Optim® 2

High throughput protein analysis instrument for stability studies

Optim is a Registered Community Trademark
Introduction to Optim® 2

The Optim 2 from Avacta Analytical is a compact and robust, high throughput microvolume protein analysis and characterization instrument, that combines data from multiple analytical methods to generate a wide range of stability-indicating parameters.

The Optim 2 provides considerable benefits, allowing you to take advantage of the following stability indicators earlier in your decision-making process in order to build a comprehensive picture of, and provide greater insight into, the stability of a protein or formulation:

- thermal unfolding midpoint ($T_m$);
- onset temperature of unfolding;
- rate of unfolding;
- aggregation onset and rates of aggregation;
- mean molecular mass and relative degree of aggregation;
- relative degree of tertiary structure.

Reducing development risk

Taking the total investment for all candidates, both successes and failures, into account, the cost of bringing a new drug to market varies from between 3 and 12 billion US dollars. A solution that can potentially reduce this risk of failure has significant value to the industry and biopharmaceutical developers.

The Optim 2 platform is just that solution, presenting a new approach to biopharmaceutical stability screening that significantly reduces development risk associated with stability-related failure. By screening candidates for stability with the Optim 2 as part of a preformulation candidate selection, or as part of the formulation of the drug substance and drug product, you can help to predict which candidate and which formulation is a low risk development opportunity. Used as part of a process development strategy, the Optim 2 can also help to predict potential losses of yield that might occur during a scaled-up manufacturing process, and guide the selection of processing conditions, such as prefiltration requirements or excipient choices.
The Optim 2 provides multiple measurement modes probing different aspects of protein stability from 48 micro volume samples at high throughput, using 30 times less sample and performing analytical experiments 30 times faster than classical instrumentation for thermal stability and aggregation analysis.

For example, a typical formulation project lasting three months might include one month of data generation and two months of data analysis. Classical non-automated instrumentation would be able to perform around 70 experiments in this time, generating basic aggregation onset and thermal unfolding data. Automating this process with instrumentation running 24 hours a day would increase this to 260 experiments. In contrast, the Optim 2 can perform approximately 1,890 experiments in the same time, running during typical normal working hours only.
How the measurement is made
A temperature ramp is applied to the sample while the conformation of the protein is monitored by observing changes in the intrinsic fluorescence spectrum (label free). The midpoint temperature of the unfolding transition is described as the unfolding ‘transition midpoint’ or $T_m$.

How the measurement is used
Unfolded molecules in solution are generally highly prone to aggregation and are expected to lose functionality. Molecules or formulations which have a high $T_m$ are expected to display a greater resistance to unfolding and thus have a reduced chance of forming aggregates or losing efficacy during manufacture, storage and use. $T_m$ is widely used as a screening measurement of molecule or formulation stability.

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Thermal ramp aggregation experiments to measure protein aggregation stability

How the measurement is made
A temperature ramp is applied to the sample and light scattering is used to monitor the thermally-induced aggregation of the protein. The temperature at which the protein begins to aggregate, the $T_{agg}$, and the subsequent magnitude of the aggregation can be observed.

How the measurement is used
The temperature at which the protein begins to aggregate is often, but not always, correlated with the temperature at which the protein begins to unfold and with the rate at which the proteins aggregate – typically a high $T_{agg}$ is preferred. The magnitude of aggregation after onset gives an indication of the propensity of unfolded proteins to aggregate – typically lower levels of aggregation are preferred.
How the measurement is made
The samples are held at a fixed temperature and rates of unfolding and aggregate formation are monitored using intrinsic fluorescence and light scattering.

How the measurement is used
The time-dependant unfolding gives a useful orthogonal measure of the susceptibility of a protein to unfold – molecules or formulations which unfold very slowly may be preferred. Refolding kinetics may also be studied. Rates of aggregation at elevated temperature give an indication of the propensity of unfolded protein molecules to aggregate – slow rates of aggregate formation are preferred.

Different concentrations of excipients (GuHCl) can cause unfolding to occur at different rates.

How the measurement is made
The samples are held at a fixed temperature and rates of unfolding and aggregate formation are monitored using intrinsic fluorescence and light scattering.

How the measurement is used
A thermodynamic function is fit to the data and an indication of the relative stability of the folded to unfolded protein is obtained, as well as a relative measure of the solvent accessible surface area.

GuHCl affects the tertiary structure composition of IgG at 25 °C.

How the measurement is made
The Optim 2 may be used as a general, highly sensitive, very small sample volume plate-based light scattering and fluorescence instrument.

How the measurement is used
The Optim 2 may be used for high throughput analysis of protein samples subjected to accelerated stress conditions outside the instrument, such as freeze/thaw or shear forces. Intrinsic fluorescence gives information about loss of conformational structure, while light scattering data monitors aggregate formation. A range of extrinsic fluorescent probe dyes may also be used.

