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# Optimization of injectable PLGA *in-situ* forming implants of anti-psychotic risperidone via Box-Behnken Design

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ABSTRACT

The current study aims to develop *in-situ* forming implants (ISFI) of anti-psychotic risperidone to customize schizophrenia therapy. Non-adherence to treatment is the most prevalent unsuccessful clinical outcome in schizophrenic patients. Poly(lactic-co-glycolic acid) (PLGA) was utilized to prepare ISFI using various solvents having different drug solubilization capacity. $3^3$ Box-Behnken Design (BBD) was used to study the effects of independent variables; lactide concentration (A), solvent type (B) and solvent/polymer ratio (S/P) (C) on burst release after 6h ($Y_1$), cumulative release after 40 days ($Y_2$) and injectability time ($Y_3$). Statistical analysis was followed by optimization process to minimize burst release and injectability time with targeted cumulative release to 50% at 40 days. BBD results represented the critical impacts of increasing lactide and PLGA concentrations on reducing drug burst and percent release. Rapid ISFI solidification using DMSO supported the high dispersed drug encapsulation and sustained release. Triacetin demonstrated a lag time between formulation injection and solidification that negatively affected the studied responses. The optimized formulation manifested burst (8.1%), cumulative release (51.08%) and 18 s for injection by mixing PLGA (75:25) and DMSO (S/P = 2.851). Compared with injectable commercial microspheres, ISFI emphasized their potential to enhance compliance and eliminate costs issues of additional oral therapy.

## 1. Introduction

Schizophrenia is a severe, heterogeneous behavioral and cognitive syndrome that generates from disturbance of brain development [1]. Schizophrenic patients are exposed to premature death with a rate of 40–60% greater than general population owing to unattended physical health problems and suicide [2]. Schizophrenia is characterized by various psychopathological disorders such as positive symptoms (delusions and hallucinations), negative symptoms (emotional apathy, impaired motivation, and social withdrawal) and cognitive impairments (poor performance and trouble focusing) [3]. The appearance of oral conventional anti-psychotics such as haloperidol proved a noteworthy progress in the treatment of acute schizophrenia and prevention of relapse. On the other hand, these drugs in injectable forms cause insufferable motor side effects, injection-site reactions and pain that restrained their use [4,5]. Therefore, the recent oral atypical anti-psychotics (e.g. risperidone) are recommended as first choice medications for treatment of acute schizophrenic attacks.

Risperidone, a benzisoxasole derivative, is a new potent anti-psychotic drug with a powerful blocking efficacy of 5-$HT_2$ and $D_2$ receptors [6]. Generally, atypical agents have more effective impact on relapse prevention with a broader spectrum of activity causing less extra-pyramidal adverse effects and tardive dyskinesia when compared with conventional typical anti-psychotic therapy [7,8]. Although oral atypical drugs are efficacious in enhancing clinical outcomes and minimizing relapse rates, non-adherence of these drugs is a great issue in the proper treatment of schizophrenia [9]. To combat the non-adherence problem of oral drugs, depot formulations have been designed to hold lower drug content bypassing hepatic metabolism with comparable therapeutic efficacy, thereby reducing risks of hepatotoxicity and hyperprolactinemia [10]. Hence, implantable anti-psychotic delivery systems including poly(lactic-co-glycolic acid) (PLGA) matrix were demonstrated to maintain the benefits of extended release systems as well as achievement of patient adherence [11].

PLGA, a durable biodegradable polyester co-polymer, is already approved by Food and Drug Administration and European Medicines Agency for parenteral use, diagnosis and many clinical fields. Along with its biodegradability and biocompatibility, PLGA is considered as one of the most used synthetic eco-friendly polymers in the biomedical area [12,13]. An outstanding feature of delivering atypical anti-psychotics by PLGA co-polymers comprises a reduced frequent dosing that results in increase of patient adherence to therapy regimens. In

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addition, PLGA co-polymers can be adapted to achieve a sustained release of drug. This is attributed to the effective characteristics of PLGA co-polymers such as variable molecular weights and several lactide:glycolide ratios that help to monitor the rates of polymer degradation and *in-vitro* and/or *in-vivo* drug release [14].

Risperidone is traditionally administrated as daily oral tablets or as biweekly intramuscular microsphere injections named as Risperdal Consta™. Once injected, the commercial microspheres can reach the desired therapeutic levels after 2–3 weeks. Throughout this lag period, co-administration of oral risperidone is required [15]. This inconvenient combination represents greater compliance and costs troubles in psychotic patients and therefore the non-oral controlled delivery system of risperidone is ultimately desired [14]. To overcome the complicated manufacturing and scale-up obstacles of microspheres [6], it is necessary to design optimized implantable formulations possessing favorable initial release profiles in order to maintain the therapeutic drug plasma concentration and avoid extra-pyramidal adverse effects.

*In-situ* forming implants (ISFI), as promising drug delivery systems, are subcutaneously or intramuscularly injectable liquids that transform to semi-solid or solid depots (gel-like mass) in contact with aqueous body fluids, consequently release the drug in a controlled pattern [16]. The drug is dissolved or dispersed in a concentrated solution of lipophilic biodegradable polymer in a water miscible biocompatible solvent. Upon injection into aqueous body fluids, the solvent diffuses into the surrounding media while water diffuses into the polymeric matrix leading to the formation of solid ISFI and retardation of drug release [17,18].

The biodegradable ISFI systems show diverse merits over the traditional polymeric microspheres including uncomplicated manufacturing methods, ease to deliver drugs of high dose in addition to utilization of smaller diameter injection needles and less tissue irritation [19]. On the other hand, the incidence of initial burst effect after few hours of injection as a result of the lag between injecting the liquid implant and forming the solid implant, is still a deleterious problem. So far, no encapsulating matrix is successfully formed leading to poor drug loading and local or systemic side effects [20,21]. One of the most crucial factors influencing the drug release, injectability and stability of drug and polymers is the selection of solvent type [22]. Different solvents such as N-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), 2-pyrrolidone, ethyl acetate, triacetine and benzyl benzoate have been experimented to obtain various phase inversion dynamics and then modify drug release [23].

Quality by design (QbD) is a systemic proposal for pharmaceutical products development that focuses on realizing the functionality of products, optimizing and improving the process efficiency under significant quality specifications [24]. Box–Behnken design (BBD) of response surface methodology (RSM) is considered the most adequate economical technique that analyze the multivariate with few number of experiments in comparison to other designs of RSM such as central composite design and D-optimal design [25]. This technique produces an empirical model equation to understand the relation between controllable (independent) variables and quality (dependent) responses [26].

The current study aims to design risperidone-loaded PLGA ISFI formulations using BBD in order to attain an optimized sustained release formulation of risperidone with considerable physicochemical characteristics and therapeutic outputs.

## 2. Materials and methods

### 2.1. Materials

Risperidone was supplied from Corey Organics Co., India. PLGA copolymers (65:35, 75:25 and 85:15) were purchased from LACTEL Absorbable Polymers Co., USA. NMP was purchased from Central Drug House Co., India. Triacetin was supplied from Euromedex Co., France. Ethyl acetate and DMSO were purchased from SD Fine Chem Limited Co., India. All other chemicals were of analytical grade.

### 2.2. Methods

#### 2.2.1. Solubility measurements of risperidone in different organic solvents

Solubility determination of risperidone in different organic solvents of NMP, triacetin, ethyl acetate and DMSO was carried out. Inside various glass vials, excess amounts of risperidone were suspended in 5 ml of each solvent (n = 3) and then kept in a water bath shaker controlled at 25 ± 0.5 °C for 72h until equilibrium was reached. Thereafter, the contents were filtered using nylon syringe filter (0.22 μm). The filtrate was analyzed spectrophotometrically at $\lambda_{max}$ 278 nm to record the dissolved drug amount. The results were represented as mean values ± SD.

