Validation of non-invasive measurement of Total Package Oxygen

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INTRODUCTION

It is widely accepted that oxygen can break or make a wine. Oxygen management is therefore a critical aspect in the winemaking process from harvest to post-bottling. This is quite a challenging task as a good oxygen management strategy requires a good understanding of the specific needs of specific wines at specific time throughout the whole process.

For instance, oxygen exposures during fermentation (oxygen is a key nutrient for fermenting yeasts) are different from those needed during ageing (micro-oxygenation plays a role in stabilizing colour, softening tannins and diminishing herbaceous characters) or those needed after bottling (oxygen transferred through closures keeps on participating to wine evolution). And that will depend on grape varietals, winemaking styles and intended shelf-life.

In front of this very complex challenge, the need for reliable and easy-to-use oxygen analyzers is obvious. Measuring oxygen is indeed the first step towards understanding the oxygen needs of different wines.

Different oxygen analyzers had been used in the wine industry in the last decades but none of them was specifically developed for measuring dissolved oxygen in wines. The use of these analytical tools is often limited to some restricted situations. On one hand, basic and easy-to-use oxymeters are used to measure the oxygen pickups during dynamic phases (pumping, filtration…) but are not accurate enough to measure the low variations of dissolved oxygen in static phases during ageing notably (micro-oxygenation, barrels ageing…). On the other hand, some very accurate probes exist which use implies heavy protocol that are restricted to laboratory use and are not compatible with field applications.

Measuring oxygen during and post bottling is even more challenging than measuring oxygen in bulk wines as it is more difficult to measure oxygen content in a bottle without any air contamination coming from outside the bottle. Only few devices exist that allow this kind of measurements and it was only done by laboratories, research institutes or big wineries. To get a full oxygen assessment of oxygen present in a bottle (Total Package Oxygen), it is critical to measure both dissolved oxygen but also oxygen from the head space of the bottle. Vidal et al. (2006) reported indeed that the head space can represent a big reserve of oxygen (more than 3 mg) in the bottle.

Measuring head space oxygen content implies that head space gas is sampled through the closure with a needle. This needle can be directly connected to an analyzer (Dansensor Checkmate (O'Brien and Gibson, 2007), gas chromatograph (Cook and al., 1985)) or to a syringe that will be used to load the sample into a specific measurement cell (Orbisphere

probe (Vidal et al., 2004)). An alternative method consists of estimating the Total Package Oxygen by measuring dissolved oxygen only after shaking the bottle (Vilacha and Uhlig, 1984) to obtain equilibrium of oxygen pressure between the head space and the wine. However, it remains very difficult to be sure that the equilibrium is reached when performing the measure.

Recently, luminescence-based technologies have been developed. These tools are very easy to use and interestingly a new generation of analyzers had been developed with separate sensors. In the case of measurement of Total Package Oxygen, the sensors can be glued in bottles prior to filling with wine and allow for non-invasive measurement (measurements done through the glass using an optical fiber). These sensors can be used to measure dissolved oxygen as well as head space oxygen (O'Brien et al., 2009).

The objective of this study was to evaluate the performances of the PreSens technology based on luminescence with separate sensors in measuring Total Package Oxygen.

MATERIALS AND METHODS

A full validation study was performed according to the MA-F-AS1-06-PROVAL protocol proposed by the OIV. We have evaluated different parameters such as repeatability, linearity, intra-laboratory reproducibility and limits of detection and of quantification (LOD and LOQ) of three analytical methods. The performances of luminescence-, polarography- and micro gas-chromatography-based techniques were compared.

The actual objective of this study was to evaluate the performance of the PreSens non destructive technology to measure both head space and dissolved oxygen within wine bottles. A specific vessel was specially designed for that study that consists of a 19 mm diameter glass sleeve melted on a beaker and mounted with 2 gas-tight valves allowing flushing the vessel volume with selected gases. This glassware was sealed with a Nomacorc co-extruded closure.

A Fibox 3 trace fiber optic oxygen meter purchased from PreSens Precision Sensing GmbH, Regensburg, Germany, was used. The Fibox 3 measures the luminescence decay time of an immobilized luminophore. The luminophore is excited with a sinusoidal intensity-modulated monochromatic light delivered by an optical fiber and its decay time causes a time delay in the light signal emitted by the luminophore. This decay time, or phase angle, Φ, decreases in the presence of oxygen and is correlated to oxygen content.

