



## **NCL Method PCC-17**

### **Quantitation of Surface Coating on Metallic Nanoparticles Using Thermogravimetric Analysis**

**Nanotechnology Characterization Laboratory**  
Frederick National Laboratory for Cancer Research  
Leidos Biomedical Research, Inc.  
Frederick, MD 21702  
(301) 846-6939  
[ncl@mail.nih.gov](mailto:ncl@mail.nih.gov)  
<http://www.ncl.cancer.gov>

This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

Method Written By:

Jeffrey D. Clogston

Alpana A. Dongargaonkar

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## 1. Introduction

Nanoparticles are critical components in nanomedicine and nanotherapeutic applications. Some nanoparticles, such as metallic nanoparticles, consist of a surface coating or surface modification to aid in its dispersion and stability. This surface coating may affect the behavior of nanoparticles in a biological environment, thus it is important to measure. Thermogravimetric analysis (TGA) can be used to determine the amount of coating on the surface of the nanoparticle. TGA is an experimental technique to measure the change in mass of a sample as a function of temperature and/or time in a controlled atmosphere (1). TGA experiments run under inert atmosphere can also be used to determine residual metal content present in the sample.

This protocol describes the methods and related analysis to measure the amount of surface coating on colloidal metal nanoparticles using TGA. Specifically, the protocol is written for TGA analysis of polyvinylpyrrolidone (40 kDa PVP) stabilized silver nanoparticles, but it can be adapted for other coated metallic nanoparticles. The detailed protocol is published in ref. (2).

## 2. Principles

The thermogravimetric analyzer used for TGA experiments consists of a high precision thermobalance, which is connected to a pan/crucible holder inside a temperature controlled furnace. The pan/crucible holder is located on a sensor that is supported with a thermocouple to measure the sample temperature. A purge gas introduced into the furnace, such as nitrogen for an inert atmosphere or air/oxygen for an oxidizing atmosphere, controls the sample environment. TGA experiments are generally run from ambient temperature to 1,000°C (3).

The result of a thermogravimetric measurement is displayed as a mass vs. temperature or time curve, known as the thermogravimetric (TGA) curve (1). A derivative plot of the TGA curve, referred to as the derivative thermogravimetric (DTG) curve, shows the rate at which mass changes and is displayed as a rate of mass loss vs. temperature curve (4). Mass changes in a sample can occur due to processes such as evaporation, drying, desorption or adsorption, sublimation and thermal decomposition. These changes in mass are observed as step changes in the TGA curve or peaks in the DTG curve (1).

There are various factors or experimental conditions that affect TGA measurements:

1) Buoyancy. Buoyancy is the upward force exerted on the sample by the surrounding atmosphere which results in an apparent increase in mass (3). The buoyancy effect is caused by the change in density of the gas due to an increase in temperature and can be corrected by performing a blank measurement without any sample (1). The blank measurement should be performed at the same temperature program and crucible that would be used for the sample. To get a corrected final curve, the blank curve can be subtracted from the sample measurement curve.

2) Heating rate. An optimum heating rate should be used to obtain a better resolution of the thermal events/transitions occurring during a TGA measurement. Low heating rates are recommended so that individual thermal events can be resolved. If heating rates are too high, multiple thermal events may overlap (5).

3) Choice of crucible. The container used for the samples during a TGA measurement is called a crucible. The crucible material should not react with the sample nor undergo any physical changes in the temperature range of interest. Generally, aluminum oxide crucibles are used for TGA measurements. A crucible is closed at the top by a loosely fitted lid containing a very small hole, so it is vented to the atmosphere (1).

4) Furnace atmosphere. A protective gas, such as nitrogen, is introduced into the furnace to protect the balance from any corrosive gases being discharged during the measurement. Additionally, purge gas and /or reactive gas can be introduced in the furnace through separate gas lines at a rate of 30-50 mL/min to help remove gaseous products from the furnace (1).

TGA can be used to determine properties and characteristics of polymers, decomposition temperatures of polymers, absorbed moisture content or residual metal content in a sample. It can also be interfaced with infrared spectroscopy, mass spectrometry or gas chromatography to analyze the residual components or gases involved. Based on these capabilities, TGA is a useful tool to analyze the surface coating of nanoparticles.

In this protocol, we describe the methods and related analysis to measure the amount of surface coating on colloidal metal nanoparticles using TGA. We include analysis of 40 kDa PV

stabilized silver nanoparticles. The initial measurement will be the blank measurement performed on an empty crucible followed by the sample measurement at the same temperature program. For evaluation, the final TGA curve will be obtained by blank curve correction, and the DTG curve will be generated from the final TGA curve. The final TGA and DTG curves will be used for further analysis and mathematical calculations.

