AppliedPhotophysics

SX Series of Stopped-Flow Spectrometers

SX20 | SX20-LED

photophysics.com

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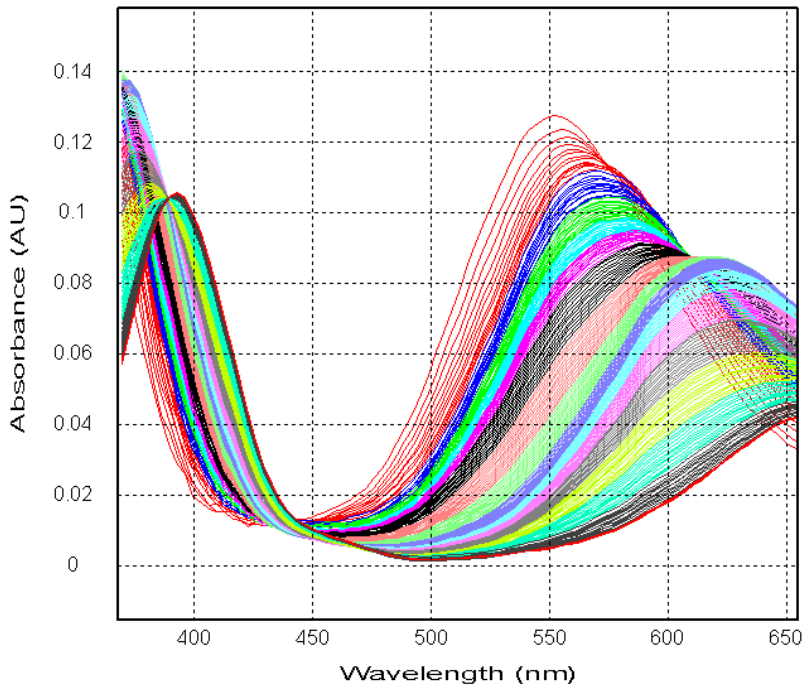
INSTRUMENT OVERVIEW

Applied Photophysics Ltd. is the world's leading producer of stopped-flow spectrometers, with thousands of stopped-flow spectrometers supplied since 1991. We have manufactured kinetic instrumentation since 1971 and have pioneered the development of modern stopped-flow instrumentation. Our ongoing development of stopped-flow applications and our large customer base are your assurance that we provide world class expertise and technical support for your kinetics research with the SX20 stopped-flow spectrometer.

SX20

- Ultra-stable Xenon light source suitable for all absorbance and fluorescence applications
- Programmable monochromator enabling acquisition of both absorbance and fluorescence kinetic traces and steady-state spectral acquisition
- Optimised detectors for fluorescence and absorbance kinetics with no reconfiguration required when switching between these techniques

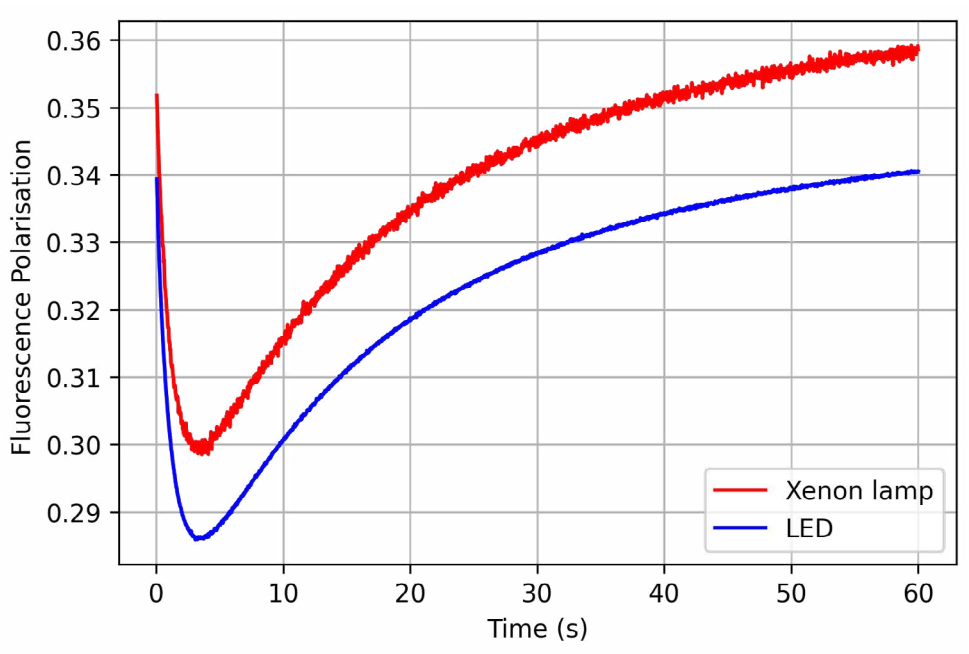


Time-dependent PDA spectra in the time range of 1 ms to 20 s

- Unique lower inner filtering / high-sensitivity cell designs
- Low dead time, low volume requirement
- ProData acquisition, display and analysis software (unlimited seats)
- Large range of upgrade options

SX20-LED

- High-intensity LED light source. Over 40 wavelengths available from 280 nm to 830 nm
- Very high fluorescence sensitivity
- Extremely high stability, rapid start-up and long lasting
- Cost effective
- Small footprint



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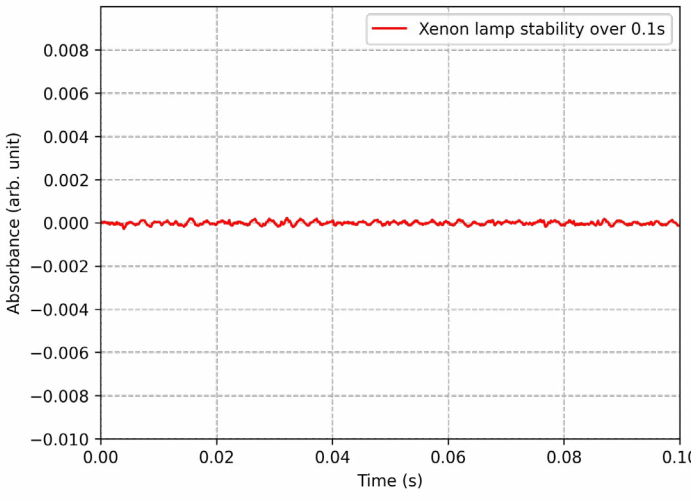
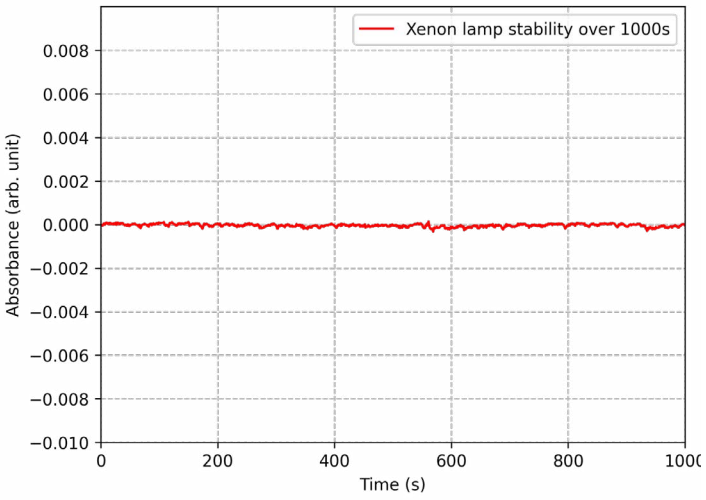
- Large range of upgrade options

SX20 STOPPED-FLOW SPECTROMETER

The SX20 includes everything required for highly sensitive fluorescence, light scattering and absorbance kinetics.

