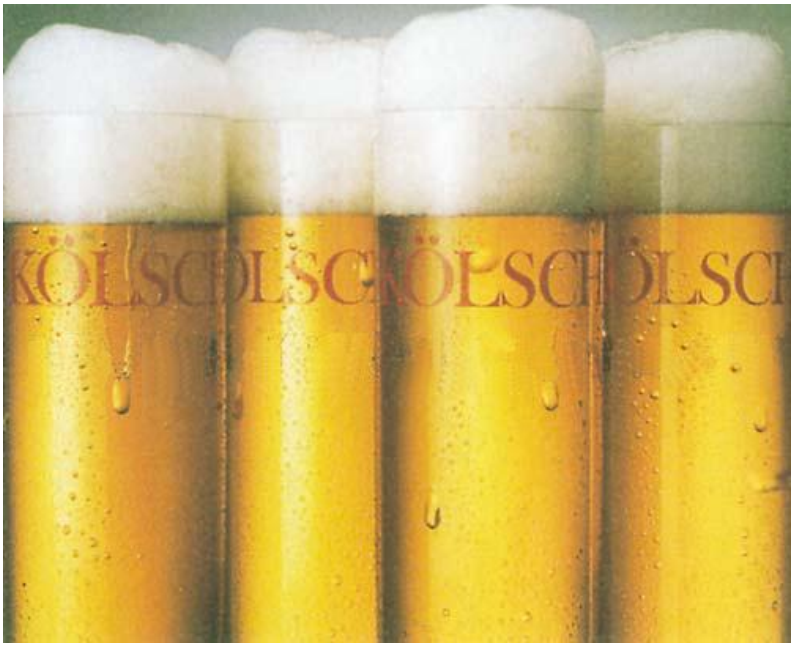
MACHEREY-NAGEL GmbH & Co. KG
Neumann Neander Str. 6-8
52355 Düren / Germany
Tel.: +49-(0)2421-969-0

NANOCOLOR® PC-Software for Spectrophotometers

Version 4.0

Rev. 7 (October, 2010)



Instructions for Brewery Analysis

Software Manual Addendum II

1 Introduction

Since version 3.0 of the ***NANOCOLOR*® PC software for Spectrophotometers** MACHEREY-NAGEL offers the possibility to conduct all photometric analysis methods (with the exception of enzymatic tests) according to the MEBAK, Volume II, 2002. Simply select the required test, insert the requested cuvette in the photometer and read the measured value.

2 Tests

The following tests can be carried out with the ***NANOCOLOR*® PC software for Spectrophotometers**:

Anthocyanins acc. to Harris and Ricketts	MEBAK 2.17.2
EBC beer colour	MEBAK 2.13.2
Bitterness units	MEBAK 2.18.1
Total carotenoids, fraction 1-4	MEBAK 3.7.2.1
Total carotenoids in carrot juice	MEBAK 3.7.2.2
Total polyphenols	MEBAK 2.17.1
Copper (cuprethol)	MEBAK 2.29.4
Nickel	MEBAK 2.29.6
Thiobarbituric acid value	MEBAK 2.4
Vicinal diketones	MEBAK 2.23
α-acids	MEBAK 2.18.2
Cyclamate	MEBAK 3.11.4
Iron	MEBAK 2.29.3
Free amino nitrogen (FAN)	MEBAK 2.8.4.1
Total carotenoids acc. to Wesergold	MEBAK 3.7.2.3
Iso-α-acids	MEBAK 2.18.2
Copper ZDBT	MEBAK 2.29.5
Photometric iodine sample	MEBAK 2.3.2
Total carbohydrates in beer	MEBAK 2.11

To conduct these tests you need all devices and chemical substances indicated in the relevant chapters of the MEBAK book "Brautechnische Analysenmethoden" (Methods of Analysis used in Brewery).

3 Perform a test

Start the ***NANOCOLOR*® PC software for Spectrophotometers**, connect the photometer to the computer and switch it on. Click on the *Measure* than chose *Brewery analysis* , as shown in Figure 1.

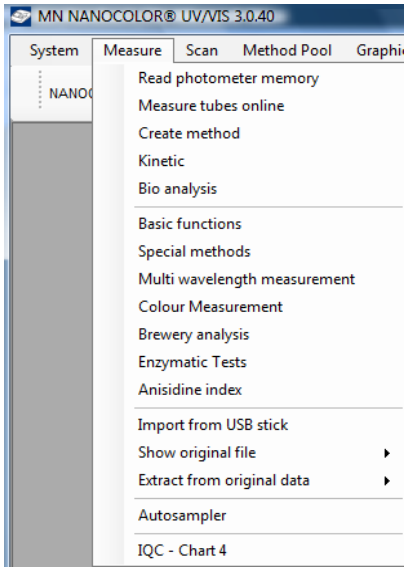


Fig. 1: Menu Measure/Brewery analysis

Figure 2 shows the *Brewery analysis* window.

