NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF ANALYTICAL CHEMISTRY

LIQUID–LIQUID MICROEXTRACTION PRIOR TO SMARTPHONE DIGITAL IMAGE COLORIMETRY AND UV/VIS FOR THE DETERMINATION OF COPPER, CURCUMIN, AND SUDAN I DYE

DOCTORATE THESIS

Mais Al-Nidawi

Nicosia
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APPROVAL

We specify that we have read the thesis submitted by Mais Al-Nidawi titled “**Liquid–Liquid Microextraction Prior to Smartphone Digital Image Colorimetry and UV/Vis for the Determination of Copper, Curcumin, and Sudan I Dye**” 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 information, documents, analysis, and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of the Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Mais Al-Nidawi
30/06/2022

Acknowledgements

I would like to convey my heartfelt appreciation and gratitude to my supervisor Assist. Prof. Dr. Usama Alshana. As the quote says, "A journey of a thousand miles starts with a single step". You guided me, trained me, taught me and provided me with knowledge at every step of this journey. I am grateful for your efforts and for all you have done.

I also want to extend my sincere thanks to my co-supervisor Prof. Dr. Mustafa Soylak, for giving me the opportunity to work in his laboratory with his research group and to learn from his knowledge.

My profound gratitude goes to my monitoring committee and jury members Prof. Dr. Nusret Ertaş and Assist. Prof. Dr. Hürmüs Refiker for their informative comments and recommendations that were useful to complete this thesis. I am also grateful to Prof. Dr. H. Eda Şatana Kara for accepting our invitation to be a jury member.

I would like to express my gratitude to the Head of the Department, Assist. Prof. Dr. Selin Işık for her consistent support and assistance, for translating my English abstract into Turkish and for being a part of the jury.

I would like to thank Near East University for providing me with the Dr. Suat Günsel Lecturer Training Program scholarship that enabled me to achieve a Ph.D. study while acquiring experience in teaching and administrative duties.

I would like to express my gratitude to the Dean Prof. Dr. İhsan Çalış, Vice-Dean Assist. Prof. Dr. Azmi Hanoğlu, Head of the Institute of Graduate Studies Prof. Dr. Kemal Hüsnü Can Başer and to my colleagues from the academic staff at the Faculty of Pharmacy for their motivation and support throughout this work.

I would like to thank the Alshana Research Group for creating a family-like environment and being there for each other in both good and bad times. Additionally, I want to thank my partner and closest friend Dr. Jude Caleb for assisting me, inspiring me and listening to me whenever I felt down, which helped me battle the stress and problems during the research.

My family and my friends I do not know how to thank you. I consider myself very fortunate to have you in my life. I appreciate your trust and belief in me. You supported me whenever I needed it the most, especially my father and mother. I will be eternally thankful to you for allowing me to pursue my goal. You never stopped assisting, supporting and praying for me. You were always proud of me. I adore and admire you all.

Abstract

Liquid–Liquid Microextraction Prior to Smartphone Digital Image Colorimetry and UV/Vis for the Determination of Copper, Curcumin and Sudan I Dye

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June 2022, 117 pages

In this work, three liquid–liquid microextraction (LLME) methods were combined with smartphone digital image colorimetry (SDIC) and ultraviolet/visible spectrophotometry (UV/Vis) for the analysis of some food samples. In the first study, reversed-phase switchable-hydrophilicity solvent liquid–liquid microextraction (RP-SHS-LLME) was combined with SDIC for the determination of copper in edible oils. To get reproducible images of the colored samples, a colorimetric box was designed and the performance of both the RP-SHS-LLME and the SDIC was improved by optimizing the factors affecting their performance at a wavelength of 400.0 nm, which include the type and volume of the switchable-hydrophilicity solvent (SHS, TEA, 80 µL), concentration and volume of nitric acid (7.5 mol L^{-1}, 140 µL), complexation pH (5.50), back-extraction solvent (1-DO) and volume of *N*,*N*-diethyl-*N'*-benzoylthiourea as a complexing agent (DEBT, 200 µL). The limit of detection (LOD) was found as 0.30 µg mL^{-1}. Good linearity was obtained with coefficients of determination (R^2) above 0.9963, percentage relative recoveries (%RR) of 92.5–105.0%, and percentage relative standard deviations (%RSD) of less than 6.9%. In the second study, synergistic cloud-point extraction (SCPME) was combined with UV/Vis for the determination of curcumin in tea and spices. Using alcohol as an additive reduced the cloud-point temperature of the surfactant below room temperature without the addition of a salt or heating, thus, reducing the extraction time. Curcumin was extracted from the food samples using SCPME with 1-pentanol, as a synergic reagent and Triton X-405, as a non-ionic surfactant, prior to its determination using UV/Vis. The optimum SCPME conditions were achieved using 500 µL of Triton X-405 and 600 µL of 1-pentanol in 10.0 mL of the sample solution. Curcumin was monitored at a wavelength of 430.0 nm. LODs were found within the range of 0.012–0.027 µg mL^{-1}, %RR ranged between 90.6 and 105.4%, R^2 of the calibration graphs was higher than 0.9974 and %RSD was below 7.5%. In the third study, switchable-hydrophilicity solvent liquid–liquid microextraction (SHS-LLME) was combined with UV/Vis spectrophotometry for the determination of Sudan I dye in spices. Sudan I dye was monitored at a wavelength of 445.0 nm using 600 µL of octylamine as the SHS and 600 µL of 10.0 M sodium hydroxide as a phase-separation trigger in 20.0 mL of the sample solution. LOD was found within the range of 0.009–0.021 µg mL^{-1}. %RR ranged between 91.0 and 104.4%, R^2 was higher than 0.9983 and %RSD was below 7.7%.

Keywords: Food, liquid–liquid microextraction, smartphone digital image colorimetry, switchable-hydrophilicity solvent, synergistic cloud-point extraction, UV/Vis.

Öz

Bakır, Kurkumin ve Sudan I Boyasının Tayini için Akıllı Telefon Dijital Görüntü Kolorimetrisi ve UV/Vis Öncesi Sıvı-Sıvı Mikroekstraksiyonu

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Haziran 2022, 117 sayfa

Bu çalışmada, gıda numunelerin analizinde kullanılmak üzere üç sıvı-sıvı mikroekstraksiyon (LLME) yöntemi, akıllı telefon dijital görüntü kolorimetrisi (SDIC) ve UV/Vis spektrofotometri ile birleştirilmiştir. İlk çalışmada, ters-fazlı değiştirilebilir-hidrofilik çözücü sıvı–sıvı mikro mikroekstraksiyonu (RP-SHS-LLME) SDIC ile birleştirerek yemeklik yağlardaki bakır derişimi belirlenmiştir. Renkli örneklerin tekrar üretilebilir görüntülerini elde etmek için bir kolorimetrik kutu tasarlanmıştır ve performanslarını etkileyen faktörler optimize edilerek hem RP-SHS-LLME hem de SDIC'nin performansı geliştirilmiştir. Değiştirilebilir-hidrofilik çözücü (SHS) tipi ve hacmi (TEA, 80 µL), nitrik asit konsantrasyonu ve hacmi (7.5 mol L^{-1}, 140 µL), kompleksleşme pH'sı (5.50), geri-ekstraksiyon çözücüsü (1-DO) ile kompleksleştirici olarak kullanılan *N,N*-dietil-*N'*-benzoiltiyoüre (DEBT, 200 µL), RP-SHS-LLME için optimum koşul olarak belirlenmiştir. Saptama sınırı (LOD) 0,30 µg mL^{-1} olarak hesaplanmıştır. 0,9963'ün üzerinde olan belirleme katsayıları (R^2) ile doğrusallık iyi olarak tayin edilmiştir. Yöntem, %92,5–105,0 oranında bağıl geri kazanım (%RR) ve %6,9'dan az bir bağıl standart sapma yüzdesi (%RSD) ile yenilebilir yağlardaki bakır miktarını belirlemek için kullanılmıştır. İkinci çalışmada ise, çay ve baharatlarda kurkumin tayini için sinerjistik bulut-noktası ekstraksiyon (SCPME) ile UV/Vis yöntemleri birleştirilmiştir. Katkı maddesi olarak alkol kullanımı, tuz ilavesi veya ısıtma olmaksızın yüzey aktif maddenin bulutlanma noktası sıcaklığını oda sıcaklığının altına düşürülmüş. Optimum SCPME koşulları, 500 µL Triton X-405 ve 10,0 mL numune çözeltisi içinde 600 µL 1-pentanol ile 445,0 nm dalga boyunda elde edilmiştir. LOD 0,012–0,027 µg mL^{-1} aralığında bulunmuştur. R^2 0,9974'ten yüksek ve %RSD %7,5'in altında bulunmuştur. Üçüncü çalışmada, baharatlarda Sudan I boyasının belirlenmesi için değiştirilebilir-hidrofilik çözücü sıvı–sıvı mikroekstraksiyon (SHS-LLME) yöntemi ile UV/Vis birleştirilerek kullanılmıştır. SHS-LLME için optimum koşullar, 20.0 mL numune çözeltisinde 600 µL oktilamin ve 600 µL, 10,0 M’lık sodium hidroksit kullanılarak, 430,0 nm dalga boyunda elde edilmiştir. LOD değerleri 0,009–0,021 µg mL^{-1} aralığında bulunmuştur. R^2 0,9983'ten yüksek ve %RSD %7,7'nin altında olarak tayin edilmiştir.

Anahtar kelimeler: Akıllı telefon dijital görüntü kolorimetrisi, değiştirilebilir hidrofilik çözücü, gıda, sinerjistik bulut-noktası mikroektraksiyonu, sıvı-sıvı mikroektraksiyon, UV/Vis spektrofotometri.

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List of Abbreviations

Abbreviation	**Definition**
%RR	Percentage relative recoveries
%RSD	Percentage relative standard deviation
1-DO	1-Dodecanol
1-UN	1-Undecanol
AA-HLLME	Aeration-assisted homogeneous liquid–liquid microextraction
AA-LLME	Air-assisted liquid–liquid microextraction
ACN	Acetonitrile
ANOVA	Analysis of variance
CE-AD	Capillary electrophoresis-amperometric detection
CHA	Cyclohexylamine
CMC	Critical micelle concentration
CMYK	Cyan, magenta, yellow and key (black)
CP	Cloud-point
CPE	Cloud-point extraction
CPT	Cloud-point temperature
DEBT	*N*,*N*-diethyl-*N’*-benzoylthiourea
DES	Deep eutectic solvent
DESME	Deep eutectic solvent microextraction
DI	Deionized
DLLME	Dispersive liquid–liquid microextraction
DLPME	Dispersive liquid-phase microextraction
DMBA	Dimethylbutylamine
DMBenA	Dimethylbenzylamine
DMCHA	*N*,*N*-dimethylcyclohexylamine
DSSBME	Dual solvent-stir bar microextraction
EE-SPME	Electrosorption-enhanced solid-phase microextraction
EF	Enrichment factor
EtOH	Ethanol

Abbreviation	**Definition**
FAAS	Flame-atomic absorption spectrometry
FDA	Food and Drug Administration
GFAAS	Graphite furnace-atomic absorption spectrometry
GRAS	Generally Recognized As Safe
HF	Hollow-fiber
HF-LPME	Hollow-fiber liquid-phase microextraction
HLLME	Homogeneous liquid–liquid microextraction
HPLC	High-performance liquid chromatography
HPLC-DAD	High-performance liquid chromatography with diode array detection
HPTLC	High-performance thin-layer chromatography
HR-CS	High-resolution continuum source
HSV	Hue, saturation and value (brightness)
ICP-MS	Inductively coupled plasma-mass spectrometry
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LDR	Linear dynamic range
LED	Light-emitting diode
LLE	Liquid–liquid extraction
LLME	Liquid–liquid microextraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid-phase microextraction
MAE-DLLME	Microwave-assisted dispersive liquid–liquid microextraction
MEE	Microemulsion extraction
MeOH	Methanol
MRL	Maximum residue limit
MR-SHS	Magnetic retrieval-switchable-hydrophilicity solvent
MS	Mass spectrometry

Abbreviation	**Definition**
MSPE	Magnetic solid-phase extraction
MW	Microwave digestion
NS	Non-ionic surfactant
OCA	Octylamine
OPP	Organophosphorus pesticide
POE	Polyoxyethylated
POP	Persistent organic pollutant
RGB	Red, green and blue
ROI	Region of interest
RP-SHS	Reversed-phase switchable-hydrophilicity solvent
RP-UALLME	Reversed-phase ultrasonic assisted-liquid–liquid microextraction
SCPME	Synergic-cloud-point microextraction
SDIC	Smartphone digital image colorimetry
SDME	Single-drop microextraction
SE-HF-LPME	Surfactant-enhanced hollow-fiber liquid-phase microextraction
SFOD	Solidification of floating organic drop
SHDS	Switchable-hydrophilicity dispersive solvent
SHS	Switchable-hydrophilicity solvent
SMS	Supramolecular solvent
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SS-LLME	Switchable solvent-based liquid-phase microextraction
SUPRAS	Supramolecular solvent
TEA	Triethylamine
THF	Tetrahydrofuran
UA-IL	Ultrasound-assisted ionic liquid
US	Ultrasound

Abbreviation	**Definition**
USA-TIL-DLPME	Ultrasound-assisted temperature-controlled ionic liquid dispersive liquid-phase microextraction
UV/Vis	Ultraviolet/visible spectrophotometry
VA-LLME	Vortex-assisted liquid–liquid microextraction
WHO	World Health Organization

CHAPTER I

Introduction

Sample preparation is a series of different steps and processes. Because of this, it is the most time-consuming part of developing an analytical method and a major cause of mistakes in analysis. In most cases, sample preparation refers to the separation and/or (on-line or off-line) preconcentration of some components of interest or target analytes from various matrices, thereby making detection of the analyte more suitable. This is achieved through a series of steps, such as separation and pre-concentration, the removal of interferences, and, if needed, derivatization, which changes the analyte into a more useful form (Sajid, 2017). It is apparent that the different things that go into sample preparation have a direct effect on the figure of merits of the analytical method, such as precision, accuracy, detection limit (LOD), etc. The field of sample preparation has grown quickly since there is a continuous need for accurate and precise measurements of analytes at trace or ultratrace levels in samples of various matrices. Numerous "green" sample preparation techniques have been included into analytical practice (Plotka-Wasylka, Szczepanska, de la Guardia, & Namiesnik, 2015). One of them is to reduce or eliminate the number of solvents and reagents used in the analysis. Otherwise, solvent recovery and reuse are strongly advised.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are two of the most well-known conventional extraction techniques used in chemical analysis. However, in recent years, there has been an exponential increase in the development of modern methods based on miniaturized sample preparation approaches (Maciel, de Toffoli, & Lancas, 2018). In general, these are based on the previously mentioned traditional ones. As a result, they can be divided into those based on sorbents that are SPE derivatives and those that use a solvent as an extraction medium based on LLE. Consequently, the current scenario highlights the rise of innovative approaches for sample preparation procedures, emphasizing their principles based on the context of green chemistry. According to this environmentally friendly concept, analytical methods should focus on reducing the volumes of non-hazardous solvents used (Jedrkiewicz, Glowacz, Kupska, Gromadzka, & Namiesnik, 2014; Maciel et al., 2018).

Liquid–Liquid Microextraction

Liquid–liquid microextraction (LLME) is commonly defined as a miniaturized version of traditional LLE in which the extractant phase volume is limited to a few microliters. In 1996, the first report appeared on miniaturized modes of LLE. Liu et al. (Liu & Dasgupta, 1996) proposed using liquid droplets as a sampling interface. A water droplet suspended in a silica capillary tube was used to extract ammonia and sulfur dioxide from an air stream. Simultaneously, but independently, Jeannot and Cantwell (Jeannot & Cantwell, 1996) introduced single-drop microextraction (SDME) as a miniaturized sample preparation method prior to chromatographic analysis of organic compounds. Since then, various modes of LLME have been developed as strategic alternatives for simplifying experimental procedures, aiming at increasing selectivity, improving analyte uptake, and allowing the extraction of analytes with a wide range of polarities (Carasek, Merib, Mafra, & Spudeit, 2018). Several methods have been reported in recent years that use novel extraction solvents such as dispersive liquid–liquid microextraction (DLLME) (Rezaee, Assadi, Hosseinia, Aghaee, Ahmadi, & Berijani, 2006), hollow-fiber liquid-phase microextraction (HF-LPME) (Shen & Lee, 2002), switchable-hydrophilicity solvent liquid–liquid microextraction (SHS-LLME) (Lasarte-Aragones, Lucena, Cardenas, & Valcarcel, 2015), supramolecular solvent-liquid–liquid microextraction (SMS-LLME) (Moral, Sicilia, & Rubio, 2009), and deep eutectic solvent-liquid–liquid microextraction (DES-LLME) (Karimi, Dadfarnia, Shabani, Tamaddon, & Azadi, 2015).

It is challenging to transfer the analyte efficiently from an aqueous sample to a small amount of the extraction solvent. Equilibrium and kinetic parameters, as well as temperature, pH, agitation type/rate, ionic strength, and sample/solvent volume ratio, etc. must all be taken into account. An appropriate extraction solvent should be water-immiscible, capable of achieving high enrichment factors, and compatible with the instrumental method utilized. Generally, the extraction solvent is chosen using the "like dissolves like" concept. Usually, the octanol/water distribution coefficient (k) is used as a reference to estimate polarity as defined in **Equation 1**.

$$logP = logk = \frac{[Analyte]_{octanol}}{[Analyte]_{water}} \qquad \textbf{(Equation 1)}$$

The distribution ratio between the two phases is affected by many parameters, such as the pH of the aqueous phase, the extraction solvent, and the volume ratio of the organic phase to the aqueous phase. Optimizing the extraction conditions is required to ensure that the analyte is distributed preferentially into the organic phase. When poor analyte recovery is achieved, multiple sample extractions are performed until acceptable recoveries are obtained. Alternatively, a significant excess of the extraction solvent is utilized to save time. Additionally, other parameters such as ultrasound, microwave, air, and electrical potential, etc., may be utilized to speed up the extraction process and increase recovery.

Supporting the Extraction Process

Ultrasound

Ultrasound (US) is just a mechanical sound wave that can't be heard. Its frequencies are between 16 kHz and 500 MHz, which is above what humans can hear but below what microwaves can do. Ultrasonic waves may travel through an elastic media such as liquid. Fundamental US radiation parameters are frequency (Hz), power (W), and intensity (W/m^2). Based on this, three ultrasonic ranges may be distinguished. Because of the “cavitation phenomena”, In various sectors, ultrasonic assist has become a prominent augmentation component. Fine droplets occur when the US is inserted into a liquid media. Inside these droplets, highly particular physical conditions are seen when the temperature and pressure are raised to around 5000 K and 2000 atm, respectively. A large amount of energy is released when the cavitation droplet collapses resulting in an increase in mass transfer. This improves the dispersion of the droplets of the extraction solvent as well as a significant reduction in time and organic solvent consumption is observed. US is often used in sample preparation (e.g., leaching, liquid–liquid extraction, derivatization, and microextraction). In 2012, ultrasound-assisted temperature-controlled ionic liquid dispersive liquid-phase microextraction (USA-TIL-DLME) was utilized for the first time to assess inorganic cobalt species in nutritional supplements (Seidi & Yamini, 2012).

Air

Air can also be used as an additional factor to aid in the extraction process. This is a low-cost, environmentally friendly medium that can significantly improve the extraction efficiency. The mixture is pulled into a syringe with a little amount of air and pushed out into a tube with a set number of rounds, creating a turbid solution with the extract dispersed as tiny droplets in the aqueous phase. The organic phase that settled out is used in the next step of the analysis, which is sped up by centrifuging. As a result, a small volume of an organic solvent is required as the extraction solvent, and no disperser solvent is required. Many different well methods have been used to facilitate the determination of various organic and inorganic analytes, such as extraction of organophosphorus pesticides (OPPs) from juice samples using the air-assisted liquid–liquid microextraction (AA-LLME) (Farajzadeh, Djozan, Nouri, Bamorowat, & Shalamzari, 2010).

Microwave

Numerous sample preparations can be conducted using microwaves. Microwaves heat the sample from the inside, resulting in enhanced mass transfer and component diffusion. The main advantages of using microwaves as an extraction assist include quick transfer of energy, targeted heating, faster throughputs, rapid on/off switching, environmentally friendly nature at the point of use, compact equipment, reduced solvent use, and rapid extraction efficiency with a maximum yield. Over the last decade, microwave radiation has been intensely studied for the extraction of a broad range of analytes from diverse samples, and novel analytical techniques have been created. Microwave-assisted dispersive liquid–liquid microextraction (MAE-DLLME) was efficiently employed for the determination of traces of polyamines in turkey breast meat samples (Bashiry, Mohammadi, Hosseini, Kamankesh, Aeenehvand, & Mohammadi, 2016).

Surfactants

Surfactants, also known as surface-active agents, are amphiphilic organic compounds with both hydrophobic and hydrophilic groups. Surfactants are soluble in both organic solvents and water. Surfactants lower the surface tension of water by adsorbing at the liquid-gas interface and lower the interfacial tension of oil and water by adsorbing at the liquid–liquid interface. Many surfactants can also form aggregates in bulk solutions (for example, vesicles and micelles). The critical micelle concentration (CMC) is the concentration of surfactant above which micelles form spontaneously. In analytical chemistry, surfactants are often utilized in extraction and separation procedures. Probably the earliest surfactant-based extraction technique is cloud-point extraction (CPE), which employs a minimal volume of surfactant-rich phase to extract and preconcentrate analytes in a single step. Surfactants have been used in liquid-phase microextraction methods, such as LPME and DLLME, more recently. In 2005, surfactant-enhanced hollow-fiber liquid-phase microextraction (SE-HF-LPME) was used for preconcentration of basic drugs of abuse in hair. To transfer target analytes from the aqueous sample to the donor phase, a non-ionic surfactant was utilized. The authors used one of the most critical aspects of surfactants, their ability to solubilize a wide variety of solutes with varying characteristics and nature (Yazdi & Es'haghi, 2005).

