VAMAS

VAMAS TWA 42 – Project 2

Identification and quantification of TiO_2 anatase and rutile particles in binary mixture by Raman Spectroscopy

Measurement protocol

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Abbreviations used in this document

ILC: interlaboratory comparison

SI: International System of Units

NPs: nanoparticles

NA: numerical aperture

S/N: signal to noise ratio

RMS: root mean square

PLS: partial least squares

A/R ratio: anatase to rutile ratio

LV: latent variable

RMSECV: root mean square in cross-validation

1. Introduction

Raman spectroscopy is a technique that has grown in the scientific community and is increasingly adopted by industry and end users searching for a reliable and rapid tool in the characterization of materials. It is a fast and ambient technique, and portable instruments are already present in the market, thus allowing in principle in-situ analysis.

TiO_2 is the most exploited semiconductor oxide and it has a wide range of applications, from painting and coating to personal care, health and food products. In nature, three TiO_2 polymorphs exist (anatase, rutile and brookite) with individual physical and chemical features that lead to distinct functional properties. Due to their different crystalline structure and subsequent different spatial distribution of the atoms, anatase, brookite and rutile provide different Raman fingerprint spectra.

A deeper knowledge of the composition of anatase and rutile TiO_2-based products will enable an improved understanding and tailoring of their functional properties. So far, no standard exists for the identification and quantification of anatase and rutile polymorphs in binary mixtures, and the development of a validated procedure based on Raman spectroscopy would guarantee both manufacturers and end users about the quality and the performances of the final products.

2. Objectives

This ILC will aim to develop validated procedures based on Raman spectroscopy and multivariate analysis methods, for the identification and quantification of anatase and rutile TiO_2 polymorphs in titanium dioxide nanoparticles binary mixtures.

3. Instrumentation

A Raman micro-spectrophotometer in a 180° backscattering configuration should be employed, given the physical characteristics of the samples.

The instrument should be capable of performing chemical maps of 20 µm × 20 µm with a step size of 2 µm. If this is not possible, the closest possible map size and pixel dimensions to these dimensions should be employed; in any case, 11×11 points maps (121 points in total) should be taken.

A laser with a 532 nm wavelength should be employed as an excitation source for these measurements. If this is not possible, the closest possible wavelength is to be used instead, such as 514 nm.

An instrument capable of measuring Raman shifts of Stokes bands in the [75 cm^{-1}; 1000 cm^{-1}] interval is optimal for this study. The system should have a minimal spectral resolution of 5 cm^{-1} or better. If it is not possible to acquire spectra in this Raman shift interval, a subrange of this window could be measured instead: in this case, contact the Project Contacts to discuss if the available subrange would yield viable data for this study.

An objective lens of magnification of 20× or below should be used, with NA ≤ 0.45 and a working distance of several millimetres (any objective with working distance of 6.8 mm or above is suitable, while objectives with working distances below 6.8 mm might be employable depending on the film thickness). The information on the objective should be reported along with the estimated laser spot size at the sample.

3.1 Calibration

The Raman system should be spectrally calibrated using a rare gas lamp emitting in the entirety of the spectral range of interest, dependent on the employed laser. Gas lamps emit at well-defined and tabulated wavelengths, and are traceable to the SI with uncertainties low enough for the purpose of Raman calibration. The lamp should be turned on and placed under the microscope objective, and at least one spectrum should be measured for the calibration after focusing on the lamp surface with an acquisition time low enough to prevent the saturation of the detector. ASTM E2529-06 ("Standard Guide for Testing the Resolution of a Raman Spectrometer") can be used for reference for this procedure. A handy database of peak positions can be accessed at the following address: https://physics.nist.gov/PhysRefData/ASD/lines_form.html

4. Samples description and handling

The samples supplied in this ILC consist in pure powders and different powdered mixtures of two phases of TiO_2 NPs (anatase and rutile). Because of the criticality of the geometry of the powder disposition for this study, the difficulty of handling of the dry powders because of electrostatic forces, and the safety concerns in the manipulation of the substances, the NPs shall be distributed in containers prepared by the provider, from which the powders are not to be removed.

4.1 Safety

Titanium Dioxide: in 2017, International Agency for Research on Cancer has classified TiO_2 as part of Group 2B, which means possibly carcinogen to humans. The use of gloves and protective mask when handling TiO_2 powder and wet suspensions is recommended.

