

Chemical Plume Tracking. 3. Ascorbic Acid: A Biologically Relevant Marker

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Dynamic characteristics of the amperometric sensing system for tracking of turbulent chemical plumes have been studied. The correlation analysis requires that such sensors respond rapidly and have long-term stability and minimal or low-flow sensitivity. Moreover, for practical purposes, such sensors must respond to an electrochemical marker that would be found in or at least be compatible with the diet of marine animals. Ascorbic acid is such a compound and can be electrochemically oxidized. Its long-term dynamic behavior on several types of electrodes has been studied. It has been found that a Pt electrode coated with polyaniline satisfies all the above requirements. However, this system has a peculiar dynamic behavior that affects the results of the correlation analysis, namely, at the higher frequencies. The behavior of such system in the virtual plume setup has been characterized and its usability for detection of fluctuating concentration of ascorbic acid in saline solution has been confirmed.

In the previous studies, we showed that the virtual plume (VP) instrument is a valuable tool in characterization of the chemical information encoded into the chemical plume structure.¹ We have shown that using flow modulation experiments we are able to mimic the flow conditions of the chemical turbulent plume and the signal in it for the sensor pair. In that work, we concentrated on the characterization of the virtual plume instrument using coherence as the parameter of choice.^{2,3} For this kind of characterization it is useful to keep all parameters as simple as possible and therefore a well-characterized and reversible electrode–electrochemical marker pair (platinum electrode–ruthenium hexamine) was used. Unfortunately, it is not reasonable to expect that ruthenium hexamine will be present in any chemical plume in the actual environment and it definitely will not be present in the plumes that animal searchers can track in their search for food. As the final goal of our work is to mimic an animal searcher in the chemical plume using an array of electrochemical sensors, it was necessary to find an electrochemical marker that

could be found in the animal's food and to identify an electrode to sense it. Our choice for electrochemical marker was ascorbic acid. In the present work, we have addressed changes in the basic parameters of the virtual plume instrument due to change of the dynamic characteristics of the working electrode.

EXPERIMENTAL SECTION

The basic elements of the virtual plume instrument and experimental conditions have been described in previous articles.^{1–4} The block diagram of the frequency-modulated virtual plume is shown in Figure 1. The basic setup was modified according to the specific requirements of the experiments at hand (see experimental descriptions in the Results section). All experiments were done at least in triplicate.

In the flow modulation experiments, the carrier and sample solutions were delivered at a constant rate in a gravity-driven system. A solenoid three-way isolation valve (Biochem-Valve 075T3) was used to inject the sample plugs with a specific frequency that will be referred to as the modulation frequency, f_s . The solenoid valve was controlled by a custom-designed driver circuit. A square waveform was delivered from the waveform generator (Hewlett-Packard 33120A) to activate the solenoid driver circuit. Data were collected for 1000 s with an acquisition frequency of 20 Hz, to avoid aliasing. Data were analyzed using the Matlab software package (Matlab 5.3 for Windows).

The delay elements were designed to provide different retention times for marker plugs while minimizing additional dispersion. Coils of Teflon tubing with inner diameter of 0.5 mm and lengths from 10 to 200 cm were used for this purpose.

The sensing elements consist of working electrodes housed in the flow-through cells. A three-electrode electrochemical configuration was used in order to prevent polarization of the silver wire reference electrode. A stainless steel outlet tube served as an auxiliary electrode. Electrodes were connected to potentiostats (OMNI 90, Cypress Systems, Inc.), and the signals were interfaced to a PC via a DAQ interface card (AT-MIO-16XE-10, National Instruments).

Bare platinum and carbon electrodes and polyaniline (PANI)-coated platinum electrodes were used as working electrodes in the VP instrument. All electrodes were disk electrodes with a diameter of 0.3 mm. Electrochemical growth of PANI on Pt working electrodes was carried out by cycling in 0.1 M aniline/2 M HBF₄ aqueous solution in the potential range of 0 to +0.9V

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(1) Kikas, T.; Ishida, H.; Roberts, P. J. W.; Webster, D. R.; Janata, J. *Electroanalysis* **2000**, *12*, 974–979.

(2) Kikas, T.; Webster, D. R.; Ishida, H.; Janata, J. *Anal. Chem.* **2001**, *73*, 3662–3668.

