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New Approaches to Direct Electroanalysis Ascorbic Acid in Bio-samples Using a Combined Ultra-microelectrode

Samira Fathi,[a] Amir Hatami(e) [a,b]*

[a] Department of Chemistry, Institute for Advanced Studies in Basic Sciences, Zanjan-Iran.

[b] Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden.

- E-mail: amir.hatami@iasbs.ac.ir, amir.hatami@gu.se

Abstrct

While direct analysis of real biosamples is a complex process compared to other systems, it also yields a set of novel and valuable results. Vitamin C plays a pivotal role in our immune functions; however, in contrast to other vitamins, human body cannot generate it. Therefore, there is a high demand for the development of new tools capable of onsite monitoring and analysis of natural sources without any treatment, even in the field and during the transfer and storage of food products. By leveraging the unique features of microelectrodes, we have developed novel combined microelectrodes by modifying carbon fiber (33 µm) coated with an Au nano-film to serve as a working electrode and tiny silver wire as reference electrode. This microscale tool allows for the direct microelectroanalysis of ascorbic acid in lemon and cactus bodies, serving as biological matrices. Beyond the potential for direct electroanalysis in these bio-matrices, our primary objectives include the examination of the distribution of ascorbic acid content across different sections of lemon fruit and parts of the cactus plant. Both lemon and cactus are recognized sources of vitamin C. Notably, the micro-size of the combined sensor provides sufficient resolution for microscale analysis in fruit and plant samples without sample treatment. Our electrochemical measurements revealed that the center of the lemon contains notably higher levels of Vitamin C compared to its sides. Also, the levels of Vitamin C are higher in the fresh arms of the cactus in comparison to the older arms and the cactus trunk. Furthermore, our observations indicated that improper storage of lemon products in the presence of day light in on week significantly reduces the vitamin's levels. Finally, we believe that these findings hold a significance and practical applicability in the agricultural, medicinal, and food industrial sectors.

Experimental

■ Fabrication, Modification, and Characterization of a Combined Microelectrode.

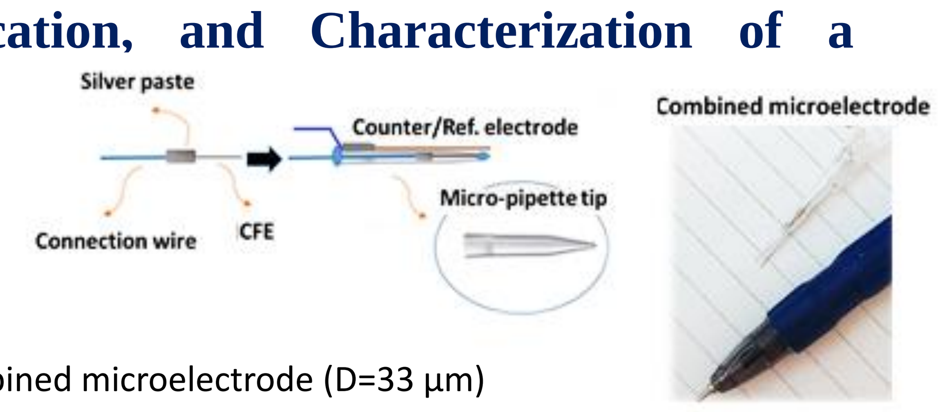


Figure 1. Schematic of fabrication of combined microelectrode (D=33 µm) and its image.

