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EXPLORING DEEP EUTECTIC SOLVENTS AS POTENTIAL SOLVENT FOR THE EXTRACTION OF FOOD ADDITIVE ALLURA RED IN FOOD TECHNOLOGY APPLICATION

BY

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LIST OF SYMBOLS

cm^3	Cubic centimetre
R^2	Coefficient of determination
°C	Degree celsius
Fe_3O_4	Iron magnetite
L	Litre
mg	Milligram
µL	Microlitre
mg	Miligram
Na_2SO_4	Sodium sulfate
nm	Nanometer
v/v	Volume per volume
w/v	Weight per volume
SS:DoAc@Fe_3O_4	Magnetic nanoparticles functionalised with deep eutectic solvents (silicone surfactant:dodecanoic acid)
mgL^{-1}	Milligram per litre

LIST OF ABBREVIATIONS

U.S. FDA	United States Food and Drug Administration
DES	Deep eutectic solvent
DoAc	Dodecanoic acid
EU	European Union
ATR-FTIR	Attenuated total reflectance-fourier transform infrared spectroscopy
GC-MS	Gas chromatography-mass spectrometry
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
ILs	Ionic liquids
LOD	Limit of detection
LOQ	Limit of quantification
MCF-7	Human breast cancer cell lines
MCF-10A	Non-tumorigenic epithelial cell lines
MSPE	Magnetic solid-phase extraction
NaOH	Sodium hydroxide
SPE	Solid phase extraction
ICP-MP	Inductively coupled plasma mass spectrometry
OVAT	One Variable at A Time
SS	Silicone surfactant
ARAC	Allura Red AC
EFSA	European Food Safety Authority

MENGEKSPLORASI PELARUT EUTEKTIK MENDALAM SEBAGAI PELARUT BERPOTENSI UNTUK PENGEKSTRAKAN PEWARNA MAKANAN ALLURA RED DALAM APLIKASI TEKNOLOGI MAKANAN

ABSTRAK

Dalam tahun-tahun kebelakangan ini, banyak pewarna makanan sintetik telah digunakan secara meluas sebagai aditif untuk menggantikan pewarna semulajadi dan pewarna azo membentuk kira-kira 65% daripada pasaran pewarna komersial. Kesan toksisiti pewarna azo yang digunakan untuk pewarna produk makanan telah membawa kepada pembangunan kaedah analisis yang sangat sensitif dan selektif untuk penentuan mereka dalam pelbagai matriks makanan yang kebanyakan melibatkan pra-konsentrasi yang kompleks, prosedur yang panjang dan kos instrumen yang tinggi. Oleh itu, kajian ini bertujuan untuk mengkaji penggunaan pelarut eutektik mendalam (DES) sebagai pelarut potensial untuk pengekstrakan Allura Red AC (ARAC). Pertamanya, adsorben nanopartikel magnetik yang difungsikan dengan pelarut eutektik mendalam (surfaktan silikon:asid dodekanoik) (SS:DoAc@Fe_3O_4) telah berjaya disintesis dan dianalisis menggunakan spektroskopi inframerah transformasi Fourier pantulan total terperlemah (ATR-FTIR) untuk mengenal pasti fungsi ikatan. Adsorben ini kemudian digunakan dalam teknik ekstraksi fasa padat magnetik (MSPE) untuk mengekstrak ARAC dalam sampel minuman ringan. Tidak seperti partikel Fe_3O_4 tulen, ARAC sasaran mudah diserap ke permukaan SS:DoAc@Fe_3O_4 kerana terbentuknya interaksi hidrofobik yang kuat antara ARAC dan adsorben, dengan itu menjadikan proses pengekstrakan lebih berkesan. Di bawah syarat-syarat yang dioptimalkan, nilai R^2 yang diperolehi ialah 0.999 manakala had pengesanan (LOD) dan had kuantifikasi (LOQ) ditemui sebagai 0.1 mgL^{-1} dan 0.4 mgL^{-1}, masing-masing.

Nilai pemulihan relatif yang diperolehi berada dalam julat 80 hingga 111% untuk ARAC yang dikaji. Kesimpulannya, teknik MSPE berasaskan SS:DoAc@Fe_3O_4 yang baru diusulkan adalah mesra alam sekitar, mudah, berpatutan, dan berkesan dalam mengekstrak ARAC dari sampel minuman.

EXPLORING DEEP EUTECTIC SOLVENTS AS POTENTIAL SOLVENT FOR THE EXTRACTION OF FOOD ADDITIVE ALLURA RED IN FOOD TECHNOLOGY APPLICATION

ABSTRACT

In recent years, synthetic food dyes have gained significant popularity as additives, replacing natural dyes, with azo dyes comprising approximately 65% of the commercial dye market. However, concerns regarding the toxic effects of azo dyes used in food colouring have prompted the development of advanced analytical methods to detect them in various food matrices. These methods typically involve intricate pre-concentration processes, time-consuming steps, and the use of expensive instruments, emphasizing the need for highly sensitive and selective approaches. Hence, this study aims to explore the application of deep eutectic solvent (DES) as potential solvent for the extraction of Allura Red AC (ARAC). Firstly, the synthesized magnetic nanoparticles functionalised with deep eutectic solvents (silicone surfactant:dodecanoic acid) (SS:DoAc@Fe_3O_4) adsorbent was successfully characterized using attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) for the identification of functional bonds. This adsorbent was subsequently applied in magnetic solid phase extraction (MSPE) technique for the extraction of ARAC in soft drink samples. In contrast to pure Fe_3O_4 particles, the target ARAC can be easily adsorbed onto the SS:DoAc@Fe_3O_4 surface due to the formation of strong hydrophobic interaction between the ARAC and the adsorbents thus amplifying the extraction efficiency. Under the optimized conditions, R^2 value obtained was 0.999 while the limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.1 mgL^{-1} and 0.4 mgL^{-1}, respectively. The relative recovery

values obtained were in the range of 80 to 111% for the studied ARAC. In a nutshell, it can be implied that the newly proposed SS:DoAc@Fe_3O_4 based MSPE technique for the extraction of ARAC in drink samples is environmentally friendly, simple, affordable, and effective.

