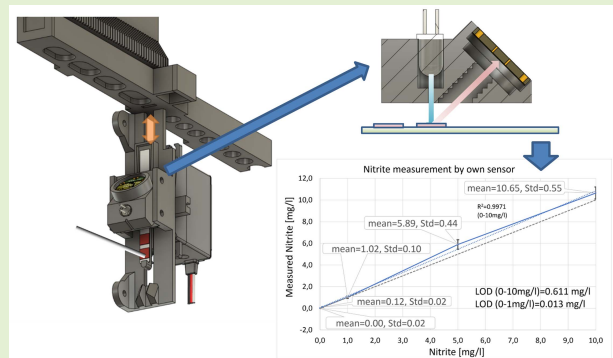


Automatic Measurement System for Nitrite and Nitrate in Water Bodies

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Abstract—Nitrate and nitrite are reaction products that result, for example, from the application of nitrogenous fertilizers to agricultural land. That part of the nitrogen not absorbed by the plants can pass into the groundwater in the form of nitrate or nitrite. This can also lead to contamination of drinking water via the groundwater, with all its negative side effects. In the present article an automatic system is presented, which performs independently the measurement of nitrate and nitrite at a water body. For the measurement of these parameters commercially available test strips are used, which are evaluated automatically. The determination of the nitrite/nitrate value is based on the Griess reaction which leads to a color change of the test pads depending on the concentration of the analyte. The sample is applied to the test pads with an automated dispenser system and the color change is measured with an RGB sensor. The nitrate/nitrite concentration present can be determined from the R/G ratio and a nomogram. The measuring accuracy of the system meets the requirements of the European Drinking Water Directive for the determination of the nitrate and nitrite value. The limit of detection (LOD) was determined for the self-designed sensor with LOD = 2.003 mg/l for nitrate over a measurement range of 0-50 mg/l nitrate and a LOD of 0.611 mg/l nitrite over a measurement range of 0-10 mg/l nitrite and 0.013 mg/l for a measurement range of 0-1 mg/l nitrite.

Index Terms—Nitrate, nitrite, optical sensor, test strips, water quality.



I. INTRODUCTION

WATER is our most important resource, and it must be protected and kept free from contamination. For this purpose, the EU Water Framework Directive specifies limit and guideline values for pollutants in order to achieve a systematic improvement and no further deterioration of the condition of all aquatic ecosystems and water bodies [1].

Especially in regions that are strongly characterized by agriculture, there is a high input of nutrients into the soils, and it is precisely there that there are considerable nutrient excesses. Liquid manure, slurry, dung, or fermentation residues from biogas plants are usually applied on site but cannot be completely consumed by the cultivated plants. Since nitrogen is only partially uptake by plants and only remains in the soil to a small extent, it is transported to a greater extent as nitrate

with the precipitation water into the groundwater. Another part of the excess nitrogen escapes into the atmosphere as ammonia, nitrogen, and nitrous oxide, thus contributing to climate change [2]–[3].

Only part of the excess nitrogen remains in the soil and the greater part is transported into the groundwater as nitrate with the precipitation water, or escapes into the atmosphere as ammonia, nitrogen and nitrous oxide. In Germany, the 5-year moving average of excess nitrogen in 2013 was 97 kg/ha/year, and this therefore contributes significantly to nitrification of soils and waters [4].

Nitrates (NO_3^-) are salts of nitric acid (HNO_3) and they are very soluble in water. Nitrates are applied in agriculture as essential fertilizers in the form of ammonium, potassium, calcium, and sodium nitrates. Organic nitrogen compounds can be converted to ammonium (NH_4) in soils and waters by biological processes. Soil bacteria can oxidize ammonium to nitrate in a multi-step aerobic process (nitrification) which is then available to plants as a nutrient. Nitrate can also be reduced by bacteria in a multi-stage anaerobic process to elemental nitrogen (denitrification) which escapes into the atmosphere.

A fundamental instrument in the EU for improving the nitrate situation in ground and surface waters is the implementation of the EU Nitrate Directive [5]. It contains specifications on the application of fertilizers to agricultural land, such as

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time and quantity restrictions on application, time and local prohibitions on the application of farm fertilizers, and record-keeping obligations for farmers.

The EU Groundwater Directive [6] and the EU Drinking Water Directive [7] specify a limit value of 50 mg/l for nitrate with measurement accuracy of $\pm 15\%$ and 0.1 mg/l for nitrite (NO_2^-) with measurement accuracy of $\pm 20\%$. In contrast to other substances, the limit value of nitrate of 50 mg nitrate per liter is not calculated for a lifetime exposure, but for an acute exposure. Especially in infants, when nitrate is reduced to nitrite by intestinal bacteria, the resulting nitrite can oxidize the blood pigment hemoglobin to methemoglobin. Methemoglobin cannot bind oxygen and consequently there is reduced oxygen uptake. This effect is known as infant cyanosis or “blue infant syndrome” [8].

In Germany, monitoring of groundwater status is carried out via a large number of monitoring sites operated by the federal states through two monitoring networks. Since 1997, the data from the groundwater monitoring stations must be reported to the European Environment Agency (EEA) in Copenhagen in accordance with the Water Framework Directive [1]. The collected measurements include a basic program with the most important physicochemical, chemical parameters, as well as volatile halogenated hydrocarbons (VHH), plant protection products (PPP) and trace metals [9]. The chemical parameters include nitrate.

