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Pulse radiolysis equipment: A setup for simultaneous multiwavelength kinetic spectroscopy

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A setup for pulse radiolysis experiments is described consisting of the following main components: an array of 15 photomultipliers attached to a spectrograph (allowing 4032 spectra to be recorded with a time resolution of 500 ns per spectrum), self-regulating high-voltage supply for the photomultipliers, computer-controlled solution mixing and dispensing system, and provisions for continuous dose variation of the FEBETRON-accelerator electron beam. The general performance of the system is discussed and construction or electronic details are given for special components.

INTRODUCTION

About ten years after the introduction of flash photolysis[1] and with the advent of accelerators allowing the delivery of short pulses of ionizing radiation,[2] the technique of pulse radiolysis (pr) came into use.[3–5] This technique requires that the energy of an accelerator pulse be deposited in a reaction cuvette, with radicals or other reactive species being produced in the solution, within a time interval short in comparison to the half-life of the reactions by which these radicals disappear.

In the beginning of pr studies a huge amount of data on radical reactivities (e.g., of the radicals produced from the irradiation of water like OH, O_2^- etc.) was obtained by radiation chemists[6–8] using experimental setups where the signal of a photomultiplier tube attached to the exit slit of a monochromator was recorded by an oscilloscope. With the discovery by McCord and Fridovich[9] that an enzyme exists within metabolizing cells that dismutates the superoxide anion radical (O_2^-), a broader scientific community became aware that O_2^- must also be a biologically relevant species and that radical reactions must also occur within living plant and animal cells. Ever since, the demand for supplying more detailed knowledge about radical reactions in biological systems has dramatically increased and methods had to be devised to meet with this need.

With the monochromator/oscilloscope technique it is rather time consuming to gather spectral data of a complex reaction mixture: all spectral and kinetic data obtained by a one-accelerator-shot/one-registration procedure have to be assembled to construct a time-dependent spectrum, usually covering a time range of a fraction of a microsecond to some hundred milliseconds. This can be overcome by combining kinetic and multiwavelength measurements into one experimental device. Early attempts have already been summarized by Porter in 1967[10] but only with the advent of fast electronic equipment and minicomputers realization of such a concept seemed really promising.

I. DESIGN CONSIDERATIONS

In principle time-dependent spectral scans can be elegantly obtained by different approaches: (1) streak cameras with TV readout of the built-in image converter tube (e.g., Ref. 11); (2) silicon diode arrays coupled to optical multichannel analyzers (which are commercially available); (3) CCDs (charge coupled devices) which are frequently used now in modern TV-cameras.

These devices provide high wavelength and time resolution. However, when pulse-radiolysis investigations of biochemical systems are intended, some additional prerequisites must be given consideration.

(*a*) *Optimal spectral performance in the far UV*: Often the most informative spectral region is between 230 and 290 nm where superoxide anions, carbonate radicals, amino acids, and many other compounds absorb; therefore, particular care should be taken to maximize sensitivity in this wavelength region.

(*b*) *There should be no time limitation to the measurements*: Reactions of O_2^- and other species of interest are at times rather slow and complex, follow-up kinetics can easily extend into the region of hundreds of milliseconds. Improvement of the light intensity of the analyzing light by pulsing the Xenon lamp (as being done in most pr laboratories to enhance performance and signal-to-noise ratio in the far UV) would set an undesired upper limit for the time of investigation since lamp pulsing is only possible for some milliseconds if destruction of the lamp is to be avoided.

