NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF ANALYTICAL CHEMISTRY

# SWITCHABLE-HYDROPHILICITY- AND DEEP EUTECTIC SOLVENT LIQUID–LIQUID MICROEXTRACTION PRIOR TO THE DETERMINATION OF LEAD AND COBALT IN FOOD SAMPLES

DOCTORATE THESIS

ALIYU BAWA ABDULLAHI

Nicosia
April, 2023

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**DOCTORATE THESIS**

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**Nicosia**
**April, 2023**

# APPROVAL

We certify that we have read the thesis submitted by Aliyu Bawa Abdullahi titled "**Switchable-hydrophilicity- and deep eutectic solvent liquid–liquid microextraction prior to the determination of lead and cobalt in food samples**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy of Science.

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# DECLARATION

I hereby declare that all documents, materials, research findings, and analysis in this thesis were obtained and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also affirm that all information and data that were not original to this work were properly cited and referenced, as required by the rules and conduct of the Institute.

Aliyu Bawa Abdullahi
13/04/2023

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# ABSTRACT

## Switchable-hydrophilicity- and deep eutectic solvent liquid–liquid microextraction prior to the determination of lead and cobalt in food samples

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The first study, deep eutectic solvent-liquid–liquid microextraction (DES-LLME), was combined with smartphone digital image colorimetry (SDIC) for the determination of cobalt as its chelate with 1-(2-pyridylazo)-2-naphthol. The final extract contained in a UV cuvette was placed in a laboratory designed colorimetric box, from which images of the extract were captured and split into their red-green-blue channels. The intensity of the red channel, which was found to have the highest intensity, was used to calculate the concentration of cobalt. Optimum SDIC performance was obtained at a distance of 8.0 cm between the cuvette and the detection camera with a 60.0% brightness of the light source at a wavelength of 560 nm and 1600 px$^2$ as the region of interest. Optimum DES-LLME conditions were found as follows: a complexation pH of 5.00, 300 μL of DES (choline chloride/phenol, 1:4 molar ratio), and 900 μL of tetrahydrofuran within a 2.0-min complexation and 2.0-min extraction time. The limits of detection ($3S_b/m$) and quantitation ($10S_b/m$) were found as 0.03 and 0.16 μg g$^{-1}$, respectively. The coefficient of determination ($R^2$) was higher than 0.9966, and the relative standard deviation was lower than 7.8%. The proposed method was applied for the determination of cobalt in milk and dairy products, with percentage relative recoveries ranging between 95.0 and 107.5%. In the second study, edible oil-based switchable-hydrophilicity solvent liquid–liquid microextraction (EO-SHS-LLME) has been proposed combining with flame-atomic absorption spectrometry for the determination of lead as it complexes with ammonium pyrrolidine dithiocarbamate (APDC). Optimum extraction conditions were achieved using 600 μL of coconut oil as the extraction solvent, a pH of the sample solution adjusted to 4.50 and containing 1.0% (w/v) APDC, within 5.0 min of complexation time, a 300 μL of 2.0 M sulfuric acid as the hydrophilicity switching-off trigger, and 150 µL of 8.0 M nitric acid as the back-extraction solution within 4.0 min of back-extraction time. The detection and quantification limits were determined to be 2.8 and 9.4 µg $g^{-1}$, respectively. Good linearity was obtained with coefficients of determination ($R^2$) higher than 0.9961. Intraday and interday precision, expressed in terms of relative standard deviation, were less than 4.0% and 4.6%, respectively, while the linear dynamic range was from 12.6 to 175.0 µg $g^{-1}$. Accuracy was checked by addition-recovery tests, and percentage recoveries were found to be within the range of 97.1–106.0%. The proposed method was applied for the determination of lead in food samples (i.e., carrots, fish, onions, potatoes, and yams) and satisfactory results were achieved.

**Keywords*:*** Cobalt, deep eutectic solvent, digital image colorimetry, edible oil, flame-atomic absorption spectrometry, food contamination, lead, liquid–liquid microextraction, milk, switchable-hydrophilicity solvent.

# ÖZ

## Gıda örneklerinde kurşun ve kobalt tayini öncesinde değiştirilebilir-hidrofiliklik- ve derin ötektik çözücü sıvı-sıvı mikroekstraksiyonu

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**Şubat, 2023, 103 sayfa**

İlk çalışmada, derin ötektik çözücü-sıvı-sıvı mikroekstraksiyonu (DES-LLME) kobaltın 1-(2-piridilazo)-2-naftol ile şelatının tayini için, akıllı telefon dijital görüntü kolorimetrisi (SDIC) ile birleştirildi. Bir UV küvetine alınan nihai ekstrakt, görüntülerinin çekildiği ve kırmızı-yeşil -mavi kanallarına ayrıldığı, laboratuvarda tasarlanmış bir kolorimetrik kutuya yerleştirildi. Kobalt konsantrasyonunun hesaplanmasında en yüksek yoğunluğa sahip olduğu bulunan kırmızı kanalın yoğunluğu kullanıldı. Optimum SDIC performansı, 1600 px$^2$ ilgi alanı ve 560 nm dalgaboyundaki ışık kaynağının %60.0 parlaklığıyla, küvet ve algılama kamerası arasındaki 8.0 cm'lik mesafeden elde edildi. Optimum DES-LLME koşulları şu şekilde bulundu: 2.0 dakikalık kompleksleşme ve 2.0 dakikalık ekstraksiyon süresi içinde kompleks oluşturma pH'ı 5, 300 µL DES (kolin klorür/fenol, 1:4 molar oran) ve 900 µL tetrahidrofuran. Gözlenebilme ($3S_b/m$) ve tayin ($10S_b/m$) sınırları sırasıyla 0.03 ve 0.16 µg g$^{-1}$ olarak bulundu. Determinasyon katsayısı ($R^2$) 0.9966'dan büyük ve bağıl standart sapma %7.8'den düşüktü. Önerilen yöntem, %95.0 ile %107.5 arasında değişen bağıl geri kazanım yüzdeleri ile süt ve süt ürünlerinde kobaltın tayini için uygulandı. İkinci çalışmada, yenilebilir yağ bazlı değiştirilebilir-hidrofiliklik çözücü sıvı-sıvı mikroekstraksiyonu (EO-SHS-LLME) alevli-atomik absorpsiyon spektrometresi ile birleştirilerek amonyum pirolidin ditiyokarbamat (APDC) ile kompleks oluşturan kurşunun tayini için önerilmiştir. Optimum ekstraksiyon koşulları, ekstraksiyon çözücüsü olarak 600 µL hindistancevizi yağı, 5 dakikalık kompleksleştirme süresi içinde pH'ı 4.50 ayarlanan ve %1.0 (w/v) APDC içeren numune çözeltisi, hidrofiliklik kapatma tetikleyicisi olarak 300 µL, 2.0 M sülfürik asit ve geri-ekstraksiyon çözeltisi olarak150 µL, 8.0 M nitrik asit kullanılarak 4.0 dakikalık geri kazanım süresi içinde elde edildi. Gözlenebilme ve tayin sınırları sırasıyla 2.8 ve 9.4 µg g$^{-1}$ olarak belirlendi. 0.9961'den büyük determinasyon katsayıları ($R^2$) ile iyi bir doğrusallık elde edildi. Bağıl standart sapma cinsinden ifade edilen gün içi ve günler arası kesinlik sırasıyla %4.0 ve %4.6'den azken, doğrusal dinamik aralık 12.6 µg g$^{-1}$ dan 175.0 µg g$^{-1}$ kadardı. Doğruluk, ekleme-geri kazanım testleri ile kontrol edildi ve geri kazanımların %97.1–106.0 aralığında olduğu bulundu. Önerilen yöntem, gıda örneklerinde (havuç, balık, soğan, patates ve yer elması) kurşun tayini için uygulandı ve tatmin edici sonuçlar elde edildi.

**Anahtar Kelimeler:** Kobalt, derin ötektik çözücü, dijital görüntü kolorimetresi, yenilebilir yağ, alevli-atomik absorpsiyon spektrometresi, gıda kontaminasyonu, kurşun, sıvı-sıvı mikroekstraksiyon, süt, değiştirilebilir-hidrofiliklik çözücü.

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| **Abbreviations** | **Definition** |
|---|---|
| %R | Percentage recovery |
| %RR | Percentage relative recovery |
| %RSD | Percentage relative standard deviation |
| ACN | Acetonitrile |
| AFS | Atomic fluorescence spectrometry |
| ANOVA | Analysis of variance |
| APDC | Ammonium pyrrolidine dithiocarbamate |
| BC | Black cumin |
| BES | Back-extraction solution |
| CE | Capillary electrophoresis |
| CC | Coconut |
| ChCl | Choline chloride |
| CO | Corn oil |
| DAD | Diode-array detector |
| DI | Deionized |
| DES | Deep-eutectic solvent |
| DES-LLME | Deep-eutectic solvent-based liquid–liquid microextraction |
| DLLME | Dispersive liquid–liquid microextraction |
| DPP | Differential pulse polarography |
| DPV | Differential pulse voltammetry |
| EF | Enrichment factor |
| EO | Edible oil |
| EPA | Environmental protection agency |
| ETAAS | Electrothermal-atomic absorption spectrometry |
| FA | Fatty acid |
| FAAS | Flame-atomic absorption spectrometry |
| HBA | Hydrogen bond acceptor |

| | |
|---|---|
| HBD | Hydrogen bond donor |
| HF-LPME | Hollow-fiber liquid-phase microextraction |
| HPLC | High-performance liquid chromatography |
| HS-LPME | Head-space liquid-phase microextraction |
| ICP-MS | Inductively coupled plasma-mass spectrometry |
| ICP-OES | Inductively coupled plasma-optical emission spectrometry |
| IL | Ionic liquid |
| IL-DLLME | Ionic liquid-based dispersive liquid-liquid microextraction |
| LED | Light-emitting diode |
| LLE | Liquid–liquid extraction |
| LLME | Liquid–liquid microextraction |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| LPME | Liquid-phase microextraction |
| MCT | Medium-chain triglycerides |
| NCEA | National center for environmental assessment |
| PAN | 1-(2-Pyridylazo)-2-naphthol |
| PF | Preconcentration factor |
| RGB | Red-green-blue |
| ROI | Region of interest |
| SDIC | Smartphone digital image colorimetry |
| SDME | Single-drop microextraction |
| SO | Sesame oil |
| SFA | Salt of fatty acid |
| SFO | Sunflower oil |
| SFODME | Solidification of floating organic drop microextraction |
| SHS | Switchable-hydrophilicity solvent |
| SHS-LLME | Switchable-hydrophilicity solvent liquid–liquid microextraction |

| | |
|---|---|
| SMS-LLME | Supramolecular solvent-liquid–liquid microextraction |
| SPE | Solid-phase extraction |
| THF | Tetrahydrofuran |
| UV/Vis | Ultraviolet-visible spectrophotometry |
| WHO | World Health Organization |
| WO | Watermelon oil |

# CHAPTER I
# INTRODUCTION

Sample preparation is an essential and critical stage in the analytical procedure (Wang et al., 2016). Direct sample introduction to analytical instruments may not be possible due to the complexity of the sample matrices and the presence of analyte at trace and/or ultra-trace concentrations (Dadfarnia et al., 2009). Therefore, sample pre-treatment is needed to improve separation, extraction, enrichment, and sample clean-up, especially in food, environmental, and biological samples (Pebdani et al., 2018; Wang et al., 2016).

Recent advancements in analytical chemistry are heavily focused on improving the quality of analytical results, incorporating new technical developments into analytical applications, and, in particular, miniaturization, simplification, and automation of the entire analytical operation (Habila et al., 2020). Remarkable developments have been made in the miniaturization and simplicity of sample pre-treatment methods on the way to achieving that ultimate goal. These developments have concentrated on reducing the consumption of chemicals and organic reagents and consequently the analysis cost, while retaining selectivity, sensitivity, preconcentration and accelerating the sample pre-treatment process, which is now regarded as the bottleneck of many analytical methods (Machado and Tissot, 2020; Mei et al., 2019).

Food, milk, and dairy products are necessary for human growth because they contain high concentrations of proteins, minerals, and vitamins (Sadeghi et al., 2013). To ensure that the sufficient amount required has been consumed, it is essential to determine the nutritional values of such analytes for better human health (Cacciola et al., 2017).

## The analytes

Through natural processes and/or anthropogenic activities such as mining, volcanoes, seawater sprays, smelting, refining, combustion, burning of fossil fuels, sewage sludge, phosphate fertilizers, transformation of metal-containing ores, and industries that produce or process them (Shirkhanloo et al., 2014; Shokri et al., 2015), heavy metals are leached from the parent material and enter the soil sediment system, from which they are partially taken up by plants and animals (Baig et al., 2013; Oral et al., 2019).

### Lead

The presence of lead, along with other toxic metals, in food products results in serious chemical risks to food safety (Russo et al., 2013). Various health effects associated with exposure to lead were reported. These include altering the hematology in the body to inhibit the synthesis of the hem group, damaging the brain and peripheral nerves, and reducing learning disabilities, behavioral, neurological, cardiovascular, and intelligence quotients (Abt and Robin, 2020; Arpa and Aridasir, 2018; Dahiya et al., 2005; Gomes et al., 2017). Thus, lead has been described as one of the most dangerous contaminants (Smith & Flegal, 1995) that can only be tolerated at extremely low concentrations (Hosseini et al., 2015).

As a consequence, the World Health Organization (WHO) established the provisional tolerable weekly intake of lead as 25 mg $kg^{-1}$ body weight for all human groups and the maximum allowable limit as 10 ng $mL^{-1}$ for lead in drinking water (Burns and Gerstenberger, 2014; Zamani et al., 2019). Therefore, the development of reliable analytical methods for the determination of lead in food and water samples is of great importance for environmental monitoring and human health.

### Cobalt

Cobalt, a biologically active element, is the primary component of cyanocobalamin, also known as vitamin $B_{12}$, which is required in trace amounts for human and animal development (Arain et al., 2016). Cobalt is required in the body for the synthesis of amino acids, nucleic acids, and the formation of white blood cells (Sorouraddin et al., 2019; Tekin et al., 2020). High concentrations of some cobalt compounds, on the other hand, revealed potential adverse health effects such as heart and lung diseases (Wang, Z.Z. et al., 2018), bone dimness, skin rashes, metabolic disorders, retarded growth, anemia, and nerve cell degeneration (Moghadam et al., 2017; Poor and Rezaei, 2020). The U.S. Environmental Protection Agency's (EPA's) National Center for

Environmental Assessment (NCEA) recommends a daily cobalt intake of 0.3 μg $kg^{-1}$ $day^{-1}$ for all age groups to be considered healthy (Tvermoes et al., 2013).

## Detection Techniques

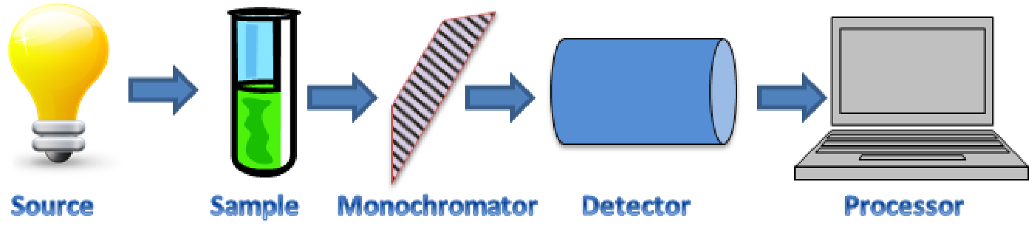
Several well-known detection techniques were used to find and measure elemental and molecular analytes in food and other samples. These included capillary electrophoresis (CE) (Alshana et al., 2015), flame-atomic absorption spectrometry (FAAS) (Bahar et al., 2017), Inductively coupled plasma-mass spectrometry (ICP-MS), Atomic fluorescence spectrometry (AFS) (Zhou et al., 2019), differential pulse polarography (DPP) (Somer et al., 2016), ultraviolet-visible spectrophotometry (UV/Vis) (Torabi et al., 2021), differential pulse voltammetry (DPV) (Ghorbani et al., 2019), high-performance liquid chromatography (HPLC) equipped with diode-array detector (DAD) (Al-Nidawi et al., 2020), electrothermal-atomic absorption spectrometry (ETAAS) (Lopez-Garcia et al., 2013; Rivas et al., 2009), and inductively coupled plasma-optical emission spectrometry (ICP-OES). Even though these techniques offer excellent analytical performance, they are unsuitable for analysis in non-centralized areas due to their high cost, requirement for specialized personnel, and inability to perform rapid on-site measurements, which require expertise and incur a high cost of maintenance. Thus, an analytical technique that would be complementary and could provide similar/comparable results becomes necessary, which will enhance the efficiency of low-income laboratories and underdeveloped countries to venture into qualitative research with the limited resources available.

## Flame-Atomic Absorption Spectrometry

FAAS is one of the most widely used instruments in elemental analysis for the determination of varying concentrations of elemental analytes in different matrices. This is attributed to the fact that AAS is among the most commonly available instruments with advantages such as ease of operation, robustness, cost-effectiveness, feasibility, high accuracy, and precision (Tekin et al., 2020). However, it has some limitations, such as low sensitivity and interference effects in some cases (Bahar et al., 2017), making it insufficiently sensitive to analyze complex matrices for metals/metalloids at low concentrations without a suitable preconcentration technique

(Tavakoli et al., 2021). The components of AAS and the different steps of how the sample solution is introduced into the flame are shown in **Figure 1** and **Figure 2**, respectively (Skoog et al., 2007).

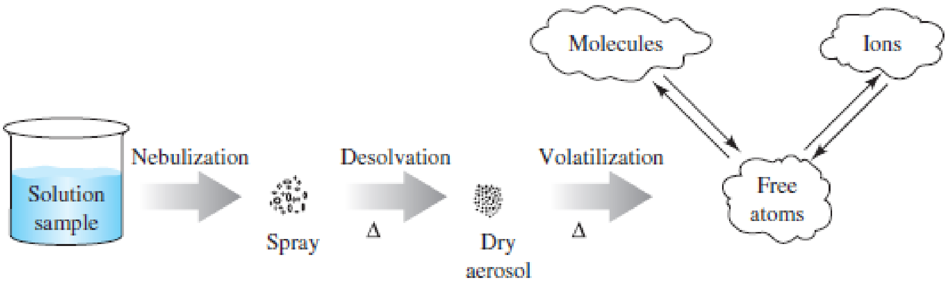
**Figure 1**: Components of AAS.



An AAS instrument must consist of the following:

- A stable light source,
- A sample introduction system (flame or graphite furnace),
- A wavelength selector that selects a specific region of electromagnetic spectrum for measurement,
- A detector that converts the radiant energy to a signal,
- A signal processor that processes and displays the signal.

**Figure 2**: Sample introduction in FAAS.



## Smartphone Digital Image Colorimetry

In recent years, and with the rapid development of mobile technology, smartphone digital image colorimetry (SDIC) has been proposed as an alternative approach aiming at lowering the analysis costs, providing easier access to instrumentation systems, and a high degree of flexibility for on-site elemental and molecular analysis (Choodum et al., 2019; Lima et al., 2020; Lopez-Molinero et al., 2013; Porto et al., 2019; Wongniramaikul et al., 2018). In addition, smartphones provide superior advantages over other tools or devices used for capturing images. This is attributed to the fact that it can capture high-quality images, have a high-speed processor, and provide instant quantification of captured data due to the extensive use of mobile applications (Fan et al., 2021).