The detection of nitrate and nitrite in the environment is usually carried out using complex analytical processes. For analysis, precise sampling must be carried out on site and the samples must be brought to the laboratory in a professional manner. This is a time-consuming and logistically not easy process that requires specially trained personnel. The samples are purified and conditioned in the laboratory in order to be able to carry out the appropriate analysis, which in turn is time-consuming and personnel intensive. The analytical techniques used can be broadly divided into spectroscopic techniques (colorimetry, fluorometry, chemiluminescence, chromatography, Raman, infrared) and electrochemical techniques (voltammetry/amperometry, potentiometry/ion-selective electrodes) [10]. The above methods for nitrate and nitrite detection are described in detail in the excellent reviews [10],[11]. For rapid and simple on-site analysis of nitrate and nitrite in surface waters, the above methods have limited applicability due to their often-high complexity. Sensor systems with test strips for the determination of soil parameters such as nitrate are discussed in [12]–[14], as well as the dependence of the measurement results on different environmental conditions.

Simple systems for the determination of water parameters based on commercial test strips have already been shown several times in the literature. In [15], a portable measurement system for the determination of arsenic in drinking water is shown. The method uses a microfluidic system designed by the authors in which the sample is mixed with the reagents. The conditioned sample is then applied to a commercial test strip and the color change is measured with a camera sensor. A comprehensive review on microfluidic sensors for overall water quality monitoring including heavy metals, nutrients, and pathogenic detection is given in [16]. In [17],

a method for easy monitoring of various water parameters using a cell phone app in conjunction with commercial test strips is shown. During this process, various environmental parameters (weather, etc.), the GPS coordinates, and a photo of the measuring strips are transferred to a database for evaluation. In [18], a pH sensor is described that is based on commercially available test strips and includes an app for cell phones in conjunction with a 3D-printed optical accessory. The system is based on digital image analysis of the colored test strips and achieves a quantification accuracy of 0.05 pH units.

Our paper describes an innovative sensor that can measure the current concentration of nitrate and nitrite in water bodies based on inexpensive test strips, on site, very quickly and with an operation time of at least one year. Test strips for different water parameters are inexpensive to purchase and easy to use. However, they have the major disadvantage that they can only be stored for longer periods under very low humidity. For a stationary sensor that uses test strips to automatically measure water parameters on a weekly basis over a one-year period, it is therefore necessary to ensure that the humidity in the vicinity of the test strips does not exceed a threshold value, for the entire operating time.

II. METHODS AND SENSOR DESIGN

For the periodic measurement of nitrate and nitrite in a river system, an automatic sensor was developed that works on the optical evaluation of commercial test strips with an integrated color sensor. The test strips and optical sensor were previously characterized and calibrated in detail.

A. Materials and Methods

The simplest method for colorimetric determination of nitrite is based on the Griess reaction [19]. The analytical principle of nitrite measurement is based on azo coupling of nitrite to a naphthylethylenediamine and photometric quantification of this color reaction. For nitrate measurement, a previous reduction of nitrate to nitrite is necessary in order to be able to detect the nitrate indirectly via the Griess reaction as well. Nitrate can be converted to nitrite with either cadmium nitrate reductase (NR) or vanadium(III) chloride (VC). Test strips for the measurement of nitrate and nitrite are available from various manufacturers and are presented in an overview in Table I.

Commercially available test strips for the combined measurement of nitrite and nitrate, which are based on the Griess reaction and a modified Griess reaction, were obtained from Macherey-Nagel GmbH & Co. KG, Germany (test strips: Quantofix Nitrate 100, order No. 91351, Fig. 1a).

As a reference for the measurements performed with our self-designed nitrate/nitrite sensor, the automatic reader for test strips Quantofix Relax (Macherey-Nagel GmbH & Co. KG, Germany, order No. 91346, Fig. 1b) was used. The reader can evaluate different test strips of the Quantofix series as well as the Quantofix Nitrate 100 test strip. The Quantofix Nitrate 100 test strip is used according to the manufacturer’s protocol by wetting the test strip with the test solution (shake off

TABLE I
A SELECTION OF COMMERCIALY AVAILABLE TEST STRIPS FOR NITRATE AND NITRITE AND THEIR PROPERTIES

Manufacturer	Type	Dev.*	Dual	Manufacturing tolerances**	NO ₃ ⁻ max. [mg/l]	NO ₃ ⁻ min. [mg/l]	NO ₂ ⁻ max. [mg/l]	NO ₂ ⁻ min. [mg/l]
Precision Labs	NIT501	N	N	+			25	0,5
Precision Labs	NIT and NAT	N	Y	+	500	10	25	0,5
WaterWorks	480009	N	Y	-	220***	2,2***	33***	0,5***
Hach Lange	2745425	N	Y	N/A	220***	2,2***	10***	0,5***
Supelco	110007	Y ^{a)}	N	N/A			80	2
Macherey-Nagel	Quantofix Nitrat 100	Y ^{b)}	Y	+	100	5	50	0,5
Macherey-Nagel	Quantofix Nitrit-Nitrat	Y ^{b)}	Y	+	50	10	80	1

* Measuring device available: ^{a)} Supelco RQFlex 20 (Merck, No. 117246), ^{b)} Quantofix Relax (Macherey-Nagel, No. 91346).
** Quality of mechanical manufacturing: tolerances, burrs etc.
*** Reference values of NO₂-N or NO₃-N converted to NO₂⁻ or NO₃⁻



Fig. 1. a) Quantofix Nitrate 100 test strip with two test pads. b) Quantofix Relax test strip measuring device.

