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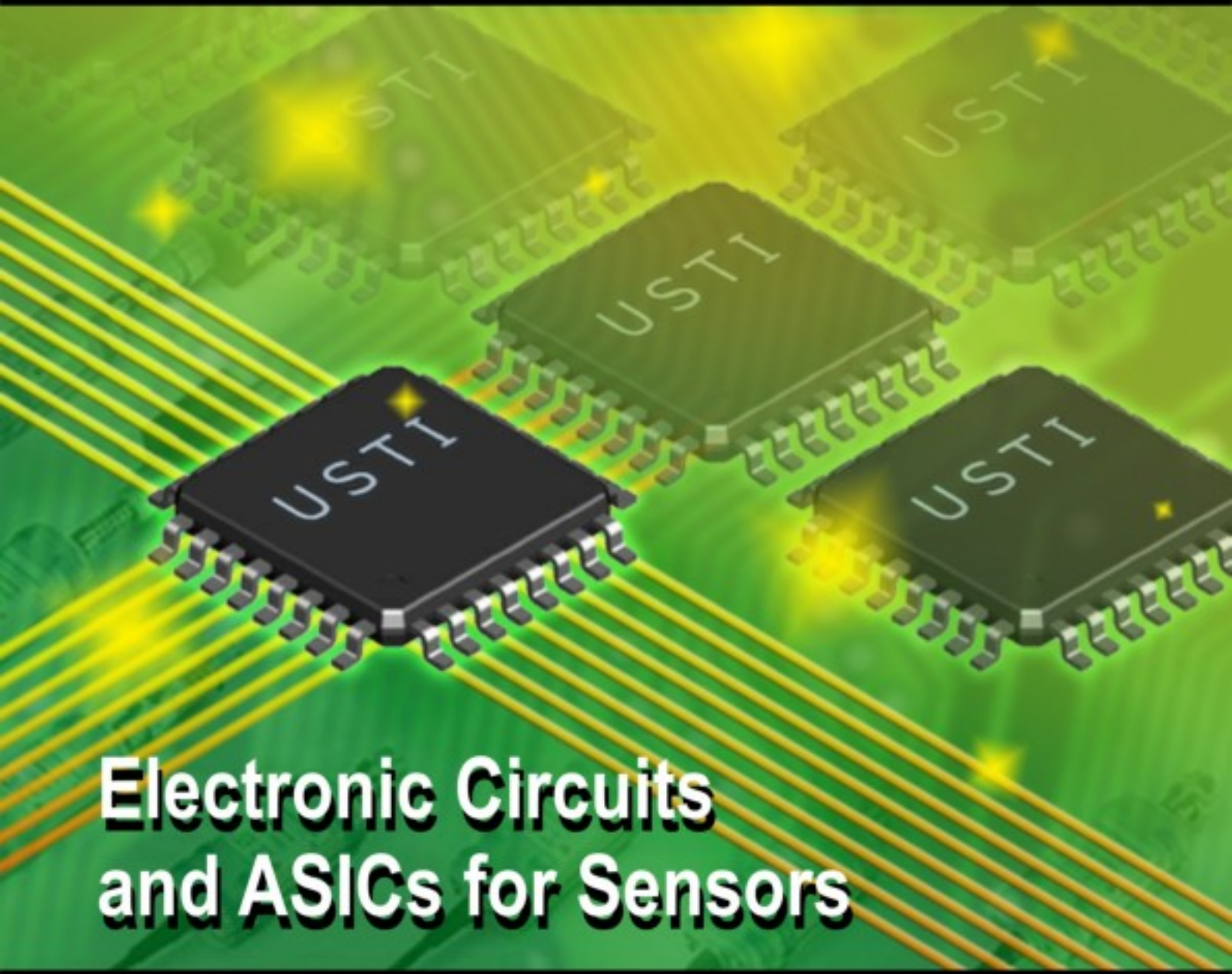

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Contents

Volume 93
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Editorial

Research Articles

Sensors & Transducers Journal, Vol. 93, Issue 6, June 2008, pp. 92-102

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Evaluation of Aquatic Environments Using a Sensorial System Based on Conducting Polymers and its Potential Application in Electrochemical Sensors

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Abstract: A sensor array consisted of interdigitated gold electrodes modified with nanostructured ultra-thin films of conducting polymers was used to evaluate different water samples from three distinct reservoirs, located in the São Paulo State, Brazil, according to their eutrophic level, i.e. oligotrophic, eutrophic and hypereutrophic. These reservoirs samples presented different eutrophic levels. The sensor array data were processed and analyzed by using PCA (principal component analysis). In the near future, this will be a reliable and straightforward method to analyze water samples based on the concept of global selectivity and electrochemical impedance.

