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Validation of an extended release tablet dissolution testing system using design and multivariate analysis

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Abstract

Felodipine is an antihypertensive substance acting as a calcium antagonist. The substance is provided as an extended release (ER) formulation obtained by a slowly eroding tablet. For the quality control of this tablet an automated dissolution testing system has been developed. The purpose of the present study was to validate the performance of the system. A chemometric approach using fractional factorial and D-optimal designs was applied. The obtained data were evaluated by projection methods, providing a validation of seven independent experimental variables and their interactions, seen as their influence on the in vitro dissolution rate of felodipine from the ER tablet in a predefined dissolution system. The benefits of such chemometric methodology were obvious, exemplified by the disclosure of synergism and quadratic relationships between descriptor variables and the responses (i.e., amount of felodipine released after a given time of dissolution). Changes in the temperature and the stirring speed had the most profound effects on the drug-release rate in the present system.

Key words: Chemometrics; Extended release; Dissolution testing; Experimental design; Felodipine; Multivariate calibration; Validation

1. Introduction

Extended release (ER) tablets with a controlled substance release (Lee and Good, 1987) disclose several clinical advantages, such as improved convenience for the patient, and less frequent dosing might lead to better compliance. In addition, a lower relative fluctuation in plasma concentrations is inherent in any ER formulation, which is essential for drugs having a narrow therapeutic window. Therefore, ER formulations have in recent times more frequently become the formulation of choice in the pharmaceutical industry. However, this generates increased demands on tablet control (Munson, 1986) as consistent quality is required for the safe use of ER tablets. In dissolution testing an in vitro release profile of the active substance in a tablet is correlated to in vivo measurements (Miller, 1977; Wingstrand et al., 1990). Such assessments are essential for intra- and inter-batch consistency control and pro-

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vide vital feedback for product improvements. As release profiles can last up to 24 h, dissolution testing performed manually is time-consuming and tedious. Thus, it is of great interest to reveal automation steps in the process. Automation may include several steps such as instrumental and communication automation, however, information handling is also important to consider (McDowall, 1992). The present automation includes: (1) a robot for instrumental operations; (2) communication between the data producing instrument (i.e., the spectrophotometer) to the information generating computer; (3) data to information conversion by multivariate calibration (Martens and Naes, 1989); and (4) information management by transport of assay results to software or printouts.

The present system has been applied to assessments of felodipine (Josefson et al., 1988), which is an antihypertensive calcium antagonist showing vascular selectivity (Ljung, 1985). Felodipine is marketed as an ER tablet formulation, which is obtained using gel forming cellulose excipients (Wingstrand et al., 1990). In contact with water solutions a hydrated gel layer is formed and substance release is provided by tablet erosion. Felodipine is a hydrophobic substance (Ljung, 1985) and requires detergents to dissolve in water solutions. The preferred method of analysis was to measure felodipine concentrations directly in the dissolution vessel, without previous sample withdrawal and filtration where the substance might adsorb to lipophilic parts of the equipment. Such measurements are possible in turbid solutions if partial least squares (PLS) multiwavelength calibrations are used (Josefson et al., 1988; Martens and Naes, 1989).

Before routine assessments could be performed by the automated system a validation was necessary, and the aim of the present study was to validate the experimental part of the system. There are several factors that are known to influence the dissolution rate in vitro (Miller, 1977; Wingstrand et al., 1990) including pH, temperature, agitation, etc. Seven independent settings were identified in the dissolution set up (Table 1), and were investigated concurrently. This was performed using experimental designs (Box et al., 1978; Johnson and Nachtsheim, 1983) and multivariate analysis (Höskuldsson, 1988), which provides a rationale for validation of several variables simultaneously, without neglecting overall supervision.

2. Materials and methods

2.1. Apparatus and software

The robotics system comprised an arm with five controlled joints. The system also controlled temperature and stirring speed in the dissolution vessels by software in a personal computer (PC). All parts of the experimental equipment were commercially available. The robotics system, software and dissolution testing equipment were purchased from Anatech AB, Mölndal, Sweden. The spectrophotometric scanner equipment, a single-beam instrument connected to an optic fiber ending with an adjustable six-to-one channel probe, was obtained from Guided Wave Inc., CA, U.S.A.

