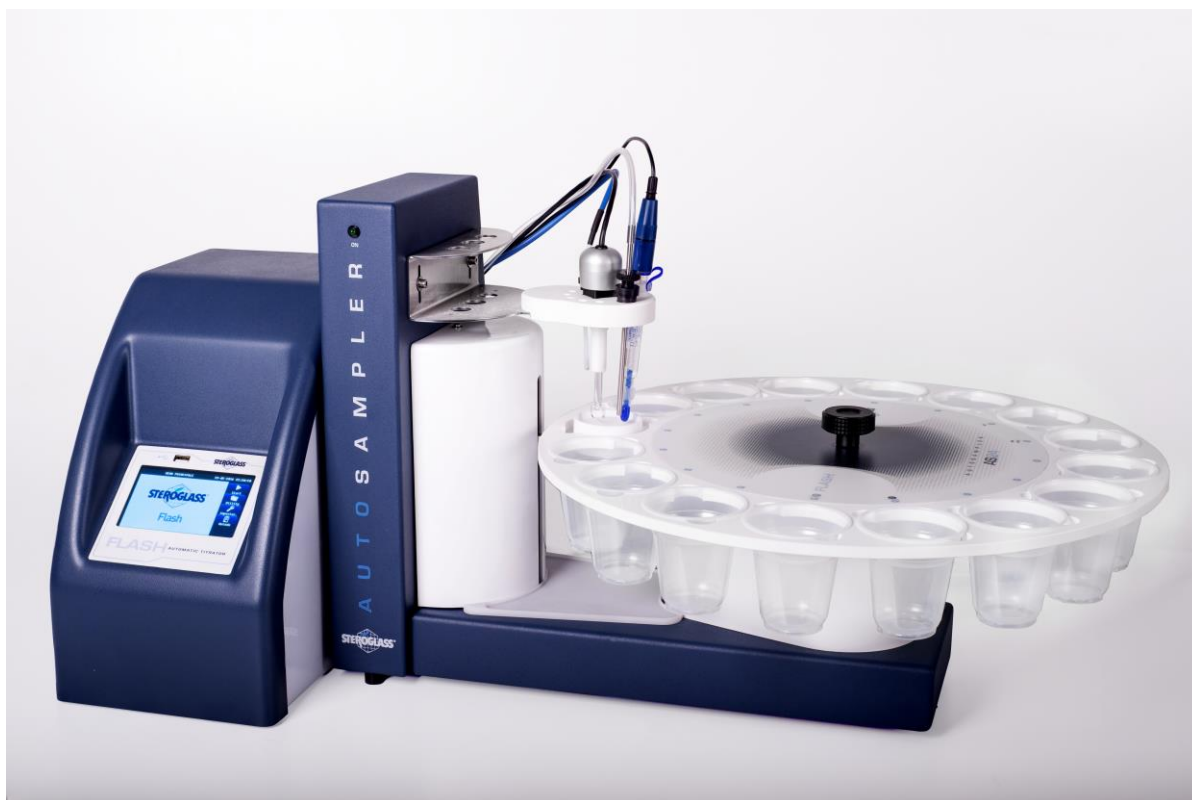


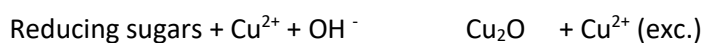


FLASH TITRATOR Reducing Sugars in Wines and Musts

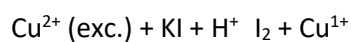


PRINCIPLE

The determination of reducing sugars in wines and musts is done following the modified Rebelein method. The aldehyde groups of the sugars are oxidized by an excess of alkaline copper sulfate solution (Fehling's solution); in a heated alkaline environment, the copper (II) is reduced to cupric hydroxide, which then precipitates as a bright red cuprous oxide:



The excess of Cu (II) is reduced by an excess of iodide (KI) in an acidic environment, with the consequent formation of iodine:



The iodine is then titrated with thiosulfate, the consumption of which is proportional to the excess of Cu (II) added to the sample, and therefore, after creating a calibration curve, it allows us to arrive at the sugar content:





INSTRUMENT AND ACCESSORIES

- Flash Titrator with at least one burette for dosing Thiosulfate 0.5M;
- 2 peristaltic pumps for dosing Sulfuric Acid 25% w/v and KI solution 30% w/v;
- If Burette 2 and a third peristaltic pump are also present, the dosing of the Fehling's solution can be automated – see APPENDIX;
- Single-position stand or AS24 Automatic Sampler;
- Combined redox electrode with Pt ring and ceramic diaphragm;
- Glass titration beakers;
- Hot plate for bringing the samples to a boil;
- Steel plate for cooling the samples.

REAGENTS

Titration solution: $\text{Na}_2\text{S}_2\text{O}_3$ 0.5 M

Fehling's A solution

Fehling's B solution

Sulfuric Acid 25% w/v

(To prepare 1 liter of solution, start from 250 grams or 140 ml of concentrated H_2SO_4 and bring to volume with distilled H_2O .)

Potassium Iodide solution (KI) 30% w/v

(To prepare 1 liter of solution, weigh 300 grams of KI technical salt, dissolve with distilled H_2O and bring to volume)

Anhydrous glucose *(for preparing standards)*

Anhydrous fructose *(for preparing standards)*

Granular pumice stone *(to promote boiling)*

SAMPLE PREPARATION

For red wines and musts, decolorizing is done with charcoal (about 1 g per 100 ml, filtering or centrifuging after a few minutes) or PVPP.

Collect 5 ml of sample (or standard) using a TD volumetric glass pipette.

Samples with a reducing sugars content greater than 25 g/l must be appropriately diluted; alternatively it is possible, using a micropipette, to dose a smaller quantity of sample (for example the dosage of 1 ml instead of 5 ml corresponds to a 1: 5 dilution of the sample).

