Optical ReagentFree (OptRF) methods with PhotoLab® 7600 UV-VIS

How to measure

Preparation of the OptRF measurement

Details on usage, optimization and limitations of OptRF methods are given in the following paragraphs.

1 Open the OptRF measurement menu.

2 Select a method

3 Carry out a zero adjustment.

For the zero adjustment, use a quartz cell.
For the highest possible measuring accuracy we recommend that you carry out the zero adjustment in the very cell you are going to use for measuring the sample.

The zero adjustment remains valid for further measurements with OptRF methods. The zero adjustment is only invalid after the menu OptRF measurement is exited.

4 If necessary, dilute the sample and enter the dilution factor.

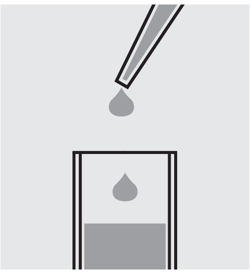
With high nitrate and nitrite concentrations, dilute the sample and enter the dilution factor. The required dilution can be different for different measured parameters.

5 Carry out a user calibration (determine and enter the raw value [#] and reference value).

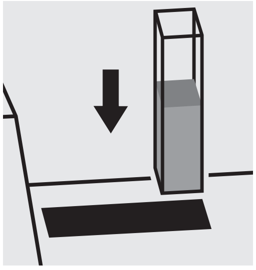
The methods are optimized for the current sample matrix with a user calibration.
Samples that do not correspond to the sample matrix of municipal waste water treatment plants are designated "Unsuitable matrix“.

<u>COD dissolved</u>: Always carry out the OptRF measurement with the unfiltered sample, even if the sample is filtered for the reference measurement.

Carrying out an OptRF measurement



Pipette the sample into a 10 mm quartz cell.



Insert the cell in the photometer cell shaft and start measurement.

6 If necessary, start a new measurement with the same or a different OptRF method.

Introduction into the measurement procedure with OptRF methods

The OptRF measurement of an aqueous sample is based on a direct, spectral absorbance measurement in the UV range 200 ... 390 nm without using any reagents. The measured spectrum is evaluated over the entire wavelength range. The concentration value is calculated by means of complex algorithms or evaluation models, which are stored in the photometer as OptRF methods. The available OptRF methods are specific for the relevant measured parameter as well as for the application and measurement location.

Measured parameters and range of application

The OptRF methods currently available were developed and optimized for the application range "Outlet of municipal waste water treatment plants“ and comprise the following measured parameters and measuring ranges referring to measurements in standard solutions:

OptRF measuring method	Parameter	Measuring range referring to measurements in standard solutions
3001 CODt_H_Outlet_10	COD_{total}[a]	0 - 75 mg/L
3002 CODs_H_Outlet_10	$COD_{dissolved}$[b]	0 - 75 mg/L
3003 NO3_H_Outlet_10	NO3-N	0 - 3.0 mg/L
3004 NO2_H_Outlet_10	NO2-N	0 - 4.0 mg/L

[a] COD_{total}:The particulate and dissolved substances of the sample are taken into account.

[b] $COD_{dissolved}$: The particulate and dissolved substances of the sample are taken into account. The impact of the particulate substances on the measured value is automatically corrected. Always carry out the OptRF measurement with the unfiltered sample, even if the sample is filtered for the reference measurement.

Due to the plant-specific composition of the waste water, the measuring ranges of the different parameters can be smaller or greater than the measuring ranges quoted for the standard solutions. A user calibration (section USER CALIBRATION FOR OPTRF MEASUREMENT) can also affect the limits of the measuring range for real samples.

OptRF methods can also be used in samples with a similar matrix such as some surface waters. This has to be verified, however, on an individual basis with user tests with the reference procedures such as cell test sets (see section OPTIMIZATION AND EVALUATION OF THE MEASUREMENT QUALITY).

If using the selected OptRF method is possible, this method can also be optimized by means of the user calibration.

Limitation of measurement

Due to interfering effects such as particles, turbidity etc. and samples with very different compositions of substances, each of the OptRF methods is only suitable for a certain sample composition (sample matrix).

Generally, only light-absorbing substances in aqueous samples can be determined. Substances such as sugar, alcohol etc, which also contribute to the sum parameter COD, cannot be determined by the spectral measurement.

Measurements in solutions with high turbidity are also restricted or impossible due to negative effects such as very high absorbance values and a sedimentation behavior.

Simultaneous measurement of several parameters - different dilution

Depending on the evaluation model, the wavelengths of the spectrum that were measured are weighted differently for the different parameters. If the maximum allowed absorbance of certain wavelengths for a parameter is exceeded, this parameter cannot be reliably evaluated. The sample has to be diluted in order to be measured reliably.

