Application Note AN-RA-008

Easy detection of enzymes with the electrochemical-SERS effect

Activation of enhanced features of silver electrodes helps obtain characteristic Raman bands of biological molecules

Raman spectroscopy is one of the most promising chemical analysis techniques. This is due to its inherent fingerprint properties which allow the identification of different species present in a studied system.

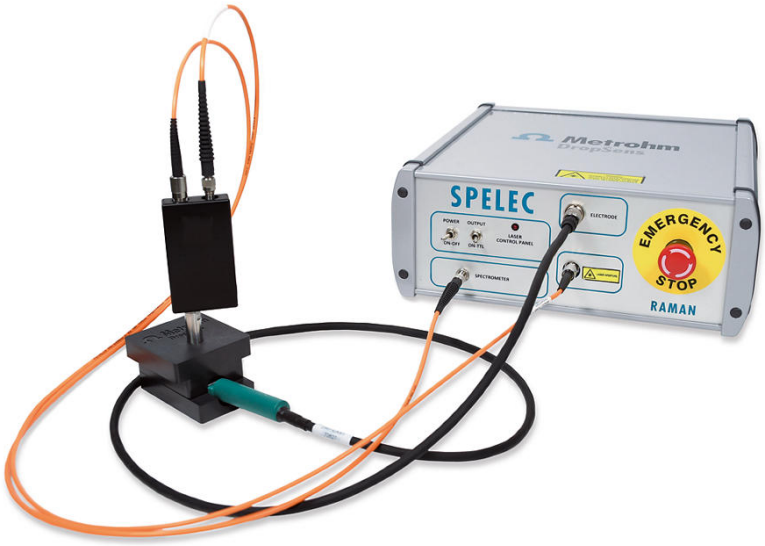
Although low sensitivity has limited its use as a detection method, the surface-enhanced Raman scattering (SERS) effect has improved its effectivity for analytical use. Enhancement of the Raman signal has allowed the development of many sensing applications. In particular, the energy provided by the 638 nm laser ensures a balance between risking damage to the sample and the generation of fluorescence, making this laser popular for most biological applications.

In this Application Note, aldehyde dehydrogenase and cytochrome c are analyzed by Raman spectroelectrochemistry as a proof of concept.

INSTRUMENTATION AND SOFTWARE

Measurements were performed using a SPELEC RAMAN 638 instrument (638 nm laser), a Raman probe corresponding to the laser wavelength, and spectroelectrochemical cells for screen-printed electrodes (Figure 1a) as well as for conventional electrodes (Figure 1b).

a)



b)

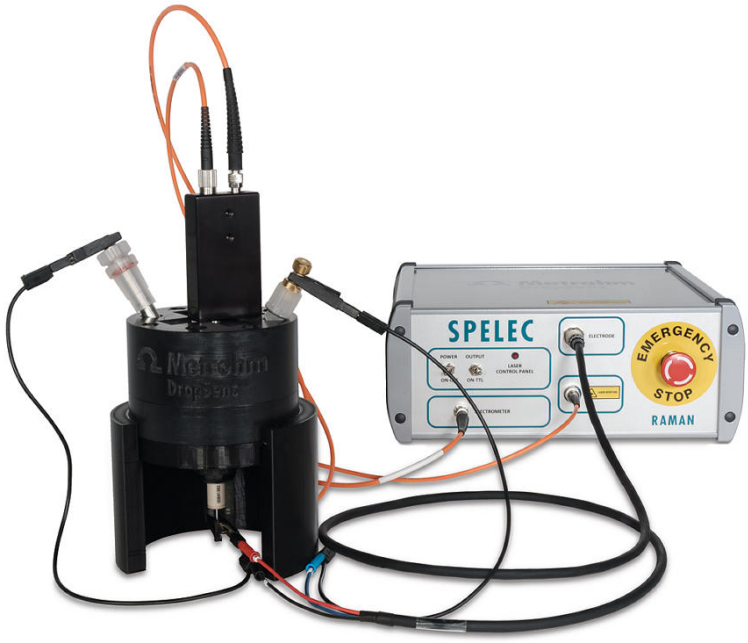


Figure 1. SPELEC RAMAN 638 instrument and Raman probe used in combination with a Raman spectroelectrochemical cell for (a) screen-printed and (b) conventional electrodes.

