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Central Composite Design for the Rapid Optimisation of Ruggedness and Chiral Separation of Amlodipine in Capillary Electrophoresis

TRACY S. SMALL, ANTHONY F. FELL, MICHAEL W. COLEMAN, AND JOHN C. BERRIDGE
Pharmaceutical Analysis Research Unit, Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, Bradford, UK (T.S.S., A.F.F.); and Analytical Chemistry Department, Pfizer Central Research, Sandwich, Kent, United Kingdom (M.W.C., J.C.B.)

ABSTRACT Systematic optimisation with central composite design offers an efficient route for rapid optimisation of resolution with multiple interacting parameters in chiral CE. This is illustrated by separations of amlodipine with α-CD as chiral selector in the running buffer, for which the predicted performance of central composite design is assessed. The utility of response surface methodology for locating optimum ruggedness in CE is also described.

KEY WORDS: central composite design, liquid chromatography, capillary electrophoresis, amlodipine, cyclodextrin, ruggedness

Systematic methods for optimisation of chiral resolution are well established in liquid chromatography (LC).[1,2] They have significant potential for rapid method development in capillary electrophoresis (CE). In LC, the use of central composite design (CCD) has already been established by Zoest et al.[3] for nitroimidazole separations in a reversed-phase system, involving the optimisation of two variables. More recently Tucker et al.[4] showed that CCD is generally applicable for the optimisation of three variables in chiral LC separations. The fact that interactions between operational parameters can be assessed by CCD, and that modelling of the response surface can be achieved in four-dimensional space to predict rapidly and reliably the experimental conditions required for optimum response (e.g., resolution), were shown to be key advantages of this approach. Indeed, in chiral LC separations of tioconazole using the experimental conditions predicted by CCD, it was shown that there was excellent correlation between the response (i.e., resolution) observed in practice, with that predicted by SAS.[4]

In the field of CE, however, by comparison with LC limited attention has so far been given to the use of systematic optimisation strategies, despite the acknowledged complexities of the separations involved. In 1991 Atamna et al.[5] explored the use of factorial design for the optimisation of two variables in capillary zone electrophoresis. In the same year Vindevogel et al.[6] demonstrated the use of a Plackett-Burman design for the first time in the micellar electrokinetic chromatography (MEKC) of steroids. In 1992 Li and co-workers[7] also examined systematic optimisation for the separation of flavonoids by MEKC. Altria et al.[8] explored the use of univariate optimisation in the chiral CE of β-aminoalcohol enantiomers, using cyclodextrins as buffer additives. In 1994 this group used a Plackett-Burman design for chiral CE in the separation of clenbuterol enantiomers.[9] These authors have recently reviewed various modes of CE with LC for drug analysis and discussed operational strategies for their optimisation.[10]

Given the advantages of CCD already identified in LC,[4] there is significant potential for its use as a novel approach to rapid method development in all modes of CE. This approach has important advantages in high-performance CE systems, particularly for chiral separations, compared with the ad hoc univariate optimisation strategies often employed.[8] The present work examines the performance of a CCD applied to 3 interacting buffer parameters, for optimising the enantiomeric separation of amlodipine (AL) using CE, with α-cyclodextrin (α-CD) as a chiral selector added to the running buffer. For the first time in CE the response surface generated by CCD in four dimensions is shown to present important features for the rational design of analytical methods for maximum robustness.

METHODS

Principles of CCD

The CCD represents a simultaneous experimental design for k factors or variables, which permits the response surface to be modelled by fitting a second-order polynomial in $(k + 1)$ dimensions. This requires $(2^k + 2k + 1)$ experiments. Thus there are 4 dimensions for 3 interacting factors. With a 3-factor design the response Y (e.g., resolution) to each of 15 experiments is obtained (Fig. 1). It is important to arrange for replication at the central point to give a measure of experi-

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Address reprint requests to Professor Anthony F. Fell, Pharmaceutical Analysis Research Unit, Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK.