(3) Kikas, T.; Ishida, H.; Janata, P.; Janata, J. *Anal. Chem.* **2001**, *73*, 3669–3673.

(4) Ishida, H.; Nakamoto, T.; Moriizumi, T.; Kikas, T.; Janata, J. *Biol. Bull.* **2001**, *200*, 222–226.

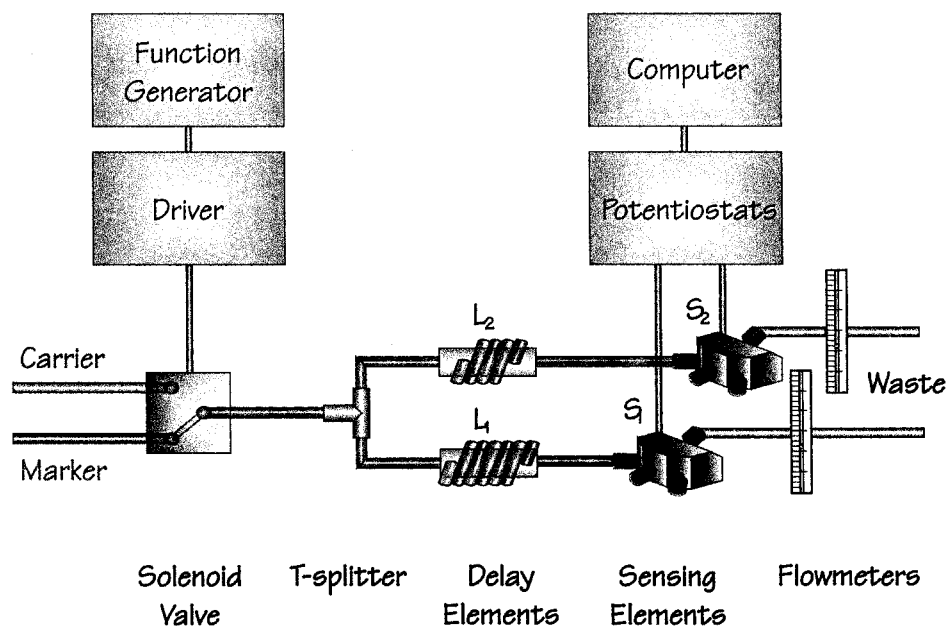


Figure 1. Schematic of the virtual plume instrument.

with the scan rate of 50 mV/s in a three-electrode cell using a platinum foil as counter electrode and an Ag/AgCl (in 3 M KCl) reference electrode. Electrodes were cycled for 100 potential cycles, which resulted in ~ 200 -nm-thick PANI layer. Electrochemical growth of PANI is known to proceed in three distinctive stages: nucleation and growth of separated islands on the electrode surface (<100 nm), two-dimensional growth of a smooth polymer layer, and three-dimensional growth of dendrites on the polymer surface (>300 nm). Therefore, PANI film has uniform morphology and characteristics only in the limited range of thicknesses. This thickness was chosen to ensure uniform coverage of the platinum surface while avoiding the dendritic growth on the surface.^{5,11}

Commercial flow meters/controllers (Accucal flow meter, Gilmont Instruments) with precision flow control valves (14 turns) were used to control the flow rate of different channels. The operating range of the flow meters was 0.0112–4.92 mL/min.

Linear scan experiments were performed in the flowing stream of the VP instrument. Scanning speed was 5 mV/s and scanning range was -0.5 to $+1.0$ V against the silver reference electrode for the polyaniline-coated platinum electrode and -0.2 to $+1.0$ V for the bare platinum electrode.

Solutions and Reagents. All solutions were made in 0.482 M NaCl solution in order to mimic the salt concentration in the marine environment and at the same time provide a supporting electrolyte. A 2.5 mM solution of hexaamineruthenium trichloride

