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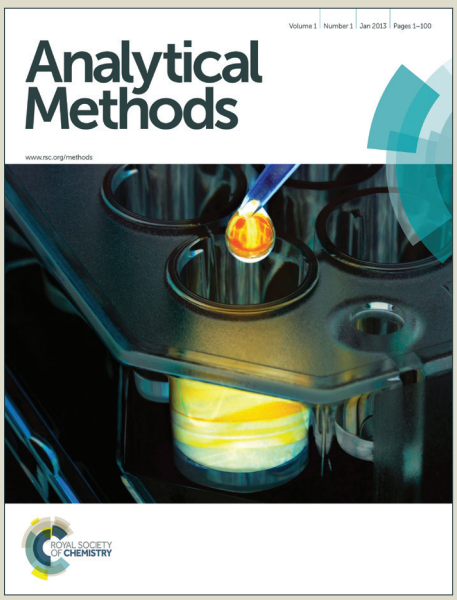

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Study of the effect of organic solvents on the fluorescence signal in a sequential injection system

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

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A study of the effect of different organic solvents and their mixtures with water on the fluorescence intensity of two model compounds (quinine sulphate as a naturally fluorescent substance and metsulfuron methyl with fluorescent properties upon UV irradiation) was carried out in a sequential injection system.

Introduction

Fluorometry is one of the most used detection techniques in a number of methods based on flow systems [1-5] due to its higher sensitivity in comparison to spectrophotometric detection, simplicity of instrumentation and lower cost of equipment in comparison to the more sophisticated detectors, such as atomic absorption or mass spectrometry, and feasibility of coupling to a sequential injection (SIA) system. Fluorescence depends, among other parameters, on the solvent used not only in the terms of emission wavelength but also intensity of the measured signal [6]. The effect of different solvents on fluorescence shift and intensity in case of several analytes, e.g. metallophthalocyanines [7], citrinin [8] or carbamate and indol compounds [9] was studied. The limitation of a wider use of fluorometry is the number of naturally fluorescent substances. However, fluorescent properties can be evoked e.g. by chemical reaction or irradiation which enable to use fluorometric detection for determination of large number of analytes. Moreover, induced fluorescence can also lead to increased method selectivity.

Recently, the use of organic solvents in flow systems has been increasing, since many novel applications, such as different modes of online sample pre-treatment automated in flow systems, mainly based on sequential injection (SIA) principle [10-12], require the use of such solvents. Moreover, several ways of handling the combination of organic solvents together with aqueous solutions in one flow system were proposed, such as wetting the Teflon tubes with organic solvent [13], using a de-bubbling device in front of the detection cell to eliminate bubbles arising from mixing aqueous and organic solutions [14], or modified flow system to separate organic and aqueous solutions handling units in case of dual-valve system (DV-SIA) [11]. In this way, possible disturbing phenomena (that could arise from adsorption of organics to lipophilic Teflon tubes and could cause a noise in signal readout because of different optical properties of different solvents) are not considered a problem but an advantage of employing organic solvents even in a SIA system. Also, due to the miniaturization related to flow techniques, only small volumes of organic solvents are commonly used, so the principles of green chemistry are adopted. However, the compatibility of the carrier stream with the sample solution must be taken into account in the development of a flow method to exclude any unwanted effects in the system such as carryover or Schlieren effect [15], resulting from different chemical and physical (optical) properties.

Flow techniques, especially SIA, are characterized by high reproducibility ensured by computer-control of all steps of the operating protocol. This makes it a convenient tool for automation of solution handling so that the sample treatment prior or during analysis, including derivatization by a reagent or even by light (UV irradiation), can be performed on-line. Automation of UV irradiation in a flow system prior to fluorescence detection of the irradiation product was reported elsewhere [16].

Notes and references

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Electronic Supplementary Information (ESI) available: Figures with the effects of different solvents, pH adjustment and UV decomposition on MSM fluorescence intensity without and after pH adjustment

In this work, the effect of several organic solvents on the fluorescent signal of two model compounds in the SIA system was studied. Quinine sulphate is a well-known substance used for treatment of malaria. As a naturally fluorescent compound in acidic pH conditions, it is used as a standard to study fluorescence quantum yield under different conditions [17, 18]. Methods based on SIA were reported for either determination of quinine [19] or using quinine as a sensitizer of a photo-oxidation [20] or chemiluminescence [21] in determination of other substances.

