Application Note UV VIS

Fields of Application/Industry:

- Chemistry/Polymer Industry
- Clinical Chemistry/Medicine/ Hygiene/Health Care
- Cosmetics
- Electronics
- Energy
- Environment/Water/Waste
- Food/Agriculture
- Geology/Mining
- Material Analysis
- Metallurgy/Galvanization
- Pharmacy
- Refineries/Petrochemistry
- Semi-Conductor Technology
- Others

紫外可见光谱法 DNA 熔点的测定

摘要：

DNA 加热到一定温度时，DNA 的双螺旋结构就会发生裂解，DNA 碱基互补的主要作用力氢键结构就会被破坏，这种变性过程称为 DNA 熔解，通常将 DNA 变性，即增色效应达到一半时的温度称为 DNA 解链温度，也称为熔解温度或者叫做 DNA 熔点(Tm)。最常用和最简单的 DNA 熔解测定是紫外可见吸收光谱法，在紫外光谱法中表现为吸光度的增加（增色效应）。吸收强度与温度关系曲线的拐点即确定的熔点。

本文利用德国耶拿分析仪器股份公司的紫外分光光谱仪 SPECORD200 PLUS 和帕尔贴控温装置，SPECORD200 PLUS 和帕尔贴控温装置相配合，具有控温精度高，升温无延迟等优点，并且根据 DNA 测定应用要求，在不同的计量模式下可以随意编程温度程序。

本文是利用超微量比色皿对质粒DNA水溶液和小牛胸腺两种样本的DNA熔点进行了测定，样品光谱在220nm和300nm处有最大吸收峰。分别采用了‘同步’测量模式和‘循环’测量模式。结果表明，在DNA样本熔化过程可以很好地显示在两个测量模式中。小牛胸腺DNA的吸光度的增加比天然DNA发生温度低。天然DNA通常在85℃以上高温下经常融化，小牛胸腺DNA熔点高于此温度。此外，碱基对比例越高，熔点也会降低。

Determination of DNA Melting Point

Introduction

DNA strands can be separated by heating. The thermal denaturation leads to a simultaneous breaking of hydrogen bonding between the bases where the double-stranded deoxyribonucleic acid unwinds into single-stranded strands. This denaturation process is called DNA melting and leads to increasing of absorbance (hyperchromic effect).

Hence one of the most commonly used and simplest techniques for the DNA melting point determination is spectroscopic determination by UV absorption. The absorption spectrum is recorded against the dependence of the temperature where the turning point of the graph describes the exact melting point. The double helix melts down to two single strands. The temperature, at which half of the DNA exists as single strands, is called melting point (T_m).

Experimental

For DNA melting point determination two different DNA samples were analyzed with SPECORD® 200 PLUS and Peltier cooled cell holder (Fig. 1). The first sample was plasmid DNA, the second sample was an animal DNA from a thymus of a calf.

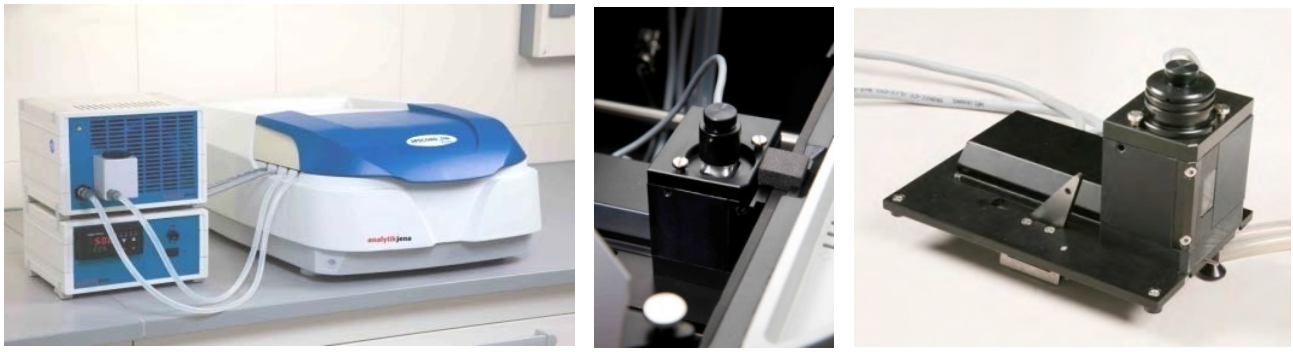

Fig. 1: SPECORD® PLUS with Peltier cooled cell holder with external heat exchanger

Melting point determination of the plasmid DNA

Sample preparation

A Lambda plasmid DNA stock solution was diluted with water. The optimum dilution is achieved when the absorption maximum lies between 0.1 and 1. The spectra of the samples are taken for this purpose between 220 nm and 300 nm and the peak maximum has been found (Fig. 2). The analysis of a 100 µL DNA solution was performed on an ultra-micro cell against molecular water which was used as blank. The measurement was carried out at 260 nm, where the DNA spectrum has its maximum.

