# DEVELOPMENT FOR BORON ANALYSIS METHOD IN SOIL USING A FLUORESCENCE SENSOR

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MASTER OF SCIENCE
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**Thesis Title:** Development for Boron Analysis Method in Soil Using a Fluorescence Sensor

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## ABSTRACT

Boron is an essential soil micronutrient critical for plant physiology, supporting fruit development, cellular growth, and cell wall integrity. Both boron deficiency and excess are highly detrimental to plant health, causing stunted growth, fruit abnormalities, or toxicity symptoms, respectively. Therefore, accurate determination of soil boron concentrations is crucial for maintaining optimal nutrient levels to enhance crop productivity and ensure overall plant health. In this work, a homemade fluorescence spectrometer consisting of an LDR sensor and 365 nm light source was developed for effective boron detection in soil samples. N-(9-Anthrylmethyl)diethanolamine was synthesized and utilized as a fluorescent reagent specifically for boron ion detection. The fluorescent reagent volume, pH conditions, and incubation time parameters were systematically studied and optimized. Under these optimized experimental conditions, the analytical system demonstrated excellent linear correlation ($R^2 = 0.9983$) over a working concentration range of $5.7\times10^{-7}$ to $5.7\times10^{-3}$ M. Method validation was conducted at the 95% confidence level using soil samples ($n = 7$), with the developed sensor results statistically compared to those obtained by the standard ICP-MS method. The results showed no statistically significant difference between the developed sensor and ICP-MS methods, with the calculated *t*-value (1.343) being less than the critical *t*-value (2.447). The method achieved lower detection limits with reagent stability successfully maintained for 30 days. The developed sensor provides a viable, cost-effective alternative to ICP-MS for routine boron analysis in agricultural applications.

**Keywords:** Fluorescence Sensor, Light Dependent Resistor (LDR) Sensor, Boron

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# ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| N-9-AMDEA 1 | 9-(anthrylmethyl)diethanolamine 1 |
| LOD | Limit of Detection |
| LDR | Light Dependent Resistor |
| LEDs | Light-emitting diodes |
| $AlCl_3$ | Aluminum Chloride |
| $CaCl_2$ | Calcium Chloride |
| $FeCl_3$ | Ferric Chloride |
| $MgCl_2$ | Magnesium Chloride |
| B | Boron |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

In modern agricultural settings, various external environmental factors impact the surrounding plants, ultimately influencing both plant growth and yield. These factors include soil quality, water availability, temperature, light exposure, air composition, and nutrient levels. The intricate balance among these elements significantly determines the overall health and productivity of plants. Minerals in the soil, which are one of the essential factors for growth, flowering, and fruiting, can be divided into 2 groups according to the amount that plants need: macronutrients and micronutrients. Macronutrients are nutrients that plants need, typically present in plants at concentrations exceeding 1000 mg $kg^{-1}$ dry weight, such as nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sulphur (S). Micronutrients are essential elements required by plants, usually found in amounts less than 100 mg $kg^{-1}$ dry weight, such as iron (Fe), manganese (Mn), boron (B), nickel (Ni), molybdenum (Mo), copper (Cu), chlorine (Cl), and zinc (Zn) (Dalcorso et al., 2014).

Boron (B) is a semi-metallic element and an essential micronutrient for humans, animals, and plants (Khaliq et al., 2018). In soil, it is generally present as boric acid, in the range of 0.0005–0.015% or 5–150 ppm (Fadayomi, 1974), while agricultural soils should contain no more than 5 mg per kg to remain beneficial (Brdar-Jokanović, 2020). Boron plays vital roles in root growth, flowering, pollination, fruit set, sugar and hormonal transport, and nutrient uptake (Landi et al., 2019; Clemente et al., 2018). Its deficiency leads to the loss of root tips, reduced water absorption, decreased drought resistance, and visible symptoms such as death of terminal and lateral buds, shortened internodes, small, thickened leaves that are curved and brittle, and clustered branches and leaves (Landi et al., 2019; Clemente et al., 2018). Therefore, continuous monitoring and management of boron levels in agricultural soils is important, particularly in areas at risk of soil degradation due to intensive cultivation and chemical fertilizer use (Angkasith, 2002).

Analyzing boron (B) typically involves complex techniques like Inductively Coupled Plasma (ICP), Atomic Absorption Spectrophotometer (AAS) (Sah & Brown, 1997a) mass spectrometry (MS), thermal ionization mass spectrometry (TIMS), and secondary ion mass spectrometry (SIMS). For isotopic measurements, TIMS and SIMS provide higher sensitivity and accuracy than ICP-MS. However, their extensive sample preparation, purification needs, and time-consuming measurements limit their practicality for routine analyses (Sah & Brown, 1997b). Various methods have been developed for boron detection, each with its own advantages and limitations, as shown in Table 1. While conventional techniques such as ICP and AAS methods offer sensitivity or selectivity, they often require expensive instruments, lengthy procedures, or complex sample preparation. In addition, the development of a selective, rapid, and low-cost fluorescent sensor is highly desirable.

There are various methods for detecting boron, each utilizing different chemical compounds or reagents to accurately identify boron in a given sample. Among these, fluorescent probes such as curcumin—a natural compound extracted from turmeric—have been widely used due to their ability to bind boron and alter fluorescence. Other commonly used probes include Alizarin Red S, BODIPY-based probes, Dansyl hydrazone derivatives, and rhodamine-based sensors. Each probe offers distinct advantages: for example, BODIPY-based probes are highly sensitive but tend to be expensive and complicated to synthesize, while curcumin and Alizarin Red S are more accessible and affordable, though they may have limitations in terms of stability and selectivity under varying environmental conditions. Ultimately, the choice of boron detection probe depends on the specific application requirements, considering factors such as cost, environmental conditions, and detection efficiency (Gangemi et al., 2022).

A green colorimetric sensor based on curcumin nanoparticles entrapped in a tapioca starch film has been developed for boron detection (Boonkanon et al., 2020). Curcumin, a widely used chromogenic reagent in spectrophotometry, was incorporated into this eco-friendly sensor, which, when combined with digital image colorimetry (DIC), offers a portable and cost-effective approach for boron quantification under field conditions, with a detection limit of 0.052 ± 0.001 mg $L^{-1}$. However, further improvement is still needed regarding sensitivity, selectivity in complex matrices, and stability of the curcumin reagent under variable environments. In a related approach, a paper-based assay was also proposed, enabling non-instrumental colorimetric detection of boron in complex water samples (Pena-Pereira et al., 2020). This study re-examined the classical curcumin reaction and integrated it with information and communication technologies, transforming a qualitative spot test into a quantitative assay. Furthermore, the use of unprocessed Curcuma longa L. extracts for boron determination was explored. Overall, merging paper-based devices with IT tools opens new opportunities for rapid, low-cost, and equipment-free field analysis.

Fluorescence techniques are highly suitable for this study because they provide extremely high sensitivity, allowing detection of even small changes in fluorescence upon binding of boronic or boric acids. These methods also offer high specificity, fast response, and molecular-level resolution, which are essential for monitoring the selective interaction between the diethanolamine recognition site and boron-containing compounds (Yorker, 2014).

Various fluorescent boron sensors have been discussed in the literature, including a new red-emitting sensor for detecting boronic acids in cells. This sensor solves the interference problems of traditional blue-emitting sensors and nuclear stains. The BS-631 sensor, which has a tetrahydroquinoxaline group, is very sensitive (detecting as low as 19.6 μM for 4-borono-l-phenylalanine, BPA) and selective for boronic acids. It allows clear imaging of boronic acid compounds in cells without disrupting nuclear stains. This makes it a useful tool for cancer therapy research, especially in Boron Neutron Capture Therapy (BNCT) (Kondo et al., 2022).

Several studies have developed fluorescent sensors for boron detection, focusing on improved sensitivity, selectivity, and applicability in biological or environmental systems with a large number of matrices. For example, Hattori et al. (2017) developed a new fluorescent boron sensor based on 2-(pyridine-2-yl)phenol. This sensor reacts with boronic acids through O,N-chelation, where the hydroxyl group of the phenol and the nitrogen atom of the pyridine coordinate to the boron atom, forming a stable boron (III) complex that exhibits enhanced fluorescence. The sensor is stable, highly fluorescent, and quickly responds to boronic acids, making it ideal for detecting these compounds in biological environments. Moreover, it offers a practical and efficient alternative to current analytical methods, such as ICP-OES or PGA, which are costly and require extensive sample pretreatment steps before measurement. Similarly, Wang et al. (2000) introduced N-(9-anthrylmethyl)diethanolamine 1, a sensor that uses an anthracene moiety modified with a diethanolamine recognition site. This compound exhibits a significant fluorescence increase up to 19-fold upon binding with boronic and boric acids. The sensor is highly selective and unaffected by other anions because boronic and boric acids form a stable boronate complex with the diethanolamine group. In this binding process, the boron atom accepts the nitrogen lone pair and interacts with the hydroxyl groups to generate two stable five-membered rings, which effectively mask the lone pair and suppress the photoinduced electron transfer (PET) quenching of the anthracene fluorophore. As a result, fluorescence is strongly enhanced, whereas other anions cannot form such complexes and thus do not interfere. This mechanism makes the sensor suitable for chemical monitoring, impurity detection, and biological studies involving boric acid in plant cells and medicinal boronic acid compounds. From the literature, functional groups that have a high possibility of binding boron include amines (N-H), carboxylates (COO-), thiols (S-H), and hydroxyls (O-H). Boron acts as a strong Lewis acid by accepting electron pairs from these groups, enabling its key roles in catalysis and synthesis. Stable boron compounds like boronic acids and boronates form strong complexes with nucleophiles, making them valuable in organic synthesis, drug design, and materials science. Boron's unique ability to form coordinate bonds is essential for selective reactions such as Suzuki coupling and for interactions with biological molecules in medicinal chemistry and sensor development (Messner et al., 2022). So, a fluorescence sensor for boron typically requires two O-H groups at the reactive site, as these can form strong bonds with boron ions, facilitating detection through changes in fluorescence.

