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Quantitative UV/VIS-spectroscopy on turbid samples

Computational correction in comparison with measurements inside an integrating sphere

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Introduction

To obtain correct results in quantitative UV/VS-spectroscopic analyses, clear samples are crucial. In case of the spectroscopic determination of formaldehyde in water-based dispersion paints according to the VdL-RL 03 [1], this prerequisite is not always fulfilled. Certainly, a dispersion paint contains relatively large amounts of pigments and fillers, representing a strongly scattering opaque system, since the paint should also provide sufficient hiding power.

The above-mentioned VdL guideline recommends the addition of acetylacetone to the undiluted dispersion paint, which reacts with formaldehyde, when present, and ammonium ions via a Hantzsch reaction to form a heterocyclic compound, the diacetyl dihydrolutidine (DDL) [2]. The DDL appears yellow due to an absorption maximum in the UV/VIS-spectrum at a wavelength of 412 nm.

Addition of the above-mentioned reagents to the paint yields a turbid dispersion with a more or less strong yellow colour in the presence of formaldehyde. After a residence time of two hours and subsequent centrifugation, a clear solution ready for spectroscopy is supposed to be obtained. For the quantitative analysis of formaldehyde, the light attenuation (absorbance) at 412 nm is then measured. In practice however, the presence of a residual turbidity is more the rule than the exception. Even additional filtration does not always lead to the result desired. Precipitation with added electrolytes may risk an underestimation of the formaldehyde content by inclusion in or adsorption onto the precipitate.

The current issue of the VdL-RL 03 proposes a solvent change from water to methanol to remove turbidity. It should be noted that the calibration must also be carried out in the corresponding solvent. In addition, a change of solvent can only be successful if the turbidity is caused by incompatibilities of certain organic components with water as solvent. In case of very fine inorganic materials, a solvent change would at best reduce turbidity but not completely eliminate it.

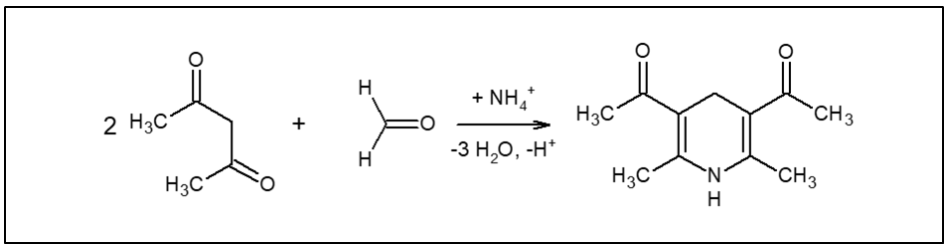

Fig.1: Reaction scheme for the formation of diactyl dihydrolutidine (DDL) from formaldehyde and acetylacetone in the presence of ammonium ions.

The problem with performing spectroscopy on turbid systems is that light propagation within the sample is no longer rectilinear and the transmitted light is partly missing the detector of the spectrometer (Figure 2). The absorbance measured in this way is then greater than that of a clear sample with the same concentration of the analyte. Without appropriate correction, depending on the extent of the turbidity, the concentration of the analyte may be considerably overestimated.

The correction method described below is based on investigations of how turbidity impacts on weakly stained human plasma samples by M. Gautschi and R. Richterich [3] and is adapted in this work to the formaldehyde determination of turbid systems, ranging from weak to strong colour.

Another possibility for obtaining correct absorbance values on turbid samples is to use a sufficiently large integrating sphere, which allows the cuvette or specimen to be placed inside that device [4, 5]. With this option, both transmitted light and scattered light hit the detector, which is also inside the sphere. This method is also known as a transflexion measurement. Both methods will be discussed and compared in the following sections.

Clear solutions

Firstly, the ideal case of quantitative analysis of formaldehyde on clear solutions by UV/VIS-spectroscopy will be presented. When such systems are placed in the sample compartment of a spectrometer, the light transmitted by the sample hits the detector in a straight line (Figure 2).

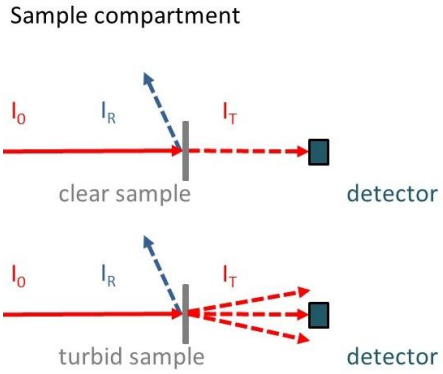

Fig. 2: Arrangement of clear and turbid samples in the sample compartment of a spectrometer. I_0 indicates the intensity of the incident light beam, I_R the intensity of the reflected portion and I_T, the intensity of the transmitted portion.