If, due to too high concentrations of some parameters in the measured sample, the absorbance values are too high, the message "dilution" is automatically generated.

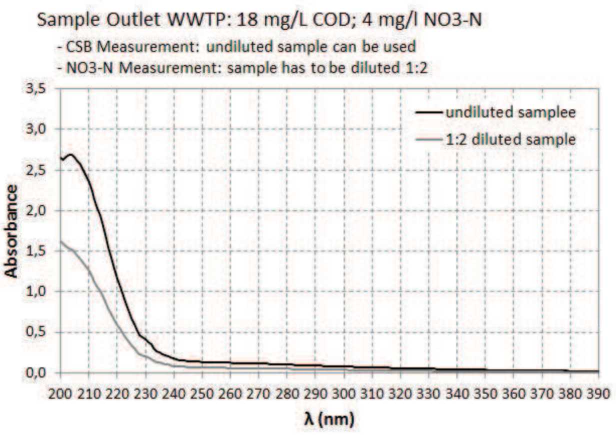
In this case, establish an individual dilution series for each OptRF method to determine the optimum dilution for this method. Depending on the sample composition, different dilutions may be required for different methods (e.g. COD, NO3-N and NO2-N).

Due to the different maximum allowed absorbance values for the different parameters, different dilution levels of the same sample can be required for the measurement of the respective parameters (see the following examples: Spectra of a sample from the outlet of a waste water treatment plant).

Example 1

COD concentration: approx. 18 mg/L
Nitrate concentration: approx. 4 mg/L NO3-N.

The COD determination can be done with the undiluted sample, the nitrate determination requires the sample to be diluted 1:2 (1+1).

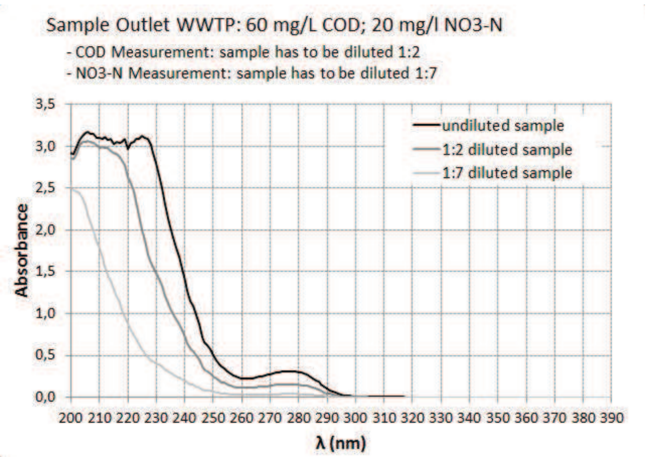


- Spectrum of the undiluted sample (for COD determination)
- Spectrum of the diluted (1:2) sample (for NO3-N determination)

Example 2

COD concentration: approx. 60 mg/L
Nitrate concentration: approx. 20 mg/L NO3-N.

The relatively high COD concentration may require a 1:2 (1+1) dilution for the determination of the COD value, though the COD concentration of the sample is still within the measuring range. To determine the nitrate concentration of this sample, it must be diluted 1:7 (1+6).



- Spectrum of the undiluted sample
- Spectrum of the diluted (1:2) sample (for COD determination)
- Spectrum of the diluted (1:7) sample (for NO3-N determination)

Optimization and evaluation of the measurement quality

The respective OptRF methods are only applicable within certain limits of variation of the sample matrix. Matrix variations can, for example, occur due to the season (e.g. increased nitrate value) or to the discharge of unusual substances. With a user calibration the OptRF methods can be optimized for the specific application location (e.g. waste water treatment plant) and the current sample composition.

User calibration for OptRF measurement

When using the OptRF measurement, you should always check whether the measurement can be used without user calibration. To do so, samples with different concentrations - if possible across the entire measuring range expected - are measured, each with OptRF measurement as well as a reference measurement (e.g. cell test sets with double determination). Normally, the entire measuring range cannot be measured and checked in one series of measurements within a short period of time. Therefore, you should make a list with the respective measurement results of representative samples. This list should contain the raw values [#] of the OptRF measurements, the reference values and the date and time of the sampling, as shown here with the example of COD_{total}:

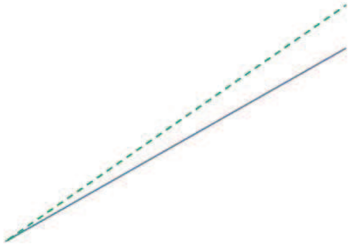
Sampling		**OptRF raw value [#]**	**Reference value**
Date	**Time**	**COD_{total} (mg/L)**	**COD_{total} (mg/L)**
03.04.2015	07:30	23.5	26
03.04.2015	09:15	28	32
05.04.2015	16:30	13.5	15
06.04.2015	14:20	19	23
…			

The raw value and reference value belonging to each other are called a value pair.

