

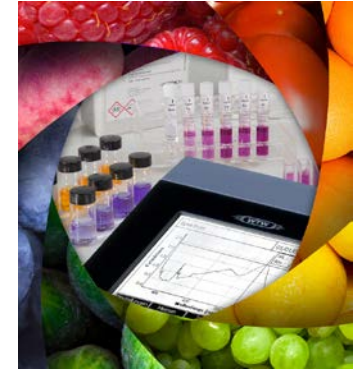
Special Photometric Procedures and Multi-step Measurements

- General Overview of Measurement & programming principles with photoLab[®] 7000 Series
- Programming of Chlorophyll method: step by step
- Wine quality factors: Colour intensity and nuance
- Overview of sugar determination principle with enzymatic test kits: Glucose, Sucrose, Fructose



Programms and Programming:

- **Concentrations measurements:**
Programmed methods for commercial test kits
User calibration curves e.g. for Uranine
- **Multi-step measurements:**
Chlorophyll methods for environmental analytics,
Food (nutrition), R&D...
- **Enzymatic test kits**
available from various manufacturers,
complex handling eased by (user-defined) programs
- **Multi-wavelength measurements**
e.g. wine colour
- **Extense Programming options**



Programming with photoLab[®] 7000



Special / Multi wavelengths | 10/16/14 14:55

Please select method for measuring!

Setup | Method list | Last method | New Method

Method numbers 2xxx: the next available number is offered automatically

Special / Multi wavelengths | 10/16/14 14:56

Display absorbance ✓

AQA

Edit method

New method

Measurement data memory

Edit existing user method:
F1 key

New method
F4 key

Edit method | 10/16/14 14:59

Number **2006**

Name _____

Version _____

Citation form _____

Unit _____

Resolution 0.01

Cell 10 mm

Lower limit of measuring range _____

Upper limit of measuring range _____

Method list | Delete | Next

Operators, Variables, Conditions...

R = Result

K = Variable in formula, to be defined

A = Absorption A_{xxxnm}

Same absorbance at different steps

=> Index to be entered for second and more absorption

measurements at same wavelength: A_{xxxnm_2} ; A_{xxxnm_3} ...

F-Keys for easy operation:

Operators via **F2**

Operators			
+	-	.	/
()	^	Pi
sin	arcsin	sinh	arsinh
cos	arccos	cosh	arcosh
tan	arctan	tanh	artanh
log	ln	10^	e

F1: Back

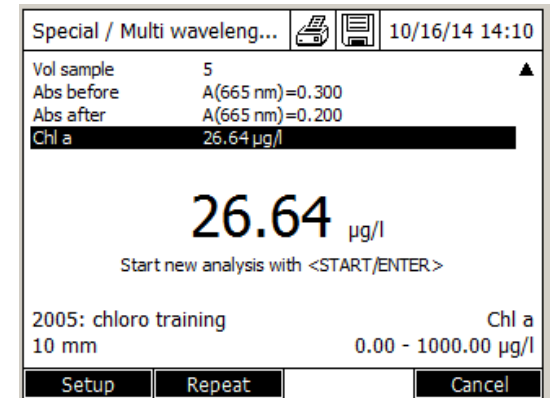
F4: Next

Variables via **F3** (A_{xxxnm} , K1, K2...)

F4: Next

Chlorophyll measurement is needed in many applications:

- Environmental Analysis
- Algae content in food & beverage industry
- Required procedures vary according to application field!



Easy programming according to application requirements

- Sample preparation according to standard methods:
Chl is extracted, and a small volume of the extract is measured photometrically
- The determination of the Chlorophyll is done by measuring the difference of the absorbance before and after adding HCl.
- The final formula of Chlorophyll take sample and extraction volume into the automatic calculation.

Programming Chlorophyll Measurement

Edit method	10/16/14 13:55
Number	2005
Name	chloro training
Version	schul
Citation form	Chl a
Unit	µg/l
Resolution	0.01
Cell	10 mm
Lower limit of measuring range	0.00 µg/l
Upper limit of measuring range	1000.00 µg/l



Wavelength	10/16/14 13:56
Wavelength 1	665 nm

Back Add Next



Procedure variables	10/16/14 13:57
<p>Procedure variables are variables whose current numerical values have to be entered during the course of the measurement (e.g. weighted sample or dilution).</p> <p>If a procedure variable is required to calculate the result: Create a procedure variable (K) with <Add>.</p>	

Back Add Next

Programming Chlorophyll Measurement

Procedure variables	10/16/14 13:56		
K1	Vol Extrak		
K2	Vol Probe		
Back	Add	Delete	Next

Formula entry	10/16/14 13:59		
This is the final formula:			
$R = 29.6 \cdot (A_{665 \text{ nm}} - A_{665 \text{ nm}_2}) \cdot (K_1/K_2)$			
Back	Operators	Variables	Next

Formeleingabe	16.10.14 13:31			
<table border="1"> <tr><td>Variablen</td></tr> <tr><td>A(665 nm)</td></tr> <tr><td>K1 (Vol Ext... K2 (Vol Pr...</td></tr> </table>		Variablen	A(665 nm)	K1 (Vol Ext... K2 (Vol Pr...
Variablen				
A(665 nm)				
K1 (Vol Ext... K2 (Vol Pr...				
R =	<input type="text"/>			
Zurück	Operatoren	Variablen	Weiter	

F4

Here, it is important to indicate the sequence of measurement steps by assigning an index to the absorbance measurement (2, 3...).

