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SX-series Stopped-flow Spectrometers

SX20 SX20-LED







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Instrument Overview

Applied Photophysics Ltd. is the world's leading producer of stopped-flow spectrometers with over 900 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.



- Ultra-stable Xenon light source suitable for all absorbance and fluorescence applications
- Programmable monochromator enabling acquisition of time-resolved absorbance spectra and steady-state spectral acquisition



- Optimised detectors for fluorescence and absorbance kinetics with no reconfiguration required when switching between these techniques
- 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 280nm to 760nm
- Very high fluorescence sensitivity
- Extremely high stability, rapid start-up and long lasting
- Cost-effective
- Small footprint



- 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 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 0.001AU 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-resolved spectra) by the point-by-point method.

The standard 20µl volume cell has a dead-time of 1.1ms and optical pathlengths of 10mm and 2mm for absorbance, and 1.5mm and 5.5mm 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 deadtime and drive volume measurement.

Instruments are supplied with the powerful ProData control and analysis software and a PC running Windows 7 with USB communication to the instrument.





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 system stopped-flow, one or more LED light sources replace the standard Xenon light source and monochromator. This reduces the cost and also the footprint.

The SX20-LED can also provide a sensitivity improvement because LEDs are highly stable, and typically around 10x more intense than the corresponding emission from the xenon lamp. In the first data example shown (right - fluorescence anisotropy kinetics) the LED at 490nm provides higher quality data that the xenon lamp even when not at the peak excitation wavelength (515nm).

The second data example (below right) shows a fluorescence test reaction where the fluorophore concentration is near the limit of detection using the standard xenon source. In this example, the signal-to-noise improvement using the 280nm LED is about a factor of 3.

LED light sources also have a quicker warmup/stabilisation time; about 5 minutes, compared with about 30 minutes with a xenon lamp.

A large range of interchangeable LED light sources are available including: 280, 290, 300, 320, 340, 360, 400, 435, 450, 470, 490, 505, 535, 565, 572, 590, 610, 625, 650, 665, 680, 720, 760nm. Please contact us for other specific wavelengths.

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





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 20µL volume quartz cell has dimensions 10mm x 2mm x 1mm, and provides optical pathlengths of 10mm and 2mm. To switch optical pathlengths, the user simply relocates the detector and light guide – a task of about 1 minute.



Fluorescence measurements and inner filtering. The stopped-flow cell is <u>uniquely</u> 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 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 20µL 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 10x1mm window (2mm port) has an optical pathlength for fluorescence of just 1.5mm. Excitation via the 2x1mm window gives a higher value (5.5mm). In both cases the entire sample is irradiated.

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



Capabilities and Performance Advantages

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 submillisecond dead-time is relatively easy to achieve simply by using a very small volume stopped-flow cell. However, a small cell also has more limited practical use because:

- o for absorbance measurements, only a small optical pathlength is available (= small signal change).
- o for fluorescence measurements, only a small volume of sample can be excited (= small signal).

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

Automatic measurement of dead-time. The dead-time can be measured directly (to within 0.1ms) 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 instrument¹. For the data shown below, 10⁻⁵M NAT was mixed with a range of NBS concentrations between 5x10⁻⁵ and 5x10⁻³M. Excitation was at 280nm and the fluorescence signal was isolated using a 305nm cut-off 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 20µL cell, and 1:1 and 10:1 mixing using the 5µL cell.

A similar chemical method using an absorbance reaction, the reduction of DCIP with ascorbic acid, is described by Tonomura et al²



¹ Peterman, Anal. Biochem., 1979, **93**, 442.

² i. Tonomura, B., Nakatani, H., Ohnishi, M., Yamaguchi-Ito, J., and Hiromi, K. (1978). Analytical Biochemistry, 84, 370-383.

Capabilities and Performance Advantages

Temperature dependant kinetics

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. Thermo Scientific Arctic series) can be controlled via the SX20 software. This enables additional functionality:

- The instrument 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 pre-set temperatures (e.g. to acquire kinetics at different temperatures for an Arrhenius plot). This includes 'pause' features to allow each set temperature to equilibriate, and 'repeat' at each temperature if required. The 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 temperaturedependent kinetics feature.

