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Deep Eutectic Solvent Based Dispersive Liquid-liquid Microextraction for the Determination of Azo Dyes in Beverages

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Azo dyes in beverages pose a major health threat to public health and restricted in many countries. To ensure the integrity of beverages, the presence of permitted azo dyes/food colourants should be monitored to be within the permissible limit. A simple, easily accessible and cheap approach for the determination of azo dyes (acid orange 20 and tartrazine) namely, dispersive liquid-liquid microextraction (DLLME) based on deep eutectic solvent (DES) was developed. DES was prepared by heating a mixture of a hydrogen bond donor (fatty acid) and a hydrogen bond acceptor (quaternary ammonium salt) at 80 °C with the ratio of 1:3. The prepared DES was added into the aqueous sample solution and followed by a disperser solvent (tetrahydrofuran). The mixture was sonicated and centrifuged to allow the formation of two-layer phases. The DES phase was transferred into a vial before analysed quantitative ly using UV-Vis spectrophotometer. Under optimum conditions, the developed DLLME method showed the limit of detection (LOD) and limit of quantification (LOQ) of 0.007–0.034 mg L^{-1} and 0.025–0.1141 mg L^{-1}, respectively for both analytes. Intra-day and inter-day precision were 2.1–3.2 % and 5.4–9.4 %, respectively. The recoveries for spiked samples were in the range of 90.9–110.1 %. Based on the results obtained, the developed DLLME-DES method has a great potential as a sample preparation technique for the determination of azo dyes in aqueous samples.

Key words: Dispersive liquid-liquid microextraction; deep eutectic solvent, azo dyes; tartazine and acid orange

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Many manufacturers use food dyes to improve the appearance and colour of beverages for the aesthetic appeal to attract customers. Synthetic dyes have been widely employed in substitute for natural dyes due to advantages such as ease of manufacture, low cost, and good stability [1,2]. Azo dye is a type of synthetic dye that often used in foods and beverages to intensify vibrant colors. However, azo dyes are easily reduced to aromatic amines (e.g., benzidine, 4-aminobi-phenyl, and 2-aminoazobenzene) under anaerobic circumstances, that exerted adverse impacts on human, including allergic reactions, asthma, DNA damage, hyperactivity in children, and cancer [3,4]. Due to the long-term negative and harmful effects, therefore, it is deemed worthwhile to attempt to develop an analytical method to determine presence of azo dyes in food samples which will not only facilitate in market monitoring but will also help protect public health.

Due to the low concentration and complexity of the matrix, sample preparation process plays an important role in enhancing sensitivity and reducing matrix interference. Liquid–liquid extraction (LLE) and solid phase extraction (SPE) are the most commonly used for sample preparation of azo dyes [5–7]. However, LLE is time-consuming and requires large volumes of toxic organic solvents. SPE is less solvent consumption method compared to LLE but requires cartridge conditioning and elution with organic solvents. Because of these limitations, liquid-phase microextraction (LPME), a miniaturised LLE, has been developed in recent decades which simple and economic as only a few microlitres of organic are needed to extract analyte from the aqueous samples. Dispersive liquid–liquid microextraction (DLLME) is one of the most recent developments in LPME procedures. To extract the target analyte in DLLME, a high-solubility extraction solvent and an emulsifier solvent are added to the sample solutions. A cloudy solution is obtained as a result of the formation of small droplets in the medium. The amount of analyte removed to the extraction phase is determined using an appropriate separation technique after centrifugation and separation of organic and aqueous layers. Of the plethora of microextraction techniques, DLLME has demonstrated various advantages over other micro-extraction procedures, including cost and time, lower organic solvent usage, and higher extraction efficiency [8].

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Choice of extraction solvent is of great significance aspect of DLLME. The commonly used extractants among DLLME were chlorobenzene, methylene chloride, tetrachloroethylene, carbon tetrachloride, trichloromethane and other toxic reagents which are harmful to the environment and human health. Over the past decade, a new type of ionic liquid solvents known as deep eutectic solvents (DESs) has garnered interest due to their unique properties. Compared to typical organic solvents, DESs are more desirable because they are of low melting point, nontoxic, cheap starting materials, easy to synthesize, biocompatible, biodegradable, low volatility and non-flammable [9]. DESs are composed of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) at room temperature and this mixture forms a eutectic with a much lower melting point than either of the individual components [10,11]. These two components self-associate via hydrogen bonding. Different combinations of HBD and HBA can be carried out to obtain required affinity with regard to extraction of target analytes [12]. Generally, DESs and their targeted analytes also interact through hydrogen bonding, electrostatic and dispersion interactions [13].

