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Using sequential injection analysis for fast determination of phosphate in coastal waters

Abstract:

A sequential injection analysis system (SIA) is described which is suited for the fast determination of filterable molybdate reactive phosphate (FRP, 0.2 µm) in coastal waters. It processes up to 270 samples per hour with a detection limit (3σ) of 0.05 µM and is used for surface mapping of phosphate in areas with steep concentration gradients like the wadden sea. The determination is based on the reaction of phosphate with acidic molybdate to phosphomolybdate which builds nonfluorescent ion pairs with rhodamine 6G. The remaining rhodamine fluorescence is detected at 550 nm with an excitation at 470 nm. Syringe pump, valve and detector were controlled by a self made python programme which was optimised for high speed SIA measurements in monitoring applications.

Introduction

Phosphorous is essential for phytoplankton growth in aquatic environments [1]. In the southern North Sea it is also the main phytoplankton growth limiting nutrient [2]. This is important because in larger seas like the North Sea phytoplankton production accounts for more than 90% of the total primary production [3]. Any change in the availability or composition of growth limiting nutrients (nitrogen (as nitrate, nitrite and ammonia), phosphorus (as phosphate) and silicon (as silicate)) has a significant influence on the composition and total amount of phytoplankton [2,4,5]. The phytoplankton dependent food web and with it the whole ecosystem is hereby influenced [6]. More direct effects are caused by increased nontoxic and toxic algal blooms [5].

Phosphate is usually determined as filterable molybdate reactive phosphate (FRP, 0.2 or 0.45 µm filter) in aquatic environments, which seems to be the most bioavailable form of phosphorous in these environments [7]. Due to the inhomogeneity and the high biological activity of the water body in coastal zones the quantification of phosphate is difficult in these areas [8]. Samples from a tight net of sampling sites have to be collected and analysed to gain reliable phosphate and phosphate distribution data. Manual approaches for sampling and analysis are expensive, time consuming and either spatially limited or wide-meshed [9]. Automated instruments ideally combined with continuous sampling are a more practical approach to gain phosphate distribution data [8]. However depth information gets lost using this kind of system, because the samples are all drawn in the same water depth. A nutrient analyser mounted on an undulating towed vehicle [10] could be used to overcome this problem. Instruments mounted on such a towfish must have the following characteristics: high sampling frequency, suitable sensitivity, reliability and low reagent consumption. In this paper the method for the phosphate part of such an analyser is described.

Most automated methods for the determination of FRP are flow methods [11] based on the segmented flow analysis (SFA) [12] or the flow injection analysis (FIA) [13,14]. Neither instruments based on the the SFA nor the FIA are suitable for the integration into a towfish due to the high reagent consumption. One possibility to reduce the reagent consumption is the reverse FIA (rFIA) which was successfully used for the determination of phosphate in seawater [14]. Another approach to reduce reagent consumption is the μFIA technique, which works with significant lower flow rates [15]. While these methods solve the problem of high reagent consumption only Lyddy-Meaney et al. [8] offers a flow system which combines low reagent consumption with high sampling frequency and suitable sensitivity. The modified rFIA system uses direct multiple reagent injections into a sample stream. The reagents are stored in pressurised bottles and are dosed by miniature solenoid valves. The use of this system on an undulating towed vehicle, which is the aimed purpose of the device described in this work, would be problematic due to fluctuations in the outer pressure leading to variations in the reagent volumes.

The sequential injection analysis (SIA) system introduced here is fast, sensitive and has a low reagent consumption. Instead of injecting reagents or sample into a sample or reagent stream, both, sample and reagents, are injected into a carrier stream. One syringe pump assembles both, sample and reagents, via a distribution valve into a holding coil, and then reverses flow direction and pumps the mixture through a reaction coil to the detector. Though the SIA is considered to be slower than the FIA [16], it can be accelerated to an at least similar performance through appropriate choice of syringe pump, valve and software. The flexibility of the SIA in combination with the software used in this system allows the automatic and regularly repeated determination of one ore more external standards as quality control. The home made software, which is based on the python programming language, was used to perform all measurements discussed in this work.

The method used for the determination of filterable reactive phosphate (FRP) is based on a method published by Wei et al. [17]. While Wei used ammonia molybdate as molybdate compound we used potassium molybdate to avoid interferences with an ammonia method running on the same system. Potassium molybdate reacts with phosphate in acidic conditions:

$H_3PO_4 + 12\ (MoO_3) \rightarrow H_3PMo_{12}O_{40}$

The resulting molybdophosphate forms ion pairs with rhodamine 6G. While rhodamine 6G has a strong fluorescence at 550 nm if excited at 470 nm the ion pairs have no significant fluorescence around this wavelength.

