Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 291 (2005) 39–49
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Application of experimental design methodology in development and optimization of drug release method

M. Kincl*, S. Turk, F. Vrečer

Krka, d.d., Novo mesto, R&D, Šmarješka cesta 6, 8501 Novo mesto, Slovenia

Received 19 January 2004; received in revised form 7 June 2004; accepted 20 July 2004
Available online 12 January 2005

Abstract

The aim of our research was to apply experimental design methodology in the development and optimization of drug release methods. Diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid monosodium salt) was selected as a model drug and Naklofen® retard prolonged release tablets, containing 100 mg of diclofenac sodium, were chosen as a model prolonged release system. On the basis of previous results, a three-level three-factorial Box–Behnken experimental design was used to characterize and optimize three physicochemical parameters, i.e. rotation speeds of the stirring elements, pH, and ionic strengths of the dissolution medium, affecting the release of diclofenac sodium from the tablets. The chosen dependent variables (responses) were a cumulative percentage of dissolved diclofenac sodium in 2, 6, 12 and 24 h. For estimation of coefficients in the approximating polynomial function, the least square regression method was applied. Afterwards, the information about the model reliability was verified by using the analysis of variance (ANOVA). The estimation of model factors' significance was performed by Student's *t*-test. For investigation of the shape of the predicted response surfaces and for model optimization, the canonical analysis was applied. Our study proved that experimental design methodology could efficiently be applied for characterization and optimization of analytical parameters affecting drug release and that it is an economical way of obtaining the maximum amount of information in a short period of time and with the fewest number of experiments.

Keywords: Fractional factorial design; Three-level design; Box–Behnken experimental design; Drug release; Prolonged release tablets

1. Introduction

Nowadays, most of the experimentation in drug release development is still performed by changing the levels of each variable (factor) separately at a time, in an unsystematic way, keeping all other variables constant in order to study the effects of the specific variable on the selected response or to find the optimal conditions of a complete system. This methodology is based on large number of experiments and often relies merely on the experience of the analyst (Kincl et al., 2004a).

* Corresponding author. Tel.: +386 73313824; fax: +386 73312193.
E-mail address: maja.kincl@krka.biz (M. Kincl).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.07.041

The traditional changing of one factor at a time is not an efficient and economic strategy because it does not give any information about the position of the optimum and can, at its best, lead only to a local optimum of the system. The one-at-a-time optimization also ignores interactions between factors and calls for unnecessarily numerous runs. With rapidly rising costs of experiments, it is very important that the development and optimization of any analytical method is done with as few experiments and with as low costs as possible (Bolton, 1990; Bloomfield and Butler, 2000; Parojčić et al., 2001; Kincl et al., 2004a).

The objective of the present work was to evaluate and characterize physicochemical parameters, which were previously statistically determined using the two-level fractional factorial design, to have a significant effect on the drug release from prolonged release tablets. Furthermore, an optimization of the drug release method was performed using polynomial mathematical equations and response surface plots. The optimization procedure enabled us to determine the combination of analytical parameters with predictable drug release profile.

Diclofenac sodium (2-[(2,6-dichlorophenyl) amino] benzeneacetic acid monosodium salt) was selected as a model drug, and Naklofen® retard prolonged release tablets, containing 100 mg of diclofenac sodium were chosen as a model tablets.

The formulation is based on a hydrophobic matrix of cetyl alcohol and additional membrane of cetyl alcohol that surrounds the matrix. The drug exhibits a diffusion-controlled drug release mechanism and the formulation is a combination of reservoir and inert-matrix system (Kincl et al., 2004a).

First, the solubility characteristics of diclofenac sodium in aqueous media with the pH in the range of 1–8 were determined. In acidic media, the active ingredient is present mostly in its free acid form, which is even less soluble than the salt. Therefore, the active ingredient is only slightly soluble or practically insoluble in these media. As the pH of the medium increases, the solubility of the active ingredient increases due to the contribution from ionized form, until the highest solubility of ionized form is reached in phosphate buffer solution, pH 8.0. Furthermore, the solubility also depends on ionic strengths of the dissolution media. In buffer solutions with higher ionic strengths having the same pH, the solubility of diclofenac sodium is lower than in buffer solutions with lower ionic strengths (Kincl et al., 2004b).

For estimation of effects of six different factors, i.e. type of the dissolution apparatus, rotation speeds of the stirring elements, pH, relative ionic strengths of the dissolution medium, applied salt, and producer of the on-line connected dissolution apparatus and UV spectrophotometer on the release of diclofenac sodium, the resolution III two-level six-factorial design (2_{III}^{6-3}) was applied. From the results of 2_{III}^{6-3} factorial design, it was concluded that three factors: rotation speed of the stirring elements, pH and relative ionic strengths of the dissolution medium have a significant influence on all seven responses, i.e. they significantly affect the release of diclofenac sodium from prolonged release tablets after 2 (Y_1), 4 (Y_2), 6 (Y_3), 8 (Y_4), 10 (Y_5), 12 (Y_6) and 24 h (Y_7) (Kincl et al., 2004a). For further studies of these parameters at least three-level experimental designs should be applied.

