International Journal of Pharmaceutics 402 (2010) 213–220

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

# Optimization of formulation and process variable of nanosuspension: An industrial perspective

Dhananjay S. Singare[a,d,*], Seshasai Marella[a], K. Gowthamrajan[b], Giriraj T. Kulkarni[c], Rajesh Vooturi[a], Parchuri Srinivasa Rao[a]

[a] Custom Pharmaceutical Services-Formulations, Dr. Reddys Laboratories Ltd., Bachupally, Hyderabad, Andhra Pradesh, 500090, India
[b] Department of Pharmaceutics, JSS College of Pharmacy, Ooty, Tamilnadu, 643001, India
[c] Department of Pharmaceutics, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, 590010, India
[d] Department of Pharmaceutics, Karpagam College of Pharmacy, Karpagam University, Coimbatore, Chennai, 641021, India, India

ARTICLE INFO

*Article history:*
Received 8 July 2010
Received in revised form
23 September 2010
Accepted 28 September 2010
Available online 7 October 2010

*Keywords:*
Nanosuspension
Zeta potential
Bead mill
Box–Behnken design
Particle size distribution *d*(90)
Analysis of variance (ANOVA)

ABSTRACT

The objective of this study was to identify and optimize formulation and process variables affecting characteristic and scale up of nanosuspension manufacturing process on bead mill considering industrial perspective. Box–Behnken design was used for this study. Formulation factors evaluated were ratio of polymer to drug and ratio of surfactant to drug, whereas process parameters were milling time and milling speed. Responses measured in this study include zeta potential and, particle size distribution *d*(90). The ANOVA test reveals that ratio of polymer to drug and milling speed has significant effect on zeta potential whereas milling time and milling speed has significant effect on the particle size distribution of nanosuspension. The X-RD pattern of drug milled at high and low speed reveals no form conversion when compared to unmilled drug. The Box–Behnken design used in this study helped in identifying the factors affecting the particle size distribution *d*(90), zeta potential and, scalability of nanosuspension. The derived polynomial equation and contour graph aid in predicting the values of selected independent variables for preparation of optimum nanosuspension formulations with desired properties.



## 1. Introduction

As per FDA a nanoparticulate drug is not considered as "generic" to an approved product and therefore can be patented; and are considered as "new drug", because nanoparticulate drug is not bioequivalent to a microcrystalline or solubilized form of the same drug, administered at the same dosage. It also offers a unique advantage to pharmaceutical companies of product line extension for the existing drug formulations. Such product line extension can also benefit consumers by giving new drug dosage form which may provide fewer side effects, lower doses and faster onset of action (Liversidge and Ruddy, 2009). The best example of this is Rapamune®.

Nanosuspensions are liquid dispersion consisting of solid drug nanoparticles which are stabilized by polymer and or surfactant. Nanosizing has been proven to be an effective tool for an active moiety considered as "brick dust candidate". There are two main approach for formulating a nanosuspension i.e. top down and bottom up technology. The bottom up technology involves dissolving drug in a solvent which is then added to non-solvent to precipitate the crystals. The top down approach relies on mechanical attrition to render large crystalline particles into nanoparticles. The 'Top Down Technologies' include Media Milling (Nanocrystals®), High Pressure Homogenization in water (Dissocubes®), High Pressure Homogenization in non-aqueous media (Nanopure®) and combination of Precipitation and High-Pressure Homogenization (Nanoedege®). Table 1 lists some of the FDA approved products relying on nanotechnology.

Nanosuspensions for oral route are mainly characterized by particle size distribution (PSD), zeta potential, crystalline status, and dissolution velocity and saturation solubility. A particle of less than 400 nm is considered to be acceptable for a nanosuspension to be administered intravenously (Raval et al., 2006). For a physically stable nanosuspension solely stabilized by electrostatic repulsion, a zeta potential of ±30 mV is required as a minimum. In the case of a combined electrostatic and steric stabilization, as a rough guide line of ±20 mV is sufficient (Muller et al., 2001). The crystalline structure of nanosuspension is important for drugs existing in different polymorphic form. This is mainly confirmed by DSC, X-RD or wide angle X-ray analysis (WAXS). Dissolution velocity and saturation

* Corresponding author at: Dr. Reddys Laboratories Ltd., Custom Pharmaceutical Services, FTO-II, Admin. Building, Survey Nos. 42, 45 and 46, Bachupally, Qutubullapur, RR Dist. Hyderabad, Andhra Pradesh, 500090, India.
Tel.: +91 4044642379/2378; fax: +91 4044645212.
*E-mail addresses:* dhananjays@drreddys.com, dhananjaysingare@yahoo.co.in (D.S. Singare).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.09.041

**Table 1**
FDA approved products based on nanoparticles technology.

| Product, active ingredient, company | Manufacturing approach |
|---|---|
| Rapamune®, Sirolimus, Wyeth | Top-down, media milling |
| Emend®, Aprepitant, Merck | Top-down, media milling |
| Tricor®, Fenofibrate, Abbott | Top-down, media milling |
| Megace®, ES,Megestrol acetate, Par Pharmaceuticals | Top-down, media milling |
| Triglide®, Fenofibrate, Skye Pharma | Top-down, high-pressure homogenization |

solubility are generally performed using official pharmacopoeial methods.

The stability and robustness of a nanosuspension is mainly governed by various formulation and process variables. Selection of proper steric and electrostatic stabilizer and its optimum quantity plays a major role in formulating a nanosuspension. Commonly used steric stabilizer includes hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), povidone (PVP K-30), and pluronics (F68 and F127) whereas electrostatic stabilizer includes polysorbate (Tween-80), sodium laurylsulphate (SLS) and docusate sodium (DOSS). A suitable working polymer to drug ratio (steric stabilizer) is from 0.05:1 to 0.5:1 (Kesisoglou et al., 2007). At high stabilizer concentrations, well above of the plateau of the adsorption isotherm, electrostatic stabilizers can cause a decrease in the diffuse layer leading to a decreased zeta potential and a decreased physical stability. Electrolytes are present in the gastrointestinal tract and the contact of the nanocrystals with these electrolytes cannot be avoided. Electrostatic stabilization is reduced in its efficiency in an electrolyte containing environment. Therefore it is important to find the optimal concentration for a stabilizer. Processing factors for formulating a stable nanosuspension vary based on the equipment selected for manufacturing.

