Study of homogenization on media milling time in preparation of irbesartan nanosuspension and optimization using design of experiments (DoE)

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Abstract

Background: The present investigation aimed at preparing nanosuspension of irbesartan to improve its dissolution. Dissolution enhancement of irbesartan can improve the oral bioavailability. Here, it was also studied how media milling time can be reduced by subjecting irbesartan to prior homogenization and then media milling.

Results: First, homogenization of irbesartan was carried out in the presence of poloxamer 407 at 6000 rpm for 2 h. Final nanosuspension preparation was done by media milling with zirconium dioxide beads. Here, the amount of poloxamer 407 and zirconium dioxide beads was studied as statistical independent variables. Response surface plot analysis and desirability function were applied to the selected optimized batch. The prepared batches were subjected to evaluation for zeta potential value, mean particle size, PDI, dissolution study, and stability study. Target particle size was less than 500 nm, and in vitro dissolution in 10 min was more than 80%. Zeta potential value was ~27 mV for optimized nanosuspension. Desirability of 0.941 was achieved. Checkpoint batch was prepared and evaluated to confirm the validity of mathematical model. Accelerated stability study was performed on the optimized batch at 40 ± 2 °C/75 ± 5% RH for 6 months.

Conclusion: The results confirmed the stability of formulation at accelerated stability conditions. Using presuspension prepared by homogenization, media milling time primarily reduced from 24–28 h to 18 h. Future perspective is to study other factors in combination method in discrete.

Background

Solubility in aqueous media, susceptibility to exposed temperature and humidity, photostability, and compatibility with solvent and excipients plays a vital role while formulating a drug product [1–3]. While developing a formulation for new drug molecule, one needs to keep in mind the solubility of it. It presents a serious problem with regard to its dissolution and therefore the bioavailability. Lipophilic compounds are a major share in new drug product research that finally reaches to market [4, 5]. For the compounds with high log \( P \) value and water insoluble, nanosuspension with mean particle size typically between 10 to 1000 nm is preferred [6, 7]. Conventionally liposome, some emulsion based systems are formulated for lipophilic drugs, but these lipid-based formulation approaches are invalid for some drugs. The compounds with high log \( P \) value, high dose, and high melting point generally work best with nanosuspension formulation [8, 9].

Irbesartan is BCS class II drug, exerting low solubility in water and high permeability through GI membrane. Due to the poor solubility, it causes problem of dissolution [10]. Irbesartan, used orally for treatment of hypertension, is a non-peptide antagonist, specific competitive

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of the angiotensin II receptor (AT1 subtype). Unlike ACE inhibitors, angiotensin receptor blocker (ARBs) does not have the adverse effect of dry cough. These drugs may be associated with an increased risk of cancer [11]. Solid dispersion, complexation with cyclodextrins, and self-nanoemulsifying drug delivery are the approaches that have been previously utilized to resolve poor aqueous solubility of irbesartan [12, 13]. Solid dispersion has disadvantage of increasing the bulk of dose [14]. In addition, the chances of crystallization are more which in turn shadows the initial dissolution in case of solid dispersion. In case of solid dispersion, one needs to stabilize the drug molecules in dissolved form, may be by the use of polymers. This makes the process of solid dispersion formulation even complex. Cyclodextrins, when used, increases the total weight of formulation and degree of complexation and its reproducibility is again in question. Nanosuspension gives freedom on the selection of appropriate dosage form for its target delivery. Improvement in dissolution of irbesartan could lead to direct improvement in bioavailability, as unlike most drugs, its absorption is independent of food effect. Irbesartan as such is an orally active molecule and no biotransformation is required to be active. Irbesartan is available in various doses ranging from 75 to 300 mg and exhibits linear pharmacokinetics. Now, to produce ultrafine drug particles, there are top-down and bottom-up techniques that can be used [15]. Nanosuspension can improve the drug dissolution rate, after the drug is available in solubilized form in the GI tract. It could improve the absorption and bioavailability of drug. Media milling is a simple and well-established technique for nanosuspension production. Several drug nanosuspensions were already prepared by using media milling such as fenofibrate and morphine sulfate.

The present research work aimed at the investigation of formulation possibility of irbesartan nanosuspension for dissolution enhancement leading to improved drug oral bioavailability. The study is designed to determine the effect of homogenization on media milling time and its effect on mean particle size. The effects of suspension stabilizer and amount of zirconium beads were studied in the present investigation. The design of experiments was used to statistically optimize the formulation.