For quantitative analysis absorbance is used, since this quantity is proportional to the concentration of a substance according to Lambert-Beer [6]. According to the VdL-RL 03, the formaldehyde content of a sample is derived from the absorbance at 412 nm of diacetyl dihydrolutidine (DDL) formed. The absorbance is based on the transmission, which is the portion of light that has passed through the sample and strikes the detector directly:

$$Abs = -\log\left(\frac{I_T}{I_0}\right) = -\log(T) \quad (1)$$

with
Abs: Absorbance at 412 nm
I_0: Intensity of the incident beam
I_T: Intensity of the light after passing through the sample

Figure 3 shows the corresponding spectra of clear solutions obtained by employing a spectrometer with double monochromator. Double monochromators reduce the proportion of false light compared to devices with single monochromators. Hence, absorbance values > 1 can also be measured with low error.

The proportion of incident light reflected by the sample into the blackened sample compartment and not hitting the detector is:

$$R = \frac{I_R}{I_0} \quad (2)$$

with
R: Reflection
I_R: Intensity of light reflected by the sample
I_0: Intensity of the primary beam

The fraction of reflected light of a transparent solution is relatively low. The loss of reflection at the air/glass or glass/liquid interface of the cuvette containing the sample is also compensated by the water-filled reference cuvette.

From the absorbance values at 412 nm, a calibration line for the different nominal contents of formaldehyde can be plotted:

$$Abs = A \times c_{Formaldehyde} \quad (3)$$

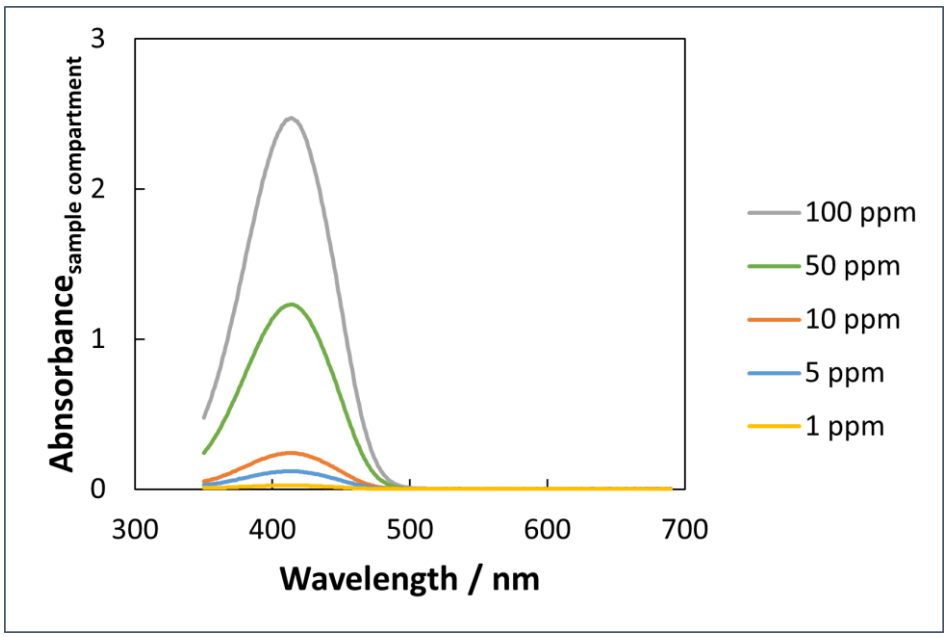

Fig. 3: UV/VIS-spectra of clear solutions with formaldehyde contents of 1-100 ppm for calibration and the cuvette placed in the sample compartment.

