Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 65–71
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Optimization of glibenclamide tablet composition through the combined use of differential scanning calorimetry and D-optimal mixture experimental design

P. Mura*, S. Furlanetto, M. Cirri, F. Maestrelli, A.M. Marras, S. Pinzauti

Department of Pharmaceutical Sciences, University of Florence, Polo Scientifico, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy

Received 17 July 2003; received in revised form 13 September 2004; accepted 22 September 2004
Available online 18 November 2004

Abstract

A systematic analysis of the influence of different proportions of excipients on the stability of a solid dosage form was carried out. In particular, a D-optimal mixture experimental design was applied for the evaluation of glibenclamide compatibility in tablet formulations, consisting of four classic excipients (natrosol as binding agent, stearic acid as lubricant, sorbitol as diluent and cross-linked polyvinylpyrrolidone as disintegrant). The goal was to find the mixture component proportions which correspond to the optimal drug melting parameters, i.e. its maximum stability, using differential scanning calorimetry (DSC) to quickly obtain information about possible interactions among the formulation components. The absolute value of the difference between the melting peak temperature of pure drug endotherm and that in each analysed mixture and the absolute value of the difference between the enthalpy of the pure glibenclamide melting peak and that of its melting peak in the different analyzed mixtures, were chosen as indexes of the drug–excipient interaction degree.

Keywords: Glibenclamide; Preformulation studies; D-optimal mixture experimental design; Differential scanning calorimetry; Drug–excipient interaction

1. Introduction

Careful selection of the excipients, integral components of all pharmaceutical products, is essential for the development of stable and effective dosage forms. Preformulation studies, aimed at the assessment of drug–excipient compatibility and identification of suitable dosage form composition, are recognized as an essential phase of the development process. Excipients are often regarded as "inert", although it is known that they can interact with drugs, giving rise to changes in their stability, solubility, dissolution rate and bioavailability [1–3]. Therefore, in order to accelerate drug development, it would be very useful to obtain knowledge rapidly about potential physical and chemical interactions between drugs and excipients. However, despite the importance of drug–excipient compatibility testing, no generally accepted method is available for this purpose.

Differential scanning calorimetry (DSC) has shown to be an important tool at the outset of any solid dosage form preformulation study to quickly obtain information about possible interactions among the formulation components, according to appearance, shift or disappearance of endothermic or exothermic peaks and/or variations in the corresponding enthalpy values in thermal curves of drug–excipient mixtures [4–7]. Moreover, DSC technique offers significant advantages over the conventional techniques of isothermal stress testing, in terms of the absence of long-term storage of samples, requirement of minimal amounts of compounds, rapid measurement and relative experimental simplicity. However, at present, DSC compatibility studies are generally carried out by comparing the thermal curves of the pure drug and examined excipients with those of the corresponding 1:1 (w/w) binary mixtures. In real formulations, instead, all the

* Corresponding author. Tel.: +39 055 4573672; fax: +39 055 4573673.
E-mail address: paola.mura@unifi.it (P. Mura).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.09.047

components and the drug are present together at the same time in complex heterogeneous mixtures and in ratios very different from 1:1 (w/w). Thus, the information obtained by classic DSC studies may be misleading, and have a limited predictive value, since it does not reflect the actual situation. It should be considered that the effect of an excipient may be strongly dependent on its relevant amount in the mixture and that the compatibility assessed in binary mixtures cannot allow for possible interactions which might occur in multicomponent mixtures, also as a consequence of mechanical treatments (such as grinding or tableting) undergone during the manufacturing process [8].

