Ion-Selective Analysis of Water Quality in the contexts of Plant Production, Biological Life Support Systems and Space Exploration

by

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ABSTRACT

ION-SELECTIVE ANALYSIS OF WATER QUALITY IN THE CONTEXTS OF PLANT PRODUCTION, BIOLOGICAL LIFE SUPPORT SYSTEMS AND SPACE EXPLORATION

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The present work explores the use of ion-selective sensors for nutrient sensing in waters relevant to plant production, biological life support systems (BLSS) and space exploration. Ion-selective detection capabilities are essential in managing environments and life support systems for humans on long-duration space missions. HPLC remains the most effective method for ion-selective analysis of water samples however the technology is large, expensive, relatively difficult to operate and requires consumable materials, making it unfavourable for use in space. Single ion-selective sensors are commercially available such as ion-selective electrodes or ion-selective field effect transistors and prototype technologies are emerging such as ion-selective optrodes. Ion-selective optrodes were selected to develop an online ion-detection system for $K^+$, $Ca^{2+}$, $Na^+$ and $NO_3^-$, using optrode sensors obtained from collaborators at the Canadian Space Agency and the National Optics Institute. They were chosen based on their small mass, compact design, simple operation, and insensitivity to electric-field interference. During characterization trials, several issues were identified using optrodes in nutrient solutions: material selection of system components is critical as contamination influences optical properties of the sensors; fabrication practices are critical and optrodes mechanically coated in a clean environment perform best; biofouling quickly impairs optrode performance; and light exposure degrades optrode dyes. Based on these results a prototype Ion-Selective Optrode System (ISOS) was developed. The ISOS was tested in the context of robotic-science exploration of Saturn’s moon Titan on the remote lake Laguna Negra, Chile. The ISOS successfully detected $Ca^{2+}$ and $Na^+$ ions in surface water samples. Light-plant-nutrient interactions were also studied and the dietary habits of Lactuca sativa cv. New Red Fire were monitored in response to blue (440 nm) and red (660 nm) light environments. Results indicate light quality influences the uptake of certain nutrients. Plants under blue light consumed more nitrogen. The uptake of certain metal ions ($Zn$ and $Fe$) also appear favoured by blue or red light treatments respectively. These results have significant implications for recirculating nutrient systems inherent in BLSS.
Dedicated to my parents,
the continuous love and support of whom,
give my words meaning.
This project could not have been possible without, more than a little, help from my friends:

Firstly I thank Dr. Mike Dixon and the exceptional team at CESRF for patiently supporting my work and facilitating my success throughout all makes of trial and tribulation. Thank you Dr. Gord Hayward for your critical guidance and support in my discovery of the sensational science of sensing (mild pun intended). Thank you Jamie Simpson for trying still to give good advice to those who never listen. Thank you Dr. Tom Graham for convincing me that getting a PhD is a great idea! (...then subsequently leaving the lab/country). Thank you Dr. Matt Bamsey for laying a firm foundation, engineered with diligent precision, for me to build upon. Thank you Dr. Pablo Sobron for the most unexpected, exciting trip of my life (so far). Merci, Dr. Serge Caron for sharing your wisdom and helping to clarify my confusion. Thank you Dr. John Phillips for your expert design advice and for always making me look good. Thank you Sarah, for your love, patience, patience, patience and patience.

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<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>ABS</td>
<td>acrylonitrile butadiene styrene</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BLSS</td>
<td>Biological Life Support Systems</td>
</tr>
<tr>
<td>BLT</td>
<td>blue light treatment</td>
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<tr>
<td>CAD</td>
<td>computer assisted drafting</td>
</tr>
<tr>
<td>CCD</td>
<td>charge-coupled device</td>
</tr>
<tr>
<td>CEA</td>
<td>controlled environment agriculture</td>
</tr>
<tr>
<td>CESRF</td>
<td>Controlled Environment Systems Research Facility</td>
</tr>
<tr>
<td>CSA</td>
<td>Canadian Space Agency</td>
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<tr>
<td>DAQ</td>
<td>data acquisition</td>
</tr>
<tr>
<td>DHL</td>
<td>Digital Haptic Lab</td>
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<tr>
<td>E.U.</td>
<td>European Union</td>
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<tr>
<td>EC</td>
<td>electrical conductivity</td>
</tr>
<tr>
<td>FCNMS</td>
<td>Feedback-Controlled Nutrient Management System</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>INO</td>
<td>l’Institut National d’Optique/Nation Optics Institute of Canada</td>
</tr>
<tr>
<td>IS</td>
<td>ion selective</td>
</tr>
<tr>
<td>ISE</td>
<td>ion selective electrode</td>
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<tr>
<td>ISFET</td>
<td>ion selective field effect transistor</td>
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<tr>
<td>ISOS</td>
<td>Ion Selective Optrode System</td>
</tr>
<tr>
<td>LED</td>
<td>light emitting diode</td>
</tr>
<tr>
<td>LIBS</td>
<td>Laser Induced Breakdown Spectroscopy</td>
</tr>
<tr>
<td>MELiSSA</td>
<td>Micro Ecological Life Support System Alternative</td>
</tr>
<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
</tr>
<tr>
<td>OPTICX</td>
<td>Optrode Immersion Multi-Chemistry Explorer</td>
</tr>
<tr>
<td>PA</td>
<td>precision agriculture</td>
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<tr>
<td>PLC</td>
<td>programmable logic controller</td>
</tr>
<tr>
<td>PLL</td>
<td>Planetary Lake Lander</td>
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<tr>
<td>PPS</td>
<td>plant production systems</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
</tr>
<tr>
<td>RLT</td>
<td>red light treatment</td>
</tr>
<tr>
<td>SETI</td>
<td>Search for Extra Terrestrial Intelligence</td>
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<tr>
<td>TTL</td>
<td>transistor-transistor logic</td>
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Chapter 1

Introduction

Interaction with the environment requires the collection and interpretation of data. Sensor networks in biological systems provide information that allows living things to respond to environmental stresses and sustain their existence. Humans have used sensor technologies to develop complex tools, and use of sensors has facilitated remarkable achievements throughout history, from medical diagnostics to the exploration of distant star systems.

This work describes the application of ion-selective (IS) sensor technologies in the analysis of water chemistry relevant to plant production systems (PPS), Biological Life Support Systems (BLSS) and space exploration. The primary focus is the ion selective-analysis of hydroponic nutrient solutions in containerized PPS at the Controlled Environment Systems Research Facility (University of Guelph). Current plant production practices using hydroponics are potentially wasteful of nutrients and water - the present work explores the integration of IS-sensor technology into PPS to improve nutrient management. Ultimately, closed-loop plant production systems will serve as critical components of BLSS and the integration of IS-sensors is a critical milestone. Three topics are discussed providing context for the present work including: (1) Plant Nutrition and Hydroponics, (2) Ion Detection and Sensors, and (3) Biological Life Support/Space Exploration.

1.1 Plant Nutrition and Hydroponics

1.1.1 Plants - Nutrition

Plants require a specific set of chemicals in their diet to maintain life functions while supporting growth and development. Most species require similar nutrients and as many as 17 essential elements have been identified. Table 1.1 lists 12 of the most critical plant nutrients obtained by root systems showing clearly that the quantity of elements required by plants varies considerably. If the concentration of a particular nutrient ion is too low the plant may develop nutrient deficiency symptoms or experience uptake inhibition for other nutrients. Conversely, if nutrient concentrations are too high, toxic conditions may arise. In plant production, it is essential that nutrient concentrations are held within acceptable ranges to maintain healthy plants.
### Macro Nutrients (ppm) | Micro Nutrients (ppm)
--- | ---
Nitrogen | 180-230 | Manganese | 0.5-1
Phosphorus | 30-50 | Iron | 0.02-0.05
Potassium | 230-400 | Copper | 0.02-0.05
Calcium | 160-250 | Zinc | 0.33-0.5
Magnesium | 45-75 | Molybdenum | 0.01-0.05
Sulfur | 65-150 | Boron | 0.5

Table 1.1: Typical concentrations of 12 important elements in plant nutrient solutions[1, 2]

and maximize their productivity. In order to manage nutrients with precision, sensor measurements must be made and modern agriculture is embracing the integration of technology and best management practices to improve the sustainability of farming operations.

### 1.1.2 Plants - Metabolism

Most environmental factors impact plant metabolism. By virtue of their stationary existence, plants are master adapters. The environmental variables that govern plant life are: light (quantity and quality), temperature, water availability, relative humidity, nutrient availability, atmospheric composition and pressure. On a global scale, Earth pressure is relatively uniform but the other factors can vary widely in time and space on both micro and macro scales. To date, a thorough understanding of plant-environment-metabolism interactions has eluded plant scientists due to the extreme complexity of the topic and a lack of affordable technology that is capable of the research. High resolution IS-sensors will generate a deeper understanding of these complex relationships.

### 1.1.3 Hydroponics

Hydroponic production is a method for growing plants that typically utilizes an inert, well-drained growing substrate in place of soil. The inert medium serves to support the plants and their root structures and ideally does not interact with nutrient feed solutions. Plants are watered, either continuously or in pulses, with nutrient rich fertigation solution (fertilizer + irrigation) and receive nutrients in dissolved forms directly from it. The most referenced instructions for hydroponic production were described by Hoagland in 1938, and the methods described have not evolved considerably since [3]. In basic terms, a balanced nutrient solution is delivered to plants until it is sufficiently depleted, at which point the solution is discarded and a fresh solution is made. The application of IS-technology to hydroponic production will further optimize the mass-transfer-processes related to plant growth by ensuring ideal nutrient concentrations are maintained continuously.
1.1.4 Research

Improved Production

Many aspects of modern agriculture are well poised for improvements. The modern age of inexpensive computer and sensor technology is ushering in an agriculture revolution. Extensive research is underway finding uses for technology that improves the sustainability of agriculture. For example, ion selective-sensors combined with geographic information systems enhance the application of fertilizer, reducing application cost and environmental pollution.

Food Security

As the world’s population increases food security becomes increasingly challenging and hydroponic production will undoubtedly increase to meet production demands [4]. This is especially true in regions where environments impose extreme pressures on agriculture (e.g. Northern Canada, deserts of the Middle East). Therefore, any benefits to hydroponic practices today will increase their sustainability and value in the future.

Environmental Impact

Intensive hydroponic production centres that exist today generate significant amounts of pollution in the form of nutrients that are discharged to natural waterways. This trend is largely due to traditional production strategies that are inherently wasteful. Precision agriculture strives to minimize or eliminate wasted nutrients and water through improved management. IS-sensor technology is critical to achieving closed-loop production systems.

Niche Benefits

Emerging applications of precision-agriculture or Controlled Environment Agriculture (CEA) are generating a demand for smart-production systems to improve and even “tune” produce quality. From custom tailored food products to fine-tuned naturopathic medicine the niche market applications for precision PPS present significant economic value.

Present Work

This thesis describes an application of ion-selective sensor technologies in the management of nutrient solution quality for hydroponic systems. Successful demonstration of a reliable, ion-selective nutrient dosing system with feedback control marks a significant milestone in the evolution of precision agriculture.
1.2 Ion Chemistry and Sensors

1.2.1 Ions in Solution

Characterizing complex chemistry is challenging. Complex solutions contain many components that interact with each other, particularly charged species (ions). As a result, the effective concentration (or, activity) of any given ion will vary with total composition of the solution. Relating measures of activity to actual concentration becomes more difficult as solution complexity increases. Several technologies exist that can characterize solution chemistry and they vary in complexity, reliability, and cost.

1.2.2 Methods of Detection

HPLC

High Performance Liquid Chromatography is a brute-force separation method that uses a solvent to carry ions through a packed column at extremely high pressure (1200 - 2000 psi). Ions of different size exit the column at different rates and can be counted using a detector. Although very accurate and precise, this technology is very expensive and labour intensive [5].

Spectroscopy

Spectroscopy methods utilize high intensity photons and physical properties of matter to illicit an energetic response from molecules. Raman spectroscopy takes advantage of the Raman effect: monochromatic photons, typically sourced from a laser, will interact with matter at the atomic level, the energy of the laser photons is increased or decreased as a result of these interactions, and the change can be measured to yield unique chemical signatures. Laser Induced Breakdown Spectroscopy (LIBS) is a destructive spectroscopy method using more powerful photons to reduce matter into a plasma state. The plasma state quickly dissipates chemical energies into photons that can be counted and related to unique chemical signatures. Both of these methods are energy intensive, require very expensive hardware, and can be hazardous.

Ion-Selective Sensors

IS-sensors are materials that respond to the presence of specific ions in a manner that is measurable. Several technologies are being developed including: IS-Field Effect Transistors (ISFET), IS-Electrodes (ISE), and IS-optrodes. Each instrument differs in the method of signal generation, but all utilize a similar chemical process for selectivity. Sensors are coated in a material that has a selective-affinity for a target ion due to its chemical composition. An ion-exchange takes place between the sensor and the measurement environment, the sensitive material experiences a physical response, and a hardware system measures it.
Biological Life Support and Space Exploration

Human and robotic missions to explore space require extensive networks of sensors. Sensors provide data that are used to interpret field observations and/or manage the status of engineered systems. In the context of human missions, sensors are essential components of Biological Life Support Systems (BLSS) designed to support life-functions of humans in space (atmosphere management, treatment of waste products, and generation of consumable biomass). Robotic missions, without direct human control, are equally dependent on sensors since long-distance communication lags create challenges for real-time decision making - robots must respond to alien environments on their own. Current projects like the Planetary Lake Lander are focused on developing adaptive-robotic intelligence, using sensors, to improve the efficacy of robotic science exploration [6].

1.2.3 What Are BLSS?

Biological Life Support Systems are engineered, closed environments capable of supporting human life for extended durations with negligible, infrequent external connection. Development of BLSS has been an on-going branch of research for space agencies since the Apollo missions. The end-goal of BLSS research is to produce a self-sustaining biological community capable of complete recirculation of wastes/nutrients. A system of this nature requires many sensors coupled with intricate control algorithms and presently a technology vacuum exists in the field of environmental sensing and management.

1.2.4 Human Space Exploration

Humanity is eager to extend its presence in the universe. Taking humans on long voyages out of Earth orbit requires months or years of life-support and resupply is not an option since shipments would not catch up to space craft in-transit. Thus the ability to produce food and treat waste products is paramount. Reliable sensor networks are necessary for the control systems that manage the complex biochemistry of BLSS.

1.2.5 Robotic Space Exploration

The field of exo-biology seeks signatures of life (past and present) on other planets and their moons. Many locations in our solar system are appealing to astrobiologists since satellite data have revealed very interesting landscapes and chemistries. Recent discoveries on Earth prove harsh environments can support life, and now missions are planned to characterize extra-terrestrial chemistries in order to assess their biological-potential. Reliable, low-mass sensors are a requirement for long-distance science projects.
Chapter 2

Background

The application of IS-sensors in plant production is a multi-disciplinary objective. To provide context for the discussion of experimental results, an overview of relevant subjects is presented. This chapter presents details on: plant nutrition and hydroponics; ions in solution; sensors and sensor theory; and applications of ion-selective sensors.

2.1 Plant Nutrition & Hydroponics

Controlled Environment Agriculture (CEA) has the potential to revolutionize modern food production by maximizing the efficiency of raising healthy crops. Technology has permitted high resolution control of most environmental variables that are important to plant physiology. However, precision management of the quality of recirculating nutrient solutions remains a significant technological gap in CEA.

2.1.1 Plant Nutrient Requirements and Physiological Significance

Plants require specific nutrients to nourish their life functions and 17 elements have been identified as essential for healthy development [7]. The concentrations of nutrient ions required by plants vary widely, as was shown in Table 1.1, and each species has unique nutritional needs, yet the majority of plants require relatively similar nutrient inputs. Plant nutrients are grouped into two categories based on their typical abundance in nutrient solutions: Macronutrients (N, P, K, Ca, Mg, S) and Micronutrients (Mn, Fe, Cu, Zn, Mo, B, Ni, Cl). The remaining essentials include water as well as carbon dioxide and oxygen that plants access from the atmosphere. Sodium is important for certain species such as halophytes. In the context of the present work, a brief overview of the nutrients taken up by roots and their functions is presented.

Macronutrients

_Nitrogen:_ Nitrogen, one of the most abundant elements in nature, is a critical building block for most plant substances and can comprise 40-50% of the dry matter in plants
It is an elemental constituent of amino acids, proteins, nucleic acids and secondary plant metabolites. As a result, nitrogen demand of crops can be very high and is often the limiting nutrient in agriculture. Nitrogen deficiency will typically result in slow or stunted growth and ultimately reduced crop yield so nitrogen fertilizer is often over applied. Most plants can use both nitrate and ammonium as a nitrogen source and fertilizers may contain both species. Due to widespread land application of fertilizers around the world nitrogen pollution is considered a major environmental impact of agriculture, driving research in best management practices for nitrate fertilization and mitigation strategies for pollution sources [8, 9, 10, 11, 12].

**Phosphorus:** Plants primarily utilize phosphorus in the production of important molecules like DNA, RNA, and the phospholipid components of cell membranes. Phosphorus is also an important ingredient for ATP production and is therefore critically important for metabolic processes of plants [1]. Uptake of phosphorus is regulated by an active \( H^+ \) co-transport system and requires energy. Owing to its diverse uses, phosphorus deficiency symptoms can be quite difficult to identify and are often misinterpreted. Several challenges currently exist in managing phosphorus application in agriculture, arising from the complex interactions between phosphorus, plants, and soils. For example, centuries of agriculture in Europe has caused widespread accumulation of insoluble phosphorus forms in soils leading to disastrous environmental effects that research is focused on quantifying and mitigating [13, 14, 15, 16].

**Potassium:** Potassium is highly-mobile in plants and is involved in most plant processes. Potassium acts as a catalyst in enzyme regulated reactions, a regulator of plant water relations, and a critical participant of cellular ion pumps. Another important role of potassium is in the regulation of stomatal function through its endogenously controlled impact on cell turgor. Potassium uptake is facilitated by several processes including ion-selective transport channels [1]. Deficiency of potassium can result in chlorosis and an overall reduction in plant growth. Potassium for agriculture comes largely from potash mined from the Earth and although large, reserves are finite [1].

**Calcium:** Similarly to potassium, calcium is primarily involved in facilitating transport of other nutrients into plant tissues and participating in enzyme reactions by affecting cellular chemistry. Calcium also contributes to structural assemblies in plant tissues [1]. Uptake of calcium occurs by facilitated diffusion and is regulated at the cellular level by active ion pumps. Calcium deficiency tends to stunt the growth of meristematic tissues and can produce noticeable effects on plant leaves. In Southern Ontario calcium is abundant in source water and can accumulate - a significant concern for hydroponic-greenhouse producers that must meet discharge criteria legislated by the government of Ontario [17, 18].

**Sulphur:** Plants mainly utilize sulphur in the form of sulphate and uptake is facilitated by an \( H^+ \) co transport system. Sulphur is an important building block of amino acids and vitamins [1]. It is integrated into the structure of plant tissues and compounds and is an immobile nutrient. The structures formed by sulphur are involved in the photosynthetic machinery of plants and its deficiency induces the breakdown of these systems since the proteins required are no longer produced. As sulphur is involved with many physiological processes plants deficient in sulphur will be stunted [19].
Metals & Metal Enzymes

The essential micro nutrients for plants are mainly metal ions that serve as components and cofactors of enzymes and enzyme catalyzed reactions. Metals with similar charges tend to be involved with similar processes, for example magnesium and manganese (both divalent cations) are involved in enzyme reactions of photosynthesis and are important components of chlorophyll. Magnesium plays a crucial role in catalyzing the phosphorylation of ATP and other important enzyme reactions related to photosynthesis [1], and manganese serves as a critical participant of the water-splitting Hill reaction[1]. Since uptake of these nutrients is facilitated by the same transport processes inhibition can occur between them as they compete for uptake pathways.

