ELSEVIER

Analytica Chimica Acta 458 (2002) 397–415

ANALYTICA CHIMICA ACTA

www.elsevier.com/locate/aca

Parallel co-ordinate geometry and principal component analysis for the interpretation of large multi-response experimental designs

Y. Vander Heyden [a,*], V. Pravdova [a], F. Questier [a], L. Tallieu [a], A. Scott [b], D.L. Massart [a]

[a] *ChemoAC, Department Pharmaceutical and Biomedical Analysis, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*

[b] *Glaxo Smith Kline, Pharmaceutical Development—Technical Operations, Park Road, Ware, Hertfordshire SG12 0DP, UK*

Received 18 September 2001; received in revised form 22 January 2002; accepted 30 January 2002

Abstract

In the evaluation of large or complex data sets the use of visualization methods can be of great benefit. In this paper, the use of parallel co-ordinate geometry (PCG) plots, principal component analysis (PCA) and N-way PCA as visualization procedures for large multi-response experimental designs was compared with the more traditional approach of calculating factor effects by multiple linear regression. PCG plots are a recent addition to the visualization tools and offer a possibility to visualize multi-dimensional data in two dimensions while no calculations are required. It was found that PCA and PCG each have their own benefits and disadvantages. Both methods can be used to some extent to select optimal conditions. Moreover, it was possible to use the PCA score plot as a Pareto optimality plot that allowed to select the experiments considered to be Pareto optimal. Therefore, the examined visualization methods can be useful and complementary aids in the interpretation of large multi-response experimental design data and they add a multivariate dimension to the more classical univariate analysis of such data. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Parallel co-ordinate geometry; Principal component analysis; Large multi-response experimental design data

1. Introduction

Due to the fast evolution in technical and computational achievements it is more and more frequent that large data sets, containing large numbers of experiments and measured variables, are acquired and need to be interpreted. Methods that allow to visualize and/or to reduce the number of variables (feature reduction) [1] are then of great benefit.

Parallel co-ordinate geometry (PCG) plots [2] are a recent addition to the tools available for the interpretation of data sets. They offer a possibility to visualize multi-dimensional data in two dimensions and require no calculations. The PCG plots can in principle be used to detect: (i) clustering of objects; (ii) correlation between variables; or (iii) which variables are affected by which (group of) objects or vice versa (Fig. 1a). When it is known which variables should be considered dependent and which ones independent, correlation between variables can be used to determine which dependent variables are influenced by which independent ones (Fig. 1b). An extreme case are experimental designs in which the objects are experiments at specific levels of the independent variables, which are now called factors, yielding results evaluated by the dependent variables, called responses (Fig. 1c).

* Corresponding author. Tel.: +32-2-477-4737; fax: +32-2-477-4735.
E-mail address: yvanvdh@fabi.vub.ac.be (Y. Vander Heyden).

0003-2670/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0003-2670(02)00080-6

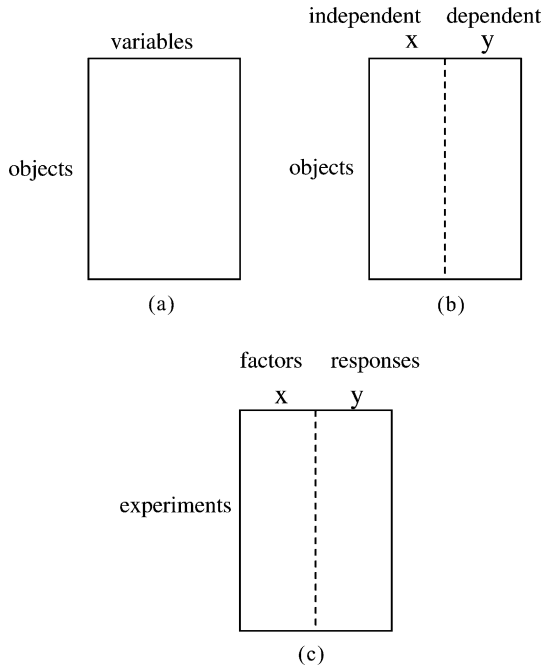

Fig. 1. Representation of data sets that could be evaluated with the PCG methodology.

PCG plots were applied in [2] to analyze the influence of different mixture and process factors on the properties of a solid dosage form. The data set used [3] studies the influence of six variables on a number of characteristics of a tablet.

In classical experimental design methodology one uses a single-response method and will calculate the effects or the regression coefficients of the examined factors on each of the responses. The significance of the estimated effects can then be determined using statistical or graphical methods [4]. Among the latter, one can find normal and half-normal probability plots [4–7]. However, these graphical procedures give only information about the effects on one response.

In this paper, we compare the use of PCG plots relative to other visualization procedures that can be used for multi-response experimental design interpretation. The other interpretation methods considered are two-way principal component analysis (PCA) [1] and N-way PCA [8,9]. An example of the use of PCA on experimental design data in the optimization of a dosage form can be found in [10]. The different techniques were applied on the data set of [3].

Other, some more complicated methods such as two-block partial least squares or non-linear methods as, for instance, non-linear mapping, could also be considered for the same purpose. However, our idea was to compare PCG with methods that can be easily applied (with the exception of N-way PCA) by users without an extensive chemometric background and, therefore, these more specialized methods were not included.

2. Theory

2.1. Parallel co-ordinate geometry

In a Cartesian representation of data the axis are plotted orthogonal to each other. This also means that one is limited to a representation of at most three variables, as is for instance the case when showing response surfaces [1,11,12]. In a PCG plot the axis are plotted parallel to each other [2]. In such a representation the number of variables (parallel co-ordinates), factors and responses in experimental design methodology, theoretically becomes infinite. This also means that, for instance, all factors (experimental conditions) and responses of an experiment can be considered in the same plot. In a PCG plot all axis are drawn with the same length (Fig. 2). This is equivalent with re-scaling all factors and responses to the same interval, e.g. $[-1, 1]$ or $[0, 1]$. The scaled values of each variable (factor/response) are then plotted on their respective

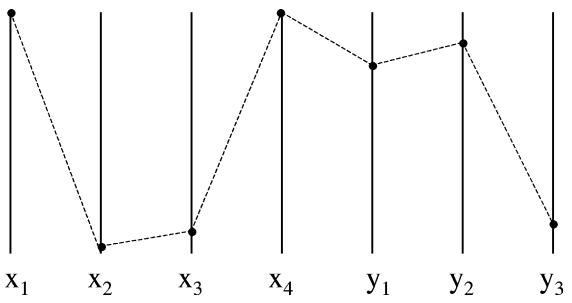

Fig. 2. PCG plot for a given experiment, representing four factors (x_1–x_4) and three responses (y_1–y_3).

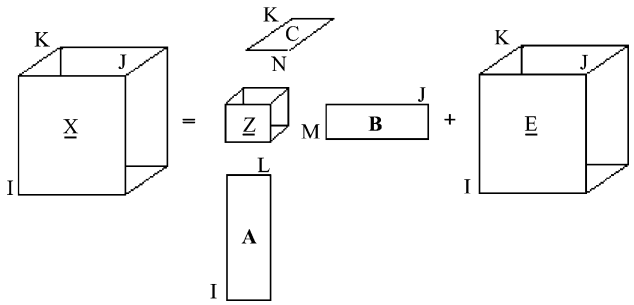

Fig. 3. Decomposition of a three-way data matrix X into three orthogonal loading matrices A ($I \times M$), B ($J \times N$) and C ($K \times L$) and into a core matrix Z ($M \times N \times L$).

axis and afterwards connected by straight lines between the different axis. In Fig. 2, a PCG plot for an experiment with four experimental conditions and three responses is represented.

2.2. Principal component analysis

PCA allows visualizing multi-dimensional data by reducing the dimensionality to only a few dimensions. The technique is widely applied in different domains. Reduction of dimensionality with PCA is only meaningful in the presence of correlated data. This property allows evaluating the effect of factors on responses. When a factor has an effect on a response both variables are namely correlated.

The theory about PCA can be found in most handbooks on chemometrics, e.g. [1,13,14], and we refer to them for further background information. Score and loading plots allow interpreting the results of experimental design data [10]. The interpretation of loading plots is based on the direction in which the variables lie on this plot as seen from its origin (examples shown in Section 4). Two variables are strongly (positively) correlated when there is a small angle between the lines connecting them with the origin. If the two variables considered are two responses one can conclude that these responses are correlated, if it is a factor and a response it means that the factor has a positive effect on the response. When the factor has a negative effect on a response the angle between the lines connecting them with the origin is close to 180°.

