

Methylene Blue - New Chemistry Experiments for University Education

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Received October 13, 2022; Revised November 20, 2022; Accepted November 29, 2022

Abstract The redox reactions of methylene blue on screen printed electrodes (SPEs) can be measured electrochemically by cyclic voltammetry (CV) and spectroscopically by optical and Raman spectroscopy. The combination of cyclic voltammetry and optical or Raman spectroscopy, known as absorptio- or Raman voltammetry, provides not only electrochemical information about redox reactions at electrodes, but also about changes in the visible and Raman properties of the substances used. Raman scattering can be enhanced by a simple electrochemical in-situ modification; this leads to an electrochemically activated surface enhanced Raman effect (EC-SERS). Therefore, chemical reactions, based on the change in vibrational states of the substances under investigation, can be characterized in a very simple way. In this article, we compare different electrodes and show that the electrochemical reactions of methylene blue strongly depend on the electrodes used. In addition, the electropolymerization of methylene blue is discussed. At the end of the article, a script for students for measuring the spectroelectrochemistry of methylene blue is presented in detail. The didactic background is that the combination of electrochemical information, such as potentials and current fluxes, with spectroscopic information, such as absorption and/or vibrational changes, leads to a better understanding of electrochemistry.

Keywords: *absorptiovoltammetry, surface-enhanced Raman spectroscopy, electropolymerization*

Cite This Article: P. Wagler, J. Scheible, and A. Habekost, "Methylene Blue - New Chemistry Experiments for University Education." *World Journal of Chemical Education*, vol. 10, no. 4 (2022): 137-148. doi: 10.12691/wjce-10-4-3.

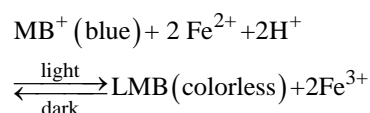
1. Introduction

In 1876, methylene blue (MB^+), a substituted phenothiazine, was first synthesized by the German chemist Heinrich Caro at BASF [1]. One year later, BASF received the first German Reich patent for a tar dye for MB^+ . Around 1900, MB^+ was applied as a drug against mental illness [2].

The electrochemical behavior of MB^+ has been intensively studied, and the SciFinder database alone yields about 3000 articles for the keywords "electrochemistry" and "methylene blue", with a marked increase in the number of publications in recent years.

In school chemistry, MB^+ is mainly known as a redox dye from the Blue Bottle experiment. In this experiment, MB^+ reacts with glucose to form leucomethylene blue (LMB). The Blue Bottle reaction is an oxidation of glucose indicated by the color change of MB^+ .

A model reaction for a light-dependent electron transfer is the photoreduction of MB^+ with iron(II) ions, where the reaction progress is indicated by the change of the color from blue to colorless.



Self-prepared Au and Ag nanoparticles [3] were compared with electrochemically activated Au and Ag electrodes to investigate their effect on surface-enhanced Raman scattering (SERS). MB^+ was used as the analyte. This was investigated spectroscopically and electrochemically by absorptiovoltammetry and Raman voltammetry. In addition, the electrochemical behavior of MB^+ was found to depend on the electrode used. The adsorption of MB^+ on different metal electrodes and electropolymerization effects at higher potentials are also discussed.

In this paper, some experiments on spectroelectrochemistry will be presented, which were carried out as a part of a Bachelor's and a Master's thesis at the Ludwigsburg University of Education, Germany. The most experiments described are integrated into a practical course of electrochemistry for advanced students at the University of Ludwigsburg. At the end of the article, we present a laboratory practical course "Spectroelectrochemical study of methylene blue", which includes an introduction and didactic background, as well as a detailed description of the experiments to be performed.

2. Didactic Objectives

A deeper understanding of electrochemistry can be achieved by combining electrochemistry and spectroscopy. This is based on the assumption of Garnett and Treagust [4] that the difficulties in understanding electrochemistry, which they empirically investigated, are due to the disconnected chemistry and physics. Therefore, Combs [5] developed a didactic-constructive model to help students to overcome this problem. To overcome this problem, the experimental investigation of electrochemical issues using computers as measurement, evaluation, and simulation systems has to play an important role. In this context Parkinson et al. [6] also determine, that "the most common feature that attracted students to science is the amount of practical work involved. Chemistry practical work received the largest number of favorable comments".

The pedagogical aspect, namely motivation of students, results from the advantages of digital learning. Therefore, Bellou et al [7] have shown that digital technologies, such as the controlling of measuring devices and the analysis of data by their visualizing, are "powerful tools to support learning" [7]. We agree with Dalgarno and Lee [8] that "digital technologies themselves do not directly cause learning to occur but can afford certain tasks that themselves may result in learning". The task of the (university) teacher is to develop meaningful digital learning environments to create incentives for learners in terms of constructivist cognitive penetration of measured / observed phenomena. Constructivist penetration of spectroelectrochemical measurements in this case means understanding the two different methods (cyclic voltammetry and optical or Raman spectroscopy) by relating them and the realization that the combination of the two methods leads to a much better characterization of molecules.

In summary, our working hypothesis is that the spectroscopic study of electrode processes is not only motivating, but that the optical and Raman effects of electrode processes also contribute to their better understanding. This is because (optical) spectroscopy is usually much easier for students to understand than electrochemistry, so it provides another "entry channel"

for the difficult understanding of electrochemistry. In this way, a broader technical basis for understanding electrochemistry is created, and it remains to be empirically investigated whether the aforementioned disconnect between chemistry and physics can be reduced by the spectroelectrochemical approach.

3. Experiments

3.1. Instruments and Chemicals

Cyclovoltammograms were recorded with the μ Stat 400 potentiostat from Metrohm/DropSens using DropView control software.

The Raman spectrometer used was the AvaRaman instrument (with a 785 nm laser) from Avantes controlled by Spectragryph software from Dr. Menges, Germany (<https://www.ffmpeg2.de/spectragryph/>).

Absorptovoltammograms were recorded using the Metrohm/DropSens potentiostat/photodiode combination μ Stat-ECL and a 660 nm LED (Thorlabs) as the light source. Bismuth, gold, platinum and silver working electrodes were used as screen-printed electrodes (Bi-, Au-Bt-, Pt-Bt- and Ag-SPE, Bt means low-temperature curing of the gold ink resulting in a large surface corrugation, all SPEs from Metrohm/DropSens), methylene blue trihydrate from Alfa Aesar, J60823.

3.2. Absorptovoltammetry at Different Electrodes

Figure 1 shows the absorptovoltammogram of MB^+ (0.1 mmol) in 0.1 mol Na_2SO_4 solution in water, $\text{pH} \approx 7$ at a Pt-SPE: The cyclovoltammogram shows two distinct peaks (black curve): A reduction peak at -0.47 V ($\text{MB}^+ \rightarrow \text{LMB}$) and a re-oxidation peak at -0.3 V ($\text{LMB} \rightarrow \text{MB}^+$). Since the redox process is a 2-electron process (Scheme 1), the peak current difference should be 29.5 mV for a reversible reaction. Since the difference is about five times as large, the process at the Pt electrode used must be quasi-reversible.

