NCL Method PCC-17

Quantitation of Nanoparticle Composition Using Thermogravimetric Analysis

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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.

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1. Introduction

Some nanoparticles, such as metallic nanoparticles, often include a surface coating or surface modification to aid in its dispersion and stability. As this surface coating may affect the behavior of nanoparticles in a biological environment, it is important to measure. Thermogravimetric analysis (TGA) can be used to determine the amount of surface coating, approximate sample component proportions, decomposition temperatures, and/or nanoparticle residues. 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 use of the TGA for a variety of nanoparticle samples, including sample preparation, data analysis, and experimental considerations. Specifically, the protocol examines the TGA analysis of polyvinylpyrrolidone (40 kDa PVP) stabilized silver nanoparticles as a detailed example of coating quantification, in addition to other examples of nanoparticles analyzed in our lab. A detailed protocol for the analysis of organic coatings on metallic nanoparticles is published in reference (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 1,100°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 composition 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. In addition, the analysis and considerations of other types of nanoparticles (beyond metal nanoparticles) are given at the end. This TGA method is applicable to many nanoparticle samples for different types of compositional analysis; data analysis should be modified accordingly.

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 µL or 150 µL 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. Transfer the sample into the crucible using a spatula or pipette. 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 the 70 µL crucible, typically 50 µL or less of a liquid sample will be used. In the 150 µL crucible, typically 125 µL or less of a liquid sample will be used. For solid samples, loading capacity depends on the density of the material; a minimum mass of 2 mg is recommended for most samples. Solid samples should fill half of the crucible at maximum to allow for potential sample expansion during decomposition. In this example, the sample is lyophilized PVP-stabilized silver nanoparticle. A detailed discussion of sample lyophilization for TGA analysis is given later in section 5.1.2.

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.

3.2.5 Place the crucible filled with sample on the crucible holder and close the furnace.

3.2.6 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 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 "1st 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.

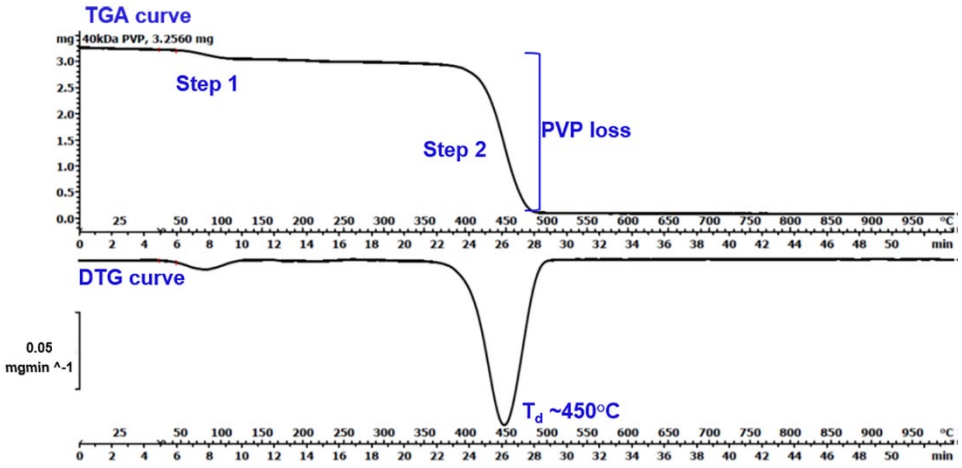

Figure 1: TGA and DTG curve for 40 kDa PVP (Control).