**Materials**

Irbesartan was a gift sample from Amneal Pharmaceuticals, Ahmedabad. Poloxamer 407 and 188, HCl, and methanol of analytical grade were purchased from SD Fine Chem, Mumbai, India. PVP K30 analytical grade was purchased from Colorcon, India. All other reagents were of analytical grade and used without further purification.

**Methods**

**Fourier-transform infrared spectrum study**

Identification of drug was done by IR spectra, taken for individual drug and in combination with excipients for the comparison. Drug to excipient ratio of 1:1 was taken to prepare the physical mixture that is subjected to 24 h desiccation. FTIR spectra were recorded with a spectrophotometer (Shimadzu, Japan), in the range 450-4000 cm⁻¹; resolution of 4 cm and 45 scans were used. The next step was to dilute the drug with KBr to form discs that were self-supporting after pressing.

**Saturation solubility study**

The solubility of irbesartan in solvent was determined after excess addition in the presence of different stabilizers. Thereafter, magnetic stirring of the mixture for 48 h at 37 °C was carried out; the content was then centrifuged at 5000 rpm, 10 min duration, and analyzed at 244 nm after apt dilution (Shimadzu, UV-1700, double beam UV-visible spectrophotometer, Japan).

**Optimization of preliminary parameters**

Different variables were selected to carry out preliminary studies for preparation of design formulations based on evaluation of particle size. Here, two methods were studied, i.e., homogenization and media milling. In preliminary study, various preliminary process parameters like homogenization speed, homogenization time, milling time, and quantity of bead were optimized by varying a factor at a time and by keeping the other parameters invariable, in order to check the effect of diverse parameter. The mean particle size in nanometers was selected as the deciding evaluation parameter for different factors. Poloxamer 407 was used as the stabilizer in preliminary studies. Homogenization speed was selected in the range of 2000–8000 rpm for studies, and the prepared suspension was evaluated for the mean particle size with homogenization time of 1 h. This study was repeated for homogenization time up to 6 h with 1 h increment. Six different studies were performed in order to determine the ideal homogenization time and speed.

The effect of media milling time on the resulting suspension particle size was studied in the range of 2–28 h. After studying the above parameters, it was decided to check the effect of the combination method, i.e., presuspension prepared by homogenization and then subjected to media milling. The aim was to check the required time to get a particle size below 500 nm for nanosuspension with and without presuspension.

**Method to prepare nanosuspension by homogenization followed by media milling**

First, the presuspension was prepared by homogenizing the suspension in homogenizer at 6000 rpm for 2 h.
These presuspension was subjected to media milling afterwards to convert it in final nanosuspension form [3]. Suspensions of 75 mg irbesartan in 20 ml doubled distilled water were prepared in 20 ml vials using ZrO$_2$ beads (0.7 mm) as a milling medium and different concentrations of stabilizer. Drug was added straight into the stabilizer-containing solution. These mixtures comminute with altered amounts of beads using a magnetic stirrer for 18 h. Decantation and washing with distilled water were carried out for nanosuspension to separate from the beads. The processing temperature was maintained at 35 ± 1 °C. Nanosuspension was freeze dried when necessary by freezing the samples followed by vacuum and degassing to get lyophilized powder.

**Design of experiments**

A 3$^2$ full factorial design was in use as the design of the experiment. Table 1 shows the independent variables, i.e., concentration of poloxamer 407 ($X_1$) with concentration in the range of 37.5 to 112.5 mg and amount of zirconium oxide ($X_2$) in the range of 3.5 to 7.5 gm on the dependent variables’ particle size ($Y_1$); % drug release after 10 min ($Y_2$) was considered for the study and optimization based on the preliminary study and prior art. A statistical model incorporating the interactive and polynomial terms was utilized to appraise the responses.

$$Y = b_0 + AX_1 + BX_2 + ABX_1X_2 + A^2X_1^2 + B^2X_2^2$$

where $Y$ is the dependent variable, $b_0$ is the arithmetic mean response of the nine runs, and $b_i$ is the estimated coefficient for the factor $X_i$. The main effects ($X_2$ and

| Amount of poloxamer 407 (mg) | 37.5 | 75 | 112.5 |
|-----------------------------|------|---|------|
| Amount of ZrO$_2$ (gm)      | 3.5  | 5.5| 7.5  |
$X_2$) represent the average result of changing one factor at a time from its low to high values. The interaction terms ($X_1X_2$) show how the response changes when two factors are simultaneously changed. The polynomial terms ($X_1^2$ and $X_2^2$) are included to investigate non-linearity. Optimum formulation prepared by response surface plot analysis and desirability function was subjected to SEM and DSC studies followed by short-term accelerated stability study.