The system was tested on an campaign in summer 2005 of which one transact is described here in detail.

Experimental

Reagents

All reagents were prepared with degassed ultra-pure water (Millipore, Milli-Q, Ion-Free). Sigma analytical grade chemicals were used, unless otherwise stated. Rhodamine stock solution was prepared by dissolving 0.20 g of rhodamine 6G in 100 ml water. Molybdate stock solution was made by dissolving 17.3 g of potassium molybdate in 100 ml water. To prepare

reagent 1 (RS1) 200 µl rhodamine 6G stock solution was added to 90 ml water. 500 µl 5% IGEPAL (Polyoxyethylene(*)octylphenyl ether, branched) was added and the solution diluted to 100 ml. Reagent 2 (RS2) was prepared by adding 8.45 ml of 30% (v/v) hydrochloric acid to about 75 ml of water. Add 4 ml of molybdate stock solution and dilute to 100 ml. The reagents RS1 and RS2 were derived from [17]. Phosphate standard stock solution was 1 g-PO_4/l from Merck.

While the rhodamine stock solution can be used for more than six months if stored in the dark, the molybdate stock solution should be renewed every two months (mainly due to crystallization at the seal). RS1 can be used for at least two months if stored in the dark. During campaigns RS2 was prepared fresh every two days, but it can be used at least one week if it is not shaken all the time (like on a ship). All reagents were stored at room temperature.

Instrumentation

A diagram of the SIA instrument used for phosphate measurement is shown in Fig. 1. The sample stream is provided by a centrifugal pump out of 1.20 m water depth. It passes a cross-flow filter (Minikros M22M-100-01N, 0.2 µm, Mixed Cellulose Ester). The filtrate passes a T-piece into which a tubing (6.5 cm long, 0.8 mm i.d.) is inserted. This tubing is used to draw fresh samples out of the filtrate stream. The other end of the tubing is connected to the 17-port/1-channel valve (Knauer Part-No. A1492). A sequence is started by aspirating 25 µl RS2 into the holding loop (0.8 mm i.d.; 120 cm long) directly followed by 50 µl sample. 10 µl RS1 and 25 µl RS2 are added (Fig. 2). The syringe pump (CAVRO xl 3000) transports the segment through the reaction loop (0.8 mm i.d.; 60 cm long) to the detector, a Hitachi F1000 fluorescence spectrometer (exc. 470 nm, em. 550 nm) with a 2 µl flow through cell. A HP 34401A digital multimeter with RS232 interface is used as A/D converter in this setup. Syringe pump, valve and multimeter are controlled by in house software.