Keywords: Aquatic environments, Conducting polymers, Sensor, Electrochemical impedance

1. Introduction

Concerning management of aquatic resources, the importance of the evaluation of water quality is undeniable. In order to identify and classify the different eutrophic level of water samples (e.g. lagoons and reservoir), two main types of eutrophic indicators have been, and still are being, used [1], which are related to biological or chemical and physical factors. The aim of the biological approach of

eutrophication is to measure its impact on the biodiversity of an environment. Thus, several classification indexes have been drawn up [2-5].

The chemical and physical approaches quantify the eutrophic level of an aquatic environment by measuring chemical and physical variables [6, 7]. These procedures present several limitations for the choice of the most appropriates analytical parameters that can be used to describe the phenomenon [8].

For the evaluation of water quality, biological, chemical and physical parameters are controlled by the inorganic chemical composition that characterizes the aquatic environment, such as nitrogen and phosphorus concentrations, which are constantly being modified by anthropic pressure [9-11].

Traditional methods were used to determine the eutrophic level of an aquatic environment requires longer times and involve relatively high costs. Therefore, the development of a relatively simple, fast and straightforward method to determine the eutrophic level of an aquatic environment is needed. The use of electrochemical sensor arrays based on the concept of global selectivity [12], which has been used to analyze the composition of the beverages [13] and to classify humic substances [14], are potential candidates to be used to determine the eutrophic level of water samples. It has been demonstrated that such sensor arrays are able to distinguish different types of humic substances, fulvic and humic acids from different sources, and to mimic the human palate to discriminate among sweet, salt, sour, bitter, and umami tastes. Sensor arrays based on this concept, i.e. global selectivity, which is based on the recognition of the system, not on discrimination of substances. Therefore, the specificity ceases to be a fundamental requirement in this sensory type of application. The materials not transducers need to be combined with particular species chemistry for the recognition of the liquid under study, because depending on the way in which they are to be contact with several, without the requirement for interaction with some chemistry in particular. Have also been used in different types of environmental analyses and water quality control, and have been demonstrated to be an efficient tool to analyze these complex systems [14, 15].

The sensor arrays based on the use of ultra-thin films of conducting polymers are generally fabricated by the use of the layer-by-layer self-assembly deposition technique of a polymeric ultra-thin film onto interdigitated gold electrodes [14, 15]. High sensitivity is achieved when interdigitated gold electrodes are coated with nanostructured ultra-thin films (~2 nm thick per deposited layer). These sensor arrays are able to detect very small changes in conductivity and dielectric properties of the materials used in each individual sensing unit that is in contact with an aqueous medium. It was also shown that low levels of impurities in water could be detected and discriminated by using these sensor arrays [15].

These sensor arrays, which use ultra-thin films of conducting polymers, work based on the changes in the capacitance of the system comprised by the ultra-thin film of conducting polymer and the interdigitated array. Changes in the thin-film structure due to the interaction with the compounds present in fluid phase induces changes in the electrical properties of the system, i.e. changes in capacitance of the system. These interactions between the sensing layer and the chemicals present in the fluid phase are not necessarily specific for any particular specie present in aqueous phase.

However, some fluid phase, such as water samples from lagoons and rivers contain many different types of chemicals in their composition. Therefore, the electrical signal pattern generated by the sensor array for each type of sample will be unique and it is considered to be the “fingerprint” of the sample, which can be related to certain features and properties of the sample. These features and properties can be determined by using different methods, such as parallel distributed processing (“neural network”) or chemometric (e.g. Principal Component Analysis, PCA). These methods are able to recognize and classify the sample that is being studied according to a database that was previously constructed using standard samples and procedures.

This work describes a new method for the evaluation of eutrophic level of an aquatic environmental sample by using a sensor array constructed using interdigitated gold electrodes modified with ultra-thin films of conducting polymers. The principle of operation of the system is based on the global selectivity concept [14-16]. Water samples from three different locations in Brazil were collected, analyzed and classified according to their eutrophic level.