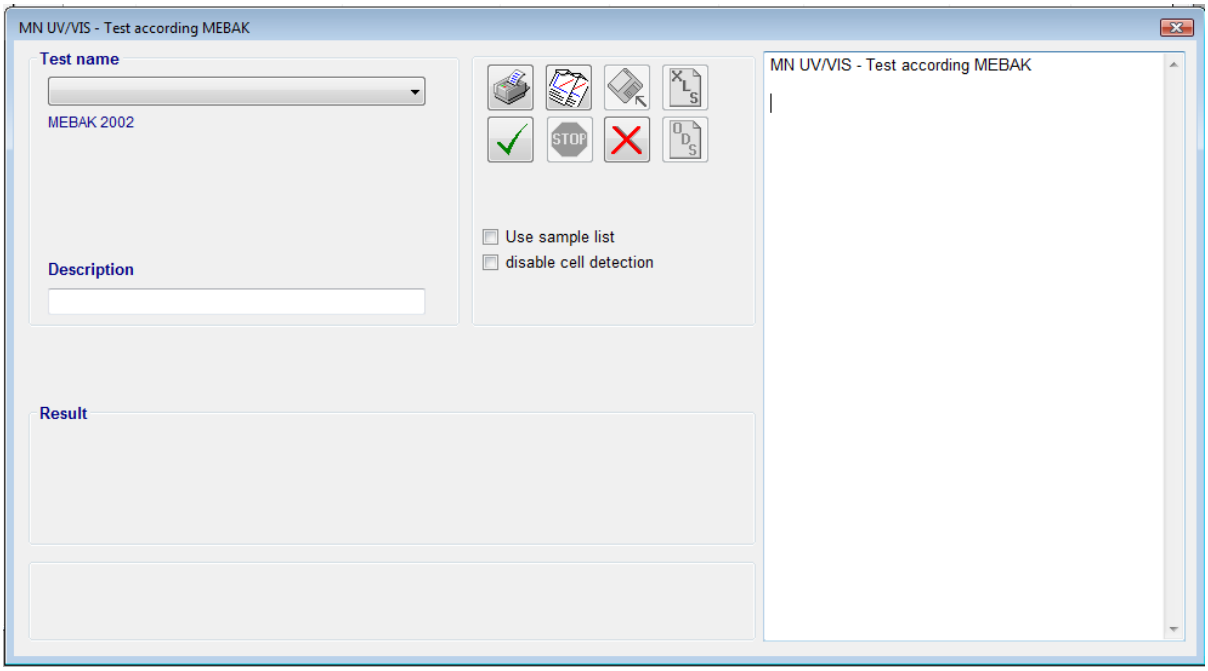


Fig. 2: Brewery analysis window

In the right section of the window, you can see the text box for the measurement log. All measurements are logged in accordance with GLP regulations. In the upper left section of the window is the *Test name* drop-down list. The required test can be selected here. Below, you will find the box *Description*. If the software security is set to **high**, this box will be compulsory. This means that if you do not enter a designation, batch number or identification for your sample, the measurement cannot be initiated. If a sample list has been created, the *Description* box will be filled automatically. At the right hand side of the *Test name* drop-down list is a row of buttons. These buttons have the following functions:

The  button prints the measurement log. This button is disabled until the first measurement has been carried out. The second button  copies the measurement log to the clipboard. This button is also disabled until the first measurement. The third button  starts the measurement. A measurement can be started only if a test has been selected and eventually a name has been assigned to the sample. The fourth button  closes the window. Just like in all tests deployed by the ***NANOCOLOR*® PC software for Spectrophotometers**, an original file is also generated for the brewery analysis tests when the software window is closed. This file contains all measured results and is saved automatically (see software manual, Chapter 3.7). The  button will only be enabled for those photometric tests in which a calibration curve has to be recorded. By clicking this button, the calibration data of the current test is saved.

The  and  buttons are disabled until the first measurement has been performed. These buttons export all results obtained during one session in tabular form to Excel or Open Office.

The last button  will be enabled only if a list of samples is being processed (see also software manual, Chapter 8.10). This button allows you to cancel processing the list.

Below this button are the *Use sample list* and *Disable cell detection* option checkboxes. If you want to perform the measurements of an existing sample list, activate the first checkbox. If you are working with disposable semi-micro or standard cuvettes recessed on both sides, choose the option *Disable cell detection* since these cuvettes do not activate the photometer's contact buttons for the cuvette recognition and, consequently, neither the photometer nor the software will detect that a cuvette has been inserted.

The other elements of the *Brewery analysis* window vary depending on the test selected and are described in the respective section (see Chapter 4).

4 The Tests in Detail

In this addendum, instructions on the analytical methods applied in the tests are not given. For questions related to the analytical methods, refer to the MEBAK book "Brautechnische Analysenmethoden" (Methods of Analysis used in Brewery). However, all tests share the same test procedure. The test "Anthocyanins acc. to Harris and Richetts" serves as an example of how to proceed when carrying out the tests. Please, read the test procedure in Chapter 4.1 carefully, **even if you are not going to work with this test**.

4.1 Anthocyanins acc. to Harris and Ricketts, MEBAK 2.17.2

This test is conducted with a 10 mm cuvette and a wavelength of 550 nm. Here, the sample is measured against a blind sample (distilled water, prepared in the same way) as zero reference solution. Select in the *Test name* drop-down list the "Anthocyanins acc. to Harris and Ricketts" test. As you can observe in Fig. 7, the test wavelength and the required cuvette size are displayed in the upper section of the window, and the test name is shown in bold face letters below the *Description* text box.

Now, enter a designation or number for the sample in the *Description* text box.

Click on the  button or press the **[ENTER]** key. The software now asks for the zero reference solution (see Fig. 3).

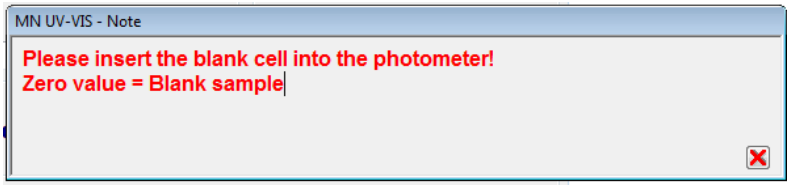


Fig. 3: Requesting the blind sample be inserted

Insert the cuvette with the previously prepared **blind sample** in the photometer. After the measurement, the software requests that the cuvette is removed, see Fig. 4.

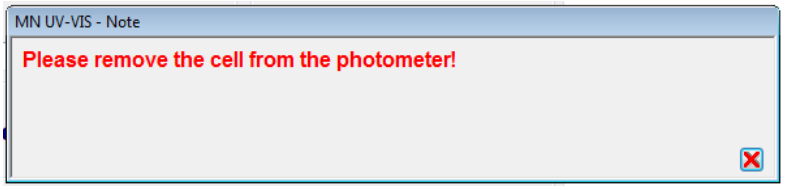


Fig. 4: Requesting the blind sample to be removed

Once the blind sample has been removed, the software asks for the sample to be inserted in the photometer (see Fig. 5).

