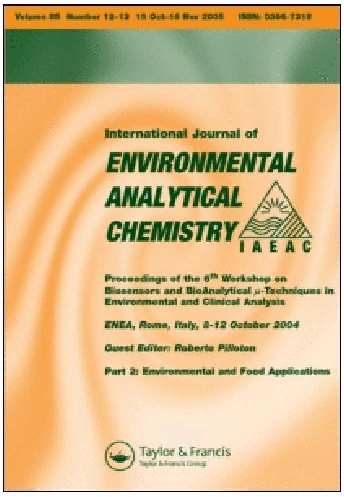

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MULTICOMPONENT ANALYSIS OF ENVIRONMENTAL MATRICES

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The paper reviews several modalities of multicomponent analysis, namely: multilinear regression by using single or multiple standards, non-linear optimization systems by using the Gauss-Newton or the Simplex methods and factor analysis, which have been used by the authors in recent papers.

These multicomponent methods have been applied to environmental samples by different analysis techniques, such as UV- visible spectrophotometry, fluorimetry, polarography-voltametry, simultaneous kinetic processes, monitoring of liquid-liquid extraction processes and HPLC (resolution of overlapped peaks). In this way, the advantages and limitations of the methodology of multicomponent analysis are shown.

KEY WORDS: Multicomponent analysis, multi-linear regression, non-linear optimization, factor analysis, environmental samples.

INTRODUCTION

Major breakthroughs in microelectronics and microcomputer science in the last few decades has brought about important changes in analytical chemistry. The development of novel instrumentation providing a wealth of experimental information in a short time, as well as the availability of increasingly faster and more powerful computers have endowed analysts with new tools for solving a wide variety of problems[1]. Thus, developing new methodologies based on several mathematical models will be essential to obtain as much relevant analytical information as possible from experimental data.

Among these novel technologies, chemometric techniques for the resolution of multicomponent mixtures have gained an important development in the last few years. These techniques essentially rely on the use of multichannel detectors to obtain a vast data set that is applied an appropriate mathematical treatment in order to determine the individual contribution of each analyte in the mixture. The methods thus developed simplify the application of analytical procedures considerably inasmuch as they allow different analytes to be determined without the need for time-consuming, error-prone separation steps.

This paper describes various computational methods for the resolution of mixtures by multicomponent analysis, as well as several applications developed by our group for the analysis of environmental samples based on a number of analytical techniques.

THEORETICAL BACKGROUND

(a) Linear-fitting resolution systems

Resolution of two components

Simultaneous equations. The spectrophotometric resolution of mixtures of two components on the basis of the well-known extended Beer's law relies on absorbance measurements at two different wavelengths and the fulfillment of the law of the additivity of absorbances[2]. This methodology has several shortcomings like the use of only two experimental data obtained at two different wavelengths. While the method could in principle be used to resolve up to *n* components by making measurements at as many wavelengths, it has not been applied to more than two components because its accuracy decreases sharply as the number of determinants involved grows[3].

Linear regression. The precision of the above-described method in the resolution of multicomponent mixtures can be increased by performing measurements at more wavelengths. Thus, for a given wavelength λ_i,

$$\frac{A_m^{\lambda_i}}{A_{s_1}^{\lambda_i}} = \frac{c_1}{c_{s_1}} + \frac{A_{s_2}^{\lambda_i} c_2}{A_{s_1}^{\lambda_i} c_{s_2}} \tag{1}$$

where $A_m^{\lambda_i}$, $A_{s_1}^{\lambda_i}$, and $A_{s_2}^{\lambda_i}$ are the measured absorbances of the sample and of the corresponding standards (S_1 and S_2) at wavelength λ_i. The C_{s1}, C_{s2}, C_1 and C_2 are the concentrations of standards and components of the mixture, respectively. From eq. (1) follows that, by plotting the $(A_m^{\lambda_i}/A_{s_1}^{\lambda_i})$ values against the $(A_{s_2}^{\lambda_i}/A_{s_1}^{\lambda_i})$ values measured at different wavelengths one will obtain a straight line whose intercept and slope will provide the sought c_1 and c_2 values. Blanco *et al.*[3] compared these two procedures for the determination of binary mixtures with highly overlapped spectra obtaining better results by the multi-wavelength linear regression method.

Resolution of more than two components

From the above considerations immediately arises the question of whether more than two components can at all be resolved. In fact, multiple linear regression methods do allow for multicomponent resolution.

Provided matrix effects and any chemical interactions involved are negligible, an overall analytical response can be assumed to consist of several additive individual responses from as many components (law of additivity of spectroscopic absorbances, polarographic currents, *etc.*). This can be expressed in mathematical terms by means of the following equation:

$$l^m = k_0 + \sum_{i-1}^{n} k_i^m \, c_i \,; \qquad \forall m = 1 \ldots n_m \tag{2}$$

where subscripts i denote the components and superscripts m the measuring channels used (*e.g.* wavelengths in asborptiometry and potentials in polarography), and k_0 is an independent term required to account for a non-zero intercept. Including this last term is advisable even if a zero intercept is to be expected (*e.g.* from Beer's law), particularly if the sample signal is fairly small. In this way the small deviations from the theoretical value will be ascribed by the fitting programme to such a term rather than the concentration values, which avoids substantial errors in the calculated concentrations. If the number of measuring channels (n_m) exceeds that of components (n), then the above system will be over-dimensioned and resolvable by multiple linear regression. Such a system can be expressed in matrix form as

$$\mathbf{L} = \mathbf{K} \times \mathbf{C} \tag{3}$$

which entails solving two analytical chemical problems, namely:

(1) First, one must determine the proportionality constants from matrix **K** by using standards of known concentration, and

(2) once matrix **K** has been determined, one must resolve the unknown mixtures so as to determine the concentration matrix, **C**, from the following equation

$$\mathbf{C} = (\mathbf{K'K})^{-1}\mathbf{K'L} \tag{4}$$

where **K'** denotes de transpose of **K**.

