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UV-1900i/UV-2600i/UV-2700i UV-Vis Spectrophotometers

Application News

Thermal Stability Analysis of Nucleic acid Drugs by New Tm Analysis System

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User Benefits

- Easily determine the temperature at which 50 % of double-stranded nucleic acids dissociate into single strands (Tm value).
- Automate annealing (pretreatment) and Tm value analysis using the average and derivative methods.
- Achieve the industry's highest data integrity when linked to the LabSolutions™ DB/CS system.

■ Analytical Method

Nucleic acid drugs are a generic term for drugs made of oligonucleic acids consisting of a dozen or more bases of nucleic acid or modified nucleic acid linked together and manufactured by chemical synthesis. Nucleic acid drugs have been actively developed in recent years as next-generation innovative therapeutics because they can target molecules such as mRNA that conventional low-molecular-weight drugs and antibody drugs cannot.

Melting temperature (Tm value), an indicator of thermal stability, plays an important role in nucleic acid drug development and quality control. The Tm value is the temperature at which 50 % of double-stranded nucleic acids dissociate into single strands, and generally the higher the temperature, the more thermally stable it is.

In this study, we performed thermal stability analysis (Tm analysis) of nucleic acids using the UV-2600i UV-visible spectrophotometer and a new Tm analysis system. Using this system enables the Tm value of nucleic acids to be easily calculated.

■ Tm Analysis System

The Tm analysis system comprises a UV-visible spectrophotometer*1 shown in Fig. 1, a TMSPC™-8i 8-cell thermoelectrically temperature-controlled cell holder, and the LabSolutions UV-Vis Tm software shown in Fig. 2. Tm analysis is a method of analysis in which the temperature of the nucleic acids is increased, and the change in absorbance (melting curve) is measured to determine the Tm value. The procedure used to determine the Tm value is (1) check the absorption spectrum, (2) anneal the sample, (3) measure the melting curve, and (4) perform analysis as shown in Fig. 3. However, using this system, the work from annealing (2) to analysis (4) can be automated using the same software, which greatly improves efficiency. In addition, data management is easy because the absorption spectrum (1), the melting curve (3), annealing (2), and analysis conditions (4) are stored as a single file. Linking this system with the Shimadzu LabSolutions DB/CS system further improves data integrity.

*1: Fig. 1 shows UV -2600i and TMSPC-8i, but it can also be combined with UV-1900i/ 2700i.

Fig. 1 UV -2600i and 8-Series Thermoelectrically-Controlled Cell Holder TMSPC™-8i

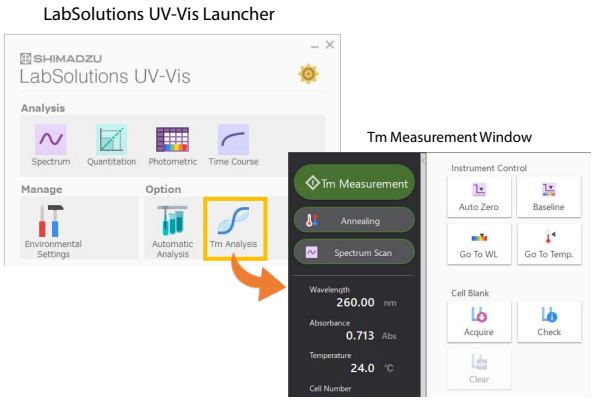

Fig. 2 LabSolutions™ UV-Vis Tm Software

Fig. 3 Tm Measurement Procedure

■ Analytical Method

This study used the nucleic acid M13 primer, prepared at 12 µM in buffer (17 mM NaCl, 10 mM phosphate buffer) as the sample. The sample solution was degassed beforehand to prevent air bubbles from forming during measurements at high temperature. Two optical pathlengths, 10 mm and 1 mm, are available with the dedicated 8-cell micro multi-cell and can be selected depending on the sample absorbance (concentration). In this experiment, a cell with a 1 mm optical pathlength was used. The samples were measured under the conditions shown in Table 1.

Table 1 Measurement Conditions

Instruments:	UV-2600i TMSPC-8i
Measuring Wavelength:	260 nm
Measuring Wavelength (for Calibration):	320 nm
Slit Width:	2.0 nm
Temperature Range:	15 – 90 °C
Acquisition Rate:	1 °C
Temperature Changing Speed:	1 °C/min

Fig. 4 shows the parameter setting window. To perform annealing, set the temperature from panel (1). Click the "Settings" button for the temperature program in panel (2) and enter parameters such as start and target temperatures and waiting time on the temperature program window (Fig. 5). This window allows the operator to simulate the temperature program and check how much time is required. To automatically analyze Tm values after measurement, the analysis method can be set in panel (3). The analysis methods available are the average method, which determines the Tm value from the intersection of the midline of the tangent to the melting curve, and the derivative method, which determines the Tm value from the maximum value of the first derivative. All the conditions set here can be saved as a template, eliminating the need to set up the conditions again the next time.

After setting the parameters, the entire process, from annealing to measurement and analysis, can be performed automatically by clicking the "Tm Measurement" button.

