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RESOLUTION OF TERNARY MIXTURES OF NITROFURANTOIN, FURAZOLIDONE AND FURALTADONE BY APPLICATION OF PARTIAL LEAST SQUARES ANALYSIS TO THE DIFFERENTIAL PULSE POLAROGRAPHIC SIGNALS

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Summary—The abilities of the Partial Least Squares (PLS) methods in the resolution of ternary mixtures of organic compounds (furaltadone, furazolidone and nitrofurantoin) by using their differential pulse polarographic (DPP) signals are reported. The applicability of these methods to resolve very overlapped peaks whose E_p also changes with concentration is demonstrated. The analysis of both synthetic and real samples has been made with satisfactory results. The relative error of prediction (REP) is 8.7% for FD, 7.7% for FZ and 6.7% for NF by application of the PLS-2 method.

Diverse chemometric methods have been applied to improve results in the multicomponent analysis by different analytical techniques, mainly spectroscopic.[1–3] In the last decade, the treatment of signals by electroanalytical techniques has been initiated. Hence, differentiation of overlapped differential pulse polarographic (DPP) peaks corresponding to reversible systems of inorganic ions cadmium(III)/indium(III) and thallium(I)/lead(II) has been reported.[4]

The use of Fourier transform in the least-squares methodology has been also reported[5] and applied to the resolution of the thallium(I)/lead(II) mixture. However, Grabaric *et al.*[6] showed this technique to be much less efficient when the overlapped peaks correspond to process involving a different number of electrons.

Another multicomponent analysis method based on least squares multiple regression procedures (MULTI3 program) has been recently applied to resolve the binary, ternary and quaternary mixtures of lead(II), thallium(I), indium(III) and cadmium(II) which present highly overlapped peaks.[7]

The applicability of multicomponent analysis to other electroanalytical techniques than DPP has been sparingly examined. Brown and Brown[8] use the Kalman filter for multicomponent analysis of the linear sweep voltammograms corresponding to cadmium(II)/indium(III)/lead(II) systems. The above mentioned MULTI3 program has also been employed in Anodic Stripping Voltammetry (ASV) for the resolution of the same metallic ions mixtures.

It must be noted that the bibliographic data are about electrochemical reversible systems, in which a linear relation exits between intensity and concentration for all the potential values selected. In this case, multicomponent analysis by multiple linear regression can be applied; hence the conditions of the additivity and linearity of the signals are satisfied.[9] PLS, however, can be used to describe non-linear systems by incorporating a larger number of latent variables than would be required for a linear system or using the non-linear or quadratic versions of the algorithms.[10] Also, PLS methods can be appropriate for the analysis of mixtures when secondary chemical reactions occur or in other instances of absence of linearity due to other different reasons as it can happen, *i.e.* in stripping voltammetry. Thus between the bibliographic references about the solution of different problems in stripping analysis by means of multivariate methods[11–13], two of them[11,12] are about the application of the Partial

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Least Squares (PLS) methods to the electrochemical analysis of inorganic reversible systems.

Partial Least Squares is a technique based in factor analysis and PLS-1 and PLS-2 types have been described. PLS-2 differs from PLS-1 in the way that it is used to perform the signal decomposition and the regression analysis. PLS-2 calculates the number of factors on all chemical components simultaneously and the overall number of regression factors is optimized. PLS-1 performs the optimization of this number of factors of only one chemical component at a time. An exhaustive mathematical treatment of the PLS algorithm has been made by Martens and Naes.[3] The bibliographic data mainly refer to its application in spectroscopic techniques.[14–17] PLS in relation with electroanalytical processes can be considered as a 'full-voltammogram' method.

The optimum number of factors depends on the number of independently varying chemical species presents, as well as on other sources of systematic signal variation, such as the presence of randomly varying baselines, detector noise, interaction between pure components, changes in the shape of the component peaks from that of its pure state, *etc.* Because of this, with an adequate design of the calibration matrix and optimization of the experiments, the above mentioned influences can be modeled.[3] In the bibliography, as we have mentioned, only data about the application of multicomponent methods, by using electroanalytical techniques, corresponding to reversible and inorganic systems have been reported.

In a previous paper,[18] we have established the applicability of PLS methods resolving the overlapped DPP peaks arising from irreversible electroanalytical processes. It must be noted that in this paper the resolution of binary mixtures of organic compound by using PLS methods is reported and applied to the analysis of a veterinary formulation with good results for the first time. The applicability of differentiation techniques is also examined and it is concluded that the derivative DPP signals are not suitable for the determination of these mixtures, by application of the zero-crossing method,[1,19] due to the failure of maintenance of the zero-crossing potential value at different concentrations for each component.

For the first time, the resolution of a ternary mixture of three organic compounds exhibiting irreversible reduction processes and the application to a real sample are presented here. The compounds of our study are the nitrofuran derivatives, furazolidone [3-(5-nitrofurfurylideneamino)-2-oxazolidinone, FZ)], furaltadone [5-morpholinomethyl-3-(5-nitrofurfurylideneamino)-2-oxazolidinone, FD], and nitrofurantoin [1-((nitro-2-furanyl)methylene)amino-2,4-imidazolidinodione, NF] which exhibit very similar chemical structures and properties. The nitrofuran derivatives are highly effective chemotherapeutic drugs, well known as antibacterial agents, and widely used to fight common infections in humans and animals or characteristic infections of domestic animals, FZ, FD and NF are sometimes formulated together in Spain. The polarographic behavior of the three compounds has been described in the bibliography.[20–22] They show a wave due to the irreversible reduction of the nitro group at very close potentials.

