Optim® 1000

A truly unique high throughput, micro-volume protein analysis and characterisation system.

Avacta
INNOVATIVE ANALYSIS

Introduction to Optim®

The Optim 1000 has been developed to reduce the time and cost of therapeutic protein preformulation studies, stability testing and formulation development. It simultaneously measures a range of stability parameters including protein unfolding transition temperature (T_m) and aggregation onset temperature (T_{agg}).

The Optim 1000 uses sample volumes as low as 1 µl and provides rapid data acquisition. Thermal unfolding and aggregation curves are simultaneously acquired for 48 samples run in a single experiment, enabling 96 samples to be analysed in one working day. No other instrument on the market offers a comparable combination of analytical versatility, measurement speed and ultra low sample consumption.

Key features include:

- Ultra low sample consumption
- Rapid, high throughput measurements
- Simultaneous optical measurements:
  - Intrinsic fluorescence to monitor tertiary structure
  - Static light scattering to detect aggregates
- Sample heating and cooling to determine:
  - Protein unfolding temperature (T_m)
  - Protein aggregation onset temperature (T_{agg})
  - Time dependent unfolding and aggregation at a fixed temperature

Ultra small sample volumes, rapid, simultaneous multi-modal measurements

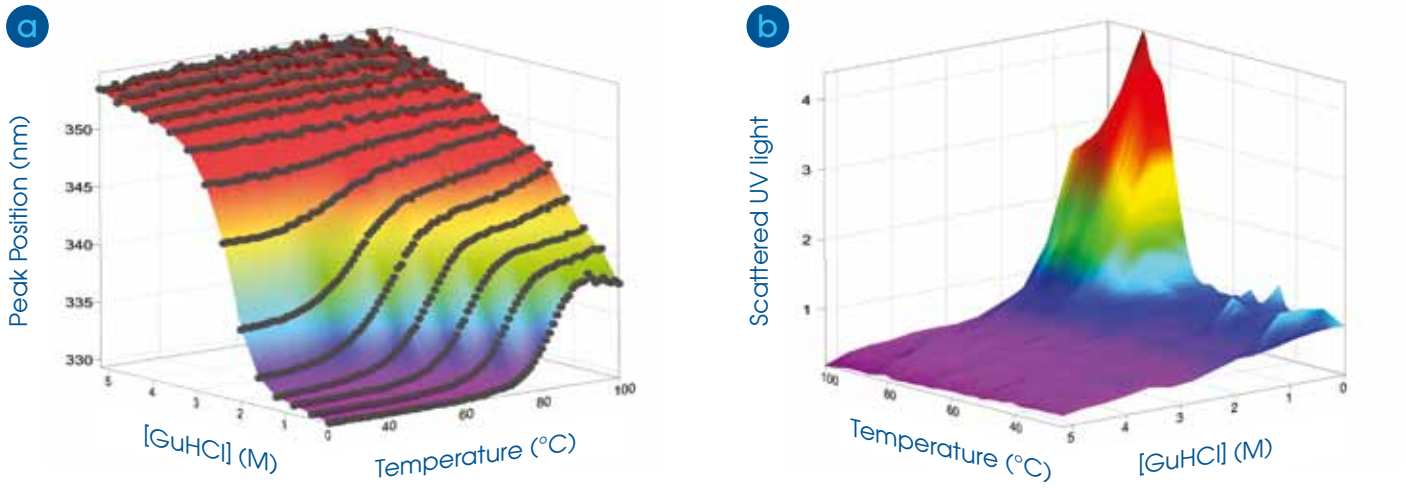


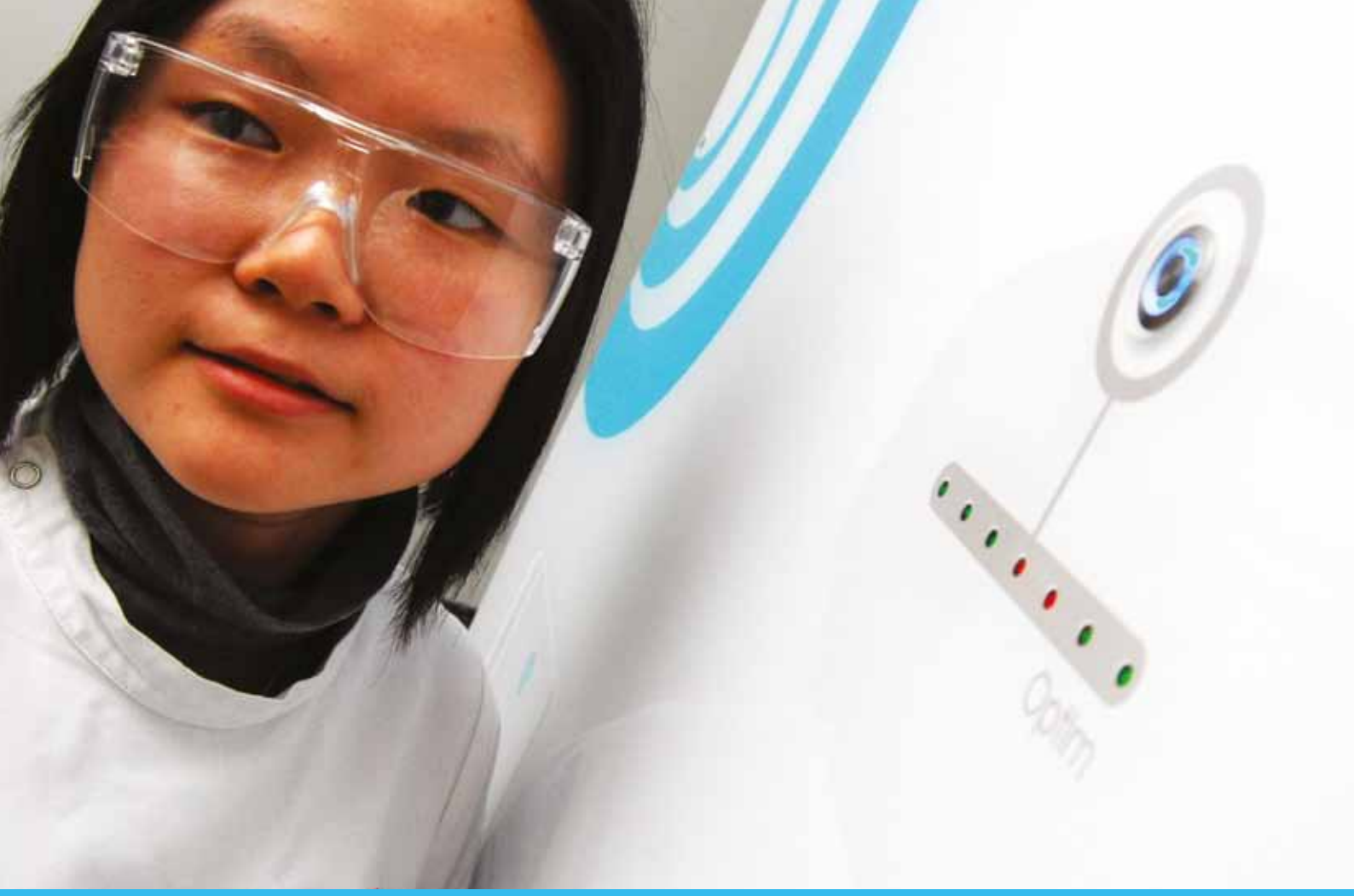
Figure 1: *Effect of temperature and denaturant concentration on*
(a) *protein tertiary structure monitored using protein intrinsic fluorescence.*
(b) *protein aggregation monitored using static light scattering.*