STANDARDS PREPARATION

Considering that working with 5 ml of sample, the response of the method is good and linear up to a concentration of 25 g/l, solutions with reducing sugar concentrations of 0, 5, 10, 15, 20 and 25 g/l are used for the control of the calibration.

The standards can be prepared by accurately weighing an exact quantity of glucose and fructose (in a 1: 1 ratio) to obtain a stock solution, and then proceeding with dilutions.

More specifically, to prepare 5 standards with respective concentrations of 5, 10, 15, 20 and 25 g/l, it is necessary to weigh exactly 12.5 g of glucose and 12.5 g of fructose, dissolve them in distilled water, and bring them to volume in a 250 ml volumetric flask.

Then take exactly 5, 10, 15, 20 and 25 ml from this stock solution and bring them to volume in five 100 ml volumetric flasks.



For the convenience of the user, STEROGLASS provides ready-made standards: the sugars calibration kit, item no. SQPE067024, which contains 6 bottles with 50, 100, 150, 200, 250 and 300 g/l of sugars.
For the control of the calibration, taking into account the linearity of the method, which is 25 g/l, just take exactly 0.5 ml of the standards with the micropipette and analyze them.

PRELIMINARY OPERATIONS

Check that the burette and the fluid circuit are rinsed with and full of titrant.

Check that the redox electrode is present in the titration holder.

Dose 15 ml of Fehling's A and 10 ml of Fehling's B into the titration vessels (in GLASS) with the maximum precision and add exactly 5 ml of sample or standard.

OXIDATION OF SAMPLES BY BOILING ON A HOT PLATE:

Place the beakers on the 8-position stainless steel holder, set them on the hot plate and bring them to a boil, continuing to boil for 2 minutes.

To promote boiling, add a few grains of fine pumice stone to each beaker. Then cool the samples in the special tray connected to the tap water and proceed with the titration using the dedicated program.

TITRATION:

- 1) Go to START from the main menu and select the SUGARS method;
- 2) If connected to the autosampler, enter the number of samples present, e.g. "3", and place the beakers in the sampler;
- 3) If you want to give a name to the samples, enter the name in SAMPLE DESCRIPTION;
- 4) Press START to start the analysis.



Method Type	<i>Inflection</i>
Method Name	<i>SUGARS WINE</i>
Descript./Sample no.	<i>Sample</i>
Degassing sec:	<i>0</i>
Pump A N:	<i>1 (sulfuric acid)</i>
Pump A sec:	<i>10</i>
Pump B N:	<i>2 (KI solution)</i>
Pump B sec:	<i>6</i>
Pump C N:	<i>0</i>
Pump C sec:	<i>0</i>
Stirrer speed	<i>6</i>
Pre-stirring time	<i>5</i>
Measurement type	<i>mv</i>
Initial auto-stability (mv)	<i>10</i>
Initial auto-stability time (s)	<i>3</i>
Initial addition	<i>0.00</i>
Initial stirring	<i>3</i>
Titration burette	<i>1</i>
Addition type	<i>Progressive</i>
Addition (ml)	<i>0.15</i>
Limit volume (ml)	<i>9.5</i>
Polarization value	<i>NA</i>
Auto-stability (mv)	<i>2</i>
Auto-stability time (s)	<i>3</i>
Max. stability time (s)	<i>60</i>
Factor	<i>35.7143</i>
Concentration (mol/l)	<i>0.5</i>
Sample volume (ml)	<i>5</i>
Result unit	<i>g/l</i>
Number decimals	<i>2</i>
Approaching factor	<i>50</i>
Blank (ml)	<i>8.000</i>
Washing type	<i>Washing position</i>
Washing time (s)	<i>5</i>
Reagent standardization	<i>NO</i>
Equation type	<i>Equat. "A"</i>
Titration direction	<i>Decreasing</i>
Minimum derivative	<i>10</i>

RESULTS

The program shown here expresses the results directly in g/l of reducing sugars.

The stoichiometric reaction involves a consumption of sodium thiosulfate titrant of:

0 g/l	8.0 ml	(Blank)
5 g/l	6.6 ml	
10 g/l	5.2 ml	
15 g/l	3.8 ml	
20 g/l	2.4 ml	
25 g/l	1.0 ml	

In calculating the result, the Flash uses "Equation A"
i.e. BLANK - Equivalent volume

NOTE

Some parameters of the program shown here have been compiled as an indication: they can be optimized according to the operating conditions and the samples analyzed, in order to improve the accuracy and/or speed of the analysis.



APPENDIX: AUTOMATIC FEHLING DOSING

Method Type	Free SO ₂
Method Name	FEHLING DOSING
Polarization value	200
Pump level N	3 (Fehling's B)
Pump level sec.	14
Acid Pump N	0
Acid Pump sec.	0
End Point value (uA)	5.000
Titration burette	2
Addition (ml)	2.00
Limit volume (ml)	13.00
Initial addition	9
Auto-stability (uA)	0.100
Auto-stability time (s)	0
Max. stability time (s)	60
Stirrer speed	2
Pre-stirring time	0
Initial stirring	0
End titration delay	0
Factor	64000.000
Concentration (mol/l)	0.01
Sample volume (ml)	50
Result unit	DOSING
Number decimals	0
Descript. Sample no.	Sample
Washing type	Washing position
Washing time (s)	5
Reagent standardization	NO

NOTES:

If you wish to increase automation and eliminate operator error in the dosing of Fehling's A and B, you can enter and launch the following program for automatic dosing BEFORE adding the sample.

Peristaltic pump number 3 must be connected to the bottle of Fehling's B and will dose 10 ml.

Titration Burette 2 must be connected to the bottle of Fehling's A and will dispense 15 ml.

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