#### 2.2.2. Experimental design

A BBD of three factors at three levels ($3^3$) was constructed to study how the selected independent variables influence the dependent responses after preparation of ISFI. The BBD was established using Design-Expert® software trial version 11. This design contained replicated center points and a group of points that existed at the midpoints of each edge of the multidimensional cube that deduced the area of interest. The response (Y) could be measured by the following polynomial equation:

$$Y = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

Where, (Y) is the measured response, ($b_0$) is the intercept of polynomial equation and ($b_1$–$b_9$) are the regression coefficients corresponding to the independent variables. The main, interacting and quadratic effects of independent variables are (A, B, and C), (AB, AC and BC) and ($A^2$, $B^2$, and $C^2$) respectively.

After preliminary experiments, three independent variables were chosen as follow; lactide concentration in the PLGA co-polymer (A), type of solvent (B) and ratio of solvent to polymer concentrations (S/P) (C). The responses to be measured were burst release after 6h ($Y_1$), cumulative amount of drug released after 40 days ($Y_2$) and injectability time ($Y_3$). Corresponding to the independent variables, three different levels were established as the lowest, the highest and central values of the tested variables (Table 1). The matrix of 15 experimental formulations was constructed as represented in Table 2.

By using analysis of variance (ANOVA), the measured responses were fitted to linear, second order and quadratic models on the basis of b-coefficients, p-values ($p < 0.05$), and F-values in addition to other statistical parameters such as multiple correlation coefficients ($R^2$),

**Table 1**
Independent and dependent variables in BBD.

| Independent variables | Symbol | Levels | | |
|---|---|---|---|---|
| | | Lowest (−1) | Central (0) | Highest (+1) |
| **Lactide concentration in PLGA co-polymer** | A | 65% | 75% | 85% |
| **Solvent type** | B | Triacetin | Ethyl acetate | DMSO |
| **Solvent/PLGA ratio (S/P)** | C | 2:1 | 3.5:1 | 5:1 |
| **Dependent variables** | **Symbol** | **Constraints** | | |
| **Burst release after 6h (%)** | $Y_1$ | Minimize | | |
| **Cumulative release after 40 days (%)** | $Y_2$ | Target to 50% | | |
| **Injectability time (sec)** | $Y_3$ | Minimize | | |

DMSO, dimethyl sulfoxide.

**Table 2**
Risperidone-loaded PLGA ISFI formulations and observed values of responses.

| Formula | Drug (mg) | A (%) | B | C | $Y_1$ (%) | $Y_2$ (%) | $Y_3$ (sec) |
|---|---|---|---|---|---|---|---|
| **F1** | 25 | 85 | Ethyl acetate | 5 | 30.82 | 49.14 | 5.00 |
| **F2** | 25 | 75 | Triacetin | 5 | 50.60 | 99.43 | 36.00 |
| **F3** | 25 | 75 | Ethyl acetate | 3.5 | 17.96 | 49.59 | 10.00 |
| **F4** | 25 | 65 | Ethyl acetate | 5 | 35.35 | 80.84 | 5.00 |
| **F5** | 25 | 65 | DMSO | 3.5 | 15.81 | 83.63 | 25.00 |
| **F6** | 25 | 75 | DMSO | 2 | 6.55 | 30.58 | 23.00 |
| **F7** | 25 | 75 | Triacetin | 2 | 12.58 | 50.20 | 84.00 |
| **F8** | 25 | 75 | DMSO | 5 | 11.32 | 63.20 | 12.00 |
| **F9** | 25 | 75 | Ethyl acetate | 3.5 | 16.95 | 42.96 | 9.00 |
| **F10** | 25 | 85 | Ethyl acetate | 2 | 4.75 | 12.12 | 17.00 |
| **F11** | 25 | 65 | Ethyl acetate | 2 | 14.75 | 38.82 | 21.00 |
| **F12** | 25 | 85 | Triacetin | 3.5 | 31.03 | 85.00 | 68.00 |
| **F13** | 25 | 75 | Ethyl acetate | 3.5 | 21.17 | 48.06 | 14.00 |
| **F14** | 25 | 85 | DMSO | 3.5 | 11.06 | 60.05 | 20.00 |
| **F15** | 25 | 65 | Triacetin | 3.5 | 36.82 | 96.50 | 65.00 |

A, lactide concentration in PLGA co-polymer; B, solvent type; C, solvent/PLGA ratio; $Y_1$, burst release after 6h; $Y_2$, cumulative release after 40 days; $Y_3$, injectability time; DMSO, dimethyl sulfoxide.

adjusted $R^2$, predicted $R^2$ and lack of fit. Subsequently, 3D response surface graphs were presented in order to estimate the relation and interaction between the studied variables and responses.

*2.2.3. Optimization of formulation components*

Optimization process was carried out by relying on desirability measurement to get the levels of tested variables that could agree with the desirable responses [27]. Based on the required criteria, a suggestion was displayed with a desirability range from 0 to 1 where the desirability value towards 1 indicated the preference of response to its ideal value [25]. Furthermore, the optimized formulation was determined and subjected to comparison of predicted and experimental values.

*2.2.4. Preparation of ISFI formulations*

To formulate risperidone-loaded PLGA ISFI, the techniques of Mashayekhi et al. [28]; Parent et al. [29] were adapted with some modifications. Formulations shown in Table 2 varied with regard to lactide concentration (A), type of solvent (B) and ratio of S/P (C). Briefly, accurately weighted amounts of PLGA with different lactide concentrations (65, 75 and 85%) were dissolved in different solvents (triacetin, ethyl acetate or DMSO) at several ratios of S/P as suggested by BBD. These mixtures were transferred in a stoppered glass vials and then placed on a hot plate magnetic stirrer at 900 rpm over 30 min at 50 °C. Risperidone was added to the polymer solution under continuous mixing until a clear gel was attained. The total amount of each preparation was adjusted to 1 gm and the risperidone amount (25 mg) was kept constant. The produced formulations were allowed to cool to room temperature and then refrigerated overnight prior to further investigation. All the *in-vitro* experiments were carried out three times (n = 3).

*2.2.5. Characterization of ISFI preparations*

*2.2.5.1. Determination of injectability time*. In order to record the force needed to push the prepared ISFI through a syringe needle, the injectability time in seconds was measured for each formulation by using a stop watch. Each formulation was withdrawn into a syringe of 21 gauge needle. The head of syringe plunger was compressed by a 1 kg weight in a downward direction allowing the syringe contents to be pushed out through the needle [30].

*2.2.5.2. In-vitro drug release study*. The cumulative release profile of risperidone from ISFI preparations was studied using a water bath shaker thermostatically controlled at 37 ± 0.5 °C. Each of the prepared ISFI formulation was injected into screw capped bottles containing the receptor medium (100 ml of Sörensen phosphate buffer pH 7.4) to maintain the necessary sink conditions. The bottles were mechanically shaken in a horizontal direction at an average speed of 100 rpm. The samples (2 ml) were withdrawn after 6 and 12h on the first day to study the burst release effect. Then, aliquots were taken after 1, 2 and 4 days with continuous withdrawal every 4 day followed by filtration using nylon syringe filter (0.22 μm). An equal volume of fresh buffer was filled back to keep the receptor volume constant. The filtered samples were subsequently analyzed spectrophotometrically at $\lambda_{max}$ 278 nm to determine the mean values of cumulative release of drug (n = 3) and compared with those of marketed Risperdal Consta™.

