FIXED DOSE COMBINATION THERAPY OF IBRUTINIB AND QUERCETIN BY SNEDDS-DEVELOPMENT AND EVALUATION BY DESIGN OF EXPERIMENT

RASHMI BAGRI1,2*, RAVOURU NAGARAJU2
1Malla Reddy Pharmacy College, Maisammaguda, Secunderabad-500100, Telangana, India. 2Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women’s University), Tirupati-517502, Andhra Pradesh, India
*Corresponding author: Rashmi Bagri; Email: rashmi.bsg1@gmail.com

Received: 13 Mar 2023, Revised and Accepted: 05 Aug 2023

ABSTRACT

Objective: Self-nano emulsifying drug delivery system (SNEDDS) comprising quercetin and ibrutinib as a fixed dosage combination therapy is being investigated to increase drug solubility and dissolution rate.

Methods: On the basis of preliminary solubility tests, castor oil, Kolliphor® RH 40, and PEG600 were utilised to construct ternary phase diagrams. The effect of the amount of Castor oil (A), Kolliphor® RH 40 (B), and PEG600 (C) on the particle size and encapsulation efficiency of ibrutinib and quercetin was evaluated and statistically analysed using multiple regression in 17 trials planned using a 3 Box-Behnken design. FTIR, XRD, DSC, SEM, and stability experiments were employed to characterise the optimised formulation. The particle size, zeta potential, polydispersity index, encapsulation efficiency, and in vitro drug release of ibrutinib and quercetin were also investigated.

Results: Ibrutinib and quercetin had encapsulation efficiencies of 61.56-87.22% (Y3) and 60.12-87.12%, respectively, according to the size range of SNEDDS formulations (1-17) of 70.18-200.56 nm. The optimised SNEDDS formulations (S1-S3) showed a particle size range of 71.12–78.38 nm, PDI of 0.126–0.312, zetapotential of 26.4–284, and encapsulation efficiencies of 88.98–90.22% and 84.96–86.78% for ibrutinib and quercetin, respectively. According to in vitro testing, the medication released from SNEDDS was released more quickly (>90%) 600 min). The formulation was further evaluated using FTIR, XRD, DSC, SEM, and stability investigations, which validated the complexation of ibrutinib and quercetin in the drug’s amorphous state and stability for six months.

Conclusion: This study revealed that SNEDDS could be used as a drug carrier for ibrutinib and quercetin due to their improved solubility and dissolution rate.

Keywords: Ibrutinib, Quercetin, Box behnken design, Solubility, SNEDDS

INTRODUCTION

The simultaneous modulation of various cell-signalling mechanisms through multimodal chemotherapy is considered a vital protocol for enhancing therapeutic efficacy and reducing systemic toxicity. In recent years, the utilization of nanocarriers for delivering a combination of chemotherapeutic medications has emerged as a promising approach in cancer treatment [1]. Co-delivery systems offer a solution to the challenges associated with poor solubility and stability of certain drugs. These systems enable simultaneous transportation of multiple medications to the intended site, controlled release of the payloads in precise doses, synchronization of drug exposure, maximization of therapeutic efficacy, and minimization of toxicity [2]. The concurrent administration of antioxidants with anti-proliferative properties along with their inherent antioxidant capabilities holds significant potential for enhancing the overall effectiveness of antitumor treatments while reducing the toxicity associated with anticancer medications. In our study, we specifically aim to investigate the combination of two medications, namely ibrutinib and quercetin. By studying the therapeutic effects of this medication combination, we hope to gain insights into how the synergistic interaction between these compounds can lead to improved antitumor outcomes and reduced side effects [3].

Ibrutinib is a B-cell malignancies because it is a specific and covalent inhibitor of the Bruton’s tyrosine kinase (BTK) enzyme [4]. Ibrutinib has a pKa of 3.74, making it a weak base. It is essentially insoluble in water (mole fraction solubility: 1.43 x 10^-7 at room temperature), easily soluble in dimethyl sulfoxide, soluble in methanol, and has a very limited oral bioavailability (2.9%).

Quercetin is a polyphenolic flavonoid molecule that has been shown to have a number of biological actions. Some of these activities include the activation of apoptosis, the prevention of angiogenesis, and an anti-proliferative effect on a number of human cancer cells. There are a few different approaches that may be taken in order to improve the bioavailability of anticancer drugs and ensure that they are effectively delivered. Some of these technologies include lipid-based delivery systems, polymeric nanoparticulate systems, crystal engineering (nanocrystals technology, co-crystal technology), liquid-solid technology, self-emulsifying solid dispersions, and P-efflux inhibition strategies [5].

