International Journal of Research in Pharmacy and Science

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Research Article

Formulation and evaluation of self emulsifying drug delivery system (SEDDS) of Indomethacin

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Received: 21-05-2014
Review completed: 31-05-2014
Accepted: 05-06-2014

Access this article online
QR Code
Website: www.ijrpsonline.com

ABSTRACT

Aim of present study was to develop a stable formulation for self emulsifying drug delivery systems (SEDDS) in order to enhance the solubility, release rate, and oral absorption of the poorly soluble drug, indomethacin. Based on the solubility of indomethacin in oil, surfactant and cosurfactant, pseudo-ternary phase diagrams were developed for SEDDS composed of labrafil, cremophor EL and transcutol P. Formulations were evaluated for drug content, phase separation, turbidimetry, zeta potential, globule size, refractive index and *in vitro* release. All formulations showed globule size in nanometric range, good stability with no phase separation, and rapidly formed emulsion which was clear. All formulations showed more than 90% of drug release at the end of 60 min. The SEDDS showed improved dissolution rate compared to Indocin (marketed product). Anti-inflammatory studies were conducted in Wistar strain male albino rats and indomethacin SEDDS showed more significant activity than the marketed product. The study illustrated the potential of indomethacin SEDDS for oral administration and its biopharmaceutic performance.

Key words : *SEDDS, Indomethacin, Cremophor EL, Anti-inflammatory studies, Turbidimetry, Transcutol P, Paw edema.*

INTRODUCTION

Most of the new drug entities are highly lipophilic and aqueous solubility of such drugs is a challenge in the pharmaceutical industry. The oral route for such drugs is associated with low bioavailability and high intra and inters subject variability[1]. Lipid based formulations, like oils, surfactants, emulsions, liposomes, self emulsifying oily preparations etc., are receiving attention for delivery of lipophilic moieties[2]. The exceipients which are pharmaceutically acceptable and applicable in the formulation of lipid and surfactant based systems is large in number. The bioavailability enhancing properties of lipid and surfactant based systems can be attributed to the ability of the vehicles to keep the compound in solution in the gastrointestinal (GI) tract[3]. Now a day's attention is drawn by self emulsifying oily formulations. Self emulsifying drug delivery systems offer advantages like faster and uniform distribution of drug, enhanced oral bioavailability, protection of drug in gut, lowering of toxicity, minimizing irritation[4,] SEDDS are isotropic mixtures of oil, surfactant and co surfactant/co solvent with drug. These form fine oil-in-water (o/w) emulsions under gentle agitation followed by aqueous dilution (i.e., the digestive motility of the GIT provide agitation required for self-emulsification *in vivo*). So drug will be in solubilised form in the GIT and with a large interfacial surface area for absorption[6]. Indomethacin a widely used NSAID having poor aqueous solubility and high permeability[7]. It is used to reduce pain/swelling involved in osteoarthritis, rheumatoid arthritis, bursitis, tendinitis, gout. Due to its poor aqueous solubility and dissolution rate limited oral absorption leads to a potential bioequivalence problem[8]. Thus, the improvement of indomethacin dissolution for its immediate release is desirable for rapid indomethacin absorption, which is prerequisite for quick onset of its pharmacological actions. The present study is to formulate indomethacin in a SEDDS to increase its solubility in water and hence improving its dissolution rate which in turn may enhance indomethacin oral bioavailability.

MATERIALS & METHODS

Indomethacin was obtained as a gift sample from micro labs, Bangalore. Cremophor EL, Labrafil, labrafac, transcutol P was obtained from Gattefosse, Mumbai, Soya bean oil was obtained from Fluka (UK) and Tween 80, PG, PEG 400, glycerol, ethyl oleate were purchased from Loba Chemie, Mumbai. All other reagents and chemicals used were of analytical grade.

