

Fast Scanning Hadamard Spectrophotometer

RONEI J. POPPI, PEDRO A. M. VAZQUEZ, and CELIO PASQUINI*

Instituto de Química, Universidade Estadual de Campinas, C.P. 6154, CEP 13081, Campinas, SP, Brazil

A simple, low-cost, multiplexed Hadamard-type spectrophotometer based on a spinning disk containing 63 separated masks has been constructed to obtain spectral data in time intervals down to 50 ms. Each mask contains 63 resolution elements. Optical localization and identification of the masks were implemented in the disk to avoid misalignment problems. The control of the spectrophotometer and data acquisition was made by using a dedicated single-board microcomputer based on an 8085 CPU. The single board microcomputer sends the data, through an RS-232C interface, to an IBM-AT compatible microcomputer where the Hadamard transform is performed to recover and display the emission, transmittance, or absorbance spectrum. The instrument has been used in the visible range to obtain absorbance data for time-resolved flow injection analysis applications.

Index Headings: Hadamard spectrometry; Fast spectral data acquisition; Flow injection spectrophotometric gradient scanning; Visible spectrometry; Multiplex spectrophotometer.

INTRODUCTION

Hadamard spectroscopy has been undergoing a revival of interest recently, with the literature reporting on its principles and applications¹ and offering at least one clear tutorial.² Hadamard spectroscopy presents advantages over the other major multiplexed-type spectroscopy, based on Fourier transform, by requiring simpler electronics and computing, while keeping the multiplex (Fellgett) advantage,³ which helps to improve the signal-to-noise ratio when the main source of noise comes from the detector.

Although the recent advances in Hadamard instruments are all based on the use of electro-optical devices to overcome mechanical problems associated with spectral data acquisition,⁴⁻⁶ such devices are still slow, extending the time interval to a few minutes for a total spectrum acquisition. Also, problems related to the transparency of the substratum for the electro-optical device in various spectral ranges are still to be solved. On the other hand, the number of analytical applications demanding time-resolved spectrometry has increased recently. The flow injection analysis (FIA) technique,⁷ for example, is capable of generating a concentration gradient (e.g., a pH gradient) that can be exploited in order to perform spectrophotometric multi-analyte determination.^{8,9} Fast and expensive diode-array spectrophotometers have been used for such a task.^{9,10} The FIA gradient scanning requires an entire spectrum to be obtained in less than 0.5 s, and a typical gradient lasts for about one minute.

This work describes a simple, low-cost, Hadamard spectrophotometer capable of acquiring spectral data in short time intervals. The mechanical approach to drive

the encoding masks, based on the previously proposed rotating encoder disk,¹¹ was adopted. Furthermore, the problems with misalignment have been minimized by using an optical mask localization that triggers the intensity measurements. As the mask is optically transparent to any spectral range, the approach could, in principle, be used in other types of spectroscopy, although the immediate interest in using it in time-resolved FIA applications leads to the development of a visible, medium-resolution instrument.

EXPERIMENTAL

Overview of the Hadamard Spectrophotometer. Figure 1 shows a diagram of the developed instrument. Simple dispersing optics based on a symmetrical Czerny-Turner arrangement were employed to obtain a compact instrument. The width of the entrance slit was fixed at 0.4 mm, and its height was 1 mm. The concave mirrors of the Czerny-Turner dispersing arrangement have 15 cm focus. The grating has 600 grooves/mm, blazed at 650 nm (Edmond Scientific). The dispersed beam is focused on the surface of the encoding disk described below. The portion of the dispersed light passing the masks is de-dispersed in a simple way by using a 3-cm-focus, 5-cm-diameter concave mirror. The mirror was located 8 cm away from the plane of the masks disk. A photodiode detector (Hamamatsu, S1226-5BQ) was placed at the focus of this mirror to follow the total light intensity passing through the mask. The photodiode was assembled in the photo-current model.³

A dedicated single-board microcomputer based on the 8085 CPU was employed to collect the 8- or 10-bit digitalized intensities and to control the data acquisition process. Data are stored in the RAM of this board and transmitted to an IBM-AT compatible microcomputer (Zenith, 10 MHz, containing an 80287 math co-processor, 512 Kbytes RAM, 40 Mbytes Winchester, and an EGA white-paper monitor) for further data transformation and treatment.