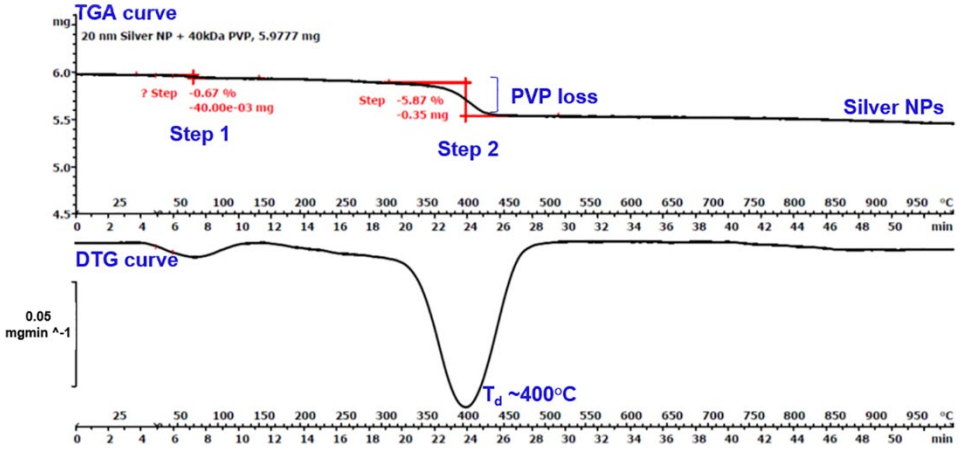

Figure 2: TGA and DTG curve for the PVP-stabilized silver sample.

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.

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

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**

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. Sample Considerations

5.1 Sample phase and form

5.1.1 Solid or liquid samples can be analyzed by TGA. Liquid sample volumes must be less than the maximum capacity of the crucible; typically, 50 µL or less is loaded with the 70 µL crucible. Liquid samples are easily pipetted into the crucible; however, the TGA curve will be dominated by the loss of liquid in the sample. Solid samples can be more difficult to transfer into the crucible (static can be a complication for some samples) but will not have a significant liquid-related loss at lower temperatures; this can be helpful if a sample component decomposes at a temperature lower than 250 °C.

5.1.2 Liquid samples can be lyophilized to yield a solid sample. This step can be considered if the sample component is present in low concentration, the evaporation of water interferes with the decomposition of a sample component, and additional TGA resolution/sensitivity is desired, to name a few. Integrating gravimetric analysis of the sample is easily done though the lyophilization of a liquid sample; in many cases, it is important to measure the total construct concentration of a sample for comparison. For

example, in the analysis described above, an aqueous suspension of the PVP-stabilized silver nanoparticle sample was measured initially (50 µL of sample was loaded into a 70 µL-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.

5.2 Samples in salt buffers

5.2.1 Samples dissolved or suspended in salt solutions (for example, phosphate-buffered saline (PBS)) will have residue from the salts of the solvent whether the sample is run as a liquid or as a lyophilized solid. One way to correct the sample residue mass for salt residue is to run a solvent-only sample on the TGA of an equal volume as your sample. For example, if your sample is dissolved in PBS and you run a 35 µL sample, you could also run a 35 µL sample of only PBS and note the residual mass (see Figure 3). For some samples, this amount could be negligible in comparison to your sample residue and masses; for others, this amount could result in significant error in your sample calculation.