Both sets of data simultaneously acquired, experiment time only 90 minutes (10 minutes hands on time), total of only 3.2 µg protein used.

Designed for biopharmaceutical analysis

Developed as part of a collaboration with a leading global biopharmaceutical company, the Optim 1000 was designed to address three critical issues that currently affect the development of therapeutic proteins:

1. Early in development protein is expensive to produce and in short supply, however large amounts of a protein are required for conventional analytical techniques used during preformulation, stability and formulation studies.
2. Measurements with conventional analytical instrumentation are often slow and labour intensive which is incompatible with large numbers of samples to be analysed and tight development timelines faced.
3. Conventional analysis methods require multiple pieces of instrumentation and considerable expertise covering each technique is required to analyse and interpret the results.

As a consequence these critical studies are often less comprehensive than would be liked or can often only be carried out in the later stages of development. This can increase the risk of costly late stage problems or failure to identify the optimum candidate or formulation.



Sensitive Analysis of Ultra-Low Sample Volumes

At the heart of the Optim 1000 is a state-of-the-art spectrograph and Peltier cooled CCD detector. This enables multiple, highly sensitive optical measurements to be made simultaneously over a broad range of wavelengths from each sample. When combined with our proprietary micro-cuvette array (MCA) sample holder, this high sensitivity means that the Optim 1000 can obtain high quality data from small volume, low protein concentration samples which means that minimal amounts of sample are used.