The application of three- or higher-level experimental designs using the response surface methodology does not appear to have been reported in development and optimization of the dissolution or drug release methods until now.

The relationship between one or more response variables and a set of quantitative parameters can be examined well by using response surface methods, such as Central composite designs or Box–Behnken designs (Box and Behnken, 1960; Ragonese et al., 2002). Response surface methods are often used once the preliminary screening has been carried out by applying the designs, such as factorial designs, to determine which factors significantly affect the response. They are also used when any curvature in the response surface is suspected. However, central composite designs usually have axial points outside the cube unless alpha, the axial spacing for ensuring orthogonality, is specified as less than or equal to one. Box–Behnken designs do not have axial points and they ensure that all factors are never simultaneously set at their high levels. Therefore, all the design points fall within the safe operating zone. Box–Behnken experimental designs have fewer design points and fewer experiments to be performed. Furthermore, each factor requires only three levels instead of five, required for central composite designs (unless alpha is equal to one), which is experimentally more convenient and less expensive to perform than central composite designs with the same number

of factors (Montgomery, 1991; Massart et al., 2001; Ragonese et al., 2002).

Considering these facts, we decided to apply the three-level three-factorial Box–Behnken experimental design for investigation, characterization and optimization of analytical parameters, affecting the release of diclofenac sodium.

2. Materials and methods

2.1. Materials

Sodium hydroxide was obtained from J.T. Baker (Phillipsburg, NJ, USA), potassium dihydrogen phosphate and potassium chloride, all analytical grade, from Fluka (Munich, Germany), and purified water for chromatography from a Milli-Q purification unit (Millipore, Milford, MA, USA). Injection syringes (10 and 20 ml) were supplied by Sartorious GmbH (Goettingen, Germany), 10 μm full flow filters and bent cannulas for dissolution sampling by Van Kel (Cary, NC, USA) and pipettes by Hirshman (Germany). Beakers, tall form, with graduation and spout (1000, 2000 and 5000 ml), graduated measuring cylinders (500 and 1000 ml), Erlenmayer flasks (100 and 250 ml) and volumetric flasks (20, 25, 50, 100, 1000 and 2000 ml) were all provided by Duran (Mainz, Germany). Diclofenac sodium (2-[(2,6-dichlorophenyl amino]benzeneacetic acid monosodium salt) was obtained from Dipharma S.p.A. (Milano, Italy). Naklofen® retard prolonged release tablets, containing 100 mg of diclofenac sodium, were produced by Krka, d.d., Novo mesto, Slovenia.

2.2. Instrumentation

For drug release tests, Van Kel VK 7010 dissolution tester (Van Kel, Cary, NC, USA), on-line connected to Cary 50 Bio UV spectrophotometer (Van Kel) was used. Furthermore, ultrasonic bath (Donaulab-sonic, DLS 700-T; Zurich, Switzerland) and analytical balance (Mettler-Toledo AT261 DeltaRange; Greifensee, Switzerland) were used. The statistical software used to evaluate the experimental design results was The SAS System, Release 8.2 (SAS Institute, Cary, NC, USA).

2.3. Drug release experiments

Technical data: all experiments were performed in 900 ml of dissolution medium with the temperature $37 \pm 0.5\,^{\circ}C$, using baskets as stirring elements (apparatus 1) at 20, 80, 110 and 200 rpm. Sample solutions were automatically withdrawn after 2, 6, 12 and 24 h of drug release and filtrated through 10 μm full flow filters for dissolution sampling (Van Kel). In all experiments, six tablets were analysed and the results are presented as the mean value of six units.

All the aqueous media used for drug release tests, i.e. phosphate buffer solutions, pH 5.8, 6.8, 7.6 and 7.8 were prepared according to the prescriptions in the USP 27 (2004). Relative ionic strengths of these media (stock buffer solutions) were marked with value 1.0. The dissolution media with pH from 5.8 to 7.8 and higher ionic strengths (relative ionic strengths of these media were marked with values 2.18, 3.65 and 6.3; which means, the absolute ionic strengths were 2.18-, 3.65- and 6.3-times higher compared to absolute ionic strengths of stock phosphate buffer solutions with relative ionic strengths 1.0) were prepared by adding potassium chloride to the stock buffer solutions, prepared according to USP 27. The amount of salt in the dissolution media was defined according to literature data on the media simulating intestinal conditions (Dressman et al., 1998; Kincl et al., 2004a). Intestinal media contain KH_2PO_4, NaOH, KCl and surfactants, Na-Taurocholate and Lecithin (Dressman et al., 1998; Hörter and Dressman, 2001). In order to prevent foaming of the dissolution media at higher rotation speeds of the stirring elements the surfactants were not used in our experiments. However, to stay as close to the media simulating intestinal conditions, potassium chloride was used as salt for achieving higher ionic strengths of aqueous media (Kincl et al., 2004a).

Calculated absolute ionic strengths and preparations of the media with 3.65- and 6.3-times higher absolute ionic strengths, used in drug release tests are shown in Table 1.

