ELSEVIER

Talanta 43 (1996) 1327–1333

Talanta

Variable-angle synchronous fluorescence spectrometry and rank annihilation methods for mixture resolution

F. García Sánchez*, M. Cedazo, J. Lovillo, A. Navas Díaz

Departamento de Química Analítica, Facultad de Ciencias, Universidad de Málaga, 29071-Málaga, Spain

Received 31 August 1995; revised 16 January 1996; accepted 19 January 1996

Abstract

The potential of variable angle synchronous spectroscopy (VASS) for fluorescent mixtures resolution was assessed and compared with the rank annihilation method (RAM). For this purpose, a set of excitation–emission matrices from three standard cyclodextrin fluorescence-enhanced solutions of the pesticides aminocarb, carbendazim and coumatetralyl and a mixture of them was obtained. Careful selection of the spectral routes to be scanned provides analyte signals that are free of interferences. Application of the rank annihilation method to excitation–emission matrices (EEMs) obtained by conventional scanning spectrofluorimetry gives quantitative results that show poor precision and accuracy when compared to those of VASS. The recoveries from ternary mixtures by VASS are within 99–104% and by RAM within 84–130%.

Keywords: Cyclodextrin-enhanced fluorescence; Mixture analysis; Rank annihilation method; Variable angle fluorescence spectrometry

1. Introduction

Although spectrofluorimetry has great sensitivity and moderate selectivity as an analytical technique, these performances in multicomponent analysis fall considerably because overlapping spectra give rise to energy-transfer processes and inner filter effects that produce spurious analyte signals. At present there are two fundamental ways to avoid this problem: modifications in analytical sampling and signal processing by mathematical algorithms after the raw data are obtained.

Simultaneous multicomponent analysis can be performed by mathematical treatment of the data such as realized by principal components regression (PCR), partial least squares regression (PLS), rank annihilation method (RAM), etc. Alternatively it is possible to act over experimental data acquisition in such a way that the contribution to the analytical signal from interfering components can be eliminated, or at least minimized. Variable angle scanning spectrometry [1–3] gives a useful approach to data acquisition for the simultaneous

* Corresponding author.

PII S0039-9140(96)01887-5

analysis of fluorescent multicomponent samples because of the reduction of the interferences associated with the design of the technique.

Mixture resolution by fluorescence spectrometry performed by mathematical procedures normally gives good results, when there are no strongly overlapped spectral profiles that would facilitate energy transfer processes between donor molecules (emission spectra) and acceptor molecules (excitation spectra). This effect produces quenching of donor and enhancement of acceptor that changes the spectral profiles of the mixtures compared with those of the standard solutions. This aspect is not a problem in VASS in which the selected routes can circumvent spectral zones subject to these energy transfer processes. The results obtained in this paper can be interpreted in this context.

Three different types of synchronous scanning have been developed: constant wavelengths (CW), constant energy (CE) and variable angle (VA). CE and CW scans maintain a constant separation (in cm^{-1} and nm, respectively) between excitation and emission beams [4]. An alternative approach, denoted variable-angle synchronous scanning (VASS) varies the separation in a controlled way. The continuous variation of the wavelength separation between the monochromators can be achieved either mechanically, by varying the scan speeds of the two monochromators, or digitally, by processing the stored data. Because mechanical control of the monochromator's speed only produces linear scan paths [5] and the generation of VASS from stored data [6] is very time consuming, a commercial digital instrument has been modified [7] to generate the variable-angle synchronous scan directly from the spectrofluorimeter output. This permits the VASS scan to be obtained in a few minutes by following a path previously selected by inspecting the contour lines.

The reduced peak-half-widths is often cited as the most important attribute of synchronous techniques for the resolution in multicomponent analysis. Recently, the theory of variable-angle synchronous spectrofluorimetry has been described in detail [8] and it has been deduced that the resolution in synchronous techniques is more related to the maximal peak intensity than with minimal peak width. Thus, the true advantage of VASS with respect to CWS is not the band narrowing effect but the capacity to go throughout maximal intensity peaks (circumvents overlapping spectral areas). This confirms the superior advantage of VASS in multicomponent analysis against CE, CW or derivative synchronous techniques due to the added flexibility in the choice of a scan path nearest to maxima.

