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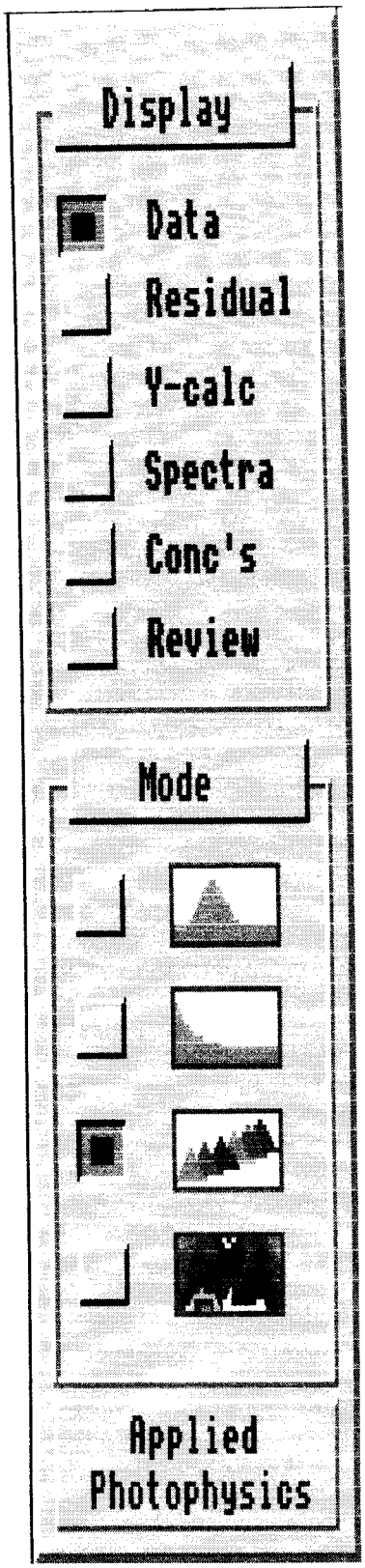
MicroVolume Stopped-flow Spectrofluorimeter

from

Applied Photophysics

SX-17MV

Applied Photophysics **DELIVERS**
what others can only talk about.



Applied SVD
SpectraKinetic Spectroscopy
Wavelength Dependent Kinetics
Reaction Kinetic Modelling
Global Analysis
Protein Folding
Circular Dichroism
Fluorescence
Sequential-mixing
Preparative Quench

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STATE-OF-THE-ART

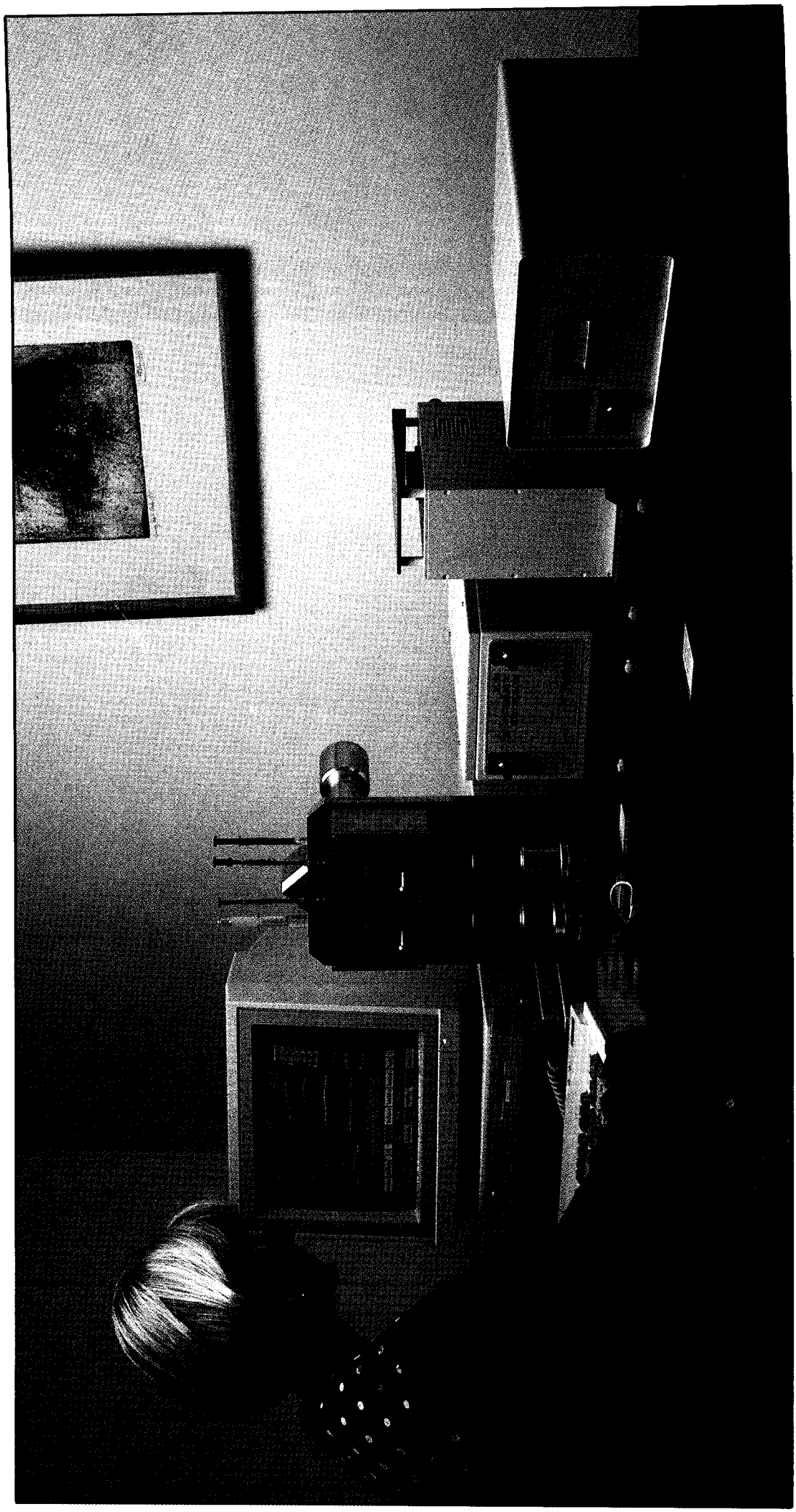
The SX.17MV is a very advanced stopped-flow spectrometer. This means that not only does it provide more facilities than conventional stopped-flow instruments but also that conventional features are implemented in a faster, more fluent, more effective way than was ever possible before.

It is a completely new design for a stopped-flow spectrometer. It may contain influences from other stopped-flow designs, but it is not a conversion from a conventional stopped-flow instrument. It has been created from scratch to be as advanced, as flexible, as adaptable and still as simple to use as advanced instrument control will allow.

The SX.17MV is more than a conventional stopped-flow spectrometer. It is used by many satisfied customers as their everyday stopped-flow spectrometer, but it can be also used for applications as varied as zero-interrupt sequential mixing, time-resolved ultraviolet and visible spectra, low temperature operation, circular dichroism detection, micro-volume quench-flow, asymmetric mixing and protein fold/unfold mixing experiments

Since the introduction of the original version of the SX.17MV, it has established itself as the clear market leader and the SX.17MV-with-ASVD is the latest and most powerful version yet. The creators of the SX.17MV come from a background of stopped-flow and advanced spectrometer design and the Company is the leading developer of software and hardware products for the study of rapid chemical and biological reactions.

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SX.17MV MicroVolume Stopped-flow Spectrofluorimeter

The SX.17MV MicroVolume Stopped-flow Spectrofluorimeter is the only stopped-flow instrument in the world with an Applied SVD technology (ASVD) option. It is also the only instrument of its kind to offer Zero-Interrupt Sequential Mixing and SpectraKinetic photometrics.

The analytical methods central to Applied SVD have been developed to accommodate the rapidly advancing capabilities of the SX.17MV in generating large quantities of accurate, multivariant experimental data.

The SX.17MV also takes full advantage of its WIMP based operating system, using the windowing/pointer/menu system to the full making it simple and intuitive to use. Its responsive windowing environment, ensures that even the most experienced researcher will gain considerable benefit.

The central processor is a 32-bit RISC-processor clocked at 25MHz (13.5 MIPS, equivalent to a Mac IIci), the memory is clocked at 12MHz and a 80Mb, IDE interface, hard disc and 3.5" floppy (720Kb and 1.44Mb DOS) are included as standard.

Stopped-flow

The stopped-flow excellence of the SX.17MV is beyond reproach. Its time resolution, sample economy and facilities are all to the very highest specification. A fresh approach, attention to detail and the combined effort of a highly experienced and motivated team of research and development instrument engineers, have produced a state-of-the-art interactive multiparametric and multifunctional instrument.

Researchers from all around the world visit our laboratory facility to use our resident instruments. We encourage the most demanding applications since this is the most effective method of demonstrating the SX.17MV unique qualities.

There is nothing comparable.

There are plenty of 'one millisecond' macro-stopped-flow instruments around, new and old. Though perfectly serviceable they are dated and thus restricted in their overall performance.

The SX.17MV is different, it is the first 100% computer controlled stopped-flow instrument. Careful integration and its interactive control system has brought benefits that previously were thought impractical or impossible. Kinetic, sequential-mixing and time resolved-spectral experiments are all under instrument control and the researcher needs only to direct each experiment. This gives the researcher better quality data and more time for sample preparation and interpretation of the experimental results.

