A ground-truth-data method for the detection of organic substances in waters

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ABSTRACT

For the remote sensing of water quality parameters (e.g organic substances), calibrations of the remotely determined data have to be carried out in general. Thus ground-truth-data of relevant substances are measured, for example, aboard a ship at defined stations synchronously to the remote sensing experiment.

Because of their large sensitivity fluorescent techniques are of great interest. Conventional fluorescence techniques detect fluorescent substances only. But there are a lot of substances, which are weak- or nonfluorescent, e.g. the nonfluorescent part of yellow substances, azocompounds from textile-, printing- and car-industry, and some nitrogen-containing aromatics.

This is a reason why a simple fluorimetric method is introduced which permits not only the detection of fluorescent substances, but also nonfluorescent ones. In principle, two different signals have to be measured:

1. The linear or conventional fluorescence (CF), which occurs at short path-lengths of the exciting radiation in the water.

2. The multi-path saturation fluorescence (MPSF), which originates at longer path-lengths, e.g. by multi-path-reflection cells, where the exciting radiation is fully absorbed by the water.

A relation between the fluorescence signals and the concentration of flucrescent and nonfluorescent substances is theoretically derived, and first experiments were performed on natural water samples in the laboratory.

It was demonstrated, that nonfluorescent substances strongly influence the MPSF especially at low concentrations. Generally, the experiments verified the theory of the CF-MPSF and showed, that there is a great potential for remote sensing purposes.

1. INTRODUCTION

Analytical data are the basis for water quality management. Analyses can be performed in a laboratory as well as in the field. Principally, there is an increasing need for monitoring large areas at short time intervals. Inexpensive and speedy methods are necessary, which can especially be used in the field. Further, there is a need to use methods, which work with small amounts of chemicals or without them. (Cammann, 1994; Schwedt, 1990; Ache, 1993).

Some water constituents can be determined by means of remote sensing: Chlorophyll, yellow substances, suspended solids, and oil. (Duursma, 1972; Hoge and Swift, 1983; Mittenzwey et al., 1992; Bartsch et al., 1993).

Remotely determined data has to be calibrated in many cases with ground-truth-data methods, which can be used, for example, aboard ships synchronously to the remote sensing experiment. In principle, there is a lack of methods, which yield water quality data in a short time and without expensive sample treatment.

Because the concentration of many organic substances falls into the ppb-range, highly sensitive methods are necessary, like fluorescence techniques.

Unfortunately, conventional fluorescence techniques can be applied to fluorescent substances only; non- or weak fluorescent substances cannot be measured. But there are a lot of such substances, e.g.: Azocompounds, nitrogen-containing aromatics and polycyclic aromatics.

This is why we introduce a fluorescence method able to yield signals from fluorescent substances as well as from nonfluorescent ones. The method is based on the conventional and the multi-path-saturation fluorescence (CF-MPSF). We investigate the response of the CF-MPSF method in dependence on non- or weakly fluorescent substances. Theoretical considerations and experiments on surface waters have been performed for this purpose. This paper contains first results in this new field of research.

2. THEORY

We consider a fluid located in a cuvette, which will be penetrated by a parallel UV-beam generating fluorescence photons with the intensity I_F. The fluorescence is detected perpendicular to the parallel beam. For simplification the sample contains a fluorescent component F with the attenuation coefficient c_F and a nonfluorescent component A with c_A. Further, we assume, that selfabsorption or inner-filter effects are of minor influence. Then, the following equation similar to Duursma (1972) and Förster (1982) can be derived:

$$I_F\,(\lambda_F) = H*\frac{a_F\,(\lambda_E)}{C_F\,(\lambda_E) + C_A\,(\lambda_E)}$$
$$* (1 - \exp\,(-\,(C_F\,(\lambda_E) + C_A\,(\lambda_E))\,d)) \qquad (1)$$

with d = cuvette-length (= path-length of the excitation radiation), λ_F = fluorescence wavelength, λ_E = excitation wavelength, a_F = absorption coefficient of fluorescent substances, H is a function of aperture, transmission of optical components, excitation intensity, and fluorescence quantum yield Q_F.