For an efficient development of stable formulations, a two-step procedure should therefore be recommended. First, classic rapid excipient compatibility screening on binary mixtures should be performed using the DSC technique; then, further studies on complete model formulation with the selected excipients, each at a realistic level, should be conducted to verify the actual stability of the drug in the final dosage form and identify the most suitable mixture composition in order to maximize drug stability. However, the second step of the proposed procedure might appear difficult to correctly and efficiently realized due to the large number of test mixtures to be prepared and analysed in order to cover, as much as possible, the different possible combinations. A favourable solution is to set up experiments according to statistical experimental design [9–11]. If the planned strategy is ideal, it is possible to obtain the desired results as quickly as possible, avoid carrying out unnecessary experiments, ensure that the results are as precise as possible, and provide a model and optimisation of the phenomena studied [9]. In particular, when the measured response is assumed to depend only on the proportions of the ingredients present in the mixture, it is possible to use experimental mixture design [12]. A mixture experiment is a special type of response surface experiment in which the factors are the components of a mixture and the response is a function of the proportions of each ingredient. The mixture components cannot range in an independent way since their sum has to be equal to 100% and specific experimental matrices and mathematic models have to be used. This approach is suitable for pharmaceutical blending problems allowing investigation, with the least number of experiments, of the effects of changes in mixture composition and selection of the optimal composition for achieving the prefixed target [12–16].

In the present study, a 20-run D-optimal mixture design was applied to the evaluation of compatibility of glibenclamide (selected as model drug) in a complete tablet formulation. After a preliminary screening, carried out by DSC analysis on 1:1 (w/w) drug–excipient binary mixtures, four classic tablet excipients (natrosol as binding agent, stearic acid as lubricant, sorbitol as diluent and cross-linked polyvinylpyrrolidone as disintegrant) were evaluated, each in adequate concentration ranges, in view of their different specific functions in tablet production. The mixtures of active ingredient and excipients were prepared according to the 20-run D-optimal mixture design. The goal was to find the mixture component proportions corresponding to the optimal drug melting parameters, i.e. its maximum stability. The responses, selected as indicative of the presence of drug–excipient interactions, were the peak temperature of drug melting endotherm and the relative enthalpy per unit of mass. Finally, the calculated empirical models were plotted as contour diagrams for revealing the optimal formulation.

2. Materials and methods

2.1. Materials

The active ingredient glibenclamide (GLI) was kindly offered by Guidotti Laboratori S.p.A. (Pisa, Italy). The excipients were as follows: hydroxyethylcellulose (Natrosol, Eigenmann & Veronelli, Milano, Italy); sorbitol (Carlo Erba, Milano, Italy); stearic acid (Fluka AG, Buchs, Switzerland); cross-linked polyvinylpyrrolidone (PVPXL) (Merck-Schuchardt, Munich, Germany).

2.2. Software

NEMROD-W software package [17] was used for generation of the experimental design and statistical evaluation of experimental data.

2.3. Preparation of samples

Each material was sieved and the respective 75–150 μm granulometric fraction was selected. Physical mixtures of GLI and the various excipients were prepared by 20 min blending in a turbula mixer. The total amount of the mixture was kept constant, and the relative amounts of the different excipients varied according to the experimental plan of the mixture design provided for by the NEMROD-W software. Tablets were produced by direct compression of mixtures. Uniformity of blending was verified by DSC measurements of three samples taken from a same mixture. For each combination, tablets of a constant weight (160 mg) were prepared using a laboratory hydraulic press for IR spectroscopy at a force of about 3 t for 2 min. The compacts obtained were then broken up and sieved, the 75–150 μm granulometric fraction being collected.

2.4. Differential scanning calorimetry

Samples of individual substances, as well as mixed systems of GLI and excipients, were weighed (Mettler M3 Microbalance) directly in pierced Al pans (5–10 mg) and scanned between 30 and 200 °C with a heating rate of 10 K min^{-1} under static air, using a Mettler TA4000 apparatus equipped with a DSC 25 cell. The instrument was calibrated using Indium as a standard (melting point, 156.61 °C; enthalpy of fusion, 28.71 J g^{-1}).

