CEITEC
MASARYK UNIVERSITY

VP-DSC calorimeter

Differencial Scanning Calorimetry (DSC)

VP-DSC calorimeter measures heat changes that occur in the sample (biomolecule solution) during a controlled increase or decrease in temperature, on the basis of a temperature difference between the sample and the reference material. It is a valuable technique for the study of samples in solution providing fast and accurate determination of the transition midpoint T_m - when 50% of the biomolecule are unfolded. In addition, a complete thermodynamic profile is generated to understand the factors that affect conformation and stability. DSC is a sensitive, easy-to-use technique that requires no assay development, labelling or immobilization.

DCS method can be used for

- characterization of the stability of proteins or other biomolecules, for elucidation the factors that contribute to the folding and stability of native biomolecules, including hydrophobic interactions, hydrogen bonding, conformational entropy, and the physical environment
- characterization of membranes, lipids, nucleic acids and micellar systems
- assessment of the effects of structural change on a molecule's stability - protein engineering or antibody domain studies
- determination of the transition midpoint T_m, enthalpy (ΔH) of unfolding due to heat denaturation, also the change in heat capacity (ΔCp) of denaturation can be determined

Technical Specifications

Instruments: VP-DSC (Malvern)

Features:

- operating temperature range is of -10°C to 130°C
- calorimetric cell - volume 500 ml, tantalum, coin-shaped

Assemblies:

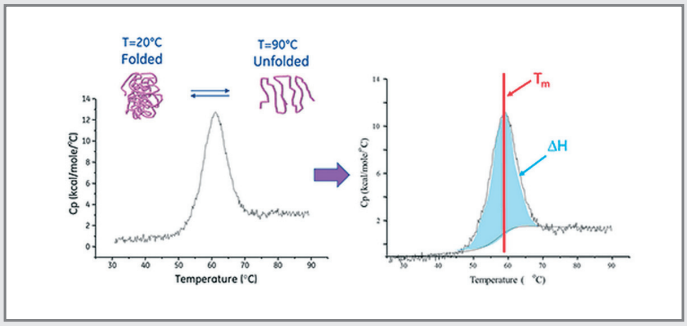
- ThermoVac - device for thermostatting and degassing samples (0-80°C)

Operational mode:

VP-DSC measurement is performed manually by the user itself after training

Data evaluation software:

Origin software (possibility to train people in data processing)



Provided services:

- instrument user training
- basic DSC data evaluation training
- consulting/assistance

Data collection:

- Conventional DSC - mode uses a linearly increasing or decreasing temperature ramp function, while measuring the differential. Scanrates fall in the range of 0°C/hr to 90°C/hr in the upscan mode and 0°C/hr to -60°C/hr in the downscan mode.
- Isothermal Scan Mode – a constant temperature is maintained for a relatively long period of time while measuring the differential power between the reference cell and sample cell.

Sample requirements - importance of sample preparation

- **Filling of the cell is crucial for the accuracy.**
- Typical sample concentration: 0.1 - 2.0 mg/ml
- Proper sample preparation is crucial for the successful DSC measurement. **Sample buffer and buffer for filling the reference cell should be exactly the same** (dialysis or lyophilisation and dissolution in the buffer for DSC). The pH should be checked before the measurement.
- The sample for filling the sample cell: 800 µl
- The buffer for filling the reference cell: 800 µl
- If the reducing agent is needed in the sample, usage of up to 5 mM b-mercaptoethanol (or TCEP) instead of DTT is recommended.
- Fluoride compounds can cause irreparable damage of the VP-DSC cell, therefore it is not possible to measure samples containing fluorides.
- Precipitation and aggregation can cause a rapid downward shift or an increase in baseline noise after the system unfolds. **Minimizing precipitation is necessary for accurate result**.

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

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