Dyes Assay for Measuring Physicochemical Parameters

ARTICLE *in* ANALYTICAL CHEMISTRY · MARCH 2009

Impact Factor: 5.64 · DOI: 10.1021/ac802482h · Source: PubMed

Anal. Chem. **2009,** *81,* 2311–2316

Dyes Assay for Measuring Physicochemical Parameters

Ewa Moczko,* Igor V. Meglinski, Conrad Bessant, and Sergey A. Piletsky

Cranfield Health, Cranfield University, Cranfield, MK43 0AL, U.K.

A combination of selective fluorescent dyes has been developed for simultaneous quantitative measurements of several physicochemical parameters. The operating principle of the assay is similar to electronic nose and tongue systems, which combine nonspecific or semispecific elements for the determination of diverse analytes and chemometric techniques for multivariate data analysis. The analytical capability of the proposed mixture is engendered by changes in fluorescence signal in response to changes in environment such as pH, temperature, ionic strength, and presence of oxygen. The signal is detected by a three-dimensional spectrofluorimeter, and the acquired data are processed using an artificial neural network (ANN) for multivariate calibration. The fluorescence spectrum of a solution of selected dyes allows discreet reading of emission maxima of all dyes composing the mixture. The variations in peaks intensities caused by environmental changes provide distinctive fluorescence patterns which can be handled in the same way as the signals collected from nose/tongue electrochemical or piezoelectric devices. This optical system opens possibilities for rapid, inexpensive, real-time detection of a multitude of physicochemical parameters and analytes of complex samples.

The development of sensor arrays for rapid, multidetection, and quantification of various analytes in complex samples is one of the major challenges faced by analytical scientists.[1] Such "intelligent sensors" are especially required in medical, biological, healthcare and food industries,[2−4] environmental monitoring,[5−7] and bioprocesses control.[8,9] Over the past decades, the majority of multidetection systems have been developed for odors characterization, so-called electronic noses consisting of array of gas sensors.[10−13] Recently sensor arrays known as electronic tongues[14−16] have been presented for liquid analysis. The operating principle of these sensors lies in the multivariate interpretation of the collective response of an array of electrochemical or piezoelectric transducers that mimics mammalian smell and taste recognition. While the concept of multivariate analysis, as an approach for examination of sensor array data and detection of analytes in complex samples, has been explored particularly for electrochemical, electrical, or piezoelectric sensors,[17,18] only limited efforts have been put into a development of equivalent optical sensors.[11,19−21] These optical sensing systems typically represent arrays of discreet optical elements which have been successfully used for measurements of diverse analytes and parameters in environmental or biological samples.[22−24] Optical methods for chemical imaging have been found advantageous as they enable direct, real time chemical imaging of samples not limited by area (distribution of oxygen and pH in seawater, human skin), visualization of intrinsically fluorescent chemical species (nicotinamide adenine dinucleotide, flavines, chlorophylls), nonfluorescent species, and analytes by using suitable molecular probes.[25,26] Optical microsensors apply commercially available fluorescent indicators and did not require expensive preparation

* To whom correspondence should be addressed. E-mail: e.moczko@cranfield.ac.uk.