CHAPTER 1

INTRODUCTION

1.1 General background

Food colorants, also known as pigments, encompass dyes or substances that impart colour to food and beverages. They are broadly utilized both in commercial food production and in home cooking settings. These colorants consist of diverse chemical substances added to food matrices, serving several purposes. They are employed to enrich or safeguard the sensory attributes of food items, which could otherwise be compromised or destroyed throughout the processes of manufacturing or storage. Additionally, food colorants are employed in food technology applications to achieve the desired appearance and colour of the final product. The visual aspects are considered a vital role in the modern consumer's food choices, colour is an integral part of food and beverages. Over the past few years, a multitude of artificial food dyes have been introduced to substitute natural colours. They are predominantly utilized to attain distinct attributes, such as enhanced visual appeal, intensified hues, increased colour stability and uniformity (Yang et al., 2011; Xing et al., 2012).

Synthetic food colours offer several commercially advantageous characteristics, such as affordability, resilience to light and pH changes, and excellent colour stability in comparison to natural colours. These colours are chemical compounds derived from coal tar derivatives, with many of them containing an azo-group. However, there are concerns in the market as some foods and beverages may contain unapproved synthetic colours or excessive amounts of approved synthetic colours. Extensive research has revealed that synthetic food colours are a significant contributor to food poisoning and can lead to severe health issues. It is also not safe to use approved synthetic colours for a long-term. The use of un-approved colours or the

indiscriminate use of approved colours are known to have adverse effects on animal and human health. There is a growing demand for the development of precise, accurate, sensitive, and selective analytical methods for the analysis and quantification of food colorants, owing to their extensive use in food manufacturing processes (Tanaka et al., 2008). Among the synthetic dyes utilized in the food industry, azo dyes dominate the commercial dye market, accounting for about 65% of the share (Ahlström, Eskilsson, & Björklund, 2005). Azo dyes are synthetic organic colorants known for their vibrant and intense colours, making them popular additives in various food products. However, these dyes possess certain characteristics that warrant attention. They are typically resistant to aerobic conditions but can be metabolized by intestinal flora, leading to the formation of aromatic amines (Rafii, Hall, & Cerniglia, 1997). Such compounds have been associated with adverse effects on health, including headaches in adults (Hawley & Buckley, 1976), neurotoxicity (Nagaraja & Desiraju, 1993), genotoxicity (Mpountoukas et al., 2010), and carcinogenic properties (Khehra, Saini, Sharma, Chadha, & Chimni, 2006). Hence, the need for accurate and sensitive analytical methods to detect and quantify these colorants in food products is of utmost importance. Most countries worldwide have taken measures to prohibit the usage of a significant number of azo dyes in food products as a response to the associated health concerns. Over the past few decades, strict regulations have been put in place to govern the use of azo dyes in both domestic food supplies and exports of various nations.

Synthetic food dyes play multiple roles in the food and beverage industries. They are utilized for enhancing natural colours, safeguarding flavours and vitamins from light-induced damage, adding artistic elements to items like cake icing, concealing natural colour variations, countering colour loss caused by light, air, temperature fluctuations, moisture, and storage conditions. Additionally, they help to

identify specific foods, offer a diverse range of nutritious options that cater to consumer demands, enhance taste, and modify the original colour of food or beverages.

In this research, we have focused on the analysis of one of the most widely used food colorant, Allura Red AC (ARAC) given its extensive application in the food industry. Allura Red (E129), also known as Red 40, is an azo dye with a dark red colour and water solubility. Originally derived from petroleum, it serves as a food colouring substitute for amaranth and is commonly added to sodas, baby medicines, and cotton candy. In the United States, Allura Red AC is the most frequently used food colouring in beverages, candy, popsicles, deli meats, cheeses, salmon, over-the-counter (OTC) medications, and liquid suspensions.

Bioactive compounds found in foods are sensitive to heat, as well as chemical, physical, and microbiological changes. Traditional food processing methods have limitations such as nutrient loss, low production efficiency, and high time and energy consumption. To address these shortcomings, new sustainable "green" techniques have emerged, which generally require less time, water, and energy. The demand for environmentally friendly solvents has led to the adoption of ionic liquids (ILs) as replacements for traditional organic solvents, which are volatile and toxic. However, the green aspects of ILs have come under scrutiny due to their poor biocompatibility and biodegradability. As a solution to this concern, deep eutectic solvents (DES) have been introduced as a green alternative to ILs since 2004. DESs can be tailored to exhibit specific properties, such as hydrophobicity or hydrophilicity, providing the advantage of selectively extracting target compounds. This selectivity leads to higher purity and concentration of the bioactive substances obtained from the extraction process (Saini et al., 2022). DESs can be easily modified by adjusting the composition

of their components, making them adaptable to meet the specific demands of the extraction process. This versatility makes DESs well-suited for various food matrices and bioactive compounds (Ünlü et al., 2019). These alternative solvents, DESs, are highly recommended for the extraction of bioactive compounds in food. They offer numerous benefits, including high solvency, low toxicity, low environmental impact, biodegradability, and cost-effectiveness, as they are derived from renewable resources. Furthermore, DESs are easy to recycle without negative effects on the environment.

