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FLUORIM: A COMPUTER PROGRAM FOR THE AUTOMATED DATA COLLECTION AND TREATMENT USING COMMERCIAL SPECTROFLUORIMETERS

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Abstract—A general purpose program that uses a PC microcomputer has been designed for the control of experiments in fluorimetry. This program allows the digital collection of the main types of scans that a commercial spectrofluorimeter can perform: emission, excitation and synchronic; as well as the measurement of the fluorescent intensity as a function of time. The program also allows a series of operations with previously stored spectra. These operations include screen and paper plots of the spectra, calculation of linear combinations of them and the nine first derivatives, as well as their relative maxima and minima.

INTRODUCTION

Automation of chemical analysis has become an actual and important subject in analytical chemistry. This is due to the large amounts of time and effort that may be saved with the use of this type of system in the analysis and data treatment steps.

In this sense, automatic titration systems (Betteridge *et al.*, 1976; Christiansen *et al.*, 1976; Wu & Malmstadt, 1978), flow injection analysis (FIA) systems (Betteridge *et al.*, 1983; Koupparis *et al.*, 1985; Prop *et al.*, 1985; Dohmen & Thijssen, 1986) and liquid chromatography (Berridge, 1986; Cela *et al.*, 1986; Lea *et al.*, 1983) seem to have been of particular interest to analytical chemistry researchers.

Several applications of chemometrics in the field of fluorescence have been described. Various algorithms have been designed with different purposes, such as: location of spectrum peaks and their height and width determination (Stieg & Nieman, 1980; Miller & Faulkner, 1976); assignment of molecular structures or substructures to unknown samples by means of the search and comparison of their spectra with pattern spectra (Miller & Faulkner, 1976; Stadalius & Gold, 1983); identification of different oil types by comparison of their spectra with those of standard compounds (Killeen *et al.*, 1981); disease diagnostics by comparison of the patient's blood spectrum with blood patterns of specific diseases (Wolfbeis & Leiner, 1985); decomposition of the spectrum of a mixture of compounds into the individual contributions of each component (Gold *et al.*, 1976; Rechsteiner *et al.*, 1977); identification and quantitative evaluation of individual fluorescent compounds in multicomponent samples (Hörer & Enache, 1986; Gold *et al.*, 1980; Sjöström *et al.*, 1983; Lindberg *et al.*, 1983; Apellof & Davidson, 1983); obtainment of corrected fluorescence spectra (Ritter *et al.*, 1981) as well as of fluorescence quantum yields (Ritter *et al.*, 1981; Rhys Williams *et al.*, 1983).

However, as can be observed, these algorithms are mainly devoted to solving specific problems. As the need for the use of fluorescence as a means for the detection and quantitative determination of polynuclear aromatic hydrocarbons (PAHs) arose at our laboratory and considering the great number of scans to be performed in this investigation, the possibility of using a microcomputer in order to perform an easy data storage and treatment of so much information was evaluated.

The high cost of commercial software for spectrofluorimetric data collection, which in most cases can only be obtained through the purchase of certain equipment sets, such as the Perkin–Elmer LS-5 and Perkin–Elmer model 3600 data station, together with the difficulties in obtaining the original source code and adapting it to other instruments have forced us to design our own software. In contrast with the foregoing, we have tried to design it to be as general and open as possible, allowing subsequent modification in a simple manner as new needs arise, and to be easily adaptable to other types of hardware.

HARDWARE

The program developed has been implemented on an IBM PC/AT and compatible PC/XT microcomputers fitted with 640 kb RAM memory and standard graphics adapters (CGA etc.). The measuring device used was a Shimadzu RF-540

spectrofluorimeter, coupled to a Shimadzu DR-3 recorder. Communication between the different computers and the fluorimeter was performed by means of an IEEE-488 interface. The paper plots have been obtained using an IBM proprinter and an IBM 7372 plotter.

SOFTWARE

In order to increase the ease of use and adaptability of the program, it has been written in BASIC, both in interpreted (IBM Basica 3.10 and Microsoft GW-BASIC 2.02) and compiled (Microsoft QuickBasic 2.0) versions. The source code for the interpreted and compiled versions takes about 61 kb. The executable code size in the compiled version is 122 kb. For both versions the core requirements are approx. 40 kb.

Figure 1 shows a diagram of the main functional blocks of the FLUORIM program.

This program has two well-defined blocks. The first is the device control block and the second allows the treatment of the results obtained with the help of the previous block. The control block allows us to perform three types of scans of the relative fluorescene intensity (i_F) as a function of the wavelength (λ): emission, excitation and synchronic scans. It also allows us to record i_F as a function of time, either with continuous or with time interval irradiation of the samples. The data obtained are stored in files for later treatment.