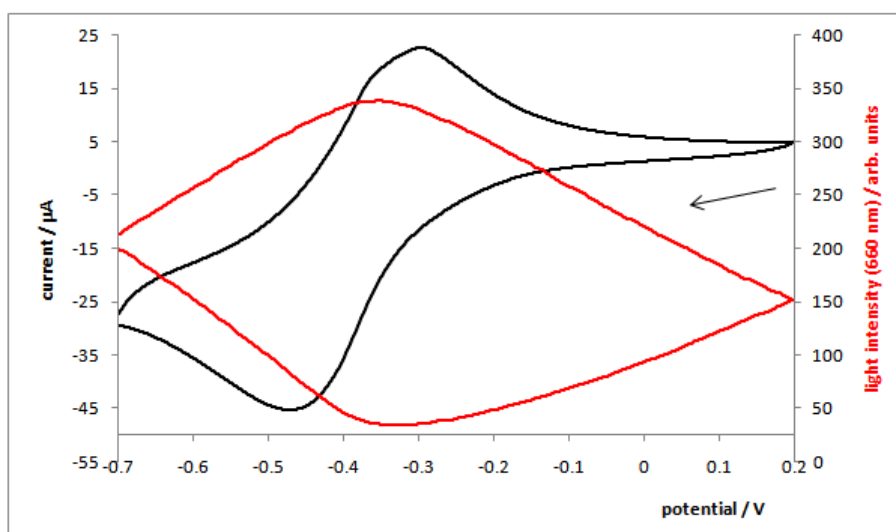
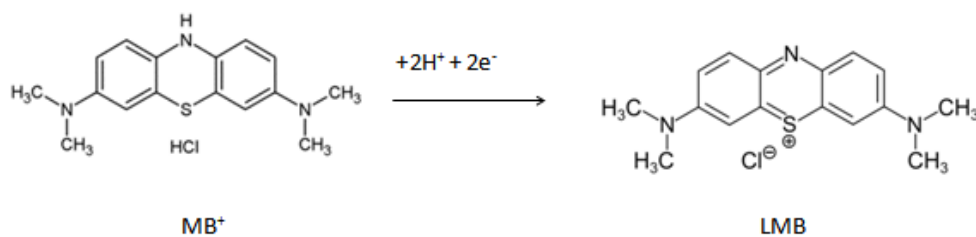


Figure 1. Absorptovoltammogram of MB^+ : Cyclovoltammogram (black) and light intensity (red) of MB^+ solution on a Pt-SPE. Scan rate: 0.01 V/s. The arrow shows the scan direction



Scheme 1. Two-electron reaction of MB⁺ to LMB

The light intensity at 660 nm (this is the absorption maximum of MB⁺) as a function of potential (red curve) shows a decreasing light intensity from 0.2 V to about -0.35 V (presence of MB⁺) and a strong increase in intensity on return to about -0.33 V. This is due to the re-oxidation of LMB to MB⁺.

However, with a Bi-SPE, additional anodic peaks between -0.3 V and -0.1 V can be observed as early as the first scan (see Figure 2, top, red). With each scan, the oxidation peak at -0.38 V decreases in intensity and a peak at about -0.24 V with a shoulder increases. The reduction peak at -0.48 V does not change, but two broad but weak peaks increase relative to the additional anodic peaks. As described by Svetlicic et al. [9,10], for platinum as a working electrode, the new anodic peaks are due to the adsorption of MB⁺ forming a film on the surface. The increase in current indicates that the film is

electrically conductive. This is also confirmed by Liu and Mu [11].

Applying a larger potential scan to about 1.2 V, another anodic peak at about 0.9 V occurs. This is shown in Figure 3, bottom.

In the following scans, another anodic peak at about 0.08 V and an additional reductive peak at about 0 V occur. When the potential range is extended to 0.9 V, two intense, broad anodic peaks are formed, which become even more intense in subsequent scans. These anodic peaks were first observed by Karyakin et al. [9]. The authors as well as Svetlicic et al. [9,10] and Liu and Mu [11] showed that these peaks are formed by the electropolymerization of MB⁺. The pairs of peaks at 0.08 V and 0 V are attributed to the oxidation and reduction of MB⁺ polymer. The polymerization occurs through the linkage of the terminal amino groups (see Scheme 2).

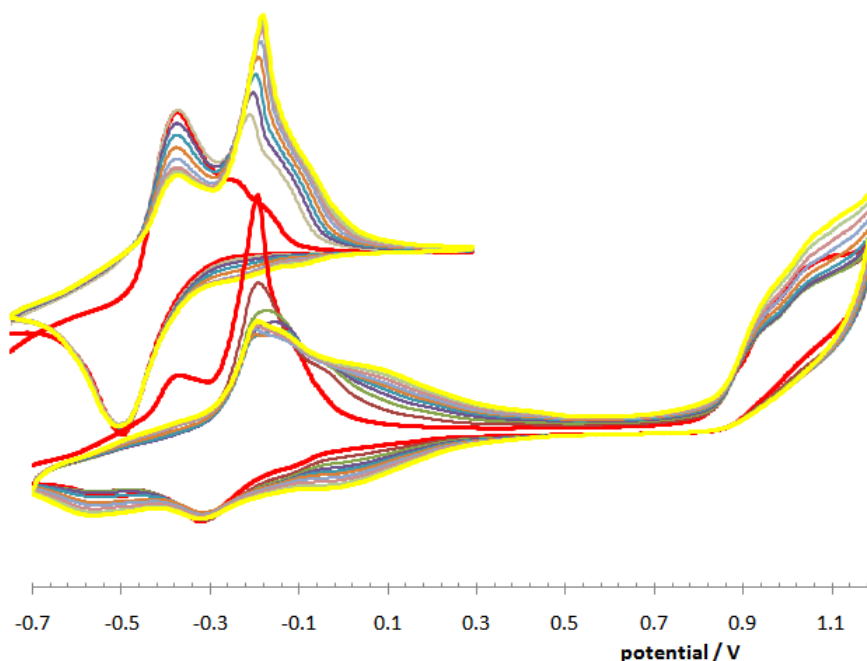
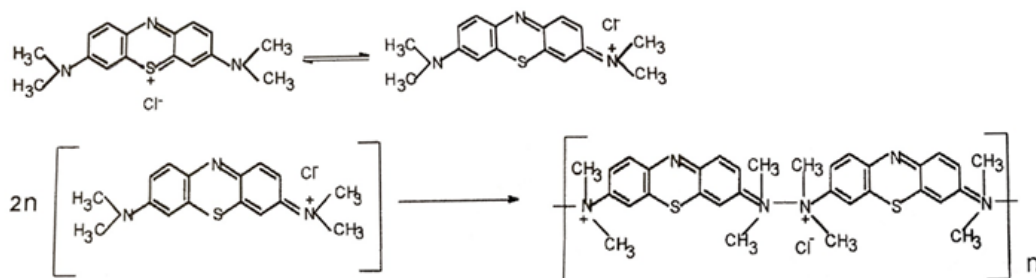


