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Use of a Neural Network for the Analysis of Fluorescence Spectra from Mixtures of Polycyclic Aromatic Hydrocarbons

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ABSTRACT

The use of a software implemented backpropagation neural network is reported for the qualitative and quantitative analysis of the fluorescence emission spectra from multicomponent mixtures of Polycyclic Aromatic Hydrocarbons (PAHs) in solution. Analysis of two types of data is described. First, a backpropagation network is developed to determine the component concentrations in a ternary mixture of PAHs. The input data provided to the network consists of sampled two dimensional (intensity vs. emission wavelength) fluorescence spectra. A second backpropagation network is investigated for the analysis of three dimensional time resolved fluorescence emission spectra for a binary PAH mixture. Both of the networks are trained to recognize preselected compounds. Each trained network is then used to evaluate unknown emission spectra and to determine the presence and relative concentration of the compounds it has learned to recognize. Results from analysis of two dimensional emission spectra show that the trained network was able to successfully identify the individual components and their concentrations in solutions containing mixtures of anthracene, chrysene and acenapthene. Analysis of three-dimensional time resolved fluorescence emission data showed that individual components could be resolved in mixtures of two spectrally similar compounds (anthracene and chrysene). Although a network could also be trained to recognize anthracene and chrysene in binary mixtures using their two-dimensional emission spectra, use of three dimensional time decay spectra reduced the learning time required to train the network by a factor of three.

1. INTRODUCTION

In recent years, work has been underway to develop techniques for remote spectroscopy using optical fibers and fiber optic based chemical sensors for real time remote *in situ* determination of toxic pollutants in natural waters and soil.[1-3] Several studies have been reported on the use of fluorescence and time-resolved fluorescence via optical fibers for environmental monitoring of polycyclic aromatic hydrocarbons (PAHs).[4-5] Laser induced fluorescence emission spectrometry is a fast and sensitive method for measuring PAH contamination in environmental samples. It provides a convenient direct method of analysis which requires no added chemical reagents and eliminates the need for discrete sampling.

The disadvantage of using fluorescence techniques for measurement of mixtures of PAHs is a lack of specificity. For *in situ* field measurements the fluorescence emission signal is a composite of the emission of all compounds present in the sample that fluoresce at the selected excitation wavelength. It is usually not possible to determine the identity or concentration of individual PAHs contributing to the observed signal. This is because many PAHs exhibit similar emission spectra or spectra that overlap significantly. Also, most PAHs are excited over a broad band of wavelengths. The problem is compounded when environmental matrix effects are considered. Fluorescent response, including both spectral and temporal intensities, may be a function of many parameters including pH, temperature, soil type, and dissolved oxygen concentration. Naturally occurring organic compounds (eg., humic

substances) and inorganic fluorophores (eg., fluorescent minerals) can also contribute to the overall signal response. Finally, because of chemical and photochemical interactions between molecular species, individual emission spectra may add nonlinearly. An example of this is when one species fully or partially quenches the emission of another. The task of accurately evaluating the fluorescence emission signals from mixtures of compounds measured *in situ* can be complex.

Methods for improving the selectivity of laser induced fluorescence by using multiple or scanned excitation wavelengths or time resolved fluorescence have been investigated.[6-7] These methods generate data arrays that contain much more chemical information than a conventional two dimensional spectrum. Whether used singly or combined, data generated by these techniques must be processed off-line using techniques that are calculation intensive and therefore not presently suitable for real time analysis.

The motivation behind the work presented here is to develop an algorithm for rapid on-line interpretation of fluorescence emission signals generated by remote fiber optic chemical sensors. Neural computing appears to offer many advantages that make it attractive for addressing pattern recognition/signal processing problems. Artificial neural systems are capable of learning complex nonlinear associations without prior knowledge of parameters or interactions of the system being learned. Neural networks are able to learn by example, thus eliminating the need to develop models as with a rule based expert system. Also, once trained neural networks can rapidly process large data arrays, allowing for real time on-line analysis. The purpose of this investigation is to provide a preliminary evaluation of the utility of using neural networks for analysis of fluorescence emission spectra from multi-component mixtures of PAHs.