Vortex and stirring

Vortex and stirring are two other elements that contribute to analyte extraction. The main benefit of mixing is the capacity to attain high analyte enrichment factors without using additional parameters, such as temperature, pressure, or ultrasonic radiation, which may, in certain instances, result in analyte deterioration. Furthermore, the vortex-mixed dispersion is thermodynamically unstable, which means that the extraction phase, containing the target analyte, can be easily separated. Moreover, the process is much cheaper than ultrasonication (Ojeda & Rojas, 2014). All of these advantages have increased chemical analysts' interest in employing this tool during the preparation step. As a consequence, several novel approaches have been used such as vortex-assisted liquid–liquid microextraction (VA-LLME) that was introduced to address the shortcomings of the DLLME. In 2010, VA-LLME was used, for the first time, to extract and enrich trace amounts of octylphenol, nonylphenol, and bisphenol A from water and wastewater samples (Ojeda et al., 2014). Using vortex agitation, an organic solvent was disseminated into an aqueous sample to generate a moderate emulsion. Due to their increased specific surface area and shorter diffusion distance, the small micro droplets created by this technique may speed the mass transfer of the desired analytes into the organic acceptor phase. Commonly, centrifugation is used to separate the two resultant stages.

Electrical potential

In the literature for the first time in 1994-1996, the use of an electric field to boost extraction process efficiency and speed was described (Huang, Seip, Gjelstad, & Pedersen-Bjergaard, 2015). Since then, a number of new techniques have been created as a consequence of the tool's many benefits. The electrosorption-enhanced solid-phase microextraction (EE-SPME) technique is a combination of electrosorption and the SPME technique that may be used to extract ionized analytes from aqueous samples. This approach removes difficulties like derivatization, acidification, and alkalinization and combines cleaning and preconcentration in a single phase. The extraction method for EE-SPME relies on the passage of counter ions into and out of the conductive polymer (e.g., polypyrrole) film to maintain charge equilibrium (J. C. Wu, Mullett, & Pawliszyn, 2002).

Food analysis

Food analysis is necessary for a variety of purposes. The most obvious of these are to ensure safety, ingredient quality, and nutritional content of commercial food items. These characteristics are critical for the customer and for adhering to federal food standards organizations' requirements. Food analysis involves the identification of a wide variety of food constituents, extending from inorganic ions to proteins. Additionally, these constituents may exist at trace level in a complicated sample matrix. National governments and supranational organizations such as the European Union (Cacciola, Dugo, & Mondello, 2017) set maximum residue limits (MRL) for hazardous substances to keep people from being exposed to them, make trade easier, and improve the health of the consumer (Maciel et al., 2018). The majority of regulatory standards are created only when analytical methodologies have been verified in interlaboratory trials or shown to meet specific analytical requirements. As a consequence, it is important to develop analytical procedures that are effective, robust, quick, and simple in order for them to be applied in food analysis.

Copper

Copper is the most crucial element for all living beings. A minute quantity of approximately 40.0 ng mL^{-1} is necessary for lipid and carbohydrate metabolism as well as to regulate heart and blood vessel activity (T. Li & Yang, 2015). It is important for the human body as a cofactor for many important enzymes and helps the body to get rid of iron through ceruloplasmin (M. Soylak, Elci, & Dogan, 2001). It has both vital and harmful properties. Deficiency of copper in the body can cause anemia and arteriosclerosis, an excess of the element, on the other hand, can cause diseases like Alzheimer's and Parkinson's (Zhang et al., 2014). Several industries, including paper, petroleum, dyeing, copper-ammonium rayon, and copper/brass plating, are major sources of copper in the environment. The unwanted quantity of copper discharged into the environment by these enterprises is detrimental to water plants and marine creatures such as fish and invertebrates, and is eventually incorporated into the food chain via bioaccumulation (Elekes, Busuioc, & Ionita, 2010). Overexposure causes a variety of disorders, including nausea, diarrhea, vomiting, gastrointestinal hemorrhage, hepatic neurosis, dermatitis, and hypertension (Mustafa Soylak & Do° an, 1996). The World Health Organization (WHO) has set the required amount of copper at 50 $\mu g\ kg^{-1}$ of body weight per day for infants and 12.5 $\mu g\ kg^{-1}$ of body weight per day for adults, while the Institute of Medicine (IOM) has proposed 10 $mg\ day^{-1}$ as a tolerable upper intake level (UL) for adults from food and supplements. The Food and Agriculture Organization (FAO) suggested that the concentration of copper in edible fats and oils should not exceed 0.1 $\mu g\ g^{-1}$ for refined fats and oils and 0.4 $\mu g\ g^{-1}$ for virgin or cold-pressed fats and oils [Food (Amendment) Regulations, 2019]. Copper is found in water, biological and environmental materials at trace levels. Because of these consequences on the human health and the environment, analytical chemists are urged to develop sensitive, reliable and rapid methods for the determination copper. Copper has been determined by flame-atomic absorption spectrometry (FAAS) (Ozkantar, Yilmaz, Soylak, & Tuzen, 2020), graphite furnace-atomic absorption spectrometry (GFAAS) (Gamela et al., 2017), inductively coupled plasma-mass spectrometry (ICP-MS) (Martin-Camean et al., 2015), and UV/Vis spectrophotometry (Sabel, Neureuther, & Siemann, 2010), and others.

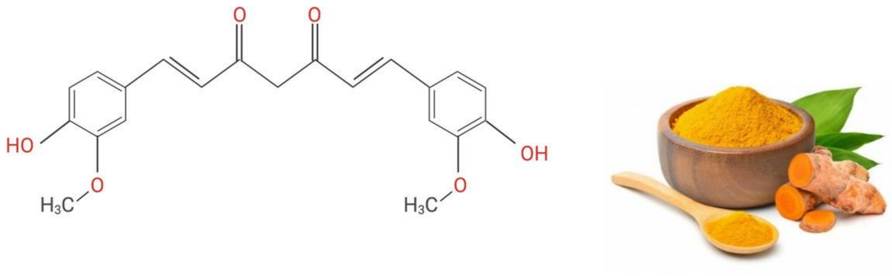
Curcumin

Curcumin is derived from the rhizomes of turmeric (*Curcuma longa* L.), which belongs to the ginger family, Zingiberaceae (Hwang, Son, Jo, Kim, Seong, & Moon, 2016). It is native to Eastern India and is known as the "Queen of Spices." It is also known as the "Indian solid gold" because of its bright yellow color that is due to the presence of polyphenolic curcuminoids, including curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%) (Mehanny, Hathout, Geneidi, & Mansour, 2016). The chemical structure of curcumin is given in **Figure 1**. The Food and Drug Administration (FDA) has declared curcumin to be "Generally Recognized as Safe (GRAS)" (M Yallapu, Jaggi, & C Chauhan, 2013), and as a result, numerous studies have confirmed the safety of curcumin dosages as high as 12.0 $g\ day^{-}$

^{1} for three months (Chainani-Wu, 2003). Curcumin, the major bioactive component of curcuminoids, has garnered serious attention in the last few years. It has a long history of use in herbal remedies, particularly in China, India, and Indonesia. It has been used in the treatment and prevention of numerous diseases, including Alzheimer's, diabetes, cancer, allergies, and other chronic ones due to its antioxidant, anti-inflammatory, anti-microbial, anti-parasitic, and anti-cancer properties (Moghadamtousi, Kadir, Hassandarvish, Tajik, Abubakar, & Zandi, 2014; Ramalingam & Ko, 2014). A number of studies on the determination of curcumin in various matrices have been reported, including spectrofluorometric (Jasim & Ali, 1992), high-performance thin-layer chromatography (HPTLC) (Paramasivam, Poi, Banerjee, & Bandyopadhyay, 2009), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (W. Z. Ma, Wang, Gun, & Tu, 2015), high-performance liquid chromatography (HPLC) (J. Li, Jiang, Wen, Fan, Wu, & Zhang, 2009), and others.

Figure 1.

Chemical Structure of Curcumin.

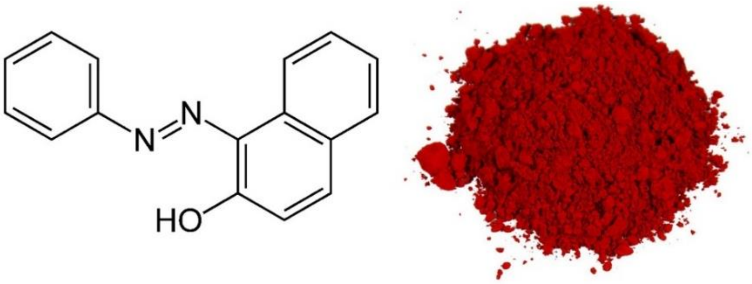


Sudan I dye

Sudan I (or 1-[(2,4-dimethylphenyl)azo]-2-naphthalenol) is one of the synthetic lipophilic red azo (-N=N-) dyes, which has aromatic rings in its structure as shown in **Figure 2**. It is used as a coloring agent in different industries, including spices, oils, medicine, colorful waxes, food, paper, printing inks, and clothes etc. due to its low price and bright color (Ghasemi & Kaykhaii, 2016). Excessive use of illegal dyes in foodstuff has countless negative effects on the human health (Rao, Bhat, Sudershan, Krishna, & Naidu, 2004). The Federal Risk Assessment Institute announced in 2003 that the European Union would not allow Sudan I-IV azo dyes to be used as food additives due to their carcinogenic effect. They have been classified as carcinogenic by the International Agency for Cancer Research due to their ability to degrade into amines after entering into the human body, and as a result, when their concentration exceeds 0.5-1.0 mg kg^{-1} in food, they should be removed from the market (Ozak & Yilmaz, 2020). Unfortunately, in some countries, these dyes are still used as additives in some foodstuff to improve the color for commercial purposes. Based on this, simple, fast, selective and sensitive analytical methods are needed for the determination of Sudan dyes in food products. many methods have been proposed for the determination of these synthetic dyes in food products including liquid chromatography-mass spectrometry (LC-MS) (Guerra, Celeiro, Lamas, Llompart, & Garcia-Jares, 2015), high-performance liquid chromatography diode array detection (HPLC-DAD) (M. Ma, Luo, Chen, Sub, & Yao, 2006), voltammetry (Mo, Zhang, Zhao, Xiao, Guo, & Zeng, 2010), capillary electrophoresis (Mejia, Ding, Mora, & Garcia, 2007), and others.

Figure 2.

Chemical Structure of Sudan I.



Various instrumental techniques have been proposed for the determination of different analytes in food samples such as flame-atomic absorption spectrometry (FAAS) (Ozak et al., 2020), inductively coupled plasma-mass spectrometry (ICP-MS) (Nardi et al., 2009), high-performance liquid chromatography with diode-array detection (HPLC-DAD) (Al-Nidawi et al., 2020), high-performance thin-layer chromatography (HPTLC) (Paramasivam et al., 2009), capillary electrophoresis (Mejia et al., 2007), graphite furnace-atomic absorption spectrometry (GFAAS) (Gamela et al., 2017), UV/Vis spectrophotometry (Hassan, Uzcan, Alshana, & Soylak, 2021), and liquid chromatography-mass spectrometry (LC-MS) (Guerra et al., 2015), among others. However, these instruments require arduous maintenance, highly qualified experts, difficult operations, and high prices. In addition, the final extract is in most cases incompatible with the analytical instrument. Therefore, there is a need to develop innovative alternative analytical techniques that are simple, easily accessible, and have low operating and maintenance costs, which can benefit low-income laboratories and people of developing countries in particular.

Smartphone digital image colorimetry

Digital image colorimetry (DIC) is a simple, quick, and cost-effective alternative to existing quantitative and qualitative analysis approaches that have been investigated in several disciplines (Caleb & Alshana, 2021). Data obtained as digital images with widely available devices such as digital cameras (Firdaus, Alwi, Trinoveldi, Rahayu, Rahmidar, & Warsito, 2014), scanners (Meng, Schultz, Cui, Li, & Yu, 2015), webcams (Urapen & Masawat, 2015) and smartphones (Franco, Suarez, & dos Santos, 2017) are processed to obtained analytical information.

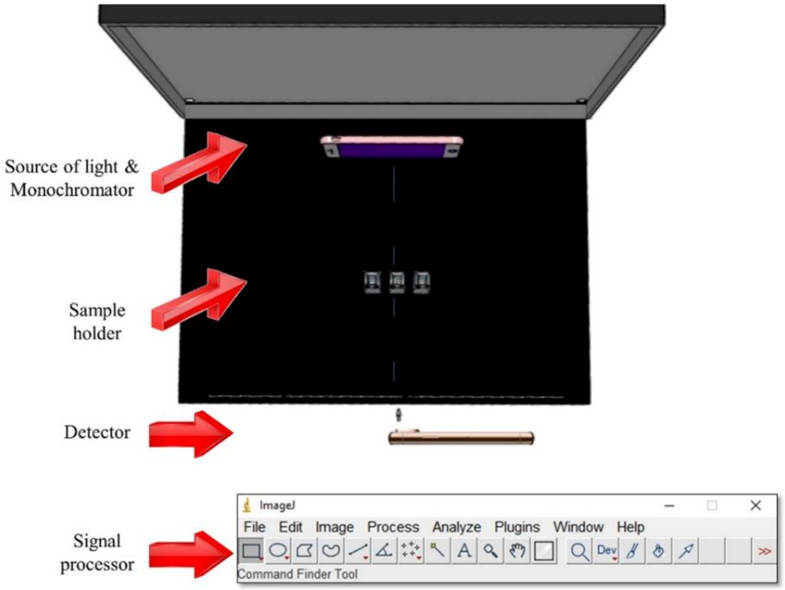
In smartphone digital image colorimetry (SDIC), most of researchers so far used light-emitting diodes (LED) as a white light source to illuminate the sample solution that is placed inside a colorimetric box (Caleb, Alshana, & Ertas, 2021). The technique has been applied for the determination of organic and inorganic analytes in different matrices, including iodate in table salt. (Caleb, Alshana, et al., 2021), ascorbic acid in fruit juices (Porto, Neto, dos Santos, Gomes, & Ferreira, 2019), speciation analysis of iron (II/III) in white wine (Abadi, Ashraf, Chamsaz, & Shemirani, 2012), calcium in water and food samples (Peng et al., 2019), titanium in plastics (Lopez-Molinero, Linan, Sipiera, & Falcon, 2010), copper in river water, rice and lettuce (Khoshmaram, Saadati, & Karimi, 2020). However, the application of a continuous light source in the lack of a monochromator is likely to result in poor selectivity and sensitivity, particularly when dealing with complex matrices. For this reason, the utilization of a monochromatic source with a low-cost monochromator along with an effective sample cleaning and preconcentration procedure for the analyte would be extremely desirable.

Construction of the colorimetric box

A suitable colorimetric box in SDIC would consist of a small hole around the size of the camera's lens drilled at the top or the side of the box to enable capturing images of the colored sample solution contained within a quartz UV/Vis microcuvette positioned at an appropriate distance from the detection camera. The light source, either continuum or monochromatic, is needed to illuminate the sample solution. A smartphone is used as the detector and the images are processed using a suitable software such as ImageJ (Rueden et al., 2017), Matlab (Lopez-Molinero et al., 2010), Adobe Photoshop (W. Li et al., 2016), Color Grab (Lima et al., 2020), among other applications. ImageJ, a product of the National Institute of Health, USA, is the most commonly applied. It can be downloaded for free from https://imagej.nih.gov/ij/download.html. A schematic diagram of an SDIC design is shown in **Figure 3**.

Figure 3.

Schematic Diagram of SDIC.



Optimization of SDIC parameters

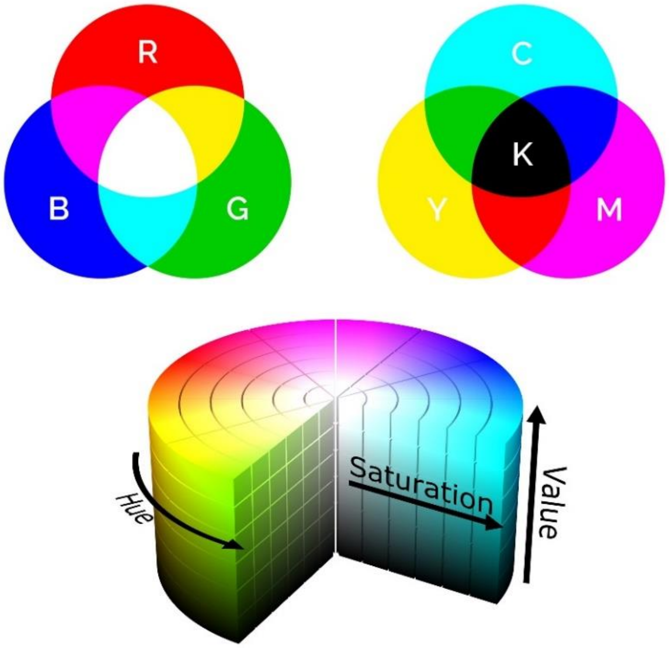
To get the best results from SDIC, you must find the best settings for the RBG channels, the detection wavelength, the location of the sample holder, the area of interest, and the intensity of the light source.

Selection of the RBG channels

Popularization of smartphones went along with the development of software and models for the analysis of colors. For example, red, green, and blue (RGB), cyan, magenta, yellow, and black (CMYK), as well as hue, saturation, and brightness (HSV) (Lima, Nascimento, & Rocha, 2017) are color models used for describing colors as shown in **Figure 4**.

Figure 4.

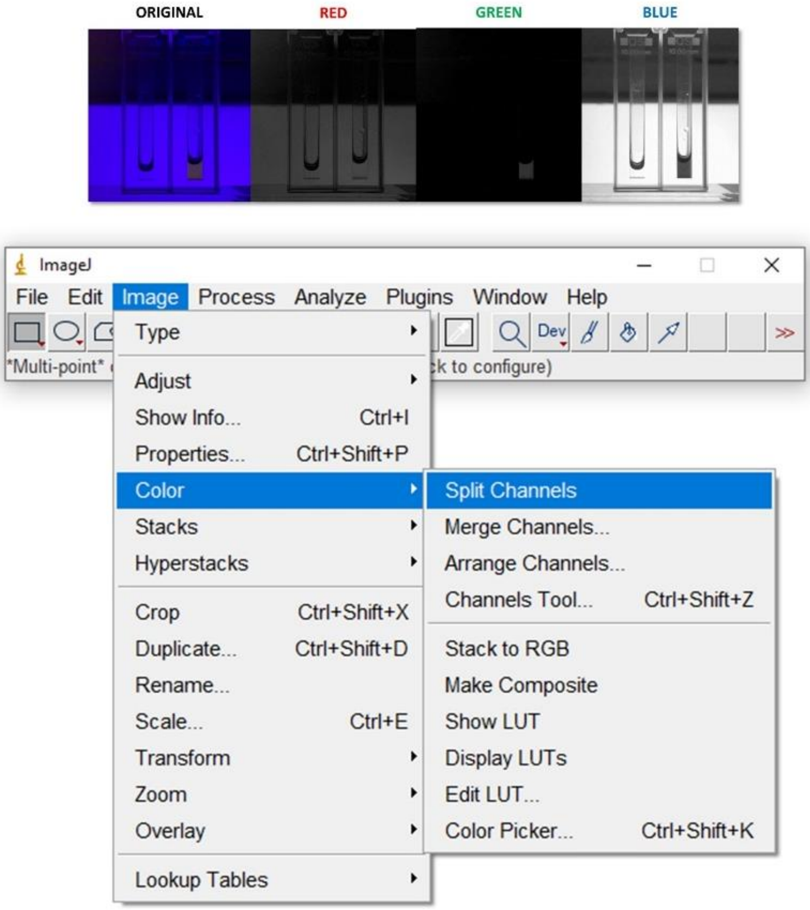
RGB, CMYK, and HSV Color Models.



The intensity and color of a digital image are often represented as 24-bit data (8-bit R + 8-bit G + 8-bit B), which adds another color space and allows for the reproduction of a broad variety of colors through a huge number of combinations of the R, G, and B intensities. Each color has 256 levels of intensity (from 0 to 255), resulting in a total of 16777216 distinct colors (i.e., 256^3 or 2^{24}). Pure black is assigned the values $R = 0$, $G = 0$, and $B = 0$. On the other hand, pure white has the values of R = 255, G = 255, and B = 255 (Choodum & Daeid, 2011). All quantitative data for analysis can be derived from a broad variety of colors (Khoshmaram et al., 2020). Based on the color of the analyte in solution, the intensity of one of the three RGB channels may be directly proportional to its concentration. The ImageJ program, for instance, offers the possibility of splitting a channel into its R, G, and B components. Choosing the best channel is, therefore, based on the intensity of the color in that channel or the difference between the sample solution and the blank (right and left image in each channel, respectively, as shown in **Figure 5**).

Figure 5.

Splitting The Image into its R, G, and B Channels.

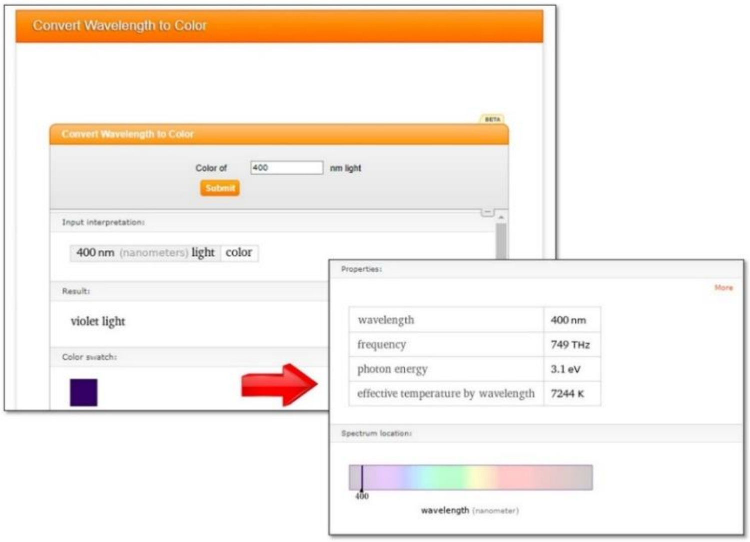


Selection of the detection wavelength

As mentioned earlier, studies applying SDIC so far have been conducted using a continuum light source, such as a white LED (Choodum, Boonsamran, NicDaeid, & Wongniramaikul, 2015; W. Li et al., 2016; Masawat, Harfield, Srihirun, & Namwong, 2017), sunlight or ambient light (Urapen et al., 2015; Yang, Sun, Bin, & Lian, 2007), a UV lamp (Lin et al., 2018), scanner light (Ledesma, Krepsky, & Borges, 2019) or more recently, a white-light-illuminating iPhone screen (Phadungcharoen et al., 2020). However, the sensitivity and selectivity would be greatly reduced in the absence of a monochromator, particularly with complicated matrices. To overcome these limitations and expand the technique's applicability to such matrices while maintaining its low cost and simplicity, a monochromatic light source is preferred. This can be done by replacing the smartphone’s white screen with a unicolored background image that can be obtained from a free online wavelength-to-color converter (https://www.wolframalpha.com) (**Figure 6**)

Figure 6.