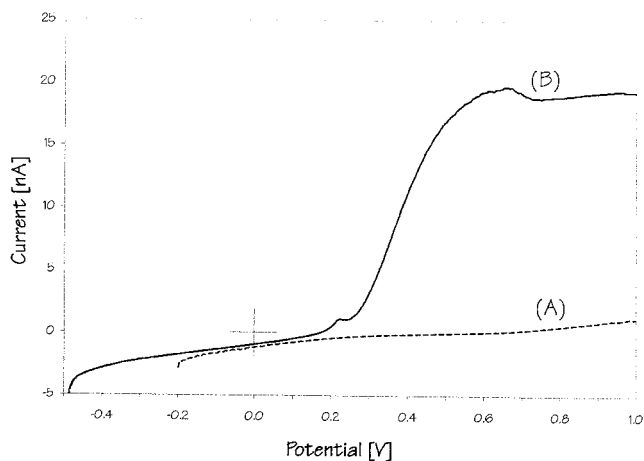


Figure 2. Linear sweep voltammograms for (A) the bare (dotted line) and (B) PANI-coated (solid line) platinum electrodes in 10 mM ascorbic acid solution in saltwater (0.5 M). The measurements were carried out in the flowing stream using the virtual plume instrument.

((NH₃)₆RuCl₃) or 10 mM solution of ascorbic acid, respectively, was used as the electrochemical marker.

RESULTS

Choosing the Electrode. The previous studies in this series^{1–4} were done with ruthenium hexamine as the electroactive marker. It was selected for its well-defined, fast electrochemical behavior. However, the overall goal of this project is to investigate the nature of chemical signaling in biological systems. Since ruthenium hexamine is not a compound that would be part of any biological scheme, we have looked for a natural substance that would be both electrochemically active, i.e., oxidizable, and be part of the diet of some marine animals. Ascorbic acid is one of the compounds compatible with the food of the marine animals and therefore could also be present in the chemical signal they use to locate food. Its electrochemical behavior has been also investigated.⁶ Unfortunately, the conventional Pt electrode does

- (5) Li, G.; Josowicz, M.; Janata, J. *Electrochem. Solid-State Lett.* **2002**, *5* (4), D5–D8.
- (6) Lyons, M. E. G.; Breen, W.; Cassidy, J. J. *Chem. Soc., Faraday Trans.* **1991**, *87* (1), 115–123.
- (7) Xu, J.-J.; Zhou, D.-M.; Chen, H.-Y. *Fresenius J. Anal. Chem.* **1998**, *362*, 234–238.
- (8) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*; John Wiley & Sons: New York, 2001.
- (9) Potje-Kamloth, K.; Josowicz, M. *Ber. Bunsen-Ges. Phys. Chem.* **1992**, *96* (8), 1004–1017.
- (10) Hartmann, W. M. *Signals, Sound, and Sensation*; Springer-Verlag: New York, 1998.
- (11) Okamoto, H.; Okamoto, M.; Kotaka, T. *Polymer* **1998**, *39* (18), 4359–4367.

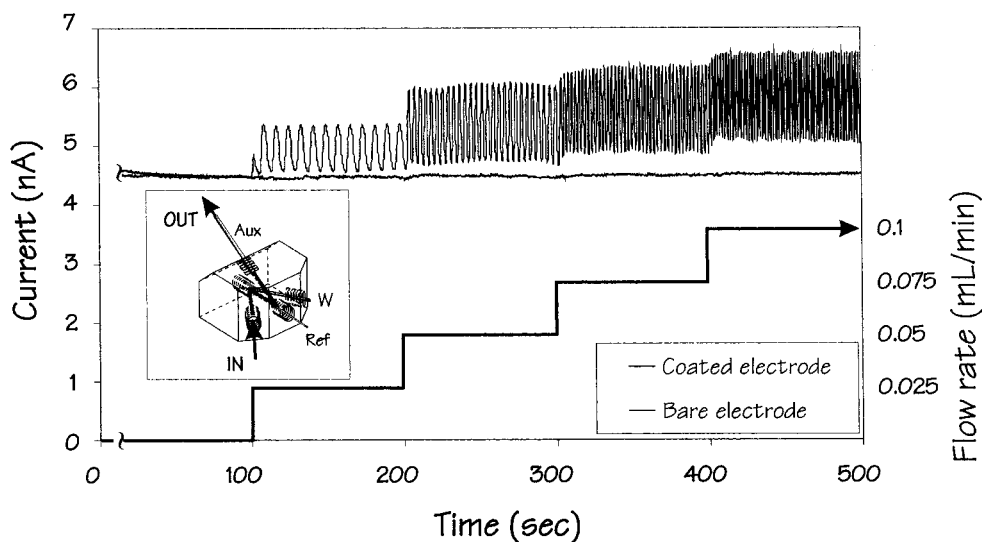


Figure 3. Flow dependence of the bare and PANI-coated (green line) platinum electrodes. The solid line represents the changes of the flow rate in the time. The figure inset shows the flow-through cell used in these experiments.