Matrix **K** can be determined in a number of ways including the following[4]:

(i) By using single or averaged standards. This is the simplest procedure and involves the use of a single or averaged standard of known concentration for each component and the calculation of the corresponding response factor from

$$k_i^m = \frac{l_s^m}{c_{s_i}} \tag{5}$$

(ii) By using several standards of the components and their mixtures. This option entails calculating the different K_i^m values by regression from standards of different concentrations of each component or mixtures of known composition. Mathematically, the procedure involves an equation system for each measuring channel of the form

$$l_s^m = z^m + \sum_{i-1}^{n} k_i^m \, c_s \,; \qquad \forall s = 1 \ldots n_s \tag{6}$$

where l_s^m denotes the reading obtained for standards s in measuring channel m, z^m the independent term of the fitting for each m value, and c_{si} the concentration of component i in standard s. This equation system can be solved by multiple linear regression provided that the number of standards used, n_s, is larger than that of components. On solving the system one obtains the k_i^m and z^m values, as well as the deviation of the fitting for each measuring channel, d^m, which can be calculated from

$$d^m = \sqrt{\frac{\sum_{s=1}^{n_s}(l^m_{s,\,exp} - l^m_{s,\,calc})^2}{n_s - n}} \qquad (7)$$

The d^m values can subsequently be used in resolving the unknown mixture so as to carry out a weigthed fitting of the initial equation in such a way that the measuring channels with the greatest deviations will have a smaller weight than the rest. Thus, the equation to be used is

$$\frac{l^m}{d^m} = k_0^l + \sum_{i=1}^{n} \frac{k_i^m}{d^m} c_i\,; \qquad \forall\, m = 1 \ldots n_m \qquad (8)$$

(iii) By using the multiple standard addition method. Equation (6) can be applied to a data set obtained by spiking the unknown samples with known amounts of the components to be determined. In this case, the c_{si} values will correspond to the added concentrations of each component in each standard. As above, the regressions performed for each measuring channel will provide the k_i^m and z^m values, which will represent estimates of the sensitivity of each component and the sample signal (in the absence of additive interferences), respectively. Finally, by using eq. (2) with the z^m values one can calculate the concentration of each component in the unknown sample.

(b) Non-linear systems

The resolution of mixtures is additionally complicated if the mathematical model of the chemical system is non-linear, which compels one to use fitting procedures other than those of linear regression. This involves the minimization of functions of the form

$$U(p_i, c_i) = \sum_{m=1}^{n_m}(l^m_{exp.} - l^m_{calc.})^2 \qquad (9)$$

where U, the function to be minimized, depends on various parameters and/or concentrations, and l_{exp} and l_{calc} denote readout values and their theoretical counterparts. The sought p_i and c_i values are obtained from starting values that are strategically changed iteratively in order to minimize U.

The minimum of function U can be determined in a number of ways. Provided that the mathematical model concerned can be readily derived with respect to the different variables that are to be calculated, then one should use the Gauss-Newton method or one of its subsequent versions[5] as it usually involves a reasonably small number of iterations. Otherwise, one should resort to the Simplex method,[6] an iteration device that uses the smallest possible number of iterations and relies on the successive values of the function at the assayed points, when the mathematical model can not be derived.

(c) Evaluation of the contribution of unknown species

The chief limitation of the above-described methods is that they require all the species contributing significantly to the measured signal to be known beforehand. This pitfall has lately been circumvented by developing new chemometric procedures. These involve using a further dimension (*e.g.* time) in order to obtain additional information on the changes undergone by the data structure along the chosen extra dimension. Obviously, the mathematical treatments involved are much more complex, particularly when more than two components are to be resolved. Among the commonest chemometric techniques used for this purpose are principal component analysis (PCA) and factor analysis (FA).

This methodology was first applied to analytical chemical problems by Lawton and Sylvestre[7] and has more recently been used in different models by other authors.[8–10] First, it allows one to determine the number of components significantly contributing to the analytical signal. Then, it permits the signal (spectrum, polarographic wave) of each individual component to be reconstructed, which finally allows the system to be resolved analytically. Different attempts made so far have only succeeded in resolving systems of three or less components, which testifies to the mathematical complexity of the problem.

Fortunately, routine analyses usually involve known components and interferences, so they rarely call for such complicated techniques.

SOFTWARE

The software required for the applications described below as regards data acquisition and processing was written in BASIC or QuickBasic for PC-compatible computers at our laboratory. The specific programmes used are listed in the Appendix.[1]

ANALYTICAL APPLICATIONS

Below are critically discussed some applications of multicomponent analysis in terms of their advantages and disadvantages, and the accuracy and precision achieved in each instance.

(a) Spectrophotometric methods

Ultraviolet-visible spectrophotometry lends itself readily to the resolution of multicomponent mixtures for several reasons, namely:

(a) The current availability of effective instrumentation for the rapid acquisition of

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[1] The software used can be obtained on request from SCIWARE, Banco de Programas, Departamento de Química, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain.

spectra—diode array spectrophotometers allow spectra to be recorded in as little as 0.2 s—with a high reproducibility in the wavelength,

(b) the typically fairly low purchase and maintance costs of such instrumentation,

(c) the high sensitivity levels it affords, which are adequate for many applications, and

(d) its ease of handling and automation.

It is therefore unsurprising that multicomponent techniques have most extensively been used in this type of application, of which some representative examples are described below.

Simultaneous spectrophotometric determination of metal ions

It is interesting that multicomponent analysis has changed some of the basic objectives of analytical chemists. Thus, analysts have traditionally strived to find a specific reagent for each species to be determined so as to avoid any potential interferences. On the other hand, the aim in multicomponent analysis is to find a reagent reacting with a number of analytes to yield products with appreciably different features (*e.g.* their spectra) that can be exploited for their determination.

Thus, while specific reagents such as dimethylglyoxime for the determination of nickel in environmental samples would in principle be the ideal choice, multicomponent analyses involving this metal are performed by using such a scarcely selective reagent as 4-(2-pyridylazo)-resorcinol (PAR) because it allows even quaternary mixtures to be readily resolved with determination limits below the μg/ml level[11]—PAR is admittedly not very selective, but it is highly sensitive. Thus, despite of the extensive spectral overlap, multiple linear regression software such as MULTIC, which uses the single-standard calibration method described above, succeeds in resolving up to quaternary mixtures without too much trouble.