excess solution) and comparing the test strip color with a reference color table after waiting 60 seconds. If the automatic reading system is used for the evaluation, the test strip must be inserted into the reader after the waiting time of 60 seconds. The Quantofix Relax system was checked and calibrated using color control test strips (No. MANA930988, Sigma-Aldrich, Germany). For all measurements with the test strip sensor and the Quantofix Relax system 1000 mg/L stock solutions for nitrate and nitrite are prepared by dissolving 75.0 mg sodium nitrite (order No. S-2252, Sigma-Aldrich Corporation, USA) in 50 ml aqua bidest (Fresenius Kabi Austria GmbH) and by dissolving 81.5 mg potassium nitrate (order No. 542040-10G, Sigma Aldrich Corporation, USA) in 50 ml aqua bidest. The Limit of Detection (LOD) was calculated according to methods described in the literature [20], [21]

$$LOD = 3.3 \frac{\sigma_y}{b} \quad (1)$$

where σ_y is the standard deviation of the y-intercept and b is the slope of the regression curve, respectively. In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected.

B. Design of the Sensor

The automatic sensor for nitrite and nitrate is to be used in a stationary system for determining water parameters on a river. For this purpose, it is necessary that the sensor automatically records water parameters over a longer period of time (>1 year) and do not require any maintenance. In order to be able to carry out a sufficient number of measurements with test strips, a supply magazine is provided for this in the sensor. The size of the magazine depends on the expected number of measurements to be performed. In the example shown, the supply magazine is designed for 33 test strips in order to be able to perform a measurement every 2 weeks within a year. The test strips used are shown in Fig. 1b, with Pad #1 being used for nitrate measurement and Pad #2 for nitrite measurement.

The measuring system consists of 3 main components: a) the magazine with the test strips (Fig. 2a,#1), b) a mechanism for transporting and placing the test strip (slider), (Fig. 2a,#2), and c) a sensor head with a drip nose for the sample application and the spectrophotometer which is shown in Fig. 2b. To perform the measurement, a storage compartment of the magazine is opened, from which the test strip falls into a slide. The slide moves the test strip to different positions: a) for priming the tubing system, b) for a reference measurement on the dry pads, c) for applying the sample to the respective pad, d) for measuring the discoloration of the pads, and e) for transporting the test strip to the waste container.

The magazine consists of small compartments with $58 \times 6 \times 1.5$ mm in size, in which the individual test strips are located. For performing the measurement, the magazine is moved by a stepper motor until a storage compartment opens and the test strip falls into the carriage (slider) of the measuring device. Loading of the test strip into the sled follows the procedure in Table II, step 1. The measurement process starts with moving the sled with the test strip in the priming position, where the test strip is still outside the measurement chamber and not in contact with liquid. During the priming process, 1ml of sample is pumped through the tubing system to flush the tubing and the drip tip (Table II, step 1). At the same time, the optical sensor is used to measure the

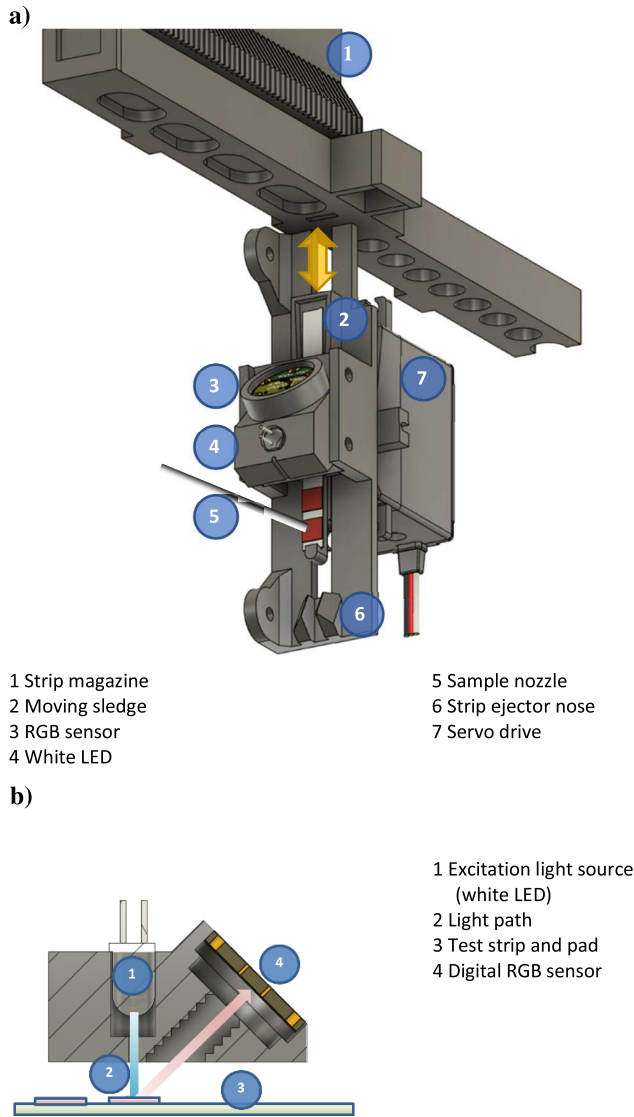


Fig. 2. a) Design of the test strip sensor. b) Optical measurement chamber for color measurement of the strip pads.

dark value for reference purposes (Table II, step 2-3). In the next step, the dry pad #1 of the test strip is placed in the measurement position and a reference value is measured with the RGB sensor (Table II, step 4). Pad #1 is then positioned under the drip tip and approximately 67 μl of sample is applied to Pad #1 (Table II, step 5). After a waiting time of 60 seconds, the slide with the test strips moves back into the optical measurement chamber, where the pad is irradiated with white light from an LED (C512A, Cree Inc. USA) and the reflected light (remission) is measured with an RGB sensor (TCS34725, Adafruit Industries, LLC, USA) (Table II, step 6, Fig. 2b). To record the nitrite value, steps 4 to 6 in Table II are performed in the same way for pad #2. After the measurements are completed, the test strip is disposed of in a waste container via an ejector nose.