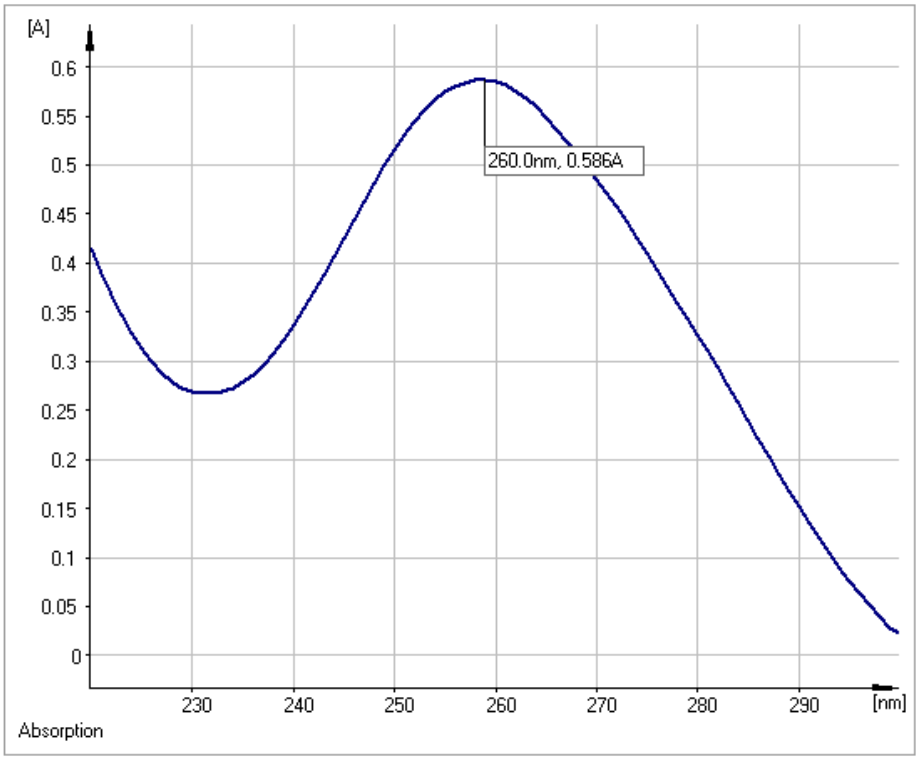

Fig. 2: Plasmid DNA spectrum

Determination

The final melting point measurement was carried out in ‘simultaneous’ mode with a start temperature of 25 °C and an end temperature of 70 °C. The heating of the sample was carried out with an increase of 1 °C per minute. The measuring data was recorded every minute respectively every 1 °C. The temperature of the DNA solution was constantly controlled by a sensor which remains in the cell during the analysis. The following table 1 gives an overview of the measurement parameters.

Table 1: Parameter settings for simultaneous measurement

Display	Absorbance	**Accessories**	Cell holder (Peltier)
Correction	Reference		
Lamp change	320 nm	**Mode** **Start temperatur** **End temperatur** **Ramp** **Measurement every**	Simultaneous 25 °C 70 °C 1 °/min 1 °C
Measurement mode **Wavelengths**	260 nm		
Integration time	0.5 s		