If the results of the two different determination methods deviate from each other considerably, a single-point or 2-point user calibration of the OptRF measurements should be carried out to achieve the highest possible measurement accuracy.

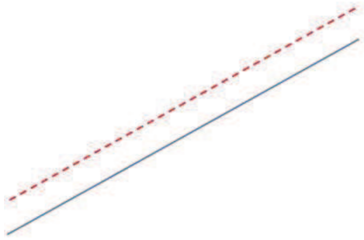
With a single-point calibration, the slope of the characteristic curve is changed while with a 2-point calibration the slope and axis intercept (offset) of the characteristic curve can be adapted (see examples).

Example 1



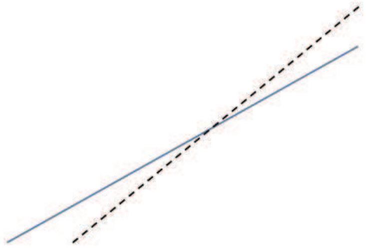
1-point calibration: Changing the value pair for the end of the measuring range (2nd value pair)

Example 2



2-point calibration:
Changing both value pairs, offset correction

Example 3



2-point calibration:
Changing both value pairs, slope correction

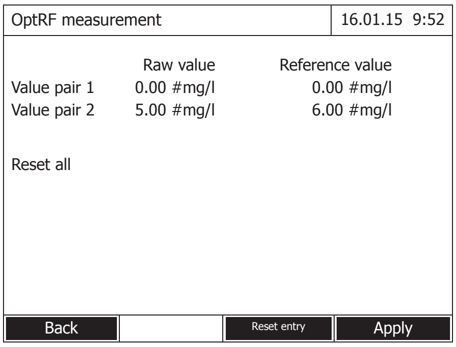
If the deviations predominantly occur in the lower **or** upper measuring range, a 1-point calibration in the relevant range of the deviation is normally sufficient. If, however, the differences are in the whole measuring range or there is an offset, a 2-point calibration has to be carried out. An offset deviation can be identified by a constant difference between raw value and reference value.

Negative raw values [#] can be displayed after the spectrum was evaluated. Negative concentration values, however, are not possible. If negative raw values are displayed, the calculated concentration value is automatically set to 0.

During a user calibration, the negative raw value [#] has to be entered with the minus sign for the lower value pair, not the adjusted concentration value "0“.

After a successful user calibration, we recommend you sporadically check the OptRF measurement with reference measurements. On the one hand, to achieve further optimization for the plant or measuring location if possible; on the other hand, to identify a possibly changing water matrix and ensure further accurate concentration measurements with a new user calibration.

Execution of a 1-point calibration

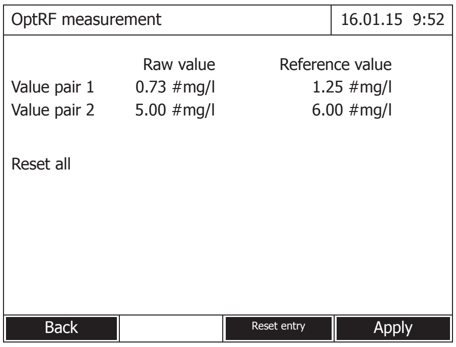

OptRF measurement 16.01.15 9:52

	Raw value	Reference value
Value pair 1	0.00 #mg/l	0.00 #mg/l
Value pair 2	5.00 #mg/l	6.00 #mg/l

Reset all

For a 1-point calibration, a suitable value pair from the beginning or end of the measuring range is entered in the input fields. The other value pair remains unchanged. In this example, the upper value pair (value pair 2) is entered. Thus only the slope but not the offset of the characteristic curve is changed:

Execution of a 2-point calibration


OptRF measurement 16.01.15 9:52

	Raw value	Reference value
Value pair 1	0.73 #mg/l	1.25 #mg/l
Value pair 2	5.00 #mg/l	6.00 #mg/l

Reset all

With a 2-point calibration, by selecting the corresponding value pairs the characteristic curve can be optimized by adjusting the slope and / or the offset.

Here, the user calibration is carried out by entering a lower (value pair 1) and an upper value pair (value pair 2).

More details on how to carry out the user calibration are given in the functional description.