Figure 2. Top: Ten cyclic voltammograms of MB⁺ on a Bi-SPE in sequence between -0.7 V and 0.2 V. Bottom: Ten cyclic voltammograms of MB⁺ on a Bi-SPE in sequence between -0.7 V and 1.2 V. Red curve: first, yellow curve: last scan, respectively. Scan rate: 0.03 V/s



Scheme 2. Electropolymerization of MB⁺ (from [12])

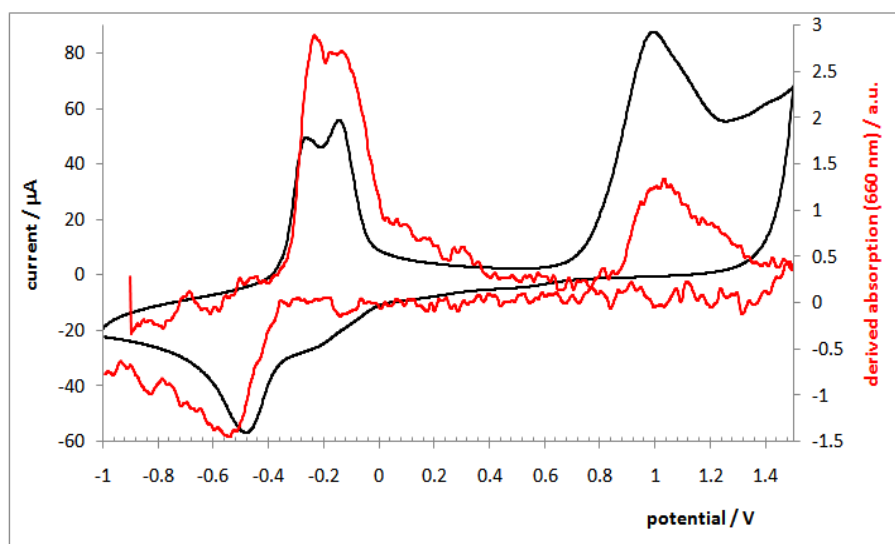


Figure 3. Derived absorptovoltammogram MB^+ on Pt. Black: CV, red: derived absorbance. Scan rate 0.01 V/s

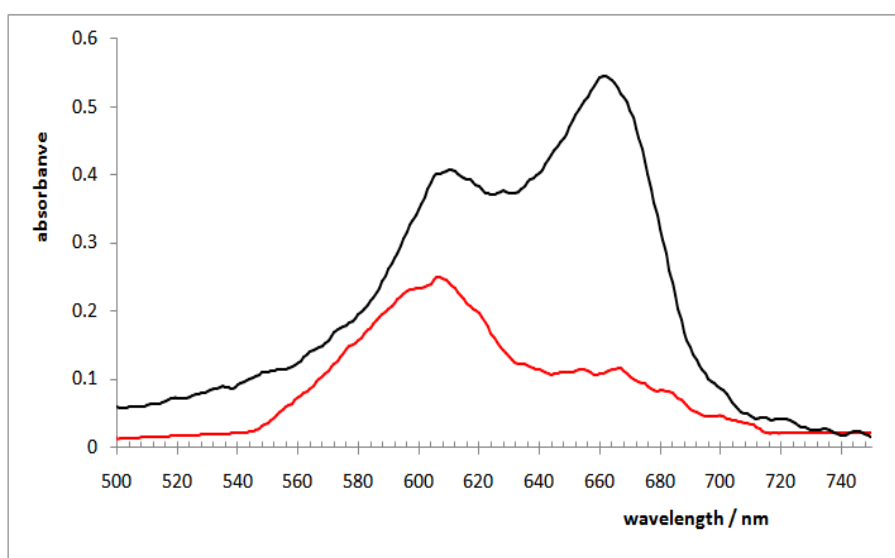


Figure 4. Absorption spectra of MB^+ : Black at 0 V, red at 0.9 V

Figure 3 shows the derived absorptovoltammogram from -1 V to 1.5 V. It is obvious that the CV correlates with the derived light intensity. The increase in the derived light intensities at 1 V means that the polymerized MB^+ must absorb 660 nm in the same way as the monomer itself (compare the light intensity at -0.2 V), but with a smaller cross-section than MB^+ itself. This is confirmed by the absorption spectra at -0.2 V (black curve) and at 0.9 V (red curve, see Figure 4). This is in agreement with Liu and Mu [8].

Table 1 gives an overview of the different processes.

Table 1. Different potential ranges and electrochemical and physicochemical processes

potential range	process
-0.7 V \leftrightarrow -0.3 V	Redox reaction $\text{MB}^+ \leftrightarrow \text{LMB}$
-0.3 V \leftrightarrow 0V	adsorption of MB^+
-0.15 V \leftrightarrow 0.3 V	Redox reaction of electropolymerised species
> 0,8 V	electropolymerization of MB^+

3.3. Raman Spectroscopy

Surface-enhanced Raman scattering (SERS) has already been described in detail by one of the authors [13]. Therefore, only a comparison of Raman intensities will be presented here. For this purpose, we compared homemade silver nanoparticles with commercial SERS substrates as well as with electrochemical activation. MB^+ was used as an analyte.

As an example, Figure 5 shows the change in Raman intensity of MB^+ during electrochemical activation of an Ag SPE before (black curve) and after electrochemical activation by potential scanning between -0.5 V and 0.5 V and vice versa (red curve).

Figure 6 shows electron micrographs of the Ag SPE used before and after electrochemical activation with a 0.1 molar KCl solution. The formation of nanoparticles in the range of 50-100 nm is clearly visible.

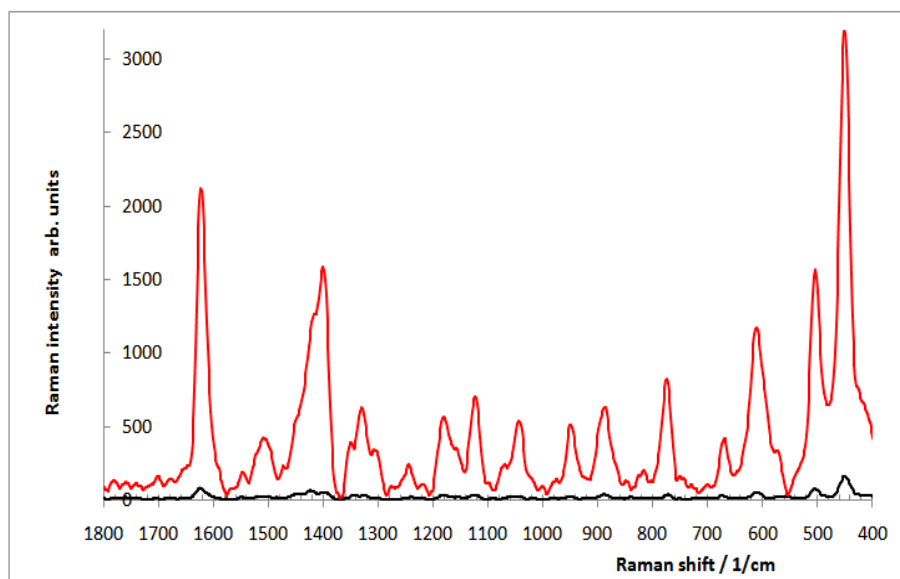


Figure 5. SERS effect due to a single CV cycle. Before (black) and after electrochemical activation of the Ag-SPE (red). Activation factor $I(\text{after}) / I(\text{before}) \approx 20$. Integration time was 1 s in both cases