The essential criteria for determining the effectiveness of a therapeutic compound as an anticancer agent are its antiproliferative and apoptosis-inducing properties. The cellular cytotoxic effects of the compound can be evaluated by determining its half-maximal inhibitory concentration (IC_{50}) (Gopal, 2020). According to the US National Cancer Institute (NCI), a lower IC_{50} value (less than 20 mgL^{-1}) indicates higher cytotoxicity against the targeted cell line (Vijayarathna and Sasidharan, 2012). To assess the cytotoxicity of the studied deep eutectic solvent (DES), MCF-7 (human breast cancer cell lines) and MCF-10A (non-tumor epithelial cell lines) were exposed to varying concentrations of DES for 72 hours in an MTT assay. The percent cell viability was plotted against DES concentration for both cell lines, and IC_{50} values were calculated and recorded. The results demonstrated that DES achieved an IC_{50} value of 12 µg/mL in MCF-7 cells after 72 hours of treatment. Conversely, DES exhibited selectivity in its toxicity, as the IC_{50} value for MCF-10A cells after 72 hours of treatment was 142 μgmL^{-1}, indicating low cytotoxicity against normal MCF-10A cells. In summary, the study showed a time-dependent reduction in cell viability in treated cancer cells (MCF-7) following exposure to DES, while normal MCF-10A cells remained unaffected. This confirms the environmentally friendly and less toxic nature of the DES used in the research.

In this study, the extraction performance of a magnetic deep eutectic solvent as sorbent employed in magnetic solid-phase extraction technique towards Allura Red AC (ARAC) will be investigated. The morphological, structural and optical properties of the DES based magnetic adsorbent will be systematically characterized. The absorbance wavelength of the target dye analyte namely Allura Red AC with different concentrations will be screened using Ultraviolet-visible (UV-Vis) spectrophotometry analysis to obtain the maximum absorbance measurements and the calibration curve will be plotted respectively. The DES based magnetic adsorbent will be synthesized using the chemical co-precipitation method that was demonstrated by Elencovan et al. (Elencovan et al., 2022). The commercial soft drink samples will be prepared according to the method described by Sanagi et al. (Sanagi et al., 2013). The magnetic solid-phase extraction (MSPE) method will be optimized for the analysis of food dye as the target analytes and it will be subjected to UV-Vis spectrophotometry analysis for the identification and quantification of the target analytes. Moreover, to evaluate the practical applicability of the proposed MSPE method combined with UV-Vis spectrophotometry analysis approach, the analytical performance test will be conducted in real complex food matrix samples. This analysis is significant to obtain the figure of merits for linearity, limit of detection (LOD), limit of quantification (LOQ), inter-precision, intra-precision and relative recovery analysis. Hence, we believe that the developed DES based magnetic adsorbent (SS:DoAc@Fe_3O_4) has a huge potential as an alternative solvent in magnetic solid-phase extraction (MSPE) towards azo dyes in food technology applications.

1.2 Scope of the study

This study explored the potential application of DES based magnetic adsorbent, a novel adsorbent synthesized by Elencovan et al. (Elencovan et al., 2022) in solid phase extraction methods for the determination of Allura Red AC in food matrices. The synthesized adsorbent has been adopted from previous work by Elencovan et al. Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) analysis was conducted to confirm the successfully re-synthesized material. Then, the newly synthesized SS:DoAc@Fe_3O_4 adsorbent was applied in MSPE technique. All the methods were optimized and validated for the analysis of Allura Red AC in soft drink samples.

1.3 Problem Statement

In recent years, a multitude of synthetic food dyes have gained widespread usage as additives, replacing natural dyes. This shift is attributed not only to their stability but also to the cost-effectiveness of their production. Among the synthetic dyes utilized in the food sector, azo dyes hold a significant share, accounting for approximately 65% of the market for commercial dyes. Azo dyes, which are synthetic organic pigments, feature azo groups (-N=N-) as a fundamental component of their molecular makeup, and they are primarily derived from coal tar derivatives. Numerous additional research investigations have demonstrated the chemical characteristic of synthetic dyes in their ability to attach to human serum albumin, thereby corroborating their theory regarding the potential harm they might pose to human well-being. The primary governing bodies such as the European Food Safety Authority (EFSA) in Europe and the United States Food and Drug Administration (U.S. FDA) are accountable for evaluating and assessing food products to improve and

uphold health-related security. However, in spite of the presence of diverse regulatory frameworks, the overall approach is grounded in similar stages that demand well-established protocols for risk assessment. Food analysis has become an exceptionally crucial procedure during the food manufacturing process, ensuring the safety and quality of food products. Furthermore, the adverse toxic effects linked to azo dyes employed for food colouring have spurred the advancement of exceptionally sensitive and selective analytical methods for their detection in various food matrices. Despite the existence of many analytical methods previously studied, regrettably, the majority of these methods entail intricate pre-concentration, lengthy procedures, and the use of expensive equipment. An appropriate approach for preparing samples is imperative before conducting instrumental analysis, aiming to eliminate interferences arising from intricate food matrices that could potentially obstruct the chromatographic system. In recent times, deep eutectic solvents (DES), a subgroup of ionic liquids (ILs), have garnered considerable interest as environmentally friendly solvents, owing to their exceptional attributes that encompass easy synthesis, affordability, non-toxicity, and biodegradability. Hence, the development of green solvents has attracted significant interests with the demand for sustainable and green processes. Based on our previous laboratory findings, a newly synthesized magnetic adsorbent named as SS:DoAc@Fe_3O_4 using non-ionic silicone surfactant and dodecanoic acid as a DES in magnetic solid-phase extraction (MSPE) was reported as a reliable approach for the extraction of organophosphorus in vegetable matrices. In this study, this magnetic adsorbent, SS:DoAc@Fe_3O_4 will be employed to explore the extraction performance towards Allura Red AC using the MSPE technique.