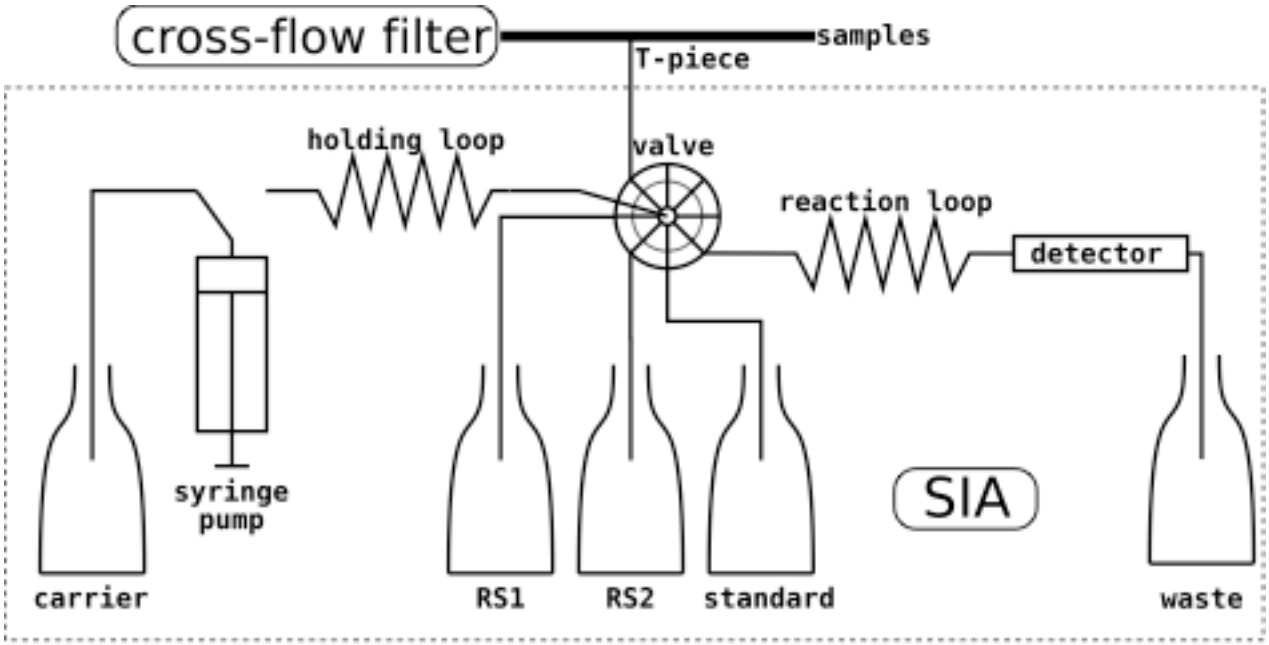

Figure 1: Schematic of the SIA used for phosphate analysis. The lab samples were taken from the sample stream leaving the cross-flow filter.

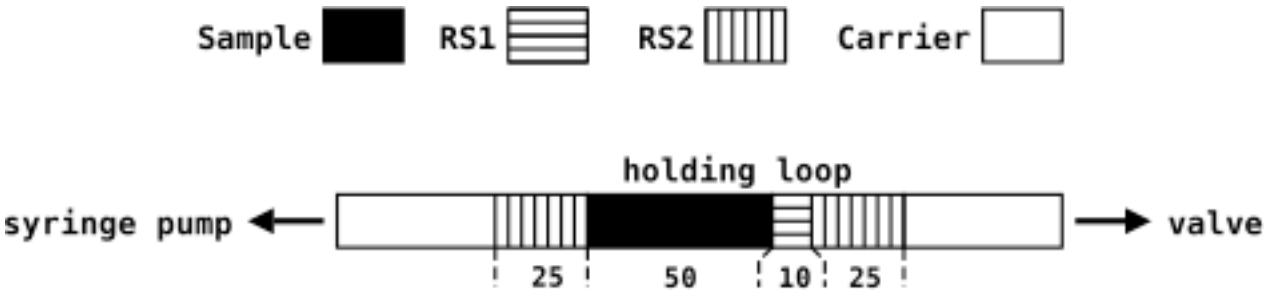

Figure 2: Sequence of reagents and sample in the holding loop (dispersion neglected). Volumes are displayed in µl.

Results and discussion

System design considerations

The sequential injection analysis system described here is designed for surface mapping applications on the North Sea, wadden sea and Elbe estuary. It has to be fast, sensitive and reliable. While the sensitivity and linear range are given by existing North Sea and wadden sea data (0.05 – 5 µM [9,18]), the speed of the system was chosen to be as fast as possible (up to 360 samples per hour were reached in some experiments) to ensure good spatial resolution even with high cruising speeds.

To achieve the required sensitivity the method described by Wei [17] was adapted to the SIA. The high speed of the SIA system introduced here was reached using a fast valve, a fast syringe pump with variable speed ramps and a home made software. This software also makes it possible integrate additional standards into the programme without interrupting the run. This standard then can determined once or on a regular base. This feature is used during campaigns to monitor the performance of the system and thus improve the reliability of the data gained with it.

While the system described here was optimized for the fast determination of FRP in coastal waters (especially the Elbe estuary and the wadden sea) it can be easily adapted for other purposes. Experiments have shown that the detection limit can be improved using more reagent and sample segments [19], other pump speeds (combined with the reversal of the flow direction) and other concentrations of the reagents. However all these measures also decrease or change the linear range and/or decrease the speed of the device.