3. Results and discussion

3.1. Planning of mixture design

The peculiar characteristic of a mixture design is that the single components cannot be changed independently of one another since their sum must add up to 100%. This means that mixture factors are expressed as the fraction of the total amount and their experimental ranges lie between 0 and 100% [11,12,18]. In the present case, where the excipient mixture composition had to be optimised, the experimental range lay between 0 and 93.75% (w/w) since 160 mg tablets were prepared with a constant drug content of 10 mg, corresponding to 6.25% (w/w) of the tablet weight. Moreover, constraints were applied to the proportions of the mixture constituents, in order to comply with the relevant amounts of them actually utilised in commercial pharmaceutical formulations. Table 1 shows the restrictions imposed on the mixture component proportions. These restrictions delimited, inside to the tetrahedron defined by the four components, an experimental region represented by an irregular polyhedron with eight vertices, twelve edges and six faces.

The responses selected as representative of drug stability and compatibility in the complete formulation were:

Y_1: the absolute value of the difference between the melting peak temperature of pure GLI endotherm and that in each analysed mixture, °C (ΔT_{fus}).
Y_2: the absolute value of the difference between the enthalpy of pure GLI melting endotherm and that in each analysed mixture, J g^{-1} ($\Delta\Delta H$).

The experimental plan was obtained according to the postulated model which, for an optimisation problem such as this, had to be predictive in order to simulate the behaviour of the studied properties inside the experimental domain of interest. In particular, it was hypothesised that the following Scheffé special cubic model [12] correlated the composition of the mixture with the characteristics of the final product:

$$Y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{14} x_1 x_4 + \beta_{24} x_2 x_4 + \beta_{34} x_3 x_4 + \beta_{123} x_1 x_2 x_3 + \beta_{124} x_1 x_2 x_4 + \beta_{134} x_1 x_3 x_4 + \beta_{234} x_2 x_3 x_4 + \varepsilon$$

where: Y represents the response; the variable x_1 represents the binder (natrosol), x_2 the diluent (sorbitol), x_3 the lubricant (stearic acid) and x_4 the disintegrant (PVPXL). For each response, 14 coefficients had to be calculated and the mixture design in this case foresaw 35 experiments, that is, 35 different mixtures. In order to reduce the number of mixtures to be prepared, a D-optimal strategy was applied [11,12]. This strategy, given the set of candidate experiments, and having defined the maximum number n of experiments to be performed, allows the subset (with the best compromise between quality of information and number of experiments to be performed) to be selected. The design found is called D-optimal design. In this case, given the set of 35-candidate experiments, a 20-run D-optimal design resulted to be the best subset according to the information matrix determinant maximisation criterion [11,12]. Table 2 reports the corresponding 20-run experimental plan in which the mixture component proportions (% w/w) are indicated. According to this experimental plan, the test mixtures were prepared and analysed by DSC for assessing drug compatibility.

Table 1
Restrictions of component proportions

Component	Proportion restriction (% w/w)
x_1 Binder (natrosol)	$42.19 \le x_1 \ge 46.87$
x_2 Diluent (sorbitol)	$36.57 \le x_2 \ge 46.87$
x_3 Lubricant (stearic acid)	$0.94 \le x_3 \ge 2.81$
x_4 Disintegrant (PVPXL)	$3.75 \le x_4 \ge 7.50$

3.2. DSC analysis

The thermal profiles of pure drug and excipients and their 1:1 (w/w) blends are presented in Fig. 1. The DSC curve of GLI indicated its crystalline anhydrous state, showing a single sharp endothermic peak corresponding to its melting point, with a peak temperature of 175.7 ± 0.4 °C and an apparent heat of fusion of 98.6 ± 1.4 J g^{-1} (average of four runs). The thermal curves of natrosol and PVPXL were characterised by a shallow, broad endothermic band in the 70–140 °C range, due to water evaporation. In contrast, the thermal profiles of stearic acid and sorbitol exhibited a sharp endothermic effect, peaked at 69.1 and 98.1 °C, respectively, due to the excipient melting process. The 1:1 (w/w) physical