1.4 Research objectives

Main objective:

To investigate the potential of deep eutectic solvent based magnetic adsorbent as green material for the extraction of Allura Red AC in food technology application.

Specific objectives:

1. To synthesize and characterize the deep eutectic solvent based magnetic adsorbent.
2. To optimise the extraction performance of magnetic deep eutectic solvent as sorbent in magnetic solid-phase extraction (MSPE) towards Allura Red AC via One Variable at A Time (OVAT) and Taguchi design of experiments.
3. To analyze the concentration levels of Allura Red AC in commercial soft drink products.

1.5 Outline of the thesis

This thesis is structured into five chapters. Chapter 1 provides an introduction to the general background, scope of the study, and research objectives. In Chapter 2, a comprehensive literature review is presented and organized in detail. Chapter 3 outlines the methodology employed in the research, starting with the characterization of SS:DoAc based DES using attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR). Subsequently, the chapter covers the determination of the maximum absorbance of Allura Red AC food dye. Following that, the optimization and procedure of MSPE technique using silicone surfactant-based DES modified Fe_3O_4 particles (SS:DoAc@Fe_3O_4) as a sorbent combined with UV-Vis

spectrophotometry analysis for the determination of Allura Red AC in drink sample was illustrated. The final part outlines the application of the MSPE technique that has been optimized and validated for the analysis of Allura Red AC in commercial soft drink products. In Chapter 4, the characterization results for the adapted SS:DoAc@Fe_3O_4 adsorbent is discussed. This chapter also includes the data for optimization and validation of MSPE technique using SS:DoAc@Fe_3O_4 adsorbent for the determination of ARAC in soft drink samples followed by its application for the analysis of ARAC in commercial soft drink products. Lastly, Chapter 5 highlights general conclusion of the study as well as the future research recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Azo food dyes

Synthetic dyes are used widely because they are stable and their production costs are far lower than those of natural colours and these are often called azo dyes (Yamjala et al., 2016). In general, synthetic food dyes can be split into two categories, namely fat-soluble and water-soluble. Allura Red AC is classified as water soluble dye (Rovina et al., 2016). In the realm of the food industry, azo dyes constitute approximately 65% of the commercial dye market among synthetic colorants (Yamjala et al., 2016). Azo dyes belong to a category of artificial food colourings widely used in the food sector, distinguished by the presence of azo groups (-N=N-) in their chemical composition (Jiao et al., 2016). These dyes consist of one or more azo linkages and are composed of a diazotized amine coupled to an amine or a phenol. Aromatic amines serve as the primary precursors for azo dyes (Chung, 2016). The majority of synthetic compounds with one or more azo groups that are frequently employed in food to enhance their appearance are called food azo dyes. There are already more than 2000 structurally distinct dyes in use, and the annual global production of food azo dyes is expected to be approximately 1 million tonnes (Elbanna et al., 2017). However, some synthetic colourants, particularly azo dyes, are limited or prohibited in food because they can readily be converted to aromatic amines in anaerobic environments. The health effects of aromatic amines include cancer, asthma attacks, allergic reactions, DNA damage, and childhood hyperactivity (Dong et al., 2021).

2.1.1 Allura Red AC

Allura Red AC, chemically referred to as the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl) azo]-2-naphthalenesulfonic acid, is an authorized colour additive widely used in foods, beverages, nutritional supplements, pharmaceuticals, and various other consumer goods worldwide. Commonly known as Food Red No. 40, it is listed as a permitted food colour with the International Numbering System (INS) number 129 (INS 129) in the Codex Alimentarius and is also approved as a colour additive with the E number 129 (E129) in the European Union (EU) (Bastaki et al., 2017). Allura Red is widely recognized as one of the most commonly used synthetic colours in the food industry (Rovina et al., 2016). It has obtained approval for use as a food additive in various countries, including the USA, Europe, Japan, and others. The safety of Allura Red AC was evaluated by the EU Scientific Committee for Food (SCF) in 1984 and 1989, following its initial assessment by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 (JECFA, 1980) (Honma, 2015).

As reported by Zhang et al. (2010), ARAC exhibits electrochemical activity and undergoes an irreversible reaction (Rovina et al., 2016). ARAC and its metabolites have been the subject of extensive research into their toxicological properties in the aspect of carcinogenicity and genotoxicity, and it has been determined that they could potentially be detrimental to human health (Bevziuk et al., 2017). According to Rovina et al., the acceptable daily intake (ADI) of ARAC is within the range of 0 to 7 mg/kg/bw/day (Rovina et al., 2016).

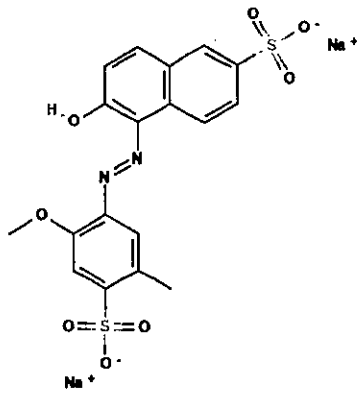

Figure 2.1 Chemical structure of Allura Red AC (PubChem, n.d.)