EXPERIMENTAL

Reagents

Furazolidone, furaltadone and nitrofurantoin obtained from Sigma Chemical Co. were used. Standard solutions of these were prepared by dissolving the appropriate amount in dimethylformamide (DMF). A stock Britton–Robinson buffer solution, which was 0.04*M* with respect to boric, ortophosphoric and acetic acids, was prepared from analytical-reagent grade reagents. From this stock solution of buffer, solutions of various pH were prepared by the addition of 0.2*M* sodium hydroxide solution. All other chemicals were of analytical-reagent grade.

Apparatus

An electroanalytical equipment formed by a computer controlled potentiostat Autolab Pstat10 and a Metrohm 663VA stand, with a Ag/AgCl electrode reference, was used. The system was controlled from a Tystar PC 486 microcomputer equipped with the 'General Purpose Electrochemical System' (GPES 3), version 3.0, software package. A basic LABDU50 home-made program[18] was used to convert the obtained ASCII DPP files into those adequate for the Lab Calc software packages. The Lab Calc software package, version A 1.01, and the PLSplus version 2.0[23] were used for the statistical treatment of the data and the application of the PLS methods.

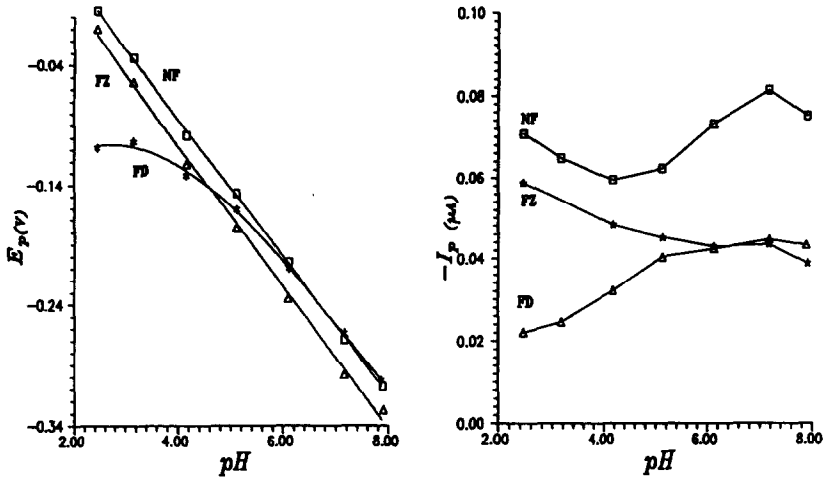

Fig. 1. Influence of pH in the E_p and I_p of $8.4 \times 10^{-6}M$ nitrofurantoin (NF), $8.9 \times 10^{-6}M$ furazolidone (FZ) and $7.5 \times 10^{-6}M$ furaltadone (FD) solutions in the presence of 0.05% gelatine and 0.6% DMF.

The cell was thermostatized by means of a Selecta Frigiterm thermostatic bath.

Solutions were purged with oxygen-free nitrogen for 7 min, before their voltammogram recording.

Procedures for the determination of furazolidone, furaltadone and nitrofurantoin by PLS methods

General. Samples were prepared in 25 ml volumetric flasks, containing between 10^{-7} and $10^{-6}M$ of FZ, FD and NF, 6% of DMF, 0.05% of gelatin, 10 ml of Britton–Robinson buffer solution (pH 2.9) and purified water (HPLC grade) to the mark. The DPP polarograms were obtained between +0.10 and −0.30 V with a 50 mV pulse amplitude, a 1 sec drop time, a 60 msec modulation time and a 4.88 mV/sec scan rate.

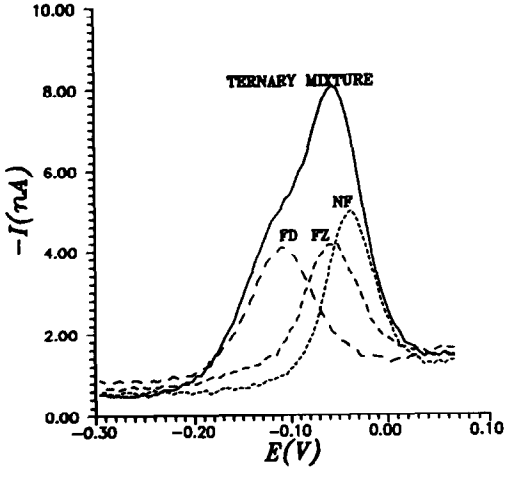

Fig. 2. DPP peaks of $5 \times 10^{-7}M$ nitrofurantoin (NF), $5 \times 10^{-7}M$ furazolidone (FZ) and $10^{-6}M$ furaltadone (FD) solutions at pH 2.8 in presence of 0.05% gelatine and 0.6% DMF. The DPP peak corresponding to the ternary mixture in the same conditions is also shown.

The polarograms were converted with the LAB-DU50 program and the previously optimized calibration matrix, calculated by the application of the PLS methods, was applied to analyze the electrochemical data and to calculate the concentration of the three components in the samples.

Procedure for the analysis of furazolidone, furaltadone and nitrofurantoin in the pharmaceutical formulation Tribactina premix (Esteve Lab.)

About 0.05 g samples of the formulation were accurately weighed and dissolved in 100 ml DMF. Suitable aliquots of these solutions (less than 1.5 ml) were pipetted to prepare the samples to carry out the analysis according to the general procedure.

