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Chemometrics and Intelligent Laboratory Systems 71 (2004) 177–193
Chemometrics and intelligent laboratory systems
www.elsevier.com/locate/chemolab

Application of multivariate calibration and artificial neural networks to simultaneous kinetic-spectrophotometric determination of carbamate pesticides

Yongnian Ni[a,*], Chunfang Huang[a], Serge Kokot[b]

[a] *Department of Chemistry, Nanchang University, Nanchang, Jiangxi 330047, China*
[b] *Inorganic Materials Research Program, School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane, Queensland 4001, Australia*

Received 5 August 2003; accepted 6 February 2004

Available online 15 April 2004

Abstract

A method for the simultaneous determination of the pesticides, carbofuran, isoprocarb and propoxur in fruit and vegetable samples has been investigated and developed. It is based on reaction kinetics and spectrophotometry, and results are interpreted with the aid of chemometrics. The analytical method relies on the differential rates of coupling reactions between the hydrolysis product of each carbamate and 4-aminophenol in the presence of potassium periodate in an alkaline solution. The optimized method was successfully tested by analyzing each of the carbamates independently, and linear calibration models are described. For the simultaneous determinations of the carbamates found in ternary mixtures, kinetic and spectral data were processed either by three-way data unfolding method or decomposed by trilinear modeling. Subsequently, 10 different RBF-ANN, PARAFAC and NPLS calibration models were constructed with the use of synthetic ternary mixtures of the three carbamates, and were validated with a separate set of mixtures. The performance of the calibration models was then ranked on the basis of several different figures of merit with the aid of the multi-criteria decision making approach, PROMETHEE and GAIA. RBF-ANN and PC-RBF-ANN were the best performing methods with %Relative Prediction Errors (RPE) in the 3–4% range and Recovery of about 97%. When compared with other recent studies, it was also noted that RBF-ANN has consistently outperformed the more common prediction methods such as PLS and PCR as well as BP-ANN. The successful RBF–ANN method was then applied for the determination of the three carbamate pesticides in purchased vegetable and fruit samples.

Keywords: Chemometrics; Multivariate calibration; Artificial neural networks; PARAFAC; NPLS; Spectrophotometry; Kinetic; Carbamate pesticides

1. Introduction

Carbofuran (CAR), isoprocarb (ISO) and propoxur (PRO) are powerful carbamate pesticides, which can be synthesized relatively simply [1,2], and hence, are widely used in agriculture, especially in the developing countries. However, they are serious environmental pollutants and can harm people and animals because the pesticides and their derivatives can remain for a long time in soils, natural waters and other environmental domains [3]. Thus, it is important to monitor and analyze the residues of these pesticides in foods, vegetables, as well as natural and environmental substances and materials. Various analytical methods have been developed for analysis of these residues with the aid of techniques such as thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) [4,5]. Since these carbamate pesticides are unstable and can be easily decomposed, especially at relatively high temperatures, it is difficult to analyze them and their derivatives by GC. HPLC is a suitable method for their determination but has to be commonly preceded by some preprocessing steps, derivatization of compounds and post column fluorimetric labeling [6]. All such steps together with the considerable consumption of solvents and reagents add to the costs, time

* Corresponding author.
E-mail address: ynni@ncu.edu.cn (Y. Ni).

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doi:10.1016/j.chemolab.2004.02.003

and complexity of analysis. Generally, GC- and LC-MS methods are the most powerful for pesticide residue analysis but the instrumentation is quite costly [7].

Sastry et al. [8] determined carbaryl and carbofuran indirectly by voltammetry with the use of a glassy-carbon electrode. The method was based on the electrochemical activity of the hydrolysis products of the pesticides produced in an alkaline medium, and PLS was applied for prediction. However, it was found that difficulties are encountered when more than two pesticides in a mixture are analyzed by voltammetry because these compounds can be easily adsorbed at the surface of a working electrode resulting in poor repeatability.

Simple spectrophotometric methods have been applied to determine carbamate pesticides. Sastry and Vijaya [9] reported a method for spectrophotometric determination of carbaryl and propoxur in insecticidal formulations, water and grains, based on the formation of coloured species with *p*-aminophenol (PAP), *p*-*N*,*N*-dimethylphenylenediamine dihydrochloride (DMPD) and 1-amino-2-naphthol-4-sulphonic acid (ANSA), respectively. Guiberteau-Cabanillas et al. [10] also reported a spectrophotometric method to determine some insecticides (carbaryl, propoxur, fenitrothion and methyl parathion) with the use of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of an oxidant (Ce^{4+} or Fe^{3+}). Additionally, several rapid spectrophotometric methods incorporating flow injection analysis (FIA) [11–14] have been used to determine the carbamate pesticides. However, it is difficult to analyze these compounds in mixtures by the above conventional spectrophotometric methods because the respective molecules have similar chemical structures, which produce seriously overlapping spectra from the pesticide mixtures. However, it is the mixtures that are commonly found in practice because it is well known that the effectiveness of these pesticides improves significantly with the application of mixtures [15].

The kinetic methodology based on the difference of reaction rates is an effective way to analyze several analytes simultaneously [16], and it has been improved with the use of chemometrics procedures for analysis of results [17–19]. This kinetic approach has been applied for the multicomponent analysis of the carbamate pesticides. Based on the degradation processes in an alkaline oxidative medium, Espinosa-Mansilla et al. [20] developed a method of simultaneous kinetic determination of chlorpyrifos and carbaryl. The statistical parameters obtained by the application of PLS methods at different reaction times were analyzed. The models constructed with the use of PLS-1 and PLS-2 methods were applied for the resolution of both pesticides in artificial samples and in commercial formulations. The concentrations of these two pesticides to be analysed were in the range of 1.5×10^{-5} and 4.0×10^{-4} mol l^{-1}. The relative standard deviation for artificial samples was 0.6–8.1%, and for commercial formulations was 1.2–13.2%. Galeano-Diaz et al. [21] compared with the aid of analysis of variance (ANOVA) the resolution ability of four chemometrics methods for the simultaneous determination of carbamate pesticides (carbofuran, carbaryl and phenamifos) in ternary mixtures after their extraction into chloroform. These approaches included taking ratios of spectral derivatives, and multivariate methods (CLS, PCR and PLS), and were successfully applied for the analysis of spiked river water samples. It was found that both PLS and PCR were preferable because data pretreatment was quite simple and better statistical parameters were obtained as compared with ratios of spectral derivatives and the CLS method. The concentrations of the three pesticides in artificial mixtures to be analysed were in the range of 5.0×10^{-6} and 1.0×10^{-5} mol l^{-1}; the method involved an extraction, and has higher sensitivity than the method in Ref. [20].

In recent years, multiway and in particular three-way methods of data analysis have been developed principally in response to progress in hyphenated detection methods such as the combined kinetic-spectrophotometric technique in this work. Three-way data are obtained from various batches, quality measures and observation times in multivariate process analysis [22], but in fact, there are many methods to construct three-way data such as simultaneous determination of spectra and pH measurements, excitation–emission spectrofluorimetry, combination of chromatograms and spectra as well as that of reaction kinetics measurements and spectra. Of these methods, simultaneous determination of metal ions [23] and pharmaceuticals [24] by spectrophotometric differential kinetic procedures combined with spectral measurements is a simple but informative method to obtain three-way data, which also improves the accuracy of prediction for multicomponent analysis. In the first example [23], analysis of ternary mixtures of iron, zinc and copper was based on the displacement reaction of their ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA) complexes by 4-(2-pyridylazo)resorcinol (PAR). The differential reaction rates and the three-way kinetic spectrophotometric data were then processed by artificial neural networks (ANN). The three-way kinetic spectrophotometric method was also applied for the simultaneous determination of acetaminophen and phenobarbital, based on their oxidative coupling reaction with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of the Fe(III) oxidant [24]. Three chemometrics methods, ANN coupled with principal component analysis (PC-ANN), conventional ANN and PLS, were used to process the data and the results showed that PC-ANN is the most efficient of the three methods applied for prediction of the two analytes. Garcia et al. applied PLS regression for the simultaneous determination of carbamate pesticides with the use of kinetic spectrophotometric data [25,26]. However, the processing methods for the three-way data described above are general multivariate calibration methods, which facilitate the transfer of the three-way data into the 'unfolded' two-way data format that is compatible with the models of common chemometrics methods (e.g. PLS). The concentrations of pesticides in the synthetic mixtures to be analysed were in

the range of 3.0 and 9.0 mg l^{-1} and the LOD was 0.15–0.3 mg l^{-1}. Mathematical critiques [27–29] of this method suggest that the unfolded data breaks up the structure and characteristics of the three-way data format with the consequence that some valuable information may be lost and the model constructed on the basis of the unfolded structure may not be satisfactory for analysis.

To address this potential problem with the unfolding of the three-way data, models based on the trilinear decomposition were developed [30–32], and some advantages, such as ease of interpretation and good predictive power of the resulting multivariate calibration methods, e.g. *n*-way partial least squares (NPLS) and parallel factor method (PARAFAC), have been recognized [33,34]. The NPLS method was applied to differential reaction kinetic-spectrophotometic data set collected for simultaneous determination of orthophosphate and arsenate by Pettersson and Karlberg [35], and no significant difference was found with respect to prediction ability, between the NPLS and the unfolded-PLS models. Also, Fernandez et al. [36] studied the multicomponent kinetic determination of metal ions with the use of first and second order multivariate calibration. The developed method provides a fast and cheap determination of the five metals at sub-ppm levels, and demonstrates that the use of temporal and spectral information increases the number of analytes that could be determined simultaneously, as well as improving the analytical figures of merit.

In this paper, we present a method for the simultaneous determination of the pesticides, carbofuran, isoprocarb and propoxur in food samples. It is based on reaction kinetics and spectrophotometry, and results are interpreted with the aid of chemometrics. The analytical method relies on the differential rates of coupling reactions between the hydrolyzed products of carbamate pesticides and 4-aminophenol with the presence of potassium periodate in an alkaline solution. Both kinetic and spectral data were used to investigate performance of the analytical method on the basis of predictions of carbamate concentrations in ternary mixtures obtained from various RBF-ANN, PARFAC and NPLS calibration models. The performance of the calibration models was then ranked on the basis of several different figures of merit with the aid of the multi-criteria decision making approach, PROMETHEE and GAIA [37].