Stopped-flow - microvolume economy

The SX.17MV excellent sample economy can be easily an order of magnitude better than most macrovolume and pseudo-microvolume instruments. Careful design has produced a SX.17MV that requires just 25µl/sample for stopped-flow and a remarkable 90µl/sample for all sequential-mixing stopped-flow experiments. This level of sample economy is

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simply not possible with alternative instruments. In addition, time resolution, mixing efficiency, sensitivity, chemical inertness, thermostatting and oxygen control are not compromised. This is a feature of the SX.17MV and applies to both time-resolved spectra and regular stopped-flow kinetics.

It is interesting to note that one 'biological' stopped-flow currently available does not mention its sample economy anywhere in its sales brochure. To do so would be unwise since it is a macrovolume instrument competing in a microvolume market.

Stopped-flow - time resolution

As mentioned before there are plenty of millisecond stopped-flow instruments around, but it is only the SX.17MV that achieves this without degrading its sensitivity (signal-to-noise) and therefore data quality.

Some manufacturers even claim sub-millisecond deadtimes! They do however fail to mention that the quality of the data and ultimate time resolution have to suffer as a consequence.

The marginal 'improvement' in dead time can only be obtained by reducing the observation volume and having the observation point very close to the mixer. Though this may improve the empirical dead time of the instrument it actually has a detrimental effect on the instrument's time resolution. The short pathlength (eg 0.8 or 1.0mm) and tiny observation volume required by these types of instruments are counter productive. Reducing the overall performance of instruments and introducing mixing and stopping artefacts.

In terms of absolute time resolution and data quality the SX.17MV comfortably outperforms all other instruments, new and old. There is nothing comparable.

Stopped-flow - syringe drives

Unique low mass syringe drives have been developed. These highly responsive drives exhibit only a small fraction (can be as low as 15%) of the inertia found on all other existing instruments. This design not only greatly improves the reliability and integrity of the sample flow circuit, but also enhances the sample economy and time resolution of the instrument. The drives are able to accelerate and then decelerate (stop) in a fraction of the time required by alternative drives.

Equally significant is the ease with which each drive can be detached from its mount. This allows the syringes to be removed in seconds, even when full with sample. Similarly the syringe pistons can be conveniently removed for maintenance and for sample flow circuit cleaning purposes.

SPEC: Low mass rapid response syringe drives. Application specific Interchangeable drives . Drives can be removed in seconds to allow rapid removal of drive syringes and access to piston tips for routine servicing.

Stopped-flow - standard temperature control

The standard operating range is from 0°C to 60°C. The sample flow circuit and optical cell are closely thermostatted and a calibrated sensor is used to record the temperature of the sample. The prevailing temperature is automatically saved to disc storage with each data file. The thermostat flow circuit is constructed exclusively from stainless steel, glass and silica (the cell).

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The standard operating range can be easily extended to -10°C using optional syringe pistons. These pistons can be used over the -10°C to 60°C range without any adjustment and are available in all syringe sizes.

Alternative syringe pistons available from other manufacturers are of conventional design, having expandable seals that require regular readjustment. NOTE: Convection artefacts as observed on the Biologic SFM-3 ("unavoidable convection") and the Durrum/Dionex are caused by inadequate thermostatting.

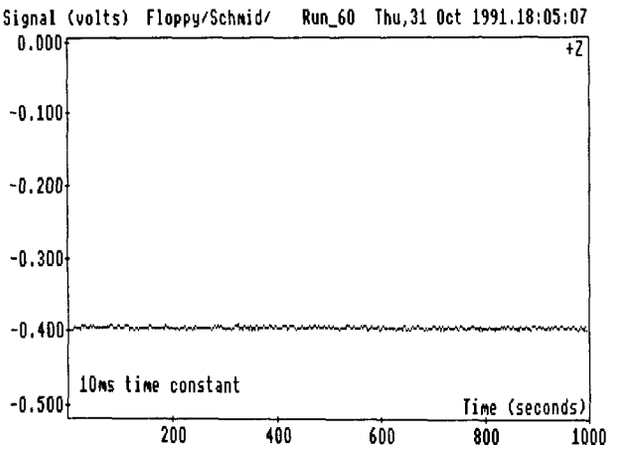
SPEC: Standard temperature range 0°C to 60°C. Sensor resolution is 0.1°C. Automatic tagging of data files. Thermostat flow circuit corrosion free and anaerobic compatible.

Stopped-flow - extended temperature control

The SX.17MV is available with an extended temperature range below -10°C.

Stopped-flow - protein folding/unfolding experiments

Such experiments often require the mixing of dense solutions such as 6 to 8M urea or guanidine chloride with aqueous buffers. The SX.17MV is the only instrument that can perform these experiments without the artefacts commonly found on other instruments.



Effect of mixing reagents with different densities (fluorescence measurement)

Reagents : 10^{-6}M tryptophan in 8M urea against water

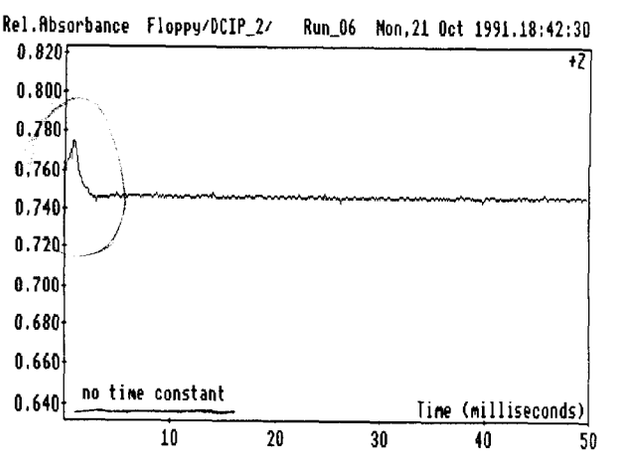
Mixing ratio of 1:10 (tryptophan/urea : water).

Standard 20µl optical cell

Wavelength : 280nm (excitation) and >305nm (emission).

NOTE: that mixing efficiency and time resolution are not compromised by asymmetric mixing

Other than the SX.17MV and almost without exception, all stopped-flow instruments are liable to suffer from significant buoyancy effects associated with the mixing of solutions of different densities. Applied Photophysics are experienced in these problems and they were designed out of the SX.17MV before it was even introduced.



Effect of mixing reagents with different densities (absorbance measurement)

Reagents : $5x10^{-4}$M DCIP in 8M urea against water

Mixing ratio of 1:10.

Standard 20µl optical cell

Wavelength : 524nm.

NOTE: that mixing efficiency and time resolution are not compromised by asymmetric mixing

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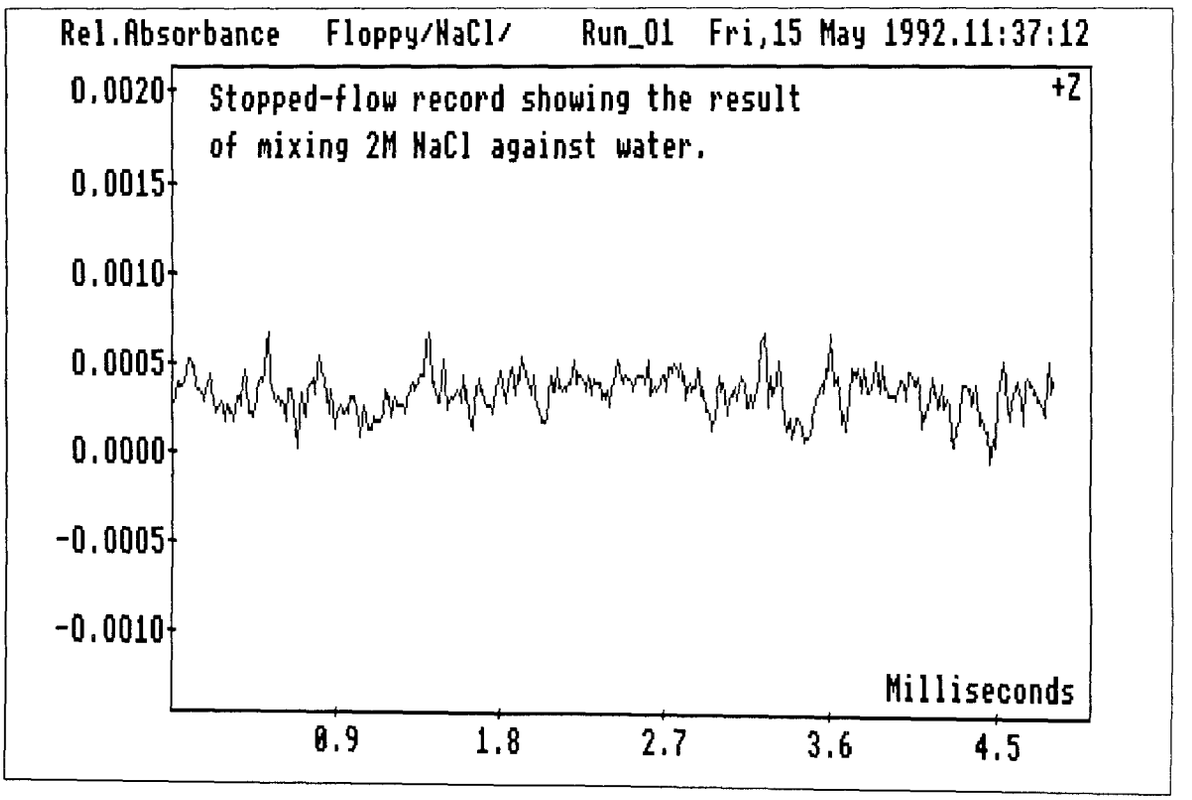
Buoyancy effects, so far only observed on the Biologic SFM-3, are simply due to inappropriate instrument design. The Biologic SFM-3 for instance requires that a special siphon device be retro-fitted to alleviate "a massive artefact ruining definitively the kinetics being recorded".