Table 2
20-run D-optimal experimental plan

Experiment no.	Mixture component proportions (% w/w)			
	Binder	Diluent	Lubricant	Disintegrant
1	42.19	46.87	0.94	3.75
2	42.19	45.00	2.81	3.75
3	42.19	43.12	0.94	7.50
4	42.19	41.25	2.81	7.50
5	46.87	42.19	0.94	3.75
6	46.87	40.32	2.81	3.75
7	46.87	38.44	0.94	7.50
8	46.87	36.57	2.81	7.50
9	42.19	45.94	1.88	3.75
10	42.19	44.99	0.94	5.62
11	44.53	44.53	0.94	3.75
12	42.19	43.13	2.81	5.62
13	44.53	42.66	2.81	3.75
14	42.19	42.19	1.88	7.50
15	44.53	40.78	0.94	7.50
16	44.53	38.91	2.81	7.50
18	46.87	40.31	0.94	5.62
19	46.87	38.45	2.81	5.62
20	46.87	37.51	1.88	7.50
27	44.53	41.72	1.88	5.62

The number of experiments is referred to the original mixture design.

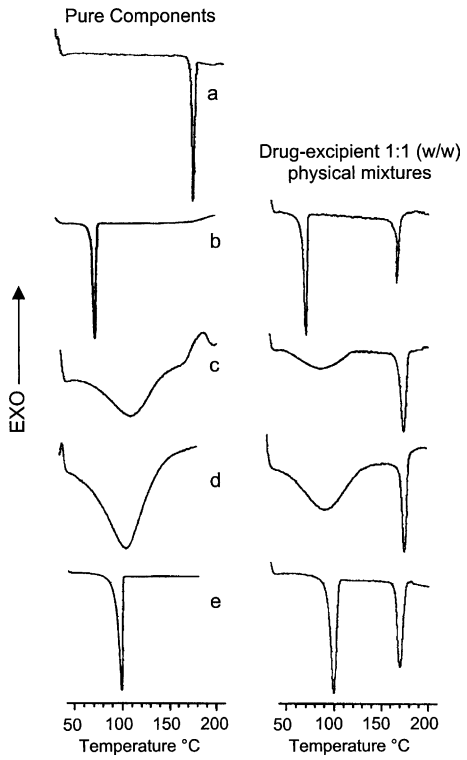

Fig. 1. DSC curves of pure components and their 1:1 (w/w) physical mixtures. Key: (a) glibenclamide; (b) stearic acid; (c) natrosol; (d) PVPXL; (e) sorbitol.

mixtures of GLI with each of these excipients, substantially reflected the characteristic features of the respective individual components. The slight lowering and/or broadening of drug melting endotherm may be attributed to the mixing process, which lowers the purity of each component in the mixture [19,20]. No problem of compatibility could therefore be detected.

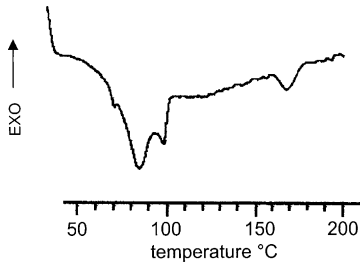

Fig. 2. Typical DSC curve of a complete mixture. Sample no. 11. For the mixture composition, see Table 2.

In order to also evaluate a possible effect of the mechanical treatment which, increasing drug–excipient contact, could favour possible interactions [5,7,8], mixtures prepared according to the 20-run experimental plan provided by the D-optimal design strategy and always containing 6.25% (w/w) of drug, were subjected to direct compression. The obtained tablets (160 mg) were then broken up and the powders sieved (75–150 μm) and analysed by DSC in a randomised order.

An example of the typical thermal curves obtained from such multicomponent mixtures is shown in Fig. 2. They were all characterized by the presence of the drug melting endotherm, preceded by an intense, broadened and "structured" endothermic band. This was essentially due to the superimposition of natrosol and PVPXL dehydration bands, which partially covered the stearic acid (≈68 °C) and sorbitol (≈98 °C) melting endotherms.

As for the GLI melting endotherm, it appeared strongly reduced in intensity and rather broadened due to the low drug content in the multicomponent mixture (6.25%). The melting peak temperature of GLI was scarcely influenced by changes in the relative proportions of the different excipients in the mixture, whereas important variations in the related fusion enthalpy were observed (Table 3).