The software was RS1-discover (BBN, Boston, U.S.A.) used for the creation of experimental designs and Unscrambler (CAMO A/S, Trondheim, Norway) which was used for development of multivariate calibrations and statistical evaluations. The in-house developed MS-Windows predictor DeTerminator 1.0 (available from the authors upon request), which reads Unscrambler calibration files, was used for enhanced prediction throughput. Response surface modeling and evaluation of the method robustness were performed with the SIMCA 4.4 software (Umetri AB, Umeå, Sweden)

The components of the liquid chromatography (LC) system were an LKB 2150 pump (Stockholm, Sweden), a Spheri-5 C18 column (Brownlee CA, U.S.A.) and a Waters model 481 spectrophotometer set at 362 nm.

2.2. Reagents

Felodipine (4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylic ethyl methyl ester) standards and tablets were produced by Astra Hässle AB. *N*-Cetyl-*N,N,N*-tri-

methylammonium bromide (CTAB) was obtained from Merck (Darmstadt Germany). Acetonitrile HPLC grade was from Rathburn (U.K.). The dissolution media were made using phosphate buffers and CTAB according to Table 1. All salts used for phosphate buffer solutions were of p.a. grade.

2.3. Robot set up and assay procedure

The robotics arm was placed on a wall-mounted rail where it could move both horizontally (500 cm) and vertically (100 cm). In addition, joints for arm and hand rotation were included. The hand comprised a grip function for basket movements, a stainless-steel sampler and a mobile spectrophotometer probe. This robotics hand could reach into 24 dissolution vessels in four different termostatted water-baths. Each vessel contained stirring propellers, mounted and shaped as specified by the US Pharmacopoeia (USP). All settings were governed from a PC connected to the system.

The tablets were positioned in baskets (Josef-

Table 1
Worksheet for validation experiment

Expt no.	Stirring speed (rpm)	pH	[CTAB] (%)	Temperature (°C)	Basket position (cm)	Ionic strength of buffer (M)	Buffer volume (ml)
1	110.0	6.0	0.50	34.0	2.0	0.05	510.0
2	110.0	6.0	0.30	34.0	2.0	0.15	490.0
3	110.0	7.0	0.50	34.0	0.0	0.15	490.0
4	110.0	7.0	0.30	34.0	0.0	0.05	510.0
5	110.0	7.0	0.50	40.0	2.0	0.15	510.0
6	110.0	6.0	0.50	40.0	0.0	0.05	490.0
7	110.0	7.0	0.30	40.0	2.0	0.05	490.0
8	110.0	6.0	0.30	40.0	0.0	0.15	510.0
9	100.0	6.5	0.40	37.0	1.0	0.10	500.0
10	100.0	6.5	0.40	37.0	1.0	0.10	500.0
11	100.0	6.5	0.40	37.0	1.0	0.10	500.0
12	100.0	6.5	0.40	37.0	1.0	0.10	500.0
13	100.0	6.5	0.40	37.0	1.0	0.10	500.0
14	90.0	7.0	0.50	34.0	2.0	0.05	490.0
15	90.0	6.0	0.50	34.0	0.0	0.15	510.0
16	90.0	6.0	0.30	34.0	0.0	0.05	490.0
17	90.0	7.0	0.30	34.0	2.0	0.15	510.0
18	90.0	6.0	0.30	40.0	2.0	0.05	510.0
19	90.0	7.0	0.30	40.0	0.0	0.15	490.0
20	90.0	7.0	0.50	40.0	0.0	0.05	510.0
21	90.0	6.0	0.50	40.0	2.0	0.15	490.0
A1	110.0	7.0	0.50	40.0	0.0	0.15	490.0
A2	90.0	6.0	0.30	40.0	0.0	0.05	490.0
A3	110.0	7.0	0.50	40.0	0.0	0.05	510.0
A4	90.0	6.0	0.50	34.0	0.0	0.05	510.0
A5	90.0	7.0	0.50	34.0	2.0	0.15	490.0
A6	100.0	6.5	0.40	34.0	1.0	0.10	500.0
A7	100.0	6.5	0.40	40.0	1.0	0.10	500.0
A8	100.0	6.5	0.40	37.0	1.0	0.10	490.0
A9	100.0	6.5	0.40	37.0	1.0	0.10	510.0

The worksheet was created as a fractional factorial design (Box et al., 1978) with a centerpoint experiment (replicated four times, i.e., Expts 9–13). The experiments were not performed in random order as stirring speed and temperature were constrained by the equipment. A1–A9 indicate additional experiments that were performed after the original design in order to resolve large, confounded cross terms of stirring speed with buffer volume and temperature with ionic strength (A1–A5), and the square terms of temperature (A6 and A7) and buffer volume (A8 and A9), respectively.