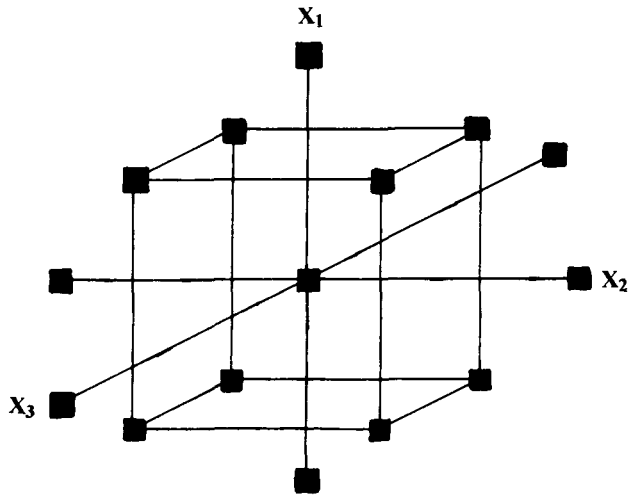
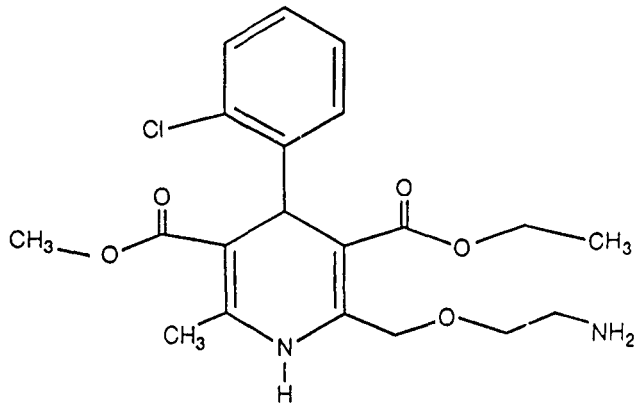


Fig. 1. Factors for CCD: X_1, pH; X_2, temperature (°C); X_3, α-CD concentration (m*M*).



Amlodipine

Fig. 2. Structure of amlodipine.

mental error. For 3 factors, χ_1, χ_2, χ_3, the design requires that the following mathematical model be fitted to the data:

$$Y = b_0 + b_1\chi_1 + b_2\chi_1^2 + b_3\chi_2 + b_4\chi_2^2 + b_5\chi_3 + b_6\chi_3^2 + b_7\chi_{12} + b_8\chi_{13} + b_9\chi_{23} + b_{10}\chi_{123}$$

where b_0 is the intercept and the b terms represent those parameters of the model which are optimised iteratively to fit or "model" the data.

The data are modelled using the SAS suite of programs (Statistical Analysis Systems Inc., Cary, NC),[11] on a bench-top PC (with 16 Mb RAM and 200 Mb hard disk) utilising the "RSREG" procedure to perform multivariate regression on the data. Of the various procedures available in SAS, it was previously shown[4] that the full regression model represented by "RSREG" was the most effective. This model permits the response surface established by the selected variables to be characterised, so that the response can be prediced at any point *within* factor space, even if that point has not been included in the design. This then permits the shape of the response surface to be modelled within factor space, subject to the limitations imposed by the use of a second-order polynomial in 4 dimensions. Thus the fine features of the response surface are readily characterised by this computational procedure in a reproducible fashion. The predicted levels of the CE buffer parameters required to obtain optimum response (i.e., chiral resolution R_s or peak separation P_i), are readily calculated as part of this suite of programs.[11]

Capillary Electrophoresis

The HP ^{3D}Capillary Elecrophoresis system with real-time UV-visible diode array detection (Hewlett-Packard GmbH, Waldbronn, Germany) was used. The system was controlled by a HP Vectra 486 PC, with math co-processor, 8 Mb RAM, and 250 Mb hard disk. Data were processed using the ^{3D}CE-ChemStation software and macro programmes based on established algorithms. Straight-walled 50-μm-i.d. uncoated fused-silica capillaries 64.5 cm in length (56.0 cm effective length) were used. Samples 1 m*M* were prepared in buffers prepared freshly each day using AnalaR-grade reagents. α-CD was used as received from Aldrich Chemical Co Ltd. (Gillingham, Dorset, UK). Temperature was found to be critical and was controlled to ±0.1°C.

The key protocol developed for the capillary conditioning-flush regime (at 50 mbar) and for sample introduction was as follows: start-up, 0.1 *M* NaOH, 2 min; new buffer, 5 min; shut-down, 0.1 *M* NaOH, 5 min; water, 5 min; sample introduction, controlled pressure profile injection at 50 mbar (typically for 4 or 8 sec) preceded by a preconditioning flush with the appropriate buffer for 3 min.

The initial conditions adopted for preliminary investigation of AL separation in CE with α-CD were buffer, phosphate (50 m*M*) at pH 2.4; α-CD, 20 m*M*; temperature, 25°C; voltage, 25 kV; wavelength, 214 nm. The buffer pH was adjusted by addition of phosphoric acid or sodium hydroxide, as appropriate, to potassium dihydrogen phosphate. The running current was constant at approximately 70 μA.