2. Theoretical background

2.1. Differential kinetic method for multicomponent analysis

Assume that when *n* analytes, M_i (i=1, 2,. . ., n) react with a common reagent R, *n* coloured products, P_i (i=1, 2,. . ., n) are obtained. If the concentration of R is much larger than that of M_i, i.e. $c_R \gg c_{Mi}$, the reaction can be expressed in terms of pseudo-first order kinetics [38], and the absorbance, A, expressed as a function of time at a set of wavelengths can be written as:

$$A_{(\lambda,t)}=\sum_{i=1}^{n} c_{Mi}\{\varepsilon^{p}_{(i,\lambda)}[1-\exp(-k_i t)]+\varepsilon_{(i,\lambda)}\exp(-k_i t)\}+e_{(\lambda,t)} \quad (1)$$

where c_{Mi} is the initial concentration of component i, k_i is the rate constant of component i, $\varepsilon_{(i,\lambda)}$ and $\varepsilon^{p}_{(i,\lambda)}$ are the molar absorbance coefficient of component i and its product P_i, respectively, with unit concentration at wavelength λ, and $e_{(\lambda,t)}$ is the corresponding background.

Eq. (1) can be further simplified to:

$$A_{(\lambda,t)}=\sum_{i=1}^{n} K_{(i,\lambda,t)}c_{Mi}+e_{(\lambda,t)} \quad (2)$$

where $K_{(i,\lambda,t)}=\varepsilon^{p}_{(i,\lambda)}[1-\exp(-k_i t)]+\varepsilon_{(i,\lambda)}\exp(-k_i t)$, which is constant for a certain component, i, when time and wavelength are also constant.

If m samples are measured, the absorbance data at p wavelengths and k time points can be expressed in matrix form as follows ($e_{(\lambda,t)}$ is omitted here):

$$\boldsymbol{A}=\begin{bmatrix} (A_{11}\cdots A_{1p})_1 & (A_{11}\cdots A_{1p})_2 & \cdots & (A_{11}\cdots A_{1p})_k \\ (A_{21}\cdots A_{2p})_1 & (A_{21}\cdots A_{2p})_2 & \cdots & (A_{21}\cdots A_{2p})_k \\ \vdots & \vdots & & \vdots \\ (A_{m1}\cdots A_{mp})_1 & (A_{m1}\cdots A_{mp})_2 & \cdots & (A_{m1}\cdots A_{mp})_k \end{bmatrix}_{m\times p\times k} \quad (3)$$

then Eq. (2) can be expressed in matrix form:

$$\boldsymbol{A}_{m\times p\times k}=\boldsymbol{C}_{m\times n}\boldsymbol{K}_{n\times(p\times k)} \quad (4)$$

According to this equation, it is possible to determine the components i individually by suitable chemometrics methods, even if there may be some degree of synergism, which arises from the interaction between the solvent and the reactants, possible changes in the activity coefficients and/or changes in the rate constants resulting from catalytic effects [39].

At a given time point, Eq. (3) reduces to the spectral form:

$$\boldsymbol{A}=\begin{bmatrix} A_{11} & A_{12} & \cdots & A_{1p} \\ A_{21} & A_{22} & \cdots & A_{2p} \\ \vdots & \vdots & & \vdots \\ A_{m1} & A_{m2} & \cdots & A_{mp} \end{bmatrix}_{m\times p} \quad (5)$$

and similarly at a given wavelength, λ, Eq. (3) reduces to the reaction kinetic form:

$$\boldsymbol{A} = \begin{bmatrix} A_{11} & A_{12} & \cdots & A_{1k} \\ A_{21} & A_{22} & \cdots & A_{2k} \\ \vdots & \vdots & & \vdots \\ A_{m1} & A_{m2} & \cdots & A_{mk} \end{bmatrix}_{m\times k} \tag{6}$$

Thus, Eqs. (3), (5) and (6) provide three possible data presentations for the building of calibration models with which to make simultaneous predictions of analytes (in the case of this work—the carbamate pesticides).

2.2. Chemometrics methods

2.2.1. Artificial neural networks

The kinetic data obtained from experiments were processed by artificial neural networks (ANN), which are generally trained with the back-propagation (BP) of errors learning algorithm. Its basic theory and application to chemical problems can be found in the literature [40–42]. The neural network performs a nonlinear interactive fit of data. The structure of the network comprised of three node layers: an input, a hidden and an output layer. The nodes in the input layer transfer the input data to all nodes in the hidden layer. These nodes calculate a weighted sum of the inputs that is subsequently subjected to nonlinear transformation:

$$o_j = f\left(\sum_{i=1}^{I} s_i w_{ij}\right) \tag{7}$$

where s_i is the input to the node i in the input layer, I is the number of nodes in the input layer, w_{ij} (weights) are the connections between each node i in the input layer and each node j in the hidden layer, and f is a nonlinear function—in this work a sigmoid function:

$$f(x) = \frac{1}{1+\exp(-x)} \tag{8}$$

The output of the network is a weighted sum of the outputs of the hidden layer and it is the calculated concentration. During the training process (i.e. calibration), the weights are iteratively calculated in order to minimize the sum of squared difference between the known concentrations and the calculated concentrations. The correction of weight Δw_{ij} was defined as follows:

$$\Delta W_{ij}(n) = \eta\delta_j o_j + \alpha\Delta W_{ij}(n-1) \tag{9}$$

where δ_j is the error term, η is the learning rate, α is the momentum and n is the iteration number. The iteration would be finished when the error of prediction reached a minimum.

Among neural networks, the multiplier feed forward networks with the back-propagation learning algorithm (i.e. BP-ANN, described above) is the most popular. Recently, a potential alternative approach has been described-radial basis function-ANN. It offers some advantages over the BP-ANN by improving the robustness and sensitivity when dealing with noisy data. The basic theory for RBF-ANN can be found elsewhere [43–45]. A background summary of the method is provided here.

RBF networks are a variant of the three-layer feed forward networks. They also contain a pass-through input layer, a hidden layer and an output layer. A different approach for modeling data is used as compared to that with the BP algorithm; the transfer function in the hidden layer of RBF is called a kernel or basis function, and thus, each node in the hidden unit contains a kernel function. The main difference between the transfer function in the BP network and the kernel function in RBF network is that the latter (usually a Gaussian function) defines an ellipsoid in the input space. RBF networks divide the input space into hyperspheres by means of the kernel function with specified width, b, and center, c. The output of a hidden unit, in the case of a Gaussian kernel function, is defined as:

$$\text{output}_j = o_j(\boldsymbol{x}) = \exp\left[-\left(|\boldsymbol{x} - c_j|/b_j\right)^2\right] \tag{10}$$

where $|\boldsymbol{x}-c_j|$ is the Euclidean distance between the input vector, $\boldsymbol{x}$, and c_j, the centroid of the Gaussian kernel function. The parameter b_j represents the width of the Gaussian function. The centroids, c_j, and the widths, b_j, of all the hidden units together define the so-called activation space, where the Gaussian function has a value larger than a given threshold value. The output of these hidden nodes, o_i, is then forwarded to all output nodes through weighted connections. The output y_i of these nodes consists of a linear combination of the kernel function:

$$y_j = \sum_{i=1}^{n} w_{ji} o_i(\boldsymbol{x}) \tag{11}$$

where w_{ji} represents the weights of the connections between the hidden layer i and output layer j, and $o_j(\boldsymbol{x})$ is obtained from Eq. (10).

2.2.2. Chemometrics for three-way data

A straightforward approach to three-way data decomposition, which avoids some of the inherent problems of three-way analysis, is to unfold the three-way data array into a two-way array, i.e. a rectangular data table, which can be processed according to the methods of general chemometrics, such as BP-ANN, RBF-ANN, PLS, etc. The operation of unfolding can be linked to spreading out of a stack of continuous forms. However, the unfolding of a three-way

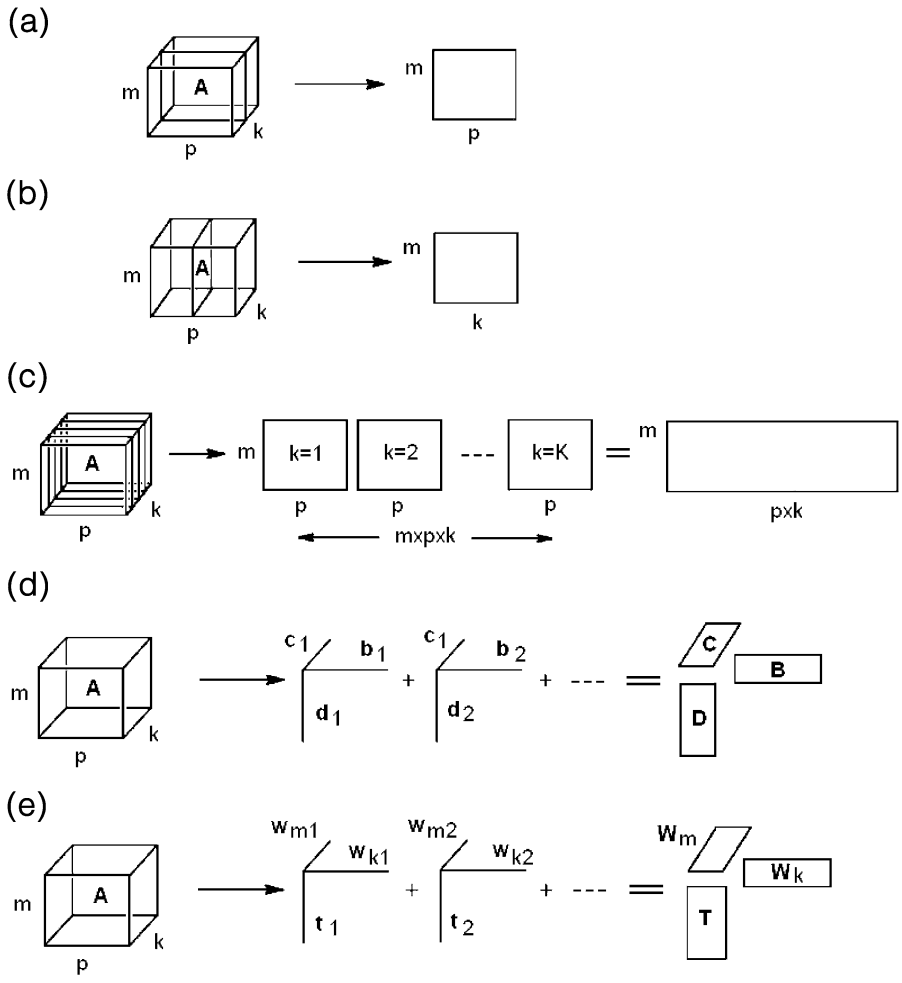


Fig. 1. Methods for decomposition of three-way data. (a) Spectra at time point, k; (b) kinetic spectra at wavelength, p; (c) unfolding method; (d) PARAFAC method and (e) NPLS method.

data array can be carried out in two different ways as shown in Fig. 1. Theoretically, an $m\times p\times k$ array will give rise to an $(m\times p)\times k$ or $(m\times k)\times p$ rectangular data table. Application of chemometrics methods to each of these two unfoldings will generally produce a different result. In particular, one obtains two different biplots for one and the same three-way table, which may be undesirable.