*2.2.5.3. Fourier transform infrared (FTIR) spectroscopy*. In order to investigate drug-excipient compatibility, comparisons of FTIR spectra of pure risperidone powder, PLGA co-polymer, physical mixture of drug and PLGA, DMSO, blank ISFI formulation and optimized ISFI formulation were conducted by using a PerkinElmer FTIR spectrophotometer with a scanning range of 4000–500 $cm^{-1}$.

*2.2.5.4. Differential scanning calorimetry (DSC)*. The DSC thermal profiles of pure risperidone powder, PLGA co-polymer, physical mixture of drug and PLGA, DMSO, blank ISFI formulation and optimized ISFI formulation were recorded on a Shimadzu DSC 60 calorimeter equipped with a computer software program. Carefully, small samples were placed into tightly sealed aluminium pans heated from 0 to 200 °C at a constant rate of 10 °C/min. The heating was under nitrogen atmosphere at a flow rate of 30 ml/min.

## 3. Results and discussion

*3.1. Determination of risperidone solubility in different solvents*

The solubility of risperidone was investigated in different organic solvents namely NMP, triacetin, ethyl acetate and DMSO that will be used to formulate PLGA ISFI. The utilized solvents have a median lethal dose higher than 2 ml/kg and are safe to be used as additives in human parenteral products as reported by Rowe et al. [31]; Ahmed et al. [32].

In the current study, the drug solubility in the NMP, triacetin, ethyl acetate and DMSO solvents was recorded as 68.05 ± 2.58, 21.85 ± 0.60, 12.24 ± 0.33 and 7.07 ± 0.36 mg/ml respectively. Despite of the safety and priority of NMP in various pharmaceutical products, it is freely miscible with water resulting in rapid burst release of large amounts of drug within minutes or hours. This may cause undesirable local tissue irritation and different systemic adverse effects [33,34]. As represented by Fig. 1, 37.62 ± 0.83% of the theoretical amount of risperidone was released in the first 6h from PLGA (75:25) ISFI containing NMP in the buffer (pH 7.4) followed by a rapid release with a maximum of 100.03 ± 0.65 after 4 days. This might be attributed to the immediate extraction of NMP to the surrounding medium and retarded solidification of polymeric gel resulting in a rapid diffusion of the dissolved risperidone [35]. In comparison, the marketed risperidone microspheres (Risperdal Consta™) showed a marked slow release of drug after 6h (1.89 ± 0.50%) followed by a rapid drug release after two weeks with a maximum release of 99.91 ± 0.72% after 44 days. Accordingly, NMP solvent was excluded from further investigation owing to its unsatisfactory results for prolongation of risperidone release when compared to the marketed drug. While, other solvents of lower solubilization capacity of risperidone (triacetin, ethyl acetate and DMSO) were selected to study their effects on the studied responses with the help of BBD.

*3.2. Statistical analysis of data*

The validity of the utilized design was examined by standard error graph shown in Fig. 2. This graph indicated the values of standard error of prediction for areas in the design space. It was satisfactory to obtain

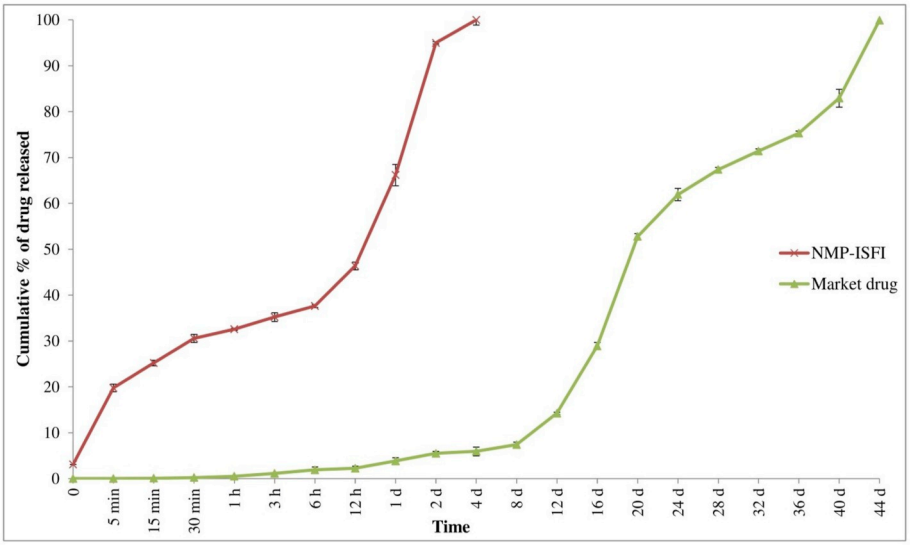


**Fig. 1.** *In-vitro* release profiles of risperidone-loaded ISFI formulation containing NMP and marketed risperidone microspheres.

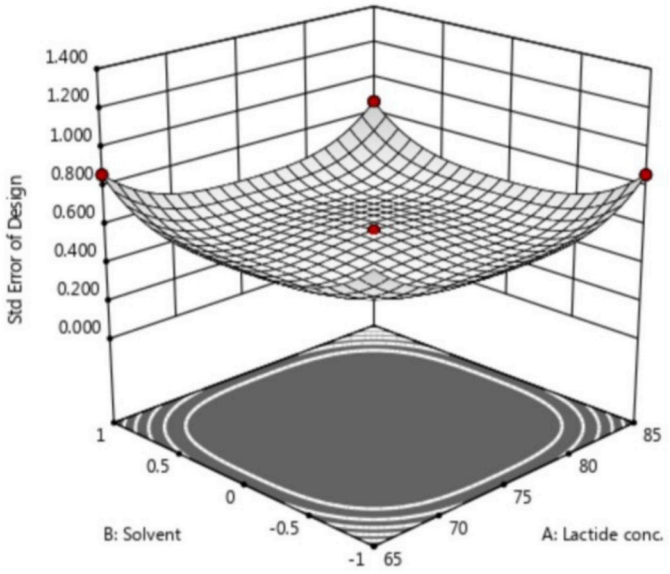


**Fig. 2.** Standard error graph of BBD design in 3D view.

relatively minimum values of standard error close to 1 or lower across the area of interest. Where, these values were only indicative of the validation of design and not of the tested responses [36]. The results revealed that the standard error was ranged between 0.562 and 0.800, hence implying the efficient potential of prediction of the design.

In the present study, ANOVA was applied at 95% confidence level to evaluate the model significance. As presented in Table 3, the model was assigned statistically significant if the p-value is less than 0.05. The model p-values observed for $Y_1$, $Y_2$ and $Y_3$ responses were 0.0001, 0.0005 and 0.0006 respectively. This declared that the independent variables manifested significant effects on the tested responses away from experimental errors or chances. Besides, this illustration would be confirmed by greater values of F-ratio where their low values elucidated more error in the model [27]. The rank order of the model predicting the capability of responses was determined as follow; $Y_1 > Y_2 > Y_3$ which was based on small p-values and high values of F-ratios.

In addition, lack of fit values could be used to inspect the efficiency of model taking into consideration of their p-values where non-significant values of lack of fit were good and fitted the satisfactory model [37]. The values of lack of fit for the observed dependent responses were 0.8266, 3.03 and 5.80 with p-values of 0.5882, 0.2578 and 0.1506 for $Y_1$, $Y_2$ and $Y_3$ respectively (Table 3). This concluded that lack of fit values were not significant and the chance for this large values due to noise were 58.82, 25.78 and 15.06% respectively.