Evaluation parameters

**Particle size, polydispersity index, and zeta potential**
The prepared nanosuspensions were characterized for particle size, using particle size analyzer (Zetasizer Ver. 6.11 Malvern) at room temperature. Three milliliters of nanosuspension sample was placed inside the sample holder. The instrument was initially allowed to reach its maximum intensity. The analysis was performed using the software provided with the instrument to get the results, and necessary conclusions were made [16].

**Scanning electron microscopy (SEM)**
Scanning electron microscopy was completed to identify the morphology of the irbesartan nanosuspension formulation. Particle morphology was examined using a Hitachi S-4700 microscope with 30 kV acceleration voltages. The samples were imaged on an aluminum mount and sputter-coated with 9 nm of gold/palladium.

**Drug content**
Drug content in the resulting nanosuspension was determined to find loss of drug during processing. The assay of weighed amount of formulations was carried out to determine the drug content. The weighed samples were to be dissolved in 10 ml methanol, stirred by vortex mixer, and filtered using Whatman filter paper. Using the calibration
curve in methanol, the drug content was estimated spectrophotometrically (UV-1700) at 244 nm [17].

**In vitro dissolution study**

In vitro drug release studies were performed by suspending the formulation in 900 ml of 0.1 N HCL as the medium, at a speed of 50 rpm and 37.0 ± 0.2 °C, in USP Apparatus 2 (paddle). Five milliliters of the sample was collected at particular time points for 60 min. Samples were withdrawn through syringe filter (PVDF 33 mm 0.2 μm) [18]. The filtered sample was then subjected to UV analysis versus a blank (0.1 N HCL). The percentage cumulative release of irbesartan was calculated at 244 nm.

**Accelerated stability study**

For stability studies, the formulations were stored in glass vials and stored in a chamber at 40 ± 2°C/75 ± 5% RH for 6 months in a photostability chamber. The samples were evaluated for zeta potential, particle size, and PDI and in vitro drug release study [19]. The results were compared with that of before stability, and conclusion was drawn accordingly of product stability.

### Results

#### Compatibility study

Drug polymer interaction was test out by comparing the FTIR spectrum of the irbesartan and mixture of the irbesartan and poloxamer 407 after due interaction time. Irbesartan spectra in Fig. 1 show the C–H stretching at 2959.72 cm⁻¹, C=O stretching at 1732.08 cm⁻¹, N-H bending at 1616.01 cm⁻¹, and aromatic C=C stretch and bend at 1565.15 cm⁻¹ which are drug characteristics of the functional groups. The physical mixture spectra shows the C–H stretching at 2941.85 cm⁻¹, C=O stretching at 1732.08 cm⁻¹, N-H bending at 1651.85 cm⁻¹, and aromatic C=C stretch and bend at 1540.17 cm⁻¹. These results indicated no interaction.

### Table 2 Characterization of designed batches F1 to F9

| Batches | Particle size (nm) | Zeta potential (mV) | PDI | Drug content (%) |
|---------|--------------------|---------------------|-----|------------------|
| F1      | 291.2 ± 8.88       | −25.89 ± 1.53       | 0.725 ± 0.21 | 98.70 ± 0.32 |
| F2      | 331.0 ± 5.48       | −26.24 ± 0.84       | 0.596 ± 0.13 | 98.89 ± 0.62 |
| F3      | 342.0 ± 11.95      | −27.82 ± 2.13       | 0.531 ± 0.21 | 99.10 ± 0.18 |
| F4      | 320.0 ± 4.87       | −28.45 ± 1.57       | 0.538 ± 0.12 | 99.36 ± 0.65 |
| F5      | 331.0 ± 9.01       | −26.73 ± 0.79       | 0.417 ± 0.26 | 98.61 ± 0.90 |
| F6      | 336.0 ± 4.53       | −28.13 ± 1.16       | 0.529 ± 0.35 | 98.74 ± 0.73 |
| F7      | 228.4 ± 6.93       | −27.32 ± 1.48       | 0.413 ± 0.29 | 100.21 ± 0.12 |
| F8      | 309.0 ± 7.85       | −26.49 ± 1.06       | 0.399 ± 0.17 | 99.80 ± 0.35 |
| F9      | 314.0 ± 6.42       | −27.34 ± 1.77       | 0.410 ± 0.30 | 99.56 ± 0.23 |