RESULTS AND DISCUSSION

The first separation of AL (Fig. 2) obtained with α-CD was not baseline (cf. Fig. 3), therefore before optimisation of the system was carried out, initial experiments were performed to analyse the effects and interactions of all the parameters. The variables involved in this separation included pH, α-CD concentration, temperature, buffer type and concentration, and voltage. These investigations showed that the first two factors dominated the chiral resolution of AL; of the others, temperature was the most significant.

Thus phosphate buffer molarity was maintained constant at 50 m*M*. It is important to use a single buffer system, in order to avoid introducing additional variables into the system. The pK_a of phosphoric acid is such as to give reasonably effective control over the pH range of interest (ca. 2.5 to 5). The applied voltage was shown to exercise a linear effect on separation and was kept constant at 25 kV.

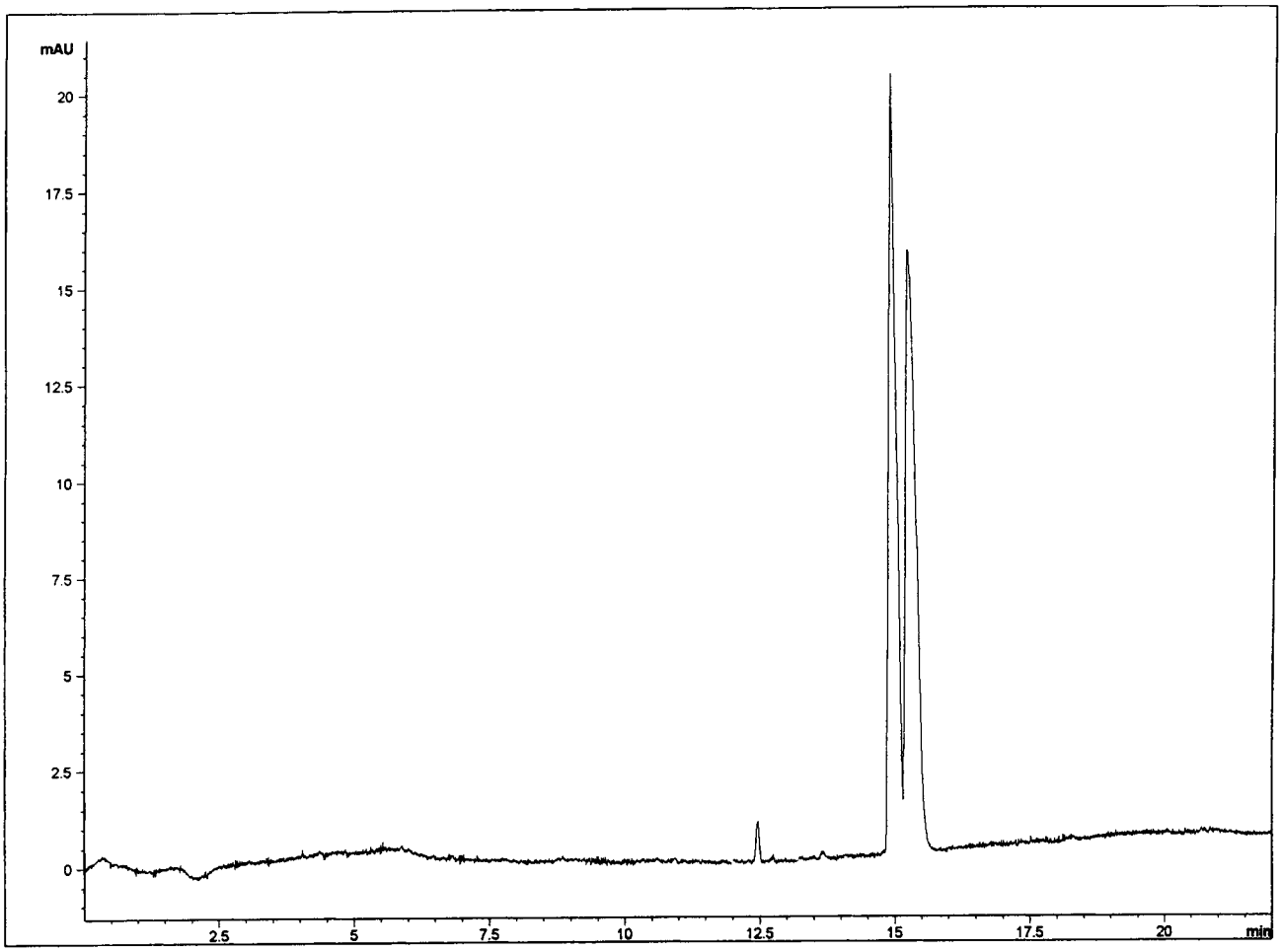


Fig. 3. Electropherogram of chiral CE separation of amlodipine with α-CD before optimisation: for conditions, see text.

