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Determining Components of Fluorophoric DOM in Narragansett Bay Using Fluorescence EEMs and PARAFAC

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By

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Abstract

Fluorophoric dissolved organic matter (FDOM) is an important component of the carbon cycle, factors in nutrient cycling, plays a role in determining the fates of trace metals and hydrophobic organic contaminants, and influences the inherent optical properties of water, affecting photosynthetic activities, productivity, and abundance of organisms in the area.

Fluorescence spectroscopy is a sensitive analytical method that can be used in conjunction with the canonical decomposition parallel factor analysis (PARAFAC) to separate component fluorescence signatures embedded in excitation-emission matrices (EEMs) of complex chemical mixtures. In this study, the methods of fluorescence spectroscopy and PARAFAC were used to determine the components of FDOM in Narragansett Bay. The PARAFAC model is input a 3-D array consisting of EEMs of unconcentrated samples from various locations in Narragansett Bay. The model deconvolves these natural mixture EEMs into individual EEM components and assigns relative concentrations for each component. Original component fluorescence signatures are not needed by the model but they improve the accuracy of the deconvolution. At least five FDOM components exist in Narragansett Bay samples. FDOM components 1, 2, 3, and 5 have been positively identified as humic substance, diesel, tryptophan and tyrosine respectively, using standards. A method was also developed to determine the concentration of identified components in mixed samples. The ability to decompose specific fluorescent signatures has the potential to allow the determination of the sources of FDOM in natural waters provided each source has a unique mix of fluorescent components. This work demonstrates that EEMs coupled

with analysis by PARAFAC is a powerful analytical technique in discriminating FDOM in natural waters.

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Preface

This thesis is prepared in manuscript style in accordance with the Guidelines set forth by the University of Rhode Island Graduate School.

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Introduction

Dissolved organic matter (DOM) constitutes the largest pool of reduced carbon in the ocean. It is a food source for oceanic micro-organisms, influences nutrient cycling, and plays a role in determining the fates of trace metals and hydrophobic organic contaminants (Minor, 2002). Dissolved organic carbon includes useable components, including nutrients, that can be released by photodegradation reactions (Rochelle-Newall, 2002; Hansell and Carlson, 2002). Bacterial utilization of dissolved organic substrates accounts for a major portion of the total carbon assimilation at the sea surface (Hedges and Farrington, 1993). Thus, DOM has been increasingly recognized as a significant component of the global carbon cycle (Hedges and Farrington, 1993).

Fluorophoric dissolved organic matter (FDOM) is the fraction of DOM that absorbs and fluoresces light. With both allochthonous and autochthonous sources, (Twardowski and Donaghay, 2001) FDOM can account for between 10% and 90% of DOM in natural water (Thurman 1985; Blough and Green 1995). The optical properties of FDOM have important ecological ramifications. In aquatic media such as seawater, the light field is variable with depth and photosynthetically available light is an important factor in determining the amount of biological activity in marine aquatic environments (Kirk 1994). FDOM can attenuate spectral components of the underwater light field, influencing the inherent optical properties of natural water and affecting photosynthetic activity. Through absorption, FDOM can decrease the amount of solar irradiance, limiting primary production. Conversely, FDOM can also

act as a shield against UV damage for aquatic organisms, increasing primary production in the surface waters (Stedmon et al. 2000; Rochelle-Newall, 2002). FDOM effects the transfer of light thereby influencing the depth distribution of primary production and the abundance of organisms found in the water column (Hansell and Carlson, 2002; Stedmon et al. 2000). FDOM is also involved in and may regulate photochemical processes occurring in the upper ocean. Photoreactions often produce free radicals and oxidants, e.g.,

$$ROOR' + h\nu \rightarrow RO\bullet + R'O\bullet$$

that then go on to participate in secondary reactions (Helz et al, 1994; Zika, 1981; Zarifou et al, 1984; Zepp, 1988). For these reasons, determining FDOM distribution and sources in natural waters enhances our knowledge of aquatic physical, biological, and chemical processes.

FDOM includes many ecologically and environmentally important chemical compounds such as humic substances, amino acids, and hydrocarbons. Coble (1996) showed marine systems have 1.) "humic-like" fluorescence consisting of two peaks at excitation 260 nm and 300-370 nm with a common emission maximum ranging from 400-500 nm 2.) a protein signature representative of amino acid-like fluorescence attributed to tryptophan and tyrosine 3.) a tryptophan-like peak at excitation of 275nm with an emission of 340 nm and 4.) tyrosine-like fluorescence at excitation 275 nm and an emission of 310 nm. While studies, including those of Coble (1996) and Determann et.al.(1994), used results from both excitation and emission spectra to identify humic substances and amino acids in seawater, there was no direct evidence of these compounds since compound specific chemical analyses were not conducted.

Since FDOM is comprised of multiple fluorophoric compounds, or moieties, capable of absorbing and fluorescing light, optical spectroscopic methods have been used as a way to categorize its characteristics and as an indicator of specific compounds. After the discovery by Kalle (1966) that organic matter in natural waters fluoresces when irradiated with UV radiation, work focused on the use of fluorescence to observe organic matter in the environment and to track the movement of water masses. Fluorescence has been used as a way to quantify the amount of DOM and the seasonal variations of its production (Laane and Koole, 1982; Laane, 1982; Laane and Kramer, 1990). Fluorescence has been used to differentiate between water masses and to determine riverine versus marine sources for this organic matter, thus providing a way to trace water masses and their sources. For example, Otto (1967) was able to differentiate between water masses based on the freshwater inputs of different rivers in the southern North Sea, and Zimmerman and Rommets (1974) determined the relative contributions of two fresh water sources to the marine environment. These and similar studies used fluorescence at single wavelengths of excitation and emission and focused on fluorescence intensity, position of intensity maximum, and salinity measurements to draw their conclusions.

In order to get more information from the fluorescence, Cabaniss and Shuman (1987) performed synchronous fluorescence measurements, whereby spectra are obtained by scanning both excitation and emission wavelengths at a fixed wavelength or energy difference. These excitation and emission measurements were used to determine sources and contributions to mixtures using linear regression analysis.

The penultimate use of scanning excitation and scanning emission fluorescence instruments allows for the measurement of excitation-emission matrices and a more complete description of the fluorescence properties of natural waters. An excitation-emission matrix (EEM) is comprised of spectral fluorescence emission as a function of spectral fluorescence excitation. It is usually constructed by acquiring consecutive emission spectra at multiple excitations. Synchronous scans are thus the equivalent of taking diagonal cross-sections through full emission excitation matrices. The use of fluorescence EEMs expands the use of maximums, minimums, and peak locations by looking at the whole 2-D spectra to determine different fluorescence patterns, and further separate water DOM into its different chemical components. EEMs produce wavelength independent data making them more useful than other traditional methods of fluorescence. EEMs characterize the number and type of fluorophoric groups comprising water masses and can be used as a sensitive measure to differentiate water masses (Del Castillo et al, 1999; Desouza Sierra et al, 1994). Analyses have applied EEMs to differentiate waters of rivers influenced by both natural and anthropogenic sources (Baker 2002, 2001).

Previous studies have used EEMs to distinguish fluorescence peaks. While each peak was assumed to represent a unique chemical component, the peaks could not be identified because their chemical identity had not been positively determined (Coble, 1996). Regardless, peaks were assigned to represent tryptophan, tyrosine, and humic substance due to the position of their fluorescence peaks (Coble,1996). Other studies have used chemical evidence to back up the interpretation of peaks being characterized as humic and protein like (Mopper and Schultz, 1993).

The common factor in all these studies is that by visual inspection and Principle Component Analysis (PCA), regions of EEMs were determined to signify chemical components responsible for the fluorescence of natural waters. The use of PCA, however has been questioned for the identification of peaks because it is a 2-way model whereas EEMs inherently have 3-way character (Bro, 1997).

Stedmon et al. (2003) used instead the canonical decomposition parallel factor analysis model (PARAFAC). By using PARAFAC, a stacked 3-D array of EEM's may be separated into the individual components present in each EEM. PARAFAC uses the differing concentrations of the components to deconvolve the EEMs. Using this method, they characterized FDOM in their samples into 5 different components that were either allochthonous or autochthonous in nature but did not further speculate on the identity or concentration of the components.

Here we show how PARAFAC can be used to determine the number of components and their relative contributions, and its extended use to determine the identity of components and their concentrations in natural complex mixtures, such as sea water, with the use of standards. For the first time, the fluorescence peaks of natural water samples are chemically identified. Peaks are separated based on changes in the concentrations of the components in various natural samples and not merely picked based on visual inspection. This work describes the development of a new chemical analytical method and its initial use in Narragansett Bay, RI.

Determining the components and concentrations of fluorescent DOM will aid in the understanding of the processes governing the cycling and distribution of carbon in natural waters. Component determination can help to identify input sources of

fluorescent DOM and will help in monitoring these inputs in coastal waters and in planning urban projects that may affect the types of inputs affecting a region.

Methods

Research Site

Narragansett Bay is a Rhode Island estuary with a volume of 706 billion gallons at mid tide. The bay reaches 25 miles in length and is about 10 miles across on average. The average salinity is 29 to 31 ppt but ranges widely. It's major tributaries include the Blackstone/Providence, Tauton, and Pawtuxet Rivers. Narragansett Bay has a daily freshwater input from rivers of 2.1 billion gallons (Ely, 1988). The average depth of the bay is 7 meters. It's average flushing time is 26 days with a tidal range of 1-1.2 meters (Ely, 1988; Pilson, 1985). Approximately 186 million gallons of treated sewage, accounting for about 7% of the freshwater input, enters the bay per day (Ely, 1998).

Narragansett Bay is an ideal site for this type of study for several reasons. First freshwater input from several different sources was expected to provide water types with several different fluorophore mixtures. Second, the coastal environment and tributaries have multiple sources of organic matter input including anthropogenic inputs and biological productivity, and estuaries are traps for organic matter. Highest organic matter concentrations are often found in estuaries due to sharp salinity and pH gradients, and areas of strong mixing and turbulence (Shi, 2001; Hansell and Carlson, 2002). Thus, FDOM, being a component of organic matter, is also higher in concentration. Third, sewage treatment plants and combined sewer overflows are another significant source of organic matter to the bay.

Field sampling

Samples were collected from various areas around Narragansett Bay on July 9, 2003 off the edge of docks on shore from East Greenwich Marina, Apponaug Harbor Marina, Oakland Beach, Highland Beach, the Pawtuxet River, Sabin Point Park, Tyler Point, and Mount Hope Bay. On December 3, 2003, sites were sampled from aboard a 22' research boat operated by WET Labs from several areas within East Greenwich Bay at both surface and at depth (Figure 1). Samples were collected in pre-rinsed bucket samples or niskin bottles and filtered through precombusted Whatman GF/F filters. Light protected polyethylene containers were used to transport samples for EEM analyses. These containers were acid and base washed, and rinsed with Nanopure water, water from a 4-cartridge Barnstead Nanopure system, to remove any organic contaminants. The samples were stored on ice, in the dark until arrival at the laboratory for fluorometric analysis. Samples were either anlayzed upon arrival at the laboratory or refrigerated and analyzed within a day.

Laboratory Procedure

Fluorometric analysis was accomplished using a Jobin Yvon – SPEX Fluorolog -3 Model FL3-21 with a double-grating spectrometer at the excitation position and a single-grating emission spectrometer. The Flourolog uses a 450 watt Xenon arc lamp as the light source and a photomultiplier tube (PMT) detector. A 1 cm^2 cuvette was used for analysis and was acid, base, solvent, and Nanopure water rinsed prior to use. Samples were allowed to warm to room temperature before analysis. An EEM was obtained for each sample by scanning at 5 nm intervals over

the spectral range of 240-360 nm excitation and 300-500 nm emission with a 1 second integration time. The excitation and emission slit widths were 5 nm each, yielding bandwidths of 10 nm. Scanning parameters were based on results from other studies (Stedmon et al., 2003; Coble et al., 1998; Blough and Del Vecchio, 2002), preliminary analysis of the bay samples, and the best compromise between the length of time needed to run the samples and minimal degradation of fluorescence intensity from photo-bleaching. Analysis time was 20 minutes per sample.