Results are average of three readings ± SD

![Fig. 4 Dissolution profile of factorial batches](source URL)
Homogenization speed and time

Batches were prepared at different speeds by keeping the time constant, and the results were as displayed in Fig. 2. Initially, the mean particle size was reduced with the increase in homogenization speed from 2000 to 8000 rpm, with 1000 rpm increment. The results are consistent across batches with a homogenization time of 1 to 5 h. The mean particle size of formulation was in the range of 1730.6 to 957.4 nm. There is a considerable effect of homogenization speed until 6000 rpm with an increment of 1000 rpm in each batch for different homogenization times. The least mean particle size in most cases was found at time 2 h.

Effect of homogenization on media milling time

To study the effect of media milling time on mean particle size, the speed of rotation, amount to drug and stabilizer and concentration of beads were unchanged. The results indicate that as the time increases, the MPS decreases due to more attrition (Fig. 3). The mean particle size of nanosuspension prepared by media milling and without homogenization was found to be 820–1782 nm when studied up to 28 h (Fig. 3). The study was carried out starting with 4 to 28 h. After 24 h, the more milling had an insignificant effect on the particle size, so further study was stopped at 28 h regarding effect of milling time.

Combination method

First, the pre-suspension was prepared by homogenizing the suspension in a homogenizer at 6000 rpm for 2 h. Then, this nanosuspension was subjected to media milling and the mean particle size was in the range of 932.5–314.2 nm. Here, it was observed that initially as the time passes, the size reduced at a rate of approximately 20%, but after 18 h, the reduction rate was reduced afterwards. After homogenization, the particle size reduced to 932.5 nm compared to 1782 nm found after 4 h of media milling. The study was continued up to 28 h for comparison. The end mean particle size of 314.2 nm was obtained after homogenization and 28 h study compared to 820 nm of without homogenization.

Characterization of nanosuspension for final batches

The results of mean particle size, zeta potential, PDI, and drug content of F1 to F9 are in Table 2. The particle size of the designed batches was in the range of 220–350 nm. It was found that the smallest particle size was obtained by formulation F7, 228.4 ± 6.93 nm, as compared to other formulations. All batches show size within the desired range, but the target here was to choose the minimum value for the optimized batch. Zeta potential values were negative 25–30 mV. It did not show significant change and so was not considered during optimization. PDI values range from 0.725 ± 0.21 to 0.399 ± 0.17. For all the

Table 3 Coefficient and $P$ value for full model for variables in the $3^2$ full factorial design

| Term  | $Y_1$       | $P$ value | $Y_2$       | $P$ value |
|-------|-------------|-----------|-------------|-----------|
| $X_1$ | 30.1667     | 0.000332  | $-3.23$     | 1.03E–05  |
| $X_2$ | $-89.8333$  | 1.27E–05  | 12.63       | 0.014     |
| $X_1X_2$ | 9         | 0.019728  | $-0.22$     | 0.00028   |
| $X_1^2$ | 4.5       | 0.205026  | 0.12        | 0.796     |
| $X_2^2$ | 18.5      | 0.006979  | $-4.13$     | 0.918     |

$R^2$ value 0.999

Fig. 5 3D mesh plot of showing the effect of concentration of stabilizer and the ratio of drug to ZrO$_2$ on particle size
formulations, values were less than one, which indicates narrow size distribution. Drug content in all formulation was found in range of 98–100%. In vitro drug release data depends on it, so it prevents the false reading during dissolution study. It also gives idea of the procedural loss of drug. Based on the results, it was decided to choose the mean particle size as one of the dependent variables for optimization. Zeta potential, PDI, and drug content results were acceptable for all batches and in narrow range so they were not considered for optimization.