RESULTS AND DISCUSSION

In the literature, exhaustive studies about the polarographic behavior of the compounds, which are the object of this paper, are found. Cathodic waves due to the reduction process of the nitro group are observed, at similar potential, for all of them. Also, the appearance of polarographic maxima by using DC techniques is reported for furaltadone and nitrofurantoin. Because of this, it is necessary to use suppressors to eliminate them. We have studied the influence of pH in the polarographic behavior of the three compounds in the presence of 0.05% gelatin with the object of selecting better chemical conditions for the simultaneous determination. In order to secure the solubilization of the

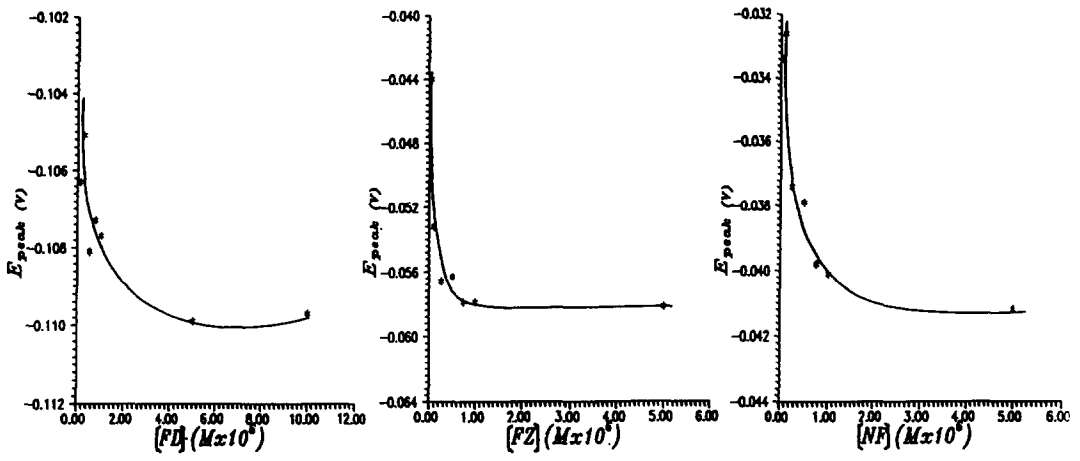

Fig. 3. Variation of E_p of furaltadone (FD), furazolidone (FZ) and nitrofurantoin (NF) with the concentration.

compounds, a 0.6% DMF proportion was retained in the samples. The influence of pH in the E_p and I_p is represented in Fig. 1. With respect to the E_p variation it can be observed that FZ and NF show a very similar behavior. Linear relationships ($E_p = -0.059$ pH $+ 0.13$ for FZ and $E_p = -0.058$ pH $+ 0.15$ for NF) were obtained. However, the E_p of FD was practically constant up to pH 4 and for higher pH values a linear relationship ($E_p = -0.052$ pH $+ 0.10$) was found. The greater differences in the E_p values were found at pH < 4 and pH 2.9 (Britton–Robinson buffer solution) was selected as optimum, although there was a remarkable decrease for the I_p of FD.

The DPP peaks obtained under the above mentioned conditions are represented in Fig. 2. A large overlapping between them was observed under these chemical conditions and, hence, no differentiated peaks were observed in the polarogram corresponding to a mixture of the three compounds. Thus the determination of each one of them in the ternary mixtures, by univariate analysis, is impossible.

The linearity, at the peak potential value (E_p), between I_p and concentration was verified separately for the three compounds in the range 5×10^{-8}–$10^{-5} M$, with the above mentioned chemical conditions. The precision in the intensity values was checked by obtaining the polarograms of 11 replicate samples containing 5×10^{-7} M of FD, FZ or NF and the obtained RSD (%) values were 2.1, 1.8 and 2.3 for FD, FZ and NF, respectively.

On the other hand, a negative exponential variation of E_p with concentration was observed for the three compounds (Fig. 3). These E_p values (zero-crossing potential in the first-derivative peaks) were obtained by differentiation of the DPP peaks with a five experimental points window corresponding to a 24 mV window. This behaviour (peak shift when the concentration changes) can be observed in irreversible systems (Fig. 4). In this figure, different intensity *vs.* concentration plots, for different potential values, before, equal to and after the peak potential, for each of the three compounds are shown. Only for the furaltadone were perfect straight lines always obtained, at the selected pH values of 2.9. For the two other compounds, no similar linearity were found; these curve lines diverge with an opposite shape depending on whether potential value was before or after the E_p.

Table 1. Training sets composition

	Matrix 1			Matrix 2		
Standard	FD*	FZ*	NF*	FD*	FZ*	NF*
M1	1	10	10	0.5	50	50
M2	4	7	10	1	10	50
M3	7	4	10	5	5	50
M4	10	1	10	10	1	50
M5	10	4	7	50	0.5	50
M6	10	7	4	50	1	10
M7	10	10	1	50	5	5
M8	7	10	4	50	10	1
M9	4	10	7	50	50	0.5
M10	3	9	9	10	50	1
M11	9	3	9	5	50	5
M12	9	9	3	1	50	10
M13	7	7	7	10	10	5
M14	5	8	8	5	10	10
M15	8	5	8	5	5	10
M16	8	8	5			

*$M \times 10^7$.