Automated flow systems could involve on-line UV irradiation to decompose the original analyte to a fluorescent product that is determined afterwards (e.g. thiamine [22]). In such applications the UV irradiation time should be short enough to obtain measurable fluorescence without the need to reach the steady state so that the advantage of a fast flow method is not neglected by a long irradiation time. Metsulfuron methyl, a sulfonylurea selective herbicide, shows fluorescence upon UV irradiation and this property was used in several analytical methods for MSM determination, even in flow systems [16, 23]. Here, it was chosen as a model compound representing molecules which are not naturally fluorescent but can be converted into a fluorescent photoproduct. The fluorescence of such photoproducts can vary in different solvents.

A thorough study of different conditions influencing fluorescence of the model compounds was performed in this work. The studied parameters such as organic solvent, pH adjustment, irradiation time, and wavelength of UV light were tested off-line to get information concerning the effect of the mentioned parameters on the decomposition pathway and the possibility to transfer such decomposition to an on-line system (the key parameter is the time of UV irradiation step) in order to obtain expected benefits in terms of determination sensitivity in compromise with quick analysis in the flow system. The compatibility of the carrier stream with the solvent used for sample preparation was studied with respect to the flow conditions and compared in terms of fluorescence signal intensity and repeatability of measurements. The study intends to give a good starting point when choosing suitable conditions in development of analytical methodologies based on fluorescence measurements in an SIA system, including the use of organic solvents for sample pre-treatment or other applications.

Experimental

Reagents

Standards of quinine sulphate (QS) and metsulfuron methyl (MSM) were purchased from Sigma-Aldrich (Prague, Czech Republic) and different solvents tested were: acetone and isopropanol (Penta, Czech Republic), acetonitrile, methanol, ethanol and toluene (Sigma-Aldrich, Czech Republic), dichloromethane (Fluka, Switzerland), trichloromethane (Lachema, Czech Republic). Demineralized water (Merck-Millipore, Czech Republic) was used as the main carrier stream if not specified otherwise, and was also tested as a solvent for fluorescent substances. Phosphoric acid and sulfuric acid were purchased from Penta (Pardubice, Czech Republic).

Apparatus

Fluorescence detection was studied in the flow system based on sequential injection analysis (SIA) principles. The setup corresponded to a commercially available FIAlab® 3500 system (FIAlab® Instrument Systems Inc., USA) with a syringe pump (syringe reservoir of 5 mL), a central eight-port Cheminert selection valve and a 0.50 mm i.d. PTFE tubing used for all connection (Figure 1). Fluorescence detection was scanned in the flow cell (3.0 mm optical length) of the PMT detector (FIAlab® Instrument Systems Inc., USA) scanning the whole spectrum without using excitation or emission filters. UV lamp (D1000 CE, Analytical Instrument Systems, Inc.) was used for excitation. The latest version of the FIAlab® software (version 5.9.312) was employed for system control and data acquisition.

UV decomposition was carried out off-line using two wavelengths – 254 and 366 nm (UV lamp, Camag, Switzerland, 50 Hz, 220 V).

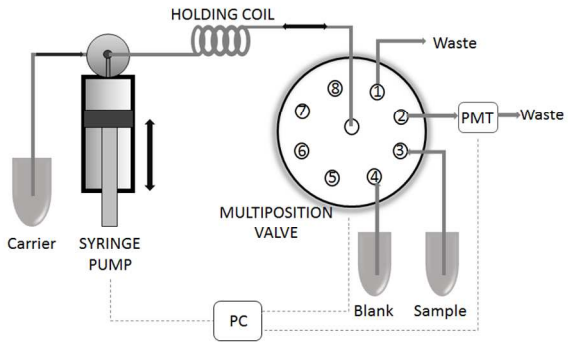

Figure 1: Set-up of the sequential injection system

Measurement procedure

QS and MSM solutions in the respective solvent or mixture of solvents were prepared and aspirated into the SIA system using a 50 µL sample zone that was transported to the detection flow cell at a flow rate of 80 µL s-1 and the fluorescence signal was scanned using 100 ms for integration time and 8 Hz as a detector frequency. To obtain statistically relevant data, all samples were measured in triplicate and the average value of peak heights was used for the final evaluation.