When a synthetic or natural oil, a surfactant, and a co-surfactant are introduced to an aqueous phase while being gently stirred, they produce a fine oil-in-water nanoeulsion that is known as a SNEDDS. SNEDDS are multi-component drug delivery systems that are isotropic. In addition to enhancing solubility and dissolving, researchers have been looking at the effects that SNEDDS have on increasing permeability, the hepatic first-pass effect, and bypassing the P-glycoproteins efflux [6].

In the area of formulation development, the Design of Experiment method has seen significant growth in popularity during the past several years [7]. In the present study, we have chosen the self-nano-emulsifying drug delivery system (SNEDDS) as the preferred method for delivering the proposed combination therapy of ibrutinib and quercetin. SNEDDS is a promising drug delivery system known for its ability to enhance the solubility, bioavailability, and therapeutic efficacy of poorly soluble drugs. By using SNEDDS, we aim to overcome the challenges associated with the limited solubility of ibrutinib and quercetin, ensuring their efficient delivery to the target site. The utilization of SNEDDS as a drug delivery method in our combination therapy approach holds the potential for achieving enhanced treatment outcomes and promoting the clinical translation of ibrutinib and quercetin as an effective therapeutic option. All of the ingredients used to make SNEDDS are nontoxic and fall into the category of excipients that are widely recognised as safe. Utilizing a 3-factor, 2-level Box Behnken design, the SNEDDS were optimised (BBD).
MATERIALS AND METHODS

Materials

The following materials were obtained from the indicated sources and used without further purification. Ibrutinib was purchased from Hetero Drugs Ltd., Hyderabad, India, and Quercetin was obtained from Research-Lab Fine Chem Industries, Mumbai, India. Linseed oil, olive oil, castor oil, sesame oil, groundnut oil, soyabean oil, Kolliphor® RH 40, and Kolliphor® EL were purchased from MSN Labs, Hyderabad, India. Tween-80, Tween-20, Span-80, Span-20, Carbitol, Triton X-100, PEG-300, PEG-400, PEG-600, Polypropylene glycol, Ethanol, Methanol, and Milli Q water were purchased from SD Fine Chemical Ltd., Mumbai.

Development of a UV-spectrophotometric method for the simultaneous estimation of ibrutinib and quercetin

Preparation of standard stock solution and calibration curve

The two drugs, ibrutinib and quercetin, showed good absorbance when dissolved in methanol. Hence, methanol was selected as the solvent for this method. Ibrutinib and quercetin (10 mg each) were separately weighed and transferred to a 100 ml volumetric flask, and the two drugs were dissolved in methanol to get a solution of 100 µg/ml of standard stock solution. Working standard solutions of 10 µg/ml were scanned in the entire UV range of 400–200 nm to determine the λmax. The λmax of ibrutinib and quercetin is 277 nm and 256 nm, respectively, from the overlap spectra. Five working standard solutions (2, 4, 6, 8, and 10 µg/ml) for each drug were prepared in methanol from stock solution. To obtain the linearity and regression equations, the absorbance of the resulting solutions were measured at their respective maximums and plotted on a calibration curve.

Two wavelengths, namely 277 nm and 256 nm, were selected, which are the λmax of two drugs, ibrutinib and quercetin, respectively. At 277 nm and 256 nm, the absorptivity of these two drugs was measured. Equations 1 and 2 were used in tandem to compute the concentrations of the two drugs in the mixture:

\[ C_1 = a_2 x_1 y_2 - a_1 x_2 y_1 - a_1 x_2 y_2 \] (1)

\[ C_2 = a_1 x_2 - a_3 x_1 y_1 - a_4 x_2 y_2 \] (2)

Where \( C_1 \) and \( C_2 \) are the ibrutinib and quercetin concentrations (µg/ml) in the known sample solution, \( a_1 \) and \( a_2 \) are the absorptivities of the sample solution at 277 nm and 256 nm, respectively. Ibrutinib’s absorptivity at 277 nm and 256 nm is represented by \( a_1 \) and \( a_2 \), whereas quercetin’s absorbivity is represented by \( a_3 \) and \( a_4 \).

Saturation solubility studies—selection of oil

Various oils, surfactants and co-surfactants were screened for their ability to dissolve ibrutinib procedure reported elsewhere [8].

