

# COMPARISON OF METHODS FOR MEASURING OXYGEN IN THE HEADSPACE OF A BOTTLE OF WINE

## COMPARAISON DE MÉTHODES DE MESURE DE L'OXYGÈNE DANS L'ESPACE DE TÊTE D'UNE BOUTEILLE DE VIN

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**Abstract :** This paper presents a protocol for the sampling of gases and the measurement of oxygen by the means of a polarographic probe after bottle corking. Analyses of certified standard gases were performed according to the standard protocol of the Office International de la Vigne et du Vin to characterize the polarographic probe and gas chromatography (GC) methods. Finally, analyses of bottles of wine were performed to compare the both methods, together with a method based on the measurement of dissolved oxygen before and after agitation of the bottle of wine.

The polarographic method displays greater sensitivity and a lower limit of quantification (LQ) than the GC method. Uncertainty was found to be 0.51 % with the probe method. Although the study showed that the results of the indirect method differed from those of the probe and GC methods, especially for high oxygen contents, it enables wine companies to measure oxygen in the wine and in the bottle headspace using the same apparatus.

**Résumé :** L'embouteillage est une source notoire et importante d'enrichissement en oxygène dissous. Une trop forte quantité d'oxygène peut provoquer une évolution négative du vin. Logiquement, les entreprises viticoles cherchent de plus en plus à quantifier et maîtriser ces contaminations. C'est pourquoi, il a paru important, afin de compléter le diagnostic de l'oxygène à dissous, de connaître la composition en oxygène de l'espace de tête de la bouteille obturée.

Or jusqu'à présent, pour la mesure de l'oxygène dans l'espace de tête, seule la chromatographie en phase gazeuse (CPG) est utilisée, mais elle ne permet pas d'effectuer les mesures *in situ*. Cet article présente un protocole de prélèvement d'un échantillon de la phase gazeuse et d'en mesurer la teneur en oxygène par sonde polarographique dès la fin du bouchage de la bouteille. Des analyses sur des gaz étalons certifiés ont été effectuées selon la norme de l'Office International de la Vigne et du Vin (OIV) pour caractériser les méthodes par sonde et par CPG. Enfin, des analyses sur des bouteilles de vin ont permis de comparer les deux méthodes, ainsi qu'une méthode basée sur la mesure de l'oxygène dissous avant et après agitation de la bouteille de vin et adaptée des travaux de VILACHA et UHLIG sur la bière.

Les résultats obtenus montrent que les mesures de l'oxygène dans l'espace de tête par sonde Orbisphère sont possibles pour des teneurs en oxygène comprises entre 0 et 21 %. La méthode polarographique se caractérise même par une meilleure sensibilité et un seuil de quantification plus bas que la méthode par CPG. L'incertitude de la méthode par sonde a été établie à 0,51 %.

Quant à la méthode Vilacha-Uhlig adaptée aux bouteilles de vin, même si cette étude a mis en évidence que les résultats s'écartent de ceux obtenus par sonde ou CPG, surtout pour les teneurs élevées en oxygène, elle permet aux entreprises viticoles de pouvoir mesurer l'oxygène du vin et de l'espace de tête avec le même matériel.

**Keywords:** bottle, headspace, oxygen, polarographic probe, GC

**Mots clés :** bouteille, espace de tête, oxygène, sonde polarographique, CPG

## INTRODUCTION

Various studies were undertaken to characterize the amounts of dissolved oxygen during the various technological steps to which wine is subjected (reception, treatments and bottling). One of the main conclusions of the work was that bottling is a critical point in the enrichment of wine with oxygen (VIDAL *et al.*, 2004; VIVAS and GLORIES, 1996; BERTA *et al.*, 1999; ALLEN, 1994).

In order to complete this diagnosis, it seemed important to know the composition of the gases in the headspace of a bottle of still wine that can form a substantial supplementary reserve with regard to further phenomena of wine oxidation. COOK *et al.*, 1985 proposed a method for sampling the gas phase combined with gas chromatography (GC).

In 1984, VILACHA and UHLIG reported that Henry's law could be used to calculate the total oxygen in bottled beer. Using agitation with appropriate apparatus to establish an oxygen balance between the beer and the headspace, measurement of oxygen in the liquid phase allows the calculation of the oxygen content in the gas phase. The oxygen contents of the two phases correspond to the total oxygen content of the bottled beer. The principle consists of measuring the dissolved oxygen content before and after equilibrium using an oximeter. To support their results, the authors verified their calculations by measuring the oxygen content in the headspace using GC.