The Pst3 sensor selected to perform this study can be used for a broad range of oxygen concentration ranging from 0 to 50 %. The sensor spot pst3 is calibrated using a conventional two-point calibration with temperature compensation. The first point of calibration is carried out at 100% of air saturation while the second point was performed under nitrogen flush (0 % oxygen). The calibration was performed under atmospheric pressure measured at 1001 mbar. A pst3 spot had been glued inside the specific vessel.

Data acquisition was performed using PST3v541 software. For each measurement, temperature was measured in the sample and oxygen measurements were compensated accordingly. Readings were performed by applying the optical fiber in front of the dot and emitting excitation light through the glass wall.

An Orbispere 31120A oxygen probe equipped with a 25 µm 2956A membrane purchased from Hach Ultra Analytics, Genève, Switzerland, was used. This membrane is compatible with measurement of gaseous oxygen ranging from 0.25 Pa to 50 kPa. A Goretex®

membrane covering the 2956A membrane is required for the analysis of gaseous oxygen, to prevent any interference due to injection of liquid. The probe tip was inserted in a circulation chamber for small gaseous samples. The probe was connected to an Orbisphere Moca 3600 single channel microprocessor analyzer for oxygen measurements.

The Orbisphere probe is based on Clark's electrode principle, basically an electrochemical reaction between oxygen and the gold cathode. A potential difference of 0.725 V is applied to allow oxygen reduction at the cathode.

$$\text{Cathode} \qquad O_2 + 2\,H_2O + 4\,e^- \leftrightarrow 4\,OH^-$$

$$\text{Anode} \qquad 4\,Ag \leftrightarrow 4\,Ag+ + 4\,e^-$$

Before each series of measurement, the probe is calibrated using one point calibration in water vapour saturated air.

The procedure of measurement was established by Vidal et al. 2004. A 10 mL glass Hamilton syringe equipped with a Teflon seal and a Luer Lock tip (Supelco, ref.26293) was used for sampling. The syringe was purged with nitrogen several times as well as the needle right before insertion through the closure. The sample was taken in the syringe and injected directly in the gas circulation chamber. To take the probe time response into account, a first injection of 1 mL was performed over 10 seconds, and the formal injection was done 10 seconds after the first one.

A micro gas-chromatograph (CP-4900) purchased from Varian Inc., Les Ulis, France, was equipped with a 10 m MolSieve 5A PLOT column and a catharometer detector. The injector, column and catharometer were maintained at 120°C. Helium (Messer) was used as carrier gas at 172 kPa. Sample volume was 400 µL. A specific system of sampling has been set up that is composed of a Genie 170 membrane to prevent any liquid contamination, a 3-ways valve to purge the needle or to load the sample into the injector, and a barometer to assess the pressure in the headspace of the sample.

The calibration of the µGC was set using a conventional two-point calibration at 5% O2 (using certified gas) and at 20.9 % (in the atmosphere). Each calibration point was measured in five replicates.

Peak integration was performed with CP Maitre Elite software.

Seven Certified gases purchased from Messer were used to have a range of oxygen content from 0 to 21% (0, 1, 1.99, 5.01, 10, 15, 21.2% v/v).

RESULTS AND DISCUSSION

Repeatability

According to the standard, a single operator must analyse q different samples, whose analytical values cover the measurement range within which the laboratory wishes to perform evaluation. Each sample must be analysed in duplicate.

The repeatability is calculated from the standard deviation of the sum of differences with the standard sample.

$$Sr = \sqrt{\frac{1}{2q}\sum_{i=1}^{q} Wi^2}$$

And r= 2.8 Sr

Table 1: Statistical results of the estimation of repeatability

	µGC	Polarographic probe	Luminescence probe
Number of samples analysed in duplicate	7	7	7
Range	0-21 %	0-21 %	0-21 %
Standard deviation of repeatability Sr	0,0422 %	0,1324 %	0,0361 %
Repeatability r	0,1181 %	0,3707 %	0,1010 %

The results (Table 1) show a better repeatability for the µGC and the luminescence methods. The repeatability of the polarographic probe is more than 3 times higher, a result that can be explained by the fact that this technique requires several steps (piercing through the closure, sampling with the syringe, priming and injecting in the gas circulation chamber) all associated with analytical error.

