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HUMAN HEALTH | ENVIRONMENTAL HEALTH

VOLUME 8 Monsoon Edition, August 2009
INDIA ANALYTICAL SCIENCES - Bio-discovery

Dear Readers,

With the advent of technology PerkinElmer has been successful in venturing in many areas of scientific research. The company known for its optical instrumentation has become a global corporation to serve the human health and environmental health. The Analytical sciences and Bio-discovery has been the pride for our customers. We are proud to celebrate the 40th year of "Man on the moon" mission of NASA as technology associate for the space station.

This FRESH edition brings you the moment of happiness as India celebrates the 62nd Independence Day on 15th of August 2009. PerkinElmer continue to offer new technologies and hyphenated techniques for the better. Your valued suggestions are welcome. Please do write to us. Happy reading.
"Long Live Independence"

WHAT'S **Fresh** inside...

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Combining Ramam Spectroscopy and Differential Scanning Calorimetery

Kevin P Menard and Richard Spragg, PerkinElmer® Inc.

Introduction

Raman spectroscopy is a powerful technique for materials characterization in its own right, having found applications in as diverse areas as polymorph identification in pharmaceuticals, crystallinity studies in polymers, and reaction monitoring to name a few. Recently the use of Raman spectroscopy with a hot stage has become common and the applications of the technique keep expanding.

Why combine Raman spectroscopy with Differential Scanning Calorimetry (DSC) then? DSC adds several capabilities to a combined instrument.

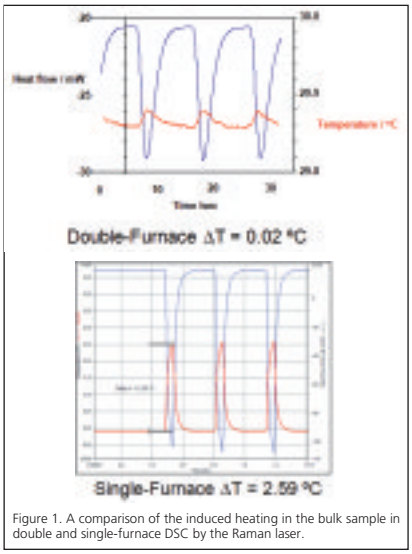

Figure 1. A comparison of the induced heating in the bulk sample in double and single-furnace DSC by the Raman laser.

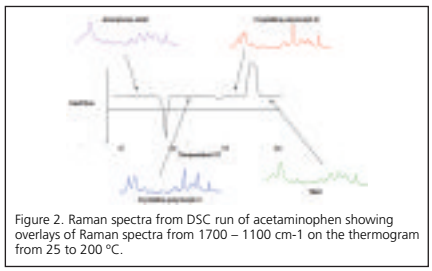

Figure 2. Raman spectra from DSC run of acetaminophen showing overlays of Raman spectra from 1700 – 1100 cm-1 on the thermogram from 25 to 200 °C.

DSC is a commonly used technique in many of the same industries as is Raman Spectroscopy. DSC requires extremely good temperature control and easily exceeds the control found on hot stages. Temperatures are extremely stable and, in double-furnace designs, isothermal performance is excellent. Since variation of a few degrees can change the form of many materials, this control and precision is important. DSC offers other advantages: the major use of DSC is to determine the precise temperature of a transition and the energy of that transition. Transition temperatures are used as indicators of changes in physicochemical properties of most polymeric and pharmaceutical materials. Measuring subtle shifts in these temperatures is important in tracking these properties. More importantly the energy of these transitions is very important in understanding material properties. For example, in a polymeric material the energy of the melting peak gives you the enthalpy of melting, which is a function of the crystallinity of the material. In pharmaceuticals, the enthalpy tells you about the type of polymorphic rearrangement you have and can be useful in determining pseudo-polymorphism.

Combining the two techniques allows one to strengthen the interpretation of the data over each technique alone. For example, Raman spectroscopy will see the conversion of one polymorphic form to another while the DSC gives you the energy needed for that transition and the precise temperature at which it occurs. In addition, as double furnace DSC controls temperature directly, it will prevent the Raman laser from causing bulk heating in the sample. As we will see, this is a significant concern.

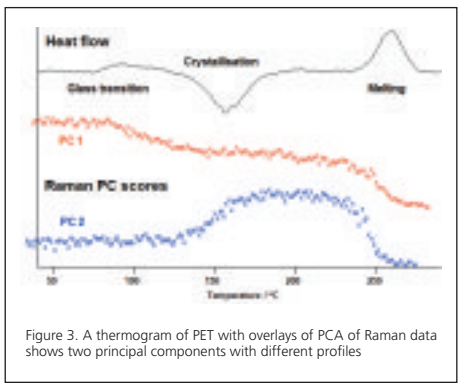

Figure 3. A thermogram of PET with overlays of PCA of Raman data shows two principal components with different profiles

Considerations in Making DSC-Raman Spectroscopy Work

Combining the two techniques allows one to apply the precise temperature control of the DSC with the ability of Raman to detect the different polymorphic structures allowing precise characterization of the material. As an example, consider the common painkiller, acetaminophen. Raman spectroscopy has identified the three solid state forms of this material found under these experimental conditions. (There is a fourth form not seen here.) Combining this technique with DSC allows us to measure precisely the temperatures at which they occur. Figure 2 shows the DSC run on a sample of acetaminophen. The thermogram shows 2 exothermic events believed to correspond to polymorphic changes in the material as well as the endothermic melt. When DSC-Raman is done, the Raman spectra show the conversion to Form II at ~90 °C and then to Form III at ~151 °C before melting. This greatly simplifies the understanding of the thermal behavior of the material and is much more conclusive than attempting to draw this information out of either conventional and/or modulated temperature DSC or Raman spectroscopy alone.