(*c*) *A maximum of information should be gained after only one accelerator pulse*: (i) Chemicals of interest to investigators in the biological field can be extremely expensive; therefore, a setup gaining information by repetitively pulsing the accelerator in order to improve the signal-to-noise ratio would for economic reasons rule out many potential experiments; (ii) Optical multichannel analyzers usually have a limited repetition frequency for spectral scans and streak cameras only allow limited "streak-time-frames" for one re-

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cording, so these devices will not record over the *whole* interval of interest with a time resolution appropriate for long-term kinetic analyses; (iii) "Repeatability" of experiments also depends on the available accelerator; whereas it is rather simple to pulse a Linac or van de Graaff many times a second, e.g., a Febetron (using a Marx generator as HV supply) can only deliver pulses every 1–2 min. Taken together it is mandatory to gain as much information as possible using a minimal number of accelerator pulses.

Evaluation of all the pertinent parameters showed that none of the quoted devices would be as sensitive in the far UV as a good photomultiplier tube which has at the same time fast time response in the order of one nanosecond. Therefore, the introduction of multichannel-spectrokinetic equipment based on photomultipliers seemed most advisable for our purposes. At the moment multichannel transient recorders with conversion times of about 500 ns per cycle are on the market at reasonable cost and if the budget permits faster AD converters could be purchased to match the inherent response speed of these photomultipliers. In the following a short description of the setup realized in recent years at the pulse-radiolysis laboratory of the GSF will be given (a preliminary report can be found in Ref. 12).

To gather spectral data with an array of PM tubes poses two problems: (a) a spectrograph has to be used with an exit slit of about 250 mm length, to mechanically allow the arrangement of an adequate number of PM tubes, each having a diameter of 38.5 mm (including mu-metal shield); (b) adjustment of the anode current of each of the photomultipliers to a reference value before the accelerator shot has to be automatized and computer controlled.

II. DESCRIPTION OF THE APPARATUS

A. Optical system

By careful design of the light path (which due to the necessary physical separation of the accelerator from the operating area could not be shorter than about 4.5 m) it was possible to avoid the need for a pulsed light source.

The specifications of the different components in the block diagram (Fig. 1) are

(a) commercially available 1.5-m spectrograph (Ebert design) (type SPN 1.5/250 Ph; RSV, Hechendorf/Germany). Two turn-table mounted gratings allow different spectral ranges to be projected on the 15 PM tubes at the 250-mm-long exit slit grating 1200 grooves/mm: spectral coverage 130 nm, 9 nm PM to PM distance (spectral bandwidth $\pm$ 1.6 nm), grating 300 grooves/mm: spectral coverage 520 nm, 37 nm PM to PM distance (spectral bandwidth $\pm$ 6.5 nm).

(b) light source: xenon high-pressure lamp with Suprasil window (Osram XBO W4); lamp housing designed to incorporate spherical mirror (for reflecting image of plasma onto position of original plasma), mu-metal shield (to avoid plasma deflection by magnetic field of accelerator beam collimating coil), and thermostat jacket (to ameliorate convectional instability of light emitting plasma).

(c) light path: Xe-lamp plasma imaged into cuvette, cuvette imaged on field lens (L4), field lens projected on entrance slit of spectrograph by cylindrical lens (L5). Suprasil lenses (Steinheil, Germany), free diameter 70 mm, were used throughout (focal lengths: L1, L2 = 120 mm, L3 = 280 mm, L4 = 888 mm, L5 = 280 mm). M1 and M2 are surface-coated (aluminum) mirrors. Between lenses L1 and L2 the beam is made parallel and a slider guide is provided allowing insertion of interference filters (F) or quartz cuvettes containing liquid cut-off filters (which at times are necessary to avoid overlay of parts of the second-order spectrum over the first order in the spectrograph or to minimize possible photolysis of the reactants).