As seen from Table 1 majority of products existing in market are based on media milling technique due its many advantages over high pressure homogenization. Therefore, scale-up of a nanosuspension using bead mill with varying operating capacity from 250 mL to 4 L and more requires a better understanding of formulation and process variables. A major emphasis should be given to increase the solid content (API) in nanosuspension which will decrease the processing time and in turn also decrease the drying time while converting it to a solid dosage form.

In order to obtain best formulation and process of nanosuspension, the relationship between controllable variable and quality variable must be understood. The traditional method used to study this relationship involves "changing one variable at a time, while keeping others as constant". This approach has been proved to be expensive, laborious and also unfavorable to fix errors that are unpredictable and at times even unsuccessful. On the other hand Design of experiment (DoE) can serve as an efficient and economical method of obtaining the necessary information to understand relationship between variables. DoE provides not only efficient use of resources, but also provides a method of obtaining a mathematical model which can be used to characterize and optimize formulation and or process.

Sudhir Verma et al. (2009) evaluated the effect of formulation and process variables affecting the preparation of nanosuspension using microfluidization technique. A $2^{(5-1)}$ factorial design was used in this study. Formulation factors such as stabilizer type, stabilizer concentration and processing factors such as microfluidization pressure, milling time and temperature were identified as significant or critical factors affecting stability of nanosuspension. A similar approach was used by Zidan et al. (2007) where they studied the formulation variables affecting nanoemulsified particles of Cyclosporine A, using a Box Behnken design. The study highlights that emulsification rate is highly dependent on drug concentration, surfactant and co-surfactant added. Apart from this several research articles are available for better understanding of nanocrystals formulated by other than media milling technology. Though most of the products existing in market are developed based on the media milling technique; there is no research work available in the public domain on the same considering the industrial perspective of nanosuspension development; which includes the cost and time of manufacturing.

High-pressure homogenization relies on the forcing of a suspension through a small gap which makes miniaturization of this technology less straightforward. Media milling, on the other hand, can be per formed by agitation of devices containing the starting suspension and milling media. Furthermore, nanosuspension production by media milling is characterized by its ease of scale-up (Date and Patravale, 2004), making results generated on nanosuspensions in downscaled designs valuable and was therefore selected for this study.

The objective of the present study was to identify formulation and process variables affecting the characteristic and scale up of nanosuspension, on bead mill using response surface method (RSM). The objective of this study also includes optimization of polymer to drug ratio and surfactant to drug ratio that could give a nanosuspension with ideal characteristic, to optimize processing factors that could affect the manufacturing process on large scale development. The objective also includes to set a guideline for a research scientist to eliminate the initial screening of process parameters while formulating a nanosuspension on bead mill.

Meloxicam was used as a model drug for this study. Responses such as particle size distribution and zeta potential were evaluated in this study. Based on several advantages of Box–Behnken design (BBD) over central composite design we decided to apply Box–Behnken experimental design.

## 2. Materials and methods

### 2.1. Materials

Meloxicam USP used in this study was manufactured by Dr. Reddys Laboratories Limited, Hyderabad, India. Hydroxypropyl methylcellulose 6cps (HPMC) was purchased from Samsung fine chemicals Co., LTD., Korea and sodium lauryl sulphate (SLS) was purchased from Stepan Co., USA. Purified water USP was used in this study.

### 2.2. Nanosuspension preparation

Nanosuspension preparation involves two main steps; the first one is uniform dispersion of drug and stabilizers in dispersion media and second one is particle size reduction in milling chamber. The uniform dispersion of drug (initial particle size $d(90)$ 5.0 μm) and stabilizer in dispersion media was prepared using Heidolph mixer (Model: RZR2051 Control, Rose Scientific Ltd., Alberta, Canada) operated at 500 rpm. This suspension was loaded in milling chamber of bead mill (Model: Lab Star 1, Netzsch Mill, Germany) for particle size reduction. The milling media used for this study was 0.2-mm yttrium-stabilized zirconium beads. The milling operation was performed in a re-circulation mode with the suspension fed at a rate of 100 mL/min. The bead mill was operated at specific speed and time as designed by the DoE. The temperature of suspension was controlled during milling by circulating cold water through the outer jacket.

### 2.3. Design of experiment

Initial screening trials were carried out for evaluating the formulation and processing aspects of nanosuspension. Various factors

**Table 2**
Variables for Box–Behnken study.

| Independent factors | | Design level | |
|---|---|---|---|
| Uncoded | Coded | Uncoded level | Coded level |
| Ratio of polymer to drug | *A* | 0.1:1.0 | −1 |
| | | 0.3:1.0 | 0 |
| | | 0.5:1.0 | +1 |
| Ratio of surfactant to drug | *B* | 0.02:1.0 | −1 |
| | | 0.04:1.0 | 0 |
| | | 0.06:1.0 | +1 |
| Milling time (h) | *C* | 3.0 | −1 |
| | | 4.5 | 0 |
| | | 6.0 | +1 |
| Milling speed (rpm) | *D* | 2500.0 | −1 |
| | | 2950.0 | 0 |
| | | 3400.0 | +1 |

like concentration of drug, ratio of polymer to drug, ratio of surfactant to drug, solvent for nanosuspension, milling media, volume of milling media, milling time and milling speed were identified as critical to give a product in nano range and with required stability. The results from the initial screening trials suggested that ratio of polymer to drug, ratio of surfactant to drug, milling time and milling speed are the main factors which affects the particle size and zeta potential of the nanosuspension.

Based on the number of factors and their level, a Box–Behnken design was used to evaluate the effect of formulation and processing parameters affecting the physical properties of nanosuspension. The four independent factors identified for this study were ratio of polymer to drug, ratio of surfactant to drug, milling time and milling speed. All these factors were operated at three levels (+1, 0 and −1). The concentration of drug, type of polymer, type of surfactant, milling media, volume of milling media, solvent i.e. purified water were kept same for all the experiments. Design-Expert® 7b1.1 software was used to conduct the study. A total of 26 experiments were designed by the software with 2 center points. Experiments were run in random order to increase the predictability of the model. A batch size of 250 g was kept constant for all experimental trials. Table 2 shows the independent factors and their design levels used in this study. Table 3 list out the formula composition of nanosuspension with respect to the ratios as mentioned in DoE.

### 2.4. Particle size measurement

The particle size of nanosuspension was measured using Malvern Zetasizer ZS200. Each sample was measured at least three times. The average values were employed for the calculations of the response surfaces.