Simultaneous spectrophotometric determination of phenol compounds

More complex mixtures than those referred to in the previous section are better resolved by using multiple-standard calibration methods and spectral derivatives, which enhances discrimination between components. By using one such method, mixtures of up to 9 phenol compounds with highly overlapped spectra have been effectively resolved.[4]

The phenol mixtures were resolved with the aid of the programme MULTI3, which allows implementation of the different types of calibration described above, as well as the mixtures to be resolved in a weighted fashion [see eq. (8)]. Table 1 summarizes the results obtained (as average errors for the 9 components) by applying MULTI3 to various mixtures of 9 phenols using the single (standarized) standard, multiple standard and weighted multiple standard methods on the absorption spectra and their first two derivatives. The two multiple standard methods provide substantially smaller errors than their single-standard counterparts in dealing with the absorption spectra. If the first spectral derivative is used, the errors made by the single-standard and multiple unweighted standard methods are of the same order, whereas those arising from the weighted multiple standard method are somewhat smaller.

Table 1 Average percent errors made in the resolution of various mixtures of 9 phenol compounds by applying different calibration methods to the absorption spectra (N) and their first (D1) and second derivative (D2).

		SS			*MS*			*WMS*	
Mixture	*N*	*D1*	*D2*	*N*	*D1*	*D2*	*N*	*D1*	*D2*
M1	20.4	10.7	22.9	9.5	11.7	16.8	8.9	7.8	10.1
M2	22.9	9.6	23.1	8.5	10.8	16.5	7.5	7.3	9.2
M3	18.3	10.3	23.5	11.8	11.7	16.2	10.5	7.7	8.7
M4	21.0	9.3	19.8	9.8	10.5	11.5	8.2	7.1	7.6
M5	20.3	9.7	17.8	10.2	10.1	9.9	9.9	8.3	9.1
M6	18.2	8.0	13.0	10.9	6.7	7.6	11.3	6.4	7.3
M7	33.7	8.8	15.3	6.1	7.2	7.6	5.9	6.1	6.7
M8	29.3	21.1	60.2	10.0	23.6	57.3	11.2	5.0	10.2
M9	22.3	7.5	13.3	14.8	8.3	8.7	14.6	7.0	7.5
M10	21.5	7.4	71.6	10.4	7.7	8.0	10.3	6.9	7.8
M11	12.2	8.4	10.2	17.7	9.4	8.3	17.6	10.3	7.9
M12	10.7	10.2	13.8	19.6	11.3	10.5	18.7	9.2	9.7

SS: Standarized single standard method
MS: Unweighted multiple standard method
WMS: Weighted multiple standard method

When the second derivative is used the errors made by using the weighted multiple standard method are similar to those resulting from its application to the first spectral derivative. Also these last results are more accurate than those provided by the single-standard and the unweighted multiple standard methods.

The spectral derivatives are subject to higher background noise, which somehow offsets the inherently enhanced spectral discrimination. The single-standard method is obviously the most sensitive to noise, whereas that of weighted multiple standards is the least as a result of the smallest weights being assigned to the noisiest wavelengths—those giving rise to the poorest regressions in the calibration step.

Simultaneous spectrophotometric determination by flow injection analysis

It is in flow analysis techniques that the assets of microelectronics and microcomputing reflect most clearly inasmuch as they take full advantage of the high speed at which data can be acquired and processed, and are most ready to automate.

By way of example we shall comment on an automatic system for the simultaneous determination of Ca and Mg in water by unsegmented flow injection analysis (FIA).[12] The set-up used can be operated at a rate of 70 samples/h.

Instrumental control and data acquisition are entrusted to the programme DARRAY, which can acquire the absorption spectrum at each point along the FIA recording. Mixtures are resolved with the aid of the programme MULTIC, which is applied to the spectra obtained at the maximum of the FIA peaks.

Table 2 lists the results obtained for a series of synthetic samples containing Ca and Mg in different proportions.

As one would expect, the determination is interfered by a number of species owing to the

Table 2 Results and standard deviations obtained in the simultaneous resolution of Ca and Mg in synthetic mixtures by applying multicomponent analysis to FIA data.

Added		*Found*	
[Ca] (mg/l)	*[Mg] (mg/l)*	*[Ca] (mg/l)*	*[Mg] (mg/l)*
10	0	9.7 (0.4)	0.04 (0.03)
0	10	0.0 (0.6)	9.8 (0.4)
1	4	1.37 (0.08)	4.15 (0.03)
1	8	1.3 (0.1)	8.2 (0.2)
1	14	0.8 (0.1)	14.1 (0.1)
4	4	3.9 (0.1)	3.77 (0.07)
4	8	3.87 (0.09)	7.50 (0.08)
4	14	3.3 (0.1)	13.23 (0.08)
8	4	7.7 (0.2)	4.0 (0.1)
8	8	7.4 (0.2)	7.2 (0.1)
8	14	6.79 (0.08)	12.34 (0.06)

low selectivity of PAR. Fortunately, determining Ca and Mg in water entails a prior strong dilution, so most potential interferents do not interfere as they are much too diluted to have any effect. Only Zn and Cu may cause an appreciable disturbing effect. The difficulty involved in their removal compelled their joint determination with the analytes (Ca and Mg).

(b) Fluorimetric methods

We applied multicomponent analysis with fluorimetric methods by using the programme package FLUOROPACK[13], also developed at our laboratory. In addition to the typical functions of fluorimeters (*i.e.* recording excitation, emission and synchronous spectra, among others), it allows one to record three-dimensional spectra, contour maps and, more interesting, variable-angle fluorescence spectra. This technique allows user-selected directions in a contour map to be followed through fluorescence peaks of the different substances potentially present. This option of the FLUOROPACK package is specially useful for multicomponent analyses.

First, we will consider the mixture of three pesticides (warfarin, carbaryl and fuberidazol)[14]. After the ideal path is chosen on the topogram of the three-dimensional spectra of mixture, obtaining the variable-angle fluorescence spectra takes only a few minutes. If the chosen path is selective enough, each of its straight portions allows the virtually specific determination of one of the pesticides with no interferences from the other two.