2. BACKPROPAGATION NEURAL NETWORKS

2.1 Overview.

Numerous implementations of the backpropagation neural network algorithm have been reported over the past five years. Applications have appeared in such diverse areas as signal processing, market analysis, pattern recognition, data compression, and text-to-speech conversion. A more thorough description of the backpropagation paradigm along with theoretical foundations and application reviews can be found in several sources.[8-10]

In most general terms, backpropagation neural networks perform a heteroassociative mapping of an n-dimensional input vector to a m-dimensional target or output vector. The greatest usefulness of backpropagation neural networks is derived from its ability to learn to perform arbitrarily complex nonlinear mappings. The network learns and stores the appropriate or desired mapping function by means of training example pairs (A1,f(A1)), (A2,f(A2)),... (Ak,f(Ak)). The input (A) and desired output f(A) pairs are presented to the network in repeated random succession. The network continuously self adapts until each of the input vectors causes the network to produce the associated desired output vector. This process is referred to as supervised learning. When no further adjustments are necessary, the network is said to be trained. At this point the error between the network's actual output and the desired output has been made acceptably small and the network may be used to process new input not previously seen by the network. Trained backpropogation networks are able to generalize. That is, given new incomplete, or noisy input data, the network can make decisions based on past experience on what the output should be.

A schematic of the generic backpropagation architecture is shown in Figure 1. The network is made up of layers (rows) of interconnected processing elements (PEs) or nodes. Associated with each pair of connected PEs is an adjustable scalar weight parameter. The first or bottom layer of PEs, the input layer, serves to distribute the input vector to the next layer of PEs. The number of nodes in this first layer corresponds to the n components of the input vector. Each PE of the input layer receives as input one component of the input vector. The next higher layer is termed the hidden layer; hidden because neither its inputs or outputs are exposed to the outside world but rather are contained within the interior of the network. There may be any number of hidden layers in a network; however, the number is usually less than three. The number of PEs in each hidden layer is a variable parameter. The optimal number can only be found by a trial and error approach. Each PE of the hidden layer receives input from each of

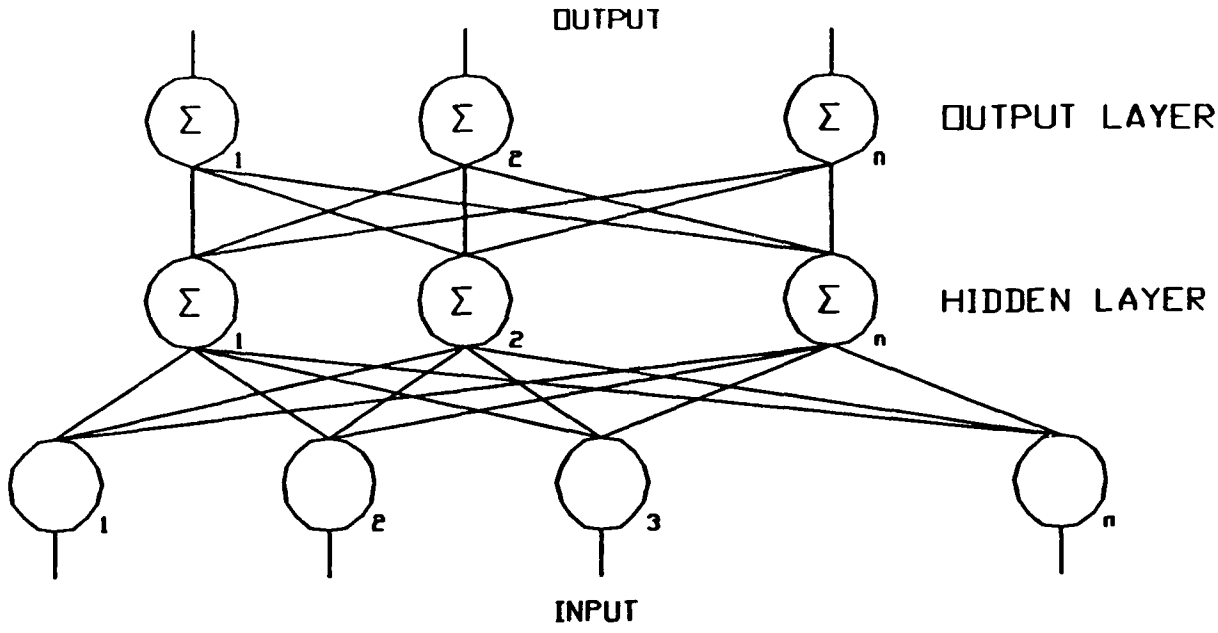
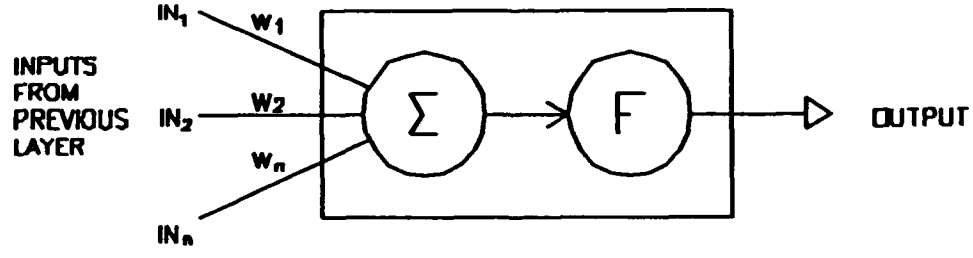