The decomposition of a three-way matrix $\boldsymbol{A}$ can also be defined by means of the parallel factor analysis (PARAFAC) model in terms of three two-way loading matrices (one for each mode) **D**, **B** and **C** [46–49]:

$$A_{mpk} = \sum_{f=1}^{f} d_{mf} b_{pf} c_{kf} + e_{mpk}$$
$$(m = 1,2,\ldots,m;\ p = 1,2,\ldots,p;\ k = 1,2,\ldots,k) \qquad (12)$$

where e_{mpk} represents a residual error term. This decomposition is also referred to as the trilinear model. The $m\times p\times k$ three-way matrix **A** is decomposed into the size of $m\times f$, $p\times f$ and $k\times f$ loading matrices **D**, **B** and **C** for the row-, column- and layer-items of **A**, respectively. In the PARAFAC model, the three loading matrices **D**, **B** and **C** are not necessarily orthogonal [30]. However, the solution of the PARAFAC model is unique and does not suffer from the indeterminacy that arises in principal components and factor analysis in the unfolding method. The number of factors in each mode is identical. This number is chosen to be much smaller than the original number of dimensions of the data matrix in order to achieve a considerable reduction of the data. The elements of the loading matrices **D**, **B** and **C** are computed so as to minimize the sum of the squared residuals.

The trilinear PLS algorithms are straightforward extensions of the PLS algorithm [50]. For the trilinear PLS regression, PARAFAC-like trilinear structure of the independent data is used. However, the trilinear components are calculated such that the scores are predictive for the dependent variables as in ordinary two-way regression. Successful applications can be found in Refs. [51,52].

2.2.3. Ranking with the use of PROMETHEE and GAIA

It is often necessary to decide the preferred order or rank of objects. This is relatively simple if only one characterizing variable is involved but when many variables need to be considered then multi-criteria (or variable) decision-making approach is required. In the present work, the performance of several chemometrics methods has been characterized by their figures of merit (%RPE and %Recovery) in predicting the three carbamate pesticides from their mixtures. Because there are only 10 chemometrics methods in the data matrix, a nonparametric method capable of multivariate ranking would be preferred to facilitate the comparison of method performance. Such a suitable chemometrics method is outlined below.

PROMETHEE (Preference Ranking Organization METHod for Enrichment Evaluation) and GAIA (Geometrical Analysis for Interactive Aid) developed by Brans (PROMCALC, version 3.1) [53] is a nonparametric multi-criteria decision-making method that has been fully described by Keller et al. [37], and sparingly applied to analytical problems, e.g. the ranking of varieties of rice [54] and the performance of organotin (IV) compounds as fungicides [55]. Here we provide a summary of this method.

PROMETHEE is a multivariate procedure that facilitates the ranking or ordering of a number of actions or objects (in this work, the chemometrics methods) according to preference and weighting conditions selected by the user. It compares the data for each criterion or variable pairwise by subtraction in all possible combinations, resulting in a difference, d, for each comparison. The first step of the procedure is to define a preference function for each criterion in the d-matrix. The linear model shown below is one of several that can be applied:

$$P(\mathrm{A},\ \mathrm{B}) = 1 \text{ for } d \leq z$$

$$P(\mathrm{A},\ \mathrm{B}) = d/z \text{ for } 0 \geq d > z$$

$$P(\mathrm{A},\ \mathrm{B}) = 0 \text{ for } d > 0 \tag{13}$$

P(A, B) characterizes the preference of object A over B. This means there is also a P(B, A) describing the preference of B over A. The difference value, d, is then compared to a threshold, z, which is chosen according to experimental conditions. Then, the P_i(A, B) values for the m criteria are summed for each pair of objects A, B and B, A. The sum can be weighted such that sum over all m criteria is 1. Also, each variable was set to 'minimize', i.e. the objects were ranked bottom–up with the smallest values being preferred.

$$\Pi(\mathrm{A},\ \mathrm{B}) = \sum_{j=1}^{m} w_j P_j(\mathrm{A},\ \mathrm{B}) \tag{14}$$

where Π is called the 'global preference index', and

$$\sum_{j=1}^{m} w_j = 1 \tag{15}$$

The preference selection process for the objects is refined by computing the φ^+ and φ^- outranking flows from the summed Π indices. The first indicates how an object is preferred over others, and the second reflects how an object is outranked by others. The outranking flows are then used to compare the objects according to the following rules: (1) one action is preferred to another, (2) there is no difference between actions, (3) actions cannot be compared. Such a process produces a partial rank order for the actions (PROMETHEE I), which is displayed in a flow chart. PROMETHEE I order can be a very useful tool to indicate objects, which perform equally well but on different variables. A normal rank order, which excludes condition 3, may be obtained from the net out ranking flow value, φ, for each action. The value of φ is defined by the equation:

$$\varphi = \varphi^+ - \varphi^- \tag{16}$$

where φ^+ and φ^- are the positive and negative outranking flows. This is known as the PROMETHEE II ranking.

GAIA is an exploratory procedure, which displays PROMETHEE II results in the form of a PCA biplot. The data matrix arises from a mathematical decomposition of the net outranking flows, such that the actions may be regarded as objects and the criteria as variables [37]. The biplot indicates relationships between objects, variables as well as objects and variables. This can often provide guidance to decide which variables have been responsible for the PROMETHEE II rank order.

3. Experimental

3.1. Apparatus

Spectra were measured at 25±0.5 °C on a UV-2501PC spectrophotometer (Shimadzu), equipped with a water TB-85 thermostat (Shimadzu). The pH of the solution was measured with a pH meter (SA-720, Orion). The obtained spectral data were recorded and processed by Pentium IV computer with programs written in MATLAB 5.3 (Mathworks).

3.2. Solution and reagents

All solutions were prepared with analytical grade reagents, and doubly distilled water was used throughout. Stock solutions of carbofuran, isoprocarb and propoxur

(100.0 mg l^{-1}) were prepared by dissolving each of the required crystalline compounds in 10-ml ethanol and diluted to 100-ml distilled water. Standard solutions of these pesticides or their mixtures were then diluted to the selected concentrations in 10-ml volumetric flasks for practical use. A *p*-aminophenol (4-PAP) solution of 0.70 mg ml^{-1} was prepared by dissolving 0.0125-g 4-PAP in 10 ml of ethanol and transferred into a 25-ml volumetric flask, then diluted to the mark with boiled and cooled water. Note that the 4-PAP solution was freshly prepared everyday because it was easily oxidized on standing. A 0.05 mol l^{-1} potassium periodate (KIO_4) solution, and 0.05 and 1.5 mol l^{-1} sodium hydroxide (NaOH) solutions were prepared by taking suitable weight aliquots of the reagents and dissolving them in distilled water, respectively.

3.3. General procedure

Appropriate amounts of the standard solution of carbamate pesticides and 50 μl of 1.5 mol l^{-1} sodium hydroxide were pipetted into a 10-ml volumetric flask, and after allowing 20 min for hydrolysis, the solution was made up to the mark with distilled water, and was ready for further use. Such analyte mixtures and other necessary reagents were added directly to a 10-mm cell with the use of micropipettes. Taking into account that the total useful volume was 2.8 ml, 1.0 ml of 0.05 mol l^{-1} sodium hydroxide and 1.0 ml of doubly distilled water were added to the cell, respectively; this was followed by 0.2 ml of potassium periodate solution and 0.4 ml of the standard solution of hydrolyzed carbamate pesticide (or a mixture of the compounds) to give a volume of 2.6 ml, and finally, 0.2 ml of 0.7 mg ml^{-1} 4-PAP solution was micropipetted to give a total volume of 2.8 ml. The mixture was then stirred as rapidly as possible by a hand-controlled micro-stirrer while the timer was started. The absorbance data of this solution were measured and recorded against a blank solution in the range of 510–710 nm every 25 s between 15 and 515 s. In total, 21 spectra were obtained from a solution and from such measurements, the kinetic-spectral data could be arranged into the three-way data matrix.

3.4. Procedure for the determination of carbamate pesticides in food samples

Samples of commercial vegetables and fruit were homogenized in a blender; then 10.0 g of this sample was transferred into a 100-ml Erlenmeyer flask (with a screw cap), and 20 ml of dichloromethane (CH_2Cl_2) was added. Because the concentration of pesticides in vegetable samples is too low for direct detection, 2.0 ml of each of the standard solutions of the pesticides was added by pipetting. Additionally, 5.0-g anhydrous sodium sulfate (Na_2SO_4) was placed into the flask to absorb the water in the sample. The sample was then mixed and stored overnight. The mixture was shaken in a laboratory shaker for 30 min (Model HY-4 oscillator), and filtered on a Buchner funnel; the residue in the funnel was further washed with 5 ml of dichloromethane. The filtrate was treated and separated with hexane–acetonitrile (1:1) in a separation funnel; the carbamates were extracted into the acetonitrile phase because of their higher polarity, while the colorants and some impurities were extracted into the hexane phase. The acetonitrile phase was treated twice with hexane to extract any residual impurities, and the acetonitrile phase, collected in an evaporating dish, was evaporated to near dryness. Finally, the residue in the evaporating dish was dissolved with ethanol and transferred into a 10-ml volumetric flask and diluted to the mark with 50% ethanol.

4. Results and discussion

4.1. Spectra and reaction kinetics

Spectra of the coloured products obtained from the reaction of the hydrolyzed pesticides with 4-PAP under the conditions described in Section 3.3 were measured in the range of 510–710 nm after 515 s of reaction (Fig. 2). They are broad, single bands with absorption maxima at 642, 614 and 606 nm. However, the spectra of the individual pesticides clearly overlap, which complicates the analysis of the spectral output from their mixtures. Kinetic curves of carbofuran, isoprocarb and propoxur were plotted taking the readings at the absorption maxima (Fig. 3). It is clear from these plots that the reaction rates of propoxur and isoprocarb are higher than that of carbofuran, and the reactions of the first two pesticides are almost complete within 515 s. Thus, differential kinetic rates may be useful for resolving a mixture of carbamate pesticides with the use of chemometrics methods.

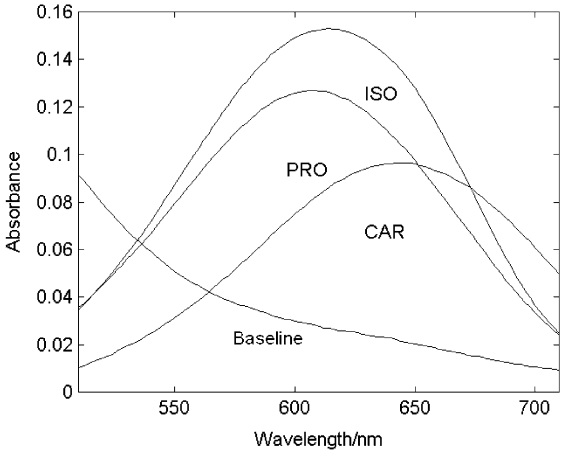


Fig. 2. Spectra of carbofuran (1.428 mg l^{-1}), isoprocarb (1.428 mg l^{-1}), and propoxur (1.428 mg l^{-1}). t=515 s, T=25 °C, $c_{4\text{-PAP}}$=50 mg l^{-1}, $c_{KIO4}=3.75\times10^{-3}$ mol l^{-1}, $c_{NaOH}=7.14\times10^{-3}$ mol l^{-1}.