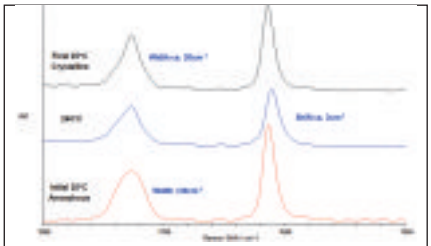

Figure 4. Band shifts in the Raman spectra of PET at selected temperatures in the DSC run.

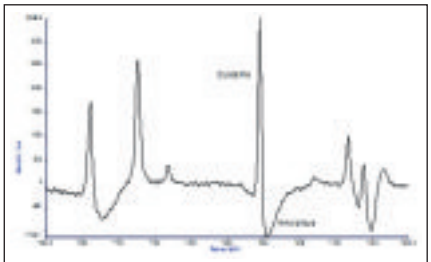

Figure 5. The PCA analysis and the bands associated with crystalline and amorphous structure in polyethylene.

One, the ideal sample geometries are quite different and this leads to several problems. The DSC sample is placed in a metal pan that is several millimeters in diameter and is best presented as a thin layer on the base of the pan for optimum heat transfer, which results in weak Raman intensities when short collection times are necessary. Additionally, the quartz windows used to contain the sample will reduce the signal. Finally the small area (typically 100µm) from which the Raman signal is collected raises the question of whether the measurement is representative. DSC sample pans are often sealed both to maintain a good sample environment and to protect the instrument from off gassing but to obtain Raman spectra the pan has to be open or covered by a quartz window. Both of these choices can adversely affect the DSC performance.

A second issue, the energy from the laser must be dissipated, thus will also affect the heat flow as well as causing localised heating that may distort the DSC data. In extreme cases the laser heating may induce transitions before they occur in the rest of the sample. These problems are much worse in single–furnace, heat flux DSC than in double–furnace, power controlled DSC. Figure 1 shows the affects of the laser induced heat gain in the bulk sample. Use of a double furnace DSC greatly decreases these effects. The presences of spikes in the heat flow data caused by the laser still must be dealt with by a software method, but this is relatively easy.

Finally, the introduction of the probe into the DSC environment creates a path for heat loss from the specimen, and in the case of sub-ambient operation, a path for moisture to get in and cause frosting. These can be addressed by proper design of the coupling system and proper choice of the purge gases used. In many cases, where sub-ambient application isn't needed, no problem is found. Heat loss at high operating temperatures is not really an issue as the black body radiation swamps the Raman spectra at temperatures higher than 350-400 °C.

Applications of DSC-Raman Spectroscopy

Combining the two techniques allows one to apply the precise temperature control of the DSC with the ability of Raman to detect the different polymorphic structures allowing precise characterization of the material. As an example, consider the common painkiller, acetaminophen. Raman spectroscopy has identified the three solid state forms of this material found under these experimental conditions. (There is a fourth form not seen here.) Combining this technique with DSC allows us to measure precisely the temperatures at which they occur. Figure 2 shows the DSC run on a sample of acetaminophen. The thermogram shows 2 exothermic events believed to correspond to polymorphic changes in the material as well as the endothermic melt. When DSC-Raman is done, the Raman spectra show the conversion to Form II at ~90 °C and then to Form III at ~151 °C before melting. This greatly simplifies the understanding of the thermal behavior of the material and is much more conclusive than attempting to draw this information out of either conventional and/or modulated temperature DSC or Raman spectroscopy alone.

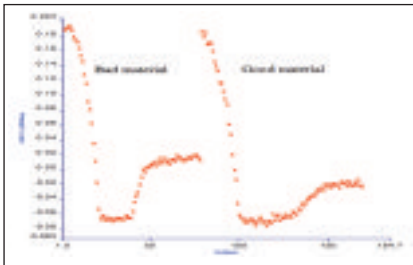

Figure 6. PCA scores for good and bad material

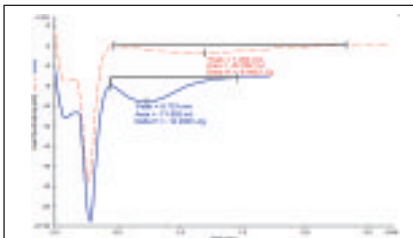

Figure 7. Thermograms for the isothermal re-crystallization of good (red) and bad (blue) PE samples. The double peak at the beginning is the transient associated with the instrument reaching isothermal.

While the transitions here are exothermic, other polymorphic materials show changes caused by the loss of water or some other solvate from the molecule that appear as endothermic transitions. While Raman spectroscopy with a hot stage might determine the changes in the material accurately, without the understanding of the thermal data and the energy of transition, it is possibly for a water loss to be seen as a polymorphic rearrange. In DSC, we could detect this is an endothermic event and hence suspect. Other DSC methods can then be used to clarify the issue.