The factor space defined for each CCD parameter is illustrated in Table 1, where X_1 represents pH, X_2 the temperature, and X_3 the additive concentration. The central point of the design corresponded approximately to the median value of each of these three parameters. The "star points" in the design were located in factor space symmetrically about the central point, at points corresponding to 50% greater than the value assigned to each factor. The parameters used as response criteria in assessing the resulting electropherograms were the Kaiser peak separation function,[12] P_i, and resolution, R_s (cf. Table 2).

The data acquired from this CCD were analysed by SAS to model the four-dimensional response surface, as noted above. The optimum conditions predicted by SAS (Table 3), were used experimentally and resulted in baseline separation of the enantiomers (cf. Fig. 4). The experimental results observed for P_i and R_s are in excellent agreement with the values predicted by SAS. The slight differences in optimum conditions predicted for the P_i and R_s models probably indicate that the optimum zone identified on the response surface is fairly flat. Although the response surface is four-dimensional, it can be readily visualised as a three-dimensional graphic by presenting the response to two factors, while the third is kept constant at its optimum value. Multivariate regression with the second-order polynomial function permits the detailed shape of the response surface to be modelled,[4] as noted above. RSD values (n = 9) were: P_i 0.73%; R_s 3.9%.

TABLE 1. Experiments for a 3-factor CCD for optimising the separation of amlodipine by CE with α-CD

Expt. No.	pH	Temperature (°C)	Additive (m*M*)
1	2.75	15	10
2	2.75	15	20
3	2.75	25	10
4	2.75	25	20
5	4.25	15	10
6	4.25	25	20
7	4.25	15	10
8	4.25	25	20
9	3.50	20	5
10	3.50	20	25
11	3.50	10	15
12	3.50	30	15
13	2.00	20	15
14	4.00	20	15
15	3.50	20	15

TABLE 2. Experimental results for a 3-factor CCD for optimising amlodipine separation by CE with α-CD

Expt. No.	R_s	P_i
1	1.216	0.983
2	1.455	0.988
3	1.258	0.973
4	1.331	0.983
5	1.247	0.983
6	1.407	0.988
7	0.903	0.870
8	1.115	0.940
9	0.966	0.892
10	1.365	0.995
11	1.500	0.988
12	1.206	0.950
13	1.585	0.990
14	0.903	0.767
15	1.324	0.990
	1.290	0.990
	1.275	0.990
	1.242	0.985
	1.313	0.985
	1.231	0.966
	1.391	0.980
	1.355	0.987
	1.351	0.985

Figure 5 shows the three-dimensional plot generated for the P_i model, where the temperature, X_2, is maintained at its optimum value, 17.2°C. This portion of the four-dimensional response surface shows a strong degree of curvature, where the optimum can be readily discerned. A sharp incline is observed as the additive concentration increases to its optimum value, beyond which it becomes fairly flat. This suggests that the concentration of α-CD (X_3) in the running buffer exerts a critical effect on the enantioseparation of amlodipine, up to its optimum value, beyond which response is more or less constant. However, it can be seen from Figure 5 that the relationship for pH (X_1) differs substantially from that for α-CD (X_3), in that steep gradients are observed on either side of the optimum. This indicates the critical importance of pH in the enantiorecognition process.

The three-dimensional graphic in Figure 6 shows the response to temperature (X_2) and pH(X_1), while maintaining the α-CD concentration (X_3) at its optimal value of 18.2 mM (cf. Table 3). This is markedly different from the response surface in Figure 5. The shape resembles a saddle, which actually corresponds to the prediction by the SAS program of a "saddlepoint" in this optimum zone. As the temperature of the system is varied over the factor space, there is only a relatively small change in the observed response. This suggests that for this particular system, temperature exercises a relatively small effect compared to that of the other two variables studied in this design. Once again, however, variation in pH is seen to exert a critical effect on the shape of the response surface, analogous to that observed in Figure 5.

Figure 7 represents a three-dimensional slice of the response surface for temperature (X_2) and additive concentration (X_3), with pH (X_1) maintained constant at its optimum value of 3.16 (cf. Table 3). As in Figure 5, the steep gradients observed for the additive concentration confirm that it plays a crucial role in the process of enantiorecognition. Once again, the effect of temperature is seen to be relatively small, as noted in Figure 6. Nevertheless, by analogy with chiral LC,[4] in general it is considered to be critically important to maintain a constant temperature during separation in chiral CE, where substantial amounts of energy are put into the system.