Fast Analysis of Ultra-Low Sample Volumes

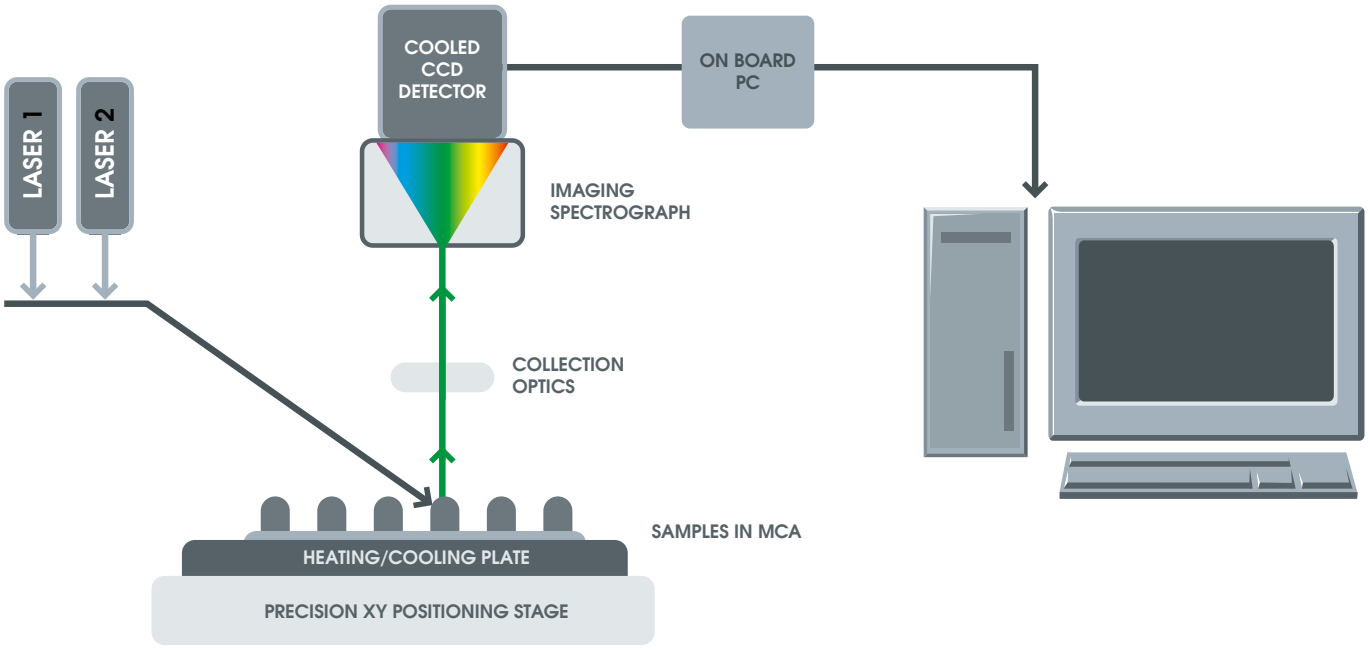
Unlike conventional scanning spectrographs, the Optim 1000 is able to acquire an entire spectrum in a single exposure. This means that data acquired using multiple optical analytical techniques can be acquired simultaneously and rapidly.

Multimodal Sample Analysis

Optim 1000 is equipped with two laser sources to enable simultaneous measurement of intrinsic protein fluorescence, static light scattering and extrinsic fluorescence from a range of probe dyes. These various optical analyses provide complimentary information and greater insight into protein behaviour than a single measurement type.

Some example data is presented in figure 1 and the system is illustrated schematically in figure 2.

Figure 2. *Schematic illustration of Optim 1000 system configuration.*



Protein Intrinsic Fluorescence to Measure Protein Conformational Stability

The higher order structure of proteins is absolutely critical to their biological function. Stresses such as elevated temperatures or extreme pH can result in the higher order structure of a protein distorting or unfolding, such that the protein no longer functions and increasing the chance of the protein aggregating. The Optim 1000 uses the protein's natural intrinsic fluorescence emission as a label-free monitor of higher order structure and can be used to explore the effects of stress or formulation on this.

In particular, Optim 1000 can be used to obtain a quantitative measure of the temperature (T_m) or denaturant concentration ($[D]_{1/2}$) at which a protein unfolds allowing the conformational stability of different proteins and/or formulations to be compared. (See figure 3 overleaf)

The Use of Static Light Scattering to Monitor Protein Aggregation

The static light scattering (SLS) signal is recorded from the samples in the MCA in order to detect the presence of aggregates. The intensity of the scattered light is sensitive to even low levels of aggregation. Two laser sources may be used to increase the dynamic range of the measurement so that both the early onset of aggregation and more severe aggregation may be measured simultaneously. The light scattering can indicate aggregation caused by, for example, formulation conditions, freeze thaw cycles or periods of time at elevated temperatures. The temperature at which the protein starts to aggregate (T_{agg}) or the time taken for the protein to aggregate after being subjected to a fixed temperature can be measured and used as metric of a given protein or formulation's resistance to aggregation.