Iron, copper and zinc are important metals in the formation of chlorophyll. Nickel also appears to be a micronutrient for certain species including cereal crops like wheat, barley and oats [1]. Only trace amounts of these elements are required by plants and elevated concentrations can quickly become toxic [1]. The larger metal ions require energy to facilitate their uptake by plants and are mediated by $H^+$ pumps driven by ATP [1]. Since they compete for the same transport machinery, excessive concentrations of any metal in the plant environment will inhibit the uptake of others. The specific roles of iron, copper, zinc, and nickel in plant physiology are widespread and poorly characterized. It is known that copper is incorporated as structural components in cell walls and deficiency of copper leads to a breakdown of metabolic processes within the plant resulting in white, twisted leaves [1]. Deficiency of zinc results in stunted leaf growth, high levels can become toxic to plants quickly and also tend to stunt leaf and root growth. Even low concentrations of nickel can be toxic to plants, however it has been demonstrated that nickel is involved with nitrogen metabolism and facilitates the decomposition of urea into useful forms of nitrogen [1].

Molybdenum is an important component of enzymes that facilitate the assimilation of nitrogen by plants and are thus important for protein synthesis [1]. This element is part of the nitrate-reductase enzyme, a critical enzyme responsible for the reduction of nitrate to ammonia [7]. Without it, plants can suffer symptoms of nitrogen deficiency. Like other metal-micronutrients only trace amounts are needed for healthy plants. Another important micronutrient is the the metalloid element boron. Primarily it acts as a component in cell walls but may also contribute to sugar transport and production of some enzymes [1]. Boron also facilitates the uptake of calcium and is involved with many physiological processes in plants. Excessive concentrations can quickly become toxic to plants resulting in leaf tip yellowing and eventual necrosis [1].

Spectator Ions

Sodium: Although not typically considered an essential element, the abundance of sodium has significant implications for plant physiology. Certain plants require sodium to complete their photosynthetic processes (CAM and C4 plants) [1]. Saline conditions are often toxic to plants, so sodium management can be of paramount
concern for CEA. A select set of plant species called Halophytes are adapted to highly-saline conditions and interact with sodium in specialized ways. It is suggested that, albeit not essential, sodium could have beneficial impacts on plant production, particularly when potassium is deficient [1].

Chloride: Chloride is an important regulatory ion in biological systems. It is a highly mobile and relatively simple element and can lend itself to many biological processes. Most critically, chlorine is responsible for maintaining charge balance at the cellular level in plants - a role that is extremely significant in the control of stomatal functions and the transportation of nutrient ions within plants [1]. Although not considered an essential plant nutrient, the concentration of chloride in the plant environment has significant biological implications.

2.1.2 Plant-Environment Interactions

Environmental conditions regulate the forms and life functions of plants. The influence of seven variables define the types and growth patterns of plants in any given environment. These variables include: light quality and quantity, temperature, water availability, relative humidity or vapour pressure deficit, nutrient availability, atmospheric composition, and air pressure. Aside from air pressure, most of the factors listed vary widely on Earth, resulting in the diverse forms of plant life that populate it. Figure 2.1 demonstrates clearly that different environments can create very different forms.

Figure 2.1: A: Photo of *Tropaeolum polyphyllum*, a high altitude flower of the Chilean Andes. Typically bright yellow, this individual shows orange pigmentation, signs of beta-carotene production as a defense from increasing UV light. B: Photo of *Amorphophallus titanium*, the Corpse Plant native to Sumatra, taken at the University of Guelph. This tropical plant grows very large and produces a very pungent odour when flowering.
Light

Light provides the energy that plants require to carry out their life functions. Plants use photosynthesis to capture light-energy and store it as chemical-energy in the form of carbohydrates to be later consumed as food. The absorption activity of the root system is dependent on this continuous feed of carbohydrates from the photosystems [7]. The relationship between light and plant physiology has long been appreciated, as the quality and quantity of light provided to plants has significant implications for plant health and productivity. Recent innovations in lighting technologies have permitted a more controlled approach to studying plant-light interactions and many interesting trends have been revealed. It has been demonstrated that blue light seems to be critical for plant production and generally enhances the growth and output of plants [20, 21, 22] and also appears to be important for the phototropism response whereby plants bend towards the light [23]. The study of plant light interactions has been ongoing for some time, as demonstrated by the work of Lewis Flint published in 1934 [24] yet still a firm understanding of the complex relationships has not been achieved. “It is clear that photosynthetic rates, shoot growth, carbohydrate export, nutrient demand, root respiration, root growth and nutrient absorption are all influenced in a coordinated manner in response to light. The integration of these processes and in particular the integration of root and shoot activity is complex and poorly understood” [7].

Temperature

Most plants do not thermoregulate and as a consequence, temperature has very strong impacts on plant physiology. This is mainly owed to the pronounced effect of temperature over enzyme activity [7]. In general, plants slow down in cold climates due to slower chemical reactions, while in tropical climates plants can grow with astonishing speed. For example, the tropical Amorphophallus titanium flower (pictured above) produces the world’s largest leaves reaching upwards of 6 meters within 24 months of growth [25]. Significant temperature changes can disrupt plant metabolism, however prolonged changes result in acclimation of plants to new temperature environments, and metabolic activity can be restored [7]. Freezing temperatures or extreme heat can have disastrous impacts on plants that are not adapted to prevent their internal water from rapidly freezing or vaporizing.

Water

Access to water is essential to plant life. Water is drawn across the root system and through the plant by suction pressure generated by osmotic potential gradients and evapotranspiration from leaf tissues into the atmosphere. It terms of plant-environment interactions, water acts as the primary solvent for most materials entering the plant body. It also plays a critical role in regulating plant growth as sufficient turgor pressure is required to support cell elongation. Water availability has a direct impact on plant nutrient uptake as water deficit impairs plant growth and alters the
demand for nutrients in growing tissues [7, 26]. Plants have many strategies for coping with water shortages and so, water stress can have very significant impacts on plant physiology that can impact the quality and size of crops [27, 28]. The responses of plants to water stress are numerous, multi-faceted, and interrelated thus specific impacts of water stress on plant systems and their interaction with the environment are very difficult to identify [29]. Due to the impacts of climate change, maintaining water budgets in support of agriculture is a primary focus of research. Many irrigation strategies have been implemented around the world to optimize the duration and frequency of irrigation events to improve plant health and water use efficiency [30, 31, 32]. Differences between these methods will influence plant-environment interactions in unique ways that must be considered from a management perspective.

**Vapour Pressure Deficit**

The main driving force for moving water against gravity in plants is the pull imposed by evapotranspiration at the canopy. The strength of this force is strongly related to the ambient vapour pressure deficit or atmospheric demand for water. If air becomes saturated evapotranspiration stops along with the majority of movement of water through plants other than redistribution along osmotic and total water potential gradients. Thus, plants regulate their transpiration rates continuously in effort to stabilize their physiology [1].

**Atmosphere**

Plants sequester carbon from the atmosphere through photosynthetic processes and require oxygen for respiration and other life functions. Other components of the atmosphere are important for plant physiology, as in the case of soy plants that obtain nitrate from symbiotic bacteria who fix nitrogen directly from the gaseous form. The composition of Earth’s atmosphere is relatively constant, however since the industrial revolution trends of increasing CO₂ have been observed [33].

The impacts of atmosphere chemistry on plant physiology are very significant in the context of CEA and BLSS. Research at CESRF has characterized some of the parameters relevant to BLSS with respect to plant production including reduced oxygen and elevated carbon dioxide [34, 35, 36, 37]. The influence plants have on their atmosphere is particularly significant in the context of CEA and BLSS and exploratory investigations of the relationship have conducted at CESRF. Studies of this nature demonstrate that interactions can become quite complex as the number of species increases in closed-production environments [38].

**Pressure**

Atmospheric pressure has some implications for mass transfer processes that govern the interactions between plants and their environment. In nature the movement of materials to root systems is mainly governed by diffusion while heat and mass transfer processes from leaf systems are dominated by convection [39]. The rate of transfer of materials across the plant-environment interface will be dependent on the
ambient pressure in soils and air. Although air pressure on earth is relatively constant, pressure changes in the context of BLSS in space or on other planetary bodies will have operational implications. Research conducted at the CESRF has explored some of the effects of low-pressure cultivation of crops and results suggest that life-functions of plants can be supported in pressures as low as 0.1 atm provided sufficient oxygen is available (greater 7 kPa partial pressure)[34, 35, 36]. Other studies have shown that plants can survive rapid decompression events, which has significant implications for plant production in low pressure environments such as space, the moon, or other planets [40].

**Nutrients**

Plants acquire food from their environment through their root and leaf systems. Most plant nutrients travel between the root zone to growing tissues via transport by water and sap through the Xylem and Phloem. To move nutrients into cells plants employ both active and passive transport processes. Passively, nutrients will diffuse down concentration gradients while plants may actively transport certain ions against gradients using chemical energy. Thus, the external concentration of nutrients is one of the most important factors that influence nutrient uptake rates by plants [7]. Nutrient ions are also stored in plant vacuoles for later consumption. In general, movements of nutrients from the environment into plants and subsequent distributions of nutrients within plant bodies are extremely dependent on nutrient availability and deficits of certain nutrients can impact the uptake of others.

Interactions between nutrients in the external environment can significantly impact the uptake of nutrient by plants. Some interactions between nutrient species are synergistic ($H^+$ ions aid the influx of large ions like $NO_3^-$, $SO_4^{2-}$ and $PO_4^{3-}$) while others are antagonistic (presence of $Rb^+$ inhibits $K^+$ uptake as its similar size and charge permits competition for binding sites of $K^+$ ion carriers)[7]. Interactions involving highly mobile ions like potassium or chlorine are important in maintaining the charge balance of membranes, and their accumulation or deficit can have significant impacts on mass-transfer processes at the cellular level [7].

### 2.1.3 Plant Production

With the world’s population pushing seven billion, food security has never been a more pressing issue. Historically, agriculture practices have advanced to support swelling populations, yet have also proven to be among the most environmentally destructive human practices. As the demand for food continues to grow, pressure on the environment must be balanced to ensure the sustainability of the planet and the global community.

The application of technology can improve the efficiency of agriculture. Affordable, robust electronics available today permit the development of sensor-based information systems to assist in crop management. This use of sensor-based feedback to assist decision making is a critical component of Precision Agriculture (PA)[41] and its application optimizes farm inputs for optimal returns. Major industrial organiza-
tions are investing heavily in PA, highlighting a growing awareness of threats to food
security from climate change [42].

One of the most critical components of PA is the management of nutrients in crop
production [43]. Traditional land application of fertilizer often leads to over fertil-
ization resulting in contamination of surface and ground water from nutrient runoff
and leaching. Greenhouse producers also generate a significant amount of waste nutri-
ents. This problem is pronounced in areas of intensive agriculture, as demonstrated by
long-standing nutrient pollution issues in the E.U., impressing that the management
of nutrient applications must be improved to protect natural systems. It is estimated
that by 2020 19.8 billion USD worth of nitrogen fertilizer will be wasted worldwide
[44]. In addition, optimizing nutrient management will yield healthier crops while
reducing fertilizer use, both of which are positives for the farm economy. Automated
nutrient management will also have applications in urban greenroof systems that will
have to maintain tight control of nutrient budgets to maintain plant health and meet
impending discharge requirements [45].

2.1.4 Hydroponic Method

The production of crops was advanced significantly by the development of hydroponic
production methods, and the first use of soil-less plant culture was documented as
early as the 17th century [3]. Instead of soil, plants are grown in an inert, well-drained
medium that is washed (periodically or continuously) with nutrient rich water. The
key advantage is that root systems have easy access to readily mobile nutrients,
resulting in fast growing plants and fruit if nutrient supply is maintained. In soil
systems nutrient uptake by plants is complicated by interactions with the soil matrix.

A critical challenge of hydroponic plant production is management of nutrient
solutions. Nutrient solutions are a complex mixture of ions in a predictable range of
concentrations. Many formulas and application strategies exist and are promoted by
the various companies that produce them, however all must supplement the nutritional
requirements of plants and have similar ingredients and methods. The methods
and nutrient solutions described by Hoagland in 1938 remain the benchmark for sci-
entific study of hydroponics [3]. For plant producers simple methods are preferred
and nutrient solutions are typically created from concentrated nutrient stocks that
are mixed to produce the desired fertigation solution. Often this method uses two
stock solutions (termed A and B) that separate species that would precipitate if com-
bined at high concentration. The pH of a nutrient solution must also be maintained
within an acceptable range and most plants require a pH of 5.5-6.5 in order to access
nutrients from their environment [46]. Using traditional practices, nutrient status
is monitored by making electrical conductivity (EC) measurements to track the de-
pletion of ion species from the solution - weak solutions are discarded and replaced
with fresh solutions. This process is inherently wasteful because discarded solutions
contain significant quantities of unused nutrients.

The evolution of hydroponic methods is focused on developing nutrient manage-
ment systems capable of closed-loop solution recycling to eliminate wasted water and
nutrients [47]. By monitoring nutrient status on an ion by ion basis, nutrients can be
added back into the feed solution as they are depleted. Ultimately, sensor data can be used as feedback to a control system for automated nutrient dosing. Achieving precision management of nutrient solutions would close the largest remaining technological gap in controlled environment plant production [48]. New ion-selective detection technologies have the potential to overcome previous development limitations bringing the goal of online nutrient management closer to reality.

\section*{2.1.5 The Future of Plant Production}

Research conducted at the CESRF has served to advance the understanding of plant-environment interactions through the development of controlled environment plant production technologies. Recent advances in LED lighting technology and nutrient management systems have positioned researchers to significantly advance our understanding of plants and their responses to light and nutrient inputs, potentially yielding a new level of understanding of plant physiology that has not been possible in the past.

Future applications of the science are widespread, but the most notable is the fine-tuning of crop quality through optimized light-nutrient-environment recipes. It is feasible that fine-tuned environmental control can be used to influence many crop traits including: taste, formation of bio-products, growth rate, size, sex, maturation, energy requirements, nutrient use, and appearance to name a few.

\section*{2.2 Ions in Solutions}

The properties of solutions become quite complicated as the number of dissolved species increases. As the complexity of a mixture is increased, the interactions between components of the mixture are increased exponentially. These complex interactions at the molecular scale represent the most significant challenge facing the quantification of individual elements in a solution without separation. To address the issue, an understanding of solution chemistry is required and the following section describes the physical properties that are relevant.

\subsection*{2.2.1 Activity vs. Concentration}

Possibly the most critical concepts to address are concentration and activity. Concentration is defined as the quantity of a substance per unit volume. In this work, it is convenient to discuss concentration in terms of Molarity, thus:

\[ c = \frac{n}{V} \]  \hspace{1cm} (2.1)

where \( c \) is concentration (\( \text{mol L}^{-1} \)), \( n \) is number of moles of a species, and \( V \) is the volume of solution (\( L \)). It would be most appealing to directly measure the concentration of a particular species, however as more species are added to a solution, molecular interactions influence the activity of a particular species. Activity, or “effective concentration”, is defined as the “effective quantity” of a substance per unit
volume that is observed in a complex solution, and is factored into concentration calculations using an activity coefficient as shown:

\[ a = \gamma \cdot c \tag{2.2} \]

where \( a \) is the activity of the species and \( \gamma \) is the activity coefficient. Activity coefficients are often determined experimentally since theoretical methods can be quite cumbersome for complex solutions. In the case of ionic solutions it is in fact impossible to measure the activity coefficient of individual ions in solution experimentally since all ions exist as dissociated pairs and their counter-ion partner will always be present. However, methods to determine a mean activity coefficient for ion pairs have been developed. For dilute solutions the Debye-Huckel model is useful for predicting mean activity coefficients, while other models have been presented for more complex solutions - these models are highlighted by Bamsey [49]. Developing a multi-ion sensor system capable of in situ measurements in complex solutions will require a careful appreciation of the relationship between concentration and activity.

### 2.2.2 Ionic Strength

The ionic strength of a solution offers a measure of its complexity. Specifically, ionic strength is a function of the total concentration of all ions in a solution as shown:

\[ I = \frac{1}{2} \sum_{i=1}^{n} c_i z_i^2 \tag{2.3} \]

where \( I \) is ionic strength, \( z \) is the charge number and \( n \) is the total number of dissolved species. From the definition of ionic strength it is clear that more ions in solution contribute to a greater ionic strength and ions with more charge contribute exponentially. In biological systems, the relative strengths of ionic solutions impart very significant influence on important mass-transfer processes as the electro-chemical properties of a solution determine the types of electrokinetic phenomena that dictate the movement of species within it.

### 2.2.3 Electrical Conductivity

Although it is ideal to determine the ionic strength of solutions to understand their properties, unless an ingredient list is provided it is typically costly and impractical to determine the complete composition of solutions. The nature of ionic solutions allows them to conduct electrical current and the Electrical Conductivity (EC) of ionic solutions is a measurable quantity. An EC probe uses a small alternating current applied over two immersed electrodes to measure the resistance of ionic solution to the flow of electrons, which is inversely correlated to conductivity. Solutions of high ionic strength have a low resistance value and thus a high electrical conductivity while pure water having a very high resistance (18MΩ) is a poor conductor.

Measures of electrical conductivity can be correlated to ionic strength, however they do not provide information on the composition of solutions. In the context
of hydroponic production, measures of EC can be used to crudely assess quality of nutrient solutions. As nutrients are depleted from fertigation water EC will decrease and growers can use that information, coupled with experience, to schedule solution replacement. In the pursuit of ion-selective management of nutrient solutions, EC will be useful to provide approximate measures of ionic strength for the determination of activity coefficients. Previous work by Bamsey describes the logistics of using EC measurements to approximate activity coefficients so that activities measured by ion-selective sensors can be converted to concentration values [49].

2.2.4 Acidity and Alkalinity

The pH of a solution is a measure of its acid or base character. In an aqueous solution, the degree of dissociation of the water molecule into $H^+$ and $OH^-$, as dictated by the solution chemistry (dissolved species), determines pH. The definition of pH is the negative logarithm of hydrogen ion activity as shown:

$$pH = -\log(a_{H^+})$$  \hspace{1cm} (2.4)

Acidic conditions exist when $pH < 7$ meaning hydrogen ion activity is high and basic conditions exist when $pH > 7$ meaning hydrogen ion activity is low. The pH of a solution holds strong implications for the solubility and mobility of dissolved chemical species. In the context of plant physiology, pH of waters, soils and nutrient solutions is a critical parameter in the determination of nutrient availability. Most plants require a slightly acidic pH (5 ↔ 6) [1] in their root zone to facilitate uptake of nutrients. Basic conditions tend to bind inorganic nutrients into insoluble forms and low $H^+$ activity inhibits co transport of large ions like nitrate, phosphate and sulphate, making them inaccessible to plants [1].

Hydroponic plant production requires pH control to maintain a suitable range since plant roots interact with their environment and produce substances that influence pH. Sensors for pH measure $H^+$ activity using a glass probe in tandem with a reference electrode. Small changes in electro-chemical potential at the glass surface are compared with the stable reference electrode using a sensitive meter. Over time pH will change in recirculating systems and pH measurements allow corrective adjustments to be made by adding acid or base depending on the case.

2.2.5 Mass Transfer Processes

Diffusion & Osmosis

The process of particles moving from an area of high concentration to an area of low concentration is termed diffusion. Diffusion is responsible for movement of nutrients in waters, soils, and tissues. Another important transport process in plants is osmosis, the movement of water across a membrane from an area of low solute concentration to an area of high solute concentration. Biological systems rely extensively on osmosis to maintain cell pressure since water tends to move into cells where solute concentrations
are high. Diffusion and osmosis both occur spontaneously in response to chemical energy gradients.

**Convection**

When aggregate groups of molecules in a fluid move together creating a flow current, convection processes take place. Convection currents have significant effects on mass transfer processes due to the creation of boundary layers when currents pass over stationary objects. Boundary layers dramatically influence mass transfer between flow fields and stationary surfaces. Ultimately, this project strives to quantify the uptake of nutrients by plants and the resulting influence of solution quality. Understanding that plants are stationary organisms implies that certain mass transfer processes will dominate their acquisition of materials from their environment. In the context of hydroponic systems, flowing nutrient solutions will induce boundary layer effects at physical structures like roots or sensors. The implications for measuring rates of mass transfer must be considered in the design of sensor systems. Biological systems exploit boundary layer effects to maximize or minimize rates of heat and/or mass transfer with the environment.