2. Experimental Section

2.1. Sensor Array

A sensor array consisting of nine sensor units were prepared using the self-assembly [17] technique (Fig. 1). The intercalated systems consisted of the layer-by-layer deposition of the following layers: (i) poly(*o*-ethoxyaniline) (POEA) 1 x 10^{-3} M / sulfonated lignin (SL) 1 x 10^{-3} M, and (ii) POEA 1 x 10^{-3} M / caproic acid 1 x 10^{-3} M. The complex systems consisted of an aqueous solution containing a mixture of the following compounds: (i) POEA 1 x 10^{-3} M + SL 1 x 10^{-3} M, and (ii) POEA 1 x 10^{-3} M + caproic acid 1 x 10^{-3} M. Single layers of the following compounds were also deposited: (i) POEA 1 x 10^{-3} M, (ii) stearic acid 1 x 10^{-3} M, and (ii) caproic acid 1 x 10^{-3} M.

All depositions described above were carried out in an aqueous solution at pH = 5.0, which was achieved by adding a diluted aqueous solution of hydrochloric acid. The deposition time used for each layer was 10 minutes. The detailed description of each sensor unit is presented in Table 1. A bare interdigitated gold electrode was used as a reference unit.

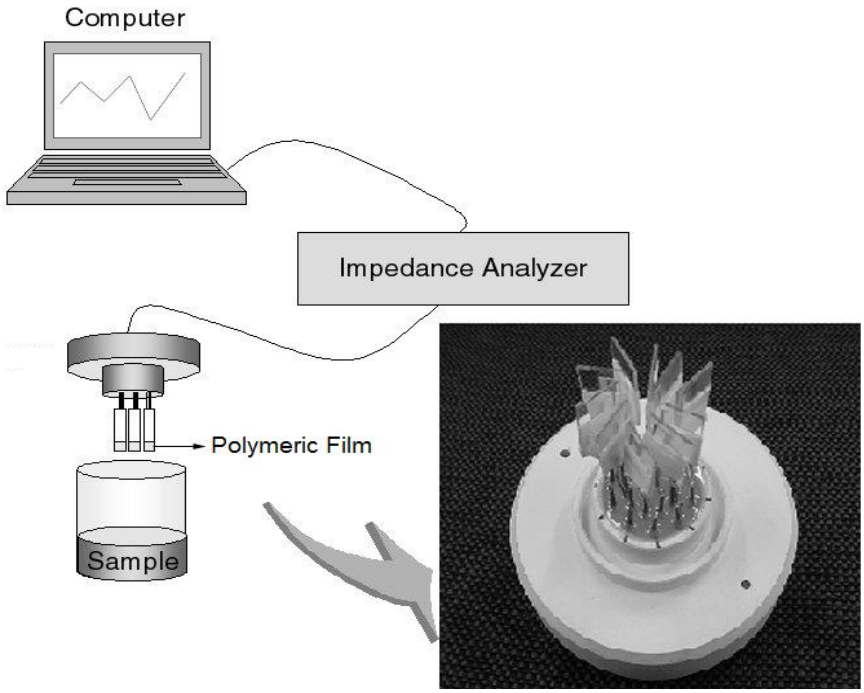


Fig. 1. Sensor array consisted of nine sensor units.

Poly(*o*-ethoxyaniline) (POEA) 1 x 10^{-3} M was chemically synthesized by the aqueous oxidative polymerization of *ortho*-ethoxyaniline in the presence of ammonium peroxydisulfate and 1.0 M HCl at ~0 ºC. The monomer to oxidant ratio was 4:1 [18].

The morphology of the ultra-thin films of conducting polymers was characterized by using a scanning electron microscope (SEM) model Zeiss DSM 960 from Zeiss. The thickness of the conducting polymer ultra-thin films was evaluated by using UV-VIS spectroscopy (Shimadzu – 1601PC).

The sensor array consisted of gold interdigitated electrodes supported on glass and containing 50 pairs of digits each (5 mm long, 10 μm wide and with a 10 μm gap between the digits).

Table 1. Detailed description of each sensor unit used in the sensor array based on the use of ultra-thin films of conducting polymers.