The majority of the proposed DESs are hydrophilic and water-soluble, making analyte extraction from aqueous samples difficult, and hydrophilic DES leaching has been detected interfering the detection of the target analytes. In order to address this shortcoming, hydrophobic DES is reported by combining two hydrophobic components of DESs, such as tetrabutylammonium bromide, decanoic acid, pelargonic acid, capric acid, and lauric acid [14,15]. Hydrophobic DES were used as extraction solvents for the determination of dyes (acid blue 29 and malachite green) and organophosphorus insecticides [16], methylene blue [17], bisphenol A [18] and dyes [19,20]. Due to remarkable properties, hydrophobic DES are being used in various food and biological fields, which confirms their appropriate pre-concentration and enrichment capabilities of the microextraction method based on the DES.

Given by the advantages of hydrophobic DES, in this study different DES types were prepared based on different compositions of tetrabutylammonium bromide (TBAB) as the HBA and fatty acids (decanoic acid, nonanoic acid and octanoic acid) as the HBD. Their performance as an extraction solvent in DLLME was evaluated based on extraction performance and pre-concentration of tartrazine and acid orange 20 (AO20) as model analytes. Finally, the developed DLLME method was successfully applied to the determine phenoxy tartrazine and acid orange 20 (AO20) in fruit juice samples under optimum conditions. Our work provides insight to contribute to the urgent need for rapid sample preparation procedure for tartrazine and AO20 extraction in aqueous samples.

EXPERIMENTAL

Chemicals and Reagents

Tartrazine ($C_{16}H_9N_4Na_3O_9S_2$), acid orange 20 (AO20, $C_{16}H_{11}N_2NaO_4S$), sodium acetate, acetic acid, disodium hydrogen phosphate, monosodium phosphate, tetrabutylammonium bromide (TBAB), decanoic acid (C_{10}), nonanoic acid (C_9), octanoic acid (C_8), tetrahydrofuran (THF) and analytical grade methanol were purchased from Merck-Sigma Aldrich (Darmstadt, Germany). Acetate and phosphate buffers were prepared and used for the pH adjustment. Stock standard solutions of 1000 mg L^{-1} tartrazine and AO20 were prepared in ultrapure water, and working standard solutions were obtained by appropriate dilution of the stock solution. The ultrapure water was prepared by a model Milli-Q Direct-Q 8 UV Remote water purification system from Merck (Darmstadt, Germany).

Instrumentation

The Fourier Transform infrared (FT-IR) spectra of the three DESs and their starting materials were recorded using FT-IR KBR. Perkin Elmer FT-IR was used for the scanning between 4000 and 600 cm^{-1}. Extraction studies were evaluated bs employing Shimadzu UV-Vis spectrophotometer provided with 1 cm quartz cell (Shimadzu, Kyoto, Japan). The centrifugation process to separate phases was carried out with a centrifuge machine model Kubota 2100 (Kubota, Tokyo, Japan). To obtain nano- and/or microsize emulsions, an ultrasonic bath model Branson 3510 (Branson, CT, USA) was employed.

Preparation of DESs

Three DESs were synthesized by mixing tetrabutylammonium bromide (TBAB) as the HBA with decanoic acid (DES 1), nonanoic acid (DES 2) and octanoic acid (DES 3) as the HBD at molar ratio of (1:3) each. In separate vials, the mixtures were stirred by using magnetic stirrer at a temperature of 80 °C until transparent, homogeneous clear liquids were obtained. After cooling, the liquid DESs were stored at room temperature.

DLLME Procedure

DLLME procedure was carried out according to Soylak and Uzcan [21] with a slight modification. In a 15 mL centrifuge tube, about 10 mL of azo dye solution (10 ppm) and 2 mL acetate buffer solution at pH 4 were added. The mixture was then added with 0.4 mL of the previously synthesized DES and followed by 0.2 mL of THF. The mixture was left in the ultrasonic bath for 4 min at a frequency of 20 kHz. To separate the DES-rich phase from the aqueous phase, the cloudy/turbid solution was centrifuged at 4000 rpm for 4 min. The aqueous phase was separated from the residual DES-rich phase after phase separation.