son et al., 1988) and inserted into the dissolution vessels, into a fixed position, by the robot. The vessel normally contained 500.0 ml dissolution buffer with 0.40% (w/v) CTAB in a phosphate buffer of pH 6.5 and ionic strength of 0.10 M. The dissolution medium was thermostated to 37°C and agitation was effected by paddle rotating at 100 rpm (Wingstrand et al., 1990). Thereafter, at preset times and intervals, the robot inserted the sampling device and/or the spectrophotometer probe. The absorbance was measured for 68 wavelengths ranging from 326 to 460 nm which covers the absorption maximum (362 nm) for felodipine. The data were stored in a second PC for subsequent data processing (i.e., multivariate calibration) or immediate computing of felodipine concentrations.

2.4. Multivariate calibration and statistics for system validation

The experimental design for the multivariate calibration was obtained by dissolution of tablets with various amounts of felodipine (2.5, 5.0 and 10.0 mg felodipine, respectively) based on a similar composition. The spectral data were collected at three different times (after 1, 4 and 7 h, respectively) of each dissolution experiment. Samples were withdrawn from the vessels simultaneously (handled by glass and steel tools only to avoid hydrophobic interactions with felodipine) for immediate determination of felodipine concentration using LC. The spectral data were then correlated to the felodipine concentrations (determined by the LC analyses) by partial least squares (PLS) (Höskuldsson, 1988; Martens and Naes, 1989).

Validation of the system robustness was based on experiments performed according to a fractional factorial design of resolution IV (Box et al., 1978). The investigated variables and the experimental worksheet are outlined in Table 1. The expansion of the fractional factorial design for the resolution of certain square and interaction terms was performed using D-optimal design (Johnson and Nachtsheim, 1983) and subsequent

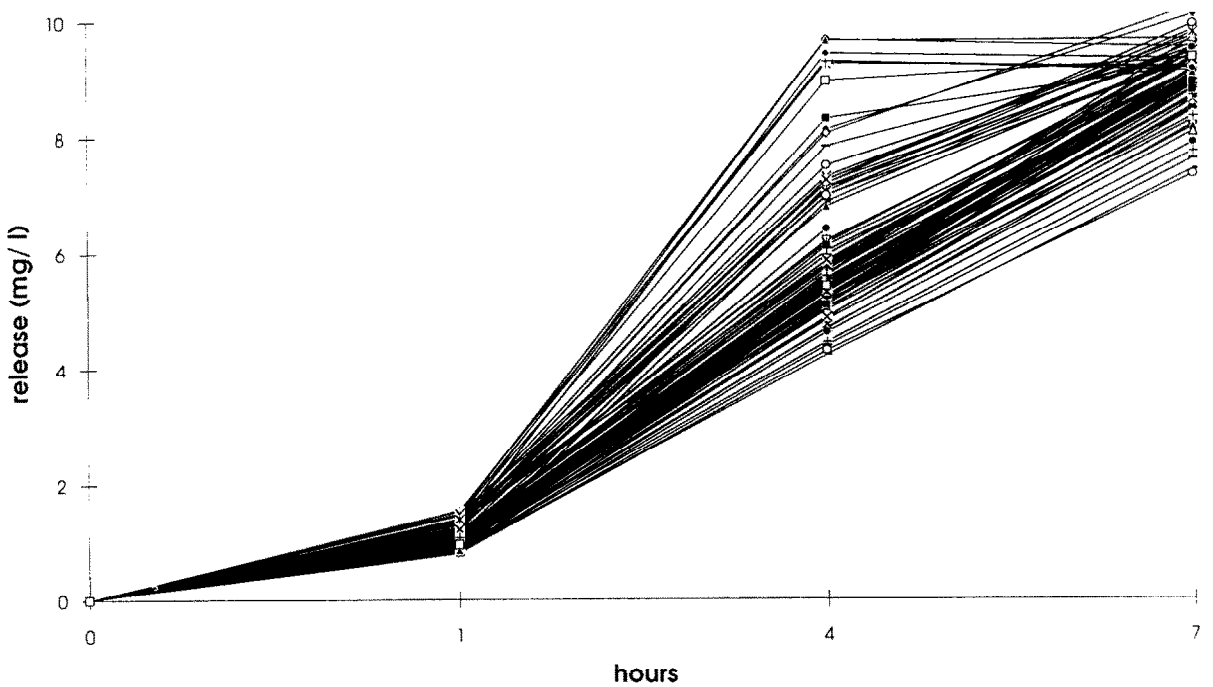

Fig. 1. Graphical representation of felodipine concentrations (mg/l, ordinate) after 1, 4 and 7 h in vitro tablet dissolution (abscissa), respectively, in experiments performed as described in Table 1.