The present article first describes a protocol for the sampling of the headspace in a bottle with a cork, capsule or cap. Assays of gaseous oxygen by polarographic probe, GC and the indirect method proposed by VILACHA and UHLIG adapted to still wines are then compared using the OIV protocol for validation of the method of analysis (OIV, 2000).

## MATERIALS AND METHODS

### I- PROTOCOL FOR THE VALIDATION OF POLAROGRAPHIC PROBE AND GC METHODS

The measurement method using a polarographic probe (Orbisphere) was compared with a GC method, an older technique more frequently mentioned in the scientific literature on the subject (COOK *et al.*, 1985; MEKHUZLA, 1977).

It is only possible to take one headspace sample per bottle, since perforation of the cork or screw cap destroys irremediably the seal. Furthermore, the variation factors even in a single batch of bottles of wine are potentially too numerous to consider that the headspace gas contents are identical in all the bottles. For these reasons, the analyses are not repeatable with regard to the statistical evaluation of an analytical method. Characterisation of the

probe and GC methods (repeatability, linearity, sensitivity, intra-laboratory reproducibility, limits of detection and quantification) was therefore only possible using certified standard gases supplied by Air Liquide.

Subsequently, 82 wines were used for comparison of the probe and GC methods, 47 for the comparison of the probe and Vilacha-Uhlig methods and 36 for that of the GC and Vilacha-Uhlig methods. In a given batch of wine, one bottle was used for measurement of gaseous oxygen by probe and/or GC methods. A second bottle was used for measurement of dissolved oxygen before agitation. A third bottle was used for measurement of dissolved oxygen after agitation. All the measurements were made one after the other. Thus, the incidence of an eventual variation of the barometric pressure is insignificant on such a short lapse of time. Each measurement requires the use of a bottle; so replicates cannot be done on the same sample, and thus the repeatability could not be calculated.

The results for gaseous oxygen were expressed as the percentage volume of oxygen in relation to the sample volume.

The bottles of wine and of certified standard gas were thermostated at 20 °C the day before the measurements. The atmospheric pressure during these tests was of 1013 ± 10 hPa. For example, according to the table given by the manufacturer (ORBISPHERE, 2002) the fugacity of oxygen at 20° C in the air saturated with water steam passes from 20.65 to 20.86 KPa when the barometric pressure passes from 1010 to 1020 hPa, that is to say 20.65 KPa + 1 %. With the atmospheric pressure of 1010 hPa, the fugacity passes from 20.65 to 20.59 KPa when the temperature passes from 20 to 22° C, that is to say 20.65 KPa - 0.3 %.

In order to compare the methods tested with different oxygen contents, the wine samples were selected so that they cover a broad range of concentrations between 0 and 21 % v/v. The other factors of variability such as the nature and the length of the cork, the type of screw cap, the volume of the headspace, the operating conditions during the filling and the obturation were not taken into account.

### II- POLAROGRAPHIC PROBE ASSAY

#### 1) MATERIALS

##### a) Polarographic probe

An Orbisphere 31110A oxygen probe equipped with a 25 µm 2956A membrane was used. The membrane range of measurement of gaseous oxygen was 0.25 Pa (2.5 10<sup>-3</sup> mbar) to 50 kPa (500 mbar). The response time for 90 % of the signal at 25 °C was 7.2 seconds (manufacturer's data). A Goretex® membrane covering the 2956A membrane is required for the analysis of oxygen

in a gaseous environment. 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 analyser for oxygen measurements. The Moca apparatus was in turn connected to a computer that recorded the measurements transmitted by the probe; version 1.03 of the Moca software was used.

b) Syringes

The method requires two syringes. As there is a slight vacuum or atmospheric pressure in the bottle, a liquid must be injected so that as much gas as possible can be collected with the sampling syringe.

The 10 mL injection syringe was fitted with a Luer Tip (Terumo, ref. 0104201) ; the liquid injected was distilled water equilibrated at 20 °C. The needle used with this syringe was bevelled, with 1.27 mm an outside diameter and a length of 76.20 mm.

A 10 mL glass Hamilton syringe with a Teflon seal and a Luer Lock tip (Supelco, ref. 26293) was used for sampling. It was gastight with an integrated valve to prevent any contamination by ambient air. The needle used with this syringe was bevelled, 0.71 mm outside diameter, 76.20 mm length.