2.3. N-way PCA

Two-way PCA is performed on data matrices with dimensions as shown in Fig. 1 and which takes the form of a two-dimensional data table. Three-way PCA, the simplest situation of N-way PCA is performed on a three-dimensional data table (Fig. 3). Each of the cubes of Fig. 4 forms such a table. How these cubes are formed starting from the two-dimensional data table of [3] is explained in Section 4.

A Tucker3 model [15] can be considered as a generalisation of PCA for N-way data arrays. In case of a

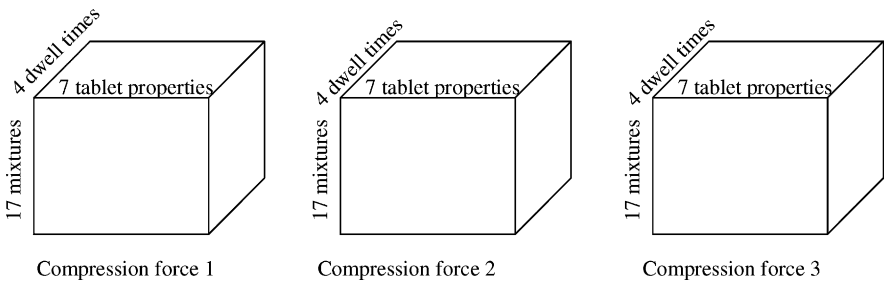

Fig. 4. Representation of the data set as used in the N-way PCA.

three-way data matrix X, the Tucker3 model decomposes matrix X (dimensions $I \times J \times K$) into three orthogonal loading matrices, called A ($I \times M$), B ($J \times N$) and C ($K \times L$) and into a core matrix Z ($M \times N \times L$), which describes the relationship between the loading matrices (Fig. 3). The results are usually presented in the form of loading plots (Section 4). The Tucker3 model can easily be extended to higher order models (e.g. a four-way model as was used here). For more theoretical background we refer to [9].

3. Experimental

3.1. Experimental data

The data set given in [3], containing the experimental conditions for 205 different experiments and representing six factors and eight responses, was used. It concerns the formulation of a 280 mg tablet consisting of 200 mg active ingredient and 80 mg excipients. The factors examined for their influence on the tablet properties are described below and details about the tablet manufacturing can be found in [3].

3.2. Software

The PCG plots were drawn by an in-house written program in Matlab 4.2 (MathWorks, Natick, MA). In this program all variables were scaled in the interval [−1, 1]. Commercial software that allows the drawing of PCG plots, curvaceous visual explorer (CVE) (Curvaceous Software, Gerrards Cross, UK), is also available and was used in [2]. PCA calculations and plots were made in Matlab 4.2. The algorithms applied for the Tucker3 model are implemented in the N-way toolbox [16] for the Matlab 5.0 (MathWorks) computing environment.

4. Results and discussion

Four of the six examined factors in [3] are excipient concentrations in the tablet, namely those of (i) silica aerogel (SA); (ii) micro-crystalline cellulose (MC); (iii) magnesium stearate (MgS); and (iv) sodium carboxymethyl cellulose (NaCMC), while the two remaining factors are instrumental variables; (v) the dwell time or compression speed (CS) and (vi) the compression force (CF). Eight responses were measured for tablets produced with a given combination of the above mentioned factors: (i) the tensile strength; (ii) the disintegration time; (iii) the friability; (iv) the capping tendency; and (v–viii) the dissolution profile, given by the dissolved percentage of drug compound after 15, 30, 45 and 60 min.

This paper focuses on the comparison of different interpretation or evaluation methods for the considered data set. A thorough discussion on the properties of the formulated tablets is, therefore, considered outside the scope of this work.

4.1. Classic evaluation of screening designs: effects or regression coefficients estimation

Before considering the graphical multi-response approaches we will first discuss the outcome of the classic approach, namely the calculation and interpretation of factor effects or regression coefficients which are then statistically or graphically evaluated for significance. It has to be remarked that our aim here is only to estimate effects and not to model the responses. Graphic evaluation of effects is namely one of the possibilities of the PCG plots and it will be compared with the outcome of the mathematical effect estimation.

For a screening design in which a relative large number of factors ($\geq$4) is examined, one first calculates the effects of the factors or the corresponding regression coefficients [11]. Since, more than two levels occur for each of the factors, the estimation of the regression coefficients (b_1–b_6) was calculated as an estimate for the effects of the factors [17]. The model built for main effect estimation is in principle:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 \quad (1)$$

where x_1–x_6 represent the factors (SA–CF) and b_0 the intercept, i.e. the predicted response with all factors at 0 level. In the regression, the x variables, i.e. the factors, are re-scaled in the interval [−1, 1] which allows obtaining coefficients that are directly comparable. The confounding matrix X^TX gives an indication about the quality of the estimation of coefficients from the considered data set. We namely are not dealing

with an orthogonal design.

$$X^{\mathrm{T}}X = \begin{array}{c|ccccccc|c} b_0 & \mathrm{SA} & \mathrm{MC} & \mathrm{MgS} & \mathrm{NaCMC} & \mathrm{CS} & \mathrm{CF} & \\ \hline 205 & -1 & 23 & -26 & -17 & -4 & -1 & b_0 \\ -1 & 167 & -46 & 1 & 9 & 0 & 0 & \mathrm{SA} \\ 23 & -46 & 97 & -50 & -123 & -1 & 0 & \mathrm{MC} \\ -26 & 1 & -50 & 149 & 38 & 0 & 0 & \mathrm{MgS} \\ -17 & 9 & -123 & 38 & 180 & 1 & 1 & \mathrm{NaCMC} \\ -4 & 0 & -1 & 0 & 1 & 114 & 1 & \mathrm{CS} \\ -1 & 0 & 0 & 0 & 1 & 1 & 137 & \mathrm{CF} \end{array}$$

The confounding matrix for an orthogonal experimental design shows a diagonal matrix in which all off-diagonal elements are equal to zero, indicating that the estimation of the effect of a factor is not affected by any of the other factors, i.e. the factor effects can be estimated unconfounded [18]. If the off-diagonal elements are different from zero this means that the main (factor) effects cannot be estimated unconfounded anymore, i.e. the effect considered is to a certain extent affected by the effect of one or more of the other factors. The larger the off-diagonal value is, compared to the diagonal one (i.e. the one of the considered factor), the more the factor effect is affected by that other factor. From the $X^{\mathrm{T}}X$ matrix one sees that the effects for CS and CF can be estimated practically unconfounded from the other factors, while those for the mixture factors are confounded among each other. Especially MC is largely confounded with the other factors. This makes the estimation of the MC effect completely unreliable. The reason for what is observed in the confounding matrix is due to the fact that the factors SA, MC, MgS and NaCMC form a mixture and that MC was used as an adjusting compound to bring the mixture to the same final weight (80 mg/tablet or 28.57% of excipients). When mixture and instrumental variables are examined in one design, the adjusting compound is not to be considered in the design, since, it ruins the orthogonality of the experimental design [19]. Leaving out the factor MC does not mean that one assumes that it has no effect on the response variables. In such situation one always has to be careful with the interpretation of the effects estimated for the remaining mixture factors, since, they in reality could be caused by the opposite effect of the adjusting compound. This item is discussed in more detail in [19]. The model then becomes:

$$Y = b_0 + b_1x_1 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 \qquad (2)$$

in which variable x_2 (MC) is not considered anymore in the design. The confounding matrix now becomes:

$$X^{\mathrm{T}}X = \begin{array}{c|cccccc|c} b_0 & \mathrm{SA} & \mathrm{MgS} & \mathrm{NaCMC} & \mathrm{CS} & \mathrm{CF} & & \\ \hline 205 & -1 & -26 & -17 & -4 & -1 & b_0 \\ -1 & 167 & 1 & 9 & 0 & 0 & \mathrm{SA} \\ -26 & 1 & 149 & 38 & 0 & 0 & \mathrm{MgS} \\ -17 & 9 & 38 & 180 & 1 & 1 & \mathrm{NaCMC} \\ -4 & 0 & 0 & 1 & 114 & 1 & \mathrm{CS} \\ -1 & 0 & 0 & 1 & 1 & 137 & \mathrm{CF} \end{array}$$

From this new matrix it is observed that now only MgS and NaCMC are somewhat confounded.