In applying DSC-Raman spectroscopy to polymers, the ability to understand crystallinity is an obvious area. Semi-crystalline and crystalline polymers are of great economic importance and understanding their behaviour is of great concern. For example, PET (polyethylene terephthalate) that has been rapidly cooled from the melt has a considerable amorphous content, which makes it clear. On heating it undergoes a glass transition and crystallisation. This example was cooled in the DSC at 200 °C/minute. The glass transition is clearly visible in the heat flow curve but is not seen in the Raman data (Figure 3). The spectral changes are relatively small consisting of band broadening and small band shifts. The curve derived from PCA does show the crystallisation and melting events, but is very noisy. The major change associated with these is broadening of the 1726cm-1 C=O band. There are also band shifts that vary almost linearly with temperature (Figure 4). The DSC data clarifies the situation by clearly showing the strong endothermic peak that corresponds to crystal formation on heating. Here we see the reason the material is so much more crystalline before the melt than at the start of the run is the large amount of it that crystallizes during the run.

DSC-Raman can also be done using both controlled cooling and isothermal re-crystallization experiments. These approaches as well as fast scan DSC and modulated temperature DSC allow us to change the way the sample is heated and cooled to manipulate its properties and to trap or exclude certain transitions. In the case study here, two PE samples, a good and a bad, were first fast heated to above their melting temperature and then cooled slowly to room temperature. All the data from each of the runs of the 2 samples was put into PCA models. The data were normalized to allow for differences between the samples. There is a single important PC which shows narrow bands from the crystalline phase and broad bands from the amorphous. (Figure 5) The scores for the two samples show crystallization for both samples. For the controlled cooling, the scores plots for heating and cooling of the two samples are very similar. The top two pictures are direct from the PCA program. The noise levels in the plots are too high for any major differences to be seen but the general shape of the curves suggests the bad material is faster at crystallizing (Figure 6). The associated DSC runs show that the material gives a nice distinct crystallization peak on cooling and small differences are noted here that

were not as apparent in the Raman spectra. These are very small however and a more aggressive type of cooling was then used to enhance these differences.

For the isothermal studies, the samples were heated to above the melt as before, but then cooled at 200 °C/min to a temperature where polyethylene was known to re-crystallize. The Raman Spectrometer can collect data during the rapid heating of the material to the melt – although the fast heating will limit the number of spectra collected - and then on both the cooling and isothermal hold stages. After this experiment, the data is more conclusive in the Raman as well as in the DSC. The scores for the two samples show crystallization for both samples. For the starting materials and the high temperature form the scores are similar for both samples (Figure 7). After the re-crystallization, it appears that the degree of crystallization is greater for the 'bad' sample than for the 'good'. The final spectra clearly show that the 'bad' sample ends up more crystalline. The sharp bands are stronger relative to the broad bands from the amorphous material. Taking the melt as a starting point, we obtain a value of about 50% more amorphous in the good, confirmed by subtraction of the melt spectrum from the final spectra of each.

There is an isolated band from crystalline material at 1125cm^{-1} that can be used to normalize between the two samples. The normalization limits quantitative interpretation but on a relative scale it appears that the final crystalline content of the 'bad' sample is about 30% higher than that of the good one.

In addition, the material responds re-crystallizes faster as seen in Figure 7. Looking at the enthalpy of crystallization, we find the bad material has a higher value of -71.00 mj/gram while the good is much lower at -44.96 mj/g. This gives us roughly a 35% difference in crystallinity between the two materials. Interesting, neither sample comes close to the crystallinity of the material at the start. Some of this is probably due to orientation in the material, but a large part is due to higher cooling rates used in making the films that were used here.

Together the DSC and Raman data explain the problems with the bad sample are due to the material responding differently to cooling and becoming more crystalline.

Conclusions

DSC-Raman spectroscopy is a powerful tool for gaining greater understanding of material behavior, whether it is a polymer or pharmaceutical. Combining Raman spectroscopy's ability to elucidate chemical and structural information with DSC's precise measurement of temperatures and the energy of events allows us to gain a fuller picture of how a material behaves.

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INDIA ANALYTICAL SCIENCES - Bio-discovery

The Determination of Iodine Value, Free Fatty Acid and and trans-unsaturation in the major commodity vegetable oils Using a FT-IR Edible Oil Analyzer.

Re-published: Dr. P.S. Jain-General Manager
Material Characterization

Traditionally, analytical methods are labor intensive and time consuming and are being replaced by new, faster and easy to use systems as well as on-line methods. These new analyzers are measuring closer to production process and use the latest computer technology for optimal flexibility. This article highlights how an integrated multi-parameter Edible Oil Analyzer changes traditional laboratory wet chemistry activities into a single method, OneTouch operation Analyzer. Peanuts, popcorn and potato chips. Though we may enjoy these simple snacks, determining which oil to use to make or bake these foods, and ensuring its quality requires a great deal of preparation. From the seed crusher to the food manufacturer and ultimately the consumer who buys cooking oil off the shelf, the edible oil industry is a diverse market with many characteristics at each step of production.

Edible oils contain fatty acids which are essential to maintaining a healthy diet, so they are vital to the food processing industry. Consumers and manufacturers have a variety of oils to choose in different foodstuffs including soybean, palm, sunflower, and cottonseed and also fish oil to name just a few.