Figure 1. Schematic of a generic backpropogation neural network.

the nodes in the previous layer. The output from each hidden PE is a function of the weighted summation of its inputs.

The transfer function F can be any differentiable function. It is most often either a sigmoid, hyperbolic tangent, or sine. Figure (2) shows an individual PE. The output of the hidden layer PEs feedforward into the PEs of the final or output layer which perform the same operation on their summed input. The number of PEs in the output layer correspond to the dimensionality of the output vector. The output of this layer is the associated mapping of input vector **A**.



$$OUTPUT = F\left(\sum_{i=1}^{n}(W_i * IN_i)\right)$$

$$F(x) = \frac{exp(x) - exp(-x)}{exp(x) + exp(-x)}$$

Figure 2. Individual processing element showing equation for ouput parameter and expression for hyperbolic tangent transfer function.

2.2 Training.

The objective of training is to find a combination of connection weight values which causes the network to produce the desired output vector in response to a given input. This is accomplished by searching the set of all possible weight combinations in order to locate an optimal combination. Initially, the weights of an untrained network are assigned small random numbers. An input vector from the training set is presented to the network which produces an output as a function of the current weight values. The value at the output of the network is compared to the corresponding target output value provided by the training set. The weights feeding from each hidden layer PE into the output layer are then adjusted according to the following rule:

$$\Delta W = n \delta \, OUTh$$

where:

$$\Delta W = Wnew - Wold$$

$$\delta = f'(\text{target output} - \text{actual output})$$

$$f' = \text{derivative of the transfer function}$$

$$n = \text{learning coefficient}$$

$$OUTh = \text{output from hidden layer}$$

Making adjustments to the weights feeding into the hidden layer is done in a similar manner. However, no target output value is assigned to the hidden layer PEs so δ for these PEs is determined by backpropagating the output error down through the network. This is computed as follows:

$$\delta \text{ hidden} = f'(\Sigma \delta W)$$

The output error is fed back through the network's weights to determine the error attributable to each lower PE.

3.0 APPLICATION TO TWO DIMENSIONAL FLUORESCENCE SPECTRA

For the initial study, fluorescence emission spectra measured for mixtures of different concentrations anthracene, chrysene, and acenapthene were used as input to the neural network. These three PAHs were chosen for this study because concentration dependent spectral nonlinearities in the mixtures provide a challenging task of analysis. Figure 3 shows the fluorescence emission spectrum of each of the three compounds as well as that of a mixture of the three at the same concentrations. It can be seen that acenapthene fluorescence is quenched by the presence of chrysene and anthracene. This is due to the absorption bands of the latter coinciding with the emission band of acenapthene. Fluorescence emission of acenapthene is absorbed by the other two compounds. This is further demonstrated in Figure 4 which shows a plot of intensity vs. concentration for acenapthene in the presence of different concentrations of chrysene. Increasing amounts of chrysene result in increasing suppression of fluorescence emission response from acenapthene.