**Active Transport**

Active transport refers to the use of energy to move species against concentration gradients. In biological systems, this typically means the use of ATP or electrochemically driven ion pumps. Ion pumps are proteins that catalyze transportation of ions across membranes [1] and are a major player in the uptake of ions in the root zone. Active transport can be primary (directly using ATP) or secondary in the case of ion co-exchange pumps.

### 2.3 Sensors & Methods of Ion-Selective Detection

Sensors are essential for process management. Sensors serve as an interface between systems and their environment and living systems depend on sensory input for survival. Evolution has crafted remarkable sensory systems for all forms of life, allowing them to monitor and respond to changes in their environment. Humans have developed many mechanical sensors to monitor and control the operation of engineered systems. From the float switch to radio receivers, sensors have helped humans solve many technical challenges. Presently, a revolution in sensor technologies is taking place. Low cost electronics and development platforms coupled with advances in chemistry and material science have created an ideal atmosphere for generating new sensors and applications.
2.3.1 Sensors

Sensor Theory

The proficient use of sensors requires an understanding of fundamental principles of their operation. Sensor parameters related to the present work are summarized in Table 2.1.

<table>
<thead>
<tr>
<th>Sensor Parameter</th>
<th>Definition and Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Detection Limit (UDL)</td>
<td>The highest limit of quantitative results that can be obtained beyond which sensor response cannot be distinguished from signal noise.</td>
</tr>
<tr>
<td>Lower Detection Limit (LDL)</td>
<td>The lowest limit of quantitative results that can be obtained in comparison with a blank sample, beyond which sensor response cannot be distinguished from signal noise.</td>
</tr>
<tr>
<td>Sensitivity (S)</td>
<td>$S = \frac{\Delta \text{Response}}{\Delta \text{Property}}$; The change in sensor response with respect to the change in the property being measured.</td>
</tr>
<tr>
<td>Range (R)</td>
<td>$R = UDL - LDL$; The difference between the upper and lower detection limits.</td>
</tr>
<tr>
<td>Noise, Signal-to-Noise Ratio</td>
<td>Noise refers to the natural deviation of a sensor signal about the actual response value. The signal to noise ratio is an important sensor parameter as it defines the limits of detection.</td>
</tr>
<tr>
<td>Selectivity and Interference</td>
<td>Selectivity is the relative ability of a sensor to respond exclusively to the property of interest. An interference is a property that influences the sensor response that is not the property of interest.</td>
</tr>
<tr>
<td>Drift</td>
<td>Drift refers to the natural deviation of a sensor signal from the calibration standard over time.</td>
</tr>
</tbody>
</table>

Table 2.1: Table listing important sensor parameters, their definition, and a general description of their relationship to sensor operation.

Calibration

Most sensors must be calibrated in order to relate the quantified sensor response to the property being measured - in other words, in order to be useful. A typical calibration involves subjecting a sensor system to known property values and recording
the sensor response to create a calibration curve. Considering a thermometer as an example, the scale (calibration) can be set by exposing the thermometer vessel to known temperatures and marking the fluid expansion at each temperature graduation - the number of graduations will determine the resolution of the sensor. Using the calibration the response of a sensor system to a sample with unknown properties can be related to a known benchmark.

**Error**

By definition a sensor error is the difference of a sensor response from the actual value, as shown:

\[
E = a_m - a_a
\]  

(2.5)

where \(E\) is the measurement error, \(a_m\) is the measured property value and \(a_a\) is the actual property value. Minimizing error is a primary concern in the design of sensor systems and understanding sources of error is the first step to mitigating their effect. Errors can be introduced in several forms: insertion error refers to influence on the system from the sensor itself; sensitivity error occurs when the calibration curve differs from specification; range errors arise when an instrument reaches the physical limits of sensing; quantization and interpolation error reflect the size of the sensors measurement steps that determine the resolution. Operational errors can also be introduced in the form of hysteresis effects or dynamic errors. Hysteresis refers to the directional uniformity of a sensor response and is an artifact of dynamic response characteristics of sensors such as time to stabilization. Characterizing error parameters is critical to minimizing their influence in the development of a useful measurement device.

### 2.3.2 Detecting Chemicals Selectively

As previously stated, complexity is a confounding factor in the effort to quantify individual ion species in a solution. This fact is largely owed to the small-scale interactions between species (particularly ions) that dynamically buzz around one another, more or less freely depending on their size and charge, in their dissolved state. Current methods to quantify individual species in complex solutions employ one of three strategies: direct measurement by separation; direct measurement by spectroscopy; or indirect measurement by transduction.

**Separation (Direct)**

The most effective way to measure concentration of individual species in solution is to sort and count them. High Performance Liquid Chromatography or HPLC is a method for quantifying individual chemical species in solution by separating them using chemical processes and counting them using detectors. As applied to ion detection, HPLC can be termed Ion Chromatography. These systems are very reliable and provide the benchmark detection method for quantifying nutrient chemistry, however
operation requires expensive hardware, considerable consumable material and energy inputs, and an experienced operator. The important components of an HPLC system are highlighted in Figure 2.2. A comprehensive review of HPLC systems as applied to nutrient ion detection is presented in a review paper by Bamsey [5].

![Image of HPLC system](image)

**Figure 2.2:** Photo of the Simadzu HPLC system used to perform ion-analysis of water samples with important components labeled

**Spectroscopy (Direct)**

Light can be used to measure the presence of species directly if the target absorbs light at a wavelength that can pass through water samples. A detailed summary of spectroscopic methods as they apply to nutrient status assessment is presented by Bamsey et al. [5] and it is stated that direct identification of many nutrient ions by spectroscopy is not feasible as they do not have suitable absorption properties. Only iron, manganese, copper, and zinc form complexes in hydroponic solutions that absorb light sufficiently to be relatable to ion activity. Methods have been developed for spectrographic analysis of other ions by means of adding secondary chemicals to selectively react with targets to form absorptive compounds.

High energy spectroscopy involving the use of high-energy photons from a laser source can provide more detailed information. Methods like Laser Induced Breakdown Spectroscopy (LIBS) use light energy to break apart chemical bonds and the subsequent photon emissions can be measured and related to composition. The use of LIBS has shown promise in the analysis of agricultural solutions [5].

**Transduction (Indirect)**

The conversion of a signal into some other form of signal is called *transduction* [50]. As mentioned previously, a sensor responds to specific stimuli by producing some measurable output. All of the detection methods discussed utilize transduction in
final counting or measuring. HPLC uses EC measurements of the effluent mobile phase to count separated species and all spectroscopy methods utilize digital spectrometers that employ arrays of electronic photoreceptors to count incident photons. Ion-Selective sensors are designed to respond to the activity of specific ions in solution and generate a measurable response, and several IS-Technologies have evolved in the past half-century.

**IS-FETs**
Ion-Selective Field Effect Transistors (IS-FETs) incorporate materials that bind selectively to target ions into electronic devices. This type of sensor responds to analyte changes with a change in resistance in some system component, and this can be quantified with a sensitive meter measuring the current through the FET. In the case of the IS-FET, the metal-oxide semiconductor that throttles the flow of electrons through the system is coated with an ion-selective material, which can be made of any inert matrix that supports ionophore chemistry, and the behaviour of the coating will influence the response of the FET. A schematic of an IS-FET sensor, as applied to nutrient sensing, is shown in Figure 2.3 highlighting critical components of the system. It is important to recognize that an ISFET sensor would require water-proofing for use as an immersion-style device.

**ISEs**
Ion-Selective Electrodes (ISEs) can utilize the same ion-selective materials as IS-FETs (often using plasticized PVC membranes) but use a different method of transduction. An electrolyte solution contained in an electrode housing provides a stable reference of electro-chemical potential (voltage), and this reference is compared to the potential measured at the sensor-solution interface. The difference between these measures is the sensor response and can be measured with a voltmeter. One drawback of ISEs is their high impedance that requires voltage signals to be amplified by additional electronics. A schematic diagram of a typical ISE is shown in Figure 2.3 and critical components are identified.

**IS-Optrodes**
Ion-selective optrodes use similar selective materials as other IS-sensors but take advantage of the absorptive properties of dyes to generate a sensor signal. The IS-materials used for optrode sensors feature colour-changing compounds that alter their colour in response to analyte activity. The response is measured by projecting known light spectra onto the sensor and measuring absorption using a spectrometer. A schematic of an optrode system as applied in hydroponic nutrient quality monitoring is presented in Figure 2.3.
Figure 2.3: Schematic diagram of an Ion-Selective Field Effect Transistor, Ion-Selective Electrode and Ion-Selective Optrode as applied to hydroponic nutrient solution monitoring illustrating critical components.
2.3.3 Suitability for Online Ion Detection

To compare the merits of technologies for ion-selective detection in the context of automated nutrient management systems, several comparisons metrics were devised. The detailed comparison presented by Bamsey et al. is summarized in Table 2.2 and has been simplified [5].

<table>
<thead>
<tr>
<th>Metric</th>
<th>HPLC</th>
<th>Absorption</th>
<th>ISE</th>
<th>ISFET</th>
<th>Optrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion Detection</td>
<td>Multi</td>
<td>Multi</td>
<td>Single</td>
<td>Single</td>
<td>Single</td>
</tr>
<tr>
<td>On-line</td>
<td>No</td>
<td>Most</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cost</td>
<td>High</td>
<td>Low-Med</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Accuracy</td>
<td>High</td>
<td>High</td>
<td>Med</td>
<td>Med</td>
<td>Med</td>
</tr>
<tr>
<td>Calibration Requirements</td>
<td>High</td>
<td>Low-Med</td>
<td>Med</td>
<td>Med</td>
<td>Low</td>
</tr>
<tr>
<td>Training Requirements</td>
<td>Med-High</td>
<td>Low-Med</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Hazards</td>
<td>Chemicals</td>
<td>Laser sources</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Established Technology</td>
<td>High</td>
<td>Med-High</td>
<td>Med</td>
<td>Med</td>
<td>Low</td>
</tr>
<tr>
<td>Mass, Power, Volume</td>
<td>High</td>
<td>Low-High</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table 2.2: A simplified version of the technology comparison of HPLC, Absorption Methods, Ion-Selective Electrodes, Ions-Selective Field Effect Transistors, and Ion-Selective Optrodes presented by Bamsey et al. [5]

Based on the metrics presented, ion-selective optrode sensors present the most appealing solution to the problem of assessing nutrient solution status in real-time. However, their low technology readiness level means that significant investment must be made to refine the technology. Specifically, the fragile nature of the optical sensors requires that a housing be developed to protect the delicate sensors from the harsh environment of hydroponic systems. Also, methods to integrate multiple ion sensors into one measurement device must be characterized in terms of functionality and cross-sensitivity including protocol for: calibration, operation, storage and care, and servicing. Efforts to use optical sensors in plant growth systems have been documented including the monitoring of pH, ammonia and ethylene [51] as well as carbon dioxide and relevant metal ions [52]. Promising results from these investigations support the development of a monitoring system for nutrient management using optical sensors. This work explores the technical challenges related to implementing optrodes in hydroponic systems.
2.4 The Ion Selective Tech Vacuum

The demand for affordable sensors capable of *in situ* assessment of solution chemistry has created a technology vacuum. The advent of inexpensive electronic components and controls along with ionophore chemistry has made ion specific sensing a viable technical and economic reality. Applications for ion selective sensor technology are widespread, and in particular plant science, agri-food and space sectors can benefit extensively.

2.4.1 Plant Science

Application of IS-Tech in plant science can yield vast insight into plant-environment interactions. Although the essential elements for healthy plants are known, due to the complex relationship between plants and their environment, the fine details of plant nutrition have eluded science to-date. Inexpensive IS sensors will permit the development of real-time nutrient monitoring systems that will provide an unprecedented look at plant-nutrient relations. Combined with other modern plant science tools (for example, multi-band LED lighting systems) IS nutrient monitoring will both answer and produce many plant-physiology questions.

Current research on plant-environment interactions at CESRF is focused on the effect of variable light recipes on plant growth. It is well established that plants respond to different light colours by altering their physiology, and it is reasonable to assume that these alterations will influence their dietary habits. Application of IS sensors in plant science will permit novel high-resolution investigations of plant nutrient uptake in response to changes in any of the major environmental variables.

2.4.2 Precision Agriculture

The single most important environmental variable in controlled environment agriculture that has yet to be successfully addressed with reliable and economical sensor technology is the quality of the nutrient solution. Since agricultural jurisdictions in Ontario and around the world are being subjected to legislative pressures to address environmental impacts of wastes such as hydroponic nutrient solutions, there is a desperate need for an economical technical solution to water quality management. Growers are limited to conventional pH and EC sensors which provide only limited information on the quantity of the dissolved nutrients. The development of affordable IS sensors will provide reliable feedback control of nutrient solutions.

The controlled environment agriculture industry requires the capability to address the environmental impact of their industry’s effluent management as well as quality control of nutrient solutions to benefit greenhouse production and quality. The most significant benefits offered to precision agriculture by IS-technology include: improved crop quality through smart-fertilization; reduced water consumption through efficient fertigation; complete re-circulation in hydroponic production thus mitigating environmental impacts from discharge; improved control of crop quality; and potential early identification of crop health problems.
2.4.3 Environmental Monitoring & Compliance

Ion selective sensor technologies have widespread applications for environmental monitoring and compliance enforcement. A major challenge in combating environmental pollution is the ability to efficiently identify and quantify pollutants. This is particularly difficult in the field setting where environment factors can complicate science objectives. Issues relating to climate change and the contamination of ecosystems are generating a huge demand for robust, affordable sensors capable of quantifying environmental chemistry.

Climate change research typically requires the long-term monitoring of environmental systems supported by dense networks of sensors. In order to predict the fate of Earth’s climate with any certainty, very large sensor networks are required and affordable sensor systems support higher resolution measurements. Current tech-development projects studying climate change effects on oceans are incorporating IS sensors into robotic research submersibles designed to monitor geo-spatial trends in water chemistry [53]. Affordable IS sensors would benefit both industries and regulators. By making measurements efficient and affordable, industries can develop more economical control methods while regulators can develop more economical enforcement practices.

2.4.4 Biological Life Support Systems

Human space exploration beyond Earth orbit requires life support systems capable of producing edible biomass while treating waste streams. These complex systems inherently require networks of sensors to manage environmental variables and coordinate system processes. A framework for Biological Life Support Systems (BLSS) has evolved from a series of projects focused on the development of technology for human life support in space and all compartments of the BLSS process require sensor feedback to maintain control of their chemistry. A simplified version of the life support system outlined by the MELiSSA Project[54], a recent international effort in the advancement of closed-loop life-support systems, is presented in Figure 2.4. The MELiSSA project (Micro-Ecological Life Support System Alternative) was conceived to further the science of artificial ecosystems that incorporate micro-organisms and higher plants, started by the European Space Agency in 1989. The project identified the critical compartments for BLSS and research areas that require attention, generating an extensive bibliography [54]. The moral of the BLSS story is that gases, liquids, solids and the substances they carry must be continuously recirculated within a closed loop and several biological processes are required to convert waste products from microbes, plants and crew into nutrients to be reused in the system. Careful control of environmental variables is essential to the health of these biological units, highlighting the demand for robust sensors. Current directions in BLSS research are focused on plant production and development of a comprehensive, closed-environment production unit as a critical milestone [55].
2.4.5 Exoplanetary Biology

Several destinations in our local solar system appeal to astrobiologists in the search for extra-terrestrial life. Characterizing the chemistry of the lakes and icy surface of moons like Titan, Europa or Enceladus is vital to understanding their astrobiological potential. The extreme distances to these targets make real time communication with experiments impossible (refer to Table 2.3) and the costs of space missions demand excellence. Landed missions to these moons and planets inherently require chemistry data to be recorded and managed autonomously in a reliable and efficient manner. Self-sufficient robotic explorers will use adaptive-science algorithms to make autonomous decisions in order to maximize scientific returns. Projects focused on developing these systems are underway, such as the Planetary Lake Lander project of the SETI Institute [6, 56, 57].

Many chemical species are considered pre-cursors to life based on their role in terrestrial systems. In particular, certain species like perchlorate indicate oxidative potential and in the presence of metals ions can provide a driving force for cellular metabolism [58]. Identifying specific elements on other worlds is a critical first-step in assessing their potential to support life. Robotic explorations are proposed for the near future and include missions to Mars, Titan, and Europa as summarized in
Table 2.3. Reliable sensor technology is a requirement of successful space exploration missions and ion-selective sensors will play an important role. In order to survive the challenges of travel to and through space, technologies must be lightweight, robust and reliable.

<table>
<thead>
<tr>
<th>Planet or Moon</th>
<th>Mean Communication Time (h:min)</th>
<th>Chemistry of Interest</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mars</td>
<td>0:14</td>
<td>NO$_3$, ClO$_4$, Fe, Cl, CaCO$_3$, MgCO$_3$, FeCO$_3$, SO$_4$, K, P, Mg(ClO$_4$)$_2$, As, pH</td>
<td>[58, 59]</td>
</tr>
<tr>
<td>Europa</td>
<td>0:35</td>
<td>H$_2$O$_2$, SO$_2$, H$_2$SO$_4$, nH$_2$O, SO$_4$, Mg and Na Salts, K, Cl, O$_2$, Ca</td>
<td>[60]</td>
</tr>
<tr>
<td>Enceladus</td>
<td>1:20</td>
<td>CO$_2$, N, CH$_4$, C$_3$H$_8$, OH</td>
<td>[61, 62, 63]</td>
</tr>
<tr>
<td>Titan</td>
<td>1:20</td>
<td>NH$_3$, CH$_3$O, CO$_2$, C$_2$H$_6$, C$_3$H$_8$, Ionic Salts</td>
<td>[64, 65, 66]</td>
</tr>
</tbody>
</table>

Table 2.3: Summary of characteristics significant to exo-planetary biology at Mars, Europa, Enceladus and Titan.
Chapter 3
Technology Development

3.1 System Design

Developing an automated system for nutrient management in closed-loop hydroponic systems requires reliable input from ion-selective sensors to provide nutrient status information. Ideally a hydroponic nutrient management system would account for all essential plant nutrients (15+ species) suggesting that a comprehensive nutrient control system could become complex, so simple robust sensors are preferred.

3.1.1 Design Constraints and Criteria

Constraints - the design must...

- facilitate repeatable measurements of ions in solution using at least the 4 optrode sensors available to the project
- be reliable for at least the duration of typical growing cycles of food crops (6-12 weeks)
- not contaminate nutrient streams with ion species or toxic chemicals
- be field-deployable (must support transportation to/operation at field sites, ruggedized to survive travel in planes, trains, and automobiles)
- be suitable for operation in micro-gravity (cannot rely on gravity for draining, must ensure fluid containment)

Criteria - the design should...

- minimize cost
- maximize accuracy of ion-selective measurement
- minimize size, mass and power requirements
- minimize calibration requirements
• minimize training requirements
• maximize reliability and ruggedness

3.1.2 Sensor Evaluations

Evaluation of the sensors available for selective ion quantification was performed and summarized in detail by Bamsey et al. [5] and the results of this summary were presented in Table 2.2 of Chapter 2. A decision matrix used to quantify the evaluation of ISFET, ISE and IS-Optrode sensors is presented in Table 3.1. It was created to aid in the selection of the most appropriate technology for development of an automated nutrient management system for use in hydroponic agriculture.