To decide on the significance of the estimated coefficients, half-normal probability plots [4,5,7] were drawn. These are plots that allow evaluating visually the significance of mathematically estimated factor effects on a given response. Non-significant factor effects are normally distributed around zero and tend to fall on a straight line in these plots, while significant effects, which do not belong to this distribution, deviate from the straight line. However, these plots require

effect sparsity, i.e. that the majority of estimated effects is non-significant. With only five effects calculated for each response and the possibility that the majority is significant, i.e. has an effect, this requirement is not always fulfilled. Therefore, also the two-factor interaction effects [17] were calculated. Ten interaction effects can be considered, leading to the model:

$$Y = b_0 + b_1x_1 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{16}x_1x_6 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{36}x_3x_6 + b_{45}x_4x_5 + b_{46}x_4x_6 + b_{56}x_5x_6 \quad (3)$$

where b_{ij} represents a two-factor interaction coefficient. From the X^TX confounding matrix it is seen that the interaction coefficients can be estimated relatively unconfounded from other main or two-factor interaction coefficients.

$\boldsymbol{X^TX}=$

	b_0	b_1	b_3	b_4	b_5	b_6	b_{13}	b_{14}	b_{15}	b_{16}	b_{34}	b_{35}	b_{36}	b_{45}	b_{46}	b_{56}	
	205	-1	-26	-17	-4	-1	1	9	0	0	38	0	0	1	1	1	b_0
	-1	167	1	9	0	0	-27	-8	-3	0	-12	0	0	0	0	0	b_1
	-26	1	149	38	0	0	0	-12	0	0	-14	-2	0	0	0	0	b_3
	-17	9	38	180	1	1	-12	-6	0	0	-13	0	0	-3	0	0	b_4
	-4	0	0	1	114	1	0	0	-1	0	0	-15	0	-10	0	-1	b_5
	-1	0	0	1	1	137	0	0	0	-1	0	0	-17	0	-12	-3	b_6
	1	-27	0	-12	0	0	121	13	0	0	10	0	0	0	0	0	b_{13}
	9	-8	-12	-6	0	0	13	150	0	0	11	0	0	0	0	0	b_{14}
	0	-3	0	0	-1	0	0	0	93	0	0	1	0	5	0	0	b_{15}
	0	0	0	0	0	-1	0	0	0	111	0	0	1	0	6	0	b_{16}
	38	-12	-14	-13	0	0	10	11	0	0	132	0	0	0	0	0	b_{34}
	0	0	-2	0	-15	0	0	0	1	0	0	83	0	21	0	0	b_{35}
	0	0	0	0	0	-17	0	0	0	1	0	0	99	0	25	0	b_{36}
	1	0	0	-3	-10	0	0	0	5	0	0	21	0	101	0	0	b_{45}
	1	0	0	0	0	-12	0	0	0	6	0	0	25	0	120	1	b_{46}
	1	0	0	0	-1	-3	0	0	0	0	0	0	0	0	1	76	b_{56}

The estimated coefficients for the model of Eq. (3) are shown in Table 1. The half-normal plots of the coefficients estimated for the different factors and interactions on the responses were drawn. The plot for the tensile strength is shown in Fig. 5 and the important coefficients identified. In Table 1, for each response all coefficients deviating from the straight line are indicated.

4.2. Optimal tablet processing conditions predicted from regression analysis

The optimal tablet shows a high tensile strength, a low disintegration time, a low friability and a fast dissolution. Based on the (significant) effects found in Table 1 the optimal levels for each factor can be predicted. However, one has to remark that curvature of response surfaces was not taken into account, since, only main effects were estimated and that the "best" level of a factor as predicted from this approach is always situated at an extreme level of the examined interval. The first factor, SA, has significant effects (b_1) on disintegration time and dissolution and should be at high level to obtain these responses at their best values. By similar reasoning it is seen that MgS should be at low level. The factors NaCMC and CF

Table 1
Regression coefficients (b_i), estimated according to Eq. (3), on the different responses

	Responses						
	Tensile strength	Disintegration time	Friability	Dissolution (15 min)	Dissolution (30 min)	Dissolution (45 min)	Dissolution (60 min)
b_0	1.287	35.7	0.912	51.48	59.65	65.83	70.93
b_1	0.048	−12.4[a]	−0.191	2.86[a]	3.89[a]	4.28[a]	4.48[a]
b_3	−0.250[a]	13.5[a]	0.191	−1.71[a]	−1.23	−0.80	−0.43
b_4	−0.018	8.0	−0.164	0.30	−1.12	−1.79	−2.33
b_5	0.321[a]	0.8	−0.626[a]	−2.56[a]	−2.24	−1.92	−1.67
b_6	−0.053	2.4	0.033	1.10	1.59	1.77	1.85
b_{13}	0.088	−12.0[a]	−0.305[a]	−0.26	−0.70	−0.93	−1.08
b_{14}	0.035	2.6	0.025	−0.13	−0.92	−1.22	−1.41
b_{15}	−0.042	−1.1	0.176	−0.35	−0.32	−0.34	−0.43
b_{16}	−0.041	−6.0	0.040	1.60[a]	1.90	1.88	1.83
b_{34}	0.009	−3.9	0.028	0.03	−0.07	−0.24	−0.38
b_{35}	−0.047	−0.3	−0.123	0.36	0.24	0.12	−0.02
b_{36}	−0.015	5.8	0.070	−0.98	−1.18	−1.22	−1.23
b_{45}	−0.043	−1.2	0.089	−0.20	−0.11	−0.04	0.08
b_{46}	0.071	−4.5	−0.069	0.28	−0.10	−0.22	−0.20
b_{56}	0.068	−0.7	0.105	0.01	0.05	0.13	0.18

[a] Considered significant from half-normal probability plots.

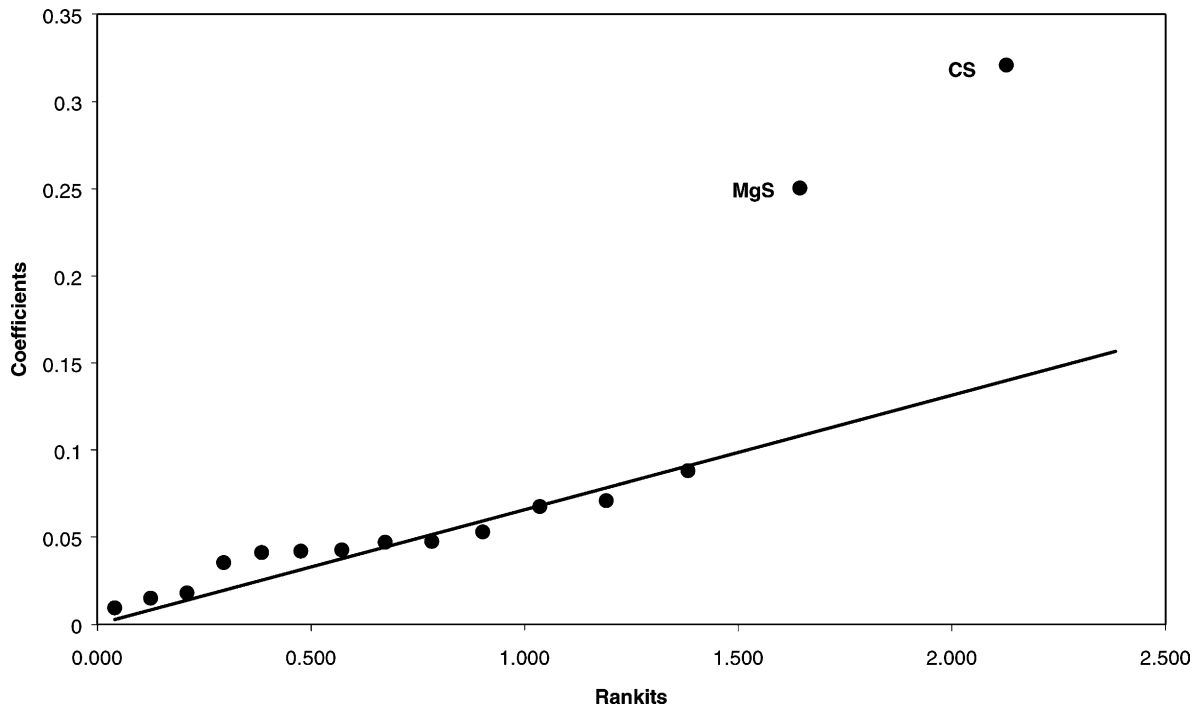

Fig. 5. Half-normal probability plots for the coefficients estimated on the tensile strength.