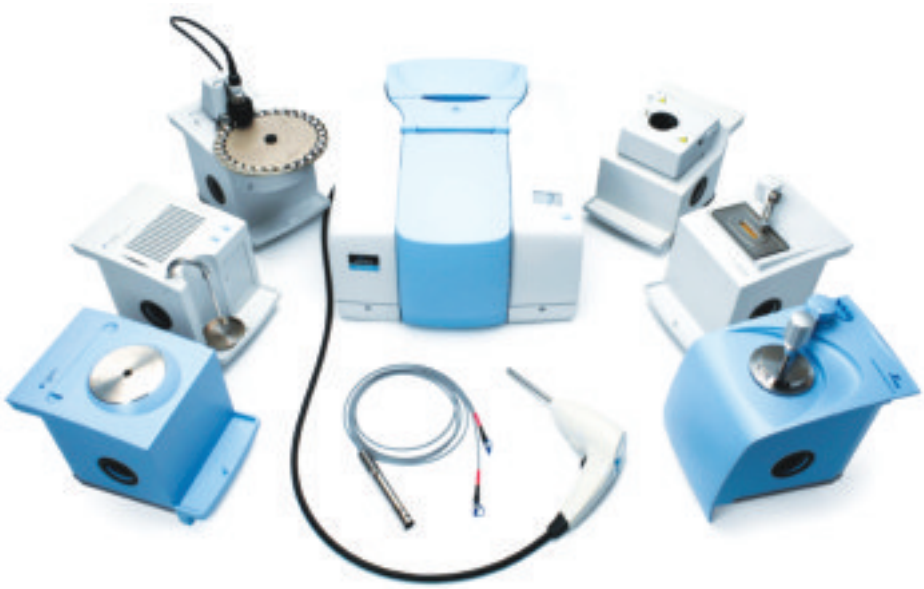
A number of consumer's health concerns, whether valid or perceived, include cis-trans saturation, lower cholesterol levels and the need to determine authenticity, functionality and nutrition labeling, quality and safety. The primary reasons to perform authenticity analyses within this industry are to detect economic fraud and determine purity. The prices of edible oils vary with supply and demand. Higher prices may be charged based on plant type, crop origin, seasonal availability, and the processing of the crude oil. Analytical techniques to determine the purity and source of the oil can help prevent both economic fraud as well as assure product quality. These techniques also allow food manufacturers -- who buy specific oils to meet product recipe requirements -- to confirm the identity of the raw ingredients and determine if adulteration - substitution of a less expensive oil - has taken place.

Parameter	Current Technique	Constraints
Free fatty acids	Sodium hydroxide titration	Requires skilled operator
Iodine Value	Iodine titration	Requires skilled operator, uses expensive reagents, and generates toxic waste.
Iodine Value	Fatty acid prpfile by GC	Time consuming (1hour)
Iodine Value	Refractive Index	Very imprecise.
Trans-unsaturatin	Fatty acid prpfile by GC	Time consuming (1hour)
Peroxide value	Titration	Requires skilled operator, slow (1hour)

Hydrogenation is the process that changes natural fats and oils into forms that are more suitable for use in recipes. Some of the functional characteristics attained by this include creaming capabilities, frying stability and sharp melting properties. A wide range of specialty products can be produced through the hydrogenation of natural oils. Hydrogenation increases the hardness of fats by reducing the level of unsaturation. Iodine Value (IV) is the traditional method used to measure the degree of unsaturation of an oil or fat and is a key parameter used in the industry today. However, many of the official IV methods use carbon tetrachloride or other chlorinated hydrocarbons as the solvent for titration. Due to the global safety issues associated with these compounds many government agencies have banned the use of these materials. Alternate methods to monitor IV include refractive index (RI), GC, Fourier-Transform -Nuclear Magnetic Resonance (FT-NMR) and Near Infrared (NIR) spectroscopy. None of these techniques are suitable for process monitoring. While RI and NIR are quick they lack precision; the

others methods are either time consuming or expensive.
Hydrogenation can lead to increased levels of trans fatty acids (tfa). This has become undesirable as consumption and the resulting high levels of tfa's in human diets have been linked to an increased risk of cancer and heart disease. An outline of the traditional techniques and some of the associated constraints currently employed by the industry are given in the table below
It has long been known that infrared spectroscopy can successfully be used to analyze oils and fats.
Initial research1 focused on the Iodine Value (IV) and Saponification Number (SN); two of the more commonly used analyses for characterizing fats and oils. The calibration standards were devised artificially from pure triglyceride oil rather than 'real' industrial samples and the spectra were all generated using Attenuated Total Reflectance (ATR). This initial work used the Partial Least Squares (PLS) technique to achieve quantitative results. Several papers on the analysis of edible oils using IR spectroscopy exist detailing this work including the determination of free fatty acids, trans unsaturation and peroxide value4. Perkin-Elmer's Edible Oil Analyzer uses Fourier Transform infrared spectroscopy to measure simultaneously a range of key quality parameters, as detailed above, which are important at various stages of the edible oil production process. The analyzer is pre-calibrated to determine the Free Fatty Acid (FFA), Iodine Value (IV) and trans-unsaturation (tFA) values for most major commodity vegetable oils including Soybean, Palm, Rape/Canola and Sunflower

The Edible Oil Analyzer based on Spectrum 100 FTIR to be used to



generate all the spectroscopic data. The sample introduction is comprised of a transmission cell, sample pump, and transfer line, all of which are heated. All spectra were recorded at 80°C with a transmission path length of 0.1mm. The analyzer first generates an infrared transmission spectrum of the sample and by maintaining the sample in a liquid state throughout the measurement, high melting point fats can be analyzed. Further calculations are based on the chemometry module and the QUANT + software. This eliminates the factors which are not responsible for determining the specific Iodine Value.

Conclusion
The goal of developing an FT-IR based solution to analyzing edible oils is to provide the industry with an accurate and reliable alternative to traditional methods. Such methods have proven to be slow and expensive. By developing a system that is easy to use and performs consistently, considerable savings can be observed with respect to analysis time and production. Using spectroscopy to analyze key quality parameters provides a considerably faster route to accurate information. This method can help overcome some of the key production issues faced by the industry today.

The detail methodology and experimental conditions are available from PerkinElmer along with the system.