Preliminary screening of surfactant and co-surfactant

Based on the solubility study results, two each of surfactants and co-surfactants were selected for the emulsification study reported elsewhere [9]. The obtained emulsions were kept aside for 2 h and percent transmittance was measured at 638.2 nm using a UV-spectrophotometer against distilled water as blank.

Construction of Pseudo ternary phase diagram

Based on the results of the solubility study and the emulsification tendency, Castor oil, Kolliphor® RH 40 and PEG-600 were selected as oil, surfactant and co-surfactant, respectively. To identify the self-nano emulsifying region, ternary diagrams of oil, surfactant, and co-surfactant were prepared, each representing the apex of the triangle. A visual test method reported by Craig et al. was modified and used in the study [10]. Ternary mixtures with varying compositions of oil, surfactant, and co-surfactant were prepared. The oil composition was varied from 40 % w/w to 80 % w/w, surfactant concentration was varied from 20 % w/w to 60 % w/w, and the co-surfactant concentration was varied from 0 % w/w to 20 % w/w. For all mixtures, the total of oil, surfactant, and co-surfactant was always 100 %.

The extreme and middle levels of the independent variables, consisting of the oil, surfactant, and co-surfactant, were selected for further study [11, 12].

Optimization of the SNEDDS by experimental design

Box-behnken experiment design

Table 1 and 2 shows 17 randomized experimental runs for the selected independent variables, including five replicates at the centre point (asterisk-marked) generated from a three-factor, three-level BBD and their corresponding responses [13]. A three-factor, three-level Box-Behnken Design was used for constructing the models with Design Expert® software (Version 8.0, Stat-Ease Inc., Silicon Valley, CA, USA). To explore and optimize the main, quadratic and interaction effects of the formulation ingredients on the performance of the SNEDDS. To determine the experimental error and the precision of the design, according to the three-factor and three-level design, BBD requires 17 randomized experimental runs with six replicates at the centre point. The percentages of independent variables, i.e., percentage of the oil phase (castor oil; \( X_1 \); 50–58%), surfactant (Kolliphor® RH 40; \( X_2 \); 22–38%), and co-surfactant (PEG-600; \( X_3 \); 12–18%), were chosen based on the results obtained from the phase diagram. Droplet size (\( Y_1 \)), ibrutinib encapsulation efficiency (\( Y_2 \)), and quercetin encapsulation efficiency (\( Y_3 \)) were the main response variables utilized to evaluate the quality of the SNEDDS formulation (table 1). The second-order quadratic or polynomial equation can be approximated by the following mathematical model:

\[ Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \] (3)

Where \( Y \) is the level of the measured response, \( \beta_0 \) is the intercept, \( \beta_i \) to \( \beta_9 \) are the regression coefficients, \( X_1 \), \( X_2 \), and \( X_3 \) stand for the main effects; \( X_1 X_2 \), \( X_1 X_3 \), and \( X_2 X_3 \) represent the interaction between the main effects; and \( X_1^2 \), \( X_2^2 \), and \( X_3^2 \) are the quadratic terms of the independent variables that were used to simulate the curvature of the designed sample space. The models were validated by lack of fit, ANOVA, and multiple correlation coefficient (R²) tests.

Optimization using the desirability function

In the present study, all three responses were simultaneously optimized by a desirability function that uses the numerical optimization method introduced by Derringer and Suich in the design-expert software (Version 8.0, Stat-Ease Inc., Silicon Valley, CA, USA) [14].

Table 1: List of dependent and independent variables in the inbox-behnken design

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Name</th>
<th>Units</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variable</td>
<td></td>
<td>Low (-1)</td>
</tr>
<tr>
<td>A</td>
<td>Amount of castor oil</td>
<td>mg</td>
<td>54</td>
</tr>
<tr>
<td>B</td>
<td>Amount of Kolliphor® RH 40</td>
<td>mg</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>Amount of PEG-600</td>
<td>mg</td>
<td>10</td>
</tr>
<tr>
<td>Dependent variable</td>
<td></td>
<td></td>
<td>Goal</td>
</tr>
<tr>
<td>Y1</td>
<td>Droplet size</td>
<td>Nm</td>
<td>Minimize</td>
</tr>
<tr>
<td>Y2</td>
<td>Encapsulation efficiency of ibrutinib</td>
<td>%</td>
<td>Maximize</td>
</tr>
<tr>
<td>Y3</td>
<td>Encapsulation efficiency of quercetin</td>
<td>%</td>
<td>Maximize</td>
</tr>
</tbody>
</table>
Preparation of plain SNEDDS (Placebo formulation)

SNEDDS formulation was prepared by stirring the components in optimized concentrations (Castor oil, Kolliphor® RH 40 and PEG-600) by stirring, vortex mixing and heating at 37°C on a magnetic stirrer.