Total 15 formulations were prepared for optimization of the 3 independent variables (A, B and C) and then characterized to analyze the influence exerted on the observed dependent responses ($Y_1$, $Y_2$ and $Y_3$). The relationship between independent and dependent variables was studied by plotting the 3D response surface graphs (Figs. 3, 7 and 8).

### *3.2.1. Effect of independent variables on burst release after 6h ($Y_1$)*

The release profile of risperidone from the developed ISFI systems showed a tri-phasic pattern as depicted in Figs. 4–6. At first 6h, the release profile exhibited an initial release period known as burst release. The possible mechanism for this stage could be due to the lag time between administration of the liquid drug-loaded polymeric system and solidification of the ISFI in the aqueous medium. During the burst interval, the solvent and dissolved drug rapidly transited out of the ISFI resulting in the initial burst release [6,38]. In addition, free risperidone might be released from the outer surface of solidified ISFI or from irregularly distributed drug-loaded polymeric matrix [27,39]. The burst release period was then followed by slow or approximately constant release phase, called diffusion phase, which persisted for several weeks. Finally, a rapid release of drug took place owing to the fast erosion or degradation of PLGA co-polymer [40].

Results mentioned in Table 3 showed the significance of the model because of the high F-ratio (62.76) with p-value of 0.0001. This revealed that the chance for this large F-ratio to occur due to noise is only 0.01%. In our study, A, B, C, BC, $A^2$ were significant terms owing to their significant p-values. Otherwise, insignificant p-values greater than 0.1 were indicative for insignificant model terms. The predicted $R^2$ of 0.9135 was in feasible agreement with the adjusted $R^2$ of 0.9754 where the difference between them was less than 0.2. Also, adequate precision quantified the ratio of signal to noise. The desirable adequate precision of 26.5342 (greater than 4) indicated an adequate signal and the model could navigate the design space.

The polynomial equation attained for this model was:

$$Y_1 = 18.69 - 2.51A - 11.41B + 11.81C - 0.99AB + 1.37AC - 8.31BC + 3.70A^2 + 2.54B^2 - 0.91C^2$$

According to the regression equation, the positive sign in the equation indicates synergistic effects and the negative sign means antagonistic effects on the studied response.[24] This equation stated that

**Table 3**
Statistical analysis results of responses.

| Source | $Y_1$ | | $Y_2$ | | $Y_3$ | |
|---|---|---|---|---|---|---|
| | F-ratio | p-value | F-ratio | p-value | F-ratio | p-value |
| **Model** | 62.76 | 0.0001* | 36.30 | 0.0005* | 34.76 | 0.0006* |
| **A** | 11.57 | 0.0192* | 40.85 | 0.0014* | 0.1657 | 0.7008 |
| **B** | 239.46 | < 0.0001* | 41.01 | 0.0014* | 137.79 | < 0.0001* |
| **C** | 229.96 | < 0.0001* | 121.00 | 0.0001* | 34.85 | 0.0020* |
| **AB** | 0.9012 | 0.3861 | 1.36 | 0.2955 | 0.5893 | 0.4774 |
| **AC** | 1.72 | 0.2467 | 0.2337 | 0.6492 | 0.1473 | 0.7169 |
| **BC** | 63.53 | 0.0005* | 2.58 | 0.1692 | 12.61 | 0.0164* |
| **$A^2$** | 11.59 | 0.0191* | 12.20 | 0.0174* | 1.55 | 0.2684 |
| **$B^2$** | 5.48 | 0.0663 | 86.46 | 0.0002* | 123.42 | 0.0001* |
| **$C^2$** | 0.8013 | 0.4117 | 16.83 | 0.0093* | 0.7671 | 0.4212 |
| **Lack of fit** | 0.8266 | 0.5882 | 3.03 | 0.2578 | 5.80 | 0.1506 |
| **$R^2$ analysis** | | | | | | |
| **$R^2$** | 0.9912 | | 0.9849 | | 0.9843 | |
| **Predicted $R^2$** | 0.9135 | | 0.7962 | | 0.7706 | |
| **Adjusted $R^2$** | 0.9754 | | 0.9578 | | 0.9560 | |
| **Adequate Precision** | 26.5342 | | 20.6018 | | 18.7159 | |

A, lactide concentration in PLGA co-polymer; B, solvent type; C, solvent/PLGA ratio; $Y_1$, burst release after 6h; $Y_2$, cumulative release after 40 days; $Y_3$, injectability time; $R^2$, multiple correlation coefficient; *, significant.

the variables (A and B) denoted a negative effect on the burst release, whereas the variable (C) conferred a positive effect on the burst release.

As per data of Table 2, the burst release varied from 4.75% (F10) to 50.60% (F2). In order to control the release of risperidone from ISFI, it was noteworthy to study different PLGA grades with various hydrophobic lactide contents (65, 75 and 85%). The proportion of lactic acid was fundamental for studying the crystallinity and water uptake of the formulation, hence the rate of *in-vivo* degradation [41]. The initial burst release was reduced by increasing the lactic acid concentration in ISFI systems (Fig. 3). As shown in Table 2, the burst release rate after 6h decreased by the following order; F4 > F1, F5 > F14, F11 > F10 and F15 > F12. The formulations showing higher burst release after 6h (F4, F5, F11 and F15) had lower ratio of lactide (65%), while others showing lower burst release contained higher lactide ratio (85%) (Fig. 4). These results would be ascribed to the higher lipophilicity of PLGA co-polymer absorbing less amount of water and the faster solidification of ISFI after increasing the lactide ratio. Consequently, the solvent diffused at a slower rate and more amount of drug remained in the ISFI.

The nature of the tested solvent is also a key factor that impacts on the burst release. When the drug solubility increases in the solvent, a higher burst release is observed due to the elevated free drug present in the ISFI system [42]. As represented by Fig. 3, the burst release rate decreased as follow; triacetin > ethyl acetate > DMSO. This might be ascribed to the higher solubilization capacity of triacetin than other solvents and more amounts of free risperidone diffused into the liquid medium during the ISFI solidification. In addition, the lower miscibility of triacetin and ethyl acetate with water enabled a lag period between the administration and solidification of ISFI systems that accelerated the diffusion of risperidone into the surrounding medium. These results were in accordance with Wang et al. [35]; Enayati et al. [43]. On the other hand, the rapid solidification of DMSO ISFI formulations supported the entrapment of dispersed drug followed by a lower burst effect. Kilicarslan et al. [44] reported that the water misciblilty of the solvent would facilitate the faster phase inversion dynamics. Where, the faster influx of water into the polymer solution and the faster out flux of DMSO caused a rapid solidification of the water-immiscible polymer. As a result, a more efficient drug entrapment, a lower initial burst and a more continuous release could be accomplished.

As per data of Table 2, the burst release rate after 6h reduced by the following order; F2 > F8, F15 > F5, F7 > F6 and F12 > F14. The formulations showing higher burst release after 6h (F2, F15, F7 and F12) were formulated by triacetin that possessed higher solubilization capacity of risperidone and lower miscibility with water. While, other formulations (F8, F5, F6 and F14) represented characteristic lower burst effect owing to the distinctive properties of DMSO such as lower solubilization capacity of risperidone and higher miscibility with water (Fig. 5).