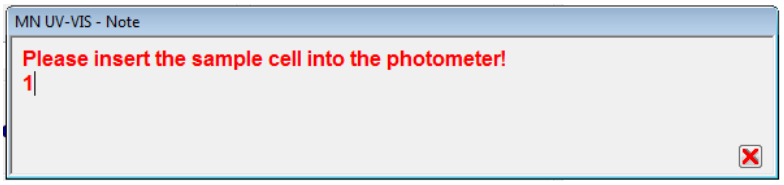


Fig. 5: Requesting the sample to be inserted

After completing the measurement, you will be asked again to remove the cuvette from the photometer. Once the cuvette has been removed, the absorbance measured is shown in the *Result* window area (see Fig. 6).

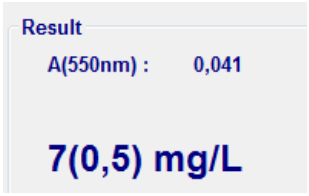


Fig. 6: Displaying the absorbance

The result is displayed in mg/l without decimals (see Fig. 7). The calculation procedure specified by the MEBAK is shown below the *Result* window area.

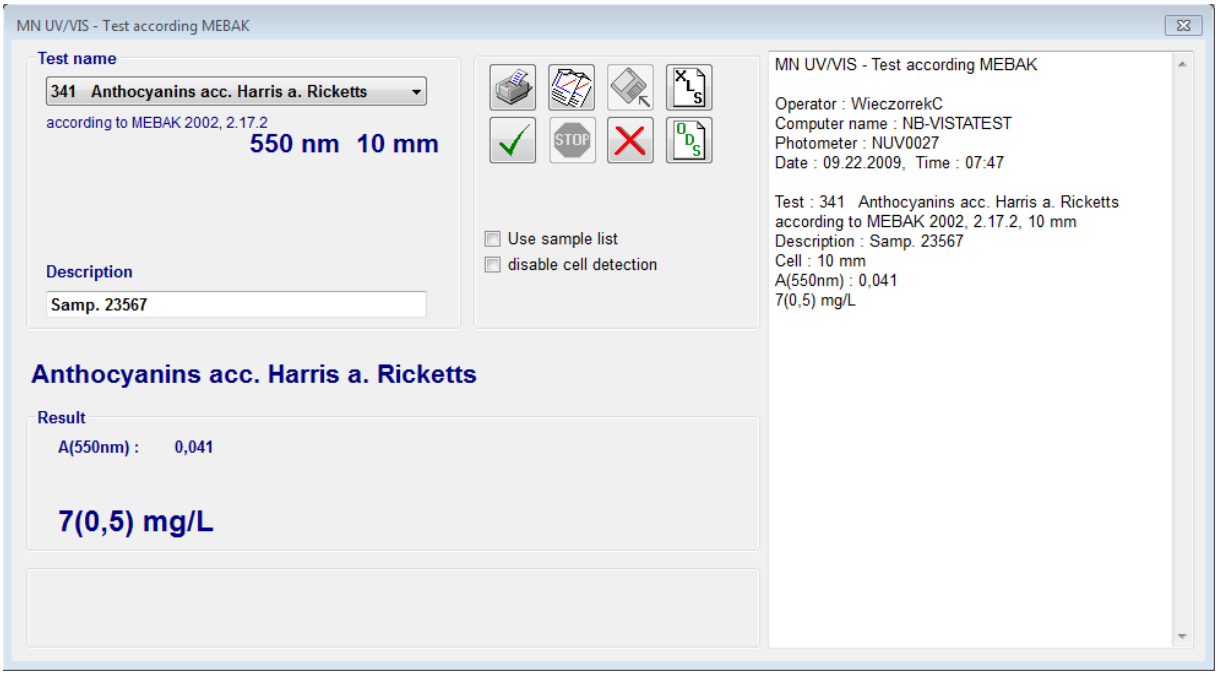


Fig. 7: Displaying the measurement result

The value displayed in this example is the result of a measurement made with a water cuvette and therefore not an actual value for the Antocyanins test. All relevant measurement data is registered in the measurement log shown in the right hand window area. The result can be printed or copied to the clipboard. If further tests are conducted, the new results are added to the log.

4.2 EBC Beer Colour, MEBAK 2.13.2

This test is conducted with a 10 mm cuvette at a wavelength of 430 nm. Here, the sample is measured against water as zero reference solution. Select "Beer colour EBC" test from the list. A second text box, namely *Factor*, appears below the list (see Fig. 8).

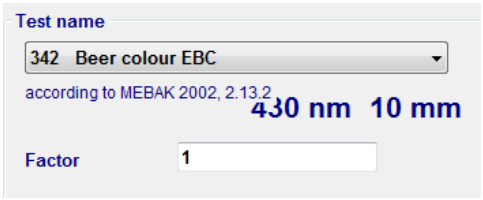


Fig. 8: Entering a dilution factor

For this test, a dilution factor can be entered. The value set here by default is "1", for undiluted solutions. In this box, enter the dilution value you are using for your experiment, i.e. "10" for a 1:10 dilution. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette be inserted. Insert the **water cuvette** in the photometer. Then, you are asked to insert the sample cuvette. The result is shown in EBC units with two decimals in the same way as in Fig. 6.

4.3 Bitterness Units, MEBAK 2.18.1

This test can not be measured with the ***NANOCOLOR*® *VIS*** photometer.

This test is conducted with a 10 mm cuvette at a wavelength of 275 nm. Since this test involves UV light, quartz cuvettes are required! The sample is measured against iso-octane as zero reference solution. Select the "Bitterness units" test from the list. There are two methods available for the calculation of the bitterness units, depending on the kind of sample being analysed: beer and wort. Before performing the measurement, you have to specify which kind of sample is going to be analysed. To do this, the software displays two option buttons below the test selection list, namely *Beer* and *Wort* (see Fig. 9).