In SDIC, the sample solution is placed in a colorimetric box, and images are captured with a mobile phone camera and processed to obtain a linear relationship between the analyte concentration and the color intensity. For quantitation of the analyte in the sample, reflectance or absorbance can be used to plot a calibration graph. So far, researchers have applied a continuum light source such as a fluorescent lamp (Soares et al., 2017) or a white light-emitting diode (LED) (Porto et al., 2019) to illuminate the sample solution in the colorimetric box. Recently, a remarkable improvement was achieved by switching from a continuum to a monochromatic source, which improves the sensitivity, selectivity, and linear dynamic range of SDIC (Al-Nidawi, M. and Alshana, U., 2021; Caleb and Alshana, 2021b), making it applicable to the analysis of complicated matrices.

The main drawback of SDIC was its poor selectivity and low sensitivity. However, researchers have recently developed and reported the use of SDIC for the determination of different analytes from simple and complex matrices. The poor selectivity was improved by using a monochromatic light source instead of a continuum light source, and the technique's low sensitivity was overcome by coupling the technique with microextraction techniques such as reversed-phase switchable-hydrophilicity solvent liquid–liquid microextraction (Al-Nidawi, Mais and Alshana, Usama, 2021), dispersive liquid–liquid microextraction (DLLME) (Peng et al., 2019), Supramolecular solvent-liquid–liquid microextraction (SMS-LLME) (Caleb and Alshana, 2021a), single-drop microextraction (SD-ME) (Bagheri and Saraji, 2019), liquid-phase microextraction (LPME) (Nepomuceno et al., 2022), liquid–liquid microextraction (LLME) (Acevedo et al., 2018), switchable-hydrophilicity solvent liquid–liquid microextraction (SHS-LLME) (Ismail et al.) and on-chip electro-membrane extraction (Zarghampour et al., 2020).

## Statement of the Problem

Among the challenges faced by analytical chemists is to develop a method that will conform to green analytical chemistry. With reference to the three main steps in the analytical method development process, i.e., sample preparation, separation, and detection, sample preparation is still considered as the bottleneck in most analytical protocols. It is a critical step especially in complex sample analysis that affects the sensitivity, selectivity, speed, and accuracy of analytical results. Conventional extraction methods, such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE), have several disadvantages and weaknesses, which include the use of large volumes of organic solvents, excessive chemical waste that not being friendly to the environment, long analysis times, low enrichment factors, and tedious experimental steps. Therefore, the need for developing alternative miniaturized and eco-friendly extraction techniques to overcome these problems becomes necessary.

## Aim of the Study

This research was aimed at developing two eco-friendly and cost-effective methods that would conform to the green analytical chemistry standard. It was focused on the development of edible oil-based switchable-hydrophilicity solvent liquid–liquid microextraction using FAAS (EO-SHS-LLME-FAAS) for the determination of lead in food samples and deep eutectic solvent-liquid–liquid microextraction using SDIC (DES-LLME-SDIC) for the determination of cobalt in milk and dairy products.

The goals of the proposed methods include the following:

- to develop an EO-SHS-LLME-FAAS method for the preconcentration and determination of lead in food samples.

- to employ EOs as a substitute to conventional SHS, which are green, biodegradable, cheap, and available.

- to highlight the use of greener solvents that would replace the toxic solvents for the purpose of environmental safety.

- to verify the accuracy of the developed methods using addition-recovery experiments with an established technique such as HPLC-DAD.

- to come up with a DES-LLME-SDIC method for preconcentration and determination of cobalt in milk and dairy products.

## Research Questions

The following are some research questions considered:

- Will the EOs act and/or be able to extract the analyte better than the standard SHS?

- Were the results of SDIC comparable with the established techniques?

## Significance of the Study

- ➢ The research focused on combining microextraction techniques with SDIC as an alternative detection technique, as well as the use of EOs as extraction solvents to investigate their suitability as alternative SHS.

- ➢ The use of the SDIC detection method will represent a complementary technique to the expensive and complicated instrumental ones that require high operational training and handling. In addition, low-income laboratories will benefit from the advantages of low cost, the least dependence on electricity, and compatibility with different types of extraction solvents.

- Also, employing EOs as an alternative extraction solvent to SHS will improve green analysis as the solvents are green, available, affordable, non-toxic, biodegradable, and most importantly, can be switched on and off on demand, for the preconcentration and extraction of molecular and elemental analytes from different matrices.

# CHAPTER II

# Literature review

## The microextraction concept

Not only instrumentation but also good methodologies are required to improve the quality of chemical analyses. However, environmentally friendly laboratory practices are imperative for an analytical chemist (Armenta et al., 2008). It is also important to note that the use of a small or minimal amount of solvent (miniaturization), appropriate management of analytical waste, avoidance of toxic reagents and use of renewable resources, and safety of the analytical chemist, among others, are highly recommended as part of green analytical chemistry (Galuszka et al., 2013; Nowak et al., 2021). It is therefore necessary for an analytical chemist to find alternatives for replacing or avoiding the use of toxic organics with greener solvents so as to have an eco-friendly and safe environment.

## Liquid–liquid microextraction

In routine analysis, LLE is still one of the most widely used sample preparation techniques, with goals including sample cleanup, preconcentration, and signal enhancement. However, some shortcomings including the use of large sample volumes and considerable amounts of hazardous organic solvents make this procedure time-consuming, low-sensitive, expensive, harmful to the environment, tedious, and potentially prone to sample contamination when trace determinations are required (Anthemidis and Ioannou, 2009; Farajzadeh et al., 2014). The basic principle of liquid–liquid microextraction (LLME) is based on the partition coefficient of a given analyte between an extraction solvent (octanol) and water, commonly known as log*P*, according to the general principle that “like dissolves like and unlike dissolves unlike”. However, an ideal extraction solvent should be immiscible with water while maintaining a high affinity toward the analyte. This enables the analyte to migrate from the organic (acceptor) phase to the aqueous (donor) phase.

$$logP = \frac{[Analyte]_{octanol}}{[Analyte]_{water}}$$

where, $[Analyte]_{octanol}$ and $[Analyte]_{water}$ are the equilibrium concentrations of the analyte in octanol and water, respectively.

During an extraction process, it is important that the *logP* value of an analyte be known. This enables the proper development of the LLME method and also will ascertain high hydrophilicity (low *logP* value) or high hydrophobicity (high *logP* value) of the analyte (Burato et al., 2020). Thus, an appropriate method of extraction, solvent type and volume, pH, temperature, ionic strength, and time of agitation for extraction would enable the extraction of the analyte into the appropriate extraction solvent.

Owing to its progressive expansion and advantages in the use of small volumes (microliters) of extraction solvents, quite a high number of studies have been reported on the use of various LLME methods to extract elemental and molecular analytes from different matrices. LLME (Kokosa, 2021), which include hollow-fiber liquid-phase microextraction HF-LPME (Khan et al., 2020), in-vessel head-space liquid-phase microextraction (HS-LPME) (Tamen and Vishnikin, 2021), single-drop microextraction (SDME) (Kailasa et al., 2021; Li and Row, 2022), solidification of floating organic drop microextraction (SFODME) (Akkaya et al., 2017; Silva et al., 2021), dispersive liquid-liquid microextraction (DLLME) (Alexovic et al., 2017; Martinez-Rubio et al., 2020), supramolecular solvent-based liquid–liquid microextraction (SMS-LLME), ionic liquid-based dispersive liquid-liquid microextraction (IL-DLLME) (Rykowska et al., 2018), switchable-hydrophilicity solvent-based liquid–liquid microextraction (SHS-LLME) (Alshana et al., 2020; Hassan et al., 2021), and deep-eutectic solvent-based liquid–liquid microextraction (DES-LLME) (Khan and Soylak; Xie et al., 2020).

### Switchable hydrophilicity solvent-based liquid–liquid microextraction

Switchable hydrophilicity solvents (SHS) have gained wide acceptance since their introduction by Philip G. Jessop in 2005. Miscibility of SHS with aqueous solutions can be switched on and off reversibly, from a liquid with one set of properties to another (Jessop et al., 2012). Jessop et al. found that, when 1,8-diazabicyclo-undec-7-ene and 1-hexanol were mixed, a polar ionic liquid was formed, and the process was reversed rapidly once carbon dioxide was removed from the media using an inert gas such as nitrogen (Jessop et al., 2005).

This outstanding property enabled SHS to have a wide potential application for the extraction of different categories of analytes. In addition, it would encourage separation processes and eliminate several tedious and time-consuming steps during extractions and allow the reuse of the same solvent for other purposes (Alshana et al., 2020; Yilmaz and Soylak, 2015). SHS are generally synthesized by purging carbon dioxide or adding dry ice to a mixture of water and usually a tertiary amine (Jessop et al., 2011). The amine is then converted to its hydrophilic form, and a single phase is observed. It is worth mentioning that in their hydrophilic form, these solvents are completely miscible with water, thereby creating an infinite surface area, whereas in their hydrophobic form, they are totally immiscible, which facilitates both mixing and recovery. Among the advantages of SHS is that they allow the extraction of analytes in a homogeneous phase without the need for a dispersive solvent (Wang et al., 2019). This emphasizes minimum usage of organic solvents, which conform to green analytical chemistry. A summary of some studies with SHS for both molecular and elemental analytes is presented in **Table 1**.

**Table 1:** Studies involving SHS method in molecular and elemental analysis.

| Method[a] | Matrix | Analytes | Detection[b] | Ref. |
|---|---|---|---|---|
| CSHS-SAP | Milk | Lead | GFAAS | (Wang et al., 2019) |
| SDLLME | Water | Lead | FAAS | (Naeemullah et al., 2016) |
| SHS-LLME-DSPME | Food | Quercetin | UV/Vis | (Hassan et al., 2021) |
| EA-SS-LPME | Food | Lead and cadmium | HR-CS-FAAS | (Chaikhan et al., 2022) |
| VA-SS-LPME | Egg yolk | Vitamin B12 and Cobalt | FAAS | (Tekin et al., 2019) |
| SHS-BME | Food and Drink | Bisphenol | HPLC-UV | (Wang, X.R. et al., 2018) |
| SHS-LPME | Water samples | Bisphenol A | GC-MS | (Bodur et al., 2020) |

[a] CSHS-SAP: Controlled-switchable hydrophilicity solvent solidification of aqueous phase, SDLLME: Switchable-dispersive liquid–liquid microextraction, SHS-LLME-DSPME: Switchable-hydrophilicity solvent liquid–liquid microextraction dispersive solid-phase microextraction, EA-SS-LPME: Effervescence tablet-assisted switchable solvent-based liquid-phase microextraction, VA-SS-LPME: Vortex-assisted switchable solvent-based liquid-phase microextraction, SHS-BME: Switchable hydrophilicity solvent-based microextraction, SHS-LPME: Switchable hydrophilicity solvent-based-liquid-phase microextraction.

[b] GFAAS: graphite furnace-atomic absorption spectrometry, FAAS: Flame-atomic absorption spectrometry, UV/Vis: Ultraviolet/visible spectrophotometry, HR-CS: High-resolution continuum source, HPLC: High-performance liquid chromatography, GC-MS: Gas chromatography-mass spectrometry.

## Edible oil-switchable hydrophilicity solvents

Edible oils (EO) are mostly triglycerides of fatty acids (FA) that can be easily converted into their corresponding hydrophilic salts of the fatty acids (SFA) through a simple saponification reaction (Tan et al., 2013). SFA are thus promising solvents for SHS-LLME, which form a stable emulsion in aqueous solution, providing an infinitely large surface area for the analyte, improving extractability, and reducing the extraction time significantly. The use of EO, in their intact form, has been reported in several studies in the context of LLE. They have been shown to be good alternatives to conventional organic solvents due to their high degree of greenness, renewability, sustainability, biodegradability, non-volatility, affordability, and compatibility with many analytical techniques.

FA can be classified as short-chain (C2–C6), medium-chain (C6–C12), and long-chain (C14–C24) (Deen et al., 2021). Medium-chain triglycerides (MCT) are mixed triglycerides of saturated FA that have a chain length of 6–12 carbons (Sung et al., 2018). MCT differ from long-chain triglycerides in their physical and chemical properties, such as their smaller molecular size, their ability to remain in the liquid form at room temperature, as well as their lower viscosity and melting point (Marten et al., 2006). MCT are superabundant in coconut oil since the FA profile is dominated by medium-chain FAs. For instance, coconut oil contains 90% saturated FA, with lauric acid being the most abundant. Because lauric acid dominates the total FA profile (35.3–52.5%), coconut oil is also marketed as lauric oil. Other saturated FA include capric acid (7.0%), myristic acid (20.4%), palmitic acid (11.2%), stearic acid (2.6%), and arachidic acid (1.4%) (Sandupama et al., 2022). A recent study employed peanut oil for the extraction of poly aromatic hydrocarbons (Pannu et al., 2004). A similar study reported the use of palm oil to extract phenolic compounds from waste samples (Venkateswaran and Palanivelu, 2006). Chemical dyes from the waste of textile industry were also extracted using vegetable oils as supported liquid membrane (Muthuraman and Palanivelu, 2006). The extraction of copper(II) ions from copper plating effluents was also reported through the use of coconut oil as a diluent in supported liquid membrane (Venkateswaran et al., 2007). Vegetable oil was also used as a transport carrier for the extraction of Rhodamine B from aqueous feed solutions through an oil-supported membrane into an aqueous strip (Muthuraman and Teng, 2009). A summary of some studies that used EO for both atomic and molecular analytes is given in **Table 2**.

**Table 2:** Studies EO in molecular and elemental analysis.

| Method[a] | Matrix | Analytes | Detection[b] | Ref. |
|---|---|---|---|---|
| VOS | Aqueous solution | Cu | FAAS | (Chang et al., 2010) |
| LLE | Waste water | Phenol | UV/Vis | (Othman et al., 2017) |
| VO-SLM | Aqueous solution | Dye | UV/Vis | (Muthuraman and Palanivelu, 2006) |
| VO-SLM | Dye solution | Rhodamine B | UV/Vis | (Muthuraman and Teng, 2009) |
| VO-SLE | Soil | PAH | GC | (Pannu et al., 2004) |
| VO-BR | Soil | Pb | AAS | (Sangeetha et al., 2021) |

[a] VOS: Vegetable oil-based organic solvent, LLE: Liquid–liquid extraction, VO-SLM: Vegetable oil-based supported liquid membrane, VO-SLE: Vegetable oil-based solid-liquid extraction, VO-BR: Vegetable oil-based biosurfactant reactor,

[b] ELM: Emulsion liquid membrane, FAAS: Flame-atomic absorption spectrometry, UV/Vis: Ultraviolet-visible spectrophotometry, GC: Gas chromatography, AAS: Atomic absorption spectrometry.

### Deep eutectic solvent liquid–liquid microextraction

Green chemistry aims at minimizing the use and generation of hazardous substances in order to reduce environmental pollution; hence the primary efforts of many researchers have focused on the development of green alternative solvents. Ionic liquids (ILs), as a class of green solvents, have attracted considerable attention owing to their special physical and chemical properties (Abbott et al., 2003). Similarly, some studies have highlighted the limitations of ILs, such as their toxicity, poor biodegradability, and high cost (Kareem et al., 2010). To overcome these limitations, a new generation of solvents called deep eutectic solvents has emerged. Owing to their advantages, DES have found widespread applications in LLME (Espino et al., 2016). They are typically formed by combining a hydrogen bond donor, HBD (e.g., urea, phenols, carboxylic acids), and a hydrogen bond acceptor, HBA, such as quaternary ammonium salts (e.g., choline chloride, ChCl) at different molar ratios (Alshana et al., 2021). The most common component used for the formation of DES is ChCl, a biodegradable, inexpensive, and non-toxic quaternary ammonium salt (Habibi et al., 2013).

Early generations of DES were mostly hydrophilic in nature and thus were used for extracting hydrophilic analytes until 2015, when van Osch and his co-workers (van Osch et al., 2015) introduced the concept of hydrophobic DES, by combining different ammonium salts with decanoic acid to extract hydrophobic analytes from aqueous samples. This opened the door for the synthesis of a wide range of hydrophobic DES for analytical applications (Lee et al., 2019).

DES-LLME was first applied in 2015 by Dadfarnia (Karimi et al., 2015), who used ChCl/urea (1:2 molar ratio) for the ligandless extraction and preconcentration of lead and cadmium from EO prior to their determination using ETAAS (Karimi et al., 2015). A variety of DES-based LLME methods have been developed and modified for the extraction of inorganic and organic analytes, and combined with a wide range of analytical techniques for detection of the analyte(s). A summary of studies involving DES-LLME in molecular and elemental analysis is presented in **Table 3**.

**Table 3:** Studies involving DES-LLME in molecular and elemental analysis.

| Method[a] | Matrix | Analytes | Detection[b] | Ref. |
|---|---|---|---|---|
| DLLME-DES | Milk | Cd, Cu, Pb | FAAS | (Sorouraddin et al., 2020) |
| DES-LLME | Oil | Pb, Cd | ETAAS | (Karimi et al., 2015) |
| DES-LLME | Milk | Tetracycline | HPLC-DAD | (Cherkashina et al., 2021) |
| NADES-UA-SLE | Vegetables | Quercetin | HPLC-VWD | (Dai and Row, 2019) |
| DES-UA-LLME | Biological, Water | Ibuprofen | HPLC-UV | (Cidem et al.) |
| DES-MAHD | Clove buds | Essential oil | GC-MS | (Chen et al., 2022) |

[a] DLLME-DES: Dispersive liquid–liquid microextraction based on deep eutectic solvent, DES-LLME: Deep eutectic solvent-based liquid–liquid microextraction, NADES-UA-SLE: Natural deep eutectic solvent-ultrasound-assisted-solid-liquid extraction, DES-UA-LLME: Deep eutectic solvent-based ultrasonic-assisted liquid–liquid microextraction, DES-MAHD: Deep eutectic solvent-based microwave-assisted hydrodistillation.

[b] FAAS: Flame-atomic absorption spectrometry, ETAAS: Electrothermal-atomic absorption spectrometry, HPLC-DAD: High-performance liquid chromatography-diode-array detector, VWD: Variable wavelength detector, UV: Ultraviolet, GC-MS: Gas chromatography-mass spectrometry.

# CHAPTER III
# EXPERIMENTAL

## EO-SHS-LLME-FAAS

### Chemicals and Reagents

All chemicals and reagents used were of analytical grade unless otherwise stated. Deionized (DI) water (18.2 MΩ.cm), treated with Purelab Ultra Analytic (ELGA LabWater, UK), was used for preparations of all aqueous solutions. Ethanol and nitric acid were purchased from Riedel-de Haën (Germany), hydrochloric acid and sodium chloride were from Merck (Germany), acetic acid, ammonium pyrrolidine dithiocarbamate (APDC), hydrogen peroxide, lead(II) nitrate, sodium acetate trihydrate, sodium hydroxide and sulfuric acid were acquired from Sigma-Aldrich (Germany).

### Apparatus

A Thermo scientific iCE 3000 series (USA) atomic absorption spectrometer was used that was equipped with deuterium lamp background correction. A lead hollow cathode lamp monitored at 217.0 nm and an air-acetylene flame as an atomizer operated at a flow rate of 0.8 L min$^{-1}$, were applied in all the measurements. An electronic balance (Mettler-Toledo, Switzerland) was used for weighing the solid reagents. Vortex mixing was performed using an IKA MS 3 digital vortex (USA). Centrifugation was carried out using EBA20 Portable Centrifuge C2002 from Hettich (Germany). All measurements were carried out with micro-injection (read time, 4 s) with peak heights being used for calibrations. The micro-injection assembly was manually constructed using a plastic micro funnel (Top I.D. 28 mm and stem I.D. 6 mm) connected to the nebulizer capillary through a rubber tubing (I.D. 6 mm) and an inverted 1000-µL pipette tip. The funnel was held by a clamp on a stand placed 10.0 cm from the nebulizer.

