

## Article

# Efficient Extraction of Methylene Blue from Aqueous Solution Using Phosphine-Based Deep Eutectic Solvents with Carboxylic Acid

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**Abstract:** Methylene blue (MB), an organic thiazine dye, has numerous industrial and medical applications. However, MB is a wastewater contaminant that is harmful to humans and aquatic life. Hence, its removal from water bodies is essential. In this work, five novel deep eutectic solvents (DESs) were synthesized using different precursors, screened, and studied for the extraction of methylene blue (MB) from aqueous solution using liquid–liquid extraction. The first, TOP-SA, was synthesized using trioctylphosphine (TOP) as a hydrogen bond acceptor (HBA) and 2-hydroxy benzoic acid as a hydrogen bond donor (HBD). Among these, TOP-SA had the highest MB removal efficiency. The effects of pH, contact time, initial MB concentration, volumetric ratio, temperature, and ionic strength were studied and optimized. A 99.3% removal was achieved in 5 min for a 200 mg dm<sup>-3</sup> MB solution mixed in a 1:10 ratio with TOP-SA at 25.0 °C. The structural properties of TOP-SA and its interactions with MB were investigated using FTIR. TOP-SA's toxicity was investigated using human cells in vitro. TOP-SA was found to be comparatively less toxic and is a more efficient MB remover than other literature reported ionic liquids (ILs).

**Keywords:** wastewater; methyl orange; deep eutectic solvent; ionic liquid; extraction efficiency



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## 1. Introduction

Methylene blue (MB) is an organic thiazine cationic synthetic dye with the formula [7-(dimethylamino)phenothiazin-3-ylidene]-dimethylazanium;chloride;hydrochloride. It has numerous industrial and medical applications that include dyeing leather, textiles, wood, and papers [1]. However, MB is a wastewater contaminant that is harmful to humans and aquatic life; inhalation of its aerosols causes breathing difficulty, nausea, vomiting, diarrhea, and chest and abdominal pains [2]. Decontamination of aqueous solutions containing MB has thus become a prime focus of environmental research.

Numerous physical, chemical, electrochemical, and biological techniques have been tested and are being successfully applied for dye decontamination in industrial wastewater [3]. These techniques mainly include liquid–liquid extraction (LLE) [4], adsorption [5], ultrasonic-assisted adsorption [6], membrane filtration [7], coagulation [8], irradiation [9], ion exchange [10], photocatalytic degradation [11], and microbial degradation [12]. Most of these techniques have drawbacks such as high cost, lengthy duration, extensive labor, and low removal efficiency. Among these tested techniques, liquid–liquid extraction (LLE) has the potential to effectively remove a dye using small amounts of solvent. Among the

various types of solvents used in dye LLE processes, ionic liquids (ILs) and deep eutectic solvents (DESs) have been gaining significant popularity.

ILs are ionic solvents that generally have high thermal and chemical stabilities, low vapor pressures, high melting points, and high miscibility with water. ILs with desired properties can be synthesized by pairing specific anions and cations. Such ‘tunable’ ILs have the potential to efficiently remove dyes from the aqueous phase via LLE [13,14]. However, most hydrophobic ILs contain fluorine and produce toxic HF upon hydrolysis [15]. Moreover, synthesis of ILs is difficult and they are more expensive than DESs. Their toxicity and high cost limit their use as MB removers. Researchers have thus been seeking alternatives to hydrophobic ILs in LLE applications.

DESs, which can be considered alternate versions of ILs, have recently emerged as potential solvents in LLE for various pollutants, including MB. They consist of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) mixed in specific molar ratios. They are superior to ILs in that they are less expensive and more ecofriendly [16,17], are convenient to synthesize, and have comparable or better physiochemical properties, in addition to being rich in hydrogen and ionic bonds [18]. DESs tend to have low melting points and vapor pressures, high solubility, good biodegradability, and high thermal and chemical stability [18–20]. These properties explain the recent expansion of their use to applications spanning several fields [21]. Hydrophobic DESs are extensively used in LLE due to their immiscibility in water. Recently, they have been used in extraction of various types of metal ions and dyes from aqueous solutions [22,23]. Their potential applications for dye removal via LLE as a solvent or surface modifier have also been explored. For example, DESs based on glycolic acid and choline chloride have been used in the extraction of Eriochrome black T [4].

In this work, five hydrophobic DESs were synthesized using three HBAs: trioctylphosphine (TOP), trioctylamine (TOA), and trihexylamine (THA); and two HBDs: salicylic acid (SA) and malonic acid (MA). The respective abbreviations of these DESs were TOP-SA, TOP-MA, TOA-SA, THA-SA, and TOA-MA; these are referred to as DES-1, DES-2, DES-3, DES-4, and DES-5, respectively, throughout the manuscript. In order to study the effect of HBA on MB extraction, DES-1, DES-3, and DES-4 were prepared in a 1:1 molar ratio. Similarly, for the effect of HBD, DES-2 and DES-5 were also prepared in the same ratio. Among the five DESs, DES-1 was found to have the highest efficiency for MB extraction from the aqueous phase. It was thus selected for the optimization studies. LLE process parameters including pH, contact time, initial MB concentration, volumetric ratio, temperature and ionic strength, DES/MB volume ratio ( $V_{DES}:V_W$ ), and temperature were optimized. FTIR was used to assess DES and MB interactions. The thermodynamics of the transfer of MB from the aqueous to the DES phase was also investigated. The toxicity of TOP-SA was studied *in vitro* using human cells.