B. Photomultipliers and associated electronics

Fifteen EMI side window type 9783R, extended S5 spectral response (wavelength range 165–750 nm),

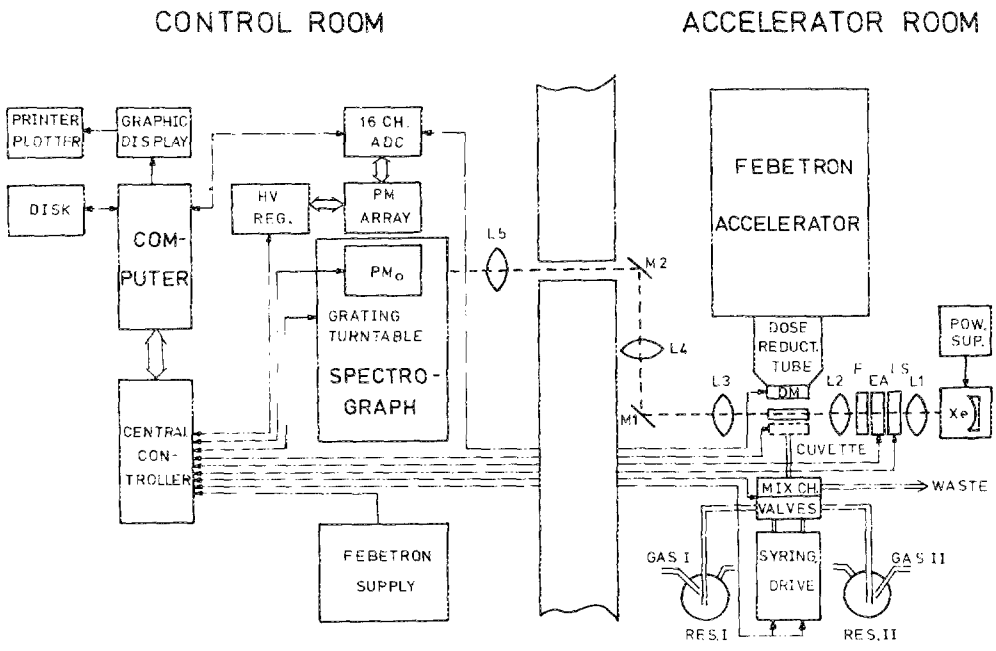

FIG. 1. Block diagram. 1.8-MeV electrons from the accelerator are deposited into the cuvette after passing the dose reduction tube and the dose monitor (DM). Analyzing light from the xenon lamp (Xe) is, at a right angle to the electron beam, focused through lenses L1–L5 via mirrors M1 and M2 onto the spectrograph entrance slit. LS, EA, and F are light shutter, entrance aperture and filters. PM_0 is a high-quality photomultiplier (mounted at a right angle to the PM array) which can be connected to a very fast single channel digitizer.

Zener diode stabilized voltage divider chain designed for maximum sensitivity and gain linearity,

current-to-voltage conversion by high-speed FET operational amplifiers (LH0062CH),

self-regulating circuitry for high-voltage supply (see below).

For high-precision measurements a separate high-sensitivity PM tube (EMI 9558 QB) was mounted in a position perpendicular to the array; it can either be connected to channel 16 of the normal digitizer or to a separate digitizer, allowing faster scan speeds (DL 905, Datalab, UK, being currently in use). Since it was not physically possible to arrange all PMs in a row in the focal plane behind the spectrograph exit slit, an array of mirrors was designed that alternatingly deflects the beams for consecutive PM tubes (one up the next down) to be focused on the photocathodes of the sidewindow PMs. To each of the PM sockets a small box is attached incorporating voltage divider chain, current-to-voltage converter, etc.

C. Experimental control

CAMAC compatible mainframe with circuitry for operating light shutter (LS), entrance aperture of light beam (EA), cuvette support, grating turntable of spectrograph, solvent delivery valves, high voltage regulation (see below), step-motor operated syringe drives (see below).

D. Data acquisition and evaluation

Sixteen channel transient digitizer VK-MC (Vuko, Germany), computer controllable via IEEE 488-Interface, input bandwidth 0.6 MHz, vertical resolution 8 bits, time resolution 500 ns/cycle;

WANG 2200 VP computer with periphery: CRT, graphic display, plotter, diskette drives.