EEM Corrections

The Fluorolog spectrofluorometer automatically provides quantum normalized EEMs. Each day the instrument was used, an EEM of Nanopure water was taken to be used for data correction. The daily EEM of Nanopure water is representative of any instrument specific changes and allows for better correction of the spectra. For each sample EEM, the area under the Raman peak of the sample EEM is compared to the Raman peak area of the EEM of Nanopure water at an excitation 305 nm using a baseline subtraction. The excitation at 305 nm was chosen as a middle value of the excitation wavelength range. These areas are used to derive a scaling factor which is then modified per excitation wavelength based on a typical UV absorption spectrum. The sample EEM is multiplied by these scaling factors to account for any inner filter effects that are wavelength dependent to make the spectra comparable to the Nanopure water EEM. These so-called "inner filter" effects result from scattering and absorption within the sample and reduce fluorescent intensity. The Nanopure water EEM is then subtracted from the scaled sample EEM to remove the Raman scatter

ridge. The EEMs are normalized to the integrated area of the Nanopure water Raman peak at an excitation of 350 nm, consistent with previous studies (Determann,1994; Stedmon et al, 1998). Figure 2 shows the EEMs obtained from each step of the correction process. The resulting data is Raman corrected and the EEM is in Raman units which can be directly compared to corrected spectra from other instruments since Raman normalization removes biases from excitation and emission slit windows, etc. specific to an instrument (Stedmon et. al. 2003). Using the areas of the peaks provides more accurate results and is more sensitive to instrument and sample affects.

PARAFAC Method

By employing the capabilities of the canonical decomposition parallel factor analysis (PARAFAC), a Matlab program consisting of the algorithms of this method, originating from psychometrics (Bro, 1997), was used to decompose the EEM components of complex mixtures. For EEM applications, the 3- way array has variables of excitation, emission, and sample number. In other words, each EEM, consisting of excitation and emission intensities, is loaded into a composite. This composite consists of each sample EEM stacked on top of each other. For these analyses, a nonnegativity constraint is used which prevents any negative spectra or contributions since these results would not make physical sense with this form of data. PARAFAC uses the equation:

$$E_{ijk} = \sum_{r=1}^{R} z_{ir} x_{jr} m_{kr} + b_{ijk}$$

where E_{ijk} is the samples input as a composite array with dimensions consisting of sample number (i), excitation(j), and emission(k). R is the number of components, and

b_{ijk} is the instrument background. PARAFAC minimizes the sum of the square of residuals to model z, x, and m, the relative contribution, excitation, and emission, respectively, of each component (r) (Bro, 1997; Stedmon et al., 2003). In other words the model separates mixed EEMs into individual EEM components and assigns relative proportions/contributions for each component.

PARAFAC requires that the suspected number of contributing components be input to the model. This is done by trial and error, based on 1) a count of the number of peaks and shoulders on an EEM, 2) the uniqueness of the EEMs from the possible components. For example, if it appears there are 3 possible components in a given sample, but the EEMs for 2 of them are very similar, modeling the unknown sample EEMs as the combination of 2 components may be most appropriate. This applies in the instance of humic and fulvic acid. These two peaks look the same, cannot be deconvolved by PARAFAC and are counted as one. Residuals, trends in contributions, and comparison with standards are used as determining factors for the validity of the component number. Residuals are the calculated difference between the results from PARAFAC and the original corrected EEMs. They are constructed by subtracting the simulated EEM constructed based on the results from PARAFAC and the original corrected EEMs obtained from the sample/standard. Trends in contributions are determined from the plot of relative contributions provided by PARAFAC.

Standard Calibration

For suspected components a standard solution was made and analyzed to determine if there was a linear relationship between the intensity of fluorescence and the concentration of the anlayte. A small amount of the solid standard, either fulvic acid, humic acid, tryptophan, or tyrosine, was weighed and diluted to make desired concentrations. The EEM of this stock solution was recorded along with EEMs of subsequent dilutions. Nanopure water at a pH of approximately 8 was used to make up and dilute the standard solutions. An EEM of this pH 8 water was taken as a blank for the standards and used for the corrections.

Results

Narragansett Bay Samples

Figure 2 shows the results of the EEM corrections on the samples. It can be seen that these Raman corrections removed the water, Raman and inner filter effects. Only minor residuals were noted in a few samples. Values obtained for intensity ranges in Raman units (nm^{-1}) agreed with those reported for previous studies (Stedmon et al., 2003).

Initial inspection of EEMs from Narragansett Bay showed some differences in the number of peaks and their relative intensity. The most noticeable difference was the presence or absence of a peak within the excitation range of 240-305 nm and the emission range of 320-400 nm. This range falls in the area expected for protein and hydrocarbon signatures (Coble, 1996). The samples from Apponaug (Figure 3) and East Greenwich Marina exhibited this peak most noticeably. This peak was most evident in samples with lower overall fluorescence intensity.

Fluorescence peaks are also seen in the excitation/emission ranges of 240-295/375-500 nm and 305-340/435-480 nm. These peaks are visible in each sample and vary in intensity depending on the sample.

EEMs of the Narragansett Bay samples (Table 1) were loaded into PARAFAC and results for 2-, 3-, 4-, 5-, 6- and 7-component models were compared (Figures 4, 5, 6, 7, 8, 9). To determine which model provided the best fit, the plot of relative contributions and the components of each model were considered. When the correct number of components is exceeded, the contribution of the last component is very

minor and changes very slightly from sample to sample. The plots of relative contribution were compared. As the number of components was increased, subsequent component contributions decreased for each additional component (Figures 4,5,6,7). However, the contribution was still significant. In the 6-component model the plot of relative contribution for this 6^{th} component was very small relative to the other components to the point that it resembled background noise (Figure 8). Comparing the spectral shape of the components reveals that two of the components, components 1 and 3, appear very similar. These results lead to the conclusion that the 6-component model had exceeded the number of components existing in the mixture. Based on these results the 5-component model provided the best fit (Figure 7).

Standards

Many of the natural peaks and deconvolved components are consistent with published EEMs for compounds of fulvic acids, humic acids, and biologically derived amino acids. Based on these observations, standards of humic substances and amino acids were analyzed using the Fluorolog 3. International Humic Substances Society (IHSS) Suwannee River Humic Acid Standard 1S101H, IHSS Suwannee River Fulvic Acid Standard 1S101F, Tyrosine, and Tryptophan were the standards chosen. Tyrosine and Tryptophan were chosen as the amino acids since they are associated with biological production, are known to be fluorescent due to their aromatic nature, in the EEM region of the excitation/emission peaks from the samples (Stedmon, 2003; Determann et. al. 1994). Phenylalanine is also a fluorescent biological amino acid, however, it's fluorescence falls outside the EEM range of these experiments and at its

typical concentrations, phenylalanine is undetectable (Determann et. al. 1994). Due to the coastal location and the proximity of the samples to marinas an EEM of diesel was also analyzed.

Fluorescence EEMs of the humic acid and fulvic acid are almost identical in shape (Figure 10) with only a slight difference with lower excitation and emission values for the fulvic acid on the primary peak. The emission range for this peak was between 380-500 nm. The excitation range was between 240-395 nm. A secondary peak was noted at an emission range between 435-480 nm and an excitation range of 305-340 nm. This result could be due to impurities within the standards resulting from the manor in which they are extracted and processed, or the fluorescence of the two components may in fact be very similar and almost indistinguishable, both possibly containing the same types of fluorescent components. Determann et al. (1994) also found the peaks of humic and fulvic acid fluorescence to be indistinguishable. Further investigation into this topic is outside the scope of this research.

The peak for tryptophan had an excitation range of 240-305 nm and an emission range between 310-430 nm. This is shown in figure 11. The excitation range for the tyrosine peak was 255-290 nm with an emission range between 300-340 nm. This is also shown in figure 11. The EEM for a diesel sample is also shown in figure 11.

Visual comparison of the components derived from the Narragansett Bay sample based loadings only, and the standards run in the laboratory suggested the humic and fulvic acid peaks match component 2 from the 5-component model (Figure

7). The tryptophan/tyrosine peaks appear to be combined and represented by component 3 from the 5-component model without much detail given to tyrosine. None of the other individual standard peaks appear to match with the other sample components.

Samples and Standards

To better determine the true components of the Narragansett Bay samples, PARAFAC was rerun, including both the standard and sample EEMs. Several component models were compared. In the 6-component model plot of relative contributions the 6^{th} component is very minor and may be associated with instrument background (Figure 12). This indicates that the number of components has been exceeded. Based on these results, the 5-component model provides the best fit (Figure 13). Visual inspection of the 5-component model reveals that components 1, 2, 3, and 5 correspond to humic, diesel, tryptophan, and tyrosine standards respectively. By using the standards, PARAFAC was better able to derive the components and confirm their identities, even resolving the tyrosine peak from the tryptophan peak. To further confirm the identity of the components beyond visual comparison, the plot of relative contributions was converted into a graph showing percentage composition of each component in the samples and standards loaded into PARAFAC (Figure 14). Based on these results it can be seen that the hypothesized components comprise the largest contribution to their suggested standard. Another way to view these results is to use the relative contribution plot from PARAFAC to reconstruct EEMs of the standards based on the proportion of the respective component and to look at the difference

between the two. This "residual" view is the reconstructed EEM subtracted from the original standard EEM. For the standards, only its respective component was used, in the proportion given by PARAFAC, to reconstruct the EEM of the standard (Figures 15,16, 17). This technique can also be used to determine how well each sample was modeled by PARAFAC. In the case of the samples, however, all components and their relative contributions are used in the reconstructions (Figure 18). As can be seen in the residual plots this technique is rather accurate with the percent difference under 40% for the majority of the plot. Larger percentages approaching infinity are seen on the very edge of the peaks and other areas of the EEMs where the intensity approaches zero.

Calibrations

Furthering the idea of component identification, calibration experiments were conducted to determine if the fluorescence of each of the standards correlated with their concentration. Calibration curves of the standards were constructed by making solutions of each standard with known concentrations and recording the EEM for that solution and subsequent dilutions of known concentrations (Figure 19). Several points on the resulting corrected EEMs were then chosen to test for linearity between the fluorescence intensity and concentration of the standard. One of the excitation/emission points chosen was the maximum of the peak. The other points were arbitrarily chosen. All points were plotted versus the concentration of the solution. Linear regression lines through the data show good correlation based on the R^2 value with R^2's greater than .991 for all but the tryptophan standard.

Mixtures

In order to determine if PARAFAC would be a viable method to determine the concentrations of components in samples, 2 mixtures of 3 of the standards, humic acid, tryptophan, and tyrosine, with known concentration were made and the resulting EEMs (Figure 20) loaded into PARAFAC with 5 other EEMs of the individual standards in various known concentrations. The mixture was run using 3-, 4-, and 5-component models. In the 5-component model two of the components, components 2 and 3, looked very similar (Figure21). The only difference between the two components is an extra shoulder along the peak at the excitation of 240 nm and is an artifact of the fluorescence instrument used. Therefore, the 4-component model provides the best fit and components 1 and 3 are seen as humic substance subfractions (Figure 22). This is supported further by the plot of relative contributions which shows each of these components mimicking in smaller contributions the contribution of component 1 which corresponds with the humic acid standard. The EEMs of the 3 components provided by the PARAFAC 3-component model correspond in shape and peak position with the EEMs of the standards used to make the solutions (Figure 23). The mixture EEMs deconvolved by PARAFAC using a 3-component model were used for comparison of concentrations in the mixtures. Concentrations for each of the loadings were compared to those used in the mixtures, along with the relative contributions of the components in the mixtures versus the standards.

Table 4 is the concentration data, the output of relative proportions and the concentration ratios of the deconvolved mixtures compared to the ratios of component

contributions for the mixtures versus the standards, along with the plot of relative proportions. These ratios should be equal to one another. The results were very encouraging and there was generally very good agreement between the concentrations resolved by PARAFAC and the actual concentrations used in the mixture. Any discrepancies can be accounted for by the humic subfractions in the standard and the very low concentrations used in the samples and the reality that when making these solutions, error by a fraction of a drop at these low concentrations can throw off the calculations entirely. This suggests this is an applicable method for determining component concentrations in natural samples. The resulting PARAFAC plot of contributions thus, is a rather accurate gauge of the concentrations of components with the largest difference of 9.22% seen in mixture 2 for the humic concentration.

Discussion

This study, consistent with other studies, reveals the presence of fluorescence EEMs characteristic of DOM in natural waters and consistent with EEMs expected for humic substances and amino acids. Unique in this study, EEMs have been used to positively identify the components responsible for producing these EEMs, and a method using PARAFAC has been developed that can be used to determine the concentrations of these components in natural water samples. The samples collected from the limited number of sites in Narragansett Bay are found to be described best by a 5-component model. Other regions of the bay closer to sewage treatment plants and combined sewer overflows could contain many other components and require higher component numbers.

While the 3-component model had components that corresponded exactly with those standards used to make up the solutions, the mixtures were best fit by a 4-component model. Knowing that only 3 standards were used to make the solutions leads to the conclusion that one of the standards must have some type of subfraction in it. Coble et al. (1998) has shown peaks that are blue shifted in relation to the humic peaks and inferred that these peaks are representative of fresher humic material. This type of reasoning could explain why the contributions of components 1 and 3 mimic each other, with component 3 contributing a smaller amount. The only explanation for a 4-component model in the case of the mixture, is that the humic material standard is comprised of some humic subfractions and produces an extra peak. This confirms the

idea that the Narragansett Bay samples and standards could have similar diverse populations of humic complexes and could be best modeled by a 5-component model.

