Introduction
Carbon dioxide (CO$_2$) is generally present in wines. This is due to fermentation and the use of CO$_2$ for transfer operations during winemaking. The principle is to make the sample strongly alkaline, by adding concentrated sodium hydroxide solution that converts all the CO$_2$ into CO$_3^{2-}$ and titrating with 0.1M hydrochloric acid.

Principle
As the wine made strongly alkaline (pH 10-12) contains only CO$_3^{2-}$, the titration occurs in two steps

\[
\text{CO}_3^{2-} + \text{H}^+ \rightarrow \text{HCO}_3^- \quad \text{(end point at pH 8.6)}
\]

\[
\text{HCO}_3^- + \text{H}^+ \rightarrow \text{CO}_2 + \text{H}_2\text{O} \quad \text{(end point at pH 4.0)}
\]

The end point titration is then performed with two successive end points (pH 8.6 and pH 4.0) and the titrant volume delivered from pH 8.6 to pH 4.0 is used to quantify the CO$_2$ level in the wine in g/l. The molar weight of CO$_2$ is 44.0 g/mol.

A blank titration run with the same, but degassed, wine is necessary.

Electrode and Reagents
pH2401-8 Combined pH Electrode (part no. E16M400)
HCl 0.1 mol/l solution in distilled water (see Application Note TTEP01-01MIN)
NaOH 50% w/v in distilled water

This solution is also commercially available.

Distilled water

Dilution of 50 g of NaOH pellets in 100 ml of freshly boiled distilled water is highly exothermic. The solution is also very caustic for the skin and eyes. Observe laboratory safety regulations.

Distilled water

In order to have a reproducible blank result, use freshly boiled, distilled water which has been cooled to room temperature.

IUPAC Series pH standards
pH 4.005 (part no. S11M002) and pH 10.012 (part no. S11M007)

End Point Titration Settings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
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</thead>
<tbody>
<tr>
<td>Burette volume:</td>
<td>25 ml</td>
</tr>
<tr>
<td>Stirring speed:</td>
<td>500 rpm</td>
</tr>
<tr>
<td>Working mode:</td>
<td>pH</td>
</tr>
<tr>
<td>Blank:</td>
<td>YES (see notes)</td>
</tr>
<tr>
<td>Predose:</td>
<td>0 ml (see notes)</td>
</tr>
<tr>
<td>Stirring delay:</td>
<td>10 seconds</td>
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<tr>
<td>Maximum volume:</td>
<td>40 ml</td>
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<tr>
<td>Minimum speed:</td>
<td>0.2 ml/min</td>
</tr>
<tr>
<td>Maximum speed:</td>
<td>10 ml/min</td>
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<tr>
<td>Direction:</td>
<td>Decreasing pH</td>
</tr>
<tr>
<td>Number of end points:</td>
<td>2</td>
</tr>
<tr>
<td>End point 1:</td>
<td>8.6 pH</td>
</tr>
<tr>
<td>Proportional band:</td>
<td>2.0 pH</td>
</tr>
<tr>
<td>End point delay:</td>
<td>5 seconds</td>
</tr>
<tr>
<td>End point 2:</td>
<td>4.0 pH</td>
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<tr>
<td>Proportional band:</td>
<td>2.0 pH</td>
</tr>
<tr>
<td>End point delay:</td>
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</tr>
<tr>
<td>Sample unit:</td>
<td>ml</td>
</tr>
<tr>
<td>Dilution:</td>
<td>YES</td>
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<tr>
<td>Sample amount:</td>
<td>100</td>
</tr>
<tr>
<td>Final dilution amount:</td>
<td>102 (see notes)</td>
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<tr>
<td>Aliquot:</td>
<td>10</td>
</tr>
<tr>
<td>Result No of results:</td>
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</tr>
<tr>
<td>Result by:</td>
<td>difference</td>
</tr>
<tr>
<td>Calculate with EP:</td>
<td>2</td>
</tr>
<tr>
<td>Result unit:</td>
<td>g/l</td>
</tr>
<tr>
<td>Molar weight:</td>
<td>44.0 g/mol</td>
</tr>
<tr>
<td>Reaction:</td>
<td>1 smp + 1 titr</td>
</tr>
</tbody>
</table>

Procedure

Calibrate the combined pH electrode. Let the wine cool to 5°C

Blank preparation

Degassing a wine sample. Pour the wine into a Buchner flask and connect it to a vacuum system for 3 minutes.

