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## MONITORING THE EVOLUTION OF REDOX CHANGES IN SEDIMENTS MADE POSSIBLE BY ELECTROCHEMICAL MULTILAYER PROBES

Marine sediments with rapid oxic/anoxic transitions are difficult to monitor in real time. Organic overload that may lead to anoxia and buildup of hydrogen sulfide can be caused by a variety of factors such as sewage spills, harbor water stagnation, algal blooms and the vicinity of aquaculture operations. We have tested a novel multiprobe technology (named SPEAR) on marine sediments to evaluate its performance in monitoring sediments and overlaying water. Our results show the ability of the SPEAR probes to distinguish electrochemical changes at 2-3 mm scale and at hourly cycles. SPEAR probes have the ability to identify redox interfaces and redox transition zones in sediments, but do not use micromanipulators (which are cumbersome in field and underwater applications). We propose that the best target habitats for SPEAR-type monitoring are rapidly evolving muddy deposits and sediments near aquaculture operations where pollution with organics stresses the ecosystem.

*Keyword:* sensor; sediments; SPEAR; multielectrode; redox interface

### 1. Introduction

When marine sediments are polluted with organic biomass, the dissolved oxygen is consumed, organisms are killed and severe ecological disruptions ensue [1-4]. This type of pollution may come from algae blooms, mariculture, sewage spills, plankton settling, sedimentation at river mouths and industrial pollution in harbor areas. Episodic hydrographic events can also contribute with sizable sedimentation rates in the form of particulate organic matter leading to rapid installation of hypoxia. Among marine ecosystem inhabitants, fish and invertebrates are some of the most sensitive to oxygen depletion, and to the buildup of ammonia and sulfide [5-7]. Patterns of hypoxia and toxic compounds effects on sediments, such as the Pearson-Rosenberg model, are known since the 1980s, but these models are more descriptive than predictive [8,9]. Surveying organically-polluted coastal zones requires advances in electrochemical monitoring and networks of sensors [9]. The purpose of this study is to evaluate how CMOS-multiplexing of electrochemical signals can help analyze the evolution of redox changes in sediments. The electrochemical system described in this paper allows permanent monitoring of pore-water and makes it possible to correlate micro-zone – measurements with predictive models.

Current probe technologies (with single or only a small number of electrodes used, and electrochemical systems that employ micromanipulators) do not achieve true monitoring or the evolution of chemical gradients. These techniques can only do one-time gradient descriptions or monitor chemical evolution in a limited number of locations and sediment layers. The multilayer approach used in this study, and the monitoring arrays this system can be part of, will greatly increase the capacity of monitoring the evolution of electrochemical gradients in sediments. In doing this, the SPEAR methodology can also track effects of anoxia enrichment in marine ecosystems. Such capabilities would also function as tools for rapid environmental survey, indicating where more detailed investigations and timely interventions are necessary in order to avoid, quantify or mitigate effects of organic pollution.

A next generation multi-electrode instrument (called SPEAR) was constructed as part of a UEFISCDI (Romania) grant named SEMSED. SPEAR-type instruments detect and report when sediment pore-water or the water above sediments become anoxic or when redox-active chemicals such as H<sub>2</sub>S accumulate. The focus of this work was to assess the capacity of this instrument to study vertical electrochemical profiles in marine sediments.

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## 2. Materials and methods

Numerous potentiostat platforms have been developed for cyclic voltammetry (CV) studies [10-16]. The closest precursors to the SPEAR system used here are multichannel potentiostats produced by GAMRY, Bio-Logic, Corrtest and Palm Sense. These are however very expensive solutions relative to the number of electrodes they carry (e.g. tens of thousands of dollars for 8-32 electrodes). SPEAR instruments use low-cost custom-made probes and multiplexers, and each potentiostat can control many probes having very large number of electrodes (for example between 64 to 512).

The SPEAR architecture probe from Fig. 1 has a 3D printed holder for the graphite electrodes, a multichannel switching board with MAX4781 complementary metal-oxide-semiconductor (CMOS) multiplexers [17] and a Keithley 2450 EChem potentiostat. This technical solution dramatically decreases the cost of CV analyses to less than \$1 per electrode in multiprobe architectures.