The procedure for measurement of nitrate and nitrite by a test strip is described in detail in Table II.

A picture of the developed instrument is given in the supplementary material Fig.1S. The sample transport and dispensing

TABLE II
STRIP LOADING AND NITRATE/NITRITE MEASUREMENT PROCEDURE

Nitrate/nitrite measurement procedure	Position of the test strip
1) Strip loading	Strip glides into priming position
2) Priming of tubing system for 15 seconds (~1 ml vol.)	Priming position
3) Dark reference measurement with LED off	Priming position
4) Nitrate/nitrite reference measurement of dry pad	Pad #1/#2 is in the optical sensor
5) Applying sample on nitrate/nitrite pad (1 sec. ~67 μl vol.)	Pad #1/#2 is below drip nose
6) Color measurement of nitrate/nitrite pad	Pad #1/#2 is in the optical sensor
7) Repeat step 4 to 6 for pad #2	
8) Wasting the strip	Wasting position

system consists of a thin silicone tube with an inner diameter of 0.1 mm and an outer diameter of 0.3 mm. The sample is transported from the sampling point to the sensor by a roller pump (WPM1-P3BA-BP, WELCO Co., Ltd., Japan) in which the tubing system is inserted. Details can be taken from Fig.1S (supplementary material).

A key challenge in the development of the sensor was that the test pads are sensitive to moisture, causing the sensor pad to discolor, which makes reliable sample determination no longer possible. However, a sample volume of 70 μl is applied to the test pads in the sensor for each measurement, causing water to accumulate in the sensor and moisture to form. Our investigations have shown that the humidity in the sensor can be kept constantly low if a hydrogel or a molecular sieve with sufficient absorption volume is used to adhere the water vapor. For this purpose, a hermetically sealed housing was designed for the sensor to prevent intrusion of external moisture and thus ensure long-term stability of the nitrate/nitrite test pads.

III. RESULTS

For all measurements, the Quantofix Nitrate 100 test strips were used for nitrate and nitrite determination. The test strips were measured with the self-made sensor system and for comparison, with the Quantofix Relax (Macherey-Nagel GmbH, Germany) commercial system. The measurement results were compared and verified, and the measurement uncertainties to be expected were specified. The nitrate/nitrite reading is calculated from the intensities of the red and green spectral components reflected from the respective test pad (remission value). To account for variations in the inherent color of the test pads, the measured intensities are related to the inherent color of the test pads. The calculation formula for the normalized remission x is given in Eq. 2.

$$x = 1 - \frac{I_{redref}/I_{greenref}}{I_{red}/I_{green}} \quad (2)$$

In order to assign the measured and referenced ratio of red and green intensity to a nitrate/nitrite value, the standard curves for nitrate and nitrite were measured using nitrate and nitrite calibration solutions (see chapter IIA) and depicted in Fig. 3a,b. The optical sensor provides the remission value x

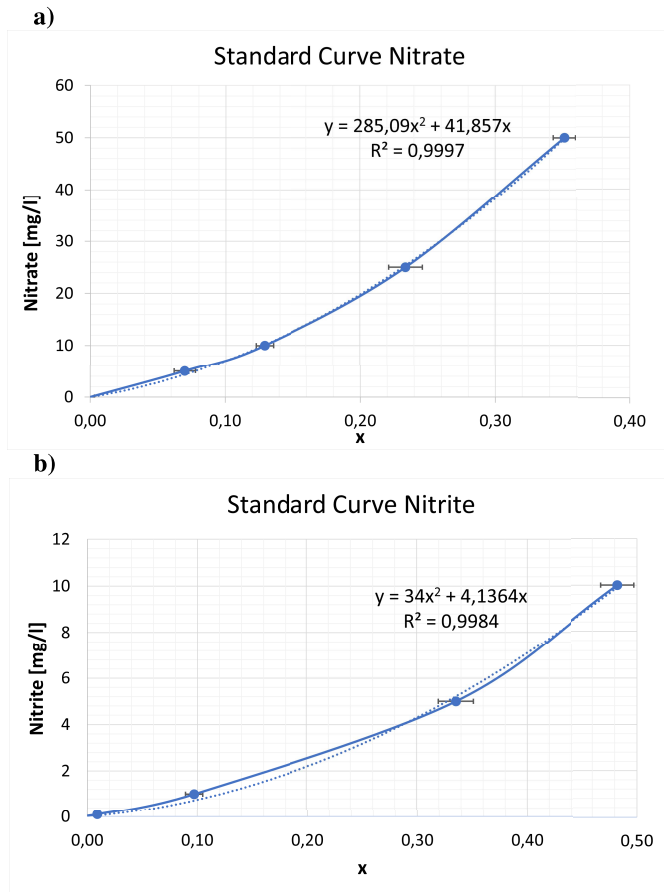


Fig. 3. a) Standard curve for nitrate, b) standard curve for nitrite (N = 6.)

according to Eq. 2, from which the nitrate and nitrite values can be determined from the standard curves by the regression functions given in Eq. 3 and Eq. 4.

$$\text{Nitrate} \left[\frac{\text{mg}}{\text{l}} \right] = 285,09x^2 + 41,857x \quad (3)$$

$$\text{Nitrite} \left[\frac{\text{mg}}{\text{l}} \right] = 34x^2 + 4.136x \quad (4)$$

The measured remission values x as a function of the calibration solutions for nitrate and nitrite can be excellently approximated in both cases by 2nd order regression polynomials. The calculated standard deviations to the obtained remission values are shown as black bars at the respective measuring points.