Future research directions should include the use of PARAFAC as a way to characterize waters and areas within the bay and to track the distribution of FDOM. Different regions or environments should be examined including rivers, shaded regions, areas near sewage treatment plants and combined sewer overflows, areas influenced by large amounts of runoff, coastal waters, and the open ocean. In future work, it is expected that these results will help to discriminate marine and terrestrial inputs to an area and possibly pinpoint rivers or other sources of this organic matter to the region, provided the EEMs of the sources are distinct. The ability to deconvolve specific fluorescent signatures has the potential to allow the determination of the sources of FDOM to Narragansett Bay provided each source has a unique mix of fluorescent components.

Determination of the components responsible for the organic matter will allow a better understanding of the carbon cycle in Narragansett Bay and the types of events and activities that might increase or decrease the amount of organic matter seen in the area. These results, once related to other environmental factors, can be used to predict which parameters and sources will have the largest effect on photosynthetic processes making it an important tool for monitoring of water quality.

In conclusion, PARAFAC is a useful tool that can discriminate component EEM signatures without having the original component EEM, and can also be used to identify components, their relative contributions, and when possible, concentrations, if a standard of known concentration can be provided. The use of standards improves

resolution of peaks and the identification of components within samples. This ability makes PARAFAC a useful and potentially affordable tool for mapping components of natural waters and could be used to differentiate between water masses to track and study DOM inputs and distributions. This type of technique will be a valuable method in the lab saving time and money in analysis.

Table 1

Sample number	Sample Identity
1	Apponaug
2	Highland
3	Mt. Hope Bay
4	East Greenwich Marina
5	Oakland
6	Tyler Point
7	Sabin Point
8	Pawtuxet
9	East Greenwich Marina surface
10	East Greenwich Marina bottom
11	East Greenwich Bay
12	Apponaug surface
13	Apponaug bottom

Table 2

Sample number	Sample Identity
1	Apponaug
2	Highland
3	Mt. Hope Bay
4	East Greenwich Marina
5	Oakland
6	Tyler Point
7	Sabin Point
8	Pawtuxet
9	East Greenwich Marina surface
10	East Greenwich Marina bottom
11	East Greenwich Bay
12	Apponaug surface
13	Apponaug bottom
14	Fulvic acid
15	Humic Acid
16	Tryptophan
17	Tyrosine
18	Diesel

Table 3

Sample number	Sample Identity
1	Mixture 1
2	Tryptophan
3	Humic Acid
4	Tyrosine
5	Mixture 2
6	Tyrosine
7	Tyrosine

Table 4
Results showing concentrations of each standard predicted by PARAFAC for each mixture and the real concentrations of standards in each mixture. See Table 3 for sample identities. The tyrosine standard used for the calculations is sample number 4.

From Standard solutions and mixture made in lab					
			Humic	Tryptopha	Tyrosine
concentration in mixture 1 (mg/L) (1)			16.91	0.19	0.17
concentration in mixture 2 (mg/L) (2)			20.30	0.11	0.21
Concentration of pure standard run (mg/L) (3)			16.91	0.14	0.17
From PARAFAC plot of relative contributions (See Figure 23)					
component # contribution in mixture 1 (4)			25.79	28.24	3.04
component # contribution in mixture 2 (5)			34.59	17.55	3.62
component # contribution in standard run (6)			26.39	20.09	2.96
Mixture 1 ratio of concentration of mix/standard (1/3)			1.00	1.40	1.00
Mixture 1 ratio of contributions of mix/standard (4/6)			0.98	1.41	1.03
Mixture 2 ratio of concentration of mix/standard (2/3)			1.20	0.84	1.20
Mixture 2 ratio of contributions of mix/standard (5/6)			1.31	0.87	1.22

	Concentration in mixture 1 (mg/L)	**Predicted concentration (mg/L)**	**% difference**
Humic	16.91	16.52	2.30
Tryptophan	0.19	0.19	0.43
Tyrosine	0.17	0.18	2.79
	Concentration in mixture 2 (mg/L)	**Predicted concentration (mg/L)**	**% difference**
Humic	20.30	22.17	9.22
Tryptophan	0.11	0.12	3.98
Tyrosine	0.21	0.21	1.97

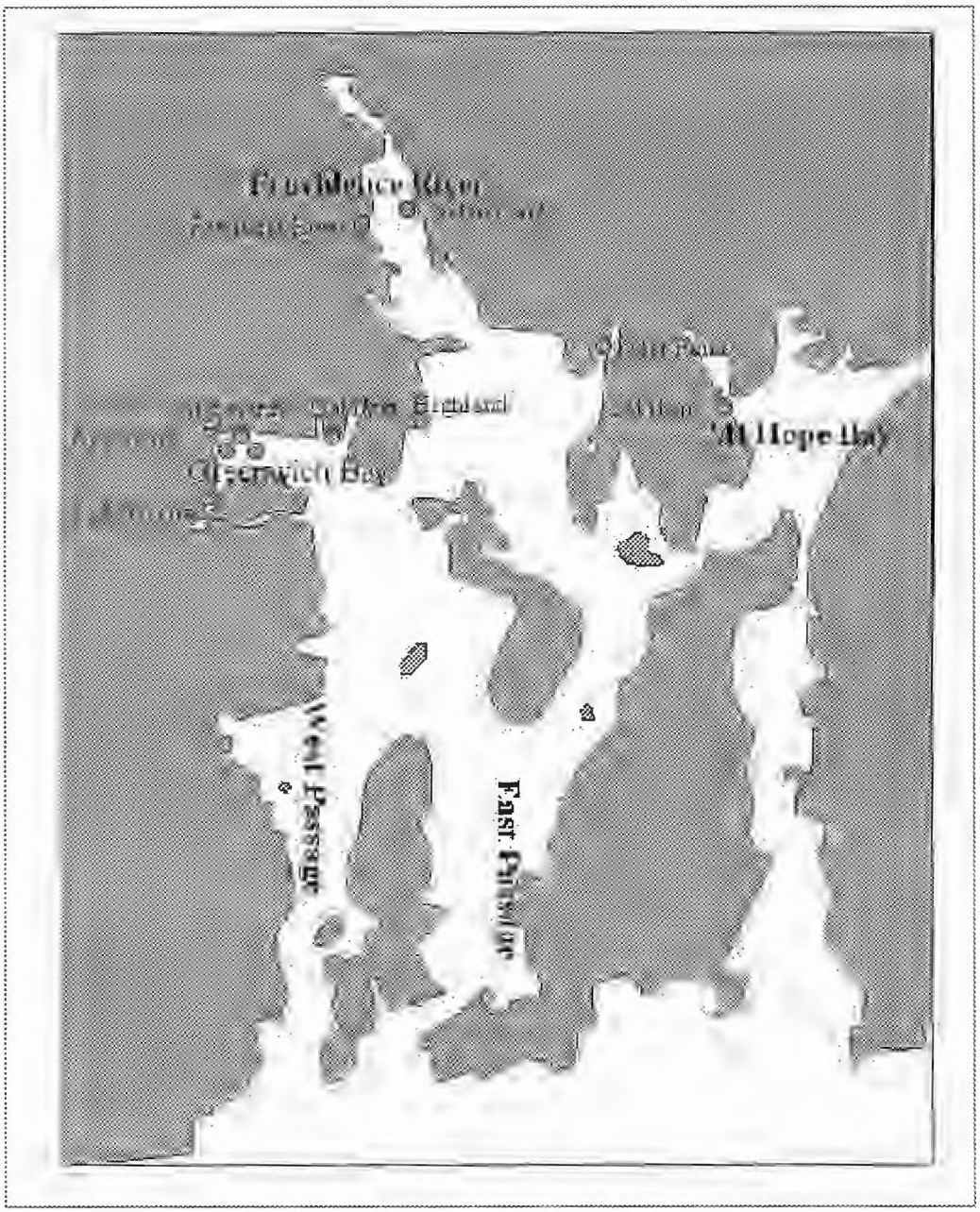

Figure 1 Map of Narragansett Bay Sites

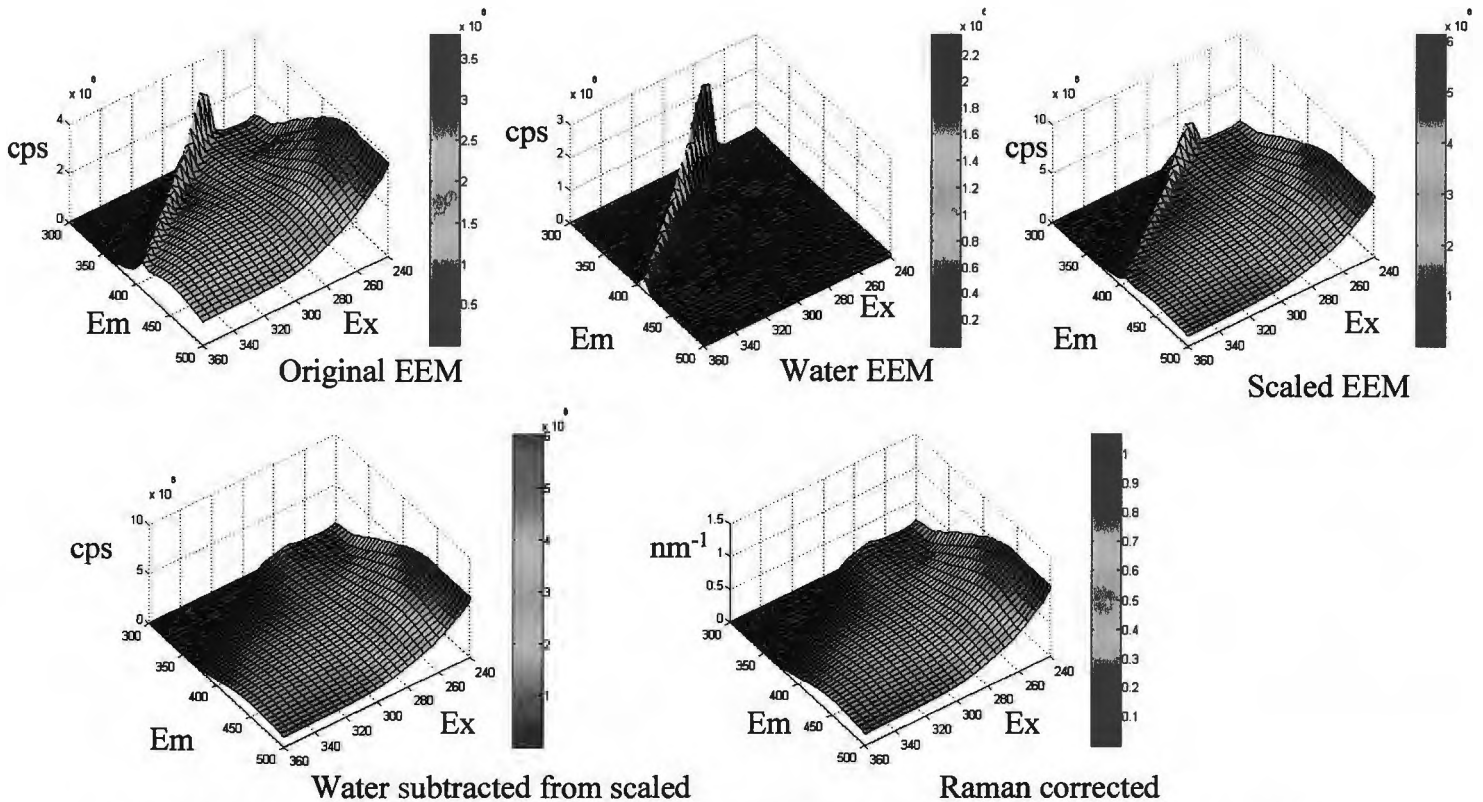

Figure 2 Original EEM in counts per second (cps) obtained from the Sabin Point Narragansett Bay sample versus the same EEM Raman corrected in Raman units (nm^{-1}) and progression of steps in between. See text for more details.

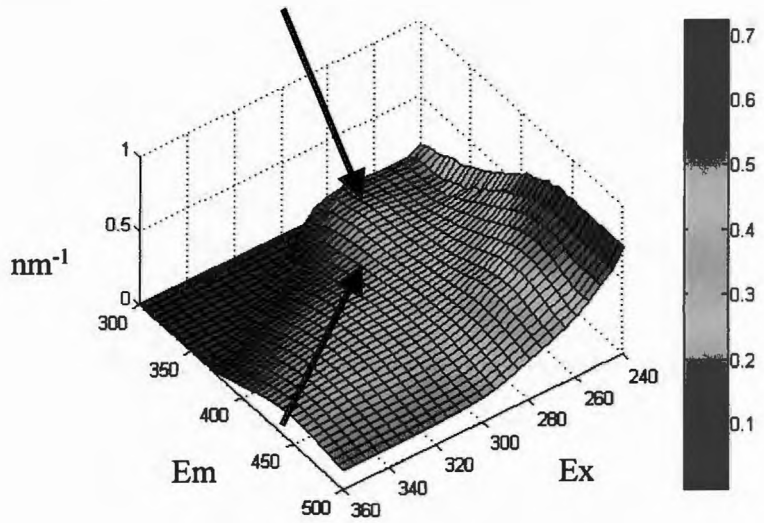

Figure 3 Corrected EEM from Apponaug Cove sample. The amino acid peak is located at excitation 240-305nm and emission 320-400nm.

