# Quantifying anhydrate/ hydrate using potential PAT *in situ* techniques

**Benefits at a glance**
- Quantifying the hydration states of four APIs
- Comparison of potential *in situ* spectroscopic PAT techniques
- Benefits of representative Raman sampling

## Introduction

The need to identify and determine the hydration states available to an active pharmaceutical ingredient (API) has been determined to be as critical as the need to assess polymorphism.[1] However, even when understood and controlled during API manufacture, process-induced changes in the preferred form can occur during drug product manufacturing. These changes can affect the efficacy of the drug product and consequently there is a requirement to quantify the solid-state form during manufacturing unit operations. Historically, off-line x-ray diffraction (XRD) methods have been used to identify the resulting powder to either release or reject the batch. XRD is invasive, time-consuming, and susceptible to subsampling (analyzing an unrepresentative fraction of a heterogeneous mixture). The FDA's recent PAT initiative strives to improve pharmaceutical manufacturing by providing a framework for in-process testing to alleviate the inefficiencies and potential errors inherent with off-line testing.

Raman spectroscopy is recognized as a PAT technique. In this study, Raman was used to quantify the hydration state (anhydrate vs monohydrate) of four model API compounds: nitrofurantoin, theophylline, caffeine, and carbamazepine.

## Experimental

Two approaches were employed to form the hydrates. Solid-phase transformation of the anhydrate by exposure to high relative humidity was used for nitrofurantoin and theophylline. For caffeine and carbamazepine, cooled recrystallization was used as the conversion process. Binary calibration mixtures containing different amounts anhydrate and hydrate were subsequently prepared. The composition of the mixtures was determined using XRD as the primary method.

Raman spectra were collected using a Raman analyzer equipped with a probe for large volumetric sampling. Spectra were collected using 1 second of integration time and 8 accumulations using approximately 100 mW of laser power.

The NIR system used was a compact NIR spectrometer from Control Development, Inc., with a fiber optic probe using six illumination fibers and a single collection fiber. Spectra were collected using 5 milliseconds of integration time and 64 accumulations.

## Results

NIR spectra of increasing amounts of hydrate for mixtures of nitrofurantoin are shown in Figure 1. The dominant features are related to the water of crystallization. Bands at 1420 and 1920 nm are assigned to water molecules incorporated into the crystal lattice. Band positions in the ranges

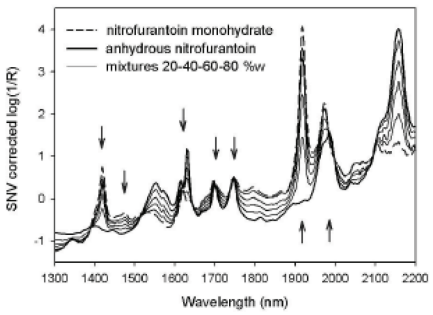


Figure 1: NIR spectra of nitrofurantoin binary mixtures are shown. Major features assigned to the monohydrate are identified.
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ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

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1600–1800 nm and 1960–2000 nm were also observed. It should be noted that distinguishing spectral features were observed for all four of the anhydrate/hydrate drug pairs.

Characteristic Raman features for all the drug pairs were observed. Raman spectra of varying amount of nitrofurantoin are shown in Figure 2. Peak shifts between the anhydrate and the hydrate form of nitrofurantoin were observed at approximately 1348, 1383, 1431, 1565 and 1610 $cm^{-1}$.

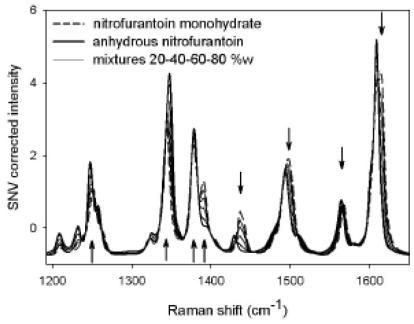


Figure 2: Raman spectra of nitrofurantoin binary mixtures are shown. Major features assigned to the monohydrate are identified.
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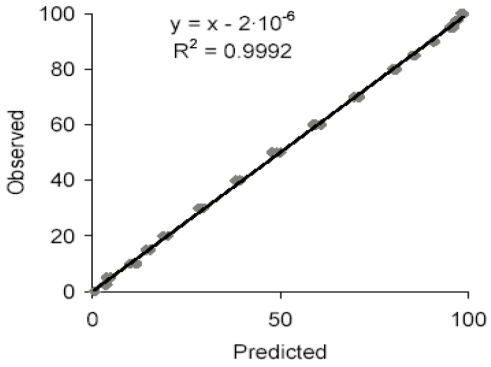


Figure 3. Observed-vs.-predicted plot of nitrofurantoin using a Raman probe with a large spot size and a stationary sample.
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Prior to modeling, the data were preprocessed and normalized. Several approaches were investigated, and standard normal variate (SNV) methods followed by data centering was selected.

Both univariate and multivariate data analysis and calibration approaches were employed for the analysis of the binary hydrate mixture. The observed-versus-predicted plots for nitrofurantoin obtained from the Raman system are shown in Figure 3. The correlation coefficient and cross-validation coefficients were found to be equivalent to the XRD and NIR results.

## Conclusion

In additional experiments (not reported here), it was found that Raman methods utilizing traditional small-spot sampling were prone to sub-sampling that limited the method's accuracy.[2] In the experiments reported here, the use of a large-spot (3 mm) Raman probe–based system with sample rotation yielded comparable results to the NIR approach employing a 6 mm spot size and sample rotation. This result indicated that representative sampling was critical to this application's success. In addition, by increasing the sample volume analyzed, the applicability of Raman spectroscopy for the study of solid-state mixtures was demonstrated.

Both spectroscopic methods investigated were found to be able to characterize the concentrations of anhydrate and hydrate for all the four drug compounds studied. Despite these findings, it should be noted that prior to selection of one or the other spectroscopic approaches for integration with a particular drug product unit operation, the operation parameters and the specific chemical makeup of the API should be reviewed for approach compatibility.

## References:


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