not yield a suitable signal from ascorbic acid in the potential window provided by the supporting electrolyte, NaCl. Curve A in Figure 2 is the linear scan of the 10 mM solution of ascorbic acid in the salt solution (0.5 M) recorded at the Pt disk electrode. It has been reported⁷ that it is possible to oxidize ascorbic acid at a Pt electrode coated with PANI (Figure 2, curve b). Indeed, for such electrode there is a distinct plateau above +0.6 V. Based on this observation, the PANI-coated platinum electrodes were used in flow modulation experiments with ascorbic acid as a chemical marker. The working potential was chosen +0.7 V against the silver reference electrode.

The carbon electrode was also studied as a possible sensor for the flow modulation. Similar to the PANI-coated platinum electrode, the carbon electrode showed a plateau in the NaCl potential window. However, the signal of the carbon electrode deteriorated over time while that of the PANI-coated electrode remained constant over several days of continuous use. Since the purpose of this study is correlation analysis performed in the frequency domain, particular attention was paid to the dynamic characteristics of these electrodes.

Flow Rate Dependence of the Electrode. It is known that the mass transport properties and therefore the dynamic behavior of coated macroscopic electrodes are different from those of bare electrodes.⁸ Since the dynamic behavior of the system is of prime importance in our studies, the effect of the PANI layer on flow rate dependence and on the experimental bandwidth was investigated. Flow-dependence experiments of amperometric sensors were conducted using hexamine ruthenium chloride (2.5 mM in 0.5 M NaCl solution) as a chemical marker. A peristaltic pump was used to propel the solution through the flow system. The pulsation of the flow due to the peristaltic pump was a convenient stimulus of the signal for these studies. The mean flow rate of the electrolyte with constant concentration of the ruthenium hexamine was varied from 0.025 to 0.1 mL/min. Flow rate was changed every 100 s. The flow dependence of current for bare and coated electrodes is very different, as can be seen in Figure 3. In this figure, the current at zero flow does not reach a steady-state value, as expected from the Cottrell equation.⁸ Modifying

platinum electrodes with the PANI coating greatly suppresses the flow sensitivity of the electrode. In fact, there is virtually no change in the mean current for the PANI-coated electrode in the tested flow region. Similar results were obtained from the experiments with the platinum electrodes coated with electrochemically inactive polyoxyphenylene film.⁹ This suggests that the polymer film acts as a stagnant layer on the surface of the electrode and changes the mode of mass transport to the electrode surface.

Effects of the Electrochemical Marker and of the Coating on the Coherence Spectra. In our previous studies, we showed that the fluctuating part of the chemical signal in the carrier stream contains information that allows us to estimate the distance to the source and the geometry of the source.^{2,3} Fluctuations introduced by the source of the chemical marker into the plume structure are common to every point in the plume. Therefore, when we look at two different points in the plume and see commonality in the signal, it is due to the source. This kind of common part in the signals of two independent points (sensors) can be found using coherence analysis. Coherence is a frequency-dependent correlation coefficient defined as

$$\alpha^2(\omega) = \frac{|X_{jk}(\omega)|^2}{|X_{jj}(\omega)| |X_{kk}(\omega)|}$$

where $|X_{jk}(\omega)|^2$ is cross-power spectral density and $|X_{jj}(\omega)|$ and $|X_{kk}(\omega)|$ are autopower spectral densities of sensors j and k , respectively.¹⁰ It shows how much commonality two signals have at a particular frequency. We have just shown that modifying the electrode changes its dynamic characteristics. Now the question rises of if and how it would affect the coherence spectra of a time series of some fluctuating signal.

The electroactive chemical marker is delivered to the carrier stream as a series of concentration pulses at the modulation frequency, f_s , using the solenoid valve. The stream is divided into two channels of length L_1 and L_2 , containing amperometric sensors 1 and 2. The time sequence of the modulated amperometric current is recorded.