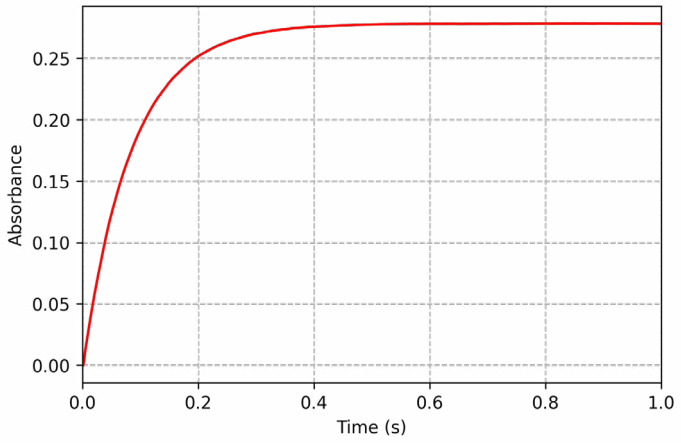


The versatility of the SX20 makes it an ideal choice for a wide variety of stopped-flow applications, as well as instruments intended for use by several research groups.



It has photomultiplier detectors optimised for both absorbance and fluorescence detection and a Xenon light source that is stable to within an absorbance of 0.001 over any time range up to 1000s. Switching between absorbance and fluorescence detection is always straightforward, with no realignment or optimisation required (e.g., cell changing), and for dual-channel instruments, a single mouse click in the control software is all that is required. A fully programmable monochromator is included as standard, enabling UV-Vis scanning and automated acquisition of multi-wavelength kinetic data sets (time-dependent spectra) by the point-by-point method.

The standard 20 µl volume cell has a dead time of 1.1 ms and optical pathlengths of 10 mm and 2 mm for absorbance, and 1.5 mm and 5.5 mm for fluorescence (the lower pathlength is particularly useful for minimising the inner filter effect without compromising sensitivity). Stopped-flow cells are rapidly interchangeable and shorter dead time cells are available.



Other standard features include automatic dead time and drive volume measurement.

Instruments are supplied with the powerful ProData control and analysis software and a PC running Windows 11 with USB communication to the instrument.

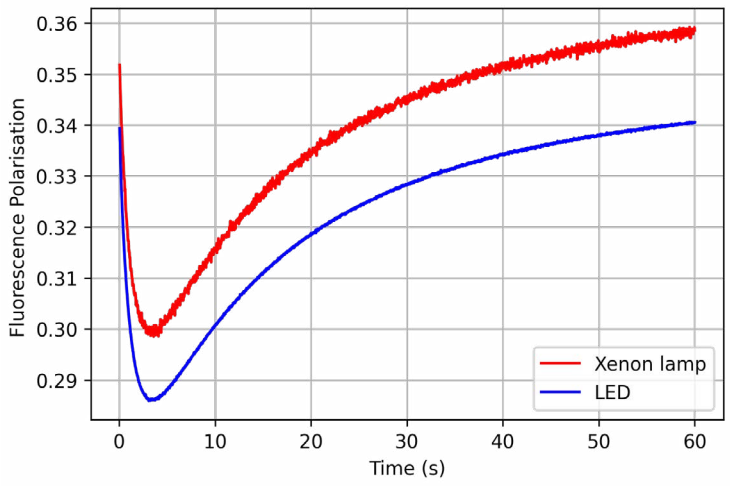
SX20

SX20-LED STOPPED-FLOW SYSTEM

The SX20-LED stopped-flow is a recently introduced version of the SX20 that is ideally suited for applications that are likely to involve sample irradiation at just a few specific wavelengths, such as fluorescence applications.

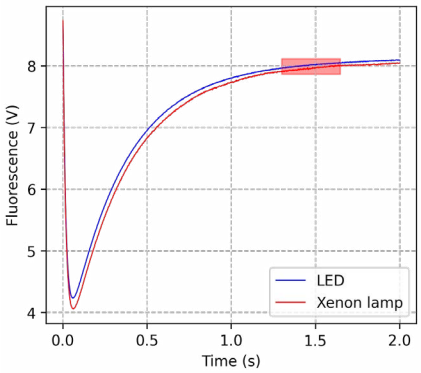
With the SX20-LED stopped-flow system, one or more LED light sources replace the standard Xenon light source and monochromator. This reduces both the instrument's cost and bench footprint.

The SX20-LED can also provide a sensitivity improvement because LEDs are highly stable and typically around ten times more intense than the corresponding emission from the Xenon lamp.

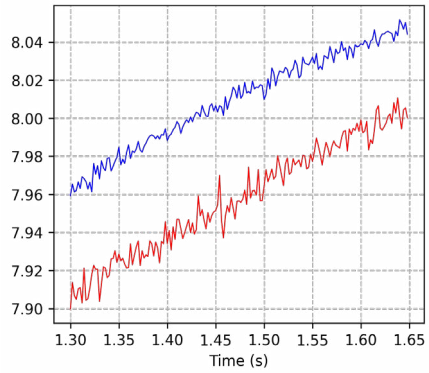


In the first data example shown at right (fluorescence polarisation kinetics), the 505 nm LED provides higher quality data than the Xenon lamp recorded at the same wavelength.

The second data example, shown at right, shows the refolding kinetics of lysozyme recorded both with and LED and a Xenon lamp at 280nm. In this example, the signal-to-noise improvement using the UV LED is about a factor of 2. The difference in noise can be seen in the zoomed in region in the lower plot.



LED light sources also have a quicker warm-up/ stabilisation time: about five minutes, compared with about 30 minutes for a Xenon lamp.



The SX20-LED system also has a much reduced footprint over the standard SX20 spectrometer: 1 m of bench space is all that is required. The LED power supply (pictured below) can be placed on the benchtop or on the back of the sample handling unit.