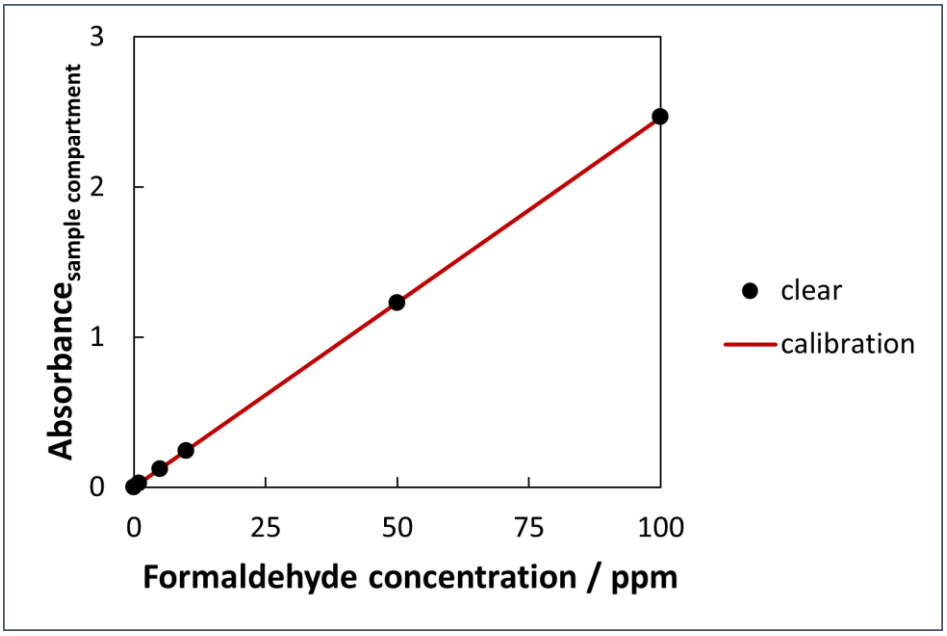

Fig. 4: Absorbance values of clear solutions with formaldehyde contents of 1-100 ppm for calibration and the cuvette placed in the sample compartment.

with
Abs: Absorbance at 412 nm
A: Parameter
$c_{Formaldehyde}$: Formaldehyde concentration in ppm

Turbid systems

The clear solutions and turbid suspensions, which will be discussed in the next section, were prepared starting from 50 ppm and 100 ppm formaldehyde calibration solutions by adding different amounts of a turbid emulsion and adjusting with demineralised water up to the mark. Hence, the impact of three different stages of turbidity (T1 = slightly turbid, T2 = turbid and T3 = very turbid) could be investigated at a known formaldehyde content.

When placing a turbid sample in the sample compartment of a spectrometer, then a fraction of the incident light is scattered from the sample into the blackened sample compartment and is therefore lost for the detector. Another fraction passes the sample in transmission. Due to turbidity, the portion of light hitting directly the detector is reduced, since light scattered

by the sample is partially radiated past it (Fig. 2) and also scattered back (not shown). The absorbance thus appears to be greater than that of a clear solution at the same level of the analyte, as already described in the introduction. Since the scattered light intensity varies with the wavelength depending on the particle size [7], the absorbance usually increases with decreasing wavelength, as shown in Fig. 5. Even in the long-wavelength range around approx. 700 nm, the impact of turbidity on the spectra is noticeable by an increasing off-set of the baseline with increasing turbidity (Figure 5).

Based on these observations, a computational correction method is suggested, which empirically captures the scattering properties of the samples in the wavelength interval 550 nm ≤ λ≤ 690 nm, in which the sample does not absorb, using a power approach as proposed in [3]:

$$Abs_{scattering} = A \times \lambda^{-B} \quad (4)$$

with

$Abs_{scattering}$: Scattering curve in absorbance units
A: Parameter
B: Exponent
λ: Wavelength in nm

For the 15 systems with differing turbidity and formaldehyde content, the exponents according to equation 4 were computed individually. These scatter around an average value of B = -2.27 ± 0.07 which is essentially due to the particle size of the emulsion used, which is the same for all systems.

By subtracting the absorbance caused by scattering via Eqn. 4 from the absorbance values of the turbid samples, corrected absorbance values can be calculated which are caused solely by the absorption of the DDL. These values are depicted in Figure 7 together with those obtained on clear solutions. All absorbance values fall onto practically the same calibration line as those displayed in Figure 4. The scatter of the measured values is mainly due to preparation errors and a possible overestimation of the impact of scattering in very turbid samples at very low formaldehyde content.

