Development of Irinotecan Dissolution Rate by Snedds

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ABSTRACT

Introduction: Irinotecan being anti-neoplastic agent belongs to BCS class II drug with low solubility and permeability that undergoes first-pass metabolism, leading to reduced bioavailability of 9%.

Aim: The main objective of this study is to develop irinotecan supersaturable-self nano emulsifying drug delivery system (S-SNEDDS) for enhancing solubility and oral bioavailability.

Methodology: An oil, surfactant and co-surfactant (canola oil – caproic acid – propylene glycol) are chosen based on the maximum solubility of irinotecan and ratios optimised by constructing pseudo ternary phase diagram and evaluated. The best formulation was chosen for screening of precipitation inhibitor (PI), and based on in-vitro release studies best S-SNEDDS is finalised. Final optimised formulation was characterised for FTIR, particle size, zeta potential, SEM and stability studies.

Results: From evaluation studies of irinotecan, formulation F12 displayed maximum drug content(99%), maximum entrapment efficiency(99%) and maximum drug release of 99.96% in 60 min, hence chosen for screening precipitation inhibitor(PI). The F12 containing 2% HPMC AS as PI was found to show high release profile. The particle size for the optimized formulation of S-SNEDDS (F12) was found to be 128.23nm with PDI 0.137 and the zeta potential value of -23.45 mV. The FTIR and SEM studies did not indicate any drug excipient interaction and confirm nanosized particles that are stable.

Conclusion: Hence the results reveal that, application of S-SNEDDS formulation technique for irinotecan increased solubility and oral bioavailability.

Key Words: Irinotecan, S-SNEDDS, precipitation inhibitor, Solubility, Particle size, Zeta potential

INTRODUCTION

Nanotechnology is a rapidly emerging scientific field that is defined as the production of devices with atomic or molecular scale precision, but it also includes all devices with size less than 100 nm. Since their introduction, nanoparticles (NPs) have been proposed as drug carriers, most notably due to their stability-related aspects, their ability to load drugs efficiently, and their control over physiochemical properties. Furthermore, localized drug delivery may be achieved by using macroscopic drug depots close to the target site in addition to systemic administration.¹

The chemical structure of irinotecan is equivalent to the natural alkaloid camptothecin. Irinotecan is a chemotherapy agent that is a topoisomerase inhibitor. Patients with metastatic carcinomas of the colon or rectum who have failed to respond to initial fluorouracil-based antineoplastic therapy can receive this drug as a single agent.

It has poor aqueous solubility (0.107 mg/ml) in water at 25°C and it is classified under to BCS class II. To overcome these inherent drawbacks an attempt will be made to formulate self nano-emulsifying drug delivery system (SNEDDS).²,³,⁴,⁵

The present study is pointed to develop canola oil-based irinotecan S-SNEDDS for increasing solubility and thereby release characteristics.
Shekhar et al: Development of irinotecan dissolution rate by snedds

MATERIALS AND METHODS

Materials
Irinotecan is a gift sample from Hetero Labs Limited, Hyderabad, India. The oils used were purchased from local market. Surfactants and cosurfactants purchased from Gattefosse, Mumbai.

Methods

Solubility of irinotecan in vehicles
An excess amount of irinotecan mixed with 1 g of vehicle in glass vials and vortexed for 10 min followed by shaking reciprocally for 48 h at 25°C. The contents and allowed to stand undisturbed for 24 h at 25°C. The contents centrifuged at 3000 rpm for 10 min, supernatant filtered, diluted with methanol and analysed for irinotecan spectrophotometrically at 365 nm.

Construction of pseudo-ternary phase diagrams
The chosen vehicles were mixed in various ratios ranging from 1:9 to 9:1 (oil: S\text{mix}). S\text{mix} is the mixture of surfactant and co-surfactant prepared in defined ratios of 1:1, 2:1, and 3:1. Phase diagrams of surfactant, co-surfactant, and oil were plotted, with each representing an apex of the triangle. Varying ratios of oil:S\text{mix} were mixed with 100 ml of water and ratios with no phase separation and turbidity were checked for the transmittance (The samples with transmittance > 90 were used for plotting pseudo-ternary phase diagram using CHEMIX software).

Effect of irinotecan loading
Twenty compositions of varying ratios of canola oil – caprylic acid – propylene glycol were taken and in 1ml composition of each ratio were incorporated with 25 mg and 50 mg of irinotecan. Contents vortexed at 40°C and transmission determined at 365 nm. The area of nano emulsification region was identified as described above by constructing ternary phase diagrams.

