

**Water content in industrial food –
Is scientific evidence enough to
establish an analytical standard method?**

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6th International Conference on Water in Food
Reims/France, 22-23 March 2010

**In trade,
the value of goods depends on
quality and
on the contents
of certain components.**

It is therefore necessary

to analyse products

and to determine

the content or

concentration of components

that decide the quality and the price.

Why is this problematic ?

Differences in analytical methods

may lead to

seemingly (!) different concentrations

and different quality grades.

Consequence:

Analytical methods should be harmonised.

Why can this be problematic ?

Official methods are usually adopted by heterogeneous bodies that do not consist of scientists only.

Economic and political reasons may influence decisions.

**A certain group
or even a single powerful company**

may influence

**the introduction or the refusal of a new
method,**

**if this method affects the price of their
products.**

**This may lead to the situation
that an official method is
scientifically incorrect.**

**Here we have the clash of
scientific truth
and economic interests !**

An example of

such a situation is given.

The price of dairy powders is fixed on the basis of dry matter.

Determination of water content is therefore an important analysis,

particularly for economic reasons:

The vendor wants to „find“ a water content as low as possible, the buyer as high as possible.

Methods to analyse water content should determine:

Water,

all the water and

nothing but the water.

Water content is very often determined by drying techniques.

Principle problems:

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1. Drying techniques do not distinguish between water and other volatile substances.

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1. Drying techniques do not distinguish between water and other volatile substances.
2. Strongly bound water may not be detected.

Consequence:

Result of drying techniques is not water content (!),

but mass loss under the applied (and principally arbitrary) conditions !

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Result of drying techniques is not water content (!),

but mass loss under the applied (and principally arbitrary) conditions !

This mass loss is often called moisture.

**Coming back to the question of
an analytical method for
determining water
in dairy powders**

**Practically all dairy powders
contain lactose.**

Lactose problem

Scientific aspect:

Lactose exists in different forms:

α -lactose: 1 mole water of crystallisation per mole (5% by mass), so-called lactose monohydrate,

β -lactose: anhydrous,

amorphous lactose: anhydrous, but water in interstices.

Real lactose samples may be mixtures (depends on production conditions).

In all cases, additional water can be adsorbed to the surface.

For dairy products,
drying at 102 °C for 2 hours is common.

Problem:

Water of crystallisation in lactose
is only partially detected.

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is only partially detected.

Consequence:

**Result of drying (mass loss) of dairy powders is
neither the surface („free“) water
nor the total water content.**

Lactose problem

Economic aspect:

Dry matter (DM) is calculated from the total mass (m_0) and the water mass (m_W) contained in the product:

$$DM = m_0 - m_W$$

Water mass can be calculated from water content (WC):

$$WC = m_W / m_0 \quad \rightarrow \quad m_W = WC \cdot m_0$$

$$DM = m_0 - WC \cdot m_0 = m_0 \cdot (1 - WC)$$

Dry matter (price!) falls with rising water content.

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The results of an international collaborative study were not taken into account.

How does the reference method work ?

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**5 g sample are heated
in containers
at 87 °C for 5 h
in a stream of
dried air (33 ml/min).**



How does the reference method work ?

The mass loss is defined as moisture content (without checking mass constancy).



As alternative,

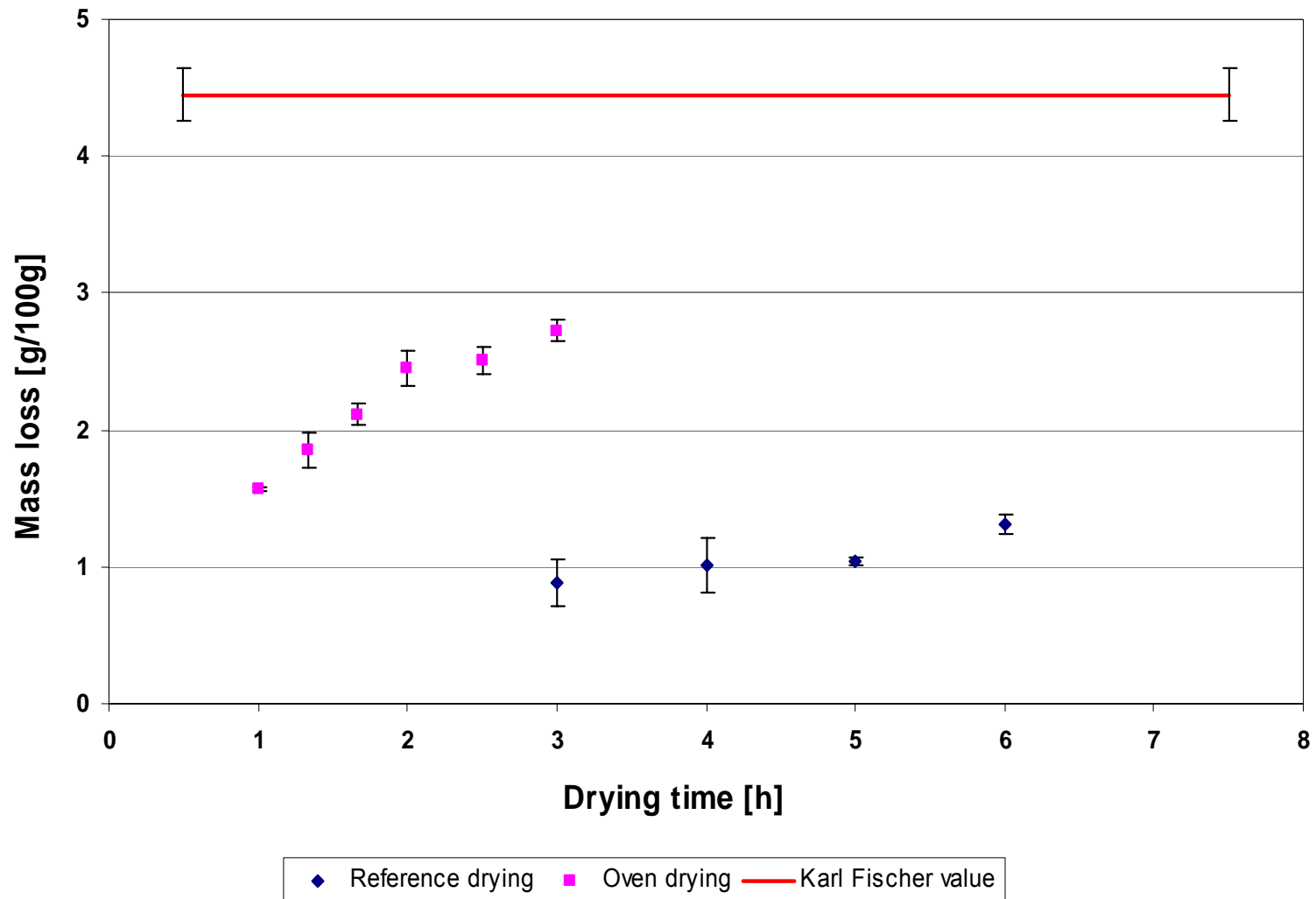
**a Karl Fischer method,
based on a selective chemical reaction,
was developed and suggested.**

**Investigations have been made to compare
classical oven drying (OD),
the reference drying method (RD),
the Karl Fischer titration
for various dairy products.**

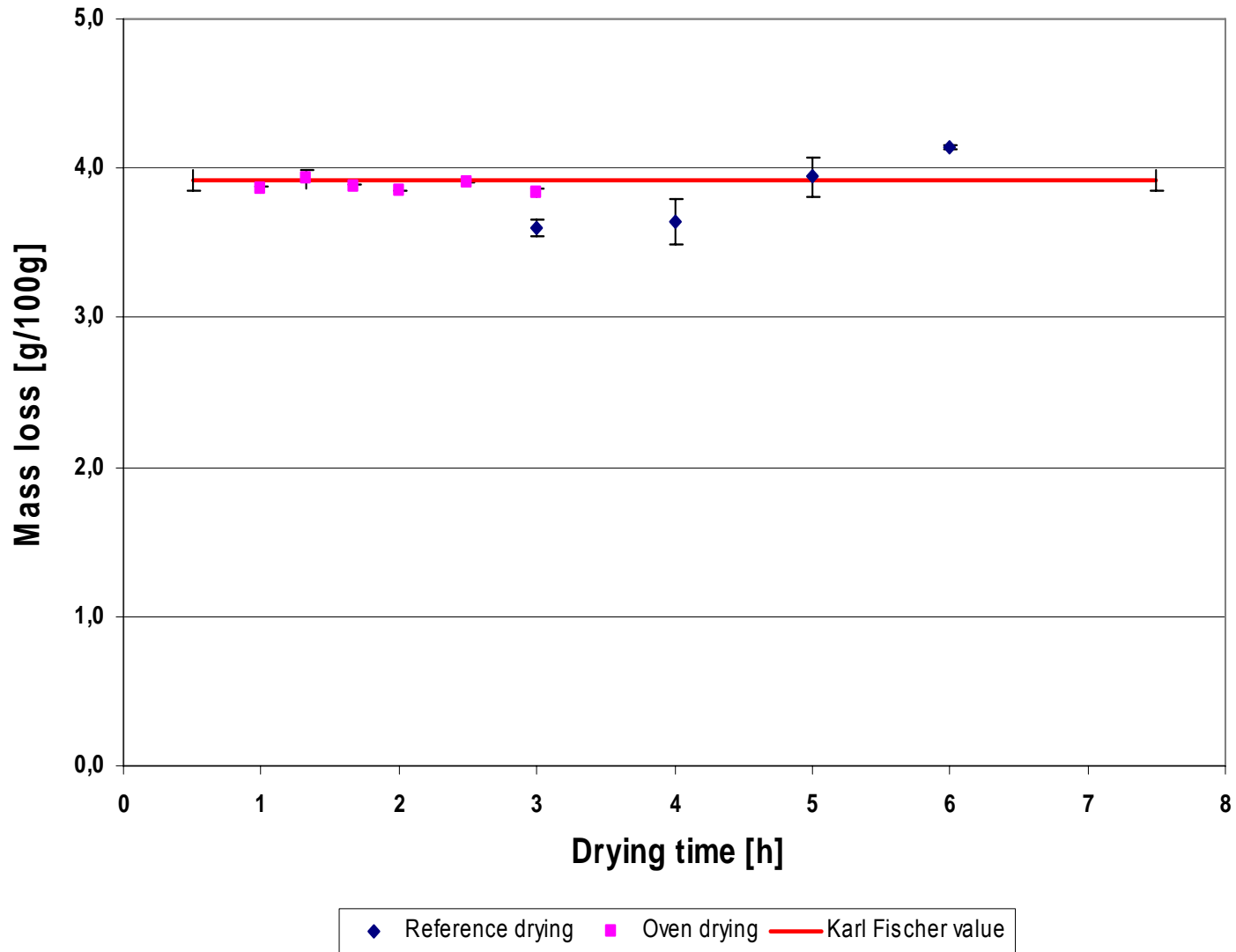
**The drying results were observed
after various drying times.**

**Results for water content by Karl Fischer titration (KFT) and for mass loss by oven drying (OD) after 2 h and by “reference drying” (RD) after 5 h;
n = number of replicates**

