Fluorescence Spectroscopy as a Rapid, Cost Effective Method to Monitor and Analyze Low Levels of Pharmaceuticals and Personal Care Products in Environmental Water Samples

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Outline

1. Introduction
2. Spectroscopy
3. Modeling
4. Conclusions and Future Directions
Part I

Introduction
• PPCP’s in drinking water, cause for concern?

Cost Analysis

• EPA has 109 compounds on Contaminant Candidate List 3 (CCL3)

• Contract GC/MS—LC/MS cost is roughly $125 per contaminant:
  
  • $109 \times $125 = $13,625

• Testing over one year on a weekly basis:
  
  • $13,625 \times 52 = $708,500

• New, less-expensive methods are needed to compliment current techniques
Using Molecular Fluorescence to Detect

- Fluorescence (photon emission) is a naturally occurring phenomenon in many molecules.
Introduction

Fluorescence Spectroscopy

• Fluorescence spectroscopy

  • Very sensitive and signals can be detected at very low compound concentrations

  • Relatively inexpensive when compared to other analytical techniques

  • Is a well described technique used for numerous applications

  • Can analyze mixtures without prior separation
Part II

Spectroscopy Data
• Two fluorescence techniques being explored

1. Synchronous Fluorescence Spectroscopy (SFS)
   • Narrow peak width
   • Easier identification in mixtures

2. Excitation Emission Matrix (EEM) Spectroscopy
   • Three dimensional ‘spectral landscape’ plots
   • Analyzed via multivariate data tools such as parallel factor analysis (PARAFAC)
SFS Data: 17α-ethinylestradiol

- **SFS graphs:**
  - SFS Peak is narrower than ordinary emission peak
  - Each peak has maximum $\Delta \lambda$ intensity value
    - $\Delta \lambda$ is the wavelength difference between excitation and emission wavelengths
  - Intensity is related to compound concentration
EEM Data: 17α-ethinylestradiol

- EEM Graphs
- 3 dimensional representations
  - Color represents intensity (red highest, blue lowest)
  - Graph 1: $4.6 \times 10^{-6}$M, Graph 2: $4.6 \times 10^{-7}$M concentrations
• Filtered Environmental Samples with no added compound
Part III

Modeling
Parallel Factor Analysis (PARAFAC)

• Multi-way data method

• Analyzes several sets of categorical variables measured in a crossed fashion

• Fluorescence emission spectra measured at several excitation wavelengths for several samples varying by concentration of $17\alpha$-ethinylestradiol

• data is three-way: arranged in a cube instead of a matrix as in standard multivariate data sets
Multi-way Data
4 Dimensions, 3 Modes, 3 Ways

Intensity Values

Emission

Excitation

Concentration

Emission

Ex

citation

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<tbody>
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<td>7 41 7 80 23 4 41</td>
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Excitation

I

J

K

Dataset

EEM Stack

EEM
Parallel Factor Analysis

PARAFAC

Factor 1

Factor 2

Score
Concentration
Loading
Emission
Excitation

Score
Concentration
Loading
Emission
Excitation

Factor 1

Factor 2

I
J
K

X

a_1
b_1
c_1

a_2
b_2
c_2
Spiked Environmental Sample Experiment

• Experimental Set-up
  
  • Water sample taken from Penobscot River Orono, ME and 0.45 µm filtered.
  
  • Aliquots of sample spiked with 12 different concentrations of 17α-ethinylestradiol ranging from $4.6 \times 10^{-6}$M to $4.4 \times 10^{-11}$M.
  
  • 1:10 dilution of natural water sample with deionized water. Concentration of natural water sample held constant throughout the experiment.
  
  • EEM data generated for each concentration sample, entered in MATLAB and PARAFAC analysis performed using PLS_Toolbox.
Part IV

Conclusions and Future Directions
Conclusions

- Due to the large number of chemical compounds in water supplies, reliable cost-effective methods are needed for monitoring purposes.

- Fluorescence Spectroscopy is a well described technique used in numerous applications. Applications with regard to water quality analysis should be further explored.

- Our spectroscopy research demonstrates that:
  1. Compounds can be detected at the ppb-ppt range
  2. Analysis can be performed without prior separation
  3. Compound detection and quantification can be determined using statistical modeling technique
Future Work

- Further PARAFAC analysis on different model chemicals and evaluating additional variables in our model:
  - Location
  - Time
  - Multi-chemical factors

- Evaluate EEM data using N-way partial least square discriminate analysis (NPLS-DA):
  - Regression analysis
  - Pattern recognition for separation of classes with high degree of similarity

- Further exploration of SFS:
  - Excitation resolved synchronous fluorescence
  - Derivative SFS
Thanks To....

1. The Patterson research group

2. My undergraduate researchers

3. My fellow authors on this talk

Parallel Factor Analysis (PARAFAC)

\[ x_{ijk} = \sum_{r=1}^{R} a_{ir} b_{jr} c_{kr} + e_{ijk} \]

\[ X_{IJK} = B_{RJK}^{\text{R}} + C_{IKJ}^{\text{I}} + E_{JK}^{\text{K}} \]

\[ X_{IJK} = A_{IKJ}^{\text{I}} + b_1 c_1 a_1 + b_2 c_2 a_2 + b_3 c_3 a_3 + E_{JK}^{\text{K}} \]
Synchronous Scan Fluorescence Spectroscopy

Excitation acquisition at $\lambda_{em}$ 310nm

Emission acquisition at $\lambda_{ex}$ 280nm

$\Delta\lambda=30\text{nm}$  $\Delta\lambda=60\text{nm}$
Synchronous Scan Fluorescence Spectroscopy

Diagram showing energy levels and transitions:
- $S_n$ to $S_2$ to $S_1$ to $S_0$
- ISC (intersystem crossing)
- A (photon absorption)
- F (fluorescence (emission))
- P (phosphorescence)
- S (singlet state)
- T (triplet state)
- IC (internal conversion)

Legend:
- $S_0$: electronic ground state
- $S_1$, $S_2$, $S_n$: excited states
- $T_1$, $T_2$: triplet states

Excited vibrational states (excited rotational states not shown)
Synchronous Scan Fluorescence Spectroscopy

Synchronous Scan Fluorescence Spectroscopy

- Jobin Yvon Fluorolog-3 Spectrofluorometer