Identification of Additives used in the Pharmaceutical and Food Industries with the NanoRam Handheld Raman Spectrometer

Today's Raman instrumentation is faster, more rugged, and less expensive than previous instrumentation. Now, with the advances in component miniaturization, the design of high performance, portable and handheld devices has introduced the technology to new application areas that were previously not possible with older, more cumbersome instruments. Handheld Raman instruments such as the NanoRam® from B&W Tek are very well-suited for pharmaceutical applications such as the testing of raw materials, verification of final products and the identification of counterfeit drugs due to the technique's extremely high molecular selectivity.

Current Challenges

The performance of handheld Raman instrumentation has improved dramatically with the release of the NanoRam because it can test far more complex compounds and differentiate between different binding/tableting materials used in the pharmaceutical industry such as cellulose, microcrystalline cellulose, and hydroxypropyl methylcellulose (HPMC). Historically, identification of these types of compounds have only been achievable using research-grade, laboratory systems, which offer high resolving power and better signal to noise capability. The inherent problem is that the Raman characteristics of cellulose materials and saccharide-based food additives are not distinctive enough due to the high fluorescence nature of compounds like HPMC. The challenge that the industry currently faces using traditional handheld Raman systems is relatively poor selectivity and extremely long testing times, which leads to reliability problems and inconsistencies in the testing methods.

However, recent breakthroughs in the areas of optical design, detectors, thermo-electric cooling technology and intelligent software algorithms are proving that high performance Raman spectroscopy can be combined with simplicity and ease of use in a compact design. To exemplify this, we carried out an investigation using the NanoRam handheld Raman spectrometer, to see if it could differentiate between a group of cellulose materials and food additives.

The NanoRam is a compact, handheld Raman spectrometer and integrated computing system for material identification and verification within cGMP compliant facilities. Weighing less than 2.2lbs, it allows rapid development of standardized and validated methods for purity and quality control applications. At the heart of the device is a 785 nm wavelength laser excitation source with a crossed Czerny-Turner spectrograph and a thermoelectrically (TE)-cooled CCD detector, providing a very stable signal with low background noise. The benefits of a temperature-controlled detector to reduce background noise is emphasized in Figure 1, which shows the dark counts (noise) for a non-cooled CCD spectrometer at room temperature and a TE cooled spectrometer, operated at 18ºC, using an integration time of 30 seconds. The calculated RMS (root mean square) noise level of the TE cooled spectrometer on the right is approximately five-fold lower than the non-cooled unit.

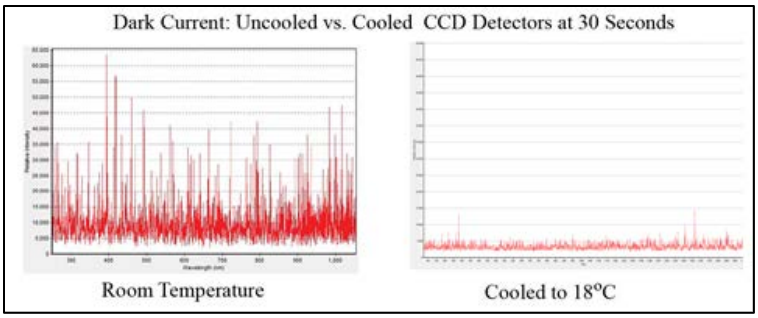

***Figure 1**: Dark current noise for a non-cooled CCD spectrometer at room temperature (left) and a TE cooled CCD spectrometer at 18°C (right)*

Coupling thermoelectric cooling with patented laser stabilization technology and a high speed microprocessor, this technology provides laboratory grade performance in a convenient handheld package. It has the capability of generating a signal with high signal to background noise specification, which is required for the successful testing and confirmation of different pharmaceutical cellulose compounds and food additives.

Characterization of Different Materials and Additives used in the Pharmaceutical and Food Industry

The following materials (used as binding agents, fillers, additives and supplements, all similar-looking white powders) were selected for this investigation:

- Cellulose (binders/fillers)
- Hydroxypropyl Methylcellulose (HPMC) (binders/fillers)
- Lactose (sweetening agent/filler)
- Maltodextrin (sweetening agent/food additive)
- Calcium Monohydrogen Phosphate dihydrate (CaHPO4.H2O) (binder/dietary supplement)

Testing Procedure

The standard operating parameters used for this study are shown in Table 1.