2) PROCEDURE

- On each day of analysis, the probe was calibrated with air saturated with water vapour and checked against the oxygen fugacity table for air saturated with water vapour according to temperature and atmospheric pressure. The probe was then flushed with inert gas (argon or nitrogen) to remove all traces of oxygen from the mea-

surement circuit. The O<sub>2</sub> content must be lower than 0.01 % for measurements to be made.

Corked bottle

- Two preliminary holes were drilled in the cork and stopped approximately 1 cm from the base to prevent any contamination by ambient air. This made it easier to insert the needles and prevent them from being bent, broken or fully plugged with cork fragments.

- 10 mL distilled water was taken up in the injection syringe, with care taken to fill the needle and the valve. The sampling syringe was flushed several times with inert gas to remove all traces of oxygen. The inert gas was conserved in the last flushing operation to flush the sampling needle.

- The injection syringe needle (IS) was pushed into the cork until the tip went as far as possible into the wine (fig. 1). This was necessary to prevent too great a disturbance during injection from causing a change in the composition of the surface liquid in contact with the gas. Variation in the surface liquid would cause liquid:gas exchanges and distort the measurements. The ideal would be to use a syringe fitted with a needle long enough to reach the bottom of the bottle in order to avoid any variation.

- The sampling syringe (SS) was driven into the cork to just reach the base of the latter.

- A gas leak detection product (Air Liquide, ref. 26624701) was used to check that the set-up was gas-tight. The bottle of wine was changed if a gas leak was detected.

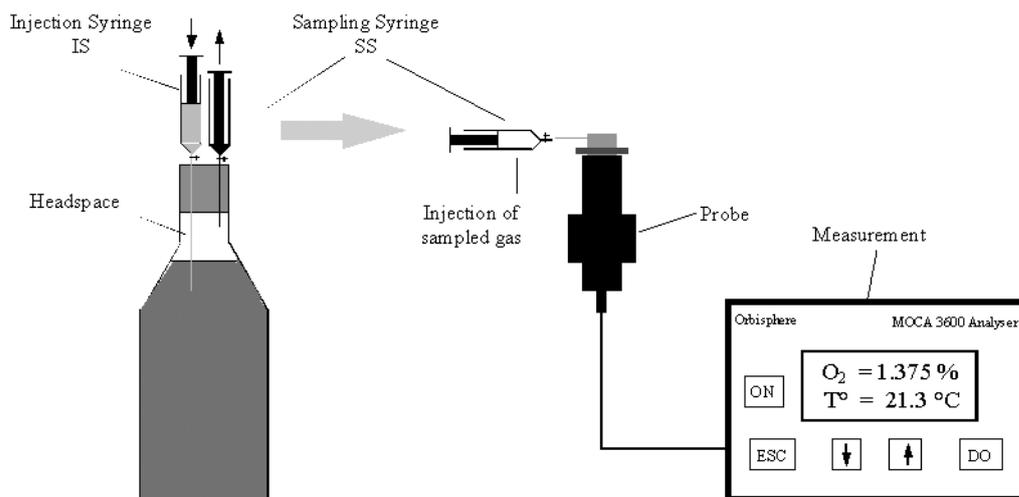


Fig. 1 – Diagram of sampling and measurement of the headspace sample using a polarographic probe

Fig. 1 - Schéma du prélèvement et de la mesure de l'échantillon de l'espace de tête par sonde polarographique.

- The valves of both syringes were opened and it was checked that there was no excess pressure (see remark below on the subject).

- The water in the IS was injected until the piston of the SS reached a volume of 3 to 7 mL (a minimum of 3 mL was required for measurement).

- The sampling syringe valve was closed when the sample had been taken. The needle was removed from the syringe so that the gas could be injected into the Orbisphere probe circulation chamber.

- The gas sampled was injected discontinuously into the probe circulation chamber.

- first injection : 1 mL for 10 sec.
- latency period : 10 sec. No injection.

The first injection was used to set the probe as the real response time under our operating conditions was approximately 19 sec, that is to say the duration of the first injection and the latency period.

- second injection : 2 to 6 mL for 20 seconds continuously, according to the volume removed initially.

The measurement is the average of the values for the second injection.