Now the idea of detecting nonfluorescent substances will be presented in principle.

At values of $(c_F(\lambda_E) + c_A(\lambda_E))*d$ lower than 0.2, e.g. at short path-lengths (d = 1-2 cm) of the exciting radiation Eq. (1) can be transformed into a linear one yielding the linear or conventional fluorescence CF:

$$CF = H*a_F(\lambda_E)\,*d \qquad (2)$$

In contrast to this, the exponential function reaches a saturation level at values greater than around 2.5. This can be achieved by long path-lengths, e.g. 0.3-2.5 m. Multi-path-cuvettes with high reflecting mirrors are appropriate for this purpose. Equ. (1) can then be transformed yielding the multi-path-saturation fluorescence MPSF:

$$MPSF = H*\frac{a_F\,(\lambda_E)}{c_A\,(\lambda_E) + c_F\,(\lambda_E)} \qquad (3)$$

The ratio between Equ.2 and Equ.3 yields the total attenuation coefficient c:

$$c\,(\lambda_E) = \frac{CF}{MPSF*d} \qquad (4)$$

Thus, CF/MPSF represents the total attenuation $A = c(\lambda_E)*d$.

Further, if we assume, that $c_F = a_F$ the attenuation coefficients can be separated according to Equ.2 and 3:

$$c_F(\lambda_E) = \frac{C_F}{H*d} \qquad (5)$$

and

$$c_A(\lambda_E) = CF* (\frac{1}{MPSF*d} - \frac{1}{H*d}) \qquad (6)$$

That means, conclusions can be drawn on the basis of CF and MPSF concerning the attenuation coefficients of fluorescent as well as of nonfluorescent substances. Of course, the assumption $c_F = a_F$ is only valid in certain cases, especially for dissolved organics, e.g. yellow substances.

3. EXPERIMENTS AND METHODS

The optical measurements were performed in the laboratory after sampling. In a first experiment the CF-MPSF method was applied to unfiltered and filtered samples of surface waters around Berlin. Chlorophyll-a (Chla) and the chemical oxygen demand (COD) were determined. Chla is a measure of the phytoplankton content. The COD of filtrates characterizes the dissolved organic load, which is dominated by yellow substances in many cases. Phytoplankton and yellow substances show strong absorption and fluorescence, therefore, correlations with the optical parameters CF and MPSF can be expected. In a second experiment we investigated a filtered sample taken from an eutrophicated canal in the city of Berlin. This sample

was "polluted" by weakly- or nonfluorescent organic substances at defined concentrations. We used nitroaniline and azobenzene because of its environmental relevance. These aromatic substances are toxic and harmful, respectively. They are very important in many industrial processes (e.g. textile-, printing- and car-industry).

3.1 Characterization of investigated waters and sample treatment

We investigated three different waters: the lakes Stechlinsee (SS) and Dagowsee (DS) in the north of Berlin and the canal Neuköllner Schiffahrtskanal (NSK) situated in Berlin. Lake SS is of oligotrophic status. Chlorophyll-a concentrations amount to typically 1 µg/l. The maximum depth is 68 m. DS is characterized by an eutrophic status at chlorophyll-a concentrations of typically 30 µg/l. Its maximum depth amounts to around 10 m. The canal NSK is connected with the eutrophic-polytrophic river Spree and is used for transports by ships. The water of a waste water treatment plant flows into the canal.

Two samples were taken from lake SS at the depths of 2 and 13 m; four samples from lake DS at depths of 1, 3, 6, and 8 m; and one sample from the canal NSK at a depth of 0.10 m. Samples were transported in glass bottles and cooled in a refrigiator at around 5°C.

The chlorophyll-a concentration was determined with ethanol according to a method after Nusch (1980). Further, the chemical oxygen demand of the filtered samples were determined on the basis of potassium-bichromate. Filtrations were done using glass-fiber filters with pore sizes of around 1-2 mm.

We also investigated a sample taken from the canal NSK., which was polluted in the laboratory by nitroaniline NA and azobenzene AB in a concentration range of 1-200 µg/l.