(1) LaFratta, C. N.; Walt, D. R. *Chem. Rev.* **2008**, *108*, 614–637.
(2) Castillo, J.; Gáspár, S.; Leth, S.; Niculescu, M.; Mortari, A.; Bontidean, I.; Soukharev, V.; Dorneanu, S. A.; Ryabov, A. D.; Csöregi, E. *Sens. Actuators, B* **2004**, *102*, 179–194.
(3) Röck, F.; Gurlo, A.; Weimar, U. *Anal. Chem.* **2005**, *77*, 2762–2769.
(4) Shepard, J. R. E.; Danin-Poleg, Y.; Kashi, Y.; Walt, D. R. *Anal. Chem.* **2005**, *77*, 319–326.
(5) Wanekaya, A. K.; Chen, W.; Mulchandani, A. *J. Environ. Monit.* **2008**, *10*, 703–712.
(6) Rodriguez-Mozaz, S.; de Alda, M. J. L.; Barcelo, D. *Anal. Bioanal. Chem.* **2006**, *386*, 1025–1041.
(7) Rogers, K. R. *Biosens. Bioelectron.* **1995**, *10*, 533–541.
(8) Rudnitskaya, A.; Legin, A. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 443–451.
(9) Esbensen, K.; Kirsanov, D.; Legin, A.; Rudnitskaya, A.; Mortensen, J.; Pedersen, J.; Vognsen, L.; Makarychev-Mikhailov, S.; Vlasov, Y. *Anal. Bioanal. Chem.* **2004**, *378*, 391–395.
(10) Dickinson, T. A.; White, J.; Kauer, J. S.; Walt, D. R. *Trends Biotechnol.* **1998**, *16*, 250–258.
(11) James, D.; Scott, S. M.; Ali, Z.; O'Hare, W. T. *Microchim. Acta* **2005**, *149*, 1–17.
(12) Rock, F.; Barsan, N.; Weimar, U. *Chem. Rev.* **2008**, *108*, 705–725.
(13) Göpel, W. *Sens. Actuators, B* **1998**, *52*, 125–142.
(14) Gutierrez, M.; Algret, S.; del Valle, M. *Biosens. Bioelectron.* **2008**, *23*, 795–802.
(15) Gutierrez, M.; Gutierrez, J. M.; Alegret, S.; Leija, L.; Hernandez, P. R.; Favari, L.; Munoz, R.; Del Valle, M. *Int. J. Environ. Anal. Chem.* **2008**, *2*, 103–117.
(16) Ciosek, P.; Wroblewski, W. *Analyst* **2007**, *132*, 963–978.
(17) Pravdova, V.; Pravda, M.; Guilbault, G. G. *Anal. Lett.* **2002**, *35*, 2389–2419.
(18) Penza, M.; Cassano, G.; Tortorella, F. W. *Meas. Sci. Technol.* **2002**, *13*, 846–858.
(19) Bruno, A. E.; Barnard, S.; Rouilly, M.; Waldner, A.; Berger, J.; Ehrat, M. *Anal. Chem.* **1997**, *69*, 507–513.
(20) Dickinson, T. A.; White, J.; Kauer, J. S.; Walt, D. R. *Nature* **1996**, *382*, 697–700.
(21) Johnson, S. R.; Sutter, J. M.; Engelhardt, H. L.; Jurs, P. C.; White, J.; Kauer, J. S.; Dickinson, T. A.; Walt, D. *Anal. Chem.* **1997**, *69*, 4641–4648.
(22) Kocincova, A. S.; Nagl, S.; Arain, S.; Krause, C.; Borisov, S. M.; Arnold, M.; Wolfbeis, O. S. *Biotechnol. Bioeng.* **2008**, *100*, 430–438.
(23) Hua, P.; Hole, J. P.; Wilkinson, J. S.; Proll, G.; Tschmelak, J.; Gauglitz, G.; Jackson, M. A.; Nudd, R.; Griffith, H. M. T.; Abuknesha, R. A.; Kaiser, J.; Kraemmer, P. *Opt. Express* **2005**, *13*, 1124–1130.
(24) Baleizaao, C.; Nagl, S.; Schaferling, M.; Berberan-Santos, M. N.; Wolfbeis, O. S. *Anal. Chem.* **2008**, *80*, 6449–6457.
(25) Wolfbeis, O. S. *Adv. Mater.* **2008**, *20*, 3759–3763.

10.1021/ac802482h CCC: $40.75 © 2009 American Chemical Society
Published on Web 02/16/2009

