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Didanosine extended-release matrix tablets: optimization of formulation variables using statistical experimental design

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Abstract

Statistical experimental design was applied to evaluate the influence of some process and formulation variables and possible interactions among such variables, on didanosine release from directly-compressed matrix tablets based on blends of two insoluble polymers, Eudragit RS-PM and Ethocel 100, with the final goal of drug release behavior optimization. The considered responses were the percent of drug released at three determined times, the dissolution efficiency at 6 h and the time to dissolve 10% of drug. Four independent variables were considered: tablet compression force, ratio between the polymers and their particle size, and drug content. The preliminary screening step, carried out by means of a 12-run asymmetric screening matrix according to a D-optimal design strategy, allowed evaluation of the effects of different levels of each variable. The drug content and the polymers ratio had the most important effect on drug release, which, moreover, was favored by greater polymers particle size; on the contrary the compression force did not have a significant effect. The Doehlert design was then applied for a response-surface study, in order to study in depth the effects of the most important variables. The desirability function was used to simultaneously optimize the five considered responses, each having a different target. This procedure allowed selection, in the studied experimental domain, of the best formulation conditions to optimize drug release rate. The experimental values obtained from the optimized formulation highly agreed with the predicted values. The results demonstrated the reliability of the model in the preparation of extended-release matrix tablets with predictable drug release profiles.

Keywords: Statistical experimental design; Response surface methodology; Multiple response optimization; Didanosine; Extended-release inert matrix tablets

1. Introduction

Didanosine (DDI) is a nucleoside analog reverse transcriptase inhibitor used in AIDS treatment to suppress HIV replication. Patients

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infected with HIV, at least at present, have to be polymedicated for the rest of their lives. This fact explains the constant search, not only for new and more powerful drugs, but also for more effective formulations of already known drugs (Sánchez-Lafuente et al., 1999a). Sustained-release dosage forms can significantly improve patient compliance, especially in case of drug chronic use, also reducing the total dosage of administered drug and, consequently, the possible side effects (Vernaug, 1993). DDI can be considered a suitable candidate for sustained-release formulations from both the biopharmaceutical (Sánchez-Lafuente et al., 1999b) and pharmacokinetic (Parfitt, 1999) points of view.

In preliminary studies, some of us showed the suitability of using blends of Eudragit®RS-PM (acrylic–methacrylic acid copolymer) and Ethocel®100 (an ethylcellulose) for obtaining directly-compressed DDI sustained-release matrix tablets (Sánchez-Lafuente et al., 1999b,c, 2000). The purpose of the present study was the optimization of drug release profiles from such matrix tablets developed previously, by using statistical experimental design methodologies to quickly and efficiently evaluate the influence of some process and formulation variables and possible interactions among such variables on DDI release. In fact, several variables usually need to be optimized during development of a pharmaceutical product, some of which have to be maximized and others, on the contrary, minimized; moreover, competition may exist among them. Therefore, all the responses that may affect the quality of the product should be simultaneously taken into account. Consequently, classic preformulation studies require great expertise and experience, are expensive and time-consuming and, moreover, while through successive approximation experiments a progressive incremental improvement can be achieved, it is not possible to establish when and whether the optimal formulation has been actually obtained. Statistical experimental design has been recognized as a useful technique to find optimal parameters and conditions for various processes, particularly when multiple factors are involved (Goupy, 1993; Renoux et al., 1996; Porter et al., 1997; Lewis et al., 1999). In particular, optimization by means of statistical experimental design methodologies has been successfully applied in developing and optimizing extended-release dosage forms (Khan et al., 1996; Sastry et al., 1997; Takahara et al., 1997; Bodea and Leucuta, 1998; Geoffroy et al., 1998; Hamed and Sakr, 2001).

Single response optimization, even though widely used, could lead to misleading results, since different release curves could show the same percent of drug released at a single reference time (Hamed and Sakr, 2001). Therefore, in our study a multiple response optimization approach was considered more useful and suitable for optimizing DDI release profile from the Eudragit–Ethocel extended-release matrix tablets. The selected response variables were the percent of drug released after three different times, the dissolution efficiency (DE) after 6 h (i.e. the area under the dissolution curve at this time) and the time necessary to dissolve 10% drug. As for the several possible factors affecting drug release rate from matrices, the formulation variables investigated in the present work were the tablets compression force, the ratio between the matrix polymers used, the size fraction of the polymers and the drug-to-polymer(s) ratio. The first step was a preliminary screening phase, performed according to a D-optimal design strategy, for evaluating the effects of different levels of each variable. The Doehlert design was then applied for the response-surface study of the factors selected in the preliminary phase as the most important for drug release rate optimization. Finally, the desirability function was used to simultaneously optimize the five considered response variables, each having a different target, and to find the optimum formulation conditions in the studied experimental domain.