Online Wavelength-To-Color Converter.



Color of 400 nm light

Input interpretation:

400 nm (nanometers) light | color

Result:

violet light

Color swatch:

Properties:

wavelength	400 nm
frequency	749 THz
photon energy	3.1 eV
effective temperature by wavelength	7244 K

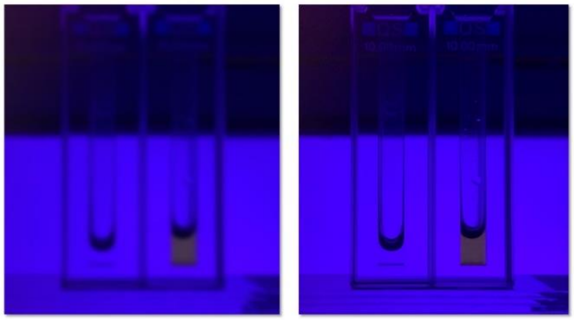
Spectrum location:

Position of the sample holder

In the case that a smartphone is employed as the light source, the sample holder is oriented in the center of the colorimetric box between two smartphones, one of which serves as the light source and the other as the detector. The distance between the detector and the analyte should then be optimized since it affects autofocus of the detection camera. Poor autofocus produces blur images as shown in **Figure 7**.

Figure 7.

Detection Camera's Autofocusing Effect.



Blurred photo

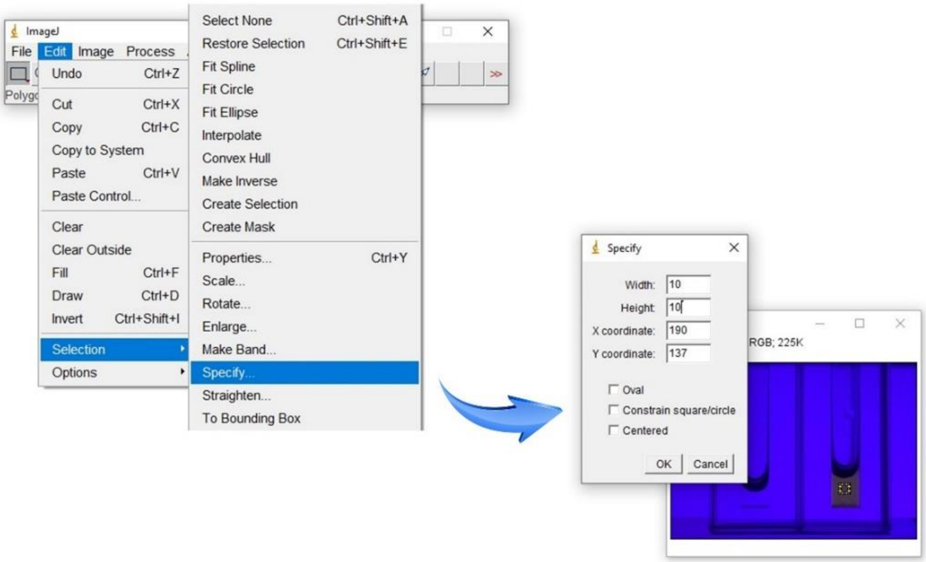
Focused photo

Region of interest

The region of interest (ROI) is defined as the cropped area selected by the software to convert the pixel intensity to a number to be used to quantify the analyte. The ImageJ program enables the user to analyze a picture by selecting an ROI within the image, measured in px^2. The steps are shown in **Figure 8**.

Figure 8.

Selection of ROI in ImageJ.



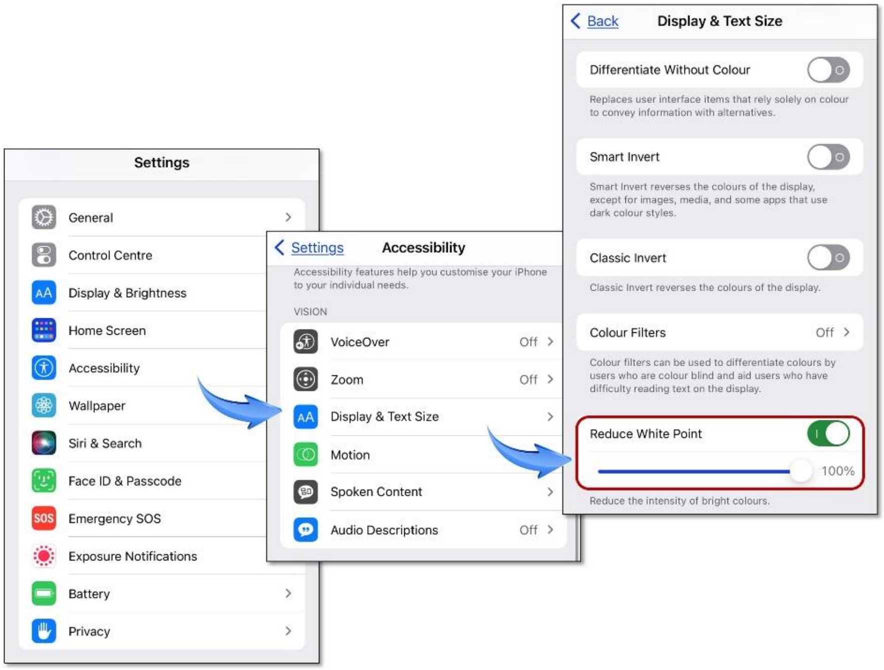
Width: 10
Height: 10
X coordinate: 190
Y coordinate: 137
☐ Oval
☐ Constrain square/circle
☐ Centered

Brightness of the light source

Although absorption should increase as the light source's brightness increases, dimming the light source can boost the response up to a point. On most iOS devices, the brightness can be adjusted by navigating to settings, accessibility, display, and text size, then selecting "decrease white point" and selecting the appropriate brightness (e.g., 100%). Because iOS devices lack a numerical scale for brightness, a scale can be assigned manually to the screen that ranges between 0 and 100%. The steps are shown in **Figure 9** and **Figure 10**.

Figure 9.

Adjusting The Intensity of The Bright Color.



Accessibility features help you customise your iPhone to your individual needs.

Replaces user interface items that rely solely on colour to convey information with alternatives.

Smart Invert reverses the colours of the display, except for images, media, and some apps that use dark colour styles.

Classic Invert reverses the colours of the display.

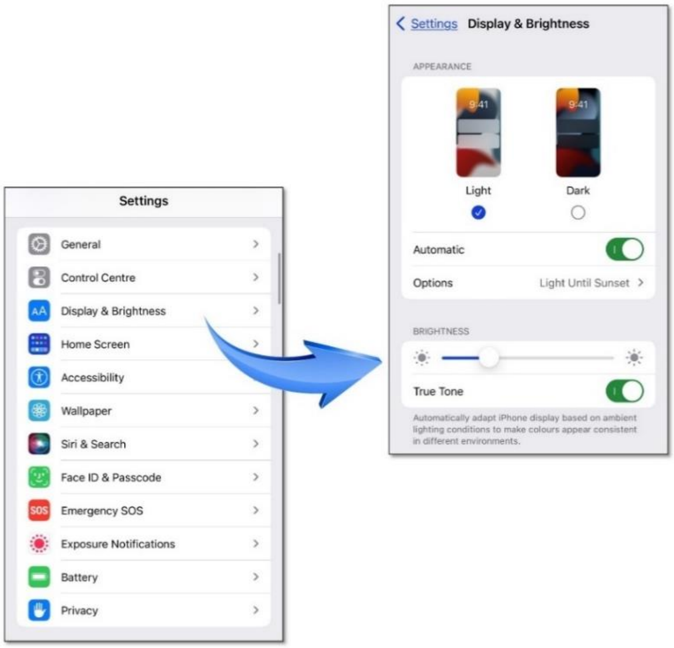
Colour filters can be used to differentiate colours by users who are colour blind and aid users who have difficulty reading text on the display.

Reduce White Point: on, 100%

Reduce the intensity of bright colours.

Figure 10.

Adjusting The Brightness.



Automatically adapt iPhone display based on ambient lighting conditions to make colours appear consistent in different environments.

Data processing

Images obtained with the detection smartphone can be saved in a JPG format, uploaded to a Drive, and then extracted from the computer for image processing. Each image can then be split into its RGB channels and the most sensitive channel can be utilized for calibration and quantification. An example is given in **Figure 11.**

In the literature, several equations have been proposed to obtain a linear correlation between the response (color intensity) (R) and the analyte concentration. In some, the blank was assumed to be absolute white (i.e., 255) and S was the signal obtained from the sample solution from a specific RGB channel (Jing, Wang, Huang, Chen, Zhu, & Wang, 2021). However, this method is not completely accurate because the blank produces a signal that is less than the ideal of 255, which eventually produces a high intercept in the calibration graph. Another method was proposed by Lima et al. (Lima et al., 2020), which referred to the intensities of the reflected radiation for the blank and sample solution, respectively.

The channel giving the highest response, say the blue (B), would have the smallest value as determined by the histogram of that channel. Beer's Law can then be applied to get a calibration graph with a negative slope because the histogram signal and color intensity are inversely proportional to each other. Multiplication with a minus sign would yield a positive calibration.

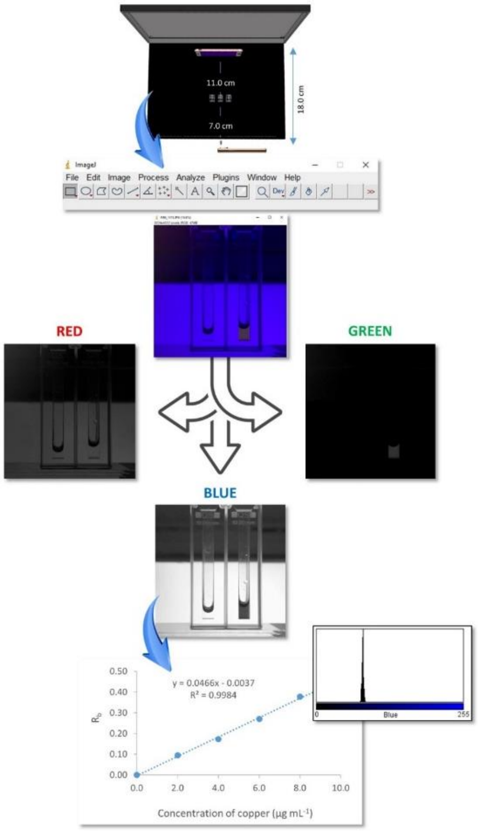
The response from the blue channel (R_b) can then be defined as shown in **Equation 2**.

$$R_b = -A_b = \log{(B_b/S_b)} \qquad \textbf{(Equation 2)}$$

Where, R_b is the response calculated from the histogram's mean values for the blank (B_b) and the sample solution (S_b).

Figure 11.

Data Processing.



Ultraviolet/visible absorption spectrophotometry (UV/Vis)

Ultraviolet and visible absorption spectrophotometry (UV/Vis) is based on the attenuation of electromagnetic radiation by an absorbing material. Attenuation occurs as a consequence of reflection, scattering, absorption, and/ or the presence of other species as interferences. This radiation has a spectral range of around 190–800 nm, which is also distinct from other similar areas in terms of energy ranges and kinds of excitation. Within certain limitations, absorbance is proportional to both the concentration of the analyte being determined and the distance traveled by the light as it travels through the sample during irradiation. Beer's Law is often written as $A = \varepsilon \times b \times c$, where A is the absorbance, ε is the molar absorbance coefficient (wavelength dependent) in $\mathrm{mol^{-1}}$ L $\mathrm{cm^{-1}}$, b is the path length in cm, and c is the concentration in mol $\mathrm{L^{-1}}$. This linear relationship is affected by a variety of factors, including the spectrophotometer's characteristics, the molecules' photodegradation, the presence of scattering or absorbing interferences in the sample, presence of fluorescent compounds in the sample, interactions between the analyte and the solvent, sample pH, etc. (Tissue, 2002). Due to its adaptability for regular quantitative and qualitative analysis of diverse analytes, UV/Vis has become a significant analytical method in modern-day laboratories, especially in less developed countries (Shokoufi, Shemirani, & Memarzadeh, 2007). A block diagram of a double-beam-in-space UV/Vis spectrophotometer is given in **Figure 12**.

Figure 12.

A double-beam UV/Vis spectrophotometer.

Direct application of both SDIC and UV/Vis spectrophotometry is not possible, especially when the analytes are present at low concentrations in complex matrices. Thus, to improve their selectivity and sensitivity, an efficient sample preparation method is unavoidable prior to the determination by both techniques. Microextraction methods are simple, cost-effective and reduce the steps involved in traditional solid- and liquid-phase extractions. A few milligrams of an adsorbent or a few microliters of an organic solvent are enough to extract the analytes from sample solutions. Nevertheless, the widespread use of toxic chemicals and solvents has caused numerous problems for the earth's natural resource system. As a result, today's analytical chemistry demands are directed towards more environmentally friendly procedures (Altunay, Elik, & Gurkan, 2020). Several modes of liquid–liquid microextraction (LLME) are nowadays actively in use by many researchers worldwide, the most recent of which are switchable-hydrophilicity solvent liquid–liquid microextraction (SHS-LLME) and synergistic cloud-point extraction (SCPME).

Switchable-hydrophilicity solvent liquid–liquid microextraction

Philip G. Jessop proposed switchable-hydrophilicity solvents (SHSs) in 2005, often known as smart solvents (Jessop, Heldebrant, Li, Eckert, & Liotta, 2005). Certain ecologically benign solvents, such as tertiary amines, are completely soluble in water in the presence of carbon dioxide or an acid. Upon mixing the tertiary amine with any of these, an acid-base reaction occurs between them to produce a protonated SHS that is polar and, hence, water-miscible. By adding 10-20 M sodium hydroxide, the reaction is reversed to produce the original water-immiscible non-polar form of the SHS. As a result, this feature enables the extraction of moderately hydrophobic and hydrophobic organic analytes or metal(loid) ions through complexation with a suitable ligand. This microextraction mode is referred to as SHS-LLME. Reversed-phase SHS-LLME (RP-SHS-LLME) is also possible, in which the tertiary amine is added to hydrophobic samples (e.g., oil) in its original water-immiscible non-polar form to extract hydrophilic analytes or metal(loid) ions without the need for a ligand. There are many advantages to using SHS. They are environmentally friendly, easy to prepare and switch, and they can be used to extract both organic and inorganic analytes from hydrophilic as well as hydrophobic samples (Usama Alshana, Hassan, Al-Nidawi, Yilmaz, & Soylak, 2020; Usama Alshana, Yilmaz, & Soylak, 2020a).

Cloud-point extraction

Cloud-point extraction (CPE) is a classical eco-friendly liquid–liquid extraction method that has been widely for the extraction of a wide range of analytes (Samaddar & Sen, 2014). In 1978, Watanabe and coworkers (Watanabe & Tanaka, 1978) employed CPE, for the first time, to extract zinc ions from aqueous solutions using 1-(2-thiazolylazo)-2-naphthol (TAN) as a ligand and Triton X-100 as a micelle-mediated extracting agent. Since then, the method has been utilized to extract metal ions and a variety of other organic analytes, including albumin. (da Silva & Arruda, 2009), persistent organic pollutants (POPs) (S. P. Xie, Paau, Li, Xiao, & Choi, 2010), fluoroquinolone antimicrobial agents (H. Wu, Zhao, & Du, 2010), pesticides (Zhou, Chen, Zhao, & Yang, 2009), and insecticides (Santalad, Srijaranai, Burakham, Glennon, & Deming, 2009). The phenomenon was based on water-soluble polyoxyethylated (POE) non-ionic surfactants (NSs) that form micelles in an aqueous solution as a result of salt addition (salting-out phenomenon) (Wael I. Mortada, Hassanien, & El-Asmy, 2014) or by raising the temperature of the solution above the cloud-point or cloud-point temperature (CPT) (Pytlakowska, Kozik, & Dabioch, 2013). Centrifugation is used to separate the mixture into two phases, a surfactant-rich phase and a surfactant-dilute phase. The dilute phase is an aqueous solution containing surfactant monomers or micelles, whereas the surfactant-rich phase contains the analyte (Akbas & Batigoc, 2009). Despite its relatively higher greenness as compared to other LLE methods, the traditional CPE procedure requires time and energy, heating, incubation, and cooling usually take up to 40 min on average (Wen, Wu, Chen, & Hou, 2009). Hence, Wen et al. (Wen, Deng, Ji, Yang, & Peng, 2012; Wen, Ye, Deng, & Peng, 2011) simplified the method by employing 1-octanol or 3,5-dichlorophenol as synergic reagents to lower the CPT of the NSs below room temperature (about 20 °C) and form the micelles without the need for heating or adding salts.

Factors affecting CPE

Effect of temperature and salting-out

Temperature is one of the significant factors that influence the CPE. The cloud-point phenomenon refers to the hydration of the POE chain at CPT (Schick, 1962). At this temperature, the surfactant is no longer soluble in the water, and the solution becomes turbid. After the micelles have aggregated, centrifugation is done and two layers are obtained (Akbas et al., 2009).

According to the salting-out phenomenon, in the presence of a relatively high concentration of electrolyte, the non-electrolyte becomes less soluble. When a salt is added to a micellar solution, it improves the hydrophobic interactions between micelles by increasing their dehydration. When the surfactant concentration is sufficiently high enough, turbidity forms and phase separation is possible (Hyde, Zultanski, Waldman, Zhong, Shevlin, & Peng, 2017). The use of the salting-out phenomenon in CPE eliminates the heating step, hence shortening the separation time (Wael I. Mortada et al., 2014).

pH of the sample solution

pH of the medium has a substantial effect on CPE, particularly for ionizable species such as amines and phenols, etc. Each CPE method has an optimal pH range for quantitative analyte extraction. Within this range, the analyte exists in an uncharged form that may be integrated into the micelles (Paleologos, Giokas, & Karayannis, 2005). pH may also affect the stability constant of metal complexes, which has a significant effect on the separation of metal ions by CPE (W. I. Mortada, Kenawy, Abdel-Rhman, El-Gamal, & Moalla, 2017). The phenomenon of pH-dependent stability constants was employed to separate platinum(II) and platinum(IV) using CPE (W. I. Mortada, Hassanien, & El-Asmy, 2013). At pH 7, Pt(II) formed a stable complex with 4-(*p*-chlorophenyl)-1-(pyridin-2-yl) thiosemicarbazide, but Pt(IV) did not. As a result, Pt(II) could be extracted as its complex into the surfactant-rich phase. The method was used to speciate platinum in biological materials.

Statement of the Problem

To begin with, sample preparation has remained the most difficult and critical step in the entire analytical process, particularly for complex matrices such as environmental, biological and food samples. Traditional extraction methods such as LLE and SPE, have several drawbacks, which include the use of large amounts of organic solvents, a long analysis time, low enrichment factors, and excessive chemical waste that is harmful to organisms, the researcher and the environment. As a result, to overcome these drawbacks, the development of alternative miniaturized extraction methods is highly required. In addition, the high cost of analytical instruments is another major problem especially for researchers working in low-income laboratories or in developing countries, necessitating the search for low-cost alternative techniques.

Aim of the Study

This study aims at combining three LLME methods with SDIC and UV/Vis for the determination of molecular and elemental analytes in food samples.

The objectives include, but are not limited to, the following,

- Developing a reversed-phase switchable-hydrophilicity solvent liquid–liquid microextraction (RP-SHS-LLME) method for preconcentration of copper prior to its detection by SDIC and determination in edible oils.
- Developing an SCPME-UV/Vis method for preconcentration and determination of curcumin in tea and spice samples.
- Developing an SHS-LLME-UV/Vis method for preconcentration and determination of Sudan I dye in spices.

- Improving sensitivity and selectivity of SDIC by replacing continuum light sources with a monochromatic light source.

Research Questions and Hypothesis

Some research questions considered include,

- Will microextraction improve SDIC's sensitivity and selectivity?
- Will the combination of microextraction and SDIC be efficient enough to detect analytes at trace and ultra-trace concentration levels?
- SHS solvents are active solvents; will they be applicable to all kinds of analytes?
- Can polar analytes be extracted with SHS-LLME?

Significance of the Study

Combining RP-SHS-LLME with SDIC methods resulted in significant cost reduction, low energy use, and improved flexibility for on-site analysis, making it an attractive option for developing countries and small laboratories.

Due to its low cost, simplicity and adaptability for routine quantitative and qualitative analysis of a broad range of analytes, UV/Vis has become an essential analytical method. However, low sensitivity and selectivity hinder its direct application to complicated samples therefore an efficient sample cleanup and preconcentration methods such as SCPME and SHS-LLME are needed.

CHAPTER II

Literature Review

Related Research

Several microextraction and analytical methods have been proposed in the literature to extract different organic and inorganic analytes from various food samples. Some of these studies were carried out with normal and reversed modes of SHS-LLME and SCPME in food matrices for molecular and elemental analysis.

Switchable-hydrophilicity solvent liquid–liquid microextraction

Significant efforts have been recently made to improve and broaden the applications of SHS-LLME. One of the main differences between the latter and dispersive liquid–liquid microextraction (DLLME) (Campillo, Vinas, Sandrejova, & Andruch, 2017) is that the use of an auxiliary tool/step to aid the dispersion of the extraction solvent, common in DLLME, would be less necessary owing to the complete water miscibility of SHS upon the addition of carbon dioxide or an acid. Yet, some researchers utilized auxiliary tools such as air (Ezoddin, Abdi, & Lamei, 2016), vortex (Tekin, Erarpat, Sahin, Chormey, & Bakirdere, 2019), and ultrasonic energy (Habibiyan, Ezoddin, Lamei, Abdi, Amini, & Ghazi-khansari, 2017). The use of SHS-LLME for sample cleanup has also been proposed (Hassan, Uzcan, Shah, Alshana, & Soylak, 2021). Cabuk & Kavaraci also used unmodified Fe_3O_4 magnetic nanoparticles as a carrier to separate and retrieve the SHS from the sample solution (Cabuk & Kavaraci). Hassan et al. (Hassan, Erbas, Alshana, & Soylak, 2020) suggested the use of RP-SHS-LLME for the extraction of copper from oil samples prior to its determination by flame-atomic absorption spectrometry. These studies are summarized in **Table 1**.