Preparation of ibrutinib-quercetin loaded SNEDDS

Drug-loaded SNEDDS formulation was prepared by dissolving specified quantities of both drugs in the mixture of Castor oil, Kolliphor® RH 40 and PEG-600. The components were mixed by stirring, vortex mixing and heating at 37°C on a magnetic stirrer, until both the drugs were dissolved completely. The SNEDDS formulations were stored at room temperature for further studies.

Physicochemical characterization of ibrutinib, quercetin and SNEDDS formulation

Determination of melting point

Ibrutinib and quercetin melting points were determined using the open capillary tube method on the melting point apparatus (MP30, Mettler Toledo with LabXTM software). The procedure was carried out in triplicate.

Differential scanning calorimetry (DSC)

DSC is one of the basic techniques used to determine the purity of drugs and investigate drug-excipient compatibility. The steps involve taking around 5 mg of each sample, sealing it in a DSC aluminum pan, and heating the sample at a rate of 10°C per minute at a temperature range of 30-400°C. As a reference standard, an empty aluminum pan was used. Five milligram's of samples were subjected to analyses in triplicate while being nitrogen-purged. The thermograms for pure ibrutinib, quercetin, and the SNEDDS formulation were obtained using a DSC instrument (DSC-60 Shimadzu, Japan).

Fourier-transformed infrared (FTIR) spectroscopy

The physicochemical compatibility between ibrutinib, quercetin, and the excipient used to prepare SNEDDS was studied using FTIR (FTIR-9400, Shimadzu, Japan) spectroscopy. The spectra were recorded in the wavelength region between 400 cm⁻¹ and 4000 cm⁻¹.

X-Ray powder diffraction study (XRPD)

Powder X-ray diffraction patterns of ibrutinib and quercetin, SNEDDS formulation were traced employing XRD (XRD 7000, Shimadzu, Japan). An X-ray diffractometer with Ni-filtered CuK radiation, a voltage of 40 K, and a current of 30 mA, radiation scattered in the crystalline portions of the sample. At ambient temperature, XRD patterns were obtained using a step width of 0.04°C and a detector resolution of 20 (diffraction angle) between 10° and 80°.

Table 2: Box behnken design with observed responses

<table>
<thead>
<tr>
<th>Run</th>
<th>Amount of castor oil (mg)</th>
<th>Amount of Kolliphor® RH 40 (mg)</th>
<th>Amount of PEG-600 (mg)</th>
<th>Droplet size (nm)</th>
<th>Encapsulation efficiency of ibrutinib (%)</th>
<th>Encapsulation efficiency of quercetin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>24</td>
<td>15</td>
<td>128.82</td>
<td>63.48</td>
<td>63.12</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>24</td>
<td>15</td>
<td>189.92</td>
<td>61.56</td>
<td>63.12</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>36</td>
<td>15</td>
<td>118.85</td>
<td>80.23</td>
<td>79.18</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>36</td>
<td>15</td>
<td>176.89</td>
<td>70.12</td>
<td>65.56</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>30</td>
<td>10</td>
<td>138.82</td>
<td>72.48</td>
<td>73.12</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>30</td>
<td>10</td>
<td>200.56</td>
<td>69.92</td>
<td>67.78</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>30</td>
<td>20</td>
<td>88.53</td>
<td>85.68</td>
<td>83.59</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>30</td>
<td>20</td>
<td>148.56</td>
<td>75.66</td>
<td>78.78</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>24</td>
<td>10</td>
<td>133.89</td>
<td>65.34</td>
<td>64.89</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>36</td>
<td>10</td>
<td>108.26</td>
<td>82.84</td>
<td>79.12</td>
</tr>
<tr>
<td>11</td>
<td>57</td>
<td>24</td>
<td>20</td>
<td>70.18</td>
<td>80.58</td>
<td>79.28</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>36</td>
<td>20</td>
<td>71.84</td>
<td>86.38</td>
<td>87.12</td>
</tr>
<tr>
<td>13</td>
<td>57</td>
<td>30</td>
<td>15</td>
<td>77.86</td>
<td>87.22</td>
<td>85.76</td>
</tr>
<tr>
<td>14</td>
<td>57</td>
<td>30</td>
<td>15</td>
<td>79.58</td>
<td>86.12</td>
<td>86.14</td>
</tr>
<tr>
<td>15</td>
<td>57</td>
<td>30</td>
<td>15</td>
<td>76.88</td>
<td>85.26</td>
<td>85.98</td>
</tr>
<tr>
<td>16</td>
<td>57</td>
<td>30</td>
<td>15</td>
<td>78.12</td>
<td>86.78</td>
<td>86.38</td>
</tr>
<tr>
<td>17</td>
<td>57</td>
<td>30</td>
<td>15</td>
<td>76.54</td>
<td>84.42</td>
<td>87.12</td>
</tr>
</tbody>
</table>