2. Materials and methods

2.1. Materials

DDI was a gift from Bristol-Myers Squibb Company (Princeton, NJ, USA). Eudragit®RS-PM (acrylic and methacrylic acid copolymer with low content in quaternary ammonium functions

(1:40) was supplied by Degussa (Barcelona, Spain), and Ethocel®100 (ethylcellulose with a high degree of polymerization, used for direct compression) by Dow Chemical Company (MI, USA). Both the polymers are water-insoluble and pH independent. Before use, the polymers were sieved (Retsch, type Vibro, Arlesheim, Switzerland) and the 50–100 and 150–200 μm granulometric size fractions were selected.

2.2. Software

NEMROD®-W software (Mathieu et al., 2000) was used for generation and evaluation of the statistical experimental design.

2.3. Tablet manufacturing and controls

Different lots of tablets were prepared according to the formulation and process conditions provided for by the experimental plan utilized in each step of optimization. Matrix tablets with a constant theoretic weight of 500 mg were obtained using an eccentric machine (Bonals A-300, Barcelona, Spain) with flat-faced punches of 12.00 mm diameter. Compaction was accomplished by direct compression of drug-polymers blends previously mixed for 15 min using a tumbler mixer. For each batch, 10 randomly taken tablets were checked for weight uniformity (Mettler AE-50 electronic balance, Greifensee, Switzerland), diameter and thickness (Export-Pel precision micrometer, Madrid, Spain), and strength (Schleuniger durometer mod. 2E/205, Greifensee, Switzerland).

2.4. In vitro dissolution studies

The in vitro release studies were carried out at 37 ± 0.5 °C for 6 h, using the USP XXIII basket apparatus (Turu Grau, mod. D-6, Barcelona, Spain). The stirring rate was 50 rpm. The dissolution medium was a pH 7.4 phosphate buffer (700 ml). At predetermined time intervals, samples were withdrawn and spectrophotometrically assayed (Hitachi, mod. U-2000, Tokyo, Japan) for drug concentration according to a previously developed technique (Sánchez-Lafuente et al., 1999b). Each data point represents the mean of three different samples for each lot (C.V. < 3%). The calibration curve for DDI (absorption maximum at 248 nm) was linear from 0.3125 to 10.00 μg/ml giving $R^2 = 0.9999$ as correlation coefficient ($n = 30$) and $F = 422409.25$ as Snedecor ratio ($P < 0.0001$). DE was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time (Khan, 1975).

3. Results and discussion

Two steps were used to optimize the DDI matrix tablet formulation: a preliminary screening phase and then a response surface study. Among the different release parameters utilized as response variables to describe and optimize drug release behavior (Sastry et al., 1997; Takahara et al., 1997), the following were selected as the most representative:

Y_1: concentration of DDI released after 60 min (% w/w), (C60)

Y_2: concentration of DDI released after 180 min (% w/w), (C180)

Y_3: concentration of DDI released after 360 min (% w/w), (C360)

Y_4: DE at 360 min (%), (DE360)

Y_5: time to dissolve 10% of drug, ($t_{10\%}$)

In particular, the percent of drug released after certain time points is considered as the key parameter for any in vitro/in vivo correlation process (FDA Guidance for Industry, 1997) and, moreover, the USP XXIV monographs for per-oral extended release dosage forms specify the percent of drug to be released after different time points (USP XXIV, 2000). Accordingly, we selected as response variables the above three time points of percent drug released, at intervals such as to achieve full description of drug release curve (Hamed and Sakr, 2001). Moreover, the final DE was chosen as indicative of the total amount of drug released and the $t_{10\%}$ to better describe the initial phase of drug release.

In the first step the effect of different levels of each independent variable on the considered responses was studied. In particular, two factors were studied at two levels, one factor at three levels and one factor at five levels. The independent variables evaluated and their levels were:

U_1: applied compression force: 0.5 and 1.5 tons.

U_2: granulometric fraction of Eudragit and Ethocel: 50–100 and 150–200 μm.

U_3: drug content (%): 5–10–15% (w/w).

U_4: Eudragit–Ethocel ratio (%): 100/0, 75/25, 50/50, 25/75 and 0/100% (w/w).

In order to evaluate the effects of variations in factor levels, a 12-run asymmetric screening matrix ($2^2 3^1 5^1 // 12$) was used. This matrix was obtained by means of a D-optimal design strategy (Mathieu et al., 1996), starting from an asymmetric screening matrix $2^2 3^1 5^1 // 18$. D-optimal design is an efficient tool in experimental design that makes it possible to detect the best subset of experiments from a set of candidate points. Starting from an initial set, several subsets with different type and number of experiments are selected. Analysing the quality criteria (i.e. determinant of the information matrix, inflation factors) of each subset of different size it is possible to find a good compromise between the quality of information obtained and the number of experiments to be performed (Frank and Todeschini, 1994). In our case, the quality of the information, obtained from the experimental matrix containing 12 experiments, was considered sufficient for this screening step, and thus the selected 12-run matrix was used. The experimental plan and the responses observed in the screening phase, carried out in a randomized order according to the 12-run matrix provided for by the D-optimal design strategy, are illustrated in Table 1, whereas the DDI release profiles from these 12 formulations are shown in Fig. 1.