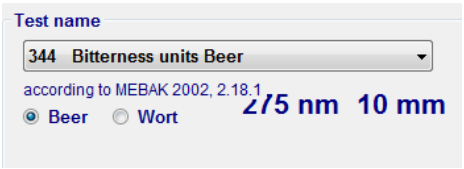


Fig. 9: Selecting the type of sample

The default setting is *Beer*.
Now, enter a designation or number for the sample in the *Description* text box. Click on the button or press the **[ENTER]** key. The software first requests the zero reference cuvette be inserted. Insert the **iso-octane cuvette** in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in bitterness units without decimals, analogously to the result shown in Fig. 7.

4.4 Total Carotenoids Fraction 1-4, MEBAK 3.7.2.1

This test is conducted with a 10 mm cuvette at a wavelength of 450 nm. The measurement is performed against petroleum ether as zero standard. Select the "Total carotenoids fraction 1-4" test from the list. For this test, a dilution factor can be entered (see Fig. 8). Now, enter a name or number for the sample in the *Description* text box. Click on the button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **petroleum ether** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l with one decimal, analogously to the result shown in Fig. 7.

4.5 Total Carotenoids in Carrot Juice, MEBAK 3.7.2.2

This test is conducted with a 10 mm cuvette at a wavelength of 450 nm. The measurement is performed against n-hexane as zero standard. Select the "Total carotenoids in carrot juice" test from the list. For this test, a dilution factor can be entered (see Fig. 8).

Now, enter a designation or number for the sample in the *Description* text box. Click on the button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **n-hexan cuvette** in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7.

4.6 Total Polyphenols, MEBAK 2.17.1

This test is conducted with a 10 mm cuvette at a wavelength of 600 nm. The measurement is performed against a blind sample (distilled water, prepared in the same way) as zero reference. Select the "Total polyphenols" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7.

4.7 Copper (Cuprethol), MEBAK 2.29.4

This test is conducted with a 10 mm cuvette at a wavelength of 445 nm. The measurement is performed against a blind sample (distilled water, prepared in the same way) as zero reference. Select the "Copper cuprethol" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Now, insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l with two decimals, analogously to the result shown in Fig. 7.

4.8 Nickel, MEBAK 2.29.6

This test is conducted with a 10 mm cuvette at a wavelength of 530 nm. The measurement is performed against a blind sample (distilled water, prepared in the same way) as zero reference. Select the "Nickel" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Now, insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l with two decimals, analogously to the result shown in Fig. 7.

4.9 Thiobarbituric Acid Value, MEBAK 2.4

This test is conducted with a 10 mm cuvette at a wavelength of 448 nm. For the calculation, a blank and a main value must first be determined. Select the

"Thiobarbituric acid value" test from the list. The measurement is performed against distilled water as zero reference. For this test, a dilution factor can be entered (see Fig. 8). Now, enter a name or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **water cuvette** in the photometer (see Fig. 10).

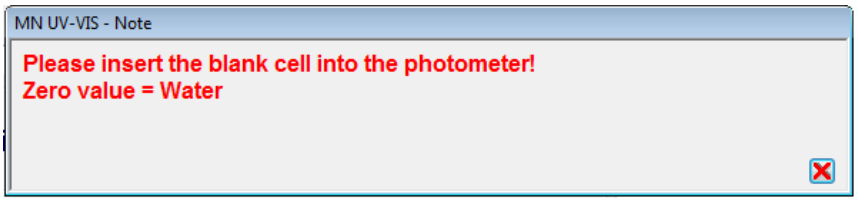


Fig. 10: Requesting the water cuvette for zero measurement

Then, you are asked to insert the blank sample cuvette (see Fig. 11).

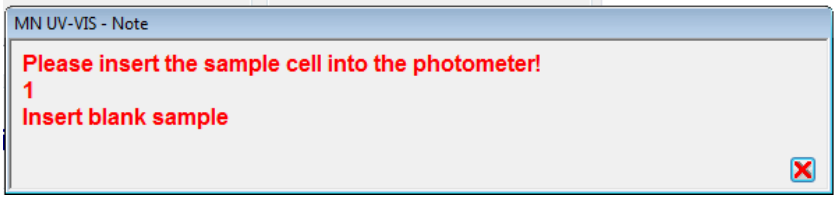


Fig. 11: Requesting the blank sample cuvette to be inserted

Insert the cuvette with the **blank solution** in the photometer. After completing the measurement, you will be asked to insert the sample cuvette, as shown in 12.

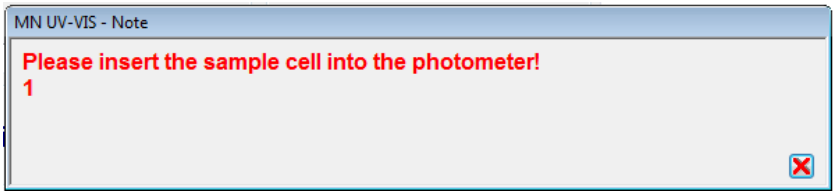


Fig. 12: Requesting the sample cuvette to be inserted

The result is displayed without units and decimals, analogously to the result shown in Fig. 7.

4.10 Vicinal Diketones, MEBAK 2.23

This test can not be measured with the ***NANOCOLOR*® *VIS*** photometer.

This test is conducted with a 20 mm cuvette at a wavelength of 335 nm. Since this test is conducted with UV light, quartz cuvettes are required! Here, the sample is measured against a blind sample (distilled water, prepared in the same way) as zero reference solution. Select the "Vicinal diketones" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Now, insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/kg with two decimals, analogously to the result shown in Fig. 7.

4.11 α-acids, MEBAK 2.18.2

This test is conducted with a 10 mm cuvette at a wavelength of 360 nm. Here, the sample is measured against a blind sample (distilled water, prepared in the same way) as zero reference solution. Select the "Alpha acids" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Now, insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7.

