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Experimental design in the development of voltammetric method for the assay of omeprazole[1]

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Abstract

A multivariate strategy was used to optimize an adsorptive stripping voltammetric method for the determination of the antiulcer drug omeprazole. A 3/4 matrix was used for the variable screening while a central composite design was chosen in the subsequent step to evaluate the response surfaces. Simultaneous optimization of the response peak height (h_p) and peak half width $w_{1/2}$), the latter being a peak shape measure, was achieved. The factors accumulation time, pulse amplitude, scan rate and stirring rate were all found to be statistically significant for the response h_p, while for the response $w_{1/2}$ only the stirring rate was found to be significant. The optimized method shows a good linearity between peak height and analyte concentration in the concentration range from 8.33×10^{-9} M to 1.42×10^{-7} M with a LOD of 6.5×10^{-9} M. The mean recovery of omeprazole in capsules was 101.9% with a SD of 2.04 (RSD = 200).

Keywords: Adsorptive stripping voltammetry; Omeprazole determination; Experimental design; Response surface plot; Composite response surface study

1. Introduction

Omeprazole, a substituted benzimidazole sulphoxide, is the first of a new class of drugs, the acid pump inhibitor, which control gastric acid secretion by inhibition of gastric H^+, K^+–ATPase, the enzyme responsible for the final step in the secretion of hydrochloric acid by the gastric parietal cell [1]. Omeprazole is an acid-labile lipophilic weak base ($pK_{a_1} = 4.2$; $pK_{a_2} = 9$) [2,3]. Unprotected exposure to acidic gastric contents results in inactivation of > 50% of an oral dose (20–40 mg daily) leading to poor bioavailability [3]. Following adsorption, omeprazole is eliminated rapidly and almost completely metabolized in the liver. Eighty percent of the metabolites (omeprazole sulphoxide and hydroxy omeprazole) is ex-

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creted in the urine while the other 20% is excreted in the feces after biliary secretion. At concentrations covering the normal theraputic range (0.19 to 19.4 μmol l^{-1}) [4] omeprazole protein binding was calculated to be between 95 and 96% in human plasma. Due to the very low plasmatic concentration of the drug (5.5×10^{-10}–5.6×10^{-8} M) the development of a sensitive analytical method such as an adsorptive stripping voltammetric method for omeprazole determination is highly desirable. Other methods already exist—liquid chromatography [2], spectrophotometry [5], radioactivity [6] and polarography [7]—but with the exception of the first, they have a LOQ which does not allow the assay of the molecule in biological fluids. The adsorptive stripping voltammetric method, which was used for the work described in this paper, was set up using a multivariate strategy by means of experimental design tools. To evaluate its application, it was tested on the assay of omeprazole pharmaceutical dosage forms (coated granule capsules) while its study on human plasma and urine was in progress in this laboratory. With respect to the HPLC mehtod, the adsorptive stripping voltammetric method is less time-consuming and allows the determination of the LOQ with RSD less than the value reported for the chromatographic technique (10–15%).

2. Experimental

2.1. Apparatus

All experiments were performed on a 433 polarographic analyser (Amel, Milan) incorporating a magnetic stirrer and three-electrode system consisting of a mercury electrode (DME or HMDE) as working electrode, a platinum wire as auxiliary electrode, and a Ag/AgCl electrode as reference electrode. All potentials quoted are relative to the Ag/AgCl electrode. The experiments were always carried out at room temperature in a special glass cell designed to accommodate a three-electrode potentiostatic unit. A host computer was used for instrumental control and data handling by means of special Amel software. An ultrasonic 300 sonicator (Ney Company, Bloomfield, USA) and 691 pH meter (Metrohm, Herisau, Switzerland) were also used. The software NEMROD by LPRAI (Université de Marseille III, Marseille, Le Merlan, France) was used for generation and evaluation of the statistical experimental design.

2.2. Materials

Omeprazole working standard was kindly supplied by Menarini Pharmaceutical Industries (Florence); 20 mg omeprazole capsules (Losac®) were purchased in a pharmacy. 3 M KCl (Metrohm, Herisau, Switzerland) and 1 M NaOH (E. Merck, Darmstadt, Germany) were used without any further treatment. All solutions were prepared using water of Milli-Q quality.