Preparation and evaluation of irinotecan SNEDDS
A series of SNEDDS (F1- F16) were selected from 25 mg loaded irinotecan system and prepared as described above. About 1 ml of the formulation (equivalent to 25 mg of the irinotecan) was filled in size ‘00’ hard gelatin capsules, sealed and stored at ambient temperature (25°C) and evaluated.

EVALUATIONS OF SNEDDS

Visual observations
To assess the self-emulsification properties, irinotecan SNEDDS (25 mg) was introduced into 50 mL of distilled water in a glass Erlenmeyer flask at 37°C and the contents were gently stirred manually. After equilibrium, time of self-emulsification, dispersibility and appearance were observed and rated according to grading system.

Turbidity measurement
Turbidity of the prepared dispersions was measured using Nephelo Turbidity Meter using 30 mL of the dispersion.

Robustness to dilution
Robustness of irinotecan SNEDDS to dilution was studied by diluting 25 mg of SNEDDS with 50, 100 and 1000 mL of distilled water, 0.1N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer. The diluted nanoemulsions were stored for 24 h and observed for any signs of phase separation or drug precipitation.

Percentage drug content
Individually weighed samples were dissolved in 10mL of methanol using a vortex mixer and agitated for 10 minutes. Each of the solutions was filtered, and the drug content of each filtrate was estimated UV spectrophotometrically against blank at 365 nm.

Entrapment efficiency
A known quantity of SNEDDS mixed with 100 mL phosphate buffer (pH 7.4) and kept in dark for 24 h. The contents filtered, filtrate diluted and analysed for drug content by UV for the drug content at entrapment efficiency was calculated by formula

\[
\text{Drug entrapment efficiency} \times 100 = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}}
\]

In vitro dissolution study
In vitro dissolution studies performed using USP dissolution Apparatus II (Lab india DS 8000, Mumbai, India). Hard gelatin capsules, size “1” filled with SNEDDS formulation were mixed with 900 mL of freshly prepared pH 7.4 phosphate buffer maintained at 37 ± 0.5°C and centrifuged at 100 rpm. At pre-determined time intervals, 5 mL of samples were withdrawn and contents replaced with 5mL of fresh medium. The samples evaluated spectrophotometrically at 365 nm.

Screening for a precipitation inhibitor (PI)
In vitro precipitation experiments were used to estimate the apparent drug concentration-time profile and the duration of the super- saturated state. Polymers such as microcrystalline cellulose, HPMC E50LV, HPMC AS and Poloxamer 407 were employed to stabilize the supersaturated irinotecan solution.
CHARACTERIZATION OF OPTIMISED IRINOTECAN SNEDDS
The samples were analyzed by FT-IR spectrophotometer with data acquisition system OPUS. The globule size and zeta potential determined by a Zetasizer Nano ZS90 dynamic light scattering particle size analyzer (Malvern Instruments, Malvern, Worcestershire, UK). The Scanning electron microscopy (SEM) studies carried out using JEOL JEM 2100 F, USA equipment.

Forced degradation and accelerated stability studies
All solutions for use in forced degradation studies were prepared by dissolving samples methanol and diluted with the respective forced degradation medium (table 3). The samples analysed at various time intervals spectrophotometrically.

Accelerated stability studies
All formulations filled in hard gelatin capsules were packed in HDPE screw capped bottles and kept in humidity chambers maintained at 40 ± 2°C/75 ± 5% RH as per ICH guidelines for Zone III and stored for 6 months.

RESULTS AND DISCUSSION
Determination of irinotecan solubility in various excipients
Canola oil was selected as oil phase due to its higher solubilization (19.54±0.26mg/ml) of irinotecan compared to other oils (figure 1). Surfactant (caproic acid) and co-surfactant (propylene glycol) was selected for further studies due to their higher solubilizing capacity towards irinotecan (figure 2,3).

Construction of ternary phase diagrams
The region of nano emulsification was indicated as shadow area encircled by a solid line and the points indicate the compositions of the system explored. Canola oil – caproic acid – propylene glycol system with $S_{\text{mix}}$ ratio in 3:1 exhibited larger nanoemulsification region as compared to 1:1 and 2:1 $S_{\text{mix}}$ ratio (figure 4).

Effect of irinotecan loading
The area of nano emulsification was considerably reduced with increase in irinotecan loading in to the canola oil – caproic acid – propylene glycol system with 3:1 $S_{\text{mix}}$ ratio hence for the stability reasons of the SNEDDS, system containing 25 mg of irinotecan was chosen for formulation of irinotecan SNEDDS and further studies. (Figure 5)

From the results it was found that canola oil concentration in the range of 22-75% w/w, caproic acid in the range of 18-59% w/w and propylene glycol in the range of 6-19% w/w in 3:1 of oil: $S_{\text{mix}}$ ratio with 25 mg loaded irinotecan drug produced the SNEDDS having the transmittance > 90, with good stability.