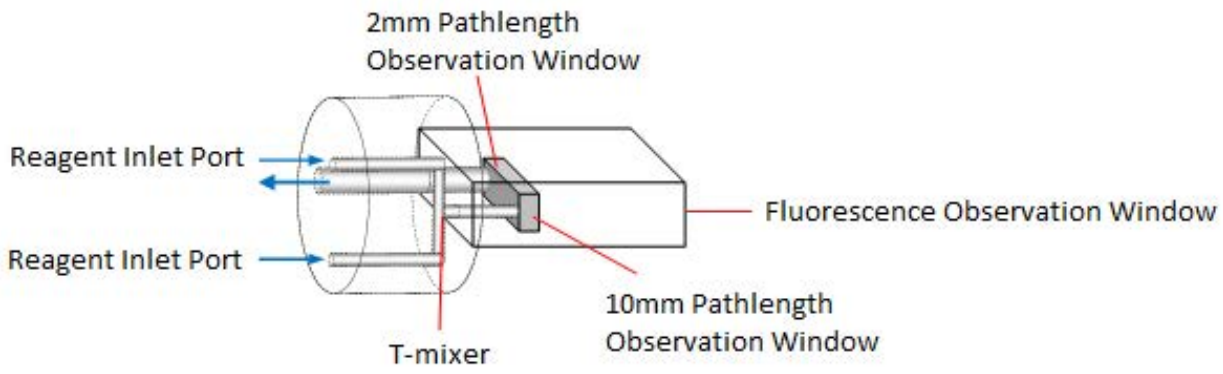
SX20-LED

CAPABILITIES AND PERFORMANCE ADVANTAGES

Stopped-Flow Cell Design

Absorbance measurements

At the heart of the SX20 / SX20-LED is a removable cell cartridge housing the stopped-flow cell. The standard 20 µL volume quartz cell has dimensions 10 mm x 2 mm x 1 mm, and provides optical pathlengths of 10 mm and 2 mm. To switch optical pathlengths, the user simply relocates the detector and light guide – a task that takes about one minute to complete.



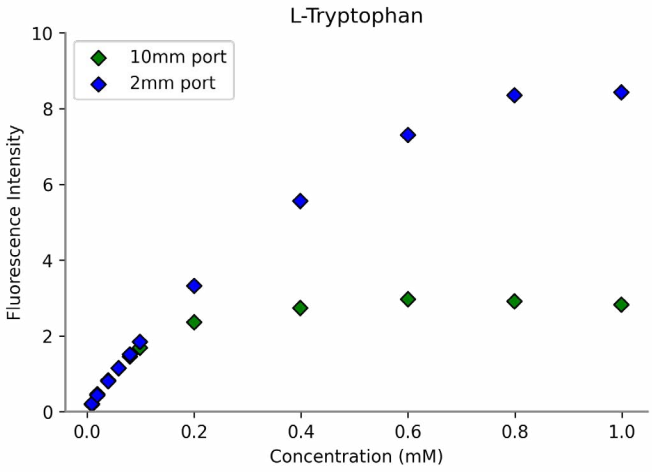
Fluorescence measurements and inner filtering

The stopped-flow cell is uniquely optimised for fluorescence detection because the 'fifth' side of the cell is dedicated to this purpose. This means

- **The cell is able to incorporate a light pipe specifically designed to maximise collection of fluorescence emission.**
- **The inner filtering effect (see below) can be low without having to compromise sensitivity by reducing the cell volume.**
- **No reconfiguration is required when switching between absorbance and fluorescence detection.**

The inner filter effect is caused by reabsorption of light emitted by the sample. It must be considered before assuming that the measured fluorescence signal is directly proportional to the concentration of a chemical species. The effect is caused by progressive absorption of the excitation light as it penetrates the solution being studied, thereby producing progressively less fluorescence signal. Hence, a change in the total absorbance during the reaction can produce a non-exponential fluorescence change. This effect is minimised by using low sample concentrations and/or a low optical pathlength. The 20 µL SX series cell is unique in minimising the inner filter effect without having to use a small volume cell (which would reduce sensitivity). Sample excitation via the 10 x 1 mm window (2 mm port) has an optical pathlength for fluorescence of just 1.5 mm. Excitation via the 2 x 1 mm window gives a higher value (5.5 mm). In both cases the entire sample is irradiated.

The data at right shows the measured fluorescence signal for increasing concentrations of tryptophan (excitation at 285 nm, using a 305 nm cutoff filter to block scattered light). Excitation via the 2 mm port (1.5 mm pathlength) shows linearity up to 0.2 mM, compared to just 0.07 mM when using the 10 mm port (5.5 mm pathlength)



Instrument Dead Time

The instrument dead time can be defined as the earliest time at which valid measurements of the reaction can be made. A short dead time is required for measurement of very fast reactions. A sub-millisecond dead time is relatively easy to achieve by simply using a very small volume stopped-flow cell; however, a small cell also has more limited practical use because

- **For absorbance measurements, only a small optical pathlength is available, producing only a small change in signal.**
- **For fluorescence measurements, only a small volume of sample can be excited, resulting in low signal.**

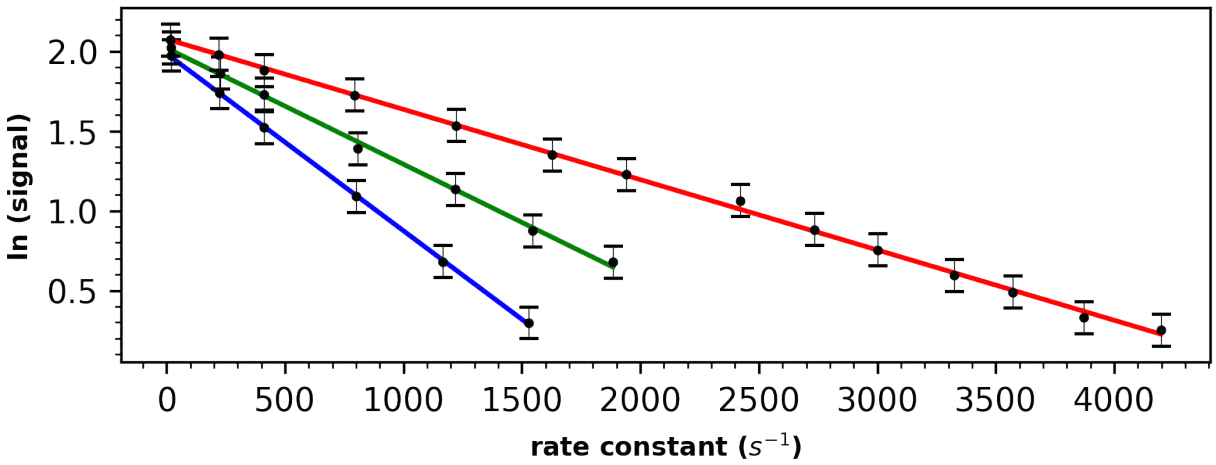
The standard 20 µL volume stopped-flow cell has a dead time of 1.1 ms. As described in the previous section, this cell is highly suitable for almost all stopped-flow requirements; however, cells are rapidly interchangeable and a 5 µL volume cell with a shorter dead time is available.