### Preparation of standard and reagent solutions

A stock solution of lead(II) ions at a concentration of 1000 µg $mL^{-1}$ was prepared from lead(II) nitrate in 1.0 mol $L^{-1}$ nitric acid. Working standard solutions were freshly prepared by diluting this stock solution in the same solvent. A 1.0% (w/v) APDC was prepared by weighing an appropriate amount and dissolving it in ethanol. Acetate buffer was prepared using sodium acetate trihydrate and adjusted to pH 4.50 with appropriate volumes of acetic acid.

### Sample preparation

Fresh samples of canned tuna fish, carrot, onion, potato and yam were purchased from local markets in Nicosia, TRNC, and were cut into small pieces, dried in an oven overnight at 60 $^{o}$C and ground using a pestle and mortar. For standard-addition calibrations, the lead standards were spiked at this stage. Then, 100 ± 0.1 mg of each sample were carefully weighed and placed in a Teflon cup, followed by the addition of 1.50 mL of nitric acid/ hydrogen peroxide mixture (5:1, v/v) and allowed to stand for 30 min before being digested at 120 $^{o}$C for 2.0 h in a closed-vessel digestion block. The sample digestate (ca. 1.5 mL) was transferred into a 15-mL polypropylene centrifuge tube and its pH was adjusted to 4.50 with 1.0 mol $L^{-1}$ acetate buffer (ca. 200 µL). Finally, the mixture was transferred into a 5.0 mL volumetric flask and was considered as the “sample solution”.

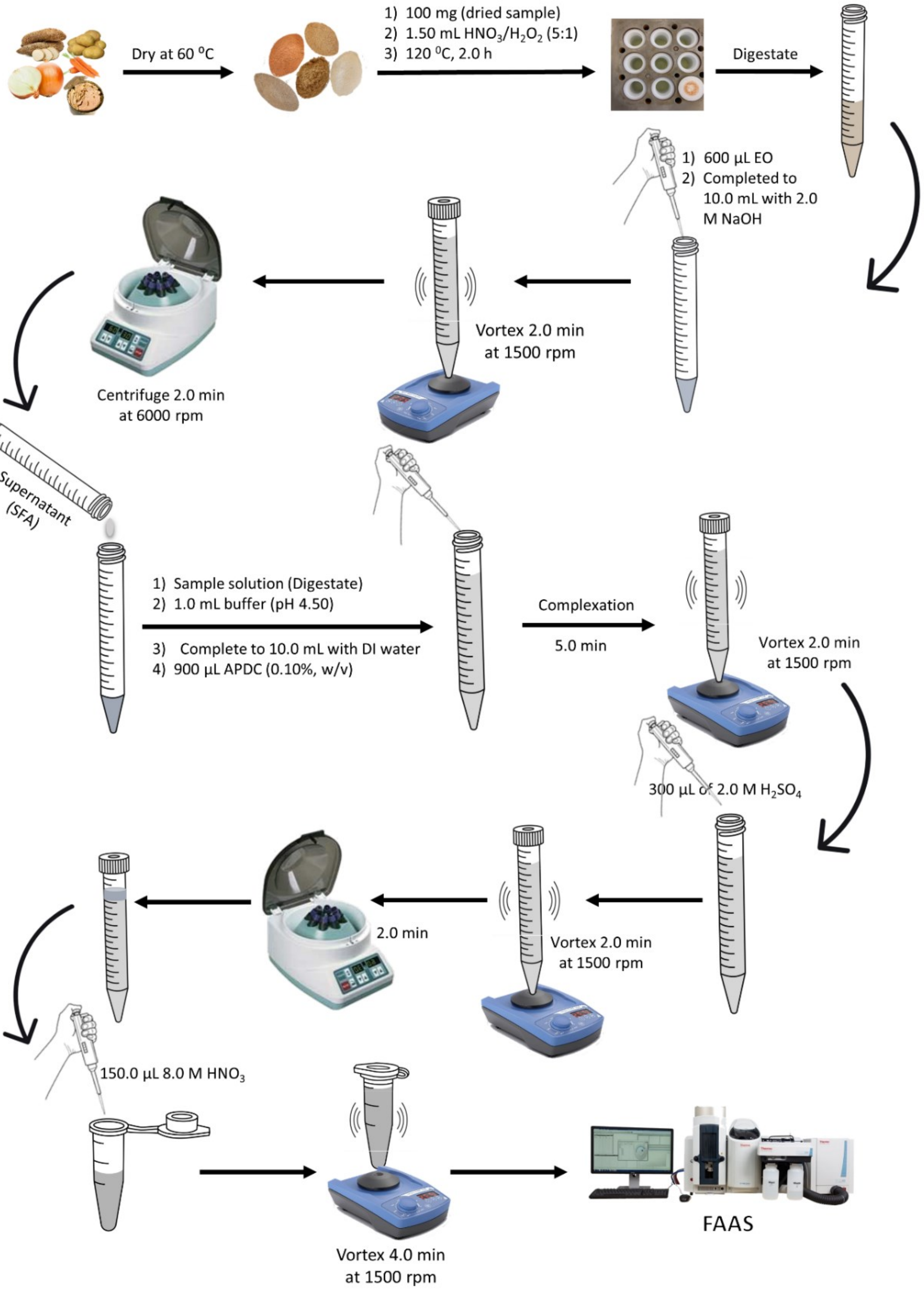
### Preparation of EO-SHS

Black cumin (BC), coconut (CC), corn (CO), olive (OO), sesame (SO), sunflower (SFO) and watermelon (WO), were purchased from pharmacies in Nicosia, TRNC and were used as the extraction solvents. A portion of the EO (i.e., 600 µL) was transferred into a 10.0 mL volumetric flask and the volume was made up to the mark with 2.0 mol $L^{-1}$ sodium hydroxide. The mixture was transferred into in a 10.0-mL centrifuge tube, vortexed for 2.0 min and centrifuged for 2.0 min at 6000 rpm (RCF: 3421×*g*). The supernatant (SFA, 550 ± 20 µL) was then transferred into a 15-mL centrifuge tube and used for EO-SHS-LLME.

### EO-SHS-LLME procedure

A 3.5 mL of the sample solution was completely transferred into a test tube containing 600 μL of the EO-SHS. Into each test tube, 2.0 mL of 1.0 mol $L^{-1}$ acetate buffer (pH 4.50) were added and the volume was made up to 10.0 mL with DI water. Complexation started immediately upon the addition of 900 μL of 0.10% (w/v) APDC and the mixture was vortexed for 5.0 min at 1500 rpm for the complexation reaction to reach an equilibrium. Afterwards, 300 μL of 2.0 mol $L^{-1}$ sulfuric acid were added to trigger the switching-off of the EO-SHS from the sample solution upon a 2.0-min vortex and a 2.0-min centrifugation at 6000 rpm. Finally, the analyte-rich final extract was back-extracted into 150 μL of 8.0 mol $L^{-1}$ nitric acid, and the concentration of lead was determined via microinjection in FAAS. Standard-addition calibrations were constructed by spiking real samples with increasing concentrations of the analyte over the range of 15.0–175.0 μg $g^{-1}$ and applying the proposed extraction procedure, with the first standard being unspiked which was considered as a blank. A schematic diagram of the proposed EO-SHS-LLME-FAAS method is presented in **Figure 3**.

**Figure 3:** EO-SHS-LLME-FAAS procedure.



## DES-LLME-SDIC

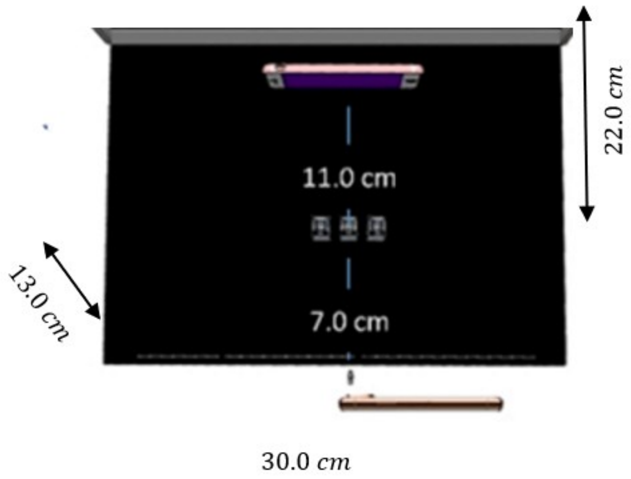
### Chemicals and reagents

All chemicals and reagents used were of analytical grade unless otherwise stated. Deionized (DI) water (18.2 MΩ.cm), treated with Purelab Ultra Analytic (ELGA LabWater, UK), was used for the preparation of aqueous solutions. 1-(2-Pyridylazo)-2-naphthol (PAN, log*P* 4.45, p$K_{a1}$ 3.94, p$K_{a2}$ 9.84), cobalt(II) nitrate hexahydrate, sodium acetate trihydrate, sodium hydroxide and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetic acid, acetone, HPLC-grade acetonitrile (ACN), ChCl, ethanol, phosphoric acid and sulfuric acid were obtained from Sigma-Aldrich (Darmstadt, Germany). Nitric acid and phenol were acquired from Reidel-de Haën (Seelze, Germany), whereas hydrochloric acid was acquired from Merck (Darmstadt, Germany).

### Apparatus

For capturing reproducible images of the sample solution, a laboratory-designed colorimetric wooden box with the dimensions of 30 × 22 × 13 cm was used, with the interior of the box painted black to minimize light scattering. A small hole was drilled in the side of the box to capture images of the sample solution that was placed in a quartz UV/Vis microcuvette (Hellma, Kruibeke, Belgium) 8.0 cm away from the detection camera (**Figure 4**). A Samsung J8 smartphone (SM-J810Y/DS), equipped with a 16 MP rear camera and a 6.0-in display screen with a resolution of 720 × 1480 px, 1.8 GHz Octa-core, 3 GB of RAM, and 32 GB of internal memory, was used as the detector. A Redmi Note7 (M1901F7G) smartphone, having a 6.3-in. display screen with a resolution of 1080 × 2340 px, 2.20 GHz Octa-core, 4 GB RAM, and 128 GB of internal memory, was used as the monochromatic light source. Images were processed using a free version of ImageJ software for PC, version 1.53 j, Java 1.80_112, 64-bit (National Institute of Health, USA) (Rueden et al., 2017). log*P* and p$K_a$ values were calculated using MarvinSketch software (Rev. 20.11.0, ChemAxon Ltd., Cambridge, MA, USA). Vortex mixing was performed with an MS-3 digital vortex (IKA, Staufen, Germany), while centrifugation was done with an EBA20 portable centrifuge (C2002; Hettich, Tuttlingen, Germany). A 10–100 and 100–1000 µL micropipette (Isolab, Eschau, Germany) were used for volume transfer. The samples and standards were weighed using an electronic balance (Mettler-Toledo, Greifensee, Switzerland). An Agilent Technologies 1200 series high-performance liquid chromatography-diode array detector (HPLC-DAD) system (CA, USA) was used for comparison and accuracy checking. A Thermo Scientific iCE 3000 series (USA) flame-atomic absorption spectrometer (FAAS), equipped with an air-acetylene flame as an atomizer, a lead hollow cathode lamp at 217.0 nm wavelength, and a deuterium background correction. All measurements were carried out with microinjection, and peak heights were used for calculations.

**Figure 4:** The colorimetric box.



## Optimization of the SDIC parameters

### Selection of the RGB channels

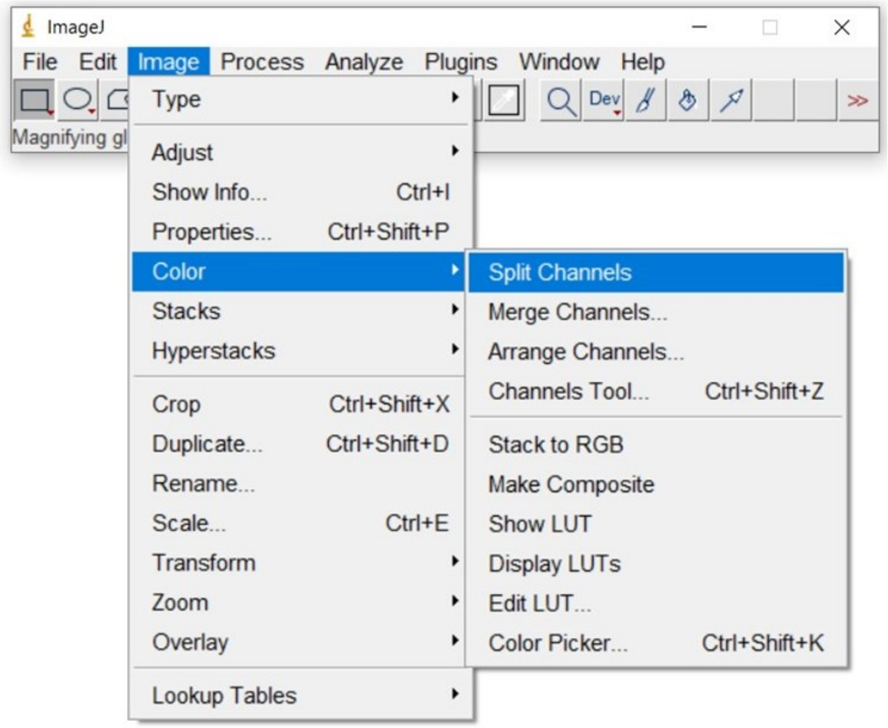
The most common approach for extracting analytical correlations is to split the image into its red, green-blue (RGB) channels and obtain the highest response at one of these channels. The sample images are captured with a smartphone camera and processed with suitable software by splitting the images into their red-green-blue components (RGB) based on a standard scale by assigning a whole number value from 0 to 255 for each of the channels. The number [0,0,0] represents absolute black, while [255, 255, 255" represents absolute white (Quesada-Gonzalez and Merkoci, 2017). ImageJ was used to split the images into their RGB channels, and the average mean value of the signal from each channel was recorded. Equation 1 was used to calculate the response from the R channel, giving the highest difference between the sample solution and the blank.

$$R_r = -A_r = \log(B_r/S_r) \qquad \text{Equation 1}$$

where, $R_r$ is the response calculated using the mean value from the histogram for the blank ($B_r$) and the sample solution ($S_r$), while $A_r$ is the absorbance.

A diagram illustrating how to select the appropriate channel is shown in **Figure 5**.

**Figure 5:** Selection of the RGB channel.



## Detection wavelength

Previous studies with SDIC have reported the use of continuum light sources such as LED (Shahvar et al., 2021) in the absence of a monochromator, which is expected to lower the sensitivity and selectivity. In order to overcome this limitation, a monochromatic light source was used with an analyte-specific color displayed on the full screen of the source smartphone. The color corresponding to each wavelength was obtained from a free online wavelength-to-color converter (https://www.wolframalpha.com). The wavelength was then scanned within the range of wavelength (nm) where the complementary color of the analyte would be found. A maximum response obtained at a particular wavelength (nm) was selected and used throughout the experiments.

### Area of the region of interest

This is a portion of the image that has been cropped. The pixel intensity was converted into a specific number that was used for quantitation of the analyte concentration in the sample. To obtain an ROI, click on "edit" to select the ROI, followed by "selection," then "specify" in the drop-down list. An auto menu will appear, which allows you to choose the shape and size of the ROI; in this case, a square is selected and cantered. Adjust the X and Y coordinates of the sample solution, followed by taking the height and width of the ROI. If 50 × 50 is selected, then it implies 2500 px$^2$, which is the ROI. In other words, a shortcut method can be followed by pressing the keys Ctrl + Shift + E from the keyboard, and the ROI will emerge automatically with the specified px$^2$.

### Distance of the sample solution to the detection camera

Although, smartphones with high pixels captures high resolution images. However, it is important to note the distance between the sample solution and the detection camera. High-resolution images were captured at an optimized distance, whereas blurred images were resulted as the distance and the target analyte were farther away.

### Statistical analysis

A single-factor analysis of variance (ANOVA) test was used in the statistical studies by using Microsoft Office Excel 2013 software for Windows (Microsoft Corporation, USA). A P value less than 0.05 was considered statistically significant.

### Preparation of standards and sample solutions

A stock solution of cobalt(II), prepared at a concentration of 1000 μg $mL^{-1}$ from cobalt(II) nitrate hexahydrate in 1.0% (v/v) nitric acid, was diluted daily with DI water to obtain the working standards (0.1–2.5 μg $g^{-1}$). PAN, used as the chelating agent, was prepared at a concentration of 0.05% (w/v) in THF. pH of the sample solutions was adjusted to 5.00 with an acetate buffer solution (1.0 M) prepared by dissolving sodium acetate trihydrate in DI water and adjusting the pH with an appropriate volume of acetic acid.

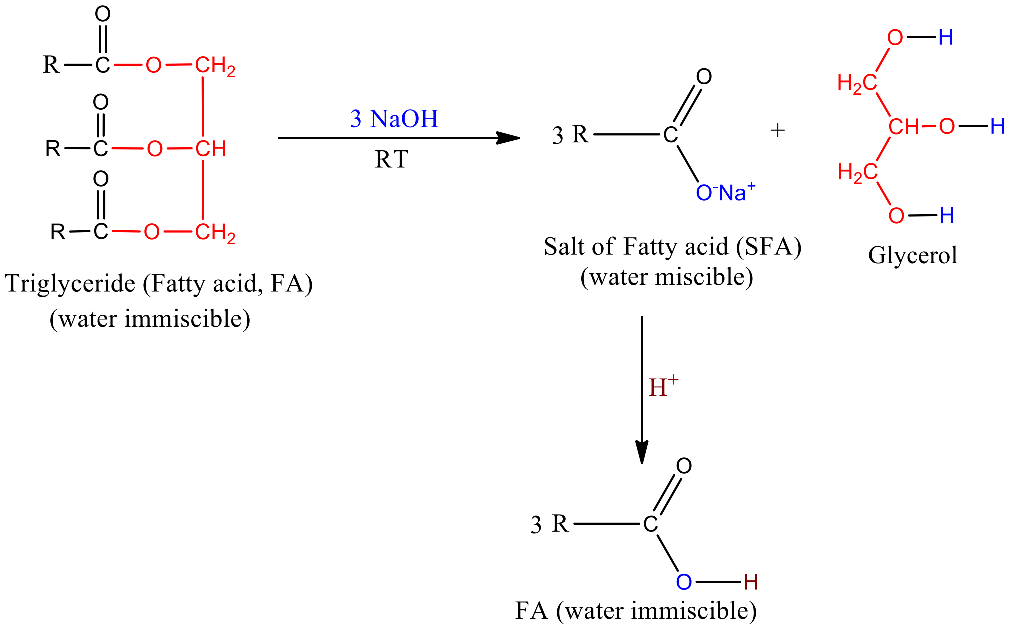
### Sample preparation

Raw cow and goat milk samples were obtained from a local farm, while ayran, pasteurized milk and yoghurt were purchased from a local market in Nicosia, TRNC. Three samples of each type were analyzed. The samples were stored in the freezer at –15 °C and were left to thaw at room temperature prior to analysis. A 1.0-mL milk sample was mixed with 300 μL concentrated acetic acid and the mixture was vortexed for 15 s. Next, 1750 μL ACN were added and the mixture was vortexed for 1.0 min and centrifuged for 2.0 min at 6000 rpm (RCF: 3461×*g*). The supernatant was transferred into a 15-mL polypropylene graduated conical centrifuge tube with a screw cap (Isolab, Eschau, Germany), which was then used in DES-LLME. Similarly, 1.0 g of yoghurt was accurately weighed and transferred into a 15-mL centrifuge tube, mixed with 1.0 mL DI water and the mixture was homogenized by vortex. Subsequently, a portion of 1.0 mL from this solution was used in the prescribed DES-LLME procedure.