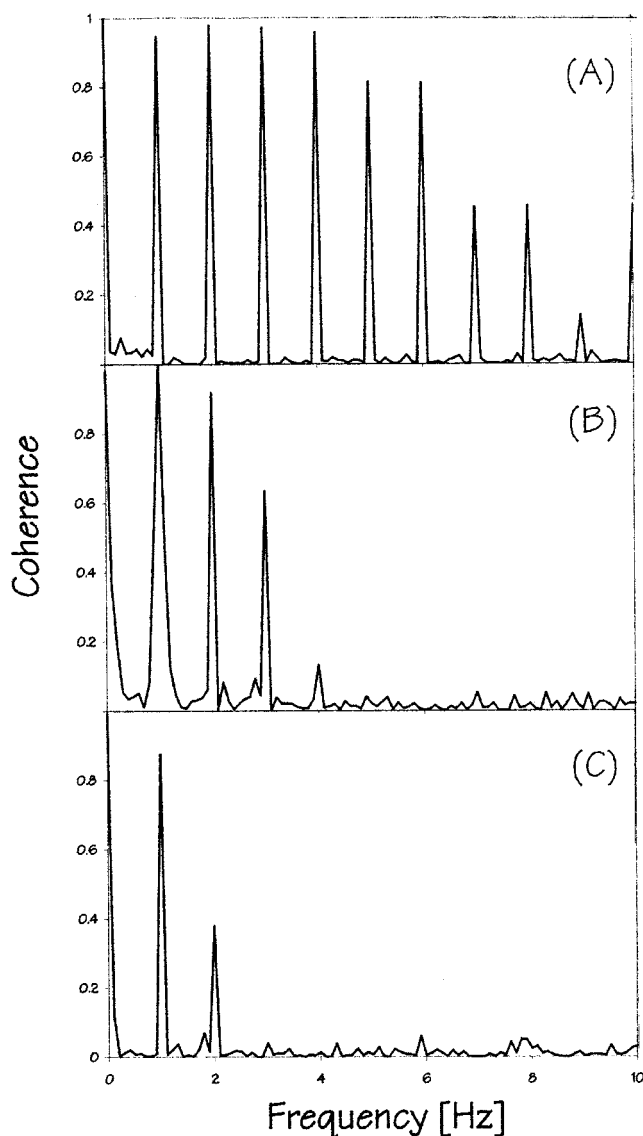


Figure 4. Effects of the electrode dynamics on the coherence spectrum. Coherence spectra: (A) bare platinum electrode and ruthenium hexamine; (B) the bare carbon electrode and ascorbic acid; (C) the PANI-coated platinum electrode and ascorbic acid.

Modulation frequency of the solenoid valve (1 Hz) and length of the delay elements (10 cm) were kept constant in this set of experiments. Electrochemical markers used were hexaamineruthenium chloride and ascorbic acid. Electrodes used were platinum and carbon as the bare electrodes and PANI/platinum as the coated electrode. Figure 4A shows the coherence spectrum of the two-channel flow modulation experiment where bare platinum electrodes were used in combination with the ruthenium hexamine redox couple. The bare carbon electrode in combination with the ruthenium hexamine redox couple was also studied. These experiments yielded results similar to ones from the platinum electrode. In Figure 4B, the coherence spectrum for bare carbon electrodes in combination with an ascorbic acid electrochemical marker for the similar experimental setup is presented. Figure 4C shows the coherence spectrum of the two-channel flow modulation experiment where PANI-coated platinum electrodes in combination with the ascorbic acid electrochemical marker were used.

Panels A and B of Figure 4 show that switching the electrochemical marker from reversible (fast) to irreversible (slow) decreases the power in higher harmonics. On the other hand, it does not significantly diminish the power in the fundamental frequency (1 Hz). As can be seen from Figure 4C, using the coated electrode as opposed to the bare one even further reduces the number and intensity of higher harmonics of the fundamental frequency.

Effect of the PANI Coating on the Bandwidth and Critical Length. In previous work, we showed^{2,3} that our virtual plume system is limited by two major parameters: critical length (a distance from the source at which the fluctuating part of the signal disappears) and fluidic bandwidth (a frequency at which the fluctuating part of the signal disappears). To evaluate how change of the electrode and of the electroactive compound affects the system, these two parameters should be the most illustrative.

