VOLTAMMETRIC DETECTION AND QUANTIFICATION OF LEAD AND CADMIUM IN TRADITIONAL HERBAL MEDICINES USING SCREEN-PRINTED ELECTRODES

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LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AOAC	Association of Official Analytical Chemists
ASV	Anodic Stripping Voltammetry
Cd	Cadmium
CV	Cyclic Voltammetry
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPV	Differential Pulse Voltammetry
GCE	Glassy Carbon Electrode
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
NPRA	National Pharmaceutical Regulatory Agency
OFN	Oxygen-Free Nitrogen
Pb	Lead
RSD	Relative Standard Deviation
SPE	Screen-Printed Electrode
SWASV	Square Wave Anodic Stripping Voltammetry
THM	Traditional Herbal Medicine
WHO	World Health Organization

ABSTRACT

Traditional herbal medicines (THMs) are widely used as complementary healthcare products because of their natural origin and easy accessibility, yet serious safety concerns remain due to possible contamination with toxic heavy metals such as lead (Pb) and cadmium (Cd). In this study, a simple, low-cost, and portable electrochemical method for the simultaneous detection and quantification of Pb and Cd in traditional herbal medicines was developed using differential pulse anodic stripping voltammetry (DPASV) with bare screen-printed carbon electrodes (SPEs). Herbal samples were subjected to microwave-assisted acid digestion, followed by electrochemical analysis in acetate buffer (pH 4.5) using a portable potentiostat. A screening method using cyclic voltammetry was first used to identify the potential range of Pb and Cd; method validation was then performed in accordance with AOAC guidelines by evaluating linearity, limits of detection, repeatability, and reproducibility. The method demonstrated good linearity for Pb (1–10 mg/L, $R^2 = 0.9938$) and Cd (5–30 mg/L, $R^2 = 0.992$), with limits of detection of 0.94 mg/L and 3.75 mg/L, respectively. Precision studies showed acceptable repeatability and reproducibility. Analysis of ten commercially available herbal medicine samples revealed that nine samples contained no detectable Pb or Cd within the method's detection limits. One sample, Tongkat Ali (Eurycoma longifolia), exhibited detectable Cd at 2.47 mg/L, while Pb was not detected in any sample. In conclusion, the developed DPASV using SPEs provides a reliable and practical approach for screening heavy metal contamination in traditional herbal medicines.

ABSTRAK

Ubat herba tradisional (Traditional Herbal Medicines, THMs) digunakan secara meluas sebagai produk penjagaan kesihatan kerana semula jadi dan mudah diperoleh, namun kebimbangan serius terhadap keselamatan produk masih wujud akibat kemungkinan pencemaran seperti plumbum (Pb) dan kadmium (Cd). Dalam kajian ini, satu kaedah elektrokimia yang ringkas, berkos rendah dan mudah alih telah dibangunkan untuk pengesanan dan penentuan serentak Pb dan Cd dalam ubat herba tradisional menggunakan voltametri pelucutan anodik denyutan pembeza (DPASV) dengan elektrod karbon bercetak skrin (SPE) tanpa pengubahsuaian. Sampel herba telah menjalani pencernaan asid berbantukan gelombang mikro, diikuti dengan analisis elektrokimia dalam penimbal asetat (pH 4.5) menggunakan potensiostat mudah alih. Kaedah saringan menggunakan voltametri kitaran terlebih dahulu dijalankan bagi mengenal pasti julat potensi Pb dan Cd, seterusnya kaedah pengesahan dilakukan mengikut garis panduan AOAC dengan menilai kelinearan, had pengesanan, kebolehulangan dan kebolehulangan semula. Kaedah ini menunjukkan kelinearan yang baik bagi Pb (1–10 mg/L, R^2 = 0.9938) dan Cd (5–30 mg/L, R^2 = 0.992), dengan had pengesanan masing-masing sebanyak 0.94 mg/L dan 3.75 mg/L. Kajian ketepatan menunjukkan kebolehulangan dan kebolehulangan semula yang boleh diterima. Analisis terhadap sepuluh sampel ubat herba komersial mendapati sembilan sampel tidak mengandungi Pb atau Cd yang boleh dikesan dalam had pengesanan kaedah ini. Satu sampel, Tongkat Ali (Eurycoma longifolia), menunjukkan kehadiran Cd pada kepekatan 2.47 mg/L, manakala Pb tidak dikesan dalam mana-mana sampel. Kesimpulannya, kaedah DPASV menggunakan SPE yang dibangunkan ini menyediakan pendekatan yang boleh dipercayai dan praktikal untuk saringan pencemaran logam berat dalam ubat herba tradisional.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Traditional Herbal Medicine (THM) continues to serve as a primary healthcare resource for a larger proportion of the global population, with usage increasing due to its perceived natural and widespread accessibility (Ekor, 2014). However, the growing trust in THM has increased concerns regarding product safety, particularly in relation to contamination by toxic heavy metals such as Cd and Pb (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014; C. Okpara, E. Fayemi, B. Wojuola, C. Onwudiwe, & E. Ebenso, 2022). Heavy metals enter herbal plants through polluted soil, irrigation water, and atmospheric deposition, allowing gradual accumulation over time (Street, 2012). Numerous investigations have reported that a significant proportion of herbal medicine samples collected worldwide exceed established permissible limits for heavy metals, underscoring serious deficiencies in quality assurance, regulatory oversight, and routine monitoring practices (Fu, Zhang, Zheng, & Jin, 2020).