Table 1.

Application of SHS-LLME for in The Analysis of Food Samples.

SHS-LLME Method[a]	Matrix	Analyte	Detection[b]	LOD[c] (ng mL^{-1})	Ref.
AA-SHS-LPME	Water, road dust, and catalytic converter samples	Pd	ETAAS	0.07	(Ezoddin et al., 2016)
Vortex assisted-SS-LPME	Egg yolk	Vitamin B_{12} and cobalt	SQT-FAAS	75 ng mL^{-1}	(Tekin et al., 2019)
Ultrasonic assisted-SHS-LLME	Water, urine, and infusion tea samples	Cd, Ni, Pb, and Co	FAAS	0.76	(Habibiyan et al., 2017)
SHS-LLME - DSPME	Apple, pepper, red and white onion	Quercetin	UV/Vis	11.9	(Hassan, Uzcan, Shah, et al., 2021)
MR-SHS-HLLME	Environmental waters	Benzophenone (BP)-type UV filters	LC-UV	0.8	(Cabuk et al.)
RP-SHS-LLME	Oil samples	Cu	FAAS	6.9	(Hassan et al., 2020)
SHS-LLME	Spices	Piperine	HPLC-UV	0.6 µg mg^{-1}	(Al-Nidawi et al., 2020)
SHDS-LLME	Meat and fish	Amphenicols	HPLC-UV	0.03 µg kg^{-1}	(W. X. Li, Chen, Huang, Zeng, & Zhu, 2019)
SHS-HLLME	Wolfberry	Pyrethroid insecticides	HPLC-UV	0.5	(Di, Zhang, Yang, & Guo, 2021)
RP-SHS-LLME	Oil	Cu	SDIC	300	(Al-Nidawi & Alshana, 2021) **This study**

[a] AA-SHS-LPME: Air-assisted switchable-hydrophilicity solvents liquid–liquid microextraction; Vortex assisted-SS-LPME: Vortex assisted switchable solvent-based liquid phase microextraction; Ultrasonic assisted-SHS-LLME: Ultrasonic assisted switchable-hydrophilicity solvent liquid–liquid microextraction; SHS-LLME-DSPME: Switchable-hydrophilicity solvent liquid–liquid microextraction-dispersive magnetic solid-phase microextraction; MR-SHS-HLLME: Magnetic retrieval of a switchable-hydrophilicity solvent-homogeneous liquid–liquid microextraction; RP-SHS-LLME: Reversed-phase switchable hydrophilicity solvent liquid–liquid microextraction; SHDS-LLME: Switchable-hydrophilicity dispersive solvent-based liquid–liquid microextraction; SHS-HLLME: switchable-hydrophilicity solvent-based homogeneous liquid–liquid microextraction.

[b] ETAAS: electrothermal atomic absorption spectrometry; SQT-FAAS: slotted quartz tube-flame-atomic absorption spectrometry; FAAS: Flame-atomic absorption spectrometry; UV/Vis: Ultraviolet/visible spectrophotometry; LC-UV: Liquid chromatography-ultraviolet; HPLC-UV: High-performance liquid chromatography-ultraviolet; SDIC: Smartphone digital image colorimetry.

[c] Limit of detection.

Cloud-point extraction

CPE is an extraction method that has been extensively applied to preconcentrate elemental and molecular analytes. Different types of surfactants have been used in the literature. The most widely utilized surfactants are Triton X-114 and Triton X-100. Triton X-114 has been used in CPE of estrogens (Ezoddin et al., 2016), disulfoton (Faria, Dardengo, Lima, Neves, & Queiroz, 2007), and lead in water samples (Manzoori & Karim-Nezhad, 2003), as well as silver and lead in wastewater and soil samples (Tavallali, Yazdandoust, & Yazdandoust, 2010). Triton X-100 has been used in CPE of zirconium in rat liver, kidney and urine (Gui et al., 2018), molybdenum in water and plant samples (Snigur, Chebotarev, Dubovyiy, Barbalat, & Bevziuk, 2018), as well as for extraction of chromium and reactive black 5 from environmental samples (Kiran, Kumar, Prasad, Suvardhan, Babu, & Janardhanam, 2008; Mousavi & Nekouei, 2011). These studies are summarized in **Table 2**.

Table 2.

Application of CPE for Extraction of Molecular and Elemental Analytes from Food Samples.

Type of surfactant	**Matrix**	**Analyte**	**Detection**	**LOD[a] (ng mL^{-1})**	**Ref.**
Triton X-114	Water	Estrogens	HPLC–UV	1.45	(Ezoddin et al., 2016)
Triton X-114	Water	Pb	FAAS	0.56	(Manzoori et al., 2003)
Triton X-114	Water	Disulfoton	GC	1.2	(Faria et al., 2007)
Triton X-114	Wastewater and soil	Ag, Pd	FAAS	10 25	(Tavallali et al., 2010)
Triton X-100	Rat liver, kidney and urine	Zr	ICP-MS	0.055	(Gui et al., 2018)
Triton X-100	Water and plant samples	Mo	UV/Vis	50	(Snigur et al., 2018)
Triton X-100	Environmental samples	Cr	FAAS	0.18	(Kiran et al., 2008)
Triton X-100	Wastewater	Reactive black 5	UV/Vis	-	(Mousavi et al., 2011)

[a] Limit of detection.

CHAPTER III

Materials and Methods

Chemicals and Reagents

Analytical-grade chemicals were used throughout the analysis unless otherwise stated. 1-Butanol, 1-dodecanol (1-DO, $logP$, 4.36, density, d: 0.83 g mL^{-1}; melting point, m. p.: 24 °C), 1-pentanol, 1-undecanol (1-UN, $logP$ 3.92, d: 0.83 g mL^{-1}; m. p.: 11 °C), acetonitrile (ACN), copper(II) sulfate pentahydrate, curcumin ($logP$ 4.85, pK_{a1} 8.8), cyclohexylamine (CHA, $logP$ 1.17, pK_a 10.45), dimethylbenzylamine (DMBenA, $logP$ 1.91, pK_a 8.99), dimethylbutylamine (DMBA, $logP$ 1.40, pK_a 10.42), ethanol (EtOH), methanol (MeOH), *N*,*N*-dimethylcyclohexylamine (DMCHA, $logP$ 1.99, pK_a 10.2), nitric acid, octylamine (OCA, $logP$, 2.48, pK_a 10.20), sodium hydroxide, Sudan I dye ($logP$ 5.07, pK_a 11.8), Tergitol, triethylamine (TEA, $logP$ 1.26, pK_a 10.2), Triton X-114, and Triton X-405 were obtained from Sigma-Aldrich (Germany). Deionized (DI) water (18.2 MΩ.cm), obtained from Purelab Ultra Analytic (ELGA LabWater, High Wycombe, UK), and from a Millipore purification system (Danvers, MA, USA), was used for the preparation of all aqueous solutions. *N*,*N*-diethyl-*N'*-benzoylthiourea (DEBT, $logP$ 2.34, pK_a 14.0) was previously synthesized and characterized by our group (U. Alshana & Aygun, 2011).

Apparatus

A laboratory-made colorimetric box was designed using an aluminum box with dimensions of $25 \times 18 \times 9$ cm. The colored extract was placed in a quartz UV/Vis microcuvette (Hellma, Kruibeke, Belgium) 7.0 cm away from the detection camera. A small hole was drilled on one side. An iPhone 6S smartphone (model number MKQR2QN/A) was used as a monochromatic light source, equipped with a 12 MP rear camera, a 4.70-inch touch screen display with a resolution of 750×1334 pixels, 64 GB of internal memory, a 1.84 GHz dual-core Apple A9 Fusion processor, and 2 GB of RAM. Images of the sample solutions were captured with an iPhone XS smartphone (model number MT9K2AH/A), equipped with a 12 MP rear camera, a 5.8-inch touch screen display with a resolution of 1125 × 2436 pixels, and 256 GB of internal memory, an Apple A9 Fusion processor, and 2 GB of RAM. Image processing was done using the freely available ImageJ software for PC, version 1.53 j, Java 1.8.0_112, 64-bit (National Institutes of Health, USA) (Rueden et al., 2017). MarvinSketch software (Rev. 20.11.0, ChemAxon Ltd., Cambridge, MA, USA) was used to calculate $logP$ and pK_a values.

A Hitachi 150-20 double-beam UV/Vis spectrophotometer (Hitachi, Ltd., Tokyo, Japan) was used for absorbance measurements at 445.0 nm and 430.0 nm. Ethanol was used as a blank solution for blank corrections. The pH values were measured using a pH meter equipped with a Sartorius PT-10 model glass electrode. Centrifugation was done with a Nuve NF400 (4X50 mL/4100 rpm) centrifugation machine. A Fisons WhirliMixer Vortex Shaker Cyclone Mixer model-12665 was used to vortex mix the solutions.

Statistical Analysis

All statistical analyses were carried out in Microsoft Office Excel 2016 for Windows using the single-factor analysis of variance (ANOVA) test (Microsoft Corporation, USA). The difference was considered statistically significant at a P value of 0.05 or less.

RP-SHS-LLME-SDIC for the determination of copper in edible oils

Copper standard solutions

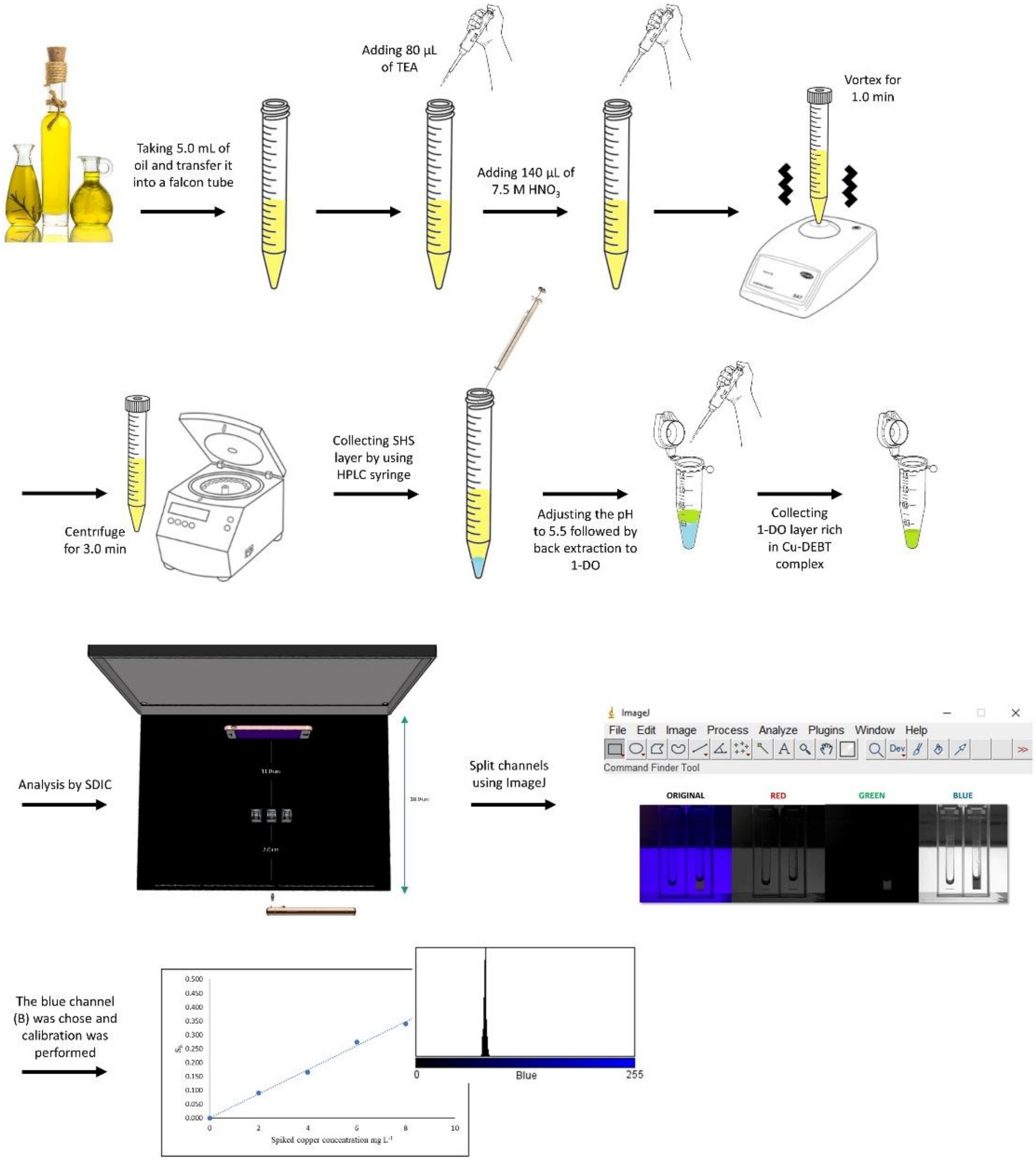
An appropriate amount of copper(II) sulfate pentahydrate was dissolved in 1.0 % (v/v) nitric acid to obtain 1000 μg mL^{-1} of copper(II) stock solution. Working standard solutions ranging from 0.3–25.0 μg mL^{-1} were prepared by diluting the stock solution in 1.0 % (v/v) ethanolic nitric acid. DEBT was prepared at 1000 μg mL^{-1} in 1-DO.

RP-SHS-LLME

A 5.0 mL spiked oil sample was transferred into a 15.0 mL screw-cap centrifuge graduated polypropylene test tube and vortexed for 3.0 min to ensure the miscibility of the standard, prepared in acidic ethanol, with the oil samples. Before extraction, the mixture was allowed to stand at least for 15.0 min for equilibrium to occur. Following that, 80 μL of TEA in its unprotonated form was added as an extraction solvent and the mixture was vortexed for 10 s. Then, 140 μL of 7.5 mol L^{-1} nitric acid was added and the mixture was vortexed for 1.0 min to protonate the TEA. After centrifuging the mixture for 3.0 min at 6000 rpm, 200 μL of the switched-off layer of TEA were collected with a 100-μL HPLC syringe and transferred into a 1.5 mL micro tube. pH of the collected layer was then adjusted to 5.50 using a few drops of 10.0 mol L^{-1} sodium hydroxide followed by a pH 5.50 phosphate buffer. The addition of 200 μL of 1000 μg mL^{-1} DEBT solution resulted in the simultaneous formation of a green complex with copper for detection with SDIC. A schematic diagram of the proposed RP-SHS-LLME method is shown in **Figure 13**.

Figure 13.

Schematic Diagram of The Proposed RP-SHS-LLME-SDIC Method.



Data processing

The captured images were saved in JPG format and uploaded to Google Drive, where they could be downloaded and analyzed on a personal computer using ImageJ software. The pictures were split into their RGB channels, and the B channel, which had the highest response, was used for all studies. The absorbance was calculated as shown in **Equation 3** by using the mean values from the B channel of the histogram for the blank (B_b) and analyte solution (S_b).

$$R_b = -A_b = log\,(B_b/S_b) \qquad \textbf{(Equation 3)}$$

SCPME-UV/Vis for the determination of curcumin in food samples

Curcumin standard solutions

An appropriate amount of curcumin was dissolved in ACN to prepare 500 µg mL^{-1} of a curcumin stock solution. Working standard solutions ranging from 1.0 to 15.0 µg mL^{-1} were prepared by dilution of this stock solution with DI water.

Sample preparation

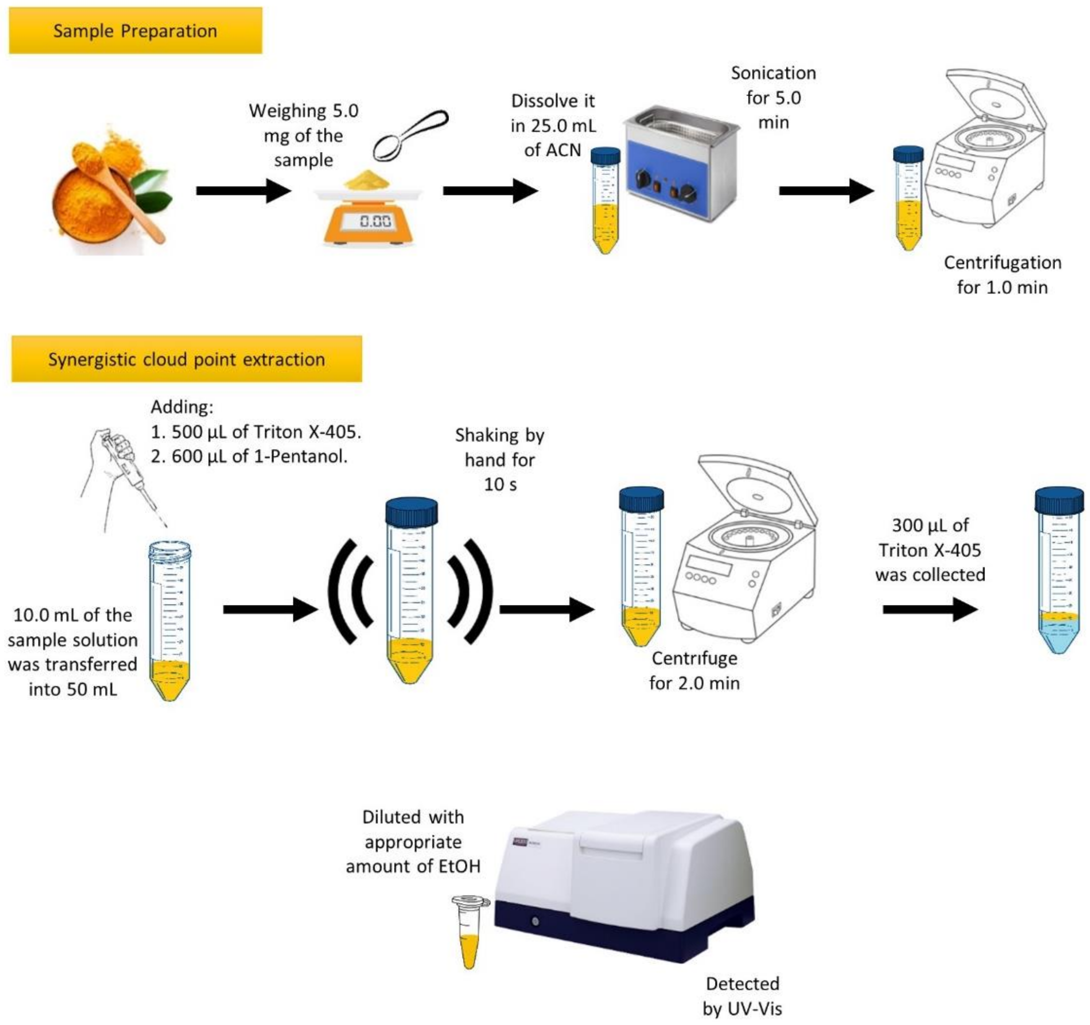
Different food samples containing curcumin were analyzed in order to test the applicability of the method. The spices and herbal tea were obtained from local markets in Kayseri, Turkey. A portion of 5.0 mg of each sample was dissolved in ACN and the solution was made up to 25.0 mL with ACN. Following filtration, the sample solution was appropriately diluted with DI water and was ready for SCPME.

SCPME procedure

An aliquot (i.e., 10.0 mL) of the sample solution was transferred into a 50 mL screw-capped centrifuge tube and 500 µL of Triton X-405 were added to the sample solution, followed by the addition of 600 µL of 1-pentanol. A period of 10 s shaking by hand was sufficient to form a cloudy solution due to micelle formation. Phase separation occurred upon centrifugation for 2.0 min at 4500 rpm, and the aqueous phase was carefully withdrawn into a syringe. The deep yellow surfactant-rich phase was collected and diluted (1:1, v/v) with EtOH to decrease viscosity and the final extract was analyzed by UV/Vis. A schematic diagram of the proposed SCPME-UV/Vis method is shown in **Figure 14**.

Figure 14.

Schematic Diagram of The Proposed SCPME-UV/Vis Method.



SHS-LLME-UV/Vis for the determination of Sudan I dye in spices

Sudan I dye standard solutions

A suitable amount of Sudan I dye was dissolved in EtOH to obtain a 100 µg mL^{-1} stock solution. Working standard solutions ranging from 1.0 to 30.0 µg mL^{-1} were prepared by diluting the stock solution with DI water.