Intrinsic protein fluorescence:
A sensitive, rapid and label free probe of conformational stability.

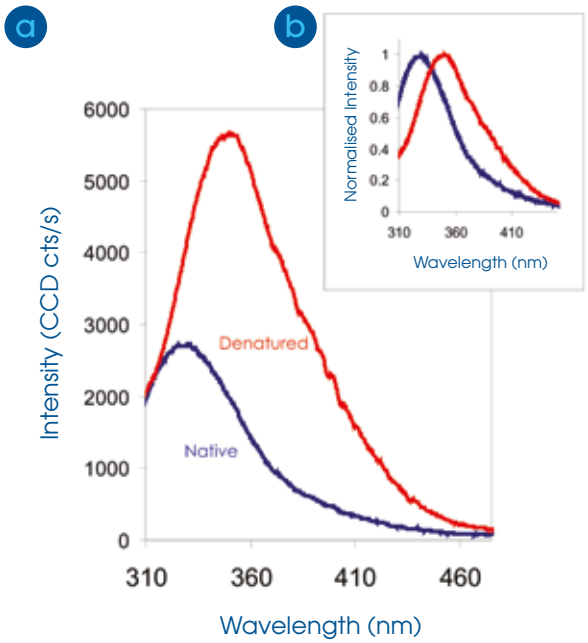


Figure 3: *The effect of unfolding on protein intrinsic fluorescence spectrum recorded in Optim 1000.*
(a) *Comparison of spectrum from native (blue) and denatured (red) IgG sample.*
(b) *Normalised spectra to highlight peak shift on unfolding.*

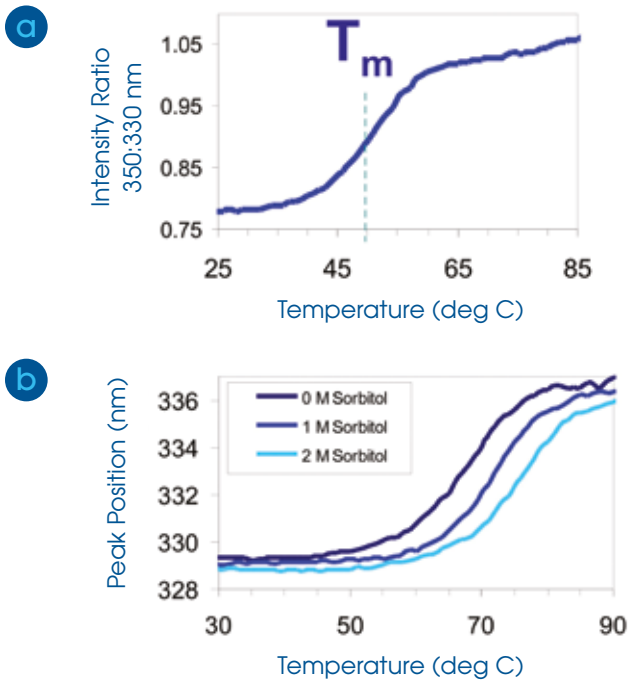


Figure 4: *Optim primary analysis data obtained from spectra recorded as a function of temperature.*
(a) *Ratio of fluorescence intensities at 350 nm and 330 nm as a function of temperature. Transition temperature, T_m, is indicated.*
(b) *Thermal unfolding curves for IgG sample with increasing concentrations of added sorbitol. Stabilising effect of sorbitol shown by shift of unfolding transition to higher temperature.*

Static light scattering:
A sensitive, rapid and label-free monitor of protein aggregation.