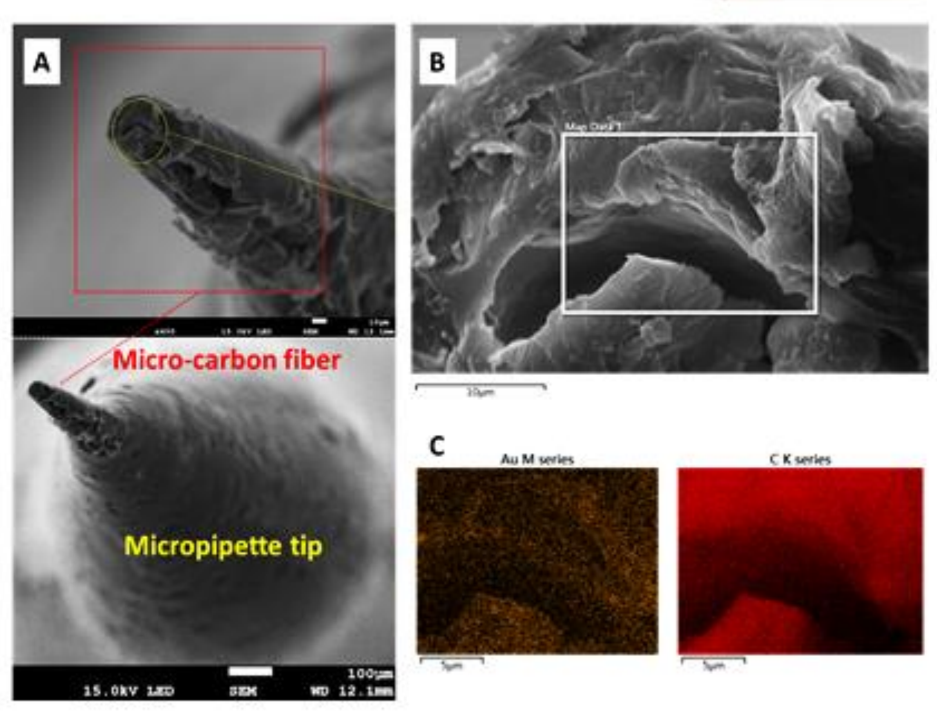
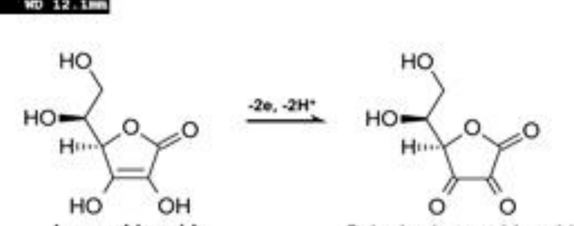


Figure 2. SEM images of modified carbon microelectrode with Au film (CME@Au).



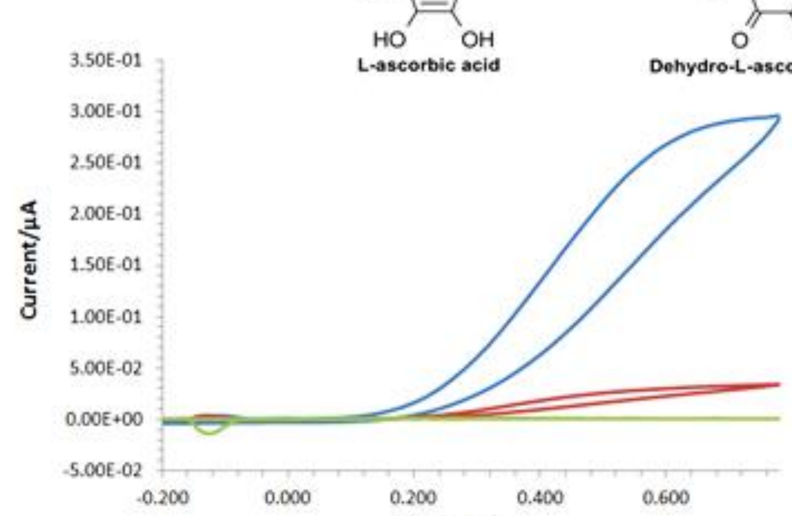


Figure 3. CVs of CME@Au microelectrode in 1.0 mM PBS in the absence (green) and presence (blue) of 0.5 mM AC, CVs of MCFE in 1.0 mM PBS and 0.5 mM AC. (Scan rate:100 mV/s.)

