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RESEARCH PAPER

Critical Dissolution Tests of Oral Systems Based on Statistically Designed Experiments. II. In Vitro Optimization of Screened Variables on ER-Coated Spheres for the Establishment of an In Vitro/In Vivo Correlation

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ABSTRACT

The study was designed to optimize the effects of the screened in vitro dissolution variables agitation, temperature, osmolality, and polarity on the release of the neuroleptic compound remoxipride from extended release coated spheres. The variables were varied independently by means of a fractional factorial design. The in vitro tests were performed with the Basket method (USP). The polarity and the osmolality of the medium had significant effects on the dissolution rate of remoxipride. A statistical model was calculated based on the obtained dissolution in vitro. The model was then used to predict the in vitro conditions that most closely correlated with the dissolution rate of remoxipride in vivo, after administration of the formulation to 16 volunteers. The predicted in vitro conditions were experimentally verified, and an excellent association with the in vivo behavior of the formulation was found. Validation of the optimal in vitro conditions was performed on another batch of the formulation. The dissolution profile obtained showed a significant association with the corresponding dissolution profile in vivo. The use of statistically designed experiments in the development of critical dissolution tests for the establishment of in vitro/in vivo correlations seems to be a useful working approach, and supports further application to other oral solid systems.

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INTRODUCTION

Knowledge of how a solid system releases its drug content in the gastrointestinal tract is important and has to be built up from the early phase of product development. A simple aid for this might be in vitro tests which reflect the in vivo behavior of the formulation. However, it is difficult and time consuming to find associations between release in vitro and in vivo, and a number of guidelines and strategies for developing physiologically relevant dissolution tests have been proposed (1–4).

One way to identify factors that are critical for drug dissolution is to screen a variety of possible dissolution variables and go on to optimize those that are identified as having some effect. This statistically designed experimental procedure is known as chemometrics. This working approach is commonly applied in the optimization of compositions and manufacturing methods as well as in the validation of processes (5–11).

In a previous study we identified some variables that were critical for the in vitro dissolution of remoxipride from extended release (ER)-coated spheres (12). By using a fractional factorial screening design we found that these variables were the agitation, temperature, and polarity of the dissolution medium. The following optimization is best performed by means of a full factorial design or a response surface methodology (RSM). However, we have chosen to continue with a screening design in order to minimize the number of experiments. Such a model might not explain a sufficiently large fraction of the data, but in that case it is possible to extend the design and include additional experiments.

The objective of this study was thus to optimize the variables previously found to affect the in vitro dissolution of the model neuroleptic compound remoxipride from ER-coated spheres and thereby improve the statistical model which predicts the in vitro conditions that are most closely correlated with the in vitro dissolution behavior of the formulation.

MATERIALS AND METHODS

Materials

Remoxipride is a base with a pK_a of 8.9 and a solubility of 0.30 g/ml in water and 0.40 g/ml in ethanol (22°C). The partition coefficient of remoxipride is 2.1 (log *P*, octanol/buffer, pH 7.5) (13). The ethylcellulose ER-coated spheres of remoxipride were manufactured as described previously (12).

Milli-Q water was used in the dissolution medium. Other substances used were of analytical grade.

In Vitro Dissolution Test

The in vitro dissolution tests were performed using the Apparatus I method (basket, USP). The tests were carried out in a randomized order according to Table 1. Six parallel vessels were used in each test. Samples were drawn at 30, 60, 120, 360, and 720 min. The amount of dissolved remoxipride was detected spectrophotometrically at 286 nm.

Table 1

Investigated Variables and Their Levels

Exp. No.	Run Order	Agitation (rpm)	Temperature (°C)	Osmolality (mmol/kg)	Polarity (%)
1	5	25	37	300	0
2	9	75	37	300	8
3	6	25	38	300	8
4	10	75	38	300	0
5	8	25	37	600	8
6	4	75	37	600	0
7	3	25	38	600	0
8	7	75	38	600	8
9	1	50	37.5	450	4
10	2	50	37.5	450	4
11	11	50	37.5	450	4
12	12	50	37.5	450	4

Dissolution Media

The media consisted of 900 ml phosphate buffer of pH 6.8 and the ionic strength was 0.1 (USP), with additives according to Table 1. All components were mixed by means of a magnetic stirrer. Deaeration of the media was performed with helium for 20 min.