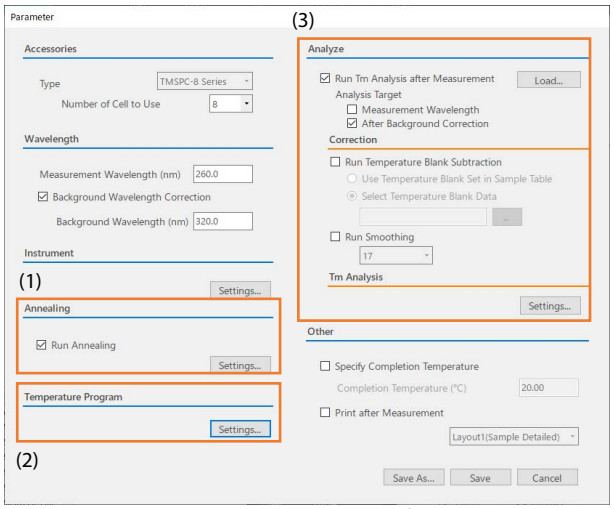

Fig. 4 Parameter Setting Window

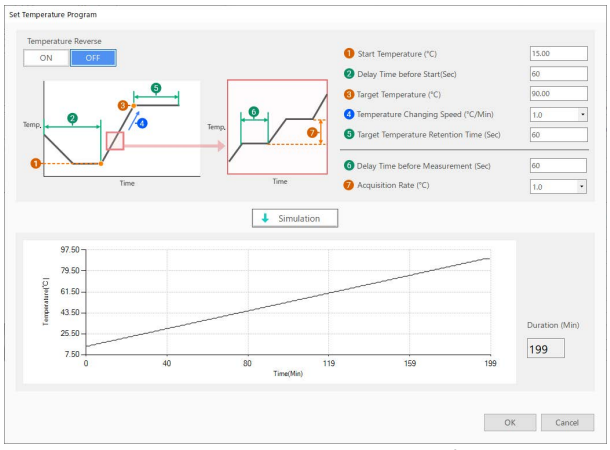

Fig. 5 Temperature Program Setting Window

■ Measurement Results

Fig. 6 shows the absorption spectrum of the sample. Tab switching ((4) in Fig. 6) allows the user to switch between the absorption spectrum and the melting curve and check them.

Fig. 7 shows the melting curves analyzed by the two methods, the average method and the derivative method, and Table 2 shows the Tm values calculated by each analysis method.

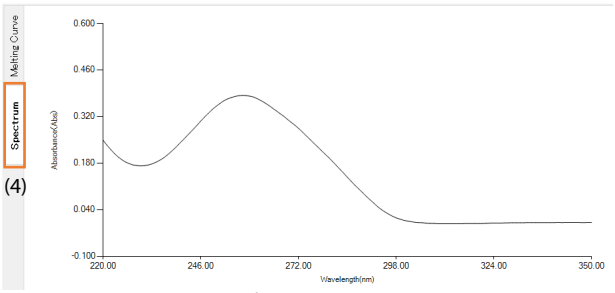

Fig. 6 Absorption Spectrum

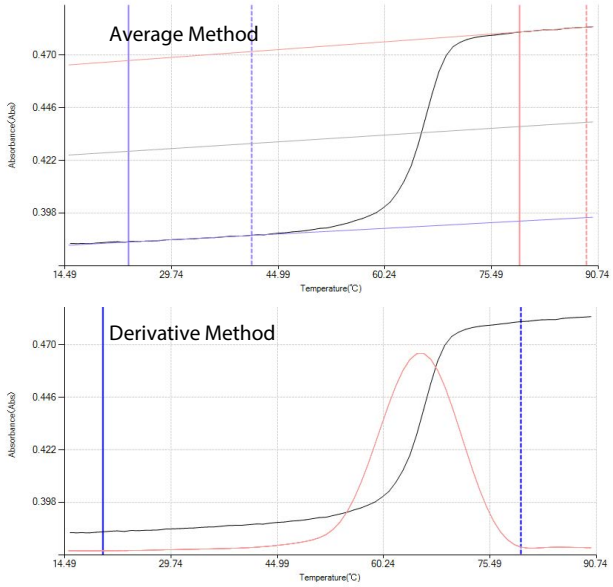

Fig. 7 Melting Curve

Table 2 Measurement Results of the Sample

Analysis Method	Tm value (°C)
Average Method	65.47
Derivative Method	65.98

As shown in Table 2, the Tm values obtained by the average method were 65.47 °C, and those obtained by the derivative method were 65.98 °C. The Tm values obtained by both methods were very close.

■ Conclusion

In this study, we performed thermal stability analysis (Tm analysis) of nucleic acids using a UV-2600i UV-vis spectrophotometer and the Tm analysis system. This system can calculate the Tm value of nucleic acid drugs efficiently and easily because the process from annealing to analysis can be performed automatically.

This system, which streamlines the procedure for determining Tm values, is expected to dramatically accelerate the development of nucleic acid drugs.

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