4.12 Cyclamate, MEBAK 3.11.4

This test can not be measured with the ***NANOCOLOR*® *VIS*** photometer.

This test is conducted with a 10 mm cuvette and a wavelength of 314 nm. Since this test is conducted with UV light, quartz cuvettes are required! The sample is measured against n-hexane as zero reference. The photometer factor has to be determined by preparing a calibration series with a standard solution containing cyclamic acid (cyclohexanesulfamic acid). Select the "Cyclamate" test from the list. Now, enter a name or number for the sample in the *Description* text box.

In the middle window area below the command buttons, another option checkbox appears, namely *Use saved standards* (see Fig. 13). This option will be enabled only if the calibration data for the test has been saved. If you activate this checkbox, the software uses the previously saved cyclamate data.

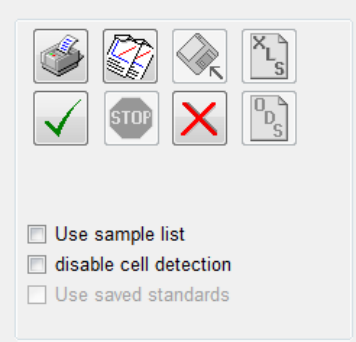


Fig. 13: Use saved standards checkbox

Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **n-hexane cuvette** in the photometer (see Fig. 14).

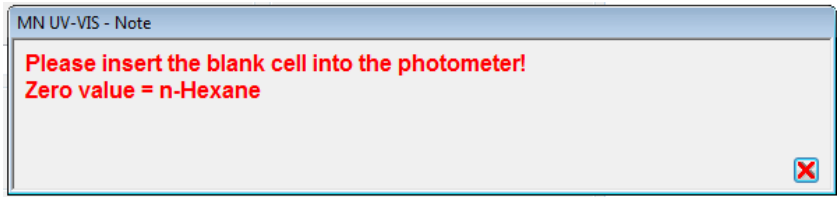


Fig. 14: Requesting the sample cuvette to be inserted

If the *Use saved standards* checkbox is not activated, the software consecutively requests the 4 cyclamate standard solutions, i.e. 50 mg/l, 100 mg/l, 200 mg/l and 300 mg/l, to be inserted in the photometer (see Fig. 15).

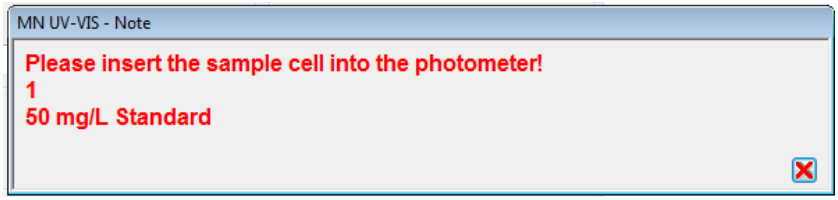


Fig. 15: Requesting the standard solution to be inserted

You will be then asked to insert the sample cuvette, as shown in Fig. 16.

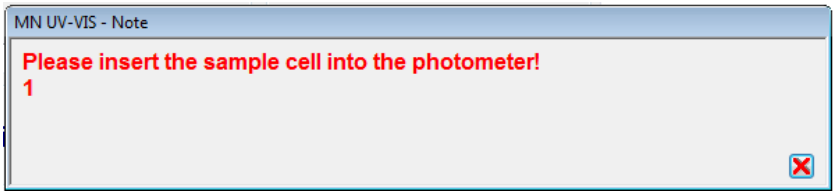


Fig. 16: Requesting the sample cuvette to be inserted

The required photometer factor is calculated automatically. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7. Once the measurement has been completed, the  button is enabled. This button saves the calibration data.

4.13 Iron, MEBAK 2.29.3

This test is conducted with a 40 mm cuvette and a wavelength of 560 nm. Here, the sample is measured against a blind sample (distilled water, prepared in the same way) as zero reference solution. The photometer factor has to be determined by preparing a calibration series with an iron(III) standard solution. Select the "Iron" test from the list. Now, enter a designation or number for the sample in the *Description* text box. A further text box named *Volume* appears below the *Factor* text box (see Fig. 17).

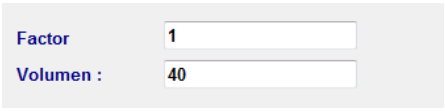


Fig. 17: Volume text box is displayed

Please enter the exact volume of your beer sample in ml. In the window area with the command buttons, the *Use saved standards* option checkbox appears.

Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **blind sample** cuvette in the photometer (see Fig. 18).

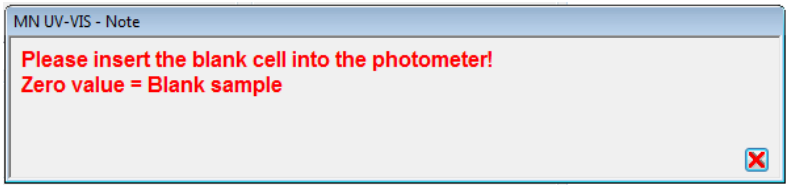


Fig. 18: Requesting the blind sample cuvette (zero reference) to be inserted

If the *Use saved standards* checkbox (see Chapter 4.12) has not been activated, the software consecutively requests the 4 iron standard solutions to be inserted in the photometer (see Fig. 19).

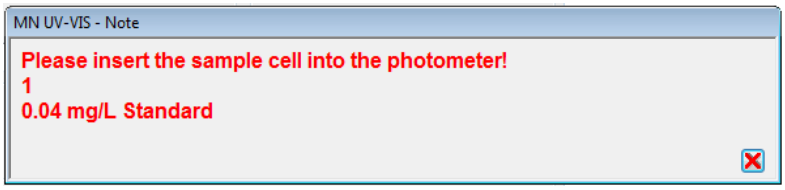


Fig. 19: Requesting for the standard solutions to be inserted

Then, you will be asked to insert the sample in the photometer (see Fig. 20).