Osmolality

The osmolality was adjusted by adding glucose. A vapor pressure osmometer (5520 Vapor pressure osmometer, Wescor, Logan, UT) was used in the measurements which were carried out at 20°C, as described previously (2).

Polarity

The polarity of the dissolution medium was adjusted by adding ethanol 99.5%. A Q-meter (Q-meter type M803A, no. 1833, voltmeter de Crete, type AC 103A, no. 332, France) was used to check the dielectric constants of the fluids. This apparatus does not allow measurements of the complete buffer solutions, but the measurements were instead carried out on pure water–ethanol mixtures (4 and 8% ethanol) at 37°C.

Statistical Experimental Design

The creation of the statistical experimental design and the evaluation of the in vitro tests were performed using the computer program Modde 3.0 (Umetri AB, Umeå, Sweden). Although this was an optimization study, a screening design was initially chosen with the ability to extend the design and include additional experiments. A resolution IV design was used, which shows unconfounded main effects and confounded two-factor interactions (14). The fractional factorial design used was a 2^{4-1} linear screening design, leading to eight runs. Four replicates were added to the center of the design in order to investigate the reproducibility. The variables investigated were agitation, temperature, osmolality, and polarity of the medium. The variables and their levels were selected on the basis of our earlier screening study (12). The experimental worksheet is outlined in Table 1. The responses were expressed as percent dissolved remoxipride at specified time-points from 30 to 720 min. A partial least squares (PLS) model was constructed for the evaluation of the in vitro tests, based on the experimental settings in Table 1 as X (variables) and the percent dissolved remoxipride at 30–720 min as Y (responses) (15). The main advantage of using this method of analysis is that it fits all the responses simultaneously, representing the variation of the responses to the variation of the variables. The more commonly used multiple linear regression (MLR) separately fits one response at a time, and hence the responses are assumed to be independent of each other. Since dissolution data at a certain time-point depend on the value obtained at a previous time-point, PLS is preferable to MLR.

In Vivo Studies

The two in vivo studies performed are described elsewhere (12,16). In the first study (12), the mean in vivo release of remoxipride from the ER-coated spheres was calculated by numerical deconvolution of the individual plasma concentration versus time curves (17) after administration of the formulation to 16 healthy volunteers. A plain aqueous solution of remoxipride was used as a reference. The time module used in the calculations of percent released remoxipride in vivo was 60 min. In the second study, comprising another 16 healthy volunteers, the mean percent absorbed remoxipride was calculated by means of the Wagner-Nelson method (18) since the reference product was another ER formulation of remoxipride and not a plain solution. The calculations were performed by a program developed in the RS/1 Command language (BBN Software Products Corp.).

RESULTS AND DISCUSSION

In Vivo Dissolution Rate

The in vivo dissolution of remoxipride from the ER-coated spheres (I) is included in Fig. 1. Within 1.5, 6, and 12 hr almost 15, 45, and 65% of the dose is dissolved, respectively.

Evaluation of In Vitro Dissolution Rate

The 12 in vitro dissolution profiles of 300 mg remoxipride spheres are presented in Fig. 1. An average of 25, 50, and 70% of the dose is dissolved within 1.5, 6, and 12 hr, respectively.

PLS Modeling

All responses were found to be normally distributed and a transformation was therefore not needed. A relatively good model was obtained without any significant model error ($p \geq 0.05$). The model explained a large