Sensor Unit	Characteristic
1	Bare electrode – gold interdigitated electrode
2	Stearic acid film, pH 5.0
3	Intercalated layers of POEA and SL both in pH 5.0
4	Layer of POEA in pH 5.0
5	Layer of POEA-caproic acid complex, both in pH 5.0
6	Layer of caproic acid in pH 5.0
7	Intercalated layers of POEA and caproic acid in pH 5.0
8	Layer of POEA-stearic acid complex, both in pH 5.0
9	Layer of POEA-SL complex, in pH 5.0

2.2. Experimental Details

The analysis of the samples of water was carried by using electrochemical impedance spectroscopy, where a Solartron impedance analyzer SI 1260, controlled by the Z-Plot electrochemical impedance software was used. The sensor array operating conditions were optimized and the frequency and the amplitude of the potential signal (peak-to-peak) were fixed at 1 kHz and 50 mV, respectively.

The experimental sensing data were processed and analyzed using chemometrics, i.e. principal component analysis (PCA). PCA is a mathematical method used to correlate variance data with properties of interest, such as water sample eutrophicity. This mathematical tool allows the reduction of variables that can describe a behavior of a system that is being studied. Therefore, a reduced number of variables related to a characteristic of interest of a system can be used to create a database containing key information that describe such characteristic of interest, which can be easily recognized by using the PCA method [18-20]. The global selectivity [16] is the concept that better described the approach used in this work.

The chemical and physical characterization of the water samples were carried out as follows: the transparency measurement of the water sample was done using a Secchi disk; biochemical oxygen demand (BOD5) [21], dissolved organic carbon (DOC) were measured by using the method of the Pt-catalyzed non-dispersive combustion and detection using infrared gas analysis (Shimadzu, TOC-5000A); nitrogen and phosphorus [22], alkalinity [23], chlorophyll [24], pH, electrical conductivity and dissolved oxygen (DO), were measured in situ by using a multiple probe device (Horiba – U10). These samples were used as standard samples to generate a database with the chemical and physical properties that describe the different types of eutrophic level, i.e. oligotrophic, eutrophic, and hypereutrophic.

2.3. Water Samples

In a partnership between Embrapa (Brazilian Agricultural Instrumentation Research Center) and UFSCar (Federal University of São Carlos, SP, Brazil), water samples were collected at three different places located in the São Paulo state, Brazil: Barra Bonita reservoir (22º29'S and 48º34'W), Óleo lake (21º36'S and 47º49'W) and Monjolinho reservoir (20º00'S and 47º54'W). The water samples were collected at three distinct depths, at the surface, at an intermediate position and at the bottom of water column, using a Van Dorn sampler. Immediately after the water sampling process, the samples were filtered through 0.4 mm pore size nylon net and fiberglass wool filter in order to remove large live organisms. To evaluate the reproducibility and repeatability of the results that were obtained using the sensor array, water samples were collected and analyzed in three distinct dates, i.e. on October 16, on November 26, and on December 16, 2006.

3. Results and Discussion

3.1. Chemical and Physical Analysis

The chemical and physical analyses indicate that the different water samples analyzed presented different eutrophic level (Table 2). The samples can be classified in three distinct categories as oligotrophic (Óleo lake), eutrophic (Monjolinho reservoir) and hypereutrophic (Barra Bonita reservoir). This classification was based on Volleinweider´s, 1968 work [25], which is mainly due to the phosphorous and nitrogen concentrations. The nitrogen content in the water samples constitutes an important indicative of recent organic matter content changes. The phosphorous containing compounds are an important factor for the aquatic organisms, and they are important for the control of the algae growth. The organic matter discharge that comes from different sources, especially those from domestic wastewater, as well as some types of industrial wastewater, can enhance the concentration of phosphorous containing compounds in the water, as it was observed in the water samples from the Barra Bonita and Monjolinho reservoirs.

Table 2. Chemical and physical variables from water samples collect at three different environments.

Variables	Óleo lake	Monjolinho reservoir	Barra Bonita reservoir
Temperature (ºC)	24.20	22.40	24.70
Alkalinity (meq L^{-1})	-	0.33	0.77
Secchi Disk (m)	1.74	0.69	1.85
BOD_5 (mg L^{-1})	-	1.57	2.20
DOC (mg L^{-1})	3.03	2.12	15.60
Chlorophyll (µg L^{-1})	35.56	34.8	76.20
pH	5.49	6.34	6.93
Conductivity (mS cm^{-1})	16.00	36.00	195.00
Dissolved Oxygen (mg L^{-1})	3.57	5.92	5.00
Nitrogen (mg L^{-1})	0.02	1.01	1.84
Phosphorous (µg L^{-1})	36.00	69.00	168.00
Eutrophic Level	Oligotrophic	Eutrophic	Hypereutrophic