Phosphate buffer solution pH 7.6 with relative ionic strength 2.18 was prepared by adding 5.8 g KCl/l of the stock phosphate buffer solution with pH 7.6, prepared according to USP 27. Calculated absolute ionic strength of the stock phosphate buffer solution pH 7.6 was 0.13 mol/l, therefore calculated absolute ionic

Table 1
Calculated absolute ionic strengths of the dissolution media used in the experiments in mol/l and preparations of the media with 3.65- and 6.3-times higher absolute ionic strengths

Medium	pH	KCl (g)/liter of dissolution medium			Absolute ionic strength (mol/l)		
		1.0-I	3.65-I	6.3-I	1.0-I	3.65-I	6.3-I
Phosphate	5.8	–	5.4	10.8	0.055	0.20	0.35
Buffer	6.8	–	8.2	16.4	0.08	0.29	0.50
Solution	7.8	–	13.0	26.1	0.14	0.47	0.82

strength of the phosphate buffer solution pH 7.6 with 2.18-times higher ionic strength was 0.29 mol/l.

2.4. Ultraviolet spectrophotometry

In drug release experiments, the sample solutions were automatically withdrawn at specified time intervals from each dissolution vessel respectively, and filtered. The absorbances were measured on an on-line connected UV spectrophotometer at the wavelength of maximum absorbance at 276 ± 2 nm, using 1 mm quartz cells. The standard linear calibration curve was applied and linear relationship in the concentration range of 0.011–0.133 mg/ml of standard solutions (0.111 mg of diclofenac sodium/ml = 100% working concentration) was attained.

2.5. Experimental design

In the present study, a three-level three-factorial Box–Behnken experimental design was used to evaluate the effects of selected independent variables on the responses, to characterize the drug release process and to optimize the procedure. This design is suitable for exploration of quadratic response surfaces and for construction of second order polynomial models, thus helping to optimize the process by using a small number of experimental runs. For the three-level three-factorial Box–Behnken experimental design, a total of 15 experimental runs, shown in Table 2, are needed.

The generated model contains quadratic terms explaining the non-linear nature of responses, which is well indicated already in the prediction plot (Fig. 1).

This design also resolves the two-factor interaction effects of individual terms and allows a mid-level setting (0) for the combination of factors (Montgomery, 1991; Singh et al., 1995). The design consists of replicated center points and a set of points lying at the mid-points of each edge of the multidimensional cube that defines the region of interest. The model is of the following form:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2 + b_5x_2x_3 + b_6x_1x_3 + b_7x_1^2 + b_8x_2^2 + b_9x_3^2 + E \quad (1)$$

where y is the selected response, b_0–b_9 are the regression coefficients, x_1, x_2 and x_3 are the factors studied and E is an error term. Box–Behnken experimental design is an orthogonal design. Therefore, the factor levels are evenly spaced and coded for low, medium and high settings, as −1, 0 and +1 (Montgomery, 1991; Singh et al., 1995; Karnachi and Khan, 1996).

The preliminary solubility and drug release studies provided the factors and settings of factor levels (Kincl et al., 2004b). Factors studied in the Box–Behnken experimental design were: rotation speeds of the stirring elements—baskets (x_1), pH of the dissolution

Table 2
Presentation of 15 experiments (BB1–BB15) with coded values for factor levels for the Box–Behnken experimental design

Experiment (run)	Factor and factor level		
	x_1	x_2	x_3
BB1	−1	−1	0
BB2	−1	+1	0
BB3	+1	−1	0
BB4	+1	+1	0
BB5	−1	0	−1
BB6	−1	0	+1
BB7	+1	0	−1
BB8	+1	0	+1
BB9	0	−1	−1
BB10	0	−1	+1
BB11	0	+1	−1
BB12	0	+1	+1
BB13	0	0	0
BB14	0	0	0
BB15	0	0	0

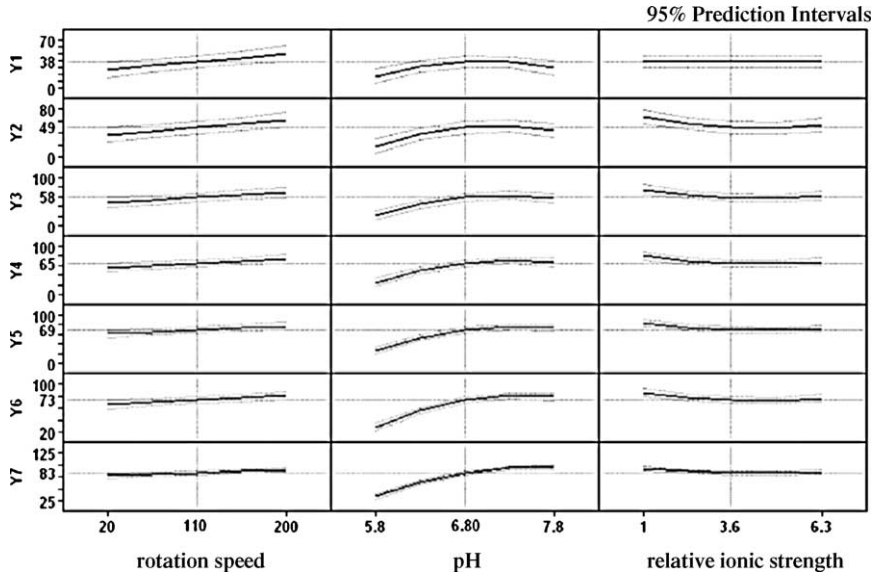

Fig. 1. Prediction plot showing effects of analytical parameters: rotation speed of the stirring basket (x_1), pH of the dissolution medium (x_2) and relative ionic strength of the dissolution medium (x_3) on the release of diclofenac sodium after 2 (Y_1), 4 (Y_2), 6 (Y_3), 8 (Y_4), 10 (Y_5), 12 (Y_6) and 24 h (Y_7).