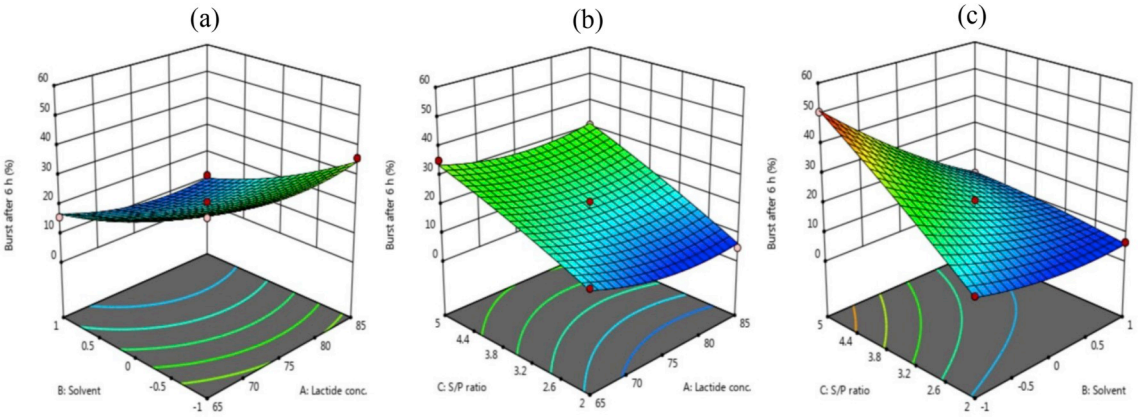


**Fig. 3.** 3D response surface graphs of influence of (a) A and B on $Y_1$ (b) A and C on $Y_1$ (c) B and C on $Y_1$.

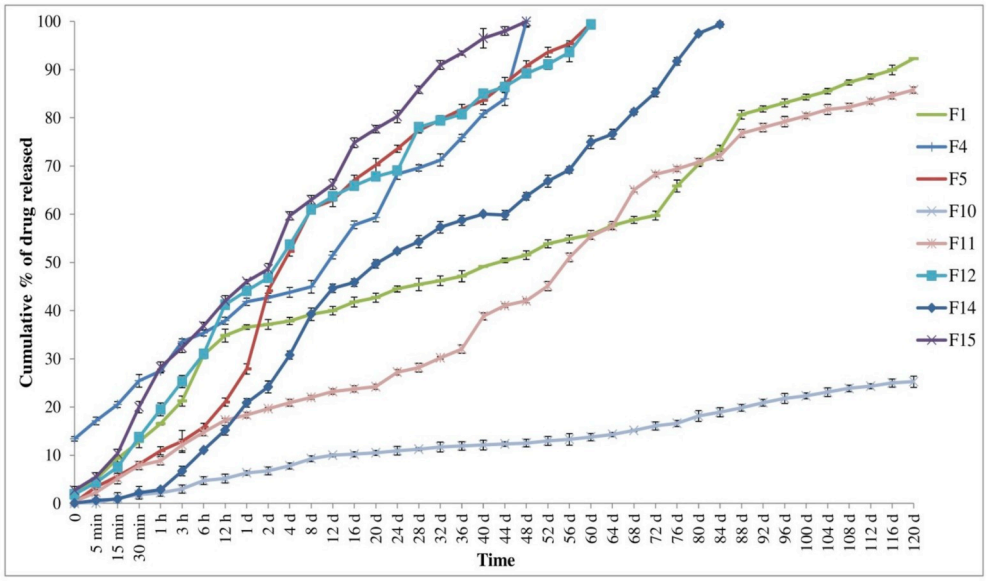


**Fig. 4.** *In-vitro* release profiles of risperidone-loaded ISFI formulations: influence of A on $Y_1$ and $Y_2$.

Another modulated way to minimize the burst release from ISFI is by increasing the system viscosity by increment of PLGA concentration or reduction of solvent concentration (Fig. 3). This could result in thickening the co-polymer skin, delaying the exchange of solvent and water and thus creating a less porous surface [45,46]. In addition, burst effect could be affected in a positive way by the concentration of solvents used in the ISFI formulations. This was consistent with Ibrahim et al. [27] who confirmed the higher drug release from the implant system that has high amount of solvent compared to that with less solvent concentration.

The aforementioned explanations could be supported by the results mentioned in Table 2 in which the burst release reduced by this sequence; F1 > F10, F2 > F7, F4 > F11 and F8 > F6. The formulations (F1, F2, F4 and F8) presented lower burst release because of their high S/P ratio at 5:1 when respectively compared to the F10, F7, F11 and F6 formulations that included lower S/P ratio (2:1) (Fig. 6).

*3.2.2. Effect of independent variables on cumulative release after 40 days ($Y_2$)*

As presented by Table 3, the high F-ratio of 36.30 with p-value of 0.0005 implied that the model was significant and there was only a 0.05% chance that this F-ratio occurred due to noise. In this model, A, B, C, $A^2$, $B^2$, $C^2$ were significant model terms because of their significant p-values, while other terms were not significant. Also, the predicted $R^2$ (0.7962) was in reasonable agreement with the adjusted $R^2$ (0.9578). The desirable adequate precision of 20.6018 pointed out that the model could express the design space.

The polynomial equation was determined as follow:

$$Y_2 = 46.87–11.69A – 11.71B + 20.11C–3.02AB – 1.25AC – 4.15BC + 9.40A^2 + 25.02B^2 – 11.04C^2$$

After the initial burst release of risperidone, the slower sustained diffusion phase was followed up. The cumulative release of risperidone after 40 days differed within the range of 12.12% (F10) to 99.43% (F2) as presented in Table 2. The lactide ratio in the PLGA co-polymer