Variable-angle fluorescence thus usually allows each individual component in a mixture to be conveniently determined. However, there are some exceptions. Thus, finding a given pathway crossing zones where only one polycyclic aromatic hydrocarbon (PAH) in a mixture of benz[a]anthracene, anthracene, phenanthrene and pyrene contributes to the signal is virtually impossible, as can be seen from Figure 1[15]. The figure 1 c shows the path chosen as a compromise between acceptable selectivity and sensitivity. Even though the path in question is the best of all possible in this instance, there is still extensive overlap in the variable-angle fluorescence spectra, though not to the extent obtained in the conventional

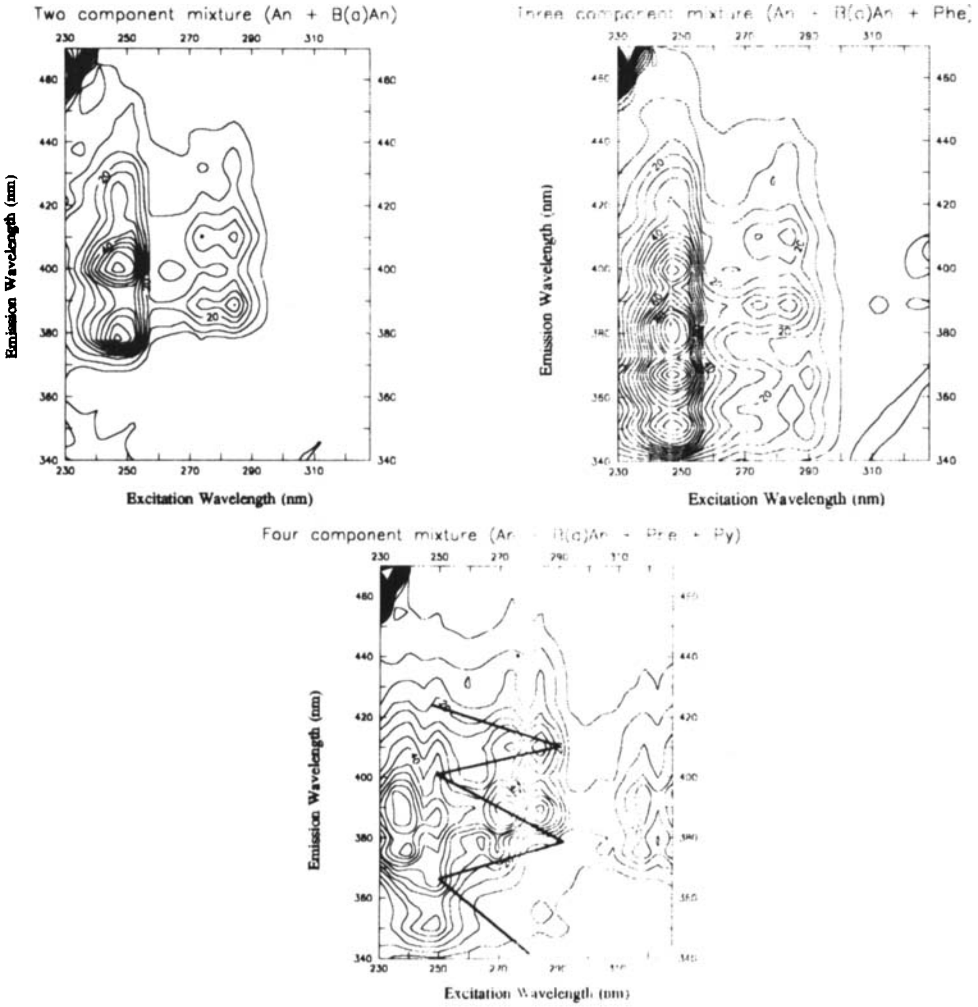

Figure 1 Topograms of the fluorescence spectra of mixtures of PAHs. (a) Binary mixture of BAN (16 ng/mL) and AN (8 ng/mL). (b) Ternary mixture of BAN (20 ng/mL), AN (2 ng/mL) and PHE (8 ng/mL). (c) Quaternary mixture of BAN (16 ng/mL), AN (16 ng/mL), PHE (40 ng/mL) and PY (80 ng/mL). The variable-angle path used to resolve the mixtures is also shown.

spectra. The above spectra can be processed by using a multicomponent programme as described for UV-visible spectra. Table 3 gives some results obtained in the analysis of quaternary mixtures of the aforementioned PAHs in different proportions by using the programme MULTIC for resolution.

(c) Electrochemical methods

The currents involved in some electrochemical techniques such as differential pulse polarography (DPP) and anodic stripping voltammetry (ASV) obey the law of additivity, so they

Table 3 Results obtained in the analysis of quaternary mixtures of PAHs by applying multicomponent analysis to asynchronous fluorimetric spectra.

Added (ng/ml)				*Found (ng/ml)*			
B(a)a	*Ant*	*Phen*	*Pyr*	*B(a)a*	*Ant*	*Phen*	*Pyr*
16	1.6	40	80	15 ± 0.2	1.4 ± 0.05	38 ± 0.7	82 ± 1.5
40	4.0	80	80	38 ± 0.4	3.4 ± 0.09	85 ± 1.5	81 ± 3.0
80	8.0	80	32	74 ± 0.6	7.0 ± 0.16	76 ± 2.5	34 ± 5.1
16	3.2	200	160	16 ± 0.4	3.2 ± 0.00	181 ± 1.5	163 ± 3.1
80	3.2	120	112	76 ± 0.6	2.6 ± 0.14	113 ± 2.3	111 ± 4.6
120	1.6	40	80	110 ± 0.8	1.5 ± 0.19	39 ± 3.1	85 ± 6.4
50	2.0	100	140	48 ± 0.3	1.6 ± 0.08	97 ± 1.3	139 ± 2.7

are suitable for application of eq. (2). However, matrix effects on the sensitivity of electrochemical methods are much more marked than they are on spectrophoto metric methods, which suggests the advisability of using the multiple standard addition method to resolve mixtures.

A representative application in this context is the resolution of quaternary mixtures of Pb(II), TI(I), In(III) and Cd(II) by DPP and ASV.[16] Electrochemical measurements were made by using a polarographic analyser that was controlled via a computer running software. The data files obtained were processed by the programme MULTI3 for multicomponent resolution.

Satisfactory results were obtained for synthetic mixtures of the above-mentioned ions by using the DPP technique and the multiple standard method for calibration. However, such a calibration method was found to be inappropriate for the ASV technique because the waves of the different ions—particularly that of lead—under-went small shifts along the potential axis on each stripping, which precluded usage of individual standards. Nevertheless, the multiple standard addition method provided acceptable results with this technique.

Table 4 shows the results obtained by analysing water samples spiked with various amounts of the above-mentioned ions using both DPP and ASV, and the multiple standard addition method. We should note here the marked matrix effect observed in the analysis of the wastewater by ASV. Figure 2 shows the voltammograms of a synthetic mixture of the four ions (a) as well as a wastewater sample spiked with the same amounts of the ions (b). In both cases the voltamograms of the standard additions are also shown. The wastewater

Table 4 Results and confidence ranges obtained in the analysis of various water samples spiked with Pb, Tl, In and Cd by using the multiple standard addition method.