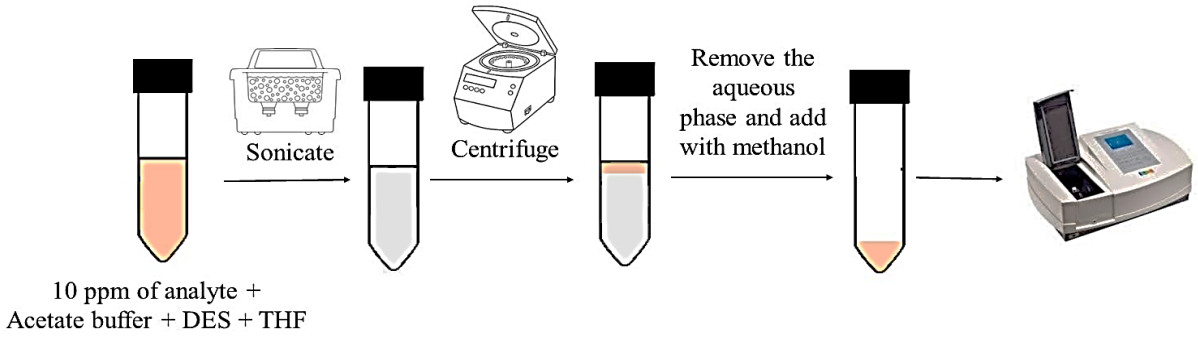

Figure 1. Schematic diagram of the proposed DLLME procedure

The DES phase consisted of a viscous liquid. Methanol was added to the DES phase to lower its viscosity. UV–Vis spectrophotometer measurements were taken at 430 and 476.5 nm for tartrazine and AO20, respectively. The schematic diagram of the DLLME procedure was depicted in Figure 1.

The recovery was calculated based on the measured azo dyes concentration as shown below:

$$\text{Recovery (\%)} = \frac{\text{Recovered analyte concentration}}{\text{Initial analyte concentration}} \times 100$$

Sample Preparation

To assess reliability of the suggested approach for extracting azo dyes from real samples, lemon tea and orange juices. The samples were purchased from local supermarket. About 30 mL of fruit juice samples were diluted in 100 mL volumetric flasks, separately. Un-spiked and spiked (10 mg L^{-1}) samples were subjected to the mentioned microextraction procedure under optimized conditions. All samples were analyzed in triplicate.