Sample preparation

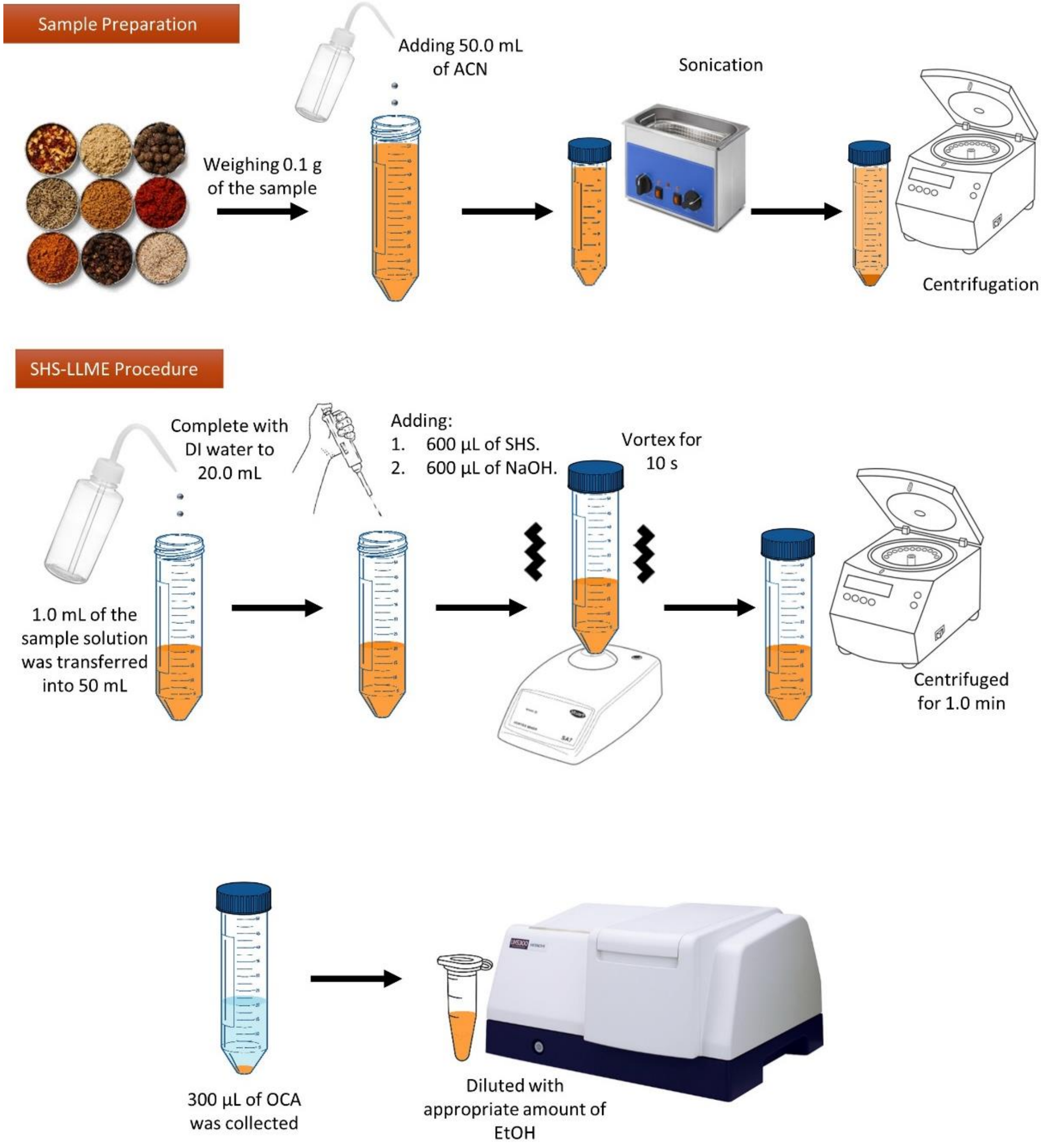
Different samples containing Sudan I dye were analyzed using the proposed SHS-LLME-UV/Vis method. Spices and soy sauce were obtained from local markets in Kayseri, Turkey. A portion of 0.1 g of each solid sample and 1.0 mL of liquid samples were mixed with 50.0 mL of ACN. The sample solution was properly diluted with DI water after filtration and was then suitable for further extraction with SHS-LLME.

SHS-LLME procedure

The sample solution (i.e., 1.0 mL) was transferred into a 50 mL screw-capped centrifuge tube and diluted with water to 20.0 mL. SHS (600 µL) was added to the sample solution followed by the addition of 600 µL of 10.0 M sodium hydroxide, vortexing for 10 s, then centrifuged for 1.0 min at 4500 rpm. Phase separation occurred and the aqueous phase was removed carefully using a syringe. The deep orange amine layer was collected and diluted with ethanol (1:1, v/v) to decrease the viscosity. Sudan I was determined in the final extract using UV/Vis. A schematic diagram of the proposed SHS-LLME-UV/Vis method is shown in **Figure 15**.

Figure 15.

Schematic Diagram of The Proposed SHS-LLME-UV/Vis Method.



CHAPTER IV

Results and Discussion

RP-SHS-LLME-SDIC for the determination of copper in edible oils

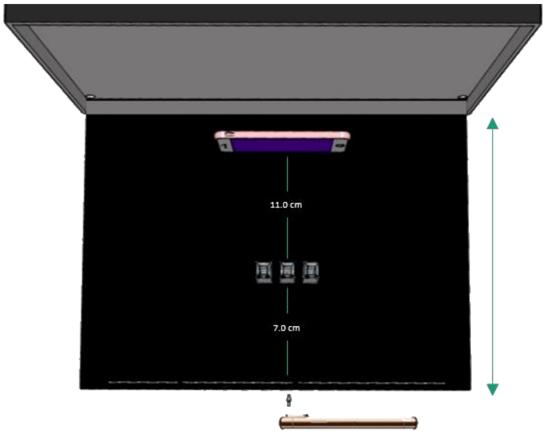
Optimization of SDIC conditions

Construction of the co1orimetric box

A colorimetric box was constructed from a rectangular aluminum box with dimensions of 25 × 18 × 9 cm. A hole was punched in the side of the box to fit the camera lens to capture reproducible images of the sample solution inside the box. The sample was placed in a quartz UV/Vis microcuvette (Hellma, Belgium) at 7.0 cm from the camera. A monochromatic source was used by placing a unicolored backlight on another smartphone screen, illuminating light that is equivalent to 400 nm, the wavelength of the metal complex. As shown in **Figure 16**, three cuvettes were placed in front of the monochromatic light source to uniform the illumination of the cuvettes. This also ensured that the blank and sample were captured at the same time, which helped to improve repeatability.

Figure 16.

Schematic Diagram of The Colorimetric Box with a Monochromatic Light Source.

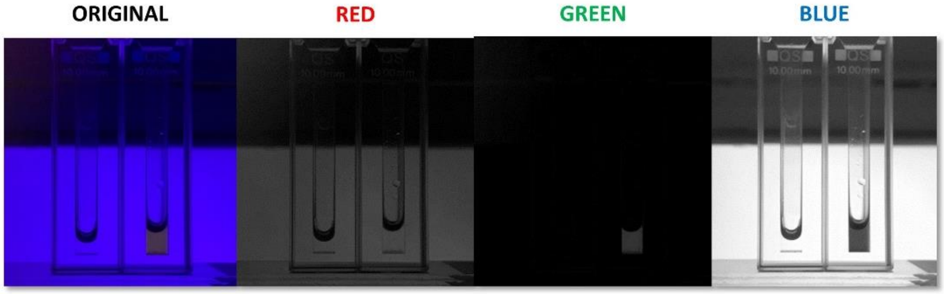


Selection of the RGB channel

The color of the solution affects the intensity of the mean of the RGB model's histogram of channels. When the RGB channels of the solution's image were split, the B channel had the maximum intensity and was thus used for all studies (**Figure 17**).

Figure 17.

Channel Splitting.



Selection of the detection wavelength

In this study, a monochromatic light source was used and the effect of the wavelength was studied by scanning the visible region in the range of 400–460 nm within a 5.0 nm interval, which is within the complementary color range of the green analyte complex (**Figure 18**). As shown in **Figure 19**, the maximum absorption wavelength was found to be 400 nm, which was used to measure the analyte concentration during the experiments.

Figure 18.

Complimentary Colors.

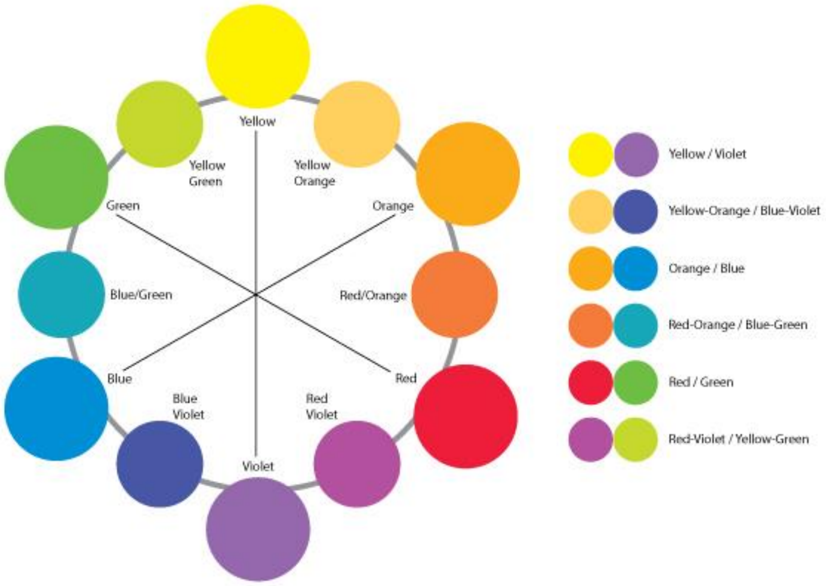
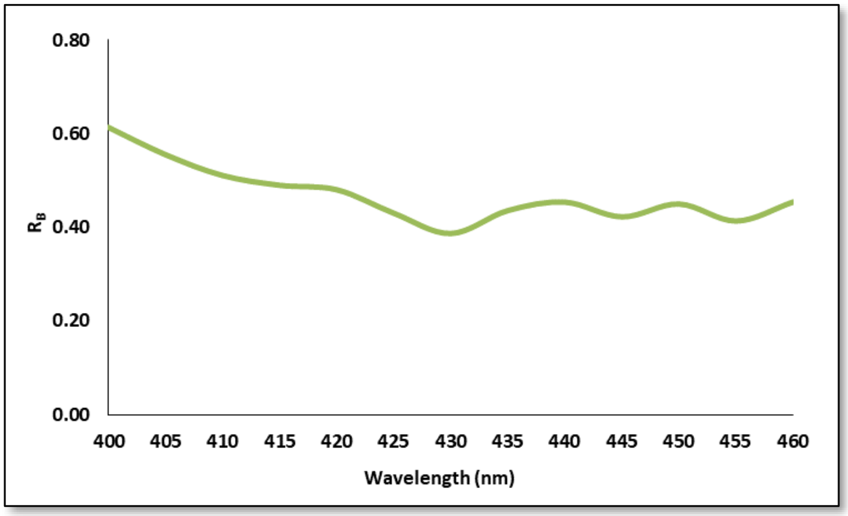


Figure 19.

Optimizing The Detection Wavelength.

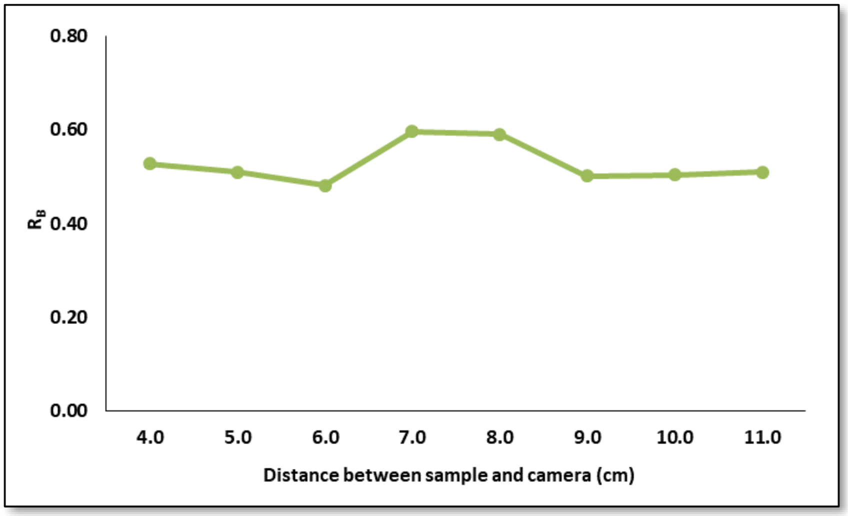


Position of the sample holder

The distance between the sample holder and the detection camera was studied over the range of 4.0–11.0 cm. Blur images were captured at 4.0 cm and below because this distance was believed to be insufficient for the detection camera to achieve its maximum autofocus efficiency. The response increased up to 7.0 cm, where it stayed virtually steady until 8.0 cm, where it started to decrease. For subsequent experiments, the optimum distance was thus determined to be 7.0 cm as shown in **Figure 20**.

Figure 20.

Optimizing The Distance Between The Sample and The Detection Camera.

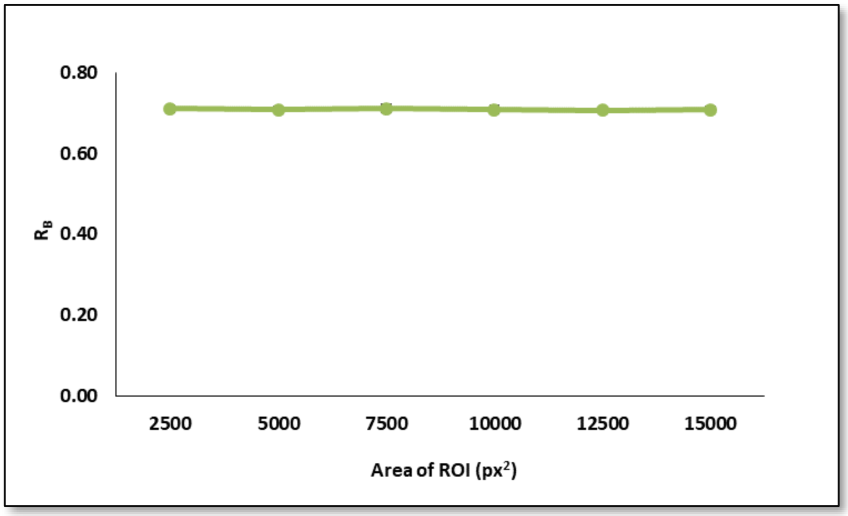


Region of interest

The more homogenous the color distribution within the area of interest (ROI), the smaller the standard deviation. Due to the extract's high homogeneity, and hence its color, it was observed that the ROI region did not have a significant influence over the response (**Figure 21**). A ROI of 2500 px^2 was employed in the following experiments.

Figure 21.

Optimizing The Region of Interest.

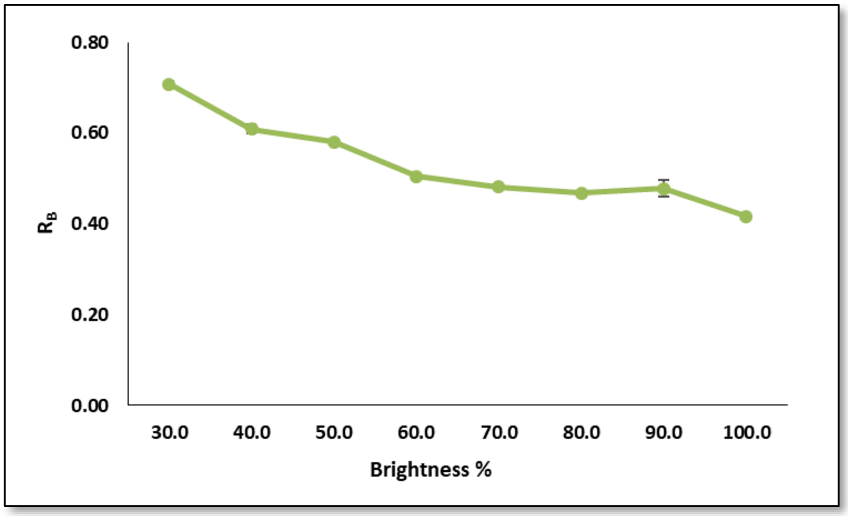


Brightness of the light source

The light source brightness, measured in the range of 30.0–100.0 %, was found to cause a rapid decrease in the response. However, below 30.0%, the image was too dark to be used for reproducible measurements. As a result, 30.0% was chosen as the optimum brightness (**Figure 22**).

Figure 22.

Optimizing The Brightness of The Light Source.



Optimization of RP-SHS-LLME conditions

Complex matrices and high hydrophobicity characterize oil samples. Additionally, since the analyte is present at an ultra-trace level, an effective extraction and preconcentration step are required to reduce matrix effect and raise the analyte concentration to a detectable level. Metal ions are usually extracted using LLME as metal chelates with a suitable ligand. However, ligandless extraction of metal ions from such matrices is desirable to minimize chemical consumption, waste, expense, and the number of processes required. In RP-SHS-LLME, SHS in its unprotonated form is added to the hydrophobic sample. The hydrophilicity of the solvent can then be switched by adding a strong acid as a switching trigger, protonating the SHS, and switching it to its hydrophilic oil-immiscible form into which the metal ion can be extracted (Hassan et al., 2020). The type and volume of SHS, concentration, and volume of nitric acid, complexation pH, volume of DEBT, type and volume of the back-extraction solvent, and complexation time were all optimized to improve the extraction efficiency of RP-SHS-LLME.

Type and volume of SHS

Due to their hydrophobic nature, tertiary amines such as TEA and DMCHA are suitable solvents for metal ion extraction from hydrophobic matrices, since they offer an enormously large surface area of contact between the sample solution and the extraction solvent in a homogenous medium. To switch off such solvents, an acid-base reaction in the presence of an acid might be used. A suitable SHS for RP-SHS-LLME must be miscible with the oil sample and possess a high extractability toward the hydrophilic analyte. The hydrophilic form of the SHS, on the other hand, must not be miscible with the oil sample. To determine the influence of SHS type on the extraction efficiency, TEA, DMCHA and a 50:50 (%, v/v) combination of both were examined. TEA showed the greatest recovery due to its lowest $logP$ value (Page 49). As a result, TEA was selected as the optimal extraction solvent (**Figure 23**). The volume of TEA was evaluated between 20.0 and 100.0 µL. The analyte recovery increased up to 80.0 µL, after which it remained nearly constant. As a result, the optimal TEA volume was determined to be 80.0 µL (**Figure 24**).

Figure 23.

Effect of The Type of SHS.

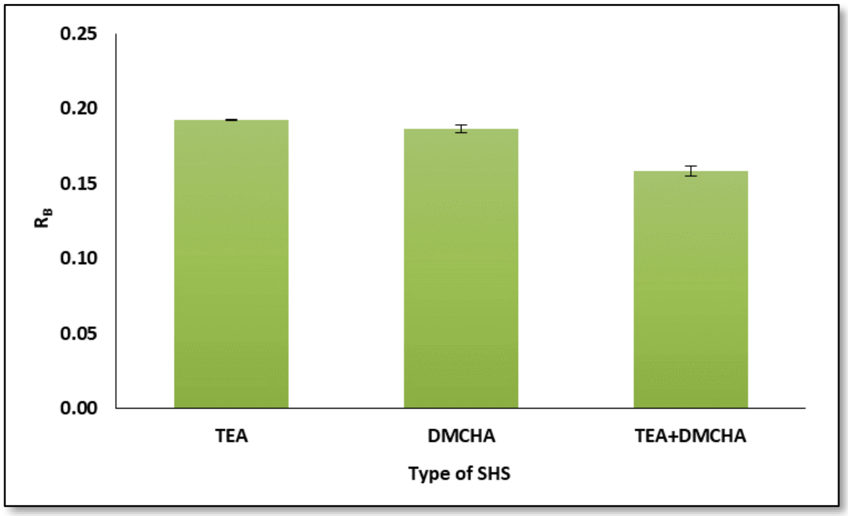
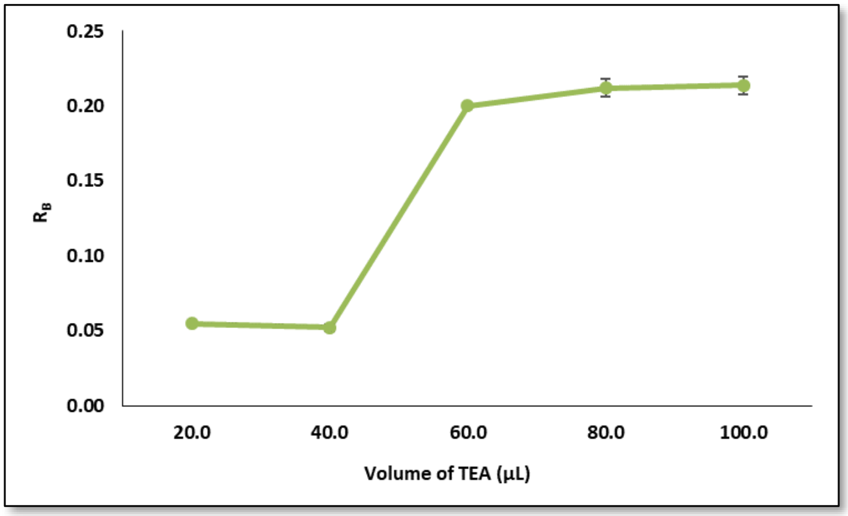


Figure 24.

Effect of The Volume of TEA.



Concentration and volume of nitric acid

Although carbon dioxide is the most frequently used switching agent in SHS-LLME (Usama Alshana, Yilmaz, & Soylak, 2020b), using a strong acid speeds up the process and prevents analyte loss. This can even facilitate freeing the metal ion from its conjugated form, which can then be extracted into the hydrophilic SHS layer. Several concentrations of nitric acid ranging from 5.5–9.5 mol L^{-1} were evaluated. It was seen that 7.5 mol L^{-1} nitric acid provided an optimal reaction (**Figure 25**) that decreased with time, most likely owing to acid-deproteinization (i.e., protein removal/precipitation) of the samples, which lowered the complex's color intensity. The effect of the volume of nitric acid was investigated between 100 and 180 µL at 20 µL intervals. Recovery rose with up to 140 µL of the acid, at which point it began to decrease owing to dilution of the analyte by the extra volume of nitric acid. As a result, 140 µL was chosen as the optimum volume for the next experiments (**Figure 26**).

Figure 25.

Effect of The Concentration of Nitric Acid.