2.3. Sample solutions

Omeprazole is unstable under acidic conditions while it can be stored at pH 7.5–9.0 for 4 days at room temperature without any degradation [2]. Thus, standard solutions (0.04 mg ml^{-1}) of omeprazole were prepared every 4 days by dissolving about 2 mg of omeprazole, accurately weighed, in a 50 ml volumetric flask adding 100 μl of 0.2 N NaOH and a little water. Then the solution was diluted to the mark with water. The pH of the resulting solution was 9. Working solutions of 1.4×10^{-3} mg ml^{-1} were prepared daily by diluting 0.35 ml of standard solution to 10 ml with water.

2.4. Stripping procedure

The following optimized stripping procedure was employed: 10 ml of 0.01 M KCl used as supporting electrolyte was transferred to the voltammetric cell and deaerated by bubbling nitrogen-free oxygen for 10 min in the initial cycle (30 s for each successive cycle); the nitrogen stream was then kept above the solution. The accumulation on the electrode surface was performed at 0 V for 68 s and during this step the solution was stirred at a fixed rate (400 rev min^{-1}) with a Teflon magnetic stirrer. At the end of the accumulation period, the stirrer was switched off and

after a quit time of 10 s a cathodic differential pulse potential scan was applied between −0.7 V and −1.5 V with the following settings: scan speed, 40 mV s^{-1}; pulse amplitude, 70 mV; drop size, 40 arbitrary units (a.u.). The adsorptive stripping voltammetric cycle was repeated with a new drop for each solution analysed and the mean of these voltammograms was used for subsequent data handling. Peak heights were evaluated as the difference between each voltammogram and the background electrolyte voltammogram.

2.5. Calibration procedure

After a background stripping voltammogram was obtained under the experimental conditions described above, eight aliquots (the first of 20 μl, the second of 30 μl, and the remaining ones of 50 μl) of omeprazole working solution (1.4×10^{-3} mg ml^{-1}) were introduced into the voltammetric cell and the voltammograms were recorded. A linear relationship between peak height (μA) and analyte concentration (M) was found and a calibration graph was obtained.

2.6. Analysis of capsules

The average weight of 10 hard capsules was determined. An accurately weighed quantity of film-coated granules containing about 2 mg of omeprazole was transferred to 50 ml calibrated flask, dissolved with 100 μl of 0.2 N NaOH and a little water and then made up to volume with water. The resulting mixture was sonicated at room temperature for 3 min; 0.35 ml of this mixture was transferred to 10 ml volumetric flask, diluted to the mark with water and the resulting working sample solution was used for the voltammetric analysis.

After a blank stripping voltammogram was obtained, 100 μl of working sample solution (1.4×10^{-3} mg ml^{-1}) was added to the voltammetric cell and the stripping procedure described above was applied. The quantitative assay of omeprazole was carried out by means of the calibration graph method using a calibration graph obtained in the 40–120% range with respect to the theoretical content of omeprzole in the voltammetric cell.

3. Results and discussion

In general, experiments are performed to measure the effects of one or more variables on one or more responses, and to find the set of variable combinations that gives the best result. Several ways exist to perform such an optimization process even though experimental design has become a rather widely used optimization tool in the chemical literature [8–11]. Experimental design counteracts the classical optimization technique called one-variable-at-a-time (OVAT) which considers the adjustment of one variable at a time for determining the optimum experimental conditions. However, only a method which considers the simultaneous variation of all the variables involved in the optimization process makes it possible to point out the possible interactions existing among the variables and thus to achieve the correct conclusions about the best set of experimental conditions. Therefore the OVAT approach, which does not consider the dependence of the effect of one variable on the value assumed by another factor (i.e. interaction), is only a good approach if variables are independent of each other. As it is nearly impossible to know if the variables are independent at the begining of a study and because, it is frequently the case that the effect of the variation of a variable (for instance an increase) on the response may be different depending on the vlaue assumed by another variable, an experimental design which considers all the factors simultaneously and estimates both the main effects (i.e. the effect on the response due to only one variable) and the interactions is required. For these reasons, experimental design tools were used to set up the omeprazole voltummetric assay.