Silver screen-printed electrodes (Ag SPEs, C013) were used as SERS substrate because of their electrochemical activation properties. In addition, a conventional silver working electrode (6.09395.044) was also used as SERS substrate in combination with steel (6.0343.110) and Ag/AgCl (6.0728.120) counter and reference electrodes, respectively.

The SPELEC RAMAN 638 was controlled with DropView SPELEC, a dedicated software that provides spectroelectrochemical information and includes tools to perform adequate treatment and analysis of the collected data. All hardware and software used for this study is compiled in Table 1.

Table 1. Hardware and software equipment overview.

Equipment	Article number
Instrument	SPELECRAMAN638
Probe	RAMANPROBE638
Raman spectroelectrochemical cell for SPEs	RAMANCELL
Raman spectroelectrochemical cell for conventional electrodes	RAMANCELL-C
Silver SPE	C013
Connection cable for SPEs	CAST
Silver working electrode	6.09395.044
Steel counter electrode	6.0343.110
Ag/AgCl reference electrode	6.0728.120
Connection cable for conventional electrodes	CABSTAT
Software	DropView SPELEC

EC-SERS EFFECT OF SCREEN-PRINTED ELECTRODES: DETECTION OF ALDEHYDE DEHYDROGENASE

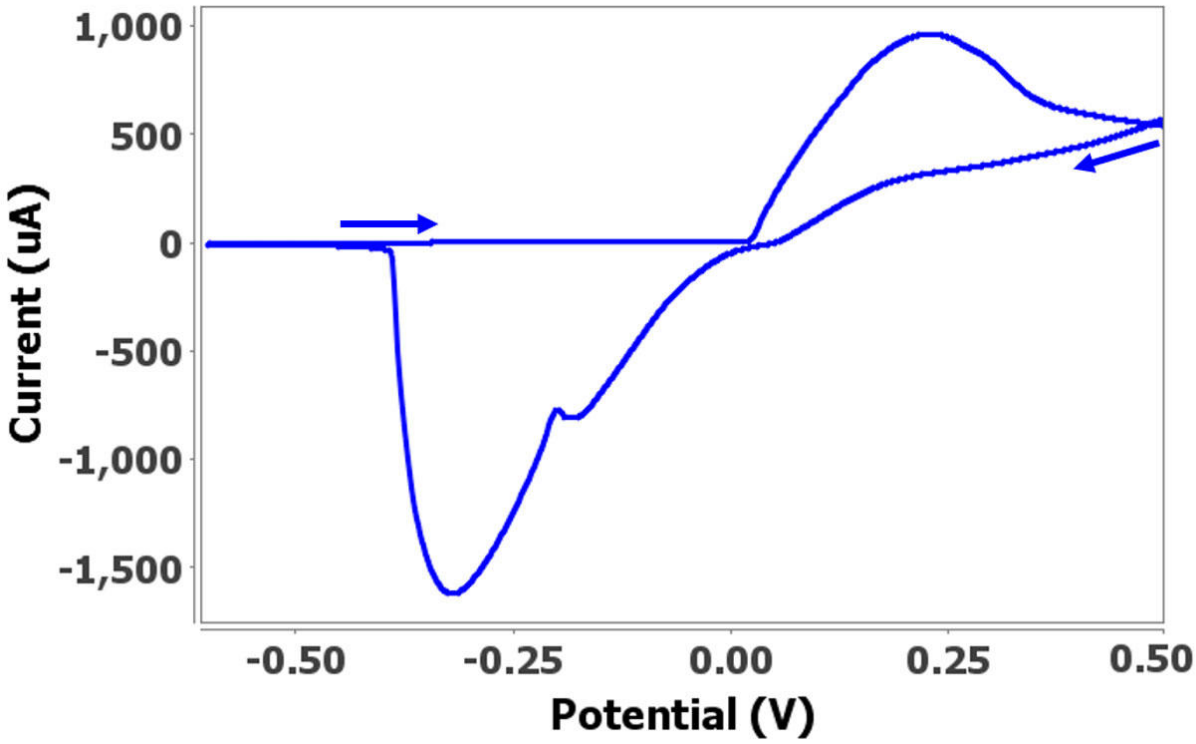
Raman spectroelectrochemistry was employed for the detection of aldehyde dehydrogenase (ALDH) in aqueous solution. Electrochemical SERS (EC-SERS) protocol requires two steps in a single experiment: the electrochemical activation of SERS features of Ag SPEs, and then the spectroscopic detection of the sample.