3.2 Optical equipment

The experimental set up is illustrated in **Fig.1**. A 100W-mercury lamp (high-pressure arc) generates radiation in the UV/VIS. By means of an optical system (quartz condenser and lens with f=200mm), the arc is imaged onto the entrance slit of a grating (1200 lines per mm). A chopper is located between lens and grating to modulate the exciting radiation. A slit width of 1mm causes a spectral bandwidth of 6nm. We used excitation wavelengths of
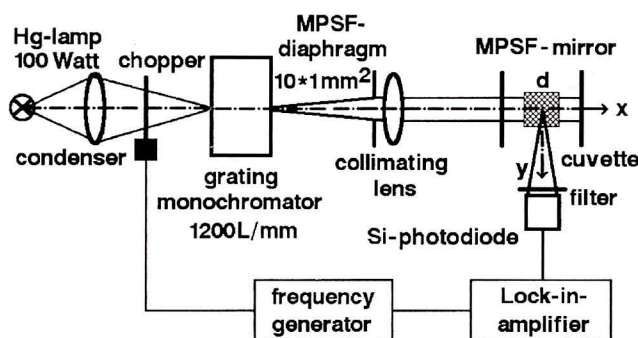
Figure 1 - Optical equipment

254, 313, 365, and 435 nm to generate CF and MPSF. Two quartz lenses with focal lengths of 160 and 500 mm, respectively, were located behind the grating yielding an effective focal length of 121 mm. So the divergent radiation is transformed into a collimated beam.

This beam enters a quartz-cuvette (10 x 10 x 45 mm³) positioned between two plane mirrors coated with high reflecting dielectric layers. The radiation falls through a 10x1 mm²-diaphragm to generate the MPSF signals before entering the cuvette under a small angle. The maximum number of reflections between the mirrors amounts to 30 yielding a maximum path-length of exciting radiation in the cuvette of 30 cm. The CF signals are generated without diaphragm and mirrors. Thus, a geometry factor has to be considered to be able to compare CF and MPSF. Under our experimental conditions, this factor amounts to 63.3. The CF-MPSF signals were measured with a silicon detector (area: 0.78 cm²) in lock-in technique at different wavelengths: blue band-pass filter with a cut-on 380 nm-filter (380-460 nm), and a red interference filter (680-705 nm).

The spectral attenuation is determined simultaneously, using cuvette lengths of 5 cm. Attenuation values are referred to the exponential basis.

4. RESULTS

4.1 Chlorophyll-a of natural unfiltered samples

Figs.2a-c show the conventional fluorescence CF in dependence on the chlorophyll-a concentration (Chla) of each water investigated. CF was measured in the spectral band from 680 to 705 nm at different excitation wavelengths. It can be seen, that the correlation is poor at the excitation wavelength of 435 nm, although there is an absorption maximum of chlorophyll-a. Obviously, a good correlation occurs only at the excitation wavelength of

313 nm. One reason could be that possible changes of the fluorescence quantum yield Q_F ($CF \sim Q_F \cdot a_F$) are of minor influence at 313 nm, but of high influence at the other excitation wavelengths. Note, that corrections concerning the red fluorescence of the filtrate failed.

Figs.2d-f show the ratio of CF/MPSF in dependence on Chla. A little decrease of the correlation at 313nm can be observed, r^2 falls from 0.94 to 0.79. This is perhaps caused by an influence of dissolved organics, e.g. yellow substances. In contrast to this, there is an essential increase at the excitation wavelength of 365 nm, r^2 rises from 0.52 to 0.81. This could be caused by the fact, that CF/MPSF is only a function of the total attenuation coefficient (CF/MPSF $\sim$ c, see Equ.4) and not of the quantum yield. Thus, there is no disturbing influence due to changes of Q_F. CF/MPSF measured at 435 nm could not cause a better correlation. Different specific attenuations due to different waters containing different algae, perhaps have an effect especially at 435 nm.