Visual observations
Visual observations indicated that at higher levels of surfactant, the spontaneity of the self-emulsification process was increased. The formulations that have low turbidity (<20) gave a transmittance values of more than 90 indicating rapid and spontaneous emulsification within 1 min, hence it gives a good correlation between transmittance and turbidity values. All the formulations were found robust towards dilution with water, 0.1N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer with drug precipitation within 24 h of storage. The drug content of all formulations ranged between 95.80±1.59 to 99.32±1.62% with maximum value exhibited by F12 (table 4). The entrapment efficiency of all formulations varies between 95.75±1.43 to 99.27±1.95 % with maximum value displayed by F12 (table 2).

In vitro dissolution tests
The formulations F1-F16 released more than 60% of drug within 30 min, whereas, pure drug released 31.92% of drug in 60 min. Formulation F12 exhibited highest drug release of 99.06% in 60 min.

In vitro evaluation of precipitation
In this study, the degree of supersaturation of the S-SNEDDS was determined using microcrystalline cellulose, poloxamer 407, HPMC E50LV and HPMC AS as precipitation inhibitors under non-sink conditions. There was 2% precipitation inhibitor in each formulation relative to the SNEDDS vehicle. Precipitation profiles indicated that the S-SNEDDS had more effective inhibition of irinotecan precipitation than the SNEDDS (same composition but without precipitation inhibitor) during the 60-minute experimental period. The HPMC AS displayed superior inhibition with highest drug concentration (421.61μg/mL after 60 min). In vitro dissolution studies for S-SNEDDS of formulation F12 with 2% HPMC AS precipitation inhibitor was studied. Comparative dissolution profiles of irinotecan pure drug, irinotecan SNEDDS and irinotecan S-SNEDDS which indicates the release of drug from irinotecan S-SNEDDS was highest with 99.96% at the end of 60min. (figure 6)
Drug compatibility study by FTIR
The pure Irinotecan spectrum showed the main characteristic bonds at 692.47 cm⁻¹ (C-F bending) 1039.67 cm⁻¹ (C-O stretching), 1190.12 cm⁻¹ (C-F stretching), 1280.78 cm⁻¹ (C=O stretching), 1448.59 cm⁻¹ (aromatic stretching), 1639.55 cm⁻¹ (amide stretching), 1689.7 cm⁻¹ (C=C stretching), 1745.64 cm⁻¹ (C=O stretching), 3026.41 cm⁻¹ (C-H stretching), 3250.16 cm⁻¹ (O-H stretching), 3319.6 cm⁻¹ (N-H stretching). The presence of prominent characteristic peaks in FTIR of SNEDDS confirming the compatibility between drug and excipient.(figure 7,8)

Globule size and zeta potential
The particle size for the optimized formulation of S-SNEDDS (F12) was found to be 128.23nm with PDI 0.137 and the zeta potential value of -23.45 mV. This might be the result of the addition of precipitation inhibitor HPMC AS (in S-SNEDDS) which formed a physical barrier around oil droplets and prevented them from aggregating into larger nanoemulsions. There is evidence that S-SNEDDS are more stable than plain SNEDDS due to their higher zeta potential. (figure 9A,9B)

SEM studies
The formulation appeared as spherical and smooth-surfaced and analysis of globule size was in accordance with these results with size of all droplets less than 100 nm.

Forced degradation studies
Optimized irinotecan SNEDDS, pure drug showed no degradation even after 24 h storage in methanol, distilled water and pH 7.4 phosphate buffer. Pure drug present in 0.1N HCl solution showed 26.72% degradation within 4 h and the degradation was increased with time (61.82 % degradation was found at 24h). Irinotecan showed very less decomposition (<1% degradation) for up to 4 h and then decomposed with the time in 0.1N HCl solution. Irinotecan showed 12.57, 27.83 and 33.86 % degradation in 6h, 12h and 24h respectively.(table 3)

Accelerated stability studies
No visible physical changes were observed in all the formulations withdrawn from the humidity chambers. The samples were assayed for % entrapment efficiency, % drug content and in-vitro drug release and the results are shown in Table 4.