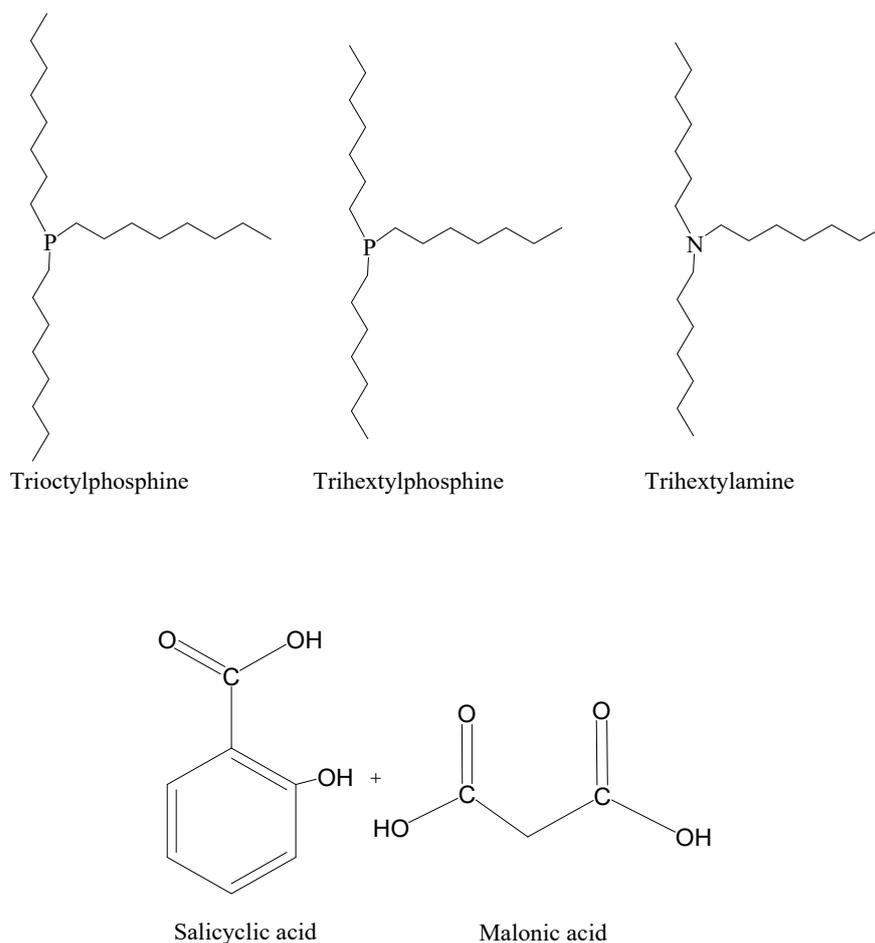
## 2. Materials and Methods

All chemicals were of reagent grade and were used without further purification. MB, trioctylphosphine ( $C_{24}H_{51}P$ ), trioctylamine ( $C_{24}H_{51}N$ ), trihexylamine ( $C_{18}H_{39}N$ ), dimethyl sulfoxide (DMSO), and salicylic acid ( $C_7H_6O_3$ ) were obtained from Sigma Aldrich (Germany). Malonic acid ( $C_3H_4O_4$ ) was obtained from HiMedia Laboratories (India). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) was obtained from Roche, Germany. Distilled deionized water from Aquatron A4000D (Water Still, UK) was used in all of the MB dye solution preparation and phase ratio studies.

### 2.1. DES Synthesis

All five DESs were prepared by mixing HBA with HBD in a small glass vial followed by stirring. In a typical experiment, DES-1 was prepared by mixing equimolar ratios of TOP with SA in a 20 mL glass vial maintained at 70.0 °C and stirred for 120 min at 250 rpm. Similarly, DES-2, DES-3, DES-4, and DES-5 were prepared by mixing and stirring equimolar ratios of the respective HBAs and HBDs. Moreover, DES-1 was prepared in four

HBD ratios of 1:0.5, 1:1, 1:1.5, and 1:2. The structural formulas of HBA and HBD are shown in Figure 1. The composition and properties of the prepared DESs are given in Table 1.



**Figure 1.** HBA and HBD of prepared DESs.

**Table 1.** DESs precursors, preparation ratios, and properties.

HBA	HBD	Molar Ratio HBA:HBD	Abbreviation	Property
Trioctylphosphine	Salicylic acid	1:0.5, 1:1, 1:1.5, 1:2	DES-1	Hydrophilic
Trioctylphosphine	Malonic acid	1:1	DES-2	Hydrophilic
Trioctylamine	Salicylic acid	1:1	DES-3	Hydrophilic
Trioctylamine	Malonic acid	1:1	DES-4	Hydrophilic
Trihexylamine	Salicylic acid	1:1	DES-5	Hydrophilic

## 2.2. Instrumentation

The pH was measured on an Oakton pH meter 510 series (Malaysia). The DES and MB were mixed in a Stuart vortex mixer (UK). The DES and MB aqueous solutions were separated using a Hermle centrifuge (LTF Labortechnik, Wasserburg, Germany). An Evolution 220 UV-Vis spectrophotometer (Thermo Scientific, Shanghai, China) was used to determine the MB concentrations remaining in the aqueous phase after extraction using DES (see Supplementary Materials). The FTIR spectra were obtained with a Perkin Elmer Fourier-transform infrared spectrometer equipped with ZnSe optics and a DTGS detector (Bomem MB-3000, Zurich, Switzerland).