Electrochemical activation consists of scanning the potential from +0.50 V to produce the initial oxidation of the silver surface, followed by its reduction up to -0.60 V to generate nanostructures with the SERS effect. The spectroelectrochemical experiment is performed in presence of the analyte to be detected (in this case ALDH), but chloride ions are also present in solution to favor the generation of silver SERS substrate [1].

In order to evaluate the evolution of Raman bands during the entire experiment, and therefore to optimize the optical detection, SPELEC RAMAN 638 performs measurements in operando mode. This way, spectra are recorded continuously during the whole experiment and not only at certain potentials.

Figure 2a displays the cyclic voltammogram of ALDH, and the characteristic Raman spectrum of ALDH is shown in Figure 2b. The results were obtained for 1 mg/mL ALDH in 0.1 mol/L KCl aqueous solution. Although the acquisition of spectra was continuously performed, the Raman spectrum in Figure 2b was recorded at -0.50 V as this potential provides the highest Raman intensity.

a)


1,000
500
0
-500
-1,000
-1,500
Current (uA)
-0.50
-0.25
0.00
0.25
0.50
Potential (V)

b)

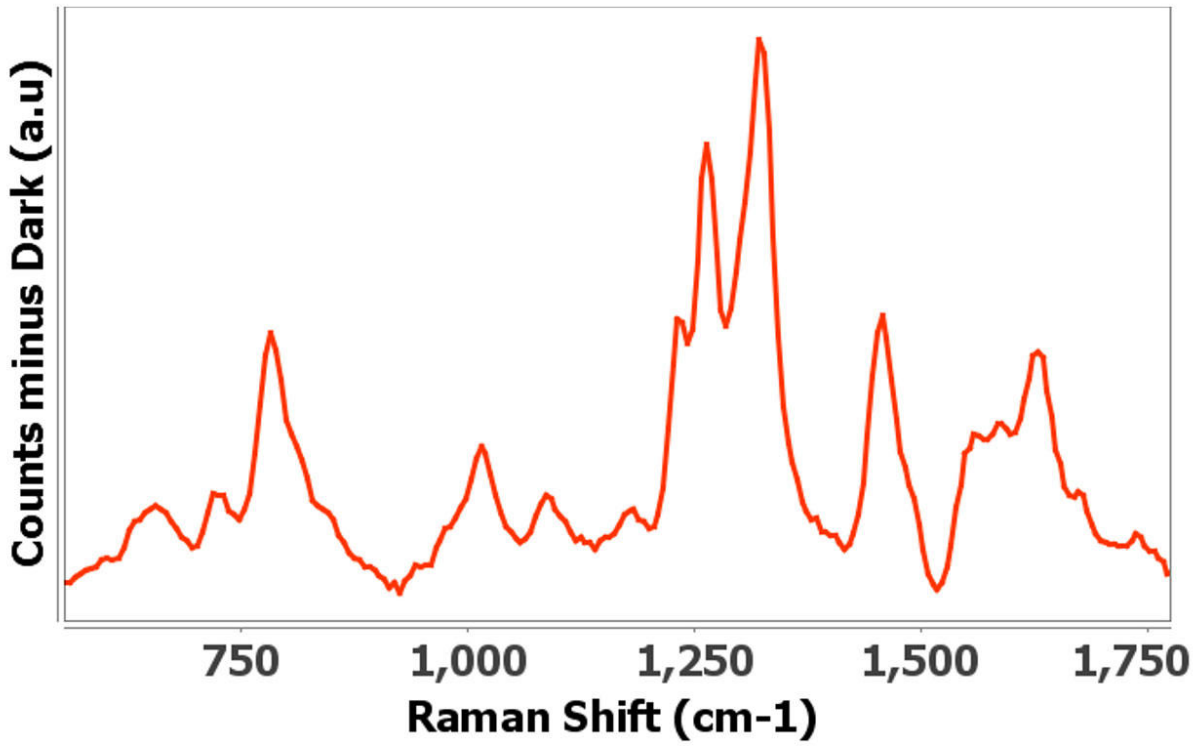


Figure 2. (a) Cyclic voltammogram and (b) Raman spectrum of 1 mg/mL aldehyde dehydrogenase in 0.1 mol/L potassium chloride aqueous solution using C013 electrodes. Integration time was 2000 ms.