SOLUBILITY STUDIES

The solubility of indomethacin was determined in various oils (soya bean oil, ethyl oleate, labrafil, labrafac), surfactants (cremophor EL, tween 80), co surfactants (transcutol P, propylene glycol, polyethylene glycol 400, glycerol). Excess Indomethacin was added to 2 ml of the selected vehicles taken in screw capped vials and the mixtures were kept at room temperature for 4 hr to reach equilibrium[9]. Each vial was then centrifuged at 1000 rpm for 10 min to separate undissolved drug. Aliquots of supernatant were diluted and the concentration of indomethacin was quantified by UV spectroscopy at 310 nm[10].

CONSTRUCTION OF PSEUDO TERNARY PHASE DIAGRAM[11]

The Pseudo ternary phase diagrams were constructed using different ratios of surfactant & co surfactant, oil (Labrafil/Labrafac) and water, by water titration method (percent by weight). The mixtures were diluted with water by drop wise addition under gentle stirring to examine self emulsification region, which was identified by visual clarity and were marked as points over the phase diagram. The area under these points indicates self emulsification region and used for further development of formulations.

PREPARATION OF SEDDS

Various self emulsifying systems were formulated using cremophor EL, transcutol P as surfactant and co-surfactant with labrafil as oil in different ratios, keeping the concentration of indomethacin (25 mg) constant. Indomethacin was dissolved in the transcutol P to which cremophor EL and labrafil were added slowly with constant stirring maintaining the temperature at 40ºC, till the drug was dissolved completely[6]**.** The mixtures were cooled to ambient temperature. The self emulsifying systems prepared (containing 25 mg indomethacin) were filled into hard gelatin capsules.

ISOTROPICITY STUDY

The prepared formulations were stored for 72 h at ambient temperature and were observed for phase separation, precipitation and isotropicity. Mixtures exhibiting a negligible phase separation were used for subsequent study[12].

VISUAL OBSERVATION OF SELF EMULSIFICATION EFFICIENCY[13]

A visual test to assess the self emulsification property was performed in this study. Efficiency of self emulsification was assessed using USP XXII dissolution apparatus 2. For this 1ml of each formulation was added to 200 ml water maintained at temperature 37±0.5°C. Gentle agitation was provided by dissolution apparatus paddle rotating at 50 rpm. The tendency to emulsify spontaneously was monitored visually and assessed using grading system as in table 1.

Table No. 1: Visual assessment of efficiency of self emulsification

Grade	Dispersibility and appearance	Time of self emulsification
I	Rapid forming microemulsion which is clear or slightly bluish in appearance	< 1 min
II	Rapid forming, slightly less clear emulsion which has a bluish white appearance	< 2 min
III	Bright white emulsion (similar to milk in appearance)	< 3 min
IV	Dull, greyish white emulsion with a slightly oily appearance that is slow to emulsify	> 3 min
V	Exhibits poor or minimal emulsification with large oil droplets present on the surface	> 3 min

PHASE SEPARATION STUDY

The self emulsifying formulation was diluted with distilled water up to 5 times and the temperature was maintained at 25°C. The mixture was then mixed for 2 min, stored for about 2 hr and visually observed for any phase separation[14].

MEASUREMENT OF DROPLET SIZE AND ZETA POTENTIAL[15, 16]

Droplet size distribution and charge on the SEDDS was determined using a photon correlation spectrometer ((Nicomp 388 ZLS; PSS Nicomp Particle Sizing Systems, USA).) based on the laser light scattering phenomenon. Samples were diluted in the ratio 1:200 with purified water and the measurements were made after 2 min stirring.

PERCENTAGE TRANSMITTANCE & REFRACTIVE INDEX[10, 17]

Percentage transmittance was observed at 310 nm after dilution with distilled water using a UV spectrophotometer (Shimadzu, Japan). The refractive index of the system was measured using Abbe's refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing drop of solution on slide.

DIFFERENTIAL SCANNING CALORIMETRY

The samples (about 3.00 mg) were placed in standard aluminum cups, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5°C /min and the heat flow from 0 to 300°C.

DRUG CONTENT

Indomethacin from pre weighed SEDDS was extracted into 100 ml of Phosphate buffer pH 7.2: water (1:4). The extract was then analyzed after suitable dilutions spectrophotometrically at 320 nm[10].