### 3. Methods

#### 3.1. MB Extraction

A  $15 \text{ g dm}^{-3}$  stock MB solution was used to make up the standard solutions. A calibration curve was prepared using the maximum absorbance at 665 nm. The MB solutions were placed in 15 mL centrifuge tubes and known amounts of DES were added. Solutions were vigorously mixed at 2500 rpm using a vortex. The aqueous MB and DES phases were then separated via centrifugation for 10 min at 3000 rpm. The MB concentrations in the aqueous phase were determined using the calibration curve. The extraction efficiency (% *E*) and experimental partition coefficient (*P*) of MB in DESs were calculated using Equations (1) and (2), respectively:

$$\% E = \frac{(C_i - C_f)}{C_i} \times 100 \quad (1)$$

$$P = \frac{(C_i - C_f)}{C_f} \left( \frac{V_{aq}}{V_{DES}} \right) \quad (2)$$

where  $C_i$  and  $C_f$  ( $\text{mg dm}^{-3}$ ) are dye concentrations in the aqueous phase before and after extraction, respectively;  $V_{aq}$  (mL) is the volume of aqueous solution containing dye at its initial concentration; and  $V_{DES}$  is the DES solution volume.

#### 3.2. Single Parameter Optimizations

The MB extraction from the liquid to the DES phase was influenced by process parameters that included pH, vortex mixing time, DES/MB solution phase ratio, initial MB concentration, temperature, and ionic strength. To maximize MB transfer from the aqueous phase, it was necessary to optimize these parameters. The optimization of these individual parameters is described below.

Recent reports indicated that the initial pH of MB solutions considerably influences the extraction efficiency [24–26]. This effect arises from the pH dependence of the MB ionization. Optimization experiments were conducted in a pH range of 2–10 after mixing for 5 min at 25.0 °C using an already-optimized MB concentration and  $V_{DES}:V_w$  (see below). The initial pH of the MB solutions was adjusted using analytical grade HCl and NaOH solutions. Vortex mixing time experiments were performed for 1–7 min at an optimum MB concentration,  $V_{DES}:V_w$ , and pH at 25.0 °C. The initial MB concentrations varied in the range of 200–1500  $\text{mg dm}^{-3}$ . The MB solutions and DES were mixed at 25.0 °C at the optimum pH and  $V_{DES}:V_w$  and stirred for 5 min. In the  $V_{DES}:V_w$  optimization study, the  $V_{DES}$  was kept at 0.15 mL while varying the  $V_{DES}:V_w$  in the range of 1:10–1:65. Already-optimized parameters for the pH, vortex time, and aqueous MB concentration were used at 25.0 °C. To study the effect of the ionic strength, NaCl was added to the MB solutions in concentrations in the range of 0.34–2.05 M. These saline solutions were mixed with DES at the optimum  $V_{DES}:V_w$  and vortexed for 5 min at room temperature. The effect of the temperature was studied in the range of 25.0–80.0 °C.

#### 3.3. Cell Viability Assays

Cellular metabolic activity was followed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as previously described [27,28]. Briefly, human cells with 80–85% confluent cell monolayers were challenged with 0.125, 0.25, 0.5, 1.0, and 2.0 mM of DES at 37.0 °C, 95% humidity, and 5%  $\text{CO}_2$  for 24 h. Following day, the media were replaced with 90  $\mu\text{L}$  of serum-free RPMI-1640 (Roswell Park Memorial Institute) media. Then, 10  $\mu\text{L}$  of freshly prepared MTT solution was added to each well of the 96-well plate and incubated for 3–4 h at 37.0 °C. Finally, 100  $\mu\text{L}$  of DMSO was added to each well to fully dissolve the formazan crystals made by viable cells. Human cells incubated with

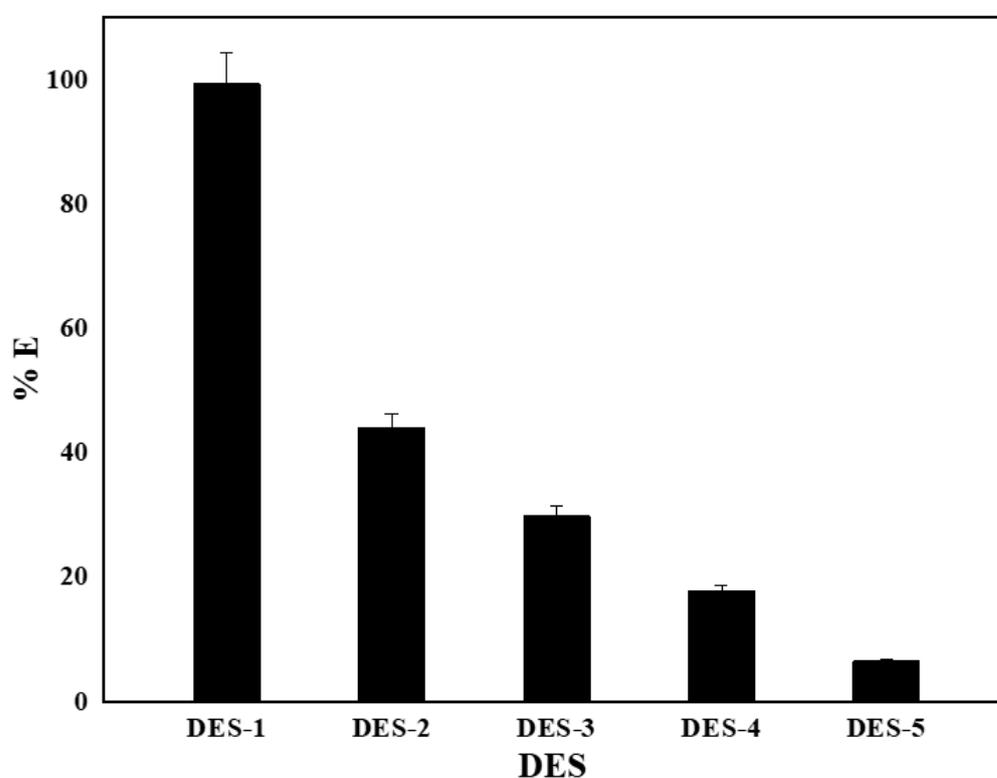
DMSO were used as negative controls [28]. The assay absorbance was calculated at 540 nm and the cell viability was evaluated using Equation (3):

$$\% \text{ viability} = (\text{mean OD}_{\text{test sample}} / \text{mean OD}_{\text{negative control}}) \times 100 \quad (3)$$