Table 3
Experimental matrix in pseudo-components and obtained responses

Experiment no.	x'_1	x'_2	x'_3	x'_4	ΔT_{fus} (°C)	$\Delta\Delta H$ (J g^{-1})
1	−0.0002	0.9996	−0.0002	0.0000	165.7	39.98
2	−0.0002	0.8184	0.1818	0.0000	164.0	75.20
3	−0.0002	0.6355	−0.0002	0.3641	167.3	56.49
4	−0.0002	0.4544	0.1818	0.3641	164.3	69.48
5	0.4539	0.5454	−0.0002	0.0000	166.3	66.15
6	0.4539	0.3643	0.1818	0.0000	165.7	61.69
7	0.4539	0.1813	−0.0002	0.3641	167.3	85.00
8	0.4539	0.0002	0.1818	0.3641	166.7	54.54
9	−0.0002	0.9095	0.0908	0.0000	167.3	63.15
10	−0.0002	0.8175	−0.0002	0.1820	167.7	51.89
11	0.2273	0.7729	−0.0002	0.0000	166.7	39.21
12	−0.0002	0.6364	0.1818	0.1820	164.6	90.15
13	0.2273	0.5909	0.1818	0.0000	166.3	60.50
14	−0.0002	0.5454	0.0908	0.3641	166.7	78.63
15	0.2273	0.4089	−0.0002	0.3641	167.0	63.51
16	0.2273	0.2268	0.1818	0.3641	168.3	46.50
18	0.4539	0.3633	−0.0002	0.1820	168.7	79.42
19	0.4539	0.1822	0.1818	0.1820	167.7	67.48
20	0.4539	0.0912	0.0908	0.3641	168.3	79.63
27	0.2273	0.4999	0.0908	0.1820	166.7	65.51

3.3. Analysis of mixture data

Before carrying out the statistical treatment of the obtained responses, the coordinates of the original tetrahedron defined by the four components (x_i) were redefined in terms of pseudo components (x'_i). Pseudo components are combinations of the original components and one of the advantages of their use is that the fitting of models is more accurate and easier when done in pseudo component system than when done in the original component system. This transformation is similar to introducing coded variables into the place of original variables in a standard independent factor design [12]. Table 3 reports the experimental matrix in pseudo components and the obtained responses for each tested formulation.

The treatment of the obtained responses led to a significant and valid model for the response $\Delta\Delta H$, while the assumed regression model did not explain the variation of the response ΔT_{fus}. Consequently, the study of this response was omitted. In particular, the significance of the model was assessed through by means of the analysis of variance while the validity of the proposed model for the response $\Delta\Delta H$ was confirmed by means of residual analysis using the three-point test [11]. By means of residual analysis [11], a measurement of the closeness of the mixture surface predicted, by the observed values of the response at the design points, was also obtained, showing that the proposed model for the response $\Delta\Delta H$ was correct and could be accepted. The comparison between observed and predicted responses is shown in Table 4. The calculated model in pseudo components for the response $\Delta\Delta H$ is reported:

$$\begin{aligned} Y_2 = {} & 208.918x'_1 + 38.746x'_2 - 269.761x'_3 + 32.180x'_4 \\ & - 202.912x'_1x'_2 - 27.966x'_1x'_3 + 636.567x'_2x'_3 \\ & - 71.986x'_1x'_4 + 96.713x'_2x'_4 + 456.983x'_3x'_4 \\ & - 137.852x'_1x'_2x'_3 - 141.964x'_1x'_2x'_4 \\ & + 1317.580x'_1x'_3x'_4 + 1742.358x'_2x'_3x'_4 \end{aligned}$$

Table 4
Comparison between observed and predicted responses by the calculated model

Experiment no.	$Y_{observed}$	$Y_{calculated}$
1	39.980	38.264
2	75.200	77.243
3	56.490	59.011
4	69.480	71.050
5	66.150	65.490
6	61.690	61.921
7	85.000	86.932
8	54.540	52.634
9	63.150	63.421
10	51.890	52.008
11	39.210	41.754
12	90.150	88.013
13	60.500	57.770
14	78.630	75.962
15	63.510	60.507
16	46.500	47.662
18	79.420	77.685
19	67.480	69.247
20	79.630	80.003
27	65.510	67.534
–	–	–
17[a]	57.455	56.532
21[a]	60.447	64.596
22[a]	63.328	63.862

[a] Test point.