Stopped-flow - mixing efficiency (Dr K A Johnson)

Dr K A Johnson, of Penn. State University, has developed a mixing efficiency test that distinguishes between a stopped-flow instrument where mixing is complete before the reaction mixture enters the observation cell and one where mixing continues in the cell itself. He claims that this is a demanding test!

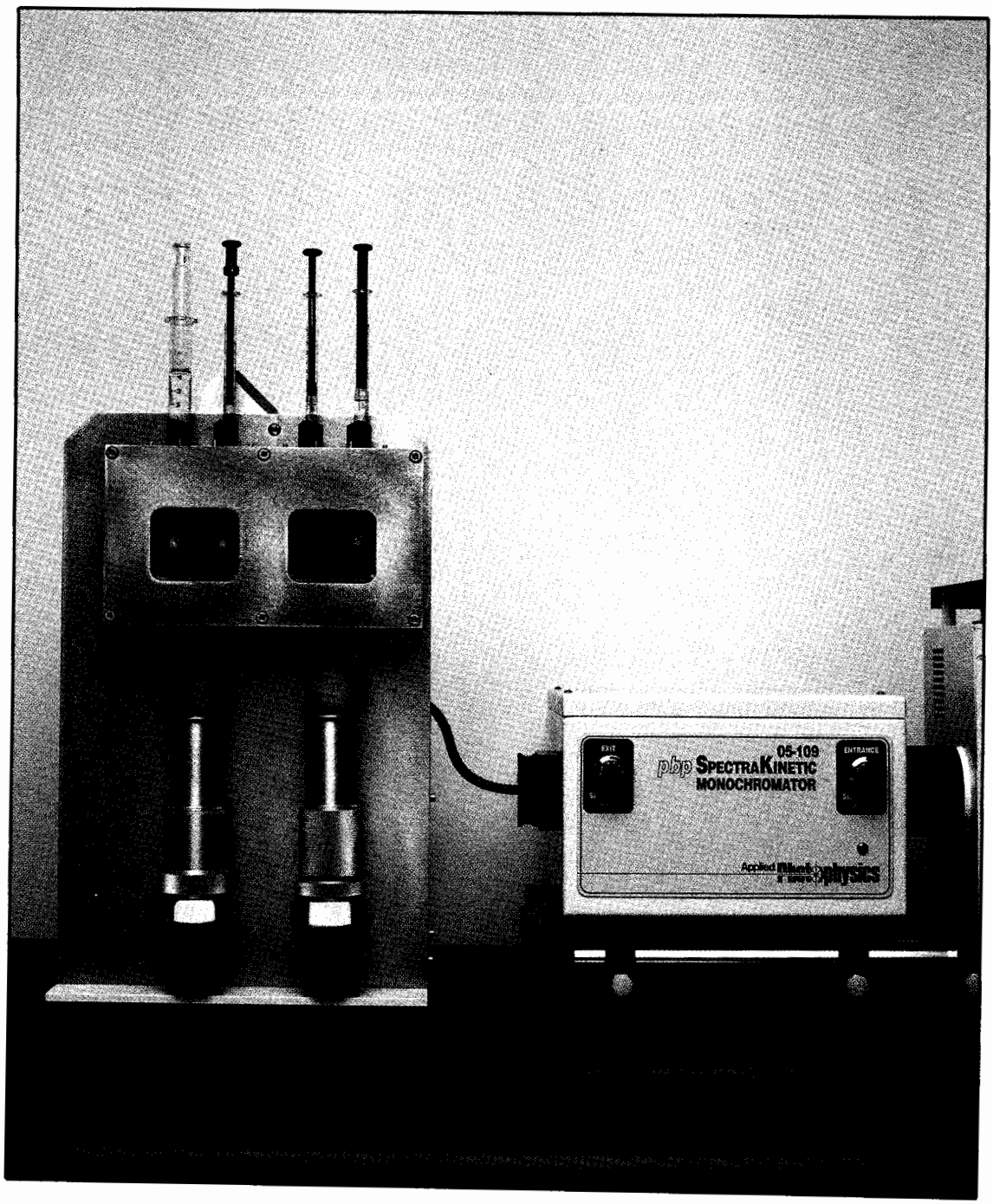
He recommends "mixing 2 M NaCl with water", predicting that "you will be surprised to see a large signal occurring at a rate of 1500 s^{-1}" and explains, that this is the "rate at which solutions finally mix *in the observation cell!*" This test is in fact quite undemanding, particularly when compared with the demands of topical applications such as protein folding (see above).

His claims do put into question the capability of the instruments to which he has had access. Dr Johnson has some "15 years of research experience in stopped-flow kinetics" and we felt that it was necessary to reassure others that a modern stopped-flow instrument does mix before the observation cell and fills the cell with fresh unadulterated reaction mixture.



This trace demonstrates that the mixing of 2M NaCl against water is complete before the reaction mixture enters the observation test. There is no large signal occurring at a rate of $1500s^{-1}$.

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Stopped-flow - asymmetric mixing

The most precise and reproducible method of varying the mixing ratio is to substitute a standard drive syringe with another syringe of the appropriate size. Time resolution and mixing efficiency are maintained at all times regardless of the mixing ratio. This is different to other instruments.

Flexible rubber grommets and readily detachable syringe drives allow all the drive syringes to be quickly substituted. A wide range of ratios is available as standard. The largest is 25:1, larger ratios to special order.

Stopped-flow - dissolved oxygen control

Outside of a sophisticated environmental glove box, the SX.17MV offers the most convenient and effective control of oxygen. A unique unilayer of chemically inert LAFT tubing allows the close control or elimination of oxygen. The thermostatting flow circuit is sealed to the atmosphere, and for the extreme control of oxygen, it is compatible with sodium dithionite solutions (removes dissolved oxygen from the thermostat fluid).

SPEC: Control and elimination of oxygen. LAFT flow lines. Thermostat flow circuit and temperature controller/cooler are compatible with solutions sodium dithionite.

Stopped-flow - automatic reset

The SX.17MV stop syringe is fitted with an automatic reset mechanism. This can be operated directly via the keyboard or automatically from within a RISC-OS sequence. This facility reduces operator intervention and greatly enhances the productivity and versatility of the whole instrument.

Sequential-mixing Option

Sequential mixing - Zero-Interrupt method

Reaction incubation (age) times over many orders of magnitude can be selected directly without having to make any changes to the sample handling procedure. The desired reaction incubation time is simply entered at the instrument's keyboard.

During each determination the sample flow rate profiles are recorded and the incubation time (age) of the reaction mixture 'stopped' in the optical cell is calculated, displayed and tagged to the data file. Parameters such as sample flow rate, sample volumes, dead time and mixing efficiency are kept constant for all determinations and only the interval between the first mix and the second mix is changed. This eliminates the usual experimental errors and poor productivity that is the norm with existing conventional instruments.

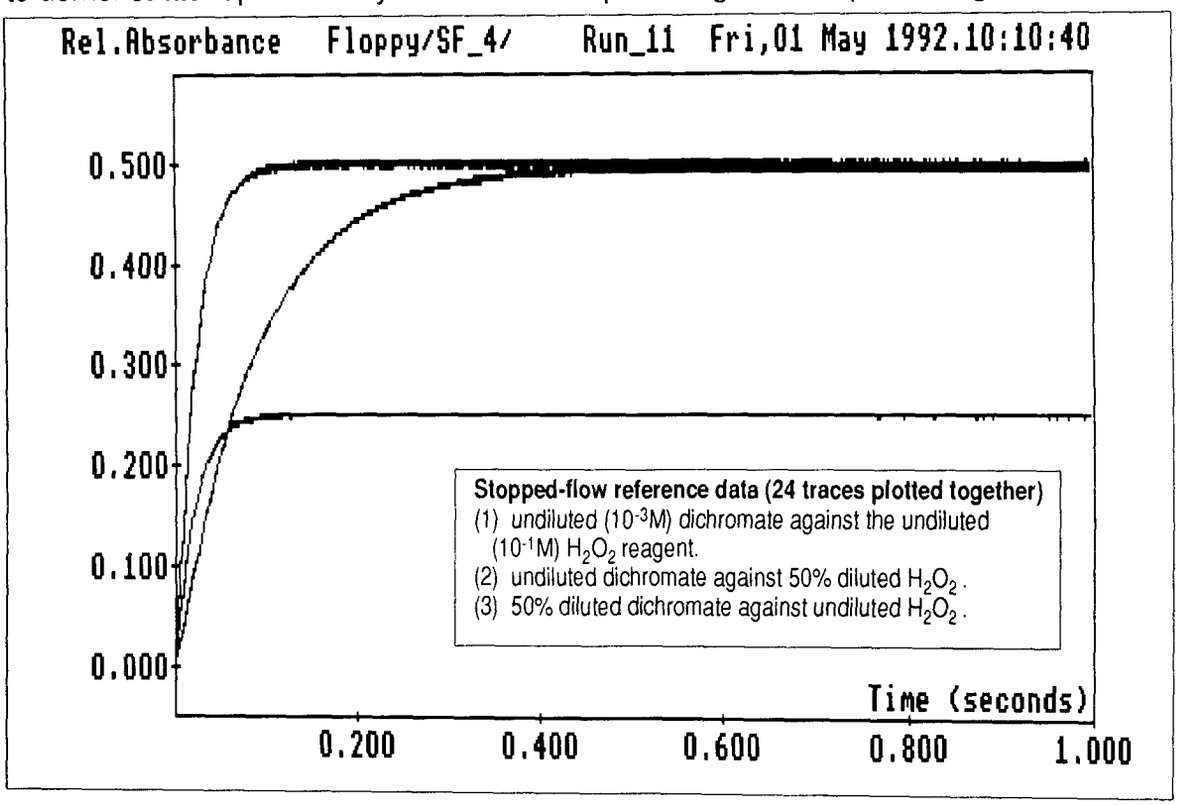
Conventional instruments use interchangeable and/or switcheable lengths of flow line, variable sample flow rates and different methods to achieve a wide spread of age times.