Scanning electron microscopy (SEM)

Scanning electron microscopy (JEM-2000 EXII; JEOL, Tokyo, Japan) was used to observe the SNEDDS formulation's morphology. A film-coated copper grid was coated with a drop of diluted emulsion, which was then stained with a drop of 2% (w/v) phosphotungstic acid aqueous solution before being allowed to dry for contrast enhancement. The samples were examined under Scanning electron microscopy at a magnification of 72,000x.

Determination of droplet size and zeta potential

The mean droplet sizes and polydispersity index of the globules were analyzed using dynamic light scattering with a scattering angle of 90° using Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK) equipped with MAS OPTION particle sizing software. Zeta potential measurements were also made using an additional electrode in the same instrument.

Thermodynamic stability studies

Under varied stress conditions, six cooling (4°C) and heating (40°C) cycles, as well as freeze-thaw cycles (-21°C and 25°C), were used to test the prepared SNEDDS formulations thermodynamic stability. These cycles were carried out for at least 48 h. The stable formulation was tested for centrifugation, which involved centrifuging the formulations for 30 min at 3500 rpm and checking for any phase separation visually [15]. Within 2-3 min, the thermodynamically stable nano-emulsions regained their original form, and they were chosen for further investigation.

Accelerated stability study

The ICH guidelines Q1A (R2) for stability testing were followed when conducting accelerated stability studies. The optimized formulations were stored in a temperature and humidity-controlled stability chamber for three months at 40°C and 75% RH. Before and after accelerated stability studies, the formulations droplet size, zeta potential, and PDI were measured.

In vitro dissolution studies

The USP type II apparatus (paddle type; Electrolab, TD L8, Mumbai, India) was used for the relative in vitro dissolution studies under sink conditions, with the paddle rotating at a speed of 50 rpm. 500 ml of dissolution medium (simulated gastric fluid, pH 1.2), which had been equilibrated at 37°C, had samples of SNEDDS and individual drug suspensions comprising 10 mg of each drug added to it. In order to maintain a consistent volume of dissolution medium, an aliquot of 5 ml was removed and reintroduced at specified time periods of 5, 10, 15, 30, 45, 60, 90, and 120 min. All samples were filtered and properly diluted, and their drug concentrations were measured using a UV spectrophotometer. 250 ml of 0.3 M dibasic sodium phosphate was added to the dissolution medium after two
hours to change the pH and mimic the gut pH (SIF, pH 6.8). The samples were collected on a regular basis and examined as previously described. There were three triplicates of each measurement.

**Kinetic analysis**

The data from the in vitro release study were fitted into different kinetic models, such as the zero-order, first-order, Higuchi’s, and Korsemeyer Peppa’s models, to understand the mode and mechanism of drug release. By using the curve fitting method, the release of both drugs from the SNEDDS formulation was calculated. Several kinetic equations were fitted to the data from in vitro release studies.

**RESULTS AND DISCUSSION**

**Solubility study**

Out of different oils screened; castor oil has a maximum solubility of ibrutinib (3456.28 μg/ml±216.78) and quercetin (2897.93 μg/ml±188.76). Among surfactants and co-surfactants, Kolliphor® RH 40 (9789.28±786.42 μg/ml), Kolliphor® EL (7126.67±342.76 μg/ml), tween-80 (6472.82±538.42 μg/ml) and PEG-600 (5973.08±212.32 μg/ml) could solubilize maximum amount of ibrutinib, respectively. For quercetin, Kolliphor® RH 40 (8734.64±423.66 μg/ml) and PEG-600 (7144.85±618.25 μg/ml) provided the highest solubility potential (fig. 1). These results are correlating with the previous works of Tang et al. and Zhang and his co-workers [16, 17].