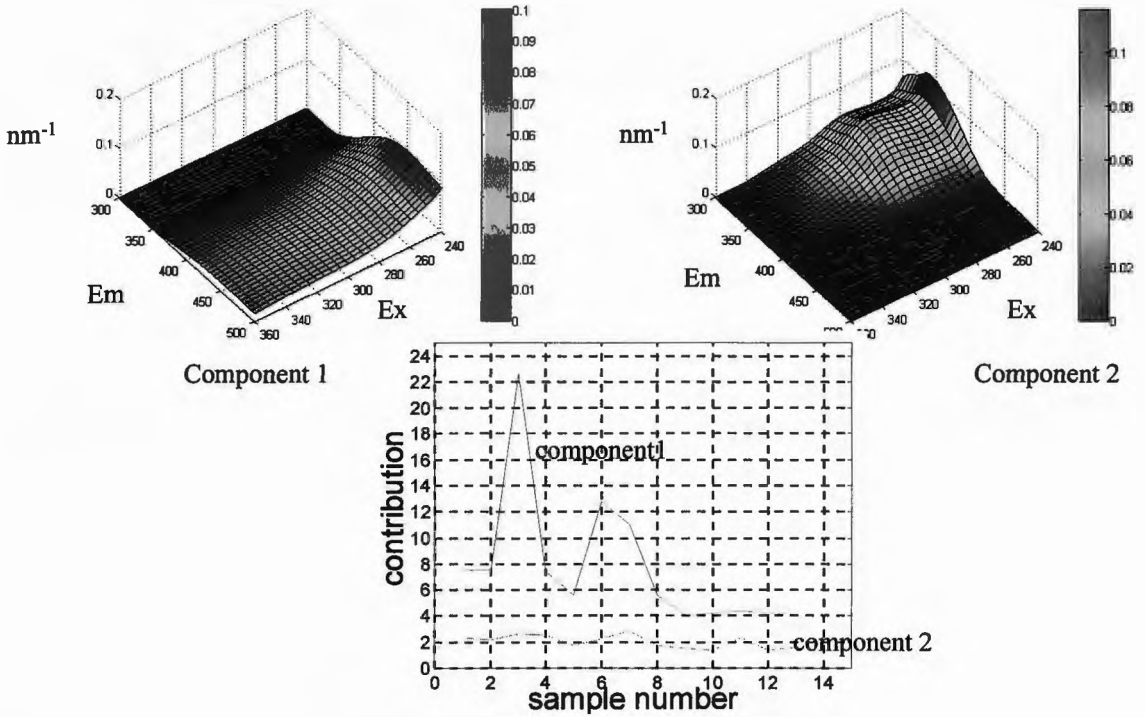

Figure 4 Results from PARAFAC of a 2 component model of samples from Narragansett Bay. See Table 1 for sample identities. Component 1 resembles humic material, component 2 resembles amino acids. See figures 10 and 11 for comparison.

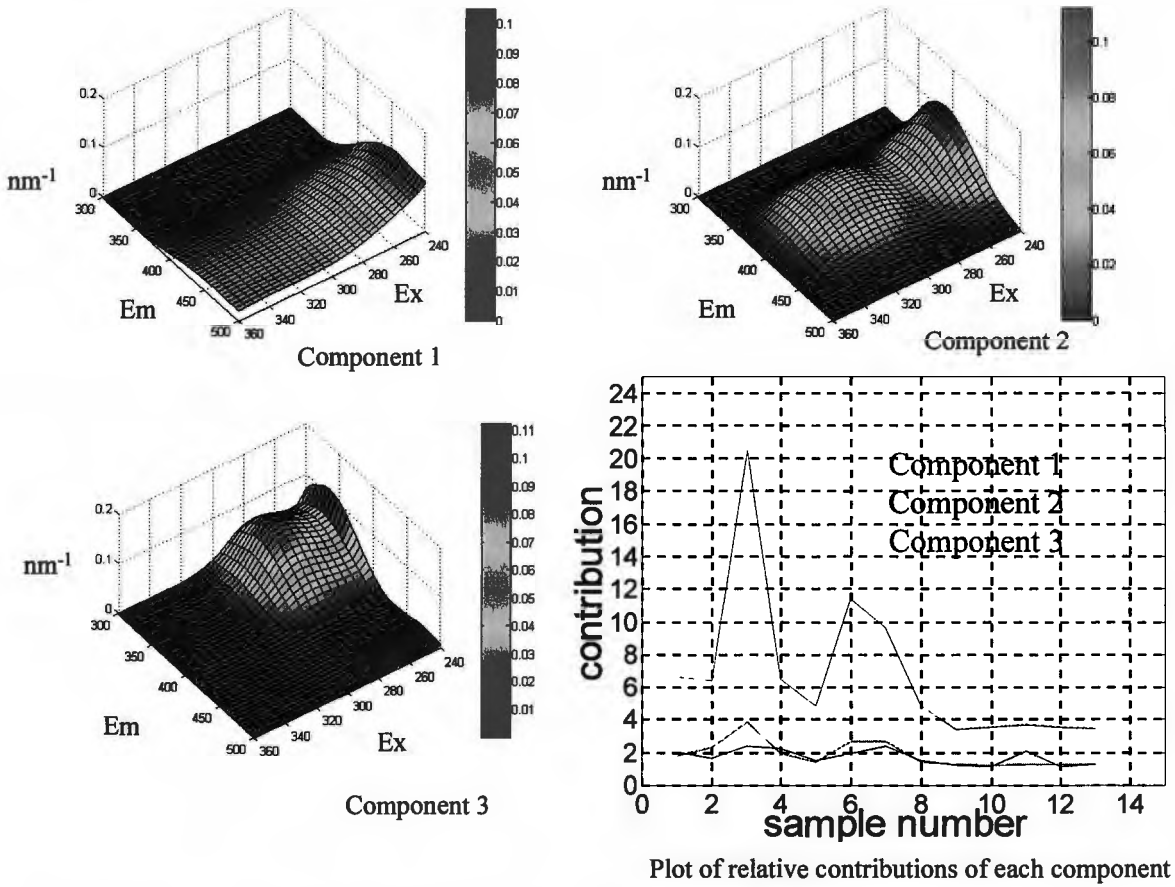

Figure 5 Results from PARAFAC of a 3 component model of samples from Narragansett Bay. See Table 1 for sample identities.

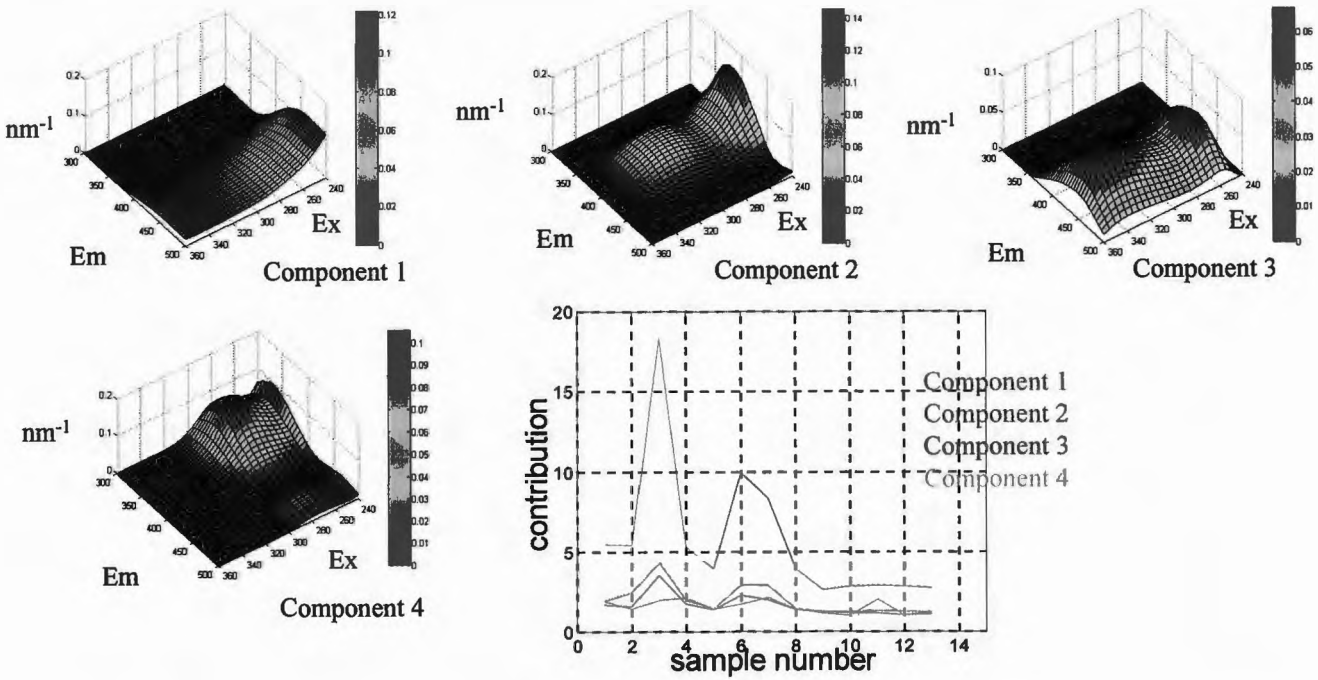

Figure 6 Results from PARAFAC of a 4 component model of samples from Narragansett Bay. See Table 1 for sample identities.

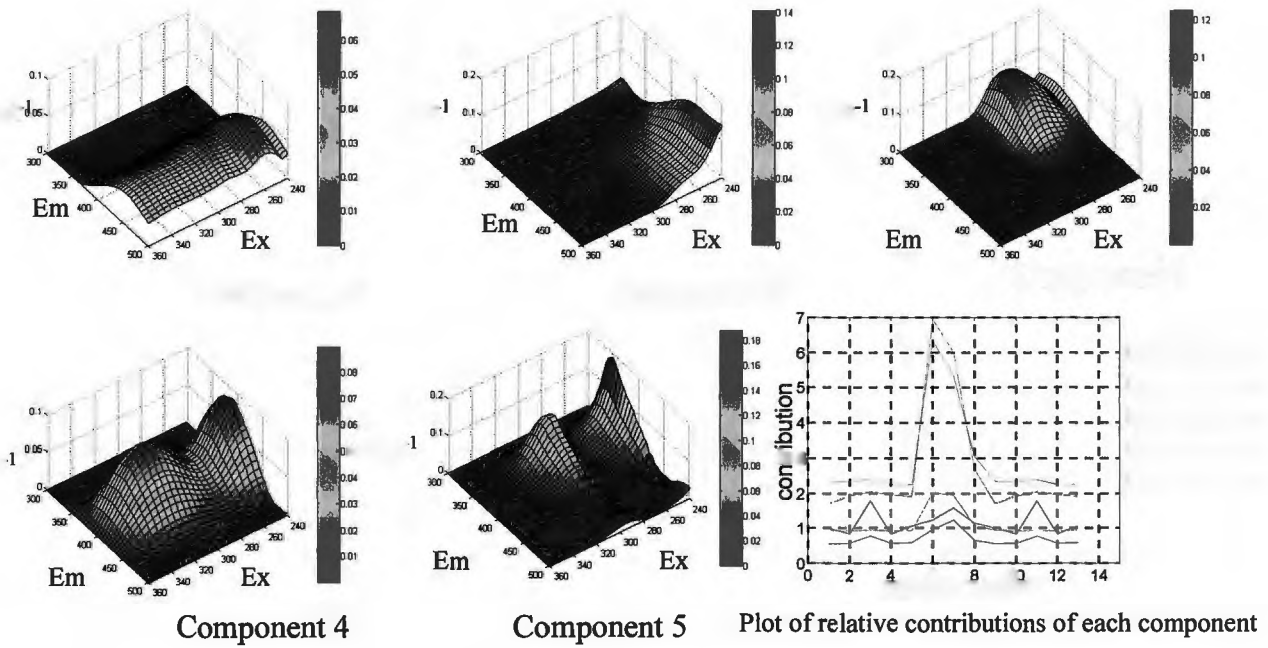

Figure 7 Results from PARAFAC of a 5 component model of samples from Narragansett Bay. See Table 1 for sample identities.