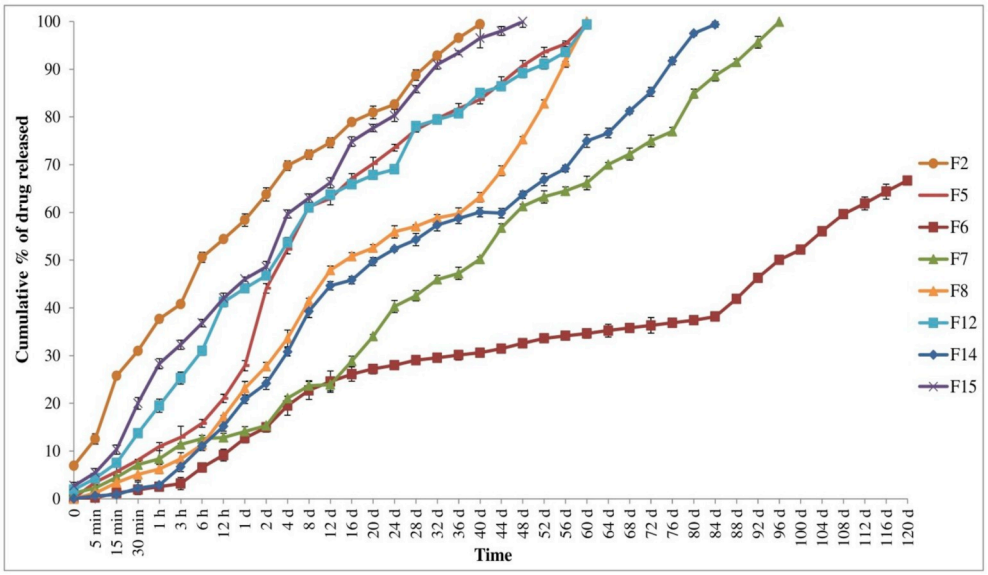


**Fig. 5.** *In-vitro* release profiles of risperidone-loaded ISFI formulations: influence of B on $Y_1$ and $Y_2$.

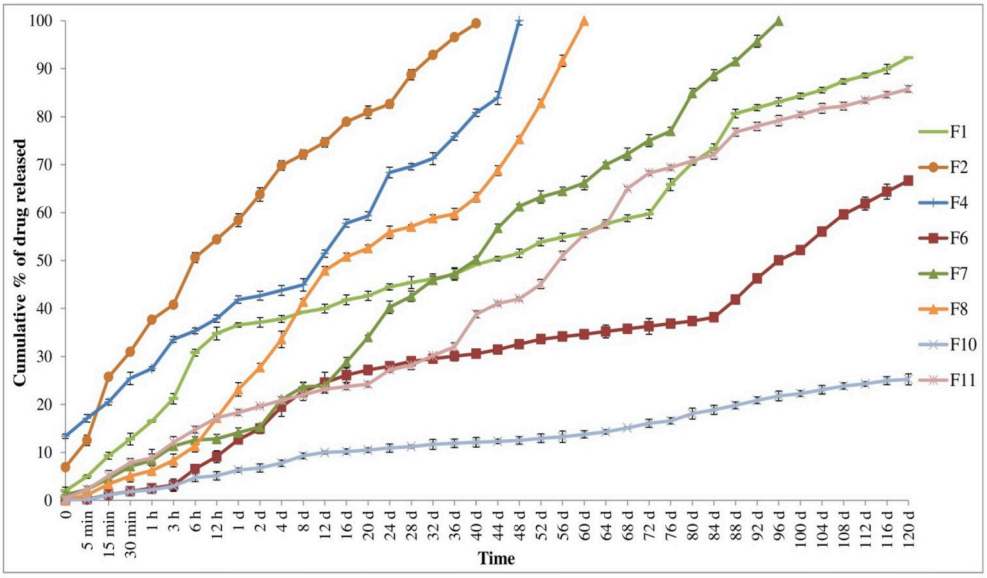


**Fig. 6.** *In-vitro* release profiles of risperidone-loaded ISFI formulations: influence of C on $Y_1$ and $Y_2$.

displayed a critical role in influencing the extended release of risperidone from ISFI [47] (Fig. 7). As mentioned in Table 2, the cumulative release after 40 days decreased by the following order; F4 > F1, F5 > F14, F11 > F10 and F15 > F12. The formulations (F4, F5, F11 and F15) showed higher cumulative release after 40 days because of their lower lactide concentration at 65%. While, other comparable formulations (F1, F14, F10 and F12) having lactide concentration of 85% exhibited lower cumulative release after 40 days (Fig. 4). These results could explain the limited critical water concentration at a lactide concentration of 85% in comparison to 75 and 65% levels of lactic acid. By a greater ratio of lipophilic lactic acid moiety in PLGA co-polymer, decreased proportions of water absorption would be exhibited followed by a slower drug release rate. These explanations were in agreement with the research work of Patel et al. [20].

According to the data of risperidone solubility in the studied organic solvents, lower solubility of drug in ethyl acetate and DMSO favored the risperidone drug to be more entrapped in the ISFI systems during the solution-gel turnover achieving a distinct sustained release (Fig. 7). On the other hand, Wang et al. [35] reported that the noticeable fast solidification of ISFI using DMSO owing to the immediate diffusion of DMSO that reflected the high retention of risperidone. As per data of Table (10), the order of cumulative release after 40 days could be displayed as follow; F2 > F8, F15 > F5, F7 > F6 and F12 > F14. The cumulative release rates of the F2, F15, F7 and F12 formulations after 40 days were raised owing to the higher drug solubilization capacity of triacetin followed by higher burst release of risperidone. In comparison, other formulations (F8, F5, F6 and F14) showed a noticeable lower cumulative release after 40 days as a result of lower solubilization and higher retention of risperidone caused by DMSO (Fig. 5).

Furthermore, the negative impact of PLGA concentration can be observed on the $Y_2$ response as shown in Fig. 7. This could be illustrated by the basis of diffusion path length where increasing the proportion of PLGA concentration in ISFI represented an increase in PLGA molecules compared to drug molecules. This result explained the prominent tendency of PLGA co-polymer for more entrapment of risperidone molecules, reduction of the amount of drug escaped to the surrounding phase and thus the reduction of the cumulative drug release [48]. In addition, the viscosity of ISFI would be controlled by modulating the concentration of solvent. Parent et al. [34] pointed out the effectiveness of lesser amounts of solvents on yielding high viscosity and delaying the liquid-liquid mixing while formulating the ISFI preparations. Thus, retardation of entrance of water and deceleration of drug diffusion were resulted.

The data of Table 2 highlighted the effect of alteration of S/P concentration on the cumulative release percent that reduced by the following order; F1 > F10, F2 > F7, F4 > F11 and F8 > F6. The higher ratio of S/P at 5:1 in the formulations (F1, F2, F4 and F8) produced higher cumulative release rates than those of the F10, F7, F11 and F6 formulations respectively due to their lower S/P ratio (2:1) (Fig. 6).

### 3.2.3. *Effect of independent variables on injectability time ($Y_3$)*

The model was significant because the F-ratio was high (34.76)

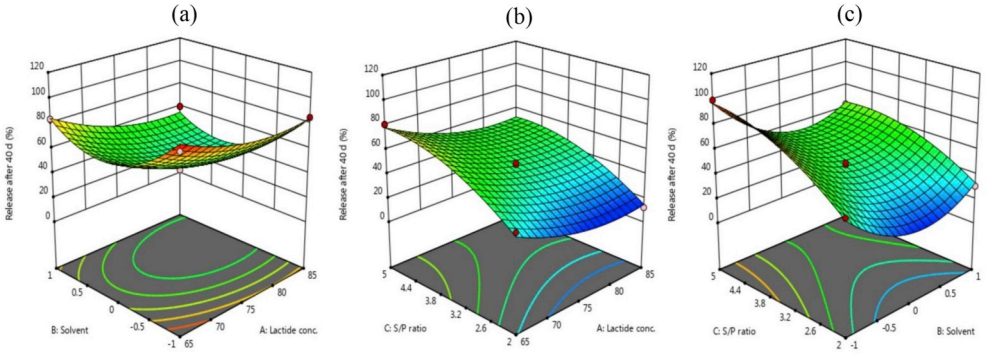


**Fig. 7.** 3D response surface graphs of influence of (a) A and B on $Y_2$ (b) A and C on $Y_2$ (c) B and C on $Y_2$.

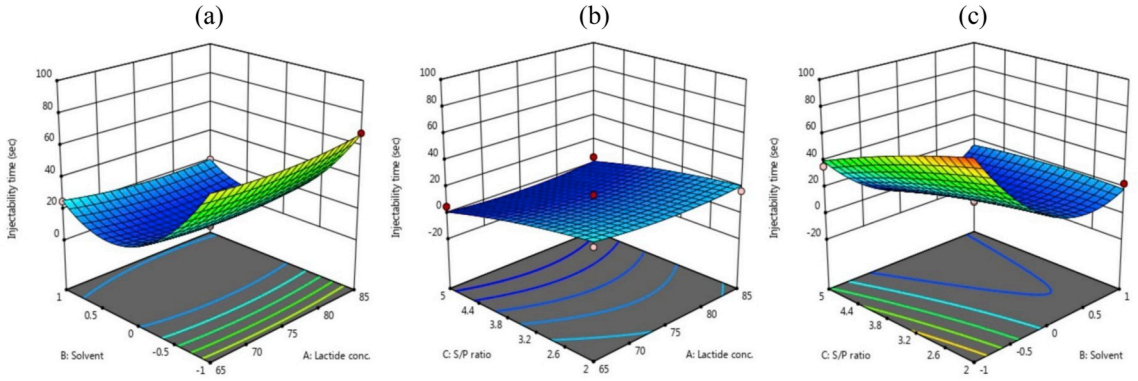


**Fig. 8.** 3D response surface graphs of influence of (a) A and B on $Y_3$ (b) A and C on $Y_3$ (c) B and C on $Y_3$.

having p-value of 0.0006. This implied the chance for this large F-ratio to occur due to noise is only 0.06%. Only B, C, BC, $B^2$ with significant p-values were significant terms while others were insignificant. The predicted $R^2$ of 0.7706 showed a good correspondence with the adjusted $R^2$ of 0.9560. In addition, the signal to noise ratio at 18.7159 provided an appropriate signal and the ability of model to navigate the design space (Table 3).