Results

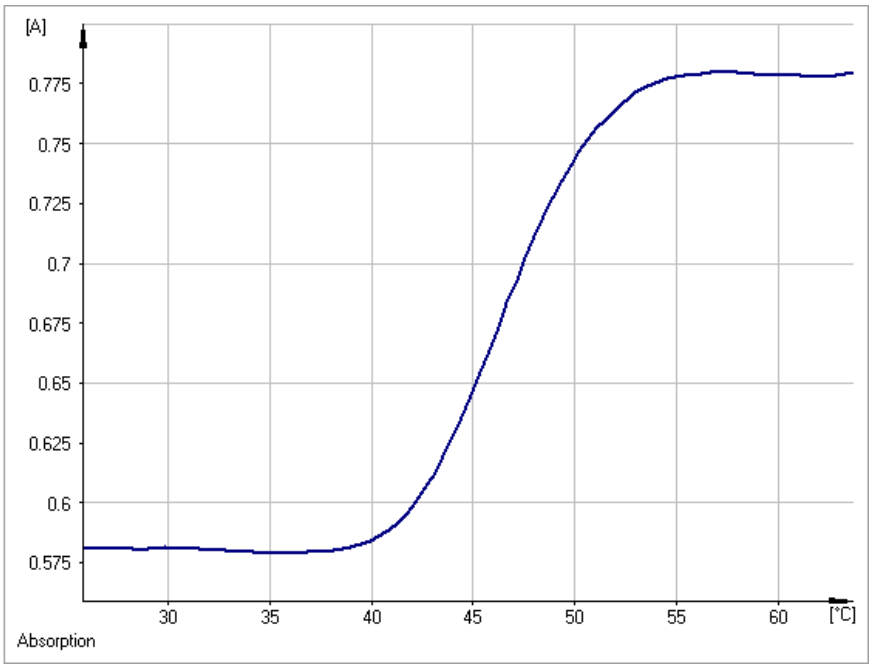

Fig. 3: DNA melting curve of the plasmid DNA

At a defined temperature the double helical structure breaks down very fast while the absorbance increases rapidly (Fig. 3). The melting point is being determined by using the module Data handling/Deviation of WinASPECT® PLUS software in which the peak maximum of the first deviation corresponds to the melting point (Fig. 4).

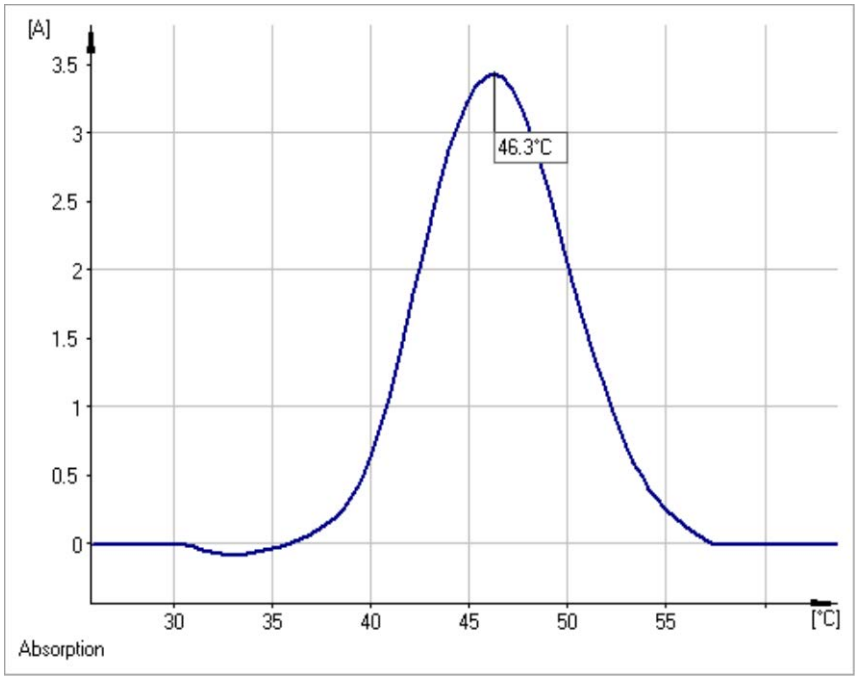

Fig. 4: First deviation with maximum of the melting curve of the plasmid DNA

Melting point determination of the calf thymus DNA

Sample Preparation

The sample preparation and recording of the absorbance spectrum were carried out analogously to the plasmid DNA.

Determination

The melting point determination was carried out in the ‚cyclic' measurement mode with a start temperature of 70 °C and an end temperature of 95 °C. Whereby in the range from 70 °C to 80 °C the optical measurement was performed every 0.2 °C; in range from 80 °C to 90 °C every 0.1 °C and from 90 °C to 95 °C again every 0.2 °C. The temperature was controlled with an accuracy of 0.1 °C. Table 2 shows the measurement parameters.