Encoding Disk and Optical Mask Positioning. Figure 2 shows the disk containing 64 radially disposed encoding masks. The disk has a 10-cm diameter and was obtained by chemical corrosion of a 0.3-mm-thick stainless-steel sheet. One totally open mask is present to be used for the maximum-intensity estimation in order to allow, in the future, automatic gain adjustment of the photodiode amplifier stage. Each one of the other 63 encoding masks has 63 resolution elements distributed following the pattern of rows of an S-matrix generated as previously described.¹² Ones and zeros in the matrix rows are translated as an open or a shut element, respectively, in the masks. Each resolution element is 1.8 mm high and 0.4 mm wide. The total length of each encoding mask is,

Received 14 April 1992; revision received 24 July 1992.

* Author to whom correspondence should be sent.

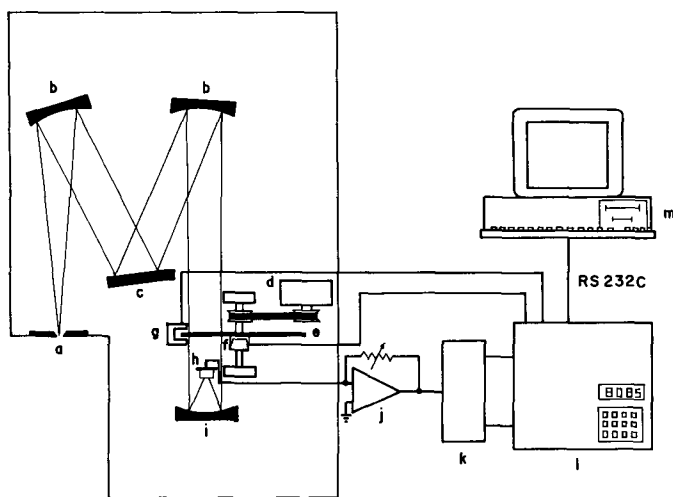


FIG. 1. Overview of the Hadamard spectrophotometer. (a) entrance slit; (b) concave mirrors; (c) dispersion grating; (d) step or dc motor; (e) disk containing the masks; (f) reflective opto-switch; (g) transmissive opto-switch; (h) photodiode sensor; (i) dedispersing concave mirror; (j) gain stage for the photodiode signal; (k) analog-to-digital converter; (l) single-board microcomputer; and (m) external microcomputer.

therefore, 25.2 mm. The disk was manufactured by Metalfoto SA, SP, Brazil, on the basis of the pattern supplied by this laboratory. An adequate correction (which depends on the material, its thickness, and the chemicals employed) was applied to the size of each element in the original drawing to ensure equal width of shut and open elements after the chemical corrosion process.

There are 64 slits, 0.9 mm high and 2.0 mm wide, one for each of the 64 masks at the border of the disk. At the central part of the disk, near the totally open mask, there is another slit of equal size. The external slits are used to sign the correct position of the masks in the dispersed beam, triggering the measurement routine software. The internal slit is used to establish the correct sequence of masks passing through the dispersed light. The external slits are monitored by a transmission optical switch (PCST-2103). The position of the internal slit is monitored by a reflective device (RS, Cat. #307-913).

The disk was fixed in a shaft that rotates under control of a stepper ($1.8^\circ/\text{step}$, maximum of $600 \text{ steps} \cdot \text{s}^{-1}$) or a dc motor (rotation speed controlled from 1 to 40 rotations per second). The transmission of the motor movement to the shaft was made with a teathed belt.

Dedicated 8085 Microcomputer and Data Acquisition Hardware. The Hadamard spectrophotometer was constructed around a dedicated single-board microcomputer based on the 8085 CPU, similar to the SDK-8085.¹³ An expansion area is available on the board to implement user-selected applications. Some simple modifications were implemented in order to fit the original board to the instrument. The RAM capacity was expanded to 4 Kbytes. An RS-232C bidirectional standard serial interface was included, based on the CI 8251A. The baud rate can be selected from 1200 to 9600 bits per second. A 10-bit ZN502E analog-to-digital converter ($15 \mu\text{s}/\text{conversion}$) was also included in the expansion area of the board.