Automatic measurement of dead time.

The dead time can be measured directly (to within 0.1 ms) simply by doing a stopped-flow drive which enables the instrument's performance to be assessed very quickly. The effects on dead time of parameters such as drive pressure, drive volume and reagent viscosity can also be easily assessed.

Chemical determination of dead time.

The fluorescence quenching reaction between N-acetyltryptophanamide (NAT) and N-bromosuccinimide (NBS) is described by Peterman as a method for measuring the dead time of a stopped-flow instrument . For the data shown below, 10 µM NAT was mixed with a range of NBS concentrations between 5 µM and 5 mM.
The sample was excited at 280 nm, and the fluorescence signal was isolated using a 305 nm cutoff filter. The rate constant was measured in each case. The dead time can be calculated as the slope of a linear plot of ln (initial signal) vs. rate constant. The figure below shows the plots and the corresponding dead times for 1:1 mixing using the standard 20 µL cell, and 1:1 and 10:1 mixing using the 5 µL cell.



deadtime = 1.10 ms (20 µl stopped-flow cell; 1:1 reagent mix)
deadtime = 0.73 ms (5 µl stopped-flow cell; 1:10 reagent mix)
deadtime = 0.43 ms (5 µl stopped-flow cell; 1:1 reagent mix)

[1] *Peterman, Anal. Biochem., 1979, 93, 442. described by Tonomura et al.*

Temperature-Dependent Kinetics

Absorbance measurements

The standard SX20 instrument can operate over the temperature range +60 °C to -20 °C with no requirement for additional accessories. The upper temperature limit can be extended to +80 °C when fitted with high-temperature drive syringes. (option SX/HT).

A temperature-controlled water circulator for temperature regulation of the reagents and stopped-flow is recommended and can be purchased from us or from a local supplier; we are happy to advise on suitable models. No instrument reconfiguration is required when operating at low temperatures, and the sequential-mixing option can also be utilised over this temperature range.

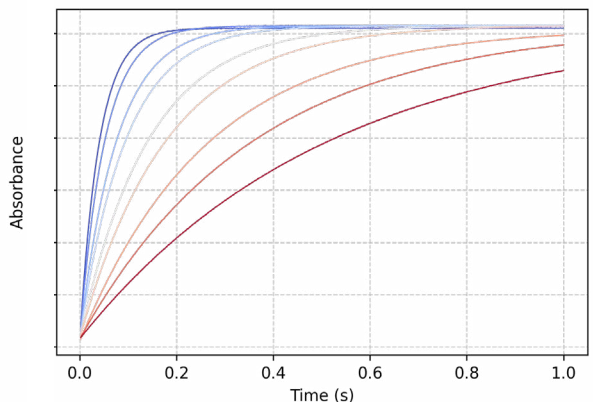
Some circulators (e.g. ThermoScientific Arctic series) can be controlled via the SX20 software. This enables additional functionality:

- **The temperature controller can be set via the stopped-flow control software (ProData)**
- **The instrument can be set to perform an automated series of stopped-flow drives at a series of preset temperatures (e.g., to acquire kinetics at different temperatures for an Arrhenius plot). The included "pause" feature allows each set temperature to equilibrate and "repeat" at each temperature if required. These capabilities enable unattended operation for the duration of the experiment.**

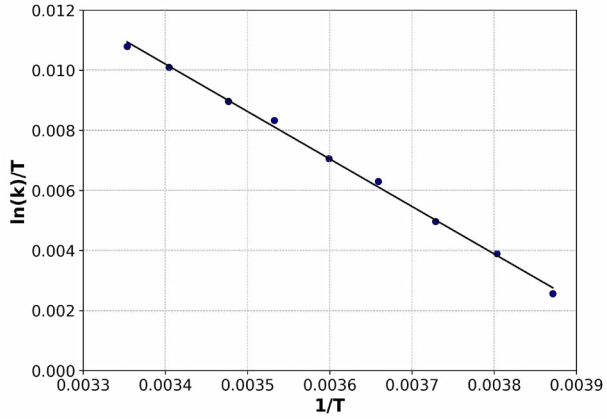
This experiment described below was performed using a Thermo Neslab Arctic SC150-A25 circulator using the SX20's automated temperature-dependent kinetics feature.

The kinetic data at left is from the base-catalysed hydrolysis of 2,4-dinitrophenylacetate (2,4-DPNA) in methanol. 30 µM 2,4-DPNA was mixed with 0.3 mM sodium methoxide (NaOMe); an automated series of stopped-flow kinetic acquisitions were recorded at 5°C intervals as the temperature was cooled from 25 °C to -15 °C. The standard 20 µL cell was used (pathlength 10 mm) at a wavelength of 360 nm. Three repeats were recorded per temperature and each averaged trace was fitted to a single exponential.

The figure at right shows the fitted data plotted as ln (kobs/T) vs. reciprocal temperature. There is a linear relationship (R2 = 0.9978), as would be expected from the Eyring equation.



Kinetics at temperatures from -15°C to 25°C



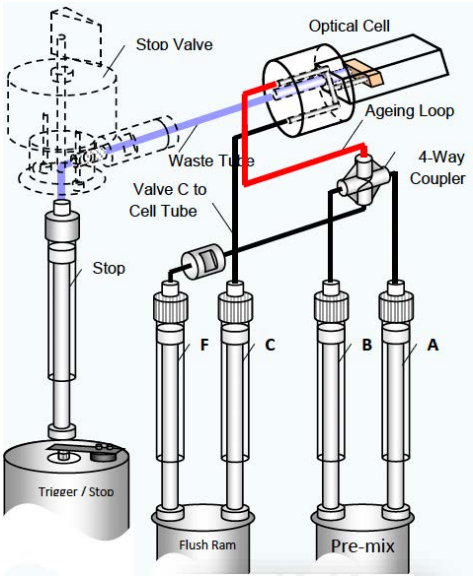
Eyring plot of the fitted data

ACCESSORY OPTIONS

Absorbance measurements

The sequential- (or double-) mixing system is used to study the reactivity of intermediate and transient species. This accessory equips the sample handling unit with two drive rams (and 4 syringes). The first drive mixes two reagents (A and B) into an aging loop and, after a user defined aging period, a second drive mixes the aged solution with a third reagent (C) in the stopped-flow cell. Asymmetric double-mixing experiments are also fully supported (e.g. for protein folding/ unfolding reactions). The fourth syringe is for a buffer rinse.

No hardware reconfiguration is required when switching between short and long aging times; the required aging time is simply entered by the user (in the range 14 ms to 1000 s). Set and calculated aging times are tagged to each data file. Full drive information is provided with each experiment including drive profiles, calculated aging time, drive volume per syringe, and a measurement of the dead time. Aging times are reproducible to within 1 ms.