In vitro dissolution study

Figure 4 shows the percentage cumulative drug release of F1 to F9 after 1 h in simulated gastric fluid, and it was in the range of 62.46 to 95.03%. The results at 10 min were in the range of 49.17 to 81.87%. Hence, the final conclusion was that the assessment and comparison of drug release results at 10 min \(Q_{10}\) could give more insight than the results at 60 min. \(Q_{10}\) was selected as the dependent variable for further optimization analysis. The amount of poloxamer 407 was evaluated at 37.5, 75, and 112.5 mg while the amount of ZrO\(_2\) was at 3.5, 5.5, and 7.5 gm. The result of the multiple regression analysis stated that both factors had statistically significant influence on all dependent variables \((P < 0.05, \text{Table 3})\). The high value of multiple regression analysis coefficient clearly indicates that the response are strongly dependent on the factors studied (Table 3). To demonstrate graphically the influence of factors, the contour plots were generated for all dependent variables (Figs. 5 and 6). To evaluate relative contribution of different levels of each factor, two-way ANOVA was performed (Table 4).

From the equation, it was clear that the selected independent variable has a significant effect on the

![Fig. 6 3D mesh plot of showing the effect of concentration of stabilizer and the ratio of drug to ZrO\(_2\) on in vitro drug release](image)

**Table 4** ANOVA for full model

| Source of variation       | DF | SS        | MS       | F         | Significant F value | Ftab |
|---------------------------|----|-----------|----------|-----------|---------------------|------|
| Particle size (nm) (\(Y_1\)) |    |           |          |           |                     |      |
| Regression                | 5  | 54929.3   | 10985.9  | 706.23    | 8.4E−05             | 9.01 |
| Residual                  | 3  | 46.66     | 15.55    |           |                     |      |
| Total                     | 8  | 54976     |          |           |                     |      |
| In vitro drug release at 10 min (\(Y_2\)) |    |           |          |           |                     |      |
| Regression                | 5  | 1054.40   | 210.88   | 86.34     | 0.0019              | 9.01 |
| Residual                  | 3  | 7.32      | 2.44     |           |                     |      |
| Total                     | 8  | 1061.73   |          |           |                     |      |
independent variable. All terms showed a significant effect on the dependent variable so there is no need to develop a reduce model. The value of correlation coefficient ($R^2 = 0.999$) for the dependent variable indicates a good fit of the mathematical model. The result of the analysis of variance is in Table 4.

The result of ANOVA for reduced model suggested that the $F$ value calculated for particle size ($Y_1$) and in vitro drug release in 10 min ($Y_2$) was $86.34$ and $706.23$, respectively. Tabulated $F$ values at $(5, 3)$ was $9.01$ for $Y_1$ and tabulated $F$ value at $(5, 3)$ was $9.01$ for $Y_2$. Both dependent variables were found to have a
calculated $F$ value significantly higher than $F$ tabulated. Therefore, the selected factors have a significant effect on all dependent variables.

**Statistical optimization based on desirability**
Desirability constraints were minimum for particle size and maximum for in vitro drug release. Desirability was found highest in formulation 7 having a value of 0.9401 (Fig. 7). The composition and predicted values were 233 nm for the mean particle size and 81.87% for the cumulative drug release. When compared with the experiment value, percentage bias was found to be $-0.85\%$ and $-0.79\%$, respectively, for the size and percentage of drug release. Optimized batch of nanosuspension was compared to that of simple drug suspension for cumulative percentage release, and the results obtained are depicted in Fig. 8. The comparison clearly indicates that the dissolution of nanosuspension for the drug was marginally increased when compared to the simple suspension.

**Scanning electron microscopy**
Scanning electron image of nanosuspension formulation revealed a change in the appearance of the surface upon formulating the nanosuspension that indicated the formation of precipitate during media milling technique [20]. Figure 9 shows the nanosuspension with defined shape and narrow size distribution. The enlarged size of the particle could be due to lyophilization-triggered aggregation.

**Mathematical model validation using checkpoint batch**
To determine the reliability of developed mathematical model, optimize formulation was performed as the checkpoint batch. As the optimized batch happens to be study batch F7, random selection of independent variable other than the study batches was done. For this, both dependent responses of the formulation were estimated and their percentage bias was 0.86% for particle size and 1.98% for $Q_{10}$, respectively, as shown in Table 5.

**Saturation solubility**
The saturation solubility of irbesartan was evaluated after solubilizing the drug in plain water, with poloxamer 407 and of optimized nanosuspension for comparison. The water solubility of irbesartan was found to be $4.63 \pm 0.235 \mu g/ml$ while for poloxamer 407, the solubility was $30.54 \pm 0.738 \mu g/ml$. The saturation solubility of optimized nanosuspension was found to be $609.56 \pm 21.756 \mu g/ml$. The results of the solubility studies of irbesartan are depicted in Fig. 10.