2.2 Analytical determination of Allura Red AC

The identification of the physical, chemical, and acid-base properties of dyes in a solution is a crucial analytical step. The basis for forecasting the reactivity of the compounds, for instance, is the dissociation constant value (pK) (Bevziuk et al., 2017). Analytical techniques have difficulty detecting Allura Red in possibly interfering substances (Rovina et al., 2016). Given the intricate nature of food product compositions and the limitations of the experimental technique, it is often essential to include sample pretreatment to achieve accurate analysis of synthetic dyes present in food items (Piton et al., 2021). The concentration of synthetic food colours in food products has been detected and measured using a variety of techniques, including thin-layer chromatography, adsorptive voltammetry, differential pulse polarography, and electrophoresis. However, the majority of them demand a considerable amount of time and are unable to isolate dye from dye mixtures (Zahedi et al., 2020). Although spectrophotometric methods are straightforward, enrichment procedures are often required before analysis. Due to their high sensitivity, HPLC procedures are the most often applied methods. However, the chromatographic procedure and dye extraction process can be tedious (Alp et al., 2018). Analysis using spectrophotometry is quick, easy, and economical (Thani, 2017). The most straightforward, time and energy

efficient approach uses solid-phase extraction (SPE) paired with UV-Vis spectrophotometry. It is not dependent on highly experienced or professional personnel, and its instrumentation expenses are minimal. Additionally, the method offers higher preconcentration factors and significantly lower detection limits compared to other methods (Bişgin, 2019).

2.3 Deep eutectic solvent

In a study conducted by Abbott et al., it was observed that certain hydrogen bond donors (HBDs) and acceptors (HBAs) exhibited an unusually significant reduction in melting point at the eutectic composition. This intriguing finding paved the way for the discovery of deep eutectic solvents (DESs), a novel category of environmentally friendly solvents closely related to ionic liquids (ILs). Over the last two decades, DESs have garnered increasing attention in the scientific community, as highlighted by the work of Hansen et al. (2021). Ionic liquids have been reported to be have certain drawbacks in terms of being costly, its toxicity and poor biodegradability. Deep eutectic solvents have appeared to address the shortcomings of ILs. DESs have been used extensively to extract or separate targeted components from mixtures since they are environmentally friendly solvents (Tang et al., 2015). The physicochemical characteristics of DESs are similar to those of conventional ILs, such as low vapour pressure, but they are less expensive to make since the raw ingredients needed are cheaper, and the synthesis is not as complex (Li and Row, 2016). DESs are experiencing a growing presence in analytical studies, extending beyond their role as efficient extractants in extraction methods. They are finding utility in various other domains of analytical science, such as chromatographic separation, electrochemical analysis, the disintegration of liquid and solid samples, as well as the synthesis and customization of

novel sorption materials (Shishov et al., 2020). An investigation was conducted to examine the extraction of aromatic hydrocarbons from contaminated water samples using a hydrophobic deep eutectic solvent (DES). This study resulted in no significant decline in their individual extraction efficiency and DES recovery. Consequently, yields the overall performance of HDES for the extraction process (Yakubu et al., 2022).

2.4 Magnetic solid-phase extraction (MSPE)

Due to its excellent selectivity, sensitivity, and effectiveness, magnetic solid-phase extraction (MSPE) is a sample preparation method that has become increasingly prevalent in recent years. MSPE involves the selective extraction of target analytes from intricate matrices using magnetic nanoparticles (MNPs) as sorbents. The target analytes can be extracted selectively from the solution using MNPs that have been functionalized with certain ligands that can interact with them. Various types of samples, such as bodily fluids (Bladergroen and van der Burgt, 2015), environmental samples (Hagarová, 2020), and food samples (Kepekci-Tekkeli et al., 2019), have been subjected to MSPE analysis. High-throughput proteomics analysis also used the method paired with mass spectrometry (Bladergroen and van der Burgt, 2015). The use of MSPE for rapid separation of metallic nanoparticles and their ionic species is another example of recent developments in MSPE (Hagarová, 2020), along with the creation of novel materials and trends in sorbents for solid-phase extraction (Bladergroen and van der Burgt, 2015). The quick and effective extraction of target analytes from complex matrices using MSPE is a promising method, and it is anticipated that additional developments in analytical chemistry will result from its further research and use in a variety of areas (Reyes-Garcés et al., 2018).

2.5 Magnetic solid-phase extraction (MSPE) for Allura Red AC

Magnetic solid phase extraction (MSPE) holds significant promise for the separation and assessment of azo dyes within food matrices. MSPE rapidly extracts and isolates azo dyes from substantial food samples, particularly advantageous when dealing with intricate food matrix (Abbas et al., 2023). Incorporating magnetic nanoparticles in MSPE yields ample adsorption surface, resulting in enhanced extraction efficiency and increased sensitivity (Jangju et al., 2017). MSPE's simplicity stems from utilizing an external magnetic field to segregate magnetic nanoparticles from the sample matrix, thus simplifying the extraction process and economizing time (Abbas et al., 2023). MSPE effectively mitigates matrix effects in food samples, enabling precise and dependable azo dye analysis. This is critical for ensuring food product quality and safety (Aashima et al., 2019). Integration of magnetic nanoparticles in MSPE diminishes reliance on organic solvents and other chemicals, presenting an environmentally mindful extraction approach. Several studies have demonstrated the potential of functionalized magnetic nanoparticles in the extraction of azo dyes, particularly Allura Red AC. Table 2.1 provides a summary of previous studies on the application of MNPs in MSPE technique for the extraction of ARAC. These investigations show that the employment of MSPE holds great potential in extracting and quantifying ARAC within food and beverage samples. This approach offers simplicity, rapidity, remarkable sensitivity and selectivity, rendering it an appropriate substitute for conventional methods of sample preparation for the extraction of azo dyes in complex food matrices.