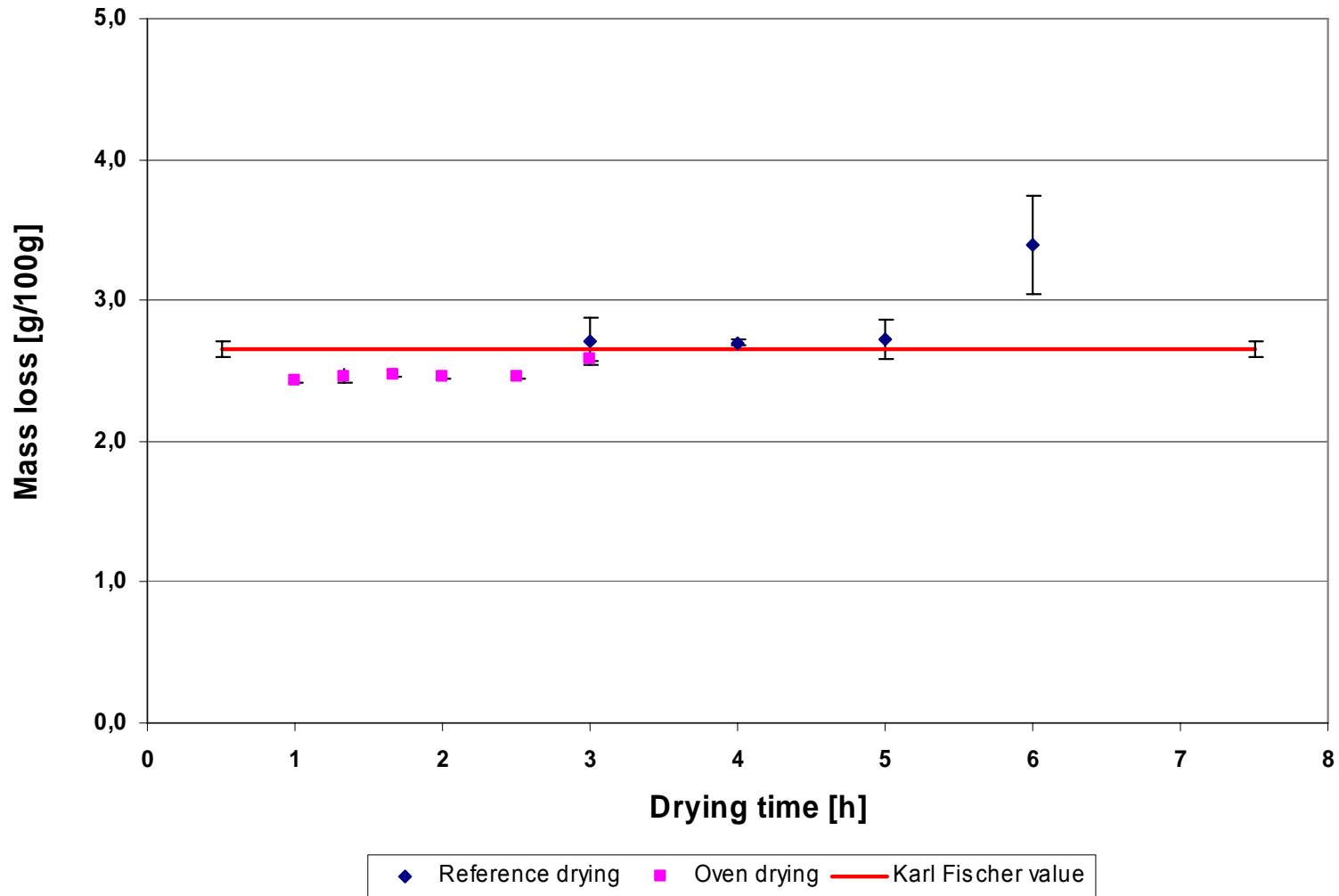
Sample	Water content by KFT (n=5) [g/100 g]	Mass loss by OD (n=2) [g/100 g]	Mass loss by RD (n=2) [g/100 g]
Lactose	4.45 ± 0.19	2.45 ± 0.13	1.04 ± 0.03
Skim milk powder	3.92 ± 0.07	3.85 ± 0.00	3.94 ± 0.13
Full cream milk powder	2.65 ± 0.05	2.46 ± 0.02	2.72 ± 0.14
Whey powder	4.46 ± 0.05	2.12 ± 0.01	2.24 ± 0.07



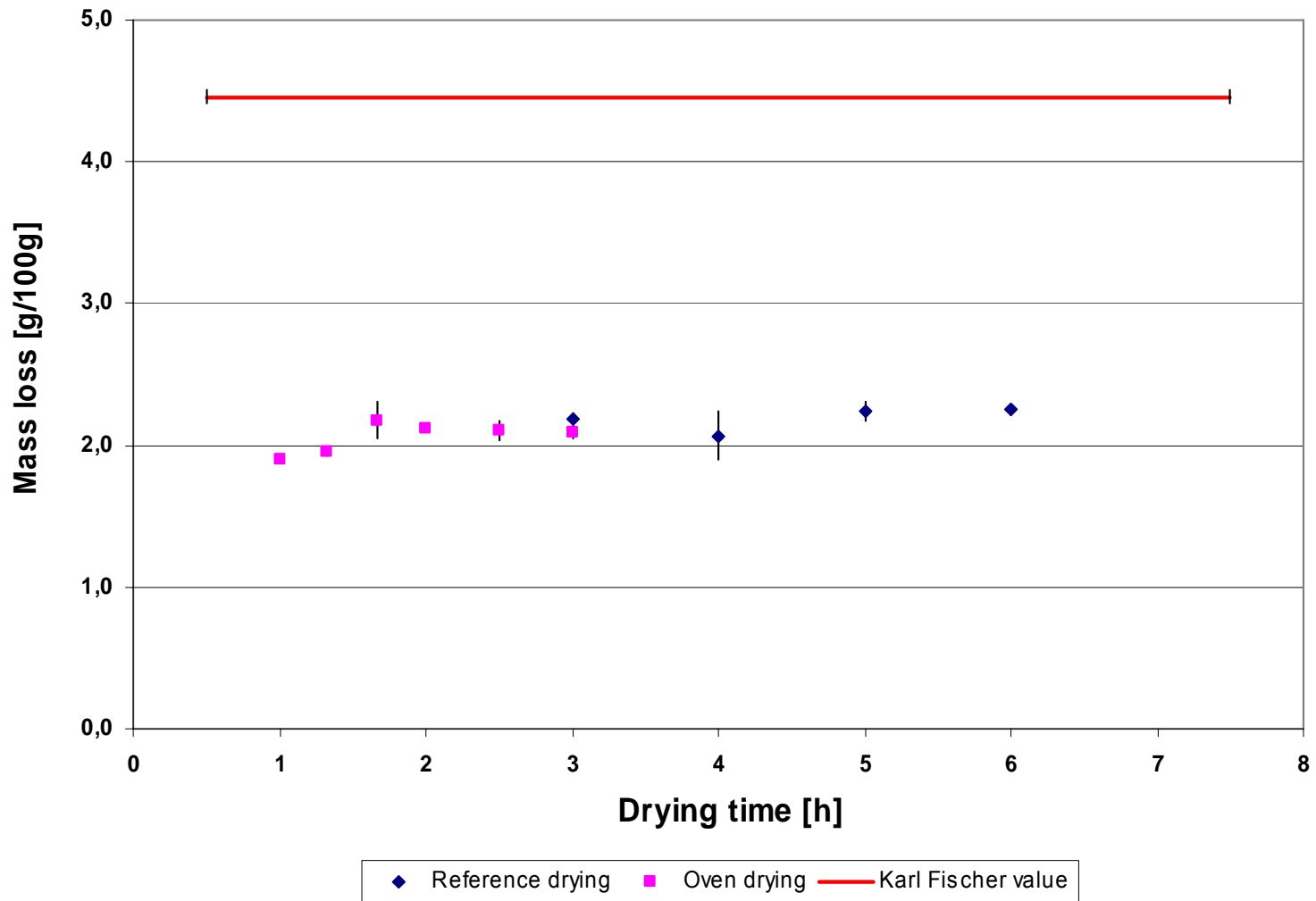
Mass loss by “reference drying” and oven drying after various drying times of ***crystallised lactose*** and – for comparison and reference – the water content by Karl Fischer titration (also in g/100 g).



Mass loss by “reference drying” and oven drying after various drying times of **skim milk powder** and – for comparison and reference – the water content by Karl Fischer titration (also in g/100 g).



Mass loss by “reference drying” and oven drying after various drying times of **full cream milk powder** and – for comparison and reference – the water content by Karl Fischer titration (also in g/100 g).



Mass loss by “reference drying” and oven drying after various drying times of **whey powder** and – for comparison and reference – the water content by Karl Fischer titration (also in g/100 g).

Moisture content of milk powder samples determined by “reference drying” at different flow rates, n = number of replicates

Sample	Moisture content in g/100 g at gas flow rate	
	33 ml/min	>33 ml/min
17	2.44 ± 0.54 (n = 3)	2.83 (n = 2)
18	3.38 ± 0.17 (n = 3)	4.35 (n = 2)
21	3.26 ± 0.34 (n = 5)	3.93 (n = 2)
28	5.91 ± 0.40 (n = 3)	6.06 (n = 2)

**Moisture content (MC) of a milk powder sample by “reference drying”
using different sample sizes;
2 replicates for sample sizes 2, 3, 4 and 6 g,
5 replicates for sample size 5 g**

Sample size in g	2	3	4	5	6
MC in g/100 g	5.31	4.21	4.43	4.10	3.73

**Moisture content (MC) of a lactose sample by “reference drying” at 102 °C in dependence of drying time,
n = number of replicates**

Drying time in h	2	3	4	5	6	7
MC in g/100 g (n)	1.79 (2)	2.37±0.56 (4)	2.65±0.51 (6)	3.00±0.32 (6)	3.17±0.30 (4)	3.35 (2)

Conclusions:

- 1. The mass loss (“moisture”) determined with the reference method (by drying) corresponds approximately to the water content found by Karl Fischer titration**

Conclusions:

1. The mass loss (“moisture”) determined with the reference method (by drying) corresponds approximately to the water content found by Karl Fischer titration

in the case of ordinary milk powders,

but not for lactose and products with high lactose content.

2. The reference drying method does not determine „free“ water only (which was claimed by the defenders of this method), but includes part of the „bound“ water.

This had already been proven by an international inter-laboratory trial !

3. The entity measured by the reference drying method is, therefore, not defined.

- 4. The Karl Fischer titration detects water, all the water and nothing but the water.**
- 5. The Karl Fischer titration is generally applicable on all dairy powders.**
- 6. The Karl Fischer titration would be a better (and scientifically correct) reference method.**

7. The customers pay an unjustified price.

They buy water at the price of dairy powder.

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They buy water at the price of dairy powder.

This was possibly the reason for the resistance of industry against a scientifically correct method.