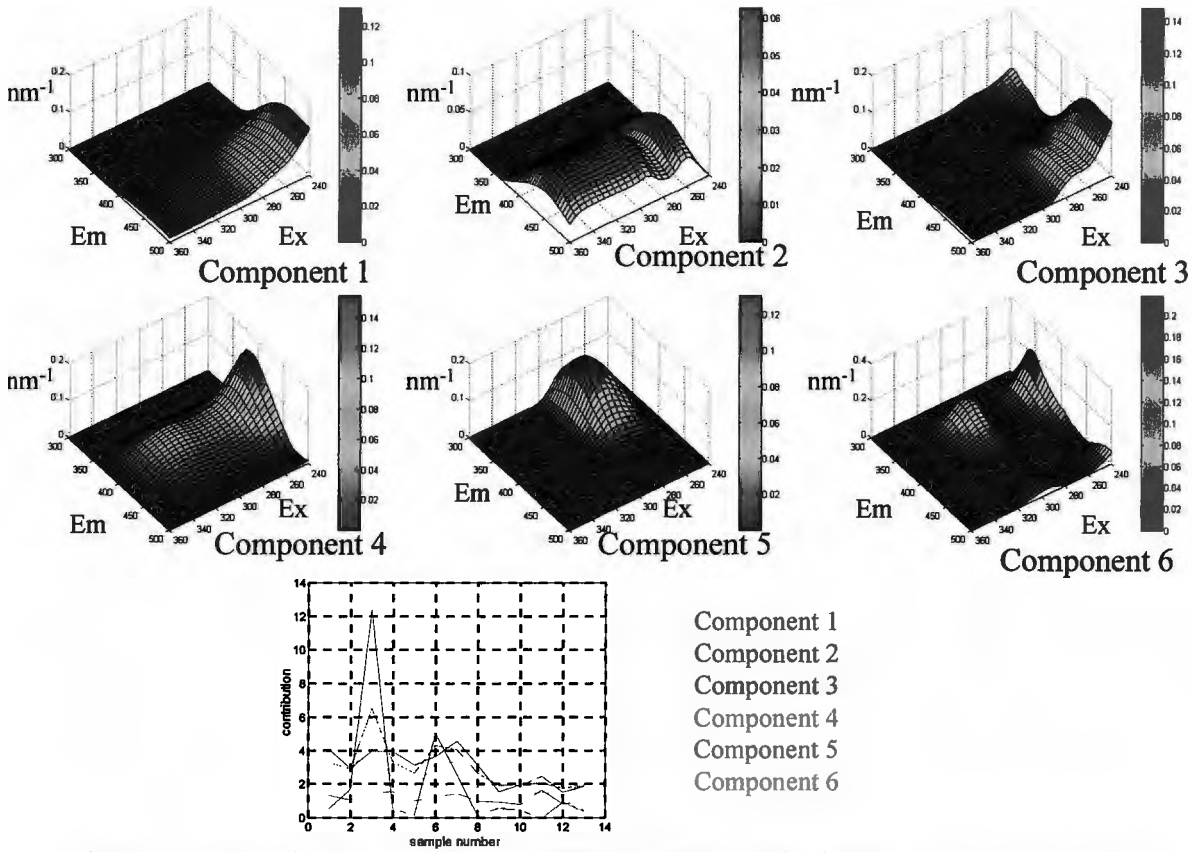

Figure 8 Results from a 6 component PARAFAC model of Narragansett Bay samples. See Table 1 for sample identities.

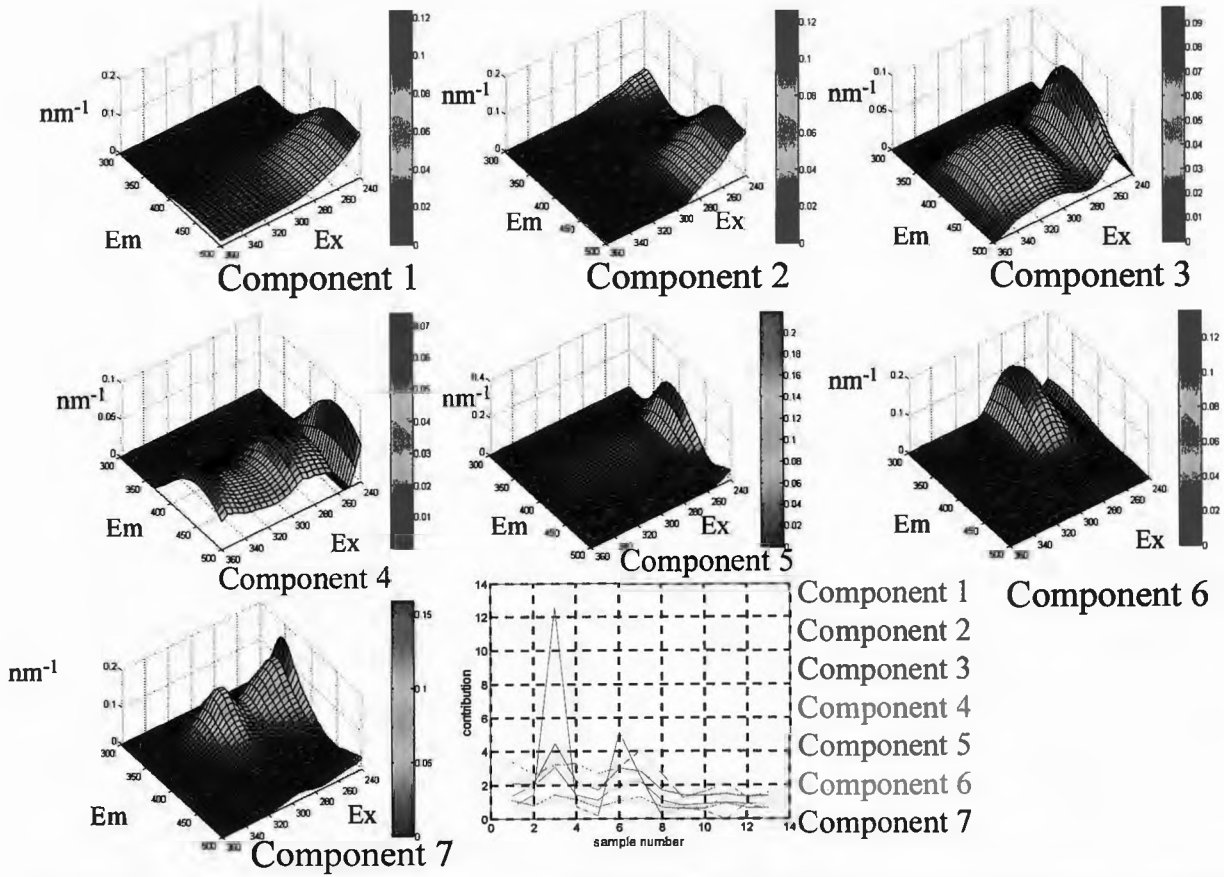

Figure 9 Results from a 7 component PARAFAC model of Narragansett Bay samples. See Table 1 for sample identities.

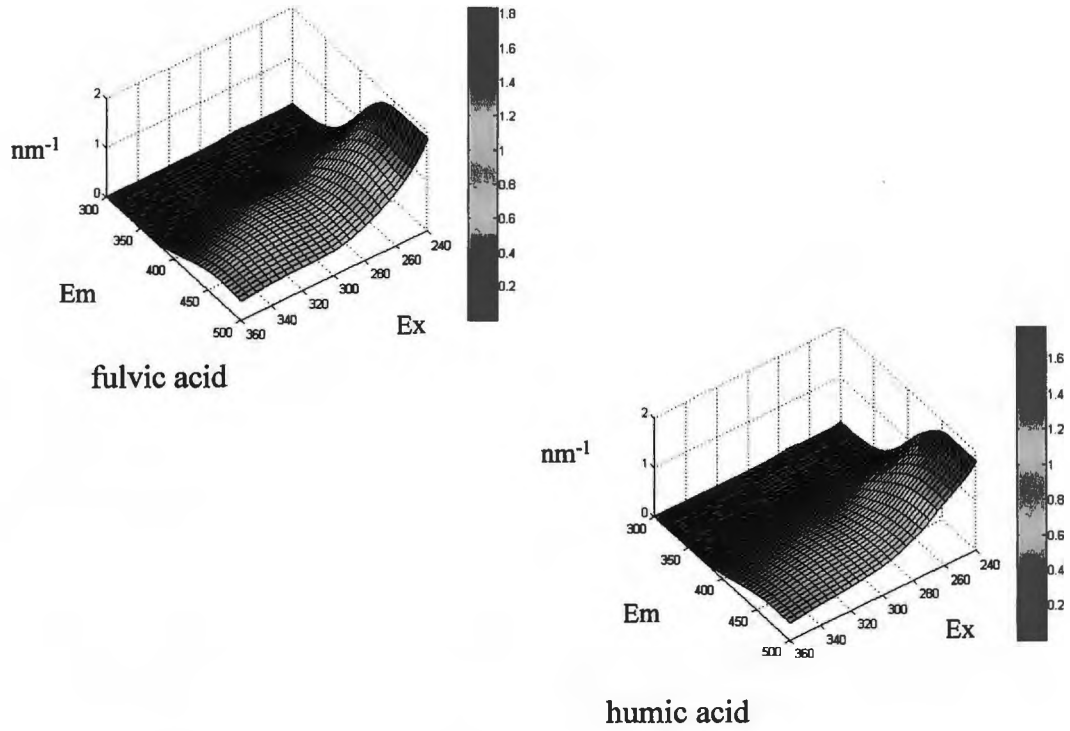

Figure 10 Corrected EEM for the fulvic acid standard and humic acid standard. Both show fluorescence peaks in similar ranges making them practically indistinguishable.

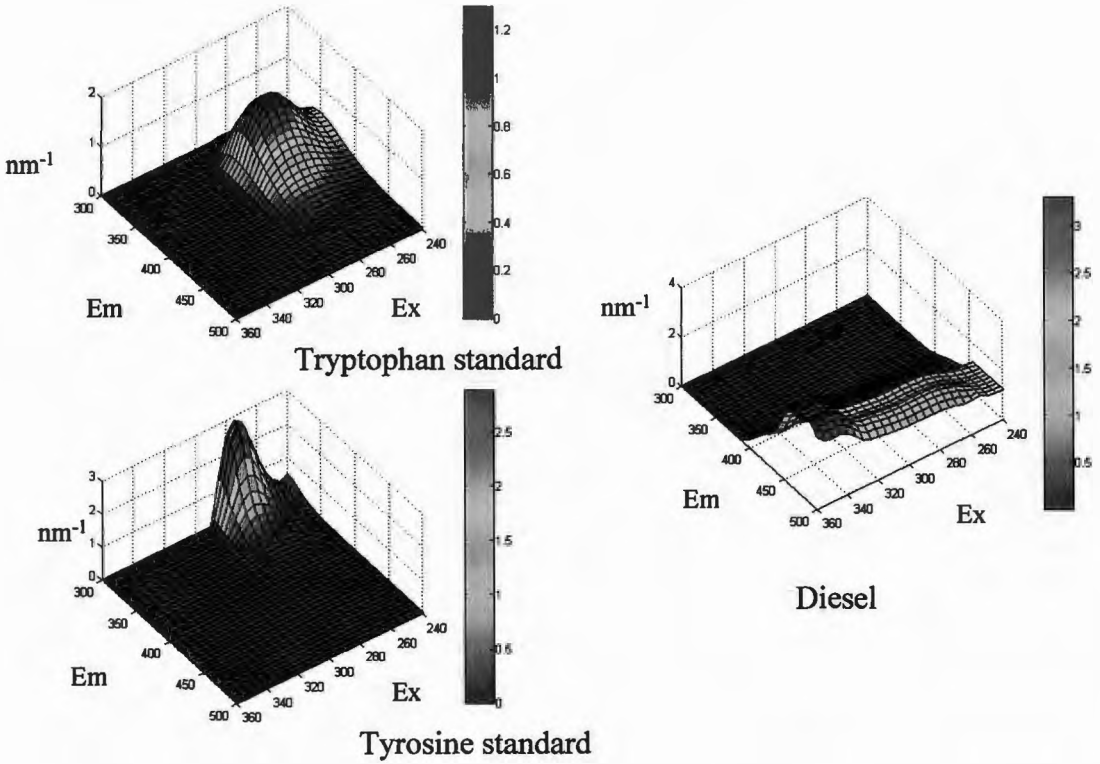

Figure 11 Corrected EEM for the tryptophan standard, tyrosine standard and diesel.