- End of measurement.

a) Bottle with internal pressure higher than atmospheric pressure

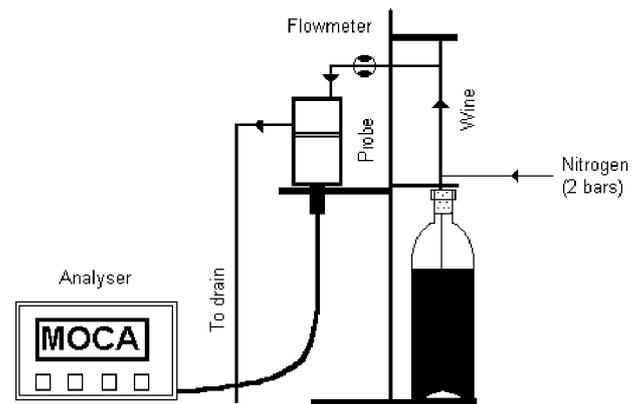
If the piston of the sampling syringe returns unaided after opening of the valves, the internal pressure is higher than atmospheric pressure (this is the case of beverages with a high CO<sub>2</sub> content. In this case, it may not be necessary to inject 10 mL distilled water when the gas is sampled. The gas in the headspace can be simply sampled with the sampling syringe.

b) Bottle with a screw cap or capsule

The cap or capsule is coated with silicone glue and the gas in the headspace is sampled with a sampling syringe fitted for the purpose with a shorter bevelled needle : 0.8 mm outside diameter, 25 mm length.

c) Certified standard gas

The sampling syringe was flushed several times with inert gas to remove all traces of oxygen. The gas sample was then taken directly at the pressure reducing valve outlet on the cylinder containing the certified standard gas.



**Fig. 2 – Assembly diagram for the measurement of dissolved oxygen in bottled wine**

**Fig. 2 - Schéma de montage pour la mesure de l'oxygène dissous dans un vin en bouteille.**

### III- GC ASSAY

#### 1) MATERIAL

An Intersmat Instruments chromatograph was parametered using the technique described by CHAMBROY *et al.*, 1984, and modified by SARRIS *et al.*, 1987. The apparatus was equipped with a Porapak Q column, outside the oven, 150 cm long and 1/8" in diameter, preceded by a non-thermostatted 13X molecular sieve and a catharometer. The injector, column and catharometer were maintained at 120 °C. The vector gas was helium U (Air Liquide) with the flow set at 35 mL/min. Injected gas sample volume was 200-1000 µL. The apparatus was connected to a Merck D2000 integrator that computes the percentage of oxygen in the injected gas mix.

#### 2) OPERATING PROCEDURE

For the comparison of methods, the sample taken was used for both measurements (polarographic probe and GC), with the polarographic probe measurement performed before GC. Only a round-tipped needle is required for GC gas injection. The probe measurement procedure was as previously described. However, 1 mL gas was kept in the sampling syringe for the GC measurement. The two measurements were performed with a short interval of time.

### IV- DETERMINATION BY CALCULATION USING THE METHOD DESCRIBED BY VILACHA AND UHLIG ADAPTED FOR STILL WINES

The bottle of wine was stirred (see below) to set up an equilibrium between the gases of the gas phase and those dissolved in the liquid phase. Determination of dis-

solved oxygen before and after agitation (at equilibrium) made it possible to calculate the total oxygen content and then the initial oxygen content of the headspace (before agitation).

### 1) MEASUREMENT OF DISSOLVED OXYGEN

The bottle was placed in an Orbisphere 29973 sampler for bottles. The cork or cap was pierced by a needle. Wine was fed to the 31120 measurement probe under a nitrogen pressure at 2 105 Pa (2 bars) in precision polyurethane tubing (Tygothane®) with very low permeability to oxygen. Movement of the wine was set at 12 L/h by a flowmeter. The wine flowed over a PFA 2956A Teflon® membrane in a 32001 circulation chamber. It was then discarded (figure 2).

### 2) BOTTLE AGITATION

The bottles were agitated in a different way to that described by Vilacha and Uhlig. A Stuart Scientific planetary agitator (3 axes) was used. The bottle was fixed on its side to the surface and the speed was set at 70 rpm for 30 minutes. Finally, the bottle was shaken vigorously by hand for 1 minute just before the measurement of dissolved oxygen.