4.2 Samples description

The following samples are provided:

- 2 pure samples of NPs, one for each phase: 100% anatase (A100) and 100% rutile (R100);
- 3 anatase/rutile binary mixtures in different ratios, 25%, 50%, 75% anatase on total ratio (AR2575, AR5050, AR7525 respectively).
- 3 binary mixtures of undisclosed ratios (X1, X2, X3).

The 8 samples are deposited as compact, well-mixed films on flat substrates, to be found at the bottom of the 8-well sample holder. The films should be compact and mostly continuous, although cracks and sparse holes on their surfaces may occur.

Should any of the samples be damaged upon receipt, please contact the Project Contacts immediately.

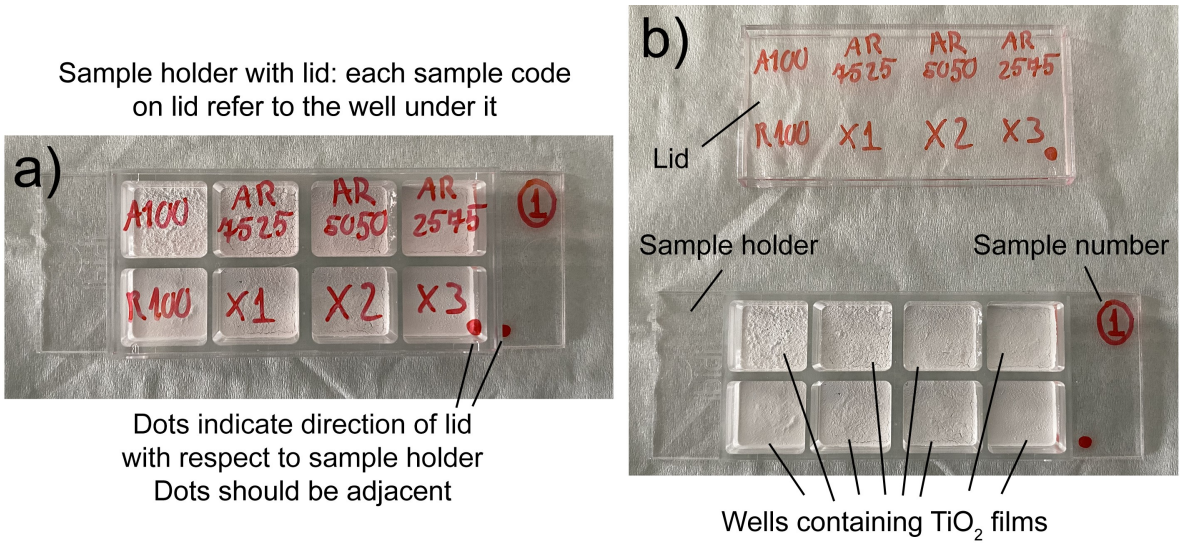

Figure 1. Photos of the sample holder with lid (a) and without lid (b). The dimensions of the sample holder are those of a microscope slide. It should be noted that the lid could be mounted in two directions: the proper placement of the lid, necessary to correctly identify the samples, is indicated by the dots, which are adjacent when the lid is positioned in the right direction.

4.3 Unpacking and handling

Before unpacking the samples, please read section 4.1 and the SDS attached to this document. Good laboratory practices should be followed in the entirety of the handling and measurement procedures.

5. Measurement

The Raman instrument should be calibrated as per section 3.1, and the calibration should be verified by measuring a well-known standard, such as a clean, monocrystalline silicon wafer or a certified polystyrene film before the measurements.

The samples have coverslip floors and it is therefore possible to measure them with an inverted microscope through the sample floors, as well as an upright system from above after removing the lid. However, the floors yield Raman signals. These signals are faint (with respect to the very Raman active TiO_2 phases constituting the films) and do not overlap with the spectral region in which the major anatase and rutile Raman peak lie. Nevertheless, it is preferred for this study to measure the films from above, and resort to an inverted microscopy measurement only if it is not possible to measure them otherwise (e.g. the sample is damaged or no available microscope objective is suitable for measurements from above).

First, a Raman parameters optimization step, described in section 5.1, is necessary to determine the working conditions to be employed in the following measurements in the ILC. This will only involve the pure anatase sample. Afterwards, the measurement protocol in section 5.2 is to be applied to each sample.