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The Analysis of Baby Foods and Juices for (As) to Protect a Sensitive Population

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Babies and small children are an especially sensitive population to exposure to environmental contaminants. Their small mass and developing systems, including brain development may show adverse health effects from even low levels of contamination on a chronic or single dose case. Foods, infant formula, milk, and water provide significant exposure routes for metal contaminants. The effect of lead exposure at low levels has been well established and levels below toxic have been shown to contribute to behavioral and learning issues. Other elements, such as arsenic and cadmium can contribute to cancer, neurological effects and diseases. This work will describe measurements of a variety of toxic Arsenic at low concentrations in fruit juices and fruit purees. Sample preparation and the effect on detection limits will be described. Analysis has been done with Graphite furnace atomic absorption (GFAA) and an overall approach to analysis will be described. Samples of a variety of fruit juices and fruit purees of different brands were purchased from a local supermarket. Two milliliters of juice or one gram of puree were taken in duplicate for digestion. Microwave digestion was used (Multiwave™ 3000, PerkinElmer®, Inc. Shelton, CT USA) to obtain clear solutions. Six mL of nitric and 0.5 mL of hydrochloric acid (GFS Chemical™, Columbus, OH USA) were added to Teflon® vessels and the digestion program shown in Table 1 applied. The digestate solutions were then transferred and diluted to 25 mL with ASTM Type I water. The samples were fairly homogeneous and in a form that allowed a representative sample to be easily taken. Preparing replicate samples will allow us to evaluate if our homogeneity assumption is accurate. The AAnalyst™ 800 atomic absorption system (PerkinElmer, Inc., Shelton, CT USA) was used for the graphite furnace measurements. The AAnalyst 800 uses a transversely heated stabilized temperature platform system and Zeeman background correction to ensure the minimum influence of matrix interferences possible. The results show very low levels of arsenic measured in the baby food and juice matrices. The standard deviations are very low showing good agreement between the three replicates measured on each sample. The relative percent differences (RPD) between the duplicate sample preparations show the material is homogeneous. Good agreement is generally considered to be less than 20% RPD, so the measurement in pear puree is likely due to the increase in variation generally seen when measuring close to the detection limit.

Metals, including arsenic can be hazardous to health, especially that of a sensitive population, such as children. Therefore materials that provide significant exposure, such as food, should be closely monitored to ensure that concentrations of elements that might be hazardous should be very low. The results measured in this

Table 1. Microwave Digestion Program.

Step	Power	Ramp	Hold	Fan
1	750	10:00	10:00	1
2	1200	10:00	10:00	1
3	0		15:00	3

Table 2. GFAA Arsenic Results.					
Sample ID	Mean (mg/kg)	SD (mg/kg)	%RSD	RPD	% Recovery
B Pear Juice	0.010	0.001	12	9.9	93.9
G Pear Juice	0.015	0.0006	4.3	3.3	90.0
B Grape Juice	0.027	0.002	8.2	0.7	85.0
B Apple Juice	0.012	0.001	7.8	3.4	92.6
G Apple Juice	0.018	0.0003	1.6	4.7	
B Cherry Juice	0.010	0.0008	7.5	23	
B Pear Puree	0.005	0.002	35	55	
95.7					
G Pear Puree	< 0.003				
B Apple Sauce	< 0.003				
HP QC TM-A*	9.995	0.051	0.51		99.9
High Purity Water Quality Control Standard = 10 ug/L As. Spike 2.5 ppb					

set of samples were very low and did not violate any of the current standards. Additional method quality checks were done to ensure both the GFAA methods was capable at the concentration levels of interest and under control during sample measurement.

GFAA has detection limit capability well below the level of concern and provides an economical choice for smaller laboratories or those with a smaller workload.

The detailed application note can be viewed at:
http://las.perkinelmer.com/Content/ApplicationNotes/APP_AnalysisofBabyFoodsJuicesforMetal.pdf





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Trace Evidence Tools Advance for Forensic

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The field of forensic science consists of evaluation of crime scene evidence to understand and attribute responsibility for a crime. The more certainty that can be attributed to the assignment of responsibility, the more likely that a trial will proceed as expected and be resolved quickly. Everyone has heard something about the ability of DNA testing to match unknown DNA from a crime scene to that of a suspect with extremely high probability. But DNA is not present at every crime scene and requires time and good reason for analysis when it is present. Other more routine analyses provide the bulk of work done in most cases. Trace evidence is the category this work generally falls into. The analysis of latent fingerprints, fibers, hair, arson evidence, gunshot residue, glass fragments and other components present at a crime scene might be in this category.

A recent forensic survey identified the largest problem in the laboratory as being a lack of sufficient personnel to perform tests requested. More productive instrumentation would help to address backlog issues. Increased analytical capability, providing additional information in each analytical run, would improve productivity and also provide more detail to distinguish and unambiguously characterize small trace evidence samples. Two new technologies seeing wider application in forensic laboratories are infrared imaging (FT-IR) and inductively coupled plasma mass spectrometry (ICP-MS). A recent market research report indicates that each forensic laboratory in the US has approximately one FT-IR, but the number of ICP-MS systems doesn't yet register in their measurements. As applications of these instruments become more well understood and productivity simultaneously becomes documented, this will likely change. Each technique will be described and applications explored.

