



Application Note AN-EC-035

## 采用便携式独立系统实现简便发酵监测

### 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.

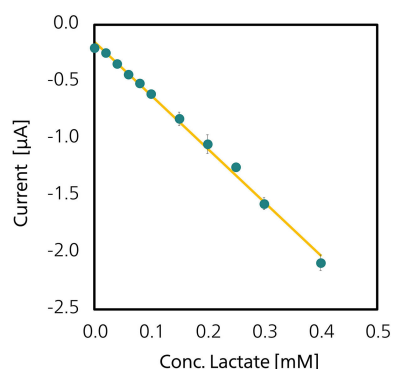
**Table 1.** Hardware and software equipment overview.

| 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  $-0.1\text{ V}$ , 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- $\text{NO}_3$  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

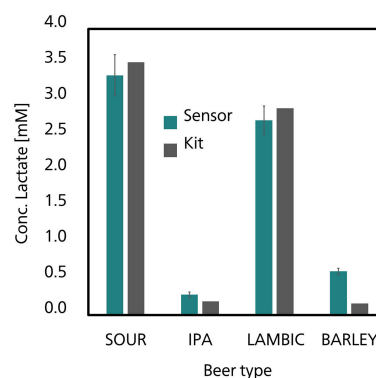
Four different types of beers from a local brewery (Cotoya) were selected to test the LACT10 sensors with real samples. These include an India Pale Ale (IPA) in which the lactic acid content is considered at normal levels, a sour beer made with an acidulated malt specially treated to acidify the beer, 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<sub>3</sub> 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**.

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



**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).

(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<sub>3</sub> 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



DropStat Plus 是一款定制化电化学读数器,旨在为那些成功开发出电化学传感器的研究人员提供优化流程后的实时结果。借助其触摸屏、锂离子电池,以及无线传输数据的能力,其可以保存高达 3 种方法以及每种方法 8 个校准值。其是 OEM 或私有贴标设备进行定制。



### μStat

可以连接单股(1 WE)SPEs 与 μStat 200、μStat 300、μStat 400、μStat 4000/P、μStat 8000/P、μStatECL、SpectroECL 和 SPELEC 仪器系列。



适用于液样中 L(+) 乳酸的测定。