However, some fluorescent sensors with the mentioned active sites, such as dihydroxycoumarins, have no reported interaction with boron. For example, there are reports that studied dihydroxy-substituted coumarins as sensitive and selective probes for detecting nitroxide radicals like 4-amino-TEMPO, not boron. Their earlier work (Zamojć et al., 2015) showed that 6,7-dihydroxycoumarin (esculetin) increased fluorescence significantly upon interaction with 4-hydroxy-TEMPO, highlighting its potential for biological studies of oxidative stress rather than boron detection (Żamojć et al., 2019). It is therefore interesting to compare fluorescent agents to find ones with better sensitivity, especially since there aren't many comparison studies in this area right now.

To detect a simple signal, various tools and methods can be employed depending on the type of signal and the desired application. RGB sensors and LDRs each have unique advantages and uses in analytical chemistry and various applications. RGB sensors, which detect red, green, and blue light, are popular for colorimetric sensing due to their cost-effectiveness, portability, and ease of use. They are used in pH measurement, analyzing analyte concentrations, food quality control, environmental monitoring, and biomedical diagnostics like blood glucose monitoring and disease detection. LDRs, which change resistance based on light intensity, are used in spectrophotometers to measure light absorbance or transmittance at different wavelengths and in colorimetric assays for many different applications in chemistry such as determining concentrations, identifying compounds, and studying reactions (El-Zomrawy, 2019). The RGB sensors are affected by environmental light on the picture's color intensity, and they require specific applications for providing accurate color analysis. At the same time, LDRs can use general light detection for ambient light sensing and basic light level measurements.

This research aims to develop a simple LDR-fluorescent sensor for boron detection with selective agents. Comparison of fluorescent agents to find ones with better sensitivity and selectivity is studied. The spectra of the commercial fluorescent agents and the synthesized N-(9-anthrylmethyl)diethanolamine 1, which contains amine (N-H) and hydroxyl (O-H) groups, are investigated. The experimental conditions, including sensor concentration, boron concentration range, pH, and pre-analysis incubation time to ensure stable and reliable fluorescence responses, are optimized. Selectivity and stability of the selected fluorescent agent are also investigated. The developed sensor is validated for boron detection in real soil samples using an ICP-MS. The developed system integrates precise simple spectroscopic measurements with practical, low-cost detection using light-dependent resistors (LDRs), enabling portable boron sensing with significant potential for real-field applications. The developed LDR-fluorescent sensor bridges the gap between laboratory accuracy and field practicality for boron detection in soils. This approach significantly reduces the cost and complexity of boron analysis, making routine soil monitoring feasible for small-scale farmers and developing regions.

## 1.2 Objectives

1.2.1 To develop boron detection with an LDR-fluorescence sensor.

1.2.2 To apply portable fluorescent LDR sensors in the real sample.

## 1.3 Scope of Research

The development of a fluorescent sensor for boron detection is the main objective of this study. To begin, 9-(anthrylmethyl)diethanolamine will be synthesized to serve as the sensor's sensing receptor, and the produced compound will be characterized using Thin-Layer Chromatography, FTIR, and NMR techniques. Subsequently, the performance of the sensor was tested by comparing the fluorescence intensity spectrum, slope, and limit of detection (LOD), as well as studying the stability of the sensor over different time periods. A prototype system was then designed by studying the chemical reactions between the sensor and boron in a matrix of PVA mixed with glutaraldehyde, alongside designing a measurement system using an RGB and LDR sensor to evaluate the detection performance of each system. Additionally, a portable prototype testing kit was developed, focusing on reducing extraction time and minimizing chemical usage, which was compared with the standard method using ICP-OES and ICP-MS. Finally, the accuracy of the developed system was validated through paired t-test statistical testing to confirm the correctness of the developed boron measurement methods

# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Boron

One of the seven acknowledged critical elements that most plants need for proper growth is boron. Maze (1914) in France was the first to discuss the importance of boron in relation to how it affected the growth of maize plants (Maze, 1914). According to research, the only element among the essential mineral nutrients that is often present in soil solution as a non-ionized molecule over the pH range necessary for plant growth is boron. Their findings indicate that the boron type that is most effectively absorbed by plant roots is boric acid. According to the report of D. Alt and W. Schwarz (1973), boron is absorbed as the molecule (Oertli & Grgurevic, 1975) and is passively dispersed with the transpiration stream, at least in high supply (Alt, 1973). The soil has a high boron level in areas with little rainfall (Eaton, 1935). These soils likely contain most of the highly soluble sodium-calcium borates that are known to contain boron (Berger, 1949), in soil was added in the form of boric acid solution. Boron deficiency in coffee plants is often linked to the depletion of soil fertility over time and the cultivation of high-yield varieties that have greater nutrient demands. This deficiency adversely impacts the growth of the root system, leading to the death of fine root tips and, subsequently, a reduction in the plant's ability to absorb both water and essential minerals. As a result, coffee plants become more susceptible to drought stress and less responsive to standard fertilization practices (Clemente et al., 2018). This can have a negative impact on the overall health and productivity of coffee crops. The present cultivation methods prioritize achieving high yields, leading to the common application of chemical fertilizers and pesticides in coffee plantations. Consequently, there is a need to be concerned about the quantity of boron available in the soil (Angkasith, 2002).

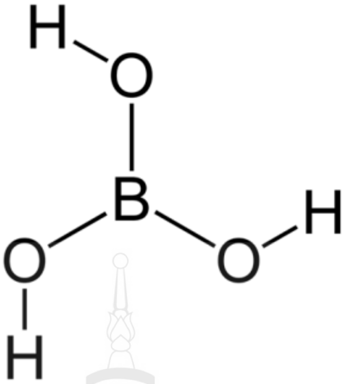

**Figure 2.1** Chemical structure of boric acid

## 2.2 Practical Methods for Boron Determination

Both atomic emission spectrometry (AES) and atomic absorption spectrometry (AAS) often involve placing samples inside of an atomizing flame, typically one made of acetylene-air or acetylene-$N_2O$-air. The basis for the AAS measurement is the idea that free atoms of an element, such as B, absorb discrete energy photons produced by a hollow cathode lamp that contains that element in its ground state (e.g., Boron). When atomized and excited species return to their ground state, the AES techniques measure emission from those species. Acceptable findings for the AES/AAS determination of boron frequently require the isolation and preconcentration of boron from the sample matrix (Bruno, 1994). The detection limit and sensitivity were enhanced by measuring the boron radical's atomic emission at 548 nm (Castillo et al., 1985) or its absorbance at 149.7 nm after boron had been extracted from the sample matrix as volatile methyl borate. Methods for boron separation and preconcentration are covered in the prior sections. Recently, Usenko (1992) and Zakhariya (1991) described how to determine the presence of boron using the AAS/AES. This approach has low sensitivity (Papaspyrou, 1994), significant memory effects of prior samples, and a lot of interference.

ICP-MS is typically used for boron determination over ICP-OES and spectrophotometric techniques (Evans & Krähenbühl, 1994). Higher sensitivity, lower detection limits, and simultaneous determination of the 10B to 11B isotope ratio and total boron concentration in a sample are advantages of ICP-MS over other techniques. ICP-MS is ideally suited for biological B tracer research due to its ability to measure B-isotope ratios (Brown & Hu, 1996). The reported detection limits range from one part per billion (ppb) to three parts per billion (ppb) for biological materials, 0.15 ppb for saline fluids (Gregoire, 1990), and 0.5 ppb for human serum, for example (Vanhoe et al., 1993). The capacity of ICP-MS to do B determination using the isotope dilution method, which is thought to be the most accurate for quantitative determination.

Mass spectrometry (MS), thermal ionization mass spectrometry (TIMS), and secondary ion mass spectrometry (SIMS). For isotopic measurements, TIMS and SIMS offer superior sensitivity and accuracy compared to ICP-MS. However, their demanding sample preparation, purification requirements, and time-intensive measurements restrict their suitability for regular analyses (Sah & Brown, 1997).

## 2.3 Boron Test Kit

For the first time, a green colorimetric sensor utilizing entrapped curcumin nanoparticles within a tapioca starch film has been created for boron detection. Curcumin, a widely used chromogenic reagent for boron determination in spectrophotometry, has been incorporated into this innovative green colorimetric sensor. The combination of this novel sensor with DIC (Digital Image Colorimetry) provides a straightforward, portable, and cost-effective method for quantifying boron under field conditions (Boonkanon et al., 2020).

Another research also introduces a novel paper-based assay (PADs) with non-instrumental detection, allowing the colorimetric assessment of boron in complex water samples. The study reexamines a traditional curcumin-based colorimetric reaction for qualitative boron detection and combines it with everyday communication and information technologies to convert a qualitative spot test into a quantitative non-instrumental colorimetric assay. Additionally, the study explores the use of unprocessed extracts from *Curcuma longa* L. for the colorimetric determination of boron. In brief, merging PADs with everyday communication and IT tools provides new opportunities to develop fast and affordable quantitative methods for field analysis, all without relying on advanced analytical equipment (Pena-Pereira et al., 2020).

## 2.4 Fluorescence Technique

Boron-containing chemical detection necessitates very costly infrastructure and/or laborious pretreatments. Boron chelating-ligands were created to create a practical way to detect boronic acid derivatives in fluorescence sensors (Hattori et al., 2015). In this communication, a novel optical sensor for measuring extracellular (serum or whole blood) potassium is described. The sensor reacts to variations in potassium concentrations typical of whole blood samples quickly and irreversibly. Clinical calcium or pH concentrations had no effect on the results, and the impact from sodium is hardly noticeable. In the visible light spectrum, there is both excitation and emission. A whole blood analyzer for sale, the Roche OPTI CCA, now uses this novel potassium sensor (He et al., 2003). A diethanolamine recognition site-based anthracene-based PET sensor that is selective for boronic and boric acids exhibits up to a 19-fold increase in fluorescence intensity upon binding (Wang et al., 2000).