### 3) SIMPLIFIED CALCULATION METHOD

Legend:

$V_c$  = volume of wine in the bottle (L)

$V_h$  = total headspace (L)

$HS = \% \text{ headspace} = \frac{V_h}{V_c} \times 100$

$T$  = temperature in °K (= °C + 273)

$m_{lo}$  = mass  $O_2$  in the liquid (mg)

$m_{Go}$  = mass  $d'O_2$  in the gas (mg)

$m_t$  = total oxygen (mg)

$X$  = dissolved oxygen in the wine shown by the Orbisphere probe in  $O_2/kg$  wine ( $\approx mg/L$ )

$k = 0.0325$  = Ostwald distribution coefficient = volumes of oxygen dissolved in one volume liquid = oxygen concentration in the liquid / oxygen concentration in the gas with a mixture of water + 12.52 % (v/v) ethanol at 25°C (SCHUKAREV and TOLMACHEVA, 1968).

= 0.0417 for a mixture of water + 11.48 % (v/v) ethanol at 12°C.

Index = 0 before agitation, 1 after agitation

### Calculations

Total oxygen in a recipient was determined as follows:

$$m_{lo} + m_{Go} = m_t \quad (\text{before agitation})$$

$$m_{lo} + m_{Go} = m_t \quad (\text{after agitation})$$

The polarographic probe was used to determine the concentrations of dissolved oxygen before and after agitation ( $X_0$  and  $X_1$ ). This gave  $m_{lo}$  ( $m_{lo} = V_c \cdot X_0$ ) and, according to Vilacha and Uhlig,  $X_1$  was used to calculate  $m_T$  using the following formula:

$$m_T(mg) = X_1 \times \left[ \frac{32000 \times HS \times K}{8,47224 \times T} + 1 \right] \times V_c$$

Finally, the differences gave the gaseous oxygen mass before agitation in the headspace  $V_h$ , and then its concentration in mg/L.

$$m_{Go}(mg) = m_T - m_{Lo} \quad X_{Go}(mg / L) = \frac{m_{Go}}{V_h}$$

### V- EVAL PROGRAM

This is a program for the evaluation of an alternative method developed by the Office International de la Vigne et du Vin (OIV) in accordance with the French standard NF V03-110 (1993) and the protocol OIV FV 1057. The program was initiated by BOUVIER (personal communication) and developed in Excel macros; it includes four applications corresponding to the OIV criteria of repeatability, linearity, limits of detection and quantification and comparison with a reference method.

### VI- FLUSHING GAS AND STANDARDS

The flushing gas used for the probe method was Nertal® argon (99.99% pure, 0.001% residual oxygen). Helium U was used as the vector gas for GC.

The Alphagaz® certified standard gases used in the study had the following molar concentrations of oxygen: 0 % (traces of oxygen: 0.00059%); 0.5081%; 1.021%; 2%; 5.04%; 10.01%; 15.01%; 15.55% and 21%.

**Table I – Statistical results of the estimation of repeatability of the polarographic probe and GC methods.**  
**Résultats statistiques de l'estimation de la répétabilité des méthodes par sonde polarographique et CPG.**

Parameter	Polarographic probe	GC
Number of samples	9	8
Range covered	0 to 21 %	0.5 to 21 %
Standard deviation of repeatability Sr	0.062 %	0.317 %
Estimation of repeatability r	0.17 %	0.89 %

**Table II - Oxygen concentration in % of certified standard gases determined by polarographic probe and GC.**  
**Concentration en oxygène exprimée en % des gaz étalons certifiés déterminées par sonde polarographique et CPG.**

GC value %	Polarographic probe		GC	
	X	X'	X	X'
0.00059	0.011	0.013	0	0
0.00059	0.008	0.008	0	0
0.5081	0.46	0.47	0.389	0.370
0.5081	0.47	0.46	0.379	0.354
1.021	0.99	1.00	0.687	1.32
1.021	1.00	1.02	0.762	0.738
1.021	1.01	1.00	0.752	0.782
2	1.863	1.875	1.859	2.235
2	1.858	1.88	1.828	1.839
5.04	4.813	4.815	4.263	4.258
5.04	4.865	4.682	4.448	5.003
10.01	9.726	9.7018	9.107	9.126
10.01	9.7135	9.6654	10.183	8.961
15.01	14.345	14.814	13.587	13.887
15.01	14.442	14.212	13.860	13.860
15.55	15.42	15.34	13.841	13.894
15.55	15.40	15.50	13.914	13.960
21.00	20.517	20.534	19.311	19.395
21.00	20.523	20.568	21.340	19.230
21.00	20.498	20.558	19.285	19.365

**Table III – Statistical results on the linearity of the polarographic probe and GC methods.**  
**Résultats statistiques de la linéarité des méthodes par sonde polarographique et CPG.**

