. If waxy excipients are used, they should be melted before weighing and then mixed with other liquid excipients. With a large variety of liquid or waxy excipients available, ranging from oils through biological lipids, hydrophobic and hydrophilic surfactants, to water soluble co-solvents, there are many different combinations that could be formulated for encapsulated in hard or soft gelatin or mixtures which disperse to give fine colloidal emulsions.^{12,13,14} The following should be considered in the formulation of a SEDDS: -

1. The solubility of the drug in different oil, surfactants and co-solvents.
2. The selection of oil, surfactant and co-solvent based on the solubility of the drug and the preparation of the phase diagram.
3. The preparation of SEDDS formulations by dissolving the drug in a mixture of oil, surfactant and co-solvent. The addition of a drug to a SEDDS is critical because the drug interferes with the self-emulsification process to a certain extent, which leads to a change in the optimal oil-surfactant ratio. So, the design of an optimal SEDDS requires preformulation solubility and phase-diagram studies. In the case of prolonged SEDDS, formulation is made by adding the polymer or gelling agent.^{15,16}

2. MATERIALS AND METHODS

2.1 Ex-vivo Skin Permeation studies

2.1.1 Preparation of Rat abdominal skin

Ex-vivo drug release study of optimized formulation was performed using male albino rat skin. The experimental protocol was designed and approved by the Institutional Animal Ethics Committee. The animal was

sacrificed by cervical dislocation of the spinal cord and then the hairs were removed using depilatory cream. Abdominal sections were excised using surgical scissors and the adhered subcutaneous fat was removed. The skin surface was observed under the microscope for existence of cuts and wounds. The full thickness skin thus prepared was soaked in distilled water at 60°C for 60 seconds, followed by careful removal of the epidermis with the intact stratum corneum. The epidermis was washed with distilled water and used.

2.1.2 Ex-vivo skin permeation studies through rat abdominal skin

The rat epidermis was mounted onto a Franz diffusion cell in such a way that the dermis side was in contact with receptor solution. Freshly excised abdominal skin was used for ex-vivo studies, whose hair had been previously removed. Subcutaneous fat and other visceral tissue were removed carefully. Franz diffusion cells with an effective diffusion area of 3.9 cm² with the diameter of 16 mm and receptor volume of 12.5 ml were used to assess in vitro drug permeation. Donor and receptor compartments were separated by freshly excised rat skin. The receptor compartment was kept at 37°C. The receptor fluid was selected as pH 7.5 phosphate buffer with 1% SLS and the hydrodynamics in the receptor compartment was maintained by stirring continuously with magnetic stirrer at 500 rpm. Each formulation equivalent to single dose was placed in the donor compartment. Permeation experiments were carried out for 24 h after application. Samples were taken from the receiver compartment at scheduled time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h) and immediately replaced with the same volume of fresh receptor fluid. The amount of amlodipine in the samples was determined by UV-visible spectrophotometer at 243 nm using fresh pH 7.5 phosphate buffer with 1% SLS as blank. Studies were repeated thrice, and results are described in mean ± SD.

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2.1.3 Permeation Data Analysis

The permeation profiles were constructed by plotting the cumulative amount of amlodipine permeated per unit rat skin area ($\mu\text{g}/\text{cm}^2$) versus time. Linear regression analysis was used to calculate the steady state flux (J_{ss} , $\mu\text{g}/\text{cm}^2/\text{hr}$) of amlodipine by using the slope of the plot. The following equation was used to determine the permeability co-efficient (K_p) of the drug through the stratum corneum:

$$K_p = \frac{J_{ss}}{C}$$

Where, C is the initial concentration of the drug in the donor compartment. The penetration enhancing effect was calculated in terms of enhancement ratio (Er) by using the following equation:

$$Er = \frac{j_{ss} \text{ of nan oemulsion gel formulation}}{j_{ss} \text{ of control formulaiton}}$$