5 µL Volume Low Dead Time Cell

Stopped-flow cells are mounted in a removable cell cartridge to enable different optical cells to be fitted according to experimental requirements. In addition to the standard 20 µL volume cell, a 5 µL cell is available with a dead time of 0.5 ms (see page 6) and which allows reliable measurement of rates in excess of 3500 s^{-1}. The 5 µL cell has optical pathlengths of 1 mm and 5 mm for absorbance and 3 mm and 1 mm for fluorescence. Changing the cell is straightforward and takes less than 5 minutes. Once fitted, no further optimisation and alignment is necessary.

Quench-Flow Adapter

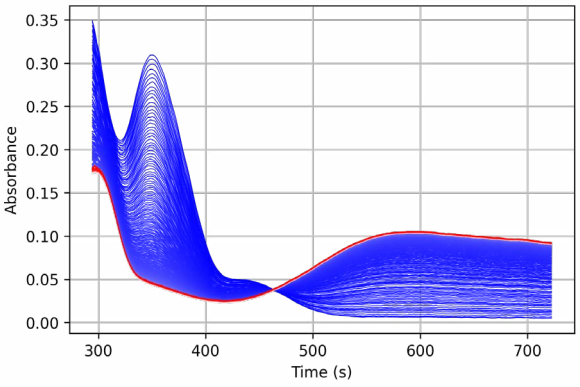
The quench-flow adapter can be fitted in a few minutes in place of the standard stopped-flow cell/cartridge. It includes a millisecond dead time mixer connected to a detachable flow line (for sample recovery). In combination with the sequential-mixing option, this accessory enables quench-flow operation, i.e., rapid mixing of reagents, incubation for a user-selected period (in the range of 15 ms to 1000 s), followed by rapid quenching of the reaction and sample recovery.

Photodiode Array Detector

The photodiode array detector (PDA) enables sets of time-dependent absorbance spectra to be acquired from a single stopped-flow drive. The accessory is a self-contained spectrograph which can be configured in a minute without the need to realign or recalibrate the instrument.

The PDA now attaches directly to the stopped-flow unit (rather than via an optical fibre). This increases light throughput and sensitivity, particularly in the UV region.
In the example shown on the right, direct coupling has extended the the lower wavelength limit from ~300 nm to ~265 nm.

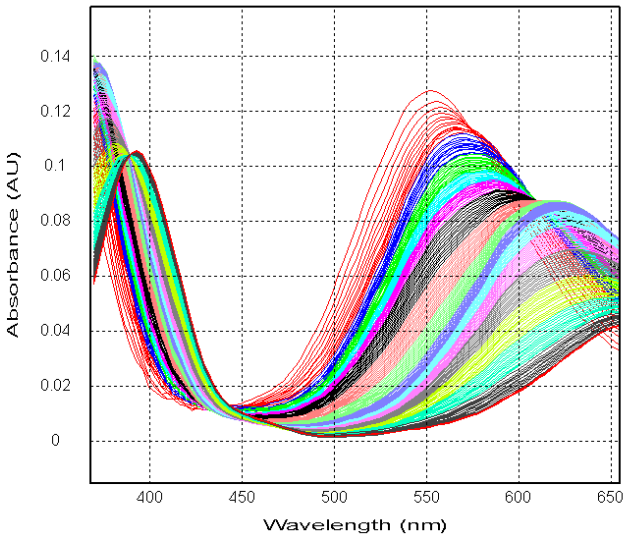
Features:



- 256 wavelength array that acquires up to 1500 spectra/s
- Up to 16000 spectra can be acquired per experiment
- Two wavelength ranges are available:
- UV: ~270-725 nm (extended to 200 nm using the boosted deuterium light source, option SX/UV)
- Vis: 330-1100 nm
- Can be fitted in 1 min - requires no calibration
- Linear, logarithmic or split time bases may be selected as appropriate to the reaction
- User-selectable digital oversampling
- The minimum 0.67 ms integration time may be increased to improve sensitivity
- Sequential-mixing experiments are supported.
- User-friendly ProData software controls all experimental aspects with straightforward data transfer to ProKIV for global kinetic analysis
- Spectral regions can be expanded for closer examination and data over discrete wavelength

Value of logarithmic acquisition

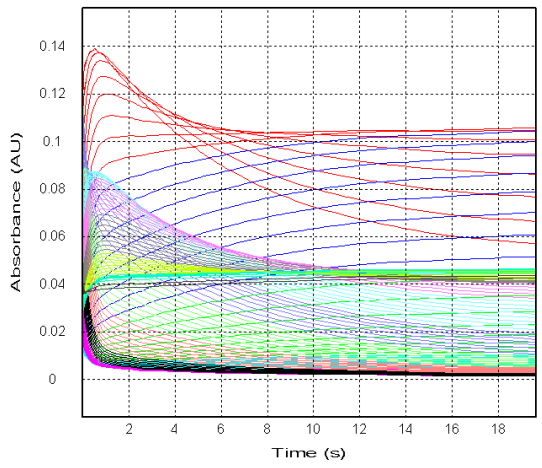
The PDA data set (right) shows the acid hydrolysis of nickel nitrate. It is a three-phase reaction with first-order rates of approximately 50 s^{-1}, 4 s^{-1} and 0.1 s^{-1}. These data were collected over 20 seconds on a logarithmic time-scale with 500 spectra in total (with oversampling).



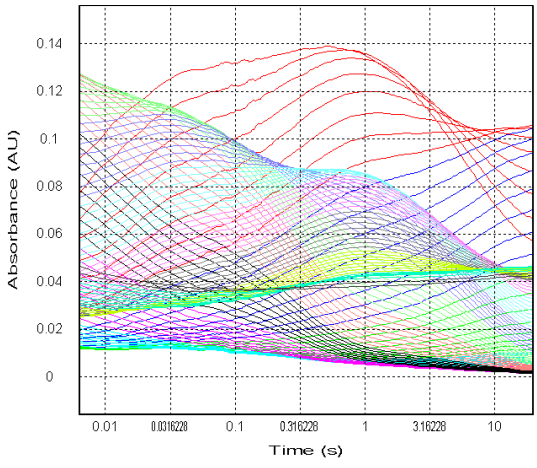
Time-dependent PDA spectra in the time range of 1 ms to 20 s

The figures below show the same dataset in terms of the kinetics at each wavelength, on both a linear and on a logarithmic timescale (i.e., each kinetic is overlaid). The advantage of acquiring spectra over a log timescale is that relatively few spectra are required to completely describe the reaction, and there are approximately equal numbers of kinetic data points (spectra) to describe each reaction phase, even when the fastest phase is around 500 times faster than the slowest phase. In contrast, a linear time base acquisition would require the scan rate to be fast enough to describe the fast phase, and therefore many thousands of spectra would need to be collected across the whole 20 seconds. The resulting data set would be very large, and almost all of the data would be describing the slowest reaction phase. In practise, most other photodiode arrays would only be able to collect 1 or 2 seconds of data before reaching their 'maximum spectra' limit.