Graphic analysis of effects allowed the different effect of factor levels to be evaluated (Fig. 2). The bars that exceed the reference lines, calculated on the basis of experimental error, (Fig. 2a, c, e, g, i), correspond to the factors for which a change among the considered levels is active on the response. Starting from Fig. 2b, d, f, h, l it is possible to select the best level for each considered factor. In particular, to maximize the response, the best level for each factor will correspond to that with maximum bar length. Bars with similar length indicate that the change in level factor is not statistically significant for the observed response (Mathieu et al., 1996). From Fig. 2, it is clear that the effect of the two levels of factor U_1 (applied compression force) on the considered responses is not statistically different; thus a compression force of 0.5 tons was selected for further studies in order to avoid possible undesirable effects due to overheating which could appear

Table 1
Experimental plan and observed responses during screening phase

Formulation	U_1	U_2	U_3	U_4	Y_1	Y_2	Y_3	Y_4	Y_5
1	0.5	50–100	5	100/0	21.20	35.60	60.99	36.80	26.46
2	1.5	150–200	15	75/25	20.15	34.99	55.37	35.09	25.84
3	1.5	50–100	10	50/50	7.11	13.37	22.31	12.30	116.60
4	0.5	50–100	10	75/25	10.78	21.93	32.50	20.82	56.65
5	1.5	50–100	5	25/75	8.61	11.01	13.02	10.11	150.30
6	0.5	150–200	15	0/100	13.83	25.84	37.99	23.51	44.70
7	1.5	150–200	10	100/0	23.84	45.68	69.17	43.19	18.30
8	0.5	50–100	15	25/75	5.77	10.95	17.85	10.54	149.22
9	1.5	50–100	5	0/100	6.96	10.13	13.55	10.04	154.22
10	0.5	150–200	5	50/50	15.90	31.64	46.11	29.46	29.34
11	0.5	150–200	10	25/75	11.81	21.88	29.79	20.04	49.33
12	1.5	50–100	15	100/0	12.79	22.83	34.54	22.13	51.84

U_1, applied compression force (ton); U_2, Eudragit and Ethocel granulometric fraction (μm); U_3, drug content (% w/w); U_4, Eudragit/Ethocel ratio (% w/w); Y_1, C60; Y_2, C180; Y_3, C360; Y_4, DE360; Y_5, $t_{10\%}$.