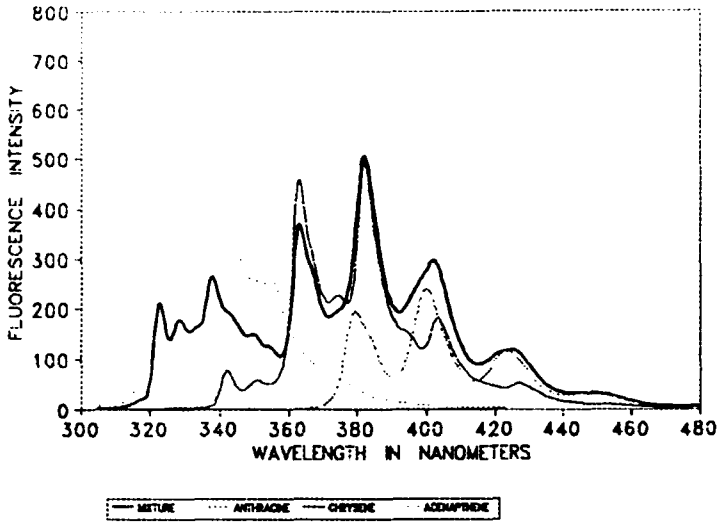


Figure 3. Fluorescence emission spectra for anthracene, chrysene, acenaphtene and a mixture of the three.

3.1 Experimental.

Emission spectra were gathered with a conventional scanning spectrofluorometer (Shimadzu RF5000U) utilizing a Xe lamp excitation source. Primary standard solutions of anthracene, chrysene, and acenapthene were prepared by dissolving a small amount of solid material in cyclohexane. All other solutions were prepared by diluting and mixing portions of the primary standards in cyclohexane. The anthracene concentration was varied between 0.0 and 60.0 ppm, chrysene and acenapthene between 0.0 and 45.0 ppm. The excitation wavelength was fixed at 290 nm with a 5 nm bandwidth. Emission was scanned between 300 and 480 nm at a 2 nm band width. Overall spectral resolution was 0.7 nm. The experimental precision was measured by preparing duplicate samples and comparing the recorded spectra. The average intensity difference was found to be less than 2%. There was no measurable error in wavelength. The collected spectra were converted to an ASCII format data file of values representing intensity every 0.7 nm using SpectraCalc (Galactic Industries, Corp.). The ACSII format spectra were sampled at 148 evenly spaced intervals between 300 and 480 nm. Thus each spectrum provided a vector of dimension 148 to be used as input data for the neural network.

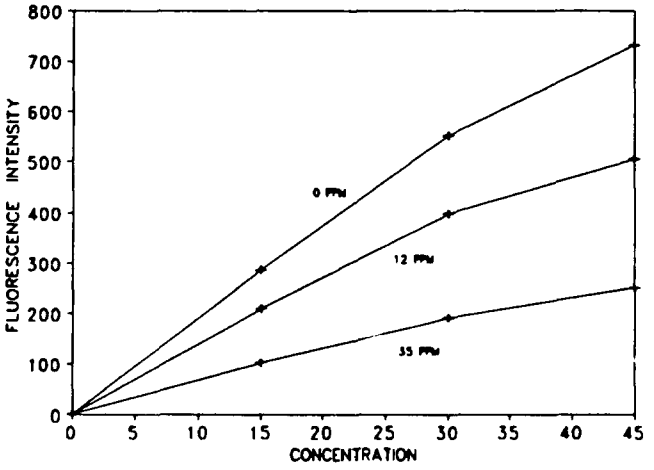


Figure 4. Standard additon curves for acenapthene in the presence of 0, 12 and 35 ppm chrysene.