3.2. Characterization of the Bare Gold Interdigitated Electrodes and of the Ultra-thin Films of Conducting Polymer

The construction of the sensor units modified with nanostructurated thin films of conducting polymers, which acts as a sensing layer, results in a sensor array capable to distinguish even differences properties of the water samples. However, a detailed characterization of these bare interdigitated gold electrodes, before the deposition of the thin film of conducting polymer, is necessary in order to improve the reproducibility and the repeatability of the experimental results. In other words, it is a procedure to control the quality of each bare interdigitated gold electrode. The sensor units were individually evaluated in a frequency range of 1 MHz to 1 Hz, at a temperature of ~20 ºC, where the sensor units demonstrated similar behavior with a relative standard deviation below 1%. Figure 2 shows the results of the characterization of the bare interdigitated gold electrodes before the deposition of the films. These results (Fig. 2) shows that the bare interdigitated gold electrodes are identical and the performance of each sensor unit, and thus of the sensor array, will depend only on the thin film of conducting polymer that is being used.

The film growth studies were carried out using a glass slide as a substrate for the film deposition. A linear growth of the film thickness, i.e. a linear increase of the value of the maximum absorption intensity of the band at ~630 nm in the UV-VIS spectrum, which is characteristic for the poly(*o*-ethoxyaniline) [15]. The maximum thin film thickness was 20 nm [17]. The scanning electron microscopy (SEM) studies done in our previous work [26] indicated that the surface of the glass slide substrate was completely covered with a homogeneous thin film of conducting polymer.

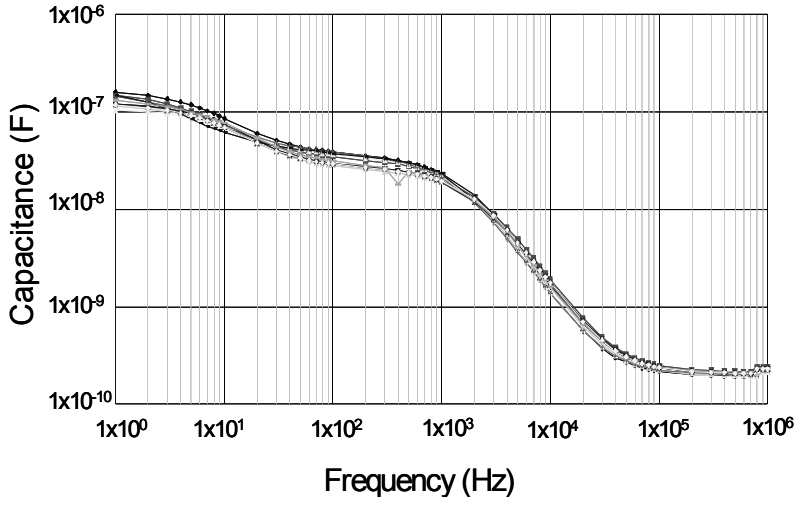


Fig. 2. Impedance spectroscopy characterization of the bare interdigitated gold electrodes. Frequency range: 1 MHz to 1 Hz. Potential signal amplitude: 50 mV. Temperature: 20 ^{o}C.

3.3. Water Analyses

Each sensor unit has a unique electrical response for a specific water sample and it gives a distinct signal for different dilution of the same sample. Consequently, the overall electric signal pattern of the sensor array can be used as a fingerprint to classify water samples according to their composition and properties, such as the level of eutrophication.

To evaluate the sensitivity of the sensor array, dilutions of the water sample from three different systems were studied. The sample from Óleo lake presented the lowest content of nutrients, e.g. nitrogen and phosphorous contents, and the sample from Barra Bonita and Monjolinho reservoirs

presented the highest content of nutrients, as it can be seen in Table 2.