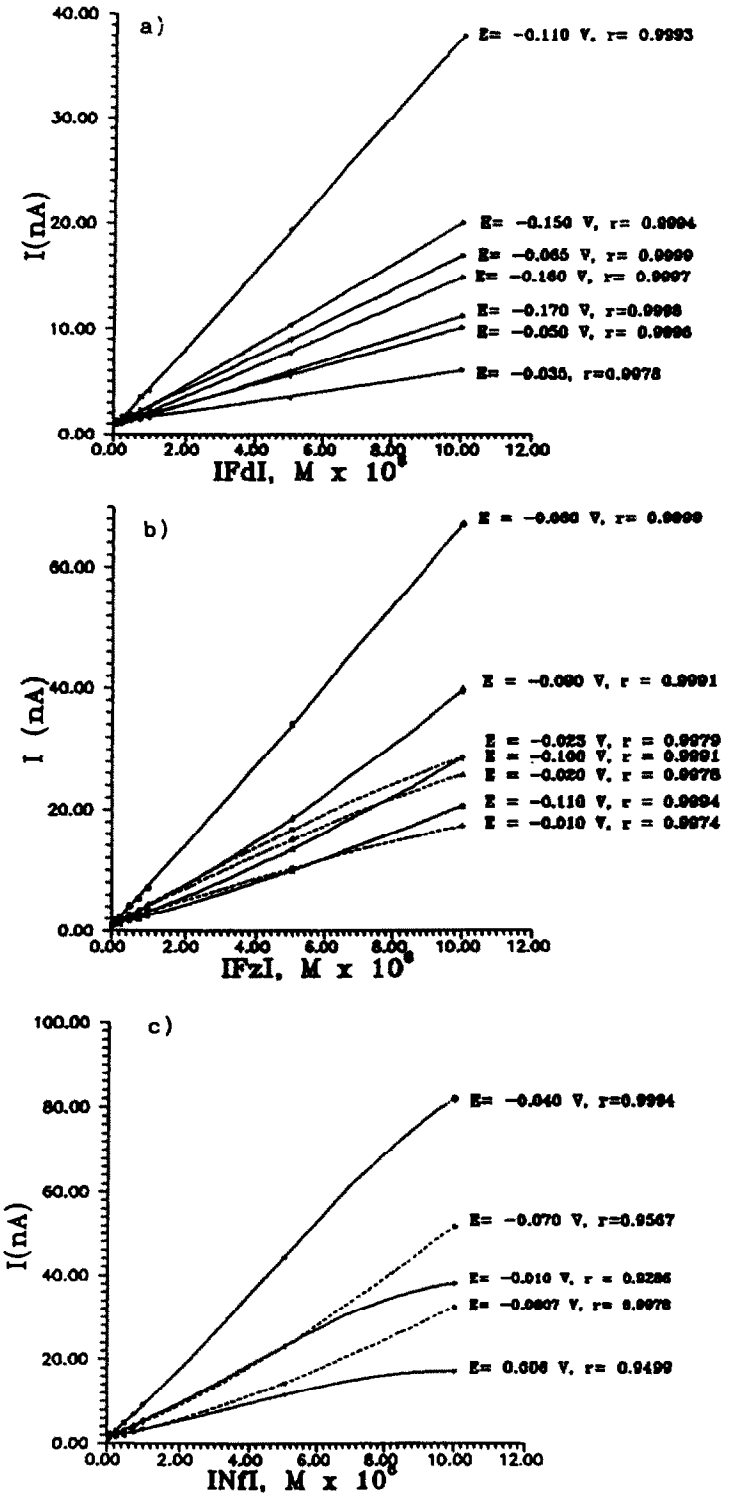

Fig. 4. Variation of reduction intensity for different potential values *vs.* concentration for (a), Fb, (b) FZ and (c) NF.

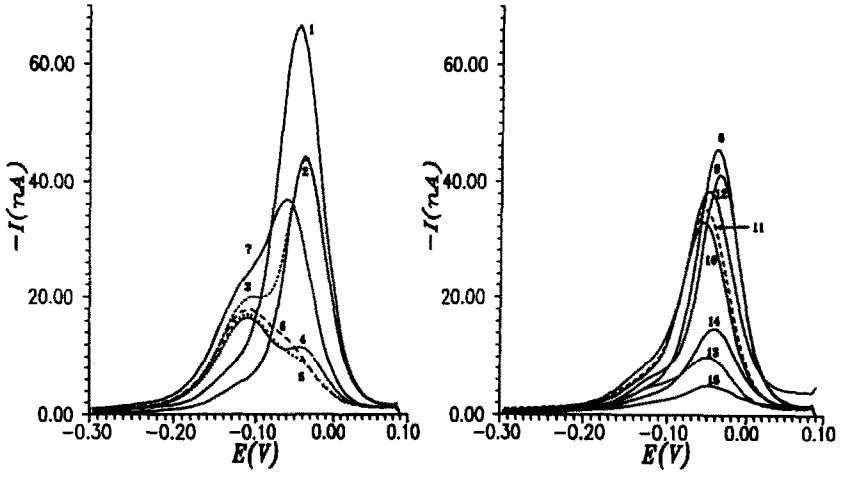

Fig. 5. DPP peak of the standard samples for Matrix 2 (composition as in Table 1: 1: 1M; 2: 3M; 3: 5M; 4: 6M; 5: 7M; 6: 8M; 7: 9M; 8: 2M; 9: 4M; 10: 10M; 11: 11M; 12: 12M; 13: 13M; 14: 14M; 15: 15M).

Application of the multivariate methods PLS-1 and PLS-2

PLS methods can be considered as 'full-voltammogram' methods when electroanalytical techniques are applied. One of the characteristics of the electroanalytical techniques is the wide range of values of analytical signal that can be measured. Hence, we have designed two training sets of 16 and 15 samples, respectively, in two different concentration ranges between 10^{-7} and $10^{-6}M$ (Matrix 1), and from 5×10^{-8} to $5 \times 10^{-6}M$ (Matrix 2). The composition of the prepared samples for the matrices is shown in Table 1. Matrix 1 was constructed according to an established experimental design; however, due to the wide range of concentrations used in the Matrix 2, the standard samples were prepared with very diverse molar ratios. The DPP peaks corresponding to the prepared samples for Matrix 2 are given in Fig. 5. The potential region used for the analysis of data was from +0.09 to −0.3 V, which results in 80 experimental points.