In order to test the performance of the sensor, the measured values obtained from the sensor were compared with the measured values of the commercial analyzer for test strips (Quantofix Relax). For this purpose, the test strips were wetted with nitrate and nitrite reference solutions and the color change was measured. The measurements were repeated 6 times independently and the results for the nitrate measurements are shown in Fig. 4a,b. The self-designed sensor shows excellent recovery and high accuracy of readings with low standard deviation of measurements. The Quantofix Relax instrument is limited in its measurement range by the manufacturer. For color changes of the test strip corresponding to nitrate

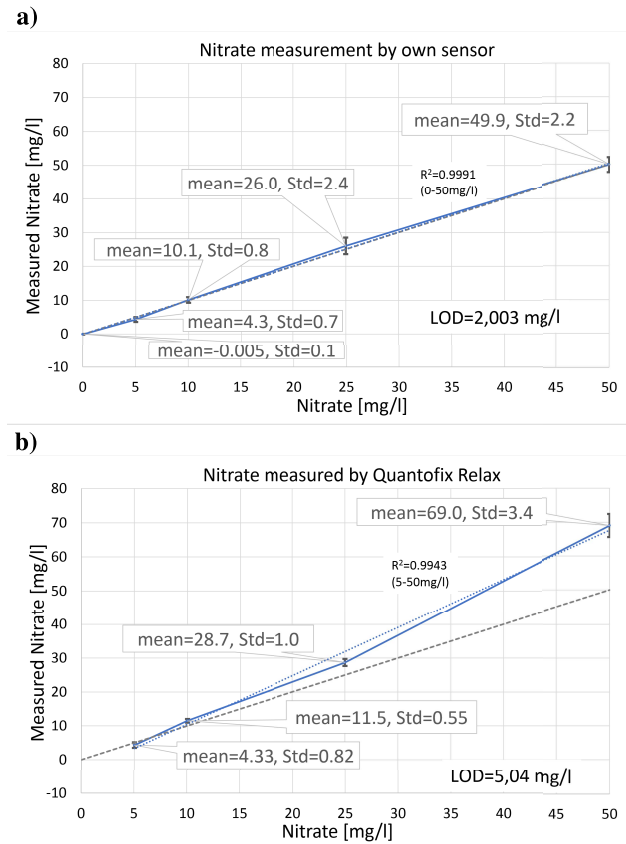


Fig. 4. Obtained measurements of nitrate calibration solutions a) by the self-designed sensor, b) by the Quantofix Relax system (N = 6.)

concentrations below 5 mg/l, the meter will not display a measurement value, but only that the measurement value is less than 5mg/l. The meter behaves in the same way for nitrate values greater than 100 mg/l where the display shows that the measured value is above 100 mg/l. In addition, the Quantofix Relax meter shows a significant systematic deviation from the reference value at concentrations of 50 mg/l nitrate. The limit of detection (LOD) was determined for the self-designed sensor with LOD = 2.003 mg/l for nitrate and for the Quantofix Relax meter with LOD = 5.04 mg/l. The LOD for our sensor is lower by a factor of approximately 2.5 and thus allows a significantly higher accuracy.

Fig. 5a,b shows the measurement results of the nitrite study for the test strip sensor and the Quantofix Relax measuring device. It can be seen that both measuring systems show a systematic deviation from the reference value at concentrations of 5 mg/l and 10 mg/l nitrite. The Quantofix Relax system did not provide a measured value for nitrite concentrations <0.5 mg/l, but an indication on the display that the measured value is less than 0.5 mg/l. The LOD was found to be 0.611 mg/l for the test strip sensor over the measurement range of 0-10 mg/l nitrite and 0.013 mg/l for the measurement range of 0-1 mg/l nitrite. For the Quantofix Relax system the LOD was determined to 1.817 mg/l over the measuring range of 0-10 mg/l nitrite. The linearity of the obtained measurement curves was checked using the correlation coefficient R^2 of a linear regression and presented to the diagrams (Fig.4, Fig.5). For all obtained data