As indicated above, some of the components are commercially available and trivial details of the electronic circuitry referred to in Sec. II C will not be given here, but some other components merit discussion in more detail.

E. Special components

1. Photomultiplier-associated electronics

The output signal of each of the PM tubes has to be adjusted before the accelerator shot to a given reference value (I_0); then the regulation has to be arrested to allow the tube to record the current changes due to absorbance changes. In principle there are dc-to-dc converters commercially available that allow the high-voltage output for PM supply to be regulated by a low-voltage dc input level, yet none of the low-cost commercial dc–dc converters tested met the requirements concerning stability, output-ripple, maximum current, or response speed. High-precision dc–dc converters were either too big to be mechanically incorporated in the available space at the spectrograph exit slit or too costly to lead to a reasonable price for the whole setup. It was, therefore, decided to use one single stabilized HV supply (HN 2500-025, Heinzinger, Germany) and to derive from this the voltages for the individual PM tubes (for electronic circuitry see Fig. 2).

2. Dose reduction tube

The FEBETRON accelerator delivers pulses of about 50-ns duration depositing an absorbed dose of about 10 kGy in the cuvette. This is too much for experimental purposes, where meaningful doses should be in the range of 5–70 Gy; within these limits the dose should be variable by small increments. Dose reduction can be achieved by introducing metal shields with holes of different size[13,14] into the accelerator electron beam. For the setup described, a different approach was chosen: within a short evacuated aluminum tube two aluminum disks with a special pattern of cone-shaped holes (Fig. 3) can be rotated one against the other allowing the "geometrical transparency" to vary from almost zero to a maximal value of 353 mm^2. This arrangement allows continuous and linear dose variation within the desired limits.

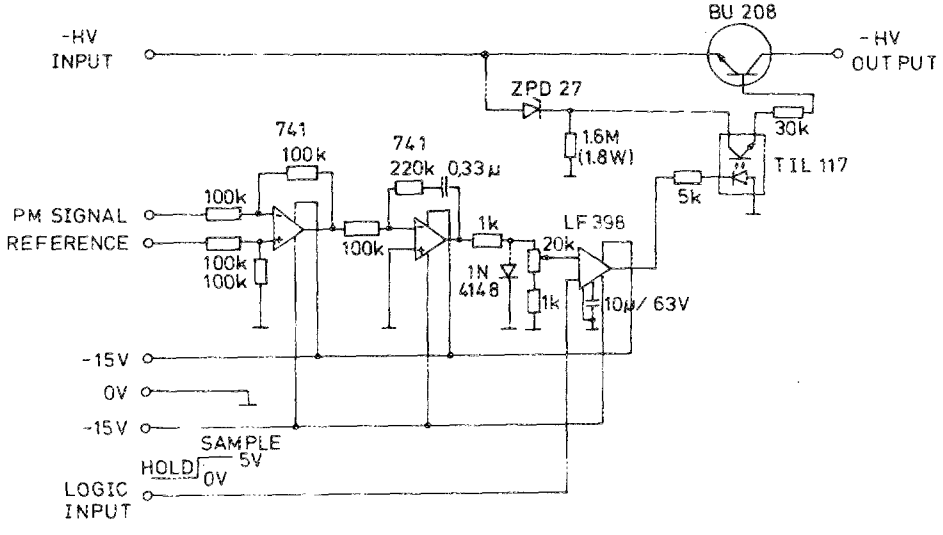

FIG. 2. HV control. The anode current of each of the PM tubes is converted to a proportional voltage which is constantly compared to a 1-V reference source; the resulting difference signal regulates, via optocoppler TIL 117, the HV supply for the PM dynode chain through the *n-p-n* transistor BU 208 (Thomson-CSF). Whenever the computer elicits a hold signal, the high voltage output is kept constant during the course of an absorbance measurement by the sample and hold circuit LF 398.