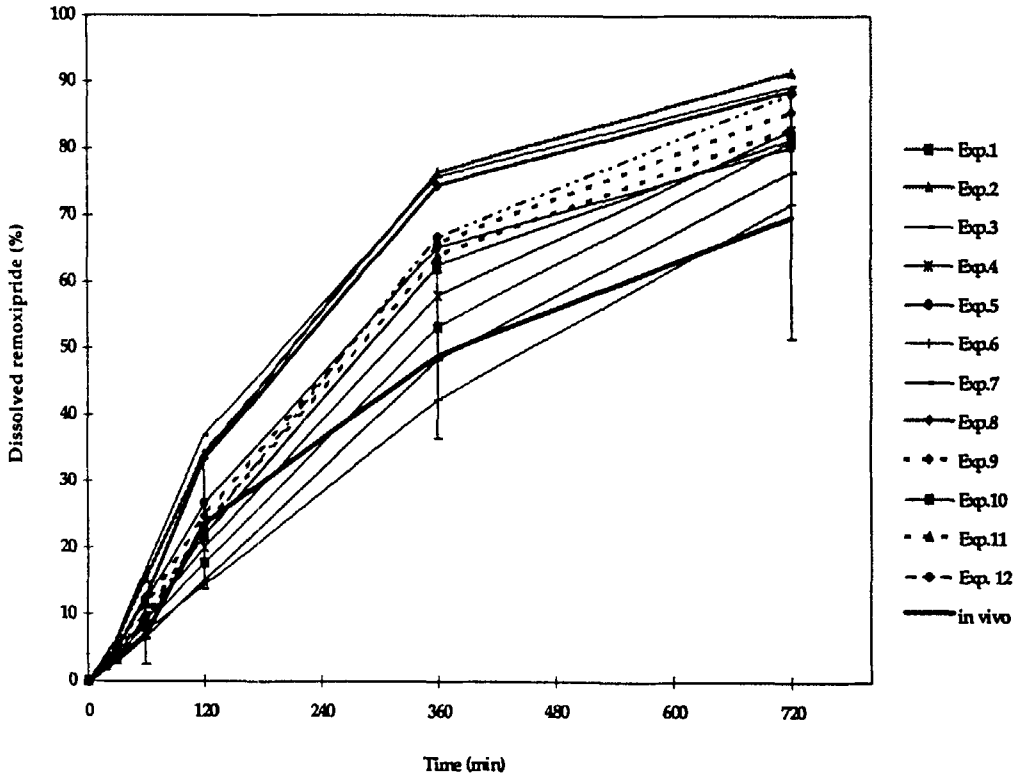

Figure 1. The obtained dissolution profiles in vitro and in vivo. The broken lines represent the replicates (experiments 9–12). Error bars denoted as standard deviation of the in vivo curve.

fraction of the data, and R^2 was found to be between 0.76 and 0.98. No additional experiments were therefore needed to investigate possible interaction terms. The predictive ability, described by the Q^2 value, was between 0.55 and 0.78. Figure 2 shows the relationship between the observed and the predicted percent dissolved remoxipride at 120 min, based on all 12 cases. The chosen time-point is highly representative of all five time responses.

Reproducibility

The variation among the four replicates can be seen in Figs. 1 and 2, experiments number 9–12. Their maximum variance was 6.9 at 12 hr, and the maximum standard deviation was 2.6%.

Effects of the Variables Investigated

Agitation and temperature did not have any significant effect on the dissolution rate of remoxipride, which is in contrast to the results of our previous screening study. This is most probably because our experimental domain has been reduced considerably. The variables found to have significant effects in the present narrowed experimental field are polarity and osmolality (see Fig. 3).

An increased polarity affects the amount dissolved positively, the maximum effect being 23.2 ± 5.6% (6 hr). The effect is no doubt a result of changed properties of the ethylcellulose film surrounding the spheres when they are exposed to the solvent. The dissolution rate of remoxipride decreased by maximally 7.9 ± 5.8% (6 hr) when the osmolality was increased from 300 to 600 mmol/kg. One explanation of this is that one of the factors governing the release out of the spheres is the presence of an osmolality gradient across the film barrier. The major release mechanism of drugs through ethylcellulose membranes is generally diffusion, although osmotic pumping may also play a role. The degree of contribution partly depends on the solubility