## 2. Materials and Equipment

*Note: The NCL does not endorse any of the suppliers listed below; their inclusion is for informational purposes only. Equivalent supplies from alternate vendors can be substituted.*

### 2.1 Materials

- 2.1.1 Polyvinylpyrrolidone (40 kDa PVP) stabilized silver nanoparticles with a 110 nm nominal size
- 2.1.2 Alumina crucibles with lids (70  $\mu$ L or 150  $\mu$ L capacity or ones compatible with the crucible holder and furnace)
- 2.1.3 Tweezers for holding the crucible
- 2.1.4 Spatula for transferring sample into crucible

### 2.2 Equipment

- 2.2.1 Thermogravimetric analyzer

## 3. Experimental Procedure

### 3.1 Performing a blank measurement using an empty crucible

- 3.1.1 Set up and save an experimental method by defining the temperature program. For the PVP-stabilized silver nanoparticle analysis, the sample is held at 25°C for 5 min, after which the temperature is increased to 1,000°C at a heating rate of 20°C/min. Nitrogen is used as the purge gas at a rate of 40 mL/min. The same temperature program and settings used for the sample is required for the blank measurement.

- 3.1.2 Start the experiment and allow the furnace and crucible holder to equilibrate to the starting temperature.
- 3.1.3 After the temperature equilibrates and the instrument's display screen reads a variation of "Insert sample", open the furnace and carefully place an empty crucible along with a lid on the crucible holder using tweezers. Close the furnace.
- 3.1.4 When the crucible weight is displayed on the screen, zero the crucible weight.
- 3.1.5 Allow the weight value indicated on the display to stabilize to zero and proceed with the experiment.
- 3.2 Performing a sample measurement after the blank measurement
  - 3.2.1 Start the same experimental method as that performed for the blank measurement. Same temperature program and crucible is required for blank curve correction.
  - 3.2.2 When the display screen on the instrument reads a variation of "Insert sample", open the furnace and carefully place the empty crucible, which was used for the blank measurement, along with a lid on the crucible holder using tweezers. Close the furnace.
  - 3.2.3 When crucible weight is displayed on the screen, tare the crucible weight and allow the weight value indicated on the display to stabilize to zero.
  - 3.2.4 Open the furnace again and remove the crucible.
  - 3.2.5 Transfer the sample into the crucible using a spatula. Cover the crucible with the lid. When loading the sample (aqueous or powdered samples) into the crucible, care should be taken to add appropriate amount of sample depending on the capacity of the crucible so as to prevent overflowing of the sample after it is covered with a lid. In this protocol, the sample is lyophilized PVP-stabilized silver nanoparticle. An aqueous suspension of the PVP-stabilized silver nanoparticle sample was measured initially (50  $\mu\text{L}$  of sample was loaded into a 70  $\mu\text{L}$ -capacity crucible for measurement). However, since the samples were in water, water loss was observed below 150°C of the TGA curve and this peak dominated the curve. Also, the

amount of PVP present in the sample was below the level of detection of the instrument. To solve this and achieve better sensitivity, the aqueous sample was lyophilized before TGA measurement. About 20-30 mL of the sample was frozen using dry ice with acetone slurry and lyophilized for 15-18 hours to remove water. The resulting lyophilized powder was used for TGA measurement.

**Note:** Standard precautions must be taken while handling nanoparticle aqueous solutions and powders. It is important to wear proper clothing, laboratory gloves and eyewear while performing the experiments. Powdered/lyophilized samples must be prepared and transferred into the crucible in a fume hood. As an extra precaution, a respiratory mask may be used.

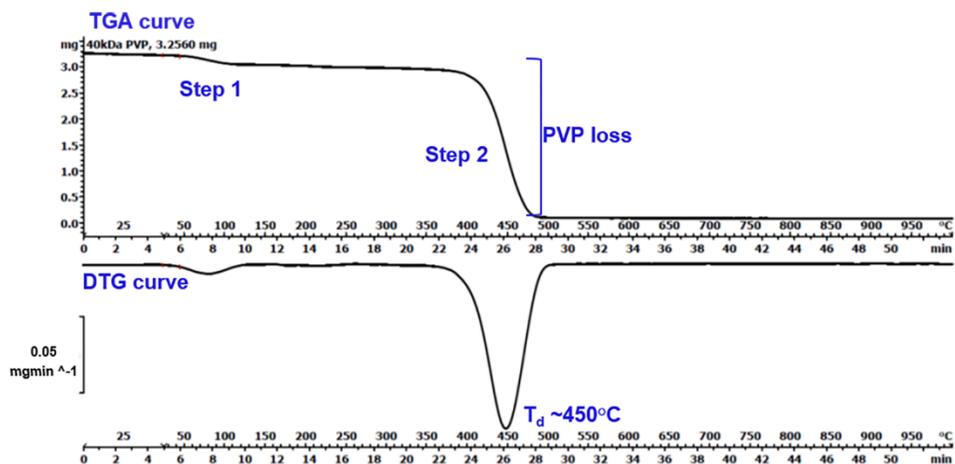
- 3.2.6 Place the crucible filled with sample on the crucible holder and close the furnace.
- 3.2.7 Allow the weight value indicated on the display to stabilize (do not press the tare button again) and proceed with the experiment.