The probe used in this project was custom-made with 64 working electrodes (WEs), each electrode 3 mm in diameter,

and distributed along 256 mm, one reference electrode (RE) and one counter electrode (CE). The probe's body was made of slow-curing epoxy resin (Epo-Tek 301). Inside the resin, each electrode's back end was inserted in a brass shield and connected with conductive graphite paste. Multi thread electrical wires were soldered to the brass end of all electrodes and the wires were embedded in the resin core of a cylinder-shaped probe. After one week of curing, the epoxy rod/probe was polished into a blade shape for easier insertion into sediments. The front edge of the blade was tapered to expose the tip of the WEs to water. Then, the exposed surface of the WEs was polished with increasingly finer sandpaper up to 400 grit. A commercial RE was used (Sigma/Aldrich). The CE was 0.2 mm diameter platinum wire (Sigma/Aldrich).

## 3. Results

A custom-made SPEAR instrument was used to perform CV measurements in marine sediment taken from Catalina Island (CA). The software was a combination of Python commands

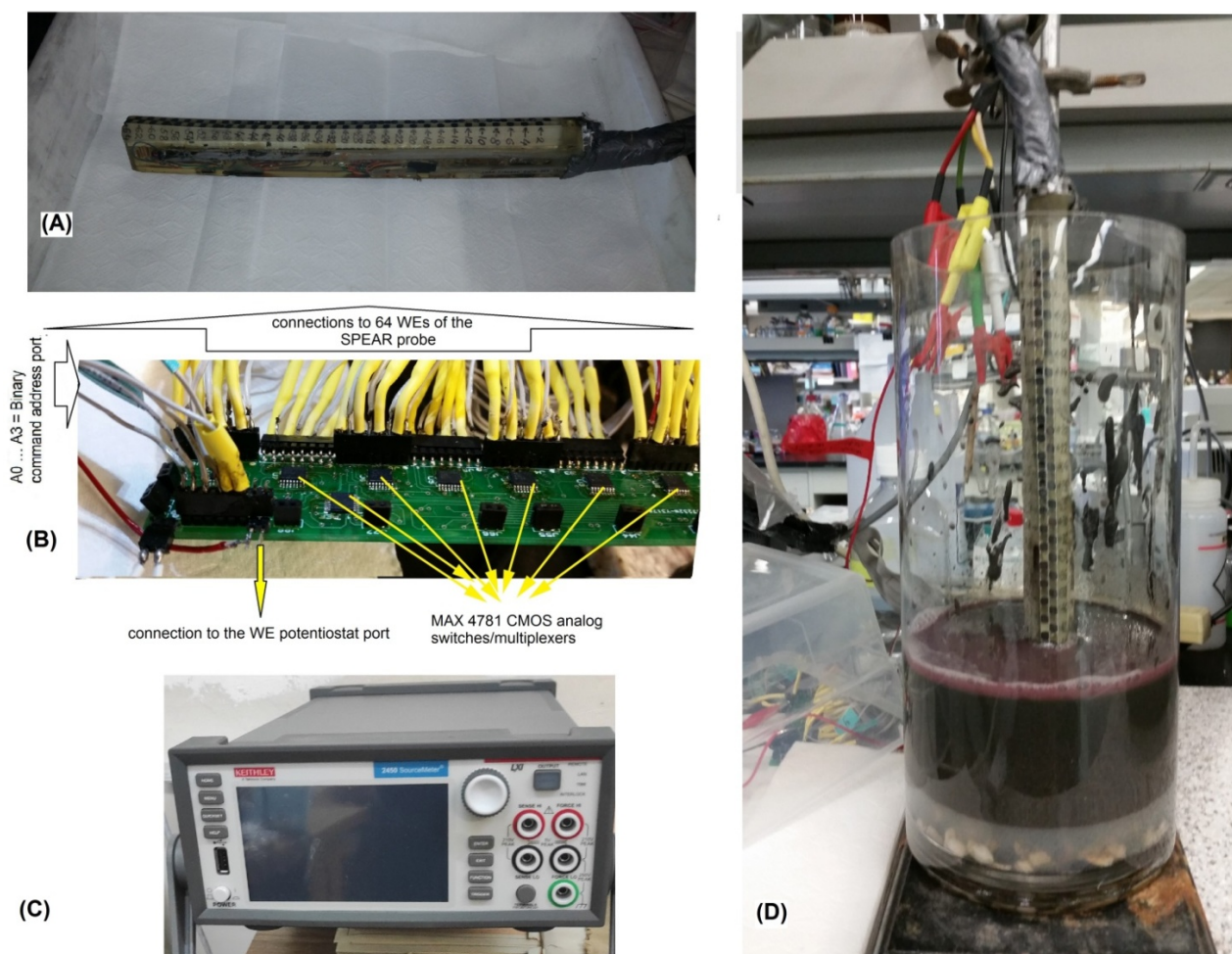


Fig. 1. The parts of a SPEAR instrument for electrochemical monitoring of water and sediments. (A) Probe with 64 electrodes. In this probe, the distance between the center of two adjacent electrodes is approximately 4 mm, and the electrodes are distributed in two parallel series to obtain a vertical resolution of approximately 2 mm. (B) Electrode multiplexing board used to select each of 64 WEs. (C) Keithley 2450 potentiostat used in this instrument variant. Each potentiostat can monitor many probes. (D) SPEAR probe with 64 working electrodes inserted approximately 25% in a marine sediment:water slurry during the onset of a series of experiments

and software already embedded in the potentiostat. Measurements were made in the laboratory and the instrument was in remote-control mode in order to verify remote control and on-line re-programming capabilities. The sediment (muddy sand) was collected by diving and was stored in the cold room until it was used. After installing the probe in a glass cylinder (Fig. 1D) the sediment was mixed with seawater into a slurry then poured around the probe. The slurry was allowed to settle overnight at room temperature (19-20°C) to create a system with sediment on the bottom and water on top. Sediment settling continued for 48 hours from the initial slurry addition.