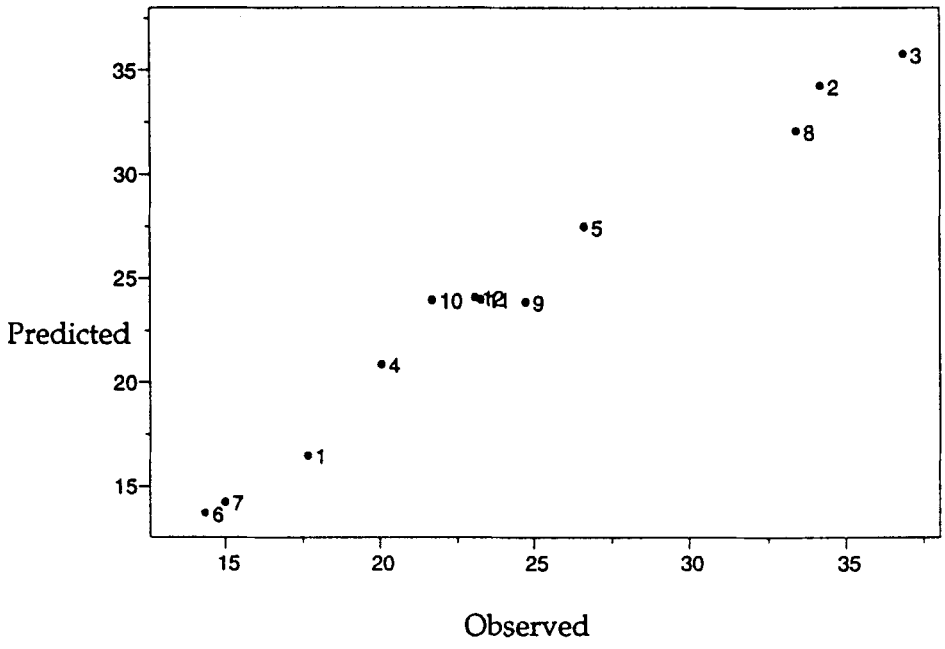

Figure 2. A plot of the observed versus the predicted values of percent dissolved remoxipride at 120 min.

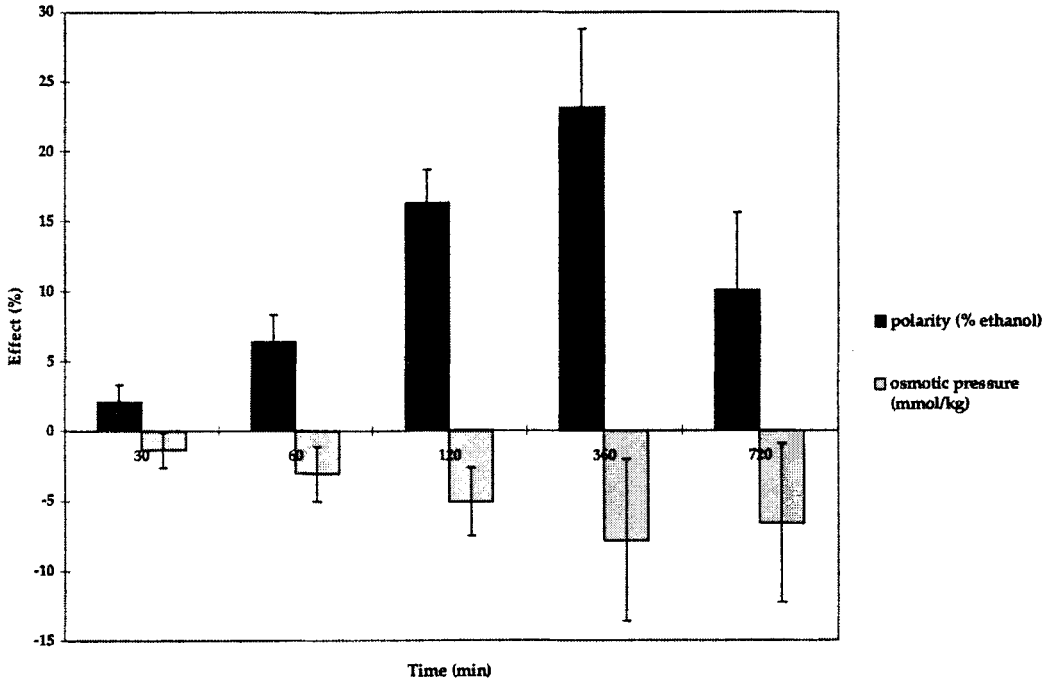

Figure 3. The effects of the significant variables from 30 to 720 min on the dissolution rate of remoxipride. The error bars indicate 95% confidence intervals.

of the drug and on the osmolality of the dissolution medium. Highly soluble drugs, such as remoxipride, create a higher drug concentration gradient across the membrane, resulting in a higher release rate of the drug. The osmolality inside the spheres was estimated to be 700 mmol/kg, based on solubility data. Hence, our dissolution medium with a high osmolality of 600 mmol/kg is subsequently expected to cause a decreased release when compared to 300 mmol/kg. The effects observed in our studies are consistent with the results of a study on other ethylcellulose ER-coated spheres, in which urea was used to increase the osmolality of the dissolution medium (19–21). However, the overall contribution of the osmolality to the release of remoxipride from the formulation investigated must be regarded as minor.