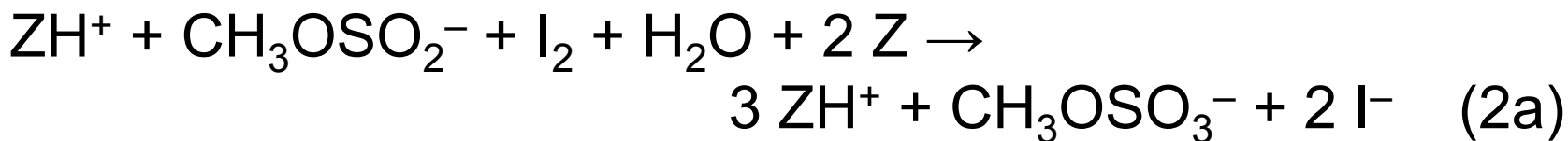
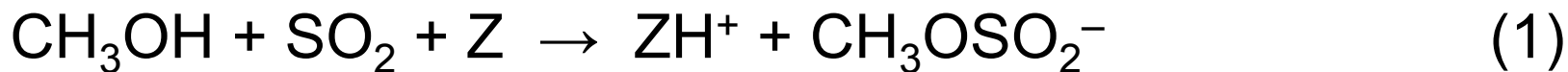
This example shows that

**scientific evidence and argumentation
may be not convincing enough
to introduce standards or
reference methods
against
commercial and economic interests.**

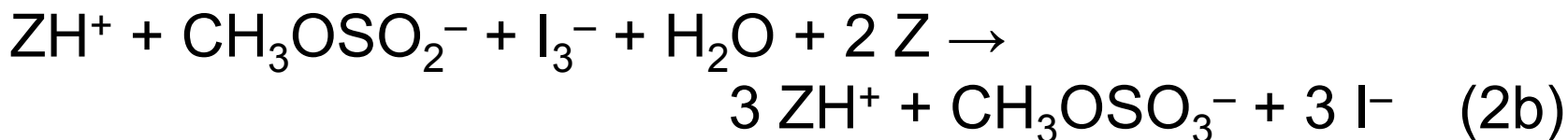
This is a very unfortunate situation.

Thank you !

Karl Fischer titration:



or



Z = Base, mostly imidazole and no longer the “historic” pyridine

1 mol I₂ indicates 1 mol H₂O.

From the consumption of I₂
the amount or the concentration of H₂O can be calculated.

End-point detection:

General:

Karl Fischer reaction takes place in the so-called working medium which is placed in the titration cell.

Before introducing a sample, this working medium is titrated to dryness (“conditioning” of the titration cell).

Then, the sample is titrated. Water content is calculated from the amount of iodine consumed and the sample size.

The titration cell is kept dry (is “conditioned”) for the next analysis.

End-point detection:

The end point is reached with the first excess of iodine.

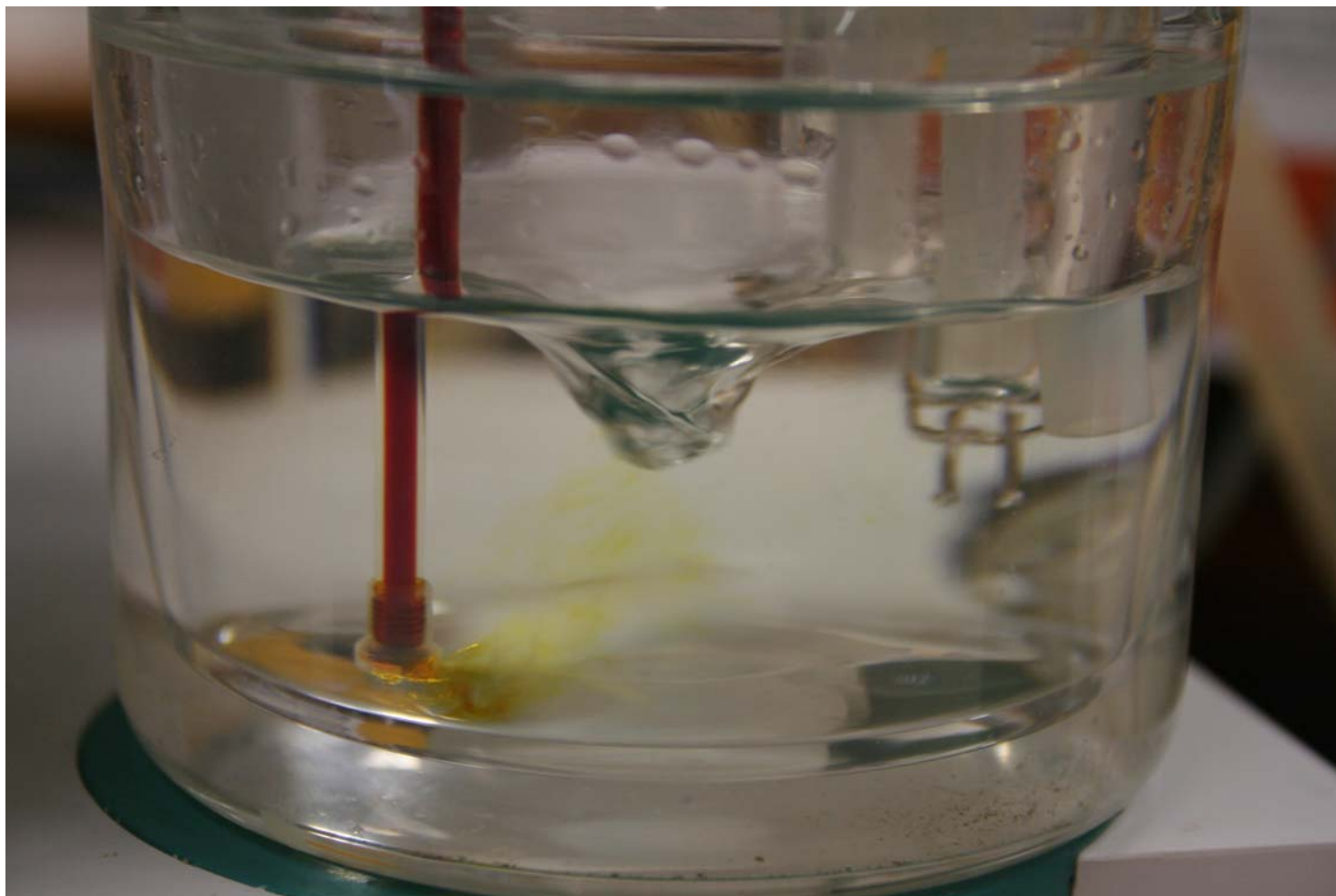
The redox couple I_2/I^- is then present in the working medium.

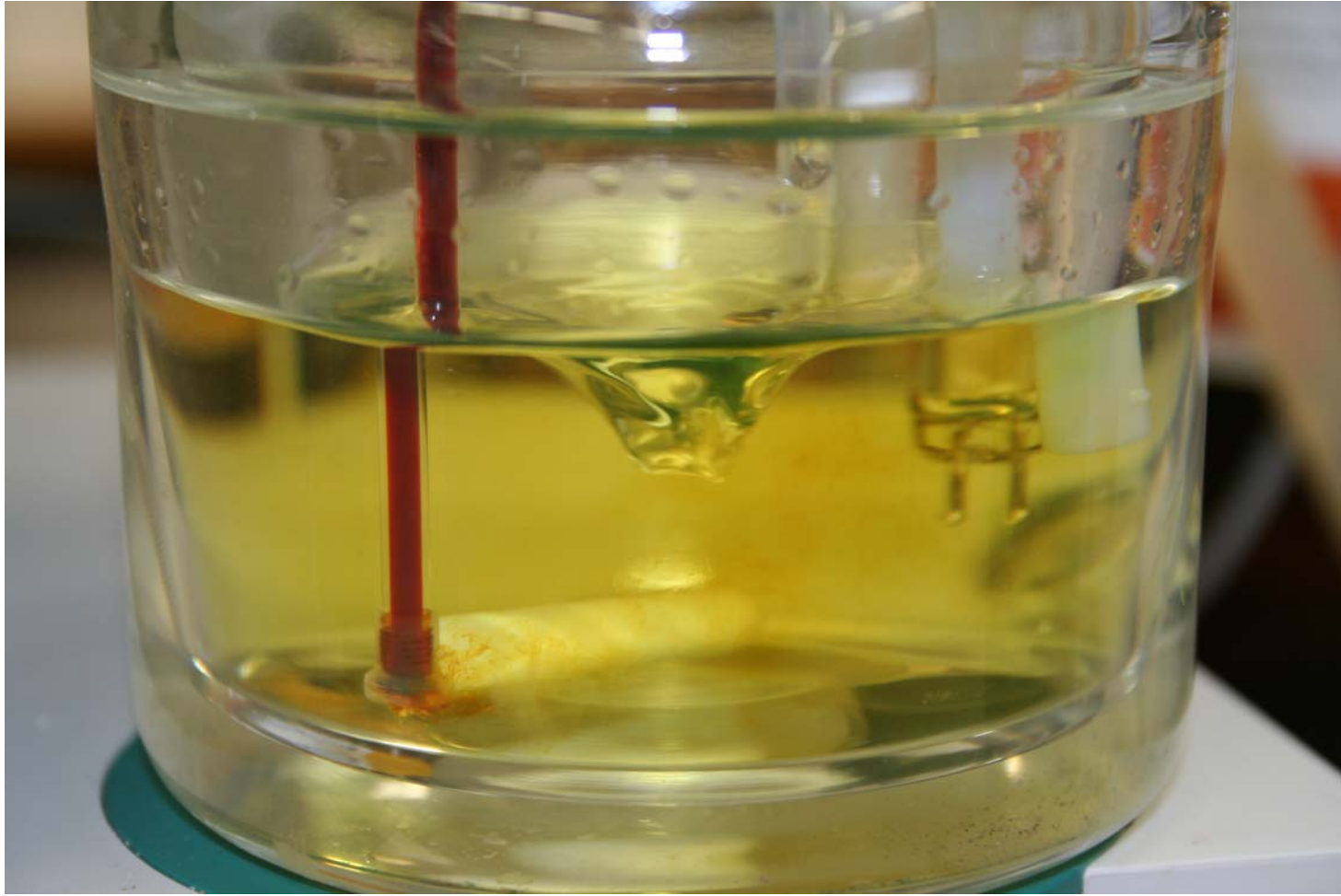
End-point detection:

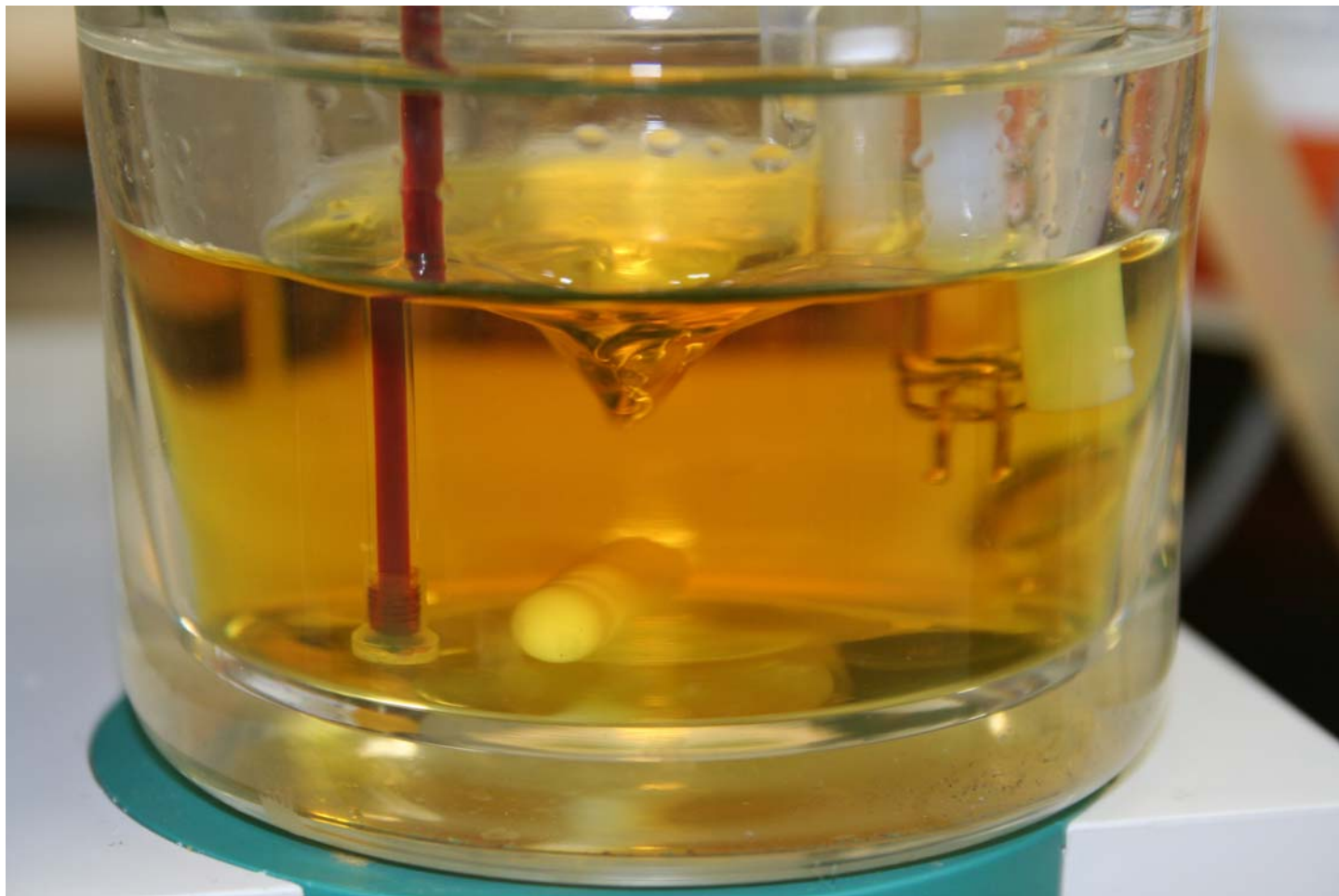
Voltametric technique:

A pair of Pt electrodes is submerged in the working medium.
It is polarised by a constant current.

The voltage to maintain this current is monitored.







End-point detection:

Before reaching the end point:

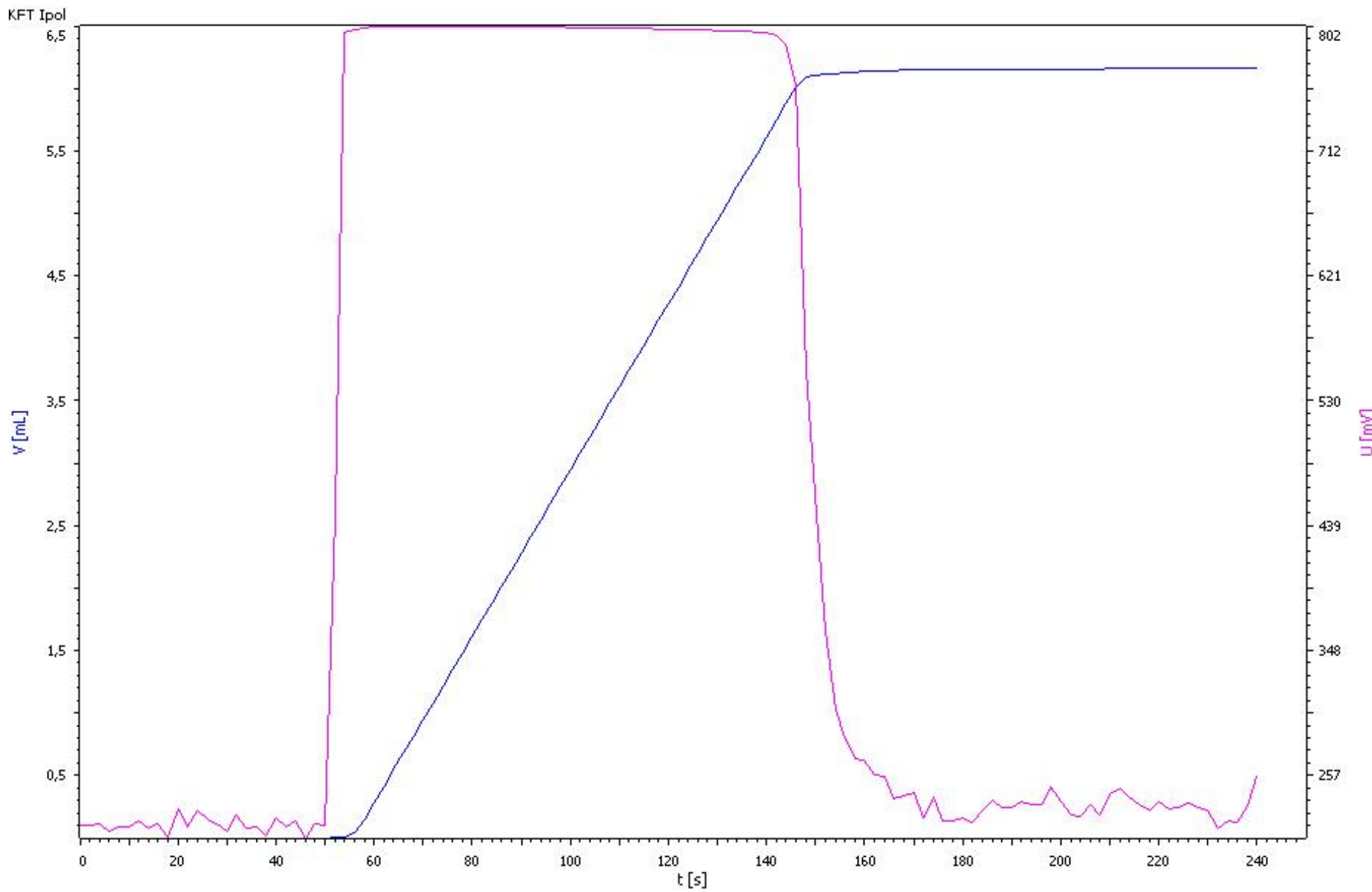
Anode reaction: oxidation of I^-

Cathode reaction: reduction of a compound (e.g. methanol)
(needs high energy)
→ voltage is high

After reaching the end point:

Anode reaction: oxidation of I^-

Cathode reaction: reduction of I_2
(needs low energy)
→ voltage is low (drops abruptly)



End-point detection:

A voltage between the value at the start of the titration and 0 V is chosen as „stop voltage“ and, thus, end-point criterion:

The titrator stops the addition of iodine, when the voltage has reached a value below the stop voltage.

End-point detection:

Water must get in immediate contact with the reagents to react.

Water of insoluble or partly insoluble samples is not immediately available.

It reaches the working medium only after some time by diffusion and extraction processes.

The titration must therefore not be stopped definitely after the first reaching of the stop voltage.

A stop delay time must be programmed.

The voltage must remain below the stop voltage during this time.

End-point detection:

Drift as end-point criterion:

During conditioning the cell very small quantities of iodine are continuously necessary to keep the cell dry, because traces of water intrude into the cell from the environment.

This consumption is the „drift“ of the cell.

The value of the drift before and after the titration is the same.

It can therefore serve as end-point criterion:

The analysis is ended, when the drift has reached the same value (which was measured) as before starting the titration.

Water equivalent or titre of the titrating solution (applies only when iodine is added in solution)

It indicates, how much water is detected by 1 ml of titrating solution.

It is determined by titrating a known amount of water.

Possible samples:

Pure water

Sodium tartrate 2-hydrate (15.66% water by mass)

Commercial water standards with certified water content.

Titration techniques:

Coulometric technique:

Iodine is produced in the working medium from iodide by anodic oxidation.

Volumetric technique:

Iodine is added in a solution (having a certain water equivalent).

Coulometric technique

Absolute method:

Water equivalent is not relevant.

The amount of iodine necessary for reaction with water is calculated from the electrical charge necessary for the oxidation of iodide to form iodine until the end point is reached.

Very sensitive method applied for traces of water. Titration cell must not be opened for sample introduction. Samples are injected through a septum and must therefore be liquid or dissolved.

Volumetric technique

Two variations exist (each with specific advantages).

One-component technique:

All the Karl Fischer components (I_2 , SO_2 , base) except methanol are in the titrating solution (dissolved in an appropriate solvent).
The working medium is methanol.

Two-component technique:

The titrating solution is a solution of iodine in methanol.
The working medium contains SO_2 and the base dissolved in methanol.

Calculation of water content in the coulometric technique

Water content is calculated from

sample size,
charge for iodine production,
(current used multiplied by time of production)
iodine produced for drift correction (subtracted),

blank value of solvent (if sample was injected in solution).

Calculation of water content in the volumetric technique

Water content is calculated from

sample size,
water equivalent of the titrating solution,
volume of titrating solution added,
volume for drift compensation (subtracted),

blank value of solvent (if sample was injected in solution).

Automation

Several techniques exist for automation.

Measures to make the sample water available

Long titration times

A minimal titration time can be imposed to guarantee that all the water has reached the working medium.

This is often called „extraction time“.

During this time the analysis must not be stopped, even if the end-point criterion should (temporarily) be fulfilled.

Measures to make the sample water available

Long stop delay time

Instead of a short delay time (5 to 10 s),
a longer time can be chosen (30 s) to “wait” longer
for delayed water.

Drift as end-point criterion

Measures to make the sample water available

External extraction

The sample water is extracted for a longer time prior to titration.

An aliquot is then titrated.