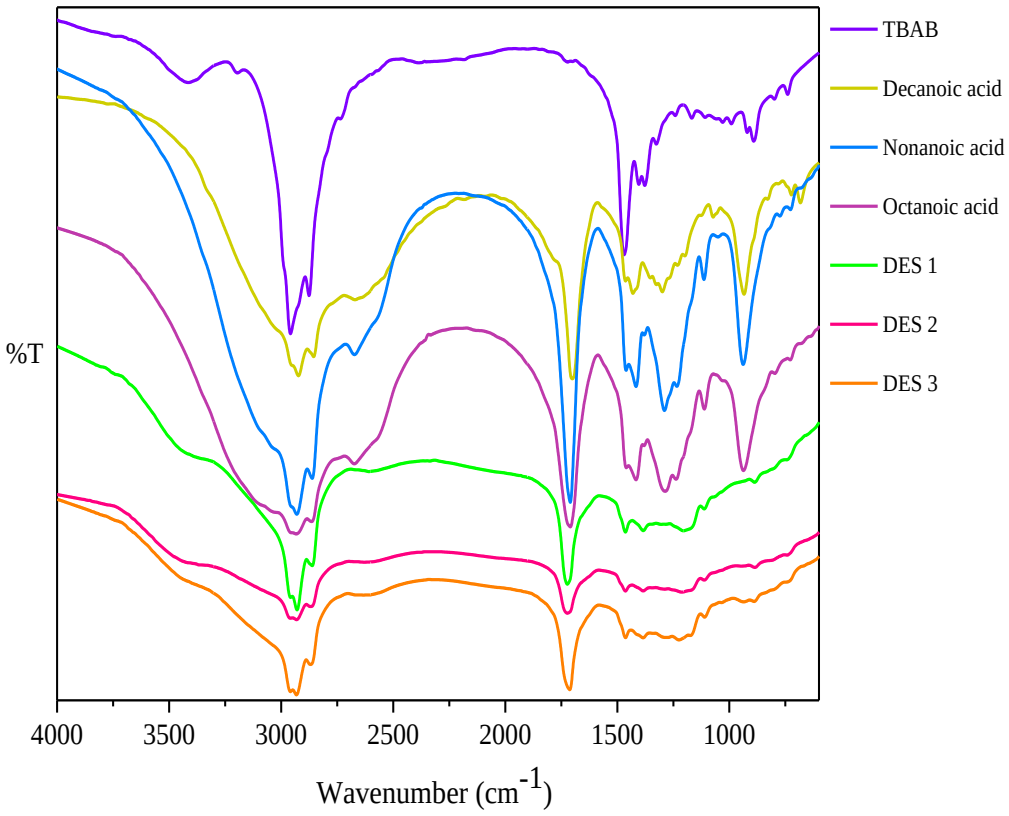

Figure 2. FT-IR spectra of TBAB, fatty acids and DESs

RESULTS AND DISCUSSION

Characterization of DESs

Figure 2 shows the FTIR spectra for the synthesized DESs and their respective starting materials. Upon the formation of DES 1 (TBAB : decanoic acid), C–H asymmetric stretching vibration band were shifted to a lower wavenumber from 2960 to 2927 cm^{-1} with low intensity, which indicated that the vibration of methyl groups ($–CH_3$) is constrained due to inclusion [8]. Similarly, C-H bond symmetric stretch of TBAB, the vibration band appears at a lower wavenumber from 2875 to 2855 cm^{-1}. When comparing decanoic acid and DES 1, the bands corresponding to carbonyl group (C=O) stretching vibrations have shifted to a higher wavenumber from 1698 to 1718 cm^{-1}, implying the introduction of new hydrogen bonds near the COOH group. Besides, when H-bonding occurred, the O-H stretch is significantly suppressed [22]. These results indicated the successful synthesis of DES 1. Likewise, shifting of asymmetric C–H, symmetric C–H, C=O and O–H stretching vibration bands for the formation of DES 2 (TBAB : nonanoic acid) and DES 3 (TBAB : octanoic acid) resembled the changes that happened for DES 1, having nearly the same wavenumber.

Optimization of DES-based Dispersive Liquid-liquid Microextraction

Selection of DES

The performance of the successfully synthesized DESs as extraction solvent were evaluated for DLLME of tartrazine and AO20. Figure 3(a) shows the effect of DES type on the recovery of azo dyes. For tartrazine, DES 2 (TBAB: nonanoic acid) provides the highest recovery while DES 1 (TBAB : decanoic acid) showed the highest recovery for AO20.

In this study, the DES was synthesized with different fatty acids as HBD. The length of the alkyl chain (decanoic acid, nonanoic acid and octanoic acid have ten, nine and eight carbon atoms, respectively) influences the lipophilicity of the synthesized DES. Meanwhile, the polarity of tartrazine and AO20 can be reflected by partition coefficient (log *P*) value. The more positive the log *P* value, the less polar the analyte is. If the analyte and the extraction solvent share similar log *P* values or at least similar hydrophobic-hydrophilic character of the DES, then the extraction recovery could be increased. Log *P* values of tartrazine and AO20 are -1.76 and 4.94, respectively [23]. Tartrazine is more polar thus, DES 2 with a lower lipophilicity compared to DES 1, could extract it more effectively. In contrast, AO20 is less polar was extracted better by DES 1 which has a high lipophilicity. Therefore, in the subsequent study, DES 1 and DES 2 was chosen for AO20 and tartrazine, respectively.

Effect of Sample pH

The extraction efficiency of an analyte with an ionizable functional group is determined by the degree of ionisation, which is greatly influenced by the pH of the aqueous phase. To ensure that the azo-dyes were in a molecular state, the pH of the sample solution must be adjusted, which may also facilitate the partitioning process and extraction efficiency. Therefore, the effect of the sample pH on the extraction efficiency was investigated over the range of pH 3–6 based on past reports [21,24,25].

As demonstrated in Figure 3(b), pH 4 and pH 5 showed the highest recovery for the extraction of tartrazine and AO20, respectively. The pH value at which a chemical species will take or donate a proton is known as the pK_a. The pK_a values of tartrazine and AO20 are 9.43 and 10.65, respectively [26]. Hence, these analytes existed mainly in anionic forms at pH < pK_a [21]. Tartrazine (pK_a values, 9.43) responded well to pH fluctuations, with no discernible change at pH 3–6. At low pH, sulfonate groups of tartrazine did not shift and remain in their anionic state [27]. Tartrazine in this form could be transferred to the DES phase. Thus, the dissolution process of target analytes is favored due to the presence of a network of HBA and HBD provided by the DES [28]. On the other hand, AO20 (pK_a values, 10.65) undergo azo-hydrazone tautomerism which the majority of the hydrazone tautomer exists in both acidic and neutral environments [29]. Therefore, at pH 5, AO20 existed in its hydrazone tautomer instead of keto tautomer which facilitate the mass transfer to DES. Thus, pH value of 4 and 5 were selected for consequence studies of tartrazine and AO20, respectively.

Based on the optimum pH conditions, the possible interaction mechanisms (hydrogen bonding, hydrophobic interaction and π–cation interactions) of DES with tartrazine and AO20 were illustrated in Figure 4(a) and (b), respectively.

Effect of Disperser Solvent Volume

To study the effect of disperser solvent, the disperser solvent volume was optimized in a DLLME by varying the volume from 100 to 500 µL. In the presence of disperser solvent such as THF, DES can readily form aggregates in aqueous sample and hence, increased the surface area between the two phases. Consequently, extraction time became shorter.