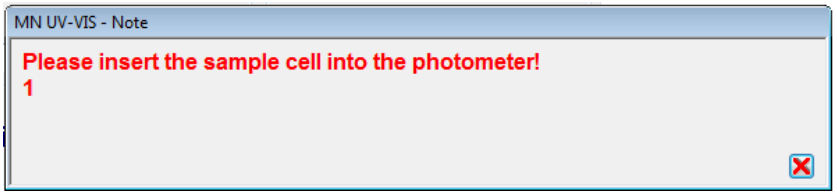


Fig. 20: Requesting the sample cuvette to be inserted

The required photometer factor is calculated automatically. The result is displayed in mg/l with three decimals, analogously to the result shown in Fig. 6. Once the measurement has been completed, the  button is enabled. This button saves the calibration data.

4.14 Free Amino-nitrogen (FAN), MEBAK 2.8.4.1

This test is conducted with a 10 mm cuvette and a wavelength of 570 nm. For the calculation, a blind value (distilled water, prepared in the same way), a standard value, a correction value and the measured value are determined. All the four measurements must be performed three times and averaged. The measurement is performed against water as zero reference. Select the "Free amino-nitrogen (FAN)" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **water cuvette** in the photometer (see Fig. 21).

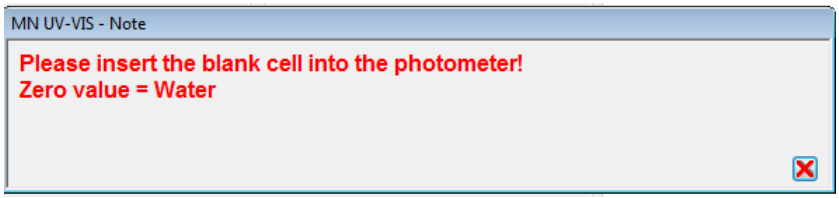


Fig. 21: Requesting the water cuvette to be inserted

Now, the software consecutively requests the samples 1 to 3 to be inserted in the photometer (see Fig. 22).

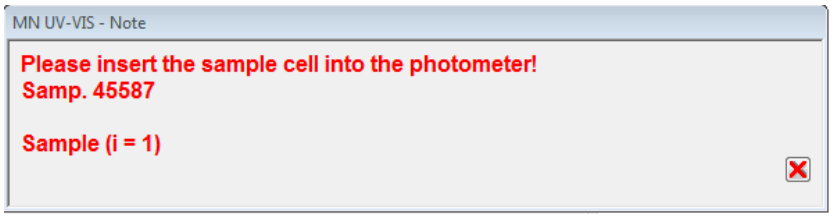


Fig. 22: Requesting sample 1 to be inserted

Insert the three samples, one after another, in the photometer.

Now, you will be asked to insert the three blind samples in the photometer (see Fig. 23).

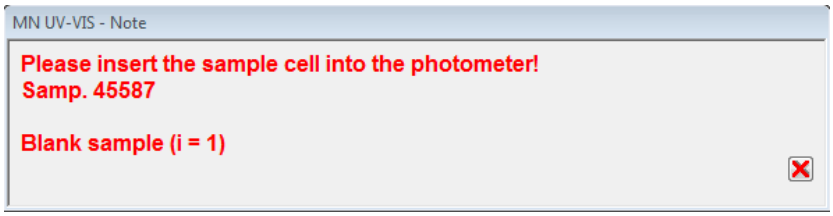


Fig. 23: Requesting blind sample no. 1

Insert the three blind samples, one after another, in the photometer. Then, the software requests the standard solutions 1 to 3 to be inserted in the photometer (see Fig. 24).

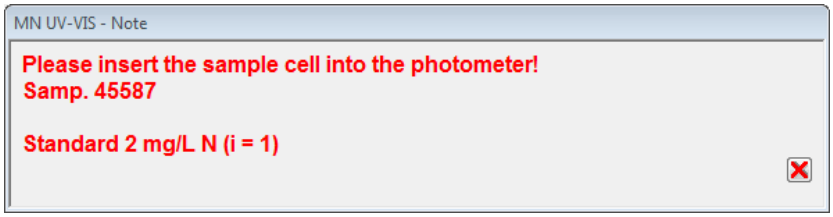


Fig. 24: Requesting standard solution no. 2 to be inserted

Insert the three standard solutions, one after another, in the photometer. Finally, the software asks you to measure the three correction values (see Fig. 25).

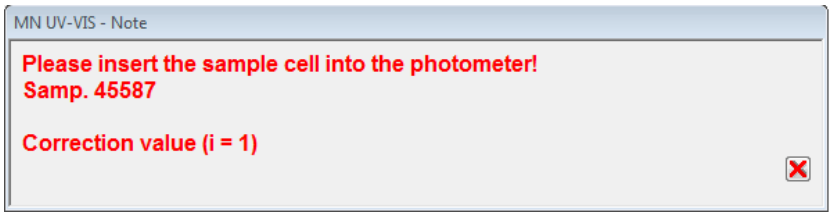


Fig. 25: Requesting correction sample no. 1 to be inserted

After removing the last cuvette, the measured values are averaged and the FAN content is calculated acc. to MEBAK 2.8.4.1.

The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7. Once the measurement has been completed, the  button is enabled. This button saves the calibration data.

4.15 Total Carotenoids acc. to Wesergold, MEBAK 3.7.2.3

This test is conducted with a 10 mm cuvette at 450 and 550 nm. Here, the sample is measured against a blind sample (distilled water, prepared in the same way) as zero reference solution. Select the "Total carotenoids acc. to Wesergold" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The photometer measures at 450 nm. The software first requests the zero reference cuvette be inserted. Insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette.

After the first measurement, the photometer makes a second measurement at 550 nm. The software asks you **again** for the zero reference cuvette. Insert the **blind sample again** in the photometer. Then, you are asked to insert the sample cuvette again as well. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7.

4.16 Iso-α-acids, MEBAK 2.18.2

This test can not be measured with the ***NANOCOLOR*® *VIS*** photometer.