Table 2.1 Application of magnetic nanoparticles in MSPE technique for the extraction of Allura Red AC.

MNP coated material	Matrix	Method	LOD	LOQ	RSD(%)	Reference
Tetraethylorthosilicate and 3-aminopropyltriethoxysilane and modified by graphene@Fe_3O_4	Beverage, candy and jelly	MSPE coupled with HPLC	2 μg kg^{-1}	7 μg kg^{-1}	3.3	(Yu and Fan, 2016)
Fullerene-activated carbon@Fe_3O_4	Water samples	MSPE coupled with capillary electrophoresis	1.0-2.0 mg/L	n.a	<10	(Abbas et al., 2023)
Elaeagnus angustifolia@Fe_3O_4	Colored sugar-coated chickpeas, child syrup and mineral water	MSPE coupled with HPLC	0.05 μg/mL	0.15 μg/mL	<8.1	(Oymak and Dural, 2023)
Zein biopolymer@Fe_3O_4	Carbonated beverage, snacks and candy	DM-μ-SPE coupled with HPLC with diode array detection	0.3 ng mL^{-1}	n.a	4.4	(Jangju et al., 2017)
Mesoporous aerogel magnetic graphene oxide@zein	Gummy candy	M-DMSPE coupled with HPLC with diode array detection	5 ng/mL	n.a	0.7	(Hamidi et al., 2021)

n.a: not applicable
[a]MSPE - Magnetic solid-phase extraction
[b]HPLC – High-performance liquid chromatography
[c]DM-μ-SPE - Dispersive μ-solid-phase extraction
[d]M-DMSPE - Magnetic-dispersive micro solid phase extraction

CHAPTER 3

METHODOLOGY

3.1 Chemicals and materials

The food dyes were purchased from R&M Chemicals (Essex, UK). The ultrapure water (18.2 MΩ/cm) supplied by Sartorius Milli-Q system (Aubagne, Germany) was used to prepare the standard stock solution for ARAC at the concentration of 1000 mgL^{-1}. Daily preparation of the standard working solutions involved dilution of a sufficient quantity of the stock solutions with ultrapure water for the extraction analysis. Additionally, the stock solution for salt was created by dissolving the requisite amount of salt in ultrapure water to achieve a concentration of 25% (w/v). Miracle S240, a non-ionic silicone surfactant, was obtained from G-Planter (Johor, Malaysia). Its IUPAC designation is 3-[methyl-bis(trimethylsilyloxy)silyl]propan-1-ol;2-methyloxirane;oxirane. Sodium sulphate (Na_2SO_4) was purchased from Qrec (Chonburi, Thailand). Methanol and acetonitrile of HPLC grade were obtained from Fisher Scientific (Seoul, Korea). R&M Chemicals (Essex, UK), Friedemann Schmidt Chemicals (Parkwood, Australia), Sigma-Aldrich (Darmstadt, Germany) and Chemiz (Shah Alam, Malaysia) supplied the Iron (II) chloride tetrahydrate (FeCl2.4H2O), iron (III) chloride hexahydrate (FeCl3.6H2O), ammonium hydroxide (25%), sodium hydroxide (NaOH), and hydrochloric acid (HCl) respectively. Absolute ethanol was purchased from Fisher Scientific (Seoul, Korea).

3.2 Instrumentation

Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) was employed to identify the functional bonds present in the newly synthesised SS:DoAc based DES. The spectra were examined using Fortier from Perkin Elmer USA

in the 4000 to 600 cm^{-1} range. The Perkin Elmer Lambda 25 UV-Vis spectrophotometer was used to analyze the analytical response of ARAC in this study. The Minitab 2022 (v21.3.1) software was used to investigate the signal-to-noise ratios of the experimental parameters that was optimized for the MSPE technique via Taguchi design of experiments.

3.3 Determination of maximum absorbance measurement of Allura Red AC via UV-Vis spectrophotometer

The maximum absorbance measurement for Allura Red AC was studied using the UV-Vis Spectrophotometer. Firstly, preparation of stock solutions for the food dye, Allura Red AC was made according to the dilution equation. The stock solution was prepared in a concentration of 1000ppm in 10mL solution. 10mg of Allura Red AC and was measured and diluted with ultrapure water to achieve the volume of final solution. Further dilutions for the working solution were made for concentrations of 2ppm, 4ppm, 6ppm, 8ppm, 10ppm and 12ppm accordingly. The different dilution concentrations were employed for the analysis of the analyte spectrum to further identify the maximum absorbance measurement for ARAC. A calibration curve was plotted according to the respective absorbance readings.

3.4 Application of magnetically modified non-ionic silicone surfactant and dodecanoic acid based DES (SS:DoAc@Fe_3O_4) in MSPE for the analysis of Allura Red AC in soft drink samples.