2.1.4 In vivo studies of amlodipine nano emulsion gel

The studies were performed as per the guidelines of the institutional animal ethics committee. The rats were deprived of food but had free access to water 24 h before the day of the experiment. Two groups of rats were used for the experiments. Each group was either administered amlodipine normal gel (control group) or amlodipine nano emulsion gel topically over a surface area of 4sq.cm after removal of superficial hair using a depilatory. Under ether anesthesia, blood samples (0.5 mL) were collected via the retro-orbital vein at 2, 4, 6, 8, 10, 12 hours after topical administration into heparinized microcentrifuge tubes. The samples were centrifuged at 15,000 rpm for 10 min at 4°C temperature. The plasma samples (100 μL) were separated, and 1ml of acetonitrile was added to each of the plasma samples to precipitate the protein. The samples were then centrifuged again at 15,000 rpm, 4°C for 5 min, and the supernatant (20 μL) was directly injected onto the HPLC (Waters.) Chromatographic column C8 (150 cm and 4.6 mm id.) with a 5 μm particle size was used Acetonitrile and methanol (55:45) were utilized as a mobile phase at a flow rate of 1.0 ml/min with total run time of 10 min. Data from these samples were used to plot curves for amlodipine absorption with time.

2.1.5 Evaluation of anti-inflammatory activity

The anti-inflammatory activity of prepared ibuprofen SEDDS was evaluated by the *carrageenan*-induced rat hind paw edema method. The experimental protocol was designed and approved by the Institutional Animal Ethics Committee (IAEC) was obtained. Wistar strain male albino rats weighing between (150-200 g) were used. The animals were in a light-controlled 12-hour cycle with free access to food and water. Animals were fasted overnight before experiment with free access to water. Anti-inflammatory activity of the ibuprofen SEDDS was compared to the marketed product. Animals were divided into three groups of six animals each. Group I (control) received normal saline. Group II

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received marketed product of ibuprofen and Group III received ibuprofen SEDDS. Paw edema was induced by injecting 50 μ l of 1% w/v carrageenan into the sub plantar region of the left hind paw. Paw volume was determined at different time intervals in all groups. The difference in the paw volume, determined before and after injection of the edema-provoking agent indicated the severity of the edema. Volumes of right hind paw of controls and treated animals were measured

with a plethysmometer and the percentage inhibition of inflammatory reaction was determined for each animal by comparison with control and calculated by the following formula.

$$\% \text{ Inhibition of edema} = \frac{(V_{\text{control}} - V_{\text{test}}) \times 100}{V_{\text{control}}}$$

where, V_{control} = mean edema of rats in control group.

V_{test} = mean edema volume of rats in tested group.



Figure 1. Male Albino Rats Figure 2. Carrageenan Injection

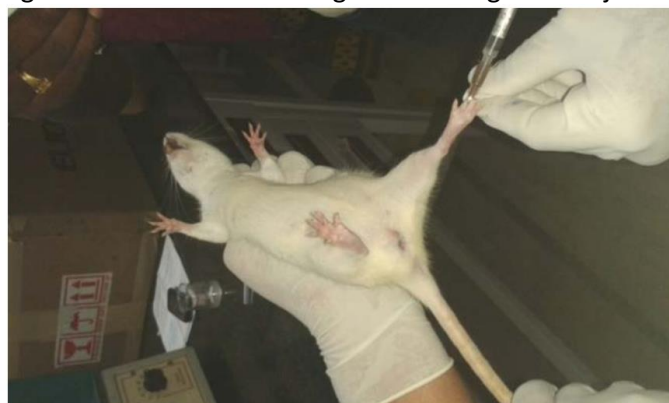


Figure 3. Carrageenan Injection

3. RESULT AND DISCUSSION

3.1 Ex-vivo skin permeation study

Ex-vivo skin permeation study was conducted on the excised abdominal skin of male Wistar rat for 12 hours.