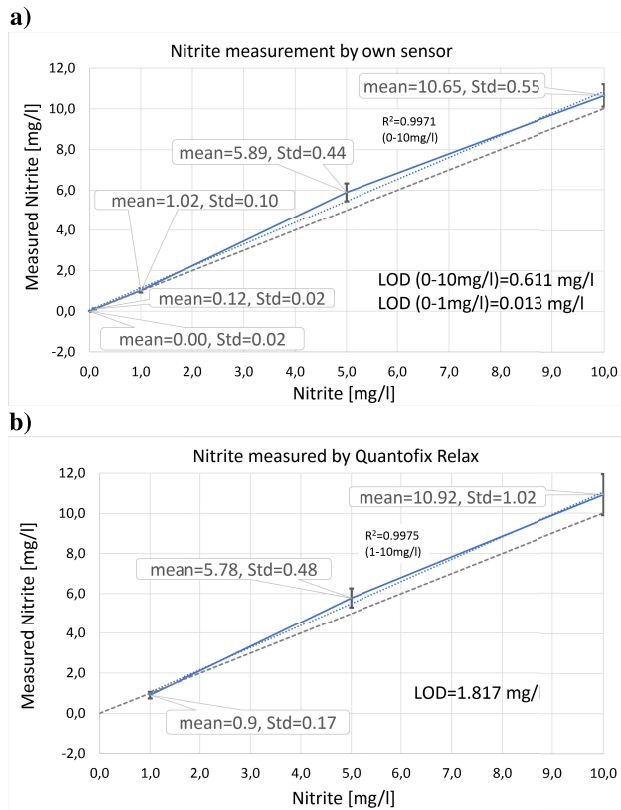


Fig. 5. Obtained measurements of nitrite calibration solutions a) by the self-designed sensor, b) by the Quantofix Relax system (N = 6).

a correlation coefficient $R^2 > 0.99$ was found, which speaks for an excellent linearity of the measurement results.

In summary, the self-designing test strip system can achieve the required measurement accuracy for the determination of the limit values for nitrate (nitrate limit value = 50 mg/l with measurement uncertainty of $\pm 15\% = \pm 7.5$ mg/l) and for nitrite (nitrite limit value = 0.1 mg/l with measurement uncertainty of $\pm 20\% = \pm 0.02$ mg/l) according to the European drinking water directive. The Quantofix Relax system can measure with sufficient accuracy the limit value for nitrate but not the limit value for nitrite.

A. Long Term Stability of the Sensor

The Quantofix Nitrate 100 test strips used employ the (modified) Griess reaction, for the detection of nitrate and nitrite, which provides a concentration-dependent color change of the test pads. However, the test pads are also sensitive to temperature and moisture, which causes discoloration of the pads and makes the test strips unusable for measurements.

1) *Influence of Moisture on the Measurements:* To reduce the influence of moisture, it is necessary to protect the test pads from moisture during the entire period of use in order not to influence the quality of the measurement results. For this purpose, the entire sensor has been installed in a hermetically sealed housing to prevent moisture intrusion. However, for priming and the measurement process, a certain amount of sample liquid is transported into the sensor and applied to the test pads. This sample volume must be bound in the sensor to

prevent moisture from rising in the housing. The entire sensor housing has a cubature $L \times W \times H$ of $40 \times 30 \times 26$ cm and a volume of 31200 cm^3 . For one measurement run, below 1 ml is required for priming and about $70 \mu\text{l}$ of the liquid sample is required for each of the nitrate and nitrite measurements, which together roughly results in a maximum of 1.2ml of liquid volume per measurement. To keep the humidity in the sensor constantly low ($< 30\%$), 750 g of silica gel (T-SC-100, KnePo Kunststofftechnik GmbH, Germany) was placed in the sensor housing. The silica gel can absorb about 30% of its own weight in liquid, which corresponds to about 225 ml. The sensor is currently designed for an operational period of 1 year over which 30 measurements will be taken. The expected volume of liquid injected into the sensor is about 36 ml and can be absorbed by the silica gel in any case.

Since the color of the sensor's test pad changes depending on the ambient humidity, the influence on the measurement accuracy was investigated. For this purpose, the nitrate/nitrite test strips were stored in a hermetically sealed housing at different humidity levels and the discoloration of the pads was measured. The humidity was measured continuously over the observation period using a humidity sensor (digital humidity sensor SHT21, Sensiron AG, Switzerland). An RGB sensor (TCS34725, Adafruit Industries, LLC, USA) was used to determine the color values of the test pad. Since the nitrate/nitrite value is calculated from the red to green (R/G) ratio (see Eq. 2), the change in R/G ratio as a function of ambient humidity over time was observed (Fig. 6a). The R/G ratio exhibits a significant variation (discoloration) over time for moisture values greater than 40%, which can be very well approximated by an exponential function. The time constant of the R/G ratio change over time was found to be $\tau = 43$ hours independent of the respective ambient humidity.

The mathematical model for the progression of the R/G ratio over time as a function of ambient relative humidity is described in Eq. 5. The position of the horizontal asymptote depends on the relative humidity in the environment of the test strip and can be approximated by another mathematical model, which is shown in Figure 6b and described in Equation 6 where t is the time, τ is the time constant of discoloration ($\tau = 43$ hours) and A models the horizontal asymptote for different relative humidities RH .

$$\frac{R}{G} = 1 - A \left(1 - e^{-\frac{t}{\tau}} \right) \quad (5)$$

$$A = 0.0003e^{0.1098 \cdot RH[\%]} \quad (6)$$

Our laboratory experiments have shown that the color change of the test pads caused by the ambient humidity is additive to the color change of the Griess reagent determined by the nitrate/nitrite concentration. This is true for low ambient humidities of $RH < 30\%$. The ambient humidity causes an offset of the zero point in the R/G ratio and can be compensated by a zero-point calibration before starting the measurement. Failure to perform this compensation will result in a deviation of the measured value according to the values listed in Table III. To keep the offset values and the resulting deviation from the zero point as small as possible ($< 1\%$), the relative humidity in the vicinity of the test strips should be kept constantly below 30%.