Synchronous scanning methods have potential for multicomponent analysis that has not been fully realized, particularly for the automatization of sampling data. We report the simultaneous analysis of the agrochemicals aminocarb, carbendazim and coumatetralyl by VASS. The studied compounds display a low fluorescence quantum yield which is enhanced by using one of the almost universal features of cyclodextrin complexes such as fluorescence enhancement [9–11] by the inclusion of an organic fluorophore in the cyclodextrin (CD) cavity.

The technique of rank annihilation, described by Ho et al. [12] utilizes the excitation–emission matrix $\boldsymbol{M}$, to determine quantitatively the concentration of a component in the presence of a mixture. The excitation–emission matrix of a standard solution of each analyte yields a series of standard reference matrices $\boldsymbol{S}_1$, $\boldsymbol{S}_2$, etc. Then, successive fractions β of $\boldsymbol{S}$ are subtracted from the mixture matrix $\boldsymbol{M}$. When the fraction of $\boldsymbol{S}$ subtracted from $\boldsymbol{M}$ equals the amount of $\boldsymbol{S}$ originally present in $\boldsymbol{M}$, the rank of the resulting difference matrix $\boldsymbol{M} - \beta\boldsymbol{S}$ is one less than the rank of $\boldsymbol{M}$ and the nth eigenvalue of the difference matrix goes to a minimum. The concentration of the analyte in the mixture is then given by β times the concentration of the analyte in the standard solution used to measure $\boldsymbol{S}$. These operations are accomplished by performing a singular value decomposition (SVD) of the difference matrix:

$$v = \mathrm{svd}(\boldsymbol{M} - \beta^{*}\boldsymbol{S})$$

and adjusting the scalar fraction β until the nth singular value $v(n)$ is a minimum.

In this paper we try to evaluate the performances of a technique based on the more precise acquisition of the signal from the sample (VASS)

so as to compare it with the results obtained by a chemometric technique (RAM) based on signal processing. The critical comparison of the results obtained by both methods is also an objective of this paper.

2. Experimental

Fluorescence spectra were obtained with a Perkin-Elmer LS-5 luminescence spectrophotometer equipped with a xenon lamp (9.9 W) pulsed at line frequency. The spectrometer was interfaced to an IBM-PC/AT computer via the RS232C serial interface. The software package FLUOROPACK [7] controlled the instrument, data acquisition and data processing. The software used for the data analysis was PC-MATLAB (MAt-Works Inc., Sherborn, MA, USA).

The solvents used were pro analysis (Merck). Water was distilled and deionized. The pesticide carbendazim (99%) was purchased from Riedel-de Haen; aminocarb (99%) and coumatetralyl (96%) were supplied by Dr. Ehrenstorfer (Augsburg, Germany); β-cyclodextrin was kindly supplied by Amaizo, Co. (USA).

The stock standard solutions of aminocarb, and coumatetralyl 1 g L^{-1} and carbendazim 0.25 g L^{-1} were prepared by dissolving the pesticide in methanol, and were stored in the dark at 4°C. Working solutions of coumatetralyl and carbendazim were prepared weekly and that of aminocarb each two days, by dilution with methanol. β-cyclodextrin was purified by recrystallization once from boiling water and 10^{-2} M aqueous solutions were prepared. A buffer solution of pH 4.5 was prepared from 0.2 M acetic acid/sodium acetate.