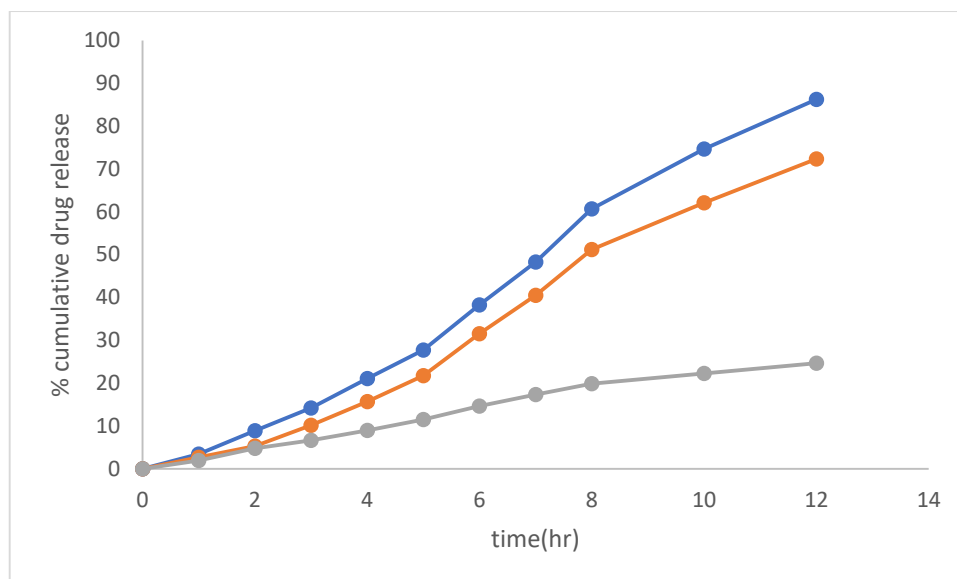


Figure 4. Ex-vivo Permeation Studies

Ex-vivo skin permeation studies were performed to compare the release rate of the drug

from the various nano emulsion formulations (amlodipine AM, NE5 and NEG5) all having the same quantity of amlodipine (20 mg). The release rate of NE5 (86.22%) was found to be more as compared to NEG5 (72.35%) and TG (12.31%). Here it was observed that the release of NE5 is slightly more than NEG because the gel formulation provides a higher diffusional resistance for drug release. The comparison between NE5, NEG and AM showed that even though the release rate of

NEG was less than NE5, it was significantly more than that of AM.

3.2 Permeation Data Analysis

The permeability parameters like steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (Er), were significantly increased in nano emulsions NE5 and the NEG formulation as compared with amlodipine gel (AM). The flux value was found to be 118.8±6.83 µg/cm²/h of normal gel (AM) in comparison to NE5 1393.2±7.14 µg/cm²/h and NEG1154.3±5.35 µg/cm²/h. Permeability coefficient (Kp) and Enhancement ratio (Er) of NE5 and NEG are described.

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Formulation Code	Jss ± SD* (µg/cm ² /h)	Kp ± SD* (cm/h)*10 ⁻²	Er*
TG	118.84 ± 6.83	0.594 ± 0.28	-
NE5	1393.2 ± 7.14	6.97 ± 0.21	11.73
NEG	1154.3 ± 5.35	5.77 ± 0.27	9.71

Table 1. Permeation Analysis Data

This result indicates higher permeability of drug through the skin because of the presence of nanocarriers in the formulations. The higher value of flux in NE5 compared to NEG2 indicates formulation provides prolonged drug release behavior as compared to nano emulsion. Moreover, this can be thought that

NE5 and NEG2 excipients contain permeation enhancers like Acrysol EL 135 and Carbitol, which was also responsible for the increased permeation ability in comparison to the normal gel.



3.3 Anti Inflammatory Activity of ibuprofen SEDDS

The study was done after induction of edema by *carrageenan* sp. Paw volume and percentage decrease in paw edema was

compared between marketed tablet of ibuprofen (control) and ibuprofen SEDDS (test) group. Paw volume in control, standard and test groups.