The polynomial equation was obtained as:

$$Y_3 = 11.00–0.75A – 21.62B–10.88C – 2.00AB + 1.00AC + 9.25BC + 3.38A^2 + 30.13B^2 – 2.38C^2$$

The time needed to push the developed formulations through the syringe needle after preparation of ISFI was recorded in the range between 5 (F1 and F4) and 84 s (F7) as mentioned in Table 2. It was noticed that the tested solvents possessed a satisfactory solvating potential for the PLGA co-polymer during the development of ISFI. However, triacetin required more time for homogeneous gel formation and more force for injecting the formulations through the syringe needle (Fig. 8). This might be attributed to the higher viscosity of triacetin (17.4 cP) than other solvents (2 cP for DMSO and 0.0261 cP for ethyl acetate) as reported by Rowe et al. [31]. These results confirmed the essential impact of controlling the formulation viscosity during the phase inversion in order to obviate the drug leakage to the external liquid medium. Where, high viscosity of triacetin demonstrated less spreading and retarded diffusion into the external buffer. Therefore, a lag time between administration and solidification of ISFI was observed. These findings resembled those obtained by Gad [49]. As per data of Table 2, the injectability time decreased by this order; F2 > F8, F15 > F5, F7 > F6 and F12 > F14. The formulations containing triacetin (F2, F15, F7 and F12) represented difficult injectability at 36.00, 65.00, 84.00 and 68.00 s respectively when compared to those prepared by DMSO solvent. These results confirmed the delayed solidification of ISFI using triacetin as a result of retarded spreading and diffusion of such solvent.

Moreover, the injectability time for the prepared ISFI formulations displayed an observed increase with the accretion of PLGA concentration (Fig. 8). This was followed by more hindrance of injectability and greater force for ISFI injection [30,41]. This could be attributed to that the viscosity of the prepared ISFI formulations could increase slightly after incorporation of specific concentration of the solvent. This was confirmed by Ahmed et al. [40] who studied the effect of low concentration of viscous polyethylene glycol solvent on reduction of injectability.

The findings could be explained by the results presented in Table 2 where the injectability time decreased as follow; F10 > F1, F7 > F2, F11 > F4 and F6 > F8. The formulations of lower S/P ratio at 2:1 (F10, F7, F11 and F6) implied difficult injectability at 17.00, 84.00, 21.00 and 23.00 s respectively in comparison to the F1, F2, F4 and F8 formulations having higher S/P ratio at 5:1 (5.00, 36.00, 5.00 and 12.00 s respectively).

### 3.3. Optimization of studied variables

The optimization technique was implemented to reproduce the optimum characteristics and desirable levels of constraints (Table 1) based on the proximity of the desirability value towards 1. The optimized ISFI formulation was selected depending on minimizing the burst release after 6h and injectability time in addition to targeting the cumulative release after 40 days to 50%.

According to the desired dependent responses, the optimized ISFI formulation was attained by the mixture of PLGA co-polymer (75:25) and DMSO with S/P value of 2.851. The predicted values given by the BBD for the optimized ISFI formulation were 7.87%, 49.99% and 19.08 s for $Y_1$, $Y_2$ and $Y_3$ respectively with a desirability value of 0.937. After preparation and characterization of optimized ISFI, the experimental values for the desirable responses were recorded as 8.1% for burst release after 6h, 51.08% for cumulative release after 40 days with injectability time of 18 s. In the meanwhile, these predicted and experimental results were in a feasible agreement implying the rationality and validity of BBD outcomes.

Compared with the optimized risperidone-loaded ISFI, the commercial microspheres (Risperdal Consta™) represented an obvious slow release of risperidone after 6h and continued for about two weeks to reach the significant drug release. As presented by Fig. 9, 1.89 ± 0.50% of risperidone was released in the first 6h followed by a rapid drug release of 99.91 ± 0.72 after 44 days. These results proved the necessity of schizophrenic patients to take oral risperidone tablets during the mentioned lag period. Therefore, utilization of ISFI systems emphasized their potential to customize therapy for patients with schizophrenia by enhancing compliance and eliminating costs issues of additional oral therapy.

### 3.4. Characterization of optimized ISFI formulation

#### 3.4.1. Fourier transform infrared (FTIR) spectroscopy

The FTIR study was carried out in order to inspect the probable interactions of risperidone with ISFI excipients by investigating the reduction, shifting or disappearance of absorption bands of studied samples (Fig. 10). Pure risperidone manifested various distinctive absorption bands of different groups as follow; aromatic C–H (3060 $cm^{-1}$), aliphatic C–H (2946 and 2758 $cm^{-1}$), C=O (1660 $cm^{-1}$), aromatic C=C (1536 $cm^{-1}$), C=N (1448 $cm^{-1}$), aliphatic C–H bending (1411 $cm^{-1}$), C–N (1350 $cm^{-1}$), C–F (1130 $cm^{-1}$) and aromatic C–H bending (860 $cm^{-1}$). The spectrum of PLGA showed absorption bands at 3499, 2950, 1758, 1458, 1169 $cm^{-1}$ for O–H, aliphatic C–H, C=O, aliphatic C–H bending and C–O stretchings respectively. The FTIR spectrum of the physical mixture prepared by mixing risperidone with PLGA displayed no change in the absorption

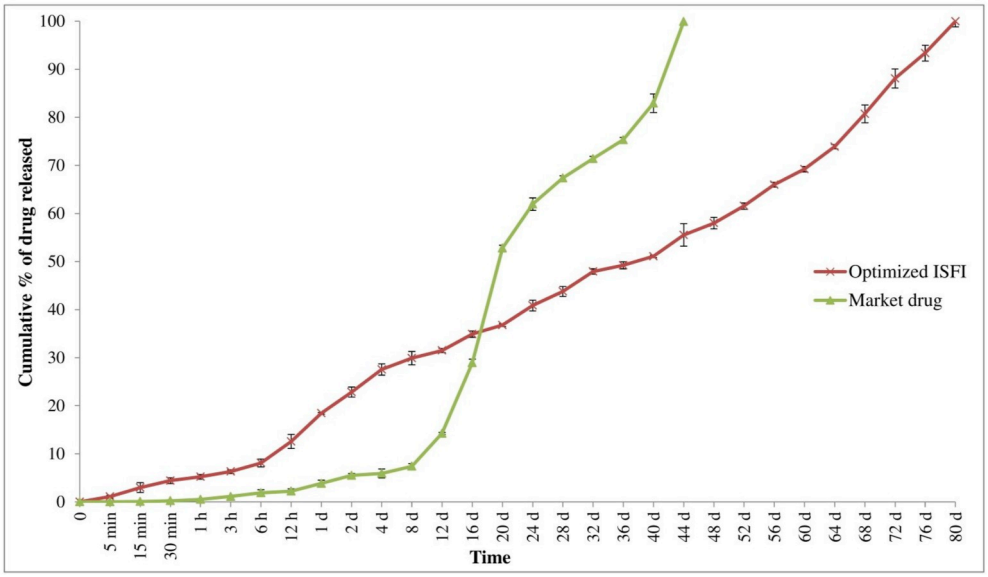


**Fig. 9.** *In-vitro* release profiles of optimized risperidone-loaded ISFI formulation and marketed risperidone microspheres.