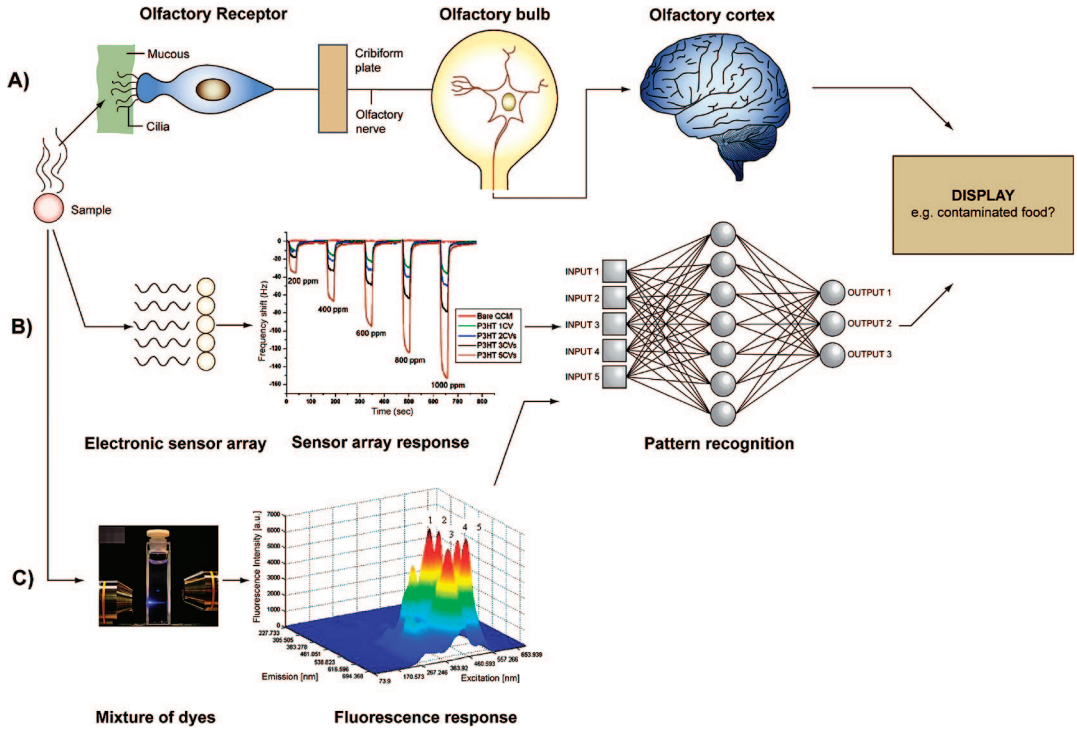

Figure 1. Schematic representation of sensing systems: (A) human olfactory system, (B) electronic analogue, and (C) our optical analogue. Principle adopted from Turner and Magan.[36]

of the array and signal processing. Optical sensor arrays have been successfully used in multianalyte sensing, e.g., in analysis of a mixture of heavy metal ions.[27] The relative selectivity in presented experiments was achieved by analyzing the pattern of the response collected from the array. Among optical techniques, fluorometry based on time-resolved imaging for detection and mapping of analyte gradients within a sample have offered a promising alternative for electrochemical sensors.[24,26] The potential of the assay has been demonstrated for monitoring of analytes such as hydroxyl peroxide, uric acid, oxygen, carbon dioxide, pH, or glucose.[28−31] Fluorometry appears to be a very promising tool because of high sensitivity and speed of measurements, low cost, and ability for simultaneous real-time detection of multiple analytes.[32−35]

This paper describes the development and testing of an optical equivalent to an electronic nose and tongue that uses fluorescence spectroscopy and a mixture of environment-sensitive fluorescent dyes where each dye of the mixture represents a different indicator. The relationship between spectral characteristics of the mixture and the changes caused by interactions with the surrounding medium provides qualitative and quantitative information about the environmental conditions. Figure 1 schematically illustrates the principle of the electronic nose that mimics the human olfactory system[36] and also the new concept where electrochemical components are replaced with fluorescent dyes. The intensity spectrum of a solution of the suitable dyes allows discreet reading of emission maxima of all dyes. Intensity variations in a response to environmental changes provide distinctive patterns which can be analyzed in the same way as complex signals collected by an electronic nose/tongue electrochemical or piezoelectric array.[37,38] The analytical information about a sample is coded in the fluorescence pattern composed of unique characteristics of individual dyes. This information is decoded using multivariate data analysis by a transformation of the optical system response into the actual physicochemical parameters and analytes of interest.

The use of a mixture of fluorescent dyes for optical sensing has a number of advantages, including easy application, high speed, and low cost of measurement but has not been documented before. It also resolves one of main problems associated with electronic nose technology wherein sensor array has to be calibrated every time it is used even for the same application. The proposed assay does not have this limitation and the mixture of fluorescent dyes can be perceived as an analytical reagent that can be tuned for various specific analytical tasks. Since the proposed assay does not base on spatially discreet sensor elements it can be potentially applied to a very small volume of a sample. The flexibility and versatility of the proposed approach is deter-

(26) Kocincova, A. S.; Borisov, S. M.; Krause, C.; Wolfbeis, O. S. *Anal. Chem.* **2007**, *79*, 8486–8493.
(27) Mayr, T.; Igel, C.; Liebsch, G.; Klimant, I.; Wolfbeis, O. S. *Anal. Chem.* **2003**, *75*, 4389–4396.
(28) Schrenkhammer, P.; Wolfbeis, O. S. *Biosens. Bioelectron.* **2008**, *24*, 994–999.
(29) Stich, M. I. J.; Nagl, S.; Wolfbeis, O. S.; Henne, U.; Schaeferling, M. *Adv. Funct. Mater.* **2008**, *18*, 1399–1406.
(30) Schaferling, M.; Wu, M.; Enderlein, J.; Bauer, H.; Wolfbeis, O. S. *Appl. Spectrosc.* **2003**, *57*, 1386–1392.
(31) Liebsch, G.; Klimant, I.; Frank, B.; Holst, G.; Wolfbeis, O. S. *Appl. Spectrosc.* **2000**, *54*, 548–559.
(32) Wolfbeis, O. S. *Trends Anal. Chem.* **1985**, *4*, 184–188.
(33) Gryczynski, Z.; Gryczynski, I.; Lakowicz, J. R. *Methods Enzymol.* **2003**, *360*, 44–75.
(34) Nagl, S.; Wolfbeis, O. S. *Analyst* **2007**, *132*, 507–511.
(35) Borisov, S. M.; Wolfbeis, O. S. *Chem. Rev.* **2008**, *108*, 423–461.
(36) Turner, A. P.; Magan, N. *Nat. Rev. Microbiol.* **2004**, *2*, 161–166.
(37) Bessant, C.; Saini, S. *Anal. Chem.* **1999**, *71*, 2806–2813.
(38) Richards, E.; Bessant, C.; Saini, S. *Analyst* **2004**, *129*, 355–358.