## 4. Results and Discussion

### 4.1. DES Selection

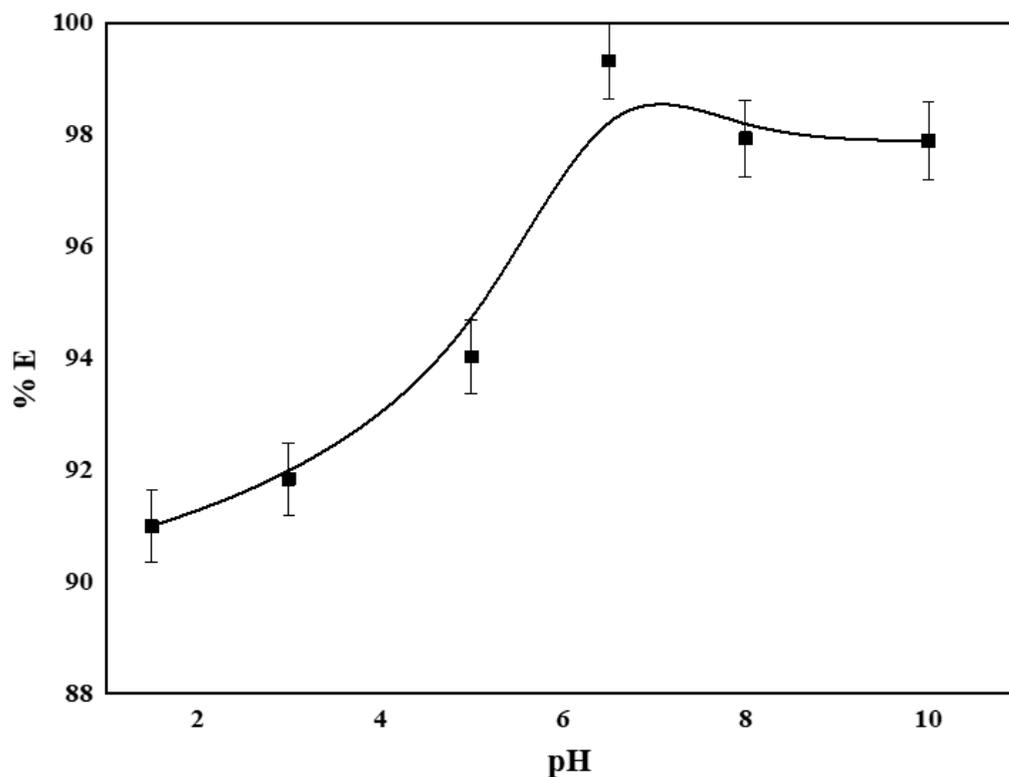
The five DESs were assessed individually for their efficiency in MB extraction from the aqueous phase. The efficiencies for DES-1, DES-2, DES-3, DES-4, and DES-5 were 99.3, 44.0, 29.9, 17.8, and 6.5%, respectively (Figure 2). Notably, these DESs were all able to extract MB to varying degrees from a water stream. DES-1, with a relatively higher hydrophobicity (arising from its longer chain length), was the most efficient, suggesting a predominant role of the interactions between the hydrophobic portions of the DES and MB. This was further corroborated by the observation that DES-1, with its higher hydrophobicity, was much more efficient than DES-2. DES-1 was thus selected for the optimization studies.



**Figure 2.** DES screening for MB extraction. [MB] = 200 mg dm<sup>-3</sup>; T = 25.0 °C.

### 4.2. Effect of Initial pH

DES-1's extraction efficiency from 200 mg dm<sup>-3</sup> MB solutions was observed to increase with pH as shown in Figure 3, reaching a maximum at pH 6.5. This observation may be explained on the basis of favorable interactions of the deprotonated MB with the positively charged phosphine-based DES-1. Above pH 5.9, which is the isoelectric point of MB, electrostatic attractions developed between MB and DES-1 [26]. Beyond pH 6.5, the efficiency decreased slightly and remained almost constant up to pH 10. Table 2 shows that the partition coefficient  $P_{\text{DES-1}}$  increased with the pH, showing a maximum of 1140 at pH 6.5.



**Figure 3.** Effect of pH on MB extraction efficiency. [MB] = 200 mg dm<sup>-3</sup>; T = 25.0 °C.

**Table 2.** The pH dependence of the partition coefficient ( $P_{DES-1}$ ) of MB in TOP-SA. [MB] = 200 mg dm<sup>-3</sup>. T = 25.0 °C.

pH	1.0	3.0	5.0	6.5	8.0	10.0
$P_{DES-1}$	5.02	86.3	120	1140	365	357

#### 4.3. Effect of Vortex Mixing Time

The vortex mixing time significantly influences the extraction efficiency in LLE processes. This parameter is also indicative of the time required to achieve equilibrium at both lab and industrial scales. This effect is presented in Figure 4. Five LLE experiments were conducted with mixing times of 1, 2, 3, 5, and 7 min. The results showed that the efficiency increased from 57.9 to 99.3% in the interval of 1–5 min. At very short mixing times, the extraction efficiency was low due to incomplete mixing of the DES-1 with the MB solutions. The MB extraction rate was thus very fast, with equilibrium attained in 5 min. To confirm the quick extraction mechanism, experiments were also conducted at 7 min but no further improvement was observed. Thus DES-1 was highly efficient and fast in extracting MB from the aqueous phase. Liu et al. reported on the effect of centrifugation on the extraction efficiency in the interval of 1–10 min, with the highest efficiency achieved after 5 min [10].