These response surfaces can be interrogated to establish the zones of optimum robustness for the method as a whole. Thus it is clear in Figure 7 that the very high enantioselectivity observed for higher values of temperature (X_2) and additive concentration (X_3) correspond to zones where the gradients of response are very high, indicating the lack of robustness associated with this high enantioselectivity. The central zone of the saddlepoint in Figure 6 clearly corresponds to the robust conditions required for analysis in CE. This in turn corresponds to the zone for optimum selectivity and robustness illustrated in Figure 5.

CONCLUSIONS

A central composite design for optimisation of three interacting parameters in CE, with data evaluated by a programme

TABLE 3. Predicted and observed data for separation of amlodipine enantiomers by CE with α-CD under predicted optimum conditions: pH (X_1), temperature (°C, X_2), CD concentration (m*M*, X_3)

	Optimum factor level	Predicted P_i or R_s	Observed P_i or R_s	Equivalent R_s *or* P_i	Migration time T_m (min)
Peak separation index (P_i)	$X_1 = 3.16$ $X_2 = 17.20$ $X_3 = 18.18$	1.000	0.995	1.364	27.8
Resolution (R_s)	$X_1 = 2.29$ $X_2 = 16.2$ $X_3 = 20.9$	1.495	1.455	0.992	33.3

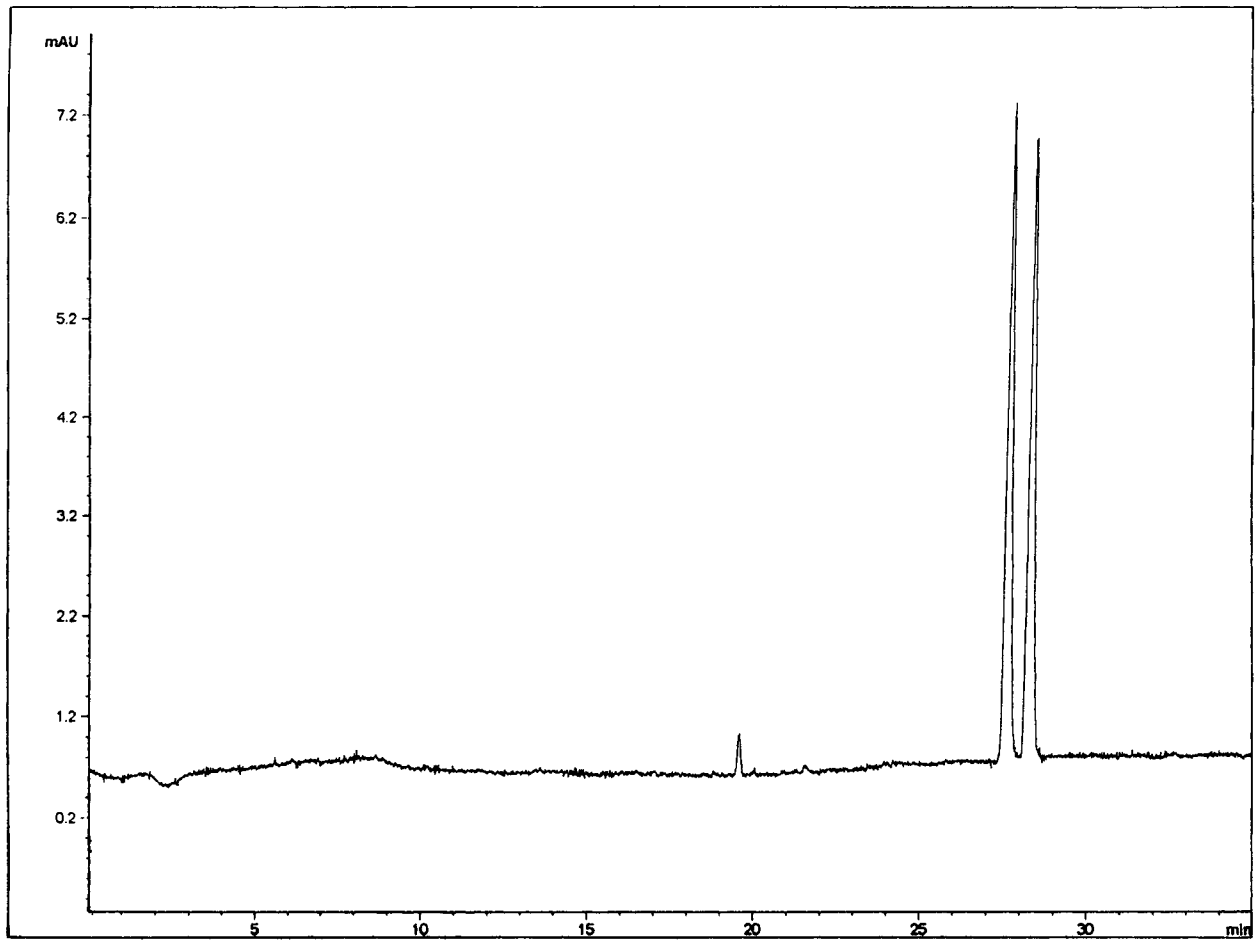