![Fig. 9 Scanning electron microscopy (SEM) of lyophilized nanoparticles](image)

| Table 5 Percentage bias for checkpoint batch |
|---------------------------------------------|
| Response | Experimental value | Predicted value | % bias |
|----------|---------------------|-----------------|--------|
| Particle size (nm) | 280.62 | 283.00 | 0.86 |
| In vitro dissolution $Q_{10}$ (%) | 81.96 | 83.58 | 1.98 |

(%b = (predicted value – experimental value)/experimental value) × 100)
Accelerated stability studies

After completion of the 6-month accelerated stability study, the formulation showed no significant difference in the mean polydispersity index, particle size, zeta potential, and in vitro drug release. The results were determined for previously mentioned parameters at specific time intervals. As it is evident from Table 6, there was no significant change in nanosuspension stability.

Discussion

The FTIR spectra of physical mixture of poloxamer 407 and drug revealed that there were no appreciable changes in the position of absorption band of the drug. These proved that there was no interaction between the drug and excipients. The homogenization speed and time were studied to find their effect of mean particle size. Speed was kept at 2000 rpm initially followed by an increment of 1000 rpm in follow-up batches. For each speed, batches were prepared and studied at five different times of homogenization starting with 1 h. The experiment was repeated four times with an increment of 1 h. Therefore, for each time, seven different speeds were studied and for each speed, five different times were studied. It was observed, for each time point after 6000 rpm, that there was no significant change in mean particle size as compared to previous rpm increments. The homogenization time of 2 h was selected based on the results, as the idea was to minimize the process time. In the range of 6000–8000 rpm homogenization speed and 2 h homogenization time, the mean particle size of below 1000 nm was achieved. The target was to get nanosuspension below 499 nm mean particle size. In another set of experiments, media milling was carried out. For that, homogenization results were kept aside and media milling was performed using irbesartan drug as such. With media milling, size below 1000 nm was achieved at 20 h. Media milling method alone was giving a particle size of 820 nm at 28 h at given set conditions. In the same conditions, after 2 h of homogenization, the particle size less than 820 nm was achieved within 8 h. Particle size after 18 h showed change of 5–10 nm, so it was decided to fix the milling time of 18 h after 2 h of homogenization. This was used for further optimization of formulation. The results imply that by using both the methods, the time required to get the smallest particle size was reduced drastically. Here, the mean particle size at 4 h shows a twofold difference that was threefold when the media milling time reaches 16 h and after that.

It was quite evident from the results that homogenization prior to media milling was able to

![Fig. 10 Saturation solubility studies comparison of irbesartan](image)

### Table 6 Accelerated stability study evaluation data

| Sample time (month) | Particle size (nm) | Polydispersity index | Zeta potential (mV) | Q_{10} release (%) |
|---------------------|--------------------|----------------------|---------------------|--------------------|
| 0                   | 227.68 ± 3.64      | 0.39 ± 0.01          | 27.34 ± 1.26        | 82.78 ± 0.55       |
| 1                   | 227.12 ± 3.91      | 0.40 ± 0.02          | 27.38 ± 1.57        | 82.83 ± 0.96       |
| 2                   | 227.41 ± 4.83      | 0.42 ± 0.01          | 27.48 ± 1.81        | 82.61 ± 1.13       |
| 3                   | 228.65 ± 3.34      | 0.42 ± 0.01          | 27.15 ± 0.97        | 82.42 ± 0.93       |
| 6                   | 227.04 ± 6.25      | 0.43 ± 0.01          | 27.45 ± 1.01        | 82.65 ± 1.08       |

Results are average of three readings ± SD
reduce the mean particle size and milling time both. Finally, it was concluded that after 18 h, media milling was not able to further reduce the particle size (Fig. 3).

The zeta potential (ZP) for all the formulations was found to be low which indicates incipient instability. This would provide only short-time stability in case of electrostatic stabilization. However, it is possible to achieve a good stability despite low zeta potential due to the adsorption of the non-ionic poloxamer 407. It is a steric stabilizer providing steric stabilization in addition to the electrostatic repulsion. The adsorption layer of steric stabilizers shifts the plane of shear in the measurement, which yields lower measured "artificial" zeta potentials. Electrostatic repulsions were actually higher than what is being reflected by zeta potential. The ZP values stayed unchanged during storage as it can be expected from the theory if no change in the composition and thickness of the stabilizer layer or to the surface charges occur [21].