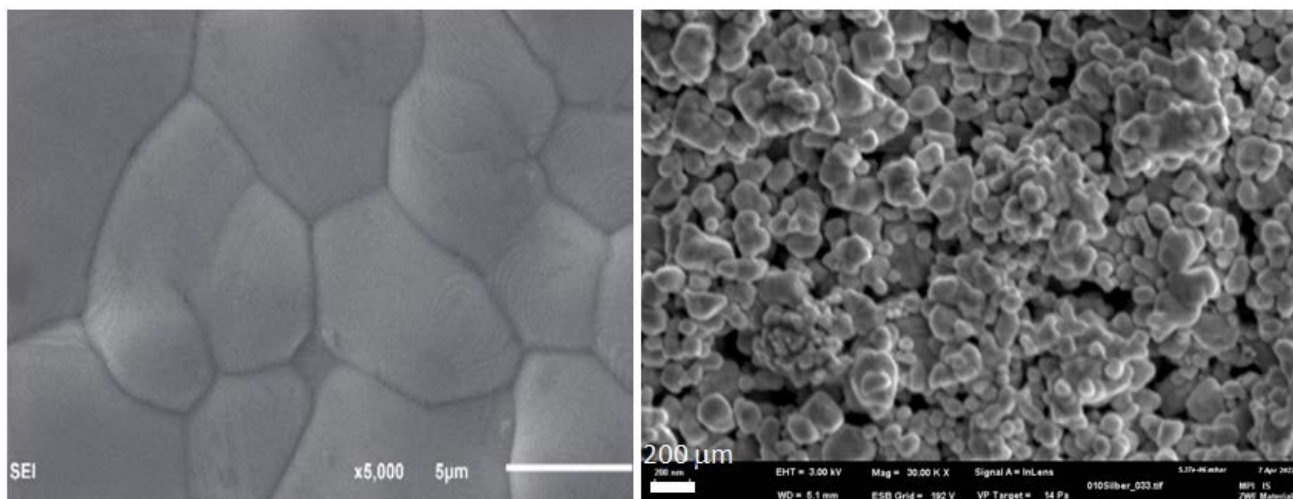


Figure 6. SEM image of an Ag SPE before (left) and after electrochemical activation (right). (Thanks to Nadine Schnabel, Institute for Physical Chemistry, University of Stuttgart)

Table 2 shows the comparison of the change in Raman intensities for different activations / SERS substrates compared to the electrochemical activation with a 0.1 molar KCl solution described above.

Table 2. Comparison of Raman intensities for different substrates/activations versus electrochemical activation for an Ag SPE

SERS substrate / activation	Rel. intensity (MB^+ , 1621 cm^{-1})
Electrochemical activation of an Ag SPE by scanning once from -0.5 V \rightarrow +0.5 V and back with a 0.1 molar KCl solution.	100 % (reference)
Etching of Ag-SPE with conc. HNO_3	17 %
Etching with conc. HNO_3 followed by electrochemical activation	200 %
RandaS (Mountain Photonics)	33 %
Sersitive (premium silver SERS substrate) (Institute of Physical Chemistry, Warsaw)	30 %
Silver P-SERS-substrate (Metrohm)	40 %
Self-prepared Ag nanoparticles and electrochemical activation	250 %

Figure 7 shows the difference in the Raman spectra of MB^+ (at 0 V) and LMB (at -0.6 V): The peaks down (top) show the decrease (increase) in Raman intensity when the voltage is switched from 0 V to -0.6 V. The results are in agreement with those of Nicolai et al. [11]. The numbers indicate the wavenumbers of the Raman transitions in cm^{-1} . The Raman transitions are characterized by Nicolai et al. [14], Xiu et al. [15] and Li et al. [16].

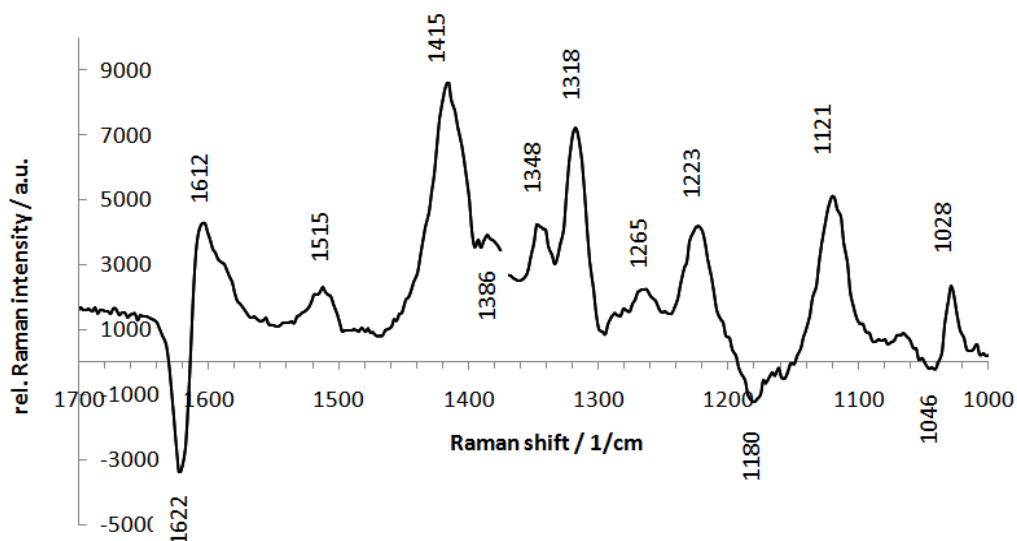


Figure 7. Difference between Raman spectra during the potential change from 0 V to -0.6 V. The Raman transitions are given in cm^{-1} . Integration times in each case 1s

Table 3 shows the assignment of the different Raman wave numbers of MB^+ and LMB by different authors.