Range switching, calibration and detection limits

Up to three different sample volumes (50, 17 and 8 µl) are used with this device depending on the expected concentration of FRP in the water body. While all three sample volumes show nearly the same limits of detection (better than 0.05 µM, calculated from the mean of the blank plus three times the standard deviation of the blank) in the laboratory, the situation is different in the field. The noise of the detector is significant higher due to vibrations and temperature differences. Therefore the 50 µl sample volume is used for phosphate concentrations up to 0.5 µM, 17 µl for the range between 0.5-1.0 µM and 8 µl for the span between 1.0 and 5.0 µM (see Fig. 3). The range switching can be done automatically or manually without interrupting the programme run. 11, 18 and 20 standards gave linear calibration graphs for the three different sample volumes ($r^2 > 0.998$) and the standard deviations were lower than 2.3 µM (3.2 µM with 8 µl sample volume; $n>10$). A second calibration is shown in Fig. 3. This calibration was performed to determine the linear ranges

for all three sample volumes.

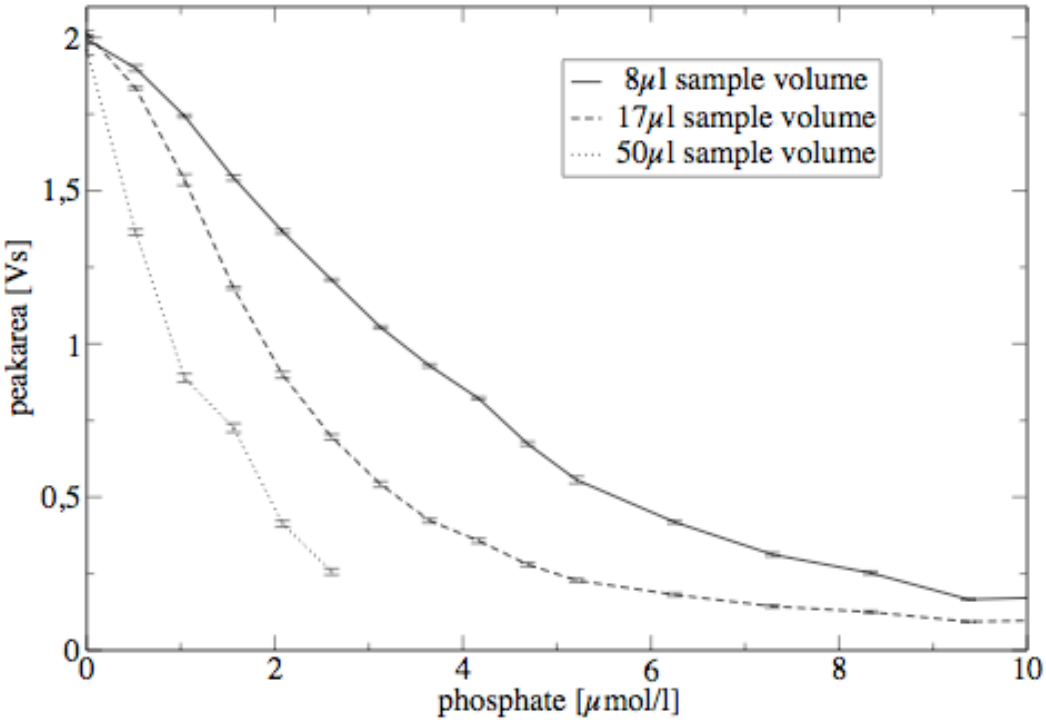

Figure 3: Calibration curves for the three different sample volumes to determine the linear range.

Effect of sample salinity

To verify the effect of the salinity of the sample two calibrations were performed for all three sample volumes using ultra-pure water and low nutrient seawater (LNSW, salinity 35). The peak areas of five standards between 0.25 and 2.0 µM were compared with each other leading to the assumption, that the salinity of the sample does have a small effect on the results of an measurement. However the correlation between the results is good (r^2>0.998, gradient 0.94 and intercept of 0.07 µM) which makes it possible to correct the data using salinity measurements. Furthermore this correction is only necessary if the salinity changes significantly and maximum precision is needed.

Application and Validation

The suitability of the SIA system for on-line rapid phosphate determination has been tested on several cruises on the research vessel Ludwig Prandtl in the North Sea, wadden sea and Elbe estuary. One transect of the last cruise from the 11th July 2005 (Husum -> Dagebuehl) is shown in Figure 4. Only one detection range was used during that trip (17 µl sample volume). The phosphate concentration decreases with increasing distance from the land, which is quite typical for the wadden sea as already known from former cruises and published data [18]. To control the noise of the detector and the quality of the data one standard and the blank were determined automatically on a regular base.