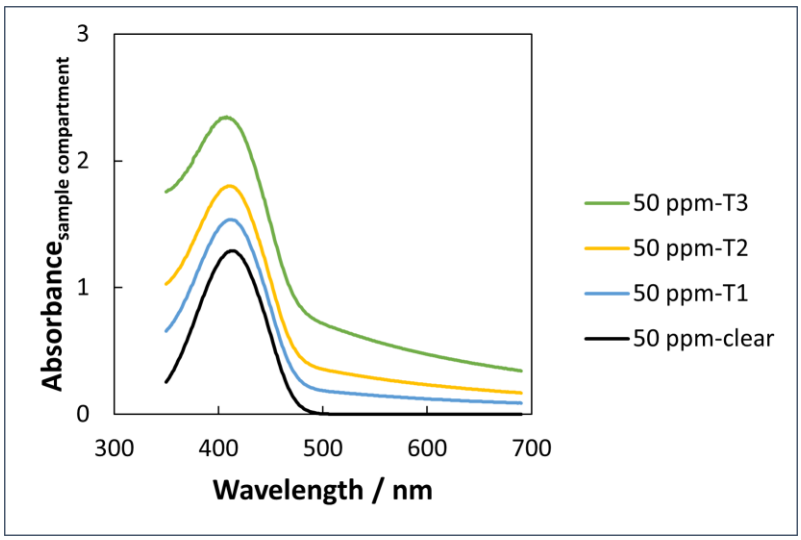

Fig. 5: UV/VIS-spectra of turbid systems at a formaldehyde level of 50 ppm and the cuvette placed in the sample compartment.

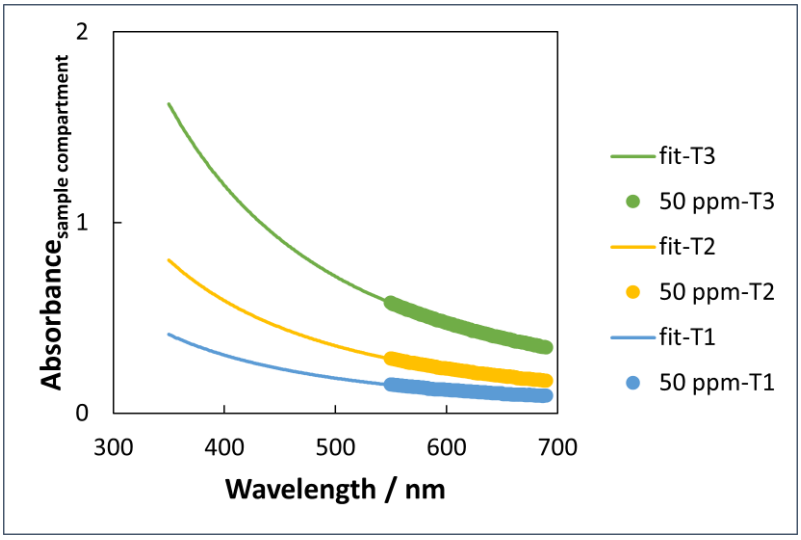

Fig. 6: Scattering curves for differently turbid systems with a formaldehyde content of 50 ppm, calculated according to Eqn. 4 and the cuvette placed in the sample compartment.

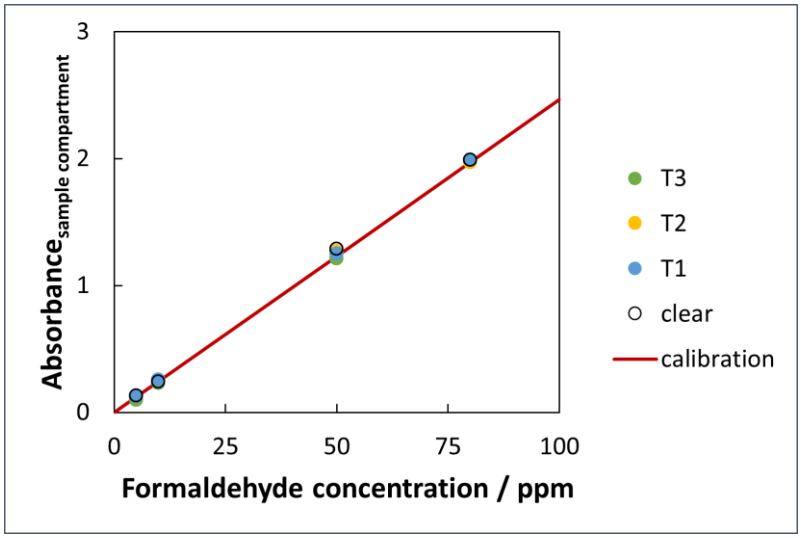

Fig. 7: Absorbance values of clear and turbid systems (corrected according to Eqn. 4) with various formaldehyde contents and the cuvette placed in the sample compartment.