![Fig. 1: Solubility of ibrutinib and quercetin in various oils and other solvents, Each value represents the mean±SD (n = 3)](image)

**Pseudo-ternary phase diagram**

A large nano-emulsion area indicates better emulsification efficacy of the surfactant toward oil. For Kolliphor® RH 40/PEG-600 systems, they showed that increasing the Kolliphor® RH 40 to PEG-600 ratio increased the nano-emulsion area, which was explained by the increase in surfactant adsorption at the emulsion interface leading to decreases in surface tension and formulation droplet sizes. As seen in fig. 2, the green region of the diagram represented the efficient self-nanoemulsifying region. Based on this diagram, the range of each component was selected as follows: 54% ≤ Castor oil ≤ 60%, 24% ≤ Kolliphor® RH 40 ≤ 36%, 10% ≤ PEG-600 ≤ 20%.

![Fig. 2: Ternary phase diagram of dual drug-loaded SNEDDS](image)
Design of experiments

The experiments were conducted as for the design and the obtained responses for the dependent variables droplet size ($Y_1$), encapsulation efficiency of ibrutinib ($Y_2$), and encapsulation efficiency of quercetin ($Y_3$) were presented in Table 2. The design summary is as shown in fig. 3 [18].

Statistical analysis of the designed experiment

The range of droplet size ($Y_1$) for all batches was 70.18–200.56 nm. Similarly, the range for encapsulation efficiency of ibrutinib ($Y_2$) was 61.56–87.22 % and the range of encapsulation efficiency of quercetin ($Y_3$) was in the range 60.12–87.12 %. All three responses were fitted to a second quadratic model and the adequacy of this model was verified by ANOVA, lack of fit, and multiple correlation coefficient ($R^2$) tests provided by Design-Expert software (Table 3).

Table 3: Regression equations for the responses—particle size, polydispersity index and percent drug release after 15 min

<table>
<thead>
<tr>
<th>Response</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1$</td>
<td>77.80+30.11 A–5.87 B+25.30 C+6.62 BC+61.95 A²+13.87 B²+4.37 C²</td>
</tr>
<tr>
<td>$Y_2$</td>
<td>85.94–3.08 A+6.08 B+4.71 C–2.05 AB–1.87 AC–2.93 BC–9.98 A²–7.13 B²</td>
</tr>
<tr>
<td>$Y_3$</td>
<td>86.31–2.60 A+5.45 B+5.48 C–4.15 AB–1.60 BC–10.53 A²–8.74 B²</td>
</tr>
</tbody>
</table>

The particle size of the nanoparticles was found to be in the range of 70.18–200.56 nm as shown in Table 2. The perturbation plot (fig. 4) showing the main effects of A, B and C on the droplet size ($Y_1$). The interaction between B and C on droplet size at a fixed level of A is shown in fig. 5. The respective contour plot is as shown in fig. 6.

Fig. 4: Perturbation plot showing the effect of A, B and C on droplet size

Fig. 5: Response surface plot showing the influence of B and C at fixed level of A
Encapsulation efficiency of ibrutinib

The encapsulation efficiency of ibrutinib within the self-nanoemulsifying drug delivery system (SNEDDS) was evaluated, and the results, ranging from 61.56% to 87.22%, are presented in table 2. Fig. 7, known as the perturbation plot, illustrates the main effects of factors A, B, and C on the encapsulation efficiency of ibrutinib (Y2). It provides a visual representation of how each factor individually influences the encapsulation efficiency. Moving on to fig. 8, it showcases the interaction between factors A and B on the encapsulation efficiency of ibrutinib at a fixed level of factor C. The corresponding contour plot, presented in fig. 9, offers a graphical representation of this interaction. Similarly, fig. 10 demonstrates the interaction between factors A and C at a fixed level of factor B, accompanied by the respective contour plot in fig. 11. Additionally, fig. 12 depicts the interaction between factors B and C on the encapsulation efficiency of ibrutinib when factor A is fixed, while the contour plot for this interaction is shown in fig. 13. Together, these fig. and contour plots provide comprehensive insights into the relationships and interactions among factors A, B, and C regarding the encapsulation efficiency of ibrutinib within the SNEDDS, aiding in the analysis and optimization of the drug delivery system for enhanced efficiency.
Fig. 9: Contour plot showing the influence of A and B at a fixed level of C

Fig. 10: Response surface plot showing the influence of A and C at fixed level of B

Fig. 11: Contour plot showing the influence of A and C at fixed level of B

Fig. 12: Response surface plot showing the influence of B and C at fixed level of A
Encapsulation efficiency of quercetin