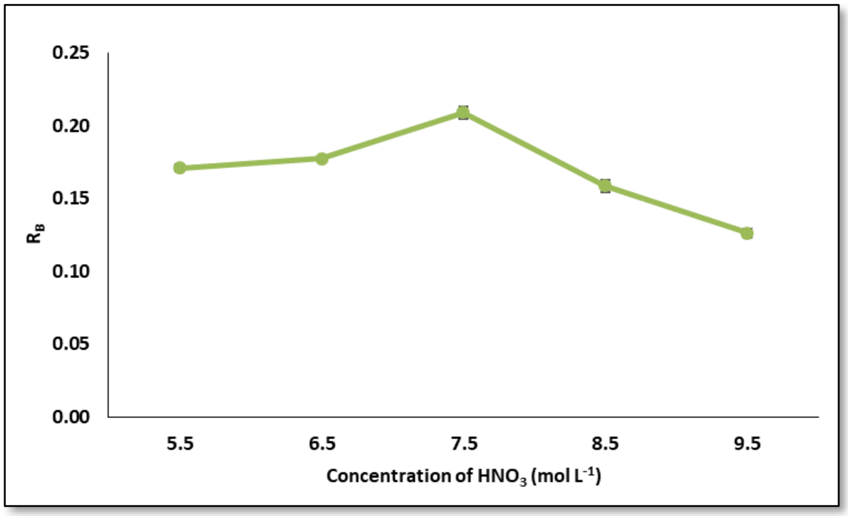
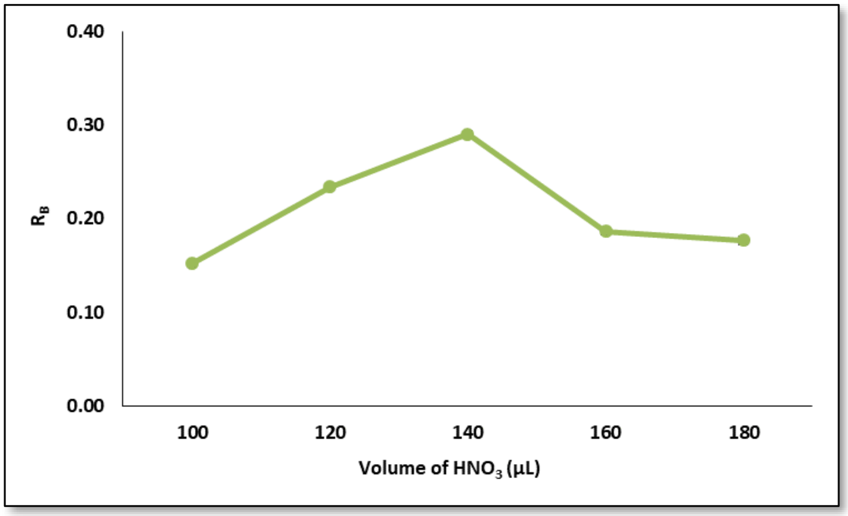


Figure 26.

Effect of The Volume of Nitric Acid.

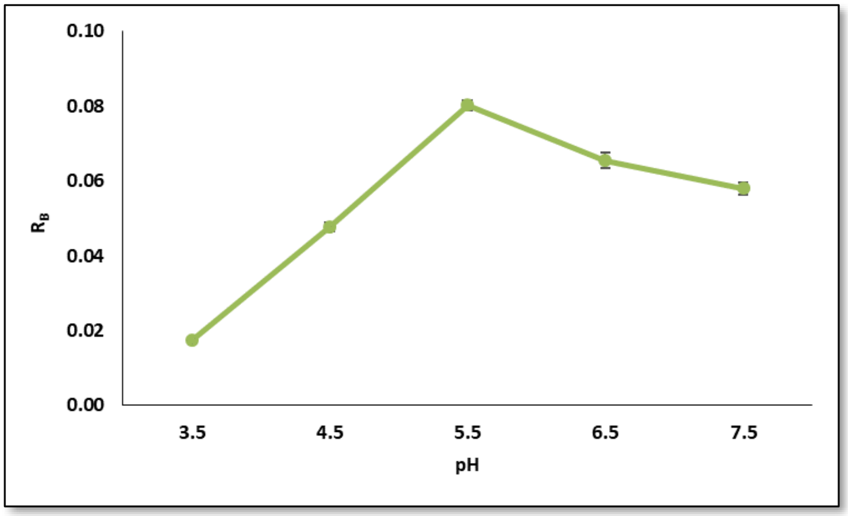


Complexation pH

pH of the sample solution is essential not only for the formation of stable complexes but also for the formation of complexes with other interfering ions that may be co-extracted with the analyte under the same conditions. Copper is predominantly bivalent in acidic conditions, where it may form a stable green complex with DEBT [Cu(DEBT)$_2$, $logP$ 8.60, pk_a 4.65]. DEBT, a highly selective ligand that can form stable complexes under acidic conditions at room temperature, can form a green complex with copper(II), pale yellow complex with palladium(III), and pale red complex with iron(III) (U. Alshana et al., 2011). Preliminary interference experiments revealed that the presence of iron (up to 30 µg mL^{-1}) and palladium (50 µg mL^{-1}) did not affect copper recovery significantly (5% signal suppression) under optimized extraction conditions. The effect of complexation pH, when scanned over the range of 3.5–7.5, was found to increase the response up to 5.5 and decrease it afterwards. Thus, pH 5.5 was chosen as optimum for the complexation reaction to take place in further experiments (**Figure 27**).

Figure 27.

Effect of The Complexation pH.

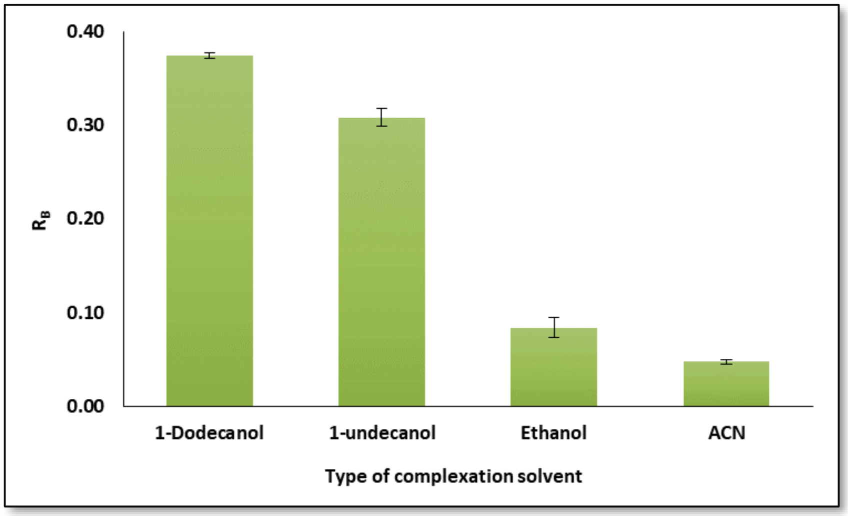


Type of complexation (back-extraction) solvent

Although DEBT and its copper complex are insoluble in water, they dissolve rapidly in 1-DO, 1-UN, EtOH and ACN. The effect of the complexation solvent type on the reaction was studied using these four solvents. Due to the high miscibility of EtOH and ACN with the switched-off form of SHS, only one phase was formed, resulting in dilution of the metal complex and a pale color. On the other hand, the immiscibility of 1-UN and 1-DO with the switched-off SHS led to the formation of two distinct layers and back-extraction of the complex into the upper layer of 1-UN or 1-DO, which increased selectivity and sensitivity further. Because of its lower polarity, 1-DO showed a higher recovery of the hydrophobic complex than 1-UN and was thus chosen as the optimum complexation solvent (**Figure 28**). It is important to keep in mind that 1-DO may solidify below 24 °C, however, which necessitates control of the laboratory temperature above 24 °C.

Figure 28.

Effect of The Type of The Back-Extraction Solvent.

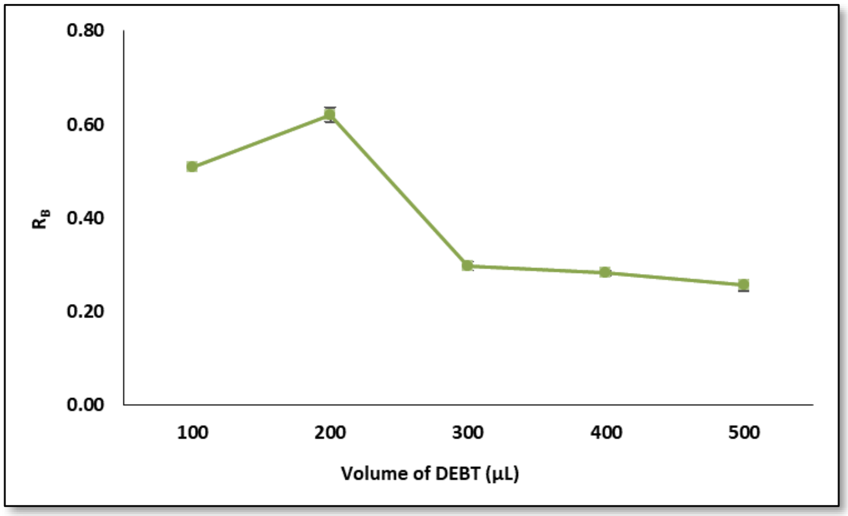


Volume of DEBT

The volume of DEBT (1000 µg mL^{-1}) was investigated in the range of 100–500 µL; volumes less than 100 µL were difficult to collect. The recovery increased up to 200 µL due to the large surface area available for the analyte, after which it decreased due to dilution. As a result, a volume of 200 µL was deemed optimal in subsequent experiments (**Figure 29**).

Figure 29.

Effect of The Volume of DEBT.

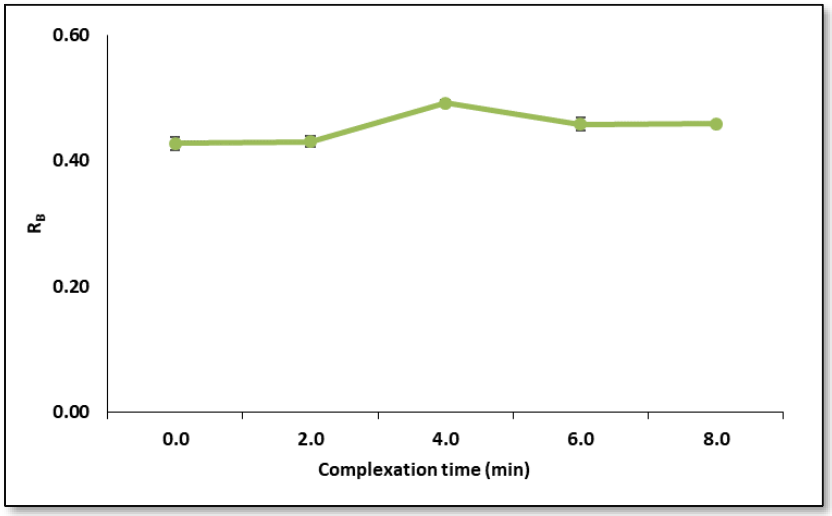


Complexation time

Complexation time was defined as the time interval between the points DEBT was added until the point the SHS layer was collected, which corresponded to the vortex time in this experiment. Investigation of the complexation time within the range of 0.0–8.0 min revealed that the maximum recovery can be achieved within 4.0 min and remain nearly constant thereafter. As a result, the best time for complexing was found to be 4.0 min in the next set of experiments (**Figure 30**).

Figure 30.

Effect of The Complexation Time.



Figures of merit of RP-SHS-LLME-SDIC

The proposed method's analytical performance was evaluated by plotting an external aqueous calibration graph with standards of copper(II) ions at concentrations ranging from 0.6–25.0 µg mL^{-1} after being complexed with DEBT and measured with SDIC without microextraction (**Table 3**). Moreover, standard-addition calibration graphs were plotted by spiking the oil samples with increasing concentrations of copper(II) ions within the range of 0.3–10.0 µg mL^{-1} and applying the optimized RP-SHS-LLME-SDIC method (**Table 3**). The coefficients of determination (R^2) ranged between 0.9963 and 0.9975, indicating good linearity. The proposed method's precision was calculated as percentage relative standard deviation (%RSD), with intra- and inter-day precisions being found as 2.9–3.9 and 5.0–6.9, respectively. The limits of detection (LOD) and limits of quantification (LOQ) based on $3S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the regression equation, were found to be 0.1 and 0.3 µg mL^{-1}, respectively. The response was linear from LOQ to 10.0 µg mL^{-1}. An enrichment factor (EF), defined as the ratio of the standard-addition calibration slopes to those of the external aqueous calibrations, ranged from 2.6 to 4.6.

Table 3.

Figures of Merit of RP-SHS-LLME-SDIC for Oil Samples.

Method	Oil sample	Regression equation[a]	R^2	LOD[b]	LOQ[e]	LDR[d]	%RSD[e]		EF
							Intraday	Interday	
SDIC	Aq.	$y = 0.0165\ (\pm 0.0002)x - 0.0014\ (\pm 0.0009)$	0.9979	0.2	0.6	0.6-25.0	1.5	2.3	-
RP-SHS-LLME-SDIC	Black seed	$y = 0.0766\ (\pm 0.0012)\ x + 0.1274\ (\pm 0.0033)$	0.9975	0.1	0.3	0.3-10.0	2.9	5.0	4.6
	Olive	$y = 0.0459\ (\pm 0.0006)\ x + 0.0022\ (\pm 0.0012)$	0.9963	0.1	0.3	0.3-10.0	3.0	5.1	2.8
	Corn	$y = 0.0433\ (\pm 0.0005)\ x + 0.0008\ (\pm 0.0012)$	0.9975	0.1	0.3	0.3-10.0	3.2	5.3	2.6
	Sunflower 1	$y = 0.0612\ (\pm 0.0008)\ x + 0.0161\ (\pm 0.0024)$	0.9965	0.1	0.3	0.3-10.0	3.9	6.8	3.7
	Sunflower 2	$y = 0.0598\ (\pm 0.0009)\ x + 0.0192\ (\pm 0.0024)$	0.9963	0.1	0.3	0.3-10.0	3.4	6.9	3.6

[a] $\text{Absorbance (mAu)} = \text{Slope}(\pm\text{SD}) \times [\text{Copper concentration } (\mu\text{g } mL^{-1})] + \text{Intercept}(\pm\text{SD})$.

[b] Limit of detection (µg mL^{-1}).

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

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

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

Recovery studies and determination of copper in oil samples

Matrix effect was investigated by spiking the oil samples at three different concentrations of copper(II) (i.e., 2.0, 4.0, and 8.0 µg mL^{-1}) and addition-recovery tests were performed using the optimized RP-SHS-LLME-SDIC procedure. The percentage relative recoveries (%RR) obtained, considering extraction yields from standard-addition calibrations, ranged from 92.5 to 105.0% (**Table 4**). By comparing the slopes of the individual standard-addition calibration graphs produced with RP-SHSLLME-SDIC to the slopes obtained with aqueous standards, the matrix effect was determined using the ANOVA test (**Table 3**). The difference between the slopes was found to be statistically significant ($P < 0.05$), indicating the presence of matrix effects due to the wide variety of the constituents of the oils analyzed. The standard addition, on the other hand, eliminated this effect. Copper concentrations in the analyzed samples were lower than the method's LOD.

Table 4.

Percentage Relative Recovery of Copper in Oil Samples.

Oil sample	Added (μg mL^{-1})	Found (μg mL^{-1})	%RR[a]
Black Seed	-	< LOD	-
	2.0	2.0	100.0
	4.0	4.2	105.0
	8.0	7.8	97.5
Olive	-	< LOD	-
	2.0	1.9	95.0
	4.0	3.7	92.5
	8.0	8.2	102.5
Corn	-	< LOD	-
	2.0	2.1	105.0
	4.0	3.8	95.0
	8.0	7.8	97.5
Sunflower 1	-	< LOD	-
	2.0	2.1	105.0
	4.0	4.2	105.0
	8.0	7.7	96.3
Sunflower 2	-	< LOD	-
	2.0	2.0	100.0
	4.0	4.1	102.5
	8.0	8.0	100.0

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

Comparison of the proposed RP-SHS-LLME-SDIC with other methods

The proposed method was compared with other methods used for the determination of copper in edible oils in terms of acid and organic solvent consumption, sample size, extraction time, sensitivity, linearity and precision (**Table 5**). These methods include microwave digestion-flame-atomic absorption spectrometry (MW-FAAS) (Mendil, Uluozlu, Tuzen, & Soylak, 2009), reversed-phase ultrasonic assisted-liquid–liquid microextraction-flame-atomic absorption spectrometry (RP-UALLME-

FAAS) (Mohebbi, Heydari, & Ramezani, 2018), microemulsion extraction-graphite furnace-atomic absorption spectrometry (MEE-GFAAS) (Gunduz & Akman, 2015), reversed-phase switchable hydrophilicity solvent-liquid–liquid microextraction-flame-atomic absorption spectrometry (RP-SHS-LLME-FAAS) (Hassan et al., 2020), and high-resolution continuum source-flame-atomic absorption spectrometry (HR-CS-FAAS) (Nunes et al., 2011). The suggested RP-SHS-LLME-SDIC method surpasses the others in terms of cost, ease of use, and minimal dependency on electricity and expertise. Additionally, acid and organic solvent use was greatly decreased. While FAAS and GFAAS are more sensitive than SDIC, they are sophisticated instruments that need extensive experience to operate and maintain them.

Table 5.

Comparison of The Proposed RP-SHS-LLME-SDIC Method with Other Methods for The Determination of Copper in Edible Oils.

Extraction method/ technique[a]	**Volume of acids (μL)**	**Volume of organic solvents (μL)**	**Sample size (mL)**	**Extraction time (min)**	**LOD**[b]	**R^{2}**[c]	**RSD**[d] **(%)**	**Ref.**
MW-FAAS	8000	-	1 g	30	-	-	<11.1	(Mendil et al., 2009)
RP-UALLME-FAAS	200	1000	10	10	0.8 ng mL^{-1}	0.9953	<1.9	(Mohebbi et al., 2018)
MEE-GFAAS	10	10.000	0.5-1.0 g	5	16.2 ng g^{-1}	-	<8.8	(Gunduz et al., 2015)
RP-SHS-LLME-FAAS	750	500	30	5	6.9 ng mL^{-1}	0.9998	<9.4	(Hassan et al., 2020)
HR-CS-FAAS	100	10.000	0.5 g	-	0.12 mg kg^{-1}	0.9995	<6.8	(Nunes et al., 2011)
RP-SHS-LLME-SDIC	140	280	5	5	0.3 μg mL^{-1}	0.9966	<6.9	This study

[a] FAAS: Flame-atomic absorption spectrometry; GFAAS: Graphite furnace-atomic absorption spectrometry; HR-CS: High-resolution continuum source; MEE: Microemulsion extraction; MW: Microwave digestion; RP-SHS-LLME: Reversed-phase switchable hydrophilicity solvent-liquid–liquid microextraction; RP-UALLME: Reversed-phase ultrasonic assisted-liquid–liquid microextraction.

[b] Limit of detection.

[c] Coefficient of determination.

[d] Percentage relative standard deviation.

Synergistic-Cloud-Point Microextraction Combined with UV/Vis Spectrometry for the Determination of Curcumin in Food Samples

Optimization of SCPME conditions

Sample pH

pH of the sample solution plays a critical role in the extraction of curcumin from aqueous solutions. To achieve maximum extraction and preconcentration efficiency, the pH was studied over the range of 3.0 to 9.0. Due to the presence of curcumin in its protonated form in acidic medium (pK_{a1} 8.79, **Figure 31**), a constant high recovery was obtained along the range of 1.0 to 8.0 (**Figure 32**), beyond which it started to decrease, which may be attributed to the ionization of curcumin in basic media. Due to the acidity of the samples, no buffer was added in subsequent experiments.

Figure 31.

Microspecies Distribution of Curcumin Versus pH.

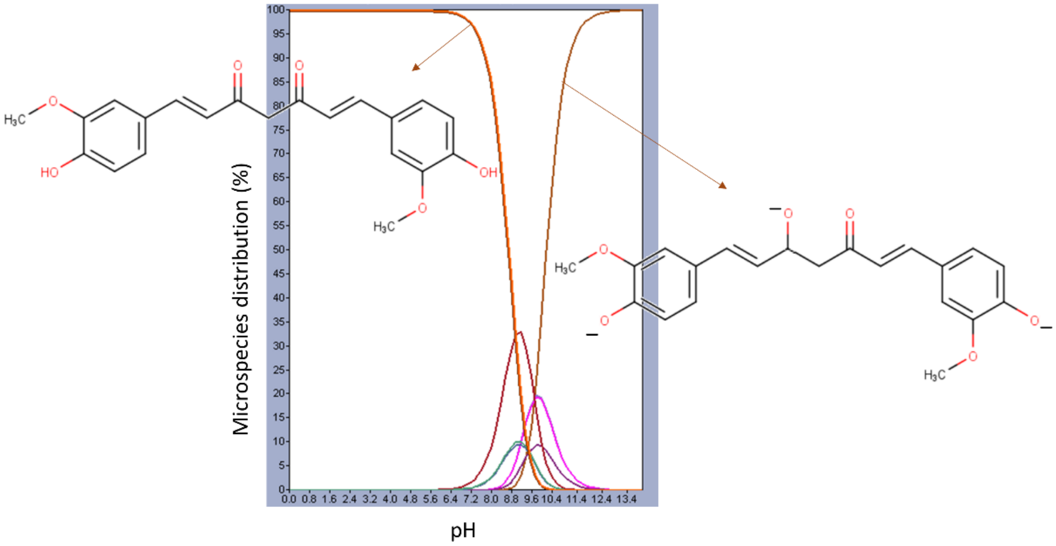
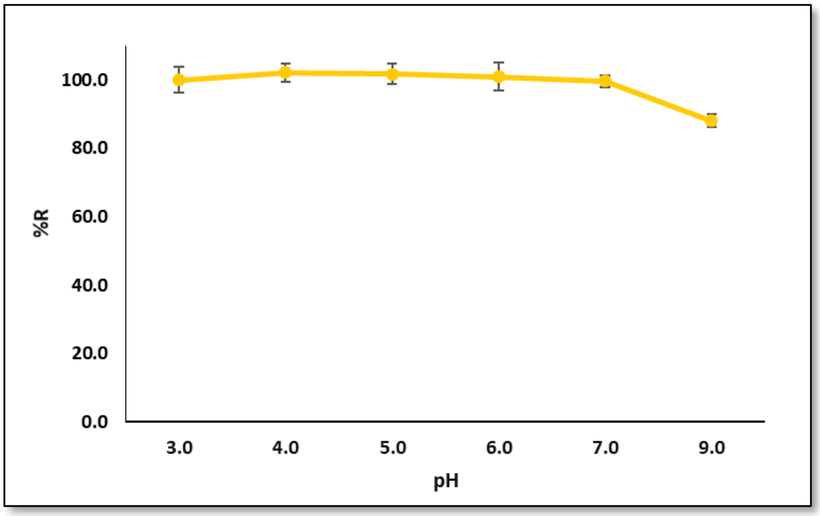


Figure 32.

Effect of Sample pH.