Two-channel flow modulation experiments were conducted. The modulation frequency was varied from 1.6 to 4.4 Hz using the virtual plume setup with constant length of delay elements (10 cm). The same setup was used for both bare platinum electrodes and coated ones. Resultant time series were analyzed for coherence, and the coherence value at fundamental frequency was plotted against the modulation frequency to evaluate the bandwidths for coated and bare electrodes. Figure 5 shows (as expected from the coherence spectra) that bandwidth for the coated electrode is considerably narrower than that for the bare electrode.

Another set of two-channel flow modulation experiments was conducted to determine the critical length of the virtual plume setup with PANI-coated electrodes. The modulation frequency was kept constant at 1 Hz, and coherence values were calculated for various delay element pairs of 10–20, 40–50, 70–80, 100–110, and 190–200 cm. As in previous experiments, bare and PANI-coated platinum electrodes were used. Coherence value at fundamental frequency was plotted against the length of the delay element. Figure 6 shows that even though critical length for a virtual plume instrument decreases when PANI-coated electrodes are used, the change in critical length relative to the absolute value was much less than that in the bandwidth. Bandwidth for coated electrodes changed almost one-third of the bandwidth for bare electrodes while critical length decreases only ~10% of the critical length for bare electrodes. This shows that in the case of critical length, unlike fluidic bandwidth, dispersion of the marker plug in the carrier flow is much more important than the response time of the electrode.

CONCLUSIONS

The results of our study show that in order to use some biologically relevant compound (e.g., ascorbic acid) as a chemical marker in our virtual plume experiments, the electrodes have to be modified. From the experiments with PANI-coated platinum electrodes, we can see that use of modified electrodes limits the performance of the virtual plume at higher frequencies. The first and most beneficial change is in the flow dependence of the electrode. Our results show that coating of the electrode virtually eliminates the flow dependence of the electrode. It creates a fixed-thickness depletion layer at the electrode surface, and in doing so, it suppresses the flow dependence. Unfortunately, this ad-

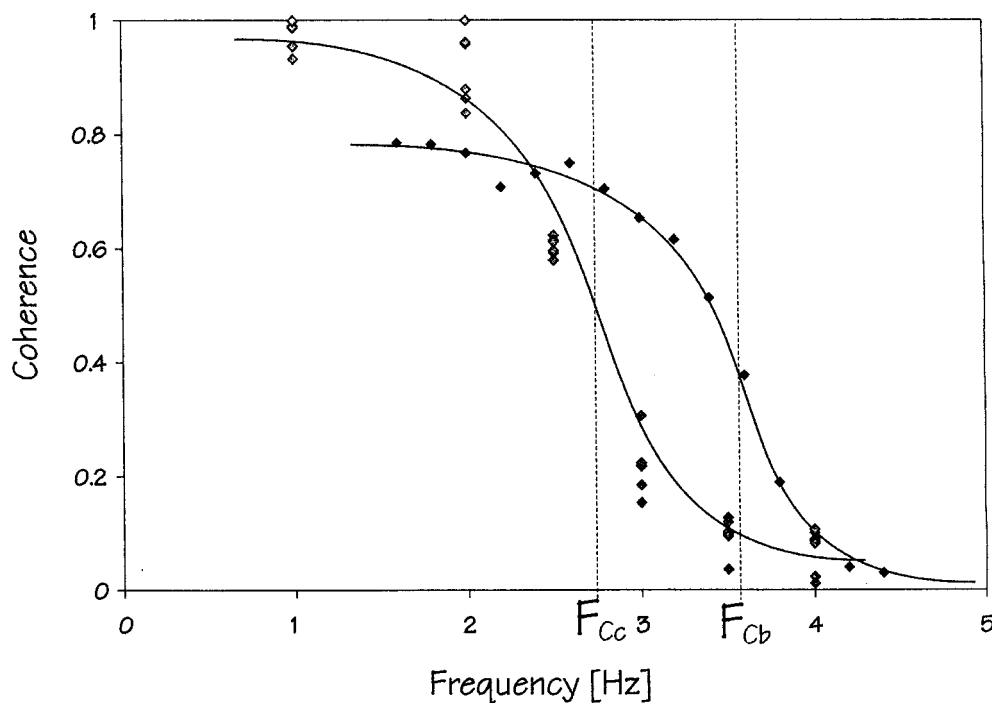


Figure 5. Effect of the coating on the fluidic bandwidth. Solid squares represent the bare platinum electrode and open squares the PANI-coated platinum electrode. F_{cb} shows the value of the fluidic bandwidth for the bare electrode and F_{cc} for the coated electrode.