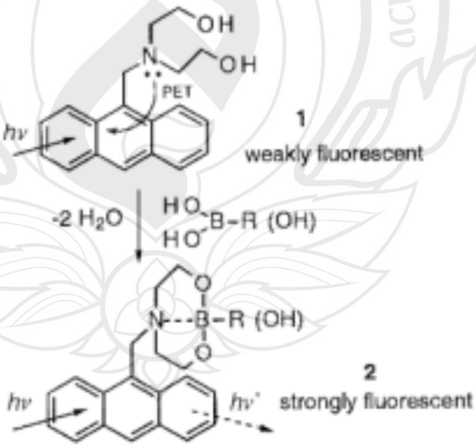

**Figure 2.2** The tight binding of boronic acid/boric acid with sensor 1

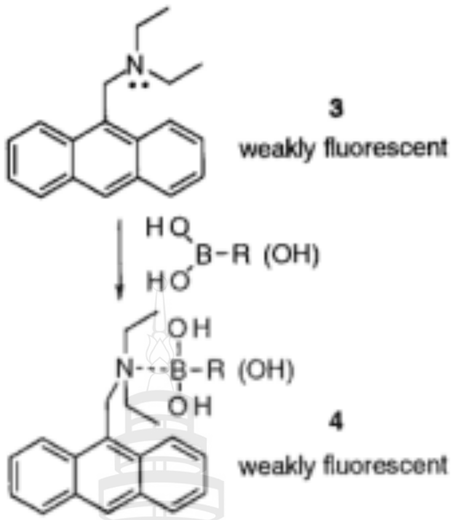

**Figure 2.3** The weak interaction of boronic acid/boric acid with control compound 3

## 2.5 Light Dependent Resistor (LDR)

Light-dependent resistors (LDRs), also referred to as photoresistors, are photoconductive devices whose resistance decreases with increasing incident light intensity due to photon-induced electron excitation in semiconducting materials such as cadmium sulfide (CdS) or cadmium selenide. Their spectral sensitivity typically spans the visible region, with a peak around 500–600 nm, which aligns with human visual perception. Although modern analytical instrumentation predominantly employs photodiodes and photomultiplier tubes for optical detection due to their superior linearity and sensitivity, LDRs continue to attract interest in low-cost, educational, and field-deployable analytical applications (Álvarez et al., 2015). A primary area of application for LDRs in analytical chemistry is low-cost spectrophotometry. LDRs are often combined with light-emitting diodes (LEDs) to construct simple spectrophotometers, where resistance changes are correlated with transmitted light intensity. Such instruments have been employed to demonstrate the Beer–Lambert law and to quantify colored analytes in solution. Álvarez et al. (2015) demonstrated the integration of an Arduino-controlled LDR detector in a teaching spectrophotometer, showing adequate performance for educational laboratories.

LDRs have also been incorporated into colorimetric detection systems, where they measure changes in solution absorbance during analyte–reagent reactions. These systems, although semi-quantitative, offer affordable and portable alternatives for resource-limited environments. For example, Suresh and Kannan (2014) reported the successful application of LDR-based colorimetric devices for analyzing food and water samples. In addition, LDRs have been evaluated as dosimetric sensors for ionizing radiation. Román-Raya et al. (2020) showed that CdS-based LDRs exhibit measurable resistance shifts upon radiation exposure, supporting their feasibility as inexpensive, reusable dosimeters. While less conventional within analytical chemistry, this application illustrates the versatility of LDRs in sensing applications beyond photometry.

Environmental monitoring represents another relevant application. LDR-based photometric devices have been utilized for turbidity and absorbance measurements in water samples, providing low-cost alternatives for field analysis. Jachimowicz and Fularz (2017) reported the construction of an LDR-based spectrophotometer for environmental laboratories, highlighting its potential in preliminary field testing where portability and cost-effectiveness are critical. Finally, LDRs hold significant value in education and outreach. Their affordability and ease of interfacing with microcontrollers make them ideal for teaching fundamental analytical concepts such as calibration, linearity, and sensitivity. McMahon (2013) highlighted the pedagogical impact of employing LDR-based spectrophotometers in demonstrating absorbance and quantitative analysis principles to students. In conclusion, while LDRs are limited by nonlinear response, slow temporal resolution, and temperature sensitivity, they remain relevant in analytical chemistry for applications emphasizing accessibility rather than precision. Their role is particularly evident in educational spectrophotometry, field colorimetric assays, radiation dosimetry, and environmental monitoring, where cost and simplicity are prioritized over advanced performance metrics.

# CHAPTER 3

# METHODOLOGY

## 3.1 Reagents, Materials, and Apparatus

Diethanolamine (QRëC, New Zealand), 9-(chloromethyl)anthracene (Alfa Aesar, Ward Hill, USA), potassium iodide (Merck KGaA, Darmstadt, Germany), and potassium carbonate anhydrous (Deajung, Gyeonggi, Korea) were used to synthesized a N-(9-anthrylmethyl)diethanolamine 1 fluorescent agent. Stock solutions of boron were prepared from boron standard solution, $H_3BO_3$ in $H_2O$ from Merck (Darmstadt, Germany). 2,2’-(4-Methylphenylimino)diethanol from Sigma-Aldrich (St. Louis, MO, USA) and 7-Diethylamino-4-methylcoumarin (Thermo Fisher Scientific, Waltham, Massachusetts, USA) were used as the studied boron-fluoresent agents. Methanol with HPLC grade, acetonitrile, chloroform, ethyl acetate were percharhed from the RCL LabScan, Pathum Wan, Bangkok, and sodium sulphate anhydrous were percharched from Merck KGaA, Darmstadt, Germany. Instuments used in this work were an Avio 220 Max Inductively coupled plasma atomic emission spectroscopy (ICP-OES) (PerkinElmer, Shelton, Connecticut, USA) with a detection limit 0.001mg/L for boron, a fluorescence spectrometer (LS55, PerkinElmer, Waltham, Massachusetts, United States) with a xenon lamp, excitation, or emission bandwidth of 10 nm, an Ocean Optics miniature spectrometer (product code: FLAME-S-VIS-NIR-ES, Ocean Optics, Orlando, Florida, USA), a 365 nm UV flashlight with 3 W power (Kooler, Bangkok, Thailand), an analytical balance, and a PH700 EUTECH benchtop pH meter (Model: pH 700 with Epoxy Electrode, Eutech, Singapore).

## 3.2 Study of Fluorescent Agents for Boron Analysis

The three fluorescent agents containing amine (N-H) and/or hydroxyl (O-H) groups that tend to be a specific active site for boron binding were selected to react with a boron standard solution for the study of fluorescence characteristics. They were 7-Diethylamino-4-methylcoumarin, 2,2′-(4-Methylphenylimino)diethanol, and N-9-AMDEA 1. The intensity and sensitivity were used as criteria for the selection of fluorescent agents. 7-Diethylamino-4-methylcoumarin and 2,2′-(4-Methylphenyl imino)diethanol were obtained from Thermo Fisher Scientific (Waltham, Massachusetts, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. N-9-AMDEA 1 was synthesized, and characterized before further performing the performance of fluorescent agents for boron analysis because it is not available commercially.

### 3.2.1 Synthesis and Characterization of N-9-AMDEA 1

The synthesis of 9-(anthrylmethyl)diethanolamine 1 (N-9-AMDEA 1) was proposed through the reaction of 9-(chloromethyl)anthracene with diethanolamine in the presence of $K_2CO_3$ and a catalytic quantity of KI following (Wang et al., 2000). This reaction was carried out in a mixture of $CHCl_3$ and $CH_3CN$ under reflux conditions for 3 hours at 60°C. The proposed method aimed to produce 9-(anthrylmethyl)diethanolamine 1 quickly. After the synthesis and purification, a yellow crystal was obtained and used to calculate the percent yield. Purification of the synthesized N-9-AMDEA 1 was performed.

The purification process involved thin-layer chromatography (TLC) and column chromatography to obtain a purified compound. During the preparation of the agent solution, distilled water and ethyl acetate were added to remove residual KI and $K_2CO_3$. To remove residual water, the organic layer was treated with anhydrous $Na_2SO_4$. Finally, TLC was performed using a mobile phase ratio of 40:60 to confirm the separation and purity of the synthesized compound. In column chromatography, fractions of the solution were collected in 45 test tubes, which then underwent TLC testing. The solution collected from test tubes 13-45 was evaporated at 60°C/60 rpm, and the resulting crude agent was recrystallized. Finally, the characterization of the synthesized N-9-AMDEA 1 was investigated by using the FTIR, fluorescence, and NMR techniques and confirmed the synthesized sensor's authenticity in alignment with existing literature reviews.

### 3.2.2 Study Fluorescence Spectra

Fluorescent agent solutions were prepared at concentrations of $1.0 \times 10^{-4}$ M. Spectral measurements were carried out using a fluorescence spectrometer (LS55, PerkinElmer, Waltham, MA, USA). A pre-scan was first performed to determine the excitation and emission profiles. The emission wavelength was further confirmed by recording spectra in %T attenuate mode with a fixed excitation wavelength. All spectra were obtained with slit widths set to 10 for both excitation (in) and emission (out). After obtaining the spectra, identify the excitation wavelength, emission wavelength, and intensity. Then, select the fluorescent agent with an excitation wavelength that is optimal for developing a test kit in the next stage.

### 3.2.3 Study of The Sensitivity of Fluorescent Agent for Boron Analysis

Quantitative analysis was performed using fluorescence spectroscopy with an Ocean Optics miniature spectrometer (product code: FLAME-S-VIS-NIR-ES, Ocean Optics, Orlando, Florida, USA). Two fluorescent agents, N-9-AMDEA 1 and 7-Diethylamino-4-methylcoumarin, were prepared as $1.0 \times 10^{-4}$ M solutions. Fluorescence spectra were recorded to construct calibration curves, and their slopes were compared to identify the fluorescent agent with higher sensitivity toward boron detection.

Subsequently, the selected fluorescent agents were evaluated for their interaction with boron. Boron solutions were prepared in methanol at concentrations ranging from $5.7 \times 10^{-3}$ to $5.7 \times 10^{-7}$ M. Equal volumes of fluorescent agent and boron solutions were mixed in a cuvette, and a prescan was performed to determine the excitation wavelength. The resulting spectra were analyzed to identify the fluorescent agent exhibiting the strongest and most stable fluorescence response toward boron. In addition, the fluorescence behavior of the selected agent (N-9-AMDEA 1) was further examined over a concentration range of $1.0 \times 10^{-5}$ to $1.0 \times 10^{-3}$ M in methanol. This was performed to establish the relationship between concentration and fluorescence intensity and to determine the optimal concentration for subsequent experiments with boron.