Type and volume of surfactant

Selection of a suitable surfactant as an extraction solvent in SCPME is important. The extractability toward the analyte of the chosen surfactant should be high enough. In this experiment, three NSs, namely, Triton X-114, Triton X-405, and Tergitol, were evaluated. Similar recoveries were achieved with the three surfactants (**Figure 33**). Triton X-405, giving slightly higher recovery and better repeatability, was selected for further study. The effect of volume of Triton X-405 was studied over the range of 50–1000 µL. Low solvent recovery was observed at 50 µL, which had a negative impact on the recovery of curcumin. The analyte recovery increased with increasing the volume of Triton X-405 up to 500 µL, where it stayed almost constant (**Figure 34**). As a conclusion, 500 µL was deemed the optimal surfactant volume for the subsequent studies.

Figure 33.

Effect of The Type of Surfactant.

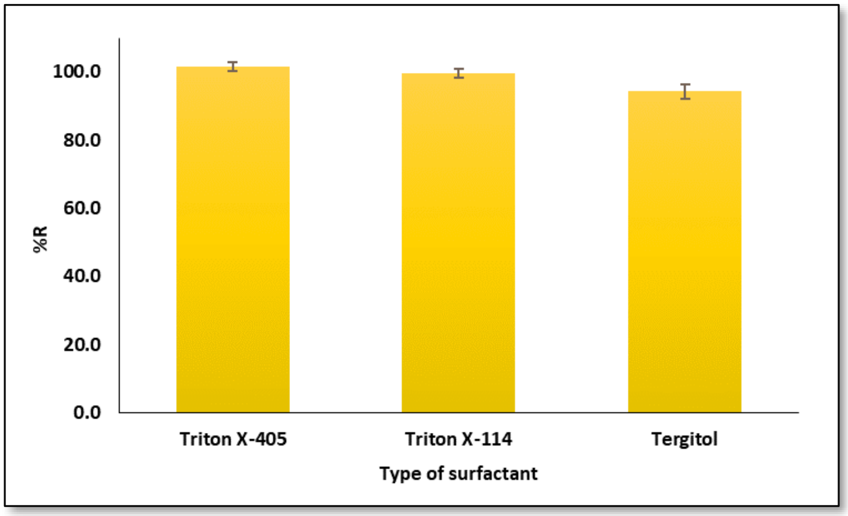
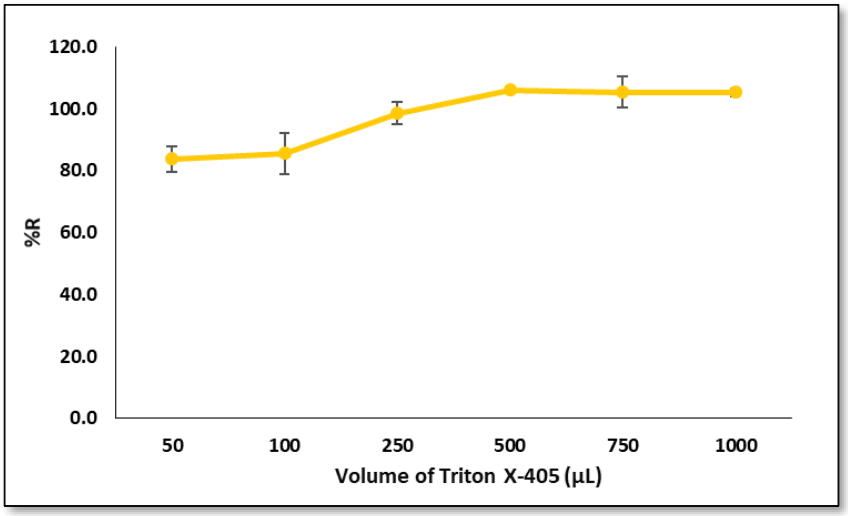


Figure 34.

Effect of The Volume of The Surfactant.



Type and volume of alcohol

Even at low concentrations, the presence of additives in the sample solution changes how the surfactant and the analyte interact by changing the critical micelle concentration (CMC), the size of the micelles, and the way the phases behave in the solution (Gu & Galera-Gomez, 1999). Such additives may include organic solvents, inorganic salts, and other surfactants (Huibers, Shah, & Katritzky, 1997; Wang, Xu, Zhang, Zhuang, & Qi, 2008). NSs, with an average number of oxyethylene units per molecule (p) ≤ 15, have a CP > 100 °C. As a result, Triton X-405 had p < 20 and a CP < 100 °C (Schott, 2001). In the proposed method, an alcohol, 1-pentanol, was added as an additive/synergic reagent to reduce the CPT below room temperature and facilitate the extraction process without the need for salt addition or heating. The type of alcohol was evaluated by using 1-butanol, 1-pentanol, and 1-decanol. 1-Pentanol showed the maximum recovery (**Figure 35**), and was thus selected for further studies. The volume of 1-pentanol was studied starting from 300.0 up to 800.0 µL. The recovery was observed to increase upon the addition of up to 600.0 µL and to decrease afterwards. Thus, a volume of 600.0 µL was set as the optimum volume in subsequent experiments (**Figure 36**).

Figure 35.

Effect of The Type of Alcohol.

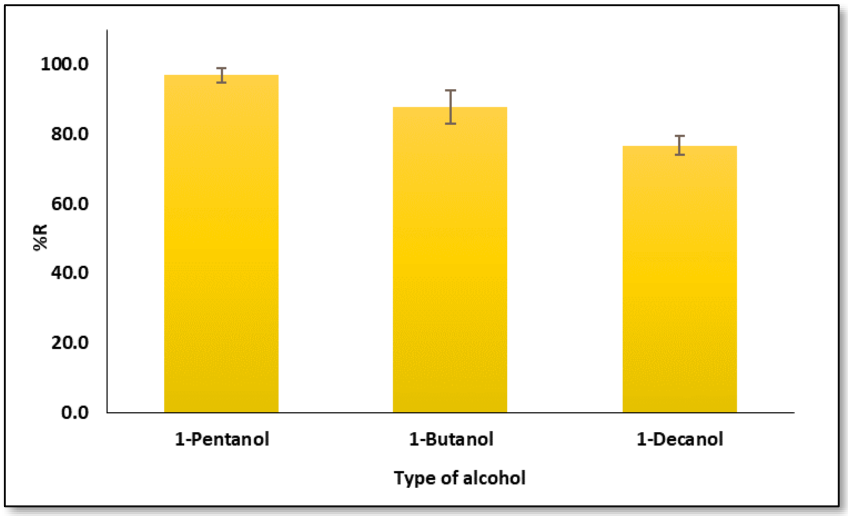
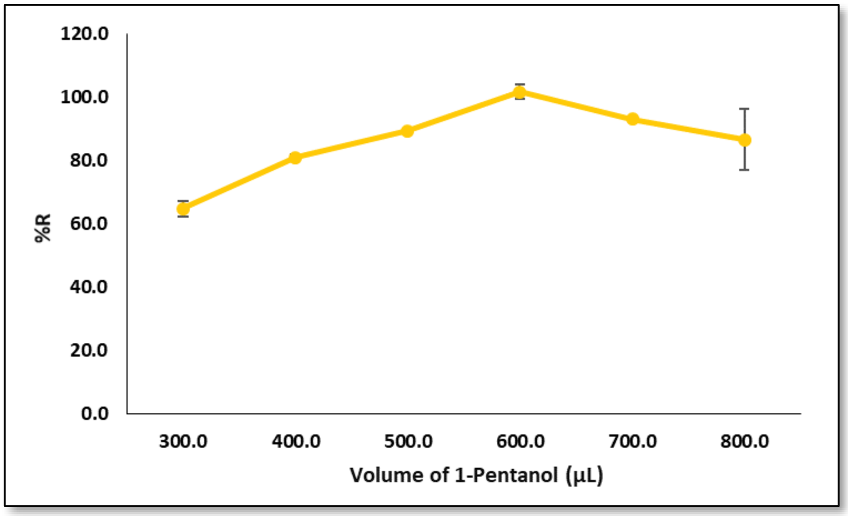


Figure 36.

Effect of The Volume of 1-Pentanol.



Figures of merit of SCPME-UV/Vis

The analytical performance was first evaluated without microextraction by plotting an external aqueous calibration graph with UV/Vis with curcumin standards at concentrations ranging from 1.0 to 15.0 µg mL^{-1}. Standard-addition calibration graphs were then plotted by spiking the food samples with increasing concentrations of curcumin in the 0.10–10.0 µg mL^{-1} range and applying the optimized SCPME-UV/Vis procedure. Analytical performance parameters of the proposed SCPME-UV/Vis method are summarized in **Table 6**. The coefficient of determination (R^2) ranged between 0.9974 and 0.9991, indicating good linearity of the method. Precision, evaluated as percentage relative standard deviation (%RSD), was found as 2.6-4.4 and 4.3-7.5, for intra- and inter-day precision, respectively. LOD values, based on $3S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the regression equation, ranged from 0.012 to 0.027 µg mL^{-1}, and LOQ, based on $10S_b/m$, ranged from 0.041 to 0.090 µg mL^{-1}. The response was linear from LOQ to 10.0 µg mL^{-1}.

Table 6.

Figures of Merit for SCPME-UV/Vis for Food Samples.

Method	Sample	Regression equation[a]	R^2	LOD[b]	LOQ[e]	LDR[d]	%RSD[e]	
							Intraday	Interday
UV/Vis	Aq.	$y = 0.1298\ (\pm 0.0023)x - 0.0434\ (\pm 0.0156)$	0.9958	0.360	1.198	1.198-15.0	4.1	6.2
SCPME-UV/Vis	Turmeric 1	$y = 0.2987\ (\pm 0.0048)\ x + 0.1014\ (\pm 0.0027)$	0.9974	0.027	0.090	0.090-10.0	2.8	5.0
	Turmeric 2	$y = 0.6643\ (\pm 0.0048)\ x + 0.0470\ (\pm 0.0027)$	0.9991	0.012	0.041	0.041-10.0	2.6	4.3
	Turmeric 3	$y = 0.6997\ (\pm 0.0089)\ x + 0.0345\ (\pm 0.0055)$	0.9980	0.023	0.078	0.078-10.0	2.7	4.4
	Turmeric Tea	$y = 1.4796\ (\pm 0.0169)\ x + 0.1295\ (\pm 0.0094)$	0.9979	0.019	0.064	0.064-10.0	4.4	7.5

[a] $Absorbance\ (mAu) = Slope(\pm SD) \times [Curcumin\ concentration\ (\mu g\ mL^{-1})] + Intercept(\pm SD)$.

[b] Limit of detection ($\mu g\ mL^{-1}$).

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

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

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

Interference studies

An interference study was performed to investigate the selectivity of the method in the presence of different concentrations of potential interfering ions and other food dyes that might be present in such samples. The tolerance level was defined as the maximum concentration of the interfering species that may cause a depression of the analytical signal not higher than 10%. The result, shown in **Table 7**, indicated good selectivity of the method toward curcumin in the presence of other interfering ions.

Table 7.

Effect of Interfering Substances on The Extraction of Curcumin[a].

Interfering Species	**Added as**	**Concentration (μg mL^{-1})**	**%R**[b]
Na^+	$NaNO_3$	5000	87.8
K^+	KNO_3	5000	91.6
Mg^{2+}	$Mg(NO_3)_2$	500	94.0
Sunset Yellow	-	1.0	94.0
Tartrazine	-	1.0	99.8
Sudan Dye III	-	1.0	94.3
Lissamine Green Blue	-	1.0	99.5
Chromotrope FB	-	1.0	99.0

[a] Concentration of curcumin was 1.0 µg mL^{-1}, (n = 3).

[b] Percentage recovery, performed with curcumin's concentration of 1.0 µg mL^{-1}.

Recovery studies and determination of curcumin in food samples

Matrix effect was studied by spiking the food samples at three different concentrations of curcumin (i.e., 0.25, 0.50 and 0.75 µg mL^{-1}) and then performing addition-recovery tests using the SCPME-UV/Vis procedure. The percentage relative recovery (%RR) ranged between 90.6 and 105.4 (**Table 8**). The ANOVA test was used to assess the matrix effect by comparing the slopes of standard-addition calibration graphs obtained with SCPME-UV/Vis (**Table 6**). The difference was found to be statistically significant ($P < 0.05$), indicating the presence of matrix effect. As a result, standard-addition to the samples was required to eliminate this effect.

Table 8.

Percentage Relative Recovery of Curcumin in Food Samples.

Sample	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	%RR[a]
Turmeric 1	-	0.34 (1.7, %w/w)	-
	0.25	0.24	94.1
	0.50	0.50	100.4
	0.75	0.72	96.1
Turmeric 2	-	0.07 (0.4, %w/w)	-
	0.25	0.26	105.4
	0.50	0.49	97.5
	0.75	0.76	102.0
Turmeric 3	-	0.05 (0.2, %w/w)	-
	0.25	0.24	98.4
	0.50	0.52	104.8
	0.75	0.72	96.6
Turmeric Tea	-	0.09 (0.4, %w/w)	-
	0.25	0.22	90.6
	0.50	0.52	104.7
	0.75	0.76	101.9

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

Comparison of the proposed SCPME-UV/Vis with other methods

The proposed method was compared with other methods used for the determination of curcumin in food samples in terms of analysis time, type and volume of the extraction solvent, sensitivity, linearity, and precision (**Table 9**). The greenest extraction solvent gains the proposed method superiority over the others, especially those applying toxic chlorinated solvents in ultrasound-assisted ionic liquid-dispersive liquid–liquid microextraction combined with UV/Vis (UA-IL-DLLME-UV/Vis) (Unsal, Tuzen, & Soylak, 2019), and aeration-assisted homogeneous liquid–liquid microextraction with HPLC (AA-HLLME-HPLC) (Khorshidi, Rahimi, & Salimikia, 2020). Despite the higher sensitivity afforded by other techniques, the suggested approach is the fastest and eco-friendliest due to its shorter analysis time and larger organic solvent volume. Supramolecular solvent-liquid–liquid microextraction followed by smartphone digital image colorimetry (SMS-LLME-SDIC) (Caleb & Alshana, 2021) had a shorter analysis time but offered more than 40 times lower sensitivity than the reported method. The linearity and precision were comparable with the others.

Table 9.

Comparison of The Proposed SHS-LLME-UV-Vis Method with Other Methods for The Determination of Curcumin in Food Samples.

Extraction method/ technique[a]	Analysis Time (min)	Type of extraction solvent	Volume of extraction solvent (µL)	R[2b]	LOD[c]	%RSD[d]	Ref.
AA-HLLME-HPLC	27	Chloroform	210	0.9970	19.0	3.0	(Khorshidi et al., 2020)
SMS-LLME-SDIC	3	SMS (THF:1-undecanol)	1000	0.9975	200.0-900.0	8.5	(Caleb & Alshana, 2021)
SPE-CE-AD	1320	-	300	0.9986	10.0	6.3	(Sun, Gao, Cao, Yang, & Wang, 2002)
UA-IL-DLLME-UV/Vis	20	Dichloromethane-[bmim][PF6]	1575	0.9995	0.51	4.1	(Unsal et al., 2019)
DES-DLLME-SFO-HPLC	1516	DES (tetrabutylammonium chloride-decanoic acid)	70	>0.99	0.09	4.2	(L. Y. Xie, Bai, Zhang, Chen, Bai, & Hu, 2020)
SCPME-UV/Vis	8.5	Triton X-405	500	0.9981	20.3	7.5	**This study**

[a] AA-HLLME-HPLC: Aeration-assisted homogeneous liquid–liquid microextraction and high-performance liquid chromatography; SMS-LLME-SDIC: Supramolecular solvent-liquid–liquid microextraction followed by smartphone digital image colorimetry; SPE-CE-AD: Solid-phase extraction-capillary electrophoresis-amperometry detection.

UA-IL-DLLME-UV–Vis: Ultrasound-assisted ionic liquid-dispersive liquid–liquid microextraction and UV/Visible spectrophotometer; DES-DLLME-SFO-HPLC: Deep eutectic solvent - dispersive liquid–liquid microextraction - Solidification of floating organic droplet with high-performance liquid chromatography.

[b] Coefficient of determination.

[c] Limit of detection (ng mL^{-1}).

[d] Percentage relative standard deviation.

Switchable-Hydrophilicity Solvent Liquid–Liquid Microextraction prior to Spectrophotometric Determination of Sudan I Dye in Spices

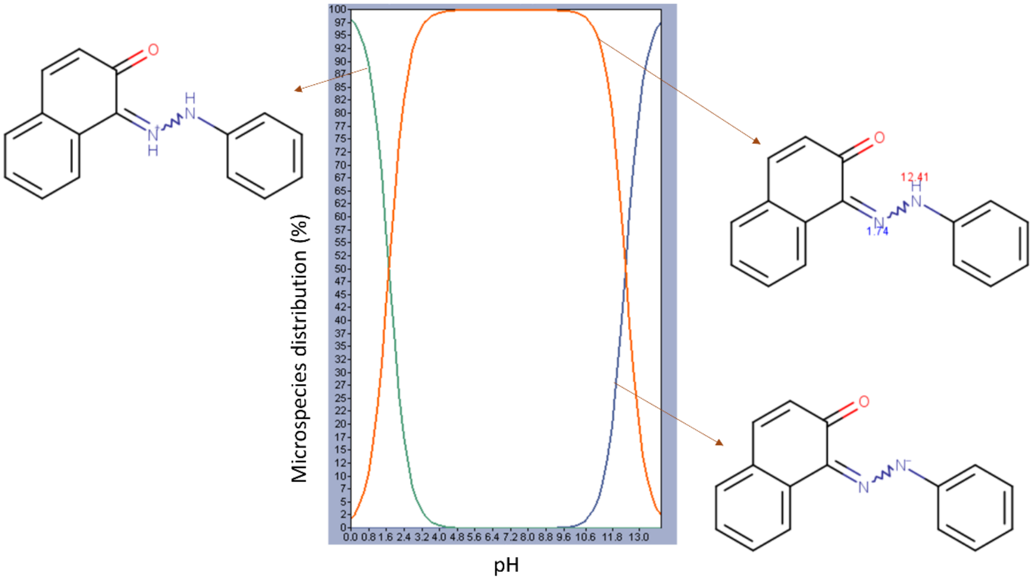
Optimization of SHS-LLME conditions

Sample pH

The effect of pH was investigated over the range of 3.0 to 9.0. Sudan I is present predominantly in its protonated form in the pH range of 4.0-10.0 (**Figure 37**), such a wide range that it was decided that no buffer would be necessary in further experiments.

Figure 37.

Microspecies Distribution of Sudan I Versus pH.



Type and volume of SHS

The extraction solvent used in microextraction is selected based on its miscibility with the aqueous solution in its switched-on and switched-off forms as well as its high analyte extraction capacity (Usama Alshana, Hassan, et al., 2020). Amines with a typical switchable behavior have $logP$ between 1.2 and 2.5 as well as pk_a values greater than 9.5 (Vanderveen, Durelle, & Jessop, 2014). In this experiment, OCA, DMBA, DMCHA, CHA, and DMBenA were used as SHS, along with equal volumes of concentrated nitric acid to switch them on. When compared to the other SHS, OCA provided the highest recovery (**Figure 38**), which was related to its highest $logP$ value of 2.48 (Page 49). As a result, OCA was selected as the optimum extraction solvent. The effect of OCA volume on the extraction efficiency was studied in the range of 400–800 µL. As its volume increased, the extraction efficiency improved, but then gradually decreased due to the dilution effect. Because the maximum recovery was obtained with 600 µL of OCA, this volume was preferred as the optimum for next experiments (**Figure 39**).

Figure 38.

Effect of The Type of SHS.

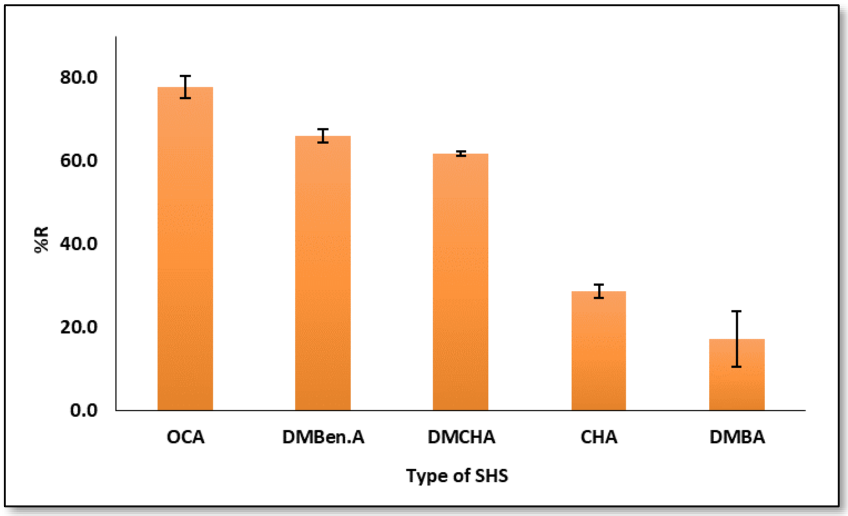
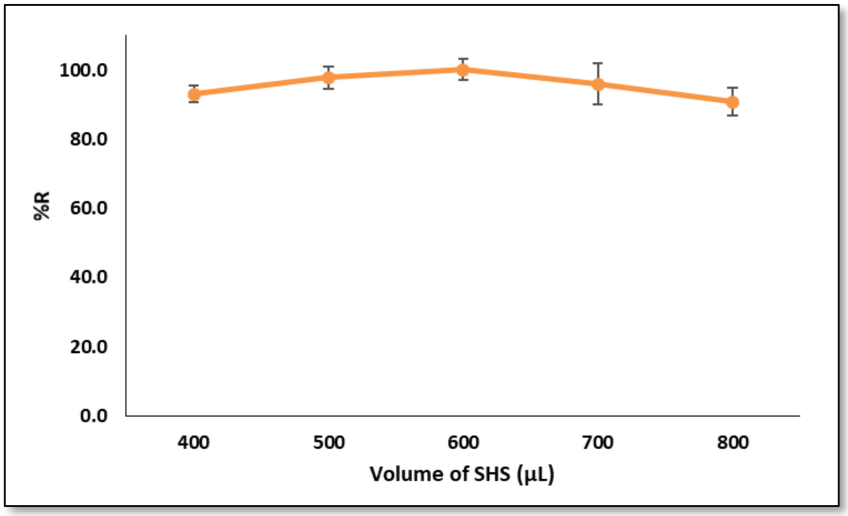


Figure 39.

Effect of The Volume of SHS.