Linear timescale

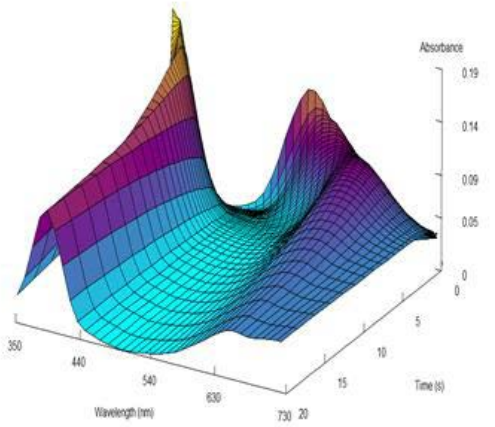


Log timescale



ProKIV Global Kinetic Analysis Software

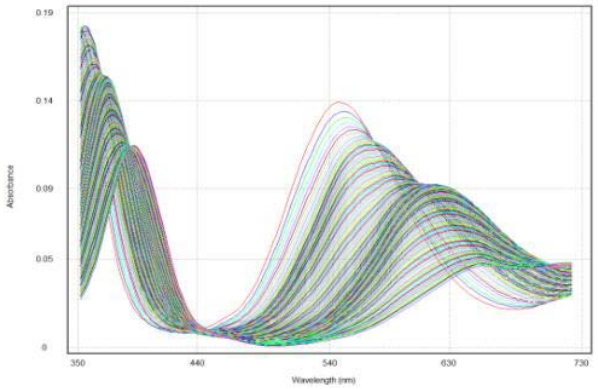
ProKIV is a fully integrated kinetic analysis package, written and supported by Applied Photophysics, that enables global fitting to multi-wavelength kinetic datasets, such as data recorded on a photodiode array detector or by the automated point-by-point method (see page 3).

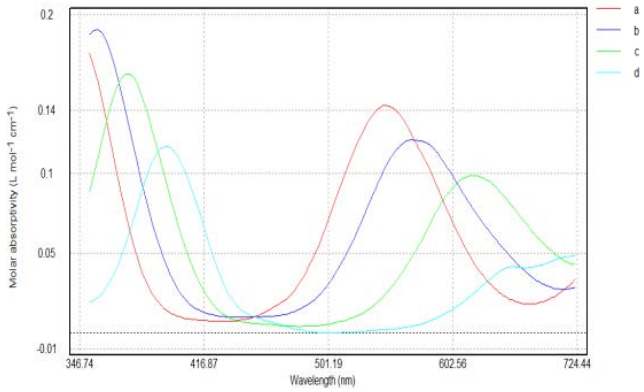


ProKIV simultaneously (globally) fits each kinetic trace in the dataset to the proposed reaction model. This provides more robust reaction rate determination and allows the study of more complex reaction mechanisms.

Features:

- Fits multi- or single- wavelength kinetic data
- Singular value decomposition (SVD) for spectral component prediction and removal of random noise if required
- Robust fitting using numerical integration and no limit to the complexity of the reaction model
- Straightforward reaction scheme editor to virtually any reaction scheme. User can save and load reaction models
- Calculates best-fit spectra and concentration vs. time profiles of all reaction components, including short-lived intermediates
- A wealth of tools for rapid assessment of the fitted data in kinetic, spectral, 2D and 3D formats
- Powerful data simulation tools for exploring kinetics and testing data analysis methodology.





Calculated best fit component spectra

Scanning Emission Monochromator

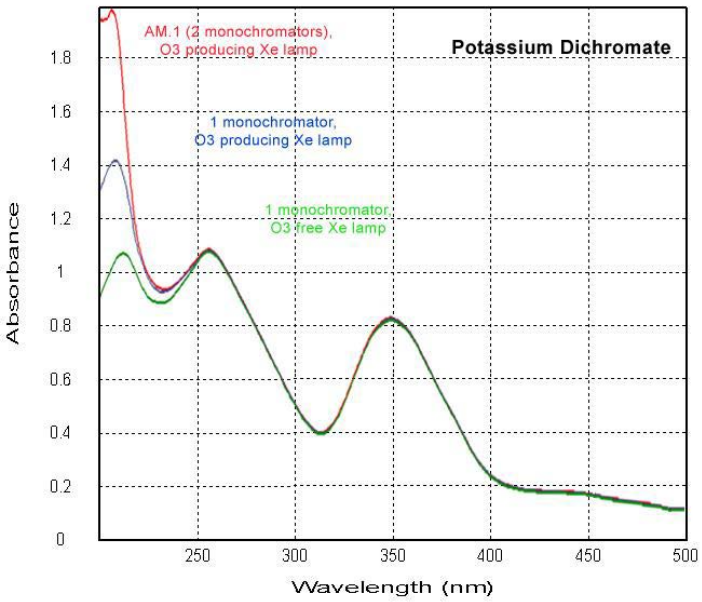
The scanning emission accessory extends the SX20's fluorescence capabilities by adding a second programmable monochromator and a light guide to connect the cell block. This configuration enables the detected emission wavelength and bandpass to be selected via the SX20 control software. This also enables

- **Automated acquisition of time-dependent emission spectra**
- **Acquisition of steady-state emission spectra**

Scanning Monochromator (far-UV) and photometric accuracy

This accessory consists of a second monochromator and a coupling unit to enable two monochromators to be connected in series as shown in the figure. The double-monochromator configuration removes stray light error (improving photometric accuracy) when recording absorbance kinetics in the far-UV wavelength region. This option allows absorbance kinetics to be recorded over the entire range of the detector (200 nm to 850 nm) and without any reconfiguration of the instrument. The second monochromator is identical to that used with the scanning monochromator option and so purchasing either of these options can, with the addition of only minor components, provide the functionality of the other.

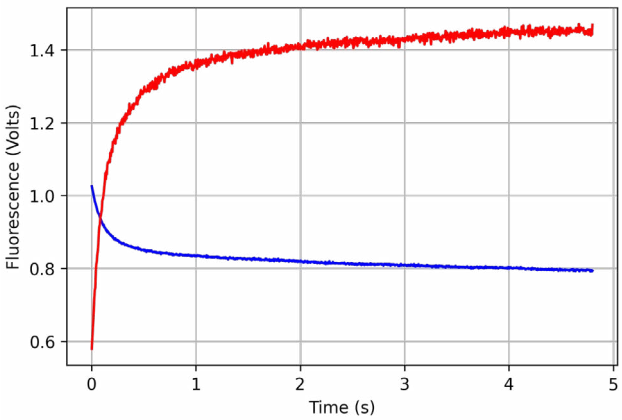
Photometric accuracy. Accurate absorbance measurements in the UV region require that adequate account be taken of spectrometer stray light error. Potassium dichromate is a useful reference material for assessing spectrometer performance. The spectral plots shown in the figure indicate that the SX20 stopped-flow spectrometer with the standard ozone-free Xenon lamp has good photometric accuracy down to ~240 nm (green trace). With the ozone-producing lamp, this is extended to ~225 nm (blue trace), and using the double monochromator configuration of the scanning monochromator option (red trace), there is excellent photometric accuracy to an absorbance of 2 at 200 nm.