During the whole trip (27. June -15. July 2005) lab samples were taken to compare the on-line method to a standard laboratory method based on the SFA [20]. These lab samples were drawn directly from the filtrate stream leaving the cross-flow filter (see Fig. 1) and stored in

the freezer using 50 ml PE bottles. The comparison does show a correlation between both datasets (Fig. 5; r^2=0.87; gradient 0.92; intercept 0.34 µM; n=32). The differences between the on-line and the lab results are most likely caused by the long sample storage of more than three months. This theory is backed by an earlier experiment with frozen samples which were split and determined from the same lab and with the system described in this paper (Fig. 6).

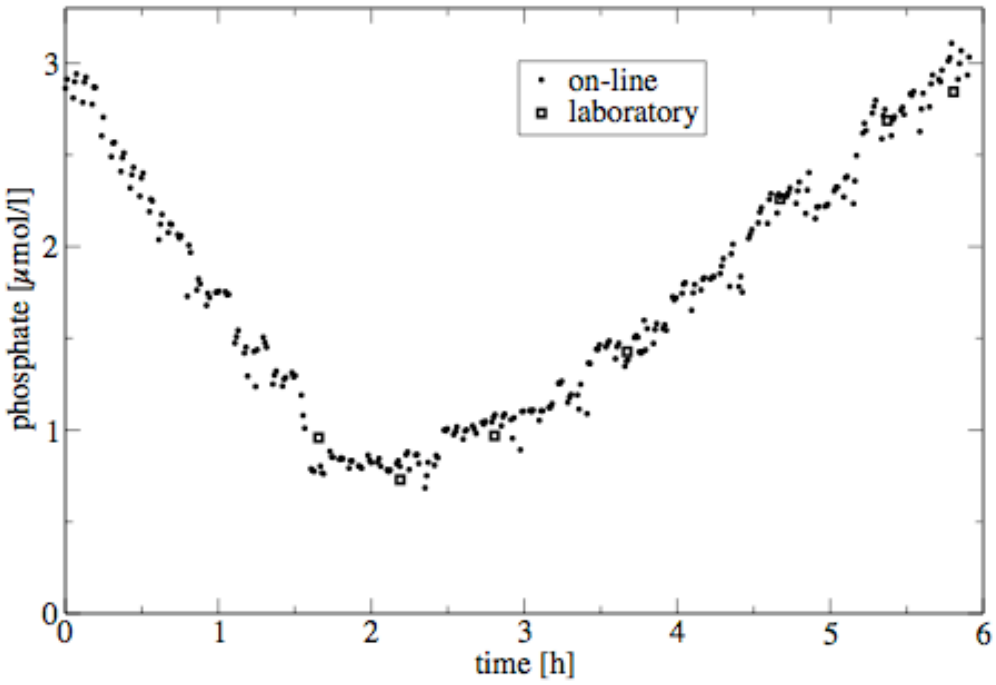

Figure 4: Transect from Husum to Dabebuehl 11. July 2005. Rectangles indicate the lab samples.