In Vitro/In Vivo Correlation

The developed statistical model was used to predict the in vitro conditions that are most highly correlated with the in vivo dissolution behavior (12). The equation used is

$$\% \text{ Dissolved} = \text{constant} + (\beta_{ag} \cdot \text{ag}) + (\beta_{te} \cdot \text{te}) + (\beta_{os} \cdot \text{os}) + (\beta_{po} \cdot \text{po}) \quad (1)$$

where β corresponds to the unscaled and regular coefficients calculated in Modde, ag is agitation, te is temperature, os is osmolality, po is polarity, and the constant represents the mean value of all the experiments. By using the Solver function in Excel 5.0c, the values of the in vitro variables were calculated so that the dissolution in vitro corresponded to the dissolution in vivo. The difference between the experimentally found in vivo curve and the predicted in vitro curve, e.g., the difference sum of squares, was calculated according to Eq. (2) and resulted in a value of 91.6 (the mean standard deviation per datapoint was 4.79%).

$$\Sigma_i = (y_{\text{in vivo}} - \hat{y}_{\text{predicted}})^2 \quad (2)$$

The best in vitro conditions predicted by the model were agitation 30 rpm, osmolality 825 mmol/kg, polarity 3.8%, and temperature 37.0°C. The pH was held constant at 6.8. The consistency between the curves in Fig. 4 suggests that the predicted in vitro conditions might be expected to serve as a substitute for the physi-

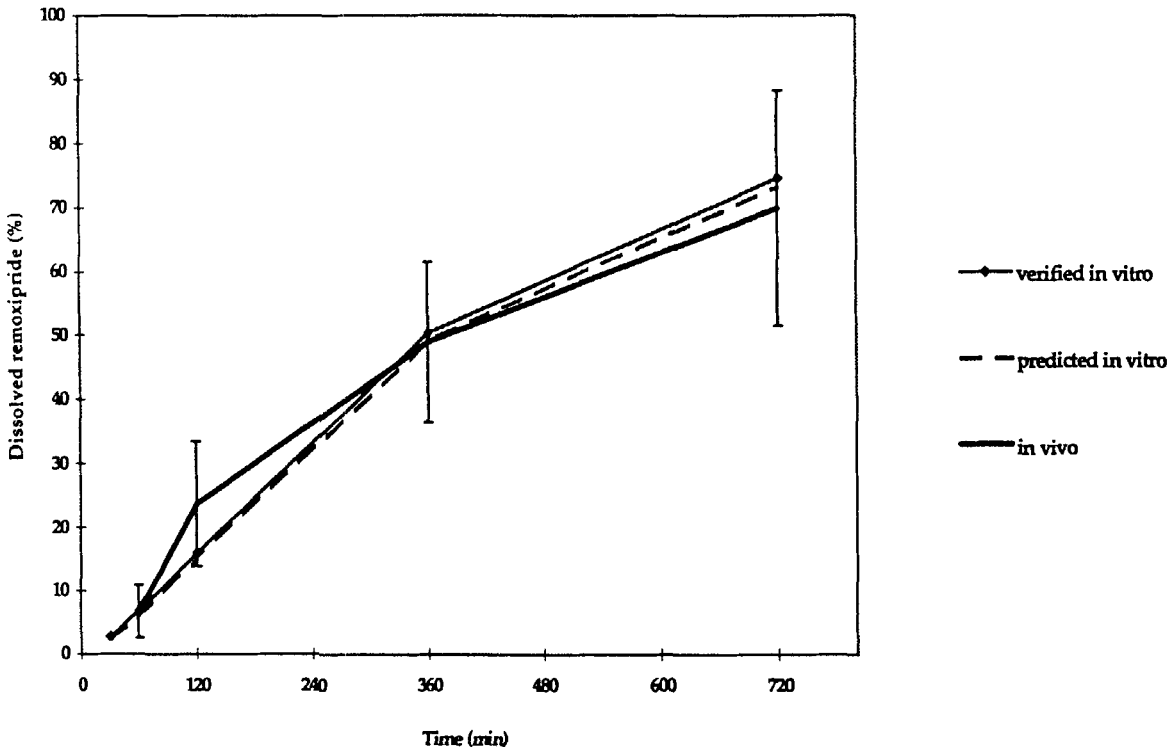

Figure 4. The association between the predicted in vitro dissolution profile from remoxipride spheres, the verifying in vitro profile, and the in vivo dissolution curve. Error bars denoted as standard deviations.