At this point, the use of this model in a predictive way was easy. Fig. 3 shows the relative tri-dimensional response surface in which the disintegrant is fixed at 4.0% with respect to the 93.75% of excipients. From this figure, which covers the complete mixture space, it is possible to state that we have two possibilities for the response minimization. It is possible to use a high percentage of diluent and a low percentage of binder and lubricant, or it is possible to use a low percentage of diluent and a higher but equal percentage of binder and lubricant. However, for mixture design the use of the isoresponse curves allows a much easier interpretation of the results. For example, Fig. 4 shows the lines of equal response in the restrained region in function of the binder, diluent and lubricant percentages, having fixed the content of disintegrant. By comparing Fig. 4a and b, in which the value of disintegrant is fixed at the low and high level, respectively, we can say that in order to minimize the response it is important that this component is fixed at the low level. In addition, Fig. 4a points out that it is important for the maximum stability of the formulation to use a low level of binder and lubricant and a high level of diluent.

In order to better evaluate the contribution of each of the four components, the response trace method was also used [12]. The response trace is a plot in which the changes in

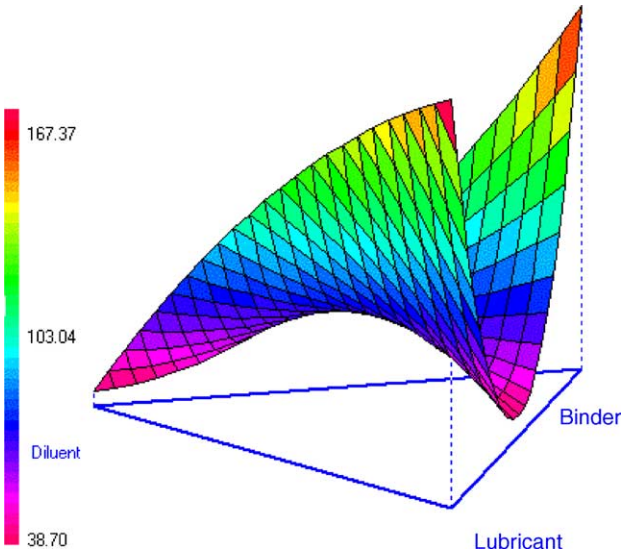

Fig. 3. Response surface predicted from the special cubic model for the response $\Delta\Delta H$. In the graph the response is a function of diluent, lubricant and binder, having fixed the disintegrant to a value of 4.0% with respect to the total 93.75% of excipients.