Infrared imaging

Infrared technology has been used in forensic laboratories for many years. Small samples are exposed to infrared light and the absorption monitored over a range of frequencies. The "fingerprint" spectrum provides a nondestructive way of identifying the material or characterizing it in anticipation of a match with a known material. Infrared technology was coupled with microscopy early on to allow visual inspection and identification of the sample portion for infrared analysis and to enhance the analysis of small samples. The development of imaging systems allowed more flexibility in acquiring a large frequency of views with high spatially-resolved spectra that could be individually viewed in detail or displayed in false color to show regions of chemical similarity. The ability to collect spectra in several dimensions automatically with excellent sensitivity and spatial resolution adds productivity. Several examples of how this has been found helpful will be described. Michigan State Police Forensic Science Division (MSPFSD), Lansing Laboratory, helped win a conviction by matching paint from one victim's clothing to the murderer's car. MSPFSD forensic scientists found a microscopic paint chip on the hit-and-run victim's clothing, and then associated each of its five layers to a chip taken from the van driven by the perpetrator. This evidence played a significant role in the killer's conviction for the third murder that he committed that day, a case that was otherwise puzzling because there was no known connection between killer and the third victim. The paint chip found on the victim was very small and five layers of paint had to be analyzed individually. So resolution and sensitivity in the spectral range of interest was important. The MSPFSD has progressed beyond finding an association between a paint sample and suspect vehicle to identifying vehicle model, make, year and color even in cases where there is no suspect vehicle. The Royal Canadian Mounted Police (RCMP) and the Federal Bureau of Investigation (FBI) have created a database consisting of FT-IR results from paint samples taken from a wide range of vehicles, extending the utility of this technique even further.

The detection of counterfeit pharmaceutical medicines as well as over the counter (OTC) products shows another strength of FT-IR imaging. The manufacturing, sale and distribution of counterfeit medicines are not only serious crimes, but also lead to failed treatment, disability, and even death. The World Health Organization (WHO) defines counterfeit medicines as "deliberately and fraudulently mislabeled with respect to identity and/or source" and that "counterfeiting can apply to both branded and generic products and may include products with correct ingredients, with wrong ingredients, without active ingredients, with insufficient quantity of active ingredient or with fake packaging." It is estimated that five to eight percent of the world's total pharmaceutical sales are counterfeit or of dubious quality.

In this case the composition may be very similar between the counterfeit and the real preparation. However the distribution of the excipient or active pharmaceutical ingredient may differ and can be detected by imaging because of the spatial information gained in addition to the composition. Figure 1 shows an FT-IR image of a genuine analgesic with 5% caffeine (top image) and a synthetic "counterfeit analgesic with 5% caffeine added" prepared in the lab (bottom image). The false color image shows the distribution of caffeine is different in the counterfeit preparation when compared to the genuine preparation, indicating differences in the manufacturing process.

FT-IR imaging is taken a step further in work done by the U.S. Federal Bureau of Investigation in examining latent fingerprints non-invasively for identification and examination of trace evidence that might be incorporated

into the fingerprint. The fingerprint can be visualized using the ability of FT-IR to discern latent chemical information found on the fingerprint. Advanced mathematical techniques, such as second derivative calculations on a particular spectral band, can even distinguish a fingerprint deposited on an absorbent paper substrate. Small trace evidence fragments or fibers can be analyzed separately by collection of FT-IR spectra at their location within the fingerprint.

Inductively coupled plasma mass spectrometry

FT-IR can help identify molecular substances, but advances have also been made in the identification of metallic components. Metals analysis has been part of the forensic laboratory tool-kit since the introduction of commercial atomic absorption in the 1960's and used for applications such as gunshot residue detection. Increased sensitivity has been important in reducing the amount of sample that may be consumed, preserving as much of the sample as possible for further analysis. New techniques such as inductively coupled plasma mass spectrometry (ICP-MS) can provide better sensitivity, look at many elements, and provide more sophisticated interference compensation to allow a wider range of matrices to be examined accurately.
ICP-MS is often coupled with laser ablation sample introduction to allow the analysis of small samples with less consumption. A small portion of the sample is vaporized and carried into the ICP-MS with a stream of helium. This is in contrast to the more usual sample introduction which requires digestion of small samples with acid, a procedure that can be difficult and time consuming with some matrices, and even impossible if the sample is too small. A number of journal articles have described the analysis of glass using laser ablation ICP-MS with good success. A large number of elements can be examined yielding more detailed information about the composition of the glass. The more elements that can be used to distinguish glass samples, the higher the probability that a match confirms that pieces of glass originate from the same source. This might allow a better match when several types of glass are collected from a suspect or crime scene.

A recent article summarizes consensus conditions for an internal standard (29Si) and sampling time to ensure consistent performance. Note that the data acquisition is divided into four time segments. This is done to account for background, instability and particle size effects in the ablated aerosol. The article indicates that the technique has potential to provide additional discrimination capability in glass analyses because of ability to provide accurate and precise analysis of major, minor and trace elements in a variety of glass types. A second article expands on LA-ICP-MS research to include the use of an advanced interference correction technique to improve results for iron determination.[7] Iron is present in many glasses at widely different concentrations making it a good candidate for a matching marker. However, as in most analytical techniques, interferences can limit the potential of the technique for detecting low concentrations. The use of a Dynamic Reaction Cell (DRC) in conjunction with the laser ablation ICP-MS allows the interferences from ArO^+ and CaO^+ to be eliminated, improving the detection limit from 9.5 µg/g (without DRC) to 0.03 µg/g (with DRC). Good interference correction options make the technique more robust and allow a wider skill level to operate instruments productively and with good results.

Conclusions

Several new technologies are expanding into forensic science allowing additional information to be productively collected on trace evidence to distinguish individual samples. The more detailed information they provide can contribute to the certainty of matches between crime scene evidence and evidence obtained from suspects.