Linearity

Linearity is defined as the ability of a method to give results proportional to the concentration of the component in a given range of concentration.

To test for linearity, a linear regression (y = b.x + a) between the reference values and measured values was performed and slope and intercept were tested though a Student test comparison.

The slope is tested against the following equation:

$$b - (t_{1-\alpha/2} * s_b) < 1 < b + (t_{1-\alpha/2} * s_b)$$

b: slope of the linear regression
$t_{1-\alpha/2}$: t value from Student table for v degrees of freedom with v = q – 2 and α=0,05 (P = 1 – α/2 = 1 – 0,025 = 0,975)
s_b: standard deviation of the estimated slope

and the intercept against the following equation:

$$Md - ((t_{1-\alpha/2})\, s_d/\sqrt{q}) < 0 < Md - ((t_{1-\alpha/2})\, s_d/\sqrt{q})$$

Md: Algebraic mean of result differences.
$t_{1-\alpha/2}$: t value from Student table for v de degrees of freedom with v = q – 2 and α=0,05 (P = 1 – α/2 = 1 – 0,025 = 0,975)
s_d: standard deviation for the difference between measured and theoretical values

Table 2: Statistical results on the linearity

	µGC	Polarographic probe	Luminescence probe
Number of samples analysed in duplicate	18	18	18
Range	0-21%	0-21%	0-21%
Linear regression equation	0,9901x -0,1882	1,0126x -0,2214	1,0016x -0,0041
S_{xy}	0,2598	0,4674	0,0361
Correlation coefficient	0,9990	0,9969	1,0000
test on the slope ($t_{1-\alpha/2}$=2,228)	0,973 < 1 < 1,007	0,981 < 1 < 1,044	0,999 < 1 < 1,004
test on the intercept ($t_{1-\alpha/2}$=2,201)	0,135 > 0 < 0,409	-0,124 < 0 < 0,358	-0,029 < 0 < 0,010

The statistical tests (Table 2) show that the three methods have a linear response (slope non-significantly different from 1). The intercept for the µGC method was significantly different of 0 in the conditions defined in this experiment. This may be related to the fact that the calibration was done at 5 and 20.9 % and not at 0 % which can induce a bias in the calibration curve.

The PreSens method equation (Tab.2) is the closest to the theoretical equation y = x indicating the best performance in terms of linearity among the three tested methods.

Reproducibility

In the frame of this validation study, the intra-laboratory reproducibility was evaluated under the following conditions: use of the same certified gases (1, 10 and 15 % oxygen), in the same laboratory, a single operator over a month period.

The reproducibility (R) was calculated with the same equation as for repeatability.

Table 3: Statistical results about intra-laboratory reproducibility

	µGC	Polarographic probe	Luminescence probe
Number of samples analysed in duplicate	60	60	60
Range	1-15 %	1-15 %	1-15 %
Standard deviation of reproducibility SR	0,3328 %	0,4288 %	0,0596 %
Intra-laboratory reproducibility R	0,9319 %	1,2005 %	0,1668 %

The results (Table 3) show a very low R value for PreSens method, a value close to those obtained for repeatability (R= 0.1668 % compared to r = 0.101 %). R is expected to be higher than r. The fact that these values are really close in this experiment can be explained by the set up of this experiment. The PreSens method is intended to be used for non-destructive monitoring of the oxygen content in bottles over time without opening the bottles. As a consequence, we decided not to change the dot calibration done at the very beginning of the reproducibility assessment. This highlights the good stability of the dots over this period.

The Orbisphere method is the less reproducible, the estimated R value being 10 times higher (1.2 %) than those of PreSens and twice as high as the value calculated by Vidal et al. in 2004 (0.51 %). The number of steps in the measurement protocol is a big source of analytical error as already mentioned in the paragraph related to repeatability. The set up of the Vidal et al. 2004 experiment was different from the one applied in this study in the sense that certified gases were directly sampled with the syringe and injected in circulation chamber.