Table 1. List of Fluorescent Dyes Selected for the Assay

	dye name	sensitivity	excitation [nm]	emission [nm]	extinction coefficient [cm^{-1} M^{-1}]	purity [%]	supplier CAS number
1	8-hydroxypyrene-1′,3,6-trisulfonic acid	near-neutral pH ionic strength	470	527	24 000 (water)	≥98	Sigma-Aldrich 6358-69-6
2	Oregon green 514 carboxylic acid	low pH	504	528	86 000 (DMF)	94	Molecular Probes
3	rhodamine B	temperature	541	576	88 000 (water)	∼95	Sigma-Aldrich 81-88-9
4	tris (4,7-diphenyl-1,10-phenanthroline) ruthenium dichloride	oxygen	541	628	14 600 (water)	99.95	Sigma-Aldrich 50525-27-4
5	thionin acetate	hydrogen peroxide	463	637	53 000 (water)	>85	Acros Organics 78338-22-4

mined by a large diversity of available fluorescent dyes selected in respect to a relevant analytical problem. The advantage of the system also lies in the possibility of extreme miniaturization.

The proposed concept is demonstrated in this paper for a simple assay combining four VIS-NIR/fluorescent dyes[39] capable of qualitative and quantitative simultaneous measurement of four common physicochemical parameters, namely, pH, temperature, and concentration of dissolved oxygen (DO) and sodium phosphate buffer (PB). These parameters have been chosen for a first instance because of their regular examination required in many areas including medicine, biotechnology, environmental diagnostics, and food and chemical industries. A sensor array for similar parameters has already been used[31] using a microtiter plate and time-resolved fluorometric measurements. However, our approach was to use fluorescent dyes in a mixture not as a spatially discreet sensor elements. In combination with multivariate data analysis, quick evaluation of complex fluorescence response of the mixture can be obtained.

EXPERIMENTAL SECTION

Composition of Dyes Mixture. The fluorescent dyes selected for the proposed assay are listed in Table 1. The mixture of fluorescent dyes was prepared as a stock solution of the following dyes: 0.15 mM 8-hydroxypyrene-1′,3,6-trisulfonic acid, 0.1 mM rhodamine B, 2 mM thionin acetate, 0.025 mM Oregon green 514, 6 mM tris (4,7-diphenyl-1,10-phenanthroline) ruthenium dichloride. Solutions of dyes were prepared in deionized water and stored at ∼5 °C, covered with aluminum foil to protect them from light. Then 200 μL of each water/dye stock solution was mixed together and used in further experiments.

Fluorescence Measurements. The measurements of fluorescence intensity have been performed using a three-dimensional spectrofluorimeter Jobin Yvon-SPEX FL-3D (Instruments SA, Stanmore, Middlesex, U.K.) at 0.5 s of time exposure. The spectra have been recorded over a range of excitation (227.7−724.5 nm) and emission (73.9−691.4 nm) wavelengths. The range of wavelengths is based on the technical specification of the spectrofluorimeter. The fluorescence measurements have been performed using 4 mL quartz cuvettes with stoppers and a light path of 10 mm. The typical spectrum of the dyes mixture is shown in Figure 2. The spectral characteristics of individual dyes have been analyzed at the intensity maxima of excitation and emission wavelengths (Table 1).

All measurements have been performed in sodium phosphate buffer (PB) consisting of sodium phosphate dibasic (Na_2HPO_4) and sodium phosphate monobasic (NaH_2PO_4) diluted in distilled water. The desired pH of the buffer and its concentration was prepared based on the ratio of monobasic and dibasic sodium phosphate and their different concentrations, respectively. Measurements have been performed at a pH range from 5.0 to 8.0, temperature from 25 to 40 °C, dissolve oxygen (DO) from 0 to 21.6 ppm, and PB concentration from 5 to 150 mM. In each measurement, 3 mL of suitable buffer was transferred into a 4 mL quartz cuvette, and then 50 μL of the mixture of five fluorescent dyes was added. The temperature of the cuvette containing the dyes solution was adjusted externally within a range of 25−40 °C using a thermostatic water-bath (Grant Instruments Ltd., model 0331, Cambridge, U.K.). The concentration of DO was controlled using an oxygen probe (World Precision Instruments Ltd., OXEL-1, Stevenage, U.K.) and potentiostat-galvanostat (Uniscan Instrument Ltd., PG580, Buxton, U.K.). The concentration of oxygen was adjusted by bubbling the solution with air or nitrogen and measured amperometrically. The number of moles of oxygen present in the solution was calculated at different temperatures using the ideal gas low.[40]

