Application of Raman Spectroscopy for Noninvasive Detection of Target Compounds

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OTSC Seminar
Raman Spectroscopy 1

- A **vibrational technique** for identification and analysis of molecular species

- Irradiate a substance with monochromatic light and to detect the scattered light with a different frequency to the incident beam

- **Raman shifts**: differences in the frequencies between the incident and scattered radiation
Raman Spectroscopy 2

- Based on the polarity of chemical bonds
- Provide information about the vibrational motions of the molecule: stretching, bending, wagging, deformation, and others
- Well-resolved bands ➔ molecular structure information of compounds

Not fully explored despite of its great possibilities and advantages over other spectroscopic techniques
Instrumentation

- PerkinElmer RamanStation™ 400F bench-top spectrometer
- Efficient & easy to use
- Unique Echelle spectrograph and CCD detector
- Motorized stage: automatic alignment of samples & high throughput
- Fiber optic probe: bring the spectrometer to the sample ➔ results easier to gather
Advantages of Raman Spectroscopy

- **Little or no** sample preparation
- **Small** portions of sample
- **Non-destructive** technique
- **No water** interference
- **Minimal glass** interference
- **Higher spectral resolution and more distinctive bands** ➔ detailed information about structural changes and role of specific components
- Provide a plenty of **qualitative and quantitative** information
- More sensitivity to the **symmetrical vibrations** of covalent bonds
- Consideration as a **routine method of analysis**
Disadvantages of Raman Spectroscopy

- Can not be used for metals or alloys
- Raman scattering is inherently weak
- Visible and ultraviolet laser excitation wavelengths → interference from fluorescence
- Sample heating through the intense laser radiation
Development of Raman Spectroscopy

- For higher sensitivity, improved spatial resolution, and very specific information

- Surface Enhanced Raman Spectroscopy (SERS)
- Resonance Raman spectroscopy
- Surface-Enhanced Resonance Raman Spectroscopy (SERRS)
- Angle Resolved Raman Spectroscopy
- Hyper Raman
- Spontaneous Raman Spectroscopy (SRS)
- Optical Tweezers Raman Spectroscopy (OTRS)
- Stimulated Raman Spectroscopy
- Spatially Offset Raman Spectroscopy (SORS)
- Coherent anti-Stokes Raman spectroscopy (CARS)
- Raman optical activity (ROA)
- Transmission Raman
- Inverse Raman spectroscopy
- Tip-enhanced Raman Spectroscopy (TERS)
Surface Enhanced Raman Spectroscopy (SERS)

- **Traditional Raman spectroscopy**: require bulk samples or concentrated solutions
- Much **more sensitive** method
- **LOD** to ppb level or even a single molecule level
- Aid of **metallic nanostructures** ➔ signal enhanced by $>10^6$ times due to the effects of electromagnetic field and chemical enhancement
- Faster, simpler, minimum sample preparation ➔ satisfactory qualitative and quantitative results
Spectroscopic amplification & Enhancement techniques

- **Chemometrics**
  - non-uniform samples: produce spectra irrelevant chemical information
  - mathematical treatments to reduce scatter effects and extract only meaningful information
  - resolution-enhancement techniques such as derivatives (curve-fitting) and deconvolution before applying chemometrics → reliable prediction

- **Chemical imaging (Hyperspectral or spectroscopic imaging)**
  - spatial and spectral information from the sample

![Image of a caplet and its chemical image](image-url)
Applications

- Applications are fast growing
- Demonstrated its superiority, or at least equality to infrared and other spectroscopic techniques
- Modified and Applied to a variety of food and feed samples
- Capable of analyzing organics, minerals, inorganics, polymers, emulsions, pharmaceuticals, and biomaterials
- Finding more use and applications: microbiology, art and archaeology, color, electronics, forensics, plant control and reaction following
Use of Raman Spectroscopy in OTSC

App 1
Detection of aflatoxin in ground corn samples

App 2
Identification & characterization of food-grade tracers for grain tracing system

App 3
Classification of Bovine Spongiform Encephalopathy (BSE) samples

App 4
Quantitation and classification of camphor in goat serum
Spectra collection

- **Raman shift range**: 200–3500 cm\(^{-1}\)
- **Laser light source**: 785 nm at 350 mW
- **256×1024 pixel CCD detector**
- **Spectral resolution**: 4 or 8 cm\(^{-1}\)
- **Large sample spot** (6 locations around centered 1 location) with an exposure time of 1 sec and 10 scans
- **Baseline correction and normalization**
Detection of *aflatoxin* in ground corn samples

- Raman spectroscopy coupled with chemometrics
- 40 samples (11–1,206 ppb)
- Training data set (30 samples) & test data set (10 samples)
- Collected spectra ➔ baseline corrected and normalized ➔ preprocessed mathematically (1\(^{st}\) derivative, 2\(^{nd}\) derivative, and deconvolution algorithms) ➔ converted to Excel and exported to SAS ➔ chemometrics (PCA, PLS, & MLR) to build calibration models
Detection of *aflatoxin* in ground corn samples 2

* PCA & PLS models ($R^2$): 0.76 - 0.86
* Paired sample t-test (HPLC vs Predicted): $p = 0.950$
Characterization of food-grade tracers

Sucrose-based tracers

* PS = pregelatinized starch, HPMC = hydroxypropylmethylcellulose
1st derivative

2nd derivative

- UN = uncoated
- PS = pregelatinized starch coating
- HC (HPMC) = hydroxypropylmethylcellulose coating
Classification of \textit{BSE} samples

* Using preprocessed 2\textsuperscript{nd} derivative data
Quantification and classification of *camphor* in goat serum 1

\[ y = 2352.1x + 4008.5 \]

\[ R^2 = 1 \]

* At Raman band 648 cm\(^{-1}\)
Quantification and classification of *camphor* in goat serum

* SERS application
* Using preprocessed 1st derivative data
Summary & Conclusions

- Raman spectroscopy: rapid, inexpensive, and convenient
- Use of SERS technique: discrimination performance $\uparrow$ & desired sensitivity and specificity
- Analytical method for quick estimation of target compounds at busy locations
- More rapid qualitative and quantitative characteristics $\Rightarrow$ real-time monitoring
- Successful implementation of a robust model $\Rightarrow$ economic benefits $\uparrow$
- Integrated spectra features into chemometrics $\Rightarrow$ a great potential for automatic detection & online-monitoring quality control
- Many new applications in the future
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