As shown in Figure 3(c), the recovery values for tartrazine and AO20 were achieved in the THF volume of 200 µL and 400 µL, respectively. For tartrazine, at the volume of THF greater than 200 µL, the recovery dropped due to the increase in solubility of analyte in water (Kachangoon et al. 2020b). For

AO20, at the volume of THF lower than 400 µL, the recovery values were comparatively low because the small volume of THF might not disperse the extraction solvent appropriately [30]. As the result, 200 µL (for tartrazine) and 400 µL (for AO20) of THF were selected for further works.

Effect of Sonication Time

By enhancing the contact between the targeted analyte in the aqueous sample phase and the extraction solvent, ultrasonication plays an essential role in the mass transport of the analyte from the aqueous sample phase to the extraction solvent [31]. Transfer of analyte requires a complete equilibrium reaction to achieve maximum sensitivity.

To determine the optimum sonication time, model solutions were placed into sonication bath for 2–8 min. Upon sonication, the cloudy solution immediately formed which indicates that the aggregates of analyte-containing DES have broken down into fine-particle droplets. Equilibrium state was achieved rapidly. Based on the results illustrated in Figure 3(d), it was found that 4 min and 6 min sonication time were sufficient to achieve the highest extraction efficiency for tartrazine and AO20, respectively. Hence, these values were chosen for the subsequent analysis.

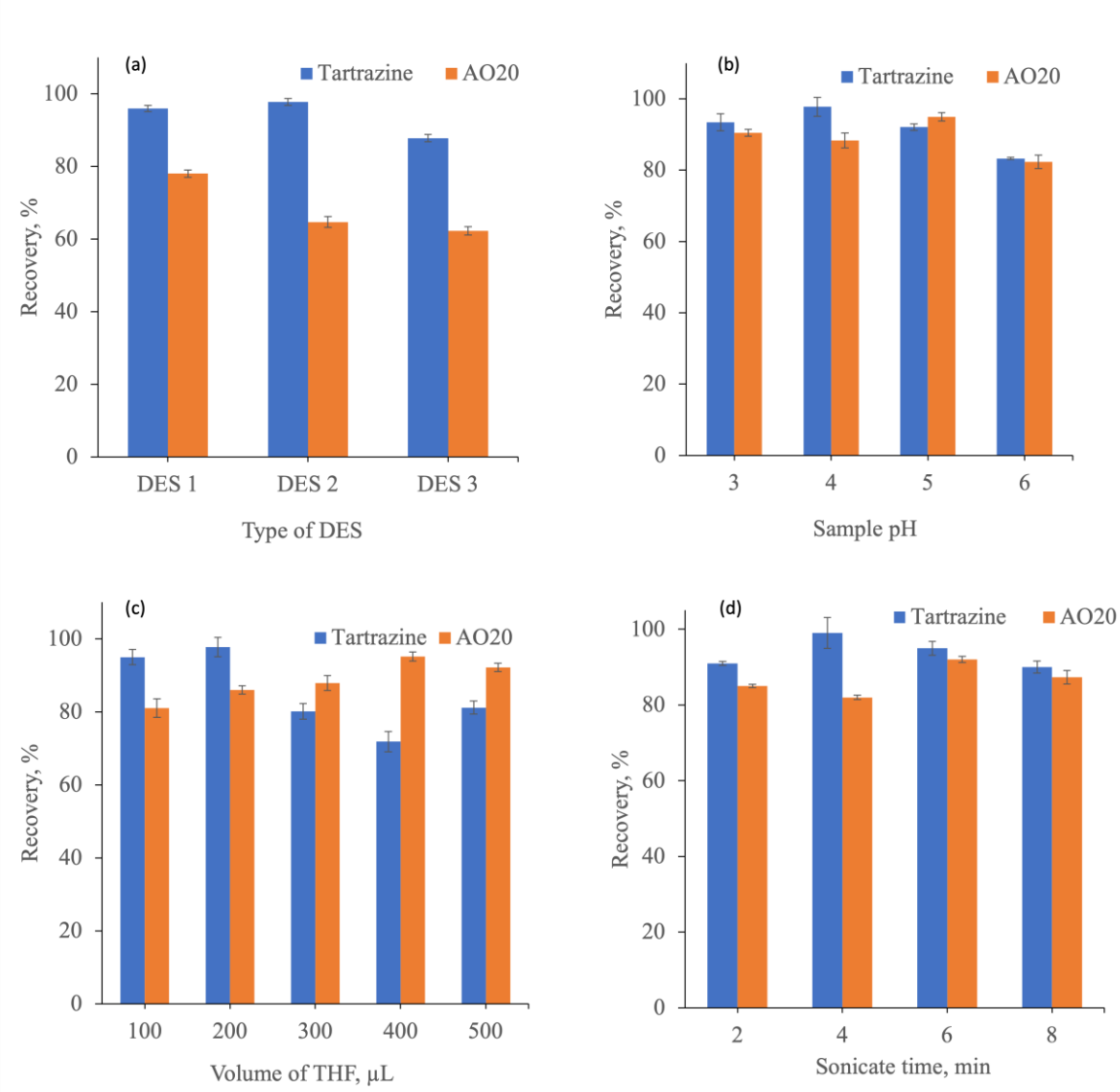

Figure 3. The effect of (a) type of DES, (b) sample pH, (c) volume of disperser solvent and (d) sonication time on recovery of tartrazine and acid orange 20.