medium (x_2) and relative ionic strength of the dissolution medium (x_3). The factor levels for pH and relative ionic strengths of the dissolution media were chosen in accordance with obtained solubility results (Kincl et al., 2004b) and considering literature data about media simulating intestinal conditions (the amount of added KCl increases the ionic strength of intestinal media 6.3-times (Dressman et al., 1998; Hörter and Dressman, 2001)). Therefore, in our study relative ionic strength 6.3 was chosen as the highest value of the factor x_3. Phosphate buffer solutions suggested in US Pharmacopoeia as appropriate media for dissolution testing were chosen as media with relative ionic strength 1.0 (the lowest value of factor x_3). According to our experience on developing drug release methods the best in vitro in vivo correlation are achieved when using rotation speeds from 20 to 200 rpm. For this reason, these two rotation speeds were chosen as the lowest and the highest value of the factor x_1. Because the factor levels in Box–Behnken experimental design are evenly spaced, 110 rpm was determined as the middle value of the factor x_1.

The selected responses were a cumulative percentage of dissolved diclofenac sodium in 2 (y_1), 6 (y_2), 12 (y_3) and 24 h (y_4). Table 3 shows the factors chosen and settings of factor levels.

Table 3
Factors and factor levels investigated in Box–Behnken experimental design

Factor	Level		
	−1	0	+1
x_1: rotation speed of the stirring basket (rpm)	20	110	200
x_2: pH of the medium	5.8	6.8	7.8
x_3: relative ionic strength of the medium	1	3.65	6.3

The responses studied and the constraints selected considering pharmacodynamic and pharmacokinetic properties of diclofenac sodium and regarding EMEA, FDA and FIP guidelines for dissolution testing are presented in Table 4 (Todd and Sorkin, 1988; EMEA, 1999a,b; FDA, 1997, 2000; FIP, 1997).

Table 4
Responses selected and the constraints used in Box–Behnken experimental design

Response	Constraints (%)
y_1: % of released diclofenac sodium in 2 h	25–35
y_2: % of released diclofenac sodium in 6 h	55–65
y_3: % of released diclofenac sodium in 12 h	70–85
y_4: % of released diclofenac sodium in 24 h	95–105

The responses were selected in accordance with preliminary drug release experiments and concerning the results presented in Fig. 1. From the prediction plot (Fig. 1), it is obvious that substantial changes in drug release profiles occurred within 2, 6, 12 and 24 h of the drug release. The differences in the release of diclofenac sodium between 2nd and 4th h, 6th and 8th h and 10th and 12th h are not considerable (Kincl et al., 2004a). Therefore, selected responses of the three-level three-factorial Box–Behnken experimental design are a cumulative percentage of released diclofenac sodium in 2, 6, 12 and 24 h.

3. Results and discussion

3.1. Experiments of Box–Behnken experimental design

Response data for all 15 experimental runs of Box–Behnken experimental design (BB1–BB15), performed in accordance with Table 2, are presented in Figs. 2–5. Regarding different combinations of factors and factor levels, a considerable difference between drug release profiles was obtained. The responses of the Box–Behnken experimental design ranged from an exceedingly low drug release profile, in run BB1 (around 30% of released diclofenac sodium after 24 h), to very fast drug release profiles, in runs BB7 and BB8 (around 65% of released diclofenac sodium in just 2 h).

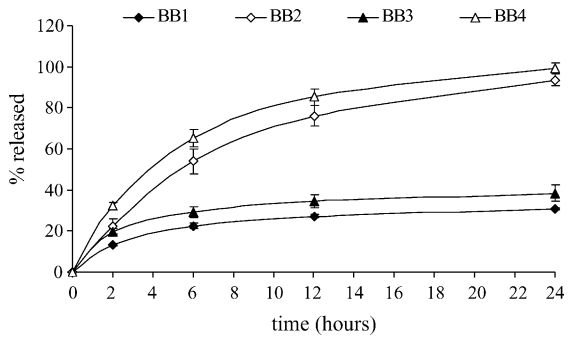

Fig. 2. Release profiles of diclofenac sodium in accordance with Box–Behnken experimental design runs BB1–BB4.