Software for the Dedicated Microcomputer. A small machine code software piece was recorded in the original

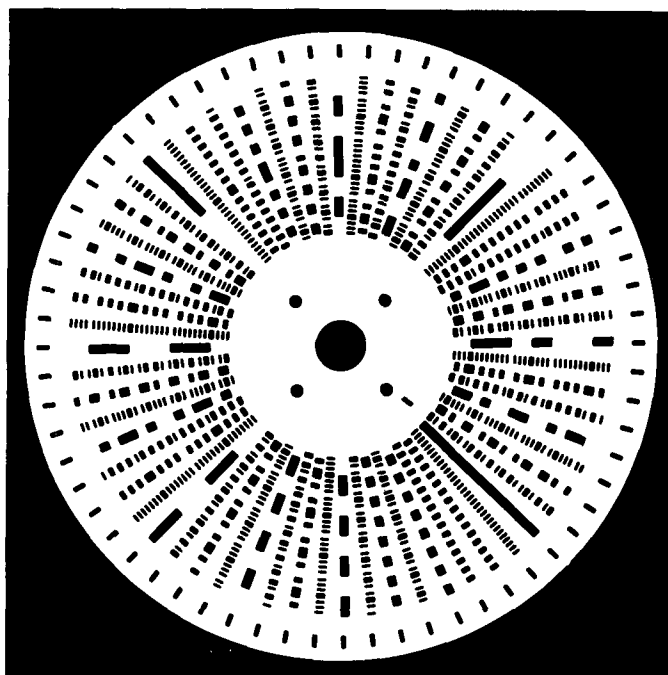


FIG. 2. Disk containing the Hadamard masks and optical encoder for mask localization.

EPROM address area below the 2 Kbytes used for the monitor program of the single-board microcomputer. When this program is run from the dedicated microcomputer keyboard, it is ready to accept the main machine code program from the external microcomputer through the RS 232C serial interface running at 9600 baud. This approach to the software manipulation was adopted to reduce the time spent in the development, as the machine code can be easily modified in the external micro, by using an Assembler for 8085, and tested quickly in the dedicated computer. When the main program is received by the dedicated micro and allocated in its RAM, it can be run from a command sent by the external microcomputer. Once the control is transferred to this main program, the external micro can request the spectrophotometer to acquire, to store, and to send spectral data. Also, an entire procedure, necessary for FIA applications, can be performed by sending only one command from the external computer.

Intensities Measurements Procedure. A fundamental piece of software that must be present in any kind of application of the spectrophotometer is that related to the acquisition of intensity data. In the developed instrument, intensity data are acquired under control of the optical position encoder present at the border of the masks disk.

Figure 3 shows a time diagram of the events occurring when the disk is spinning. A complete reading starts when a negative-going pulse, coming from the reflective opto-switch, is detected by the 8085 CPU. Therefore, it can be considered as a start-pulse control signal. The transmission opto-switch is then monitored. The software waits for a high-to-low TTL level transition followed by a low-to-high transition. When it occurs, the light intensity, passing through the mask, is at its maximum and the data acquisition software is triggered. The

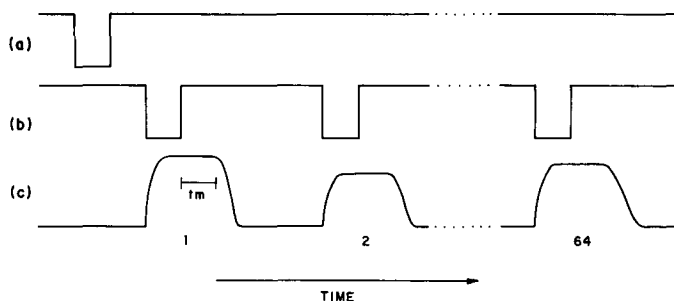


FIG. 3. Main events occurring during the measurement process in the spectro-photometer. (a) Scan start signal generated by the reflective opto-switch; (b) triggering signals generated by the transmissive opto-switches during the passage of the masks through the dispersed beam; (c) signals due to the total dispersed light passed through the masks and t_m , time interval for measurement.

software looks for 64 negative-going pulses; at the end of the 64th pulse, a complete spectral data set is acquired. If required, the negative pulse from the reflective opto-switch is again monitored to start new data acquisition. The time interval shown as t_m in Fig. 3 is available for the intensity readings. The digitalized data are stored in the dedicated microcomputer RAM. When the data collection routine is finished, the dedicated microcomputer signals the external micro. The external micro can access the data using an adequate protocol.