4.2 Chemical oxygen demand of natural filtered samples

Figs.3a-c show the conventional fluorescence CF as a function of the chemical oxygen demand (COD). CF was measured in the blue spectral range at different excitation wavelengths. Principally, correlations are relatively low, r^2 ranges from 0.36 to 0.45. This could be caused by CF, which is only a function of fluorescent substances and in contrast to this, the COD is also influenced generally by nonfluorescent ones. Furthermore, the correlations could be "disturbed" by changes of Q_F. Consequently, the correlation increases essentially, if the ratio CF/MPSF is used, which represents fluorescent as well as nonfluorescent substances, e.g. the nonfluorescent aliphathic part of yellow substances. The r^2 values cover a range of 0.37 for 254 nm to 0.88 for 365 nm (**Figs.3d-f**).

It has to be remarked that due to the little path-way of the excitation radiation within the multi-path cuvette used to generate MPSF signals, the CF/MPSF ratio is undervalued particularly at small attenuations or concentrations (see **Figs. 3d-e**). This can lead to lower correlations. Therefore, the used multi-path-cuvette with plane mirrors is not appropriate for waters with low concentrations. In further experiments, e.g. so-called Herriott cells (Kronfeldt et al., 1992) appropriate for longer path-lengths of exciting radiation have to be used.

4.3 Eutrophicated canal water polluted by aromatics

Furthermore, we show the response of CF/MPSF at varying concentrations of nonfluorescent nitroaniline (NA) and azobenzene (AB) in eutrophicated surface water.

First the **Figs.4a-b** show the conventional fluorescence CF measured in the blue spectral range at the excitation wavelength of 365 nm. Obviously, correlations between CF and the varied concentration of NA and AB do not occur. This is due to the non- or weakly fluorescent status of the substances investigated.

Figs.4c-d show the CF/MPSF of the polluted canal water in dependence on the concentration of NA and AB. Principally, it can be seen that CF/MPSF increases with arising concentration of NA or AB. The behaviour seems to be nonlinear. According to the above described theory, a linear function has to be expected. A reasonable explanation cannot be given at the moment. However, the detection of nonfluorescent organic micropollutants in filtered samples should be possible using CF-MPSF.

To compare the CF-MPSF method with the absorption spectrometry, the attenuation coefficient c was investigated. **Figs.4e-f** show c of canal water samples polluted by NA and AB measured at 365 nm by absorption spectrometry. Obviously, c does not vary in the concentration range from 0 to 40 µg/l. This is in contrast to the c values measured by CF/MPSF (**Figs.4g-h**), which show a distinct change. The conventional absorption spectrometry failed, at least in the lower concentration range. Concluding, the CF-MPSF method is a more sensitive tool.

Because of the relatively small number of measurements, these results have to be verified in further experiments. Actually, regression analyses need more measurements to yield higher reliability. Thus, regressions performed can only show a rough behaviour of the total attenuation coefficients.

5. APPLICATION TO LIDAR - THEORETICAL CONSIDERATIONS

According to Hoge and Swift (1981) and many other authors, the total remote sensing signal S of a Lidar equipped in an aero-plane can be described by a simple equation:

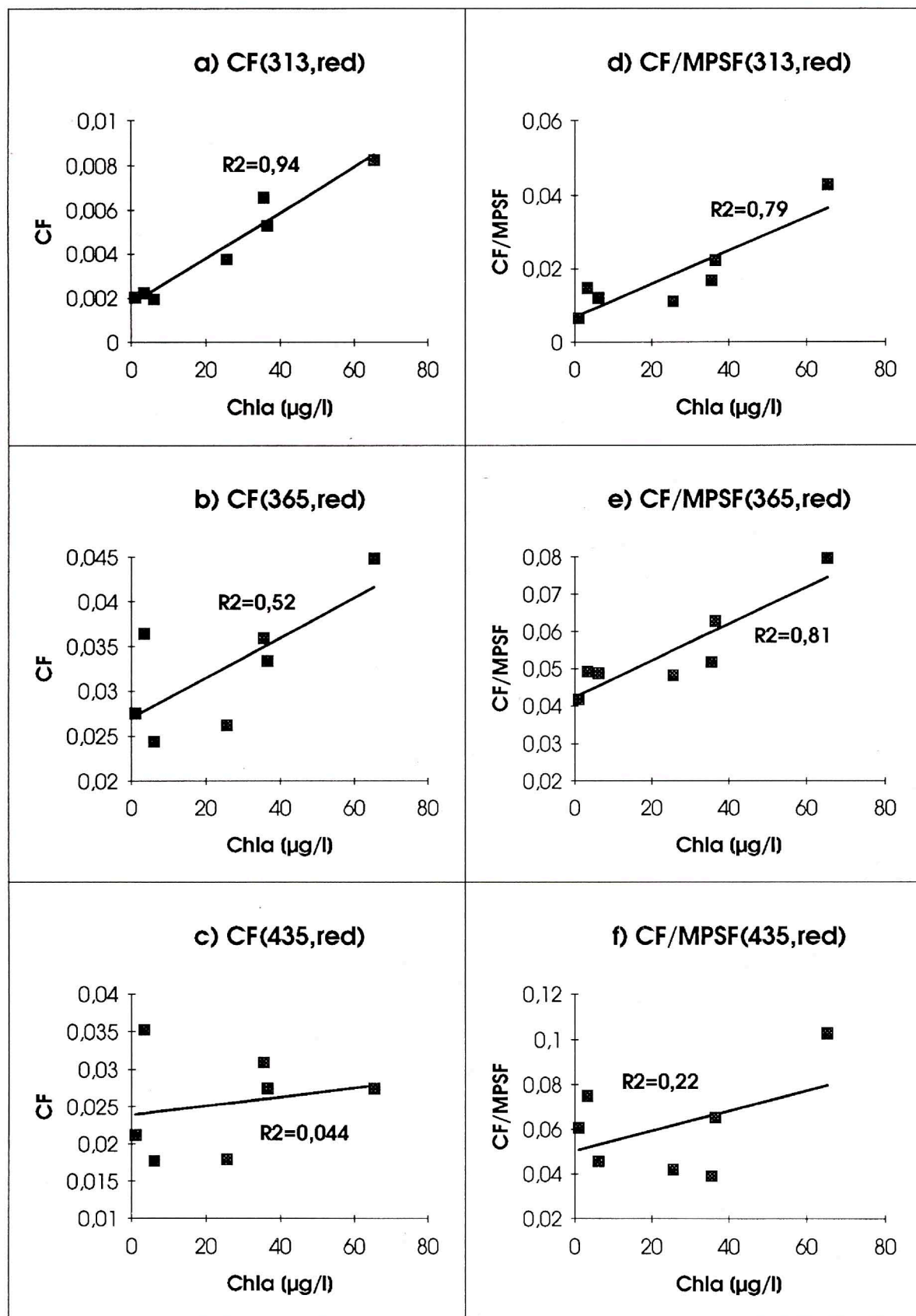$$S = f(H) * \frac{a_F(\lambda_L)}{c_1(\lambda_L) + c_2(\lambda_F)} \qquad (7)$$