<table>
<thead>
<tr>
<th>Design Criteria (weight)</th>
<th>ISFET</th>
<th>ISE</th>
<th>Optrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Points: 1 - 3 worst - best</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost (0.05)</td>
<td>Low(3)</td>
<td>Low(3)</td>
<td>Low(3)</td>
</tr>
<tr>
<td>Accuracy (0.05)</td>
<td>Med(2)</td>
<td>Med(2)</td>
<td>Med(2)</td>
</tr>
<tr>
<td>Size, Mass, Power (0.2)</td>
<td>Low(3)</td>
<td>Low(3)</td>
<td>Low(3)</td>
</tr>
<tr>
<td>Calibration Requirement (0.2)</td>
<td>Med(2)</td>
<td>Med(2)</td>
<td>Low(3)</td>
</tr>
<tr>
<td>Training Requirement (0.2)</td>
<td>Low(3)</td>
<td>Low(3)</td>
<td>Low(3)</td>
</tr>
<tr>
<td>Reliability and Ruggedness (0.3)</td>
<td>Low(1)</td>
<td>Low(1)</td>
<td>TBD(?)</td>
</tr>
<tr>
<td>DESIGN MATRIX SCORE (\sum(weight \times points))</td>
<td>2.15</td>
<td>2.15</td>
<td>2.35(1) 2.65(2) 2.95(3)</td>
</tr>
</tbody>
</table>

Table 3.1: Design decision matrix providing quantitative metrics for the qualitative technology comparison provided by Bamsey[5]. Three potential scenarios are considered reflecting low, medium and high scores for the Reliability and Ruggedness factor of optrodes that is currently unknown.

All of the prospective technologies weigh in equally for most of the design criteria, however ion-selective optrodes boast a low calibration requirement, a critical advantage in an autonomous system operating for extended periods without human interaction. Also, the reliability and ruggedness of optrodes sensors in hydroponics is unknown and characterization of optrode performance is required. Three scores for optrode sensors were calculated based on the three potential scenarios of low, medium and high ranking for reliability and ruggedness.

3.1.3 Sensor Selection

Due to a lesser requirement for calibration and the potential for a more reliable and rugged sensor system, ion-selective optrode sensors were selected for development
of an automated nutrient detection system. It should also be underscored that the technology readiness level of the optrode sensors is currently low and that further optimization of the cost and construction is anticipated [5].

3.2 Optrode Theory

The Ion-Selective Optrode System (ISOS) was designed to support the simultaneous detection of several ions in samples drawn directly from nutrient reservoirs of closed-loop plant production units. To make a measurement, the ISOS takes advantage of the selective-exchange process that occurs between a sample solution and the optrode sensor. The optrode film contains a cocktail of chemically engineered molecules working in tandem to produce a measurable response. The following sections describe the basics of optrode design and operation. A detailed review of ion-selective optrode theory in contrast with electrode theory is presented by Bakker [67].

3.2.1 Optrode Design

Ion-selective optrodes typically consist of up to five critical components:

1. an inert support matrix - serves to contain membrane components
2. a plasticizer - swells the support matrix allowing for ion mobility
3. an ionophore - a molecule possessing a selective affinity for target ions
4. a chromoionophore - a molecule that undergoes a colour change
5. an ionic/anionic charge site - a molecule that maintains charge balance in the membrane (may not be required for all optrodes)

The ISOS utilizes Bulk Optrodes, signifying that all components are dissolved in the support matrix and are not covalently bound to the structure. As result, the bulk-class optrode must rely on a competitive ion exchange or co extraction of two species to achieve a response and maintain electro-neutrality within the sensor film [67]. In the case of the optrodes used, an ion-exchange with H⁺ occurs and therefore the H⁺ activity of a sample solution must also be known. The ISOS optrodes contain the five components listed above, using polyvinyl chloride for a support matrix and dioctyl sebacate as a plasticizer. The ionophore, chromoionophore and ionic site used varies depending on the optrode and detailed cocktail recipes are often proprietary information, as in the case of optrodes obtain from collaborators at INO. By adjusting the concentration of membrane components, the sensitivity and range of an optrode sensor can be tailored to meet specific sensing requirements. To design an optrode cocktail one must select an appropriate ionophore, determine a suitable chromoionophore/ionic site combination, and balance concentrations of membrane components to obtain the desired response. Optrode cocktails tailored for hydroponic applications have been developed recently by Bamsey [68, 69] at the the Canadian Space Agency and their recipes are presented.
3.2.2 Optrode Response

The optrode response is based on a reversible ion-exchange reaction between the ionophore and the target ion. Once immersed in a solution, target ions that are present diffuse into the optrode film and bind to the ionophore. The change in ionophore charge-state induces a membrane reaction, and the chromoionophore responds by ejecting or accepting a hydrogen ion to maintain the balance of charge. As a result the chromoionophore undergoes a conformational change and the sensor changes its colour (Figure 3.1). Using a spectrometer, the degree of colour change can be quantified and related to the concentration of target ions - a process that will be described in the following sections.

![Diagram of the optrode response governed by the equilibrium of chemical species between the sensor film and nutrient solution. Ionophores in the sensor film selectively bind target ions (K⁺ above) resulting in changes to the film’s optical properties. The reactions involved are reversible and the colour of the film will reflect the dynamic concentration of the target ion in solution.](image)

Figure 3.1: Diagram of the optrode response governed by the equilibrium of chemical species between the sensor film and nutrient solution. Ionophores in the sensor film selectively bind target ions (K⁺ above) resulting in changes to the film’s optical properties. The reactions involved are reversible and the colour of the film will reflect the dynamic concentration of the target ion in solution.

3.2.3 Optrode Response Function

The theoretical model of the optrode response mechanism is derived from the equilibrium reactions between the species involved. The mechanism is a function of: membrane component concentrations, analyte and reference ion concentration and charge, reaction stoichiometry, and chemical reaction constants. A detailed derivation of an optrode response function for a potassium optrode was presented by Bamsey [49].
The general response function applied to any optrode, as described by Bakker [67], is presented here:

\[
\frac{a^z_{analyte}}{a^z_{ref}} = K_{exch} \left[ \frac{\alpha n C_{tot}^{n-1}}{v_\text{ne}} \left( \frac{p}{n} R_{tot} - (1 - \alpha) C_{tot} \right) \right]^z \left( \frac{1 - \alpha}{\alpha} \right)^v \left[ I_{tot} - \left( \frac{p}{n} R_{tot} - (1 - \alpha) C_{tot} \right) \right]^{p v}
\]  

(3.1)

where \( a_{analyte}, a_{ref}, v \) and \( z \) are the activities and charges of the target ion and reference ion; \( p \) and \( n \) are the stoichiometry coefficients for the ionophore and chromoionophore equilibrium reactions; \( R_{tot}, C_{tot} \) and \( I_{tot} \) are the concentrations of anionic site, chromoionophore and ionophore respectively; and \( \alpha \) is the absorbance ratio. The \( \alpha \) parameter is defined as the ratio of uncomplexed chromoionophore to the total chromoionophore concentration ([\( C_{uncomplexed} \]) : [\( C_{tot} \)]) and is the measured quantity that relates to target ion activity using a known calibration. From Equation 3.1 it is clear that the reference ion activity must be known to determine the target ion activity. All other parameters in the optrode response function are defined by the optrode design, including the \( K_{exch} \) constant that is defined by the reaction kinetics of the chromoionophore-ionophore system as described by Bamsey [49]. The optrode response function describes a sigmoidal curve, the mechanics of which are described in detail by Bakker [67].

Assuming the optrode system employs a chromoionophore using \( H^+ \) as a reference ion rearranging Equation 3.1 to solve for target ion activity yields a generalized optrode response function - Equation 3.2. This relationship can be used to determine target ion activity from an observed optrode response as a function of \( a_{H^+} \) and \( \alpha \), both of which are measured parameters. The activity of the hydrogen ion is measured using a pH probe and the value of \( \alpha \) is determined from the optical response of the sensor, as will be described in the subsequent sections.

\[
\frac{a^z_{analyte}}{a^z_{ref}} = (v K_{exch})^{-1} \left( \frac{\alpha}{1 - \alpha} a_{H^+} \right)^v \left( \frac{R_{tot} - (1 - \alpha) C_{tot}}{I_{tot} - \left( \frac{p}{n} R_{tot} - (1 - \alpha) C_{tot} \right)^p} \right)^v
\]  

(3.2)

### 3.3 Data Analysis Method

#### 3.3.1 Preprocessing

Using the theoretical relationships presented above, the optical properties of an optrode can be correlated to the activity of target ions in solution, provided the activity of the reference ion is known. Hydrogen was the reference ion for the optrodes used in the present work and was measured using a pH probe. Equation 3.2 describes the relationship between ion activity and optical response in terms of the absorbance ratio, being the ratio of uncomplexed chromoionophore to the total chromoionophore concentration. For the ISOS, \( \alpha \) was determined for a particular optrode by measuring the light reflected from its surface, calculating the absorbance using the known source spectra, and comparing the measured absorbance value with the known absorption of the completely uncomplexed chromoionophore (recall \( \alpha = [C_{uncomplexed}] : [C_{total}] \)). For a particular optrode, the value of \( [C_{tot}] \) is known and the value of \( [C_{uncomplexed}] \) is
inferred from the optical properties of the film. It is necessary to process the raw spectra that are collected to eliminate systemic contributions to signal noise and account for optrode variability before calculating the absorbance. This process has several steps including:

1. Averaging of spectra collected in both time and space

2. Removal of dark-current spectrum

3. Normalization of the measured spectra

4. Absorbance calculation

Averaging of the recorded spectra was achieved using built-in functions of the spectrometer (USB4000 - Ocean Optics). Time variations were accounted for by averaging a series of spectra to produce a single output - increasing the number of spectra that were averaged increased the stability of the signal at the expense of processing time. Spatial variations were accounted for by averaging the output value from each cell of the spectrometer’s CCD array over some range of its neighbouring cells using a “box-car” method - increasing the size of the averaging range or “box-car” produced a smoother output signal at the expense of spatial (wavelength) resolution.

Removing the dark spectrum refers to the subtraction of the “dark-current” output of the spectrometer from the actual measured spectra. The dark-current refers to the output from the spectrometer that is observed without any incident photons. Although relatively stable, this baseline output will vary with time and should be recorded at the time of measurement for all normal sensor operations. Allowing the spectrometer sufficient time to warm up (more than one hour) also serves to stabilize the dark current output [49]. For practical measurement purposes, a warm-up phase can be avoided by recording a reference spectra reading before every optrode reading to characterize any drift in baseline.

The light that is reflected (not absorbed) by the optrode sensor is received by the spectrometer. Any variations in the system components that influence the measured spectra can be accounted for with signal normalization, assuming that the influence is wavelength independent. Normalization is typically performed by considering specific wavelengths of the absorption waveform at which the chromoionophore does not absorb light. By this method, signal variations at the target wavelength are assumed to apply uniformly to the entire waveform and a correction factor is applied to the entire spectrum (for a detailed description of this process refer to the work of Bamsey [49]). Another method of normalization is employed by collaborators at INO that accounts for shifts in the baseline of spectra readings by considering the far-red regions of the output where the sensor materials do not absorb (between 734 and 827 nm)[70]. By subtracting the baseline from the measured absorbance spectrum, baseline shifts resulting from optical connections and other systemic errors can be corrected to preserve the shape of the signal waveform.

Once the measured spectra have been averaged, adjusted for dark-current and normalized to remove non-wavelength dependent variations, the absorbance of the
optrode can be calculated by relating the measured spectra to the reference spectra. The calculation of absorbance \((A)\) is demonstrated in Equation 3.3 where \(M\), \(D\), and \(R\) are the measured, dark and reference spectra respectively:

\[
A = -\log\left(\frac{M - D}{R - D}\right)
\]  

\(3.3\)

### 3.3.2 Ratiometric Measure of Optrode Response

Once the treated data have been converted into absorbance measurements, an estimate of the absorbance ratio can be made. The ISOS system takes advantage of the absorption properties of the chromoionophore to perform the analysis. The chromoionophore absorbs light at discrete wavelengths defined by the completely protonated and unprotonated states of the molecule. Assuming the optical properties of the sensor are only influenced by the analyte activity, the ratio of the absorbance intensity between these critical wavelengths can be related to \(\alpha\). This process is described visually in Figure 3.2.

![Example Optrode Response for Low-Ion Concentration](image)

![Example Optrode Response for High-Ion Concentration](image)

**Figure 3.2:** Diagram of absorption spectra for an optrode highlighting the response to a low concentration (green line) and high concentration (red line) of target ion in solution. The two extreme cases shown (purple and pink lines) represent the completely complexed and uncomplexed states.

The absorbance measured at wavelengths for which absorption peaks are known to occur (approximately 545 and 660 nm for the case above) are compared to yield
the absorbance ratio. Based on the previous work of Bamsey [49], the absorbance ratio is considered as the lower wavelength peak divided by the higher wavelength peak \( \frac{A_{545}}{A_{660}} \). Relating the ratiometric value to ion concentration requires a calibration curve to convert the measured ratiometric response to a concentration value.

**Isosbestic Point**

The absorbance spectra illustrated in Figure 3.2 demonstrate an important feature of spectroscopy known as the isosbestic point that is highlighted in both figures. This point refers to a specific wavelength at which the total absorbance does not change for a given system, even as it undergoes some chemical or physical reaction. In the case of an optrode sensor system, the isosbestic point provides a stable benchmark to assess the stability of the sensor and its relative performance. Under ideal conditions only changes of the analyte state will influence the form (colour) of the absorbed spectra and therefore any deviation of the isosbestic point from its starting position indicates the sensor is functioning improperly. A stable isosbestic point is an indicator that the sensor system is working well while a downward drift indicates a loss of absorbance and could suggest loss of dye due to leaching or photo-degradation.

**3.3.3 pH Correction**

The pH of the measurement environment influences the absorbance ratio of the optrode sensors used in this study, as described by Equation 3.2, signifying that pH must be known to calculate the absorbance ratio and determine analyte activity. In plant production systems, pH is typically monitored, however in environmental monitoring or field applications a pH measurement must be made along side every optrode measurement. It is interesting to note that a particular optrode cocktail will have a variable operating range depending on the pH of the measurement environment. Therefore, it is conceivable that pH can be adjusted to accommodate measurements that are out of the sensor range under normal operating conditions.

**3.4 Calibration**

The response mechanism of the optrode sensor is, by theory, completely reversible and as a result optrodes do not require the frequent calibration necessary for sensors that leach or consume critical system components. The optrode manufacturing process influences the final optrode calibration and a calibration check must be performed on new cocktails to determine actual performance; however, the calibration for single cocktail can, in theory, be applied to all optrodes produced from it since they will possess the same proportions of membrane components. Due to complications during the testing phase, the reproducibility, calibration, stability and life time of the optrode calibration from both collaborators was not characterized within the scope of this project.
3.4.1 Calibration Procedure

Calibration of optrode sensors should be performed in a background solution that is appropriate for the application. This is owed to the nature of ionic solutions as discussed in Chapter 2. Each ion-selective optrode variety must be calibrated separately however sensors made from a single membrane cocktail will yield optrodes with identical compositions and therefore identical calibrations. The optrode manufacturing process will impart some systematic variation in the physical properties of the optrodes such as membrane thickness, however normalization techniques described earlier can account for any differences. A refined production process wherein optrodes are mechanically coated (either on a mirrored cap or fibre) will help ensure the uniform application of a well mixed optrode cocktail to the support surface. The calibration procedure for the present application required the creation of a background solution containing the species known to exist in standard fertigation water, except for the target calibration ion.

The example below describes a calibration performed for a calcium optrode at the Canadian Space Agency laboratories in Montreal, Quebec. The background solution, a half-strength Hoagland solution without calcium, was pH adjusted to 6.00 as prescribed amounts of CaCl$_2$ were added to the solution at ten minute intervals. During the additions the optrode probe was continuously immersed in the solution and measurements were recorded every minute. The resulting data formed a calibration series that could now be applied to the set of calcium optrodes made from that cocktail. To relate optrode measurements to the activity of target ions, the measured $\alpha$ value is compared with the calibration $\alpha$ values. The upper plot in Figure 3.3 presents the absorbance spectra measured during the calibration and the lower is a plot of the ratiometric response value calculated by comparing the absorption peaks at 500.13 and 639.86 nm.

3.4.2 Ion-Interferences

Interference on the sensor response from non-target species is critical to consider in the development of a multi-ion detection system. The influence of interfering species can be considered on the solution-side and on the optrode-side of the sensor response mechanism. On the solution-side, increasing the concentration and/or complexity of a solution will impact the activity of dissolved chemical species. This effect serves to cloud the actual concentration of analyte species in complex solutions. The activity coefficient described in Equation 2.2 is approximated as a function of ionic strength using empirical relationships such as the Debye-Huckel method, and details of its application in the context of hydroponics are presented by Bamsey [49]. On the optrode-side, increasing the concentration and complexity of a solution will increase the potential for interference binding with ionophores in the optrode film. This effect can temporarily or permanently alter the performance characteristics of the sensor contributing to false readings and therefore ionophores for ion selective sensors must be selected with care as emphasized by Patko [43]. The inherent challenges of multi-ion detection can be overcome through systematic characterization of interference.
Figure 3.3: The upper plot presents absorbance spectra for a calcium optrode calibration run performed at the Canadian Space Agency in Montreal, Quebec. The spectra reveal a very stable isosbestic point indicating a healthy optrode. The lower plot presents the absorbance ratio calculated using the spectra above.

effects. In the context of controlled environment plant production, the challenge is simplified since the chemical components of nutrient solutions and their concentrations are known and prescribed. Existing optrodes for potassium and calcium have demonstrated selectivity and measuring ranges that meet the requirements of hydroponic solutions, as summarized in Table 3.2 [68, 69]. Optrode cross-sensitivity using multiple sensors simultaneously has yet to be assessed, however a thorough characterization of the relationships will yield a system capable of correcting for problematic interferences. Cross-sensitivity information can also be exploited as a means to confirm measurements of other sensors.

3.5 Ion Selective Optrode System Development

The use of ion-selective optrodes for online monitoring of hydroponic solution quality has not been documented. To integrate optrode sensors into an active plant production system, several important operating factors were considered. Growers require a system that is affordable, relatively simple to use, and reliable for at least the duration
of typical growing cycles (6 - 12 weeks). An ideal monitoring system would minimize maintenance requirements and cost of inputs. To determine the feasibility of creating an online monitoring system, characterization experiments were performed to assess the use optrodes in hydroponic solutions.

A hardware system for operating a suite of optrodes was required for performing characterization experiments, interfacing with hydroponic equipment, and conducting field measurements. The development of the Ion-Selective Optrode System (ISOS) was conducted at the CESRF with support of the Digital Haptic Lab Rapid Prototyping Facility [71].

This section provides details on the ISOS design process and highlights the significant findings of the prototype testing phase. Experience gained during this phase of the project drove the development of the ISOS operating procedures and the methods for system demonstration (Chapter 4).

### 3.5.1 ISOS Process Schematic

The ISOS consists of the following components shown schematically in Figure 3.4: (1) light source that is on continuously; (2) optical switch to limit exposure of light to sensors; (3) inline optical filter to reduce the quantity of incident light on optrode sensors; (4) bank of variable attenuators to balance light intensity for variations in optrode connections; (5) suite of optrode sensors encased in a light-proof housing; (6) fiber optic multiplexer for multi-optrode readings; (7) spectrometer to measure output from the optrode fibres; (8) computer data acquisition system; (9) microcontroller to coordinate switch, multiplexer, and spectrometer operations. For details on the specific hardware used in the ISOS system refer to Appendix B.
ISOS Hardware Diagram

Figure 3.4: Schematic diagram of the Ion Selective Optrode System (ISOS) showing critical hardware components and their connections. Solid arrows indicate the path of light through the system and dashed arrows indicate electronic signal connections.

3.5.2 Prototype Construction

Development of the ISOS was a multi-staged process. At the beginning of the project initial concept drawings were generated based on the project description. Optrode hardware was obtained from our project partners at the Canadian Space Agency (CSA) and National Optics Institute of Canada (INO). Two different sensor configurations were supplied: CSA optrodes featured a sensor film coated on a mirror that reflected the light signal back to a bifurcated fibre optic probe stem; INO optrodes featured an sensor film coated on an optical fibre through which a light signal was transmitted, as pictured in Figure 3.5. Rapid prototyping technologies were used to generate physical models of conceptual designs and test critical design features. 3D printed prototypes led to significant design revisions and recommendations for further prototyping. A final design for the field instrument version of the ISOS was produced in October 2013.
3.5.3 Brainstorming

The design phase considered ideas for isolating optrodes from the environment and accommodating flow-through or injection/batch-style water samples. A subset of the preliminary housing design concepts are presented below - for additional concept drawings refer to Appendix A.