		Added (μM)				*Found (μM)*			
Tech.	*Sample*	*Pb*	*Tl*	*In*	*Cd*	*Pb*	*Tl*	*In*	*Cd*
DDP	Tap	10	10	—	10	10.8±0.2	9.5±0.4	—	11.9±0.3
	Tap	10	20	10	10	10.8±0.3	19.9±0.5	10.9±0.4	10.2±0.3
	Tap	10	20	15	10	10.1±0.7	19.7±1.3	20.6±1.3	7.8±0.9
	Tap	10	20	20	10	11.0±0.6	19.2±1.2	27.4±1.1	6.7±0.7
ASV	Tap	0.05	0.30	0.10	0.15	0.060±0.002	0.271±0.005	0.106±0.003	0.144±0.003
	Waste	0.05	0.30	0.10	0.15	0.077±0.003	0.291±0.004	0.040±0.002	0.120±0.008

matrix introduced a marked effect on the sensitivity, notwithstanding which the multiple standard addition method provided more than acceptable results for this sample.

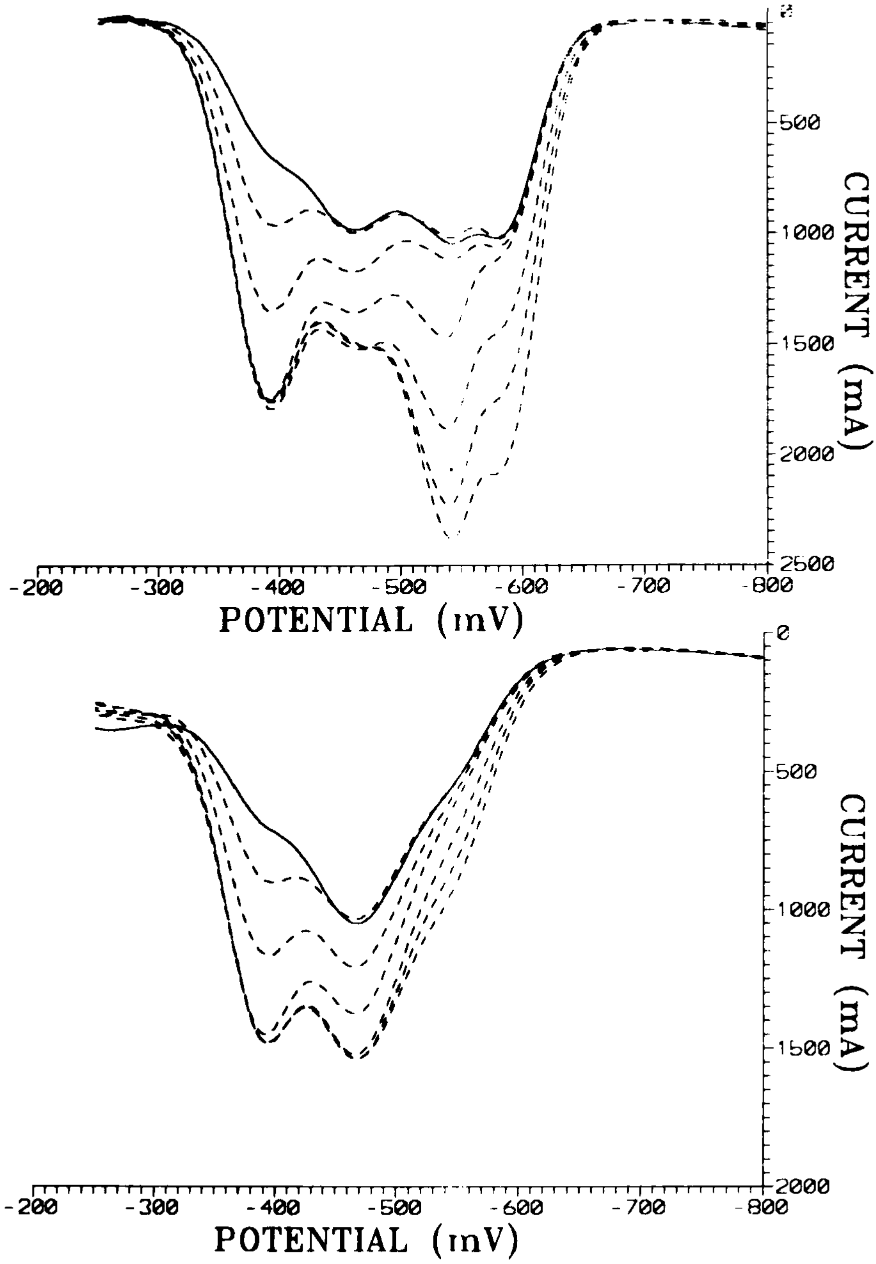

Figure 2 Anodic stripping voltammograms obtained for: (a) a synthetic sample containing 50 nM Pb(II), 300 nM Tl(I), 100 nM In(III) and 150 nM Cd(II); and (b) a wastewater sample spiked with the same amounts of the four ions.

(d) Kinetic methods

Multicomponent analysis as applied to kinetic methods has some distinct features on account of the special data structure involved.

Simultaneous determinations of catalysts and activators

There are some precedents of the individual determination of both types of species, yet there seems to be no literature reference to their joint determination.

Kinetic methods often rely in the use of calibration curves obtained by plotting the initial reaction rate (slope of the kinetic curve) against the concentration. The presence of an activator (or inhibitor) alters the slopes of the curves to an extent proportional to its concentration.

In the standard addition method, which involves the addition of gradually increasing amounts of a standard to the sample, the unknown analyte concentration is obtained by extrapolating the curve to a zero intercept. In the presence of different amounts of activator, the curves extrapolated to a zero intercept coincide at a given catalyst concentration. Therefore, the slope of the curves provides the activator concentration, while their extrapolation yields that of the catalyst. We shall term this procedure "graphical method".

The above-described behaviour can be modelled mathematically. Thus, the initial reaction rate is given by

$$v=A+(B+C[X])\,[Y] \tag{10}$$

where A denotes the rate of the uncatalysed reaction, and B and C the contributions of the activator (X) and catalyst (Y), respectively. Application of the multiple standard addition method to this equation yields

$$v=A+\{B+C\,([X]_x+[X]_i)\}\,([Y]_x+[Y]_i) \tag{11}$$

where subscripts x and i denote the analyte concentrations in the sample and the added amounts of standard, respectively.