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VOLUME 8 Monsoon Edition, August 2009
INDIA ANALYTICAL SCIENCES - Bio-discovery

Reduced Solvent Usage and Increased Throughput for PAH Analysis Using UHPLC

Wilhad Reuter, Eric Denoyer, William Goodman,
PerkinElmer

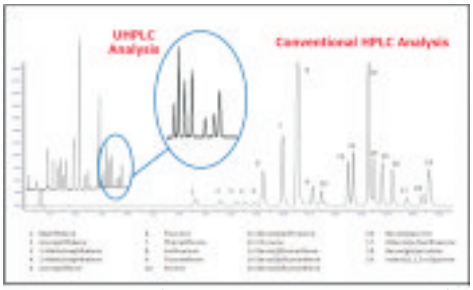

Figure 1: UHPLC analysis of 19 PAHs reduces chromatographic runtime by 3-fold, using 6 times less acetonitrile compared with conventional HPLC.

Introduction

Polynuclear aromatic hydrocarbons (PAHs) are carcinogenic condensed ring aromatic compounds widely found as trace pollutants in waters, wastes, air particulates, soil, and foods. PAHs can be routinely monitored using HPLC with a combination of UV and fluorescence detection as prescribed in EPA methods 550.1, 610 and 8310. Conventional HPLC analysis of nineteen PAHs typically requires 20 minutes and uses 25 mL of acetonitrile. However, there is a continual drive to improve productivity and reduce solvent consumption and waste in chemical analysis. Using Ultra High Pressure LC (UHPLC) with sub-2µm particle-size columns, we demonstrate a 3-fold improvement in throughput and a 90% reduction of mobile phase solvent in the determination of 19 PAHs

Experimental

A Flexar FX-15 UHPLC system comprising a Flexar FX-15 UHPLC pump with vacuum degassing, Flexar FX UV/VIS UHPLC detector (operated at 100 pt/sec data acquisition rate), Flexar Fluorescence Detector, Flexar Column Oven and Flexar FX UHPLC Autosampler (PerkinElmer Inc.), was used for UHPLC analysis. System control and data handling were performed using Chromera chromatography data system (PerkinElmer Inc.). Separation was achieved using a 1.9µm 50x2.1 mm Pinnacle DB PAH column (Restek Corp.) at a flow rate of 0.7mL/min at 45°C. A 4-minute linear gradient from 52% acetonitrile in water to 100% acetonitrile was used with an initial operating pressure of 11,800 psi. Conventional chromatography was run on a Series 200 HPLC system (PerkinElmer Inc.) outfitted with an oven as well as UV/VIS and fluorescence detectors. TotalChrom chromatography software was used for system control and data handling. A 5µm 150x3.2 mm Brownlee Analytical PAH column (PerkinElmer Inc.) was used at a flow rate of 1.2mL/min and a temperature of 30°C. An 11-minute two-step linear gradient from 40% acetonitrile in water to 100% acetonitrile was used, followed by a 9-minute hold with an initial operating pressure of 2,500 psi.

Discussion

Figure 1 shows that a significant improvement in throughput was achieved using UHPLC compared to conventional HPLC. Total analysis time (including equilibration time) was reduced 3-fold. Moreover, excellent resolution is achieved using UHPLC methodology, even while achieving significant throughput improvement. Another important factor is the reduction in solvent consumption. The UHPLC method illustrated here consumes six times less acetonitrile, representing an 83% reduction in solvent cost and waste generated. As laboratories around the world strive to achieve 'greener', more productive operations, the throughput improvement achieved, combined with the reduction in hazardous waste material generated, is a significant benefit of UHPLC.

VOLUME 8 Monsoon Edition, August 2009
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« Hook effect » and AlphaLISA assays

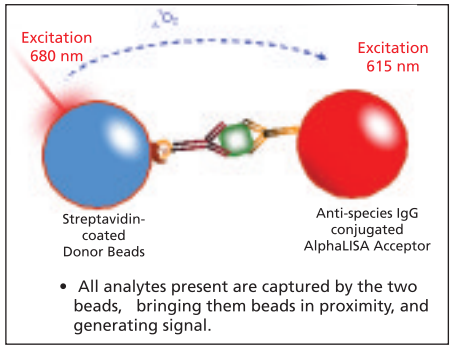
Dr. Niraj Khurana-Marketing Leader BD

Introduction

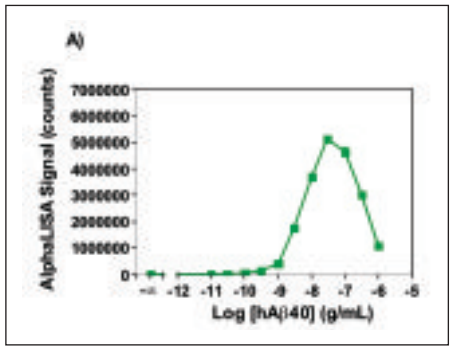
One of our most successful product line on the market is AlphaLISA. This is a technology that enables users to perform assays very similar to the well-known ELISA assay platform, but without the need to wash extensively between each step of the protocols. While very well received by clients, some resistance persists from people. One of the point mentioned as a drawback is the hook effect.

To understand the phenomenon, it is useful to understand the AlphaLISA technology. The process is based on the bringing into proximity of two latex beads. One of these beads is a "donor" bead that will generate singlet oxygen upon stimulation by 680nm laser light. This singlet oxygen will travel up to 200nm in aqueous solution. If the other bead, the "acceptor", is within range, the singlet oxygen will diffuse into that bead, generating a light-emitting chemical reaction. The fact that one donor bead can generate tens of thousands molecules of singlet oxygen upon a single activation creates a greatly amplified signal.