TURBIDIMETRIC EVALUATION

0.5 ml of self-emulsifying system was added to 150 ml of 0.1M hydrochloric acid under continuous stirring at 50 rpm on a magnetic stirrer at ambient temperature. The turbidity was measured using turbidimeter **(**Systronics, India**)** until equilibrium is reached. The increase in turbidity was measured until there was no further change in turbidity[18, 19].

VISCOSITY MEASUREMENT[20]

1 ml of self emulsifying formulation was diluted 10 to 100 times with distilled water with constant stirring on magnetic stirrer. Viscosity of the initial emulsion and diluted formulations were then determined using Brookfield LVDL 111 + CP viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA, spindle # CPE40) at 5 rpm at 25±1.0°C.

***IN VITRO* RELEASE STUDIES**[10]

The release of indomethacin from the SEDDS formulation was determined according to USP dissolution apparatus type-II. To permit the quantitative drug release from SEDDS formulation, 750 ml of 7.2 pH phosphate buffer and water in 1:4 ratio. The SEDDS formulation filled in hard gelatin capsule was placed in the dissolution medium and was agitated at 100 rpm at 37 ± 0.5^0C. At predetermined time intervals, 10 ml of the samples were withdrawn at 10, 20, 30, 40, 50, 60 min and samples were filtered, and the drug concentration was determined at 320 nm. The volume removed was replaced each time with fresh dissolution medium to maintain sink conditions. The drug content of the samples was assayed using UV visible spectrophotometric method. All measurements were done in triplicate.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of prepared indomethacin SEDDS was evaluated by the carrageenan-induced rat hind paw edema method[21]. The experimental protocol was designed and approval of Institutional Animal Ethics Committee (IAEC) (Reg. No. 1434/PO/a/11/CPCSEA/09-04-2013) was obtained. Wistar strain male albino rats weighing between (150-200 g) were used. The animals were in a light controlled 12 hours cycle with free access to food and water. Animals were fasted overnight before experiment with free access to water[22]. Anti-inflammatory activity of the optimized SEDDS was compared to the marketed product. Animals were divided into three groups of six animals each. Group I (control I) received water. Group II, received 10 mg/kg indomethacin SEDDS and Group III received 10 mg/kg Indocin (marketed product). After one hour, paw edema was induced by injecting 50 µl of 1% w/v carrageenan into the sub planar region of the left hind paw. Paw volume was determined after five hour in all groups. Difference in the paw volume, determined before and after injection of the edema-provoking agent indicated the severity of 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.

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

Where, $V_{control}$ = mean edema of rats in control group; V_{test} = mean edema volume of rats in tested group.

RESULTS & DISCUSSION

Solubility of indomethacin in various vehicles is an important parameter as the SEDDS should be clear and monophasic. The solubility of indomethacin in various oils and co surfactants are presented in table 2. Labrafil and labrafac showed good solubility and were selected as oils, cremophor EL as surfactant and transcutol P as co-surfactant.

Table No. 2: Solubility data of indomethacin in various oils & surfactants

Vehicle	Solubility (mg/ml)
Soyabean oil	6.71±1.61
Ethyl oleate	5.36±1.39
Labrafil	20.22±1.05
Labrafac	17.48±1.13
Cremophor EL	20.17±2.31
Tween 80	15.46±2.04
Transcutol P	115.34±1.51
Propyleneglycol	35.15±3.22
Polyetheleneglycol 400	26.43±2.36
Glycerol	94.23±2.19

Mean ± SD, n = 3

Pseudo ternary phase diagrams were constructed in the presence of indomethacin to identify the self emulsification region and to optimize the ratio of excipients used. SEDDS formulations are thermodynamically spontaneous and are formed with only mild agitation on addition into water. The size of self emulsification region was compared in the ternary diagrams, larger the region greater is their emulsification ability. The mixtures of cremophor EL: transcutol P (1:1, 1:2, 2:1, 1:3, 3:1, 4:1) with labrafil showed efficient self emulsification region and were used for further study.