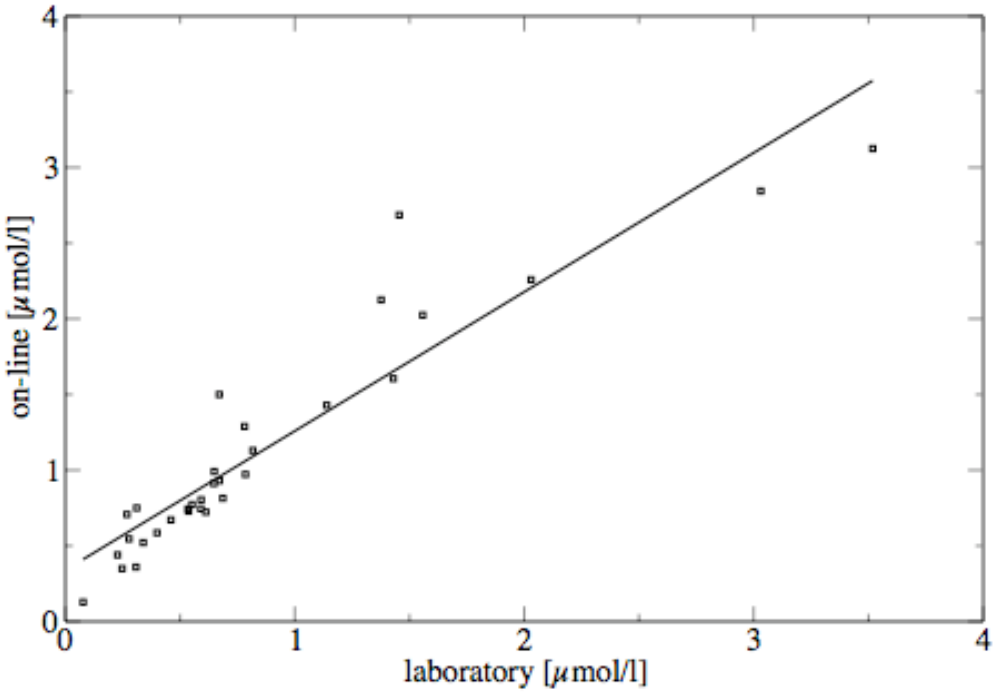

Figure 5: Correlation of on-line data with lab results.

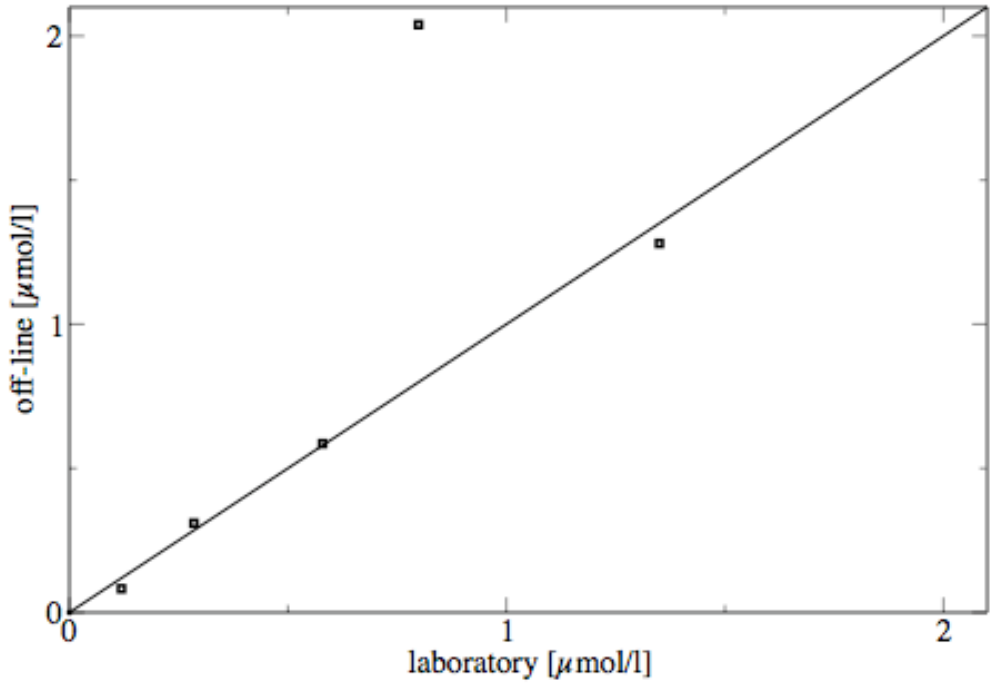

Figure 6: Off-line data correlated with lab results. One sample was contaminated.

While the SIA system described in this paper has proved to be suitable for the fast determination of filterable molybdate reactive phosphate in coastal waters it is not very portable. Recent experiments have shown, that the CAVRO syringe pump can be replaced by a small pump (Lee LPVX-series) without excessive loss in speed and precision. By also replacing the Hitachi detector with a much smaller fluorescencedetector of IPHT (Jena, Germany) the system will be portable.

Conclusions

The sequential injection analysis (SIA) is well suited for the fast determination of filterable molybdate reactive phosphate in coastal waters. It is especially suited for difficult environments with steep concentration gradients and varying salinity which are conditions found in the wadden sea and in estuaries. The flexibility of the SIA is especially advantageous for the (automatic) adaption of the system to different conditions like different concentration ranges and an improved quality control using additional standards. While other flow systems like the SFA or FIA are often difficult to reactivate after a long downtime, no such problems were observed with the SIA.

With these satisfying results work is going on to proceed with the towfish integration of the device introduced here. Future work will include the replacement of the syringe pump and the detector with significant smaller parts. After testing, that system will be integrated into the towfish.

The high speed, low reagent consumption, sensitivity and the general flexibility of this SIA instrument makes it also suitable for other applications like unattended continuous monitoring or the integration into an automated water measurement system (ferrybox [21,22]).

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