Formulation name	1 Hour	2 Hours	3 Hours	4 Hours
Normal saline (Control) (n=6)	0.32±0.005	0.35±0.016	0.35±0.016	0.53±0.021
Ibuprofen Tablet (std) (n=6)	0.26±0.005	0.24±0.006	0.23±0.003	0.18±0.01
Ibuprofen SEDDS (test) (n=6)	0.22±0.011	0.20±0.006	0.18±0.005	0.15±0.017

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Table 2. Paw Volume After Induction

Formulation	1 Hour	2 Hours	3 Hours	4 Hours
Ibuprofen tablet (Standard)	18.75	31.42	45.25	66.03
Ibuprofen SEDDS (Test)	31.25	42.85	57.14	71.69

Table 3. Percentage Inhibition of paw edema

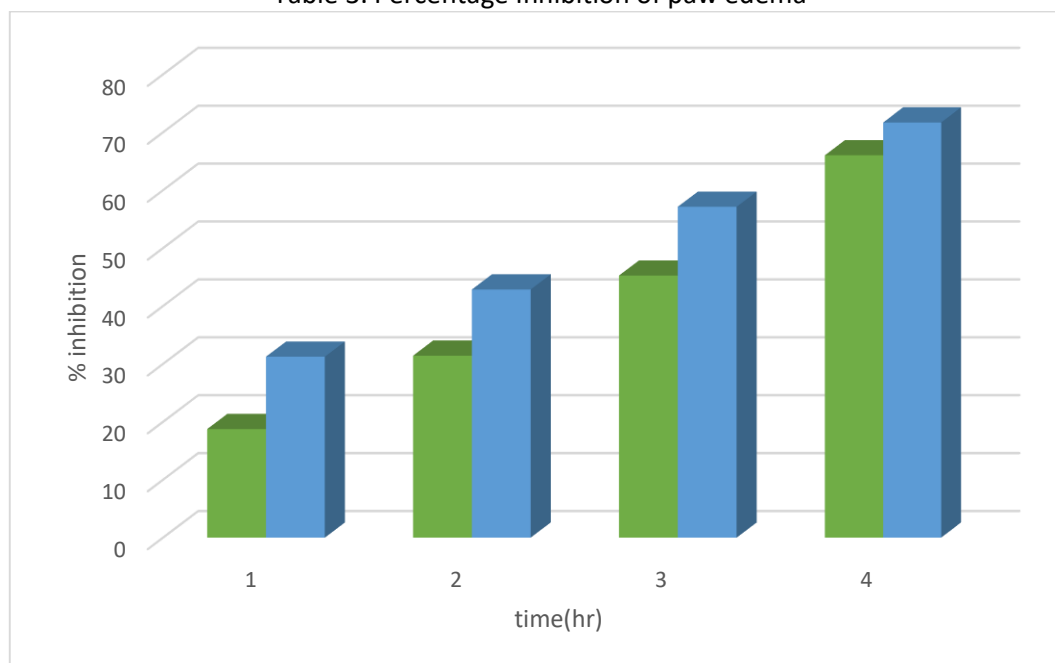


Figure 5. Comparison Of Percentage Decrease in Paw Edema

The optimized test formulation indicated a statistically significant decrease ($p < 0.05$) in paw volume. Ibuprofen SEDDS showed 71.69% inhibition in paw edema at 4hours

as compared to the standard which exhibited 66.03% inhibition at the same.

4. SUMMARY & CONCLUSION



Self-emulsifying drug delivery systems (SEDDS) are a promising approach for the formulation of drug compounds with poor aqueous solubility. In the present work SEDDS were formulated to improve the bioavailability of BCS class-II drugs. The model drugs chosen for the study were an antifungal drug itraconazole, an anti-hypertensive drug amlodipine, an anti-inflammatory drug ibuprofen. *in-vivo* which justifies the nano emulsion gel to be a promising carrier for transdermal delivery of amlodipine. Anti-inflammatory studies indicated that the test formulation showed significant inhibition in paw edema compared to the standard formulation.

