SHIMADZU
Excellence in Science

Application News

No. A618

Spectrophotometric Analysis

Thermal Stability Analysis of Nucleic Acid Drugs by TMSPC™-8 Tm Analysis System

Functional nucleic acids, beginning with nucleic acid drugs, have attracted considerable attention in recent years. In the development and evaluation of these functional nucleic acids, it is essential to understand their thermal stability, as this is a factor that controls their structure and functions. Thermal stability analysis is also indispensable in techniques based on hybridization of nucleic acids such as PCR and DNA microarray.

In this article, a thermal stability analysis (Tm analysis) of a nucleic acid was conducted using a Shimadzu UV-1900i ultraviolet-visible (UV-Vis) spectrophotometer and TMSPC-8 Tm analysis system. Simple calculation of the Tm value of nucleic acids is possible by using this system.

A. Goto

Thermal Behavior of Nucleic Acids

Nucleic acids (DNA, RNA) have a double-stranded helix structure, but the hydrogen bonds that join the strands are broken when the temperature is raised, and the double-strand structure gradually dissociates and forms a single-strand structure. This phenomenon is called "melting" of nucleic acids, and the temperature at which the ratio of the single strand to the double strands becomes equal is defined as the melting temperature Tm. Tm is an index of the thermal stability of nucleic acids, and depends on various factors such as the base sequence, nucleic acid concentration, and mismatch (non-complementary base pairs).

It is known that nucleic acids have an ultraviolet absorption peak at around 260 nm, and their absorbance at 260 nm increases when melting occurs (Fig. 1). The Tm analysis system of the spectrophotometer determines the Tm value by measuring this change in absorption.

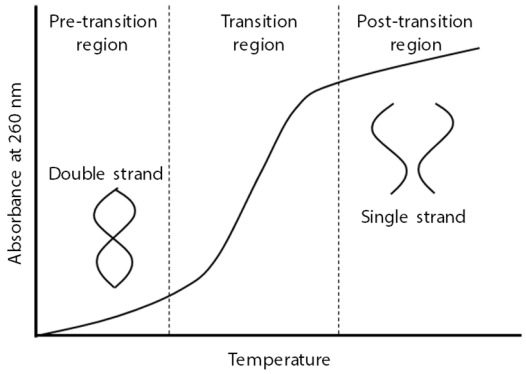

Fig. 1 Melting Curve of Nucleic Acid

TMSPC-8 Tm Analysis System

Fig. 2 shows the appearance of the UV-1900i and TMSPC-8 Tm analysis system. This system comprises an 8-series thermoelectrically-controlled micro cell holder, a dedicated 8-series micro multi-cell (pathlengths: 10 mm, 1 mm), a temperature controller, and Tm analysis software, and enables simultaneous measurement of a maximum of 8 samples.

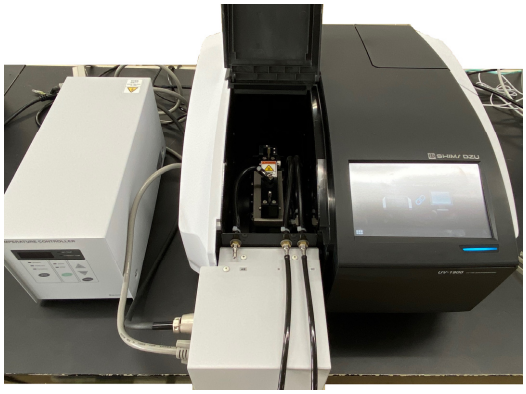

Fig. 2 UV-1900i and TMSPC™-8 Tm Analysis System

Tm Analysis of Nucleic Acid by TMSPC-8

In this experiment, the nucleic acid M13-25mer was used as the sample.

As pretreatment, the sample solution was degassed, as bubbles will form at high temperature if air is dissolved in the sample solution, making accurate measurement impossible. Annealing is also necessary to ensure complete formation of a double-stranded structure of the sense and antisense strands. Here, annealing was carried out by holding the samples at 95 °C for a minimum of 10 min, followed by cooling at a rate of 2 °C/min.

Two optical pathlengths, 10 mm and 1 mm, are available with the dedicated 8-series micro cell, and can be selected depending on the sample absorbance (concentration). In this experiment, the samples were prepared so as to have absorbance of 0.5 to 1 at 260 nm, and the cell with the 10 mm pathlength was selected (Fig. 3). The measurement was conducted under the conditions in Table 1.

