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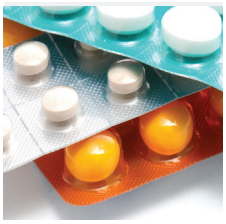
Raman Spectroscopy

Raman spectroscopy of pharmaceutical ingredients under humidity controlled atmosphere



Application Note

Pharmaceutical RA61

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Abstract
Full and detailed characterization of pharmaceutical formulations is required before release of new drugs. When exposed to warm and humid weather over time, ingredients may undergo change in their hydration level, thus affecting the properties and/or efficiency of the drug. Raman spectroscopy, combined to a temperature and humidity controller can mimic such harsh conditions and can be used to monitor the properties of the different ingredients and track their modifications induced by environmental changes.

Keywords
Raman Spectroscopy, Humidity controlled stage, Hydration, Pharmaceutical ingredients

Introduction

Before being introduced on the market, pharmaceutical products need to be fully analysed: this includes of course the detailed characterization of the active ingredient in terms of physical and structural properties. Active substances may exist as various polymorphic forms, or with different hydration levels, which may have an impact on the efficacy of the drug.
Characterization also involves stability studies of both the active substrate and the entire formulation. Several factors may affect the stability, such as temperature, light and humidity. The impact of the storage conditions of manufactured drugs must be thoroughly evaluated, considering that some tropical countries are constantly exposed to warm and humid weather, which may alter the dosage form over time.
Raman spectroscopy is commonly used in the investigation of pharmaceutical compounds and its applications have been extensively reviewed. It is highly chemically selective, and offers the possibility to monitor the chemical and structural change of ingredients when exposed to harsh conditions. Raman spectroscopy can be combined with a temperature and humidity controlled cell to understand the modifications induced by environmental changes.

Instrumentation

The Raman spectrometers from HORIBA Scientific are highly versatile, ideally suited to the analysis of many sample types. They offer full automation, with ease of use, coupled with powerful functionality via their wide range of options and accessories. All instruments from both the XploRA and LabRAM Evolution series are compatible for example with the use of temperature control cells to monitor chemical changes within a sample as a function of temperature. Mapping options in X, Y, and Z axes are available to fully analyze any sample, even when placed within specific environment cells. LabSpec6 software package offers advanced capabilities for data acquisition, treatment and display, with an intuitive and easy-to-use interface. All functionnalities are included to control the temperature of the sample or program temperature ramps.
The system used in this study is the Humidity System, from Linkam Scientific Instruments. It comprises a RH95 Humidity Controller and a THMS600 heating and freezing stage, and is a turn-key solution for temperature and humidity control for sample characterisation.
The THMS600 heating/freezing stage can be used alone to heat/cool the sample from -196°C to 600°C. Used in conjunction with the humidity controller, its temperature range is ambient to 100°C, with a 0.1°C accuracy and high temperature stability.

The THMS600-H variant has a combined humidity and temperature digital capacitive sensor within the stage chamber to enable accurate humidity control. Atmospheric air is drawn into the RH95 where it is passed through a dessicator and de-ionised water to precisely control the RH, before flowing into the chamber.

In addition to use with the Linkam stage the RH95 can be used to control humidity within a 2000cc chamber within the range 10-95% RH. Calibration ampoules can be provided to enable calibration of the probe.

Fig. 1: The Linkam RH95 Humidity Controller.

Hydration of anhydrous lactose

Anhydrous lactose is often found as an inactive ingredient in different medications. It is particularly useful because it contains no water, which means that it will not react with medications that are sensitive to moisture.

Lactose also exists in a monohydrate form, which can behave differently from the anhydrous form when formulated with other ingredients. It is therefore important to understand the conditions for which anhydrous lactose turns into the monohydrated form. Anhydrous and monohydrate lactose have different Raman spectral features as shown in Fig. 2.

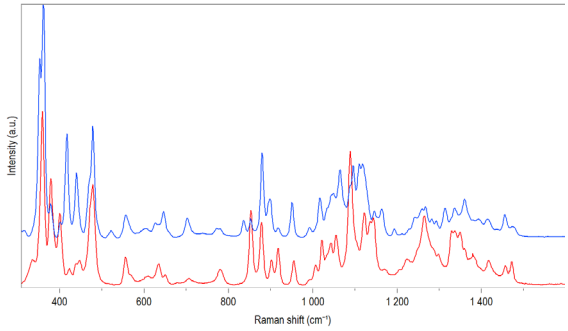

Fig. 2: Raman spectra of anhydrous lactose and lactose monohydrate.