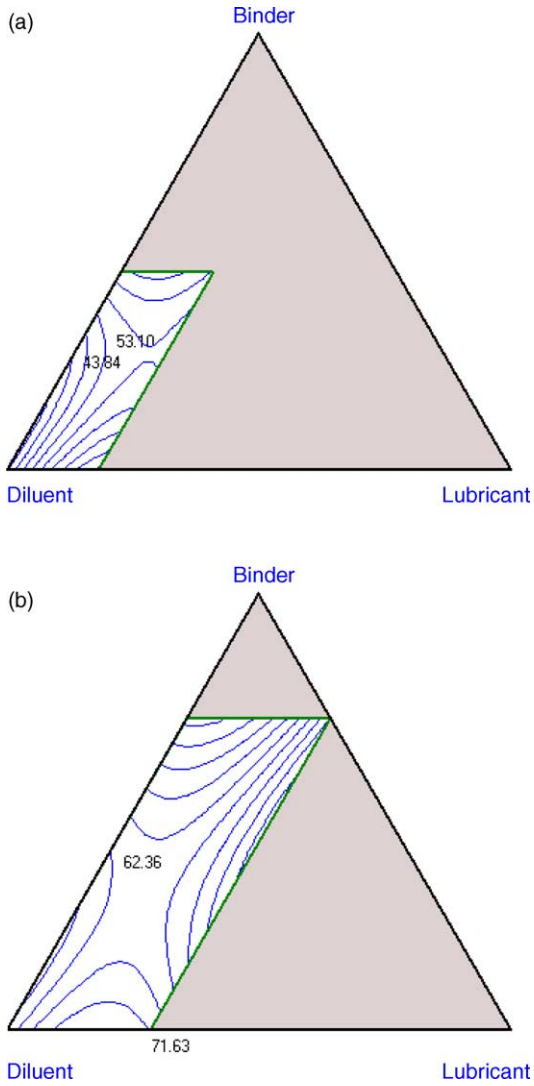

Fig. 4. Contour line plot of $\Delta\Delta H$ in the restrained region, as function of diluent, lubricant and binder, having fixed the disintegrant. The numbers reported correspond to the response value. (a) Disintegrant equal to a value of 3.75% with respect to the 93.75% of excipients. (b) Disintegrant equal to a value of 7.5% with respect to the 93.75% of excipients.

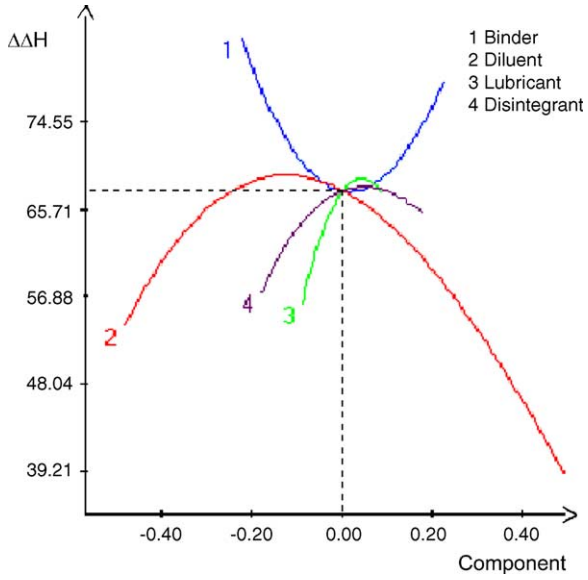

Fig. 5. Response trace plot for the response $\Delta\Delta H$ using as reference mixture the centroid of the constrained domain (binder 44.53%; diluent 41.72%; lubricant 1.88%; disintegrant 5.62%).

the estimated response due to the change of the proportion of a single component, keeping the other components at a fixed value, is shown. Once a reference mixture has been chosen (normally the centroid of the experimental region), the plot shows the variation of the estimated response moving, along the component axes, away from the reference mixture. The response trace plot reported in Fig. 5 illustrates the effects of the component proportion change on the response $\Delta\Delta H$ using as reference mixture the centroid. Keeping in mind that a response minimization is required in order to have a maximum stability of the formulation, it is possible to state that diluent has a strong effect on the response and high percentages of this excipient are required. On the other hand for lubricant and disintegrant, it is important to move towards lower levels with respect to the centroid in order to have a decrease of the response. Finally, for the binder the percentages around the centroid are suitable for a response minimization and thus for a maximum formulation stability. In particular, a desirable value of the response $\Delta\Delta H$ equal to $40.99\,J\,g^{-1}$ was predicted from the model found using the following percentages of the components: binder 42.5%, diluent 46.5%, lubricant 1.00% and disintegrant 3.75%.

The value of the measured, $\Delta\Delta H$-optimised glibenclamide tablet was not different in a statistically significant way from the predicted values, when the *t*-test at a probability level of 95% [21,22] was carried out.