Table 2: Parameter settings for cyclic measurement mode

Display	Absorbance	**Accessories**	Cell holder (Peltier)
Correction	Reference		
Lamp change	320 nm	**Mode** **Start temperatur** **End temperatur** **From to**	Cyclic 70 °C 95 °C 70 °C to 80 °C every 0.2 °C 80 °C to 90 °C every 0.1°C 90 °C to 95 °C every 0.2 °C
Measurement mode Wavelengths	260 nm		
Integration time	0.5 s		

Results

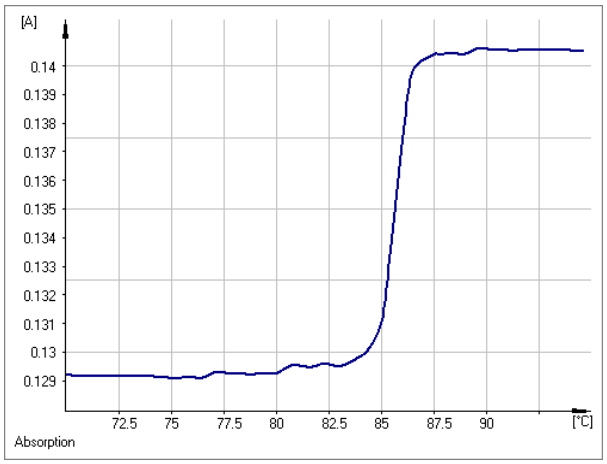

Fig 5: DNA melting curve of the calf thymus DNA

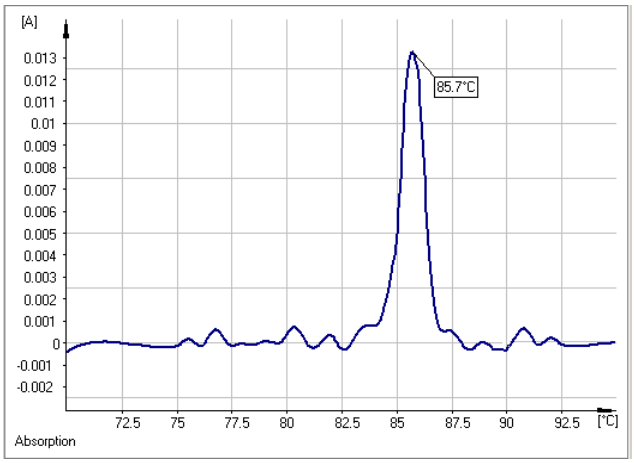

Fig. 6: First derivation with peak maximum of the melting curve

Similar to the melting curve of the plasmid DNA there is a rapid increase of absorbance during the melting process (Fig. 5). The melting point is derived from the peak maximum of the first deviation of the calf thymus DNA melting curve (Fig. 6).

Conclusion

The melting process of the DNA samples could be shown very well in both measurement modes. Because of the base stacking the structure modification of the DNA is performed within a narrow temperature interval. In the ‚cyclic' measurement mode it is possible to set different intervals of several temperature ranges. The melting curve of the thymus DNA could be shown more accurately by setting smaller measurement points in the range where absorbance increases (Fig. 5).

Compared to the thymus DNA the absorbance increase of the bacterial DNA has taken place at lower temperatures. The melting temperatures of plasmid DNA and calf thymus DNA are determined at 46.3 °C (Fig. 4) and at 85.7 °C (Fig. 6), respectively.

Native DNA in general melts at high temperatures often above 85 °C. For example the melting point of human DNA lies at 86 °C. The melting point of synthetic DNA can be estimated according to the base composition and the chain length. The lower melting point of the Plasmid DNA is a result of the short chain length of the molecule having 48.5 base pairs (bp). The thymus DNA is more complex having a molecular weight of 13 kilobase pairs (kbp). Therefore the melting point of the thymus DNA is correspondingly higher.

Furthermore the melting point of nucleic acids depends on other factors, like the chemical composition of formed hybrids, the salt concentration of the buffer solution, as well as the specific

reagents which are given to destabilize the DNA. Besides, the higher the proportions of mismatches of the base pairs, the lower the melting point will be.

Summary

Nucleic acids store life's blueprint in their special molecular structure. The hyperchromic effect, which is caused by a temperature increase while the separation of the DNA double-strands, allows the DNA melting point to be determined by UV spectroscopy. The SPECORD® PLUS, in combination with Peltier cooled accessories, allows the highly precise determination of melting points. Depending on the DNA material and the applicative requirements, temperatures can be programmed over a wide range and in different measurement modes. The use of an ultra microcell enables the work with microliter sample volumes. A temperature sensor in the cell exactly monitors the sample temperature and makes sure that it is always equal to the programmed temperature and that heating delays do not occur.

Chemicals provided by Sigma Aldrich® were used.

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