To select the number of factors for PLS methods, the full cross-validation method, leaving out one sample at a time, was used.[24] This process was repeated a total of 15 and 14 times, respectively, for Matrix 1 and 2, until each sample had been left out once. The predicted and actual composition of the samples were compared. PRESS (Prediction Error Sum of Squares) is expressed as:

$$\text{PRESS} = \sum_{i=1}^{N} \sum_{j=1}^{m} (\hat{y}_i - y_i)^2,$$

where $\hat{y}_i$ = predicted concentration and y_i = standard concentration, N = total number of samples and m = total number of components used in the prediction set. It is a measure of how well a particular PLS model predicts unknown samples. The number of factors giving the minimum PRESS can be selected from this cross-validation. However, this usually leads to some overfitting. A better criterion for selecting the optimum number of factors involves the comparison of PRESS from models (h models) with the model which involves the number of factors yielding the minimum PRESS (h^* model). The F-Statistic and the Haaland and Thomas criteria[2,3] are then used.

In our case, both criteria (minimum PRESS and F-Statistic) gave rise to identical results in the selection of the optimum number of factors both with PLS-1 and PLS-2 methods. These numbers of factors for the training set of standards (Matrices 1 and 2) are summarized in Table 2 as well as the statistical parameters (RMSD and R^2) found by PLS-1 and PLS-2. The expression of these parameters is:

$$R^2 = 1 - \left[\sum_{i=1}^{N} (y_i - \hat{y}_i)^2 \Big/ \sum_{i=1}^{N} (y_i - \bar{y})^2 \right],$$

where $\bar{y}$ is the mean of standard concentration, and

$$\text{RMSD} = \left[1/N \sum_{i=1}^{N} (\hat{y}_i - y_i)^2 \right]^{1/2}.$$

Similar and satisfactory values for R^2 were obtained for the application of PLS-1 and PLS-2 optimized matrices. However, the PRESS values were significantly smaller for Matrix 1. The obtained PRESS values using the PLS-1 and PLS-2 methods, for the different number of factors in Matrix 1 and Matrix 2, are represented

Table 2. Optimum number of factors and statistical parameters by PLS-1 and PLS-2 methods for matrices 1 and 2

	Number of Factors	R^2	RMSD $\times 10^7$	PRESS
PLS-1				
Matrix 1				
FD	3	0.9947	0.1981	0.62×10^{-14}
FZ	4	0.9807	0.3796	2.30×10^{-14}
NF	4	0.9778	0.4082	2.66×10^{-14}
Matrix 2				
FD	4	0.9981	0.9156	0.12×10^{-12}
FZ	5	0.9980	0.9434	0.13×10^{-12}
NF	4	0.9773	3.1762	1.51×10^{-12}
PLS-2				
Matrix 1				
FD		0.9940	0.1981	
FZ	4	0.9805	0.3796	5.70×10^{-14}
NF		0.9779	0.4082	
Matrix 2				
FD		0.9982	0.8927	
FZ	4	0.9883	2.2760	2.38×10^{-12}
NF		0.9777	3.1468	

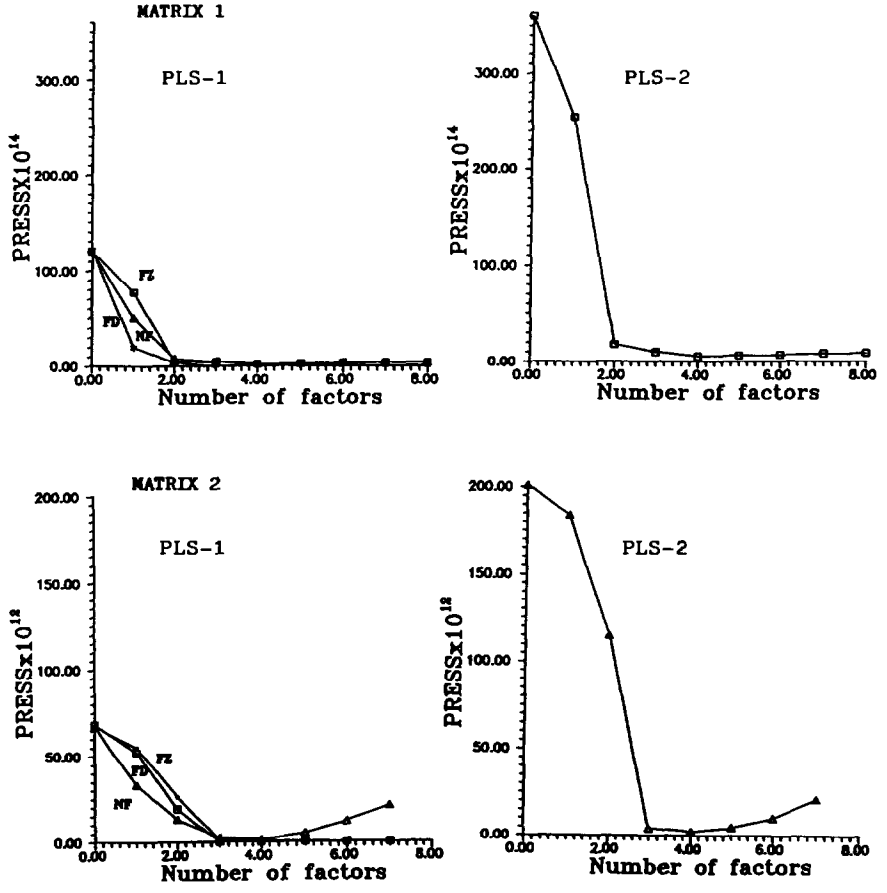

Fig. 6. Obtained PRESS values, using PLS-1 and PLS-2 methods for the different assayed number of factors in Matrices 1 and 2.