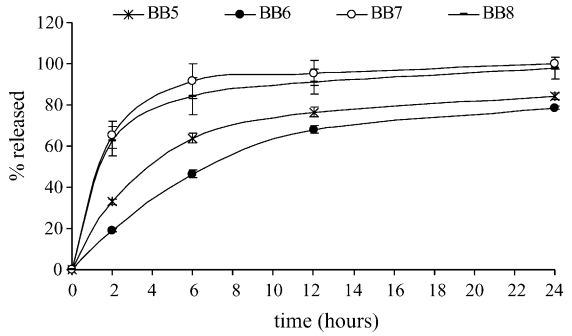

Fig. 3. Release profiles of diclofenac sodium in accordance with Box–Behnken experimental design runs BB5–BB8.

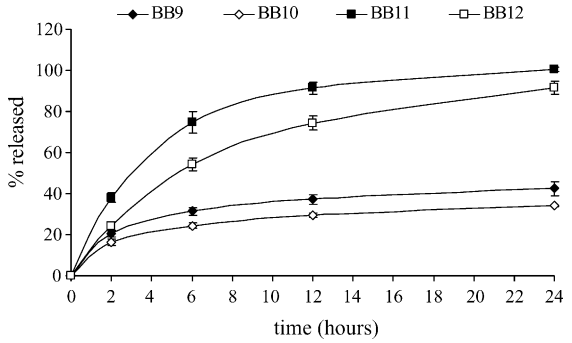

Fig. 4. Release profiles of diclofenac sodium in accordance with Box–Behnken experimental design runs BB9–BB12.

3.2. Formation of the second order model and analysis of variance (ANOVA)

For estimation of coefficients in the approximating polynomial function (Eq. (1)) applying uncoded values of factor levels, the least square regression method was

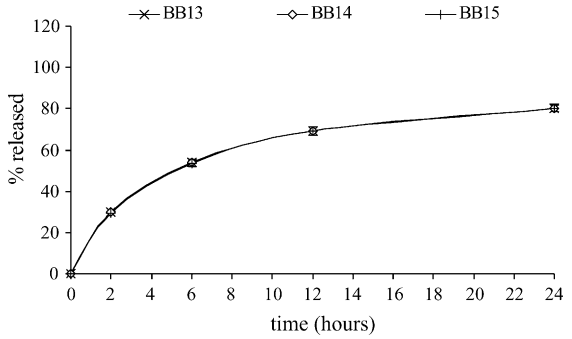

Fig. 5. Release profiles of diclofenac sodium in accordance with Box–Behnken experimental design runs BB13–BB15.

Table 5
Analysis of variance for all four responses y_1, y_2, y_3 and y_4

Source of variation	Response							
	y_1		y_2		y_3		y_4	
	F	p-value	F	p-value	F	p-value	F	p-value
Model	3.46	0.092	11.20	0.0080	40.37	0.0004	61.87	0.00014
Linear contribution	5.48	0.049	21.60	0.0027	90.93	0.0001	152.7	0.0001
Quadratic contribution	4.69	0.64	11.55	0.011	29.74	0.0013	32.82	0.0010
Cross-product contribution	0.21	0.88	0.45	0.73	0.44	0.73	0.092	0.96

performed using the SAS System statistical software, Release 8.2. The resulted equations (Eqs. (2)–(5)) for all four responses y_1, y_2, y_3 and y_4 are presented below:

$$y_1 = -648.67 + 0.16\,x_1 + 198.29\,x^2 - 6.16\,x_3 + 0.011\,x_1x_2 - 0.92\,x_2x_3 + 0.011\,x_1x_3 + 0.00077\,x_1^2 - 13.99\,x_2^2 + 1.30\,x_3^2 \quad (2)$$

$$y_2 = -896.48 + 0.21\,x_1 + 267.88\,x_2 - 6.00\,x_3 + 0.013\,x_1x_2 - 1.27\,x_2x_3 + 0.011\,x_1x_3 + 0.00089\,x_1^2 - 18.17\,x_2^2 + 1.51\,x_3^2 \quad (3)$$

$$y_3 = -970.87 + 0.13\,x_1 + 285.16\,x_2 - 4.68\,x_3 + 0.0053\,x_1x_2 - 0.88\,x_2x_3 + 0.0045\,x_1x_3 + 0.00070\,x_1^2 - 18.95\,x_2^2 + 1.15\,x_3^2 \quad (4)$$

$$y_4 = -989.40 + 0.023\,x_1 + 288.48\,x_2 - 7.18\,x_3 - 0.0056\,x_1x_2 - 0.066\,x_2x_3 + 0.0039\,x_1x_3 + 0.00052\,x_1^2 - 18.95\,x_2^2 + 0.082\,x_3^2 \quad (5)$$

For estimation of significance of the model, the analysis of variance (ANOVA) was applied. Using 5% significance level, a model is considered significant if the p-value (significance probability value) is less than 0.05. From the p-values presented in Table 5, it can be concluded that for all four responses, the cross-product contribution of the model was not significant. For response y_1, only linear contribution of the model was significant, whereas for responses y_2, y_3 and y_4 quadratic contribution of the model was also significant.