CONCLUSION
S-SNEDDS of irinotecan comprising of canola oil – caproic acid – propylene glycol system with Sₘᵟₓ ratio in 3:1 and containing 2% HPMC AS as precipitation inhibitor were prepared for enhancing the solubility and dissolution rate of irinotecan. S-SNEDDS were optimized based on the optimum globule size, increased dissolution and drug release. Close to complete drug release was achieved from the formulation F12 S-SNEDDS which was 99.96%, that is significantly higher as compared to pure drug release of 31.92 % in 60 min. The particle size for the optimized formulation of S-SNEDDS (F12) was found to be 128.23 nm with PDI 0.137 and the zeta potential value of -23.45 mV. In the present study, precipitation inhibitor HPMC AS (in S-SNEDDS) was added to prevent the aggregation of oil droplets in order to produce nanoemulsions of smaller size. Thus, the developed irinotecan S-SNEDDS can be used as an effective approach for the management of cancer with relatively low drug dose with enhanced solubility and drug release.

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Conflict of Interest
No conflict of interest

Author Contribution
All the two authors contributed equally towards the data collection, data analysis and compilation.

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Shekhar et al: Development of irinotecan dissolution rate by snedds

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Table 1: Composition of irinotecan SNEDDS

| S. No | Formulation code | Irinotecan drug (mg) | Ratios of Oil: S<sub>mix</sub> | Oil (Canola oil) | Surfactant (Caproic acid) | Co-surfactant (Propylene glycol) |
|-------|------------------|---------------------|-----------------------------|----------------|-------------------------|-------------------------------|
| 1     | F1               | 25                  | 01:01                       | 50             | 37.5                    | 12.5                          |
| 2     | F2               | 25                  | 01:02                       | 33             | 49.5                    | 16.5                          |
| 3     | F3               | 25                  | 01:03                       | 75             | 56.25                   | 18.75                         |
| 4     | F4               | 25                  | 03:01                       | 66             | 24.75                   | 8.25                          |
| 5     | F5               | 25                  | 02:01                       | 40             | 45                      | 15                            |
| 6     | F6               | 25                  | 02:03                       | 72.7           | 20.25                   | 6.75                          |
| 7     | F7               | 25                  | 08:03                       | 70             | 22.5                    | 7.5                           |
| 8     | F8               | 25                  | 07:03                       | 62.5           | 28.12                   | 9.3                           |
| 9     | F9               | 25                  | 05:03                       | 57.1           | 31.95                   | 10.65                         |
| 10    | F10              | 25                  | 04:03                       | 28.5           | 53.25                   | 17.75                         |
| 11    | F11              | 25                  | 02:05                       | 22.2           | 58.2                    | 19.4                          |
| 12    | F12              | 25                  | 02:07                       | 60             | 30                      | 10                            |
| 13    | F13              | 25                  | 03:02                       | 42.6           | 42.6                    | 14.8                          |
| 14    | F14              | 25                  | 03:04                       | 71             | 21.3                    | 7.1                           |
| 15    | F15              | 25                  | 03:07                       | 30             | 52.5                    | 17.5                          |
| 16    | F16              | 25                  | 05:02                       | 71             | 21.3                    | 7.1                           |
Shekhar et al: Development of irinotecan dissolution rate by snedds

Table 2: % drug content and % entrapment efficiency values

| Formulation code | % Drug content (mean ± SD) | % Entrapment Efficiency (mean ± SD) |
|------------------|-----------------------------|------------------------------------|
| F1               | 97.61 ± 1.21                | 97.76 ± 1.43                       |
| F2               | 98.21 ± 1.19                | 98.37 ± 1.51                       |
| F3               | 98.05 ± 1.65                | 98.02 ± 1.69                       |
| F4               | 95.80 ± 1.59                | 95.75 ± 1.43                       |
| F5               | 96.53 ± 1.19                | 96.48 ± 1.22                       |
| F6               | 98.03 ± 1.49                | 98.08 ± 1.67                       |
| F7               | 95.93 ± 1.78                | 95.88 ± 1.53                       |
| F8               | 96.14 ± 1.15                | 96.09 ± 1.79                       |
| F9               | 96.85 ± 1.66                | 96.80 ± 1.73                       |
| F10              | 97.40 ± 1.45                | 97.39 ± 1.35                       |
| F11              | 98.81 ± 1.13                | 98.76 ± 1.39                       |
| F12              | 99.32 ± 1.62                | 99.27 ± 1.95                       |
| F13              | 97.72 ± 1.89                | 97.61 ± 1.84                       |
| F14              | 97.97 ± 1.39                | 97.91 ± 1.70                       |
| F15              | 98.47 ± 0.72                | 98.32 ± 0.54                       |
| F16              | 96.07 ± 1.39                | 95.91 ± 1.70                       |