Compartmentalized Optrode Battery (COB)

The COB was conceived out of consideration that an ideal ion selective sensor system for hydroponics could require up to 16 ion sensors. A 16-sensor system has inherent complexity and the COB design was intended to support easy exchange of expired sensors by replacing individual “kernels”. The circular design enabled the COB to exploit centrifugal forces for operation in zero-gravity. A simple rendering of the concept is presented in Figure 3.6.

Due to the small scale of optical components it has been envisioned that the COB could be very compact, with individual kernals approximately 5-10 cm$^3$ each holding optrodes for single-ions. The kernal design allows individual optrodes compartments to be removed and replaced as necessary using a quick-disconnect system. A central manifold would act as a support structure for the kernals as well as provide samples of nutrient solution for analysis.
Figure 3.6: A: Original concept sketch of the Compartmentalized Optrode Battery (COB). B: CAD model of the COB created using Google Sketchup. The COB features removable optrode cartridges or “kernels” that permit easy exchange of individual sensors.

Cross (X)-Flow Chamber

The cross-flow (X-Flow) chamber was conceived to support both the probe and fibre style optrode sensors. The probe-style sensors could be included inline using standard Tee-fittings and custom caps - a simple design that could accommodate many sensors by appending additional Tees. The fibre-style optrodes could be supported by a plumbing X-fitting and custom caps. Owing to the small size of optrode fibres many could be housed in a single X-fitting provided they were restricted from contacting one another. An ideal configuration was envisioned that would promote thorough sample mixing in the chamber and is presented in Figure 3.7.

Figure 3.7: Concept sketch of the X-Flow chamber created using Google Sketchup. The X-Flow chamber features a single, removable optrode cartridge that permits easy exchange of expired sensors.
3.5.4 Preliminary Concepts

X-Flow

Owing to its simplicity and the potential for assembly from low-cost, off-the-self components, the X-Flow concept was selected as a preliminary housing design. The concept was refined and detailed sketches were generated and communicated to design specialists at the Digital Haptic Lab (DHL) for prototype construction. The design consisted of a flow through cell that directed a water stream past a bank of optrode sensors (Figure 3.7). The optrodes were supported by a cap system that held the fibres rigidly in place, which prevented movement of fibres as samples flowed past them. Studies can characterize the influence of flow rate through the sample cell on sensor performance to optimize operating parameters.

A carefully designed cap system could support multiple sensor fibres while providing a water-tight seal capable of withstanding small pressure as required for zero-g applications. In consultation with designer Dr. John Phillips of DHL, an endcap concept capable of holding up to 16 optrode fibres was realized. Achieving a reliable seal was recognized as the critical design challenge for a 16-fibre system.

Replacement of expired optrodes was also considered. It became apparent that the endcap fibre system would be cumbersome to manipulate, and increasing the number of fibres would certainly increase the challenge. This consideration spawned the concept of a fibre-cartridge that would hold multiple optrodes rigidly in place allowing the complete sensor bank to be easily removed and replaced. The X-Flow system is shown graphically in Figure 3.8 highlighting the removable sensor cartridge (an eight-fibre version holding a single fibre) and the flow-cell sample style. An intellectual property disclosure was filed with the Catalyst Centre of the University of Guelph in 2015 describing the fibre cartridge concept - the first stage in seeking patent protection. Original concept drawings of the X-Flow have been included in Appendix B.
Gasket Fibre Cartridge

Experience gained working with the X-Flow prototype and concerns regarding watertightness drove the prototype evolution in a new direction. Working with the idea of an easily removable/replaceable optrode cartridge, a simplified flow-cell concept was considered. An in-line cartridge would greatly simplify the creation of a water-tight seal at the cartridge-interface (Figure 3.9: A). A gasket that could be “sandwiched” between adjoining faces of the housing body to form a pressed-seal around the optrode fibres was conceived. The housing body was sized to accommodate 8 x 15 cm optrode fibres and was clamped together using 6 set-screws (Figure 3.9: B & C). Inlet and outlet fittings were included to accommodate flow-through measurements. Engineering drawings of the housing prototype are included in Appendix C.

Ultimately, the Gasket Fibre Cartridge system failed due to pronounced leaks from the optrode-gasket interface and from the 3D printed materials, despite attempts to close the porous structure. Solutions to these problems were conceived; however the stage of the project warranted a new approach to housing design to facilitate data collection. The leak issue could be solved by creating a gasket embedded with optrode fibres, that could be easily manufactured and ultimately sold as a sensor replacement cartridge. The small size of the optrode fibres would allow for a very small product to be created (5 x 9 cm). The form of the sensor housing was suitable, however the final prototype would be machined or moulded and leaks from the housing body would be negated.
Figure 3.9: A: Original concept sketch that generated the inline cartridge system. B: 3D rendering of the X-Flow Box with removable sensor cartridge system shown closed in operation mode. C: 3D rendering of the X-Flow Box with removable sensor cartridge system shown in exploded view to reveal the custom gaskets and bank of eight optrode fibres.

**Bench-Top System/Field Instrument**

An old concept, originally intended to support optrode storage, was reconsidered for development of a bench-top optrode measurement system. The simple idea consisted of a custom lid for a standard sample container that accepts a fibre loop and isolates the fibre from the ambient environment. A fibre lid was created to support optrode testing using single fibres early in the project (Figure 3.10 A).

In consultation with DHL, the lid was expanded to hold four fibre-loops and a quick-disconnect system was devised using magnets allowing quick assembly and removal (Figure 3.10 B). Ultimately the lid and sample vessel were outfitted with fittings to allow inflow and draining of water samples for automated operation. Detailed drawings of the lid assemblies are presented for reference in Appendix C.

A tower was designed to support the ISOS (Figure 3.11). The design created by DHL consisted of four threaded rods that stood vertically on rubberized bases connecting two aluminum plates. The plates supported the ISOS hardware: the upper plate held a bank of four light attenuators and fibre-optic couplings to connect up to four optrode sensors; the bottom plate held the sample vessel. The plates were precision cut using a water jet cutter (Flow Water-Jet Mach 2031b, [72]) - for detailed drawings refer to Appendix C.
Figure 3.10: Photo of 3D printed (A) single-optrode and (B) multi-optrode lid assemblies. The former is capable of holding a single INO-style optrode fibre in a sample container while the latter can support up to four. Both lids were designed to prevent contact between optrodes and container walls and both serve to isolate the optrodes from the ambient environment.

Figure 3.11: Photos of the ISOS support tower fully assembled. A: Shown holding four optrode fibres with the sample vessel open and key components identified. B: Shown holding four optrodes in operation mode. The three piece sample vessel lid serves to hold fibres rigidly, isolate samples from the environment, and prevent background light from affecting sensor readings.

3.5.5 Rapid Prototyping

Concept sketches were delivered to the Digital Haptic Lab and adapted into 3D-CAD models by Dr. John Phillips using SpaceClaim version 2012. Parts were printed using the Dimension 1200es 3D printer by Stratsys Inc in ABS plastic and typically took two days to complete [73, 74]. The use of 3D printing allowed for rapid turnaround on prototype production and nearly-real-time trouble shooting of production issues.
3.6 Testing

Optrodes obtained from project collaborators required exploratory testing to determine the feasibility of developing the ISOS. Studies were performed at the CESRF to explore the technical limitations of applying ion-selective optrodes in hydroponics.

3.6.1 Exploring Life Time Issues

The first tests were focused on characterizing the lifetime of optrodes in hydroponic solutions. Optrode development studies conducted by Bamsey [49] determined the life expectancy of potassium and calcium optrodes to be 70 and 40 hours respectively with continuous immersion in the sample solution [68, 69]. The hypothesis that optrode failure was most likely due to leaching of plasticizer from the sensor membrane into the sample solution was supported by in-house studies at INO. It was suggested that periodic immersion could reduce leaching and extend sensor lifetime. Experiments conducted at INO revealed that periodic immersion significantly reduced plasticizer leaching [70]. A simple experiment was designed to characterize the effect of periodic immersion on sensor lifetime at CESRF.

A testing apparatus was constructed allowing up to 8 sample reservoirs to be filled periodically based on a predefined control sequence. Figure 3.12 highlights the critical components and illustrates the principle of operation. A PLC (Moeller Easy 618-AC-RC) coordinated the activation of solenoid valves and pump service to fill pairs of sensor reservoirs in specified watering cycles, the duration and period of which were customized. Constant head was maintained in each reservoir using a two-drain system (refer to frame D in Figure 3.12). After each watering cycle was complete, the reservoir drained by gravity leaving the sensor exposed to ambient air. The blue horse trials were intended to characterize the effects of cycled immersion and trying to identify possible hysteresis or stabilization issues; however, results were plagued by unexpected results as described in the following sections. The wiring diagram for the control system is presented in Appendix C for reference.

3.6.2 Periodic Optrode Exposure

Optrodes were exposed to hydroponic solutions both continuously and in periodic watering cycles. Based on previous studies by INO, a two-hour-on/two-hour-off cycle was chosen. Results from preliminary tests identified several issues that must be considered in ISOS development including: minimization of system contamination from microbes and precipitates, protection of the optrodes from light interference and damage, the importance of proper sensor fabrication and the importance of proper optrode handling. The control code used to operate the PLC is presented in Appendix D for reference.
3.7 Data Interpretation

3.7.1 Confusing Data at Startup

Lifetime studies performed to characterize periodic optrode exposure were not successful. Optrode response was extremely unstable, repeatable measurements were not obtained, and unpredictable sensor drift was observed. Figures 3.13, 3.14 and 3.15 illustrate the types of unpredictable sensor behaviour that was observed in preliminary characterization studies.

Figure 3.13 describes a cycled immersion study wherein an optrode for potassium was subjected to a ten minute watering event every hour. Data points from the dry cycle have been removed for clarity. The solution pH was not controlled and early variations in the sensor response are difficult to explain, however the sensor response was relatively stable for the first 10 hours of operation. After the 10 hour mark the
Figure 3.13: Response of a potassium optrode periodically exposed to hydroponic solutions in cycles of 10 minute solution exposure followed by 50 minute exposure to air. Data during the dry cycle have been removed for clarity. The sensor response seemed reasonable, but began to drift significantly after approximately 10 hours.

sensor response began to drift significantly.
Figure 3.14: Response of a potassium optrode continuously immersed in hydroponic solution. The sensor exhibited a relatively constant drift for most of the exposure. The sensor did respond to changes in potassium concentration, however after 20 hours of exposure, the same changes in concentration produced a smaller response. After 24 hours the optrode began to drift unpredictably.

Results from a continuous immersion trial for a potassium optrode is presented in Figure 3.14. The optrode was immersed in a continuously recirculating sample container while the fertilizer solution was modified manually. The optrode response drifted continuously during the trial, yet seemed to respond well to changes in potassium concentration. The dilution of the reservoir and addition of potassium produced smaller response when repeated after 20 hours of continuous immersion. The sensor response began to deteriorate after 24 hours of exposure.

Figure 3.15 demonstrates the improved optrode response after implementing careful pH control and using only fresh nutrient solutions. The stability and repeatability of the sensor improved significantly however a steady degradation of the response was observed with time - in particular after resuming the trial on Day 2. It was noted that the LED light source was on continuously and the optrode was not protected its light when not in use.
Figure 3.15: Response of a potassium optrode exposed to three hydroponic solutions with variable potassium concentration. The sensor was shown solutions with medium → high → medium → low concentrations of potassium sequentially, after which the cycle was repeated. The sensor response appeared to be very stable, however decreased in amplitude with each exposure cycle, decreasing significantly between Day 1 and Day 2 of the trial.

### 3.7.2 Membrane Contamination

It was apparent that a red film was building up on the hardware of the Blue Horse, including the optrodes (Figure 3.16). The solenoid valves used in the test apparatus (Orbit 24VAC) were not corrosion resistant and rusted considerably. The contamination of the system with iron was likely a major source of widely variable optical response (Figures 3.17). The build up of rust in the system did not become apparent until the system had been operational for approximately one month. At that point, inspection of the solenoid valve revealed heavy corrosion. Replacing the valves with stainless steel components (ASCO Red-Hat 8262G7) eliminated iron contamination issues. Future ISOS development must consider material selections to avoid contamination, especially from ions significant to plant nutrition.
Figure 3.16: After preliminary testing of sensors in the Blue Horse apparatus, it became apparent that a red coating was developing on all system hardware. This contamination was likely a major contributor to irregular sensor behaviour.

Figure 3.17: A preliminary, long duration optrode lifetime study revealed a steady degradation of the optical response of the sensor that began soon after the start of the trial. The impact of contamination on the optical properties of the sensor was significant.
3.7.3 Biofouling

Eliminating metal contamination restored stable sensor behaviour initially, however the expected lifetime was cut considerably short. In most cases, fresh optrodes would maintain a stable response for roughly 8 hours before behaving unpredictably (Figure 3.18) and visual inspection of the sensor membranes revealed a cloudy appearance. Figure 3.19 demonstrates the difference between a ‘fresh’ optrode and a ‘failed’ optrode. The centre image shows a fresh optrode that is uncloudy and transparent, while the left and right images show failed optrodes that are noticeably cloudy. The left sensor film also shows a very prominent cloudy feature, indicative of a localized issue.

![K+ Optrode Response During a Continuous Immersion Study Using a Used Nutrient Solution](image)

Figure 3.18: Response of a potassium optrode exposed continuously to a hydroponic solution. Sensor response was very stable for approximately 10 hours, after which the response drifted unpredictably.

Biofouling was suspected as the source of the cloudy formation, based on the visual appearance of the failed sensors. The composition of the optrode sensors used provide ideal habitats for microorganisms, and it was understood that the hydroponic solutions were not sterile. Nutrient water sampled at the start and end of a test run was plated on agar to reveal a significant increase in microbe population. The result highlighted the importance of maintaining membrane sterility for prolonged operation. Future ISOS iterations must incorporate disinfection processes into normal operation. A UV lamp was included in the recirculation loop of the testing apparatus to reduce biological activity for future tests.
Figure 3.19: Photos of optrode sensor membranes reveal cloudy formations that, based on their shapes and colour, are likely of biological origin. B: A fresh optrode film was visibly transparent and purple. A: An expired optrode membrane after exposure to hydroponic solutions shows a cloudy formation at its top and a generally speckled complexion. C: An expired optrode membrane after exposure to hydroponic solutions shows a generally cloudy surface with concentrated white patches.

The trends observed in Figure 3.18 were not encountered during the previous investigation by Bamsey.

Figure 3.20: Sensor response for a potassium optrode exposed continuously to a fresh hydroponic solution. Optrode response was stable for long duration exposures and errors previously observed did not occur within 48 hours of continuous exposure.

3.7.4 Mechanical Failure

Once the supply of optrode caps obtained from CSA were exhausted, the optrode fibres from INO were incorporated into the ISOS development program. The remaining data sets were collected using the INO fibre-optrodes. Preliminary testing of fibre-based sensors revealed unpredictable repeatability issues, despite careful attention to avoid contamination (Figure 3.21). It was suggested that mechanical failure stemming from membrane fabrication practices could be the source of the problem [75]. Traditionally optrode films were applied by hand using a fine tip paint brush.
to coat an unclad fibre with the sensor cocktail. Once expired, optrode films were removed from the optical fibre using tetrahydrofuran, and a fresh film was repainted from a stock of fresh membrane cocktail. It is probable that contamination during this process resulted in poor adhesion of the film to the optical fibre and improper sensor function. INO produced a batch of mechanically coated fibres to rectify the issue. Performance of the new optrode fibres that were mechanically coated immediately after being drawn improved dramatically. The new fibres produced a stable, repeatable response as demonstrated in Figure 3.22.

Figure 3.21: A potassium optrode exposed to hydroponic solutions of variable strength displaying very irregular behaviour is plotted. Mechanical failure of the sensor due to manufacturing inconsistency was considered.

Figure 3.22: Response of a mechanically coated calcium optrode exposed to hydroponic solutions with different calcium concentrations. Sensor response was stable and repeatable. The sensor response was also consistent when comparing different test days, indicating that sensor performance did not degrade during storage between measurements.
3.7.5 Light Effect

A slow steady decay in the absorption of light by the optrode films was observed with continued sensor exposure. The drift rate was constant and seemed to only occur when the fibre was in use (down time did not result in a response drift) suggesting that either exposure to the solution or exposure to light were factors. It was also observed, upon visual inspection, that optrodes seemed ‘pale’ after use in comparison to fresh optrodes. To test the effect of photo bleaching on the INO fibres, a small segment of fresh fibre was placed directly in the light path of the LED light source and left for five days, after which the fibre was virtually colourless when inspected (Figure 3.23).

![Figure 3.23: Photo comparing the optical properties of an optrode fibre before and after direct exposure to an LED light source for five days. The sensitive dye in the optrode film is evidently degraded by the light exposure.](image)

An optical shutter was installed to minimize the exposure of light to the sensor membrane - opening for one second only when measurements were made. For the ISOS, a two-way optical switch (2x2FOS by Ocean Optics) was installed and timing was controlled using a TTL signal from the Arduino micro-controller. This solution was less than ideal, but took advantage of on-hand equipment - a more appropriate shutter can be installed in the future. Limiting light exposure reduced the rate of sensor drift to a negligible effect.
3.8 System Optimization

The results from preliminary optrode testing have highlighted significant operational details to be considered for the development of the ISOS. Several design features are recommended to improve the reliability of the system and facilitate the collection of useful measurements.

3.8.1 Membrane Filtration

The ISOS must include disinfection protocol to protect the sensor and improve reliability. Most plant production systems include disinfection for plant health purposes. However, secondary filtration of samples for ISOS measurements is critical. A 20 µm membrane pre-filter would be sufficient for removing bacteria and viruses from solution while permitting the flow of nutrient ions. Owing to their small scale, the fibre-optrode sensors from INO required very small volumes of water for analysis and a simple, automated pre-filtration system to supply the ISOS is readily feasible.

3.8.2 Manufacturing Changes

Changes to the manufacturing process of the fibre sensors from INO improved the consistency of the optrode sensors and measurements considerably. It stands to reason that continued refinement of manufacturing process will further optimize productive measurements from the ISOS. Specifically, streamlining the process of connecting fresh optrode segments to the ISOS will improve measurement efficiency and drive the evolution of a commercially viable system.

3.8.3 Limited Light Exposure

Limiting the exposure of optrodes to light seemed to improve the long-term stability of the sensor response. The ISOS should incorporate a shutter system to limit the exposure of optrodes to light. Considering the nature of the optrode response mechanism that will be described in the following sections, the ISOS light source could be simplified using narrow bandwidth LEDs that target the known absorption wavelengths of optrode components, further limiting exposure from unnecessary photons. If the degradation issue happens to be wavelength specific, simplifying the light source could eliminate the issue completely. This effect will be characterized in detail during the next phase of prototype development.

3.9 System Integration

3.9.1 Feedback Control

The output from the ISOS will ultimately be used as input for a Feedback Control Nutrient Management System (FCNMS). An automated nutrient management system must use data from ion-selective sensors to assess solution quality, determine necessary
nutrient adjustments, and deliver a prescribed dose of the required stock nutrients to the nutrient reservoir. The process flow diagram shown in Figure 3.24 highlights the critical system components. Ion-selective measurements will be made periodically to assess nutrient concentration status and nutrient injections will be triggered when measurements stray too far from user-defined set-points. Samples will be drawn from the fertigation network using a by-pass loop, disinfected using an ultra-filtration membrane, and injected into the ion-selective sensor module. After measurements are completed the sample is returned to the fertigation stream. Sensor data will be processed by a computer module that will generate a correction recipe if required. The correction order will be sent to the nutrient dosing controller and prescribed amounts of stock solution will be dispensed, mixed and added to the reservoir.

Figure 3.24: Critical components of an integrated Feedback Control Nutrient Management System.