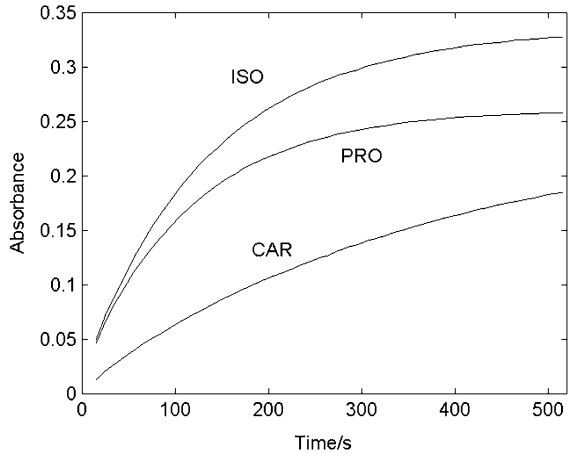


Fig. 3. Plot of absorbance vs. time for carbofuran, isoprocarb, and propoxur. Absorbances were measured at 642, 614 and 606 nm, respectively. Experimental conditions are the same as Fig. 2.

According to the pseudo-first order reaction model, the rate constant for each carbamate pesticide can be estimated by fitting the kinetic data obtained from several known single component samples to the equation of $A=a_0+a_1\exp(-kt)$ by a suitable regression method [56]. Estimates of the three rate constants for the hydrolyzed pesticide with 4-PAP obtained in this manner are presented in Table 1. There are only small differences between the three values, especially for propoxur and isoprocarb, and this is consistent with the molecular nature of the products of these reactions. The molecules are structurally similar, and have similar electron delocalisation; the ratio of the rate constants for propoxur to isoprocarb is only 1.17, and it is difficult to use a classical differential kinetic method, such as logarithmic extrapolation [57] and linear graphing [58], to resolve this mixture.

4.2. Chemical reaction mechanism and the choice of hydrolysis time

In an alkaline and oxidative medium, 4-PAP will react with a phenolate ion, which is unsubstituted in the *p*-position, resulting in the formation of a benzoquinoneanil. In general, according to the reaction scheme (Fig. 4), *p*-aminophenol undergoes oxidation in the presence of potassium periodate forming a highly reactive electrophilic reagent, which can attack the most nucleophilic site on a benzene ring [59]. The position of such a site is influenced by the nature and location of the ring substituents; if the functional group is *o*-or *p*-directing, a coupling reaction will occur. In the case of the carbamate molecules, the benzene ring (Table 1) has no suitable *o*- or *p*-activating groups, and no reaction can occur with the 4-PAP molecule. However, when hydrolyzed in an alkaline medium, the products of the carbamates react with 4-PAP (Fig. 4) to yield coloured compounds. As shown in Fig. 3, progress of the hydrolysis of the carbamates indicates that the reaction is almost complete within approximately 515 s; when the absorbance was checked after further hydrolysis up to 20 min, little change was noted from the values measured at 515 s. Thus, this observation confirms that the hydrolysis reaction is essentially complete at 515 s.

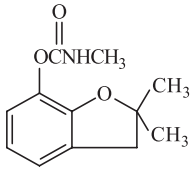
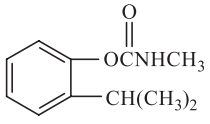
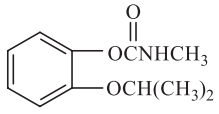
4.3. Effect of the NaOH concentration

The oxidative coupling reaction between the hydrolysis products of carbofuran, isoprocarb and propoxur and 4-PAP requires an alkaline medium (NaOH) for its development. In order to study the effect of the NaOH concentration ([NaOH] range: 7.15×10^{-3} to 7.14×10^{-2} mol l^{-1}) on sensitivity and kinetic behaviour of the reaction, repeat spectral scans were collected at 50 s intervals in a given NaOH solution. As shown in Fig. 5, the absorbance increased with increasing concentration of NaOH in the low concentration range (<0.02 mol l^{-1}) with little apparent change thereafter; the kinetic results were very similar across the whole range of NaOH concentrations. Thus, 1.79×10^{-2} mol l^{-1} of NaOH (in the cuvette) was chosen on the basis of favorable spectral sensitivity and minimal amount of NaOH.

4.4. Effect of the 4-PAP concentration

The amount of 4-PAP has to be in reasonable excess to maintain a pseudo-first order reaction. However, 4-PAP can be easily oxidized by oxygen in air. In order to find the optimal concentration of 4-PAP, the effect of its concentration on the reaction was studied in the range 3.57–57.14 μg ml^{-1}. As can be seen in Fig. 6, the concentration of 4-PAP strongly affects the spectral sensitivity and kinetic behaviour of all three compounds. The absorbance increases quickly with the increasing concentration of 4-PAP up to 40 μg ml^{-1} for each of the three compounds, and especially for carbofuran, the reaction rate of which as a function of 4-PAP

Table 1
Formulae and kinetic rate constants of the three carbamate pesticides

Pesticide	Molecular formula	Kinetic rate constant (s^{-1})	Structure
Carbofuran (CAR)	$C_{12}H_{16}O_3N$	0.0028	
Isoprocarb (ISO)	$C_{11}H_{15}O_2N$	0.0072	
Propoxur (PRO)	$C_{11}H_{15}O_3N$	0.0084	

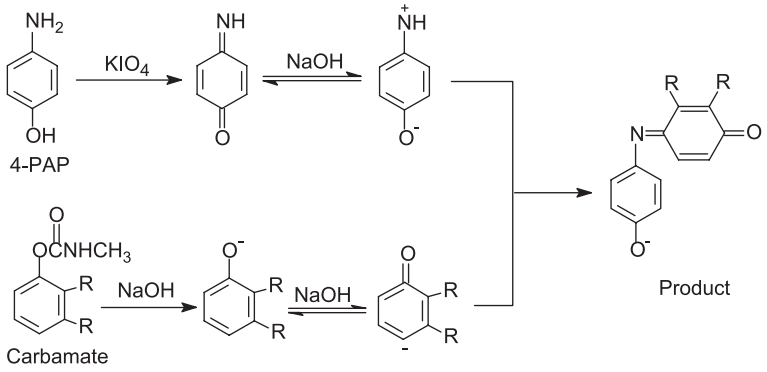


Fig. 4. Mechanism of the coupling reaction of 4-PAP with carbamate pesticides.

concentration was almost linear over the interval studied. It was also noted from Fig. 6 that the blank increased with increasing amount of 4-PAP, and therefore, to minimize this effect, 50 μg ml^{-1} (in the cuvette) of 4-PAP was chosen as a suitable concentration for analysis. However, the increase of 4-PAP concentration still had a significant effect on the kinetic behaviour of the carbamates with concentrations above 50 μg ml^{-1} (Fig. 6).

4.5. Effect of KIO_4 concentration

The 4-PAP molecule has to be oxidized to react with the hydrolysates of the carbamate in order to produce the coloured product suitable for spectrophotometric analysis. Potassium periodate, KIO_4, was used for this purpose in this work, and, consequently, it was necessary to select a suitable concentration of this reagent. The concentration range of KIO_4 was between 1.79×10^{-4} and 5.36×10^{-3} mol l^{-1}, and the dependence of the reaction on the concentration of KIO_4 is shown in Fig. 7. A relatively high reaction rate is observed with the increasing concentration of KIO_4. It is also known that some unreacted 4-PAP gives coloured products itself in an alkaline medium, and will increase the absorbance background [59]. Such products are rapidly oxidized by KIO_4 to colorless compounds, and there is no interference. Considering all of the above, 5.36×10^{-3} mol l^{-1} of KIO_4 (in the cell) was selected as an appropriate concentration of the oxidant.

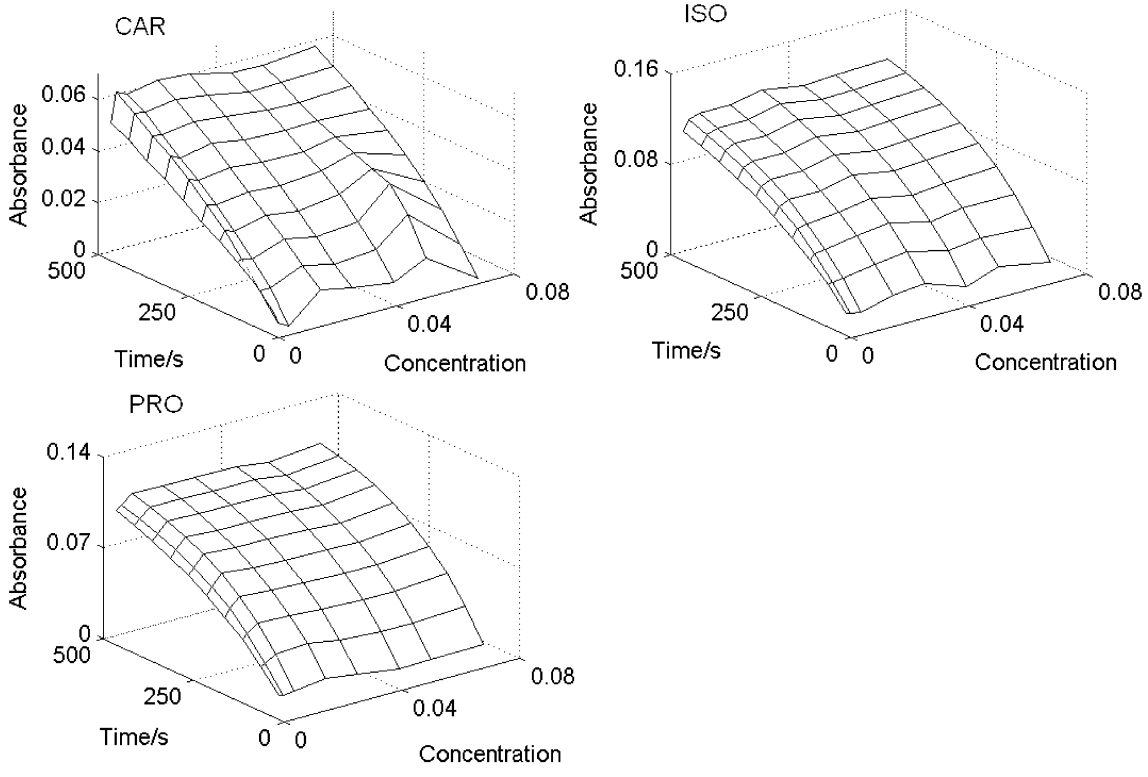


Fig. 5. Three-way plot of absorbance of each carbamate pesticide vs. time and concentration of NaOH. Experimental conditions are as in Fig. 2.