To carry out the first three types of scans, the computer sends the instructions to the fluorimeter which temporarily stores the data in its internal memory. When the operation is completed, the data are transferred to the computer, stored in a file and displayed on the screen.

The file format in which the scans are stored consists of a header, in which the type of scan and

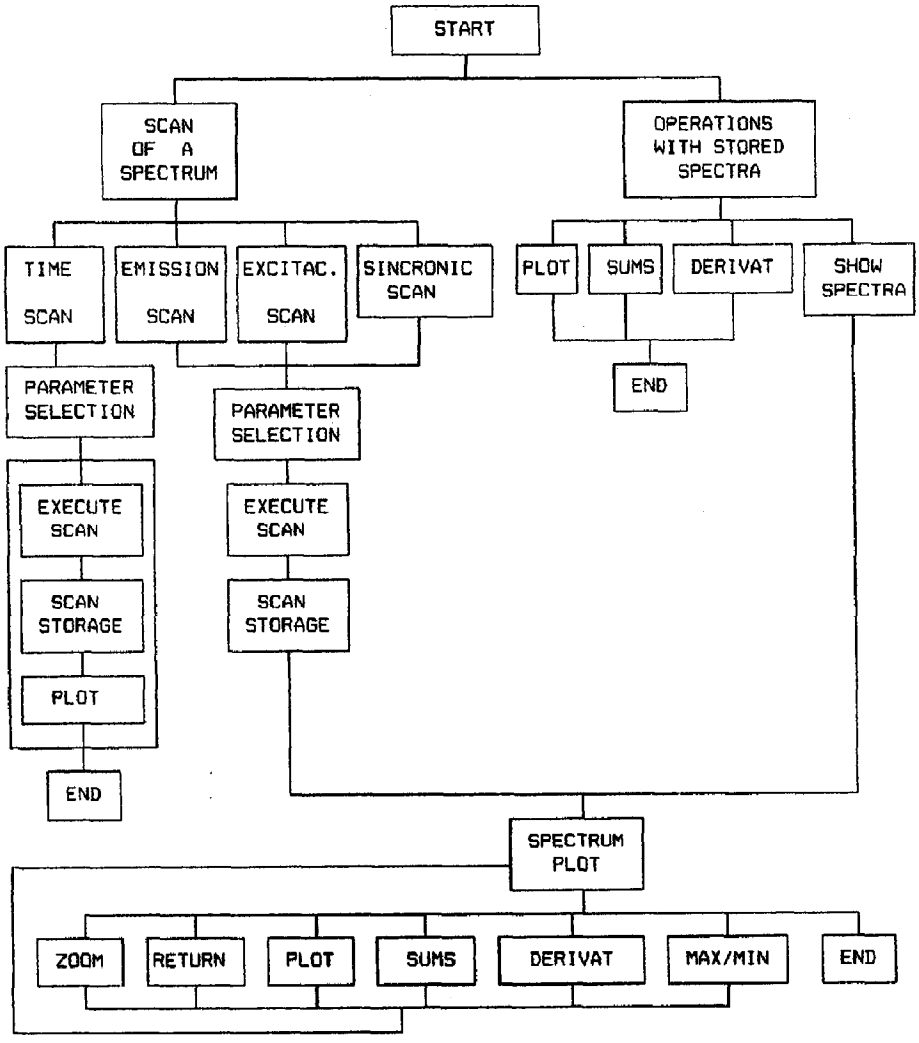


Fig. 1. Functional block diagram of the FLUORIM program.

its characteristic parameters are detailed, followed by the numeric data pairs (λ, i_F) corresponding to every scan point. The device used allows us to obtain scans with three different resolution values (0.1, 0.5 and 1 nm), defining this parameter as the inverse of the λ interval between two consecutive values.

To perform the fourth type of scan, the device is instructed to collect the i_F values at the desired time interval, store the data in a file and display them on the screen as they are sent from the measuring instrument. During the time interval between data collection the sample may or not be irradiated. The scan resolution of this fourth type is the inverse of the time interval between data collection.

The data treatment block allows us to perform the following operations with the files created in the former block.

SHOW/SPECTRA operation

This operation allows presentation on the screen of any scan or the result of any SUMS or DERIVAT operation, giving access to the SUMS, DERIVAT and MAX/MIN operations. It also allows the enlargement (ZOOM) of a zone of them. Figure 2 shows an emission spectrum of a 1017 ng/ml benzo-[*a*]pyrene solution in dioxane as well as a zoom of part of it. Both these plots were obtained by directly printing the computer screen on the printer.

