GDRP – Hints & Tips

Good Refractometry Practice Brochure

Hints & Tips

How to Achieve the Best Results

Day-to-Day Refractive Index Measurement

METTLER TOLEDO

Contents

Tips and Hints
Refractive Index Measurement

Modern digital instruments are easy to use and allow the refractive index of liquids to be determined with a high degree of accuracy. High-resolution instruments are however no guarantee for accurate results. This document explains what precautions should be taken to avoid errors when measuring the refractive index of liquids.

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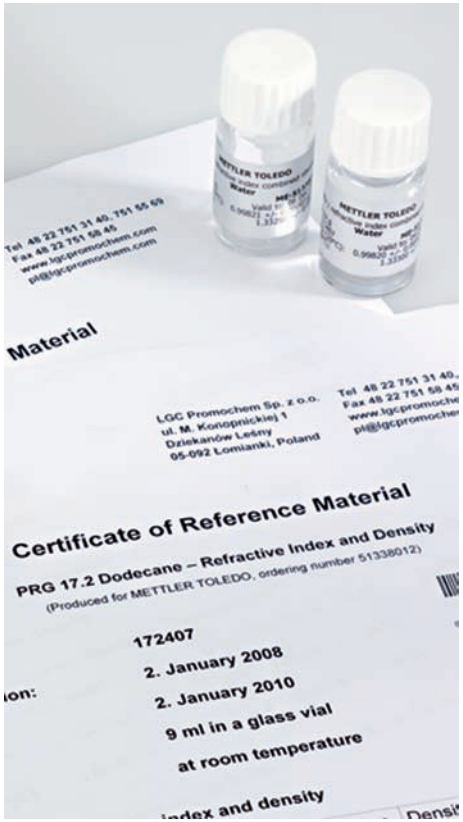
Test and Adjustment

The commonly held opinion that frequent adjustment of the instrument guarantees accurate results is not true. Any adjustment operation results in changes being made to the internal instrument settings.
If the adjustment is not properly performed, all the measurements performed afterwards will be wrong.

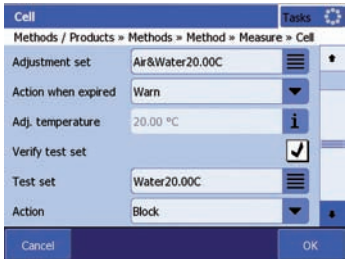
Instead of frequent adjustment, it is better to **regularly verify the measurement accuracy** of the system by measuring a sample of accurately known refractive index (e.g. distilled water or a standard) which is called test, calibration or check. Then the measured refractive index is compared to the known nominal value of the sample.



Test (Calibration)

How often?

Tests should be done routinely in relatively short intervals (days, weeks). Often a test with water is done every day, as it is done quickly and ensures that the instrument works accurately.
METTLER TOLEDO RM Refractometers offer the possibility to define fixed intervals for test sets with an automatic reminder for the operator. Measurement Methods can be set up in way that the operator gets warned again or the instrument is blocked from use if the defined test interval is expired.



Which substance?

The most frequently used test substance is deionized water as it is available in almost every laboratory and in a high and reproducible purity. Also Brix standards are often used.
A different test can be defined separately with larger intervals (months, a year), using certified and traceable standards for quality assurance and traceability purposes.
METTLER TOLEDO offers combined (density and refractive index) certified standards in different ranges:

- Water (0.99… g/cm^3; nD 1.33…)
- Dodecane (0.75… g/cm^3; nD 1.42…)
- 2,4-dichlorotoluene (1.25… g/cm^3; nD 1.55…)
- 1-bromonaphthalene (1.48… g/cm^3; nD 1.66…)

Which tolerance should be applied?

The following guidelines may help to define reasonable tolerances so that frequent error messages caused by tolerances which are too strict can be avoided.