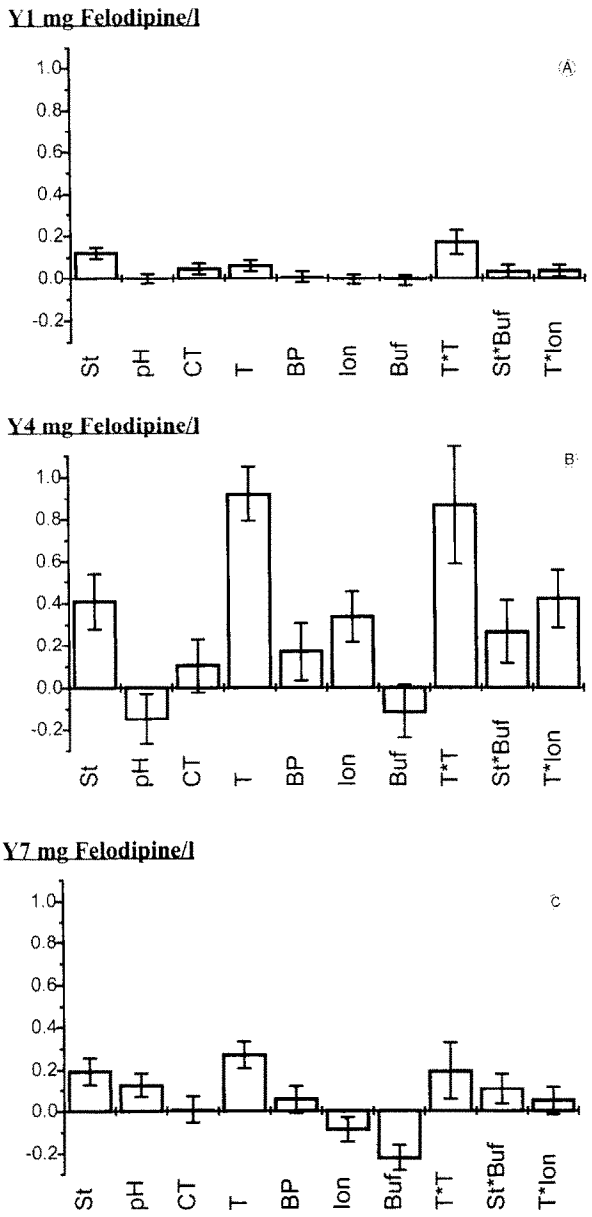

Fig. 2. (A–C) Scaled and centered PLS coefficients for felodipine tablet dissolutions after 1, 4 and 7 h (A–C, respectively). As the design matrix was close to orthogonal the coefficients were calculated assuming that they were independent. The coefficients were corrected for the weight introduced into the PLS procedure and thus the ordinate scale is comparable to Y-matrix units (i.e., mg felodipine/l dissolution medium). The bars correspond to the change in response (ordinate scale) estimated for a relative increase of the indicated descriptor variable, i.e., from median to high level in the original factorial design (Table 1). The bars indicate confidence intervals (see section 2). St, stirring speed; CT, CTAB; T, temperature; Bp, basket position; Ion, ionic strength; Buf, buffer volume.

evaluation was performed by response surface modeling using PLS (Höskuldsson, 1988). The modeled responses were the felodipine concentrations in the vessels after 1, 4 and 7 h of tablet dissolution. Cross-validation (Wold, 1978) was used for the evaluation of the PLS models. The models were further investigated by scores and loading plots (Kettaneh-Wold, 1992). Some dissolutions in the score-plot appeared as potential outliers (i.e., experiments 5, 8 and 15) and were re-run, but the original results were confirmed (data not shown). Confidence intervals were calculated as follows: $b \cdot [t_{\alpha/2} \times \mathrm{SE}(b)]$; where SE is calculated according to the proceedure in multiple regression (Box et al., 1978).