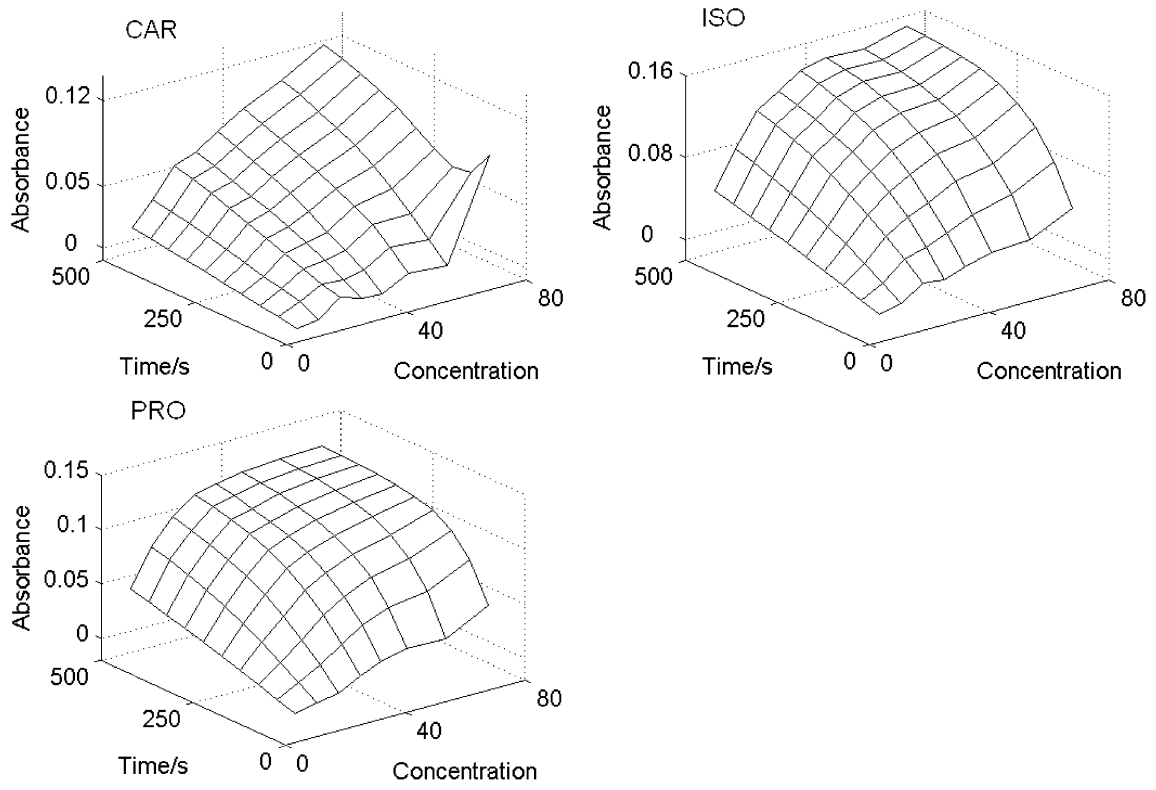


Fig. 6. Three-way plot of absorbance of each carbamate pesticide vs. time and concentration of 4-PAP. Experimental conditions are as in Fig. 2.

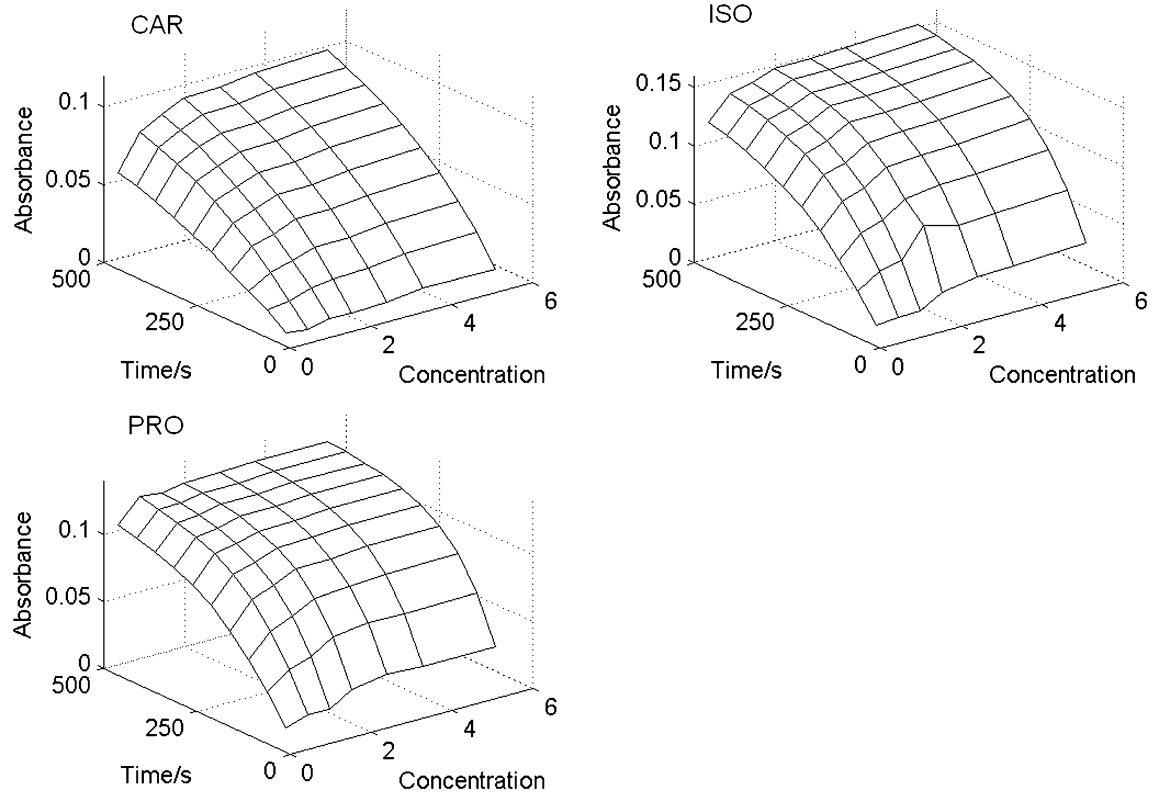


Fig. 7. Three-way plot of absorbance of each carbamate pesticide vs. time and concentration of KIO_4. Experimental conditions are as in Fig. 2.

4.6. Calibration models for single component analysis

The kinetic curves (Fig. 8) were obtained at the analytical wavelengths 642, 614 and 606 nm for different concentrations of carbofuran, isoprocarb and propoxur, respectively, under conditions discussed in the previous sections above. The linear calibration models and their relative parameters were established at the selected time point (t=515 s), and Table 2 summarizes the results for the analysis of each pesticide. The correlation coefficients suggest good linearity over the concentration range of about 0.6–10.0 mg l^{-1} (i.e. 3.0×10^{-6}–5.0×10^{-5} mol l^{-1}, on the basis of the mean molecular weight of the pesticides ~ 200), which is marginally better than that achieved in Ref. [20] and a significant improvement than on the ranges quoted in Refs. [21,25].

Table 2
Parameters of the linear calibration equations for carbamate pesticides (t=515 s)

Parameter	CAR	ISO	PRO
Number of samples (n)	8	7	8
Linear range (mg l^{-1})	0.857–11.4	0.571–7.14	0.571–10.0
Correlation coefficient	0.9997	0.9997	0.9999
Intercept ($\times10^{-3}$)	−15.7	−4.3	−2.6
Slope (l mg^{-1})	0.072	0.117	0.0921
s_I ($\times10^{-3}$)[a]	4.1	3.2	2.8
s_s ($\times10^{-4}$)[a]	6.59	7.98	5.39
LOD (mg l^{-1})[a]	0.26	0.12	0.15

[a] s_I, s_s and LOD are the standard deviation of the intercept, the standard of deviation of the slope and the detection limits, respectively. They were calculated according to Miller's method [60].

4.7. Interferences

Various organic pesticides which may be present in vegetable samples were tested for interference during the kinetic-spectrophotometric measurements under the same experimental conditions. It was found that organophosphorus pesticides such as parathion-methyl, acephate, isocarbophos, coemaphos and methamidophos do not interfere with the carbamate analysis because they do not react with 4-PAP. For the same reason, the organochloride pesticides (such as α-, β-, γ- and δ-hexachlorocyclohexane) and some carbamate pesticides (for example benthiocarb) also do not interfere with the kinetic measurements.

4.8. Prediction of carbamate pesticides in a synthetic mixture

In this work, the quantitative analysis of mixtures of carbofuran, isoprocarb and propoxur was investigated

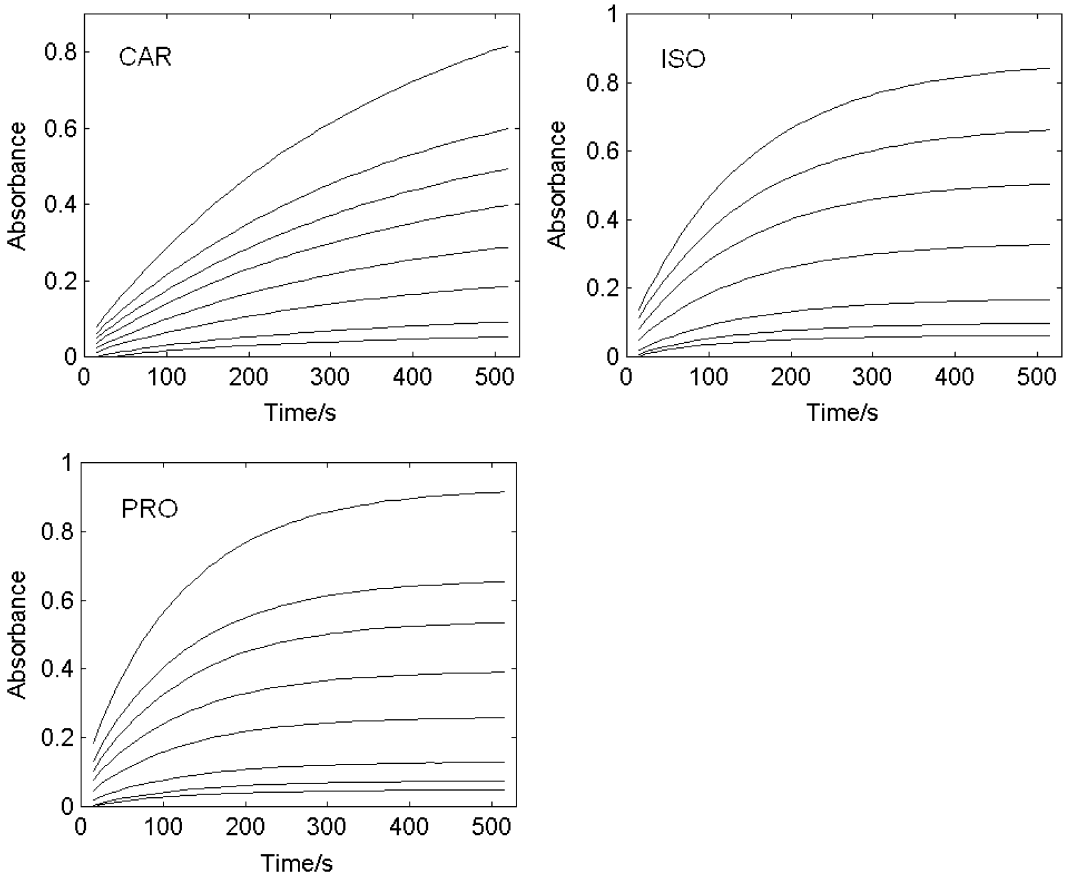


Fig. 8. Kinetic spectra of each pesticide with different concentrations (mg l^{-1}). c_{CAR}=0.857, 1.428, 2.857, 4.286, 5.714, 7.143, 8.571 and 11.428; c_{ISO}=0.571, 0.857, 1.428, 2.857, 4.286, 5.714 and 7.143; c_{PRO}=0.571, 0.857, 1.428, 2.857, 4.286, 5.714, 7.143 and 10.00.

with the use of several different types of chemometrics models in order to study their effect on the prediction of individual pesticides found in synthetic and real pesticide mixtures.