QS at a concentration of 10 mg L-1 was tested using water as a carrier stream for fluorescence detection of solutions prepared in methanol, ethanol and isopropanol. Then carrier stream was changed to pure organic solvents to avoid the effect of mixing the aqueous and organic phases. These tests were carried out with methanol, ethanol, isopropanol and dichloromethane as the carrier. Dichloromethane was not tested in combination with the aqueous carrier because of the immiscibility of these two solvents.

MSM fluorescent properties were tested with respect to different solvents following the study published by Casseli [24] where UV decomposition and an increased fluorescence of degradation products were discussed. The tested concentration of MSM solutions was 25 mg L-1. The solutions were prepared in: water, acetone, mixtures of water and acetonitrile and trichloromethane. Two different pH values were tested for each solvent, pH 2 and pH 7, adjusted by concentrated phosphoric acid. UV decomposition was carried out off-line using a low-pressure mercury arc lamp and 15 mL of each solution were placed in a beaker in the distance of 15 cm from the mercury lamp. The effect of the UV radiation on the fluorescence (detection) intensity of the obtained decomposition products was observed in intervals of 15 min for periods of 60 – 120 min.

Results and discussion

Effect of organic solvents on fluorescence detection of QS

At first a calibration of QS determination in the SIA system under the described conditions was performed using aqueous solutions in the concentration range of 1 – 100 mg L-1 prepared in 0.05 M sulfuric acid with water as a carrier stream. The linear part of the calibration was found in the range from 1 to 10 mg L-1 with correlation coefficient of 0.9966 using baseline subtraction. The effect of dilution of the acidified solutions in water without pH adjustment used for transport of the QS zone to the detection flow cell was not observed. The calibration curve for higher concentrations of QS (10 – 100 mg L-1) showed logarithmic shape because of concentration quenching of fluorescence signal. Thus the highest concentration of the linear range was chosen for further experiments to evaluate the effect of organic solvents expressed as difference in the fluorescence intensity obtained at different conditions.

The effect of organic solvents was studied for combination of different solvents used for preparation of acidified QS solutions at the 10 mg L-1 level and water used as a carrier stream. The evaluation was based on the difference of fluorescence intensity between aqueous and organic solutions together with comparison of repeatability of these measurements expressed as a standard deviation (SD) values.

Results given in Figure 2 show that in case of aqueous carrier stream, the aqueous QS solution was the most fluorescent. What is more, the increased SD values for organic solutions showed problems with mixing of aqueous and organic phases in the flow system. The repeatability of aqueous solution measurements (0.53 %) compared to the highest RSD values for methanol solution (11.73 %) was the main reason for the next assay comprising modification of the carrier stream. The solvent used as a carrier stream was identical with the solvent used for preparation of the respective QS solution and sulfuric acid content was kept at the same level as in the aqueous solution. The obtained results are summarized in Figure 2. Baseline subtraction of the respective blank solution was applied for evaluation of each measurement. In this study, the fluorescence intensity of acidified isopropanol solution was found to be higher than in aqueous solution and also the methanol solution revealed comparable fluorescence. The observed repeatability was in acceptable range for all solvents (0.21% for isopropanol – 2.75 % for ethanol).

A very large difference of QS fluorescence in different solvents showed the effect on the fluorescence intensity that could highly influence fluorescent properties even in the flow systems. Compared to the aqueous solution, dichloromethane solution expressed just 8.93 % (because of fluorescence quenching caused by chlorine atoms) and ethanol 63.50 % of the fluorescence intensity while QS prepared in isopropanol had 116.89 % of the original fluorescence of the aqueous solution. In the case of QS fluorescence, some of the tested organic solvents slightly increased the measured intensity. Their consumption, however, could be considerably large, which is a serious disadvantage. From another point of view, the use of aqueous carrier stream can decrease their consumption. Nevertheless, the combination of aqueous and organic phases led to increased RSD values which affected the measurement repeatability. Thus a compromise should be always found in optimization of flow fluorescent measurements to obtain not only the desired sensitivity but also repeatability and low consumption of organic solvents that corresponds to the green chemistry requirements.