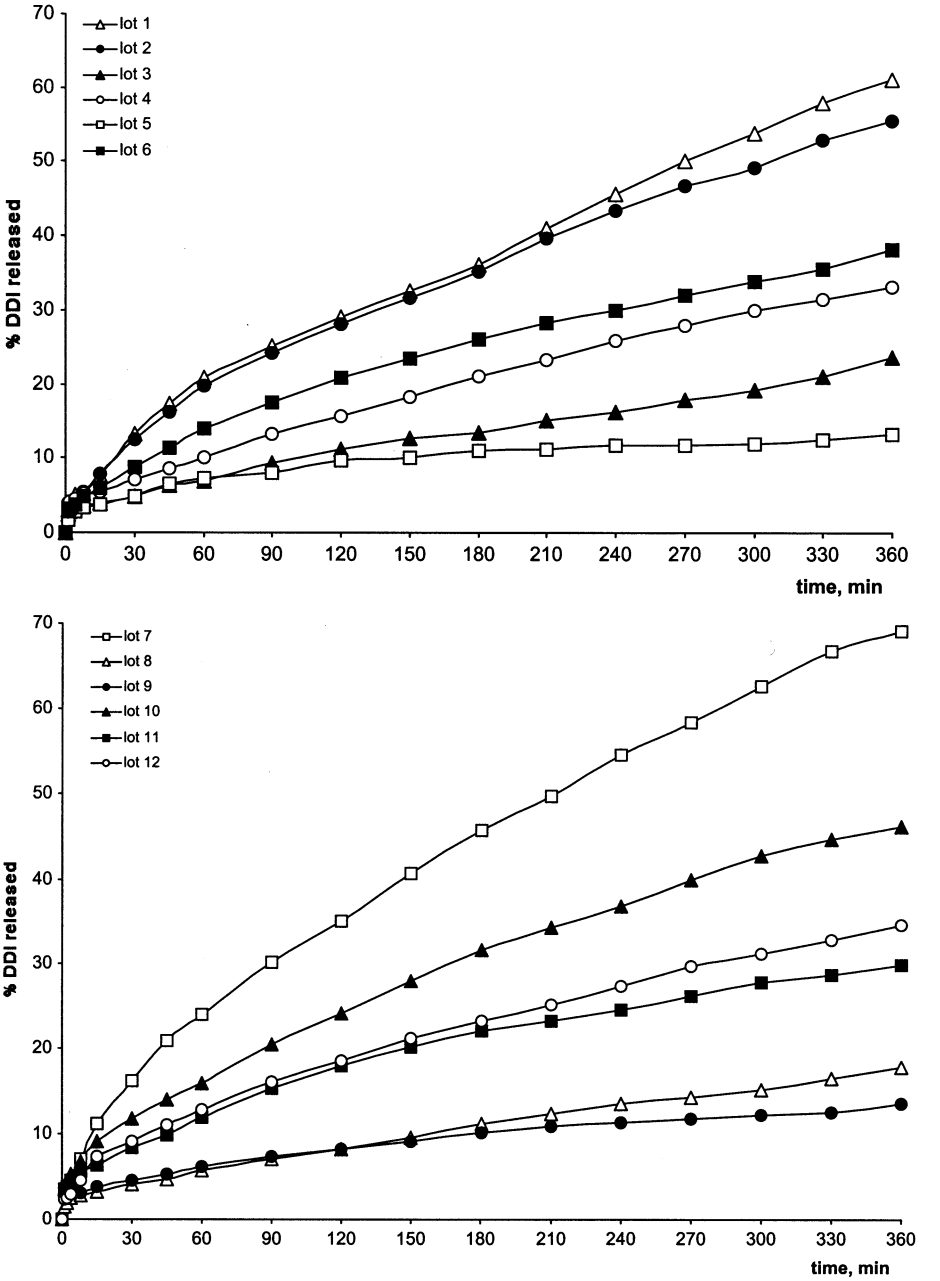

Fig. 1. Release profiles of DDI from model extended-release matrix tablets formulations (formulations 1–12 in Table 1) (mean of three experiments, C.V. < 3%, error bars omitted for the sake of clarity).

operating at higher levels of compression force. For the other factors (U_2–U_4) the change in level was statistically significant for the considered responses. However, the polymer particle size (U_2) is not a continuous factor, and thus it was excluded from the following response surface study. Therefore, because the 150–200 μm granulometric fraction was found to be the best for maximizing

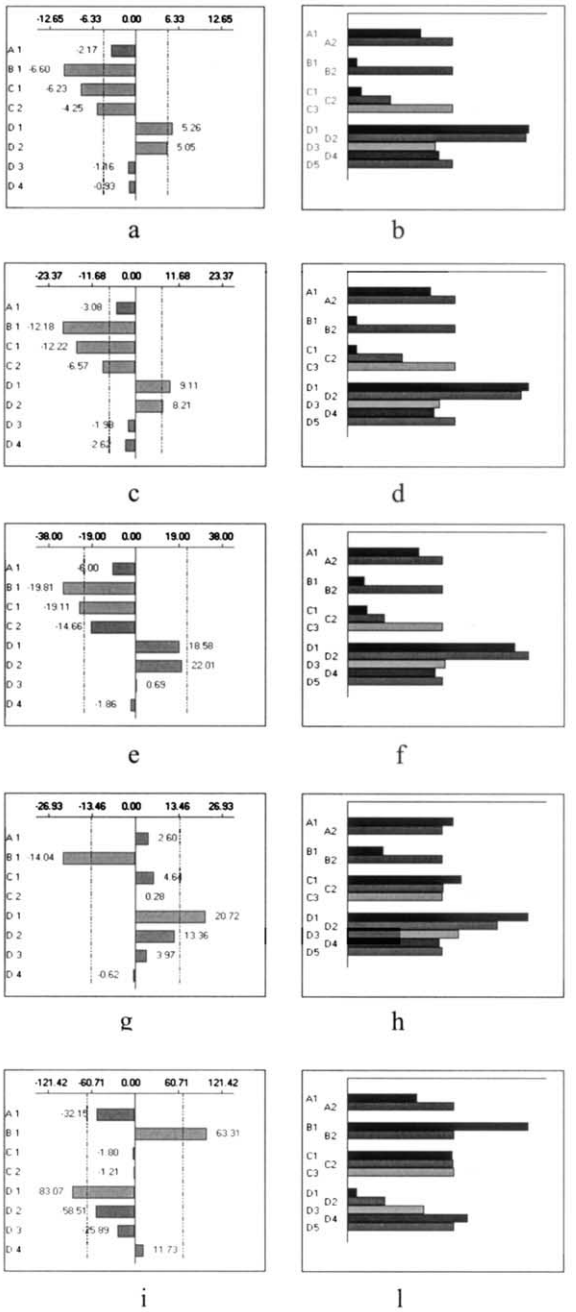

Fig. 2. Graphic analysis of the effects: (A) applied compression force; (B) granulometric fraction of the polymers; (C) drug content; (D) Eudragit/Ethocel ratio. (a, b) C60 response; (c, d) C180 response; (e, f) C360 response; (g, h) DE360 response; (i, l) $t_{10\%}$ response. The dotted lines (a, c, e, g, i) define the 95% confidence interval. The length of each bar (b, d, f, h, l) indicates the weight of the level of each considered factor.