Experimental design follows a sequential approach, which means that it obtains information about the significance of the factors on the response in successive steps. The information gained in the previous stage is then used to decide which factors should be maintained and studied in later stages. By so doing, as the study progresses it is possible to reduce the size of the problem towards a smaller number of factors and to adjust their variation range to a more promising region which can be explored more thoroughly.

3.1. Screening phase: three fourths matrix

Since preliminary studies carried out on the drug allowed potassium chloride to be selected as the best background electrolyte, seven variables were identified which could affect the response: accumulation potential (E_{acc}), accumulation time (t_{acc}), ionic strength (μ), scan rate, pulse amplitude (ΔE), drop size and stirring rate. The peak height was the response to be maximized and all the experiments were performed at an omeprazole concentration of 4.5×10^{-8} M.

Among the many possible tools of the experimental design, a three fourths matrix was chosen for the preliminary screening phase of the seven selected factors. Table 1 reports the factors and experimental domains used for each variable. The seven factors together with 11 of their possible first-order interactions (i.e. two-factor interactions) were considered to influence the response. Thus, at least 19 experiments ($7 + 11 + 1$ constant term b_0) were needed. The two-factor interactions were selected according to the authors' knowledge and the experience derived from previous work [12,13] on the problem. The three fourths matrix was chosen because it allows the smallest number of experiments to be carried out and at the same time gives quality information. The matrix allowed the desired interaction and main effect to be estimated, not confounding them, and was obtained, as the matrix name implies, by taking three of the total four parts of the suitable matrix. With seven variables, a full factorial design involves a 2^7 matrix (with 128 experiments).

Table 1
3/4 matrix: factors and experimental domain

Factor	Domain
x_1 (μ, M)	0.01–0.1
x_2 (t_{acc}, s)	40–70
x_3 (E_{acc}, mV)	0–700
x_4 (ΔE, mV)	30–60
x_5 (scan rate, mVs^{-1})	20–40
x_6 (drop size, a.u.)	20–40
x_7 (stirring rate, rev min^{-1})	200–500

Table 2
3/4 matrix: estimated effects

Effect	Estimate	Effect	Estimate
b_0	113.25	b_{14}	4.62
b_1	−5.00	b_{15}	0.88
b_2	24.12[a]	b_{16}	4.62
b_3	4.25	b_{23}	4.12
b_4	17.25[a]	b_{24}	17.37[a]
b_5	36.19[a]	b_{25}	10.62[a]
b_6	9.99	b_{26}	7.0
b_7	10.81	b_{34}	−3.25
b_{12}	4.37	b_{35}	−5.62
b_{13}	−3.37		

[a] Significant estimated effects.

Its corresponding quarter-fraction is a 2^{7-2} ($1/4 * 2^7 = 2^{-2} * 2^7 = 2^{7-2} = 2^5$) matrix (with 32 experiments), of which three parts are taken, achieving the desired matrix comprised of 24 experiments. Therefore by performing 24 experiments it was possible to obtain the main effects and the desired first-order interactions which were not confounded among them. Of course, aliases may occur among main effects and higher-order interactions (i.e. three-, four- or more factor interactions) and among first-order interactions and higher-order interactions. However, often these higher-order interactions are small in comparison to main effects and two-factor interactions so that they can be regarded as negligible or redundant. All 24 experiments were performed in randomized order in attempting to minimize the effects of some unknown and uncontrolled factors or variations which may lead to biased results. This approach has been maintained throughout the whole work.

The analysis of the estimated effects obtained from the 3/4 matrix (Table 2) indicates that only four of the estimated effects were appreciably important. These included four main effects (t_{acc}, ΔE, scan rate and stirring rate) and also the x_2x_4 and x_2x_5 interactions which were found to have an influence upon the response. At this point in the study, the values of ionic strength, accumulation potential and drop size could be fixed at 0.01 M, 0 mV and 40 a.u. respectively, and the study continued with the four relevant variables.