### 2.5. Zeta potential

The zeta potential of nanosuspension was measured using Malvern Zetasizer ZS200 at 25 ± 0.5 °C. Each sample was measured at least three times. The average values were employed for the calculations of the response surfaces.

**Table 3**
Formula composition of nanosuspension batches.

| Ingredients | Quantity/batch (g) |
|---|---|
| Meloxicam | 16.0 |
| HPMC 6cps | 1.6–8.0 |
| SLS | 0.32–0.96 |
| Purified water | qs |

qs, quantity sufficient to 250 g.

### 2.6. Solid state characterization

#### 2.6.1. Powder X-ray diffraction (PXRD)

Initial unmilled suspension of meloxicam, nanosuspension milled at high speed (3500 rpm) and low speed (2500 rpm) were dried using spray dryer (Spray Dryer Model: LU 222, Labultima, Mumbai, India) under following set of condition: inlet temperature, 85 °C; outlet temperature, 60 °C; feed rate, 2.5 mL/min; atomization pressure 2 kg/cm$^2$. The PXRD pattern of samples was recorded using a X-ray diffractometer (Bruker axs, D8 Advance) with a Cu line as the source of radiation. Standard runs using a 40-kV voltage, a 40-mA current, and a scanning rate of 0.013° min$^{-1}$ over a $2\theta$ range of 3–45° were used.

## 3. Results and discussion

### 3.1. Effect of independent factors on zeta potential ($y_1$) and particle size distribution ($y_2$)

A total of 26 experiments were carried out to study the effect of formulation and processing factors affecting the zeta potential and particle size distribution of nanosuspension. Response data for all experimental runs of Box–Behnken experimental design is presented in Table 4.

The responses obtained for this study are well modeled by a linear function of the independent variables; hence the first order polynomial was used for approximating the function.

$$y = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_3 + \beta_4\chi_4 + \in \quad (1)$$

where $\in$ represents noise or error, $\chi$ represents independent variable, $y$ represents response and $\beta$ represents coefficient.

The values of response $y_1$ (zeta potential) and $y_2$ (particle size distribution $d(90)$) ranges from −11.3 to −33 mV and 211 to 443 nm, respectively. The ratio of maximum to minimum for both the responses $y_1$ and $y_2$ is 2.92 and 2.09, respectively; therefore power transformation was not applied to the obtained values. The selection of model for analyzing the response was done based on the Sequential Model Sum of Squares, lack of fit test and Model Summary Statistics. The Prob > $F$ value of $P < 0.0001$, low standard deviation, high $R$-squared value and lower Predicted Residual Error Sum of Square (PRESS) value suggests to select linear model for analyzing the both the responses. The details of which are mentioned in Table 5.

ANOVA was applied to determine the significance and the magnitude of the effects of the main variables and their interaction. The regression model obtained was used to generate the contour plots for independent factors. The ANOVA table confirm the adequacy of the linear model (Model Prob > $F$ is less than 0.05). It also identifies the significant factors that affect the responses $y_1$ and $y_2$ of nanosuspension. For zeta potential the ratio of polymer to drug and milling speed (factors $A$ and $D$) were identified as significant model terms whereas for particle size distribution milling time and milling speed (factors $C$ and $D$) were identified as significant model terms. The details of ANOVA for response $y_1$ and $y_2$ are mentioned in Table 6. The multiple regression term were also analyzed. The details of there vaules is mentioned in Table 7. The "Pred $R$-squared" value for the responses was found to be in reasonable agreement (~0.20) with the "Adj $R$-squared" value which indicates that model has predicted the responses values well.

The final mathematical model in terms of coded factors as determined by Design-Expert software is shown below in Eqs. (2) and (3) for response $y_1$ and $y_2$, respectively.

$$y_1\,(\text{zeta potential}) = +21.02 - 5.93 * A + 0.083 * B + 0.80 * C + 2.60 * D \quad (2)$$

**Table 4**
Factor level and response data for BBD study.

| Run | Block | Factor: *A* Ratio of polymer to drug (g) | Factor: *B* Ratio of surfactant to drug (g) | Factor: *C* Milling time (h) | Factor: *D* Milling speed (rpm) | Response 1 Zeta potential (mV) | Response 2 PSD *d*(90) nm |
|---|---|---|---|---|---|---|---|
| 1 | Block 1 | 4.8 (0) | 0.64 (0) | 4.5 (0) | 2950 (0) | −21.8 | 289 |
| 2 | Block 1 | 4.8 (0) | 0.96 (+1) | 6.0 (+1) | 2950 (0) | −22.7 | 238 |
| 3 | Block 1 | 1.6 (−1) | 0.64 (0) | 3.0 (−1) | 2950 (0) | −33.0 | 265 |
| 4 | Block 1 | 8.0 (+1) | 0.64 (0) | 4.5 (0) | 2500 (−1) | −12.0 | 326 |
| 5 | Block 1 | 1.6 (−1) | 0.64 (0) | 4.5 (0) | 2500 (−1) | −25.4 | 443 |
| 6 | Block 1 | 4.8 (0) | 0.64 (0) | 4.5 (0) | 2950 (0) | −13.5 | 334 |
| 7 | Block 1 | 4.8 (0) | 0.64 (0) | 3.0 (−1) | 3400(+1) | −5.63 | 249 |
| 8 | Block 1 | 4.8 (0) | 0.64 (0) | 6.0 (+1) | 3400(+1) | −22.6 | 219 |
| 9 | Block 1 | 1.6 (−1) | 0.96 (+1) | 4.5 (0) | 2950 (0) | −25.0 | 311 |
| 10 | Block 1 | 1.6 (−1) | 0.64 (0) | 4.5 (0) | 3400(+1) | −28.3 | 240 |
| 11 | Block 1 | 8.0 (+1) | 0.96 (+1) | 4.5 (0) | 2950 (0) | −16.5 | 311 |
| 12 | Block 1 | 4.8 (0) | 0.96 (+1) | 4.5 (0) | 2500 (−1) | −23.7 | 309 |
| 13 | Block 1 | 4.8 (0) | 0.96 (+1) | 3.0 (−1) | 2950 (0) | −18.8 | 250 |
| 14 | Block 1 | 8.0 (+1) | 0.64 (0) | 6.0 (+1) | 2950 (0) | −19.1 | 288 |
| 15 | Block 1 | 8.0 (+1) | 0.32 (−1) | 4.5 (0) | 2950 (0) | −15.9 | 358 |
| 16 | Block 1 | 8.0 (+1) | 0.64 (0) | 3.0 (−1) | 2950 (0) | −17.0 | 418 |
| 17 | Block 1 | 4.8 (0) | 0.64 (0) | 3.0 (−1) | 2500 (−1) | −11.3 | 424 |
| 18 | Block 1 | 8.0 (+1) | 0.64 (0) | 4.5 (0) | 3400(+1) | −16.8 | 225 |
| 19 | Block 1 | 4.8 (0) | 0.32 (−1) | 4.5 (0) | 2500 (−1) | −17.8 | 350 |
| 20 | Block 1 | 1.6 (−1) | 0.32 (−1) | 4.5 (0) | 2950 (0) | −27.7 | 355 |
| 21 | Block 1 | 4.8 (0) | 0.32 (−1) | 4.5 (0) | 3400(+1) | −23.8 | 238 |
| 22 | Block 1 | 4.8 (0) | 0.96 (+1) | 4.5 (0) | 3400(+1) | −24.0 | 246 |
| 23 | Block 1 | 4.8 (0) | 0.32 (−1) | 6.0 (+1) | 2950 (0) | −23.3 | 236 |
| 24 | Block 1 | 4.8 (0) | 0.32 (−1) | 3.0 (−1) | 2950 (0) | −21.2 | 277 |
| 25 | Block 1 | 4.8 (0) | 0.64 (0) | 6.0 (+1) | 2500 (−1) | −15.1 | 251 |
| 26 | Block 1 | 1.6 (−1) | 0.64 (0) | 6.0 (+1) | 2950 (0) | −29.1 | 211 |