Calculating the Pearson correlation coefficients (R^2), it was found that 86.2% of the variability of experimental data could be explained using the model

Table 6
Factor effects and associated p-values for all four responses

Factor	Response							
	y_1		y_2		y_3		y_4	
	Factor effect	p-value	Factor effect	p-value	Factor effect	p-value	Factor effect	p-value
x_1	+11.49	**0.019**	+10.49	**0.011**	+7.35	**0.0058**	+6.06	**0.0083**
x_2	+5.89	0.14	+17.60	**0.0012**	+24.88	**0.0001**	+29.91	**0.0001**
x_3	−4.33	0.25	−6.51	0.059	−4.70	**0.035**	−3.18	0.078
x_1x_2	+1.00	0.84	+1.18	0.77	+0.48	0.84	−0.5	0.82
x_2x_3	−2.43	0.63	−3.38	0.41	−2.33	0.35	−0.18	0.93
x_1x_3	+2.73	0.59	+2.55	0.53	+1.08	0.65	+0.93	0.67
x_1^2	+6.21	0.26	+7.21	0.13	+5.70	0.06	+4.20	0.10
x_2^2	−13.99	**0.037**	−18.17	**0.0057**	−18.95	**0.00048**	−18.95	**0.00029**
x_3^2	+9.13	0.12	+10.61	**0.042**	+8.05	**0.019**	+5.78	**0.041**

Significant effect of factors on individual responses are shown in bold.

polynomial function y_1. For responses, y_2, y_3 and y_4, the Pearson correlation coefficients R^2 were 95.3, 98.6 and 99.1%. Therefore, it can be concluded that model functions y_2, y_3 and y_4 well interpreted the variability of data after 6, 12 and 24 h of drug release whereas, after 2 h of drug release the model polynomial function is less adequate.

3.3. Estimation of quantitative effects of the factors

For estimation of quantitative effects of the factors, Student's *t*-test was performed. A response surface regression analysis for each factor was performed using coded values of factor levels (−1, 0, +1). In Table 6, factor effects of the Box–Behnken model and associated *p*-values for all four responses are presented. A factor is considered to influence the response if the effects significantly differ from zero and the *p*-value is less than 0.05. A positive sign indicates a synergistic effect, while a negative sign represents an antagonistic effect of the factor on the selected response.

From Table 6, it can be seen that the response y_1 (% of released diclofenac sodium in 2 h) was significantly affected by the synergistic effect of rotation speeds of the baskets (x_1), with a *p*-value of 0.019 and the antagonistic effect of quadratic term of pH of the dissolution medium (x_2^2) (*p*-value of 0.037). Significant factors for the responses y_2, y_3 and y_4 were rotation speeds of the baskets (x_1), with *p*-values of 0.011, 0.0058 and 0.0083, respectively, the pH of the dissolution medium (x_2), with *p*-values of 0.0012, 0.0001 and 0.0001, respectively, and the quadratic term of relative ionic strength (x_3^2) with *p*-values of 0.042, 0.019 and 0.041, respectively. All the above-mentioned factors show synergistic effects and increase the release of diclofenac sodium from tablets. Additionally, the quadratic term of pH of the dissolution medium (x_2^2) has an antagonistic effect on all three responses (*p*-values of 0.0057, 0.019 and 0.041, respectively). However, response y_3 is also significantly affected by the antagonistic effect of relative ionic strength of the dissolution medium (x_3), with *p*-value of 0.035. Presumably, this is mostly connected with the low solubility of diclofenac sodium in media with higher ionic strength. In addition, interaction effects (cross-product terms) were not found to be significant for all four responses.

3.4. Three-dimensional (3D) response surface plots

Three-dimensional (3D) plots for the measured responses were formed, based on the model polynomial functions to assess the change of the response surface. Also the relationship between the dependent and independent variables can be further understood by these plots. Since the model has more than two factors, one factor was held constant for each diagram, therefore, a total of 12 response surface diagrams was produced—3 for each response. Response surface plots are presented

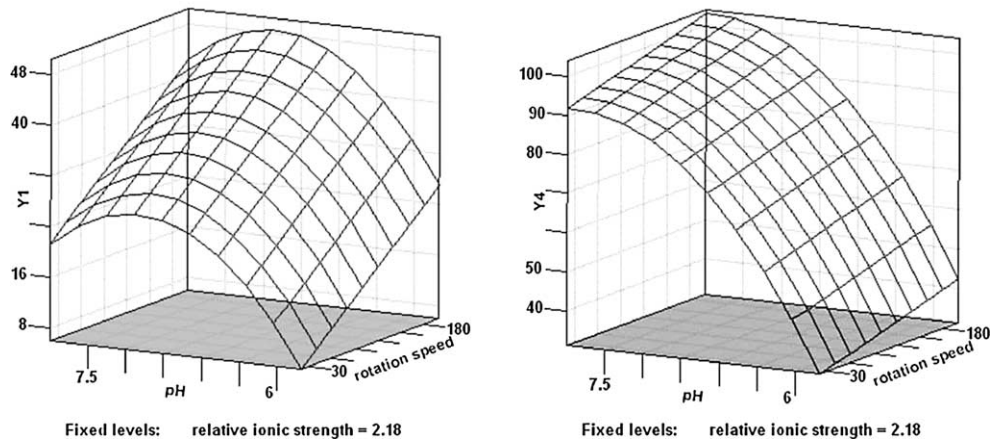

Fig. 6. Response surface plots (3D) showing the effect of rotation speed of the stirring basket (x_1) and pH of the dissolution medium (x_2) on the response y_1 (% of released diclofenac sodium in 2 h) and response y_4 (% of released diclofenac sodium in 24 h), respectively.