2.1. Sample preparation

Aliquots of sample solution to give a final concentration between 1.5–4.0 μg mL^{-1} of aminocarb, 6.7–300 ng mL^{-1} of coumatetralyl and 2.3–5 μg mL^{-1} of carbendazim were placed in a 10 mL standard flask. The contents were slowly evaporated to dryness by a nitrogen stream, and after adding 1 mL of pH 4.5 acetate buffer, the volume was adjusted to 10 mL with 10^{-2} M β-cyclodextrin solution. The solution was sonicated for 20 min and the variable angle scanning spectra (VASS) was scanned. Finally, fluorescence intensity was measured in the VASS plots at 235/390 nm, 310/380 nm and 290/335 nm, respectively, and plotted against the concentration of each pesticide.

3. Results and discussion

Separate experiments were conducted to study the behavior of aminocarb, carbendazim and coumatetralyl in water and β-cyclodextrin solutions. In Table 1 the excitation and emission wavelengths and relative fluorescence intensities (RFI) in these media are presented. An emission enhancement in cyclodextrin media compared to water is observed. The weaker complexes produce faint emission signals. The formation constants of the β-CD complexes ([aminocarb] = 2 μg mL^{-1}, [coumatetralyl] = [carbendazim] = 1 μg mL^{-1}), obtained by the Benessi–Hildebrand methods [13] were 267 L mol^{-1}, 21 L mol^{-1} and 669 L mol^{-1} for the β-CD–aminocarb, –carbendazim and –coumatetralyl complexes, respectively. The maximum RFI is achieved in 10^{-2} M solutions of β-CD, a slight modification in this concentration has little effect on fluorescence intensity. Ethanol has also a little effect on the fluorescence; low proportions of this solvent are admitted but absence gives maximum sensitivity. A pH of 4.5 is adequate for sensitivity because it permits greater emission signals to be obtained.

Table 1
Excitation and emission wavelength and relative fluorescence intensity (RFI) for aminocarb, carbendazim and coumatetralyl in water and β-CD.

Compound	λ_{exc} (nm)	λ_{em} (nm)	RFI
Aminocarb (water)	237	383	185
Aminocarb (β-CD)	238	382	472
Carbendazim (water)	281	307	138
Carbendazim (β-CD)	210	307	264
	279		199
Coumatetralyl (water)	311	387	74
Coumatetralyl (β-CD)	311	387	785

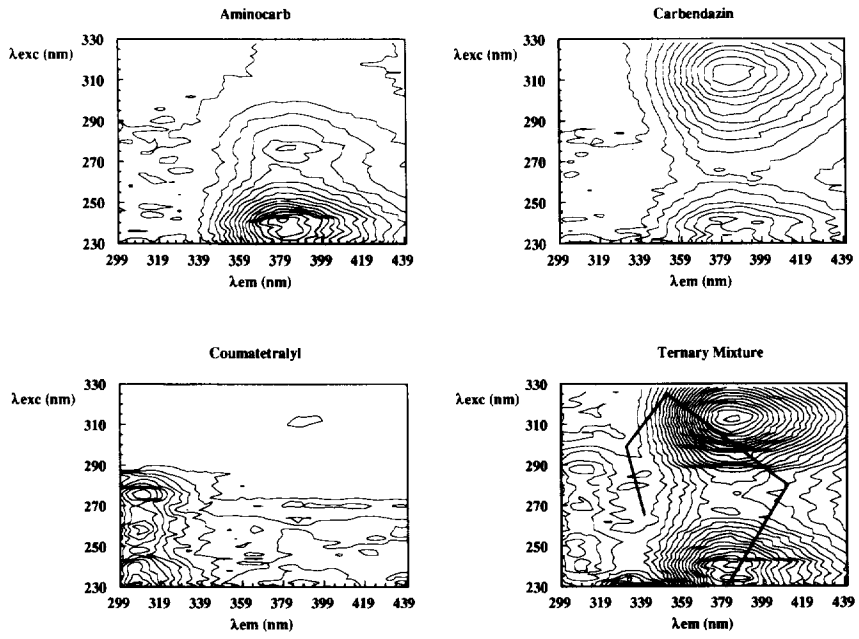

Fig. 1. Contour plots of aminocarb (3 μg mL^{-1}), carbendazim (5 μg mL^{-1}), coumatetralyl (0.5 μg mL^{-1}), and a mixture of them (approximately isoemissive, same concentrations as standards) showing the selected VASS route.