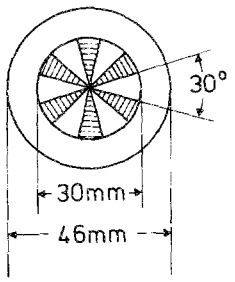

FIG. 3. Dose reduction disk. Two identical disks, mounted colinearly with the electron beam, can be rotated with respect to each other by a 1 : 6 reduction gear. This allows a near linear variation of the dose from 5 Gy to about 70 Gy.

3. Dose monitor

Variations in accelerator beam current from pulse to pulse will lead to statistical errors in absorbance measurements. It is therefore imperative to actually determine the deposited dose for each shot separately. Noninterfering measurements of electron currents by pick-up coils were described by Bess *et al.*[15] and Johnson *et al.*[16] The method was adapted to our setup by installing a toroid (ferrite-core, i.d., 65 mm, inductivity 120 mHy) at the front end of the dose reduction tube. The output signal (i.e., the damped oscillation) is directly fed into the sixteenth channel of the transient recorder and processed there by computer software. The calibration curve of the device against standard chemical KCNS dosimetry[17] is used to correct the obtained absorbance values for dose variations.

4. Solution delivery system

Most of the solutions to be investigated are not stable over long periods of time; furthermore, it is desirable to mix solutions in varying concentrations for competition experiments. A commercially available step-motor driven digital dispenser (Hamilton, Switzerland) was therefore modified and interfaced to the computer (Fig. 4).

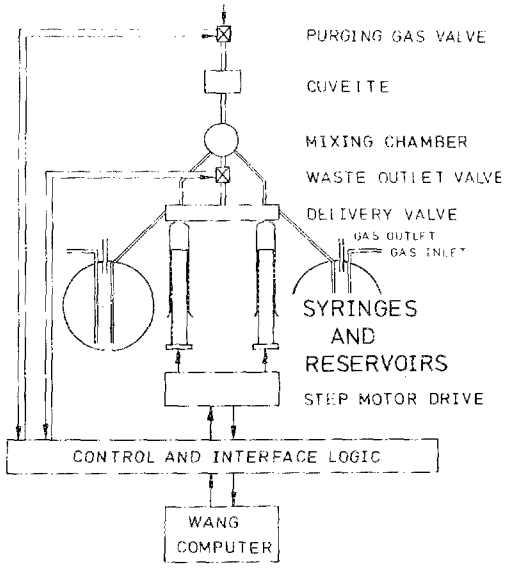

FIG. 4. Solution delivery system. Solutions from two different reservoirs are mixed and dispensed into the cuvette via step-motor driven syringes.

Two different solutions containing the reactants (occasionally saturated with different gases by bubbling with gas streams of different composition) can be pulled into the syringes and, after passing a mixing chamber, be dispensed into the cuvette. Syringe speeds (assuring correct mixing in the chamber) are computer-controlled, mixing ratios ranging from 1 : 100 to 100 : 1 can be achieved with statistical volume errors of about 1%. (The PTFE tubes connecting the syringes and valves to the cuvette were found highly permeable to oxygen. To avoid oxygen contamination by diffusion into the solution, PVC hoses with a wider diameter were put over all PTFE tubing and the space in-between is constantly flushed with an inert gas).

5. Spectrograph associated electronics

The turntable for the gratings of the spectrograph was originally driven by a one-directional motor and wavelength settings were indicated by an optically projected scale. To allow computer control, this arrangement was exchanged by a bi-directional motor drive and an incremental shaft encoder (M79, Megatron, Germany; 2000 counts/revolution, electronically doubled to 4000 counts/revolution) was attached to the axis of the turntable. The angle code is fed into the computer and the desired turntable position can be approached by an iterative software procedure.

6. Cuvette support

To allow determination of the reference spectrum of the cuvette content before the accelerator pulse the cuvette is mounted on a motor-driven dove-tail slider, which, by moving the cuvette in and out of the analyzing light beam, allows the measurement of the sample spectrum against the reference "air" (no cuvette in light beam).