Recovery rates – Sample compartment (computational correction)

Table 1 lists the recovery rates obtained by means of the computational correction of the absorbance values described. With only one exception of -20 % (5 ppm formaldehyde, very turbid), the recovery rates are quite good at < ±10 %.

Measurements inside the integrating sphere

When a sample is positioned inside a sufficiently large integrating sphere (Fig. 8), both transmitted and reflected light from the sample also will be reflected back from the inner wall of the sphere before it hits the detector, hence the name transflexion (TR). Basically, transmission and reflection are measured together inside the integrating sphere [8]:

$$TR = T + R \quad (5)$$

with
TR: Transflexion
T: Transmission
R: Reflection

The instrument software also allows a representation of the values measured in absorbance units, however, one needs to be aware that this apparent absorbance consists of two contributions that neither the software nor the integrating sphere can distinguish. Hence, these apparent absobance values are marked with an asterisk (*):

$$Abs^* = -\log(TR) = -\log(T + R) \quad (6)$$

with
Abs*: Absorbance inside the integrating sphere 412 nm
TR: Transflexion
T: Transmission
R: Reflection

For samples that fluoresce, as is the case with DDL [9], the apparent absorbance even comprises three contributions:

$$Abs^* = -\log(T + R + F) \quad (7)$$

with
Abs*: Absorbance inside the integrating sphere 412 nm
T: Transmission
R: Reflection
F: Fluorescence

Tab, 1: Recovery rates (% RR) for various clear and turbid systems at different formaldehyde levels.

condition	clear	slightly turbid (T1)	turbid (T2)	very turbid (T3)
$c_{Formaldehyde}$ / ppm	% RR	% RR	% RR	% RR
5	7,4	2,0	4,8	–20,4
10	–2,2	4,2	–0,2	–5,6
50	4,8	1,6	1,6	–1,6
80	1,1	1,1	0,2	1,1

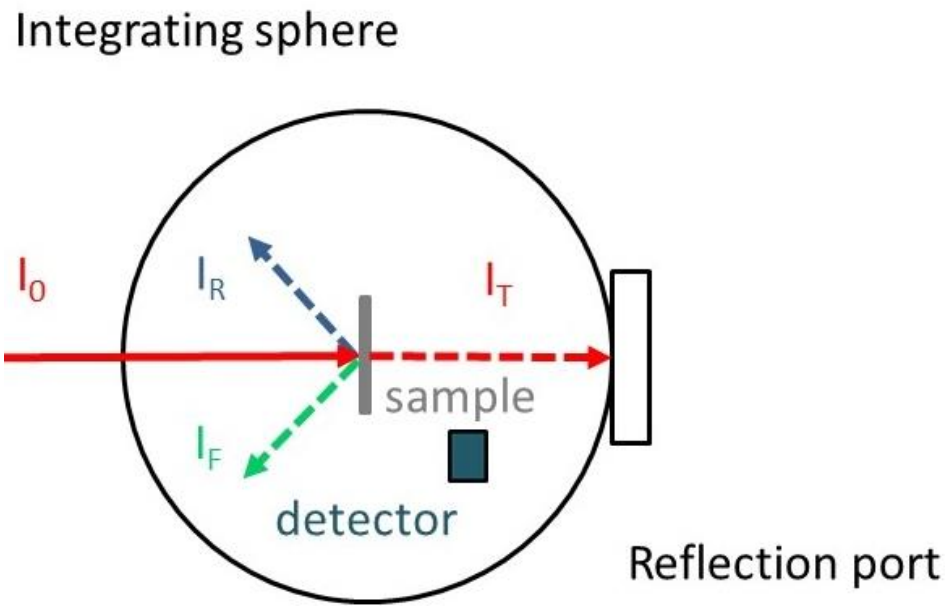

Fig. 8: Arrangement of a sample inside the integrating sphere.