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Sequential mixing - extensive evaluation

With this option installed, the SX.17MV is the only proven sequential stopped-flow instrument available. The old technology instruments that are still available, using the interrupt and continuously flow methods, are notoriously time consuming and difficult to use, and quite unproven.

The following is an extensive evaluation of the SX.17MV sequential-mixing mode of operation using a simple chemical system on a instrument taken directly from our latest production batch. The chemical system chosen was the reaction of dichromate with acidified hydrogen peroxide. Initially, this reaction was run in conventional stopped-flow mode in order to obtain reference data for subsequent comparison with data obtained using the sequential mixing mode. A set of stopped-flow reference data is shown below, to demonstrate reproducibility each curve comprises eight traces plotted together.



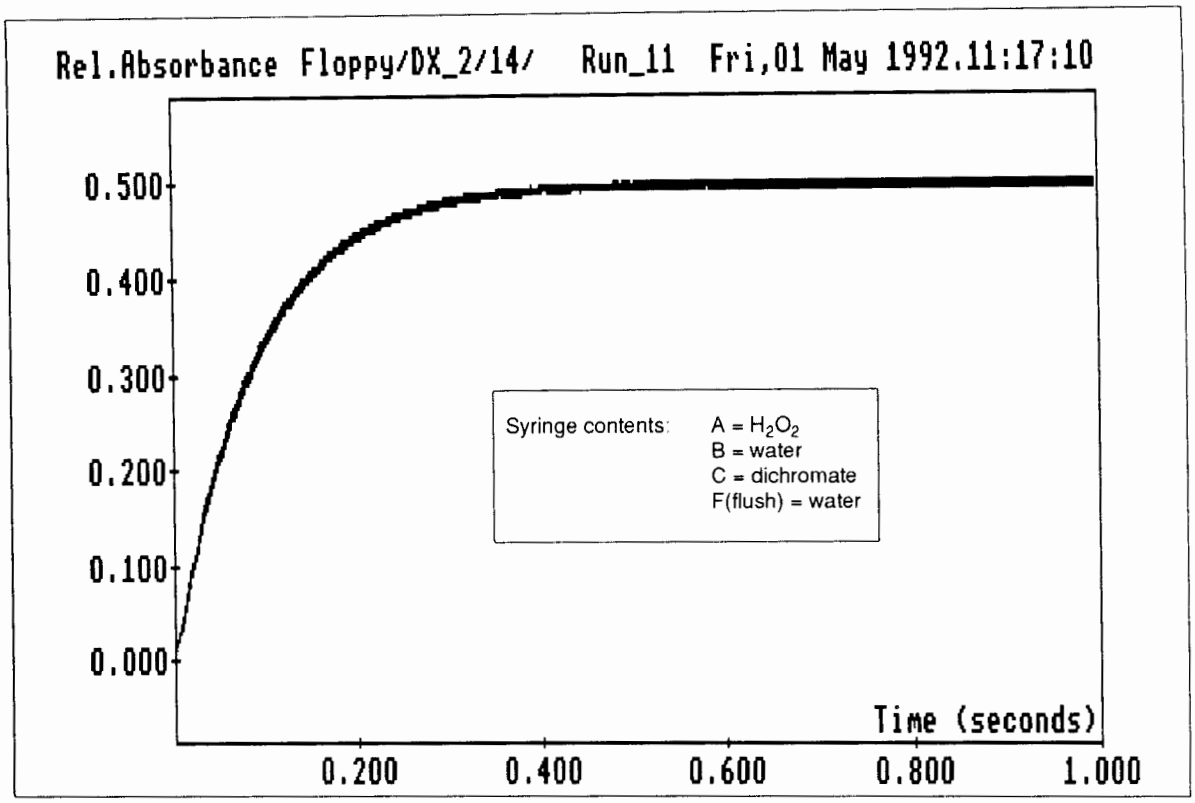
The two principal experiments performed in the sequential-mixing mode were,

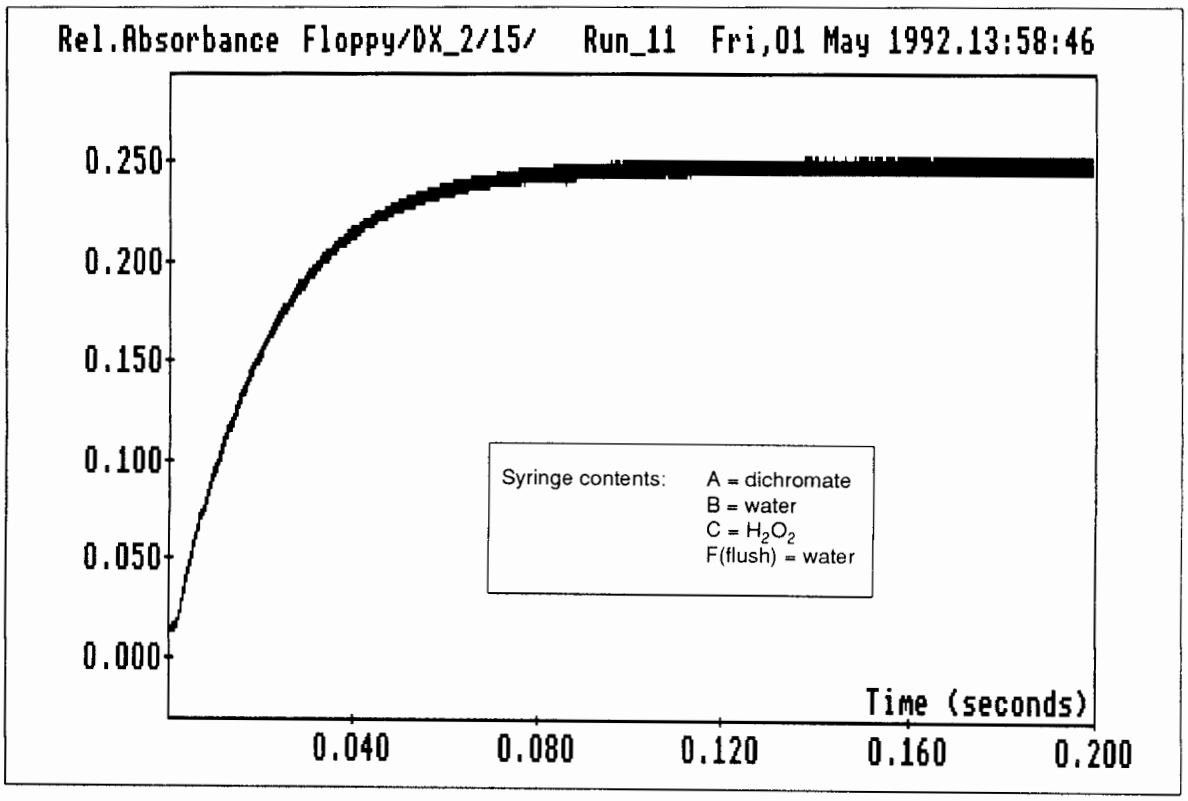
4) H_2O_2 in syringe A, water in syringe B, dichromate in syringe C and water in the flush syringe.

5) Dichromate in syringe A, water in syringe B, H_2O_2 in syringe C and water in the flush syringe.

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The two sets of data shown below were obtained with keyed in age times of 10, 15, 20, 25, 50, 100 and 1000 ms. At each age time eight traces were obtained and to demonstrate the excellent reproducibility of the SX.17MV all of these have been plotted together with the appropriate sets of reference data.





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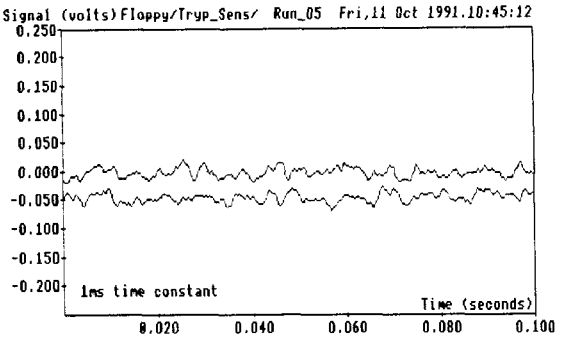
SpectraKinetic photometrics

The SX.17MV has an advanced optical cell that is crucial to its remarkable sensitivity, sample economy and time resolution. It is closely thermostatted, provides a choice of optical pathlengths, incorporates a carefully optimised turbo-mixer and is fitted with an integrated high efficiency emission optic.

This optical cell cell has been optimised further since its introduction in 1988. To this day only Applied Photophysics has the technology to produce such a high performance cell, constructed completely out of fused silica (transparent and opaque) and vacuum treated with a enhanced reflective coating to optimize its emission collection efficiency.