3. Results

3.1. PLS modeling

A PLS model was constructed using the experimental settings depicted in Table 1 as X and the respective felodipine concentrations, after 1, 4 and 7 h of dissolution (Fig. 1), as Y. The model was expanded by inclusion of the square term of temperature and the cross terms of stirring vs buffer volume and temperature vs ionic strength. The expansion was supported by the additional experiments A1–A9 (Table 1). The model comprised three significant factors. The fourth dimension had a relatively high prediction error sum of square over sum of squares (PRESS/SS) but it explained important variation in the cross terms, and was therefore included. Thus, an acceptable model with an explained variance of 75–90% for the three responses was obtained.

The temperature was the variable which had the greatest influence on the model, which was revealed by a relatively large linear term and, in addition to that a large square term combined by its synergism with the ionic strength (Fig. 2A–C). The stirring speed revealed the most important linear term for the 1 h dissolutions and also depicted interaction with the buffer volume (C17) (Fig. 2A–C). The buffer volume variable was rather the combination of the effect of a small change in volume on the dissolution rate, and that of dilution on the felodipine concentration. Calculations of the separate volume effects indi-

cated that dilution represented the measured value and the change in tablet dissolution per se was virtually zero, and the value of the combined volume coefficients (Fig. 2A–C) was close to the theoretical value for the dilution (i.e., −0.02 for a 2% dilution, from 500 to 510 ml, at the end of tablet dissolution). For all tablet dissolution times, pH, CTAB and the basket position appeared to have relatively small effects. The average confidence interval (RMS*) for the 4 h dissolutions was 2–3-times larger than the experimental noise (pure error, i.e., SE of replicates, Fig. 2B), indicating some minor additional deterministic variance in the system, that could not be explained by the included experimental variables.

3.2. Robustness of the experimental system

A maximum expected experimental error was estimated for each descriptor variable. The limits used were the USP specifications for the method except for the temperature where a deviation of less than 0.2°C was both possible and warranted. The CTAB concentration and basket position were not specified by USP and for those the estimated largest possible experimental errors were used. A full factorial design, extended with facial points (i.e., a central composite face-centered design) was created using the above limits as upper and lower settings, respectively (the actual variable settings are indicated in the legend to Fig. 3). The PLS model was used to predict felodipine concentrations at 1, 4 and 7 h tablet dissolutions for all the experimental conditions given by the design. Thus, an estimate of the worst experimental variation that might occur was simulated (Fig. 3). All dissolution simulations indicated release profiles within the specification for the method.

The independent variables containing the major influence on the dissolution rate were plotted as response surfaces. The stirring speed vs. the