Anhydrous lactose was exposed during 24h in the hermetic humidity controlled cell, set to a relative humidity (RH) value of 95%, with a constant temperature of 25 °C. The spectra were measured every minute and the spectral features of the dataset were analyzed to determine if anhydrous lactose underwent changes.

The spectra of both pure anhydrous and monohydrate forms were used with the Classical Least Squares (CLS) fitting algorithm to determine the proportion of each form during the experiment.

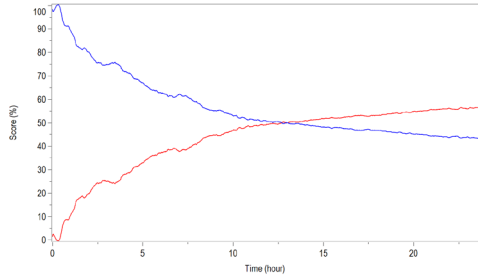

Fig. 3: Scores (%) of anhydrous lactose and lactose monohydrate obtained from CLS fitting, during a 24h exposure of anhydrous lactose to a relative humidity of 95%.

Fig. 3 indicates that the anhydrous crystals gradually (and non-linearly) absorb water to form the monohydrate form. After 24h the transformation is not complete and provided that both pure spectra of anhydrous lactose and lactose monohydrate, used as references in the CLS fitting, were acquired with the same measurement parameters than the dataset, it gives a fair estimate of the conversion to monohydrate form.

Hydration of Theophylline

Theophylline, also known as 1,3-dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases. As a member of the xanthine family, it bears structural and pharmacological similarities to caffeine.

Similarly to lactose, theophylline exists in different forms: monohydrated, anhydrous I and anhydrous II. The anhydrous II form is stable at room temperature, whereas anhydrous I isn't. The solubility – and consequently the bioavailability – of theophylline is affected by hydration, and it is thus important to understand the kinetics of hydration.

Anhydrous theophylline was placed in the moisture controlled cell maintained at 25 °C and with a RH of 95 % during 24h. Fig. 4 indicates that the anhydrous II and the monohydrated forms have rather different spectra, with notably the peaks at 1166 and 1683 cm-1 being characteristic of the monohydrated form.

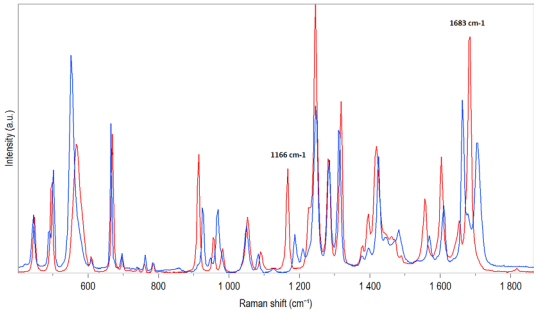

Fig. 4: Raman spectra of the anhydrous II and the monohydrated forms of the theophylline.

Spectra were taken every 30 seconds and the dataset was analysed with the CLS fitting algorithm, using both pure anhydrous II and monohydrate forms to evaluate the proportion of each form in each spectrum.

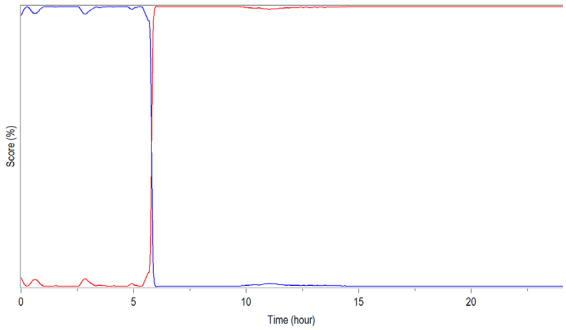

Fig. 5: Scores (%) of anhydrous theophylline and theophylline monohydrate, obtained from CLS fitting, during a 24h exposure of anhydrous theophylline to a relative humidity of 95%.