The toxicity of Cadmium (Cd) and Lead (Pb) is typically irreversible due to their non-biodegradability and their tendency to bioaccumulate in human tissues. Severe exposure leads to systemic toxicity, even at low concentrations (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). For example, Cd can cause severe nephrotoxicity, leading to kidney damage (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014; C. Okpara, E. Fayemi, B. Wojuola, C. Onwudiwe, & E. Ebenso, 2022). Pb exposure is hazardous to the central nervous system, as it can cross the blood-brain barrier and cause irreversible brain damage, encephalopathy, and edema. The presence of these highly toxic elements in herbal medicinal plants requires strict, continuous monitoring to ensure compliance with international safety standards set by the World Health Organization (WHO) (World Health Organization, 2007).

Previous studies on the determination of Pb and Cd in medicinal and herbal samples have predominantly employed laboratory-based spectrometric techniques. However, despite their analytical strengths, these techniques present several practical limitations when applied to the routine monitoring of traditional herbal medicines. The high cost of instrumentation, requirement for specialised laboratory facilities, and dependence on skilled personnel limit their accessibility for frequent quality control. These limitations highlight the need for simpler and more cost-effective screening approaches.

Hence, this study aimed to address the analytical gap by using a low-cost, portable device coupled with Screen-Printed Electrodes (SPEs) for fast, simultaneous electrochemical detection of Pb and Cd in THM extracts. It provides a simplified, cost-effective, and highly efficient solution that improves quality control procedures in the THM supply chain while also protecting public health.

1.2 Problem Statement

The contamination of traditional herbal medicine with heavy metals has become a growing and grave concern for global public health (Zhang et al., 2024). Toxic metals, such as Lead (Pb), Cadmium (Cd), mercury (Hg), and arsenic (As), are highly harmful because they are non-biodegradable and can accumulate in the environment (Alharbi et al., 2024). International bodies, such as the World Health Organization (WHO) and the National Pharmaceutical Regulatory Agency (NPRA), have established maximum permissible limits for heavy metals like Pb, Cd, Hg, and As in medicinal plants to protect public health safety (Zhang et al., 2024; Melucci, Casolari, Locatelli, & Locatelli, 2021).

Reliable and accurate methods are needed to detect and measure heavy metal contamination to protect consumers. Currently, the most used analytical techniques include Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Chaiyo et al., 2016). These techniques provide good sensitivity and accuracy in laboratory settings.

However, they also have several limitations that restrict their use, especially for time-to-time quality checks and rapid detection of contamination in traditional herbal medicine.

Firstly, high costs and specialized infrastructure are required for detecting heavy metals in herbal samples. These methods require expensive instruments, specialised laboratory space, and high sample processing costs, making them challenging to use widely across many sectors (Chaiyo et al., 2016). Additionally, they involve time-consuming, complex analytical procedures, such as sample preparation. The procedures can be lengthy and complex. Individuals with expertise must take specific steps to prevent damage to the instrument or sample (Chaiyo et al., 2016). Lastly, the instruments lack portability. Since the instruments are large and require specialised and controlled laboratory conditions, they cannot be used for rapid on-site testing (Abd El-Raheem et al., 2024).

Heavy metal contamination remains a significant safety concern, and current laboratory methods have limitations (Tian et al., 2025). So, there is a need for new analytical approaches that are low-cost, rapid, sensitive, and portable. An alternative to those high-cost techniques is electrochemical analysis, especially voltammetric methods, which use a potentiostat. Analyses such as Anodic Stripping Voltammetry (ASV), including Square Wave ASV (SWASV) and Differential Pulse ASV (DPASV), are well-known for their high sensitivity, simple procedure, and low cost in heavy metal analysis (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022). The sensitivity is further enhanced by a preconcentration step, in which the target metal ions are deposited on the electrode surface before measurement.

Since heavy metal contamination often involves multiple metals present simultaneously, such as Cd, Pb, Hg, Zn, and others, this study also focuses on using techniques that enable simultaneous detection (Jia et al., 2025). Research has shown that a modified screen-printed Electrode (SPE) incorporating bismuth films or nanocomposite materials can simultaneously measure multiple metals, such as Zn, Cd, and Pb (Zhang et al., 2024). However, in this research, bare carbon SPE was used to detect Pb and Cd.

In summary, the primary issues addressed in this research are the lack of instrument portability, affordability, and the need for fast, accurate techniques that can simultaneously detect multiple heavy metals in traditional herbal medicine. To overcome these problems, this research used a potentiostat to simultaneously detect Cd and Pb, providing fast, accurate results. The method avoids the laborious, complex procedures involved in creating nanocomposites or pre-plating metallic coatings by employing a bare surface (Li, Wang, Chen, Yi, & Wang, 2024).