## 3.3 Design the Detection System of Sensor Solution for Boron Detection

In this study, light-dependent resistors (LDRs) were designed and utilized for the fluorescent detection system. The LDRs functioned by converting light passing through the sample into electrical impulses, altering their internal resistance, which was inversely proportional to light intensity. To facilitate this process, a black-colored box was developed for the detection system because fluorescence detection requires a dark area to minimize background noise and improve the signal-to-noise ratio, ensuring that only the fluorescence emitted by the sample is measured. This increases sensitivity, prevents photobleaching of fluorescent molecules, and enhances the accuracy and reliability of the measurements by eliminating interference from ambient light. The PCR tube is used instead of a cuvette because it can handle smaller sample volumes and is more convenient for field detection. The PCR tube was placed inside the box. A 365 nm UV flashlight (Kooler, Bangkok, Thailand) is used as the light source. Specifically, holes were drilled in the box to allow the passage of the light source, ensuring accurate detection in the designated area. The designed detection system was shown in Figure 3.1. Then test the system by checking the voltage values for signal stability or consistency of the selected sensor at a concentration of $1\text{x}10^{-3}$–$1\text{x}10^{-7}$ M. After designing the detection system of the sensor solution for boron detection, the experiment was carried out to optimize the volume of the fluorescent agent solution, the concentration of the fluorescent agent solutions, the reaction time, and the pH to improve sensitivity.

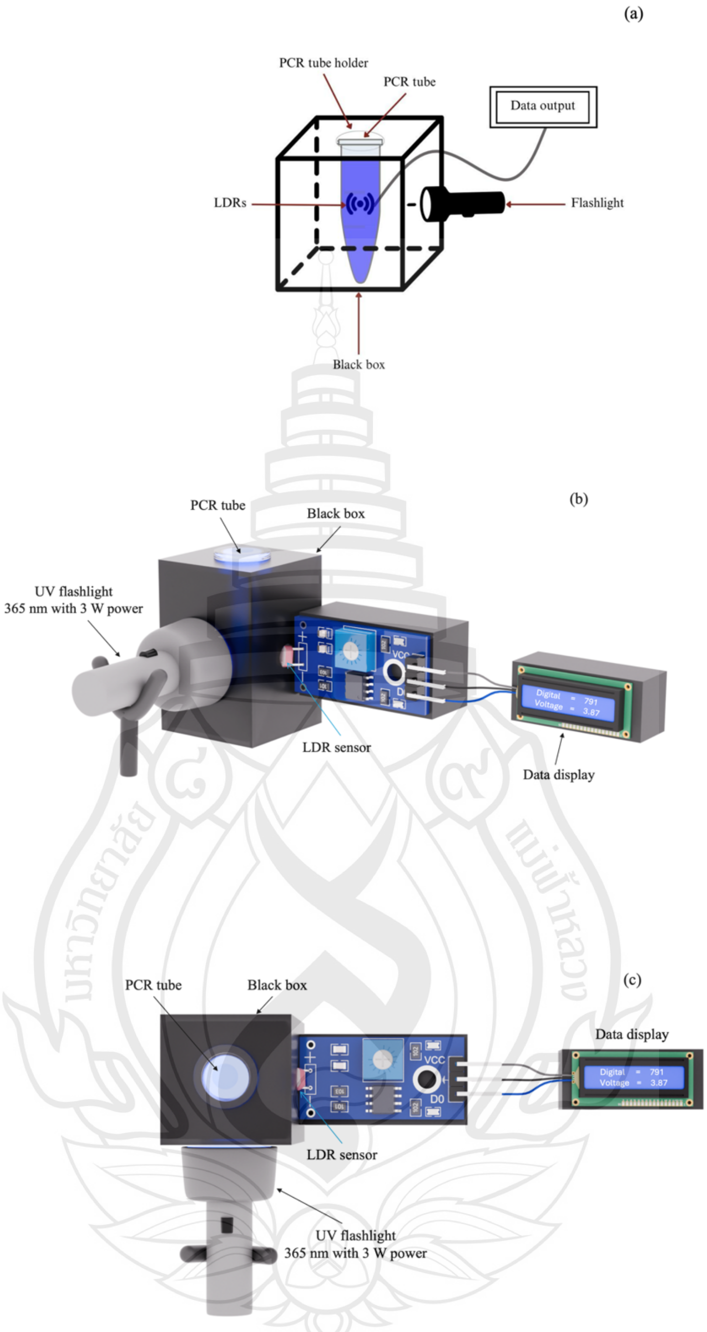

**Figure 3.1** (a) Conceptual design of the LDR detection setup with a PCR tube and light source. (b) Front view of the LDR-based fluorescence detection system. (c) Top view of the LDR-based fluorescence detection system

## 3.4 Optimization of the Developed System

In this study, the optimization of a boron detection system is being systematically pursued through variation of key parameters. The concentration of boron covers a range from $5.7\times10^{-3}$ M to $5.7\times10^{-7}$ M, facilitating the determination of detection limits. Concurrently, volumes of sensor and boron solutions are varied to evaluate their influence on system sensitivity. Detection times are evaluated at specific time points to clarify how detection works overtime. pH adjustments are implemented to investigate their impact on sensor stability and analytical performance. The analytical parameters, such as sensitivity, linearity, and reproducibility, aim to identify optimal conditions that ensure accurate boron detection capabilities.

### 3.4.1 Optimization of the Fluorescent Agent Volume

The volume of fluorescent agent used in the developed boron detection system was further studied to increase the sensitivity and efficiency of the detection. The selected fluorescent sensor N-9-AMDEA 1 was prepared at a concentration of $1.0\times10^{-4}$ M and boron standard solutions were prepared in a concentration range from $5.7\times10^{-7}$ to $5.7\times10^{-3}$ M. To study the effect of the N-9-AMDEA 1 sensor volume on the detection, namely 25 µL, 50 µL, 75 µL and 100 µL, were added to separated PCR tubes, and the prepared boron solution (100 µL) was added to each tube containing the sensor, then made volume to 200 µL with DI water. Fluorescence intensities were measured using the LDRs instrument in triplicate. For each fluorescent agent volume, calibration curves (fluorescence intensity vs. boron concentration) were constructed and linear regression performed to obtain the slope and coefficient of determination ($R^2$). The volume of fluorescent agent that produced the highest slope was selected.

### 3.4.2 Optimization of pH on Sensitivity for Boron Detection

The pH range was limited to 3.0 - 6.65 because the fluorescent sensor becomes unstable at higher pH, where deprotonation alters its structure, weakens boron binding, and reduces fluorescence. In alkaline conditions ($pH > 8$), the N-9-AMDEA 1 sensor may degrade, and boron shifts from boric acid to borate ion, which further impairs detection. For pH optimization, buffers at pH 3, 4, and 5 were prepared by combining 5.62 mL, 4.87 mL, and 2.09 mL of glacial acetic acid, respectively, with corresponding amounts of sodium acetate (0.14 g, 1.21 g, and 5.21 g) and diluting each mixture to 1 L with distilled water. In addition, a non-adjusted condition (pH: 6.65) was included by using deionized (DI) water without pH modification, representing the natural unbuffered state of the solution

After preparing the buffer solution at the desired pH, add 2.5 mL of it to a beaker, followed by the fluorescent agent solution (100 µL), and mix well. Transfer 75 µL of the mixture to a PCR tube, then add 100 µL of the boron standard solution, vortex the tube, and let the reaction occur for 30 seconds. Finally, perform the analysis using LDR detection. Fluorescence intensities were measured using the LDRs instrument in triplicate. Calibration curves (fluorescence intensity vs. boron concentration) were constructed for each pH, and linear regression analysis was applied to determine the slope and coefficient of determination ($R^2$). The obtained sensitivity values were then plotted against pH, and the pH giving the highest response was selected for further experiments.

### 3.4.3 Optimization of the Incubation Time of the Fluorescent Agent–Boron Reaction

The incubation time of the fluorescent agent–boron reaction was studied through a series of controlled experiments. Boron solutions with concentrations ranging from $5.7 \times 10^{-3}$ M to $5.7 \times 10^{-7}$ M were prepared to determine the incubation time of the sensor response. Each reaction mixture was prepared by combining the boron solutions (100 µL) with the fluorescent sensor (N-9-AMDEA 1) at a fixed concentration of $1 \times 10^{-4}$ M (75 µL). To investigate the effect of reaction time on signal development, the fluorescence intensity of the mixtures was monitored at incubation times of 0 s, 30 s, 1 min, 1.5 min, 2 min, and 2.5 min. Fluorescence measurements were conducted in triplicate using the LDRs instrument. For each incubation period, a calibration curve correlating fluorescence intensity with boron concentration was generated, and linear regression analysis was applied to determine the slope and coefficient of determination ($R^2$). The incubation time that produced the maximum slope was selected as the optimal incubation time for the sensor response.

## 3.5 The Stability of the N-9-AMDEA 1 Sensor for Boron Detection

The stability of the N-9-AMDEA 1 sensor for boron detection was investigated. Standard boron solutions were prepared, using boron concentrations ranging from $5.7 \times 10^{-7}$ M to $5.7 \times 10^{-3}$ M, and a $1.0 \times 10^{-4}$ M concentration of the N-9-AMDEA 1 sensor was prepared. The solutions were mixed by adding 75 µL of the N-9-AMDEA 1 sensor followed by 100 µL of the boron solution, then vortexed and allowed to stand at room temperature for 30 seconds. Measurements were taken using LDRs. The fluorescence signal of the boron solution with the N-9-AMDEA 1 sensor was measured every 5 days within 30 days under the same conditions of temperature, pH, and volume. The sensor was stored at room temperature and away from light. The signal intensity was recorded between signal intensity and the number of days (day vs. signal) for evaluation of the stability trend. Statistical analysis was performed to calculate the %RSD of the signal over 30 days. A check was made for any significant changes or trends in the signal.

## 3.6 Interference Study

The interference study was conducted to evaluate the selectivity of the fluorescent sensor toward boron in the presence of common metal ions found in soil. Three boron standard solutions with concentrations of $2.85 \times 10^{-3}$ M, $2.85 \times 10^{-5}$ M, and $2.85 \times 10^{-8}$ M were prepared. Interfering ions, including $Fe^{3+}$, $Ca^{2+}$, $Mg^{2+}$, and $Al^{3+}$, were separately prepared at different concentrations commonly present in soil. In the test procedure, 75 µL of the sensor reagent was pipetted into PCR tubes, followed by 100 µL of the boron solution and the corresponding interfering ion solution. The mixtures were vortexed to ensure homogeneity and subsequently measured using the LDRs instrument to evaluate fluorescence response and possible signal interference.

