VISUALIZATION OF AIR WATER GAS EXCHANGE USING NOVEL FLUORESCENT DYES

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ABSTRACT

Laser-Induced Fluorescence (LIF) techniques are applied to visualize air-water gas exchange across the aqueous mass boundary layer in wind/wave facilities. Dissolved oxygen is made visible by quenching of the fluorescence of an organic ruthenium complex (tris(4,7-diphenyl-1,10-phenanthroline disulfonic acid) ruthenium(II) dichloride, Ru(dpp ds)$_3$), and acid or alkaline volatile species such as CO$_2$, HCl or diethylamine by the fluorescent pH indicator 1-hydroxy-pyrene-3,6,8-trisulfonic acid (HPTS). Fluorescence of both dyes can be stimulated by a 473 nm DPSS laser. The spectral peaks of the fluorescence emission (510 nm for HPTS and 610 nm for Ru(dpp ds)$_3$) are different enough, so that the concentration fields of both dyes can be measured simultaneously using two cameras with different bandpass filters. The paper details the properties of the two dyes and demonstrates their usage with some preliminary visualization experiments.

1 INTRODUCTION

Gas transfer across gas/liquid interfaces are of importance in natural and technical environments. The exchange of gases between the atmosphere and the oceans determines the global distribution of many gaseous and volatile chemical species. Reaeration of lakes and rivers across the water surface is a critical process for the ecology of these environments, especially if they show high biological activity. In technical systems mass transfer across gas/liquid interfaces is an essential process in various gas/liquid reactors such as bubble columns and falling films columns.

The difficulty in studying the mechanisms of air-water gas exchange is due to the small thickness (30–300 µm) of the aqueous mass boundary layer at a free interface and the overlaying undulation by waves in the range of cm. Measuring the mean flux by mass balance techniques results only in mean values for transfer coefficient and mass boundary layer thickness. Only techniques that visualize concentration fields of the dissolved gases give direct insight into the turbulence close to the interface.

In the literature, two different kinds of visualization techniques for gases dissolved in water are reported: the pH indicator technique and the oxygen-quenching technique. The older of the two is the
pH indicator technique. The key point of this technique is the conversion of the flux of an acid or alkaline
gas from the air to the water surface. At the water surface, a rapid hydration reaction takes place that
forces an equivalent flux of a fluorescent dye to or away from the water surface.

Almost 40 years ago, this technique was used to investigate the gas transport mechanisms in falling
films \[2, 5, 6, 7\]. In 1989, the pH indicator technique was first used in a grid-stirred tank \[1\]. The
authors measured time series of the CO\(_2\) concentration fluctuations close to a gas-liquid interface at a
fixed position using dichloro fluorescein. The first successful measurements of vertical concentration
profile measurements within the aqueous mass boundary layer at a free interface in a wind/wave flume
were reported two years later in 1991 using HCl gas and fluorescein as a pH indicator \[8\]. More detailed
studies followed later \[9, 11\].

At the same symposium, also the first successful measurements were reported from wind/wave
flumes using the oxygen quenching technique \[15\]. Pyrenebutyric acid (PBA) was used, a dye known
from measurements of dissolved oxygen concentrations in cells \[13\]. PBA fluorescence was stimulated
by a N\(_2\) laser in the UV at 337 nm. Later, other authors used the same dye in wind/waves flumes \[9, 10, 16\]
and grid-stirred tanks \[3, 4\] for oxygen exchange studies.

The rather qualitative character of the previously published results indicates that the currently used
fluorescent dyes still show significant disadvantages. Although fluorescein and derivatives that have
used mostly in previous studies with the pH indicator technique are cheap dyes with a high quantum
efficiency they have two significant disadvantages. Firstly, the Stokes shift is rather small. This causes
some problems with reabsorption of the fluorescent light. Secondly, fluorescein is quite sensitive to
photobleaching.