Fig. 3 shows the effect of the sample dilution on the electrical signal profile (fingerprint) for three different samples collected at different locations, i.e. Óleo lake (Fig. 3(a)), Monjolinho reservoir (Fig. 3(b)), and Barra Bonita reservoir (Fig. 3(c)). Fig. 3(a) shows no linear trend in the electrical signal profile at more diluted samples. These results show that, as the water sample is diluted, the concentration of the nutrients (such as ions) becomes lower. Fig. 3(b) shows that no significant changes in the electrical signal pattern profile of the sensor array was observed except that for the more diluted sample, which presented much lower values of capacitance for each sensor unit. Fig. 3(c) shows a linear trend on the electrical signal profile for the different dilutions used, which might be related to the eutrophic level of this sample from the Monjolinho reservoir. The water samples from the Monjolinho reservoir presented the highest eutrophic level. Therefore, even under diluted conditions, reasonable changes in the capacitance values measured for each sensor unit were detected. However, there will be a limit of dilution for samples with lower eutrophic level, where the sensor array might not be able to detect any significant change in the eutrophic level of such diluted samples because the capacitance values depends mainly on the concentration of ions present in the sample that is being analyzed.

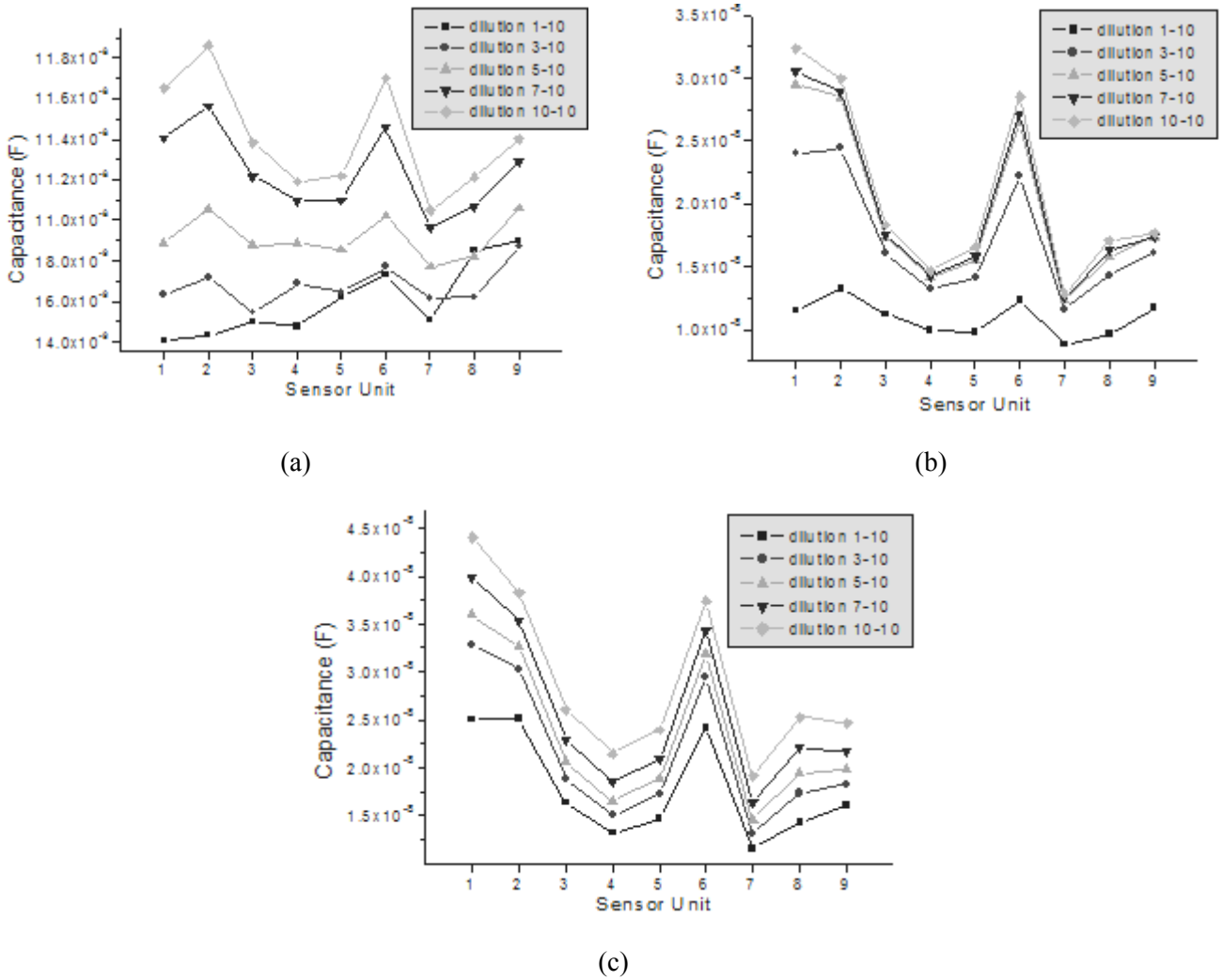


Fig. 3. Capacitance pattern profiles of the samples from: (a) Óleo lake; (b) Monjolinho reservoir and (c) Barra Bonita reservoir at different dilution conditions. The collected samples were diluted with high purity water obtained using a Millipore (MilliQ) filtering system. Potential signal amplitude: 50 mV. Temperature: 20 ^{o}C.

The samples of the three reservoirs were evaluated using the sensor array consisting of nine sensing units, where the electrical signal patterns were analyzed using the PCA technique. The sensor array was able to differentiate the three samples of water obtained from the Óleo lake, Monjolinho reservoir and from the Barra Bonita reservoir, respectively, according to their chemical and physical properties. Samples of water with different chemical and physical properties interact in a different and unique way with each sensing unit, which will then result in a unique electrical signal pattern of the sensor array for a water sample (Fig. 4).

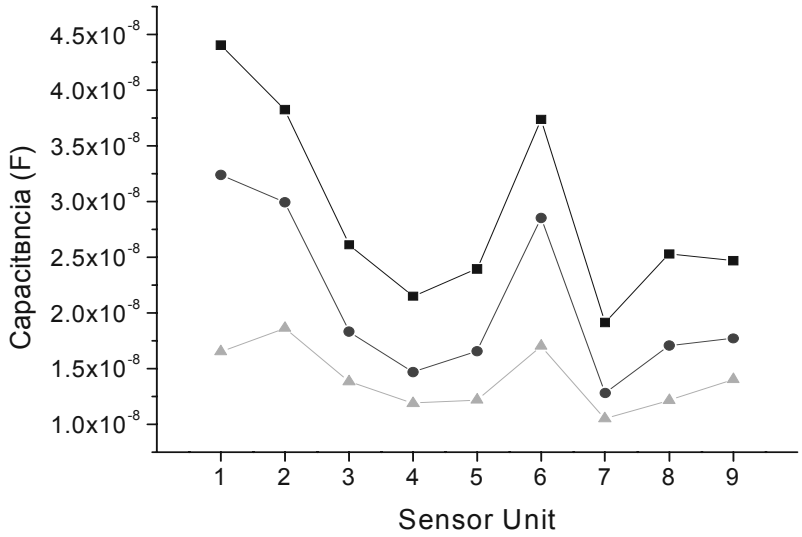


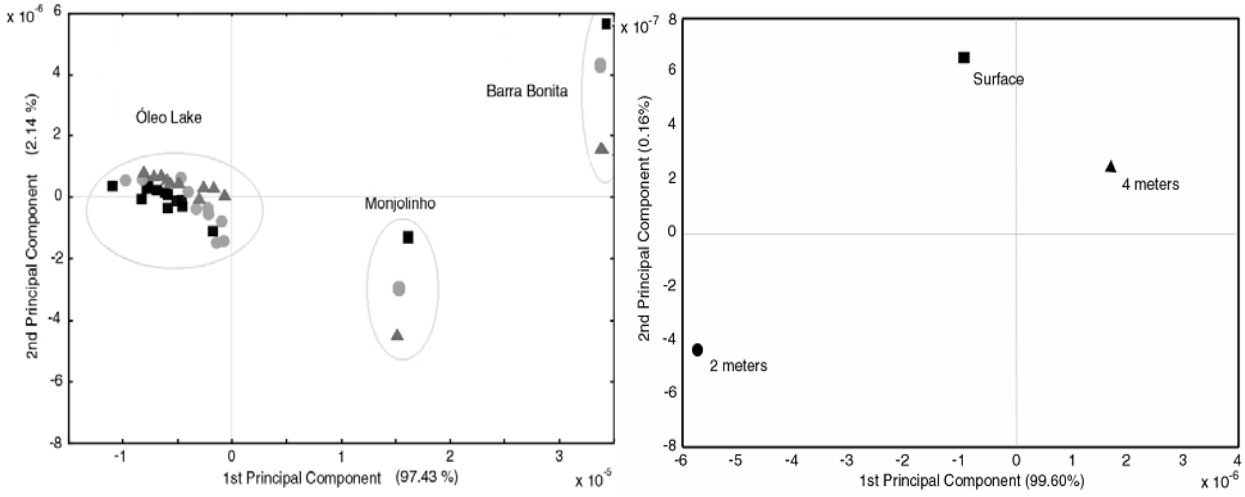
Fig. 4. Capacitance pattern profiles of the samples (■) Barra Bonita reservoir, (●) Monjolinho reservoir and (▲) Óleo lake, measured at a fixed frequency (1 kHz). Potential signal amplitude: 50 mV. Temperature: 20 ^{o}C.