## 3.7 Validation of the Developed Detection Method

### 3.7.1 Standard Calibration

A certified boron stock solution with a concentration of 1000 mg/L in deionized water was used to prepare calibration standards. Serial dilutions were performed using deionized water to obtain a range of boron concentrations from $5.7 \times 10^{-7}$ M to $5.7 \times 10^{-3}$ M. Each prepared standard was transferred in a volume of 75 µL into individual PCR tubes, each containing 100 µL of the sensor agent solution. The mixtures were vortexed gently for a few seconds to ensure homogeneity and then allowed to stand at room temperature for 30 seconds. Following this incubation period, the samples were analyzed using light-dependent resistors (LDRs) to detect and quantify the boron concentrations. The analytical characteristics of the developed method were evaluated.

### 3.7.2 Sample Preparation

The standard method for determining available boron in soil was the hot water extraction technique, originally described by Berger and Truog (1939) and later modified by Motsara and Roy (2008). In this method, 25 g of soil is placed in a beaker, followed by the addition of 50 mL of distilled water and 0.5 g of activated charcoal. The suspension is boiled for 5 minutes and then filtered through No. 1 filter paper. Finally, the filtrate volume is adjusted to 25 mL.

### 3.7.3 Validation of the Developed Detection Method

After optimizing the volume of the fluorescent agent solution, the concentration of the fluorescent agent solutions, the reaction time, and the pH of the reaction, validation of the developed detection method was conducted by preparing 75 µL of the fluorescent agent followed by adding 100 µL of extracted soil samples. Then, the samples were vortexed for 30 seconds before analysis with the LDR sensor. On the other hand, the extracted soil samples were analyzed using ICP-OES by filtering the samples with filter paper no. 1 and a 0.22 µm syringe filter, with a total volume of 5 mL before analysis with the ICP-OES analysis. The method validation was based on three main criteria to ensure the reliability of the detection method. First, the calibration curve had to show a coefficient of determination ($R^2$) greater than 0.995, indicating good linearity. Second, the relative standard deviation (RSD) for replicate measurements needed to be less than 5%, confirming the method's precision. Lastly, the accuracy of the method was evaluated using spiked samples, with acceptable recovery values ranging between 95% and 105%, demonstrating the method’s trueness.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Synthesization and Characterization of Fluorescent Agents

After the synthesis reaction was completed, the product was separated and purified to obtain pure N-9-AMDEA 1 suitable for characterization and fluorescence analysis. The purification involved liquid–liquid extraction, drying of the organic phase, column chromatography, and recrystallization. N-9-AMDEA 1 was submitted to the characterization by thin-layer chromatography (TLC; silica gel 60 F254, Merck; mobile phase, $CHCl_3$:$CH_3CN$ 60:40). The $R_f$ value obtained was 0.5625. Chromatograms were examined under UV light to confirm the existence of N-9-AMDEA 1. The yield percentage of N-9-AMDEA 1 was found to be 52%.

The FTIR spectrum serves to identify and assess the functional groups in a compound. In the spectra of 9-(chloromethyl) anthracene and N-9-AMDEA 1, specific wavenumbers corresponding to various functional groups were observed. The broad peak ~3300 $cm^{-1}$ of the OH group is missing. This indicates a change or use of this functional group in the reaction. The new or dominant peaks at ~1250 $cm^{-1}$ and ~1450 $cm^{-1}$ suggest the formation of a new C–N bond between anthracene and the ethylamine group. The peak on aromatic C=C remains, suggesting that the aromatic band has not been destroyed.

From the NMR result, the 11H NMR spectrum of the synthesized ligand is fully consistent with the proposed structure. The benzylic methylene attached to the anthracene ring appears as a singlet at $\delta$ 4.72, indicating no detectable coupling and confirming its direct connection to both nitrogen and the aromatic system. Two sets of triplets at $\delta$ 3.55 ($J$ = 5.35 Hz) and $\delta$ 2.82 ($J$ = 5.33 Hz), integrating for 4H each, correspond to the O–$CH_2$ and N–$CH_2$ groups of the two hydroxyethyl substituents, respectively. The aromatic region ($\delta$ 7.47-8.46) displays nine protons with characteristic dd and ddd coupling patterns matching those expected for a 9-substituted anthracene. No unexpected resonances were observed, indicating that the structure and substitution pattern are consistent with the target compound.

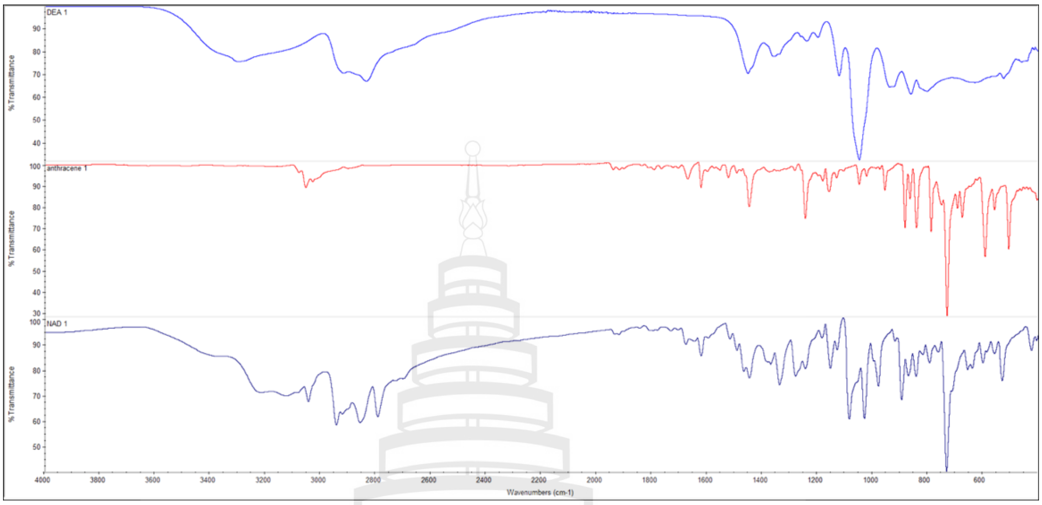

**Figure 4.1** FTIR spectra of (a) diethanolamine, (b) 9-(chloromethyl)anthracene, and (c) the synthesized fluorescent sensor 9-(anthrylmethyl)diethanolamine 1. The appearance of characteristic absorption bands corresponding to C–O and N–C stretching vibrations, together with the disappearance of the C–Cl stretching band

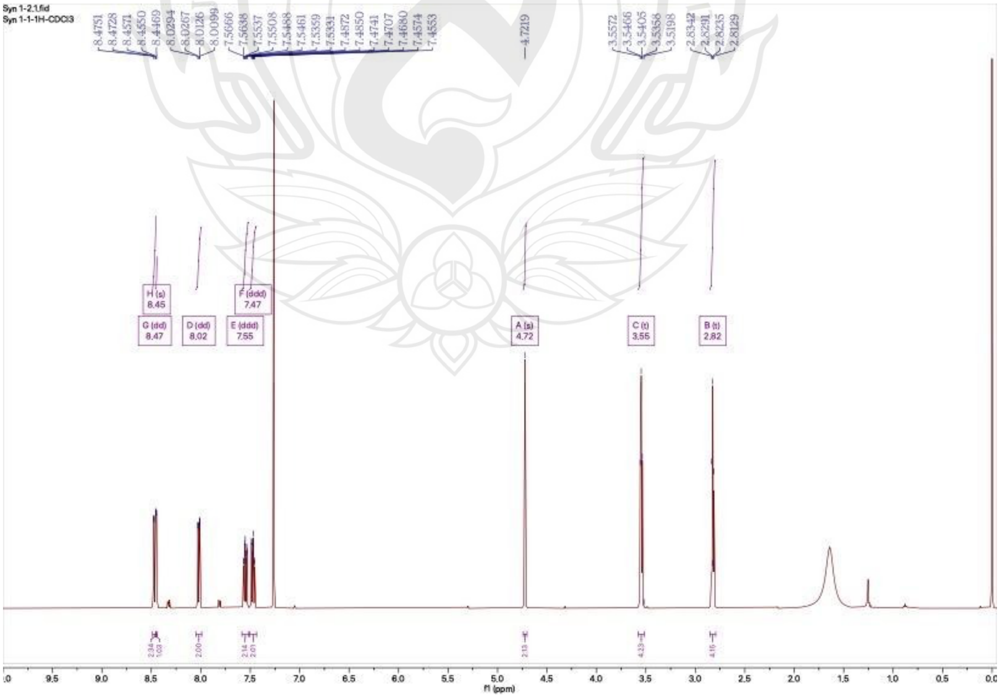

**Figure 4.2** The $^{1}H$ NMR spectrum of 9-(anthrylmethyl)diethanolamine 1 in $CDCl_3$-$d_6$

## 4.2 Selecting Fluorescent Agent for Boron Analysis

### 4.2.1 Standard Calibration

A certified boron stock solution with a concentrationThe selection of a fluorescent agent from three compounds was made based on a pre-scan using fluorescence spectroscopy. It was found that the excitation wavelength and emission wavelength of the fluorescent agents that reacted with boron are different. The results are as follows: 7-Diethylamino-4-methylcoumarin shows $\lambda_{ex}$= 374 nm, $\lambda_{em}$= 417 nm (Figure 4.3a); N-9-(anthrylmethyl)diethanolamine 1 shows $\lambda_{ex}$= 370 nm, $\lambda_{em}$= 419 nm (Figure 4.3b); and 2,2′-(4-Methylphenylimino)diethanol shows $\lambda_{ex}$= 260 nm, $\lambda_{em}$= 370 nm (Figure 4.3c). However, 2,2′-(4-Methylphenylimino)diethanol was not selected in further experiments. This compound requires UV light at 260 nm for excitation, but the flashlight source in this study can only emit wavelengths above 300 nm effectively. Therefore, the flashlight cannot provide sufficient energy to excite this fluorescent agent.