1.3 Study Objective

1.3.1 General Objective

- To develop and apply a voltammetric method using screen-printed electrodes (SPEs) coupled with a potentiostat for the detection and quantification of Pb and Cd in traditional herbal medicines

1.3.2 Specific Objectives

- To optimise sample pretreatment and voltammetric parameters for analysis.
- To prepare and extract THM samples for voltammetric analysis of Pb and Cd.
- To simultaneously detect and quantify the presence of Pb and Cd in THM samples using a potentiostat.

1.4 Significance of the Study

The findings of this research have the potential to strengthen the quality control of traditional herbal medicines by introducing a portable and cost-effective method for detecting toxic heavy metals. This study provides a valuable understanding of how electrochemical sensing can be utilised as a practical alternative to conventional laboratory-based techniques, particularly in settings where rapid and on-site testing is required. Addressing the limitations of existing

methods, this research helps ensure contaminated herbal products are identified and removed before they reach consumers.

From a forensic science perspective, this study contributes to the development of a rapid, portable analytical approach for the preliminary screening of heavy metal contamination in traditional herbal medicines. The voltammetric method developed using SPE provides a practical tool for forensic toxicology investigations involving suspected heavy metal exposure, adulteration, or contamination of herbal products. Its portability and low operational cost enable on-site screening during enforcement actions or field investigations, even when access to advanced laboratory facilities is limited. Moreover, the ability to simultaneously detect Pb and Cd enhances evidential efficiency by allowing rapid differentiation between contaminated and non-contaminated samples prior to confirmatory laboratory analysis.

Furthermore, the development of a potentiostat-based electrochemical platform enables laboratories, industries, and healthcare authorities to conduct routine monitoring. The method's ability to detect multiple heavy metals simultaneously also enhances its usefulness for evaluating the safety of herbal medicines, which often contain multiple coexisting contaminants. Additionally, by utilising the sensor with real herbal medicine samples and comparing the results with those obtained using standard techniques, this study demonstrates the practicality of the portable system. Apart from that, it also contributes to safer consumption of traditional herbal medicines and improved public health protection.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews previous studies on heavy metal contamination and the simultaneous voltammetric detection of Pb and Cd in THM samples.

2.0 Heavy Metals in Medicinal Plants

Heavy metals are metallic elements known for their extremely high density and molecular weight, which often exceed 5g/cm^3 (Jin Mei & Ainliah Alang Ahmad, 2021). The metals are toxic even at low concentrations. Anthropogenic activities, such as mining, industrial developments, and the production and use of fertilisers and pesticides in agriculture, significantly contribute to their extensive presence as pollutants (Suwannasom & Ruangviriyachai, 2011). Heavy metals are classified as hazardous pollutants due to their persistence in the environment and their tendency to bioaccumulate in living organisms. These factors lead to cumulative toxicity even at minute concentrations (Jin Mei & Ainliah Alang Ahmad, 2021).

International bodies, such as the World Health Organization (WHO), have set permissible limits to regulate these pollutants in medicinal plants. The allowable limits for Cd are 0.3 mg/L and for Pb are 10 mg/L, aimed at reducing the health risks associated with these pollutants (Palisoc, Vitto, & Natividad, 2019).

Trace amounts of heavy metals such as Pb and Cd have been reported in commercially available herbal products and food supplements in various countries (Yuan, Chapman, & Wu, 2011). Traditional remedies are often consumed over extended periods and sometimes without medical supervision. The presence of these toxic elements, even at regulated levels, also has the possibility of continuous risk of chronic intoxication and cumulative body burden (Thanh, Luyen, Thanh Tam Toan, Hai Phong, & Van Hop, 2019).

The global need for safety in traditional herbal medicine demands reliable and efficient analytical techniques to quantify trace metals in complex plant matrices. Although conventional techniques such as AAS and ICP-MS remain reference methods, their limitations have motivated the development of alternative electrochemical approaches for routine screening. (Suwannasom & Ruangviriyachai, 2011). They are therefore considered reference methods for regulatory procedures in various types of samples. However, these techniques suffer from significant drawbacks that restrict their suitability for high-throughput or decentralised screening of herbal products (Suwannasom & Ruangviriyachai, 2011).

The development of electrochemical methods, especially Anodic Stripping Voltammetry (ASV) and Differential Pulse Anodic Stripping Voltammetry (DPASV), with high sensitivity and simple operational procedures, closes the analytical gap. These analytical approaches offer easy operation, low cost, portability, and rapidity (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022). It also provides real-time measurement, making it more suitable for routine monitoring and on-site screening applications.

Although conventional methods remain the benchmark for validation and regulatory confirmation, there are challenges in using an electroanalytical platform that can deliver performance comparable to AAS and ICP-MS, particularly in terms of sensitivity, reliability, and selectivity. However, electrochemical methods need to operate within cost-effective limits and provide an easily deployable system for analysing herbal matrices.