4.8.1. Calibration and validation of the analytical method

In order to extract maximum quantitative information about the samples with the use of minimum experimental trials, the orthogonal array design was applied for the construction of the set of calibration samples [61–65]. Table 3 shows the composition of the calibration samples, which were designed according to a four-level orthogonal array design denoted by $OA_{16}(4^5)$. The concentration levels for the carbamate pesticides were: carbofuran, 0.857, 2.85, 4.57 and 6.28 mg l^{-1}; isoprocarb, 0.571, 2.28, 4.28 and 5.71 mg l^{-1}; and propoxur, 0.571, 2.28, 4.28 and 5.71 mg l^{-1}. These levels of pesticides were selected to allow for a wide distribution of concentrations, which will also cover the range of levels found in real samples. The 16 ternary synthetic mixtures of carbamate pesticides shown in Table 4 were prepared and were used to validate the different chemometrics models.

4.8.2. Comparison of various calibration models

Calibration mixtures of the carbamate pesticides were analyzed and the three-way data were obtained by the experimental procedure described in Section 2.1. From this matrix, the kinetic data at a selected wavelength, spectral data at a selected time point, and the unfolded kinetic-spectral data were extracted, and processed for calibration by the global RBF-ANN method, i.e. all the components were simultaneously trained to establish the neural network model. Sixteen ternary synthetic mixtures of the three carbamate compounds were analyzed by the proposed kinetic analytical method and global RBF-ANN. The relative prediction errors (RPE) [66] obtained from each method are listed in Table 5a (important model parameters are noted as footnotes to Tables 5a and 5b). As can be seen from the comparison of the figures of merit in the table, the unfolded URBF-ANN method (without any data pretreatment) gives much better results than the other two because the unfolded kinetic-spectral data contain much more information than the kinetic or spectral data sets individually.

It is important to select a suitable method for preprocessing the measured data in order to improve the prediction accuracy of RBF-ANN. In this work, several preprocessing methods such as normalization [67], taking derivatives, and compressing data into scores with the use of PCA were employed to investigate their effect on the %RPE and Recovery. The results listed in Table 5b indicate that the RBF-ANN models, developed with unfolded data preprocessed by normalization only, gave the best %RPE and Recovery results (RPE_T=3.2%; good and consistent Recoveries) followed closely by the method which included the

Table 3
Composition of the calibration set

Sample	Concentration (mg l^{-1})		
	CAR	ISO	PRO
1	0.857	0.571	0.571
2	0.857	2.28	2.28
3	0.857	4.28	4.28
4	0.857	5.71	5.71
5	2.85	0.571	2.28
6	2.85	2.28	0.571
7	2.85	4.28	5.71
8	2.85	5.71	4.28
9	4.57	0.571	4.28
10	4.57	2.28	5.71
11	4.57	4.28	0.571
12	4.57	5.71	2.28
13	6.28	0.571	5.71
14	6.28	2.28	4.28
15	6.28	4.28	2.28
16	6.28	5.71	0.571

Table 4
Composition of the validation set

Sample	Concentration (mg l^{-1})		
	CAR	ISO	PRO
1	1.14	1.14	1.14
2	1.14	2.85	2.85
3	1.14	4.85	4.85
4	1.14	6.28	6.28
5	2.85	0.857	2.85
6	2.85	2.85	0.857
7	2.28	4.85	6.28
8	2.28	6.28	4.85
9	4.00	0.857	4.85
10	4.00	2.85	4.28
11	4.00	4.85	0.857
12	4.00	6.28	2.85
13	5.71	0.857	6.28
14	5.71	2.85	4.85
15	5.71	4.85	2.85
16	5.71	6.18	0.857

Table 5a
Comparison of %RPE and %Recovery for the global RBF-ANN methods[a]

Methods	RPE_S for carbamate pesticides			RPE_T
	CAR	ISO	PRO	
URBF-ANN[b]	2.9(98)[c]	5.2(105)	7.4(88)	5.7
SRBF-ANN[d]	20.3(70)	16.2(107)	15.7(102)	17.3
KRBF-ANN[e]	20.6(137)	34.3(107)	41.4(132)	34.7

[a] Original raw data matrix without any pretreatment was used for all the three RBF-ANN methods. The number of nodes in the hidden layer and the spread coefficient (sc) for all the three methods were 10 and 556, respectively.

[b] Unfolded (U) three-way data matrix (as in Eq. (3)) was used.

[c] The values in parentheses correspond to the mean recoveries (%). $\text{Recovery}(\%) = 100 \times \sum_{i=1}^{n} (c_{i(\text{pred})}/c_{i(\text{real})})/n$, where n is the number of samples.

[d] Only spectral (S) data matrix (as in Eq. (5)) was used.

[e] Only kinetic (K) data matrix (as in Eq. (6)) was used.

Table 5b
Comparison of %RPE and %Recovery values for different chemometrics methods[a]

Methods	%RPE_S for carbamate pesticides			%RPE_T
	CAR	ISO	PRO	
RBF-ANN[b]	2.4(102)[c]	3.3(97)	3.7(97)	3.2
PC-RBF-ANN[d]	3.6(99)	3.3(98)	4.4(105)	3.8
URBF-ANN[e]	2.9(98)	5.2(105)	7.4(88)	5.7
DRBF-ANN[f]	15(126)	8.8(84)	7.6(113)	10
NPLS1[g]	3.3(100)	7.4(107)	7.8(88)	6.7
NPLS2[h]	3.2(102)	14(122)	5.6(105)	9.3
PARAFAC[i]	3.6(103)	18(132)	4.4(109)	11
BP-ANN[j]	6.8(109)	8.9(98)	12(82)	10

[a] Three-way data matrix (as in Eq. (3)) was used for all chemometrics methods.
[b] Data pretreatment with normalization. The number of nodes in the hidden layer and the spread coefficient (sc) were 10 and 1400, respectively.
[c] As e in Table 5a.
[d] Data pretreatment: as above in (*b*), and then the resulting matrix was submitted to PCA. The extracted scores were used as data for the neural network training. The number of nodes in the hidden layer and the spread coefficient (sc) were 10 and 910, respectively.
[e] Original raw data matrix without any pretreatment was used.
[f] Original data matrix was converted to derivatives, and this new matrix was submitted to RBF-ANN processing. The number of nodes in the hidden layer and the spread coefficient (sc) were 8 and 2800, respectively.
[g] The number of factors used in calibration models for carbofuran, isoprocarb and propoxur were 8, 8 and 6, respectively.
[h] The number of factors used was 14.
[i] The number of factors used was 6.
[j] The parameters of learning rate, momentum and nodes in the hidden layer were 0.01, 0.7 and 6, respectively.

PCA preprocessing step. Both of these approaches performed better than the method involving the unfolded matrix without any pretreatment. The matrix consisting of spectral derivatives as objects performed less satisfactorily (e.g. RPE_T=10% and poorer, less consistent Recoveries) when submitted to the RBF-ANN than the previous cases, suggesting that taking derivatives as a pretreatment by itself is insufficient to lower RPE_S and improve Recoveries significantly. In addition, one of the drawbacks of conversion to derivatives is the possible increase in noise; this could also have contributed to the poorer values for the figures of merit.

The other calibration methods in Table 5b are based on the trilinear decomposition approach. These methods use the signal matrix without unfolding, and thus reflect the raw three-way data structure. In this work, NPLS and PARAFAC are representative of such methodology and were applied directly to decompose and calibrate the kinetic-spectral three way data against the concentration matrix. The %RPE and Recovery values (Table 5b) are compared with the methodologies already considered as well as with BP-ANN, which is arguably the most commonly used ANN method in analytical chemistry. The results show that the NPLS models give better RPE, and the rate of data processing is faster than with PARAFAC and BP-ANN. On the other hand, NPLS1 calibration model gives a somewhat better result than NPLS2. This actually reflects the common finding with PLS modeling in general with predictions from PLS2 treatment being often somewhat worse than those from the corresponding PLS1 models. Additionally, none of the trilinear methods have outperformed the first three noted in Table 5b, all of which have RPE values of <6% as well as reasonable and consistent recoveries, particularly for the first two models, RBF-ANN and PC-RBF-ANN.

4.8.3. Ranking the performance of the chemometrics methods—application of PROMETHEE and GAIA

The data matrix consisted of 10 chemometrics methods as objects or actions, and each was rated on seven criteria (the 3×RPE_S (E), the 3×mean Recoveries (R) and the RPE_T). The 10 chemometrics methods with and without pretreatment (see Tables 5a and 5b) were ranked according to the PROMETHEE. The Recovery values were transformed by the expression |R-100|, i.e. the absolute value for the difference between the measured and the ideal target of 100% was calculated. Each of the seven criteria was set to minimize implying that the preferred performance occurs when the precision and |R-100| values tend to zero. A linear model was selected as the preference function (Eq. (13), Section 2.2.3) for the criteria.