In one series of experiments, the sediment:water column had ~30 cm in total height, of which the water layer was ~10 cm deep and the sediment was ~20 cm deep. The working electrodes (WE) were numbered up-down. The WEs #1 through #9 were above the water:air interface and not active in this experiment. The WEs #10 through #30 were in the water phase, with WE #30 at the sediment:water interface (Fig. 2).

The WEs #31 through #64 were embedded into the sediment with the WE #64 in the lowest position. The following reading parameters were selected on a Keithley 2450 potentiostat: EOC potential -0.428 V; number of vertices 3; Vertex 1: -0.8 V; Vertex 2: 1 V; Vertex 3: -0.8 V; Source rate 16.1 mV/sec; number of cycles 1; Current Range 0.1 A; Sampling Interval 400 pts/cycle; nplc 0.14; Step size 0.00904 V. The data from the second CV cycle reads obtained from 54 (out of 64) electrodes were saved in a data base. Fig. 2 shows a stack of voltammograms produced by 34 of these electrodes.

These results show the capacity of SPEAR instruments to generate large series of CV data from marine sediments. The entire cycle for all electrodes takes 1-3 hours (depending on the characteristics of the CV cycles) and the series are repeated

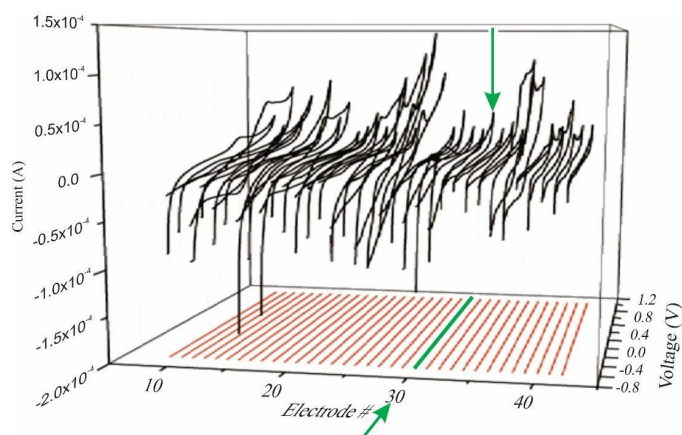


Fig. 2. CV profile stack containing a sequence of voltammograms (produced by 34 electrodes from a 64 electrode SPEAR probe). The WE#10 is just under the water:air interface. WE#30 is at the sediment:water interface (indicated by green arrows). The electrode #42 is the deepest in this stack. For graphical simplicity the voltammograms produced by the WEs #42 through #64 are not shown here

8-24 times during each day:night cycle. The best voltammograms for each electrode and for each series are saved and graphed individually and in stacks. Further analysis compares the shapes of the voltammograms and how they evolve from one electrode to another. These results inform about overall electrochemical patterns of sediments, the presence and smoothness of gradients as well as about changes during the day:night cycle.

In another series of experiments (Fig. 3) we used all 64 electrodes of a SPEAR probe.