3.2. Central composite design

The number of variables (four) that still remained to be investigated allowed a more detailed study of how the response is related to the variation in the experimental conditions of the variables. Thus, a study of the graph of the response as a function of the four variables considered (response surface) was carried out. The values of the other factors were fixed according to the indication of the first stage of the experimental design (3/4 matrix). Moreover, according to the results of the 3/4 matrix, the experimental domain of the important variables under study was adjusted. In particular, all their estimated effectrs being positive, the experimental domain of the factors (Table 3) was moved towards an increase in the variable values.

The design chosen for the study of the response surfaces was a central composite design (CCD) with $\alpha = 1$ for the necessity of studying the variables at no more than three levels. The responses considered for the optimization were both the peak height (h_p, to maximize) and the peak half width ($w_{1/2}$, to minimize), i.e. the width of the peak at the point where the peak height is half its maximum. The latter parameter was considered as a quantitative evaluation of the peak shape. The CCD design requires $2^k + 2k + n$ experiments and provides for an equation model of the type: $y = b_0 + \Sigma b_i + \Sigma b_i^2 + \Sigma\Sigma b_{ij}$; where k represents the number of variables and n the number of experiments carried out at the center point. The design consists of three parts: a factorial design (2^k) used to estimate the coefficients of the linear (b_i) and interaction (b_{ij}) terms of the model; n experiments at the center of the experimental domain which afford both an estimate of the experimental error variance and the opportunity to assess the presence of curvature in the model; and $2k$ experiments symmetrically spaced at $\pm\alpha$ along a variable axis, i.e. points whose projection falls at the center of the hyperfaces of the hypercube representing the geometrical locations of the experiments of the full factorial design. The experiments at the extremities of the star are used to estimate the coefficients of the square terms (b_i^2) in the model. Generally, the value assumed by α depends on the number of experiments (N_F) in the factorial part of the CCD, being $\alpha = N_F^{1/4}$. In this way, the shape of the experimental domain of the variables is spherical and each variable is studied at five levels: $-\alpha$, -1, 0, $+1$, $+\alpha$. Otherwise α can be 1, the experimental domain is now a hypercube and each variable is studied at three levels: -1, 0, $+1$. In this case, the design is called face centered design (FCD) because each point of the star falls at the center of a hyperface of the hypercube. The CCD used in the omeprazole response surface design was a FCD, in order to study the variables at no more than three levels, and included four center points.

Table 3
CCD matrix: factors and experimental domain

Factor	Domain
x_1 (t_{acc}, s)	50–70
x_2 (ΔE, mV)	50–70
x_3 (scan rate, mV s^{-1})	30–50
x_4 (stirring rate, rev min^{-1})	300–500

Table 4 reports the experimental matrix and the responses obtained for each run, while the model equations obtained for both responses are:

(1) hp
$$y = 0.287 + 0.032x_1 + 0.026x_2 + 0.038x_3 + 0.040x_4 + 0.005x_1^2 + 0.004x_2^2 - 0.016x_3^2 - 0.032x_4^2 + 0.013x_1x_2 + 0.001x_1x_3 + 0.005x_2x_3 + 0.013x_1x_4 - 0.001x_2x_4 + 0.005x_3x_4$$

(2) $w_{1/2}$
$$y = 1.938 + 0.036x_1 - 0.011x_2 + 0.038x_3 + 0.108x_4 - 0.103x_1^2 - 0.038x_2^2 - 0.028x_3^2 + 0.047x_4^2 - 0.004x_1x_2 + 0.015x_1x_3 + 0.009x_2x_3 + 0.015x_1x_4 - 0.041x_2x_4 + 0.052x_3x_4$$

With reference to the h_p response, the analysis of the coefficients of the model shows that all the

Table 4
CCD: experimental matrix and responses (h_p, $w_{1/2}$)