III. EXPERIMENTAL PROCEDURE

A simplified description of the computerized control of an experiment is given below:

prepare receive files on diskette, set default conditions for all peripheral devices, set wavelength, dose reduction aperture, transient recorder (according to preselected menu),

empty and rinse cuvette by purging with solvent,

fill cuvette with desired mixture from reservoirs,

open light shutter, adjust iris diaphragm for analyzing light, adjust high voltage for all PMs to give I_0, hold HV regulation,

trigger transient recorder for control of I_0,

move cuvette to sample position,

trigger transient digitizer to record spectrum of solute against the reference "air,"

readjust HV for all PMs to compensate for solute absorbance, hold HV regulation,

trigger transient recorder for control of I_0 and determination of "noise" level,

TRIGGER ACCELERATOR;

record transient spectra (according to preselected menu: time regime, input sensitivity, etc.),

close light shutter, move cuvette to reference position,

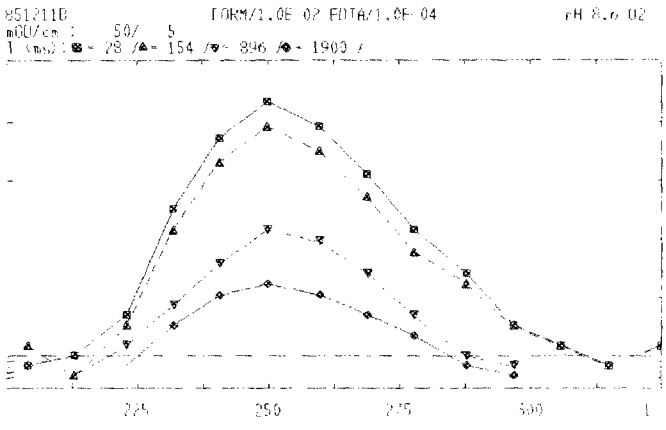

FIG. 5. Absorption spectrum of superoxide anion. Hardcopy of original computer output. The header gives besides the identifier (date of experiments, control letter) information about reactants and their molar concentrations (formate, EDTA), pH, saturating gas and the time after the pulse (T) at which spectra were taken. Coordinates are scaled automatically, maximal and minimal ordinate values (as milli-optical-densities per cm cuvette length) above and below the dashed zero line are given in the header, the wavelength scaling (L) on the abscissa is in nanometers. Default time values are displayed, other time intervals could be chosen by the operator.

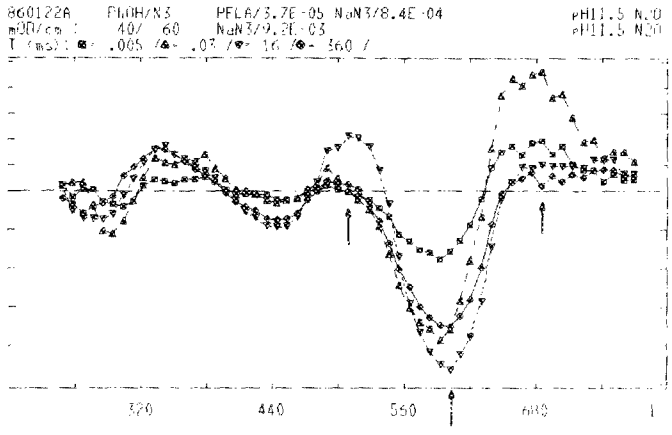

FIG. 6. Spectrum of pelargonidin chloride. The header gives the same information as for Fig. 5. The general index topic "reaction of phenols with azide" (PhOH/N3) is displayed together with two lines of information concerning the composition of the two different solutions that were mixed into the cuvette. The wavelength positions for the kinetic evaluations of Fig. 7 are indicated by arrows.

set HV adjustment to "free" mode (e.g., to prevent damage to PM tubes by accidental light exposure),

sequentially read all the channels from the transient recorder into the computer, reduce data according to algorithms depending on determined noise, calculate absorbances and assemble spectra, display time-dependent spectra on graphic monitor, kinetically process data by "standard" procedure (regression analysis for first- or second-order chemical kinetics), display calculated data (extrapolated initial absorbances, rate constants, F values for goodness of fit etc.) on screen,

save data on diskette (together with all necessary control and chemical information).