This test is conducted with a 10 mm cuvette at 255 and 360 nm. Since this test is involves UV light, quartz cuvettes are required! The sample is measured against a blind sample as zero reference. Select the "Iso-alpha-acids" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The photometer makes a measurement at 255 nm. The software first requests the zero reference cuvette be inserted. Now, insert the **blind sample** cuvette in the photometer. Then, you are asked to insert the sample cuvette. After the first measurement, the photometer makes a second measurement at 360 nm. The software asks you **again** for the zero reference cuvette. Insert **again** the **blind sample** cuvette in the photometer. Then, you are

asked to insert the sample cuvette. The result is displayed in mg/l without decimals, analogously to the result shown in Fig. 7.

4.17 Copper (ZDBT), MEBAK 2.29.5

This test is conducted with a 10 mm cuvette and a wavelength of 435 nm. A main value, a blind value (distilled water, prepared in the same way) and a correction value have to be determined for this test. All values are measured against trichloroethane as zero reference. The photometer factor has to be determined by preparing a calibration series with a copper standard solution. Select the "Copper ZDBT" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **trichloroethane cuvette** in the photometer (see Fig. 26).

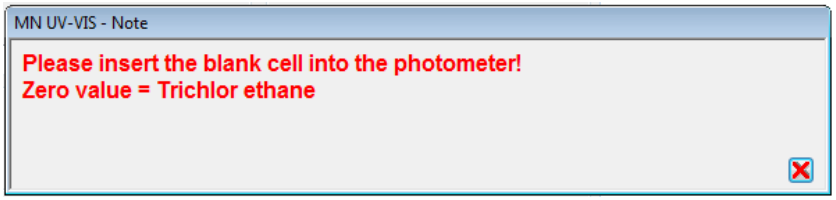


Fig. 26: Requesting the zero reference cuvette to be inserted

If the *Use saved standards* checkbox (see Chapter 4.12) has not been activated, the software consecutively requests the 6 copper standard solutions to be inserted in the photometer (see Fig. 27).

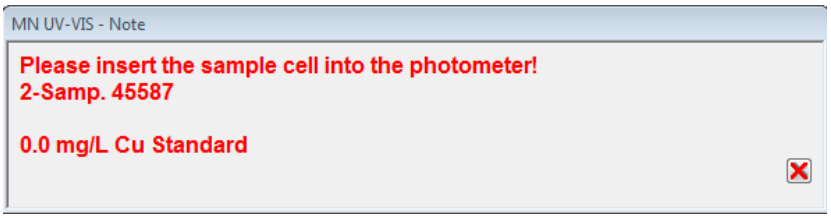


Fig. 27: Requesting the 1st. standard solution to be inserted

You will be then asked to insert the blind sample cuvette, as shown in 28.

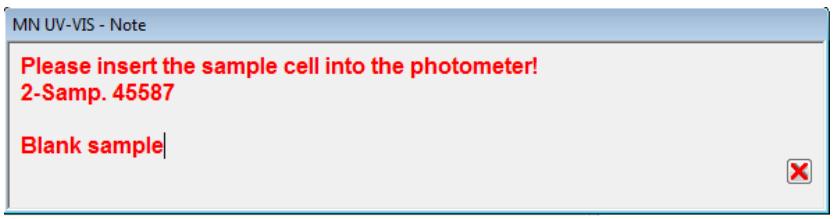


Fig. 28: Requesting the blind sample to be inserted

Subsequently, the correction value has to be measured (see Fig. 29).

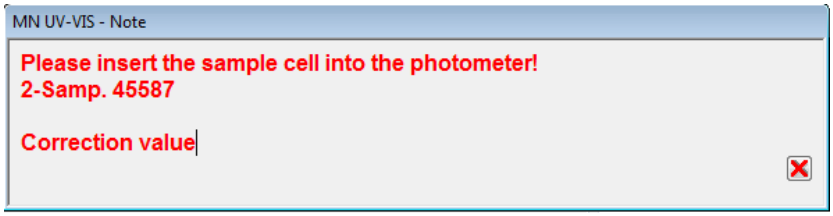


Fig. 29: Requesting the cuvette with the correction value

Finally, you are asked to insert the sample cuvette in the photometer (see Fig. 30).

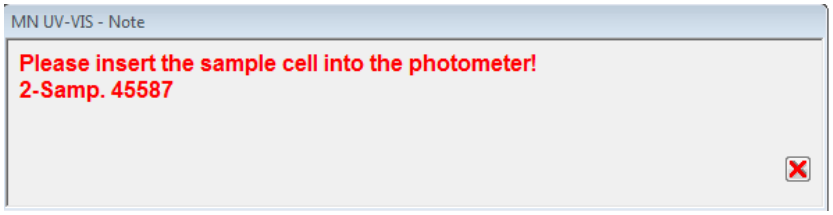


Fig. 30: Requesting the sample cuvette be inserted

The required photometer factor is calculated automatically. The result is displayed in mg/l with two decimals, analogously to the result shown in Fig. 7. Once the measurement has been completed, the  button is enabled. This button saves the calibration data.

4.18 Photometric Iodine Sample, MEBAK 2.3.2

This test is conducted with a 40 mm cuvette at a wavelength of 578 nm. An iodine blind value, a main value and a centrifuge value have to be determined for this test. All values are measured against a phosphate buffer as zero standard. Select the "Photometric iodine sample" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **phosphate buffer cuvette** in the photometer (see Fig. 31).

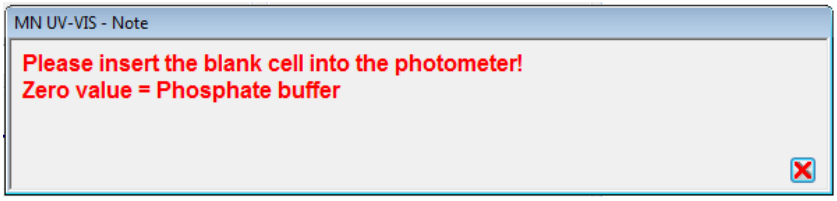


Fig. 31: Requesting the zero reference cuvette to be inserted

Afterwards, you are asked to insert the cuvette with the **iodine blank value** (see Fig. 32).