Table 1: Handheld Raman operating parameters for the characterization of pharmaceuticals

Parameter	**Value**
Laser excitation wavelength	785 nm
Laser line width	<0.3 nm
Spectrometer wavelength range	175-2900 cm^{-1}
Spectrometer resolution	9.0 cm^{-1} @ 912 nm
CCD detector type	Thermoelectric cooled (TEC) linear array
Size and number of pixels on detector	14x200 µm, 2048
Detector TEC temperature	18 ºC
Measurement time	< 20 sec

The testing procedure involves calibrating the device using pure forms of each of the compounds to develop and store methods to test and characterize all the other materials. Each instrument "method" collects a minimum of 20 scans from the specific type of material, which allows a user to include slight variations in sampling position, packaging materials/ batches, and inconsistencies associated with multiple operators carrying out testing. Included in the customized method for each material is a proprietary software algorithm where the Raman spectrum is compared and matched with that in the method to generate a numerical P-value from which the PASS/FAIL result is determined. The methods created in this way will not only be related to the unique characteristics of the materials, but will also ensure a rugged and robust method for a reliable testing procedure required for unambiguous material identification. Once a method is developed, the total scan time and decision making process takes approximately 20 seconds to make a PASS/FAIL assessment of a material.

An example of a scan using this procedure is seen in Figure 2, which shows a Raman scan of a cellulose material (blue plot) and the stored "Cellulose Method" generated from the reference standard (red plot). A P-value of 0.9982 was obtained, which is considered an extremely good fit compared to the reference material (a P-value of 1.000 is considered a perfect fit). Note that the Raman spectrum of the pure cellulose reference material has been offset by 1000 intensity units compared to the unknown sample for clarity purposes. This unknown sample would therefore be assessed as a PASS for cellulose.

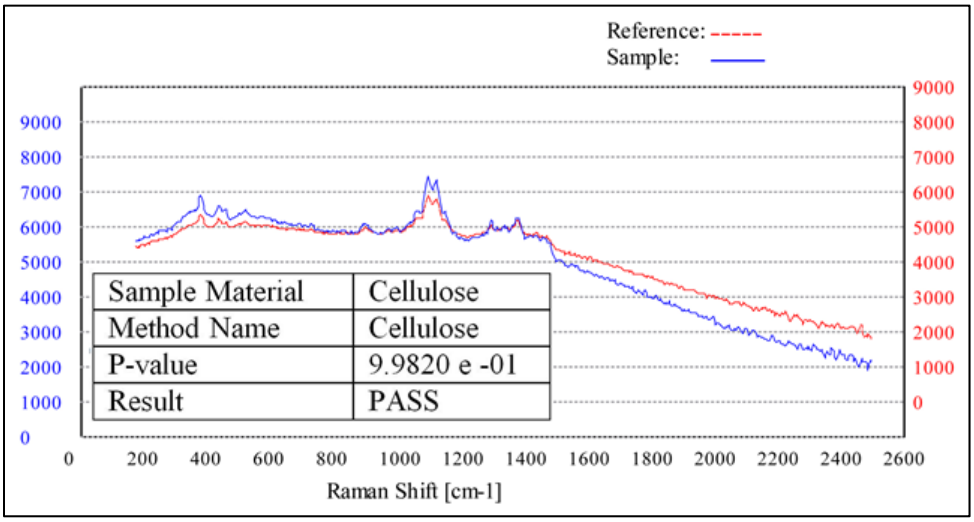

Figure 2: *Raman spectrum of a cellulose sample (blue) overlaid with the stored cellulose method (red). The P-value in the inset table shows the cellulose was confirmed as cellulose.*

By comparison, Figure 3 shows a Raman scan of cellulose using a standard method generated for HPMC. It can be seen that the Raman spectrum for the sample (blue) looks very different from the HPMC reference scan (red), as indicated by the P-value for the sample, which is 2.23716 e -06. This unknown sample would therefore be assessed as a FAIL for HPMC.