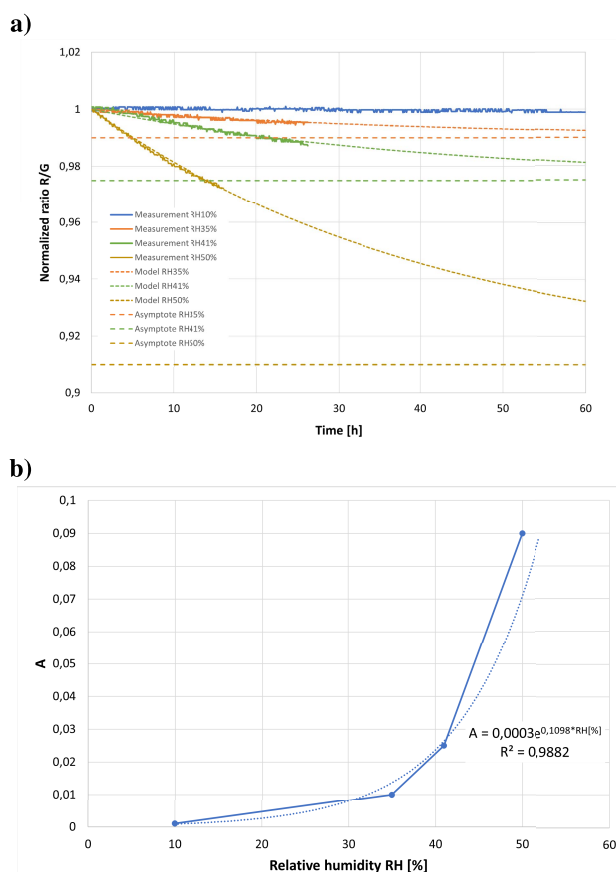


Fig. 6. a) Dependence of R/G ratio versus time for different relative humidities (RH). b) Model of the horizontal asymptote for different relative humidities.

TABLE III

MEASUREMENT UNCERTAINTY BY DISCOLORATION

RH [%]	A	Expected long term deviation without zero-point calibration
10	0.001	0.1%
35	0.01	1.0%
41	0.025	2.5%
50	0.09	9.0%

2) *Influence of Temperature on the Measurements and Reaction Kinetics:* A chemical reaction takes place on the test pads of the test strip, which represents the concentration of nitrate and nitrite in the sample as a coloring reaction. In general, almost all chemical reactions proceed faster when the temperature is increased. Conversely, this means that slower chemical reaction kinetics must be expected for lower temperatures, and thus the coloration of the test pads will only reach a steady-state value after a longer period of time. If the sensor is used over an extended temperature range, this must also be taken into account accordingly in the algorithm of the evaluation unit so that no deviating measurement results are obtained. The reaction kinetics were examined as an example for nitrite with a concentration of 5 mg/l at different temperatures (Fig. 7). Since the measurement system

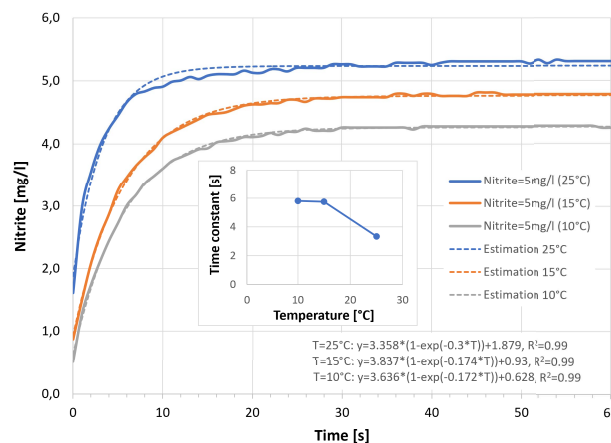


Fig. 7. Reaction kinetics of the nitrite test pad in dependency on the sample temperature.

TABLE IV
PARAMETERS FOR EQ. 7

T [°C]	a [mg/l]	b [1/s]	c [mg/l]	$\tau=1/b$ [s]	R^2
10	3.64	0.172	0.63	5.81	0.99
15	3.84	0.174	0.93	5.75	0.99
25	3.36	0.3	1.88	3.33	0.99

is used at a river, the measurements were carried out at water temperatures between 10°C and 25°C. To capture the effect of temperature on reaction kinetics, a mathematical model (Eq. 7) was formulated by approximation, and this was evaluated. Parameters for Eq. 7 are listed in Table IV.

$$y = a \cdot (1 - e^{-b \cdot T}) + c \quad (7)$$

It can be seen that the reaction kinetics of the nitrite test pad are approximately the same for temperatures of 10°C and 15°C, and the measured course of the staining reaction has the same increase (10°C: $\tau = 1/b = 5.81$ s; 15°C: $\tau = 1/b = 5.75$ s). For a sample temperature of 25°C, the time constant of the reaction kinetics reduces to $\tau = 3.33$ s. From the measurement results it follows that, especially at low temperatures, the longer duration of the coloring reaction must be taken into account and the evaluation must be carried out only after a stable measured value has been reached (min. 5τ). As described in Section II.B, the reading of the test pad coloration is taken after 60 seconds, which is in any case more than the calculated 5τ .