### Preparation of DES

DES was prepared by weighing appropriate amounts of ChCl and phenol to obtain a molar ratio of 1:4 in a 50-mL beaker and ultrasonicating the mixture for 3.0 min at 50 °C until a clear, colorless homogeneous monophasic liquid was obtained. The resulting DES was used for the extraction and preconcentration of cobalt from milk and dairy products. The reaction for the formation of SFA is shown in **Figure 6**.

**Figure 6:** Reaction for the formation of SFA.



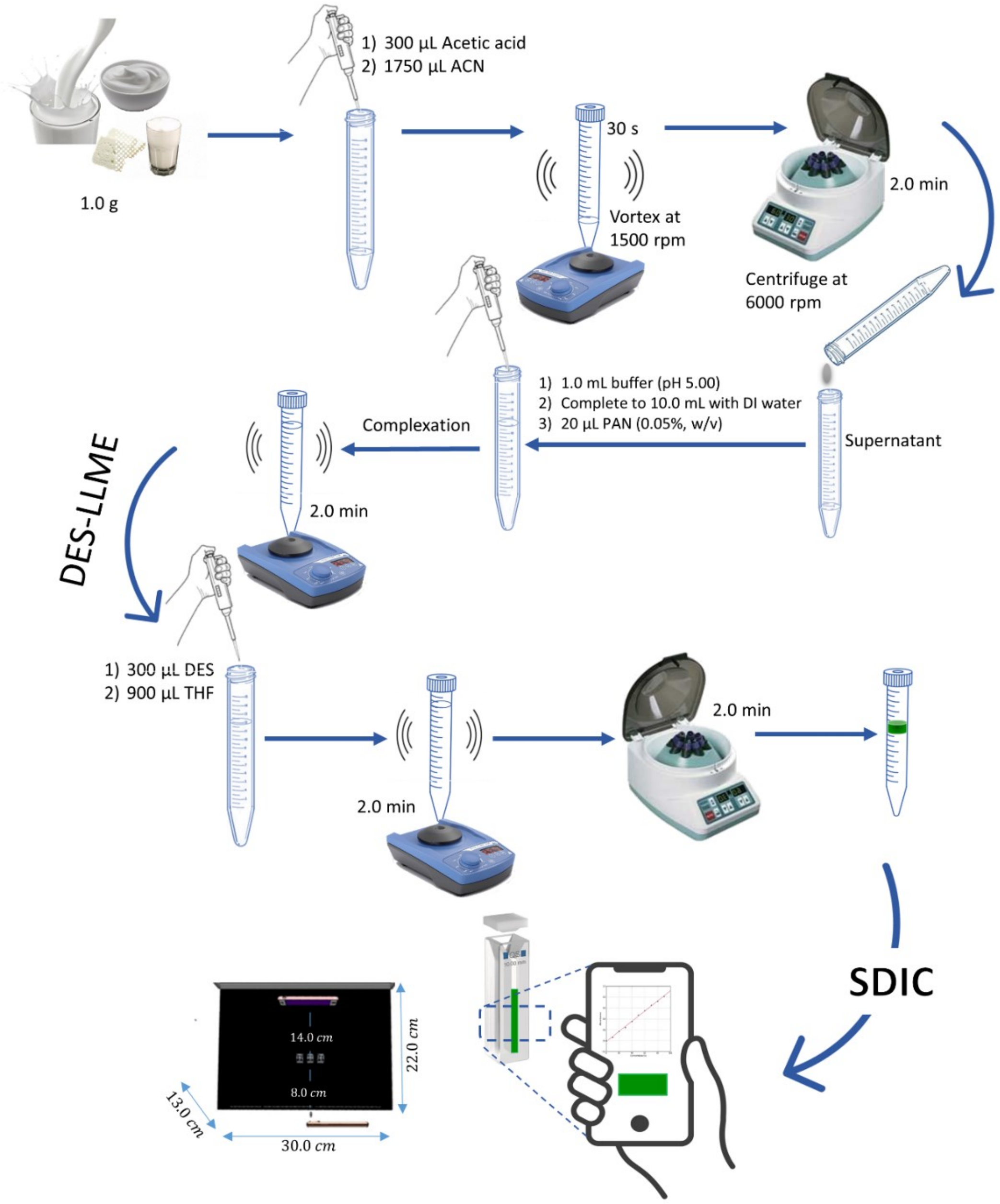
## DES-LLME-SDIC procedure

A schematic presentation of the proposed DES-LLME-SDIC procedure is given in **Figure** 7. The supernatant, obtained as described above, was mixed with 1.0 mL of 1.0 M acetate buffer (pH 5.00) and the volume was made up to 10.0 mL with DI water. A 20.0 µL portion of 0.05% (w/v) PAN was added and the mixture was vortexed at 1500 rpm for 2.0 min to allow complexation to take place. Next, 300 µL of DES were added and the mixture was vortexed for another 10 s, followed by the addition of 900 µL of THF and vortex mixing for 2.0 min. Finally, a portion (ca. 250 µL) of the floating DES layer was collected after 2.0 min centrifugation at 6000 rpm (RCF: 3461×*g*) and was transferred into a quartz micro-cuvette for detection with SDIC. The most influential factors affecting the performance of DES-LLME and SDIC were optimized using the one-factor-at-a-time method by changing one factor at a time while keeping the others constant.

## Data processing and statistical analysis

Images captured for the collected DES layer placed in the quartz micro-cuvette were saved in a JPG format and uploaded to Google Drive, from which they were retrieved to a personal computer and processed using ImageJ. Upon splitting the images into their RGB channels, the maximum response was obtained from the R channel that was selected for calibration and quantitation of cobalt. A single-factor analysis of variance (ANOVA) test was used using Microsoft Office Excel 2016 software for Windows (Microsoft Corporation, WA, USA). A P value less than 0.05 was considered to be statistically significant.

**Figure 7:** DES-LLME-SDIC procedure.



# CHAPTER IV
# RESULTS AND DISCUSSION

## EO-SHS-LLME-FAAS for the determination of lead in food samples

### Type and volume of the EO

The type and nature of the EO have a significant impact on the extraction efficiency of the analyte. Different EOs have different alkyl (R) groups, lengths of the carbon chain, degrees of (un)saturation, positions, and numbers of functional groups, which collectively affect the hydrophobic nature and the kind of interaction of the EO with the analyte. In this experiment, eight different EOs were tested for the extraction of the metal complex. All the oils investigated satisfied the switching on/off criteria. However, CC and BC gave the highest response (**Figure 8**) due to the above-mentioned reasons and could thus be selected as optimum extraction solvents. The effect of the volume of CC was studied over the range of 300–800 µL. Optimum recovery was achieved with 600 µL (**Figure 9**), beyond which the excess amount of solvent caused dilution of the analyte.

**Figure 8:** Effect of the type of EO on extraction efficiency of the analyte.

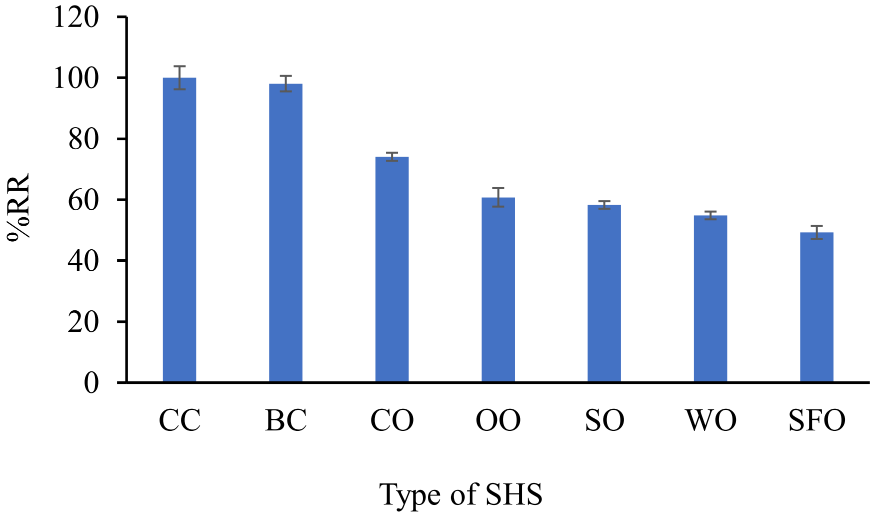
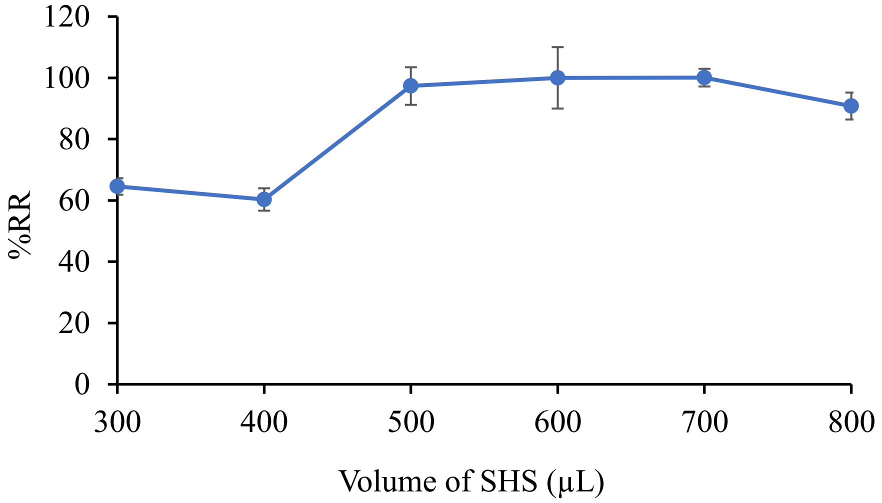


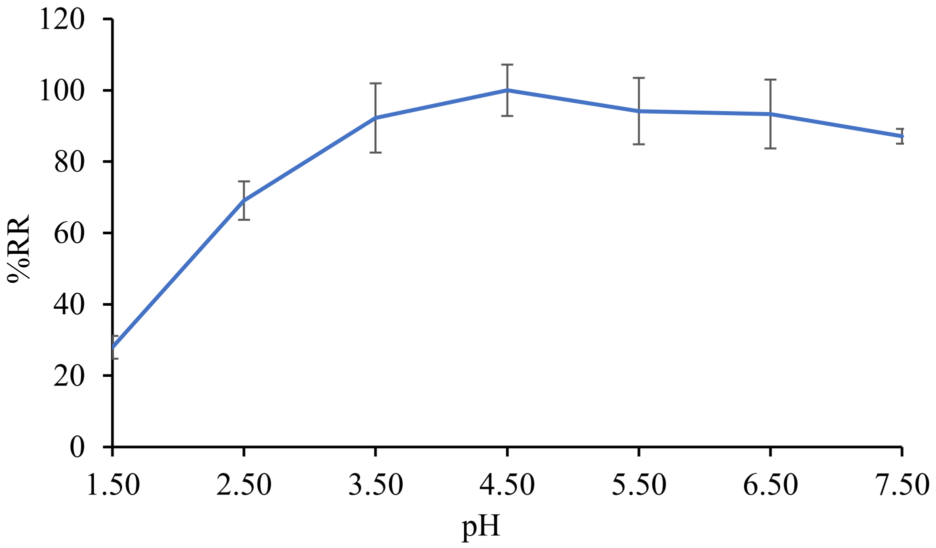
**Figure 9:** Effect of the volume of EO on recovery of the analyte.



### Complexation pH

The sample pH plays an important role in any complexation reaction. Studies revealed that lead(II) ions are generally extracted in an acidic medium to prevent the potential precipitation of lead(II) hydroxide in a basic medium (Afzali et al., 2013; Yilmaz and Soylak, 2013; Zhang, S. et al., 2018). The effect of pH was studied in the 1.50–7.50 range. A low signal was recorded at low pH values, which is attributed to the high competition between the metal and the hydrogen ions for the donor atoms of the ligand. Optimal response was observed at pH 4.50 (**Figure 10**), such that this value was considered optimum for subsequent experiments.

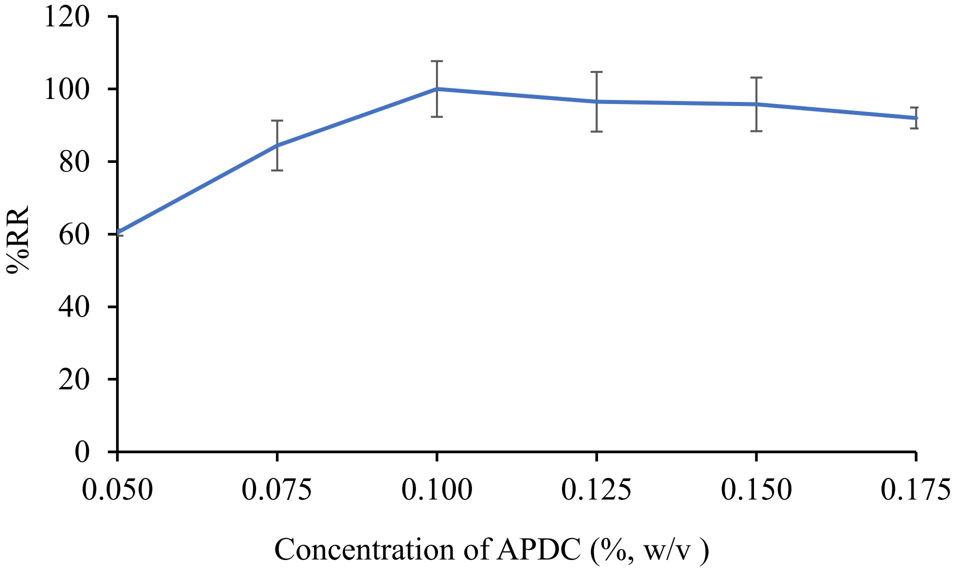
**Figure 10:** Effect of sample pH on complexation of the analyte.



### Concentration of ligand

To avoid excessive usage of chemicals and have an idea of the amount of chelating agent to be used, the concentration of APDC was studied over the range of 0.05–0.18% (w/v). The results showed that the maximum recovery was achieved with 0.10% (w/v) of the complexing agent (**Figure 11**). Further addition of the ligand yielded an insignificant effect. Based on these findings, an APDC concentration of 0.10% (w/v) was considered optimum. However, for real samples, an excess amount (ten times) was used in subsequent experiments to ensure maximum extraction efficiency of the analyte.

**Figure 11:** Effect of the concentration of ligand.



### Type, concentration and volume of the switching-off acid

Unlike in traditional SHS, where concentrated sodium hydroxide is used as a trigger for the switching-off step, in this approach, different acids have been employed for that purpose. The effect of using different acids was examined by monitoring the response produced in each case. An optimum signal was achieved using sulfuric acid (**Figure 12**), indicating the high stability of the Pb-APDC complex and its resistance in a strong acidic environment. The concentration of sulfuric acid was measured between 0.50 and 3.50 M. It was found that the recovery increased with increasing the acid concentration up to 2.0 M, after which the analytical response rapidly declined (**Figure 13**) due to deterioration of the metal complex and/or the extraction solvent itself. Similarly, when studied between 200 and 400 µL, the volume of the switching-off acid optimum was found to reach an optimum at 300 µL (**Figure 14**), beyond which dilution of the analyte took place and the signal decreased.

**Figure 12**: Effect of the type of switching-off acid.

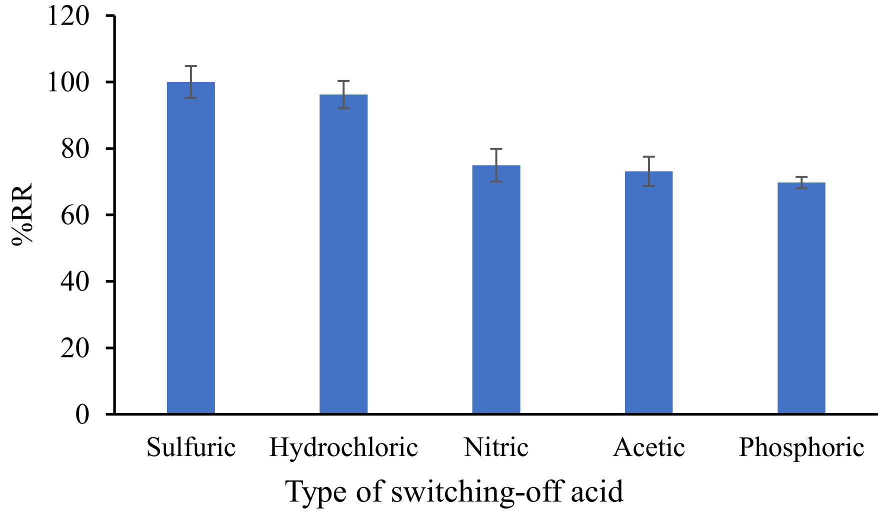


**Figure 13:** Effect of the concentration of switching-off acid.

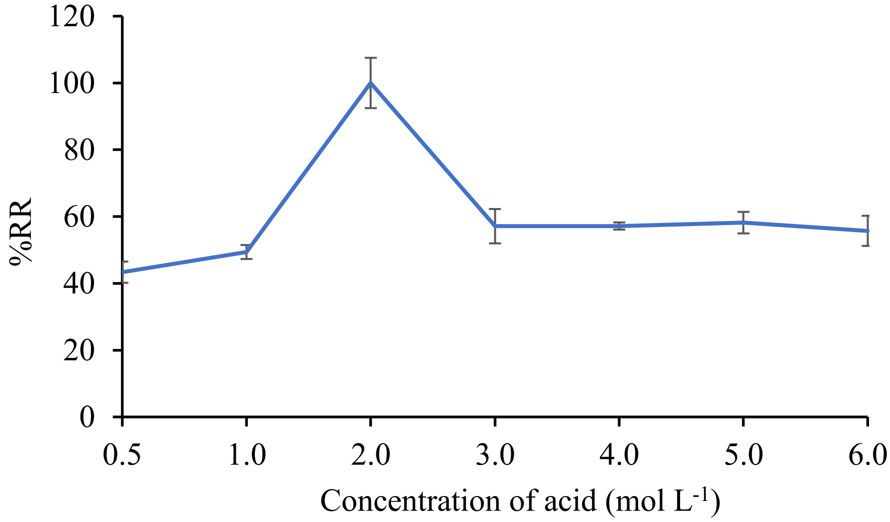
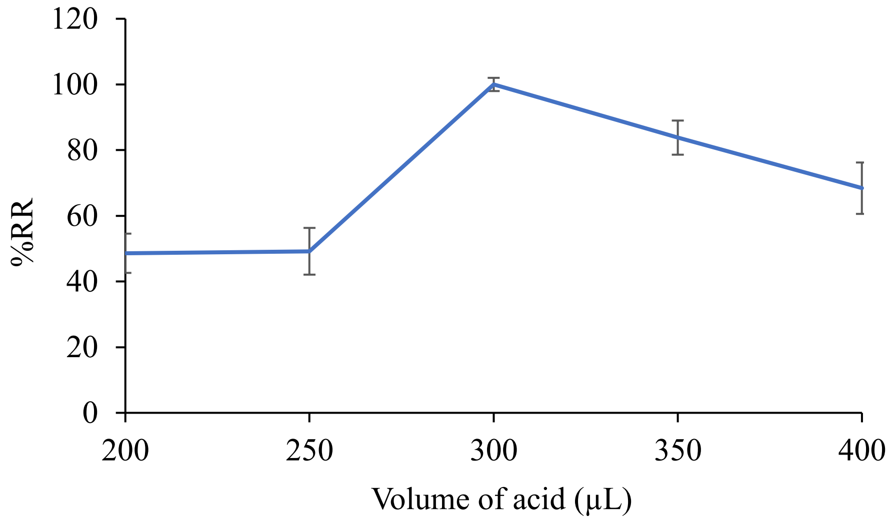


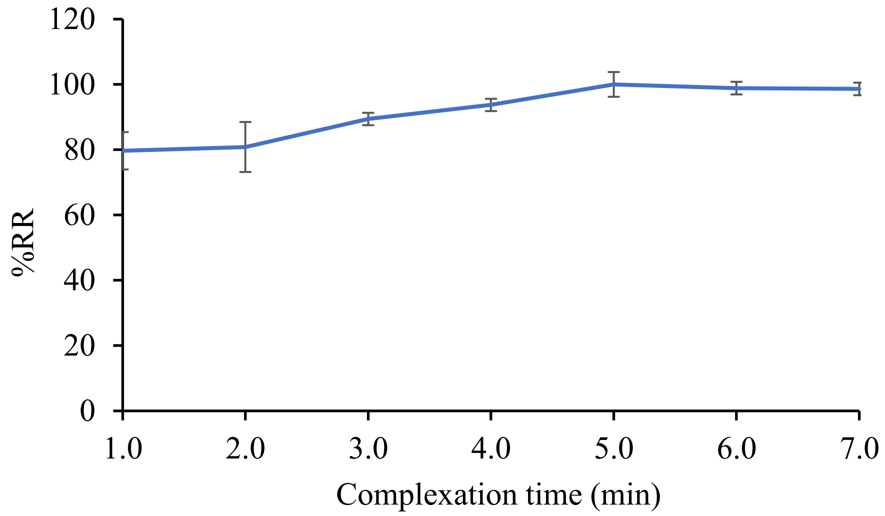
**Figure 14**: Effect of the volume of switching-off acid.