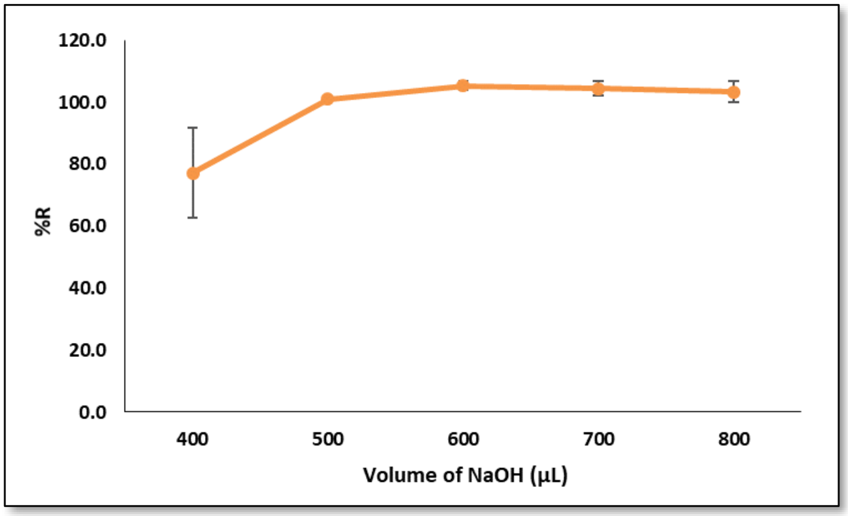


Volume of sodium hydroxide

Phase separation is an important step in this study because extraction occurs at this point. Among other triggers such as purging nitrogen, heating, and adjusting the pH of the solution, highly concentrated sodium hydroxide solutions are desired not only because they deprotonate the SHS, but also because they salt-out hydrophobic analytes that are present in the aqueous solution to the organic layer significantly due to higher ionic strength. In this respect, the effect of varying the volume of 10.0 M sodium hydroxide from 400 to 800 µL on SHS deprotonation was investigated. The recovery increased up to 600 µL and then remained constant (**Figure 40**) such that this volume was set as optimum.

Figure 40.

Effect of The Volume of Sodium Hydroxide.

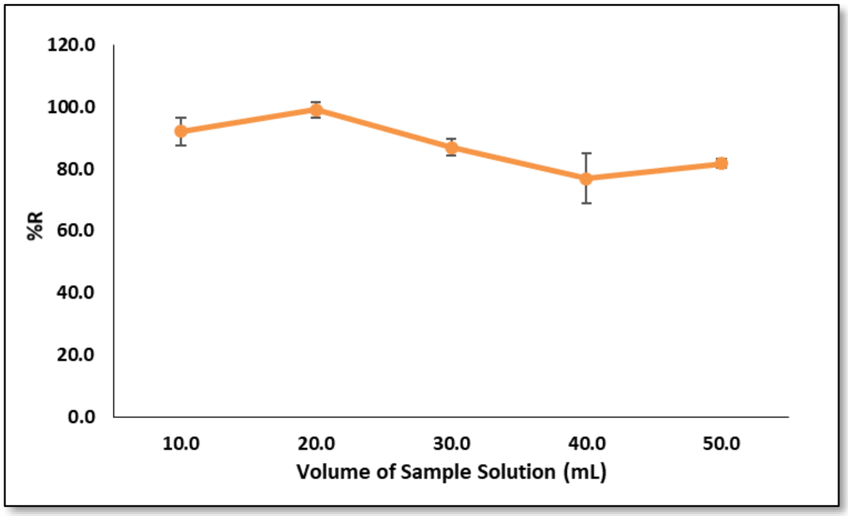


Volume of sample solution

To improve the sensitivity of the proposed method, high recovery and preconcentration factors are always desired, as they are highly dependent on the initial and final volumes. The effect of sample solution volume was evaluated between 10.0 and 50.0 mL, with 20.0 mL achieving the highest recovery (**Figure 41**). As a result, further experiments were performed with 20.0 mL of the sample solution.

Figure 41.

Effect of The Volume of The Sample Solution.



Figures of merit of SHS-LLME-UV/Vis

The analytical performance of the proposed SHS-LLME-UV/Vis method was assessed by plotting an external aqueous calibration graph using UV/Vis with standards of Sudan I dye at concentrations ranging from 1.0 to 30.0 µg mL^{-1} without microextraction. Furthermore, standard-addition calibration graphs were plotted by spiking the food samples with increasing concentrations of Sudan I ranging from 0.1 to 20.0 µg mL^{-1} and using SHS-LLME-UV/Vis. The results are summarized in **Table 10**. The coefficients of determination (R^2) ranged between 0.9983 and 0.9995, indicating good linearity. The method's precision was determined as %RSD, with intra- and inter-day precision ranging from 2.6-3.0 to 4.7-7.7, respectively. LOD values varied from 0.009 to 0.021 µg mL^{-1} and LOQ from 0.029 to 0.069 µg mL^{-1}. LDR was up to 20.0 µg mL^{-1}.

Interference studies

An interference analysis was performed to examine the selectivity of the process at 1.0 µg mL^{-1} of Sudan I dye in the presence of various concentrations of interfering ions and other food dyes that might be present in these samples. The tolerance level was established as the highest concentration of the foreign species that caused changes in the analytical signal that was not less than 10% of the dye alone. The results, summarized in **Table 11**, revealed good selectivity toward Sudan dye I.

Table 10.

Figures of Merit for SHS-LLME-UV/Vis for Spices.

Method	Sample	Regression equation[a]	R^2	LOD[b]	LOQ[e]	LDR[d]	%RSD[e]	
							Intraday	Interday
UV/Vis	Aq.	$y = 0.0472\ (\pm 0.0006)x - 0.0008\ (\pm 0.0087)$	0.9968	0.552	1.837	1.837-30.0	3.1	4.8
SHS-LLME-UV/Vis	Chili pepper	$y = 0.2503\ (\pm 0.0033)x + 0.3207\ (\pm 0.0017)$	0.9983	0.021	0.069	0.069-20.0	2.7	4.7
	Pepper flakes	$y = 0.5420\ (\pm 0.0039)x + 0.2117\ (\pm 0.00217)$	0.9991	0.011	0.038	0.038-20.0	2.9	5.5
	Sweet pepper	$y = 0.5427\ (\pm 0.0039)x + 0.1496\ (\pm 0.0020)$	0.9992	0.011	0.037	0.037-20.0	2.6	6.2
	Sumac	$y = 0.5424\ (\pm 0.0043)x + 0.0386\ (\pm 0.0022)$	0.9990	0.012	0.041	0.041-20.0	3.0	5.2
	Cumin	$y = 0.6100\ (\pm 0.0034)x + 0.0029\ (\pm 0.0018)$	0.9995	0.009	0.029	0.029-20.0	2.6	5.3
	Soy sauce	$y = 0.4632\ (\pm 0.0025)x + 0.0260\ (\pm 0.0013)$	0.9995	0.009	0.029	0.029-20.0	2.8	7.7

[a] $Absorbance\ (mAu) = Slope(\pm SD) \times [Sudan\ I\ dye\ concentration\ (\mu g\ mL^{-1})] + Intercept(\pm SD)$.

[b] Limit of detection ($\mu g\ mL^{-1}$).

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

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

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

Table 11.

Effect of Interferences on The Extraction of Sudan I Dye[a].

Interfering Species	Added as	Concentration (μg mL^{-1})	%R[b]
K^+	KNO_3	5000	101.0
Na^+	$NaNO_3$	3500	92.5
NH_4^+	NH_4Cl	3000	102.0
F^-	NaF	1000	107.6
CO_3^{2-}	Na_2CO_3	1000	95.5
Mg^{2+}	$Mg(NO_3)_2$	100	91.1
4R	-	1.00	92.0
Tartrazine	-	0.25	103.5
Sudan Dye III	-	0.25	94.4
Lissamine Green Blue	-	1.00	91.0
Allura red	-	1.00	104.9
Amaranth	-	1.00	107.0

[a] Concentration of Sudan I dye was 1.0 µg mL^{-1}, (n = 3).

[b] Percentage recovery found with Sudan I dye concentration of 1.0 µg mL^{-1}.

Recovery studies and determination of Sudan I dye in spices

The matrix effect was investigated by spiking the food samples at three concentration levels of Sudan I (i.e., 0.1, 0.3, and 0.5 µg mL^{-1}) and performing addition-recovery studies followed by the optimized SHS-LLME-UV/Vis procedure. %RR varied between 91.1 and 104.4 (**Table 12**). The ANOVA test was used to assess the matrix effect by comparing the slopes of the standard-addition calibration graphs obtained using SHS-LLME-UV/Vis (**Table 10**). It was determined that the difference was statistically significant ($P < 0.05$), showing the matrix effect. In order to counteract its impact, normal addition was necessary.

Comparison of the proposed SHS-LLME-UV/Vis with other methods

In terms of extraction time, type and volume of extraction solvent, sensitivity, linearity, and accuracy, the suggested approach was compared to previous methods used for the measurement of Sudan I in food samples (**Table 13**). Even though some other methods in the literature provided better sensitivity and used lower volumes, the proposed method offers the shortest extraction time. Large volumes of the extraction solvents were required in supramolecular solvent-based microextraction and liquid chromatography-photodiode array (SUPRAS-LLME-LC-DAD) (Lopez-Jimenez, Rubio, & Perez-Bendito, 2010) and magnetic solid-phase extraction coupled with high-performance liquid chromatography (MSPE-HPLC) (Shi, Chen, Hao, Li, Xu, & Wang, 2018) making the proposed method more environmentally friendly. The precision and linearity were comparable with the others as shown in **Table 13**.

Table 12.

Percentage Relative Recovery of Sudan I Dye in Spices.

Sample	**Added (μg mL^{-1})**	**Found (μg mL^{-1})**	**%RR[a]**
Chili pepper	-	1.3 (1.3,%w/w)	-
	0.1	0.097	97.0
	0.3	0.310	103.3
	0.5	0.472	94.5
Pepper flakes	-	0.4 (0.2,%w/w)	-
	0.1	0.094	93.6
	0.3	0.307	102.3
	0.5	0.513	102.7
Sweet pepper	-	0.276 (0.1,%w/w)	-
	0.1	0.091	91.0
	0.3	0.300	100.0
	0.5	0.490	98.0
Sumac	-	0.071 (0.03,%w/w)	-
	0.1	0.094	93.6
	0.3n	0.313	104.4
	0.5	0.509	101.9
Cumin	-	NF	-
	0.1	0.094	93.6
	0.3	0.312	104.1
	0.5	0.490	98.0
Soy sauce	-	0.056	-
	0.1	0.094	93.6
	0.3	0.305	101.8
	0.5	0.494	98.9

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

Table 13.

Comparison of The Proposed SHS-LLME-UV-Vis Method with Other Methods for The Determination of Sudan I Dye in Spices.

Extraction method/ technique[a]	Extraction Time (min)	Type of extraction solvent	Volume of extraction solvent (μL)	R^{2b}	LOD[c]	%RSD[d]	Ref.
SUPRAS-LLME-LC-DAD	10	decanoic acid/THF	4000	> 0.9960	4.2 μg kg^{-1}	< 7	(Lopez-Jimenez et al., 2010)
SFO−DLPME-HPLC	20	1-dodecanol	100	> 0.9921	0.1−0.2 ng g^{-1}	< 4.8	(Chen & Huang, 2014)
MSPE-HPLC	12	Ethyl acetate	2000	> 0.9998	0.5–2.5 μg kg^{-1}	< 9.2	(Shi et al., 2018)
DSSBME-HPLC-UV/MS	20	1-octanol	38	> 0.9945	4.8 μg L^{-1}	< 6.3	(Yu, Liu, Lan, & Hu, 2008)
U-shaped HF–LPME–HPLC–UV/MS	40	1-octanol	18	> 0.9981	90 ng mL^{-1}	5.7	(Yu et al., 2008)
SHS-LLME-UV/Vis	10 s	Octylamine	600	> 0.9983	24 ng mL^{-1}	< 8.6	This study

[a] SUPRAS-LLME-LC-DAD: Supramolecular solvent-based microextraction and liquid chromatography-photodiode array; SFO-DLPME-HPLC: Dispersive liquid-phase microextraction with solidification of floating organic droplet coupled with high-performance liquid chromatography; MSPE-HPLC: Magnetic solid-phase extraction coupled with high-performance liquid chromatography; DSSBME-HPLC-UV/MS: Dual solvent-stir bars microextraction coupled with high-performance liquid chromatography-ultraviolet/mass spectrometry; U-shaped HF-LPME-HPLC-UV/MS: U-shaped hollow fiber-liquid-phase microextraction coupled with high-performance liquid chromatography-ultraviolet/mass spectrometry.

[b] Coefficient of determination.

[c] Limit of detection.

[d] Percentage relative standard deviation.

CHAPTER V

Conclusions and Recommendations

In the first study, reversed-phase switchable-hydrophilicity solvent liquid–liquid microextraction-smartphone digital image colorimetry (RP-SHS-LLME) was used for preconcentration and determination of copper in edible oils. Selectivity and sensitivity were improved by replacing the commonly used continuum light source in SDIC with a monochromatic one and through combining it with RP-SHS-LLME, which expanded its applicability to complex food samples such as oils. Compared to other conventional techniques, this method offers several advantages such as significant reduction of cost, consumption of acids and organic solvents, and minimum dependence on electricity, making it a good alternative in developing countries and small laboratories to those sophisticated analytical techniques. The results show that RP-SHS-LLME-SDIC has the possibility of being used for the measurement of various metal ions and organic analytes in various samples. Although the method's sensitivity was improved by replacing the polychromatic light source with a monochromatic one, it is expected to be improved further in the near future as the technology of cell phones and cameras improves. Furthermore, the use of other ligands and/or microextraction methods that result in a more intense color and a lower volume of the final extract, respectively, would result in higher sensitivity.

In the second study, synergistic cloud point microextraction (SCPME) was developed and coupled with UV/Vis for the determination of curcumin in food samples. 1-Pentanol was used, for the first time, as a cloud-point revulsant of Triton X-405 and synergic reagent for extraction, which decreased the cloud point temperature of the surfactant below room temperature and assisted the subsequent extraction process. The use of the alcohol overcame the drawback of long time and/or energy consumption of conventional cloud-point microextraction by forming the micelles rapidly without heating or salt addition. The proposed method offers several advantages such as rapidness, greenness, simplicity, high sensitivity and good repeatability. SCPME was suggested as a viable option for sample cleanup and preconcentration prior to the measurement of curcumin in food samples. The superior analytical performance of SCPME, as compared to other methods reported in the literature, would gain it a high potential to be applied in routine analysis of a variety of food samples.

In the third study, SHS-LLME was proposed prior to UV/Vis for the determination of Sudan I dye in different food samples. This method provided numerous advantages over conventional sample preparation techniques, including environmental friendliness due to the use of smaller volumes of biodegradable organic solvents, simplicity, cost-effectiveness, short extraction time, and ease of operation. Analytically satisfactory results were obtained, and Sudan I dye could be detected in five different spices.

To conclude, the three studies showed that microextraction techniques improves the sensitivity and selectivity of a relatively simple detection systems such as SDIC and UV/Vis. These methods provided numerous advantages over conventional sample preparation techniques, including environmental friendliness due to the use of smaller volumes of green organic solvents, simplicity, cost-effectiveness, short extraction time, high sensitivity, and good repeatability. In particular, the use of normal- and reversed-phase modes of SHS-LLME expanded the applicability of SHS to cover the extraction of polar (copper) and non-polar (Sudan I) analytes. As compared to UV/Vis, SDIC has remarkable advantages, which include portability, speed, ease of use, almost zero reliance on electricity, cost-effectiveness and the potential for on-site analysis. Such characteristics of the technique are of main importance in developing countries that cannot afford to acquire and maintain expensive instruments.

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Curriculum Vitae

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MAIS AL-NIDAWI

PERSONAL INFORMATION	Marital status	Single
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EDUCATION	October 2009 – July 2013	BSc. in Science (CGPA: 2.75/4.00) Department of Chemistry, Baghdad University, Baghdad, Iraq
	September 2016 – May 2018	MSc. in Analytical Chemistry (CGPA: 3.86/4.00) Department of Analytical Chemistry, Faculty of Pharmacy, Near East University (NEU), T.R.N.C., Turkey Supervisor: Asst. Prof. Dr. Usama Alshana Co-supervisor: Prof. Dr. İhsan Çalış Thesis title: Switchable-polarity solvent-liquid–liquid microextraction of piperine from black and white pepper prior to its determination by HPLC.
	October 2018 – Present	Ph.D. in Analytical Chemistry (CGPA: 3.89/4.00)

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Thesis title:Liquid–Liquid Microextraction Prior to Smartphone Digital Image Colorimetry and UV/Vis for the Determination of Copper, Curcumin and Sudan I Dye.

LANGUAGES	Arabic English Turkish	
WORK EXPERIENCE	2018 – Present	Teaching Assistant Department of Analytical Chemistry, Faculty of Pharmacy, Near East University, Nicosia, TRNC, Turkey
	2013 – 2014	Presentative in Iraqi Biotechnology Co. Ltd. for medical equipments.
ADMINISTRATIVE TASKS	0ctober 2020- Present	Member of Graduation Projects and Sign-Out Committee, Faculty of Pharmacy, Near East University, T.R.N.C., Turkey. Member of Assessment Committee, Faculty of Pharmacy, Near East University, T.R.N.C., Turkey. Member of Student Rights and Advisors Committee, Faculty of Pharmacy, Near East University, T.R.N.C., Turkey.

Member of Lecture Program and Course Opening Committee, Faculty of Pharmacy, Near East University, T.R.N.C., Turkey.

Technology and Research Committee, Faculty of Pharmacy, Near East University, T.R.N.C., Turkey.

COURSES RECENTLY TAUGHT

Year	Semester	Course
2018–Present	Spring	Analytical Chemistry Lab. I (BSc.)
	Fall	Analytical Chemistry Lab. II (BSc.)

RESEARCH INTERESTS

Chromatographic Separation
Smartphone Digital Image Colorimetry
Elemental Analysis
Microextractions
Green Analytical Chemistry
Spectroscopic Techniques

TECHNICAL EXPERIENCE

High-performance liquid chromatography (HPLC)
Atomic absorption spectrometry (AAS)
Ultraviolet/visible spectrophotometry (UV/Vis)

PUBLICATIONS

JOURNAL ARTICLES

[1] **M. Al-Nidawi**, U. Alshana, Reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction of copper prior to its determination by smartphone digital image colorimetry. Journal of Food Composition and Analysis, 104 (2021) 104140. [Impact Factor: 4.556].

[2] U. Alshana, M. Hassan, **M. Al-Nidawi**, E. Yilmaz, M. Soylak, Switchable-hydrophilicity solvent liquid-liquid microextraction. Trends in Analytical Chemistry (TrAC) 131 (2020) 116025. [Impact Factor: 12.296].

[3] **M. Al-Nidawi**, U. Alshana, J. Caleb, M. Hassan, Z. U. Rahman, D. Y. Hanoğlu, İ. Çalış, Switchable-hydrophilicity solvent liquid-liquid microextraction versus dispersive liquid-liquid microextraction prior to HPLC-UV for the determination and isolation of piperine from *Piper nigrum* L.. Journal of Separation Science, 43 (2020) 3053–3060. [Impact Factor: 3.645].

CONFERENCE PRESENTATIONS

INTERNATIONAL

[1] **M. Al-Nidawi**, Ö. Özalp, U. Alshana, M. Soylak, Cloud-point microextraction combined with flame-atomic absorption spectrometry for the determination of Zineb in environmental and food samples. *3rd International Environmental Chemistry Congress (3rd EnviroChem)* (Antalya/ Turkey: 01–04 November 2021).

[2] **M. Al-Nidawi**, F. Uzcan, U. Alshana, M. Soylak, Switchable-hydrophilicity solvent liquid-liquid microextraction prior to the spectrophotometric determination of Sudan I dye in spices. *3rd International Environmental Chemistry Congress (3rd EnviroChem)* (Antalya/ Turkey: 01–04 November 2021).

[3] **M. Al-Nidawi**, U. Alshana, Smartphone digital image colorimetry combined with reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction for the determination of copper in edible oils. 3rd *International Congress on Analytical and Bioanalytical Chemistry (3rd ICABC 2021)* (Online: 22–26 March 2021).

[4] **M. Al-Nidawi**, U. Alshana, J. Caleb, Z. U. Rahman, D. Yiğit, Hanoğlu, İ. Çalış, Scaling-up of dispersive liquid-liquid microextraction for the isolation of piperine from black pepper. *1st International Congress on Analytical and Bioanalytical Chemistry (1st ICABC 2019)* (Antalya/ Turkey: 27–30 March 2019).

[5] Z. Ur Rahman, J. Caleb, **M. Al-Nidawi**, U. Alshana, Solidification of floating organic drop microextraction of piperine from black and white pepper prior to its determination by HPLC. *12th International Symposium on Pharmaceutical Sciences (ISOPS-12)* (Ankara, Turkey: 26–29 June 2018).

[6] **M. Al-Nidawi**, M. Hassan, U. Alshana, Switchable-polarity solvent-liquid-liquid microextraction of piperine from black and white pepper prior to its determination by HPLC. *8th Black Sea Basin Conference on Analytical Chemistry (BBCAC2018)* (Istanbul, Turkey: 09–11 May 2018).

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[7] **M. Al-Nidawi**, U. Alshana, J. Caleb, Z. U. Rahman, D. Yiğit, Hanoğlu, İ. Çalış, Isolating piperine from black pepper by scaled-up dispersive liquid-liquid microextraction. *31. Ulusal Kimya Kongresi* (Istanbul/ Turkey: 10–13 September 2019).

[8] **M. Al-Nidawi**, M. Hassan, U. Alshana, Centrifugeless switchable-polarity solvent liquid-liquid microextraction followed by direct injection into HPLC for the determination of piperine in black and white pepper. *30. Ulusal Kimya Kongresi* (Famagusta, North Cyprus: 05–08 November 2018).

FELLOWSHIPS AND AWARDS

1. Young Researcher Award, Near East University, TRNC, Turkey, 2020
2. Dr. Suat Günsel Lecturing Training Scholarship for Ph.D. Students, Near East University, TRNC, Turkey, 2018.
3. Near East University Scholarship for Master Degree, 2016.

REFERENCES

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