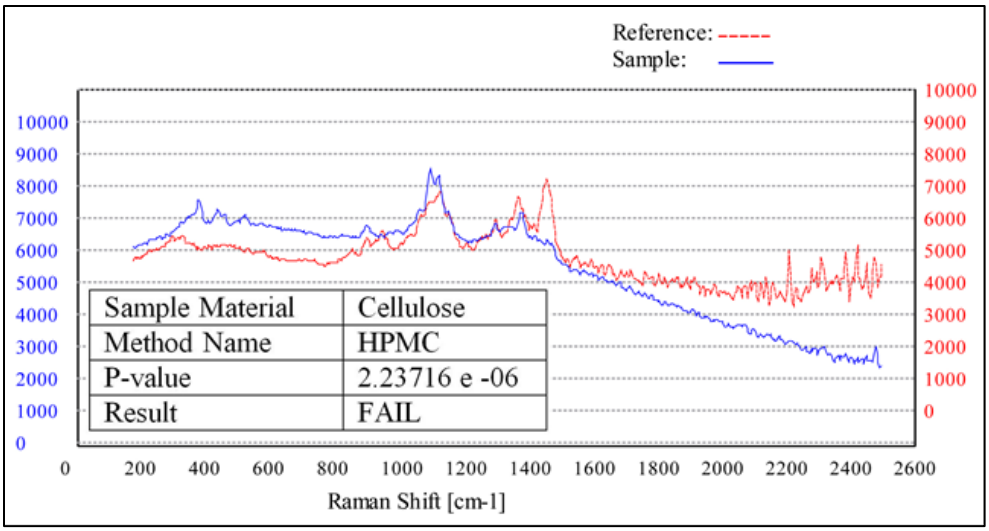

Figure 3: *Raman spectrum of a cellulose material (blue) overlaid with the stored HPMC method (red). The P-value in the inset table shows the cellulose was NOT verified as HPMC.*

If a sample material has failed, the software algorithm then makes a "probable" assessment of the material's identification based on the library of methods and stored spectral information. This is exemplified in Table 2, which shows all the materials being tested using the "Cellulose Method." Both the cellulose and microcrystalline cellulose PASS the cellulose ID test, whereas all the other materials FAIL it. The materials that failed the cellulose test are further evaluated against the on-board spectral libraries and confirmed as the correct material with almost 100% accuracy, based on the Hit Quality Index (HQI) in the final column. The HQI nomenclature is well-recognized for assessing Raman spectra and is defined as the similarity of two spectra, when comparing an unknown spectrum and a library spectrum via a correlation test. An HQI value of 1.00 (100%) indicates when the unknown spectrum is identical to the library spectrum, and gets smaller as the unknown and library spectra become less similar to one another. In fact, for all the materials that failed a specific method test, the average HQI value was >99%, indicating that the match of the unknown spectrum to a known reference spectrum in the library was extremely accurate.

Table 2: Results of all the materials being tested and assessed using the "Cellulose Method."

Method Name	**CELLULOSE**			
Material	Identification Result	P-value	Probable Match from Investigation	HQI (%)
Cellulose	Pass as Cellulose	0.9998	Cellulose Confirmed	
HPMC	Fail	7.61E-03	HPMC	99
Lactose	Fail	2.26E-03	Lactose	100
Maltodextrin	Fail	2.27E-03	Maltodextrin	100
Dihydrate $CaHPO_4$	Fail	4.79E-06	Dihydrate $CaHPO_4$	100

The final part of the investigation was to test all the powders using specific methods generated by each of the pure reference materials. The results of this test are shown in Table 3. It can be seen from the color-coded boxes that the NanoRam handheld Raman system has correctly confirmed all the materials that were tested using their respective methods as indicated by the green PASS boxes; while all the other materials FAILED the test, as indicated by the red and brown colored boxes. Note the color legend below the table that shows actual P-value ranges, emphasizing the severity of the failure – in other words, the lower the value, the worse the failure.

Table 3: Testing of all the materials under investigation using specific methods generated by each of the pure reference materials

Method / Materials	HPMC	Cellulose	Lactose	Maltodextrin	Dihydrate $CaHPO_4$
HPMC	PASS	Fail	Fail	Fail	Fail
Cellulose	Fail	PASS	Fail	Fail	Fail
Lactose	Fail	Fail	PASS	Fail	Fail
Maltodextrin	Fail	Fail	Fail	PASS	Fail
$CaHPO_4$ Dihydrate	Fail	Fail	Fail	Fail	PASS

Color Legend for P-values:

P-value > 0.05	P-value 10^{-3} - < 0.05	P-value 10^{-6} - < 10^{-3}	P-value 10^{-15} - < 10^{-6}	P-value zero

Conclusion

This investigation has clearly emphasized that raw materials and additives used in the pharmaceutical and food industries can be successfully tested and identified using the NanoRam handheld Raman spectrometer with a high degree of confidence in less than 20 seconds. It has also shown that even when a compound fails a test, the software algorithms and on-board spectral libraries can make a very accurate assessment of the probable identification of that compound. These capabilities make the technique ideally-suited for the unambiguous identification and verification of incoming raw materials in a pharmaceutical or food manufacturing environment.