The profiles in Fig. 5 suggest an instantaneous transformation of the anhydrous form after 6 hours of exposure; however this statement only applies within the few μm^3 sampling volume resulting from the microscope sampling geometry. For powders, made of grains varying in size and shape, such sampling is clearly not statistically viable, and the results in Fig. 5 may not reflect the bulk behaviour of the powder.

To check if the conversion is homogeneous across the powder, various maps were taken in a repeated analysis of anhydrous theophylline submitted to a RH of 95 %. An area of 1.6 x 1.6 mm of packed anhydrous powder in a crucible was mapped every 4 minutes over the 24h experiment time. Each map comprised 400 data points, corresponding to a map step size of 80 µm - the DuoScan™ macrospot option was used to match the laser spot size to this step, to ensure full coverage of the sample surface.

Spectral features of the monohydrate form were used to monitor its presence in each map.

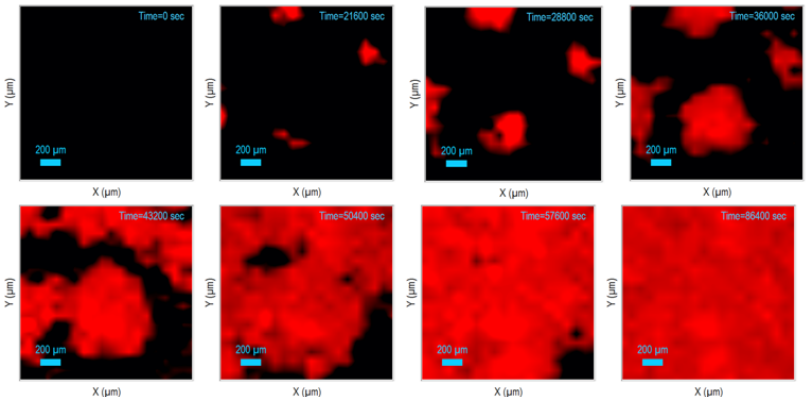

Fig. 6: Raman images of the monohydrated form of the theophylline after different exposure times to 95% RH – 0h, 6h, 8h, 10h, 12h, 14h, 16h and 24h.

The maps represented in Fig. 6 reveal that the transformation to the monohydrated form is indeed not homogeneous over the whole area. Some spots start to convert after just a few hours and the conversion to monohydrate spreads out to the rest of the area from these starting points. The transformation to monohydrate is complete after the 24 hours of the test.

By taking the average spectra of each mapped area, and applying the CLS fitting, it is then possible to plot the conversion to monohydrate form over time (Fig. 7) in a more comprehensive and rigorous way than by analyzing one single spot (Fig. 5).

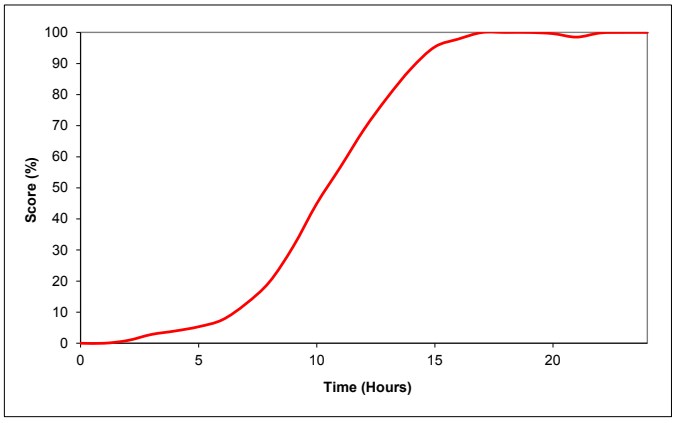

Fig. 7: Scores (%) of the monohydrated form, obtained with the CLS fitting of the averaged spectra of each mapped area.

Conclusions

The combined use of Raman microspectroscopy and the controlled humidity cell offers the possibility to accurately follow the structural changes of pharmaceutical substances when exposed to given environmental conditions (RH, temperature). Such capabilities allow not only monitoring of sample hydration (as in the case of lactose and theophylline discussed in this note), but also characterization of polymorphic or phase transitions, swelling or degradation induced by moisture. The use of the cell is also compatible with Raman imaging, and thus is ideally suited to analysis of heterogeneous samples – e.g. structural modifications in dosage forms such as tablets.

Further Reading

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