#### 4.4. Effect of Phase Ratio

The phase ratio ( $V_{DES}:V_w$ ) is a useful parameter in selecting the amount of DES required for MB extraction from a specific volume and concentration of aqueous MB. Figure 5 shows that the extraction efficiency decreased somewhat with the  $V_{DES}:V_w$ : from 99.3% for a 1:10 ratio to 85.7% for 1:65. Thus, TOP-SA was highly efficient in extracting MB from a very large volume of MB solution. The decrease in removal at the 1:65 ratio could be due to saturation of the DES with MB. Similar observations were reported previously. The efficiency of thymol- and camphor-based DES extraction of malachite green and crystal

violet dyes from aqueous solutions was significantly higher at a low  $V_{DES}:V_w$  [24]. The same trend was observed for Sudan dyes [25].

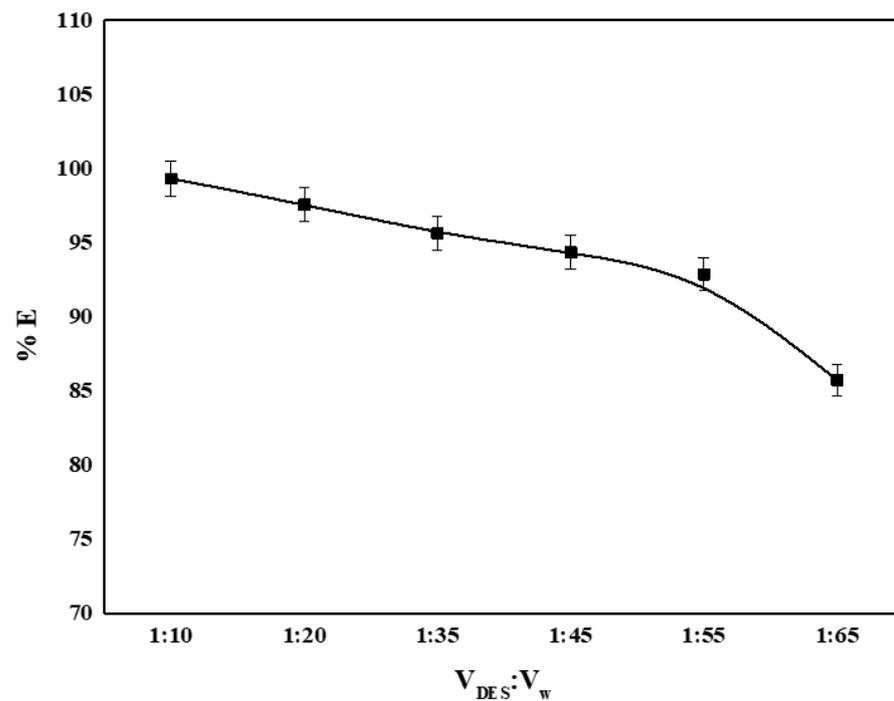


Figure 4. Effect of vortex mixing time on MB extraction efficiency. [MB] =  $200 \text{ mg dm}^{-3}$ ;  $T = 25.0 \text{ }^\circ\text{C}$ .

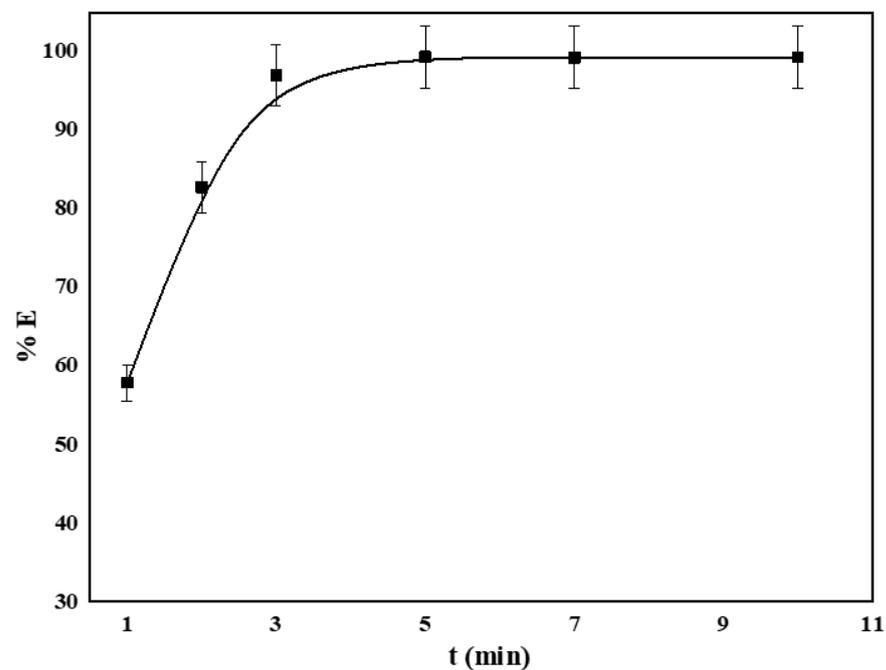


Figure 5. Effect of phase ratio on MB extraction efficiency. [MB] =  $200 \text{ mg dm}^{-3}$ ;  $T = 25.0 \text{ }^\circ\text{C}$ .