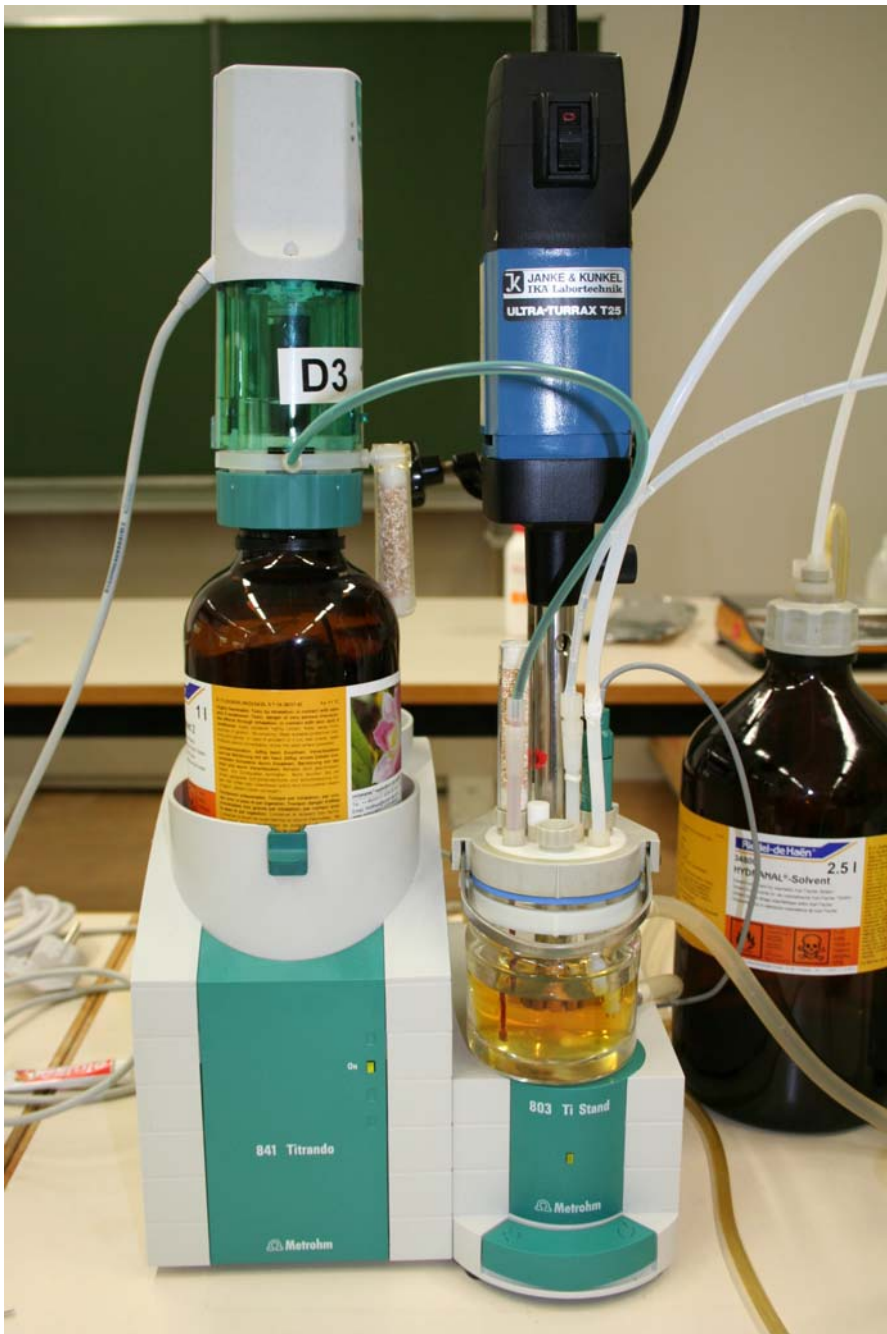
In automated systems the extraction vessel can be used as titration cell.

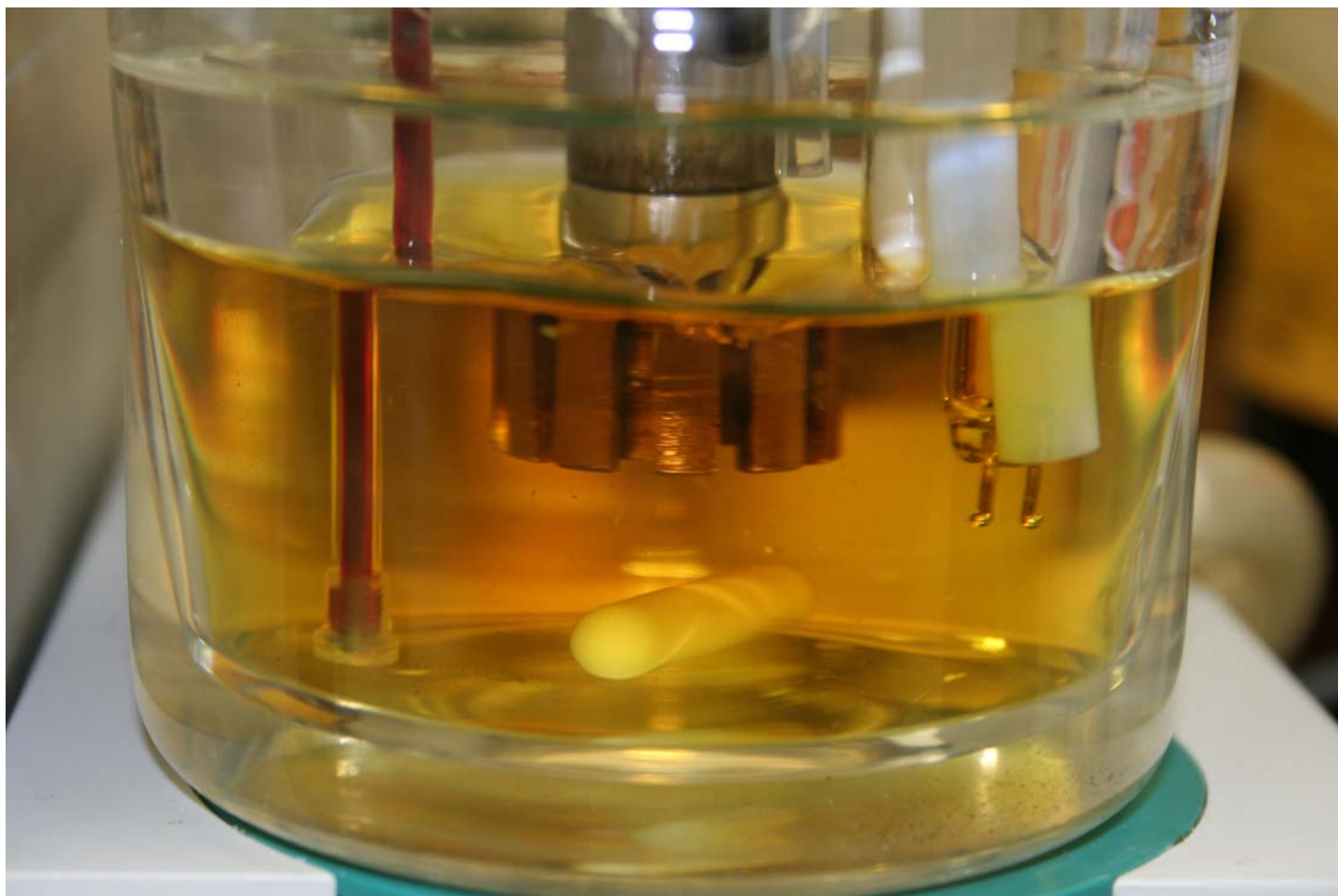
Measures to make the sample water available

Particle size

The sample can be ground or cut (risk to loose water!) before introduction into the cell.

The reduction of sample size can be carried out in the cell using an internal homogeniser.







Measures to make the sample water available

Elevated temperature

Titration cells with thermostatic jackets allow titrations at higher temperatures.

In special cells titrations are even possible at the boiling point of the working medium.

Measures to make the sample water available

Polarity of the working medium

The solubility of samples can be improved by adding appropriate polar or non-polar solvents.

In particular cases methanol can be replaced by another alcohol.

Measures to make the sample water available

The various measures can usually be applied simultaneously.

Chemical interferences:

Iodine might be reduced or iodide be oxidised by a component in the sample (e.g. peroxides).

⇒ too high result

A compound in the sample might react with a Karl Fischer reagent and produce (consume) water (e.g. carbonyl compounds, acids, esters).

⇒ too high (low) results

The sample might contain substances which react, after dissolution, with each other and produce water (carbonates and solid acids).

⇒ too high results

Possibilities to avoid chemical interferences:

- Working at lower temperatures
- Adding of buffers
- Using other alcohols
- Quantitative determination of interfering substance
- Graphic evaluation of titration curve

- Renouncing on Karl Fischer titration and use of other determination method

Zielgröße von Wassergehaltsbestimmungen:

Wasser,

das ganze Wasser und

nichts als Wasser

Maßnahmen zur Freisetzung des Wassers

Lange Zeit

Kurze Wege

Hohe Temperatur

Polarität des Arbeitsmediums

Kombination mehrerer Maßnahmen

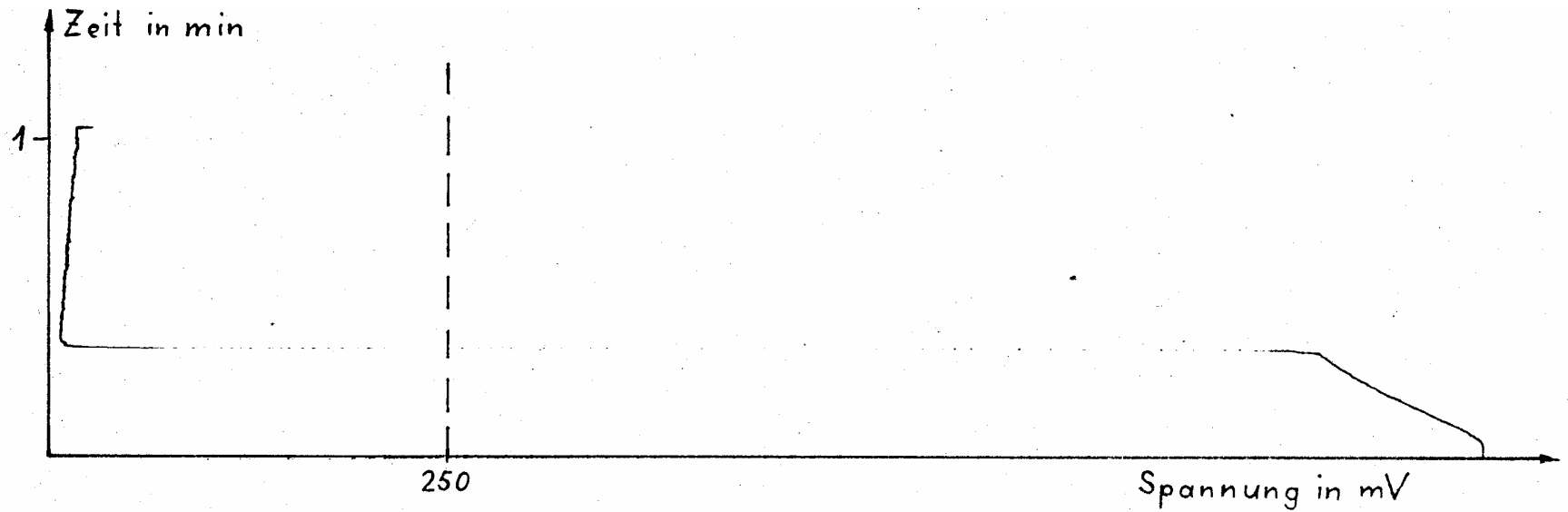
Lange Zeit

Angemessene Titrationszeit

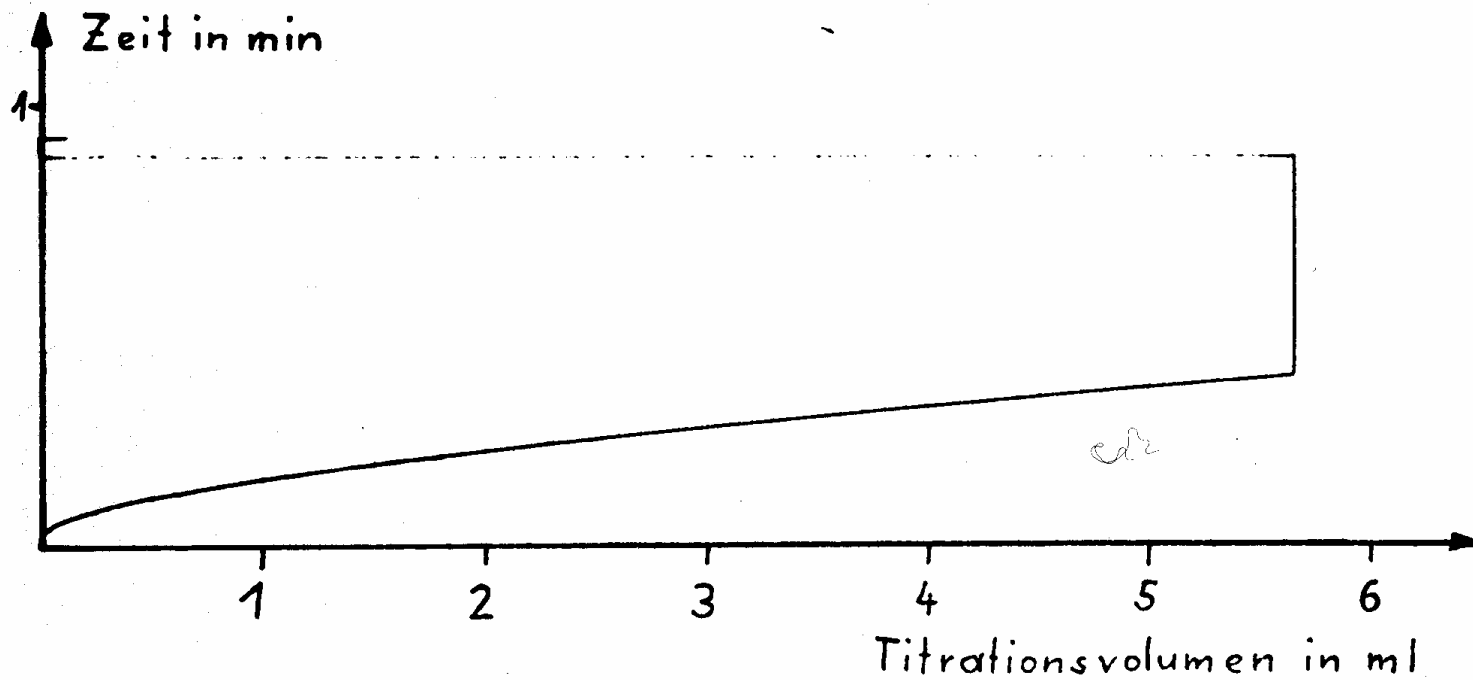
- lange Abschaltverzögerungszeit
- Drift als Abschaltkriterium