The neural network was implemented in software (NeuralWorks Professional II, NeuralWare Inc, Pittsburgh, PA) on a 80386 processor based microcomputer. The network consisted of 148 nodes in the input layer, 9 nodes in the hidden layer, 3 nodes in the output layer, and a bias node. The bias node is a separate PE which is fully connected to all other PEs. Its purpose is to supply a threshold analogous to ground in an electronic circuit. All nodes were fully connected. The transfer function was the hyperbolic tangent. This transfer function serves to limit the output of each node to values greater than -1 but less than 1. The learning rule used was a normalized cumulative delta rule with momentum. The epoch was set to 8, that is the weights were updated after every 8 vector presentations of the learning set. Each layer used a separate variable learning schedule. The hidden layer learning rate was 0.5 at the beginning of a training session. The output layer started at 0.25. Each learning rate was lowered by a factor of two after every 2000 iterations. The momentum parameter was initially set at 0.4 and programmed to diminish by a factor of two every 2000 iterations.

Spectra from 104 separate mixtures of the three PAHs were used as the training set. Each spectrum corresponded to one input vector. The concentration of each of three components used to make each training mixture was used as the output vector for supervised training of the network. A separate test set of PAH mixtures was prepared and their fluorescence emission spectra measured. The test set contained 26 spectra that the network had not previously been exposed to during training. Data was presented randomly.

3.2 Results.

The time required for the network to learn the set of 104 training spectra was approximately 20 minutes. In this time the network went through 18000 iterations and reached a final root mean square (rms) error of 0.04 for the training set. Further training up to 200000 iterations did not improve the learning. Figure 5 shows the results of testing the network with 26 previously unseen spectra.

Each plot in Figure 5 shows actual vs. network predicted concentration values for individual components in the three component mixtures. The solid line in each figure is the line where the predicted concentration is equal to the actual concentration. The observed agreement between the predicted vs. actual concentration for each component in the three component test mixture shows that the network was able to recognize the identity and concentration of individual components in the mixtures. The success of the network for separating contributions from individual PAHs

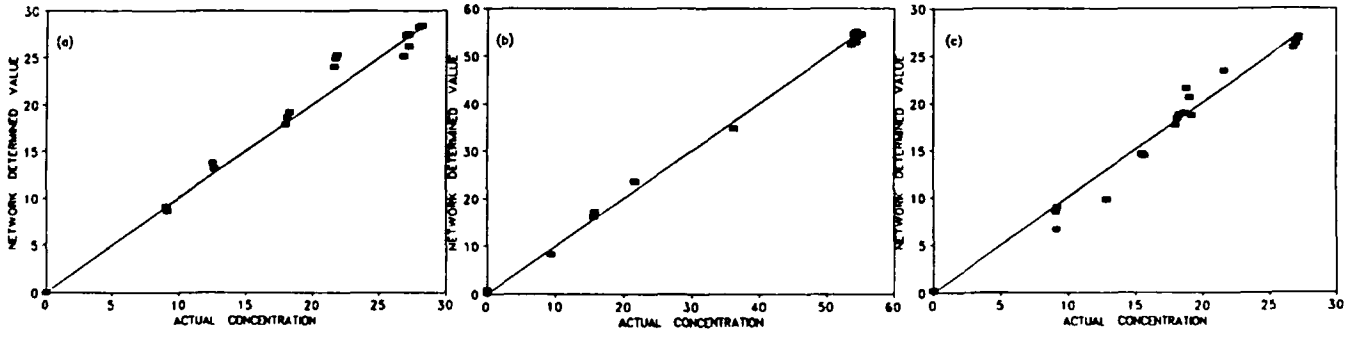


Figure 5. Network predited vs. actual concentrations of (a) acenapthene, (b) anthracene and (c) chrysene for unknown mixtures in test data set. All concentratrations in ppm.

in the spectra from the mixture is significant because, as was shown earlier, emission spectra from individual components do not add linearly. In addition, the number of components (zero, one, two or three) in each test mixture was not known.