#### 4.5. Effect of Initial MB Concentration

The extraction efficiency was studied using MB concentrations in the range of  $250\text{--}1500 \text{ mg dm}^{-3}$ . Figure 6 shows that an increase in [MB] from  $250$  to  $1500 \text{ mg dm}^{-3}$  resulted in an efficiency drop from  $99.3$  to  $67.0$ . This trend conformed with previous findings by Xu et al. [24]. The relatively low extraction efficiency for the  $1500 \text{ mg dm}^{-3}$  solution may be attributed to the presence of fewer free DES molecules.

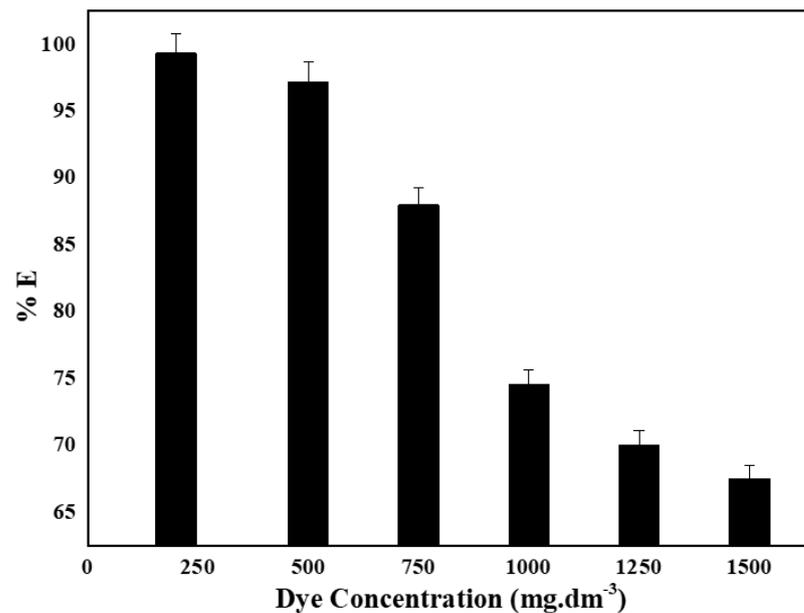


Figure 6. Effect of initial dye concentration on MB extraction efficiency. T = 25.0 °C.

#### 4.6. Effect of Temperature

Figure 7 shows the MB extraction efficiency using DES-1 at 25.0, 30.0, 40.0, and 50.0 °C. The effect of the temperature on the efficiency was seen to be marginal, with an increase in temperature from 25.0 to 50.0 °C resulting in an efficiency reduction of ca. 5%. The observation may have been due to the accompanying DES viscosity decrease with temperature. The drop in the viscosity difference between the aqueous and DES phases favored mass transfer of MB into the aqueous phase, thereby reducing the efficiency. These results were in line with recent findings [26].

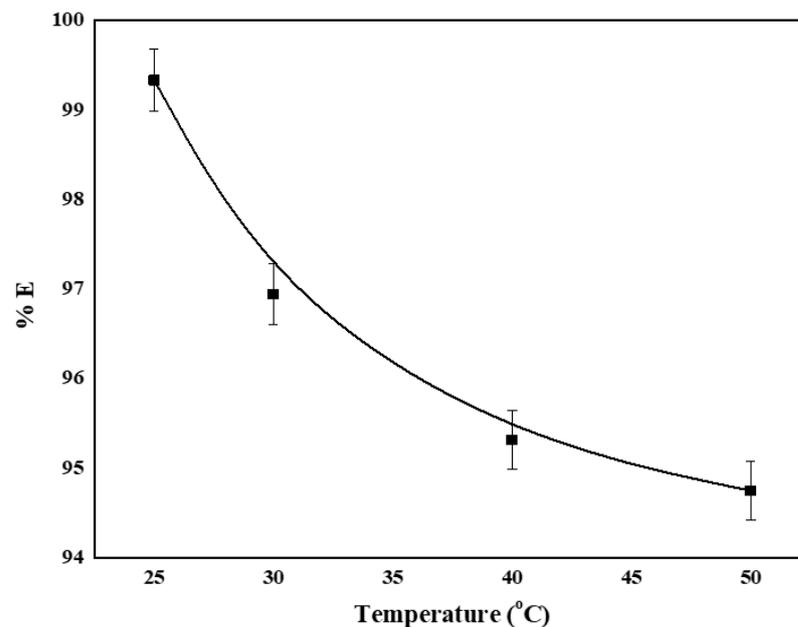


Figure 7. Effect of temperature on MB extraction efficiency. [MB] = 200 mg dm<sup>-3</sup>.

#### 4.7. Effect of the Ionic Strength of Aqueous Solution

With a view toward replicating conditions in common wastewaters, the effect of salt on the MB extraction efficiency was studied in optimum conditions (see Figure 8). At 25.0 °C, a small efficiency decreases from 99.3 to 96.9% was observed upon addition of 20 g/L NaCl

to MB solutions. Further addition of salt resulted in a small increase in efficiency, which reached 98.2% at 120 g/L NaCl (Figure 8). The general efficiency drop may have been due to the salting-out effect in which salt favors migration of MB from the aqueous to the DES phase. This increase in the MB extraction efficiency by DES with a salt addition was documented previously [26].