Subsequently a clear description to each single step can be entered for user-guidance on display.

Programming Chlorophyll Measurement

Condition	10/16/14 14:02
<p>Here you can enter a formula for a condition. The measured value is only valid if this condition is met.</p>	
Back	Operators
Variables	...

- ⇒ Using F3 will show an R for Result which can be defined: e.g. for $R < 0$ wrong the resulting display ---- mg/l
- ⇒ To correct condition, use back space until back to left screen



Edit method	10/16/14 14:06
Sequence	Designation
Measurement 1	Abs before
Measurement 2	Abs after
Back	Next

Wine Colour: An Important Quality Factor



Colour Measurement in Beverage / Wine Industry ...

... is important for:

- Wine growers
- Third party bottlers' income quality control.

Photometric colour measurement offers:

- **Accuracy and comparability** (versus human eye, comparator ...)
- **Reproducibility**

Bottlers need several routine testings besides color:

- Testing Water parameters, sugar content, ...
- Turbidity (in many other beverages)
- COD before disposing water to municipal wastewater plant

Wine Colour = Multi- λ measurement

Wine characterization by colour

Colour Intensity = Sum

Absorbance measurement @ λ 420nm, 520nm and 620nm

Abs₄₂₀ for yellow/orange/ocker

Abs₅₂₀ for red

Abs₆₂₀ for blue (not always included)

$$R = \text{Abs}_{420} + \text{Abs}_{520} + \text{Abs}_{620}$$

$$R = \text{Abs}_{420} + \text{Abs}_{520}$$

Colour nuance

Ratio of brown/red nuances

$$R = \text{Abs}_{420} / \text{Abs}_{520}$$



< 0,8 violet, 0,8-1,2 red, > 1,2 orange

Image source: perrotwein@bluewin.ch

Determinations of Sugar (Glu...) with enzymtic test kits, e.g. with



Understanding Enzymtic Test Kits

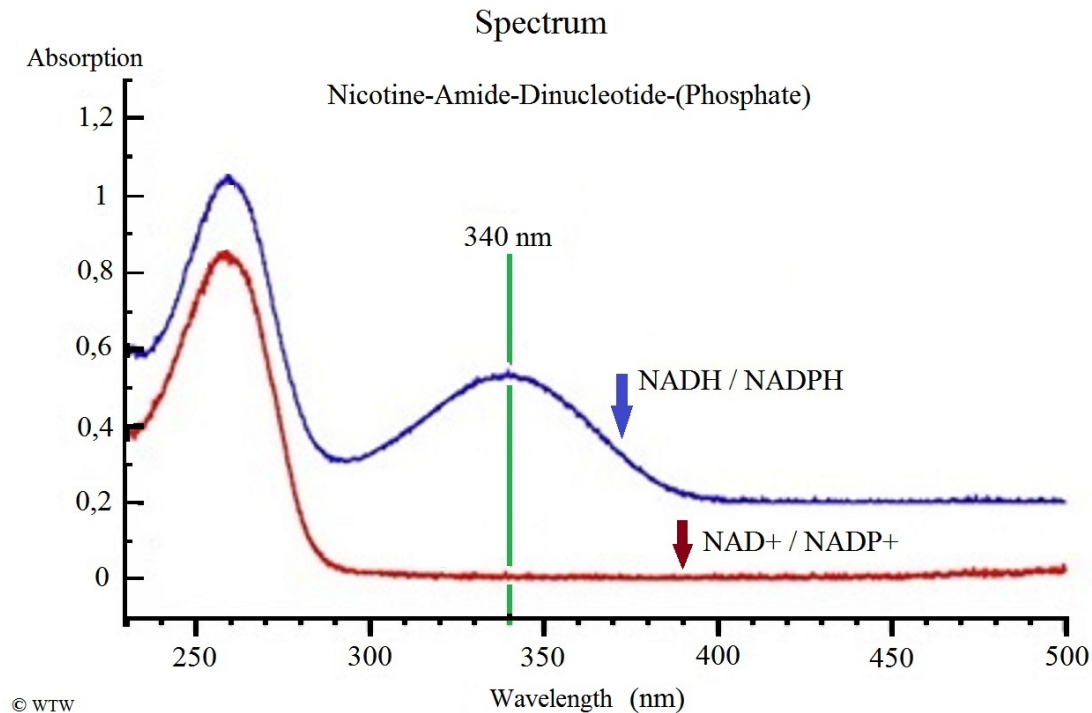
Sugar (D-Glu, D-Fru, Sucrose etc.) are important parameters in Wine, Food & Beverage