Software for the External Microcomputer. The external microcomputer runs a software written in QuickBasic 4.5. The program is menu driven and provides the necessary subprograms for data treatment. The Hadamard transform is made with the use of the algorithm previously described;¹ for the 63 resolution elements herein employed, it takes less than 0.5 seconds.

The user can select the number of readings per mask to be performed and the number of revolutions of the disk which must be monitored during a complete data acquisition routine.

Data can be manipulated in the external microcomputer to obtain an emission, an absorption, or a transmittance spectrum. All results are displayed in graphic form and can be copied to a dot matrix printer.¹⁴

For absorption measurements, the radiant power transmitted in the absence of the sample is first accessed and stored to be used in the transmittance calculation.

Two smoothing procedures were evaluated, based on the following mathematical relationships,¹⁵ applied to the intensities which resulted from the Hadamard transform:

$$SI_n = \frac{I_{n-1} + I_n + I_{n+1}}{3} \quad (1)$$

$$SI_n = \frac{1I_{n-2} + 2I_{n-1} + 3I_n + 2I_{n+1} + 1I_{n+2}}{9} \quad (2)$$

where SI_n is the smoothed n th spectral intensity, and I_n is the raw transformed intensity of the n th resolution element. To obtain an absorption spectra, one smooths both the data of the original throughput and those after absorption in the flow cell before the absorbance calculation is performed.

One of the items of the principal menu selects the FIA gradient scanning application. The user can choose the injection time (the time interval during which the sam-

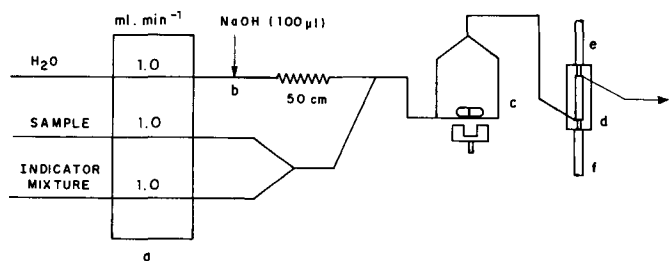


FIG. 4. Flow injection system employed in the gradient scanning technique. (a) Peristaltic pump (Ismatec MP13); (b) point where the NaOH solution is introduced; (c) gradient camera; (d) "Z"-type flow cell; (e) optical cable for light transmission to the flow cell; (f) optical fiber cable to the spectrophotometer entrance slit; (g) waste fluid line.

pling valve is turned on to introduce a sample), the time interval before starting data collection, the number of scans to be performed, and the time interval between scans. Time intervals are controlled by the dedicated microcomputer using a 0.1-s delay generated by software.¹⁶

For the application in mind, the time interval between spectra acquisition was set to 1 s. By doing this, one ensures that the external and the dedicated microcomputer can work in parallel. While a new spectrum is being acquired by the dedicated micro (this operation takes 0.3 s when the stepper motor is used to rotate the disk), the external micro performs the necessary data treatment, including the Hadamard transform, on the previously acquired intensity data. Up to 55 spectra can be acquired with the use of the present software and configuration of the external computer. Therefore, 55 s of the gradient FIA signal can be monitored.

Acquisition of a greater number of spectra is made possible by lowering the time interval between spectra acquisition, but the parallel data processing cannot be implemented anymore. The data are then saved in the external computer to be treated at a later time. The maximum speed of data acquisition is attained by storing all the spectral data in the dedicated microcomputer RAM and sending it after the data collecting routine has been finished.

Flow Injection System for Gradient Scanning. The FIA system described in Fig. 4 has been used to scan a pH gradient generated by introducing 100 μ L of a NaOH solution (0.75 M). An automatic sampling device¹⁷ is used for the NaOH introduction. The alkali solution is dispersed and mixed with the sample solution (which is pumped continuously) in the gradient camera, under magnetic stirring.