Fig. 4. Electropherogram of the chiral CE separation of amlodipine under optimised conditions (cf. Table 3).

from the SAS suite, has been used for the first time to locate optimum conditions for the chiral resolution of amlodipine in CE using a CD additive to the running buffer. CCD coupled with SAS predicted the optimum parameter values for successful chiral separation in a rapid and efficient manner. Indeed, SAS predicted optimum values for the peak separation function and also for resolution (the two measures adopted to assess response), which were in excellent agreement with those observed experimentally using the optimised parameters.

With this new approach, peak separation and resolution in CE can be predicted to an accuracy of about 1%, and migration time to better than about 5%. Moreover, the SAS programmes generate three-dimensional response surfaces which provide valuable information on the robustness of the conditions selected for an analytical method in CE. This approach is perfectly general in its application and should be of value in all modes of electrophoresis, where the resolution of solutes depends on three or more interacting parameters.

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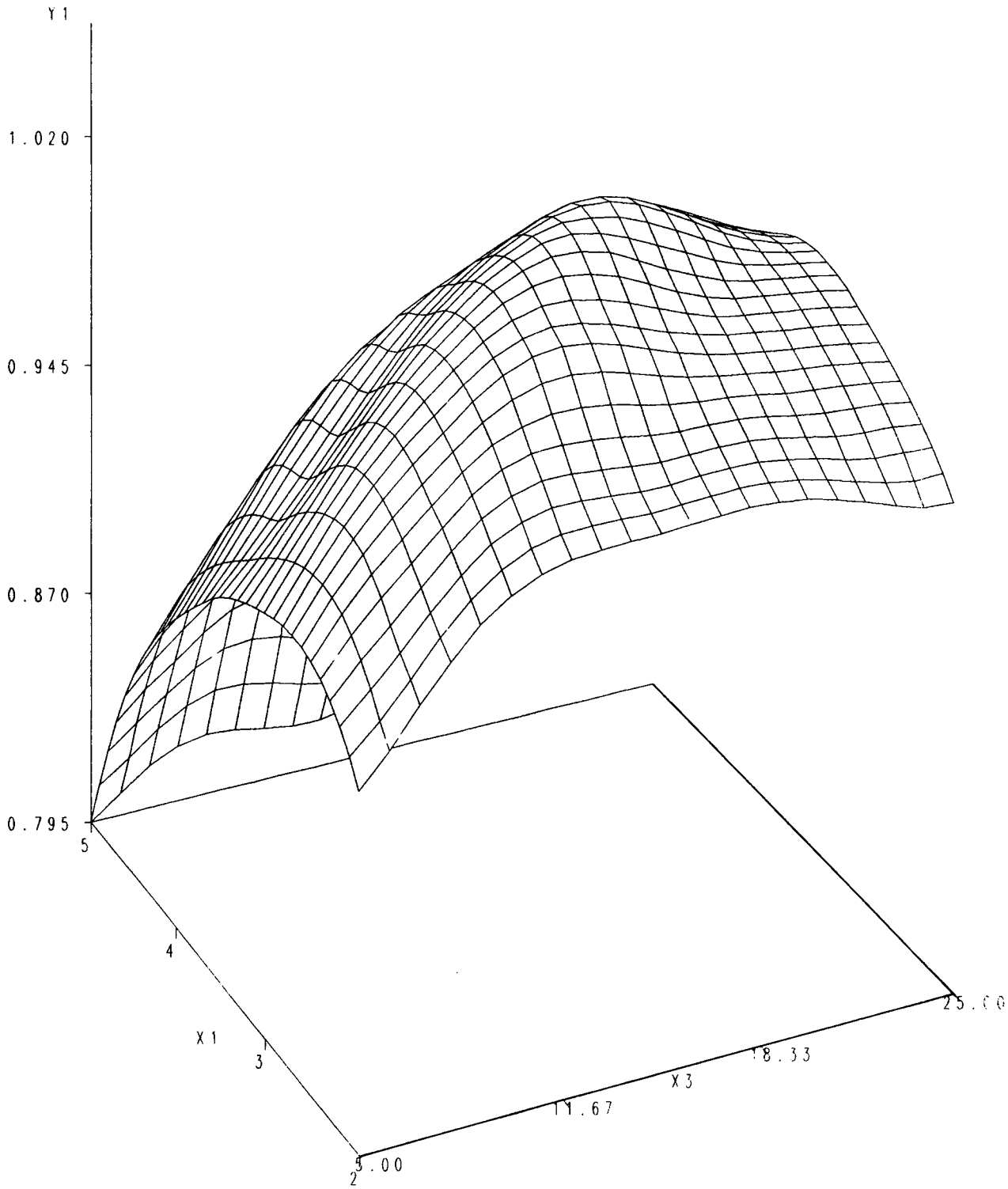