Run[a]	t_{acc} (s)	ΔE (mV)	Scan rate (mV s^{-1})	Srirring rate (rev min^{-1})	w_p (μA)	$w_{1/2}$ (mm)[b]
8	−	−	−	−	0.163	1.70
3	+	−	−	−	0.164	1.75
4	−	+	−	−	0.164	1.75
18	+	+	−	−	0.215	1.75
24	−	−	+	−	0.172	1.60
28	+	−	+	−	0.203	1.75
11	−	+	+	−	0.268	1.80
13	+	+	+	−	0.316	1.70
20	−	−	−	+	0.185	1.95
26	+	−	−	+	0.230	1.80
1	−	+	−	+	0.226	1.65
12	+	+	−	+	0.354	1.85
9	−	−	+	+	0.283	1.95
15	+	−	+	+	0.336	2.15
19	−	+	+	+	0.301	1.90
23	+	+	+	+	0.407	1.99
22	−	0	0	0	0.221	1.75
5	+	0	0	0	0.341	1.95
10	0	−	0	0	0.299	1.88
2	0	+	0	0	0.261	1.95
27	0	0	−	0	0.209	1.90
16	0	0	+	0	0.309	1.95
17	0	0	0	−	0.209	1.75
25	0	0	0	+	0.277	2.25
6	0	0	0	0	0.292	1.95
14	0	0	0	0	0.314	1.90
21	0	0	0	0	0.308	1.85
7	0	0	0	0	0.300	1.95

[a] Randomized order.
[b] 1 mm = 5.55 mV.

variables seem to be significant on the response and the x_1x_2 and x_1x_4 interactions are significant as well. Concerning the $w_{1/2}$ response, the factor which is mainly significant on the response is x_4 which has to be taken at its lower level ($w_{1/2}$ has to be minimized) and even the quadratic effect of x_1 is relevant. However, the interpretation of the results has to start from the analysis of the whole model equations rather than from the analysis of the single coefficients; i.e. it is important, for the response surfaces study, to consider also the factors whose coefficients are statistically not significant. For this reason the analysis of the response surface plot is necessary.

Fig. 1 shows the three-dimensional response surface plot for the response h_p. From Fig. 1a it is evident that the stirring rate has a quadratic effect on the response and that an interaction between accumulation time (x_1) and stirring rate (x_4) exists. Due to this interaction, the influence of x_4 is higher when x_1 is at its highest level, i.e. there is a

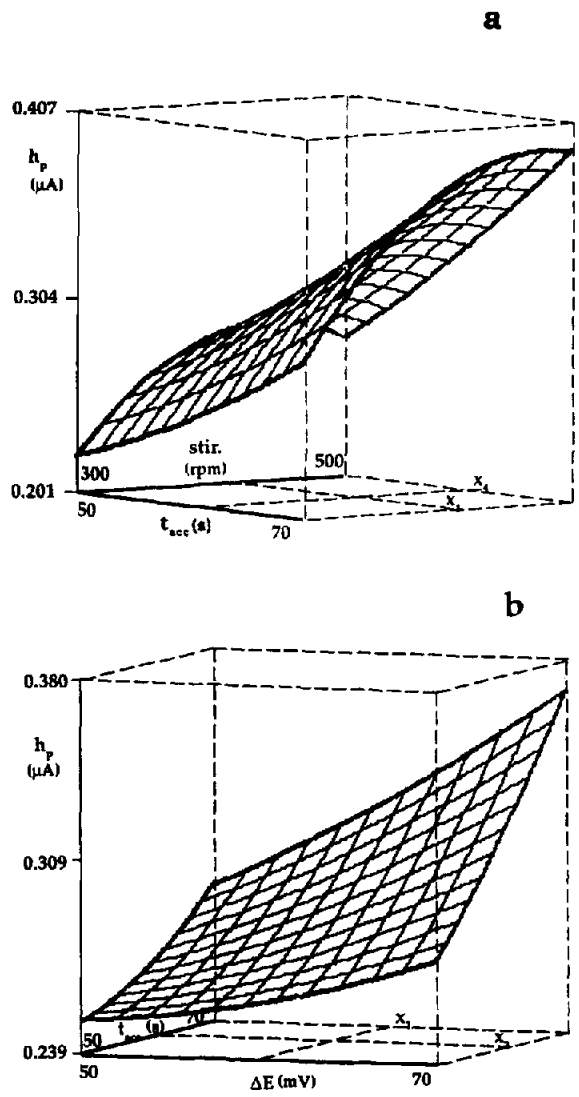

Fig. 1. Three-dimensional plot of the response surface for peak height (h_p) calculated according to Eq. (1). (a) Surface as a function of t_{acc} (x_1) and stirring rate (x_4), maintaining ΔE (70 mV) and scan rate (40 mV s^{-1}) constant. (b) Surface as a function of t_{acc} (x_1) and ΔE (x_2), maintaining stirring rate (400 rev min^{-1}) and scan rate (40 mV s^{-1}) constant.