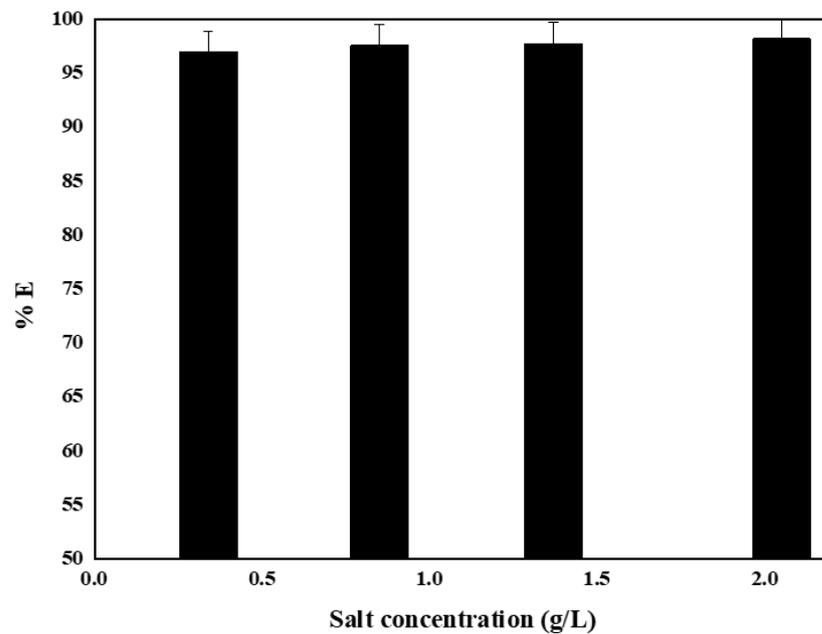


Figure 8. Effect of ionic strength on MB extraction. [MB] = 200 mg dm<sup>-3</sup>; T = 25.0 °C.

#### 4.8. Effect of Molar Ratio

Four DESs were prepared with HBA:HBD molar ratios of 1:0.5, 1:1, 1:1.5, and 1:2. TOP, an HBA, was kept constant while SA, an HBD, was varied. Figure 9 shows that a high extraction efficiency was obtained at an HBA:HBD molar ratio of 1, which was thus used as the optimal ratio. This observation was in agreement with findings by Xu et al. [26].

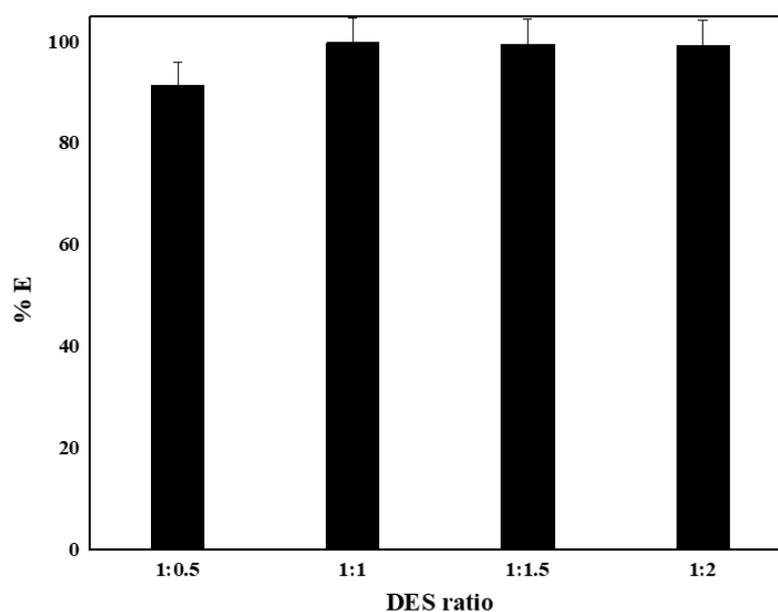


Figure 9. Effect of DES molar ratio (HBA:HBD) on MB extraction efficiency. [MB] = 200 mg dm<sup>-3</sup>; T = 25.0 °C.

#### 4.9. Thermodynamic study

To understand the effect of heat on the extraction of MB from an aqueous solution using DES-1, standard values of the enthalpy ( $\Delta H^\circ$ ), Gibbs free energy ( $\Delta G^\circ$ ), and entropy ( $\Delta S^\circ$ ) were obtained in the range of 25–50 °C. The van 't Hoff Equation (4) was used to calculate the  $\Delta H^\circ$  from a plot of  $\ln D$  vs.  $1/T$ , where  $D$  is the partition coefficient of MB between DES and the aqueous phase [29]. A third-order polynomial was then used to fit the data. Slopes of this polynomial were calculated at the given temperatures and  $\Delta H^\circ$ . The  $\Delta G^\circ$  and  $\Delta S^\circ$  were calculated using Equations (5) and (6), respectively.

$$\frac{d(\ln D)}{d(1/T)} = -\frac{\Delta H^\circ}{R} \quad (4)$$

where  $= \frac{C_i - C_f}{C_f}$ ,  $C_i$ , and  $C_f$  are defined in Equation (1).

$$\Delta G^\circ = -RT \ln D \quad (5)$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad (6)$$

