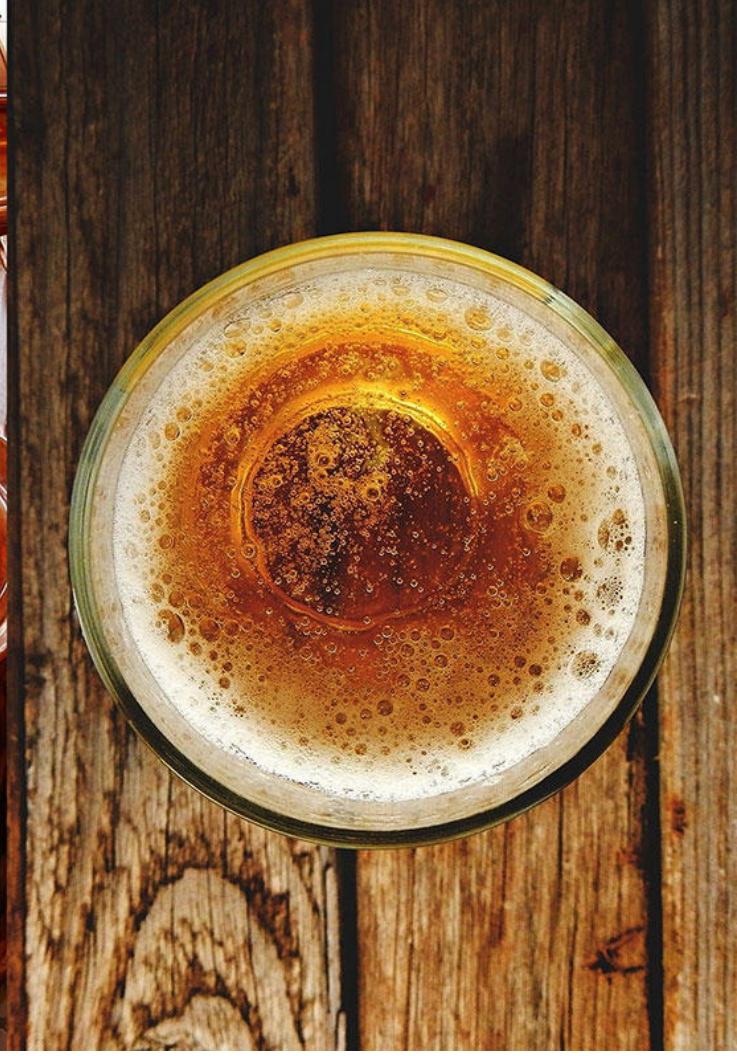




Application Note AN-EC-035

Using a portable standalone system for easy fermentation monitoring

In-situ measurements of lactic acid in beer using screen-printed electrodes (SPEs)

Lactic acid (2-hydroxypropanoic acid) is primarily found in sour dairy products. In beer, the presence of lactic acid contributes to its acidity and lactic aromas, and usually appears as the result of fermentation with lactic acid bacteria. These bacteria may be purposely introduced to the wort, or they can also appear as a result of an infection.

Although lactic acid is essential in beer up to a certain amount, in excess it is considered a defect. Its perception threshold is 0.0044 mol/L. However, in some lambic-type beers, the lactic acid concentration can reach levels higher than 0.0333 mol/L.

In this Application Note, an enzymatic sensor based on a screen-printed electrode is used to measure lactic acid in commercial beers as a proof of concept for its potential application during fermentation monitoring.

INSTRUMENTATION AND SOFTWARE

Measurements were performed using DROPSTATPLUS and CASTDIR connectors for screen-printed electrodes (Figure 1).

L-lactate oxidase-based screen-printed electrodes (LACT10) were used as the biosensing substrate because of their selectivity properties. The analytical signal corresponds to the detection of a hydrogen peroxide intermediate. This byproduct is produced while converting lactate to pyruvic acid due to the enzymatic reaction that occurs on the working electrode.

DROPSTATPLUS is a custom potentiostat-based electrochemical reader that is configured according to the specific needs of each user. By specifying the electrochemical technique and its parameters as well as the calibration curve, it is possible for a single instrument to automatically display the concentration of the analyte for which the electrochemical sensor has been developed directly on an LCD screen. All hardware used for this study is compiled in Table 1.



Figure 1. The DROPSTATPLUS instrument and CASTDIR used to connect screen-printed electrodes.

Table 1. Hardware and software equipment overview.

Equipment	Article number
Instrument	DROPSTATPLUS
Biosensor SPE	LACT10
Connection for SPEs	CASTDIR

DETECTION OF LACTIC ACID

Amperometric detection was selected as the analytical technique in this case. By applying a potential of -0.1 V, it is possible to measure lactic acid in less than 75 s as current signal reaches its steady state. Employing this particularly low potential is possible due to the mediator, and it is crucial to overcome typical interfering signals that appear when applying higher potentials [1].