Parameter	Polarographic probe	GC
Number of samples	20	20
Regression	$y = -0.04561 + 0.97926x$	$y = -0.077 + 0.92873x$
S <sub>xy</sub>	0.121	0.298
Correlation coefficient	0.99989	0.99923
Regression remainder distribution	12 > 0 and 8 < 0	8 > 0 and 12 < 0
Fobs / F1- (α = 5 %)	1.77 < 2.15	0.51 < 2.15
Sensitivity %	0.73	1.89

## RESULTS AND DISCUSSION

### I- EVALUATION OF THE PROBE AND GC METHODS

#### 1) ESTIMATE OF REPEATABILITY

According to the basic protocol of the standard, the single operator must analyse  $q$  different samples numbered from 1 to  $q$  (where  $q \geq 60$ ), whose analytical values cover the measurement range within which the laboratory wishes to perform evaluation (0 to 21%). Each sample is analysed in duplicate. However, for the technical reasons mentioned before in the validation protocol, only the use of certified standard gases is repeatable and can

cover the range of 0 to 21 % oxygen. The high price and limited number of oxygen concentrations in the certified standard gases available on the market meant that it was not possible to attain the 60 samples desired.

The results shown in table I tend to show the better repeatability of probe analysis, especially as the certified standard gas at 0% was not detected by GC, as is seen below in the study of limits.

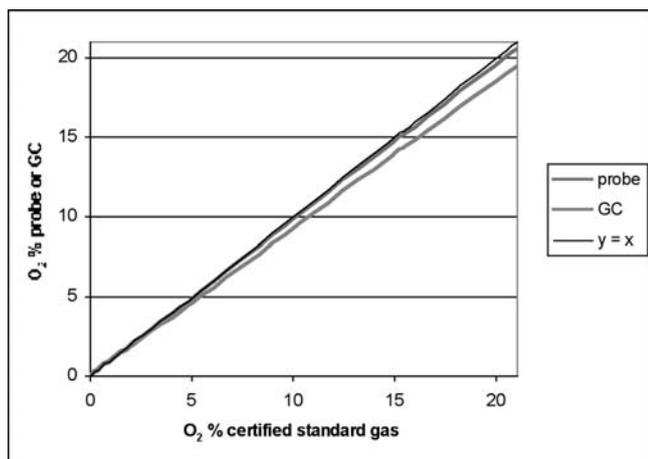
#### 2) LINEARITY AND SENSITIVITY

Linearity is the ability of a method to give results proportional to the concentration of the component sought in the wine within the measurement range chosen. The

sensitivity of the method is the smallest detectable variation in the concentration of the substance analysed.

The statistical study was performed using the results presented in table II. It was not possible to confirm the non-linearity of the two methods from 0 to 21% oxygen (table III). However, it can be seen in figure 3 that the GC regression line is further from the ideal line for high values than that of the probe.

### 3) INTRA-LABORATORY REPRODUCIBILITY



**Fig. 3 - Comparison of the linear regression lines of the polarographic probe and of GC with the line y = x.**

Comparaison des droites de régression linéaires pour la sonde polarographique et la CPG avec la droite y = x.

Intra-laboratory reproducibility in this study is the close agreement between the analytical results obtained with the method in question applied to the same certified standard gas in the same laboratory with the same operator or different operators using different calibration curves on different days.

It was calculated from analyses spread over a month using certified standard gases at 2%, 15.01% and 15.55%. The standard deviation of the data given by the probe in relation to the theoretical value of the certified standard gas analysed (SR) was used to calculate intra-laboratory reproducibility (R = 2.8 times SR). The results mentioned in table IV show that at a probability of 95% the intra-laboratory reproducibility of the probe is distinctly lower than that of GC. These values can be used as uncertainty values applicable to the two methods.

### 4) CAPABILITY

FEINBERG (2000) explains that the capability of a method makes it possible to check whether one can rely on its results. It is defined by the following formula:

$$Capability = \frac{accepted\ tolerance\ \%}{uncertainty\ \%} \times 100$$

Setting the tolerance for the method at +/- 0.5% gives measurement capability of 98% for the probe and 13% for GC. The very high value obtained with the probe means that it can be suggested that the capability of the probe method is greater than that of GC with a 0.5 % tolerance.

**Table IV – Statistical results for intra-laboratory reproducibility of the polarographic probe and GC methods.**

Résultats statistiques de la reproductibilité intralaboratoire des méthodes par sonde polarographique et CPG.