4.0 APPLICATION TO TIME RESOLVED FLUORESCENCE DATA

Combinations of a binary mixture of chrysene and anthracene was chosen as a test case for the investigation of the use of neural networks for evaluating time resolved fluorescence data. Figure 6 shows the emission spectrum of the two compounds along with that of a mixture of the two. Emission spectra for the two compounds show significant overlap, differing only in the intensity ratios of the major peaks. Although chrysene and anthracene are difficult to distinguish spectrally, fluorescence lifetimes for the two compounds differ by nearly 10 ns (5 and 15 ns, respectively). Therefore, mixtures of these two compounds could be used to evaluate the ability of the neural network to recognize compounds that are spectrally similar but have different lifetimes.

4.1 Experimental.

The instrumental arrangement for collecting time resolved fluorescence spectra is similar to that previously reported.[4] A pulsed nitrogen laser (Model 2300, Photon Technology, Inc) served as the excitation source. The laser pulse energy is 1.4 mJ at 337 nm for a duration of 0.8 ns per pulse. This light is coupled into a 45 m long 580 micron diameter fused silica optical fiber. Output at the distal end of the fiber is directed into a cuvette containing the sample solution. The fluorescence induced in the sample cell is partially collected by a second fiber of the same type and length. The fluorescence is transmitted via the receiving optical fiber through collimating optics to a 300 groove/nm holographic grating spectrograph (EG&G PARC model 1232) which disperses the light over a photodiode array detector (EG&G PARC model 1420). An optical multichannel analyzer (EG&G PARC model 1460) is used to measure and record the output response of the photodiode array as well as to control the firing of the laser.

Measurement of the fluorescence lifetime is accomplished by gating the photodoide array detector. With each pulse of the laser, a small portion of the excitation light pulse is intercepted with a separate length of optical fiber and directed onto a photodiode which serves as an optical trigger. The optical trigger activates a fast pulser (EG&G PARC model 1302) that gates the photodiode array detector on for 20 ns. Successive scans are incrementally delayed relative to the laser pulse. This provides multiple 20 ns integrations of the signal, each recorded at an

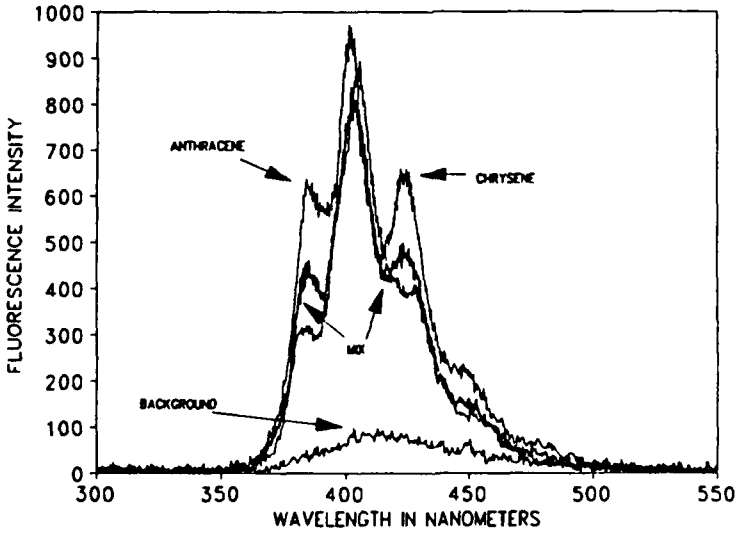


Figure 6. Fluorescence emission spectra of anthracene, chrysene and a mixture of two.

increasingly longer delay after the excitation pulse. Hence, the signal collected consists of a number of stacked spectra or spectral time slices.

The topology of the neural network used to analyze the time resolved data was similar to that presented in Figure 1. Seven PEs were used in the hidden layer. The intensity of each successive time slice is sampled along its wavelength coordinate. These values provide the input to the network. As before, the network output consists of concentration values of the components of the mixture. The training set was made up of 33 separate emission decay matrices generated from mixtures of the two components varying in concentration between 0 and 500 ppb for anthracene and between 0 and 60 ppm for chrysene. The test set consisted of six emission decay matrices.