By simply placing a drop of sample on the LACT10 SPE, it is possible to measure lactic acid in the range of 0 to 0.0004 mol/L. A typical calibration curve obtained with triplicate data is shown in Figure 2. The results were obtained in an aqueous solution of 0.1 mol/L Tris-NO_3 at pH 7.2 where lactic acid is found only in its dissociated form—that is, as lactate, not as lactic acid.

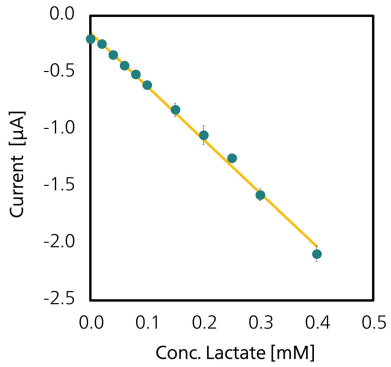


Figure 2. A typical calibration curve obtained with standard solutions of lactate between 0–0.0004 mol/L in an aqueous solution of 0.1 mol/L Tris-nitrate (pH 7.2) using LACT10 electrodes. Measuring time was 75 s.

MEASURING LACTIC ACID IN COMMERCIAL BEER

Four different types of beers from a local brewery (Cotoya) were selected to test the LACT10 sensors with real samples. These include an India Pale Ale (IPA) in which the lactic acid content is considered at normal levels, a sour beer made with an acidulated malt specially treated to acidify the beer, a barley wine beer with a high alcohol content, and a lambic beer (Al Debalu) obtained via a wild fermentation process.

To avoid matrix effects, a dilution of 1:10 was performed with each beer using 0.1 mol/L Tris-NO_3 pH 7.2 aqueous solution before measurement. It is not necessary to degas the samples since the obtained results were very similar with and without degassing the beer.

To validate the obtained data, a commercial L-Lactate Assay kit was used. Optical experiments were done with SPELEC equipment and the DropView SPELEC software. Different sample dilution was necessary with the optical kit to avoid matrix effects with each beer style. The sour beer sample was diluted 1:100, IPA 1:20, Al Debalu (lambic) beer 1:50, and barley wine was diluted 1:10.

Results obtained with both methods, electrochemical and optical, are presented in Figure 3.

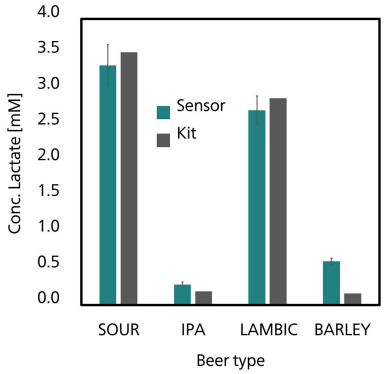


Figure 3. Data comparison obtained with the electrochemical lactate sensor from Metrohm DropSens (in green) and with an optical L-Lactate Assay kit (in grey).

Note that barley wine could not be measured correctly with the optical assay kit due to its dark brown color that interfered with the kit's measurement wavelength (450 nm). There is a compromise between the absorption of beer and the low lactate concentration it has. Therefore, there is not enough sensitivity to measure it. This can be overcome by putting the beer through a treatment with PVPP (polyvinylpolypyrrolidone) which removes the color and thus prevents it from absorbing.
Considering that data obtained with both methods are quite similar, this protocol demonstrates the possibility to detect lactate in beer in less than 75 s with a portable device without the requirements of complex pretreatments or the use of bulky optical devices.

CONCLUSION

In this study, an electrochemical biosensor for the detection of lactic acid in beer is proposed. The sample with the electrochemical method proposed only needs to be diluted 1:10 with a solution of 0.1 mol/L Tris-NO_3 at pH 7.2. Using a simple amperometric assay, it is possible in 75 s to measure the amount of lactic acid in a beer sample without any pretreatment. Measurements obtained are similar to those taken with a traditional L-Lactate Assay kit and non-portable optical devices.

REFERENCE

1. Biscay, J.; Rama, E. C.; García, M. B. G.; et al. Enzymatic Sensor Using Mediator-Screen-Printed Carbon Electrodes. *Electroanalysis* **2011**, *23* (1), 209–214. DOI:10.1002/elan.201000471

RELATED APPLICATION NOTES

AN-T-227 Determination of sodium lactate
AN-PAN-1057 Inline monitoring of fermentation processes
AN-NIR-093 Quality Control of fermentation processes

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CONFIGURATION



Standalone electrochemical reader customized for your final application

DropStat Plus is a customized electrochemical reader intended for those researchers who have successfully developed an electrochemical sensor to get immediate results based on an optimized procedure. With a touch screen, li-ion battery and the possibility of wirelessly transfer your data, it can store up to 3 methods and up to 8 calibrations per method. Ideal to be customized as an OEM or private labelled device.



µStat cable connector for screen-printed electrodes

Connects single (1 WE) SPEs to µStat 200, µStat 300, µStat 400, µStat 4000/P, µStat 8000/P, µStatECL, SpectroECL and the SPELEC line of instruments.



Lactate sensor

Suitable for the determination of L(+)-Lactate determination in liquid samples.