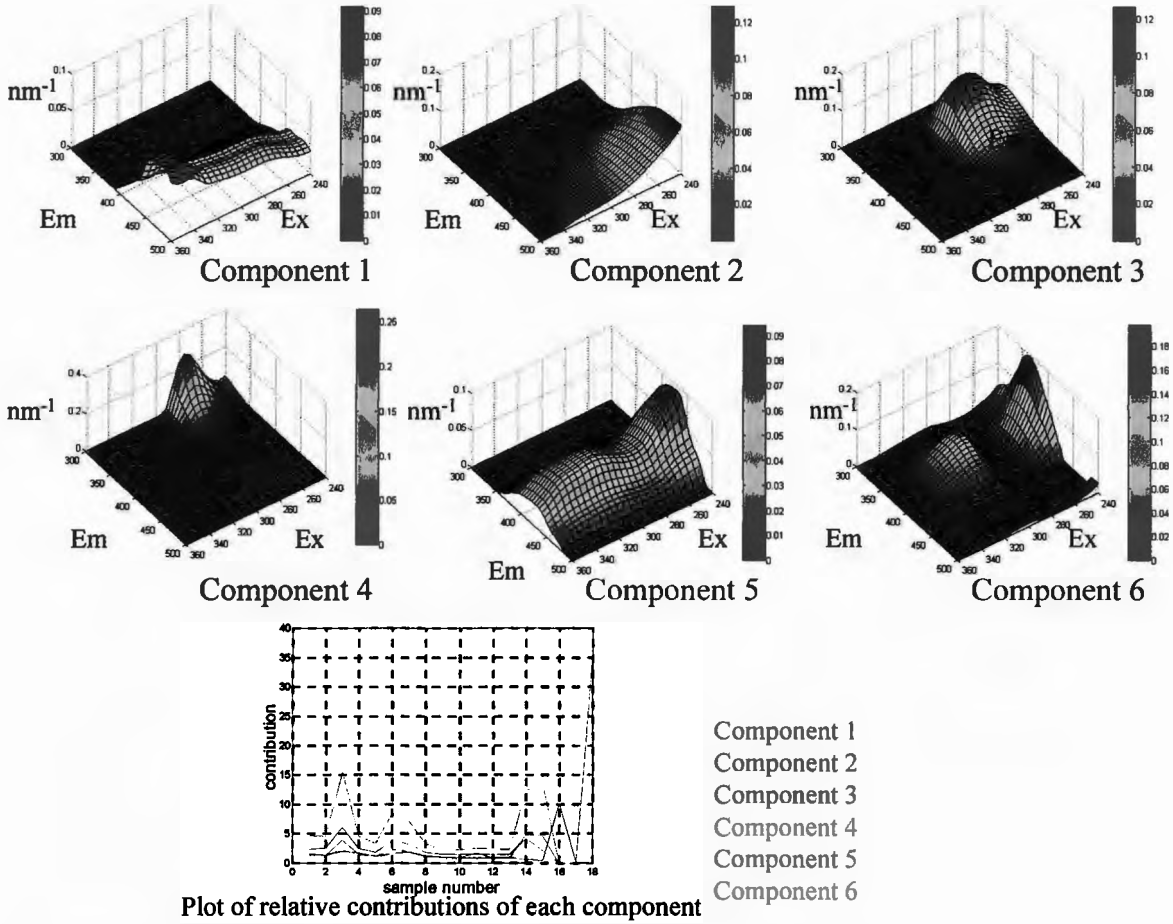

Figure 12 Results from a 6 component PARAFAC model of Narragansett Bay samples and the standards. See Table 2 for sample identities.

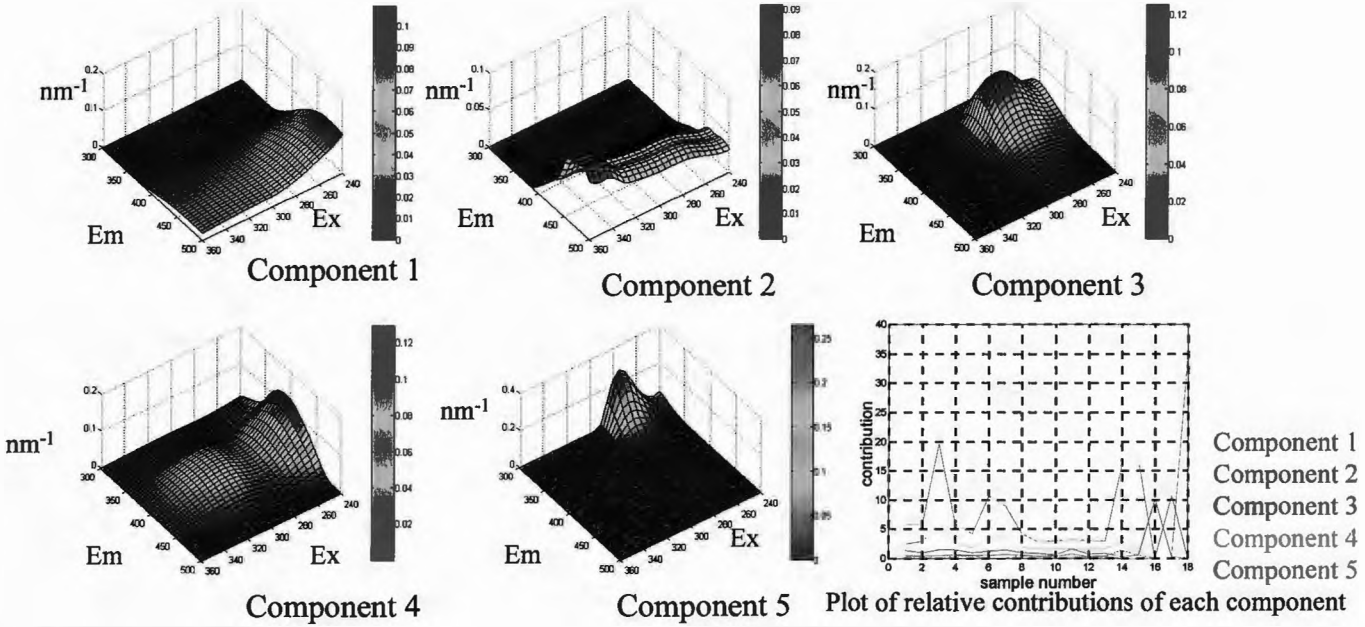

Figure 13 Results from a 5 component PARAFAC model of Narragansett Bay samples and the standards. See Table 2 for sample identities.

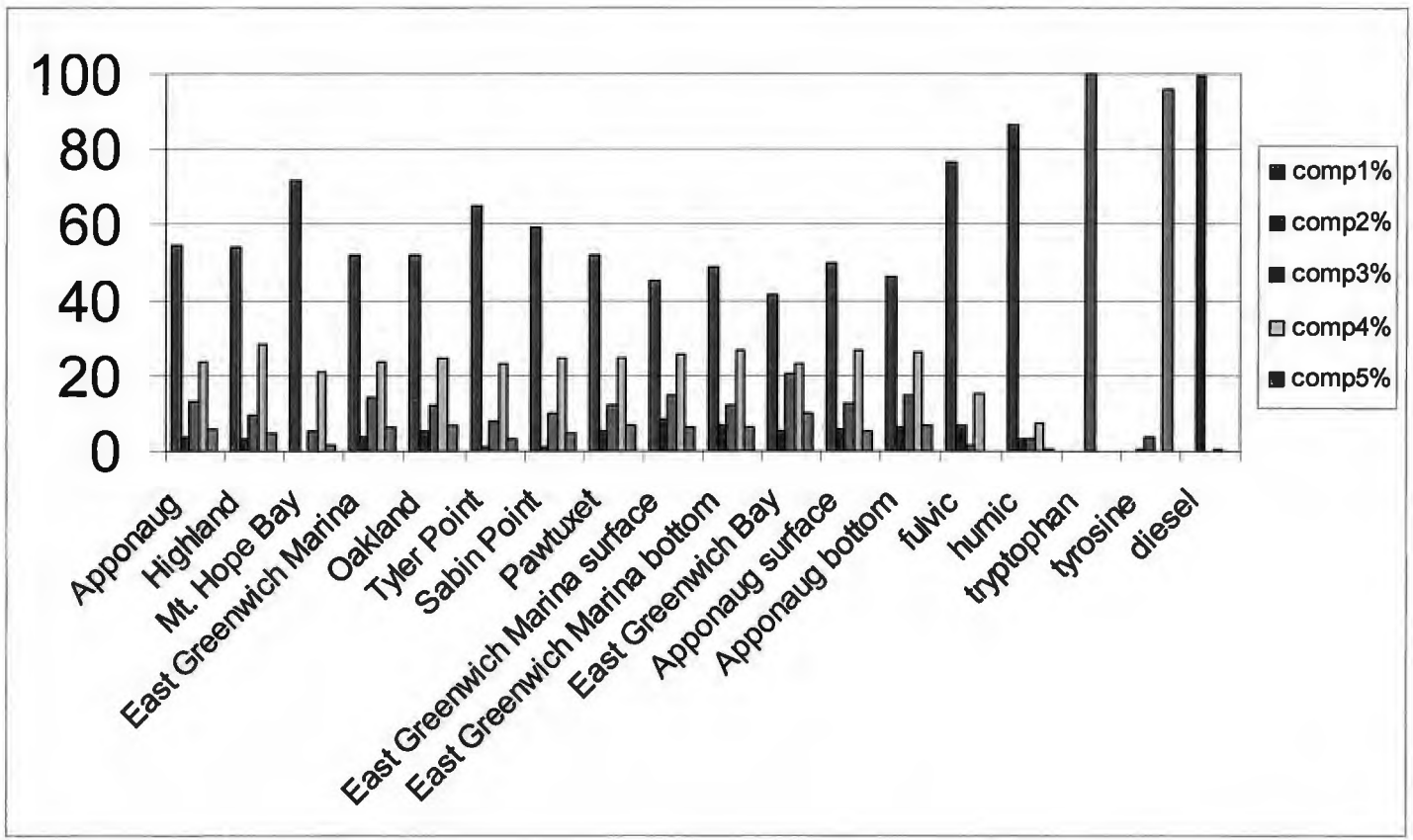

Figure 14 Percentage components in each of the Narragansett Bay samples and each standard.

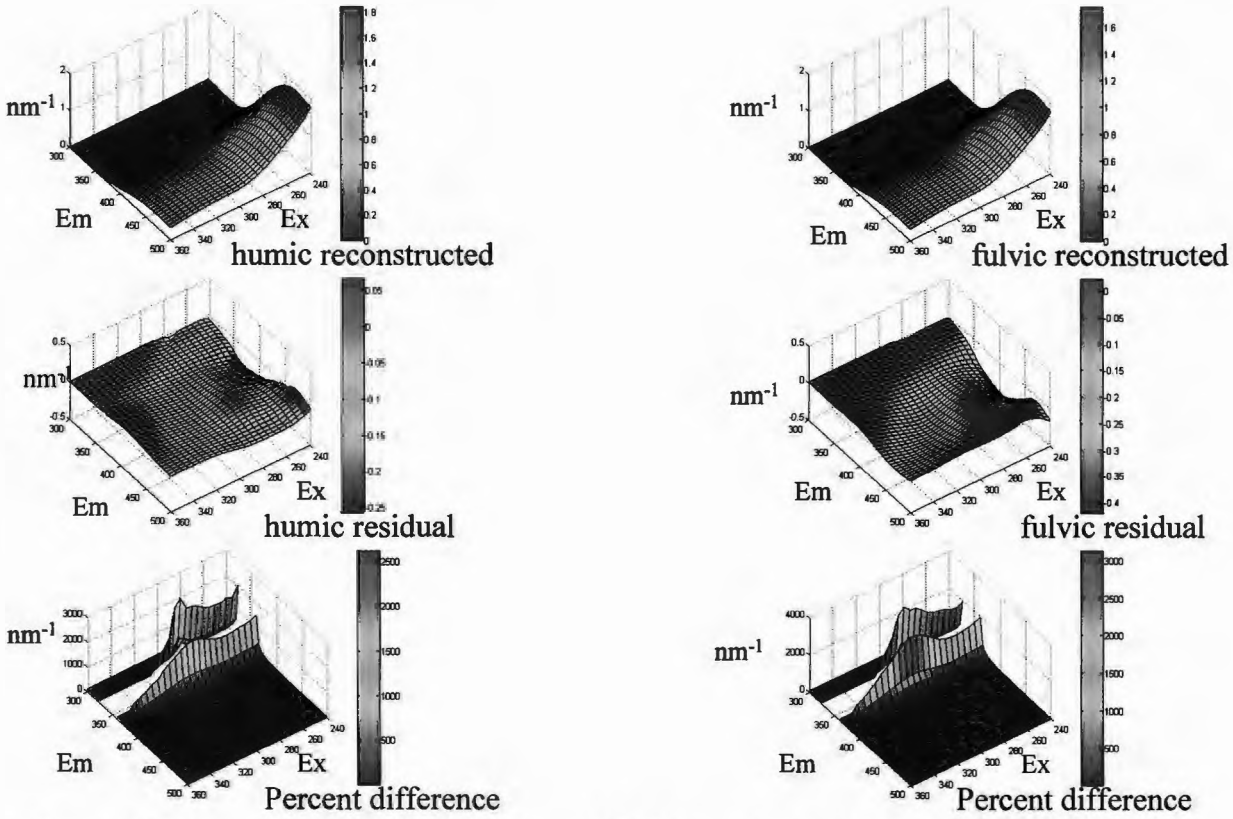

Figure 15 Reconstructions of the humic and fulvic standards, the residual difference between the reconstructions and the original corrected EEMs, and the percent difference between the originals and reconstructions.