Stray light error can also be removed by using a solar-blind detector in place of the standard absorbance detector: by limiting the detector's range (e.g., to cover the region between 200 nm and 320 nm), almost all stray light will be undetected, so absorbance measurements will be photometrically accurate in this range.

Dual Fluorescence Detection

This option comprises an additional fluorescence detector to enable simultaneous fluorescence detection at two emission wavelengths. Both detectors are mounted directly onto the cell block. In general, a cutoff filter is used with one detector to isolate emission at the higher wavelength, and an interference filter is used with the second detector to isolate

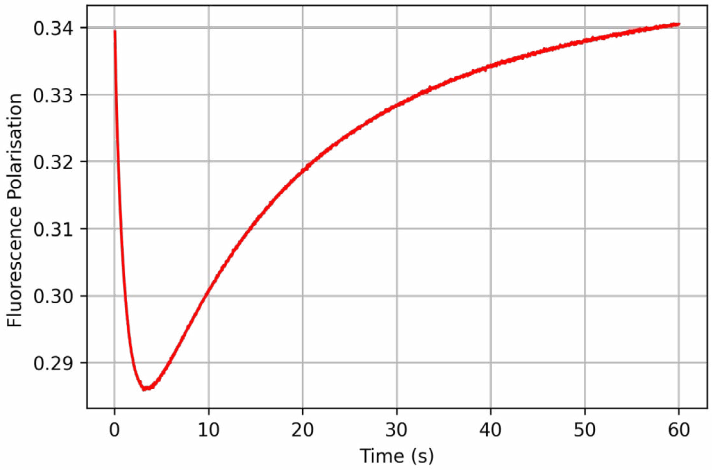


emission at the lower wavelength. This accessory finds common application in the study of FRET systems, enabling both donor and acceptor kinetics to be recorded simultaneously. Optional dual fluorescence requires a dual detection channel with the SX20 or SX20-LED (dual channel is now standard on both models).

Fluorescence Polarisation/Anisotropy

Excitation of a fluorophore with plane-polarised light results in the preferential excitation of molecules with their absorption moments oriented parallel to the plane of polarisation. Fluorescence polarisation/anisotropy can provide information about changes in the mobility and environment of a fluorophore when it interacts with other molecules.

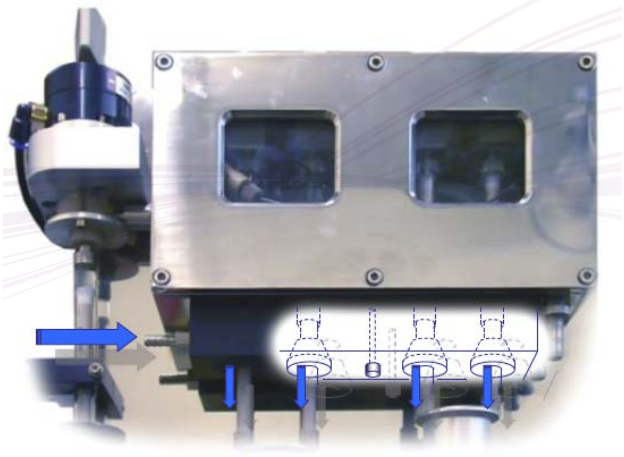
The fluorescence polarisation/anisotropy accessory is an easy-to-fit, dual-channel T-format fluorescence polarimeter. G-factor determination is controlled from the software, and both kinetics and spectra may be acquired in polarisation, anisotropy, total emission and raw data (|| and ⊥) modes, and with full post-acquisition conversion between data modes as required. The fluorescence polarisation option also requires the dual fluorescence option, and likewise requires a dual detection channel with the basic SX20 or SX20-LED system (dual channel is now standard on both models).



Anaerobic Accessory

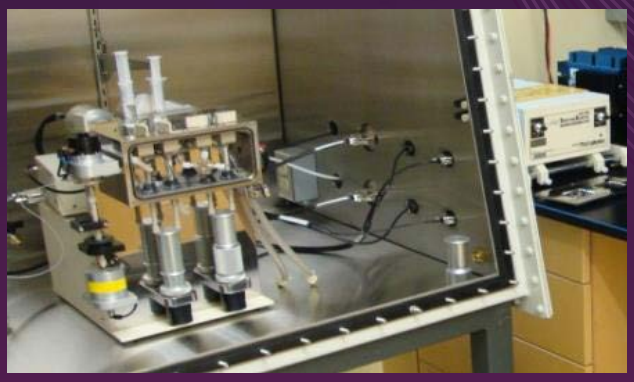
The anaerobic accessory equips the SX20 with high-performance benchtop anaerobic capability. The SX20 is designed to enable anaerobic conditions to be achieved with ease, most commonly by purging the flow circuit with a dithionite solution. The flow circuit material is composed of PEEK which enables the rapid removal of oxygen. PTFE, a material which is more difficult to purge of oxygen, is present only in the tips of the syringes.

Anaerobic conditions are maintained using the purging manifold that forms the main part of the anaerobic option. This unit mounts over the lower section of the drive syringes and is purged with a steady stream of nitrogen to maintain an oxygen-free environment in the region between the syringe pistons and the syringe barrels. This prevents oxygen diffusion across the syringe tips and contamination of the sample. Three-way valves are also provided, enabling anaerobic samples to be introduced to the sample handling unit without contact with the surrounding aerobic environment. A full protocol for anaerobic sample introduction is provided.



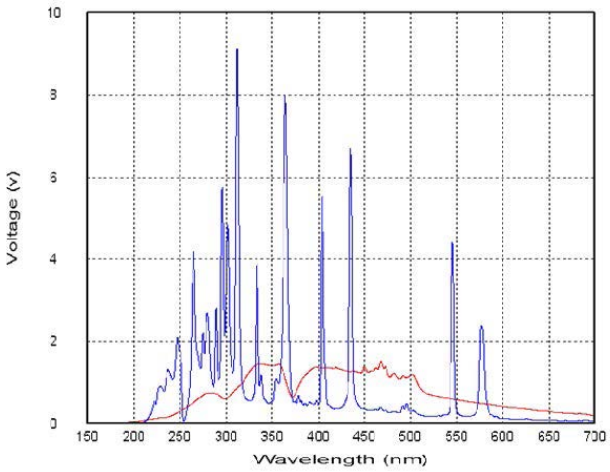
Glovebox integration

The SX20 sample handling unit can be mounted inside a glovebox for the ultimate in anaerobic performance. SX20 installations with glove boxes include those manufactured by Belle Technology, Coy Laboratory Products, MBraun, and Vacuum Atmospheres Company.