## Complexation time

Among the important analytical parameters influencing the extraction efficiency is the complexation time. Sufficient vortex time is required for the complexation reaction to reach equilibrium. This factor was evaluated by varying the vortex (complexation) time from the point of addition of the ligand to the point where the acid was added to switch off the EO-SHS from the sample solution. The optimum recovery was recorded in 5.0 min, and the response remained constant afterwards (**Figure 15**), indicating the establishment of equilibrium.

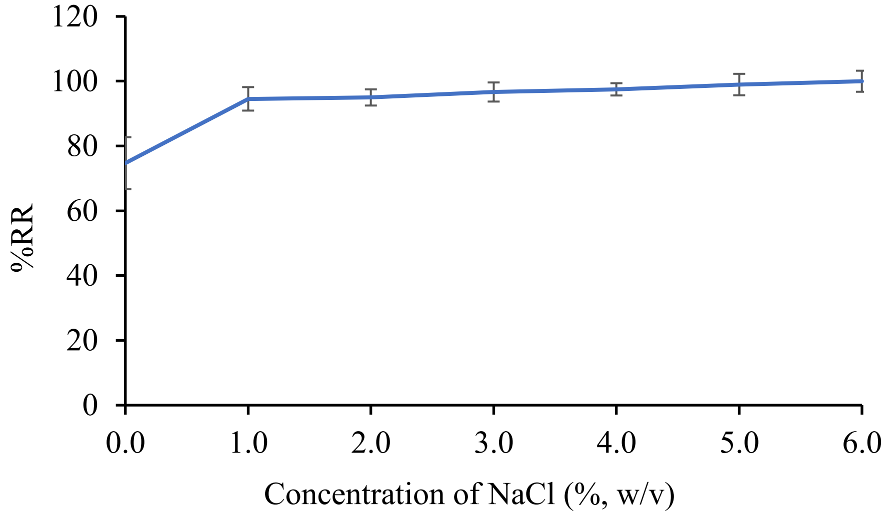
**Figure 15**: Effect of complexation time.



**Salt addition**

In most extraction procedures, addition of sodium chloride has effect on both the aqueous sample solution and the metal ion. In general, addition of salt results in increasing the ionic strength and decreases the analyte solubility in the sample solution, which improves the extraction efficiency. This effect was investigated by adding different volume of salt (0.0–6.0 %, w/v) into the aqueous solution. A 25% improvement in the recovery of analyte was achieved with 1.0% (w/v), and no significant effect was observed thereafter (**Figure 16**). Hence, 1.0% salt was used in all other experiments.

**Figure 16:** Effect of salt addition.



**Back-extraction**

Back-extracting the analyte from the more hydrophobic EO-SHS extract into an aqueous solution has a number of benefits such as better compatibility with the instrument and lower viscosity, which can both improve the nebulization efficiency and give better injection repeatability (Yao et al., 2017). The introduction of an acid as a back-extraction solution (BES) would play a significant role as it leads to the decomposition of the metal complex, resulting in the release of the metal ion into the BES. Solutions of several acids were investigated as BES, which included acetic, hydrochloric, nitric, phosphoric and sulfuric acid. Nitric acid was found to provide the highest signal (**Figure 17**) and was thus used as optimum. This can be explained based on the fact that, it is a better acid used for AAS techniques. Additionally, nitrates are stable and generally does not affect the kinetics of the experimental techniques. However, while chlorides are susceptible to vaporization, sulphates and phosphates form unstable salts. As the strength increases, the extraction efficiency increases. Although sulfuric acid is stronger, it would also result in decomposition of the oil, which can cause dilution of the analyte in the final extract. The concentration of nitric acid, when varied between 2.0 and 14.0 mol $L^{-1}$, was found to yield a maximum signal at 8.0 mol $L^{-1}$, beyond which the signal steeply decreased (**Figure 18**). The same explanation applies here, with concentrations greater than 8.0 mol $L^{-1}$ causing oil decomposition. Volume of BES was studied over the range of 50–300 µL, with 150 µL found optimum (**Figure 19**). When studied within the range of 1.0–6.0 min, back-extraction time was found to reach an optimum value at 4.0 min (**Figure 20**). This result shows that a period of 4.0 min was sufficient to reach extraction equilibrium due to the large surface area between the BES and the oil.

**Figure 17:** Effect of the type of back-extraction solvent.

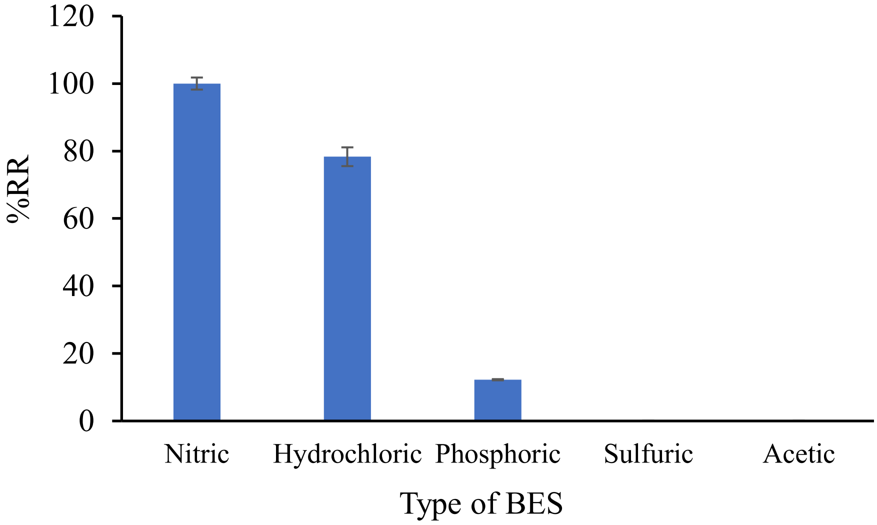


**Figure 18:** Effect of the concentration of back-extraction solvent.

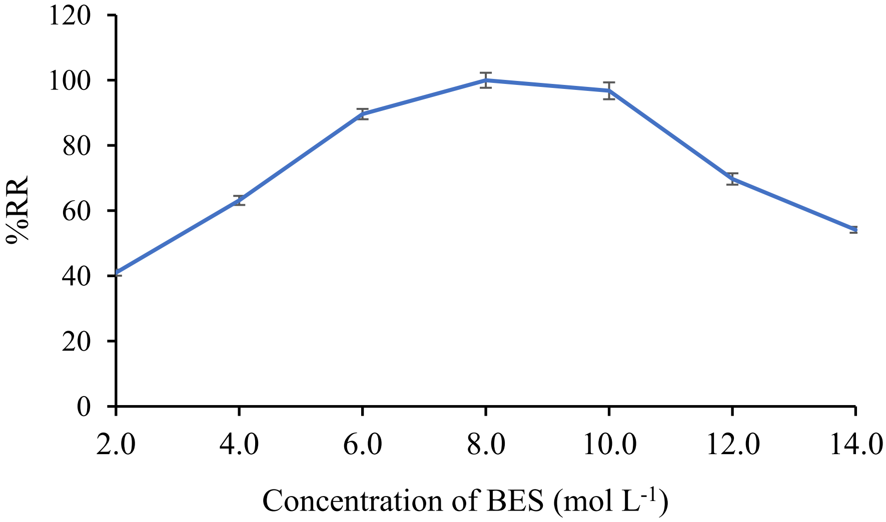


**Figure 19**: Effect of the volume of back-extraction solvent.

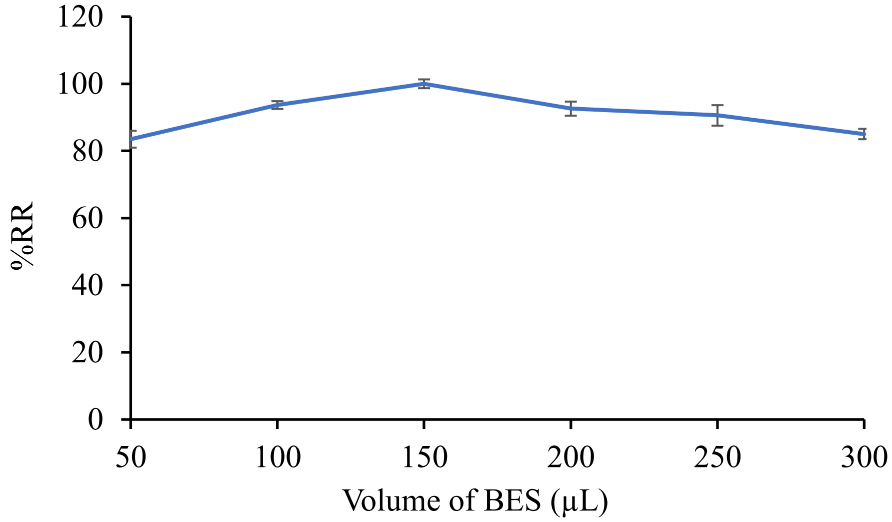
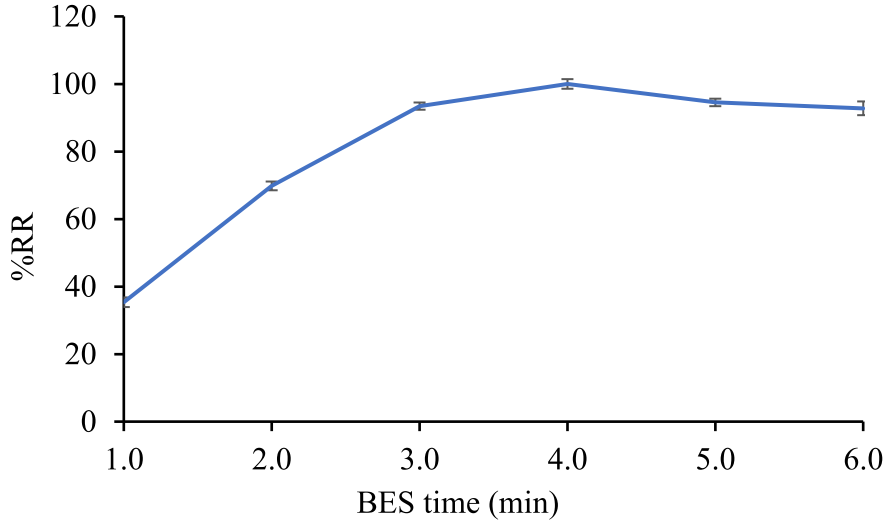


**Figure 20**: Effect of back-extraction time.



**Analytical performance of the proposed EO-SHS-LLME-FAAS method**

Analytical performance of the proposed method was assessed through plotting standard-addition calibrations with spiked samples and extraction with EO-SHS-LLME. The results, summarized in **Table 4**, show that good linearity was achieved with a coefficient of determination ($R^2$) above 0.9961. The limit of detection (LOD), evaluated based on $3S_b/m$ and the limit of quantitation (LOQ), based on $10S_b/m$, where $S_b$ is the standard deviation of the intercept and $m$ is the slope of the regression equation, were in the ranges of 2.8–3.9 and 9.4–12.9 µg $g^{-1}$, respectively. The linear dynamic range (LDR) expanded from the corresponding LOQ up to 175.0 μg $g^{-1}$. Precision, expressed in terms of percentage relative standard deviation (%RSD), was better than 4.0% and 4.6% for intraday and interday precision, respectively. The preconcentration factor (PF), calculated as the ratio of the initial volume of the sample solution to the final volume of the BES, was found as 72.7, whereas enrichment factors (EF), calculated as the ratio of the analyte concentration in the BES to the initial concentration in the sample solution, ranged between 72.5 and 74.6. Percentage recovery (%R), calculated as the percentage ratio of EF to PF, was found to range between 99.7% and 102.6%.

**Table 4:** Figures of merit of the proposed EO-SHS-LLME-FAAS method for food samples.

| Sample | Regression equation[a] | $R^2$ | LOD[b] | LOQ[c] | LDR[d] | %RSD[e] | | PF[f] | EF[g] | %R[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Intraday | Interday | | | |
| Canned tuna fish | $y = 3.4 \times 10^{-3}\ (\pm 3.9 \times 10^{-5})x - 7.7 \times 10^{-3}\ (\pm 3.2 \times 10^{-3})$ | 0.9982 | 2.8 | 9.4 | 9.4−175.0 | 2.0 | 2.9 | 72.7 | 73.2 | 100.7 |
| Carrot | $y = 3.4 \times 10^{-3}\ (\pm 4.3 \times 10^{-5})x + 7.7 \times 10^{-3}\ (\pm 3.6 \times 10^{-3})$ | 0.9976 | 3.2 | 10.6 | 10.6−175.0 | 2.8 | 3.4 | 72.7 | 72.6 | 99.8 |
| Onion | $y = 3.4 \times 10^{-3}\ (\pm 5.1 \times 10^{-5})x + 8.7 \times 10^{-3}\ (\pm 4.2 \times 10^{-3})$ | 0.9968 | 3.7 | 12.4 | 12.4−175.0 | 3.4 | 4.2 | 72.7 | 74.6 | 102.6 |
| Potato | $y = 3.4 \times 10^{-3}\ (\pm 5.4 \times 10^{-5})x + 9.0 \times 10^{-3}\ (\pm 4.4 \times 10^{-3})$ | 0.9961 | 3.9 | 12.9 | 12.9−175.0 | 4.0 | 4.6 | 72.7 | 72.8 | 100.2 |
| Yam | $y = 3.4 \times 10^{-3}\ (\pm 5.2 \times 10^{-5})x + 8.9 \times 10^{-3}\ (\pm 4.3 \times 10^{-3})$ | 0.9963 | 3.8 | 12.6 | 12.6−175.0 | 3.9 | 4.3 | 72.7 | 72.5 | 99.7 |

[a] Absorbance (mAu) = Slope (± SD) × [Concentration of lead (μg g$^{-1}$)] + Intercept ( ± SD). Regression equations with EO-SHS-LLME were obtained from standard-addition calibrations.

[b] Limit of detection (μg g$^{-1}$).

[c] Limit of quantitation (μg g$^{-1}$).

[d] Linear dynamic range (μg g$^{-1}$).

[e] Percentage relative standard deviation, $n = 3$.

[f] PF, Preconcentration factor, calculated as the ratio of the initial volume of the sample solution to the final volume of the extract.

[g] EF, Enrichment factor, calculated as the ratio of the analyte concentration in BES to the initial concentration in the sample solution.

[h] %R, Percentage recovery, calculated as the percentage ratio of EF to PF.

## Determination of lead in food samples and matrix effect

In order to evaluate possible matrix effect, addition-recovery tests were performed by spiking the samples at three concentration levels (i. e., 25.0, 100.0 and 175.0 µg $g^{-1}$) followed by digestion and subjection to the proposed EO-SHS-LLME-FAAS procedure. %R ranged from 97.1 to 106.0 as indicated in **Table 5**. None of the analyzed samples was found to contain the analyte at a concentration above the method's LOD. The matrix-to-matrix effect was analyzed by comparing the slopes of the calibration equations obtained from each sample. Calibrations with all the samples were found to possess similar slopes (**Table 4**), indicating negligible matrix-to-matrix effect, which is also due to the high extraction ability of the EO-SHS and the digestion step. The blank solution was also injected in triplicates and the analytical signal was recorded. A response similar to the unspiked samples, that were below the LOD of the method, was observed, indicating a significant reduction in matrix effect.

**Table 5**: Application of EO-SHS-LLME-FAAS to food samples ($n = 3$).

| Sample | Added concentration ($\mu g\ g^{-1}$) | Found concentration ($\mu g\ g^{-1}$) | %R[a] |
|---|---|---|---|
| Canned tuna fish | – | < LOD | – |
| | 25.0 | 25.0 | 100.0 |
| | 100.0 | 102.0 | 102.0 |
| | 175.0 | 175.0 | 100.0 |
| Carrot | – | < LOD | – |
| | 25.0 | 25.0 | 100.0 |
| | 100.0 | 101.0 | 101.0 |
| | 175.0 | 172.0 | 98.3 |
| Onion | – | < LOD | – |
| | 25.0 | 26.0 | 104.0 |
| | 100.0 | 106.0 | 106.0 |
| | 175.0 | 171.0 | 97.7 |
| Potato | – | < LOD | – |
| | 25.0 | 25.0 | 100.0 |
| | 100.0 | 103.0 | 103.0 |
| | 175.0 | 171.0 | 97.7 |
| Yam | – | < LOD | – |
| | 25.0 | 25.0 | 100.0 |
| | 100.0 | 102.0 | 102.0 |
| | 175.0 | 170.0 | 97.1 |

[a] Percentage relative recovery, a value obtained considering extraction yields from addition-recovery experiments.

## Interference study

The effect of possible interfering ions was studied by spiking various salts of some common ions at a fixed concentration of lead (i.e., 25.0 μg $g^{-1}$), and the difference between signals obtained for the spiked matrix and the unspiked ones was recorded in each case. The concentration of the interfering ions was added while monitoring the response until the response changed by ±10% from the original signal. The tolerance limits ranged between 200 and 5500 μg $g^{-1}$ as shown in **Table 6**.