## 4. Data Analysis

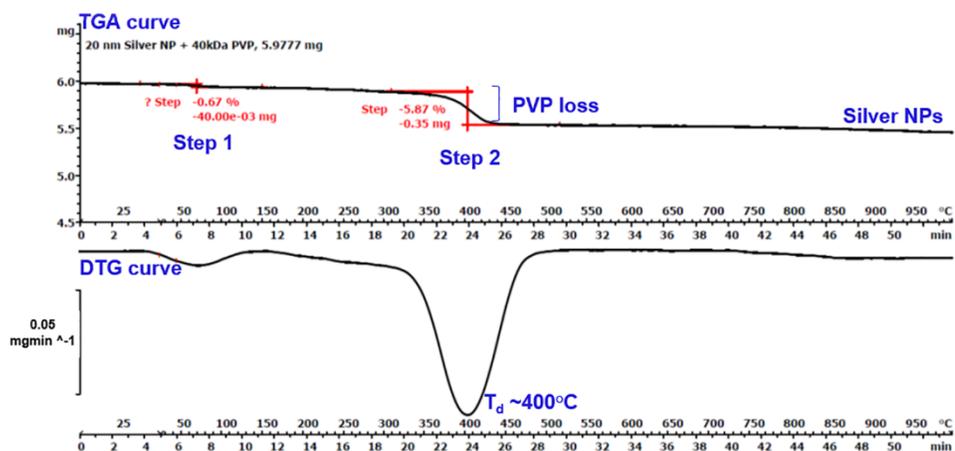
- 4.1 Performing blank curve subtraction
  - 4.1.1 Once both the blank measurement and sample measurement are collected, open the evaluation window for data analysis.
  - 4.1.2 Open the mass loss vs. temperature or time curves for both the measurements simultaneously in one window.
  - 4.1.3 For blank curve correction, subtract the blank measurement curve from the sample measurement curve using the curve subtracting function on the analyzer software.
  - 4.1.4 The subtracted curve obtained is the blank corrected sample curve and also the final TGA curve that will be used for further analysis. Remove the initial blank and sample measurement curves used for subtraction from the evaluation window.

- 4.2 Generating a derivative thermogravimetric (DTG) curve
  - 4.2.1 Select the final TGA curve obtained in 3.3.1 and generate the DTG curve using the “1<sup>st</sup> derivative” option in the analyzer software.
  - 4.2.2 The final TGA and DTG curves can be displayed in the same evaluation window.
- 4.3 Performing step evaluation to determine weight loss
  - 4.3.1 The TGA and DTG curves show the decomposition of the surface coating and the metallic content present in the sample from the residual amount left after the measurement. In this example, PVP is the surface coating and silver is the residual metal. To determine the weight loss due to decomposition of the surface coating, draw a frame around the section of the curve that shows a weight loss event; this indicates sample decomposition. Because the weight loss event is best seen in the DTG curve, use the DTG curve to draw a frame around the decomposition event and correlate that frame to the corresponding location in the TGA curve. Then, use the “Step Horizontal” option or a similar selection in the analyzer software to obtain the weight loss value.

**Figures 1 and 2** show the TGA and corresponding DTG curve for the 40 kDa PVP (control) and PVP-stabilized silver nanoparticle sample. Both the samples decompose in two stages. Initial weight loss (Step 1) is observed due to water loss (below 150°C) followed by decomposition of PVP between 300°C-500°C (Step 2). 40 kDa PVP has a decomposition temperature ( $T_d$ ) of approximately 450°C, and it decomposes completely upon heating to 1,000°C. However, the decomposition temperature of PVP in the silver sample is approximately 400°C, which is lower than that observed in the PVP control measurement; presumably, silver catalyzes the thermal decomposition of PVP. The residual weight at 1,000°C is attributed to the silver content in the sample.



**Figure 1:** TGA and DTG curve for 40 kDa PVP (Control).



**Figure 2:** TGA and DTG curve for the PVP-stabilized silver sample.

The calculation for PVP concentration in the sample is summarized in **Table 1**. The resulting weight after water loss is the total construct weight in the sample. This value can be used to calculate the concentration of PVP as mg PVP per mg total construct.

Weight after water loss (Total construct), mg	PVP loss, mg	Silver NP at 1000°C, mg	[PVP], µg PVP/mg Ag
5.93	0.35	5.46	64.1

**Table 1:** Determination of PVP concentration in the PVP-stabilized silver sample.

4.3.2 To determine the residual metal amount, select the full range of the curve and use the same software option described above to display the total weight loss and residual amount left at the end of the measurement. If the coating surface material is known, it is good practice to include that material as a control to compare weight loss events between the coated nanoparticle and the coating alone.

## 5. References

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## 6. Abbreviations

DTG	derivative thermogravimetric
NCL	Nanotechnology Characterization Laboratory
PVP	polyvinylpyrrolidone
TGA	thermogravimetric analysis