The use of PBA to measure oxygen dissolved in water is even more problematic. Firstly, the quench-
ing effect is rather weak causing a poor signal-to-noise ratio of the concentration measurements. Sec-
ondly, it is difficult to solve PBA in water. Thirdly, PBA is a surface active chemical species. In this way,
the hydrodynamic boundary conditions at the air-water interface (surface tension and surface elasticity)
are altered by PBA.

Given the rapid progress of fluorescent probes in biochemistry in the last decade, to search for more
suitable fluorescent dyes seemed promising. This paper reports that for both the oxygen-quenching
technique and the pH indicator technique better dyes could be found. Their properties and advantages
are described and demonstrated by some preliminary visualization experiments.

## 2 VISUALIZATION BY PH INDICATORS

A second method uses fluorescent pH indicators to make acid and alkaline gases visible. The idea behind
this method is the following: acid gases like HCl gas penetrate the air-sided mass boundary layer and
are solved in the water phase. Because of the rapid hydration reaction, HCl does not penetrate into the
aqueous mass boundary layer but the pH value of the solution changes. These changes can be visualized
by fluorescent pH indicators added to the solution.

This process can be modeled in the following way \[8\]: The pH indicator in its acid form is denoted
by HR\(^-\), the conjugate base by R\(^2-\) and the following chemical reversible reaction takes place:

\[
H^+ + R^{2-} \rightleftharpoons HR^-
\]  

(1)
with the equilibrium
\[ \text{pH} = \text{pK}_a + \log \left( \frac{[R^2^-]}{[HR^-]} \right) \]  

Only at pH values close to the indicator constant \( \text{pK}_a \), both forms of the indicator occur in about the same amount. If the pH value differs only by one from the \( \text{pK}_a \) value, one form is already ten times more abundant than the other. Therefore, it is obvious that an ideal indicator should have a \( \text{pK}_a \) value close to the neutral point, i.e., close to 7.

The flux of the acid gas \( \text{HCl} \) \( j_a \) into the water phase is compared to the flux \( j_w \) (Fig. 1).

\[ j_a = k_a [\text{HCl}]_a \]
\[ j_w = k_w \Delta [\text{HR}^-] \]

The quantities \( k_a \) and \( k_w \) are the transfer velocities across the air-side and water-side mass boundary layers, respectively.

Because one chemical species is completely replaced by the other in a rapid chemical reaction right at the water surface, both fluxes must be equal and the induced concentration difference of the indicator across the aqueous mass boundary layer is

\[ \Delta [\text{HR}^-] = \frac{k_a}{k_w} [\text{HCl}]_a \]  

Besides a \( \text{pK}_a \) value close to 7.0, an ideal pH indicator for the visualization of concentration fields should have the same features as an oxygen quenching indicator. It should be highly water soluble and show no surface activity. 1-hydroxy-pyrene-3,6,8-trisulfonic acid trisodium salt (HPTS) is a fluorescent pH indicator that meets these requirements. In pure water it has a \( \text{pK}_a \) value of 8.04 ± 0.07 \(^{[14]}\). When the fluorescence is stimulated at 473 nm, the alkaline form is highly fluorescent while the acid form shows almost no fluorescence (Fig. 2a). This high contrast is much better than for fluorescein. The emission spectrum maintains its shape, while only the absorption spectrum is highly pH dependent (Fig. 2a).

In order to verify the quantitative relation between the fluorescent intensity and the concentrations, conductometric titration experiments were carried out. Instead of acid/alkaline gases, the HCl and NaOH
(both in concentration of 0.1 mol/L) were used as titrants (i.e., solutions to add) as they are easier to handle quantitatively than gases in first test experiments.

The resulting titration curves are shown in Fig. 2 b for an experiment in which HCl is added continuously to a 15 µmol/L HPTS solution to which 120 µmol/L NaOH has been added in order to start the titration in the alkaline range. During the titration the conductivity of the HPTS/NaOH solution is measured together with the intensity of fluorescence as grey values of a CCD-camera. The two sharp bends of the conductivity curve (see vertical lines) mark the equivalence points of the titration. The first indicates the neutralization of the strong base NaOH (conductivity descends), the second one the neutralization of the weaker HPTS anion base (conductivity rises slowly). The strongly ascending part of the curve is due to the surplus of HCl.