Table 2
Composition of an optimal tablet as predicted from the regression and half-normal plot analysis

Factor	Level	Experimental value
SA	High	2%
MC	Adjusting compound	23.87%
MgS	Low	0.2%
NaCMC	Low	2.5%
CS	Low or high	13.2–85 ms
CF	High	28 kN

did not had significant effects on any of the responses. However, some tendencies could be seen from the intermediate effects. For NaCMC a low level could be proposed based on the intermediate effects on disintegration time and dissolution, while for CF a high level is preferred based on the effects on dissolution. For the factor CS a conflict occurs in the selection of the best level. To obtain a low friability a high level is required while a high dissolution demands a low level of this factor. The level of the adjusting compound MC depends on those selected for the other mixture variables. The composition of the optimal tablet is summarized in Table 2.

4.3. Parallel co-ordinate geometry plots

For the 205 points data set the PCG plot was drawn (see Fig. 6). The response capping tendency was eliminated from the data set and the PCG plots because only in 35 experiments (17%) a response different from zero was observed. In PCG plots it is possible to choose an arrangement of parallel axis, which emphasizes the intercorrelation or clustering of the factors and responses, if one has prior knowledge about such phenomena (e.g. in Fig. 6 the four dissolution variables have been grouped and were sequenced in order of sampling time). In Fig. 6, all variables were re-scaled independently. Sometimes, for closely related variables, e.g. the dissolution responses, they can be re-scaled together [2]. The benefit of re-scaling simultaneously is that the curve remains looking as a dissolution curve which increases the recognizability of the situation but which is not the aim of the plot, since, the effect is interpretation. Due to a combined re-scaling the responses measured at 15, 30, 45 and 60 min all are compressed in some part of their axis instead of being distributed over the complete axis, which makes it much harder to decide on the effect of a factor.

Usually in multi-response situations, each response is studied separately as a function, the factors and the influence/effect of the factors on that response is determined. The PCG plot allows a simultaneous visualization of all responses in combination with their experimental conditions (factor levels). It offers the possibility to observe which factors have an influence on which responses and provides a graphical alternative for the calculation of effects. To do so, the user indicates for a given factor all experiments where it is at a higher level (e.g. above 0 for the scaled values) in one color and all where it is at a lower one (e.g. below 0) in another. All points from the same experiment on the other parallel co-ordinate axis are then given the same color. In Fig. 6, this procedure is demonstrated for the factor SA. The effects of this factor can be evaluated from the color patterns on the co-ordinate axis. It can be seen that when factor SA is at a higher level (blue lines) the dissolution tends to be larger than when SA is at low levels (red lines), indicating that the effect of SA on dissolution is positive. In the same way the influence of SA on the other responses can be evaluated. For the disintegration time one sees that most results are rather low and that the few high results obtained are found when SA was at low level. Therefore, we conclude there is a negative effect of SA on disintegration. For the responses tensile strength and friability one sees whatever the level of SA is, the results are distributed over the complete response interval indicating no influence of SA. A disadvantage in the interpretation of PCG plots is the occurrence of a limited number of rather extreme measurements in a response which tends to obscure the effect of a factor on that response. Examples can for instance be seen for the disintegration time and the friability.

Table 3 makes a comparison between the conclusions drawn from our PCG plots, from PCG plots made in [2], from the modeling in [3] and from the regression coefficients estimated for the main factors. It can be observed that conclusions drawn from the different PCG plots are sometimes in conflict, e.g. those for SA on the disintegration time. This shows the inherent subjectivity of any visualization method.

For instance the fact that either the blue or the red lines are drawn first leads to PCG plots that visually look different. This is demonstrated in Fig. 7 in

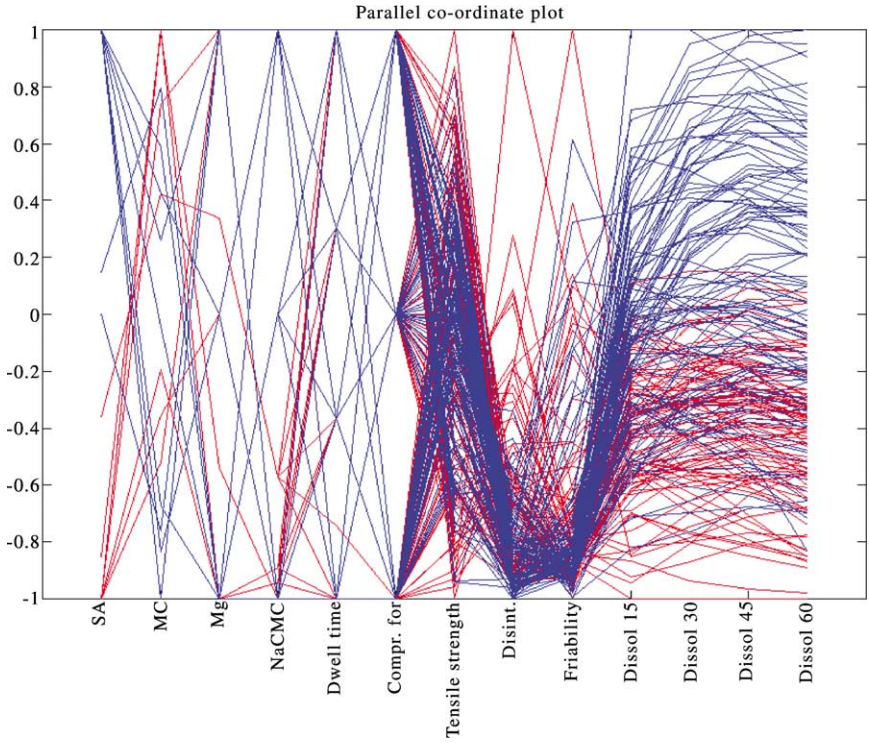

Fig. 6. PCG plot for the data set of [3] as drawn by the in-house program. Blue lines SA ≥ 0; red lines SA < 0.

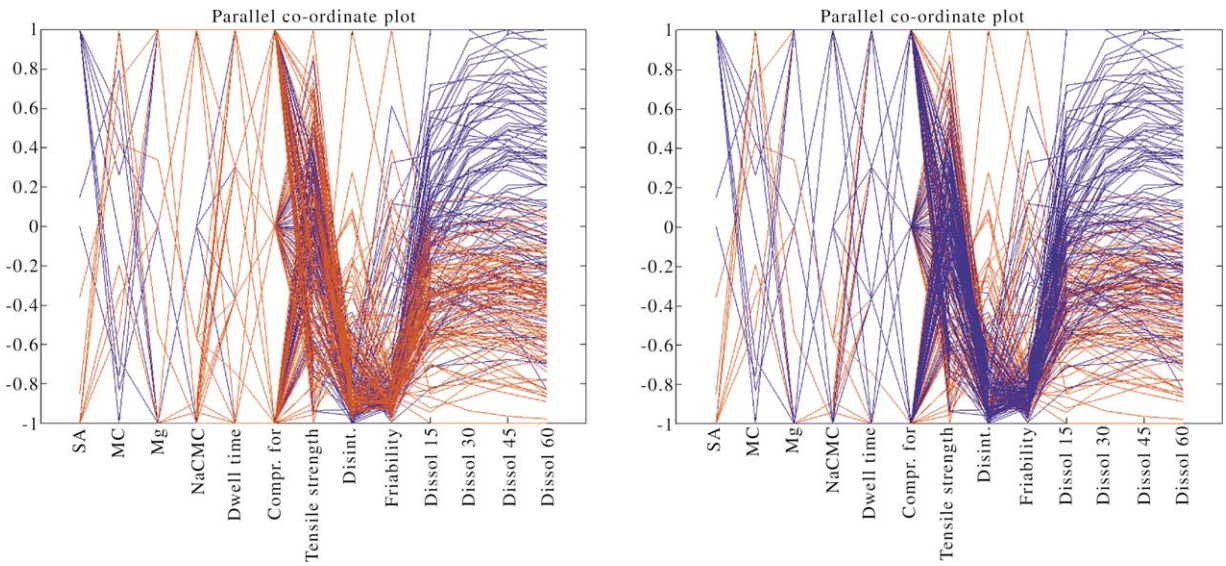

Fig. 7. PCG plot with colors selected as a function of factor SA levels: (a) SA ≥ 0 (blue lines) drawn first; (b) SA < 0 (red lines) drawn first.