Fig. 5. Response surface for P_1 of amlodipine enantiomers: temperature 17.20°C; pH (X_1) vs. α-CD (X_3, m*M*).

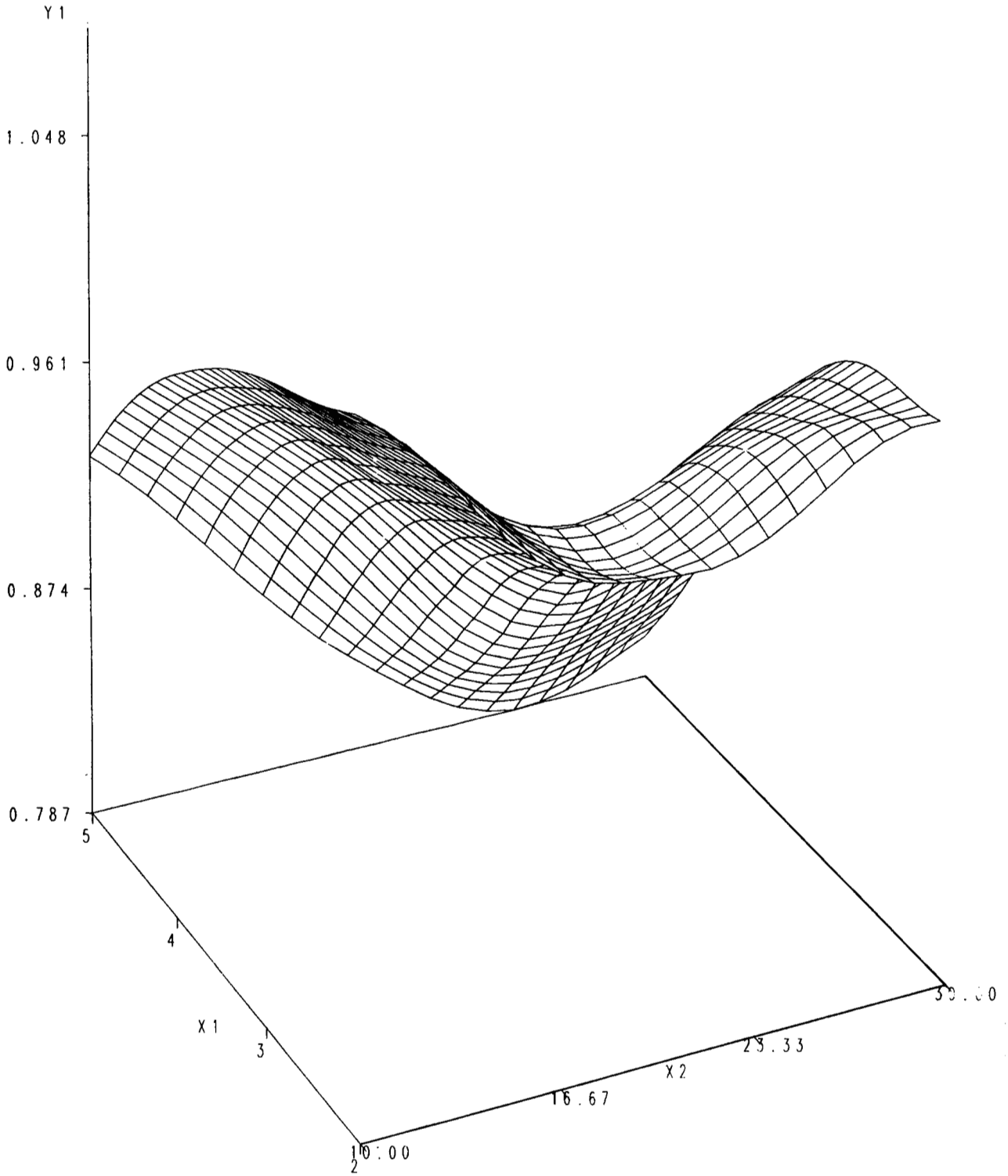

Fig. 6. Response surface for P_1 of amlodipine enantiomers: α-CD 18.2 m*M*; pH (X_1) vs temperature (X_2, °C)