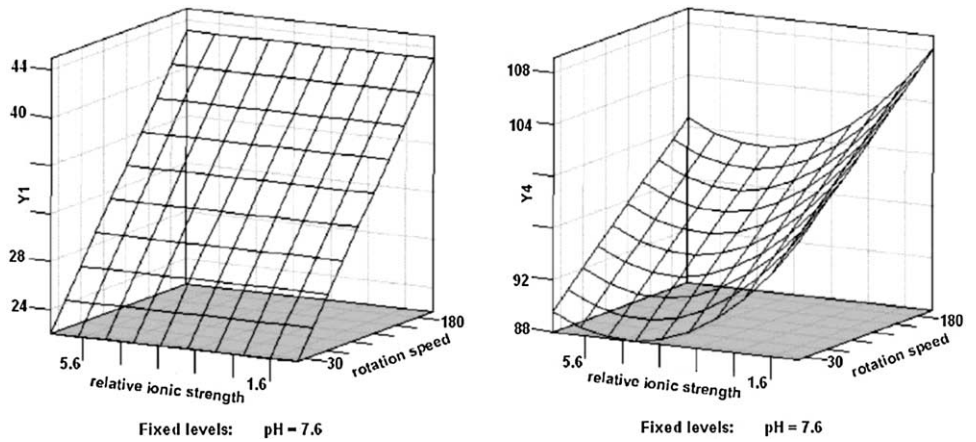

Fig. 7. Response surface plots (3D) showing the effect of the rotation speed of the stirring basket (x_1) and relative ionic strength of the dissolution medium (x_3) on the response y_1 (% of released diclofenac sodium in 2 h) and response y_4 (% of released diclofenac sodium in 24 h), respectively.

using optimal levels of the factors studied (see Section 3.5). Considering the greatest difference in model polynomial functions response, the surface plots for responses y_1 and y_4 are further presented (Figs. 6–8).

In Fig. 6, response surface plots (3D) showing the effect of rotation speed of the stirring basket (x_1) and pH of the dissolution medium (x_2) on the response y_1 (% of released diclofenac sodium in 2 h) and the response y_4 (% of released diclofenac sodium in 24 h), respectively are presented. In Fig. 7, response surface plots (3D) showing the effect of rotation speed of the stirring basket (x_1) and ionic strengths of the dissolution medium (x_3) on the response y_1 and response y_4, respectively are shown. The influence of pH of the dissolution medium (x_2) and ionic strengths of the dissolution medium (x_3) are presented in Fig. 8.

3.5. Optimization

After generating the model polynomial equations to relate the dependant and independent variables, the process was optimized for all four responses. The final optimal experimental parameters were calculated using the canonical analysis, which allows the compromise

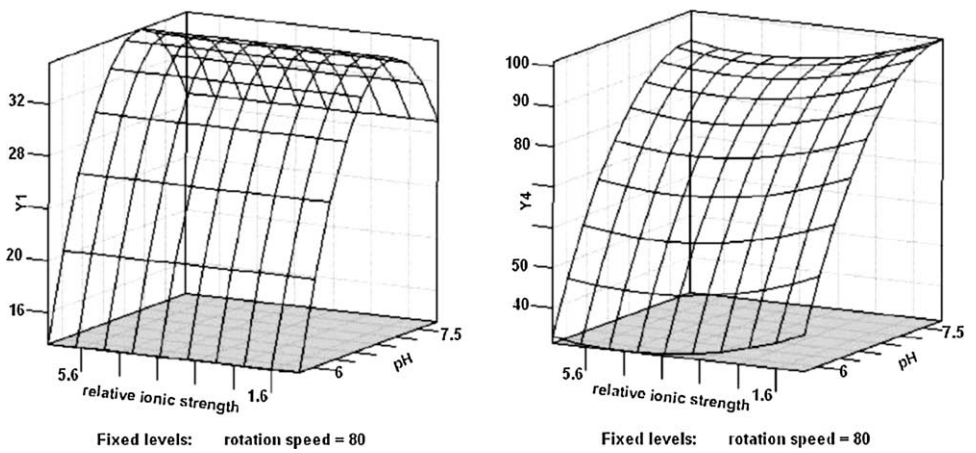

Fig. 8. Response surface plots (3D) showing the effect of pH of the dissolution medium (x_2) and relative ionic strength of the dissolution medium (x_3) on the response y_1 (% of released diclofenac sodium in 2 h) and response y_4 (% of released diclofenac sodium in 24 h), respectively.

Table 7
Observed and predicted responses (y_1, y_2, y_3 and y_4) and residual values for the drug release test, performed at optimal values of factors investigated

Response	Observed response	Predicted response	Residual
y_1	27.7	30.0	−2.3
y_2	61.8	63.8	−2.0
y_3	81.2	82.7	−1.5
y_4	97.5	95.9	+1.6

among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. In this study, the optimization was performed with constraints for all four responses, presented in Table 4. The optimal calculated parameters were:

- rotation speed of the basket (x_1): 80 rpm;
- pH of the dissolution medium (x_2): 7.6;
- relative ionic strength (x_3): 2.18.