g, gram; h, hours; rpm, rotation per minute; nm, nanometer; mV, millivolts; PSD, particle size distribution; BBD, Box Behnken design.

**Table 5**
Fit summary for zeta potential and particle size distribution.

| Source | Sum of squares | | df | | *F* value | | Prob > *F* *P*-value | |
|---|---|---|---|---|---|---|---|---|
| | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ |
| *Sequential sum of model square* | | | | | | | | |
| Linear | 511.34 | 58049.83 | 4 | 4 | 11.57 | 6.10 | <0.0001 | 0.0020 |
| 2FI | 22.77 | 9970.00 | 6 | 6 | 0.27 | 0.62 | 0.9416 | 0.7090 |
| Quadratic | 64.47 | 12199.13 | 4 | 4 | 1.22 | 1.21 | 0.3556 | 0.3613 |
| Cubic | 44.15 | 22973.17 | 8 | 8 | 0.16 | 1.80 | 0.9815 | 0.3411 |
| Residual | 10.71 | 4785.75 | 3 | 3 | – | – | – | – |
| Total | 12226.24 | 2.365+006 | 26 | 26 | – | – | – | – |
| *Lack of fit tests* | | | | | | | | |
| Linear | 197.65 | 48915.55 | 20 | 20 | 0.29 | 2.42 | 0.9234 | 0.4727 |
| 2FI | 174.88 | 38945.55 | 14 | 14 | 0.36 | 2.75 | 0.8810 | 0.4440 |
| Quadratic | 110.41 | 26746.42 | 10 | 10 | 0.32 (−1) | 2.64 | 0.8922 | 0.4479 |
| Cubic | 66.26 | 3773.25 | 2 | 2 | 0.96 | 1.86 | 0.5848 | 0.4600 |
| Residual | 34.45 | 1012.50 | 1 | 1 | – | – | – | – |

| Source | *R*-squared | | Adjusted *R*-squared | | Predicted *R*-squared | | PRESS | |
|---|---|---|---|---|---|---|---|---|
| | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ |
| *Model summary statistics* | | | | | | | | |
| Linear | 0.6878 | 0.5376 | 0.6283 | 0.4495 | 0.5444 | 0.2755 | 338.73 | 78230.62 |
| 2FI | 0.7184 | 0.6299 | 0.5307 | 0.3832 | 0.2272 | −0.2130 | 574.52 | 1.310E+005 |
| Quadratic | 0.8052 | 0.7429 | 0.5572 | 0.4157 | −0.0408 | −0.4643 | 773.75 | 1.581E+005 |
| Cubic | 0.8645 | 0.9557 | −0.1289 | 0.6307 | −12.0203 | −4.0695 | 9679.70 | 5.474E+005 |

df, degree of freedom; PRESS, Predicted Residual Error Sum of Squares; statistically significant terms are underlined (*P*-value less than 0.05).

**Table 6**
ANOVA for Response surface linear model of zeta potential and particle size distribution.

| Source | Sum of squares | | df | | *F* value | | Prob > *F* *P*-value | |
|---|---|---|---|---|---|---|---|---|
| | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ | $y_1$ | $y_2$ |
| Model | 511.34 | 58049.83 | 4 | 4 | 11.57 | 6.10 | <0.0001 | 0.0020 |
| A | 422.45 | 850.08 | 1 | 1 | 38.22 | 0.36 | <0.0001 | 0.5563 |
| B | 0.083 | 1850.08 | 1 | 1 | 7.540E−003 | 0.78 | 0.9316 | 0.3877 |
| C | 7.68 | 16133.33 | 1 | 1 | 0.69 | 6.79 | 0.4139 | 0.0165 |
| D | 81.12 | 39216.33 | 1 | 1 | 7.34 | 16.49 | 0.0131 | 0.0006 |
| Residual | 232.10 | 49928.05 | 21 | 21 | – | – | – | – |
| Lack of fit | 197.65 | 48915.55 | 20 | 20 | 0.29 | 2.42 | 0.9234 | 0.4727 |
| Pure error | 34.45 | 1012.50 | 1 | 1 | – | – | – | – |
| Cor total | 743.43 | 1.080E+005 | 25 | 25 | – | – | – | – |

Statistically significant terms are underlined (*P*-value less than 0.05).