- Test kits are based on **enzymatic reactions** for many substances
- Enzymes are specific to chemical reactions and substances (substrates)
- Some tests offer **multi-step** measurements for determinations of individual sugar molecule concentration such as Sucrose, Glu and Fru
- Enzymatic test kits are gradually replacing other standard methods
- Are **accepted by many International Organisations** in Food & Beverage
- Enzymes „co-operate“ with metabolic „energy provider“ (ATP) and Co-enzymes offering a Redox system, here **NAD⁺/NADH and NADP⁺/NADPH**

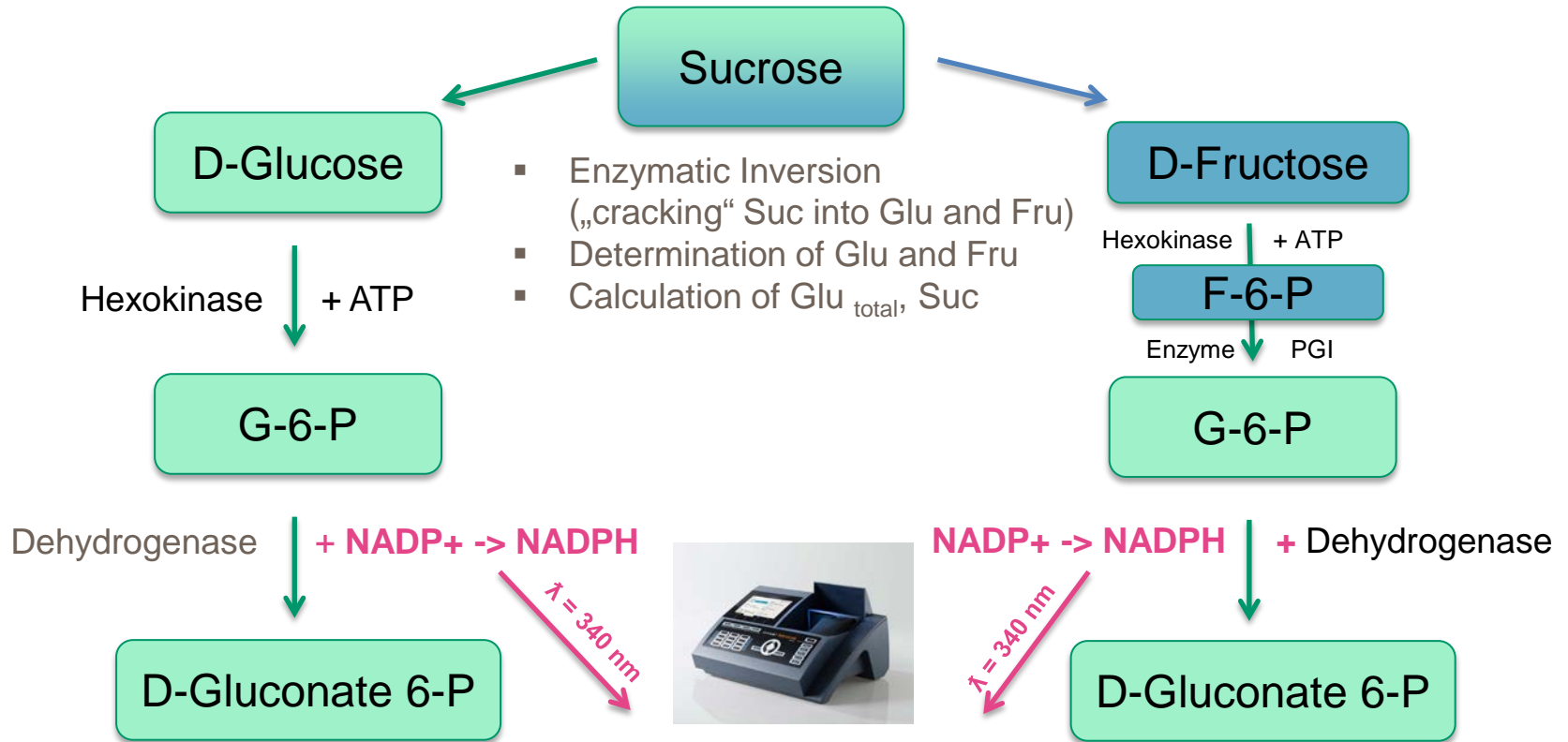
The Hub: NAD⁺/NADH co-enzyme

NAD⁺/NADH and NADP⁺/NADPH is a standard step in enzymatic reactions.

In reduced form, these redox systems show a peak at 340 nm.



Scheme of Suc, Glu, Fru determination



1 NADP is equivalent to 1 D-Glu / 1 D-Fru

Glu_{total}: Σ of Glu_{free} and Glu_{bound}

Suc / Fru: Glu_{free} determination before and after „cracking“ of Sucrose

Enzymatic Test Kits ...

.... provide:

- all necessary information on substances to enter formula
- all enzymes needed
- all co-enzymes such as NADP⁺, ATP etc.
- AQA material



The transfer of reaction into a simplified photometric routine may need several steps and formulae.