SpectraKinetic photometrics - fluorescence

This is the where the SX.17MV truly comes into its own. First seen on its predecessor, the SX.17MV has a unique **sensitivity X time resolution X sample economy combination**. No other instrument can match this combined capability.



Detection limits: 5x10^{-10}M tryptophan final concentration.

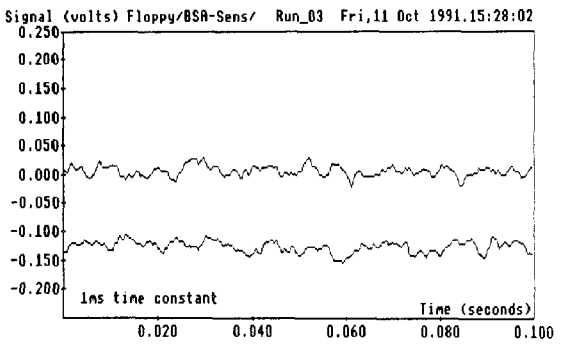
Maximum sensitivity available without loss of instrument time resolution.

Standard 20µl optical cell

Mixing ratio of 1:1.

Excitation wavelength 280nm

Emission wavelength >305nm



Detection limits: 5x10^{-10}M bovine serum albumin final concentration.

Maximum sensitivity available without loss of instrument time resolution.

Standard 20µl optical cell.

Mixing ratio of 1:1.

Excitation wavelength 280nm.

Emission wavelength >305nm.

A unique arrangement ensures that the contents of the cell are uniformly excited and that the emitted light is collected along the full length of the cell and over a wide solid angle.

This arrangement is different to most if not all other instruments where microvolume cells (25µl and less) have been introduced with little regard given to the subsequent loss of sensitivity.

SpectraKinetic photometrics - absorbance pathlengths

A 2mm optical pathlength is provided for the measurement of larger absorption signals and it is complemented by a more sensitive 10 mm pathlength. It takes just a few moments to interchange between these two optical arrangements. It is also important to note that the time resolution of the instrument is not influenced by the pathlength.

It is not unusual with alternative instruments for the time resolution to be roughly proportional to the pathlength.

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SpectraKinetic photometrics - baseline stability

A super-quiet high intensity light source is used for all absorbance measurements and fluorescence excitation. The light source has excellent long term stability and features peak-to-peak ripple of less than 0.005%.

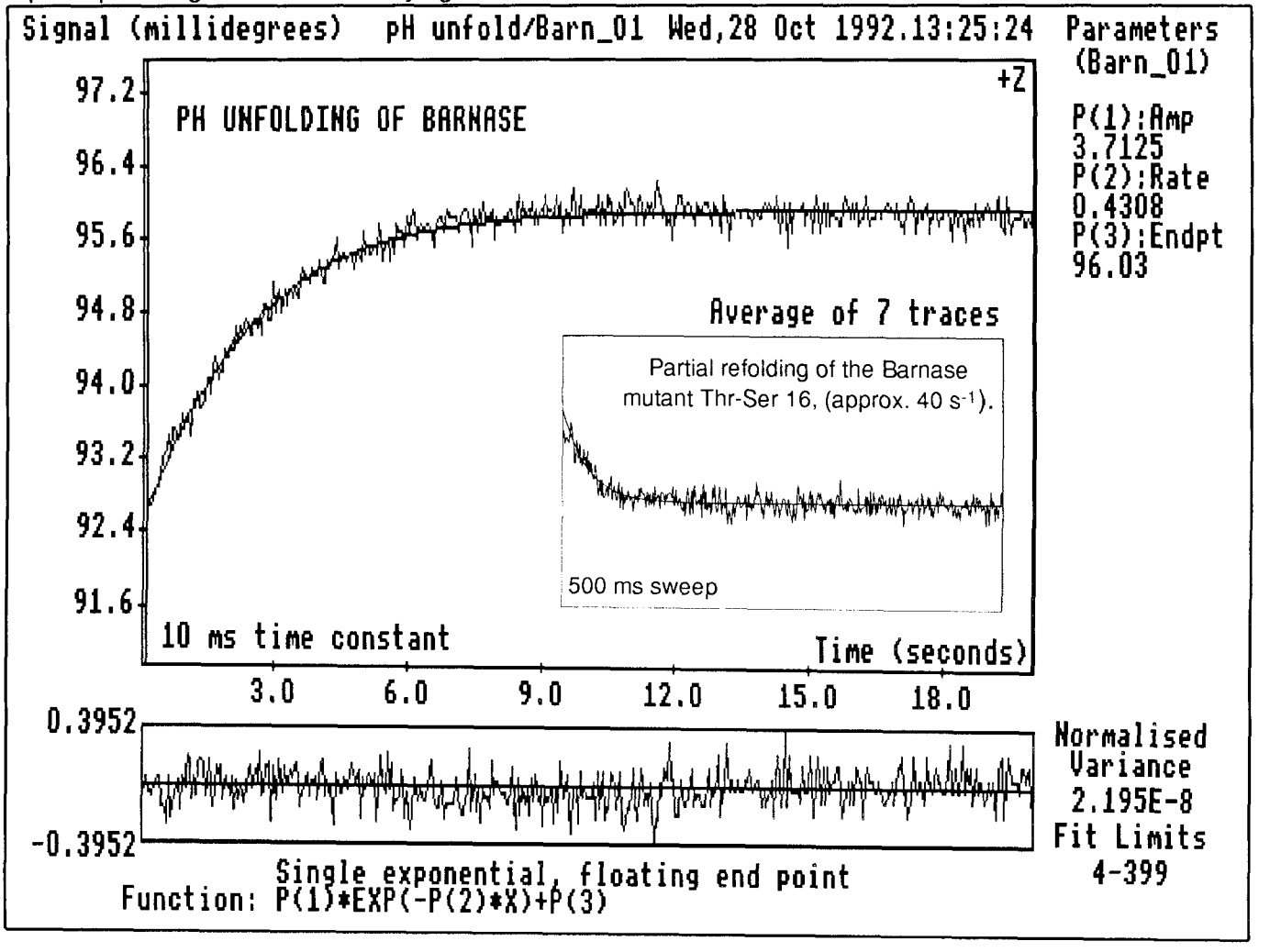
The normal operational range is 240 to 800nm. This can be extended down to 185nm and up to 1000nm.

SPEC: Wavelength reproducibility is to within 0.05nm. Wavelength linearity to within 0.1nm or better. Wavelength bandpass is continuously adjustable over the range 0.5nm to 45.0nm. Peak-to-peak ripple is better than 0.005% (power supply).

CIRCULAR DICHROISM DETECTOR

This features a detection system which was developed for the measurement of CD signals in the 215 - 310nm wavelength range. It features unsurpassed sensitivity, time resolution and low stray light levels. The standard CD stopped flow cuvette has a low birefringence and provision for the fluorescence detector. 2mm and 10mm pathlengths are provided as standard. When configured for CD detection the instrument has a dead time of 5ms. The sample flow line is chemically inert (no stainless steel) and biologically friendly (low Teflon content). The experiment shown below was performed by Dr M. Oliveberg of the MRC Unit for Protein Function and Design, Cambridge IRC foe Protein Engineering, Cambridge, England.

Experimental conditions:
Mixing ratio = 1:1. Temperature = 25°C. Wavelength = 225 nm. Bandpass = 4 nm.
Optical pathlength = 2 mm. Stray light is less than 1%.



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GLOBAL KINETIC MODELLING SYSTEM

Applied Photophysics is pleased to announce the first commercial Global analysis package which enables the scientist to obtain accurate kinetic and spectral parameters by the direct fitting of multivariate experimental data to classical chemical reaction schemes.

Modern instrumentation, such as the Applied Photophysics SX.17MV sequential spectrofluorimeter, allow the generation of large multivariate data sets. Time dependant spectra are a good example. Complete spectral and kinetic information describing a complex chemical sequence may reside in a single data set. However, it may well contain time dependant absorption changes which cannot be analysed fully by classical methods alone (except in relatively simple cases), most commonly due to the absence of appropriate fitting functions in traditional curve fitting packages. Even when mathematical functions can be found they may at best be a compromise and require careful interpretation in terms of how calculated rates and amplitudes relate to individual reaction steps in proposed chemical reaction schemes. Commonly a separate cycle of mathematical reaction simulation, using numerical integration methods, followed by systematic comparisons of synthetic and experimental data provide the only means of assessment.

Such an approach, though ultimately effective, requires a fairly high mathematical and computing literacy, but more significantly takes time and resources.

The AP Global Modelling system solves the problem fully.

By a unique intercalcation of multivariate analysis and high precision reaction simulation **all involvement of mathematical fitting functions and their interpretation in the data reduction process is eliminated.**

A typical data set derived from the DX17MV comprises high quality kinetic curves at multiple wavelengths. Analysed globally. specific reaction rates and component spectra can be routinely determined simply by providing a proposed chemical reaction scheme and initial component concentrations. In the case of a two step sequential mechanism the operator supplies the following:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad [A]_0 = 0.01M$$

Concentration profiles of each intermediate are generated and the rates k1 and k2 optimised so that the best least squares fit to the data set is obtained for this model, together with the spectra of the three proposed components.