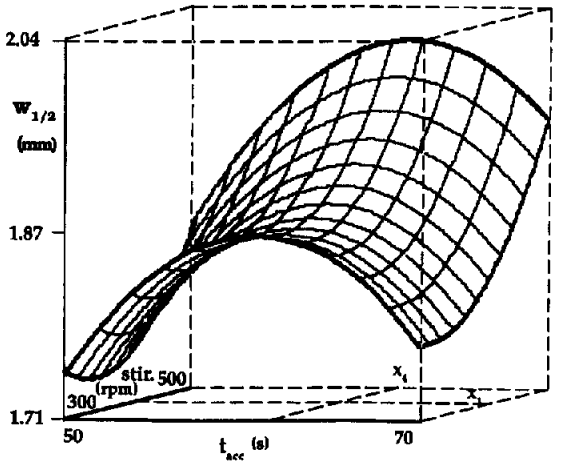

Fig. 2. Three-dimensional plot of the response surface for the peak width. The surface (x_1, t_{acc} vs. x_4, stirring rate) was determined according to Eq. (2), keeping ΔE (70 mV) and scan rate (40 mV s^{-1}) constant.

higher increase in the response increasing the stirring rate when accumulation time is at the highest level. Fig. 1b shows the interaction between x_1 (t_{acc}) and x_2 (pulse amplitude); in fact it is evident that the effect on the response of the accumulation time depends on the value of x_2 and the effect of pulse amplitude depends on the value of x_1. In particular, the effect on the response of the accumulation time is much higher when the pulse amplitude is at the highest level and at the same time the effect on the response of the pulse amplitude is much higher when the accumulation time is at the highest level. Fig. 2 shows the three-dimensional response surfaces plot for the response $w_{1/2}$. From this Figure it is evident that, to minimize the response (i.e. to obtain the minimum of the response surface), it is important to maintain the stirring rate at the lowest level and the accumulation time either at the lowest level or at the highest level, but not at its central level.

3.3. Desirability function

When the number of responses is more than one, a composite response surface study is necessary to find the best compromise between the responses. In this case it was necessary to find the best compromise between the responses h_p and $w_{1/2}$. The composite response surface study gives the coordinates of the optimum which allows the analysis of the drug to be performed. One of the techniques used to optimize several responses simultaneously is to weigh them together into one single criterion, a desirability function, which can be used as a criterion for the optimization [14]. The first step for finding the criterion is to clearly define what the desired result is for each response. This is achieved by transforming each y_i response into a d_i function called a partial or individual desirability function which is defined according to the desired variation of y_i in a defined range. Once the minimum and maximum values of y_i are selected within which the response is accepted to vary, the variation function is chosen depending on how one wants the desirability function to increase, decrease, remain constant and so on [15].

An overall desirability function, D, can then be defined as a geometric mean of all individual desirabilities $d_i = 1, 2 \ldots, m$; $D = (d_1 \times d_2 \times \cdots d_m)^{1/m}$. Depending on the importance one wants to attribute to a response, the individual d_i functions can be weighed and the total D function assumes the form: $D = (d_1^{w1} \times d_2^{w2} \times \cdots d_m^{wm})^{1/(w1+w2+wm)}$. A calculation algorithm is then applied to the D function in order to determine the set of variables that maximizes it, i.e. that results in the value for D as close as possible to 100%. This set of variables is known as the "optimal point".