The proof of concept for the ISOS represents completion of the final technical challenge facing development of a FCNMS. The remaining system components exist and need only to be sourced and configured for the specific application. Filtration membranes are readily available and sizing a filter requires knowledge of design flow rates for the system. The infrastructure for nutrient dosing systems are also readily available, are utilized in greenhouse facilities already and adapting existing hardware and controls would be a simple engineering exercise [76]. The control software for the FCNMS must be customized and will require relatively complex control algorithms as the number of ions and interferences increases, requiring the support a software developer.

3.9.2 Nutrient Dosing

The concept behind automated nutrient dosing is simple and reliable hardware and control systems capable of single-nutrient or single-salt dosing exist. Current commercialized dosing systems strive to maintain nutrient solution quality through nutrient salt injections and some systems are integrating commercially available ion-selective sensors. Often these systems are limited to the standard two-part nutrient solutions of traditional hydroponics making them prone to accumulations or deficiencies. Adapting these systems to single-salt dosing will require the expansion of controller inputs/outputs, more stock nutrient tanks, proportioning injector controls for each
tank, and dense sensor networks to ensure accurate operation [76]. The complexity of an automated nutrient dosing system will increase with the number of nutrient stock solutions, and many stock solutions are required to provide sufficient system flexibility. Due to the dynamic nature of plant nutrient uptake, the “permutations and combinations of stock solutions required to achieve a given recipe can become quite complex [76].” Identifying and quantifying the relationships between plant-environment interactions and plant nutrient uptake is a critical first step toward selecting an inclusive suite of stock solution and developing dosing algorithms.

3.9.3 Field Instrument Development

Prototype ISOS Field Instrument

The requirement for field measurements using the ISOS prompted the design a field deployable instrument to overcome challenges of field operation relating to: transportability, environmental exposure, power requirements, and ease-of-use. The bench-top system described in Section 3.5.4 was intended to support field measurements. The optical hardware were fixed into a metal component box to accommodate transportation to field sites. An Arduino (Arduino UNO) controller was used to coordinate shutter operation and spectra acquisition - for control sequence code refer to Appendix C. The bench-top system prototype was completed in October 2013.

Field Instrument Evolution

Performance of the ISOS field instrument was comparable to laboratory performance as characterized during the field demonstration that will be later described in detail (Chapter 4). The ISOS has demonstrated the potential for reliable optrode field measurements. The field instrument evolution will focus on simplification of system hardware to reduce cost, including but not limited to: replacing the light source with low-power, narrow-bandwidth LEDs targeting desired colours of chromoionophore interaction; replacing costly multiplexer-spectrometer combo with several spectrometer-on-a-chip units [77]; eliminating shutter system by using light-pulse method; replacing Arduino controller with a customized controller; replacing long fibre patch cords with compact, engineered hardware; and creating a quick-disconnect system for optrode replacement.

Interplanetary Field Applications

Another field application of the ISOS is focused on remote water quality monitoring. Based on the successful field trial of the system development has begun on a remote water quality lab called the Optrode Immersion Multi-Chemistry Explorer (OPTICX) in collaboration with the SETI Institute Figure 3.25. The primary goal of the OPTICX is development of an autonomous chemistry lab capable of in situ measurements to characterize aqueous chemistry in extra-terrestrial environments. Proposed deployment sites include the surface waters and lakes of Titan, the oceans
below the thick ice crust of Europa or within its polar plumes, and the icy grounds of Mars [53].

**OPTICX SUBMERSIBLE CHEMSITRY LAB**

Figure 3.25: Conceptual rendering of the OPTICX submersible chemistry probe, designed to autonomously perform underwater science investigations focused on characterizing chemistry in both space and time. Used with permission from Dr. Pablo Sobron - SETI Institute.
Chapter 4

Optrode Field Instrument Demonstration

To explore the application of ion-selective optrode measurements in the context of environmental monitoring, the ISOS was included in a field expedition to a remote, glacier-fed lake (Laguna Negra, Chile) as part of the SETI Institute’s 2013 Planetary Lake Lander project. The PLL project is focused on developing adaptive science technology for robotic space exploration. The following chapter details the field deployment and successful detection of relevant species using the ISOS: $Na^+$, $Ca^{2+}$, and $NO_3^-$. The results demonstrated that optrodes are a suitable technology to characterize water chemistry in the field and potentially for exploring aqueous environments on other planetary bodies within our solar system.

4.1 Background

The 2013-2022 Decadal Survey prepared by NASA identifies three themes of scientific interest in the context of planetary exploration ([59]):

1. Building new worlds - understanding the beginnings of the solar system;
2. Planetary habitats - searching for the requirements for life;
3. Workings of solar systems - revealing planetary processes through time.

Within the framework of the three themes, ten primary scientific questions were outlined along with missions to various planetary destinations designed to address them. Half of the 24 exploration missions prioritized are to be landed or sample return missions, highlighting the need for a better understanding of the surface and subsurface characteristics of planetary environments. The OPTICX system, described in section 3.9.3 is well positioned to answer several of the ten primary scientific questions under consideration, as highlighted in Figure 4.1.

The next generation of planetary exploration missions focuses on surface and subsurface processes and NASA has funded a number of mission concept studies designed to define the scope of these investigations. Some of the more prominent examples
Figure 4.1: Visual overview of the current science objectives in space exploration highlighting synergies between the science objectives of the OPTICX hardware development. (Used with permission from Dr. Pablo Sobron SETI Institute)

include: Mars Ice Breaker Life [58], Mars Polar Climate [78], Europa Lander [60], Sub-Ice Marine and Planetary Analog Ecosystems (SIMPLE) [79], Deep Pheratic Thermal eXplorer (DEPTHX) [80], Titan Mare Explore (TiME) [66], and Planetary Lake Lander [6].

There is a clear trend toward more autonomous and flexible robotic platforms for planetary exploration. However, it is important to recognize that under-sampling in time and space during any scientific mission will limit our understanding of any geochemical, physical and biological processes that operate on other worlds. The ISOS is capable of high resolution, \textit{in situ} sampling and addresses the technical challenges of providing rapid, quantitative multi-chemistry analyses of aqueous solutions with a high degree of spatial resolution.

The ISOS was included in the 2013 PLL field campaign to demonstrate the feasibility of performing optrode field measurements in extreme environments. The suitability of the optrode system for planetary exploration missions will depend on the sensitivity, precision and reliability of the sensor systems. Upon successful proof of concept, the next phase of development will quantitatively assess these parameters and initiate a technological development toward a flight instrument responsive to mission requirements.

4.2 Objectives

The ISOS was tested in the field December 12-18 2013, during the PLL project field campaign [6]. The main objective was to determine the feasibility of using optrode
technology for science investigations at an extreme field site. A critical secondary objective was to evaluate use of the ISOS in the field and determine any potential for optimization of methods and/or hardware for field operation.

4.3 Experimental Design

4.3.1 Equipment Design

The ISOS was tasked with characterizing water chemistry, in situ, in the context of the development of adaptive science for PLL. The ISOS was ruggedized to accommodate transportation to the remote mountain location and to protect sensitive optical hardware the final hardware component box is presented in Figure 4.2.

Figure 4.2: Front and back-end hardware were fitted into a component box to accommodate transportation to and operation in field sites. A desktop computer shell was retrofitted to support optical devices and patch cords.

4.3.2 Field Test Protocol

Sample Collection

Grab samples were collected from the surface waters of Laguna Negra from the side of a Zodiac in 500 mL Nalgene bottles. Locations were selected for sampling based on proximity to interesting features such as waterfall outflows and algae deposits. Samples were returned to base camp and left in the field laboratory overnight to settle.

Sample Analysis

The pH of each water sample was measured and adjusted to 7 using $HCl$ before exposure to the optrodes sensors. The samples were poured manually into the sample vessel in sequential order (#0 → #5) based on the identifiers listed in Table 4.1 below.
Once samples were loaded, the vessel lid was “flicked” several times to remove any bubbles stuck to the optrode fibres. Optrode measurements were collected at roughly one minute intervals until a stable response was observed (roughly five minutes).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>$pH_o$</th>
<th>$pH_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>CONTROL - Distilled water (pH adjusted)</td>
<td>n/a</td>
<td>6.985</td>
</tr>
<tr>
<td>1</td>
<td>Amazon Cave</td>
<td>7.3</td>
<td>6.988</td>
</tr>
<tr>
<td>2</td>
<td>West Shore</td>
<td>8.6</td>
<td>6.988</td>
</tr>
<tr>
<td>3</td>
<td>Yellow Algae Spot 2</td>
<td>8.7</td>
<td>6.956</td>
</tr>
<tr>
<td>4</td>
<td>Victoria Falls</td>
<td>8.1</td>
<td>6.866</td>
</tr>
<tr>
<td>5</td>
<td>Yellow Algae Spot 1</td>
<td>9.4</td>
<td>6.989</td>
</tr>
</tbody>
</table>

Table 4.1: Summary of water sample details including sample location on Laguna Negra (Chile) along with starting pH and the adjusted sample pH after additions of $NaOH$ or $HCl$.

4.4 ISOS Field Operations

4.4.1 Transportation

The ISOS traveled to the field site via air and truck transportation - all components were in working order upon arrival. Some components had moved around inside the ISOS box yet no damage to optical hardware or fiber connections was observed. The ISOS will be further ruggedized as the system matures, reducing the risk of transportation damage. Appreciating the small scale of optical hardware, current developments in spectroscopy (spectrometer on a chip technology, for example) and advances in LED hardware, a compact, hand-held device for measuring optrode response is certainly conceivable and would greatly improve transporability.

Fresh optrode sensors were assembled for the field study at the CESRF using freshly coated optrode fibres sent from INO. The optrode sensors were transported to site inside standard office file-folders that were covered with sheets of aluminum foil inside. The aluminum foil protected the optrodes from contact with materials that could wick membrane components. The fibres were secured within the folders using scotch tape to prevent sliding and to ensure that sensor tips did not contact one another.

4.4.2 Sample Preparation

Sensors for $Ca^{2+}$, $Na^+$ and $NO_3^-$ were tested in the lake water using samples collected from various locations. The samples were given time to settle to minimize contamination of the optrodes by suspended solids. The pH of each sample was adjusted using dilute $HCl$ or $NaOH$ to a value of approximately 7.00 (Actual pH values ranged from 6.866-6.989 as summarized in Table 4.1). The adjustment was made to bring
the sample solutions to the operating pH of the optrodes used prescribed by INO. To demonstrate the function of the ISOS the optrodes were immersed in six different water samples for roughly five minutes and readings were taken at 1 minute intervals. In all cases, the sensor response was relatively stable after 30-60 seconds. The process was performed twice to ensure that the response was repeatable.
4.4.3 Optrode Field Results

Calcium

Absorbance spectra measured from the calcium optrode channel are plotted in Figure 4.3. The plot revealed a stable isosbestic point indicating the optrode was functioning properly. Some signal noise was evident in the blue end of the absorbance curves, attributed to limited blue light in source-spectrum and limitations of the methods for spectra normalization.

Figure 4.3: UPPER: Plot of absorbance from a calcium optrode exposed to surface water samples from Laguna Negra, Chile. LOWER: Plot of ratiometric response calculated from the absorbance spectra showing repeatable measurements.
Repeated exposures of the calcium optrode to the various samples collected around Laguna Negra resulted in very stable and repeatable measurements as presented in the lower plot of Figure 4.3. The sensor was exposed to each solution until a stable reading was observed (roughly 1-2 minutes) plus an additional 2 minutes - meanwhile spectra were collected once per minute. Between solutions the sensor was quickly rinsed with distilled water and each solution exchange took 2-3 minutes.
Sodium

Absorbance spectra measured from the sodium optrode channel are plotted in Figure 4.4. The plot revealed a stable isosbestic point indicating the optrode was functioning properly.

![Sodium Optrode Absorbance Measurements and Ratiometric Response Value](image)

Figure 4.4: UPPER: Plot of absorbance from a sodium optrode exposed to surface water samples from Laguna Negra, Chile. LOWER: Plot of ratiometric response calculated from the absorbance spectra demonstrating sodium detection.

Repeated exposures of the sodium optrode to the various samples collected around Laguna Negra resulted in one relatively stable and repeatable measurement as presented in the lower plot of Figure 4.4. The sensor was exposed to each solution until a stable reading was observed (roughly 1-2 minutes) plus an additional 2 minutes - meanwhile spectra were collected once per minute. Between solutions the sensor was quickly rinsed with distilled water and each solution exchange took 2-3 minutes.
Solution #0 was the only solution to illicit a significant response from the sodium sensor.
Nitrate

Absorbance spectra measured from the nitrate optrode channel are plotted in Figure 4.5. The plot revealed a less-stable isosbestic point in comparison with the calcium and sodium optrodes, indicating the optrode was not functioning properly. The nitrate optrode did not respond to sample changes, suggesting negligible concentrations of nitrate. Reference spectra measured in weak acid and base solutions are plotted to demonstrate the range of sensor function. Some signal noise was evident in the blue end of the absorbance curves, attributed to relatively lower intensity of blue light in source-spectrum coupled with limitations of the methods for spectra normalization.

![Nitrate Optrode Absorbance Measurements](image1)

![Nitrate Optrode Ratiometric Response Value](image2)

Figure 4.5: UPPER: Plot of absorbance from a nitrate optrode exposed to surface water samples from Laguna Negra, Chile. LOWER: Plot of ratiometric response calculated from the absorbance spectra indicating samples do not contain nitrate.

Repeated exposures of the nitrate optrode to the various samples collected around Laguna Negra resulted in no noticeable response from sensor as presented in the lower
plot of Figure 4.5. The sensor was exposed to each solution until a stable reading was observed (roughly 1-2 minutes) plus an additional 2 minutes - meanwhile spectra were collected once per minute. Between solutions the sensor was quickly rinsed with distilled water and each solution exchange took 2-3 minutes. A steady drift in the sensor response was observed and could have been related to drift in solution pH or leaching of membrane components.

### 4.4.4 HPLC Analysis

Results from HPLC analysis are presented in Table 4.2. The pH of each sample was adjusted to obtain a $pH \approx 7.00$, the operating pH of the optode sensors. Sample #0 was distilled water, and both $NaOH$ and $HCl$ were added to stabilize the sample pH as indicated by the HPLC data. Sample #1 → 5 were collected from the surface waters of Laguna Negra and had a starting $pH > 7$ that was lowered using $HCl$, again indicated by HPLC data. Entries of (-) indicated that the sample range was below the lower detection limit of the HPLC. The presence of $K^+$ ions in each solution was assumed to be due to leaching from the pH probe. In particular, the concentration of $K^+$ was highest in Sample #0, the distilled water control sample, as it required significant time for pH adjustment/stabilization, and the pH probe was immersed in this solution for a much longer period.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cl ppm</th>
<th>NO$_2$ ppm</th>
<th>NO$_3$ ppm</th>
<th>PO$_4$ ppm</th>
<th>SO$_4$ ppm</th>
<th>Na ppm</th>
<th>NH$_4$ ppm</th>
<th>K ppm</th>
<th>Mg ppm</th>
<th>Cu ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.14</td>
<td>-</td>
<td>11.49</td>
<td>0.06</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>11.68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20.84</td>
<td>1.43</td>
<td>-</td>
<td>5.8</td>
<td>2.03</td>
<td>14.04</td>
</tr>
<tr>
<td>2</td>
<td>5.88</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>23.9</td>
<td>1.81</td>
<td>-</td>
<td>3.01</td>
<td>0.7</td>
<td>13.07</td>
</tr>
<tr>
<td>3</td>
<td>8.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.29</td>
<td>-</td>
<td>2.84</td>
<td>0.67</td>
</tr>
<tr>
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<td>1.55</td>
<td>-</td>
<td>1.42</td>
<td>0.69</td>
<td>9.9</td>
</tr>
<tr>
<td>5</td>
<td>6.38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.75</td>
<td>-</td>
<td>3.01</td>
<td>0.66</td>
<td>12.98</td>
</tr>
</tbody>
</table>

Table 4.2: Results from HPLC analysis of water samples collected on Laguna Negra (Chile) during the 2013 PLL Project. Analysis was performed at the Controlled Environment Systems Research Facility at Guelph University.
4.4.5 Discussion of Results

The performance of the ISOS in the field was comparable to performance in the lab. The ISOS was able to repeatedly detect ion species at very small concentrations. Interferences from other ions were negligible, which is to be expected given the very low ionic strength of the samples. The data presented in Table 4.2 validated the measured response from the ISOS. As anticipated, \( \text{NO}_3^- \) was not detected by HPLC in any sample. Small concentrations of \( \text{Ca}^{2+} \) were detected in lake samples, a fact that validated the response of the optrode sensor in the field. Trace amounts of \( \text{Na}^+ \) were detected in all samples, due to small additions of \( \text{NaOH} \) for pH adjustment, and Optrode measurements were supported by these data.

![Figure 4.6: Plot of calcium and sodium concentration in lake samples from Laguna Negra as measured by optrode sensor and HPLC.](image)

Reflecting on Figure 4.6, the theoretical optrode response based on Equation 3.2 does not align well with the HPLC results for the calcium optrode. The response of the sodium optrode was suitable, however the scale of the calibration was not correct. Several possibilities exist that can account for the errors. For calcium, there is a strong possibility that original calcium concentrations of the samples could have changed significantly between the time of collection and HPLC analysis. Owed to the crude sample collection/storage methods and relatively small calcium content of the samples, the HPLC analysis cannot be considered representative of the site conditions. This theory is supported by the fact that most samples are close to the solubility of calcium carbonate. The sodium optrode, although capable of reproducible measurements, could only detect significant sodium in one sample and thus the comparative quality of measurements was not determined. The observed deviation from the theoretical calibration could be a function of the optrode’s age or of differences in the background environment of the measurements. In either scenario, this result reinforces the fact that site-specific calibration is likely required for field applications.
4.5 Conclusions

4.5.1 Feasibility of Optrode Field Instrument

The first deployment of the ISOS in the field was a success. The technology demonstrated the ability to selectively detect ions in small concentration in a water source of unknown composition. The field environment imposed some pressure on the ISOS including transportation stress, environmental stress (temperature change, winds, high-UV), and operational stress (lack of deionized water supply, dust/dirt contamination). Effort was made to protect the system from most hazards and sources of contamination, and the field laboratory was very clean and well supported. The ISOS performed well in this challenging environment and data collected in the field were of reasonable quality compared to those collected in the lab. HPLC analysis of water samples returned to the lab demonstrated that the water samples were very clean, containing only trace amounts of ions. The successful detection of \( Ca^{2+} \) and \( Na^+ \) by the ISOS was confirmed by HPLC.

Optrode measurements in the field did not align well with the HPLC results, in particular for the calcium optrode. Some possible explanations for disagreement include age of the optrodes (eight months) and/or changes in solution chemistry between the field and lab (calcium carbonate). Results for the sodium optrode were in better alignment with the theoretical response although the calibration slope was incorrect - this result highlights the need for site specific calibration. Developing protocols for site-specific calibration will be critical to developing a successful field instrument.

The ISOS was tested from the side of a zodiac to determine the feasibility of \textit{in situ} measurements (Figure 4.7). Provided the optical hardware was shielded from ambient light, suitable reference measurements were made, and pH of target water bodies were measured, \textit{in situ} measurements with the ISOS were possible.
4.5.2 Recommendations

Based on the success of the field demonstration, further development of the ISOS is recommended. The next evolution will yield a system that is better suited to field deployment considering specifically: cost, miniaturization, optimized packaging, optimized Optrode replacement procedure, and optimized sample handling methods. Ultimately an autonomous ISOS will be developed, well suited for robotic space exploration. Specifically, many of the expensive optical hardware components can be replaced with low-cost versions as analytical precision is not required, which should also help reduce the size of hardware components and overall system footprint. Costly optical patch cords can be substituted for customize optical connections that are of appropriate length and form to optimize the assembly of hardware – this will greatly reduce the footprint of the device as the current horizontal footprint is defined by the minimum-bend radius of the patch cords. Once suitably miniaturized, an optimized casing for the system hardware can be developed and, given the small scale of optical and electronic components, a hand-held device is conceivable. Improvements to the optical connections between the optrode sensors and the ISOS detector will support efficient ion measurements and a quick disconnect system using standard optical hardware is in development with DHL.
Chapter 5

Red versus Blue Light Experiment

5.1 Introduction

5.1.1 Background

It has been demonstrated in studies conducted at CESRF that the lettuce variety *Lactuca sativa* cv. New Red Fire displays morphological variations dependent on the colour of to which it is exposed [81]. Lettuce grown in red light produces light green leaves that were larger in size, while lettuce grown in blue light produced purple leaves that were smaller. It seemed reasonable to expect that changes in physical character may be correlated to changes in nutrient usage/uptake by plants.