This example though relatively simple would traditionally require the following integral functions to have been programmed into an analysis package if the same results are to be obtained:

$$[A] = [A]_0 \exp -k_2 t$$

$$[B] = [A]_0 k_1/k_2 - k_1 \{\exp(-k_1) - \exp(-k_2 t)\}$$

$$[C] = [A]_0 [1 + 1/k_1 - k_2 \exp(-k_1 t) - k_1 \exp(-k_2 t)\}]$$

Such integrated equations though available for this model and other relatively straightforward cases either from kinetic reference books or by integration of the basis differential rate equations, cannot be obtained for more elaborate mechanisms, which inherently limits the flexibility of traditional analysis. Even if they can be found they have to be incorporated into analysis software in an appropriate form before they can be used. The diversity of chemical and biochemical mechanisms inevitably leads to

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compromises in this area (ie by fitting to inadequate of "next best" functions) and ultimately to no solution being available.

The way forward has been to compare "next best" fits on experimental data to same fits performed on synthetic data produced by numerical integration methods, a technique which generates concentration profiles of intermediates by numerical rather than analytical means. Predicted changes in simulated data sets by changing starting conditions can then be compared to those observed experimentally. Numerical integration is frequently the only means of generating concentration profiles of intermediates in complex mechanisms.

Applied Photophysics has now combined a high performance numerical integration algorithm with its multivariate global analysis system to eliminate this problem. An expert translation system requires the operator simply to enter the proposed chemical mechanism. This may contain multi-step, multi-order and multi-equilibria mechanisms. The scheme is then directly fitted to the data set and the best fitted rates and spectra of all steps and components evaluated.

Acid hydrolysis of Tris(ethylamine) Nickel(II) ion (0.05M)

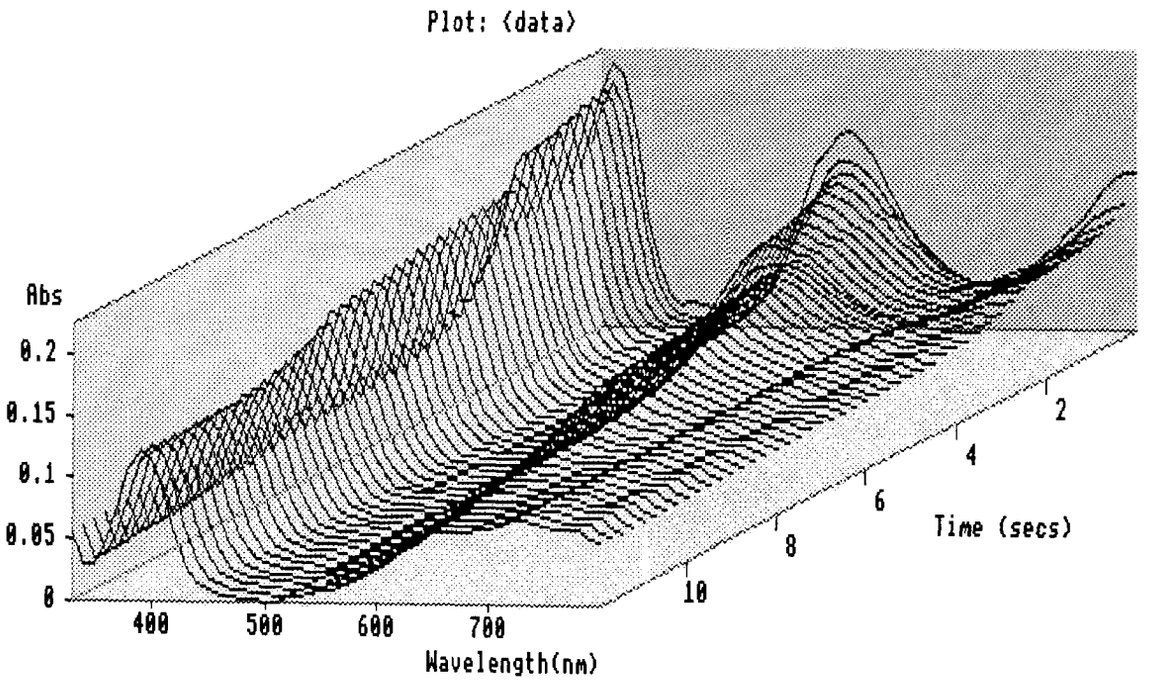


Fig 1a. Global analysis 3-D display of experimental data. 400 spectra recorded at 51 wavelengths. (Not all data shown)

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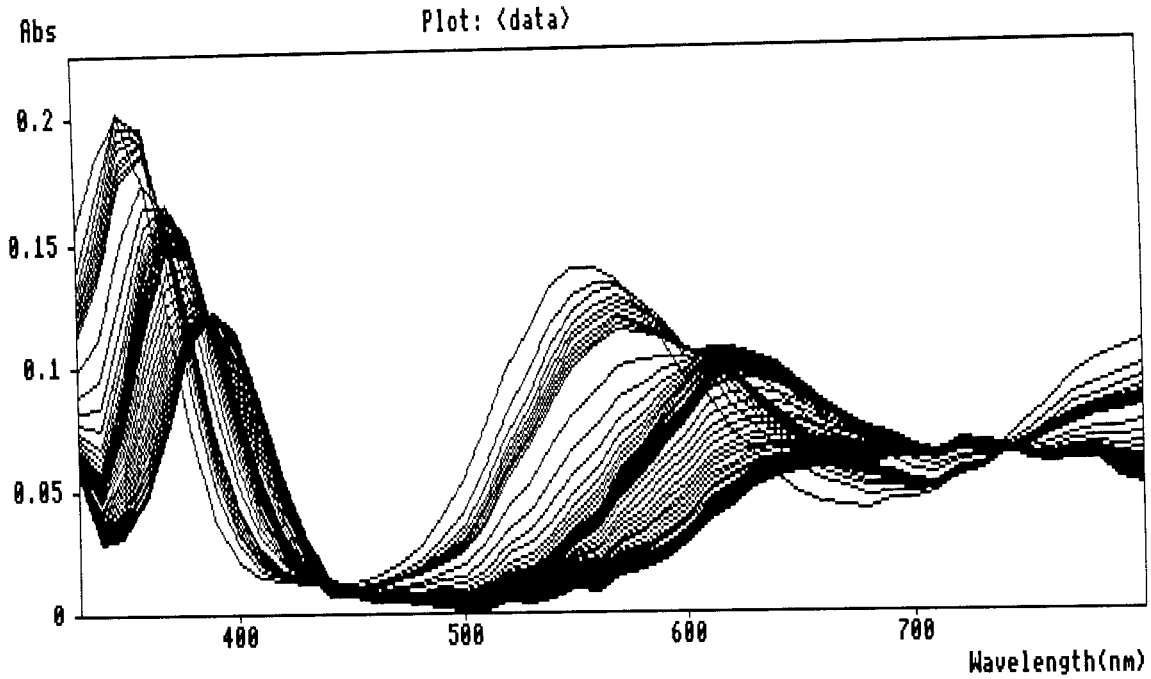


Fig 1b. Data set plotted out as Absorbance vs Wavelength

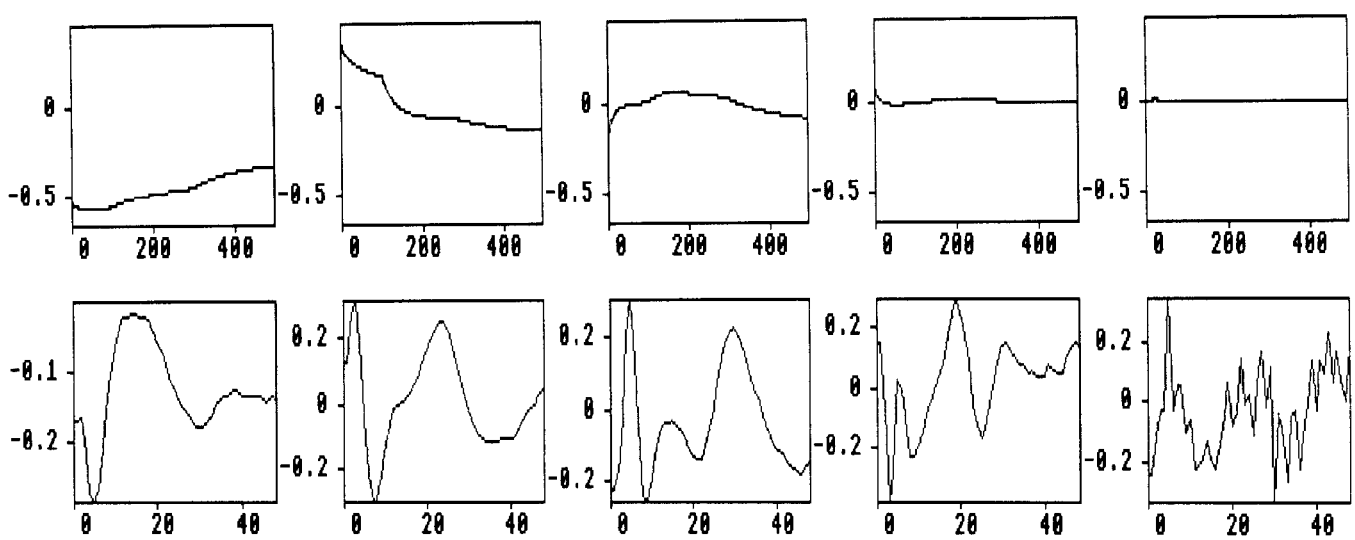


Fig. 2. Basic spectra and time courses derived from singular value decomposition of experimental data matrix. (Only first 5 vectors displayed.) These and the singular values themselves provide support for presence of 4 independent components significantly above the noise level. Analysis therefore justified to a 3 step, 4 species model. After analysis it was discovered that there were, in fact, 5 species and the final model was for a 4 step, 5 species reaction.