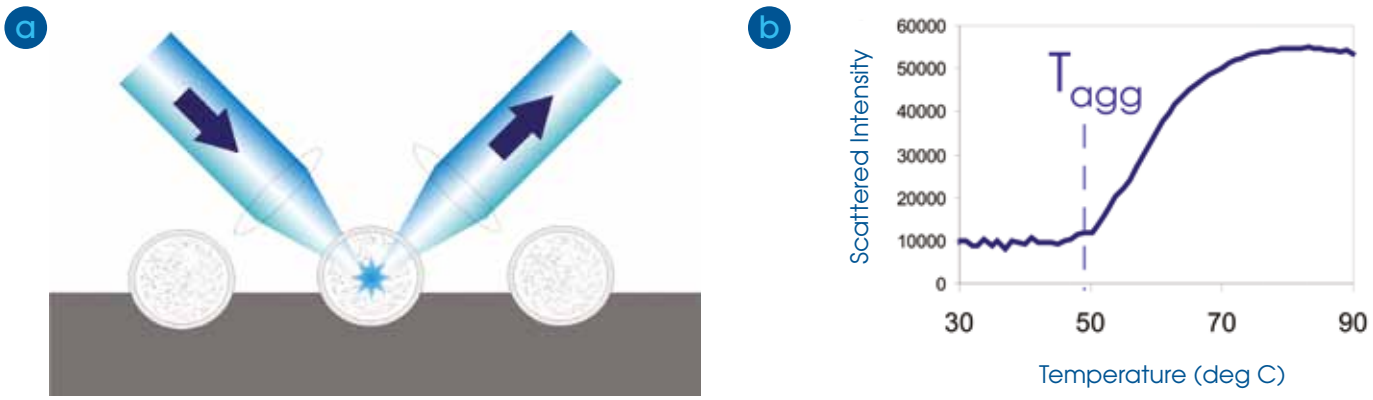
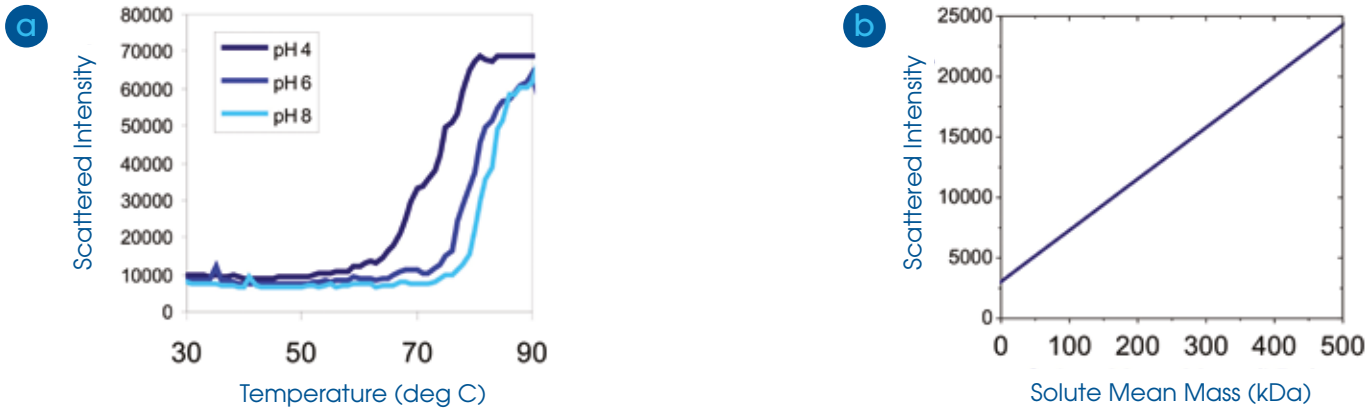


Figure 5:
(a) *Schematic illustration of 90° illumination-collection configuration for light scattering.*
(b) *Example thermally induced aggregation curve showing aggregation onset temperature (T_{agg}).*

Figure 6:
(a) *Thermally induced aggregation of IgG samples as a function of solution pH monitored with static light scattering.*
(b) *Static light scattering intensity as a function of protein molecular weight at a concentration of 1 mg/ml highlighting sensitivity of Optim's light scattering function.*



Using the Optim 1000

Loading the samples into the instrument: The spacing of the cuvettes in the Optim 1000 MCAs is compatible with a standard 384 well plate allowing quick and convenient loading of the samples with a standard 16 channel pipette. Up to three MCAs each containing sixteen samples can be loaded into the instrument.

Load or Input sample Information: Sample information can be input directly into the Optim software or imported from a previously prepared Microsoft® Excel® spreadsheet.

Select or set up experiment: The user can select from one or more pre-programmed experimental protocols or define a new experimental protocol. The instrument may then be left to automatically run and acquire data, with the acquired spectra being made available to view 'live' as they are recorded. The sample information, instrument settings and acquired data are all stored together in a searchable data base.

Once the experiment is complete the Optim software provides a range of tools with which to analyse the acquired data.

Optim Software

Full Optim Control: The Optim software includes pre-programmed experimental protocols for isothermal and temperature ramped protein analysis. These protocols can be modified to suit individual protein or experimental needs.

Data Analysis: The data analysis capability built into the Optim software extracts a range of relevant parameters from the raw experimental data and plots these as function of temperature or time. The software then determines T_m and T_{agg} from these curves.

Integrated data analysis for efficient distillation of data into knowledge.