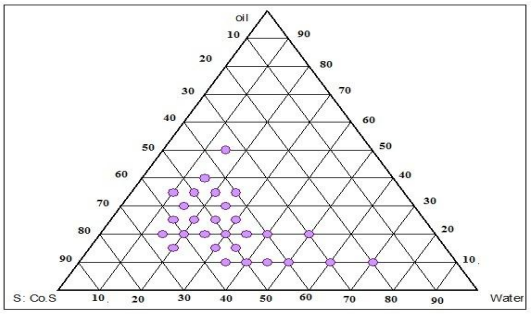

Figure No. 1: Pseudo-Ternary Phase Diagram of indomethacin

The prepared self emulsifying formulations with cremophor EL: transcutol P in 3:1 and 4:1 showed precipitation and a decreased clarity after 72 h this can be due to increased surfactant concentration leading to disruption of the interfacial barrier. From the solubility, phase diagram (figure 1) and isotropicity studies the formulations selected were shown in table 3.

Table No. 3: Composition of indomethacin formulations

Ingredients	F1	F2	F3	F4
Indomethacin	25 mg	25 mg	25 mg	25 mg
Cremophor EL (w/w)	39%	26%	52%	19.5%
Transcutol P (w/w)	39%	52%	26%	58.5%
Labrafil (w/w)	100% qs	100% qs	100% qs	100% qs

Self emulsification efficiency was observed visually by dispersibility and time of self emulsification. The grading of the selected formulations was given in table 4. The phase separation was found negligible with the mixture of indomethacin, cremophor EL, transcutol P, labrafil. So the formulations were further analysed. The average particle size and zeta values (figure 2 & 3) of the formulations were measured and tabulated in table 4. It was observed droplet size increased with increase in cremophor EL ratio to transcutol P. Addition of surfactant stabilizes the interfacial film while co surfactant causes it to expand, so ratio of surfactant to co surfactant showed varied effects on droplet sizes. F3 showed larger sized droplets which may be due to higher concentration of surfactant (more than 50%). The charge on droplets showed good stability of the formed emulsions and of the four formulations F2 and F4 showed greater stability.