the drug release from the tablets, it was chosen for the subsequent studies. In agreement with this finding, it has been reported that the ability of ethylcellulose matrix tablets to retard the drug release is inversely proportional to its particle size (Pollock, 1997).

After this first step, a response surface study, by means of a Doehlert design, was carried out in order to investigate in detail the effects of the remaining factors (polymers ratio and drug content) on the considered responses. In general the response surface study allows prediction of the response in all experimental domain studied. In this way, through an analysis of the response surfaces, it is possible to select the best combination of factor levels in order to optimize the considered response. The hypothesized relationship between factors and response was the following:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$$

In general, the Doehlert design requires $k^2 + k + n$, where k is the number of factors and n the number of central points. Replicates at the central level of the variables are performed in order to validate the model by means of an estimate of the experimental variance. An important property of the Doehlert design regards the number of levels that each variable takes. For two variables the Doehlert is a hexagon where each experiment is a vertix of the geometrical figure and the number of levels are five and three (Lewis et al., 1999). In this case the variable U_3 (drug content) was studied at three levels, and the variable U_4 (Eudragit/Ethocel ratio) at five levels. Therefore, the new series of tablets was prepared using the 150–200 μm granulometric fraction of both polymers and a constant compression force of 0.5 tons while the other two factors were changed according to the experimental plan reported in Table 2. The experiments were carried out in a randomized order and the obtained responses are reported in Table 2.

Analysis of variance (ANOVA) (Table 3) indicated that the assumed regression model was significant and valid for each considered response (Carlson, 1992; Lewis et al., 1999; Furlanetto et al., 2000). Thus the map of response surface was drawn.

Table 2
Experimental plan of Doehlert design and obtained responses

U_3	U_4	Y_1	Y_2	Y_3	Y_4	Y_5
10	100/0	20.28	34.84	50.27	33.15	4.00
10	0/100	11.83	22.44	26.30	17.60	49.27
14	75/25	15.25	28.18	35.53	22.70	31.25
6	25/75	15.74	22.83	33.43	21.18	21.00
6	75/25	18.15	29.71	43.50	27.82	21.83
14	25/75	7.54	14.63	18.30	11.28	108.0
10	50/50	14.81	21.53	28.52	18.60	43.75
10	50/50	12.70	19.46	27.33	17.57	52.75
10	50/50	12.08	20.56	27.19	17.41	54.50
10	50/50	15.08	25.02	37.78	18.41	39.50

U_3, drug content (% w/w); U_4, Eudragit/Ethocel ratio (% w/w); Y_1, C60; Y_2, C180; Y_3, C360; Y_4, DE360; Y_5, $t_{10\%}$.

The obtained three-dimensional response surfaces illustrating the simultaneous effects of the causal factors on each response variable are represented in Fig. 3. Starting from these graphs it was possible to select the best conditions to optimize each response and to point out possible interactions between factors. In particular, a response evolution map in the experimental domain under study was obtained. For example, a positive interaction between the two factors was pointed out for the responses DE360, C60, C180 and C360, while a negative interaction between the same factors existed for the response $t_{10\%}$. Thus, in order to maximize the responses DE360, C60, C180, and C360, if the factor U_4 was at its lowest level, the factor U_3 had to be set at its lowest level; while, if the factor U_4 was at its highest level, the factor U_3 had to be set at its highest level. In particular, the maximum DE was obtained with a high Eudragit/Ethocel ratio and a high drug–polymer ratio. These conditions were the best also for the response $t_{10\%}$, since this response had to be minimized. These results were attributable to both the highest hydrophobic characteristics of Ethocel and its plastic deformation properties under compression (Katikaneni et al., 1995) which gave rise to more compact tablets. In fact, tablet strength was strongly influenced by Eudragit/Ethocel ratio variations and decreased up to about 2.5 times when passing from formulations containing 100% Ethocel (16.3 Kp) to those with 100% Eudragit (6.5 Kp) as polymeric matrix, even though it always remained within values suitable to give compacts with good handling properties without breakage or excessive friability problems. On the contrary, no significant differences ($P > 0.1$) were observed between the different lots for tablet uniformities of weight (C.V. $< 0.5\%$), thickness (C.V. $< 0.5\%$), and diameter (C.V. $< 0.1\%$).