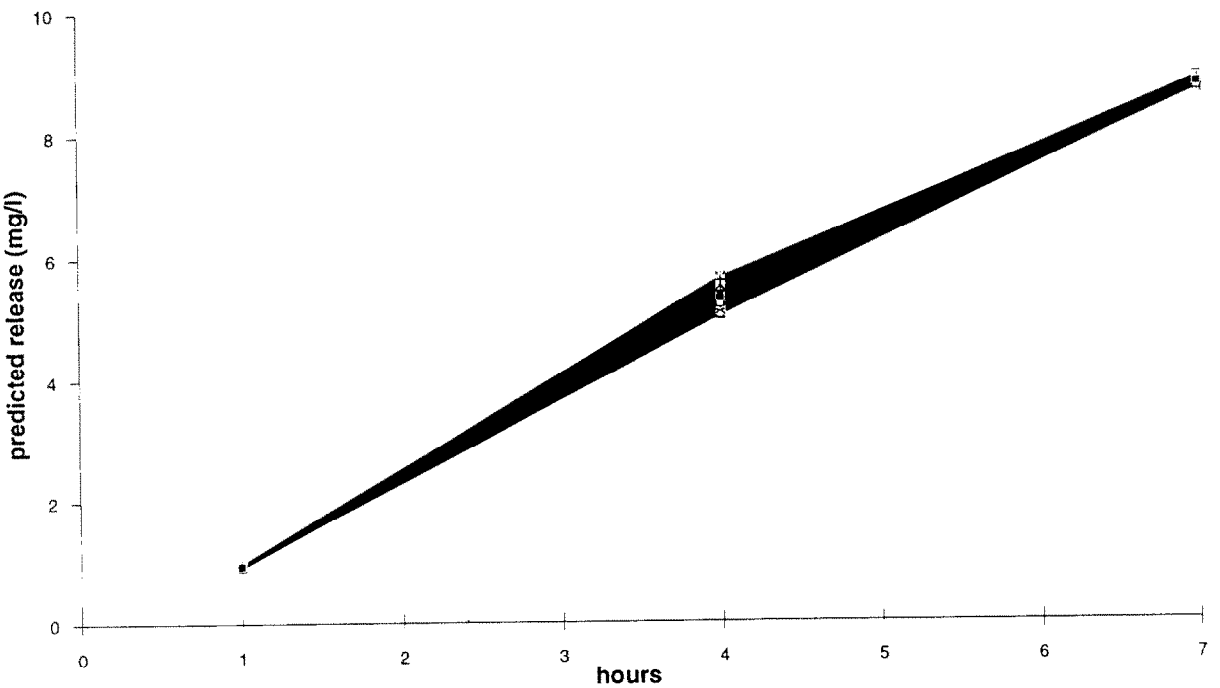

Fig. 3. Predictions of felodipine concentrations (mg/l) after 1, 4 and 7 h of tablet dissolution for simulated experiments. The simulated settings were combined as a factorial design. All possible combinations within the experimental limits were simulated. Limits as follows: stirring speed, 99–101 rpm; pH, 6.45–6.55; CTAB, 0.39–0.41%; temperature, 36.5–37.5°C; basket position, 8–12 mm above stirring paddle; buffer volume, 499.5–500.5 ml.

buffer volume response surface displayed a certain twist (Fig. 4). The temperature appeared to account for the major part of the curvature, depicted as a parabolic shape of the response surface (Fig. 5), and such information must be taken into account when the experimental conditions for routine tablet dissolution assessments are chosen.

4. Discussion

Stirring speed appeared important for the initial part of the dissolution, while the surface coat of the tablets dissolved and wetting of the tablet gel took place. However, the temperature soon became the major factor. The explanation for the parabolic shape of the dissolution of felodipine as a function of temperature (Fig. 5) is assumed to be the phase separation within the tablet gel, with increasing temperature (Carlsson et al., 1989). Thus, the intended erosion limitation of the substance dissolution, predominating during the lower temperature interval (i.e., 34–38°C, Fig. 5), was altered towards gel-dissolution control with increasing temperature (i.e., at 38–40°C, Fig. 5).

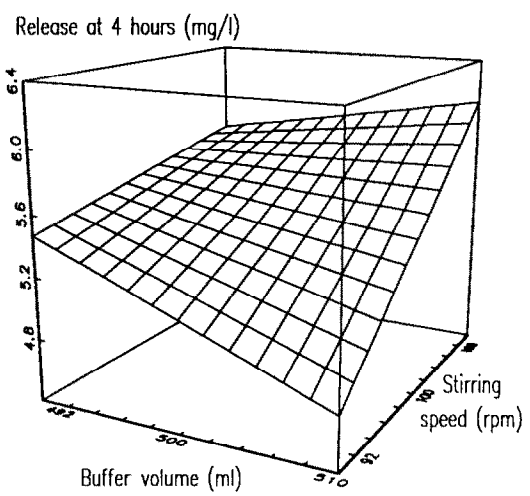

Fig. 4. Response surface of felodipine concentration (z-axis) after 4 h of dissolution of a 5 mg felodipine ER tablet. Buffer volume (x-axis) varied from 490 to 510 ml and stirring speed (y-axis) varied from 90 to 110 rpm. All other experimental variables were set constant; pH 6.5, CTAB 0.40%, temperature 37.0°C, ionic strength 0.12 M.

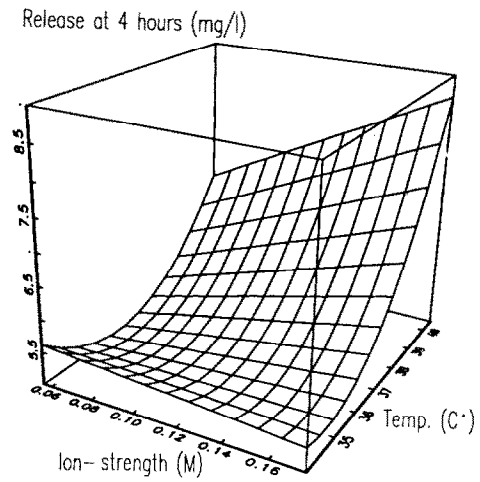

Fig. 5. Response surface of felodipine concentration (z-axis) after 4 h of dissolution of a 5 mg felodipine ER tablet. Ionic strength (x-axis) was varied between 0.05 and 0.15 M and temperature (y-axis) was varied between 34 and 40°C. All other experimental variables were set constant; stirring speed 100.0 rpm, pH 6.5, CTAB 0.40%, basket position 1.0 cm above stirrer, buffer volume 500.0 ml.