In the case of the omeprazole optimization, the responses h_p and $w_{1/2}$ were transformed into appropriate desirability functions d_i, d_1 and d_2 respectively, according to the fact that h_p had to be maximized and $w_{1/2}$ minimized (Fig. 3). In the overall function D the weights 3 and 2 were attributed to d_1 and d_2 respectively, so that D had the form: $D = (d_1^3 \times d_2^2)^{1/5}$. The three-dimensional plot of D is shown in Fig. 4 and the coordinates of the optimal point, expressed in the coded values of the variables, were: $x_1 = 0.85$ ($t_{acc} = 68$ s); $x_2 = 1$ ($\Delta E = 70$ mV); $x_3 = 0$ (scan rate = 40 mV s^{-1}); $x_4 = 0$ (stirring rate = 400 rev min^{-1}) which produced a total value for D to 80%.

Thus, the optimal conditions resulting from the experimental design were: $\mu = 0.01$ M, $E_{acc} = 0$ mV, drop size = 40 a.u., $t_{acc} = 68$ s, $\Delta E = 70$ mV,

scan rate = 40 mV s^{-1} and stirring rate = 400 rev min^{-1}.

3.4. Validation of the models

The models (1) for h_p and (2) for $w_{1/2}$ were also validated to assure their predictive capacity. The validtion process for the two responses proceeded in two steps: prediction and validation. The prediction process consisted of substituting the coded settings of the optimized factors in the equation model to obtain a prediction value of the response y^{Pred}. During validation, the response y^{Val} of the experiment, carried out with the optimized conditions, was obtained and compared with y^{Pred}. Model validation was achieved if the ratio y^{Val}/y^{Pred} was the same as the ratio y_1/y_2, where

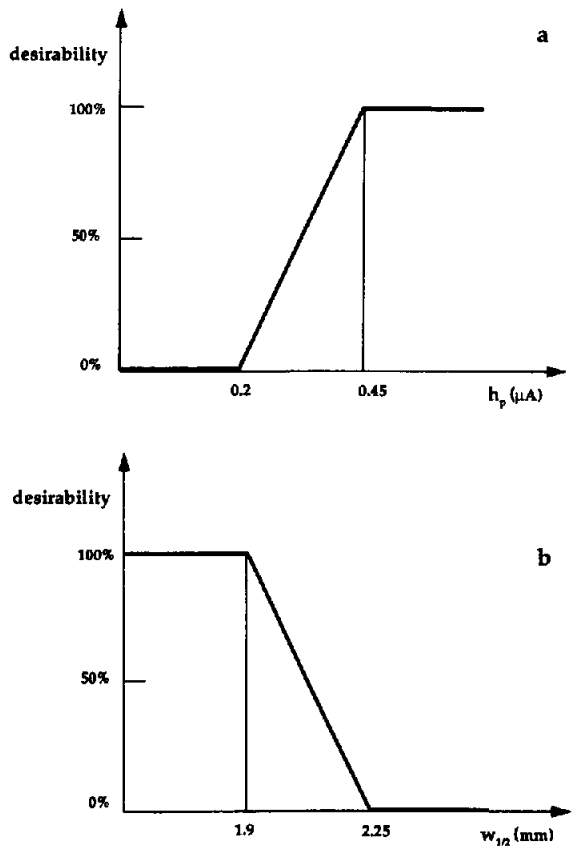

Fig. 3. (a) Transformation of (a) h_p and (b) $w_{1/2}$ in the individual desirability function.

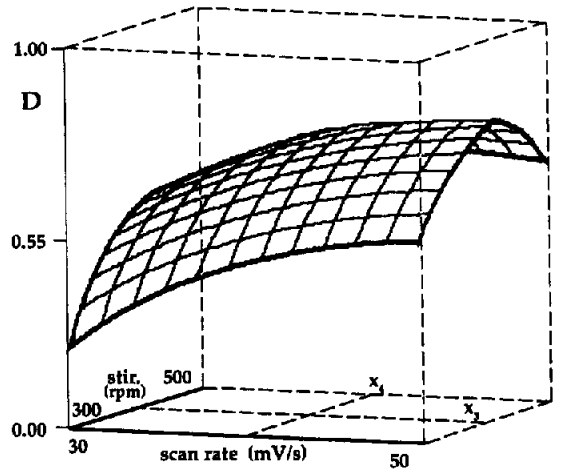

Fig. 4. Three-dimensional plot x_3, scan rate (mV s^{-1}), vs. x_4, stirring rate (rev min^{-1}), of the desirability function.

y_1 and y_2 were the responses of two experiments carried out with the same experimental conditions (e.g. at the center of the experimental domain). The former response (y_1) was obtained during the validation step, i.e. on the day of the model construction, and the latter response (y_2) during the prediction step, in such a way as to overcome any possible block effect.