Data Evaluation. The spectral characteristics of the dyes mixture and changes caused by interactions with its surroundings have been analyzed using artificial neural networks. ANNs were implemented in MATLAB (version 7.3.0, MathWorks Inc., 2006) using the Neural Network Toolbox (version 5.0.1). The prediction model used for simultaneous determination of four parameters was created based on a feedforward network trained using a backpropagation of errors.[41] The network consisted of an input layer, one hidden layer, and an output layer. The input layer was made up of five neurons corresponding to the fluorescent emission of the five fluorescent dyes (taken at wavelengths shown in Table 1) and a single neuron in the output layer corresponded to a parameter of interest. The valuation of the parameters based on changes of the height of the fluorescence peaks of all five dyes. A different network was trained with 576 samples for each parameter being tasted. The best performance was obtained for the hidden layer that consisted of 25 neurons and Bayesian regularization[41] used in the learning process. To test the ability of the trained network to predict parameters from acquired data, the network was tested on a set of previously unseen 192 samples.

(39) Umezawa, K.; Nakamura, Y.; Makino, H.; Citterio, D.; Suzuki, K. *J. Am. Chem. Soc.* **2008**, *130*, 1550–1551.

(40) World Precision Instruments Inc. Instruction Manual for Isolated Dissolved Oxygen Meter and Oxygen Electrode.

(41) Demuth, H.; Beale, M.; Hagan, M. *NN Toolbox 5*; The MathWorks Inc.: Natick, MA, 1992−2007.

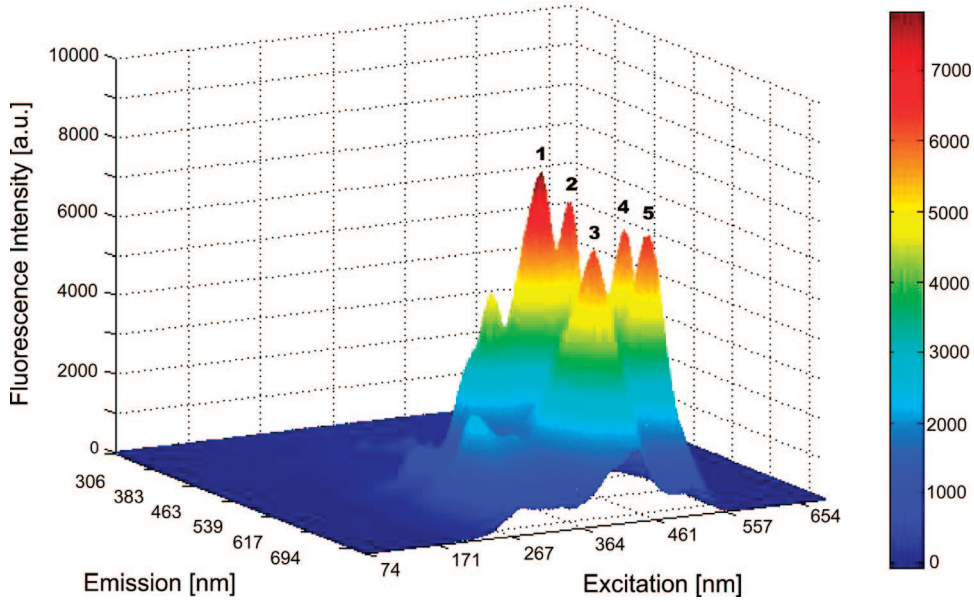

Figure 2. Three-dimensional color mapped surface diagram of five fluorescent dyes: dye 1 is 8-hydroxypyrene-1′,3,6-trisulfonic acid, dye 2 is Oregon green 514, dye 3 is rhodamine B, dye 4 is tris(4,7-diphenyl-1,10-phenanthroline) ruthenium dichloride, dye 5 is thionin acetate. Measurements were performed in 3 mL of 50 mM PB buffer in a quartz cuvette with 0.5 s of time exposure. Adopted from Piletsky, Moczko, and Meglinski.[45]

RESULTS AND DISCUSSION

Dyes Assay Characterization. The specific objective of our investigation was to create a combination of fluorescent dyes for quantitative monitoring of environmental changes including pH, temperature, ionic strength, and oxygen. On the basis of these requirements and available literature data, suitable fluorescent dyes have been selected. For the minimum interference with biological samples, our intention was to select fluorescent dyes that were responsive in the visible (VIS) and near infrared (NIR) (400−1600 nm) and that dyes have discreet emission maxima with the wavelengths separated by at least 20 nm. The selected dyes were not specific in the sense that their response can be triggered by more than one parameter. It is for this reason that multivariate calibration is required for quantitative analysis.