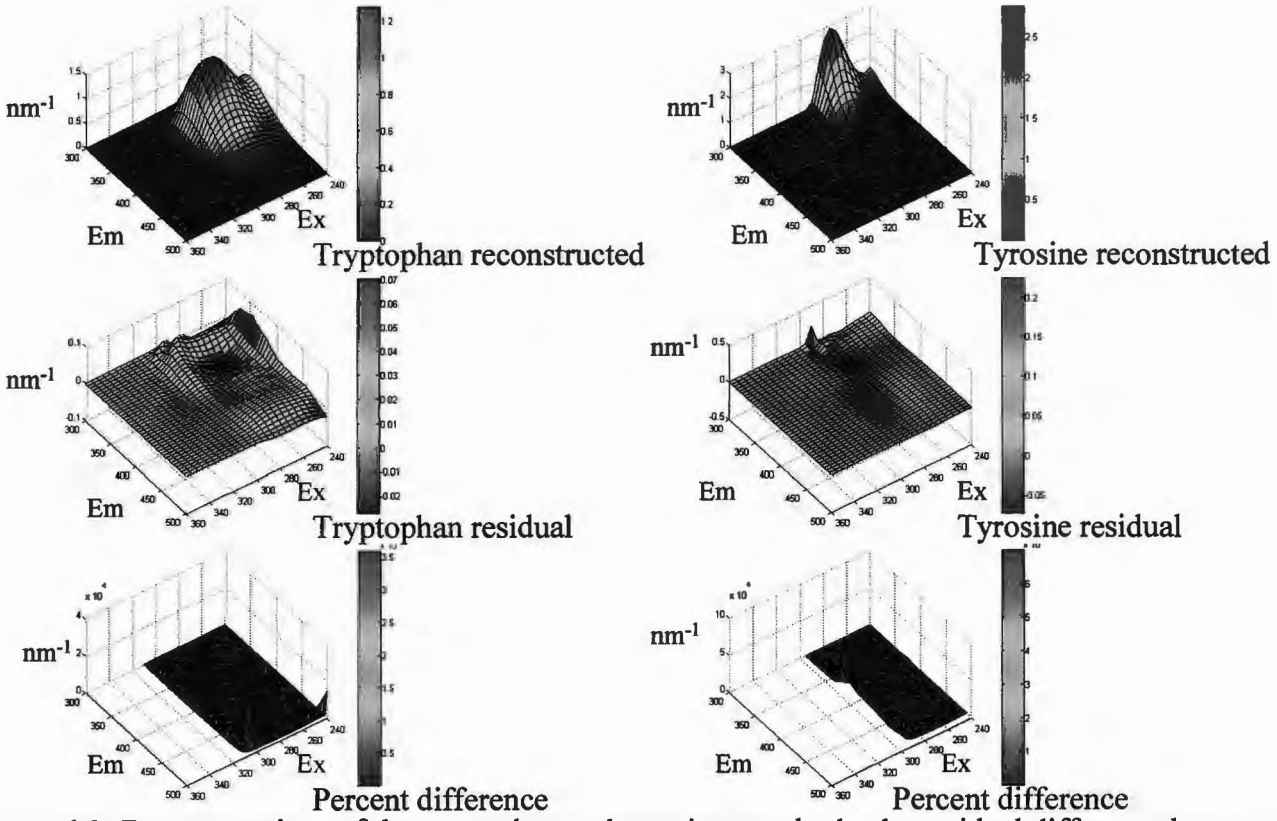

Figure 16 Reconstructions of the tryptophan and tyrosine standards, the residual difference between the reconstructions and the original corrected EEMs, and the percent difference between the originals and reconstructions.

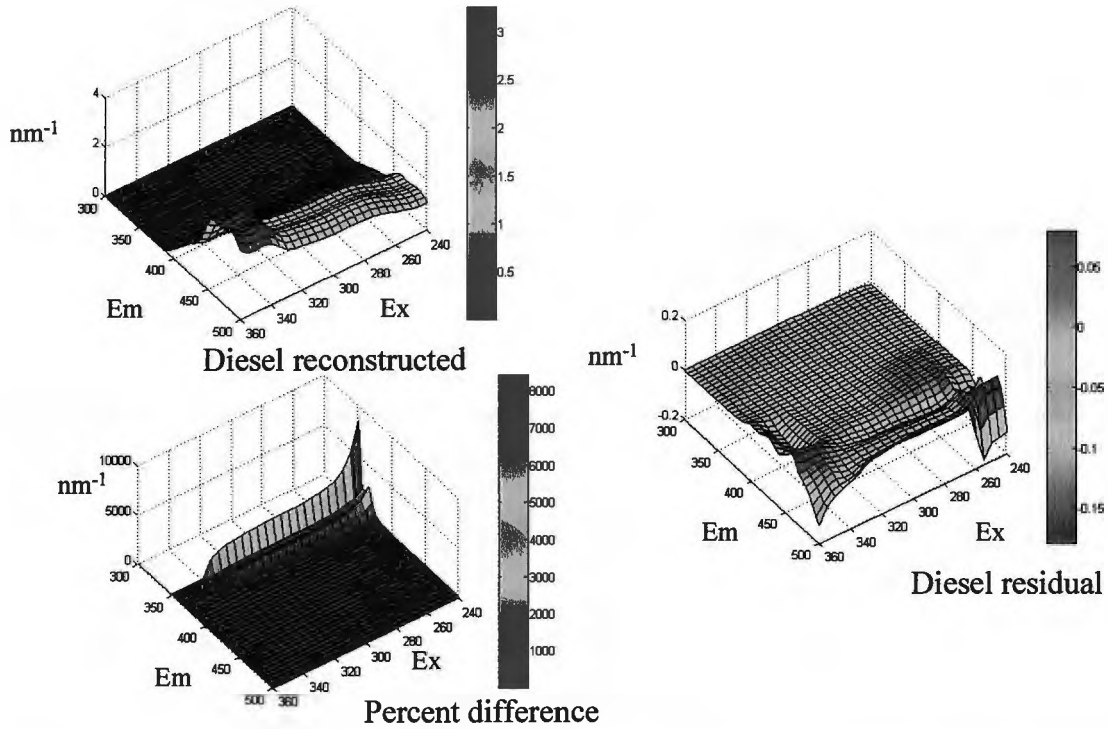

Figure 17 Reconstruction of diesel, the residual difference between the reconstruction and the original corrected EEM, and the percent difference between the original and reconstruction.

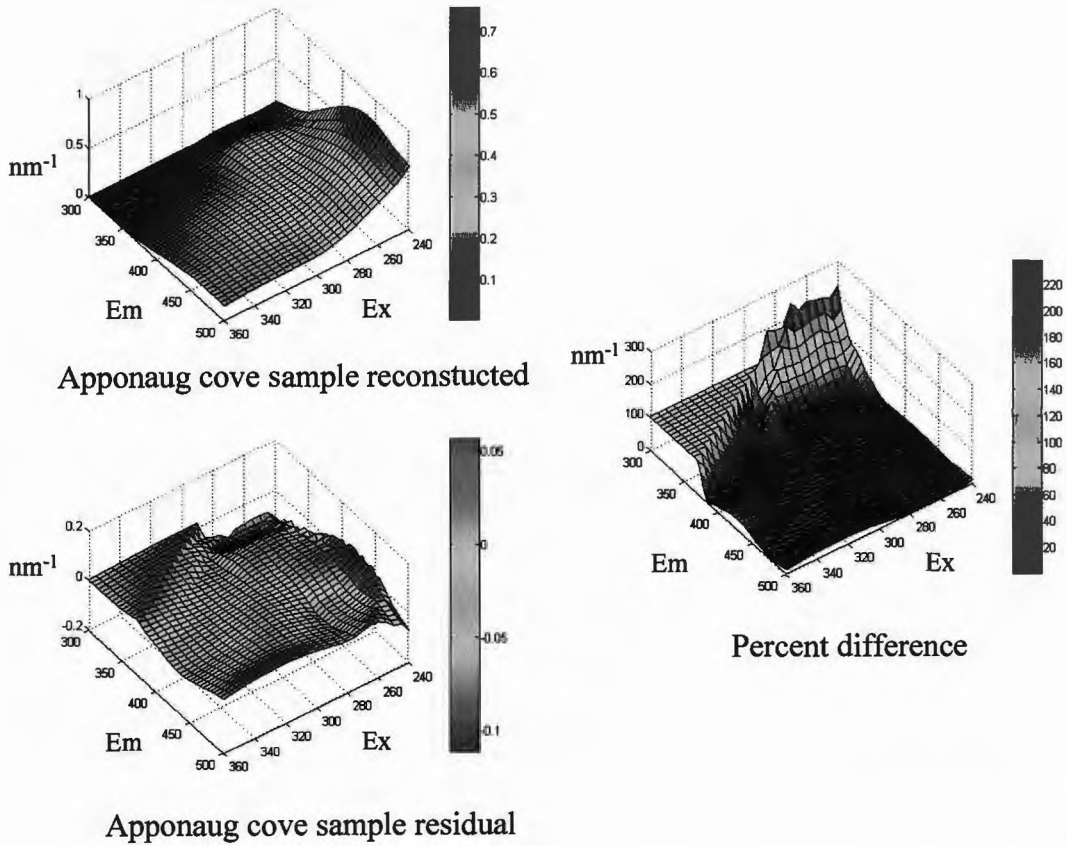

Figure 18 Apponaug cove sample reconstucted (top), percent difference (middle), and residual (bottom).

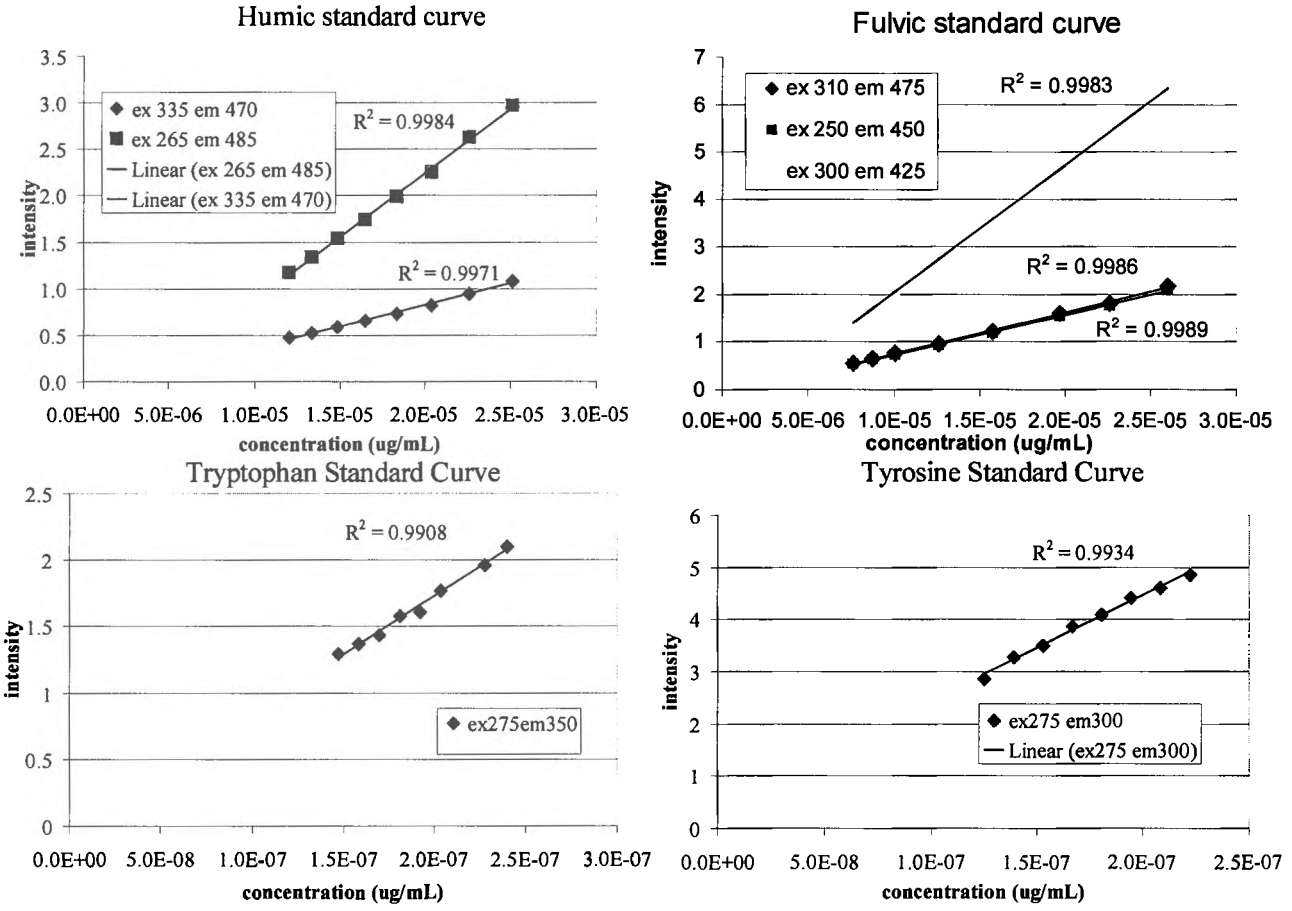

Figure 19 Calibration curves of the standards. See text for more details.