ological values of the gastrointestinal tract, except perhaps for the osmolality. The agitation intensity in the human gastrointestinal tract is found to be extremely low when the hydrodynamic flow around a tested ER dosage form is measured. Hence, it was shown that an agitation speed of 10 rpm, when using the Paddle method, best reflected the hydrodynamic flow in vivo (22). The osmolality in our model may be found to be comparatively high. However, the osmolality in the gastrointestinal tract varies depending on the type of food that is administered. After administration of, for example, a milk/doughnut meal, osmolality has been found to be 440 mmol/kg in the gastric fluids (23). Furthermore, studies in dogs have shown it to rise from 260 to 540 mmol/kg after administration of a meal consisting of a hamburger and french fries. Based on these dog studies, a medium with an osmolality of 485–535 mmol/kg has been suggested for dissolution testing (24). The polarity of our dissolution liquid measured at 37.0°C, was found to have a dielectric constant of about 76, which is very close to that of pure water, i.e., 78.

Verification of our model was performed by testing the formulation in the optimized in vitro conditions. The experimentally found dissolution in vitro was in excellent agreement with the predicted data, as demonstrated in Fig. 4. The mean difference sum of squares was 92.9 (the mean standard deviation per datapoint was 4.82%), indicating very high conformity with the predicted in vitro curve. The predicted curve and the verifying dissolution curve in vitro were the same, and were also found to be closely related to the dissolution curve in vivo. The maximum deviation between the dissolution rate in vitro and in vivo was about 8% (2 hr). This is a major improvement compared to our previous results, in which the mean difference sums of squares for the predicted and the verified data were 224.5 and 1551.1, respectively.

By plotting the percentage of remoxipride dissolved in vitro versus the percentage of remoxipride dissolved in vivo (see Fig. 5), a linear correlation was obtained. The regression line was calculated without an intercept and was $y = 0.96x$ ($R^2 = 0.97$), indicating an insignificantly faster dissolution rate in vivo than in vitro.

Validation

Our predicted optimal in vitro conditions were validated by experimentally testing another batch of the ER