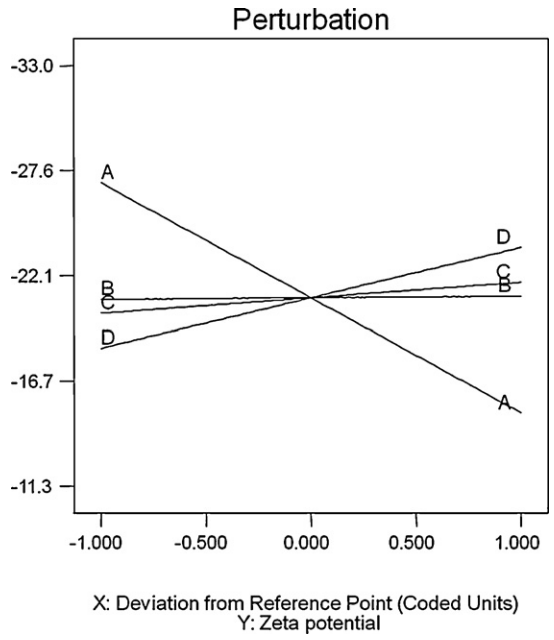


**Fig. 1.** Perturbation graph for effect of individual factor on response $y_1$ (zeta potential).

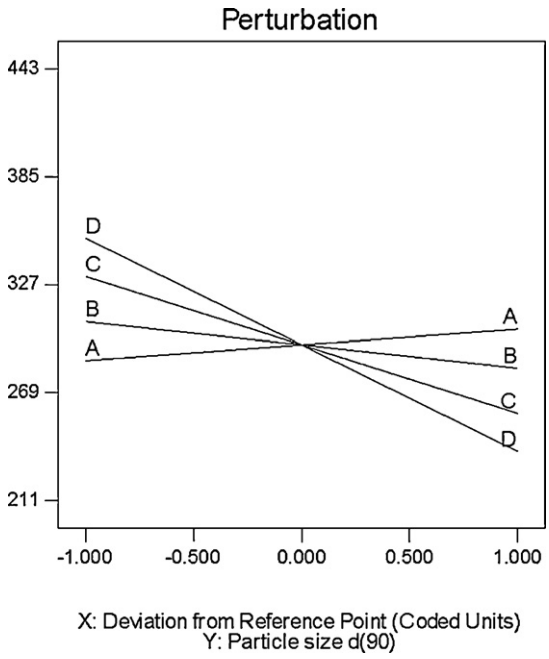


**Fig. 2.** Perturbation graph for effect of individual factor on response $y_1$ (particle size distribution $d(90)$).

$$y_2\,(\text{PSD } d(90)) = +294.65 + 8.42 * A - 12.42 * B - 36.67 * C - 57.17 * D \quad (3)$$

A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. In case of $y_1$ negative coefficients of A in the model refer to decreased zeta potential at higher level of polymer to drug ratio. Similarly, the positive coefficients of *B*, *C* and *D* indicate the increase in zeta potential with increasing rate of respective factor. Whereas for $y_2$ the negative coefficient of *B*, *C* and *D* in the model refers to decreasing the PSD $d(90)$ as the concentration of surfactant, milling time and milling speed increases. Similarly, the positive coefficients of A indicate the increase in PSD $d(90)$ with increasing concentration of respective factor.

The theoretical values were compared with the experimental values of responses using diagnostic case statistic reports (Table 8) and were found to be in reasonably close agreement.

A perturbation graph was plotted to find those factors that most affects the response. A steep slope or curvature in a factor shows that the response is sensitive to that factor. A relatively flat line shows insensitivity to change in that particular factor. In case of response $y_1$ factor *A* shows a steep slope, factors *C* and *D* exhibit a slight slope or a noticeable bend and factor *B* shows a flat line. Whereas in case of response $y_2$ factors *C* and *D* shows a steep slope compared to *A* and *B*. Figs. 1 and 2 represent perturbation plot for response $y_1$ and $y_2$. Based on ANOVA and perturbation plot factors *A* and *D* are most ideal for generating 2D contour plot for response $y_1$. But to have a better understanding factor *B* was also evaluated for response $y_1$. Factors *C* and *D* were focused more for generating 2D contour plot for response $y_2$.

The relationship between the dependent and independent variables were further elucidated using contour plots. Figs. 3–5 represent the effect of factors *A*, *B*, *C* and *D* on the response $y_1$. Fig. 3 shows the effect of factors *A* and *B* on response $y_1$ at fixed level of factors *C* and *D*. Fig. 4 shows the effect of factors *A* and *D* on response $y_1$ at fixed level of factors *B* and *C*. Fig. 5 shows the effect of factors *A* and *C* on response $y_1$ at fixed level of factors *B* and *D*. Zeta potential is the potential at the hydrodynamic shear plane and can be determined from particle mobility under an electric field. The mobility will depend on surface charge and electrolyte concentration. As can

**Table 7**
Values for regression term.

| Sr. No. | Terms | Values | |
|---|---|---|---|
| | | $y_1$ | $y_2$ |
| 1 | *R*-squared | 0.6878 | 0.5376 |
| 2 | Adj *R*-squared | 0.6283 | 0.4495 |
| 3 | Pred *R*-squared | 0.5444 | 0.2755 |
| 4 | Adeq precision | 11.706 | 8.777 |

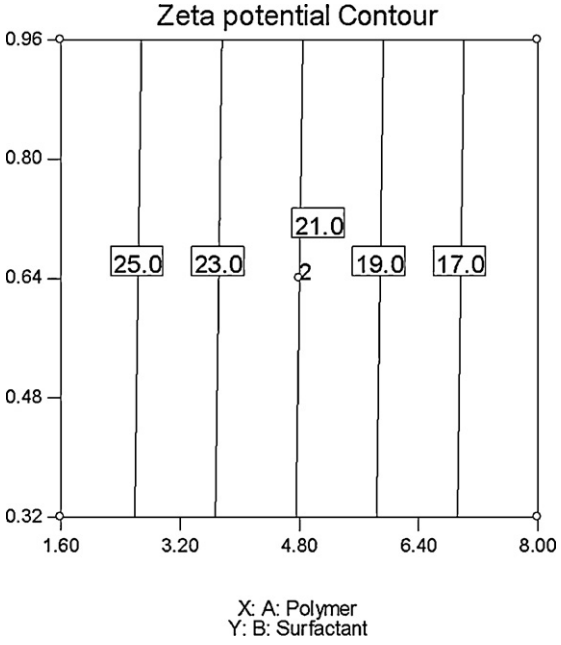


**Fig. 3.** Contour plot for effect of ratio of polymer to drug (*A*) and ratio of surfactant to drug (*B*) on zeta potential ($y_1$).