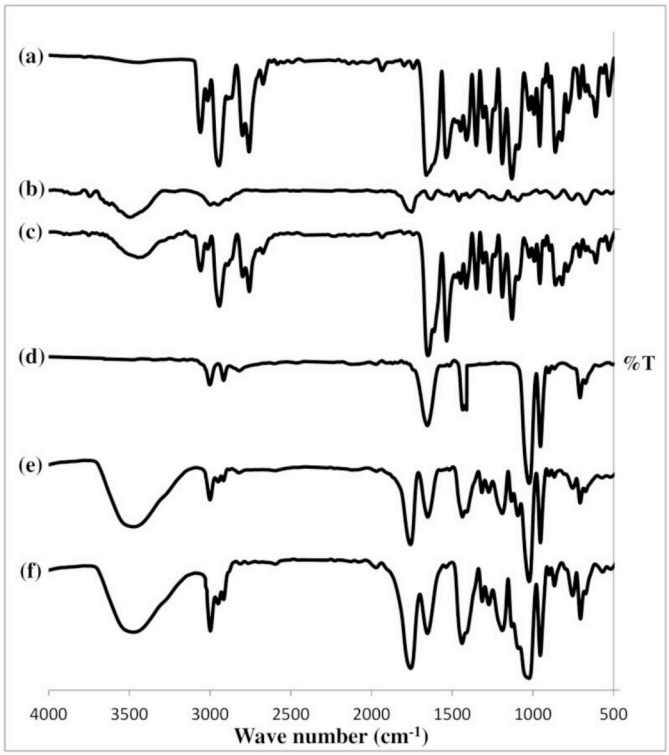


**Fig. 10.** FTIR spectra of (a) pure risperidone (b) PLGA co-polymer (c) physical mixture (d) DMSO (e) blank ISFI formulation (f) optimized ISFI formulation.

bands of the two components. This result revealed that there was no significant chemical interaction between risperidone and PLGA in the solid state.

The DMSO solvent showed various characteristic peaks for aliphatic C–H groups (3001 and 2916 $cm^{-1}$), C–S groups (1653 and 1434 $cm^{-1}$), S═O group (1029 $cm^{-1}$) and C–H bending (953 $cm^{-1}$). On evaluation of FTIR spectrum of blank ISFI formulation, considerable changes in the PLGA absorption bands were observed. This might be attributed to the formation of intermolecular hydrogen bonds between sulfoxide group of DMSO and free hydroxyl groups of PLGA. These results could be emphasized by the formation of obvious broad peak of O–H group at 3460 $cm^{-1}$ and sharp peaks of C═O and C–O groups at 1751 and 1169 $cm^{-1}$ respectively. Therefore, the possible chemical interactions between PLGA with DMSO were confirmed. In comparison, the optimized ISFI formulation showed no significant change in the absorption bands of the FTIR spectrum. These results indicated the absence of structural perturbation and chemical interactions between drug and excipients. These findings were in agreement with Fitriani et al. [50]; Kapoor et al. [51].

### 3.4.2. Differential scanning calorimetry (DSC)

The DSC study was accomplished in order to perceive the nature of risperidone in ISFI and the alteration of its crystallinity. This could be observed by the shift or disappearance of thermal peaks of the studied samples as shown in Fig. 11. Pure risperidone demonstrated a single sharp endothermic peak at 171.00 °C that confirmed its crystalline condition at this characteristic melting point. The DSC thermogram of PLGA co-polymer showed an endothermic peak at 47.15 °C corresponding to the glass-transition temperature of the polymer. The thermal profile of the physical mixture demonstrated that the endothermic peaks of PLGA and risperidone were still present indicating no interactions between the two components in the solid state.

The DMSO solvent did not exhibit any thermal peak. In addition, the blank ISFI sample represented a disappearance of the endothermic peak of PLGA co-polymer. These results indicated the possible interaction of PLGA with DMSO solvent. On the other hand, the thermal profile of optimized ISFI formulation showed the presence of the characteristic peak of risperidone with a shift to 164.66 °C. These findings indicated the encapsulation of most part of drug in a crystalline state when formulated in the optimized ISFI formulation. This might be ascribed to the lower saturated solubility of risperidone in the PLGA-DMSO mixture and consequently could result in a retardation of drug release from ISFI. These observations were consistent with Shang et al. [52].

## 4. Conclusions

In the present study, risperidone-loaded ISFI systems were successfully developed by using biodegradable PLGA co-polymer with assistance of different organic solvents. With the help of BBD, three factors at three levels ($3^3$) were selected to study how the independent variables influence the dependent responses after preparation of ISFI. Variables A and B denoted negative effects on the burst and cumulative release, whereas variable C represented its positive effect on them. In

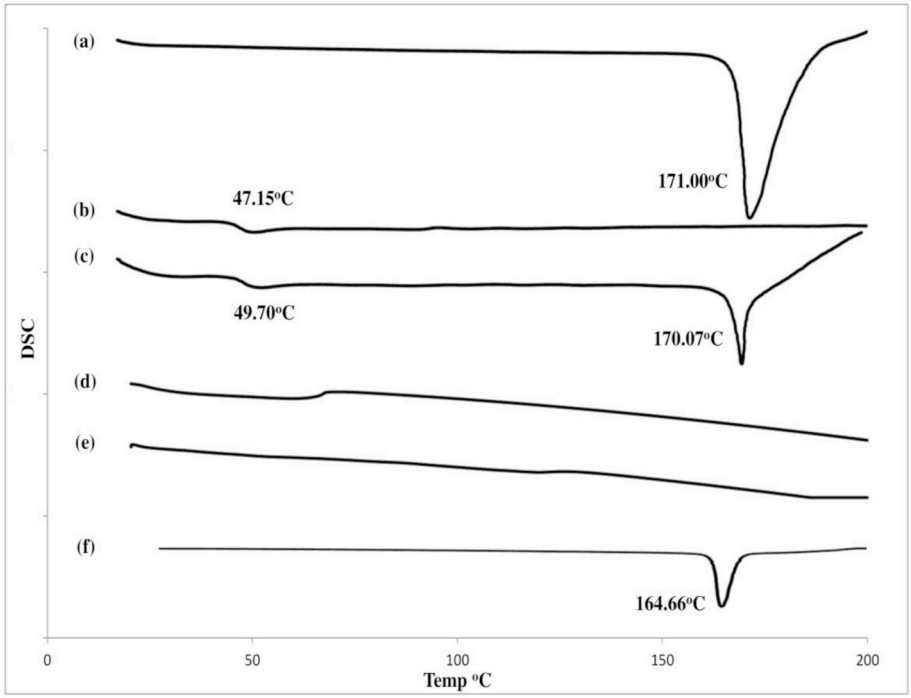


**Fig. 11.** DSC thermograms of (a) pure risperidone (b) PLGA co-polymer (c) physical mixture (d) DMSO (e) blank ISFI formulation (f) optimized ISFI formulation.

addition, the negative action of three independent variables (A, B and C) on injectability time was evident. In comparison to marketed microspheres, proper optimization and selection of ISFI components with desirable constraints levels in the study had verified the great potential to keep schizophrenic treatment regimens away from oral tablets co-administration. The identification of the behavioral and biochemical alterations in induced schizophrenic rats while being injected with the optimized risperidone-loaded PLGA ISFI formulation are in progress.

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## CRediT authorship contribution statement

**Tarek M. Ibrahim:** Conceptualization, Methodology, Software, Writing - original draft. **Nagia A. El-Megrab:** Validation, Investigation, Writing - review & editing. **Hanan M. El-Nahas:** Resources, Data curation, Writing - review & editing, Supervision.

## Declaration of competing interest

The authors report no conflicts of interest.

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