5.1.2 Objectives

Identify and quantify trends in nutrient uptake by *Lactuca sativa* cv. New Red Fire and identify changes in nutrient uptake in response to changes in light quality.

5.1.3 Experiment Overview

Lettuce seeds were sown into rockwool cubes (Delta 6.5h 42/40, Grodan Inc. Milton, Ontario) and transferred to a four-trough hydroponic growing system within a closed environment plant chamber at the CESRF. Plants were established for 33 days under a mix of red and blue light and fed from a single common nutrient reservoir using continuous Nutrient Film Technique irrigation. On Day 34 a second nutrient reservoir was added, providing two independent, equal volumes of nutrient solution each feeding two of the four troughs. The lighted environment was also divided in half using a black polypropylene sheet. On Day 35 the LED light source was reconfigured to provide red light to half of the plants and blue light to the remainder. After 1.5 weeks the plants were harvested and analyzed.
5.2 Experiment Protocol

5.2.1 Preparing Plants

1. 20 dry rockwool cubes were weighed and numbered using a permanent marker (Sharpie, Sanford Brands, Oakville, Ontario.). The cubes were left to soak overnight in deionized water prior to use.

2. 4 identical hydroponic troughs were assembled to support 5 rockwool cubes each, for a total of 20 cubes.

3. Three seeds of *Lactuca sativa* cv. New Red Fire were planted in each rockwool cube to ensure successful germination of each plot. Seedlings were thinned to one plant per cube 4-5 days after planting.

4. The planted trays were loaded into a sealed, controlled environment chamber (Hypobaric Chamber 1 at the CESRF) and grown for 5 weeks at 22°C (isothermal). Vapor pressure deficit, carbon dioxide and light intensity were maintained at 1.1 kPa, 400 ppm and 300 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) respectively for the duration of the experiment. The photoperiod was set to 16:8 hours light:dark. Lighting was provided by an internal, water cooled 80 x 90 cm LED array (Lumigreen AS, Norway). The array consisted of 512 LEDs, 128 each of Philips Inc. (Amsterdam, Netherlands) Red (638nm), Deep Red (660 nm), Blue (472 nm) and Royal Blue (440 nm), with each wavelength separately controlled with dimmable (0-10 VDC) power supplies (Figure 5.1). Lighting in this experiment was limited to Deep Red and Royal Blue only.

![Figure 5.1: Plot of power output from the lighting array for each LED type.](image)

5.2.2 System Environment Parameters

Table 5.1 summarizes the set-points values used to control the environment within the sealed chamber used to grow plants during both Growth Phase 1 & 2.
<table>
<thead>
<tr>
<th>Environmental Variable</th>
<th>Control Set Point Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>22°C</td>
</tr>
<tr>
<td>Vapour Pressure Deficit</td>
<td>1.1 kPa</td>
</tr>
<tr>
<td>Carbon Dioxide</td>
<td>400 ppm</td>
</tr>
<tr>
<td>Light Intensity</td>
<td>300 $\mu$mol $m^2s^{-1}$</td>
</tr>
<tr>
<td>Photo Period</td>
<td>16:8 hours light:dark</td>
</tr>
</tbody>
</table>

Table 5.1: Summary of control set-point values for environmental conditions within the hypobaric chamber used for Growth Phase 1 & 2.

### 5.2.3 Growth Phase 1

1. For Phase 1 the four plant troughs were all fed by a common nutrient reservoir (Figure 5.2) and nutrient solution was recirculated continuously using two submersible pumps (one pump supplied two troughs).

2. Fifty liters of nutrient solution was mixed using deionized water and a complete water soluble fertilizer (Plant-Prod 7-11-27, [82]). pH was adjusted to approximately 5.8 using 1.0 M NaOH.

3. In Phase 1 plants were grown under 300 $\mu$mol $m^2s^{-1}$ light made up of both red light (660 nm) and blue light (440 nm) for the first 35 days of plant growth. The ratio of red to blue light content was 82:18 (red:blue) and was selected based on the work of Stutte [81]. Figure 5.3 shows uniform plant growth 15 days after planting.

4. The quality of the nutrient solution was maintained by monitoring EC and pH, making adjustments at Plant-Dawn as required (pH 5.5-6.2, $E.C. > 1000\mu S$).
When E.C. values were below 1000 $\mu$S the nutrient solution was refreshed during Phase 1.

5. The uptake of nutrients by plants was monitored by sampling nutrient reservoir for offline HPLC analysis (two samples were taken at plant-dawn, and two samples were taken at plant-dusk).

![Growth Phase 1](image1)

**Figure 5.3**: Photo of *Lactuca sativa* cv. Individuals on Day 16 during Growth Phase 1 and on Day 35 during Growth Phase 2.

### 5.2.4 Growth Phase 2

1. On Day 33, plants were removed from the chamber and the hydroponic troughs were reconfigured to be fed from two independent reservoirs as in Figure 5.2. (While removed, plants were photographed under white light to document plant growth status).

2. The growth chamber was divided using a physical barrier (black plastic) to isolate the two hydroponic troughs on either side, taking care to prevent leakage of light.

3. A fresh nutrient solution was prepared and 20L was added to each reservoir.

4. On Day 35 the LED array was reconfigured and split into red and blue treatments. Two troughs of lettuce were exposed to 300 $\mu$mol $m^2s^{-1}$ red light and the remaining two troughs were exposed to 300 $\mu$mol $m^2s^{-1}$ blue light, as shown in Figure 5.3.
5. In Phase 2 nutrient solution quality was not maintained as in Phase 1 and the solution created on Day 33 was used until the end of the trial.

6. Condensed water from atmospheric moisture control was stored in a container until sample collection at plant-dawn was performed. During sample collection exercises for Growth Phase 2, the volume of condensate collected was measured and the total volume was dispensed between the two reservoirs 50:50.

7. Plant nutrient uptake was monitored by sampling each reservoir for off-line HPLC analysis (One sample was taken at plant-dawn, and one was taken at plant-dusk).

8. Sample collection was continued until plants displayed a noticeable difference in their appearance, which required 7-10 days. Upon conclusion of the experiment plants were harvested by separating shoot from root and bagging individual lettuce heads separately. Fresh weights of shoot material were measured following which all plant materials were dried at $60^\circ C$.

9. Once dried completely, plant materials were weighed to obtain the dry-mass of individual lettuce heads.

5.2.5 Analysis

1. Water samples collected during the investigation were analyzed by HPLC. (Simadzu liquid chromatograph)

2. Plant dry mass was determined for shoots and roots separately.

3. Samples were homogenized and submitted for tissue analysis to determine nutrient content in shoot material (SGS Agri-Food Laboratories)

5.3 Results

The chamber experiment clearly demonstrated the effect of light quality on *Lactuca sativa* cv. A clear distinction was observed between the plants grown in blue light compared to those grown in red light. Plants grown in blue light produced reddish-purple pigments and grew more densely while plants grown in red light were light-green in colour and produced larger leaves. Analysis of reservoir samples revealed interesting trends: the rate of nitrate uptake was enhanced by blue light exposure, while uptake rates of the other quantified ions did not change significantly. Tissue analysis confirmed an increased content of nitrogen, potassium and zinc in Blue-Light-Treatment (BLT) plants in comparison to Red-Light-Treatment (RLT) plants. The tissue content of the other nutrient elements studied did not vary as significantly between treatments; however, in general BLT plants seemed to sequester more of most nutrient ions. One exception was observed with iron where it appeared to accumulate more in RLT plants.
5.3.1 Visual Comparison

The lettuce grew very uniformly for the first 33 days. The left photo in Figure 5.4 shows the plants photographed under fluorescent lighting on Day 33. The lettuce were similar in size and colouration in all four troughs. On Day 35 the light treatments were split and plants were exposed to either red or blue light for 10 days. On Day 46 the experiment was terminated and the plants were removed for harvest. The right photo in Figure 5.4 shows the plants illuminated by white light before harvest.

![Head-to-Head Comparison of Lettuce Treatments](image)

Figure 5.4: LEFT: *Lactuca sativa* cv. individuals on Day 33 under white light. RIGHT: The same *Lactuca sativa* cv. individuals on Day 45 before harvest, also under white light. Some leaf rot was evident on individuals from both light treatments. LOWER: A lettuce head to head comparison after harvest.

The lettuce heads displayed a clear morphological difference after 10 days of exposure to their new light environment (lower image of Figure 5.4). The BLT plants had a purple colouration and appeared to grow in more tightly packed clusters. The RLT plants had a bright-green colouration and appeared to grow larger leaves that were less densely packed. The crop consisted of individuals roughly 15-20 cm in diameter. It was clear that the chamber was congested and horizontal spread of the plants was likely restricted. Some evidence of leaf rot was visible on some individuals at the
interior of the experiment, resulting from insufficient air flow.

5.3.2 Plant Weights

Individual lettuce heads were dried and weighed and results are summarized in Figure 5.5. The total dry mass produced in each treatment were not significantly different, yielding an average of 907.8 g from RLT and 877.2 g from BLT.

![Average Total Mass of Plant Matter By Treatment](image)

**Figure 5.5:** Average total dry mass of *Lactuca sativa* cv. New Red Fire individuals by light treatment for Trials 1, 2 and 3.

On average the RLT individuals were slightly heavier and typically more variable at 9.3±0.87 g than those from the Blue-Light treatment BLT at 9.0±0.78 g, although the difference was not significant. These data were supported by visual observations of the plants. Trial 2 and Trial 3 plants were grown three days longer and as a result, were significantly larger than those from Trial 1.

5.3.3 Nutrient Data

*False Results* Nutrient reservoir samples collected during Growth Phase II were analyzed using HPLC and revealed interesting trends. Figure 5.6 illustrates potassium uptake for all three trials and some significant features were observed. Analysis of Trial 1 and Trial 2 samples revealed very similar uptake trends: uptake of potassium appeared constant and similar for both treatments before the light regime was changed on Day 5. At the end of Day 9 the nutrient solution was refreshed. At 10 days after light-change, the uptake of potassium appeared to diverge with the RLT taking up more. This trend was confirmed and continued in Trial 2 when plants were
left to grow longer. A similar trend was observed for the other major nutrient ions analyzed by the HPLC system.

Before the start of the third trial, it was considered that the uptake of nutrients observed could be influenced quite significantly by errors imparted from improper dilution of nutrient reservoirs with the condensate water. It was initially assumed that any differences in transpiration between the two light treatments should be insignificant since the amount of light was balanced to 300 $\mu$mol $m^2 s^{-1}$ in each treatment. In Trial 3 the nutrient reservoirs were replaced with modified containers equipped with a site-level to improve the management of nutrient-tank volume. During Trial 3 sample collection it became apparent that the BLT was consuming more water than the RLT. If BLT transpiration was greater than RLT transpiration, while the reservoirs were being diluted equally, the nutrient concentrations would have been biased toward the trends shown by Trial 1 and 2 data in Figure 5.6.

![POTASSIUM UPTAKE DATA REVEALS VOLUME ERROR IN REP 1 & 2](image)

Figure 5.6: Plot of Potassium uptake from nutrient reservoirs for Trials 1, 2 and 3. Trends observed in Trial 3 did not match Trial 1 and 2 as volume changes of the nutrient due to differential transpiration resulted in erroneous, divergent data. Volume was strictly controlled in Trial 3 to confirm the error.

*Correction Factor Development Using Phosphate* To recover the data from Trial 1 and Trial 2 a correction factor was conceived that would correct each measurement by adjusting the volume of the BLT reservoir to an “effective volume” compensating for the confounding volume error. The correction factor was derived from the data sets for phosphate since in Trial 3, phosphate uptake was constant irrespective of light treatment as presented in the upper plot of Figure 5.7.

Developing the correction factor required a comparison of Trial 3 data with that of Trials 1 and 2. The data for phosphate was selected as a comparison benchmark as Trial 3 trends revealed that the rate phosphate uptake was independent of light
Figure 5.7: UPPER: Plot of phosphate uptake from nutrient reservoirs for Trials 1, 2 and 3. Trends observed in Trial 3 did not match Trial 1 and 2 as differences in transpiration between treatments resulted in erroneous, divergent data. Volume was strictly controlled in Trial 3 to confirm the error. Trial 3 phosphate uptake appeared to be independent of light treatment and was used to develop a correction factor for Trial 1 and 2 data. LOWER: Corrected phosphate data using a data correction factor for Trials 1 and 2.

quality. It was observed that the first part of the each trial’s data set was relatively similar (the block of data recorded before the light colour was switched until roughly three days after the switch was made – 7 days). From this time forward, data in Trials 1 and 2 seemed to diverge from the Trial 3 example (Figure 5.7). The BLT samples appeared to decrease uptake of phosphate and while phosphate uptake in the RLT did not change significantly.

During Trial 3 it was determined this change was due to the concentrating effect
of the reservoir volume error discussed previously. Since the diverging data formed relatively linear trends, a simple transformation was applied to adjust the data points to match the slope of the previous time-period (as it does in Trial 3). The correction was considered a “volume-correction” accounting for the missing water in the BLT reservoir. RLT data did not seem to diverge from the linear trend observed in Trial 3 so the data was not adjusted. The correction factor calculated to adjust the phosphate data generated trends for Trials 1 and 2 that matched those of Trail-3 indicating that the correction factor was suitable (lower plot of Figure 5.7). The same volume correction was then applied to all other ions in the data sets for Trails 1 and 2 yielding the corrected data plots presented below. In general, the correction factor was effective in bringing data from the first two trials in-line with those of the third.
Corrected Potassium Uptake Results

Figure 5.8: Plot of potassium uptake from nutrient reservoirs for Trials 1, 2 and 3 after volume correction factors were applied to data from the BLTs. Trends observed in Trial 1 and 2 now align with the trends observed in Trial 3. Data suggest potassium uptake is independent of light quality.

A plot of potassium uptake from the nutrient reservoirs, after data correction factors were applied, is presented in Figure 5.8. The divergent trends observed in Trial 1 and Trial 2 results were eliminated by the volume correction factors and align well with the data from Trial 3. The data suggest that the rate of potassium uptake for *Lactuca sativa cv.* New Red Fire is independent of light quality. It is interesting to note that in Trial 3, potassium was depleted in both treatments by Day 10. There are two significant implications to this result:

1. E.C. of the solution was still relatively high (≈ 800µS) suggesting nutrient supply should be adequate, however lettuce tend to have high demand for potassium and quickly stripped the solution of the critical ion, a fact only revealed by ion-analysis.

2. The depletion of potassium on Day 10 resulted in complementary decreases in the uptake of other important ions including nitrate, sulphate and phosphate that all rely on potassium regulated transport systems to facilitate movement into plants [1]. This result underscores the importance of ion-selective analysis of nutrient solution quality and the demand for the ISOS in recirculating systems.
Corrected Nitrate Uptake Results. Nitrate uptake trends from Trial 1, 2 and 3 are presented in Figure 5.9. Interestingly, nitrate uptake in the BLT seemed to accelerate slightly following the start of Growth Phase 2, while the uptake rate in the RLT treatment remained constant. In Trial 3 nitrate uptake in both treatments slowed down on Day 10, coinciding with the depletion of potassium from the reservoir. An increased uptake of nitrate in the BLT plants could be explained by an increase in production of pigments and secondary metabolites associated with the adaptation to blue light. As previously discussed in Chapter 2, nitrogen is an elemental component of many plant pigments, enzymes, and other proteins.

Figure 5.9: Plot of nitrate uptake from nutrient reservoirs for Trials 1, 2 and 3 after volume correction factors were applied to data from the BLTs. Trends observed in Trial 1 and 2 now align better with the trends observed in Trial 3. Data suggest nitrate uptake increased for individuals grown in blue light versus those grown in red light.
Corrected Sulphate Uptake Results Corrected uptake trends of sulphate followed similar patterns as phosphate and uptake rates were similar for both BLT and RLT treatments, as shown in Figure 5.10. Rates of uptake did not change considerably when the light regime was changed, suggesting that sulphate uptake is independent of light quality for *Lactuca sativa* cv. New Red Fire. In Trial 1 and 2, uptake was relatively constant for both light treatments throughout the 10 day period, while uptake of sulphate virtually ceased in both BLT and RLT treatments of Trial 3 on Day 10, coinciding with the depletion of potassium from the reservoir. This observation underscores the requirement of potassium for sulphate metabolism [1] and the need for ion-selective nutrient management in CEA.

![Figure 5.10: Plot of sulphate uptake from nutrient reservoirs for Trials 1, 2 and 3 after volume correction factors were applied to data from the BLTs. Trends observed in Trial 1 and 2 now align better with the trends observed in Trial 3. Data suggest sulphate uptake is independent of light quality.](image-url)
Corrected Calcium Uptake Results Corrected trends of calcium uptake from the reservoirs of BLT and RLT plants are presented in Figure 5.11. Data from Trial 3, when reservoir volume was strictly controlled, reveal calcium ions were taken up at similar rates by both BLT and RLT plants and both data sets seem to become noisier as the experiment progressed. In Trail-1 and 2 data suggest that the BLT plants may be taking up more calcium than the RLT plants. Upon termination of the trials, it was observed that mineral salts had accumulated on the reservoir walls. In the case of Trial 1 and 2, where evapotranspiration rates were higher, it is likely that more mineral scale would have accumulated on the tank walls, and could account for the differential draw down and the increase in measurement noise with time. This logic was supported by visual observations of the scale formations revealing more deposits in the BLT reservoir.

![Image](image_url)

Figure 5.11: Plot of calcium uptake from nutrient reservoirs for Trials 1, 2 and 3 after volume correction factors were applied to data from the BLTs. Trends observed in Trial 1 and 2 do not match those of Trial 3. Data from Trial 3, wherein volume was strictly controlled, suggest that calcium uptake was independent of light quality.
Corrected Magnesium Uptake Results Corrected trends of magnesium uptake from the reservoirs of BLT and RLT plants are presented in Figure 5.12 and reflect similar trends as calcium. Data from Trial 3 reveal magnesium ions were taken up at similar rates by both BLT and RLT plants and both data sets seem to become noisier as the experiment progressed. In Trail-1 and 2 data suggest that the BLT plants may be taking up more magnesium than the RLT plants. Upon termination of the trials, it was observed that mineral salts had accumulated on the reservoir walls. In the case of Trial 1 and 2, where evapotranspiration rates were higher, it is likely that more mineral scale deposits would have accumulated on the tank walls, and could account for the differential draw down of magnesium and the increase in measurement noise with time. This logic was supported by visual observations of the scale formations revealing more deposits in the BLT reservoir.

Figure 5.12: Plot of Magnesium uptake from nutrient reservoirs for Trials 1, 2 and 3 after volume correction factors were applied to data from the BLTs. Trends observed in Trial 1 and 2 do not match those of Trial 3. Data from Trial 3, wherein volume was strictly controlled, suggest that magnesium uptake was independent of light quality.
Discussion of Uptake Results

The data presented reveal several critical results from the blue versus red light growth trial with *Lactuca sativa* cv. New Red Fire:

1. The uptake rate of some nutrient ions is dependent on light quality, in particular nutrients associated with protein synthesis (nitrogen) and enzyme function (zinc and iron).


3. Quantification of divalent cations in solution can be complicated by formation of mineral scale on reservoir walls.

4. Ion-selective nutrient analysis of hydroponic nutrient solutions can yield management information that can save costly nutrients and labour, while optimizing plant growth.