However, it must be taken into account that, for optimizing a sustained-release dosage form, the goal was to obtain specific concentration values of released drug for the responses C60, C180 and C360. Thus, having to optimize five responses with different targets, a multicriteria decision approach, like desirability function, was used (Frank and Todeschini, 1994; Lewis et al., 1999; Gotti et al., 2000). Each response was associated with its own partial desirability function d_i. This varied from 0 to 1, according to the closeness of the response to its target value. Just as each response variable could be calculated over the experimental domain using the model and the calculated coefficients, so could the corresponding desirability be calculated for that variable at all points in the domain. The individual desirability functions were then combined together, as the geometric mean, to obtain the overall desirability function (D) for the system whose maximum value could then be looked for within the domain (Frank and Todeschini, 1994; Lewis et al., 1999; Gotti et al., 2000). In this case, the five partial desirability functions (d_1, d_2, d_3, d_4, d_5) for the five responses (Y_1, Y_2, Y_3, Y_4, Y_5), respectively, are presented in Fig. 4. From the overall desirability function (D) graph (Fig. 5) it is easy to see that there are a limited number of combinations among variables levels which allow the target values for all the responses to be reached, while there is a large zone in which D is 0. In particular in the experimental domain investigated, the best conditions to optimize the drug release behavior from matrix tablet formulation corresponded to an Eudragit–Ethocel ratio of 83/17 (w/w) and a drug content of 13% (w/w). This optimum point represented a predicted point; thus in order to validate the predictive ability of the hypothesized model for each response around the optimized conditions, the agreement between predicted and measured re-

sponses was verified. Therefore, DDI matrix tablets were prepared according to the optimized conditions and subjected to the release test. The confidence interval for each response at a probability level of 95% was calculated using the mean and the standard deviation obtained from replicates (S.D. $Y_1 = 1.34\%$, $n = 4$; S.D. $Y_2 = 1.93\%$, $n = 4$; S.D. $Y_3 = 3.10\%$, $n = 4$; S.D. $Y_4 = 1.93\%$, $n = 4$; S.D. $Y_5 = 3.99$ min, $n = 4$). The confidence intervals were 16.80 ± 2.13, 29.76 ± 3.07, 39.58 ± 4.93, $25.45 \pm 3.07\%$, 20.97 ± 3.99 min for Y_1, Y_2, Y_3, Y_4, Y_5, respectively. The predicted values (Y_1 15.59%; Y_2 27.61%; Y_3 41.72%; Y_4 25.25%; Y_5 21.3 min) were inside the confidence interval for each observed response, indicating statistical equivalence of the experimental drug release profile and the predicted one.

Table 3
Analysis of variance (ANOVA)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F ratio
C60				
Regression	106.47	5	21.29	11.79[a]
Residuals	7.22	4	1.81	
Lack of fit	0.46	1	0.46	0.21[b]
Pure error	6.76	3	2.25	
Total	113.69	9		
C180				
Regression	270.60	5	54.12	7.70[a]
Residuals	28.10	4	7.03	
Lack of fit	10.75	1	10.75	1.86[b]
Pure error	17.36	3	5.78	
Total	298.70	9		
C360				
Regression	705.13	5	141.02	7.10[a]
Residuals	79.42	4	19.86	
Lack of fit	1.85	1	1.85	0.071[b]
Pure error	77.57	3	25.86	
Total	784.55	9		
DE360				
Regression	336.28	5	67.25	127.43[a]
Residuals	2.11	4	0.53	
Lack of fit	1.05	1	1.05	2.97[b]
Pure error	1.06	3	0.35	
Total	338.39	9		
$t_{10\%}$				
Regression	6782.81	5	1356.56	17.44[a]
Residuals	311.13	4	77.78	
Lack of fit	156.57	1	156.57	3.04[b]
Pure error	154.56	3	51.52	
Total	7093.95	9		

[a] $> F^{\text{crit.}} = 6.26$ (with 5 and 4 degrees of freedom and $\alpha = 0.05$).
[b] $< F^{\text{crit.}} = 10.13$ (with 1 and 3 degrees of freedom and $\alpha = 0.05$).