Figure 2 - ***a-c***: *Conventional fluorescence CF versus Chlorophyll-a concentration Chla. Excitation: 313nm, 365nm and 435nm. Emission: 680-705nm. - **d-f**: CF/MPSF (MPSF = multi-path-saturation fluorescence) versus Chlorophyll-a concentration Chla. Excitation: 313nm, 365nm and 435nm. Emission: 680-705nm*
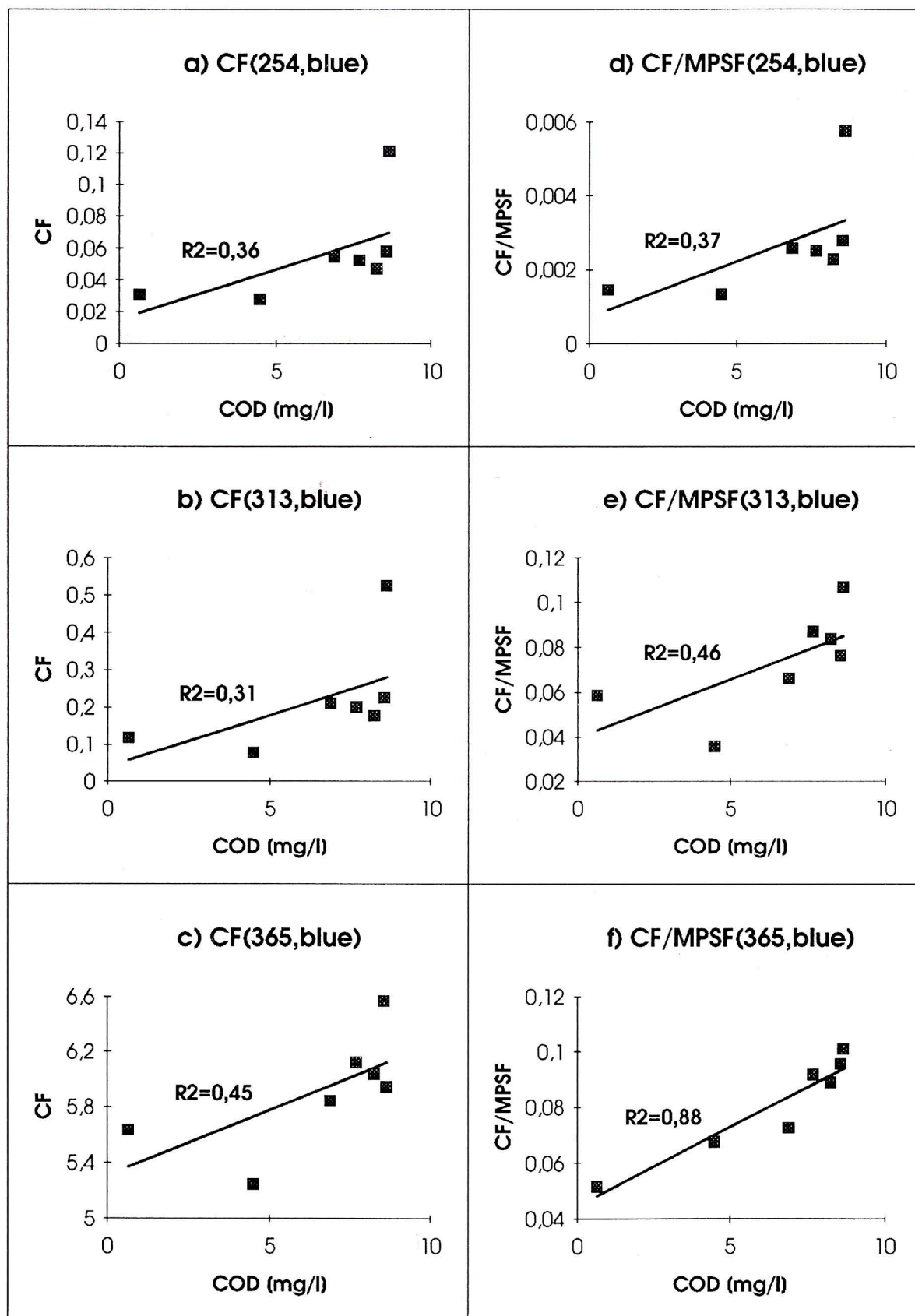
*Figure 3 - **a-c**: Conventional fluorescence CF versus chemical oxygen demand COD. Excitation: 254, 313 and 365nm. Emission: 380-460nm. **d-f**: CF/MPSF (MPSF = multi-path-saturation fluorescence) versus chemical oxygen demand COD. Excitation: 254, 313 and 365nm. Emission: 380-460nm*
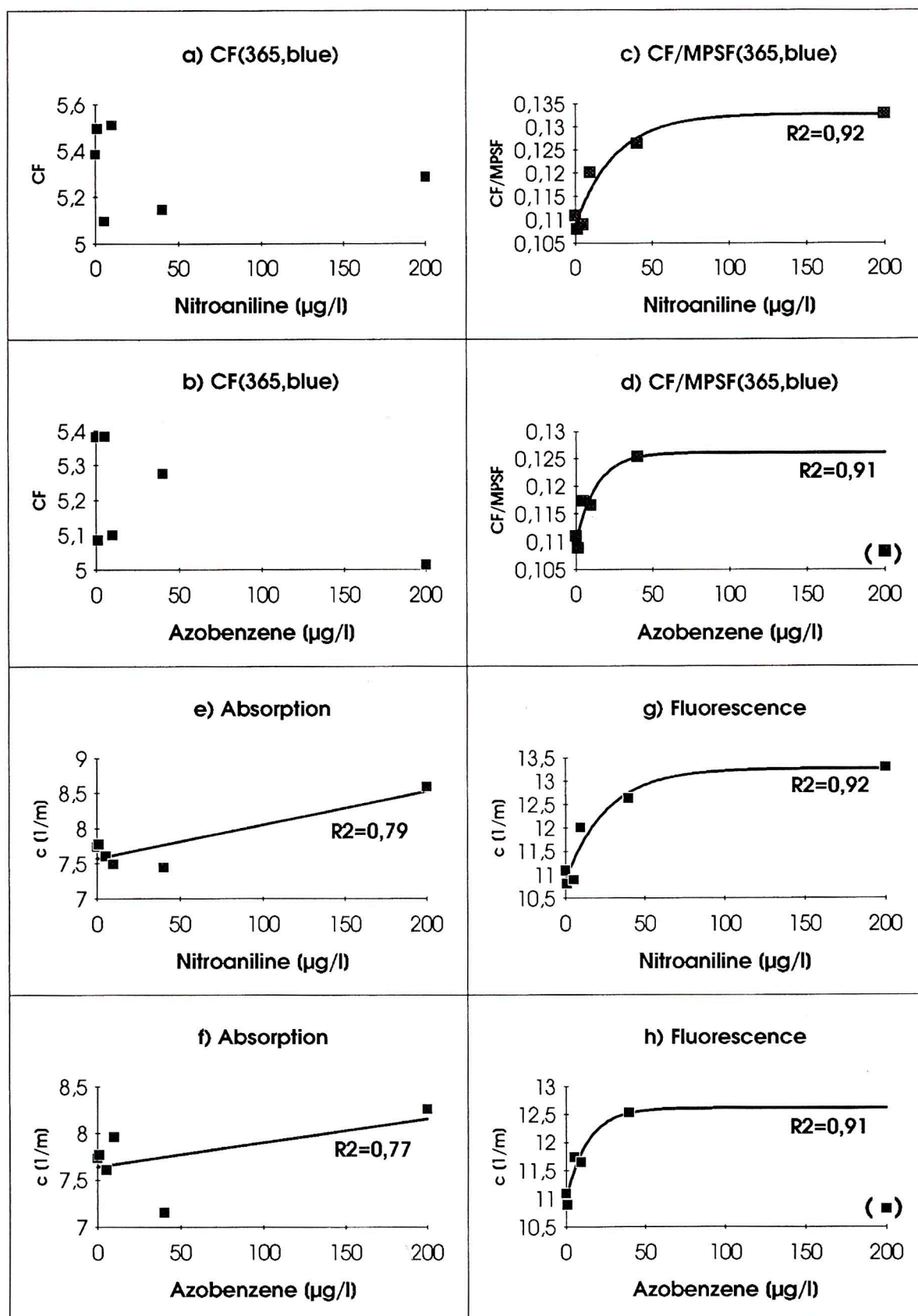
*Figure 4 - **a-d**: CF and CF/MPSF versus concentration of Nitroaniline and Azobenzene. Excitation: 365nm. Emission: 380-460nm.
e-f: Total attenuation coefficient c measured by absorption spectrometry at 365nm. **g-h**: Total attenuation coefficient c measured by the fluorescence ratio CF/MPSF. Excitation: 365nm. Emission: 380-460nm*