Table 3. Assignment of the different Raman wave numbers of MB^+ and LMB [11,12,13] (ν : symmetrical stretching, β : in-plane bending)

Raman transition $\text{MB}^+ / \text{cm}^{-1}$	Raman transition LMB / cm^{-1}	Assignment after [14]	Assignment after [15]	Assignment after [16]
1622		$\nu(\text{CC})\text{ring} + \nu(\text{CNC})\text{ring}$	$\nu(\text{CC})\text{ring}$	$\nu(\text{CC})\text{ring}$
	1612	$\nu(\text{CC})\text{ring} + \nu(\text{CNC})\text{ring}$		
	1515	$\nu(\text{CC})\text{ring}$	$\nu_{\text{asym}}(\text{CC})$	
	1415	$\nu(\text{CC})\text{ring}$	$\nu_{\text{asym}}(\text{CN})$	
	1386	$\nu(\text{CC})\text{ring} + \nu(\text{CNC})\text{ring}$		$\nu(\text{CN})$
	1348			
	1318			In-plane ring deformation of CH
	1265			
	1223			
1180			$\nu(\text{CN})$	$\nu(\text{CN})$
	1121		$\nu(\text{CH})$	
1146				$\beta(\text{CH})$
	1028		$\beta(\text{CN})$	$\beta(\text{CH})$

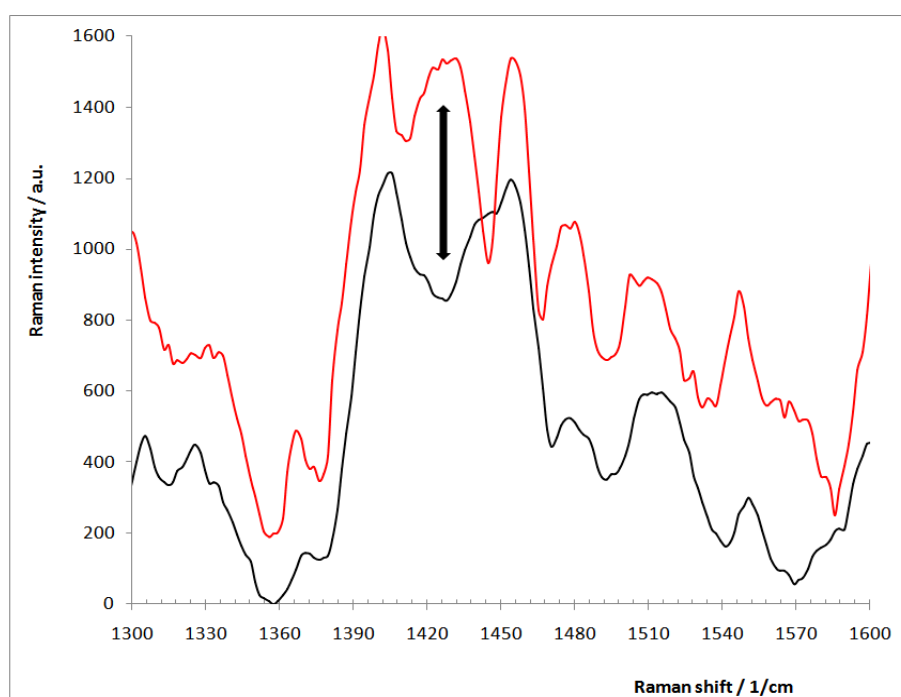


Figure 8. Raman spectrum (detail) at 0 V (black) and at 1.1 V (red). Formation of a new Raman band at 1429 cm^{-1} . Integration times 1 s each

According to Scheme 1, the linkage of the MB⁺ monomers via the terminal amino groups takes place during electropolymerization, and a new RR'-N--N-R'R bond is formed, which is at 1429 cm⁻¹ according to Liu and Mu [11]. A comparison of the Raman spectra at 1.1 V (red curve) and at 0 V (black curve) confirms this (Figure 8).

4. Summary

With the treatment of these new methods of spectroelectrochemical analysis, the existing study regulations of the Ludwigsburg University of Education have been supplemented in terms of content. For this purpose, students should acquire theoretical knowledge of these spectroelectrochemical methods in the Bachelor's phase in the lectures Physical Chemistry 1 and 2 and practical knowledge in the practical course of Physical Chemistry. In addition, a teaching module on spectroelectrochemistry (absorption and Raman voltammetry) for determining the detection limits of selected organic and inorganic compounds was developed for the Master's phase in the seminar practical course in analytical chemistry. The first step for this was preliminary work by spectroelectrochemical investigations of methylene blue. Reflecting on the practical work, advantages and disadvantages of the individual experiments can be discussed in terms of their comprehension problems and how spectroelectrochemistry can facilitate the understanding of electrode reactions. The assumption is that by following the electrode processes spectroscopically, not only the motivational aspects are conducive to learning, but also the "visibility" of the electrode processes contributes to better understanding. With the electrochemical activation of Raman scattering (EC-SERS), a significant increase in the detection limits is achieved.

Evaluation criteria for the performed experiments are

- Aesthetics and difficulty of the experiments,
- Difficulties in performing the experiments,
- Problems in understanding the corresponding theories,
- Understanding of the electrochemical processes taking place.

We expect

- an improvement in the understanding of electrochemical processes through the correlation between the change in current flow and absorption or the change in Raman transitions,
- an increase in motivation through the multidimensional measurements of redox reactions.

References

- [1] <https://de.wikipedia.org/wiki/Methylenblau> (8.9.22).
- [2] <https://de.wikipedia.org/wiki/Neuroleptikum> (8.9.22).

- [3] L.C. Lee, D. Meisel, J. Phys. Chem. 1982, 86, 3391.
- [4] P.J. Garnett, D.F. Treagust D.F., J. Res. in Sci. Teach. 1992, 29, 2, 121.
- [5] L. L. Combs, J. Chem. Educ, 1993, 70, 462-464
- [6] J. Parkinson, D. Hendley, H. Tanner, A. Stables, *Pupils' Attitudes to Science in Key Stage 3 of the National Curriculum: A Study of Pupils in South Wales*, 1998, 16, 165.
- [7] I. Bellou, N.M. Papachristos, T.A. Mikropoulos, 2018, *Digital Learning Technologies in Chemistry Education: A Review*. In: D. Sampson, D., Ifenthaler, J.M. Spector (eds.) (2018): *Digital Technologies: Sustainable Innovations for Improving Teaching and Learning*, Springer.
- [8] B. Dalgarno, M.J.W. Lee M.J.W., British J. Educ. Tech. 2010, 41, 1, 10-32
- [9] V. Svetlicic, V. Zutic, J. Clavilier, J. Chevalet, J. Electroanal. Chem. 1985, 195, 307.
- [10] V. Svetlicic, V. Zutic, J. Clavilier, J. Chevalet, J. Electroanal. Chem. 1987, 219, 183.
- [11] J. Liu, S. Mu, Synth. Metals, 1999, 107, 159.
- [12] A.A. Karyakin, A.K. Strakhova, E.E. Karyakina, S.D. Varfolomeyev, A.K. Yatsimirsky, Bioelectrochem. and Bioenerget. 1993, 32, 35.
- [13] A. Habekost, Nachr. aus der Chemie 2022, 70 (3), 19.
- [14] S.H. de Araujo Nicolai, P.R.P. Rodrigues, S.M.L. Agostinho, J.C. Rubim, J. Electroanal. Chem. 2002, 527, 103.
- [15] G-N. Xiao, S-Q. Man, Chem. Phys. Lett. 2007, 447, 305.
- [16] C. Li, Y. Huang, K. Lai, B.A. Rasco, Y. Fan, Food Control 2016, 65, 99.
- [17] T. Engel, P. Reid, 2006, *Physikalische Chemie*. Munich: Pearson Studium.
- [18] R. Holze, 2001, *Elektrochemisches Praktikum*. Stuttgart: B.G. Teubner.
- [19] C. Li, Y. Huang, K. Lai, K., B. Rasco, Y. Fan, Y. (2016). *Analysis of trace methylene blue in fish muscles using ultra-sensitive surface-enhanced Raman spectroscopy*. Food Control, pp. 99-105.
- [20] S. Roy, M. Ghosh, J. Chowdhur (2015). *Adsorptive parameters and influence of hot geometries on the SER(R)S spectra of methylene blue molecules adsorbed on gold nanocolloidal particles*. Journal of Raman Spectroscopy, pp. 451-461.
- [21] W. Walter, F. Wittko, 2004, *Lehrbuch der organischen Chemie* (24th edition). Stuttgart: S. Hirzel Verlag.
- [22] G-N. Xiao, S-Q. Man, Chem. Phys. Lett. 2007, 447, 305.
- [23] A.J. Bard, L.R. Faulkner (2001). *Electrochemical Methods* (2nd Edition), John Wiley, Hoboken, NJ.
- [24] R.G. Compton, C.E. Banks (2011). *Understanding Voltammetry* (2nd Edition), Imperial College Press, London.
- [25] T. Fox (2016). *Infrarot- und Raman-Spektren*. In S. Bienz, L. Bigler, T. Fox, & H. Meier (2016). *Spektroskopische Methoden in der organischen Chemie* (9th edition, pp. 37-74). Stuttgart: Thieme.
- [26] E. Gileadi (2013). *Physical Electrochemistry*, Wiley-VCH, Weinheim.
- [27] W. Schmickler (1996). *Grundlagen der Elektrochemie*, Friedr. Vieweg & Sohn Verlagsgesellschaft, Braunschweig.
- [28] E. Smith, G. Dent (2019). *Modern Raman Spectroscopy: A Practical Approach*, Wiley.
- [29] P. Vandabeele (2013). *Practical Raman Spectroscopy: An Introduction*, Wiley, Weinheim.
- [30] W. Vielstich, W. Schmickler (1976). *Elektrochemie II: Kinetik elektrochemischer Systeme*, Steinkopff Verlag, Darmstadt.