After this the operator is free to proceed to the next experiment or to analyze data in more detail, using special procedures (e.g., various kinetic models for parallel, successive, or competing reactions), reconstruct and display new time-dependent spectra, "normalize" spectra according to dose or correct them for G values of the radicals under investigation.

IV. PERFORMANCE AND EXPERIMENTAL RESULTS

The advantage of the setup described is not only the enormous gain of time by recording complete kinetics (from 0.5 μs to many seconds if desired) at 15 different wavelengths with one experiment but also the improvement of statistics by eliminating the usual dose variations and other statistical errors of a "one shot for each wavelength" procedure. By use of the low-resolution grating the whole spectral range of 200–700 nm can be crudely examined after only one pulse, for spectral details only 2–3 pulses with the high-resolution grating have to be taken. (If higher resolution than given by the standard PM to PM distance of 9 nm is desired, intermediate spectral points can be recorded by positioning the grating turntable at an intermediate position; to improve signal-to-noise ratios, whenever necessary, it is possible to average over consecutive accelerator pulses).

The overall characteristics of the setup are wavelength accuracy (as determined by Holmium and Didymium calibration filters): $\pm$ 2 nm, wavelength resolution: $\pm$ 1.6 nm, optical resolution:0.4% absorbance change, reproducibility of solvent mixing: maximum volume error = 1.5%.

To give an example for the performance of the system, in the following figures a few experiments are displayed as hardcopies of the original computer output (since not all data are relevant to the reader they need not be read in detail).

The O_2^- spectrum of Fig. 5 was obtained after only one accelerator pulse, for Fig. 6, the spectrum of the flavylium salt pelargonidin chloride after attack by azide radicals, taken from a current research project, four accelerator shots were combined to cover the whole spectral range from 200 to 700 nm.

The original computer output at the default time values indicated in the header gives a first overview over the spectral changes, from the same set of data other spectra can be constructed deliberately or kinetic analyses can be carried out at each of the individual wavelength points.

Figure 7 gives a set of kinetic analyses at selected wavelengths. It should be noted that the general appearance of the curves, i.e., the two apparent "discontinuities," is determined by the changeover points of three different time scales preselected by the operator.

Comparison of the kinetic plots shows that the first portion of the transmission curve, the fast decay at 603 nm in Fig. 7(b), is kinetically correlated to the absorbance buildup at 680 nm in Fig. 7(d) whereas the second part, the slower decay [Fig. 7(c)], is correlated to the absorbance buildup at 510 nm [Fig. 7(a)].