MAX/MIN operation

Scan characterization is performed with this operation. The position and values of the scan's relative maxima and minima are obtained. This operation also allows screen and printer output of a report of them.

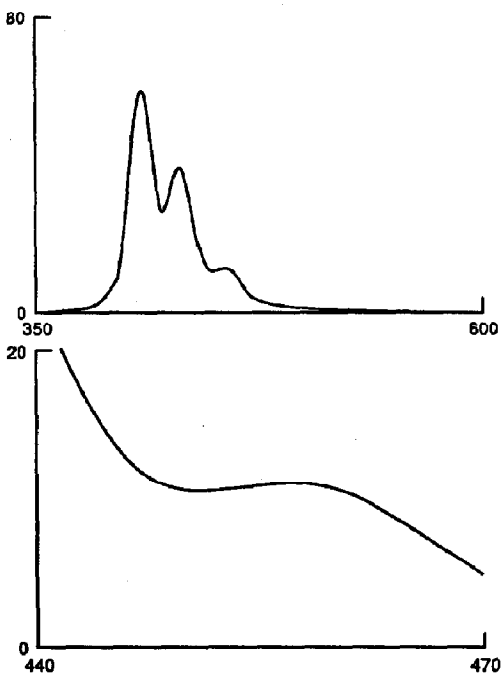
In a first approximation the points (λ, i_F) having i_F values greater than their two adjacent points are selected. In order to avoid assignation errors due to possible instrument noise, at a second stage those points having i_F values greater than their two adjacent points are chosen from amongst the points selected at the former step and given the label of maxima, provided that they are not separated by more than 10 nm from their adjacent points; the limit in which the noise is considered to have been eliminated. The minima computation is performed in a similar way.

Figure 2 also shows the printer output obtained as a result of applying the MAX/MIN operation to an emission spectrum of a 1017 ng/ml benzo[*a*]pyrene solution in dioxane.

DERIVAT operation

This operation allows the calculation of the first nine derivatives of a scan, with a graphic output of each one of them.

The algorithm used for the derivation consists of the computation of the ratio of the increments of the $y(i_F)$ and $x(\lambda)$ values for every two consecutive scan points. This quantity is assigned to an x value in the middle of the x values of the considered points.



Relative maxima		Relative minima	
Wavelength	Fluor. Int.	Wavelength	Fluor. Int.
371.0	0.6	377.0	0.5
409.0	60.6	422.0	26.1
432.0	38.8	451.0	10.5
457.0	11.0	588.0	0.0
668.0	0.0	649.0	0.0
708.0	0.0	706.0	0.0

Fig. 2. Emission spectrum at $\lambda_{exc} = 370$ nm of a 1017 ng/ml benzo[*a*]pyrene solution in dioxane and a zoom of a part of it. Both pictures have been obtained by directly printing the screen display on the printer. The printer output is obtained as a result of the application of the MAX/MIN operation to this spectrum.

The use of derivative spectra can be particularly useful in two cases:

(a) To discover the presence of shoulders in the spectra. Figure 3 shows an emission spectrum of a 4 μg/ml Cloxazolam (a psicotropic drug) solution (1) and its first derivative (2), where the presence of a shoulder is more clearly appreciated.

(b) To facilitate the measurement of some peak heights. Figure 4 shows a synchronic spectrum of 8 ng/ml benzo[*a*]pyrene solution in dioxane (1) and its first derivative (2), where peak height determination is easier.

PLOT operation

This operation allows us to obtain hard copies of previously stored scans in a rapid and easy manner with the help of a plotter. It is possible to accumulate up to 20 different scans on the same sheet of paper,