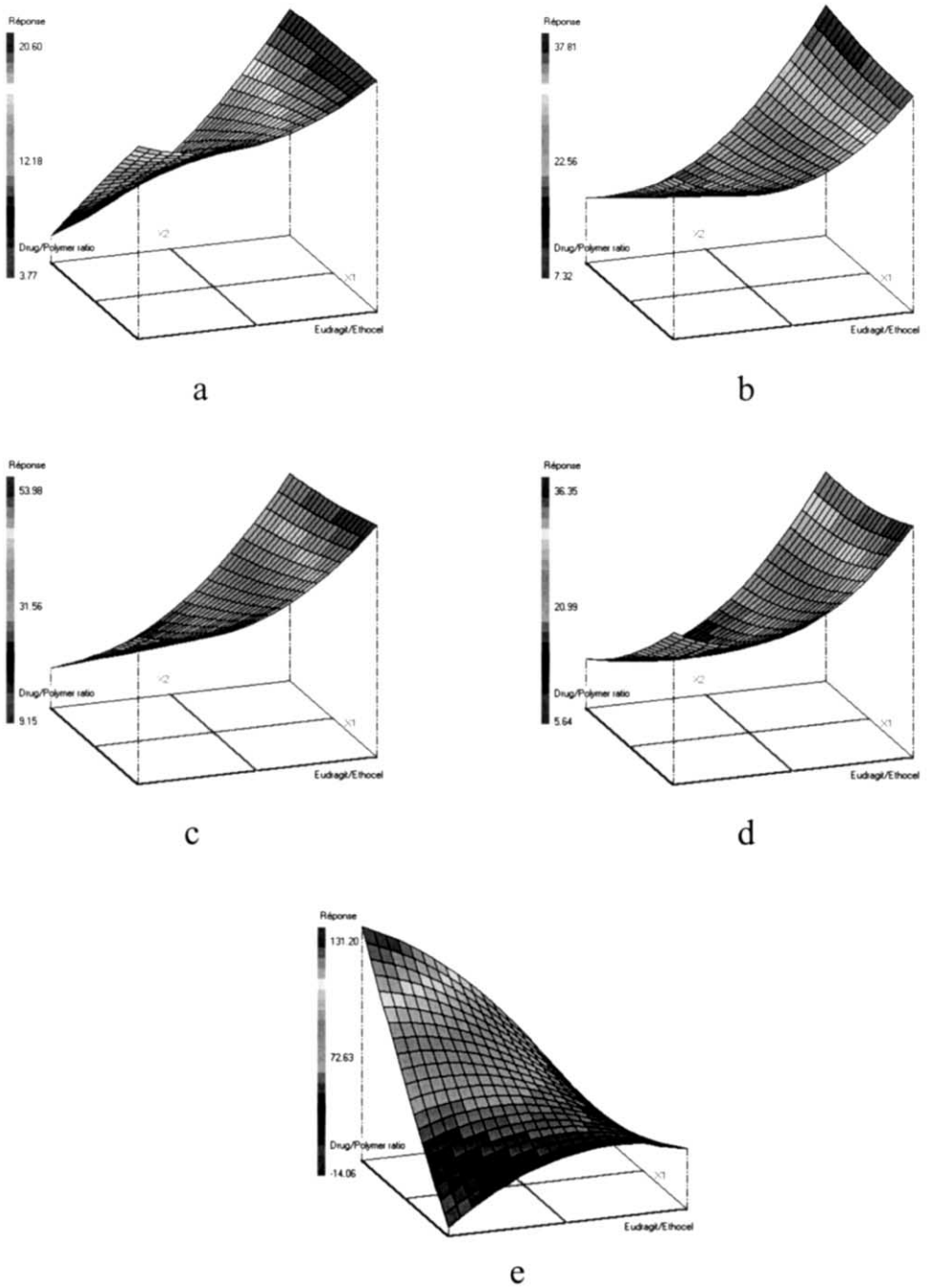

Fig. 3. Response surface plots obtained by plotting Eudragit/Ethocel (w/w) ratio against drug/polymer (w/w) ratio: (a) C60; (b) C180; (c) C360; (d) DE360; (e) $t_{10\%}$.