Parameters A, B and C in eq. (11) can be previously estimated by using standard solutions and applying an optimization procedure (*e.g.* Gauss-Newton, Simplex) to eq. (10). Once their values have been determined, the multiple standard addition method is used to resolve the unknown mixtures, which entails optimizing eq. (11). In principle, this last equation could be used to determine the above parameters and concentrations jointly. The results thus obtained, however, are somewhat worse.

Table 5 lists the results obtained by applying the above-described methodology to the determination of Mn and Pb in water from kinetic thermometric data compiled from the oxidation of Tiron by hydrogen peroxide in the presence of manganese as catalyst and lead as activator.[17] The different optimization operations involved were performed by using programmes for calculation of the parameters and for determining the concentrations by the Gauss-Newton and Simplex methods.

Similar results can be obtained by kinetic spectrophotometric monitoring[18,19] or any other suitable analytical technique.

Table 5 Comparison of the results obtained by using various calculation methods in the determination of Mn and Pb as activator and catalyst, respectively, of thermometric kinetics.

Method	*[Mn]* (ng/ml)	*[Pb]* (μg/ml)
Reference value (GFAAS)	1.9	0.80
Standard additions, Graphical	1.2	0.75
Standard additions, Gauss-Newton	1.6	0.71
Standard additions, Simplex	1.6	0.71

Resolution of simultaneous kinetic processes

The resolution of systems of several components that evolve kinetically is usually addressed by using differential reaction-rate methods, which call for appreciable differences between the rate constants of the reactants involved in order that they may be resolved satisfactorily[20]. This major constraint can be overcome by using multichannel detectors to monitor kinetic processes. This type of detector provides more than acceptable results as long as the spectral features of the reactants are sufficiently distinct.

In broad terms, for a mixture of n components each conforming to a given kinetics, the temporal evolution of the signal they yield can be expressed as

$$I_t^m = \sum_{i=1}^{n} f_i\,(c_i, t, m); \qquad \forall\, m = 1 \ldots n_m \text{ and } t = t_0 \ldots t_f \tag{12}$$

where $I^m{}_t$ is the signal measured by channel m at time t, f_i is the kinetic law obeyed by component i, and c_i is the initial concentration of such a component. As a rule, f_i will be a non-linear function dependent on various parameters (the sensitivity for each channel m, the rate constants, *etc.*). Equation (12) thus defines a system of non-linear equations that can be solved by using one of the above-mentioned optimization methods.

This methodology was applied to the resolution of Co(II)-Ni(II) mixtures on the basis of the chemical model developed by Tanaka *et al.*,[21] which relies on the kinetics of displacement of EGTA from its metal complexes by PAR[22]. The appearance of the PAR complexes was monitored by UV-visible spectrometry. The process was found to conform to a *pseudo* first-order kinetics, so eq. (12) was transformed into

$$A_t^m = \varepsilon_{Co}^m\, b c_{Co}(1-e^{-k_{Co}t}) + \varepsilon_{Ni}^m\, b c_{Ni}(1-e^{-k_{Ni}t}) \tag{13}$$

The best computational strategy was found to be that first involving the calculation of the absorptivities (ε) of the Co-PAR and Ni-PAR complexes from standard solutions in one series of experiments. Then the rate constants and concentrations of the metal ions by optimizing eq. (13) from data were obtained by multiple additions of standard to the sample in another series of experiments. Table 6 summarizes the results thus obtained in the analysis of various synthetic samples. Optimizations were carried out by using the Gauss-Newton method.

Table 6 Results obtained in the resolution of synthetic samples by simultaneously optimizing concentrations and rate constants.

Sample	*Added (μg/ml)* *Co*	*Ni*	*Found (μg/ml)* *Co*	*Ni*	$k_{Co} \times 10^4$	$k_{Ni} \times 10^5$	$RMS \times 10^3$
1	0.33	0.00	0.28	0.00	12.0	7.7	4.69
2	0.00	5.00	0.05	5.46	9.3	3.7	4.66
3	0.10	0.50	0.11	0.40	8.7	4.3	0.96
4	0.10	4.00	0.10	4.20	9.6	4.2	1.58
5	0.30	2.50	0.28	2.02	10.2	6.2	3.28
6	0.50	1.00	0.45	0.94	10.7	5.0	2.52
7	0.70	0.50	0.65	0.43	11.0	4.3	3.58
8	0.80	4.00	0.54	3.29	13.8	5.2	3.27

RMS = Mean of the sum of residuals provided by the program NGOPT

Resolution of simultaneous kinetics by factor analysis

As noted earlier, there are a number of chemometric techniques - FA included- that allow the number of components in a given sample and their individual contributions to the overall signal to be determined from the temporal evolution of spectra without the need for a prior knowledge of the individual spectra of the components concerned.

Even though these techniques have so far been applied preferentially to chromatographic data[23], those obtained by monitoring simultaneous kinetic processes with the aid of a multichannel detector—see preceding section—are equally liable for treatment, which enables such processes to be resolved even if neither the spectra of the analytes involved nor the kinetic laws they conform to are known.

In a recent paper[24] we simulated various models of formation and disappearance kinetics by means of the programme FASIMKIN and subsequently applied the displacement reaction procedure to the EGTA complexes of Co(II) and Ni(II), in which the ligand was substituted by PAR. The experimental data obtained were arranged in a matrix **D** where each row corresponded to one spectrum that was acquired at a given time and each column thus represented the kinetic curve obtained at a given wavelength. The first step involved in applying factor analysis was determining the eigenvectors and eigenvalues of the dispersion matrix (**D'D**). From the eigenvectors thus obtained was determined the number of species significantly contributing to the temporal variation of the spectra. Figure 3 shows the first three eigenvectors obtained by processing a data matrix. As can be seen, only the first two vectors provide significant information, which means that the system in question consists of only two components.

The next step involved evaluating the spectra of the two components. This required the following two constraints to be imposed:

(a) The spectra of the pure components should include no negative absorbance values, and

(b) all recorded spectra should be expressable as a linear combination—with positive coefficients only—of the pure spectra.