**Table 6:** Tolerance level of common interfering ions.

| Interfering ion | Added as | Tolerance limit (μg $g^{-1}$) |
|---|---|---|
| Calcium | $Ca(NO_3)_2.H_2O$ | 2200 |
| Copper(II) | $Cu(NO_3)_2.2.5\ H_2O$ | 2400 |
| Iron(III) | $FeNO_3.9H_2O$ | 1000 |
| Potassium | $KNO_3$ | 5000 |
| Sodium | NaCl | 5500 |
| Zinc | $ZnCl_2$ | 200 |

### Comparison of the proposed EO-SHS-LLME-FAAS with other methods

The proposed EO-SHS-LLME method was compared with other methods reported in the literature for the determination of lead in different samples in terms of type and volume of the organic solvent(s), volume of acid used per sample, sample size, sensitivity, linearity and precision (**Table 7**). While some of the methods required the use of toxic and environmentally hazardous chlorinated organic solvents like carbon tetrachloride (Khoshmaram, 2021; Rosa et al., 2015; Zhou et al., 2011), others used large volumes of less toxic organic solvents like triethylamine, 1-undecanol and undecanoic acid, which increases the analysis cost (Afzali et al., 2013; Chaikhan et al., 2022; Zhang et al., 2018). Most of the studies given in **Table 7** used large volumes of acids (e.g., 2.5–15 mL per sample), which increases the hazard for the researcher and the environment. The use of much greener extraction solvents (i.e., EO) and the significant reduction in the volume of the acid required for the digestion step in the proposed method dramatically decreases chemical waste and the analysis cost, making the proposed method superior to the others. Owing to their higher sensitivity, yet higher cost and complexity, AFS (Zhou et al., 2011) and GFAAS (Rivas et al., 2010; Zhang et al., 2018) provided lower LOD values, while sensitivity, linearity and precision were comparable with those of the others.

**Table 7:** Comparison of DES-LLME-SDIC with other methods for the determination of cobalt.

| Extraction method/technique[a] | Sample | Sample size (mg) | Type and volume of organic solvents (μL) | Volume of acid (mL) | LOD[b] (μg $^{-1}$) | $R^2$[c] | %RSD[d] | Ref.[e] |
|---|---|---|---|---|---|---|---|---|
| DLLME-AFS | Water | 50 (mL) | Carbon tetrachloride, ethanol (3000) | - | 0.95 (ng L$^{-1}$) | 0.9990 | 2.12 | (Zhou et al., 2011) |
| DLLME-FAAS | Honey | 500 | Acetone, acetonitrile, carbon tetrachloride (1340) | 0 | Acetone, acetonitrile, carbon tetrachloride (1340) | - | < 20 | (Rosa et al., 2015) |
| SHS-LPME-GFAAS | Water, tea, hair | 10 (mL),<br>200 (mg) | Triethylamine (1000) | 4.0 | 16 (ng L$^{-1}$) | 0.9990 | 4.2 | (Zhang, S. et al., 2018) |
| EA-SS-LPME-HR-CS-FAAS | Water, food | 500 | Triethylamine (200) | 9 | 0.0195 (mg L$^{-1}$) | 0.9900 | 1.25 | (Chaikhan et al., 2022) |
| D-DLLME-SFO-FAAS | Water | 10 (mL) | 1-Undecanol, dimethylformamide, ethanol (945) | - | 0.7 (ng mL$^{-1}$) | 0.999 | 1.6 | (Afzali et al., 2013) |
| AA-LLME-FAAS | Water, urine | 10 (mL) | Carbon tetrachloride, ethanol (400) | 10 | 8.5 (μg L$^{-1}$) | – | 4.1 | (Khoshmaram, 2021) |
| LPME-SFO-GFAAS | Water | 25 (mL) | Undecanoic acid (50) | 15 | 10 (ng L$^{-1}$) | 0.9985 | 2.8 | (Rivas et al., 2010) |
| EO-LLME-FAAS | Food | 100 | Coconut oil (600) | 1.0 | 2.8 | 0.9961 | 4.6 | This work |

[a] DLLME-FAAS: Dispersive liquid–liquid microextraction-flame-atomic absorption spectrometry; LPME-SFO-GFAAS: Liquid-phase microextraction-based on solidification of floating organic drop-graphite furnace-atomic absorption spectrometry; D-DLLME-SFO-FAAS: Displacement-dispersive liquid–liquid microextraction based on solidification of floating organic drop-flame-atomic absorption spectrometry; SHS-LPME-GFAAS: Switchable-hydrophilicity solvent-based liquid-phase microextraction-graphite furnace-atomic absorption spectrometry; DLLME-AFS: Dispersive liquid–liquid microextraction-atomic fluorescence spectrometry; AA-LLME-FAAS: Air-assisted liquid–liquid microextraction-flame-atomic absorption spectrometry; EA-SS-LPME-HR-CS-FAAS: Effervescent tablet-assisted switchable solvent-based liquid-phase microextraction-high-resolution continuum source-flame-atomic absorption spectrometry.

[b] Limit of detection.

[c] Coefficient of determination.

[d] Percentage relative standard deviation.

### DES-LLME-SDIC for the determination of cobalt in milk and dairy products

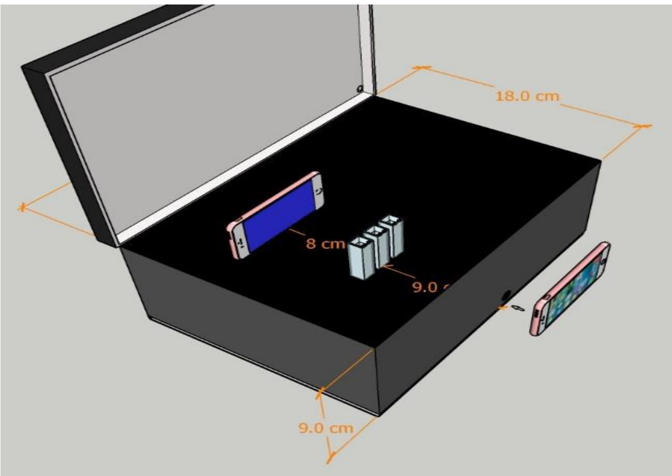
Various parameters were optimized following the one-parameter-at-a-time approach. This section provides the details for the optimization of DES-LLME-SDIC conditions for the determination of cobalt in milk and dairy samples.

### Optimization of SDIC conditions

### The colorimetric box

A homemade colorimetric wooden box with the dimensions of 30 × 22 × 13 cm (**Figure 21**) was designed for taking reproducible images of the sample solution, and the interior of the box was painted black in order to minimize light scattering. A small hole was drilled on the side of the box for capturing images of sample solutions placed in a quartz UV/Vis microcuvette (Hellma, Kruibeke, Belgium) at 8.0 cm from the detection camera.

**Figure 21:** The colorimetric box with monochromatic light source.



### Selection of the RGB channel and data processing method

ImageJ was used to split the images into their RGB channels, and the average mean value of the signal from each channel was recorded. Equation 1 was used to calculate the response from the R channel, giving the highest difference between the sample solution and the blank. The R channel showed a good linear response with a satisfactory coefficient of determination when applying Beer's law because the red color is just the complementary color of the green Co-PAN complex (Peng et al., 2019).

$$R_r = -A_r = \log(B_r/S_r) \qquad (1)$$

where, $R_r$ is the response calculated using the mean value from the histogram for the blank ($B_r$) and the sample solution ($S_r$), while $A_r$ is the absorbance.

### Selection of the wavelength of the monochromatic light source

Previous studies with SDIC have reported the use of continuum light sources such as LED (Shahvar et al., 2021) in the absence of a monochromator, which is expected to lower the sensitivity and selectivity. In order to overcome these limitations, a monochromatic light source was used with an analyte-specific color turned on as a full screen on the source smartphone. The color corresponding to each wavelength was obtained from a free online wavelength-to-color converter (https://www.wolframalpha.com). The wavelength was then scanned within the range of 555–610 nm, with the maximum response obtained at 560 nm (**Figure 22**) that was used throughout the experiments. The UV/Vis spectra of PAN and the Co-PAN complex are given in **Figure 23** and **Figure 24**, respectively. It is noteworthy that at 560 nm, excess PAN would not cause spectral interference with Co-PAN. This was also in a good agreement with the literature (Gharehbaghi et al., 2008). The colors of the solutions containing PAN and the Co-PAN complex are given in **Figure 25**.

**Figure 22:** Scanning the wavelength of the monochromatic light source.

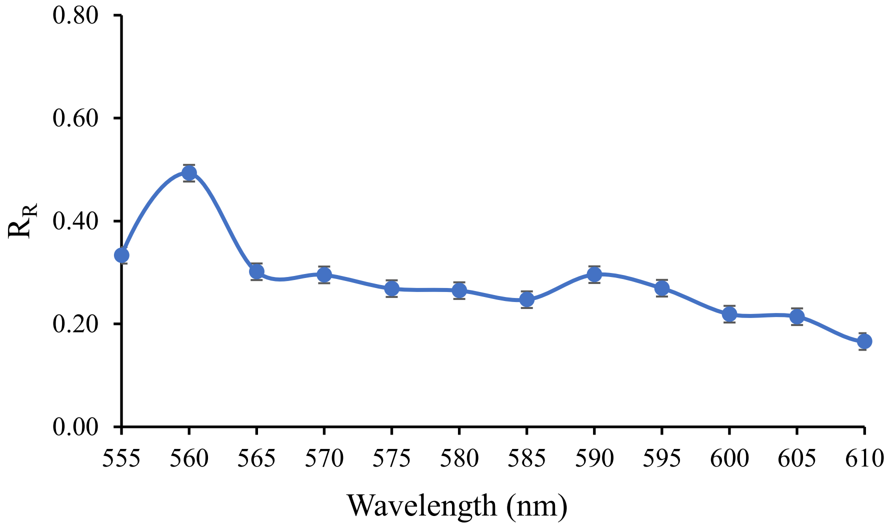


**Figure 23:** UV/Vis spectrum of PAN.

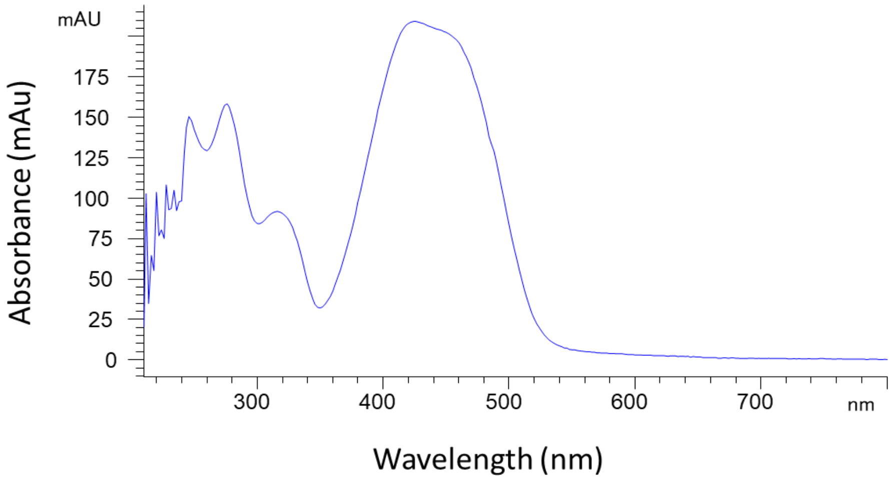


**Figure 24:** UV/Vis spectrum of Co-PAN complex.

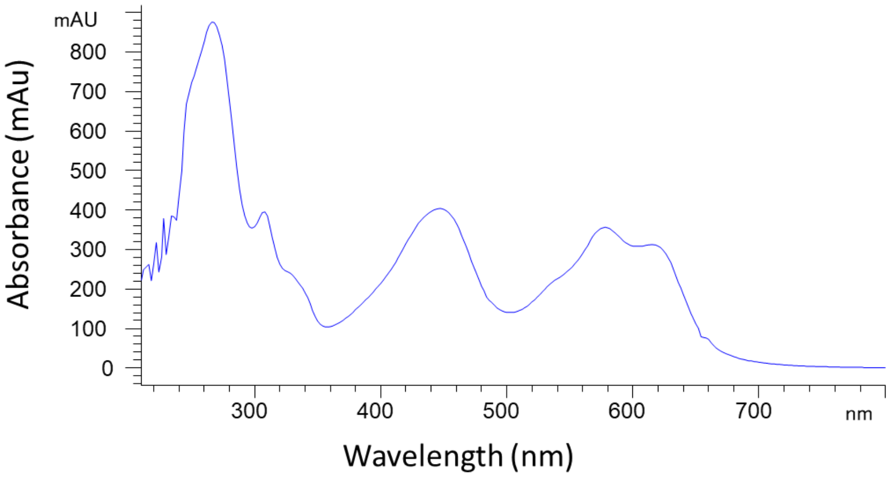
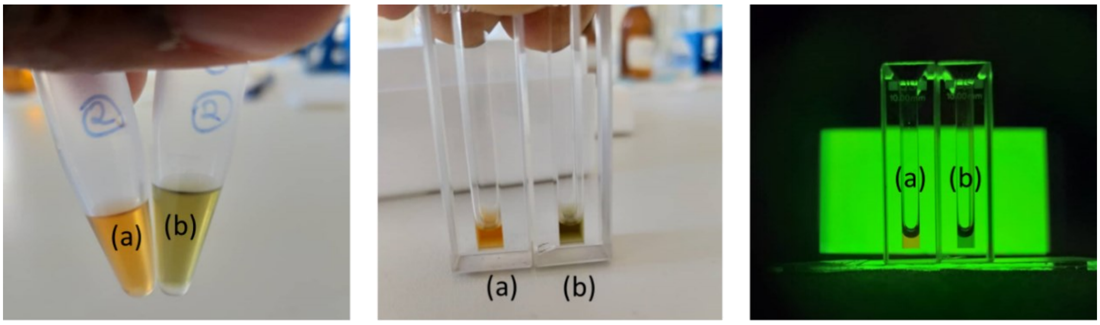


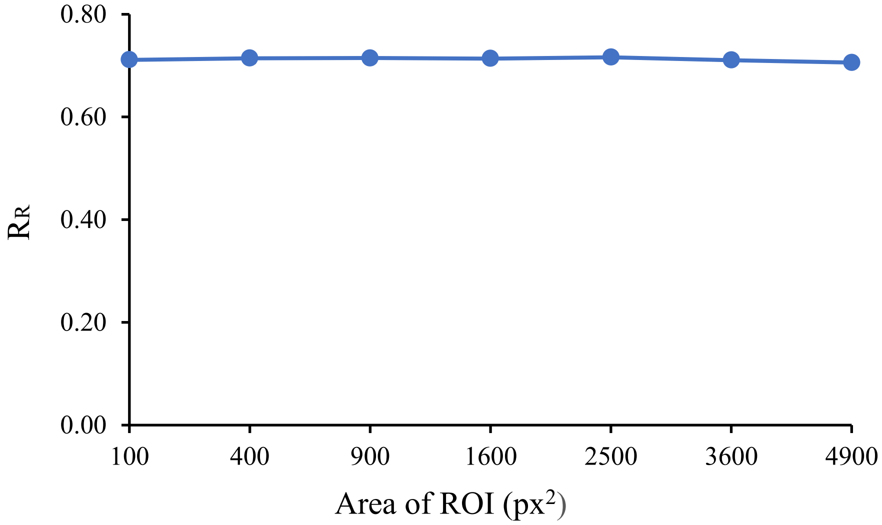
**Figure 25***:* Colors of (a) PAN; and (b) Co-PAN complex.



**Selection of the region of interest**

A specific area was cropped using the ImageJ options, termed as the region of interest (ROI), and signals were obtained from the corresponding histogram. No significant effect was observed when the ROI was varied from 100 to 4900 px$^2$ (**Figure 26**). The greater the color homogeneity of the final extract, the lower the standard deviation of the pixel intensities measured from the histogram within the selected ROI, and the lower the effect on the response (Caleb and Alshana, 2021a; Porto et al., 2019). An area of the ROI of 1600 px$^2$ was used throughout the experiments.

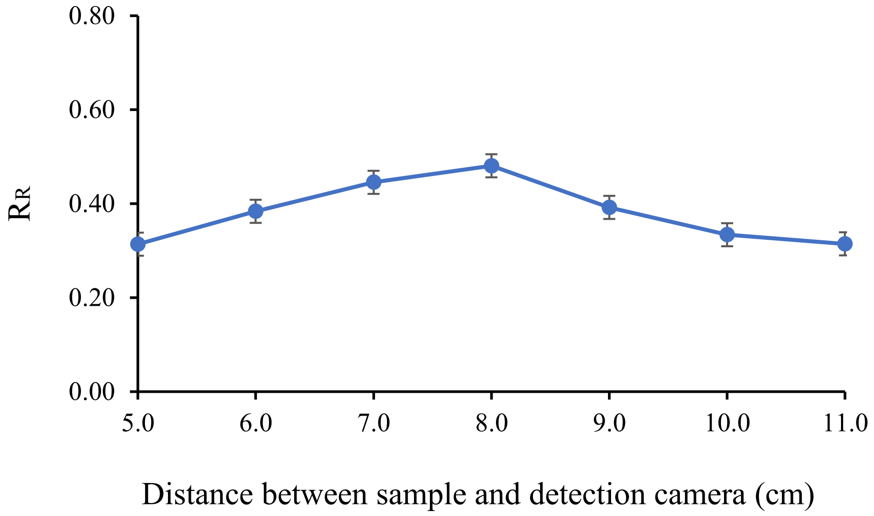
**Figure 26**: Effect of ROI.



**Position of the sample holder**

The sample holder was positioned between two smartphones, one acting as the light source and the other as the detector. Several factors can influence the detection camera's autofocus performance, including the camera's model, lenses used, focus settings, light level, subject (sample) contrast and size, and camera's motion. These variables are indeed not independent (Zhang, Y.P. et al., 2018). However, assuming that all of them remain constant during the measurement, the distance between the sample holder and the detection camera would be the most important factor influencing the autofocus of the detection camera (Caleb and Alshana, 2021a). The effect of the distance between the sample and the detector was studied within the range between 5.0 and 11.0 cm. It was observed that below 8.0 cm, the images captured were blurred due to poor autofocus of the detection camera, whereas at higher distances, the image resolution decreased (**Figure** *27*). Therefore, a distance of 8.0 cm was chosen as optimum in subsequent experiments.

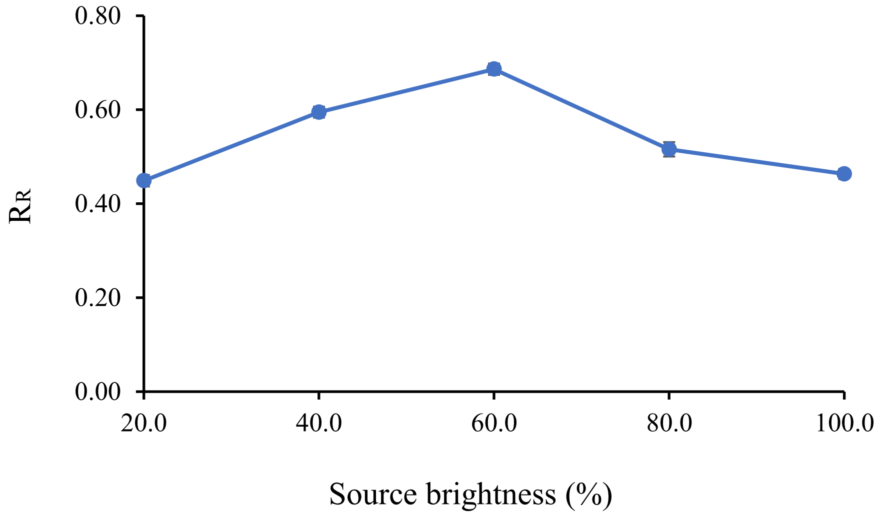
**Figure 27:** Effect of the distance between sample and detection camera.



**Brightness of the light source**

The brightness of the light source can have a significant impact on the detection camera's autofocus as well as the degree of saturation of the detection camera and thus the response (Caleb and Alshana, 2021a). The effect of the brightness of the light source was studied over the range of 20.0–100.0%. Whereas a low response was obtained below 60.0% due to the darkness of the images, saturation of the detection camera was thought to have reduced the response thereafter (**Figure 28**). Hence, 60.0% was chosen as the optimum brightness of the light source.