The mixtures of selected fluorescent dyes were tested and optimized experimentally using a 3D spectrofluorimeter that allows rapid measurements over multiple excitation/emission wavelengths. The most suitable mixture composed of five fluorescent dyes (see Table 1) was chosen and used in the following experiments. The fifth dye (thionin acetate), which is sensitive to hydrogen peroxide, allows further expansion of possible applications into enzymatic assays, where hydroxyl peroxide is a product or substrate of the reaction.[42] The spectrum of the fluorescent dye mixture used in the study is shown in Figure 2. The concentration of dyes was optimized to allow discreet reading of emission peaks for each fluorescent dye and to provide visualization of collective fluorescent patterns of dyes mixture. To exclude possible influence on the detected signal response, the concentrations were kept constant in all experiments. Therefore, it was expected that any changes of fluorescence emission of the solution of dyes were caused only by interaction with the surrounding medium. Figure 2 shows that fluorescence emission bands of proposed dyes overlap each other but the intensity maxima remain reasonably separated. This gives the possibility to analyze the comprehensive response of the whole mixture, along with each dye's individual impact on the signal. In further ANN simulations, the value of the fluorescence emission of each dye at the maximum of intensity were used. The dyes mixture in a solution appeared to be stable showing no variation in spectral properties over at least a 1 month period. Practically, no photobleaching was observed in the process of measurement.

The ability of fluorescent dyes to simultaneously monitor the changes of pH, temperature, and concentrations of DO and PB has been investigated. The response time of the dyes mixture to all analytes is very short (<1 min); therefore, the assay is suitable for real-time measurements. The initial experiments were performed to establish the influence of each single parameter on the spectrum response while other parameters of the dye assay were kept constant. Spectroscopic profiles of the selected fluorescent dyes were studied separately and in a mixed solution with others. An example of an effect on fluorescence characteristic of the mixture caused by pH changes is shown in Figure 3. Three-dimensional color mapped surface diagrams (on the left) and color filled contour diagrams (on the right) illustrate changes of the fluorescence signal of the dyes due to the different pH of a PB. Since the excitation/emission maxima of the intensity of fluorescent dyes are well separated, their individual characteristics due to decreasing pH are easily distinguished. The fluorescence intensity of dyes changing differently depends on their sensitivity to pH. The most significant changes appear for 8-hydroxypyrene-1′,3,6-trisulfonic acid (HPTS), which is specified as a near-neutral pH indicator.[43] The emission intensity of other dyes is also changed, very slightly in comparison with HPTS. A similar

(42) Cardosi, M. F. In *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*; Meyers, R. A., Ed.; John Wiley & Sons Ltd: Chichester, U.K., 2000; pp 1336−1348.

(43) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, 1999.