Light from a continuous source (tungsten-iodine) is guided to the flow cell and from there to the spectrophotometer entrance by using two optical fiber cables. The flow cell has a 2-cm-long optical pass and 2-mm inner diameter.

Samples used for testing the capacity of gradient scanning of the Hadamard spectrophotometer consisted of mixtures of acetic and hydrochloric acids. These mixtures merge with another solution of two acid-base indicators: phenolphthalein and bromocresol green. When exposed to the alkaline gradient in the camera, the acids are sequentially titrated, and the color change of the indicators can be monitored in the tail portion of the FIA signal.

RESULTS AND DISCUSSION

This spectrophotometer was developed to be used for gradient scanning in FIA applications. As stated before, the scanning speed does not need to be greater than 0.5 s. However, the instrument was evaluated for its performance in terms of its scanning speed. The dc motor was employed, and the rotation speed was changed in order to obtain a complete spectral set of data (one complete disk revolution) in time intervals of 30, 50, 100, 200, 300, and 600 ms. The results show that the scan speed limit for the present electronics is 50 ms. With this scan speed, two measurements of the intensity are possible during the short t_m time interval within which the dispersed beam is passing through each mask (see Fig. 3). The absorption spectra of a 1.0×10^{-4} M KMnO_4 solution do not differ substantially from those obtained at longer revolution times, such as 200 or 300 ms. The main limiting factor observed when the scan speed was increased is due to the present electronics around the photodiode. As the revolution speed was increased above one per 30 ms, the mask intensity signal, which usually displays the form shown in Fig. 3, did not return to zero in between two consecutive masks and could not reach the same amplitude as obtained at slower rotation speed. In fact, the intensity signals show a peak-like format, and the safe range for data acquisition (t_m) is not observed anymore. Another limiting factor could be the data capture time interval. However, on the basis of the current $20 \mu\text{s}$ per complete reading (8-bit analog-to-digital conversion and digital data storage in the dedicated microcomputer RAM), the spectral data acquisition time interval could be reduced (if only one intensity measurement is taken for each mask) to about 1 ms. Obviously, well-balanced mechanics must also be employed to couple with the higher-revolution speed that will be necessary. For the applications addressed, the scanning rate obtained is far more than sufficient.

The scanning speed selected for FIA applications was 300 ms. The stepper motor was employed to maintain a constant rotation speed, ensuring good precision of the scanning time interval.

Raw intensities for each resolution element, found after the Hadamard transform has been applied, form a profile like the one shown in Fig. 5A. The irregular profile is produced mainly by the defects in the size of the mask elements. However, the intensities for each element are reproducible. A mean relative standard deviation of 1.3% was observed for the 63 intensities obtained from ten consecutive 300-ms scans taken at ten measurements per mask.

Raw data were submitted to two smoothing procedures represented by Eqs. 1 and 2 in the experimental section. The resulting 61 or 59 intensities are shown in Fig. 5B and 5C. A substantial decrease in the mean relative standard deviation of the smoothed intensities was observed. With the same raw intensities obtained as described above, the values were 0.8 and 0.7% for smoothing procedures 1 and 2, respectively. The profiles of emission spectra are improved as they look more like those expected for a continuous source. Procedure 2 appears to be the better one. However, it was observed that this approach introduces substantial distortion in the ab-