Supporting Information

Script "Spectroelectrochemical investigation of methylene blue"

S1. Introduction and Didactic Background

Spectroelectrochemistry is two-dimensional or hybrid spectroscopy that combines spectroscopic and electrochemical measurement methods. It allows a detailed study of redox processes at electrodes.

In the following experiments, the electrochemically induced decoloration of methylene blue by reduction to leucomethylene blue is studied using spectroelectrochemical methods. This is done on the one hand by absorptovoltammetric measurement, i.e., the simultaneous investigation of the redox process by absorption spectroscopy and cyclic voltammetry, and on the other hand by ramanvoltammetric measurement, in which the Raman spectra of methylene blue and leucomethylene blue are compared.

The didactic background of the experiments consists in the assumption that redox reactions at electrodes are greatly facilitated by multidimensional spectroelectrochemical methods, i.e., by supplementing electrochemical information, such as potentials and current fluxes, with spectroscopic methods to identify electrode reactions.

S1.1. Cyclic Voltammetric

Cyclic voltammetry is the "spectroscopy of electrochemistry". In this method, an electrode potential is applied to an electrode containing the analyte under investigation; this potential changes in time between a predetermined potential limit at a characteristic scan rate. From the reversal point, the potential change proceeds in the reverse direction back to the starting point. As a result of the potential change, a redox process takes place at the analyte in which electrons are released to or accepted from the electrode. This process is represented in a cyclic voltammogram by the formation of a peak. Positive (negative) peaks correspond to oxidations (reductions). In case of a reversible reaction, the potential difference between the oxidation and reduction peaks is 59 mV (for a one-electron transfer).

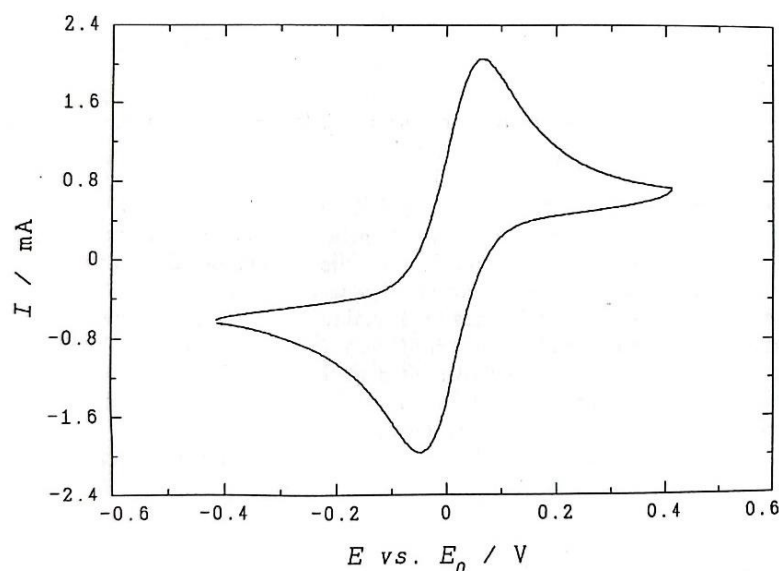


Figure S1. Typical course of a CV. Recorded in the redox system $\text{Fe}^{2+}/\text{Fe}^{3+}$ with a potential sweep rate of 0.05 V/s. [18]

A three-electrode arrangement consisting of a working electrode, a counter electrode and a reference electrode is used to measure a cyclic voltammogram. The actual redox reaction takes place at the working electrode. A potentiostat prevents the potential from changing due to the currents flowing through the redox reaction by means of rapid readjustment. The reference electrode is a 2nd type electrode.

Figure S1 shows the cyclic voltammogram of a reversible reaction. Based on this example, the electrode processes in the course of a cyclic voltammogram will be explained as follows. For the following description, it is assumed that the substance to be investigated is initially in its reduced form. The starting point of the measurement is therefore at -0.4 V. For a substance in oxidized form, the starting point would be at 0.4 V. At the start time of the measurement, a cathodic (negative) current initially flows, i.e., electrons are predominantly released from the electrode into the redox system. The reduction reaction predominates. This process initially continues with increasing positive potential. Since the number of

reduced particles at the electrode decreases with further potential passage, the cathodic current also decreases increasingly. From about - 0.07 V, the current flow approaches 0 mA. Thereafter, an anodic current predominates, i.e., the system now predominantly donates electrons to the electrode and the substance is increasingly oxidized. Since the proportion of reduced particles at the electrode is very high at this point, the oxidation reaction initially proceeds very rapidly and the current increases sharply. At about 0.06 V, the current curve passes through a maximum and a peak is formed. At this point, the reduced species at the electrode is consumed. The further oxidation reaction now depends only on mass transport (diffusion) of the reduced species to the electrode (there is no stirring during the uptake of the CV). Since diffusion is a slow process, the reaction now proceeds only slowly. Therefore, the current flow also decreases more and more. At 0.4 V the reversal point of the measurement is reached. From here on, the potential runs back to the starting point. In the case of the reversible reaction described here, the reduction process now takes place analogously to the oxidation process. In the case of an irreversible reaction, no second peak would form in the return path.

The course of a cyclic voltammogram can be influenced not only by the redox processes themselves, but also by other factors, such as adsorption of the analyte on the electrode or redox processes of the solvent (especially at high negative and positive potentials) or by the electrode material itself.