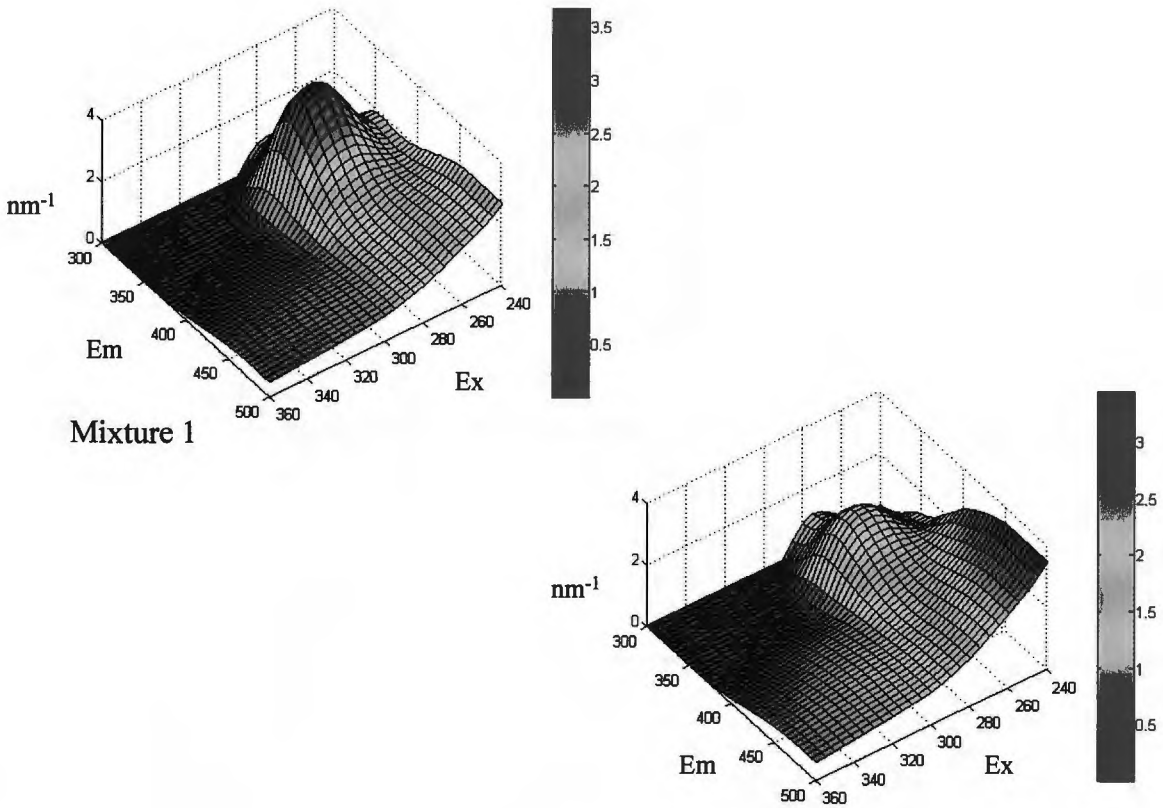

Figure 20 EEMs of the mixtures

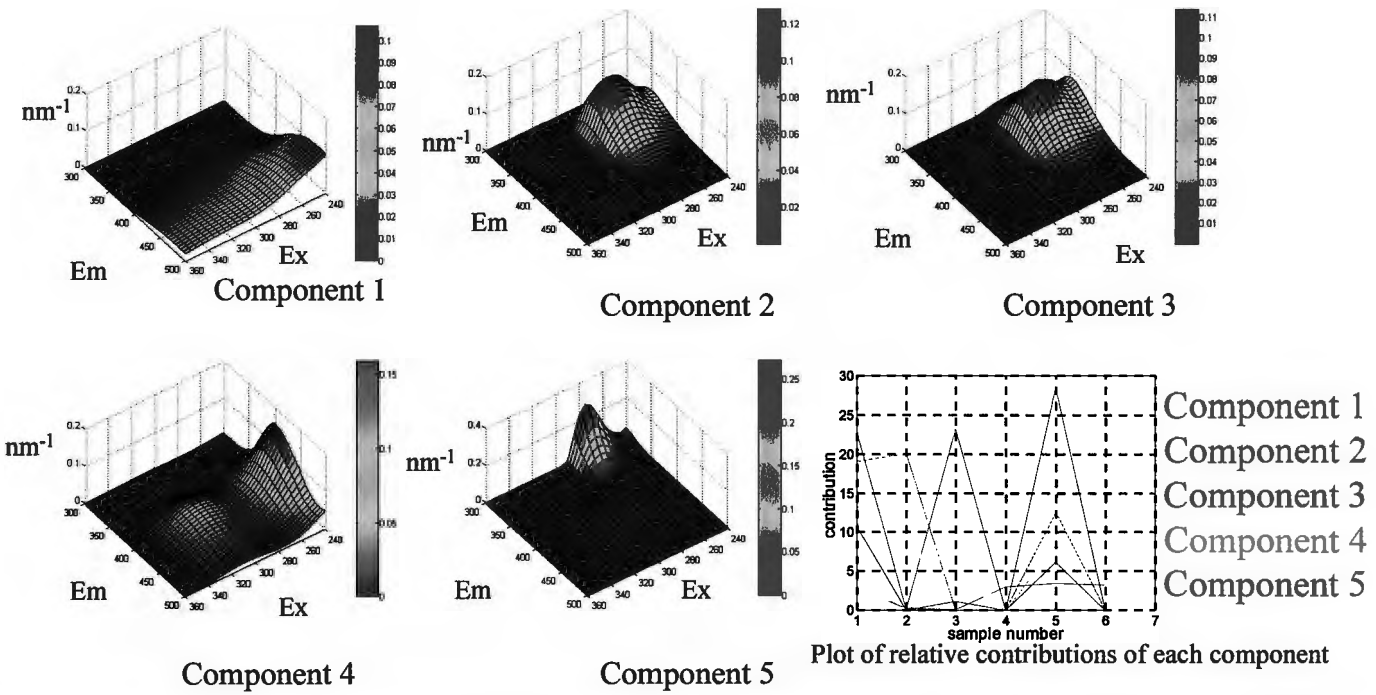

Figure 21 Results from a 5 component PARAFAC model of the mixtures and standards. See Table 3 for sample identities.

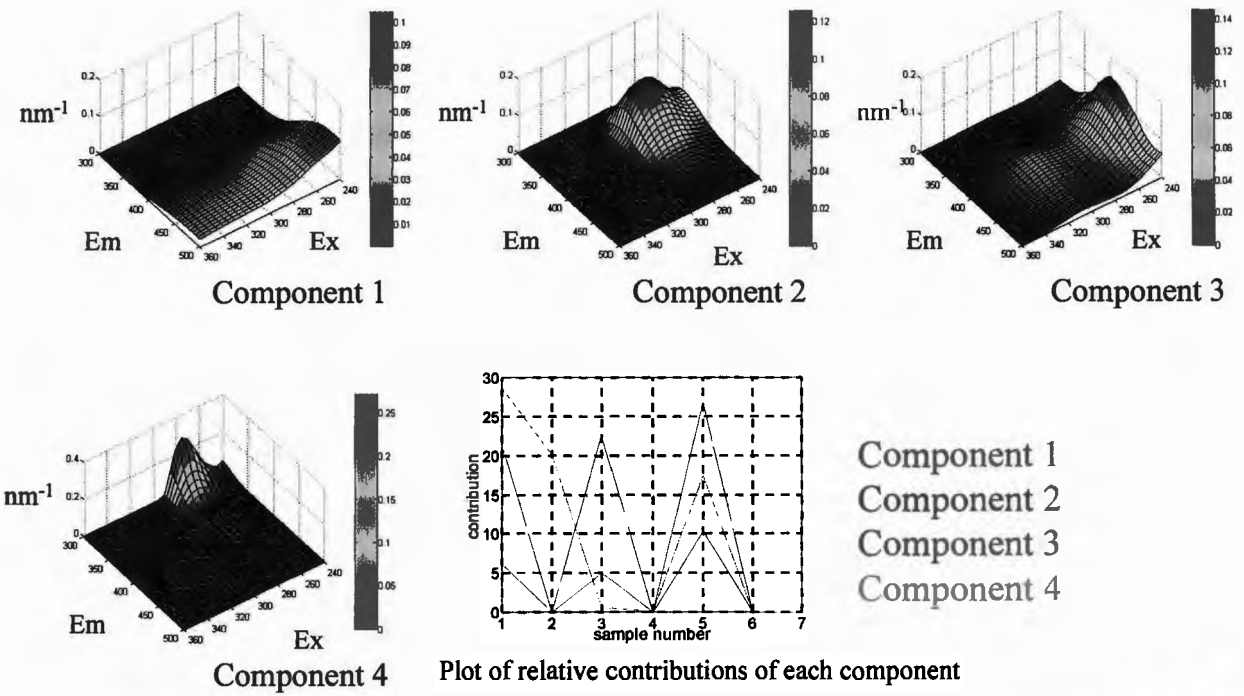

Figure 22 Results from a 4 component PARAFAC model of the mixtures and standards. See Table 3 for sample identities.

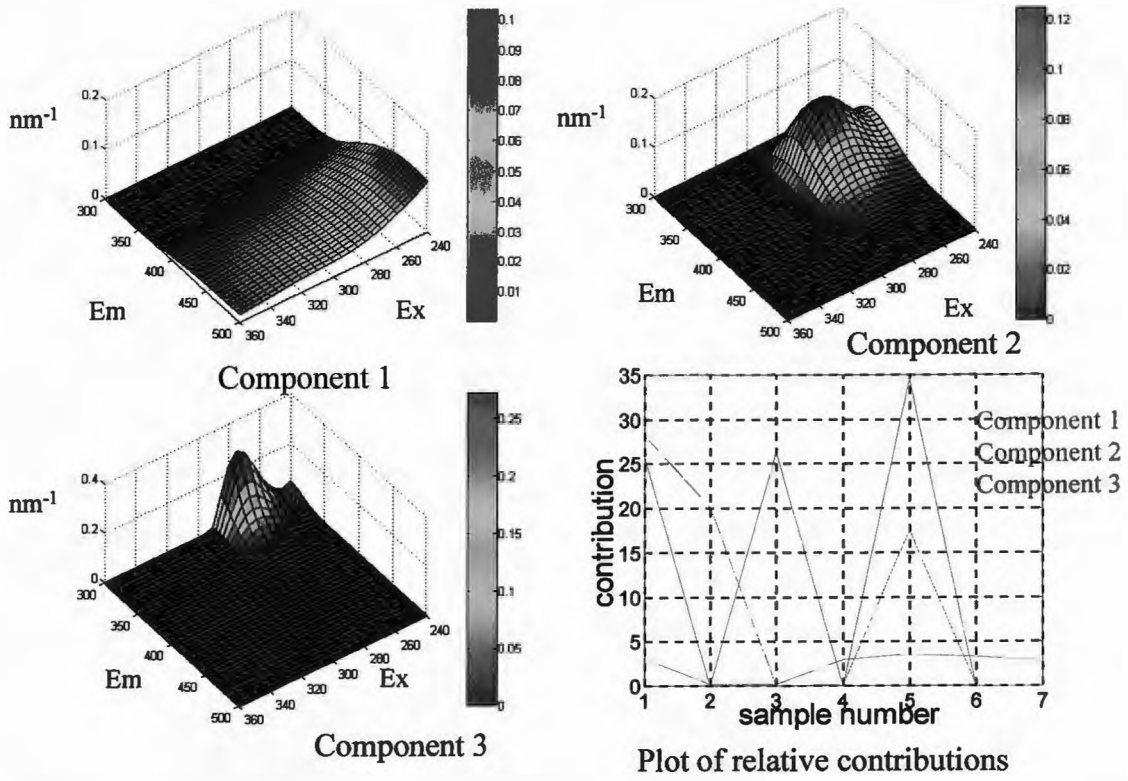

Figure 23 Results from a 3 component PARAFAC model of the mixtures and standards. See Table 3 for sample identities.

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