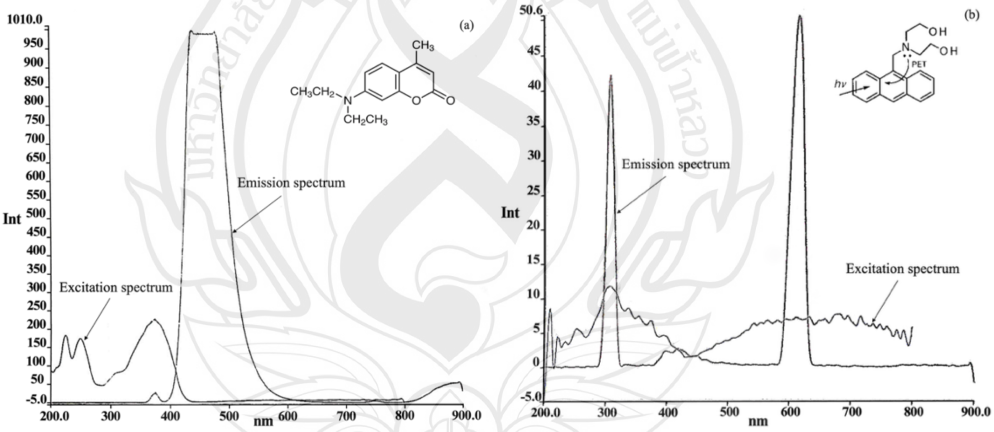

**Figure 4.3** Fluorescence spectra of three fluorescent agents (1x$10^{-4}$ M) after reaction with boron (5.7x$10^{-7}$ M) in prescan mode, showing their excitation and emission wavelengths: (a) 7-diethylamino-4-methylcoumarin (a), N-9-(anthrylmethyl)diethanolamine 1 (b), and 2,2′-(4-methylphenylimino) diethanol (c)

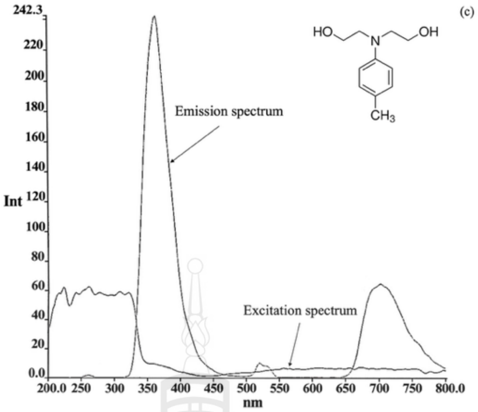

**Figure 4.3** (Continued)

### 4.2.2 The Sensitivity of Fluorescence Spectroscopy with Boron

The fluorescence sensitivity toward boron standard solution was compared using two fluorescent agents: N-9-AMDEA 1 and 7-Diethylamino-4-methylcoumarin. The comparison was performed using an Ocean Optics miniature spectrometer (product code: FLAME-S-VIS-NIR-ES). Results showed that N-9-AMDEA 1 exhibited a higher slope than 7-Diethylamino-4-methylcoumarin, as shown in Figure 4.4. Additionally, when N-9-AMDEA 1 was reacted with boron, it demonstrated enhanced fluorescence intensity, changing from a weak to a strong fluorescence signal. In addition, the fluorescence intensity of N-9-AMDEA 1 was measured across a range of concentrations in methanol to establish a working concentration for subsequent experiments. It was found that increasing the concentration of N-9-AMDEA 1 from $1.0\times10^{-5}$ M to $1.0\times10^{-3}$ M increased the fluorescence intensity, while the blank sample (pure methanol) showed little or no change (Figure 4.5). The shape of the emission spectra remained the same in all solutions, with a distinct peak at 410–420 nm, which is consistent with the fluorescence behaviors of the anthracene structure, the main component of this molecule. These results indicate that N-9-AMDEA 1 has a response to concentration in the measured range, making it suitable for quantitative fluorescence analysis in the low-to-medium concentration range before the signal starts to saturate at higher concentrations. This work, at a concentration of $1.0\times10^{-4}$ M, the fluorescence intensity was sufficiently strong to clearly detect the changes induced by boron binding.

In contrast, at 1.0×10⁻⁵ M, the fluorescence signal was considerably weaker, resulting in a lower signal-to-noise ratio and reduced measurement accuracy.

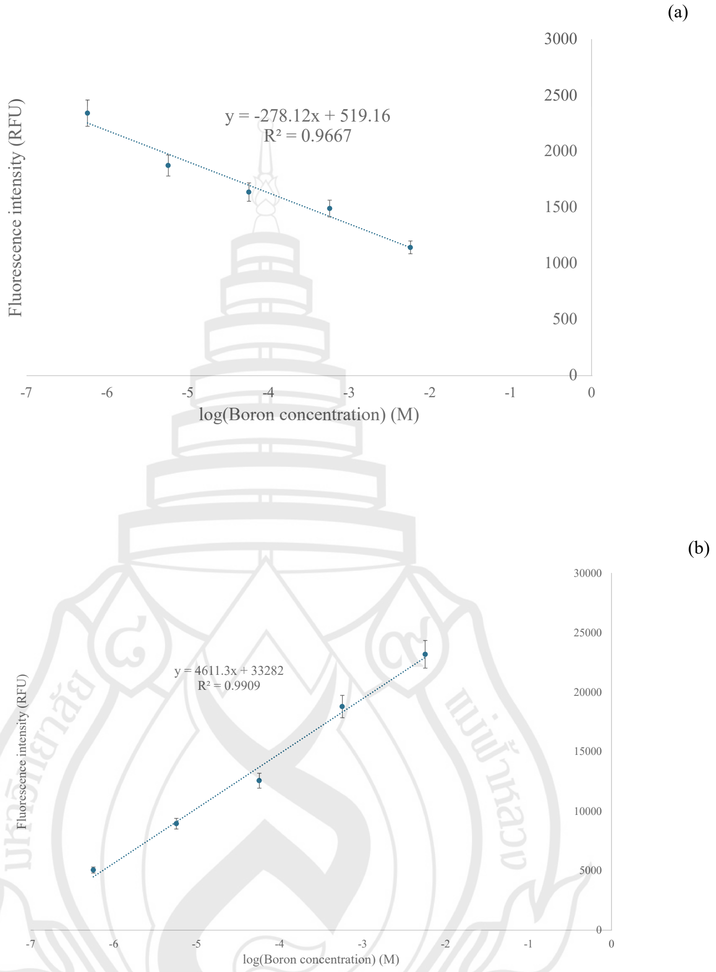

**Figure 4.4** Calibration curve of boron analysis measured by an Ocean Optics miniature spectrometer (FLAME-S-VIS-NIR-ES) with 1x$10^{-4}$M of N-9-AMDEA 1 (a), 1x$10^{-4}$M of 7-Diethylamino-4-methylcoumarin (b)

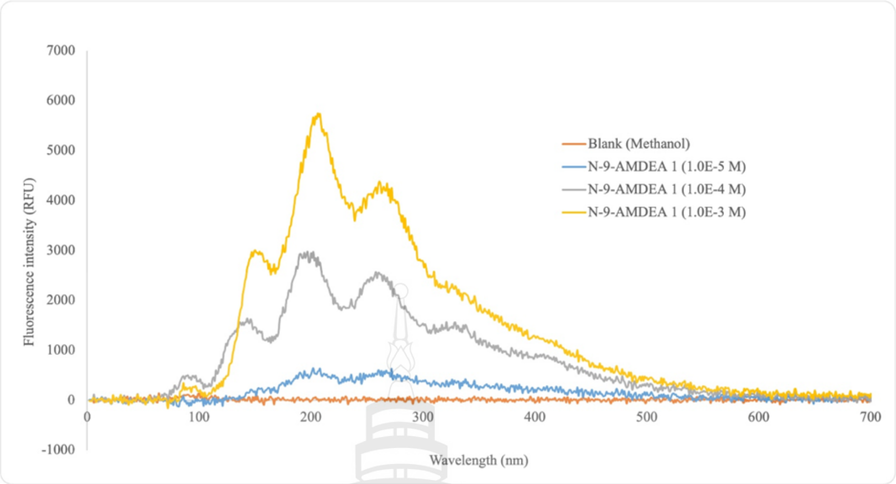

**Figure 4.5** Fluorescence emission spectra of N-9-AMDEA 1 at different concentrations ($1.0 \times 10^{-5}$, $1.0 \times 10^{-4}$, and $1.0 \times 10^{-3}$ M) in methanol compared with blank, measured using an Ocean Optics miniature spectrometer (product code: FLAME-S-VIS-NIR-ES)

The fluorescence enhancement observed in this study agrees well with the PET sensing mechanism proposed by Wang et al. (2000). In our experiment, the addition of boric acid ($H_3BO_3$) led to an increase in fluorescence intensity, consistent with the suppression of PET upon coordination between boric acid and the diol-containing amine group of N-9-AMDEA 1. However, it is important to note that the original work by Wang et al. (2000) did not specifically investigate borate ions ($B(OH)_4^-$) but rather discussed the possible conversion of boric acid to borate species at higher pH as part of the coordination process. In our case, the experiment was conducted with boric acid as the analyte, and the obtained fluorescence response is therefore attributed to the interaction between N-9-AMDEA 1 and boric acid itself under the tested conditions.

## 4.3 Optimization of the Boron Detection Sensor

### 4.3.1 Optimization of the Volume of the Fluorescent Agent Solution

The results of slope and $R^2$ obtained from the calibration graph of each studied volume are shown in Figure 4.6 The volume of the fluorescent agent increased, the system became more sensitive (the slope increased). The fluorescent agent also increased, leading to a higher baseline light intensity and a clearer reaction to boron. At a fluorescent agent volume of 75 µL, the highest slope value was reached; at 100 µL, the slope value did not change much. This decrease might have been due to self-quenching or the signal starting to saturate (Lakowicz, 2006) In summary, it was concluded that 75 µL was an appropriate volume of fluorescent agent to use.