The combination of the proposed EC-SERS procedure for the easy activation of Ag SPEs with a Raman spectroelectrochemical instrument (638 nm laser) offers a fast and interesting alternative for the characterization of ALDH in solution not previously reported in the literature.

EC-SERS EFFECT OF CONVENTIONAL ELECTRODES: DETECTION OF CYTOCHROME C

Conventional electrodes were also used for the EC-SERS detection of different enzymes such as cytochrome c. The protocol for this type of electrode follows the same steps as for SPEs. An initial oxidation of the surface is required followed by the subsequent reduction to generate silver nanostructures with SERS effect. The potential window was adjusted according to the electrodes used in this experiment. The best results were obtained by scanning the potential of 0.1 mg/mL cytochrome c in 0.1 mol/L KCl aqueous solution from +0.80 V to -0.80 V. The Raman spectrum with the highest intensity (Figure 3) was recorded at -0.70 V.

Assignments of vibrational modes of each Raman band of cytochrome c are listed in Table 2.

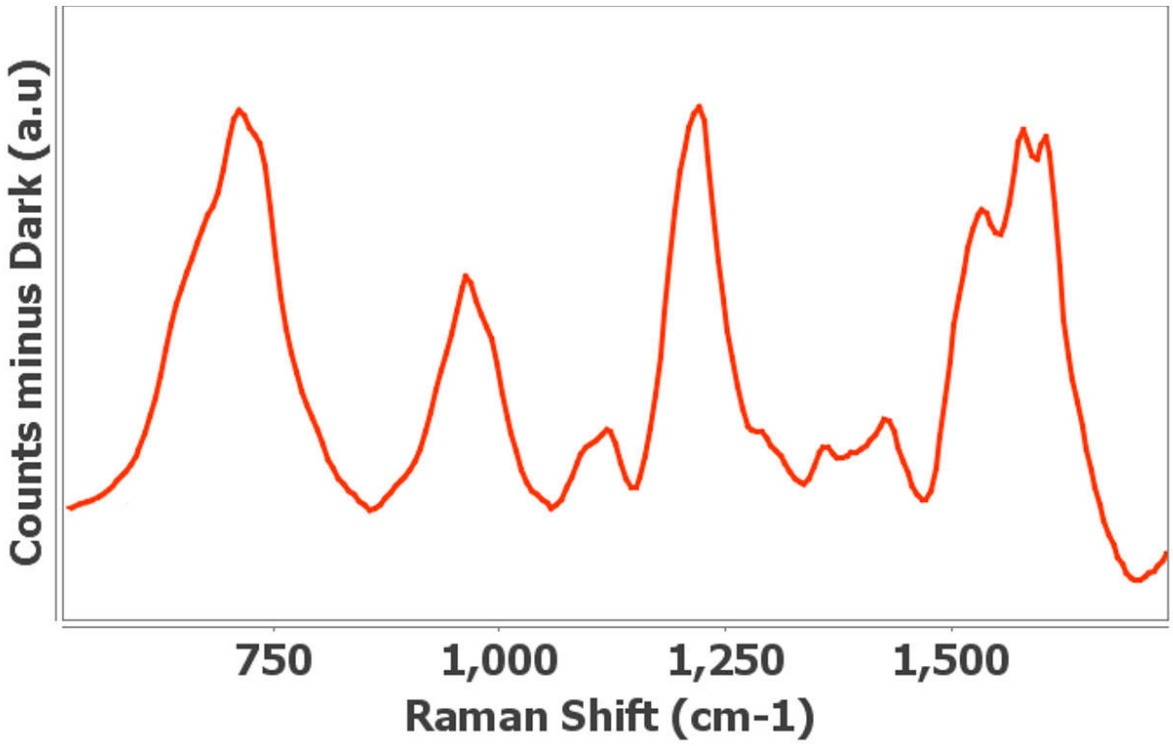


Figure 3. Raman spectrum obtained of 0.1 mg/mL cytochrome c in 0.1 mol/L potassium chloride aqueous solution using a conventional silver electrode. Integration time was 2000 ms.

Table 2. Vibrational assignment of cytochrome c [2,3].