The dissolution response variable showing the largest noise was the amount of substance released after 4 h dissolution. The alteration in this variable was estimated to vary between 50 and 56% of substance release, assuming that the experimental error, as suggested in the legend to Fig. 3, was relevant. Such simulations are suitable as a background for decisions on whether or not the variability of a method is acceptable. The present example indicated acceptable noise for the application of felodipine ER tablet in vitro dissolution assessments. However, the coefficients and response surfaces depicted in Fig. 2A–C, 4 and 5, respectively, emphasize that particular attention to the temperature control might be advantageous for optimal assessments. Also, the stirring speed, buffer volume and ionic strength have an influence on crucial parts of the dissolution.

Therefore, the present validation of a tablet dissolution testing system indicated safe use for eventual routine assessments and the fully auto-

mated system was advantageous in several aspects. The timing of the individual assessments were invariably exact, as they were triggered by a computer and needed no survey. In addition, the automation anticipated a substantial time gain for the experimenter. The use of a multivariate calibration made removals of aliquots during dissolution superfluous, leading to less manipulations with the dissolution media during the entire dissolution, and thus computations could be made without extrapolations.

The use of chemometric methodology for validation purposes accomplished valuable benefits, beginning with the constraining of the experimental effort. An acceptable validation of seven experimental variables in 30 experiments is highly economical. In spite of that, the major gain from the multivariate methods was the demonstration of the complex dependence of the dissolution rate as functions of the descriptor variables, including quadratic and synergistic effects (Fig. 4 and 5). Such an overview is impossible when univariate validation methods are applied. Thus, a relatively small quantity of experiments provided high-quality information, which was essential for appropriate interpretations.

5. References

Box, G.E.P., Hunter, W.G. and Hunter, J.S., *Statistics for Experimenters*, Wiley, New York, 1978, pp. 291–432.

Carlsson, A., Karlström, G. and Lindman, B., Characterization of the interaction between a nonionic polymer and a cationic surfactant by the Fourier transform NMR self-diffusion technique. *J. Phys. Chem.*, 93 (1989) 3673–3677.

Höskuldsson, A., PLS regression methods. *J. Chemometrics*, 2 (1988) 211–228.

Johnson, M.H. and Nachtsheim, C.J., Some guidelines for constructing exact D-optimal designs on convex design spaces. *Technometrics*, 25 (1983) 271–277.

Josefson, M., Johansson, E. and Torstensson, A., Optical fiber spectrometry in turbid solutions by multivatiate calibration applied to tablet dissolution testing. *Anal. Chem.*, 60 (1988) 2666–2671.

Kettaneh-Wold, N., Analysis of mixture data with partial least squares. *Chemomet. Intell. Lab. Syst.*, 14 (1992) 57–69.

Lee, P. and Good, W.R., *Controlled-Release Technology: Pharmaceutical Applications; ACS Symposium Series 348*, 1987, pp. 1–13.

Ljung, B., Vascular selectivity of felodipine. *Drugs*, 29 (1985) 46–58.

Martens, H. and Naes, T., *Multivariate Calibration*, Wiley, New York, 1989, pp. 73–265.

McDowall, R.D., Strategic approaches to laboratory automation. *Chemomet. Intell. Lab. Syst.*, 17 (1992) 265–282.

Miller, J.A., Apparatus and instrumentation for tablet dissolution testing. *Pharm. Technol.*, 1 (1977) 19–42.

Munson, J.W., Analytical techniques in the dissolution testing and bioavailability studies. *J. Parmacol. Biomed. Anal.*, 4 (1986) 717–724.

Wingstrand, K., Abrahamsson, B. and Edgar, B., Bioavailability from felodipine extended-release tablets with different dissolution properties. *Int. J. Pharm.*, 60 (1990) 151–156.

Wold, S., Cross-validatory estimation of the number of components in factor and in principal components models. *Technometrics*, 20 (1978) 397–405.