3.4.1 Synthesis of SS:DoAc based DES

The SS:DoAc based DES used in this study was synthesized according to the procedure mentioned in previous work (Elencovan et al., 2022). The chemical co-precipitation method was used to synthesise SS:DoAc@Fe_3O_4 adsorbent. The DES was made by combining silicone surfactant (SS) as a hydrogen bond acceptor (HBA) and dodecanoic acid (DoAc) as a hydrogen bond donor (HBD) at a molar ratio composition of 1:5 respectively. A clear, homogeneous liquid of SS:DoAc-based DES was produced by heating the mixture at 70°C for 15 min while stirring continuously. Then, 150 µL of SS:DoAc based DES was added to the mixture of 1.4576g of $FeCl_2 \cdot 4H_2O$ (Iron(II) chloride tetrahydrate) and 3.7306g of $FeCl_3$. $6H_2O$ (Iron (III) Chloride Hexahydrate). Subsequently, 75mL of ultrapure water was incorporated into the mixture. The mixture was subjected to vigorous stirring using a magnetic stirrer for 30 minutes at 85°C under nitrogen gas stream protection. Next, 10 mL of ammonium hydroxide (25%) was added dropwise to the mixture and agitated constantly for 60 minutes. After that, the synthesized SS:DoAc@Fe_3O_4 adsorbent was collected using an external magnet. Then, the SS:DoAc@Fe_3O_4 adsorbent was rinsed repeatedly, alternating between ethanol and ultrapure water to ensure complete removal of any residual unreacted chemicals. Lastly, the isolated SS:DoAc@Fe_3O_4 adsorbent was placed to dry in an oven for 24 hours at 80°C.

3.4.2 Proposed SS:DoAc based DES in MSPE technique

The magnetic solid-phase extraction (MSPE) method was conducted as described by Elencovan et al. altered with some modifications (Elencovan et al., 2022). Firstly, a mass of 25 mg of SS:DoAc@Fe_3O_4 adsorbent was weighed precisely and incorporated into 10 ml of sample solution containing target dye analytes, followed by the addition of 800 µL of 25% (w/v) sodium sulfate (Na_2SO_4) in order to increase the ionic strength. Then, the sample vial was vortexed for 2 minutes at 2500 rpm using a vortex mixer. This step is to promote the adsorption of target dye analytes onto SS:DoAc@Fe_3O_4 adsorbent. Next, an external magnet was used to separate the SS:DoAc@Fe_3O_4 adsorbent while the supernatant obtained was discarded. Afterwards, 700 µL of methanol that acts as a desorption solvent was transferred to the isolated SS:DoAc@Fe_3O_4 adsorbent. The mixture was then subjected to sonication for 2 minutes to facilitate the elution of target dye analyte from SS:DoAc@Fe_3O_4 adsorbent into methanol. Subsequently, SS:DoAc@Fe_3O_4 adsorbent was separated using an external magnet and the methanol was carefully retrieved with the aid of a micropipette tip and filtered using a 13mm nylon syringe filter with a pore size of 45µm. The final solution was then transferred into a 20mL vial. Lastly, the methanol containing target dye analyte was subjected to UV-Vis spectrophotometry analysis for further identification and quantification of the target analyte.