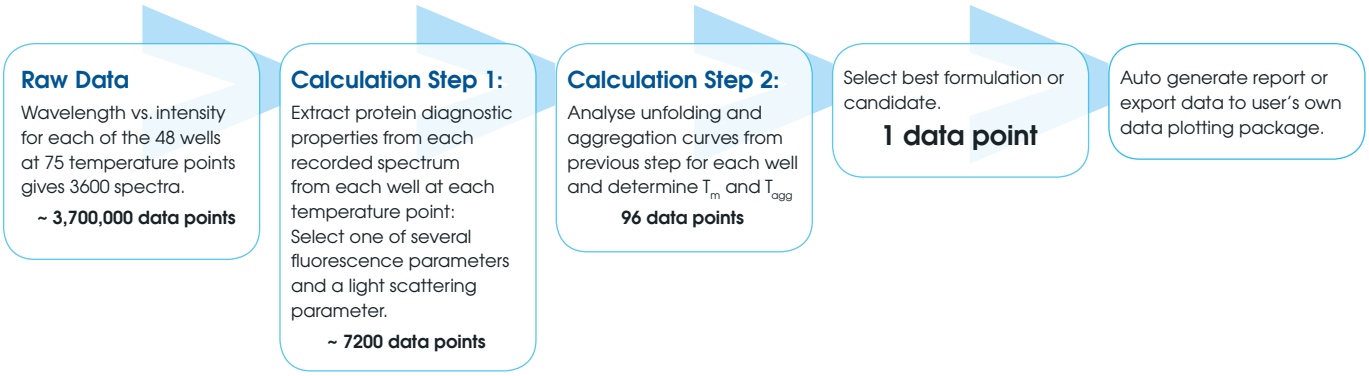


Figure 7: *Schematic Illustration of typical Optim 1000 data analysis workflow.*

Optim Software Continued

Data Storage and Report Generation: The raw and analysed data is stored automatically from all experiments in the Optim database and can be exported into Microsoft® Excel® format or another tab-delimited file format. The software also has the facility to auto-generate a report summarising the experiment and results.

Security: The Optim software is password protected and can be run only by authorised users, each have an administrator configured user profile that defines which experiments they can access, which protocols they can run, whether they can modify protocols and which data they can access, export and analyse.

Optim Experimental Versatility

In addition to measuring parameters such as T_m and T_{agg} the Optim 1000 is capable of obtaining a much wider range of other stability indicating parameters from proteins than alternative technologies.

Label free measurement: No need to add dyes that bind to the protein or aggregates and may affect protein stability or the effect of added excipients.

Denaturant and pH unfolding curves: The instrument provides a convenient and rapid means to obtain 'chemical' unfolding curves by exposing the protein to increasing concentrations of denaturant.

Isothermal measurements: The samples are held at a fixed temperature and the unfolding and aggregation of the proteins is recorded as a function of time. The kinetics of unfolding and aggregation can provide an additional useful insight into protein or formulation stability.

Extrinsic probe dyes: A range of dyes are available to probe protein parameters such as 'hydrophobicity' and aggregation.

Accurate and reproducible determination of protein T_m.