3.5. Calibration curve

Using the optimized conditions the assay of the antiulcer drug omeprazole in pharmaceutical dosage form was performed. The linear dependence existing between the peak height and the antiulcer concentration leads to a calibration range extending from 2.88 to 48.9 μg ml^{-1} (8.33×10^{-9} M–1.42×10^{-7} M) according to the equation: $y = 0.016(\pm 7.6 \times 10^{-4})(\mu A\ l\ \mu g^{-1})x + 0.0231(\pm 0.018)(\mu A)$. Fig. 5 reports typical voltammograms for increasing omeprazole concentrations and shows a well-defined signal with a peak potential (E_p) of −954 mV. The LOD, i.e. the analyte concentration whose signal was calculated as $y\mathrm{LOD} = b + 2s_b$, where b = intercept and s_b = standard deviation of intercept, was found to be 2.25 μg ml^{-1}.

The lack of fit of the model was checked using the F distribution to detemine the probability that two independent estimates, s_1^2 and s_2^2, are mea-

sures of the same variance σ^2. s_1^2 was calculated as $s_1^2 = \Sigma e_i^2/df_1$ where e_i = residuals = $y_i^{Obs} - y_i^{Pred}$, df = degree of freedom and s_2^2 was calculated as $s_2^2 = (x_i - \bar{x})^2/df_2$, where x_i = repeated measurements of the same sample concentration performed on different samples. The F ratio ($F^{lack\ of\ fit} = s_1^2/s_2^2$) for testing the lack of fit did not exceed the F critical value for df_1 and df_2 degrees of freedom at a probability level of $p = 0.05$. Therefore the model provides an adequate fit of the data [11].

3.6. Quantitation

The assay of omeprazole in capsules was obtained by the calibration graph method applied over a more restricted range than that described for the linearity, and corresponded to 40–120% of a middle point of the calibration range taken as representative of the claimed in-cell omeprazole concentration. At this concentration level the recovery attained for astandard omeprazole solution was 99.4% (RSD = 2.78%, $n = 5$). The assay of the drug in capsules led to an average recovery of 101.9% (RSD = 2.00%, $n = 5$).

4. Conclusion

The promising results obtained in the assay of the omeprazole dosage forms led the authors to the application of the set-up method for the determination of the drug at the low concentration level of biological fluids. In fact, the optimized method shows an elevated sensitivity and good accuracy, according to the authors' needs, thus showing that the correct use of an appropriate experimental design is of considerable benefit in the optimization of analytical methods.

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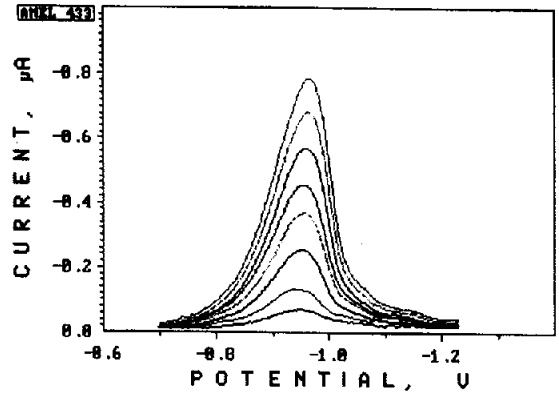

Fig. 5. Adsorptive stripping voltammograms of omeprazole obtained for successive additions of omeprazole working solution (1.45 $\mu g\ ml^{-1}$): the first addition of 20 μl, the second of 30 μl and successive additions of 50 μl. The experimental optimized conditions were: KCl, 0.01 M; E_{acc}, 0 V; t_{acc}, 68 s; v_{scan}, 40 mV s^{-1}; ΔE, 70 mV; drop size, 40 a.u.; stirring rate, 400 rev min^{-1}.

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