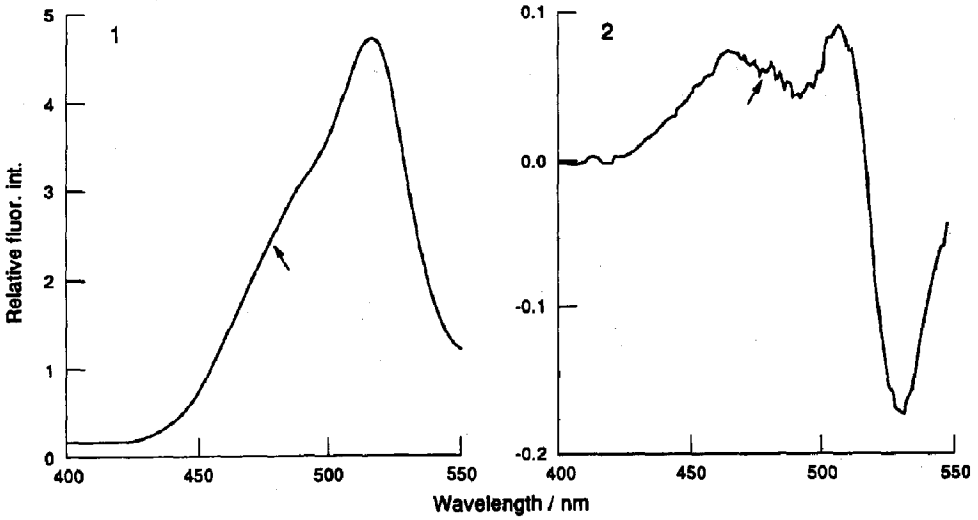


Fig. 3. Emission spectrum at λ_{exc} = 258 nm of a 4 μg/ml Cloxazolam solution in an HCl 0.05 M, KCl 0.5 M and (98 eq : 2), water–methanol medium (1) and its first derivative spectrum (2), where the presence of a shoulder is more clearly appreciated.

providing a useful way for visual comparison of them. This operation is based on the creation of files with adequate format to be introduced in the PLOTIT and PLOT-HP programs (Puigdomenech, 1986). Figures 3–6 have been obtained by means of this operation.

SUMS operation

With this operation it is possible to obtain any linear combination of up to 20 scans in such a way that: resulting scan = α_1*scan1 + α_2*scan2

$$+ \cdots + \alpha_{20}*\text{scan20}.$$

The result of this operation is shown on the screen, and with it it is possible to perform any of the program data treatment block operations.

This operation can be particularly useful in two cases:

(a) When it is necessary to subtract the blank spectrum from the sample spectrum. As an example, Fig. 5 shows the emission spectrum of 4 μg/ml Cloxazolam solution in an HCl 0.05 M, KCl 0.5 M and (98 : 2) water–methanol medium (1) and that corresponding to the blank solution (2) as well as the result of subtracting the second from the first with the help of the SUMS operation (3).

(b) When the simulation of the spectrum of a compound mixture is desired. This simulation will be reliable when it is possible to assume

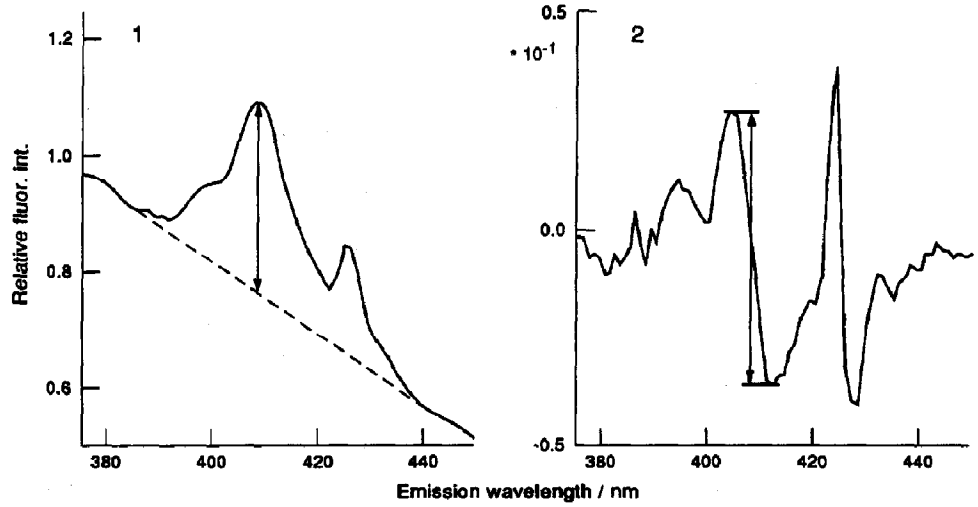


Fig. 4. Synchronic spectrum at $\Delta\lambda$ = 6 nm of a 8 ng/ml benzo[*a*]pyrene solution in dioxane (1) and its first derivative spectrum (2), where peak height determination is easier.