**Table 8**
Actual and predicted values of the responses.

| Std order | Actual value | | Predicted value | | Residual | |
|---|---|---|---|---|---|---|
| | $y_1$ (mV) | $y_2$ (nm) | $y_1$ (mV) | $y_2$ (nm) | $y_1$ (mV) | $y_2$ (nm) |
| 1 | −27.7 | 355 | −26.87 | 298.65 | 0.83 | 56.35 |
| 2 | −15.9 | 358 | −15.00 | 315.49 | 0.90 | 42.51 |
| 3 | −25.0 | 311 | −27.03 | 273.82 | −2.03 | 37.18 |
| 4 | −16.5 | 311 | −15.17 | 290.65 | 1.33 | 20.35 |
| 5 | −11.3 | 424 | −17.62 | 388.49 | −6.32 | 35.51 |
| 6 | −15.1 | 251 | −19.22 | 315.15 | −4.12 | −64.15 |
| 7 | −21.0 | 249 | −22.82 | 274.15 | −1.82 | −25.15 |
| 8 | −22.6 | 219 | −24.42 | 200.82 | −1.82 | 18.18 |
| 9 | −25.4 | 443 | −24.35 | 343.40 | 1.05 | 99.60 |
| 10 | −12.0 | 326 | −12.48 | 360.24 | −0.48 | −34.24 |
| 11 | −28.3 | 240 | −29.55 | 229.07 | −1.25 | 10.93 |
| 12 | −16.8 | 225 | −17.68 | 245.90 | −0.88 | −20.90 |
| 13 | −21.2 | 277 | −20.13 | 343.74 | 1.07 | −66.74 |
| 14 | −18.8 | 250 | −20.30 | 318.90 | −1.50 | −68.90 |
| 15 | −23.3 | 236 | −21.73 | 270.40 | 1.57 | −34.40 |
| 16 | −22.7 | 238 | −21.90 | 245.57 | 0.80 | −7.57 |
| 17 | −33.0 | 265 | −26.15 | 322.90 | 6.85 | −57.90 |
| 18 | −17.0 | 418 | −14.28 | 339.74 | 2.72 | 78.26 |
| 19 | −29.1 | 211 | −27.75 | 249.57 | 1.35 | −38.57 |
| 20 | −19.1 | 288 | −15.88 | 266.40 | 3.22 | 21.60 |
| 21 | −17.8 | 350 | −18.33 | 364.24 | −0.53 | −14.24 |
| 22 | −23.7 | 309 | −18.50 | 339.40 | 5.20 | −30.40 |
| 23 | −23.8 | 238 | −23.53 | 249.90 | 0.27 | −11.90 |
| 24 | −24.0 | 246 | −23.70 | 225.07 | 0.30 | 20.93 |
| 25 | −21.8 | 289 | −21.02 | 294.65 | 0.78 | −5.65 |
| 26 | −13.5 | 334 | −21.02 | 294.65 | −7.52 | 39.35 |

be seen from Fig. 3 the zeta potential values increases at low level of factor *A* where the level of factor *B* is high. HPMC is a nonionic polymer and SLS is an anionic surfactant. At low level of factor *A* the particle surface of drug is not covered so densely with HPMC, due to which SLS diffuses faster to the particle surfaces; since it has excellent dispersion properties. Adsorption of SLS onto the particle surface leads to high zeta potential value. The zeta potential value of nanosuspension decreases at high level of factor *A*, irrespective of factor level *B*. At this stage due to increased concentration of HPMC in nanosuspension its adsorption on drug particles increases which leads to a reduction of the measured zeta potential. The adsorption layer of the stabilizer shifts the plain of shear, at which the zeta potential is measured, to a larger distance from the particle surface. Consequently the measured zeta potential is lower. In such cases zeta potentials of about 20 mV are still sufficient to fully stabilize the system in combination with steric stabilization.

Fig. 4 shows the effect of factors *A* and *D* on response $y_1$. It was observed from the figure that zeta potential value increases at high milling speed and lower polymer concentration. The probable reason for this may be that due to high milling speed the adsorption of steric and electrostatic stabilizer is more which increases the particle mobility and zeta potential value. The zeta potential values