5.1 Parameters optimization protocol

1. Unpack the sample holder, remove the lid and verify that the chosen microscope objective has enough working distance to put in focus the surface of each film (as the internal height of the empty wells is 6.8 mm, objectives with a working distance above that should be suitable). Because of variability in the physical properties of the films and the powder suspension during film preparation, the films may vary in height, therefore it should be verified before measurements that the chosen microscope objective can be employed in this study. If it is not possible to focus on the surface of each film, employ an objective with a longer working distance. If no suitable objectives are available, please contact the Project Contacts.
2. Locate the pure anatase film (sample A100), and face it towards the microscope objective placing it on the microscope stage.
3. Choose an area of the sample near the centre and roughly focus on the surface employing brightfield optical microscopy with the chosen objective lens. The chosen area should be flat and horizontal in the range of the expected laser spot size.

4. After setting a laser power ≤1.0 mW, illuminate the sample with the laser and finely focus on the sample by registering Raman spectra and maximising the most intense anatase Raman signal (E_g at 144 cm^{-1}) while changing the position of the sample along the optical axis.
5. Optimise laser power, exposure time and number of scans per spectrum. The laser power incident on the sample should never surpass 1.0 mW, in order to avoid thermal degradation of the phases. A signal to noise ratio (S/N) of at least 10 on the anatase B_{1g} peak at 400 cm^{-1} should be sought. S/N is calculated dividing the peak height (after baseline subtraction) by the root mean square (RMS) of the signal in a spectral region devoid of noticeable Raman peaks, such as the 850 cm^{-1} – 1000 cm^{-1} interval. The main issue other than seeking a good S/N is to avoid CCD saturation in samples with high anatase content in the spectral region of the E_g peak at 144 cm^{-1}: if saturation occurs in the spectral region near this Raman band during measurement, change the parameters to prevent this.

If the centre of the anatase E_g peak at 144 cm^{-1} falls outside of the employed spectral acquisition window and is therefore not measurable, the following variations to the aforementioned procedure should be made:

- in step 4, another anatase Raman signal should be employed to optimise focus instead of the E_g peak at 144 cm^{-1};
- in step 5, to avoid CCD saturation, the optimisation of the spectral parameters should take into consideration other Raman bands instead of the non-measurable anatase E_g peak at 144 cm^{-1}: the optimised parameters sought in step 5 should be tested on pure rutile sample R100 as well as pure anatase sample A100, observing the two most intense rutile signals (E_g and A_{1g} in the spectral interval [400 cm^{-1}; 650 cm^{-1}]) and the other visible anatase signals respectively to prevent CCD saturation.

In our setup (upright microscopy system, 10× objective with 0.25 NA, 532 nm Raman excitation wavelength, 50 µm slit confocal aperture, ~1 cm^{-1} mean CCD pixel spacing, spectral acquisition interval larger than [75 cm^{-1}; 1000 cm^{-1}]), 0.5 mW laser power, 0.2 seconds per scan, and 50 scans per spectrum yielded satisfactory spectra for this study.

5.2 Measurement protocol

This procedure has to be repeated for each of the 8 titanium dioxide films.

1. Remove the sample holder lid, locate the selected film and face it towards the microscope objective placing it on the microscope stage.
2. Choose an area of the sample near the centre and roughly focus on the surface employing brightfield optical microscopy with the chosen objective lens, seeking a flat, horizontal area of the film surface in the 20 µm × 20 µm range.
3. Set the Raman parameters (laser power, exposure time, number of scans) to the optimised parameters found in section 5.1, and then finely focus on the sample surface by maximising the most intense TiO_2 signal visible in the spectrum.
4. Measure a Raman map around the measured point, with the optimised parameters found in section 5.1 as the conditions for each point of the map. The map should be 20 µm × 20 µm with a step size of 2 µm. If this is not possible, the closest possible map size and pixel dimensions to the specifications should be employed; in any case, 11×11 points maps (121 points in total) should be taken. Calculate the mean spectrum for the map by calculating the mean spectrum of all the point spectra of the map.
5. Repeat steps 2, 3 and 4 for at least two other different areas of the sample (for a total of at least 3 areas per sample). The chosen areas should be flat, and distant at least 500 µm from one another if possible, avoiding areas very close to the walls of the wells of the sample holder, where the surfaces of the TiO_2 films are often very sloped.

In our setup (10× objective with 0.25 NA, upright microscopy system, 532 nm Raman excitation wavelength, 50 µm slit confocal aperture, ~1 cm^{-1} mean CCD pixel spacing), 0.5 mW laser power, 0.2 seconds per scan, and 50 scans per spectrum were chosen as optimised parameters, therefore each Raman map required approximately 10 minutes.