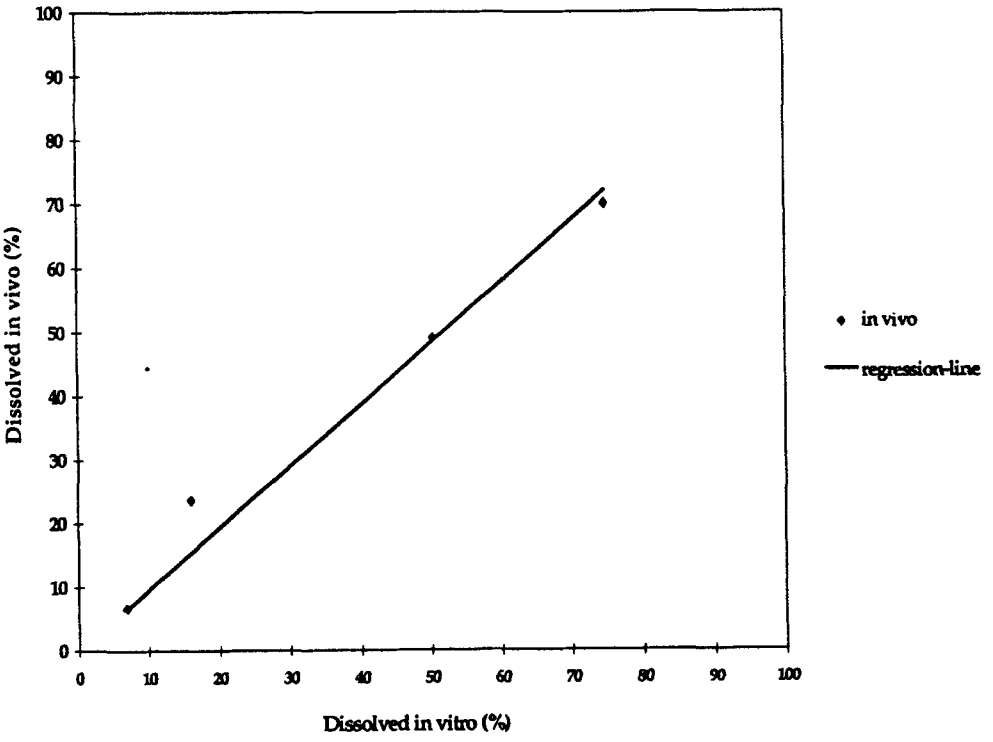

Figure 5. A linear correlation between the dissolution from remoxipride spheres in vitro and the mean dissolution in vivo calculated by numerical deconvolution ($n = 16$).

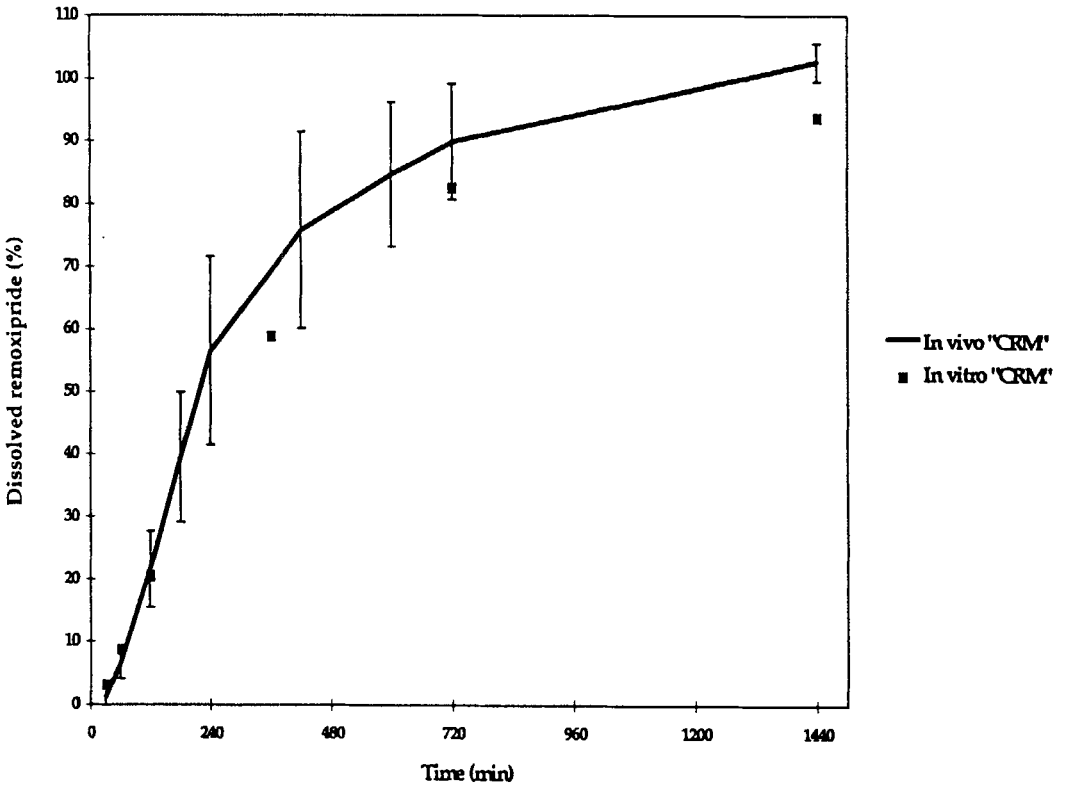

Figure 6. The association between the in vitro dissolution profile from remoxipride spheres CRM and the corresponding in vivo absorption curve calculated by the Wagner-Nelson method (n = 16). Error bars denoted as standard deviations of the in vivo curve.

formulation, called CRM, which was used in a second in vivo study comprising 16 healthy volunteers (16). The in vitro conditions were applied in two separate tests and a mean dissolution profile was calculated. The mean in vivo profile used to compare the data was calculated by the Wagner-Nelson method.

The relationship between the in vitro dissolution rate from the validation batch (CRM), and its corresponding absorption profile in vivo is shown in Fig. 6. The profiles are similar, but not superimposable.