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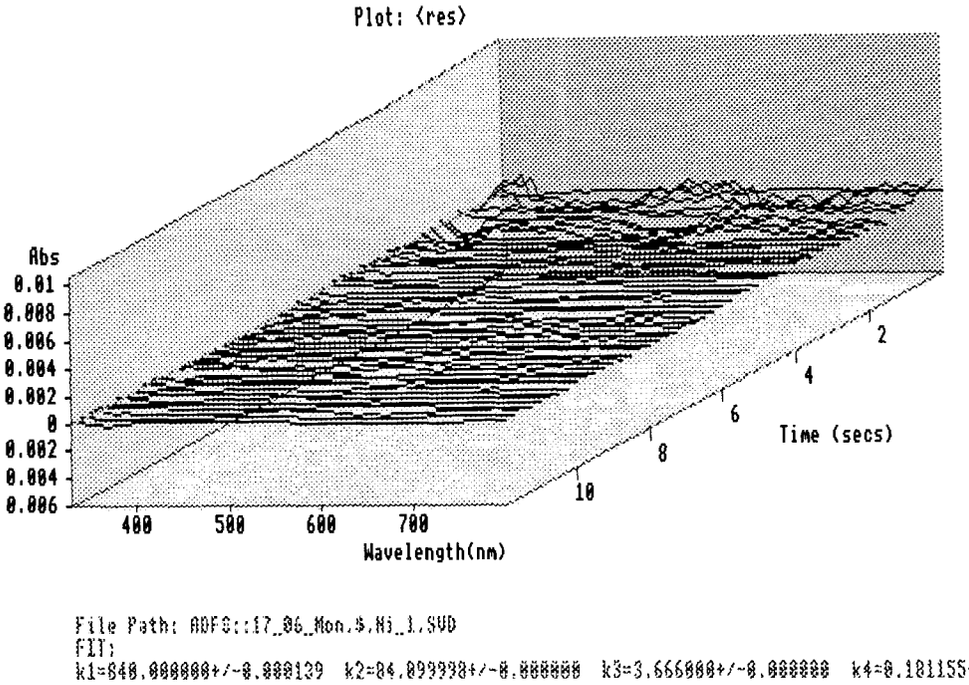


Fig. 3. 3-D plot of residuals following fitting of the significant time courses of Fig. 2. to the reaction scheme A>B>C>D>E. The use of the SVD time vectors for fitting, (which taken with there corresponding basic spectra provide the best n-component approximation to the original data) reduces the computational overhead in this case from fitting 207 parameters to 20400 data points to 19 parameters to 1600 data points.

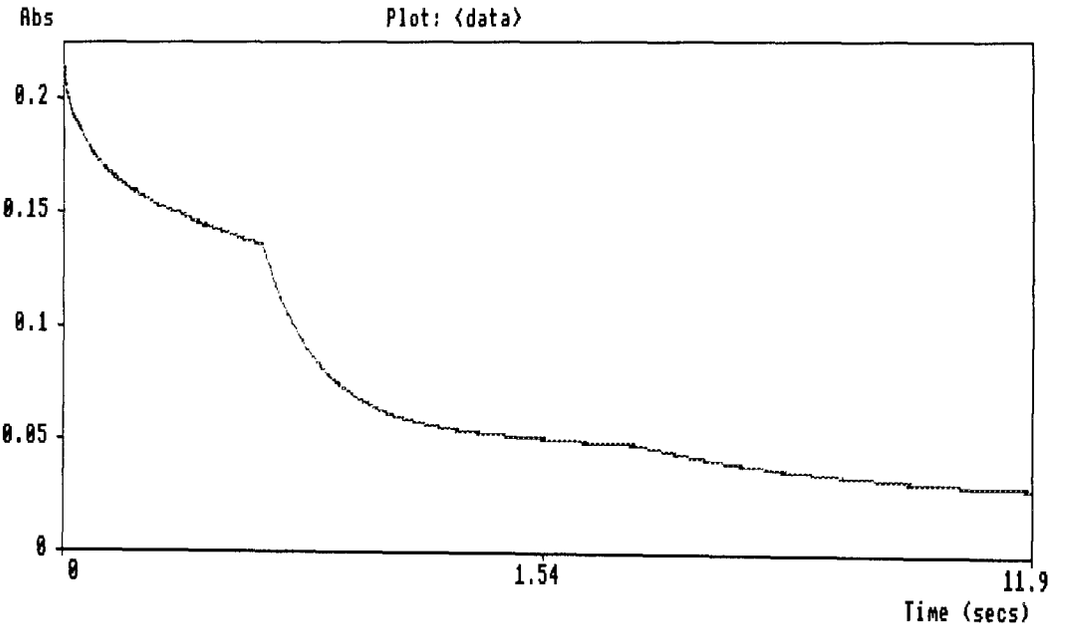


Fig. 4. Example experimental time course at 340nm with overlayed fitted curve. Non linear X-axis shows following phases: 0–20ms, 20ms–2secs, 2secs–~11secs.

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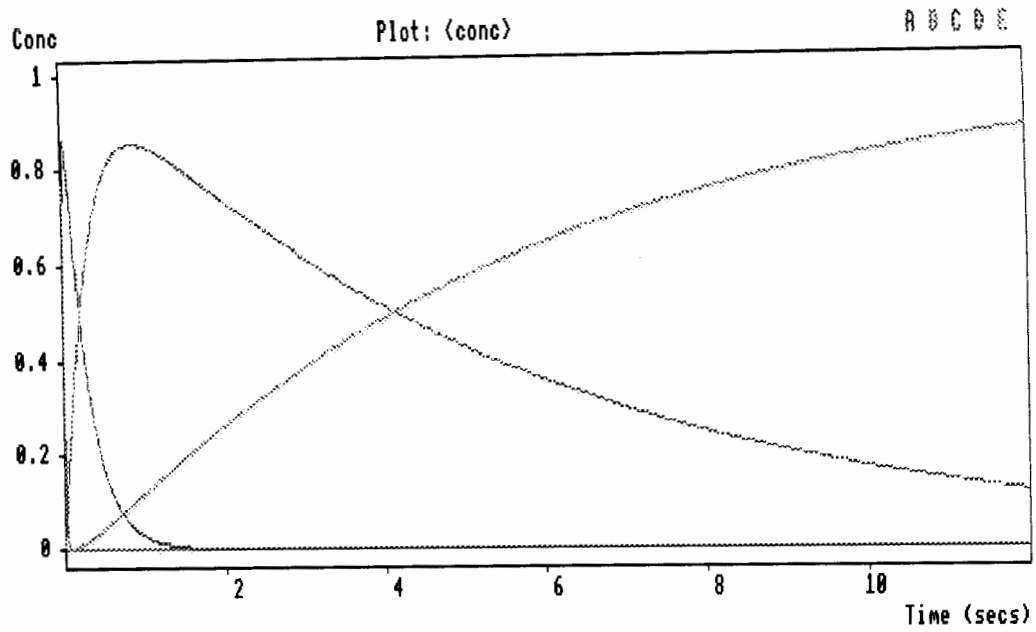


Fig. 5a. Time dependant concentration profiles of fitted components A to E on linear time scale.

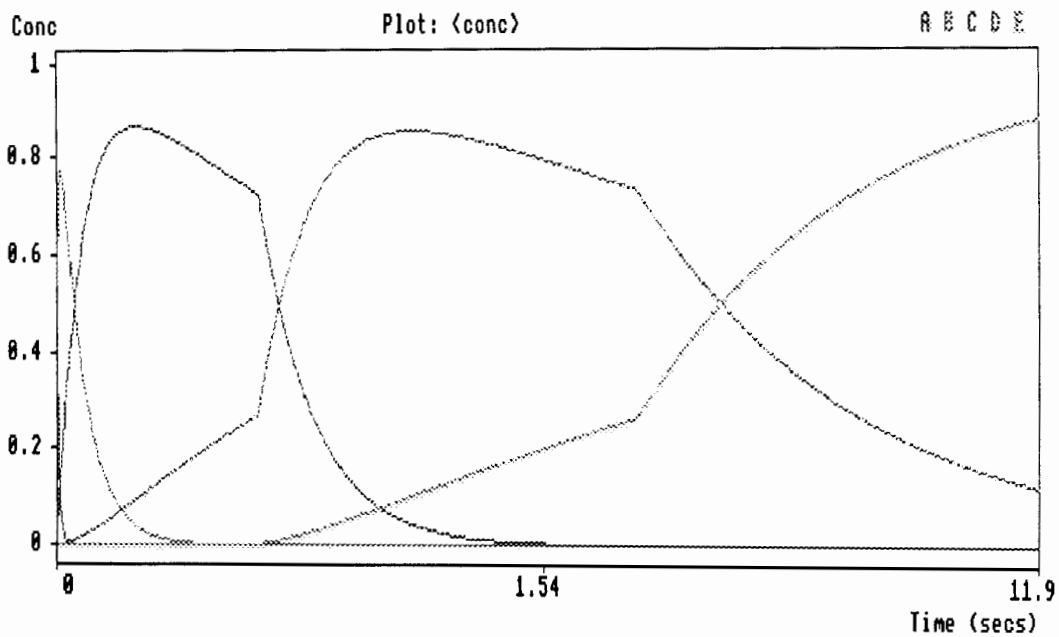


Fig. 5b. Time dependant concentration profiles of fitted components A to E on non-linear time scale.