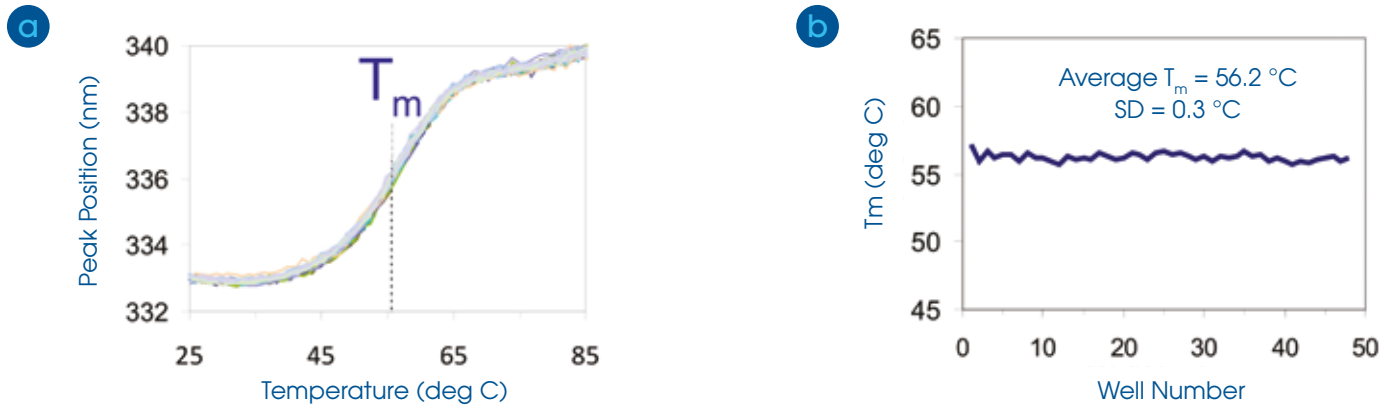
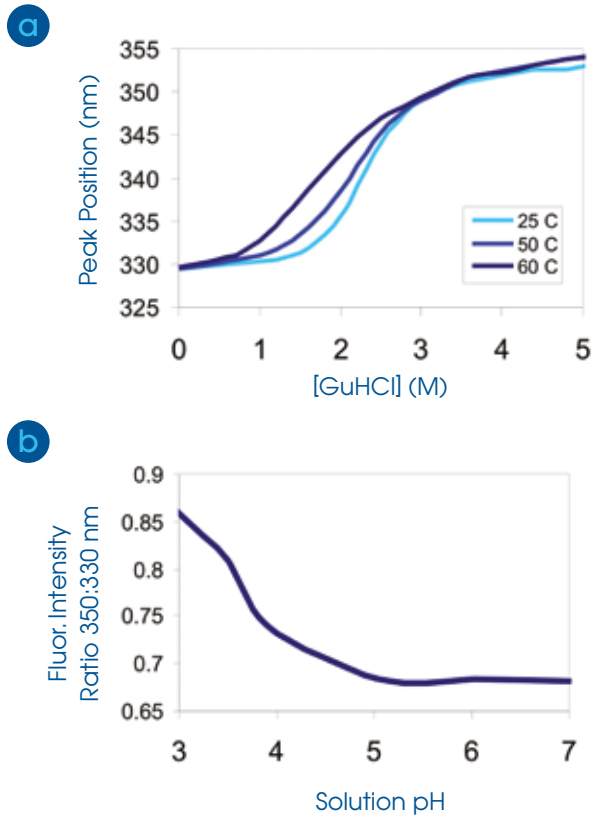
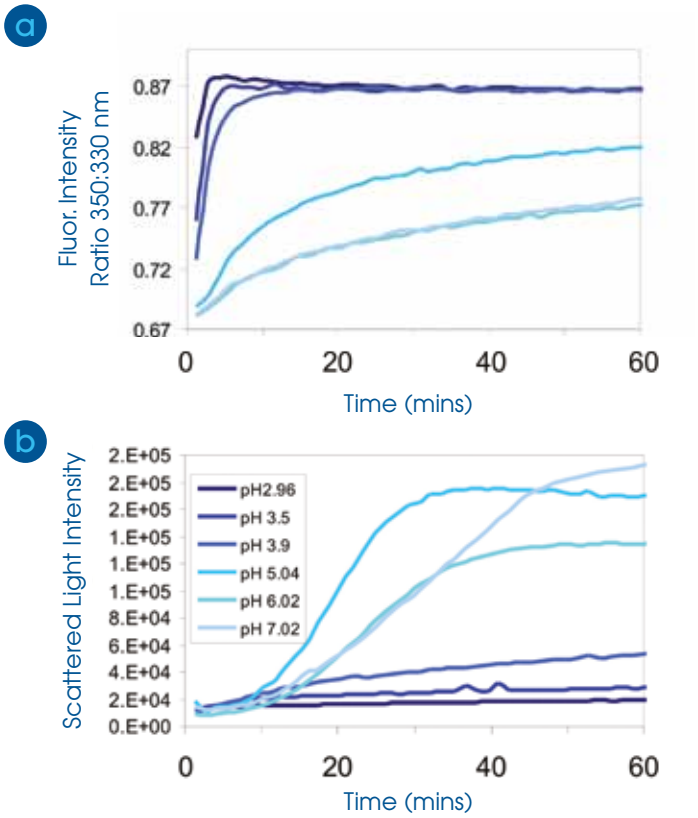


Figure 8:
(a) *Overlay of all 48 unfolding curves from an Optim run where the same IgG sample was placed in all wells showing excellent reproducibility well to well and across the sample plate.*
(b) *Plot showing T_m determined from unfolding curves as a function of well number.*

Measurement of wide range of stability indicating parameters.





Denaturant and pH Unfolding curves.

Figure 9:
(a) *Denaturant (GnHCl) unfolding curves for IgG sample at 3 example temperatures.*
(b) *pH induced unfolding of IgG sample at 25 °C.*

Isothermal (kinetic) measurements.