Selection of the normalizing *z* value in this model for each criterion is important. For *z* values for the RPE variables, we

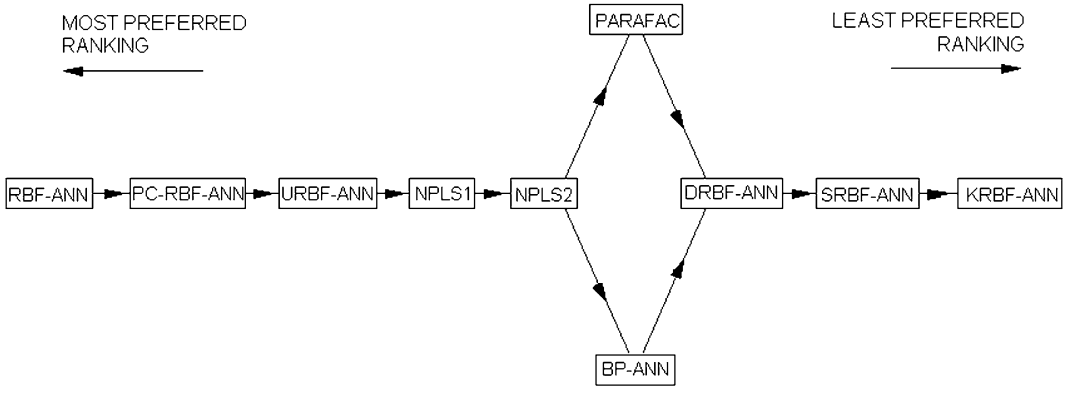


Fig. 9. PROMETHEE I partial ranking of chemometrics methods.

referred to the well known Horwitz's Trumpet curve (HTC) as a guide. Given the concentration range of analytes in this work (several mg/kg), we chose precision values at somewhat tighter levels than that suggested by the intra-laboratory HTC reference ($\sim \pm 10\%$). We estimate the RPE_S precision at $\pm 5\%$, and the RPE_T at $\pm 8\%$; in general, the commonly acceptable Recovery values fall in the $\pm 5-15\%$ range—we chose a value of $\pm 6\%$.

The results of PROMETHEE I partial ranking for the data set are shown in Fig. 9. The best performing methods are on the left side of the diagram, i.e. RBF-ANN, PC-RBF-ANN and URBF-ANN. The RBF-ANN with only spectral or kinetic data (i.e. SRBF-ANN and KRBF-ANN) fill the last two places. The PROMETHEE I method shows a rank order for the chemometrics methods as: RBF-ANN>PC-RBF-ANN>URBF-ANN>NPLS1>NPLS2>(PARAFAC, BP-ANN)>DBRF-ANN>SRBF-ANN>KRBF-ANN. The PROMETHEE II full ranking method offered quantitatively little extra information. The calculated ranking values of φ in Eq. (16) are 0.64, 0.58, 0.32, 0.22, 0.08, −0.06, −0.16, −0.22, −0.52 and −0.89 for RBF-ANN, PC-RBF-ANN, URBF-ANN, NPLS1, NPLS2, PARAFAC, BP-ANN, DRBF-ANN, SRBF-ANN and KRBF-ANN, respectively.

The methods follow more or less the discussion in the previous Section 4.8.2 but of interest is the pairing of the PARAFAC and the BP-ANN being classified in Section 2.2.3 under rule 3 as methods that cannot be compared.

The performance of some of the chemometrics methods in this study can be compared with that in our similar recent investigations [68–70]. The first of these [68] involved a kinetic-spectrophotometric determination of reducing sugars in ternary mixtures; the second [69] was concerned with the spectrophotometric prediction of five metals ions commonly found in electroplating baths; and the third [70] described the application of stripping voltammetry for the prediction of organophosphorus pesticides in ternary mixtures. In each case the performance of a chemometrics method was assessed on the basis of $\%RPE_T$ (or in the case of Ref. [69], by the global mean relative error of prediction (RPE_M)) and %Recovery. The RBF-ANN method was the best performing method on the basis of the RPE estimates in all these three studies with values typically falling into the range of 7–8%. Thus, in the present study the performance of the two leading methods, RBF- and PC-RBF-ANN, echoes and improves on the previous findings with $\%RPE_T$ values of about 3–4, respectively. With regards to %Recovery, in most studies, especially with the kinetic spectrophotometric determination of ternary reducing sugars [68], the performance is high, and recoveries are typically over a range of 95–110% with quite consistent sets of $\%RPE_S$ values for the individual analytes. This contrasts to the %Recovery sets with rather scattered values for many other methods, e.g. PLS/PCR typical ranges—103–125 [70] and 85–110 [68], and in extreme cases CLS with ranges of 46–155 [68], and 75–103 [70]. It is noted that %Recovery from the RBF-ANN results for the analysis of the reducing sugars is about 97, and is similar in values and the quite narrow range to that is found in the present work (97–105). It useful to observe that the more common BP-ANN methodology generally performs moderately well but has rather

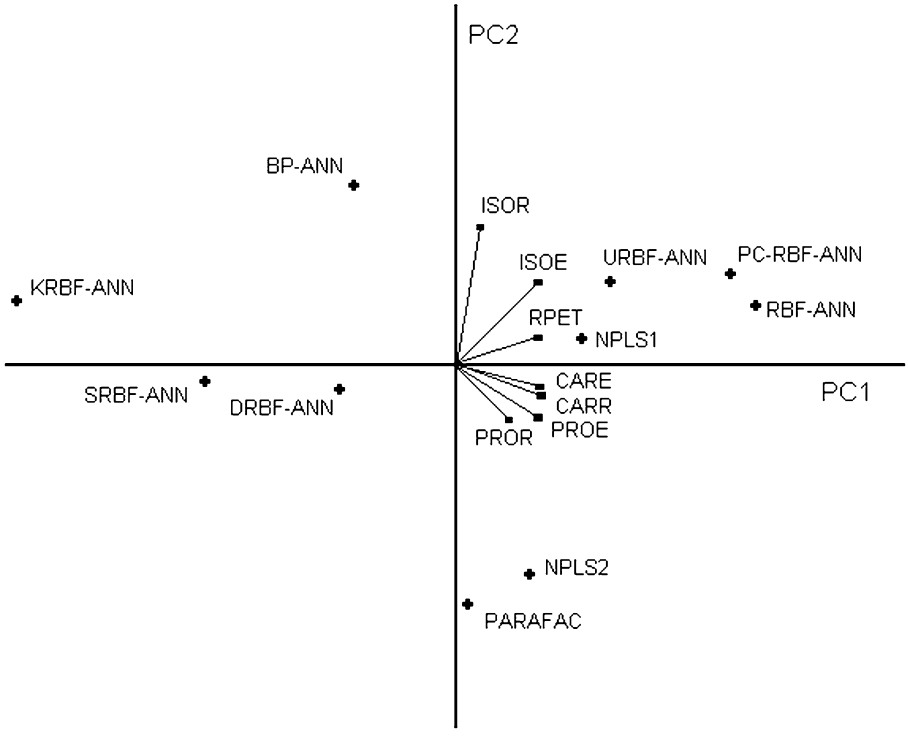


Fig. 10. GAIA biplot for the chemometrics methods. Symbols E and R attached to the criteria labels refer to the RPE_S and %Recoveries, respectively.

poorer %RPE_T values, e.g. 15 [68], and inconsistent %Recovery ranges, e.g. a tight set of values averaging 97 [68], contrasting with the rather wide range of 82–109 in this work. Finally, it has been observed that spectral derivative pretreatment can be sometimes beneficial for the performance of a method; a singularly emphatic example was found in the performance of the CLS method [68], with %RPE_T (62) and %Recovery range (46–155) improving after derivative data matrix pretreatment to %RPE_T (8) and a %Recovery range of 92–104. Thus, this current assessment of performance of the chemometrics methods indicates that RBF-ANN procedure is consistently outperforming the common prediction methods such as PLS and PCR as well as the BP-ANN on the basis of figures-of-merit criteria obtained from analysis of different organic and inorganic analytes with the use of hyphenated and simple spectrophotometric as well as voltammetric techniques.

GAIA-PCA biplot (Fig. 10) was used to investigate any relationships between the chemometrics methods and the ranking criteria. Only three PCs were needed to account for about 95% of data variance. Projection of objects on to PC1 closely reflected the ranking by PROMETHEE I. Projection of loading vectors on PC1 shows that positive loadings responsible for the discrimination of the better performing methods include all the seven criteria. The poorer performing ANN methods (including KRBF-ANN, SRBF-ANN, DRBF-ANN and BP-ANN) have negative scores. PC2 essentially discriminates BP-ANN (positive scores) from PARAFAC and NPLS2 (negative scores) methods. The criteria, ISOR, and to a lesser extent ISOE have significant positive loadings on PC2, and are principally responsible for the separation of BP-ANN from the other two objects since the other five criteria have relatively small loadings on the same PC. It is noted that in Table 5b the ISOR %Recovery values for PARAFAC and NPLS2 are 132 and 122, respectively, as compared to a good value of 98 for BP-ANN; the poor values for %RPE_T of the former two methods also stand out.

Thus, in summary the PROMETHEE I and GAIA analysis indicates that in addition to the particularly well performing chemometrics methods RBF-ANN and PC-RBF-ANN, URBF-ANN and NPLS1 should be regarded as adequate alternatives for the determination of carbamate pesticides by the described kinetic spectrophotometric method.

4.9. Application to food samples

The proposed kinetic-spectrophotometric method was applied for the determination of the three carbamate pesticides from fruit and vegetable samples such as apples, lettuce, Chinese cabbage and tomatoes. Since the RBF-ANN method with unfolding and data pretreatment (normalization) gave the best prediction results, it was the method applied for the determination of the pesticides in real samples. The analytical results obtained by this method are summarized in Table 6, in which the recoveries obtained by standard additions to each sample are listed and compared with the results obtained before and after adding them to sample. No carbofuran was found in any of the food samples, and RBF-ANN with unfolded data gives satisfactory results, with %Recovery values that are generally in the range of 92–109 with only two samples outside the upper threshold.

5. Conclusion

In this paper, we have investigated the chemistry, analytical methodology and the chemometrics interpretation of results for the determination of carbofuran, isoprocarb and propoxur carbamate pesticides in food samples by the kinetics-spectrophotometric method employing the coloured coupling reaction of the alkaline hydrolysis products of the carbamates with *p*-aminophenol (PAP).

After studying the important chemical variables such as the reaction time and concentration effects of the alkali, the 4-PAP reagent and the potassium periodate oxidant, as well as the spectro-kinetic issues, a method of analysis was proposed and tested satisfactorily for the analysis of the individual carbamates. The LOD was found to be in the range of 0.1–0.3 mg l^{-1}, which compares well with LOD from previous work (0.15–0.3 mg l^{-1} [25]). In addition, we showed that many other commonly found organophosphorus and organochloro pesticides do not interfere with the analysis because they essentially do not react with the coupling reagent 4-PAP.