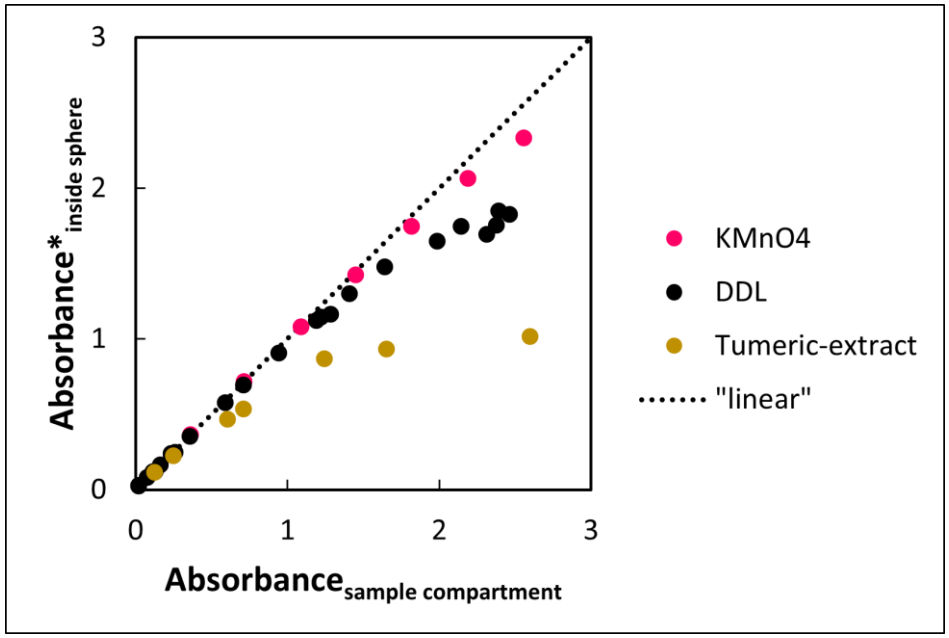

Fig. 9: Absorbance values measured on different samples placed inside the integrating sphere compared to those placed in the sample compartment.

The spectra of the clear solutions placed inside the integrating sphere with a highly reflective inner coating differ from those located in the sample compartment. The spectrometer employed registers from long wavelengths to short ones. This means for the DDL that when the excitation wavelength of 412 nm is reached, the fluorescence emission at approx. 510 nm is triggered and radiates in all directions within the sphere. Hence, a lower absorbance is obtained at 412 nm than with a measurement where the cuvette is placed in the sample compartment, since at this wavelength the fluorescent light is simply additional light (Figure 9). To put it bluntly, the impact of fluorescence on the absorbance recorded can be described as if a lamp were switched on for a short time inside the sphere, thereby reducing the absorbance.

Figure 9 presents the evolution of absorbance values of samples placed inside the integrating sphere with increasing dye concentration and thus with increasing optical density. For potassium permanganate ($KMnO_4$), the absorbance values were read at 528 nm, for DDL at 412 nm and for the ethanolic turmeric-extract at 425 nm. Solutions of $KMnO_4$ in water do not fluoresce. Those of DDL and turmeric do fluoresce [7, 10]. As the colour strength increases, the absorbance values of samples placed inside the sphere fall below those where the samples were mounted in the sample compartment. As the colour strength increases, the transmission approaches zero – an absorbance of 2 for a specimen located in the sample compartment corresponds to a transmission of only 1% – the sample becomes opaque and ultimately rather a reflection than a transmission spectrum is obtained. When fluorescence takes place, the light attenuation by absorption is increasingly reduced by fluorescent emission, whereby the absorbance values deviate more or less strongly from a linear behaviour. Such a behaviour has also been reported for various non-fluorescent stained solutions in the presence and absence of scattering particles up to high absorbance values [11].

Nevertheless, a calibration of the absorbance values at 412 nm can be performed reproducibly by positioning clear solutions inside the integrating sphere, using a non-linear relationship:

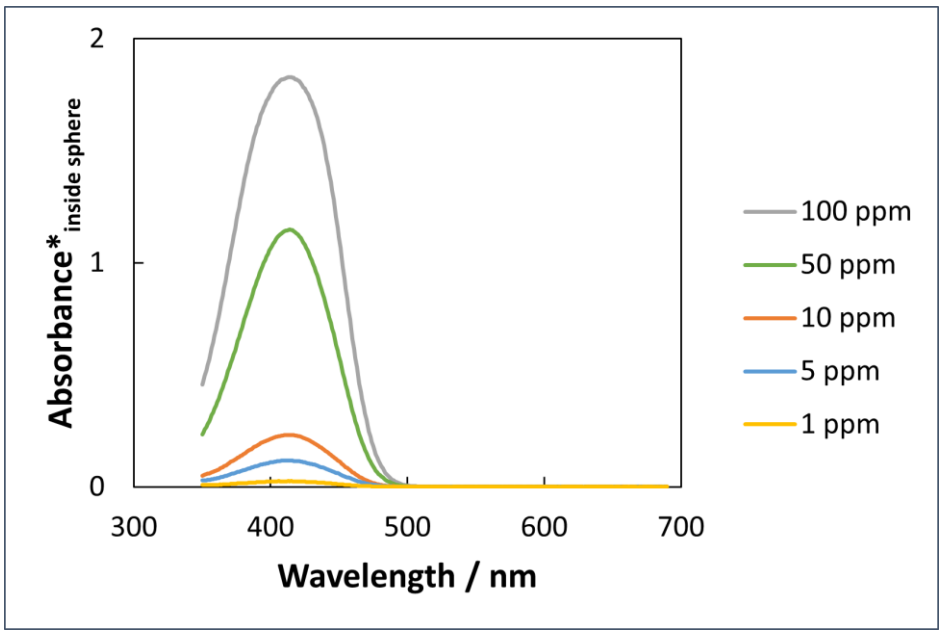

Fig. 10: Spectra of clear solutions with different formaldehyde contents and the cuvette placed inside the integrating sphere.

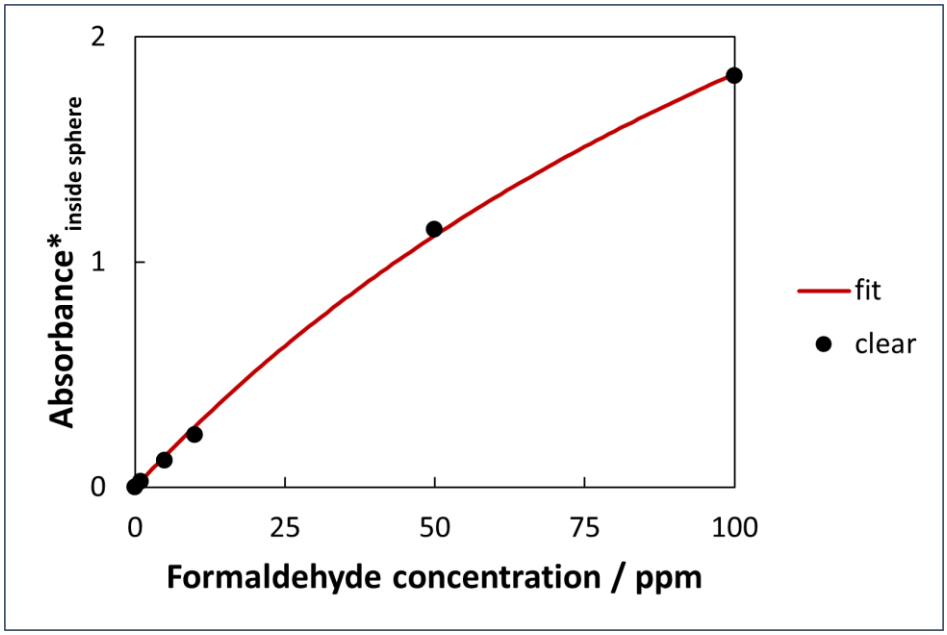

Fig. 11: Absorbance values of clear solutions with formaldehyde contents of 1-100 ppm for calibration and the cuvette placed inside the integrating sphere.

$$Abs^* = \frac{A \times c_{Formaldehyde}}{c_{Formaldehyde} + B} \quad (8)$$

with

Abs*: Absorbance inside the integrating sphere 412 nm
A: Parameter
B: Parameter
$c_{Formaldehyde}$: Formaldehyde concentration in ppm

When placing turbid samples inside the integrating sphere, their spectra do not differ from those of clear solutions, since scattered light, reflected light and possibly fluorescent light remain within the sphere and hit all together the detector, which is also located inside the sphere (Figure 12).

According to Figures 12 and 13, a single calibration with clear solutions positioned inside the integrating sphere is therefore sufficient. The scatter of the absorbance values in Figure 13 is mainly due to preparation errors in the samples.

Recovery rates – Inside the integrating sphere

The recovery rates of the transflection measurements on samples mounted

inside the integrating sphere (Table 2) are comparable to those of the transmission measurements on samples placed in the sample compartment (Table 1).

Summary

Turbid samples lead to an overestimation of the analyte and thus to incorrect results if the measured absorbance values are not corrected accordingly. A computational correction of the spectra of turbid samples can be performed by applying a power-law approach and provides predominantly good recovery rates. When using an integrating sphere of appropriate size, which allows the sample to be placed inside this sphere, correct absorbance values and good recovery rates are obtained as well. For the samples tested, both methods can be considered as equivalent.