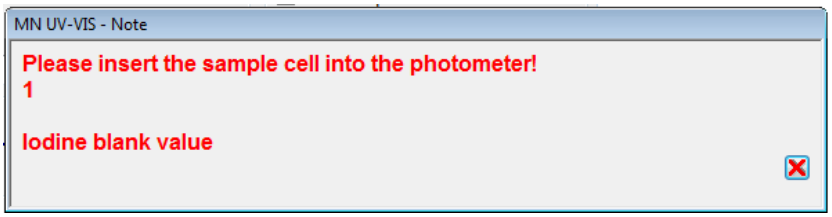


Fig. 32: Requesting the cuvette with the iodine blank value

You are now asked to insert the cuvette with the centrifuge value sample (see Fig. 33).

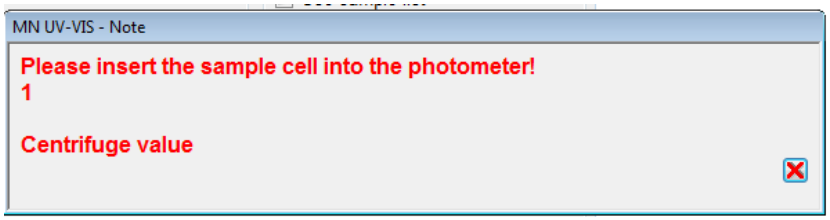


Fig. 33: Requesting the cuvette with the centrifuge value sample

Afterwards, you will be requested to insert the **iodine main value** cuvette (see Fig. 34).

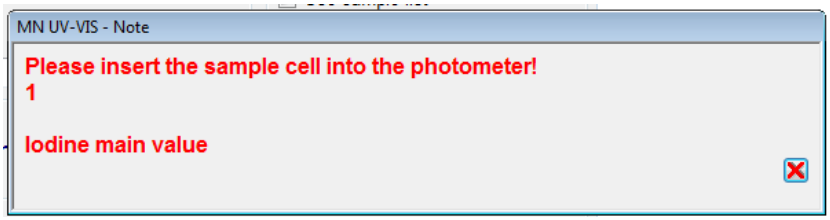


Fig. 34: Requesting the iodine main value cuvette to be inserted

The result is displayed without units (absorbance) and with two decimals, analogously to the result shown in Fig. 7.

4.19 Total Carbohydrates in Beer, MEBAK 2.11

This test is conducted with a 10 mm cuvette at a wavelength of 625 nm. Three D-glucose standard solutions and the measured value have to be determined for this test. All values are measured against water as zero reference. Select the "Total carbohydrates beer" test from the list. Now, enter a designation or number for the sample in the *Description* text box. Click on the  button or press the **[ENTER]** key. The software first requests the zero reference cuvette to be inserted. Insert the **water cuvette** in the photometer (see Fig. 35).

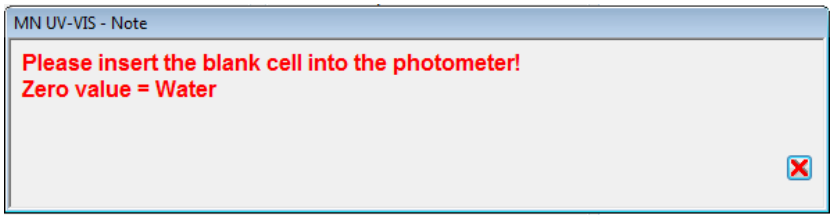


Fig. 35: Requesting the zero reference cuvette be inserted

Afterwards, you are asked to insert standard solution no. 1 (see Fig. 36).

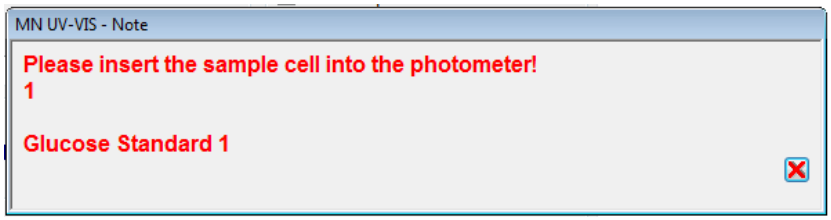


Fig. 36: Requesting the glucose standard to be inserted

Now, insert the three glucose standard solutions, one after another, in the photometer. Then, you are asked to insert the sample cuvette (see Fig. 37).

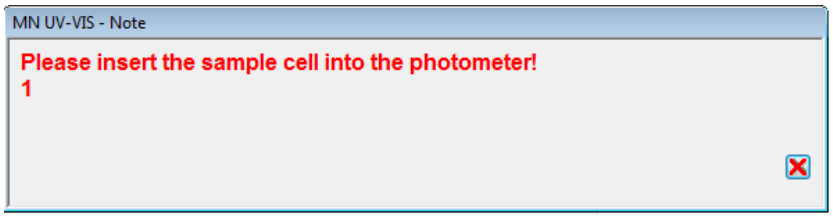


Fig. 37: Requesting the sample cuvette to be inserted

The result is shown in g/100 ml with two decimals, in the same manner as in Fig. 7.

5 Important Notice

***NANOCOLOR*®** Spectrophotometers are fitted with an automatic standard cuvette detection system. This automatic system recognizes 10 mm, 14 mm, 20 mm and 50 mm cuvettes. When using 40 mm cuvettes, a 50 mm cuvette erroneously appears in the measurement log. The user has to ensure that the correct cuvette size (= 40 mm) is being used for the "Photometric iodine sample" and "Iron" tests. For technical reasons, neither the photometer nor the software can ensure that the correct cuvette size has been inserted in these tests.