4.2 Results.

Emission spectra at two delay times (two time slices, separated by 12 ns) were used as input to the network. This proved to be sufficient to distinguish the two compounds of interest. Training time for the small training set was less than five minutes. After 6000 iterations the network had learned to recognize the spectra of the training set to an accuracy of better than 1.0% (rms error). Results of processing the test set consisting of emission-decay matrices from six binary mixtures of anthracene and chrysene not previously seen by the network are shown in Figure 7. Each set of four bars in Figure 7 gives the predicted vs. actual concentration for anthracene followed by the predicted vs. actual concentration for chrysene. It is interesting to note that similar results were obtained with the same data using only the two dimensional emission spectra; however, the network took three times as long to train. Confidence limits (99%) on predicted concentrations equals ± 23%. The network did not do as good a job of predicting concentrations in this case as it did in the case of ternary mixture discussed previously because a much smaller training set was used to train the network and the precision of the experimental data was not as good for the time decay data as for the emission spectra.

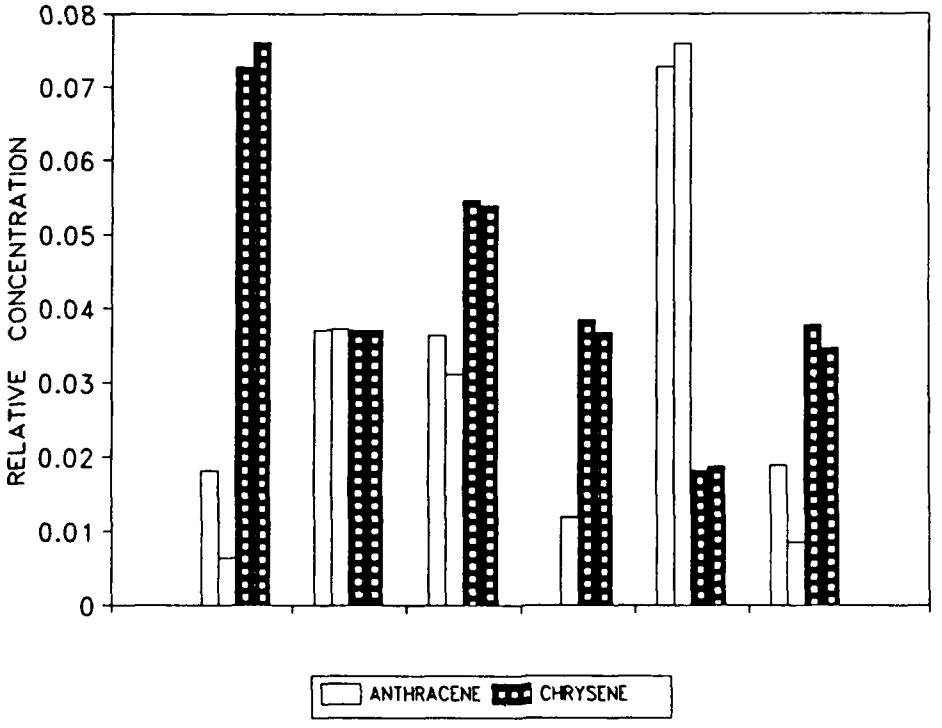


Figure 7. Network predicted vs. actual concentration for six test mixtures of anthracene and chrysene. The first bar in each series is the actual anthracene conentraton, the second bar is the predicted anthracene concentation, the third and fourth bars are the actual and predicted concentations of chrysene.

5.0 CONCLUSIONS

Results from this investigation suggest that artificial neural networks may be useful for on line analysis of data from fluorescence based optical fiber sensor systems. Studies presented here show that neural networks could readily resolve (qualitatively and quantitatively) individual compounds in ternary and binary mixtures through analysis of fluorescence emission spectra and fluorescence time decay data. Training time was quite rapid for the examples studies here (tens of minutes). Once trained the networks could process data on time scales that are compatible with on line processing (seconds). Future successful use of neural networks for analysis of sensor data from environmental samples will depend on the ability to generate representative training data sets and o the ability of the network to generalize when presented data that deviates from the training set. We are presently investigating how the performance of the network is effected when previously unseen compounds are added and/or ancillary environmental parameters are changed.

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