An EEM of the mixture (Fig. 1) was obtained and maxima of analytes were identified. The excitation and emission maxima pairs for β-CD complexes with aminocarb, coumatetralyl and carbendazim are 238–375 nm, 315–387 nm and 280–307 nm, respectively. This type of mixture cannot be resolved either by conventional fluorimetry (there are no excitation–emission pairs for selective excitation) nor synchronous scanning fluorimetry because the almost complete overlap of the spectral shapes impedes the detection of an interference-free signal. Moreover, using VASS, we can find those regions with minimum overlap and reduced cross interference between analytes.

The variable-angle scanning route was carefully determined by trial and error to traverse those parts of the three-dimensional (3-D) spectral zones with the least overlap. The scan was selected to transverse those parts of the 3-D data matrix with the least overlap and the nearest of the maximum peaks. In spite of the fact that losses in sensitivity occur because no maximum peaks are traversed, interference-free signals of the three components may be obtained from the chosen routes that scan the three-dimensional zones by skirting the slopes of the peak and avoiding the areas of interference between the three compounds. Fig. 2 shows a 3-D variable angle scanning spectrum for a ternary mixture.

The superior advantage of VASS in multicomponent analysis against CE, CW or derivative synchronous technique is due to the added flexibility in the choice of a scan path nearest to

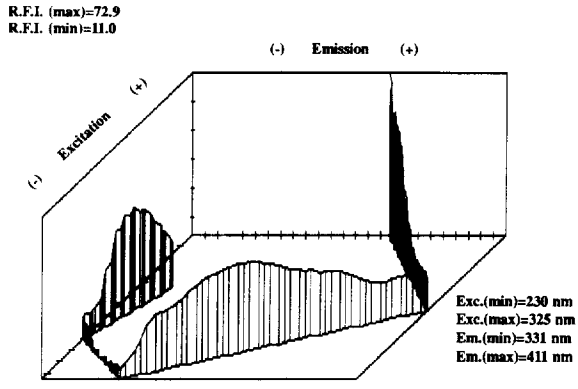

Fig. 2. Three-dimensional VASS of a ternary mixture (concentrations as in Fig. 1).

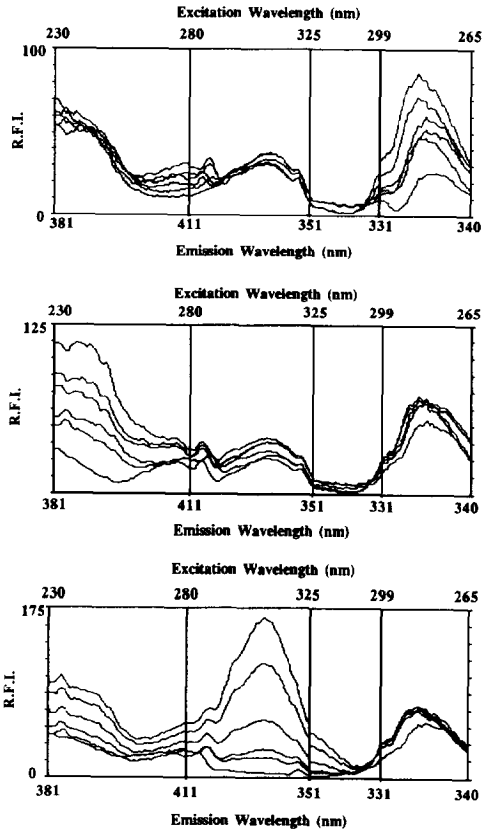

Fig. 3. Two-dimensional projection of several ternary mixtures. (A) [aminocarb] = 1 μg mL^{-1}; [coumatetralyl] = 0.05 μg mL^{-1}; [carbendazim] = 0, 1.5, 3 and 5 μg mL^{-1}. (B) [coumatetralyl] = 0.05 μg mL^{-1}; [carbendazim] = 5 μg mL^{-1}; [aminocarb] = 0, 1 > 1.5, 2.5, 3 and 5 μg mL^{-1}. (C) [carbendazim] = 5 μg mL^{-1}; [aminocarb] = 1 μg mL^{-1}; [coumatetralyl] = 0, 0.025, 0.05, 0.1, 0.2 and 0.3 μg mL^{-1}.