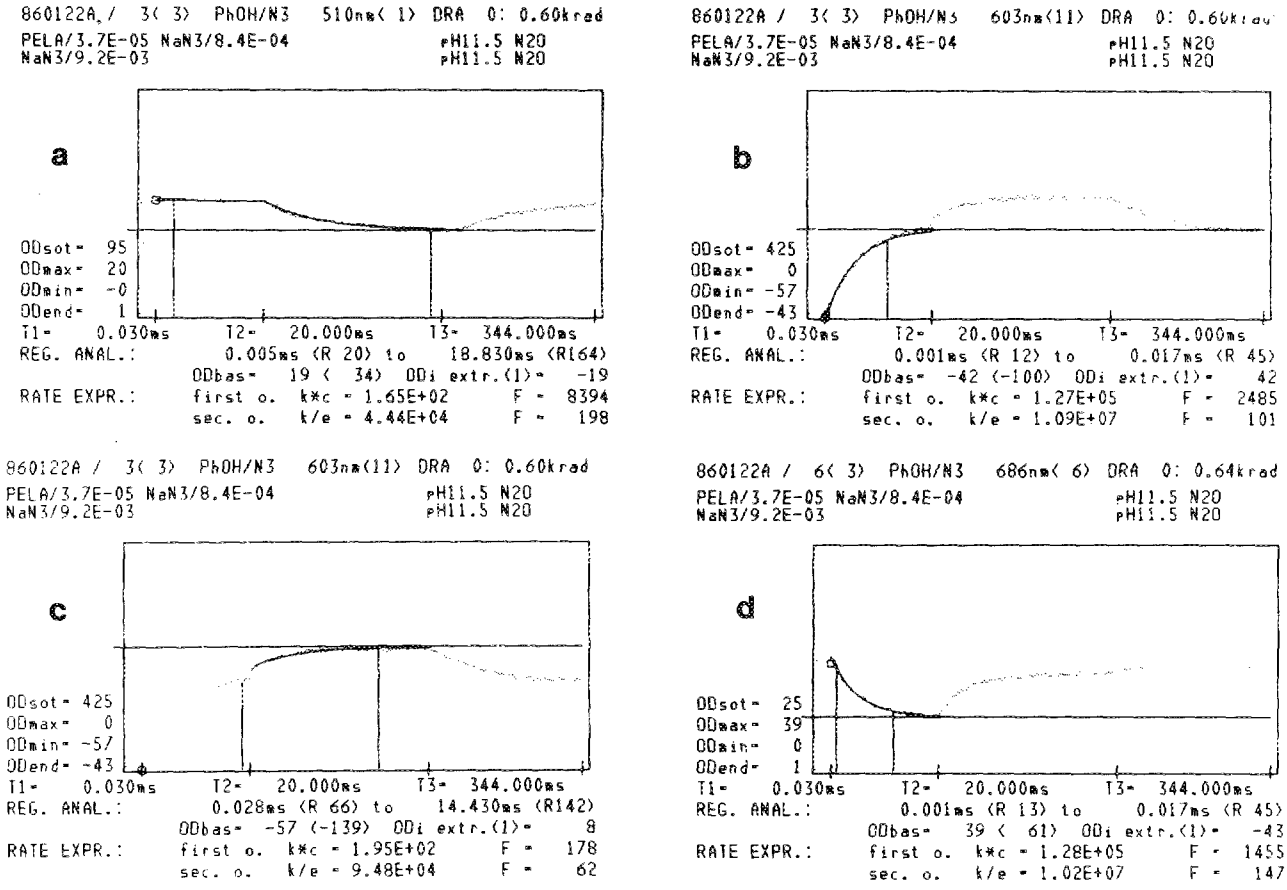

FIG. 7. Selected kinetic evaluations (pertaining to spectrum Fig. 6). The header tells us, e.g., in (a) that three separate experiments are averaged concerning the reaction of phenols with azide. The transient at 510 nm (from PM-tube #1) at a dose reduction aperture (DRA) setting of 0 (corresponding to 0.6 krad) is displayed. Besides the usual chemical information the following items are given: ODsot, absorption of solution against air; ODmax, ODmin, ODend are maximal, minimal, and end absorption (all given in milli-absorbance-units); T1–T3, changeover points of the transient recorder; REG. ANAL., time interval between vertical cursors; RATE EXPR., computer calculated rate expressions first and second order for the interval between cursors; F, F-values for goodness of fit. The small dots are experimental points from the digitizer. The full line between the two vertical cursors, the position of which is chosen by the operator, is the computer-processed analytical curve superimposed on the experimental points.

The whole pr setup as described here has now been in continuous use for more than a year and operates to the full satisfaction of all experimentalists. Experimental results are currently being published and can be found, e.g., in Refs. 18–20.

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