Parameter	Polarographic probe	GC
Number of GC analyses, 2%	20	10
Number of GC analyses, 15.01%	6	5
Number of GC analyses, 15.55%	10	10
Number of samples analysed in duplicate	36	25
Standard deviation of reproducibility SR	0.184 %	1.345 %
Intra-laboratory reproducibility R	0.51 %	3.77 %

**Table V – Limits of detection and quantification in the polarographic probe and GC methods.**

Seuils de détection et de quantification des méthodes par sonde polarographique et CPG.

Parameter	Probe	GC	GC
O2 concentration of certified standard gas	5.9 ppm *	5.9 ppm	5081 ppm
Number of measurements	20	20	20
LD ppm	89	not detected	5900
LQ ppm	154	not detected	12600

\* 10000 ppm = 1%

**Table VI – Raw data concerning oxygen in the headspace of bottles of wine for comparison of the three methods**

Données brutes en oxygène dans l'espace de tête de bouteilles de vin pour la comparaison des trois méthodes.

N°	O <sub>2</sub> %			N°	O <sub>2</sub> %		
	Probe	GC	Vilacha		Probe	GC	Vilacha
1	0.02	0.63	0.24	48	7.88	7.01	
2	0.02	0.43		49	8.57	8.24	
3	0.03	0.00		50	9.20	9.16	
4	0.03	0.42		51	9.47	9.02	
5	0.03	0.00	0.08	52	9.49	9.09	
6	0.03	0.00	0.21	53	9.76	9.89	
7	0.04	0.00	0.22	54	9.91	10.58	
8	0.04	0.62	0.18	55	10.17	10.12	
9	0.04	0.78		56	10.62	11.00	
10	0.04	0.75		57	10.78	10.09	
11	0.05	0.69	2.80	58	11.00	10.65	12.26
12	0.05	0.96		59	11.56	11.76	
13	0.08	0.77	0.19	60	11.62	11.81	
14	0.08	0.00	0.36	61	11.86	11.77	
15	0.12	0.80	0.94	62	11.87		11.40
16	0.14	0.80		63	11.93	12.04	
17	0.16	1.12	0.15	64	12.05		11.16
18	0.16	1.30	0.16	65	12.07		11.35
19	0.18	1.08		66	12.12	12.48	10.04
20	0.19	1.39	0.30	67	12.29	12.24	15.79
21	0.20	0.57	0.24	68	12.30	12.55	
22	0.20	1.28	0.36	69	12.34	11.89	11.33
23	0.22	1.03		70	12.35		11.83
24	0.23	0.47	0.24	71	12.46	11.94	14.50
25	0.24	1.46	0.29	72	12.80	12.95	
26	0.25	0.43	0.16	73	12.81	12.51	13.07
27	0.26	0.72	0.12	74	12.86		11.46
28	0.26	1.33	0.19	75	12.89	12.38	17.67
29	0.28	0.85	0.22	76	12.99		15.68
30	0.49	1.30	0.35	77	12.99	12.70	12.61
31	0.50	2.06	0.15	78	13.17		15.55
32	0.82	1.40	0.00	79	13.46	14.61	
33	1.05	1.30	0.21	80	13.69		14.48
34	2.43	3.18		81	13.80		18.00
35	2.66	3.48		82	13.80		16.56
36	2.96	3.62		83	13.82	13.11	
37	3.20	3.17		84	14.30	14.40	15.32
38	3.97	4.16		85	14.33	14.23	
39	4.53	4.37		86	15.31	16.14	
40	4.58	4.80		87	15.56	15.27	
41	4.82	4.57	2.99	88	16.42	16.38	
42	5.02	5.76		89	16.63	17.18	
43	5.38		6.91	90	17.63	18.02	
44	5.60	5.57		91	17.96	19.03	
45	6.29	6.06		92	18.60	18.24	26.81
46	7.53	7.85		93	20.04	19.60	31.00
47	7.74	8.07					

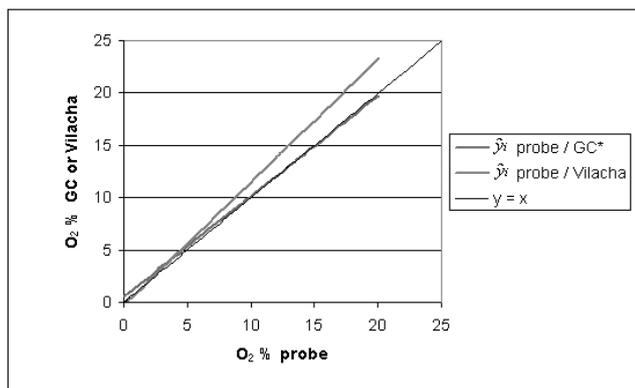
**Table VII – Statistical results of comparisons of the three methods.**

