Portable Fluorescent Sensing Array for Monitoring Heavy Metals in Water

Simon Maher*, B. Bastani, B. Smith, F. Jjunju, S.Taylor
Department of Electrical Engineering & Electronics,
University of Liverpool,
Liverpool, UK
*e-mail: s.maher@liv.ac.uk

Iain S. Young+
Institute of Integrative Biology,
University of Liverpool,
Liverpool, UK
+e-mail: isyoung@liv.ac.uk

***Abstract*—The availability of clean water is of fundamental importance to human health and survival, as a habitat for aquatic species, for farming and industrial use. Water quality is likely the most important factor in maintaining the health in both farmed and wild fish. Understanding water quality is critically important in facilities which reuse a large percentage of system water. Heavy metals can have a major impact on aquatic health and have a tendency to accumulate in the food chain. In this study we describe a novel fluorescence sensor for the detection of heavy metal ions in water. Fluorophores immobilized in a polymer membrane were excited using an array of LEDs and the fluorescent emission measured via a CCD camera. This novel portable device is comprised of a 3x3 array of sensing elements across which the water sample is pumped and the fluorescence image generated is used to determine the metal ion content. Herein we demonstrate this approach for the detection of Cu (II) ions in water.**

Keywords—fluorescence; fluorophores; heavy metals; water quality; aquaculture; fluorescence imaging; portable optical sensor

I. INTRODUCTION

Heavy metals play an important role in many industries and applications but their increasing prevalence in modern society is a cause for concern regarding their environmental impact and associated health implications not least because they readily enter water supplies. Metals may enter aquatic systems by natural geochemical action or via anthropogenic means including mining and other industrial processes [1]. These water borne pollutants can have serious environmental impacts; they are directly toxic to most fish species at all stages of the life cycle [2]. Recirculating aquaculture systems (RAS) reuse a large percentage of the system water (~ > 99%), while reducing the impact of water extraction and discharge, this can inadvertently concentrate toxins and intensify exposure of sensitive hatchery stock to pollutants [3]. Water supplies to salmon smolt hatcheries can be contaminated with natural iron and aluminum pollutants. In RAS, the accumulation of such heavy metals may be intensified by metals released from feed and metabolic waste breakdown products. Similarly for the capture fishery, heavy metals may accumulate in the food chain and contaminate seafood products [4]. Many heavy metals such as zinc, copper and nickel are essential for health in small amounts but can be very toxic in excess. Accumulation of these metals can result in high concentrations that affect human health, causing serious damage to the central nervous system, liver, kidneys and bones [5]. Therefore, there is a demand to be able to rapidly and sensitively detect trace amounts of such elements in aquatic environments.

Commercial fish production in the EU requires high standards of water quality monitoring in turn, necessitating regular and effective measurement of multiple parameters. These typically include pH, salinity, temperature, and concentrations of dissolved gases, phosphate and nitrogenous compounds. However, while fish health, production efficiency and product quality can be detrimentally affected by accumulation of toxins, heavy metals are often measured less frequently than other parameters, largely because their determination is generally made by labour-intensive wet chemistry techniques. Therefore, there exists a need for advanced technologies and sensors capable of simple, fast, accurate on-line measurement of hazardous substances in the aquatic environment.

This paper describes the development of a fluorescence optical sensor that comprises an array of sensing elements functionalized with suitable photochemical receptors (fluorophores), whereby the binding of the target analyte with the fluorophore modifies the fluorescent light emissions. This is detected by a CCD and represents a measurable concentration of the analyte. As the analytical signal is provided by the fluorescence signal upon the chemical binding between the analyte and the fluorophore the sensor element is chemically functionalized by the fluorophores prior to application.

II. FLUORESCENCE INDICATOR

Fluorescent indicators selectively bind to target analyte(s) and, when excited, produce a measurable difference in the emission spectrum. We considered a number of factors when identifying suitable fluorescent indicators for this device. These factors included: 1) producing a sufficiently large change in the fluorescence signal, 2) high photostability, 3) suitable excitation and emission maxima to allow use of LEDs as stable light sources, 4) large Stokes' shift, 5) ease of immobilization, and 6) good water solubility. In accordance with these criteria, Phen Green SK (Life Technologies Ltd.) was selected as an appropriate indicator for construction of the prototype sensor.

III. CHARACTERIZATION *IN-AQUA*

The behavior of the fluorophore in response to excitation by light and exposure to ionic solutions was investigated. Initially, Phen Green was selected as a quencher of fluorescence when exposed to copper (II) ions. Response of the fluorophore to the

various concentrations of copper ions was characterized using a Hitachi F-2700 fluorescence spectrophotometer. Three concentrations of the fluorophore Phen Green (1 µM, 5 µM, 10 µM) were tested with a range of concentrations of copper (II) ions spanning from 1 nM to 20 µM. For example, figure 2 shows the linear response of 5 µM dye concentration over a range of 100 nM to 1000 nM additions of copper chloride solution.