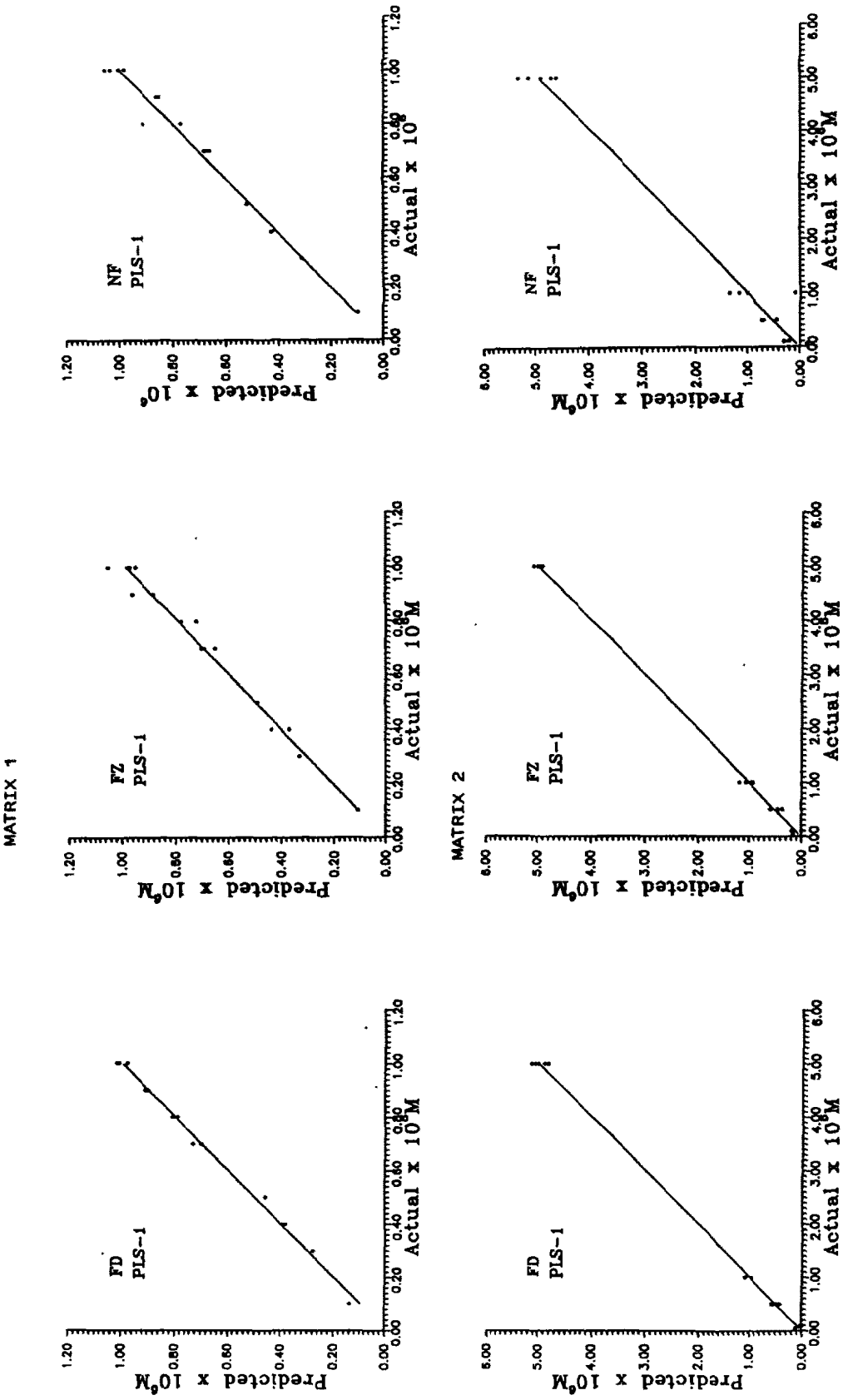

Fig. 7. Predicted *vs.* actual concentrations for FD, FZ and NF by using Matrices 1 and 2 and PLS-1 algorithm.