In this series, the water:sediment column was >35 cm deep and all electrodes were submerged (numbered from top to bottom). The water:sediment interface was between WE #29

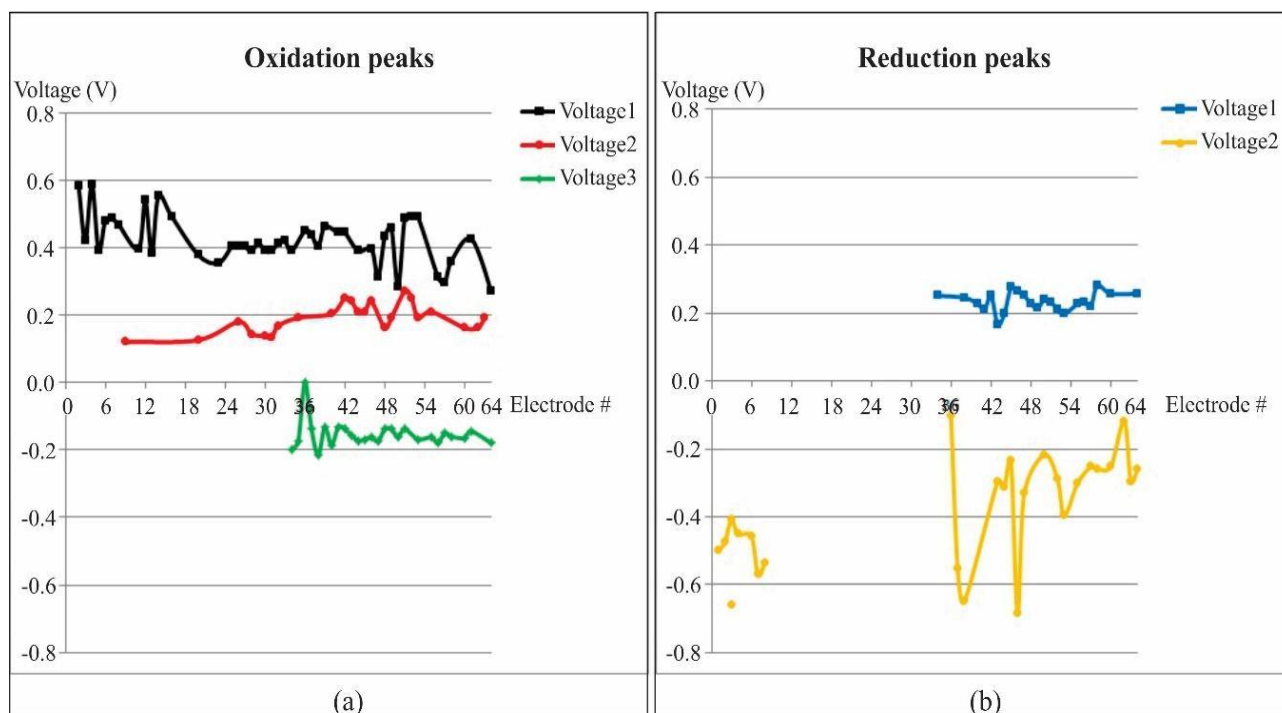


Fig. 3. (a) Oxidation and (b) reduction peaks identified in a sequence of SPEAR CV measurements using a SPEAR probe with 64 electrodes



and WE #30. As in earlier measurements for each electrode we have performed three full CV cycles and the second cycle was selected for further analyses. The following measurement conditions were applied on a Keithley 2450 potentiostat: EOC potential  $-0.42806$  V; number of vertices 3; Vertex 1:  $-0.75$  V; Vertex 2:  $0.75$  V; Vertex 3:  $-0.75$  V; Source Rate  $300$  mV/sec; number of cycles 2; Current Range  $0.1$  A; Sampling Interval  $500$  pts/cycle; nplc  $0.14$ ; Step size  $0.001$  V and Source Delay  $3.33$  ms. As this work is but an example of how the SPEAR instrument is used, the three CV voltammograms of each electrode were not compared to select the best. The commonly cleaner, second CV cycle, of all reads was analyzed to identify oxidation and reduction peaks along the forward and reverse path respectively (Fig. 3).