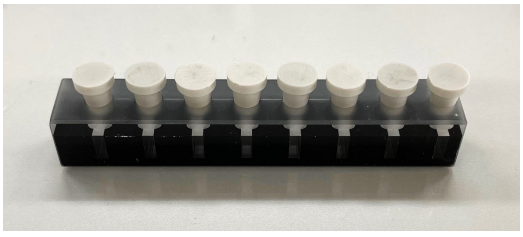

Fig. 3 Dedicated 8-Series Micro Cell (Pathlength: 10 mm)

Table 1 Measurement Conditions

Instruments	: UV-1900i, TMSPC-8 Tm analysis system
◆ Measurement parameters	
Wavelength	: 260 nm, 320 nm*1
Slit width	: 1.0 nm
Cell blank correction	: Enabled
◆ Temperature parameters	
Start temp.	: 15 °C
Start retention time	: 1,800 s
Ramp rate	: 1.0 °C/min
Measurement wait time	: 45 s
Measurement interval	: 0.5 °C
End temp.	: 95 °C

*1 Absorbance at 320 nm, which is not absorbed by nucleic acids, was subtracted as the baseline in order to mitigate variations in the baseline of each cell and obtain data with fewer variations.

Fig. 4 shows the melting curves obtained by the Tm analysis (curves in which absorbance at 260 nm is plotted against temperature). The figure shows the results for both temperature ramp-up and ramp-down. From Fig. 4, it can be understood that absorbance increases as the temperature rises. Moreover, the melting curves can be divided into three regions from the low temperature side, i.e., the pre-transition region, transition region, and post-transition region. Larger ratios of the double-strand structure and the single-strand structure exist in the pre-transition region and the post-transition region, respectively.

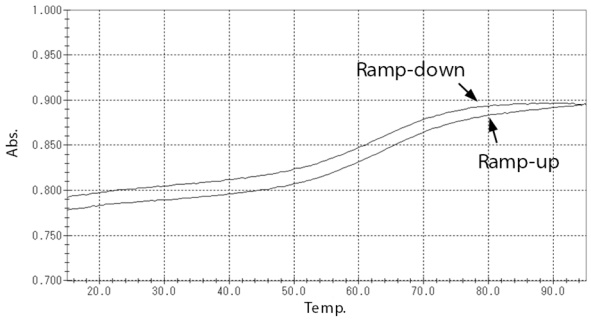

Fig. 4 Melting Curves of Samples

The Tm value was calculated by two calculation methods, the average method and the derivative method. Table 2 shows the results of Tm value obtained by these two analysis methods. Similar results were obtained for ramp-up and ramp-down, respectively.

Table 2 Results of Tm Analysis

Calculation method	Tm value (°C)	
	Ramp-up	Ramp-down
Average method	63.58	63.56
Derivative method	63.68	64.10

In the average method, tangents are drawn in each selected section in the pre-transition region and the post-transition region, and the point of intersection of the median between those two tangents and the absorbance curve is calculated as the Tm value (melting temperature). The pre-transition region and the post-transition region were set arbitrarily as shown in Fig. 5, and the Tm value was obtained.

On the other hand, in the derivative method, a first derivative calculation is made for each point number set in each determined section, and the position which shows the largest value is calculated as the Tm value (melting temperature). In this case, the Tm value was obtained by setting the judgment regions arbitrarily as shown in Fig. 6.

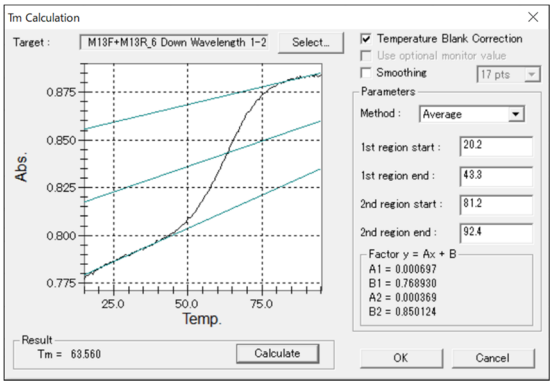

Fig. 5 Analysis by Average Method

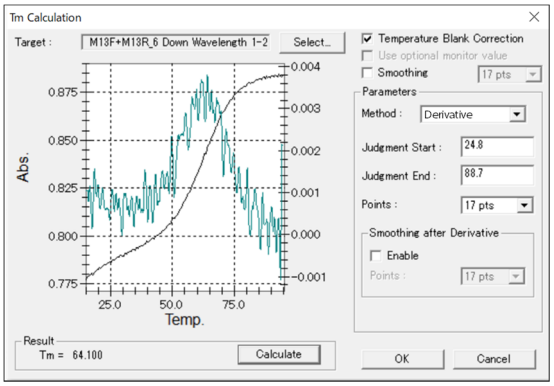

Fig. 6 Analysis by Derivative Method

Conclusion

In this article, a thermal stability analysis (Tm analysis) of a nucleic acid was conducted by using a UV-1900i UV-Vis spectrophotometer and a TMSPC-8 Tm analysis system. With this system, the Tm value is calculated by measuring the transition of absorbance at 260 nm accompanying temperature increase. A maximum of 8 samples can be measured simultaneously by using the dedicated 8-series micro cell.

The Tm values were calculated from the melting curves obtained by the measurements by two calculation methods, the average method and the derivative method. As demonstrated by this experiment, simple calculation of Tm values is possible by using this system.

First Edition: May 2020

Shimadzu Corporation
www.shimadzu.com/an/