Table 3
Comparison of conclusions drawn about the effect of the different factors on the considered responses

Factor	PCG plots	PCG plots [2]	Neural networks [3]	Regression coefficients
(a) Tensile strength				
SA	No effect	No effect	No effect	No significant effect
MC[a]	–[b]	(Small positive)	(Small positive)	–
MgS	Negative effect	Negative effect	Negative effect	Negative effect
NaCMC	Small negative	Small negative	No effect	No significant effect
CS	Positive effect	Positive effect	Positive effect	Positive effect
CF	No effect	No effect	No effect	No significant effect
(b) Disintegration time				
SA	Negative effect	No effect	–	Negative effect
Mc[a]	–	(Small negative)	–	–
MgS	Positive effect	Small positive	Small positive	Positive effect
NaCMC	Small positive	Small positive	–	No significant effect
CS	No effect	No effect	No effect	No significant effect
CF	Small positive	Small positive	No effect	No significant effect
(c) Friability				
SA	No effect	–	–	No significant effect
Mc[a]	–	–	–	–
MgS	No effect	–	–	No significant effect
NaCMC	No effect	–	–	No significant effect
CS	Negative effect	–	–	Negative effect
CF	No effect	–	–	No significant effect
(d) Dissolution rates				
SA	Positive effect	Positive effect	Positive effect	Positive effect
Mc[a]	–	(No effect)	–	–
MgS	No effect	No effect	–	No significant effect
NaCMC	No effect	No effect	–	No significant effect
CS	Small negative	Small negative	–	No significant effect
CF	No effect	No effect	–	No significant effect

[a] Reasons for not considering given in the text.
[b] Not performed or not given.

which once the blue lines (a) were drawn first and once the red (b). The user can also be influenced in his interpretation by the lines connecting two parallel co-ordinate axis, although his decision should only be based on the color patterns on the parallel co-ordinate axis. In Fig. 8, the PCG plots of Fig. 7 are repeated but without interconnection of the parallel co-ordinate axis, only the colored symbols are indicated on the axis. From Fig. 8 we draw the same conclusions about factor SA as before, namely that it affects the disintegration time and the dissolution responses. However, Fig. 8 gives us a clear idea about the variation of responses. For instance, for the dissolution responses one can see that even though a high level of SA can lead to high dissolution results, the variation of the results is widespread and covers almost the whole response interval. This is observed best in Fig. 8b.

An effect can be hidden in PCG plots based on the decision which lines to draw in one color and which in the other. In the above, levels ≥ 0 were in one color and those <0 in another. Since, most factors are examined at several levels not always a clear interval/range is occurring between 'low' (red) and 'high' (blue) levels. In comparison, the effect of a factor is estimated from screening designs for a change from level -1 to $+1$, while the regression coefficients represent a change between levels 0 and $+1$, i.e. a clear distinction between the considered levels is made. To easier evaluate the influence of a factor by means of PCG plots,

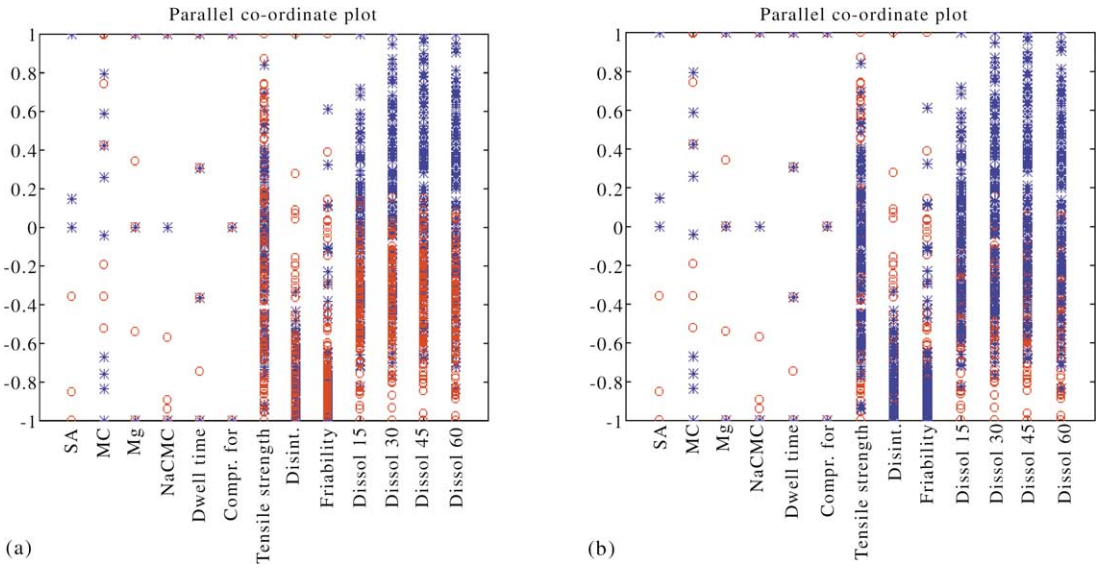

Fig. 8. PCG plot without interconnection of the parallel co-ordinate axis: (a) SA ≥ 0 (blue symbols) drawn first; (b) SA < 0 (red symbols) drawn first.

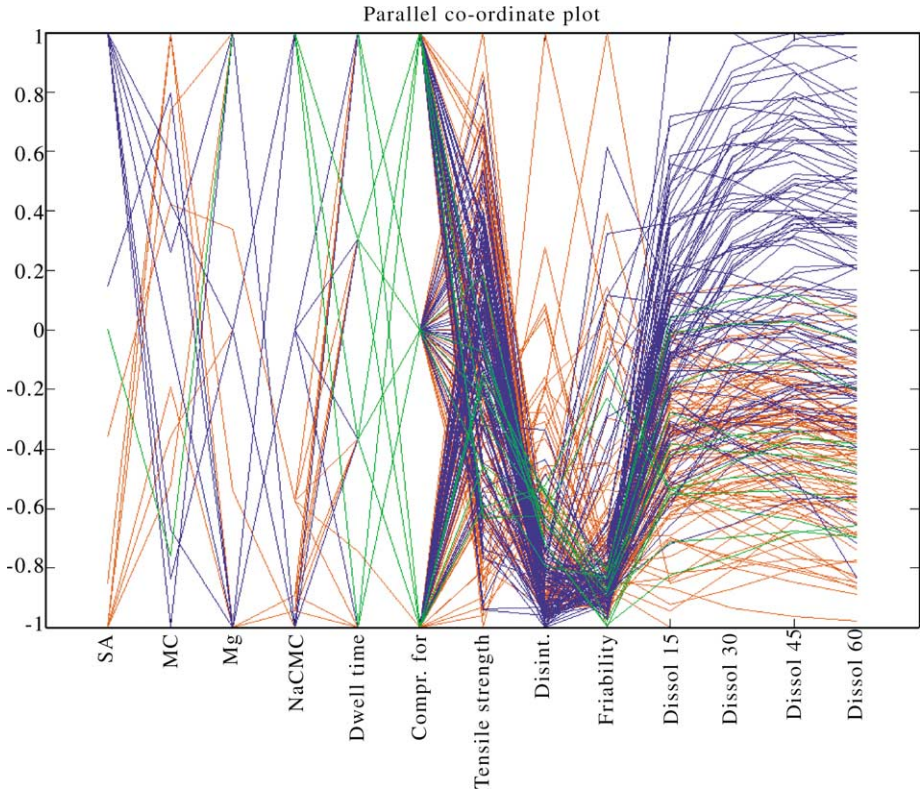

Fig. 9. PCG plot with three colors selected as a function of factor SA levels: SA > 0.3 (blue lines), SA < -0.3 (red lines) and $-0.3 <$ SA < 0.3 (green lines).

i.e. with a clearer distinction between the levels for a given factor it is better to consider three intervals, e.g. what is above factor level 0.3 is plotted in one color (e.g. blue), what is below −0.3 in another (e.g. red) and what is between in a third (e.g. green) (see Fig. 9). This figure indicates that the large variation observed for the blue lines in the low dissolution rates in Figs. 7 and 8 is due mainly to the intermediate levels of the SA concentration. The decision on the occurrence of an effect is then made by considering the tendency of the lines representing the extreme levels of the studied factor.