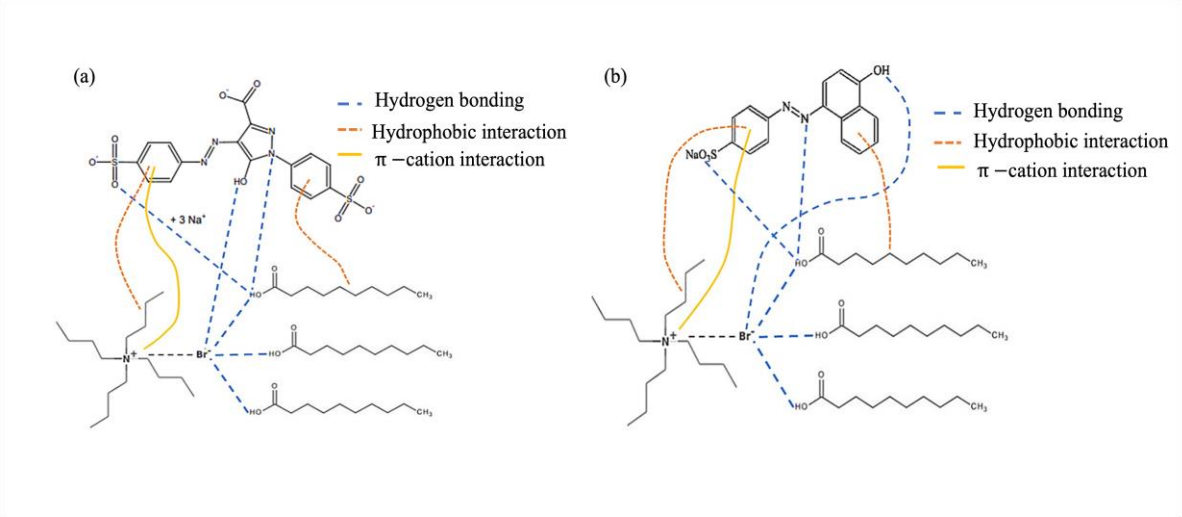

Figure 4. Potential extraction mechanism of (a) DES 2 and tartrazine, (b) DES 1 and acid orange 20.

Effect of Centrifugation Time

To obtain the phase separation between aqueous and organic/DES layers, the mixtures were centrifuged. Centrifugation time between 2 to 8 min at 4000 rpm was investigated. After centrifugation, the DES phase appeared on top of the aqueous phase. Based on Figure 5(a), 4 min and 6 min centrifugation time provide the highest recovery for tartrazine and AO20, respectively. The overall microextraction procedure can be completed more rapidly with less centrifugation time.

Effect of Sample Volume

As the concentration ratio of the analyte in the extraction solvent to the analyte in the initial sample solution has a substantial impact on analytical performance parameters, determining the largest sample volume possible is crucial [32,33]. To evaluate the effect of sample volume on recovery of azo dyes, the sample volumes were varied from 5 and 25 mL under optimal conditions. The results are shown in Figure 5(b). Based on the trend shown, quantitative recovery values of tartrazine and AO20 were obtained at 20 mL and 25 mL of sample volume, respectively. It is still possible to get a trend which shows increasing recovery percentage with increased sample volume [34,35]. Also, larger sample volumes are advantageous in DLLME as the method's enrichment factor (EF) rises [36].