S1.2 Absorption Spectroscopy

Absorption spectroscopy works with electromagnetic radiation - often in the wavelength range of light visible to humans between 380 nm and 800 nm. Radiation in this wavelength range is able to resonate with the valence electrons or delocalized electrons of an atom or molecule. In this process, the electron absorbs radiation of a specific wavelength from the radiation that is specific to the molecule and is thereby transferred from the ground state S_0 (singlet state) to a state of higher energy S_1 . The electron is excited. Figure S2 shows the excitation of an electron to a higher energy state.

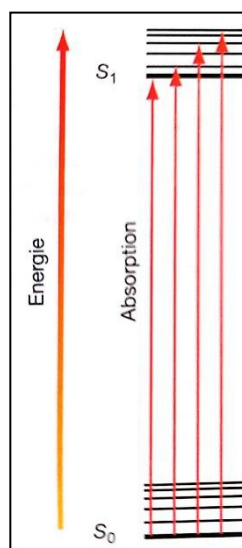


Figure S2. Excitation of a valence electron [17]

The figure also shows vibration levels. These are caused by the movement of atoms or molecule groups within a molecule.

In this experiment, absorption spectroscopy is measured in reflection, i.e., the excitation light is directed onto the sample and reflected from the surface under investigation. In the process, part of the light is absorbed by the sample. This reflection is detected by an optical detector. If the analyte decolorizes as a result of the reaction, the intensity of the reflected light increases and less light is absorbed by the sample. Therefore, absorption is measured indirectly via the change in light absorption as a result of an electrochemical redox process.

S1.3. Raman Spectroscopy

Raman spectroscopy is a method of vibrational spectroscopy. It measures the vibrations of individual bonds within a molecule and can thus provide information about the structure of molecules.

When a molecule is irradiated with an intense laser, a small fraction of the photons brings the molecules into a so-called virtual vibrational state (in resonant Raman spectroscopy, a real energy level is occupied). The subsequent relaxation of this state occurs by scattering in all spatial directions. A small part of this scattered radiation has different frequencies than the incident radiation. This scattered light is called Raman scattering and is produced by the vibration of individual bonds in the molecule. Raman spectroscopy detects this Raman scattering (Usually only the long wavelength Stokes shift, the anti-Stokes shift is even weaker in intensity). Certain vibrations cause Raman scattering with a specific wavenumber ($\nu^* = 1/\lambda$ in cm^{-1} , with λ : wavelength). This allows the identification of molecules by their vibrational states. (Electro-)chemical reactions can be followed by changing these vibrational bands.

The vibrations of a molecule are diverse: for example, molecular groups can perform valence vibrations and deformation vibrations. These vibrations can be symmetric or asymmetric. There can also be various deformation vibrations with respect to the plane of the molecule, as well as vibrations that arise from coupling effects of individual vibrations and involve multiple bonds within the molecule, known as framework vibrations. Cyclic molecules also show so-called ring vibrations, which result from the coupling of individual valence vibrations within the ring.

Since only a very small fraction of the photons of the incident laser beam are scattered, and an even smaller fraction of this scattered light is due to Raman scattering (as inelastic scattering), the intensities measured in Raman spectroscopy are usually very low. To increase the Raman intensities, the surface-enhanced Raman effect (SERS effect) is used in this experiment. By using a surface that is as rough as possible and by adsorbing the analyte on this surface, the intensity of Raman scattering can be increased by several orders of magnitude. This is done by scanning the gold electrode used through several cyclic voltammograms with the analyte in an aqueous chloride solution. This causes nanostructuring of the surface and a better absorption of the analyte. Therefore, at the end of this activation the surface corrugation is increased and gold nanoparticles are present on the surface onto the methylene blue is adsorbed.

S1.4. Methylene Blue

In this lab script, the molecule methylene blue is studied. Methylene blue is a dye that exhibits an intense blue color in aqueous solution. The color is due to the large delocalized π -electrode system, which consists of three aromatic rings. Reduction of methylene blue gives the colorless leucomethylene blue by interrupting the π -system (see Figure S3). The reaction is reversible.

In the absorptovoltammetric experiment, methylene blue is electrochemically reduced at the electrode and then re-oxidized. The resulting decolorization and subsequent coloration is measured spectroscopically.

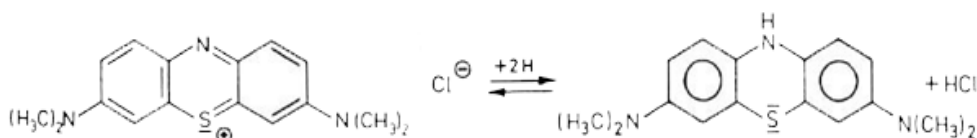


Figure S3. Reduction von Methylene blue to form colorless Leukomethylene blue [21]

S2. Experiments

S2.1. Absorptovoltammetry

Task: Perform an absorptovoltammetric measurement on methylene blue. Then convert the measured cyclic voltammogram and the light intensity into an absorptovoltammogram. Explain the processes at the electrode with respect to the cyclic voltammogram.

Materials: Au-BT-SPE, potentiostat, spectrometer, bifocal light guide, reflection cell, computer with DropView and Avasoft 8 software, 660 nm LED, 5 mL syringe.

Chemicals: aqueous methylene blue solution in ethanol / water ($c = 0.625$ mmol/L).

Experimental setup:

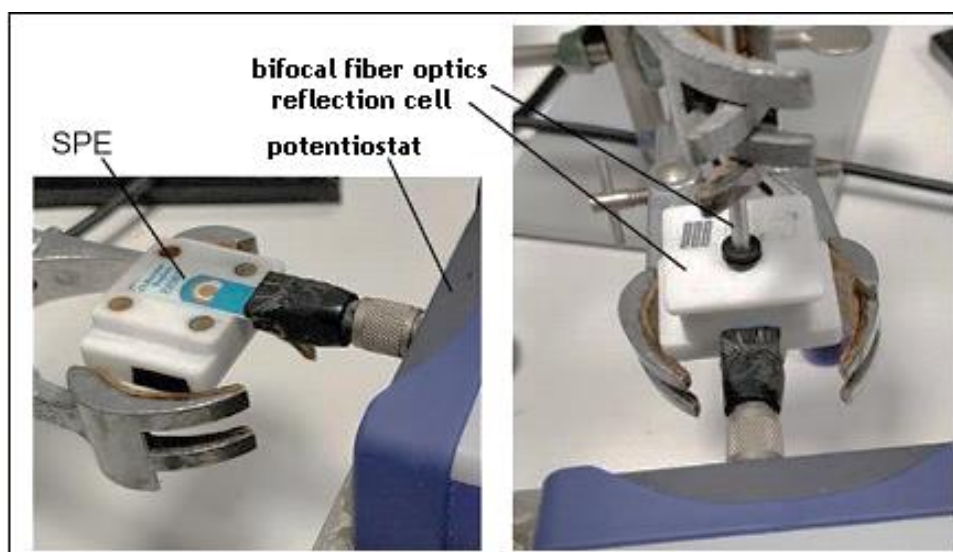


Figure S4. Experimental setup of the absorptovoltammetric measurement in reflection

Experimental procedure: Connect the Au-BT electrode (SPE) with the potentiostat, place the SPE in the lower part of the reflection cell and set the upper part as shown in the experimental setup.