**Résultats statistiques des comparaisons des trois méthodes.**

Methods compared	Number of wines	Bias between methods*	Regression	Statistical interpretation**	Range of concentrations
Probe / GC	82	-0.28 ± 0.52%	$O_{2_{GC}} = 0.54 + 0.959 O_{2_{probe}}$	Not significantly different to $y = x$	0.02 to 20.04%
Probe / Vilacha	47	-0.84 ± 2.38%	$O_{2_{Vilacha}} = -0.29 + 1.18 O_{2_{probe}}$	Significantly different to $y = \frac{x}{x}$	0.63 to 19.60%
Vilacha / GC	36	0.49 ± 2.81%	$O_{2_{GC}} = 1.03 + 0.714 O_{2_{Vilacha}}$		

\* Bias: mean deviation between the two methods for the same wine ± the standard deviation.

\*\* error risk : 5%.



**Fig. 4 - Comparison of the probe method with the GC and Vilacha-Uhlig methods.**

**Fig. 4 - Comparaison de la méthode par sonde aux méthodes par CPG et Vilacha-Uhlig.**

\* probe / GC: estimate of oxygen using the GC method from the probe / GC regression.

### 5) LIMITS OF DETECTION AND QUANTIFICATION

The limits of detection (LD) is the smallest quantity of the substance to be assayed that can be detected but not necessarily quantified as precise. It is given by the formula:

$$LD = M_{blank} + 3.S_{blank}$$

where  $M_{blank}$  is the average of blanks measured and  $S_{blank}$  is the standard deviation of repeatability.

The limit of quantification (LQ) is the smallest quantity of the substance that can be assayed using the method. It is given by the formula:

$$LQ = M_{blank} + 10.S_{blank}$$

It is seen in table V that the values obtained using the probe were distinctly higher than the quantity of the certified standard gas used. These differences are explained to a considerable extent by contamination at injection and by the plastic tube at the connection between the syringe and the probe. Furthermore, the background noise during flush is not negligible (20 ppm). However, these values are very low in relation to current requirements in oeno-

logy (see Capability above) and in comparison with those obtained by GC, even if COOK *et al.*, 1985, found 150 ppm v/v at the limit of detection. The use of a certified standard gas with an oxygen content between those of the two used should make it possible to lower the limits for the chromatographic method.

### II- COMPARISON OF THE ACCURACY OF THE THREE METHODS

Accuracy is the closeness of the agreement between the values obtained by comparison of pairs of methods, irrespective of the errors in accuracy of the two methods.

The raw data used for the comparison of the methods are shown in table IV. The statistical methods synthesised in table VII and illustrated in figure 4 show that the probe and GC methods give similar results as the linear regression slope ( $0.959 \pm 0.008$ ) is not significantly different from 1. Nevertheless, it is seen that the difference increases for oxygen contents approaching zero. Indeed, the GC responses were greater than those of the probe as a result of the greater sensitivity and higher limits in GC (see study of GC linearity and limits) and possibly the order of injection operations (increased contamination).

In contrast, the study reveals that the indirect method adapted from the work of Vilacha and Uhlig gives better results than the other two methods, especially at higher oxygen contents (see bias in table VII and figure 4). Indeed, removal of the values higher than 14.3% from the statistical analysis considerably improves the statistical parameters for comparison with the probe method (45 wines, bias  $-0.45 \pm 1.48 \%$ ,  $O_{2_{Vilacha}} = 0.03 + 1.074 O_{2_{probe}}$ ).

### CONCLUSION

The polarographic probe method allows the measurement of oxygen in the headspace of bottles closed with corks, capsules or caps. The sampling method proposed, its higher sensitivity and lower uncertainty and quantification limits than those measured for GC make the polarographic method an effective alternative to meet current demand in oenology, even if it cannot be used for the

simultaneous assay of oxygen and carbon dioxide. The method is mobile and can be used in situ at the end of the bottling line and allows the checking of the quality of inert blanketing of bottles and of gas exchanges during the keeping of wine in bottles.

Although the study showed that the results of the Vilacha-Uhlig method adapted for wine bottles differed from those using the probe and GC methods, especially at high oxygen contents, it enables wine making companies to measure dissolved and gaseous oxygen with the same apparatus.

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