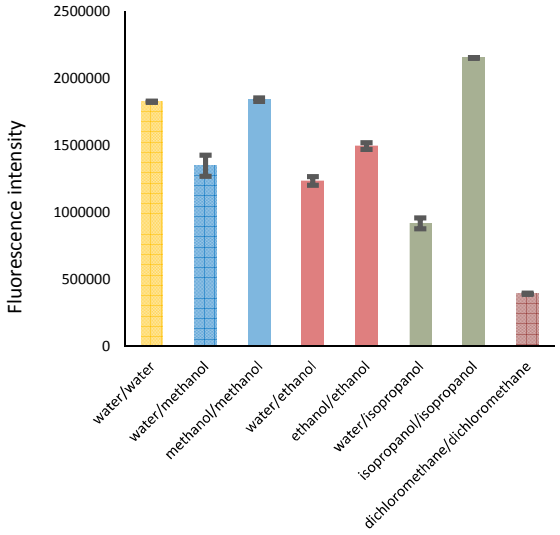

Figure 2: Fluorescence intensity and standard deviation of QS measured in combination of different solvents - effect of the carrier stream/acidified QS solution composition

Effect of UV decomposition of MSM solution in different organic solvents

In the following experiments, MSM was chosen to study the influence of organic solvents on the UV decomposition that is commonly used in case of light-induced fluorescence determinations. At first, the fluorescence of MSM aqueous solution at a concentration of 25 mg L-1 was measured, using water as a carrier stream. Figure 3 shows the effect of UV (254 nm) decomposition applied to a solution without and with pH adjustment (pH 2) following the literature [24] where mainly the effect of UV decomposition was stressed over the effect of pH. In the tested solution, UV decomposition increased the fluorescence relatively slowly. This is not advantageous for flow systems where this step could be carried out even on-line using a reaction tube coiled and fixed around an UV lamp. Also, much higher effect of pH adjustment on the fluorescence intensity was found which was demonstrated by 3.86-times higher signal for acidified solution compared to signal of the solution without a pH adjustment.

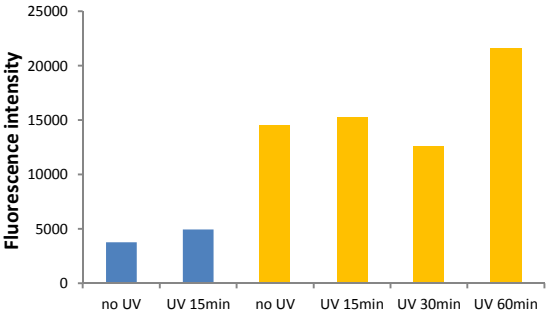

Figure 3: Fluorescence intensity of MSM in aqueous solution - blue (without pH adjustment), yellow (pH 2)

In the next experiments, organic solvents or mixtures tested for UV decomposition were used as carrier stream. In case of acetone solution, UV decomposition showed effect on the solution without pH adjustment and also the effect of prolonged decomposition was visible (2-times higher fluorescence was obtained after 30 min of degradation). The mixture of water and acetonitrile that was described as the most suitable in the literature [24] was tested in different ratios (7:3, 1:1, 3:5 and 1:9), with water as a carrier stream including the pH adjustment and UV decomposition. Comparison of the obtained results for the ratio of 7:3 is shown in Figure 4 where a significant difference caused by pH adjustment (4.5-times increased fluorescence) was proved. However, the UV decomposition had only a slight effect on the fluorescence intensity – even after 60 min of irradiation at 254 nm UV, the measured solution showed a comparable signal to the original one.

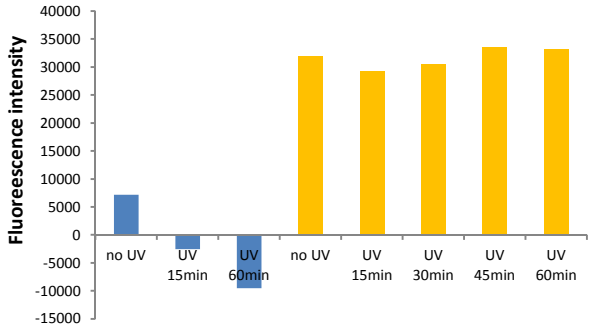

Figure 4: Fluorescence intensity of MSM in the mixture of water and acetonitrile (ratio of 7 : 3) - blue (without pH adjustment), yellow (pH 2)