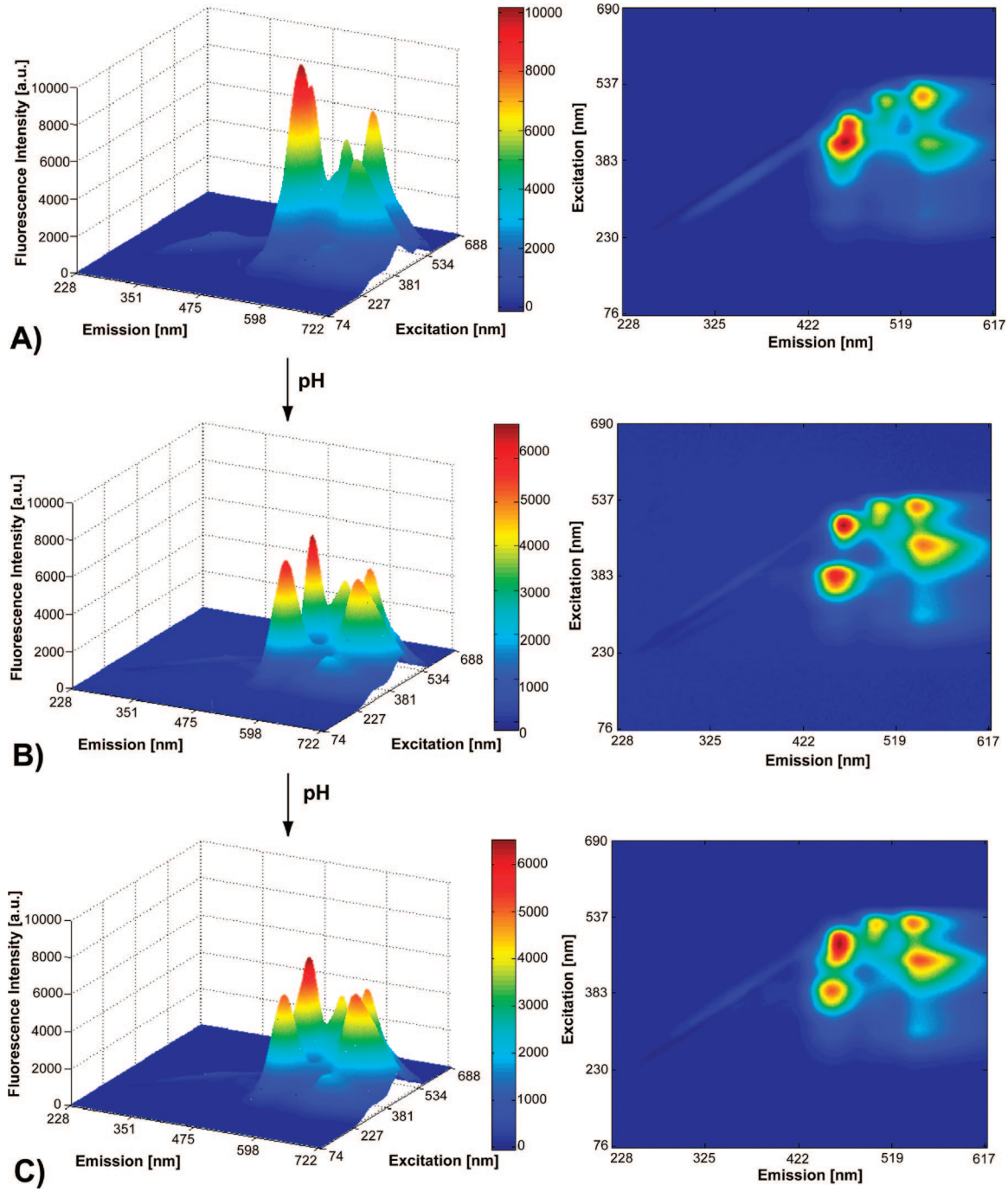

Figure 3. Three-dimensional color mapped surface diagrams (left) and color filled contour diagrams of the fluorescence signal of dyes mixture at pH 8 (A), pH 7 (B), and pH 6 (C) in 50 mM PB buffer at 30 °C and 20.6 ppm of oxygen concentration. Measurements were performed with 0.5 s of time exposure.

response of the dyes mixture was recorded for temperature and concentrations of DO or PB. As expected, reasonable changes in the fluorescence spectrum of the dyes mixture were observed in response to change of all parameters tested in this study. However, the signal response is not just a summation of individual spectral signatures of fluorescent dyes but also the product of their interaction and cross-interferences. This obviously complicates the analysis especially in the case when several parameters are changing simultaneously.

To study the effect of the parameters' variations, the fluorescence emission values were recorded for a number of samples with different values of pH, temperature, and DO and PB concentrations. The fluorescence signals were analyzed based on ANN as a pattern recognition method.

ANN. The key ability of the network has been identified and tested. This included training the ANN on patterns from samples of known identity and using them to predict the characteristics of new unknown samples with the smallest prediction error. Thus, the optimized network of the best performance was selected and applied for simultaneous determination of four parameters. The input data set consisted of spectral responses of the fluorescent dyes corresponding to known values of output parameters: pH, temperature, and DO and PB concentrations. Although, it was expected that the use of the whole fluorescence spectra in the