- For deionized water (where the uncertainty of the water is usually not known) the tolerance should be defined at 2 times the instrument resolution plus the operator repeatability (the non-linearity of the cell has been set to zero for deionized water, if it was used for the adjustment as well)
  - ➔ Never go below that value range, otherwise there is a high risk of frequently producing false failed tolerance tests, which are only caused by internal rounding. But keep it in general as narrow as possible according the instrument resolution and operator repeatability.
  - ➔ Example: RM40 Refractometer with a resolution of 0.0001
    Operator repeatability (as example) = 0.00005 (standard deviation when the operator measures the same sample 3 times in a row. If an operator works properly, he should not get a S.D. more than that of the instrument's rounding capability).
    Tolerance = 2 x instrument resolution + operator repeatability = 0.0002 + 0.00005
    → round up to a tolerance of **± 0.0003**.
- When using certified organic standards which usually have a relatively high temperature coefficient (refractive index change with temperature change), please also allow for the specified temperature error of the instrument. So there are four components which normally have to be summed up to form the tolerance, in order to avoid establishing tolerances which are too strict:
  Uncertainty of the standard, limit of error instrument, temperature error and repeatability.
  - ➔ Example: certified standard dodecane with the following given values:

Temperature	Refractive Index
15 °C	1.42382 ± 0.00002
20 °C	1.42164 ± 0.00002
25 °C	1.41955 ± 0.00002

Instrument = RM50 Refractometer with a resolution of 0.00001, limit of error of 0.00002 and limit of error for the temperature of 0.03 °C.
(a) Uncertainty of the standard: ± 0.00002
(b) Limit of error instrument: ± 0.00002
(c) Temperature error: ± 0.00001
→ 0.03 °C (limit of error for the temperature) * 0.000427 [1/°C]
(α calculated from given values at different temperatures of the standard = 1.42382 – 1.41955 / 25 – 15 °C)
(d) Operator Repeatability: ± 0.00001 (example, has to be determined)
Tolerance = sum of the 4 components = **± 0.00006 g/cm³**

This is an example and the tolerance has to be calculated specifically for each combination of standard and instrument. The tolerance for a certified standard may become larger than the 2 to 5 times instrument resolution as it is the case for a normal test with local deionized water.

What to do if the test fails?

If the value obtained deviates from the expected (true) value more than the defined tolerance, proceed as follows:

1. Check if the correct substance has been used, e.g. pure fresh deionized water
2. Clean the prism thoroughly
3. Repeat the Test
4. If the test continues to fail with a difference which varies from test to test (i.e. not stable), then the cleaning should be continued with more care, also using other and more powerful types of solvents (as maybe residues on the prism have built up over time), until the test show perfectly repeatable behavior. If this repeatable behavior is reached (only rounding difference between the results) but the test still fails, a new adjustment is required. This can be caused by a normal instrument drift over time (usually over months or years).

Adjustment

Which temperature and substances?

Usually refractometers are adjusted with air and water. During the air part of the adjustment the instrument simply checks the response of the CCD detector to mark defective positions (the refractive index of air is out of the measuring range of digital refractometers).
The temperature for the adjustment should be the same as the usual measurement temperature. E.g. if samples are measured at 40 °C the adjustment should be done at 40 °C as well.

How to proceed?

Only adjust the instrument if the test (calibration) fails but in a repeatable way (see chapter Test).
Prior to the adjustment the prism must be perfectly clean and dry. If the adjustment is performed on a contaminated cell this will cause an error (shift) in the water point. Such a shift will be constant and systematic throughout the whole measuring range!

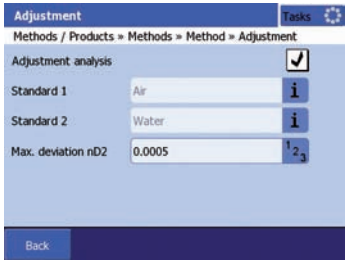
Every error or offset caused by a wrong adjustment is a systematic error - it will add on top of the error specification of the instrument!