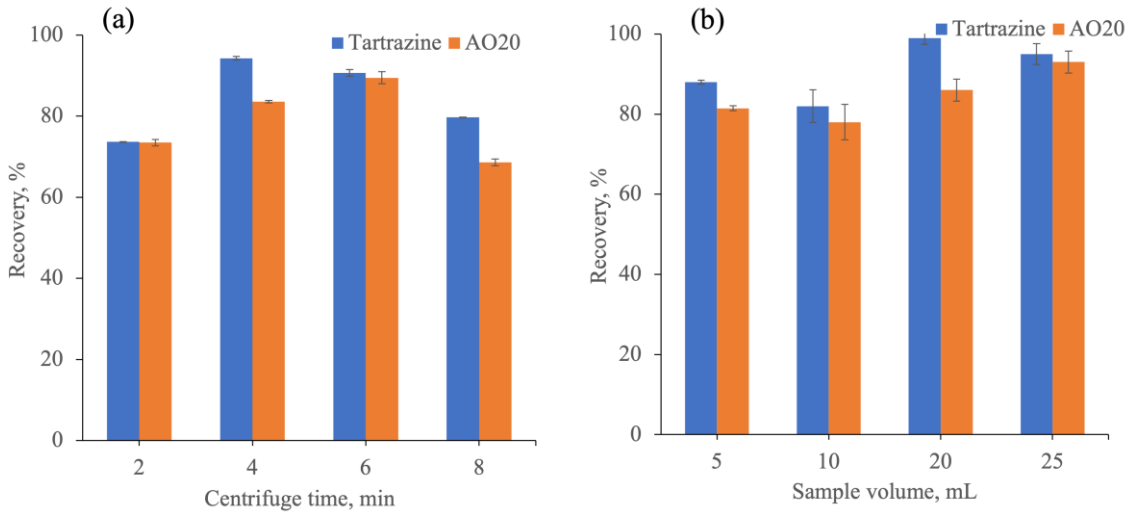

Figure 5. The effect of (a) centrifuge time and (b) sample volume on recovery of tartrazine and acid orange 20.

Method Validation

The optimized conditions obtained for the extraction of tartrazine were DES 2 as the extraction solvent, pH 4 sample solution, 200 µL of THF, 4 min sonication time, 4 min centrifugation time and 20 mL of sample solution. The optimal conditions for extraction AO20 were DES 1 as the extraction solvent, pH 5 sample solution, 400 µL of THF, 6 min sonication time, 6 min centrifugation time and 25 mL of sample solution.

Linearity, limit of detection (LOD), limit of quantification (LOQ), and precision tests were done under ideal conditions to examine the validity of the developed DLLME-DES method. The linear regression method was employed for this investigation and it can be modelled as follows;

$$y = mx + c$$

The sensitivity of the LOD and LOQ is determined using this model. As a result, the LOD and LOQ can be stated as follows;

$$LOD = \frac{3\,s.d}{m}$$

$$LOQ = \frac{10\,s.d}{m}$$

where s.d is the standard deviation of ten successful blank and m is the slope of the calibration curve. The calibration curves attained for the understudy azo dyes were linear in the range of 1–10 mg L^{-1} with R^2 more than 0.9816 indicating a good correlation between absorbance and concentration within a wide concentration range. LOD of this technique lies within the range of 0.007–0.034 mg L^{-1} which suggested that this technique is satisfactory for azo dyes extraction because of the low LOD value. LOQ of this technique ranged between 0.025 to 0.114 mg L^{-1}. A good precision with RSD value of 2.1–3.2 % (intraday) and 5.4–9.4 % (interday) were obtained. In addition, the EF values were 10 and 12.5 for tartrazine and AO20, respectively, when the final volume was 2 mL and initial volumes were 20 mL (tartrazine) and 25 mL (AO20). Table lists the analytical performance numbers of merits.

Analysis of Real Samples

The developed DLLME-DES technique was applied in fruit juice samples to assess the interference effect for the extraction of tartrazine and AO20 prior to UV-Vis spectrophotometric determination. Lemon tea and orange juices were selected as the real samples because the bright colour (yellow and orange) of the beverages might indicate the presence of tartrazine or AO20.

By using the developed DLLME-DES technique, tartrazine and AO20 were found presence in both fruit juice samples. Table shows the concentration and recovery of tartrazine and AO20 in lemon tea and orange juices under the optimized conditions described. As tabulated in the table, concentrations of tartrazine in lemon-tea and orange juices were 2.73 and 4.51 mg L^{-1}, respectively. After multiplying with dilution factor, which is 3.3, the concentrations were 9.00 and 14.88 mg L^{-1}, respectively. Based on the acceptable daily intake (ADI) of tartrazine, 7.5 mg kg^{-1} of body weight, it can be said that the concentrations of tartrazine in the beverage samples are still safe for adult consumption if it is consumed in moderation [37].

For AO20, its concentrations in lemon-tea and orange juices were 2.97 and 15.81 mg L^{-1}, respectively. After multiplying with dilution factor, which is 3.3, the concentrations were 9.80 and 52.17 mg L^{-1}, respectively. In addition, there is no ADI allocated for AO20.