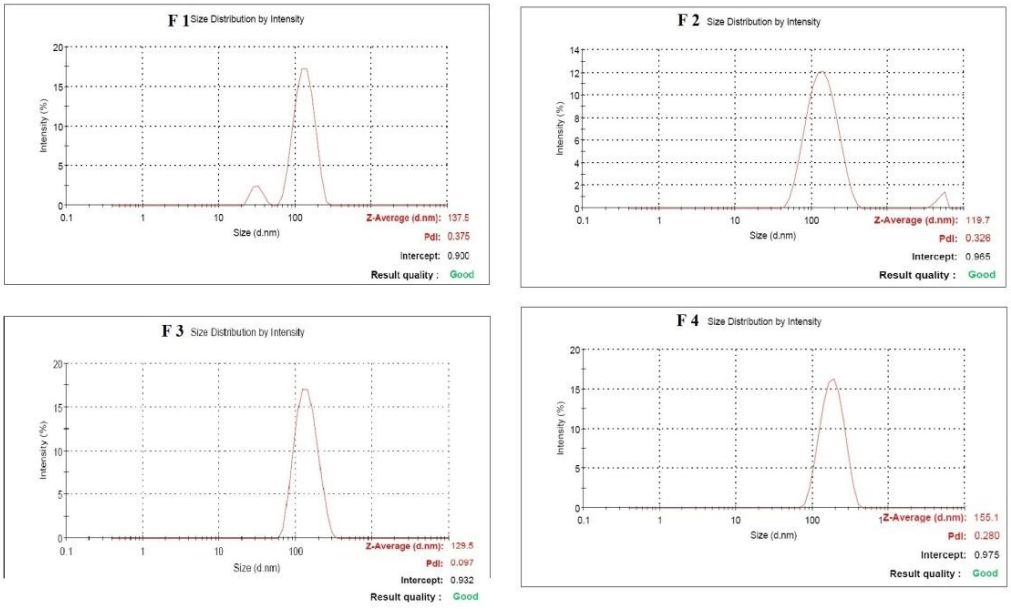

Figure No. 2: Globule size distribution of F1, F2, F3 & F4

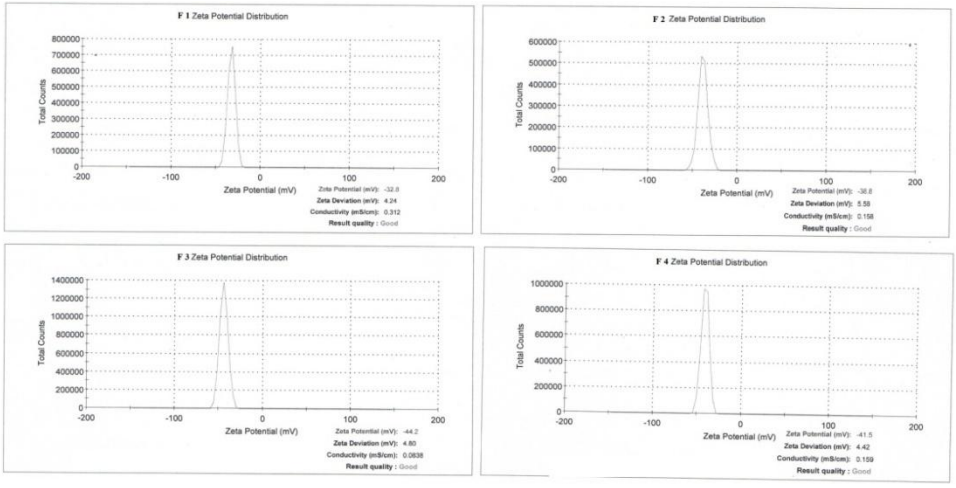

Figure No. 3: Zeta potential of F1, F2, F3 & F4

Developed formulations showed >99% transmittance and the refractive index was similar to water (1.333) which prove the transparency of the prepared systems table 5. Drug content of formulations was within the specified limit and was given in table 5. Formulation 1 and 2 does not show any detectable values which can be due to formation of very fine droplets with the increase in surfactant concentration. F3 and F4 showed low turbidity values of 15.22 and 10.38 NTU respectively.

Table No. 4: Globule size, zeta potential, poly dispensability index of formulations

Form-ulation	Droplet size (nm)	Zeta potential	Poly dispersibilty index	Visual grading of dispens - ability
F1	120.23	-28.32	0.273	II
F2	131.11	-36.11	0.257	II
F3	151.71	-42.37	0.269	II
F4	172.31	-38.16	0.285	II

Table No. 5: Viscosity, refractive index, % transmission of formulations

Form-ulation	Viscosity (cps)	Refractive index	Percentage Transmission	Drug content
F1	23.24±0.91	1.334	100.9±2.01	100.6±0.06
F2	25.72±1.07	1.334	99.6±1.21	101.3±0.04
F3	27.75±1.28	1.331	100.6±0.34	99.9±0.10
F4	28.65±1.37	1.335	99.8±1.32	99.6±0.09

Mean ± S.D, n=3

Viscosity of initial undiluted emulsion was high making it suitable for filling into capsules. Diluted emulsions showed lesser viscosity and hence can be expected to show better release and absorption in the gastrointestinal tract. Results are indicated in table 5.