2.1 Electrochemical Detection of Heavy Metals

The analytical determination of trace heavy metals has undergone significant evolution with the advancement of electrochemical techniques, particularly stripping voltammetry (SV). This methodology has emerged as a widely used alternative in trace metal analysis. Anodic Stripping Voltammetry (ASV) is typically suitable for trace analysis because it involves a two-step process that efficiently concentrates the analyte before measurement, involving electrodeposition and anodic stripping (Suwannasom & Ruangviriyachai, 2011). Firstly, DPV

starts with electrodeposition, where the target metal ions (Pb, Cd) are reduced onto the surface of the working electrode (WE) at a carefully controlled negative potential (E-*dep*) for a specific accumulation time (t-dep). The process deposits the ions as an electrically neutral metallic film (M0) onto the electrode. Stirring the solution is crucial during this phase to maximise ion mass transfer to the electrode surface (Suwannasom & Ruangviriyachai, 2011).

Next is anodic stripping, where the potential is scanned rapidly in a positive (anodic) direction after a brief period. This strips the accumulated metal back into the solution (M0 • Mn+ + ne−), generating a sharp current peak proportional to the metal's concentration in the original sample (Suwannasom & Ruangviriyachai, 2011). Due to its preconcentration effect, which concentrates metals from a large volume of solution onto a small electrode surface, ASV is effective for detecting trace metals. Compared to traditional voltammetry, this method enables detection limits that are much lower, frequently in the parts per billion (ppb) or even parts per trillion (ppt) range (Chaiyo et al., 2016).

2.1.1 Differential Pulse Voltammetry (DPV)

Differential Pulse Voltammetry (DPV) is the favoured stripping method due to its advantages over simpler linear sweep voltammetry (LSV) (Jin Mei & Ainliah Alang Ahmad, 2021). Small potential pulses are applied to a staircase potential ramp using DPV, which measures the current twice during each pulse cycle: right before the pulse (T) and right after the pulse (T') (Jin Mei & Ainliah Alang Ahmad, 2021).

The key advantage of DPV is that it effectively measures the difference in current ($\Delta I = I_{T'} - I_T$), which minimises the non-faradaic (capacitive) current resulting from the electrical double layer at the electrode surface as it is charged and discharged (Jin Mei & Ainliah Alang Ahmad, 2021). Since the faradaic current decays more slowly than the capacitive current, DPV improves the signal-to-noise ratio and provides better sensitivity than linear sweep voltammetry (Jin Mei & Ainliah Alang Ahmad, 2021).

2.1.2 Simultaneous Detection of Pb and Cd

The electrochemical method is suitable for the simultaneous quantification of Pb and Cd, provided that their metallic stripping species oxidise at sufficiently separated potentials (Ouyang, Zhu, Tatum, Chambers, & Xue, 2011). In a typical stripping voltammogram, Pb and Cd are resolved by their characteristic oxidation potentials, yielding distinct, well-separated current peaks. Using a carbon electrode in an appropriate supporting electrolyte (pH 4.5–5.5), the stripping peak of Pb generally appears at a less negative potential (approximately −0.52 V) compared to Cd, which appears at a more negative potential (approximately −0.68 V) (Ouyang, Zhu, Tatum, Chambers, & Xue, 2011). For reliable simultaneous quantification, a minimum peak separation of roughly 100–200 mV is required to prevent peak overlap and ensure accurate individual metal determination (Jin Mei & Ainliah Alang Ahmad, 2021).

Effective simultaneous detection of Pb and Cd requires careful optimisation of experimental parameters to maintain distinct, non-overlapping peaks. The supporting electrolyte plays a critical role, particularly the solution pH. Weak acid buffers such as acetate, typically maintained at pH 4.5–5.0, are commonly used to stabilise metal ions while minimising hydrolysis or precipitation at higher pH values and suppressing hydrogen evolution at strongly acidic conditions (Suwannasom & Ruangviriyachai, 2011; Baldrianova et al., 2011).

In addition, the deposition potential (E-dep) must be sufficiently negative to reduce both Pb and Cd simultaneously during the preconcentration step. Deposition potentials are commonly optimised within the range of −1.2 to −1.6 V, depending on the electrode material and matrix composition (Baldrianova et al., 2011). The selection of Differential Pulse Anodic Stripping Voltammetry (DPASV) is therefore justified by its ability to provide highly sensitive, simultaneous multi-element detection. The use of a potentiostat allows precise control of the deposition and stripping waveforms, ensuring reproducible and reliable voltammetric measurements suitable for high-throughput screening in herbal medicine quality control.

2.2 Working Electrodes for Heavy Metal Sensing

In previous years, the benchmark for sensitive stripping analysis was the mercury-based electrode, such as the Hanging Mercury Drop Electrode (HMDE), which is highly regarded for its high hydrogen overpotential, clean, uniform surface, and tendency to form stable amalgams with heavy metals. However, the toxicity of mercury severely limits its use in modern, environmentally conscious analysis (Chaiyo et al., 2016).

The modern standard alternative is the Screen-Printed Electrode (SPE), which utilises non-toxic bismuth (Baldrianova, Agrafiotou, Svancara, Jannakoudakis, & Sotiropoulos, 2011). Because bismuth forms intermetallic alloys or "fused alloys" with numerous heavy metals, including Cd and Pb, during the deposition step, it exhibits analytical properties that are very similar to those of mercury (Chaiyo et al., 2016). This process facilitates the electrodeposition of metals, thereby improving overall analytical performance (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022). However, to assess the sensitivity and selectivity of the carbon electrode, a bare carbon SPE was used in this study without bismuth modification.