$a_F(\lambda_L)$ - absorption coefficient of fluorescent substances at the laser wavelength

$c_1(\lambda_L)$ - total attenuation coefficient of the water at the laser wavelength

$c_2(\lambda_F)$ - total attenuation coefficient of the water at the emission wavelength

The function f(H) considers, e.g., laser intensity, flying altitude, aperture, optical transmission of the boundary water-air and optics used.

To reduce disturbances like changes of the laser power and of the optical proporties of the water investigated, S will usually be normalized to the Raman signal R of the water body:

$$R = f(I) * \frac{1}{c_1(\lambda_L) + c_3(\lambda_R)} \qquad (8)$$

$c_3(\lambda_R)$ - total attenuation coefficient of the water at the Raman wavelength

The function f(I) involves f(H) as well as the raman cross-section and the number of water molecules. Assuming the ratio J=f(H)/f(I) remains constant, we get after normalization:

$$\frac{S}{R} = constant * a_F(\lambda_L) * \frac{c_1(\lambda_L) + c_3(\lambda_R)}{c_1(\lambda_L) + c_2(\lambda_F)} \qquad (9)$$

In some cases the ratio of the attenuation coefficients (third term) remains constant, in particular at small differences between λ_F and λ_R. Then, S/R yields informations about fluorescent substances only, e.g. algae, oil, yellow substances. Equ.9 is similar to CF in Equ.2.

If we now separate the total attenuation coefficient into their fluorescent and nonfluorescent parts Equ.7 produces:

$$S = f(H) * \frac{a_F(\lambda_L)}{c_{1F}(\lambda_L) + c_{1A}(\lambda_L) + c_{2F}(\lambda_F) + c_{2A}(\lambda_F)} \qquad (10)$$

The indices F and A represent fluorescent and nonfluorescent substances, respectively. Evidently, the signal S is controlled both by fluorescent and nonfluorescent substances. Thus, nonfluorescent substances could also be detected by means of Lidar.

Of course, in contrast to the MPSF signal, the remote signal S contains additional unknown parameters, namely the attenuation coefficient of fluorescent and nonfluores-

cent substances at the emission wavelength λ_F. This could lead to some trouble. But using Equ.9 and in case of correlations of $a_F(\lambda_L) \sim c_{1F}(\lambda_L) + c_{2F}(\lambda_F)$ and $c_{1A}(\lambda_L) \sim c_{2A}(\lambda_F)$, Equ.10 could yield information about nonfluorescent substances. In further research it should be verified under which natural conditions such a procedure can be applied.

6. CONCLUSIONS AND OUTLOOK

1. Principally, the experiments verified the theory of CF-MPSF that both fluorescent and nonfluorescent substances yield a fluorescence response.

The ratio CF/MPSF does not depend on the fluorescence quantum yield and is controlled by fluorescent as well as nonfluorescent substances. This can lead to an improved detection of chlorophyll and dissolved organic load.

A detection of organic micro-pollutants on the basis of the CF-MPSF method also seems to be possible without any effort concerning sample treatment; ppb-substances contained in ppm-matrices can be detected, e.g. nitroaniline (ppb) in eutrophicated waters with yellow substances falling into the ppm concentration range.

2. The CF-MPSF method combines the advantage of fluorescence techniques to be very sensitive and the advantage of absorption methods to detect a wide range of substances. The disadvantages like the small sensitivity (absorption) and the limited detection of only fluorescent substances (fluorescence) will be eliminated. Because there are a lot of nonfluorescent substances of interest, CF-MPSF should have a great analytical potential for monitoring organics in-situ. Accordingly, CF-MPSF could be used for remote sensing purposes, e.g. for measurements of ground-truth-data aboard ships at short time intervals.

3. Further investigation and experiments are necessary to verify CF-MPSF. Multi-path-cuvettes with plan mirrors are not appropriate for pure waters (e.g. off-shore). Use of so-called Herriott cells leading to longer path-lengths of the excitation radiation is recommended.

4. Moreover, the problem has to be solved if the water sample contains unknown organic micro-pollutants. Principally, we think that only substance-classes can be detected, which could be accomplished in the following way. In a first step a "natural optical standard" NOS of the water investigated is determined by measuring CF and MPSF at a defined time interval in unpolluted regions. In

many cases NOS of waters should be dominated by the optical proporties of phytoplankton and yellow substances. Knowing the NOS behaviour, deviations from NOS measured indicate substances, e.g. organics, which do not belong to NOS. Detection of different substance classes should be possible because many aromatic substances show different optical behaviour.

5. It should be verified under which conditions the CF-MPSF method can be applied to Lidar remote sensing.

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