Externe oder interne Extraktion des Wassers

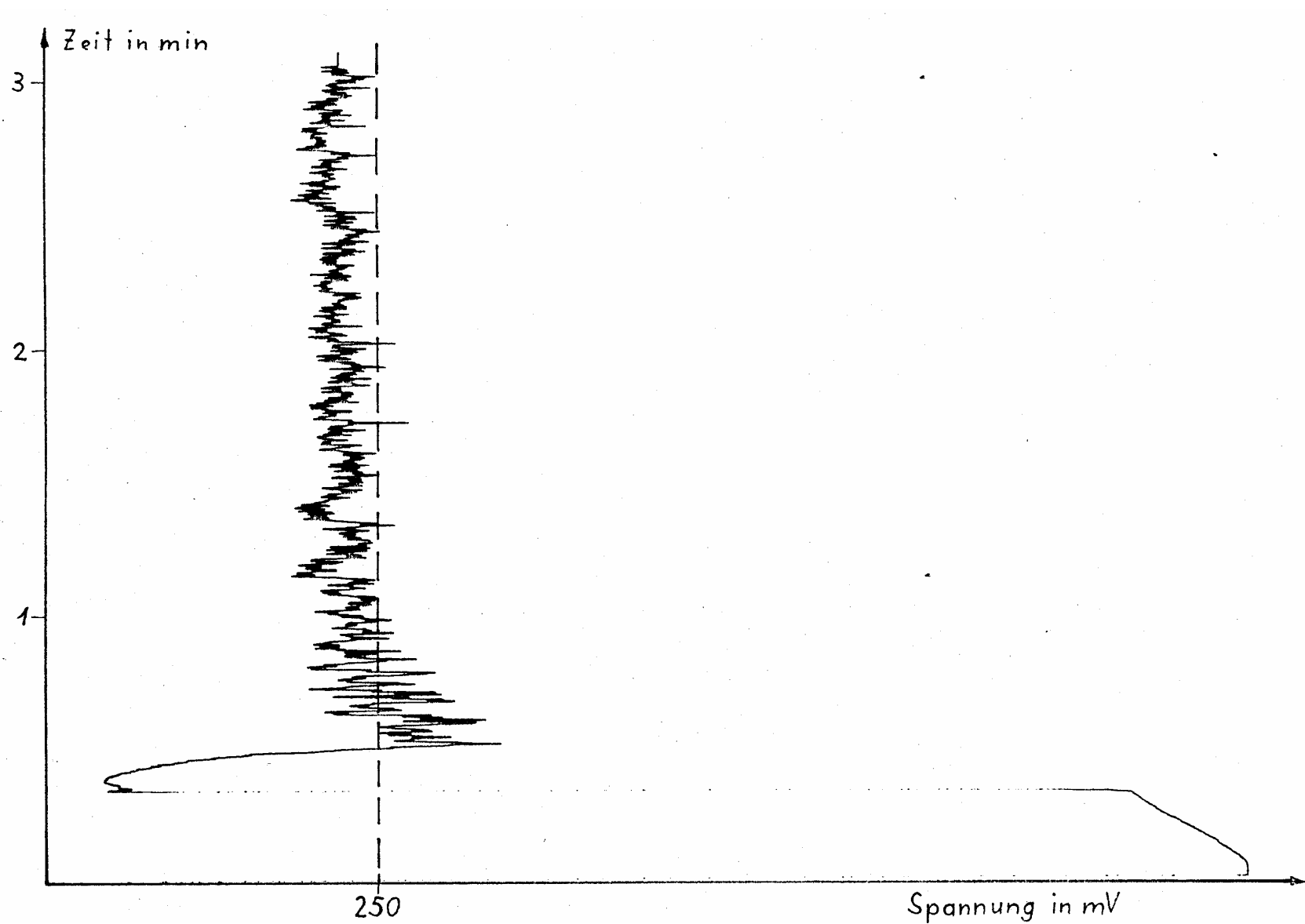
U/t-Kurve frei vorliegendes Wasser



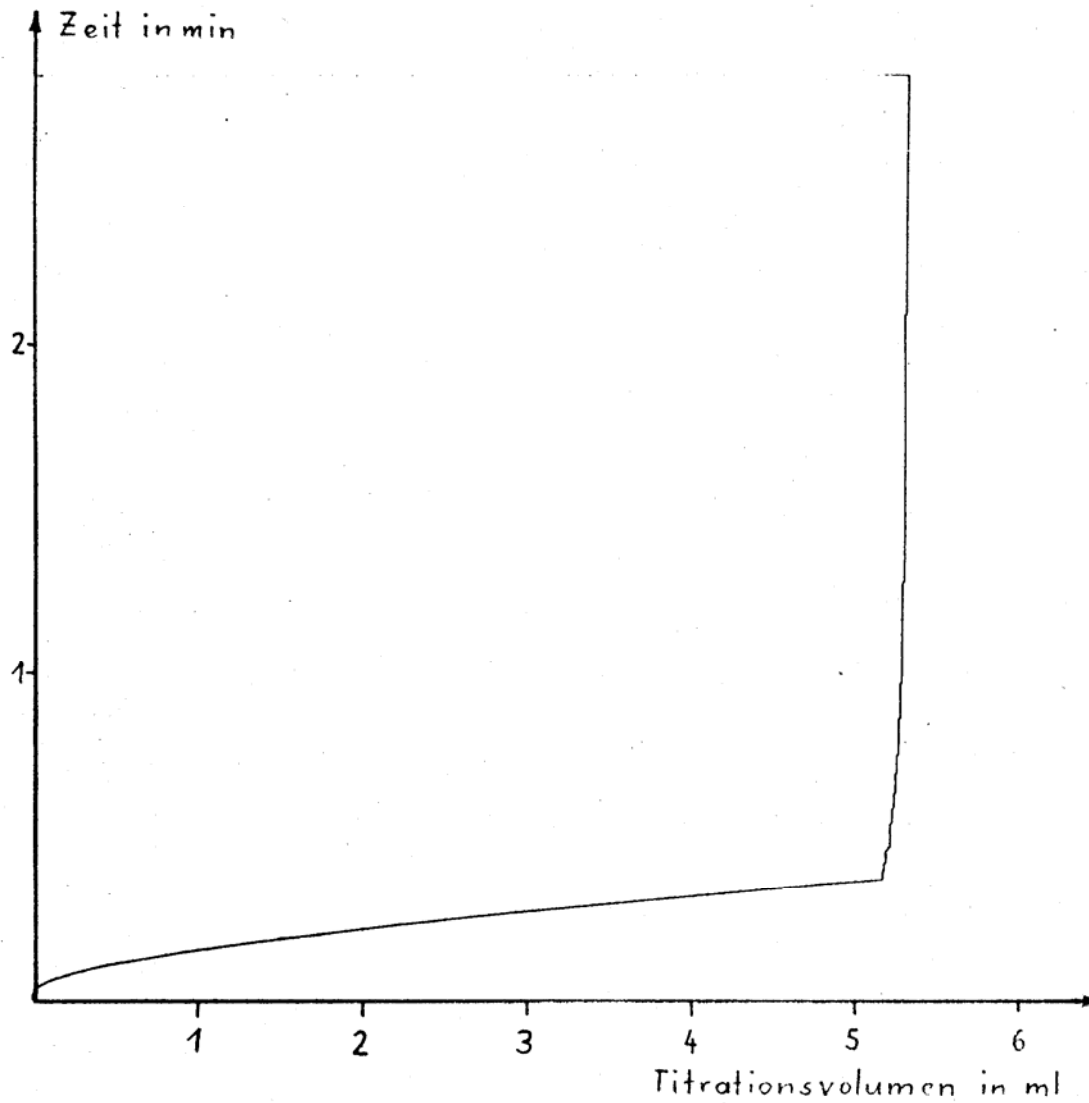
V/t-Kurve frei vorliegendes Wasser



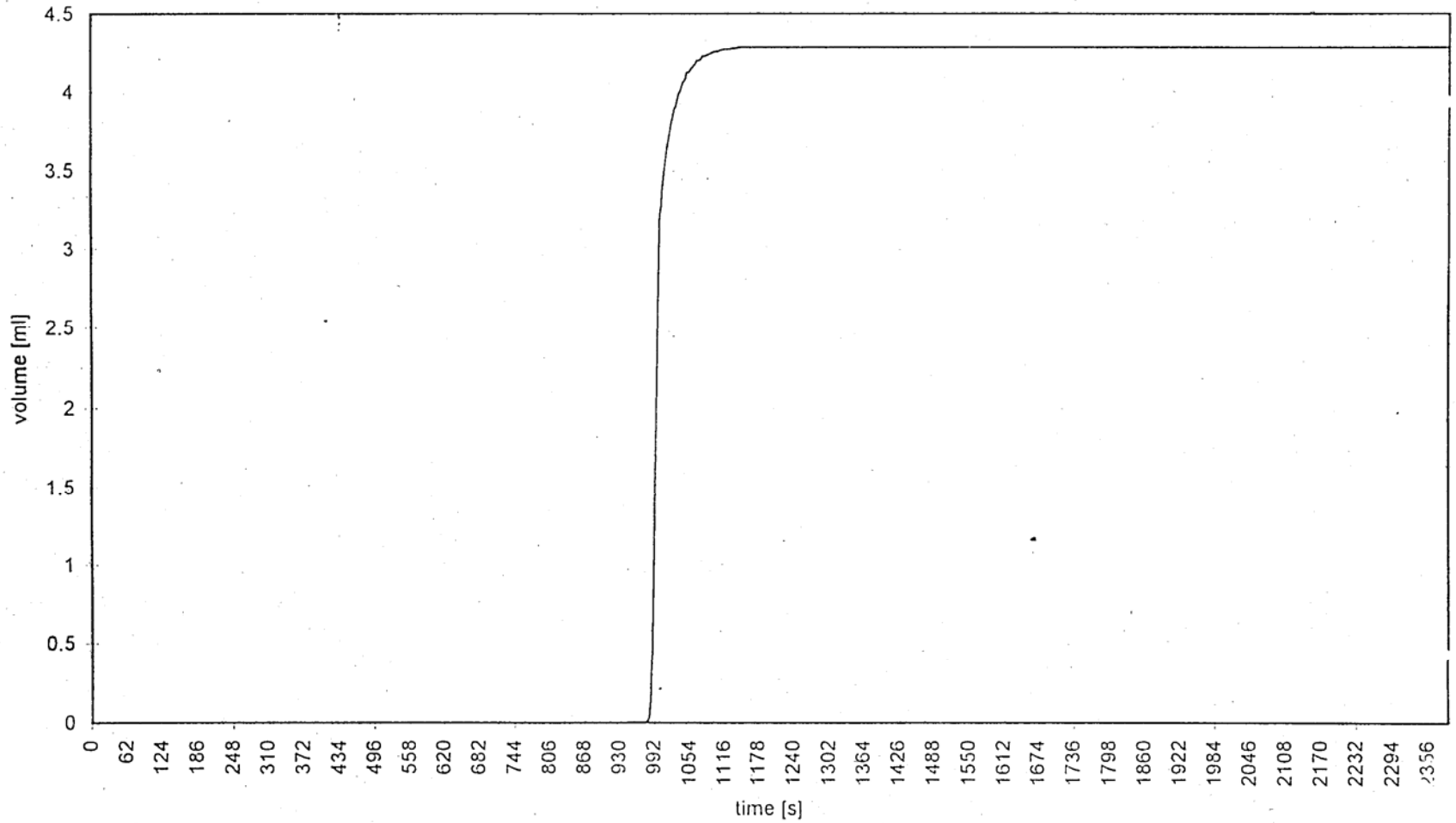
U/t-Kurve feste Probe



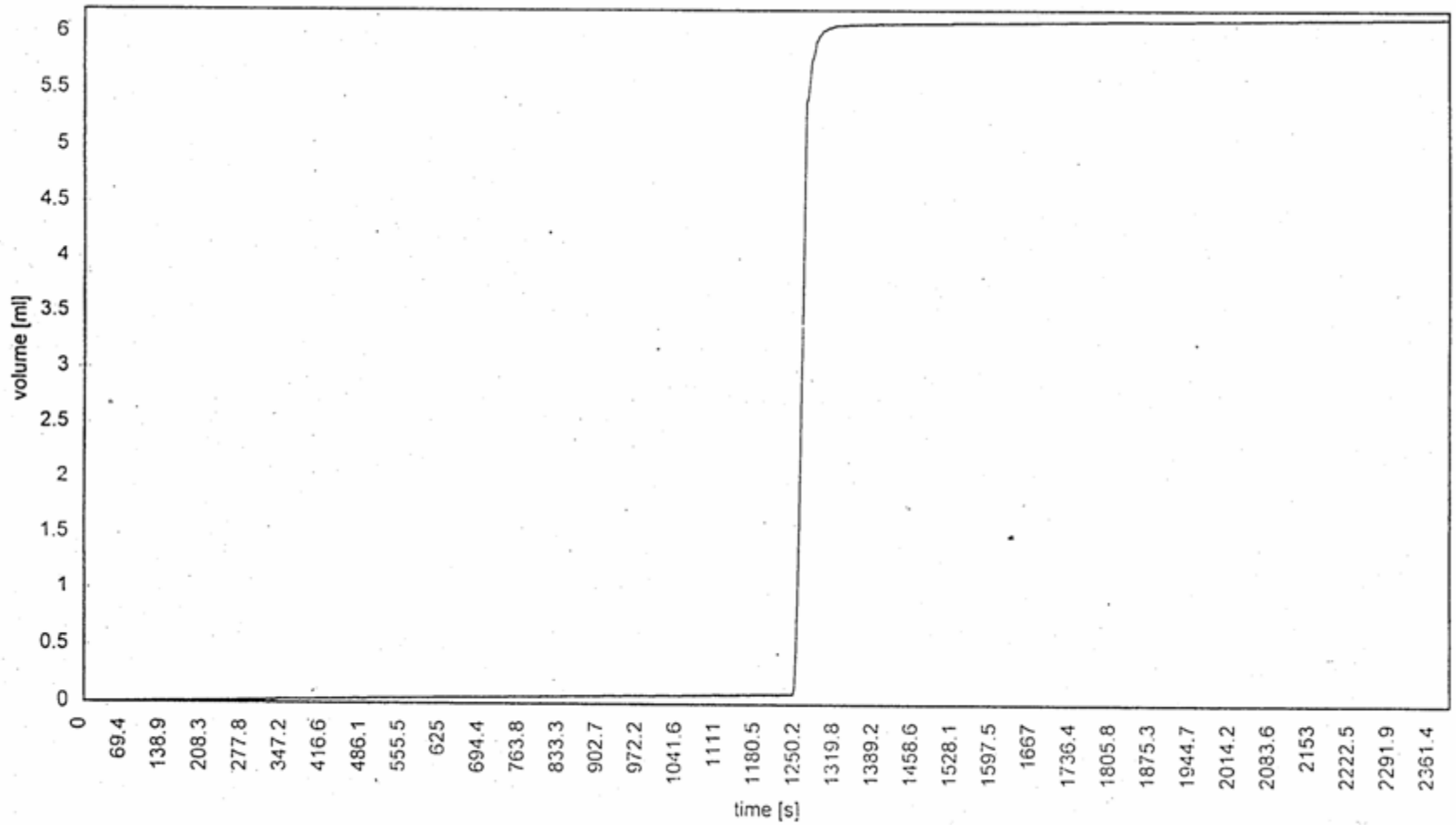
V/t-Kurve feste Probe



Drift praktisch 0



Drift vorhanden

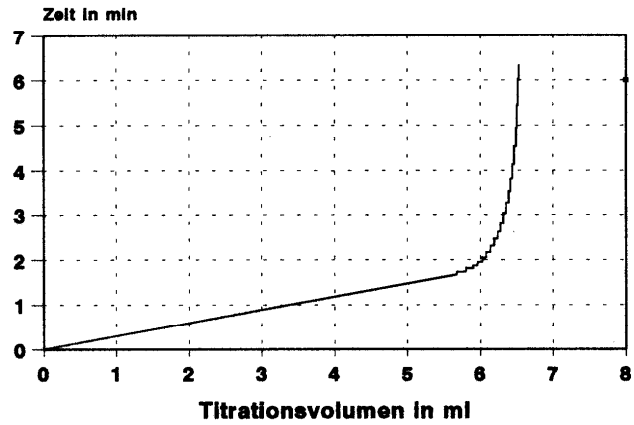


Extraktion des Wassers

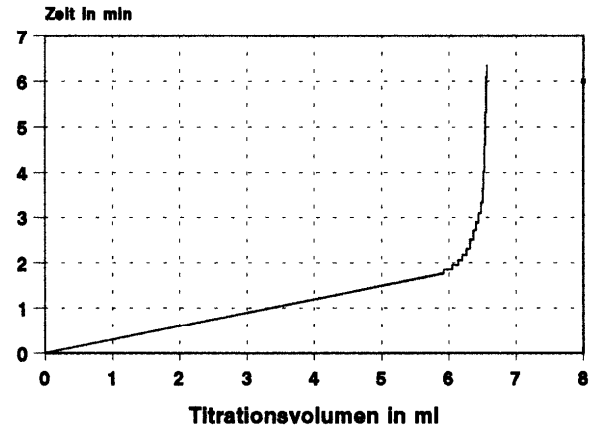
intern als Vorgabe bei der Titration

extern mit Überführung eines Aliquots in die Zelle

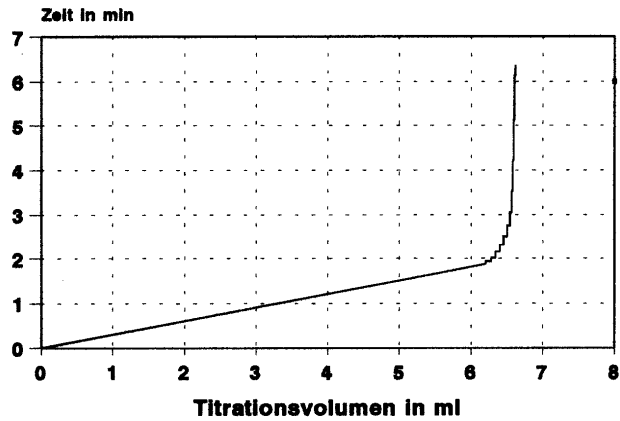
extern in Probenwechsler



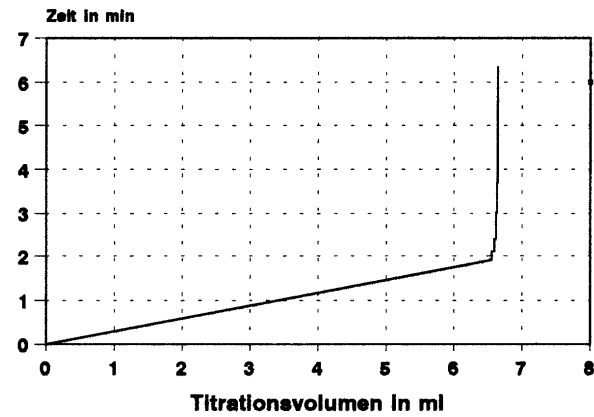
**Titration curve of Sultanin
after 15 min extraction at 40 °C**



**Titration curve of Sultanin
after 30 min extraction at 40 °C**



**Titration curve of Sultanin
after 45 min extraction at 40 °C**



**Titration curve of Sultanin
after 60 min extraction at 40 °C**

Kurze Wege

Probenzerkleinerung

extern als Probenvorbereitung

im Titriergefäß vor oder während der Titration

Dispergierung der Probe im Titriergefäß vor oder während der Titration

Wassergehalt bzw. Trocknungsverlust in g/100 g und Analysezeit

Produkt	Normal-KFT	1 min Ultra-Turrax	Trockenschrank
Emmentaler	32,45±0,08 20 min	32,44±0,29 3 min	31,45±0,17 240 min
Sultaninen	14,50±0,01 30 min	14,48±0,06 3 min	15,52±0,18 240 min
Kakaopulver	3,78±0,05 30 min	wie Normal-KFT	3,75±0,02 240 min
Roggenmehl	11,50±0,04 30 min	wie Normal-KFT	11,27±0,14 120 min