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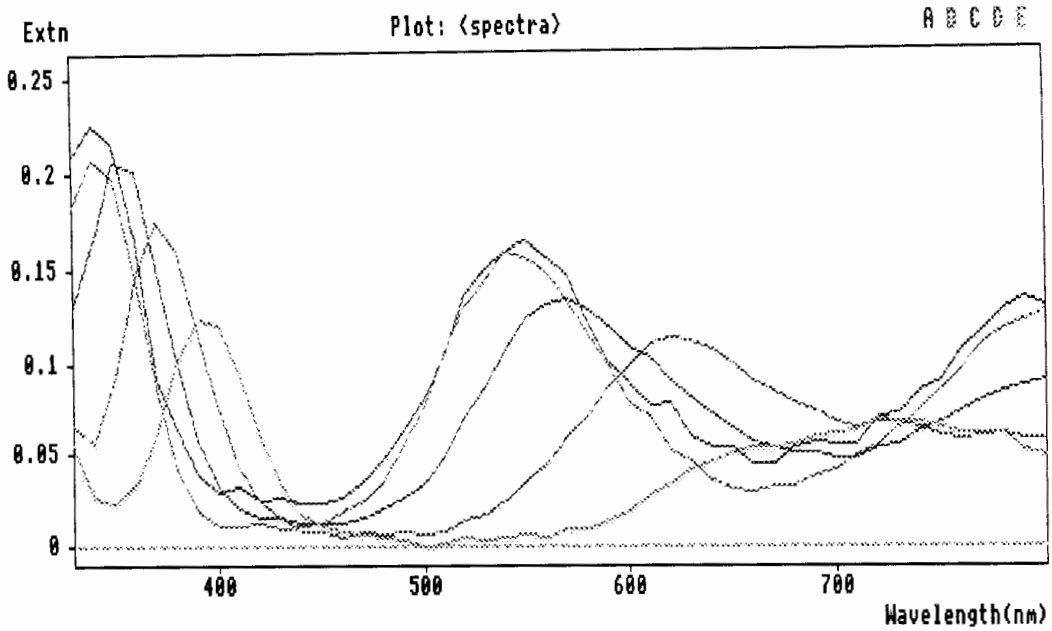


Fig. 6. Intermediate Spectra of components A to E obtained by linear regression of fitted concentration profiles to the original data.

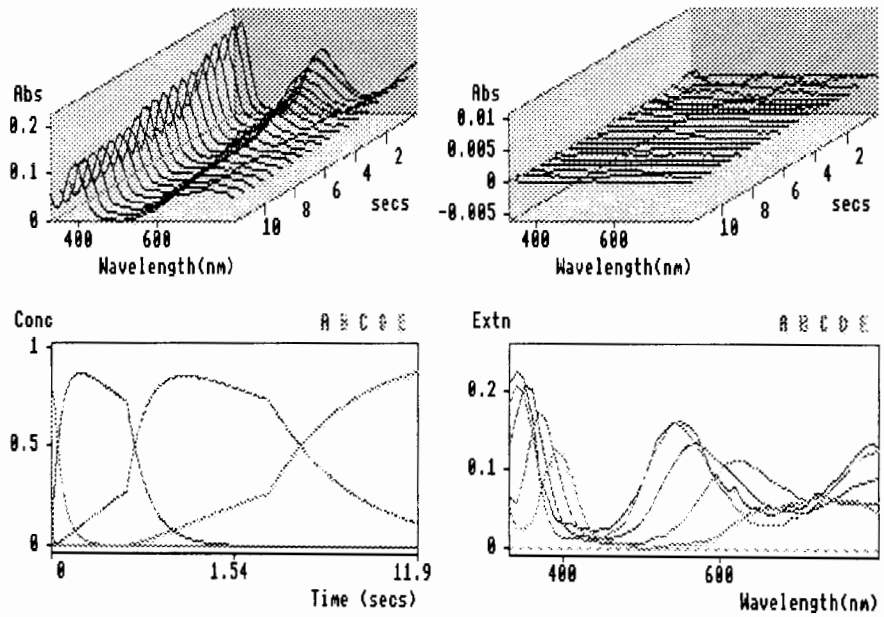


Fig. 7. Review printout showing a combination of the earlier figures. Note that the concentration display is shown with a non-linear time axis.

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RISC-OS operating system

An advanced operating system (RISC-OS) has been developed that communicates with and instructs all aspects of the instrument. When you first switch on the SX.17MV, both the look and feel of the operating system are impressive. Not only does it support multitasking, but individual elements of the operating system itself are multitasking, the formatting of a floppy disc for example.

RISC-OS operating system - hardware

The instrument incorporates a RISC-processor running at 13.5 MIPS, 2Mb RAM, a 3.5" floppy drive (1.44Mb and 720Kb DOS formats) and an 80 Mb hard drive. An enhanced IBM style keyboard is provided accompanied by a 3 button mouse, multi-sync colour monitor and NLQ printer.

Wavelength, choice of detection method, sensitivity and signal conditioning are controlled by slave microprocessors that take their operational instructions from the operating system (RISC-OS). Such an arrangement releases the RISC-processor from intensive instrument control and so making the SX.17MV very much more responsive and capable.

RISC-OS operating system - multitasking

The multitasking operating system and advanced disc filing system are proprietary and written in high speed RISC assembler. The operator environment is in a style made popular by the Apple Mac using windows for applications and icons to represent files and tasks. It is extremely easy to use and is supplemented by various software applications for Drawing, Painting and text editing. IBM software can be run in a window concurrently with native SX.17MV software.

RISC-OS operating system - IBM compatibility

100% compatibility equivalent to a good PC or PC clone running MS-DOS. Supports CGA, EGA and VGA colour graphics, maths co-processor, 3.5 inch floppy drive (5.25 inch - optional extra) and hard disc. This allows the workstation to utilise the vast range of IBM software available.

RISC-OS operating system - external communication

A number of communications packages are available for direct file transfer using the RS-232 port. Several popular file transfer protocols are supported: X-modem, Y-modem, Kermit etc. These packages also support a range of terminal emulation modes allowing interaction with host computer systems. It is also possible to include the SX.17MV in an Ethernet network.

RISC-OS operating system - user interface

The operating system is entirely mouse driven with a 'point and click' operating mode to pick and execute tasks or objects displayed on the screen. The approach adopted is based on features of the Apple Macintosh user interface.

The mouse linked pointer system and use of windows allows very much greater flexibility and control than other alternative menu drivers. The other principal advantage is that the open, random access structure of the interface means that the operator can move arbitrarily and instantly from one context environment to another and perform numerous specific tasks without disorientating route searches or restrictive pathway sequences.

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RISC-OS operating system - data presentation

To display a data set it is simply necessary to point at the occupied data block symbol (which displays the occupying file name) and click the (left) mouse button.

A full screen graph of the selected data appears instantly. This is automatically scaled and labelled with key file information. The display can be zoomed using a simple 'rubber-band box' that is sized over the data region of interest. The zoom process can be repeated or reversed as necessary.

At any time the display can be put into overlay mode, whereby subsequent display selections are superimposed over or removed from the existing graph scales (using the left and right mouse buttons respectively). Data sets can thus easily be compared. Every data set is plotted in a separate colour, the key to which is displayed next to the data block symbols on the screen.

RISC-OS operating system - non-global analysis

During the fit any combination of fit parameters can be locked at a constant value ie prevented from adjustment, an option not normally available in standard analysis packages. This facility is clearly required when providing fixed concentration terms for second order curve fits, but is additionally of great value when certain mechanistic parameters are known in advance of a fit and therefore the degrees of freedom are fewer than normal. (eg in the case of a double exponential where on rate and /or amplitude is known and it is only the second that must be iteratively optimised. This parameter locking is performed simply by appending an '!' to the appropriate numeric entries in the starting list of estimates.

The other advanced feature available within the data analysis is the provision of a user function library, for adding and filing new fitting equations.

The majority of functions used routinely for fitting are provided in what is called the 'standard' library. This includes single, double and treble exponentials, single exponential with steady state, rectangle hyperbola, and 2nd order reaction curves. These are supplemented by a simple second order polynomial and a straightforward linear regression option. All can be fitted with a floating end point, and can be of any or mixed polarity. This set will probably suffice for routine analysis on many occasions, and as mentioned above any combination of the constituent parameters may be fitted or locked. This standard library resides permanently in memory and may be modified in the future depending on customer demand.

The situation may well arise, however, when the particular reaction mechanism under investigation warrants analysis using an unusual fitting function, one that is not provided in the standard library.

A facility has therefore been created which allows the operator to add new functions to supplement those there already. These are introduced into what is termed a 'user' library. The operator first selects the user option and then makes a request to add a new equation to the this library (which is empty at first). After providing a simple name for the equation all that is required is that the appropriate equation be typed in as it would be written on a page. No programming skills are required. The software then prompts the user for trivial names for the parameters in the entered equation. Any errors or mistakes made during this process can simply be corrected using an editing facility.

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Any co-ordinate data can be typed directly into a data block from the keyboard.

Although not supported as standard the RS-423/232 port or expansion IEE-488 interface could easily be configured to acquire data from a range of external devices. ASCII data files on disc can be loaded after transformation. Biologic data files are supported.

The source, scales and units of each set of data are however arbitrary, so that stopped flow records may be intermingled freely with co-ordinate data from any alternative source. Such data could be derived from an on-line RS-232 or IEE-488 instrument, or simply have been typed in at the keyboard.

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