**Figure 28:** Effect of the brightness of the monochromatic light source.



## Optimization of DES-LLME conditions

A standard solution of 0.1 µg mL$^{-1}$ of cobalt was used for the optimization. The microextraction conditions were optimized by pooling an appropriate volume of each of the samples and spiking them with the cobalt standard solution.

### Composition and volume of DES

In general, DES that are suitable as extraction solvents for LLME must have certain physicochemical properties, such as high affinity for the analyte, low solubility in water, and ease of dispersion in aqueous solutions (Zounr et al., 2018). Therefore, the choice of an appropriate DES composition is important in order to achieve maximum recovery of the analyte. ChCl/phenol is the most commonly used DES in LLME as a hydrogen bond acceptor and donor, respectively (Alshana et al., 2021). In this experiment, ChCl and phenol were used to prepare five DES compositions at molar ratios of 1:1 and 1:5, respectively. In all cases, a clear, colorless, homogeneous, monophasic extraction solvent was obtained. An optimum response was reached with a ChCl/phenol molar ratio of 1:4 (**Figure** *29*). Other ratios, such as 2:1 and 3:1, were also considered. However, it was observed that at these two compositions, the solvent mixture solidified upon standing at room temperature and was therefore considered not suitable for extraction. In addition, the effect of the volume of DES was studied over the range of 200–450 µL. While no separation was observed with 200 µL and lower volumes, increasing the volume above 300 µL caused a reduction in the response due to dilution of the analyte in the excess DES (**Figure *30***). Therefore, a ChCl/phenol DES (1:4, 300 µL) was considered optimum for further experiments. It is noteworthy that the collected volume was 350 ± 20 µL.

**Figure 29**: *Effect of the composition of DES.*

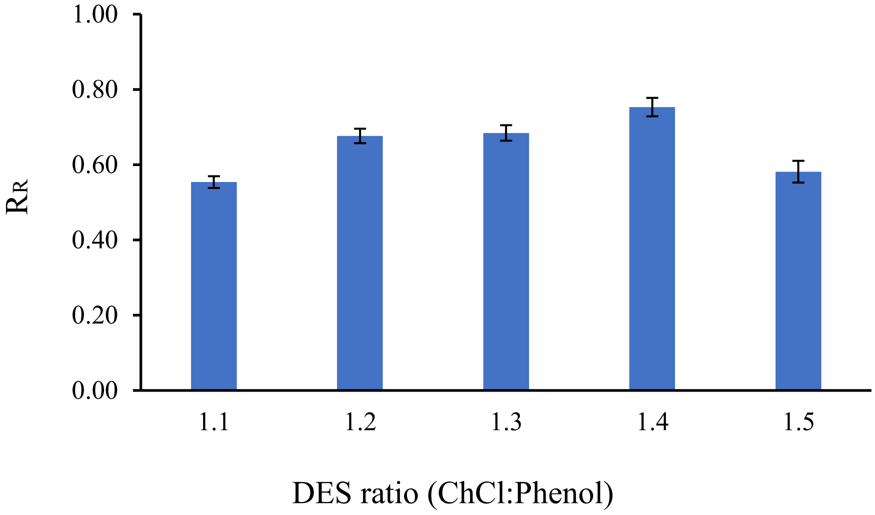
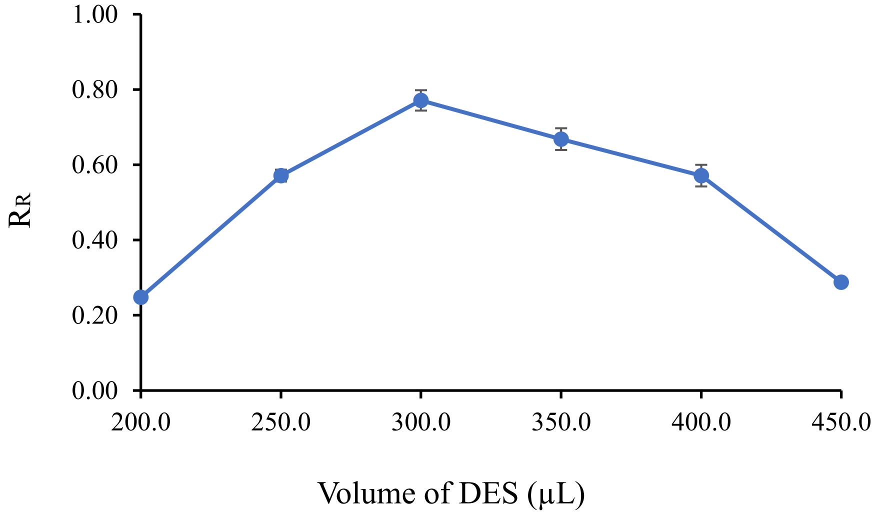


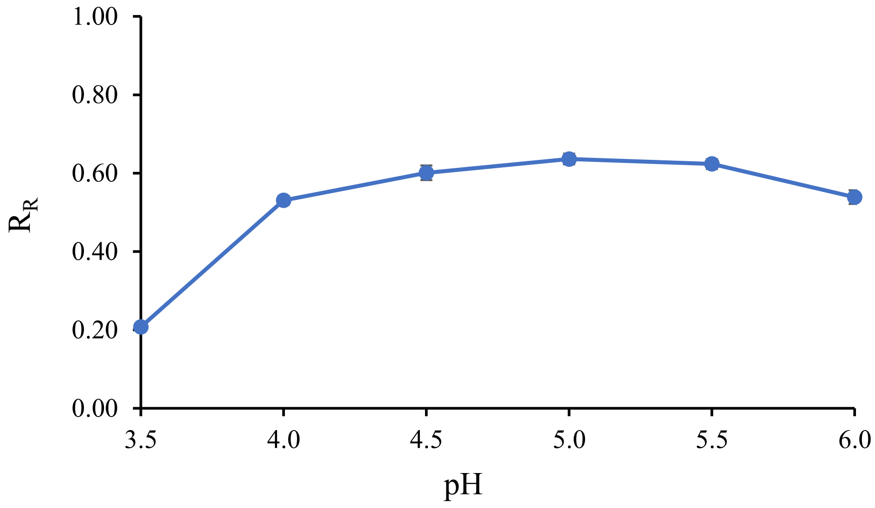
**Figure 30:** *Effect of the volume of DES.*



### Sample pH

pH of the sample solution is an important factor in metal ion complexation and extraction. The complexation pH was studied between 3.50 and 6.00. At low pH, competition between hydrogen ions and the metal ion for the ligand would be expected. On the other hand, increasing the pH would result in higher hydrolysis of the metal ions(Werner, 2020). Moreover, the complexation of cobalt by PAN is affected by the pH of the sample solution. According to the p$K_a$ values of PAN (p$K_{a1}$ 3.94, p$K_{a2}$ 9.84), it would be predominantly present in its ionized forms at low (< 3) and high pH (> 11) values, and in its neutral form over the pH range of 5.5-8.5. Based on the above, the maximum extraction efficiency was achieved at pH 5.00 (**Figure 31**), which was chosen as the optimum for further experiments.

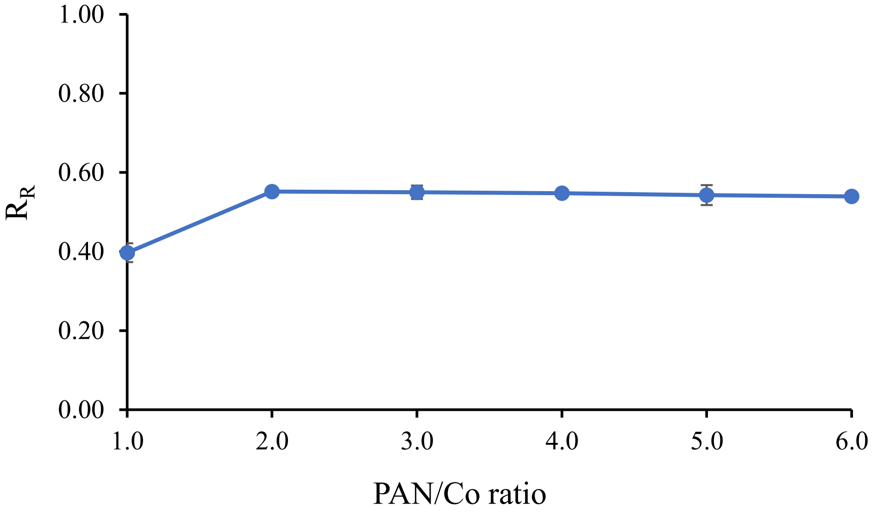
**Figure 31:** Effect of the sample pH on complexation of the analyte.



### Concentration of the ligand, PAN

The effect of the amount of PAN as a chelating agent was studied. A solution of PAN (500 µg mL$^{-1}$) was used to chelate the metal at different mole ratio. A Co-PAN mole ratio of 1:1 to 1:6 was studied (**Figure 32**), where a lower response was observed with 1:1. However, at 1:2 and beyond, the response was found to be almost constant, and this quite agreed with the theoretical metal-ligand ratio. Based on this, excess ligand was added in order to have maximum extraction of the analyte in the real samples. Therefore, 1:4 was selected as the optimum and used in all the experiments.

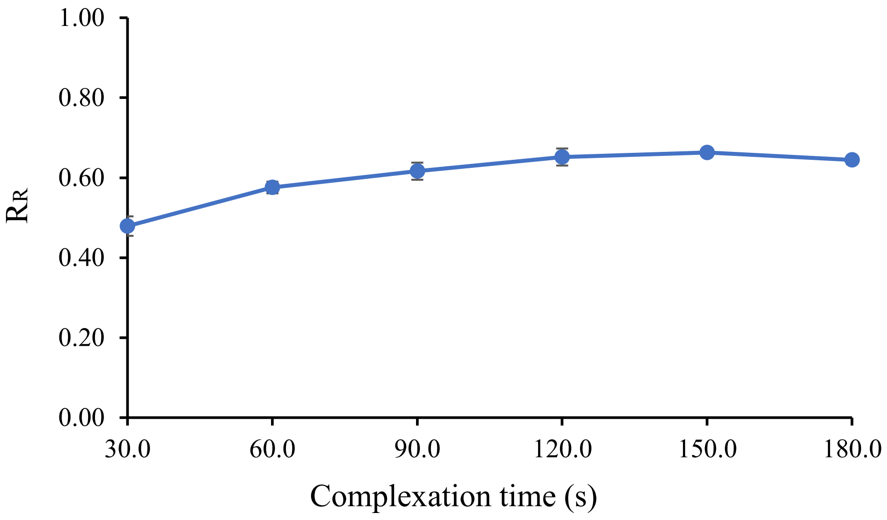
**Figure 32:** Effect of the amount of PAN for analyte complexation.



**Complexation time**

To achieve maximum analyte extraction efficiency from the sample solution, sufficient vortex time is required to allow chelation between the analyte and the ligand. Vortex agitation of the sample solution increases the surface area of interaction between them and hence the complexation yields as well as the extraction efficiency. The effect of complexation time was studied in the range of 30–180 s, where a gradual increase in the response was observed up to 120 s (**Figure 33**), which was chosen as the optimum.

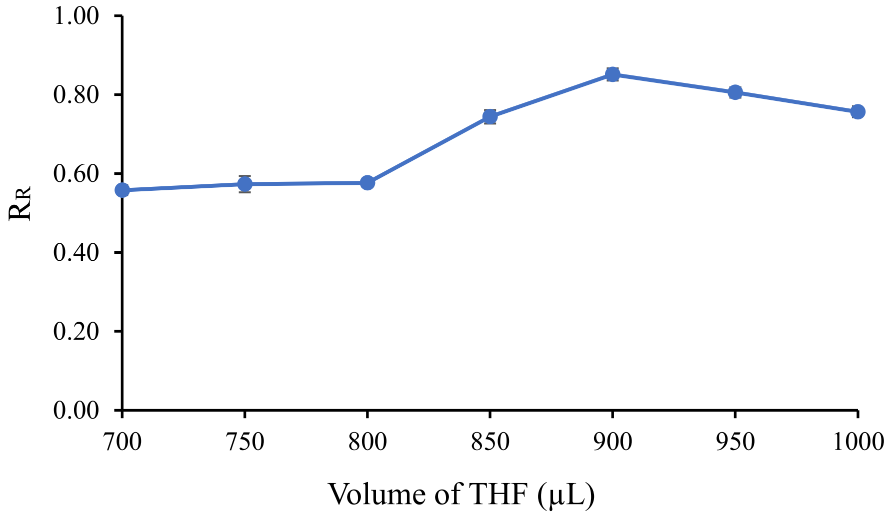
**Figure 33:** Effect of complexation time.



### Effect of type and volume of demulsifying solvent

In DES-LLME, adding a demulsification solvent such as acetone, ACN, ethanol, and THF is a common approach to demulsify the cloudy solution after extraction is complete to ensure a successful phase separation (Abdi et al., 2021). Expectedly, the volume of the demulsification solvent would influence the recovered volume of DES upon centrifugation. Acetone, ACN, ethanol, and THF were investigated for this purpose. It was found that THF was the only solvent to cause significant phase separation. Volumes of THF were investigated within the range of 700 to 1000 µL. While low solvent recoveries were observed with THF volumes less than 700 µL, quantitative recoveries were obtained with volumes greater than 900 µL. The extraction efficiency increased up to 700 µL of THF but decreased afterwards due to dilution of the analyte in an excess amount of the solvent (**Figure** ***34***). Consequently, an optimum THF volume of 900 µL was chosen for subsequent experiments.

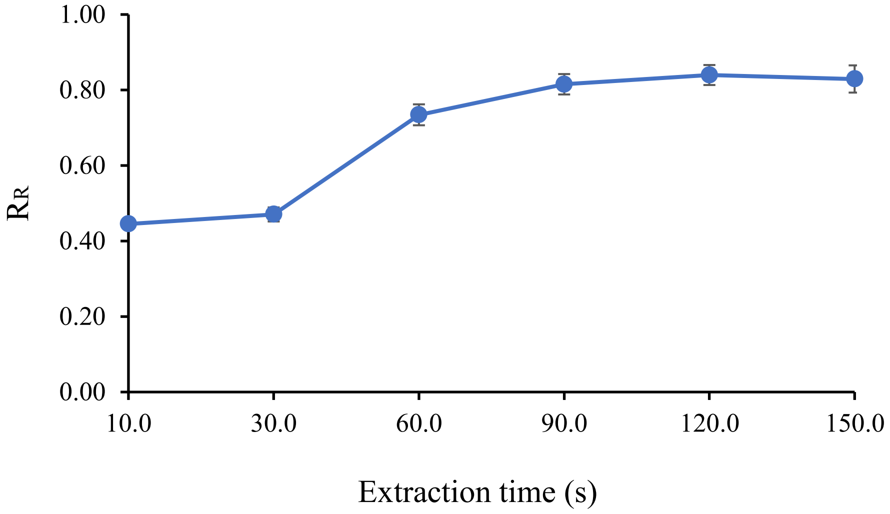
**Figure 34**: Effect of the volume of THF for demulsification of the sample solution.



## Extraction time

Extraction time has a significant impact on the overall analysis time and sample throughput. It is, therefore, critical to choose an extraction time that confirms the achievement of equilibrium between the analyte and the extraction solvent for maximum recovery to be reached. In this study, the extraction time was defined as the time interval between the addition of the extraction solvent to the end of the centrifugation step. The effect of extraction time on the extraction efficiency was examined by varying the vortex time from 10 to 150 s. The results indicated that maximum extraction of the analyte was achieved within 120 s (**Figure 35**), which was used in further experiments.

**Figure 35:** Effect of the extraction time.



### Analytical performance of the proposed DES-LLME-SDIC method

In order to assess the method's analytical performance, standard-addition calibration graphs were plotted with the samples being spiked with cobalt at the concentration range of 0.20–2.50 µg g$^{-1}$ and applying the DES-LLME-SDIC procedure. **Table *8*** shows the achievement of good linearity with R$^2$ above 0.9966. Precision, expressed in terms of %RSD, was found to be within the ranges of 2.2–3.8 and 3.7–7.8 for intraday and interday precision, respectively. LOD, also evaluated based on $3S_b/m$ and LOQ based on $10S_b/m$, where, $S_b$ is the standard deviation of the intercept and m is the slope of the regression equation, were found to be within the ranges of 0.02–0.05 and 0.08–0.16 µg g$^{-1}$, respectively. Wide LDR were obtained starting from the corresponding LOQ up to 2.50 µg g$^{-1}$. EF, calculated as the ratio of the slope of the calibration equation obtained with SDIC to that obtained with DES-LLME-SDIC, were found to fall within the range of 1.6–2.5.

**Table 8:** Figures of merit for DES-LLME-SDIC for milk and dairy samples.

| Method | Sample | Regression equation[a] | $R^2$ | LOD[b] | LOQ[c] | LDR[d] | %RSD[e] |  | EF[f] |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  | Intraday | Interday |  |
| SDIC | Aq. Calibration | $y = 0.18\ (\pm 2.0 \times 10^{-3})x - 0.01\ (\pm 3.1 \times 10^{-3})$ | 0.9979 | 0.05 | 0.17 | 0.17–2.5 | 2.9 | 4.8 | – |
| DES-LLME-SDIC | Ayran | $y = 0.45\ (\pm 4.5 \times 10^{-3})x + 0.06\ (\pm 3.8 \times 10^{-3})$ | 0.9977 | 0.03 | 0.08 | 0.08–2.5 | 2.5 | 4.5 | 2.5 |
|  | Cow milk | $y = 0.45\ (\pm 5.5 \times 10^{-3})x + 0.06\ (\pm 3.9 \times 10^{-3})$ | 0.9972 | 0.03 | 0.09 | 0.09–2.5 | 2.2 | 3.7 | 2.5 |
|  | Goat milk | $y = 0.39\ (\pm 4.3 \times 10^{-3})x + 0.08\ (\pm 3.0 \times 10^{-3})$ | 0.9977 | 0.02 | 0.08 | 0.08–2.5 | 3.8 | 6.8 | 2.2 |
|  | Pasteurized milk | $y = 0.44\ (\pm 5.9 \times 10^{-3})x + 0.05\ (\pm 4.2 \times 10^{-3})$ | 0.9966 | 0.03 | 0.10 | 0.10–2.5 | 3.5 | 6.9 | 2.4 |
|  | Yoghurt | $y = 0.28\ (\pm 3.9 \times 10^{-3})x + 0.04\ (\pm 4.5 \times 10^{-3})$ | 0.9967 | 0.05 | 0.16 | 0.16–2.5 | 3.7 | 7.8 | 1.6 |

[a] Absorbance (mAu) = Slope (±SD) × (Cobalt concentration (μg $g^{-1}$) + Intercept (±SD).

[b] Limit of detection (μg $g^{-1}$).

[c] Limit of quantitation (μg $g^{-1}$).

[d] Linear dynamic range (μg $g^{-1}$).

[e] Percentage relative standard deviation, $n = 3$.

[f] EF, Enrichment factor, calculated as the ratio of the slope of the calibration equation obtained with SDIC to that with obtained with DES-LLME-SDIC.