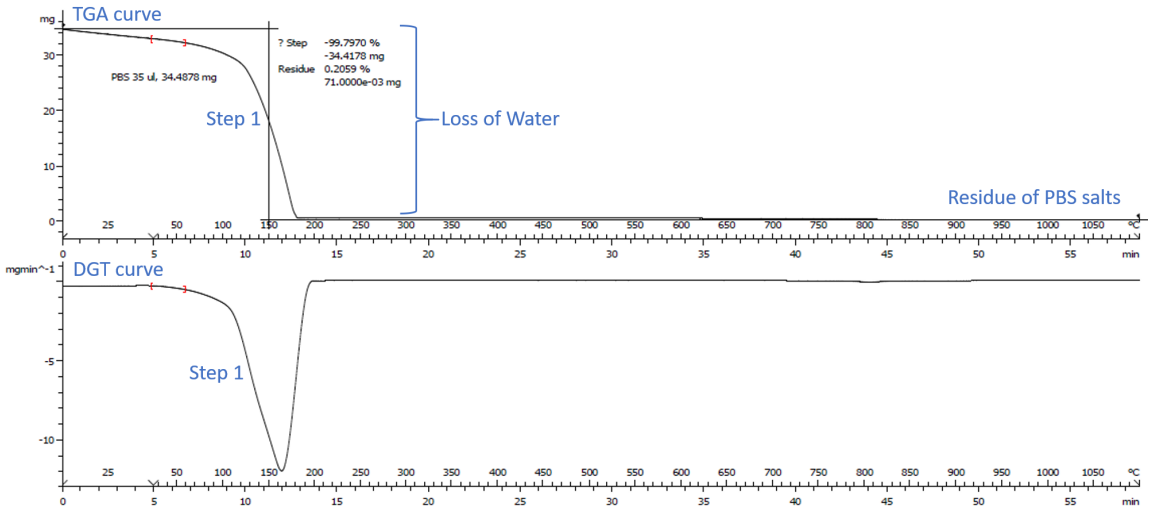

Figure 3: TGA and DTG graphs of a 35 µL aliquot of 1x PBS. For a 35 µL sample of PBS, 0.071 mg solids remains. This corresponds to approximately 2 mg/mL PBS .

5.3 Multi-component samples

5.3.1 For a sample comprised of multiple known components, TGA analysis can be used to determine the approximate concentration or ratio of the components in the sample. For this technique to be applicable, pure samples of each component must be analyzed on the TGA to determine each component's decomposition temperature and degradation profile. This is done to make sure that the individual components do not have overlapping decomposition events over the course of the TGA temperature program. Given that the components have baseline resolvable decomposition events (i.e. mass loss rate returns to near-zero at some point between the completion of the first mass loss event and the onset of the second mass loss event), the mass losses can be used to calculate the original starting masses of the components in the original sample as well as the ratio of the components to one another in the original sample.

5.3.2 It is also important to note that it is frequently helpful to have pure samples of the known components of a complex sample to help identify the likely sources of each decomposition event in a TGA thermogram. While covalent bond formation between components and other types of

molecular interactions can slightly shift the exact decomposition temperature of a sample component (as in the silver PVP sample above where the silver acted as a catalyst), knowing the approximate decomposition temperature of all of the sample components (or even some of the components) can be a helpful tool in analyzing sample composition, understanding decomposition progress, and examining sample stability.

5.4 Samples with incomplete decomposition

5.4.1 Many of the assumptions made in TGA calculations and analysis rely on the assumption that the sample (or sample component of interest) decomposes completely; however, there are many samples and sample components that do not completely decompose over a temperature program. For these samples, pure components of the sample are necessary to determine which of the components are the source of the incomplete degradation and to determine the percentage of that component that does decompose. After determining the component's decomposition percentage, the sample containing that component can be analyzed to determine the amount of the partially degrading component in the original sample by using the determined percentage in combination with the component's mass loss in the original sample to back calculate the mass of the component in the original sample.

5.5 Metal and metal oxide nanoparticles

5.5.1 In general, metals and metal oxides under an inert atmosphere will be relatively stable in a TGA analysis and therefore have little to no overall degradation. Thus, as in the example above with the silver-PVP particles, it is easy to analyze organic coatings on these types of nanoparticles as organic components will often completely decompose under a typical TGA temperature program. If a coating degrades entirely, it can be assumed that any remaining sample mass can be attributed to the metal or metal oxide particle and can also be used to calculate metal or oxide concentration in the original sample.

5.6 Organic samples and sample components

5.6.1 As discussed above, not all sample components will degrade completely; however, TGA is still often a good analytical tool for complex organic samples, particularly when all or some of the sample components are known and available to be analyzed individually. Despite its analytical benefits, TGA is not ideal for identifying unknown samples and in most cases, it is important to know as much as possible about the sample before examining the sample using the TGA method.

6. References

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7. Abbreviations

DTG	derivative thermogravimetric
PVP	polyvinylpyrrolidone
TGA	thermogravimetric analysis
PBS	phosphate-buffered saline