Other Accessories

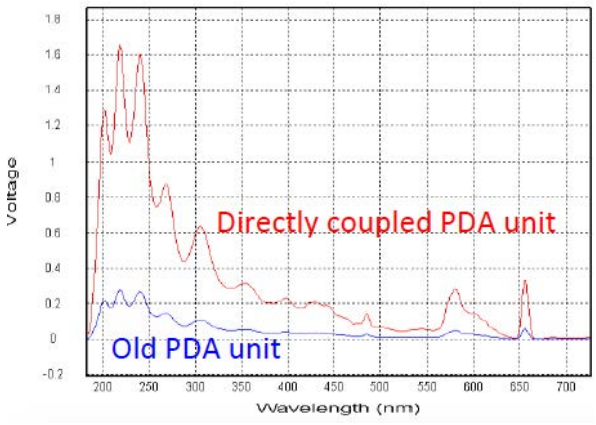
AP150HG Xe-Hg Lamp



The Mercury-Xenon lamp has strong mercury emission lines over the Xenon spectrum. These can improve fluorescence sensitivity when a mercury line is used for excitation. For example, there are several intense mercury lines that can be used for tryptophan excitation. The figure at right shows the output profiles for a Xenon lamp (red) and a Mercury-Xenon lamp (blue). Lamps can be interchanged, but if this will be regularly required, we recommend purchasing a second "lamp mount" for the lamp housing: this enables lamps to be changed in a few minutes without the requirement to handle the lamp itself or realign the lamp when it is fitted.

Boosted Deuterium Light Source

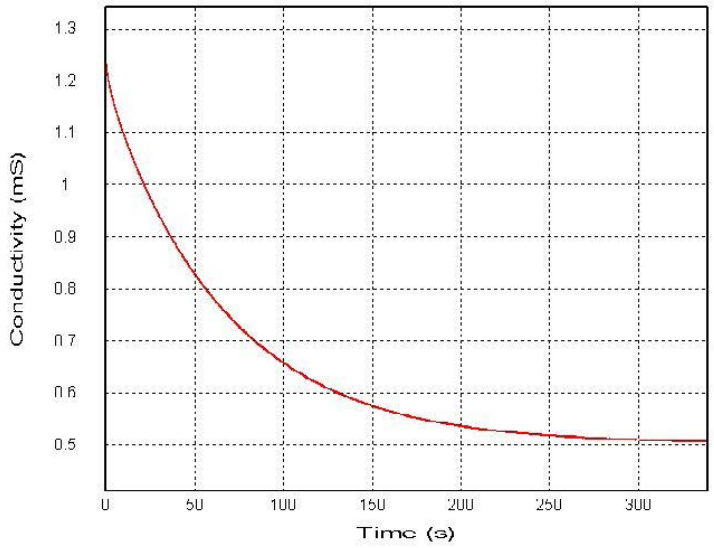
Designed for use specifically with the UV photodiode array, the boosted deuterium source extends the useable wavelength range in the UV down to 200 nm, enabling collection of time-dependent spectra in the range of 200 to 400 nm (compared with ~270-725 nm for the standard Xenon light source). The new directly coupled PDA design (see page 9) also improves the light throughput when used with the deuterium source as shown in the figure at right.



Stopped-flow conductivity accessory

The stopped-flow conductivity accessory enables measurement of time-dependent conductivity with the SX20 and SX20-LED stopped-flow systems. The conductivity accessory can be useful for monitoring reactions where there is no spectral change observable. The conductivity cell cartridge is interchangeable and can be installed and removed from an SX20 stopped-flow spectrometer with ease. The cell cartridge contains a mixer directly upstream of a flow-through conductivity cell, allowing rapidly mixed solutions to be analysed in the cell. A stand-alone conductivity meter and data acquisition unit are used to record the conductance through the cell as triggered by the SX20 electronics unit.

Range:	0-2 mS (0-20 µS, 0-200 µS, 0-2 mS)
Sampling rate	1000 points per second.
Dead time	< 10 ms
Cell constant	1 (approx.)



Other accessories include: temperature-controlled water circulators (including programmable circulators), filters, spares kits, alternative photomultiplier detector options, Service Level Agreements which include PM visits and other benefits.

Key Specifications

	SX20	SX20 - LED
Light Source	150 W Xe – ozone-free (Standard) 150 W Xe - ozone-producing (optional) 150 W Hg-Xe lamp (optional)	LED power supply with one or more LED light sources
Lamp Ignition	Safe start	
Monochromator	Fully programmable diffraction grating Symmetrical Czerny-Turner	N/A
Lamp stability	<0.001 peak-to-peak over any time range between 10 ms and 1000 s	
Measurement modes	Optimised absorbance and fluorescence photomultiplier detectors	
Cell Volume (in the light path)	20 µL (standard) 5 µL (option)	
Dead Time	**20 µL cell:** 1.1 ms **5 µL cell:** 0.5 ms	
Automatic dead time measurement	Yes	
Optical pathlength (absorbance)	**20 µL cell:** 10 mm and 2 mm **5 µL cell:** 5 mm and 1 mm	
Optical pathlength (fluorescence)	**20 µL cell:** 5.5 mm and 1.5 mm **5 µL cell:** 3 mm and 1 mm	
Minimum Drive Volume (per syringe per drive)	**20 µL cell:** 40 µL and 30 µL with option SX/NRV **5 µL cell:** 40 µL and 20 µL with option SX/NRV	
Dead volume (volume held in loading value)	<30 µL	
Priming Volume	~150 µL per flow line	
Temperature range	+60 °C to -20 °C. Extendable to +80 °C	
Main accessories	Sequential mixing, dual fluorescence, fluorescence polarisation (anisotropy), quench-flow, short dead time cell, emission monochromator, anaerobic options and glove box interface, ProKIV global kinetic analysis and data simulation software	
Accessories (SX20 only)	Photodiode array detection, deuterium (PDA) light source, 2nd monochromator for far-UV kinetics, steady-state sample housing. LED power supply	
Other Standard Features:	ProData Viewer data display and kinetics analysis software can be installed on unlimited PCs/Laptops. USB communications.	

Free Direct Evaluation - put the SX20 or SX20-LED to the test!

We invite researchers to visit our demonstration labs or to send samples to us to run on the SX20 and/or SX20-LED stopped-flow systems. We have demonstration labs at our factory in **Leatherhead, UK** and in **Charlotte, North Carolina, USA.**

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