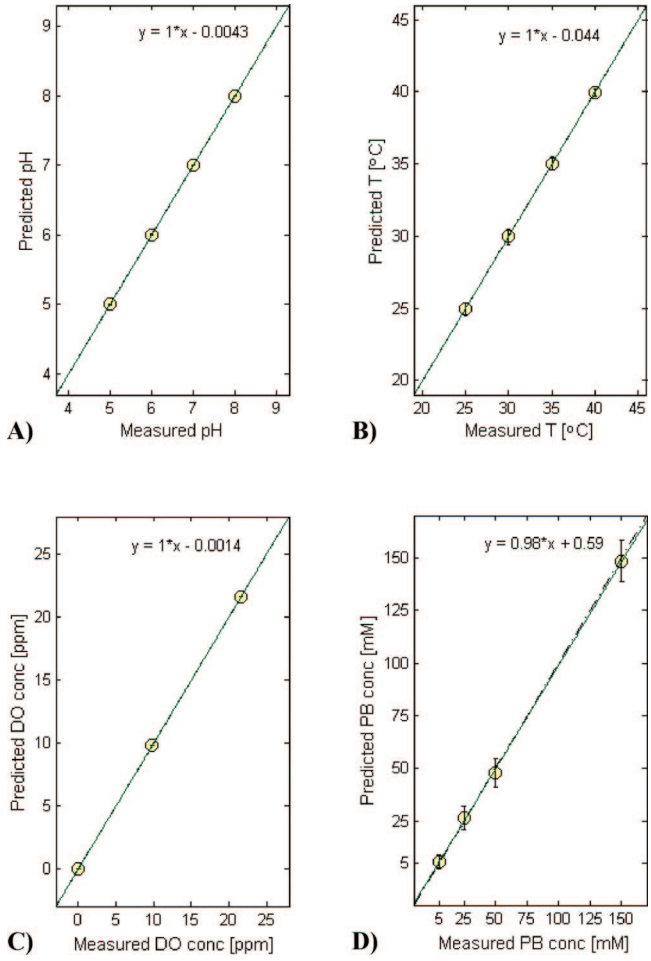

Figure 4. Correlation between actual (measured) and determined by ANN values of pH (A), temperature (B), dissolved oxygen (DO) concentration (C), and PB concentration (D). Points marked as circles (○) indicate mean values of the data points of ANN simulations of unseen samples. The diagonal line (—) indicates the best linear fit of data points and the line (— · —) indicates ideal response with zero error. Error bars indicate standard deviation.

training process would give the best contrast of unique responses of the dyes due to changes in a solution, it would also increase the amount of processing data and training time of the network. To minimize the workload, five wavelength points at the maxima of the fluorescence intensity of the dyes were chosen for a representation of the profile of the original spectra and used as actual input data for the ANN. This decision has been found sufficient for accurate prediction of target parameters.

Once the ANN was trained, an unseen set of data, called the test set, was used for the model evaluation. On the basis of learning experience, the network was capable to find the most likely identity of the unknown data pattern and predict output values of parameters of test solutions. Results of ANN predictions are shown in Figure 4. The graphs clearly illustrate the correlation between real (measured) values (*x* axis) and the values determined by ANN (*y* axis). Circles (○) indicate the mean values of data points of the network predictions. The standard deviation was calculated for 48 test samples for pH, temperature, and PB concentration and 64 samples for DO for each point. Accurate prediction of four parameters is demonstrated with relatively low root means square error (RMSE) of only 0.0038 for pH (Figure 4A), 0.4369 for temperature (Figure 4B), 0.0494 for DO (Figure 4C), and 6.8178 for PB concentration (Figure 4D). The slightly higher RMSE obtained for PB concentration is probably because the sensitivity of the fluorescent dye selected for salt detection is lower than sensitivities of other dyes. The RMSE values seem acceptable especially for measurements at the low PB concentration range. Additional information on the chemical selectivity of the calibration model and the analytical performance for particular parameters can be obtained using a pure component selectivity analysis combined with analysis of the net analyte signal for each analyte.[44] In this study, the combination of fluorescence signals of dye assay as a response to simultaneous changes of four parameters in the environment together with ANN allows quick and accurate qualitative and quantitative analysis of all these parameters.

CONCLUSIONS

An assay composed of five fluorescent dyes for a rapid, simultaneous determination of pH, temperature, dissolved oxygen, and a buffer concentration has been developed. The proposed optical system was combined with an ANN for the processing of spectral responses similar to electronic nose devices. The suitable mixture of fluorescent dyes has been designed based on requirements and the dye's sensitivity to the parameters of interest. The selected fluorescent dyes were stable, soluble in water, and had emission in the longer wavelengths region that makes them attractive for various biological applications. The result of our spectroscopic investigations clearly demonstrate that five fluorescent dyes can be used simultaneously along with discreet reading of the emission intensity changes in response to the changes of the tested environment. The ANN has shown great ability in adapting and modeling the nonlinear changes in the investigated system and provided accurate data analysis for measured solutions. As a result, high accuracy of simultaneous determination and quantification of pH, temperature, DO, and PB concentration were obtained. The potential of the proposed analytical tool is wide and diverse and can have great impact on a variety of applications including biological, clinical, and environmental analysis.

Received for review November 24, 2008. Accepted January 29, 2009.

AC802482H

(44) Ren, M.; Arnold, M. A. *Anal. Bioanal. Chem.* **2007**, *387*, 879–888.
(45) Piletsky, S.; Moczko, E.; Meglinski, I. U.K. Patent Application 0719373.3, 2002.