Cytochrome c SERS bands (cm^{-1})	Assignment
713	Heme breathing
969	Asymmetric pyrrole deformation
1123	C_β-C_1
1220	Asymmetric pyrrole half-ring
1358	Symmetric pyrrole half-ring
1426	Pyrrole quarter-ring
1528	$C_\alpha C_m$, $C_\alpha N$
1578	$C_\beta C_\beta$, $C_\alpha C_m$,
1604	$C_\alpha C_m$, $C_\alpha C_\beta$

Considering that cytochrome c exists as interconvertible reduced and oxidized forms, the oxidation state of the Fe ion can be determined according to the position of their characteristic Raman bands. Reduced cytochrome c shows one Raman band centered at 1604 cm^{-1}, while the oxidized form displays an upshifted band centered at 1636 cm^{-1}. According to the Raman spectrum (**Figure 3**) and the vibrational assignments (**Table 2**), the reduced form of the analyte is detected during the EC-SERS experiment. This experiment demonstrates the possibility to detect cytochrome c as well as characterize its oxidation state.

CONCLUSION

Raman spectroelectrochemistry is a multi-response technique that provides outstanding results when studying a wide variety of systems. In the particular case of the 638 nm laser, the energy provided at this wavelength is suitable for biological applications.

In this work, the proposed electrochemical protocols for the activation of SERS properties of different silver electrodes (screen-printed as well as conventional) offers an easy and fast procedure to enhance the Raman intensity. This enhancement of Raman intensity allows the detection of different molecules present in solution. Fingerprint Raman bands of ALDH, not previously reported, are defined in this work. In the study of cytochrome c, the characteristic Raman bands are defined in addition to characterization of the redox state of the Fe ion.

REFERENCES

1. Martín-Yerga, D.; Pérez-Junquera, A.; González-García, M. B.; et al. Quantitative Raman Spectroelectrochemistry Using Silver Screen-Printed Electrodes. *Electrochimica Acta* **2018**, *264*, 183–190. https://doi.org/10.1016/j.electacta.2018.01.060.
2. Brazhe, N. A.; Evlyukhin, A. B.; Goodilin, E. A.; et al. Probing Cytochrome c in Living Mitochondria with Surface-Enhanced Raman Spectroscopy. *Sci Rep* **2015**, *5* (1), 13793. https://doi.org/10.1038/srep13793.
3. Hu, S.; Morris, I. K.; Singh, J. P.; Complete Assignment of Cytochrome c Resonance Raman Spectra via Enzymic Reconstitution with Isotopically Labeled Hemes. *J. Am. Chem. Soc.* **1993**, *115* (26), 12446–12458. https://doi.org/10.1021/ja00079a028.

RELATED APPLICATION NOTES

AN-RA-006 New strategies for obtaining the SERS effect in organic solvents

AN-SEC-001 Spectroelectrochemistry: an autovalidated analytical technique – Confirm results via two different routes in a single experiment

AN-SEC-002 Gathering information from spectroelectrochemical experiments – Calculation of electrochemical parameters from data

CONTACT

メトロームジャパン株式会社
143-0006 東京都大田区平和島6-1-1
null 東京流通センター アネックス9階
metrohm.jp@metrohm.jp

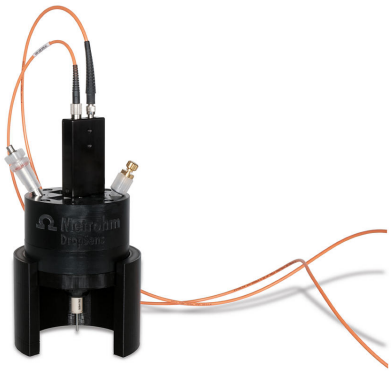
CONFIGURATION

Spectroelectrochemical Raman instrument (638 nm laser)

SPELECRAMAN638 is an instrument for performing spectroelectrochemical Raman measurements. It combines in only one box a laser class 3B (638 nm ± 0.5), a bipotentiostat/galvanostat and a spectrometer (wavelength range 640 – 885 nm and Raman shift 50 – 4370 cm-1) and includes a dedicated spectroelectrochemical software that allows optical and electrochemical experiments synchronization.

Raman Cell for Screen-Printed Electrodes

Black teflon reflection cell for performing Raman Spectroelectrochemistry with screen-printed electrodes in combination with ref. RAMANPROBE.



Raman spectroelectrochemical cell for conventional electrodes

Cell in PEEK for Raman spectroelectrochemical measurements. Designed be used with ref. RAMANPROBE and conventional Metrohm electrodes.

DropView SPELEC Software

DropView SPELEC is a Spectroelectrochemical software that controls SPELEC instrument, offering a perfect synchronization of the optical and electrochemical measurements, as well as advanced tools for data treatment.