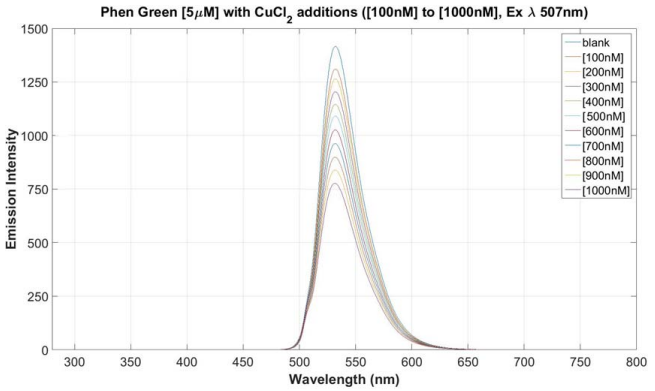

Fig. 1 Phen Green (5 µM) response to a range of 100 nM to 1000 nM of copper (II) ions

Figure 1 shows that there is no shift in peak emission wavelength but there is a clear reduction in intensity with increasing copper concentration (quenching). This indicates the suitability of the fluorophore Phen Green for detection of trace concentrations of copper ions in water. However, in order to create a robust sensor the indicator must be incorporated into a sensing membrane. This will facilitate portability, field operation and simplicity of operation. Therefore, a non-liquid sensing medium (i.e., a thin membrane) was functionalized with the target fluorophore via a suitable immobilization technique (section IV).

IV. Immobilization

Organic and inorganic polymers can be utilized in chemical and biological sensors providing a rigid support matrix for immobilization of active chemical reagents and allowing selective interaction between the target analyte and the reagent. The sensor's response time and sensitivity will be dependent on the diffusion characteristics of analyte in the polymer. In liquid phase, ionic species diffuse through hydrophilic polymers while hydrophobic polymers permeate gases.

We can consider two broad categories of immobilization: physical and chemical. The former includes adsorption, entrapment and ion exchange (electrostatic) procedures, the latter involves covalent bonding between the reagent and the polymeric support (host) matrix. A number of polymers were investigated as host matrices. For construction of the sensor, we considered that the most important characteristic was their hydrophilicity, allowing the immobilization of water soluble fluorophores. This enables the target ionic species to readily diffuse into the polymer matrix and interact with the entrapped fluorophores thus generating the characteristic fluorescence signal when excited by an appropriate light source.

Initially standard 96-well plates (Costar 3603, Corning Inc.) with flat clear well bottoms were used to optimize the fluorescence response from various membrane formations (i.e., to find the optimum ratio of fluorophores and polymers). Different volumes of fluorophore embedded polymers were used to form thin functional layers of membranes in the wells. Ideally very thin membranes were preferred to ensure fast diffusion of the target metal ions into the membrane leading to the interaction of ions with the dye and resulting in fluorescence upon illumination.

Three different hydrophilic polymers (agarose, sol gels, polyurethane D4) with good mechanical stability to form functionalized membranes for the sensing were selected for experiments with the fluorophore. Various molar concentrations (0.2, 0.33, 0.5, 0.83, 1.25, 2.5, 5.0 µM) of the fluorophore in the polymers were prepared and tested. The synthesis and fluorophore immobilization processes for sol-gels proved to be challenging. Forming stable and uniform membranes from the functional sol-gels inside the wells of the microtiter plates, used for characterization of the fluorescence indicator, was particularly difficult. During the drying process, solvent evaporation leaves a thicker/denser layer around the periphery of the well. Furthermore, as there is poor adhesion to the plastic surface of the well the sol-gel layer tends to crack producing a non-uniform sensing membrane resulting in an inconsistent fluorescence signal. Despite forming a more uniform membrane, polyurethane polymer also yielded inconsistent signals due to the aggregation of fluorophores within the polymer and a non-homogenous mixture of the dye and polymer. Agarose however, formed suitably thin membranes with a homogeneous distribution of fluorophores.

V. Integrated Sensor with Functional Membranes

A prototype 3x3 well array sensor configuration was designed and constructed in order to make a portable fluorescence metal ion sensor suitable for field operation (Fig. 2). The following design criteria were specified:

- Compact size to render portable
- Low cost light source for illumination
- Fluidic provisions to apply the sample and allow future automation
- Detection of fluorescence by imaging to simplify the sensor hardware and improve sensing flexibility.