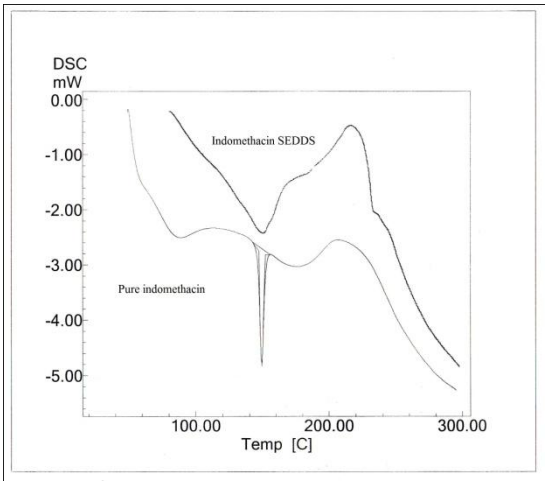

Figure No. 4: DSC thermograms of indomethacin and indomethacin SEDDS

DSC thermograms of pure drug and physical mixture are shown in figure 4. Pure drug show sharp endothermic peak at 155.3ºC, that corresponds to the melting point of indomethacin. The physical mixture didn't show sharp endothermic peak for indomethacin, as drug is in molecularly dissolved state in the lipid excipients. Drugs of class II (BCS classification) exhibit poor solubility and hence may cause low oral bioavailability. Dissolution studies were compared for the selected 4 formulations with the marketed product of indomethacin (Indocin capsule 25 mg). All formulations showed above 95% of drug release in 60 min (figure 5). F1, F2, F3, F4 and Indocin showed 99.12, 99.77, 98.12, 98.86 and 98.79% of drug release respectively. F2 showed better release compared to other formulations and the marketed formulation. Release was slower in F3 may be due to higher surfactant concentration while in F4 cremophor concentration was too less which might have effect on emulsification and hence drug release. F2 showed the maximum release hence it's selected for anti-inflammatory activity.

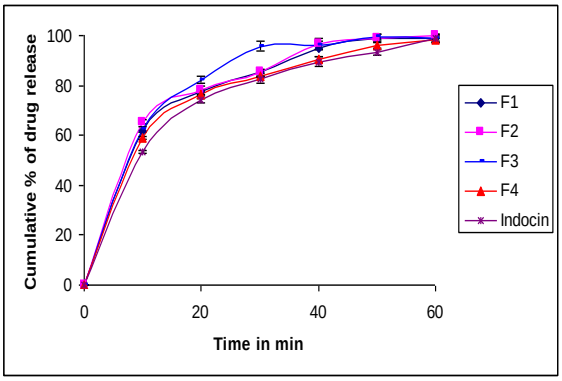

Figure No. 5: *In vitro* drug release profile of Formulations

Table No. 6: Anti-inflammatory activity of ibuprofen SEDDS and marketed product.

Group	Percentage inhibition of edema at various time intervals				
	1h	2 h	3 h	4 h	5 h
II (F2 treated)	61.28±1.93	68.91±1.37	75.18±3.61	83.43 ±2.72	86.14 ±4.48
III (Indocin treated)	55.28±3.1	61.94±3.28	70.87±3.17	79.34 ±4.71	83.07 ±4.36

Mean ± S.D, n=6

Table 6 shows the results of percentage inhibition of carrageenan-induced paw edema in rats treated with Indocin and F1. Significant ($p<0.05$) inhibition of carrageenan induced paw edema was observed in animals treated with F1 in comparison with Indocin during the entire 5 h duration of the study. This may be due to increased permeation (solubilised indomethacin in lipids) of indomethacin from F1 over Indocin, leading to better absorption and onset of action of drug. Hence, SEDDS

showed better anti-inflammatory activity over the marketed product. Therefore, the results of the *in vivo* studies clearly demonstrate that the SEDDS showed better anti-inflammatory activity over the marketed product, thus confirming the better therapeutic efficacy of the SEDDS.

CONCLUSION

Self-emulsifying drug delivery systems containing indomethacin were formulated using various ratios of oil, surfactant and co surfactant mixture in an attempt to increase its release rate and bioavailability. SEDDS of indomethacin showed improved dissolution rate and absorption. Indomethacin SEDDS showed more significant anti-inflammatory activity than the Indocin (marketed product). The present study demonstrated successful preparation of self-emulsifying drug delivery systems of indomethacin.

ACKNOWLEDGEMENT

The authors are thankful to M/S Microlabs, Bengaluru, India for the gift sample of indomethacin. We also thank the Quality Assurance Deputy Manager, Macleods Pharmaceuticals, Vapi, Gujarat for Zeta Potential determination.

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