The intensity of fluorescence plotted in the upper part shows a linear range near the first equivalence point of the conductivity. In this area the intensity changes not only linear but is also very sensitive to concentration changes. Therefore, this range is used for the visualization experiments.
3 VISUALIZATION BY OXYGEN QUENCHING

A dye with a long lifetime of its excited state is needed as a oxygen sensible luminophore in order to increase the probability of a successful hit inducing the so called quenching: a collision between the excited luminophore and the quencher gives way to the energy dissipation of the excited electronic state by collisions instead of the photon emission.

The Stern-Volmer equation \[ I(c)/I_0 = \frac{1}{(1 + K_{SV} \cdot c)} \] describes the quenching of the luminescence by the concentration of a quencher such as dissolved oxygen:

where \( I(c)/I_0 \) is the fraction of the actual luminescence related to the luminescence in absence of the quencher; \( c \) is the concentration of the quencher in the liquid which is oxygen in our case; and \( K_{SV} \) is the Stern-Volmer quenching constant in units of [L/mol].

As the quenching process is fast compared to mass fluxes, the concentration at one point of the quencher \( O_2 \) can be evaluated reliably by measuring the intensity of the light emission. Other influencing conditions like intensity of the exciting light and temperature have to be the same as in the calibration procedure.

An ideal quencher for oxygen gas transfer measurements should have a high quenching constant, be readily soluble in water, and show no significant surface activity. A high quenching constant, and
thus high sensitivity according to Eq. (4) requires a long fluorescent life time in the order of some µs. A family of diimine ruthenium complexes, especially tris(4,7-diphenyl-1,10-phenanthroline disulfonic acid)ruthenium(II) dichloride complex (Ru\((\text{dpp ds})_3\)) has these desired properties.

The following is a list of the advantages of Ru\((\text{dpp ds})_3\) as compared to the widely used pyrenebutyric acid (PBA):

- PBA requires a UV laser for excitation (e.g., a N\(_2\) laser at 337 nm), while the ruthenium complex absorbs in the visible blue where cheap and handy light sources for the stimulation of the fluorescence are available. Fig. 3 shows a maximum of absorption at 465 nm. Above 520 nm it is low enough so that no significant self absorption of the fluorescent light occurs.

- The Stokes shift of Ru\((\text{dpp ds})_3\) for stimulation with 473 nm and an emission maximum of 610 nm (Fig. 3b) is 137 nm. This is much larger than the Stokes shift for PBA: stimulation at 337 nm, emission maximum at 375 nm, Stokes shift 38 nm [13].

- The quenching constant \(K_{SV}\) of Ru\((\text{dpp ds})_3\) is about 8700 ± 1300 L/mol (cf. Fig. 3b), while the quenching constant for PBA is more than ten times lower (683 ± 70 L/mol [9] or 645 ± 79 L/mol [13], Fig. 3b). Therefore, measurements are more than ten times more sensitive at small oxygen concentrations. At the saturation concentration of 8 mg/l, the fluorescence quenching for Ru\((\text{dpp ds})_3\) is 63% while it is only 13% for PBA.

- Because of the six sulfonic groups, Ru\((\text{dpp ds})_3\) is excellently soluble in water and shows no surface activity. In contrast, PBA is almost insoluble in water. It can only be solved in NaOH before mixing with water to obtain only much lower concentrations.

4 COMPATIBILITY OF THE TWO TECHNIQUES

Comparing the properties of Ru\((\text{dpp ds})_3\) and HPTS, it is obvious that both techniques can be used simultaneously. The fluorescence of both dyes can be stimulated at 473 nm. The emission signals overlap only little and can easily be separated by interference filters or a dichroitic mirror (Fig. 4a).