2.2.1 Screen-Printed Electrodes (SPEs)

SPEs, which are usually made of carbonaceous materials, change the sensing process from a costly laboratory method to an economical, disposable one. SPEs integrate the complete three-electrode system, which includes working, reference, and counter electrodes, onto a single substrate. Low manufacturing costs, batch production compatibility, convenience of use, and disposability, which eliminate the time-consuming reconditioning or polishing required by traditional electrodes such as Glassy Carbon Electrodes (GCEs), are their primary advantages (Chaiyo et al., 2016). Additionally, cross-contamination is naturally prevented in complicated samples by this disposability. Additionally, their small size makes them ideal for merging with a portable potentiostat for in-situ, decentralised analysis (Li, Wang, Chen, Yi, & Wang, 2024).

Compared with conventional electrodes, SPEs offer rapid use and require less maintenance, making them preferable to approaches that employ GCEs or costly spectrometric procedures. By increasing the carbon substrate's surface area and conductivity, often through the use of modifiers, the high sensitivity required for trace analysis can be achieved (Chaiyo et al., 2016).

2.2.2 Challenges and Non-Additive Activation

Although bare carbon electrodes are easy to work with, they pose unique analytical challenges that require method optimisation rather than chemical modification (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022). In contrast to modified surfaces, a pair of weak redox peaks is often observed for model systems on a bare SPE, indicating electron transport at the interface (Chaiyo et al., 2016). Because it is more difficult to deposit target metals directly onto a plain carbon surface without an intermediate coating, small stripping current responses are typical on bare carbon (Chaiyo et al., 2016).

Next, for electrochemical activation, instead of adding external chemical modifiers, the bare carbon surface can be improved through pre-anodisation (Li, Wang, Chen, Yi, & Wang, 2024). To enhance the amount of carbon-oxygen functional groups and remove organic binders from the carbon ink, this electrochemical treatment involves applying a constant voltage to the electrode in a buffer solution. This activation accelerates electron transfer rates and increases the effective active surface area of the electrode without altering its fundamental "bare" composition (Li, Wang, Chen, Yi, & Wang, 2024).

The need for a cost-effective, efficient platform for large-scale testing of traditional medicine extracts supports the use of bare, screen-printed carbon electrodes. This research aims to provide a straightforward and reliable alternative to highly customised sensors by leveraging the inherent kinetic benefits of graphite flakes and enhancing the system through pre-anodisation.

2.3 Sample Preparation for Herbal Matrices

Complex matrices, such as powdered traditional medicines or herbal dietary supplements, need extensive preprocessing before electroanalytical analysis (Chandrasiri, Mubarak, Mahatantila, & Mahanama, 2019). High levels of organic materials, such as proteins, carbohydrates, and humic compounds, are the main barrier. The sample needs complete mineralisation. The remaining organic chemicals and surfactants will seriously interfere with the electrochemical analysis if the organic matrix is not entirely broken down (mineralised) (Suwannasom & Ruangviriyachai, 2011). Electrode surface blocking is one of the primary mechanisms by which these interfering chemicals function. They effectively block the active sites required for metal ion deposition by attaching to the working electrode surface (Xing et al., 2024).

The anodic metal peak produced by DPASV can be significantly compromised by signal deterioration caused by organic debris and chloride ions. This creates inaccurate and irreproducible outcomes (Suwannasom & Ruangviriyachai, 2011). DPASV produced incorrect results due to interference from organic matter, which occurred when proper digestion was not achieved, as reported in research on the determination of metals in fermented foods (Suwannasom & Ruangviriyachai, 2011). Therefore, the destruction of the organic matrix is essential to ensure accurate electrochemical quantification.

2.3.1 Digestion Methods

A vital laboratory procedure required before electrochemical analysis is digestion, which involves breaking down organic materials to release metal ions. The accuracy and recovery rates of heavy metals, such as Pb and Cd, are significantly influenced by the choice between traditional and modern analytical methods.

The first digestion method is dry ashing. In this conventional technique, organic materials are combusted at high temperatures, typically 450–500 °C. However, it is considered time-consuming and poses a significant risk of losing hazardous materials during heating, particularly Pb (Chandrasiri, Mubarak, Mahatantila, & Mahanama, 2019). Next is wet digestion. This involves treating samples with concentrated acids in open vessels, such as beakers or flasks. The low boiling points of reagents, such as nitric acid at 122 °C, frequently limit their effectiveness and may not be sufficient to oxidise complex matrices entirely (Wale, 2024). Furthermore, open systems are more prone to systematic errors and contamination from laboratory air.

Lastly, Microwave-Assisted Wet Digestion. Due to its speed and accuracy, this method is highly recommended for trace and ultra-trace analysis (Wale, 2024). It reduces the possibility of external contamination and stops volatile analytes from being lost by employing a closed system (Wale, 2024). The procedure utilises ionic migration and dipole rotation, facilitated by microwave radiation, to provide rapid, uniform heating that enables the oxidation of nearly all organic materials (Wale, 2024).