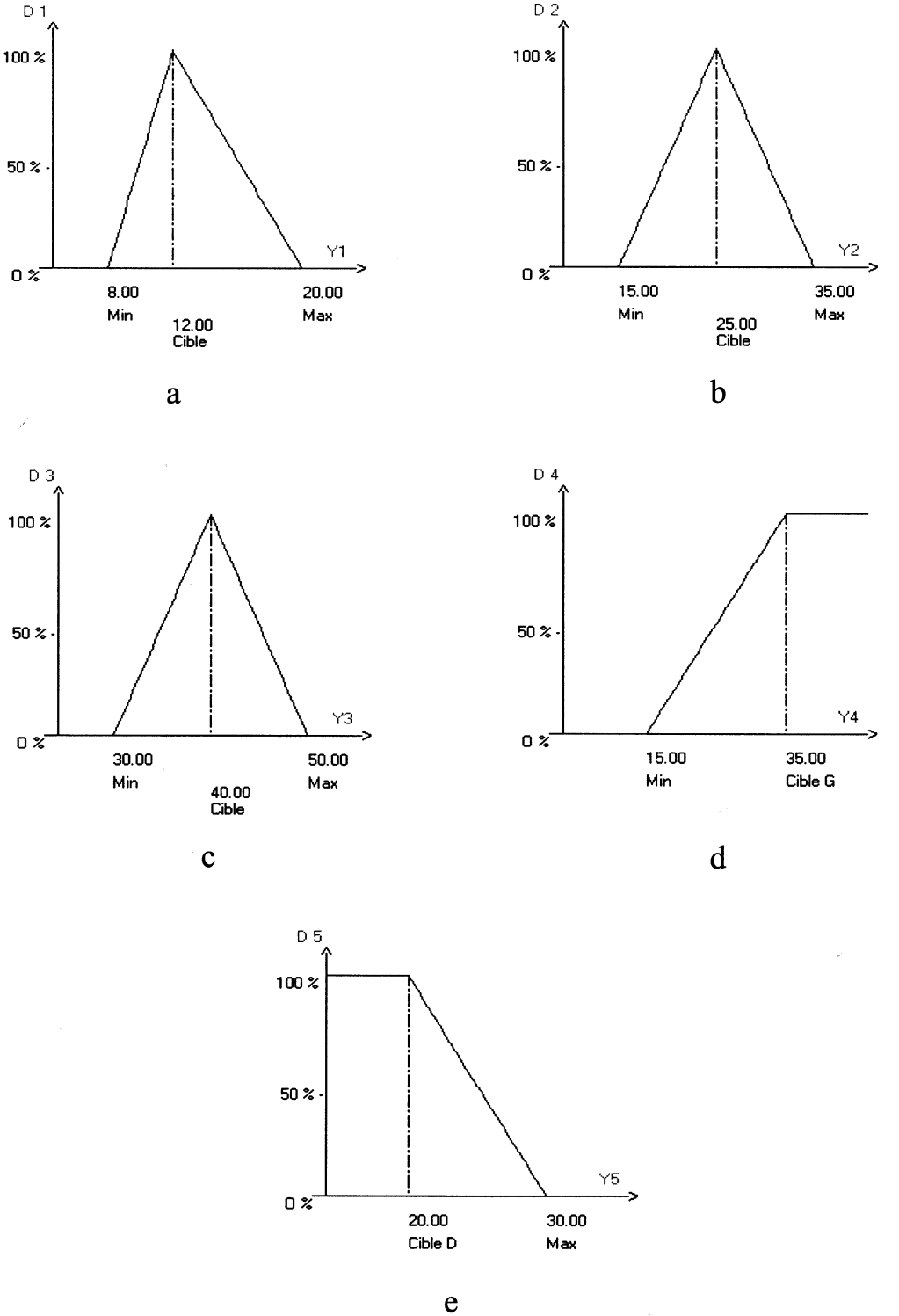

Fig. 4. Transformation of: (a) C60; (b) C180; (c) C360; (d) DE360; (e) $t_{10\%}$ response variables in their individual desirability functions.

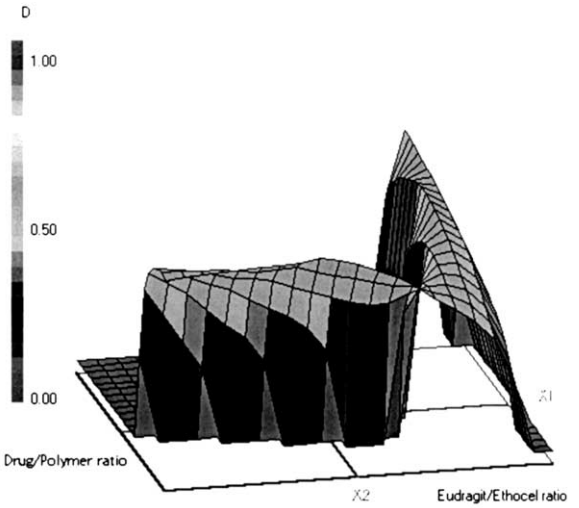

Fig. 5. Graphical representation of the overall desirability function (D). Eudragit/Ethocel (w/w) ratio, (x_1) is plotted against drug/polymer (w/w) ratio (x_2).

4. Conclusions

It was shown that appropriate statistical design and optimization technique can be successfully used in the development of extended-release tablets with predictable drug release properties. Response surface methodology and multiple response optimization enabled formulation of Eudragit–Ethocel matrix tablets with the desired DDI release rate. In particular, a response evolution map in the experimental domain under study was obtained for the variables which had the most important effect on drug release and the use of desirability function allowed all the considered responses to be simultaneously optimized. The experimental values of the response variables obtained from the optimized formulation were very close to the predicted values. The results demonstrated the reliability of the assumed model in the preparation of extended-release matrix tablets having predictable drug release. Moreover, the information obtained by response surface studies, supported by multicriteria decision approach, can be expected to be useful for further formulation studies, when matrix tablets with different drug release profiles could be required.

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