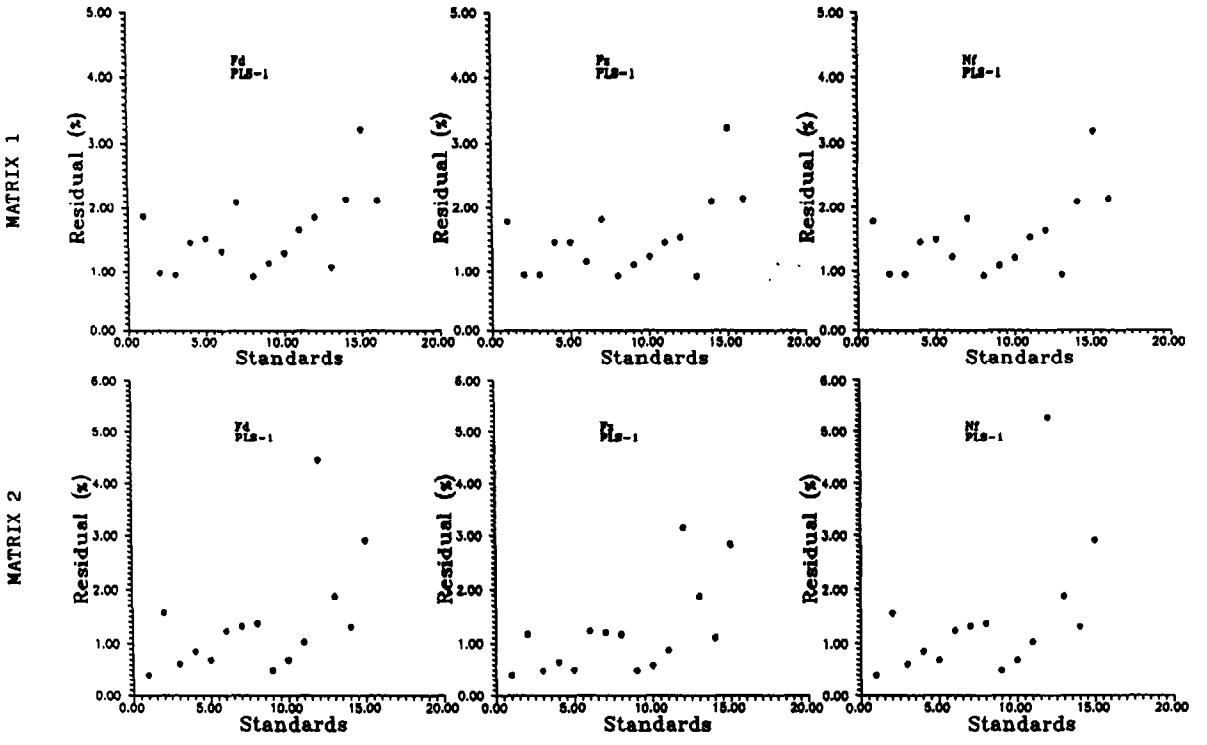

Fig. 8. Residual plots for FD, FZ and NF using Matrices 1 and 2 and PLS-1 algorithm.

in Fig. 6. In Fig. 7 a representation of the predicted using the cross-validation number of factors *vs.* actual for the training set data which is standard in each matrix, by means of PLS-1 is shown and in Fig. 8 the residual plots for FD, FZ and NF using Matrices 1 and 2 equally by means of PLS-1 are shown. The results obtained with PLS-2 were very similar.

The abilities of optimized matrices using both mathematical algorithms were examined in the resolution of synthetic ternary mixtures. The better matrix (Matrix 1) was applied to nine problems (Table 3). On the other hand, the more unfavorable Matrix 2 was assayed with only four problems (Table 3).

Table 3. Synthetic samples composition

Matrix	Samples	FD*	FZ*	NF*
1	1P	2	9	9
	2P	9	2	9
	3P	9	9	2
	4P	5	8	8
	5P	8	5	8
	6P	8	8	5
	7P	6	6	9
	8P	9	6	6
	9P	6	9	6
2	P1	5	25	7.5
	P2	5	7.5	25
	P3	10	7.5	7.5
	P4	25	5	7.5

*Concentration expressed in $M \times 10^7$.

Two diagrammatic representations of the recovery values are shown in Figs 9 and 10. In the case of the results obtained using Matrix 1, similar and satisfactory values were obtained by applying PLS-1 and PLS-2 methods, in the simultaneous resolution of the three compounds. It must be noted that for the smallest molar ratio of FD with respect to FZ and NF assayed, unsatisfactory results were obtained for FD determination. In all the other instances, the recovery values for the three compounds were between 90 and 110%—acceptable values taking into account the low concentration range of analyzed samples.

In the application of Matrix 2, no better results were obtained for the determination of FD compound in the presence of a molar excess of another compound in the mixture.

Analysis of a veterinary formulation by PLS-1 method

The optimized Matrix 1 has been used to analyze FD, FZ and NF simultaneously in a veterinary formulation (Tribactina Premix from Steve lab.) which contains only the three analyzed nitrofurans. In Table 4, the results are summarized and compared with those obtained by HPLC. The overall agreement is very

satisfactory for both FZ and NF; FD concentration falls in the low interval discussed above.

CONCLUSIONS

PLS methods were applied to resolve a ternary mixture of organic compounds in base by the analysis of the DPP signals generated by their irreversible electrochemical reductions. It must be noted that the reduction peaks appeared at very close potential values and the peak potential changed to more negative values when increasing the concentration for each component. The abilities of PLS methods in the