Then the UV wavelength was changed to 366 nm to test the effect of wavelength on the decomposition step but only decrease of the fluorescence intensity in the period of 30 min by 18.66 % was observed. The effect of UV decomposition that caused decrease of the measured fluorescence was similar for solution without pH adjustment using 254 nm and with acidified solution in case of 366 nm. The other solutions were not affected by the applied UV degradation. It was found that pH adjustment affected the fluorescence more significantly, since 8-times higher fluorescence signal was observed. This corresponded to the higher effect of ionization of the MSM molecule which is related to increased solubility but suppressed fluorescent properties. The other previously mentioned ratios of water and acetonitrile did not show any fluorescence either for original solutions or after UV decomposition.

The last solvent tested was trichloromethane in which higher effect of acidification of MSM solution was also proved. Figure 5 shows that 10-times higher fluorescence could be found for acidified solutions prepared in this solvent and again only a small effect of UV decomposition was observed leading even to lower signals. Comparing the same conditions with aqueous solution in case of pH adjustment, 54-times higher and for pH adjustment with UV decomposition using 30 min at 254 min even 66-times higher fluorescence was found. Since the most intense fluorescence was obtained with trichloromethane solution, calibration using this solvent with pH adjustment was also measured with linearity in the range of 5 – 25 mg L-1 and a correlation coefficient of 0.999. Limits of detection and quantitation were calculated based on the 3-times and 10-times baseline noise and were found to be 1.52 and 5.13 mg L-1, respectively.

Transfer of UV decomposition step to an on-line procedure in the flow system should provide the compromise between benefits of determination sensitivity and analysis time (sample throughput). Although in this tested determination simple pH adjustment showed lower fluorescence signal, the automated determination of MSM is not expected to be prolonged by 30 min decomposition. Thus fast and simple SIA determination was found to be more suitable for practical application in this example.

The detailed comparison of fluorescence intensity obtained in different organic solvents and after different degradation times is given also in Supplements 1-3.

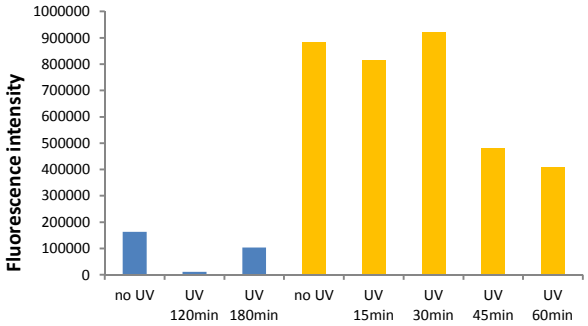

Figure 5: Fluorescence intensity of MSM in trichloromethane - blue (without pH adjustment), yellow (pH 2)

Conclusions

Effect of organic solvents that are commonly used in flow systems based on sample pre-treatment automation (extraction techniques) was studied in the SIA system. Mainly carrier stream composition was taken into account and combination of organic solvents used for sample preparation with aqueous carrier was tested and compared with respect to fluorescence intensity and measurement repeatability. Varying fluorescence was found for respective combinations and decreased repeatability (increased RSD values) were proved in case of combination of small volumes of samples in organic solvents aspirated into the aqueous carrier stream.

Testing organic solvents for UV irradiation as a preliminary information for transfer of this step into the on-line procedure in the flow system was carried out, too. In this case different organic solvents show very big differences that could be expected because of different pathways of decomposition. Thus choice of solvent could be the key factor to get highly fluorescent decomposition product for detection. As the other important parameter, time of irradiation should be optimized to get reasonably high sensitivity together with acceptable analysis time.

Crucial parameters should be studied in detail during optimization of fluorometric measurements in flow systems using organic solvents to take advantage of all benefits of such methodology, such as sensitivity, speed and precision of analysis.

Acknowledgement

The authors gratefully acknowledge financial support of the bilateral cooperation between Argentina and the Czech Republic, project No. 7AMB14AR029 (ARC/13/09) and is co-financed by the European Social Fund and the state budget of the Czech Republic, project No. CZ.1.07/2.3.00/20.0235, the title of the project: TEAB. I. Šrámková would like to acknowledge financial support of the project of specific research, No. SVV 260 063. B. Fernández Band and C. Acebal also would like to express their gratitude to National University of the South and National Council of Scientific and Technical Research (CONICET).

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