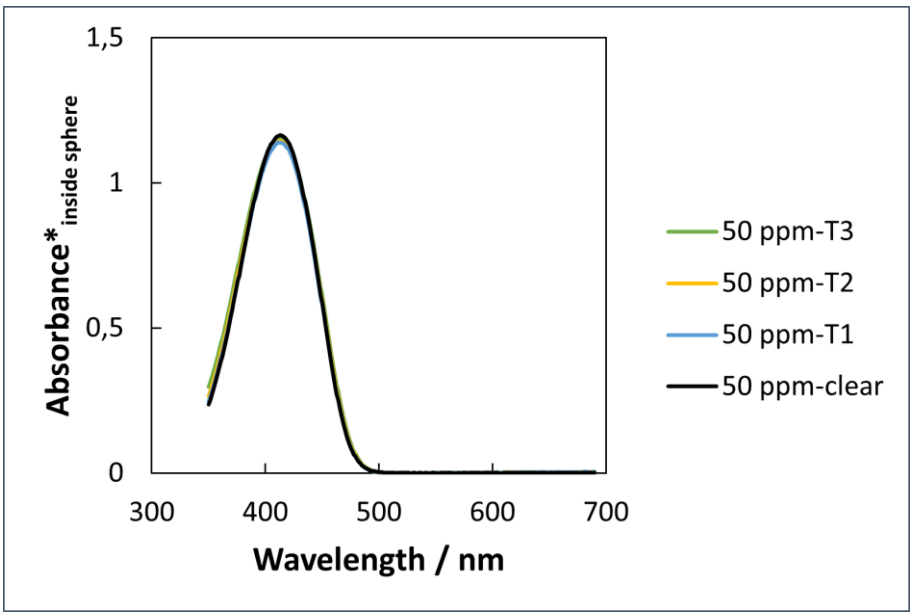

Fig. 12: Spectra of clear and turbid samples with a formaldehyde content of 50 ppm and the cuvette placed inside the integrating sphere.

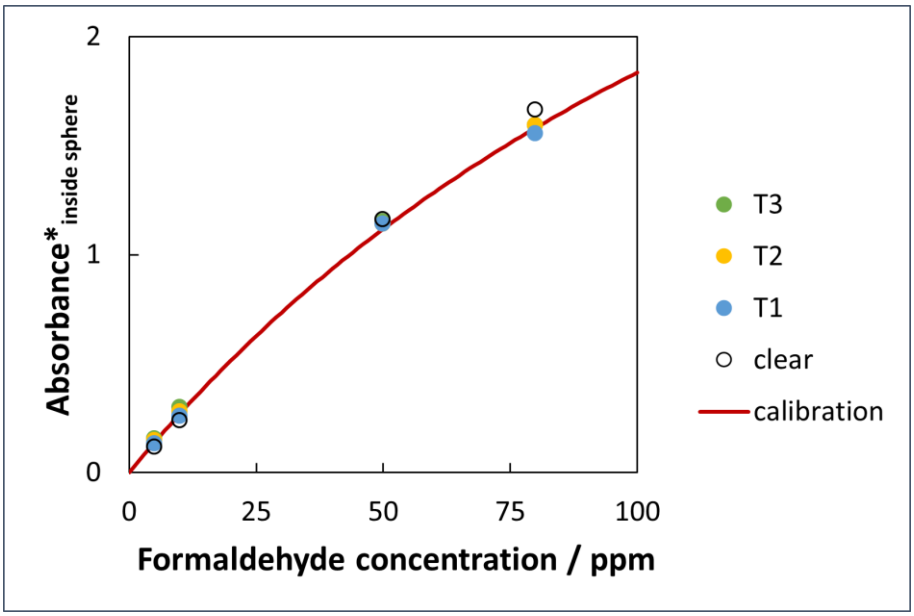

Fig. 13: Absorbance values of clear and turbid systems with different formaldehyde contents and the cuvette placed inside the integrating sphere.

Tab. 2: Recovery rates (% RR) for various clear and turbid systems at various levels of formaldehyde.

condition	clear	slightly turbid (T1)	turbid (T2)	very turbid (T3)
$c_{Formaldehyde}$ / ppm	% RR	% RR	% RR	% RR
5	–15,8	–5,6	6,0	12,4
10	–12,3	–4,6	2,9	10,9
50	4,6	–2,1	2,3	3,3
80	7,2	–2,9	0,5	0,6

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