However, in field practice the pesticides are applied as a mixture, and, consequently, the methodology required for the simultaneous determination of the pesticides was

Table 6
Determination of three carbamate pesticides in commercial vegetable samples by the RBF-ANN method[a]

Sample[b]	Found (μg g^{-1})			Added (μg g^{-1})			Found (μg g^{-1})			Recovery (%)		
	CAR	ISO	PRO	CAR	ISO	PRO	CAR	ISO	PRO	CAR	ISO	PRO
Apples	Nd[c]	2.51	1.59	10.0	10.0	8.0	10.1	14.6	9.8	101	120	103
Lettuce	Nd	2.75	0.86	10.0	10.0	8.0	10.7	14.2	8.2	107	115	92
Chinese cabbage	Nd	4.02	1.19	10.0	10.0	8.0	10.1	13.2	9.1	101	92	98
Tomato	Nd	4.02	0.99	10.0	10.0	8.0	10.0	13.5	9.8	100	96	109

[a] Unfolded data matrix was used, and the parameters used for RBF-ANN were as in Tables 5a and 5b.
[b] All the samples were obtained from a supermarket in Nanchang.
[c] Not detected.

investigated. In this context, the data from the hyphenated kinetic-spectrophotometric technique may be processed by either three-way data unfolding or decomposition by trilinear modeling. Both methodologies were applied in this study—the first in conjunction with a number of different RBF-ANN models and the second with the PARAFAC and the NPLS procedures. In addition, spectral and kinetic data matrices were studied separately, and some common pretreatments of the data matrices such as *y*-mean centering and normalization as well as spectral derivatives were applied. In all, 10 different calibration models were constructed with the use of RBF-ANN, PC-RBF-ANN, URBF-ANN, NPLS1, NPLS2, PARAFAC, BP-ANN, DBRF-ANN, SRBF-ANN and KRBF-ANN chemometrics methods. The models were validated with the use of synthetic ternary mixtures of carbamates and figures of merit, %RPEs and %Recoveries were collected. On this basis, the model performance was investigated with the aid of the multi-criteria ranking program, PROMETHEE and GAIA. This analysis showed that the best performing chemometrics methods were RBF-ANN and PC-RBF-ANN, followed by two possible alternative but somewhat less well performing methods, URBF-ANN and NPLS1. In addition, the current method performance was compared with recent similar past studies, and suggested that the RBF-ANN methodology has consistently outperformed the more common prediction methods such as PLS and PCR as well as BP-ANN.

Finally, the most successful RBF-ANN calibration model was applied for the prediction of the carbamate pesticides in a selection of locally purchased vegetables and fruit with satisfactory results.

Acknowledgements

The authors gratefully acknowledge the financial support of this study by the National Science Foundation of China (NSFC Nos. 20065001 and 20365002), the State Key Laboratory of Chemo/Biosensing and Chemometrics of Hunan University (No. 2002-18), the Jiangxi Province Natural Science Foundation (JXNSF No. 0320014), the Key Laboratory of Food Sciences of MOE and the Analytical and Test Centre of Nanchang University.

References

[1] Y. Zhang, Z. Zhang, Pesticides, Chinese Material Press, Beijing, 1997.
[2] United Nations Environmental Program, Convention on Trade in dangerous chemicals and pesticides: final round of talks started, homepage: http://www.unep.ch/iuc/submen/press/press/pic/pr3-98.htm.
[3] W.H.O. (World Health Organization), Environ. Health Criteria 63 (1986) (Geneva).
[4] D. Fang, Analysis and Detection for Residues of Pesticides, Shanghai Science and Technology Press, Shanghai, 1982.
[5] H.J. Stan, P. Klaffenbach, Fresenius' J. Anal. Chem. 339 (1991) 151.
[6] W. Bla Beta, Fresenius' J. Anal. Chem. 339 (1991) 340.
[7] S. Chiron, A. Valverde, A. Fernandez-Alba, D. Barcelo, J. AOAC Int. 78 (1995) 1346.
[8] A.S.P. Sastry, D. Vijaya, D.S. Mangala, Analyst 112 (1987) 75.
[9] C.C.S.P. Sastry, D. Vijaya, Talanta 34 (1987) 372.
[10] A. Guiberteau-Cabanillas, T. Galeano-Diaz, F. Salinas, J.M. Ortiz, Anal. Chim. Acta 305 (1995) 219.
[11] J. Ferre, R. Boque, B. Fernandez-band, M.S. Larrechi, F.X. Rius, Anal. Chim. Acta 348 (1997) 167.
[12] K.D. Khalaf, J. Sancenon, M. de la Guardia, Anal. Chim. Acta 266 (1992) 119.
[13] K.D. Khalaf, A. Morales-Rubio, M. de la Guardia, Anal. Chim. Acta 280 (1993) 231.
[14] J. Wang, L. Wang, X.L. Huang, Test and Analytical Techniques for Food Safety, Chinese Chemical Engineer Press, Beijing, 2002.
[15] A. Espinosa-Mansilla, F. Salinas, A. Zamoro, Mikrochim. Acta 113 (1994) 9.
[16] D. Perez-Bendito, Analyst 115 (1990) 689.
[17] S.R. Crouch, Anal. Chim. Acta 283 (1990) 453.
[18] M. Otto, Analyst 115 (1990) 685.
[19] T.F. Cullen, S.R. Crouch, Mikrochim. Acta 126 (1997) 1.
[20] A. Espinosa-Mansilla, F. Salinas, A. Zamoro, Analyst 119 (1994) 1183.
[21] T. Galeano-Diaz, A. Guiberteau, J.M. Ortiz-Burguillos, F. Salinas, Analyst 122 (1997) 513.
[22] T. Kourti, J.F. MacGregor, Chemom. Intell. Lab. Syst. 28 (1995) 3.
[23] Y. Ni, C. Liu, Chem. J. Chin. Univ. 20 (1999) 529.
[24] Y. Ni, C. Liu, S. Kokot, Anal. Chim. Acta 419 (2000) 185.
[25] J.M. Garcia, A.I. Jimenez, J.J. Arias, K.D. Khalaf, A. Morales-Rubio, M. de la Guardia, Analyst 120 (1995) 313.
[26] K.D. Khalaf, A. Morales-Rubio, M. de la Guardia, J.M. Garcia, F. Jimenez, J.J. Arias, Microchem. J. 53 (1996) 461.
[27] A.K. Smilde, P. Geladi, R. Bro, Multiway Analysis in Chemistry, Wiley, Chichester, 2000.
[28] R. Bro, J. Chemom. 10 (1996) 47.
[29] R.G. Brereton, Analyst 125 (2000) 2125.
[30] P. Geladi, Chemom. Intell. Lab. Syst. 7 (1989) 11.
[31] A.K. Smilde, Chemom. Intell. Lab. Syst. 15 (1992) 143.
[32] R. Henrion, Chemom. Intell. Lab. Syst. 25 (1994) 1.
[33] R. Bro, Chemom. Intell. Lab. Syst. 46 (1998) 133.
[34] R. Bro, Chemom. Intell. Lab. Syst. 38 (1997) 149.
[35] A.K. Pettersson, B. Karlberg, Anal. Chim. Acta 354 (1997) 241.
[36] F.M. Fernandez, M.B. Tudino, O.E. Troccoli, Anal. Chim. Acta 433 (2001) 119.
[37] H.R. Keller, D.L. Massart, J.P. Brans, Chemom. Intell. Lab. Syst. 11 (1991) 175.
[38] R. Kellner, J.-M. Mermet, M. Otto, H.M. Widmer, Analytical Chemistry, Wiley-VCH, New York, 1998, p. 229.
[39] D. Perez Bendito, M. Silva, Kinetic Methods in Analytical Chemistry, Ellis Horwood, Chichester, 1988.
[40] J. Zupan, J. Gasteiger, Anal. Chim. Acta 248 (1991) 1.
[41] J. Zupan, J. Gasteiger, Neural Network for Chemists, An Introduction, VCH, Weinheim, 1993.
[42] B.G.M. Vandeginste, D.L. Massart, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part B, Elesevier, New York, 1998, p. 649.
[43] J. Park, I.W. Sandberg, Neural Comput. 3 (1991) 246.
[44] B. Walcza, D.L. Massart, Anal. Chim. Acta 331 (1996) 177.
[45] A. Pulido, I. Ruisanchez, F.X. Ruis, Anal. Chim. Acta 388 (1999) 273.
[46] C.A. Andersson, R. Bro, Chemom. Intell. Lab. Syst. 52 (2000) 1.
[47] Z.P. Chen, H.L. Wu, J.H. Jiang, Y. Li, R.Q. Yu, Chemom. Intell. Lab. Syst. 75 (2000) 52.
[48] Z.P. Chen, H.L. Wu, R.Q. Yu, Anal. Chim. Acta 87 (2000) 423.
[49] A.K. Pettersson, B. Karlberg, Anal. Chim. Acta 354 (1997) 241.
[50] R. Bro, J. Chemom. 10 (1996) 47.
[51] J.L. Beltran, R. Ferrer, J. Guiteras, Anal. Chim. Acta 373 (1998) 311.
[52] R. Bro, Chemom. Intell. Lab. Syst. 34 (1996) 85.

[53] J.P. Brans, PROMCALC (Version 3) Software, Centre for Statistics and Operations Research, Free University of Brussels, Brussels, Belgium, 1991.
[54] S. Kokot, T.D. Phuong, Analyst 561 (1999) 124.
[55] G.A. Ayoko, J.J. Bonire, S.S. Abdulkadir, P.F. Olunrinola, J.O. Ehinmidu, S. Kokot, S. Yiasel, J. Appl. Organo-Met. Chem. 17 (2003) 749.
[56] N. Draper, H. Smith, Applied Regression Analysis, 2nd ed., Wiley, New York, 1981.
[57] S. Siggia, J.G. Hanna, N.M. Serencha, Anal. Chem. 35 (1963) 362.
[58] J.B. Worthington, H.L. Pardue, Anal. Chem. 44 (1972) 767.
[59] M. de la Guardia, K.D. Khalaf, B.A. Hasan, A. Morales Rubio, J.J. Arias, J.M. Garcia Fraga, A.I. Jimenez, F. Jimenez, Analyst 121 (1996) 1321.
[60] J.N. Miller, Analyst 116 (1991) 3.
[61] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1659.
[62] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 119 (1994) 1669.
[63] W.G. Lan, M.K. Wong, K.K. Chee, Y.M. Sin, Analyst 120 (1995) 273.
[64] W.G. Lan, K.K. Chee, M.K. Wong, H.K. Lee, Y.M. Sin, Analyst 120 (1995) 281.
[65] W.G. Lan, M.K. Wong, N. Chen, Y.M. Sin, Analyst 120 (1995) 1115.
[66] M. Otto, W. Wegscheider, Anal. Chem. 57 (1985) 63.
[67] P. Geladi, B.R. Kowalski, Anal. Chim. Acta 185 (1986) 1.
[68] Y. Ni, C. Huang, S. Kokot, Anal. Chim. Acta 480 (2003) 53.
[69] Y. Ni, S. Chen, S. Kokot, Anal. Chim. Acta 463 (2002) 305.
[70] Y. Ni, P. Qiu, S. Kokot, Anal. Chim. Acta (accepted).