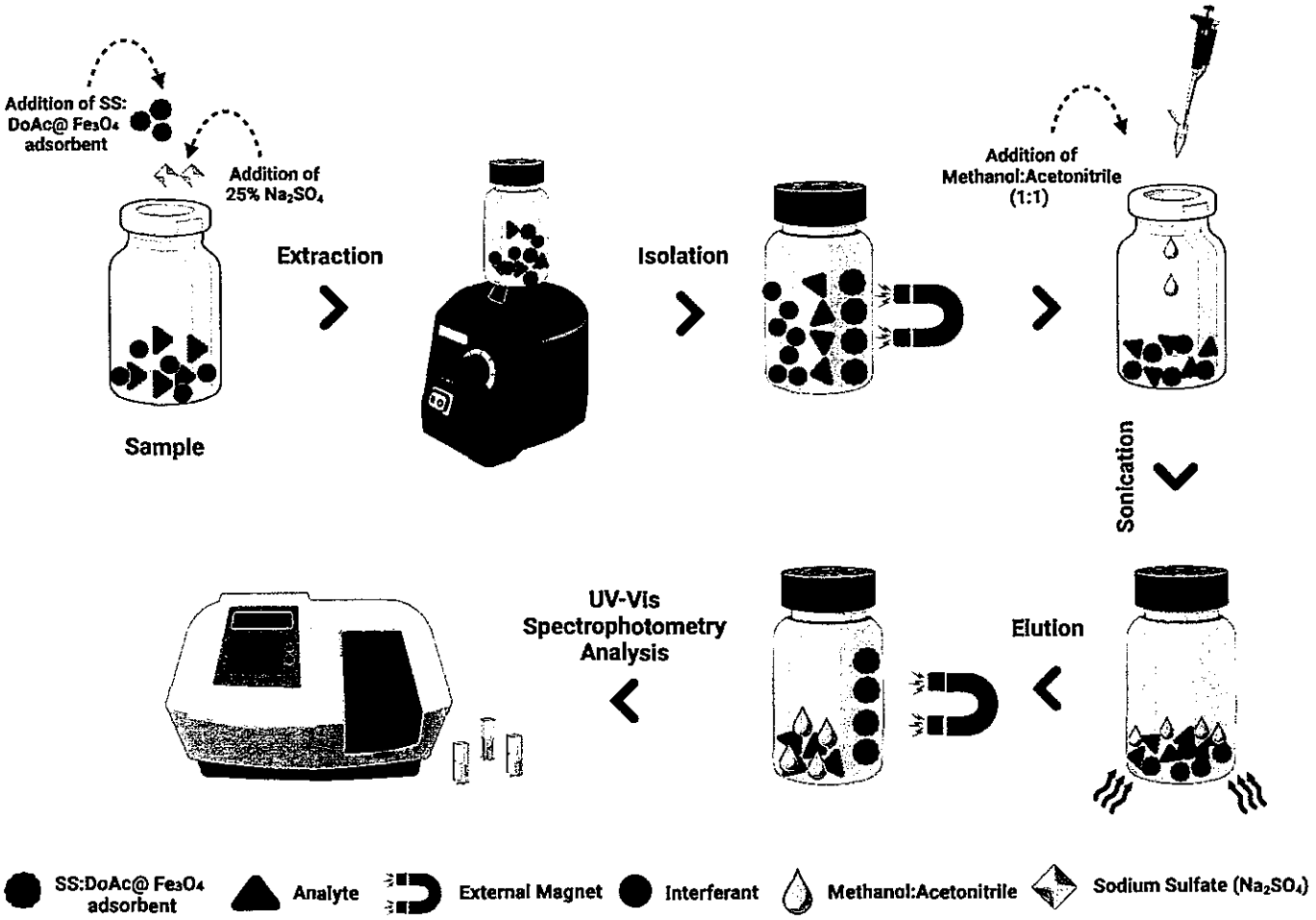

Figure 3.1 Schematic diagram of MSPE technique.

3.4.3 Preliminary screening extraction performance of Allura Red towards SS:DoAc based DES in MSPE

Using the MSPE approach, a preliminary study was conducted to compare the performance of newly synthesized SS:DoAc@Fe_3O_4 adsorbent with control (Fe_3O_4 particles). Other extraction parameters include 25 mg of adsorbent, 10 mL of sample solution, 2 minutes of extraction time, 800 µL of 25% (w/v) Na_2SO_4 and desorption parameters consisting 700 mL of methanol and 2 minutes of sonication.

3.4.4 Optimization of SS:DoAc based DES in MSPE technique via One Variable at A Time (OVAT) design of experiments

The OVAT (One Variable at a Time) design of experiments is a simple and straightforward approach in experimental design where one independent variable is varied while keeping all other variables constant. This method involves changing one factor at a time to observe its individual effect on the dependent variable. It is often used in preliminary stages of research. This approach allows researchers to quickly evaluate the impact of individual factors on a system without the complexity of considering interactions between multiple variables. In this study, in-depth analysis was conducted on the types of desorption solvent, addition of salt, volume of salt, volume of sample solution and also point zero charge as well as how they affect the extraction performance of SS:DoAc-MSPE method. All the experiments were conducted in triplicates (n=3).

3.4.4(a) Type of desorption solvent

Four different types of desorption solvents, including methanol, acetonitrile, a 1:1 mixture of methanol and acetonitrile, and isopropanol were evaluated in this study. The following extraction conditions were present: 10 mL of sample solution, 800 µL of 25% (w/v) Na_2SO_4, 2 min of extraction time, 25 mg of SS:DoAc@Fe_3O_4 adsorbent, while 700 µL of desorption solvent, and 2 mins of sonication were applied for the desorption process.

3.4.4(b) Salt addition

To assess the effectiveness of target analyte extraction and recovery with and without the addition of salt, 25% (w/v) Na_2SO_4 was used to study the effect of salt. The following extraction conditions were present: 10 mL of sample solution, 800 µL of 25% (w/v) Na_2SO_4, 2 min of extraction time, 25 mg of SS:DoAc@Fe_3O_4 adsorbent, while 700 mL of desorption solvent, and 2 mins of sonication were applied for the desorption process.

3.4.4(c) Volume of salt

The effect of salt volume was investigated using volumes of 25% (w/v) Na_2SO_4 in the range of 200 µL to 800 µL. The following extraction conditions were present: 10 mL of sample solution, 2 mins of extraction time, 25 mg of SS:DoAc@Fe_3O_4 adsorbent, while 700 µL of desorption solvent, and 2 mins of sonication were applied for the desorption process.

3.4.4(d) Volume of sample solution

Different volumes of sample solution were investigated in this procedure with the range of 10 mL to 25 mL. The following extraction conditions were present: 800 µL of 25% (w/v) Na_2SO_4, 2 mins of extraction time, 25 mg of SS:DoAc@Fe_3O_4 adsorbent, while 700 µL of desorption solvent, and 2 mins of sonication were applied for the desorption process.

3.4.4(e) Point of zero charge

The point of zero charges (pzc) of the adsorbent were determined using this approach. 25 mg of newly synthesized SS:DoAc@Fe_3O_4 adsorbent and control (Fe_3O_4 particles) were added to 10mL solutions with pH levels of pH 2, 4, 6, 8 and 10 respectively. An orbital shaker was used to agitate the mixture for 24 hours. Subsequently, the final pH of the mixtures was determined using a pH meter and the graph of ΔpH versus the initial pH was plotted.

3.4.5 Optimization of SS:DoAc based DES in MSPE technique via Taguchi design of experiments

A process can be optimised using the Taguchi design of experiments method by identifying the crucial parameters that influence the output and determining the ideal levels of these parameters. This strategy uses the one variable at a time method, which is comparable to the conventional approach known as OVAT. In this strategy, five different parameters were analysed simultaneously where only one parameter was manipulated at a time whereas the rest were held constant. The parameters that were analysed in this work includes pH of sample solution, mass of adsorbent (mg), extraction time (min), desorption time (min), and volume of desorption solvent (µL).