Figure 7 presents a plot of the percentage dissolved from the formulation in vitro versus the percentage dissolved from the formulation in vivo. The plot reveals a very high correlation. The regression line, which was calculated without an intercept, was $y = 1.10x$ (R^2 = 0.99), indicating an insignificantly higher dissolution rate in vitro in this case.

CONCLUSIONS

The present study was designed to optimize the effects of the variables agitation, temperature, osmolality, and polarity on the dissolution rate of remoxipride from extended released coated spheres. It was concluded that polarity and osmolality have significant effects on the drug dissolution rate from the formulation. The dissolution data obtained in vitro were used in a statistical model to predict the optimal in vitro conditions most closely correlated with in vivo dissolution data obtained after administration of the drug formulation to 16 healthy volunteers. The experimental verification of the predicted in vitro conditions produced a high degree of correlation between both the predicted and the verified in vitro dissolution profiles, and between the verified in vitro and the in vivo dissolution curve. The optimal in

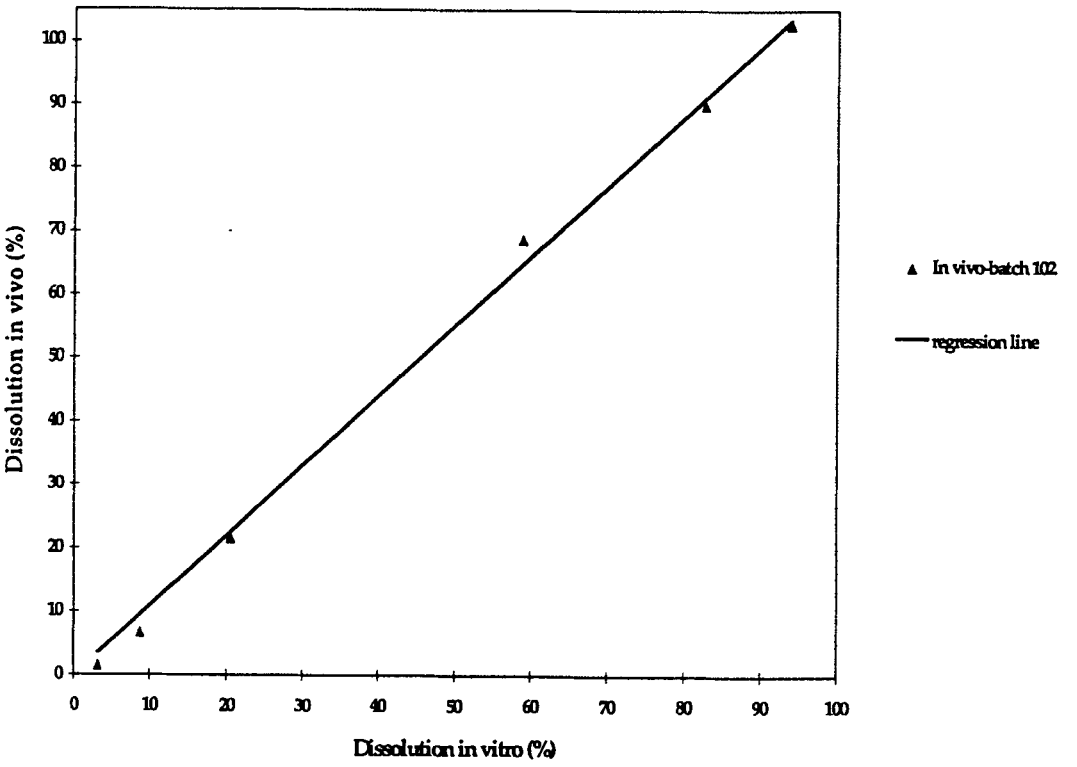

Figure 7. A linear correlation between the in vitro dissolution of remoxipride from ER spheres (CRM) and the corresponding mean in vivo absorption profile, calculated by the Wagner-Nelson method (n = 16).

vitro conditions were successfully validated on another batch of the formulation, which had been given to another group of volunteers.

Based on the presented results, we conclude that the chemometric working approach is useful for the development of critical dissolution tests and for the establishment of in vitro/in vivo correlations. It therefore seems promising to apply the working strategy used to other solid systems.

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