The kinetic data (right) is from the base catalysed hydrolysis of 2,4-dinitrophenylacetate (2,4-DPNA) in methanol. 30μ M 2,4-DPNA was mixed with 0.3mM 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 20µL cell was used (pathlength 10mm) at a wavelength of 360nm. Three repeats were recorded per temperature and each averaged trace fitted to a single exponential.

The figure (right) shows the fitted data plotted as $ln(k_{obs})$ vs. 1/(Temperature) There is a linear relationship (R²=0.9978) as would be expected from the Eyring equation.



SX/SQ Sequential-Mixing Accessory

The sequential- (or double-) mixing accessory 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 protein also fully supported (e.g. for folding/unfolding reactions).

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 14ms to 1000s). Set and calculated age times are tagged to each data file. Full drive information is provided with each experiment including: drive profiles, calculated age time, drive volume per syringe, and a measurement of the dead-time. Age times are reproducible to within 1ms.

Single-mixing stopped-flows can be later upgraded to sequential-mixing.

SX/RC5 5µL 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 20µL volume cell, a 5µL cell is available with a dead-time of 0.5ms (see page 6) and which allows reliable measurement of rates in excess of $3500s^{-1}$. The 5µL cell has optical pathlengths of 1mm and 5mm for absorbance and 3mm and 1mm for fluorescence. Changing the cell is straightforward and takes less than 5 minutes. Once fitted, no further optimisation and alignment is necessary.

SX/QFA 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 milli-second dead-time mixer connected to a detachable flow line (for sample recovery). In combination with the sequential-mixing capability option (SX/SQ), this accessory enables quench-flow operation i.e. rapid-mixing of reagents, incubation for a user selected period (in the range 15ms to 1000s), followed by rapid-quenching of the reaction and sample recovery.



SX/PDADC Photodiode Array Detector

The SX/PDADC photodiode array detector (PDA) enables sets of time-resolved 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 NEW 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 lower wavelength limit from ~300nm to ~265nm.

Features:

- 256 array that acquires up to 1500 spectra/s
- Up to 16000 spectra can be acquired per experiment
- Two wavelength ranges are available:
- SX/PDADC-UV:~270-725nm (extended to 200nm using the boosted deuterium light source, option SX/UV)
- o SX/PDADC-Vis 330-1100nm
- 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 0.67ms 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 ranges can be selected for export or analysis

Value of logarithmic acquisition

The PDA data set (right) shows the acid hydrolysis of Nickel Nitrate. It is a 3 phase reaction with first order rates of approximately 50s⁻¹, 4s⁻¹ and 0.1s⁻¹. These data were collected over 20 seconds on a logarithmic time-scale with 500 spectra in total (with oversampling).

The figures below show the same dataset in terms of the kinetics at each wavelength, on both a linear and a logarithmic timescale (i.e. each kinetic is



The PDA detector is directly coupled to the stopped-flow unit



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.

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 (a + b > c, c <> d etc.) 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.









SX/SM Scanning Emission Monochromator

The SX/SM accessory extends the 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-resolved emission spectra



Acquisition of steady-state emission spectra

SX/AM Scanning Monochromator (far-UV) and photometric accuracy

The SX/AM accessory consists of a second monochromator and a coupling unit to enable two monochromators can be connected in series as shown in the figure. The double-monochromator configuration removes stray light error (improves photometric accuracy) when recording absorbance kinetics in the far-UV wavelength region. With the SX/AM accessory absorbance kinetics can be recorded over the entire range of the detector (200nm to 850nm) and without any reconfiguration of the instrument. The second monochromator is identical to that used with option SX/SM (see above) 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 ~240nm (green With the ozone-producing lamp this is trace). extended to ~225nm (blue trace) and using the double monochromator configuration of option SX/AM (red trace) there is excellent photometric accuracy to 2AU at 200nm.