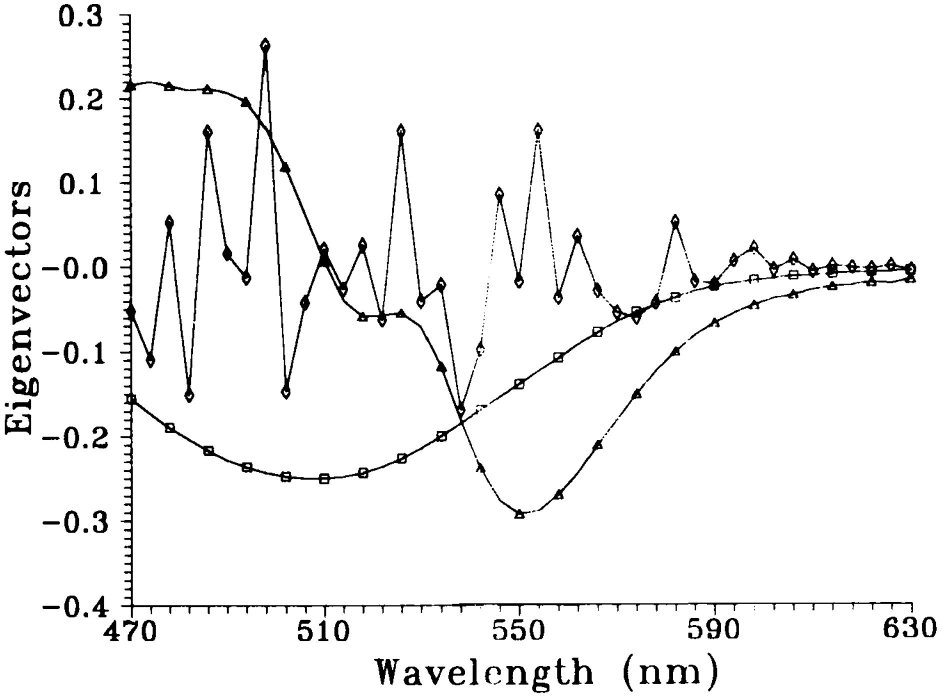

Figure 3 Resolution of Co(II)-Ni(II) mixtures by factor analysis: plot of the first three eigenvectors of the data matrix [(□) first eigenvector; (△) second eigenvector (◇); third eigenvector].

Application of these two constraints yielded a set of solutions for the pure spectrum of each component. In the best of cases, *viz.* when neither the concentration profiles nor the spectra of the components overlap, such solution sets were very narrow. Otherwise, broader sets were obtained which by themselves did not allow the pure spectra of the components to be determined, so additional information was required.

Finally, after the spectra of the pure components had been obtained, the individual kinetic curves were reconstructed and subsequently processed by using one of the classical methods for calculating concentrations.

(e) Monitoring of liquid-liquid extraction processes

One interesting application of multicomponent techniques is monitoring processes involving several components that cannot be reproduced from measurements on the individual species. Such is the case with liquid-liquid extraction processes, where the extraction of a given component may depend on the presence or absence of others. In one such application our group developed a continuous extraction/back-extraction system in which the compounds of interest were extracted by means of a solvent that was propelled through a closed circuit into a back-extracting solution. The extraction process was followed by monitoring the changes in this last solution with the aid of a diode array spectrophotometer. This set-up was applied to the extraction of phenol compounds from an aqueous matrix.[25] Phenols in a previously acidified sample were extracted into n-hexane and subsequently back-extracted into 1 M NaOH. The procedure provided appreciable extraction of six of the phenols assayed, *viz.* phenol, 2,4-dinitrophenol, 2-chlorophenol, 2-nitrophenol, 4-bromophenol and 2,3,5-trimethylphenol. The spectra obtained at different extraction times were processed by the programme MULTI3 in order to calculate the amount of each phenol extracted at each time.

(f) Resolution of overlapped HPLC peaks

A number of mathematical procedures for assessing the individual contributions of substances to overlapped chromatographic peaks have been reported in recent times. Among them, those based on the second derivative of the chromatographic peaks (the limitations of which were recently reviewed by Gurshka and Israeli[26]) and on their deconvolution (which rely on the assumption that all the peaks involved conform to a given function[27-29]) are worth special note.

The inception of multichannel detectors for monitoring chromatographic processes has opened up new prospects for the resolution of overlapped peaks[9]. This type of detector expands the available information on the mixture components that yield the chromatographic peaks.

Because the qualitative nature of the compounds that may co-elute in a given chromatographic peak is usually quite well-known, so the mathematical treatment required is normally quite simple. The above-described multicomponent analysis methods allow one to readily calculate the contribution of each component to a chromatographic peak from the spectra measured at it. This can be accomplished in various, ways, namely:

(a) Inasmuch as the spectrum at each point along the chromatogram is known, the multicomponent analysis method of choice can be applied to every spectrum so as to obtain the individual elution profiles of the compounds. However, this involves an inordinate number of calculations even though only the concentrations of the different components need be calculated as is usually the case in most practical instances.

(b) Application of multicomponent analysis to a single point of the chromatographic peak (usually the maximum) may directly provide the concentration of each component. This has the disadvantage of potential irreproducibility in the measuring point and the problem involved in acquiring the spectra of the standards, which should be obtained from the pure components at the same point as that used for the analysis—this is not always easy.

(c) One plausible solution to the above-described problems entails applying multicomponent analysis to the so-called "area spectrum" of the chromatographic peak, which is obtained by calculating the area of the peak for each measuring channel (*viz.* each wavelength in absorptiometric techniques). This can be done quite reproducibly inasmuch as it is whole peaks (of the sample and standards) rather than single points that are used. Since peak areas are proportional to the amounts of analyte, eq. (2) is accurately applicable to this type of data in much the same way as with conventional spectra.

Table 7 Results and confidence limits (95%) obtained by applying the programme MULTI3 to the chromatographic peak recorded on injection of mixtures of 3-nitrophenol (3NP) and 4-nitrophenol (4NP).