Figure 10:
(a) *Time dependent unfolding of IgG protein in a range of pH conditions. Sample held at 65 °C. Larger intensity ratio corresponds to increased solvent exposure of hydrophobic residues (loss of tertiary structure). Key to curves shown in (b).*

(b) *Time dependent aggregation of IgG in range of pH conditions recorded simultaneously with data in (a). Increased SLS intensity indicates increased aggregation.*

Optim 1000 Performance in Perspective: A Formulation Study

An example formulation study using 3 buffers, 3 pH levels, 3 ionic strengths and 10 excipients, each running in triplicate, would create a sample matrix of up to 810 samples.

The Optim 1000 can analyse 810 samples, generating 810 T_m and T_{agg} measurements in under six working days, using less than 90 µg of protein. In order to generate the same information alternative high throughput analysis techniques would take over 50 times longer, almost half a year working 24 hours per day, and use more than 30 – 600 times the amount of protein.

PRE-FORMULATION STUDY OF 810 SAMPLES	TIME	PROTEIN SAMPLE
Alternative instrumentation	4000 hours	2.6mg – 54mg
Optim 1000	**74 hours**	**0.081mg**

Optim 1000 is ideal for:

- Candidate screening.
- Optimising formulation parameters.
- Determining the effects of process holds and elution conditions as part of process optimisation.
- Measuring protein stability during freeze-thaw cycles, mechanical stress testing and other forced stability trials.

Optim 1000 has been developed in order to bring preformulation and formulation forwards in the drug development process, providing dramatic time and cost savings, reducing risk and improving product performance.

Contact Avacta to see how Optim 1000 can help you to deliver faster, better and cheaper drug development.

SPECIFICATION	
Optim micro-cuvette array (MCA)	Single use micro-cuvette array with 16x micro-cuvettes. Instrument accommodates up to 3 MCAs (48 samples). Available in 1 µl or 9 µl volume versions. Micro cuvette pitch compatible with 384 well plates and standard 16 channel pipettes.
Minimum Protein concentration	Protein dependant. For typical IgG < 0.1 mg/ml.
Detector	TE cooled, scientific grade CCD camera, 1024 x 256 pixels. Operational wavelength range ~250 – 1000 nm .
Imaging Spectrograph	Achromatic, aspheric optics f/4.0. Standard grating with optimised efficiency in UV-blue region of spectrum. User selectable centre wavelength. Software controlled motorized entrance slit.
Proprietary Optical Configuration	Optimised for simultaneous high quality multi-modal optical measurements from small sample volumes.
Laser source 1	266 nm laser to excite intrinsic protein fluorescence and for sensitive light scattering.
Laser source 2	473 nm laser for light scattering and excitation of some dyes.
Sample temperature control	TE control of temperature of all 48 samples simultaneously. Temperature range: 10 to 100 °C. Temperature accuracy +/- 0.1°C at room temperature, +/- 0.5 °C at 100°C.
Optim Software	Full instrument control. Integrated data analysis. Database for data storage. Data export and auto report generation.
External Computer	Desktop PC supplied pre-loaded with Optim software. W x D x H 17 cm x 43 cm x 37 cm, Standard LCD monitor, keyboard and mouse.
Physical parameters	Width x Depth x Height 85 cm x 69 cm x 76 cm (fits on standard 60 cm deep bench) Weight 75 kg
Environmental conditions	Temperature range: 18 to 28 °C. Humidity: 40 to 60% relative humidity (non-condensing). Temperature and humidity should be kept constant during operation.
Electrical	Factory set at one of the following options:

Voltage	Frequency	Current Drawn	Fuse rating	Max Power
220 – 240 V AC	50-60 Hz	8-9 A	10 A	2 kW (VA)
110 – 125 V AC	50-60 Hz	16-18 A	20 A	2 kW (VA)

Specifications are subject to change without notice.

About Avacta Analytical

Avacta Analytical provides innovative analysis and detection solutions to the biopharmaceutical, pharmaceutical and healthcare industries through leveraging its broad ranging expertise in analytical techniques and protein science.

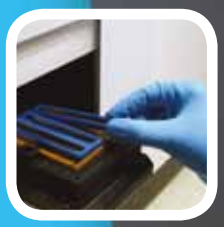
Avacta Analytical's unique skill lies in making advanced analytical technology more broadly accessible through a range of market-centric products and services. Our primary focus is to equip pharmaceutical developers and manufacturers with tools to get their products to market more quickly, at reduced cost, and to optimise the critical-to-performance properties of these new drugs.

Avacta Analytical's products and services make it possible for drug developers to analyse their compounds in much greater detail at an earlier stage in the drug development pipeline than is now possible, thereby providing timely identification of problems that can cause costly late stage failures or poor product performance.

Based in York, UK, Avacta Analytical is a wholly-owned subsidiary of Avacta Group plc, and occupies purpose built laboratories within the Biocentre situated in York Science Park.

Avacta Analytical is ISO9001 registered.





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