Voltammetric Analysis of AC by Combined CME@Au

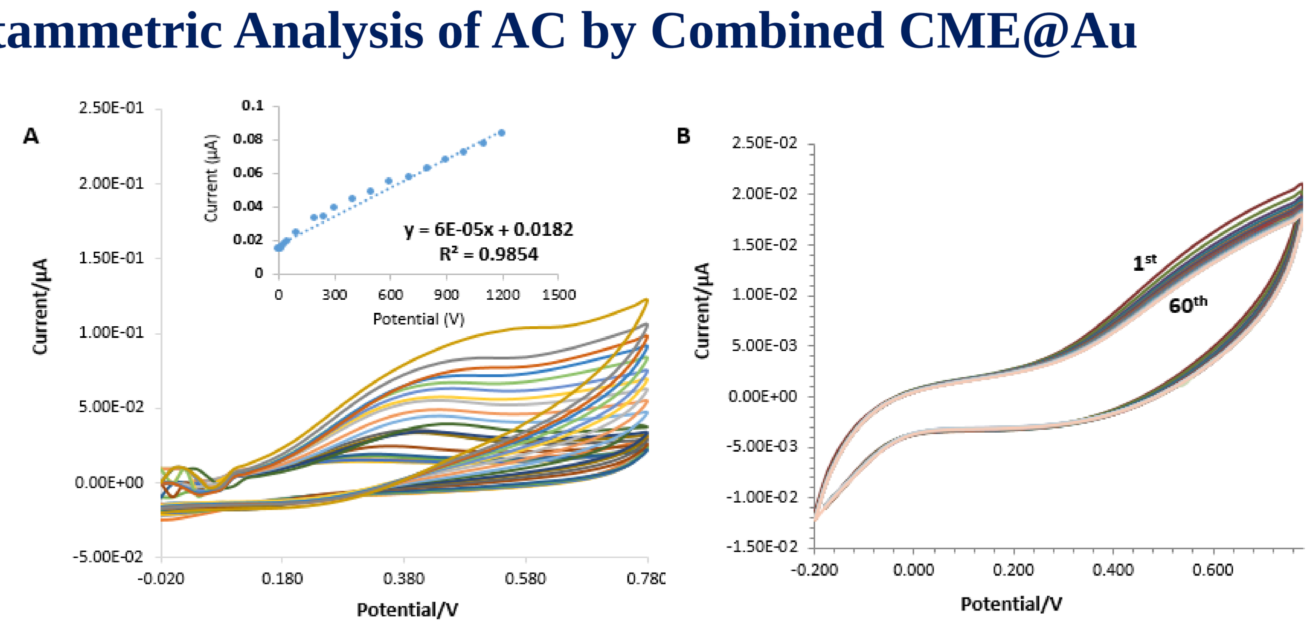


Figure 4. . (A) CVs of AC in 0.1 M PBS (pH 6.0) containing various concentrations of AC, listed from inner to outer: 5, 10, 20, 50, 35, 100.0, 150, 200, 300, 110, 500.0, 150.0 + 700.0, and 1200.0, respectively. Inset: Plots of I_{lim} vs. AC concentrations.

Results and discussion

Analytical applications

In the following sections, the ability of fabricated sensor to direct analysis of AC has been evaluated in different samples such as fruit, single drop (microvolume sample) and plant parts.

►On site Microanalysis in Lemons Reveals that the Fresher Parts Exhibit Higher AC Content

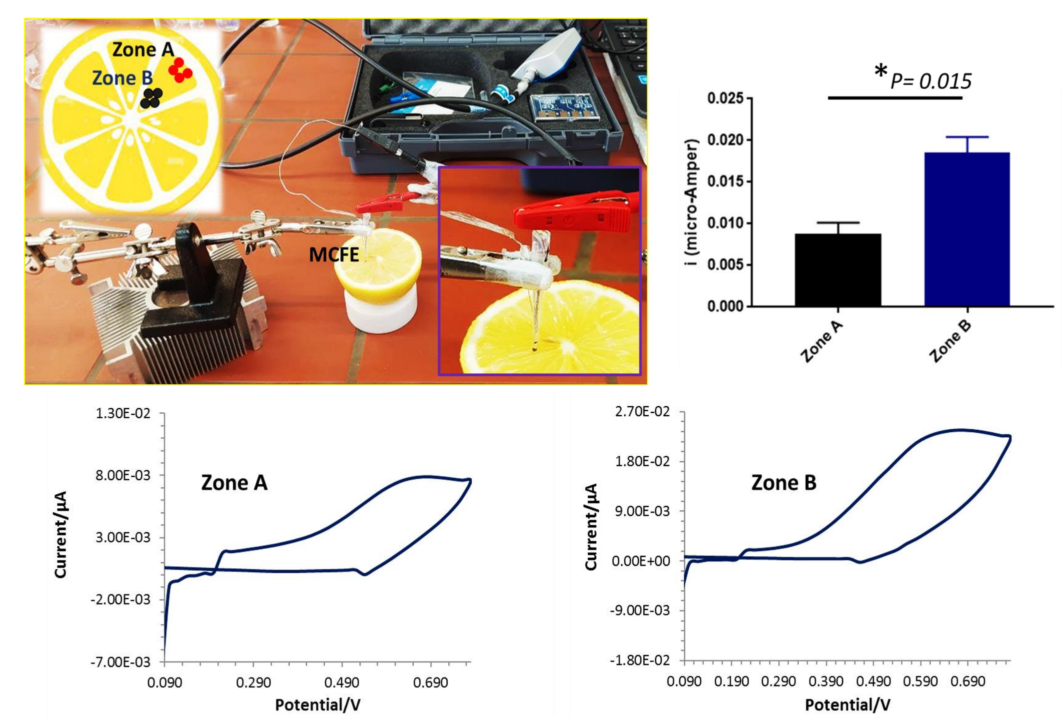


Figure 5

In this test, fresh lemons cut into two slices using a knife. Next, the CME@Au was gently inserted into zone A and zone B (See Figure 5) with 2 mm of the tip inserted into the lemon tissue at three different points (2–3 mm apart) in each zone. During these experiments, the average limiting current intensity for each zone was calculated and compared. It can be observed that the average peak height of the CVs taken at Zone A (near the border) is lower than the average for Zone B (near the center).also, t-test analysis was applied to analyze this data, and shows the obtained signals are significantly different (See Fig.5). We can also hypothesize that Zone B, being the younger part of the lemon, has a higher level of AC compared to the border layers or the older part.