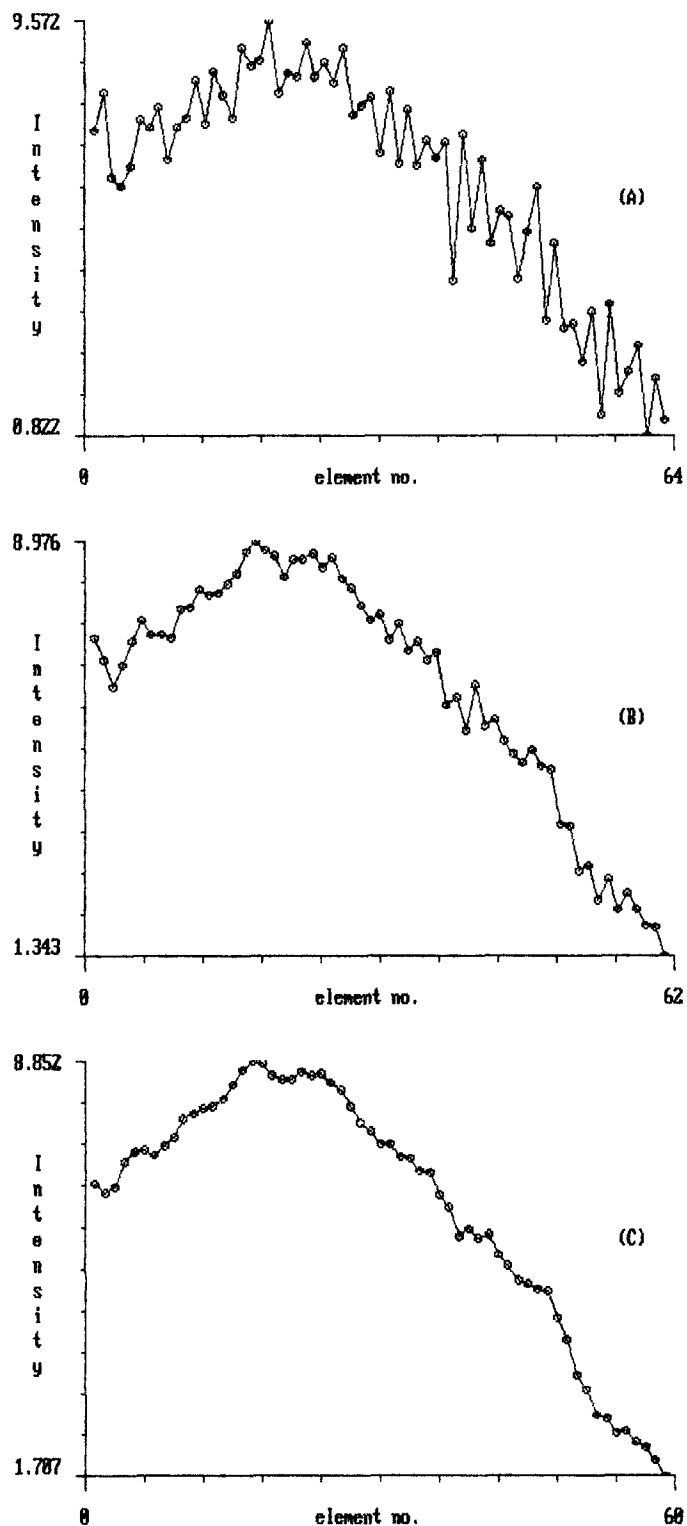


FIG. 5. Emission spectra obtained from a tungsten-iodine light source. (A) Raw intensity data; (B) smoothed intensity data after process 1; (C) smoothed intensity data after process 2 (see text for additional data on smooth processes 1 and 2).

sorption spectra of a KMnO_4 solution, producing an excessive smoothing with some spectral details being absent. Therefore, all absorbance results described below were obtained after passing the raw intensity data through filter 1.

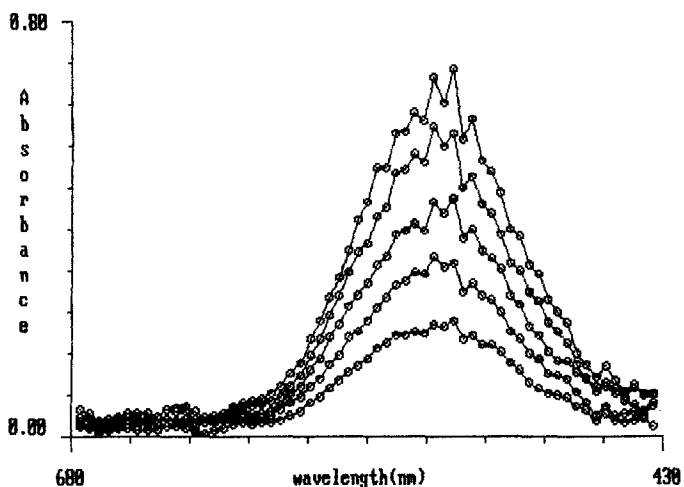


FIG. 6. Absorption spectra for KMnO_4 solutions with the following concentrations: 5.0×10^{-5} , 7.5×10^{-5} , 1.0×10^{-4} , 1.25×10^{-4} , and 5.0×10^{-4} M.