In contrast to samples analysed without microwave treatment, which frequently yield irreproducible results because organic matter deteriorates the anodic metal peaks. Comparative studies have demonstrated that microwave digestion yields results that are well-correlated with those obtained using reference methods, such as Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (Suwannasom & Ruangviriyachai, 2011).

2.3.2 Application to Herbal and Food Matrices

Applying digestion protocols to complex herbal matrices requires precise control over sample mass, acid volume, and thermal programs to ensure total mineralisation. For plant-based foods and herbal food supplements (HFS), standard procedures require precisely weighing a homogenised sample between 0.1 and 0.5 grams (Suwannasom & Ruangviriyachai, 2011). An optimal reagent mixture, such as 6 mL of HNO_3, 2 mL of H_2O_2, and 2 mL of HF, is frequently

used for complete mineralisation (Suwannasom & Ruangviriyachai, 2011). Using ultra-pure HNO_3 alone is effective in certain situations, such as analysing rice. It eliminates the possible over-recovery problems occasionally connected with the addition of hydrogen peroxide (Chandrasiri, Mubarak, Mahatantila, & Mahanama, 2019).

Next, for the Thermal Programs, which utilise microwave systems for sample preparation. In a typical two-step program, the temperature might be raised to 110 °C at 600 W, then ramped again to 200 °C at 1200 W with a 10-minute hold. A 30-minute hold time at 180 °C is used in other verified rice-specific procedures (Chandrasiri, Mubarak, Mahatantila, & Mahanama, 2019). The World Health Organization describes a wet digestion method for plant samples using a mixture of nitric and perchloric acids (2:1). The mixture is then heated slowly to 240°C and maintained at this temperature for 4 hours to ensure a clear inorganic residue.

After digestion is complete, the resulting clear solution is typically filtered and diluted with a supporting electrolyte, such as 0.025 M acetate buffer (pH 4.5), to a fixed volume, such as 25 mL (Suwannasom & Ruangviriyachai, 2011). To prevent dissolved oxygen from interfering with the measurement of metal stripping signals, the solution is purged with oxygen-free nitrogen (OFN) for approximately 300 seconds before initiating the voltammetric scan using a potentiostat (Suwannasom & Ruangviriyachai, 2011).

The effective use of these techniques ensures that Pb and Cd levels in traditional medicines remain within acceptable regulatory limits, such as 0.3 mg/L for Cd and 10 mg/L for Pb, by providing a verified alternative to standard AAS for routine quality control (Wale, 2024).

2.4 Supporting Electrolyte

The stability of Pb and Cd ions is directly affected by the supporting electrolyte, which is essential for regulating the pH and conductivity of the analytical solution (Suwannasom & Ruangviriyachai, 2011). The best medium for the simultaneous measurement of Cd, Pb, Cu, and Zn is generally acknowledged to be the acetate buffer. Typically, a pH range of 4.4 to 5.5

is considered optimal (Chaiyo et al., 2016). A strong acidic medium (low pH) can lead to excessive hydrogen gas development at the working electrode, physically restricting metal deposition, making this range a critical balance. On the other hand, Pb inos are prone to hydrolysis at higher pH, which eliminates them from the electrochemically active pool (Xing et al., 2024).

Signal intensity is strongly affected by the concentration of the acetate buffer. Research shows that at very low or zero acetate levels, stripping signals are significantly increased. This is due to metal ions forming stable acetato-complexes at high acetate concentrations (Baldrianova, Agrafiotou, Svancara, Jannakoudakis, & Sotiropoulos, 2011). The rate at which these complexed ions deposit onto the electrode during the preconcentration step was decreased because their diffusion coefficients are lower than those of their free ionic forms (Baldrianova, Agrafiotou, Svancara, Jannakoudakis, & Sotiropoulos, 2011).

2.4.1 Deposition Parameters

Preconcentration efficiency is determined by deposition potential (Edep) and deposition time (tdep). To ensure a simultaneous, quantitative reduction of Pb and Cd ($Pb \rightarrow Pb^0$ and $Cd \rightarrow Cd^0$), the E-dep must be sufficiently negative (Chaiyo et al., 2016). Optimised potentials typically range from −1.15 V to −1.6 V (vs Ag/AgCl). A potential that is too negative can expose the electrode surface to hydrogen gas evolution, disrupting metal deposition and affecting outcomes (Chaiyo et al., 2016). For example, Cd, Pb, Cu, and Zn were successfully analysed simultaneously at a deposition potential of −1.15 V (Suwannasom & Ruangviriyachai, 2011).

Additionally, the deposition time is crucial to this analysis. During the accumulation phase, the signal strength (peak current, Ip) is directly and linearly related to the deposition time (t-dep) (Suwannasom & Ruangviriyachai, 2011). Extending t-dep directly lowers the detection limit. However, this benefit involves a critical compromise. For example, between sensitivity and efficiency, a longer duration results in higher sensitivity but longer overall analysis times.