The principal component analysis results (Fig. 5a) for the water samples analyzed in Fig. 4 shows that the eigenvalues of the two first principal components explain approximately 100% of total variance (PC1 = 97.43%; PC2 = 2.14%) of the observations. A plot of the factors scores on a PC1-PC2 axes plane (Figure 5a), shows an excellent discrimination among the different water samples used. The first principal component is mainly influenced by the eutrophic level of the water sample, where the water samples located towards the right side of the PCA plot present a higher eutrophicity degree.

The water samples that were collected at the same location, but at different depth, were identified and classified by the sensor array, showing that changes in chemical composition that occur due to different factors, such as the effect of the luminosity and the temperature of the water can be evaluated. The PCA results for water samples collected at different depths are presented in Fig. 5b. It can be seen that the eigenvalues of the two first principal components contains approximately 100% of total variance (PC1 = 99.60%; PC2 = 0.16%) of the observations. These results show that the studies related to the stratification dynamics and mixtures of lakes can be easily evaluated by measuring the eutrophic level of the reservoirs. It is also important to point out that the adsorption of organic matter to the nanostructured thin film on each sensing unit may significantly influence the double layer capacitance.

It is well known that organic constituents of natural waters are surface active and thus adsorb at the electrode/solution interface and change the double layer capacitance [27]. In Fig. 3 the effects of the sample dilution on the electrical signal profile clearly show that there is a potential influence of adsorbable organic substances on the measured signal. For example in more diluted samples of Oleo lake (Fig. 3(a)) and Monjolinho reservoir (Fig. 3(b)), which contain lower DOC concentrations, the signal is changed for dilution 1-10. However, Fig. 3(c), which presents the highest DOC content, even at lower dilution levels no significant change on the capacitance signal observed. In addition, from our

experimental results it was observed that these organic matter adsorption processes were reversible since the sensor array electrical signal for high purity water, the reference sample, which was done before and after the reservoir sample analysis was reproducible. Therefore, temporal and local changes in the chemical and physical properties of aquatic systems could be monitored using a relatively simple, fast and straightforward method.



(a) (b)

Fig. 5. (a) Principal component analysis of samples collected in three different dates: (■) October 10, 2006, (●) November 10, 2006, and (▲) December 10, 2006, the samples were collected at the following locations: Barra Bonita reservoir, Monjolinho reservoir and Óleo lake; (b) Principal component analysis of samples of Óleo lake collected at three different in depth location (■) surface, (●) 2 meters, and (▲) 4 meters.

4. Conclusions

The sensor array presented good stability and reproducibility during the experiments done with water samples, i.e. it was stable when in the presence of different water samples and it did not show any type irreversible chemical reaction or degradation process. The results obtained in this work showed that the sensor array was able to recognize and classify different water samples. The sensor array was also able to distinguish among samples of water that were collected at the same location but at different dates. The polymeric ultra-thin films interact in a unique way with each type of water sample. The combination of the electrical signal of each sensing unit contained in the sensor array resulted in a fingerprint profile that is characteristic and unique for each different water sample analyzed. However, after some adjustments, the approach presented in this work will be a new and straightforward method to identify and classify water samples according to their eutrophic level.

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Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
- Digital, frequency, period, duty-cycle, time interval, PWM, pulse number output sensors and transducers;
- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
- Applications.

Submission of papers

Articles should be written in English. Authors are invited to submit by e-mail editor@sensorsportal.com 6-14 pages article (including abstract, illustrations (color or grayscale), photos and references) in both: MS Word (doc) and Acrobat (pdf) formats. Detailed preparation instructions, paper example and template of manuscript are available from the journal's webpage: http://www.sensorsportal.com/HTML/DIGEST/Submition.htm Authors must follow the instructions strictly when submitting their manuscripts.

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