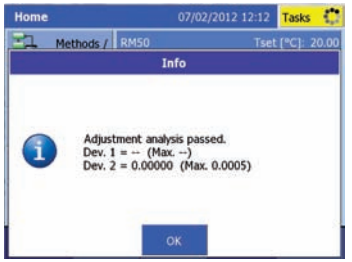
Follow the instrument instruction to perform the adjustment.
After performing an adjustment:

- Always question the adjustment which you have performed – do these values make sense?
- Verify the validity by measuring deionized water afterwards.
  If it is not possible the reproduce the nominal value for water which has just been assigned, something went wrong, either in the adjustment or in the following measurements.
- When deviations are observed, repeat the adjustment (or the measurement of water) after correcting the problems (usually a more thorough cleaning is needed)

Adjustment analysis and automatic adjustments

METTLER TOLEDO RM Refractometers offer the possibility to activate an adjustment analysis. This brings a warning if the deviation turned out too high and the adjustment can be rejected. In addition, the adjustment history can be viewed anytime, also as a graph.





The METTLER TOLEDO SC1 and SC30 automation units help to avoid erroneous adjustments:

- Adjustments are very easy to perform by just clicking the Adjust shortcut on the home screen.
- Prior to each adjustment, the measuring cell can be thoroughly cleaned and completely dried (if selected).

Samples

Pasty samples

Pasty samples, for instance tomato puree, bear the risk of air cushions between the prism and the sample. Make sure that the sample is in full contact with the prism by "pressing" it down.
METTLER TOLEDO RM Refractometers can be equipped with an easy mountable presser. When the lid is being closed, the sample is automatically pressed to the prism.

Sticky / viscous samples

Samples such as concentrated glycerol, syrup concentrates etc. often do not adapt readily or quickly to a temperature change. In such samples, even though the instrument measures the correct temperature, the sample in contact with the prism is still slowly adapting to the temperature, causing a very slow but constant drift of the measured refractive index. Thus, for such samples, a waiting time (20 to 60 seconds) prior to starting the measurement is recommended to ensure absolutely correct readings. The required duration has to be validated quickly in a series of measurements.

Aggressive samples

Make sure that all parts which come in contact with the sample are resistant!
Most refractometer cells are made of stainless steel which would be attacked by strongly oxidizing samples.
If the concentration of relatively concentrated acids (sulfuric acid, nitric acid, hydrochloric acid) has to be determined, a refractometer with a resistant cell or a density meter should be used (the materials of density meters which are in contact with sample are typically borosilicate glass, PTFE, PP)

Volatile samples

The measurement of volatile samples is usually performed at low temperatures to reduce evaporation (take care about condensation on the cell!). Depending on the sample, the evaporation can preclude a stable temperature and a reliable measurement would not be possible.
For very volatile (e.g. hexane) or toxic samples a METTLER TOLEDO RM Refractometer can be equipped with a flow-through cell and used together with a SC1 or SC30 automation unit. This has following advantages:

- Sample will not evaporate during measurement and can be measured at room temperature (quicker!).
- High occupational safety as the handling of these materials is limited to a minimum and no evaporation occurs (closeable vials)
- Sample recovery

Non-homogeneous samples/suspensions

If solutions or suspensions are allowed to stand, part of the solid material may settle or a concentration gradient may form. Stir the sample well before taking a sample. Make sure that no air bubbles are introduced during stirring. If you have to measure suspensions, it is often not possible to completely homogenize the sample (e.g. ketchup). In such cases, the measurement should be repeated several times and the mean value of the individual measurements calculated in order to obtain reliable values.

Sampling

With a syringe

Use plastic syringes with luer tip, preferably 3-component syringes (with rubber O-ring) as they allow a much better speed control than cheap 2-component syringes.