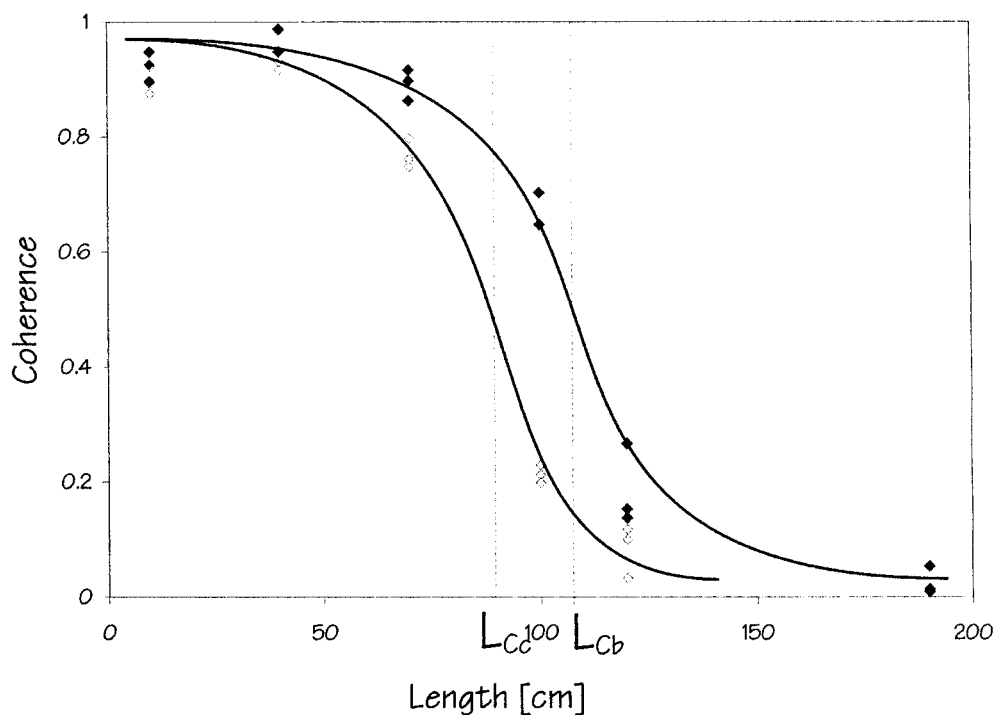


Figure 6. Effect of the coating on the critical length. Solid squares represent the bare platinum electrode and open squares the PANI-coated platinum electrode. L_{cb} shows the value of the critical length for the bare electrode and L_{cc} for the coated electrode, respectively.

ditional diffusion layer also has adverse effects. As the diffusion of the marker to the electrode surface rather than kinetics of the electrochemical reaction becomes more important, it smooths out the signal profile and therefore suppresses higher harmonics in the coherence spectrum of the signal. It also decreases considerably the fluidic bandwidth of the virtual plume instrument. Our results allow us an alternative of using a bare carbon electrode which would not have the additional diffusion layer. The downside

of using the carbon electrode is that the carbon surface becomes coated in the electrochemical oxidation of ascorbic acid and therefore the signal is decreasing with time.

Change of electrode and electroactive compound also leads to shorter critical length of VP. Unlike the fluidic bandwidth, the critical length is decreased only by 10%, which implies that the critical length as a limiting parameter of the VP instrument has more to do with the dispersion of the sample plugs in the flow

channel than with the diffusion of the electrochemical marker to the electrode surface.

Despite the somewhat negative impact on information content from loss of higher harmonics, the ascorbic acid and PANI-coated electrode is a viable system for use in the natural environment. Most of the information content encoded in the plume structure is in the coherence value at the fundamental frequency. Therefore, loss of the higher harmonics is not of primary concern. The impact of the system change on the critical length was minor. And even though the fluidic bandwidth decreased considerably, it still covers

the range of frequencies that one would expect to encounter in the natural environment.

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