Design of autonomous in-situ spectrophotometric systems for measurement of nutrients and CO₂-system parameters

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ABSTRACT

A compact spectrophotometric analysis system (SEAS) has been developed for autonomous analyses of the upper ocean. SEAS is capable of spectral analysis from 400 to 750nm in both absorbance and fluorescence modes. The sample cell is configured to use long pathlength liquid core waveguides (10-500cm) whereby analyte detection limits are on the order of one nanomolar or better. Internal memory of SEAS is sufficient to store 1000 complete measurements. A serial port or LonWorks interface can be used to access stored data and/or control the instrument’s operation. Field deployments have measured in-situ nitrite profiles to 200 meters and surface seawater pH on a long-term mooring. SEAS is currently being configured for measurements of ferric and ferrous iron in rainwater. Demonstrated measurement capabilities using our liquid core waveguide systems in absorbance and fluorescence modes include ammonia, nitrate, nitrite, iron, copper, chromate, molybdate, hydrogen sulfide, pH, pCO₂, total inorganic carbon, total alkalinity and fluorescence of natural organics. In this work we present an overview of SEAS, discuss applications for our sensor and present calibration and field data.

Keywords: colorimetry, spectrophotometry, nutrient, chemical concentration, fluorescence, in-situ

1. INTRODUCTION

Variation of oceanic parameters on vast spatial scales, and temporal scales encompassing hours and decades, necessitates robust autonomous monitoring capabilities. Fluorometric and colorimetric analyses are well suited to in-situ analysis. The simplicity, sensitivity and specificity of colorimetric and fluorometric procedures has led to their widespread use in analysis of natural waters. Biogeochemically important measurements commonly obtained by fluorimetry and colorimetry include phosphate, nitrate, nitrite, ammonia, silicate, iron, pCO₂, pH and CDOM \(^1\). In support of global autonomous monitoring capabilities, the Center for Ocean Technology (within the USF College of Marine Science) has been developing spectral elemental analysis systems (SEAS) for autonomous analysis of the upper ocean. SEAS has been designed for operation in a number of deployment modes including: moorings, autonomous underwater vehicles, and diver-directed operations. Since key oceanic nutrients generally occur in the upper ocean at very low concentrations, sensitivity issues are very important. The spectrophotometric system used in SEAS is capable of analysis over a range of concentrations between one nanomolar and several hundred micromolar.

2. SYSTEM DESIGN

In its current configuration, each SEAS instrument is housed in an anodized aluminum pressure vessel that is capable of withstanding pressures of at least 500 bar. The instrument shown in Fig. 1 is 10 cm in diameter with a length of 50 cm. The system’s maximum power consumption is 6 watts at 8-24 Volts DC. System weight is approximately 3.5 kg. The rate of data communication, through an RS-232 port, is on the order or 58 kb per second. SEAS can be operated autonomously or under user control. In its “sleep” mode the SEAS power consumption is less than 1.0 milliwatt. An external battery pack, with physical dimensions comparable to seas, allows operation over a period of several months with an acquisition rate of six samples per day.
Figure 2 shows the SEAS instrumental layout. The SEAS electronics, spectrometer and lamp are enclosed within the pressure housing while the sample and reagent pumps, as well as the optical cell (LCW) are exposed to ambient seawater. A redesigned layout (SEAS-III) will incorporate multiple reagent pumps and reservoirs, allowing reagents and samples to be combined in a multi-step process.
Overall control of SEAS electrical and mechanical operations is directed by a Motorola HC16 microcontroller. SEAS has a four megabyte data storage capacity (upgradable to sixteen megabytes). Data are stored in an array of 16-bit elements. Complete scans (400 – 750 nm) then require approximately 4 kilobytes of memory. At the user’s discretion, however, data can be stored for only a specific number of wavelengths or on-board data processing can be used to provide final chemical concentrations for storage and transmittal.

The spectrometer used in SEAS is an Ocean Optics S2000 CCD array spectrometer. Analog signals are converted to 12-bit digital information using an Analog Devices converter (250 kHz conversion rate). External communication is effected using an RS-232 connection or a LONWorks interface. LONWorks consists of a two-wire connection, a unique identification code processor and a transceiver module. LONWorks provides distributed control and communication of multiple networked sensors without a master control. As such, the data acquisition, processing, storage and transmittal of multiple sensors is coordinated under user control or a user-defined protocol.