The encapsulation efficiency of quercetin within the self-nanoemulsifying drug delivery system (SNEDDs) was assessed, with results ranging from 60.12% to 87.12% as presented in Table 2. Fig. 14, known as the perturbation plot, illustrates the main effects of factors A, B, and C on the encapsulation efficiency of quercetin (Y2). This plot provides a visual representation of how each factor individually influences the encapsulation efficiency. Additionally, Fig. 15 showcases the interaction between factors A and B on the encapsulation efficiency of quercetin at a fixed level of factor C, while the corresponding contour plot is presented in Fig. 16. Similarly, Fig. 17 depicts the interaction between factors B and C on the encapsulation efficiency of quercetin at a fixed level of factor A, accompanied by the respective contour plot in Fig. 18. These fig. and contour plots provide comprehensive insights into the relationships and interactions among factors A, B, and C concerning the encapsulation efficiency of quercetin within the SNEDDs. The findings aid in the analysis and optimization of the drug delivery system, contributing to improved efficiency and performance.

Fig. 13: Contour plot showing the influence of B and C at fixed level of A

Fig. 14: Perturbation plot showing the effect of A, B and C on encapsulation efficiency of quercetin

Fig. 15: Response surface plot showing the influence of A and B at fixed level of C
Fig. 16: Contour plot showing the influence of A and B at fixed level of C

Fig. 17: Response surface plot showing the influence of B and C at fixed level of A

Fig. 18: Contour plot showing the influence of B and C at fixed level of A

Fig. 19: 3D-response surface plot showing the global desirability value with point prediction
Optimization by desirability function

The responses: droplet size ($Y_1$), encapsulation efficiency of ibrutinib ($Y_2$), and encapsulation efficiency of quercetin ($Y_3$) were transformed into the desirability scale, respectively. Among them, $Y_1$ has to be minimized, while $Y_2$ and $Y_3$ have to be maximized. For individual desirability function, $Y_{max}$ and $Y_{min}$ were taken as the highest objective function (D) was calculated by Equation (3) for each response. The prediction model is as shown in fig. 19 and 20 (table 4).

Evaluation of SNEDDS

Droplet size, polydispersity index and zeta potential

The droplet size for dual drug-loaded SNEDDS ($S_1$-$S_3$) ranged from 71.12-76.38 nm with PDI 0.126-0.312. The negative value of zeta potential ranging between -24.6 to -28.4 mV might be due to the presence of anionic groups of free fatty acids and glycols present in the oil, surfactant and co-surfactant [19, 20]. The particle size and zeta potential values were presented in table 5.

Table 4: Optimized values obtained by the constraints applies on $Y_1$, $Y_2$ and $Y_3$

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Nominal values</th>
<th>Predicted values</th>
<th>Observed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of castor oil</td>
<td>56.32 mg</td>
<td>69.32</td>
<td>88.27</td>
</tr>
<tr>
<td>Amount of Kolliphor RH 40</td>
<td>31.32 mg</td>
<td>76.30±5.26</td>
<td>87.8±2.25</td>
</tr>
<tr>
<td>Amount of PEG-600</td>
<td>15.91 mg</td>
<td>S3</td>
<td>86.58±3.17</td>
</tr>
</tbody>
</table>

(All determinations were performed in triplicate and values were expressed as mean±SD n=3)

Table 5: Droplet size, polydispersity index and zeta potential of S-SNEDDS formulations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Particle size±SD (nm)</th>
<th>Polydispersity Index</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>75.34±4.24</td>
<td>0.19±0.005</td>
<td>-24.6±2.1</td>
</tr>
<tr>
<td>S2</td>
<td>76.38±5.26</td>
<td>0.12±0.005</td>
<td>-25.8±1.6</td>
</tr>
<tr>
<td>S3</td>
<td>71.12±3.14</td>
<td>0.31±0.005</td>
<td>-28.4±2.3</td>
</tr>
</tbody>
</table>

(All determinations were performed in triplicate and values were expressed as mean±SD, n=3)

Thermodynamic stability studies

The selected formulation passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternate temperature cycles (4 °C and 40 °C), freeze-thaw cycles [-21 °C and+25 °C] and centrifugation at 3500, indicating good stability of the formulation.