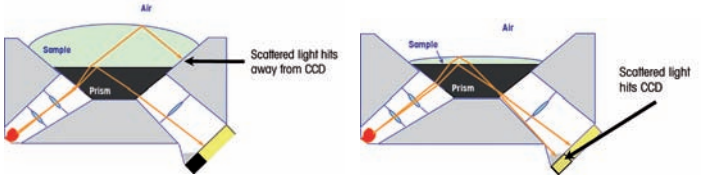
Avoid air cushions

Add the sample to the prism and – especially important for sticky samples – stir (scratch) with the tip of the syringe to removes air cushions between the prism and the sample. This also Homogenizes the sample (dilutes contamination in sample).

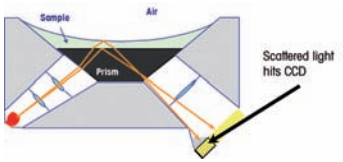
Add Enough Sample

Add enough sample (not just a few drops) to avoid erroneous results.

Proper filling: some light is reflected at the prism/sample interface (total reflection as needed to calculate the refractive index) and some light is reflected at the sample/air interface. The latter does not hit the CCD sensor, thus the result is correct (left picture).
If only a few drops are added to the prism, the scattered light (reflected at sample/air interface) hits the CCD sensor and falsifies the result (right picture)!



For most bench-top refractometers the minimum sample volume is around 0.5 mL. METTLER TOLEDO RM Refractometers have a mark which indicates this volume.
The surface tension of organic solvents or samples containing organic solvents such as ethanol or acetone is low compared to water. Such samples act as wetting agents and form a concave meniscus. Thus, add more sample to achieve sufficient filling height of the prism, minimum ~ 1 mL:



Automatic filling

A METTLER TOLEDO RM Refractometer can be equipped with a flow-through cell and used with following automation solutions:

- FillPal™ Food (aqueous products) and FillPal™ Chem (acids, bases, solvents): sampling pumps for low viscous samples (max. viscosity 1'000 mPa·s)
- SC1 and SC30 automation units: even possible to fill very highly viscous or sticky samples into measuring cell such as highly viscous oils, creams, molasses or liquid honey. (max. viscosity 30'000 mPa·s)

Cleaning

Procedure for manual use of refractometer

Remove old sample

To remove the sample (and the solvents) from the refractometer cell, it is suggested to use a syringe. This "waste syringe" can be used over and over again (tip: mark this syringe, for instance with black tape).
Using a syringe saves a lot of soft tissue cleaning wipes and reduces waste.

Rinse

Clean with an ideal solvent a few times. The solvent must be able to quickly dissolve the sample.
- Add the solvent
- Stir with the "waste syringe"
- Remove all with the "waste syringe"

A second solvent which allows quick drying (e.g. Acetone) often bears the risk for contamination!

Dry

Wipe the prism/cell dry with a soft tissue.
Wait 10 seconds, before adding next sample

Cleaning with automation devices

If a FillPal™ sampling pump is used and measured samples are able to dissolve the residues in the measuring cell (e.g. when the refractometer is used to measure different juices), it is also possible to skip cleaning and do a large over-sampling with the new sample to ensures a complete removal of the old one ("analytical rinse"):
- Immerse the sampling tube of the pump in the sample, then remove it so that air is sucked in the tube (~2–3 cm air in the tube) and immerse it again in the sample. Repeat this procedure approx. 5 times before the cell is filled for the measurement. This ensures that the old sample is properly flushed out of the cell.
- Verify the procedure to make sure that the required repeatability and limit of error are maintained. Measure the most critical sample first (for instance, the one with the highest sugar content) followed by deionized water then repeat this step a couple of times.
- If for instance sugar containing products are measured, make sure that the flow-through cell remains filled with either sample or with water between measurements to avoid the sample drying out and crystallizes sugar on the prism.
- Completely clean the cell/prism at least once after each working day.