## Determination of cobalt in milk and dairy products

The effect of the sample matrix on the response was studied using addition-recovery experiments. The samples were spiked at three different concentration levels (i.e., 0.40, 0.60, and 1.00 µg $g^{-1}$) before being analyzed with DES-LLME-SDIC. The percentage relative recoveries (%RR) ranged from 95 to 108% (**Table 9**). The significance of the matrix effect was assessed using the analysis of variance (ANOVA) test by comparing the slopes of the standard-addition calibration graphs obtained with the spiked samples (**Table 8**). It was observed that the slopes were statistically different ($P < 0.05$), which indicated the need for standard addition to eliminate this matrix effect. This was believed to be due to differences in the fat and protein content of the analyzed samples. It was observed that sensitivity decreased as the fat and/or protein content increased in the sample (**Figure 36**), which is due to a higher interaction of the metal ion with the macromolecules and a lower degree of ACN deproteinization at higher concentrations (Hassan and Alshana, 2019; Wang et al., 2011). All milk and dairy products analyzed were found to contain cobalt at concentrations ranging between 0.11 and 0.20 µg $g^{-1}$ (**Table 9**). The accuracy of these results was also checked with high-performance liquid chromatography-diode array detection (HPLC-DAD) and the results were statistically in good agreement as determined by a single-factor ANOVA test ($P > 0.05$) (**Table 9**). The chromatograms of PAN and Co-PAN complex as well as the chromatographic conditions are given in **Figure 37**.

**Figure 36:** Effect of fat and protein content on sensitivity (slope).

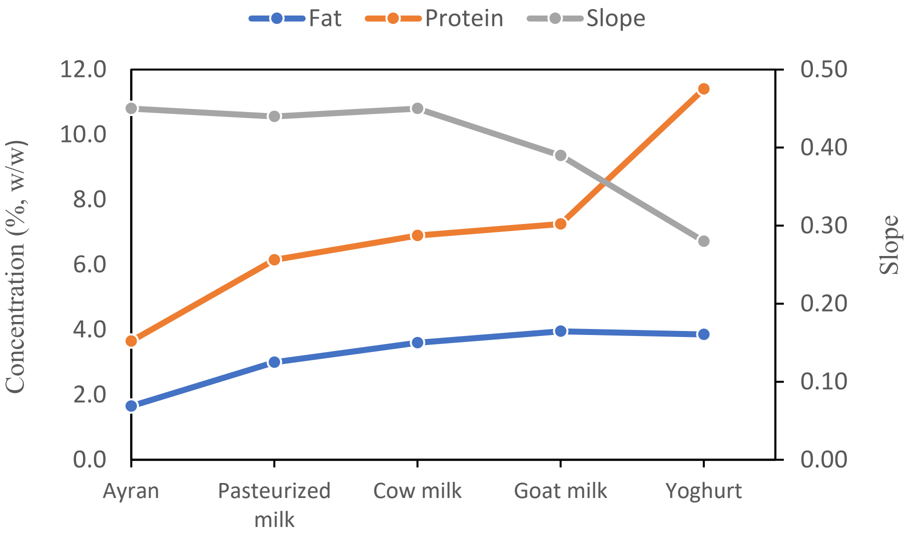
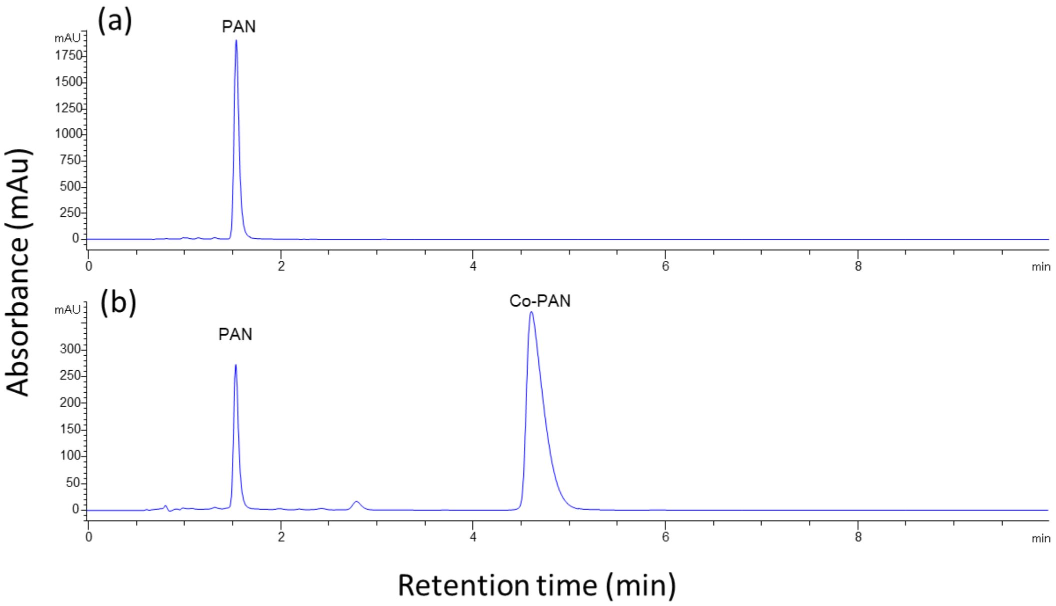


**Figure 37:** HPLC-DAD chromatograms of (a) PAN; and (b) Co-PAN complex



**Chromatographic conditions**: Column, reversed phase, ACE 5 C18, 125 mm × 3.0 mm I.D. (5 µm), Flow rate: 1.0 mL min$^{-1}$, Column temperature: 35 °C, Sample injection volume: 20 µL, Mobile phase: 50:50 (%, v/v) acetonitrile/water (containing 1.10 % w/v trichloroacetic acid); Instrument: HPLC-DAD (Agilent Technologies 1200 series, USA) equipped with an online vacuum degasser, a quaternary pump, an autosampler, a thermostatted column compartment, DAD, and ChemStation software (Rev. B.03.01).

**Table 9*:*** Application of DES-LLME-SDIC to milk and dairy product samples ($n = 3$).

| Sample | Fat content (%, w/w) | Protein content (%, w/w) | Added concentration (μg g$^{-1}$) | Concentration found with DES-LLME-SDIC (μg g$^{-1}$) | %RR[a] | %RSD | Concentration found with HPLC-DAD (μg g$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Ayran | 1.7 | 2.0 | – | 0.14 | – | 0.6 | 0.13±0.1 |
| | | | 0.40 | 0.42 | 105 | 1.4 | |
| | | | 0.60 | 0.60 | 100 | 2.4 | |
| | | | 1.00 | 0.98 | 98 | 4.5 | |
| Cow milk | 3.6 | 3.3 | – | 0.14 | – | 0.3 | 0.14±0.2 |
| | | | 0.40 | 0.41 | 103 | 1.9 | |
| | | | 0.60 | 0.61 | 102 | 2.0 | |
| | | | 1.00 | 0.98 | 98 | 3.8 | |
| Goat milk | 4.0 | 3.3 | – | 0.20 | – | 0.7 | 0.21±0.1 |
| | | | 0.40 | 0.38 | 95 | 2.6 | |
| | | | 0.60 | 0.59 | 98 | 1.7 | |
| | | | 1.00 | 0.97 | 97 | 2.4 | |
| Pasteurized milk | 3.0 | 3.2 | – | 0.11 | – | 0.4 | 0.13±0.2 |
| | | | 0.40 | 0.41 | 102 | 2.9 | |
| | | | 0.60 | 0.59 | 98 | 4.8 | |
| | | | 1.00 | 1.02 | 102 | 0.8 | |
| Yoghurt | 3.9 | 7.6 | – | 0.13 | – | 0.4 | 0.12±0.2 |
| | | | 0.40 | 0.43 | 108 | 1.7 | |
| | | | 0.70 | 0.74 | 106 | 3.5 | |
| | | | 1.00 | 1.00 | 100 | 2.6 | |

[a] Percentage relative recovery, a value obtained considering extraction yields from standard-addition calibrations.

## Interference study

The effect of the existing ions was investigated for those ions commonly found in milk and dairy products, such as $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Fe^{3+}$, that would compete and interfere with the extraction and determination of Co(II). Varying concentrations of interfering ions were added to the solution containing 0.075 μg g$^{-1}$ $Co^{2+}$ until the signal of the original solution was affected by ±10%. The response decreased as the concentrations of $Cu^{2+}$ and $Zn^{2+}$ were added, while $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $Fe^{3+}$ showed positive interference. The results, shown in **Table *10***, indicated that under the optimum conditions described above, the proposed method has good selectivity for Co(II) ions up to 0.5 µg $g^{-1}$ of $K^+$, $Cu^{2+}$ and $Zn^{2+}$, 1.0 µg $g^{-1}$ of $Mg^{2+}$ and $Ca^{2+}$, and 0.3 µg $g^{-1}$ of $Fe^{3+}$. At higher concentrations, masking agents can be used to suppress their effect, such as the oxalate ion (for $Ca^{2+}$ and $Fe^{3+}$), ascorbic acid, or the iodide ion (for $Cu^{2+}$) (Gharehbaghi et al., 2008).

**Table 10*:*** Tolerance level of common interfering ions.

| Interfering ion | Added as | Tolerance limit (µg $g^{-1}$) |
|---|---|---|
| $K^+$ | $KNO_3$ | 0.5 |
| $Mg^{2+}$ | $Mg(NO_3)_2.6H_2O$ | 1.0 |
| $Ca^{2+}$ | $Ca(NO_3)_2.4H_2O$ | 1.0 |
| $Cu^{2+}$ | $Cu(NO_3)_2.5H_2O$ | 0.5 |
| $Zn^{2+}$ | $Zn(NO_3)_2.6H_2O$ | 0.5 |
| $Fe^{3+}$ | $Fe(NO_3)_3.9H_2O$ | 0.3 |

### Comparison of the proposed DES-LLME-SDIC with other methods

The analytical performance of the proposed DES-LLME-SDIC method was compared with that of other reported methods in terms of the type and volume of the extraction solvent, extraction time, sensitivity, linearity, and precision (**Table *11***). While some of the methods required the use of chlorinated organic solvents that are toxic and environmentally hazardous, like carbon tetrachloride, chloroform, and trichloroethylene (Baliza et al., 2009; Barreto et al., 2020; Farajzadeh et al., 2009), others used large volumes of trimethylamine (900 µL per sample) (Habibiyan et al., 2017). The synthesis of the ferrofluid, used in ultrasound-assisted ferrofluid dispersive liquid-phase microextraction (USA-FF-DLPME), involved tedious steps and required more than 36 h of preparation (Ghasemi et al., 2021). Similarly, an elevated temperature, long hours of sample preparation coupled with the tedious process towards manufacturing the samples (Vural et al., 2008), also the use of large volume of reagents and long analysis time (Temel et al., 2018) were observed for these studies. Hexanoic acid, used in effervescence assisted dispersive liquid–liquid microextraction (EA-DLLME), is a green solvent (Moghadam et al., 2017). The extraction time of the proposed method, defined as the time interval between the injection of the extraction solvent into the sample solution until collection, was only 2 min, which was comparable to those methods applying DLLME (Baliza et al., 2009; Ghasemi et al., 2021; Moghadam et al., 2017) and trimethylamine as a switchable-hydrophilicity solvent (Habibiyan et al., 2017). Pressure variation in-syringe dispersive liquid–liquid microextraction (PV-IS-DLLME) (Barreto et al., 2020) required only 10 s. The proposed method provided the highest sensitivity among the others. Good linearity and acceptable repeatability were obtained with such highly complicated food matrices.

**Table 11:** Comparison of DES-LLME-SDIC with other methods for the determination of cobalt.

| Extraction method/technique[a] | Sample | Extraction solvent | Volume of extraction solvent (μL) | Extraction time (min) | LOD[b] | R$^2$[c] | %RSD[d] | Ref.[e] |
|---|---|---|---|---|---|---|---|---|
| DLLME-FAAS | Water | Carbon tetrachloride | 50 | 2 | 0.9 μg L$^{-1}$ | – | 5.8 | (Baliza et al., 2009) |
| DLLME-HPLC-UV | Water | Chloroform | 250 | 5 | 3 μg L$^{-1}$ | 0.999 | 3.3 | (Farajzadeh et al., 2009) |
| SS-LPME-FAAS | Food and biological | Triethylamine | 900 | 2 | 0.7 μg L$^{-1}$ | 0.9921 | 2.9 | (Habibiyan et al., 2017) |
| EA-DLLME-FA-FAAS | Water, beverages | Hexanoic acid | 300 | 3 | 3.0 ng mL$^{-1}$ | 0.98 | 4.2 | (Moghadam et al., 2017) |
| PV-IS-DLLME-DIC | Food | Trichloroethylene | 115 | 10 s | 0.08 μg g$^{-1}$ | – | 4.6 | (Barreto et al., 2020) |
| USA-FF-DLPME-FAAS | Water | Ferrofluid | 200 | 3 | 0.7 μg L$^{-1}$ | – | 2.6 | (Ghasemi et al., 2021) |
| HPLC-ICP-OES | Kefir and milk samples | Ethanol | 1.0 (g) | 3.5 min (Retention time) | 0.06 mg/kg | 0.9998 | 10.8 | (Bodur et al., 2021) |
| UA-CPE-FAAS | Chocolate | HNB/CTAB* | 6.0 mL | 7 | 0.56 μg L$^{-1}$ | 0.9936 | 3.8 | (Temel et al., 2018) |
| DES-LLME-SDIC | Milk and dairy products | DES (ChCl/phenol, 1:4) | 300 | 2 | 0.03 ng g$^{-1}$ | 0.9966 | 7.8 | This work |

[a] DLLME-FAAS: Dispersive liquid–liquid microextraction-flame-atomic absorption spectrometry; DLLME-HPLC-UV: Dispersive liquid–liquid microextraction-high-performance liquid chromatography-ultraviolet spectrophotometry; SS-LPME-FAAS: Switchable solvent-liquid phase microextraction-flame-atomic absorption spectrometry; EA-DLLME-FA-FAAS: Effervescence-assisted dispersive liquid–liquid microextraction based on a medium-chain fatty acid-flame-atomic absorption spectrometry; PV-IS-DLLME-DIC: Pressure variation in-syringe dispersive liquid–liquid microextraction-digital image colorimetry; USA-FF-DLPME-FAAS: Ultrasound-assisted Ferro fluid dispersive liquid-phase microextraction-flame-atomic absorption spectrometry; HPLC-ICP-OES: High-performance liquid chromatography-Inductively-coupled plasma optical emission spectrometry; UA-CPE-FAAS: Ultrasound-assisted cloud point extraction flame-atomic absorption spectrometry; DES-LLME-SDIC: Deep eutectic solvent-liquid–liquid microextraction-smartphone digital image colorimetry.

[b] Limit of detection.

[c] Coefficient of determination.

[d] Percentage relative standard deviation.

[e] References are sorted by date, oldest first.

*Hydroxynaphthol blue disodium salt/cetyltrimethylammonium bromide.

# Chapter V

## Conclusions and Recommendations

The first study extends the applicability of smartphone digital image colorimetry (SDIC) to more complex food samples like milk and dairy products by combining it with deep eutectic solvent-liquid–liquid microextraction (DES-LLME) and using a monochromatic light source. This resulted in further enhancements to SDIC's selectivity, sensitivity and linearity. Superior advantages of DES, such as low cost, biodegradability, and biocompatibility, as well as ease of preparation, and those of SDIC, such as low dependency on electricity, cost-effectiveness, and low level of expertise requirement, make the proposed method a good alternative, particularly for low-income laboratories and those in developing countries. Other manifestations of its usefulness include the method's adaptability to liquid, solid, and semi-solid samples with minimal modification of the procedure and compatibility of the final extract with the detection system. With the rapid advancement of technology, especially high-resolution smartphone cameras and software, it is expected that the analytical performance of SDIC will be improved even further for the determination of other metal ions and molecular analytes.

In the second study, a method called "edible oil switchable-hydrophilicity solvent liquid–liquid microextraction" was suggested for the determination of lead from food samples as it combined with flame-atomic absorption spectrometry. Using edible oils as switchable-hydrophilicity solvents has many benefits, like being easy to use, biodegradable, cheap, renewable, long-lasting, and green. In comparison to other recently published methods, the proposed method eliminates the use of conventional organic solvents and reduces the amount of acids used while maintaining comparable analytical performance. Due to these benefits, the proposed method could be used to get a wide range of molecular and elemental analytes out of different matrices.

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# CURRICULUM VITAE

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- Place of Birth: Zarogi
- Local Government: Tsanyawa
- State of Origin: Kano
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**Institutions Attended**

- Fatih University, Istanbul 2012– 2014
- Bayero University, Kano 2002 – 2005
- Kano State College of Arts, Science and Remedial Studies (CAS) 2000 – 2001
- CAS Staff School 1998 – 2000
- GSS, Danbatta 1992– 1997
- Batta Primary School, Danbatta 1986 – 1991

**Educational Qualifications**

- M.Sc. Chemistry 2014
- B.Sc. Chemistry (Upper Second-Class Honors) 2005
- I.J.M.B.E Certificate 2002
- NECO Certificate 2000
- WAEC Certificate 1997
- Primary School Certificate 1991

**Membership**

- Member Institute of Chartered Chemist of Nigeria (ICCON)
- Member Student Chemical Society of Nigeria (SCSN)

**Experiences/**

- Organic compounds synthesis & characterization

**Skills**

- Kano State College of Education and Preliminary Studies, (KASCEPS Kano). 2007 to date
- Education Officer at Girls Science College, Garko. 2007
- Students Industrial Work Experience Scheme (SIWES) at Aminu Kano Teaching Hospital, Kano. 2003
- NYSC Scheme: Served at Girls' High School Umunkolo, Abia State. 2006 – 2007

**Hobbies**

- Reading and traveling

**Conferences/ Workshops**

- S. Ismail, **A. B. Abdullahi**, U. Alshana, Edible oil-based switchable-hydrophilicity solvent liquid–liquid microextraction prior to smartphone digital image colorimetry for the determination of total curcuminoids in food samples. IMA-2023- International Conference on InstrumentalMethods of Analysis: Modern Trends and Applications (Chania, Crete, Greece: 17–20 September 2023).

- **Aliyu B. Abdullahi**, Salihu Ismail, Usama Alshana, Deep eutectic solvent-liquid–liquid microextraction prior to smartphone digital image colorimetry for the determination of cobalt in milk and dairy products, 4th ICABC 2022.

- Salihu Ismail, **Aliyu B. Abdullahi**, Usama Alshana Switchable-hydrophilicity solvent liquid–liquid microextraction combined with smartphone digital image colorimetry for the determination of palladium in catalytic converters, 4th ICABC 2022.

- **Aliyu B. Abdullahi**, Chemical Society of Nigeria (Chemsearch conference 2009).

- **Aliyu B. Abdullahi,** 1st IJMB Training Workshop (Federal University, Gashua) 2015

**Publications**

- **Aliyu B. Abdullahi**, Salihu Ismail, Usama Alshana, Nusret Ertaş (2023) Smartphone digital image colorimetry combined with deep eutectic solvent liquid–liquid microextraction for the determination of cobalt in milk and dairy products, 119 (2023) 105263.

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- **Aliyu B. Abdullahi**, Iliyasu, S.M, Atiku, U.A, (2017) Synthesis of Electrically Conducting Organic Polymer. Kano State College of Arts and Science Journal of Academic Research, 1(1): 99 – 104.

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- Gumel, B.U, Gumel, S.M, **Aliyu B. Abdullahi**, (2017) Salt and Alkali Free Vat-Dyeing of Grey Cotton Fabric as an Environment Friendly Route of Improving Local Dyeing. Accepted paper in the 3rd Annual International Conference, Northwest University, Kano.

**Committees/ Responsibilities**

- Member IJMB mid-session examination 2008 - 2009
- Examination officer, School of Sciences 2014 - 2015
- Member Result verification committee 2015 - 2017
- Member examination irregularities/malpractice 2015 - 2017
  CEC examination officer/Secretary, examination standing committee, 2015 – 2017.

**Referees**

- Assist. Prof. Dr. Usama Alshana
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