In vitro release study shows increase in drug release as a decrease in poloxamer 407 concentration and increased quantity of ZrO\textsubscript{2}, so the particle size decrease results in increase in drug release. F7 with the smallest particle size, among the nine batches, shows the highest drug release. Decrease in drug release was due to the increase in polymer concentration. This increased concentration results in increased particle size which in turn affects drug release from nanosuspension. Out of all the nine batches, batch 7 had highest drug release (95.03%) in 1 h that also has the smallest particle size.

From the polynomial data, it was apparent that all main terms including X\textsubscript{1} and X\textsubscript{2} have a significant effect on particle size. Negative sign in term X\textsubscript{2} showed reduction of particle size. Interaction term X\textsubscript{1}X\textsubscript{2} has a significantly higher value of coefficient that indicates increase in particle size (Fig. 5). The stabilizer concentration decrease and ratio of drug to ZrO\textsubscript{2} increase resulted in particle size decrease up to 228.4 nm. The data clearly indicated that the value of in vitro drug release strongly depends on the select independent variables. The results of the statistical analysis (Table 3) show the significant effect on dependent variable, so there is no need to develop a reduce model. The equation showed that the main coefficient bear X\textsubscript{2} positive sign indicated increase in drug release and X\textsubscript{1} negative sign indicated reduction in drug release, while the coefficient of X\textsubscript{1}X\textsubscript{2} showed negative sign indicating retardation in drug release. The effect of various independent variables can be explained using 3D mesh plot as shown in Fig. 6 which shows the 3D mesh plot for the effect of independent factors on the percentage cumulative drug release at 10 min. In the plots, moving from the blue region to yellow orange region, the drug release from nanosuspension increases. Therefore, from the plot, it could be concluded that the decrease in stabilizer concentration and increase in the ratio of drug to ZrO\textsubscript{2} increases drug release. The results indicate that not only the increased surface area is responsible for dissolution enhancement but the increased saturation solubility (Fig. 10) also contributed as illustrated by the Freundlich-Ostwald equation [22]. Saturation solubility not only depended on the temperature but also on the drug particle diameter, so when the drug particle is below 1000 nm, the saturation solubility increases [23–26].

Statistical optimization was carried out using desirability function. It was performed for two dependent variables Y\textsubscript{1} (particle size) and Y\textsubscript{2} (in vitro drug release at 10 min). Low value of percentage bias (< 5%) indicates an agreement between predicted and experimental values. It also shows the robustness of formulation and high predictive power of the generated mathematical model. It could be concluded that the generated equation describes adequately the influence of the selected formulation compositions on the responses under study and indicates the robustness of the model.

Conclusion

The FTIR study pointed out that irbesartan is compatible with the selected excipients. Media milling technique and prior homogenization for 2 h were prepared for the nanosuspension of irbesartan. The optimized nanosuspension was of 280.62 nm mean particle size and more than 80% drug release was achieved within 10 min. In the present study, the amount of ZrO\textsubscript{2} beads and poloxamer 407 as stabilizer was found to affect the character of nanosuspension. Nano-sized irbesartan dissolves at a much faster rate than its counter form of micronized size. The experimental design results indicated the suitability of the formulation procedure for the preparation of irbesartan nanosuspension by means of noteworthy increase of the in vitro drug dissolution rate which possibly improved oral bioavailability.

Abbreviations

BCS: Biopharmaceutical Classification System; ACE: Angiotensin-converting enzyme; GI: Gastrointestinal; IR: Infrared; FTIR: Fourier-transform infrared; KBr: Potassium bromide; ZrO\textsubscript{2}: Zirconium dioxide; PDI: Polydispersity index; SEM: Scanning electron microscopy; USP: United States Pharmacopoeia; ANOVA: Analysis of variance; ZP: Zeta potential

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None

Authors’ contributions

CB and SB carried out most of the practical work and data collection. DP, NP, KP, and RM contributed in the analytical part, i.e., estimation of drug in samples, interpretations, and reporting of the same. Collectively, all contributed to this work at some point of research and drafted the manuscript. The manuscript was prepared by CB and DP. All authors have reviewed the manuscript and finally settled on its communication with this relevant journal.
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