To confirm the validity of the calculated optimal parameters and predicted responses, the drug release profile at optimal combination of physicochemical parameters was carried out. Table 7 illustrates the observed and predicted response and residual values for the drug release test, performed at optimal values of the analytical parameters investigated in this study. From the results presented in Table 7, it can be concluded that optimized combination of investigated physicochemical parameters ensured the release profile, which was very close to the predicted values.

4. Conclusions

The method for drug release of diclofenac sodium from prolonged release tablets with optimal release properties was determined using experimental design methodology. After determination of significant parameters by using resolution III two-level six-factorial design, the three-level three-factorial Box–Behnken experimental design was applied. Analytical parameters investigated in this study were: rotation speed of the stirring basket (x_1), pH of the dissolution medium (x_2) and ionic strengths of the dissolution medium (x_3). The chosen responses were a cumulative percentage of dissolved diclofenac sodium in 2, 6, 12 and 24 h. The model reliability and estimation of quantitative effects of different levels of investigated factors was performed using the SAS System statistical software, Release 8.2. The levels of these factors were predicted to obtain an optimal response with reference to set constraints. The observed responses were close to the predicted values for the optimized drug release method. From the above results, it can be concluded that characterization and optimization of the drug release method was performed in a very short time period and with a small number of experimental runs.

It is essential that experimental design methodology is a very economic way for extracting the maximum amount of complex information, a significant experimental time saving factor and moreover, it saves the material used for analyses and personal costs as well.

References

Bolton, S., 1990. Pharmaceutical Statistics: Practical and Clinical Applications, second ed. Marcel Dekker, New York, Revised and expanded.

Box, G.E.P., Behnken, D.W., 1960. Some new three level designs for the study of quantitative variables. Technometrics 2, 455–475.

Bloomfield, M.S., Butler, W.C., 2000. Robustness testing, using experimental design, of a flow-through dissolution method for a product where the actives have markedly differing solubility properties. Int. J. Pharm. 206, 55–61.

Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15, 11–22.

EMEA, Committee for Proprietary Medicinal Products (CPMP), 1999a. Note for guidance on quality of modified release products.

EMEA, Committee for Proprietary Medicinal Products (CPMP), 1999b. Note for guidance specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances.

Hörter, D., Dressman, J.B., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv. Drug Dev. Rev. 46, 75–87.

Karnachi, A.A., Khan, M.A., 1996. Box–Behnken design for optimization of formulation variables of indomethacin coprecipitates with polymer mixtures. Int. J. Pharm. 131, 9–17.

Kincl, M., Vrečer, F., Veber, M., 2004a. Characterization of factors affecting the release of low solubility drug from prolonged release tablets. ACA 502, 107–113.

Kincl, M., Meleh, M., Veber, M., Vrečer, F., 2004b. Study of physicochemical parameters affecting the release of diclofenac sodium from prolonged release tablets. ACSi 51, 409–425.

Massart, D.L., Vandenginste, B.G.M., Buydens, L.M.C., De Jong, S., Lewi, P.J., Smeyers-Verbeke, J., 2001. Data Handling in Science and Technology 20A: Handbook of Chemometrics and Qualimetrics. Part A. Elsevier, Amsterdam.

Montgomery, D.C., 1991. Design and Analysis of Experiments, third ed. Wiley, New York.

Parojčić, J., Đurić, Z., Jovanović, M., Ibrić, S., Nikolić, L., 2001. Influence of pH and agitation intensity on drug dissolution from tablets evaluated by means of factorial design. Pharm. Ind., 774–779.

Ragonese, R., Macka, M., Hughes, J., Petocz, P., 2002. The use of Box–Behnken experimental design in the optimization and robustness testing of a capillary electrophoresis method for the analysis of ethambutol hydrochloride in pharmaceutical formulation. J. Pharm. Biomed. Anal. 27, 995–1007.

Singh, K.S., Dodge, J., Durrani, M.J., Khan, M.A., 1995. Optimization and characterization of controlled release pellets coated with an experimental latex. I. Anionic drug. Int. J. Pharm. 125, 243–255.

The section for official laboratories and medicines control services. The section of industrial pharmacists of the FIP, 1997. FIP guidances for dissolution testing of solid oral products. Drugs made in Germany 40, 123–128.

Todd, P.A., Sorkin, E.M., 1988. Diclofenac sodium: a reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. Drugs 35, 244–285.

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 1997. Extended release oral dosage forms: development, evaluation and application of in vitro/in vivo correlations. In FDA Guidance for Industry, Rockville (http://www.fda.gov/cder/guidance/index.htm).

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2000. Waiver and in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms containing certain active moieties/active ingredients based on biopharmaceutical classification system. In FDA Guidance for Industry, Rockville (http://www.fda.gov/cder/guidance/3618fnl.pdf.).

US Pharmacopoeia 27, NF-22, 2004. United States Pharmacopeial Convention Inc., Rockville, pp. 2724–2725.