maxima. So, as expected the generation of VASS by following rigid routes, or by introducing a circular, elliptical or other function into the software routine, did not improve the method. The 2-D profiles of the optimized routes were less pronounced when rigid geometric functions were introduced.

Several 3-D variable angle spectra of a three-component mixture of the pesticides are shown in Fig. 3 to illustrate the potential utility of this technique for mixture analysis. The software program produces and displays the required data in 4–5 min. The fact that both monochromators of the spectrofluorimeter cannot be set to difficult scan speeds does not matter because the program signals override and differentially vary the excitation and emission monochromator drive motors of the spectrofluorimeter by pulsing their power input.

Table 2
Analytical parameters

Compound	Linear dynamic range (mg L^{-1})	Limit of detection (ng mL^{-1})	RSD (%)
Aminocarb	0.29–5.0	89	4.32
Carbendazim	0.18–5.0	55	8.99
Coumatetralyl	0.06–0.3	17	4.78

RSD, relative standard deviation.

Once the VASS spectra were obtained, fluorescence intensity readings at the maximum of VASS spectra and setting of the excitation and emission monochromators at the adequate wavelengths were performed.

Linear calibration graphs were obtained by plotting the fluorescence intensity against standard concentrations of aminocarb, carbendazim and coumatetralyl. Table 2 gives the linear dynamic range, detection limits and precision of the method. The accuracy was evaluated through a interferences study; the effect of some pesticides (fenitrotion, chlorpiryfos, dicofol and tetradifon) on the determination of a synthetic sample of composition 2 μg mL^{-1} aminocarb, 2 μg mL^{-1} carbendazim and 0.12 μg mL^{-1} coumatetralyl was evaluated. Various volumes of stock solutions of the different potential interferents were added to the standard solution in order to obtain different interferent-to-analyte ratios in the final solution. The results obtained are given in Table 3. Acceptable recoveries of coumatetralyl are obtained. Recoveries are low and high for

Table 3
Recoveries in the ternary mixture by VASS

Compound	Taken (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD (%)
Aminocarb	2.0	1.99	99.5	4.56
Carbendazim	2.0	2.067	103.8	8.25
Coumatetralyl	0.11	0.115	106	3.09

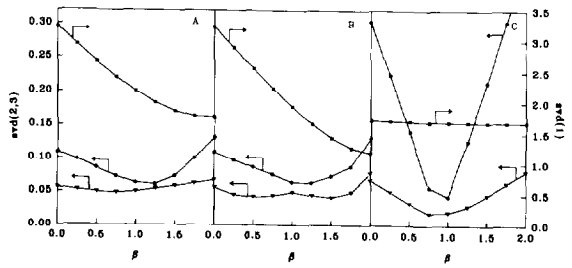

Fig. 4. First (■), second (●), and third (▽) singular values as a function of β. (A) Aminocarb, 3 mg L^{-1}; (B) Carbendazim, 5 mg L^{-1}; (C) Coumatetralyl, 0.3 mg L^{-1}.

aminocarb and carbendazim, respectively, when the interferent is twice or higher than the analyte concentration.

To apply the RAM method to the three-component mixture under study, we used four data sets, each composed of an EEM of the single standard component and a mixture of the three standards. The EEM were smoothed and blank-subtracted to remove scattered light. The resulting 48×48 matrices were then analyzed. Although blank substraction does not totally eliminate high scattered light, the regions of analytical interest are situated far away from the scatter emission; thus, this event will not affect the estimated concentrations of the analytes.