The R value obtained for the µGC method was approximately 8 times higher than the r value which tends to point out that the calibration performance can vary from time to time. It is important to note that this is the only method for which temperature compensation could not be applied.

Limit Of Detection – Limit Of Quantification

The limit of detection (LOD) is the smallest quantity of the substance that can be detected whereas the limit of quantification (LOQ) is the smallest quantity of the substance that can be accurately quantified by the considered method.

Twenty blank samples had been run in duplicate to assess the noise background associated with each method.

The LOD and LOQ were then estimated using the two following formula:

$$LOD = M_{blanc} + (3.\ S_{blanc})$$

$$LOQ = M_{blanc} + (10.\ S_{blanc})$$

M_{blanc}: mean of measurements for blank samples
S_{blanc}: standard deviation for blank samples

Table 4: Estimated limits of detection and quantification

	µGC	Polarographic probe	Luminescence probe
Number of samples analysed in duplicate	20	20	20
Oxygen content of gas (%O_2)	0,0006	0,0006	0,0006
LOD	0,09 %	0,13 %	0,02 %
LOQ	0,25 %	0,31 %	0,03 %

The PreSens method allows for the lowest LOD and LOQ (Table 4). It is important to note that the LOD determined by the supplier is 0.03 %, a result in good accordance with the results from this validation study.

In conclusion, these three methods represent accurate analytical tools to assess the oxygen content in the headspace of the bottle. Among the three methods, the PreSens method is the most accurate and sensitive one. Furthermore, the handiness of this method makes it a preferred choice as a lot of applications can be conceived using this tool in laboratories but also in wineries.

Notably, one of the applications that can be mentioned is the measurement of Total Package Oxygen (Dissolved oxygen (DO) + oxygen from the Head Space (HS)) in wine bottles. To verify that this technique is suitable for such an application, we decided to run a quick verification of the performances of this method to measure DO.

The same kind of approach was followed to evaluate the repeatability and the linearity of DO measurements with PreSens. In order to obtain a range of wines with known DO values, the same certified gases were flushed through a porous ceramic in bottled wine long enough to reach equilibrium between the wine and the gas phases. We considered that the equilibrium was reached when the measurement with the PreSens technique was steady for several minutes. After removal of the ceramic, the formal measurement was carried out with the same device.

Table 5: statistical results about dissolved oxygen measurements

	Luminescence
Number of samples analysed in duplicate	8
Range	0 – 9.14 ppm
Repeatability r	0.02 ppm
Linear regression equation	0.99574 x – 0.0384
S_{xy}	0,07766
Correlation coefficient	0.99975
Fobs<Ftheo ; (α=0.05)	1.66 < 3.58
Test on the slope ($t_{1-\alpha/2}$=2,228)	0,9736 < 1 < 1,0179
Test on the intercept ($t_{1-\alpha/2}$=2,201)	-0,0093 < 0 < 0,1129

The performances of the method for DO measurement are very close to those obtained for the HS measurement and we can conclude that the PreSens method is repeatable, accurate and linear.

The intra-laboratory reproducibility R can be estimated to be very close to the repeatability r and to be less than 0.05 ppm based on the observation made during the assessment of the reproducibility for HS measurement.

BIBLIOGRAPHY

Cook J-M. and al., 1985. Measurement of oxygen, nitrogen and carbon dioxide in beverage headspace. J. chromatogr. Sci, 23, 57-63.

O'Brien V., Gibson R., 2007. Is your head space vacant? Australian & New Zealand Grapegrower & Winemaker, 513, 66-72.

O'Brien V. et al., 2009. Managing oxygen ingress at bottling. Wine Ind. Journal, 24, n°1, 24-29.

Vidal J-C. and al., 2004. Comparison of methods for measuring oxygen in the headspace of a bottle of wine. J. Int. Sci. Vigne Vin, 38, n°3, 191-200.

Vidal J-C. and Moutounet M., 2006. Suivi des phases gazeuse et liquide de bouteilles de vin à l'embouteillage et en conservation. J. Int. Sci. Vigne Vin, 40, n°1, 35-45.

Vilacha C. and Uhlig K., 1984. Die messung niegriger gesamtsauer-stoffgehalte im abbgefüllten bier. Brauwelt, 18, 754-758.