From the above, we would recommend for evaluation of correlations in experimental design data (effects of factors on responses or correlations between responses): (i) to draw always two PCG plots for a same situation, namely plotting once one color first and once the other; (ii) not necessarily to interconnect the axis, because it overloads the plots when large data sets are involved and distracts the attention from the distribution on the axis; and (iii) occasionally to use a third color for intermediate factor levels. In contrast with modeling, where one model per response is built, here one will make a PCG plot (or preferably two) for each factor, or even more general for each variable (e.g. when correlations between responses also are examined).

Drawing PCG plots to interpret the two-factor interactions involves incorporation of additional parallel axis in the plot and drawing additional plots with the colors selected based on the interaction levels. The values plotted on the interaction axis are those obtained by multiplying the relevant scaled values of the involved factors, similarly as interaction columns are defined in statistical experimental design [17]. However, since two-factor interactions are normally smaller than the significant main effects and, since, the interpretation of the main effects sometimes is already rather difficult, the evaluation of two-factor interaction effects from the PCG plots is not evident and we would reserve their use rather as a first estimate for main effects.

It could be remarked that the visual inspection of a table of numerical inter-correlations might be as effective as the evaluation of a series of PCG diagrams. The preference for one or the other will depend on the analyst. However, to our opinion a majority will prefer a visual interpretation to the study of a matrix table. For comparison purposes, in Table 4 the correlation coefficients between the factors and responses are shown. Indeed, from this matrix the same conclusions can be drawn as from the PCG plots (Table 3).

In Table 3 also some illustrations can be found concerning the remark made earlier about the effect of the adjusting compound. In regression analysis, the adjusting compound has to be removed from the data set for calculation purposes. However, in the PCG plots this factor can be maintained. From the interpretation of these plots it can be observed that if an effect of a mixture factor on a response is found that this effect in reality also could be caused by the opposite effect of the adjusting compound. This can for instance be seen from the effects on the tensile strength and disintegration time.

Concerning the optimal tablet processing conditions, the conclusions from the PCG plots (Table 3) are similar to those drawn from the regression coefficients interpretation (Table 2).

It can be concluded that PCG plots can be used as a visual interpretation tool that allows a fast identification of correlations in large experimental design data sets. Another application of PCG plots is to follow in a production process the quality of produced tablets as a function of time. If for each of the quality parameters (responses) desirability limits are defined (e.g. for dissolution after 60 min, for friability, for disintegration time and for tensile strength), then plotting the measured values for a manufactured tablet on a PCG plot immediately allows to evaluate whether all property requirements are fulfilled.

4.4. Principal components analysis

PCA score and loading plots can be used in the evaluation of the results of an experimental design data set [10]. Score and loading plots were drawn from the auto-scaled values. Fig. 10a shows the PC1/PC2 loading plot for the auto-scaled matrix containing the factors and responses, while in Fig. 10b the loading plot is given from the matrix that also contains the two-factor interaction columns. From these loading plots, in general, similar conclusions about the influence of the factors can be drawn as from the normal probability plots of the regression coefficients or from

Table 4
Correlation coefficients between the examined factors and responses

	SA (%)	MgS (%)	NaCMC (%)	CF (ms)	CS (kN)	Tensile strength (N/mm^2)	Disintegration time (s)	Friability (%)	Dissolution (15 min) (%)	Dissolution (30 min) (%)	Dissolution (45 min) (%)	Dissolution (60 min) (%)
SA	1.000											
MgS	0.008	1.000										
NaCMC	0.053	0.221	1.000									
CF	0.002	−0.003	0.003	1.000								
CS	0.002	−0.003	0.003	0.006	1.000							
Tensile strength	0.066	−0.546	−0.169	0.621	−0.115	1.000						
Disintegration time	−0.337	0.497	0.394	0.026	0.064	−0.363	1.000					
Friability	−0.132	0.118	−0.105	−0.433	0.016	−0.435	0.205	1.000				
Dissolution (15 min)	0.526	−0.271	0.022	−0.384	0.195	−0.064	−0.317	0.110	1.000			
Dissolution (30 min)	0.621	−0.203	−0.175	−0.290	0.245	−0.058	−0.376	0.086	0.928	1.000		
Dissolution (45 min)	0.642	−0.149	−0.245	−0.231	0.254	−0.049	−0.376	0.070	0.848	0.983	1.000	
Dissolution (60 min)	0.639	−0.108	−0.295	−0.190	0.251	−0.042	−0.366	0.061	0.774	0.948	0.990	1.000

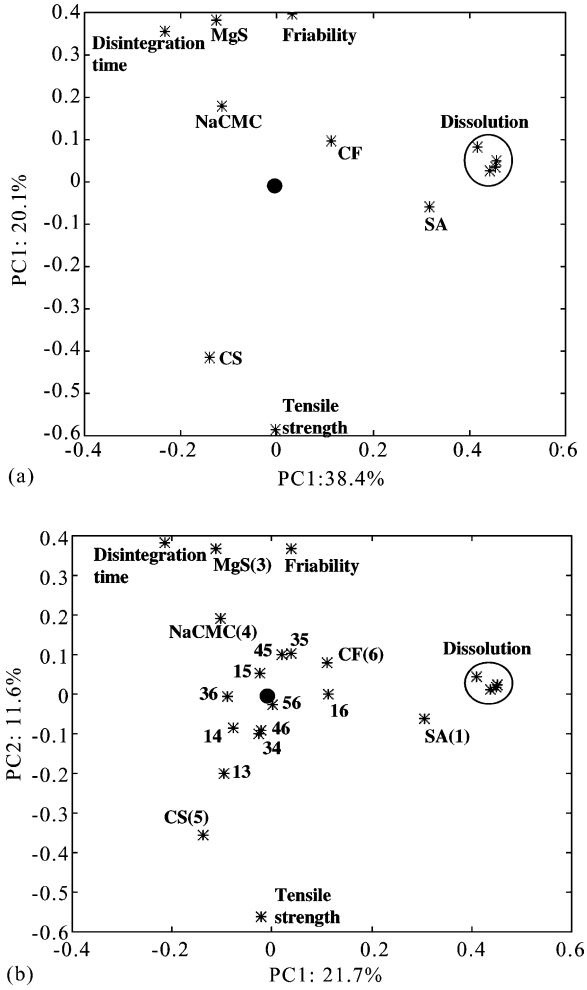

Fig. 10. PC1–PC2 loading plot from: (a) auto-scaled matrix of factors and responses; (b) auto-scaled matrix of factors, interactions and responses; center point: (●), (0,0).

the table of correlation coefficients. It is observed that SA is positively correlated with the dissolution, i.e. has a positive effect, and negatively with the disintegration time. MgS is negatively correlated with the tensile strength and positively with disintegration time and friability. CS is positively correlated with the tensile strength and negatively with the friability. It is also observed that the loadings of the interaction terms are situated close to the origin. The loadings of the factors NaCMC and CF for which no significant effects were observed from the regression analysis do not largely exceed those of the interactions. However, the intermediate effects of NaCMC on the disintegration time and of CF on the dissolution, which were found earlier, could also be expected from the loading plots. The interactions 13 and 16 that were found to have significant effects on some responses were found to have to the largest loadings among the interactions and their intermediate effects on dissolution (16), and on friability and tensile strength (13) could also be derived. The interaction effect of 13 on disintegration time is less evident to be found from the loading plots. Though PC1 and PC2 in Fig. 10a and b explain only 58.5 and 33.3%, respectively, of the total variability, higher PCs did not give additional useful information. The loading plots of Fig. 10 also demonstrate the high correlation between the different dissolution responses as was already observed from the PCG plots, and a negative correlation between the friability and the tensile strength.

Fig. 11 shows the PC1/PC2 score plot for the data set, once numbered according to the tablet powder mixture used and once according to the experiment number given in [3]. In the data set, 17 different powder mixtures could be distinguished. They were numbered after sorting in ascending order for SA, MgS and NaCMC, respectively. In Fig. 11a the properties of the tablets originating from these mixtures were indicated as they were derived from the loading plots. The correctness of the awarded properties on the score plot was confirmed by performing an analysis of variance (ANOVA) followed by a Student–Newman–Keuls test [20] on each response after grouping the results based on the different powder mixtures. From this Figure it can be observed that the tablets with the desired properties should have high PC1 and low PC2 scores, and should be situated in the bottom right quadrant of the score plot.