Three probes to monitor unfolding, self-association and fibrillation of human insulin.
Inside the Optim® 2

Speed and power, yet sensitive and intelligent
The Optim 2 uses light scattering and fluorescence technology in a configuration never seen before, featuring a sophisticated optical measurement system and a proprietary disposable sample holder. This outstanding solution uses two separate analytical techniques to provide at least five different metrics of stability simultaneously from forty eight 9 µl samples.

Speed and power
- Parallel sample temperature control – uses a thermal ramp to generate the transition temperatures of up to 48 samples in parallel.
- Multiple lasers for multiple measurements – different lasers provide different sensitivities of light scattering and excite different optical probes, a 266 nm laser excites intrinsic fluorescence for conformational measurements, a 473 nm laser excites multiple dyes for complementary information.
- Rapidly responding and tightly controlled temperature – great reproducibility and confidence that your samples are at a specified temperature.

Sensitive and intelligent
- Proprietary micro cuvette array – innovative sample presentation to provide sensitive measurement of light scattering and fluorescence from small volume samples.
- Laser excitation for tighter optical control – no need to change lamps or worry about slow and inefficient monochromators typical with spectroscopy-based solutions.
- Achromatic laser focus system – equally good performance, whatever your application.
- Cooled spectrograph for better signal-to-noise ratio – high performance detector generating a full spectrum in a single shot for rapid data acquisition.
Optim 2 software is easy to use, with exceptional functionality and performance, and is comprised of two packages:

**Optim Client software** provides instrument control and experimental set-up functionality, focusing on application rather than spectroscopy and providing a solution rather than a tool.

**Optim Analysis software** is a stand-alone data analyser that brings the potential of off-site and multi-seat flexibility to the Optim 2.

**Optim Client software** allows you to choose from a selection of the most frequently used pre-programmed protocols and analysis algorithms, as recommended by our customers or, alternatively, a completely independent advanced focus allows you to design your own experiments and analyses.

Whichever approach you take, you can use a simple, flowthrough methodology to quickly achieve your goal, featuring an intuitive and user-friendly workflow with just nine mouse clicks from turning the instrument on to starting to measure samples.

Easy-to-use **Optim Analysis software**, featuring drag-and-drop sample management, allows you to automatically analyse trends between different components or conditions of your samples. **Optim Analysis software** comes as standard with the instrument and is able to automatically process data, assigning thermal midpoints and aggregation onset values, as well as calculating, averaging and analysing trends in your data. This level of automated analysis allows you to quickly process large amounts of data and leaves you free for higher value tasks, such as interpreting results and drawing conclusions.
About
Avacta Analytical

Biopharmaceutical scientists need to equip customers with a broad range of capabilities, enabling them to develop their therapeutics quicker, cheaper and better.

The ground-breaking Optim 2 instrument combines the powerful analytical capabilities of fluorescence and static light scattering technologies to provide valuable new insights.

Our services business specialises in supporting characterisation, formulation, comparability and stability studies of therapeutic proteins. We are able to draw upon a wide range of analytical capabilities and aim to give our customers a flexible value-added service.

Avacta Analytical is an ISO 9001 2008 registered company.

### SPECIFICATION*

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum sample volume</td>
<td>9 µl</td>
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<tr>
<td>Simultaneous samples per experiment</td>
<td>48</td>
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<tr>
<td>Heating rate</td>
<td>0.01 – 10 °C/minute (relevance is sample dependent)</td>
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<tr>
<td>Protein concentration range</td>
<td>Protein dependent: 0.1 mg/ml – 150 mg/ml (typical IgG)</td>
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<tr>
<td>SLS sensitivity</td>
<td>12 kDa.mg/ml – 22500 kDa.mg/ml (Dextran and IgG)</td>
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<tr>
<td>SLS precision</td>
<td>&lt;20 % CV</td>
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<tr>
<td>SLS resolution</td>
<td>~15 kDa mean molecular mass</td>
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<tr>
<td>Detector</td>
<td>Spectrometer 250 – 720 nm spectral range</td>
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<tr>
<td>Sample temperature range</td>
<td>15 – 95 °C</td>
</tr>
<tr>
<td>Sample temperature accuracy</td>
<td>~1 °C (&lt;70 °C) ~1.5 °C (&gt;70 °C)</td>
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<tr>
<td>Sample temperature precision</td>
<td>± 0.3 °C (SD, 48 identical samples, Tm~42 °C)</td>
</tr>
<tr>
<td>Environmental conditions</td>
<td>Temperature range: 18 – 28 °C Humidity: 40 to 60 % relative humidity (non-condensing) Environmental conditions must be kept constant to maintain calibration</td>
</tr>
<tr>
<td>Physical parameters</td>
<td>Dimensions: width x depth x height 54 cm x 50 cm x 58 cm Weight 46 kg</td>
</tr>
<tr>
<td>Electrical</td>
<td>Auto switching power supply Voltage 110 – 240 V ac, 50 – 60 Hz Fuse rating 6 A anti-surge Max power 600 W</td>
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*S*pecifications are subject to change without notice.

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