The next obstacle is electrode fouling**.** A long deposition time, exceeding 200 seconds, may saturate the electrode surface or degrade electrode modifications (fouling), leading to signal leveling off or irreversible damage (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022). The optimal tdep must be determined empirically for each electrode or matrix. To achieve high throughput while maintaining sufficient sensitivity for trace analysis, this is frequently set to a bit shorter duration, such as 90 or 110 seconds (Suwannasom & Ruangviriyachai, 2011).

2.4.3 DPV Scanning Parameters

The collected metal is converted back into a detectable current signal by the stripping scan. The potentiostat controls the parameters of the stripping waveform, which in turn impacts the signal magnitude and resolution. The peak height is significantly influenced by the amplitude of the potential pulse (ΔE). In general, larger amplitudes produce higher signals; however, if they are huge, they may reduce resolution and broaden the peak. For optimal DPASV processes, amplitudes of approximately 50 mV are typically selected (Suwannasom & Ruangviriyachai, 2011).

Additionally, the setting of the step potential and scan rate for the DPV. These parameters determine the resolution of the voltammogram. Larger steps, up to 30 mV, enable high-speed analysis, while smaller step potentials, such as 2-5 mV, offer good resolution (Suwannasom & Ruangviriyachai, 2011).

Electrode regeneration, involving a conditioning pulse (or cleaning step), is frequently used between measurements to ensure reliability. For 60 to 90 seconds, the electrode must be polarised at a positive voltage, such as +0.3 V to +0.8 V, to eliminate any last traces of metal and memory effects (Chaiyo et al., 2016).

To balance the stability of Pb against the interference of hydrogen evolution, an acetate buffer (pH 4.5) was chosen for this research. Additionally, a 180-second deposition time was selected to achieve high sensitivity while keeping the overall testing duration at about 3 minutes, which is ideal for quick screening of herbal samples (Li, Wang, Chen, Yi, & Wang, 2024).

2.5 Method Validation

The process of proving that the suggested DPASV-SPE method is accurately and consistently "fit for its intended purpose" of evaluating Pb and Cd in complex herbal matrices is known as method validation (Thompson, Ellison, & Wood, 2002). This usually follows the standards established by groups such as AOAC International.

2.5.1 AOAC Guidelines for Analytical Methods

AOAC International establishes Standard Method Performance Requirements (SMPRs®). To define the minimum analytical performance for classes of analytical techniques, single-laboratory validation (SLV) techniques evaluate critical performance attributes for trace contaminants in complex matrices, such as accuracy. Precision is evaluated in terms of repeatability and reproducibility. Besides that, Linearity demonstrates a predictable response across the concentrations of interest. Lastly. Limits of Detection (LOD). Defining the lowest reliably measured concentrations (AOAC International, 2024).

2.5.2 Linearity

Outside the known working range, linearity ensures that the measured signal is directly proportional to the analyte concentration, a key requirement for the calibration curve. A regression coefficient (R^2) of more than 0.99 is a standard criterion for determining the linearity of the calibration plot (Suwannasom & Ruangviriyachai, 2011). The necessary R^2 values can be considerably more stringent for high-performance techniques, such as ICP-MS (e.g., >0.9996) (Chandrasiri, Mubarak, Mahatantila, & Mahanama, 2019). Electrochemical stripping techniques regularly report excellent linearity.

2.5.3 Precision

Precision, commonly expressed as Relative Standard Deviation (RSD), quantifies the repeatability (within-day variance) and reproducibility (laboratory variation) of data. The concentration level determines the permissible RSD. The desired RSD is ≤7.3% for analytes at 10 mg/L (AOAC International, 2024). Excellent reproducibility precision, with RSD values of less than 4.0%, was obtained when DPASV was applied to food matrices (Suwannasom & Ruangviriyachai, 2011). In addition, repeatability RSDs of typically less than 5.0% have been achieved using DPASV techniques (AOAC International, 2024).

2.6 Applications in Herbal Medicine Analysis

The widespread use of traditional remedies and herbal medicines globally underscores the importance of rigorous quality control procedures, particularly regarding hazardous heavy metal contamination (Yuan, Chapman, & Wu, 2011). In addition to pesticide residues or industrial processing artifacts, Pb and Cd can enter medicinal plants through soil uptake, which is the main pathway for accumulation (Yuan, Chapman, & Wu, 2011). It is crucial to test source materials and final products using techniques that are both sensitive and appropriate for high-volume screening, as bioaccumulation poses significant health risks, including kidney deterioration and brain damage (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014).

2.6.1 Electrochemical Methods for Herbal Products

For the routine screening of pharmaceuticals, electrochemical techniques, especially variations of anodic stripping voltammetry (ASV), offer clear advantages over conventional spectrometric methods. Frequent screening is one of the benefits. While traditional methods, such as AAS and ICP-MS, are highly accurate, they are not suitable for quick field investigations due to their reliance on costly equipment, complex procedures, and lengthy

detection times (Chaiyo et al., 2016). Potentiostat-based electrochemical techniques are simple, rapid, and cost-effective.