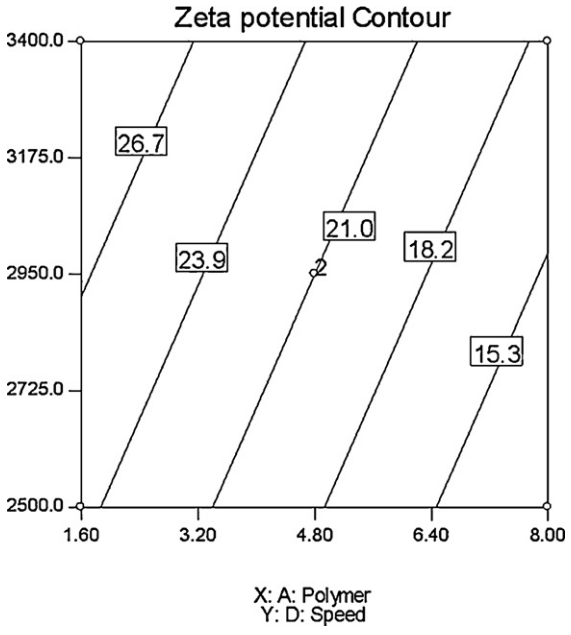


**Fig. 4.** Contour plot for effect of ratio of polymer to drug (*A*) and milling speed (*D*) on zeta potential ($y_1$).

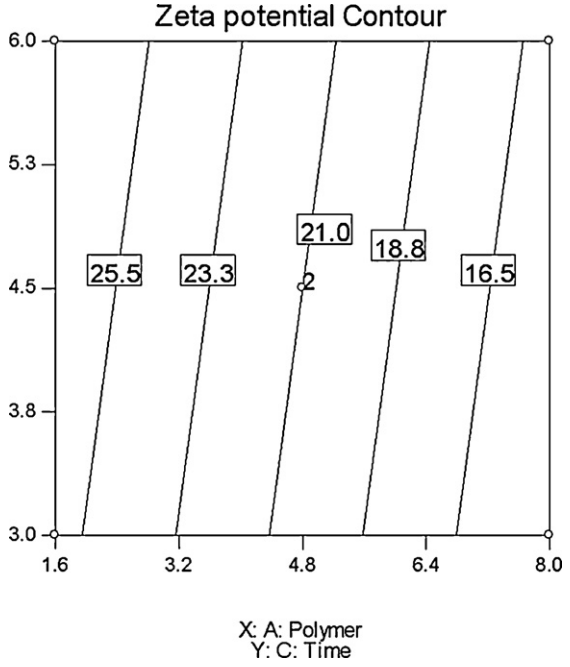


**Fig. 5.** Contour plot for effect of ratio of polymer to drug (*A*) and milling time (*C*) on zeta potential ($y_1$).

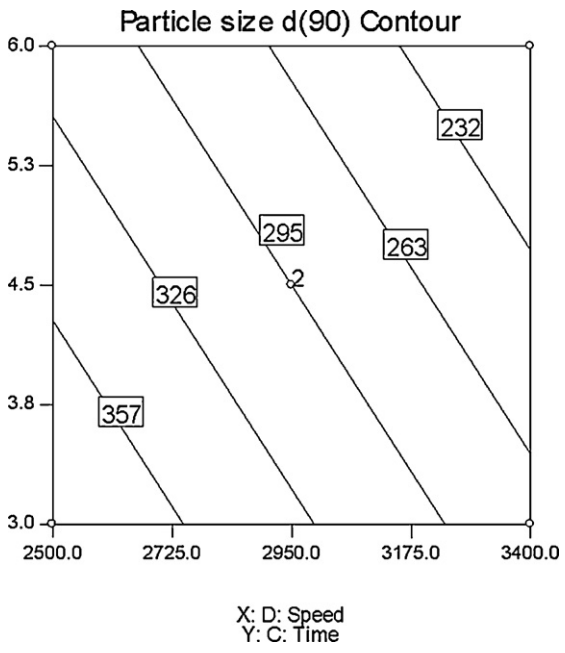


**Fig. 6.** Contour plot showing effect of milling speed ($D$) and milling time ($C$) on PSD $d(90)$ ($y_2$).

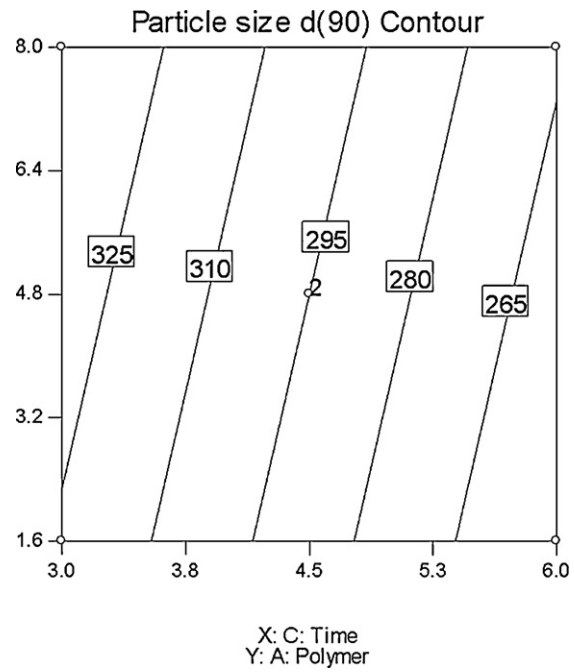


**Fig. 8.** Contour plot showing effect of ratio of polymer to drug ($A$) and milling time ($C$) on PSD $d(90)$ ($y_2$).

increases at low level of factor $A$ where the level of factor $C$ is high (Fig. 5).

The effect of factors $D$, $C$, $A$ and $B$ on $y_2$ is shown in Figs. 6–8. The effects of milling speed, $D$ and Milling time, $C$ on PSD $d(90)$ is shown in Fig. 6. Fig. 7 represents effect of ratio of polymer to drug, A and milling speed, D on the PSD $d(90)$ of nanosuspension.The effects of ratio of polymer to drug and Milling time, $C$ on PSD $d(90)$ is shown in Fig. 8. In Fig. 6, the PSD $d(90)$ response surface varies in linear pattern with increasing milling speed and milling time. PSD $d(90)$ of nanosuspension decreases as the milling time and milling speed increases. The minimum value of PSD $d(90)$ 211.0 nm is at the higher milling time and milling speed. The probable reason for this may be, high energy and shear forces generated as a result of the impaction of the milling media with the drug which provides the energy input to break the the microparticulate drug into nano-sized particles.

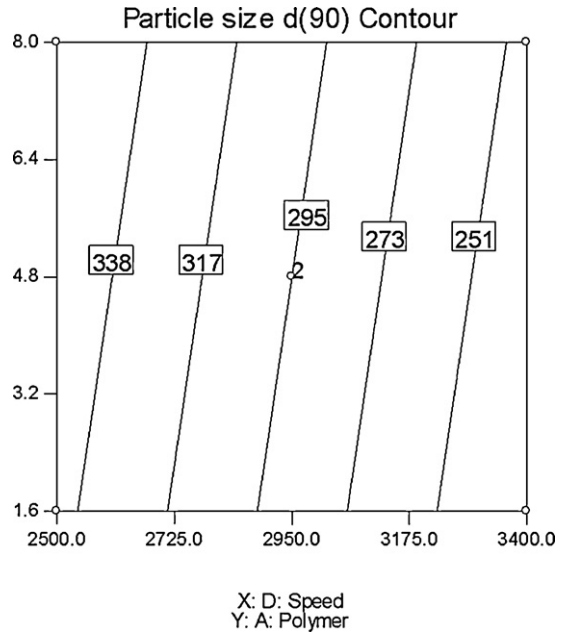


**Fig. 7.** Contour plot showing effect of milling speed ($D$) and ratio of polymer to drug ($A$) on PSD $d(90)$ ($y_2$).

As can be seen from Fig. 7 the PSD $d(90)$ of nanosuspension decreases at the lower polymer concentration. The probable reason for this may be, at lower polymer concentration collision of drug particles due to high impaction of milling media is increased which in turn decreases the PSD $d(90)$ of nanosuspension. Moreover at higher polymer concentration the viscosity of polymer increases drastically, which alters or hinders the processing of nanosuspension on bead mill. Issues such as increase in product temperature and increase in pressure on the bead mill were also observed during manufacturing nanosuspension with high polymer to drug ratio and high milling speed. These states that nanosuspension with high polymer to drug ratio to be milled at higher milling speed are unsuitable for manufacturing and scale up. But an attempt was carried out to overcome this issue by operating the bead mill at lower speed for initial 10 min and further increasing the speed slowly. The milling time also shows a prominent effect on the PSD $d(90)$ of nanosuspension even at high polymer concentration (Fig. 8).