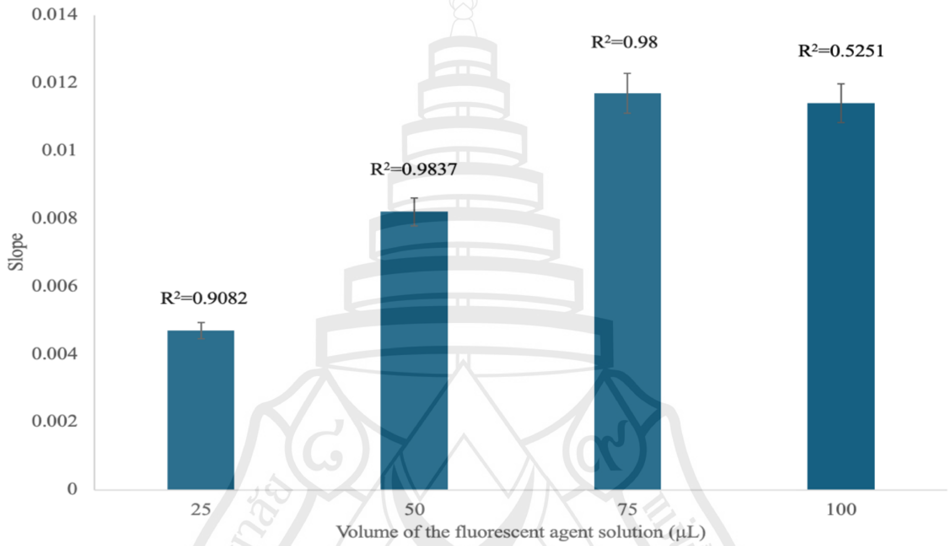

**Figure 4.6** Effect of fluorescent agent volume on sensor sensitivity. The fluorescent reagent (N-9-AMDEA 1) concentration was $1.0 \times 10^{-4}$ M, and the boron standard solutions ranged from $5.7\times10^{-7}$ to $5.7\times10^{-3}$ M. Measurements were performed in triplicate (n = 3) using the LDRs instrument

### 4.3.2 Optimization of the Reaction Time for Boron Detection

From the experimental results shown in both graphs, it was found that the 30-second period gave the most suitable result for the measurement of boron with N-9-AMDEA 1 fluorescent material, giving an $R^2$ value of 1 in the low concentration range, indicating high measurement accuracy. It also gave a relatively high slope value, reflecting good sensitivity to changes in boron concentration. Although some periods, such as 2 minutes, gave slightly higher slope values, the $R^2$ value was lower and the measurement took longer. Therefore, choosing a 30-second period is the most suitable choice because it provides a balance between accuracy, sensitivity, and short measurement time, suitable for use in systems that require fast and efficient results.

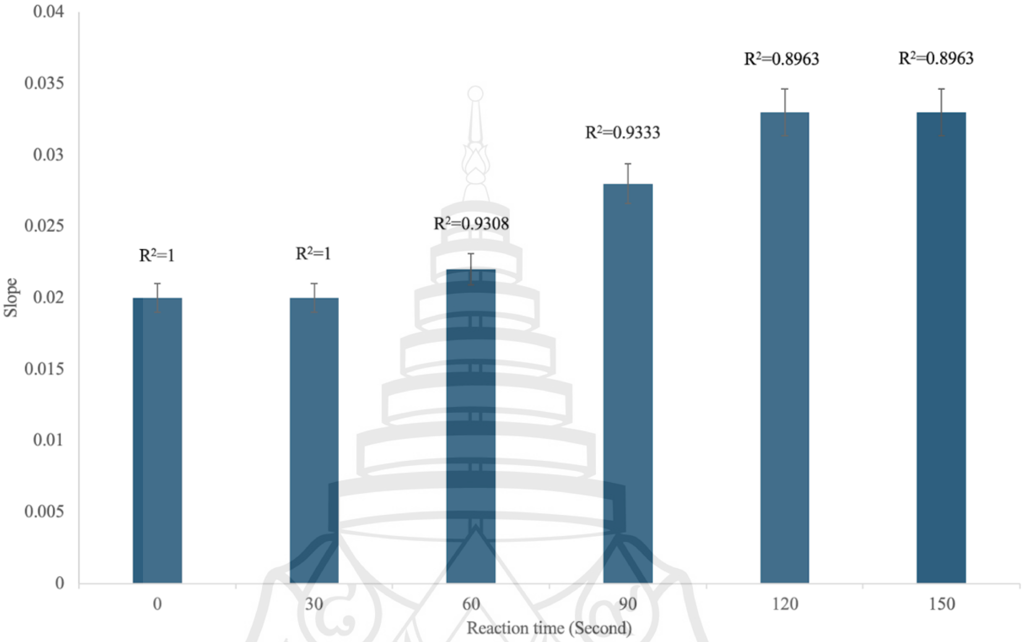

**Figure 4.7** Effect of reaction time on the sensitivity of boron detection using N-9-AMDEA 1. A reaction time of 30 s gave the most suitable result, with the highest $R^2$ value (1.0) and a relatively high slope, indicating high accuracy and sensitivity while maintaining a short measurement time

### 4.3.3 Optimization of pH Conditions for Boron Detection

The pH conditioning study revealed that the sensitivity of the boron detection system, as judged by the slope of the standard curve, was significantly different at different pH values, with pH 6.65 providing the highest slope, indicating the efficient interaction between boron and the fluorescent sensor under this condition. Meanwhile, the slope decreased at lower pH values (3–5), possibly due to the protonation of the important functional groups of the sensor, which affected the binding of boron or resulted in a decrease in the fluorescence signal. Therefore, pH 6.65 was the most suitable condition to enhance the detection efficiency in the developed system. The pH range was limited to 3.0–6.65 because of several important factors that affect how well the sensor works. At higher pH values ($pH > 8$), the fluorescent sensor becomes unstable due to excessive deprotonation of amine or phenolic functional groups, which alters the conjugated structure and quenches fluorescence (Di Cesare, 2002; Liu et al., 2023). In basic conditions, this deprotonation process disrupts the optimal configuration required for efficient boron binding and light emission (Lakshmi, 2016). In addition, boron speciation changes significantly at higher pH values (Zeebe, 2001). At neutral to slightly acidic conditions, boron mainly exists as boric acid ($H_3BO_3$), whereas above pH 9, it converts into borate ions ($B(OH)_4^-$), which can alter the binding equilibrium and fluorescence response. These chemical stability issues explain why pH 6.65 was selected as the optimum working condition and why the study did not include higher pH levels.

The equilibrium between boric acid ($H_3BO_3$) and borate ion ($B(OH)_4^-$) is strongly dependent on the pH of the solution. At pH values lower than 7, boron is predominantly present in the form of undissociated boric acid, which behaves as a weak Lewis acid and is more reactive in complexation and extraction processes. Conversely, when the pH exceeds 7, boric acid undergoes hydrolysis to form borate ions. These ions are generally more stable and exhibit lower reactivity in subsequent reactions. Therefore, controlling the pH below 7 prior to extraction or reaction is a critical step to ensure that boron remains in its active boric acid form, which can effectively participate in the intended chemical interactions. (Adavodi et al., 2025).

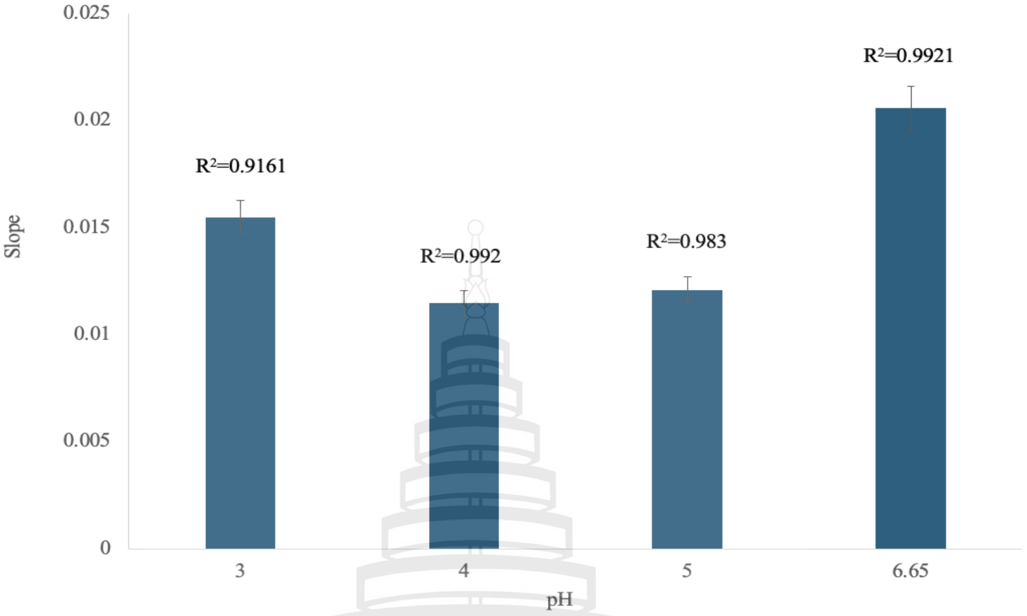

**Figure 4.8** Effect of pH on the sensitivity of the boron fluorescent sensor. The slope of calibration curves obtained at different pH values (3, 4, 5, and 6.65). The results indicate that the highest slope, and thus the greatest sensitivity, was observed at pH 6.65, while lower slopes were obtained at acidic conditions (pH 3–5), suggesting reduced sensor performance due to protonation effects

## 4.4 The Stability of the N-9-AMDEA 1 Sensor for Boron Detection

The stability of the N-9-AMDEA 1 sensor for boron detection was tested for 30 days, as shown in Figure 4.9, providing an average slope of 0.0134 with a standard deviation (SD) of 0.0028. A stable system is demonstrated by the slope value, which shows little variation in sensor response over time. Furthermore, the low SD shows the measurements are highly consistent and have few changes. These findings demonstrate that the N-9-AMDEA 1 sensor is a viable choice for long-term applications since it maintains its stability and dependability for boron detection during the tested duration. Stability testing uses statistical analysis, specifically ANOVA (Analysis of Variance), as a method to determine whether the values obtained from different variables show significant differences. When comparing how a variable changes over time, this is especially helpful. The results show that the F-value (0.4018) is less than the *F-critical* (3.6823), indicating that the groups under comparison do not differ statistically significantly.