What bring the beads into proximity are molecules interacting between them. In AlphaLISA, the acceptor bead is directly labeled with an antibody against the analyte of interest, while the donor beads bears streptavidine reacting with a second antibody against the same analyte. This creates a sandwich effect, trapping the analyte between both antibodies, and bringing the beads together. (Figure 1)



There could be an "autocompetition" owing to the presence of an antibody pair but it is easy to counter in AlphaLISA and this will actually cause a lowering in signal (figure 2) Similar technologies based on an antibody pair to detect an analyte are also sensitive to hook effects, including ELISA (and derivatives) assays and TR-FRET.



The following figures illustrate the general assay phenomenon
Figure 1 Regular AlphaLISA assay configuration.
Figure 2 Actual data set for Ab40 showing a hook effect over 30ng/mL

Solution to the hook effect

There are several solutions possible to the hook effect.

- The first is to try to increase the amount of antibody that is saturated. In AlphaLISA, that means titration of the amount of beads in the assay.

- The second solution is to test various antibodies to find some with slightly less affinity (more difficult to saturate), especially if the user knows that his material contains large amount of analyte.
- Finally, the best way to reduce the influence of the hook effect is to actually design an assay with the largest possible dynamic range. In an optimal assay, the upper limit of detection should be above what users should expect out of most (if not all) physiological samples. This will allow users to perform assays without the actual need for dilution of their samples.

Things to remember about the hook effect

- The hook effect is present in all assays using an antibody pair, including ELISA and variants, RIA and variants and most TR-FRET assays (but not LANCE Ultra assays).
- The chances of hook effect in our AlphaLISA assays only occur at very large amounts of analytes in samples (usually in the µg/mL), which are way above what is expected in a physiological sample.
- Importantly, researchers inclined on using other technologies due to their fear of the hook effect must be aware that saturation will also happen in their assay, creating a plateau. Data in this plateau region cannot give reliable amount results and will still force users to redo the assay with sample dilutions.

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VOLUME 8 Monsoon Edition, August 2009
INDIA ANALYTICAL SCIENCES - Bio-discovery

Say Goodbye to washing!!!!!

Authors: Uday Mishra & Dr. Niraj Khurana- PerkinElmer (I) Pvt. Ltd. –Bio-Discovery

About 38 years ago, in year 1971 two Swedish scientists, Eva Engvall and Peter Perlman published a paper which revolutionized the way assays were performed. Before this for 11 years (from 1960 to 1971) RIA assays were the only option available to scientists but this paper brought a new, sensitive, simple, easy & non radioactive platform which made the research and even routine assay much simpler and reproducible at the same time. This technology today we know a**s Enzyme Linked ImmunoSorbent Assay (ELISA or EIA). Once biologists started exploring it further some of the inherent limitations of this biotechnique came to light** which in turn prompted scientist to look out for better alternatives based on different detection chemistries/ technologies like fluorescence, time resolved fluorescence, luminescence and fluorescence polarization. Meanwhile a small group of scientists trying to answer all the limitations of ELISA and the questions raised by other alternatives, hit the jackpot…… Let's start with a string of questions that would plague any lab before they can switch their assay technology.

I'm giving a few relevant questions herein, but please logon to the next issue to know more about this what could this "small group of researchers" could eventually come out with:

Are you performing very sensitive assays wherein low levels of physiological analytes need to be measured?

Antibodies are not cheap, we all know. Is your present assay capable of working with low affinity antibodies?

Polyclonal antibodies are solution to various problems, but do your assay is still able to maintain the sensitivity?

I understand that sometime you need to use your own antibody. Does your present assay give you flexibility to use your own antibodies or to compare them with the ones available commercially for your assays?

What if you want to measure a wide range of concentrations and you don't want to dilute your samples? What if I say an assay which works on broad substrates from small molecules to full length proteins to Viruses?

Do you work on biochemical and cell based assays both and are looking for a platform on which both work fine?

What if I say you work on 96 well and move to 384 and then to 1536 by just miniaturizing the volume of components factorially, without validating and exploring the assay parameters again?

Science is race and your research matters if it is reported before anyone else do that. Many labs have moved to automation to achieve this.

What about an assay which is completely automation friendly and involves fewer steps than your present assays?

Are you concerned about S/B ratio? Do you want higher then given by your present assays?

How much Z' value holds significance to your assay? Are you getting somewhere between 0.6 to 0.9?

Is your assay interference proof? I thought of an assay where signals are stable for more than 24 hours. You can prepare your plate and need not look at your clock to read or in case you are in hurry and want to read next day. Does it excite you? And above all what if you can get rid of all those washing steps?????????

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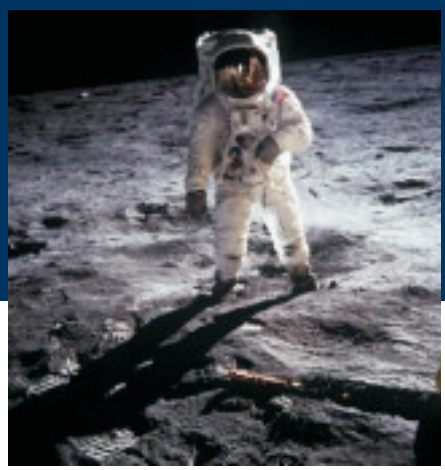

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