Erhöhte Temperatur

Titration bei Temperaturen bis 50 °C in doppelwandigen Titrierzellen

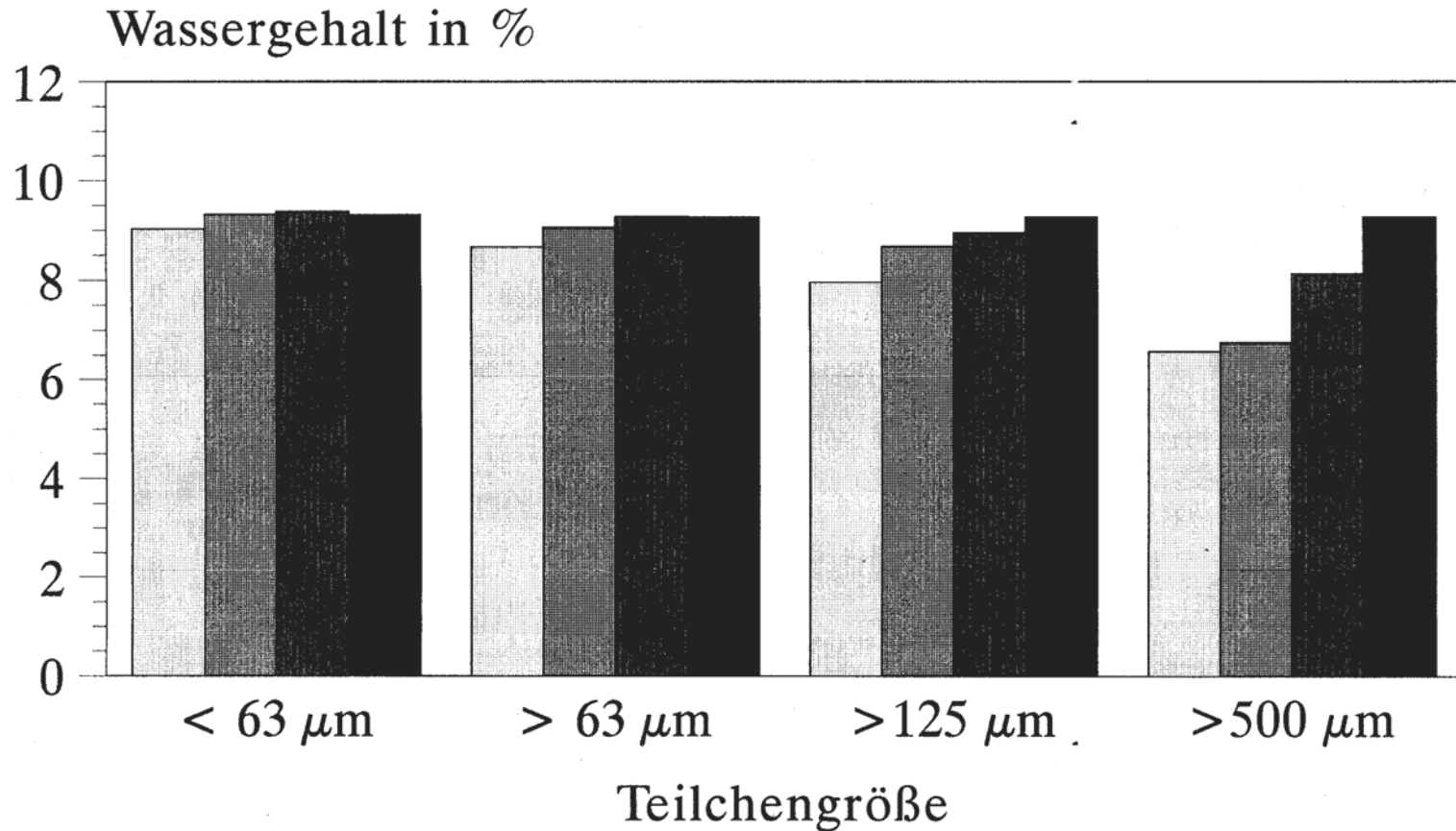
Titration am Siedepunkt des Arbeitsmediums in speziellen Titriezellen

Austreiben des Wassers im Röhrenofen in die Titrierzelle

Austreiben des Wassers aus Vials in Probenwechslerstation

Wassergehaltsbestimmung bei verschiedenen Temperaturen

Produkt	Temp. [°C]	WG [g/100 g]	Zeit [min]
Weizenmehl	25	10,41 ± 0,01	14 – 15
	50	10,48 ± 0,01	4 – 5
	65	10,53 ± 0,02	2 – 3
Zwieback	50	3,42 ± 0,03	19 – 21
	65	3,62 ± 0,01	11 – 13
Nudeln	50	10,38 ± 0,03	8 – 9
	65	10,43 ± 0,02	4 - 5



50 °C ohne UT
 50 °C mit UT
 65 °C ohne UT
 65 °C mit UT

Wassergehalt von Weckmehl in Abhängigkeit von Teilchengröße, Temperatur und Einsatz eines internen Homogenisators (UT)

Polarität des Arbeitsmediums

Zusatz von Lösemitteln entsprechend der Polarität der Probe

Ersatz von Methanol durch andere Alkohole

Bestimmung von Gesamt- und Oberflächenwasser von Saccharose verschiedener Teilchengröße

	Titrant 2/ Solvent:Formamid 2:1; 40 °C	Titrant 2/ Solvent:Chloroform 2:1; 20 °C
	Gesamtwasser [g/100 g]	Oberflächenwasser [g/100 g]
Raffinade	0,122 ± 0,0039 (n=10)	0,026 ± 0,0023 (21%) (n=9)
Puderzucker	0,046 ± 0,0039 (n=6)	0,033 ± 0,0036 (72%) (n=7)

Wasserbestimmung nach Karl Fischer und Bestimmung des Trocknungsverlusts mit verschiedenen Techniken

Methoden	Vollmilchpulver	Magermilchpulver	α -Lactose	Na-tartrat
KFT	3.98 ± 0.011 $s_{rel} = 0.28 \%$	4.80 ± 0.014 $s_{rel} = 0.30 \%$	5.13 ± 0.015 $s_{rel} = 0.29 \%$	15.68 ± 0.017 $s_{rel} = 0.11 \%$
2 h oder länger, 103 °C	3.58 ± 0.138 $s_{rel} = 3.86 \%$	4.34 ± 0.142 $s_{rel} = 3.26 \%$	0.97 ± 0.284 $s_{rel} = 29.2 \%$	6.21 ± 0.191 $s_{rel} = 3.08 \%$
N ₂ -Strom, 2 h, 103 °C, 100 mbar	4.11 ± 0.048 $s_{rel} = 1.16 \%$	4.88 ± 0.105 $s_{rel} = 2.15 \%$	2.47 ± 0.112 $s_{rel} = 4.55 \%$	5.75 ± 0.101 $s_{rel} = 1.75 \%$
Luftstrom, 5 h, 87 °C	3.76 ± 0.032 $s_{rel} = 0.85 \%$	4.64 ± 0.149 $s_{rel} = 3.21 \%$	0.49 ± 0.049 $s_{rel} = 9,97 \%$	4.54 ± 0.319 $s_{rel} = 7.03 \%$

Sample	MC by OD (n)	MC by RD (n)	WC by KFT (n)
1	2.49 ± 0.25 (3)	2.88 ± 0.33 (5)	2.52 ± 0.09 (10)
3	4.64 ± 0.05 (3)	4.57 ± 0.82 (3)	4.81 ± 0.07 (10)
4	3.63 ± 0.22 (3)	3.54 ± 0.41 (5)	2.88 ± 0.04 (10)
6	3.08 ± 0.03 (3)	3.80 ± 0.28 (5)	3.02 ± 0.08 (10)
9	4.01 ± 0.54 (3)	4.10 ± 0.49 (5)	3.26 ± 0.03 (3)
11	3.03 ± 0.26 (3)	2.64 ± 0.31 (3)	2.97 ± 0.07 (9)
14	2.64 ± 0.37 (3)	2.78 ± 0.26 (5)	2.30 ± 0.04 (8)
17	3.18 ± 0.56 (3)	2.44 ± 0.54 (3)	2.59 ± 0.07 (10)
18	4.47 ± 0.46 (3)	3.38 ± 0.17 (3)	3.36 ± 0.03 (10)
21	4.15 ± 0.19 (3)	3.26 ± 0.34 (5)	2.80 ± 0.00 (3)
22	2.72 ± 0.27 (3)	2.49 ± 0.55 (3)	2.21 ± 0.03 (4)
24	3.90 ± 0.32 (3)	3.26 ± 0.25 (3)	2.43 ± 0.03 (3)
26	4.22 ± 0.05 (3)	3.55 ± 0.39 (5)	2.91 ± 0.02 (4)
27	3.19 ± 0.33 (3)	1.96 ± 0.13 (8)	4.47 ± 0.09 (7)
28	6.44 ± 0.15 (3)	5.91 ± 0.40 (3)	5.76 ± 0.14 (4)
30	2.19 ± 0.20 (3)	1.88 ± 0.34 (3)	4.75 ± 0.04 (3)
32	6.77 ± 0.46 (3)	6.31 ± 0.17 (3)	7.16 ± 0.14 (4)
Lactose 1	0.67 ± 0.47 (3)	1.08 ± 0.14 (3)	4.48 ± 0.02 (4)
Lactose 2	0.67 ± 0.11 (3)	1.46 ± 0.07 (3)	5.05 ± 0.06 (4)

Moisture content determined by “reference drying” at different flow rates,
n = number of replicates

Sample	Moisture content in g/100 g at gas flow rate	
	33 ml/min	>33 ml/min
17	2.44 ± 0.54 (n = 3)	2.83 (n = 2)
18	3.38 ± 0.17 (n = 3)	4.35 (n = 2)
21	3.26 ± 0.34 (n = 5)	3.93 (n = 2)
28	5.91 ± 0.40 (n = 3)	6.06 (n = 2)

Moisture content (MC) by “reference drying” for the same sample using different sample sizes; 2 replicates for sample sizes 2, 3, 4 and 6 g, 5 replicates for sample size 5 g

Sample size in g	2	3	4	5	6
MC in g/100 g	5.31	4.21	4.43	4.10	3.73

Moisture content (MC) of a lactose sample by “reference drying” at 102 °C in dependence of drying time, n = number of replicates

Drying time in h	2	3	4	5	6	7
MC in g/100 g (n)	1.79 (2)	2.37±0.56 (4)	2.65±0.51 (6)	3.00±0.32 (6)	3.17±0.30 (4)	3.35 (2)

Results for water content by Karl Fischer titration (KFT) and for mass loss by oven drying (OD) after 2 h and by “reference drying” (RD);

n = number of replicates

Sample	Water content by KFT (n=5) [g/100 g]	Mass loss by OD (n=2) [g/100 g]	Mass loss by RD (n=2) [g/100 g]
Lactose	4.45 ± 0.19	2.45 ± 0.13	1.04 ± 0.03
Skimmed milk powder	3.92 ± 0.07	3.85 ± 0.00	3.94 ± 0.13
Full cream milk powder	2.65 ± 0.05	2.46 ± 0.02	2.72 ± 0.14
Whey powder	4.46 ± 0.05	2.12 ± 0.01	2.24 ± 0.07
Calcium caseinate	6.19 ± 0.11	5.62 ± 0.03	5.73 ± 0.02

If the Karl Fischer method was adopted as a standard reference method for water/moisture and utilized as an alternative method within existing unrevised product standards of identity, the result would be a decrease in the apparent dairy solids content of the product. This negative impact on trade and the commercial value of the dairy product or ingredient could be significant and would be contrary to IDF's mission in regard to providing scientific and technical advice while representing the dairy sector's interest in trade.

The Karl Fischer method is appropriate for quality control/shelf life evaluation, but it is not appropriate as a standard reference method without review and necessary revision of all standards which include compositional requirements for moisture or water or dry matter. This could prove to be an overwhelming task.

Chemical methods :

Calcium carbide method



Calcium hydride method



Chemical method :

Diphosphorus pentoxide method (in the gaseous phase)