Example 1: COD_{total} measurement in the outlet of a municipal waste water treatment plant

Sampling		**OptRF raw value [#]** NO_3**-N (mg/L)**	**Reference value** NO_3**-N (mg/L)**
Date	**Time**		
03.03.2015	07:30	23.5	26
03.03.2015	09:15	28	32
05.04.2015	16:30	13.5	15
05.04.2015	14:20	19	23
…			

In this example, we recommend for the OptRF method 3001 CODt_H Outlet a 2-point user calibration with the following value pairs:

	OptRF # raw value COD_{total} **(mg/L)**	**Reference value** COD_{total} **(mg/L)**
Value pair 1	13.5	15
Value pair 2	28	32

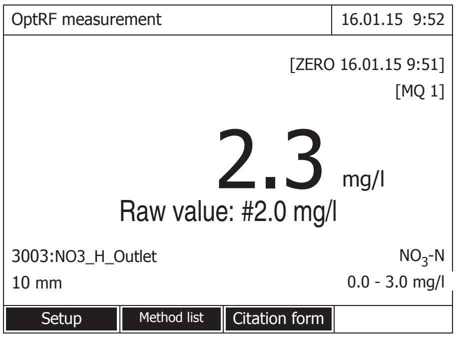
Example 2: NO3-N measurement in the outlet of a municipal waste water treatment plant

Sampling **Date**	**Time**	**OptRF raw value [#]** **NO_3-N (mg/L)**	**Reference value** **NO_3-N (mg/L)**
03.03.2015	07:30	12.1	10
10.03.2015	09:15	9.2	7
01.04.2015	16:30	7	5
15.04.2015	14:20	3.1	1
…			

In this example of a concentration offset of approx. 2 mg/L NO_3-N we recommend for the OptRF method 3004 NO3_H_Outlet a 2-point user calibration with the following value pairs:

	OptRF raw value [#] **NO_3-N (mg/L)**	**Reference value** **NO_3-N (mg/L)**
Value pair 1	3.1	1
Value pair 2	12.1	10

Evaluation of the measurement quality


OptRF measurement 16.01.15 9:52

[ZERO 16.01.15 9:51]

[MQ 1]

2.3 mg/l

Raw value: #2.0 mg/l

3003:NO3_H_Outlet NO_3-N

10 mm 0.0 - 3.0 mg/l

In addition to the measured value, an evaluation number for the measurement quality (MQ) is shown on the display.

The evaluation number (1 - 4) gives information about how suitable the evaluation model of the OptRF method is for the current sample matrix. The better the evaluation model matches the sample matrix, the besser the quality of the measured values will be.

For the determination of the evaluation number, the recorded spectrum is evaluated without taking the user calibration into account.

Rating number	**Meaning**
MQ 1	Excellent measured value quality
MQ 2	Good measured value quality
MQ 3	Sufficient measured value quality
MQ 4	Insufficient measured value quality, measurement invalid, display of "Unsuitable matrix"

The user calibration optimizes the calculated concentration values. The determined evaluation number, however, is not affected by a user calibration.

Characteristics and sources of error with OptRF measurement

With OptRF methods, the preparation and execution of the measurement can have a considerable effect on the measured value. Please make sure to exclude the following sources of error.

Foreign bodies, particles and air bubbles

Very turbid samples or samples with air bubbles (e.g. samples that were pipetted too quickly) cause the absorbance to be higher and thus the measured values to be incorrect (too high), or the message "Unsuitable matrix“ is displayed.
COD dissolved: Always carry out the OptRF measurement with the unfiltered sample, even if the sample is filtered for the reference measurement.

Quartz cell

The cell must be clean, without scratches and have opaque sides (automatic cell recognition)

If the quartz cell is very dirty, it has to be cleaned according to good laboratory practice with a small brush and/or suitable cleaning solutions such as ethanol, Edisonite etc.

Zero adjustment

To achieve optimum measurement results, the necessary zero adjustment and the sample should be measured in the same cell with the same orientation. According to good laboratory practice, water that is free of nitrogen and COD has to be used for the zero adjustment (i.e. no bacteria or algae intrusion from ion exchangers seldom used).

Errors with user calibration

Please make sure that the sample to be measured is a sample representative of the application environment and that the concentration values of the required parameters of this sample are promptly determined with OptRF measurements and a suitable reference measurement.

Known sources of error during the execution of cell test sets have to be excluded (sample preparation, pipetting volume etc.). According to good laboratory practice, the measurement should at least be double plus a control standard should be measured. Thus, outliers can be identified, measured value plausibility secured and the highest possible measuring accuracy achieved. For the determination of the reference value, the value should be in the center of the measuring range of the test set used.

For user calibration, suitable value pairs have to be selected. Negative raw values [#] have to be entered with a minus sign for the lower value pair (see section USER CALIBRATION FOR OPTRF MEASUREMENT).

Seasonal fluctuations of the water matrix

With higher nitrate values (e.g. in winter), the methods used may produce different results and messages. Then, higher dilutions may possibly be required depending on the parameter and in addition to optimization with user calibration. These dilutions may partly be different for each method.