4. Conclusion

In developing a new drug solid dosage form, it is important to rapidly identify the best component composition allowing optimisation of drug stability. The obtained results underline the utility of the use of DSC technique combined with experimental mixture design strategy in the final stage of a preformulation compatibility study. The proposed approach enabled investigation of complete tablet formulations in order to evaluate compatibility among the components and was successful in finding the best component proportions corresponding to minimum interaction and therefore maximum drug stability. In fact, with a limited number of experiments the quaternary mixtures were modelled and the obtained contour plots were extremely useful in studying the effects of the different component proportions on the drug stability.

Acknowledgment

This work was supported by a grant from MIUR (Italian Ministry of Instruction, University and Research).

References

[1] D.C. Monkhouse, Drug Dev. Ind. Pharm. 10 (1984) 1373–1412.
[2] J.I. Wells, Pharmaceuticals Preformulation—The Physicochemical Properties of Drug Substances, Ellis Horwood, Chichester, UK, 1988.
[3] A.T.M. Serajuddin, A.B. Thakur, R.N. Ghosal, M.G. Fakes, S.A. Ranadive, K.H. Morris, S.A. Varia, J. Pharm. Sci. 88 (1999) 696–704.
[4] D. Giron, J. Pharm. Biomed. Anal. 4 (1986) 755–770.
[5] P. Mura, A. Manderioli, G. Bramanti, S. Furlanetto, S. Pinzauti, Int. J. Pharm. 119 (1995) 71–79.
[6] C.E.P. Malan, M.M. De Villiers, A.P. Lotter, J. Pharm. Biomed. Anal. 15 (1997) 549–557.
[7] P. Mura, M.T. Faucci, A. Manderioli, S. Furlanetto, S. Pinzauti, Drug Dev. Ind. Pharm. 24 (1998) 747–756.
[8] D.C. Monkhouse, A. Maderich, Drug Dev. Ind. Pharm. 15 (1989) 2115–2130.
[9] J.L. Goupy, Methods for Experimental Design, Elsevier, Amsterdam, 1993.
[10] S.C. Porter, R.P. Verseput, C.R. Cunningham, Pharm. Technol. 21 (1997) 60–70.
[11] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, Pharmaceutical Experimental Design, Marcel Dekker, New York, 1999.
[12] J.A. Cornell, Experiments and mixtures, in: Designs Models and the Analysis of Mixture Data, 2nd ed., J. Wiley and Sons, New York, 1990.
[13] B. Campisi, D. Chicco, D. Vojnovic, R. Phan-Tan-Luu, J. Pharm. Biomed. Anal. 18 (1998) 57–65.
[14] S. Cafaggi, R. Leardi, B. Parodi, G. Caviglioli, G. Bignardi, Chemometr. Intell. Lab. Syst. 65 (2003) 139–147.
[15] A. Kamoun, M. Chaabouni, M. Sergent, R. Phan-Tan-Luu, Chemometr. Intell. Lab. Syst. 63 (2002) 69–79.
[16] B. Campisi, D. Vojnovic, D. Chicco, R. Phan-Tan-Luu, Chemometr. Intell. Lab. Syst. 48 (1999) 59–70.
[17] D. Mathieu, J. Nony, R. Phan-Tan-Luu, NEMROD-W, LPRAI sarl, Marseille, F-13331, France, 2000.
[18] L. Eriksson, E. Johansson, C. Wikstrom, Chemometr. Intell. Lab. Syst. 43 (1998) 1–24.
[19] A. Smith, Anal. Proc. 19 (1982) 559–561.
[20] A.A. Van Dooren, B.V. Duphar, Drug Dev. Ind. Pharm. 9 (1983) 43–55.
[21] S. Furlanetto, S. Orlandini, G. Aldini, R. Gotti, E. Dreassi, S. Pinzauti, Anal. Chim. Acta 413 (2000) 229–239.
[22] J.C. Miller, J.N. Miller (Eds.), Statistics for Analytical Chemistry, 3rd ed., Ellis Horwood, Chichester, 1993, pp. 110–117.