

PHOSPHATE BASED PROOF OF CONCEPT FOR A MICROFLUIDIC
MULTI-NUTRIENT IN-SITU AUTO-ANALYZER

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I AT MĀNOA IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE
in
OCEANOGRAPHY

DECEMBER 2020

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Keywords: Phosphate, In-Situ, Auto-Analyzer, Sequential Injection

Abstract

Progress and discovery in the understanding and modeling of biogeochemical processes in natural waters has been limited by our ability to make measurements at relevant spatial and temporal scales. Innovations in microfluidics have led to the miniaturization of traditional reagent-based assays without loss in sensitivity. Micro Sequential Injection Analysis (μ SIA) represents the second generation of flow injection analysis (FIA) techniques and solved many of the problems inherent to Continuous Flow Analysis (CFA) and first generation of FIA. The μ SIA system comprises (one or two) high precision pump (s), holding coil(s), a multi-channel Lab-on-Valve (LoV) module with an external flow cell, a light source and a spectrophotometer. All components are fully computer controlled, and by using these high precision pumps and the capability of the discontinuous bi-directional flow one can obtain reproducible results. The high precision pumps not only allow for control of the mixing between small volumes of sample and reagents for each analysis but also a low standard deviation of the determinations, which then leads to nanomolar level of the detection limits (Hatta et al., 2018; Hatta et al., 2019; Ruzicka et al., 2019). The greatest advantage of the μ SIA platform comes from its ability to perform any spectrophotometric, fluorometric, or chemiluminescence assay without any major reconstruction of the system simply by using a programmed sequence for each method using appropriate reagents. All of these advantages make it an ideal platform for in-situ autonomous applications. To this effect the objective of this work is to design and deploy an autonomous field deployable μ SIA system using phosphate as a proof of concept

to demonstrate the viability of this equipment for further development introducing multiple assays on the same system.

Achieving the objective of this work required development and adaptation of the chemical analytical methodology and the development of an electrical control system and a housing system to deploy it. Through experimentation it was found that traditional phosphate methodology required optimization for use in an autonomous system. Thus, presented here are two viable versions of a phosphate based single-pump μ SIA chemical assay adapted from recent publications in programmable flow injection methods (Hatta et al., 2019; Ruzicka et al., 2019). The first assay uses sulfuric acid (H_2SO_4) as a source of protons to the reaction while the second utilizes hydrochloric acid (HCl), and both incorporate the surfactant sodium dodecyl sulfate. The H_2SO_4 based assay requires a 600sec reaction time, achieves a limit of detection of 45nM P, and is unaffected by changes in salinity. The HCl based method has a reaction time of 50sec or 100sec in fresh and sea water respectively and a limit of detection of 14nM PO_4 . Due to the difference in reaction times between fresh and sea water the HCl method is subject to a salinity matrix effect. The matrix effect observed in the HCl based method may be overcome by comparing the slopes of the reaction curve as opposed to the absorbance, though more study is needed to verify this. In order to build the new autonomous platform a control scheme was developed to facilitate communication and distribute power to the different components using a Raspberry Pi single board computer. A new protocol sequence was developed using the python programming language to perform the assay and clean the μ SIA manifold. Two housing systems were developed for use in dockside and submerged environments. Using the dockside housing numerous laboratory-based experiments were conducted to optimize the

chemistry of the phosphate assay for continuous determination. Two deployments (dockside and reef) and submergence tests were conducted to evaluate the capability of the autonomous platform. The successful dockside deployment was conducted in the Hawaii Kai marine embayment located on the south-east coast of the island Oahu, Hawaii USA, using the H_2SO_4 method over the course of 3 days. Unfortunately, the reef deployment was unsuccessful and resulted in loss of equipment. Despite the loss of equipment, this work achieves its objectives in providing a proof of concept for a microfluidic multi-nutrient in-situ auto-analyzer.