Fig. 3 shows how analysis of the voltammograms produced by a multielectrode SPEAR probe can help study electrochemical gradients in water:sediment columns. In this analysis, the tip of oxidation and reduction peaks from each voltammogram were first identified then plotted. Fig. 3 summarizes the distribution of the main CV peaks (oxidation and reduction) from each voltammogram. In these graphs, the peaks represent independent electrode reads and therefore, the lines that connect the peaks bear no significant relevance and are only drawn for graphical simplicity or are used to better visualize variability among voltammograms. The peaks connected by lines can be proposed as first-hand approximation of being common in a group of electrodes. In the CV theory, each redox active class of chemicals that respond to applied currents shows one peak along the oxidation part of the voltammogram and one peak along the reduction path. Sediments contain many redox pairs, and sediment voltammograms are often too complex to single out any specific chemical. For this reason, here we do not discuss the means to identifying peak pairs and the middle point of a pair (i.e. the redox potential of a chemical redox couple). The location of these peaks and changes in their vertical distribution from one electrode to another and between series of reads informs however about changes in the redox properties of sediments (for example installation of anoxia caused by accumulation of organics).

To give a simple example of SPEAR-derived data analysis in Fig. 3, the lowest cluster of peaks (that is only revealed by the WE #34 through WE #64, between  $0.0$  V to  $-0.2$  V) from the oxidation graph, appears to be paired with another cluster of peaks in the reduction graph (between  $0.18$  V and  $-0.24$  V). It is not the purpose of this study however to identify the chemicals that are producing these peaks. Still, from this analysis one can propose that these clusters of peak pairs (at approximately  $-0.15$  V and  $0.22$  V) represent a chemical couple with redox potential close to  $0.035$  V. The volume of data generated by SPEAR instruments is very large and cannot be processed by hand. Therefore, it is not practical to aim identifying each the chemical that may be present. Rather, the purpose here is identifying electrochemical patterns and how these patterns change in space and time, as well as from one probe to another. We repeat here, that in SPEAR instruments one potentiostat can monitor many probes, each

probe may have hundreds of electrodes and reading series are repeated over and over, 24 hours per day.

#### 4. Discussions and conclusions

A novel electrochemical instrument called SPEAR was used for laboratory tests on marine sediments. Our results show that the SPEAR instrument has functioned as predicted. We have verified the basic functions of a 64-electrode probe, the ability of the instrument to perform measurements of redox potential and cyclic voltammetry, easy switching between electrodes in any sequence desired and the capacity to re-program and control the instrument remotely.

The main benefit of the SPEAR methodology is the ability of analyzing how electrochemical gradients evolve in time and space, without the need to push/pull electrodes through sediments by means of micromanipulators. Also, this instrument allows selecting or skipping electrodes, as well as altering the timing between reads to compensate for diffusion effects. This capability can also help identify artifacts caused by earlier, or neighbor electrode, reads. By combining measurements of CV, conductivity, temperature and chronoanalyses, SPEAR instruments will also allow studying diffusion effects and the kinetics of secondary reactions. Another benefit of the SPEAR methodology is monitoring how chemoclines and chemical interfaces evolve in space and time (a feature not available to any other electrochemical technique).

SPEAR instruments have one obvious weakness. It is not possible to rotate the electrodes nor to agitate the solution in the vicinity of the electrodes. Therefore, readings will be influenced by reagent consumption near the electrode surface and by diffusion. Hence, when electrodes are close to each other artifacts are expected in nearby subsequent readings. This however, also underlines some chief strengths of the SPEAR approach. Each SPEAR protocol can be unique, in that readings can be adjusted to the shape of earlier voltammograms and to local patterns and some electrodes can be skipped to compensate for diffusion limitations, or to study diffusion rates.

SPEAR instruments are sufficiently versatile to allow additional probes for example temperature, pH and redox-active and redox-inactive chemicals. Electrochemical analyses require parallel validation to associate specific peaks with specific chemicals. It is recommended that probes are added to SPEARS to confirm the presence  $H_2S$  and  $O_2$ . Some chemicals used, or produced, by respiratory activities are not detectable by CV. Nitrate, nitrite and ammonia probes added to SPEAR probes can complement CV electrochemistry and create a better picture of local chemical patterns. Light sensors can help localize the sediment:water interface, monitor the day/night cycle, level of light penetration and also to study sedimentation rates. Lastly, desirable developments for the SPEAR technology include: increasing the spatial resolution of the working electrodes; 3D printing of the probes, solid state reference electrodes and graphene coating of working electrodes. Upgrades are also

needed in software and hardware to frequently verify the degree of electrode fouling and to adjust reading protocols to electrodes' state and voltammogram' behavior. This means implementing machine learning algorithm to adapt readings to local conditions and to previous reads.

#### Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CCDI- UEFISCDI, project number PN-III-P2-2.1-PTE-2019-0198, within PNCDI III.

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