With future development of this technology, SEDDSs will continue to enable novel applications in drug delivery, and solve problems associated with the delivery of poorly soluble drugs.

5. REFERENCES

- 1) Bachynsky MO, Shah NH, Patel CI, Malick AW. Factor affecting the efficiency of self-emulsifying oral delivery system. *Drug Dev Ind Pharm* 1997; 23: 809-16.
- 2) Zhang P, Liu Y, Feng N, Xu J. Preparation and evaluation of self microemulsifying drug delivery system of oridonin. *Int J Pharm* 2008; 221: 375-82.
- 3) Pongcharoenkiat N, Narsimhan G, Lyons RT, Hem SL. The effect of surface charge and partition coefficient on the chemical stability of solutes in O/W emulsion. *J Pharm Sci* 2002;91: 559-70.
- 4) Negi LM, Tariq M, Talegaonkar S. Nano scale self-emulsifying oil based carrier system for improved oral bioavailability of camptothecin derivative by P-Glycoprotein modulation. *Colloids Surf B Biointerfaces* 2013; 111(1):346-53.
- 5) Patel PV, Patel HK, Panchal SS, Mehta TA. Self micro-emulsifying drug delivery system of tacrolimus: Formulation, in vitro evaluation and stability studies. *Int J Pharm Investig* 2013; 3(2): 95-104.
- 6) Kang BK, Lee JS, Chon SK, Jeong SY, YuK SH, Kahng G, Lee HB, Cho SH. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *Int J Pharm* 2004; 274: 65-73.
- 7) ICH, Q1A, Stability testing of new drug substances and product in proceedings of international conferences on harmonization, Geneva, October 1993.
- 8) Chambin O, Jannin V, Champion D, Chevalier C, Rochat-Gonthier MH, Pourcelot Y. Influence of cryogenic grinding on properties of self-emulsifying formulation. *Int J Pharm* 2004; 278: 79-89.
- 9) Rahman MA, Hussain A, Hussain MS. Role of excipients in successful development of self-emulsifying/microemulsifying drug delivery system (SMEDDS/SMEDDS). *Drug Dev Ind Pharm* 2013; 39:1-19.
- 10) Craig DQ, Barker SA, Banning D, Booth SW. An investigation into mechanism of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. *Int J Pharm* 1995; 114:103-10.
- 11) Baboota S, Shakeel F, Ahuja A, Ali J, Shafiq S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. *Acta Pharm* 2007; 57:315-32.
- 12) Bajaj A, Rao MR, Khole I, Munjapara G (2013) Self-nano emulsifying drug delivery system of cefpodoxime proxetil containing tocopherol and polyethylene glycol succinate. *Drug Dev Ind Pharm* 2013; 39:635-45.
- 13) Elnaggar YS, El-Massik MA, Abdallah OY. Self-nanoemulsifying drug delivery system of tamoxifen citrate: design



- and optimization. Int J Pharm 2009; 380:133-41.
- 14) Seo YG, Kim DH, Ramasamy T, Kim JH, Marasini N, Oh YK, Kim DW, Kim JK, Yong CS, Kim JO, Choi HG. Development of docetaxel-loaded solid selfnanoemulsifying drug delivery system (SNEDDS) for enhanced chemotherapeutic effect. Int J Pharm 2013; 452: 412-20.
- 15) Wei W, Yang W, Li Q. Enhanced bioavailability of silymarin by selfmicroemulsifying drug delivery system. Euro J Pharm Biopharm 2006; 63 (3): 288-94.
- 16) Tiwari G, Rai A, Tiwari R. Self-emulsifying drug delivery system: an approach to enhance solubility. Syst Rev Pharm 2010;1(2):133-40.