Phosphate Salts: Qualitative Identification and Quantitative Contamination Analysis with Near-Infrared Spectroscopy

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Introduction

Confirmation of raw material quality is a vital part of any industrial process. Incorrect or poor quality vendor materials account for a considerable portion of failed and recalled product. To avoid these expensive problems, strict quality control procedures are often implemented and used to screen for inappropriate incoming materials. The quality control procedures commonly used are chemical tests that involve removing samples to a laboratory and performing, what can at times be, complex, time consuming, and laborious protocols.

One common test procedure that is considerably easier and quicker to perform than laboratory chemical tests uses near-infrared (NIR) spectroscopy. NIR analysis provides spectral information that can be used to identify materials, determine the quality of those materials, and quantitatively measure component concentrations. Robust and fast NIR instruments are particularly amenable for raw material analysis right at the receiving dock as the materials are being unloaded. This precludes the need for bringing samples to a lab for analysis.

NIR spectroscopy relies on changes in dipole moments caused by molecular vibrations. Typically these NIR active vibrations are found in organic compounds where bonds between carbon and other atoms allow for relatively easy analysis. Emphasis on organic analysis has led to the often incorrect assumption that inorganic materials like salts are not suitable for NIR analysis. While it is true that some inorganic salts will not have strong NIR spectra, many will have vibrations that are sufficiently active in the NIR region to do proper analysis and measurement. Hydrated inorganic salts are particularly easy to analyze. Additionally, studies that work to separately identify different polyatomic ions from each other can be successful. This application note demonstrates this ability by describing methods to identify and qualify phosphate salts used for the manufacture of ultrahigh purity cell culture buffers.

The Thermo Scientific Antaris Method Development Sampling (MDS) system (Figure 1) is a versatile NIR analyzer commonly used in pharmaceutical, biopharmaceutical, chemical, polymer and other industries. It is specifically designed to be rugged enough for placement in industrial and production settings where mechanical vibrations or extreme temperatures and conditions are unsuitable for other delicate scientific equipment. Furthermore, the software that drives the Antaris™ MDS is easy enough to be operated by non-technically trained personnel. A variety of fiber optic probes can be coupled to the system and interface with raw materials, intermediates or finished product directly in pipelines or in process tanks. In this particular application, a hand-held trigger-controlled Thermo Scientific SabIR probe was used to analyze batches of phosphate salts directly in their original containers. Additionally, quantitative studies utilized the integrating sphere for analyzing samples collected in glass vials.
Case Study

This application note resulted from a case study whereby a bioprocessing company was provided subquality raw materials for preparing complex cell culture buffers. These inferior raw materials were undetectable using the standard quality assurance methods and eventually resulted in over $3 million dollars in lost finished product, investigations and recovery as well as a damaged reputation. Further investigation traced the subquality raw material to a batch of poorly refined sodium phosphate dibasic (Na₂HPO₄). Specifically, the poorly refined material contained significant quantities of pyrophosphate ion (P₂O₇⁻⁴). The presence of this pyrophosphate led to incorrectly formulated synthetic cell culture media, which subsequently damaged, destroyed and invalidated healthy bioreactor runs. Complex analysis using inductively coupled plasma mass spectrometry (ICP-MS) on the finished cell culture media was able to detect the presence of pyrophosphate. Eventually this was traced to the sodium phosphate dibasic raw material. Subsequent NIR analysis proved that the inferior raw materials provided by the vendor would have been quickly and positively identified and quantified at the receiving dock, if the Antaris NIR system had been in place.

Methods

As part of this evaluation, the Antaris NIR system was used to successfully identify various phosphate powders and quantitatively determine the mass percent of pyrophosphate in sodium phosphate dibasic raw material. Two separate studies were carried out for these two separate goals. Both the qualitative identification and the quantitative studies were performed using an Antaris MDS system.

Qualitative Identification

Five different classes of phosphate materials were selected based on their use and potential for use as incoming raw materials. These materials are representative of the variety of minerals that may be refined from phosphoritic ores. Table 1 lists the materials analyzed in this study. The phosphates were analyzed directly inside their original containers using the SabIR™ fiber optic probe. Spectra were averaged from 16 scans using 8 cm⁻¹ resolution. A total of 11 samples from each material were used in the analysis.

The spectra were subjected to chemometric treatment using Thermo Scientific TQ Analyst software. A Discriminant Analysis algorithm was selected using Multiplicative Signal Correction in the pathlength. The raw spectra were used without pre-processing or derivatives except for a linear removed baseline to account for baseline shifts due to differences in sample reflection. The spectral regions used in the method were from 7400 to 4100 cm⁻¹. Example spectra from each of the classes are shown in Figure 2. Note that even though there are only subtle absorbing bands and spectral characteristics in the samples, robust calibration methods were developed.