Performing a singular value decomposition of the difference matrix $(\boldsymbol{M} - \beta \boldsymbol{S})$ and adjusting the scalar fraction β until the nth singular value $v(n)$ is a minimum, we can obtain the optimum β value that multiplied by the standard matrix $\boldsymbol{S}$ (or the concentration of standard equivalent) gives the concentration of each standard in the mixture.

Fig. 4 shows the plot of the logarithm of the singular values (v) against the β parameter, showing the minimum value that gives the correct β value. It must be stressed that adequate selection of the svd values is critical to obtain good results.

The results of the rank annihilation method are summarized in Table 4. It can be observed from Table 3 that the recovery values are closest to 100% in the case of VASS indicating that interferences are avoided, while RAM suffers the interference of the other components in the mixture to a considerable extent. This result can be assigned to the incapacity of RAM, a method that uses all the spectral data to calculate the individual concentrations in the mixture, to eliminate the interferences arising from energy transfer or inner filter effects.

Finally, it must be pointed out that chromatographic resolution of this type of mixtures with severe spectral overlap, may be better and faster provided that no degradation of the products, during the chromatographic elution occurs. Moreover, in this case the use of cyclodextrin may be troublesome in the chromatographic elution.

4. Conclusions

Cyclodextrin-enhanced fluorescence determination of the pesticides aminocarb, carbendazim and coumatetralyl was performed by VASS and RAM. Fluorescence spectral distribution show great overlap that precludes the direct determination of this type of mixture. VAS shows better analytical performances than RAM due to the resolution of mixtures of overlapping fluorescence compounds.

Acknowledgements

We thank the Dirección General de Investigación Científica y Técnica (Project BIO94-0548) for supporting this study.

Table 4
Recoveries of three ternary mixtures by RAM

Mixture	Compound	Taken (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
1	Aminocarb	3	3.084	102.8
	Carbendazim	5	5.4	108
	Coumatetralyl	0.3	0.253	84.49
2	Aminocarb	1.5	1.569	104
	Carbendazim	2.5	2.33	93.16
	Coumatetralyl	0.15	0.178	118.72
3	Aminocarb	1.5	1.908	127.2
	Carbendazim	2.5	2.55	102
	Coumatetralyl	0.3	0.39	130

References

[1] F. García Sánchez, A.L. Ramos Rubio, V. Cerdá and M.T. Oms, Anal. Chim. Acta, 228 (1990) 293.

[2] M.T. Oms, R. Forteza, V. Cerdá, F. García Sánchez and A.L. Ramos, Int. J. Environ. Anal. Chem., 42 (1990) 1.

[3] F. García Sánchez, A. Fernández Gutiérrez and C. Cruces Blanco, Anal. Chim. Acta, 306 (1995) 313.

[4] T.T. Ndou and I.M. Warner, Chem. Rev., 91 (1991) 493.

[5] J.N. Miller, Analyst, 109 (1984) 191.

[6] B.J. Clark, A.F. Fell, K.T. Milne, D.M.G. Pattie and M.H. Williams, Anal. Chim. Acta, 70 (1985) 35.

[7] F. García Sánchez, A.L. Ramos Rubio, V. Cerdá and M.T. Oms, Talanta, 35 (1988) 335.

[8] S.E. Cabaniss, Anal. Chem., 63 (1991) 1323.

[9] S. Scypinski and L.J. Cline Love, Anal. Chem., 56 (1984) 322.

[10] I.M. Warner and L.B. McGown, Anal. Chem., 64 (1992) 343R.

[11] F. García Sánchez, A. Navas Díaz and L. Sánchez Feria, Anal. Lett., 27 (1994) 2171.

[12] C.-N. Ho, G.D. Christian and E.R. Davidson, Anal. Chem., 50 (1978) 1108.

[13] A. Benessi and J.H. Hildebrand, J. Am. Chem. Soc., 71 (1949) 2703.