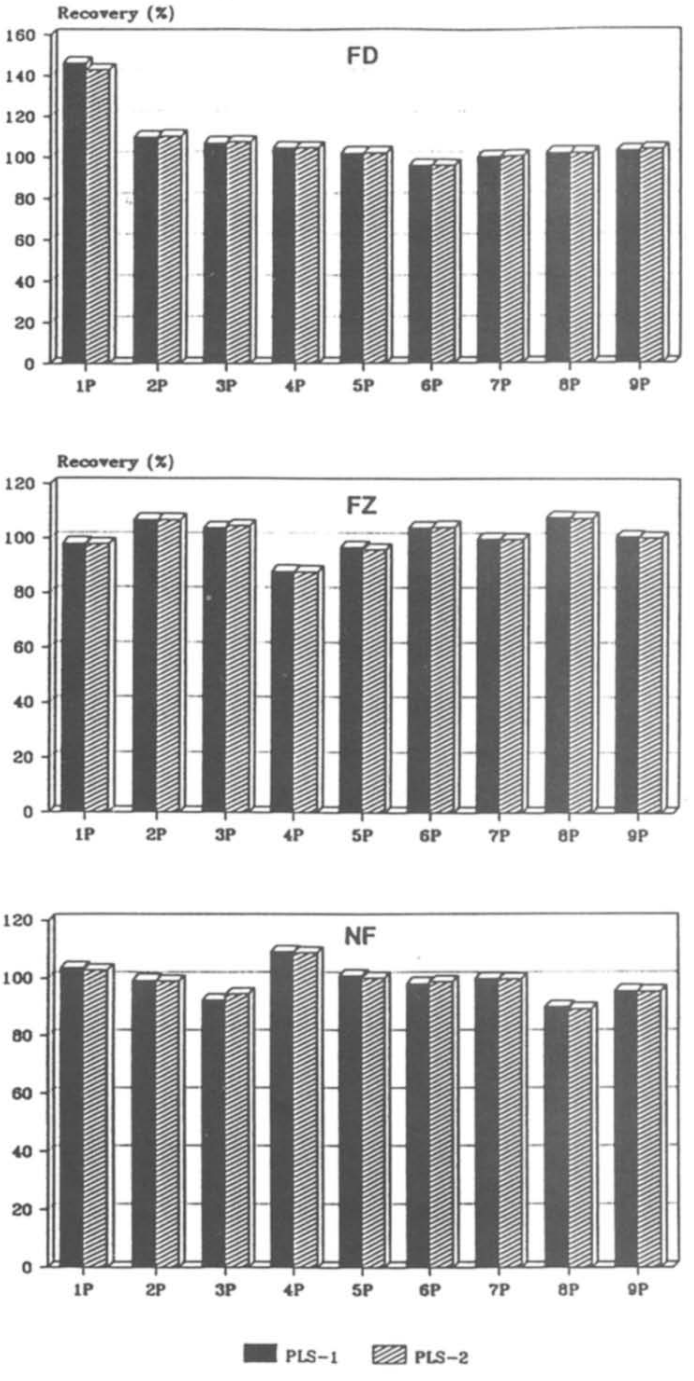

Fig. 9. Diagrammatic representation of the results obtained in the determination of ternary mixtures of furaltadone (FD), furazolidone (FZ) and nitrofurantoin (NF) by PLS-1 and PLS-2 methods with Matrix 1 composition in Table 3).

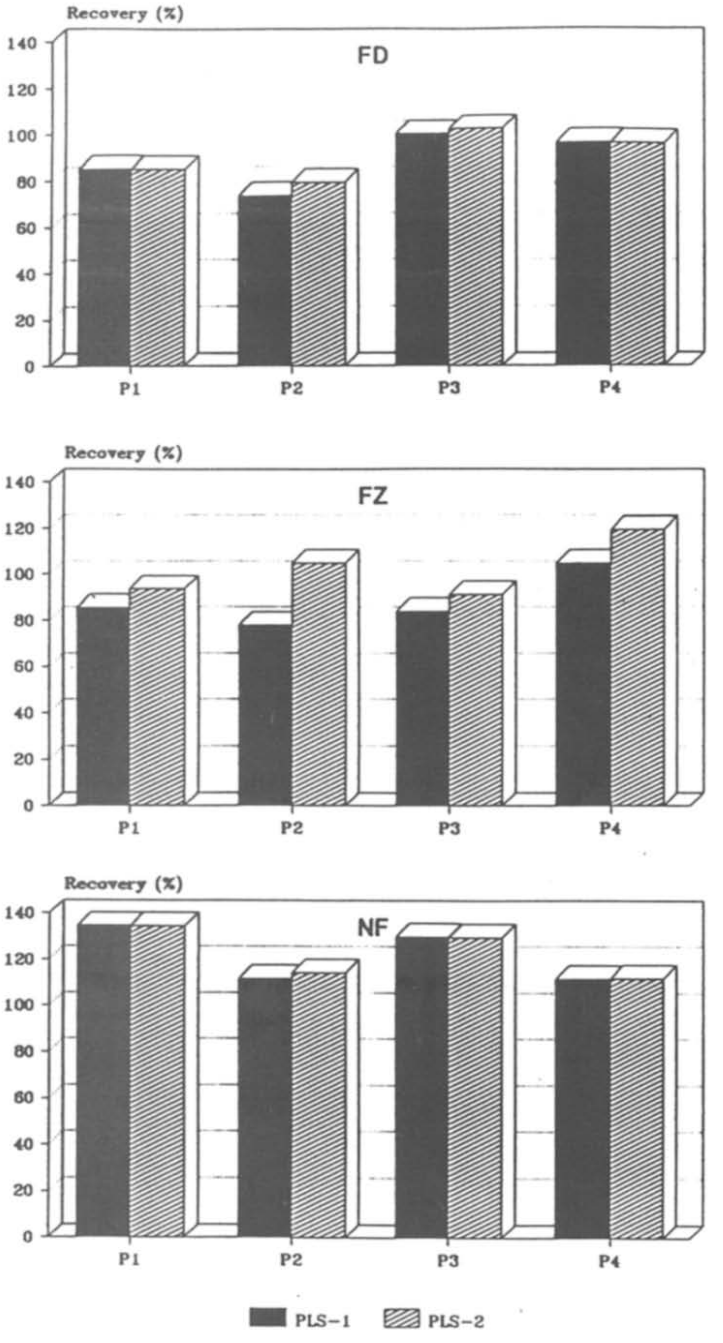

Fig. 10. Diagrammatic representation of the results obtained in the determination of ternary mixtures of furaltadone (FD), furazolidone (FZ) and nitrofurantoin (NF) by PLS-1 and PLS-2 methods with Matrix 2 (composition in Table 3).

Table 4. Simultaneous determination of furaltadone (FD), furazolidone (FZ) and nitrofurantoin (NF) in Tribactina Premix

Compound	Found (*mg/g*)	HPLC* (*mg/g*)
FD	35.4	37.3
FZ	122.8	124.0
NF	60.4	60.4

*Mean of three independent determinations. Claimed level: FD, 41 mg/g; FZ, 120 mg/g; NF, 60 mg/g.

resolution of the DPP peaks corresponding to irreversible reduction processes for three very similar compounds were satisfactorily demonstrated. The application of the calibrated method to the analysis of both synthetic samples and a real sample (veterinary formulation) gave rise to good results, comparable to those obtained by HPLC.

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