Quantitative Measurement

This quantitative study used 26 samples of sodium phosphate dibasic (Na₂HPO₄) contaminated with various amounts of potassium pyrophosphate (K₄P₂O₇) ranging from 0 to 15% (0 to 7.9% pyrophosphate ion). Potassium pyrophosphate was measured as the contaminant because, unlike the sodium salt, there are no waters of hydration that would provide strong absorption bands. These strong water bands would allow easy quantitation of the contaminant, but would not be representative of the type of contamination found in the case study.

The 26 samples were collected in two dram vials which were placed on the integrating sphere module of the instrument (Figure 3). Spectra were averaged from 64 scans using 8 cm⁻¹ resolution. Typically, spectra from each sample were collected twice and the two examples were merged in the chemometric analysis. A Partial Least Squares (PLS) method was developed that allowed for quantitative prediction of the weight percent of pyrophosphate in the Na₂HPO₄. Most of the samples were used to build and develop the PLS chemometric calibration. Six samples were not directly used to build the model but were later used as validation standards to test its robustness and reliability. The standards spectra were subjected to a second derivative pre-processing treatment with a Norris derivative smoothing algorithm (segment 15, gap 9). The region of analysis ranged from 7300 to 4700 cm⁻¹.
Results

Qualitative Identification

The fully developed Discriminant Analysis method identified the various raw materials successfully without any errors. There was sufficient spectral variability between each of the classes that they were clearly separated. Mahalanobis distances were reported for the spectra which indicate how closely each spectrum clusters around the class average. Smaller Mahalanobis distances indicates the sample is spectrally close to the class average, while larger Mahalanobis distances indicates the sample falls far away from a particular class average. Ideally, each spectrum’s lowest Mahalanobis distance will be to its correctly identified class, while the next highest Mahalanobis distance will be relatively large. Mahalanobis distances can be thought of as the number of standard deviations a spectrum might fall from the class average. Distance values of less than one indicate the spectrum is very similar to the class average. Table 2 lists the average Mahalanobis distance ratios of the closest incorrect classes to the correct classes. Ratios higher than three suggest there is a substantial separation between the clusters of spectra assigned to each class.

Quantitative Measurement

The PLS method developed from the second derivative spectra provided a calibration curve with excellent correlation between the NIR predicted values and the mass percentages determined gravimetrically. A calibration curve for the pyrophosphate ion mass percent (Figure 4) demonstrates this high correlation. In addition to the high correlation, the PLS method provides low errors. Root Mean Square Error of Calibration (RMSEC) is calculated from the standards used to develop the method. Root Mean Square Error of Prediction (RMSEP) is calculated from the six validations standards used to predict the robustness of the method. The RMSEC and RMSEP values are 0.25% and 0.26% respectively. This indicates that the method can easily predict the amount of pyrophosphate contamination in Na$_2$HPO$_4$ between 0 to 7.9% and be accurate, on average, to within about a quarter of a percent. In addition to high correlation and low errors, a Predicted Residual Error Sum of Squares (PRESS) plot was used to evaluate the quality of the

<table>
<thead>
<tr>
<th>Material</th>
<th>Ratio</th>
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<tbody>
<tr>
<td>Ca$_2$HPO$_4$·2H$_2$O</td>
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</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>6.1</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
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<td>Na$_2$HPO$_4$</td>
<td>47.8</td>
</tr>
</tbody>
</table>

Table 2: Ratios of the nearest incorrect Mahalanobis distance to the correct class. Results greater than three indicate there is excellent separation between the different groups.

Conclusions

The methods described above demonstrate that inorganic phosphate salts commonly used in buffers can be easily distinguished from each other using the Antaris FT-NIR analyzer. Despite misconceptions that these materials do not have sufficient signal to be analyzed with NIR, there is clearly enough differences in the absorbing regions to identify and classify these incoming raw materials. Additionally, the quality of refined Na$_2$HPO$_4$ can be monitored with respect to contaminating materials like pyrophosphate. NIR is a rapid, easily implemented technique that can be used directly on receiving docks and production facilities by non-technically trained people to analyze the quality of raw materials and product. Specifically, this method was able to determine concentrations of contaminating pyrophosphate to within 0.25% quickly without bringing samples back to the lab for expensive, time consuming analysis. Had this method been in place in the case study described above, several million dollars in lost product and revenue would have been avoided.