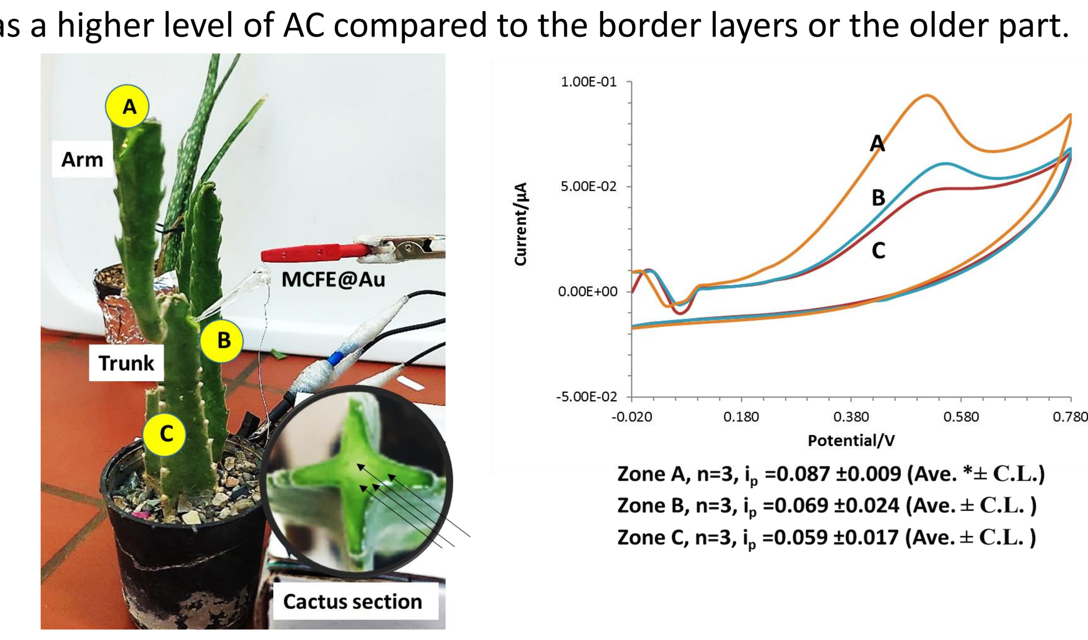


Figure 6

►In Vivo Electrosensing of AC within a Plant, Cactus as a model

We assessed AC levels in three different parts of the cactus plant (A, B, C zones) as a model (see Figure 6) and compared the recorded current intensities from these points (See Figure 3). The comparison of recorded signals revealed that the AC levels at points A and B differed but were not statistically significant. Interestingly, the amount of AC in zone A is significantly higher than in Zone C. This finding is consistent with other reports that suggest different parts of cacti have different levels of AC due to factors such as growing conditions and maturity.

Conclusion

In this study, we have developed a cost-effective miniaturized analytical tool using micro-carbon fiber coated with a gold film. This tool allows for the monitoring of ascorbic acid (AC) in biosamples both in vivo and in vitro. Our aim was to directly assess the AC content in plants, specifically lemon and cactus, without the need for any sample treatment or separation steps.
The proposed microsensor is accurate, practical, portable, and exhibits acceptable stability and selectivity. It can be utilized for AC analysis in various samples. Its small size enables analysis with only a small sample volume, such as a single micro-droplet. Furthermore, it can directly analyze small zones within fruit and plant samples without the requirement of any sample preparation steps.
We employed this electrode to analyze different sections of a cut lemon and to perform in vivo analysis of a cactus plant. The results revealed that the AC level is higher in the center of the lemon, specifically in the younger part. In the cactus sample, we observed a higher AC level in the new cactus arm compared to the old part. Additionally, this electrode was used to monitor AC in fresh droplet lemon juice samples during oxidation by air. It was also utilized to assess AC levels in samples that were improperly stored in front of daylight.
Simplicity and fast response are the key advantages of this combined sensor in all applications. We believe that this sensor can be valuable for agriculture and food analysis, as it can address complex questions regarding the distribution of chemicals within plants or fruit samples. These questions are often challenging or expensive to answer using other analytical techniques.

References

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