The score plot then resembles very much a Pareto optimality plot to simultaneously optimize two responses [21,22]. However, in this situation the combination of seven responses is visualized in a two-dimensional plot. The ‘Pareto optimal points’ in the score plot were identified (Fig. 11b) to evaluate whether they were representing tablets with acceptable properties. The factor levels and their properties are summarized in Table 5. It can be seen that the selected experiments can be considered as leading to tablets with good properties. They have dissolution

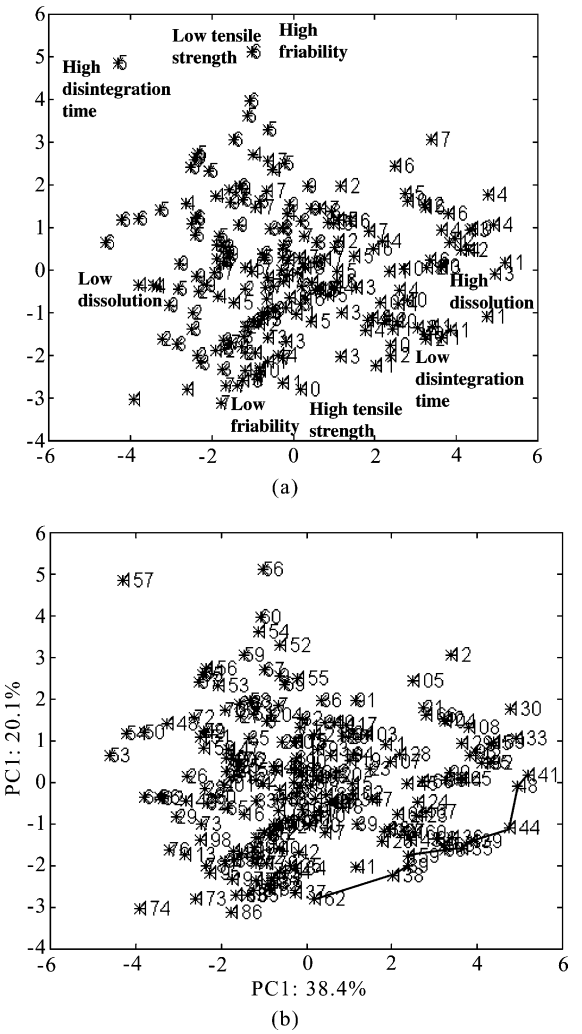

Fig. 11. PC1–PC2 score plot: (a) experiments numbered based on powder mixture composition, and (b) experiments numbered based on experiments number given in [3]. Pareto optimal points are connected.

profiles that are above the average, show short disintegration times, have a low friability and a tensile strength that in general is above the average of the complete data set.

If we have a look at the factor levels leading to these Pareto optimal experiments it is observed that SA usually is at high level (2%) which is in agreement with the prediction from the regression coefficients. The levels for MgS are low (0.2%) as predicted from the effects. For NaCMC, usually low levels are selected, for CS no tendency is observed in the selected levels and for CF intermediate and high levels were selected, which all is in accordance with the interpretation of the regression coefficients.

5. N-way PCA

The data set as it is used for the N-way analysis is represented in Fig. 4. In the first mode the 17 different mixtures are considered, in the second the tablet properties, in the third the dwell time (CS) and in the fourth the compression force. Three cubes, in which each cube contains the first three modes, while a cube for each compression force is considered, represent this situation. The model complexity was determined as [2 2 1 1], i.e. two factors in first and second modes and one factor in the third and fourth modes. For more details about model construction we refer to [9]. This model explained 99.2% of the variability in the data. The N-way analysis was performed on the standardized X data matrix. The results are shown in the loading plots of Fig. 12 and in the core matrix of Fig. 13. In loading plot A1 and A2 the concentration SA determines the distribution of the mixtures along A1 while along A2 it is the MgS concentration as was seen from relating this plot with the original data table. Loading plot B1 and B2 shows the properties of the tablets. Axis B1 differentiates the dissolution from the other properties. The figure also shows the high correlation between the dissolution responses. Along B2 differences between disintegration, friability and tensile strength are revealed. In loading plot C1, the loadings are not ranked in increasing order of the dwell time (CS). It can be caused by the fact that two instruments each with two specific dwell times were used in [3]. An opposite trend is seen between the two instruments. However, this did not lead to significant interaction effects of CS with another factor on any of the examined responses (Table 1). In loading plot D1 an increasing order of compression force is observed. The loading for CF = 20 kN is much closer to the one of 28 kN than to the one of 12 kN, which indicates that the compression force has a non-linear effect on the tablet properties. It also confirms the levels found in for instance Table 5.

Table 5
Factor levels and properties of the Pareto optimal experiments derived from the score plot of Fig. 11

Experiment no.	Factors					Responses						
	SA (%)	MgS (%)	NaCMC (%)	CF (ms)	CS (kN)	Tensile strength (N/mm^2)	Disintegration time (s)	Friability (%)	Dissolution (15 min) (%)	Dissolution (30 min) (%)	Dissolution (45 min) (%)	Dissolution (60 min) (%)
141	2.00	0.2	2.5	13	20	1.146	10	0.57	62.5	74.8	81.2	86.3
48	2.00	0.2	7.5	36	28	1.598	34	0.09	69.1	75.5	79.4	82.6
144	2.00	0.2	2.5	36	20	1.730	12	0.43	60.4	72.4	80.9	87.7
139	2.00	0.2	2.5	85	28	1.549	10	0.89	57.4	71.1	78.4	83.4
135	2.00	0.2	2.5	60	20	1.640	8	0.27	56.2	68.7	78.1	85
90	2.00	0.2	5.0	85	28	1.754	22	0.22	59.8	68.9	75.6	80.9
159	1.19	0.2	2.5	60	20	1.908	16	0.63	54.9	66.4	74.1	80.6
89	2.00	0.2	5	85	20	2.045	40	0.19	58.1	67.5	73.5	78.4
138	2.00	0.2	2.5	85	20	1.771	12	0.27	53.3	65.3	72.3	77.5
162	1.19	0.2	2.5	85	20	2.175	15	0.44	54.3	60	64.6	68.4
Minimal response						0.554	8	0	40.6	47.7	53.5	58.6
Maximal response						2.316	210	6.95	69.1	75.5	81.2	87.7