Table 3: Percent Degradation of irinotecan from Pure Drug and Optimized irinotecan SNEDDS in Forced Degradation Study

| Formulation code | Time (hr) / Diluting Solvent | % Drug Degraded (% mean ± SD, n=3) |
|------------------|------------------------------|-----------------------------------|
|                  | 0 Hour                       | 4th Hour                          | 6th Hour                          | 12th Hour                         | 24th Hour                         |
| Pure drug        | Methanol                     | 0.02 ± 0.26                       | 0.012 ± 1.02                      | 0.03 ± 0.29                       | 0.012 ± 2.02                      | 0.10 ± 1.09                       |
|                  | Water                        | 0.02 ± 1.03                       | 0.01 ± 1.19                       | 0.03 ± 0.19                       | 0.04 ± 1.02                       | 0.04 ± 2.00                       |
|                  | 0.1 N HCl                    | 0.07 ± 1.35                       | 26.72 ± 1.72                      | 29.83 ± 0.27                      | 42.01 ± 0.37                      | 61.82 ± 0.27                      |
|                  | pH 7.4 phosphate Buffer      | 0.02 ± 0.93                       | 0.03 ± 1.36                       | 0.11 ± 1.92                       | 0.06 ± 1.27                       | 0.08 ± 1.72                       |
| F12 (SNEDDS)     | Methanol                     | 0.02 ± 1.27                       | 0.12 ± 0.29                       | 0.17 ± 1.73                       | 0.03 ± 1.08                       | 0.01 ± 0.72                       |
|                  | Water                        | 0.01 ± 1.57                       | 0.22 ± 0.37                       | 0.02 ± 1.22                       | 0.11 ± 1.17                       | 0.07 ± 1.27                       |
|                  | 0.1 N HCl                    | 0.02 ± 1.94                       | 0.04 ± 1.23                       | 12.57 ± 1.06                      | 27.83 ± 0.03                      | 33.86 ± 0.72                      |
|                  | pH 7.4 phosphate Buffer      | 0.01 ± 0.86                       | 0.05 ± 0.27                       | 0.07 ± 0.28                       | 0.02 ± 0.27                       | 0.12 ± 0.86                       |

Above parameters are communicated as Average ± Standard Deviation; (n=3)

Table 4: Storage at 40±2°C /75±5% RH for 6 months

| Retest time for optimized formulation F12 (SNEDDS) | % Drug content (mean ± SD) | Entrapment efficiency (%) | In-vitro drug release (%) |
|---------------------------------------------------|-----------------------------|----------------------------|---------------------------|
| 0 days                                            | 99.32 ± 1.50                | 99.27 ± 0.50               | 99.96 ± 0.93              |
| 30 days                                           | 99.06 ± 0.15                | 99.01 ± 0.39               | 99.63 ± 0.37              |
| 60 days                                           | 98.72 ± 0.96                | 98.82 ± 1.60               | 99.32 ± 1.26              |
| 90 days                                           | 98.41 ± 0.48                | 98.47 ± 1.72               | 98.97 ± 0.67              |
| 180 days                                          | 98.09 ± 0.75                | 98.05 ± 0.41               | 98.75 ± 0.24              |

Above parameters are communicated as Average ± Standard Deviation; (n=3)
Shekhar et al: Development of irinotecan dissolution rate by snedds

Figure 1: Solubility of irinotecan in various oils.

Figure 2: Solubility of irinotecan in various surfactants.

Figure 3: Solubility of irinotecan in various co-surfactants

Figure 4: Ternary phase diagram for Canola oil – caproic acid – propylene glycol with $S_{\text{mix}}$ in 1:1 ratio (A), 2:1 ratio (B), and 3:1 ratio (C) (Key: the filled region within the ternary phase diagram indicates nanoemulsification area where the transmittance is greater than 90).

Figure 5: Ternary phase diagram for 25 mg (A) and 50 mg (B) of irinotecan loaded in Canola oil – Caproic acid – Propylene glycol system with $S_{\text{mix}}$ in 3:1 ratio (Key: the filled region within the ternary phase diagram indicates nanoemulsification area where the transmittance is greater than 90).

Figure 6: Comparative dissolution profiles of irinotecan pure drug, irinotecan SNEDDS and irinotecan S-SNEDDS.

Figure 7: FTIR spectrum of pure drug irinotecan.

Figure 8: FTIR spectrum of irinotecan S-SNEDDS (F12).
**Figure 9A:** Particle size of optimised SNEDDS formulation of irinotecan (F12).

**Figure 9B:** Zeta potential of optimised SNEDDS formulation of irinotecan (F12).

**Figure 10:** SEM images of optimised formulation of irinotecan SNEDDS F12 (A, B & C).