The SEAS optical cell is a liquid core waveguide (LCW) constructed of Teflon AF-2400. Light from one or more source lamps is inserted into the LCW through a small coupling device that also allows introduction of fluids (seawater or seawater mixed with reagents). Light is collected at the distal end of the waveguide via optical fibers located within a second coupling device, and is transmitted to the CCD array spectrometer. Teflon AF-2400 has a lower index of refraction than seawater \((n/\text{Teflon AF 2400}) \approx 1.29, (n/\text{seawater}) \approx 1.34\), whereby light intersecting the waveguide walls at angles less than 15 degrees is confined within the liquid core by internal reflection. The LCW in current versions of SEAS has an 800 µm inner diameter and an internal volume of 0.5 cm³ per meter of optical path. Thus, even the use of a two meter pathlength creates a very low reagent consumption rate.

The LCW configuration in SEAS is typically an eight cm diameter coil with pathlengths between 30 and 200 cm. Longer pathlengths can be used to further increase measurement sensitivity. Calibrations of analyte/reagent absorbance versus concentration shows, in all cases, excellent conformity to Beers law

\[
\lambda A_i = \epsilon_i [i] \cdot l, \tag{1}
\]

where \(\lambda A_i\) is the absorbance of analyte \(i\) at wavelength \(\lambda\), \(\epsilon_i\) is the molar absorbance of the analyte/reagent moiety, \([i]\) is analyte concentration and \(l\) is physical pathlength. Molar absorbances measured with Teflon AF-2400 LCWs are essentially identical to the molar absorbances of chemical species measured with 1 cm optical paths 3,5,6.

SEAS spectrophotometric measurements typically begin with dark spectral scans while the light source is turned off. The light source is then turned on and the sample pump is activated. After complete flushing of the LCW, the lamp intensity is adjusted and a reference scan is recorded. The reagent pump is then activated and the combined sample/reagent mixture is pumped through the waveguide. Sample spectra are then recorded and the analyte concentration is calculated from the absorbance characteristics of the analyte \((\lambda \epsilon_i)\) and the physical pathlength of the LCW.

Although SEAS was primarily designed as an absorbance based measurement system, it is readily adaptable for use in fluorescence measurements. Fig. 3 shows SEAS in the configuration used for fluorescence measurements. During fluorescence measurements the visible lamp is turned off and an UV excitation lamp is turned on. The LCW is wound in a helix around the UV lamp and the fluorescence stimulated within the LCW is transmitted to the CCD spectrometer in the normal manner. Using such a system, SEAS can be used to make both fluorescence and long pathlength absorbance measurements of colored dissolved organic matter. The reagent reservoir in this case would contain a CDOM-free reference seawater.
3. DEPLOYMENTS AND FUTURE APPLICATIONS

SEAS has been deployed previously on moorings and AUVs, in diver-directed operations, and in vertical profiling operations. In-situ analyses to date have included pH, iron and nitrite. Vertical profiles have been obtained only in case of nitrite. Fig. 4 shows a typical NO$_2^-$ profile obtained via the R/V Longhorn in the Gulf of Mexico (26° N, 94° W) in August 2000. The diurnally variable profiles obtained at this station typically exhibited double maxima, or a single maximum with a prominent shoulder. A capability for real time observation of nitrite profiling results is anticipated during 2001, and protocols for rapid in-situ nitrate measurements are currently being developed. A capability for ammonia measurements at nanomolar concentration levels is also expected in 2001. Ammonia measurements will be obtained using SEAS in fluorescence mode.

Fig 3. SEAS can easily be configured to measure both absorbance and fluorescence by placing an ultraviolet source in the center of the LCW coil.

Fig 4. Nitrite profile measured with SEAS in the Gulf of Mexico.
In addition to the use of SEAS for observations of macronutrients, SEAS is currently being developed for measurements of iron in rainwater. This project with Woods Hole Oceanographic Institution will use the rain collector being developed by Sholkovitz and associates (http://www.whoi.edu/science/MCG/dept/). The WHOI collector has been designed to activate (open) during rain events, and will include a washdown capability between rain events. Collected water will be analyzed for both ferric and ferrous iron. Future moored deployments for measurements of iron in rainwater will include the ECOHAB study site off the West Coast of Florida. The performance of SEAS during a series of ferric iron calibrations is shown in Fig 5.