In the case of nitrate, the rate of uptake increased for both treatments once the light regime was switched to either blue or red and uptake of nitrate increased more for the BLT plants. Uptake for potassium appears to be independent of light quality for the species under consideration. Trends in uptake of calcium and magnesium seemed to align, and both showed a jump in uptake for the BLT treatment plants following the change in light quality; however this increase can likely be attributed to an increase in precipitate scale formation due to the higher evapotranspiration rates observed. Phosphate and sulphate uptake trends also appeared to align and the uptake of both ions appeared to be independent of light quality. In Trial 3 the uptake of nitrate, phosphate and sulphate slowed on day 10, coinciding with the depletion of potassium from the reservoir. Potassium is involved in many ion-mediated transport processes, and this experiment demonstrated that potassium depletion in Trial 3 contributed to decreased uptake rates of nitrate, phosphate and sulphate.

In all cases, data from Trial 1 and 2 were severely influenced by the changes in reservoir volume owed to the difference between RLT and BLT evapotranspiration rates. This issue is a consequence of the relatively low concentration of nutrients in hydroponic solutions and the relatively slow rates of plant nutrient uptake compared with the rates of water consumption. So, unless the nutrient reservoir is extremely large, volume changes will impact concentration measures in a significant way.

Build-up of mineral scale on the walls of nutrient reservoirs can have significant impacts on the nutrient balance of hydroponic solutions, especially if water levels change frequently and the reservoir is small. In particular, measurements of calcium and magnesium became increasingly noisy as plant growth trials progressed, a trend which is likely due to the buildup of scale. This logic is supported by Trial 3 results, wherein reservoir volume was strictly controlled and measurement sample noise decreased.
5.3.4 Tissue Analysis

Harvested plant material was dried and homogenized for tissue analysis performed off-site at SGS Agrifood Laboratories (Guelph, ON). Samples from each treatment were combined to provide close to the required 100 g for sample analysis.

Light Independent Ions

*Phosphorus* The uptake of phosphorus did not seem to vary significantly based on light treatment (Figure 5.13). This result is supported by the nutrient uptake data presented previously that showed uptake of phosphate to be independent of light quality. The roles of phosphorus in plant metabolism are widespread, and direct correlations between light quality and phosphorus uptake are likely harder to discern.

![Phosphorus Dried Plant Tissue](image)

Figure 5.13: Phosphorus content in dried plant shoot. Phosphorus content in plants did not differ significantly between plants grown under blue or red light treatments.
Magnesium & Calcium The uptake of magnesium and calcium did not seem to vary significantly based on light treatment (Figure 5.14). This result is in agreement with the nutrient uptake data presented previously that showed uptake of these ions appeared to be independent of light quality. Blue light plants seem to sequester more of both magnesium and calcium, however the differences are not significant. It is interesting to note that both divalent cations seem to produce similar uptake and tissue analysis results.

![Figure 5.14: Magnesium and calcium content in dried plant shoots. Magnesium and calcium content appeared to be greater in BLT plants but the difference was not significant.](image)

Manganese and Copper The uptake of manganese did not seem to vary significantly based on light treatment nor did the uptake of copper (Figure 5.15).
Figure 5.15: Manganese and copper content in lettuce grown under blue or red light only. The amount of both manganese and copper appeared to be greater in BLT plants but the difference was not significant.

**Light Dependent Ions**

_Nitrogen_ Analysis of dried plant matter from the blue versus red light plant growth trials revealed interesting trends. Data suggest that plants sequester nutrient ions at different rates based on their light environment. In particular, the sequestering of nitrogen is pronounced in _Lactuca sativa_ cv. New Red Fire individuals that are grown under blue light as shown in Figure 5.16. This result is supported by the nutrient uptake data measured by HPLC analysis of nutrient samples collected during the second growth phase, and by classical understanding of plant nutrition.

_Potassium_ Tissue analysis revealed that potassium concentration in plant tissue was higher for BLT plants, although the difference between treatments was not conclusive (Figure 5.16). It is conceivable that the increased demand for nitrate and other elements important for the production of secondary metabolites could have promoted potassium sequestering. Also, the relationship between potassium and stomatal operation may play a role in the elevated content of potassium in tissues of BLT plants, since potassium serves an important regulatory role in stomatal function [1]. It has been demonstrated that blue light supplementation tends to increase stomatal opening and boosts transpiration [83], perhaps to dissipate the increased energy load, and the elevated potassium in tissues may be attributed to this activity.
Zinc and Iron The BLT plants appeared to sequester zinc more readily than plants grown in the RLT (Figure 5.17). Considering the involvement of zinc in the production of plant pigments and enzymes, this result is logical. It is interesting that utilization of zinc in particular was favoured by BLT plants. Zinc has been shown to play critical roles in gene regulation and transcription, in particular association with DNA repair. Zinc finger formations have been identified as critical players in the identification of specific DNA sequences to which they can bind and manipulate [84, 85, 86]. Neither HPLC nor IS-sensor data was collected for zinc and uptake data is not available to compare tissue analysis with uptake trends.

The relative abundance of all species studied in dry-matter analysis was greater in BLT plants except for iron (Figure 5.17). RLT plants sequestered significantly more iron than BLT plants. This result is significant since large metal ions are important for the formation of macro molecules and their participation in plant enzymes and reactions. It is interesting to note that the light environment appears influence which metal ions are prioritized/favoured.
Figure 5.17: Zinc and iron content in lettuce grown under blue or red light only. Significantly more zinc was measured in the plants grown under blue light while significantly more iron was measured in plants grown under red light (It is notable that this is the only nutrient ion studied in plant tissue that was more abundant in plants grown under red light).

Sulphur & Boron The uptake of sulphur and boron did not vary conclusively based on light treatment, although more of both seemed to be sequestered by plants in the BLT (Figure 5.18). This result is supported by the nutrient uptake data presented previously that showed uptake of sulphur appeared to be independent of light quality (no uptake data is available for boron). Given that sulphur and boron both have many elementary roles in plant physiology [1], the result is not unexpected.

Summary

The types of nutrients that plants prioritize seem to shift based on their light environment. The BLT plants contained higher concentrations of nitrogen, potassium, magnesium, sulphur and zinc. In contrast, the RLT plants contained significantly more iron. The concentration of some elements did not differ considerably between the light treatments and these include calcium, copper and boron.

5.4 Summary and Conclusions

The data generated in the blue versus red light experiments provide a quantitative description of nutrient-plant-light relationships that must be considered in the development of closed-loop nutrient management systems. Changes in light quality will impact the dietary habits of plants, and in closed-environment production systems the ability to recycle and recirculate nutrient solution requires careful attention to
Figure 5.18: Sulphur and boron content in lettuce grown under blue or red light only. The amounts of both sulphur and boron appeared to be greater in BLT plants but the difference was not conclusive.

changes in nutrient consumption. The ISOS is ideally suited to provide real-time nutrient status information to controllers allowing informed nutrient adjustment decisions to be made.

5.4.1 Light Quality and Plant Physiology

It is clear from the results presented that plants alter their physiology to adapt to new light environments. These adaptations take many forms and have implications for nutrient uptake. In the case of light quality, *Lactuca sativa* cv. experiences an increase in blue light responded by producing pigment molecules to darken the colour of plant tissue resulting in purple leaves. This response typically serves to protect the plant from damage from higher-energy photons. Under less energetic red-light exposure production of dark pigment molecules was apparently not required for *Lactuca sativa* cv. resulting in bright green leaves. The production of plant pigments inherently impacts the energy and nutrient budget of plants. The synthesis of pigments (proteins) requires nitrogen and the observed colour change in BLT plants was supported by the increased uptake of nitrate and other elements from solution.

The understanding that quality of light influences plant physiology is no revelation. Yet, the results from this investigation highlight important relationships between the environment and the dietary habits of plants and demonstrate that quantification of nutrient uptake is absolutely essential to developing a comprehensive nutrient management system. The high-resolution nutrient status data that is required for such systems would be cumbersome to manage without reliable sensor technology that is
robust, affordable and easy to use.

5.4.2 Implications for Nutrient Management

The uptake rate of nutrients by plants is dependent on most environment variables. In natural systems environmental factors are extremely variable and notoriously unpredictable, rendering the real-time application of nutrients in response to the environment unfeasible. In the context of controlled environment plant production however, most environmental variables are under strict control, which affords a greater ability to manage nutrient application in real-time. The advantages of real-time nutrient management in hydroponic systems have yet to be fully explored, however obvious gains such as minimizing waste and optimizing water budgets offer immediate financial benefits to producers. Additional benefits can be realized through the use of light quality to influence the nutritional quality of crops, making it possible to optimize the nutrition of plants by tailoring nutrient and light recipes.

The justification for ion-selective management is apparent in hydroponic plant production systems and results of the red versus blue experiment demonstrate the utility of high resolution nutrient status information. For example, potassium depletion in Trial 3 could have been avoided by adding $KCl$ on Day 7, referring to Figure 5.8. Avoiding the traditional need to refresh the nutrient solution completely, nutrients that remain in ample supply are conserved.
Chapter 6

Conclusions and Recommendations

6.1 Implications of Ion-Selective Nutrient Sensing

The success of the ISOS prototype demonstrates that online ion-selective detection is possible for at least four nutrient ions that are significant for plant production. Further development of the technology will improve the quality and reliability of measurements and expand the suite of available optrode sensors. There are virtually limitless applications for IS-Sensors and many industry and research sectors can benefit from affordable ion-chemistry analysis. HPLC remains the only technology capable of reliable, routine quantification of solution chemistry but its large capital and operating costs prohibit the widespread application of IS-tech. Thus producing ion-selective sensors that are simple and reliable while remaining cost effective will permit widespread application in many fields.

Several critical industries including water and waste water treatment, mining, and agriculture could benefit extensively from low-cost IS-sensor technologies. As human populations grow, their impact on the environment increases and managing the effluents of the industries that support large populations becomes more important. Low cost IS-tech can greatly improve the efficacy of pollution control systems while simultaneously supporting the effectiveness of environmental monitoring programs. The present work focused on the use of IS-tech in hydroponic plant production systems, with the specific goal of developing nutrient management controls for integration into Biological Life Support Systems for human space travel. However, terrestrial applications of hydroponic nutrient management stand to yield considerable benefits. Apart from improving the efficacy of nutrient use in plant production, it is readily conceivable that application of high-resolution nutrient solution management in the connoisseur food and medicinal plant industries could yield unprecedented control of end product quality.

6.1.1 What does this mean for plant production?

The blue versus red light investigation demonstrated conclusively that light colour will influence the nutrient uptake of plants. Due to the fact that plants modify their dietary habits depending on their light environment, it stands to reason that
wavelength-specific feeding optimization is realizable. Feed solutions can be tailored to improve the efficacy of hydroponics when multi-band LED lights are used. In the context of closed-loop fertigation systems, the influence of light quality on nutrient uptake must be accounted for to properly manage feed solution quality. In systems using multi-band LEDs, trends in plant nutrient uptake could vary considerably if output spectra are modified. This creates the demand for the ISOS as IS-sensors are required to quantify these dynamic trends. Using IS-sensors the question of, “What to feed and when to feed it,” can be answered in real time. Ultimately sensor feedback from the monitoring system can be used to tune the quality of nutrient solutions continuously in response to plant and environment dynamics.

As the science of plant-nutrient uptake relationships matures, it is conceivable that established trends in light-plant-nutrient interactions can be employed in the management of feed solutions. For example, if it has been shown that increasing the amount of incident red-light enhances iron uptake, growing a “catch-crop” under red-light could help a system reduce iron in excess, while reducing the amount of incident red-light could help alleviate iron deficiency. This concept could be very useful in the management of ion-accumulations in closed systems.

### 6.1.2 What does this mean for space exploration?

The extension of the human presence in Earth’s solar system will require robust life support systems capable of feeding crew while treating their waste products. The sophistication of controlled environment plant production systems has advanced considerably since its inception and the remaining technical gaps to achieving completely autonomous, closed environment agriculture are all but closed. Advancing the state of IS-sensor technology while developing a knowledge base for light-plant-nutrient interactions is a critical and preliminary step in the evolution of BLSS. IS-tech offers benefits to other avenues of BLSS such as the monitoring and control of atmospheres and water/waste streams, and can also supplement scientific research activities.

The successful demonstration of the ISOS in an extreme, remote environment (despite low technology-readiness) indicates that optrode sensors could be well suited for in situ water chemistry analysis at challenging field sites. Further system and protocol development is required to generate a measurement system that is conducive to field science. Unmanned space probes performing science-at-a-distance will continue to rely exclusively on sensors to study distant worlds. Low-cost, low-mass IS-sensors suitable for long-duration space travel are critical components of these missions and their continued evolution will facilitate our understanding of the solar system.

### 6.1.3 Recommendations

Based on the demonstrated success of the ISOS in hydroponic nutrient management and the field instrument proof of concept, continued refinement of the ISOS is recommended. Incorporation of the ISOS into an automated sample loop is the next major milestone toward achieving online management of nutrient solution quality.
It was identified that knowing the volume of a nutrient reservoir is critically important in the determination of plant nutrient uptake rates and ultimately nutrient budgets. For future experiments investigating nutrient uptake by plants, consideration for accurate control of reservoir volume must be made. Use of a sight-tube can be useful to accurately monitor reservoir volume, or a weigh scale can be used for more precision.

In pursuit of understanding light-plant-nutrient interactions an extensive experimental program must be created. Developing optimized light and nutrient recipes for individual plant species will be a truly epic undertaking, owed to the complexity and diversity of environmental permutations that are possible. However, as the science evolves new discoveries will undoubtedly streamline the development of improved management systems and practices. The successful field test of the ISOS has also sparked interest in further refining the optrode system for use in field applications. Further investment in prototype development is recommended to streamline the use of optrode sensors for making chemistry measurements in the field.

6.2 Current Science Objectives - CESRF

6.2.1 Determine optimized light recipes

The research program at CESRF is currently focused on developing optimal light recipes for specific crops. It is anticipated that applying optimized light recipes will also yield characteristic nutrient uptake trends. The relationships between light quality and nutrient uptake by plants are evident and their exploration is a primary focus utilizing state-of-the-art technologies (Figure 6.1).

Figure 6.1: On going work at the controlled environment Systems Research Facility (University of Guelph) will continue to explore the relationships between light quality and plant nutrition.
6.2.2 Medicinal plant optimization

Increasing public interest in medical plants is driving research to characterize the production of pharmaceutically significant plant compounds in relation to light quality. In particular, production of medically active substances in plants (anti-oxidants, for example) can be stimulated by certain colours of light. It is logical to assume that this work will also yield optimal nutrient recipes to support the manufacture of specific plant products or responses.

6.2.3 Hands-off nutrient solution management

The “holy grail” of closed environment plant production is achieving complete autonomy in all aspects of environmental control. Nutrient management has always been a major obstacle to this goal, since useful sensor technologies have not been available in the past. The advent of low-cost electronics and optrode chemistry is permitting the first technological solutions to autonomous nutrient management. Developing comprehensive nutrient management systems is an ongoing branch of the CESRF research program. In the past 20 years, several technologies have been explored, and IS-optrodes show the greatest potential for successful implementation.
Bibliography


# Appendix A

## ISOS Equipment List

<table>
<thead>
<tr>
<th>No.</th>
<th>Quantity</th>
<th>Equipment Name &amp; Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Ocean Optics - bluLoop multi-LED light source</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Ocean Optics - FOS-2X2-TTL fiber optic dual switch</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Ocean Optics - inline fiber optic filter</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Ocean Optics - FVA-UV fiber optic variable attenuator</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>INO - ion-selective optrode sensors</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Avantes - FOM-UVIR400-1X16 fiber optic multiplexer</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Ocean Optics - USB4000 spectrometer</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Laptop PC with SpectraSuite, LabView and MATLAB</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Arduino Microcontroller</td>
</tr>
</tbody>
</table>

Table A.1: List of hardware components used in the ISOS system presented with model and manufacturer information.
Appendix B

Concpet Sketches

Figure B.1: Sketch of the X-Flow chamber concept that focuses on the details of construction and operation. Critical features include the water tight seals at fibre connections and secondary inlet/outlet ports for the introduction of rinse, storage or calibration solutions.
Figure B.2: A detail sketch of the endcap design to support up to 16 optrode fibres, conceived by Dr. John Phillips. For simplicity the sketch only shows an endcap disc that hold 8 fibres.

Figure B.3: 3D rendering of the optrode fibre cartridge concept to be integrated into the Gasket Fibre Cartridge prototype. (Created using SpaceClaim version 2012)
Figure B.4: Concept sketch of the Blue Horse sensor characterization apparatus. The Blue Horse facilitates periodic watering cycles for sensors housed in sample vessels with adjustable watering frequency and duration. The system can simulate up to four watering treatments simultaneously.
Figure B.5: As-built wiring diagram for the Moeller618 EASY AC-RC PLC controller used to operate the Blue Horse testing apparatus. Diagram indicates control wiring for the two 120 VAC power outlets and the bank of four 24VDC solenoids.
Appendix C

CAD
Figure C.1: CAD diagram of the prototype, multi-optrode fibre housing designed in consultation with the Digital Haptic Lab (University of Guelph) and produced using 3D ABS printer.
Figure C.2: CAD diagram of the storage container lid designed in consultation with the Digital Haptic Lab (University of Guelph) to protect optrode fibres when not in use. The lid holds fibres rigidly so they do not contact walls of the storage container.
Figure C.3: CAD diagram of the sample vessel lid designed in consultation with the Digital Haptic Lab (University of Guelph) to support optrode fibres while analysing samples. The lid holds up to four fibres rigidly ensuring they do not contact walls of the sample vessel or other optrodes.
Appendix D

Code
Basic Moeller Easy618AC-RC PLC Program for Blue Horse

definitions
-------------
Q = outputs
M = Make-contact
R = Reset-contact
P = input button on PLC display (1-4)
T = Timer
S = flag/marker
% = comments
"-" or "|" = wire connections

code
-------------
% Set up call contacts for pump
M13----------------Q1
P2 ----------------RM1
M14-                      RM2
M15-                      RM3
M16-                      RM4
P1-----------------SM1
M1-----------------TT1
T1-----------------M13
S1-M1--------------TT2
T2-----------------RM1
SM2
SM3
SM4
M2-----------------TT3
T3-----------------M14
S2-M2--------------TT4
T4-----------------RM1
RM2
RM3
RM4

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Figure D.1: Example code for the control of systems for the Blue Horse sensor characterization system that coordinates pump and valve operation to perform schedule watering events simulating typical hydroponic irrigation.
SHUTTER AND AUTO-MEAURE CONTROL CODE - ARDUINO

This program operates the Ocean Optics 2x2FOS switch in coordination with spectrometer. The code operates in a continuous loop until stopped by the user.

Two outputs are used to send a signal:
first to the FOS to open the light shutter
second to the spectrometer to trigger a spectra acquisition

Once the spectra is acquired, the shutter is closed

The program then waits until the next sample acquisition is required

This version of the program accommodates 3 sensor readings before waiting for the next.

// Pin 13 has an LED connected on most Arduino boards.
// give it a name:
int shutter = 13;
int specaq = 12;

// the setup routine runs once when you press reset:
void setup() {
    // initialize the digital pin as an output.
    pinMode(shutter, OUTPUT);
    pinMode(led2, OUTPUT);
}

// the loop routine runs over and over again forever:
void loop() {

    // Start Data collectons
    //--------------------------------------------------------------------------------
    //---READ SEBNSOR 2-------------------------------------------------------------
    //--------------------------------------------------------------------------------

digitalWrite(shutter, HIGH);  // Open Light Shutter
delay(200);  // wait 200 ms for shutter to open
digitalWrite(specaq, HIGH);  // Send singal to start integration
delay(100);  // Small signal pulse length
digitalWrite(specaq, LOW);  // Reset signal
delay(1000);  // wait 1 second for reading
digitalWrite(shutter, LOW);  // Close shutter
delay(58700);  // wait until end of this minute
Figure D.2: Example code for the control of systems for the Ion-Selective Optrode System that coordinates shutter operation and spectra acquisition by sending output signals to TTL inputs on the OceanOptics 2x2FOS and USB4000 spectrometer.