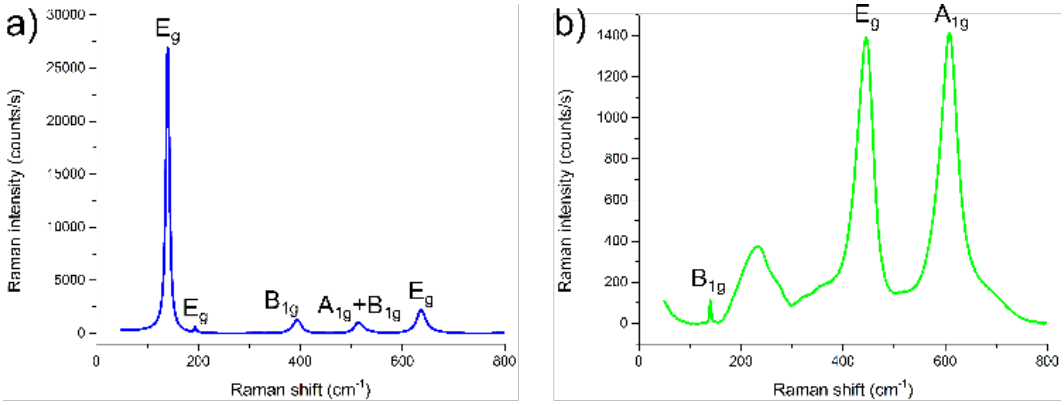

Figure 2. Raman spectra of anatase (a) and rutile (b) acquired with a 532 nm excitation wavelength.

6. Data analysis

6.1 Data elaboration

For each Raman map, the average spectrum must be calculated. Calculate the standard deviation of the most intense peak of the average spectra obtained from three different maps collected in three different regions. Should this be higher than 15%, please select other areas of analysis for the collection of the Raman maps and repeat the measurements.

The average Raman spectra of all Raman maps should be saved in .csv format (using "." as decimal separator and ";" as field separator, one line per sample). Named with a code of alphanumeric symbols (as an example "A100a" for anatase 100% first replicate, or "AR2575b" for the mixture anatase 25% – rutile 75% second replicate). The spectra should be arranged in a single data matrix. Two separate matrices should be prepared, one for the known standards (named "training set" containing in the following, each spectrum belonging to the training set is named "calibration standard" in the following), and one for the unknown samples ("prediction set" in the following). The first row of each of the matrices should contain the Raman shift indication (in cm^{-1}), the first column must contain the spectrum name, the second column the percentage of anatase, while from the third column on the Raman intensity corresponding to the frequency reported in the first row in the corresponding column should be entered. Tables 1 and 2 show the structures of these files.

Table 1. Structure of "training set.csv" shown as a table. Green cells indicate Raman shift values of the respective column.

Sample	Anatase percentage	75.40	75.88	76.37	…	1000.10
A100a	100	2.56702	3.12786	3.98425	…	20.9839
A100b	100	5.78057	3.25425	4.8987	…	19.4890
A100c	100	1.78581	4.11436	4.47461	…	25.0697
AR7525a	75	3.74861	2.09417	5.96517	…	22.6401
…	…	…	…	…	…	…

Table 2. Structure of "prediction set.csv" shown as a table. Green cells indicate Raman shift values of the respective column.

Sample	Anatase percentage	75.40	75.88	76.37	…	1000.10
X1a		1.09784	1.80854	2.64166	…	23.2171
X1b		4.4189	1.50997	3.41934	…	25.8261
X1c		2.19562	2.51067	5.98755	…	18.9345
X2a		5.16458	1.53277	2.38641	…	20.4834
…	…	…	…	…	…	…

6.2 Spectra preprocessing

The spectra should be subjected to cosmic ray spikes removal if necessary.

7. Report

The following data should be uploaded to the VAMAS sharepoint:

- Raman apparatus maker, model, and excitation/collection geometry;
- Microscope objective magnification and numerical aperture;
- Estimated laser spot size at the sample;
- Acquisition Raman conditions including laser power, acquisition time per scan, and number of scans;
- Data in the form stated in section 6.1: 2 .csv files of the mean spectra for each map after preprocessing containing the two matrices “training set” and “calibration standard”.

8. Acknowledgements

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