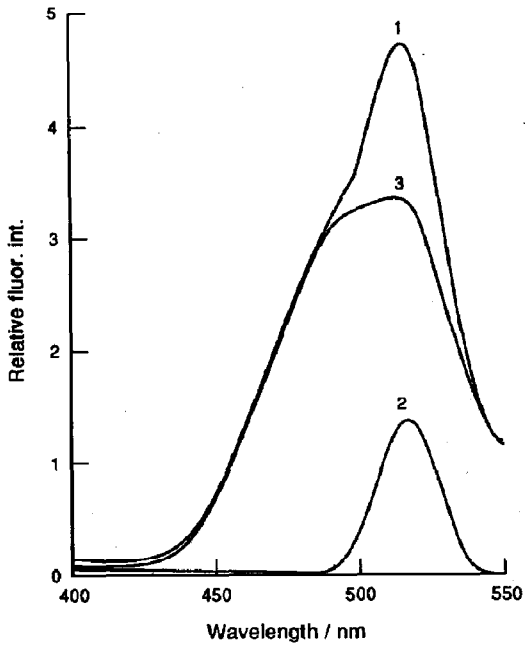


Fig. 5. Emission spectrum at $\lambda_{exc} = 258$ nm of a 4 μg/ml Cloxazolam solution in an HCl 0.05 M, KCl 0.5 M and (98 eq : 2) water–methanol medium (1) and that corresponding to the blank solution (2), together with the spectrum resulting from subtraction of the second from the first with the help of the SUMS operation (3).

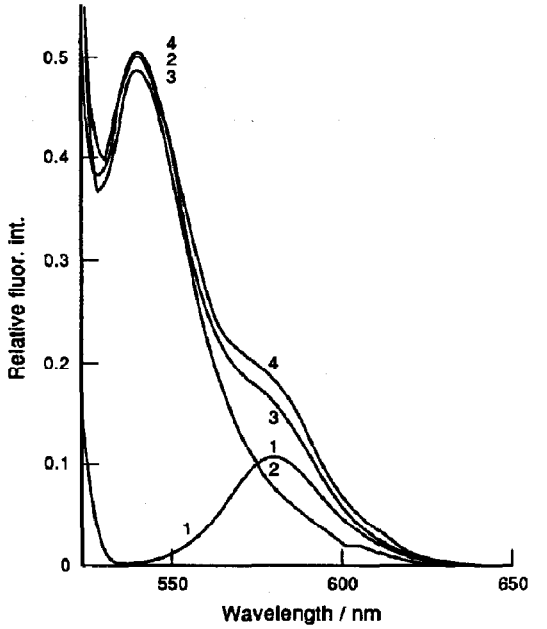
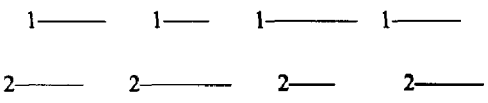


Fig. 6. Emission spectra at $\lambda_{exc} = 510$ of 11.4 ng/ml rhodamine B (1), 13.9 ng/ml eosine (2) and an 11.4 ng/ml rhodamine B and 13.9 ng/ml eosine mixture (3) solutions in NaOH 0.1 M, together with the spectrum resulting from the application of the SUMS operation to the first two (4).

that the fluorescence intensity of a fluorophores' mixture is the sum of the fluorescence intensities of each of them individually. Figure 6 shows the emission spectra at $\lambda_{exc} = 510$ nm of solutions 11.4 ng/ml rhodamine B (1), 13.9 ng/ml eosine (2) and a 11.4 ng/ml of rhodamine B and 13.9 ng/ml eosine mixture (3) in NaOH 0.1 M, together with the spectrum resulting from the application of the SUMS operation to the first two (4). Good agreement between the spectra corresponding to the rhodamine B and eosine mixture solutions and the simulated spectra obtained by means of the SUMS operation is seen.

In relation to the way in which operations with scans are carried out, it is necessary to indicate that in order to perform the PLOT and SUMS operations, the scans may overlap in any of the following ways:



In any of these cases the result of the PLOT operation includes the operated registers completely, enclosing the no overlapping zones. However, the result of the SUMS operation is only obtained for the overlapping zones.

The PLOT and SUMS operations may be applied to any type of scan provided that it fulfils two conditions. First, scan data (x, y) must be separated by a constant difference in the x values (resolution was defined as the inverse of this difference). Second, the resolutions of the different scans to be operated must be an integer number of times the smaller of them. The resolution of the result of both operations will be that of the lower of the operated registers.

CONCLUSIONS

An algorithm as general and useful as possible has been designed. This program, applied to the specific case of fluorescence intensity measurements performed with a Shimadzu RF-540 instrument, can be easily adapted to other commercial spectrofluorimeters and to other analytical techniques, such as u.v.–vis spectrophotometry, with the only requirement being modification of the specific communication codes between the device and computer.

The modular program structure used easily allows the inclusion of other subroutines that may perform new operations, whenever this may be considered convenient, without the need to make important changes in the remaining program.

The program is available upon request from the authors.

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