In addition to the influence of temperature on reaction kinetics, the influence of sample temperature on measurement accuracy was also investigated. For this purpose, the sensor and the samples were exposed to different temperatures and measurements were carried out on standard solutions for nitrate and nitrite in triplicate. Over the investigated temperature range from 5°C to 25°C, an almost linear dependence of the nitrate measured value on the temperature was found [Fig. 8]. In the range around 20°C the measured value corresponds to the reference value, which indicates that the chemical reaction in the test pad was calibrated to this temperature by the manufacturer. Above 20°C the measurement is overestimated

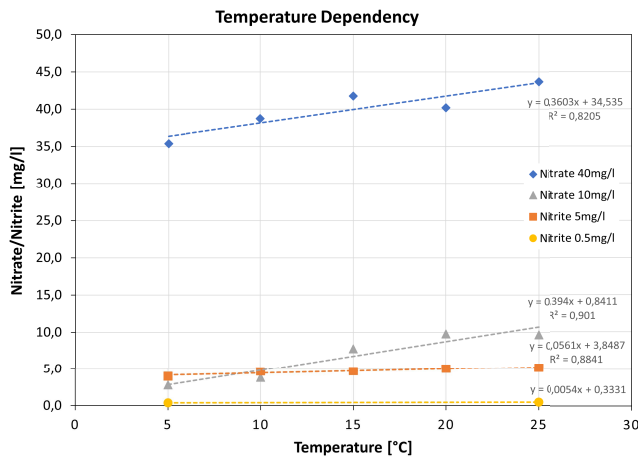


Fig. 8. Dependency of nitrate and nitrite measurements on temperature.

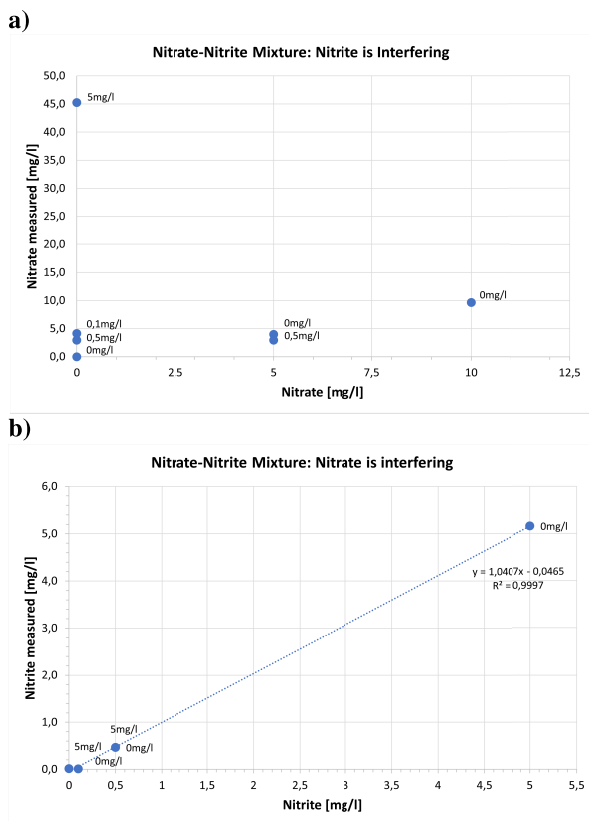


Fig. 9. a) Interference of nitrite on the nitrate measurement, b) interference of nitrate on the nitrite measurement. The interfering nitrite or nitrate concentrations are depicted at the measuring points.

and below 20°C it is underestimated. Quite similar results for the temperature dependence of the nitrate reading of Quantofix test strips were also found in [22]. The slope of the regression line for the analyzed nitrate concentration versus temperature is almost identical. This allows a parallel shift of the regression lines for different nitrate concentrations for temperature compensation. Thus, the temperature dependence of the nitrate readout can be compensated with a very simple algorithm, if required. The nitrite measured value shows almost no temperature dependence in the temperature range investigated.

3) *Influence of Nitrite on the Nitrate Measurement:* The test strip used for the measuring system consists of a test field for nitrite, which is determined via the Griess staining reaction, and a test field for nitrate, which is determined via conversion to nitrite and subsequent determination with the Griess staining reaction (modified Griess reaction, see section II.A). Since the modified Griess reaction is used for the nitrate determination, an influence of nitrite on the measurement result is to be expected. The influence of nitrite on the nitrate measurement is shown in Fig. 9a. It is shown that an addition of nitrite, as expected, leads to a clear overestimation of the nitrate concentration actually present in the sample. Conversely, addition of nitrate shows no effect on a nitrite measurement (Fig. 9b) as expected. It is therefore recommended to discard the measured nitrate value if nitrite is present in the sample. As noted in the data sheet of the Quantofix test strips manufacturer, the influence of nitrite on the nitrate measurement can be avoided by adding sulfamic acid to the sample to overcome this problem [14].

IV. CONCLUSION

In this paper an automated measuring system for the determination of nitrate and nitrite in surface waters is shown. The measuring system consists of a storage magazine in which commercial test strips for nitrate/nitrite determination are stored and an automatic device for carrying out the measurement. The determination of the nitrite/nitrate value is based on the Griess reaction which leads to a color change of the test pads depending on the concentration of the analyte. The sample is applied to the test pads with an automated dispenser system and the color change is measured with an RGB sensor. The nitrate/nitrite concentration present can be determined from the R/G ratio and a nomogram. The measuring accuracy of the system meets the requirements of the European Drinking Water Directive for the determination of the nitrate and nitrite value. To ensure automated use of the system over a period of 1 year, the test strips were subjected to artificial aging and the influence of ambient humidity and temperature on the measurement result were investigated. From this, a recommendation could be derived that the relative humidity in the environment of the test strips should be kept constantly below 30% in order to keep the measurement deviations low. A second recommendation is to take the temperature of the sample into account when evaluating the measurement results in order to keep the temperature influence low.

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