Degassing is also possible by heating the wine and boiling for few seconds only. Then allow the wine to cool to room temperature. Pipette 100 ml of this degassed wine; add 2 ml or more of the NaOH 50% solution as accurately as possible. Make sure that the pH of this solution is around pH 10-12. Pipette 10 ml of this solution. Add the necessary volume of distilled water; dip electrode and delivery tip in the solution. Run a blank titration. You can run more than one test on the blank; in this case the Titration Manager takes
into account the mean value.

**Sample preparation**
Taking care not to lose carbon dioxide, take 100 ml of the wine at 5°C using a measuring tube. Pour it in a conical flask, add the same volume of the NaOH 50% solution as for the blank, seal the flask and mix thoroughly. To avoid losing carbon dioxide, ensure that the flasks handling the sample are always at low temperature (near 0°C). Use an ice bath or refrigerator. Make sure that the pH of this solution is around pH 10-12. Pipette 10 ml of this solution. Add the necessary volume of distilled water, dip electrode and delivery tip in the solution. Run the titration.

**Results**
Expressed as g/l of CO2 (molar weight of CO\(_2\) = 44 g/mol)

\[
R_{CO2} = (V_{titr} - V_{blk}) \times C_{titr} \times 44 / V_{smp}
\]

\(V_{titr}\) = Delivered titrant volume between pH 8.6 and pH 4.00 for sample

\(V_{blk}\) = Delivered titrant volume between pH 8.6 and pH 4.00 for blank

\(C_{titr}\) = Titrant concentration in mol/l (generally 0.1)

\(V_{smp}\) = Sample volume (generally 10 ml)

The above-mentioned END POINT SETTINGS take in account:
- the blank calculation,
- the dilution factor due to the addition of 2 ml of NaOH 50% solution.

**Result on red wine**
No carbon dioxide measured

Verification of the method by addition of Na\(_2\)CO\(_3\) directly in the sample beaker before titration with the same wine.

<table>
<thead>
<tr>
<th>Na(_2)CO(_3) added in mg</th>
<th>V(<em>{titr}) - V(</em>{blk})</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.57 ml</td>
</tr>
<tr>
<td>45</td>
<td>4.5 ml</td>
</tr>
<tr>
<td>61</td>
<td>6.08 ml</td>
</tr>
</tbody>
</table>

As indicated in this table the method allows the added Na\(_2\)CO\(_3\) be measured with a recovery ratio close to 98%.

**Results on sparkling white wine**
Sample dilution settings for this wine

- Sample amount: 50 ml
- Final dilution amount: 52 ml
- Aliquot: 10 ml

Blank on degassed wine

5.37 ml of titrant (mean on 3 determinations)

Results (3 determinations)

- Mean: 6.83 g/l CO\(_2\)
- Standard deviation: 0.02 g/l CO\(_2\)

**Working Range**
According to the formula mentioned under "Results":

1 ml for (\(V_{titr} - V_{blk}\)) corresponds to 0.44 g/l of CO\(_2\)

Note that this result is calculated for 10 ml of sample and does not take into account the dilution factor due to addition of NaOH 50% solution.

**Notes**

**Note regarding the "blank"**
As the behaviour of every wine is different, it is necessary to run a blank titration for every different type of wine.

As the blank titration depends on the composition of the studied wine, do not forget to use the same sample amount of wine and dilution settings for the blank determination and for the sample.

**Note regarding the "predose"**
To save time it is possible to use the "predose" function of the Titration Manager.

Bear in mind that the "predose" volume of titrant is effective during sample titration and also "blank titration."

Note that using "predose" can eliminate the first end point.

**Note regarding the final amount dilution**
This amount is the sum of the volume sample (generally 100 ml) and the volume of the NaOH 50% solution added (in this application note 2 ml).

The volume of the NaOH 50% can change according to the wine.

**Note regarding the result**
As the method itself offers good reproducibility, the accuracy of the result depends on the handling of the non-degassed sample. It is important to avoid losing carbon dioxide especially with sparkling wines.

Ensure that a sufficient volume of NaOH 50% solution is added.

**Curves**

![Blank](attachment:blank.png)
Sample: sparkling wine

Bibliography
Techniques for chemical analysis and quality monitoring during winemaking
Ed: Patrick ILAND wine promotion
Campbelltown AUSTRALIA