The range of the visible spectrum encompassed by the spectrophotometer is 250 nm. The range selected for FIA gradient scanning applications goes from 430 to 680 nm and was found with the use of two interference filters with cutoff frequencies at 630 and 515 nm.

The use of the instrument for visible absorbance measurements was evaluated by measuring the absorbance related to five solutions of KMnO_4 (5.0×10^{-5} – 1.5×10^{-4} M) pumped through the FIA flow cell. Figure 6 shows the absorbance spectra obtained. A linear relationship ($r = 0.9997$) was found to exist between the KMnO_4 concentration and the maximum absorbance value observed at resolution element 40. The average and standard deviation of ten consecutive absorbance measurements for a KMnO_4 solution of 5.0×10^{-5} M was 0.250 ± 0.003 absorbance units.

Figure 7 is a three-dimensional representation of an FIA gradient scanned during 55 s. The spectra start to be acquired just after the peak value of the alkaline gradient is achieved. A mixture of acetic acid (0.05 M) and hydrochloric acid of equal concentration has been titrated by the NaOH gradient generated in the FIA system. The results demonstrate how the Hadamard fast-scanning spectrophotometer can be used to monitor the titration of the mixture, showing where each indicator employed undergoes color change. Analytical procedures for simultaneous determination of acid mixtures are being developed on the basis of data like those shown in Fig. 7.

CONCLUSION

The spectrophotometer here described demonstrates that Hadamard spectrometry can be used where time-resolved data are necessary. In the present configuration, a complete scan can be obtained in 50 ms. However, it appears reasonable to suppose that the time scale in which an instrument could operate might be reduced to a few milliseconds. Obviously, last generation photo-sensor analog and digital electronics must be employed, along with improved mechanical parts.

The inherent advantage of the Hadamard approach,

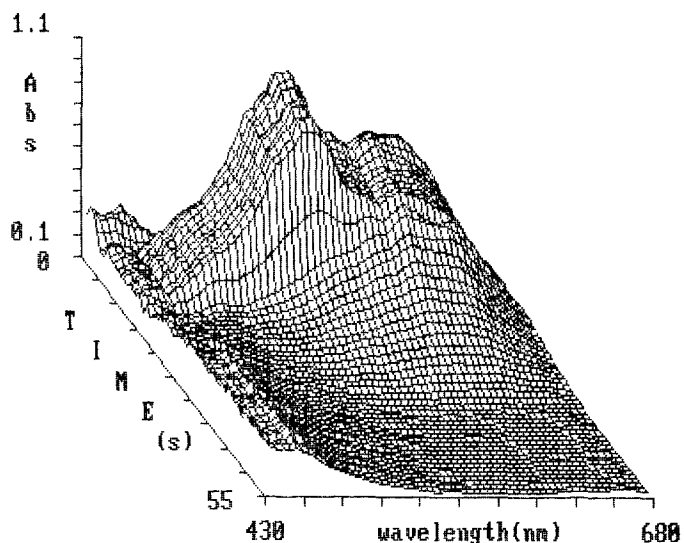


FIG. 7. Time-resolved FIA gradient scanning for a mixture of hydrochloric and acetic acids titrated with a pH gradient generated by injecting a 0.75 M NaOH solution.

which takes half of the total intensity of the dispersed light, when applied to time-resolved spectroscopy, can result in better performance in relation to small-area detector arrays, which could demand larger integration times. For common time-resolved spectrophotometric applications, such as FIA gradient scanning, the Hadamard approach can give results similar to those obtained with the use of more expensive diode-array based spectrophotometers.

The use of the optical encoder to mask localization and identification and the ruggedness of the mechanical moving parts ensure the long-term operation of the proposed instrument. Also, the use of simple dc motors is allowed.

The resolution obtained at present by using 63 elements per mask could be improved for applications demanding monitoring of sharper spectral features. However, the resolution achieved appears to be enough for the visible and UV region, where the absorption spectra are wide. The use of simple smoothing procedures produces a good-quality data presentation for these wide spectra.

ACKNOWLEDGMENTS

The authors are grateful to FAPESP for financial support (process No. 91/4876) and to Drs. C. Kascheres and H. Pastore for manuscript revision. R.J.P. is indebted to CNPq for a doctoral fellowship.

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