Research shows that specialised bare carbon surfaces, like edge plane pyrolytic graphite (EPPG), can achieve high sensitivity for the simultaneous determination of Cd and Pb while remaining simpler and less expensive than modified alternatives, even though many prior voltammetric studies on medicinal plants have used modified electrodes to increase sensitivity (Roohollah Torabi Kachoosangi, Banks, Ji, & Compton, 2007). By eliminating the possibility of uneven modifier distribution or surface deterioration, the use of bare Screen-Printed Electrodes (SPEs) considerably simplifies the procedure. It offers a more reliable platform for high-throughput screening of herbal extracts (Chaiyo et al., 2016).

2.6.3 Current Gaps and Future Directions

Despite the analytical success of laboratory-based voltammetry, numerous challenges remain in the way of the commercial and widespread application of these technologies. The method requires simplicity and portability. Low-cost, portable devices with quantitation capabilities are crucial (Li, Wang, Chen, Yi, & Wang, 2024). Many current techniques still depend on complex sample pretreatment or the complex production of electrode modifications, both of which require skilled workers (Zhao, Wang, Liu, & Thi Dieu Thuy, 2022).

This study fills the need for a significantly simplified screening tool by creating a verified technique utilising bare screen-printed carbon electrodes. This strategy brings the herbal medicine sector several significant advancements. First, surface modification must be eliminated. Secondly**,** utilising mass-produced SPEs lowers test costs and ensures excellent batch reproducibility (Chaiyo et al., 2016). On-site potential comes in third. As a valuable alternative to the conventional AAS approach for routine quality assurance, the technique can be customised for use with a portable potentiostat, enabling on-site heavy metal analysis immediately after sample digestion (Li, Wang, Chen, Yi, & Wang, 2024).

CHAPTER 3

METHODOLOGY

3.1 Overview

This study employed an experimental research methodology to validate and use an electroanalytical method for the simultaneous detection and quantification of Pb and Cd in herbal medicine samples. A comprehensive analytical approach was employed, incorporating sample digestion, electrochemical detection, and statistical validation, to develop a reliable and cost-effective alternative to traditional spectrometric techniques for measuring heavy metals in complex herbal matrices.

3.2 Chemicals and Reagents

Table 3.1 lists the standard analytes, chemicals, and reagents used in this research.

Table 3.1: Standard, chemical, and reagents used in this study.

No.	Chemical and reagent	Source / Manufacturer
1	Pb standard solution, 1000 mg/L (AAS grade)	Thermo Scientific Chemicals
2	Cd standard solution, 1000 mg/L (AAS grade)	Thermo Scientific Chemicals
3	Sodium hydroxide pellets (NaOH, EMSURE ISO)	Merck KGaA, Darmstadt, Germany
4	Glacial acetic acid, min 99.8%	HmbG Chemicals, Germany
5	Sodium acetate, analytical grade	HmbG Chemicals, Germany
6	Hydrochloric acid, ~37%, analytical reagent grade	Fisher Scientific (Fisher Chemical), UK
7	Deionised water	Milli-DI (Merck Millipore) purification system

Table 3.2 shows the materials and apparatus utilised in this study.

Table 3.2: Materials and Apparatus used in this study

No.	Material/apparatus	Manufacturer / Model
1	Screen-printed carbon electrodes (SPE)	As supplied with the PalmSens system
2	Filter paper	Whatman or equivalent (specify grade used)
3	Volumetric flasks, pipettes, and beakers.	Standard laboratory glassware (various suppliers)

Table 3.3 shows the equipment and instruments used in this study.

Table 3.3: Equipment and instruments used in this study

No.	Equipment/instrument	Manufacturer / Model
1	Potentiostat	PalmSens4, PalmSens BV, Houten, Netherlands
2	pH meter	pH 700, Eutech Instruments, Thermo Fisher Scientific
3	Microwave digestion system	ETHOS EASY, Milestone Srl, Italy
4	Analytical balance	HR-251AZ, A&D Company Ltd, Japan

3.3 Calibration Series

A six-point calibration series was set up at the following concentrations: 1, 2, 4, 6, 8, and 10 mg/L for Pb and 5, 10, 15, 20, 25, and 30 mg/L for Cd. Each calibration level was examined three times to evaluate measurement precision and to develop reliable calibration curves.

Blank solutions (acetate buffer only) were examined before and after each calibration series to assess baseline drift and potential carryover effects.

3.4 Sample Collection

Ten herbal medicine products, in the form of dried leaves and stems, were purchased from traditional medicine shops in Kota Bahru, Malaysia. Samples were selected to represent various plant species across diverse herbal matrices. The samples that were used were (Hempedu bumi) Andrographis paniculata, Halia Bara (Zingiber officinale var. rubrum), Kacip Fatimah (Labisia pumila), Rose buds (Rosa damascene), Ginseng (Panax quinquefolius), Misai Kucing (Orthosiphon stamineus), Dried chrysanthemums (Chrysanthemum morifolium), Tongkat Ali (Eurycoma longifolia), Astragalus root (Astragalus membranaceus), and Codonopsis (Codonopsis pilosula).

Table 3.4: The sample used in this research.

Hempedu bumi (Andrographis paniculata)	
Halia Bara (Zingiber officinale var. rubrum)	

Kacip Fatimah (Labisia pumila)	
Rose buds (Rosa damascene),	
Ginseng (Panax quinquefolius),	
Misai Kucing (Orthosiphon stamineus),	