With the METTLER TOLEDO SC1 and SC30 automation units, the measuring cell and prism is fully automatically cleaned and dried after the measurement. The two rinsing liquids for cleaning (e.g. water and acetone) are mixed with lots of air and pumped through system at high speed. This results in a pulsating flow which provides very efficient near-mechanical cleaning.
As the inside and outside of the SC1/SC30 sampling nozzle is thoroughly cleaned and dried after each measurement, sample carryover is not possible!

Result verification and documentation

Automatic result conversion

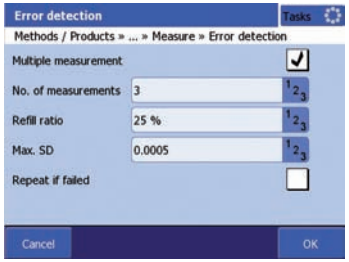
Often the result has to be converted using a table. Looking up in or interpolating from a table is error-prone and time-consuming. Automatic conversion using built-in tables (e.g. alcohol, Brix, temperature compensation according to API) prevents reading or calculation errors and saves time. A digital refractometer of the latest generation allows the use of built-in conversion tables to show the result directly in the desired unit. METTLER TOLEDO RM Refractometers have to following built-in result units / concentration tables:

- nD, Zeiss (14.45), Zeiss (15.00)
- Brix, HFCS 42/55, Invert Sugar, Oechsle
  If Brix is measured between 10 °C and 40 °C it can be automatically compensated up 20 °C (as per ICUMSA)
- Up to 30 user-defined concentration tables (can be entered as tables or formulas)

Error detection

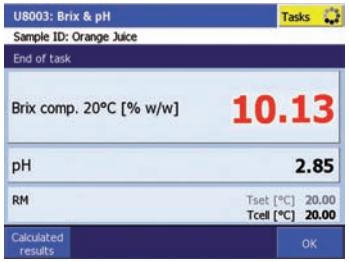
METTLER TOLEDO RM Refractometers offer automatic error detection for critical/problematic samples:
The Error detection will perform an n-fold measurement out of the same vessel, moving the sample for each new measurement automatically if you have connected a FillPal (aspiration pump) or an SC1/SC30 automation unit.
In the end the refractometer reports the mean refractive index and also the standard deviation.If the defined maximum standard deviation is exceeded, a warning message is displayed, and the result marked as suspicious.

The Error detection allows securely and automatically detects bubbles, sample inhomogeneity, and contaminations.



Result limits

METTLER TOLEDO RM Refractometers offer a product approach. The same measurement method might be used for several samples, but some sample specific parameters (like filling speed) as well as the lower and upper result limits can be defined for each product. Measured products are then automatically verified with the limits and the color of the result(s) indicates if specifications are met: black = passed, red = failed.



Proper Documentation

Hand-written results bear the risk of transcription errors. Depending on the environment, there is no guarantee that this kind of documentation fulfills regulatory requirements.
Regulatory requirements are easy fulfilled when using a printer or PC software. GLP-compliant printouts contain all of the necessary information for complete documentation. METTLER TOLEDO RM Refractometers can be used with the USB-P25 dot matrix printer for GLP-compliant printouts. Printouts must be manually managed and filed, which is a potential source of errors and incompleteness. Using PC software for data collection and management has the advantage of greater data reliability and easier future accessibility.
The software LabX® in connection with RM Refractometers guarantees to have all data stored centrally and securely in a database. It offers ERP/LIMS connectivity, customized reports, secure traceability and 21 CFR 11 support. The software can be connected remotely via Ethernet saving valuable bench space and enabling the operator to perform all operations from the instrument terminal.

Define your Test procedures (interval, substances, tolerances) and your Adjustments (substances, temperatures):

Notes:

- Very easy to use thanks to One Click® operation
- 3 built in thermistors for most accurate temperature control
- Automatic error detection and result verification (product database with stored limits)
- Automatic filling, cleaning & drying. Also for difficult and viscous samples
- Automatic storage of results in database, LIMS export etc. with software LabX

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