![Graph showing calibration of SEAS for ferric iron measurements.](image)

Fig. 5. Calibration of SEAS for ferric iron measurements.

A number of SEAS deployments are planned in conjunction with the Bottom Stationed Ocean Profiler program at USF. BSOP devices are autonomously profiling floats with an endurance in coastal areas on the order of 150 profiles. These devices have been designed to carry SEAS and other instruments, and telemeter chemical and physical data after each cycle. A BSOP diagram is shown in Fig. 6. SEAS measurements during initial BSOP deployments will include pCO₂ and nutrients.

![Diagram of a Bottom-stationed ocean profiler (BSOP) outfitted with SEAS.](image)

Fig. 6. A Bottom-stationed ocean profiler (BSOP) outfitted with SEAS.
SEAS is currently being configured and tested for measurements of pH, pCO₂, total inorganic carbon
($C_T$) and total alkalinity ($A_T$). Seawater pH measurements are made using calibrated sulfonephthelein
indicators. Equilibrium quotients of the form

$$I^- + H^+ \xrightleftharpoons{K_i} HI^-$$

have been obtained for indicators suitable to the pH range of seawater. Solution pH is precisely measured
via observations of absorbance ratios ($R$):

$$pH = -\log K_i + \log \left( \frac{R - e_1}{e_2 - Re_3} \right),$$

where $K_i$ is an equilibrium quotient, $e_i$ are molar absorbance ratios and $R$ is equal to a ratio of indicator
absorbances at the absorbance maxima of $I^2$ and $HI^-$. Spectra of a sulfonephthalein indicator, meta cresol
purple, are shown over a range of seawater pH in Fig. 7.

![Fig. 7. Spectra of the sulfonephthalein indicator, meta cresol purple, are shown over a range of seawater pH.](image)

Seawater pH measurements can be made with sulfonephthaleins in seawater at concentrations on the
order of 1 micromolar or less. Measurements of pCO₂ can be made using LCWs that contain
sulfonephthaleins in solutions of known alkalinity that are equilibrated with ambient seawater. Teflon AF-
2400 is permeable to CO₂. Accordingly, when an inner solution of known alkalinity is equilibrated with
seawater, the pCO₂ of the inner solution (and the external seawater) can be calculated from $A_T$ and pH.
The total carbon content of seawater can be calculated in a similar manner. Fig. 8 shows a section of
Teflon AF-2400 waveguide within a section of somewhat larger diameter impermeable tubing. In this case,
for measurements of $C_T$ (Fig. 8a), the core of the LCW contains a solution of known alkalinity and a
sulfonephthalein indicator. The outer solution contains seawater that has been acidified to about pH 2.5.
Equilibration of the inner and outer solutions then allows calculation of the pCO₂ of the inner solution from
At(inner) and pH(inner). Since pCO₂(inner) equals pCO₂(outer) at equilibrium, and Cₜ equals [CO₂*] at pH 2.5, Cₜ is calculated from pCO₂ using the Henry’s law constant for the outer solution.

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pCO₂(A_T, pH) \cdot K_H(\text{outer}) = [CO₂^\text{eq}](\text{outer}) = C_T(\text{outer})
\]  

(4)

**Fig. 8.** LCW configurations for measurement of Cₜ (a) and Aₜ (b).

The total alkalinity of a seawater sample can be measured in a similar manner (Fig. 8b). In this case the inner tubing contains ambient seawater and a sulfonephthalein indicator. The outer solution contains a solution whose total carbon content is accurately known. Acidification of the outer solution produces a known pCO₂ at which the inner and outer solutions equilibrate. A measurement of pH within the LCW in conjunction with the known pCO₂ of the system allows calculation of the alkalinity of the inner solution.

4. PROSPECTS AND CONCLUSION

SEAS is a versatile system for measurement of a wide variety of nutrients, trace metals and CO₂ system parameters. Improvements envisioned for SEAS in the near future principally involve the reagent delivery system. A capacity for delivery of multiple reagents will allow a single SEAS instrument to perform a variety of types of analyses. The multiple reagent delivery capabilities of SEAS will eventually include washdown capabilities to suppress biofouling.

5. ACKNOWLEDGMENTS

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6. REFERENCES