Physicochemical characterization

The interactions between the drugs and other components were determined by FT-IR spectroscopy. The characteristic peaks of ibrutinib are seen at 3396.76, 1741.78, 1687.77, 1587.47, 1506.67, 1462.09, 1375.29, 1244.13, 1155.4, 1033.88, 972.16, 835.21, 746.48 and 704.04 cm⁻¹. Similarly, the characteristic peaks of quercetin are seen at 3406, 3283, 1666, 1610, 1560, 1510, 1379, 1317, 1263, 1200, 1165, 933, 820, 679 and 600 cm⁻¹. It is anticipated that ibrutinib and quercetin peaks were masked by the formulation absorption peaks due to the encapsulation of drug molecules within the SNEDDs (fig. 21).

In the XRPD pattern of the dual drug-loaded SNEDDS was completely amorphous, lacking characteristic peaks of both drugs (fig. 22).

DSC curves of ibrutinib, quercetin and SNEDDS formulation are shown in drugs have shown sharp endothermic peaks (figure). However, the SNEDDS formulation showed no specific peaks from 40 °C to 400 °C. This confirms that both drugs are presented in an amorphous form within the SNEDDS (fig. 23).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) studies showed that the regular spherical shape and size of all the SNEDDS formulations, as shown in fig. 24.

In vitro dissolution studies

The comparative dissolution profiles of pure ibrutinib, quercetin and SNEDDS formulations were carried out both in SGF and SIF as dissolution medium is presented in fig. 25. The dissolution profile shows that the SNEDDS showed faster drug release (>50 % in 240
min) when compared with both pure drug dispersions. Rapid drug dissolution from the SNEDDS may be due to low surface free energy of the self-emulsifying systems, which favours rapid emulsification by quick establishment of an interface between dissolution medium and oil. The improved dissolution can be attributed to the greater surface area of the nanosized globules, the transformation of the drugs physical state from low water-soluble crystalline form to a non-crystalline amorphous or disordered crystalline phase of a molecular dispersion in SNEDDS. The in vitro dissolution studies revealed that both drugs are hydrophobic, in particular, Quercetin showed negligible drug dissolution at both SGF and SIF environments. These data emphasize the need for enhancing oral quercetin delivery by proper formulation design. Interestingly, ibrutinib-Quercetin SNEDDS showed superior (p<0.05) dissolution enhancement of both drugs at SGF and SIF. This was owing to the efficient self-nano emulsification process that formed a favorable environment to maintain the drug solubilized, within the nano-sized oil droplets, upon exposure to GI fluids.

Fig. 21: FTIR spectra of pure ibrutinib, quercetin and SNEDDs formulation

Fig. 22: XRD pattern of pure ibrutinib, quercetin and SNEDDs formulation

Fig. 23: DSC thermograms of pure ibrutinib, quercetin and SNEDDs formulation
Fig. 24: SEM images of SNEDDS formulations (S1, S2 and S3 under 21K, 10K and 5k magnification respectively)

Fig. 25: Dissolution profile of ibrutinib and quercetin from SNEDDS formulations

Table 6: Droplet size, Zeta potential and PDI of ibrutinib S-SNEDDS formulation after 90 d of storage at refrigerated and room temperature

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Particle size (nm)</th>
<th>Zeta potential</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 mo</td>
<td>3 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>4±1 ºC</td>
<td>76.38±5.26</td>
<td>80.12±3.32</td>
<td>81.18±2.76</td>
</tr>
<tr>
<td>25±2 ºC</td>
<td>76.38±5.26</td>
<td>81.56±2.78</td>
<td>83.50±3.32</td>
</tr>
</tbody>
</table>

(All determinations were performed in triplicate and values were expressed as mean±SD n=3)

Stability study

The purpose of stability testing is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Table 6 indicates that no significant difference (p<0.05) was found in droplet size, zeta potential and PDI of optimized formulation stored at refrigerated conditions and at room temperature.
CONCLUSION

The combined dosage form of Ibrutinib-Quercetin SNEDDS formulation was successfully developed with increased drug solubilization and enhanced dissolution rate. The formulation variables were optimized by response surface methodology. The optimized loaded formula consisted of 56.32 % Castor oil (oil; X1), 31.32 % Kolliphor® RH 40 (Surfactant; X2) and 15.91 % PEG-600 (Co-surfactant; X3) which formed aqueous thermodynamically stable nanoemulsion upon contact with aqueous medium without being affected by change in pH of media. In vitro release studies showed that the optimized formula had faster release than that of pure drugs, confirming the efficiency of SNEDDS for improving the solubility and dissolution rate of poorly water-soluble drugs (Ibrutinib/Quercetin) combination. In vivo studies proved the superior efficiency of optimized-SNEDDS to enhance oral bioavailability of Ibrutinib/Quercetin combination.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

REFERENCES


R. Bagri & R. Nagaraju