The formol number, or formol titration, measures the total amino acid (NH$_2$-R-COOH) concentration. Using 2 successive end point titrations, it is possible to determine total titratable acidity and formol number.

**Principle**

The determination of formol number takes place in three steps:

1) Neutralisation of titratable acidity by means of an end point titration at pH 8.2 with NaOH 0.1 eq/l  
2) Addition of an excess of formol (HCHO) to the solution. This operation locks the NH$_2$ groups of amino acids due to the decrease in pH and allows titration of the COOH groups of amino acids with an end point titration at pH 8.2  
3) Second endpoint titration at pH 8.2 to determine total amino acid content. The result is then expressed as milliequivalents/100 ml or milliequivalent/l

**Electrode and Reagents**

- pH2401-8 Combined pH Electrode (part no. E16M400)  
- NaOH 0.1 eq/l solution in distilled water  
- Distilled water  
- Check the pH of the distilled water.  
  Using the titration manager as a pH meter, adjust the distilled water to pH 8.2, by means of few drops of base or acid solution, with NaOH 0.1 as titrant  
- Formol (HCHO) solution at 37% adjusted to pH 8.2  
- IUPAC Series pH standards  
  - pH 4.005 (part no. S11M002) or  
  - pH 7.000 (part no. S11M004) and  
  - pH 10.012 (part no. S11M007)

**End Point Titration Settings**

<table>
<thead>
<tr>
<th>First method (can be used as titratable acidity)</th>
<th>Second method (Formol number determination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burette volume: 10 ml</td>
<td>Burette volume: 10 ml</td>
</tr>
<tr>
<td>Maximum volume: 30 ml</td>
<td>Maximum volume: 10 ml</td>
</tr>
<tr>
<td>Stirring speed: 400 rpm</td>
<td>Stirring speed: 400 rpm</td>
</tr>
<tr>
<td>Working mode: pH</td>
<td>Working mode: pH</td>
</tr>
<tr>
<td>Number of end points: 1</td>
<td>Number of end points: 1</td>
</tr>
<tr>
<td>End point: 8.20 pH</td>
<td>End point: 8.20 pH</td>
</tr>
<tr>
<td>Stirring delay: 30 seconds</td>
<td>Stirring delay: 60 seconds</td>
</tr>
<tr>
<td>Minimum speed: 0.2 ml/min</td>
<td>Minimum speed: 0.2 ml/min</td>
</tr>
<tr>
<td>Maximum speed: 10 ml/min</td>
<td>Maximum speed: 5 ml/min</td>
</tr>
<tr>
<td>Proportional band: 3.00 pH</td>
<td>Proportional band: 2.00 pH</td>
</tr>
<tr>
<td>End point delay: 10 seconds</td>
<td>End point delay: 10 seconds</td>
</tr>
<tr>
<td>Sample unit: ml</td>
<td>Sample unit: ml</td>
</tr>
<tr>
<td>Sample amount: 10</td>
<td>Sample amount: 10</td>
</tr>
<tr>
<td>Result expression: g/l (of tartaric acid or citric acid)</td>
<td>Result expression: meq/100 ml</td>
</tr>
<tr>
<td>Titration: Increasing pH</td>
<td>Titration: Increasing pH</td>
</tr>
</tbody>
</table>

**Procedure**

Connect the electrode to the electrode input.  
Calibrate the electrode using the two IUPAC standards above.  
Stir and pipette 10 ml of sample.  
Always dilute the sample with the same volume of distilled water (25 ml for example).  
Dip electrode and delivery tip in the solution  
Start titration by pressing the RUN key.  
At the end of the first method manually add 5 ml of HCHO solution for 10 ml of sample to the sample solution.
Results
For titratable acidity expressed as g/l of tartaric acid (HOOC-(CHOH)2-COOH)
Result is normally expressed as g/l of tartaric acid (MW= 150.09 g/mol and 2 acid functions).
As 2 molecules of NaOH react with 1 molecule of tartaric acid: 
\[ R = \frac{V(\text{titr}) \times C(\text{titr}) \times 150.09}{V(\text{sample}) \times 2} \]
\[ V(\text{titr}) = \text{total volume of titrant to reach the end point in ml} \]
\[ C(\text{titr}) = \text{Titrant concentration in eq/l (currently 0.1)} \]
\[ V(\text{sample}) = \text{sample volume} \]
\[ 2 = \text{Ratio between titrant and sample} \]

For titratable acidity in g/l of tartaric acid
Enter in the first method
The actual sample amount in the SAMPLE screen in ml
The titrant concentration in the TITRANT screen in mol/l or eq/l
2 Titrants and 1 Sample in the COEFFICIENTS display
150.09 as molecular weight
The Titration Manager gives a result according the above formula.

For titratable acidity in g/l of citric acid (that has 3 acid functions with a MW of 192.4)
Enter in the first method
The actual sample amount in the SAMPLE screen in ml
The titrant concentration in the TITRANT screen in mol/l or eq/l
3 Titrants and 1 Sample in the COEFFICIENTS display
192.4 as molecular weight
The Titration Manager gives a result according the above formula.

For Formol number expressed as meq/l
\[ \text{Formol no.} = \frac{V(\text{titr}) \times C(\text{Titr}) \times 1000}{V(\text{sample})} \]
\[ V(\text{titr}) = \text{total volume of titrant to reach the end point in ml} \]
\[ C(\text{Titr}) = \text{Titrant concentration in eq/l or mol/l (currently 0.1)} \]
\[ V(\text{sample}) = \text{sample volume in ml} \]

For formol number in meq/100ml
Enter in the second method
The actual sample amount in the SAMPLE screen in ml
The titrant concentration in the TITRANT screen in mol/l or eq/l
1 Titrant and 1 Sample in the COEFFICIENTS display (if necessary)
Enter in the RESULT screen
Result: 1
Unit: eq/l
Equation: 1
Formula: R1/10
Name: meq/100 ml
The Titration Manager gives 2 results: Expressed in meq/l; From equation formula, expressed in meq/100 ml

3 Determinations on Grapefruit Juice

<table>
<thead>
<tr>
<th>Acidity</th>
<th>Mean: 15.28 g/l tartaric acid</th>
<th>Formol Number Mean: 2.19 meq/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation: 0.02 g/l tartaric acid</td>
<td>Standard deviation: 0.038 meq/100 ml</td>
<td></td>
</tr>
<tr>
<td>Rel. standard deviation: 0.13%</td>
<td>Rel. standard deviation: 1.7%</td>
<td></td>
</tr>
</tbody>
</table>

Working Range
Related to the calculation formula, using 10 ml for sample volume and 0.1 eq/l titrant concentration. For acidity determination 1 ml of titrant corresponds to 0.75 g/l of tartaric acid. For formol number determination: Formol no. (meq/100 ml) = V(titr) in ml.
For a 10 ml burette, formol number range can be estimated between 0.2 (experimental low limit corresponding to 0.2 ml) and 10 (nominal value of cylinder capacity): Formol no. (meq/l) = Formol no. (meq/100 ml) * 10