Performing simultaneous quantitative concentration field measurements with both dyes is of interest because oxygen has a significantly higher diffusion coefficient than the chemical species diffusing with the pH indicator technique. Therefore, the dependency of the transfer process on the molecular diffusivities can be studied.

5 EXPERIMENTAL SETUP

The setup shown in Fig. 4b uses a vertical laser sheet and is suitable for a measurement footprint of up to several centimeters down from the water surface. The resolution thereby is between 20 – 50 µm/pixel. Illumination by a 50 mW laser at 473 nm is sufficient to acquire image sequences with 200 frames/s from the side with a Dragonfly CCD-camera (Point Grey Research, Canada). The camera was tilted by 10 degree to avoid interference with the moving surface. This generates a mirror image above the surface line by total reflection at the water surface.

The measurements are carried out in a circular wind wave flume with 12 cm water height and a water volume of 70 L. The channel exhibits a width of 20 cm, a height of 41 cm, and a diameter of 1.2 m. Wind is generated by a rotating paddle wheel. The concentration of the dyes were \(10^6 – 10^5\) mol/L.
In order to strip dissolved oxygen from the water, a ”JOSTRA QUADROX” gas exchanger module from Maquet is installed containing membrane capillaries with a total membrane surface of 1.8 m² and a water volume of 250 ml. This device is normally used in cardio-pulmonary machines to load blood with oxygen and extract carbon dioxide. The gas exchanger can be used for other gases as well. In the work herein described, we employed it with vacuum (160 mbar) to degas the water to oxygen concentrations as low as 0.8 mg/L. The oxygen concentration is measured constantly by a commercial oxygen sensor.

### RESULTS

In Fig. 5 images of the luminescence profiles demonstrate the good contrast in the boundary layer. In Fig. 5a+b for HPTS and ruthenium complex, respectively, the decrease of the luminescence near the surface is evident where gases penetrate the surface in both cases.

In the case of HPTS, it is seen how HCl solves in the liquid phase changing the pH of the water. In the case of the ruthenium complex the dissolution of oxygen is visualized. The more oxygen is present the less intensity of phosphorescence is observed. In the time/space image, 300 lines are gathered one after the other. These lines were taken with 200 Hz to add up to a total time of 1.5 seconds. Turbulence near the surface and intrusion events appear in some pictures like the one displayed here.

To extract quantitative values some steps of image processing are needed. In Fig. 5c a line profile of the ruthenium complex is shown. Towards greater deepness a decrease of phosphorescence is due to the Beer-Lambert extinction of the incident light by the absorption of the ruthenium dye. Above the surface, a mirror effect is evident. This is because the camera looks at the surface with a slightly tilted angle so that a total reflection on the water surface is observed.
**Fig. 5** Measured profiles in false-colors.  

*a* Fluorescence of HPTS excited by the laser sheet at a wind speed of 3 m/s. In the air space small concentrations of HCl were present and no surface film was added.  

*b* Time/space image of the fluorescence profile emitted by the ruthenium complex near the surface with surfactant at a wind speed of 7 m/s.  

*c* Line profile of the luminescence near the surface. Fitting lines are for Beer-Lambert extinction and the boundary layer.

Because of an optical blurring, the function of the light intensity appears not to be pointed that much as a flat mirror would suggest. This blurring is a result of the quality of the optical path at the high magnification used. It reduces the effective resolution of the imaging.

### 7 CONCLUSIONS AND OUTLOOK

The two new fluorescent dyes for quantitative visualization of concentration fields within the aqueous mass boundary layer investigated in this paper show significant advantages over the previously used dyes. Thus much better results can be expected than with previous studies.

Currently, a new inert an chemically clean linear wind/wave facility is being constructed that is specifically designed for the visualization techniques described in this paper. The air space and the water channel will be coated with Teflon and the whole facility is gas-tight. Therefore acid and alkaline gases can be used in this facility. The water channel will be 4 m long, about 0.4 m wide and 0.1 m high and can be filled with ultraclean water.

### 8 REFERENCES


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