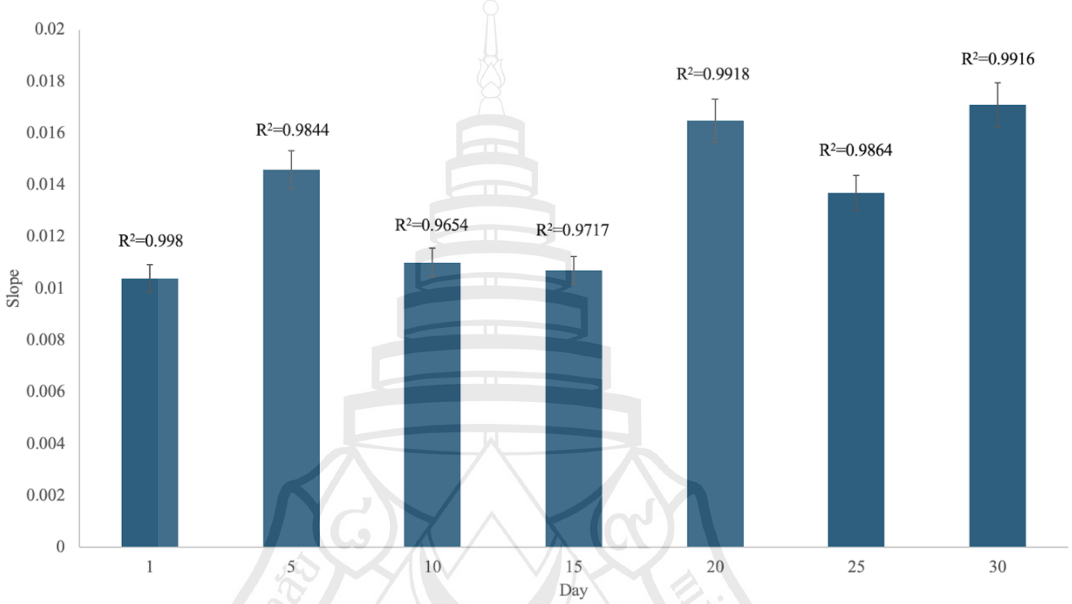

**Figure 4.9** Long-term stability of the N-9-AMDEA 1 sensor for boron detection. The sensor maintained consistent slope values over 30 days, confirming stable performance for long-term applications

The stability of the fluorescence–LDR system was statistically evaluated using slope data obtained over a 30-day period. The mean slope (0.0134 ± 0.0028, n = 7) showed a 95% confidence interval of 0.0108–0.0160 and a 95% prediction interval of 0.0061–0.0207. All measured values were within the prediction interval, and Grubbs' test ($G = 1.31 < G_{crit} = 2.02$) indicated no significant outlier. These results confirm that the system maintained consistent performance and exhibited no detectable drift, demonstrating good precision and reliability for long-term measurements.

## 4.5 Interference Study

The impact of different metal ions on detecting boron was evaluated, which displayed how the log concentration of various metal chlorides related to the voltage response. A one-way analysis of variance (ANOVA) was performed to examine the effect of different metal ions ($AlCl_3$, $CaCl_2$, $FeCl_3$, $MgCl_2$, and B as control) on the fluorescence response of the sensing compound. The results revealed a statistically significant difference among the ion groups, $F(4,10) = 193.42$, $p < 0.001$, indicating that the type of metal ion significantly influenced the fluorescence intensity. Among the tested ions, $MgCl_2$ exhibited the highest mean fluorescence response, suggesting that $Mg^{2+}$ may enhance fluorescence emission more effectively than other ions.

**Table 4.1** Effect of Metal Ions on the Fluorescence Response of the Boron Sensor at Different Boron Concentrations.

| **Concentration (M)** | **$AlCl_3$** | **$CaCl_2$** | **$FeCl_3$** | **$MgCl_2$** | **B (Control)** |
|---|---|---|---|---|---|
| $2.85 \times 10^{-8}$ | 0.98x | 0.97x | 0.99x | 1.14x | 1.00x |
| $2.85 \times 10^{-5}$ | 0.98x | 0.97x | 1.00x | 1.12x | 1.00x |
| $2.85 \times 10^{-3}$ | 0.98x | 0.97x | 1.00x | 1.11x | 1.00x |

The amount of interference appeared to follow the order Magnesium > Aluminium > Iron (III) ≈ Boron > Calcium. Unexpectedly, our fluorescence measurements coupled with a light-dependent resistor (LDR) detector showed interference effects attributable to $Mg^{2+}$. This observation can be rationalized by possible mechanisms. Trace amounts of $Mg^{2+}$ may still be solubilized during hot water extraction under the elevated temperature conditions, despite their low solubility compared with boron.

To reduce such interference, sample dilution (e.g., 1:50) was used to isolate interfering ions. Hot-water extraction has long been accepted as a reliable method for quantifying water-soluble boron in soils, since boron occurs mainly in readily soluble forms and can be efficiently released under such conditions (Mahler et al., 1984; Gupta, 1979). In contrast, divalent cations such as magnesium ($Mg^{2+}$) are strongly retained on soil exchange sites and are not released into solution by hot water alone (Food, 2008).

Therefore, conventional soil analysis protocols recommend ammonium acetate extraction as the standard procedure for determining exchangeable magnesium, whereas water extraction does not yield appreciable amounts of this cation (Cheng, 1973; Rutter et al., 2021).

## 4.6 Validation of the Developed Detection Method

The developed method shows excellent analytical performance, with %RSD at 0.14%. The linearity of the standard curve had an $R^2$ value of 0.998, indicating a strong relationship between the concentration of the substance and the measured signal. In addition, with a total preparation time of 2 minutes (30 seconds of LDR analysis per sample), the method could analyze up to 30 samples per hour, demonstrating its high efficiency and suitability for use in high-throughput analysis.

**Table 4.2** Analytical Performance Summary

| Parameter | Value |
|---|---|
| Linear range | $5.710^{-7}$ – $5.710^{-3}$ M |
| Correlation coefficient | 0.998 |
| Precision (%RSD, n=6) | 0.14% |
| Analysis time | 30 seconds |
| Stability | > 30 days |
| Sample throughput | 30 samples/hour |

The developed fluorescent sensor demonstrated excellent performance when compared with previously reported boron detection methods (Table 4.2). Wang et al. (2020) reported a limit of detection (LOD) of 0.5 µM. In terms of analysis time, Recent research has reported significant progress in the design of boron-responsive fluorescent sensors. Nehra et al. (2019) developed a boronic acid–based fluorescent probe for boric acid detection that exhibited good selectivity and a measurable fluorescence response under aqueous conditions. The system developed in this study, however, achieved a much faster detection response, completing the analysis within seconds while maintaining comparable sensitivity. In addition, Liu et al. (2023) summarized that boronic acid–based biosensors generally possess moderate fluorescence stability depending on the composition of the sensing matrix. In contrast, the developed sensor maintained consistent fluorescence intensity for over 30 days, indicating superior long-term stability and usability. Overall, the proposed method demonstrates clear improvements in sensitivity, response rate, and stability compared with previously reported boron-based fluorescent sensors.

**Table 4.3** Comparison of Boron Detection Methods

| Method | LOD | Time | Cost | Portability | References |
|---|---|---|---|---|---|
| ICP-MS | 0.01 mg/L | ~5-10 min | High | No | (Pintilie, 2021) |
| AAS | 60 µg/L | ~10-15 mins | Moderate | No | (Driscoll, 1996) |
| Colorimetric (Azomethine-H) | 0.02 mg/L<br>(20 µg/L) | 90 min | Low | Yes | (Zaijun, 2006) |
| Colorimetric (Curcumin) | 0.010 mg/L<br>(10 µg/L) | ~70-75 min | Extremely low | Yes | (Sah & Brown, 1997a) |
| LDR-coupled fluorescent sensor | | 30 s | Low | Yes | This work |

Boron (B) in soils exists in various forms, including water-soluble, adsorbed, organically bound, and insoluble mineral fractions, with the water-soluble form being the most available to plants (Goldberg, 1997; Gupta, 1979). Conventional soil B analysis typically employs hot-water extraction, which targets the soluble and partly adsorbed fractions (Keren & Bingham, 1985). The developed fluorescence–LDR sensor, showing high sensitivity in this study, is therefore most applicable to detecting water-soluble B. For soil applications, coupling the sensor with an appropriate extraction step would allow reliable field estimation of plant-available B, providing a rapid and portable alternative to laboratory-based spectrophotometric or ICP methods.

The developed detection method was validated by comparing its results with those obtained using the standard ICP-MS technique on seven samples. A statistical analysis was performed to determine whether there was a significant difference between the two methods. The absolute test statistic value was |1.343|, which is less than the critical value of 2.447 at the 95% confidence interval. Because this value falls within the acceptable range, the difference between the two methods is statistically insignificant. These findings imply that hot-water extraction of boron at pH values below 7 can convert other potential boron species into boric acid, thereby enabling accurate detection using the fluorescence-based method (9-N-AMDEA 1). This further suggests that the newly developed method employing LDRs provides results comparable to the standard ICP-MS technique and may be regarded as a reliable and cost-effective alternative for certain applications.

## 4.7 Limitations and Future Work

The current method demonstrates effective analyte detection; however, it faces several limitations that impact its broader applicability. Complex soil matrices often require sample dilution due to matrix effects, and the performance has only been validated at room temperature, limiting use under varying environmental conditions. Additionally, high concentrations of $Fe^{3+}$ and $Mg^{2+}$ ions interfere with selectivity, reducing accuracy. To address these challenges, future work will focus on developing a temperature compensation algorithm to improve robustness across different conditions, investigating matrix-matched calibration methods to better handle complex samples, and exploring multiplexed detection approaches for simultaneous multi-element analysis, thereby enhancing both sensitivity and throughput.

# CHAPTER 5

# CONCLUSION

The present work reports on the development of a rapid and ultra-sensitive portable fluorescent sensor for detecting nanomolar levels of boron ions in soil samples. The sensor, based on the fluorescent compound N-9-AMDEA 1, was successfully synthesized and its structure was confirmed by FTIR and NMR analysis. It demonstrated high sensitivity and specificity for boron, with optimal detection conditions found at pH 6.65 and a reaction time of 30 seconds. The sensor showed excellent stability for over 30 days and minimal interference from common ions, delivering results comparable to standard ICP-MS methods but with much faster analysis with a linearity of the standard curve $R^2$ value of 0.998 and a high throughput capacity of 30 samples per hour. The portable design enables cost-effective, on-site monitoring that reduces analysis costs by over 90% compared to ICP-MS. Statistical validation confirmed no significant difference from ICP-MS results ($p > 0.05$), supporting the sensor's reliability. This method offers a practical and efficient solution for real-time boron monitoring in agriculture, helping prevent crop losses caused by boron imbalances, and represents a significant advancement in portable analytical tools for environmental and agricultural applications.

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