### *3.2. Effect of milling speed on the powder X-ray diffraction pattern of API*

The powder X-ray diffraction study of spray dried nanosuspension operated at high milling speed and low milling speed showed no significant shift in the main peaks when compared with initial unmilled drug. The characteristic peaks for milled drug was observed at same $2\theta$ value as that of unmilled drug. A slight decrease in intensity of peaks was observed with spray dried nanosuspension operated at higher milling speed. Fig. 9 shows the XRD pattern of spray dried nanosuspensions.

### *3.3. Optimization of formulation and processing parameters using graphical optimization method*

Optimization of nanosuspension was performed to find the levels of factors $A$–$D$ which gives $y_1$ in −20 to −25 mV range and $y_2$

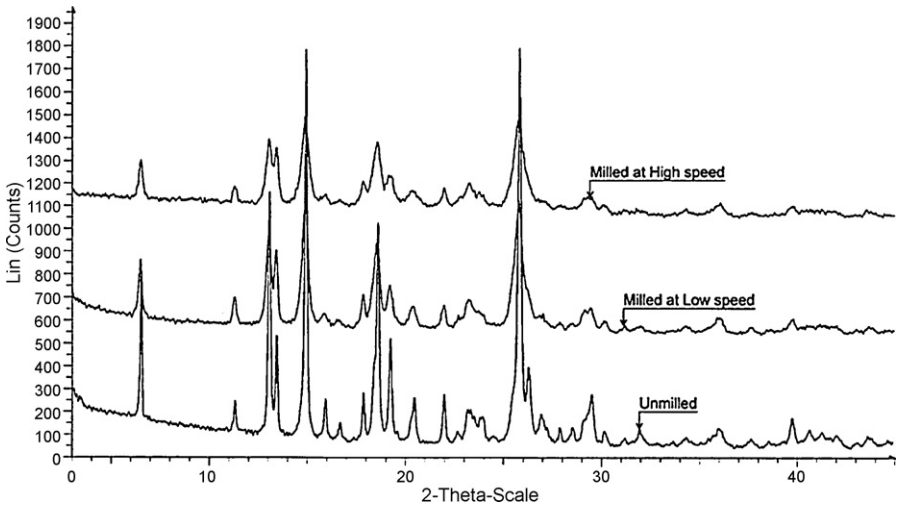


**Fig. 9.** XRD patterns for milled and unmilled drug.

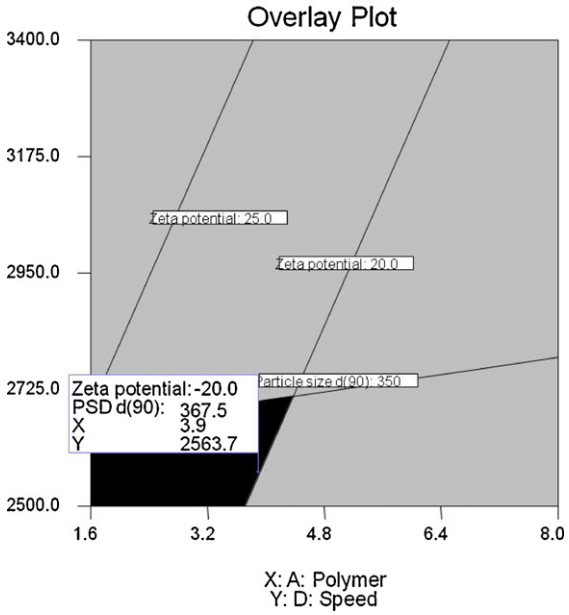


**Fig. 10.** Overlay plot for optimized parameters of nanosuspension.

$d(90)$ of 350–400 nm. Under this the model predicted $y_1$ and $y_2$ in required range at *A*, *B*, *C* and *D* values of 3.9 (g), 0.40(g), 3.8 (h) and 2563.0 (rpm), respectively for a batch size of 250 g. By using these values of factors three different batches of nanosuspensions were prepared. The values of observed $y_1$ and $y_2$ were in very close agreement to the predicted one. By this the reliability of the optimization procedure was established. Fig. 10 represents an overlay plot showing the optimized parameters suggested by DoE software to get the responses in required range.

## 4. Conclusion

Optimization of nanosuspension using media milling approach is a complex process since it involves large number of factors that affects the characteristic of nanosuspension. The RSM was been applied successfully to optimize formulation and process parameters for nanosuspension. From this study it was concluded that polymer concentration (ratio of polymer to drug) and milling speed plays a significant role in controlling the zeta potential of nanosuspension. Milling time and milling speed were considered to be significant factors which affected the PSD $d(90)$ of nanosuspension. The graphical optimization method helped in finding the sweet spot or design space to get nanosuspension with desired physicochemical properties. The study also helped in identifying certain formulation and processing parameters, such as high polymer concentration and high milling speed which may affects the manufacturing of nanosuspension at higher scale. Zeta potential of the nanosuspension was found to dependent more on the polymer concentration compared to surfactant concentration. Milling of nanosuspension at high speed and for long period of time didn't show any form conversion of drug.

## Acknowledgements

The author wishes to thank Dr. Ganesan M for providing valuable review comments on the research work. We also would like to thank Dr. Ravi Pillai who has always stressed for enhanced scientific understanding and been a rich source of inspiration.

## References

Date, A.A., Patravale, V.B., 2004. Current strategies for engineering drug nanoparticles. Curr. Opin. Colloid Interface Sci. 9, 222–235.
Kesisoglou, F., Panmai, S., Wu, Y., 2007. Nanosizing—oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.
Liversidge G., Ruddy S.B., 2009. Will nanoparticles deliver? http://www.dddmag.com/Article-Nanoparticles-For-Drug-Delivery-050509.aspx.
Muller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy Rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.
Raval, J.A., Patel, J.K., Patel, M.M., 2006. Nanosuspensions as Particulate Drug Delivery Systems, vol. 4, http://www.pharmainfo.net/reviews/.
Verma, S., Lanb, Y., Gokhale, R., Burgess, D.J., 2009. Quality by design approach to understand the process of nanosuspension preparation. Int. J. Pharm. 377, 185–198.
Zidan, A.S., Sammour, O.A., Hammad, M.A., Megrab, N.A., Habib, M.J., Khan, M.A., 2007. Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function. Int. J. Pharm. 332, 55–63.