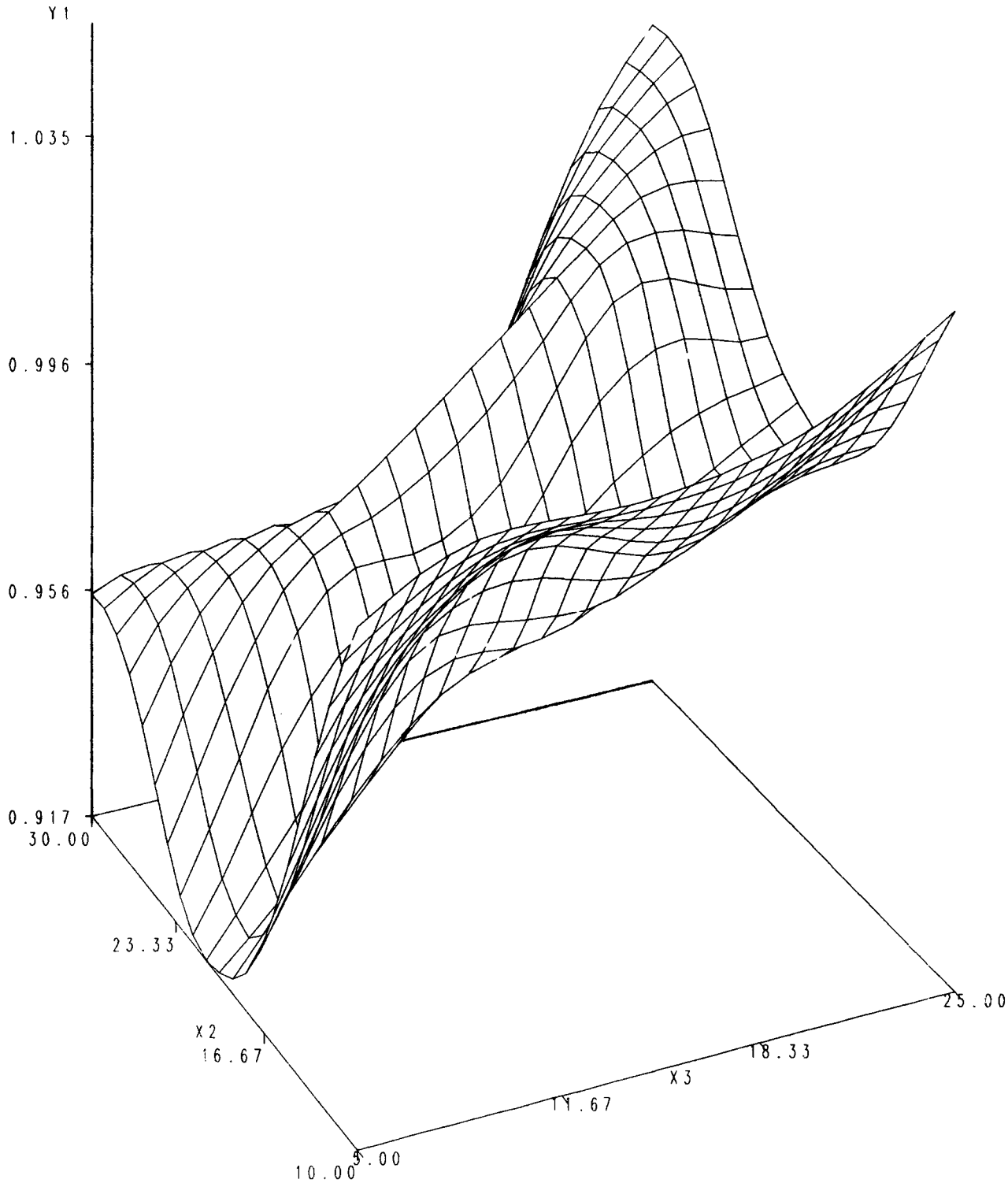

Fig. 7. Response surface for P_i of amlodipine enantiomers: pH 3.16; temperature (X_2, °C) versus α-CD (X_3, m*M*).

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