Injected volume (μL)	*Added (μg)* *4NP*	*3NP*	*Found (μg)* *4NP*	*3NP*
10	0.88	0.07	0.86 ± 0.01	0.06 ± 0.01
50	0.29	5.43	0.25 ± 0.01	5.56 ± 0.01
50	1.47	1.81	1.46 ± 0.01	1.91 ± 0.01
30	0.88	1.09	0.95 ± 0.01	1.17 ± 0.01
50	1.47	0.00	1.52 ± 0.01	0.04 ± 0.01
30	0.88	0.00	0.92 ± 0.01	0.00 ± 0.01
50	0.00	1.81	0.04 ± 0.01	1.85 ± 0.01
30	0.00	1.09	0.04 ± 0.01	1.12 ± 0.01
10	0.00	0.36	0.05 ± 0.01	0.37 ± 0.01
30	1.76	1.09	1.73 ± 0.01	1.17 ± 0.01
10	0.59	0.36	0.59 ± 0.01	0.36 ± 0.01
50	1.47	3.62	1.45 ± 0.01	3.77 ± 0.01
30	0.88	2.17	0.91 ± 0.01	2.34 ± 0.01
10	0.29	0.72	0.31 ± 0.01	0.76 ± 0.01
50	2.06	0.72	2.08 ± 0.01	0.78 ± 0.01
50	0.88	1.81	0.85 ± 0.01	1.88 ± 0.01
30	0.53	1.09	0.57 ± 0.01	1.18 ± 0.01

This last methodology was used for the resolution of highly overlapped peaks obtained by applying the HPLC method of Alarcón and Bustos[30] to the determination of 20 phenol compounds that were monitored by means of a diode array spectrophotometer[31]. The different overlapped peaks involved were processed as described above. Data were acquired and the "area spectra" calculated by using the programme DARRAY, the spectra in turn being processed with the programme MULTI3. Tables 7 and 8 list the results obtained by processing two overlapped peaks among those acquired on injection of various mixtures of the phenols.

Because of the nature of this methodology, its performance does not depend on the degree of overlap of the chromatographic peaks, nor on small oscillations in their shapes or retention times, but only—and largely, in common with other multicomponent analysis methods—on the degree of spectral overlap.

CONCLUSIONS

Multicomponent analysis techniques have opened new prospects for the resolution of diverse analytical systems. Frequently the application of these techniques requires some chemical or instrumental innovations respect the previous procedures.

Among the referred algorithms, the multi-linear regression method has often been used because of its easy implementation and the good results obtained in most cases. The chemical interferents are the main limitation of such techniques because a prior knowledge of each

Table 8 Results and confidence limits (95%) obtained by applying the programme MULTI3 to the chromatographic peaks recorded on injection of mixtures of 2,4-dichlorophenol (24DCLP), 2,3,5-trimethylphenol (235TMP) and 2,3,6-trimethylphenol (236TMP).

Injected Volume (µL)	*Added (µg)*			*Found (µg)*		
	24DCLP	*235TMP*	*236 TMP*	*24DCLP*	*235TMP*	*236 TMP*
50	2.03	2.74	2.77	1.92 ± 0.01	2.89 ± 0.03	2.42 ± 0.14
30	1.22	1.64	1.66	1.21 ± 0.01	1.62 ± 0.01	1.76 ± 0.06
10	0.41	0.54	0.55	0.39 ± 0.01	0.45 ± 0.01	0.64 ± 0.06
50	0.81	3.04	0.00	0.81 ± 0.01	3.12 ± 0.02	0.09 ± 0.08
30	0.49	1.82	0.00	0.51 ± 0.01	1.92 ± 0.01	0.06 ± 0.06
50	0.00	3.04	0.79	0.00 ± 0.01	3.23 ± 0.02	0.74 ± 0.07
10	0.00	0.12	0.79	0.03 ± 0.01	0.14 ± 0.01	0.83 ± 0.05
50	4.06	0.00	0.79	4.07 ± 0.01	0.00 ± 0.01	0.84 ± 0.06
30	2.44	0.00	0.48	2.37 ± 0.01	0.00 ± 0.01	0.55 ± 0.06
50	0.81	0.00	5.94	0.72 ± 0.01	0.28 ± 0.02	5.43 ± 0.11
30	0.49	0.00	3.56	0.45 ± 0.01	0.07 ± 0.01	3.58 ± 0.05
30	0.24	2.74	0.24	0.23 ± 0.01	2.78 ± 0.01	0.27 ± 0.06
50	4.06	3.04	0.40	4.07 ± 0.01	3.12 ± 0.02	0.38 ± 0.09
10	0.81	0.61	0.08	0.81 ± 0.01	0.70 ± 0.01	0.08 ± 0.05
50	4.06	0.30	3.96	3.99 ± 0.01	0.29 ± 0.02	4.19 ± 0.09

substance which contributing to the overall signal is needed. In this case multiplicative interferences can easily be addressed using the multiple standard addition method. The elimination of additive interferences has not been achieved.

The problems due to additive interferences are removed in kinetic methods since the unknown species contribution may be evaluated by extrapolation to zero time. Non-linear fitting is frequently used in kinetic methods. However it produces worse results when the number of parameters to determine is increased. Working concentrations ranges are also narrower.

On the other hand, factor analysis techniques allow resolution of multicomponent mixtures when individual contribution of each component is unknown. In broad terms, this methodology yields a solutions set for each component whose width depends on the data supplied. Nevertheless, the complexity of the mathematical treatment has actually prevented the resolution of chemical systems with more than three components.

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APPENDIX

List of programs quoted in this work

DARRAY	Universal programme for the acquisition and pre-processing of data from a Hewlett-Packard HP 8452A diode array spectrophotometer obtained by flow (HPLC, FIA, *etc.*) and kinetic methods.
FLUOROPACK	Software package for controlling the operation of a Perkin-Elmer LS-5 fluorimeter and acquiring data from it (a version for model LS-50 is currently being developed).
MULTIC	Programme for multicomponent resolution by least-squares multiple linear regression from spectra provided by the above spectrophotometric and fluorimetric detectors.
MULTI3	Extension of previous programme including all the calibration methods described under "Theoretical background".
CONVPOL	Programme for translation of data files provided by the commercially available programme POL (AMEL) to a format compatible with MULTI3.
CONVANS	Program for translation of data files provided by the commercially available programme STRIP (AMEL) to a format compatible with MULTI3.
NGOPT, GKCAL, GKAD	Programmes for optimizing functions by the Gauss-Newton method (see Applications section)
SIMKINCA, SIMKINAD	Programmes for optimizing functions by the Simplex method (see Applications section).
FASIMKIN	Programme for simulating kinetic data from theoretical functions subjected to a given noise level. With the simulated or real data calculates the eigenvectors and eigenvalues of the data covariance matrix. Finally the spectra and kinetics of the pure components by factor analysis are reconstructed.