The sensor array flow cell assembly was fabricated using a 3D printer in polylactic acid. The overall size of the sensor array was approximately 60x60 mm. The sample reservoirs (wells) had a depth of 7 mm and a diameter of 5 mm (volume ~550 µL). Illumination was provided by an array of nine blue LEDs mounted below the flow cell. Sensing spots of the equal diameter were coated with a uniform thin membrane of thickness 1 µm on a thin (188 µm) transparent polymer sheet (zeonor polymer, MicroFluidic Chipshop GmbH, Germany). A compact peristaltic pump (200 Series with DC powered motor, Williamson Pumps Ltd, UK) was used to pump the samples into the sensor array. Images of the array were captured using a sensitive CCD camera (DCU223M, Thorlabs) with a 3.5 mm focal length lens and 130º field of view (MVL4WA, Thorlabs). An Arduino Uno microcontroller was used to provide power and control for the LEDs and pump. Silicone tubing of 3.0 mm inner diameter (Williamson Pumps Ltd, UK) was used for fluidic

connections between the pump, the sample reservoir, the sensor array and the waste reservoir.

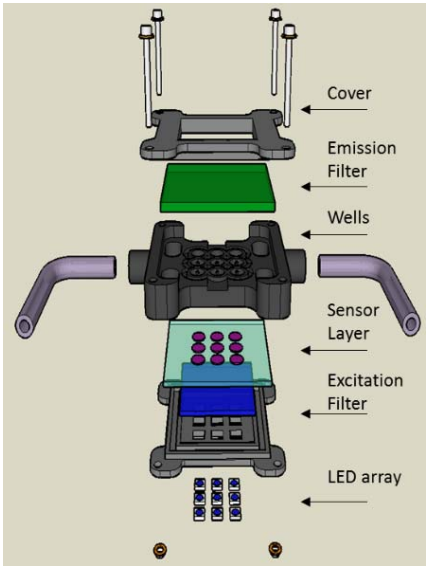

Fig. 2 CAD design of prototype 3x3 well sensor array (exploded view)

The fluidics were designed to allow filling of the sensor wells sequentially via the inter-well through holes located between the inlet and outlet ports. The assembled sensor array, associated pump electronics and CCD camera are shown in figure 3.

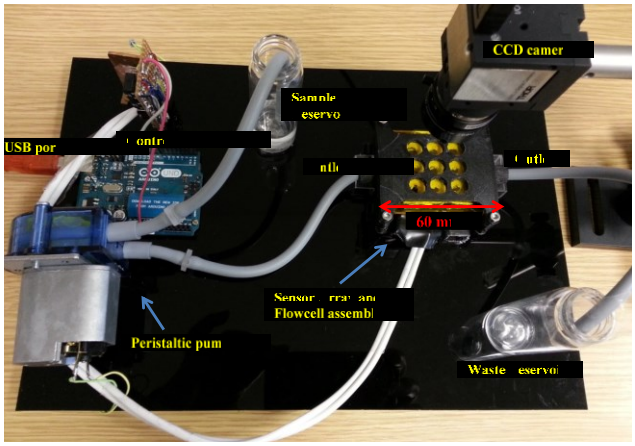

Fig. 3 Prototype fluorescence imaging sensor array

A typical fluorescence response to three water samples with Cu (II) concentrations of 1, 5 and 10 µM (each applied in a separate row of the sensor matrix) was obtained (figure 4). The sensor membranes were fabricated by immobilizing 5 µM Phen Green throughout the entire array as shown in figure 4. For this case the sensor was modified to allow three samples to pass through the array in parallel. This was carried out to illustrate the average fluorescent intensity changes received for three distinct concentration of Cu (II) in water. The sensor has a detection limit in the low nM range. The response time was ~2 seconds and this was believed to be due to the very low thickness of the sensing membranes allowing fast diffusion of the analyte into the membrane, to form the fluorescent complex with the immobilized fluorophore.

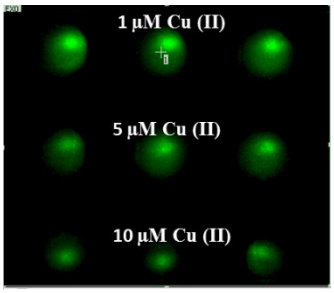

Fig. 4 A typical fluorescence response image from the sensor array illustrating the response for three water samples spiked with Cu (II) of 1, 5 and 10 µM concentrations (top, middle and bottom respectively)

VI. CONCLUSIONS

The fluorescence response of fluorophore Phen Green and its response to various concentrations of copper (II) ions was determined. A range of polymers with various concentrations and molar ratios were investigated for immobilization of the fluorophore within a polymer matrix. This allowed the fabrication of functionalized sensing membranes which can be arranged in an array format for detection of various concentrations of the specified metal ions in water. Three polymers were investigated (polyurethane, sol gel and agarose). Agarose hydrogel was found to be a suitable polymer for embedding of fluorophores and forming membranes. Calibration plots show good linearity response of Phen Green to copper ions. Integrating different fluorophore concentrations in to the array effectively increases the sensing concentration range by several orders of magnitude. The integrated 9-element functionalised sensor array device was designed to allow water to continuously flow across the sensor platform. This produced fluorescence images which can give a rapid, holistic and single shot indication of heavy metal content in water.

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