Connect the two ends of the bifocal light guide to the spectrometer and the LED. Now, using the Eppendorf pipette, dispense 60 μL of the methylene blue solution through the upper opening of the reflection cell onto the electrode. The electrode should still be clearly visible through the solution afterwards. Then insert the third end of the light guide into the opening of the reflection cell as shown in the experimental setup. Now start the two measuring programs. The DropView program measures the cyclic voltammogram. Set this via "Nodes" to the electrochemical method "Cyclic Voltammetry". The start and end point of the measurement is set to 0.5 V, the reversal point to -1 V. The potential sweep rate is set to 0.01 V/s. The AvaSoft 8 program measures the light intensity. To do this, first turn on the LED and run the AutoConfigure function of the program. The light from the LED is now detected by the spectrometer and should be visible by a peak in the spectrometer at 660 nm. Now stop the measurement in AvaSoft 8 and run the Dark function of the program. The measured spectrum is now set to zero so that changes due to the reduction can be detected. Now start the measurement in the DropView program. As soon as the measurement starts, restart the measurement in AvaSoft. After the measurement save the values in an Excel spreadsheet. Clean the electrode and the reflection cell with ethanol.

Evaluation: The evaluation of the results is done with an Excel file. The oxidation and reduction peaks in the cyclic voltammogram are correlated with the change in light intensity. The shape of the cyclic voltammogram can be plotted analogously to the cyclic voltammogram described in section 1.1, comparing the processes at the electrode with the data from the spectroscopic measurement.

Note: Since the measurement does not measure the absorption directly, but only the change in reflected light, an increase in light intensity during the measurement means a decrease in the light absorption of methylene blue, since less radiation is absorbed by the sample.

S2.2. Ramanvoltammetry

Task: Perform a Raman voltammetry measurement on methylene blue (0 V) and leucomethylene blue (-0.6 V). Then evaluate the spectra obtained by assigning the measured bands using the literature values from Table S1. Describe the changes that occur when methylene blue is reduced to leucomethylene blue.

Materials: Raman spectrometer (Avantes) with Raman probe (Inphotonics) and optical fiber (Metrohm/DropSens), potentiostat (μStat 400 Metrohm/DropSens), electrochemically activated Au-BT SPE, SPE electrode connector (Metrohm/DropSens).

Chemicals: Aqueous chloride-containing methylene blue solution ($c = 0.625 \text{ mmol/L}$), dist. Water.

Experimental setup:

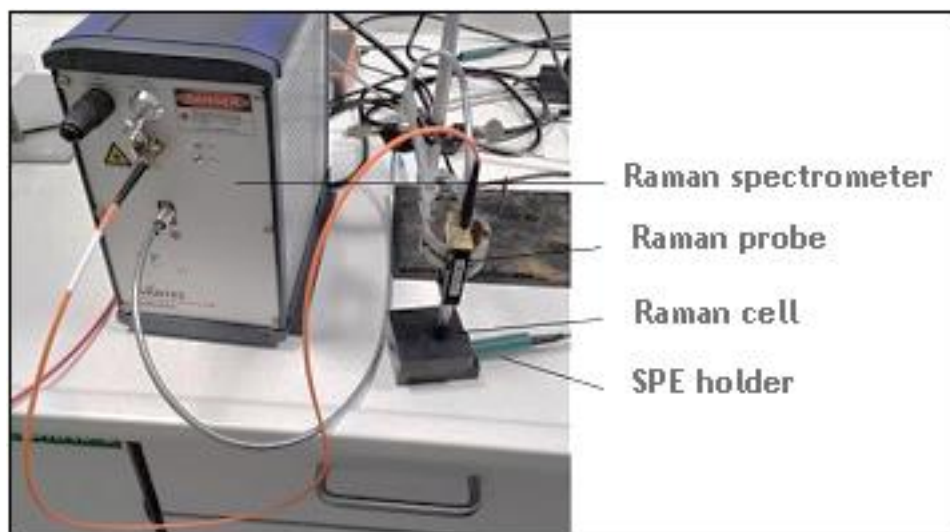


Figure S5. Experimental setup Raman spectroscopy

Experimental procedure: Place the Au-BT electrode in the Raman cell and connect it to the potentiostat via the SPE connector. Now add 60 μL of the methylene blue solution to the electrode using the Eppendorf pipette and insert the Raman probe into the hole of the Raman cell. In AvaSoft 8, change the measurement method to Ramanscope. Now execute the AutoConfigure function. Via "Start" you can now perform a single measurement of the Raman spectrum of methylene blue under "Single Measurement". Select 10 s as the integration time. Then set a potential of -0.6 V at the electrode via DropView. After approximately 60 seconds, perform the second Raman measurement. This is now the Raman spectrum of the leucomethylene blue. Then clean the electrode with distilled water.

Evaluation: Transfer the two Raman spectra into an Excel file. The file now shows you the two Raman spectra of methylene blue and leucomethylene blue as well as the wave numbers of the corresponding peaks. Assign individual characteristic bands of the spectrum of methylene blue to the respective vibrations by comparing the values with the

literature values from Table S1. A deviation of $\pm 10 \text{ cm}^{-1}$ (resolution of the Raman spectrometer) is possible. Compare the two spectra of methylene blue and leucomethylene blue. Look for a shift in bands or a change in intensity. Explain at which bands changes occur and discuss this with the structural differences of leucomethylene blue and methylene blue (see Figure S3).

Table S1. Raman wavenumber assignments of methylene blue. α = ring or framework vibration; β = deformation vibration; ν = valence vibration (Adapted from: [19,20,22])

Raman wavenumber (cm^{-1})	assignments	note	Raman wavenumber (cm^{-1})	assignments	note
445	$\alpha(\text{C-N-C})$	substituted $\text{N}-(\text{CH}_3)_2$ group	1219	$\nu(\text{C-N})$	
498	$\alpha(\text{C-N-C})$	substituted $\text{N}-(\text{CH}_3)_2$ group	1248	$\beta(\text{C-H})$	
595	$\alpha(\text{C-S-C})$		1306	$\nu(\text{C-N})$	C-N-ring vibration
669	$\alpha(\text{C-C-C})$	ring vibration	1318	$\nu(\text{C-N})$	substituted $\text{N}-(\text{CH}_3)_2$ group
772	$\beta(\text{C-H})$		1394	$\nu(\text{C-N})$	C-N-ring
882	$\alpha(\text{C-C-C})$	ring vibration	1436	$\nu(\text{C-N})$	
948	$\beta(\text{C-H})$		1493	$\beta(\text{C-H})$	
1035	$\nu(\text{C-S})$		1623	$\alpha(\text{C-C})$	ring vibration
1121	$\beta(\text{C-H})$		1248	$\beta(\text{C-H})$	
1184	$\nu(\text{C-N})$				



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