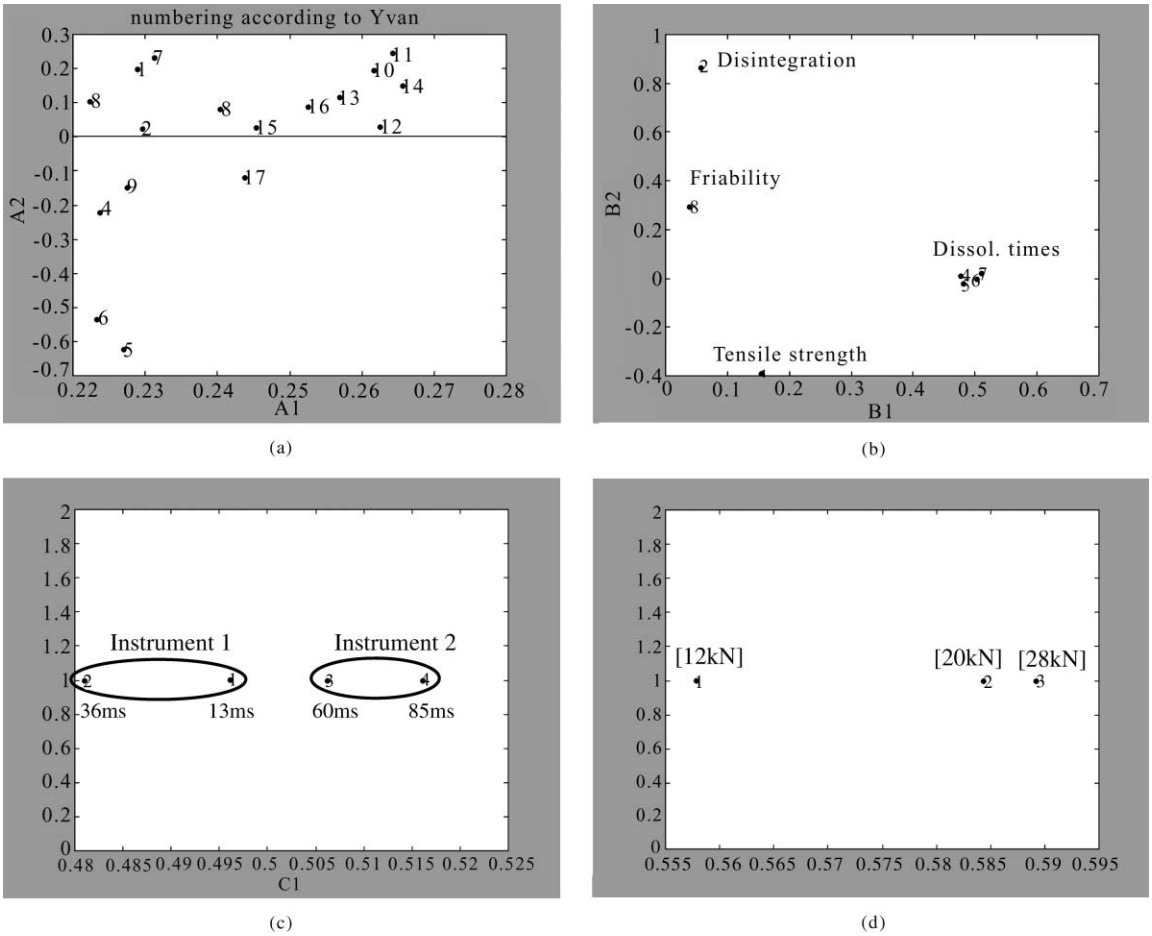

Fig. 12. Loading plots obtained from the N-way PCA on the data set studied: (a) A1–A2, 17 mixtures; (b) B1–B2, 7 tablet properties; (c) C1, four dwell times; (d) D1, three compression forces.

Element (1, 1, 1, 1) of the core matrix (Fig. 13) with value 303 indicates a very strong positive interaction between A1–B1–C1–D1. From this one can conclude that to obtain high values of dissolution one should use the mixtures 10, 11, 12 and 14 (in which all have a high concentration of SA), a dwell time of 85 ms and a compression force of 20 or 28 kN. The core element (2, 2, 1, 1) with value −14.3 indicates a strong negative interaction between A2–B2–C1–D1 and low values for the disintegration time and friability are observed for mixtures 1, 7, 10 and 11 (all with low MgS concentration), for a dwell time of 85 ms and for a compression force of 20 or 28 kN.

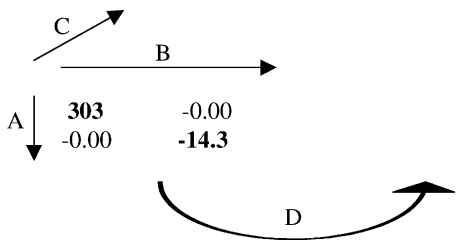

Fig. 13. Core matrix with dimensionality [2 2 1 1].

The experimental conditions predicted from the N-way PCA confirm those found earlier with the regression analysis and with the common PCA analysis.

6. Conclusions

This paper investigated the use of PCG as a multivariate method for analyzing multi-way experimental design data and compares it to two-way and to (the more sophisticated) N-way PCA for this purpose. PCG plots are found to be a useful tool for the visual evaluation of the effects of the factors of an experimental set-up on the responses considered. They also allow evaluating correlations among the different responses. A benefit is that they do not require calculations and simply can be interpreted. This is for instance the case to verify if tablet properties fall within predefined intervals.

The two-way PCA approach too allows identifying the correlation between factors and responses (effects), and between responses themselves. The N-way PCA approach yields similar conclusions as the two-way PCA method. The loading plots and the important elements of the core matrix allow interpreting the influence of the factors on the tablet properties and the prediction of optimal conditions for tablet production. However, the interpretation of the N-way results requires more expert knowledge than the other techniques.

The PCA methods can be used to some extent to select optimal conditions. Of course, it is not possible to predict intermediate optimal conditions, but it is possible to use the score plot as a kind of Pareto optimality plot and to select the experiments considered to be Pareto optimal. Those experiments lead to tablet properties, which were among the best in the data set.

To evaluate effects more thoroughly half-normal probability plots of the estimated factor effects and/or significance testing should be used, but it is observed that the important effects found usually agree with those observed with PCA and/or PCG. Moreover, correlations between responses can be observed with the latter methods, so that a more general evaluation of the data is performed when they are included.

It can be concluded that PCA and/or PCG each have their own benefits and disadvantages. They are found to be useful and complementary aids in the interpretation of large multi-response experimental design data and they add a multivariate dimension to the more classical univariate analysis of such data.

Acknowledgements

Y. Vander Heyden is a post-doctoral fellow of the Fund for Scientific Research (FWO), Vlaanderen, Belgium.

References

[1] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Part A, Elsevier, Amsterdam, 1997.
[2] R. Brooks, F. Foo, R. Rowe, Pharm. Technol. Eur., 2000, 40–50.
[3] J. Bourquin, H. Schmidli, P. van Hoogevest, H. Leuenberger, Eur. J. Pharm. Sci. 6 (1998) 287–300.
[4] Y. Vander Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M. Vandeginste, D.L. Massart, J. Pharm. Biomed. Anal. 24 (2001) 723–753.
[5] K. Jones, International Laboratory, November 1986, pp. 32–45.
[6] E. Morgan, Chemometrics: Experimental Design Analytical Chemistry by Open Learning, Wiley, Chichester, 1991, pp. 118–188.
[7] A. Nijhuis, H.C.M. van der Knaap, S. de Jong, B.G.M. Vandeginste, Anal. Chim. Acta 391 (1999) 187–202.
[8] R. Henrion, Chemom. Intell. Lab. Syst. 25 (1994) 1–23.
[9] R. Bro, Multi-way analysis in the food industry. Models, algorithms and applications, Ph.D. thesis, University of Amsterdam, The Netherlands, 1998.
[10] R. Bergman, M.E. Johansson, T. Lundstedt, E. Seifert, J. Åberg, Chemom. Intell. Lab. Syst. 44 (1998) 271–286.
[11] Y. Vander Heyden, C. Perrin, D.L. Massart, Optimization strategies for HPLC and CZE, in: K. Valko (Ed.), Handbook of Analytical Separations, Separation Methods in Drug Synthesis and Purification, Vol. 1, Elsevier, Amsterdam, 2000, pp. 163–212.
[12] E. Smet, L. Staelens, Y. Vander Heyden, H.Y. Aboul-Enein, G. Van der Weken, A.M. García-Campaña, W.R.G. Baeyens, Chirality, submitted for publication.
[13] B. Vandeginste, L. Buydens, S. De Jong, P. Lewi, D.L. Massart, A. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Part B, Elsevier, Amsterdam, 1998.
[14] D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte, L. Kaufman, Chemometrics: A Textbook Elsevier, Amsterdam, 1988, pp. 101–106.
[15] L.R. Tucker, Psychometrica 31 (1996) 279–311.
[16] R. Bro, C. Andersson, N-way toolbox, http://www.models.kvl.dk/source.
[17] Y. Vander Heyden, D.L. Massart, Review of the use of robustness and ruggedness in analytical chemistry, in: A. Smilde, J. de Boer, M. Hendriks (Eds.), Robustness

of Analytical Methods and Pharmaceutical Technological Products, Elsevier, Amsterdam, 1996, pp. 79–147.
[18] Y. Vander Heyden, M.S. Khots, D.L. Massart, Anal. Chim. Acta 276 (1993) 189–195.
[19] Y. Vander Heyden, F. Questier, D.L. Massart, J. Pharm. Biomed. Anal. 18 (1998) 43–56.
[20] P. Armitage, G. Berry, Statistical Methods in Medical Research, 2nd Edition, Blackwell, Oxford, 1987, p. 559.
[21] A.K. Smilde, A. Knevelman, P.M.J. Coenegracht, J. Chromatogr. 369 (1986) 1–10.
[22] H.R. Keller, D.L. Massart, J.P. Brans, Chemom. Intell. Lab. Syst. 11 (1991) 175–189.