Table 1. Figures of merit for DLLME of azo dyes using DES

Analyte	R^2	Linear range (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})	RSD (%) Intraday, n = 3	RSD (%) Interday, n = 3	EF
Tartrazine	0.9816	1–10	0.0342	0.1141	2.1	9.4	10
AO20	0.9938	4–10	0.0076	0.0250	3.2	5.4	12.5

Table 2. The detected amount and recovery of tartrazine and AO20 in fruit juice samples (n = 3)

Sample	Analyte	Concentration (mg L^{-1})		Recovery (%)
		Added	Found	
Lemon tea juice	Tartrazine	0	2.73 ± 0.49	–
		10	13.74 ± 0.83	110.1 ± 3.6
	AO20	0	2.97 ± 0.56	–
		10	12.50 ± 0.25	95.3 ± 3.2
Orange juice	Tartrazine	0	4.51 ± 0.40	–
		10	13.70 ± 0.63	91.9 ± 4.5
	AO20	0	15.81 ± 0.57	–
		10	24.90 ± 0.16	90.9 ± 5.1

Table 3. Comparison between the proposed method with other procedures from literature for azo dyes determination.

Analyte	Method	Sample	LOD	RSD (%) Intraday	Ref
Tartrazine	Modified silver nanoparticles-enhanced SDME coupled with diffuse reflectance FTIR	Beverages and candies	0.002 mg L^{-1}	±1.76	[38]
Tartrazine	Ultrasound-assisted IL-based floating organic droplets microextraction with UV-Vis detection	Soft drinks, candies, lollipop and icing sugar	0.0032 mg L^{-1}	±1.8–3.5 (three different concentrations)	[39]
Tartrazine, amaranth, sunset yellow, allura red, ponceau 4R, and erythrosine	Rapid shaking-based IL DLLME followed by HPLC	Soft drinks, sugar- and gelatin-based confectionery	0.015–0.32 ng mL^{-1}	-	[40]
Tartrazine and allura red AC	Hydrophobic DES (TBAB/fatty acid) vortex-assisted LLME with UV-Vis detection	Candies and powder juice	0.004 and 0.005 mg L^{-1}	≤5	[41]
Tartrazine, sunset yellow, amaranth, ponceau 4R and brilliant blue	Aqueous two-phase based on IL LLME followed by HPLC	Carbonated drinks, lollipop, jelly, candy, milk tea powder and papaya powder	0.051–0.074 ng mL^{-1}	±1.6–3.2	[42]
Tartrazine and AO20	DES-based DLLME with UV-Vis detection	Beverages	0.007–0.034 mg L^{-1}	±2.1–3.2	This work

Based on Table , the recoveries were between 90.9 and 110.1 %. The findings obtained for the real samples analysis demonstrated that there was no interference effect when the developed DLLME-DES method was applied. The proposed DLLME-DES method was effective in the applied matrix environment and appropriate for tartrazine and AO20 preconcentration and analysis in real samples.

Comparison with Previous Methods

Table 3 shows comparative studies on the analytical performance of the proposed approach and previously developed methods. The developed DLLME-DES method was comparable to other methods and provides good sensitivity and precision. In addition, the developed DLLME-DES method was greener than most of the mentioned methods.

CONCLUSION

A simple and rapid (4 min sonication and centrifugation time for tartrazine; 6 min sonication and centrifugation time for AO20) dispersive liquid-liquid microextraction (DLLME) procedure by using DES followed by UV-Vis spectrophotometry has been developed and validated for the preconcentration and determination of tartrazine and AO20 in fruit juice samples. The synthesized DESs were characterized by Fourier Transform Infrared (FT-IR) spectroscopy. Shifting of C–H asymmetric and symmetric stretching vibrations, O–H as well as C=O have proposed the formation of DESs. The optimized conditions of tartrazine were as follows: DES 2 as the extraction solvent, sample at pH 4, 200 µL of THF as the disperser solvent, 4 min sonication time, 4 min centrifugation time and 20 mL of sample volume. The optimized conditions of AO20 were as follows: DES 1 as the extraction solvent, sample at pH 5, 400 µL of THF as the disperser solvent, 6 min sonication time, 6 min centrifugation time and 25 mL of sample volume. Under optimized conditions, linearity of the studied azo dyes was in the range of 1–10 mg L^{-1}. The LOD of the developed DLLME-DES method lies within the range of 0.007–0.034 mg L^{-1} while LOQ ranged between 0.025–0.114 mg L^{-1}. The developed DLLME-DES method portrayed a good precision with intraday and interday RSD values of 2.1–3.2 % and 5.4–9.4 %, respectively. The proposed method was executed for the analysis of lemon-tea and orange juices. The recovery was 90.9–110.1 %. The developed DES as the extraction solvent was proven to provide great ability for the pre-concentration of azo dyes in aqueous food sample. The developed DLLME-DES provide the potential in the concentration and separation of other additives or pollutants in food samples.

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