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

SX/DF Dual Fluorescence Detection

The SX/DF 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 cut-off 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. The SX/DF accessory finds common application in the study of FRET reactions enabling both donor and acceptor kinetics to be recorded simultaneously. Option SX/DF requires a dual detection channel with the SX20 or SX20-LED.

SX/FP Fluorescence Polarisation/Anisotropy

Excitation of a fluorophore with plane polarised light results in the preferential excitation of molecules with their absorption moments orientated 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 SX/FP 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 (\parallel and \perp) modes, and with full post-acquisition conversion between data modes as required. Option SX/FP includes option SX/DF described above and likewise, requires a dual detection channel with the basic SX20 or SX20-LED system.

SX/APSSH01 Steady-State Sample Housing

The Steady State Sample Housing enables temperature controlled UV-Vis or fluorometric measurements using a standard cuvette – using the SX20 light source and monochromator(s). It is attached to the monochromator either directly or via the standard fibre optic light guide and coupled to either the Absorbance/Fluorescence PMT detectors or the PDA detectors.









SX/AN Anaerobic Accessory

The anaerobic accessory (SX/AN) equips the SX20 with a high performance bench-top 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 of PEEK (polyaryletheretherketone), which eases the rapid removal of oxygen, and PTFE (a material from which it 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 SX/AN 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. 3-way valves are also provided to enable anaerobic samples to be introduced to the sample handling unit without coming into contact with the outside environment (air). A full protocol for anaerobic sample introduction is provided.

Glove box integration

The SX20 sample handling unit can be mounted inside a glove box 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 Hg emission lines over the xenon spectrum and these can improve fluorescence sensitivity where a Hg line can be used for excitation. There are for example several intense Hg lines that can be used for tryptophan excitation. The figure right shows the output profiles for a xenon lamp (red) and a mercury-xenon lamp (blue). Lamps can be interchanged but if this is likely to be regularly required, we recommend purchasing a second 'lamp mount' for the lamp housing: this enable lamps to be changed in a few minutes without the requirement to handle the lamp itself or realign the lamp when it is fitted.

SX/UV Boosted Deuterium Light Source.

Designed for use specifically with the SX/DCPDA-UV photodiode array, the deuterium sources extends the useable wavelength range in the UV down to 200nm. Enabling collection of time-resolved spectra in the range 200-400nm (compared with ~270-725nm for the standard xenon light source). The new directly coupled PDA design (see page 9) has also improved the light throughput when used with the deuterium source as shown in the figure (right).

SX/CM stopped-flow conductivity accessory.

For measurement of time-dependent conductivity with the SX20 and SX20-LED stopped-flows. The SX/CM 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 with triggering being provided from the SX20 electronics unit.

Range:	0-2mS (0-20µS, 0-200µS, 0-2mS)	
Sampling rate	1000 points per second.	
Dead time	< 10ms	
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	150W Xe – ozone-free (Standard) 150W Xe - ozone-producing (optional) 150W 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	<0.001AU peak-to-peak over any time range between 10ms and 1000s	~ 0.0001AU over any time range	
Measurement modes	Optimised absorbance and fluorescence photomultiplier detectors	Optimised absorbance and/or fluorescence photomultiplier detector	
Cell Volume (in the light path)	20μL (standard) 5μL (option)		
Dead-time	20μL cell : 1.1ms 5μL cell: 0.5ms		
Automatic dead-time measurement	Yes		
Optical pathlength (absorbance)	20μL cell : 10mm and 2mm 5μL cell : 5mm and 1mm		
Optical pathlength (fluorescence)	20μL cell : 5.5mm and 1.5mm 5μL cell : 3mm and 1mm		
Minimum Drive Volume (per syringe per drive)	20μL cell: 40μL and 30μL with option SX/NRV 5μL cell: 40μL and 20μL with option SX/NRV		
Dead volume (volume held in loading valve)	<30µL		
Priming Volume	~150µL per flow line		
Temperature range	+60°C to -20°C. Extendable to +80°C with option SX/HT		
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, 2 nd 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 **London**, **UK** and in **Boston**, **USA**.

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