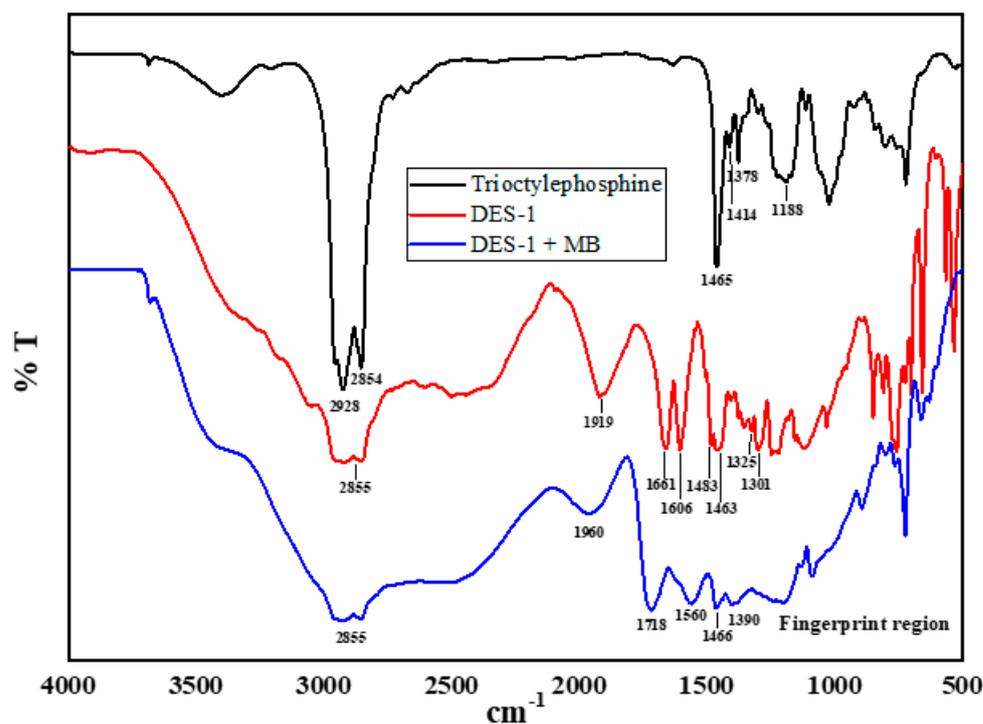
The thermodynamic parameters are listed in Table 3. Negative values for  $\Delta G^\circ$  at all temperatures confirmed the spontaneity of the MB extraction by DES-1. The drop in  $\Delta G^\circ$  with temperature indicated that heating reduced spontaneity. The negative value of  $\Delta H^\circ$  promoted MB interactions with DES-1. The decrease in entropy at higher temperatures also favored low-temperature extraction.

**Table 3.** Thermodynamic parameters for MB extraction from aqueous solutions using DES-1.

T (K)	$\Delta G^\circ$ (kJ mol <sup>-1</sup> )	$\Delta H^\circ$ (kJ mol <sup>-1</sup> )	$\Delta S^\circ$ (J K <sup>-1</sup> mol <sup>-1</sup> )
298	-12.39	-6.40	20.0
303	-8.71	-17.9	-3.0
313	-7.84	-116	-35.0
323	-7.76	-541	-165

#### 4.10. FTIR Spectra

The FTIR spectra of the HBA and TOP-SA before and after MB extraction were collected. Figure 10 shows a deep stretch in the DES-1 spectrum at 3300 cm<sup>-1</sup> that was attributed to the -OH group [30]. Peaks in the 3000–2900 cm<sup>-1</sup> and 2900–2800 cm<sup>-1</sup> regions were due to the -CH<sub>3</sub> and -CH<sub>2</sub> groups, respectively. The presence of C-P of TOP was indicated by bands in the 1200–900 cm<sup>-1</sup> region [31]. The -CH<sub>3</sub> and -CH<sub>2</sub> groups were present in the long alkyl chains of TOP [32]. The DES-1 spectra exhibited a sharp peak in the 1900–1600 cm<sup>-1</sup> region that was attributed to the carbonyl group of salicylic acid [33]. The DES-1 spectrum after MB extraction contained shifted band positions. For the carbonyl group, the 1919 cm<sup>-1</sup> band shifted to 1960 cm<sup>-1</sup> and the 1661 cm<sup>-1</sup> band shifted to 1718 cm<sup>-1</sup>. Such shifts were indicative of the ability of DES-1 to extract MB from the aqueous solution. These observations conformed to reported results [34].



**Figure 10.** FTIR spectra of TOP, DES-1, and DES-1 + MB.

#### 4.11. Cell Viability Assays

The MTT assay was performed using several DESs against human cell lines. Figure 11 shows that these DESs possessed a negligible cytotoxicity. According to ISO10993-5, if a drug viability is  $\geq 80\%$ , it is considered safe. In addition, a drug is considered weakly cytotoxic if the viability is in the range of 60–80% and moderately so, if the range is 40–60%. A potent cytotoxin has a viability of less than 40% [28,35]. DES-1 and DES-3 had no cytotoxic effects, at all the tested concentrations. For DES-2, DES-4, and DES-5 at concentrations of 0.125, 0.25, 0.50, and 1.0 mM, minimal cytotoxic activity was observed. However, there was a moderate effects at 2.0 mM. Our synthesized DESs had less cytotoxic effects in comparison with the commercial ones and the outcomes conformed to already-published data. For instance, Sajadiyeh et al. found that imidazolium-based ILs had low cytotoxic effects on human cells [36]. In addition, several DESs and ILs had reasonable cell viability effects on fish cell lines [37]. McLaughlin et al. reported that ILs showed a moderate to higher cytotoxicity at higher concentrations on human cell lines [38]. Other studies revealed that imidazolium-based ILs exhibited a higher cytotoxicity on human cell lines [27,39].

#### 4.12. Comparison with Other DES and ILs

The MB extraction efficiency of the synthesized DESs was compared with those of previously reported DESs and ILs. As shown in Table 4, DES-1 clearly had a higher MB extraction efficiency than the reported DESs and ILs. However, the [HPhIm][NTf<sub>2</sub>] IL reported in the literature had a comparable extraction efficiency (97.9%) to that of the DES-1 prepared in this work, but extraction with IL could have a high cost and be a non-user-friendly process due to its complicated preparation and unknown toxicology information [40]. Moreover, contact time is another important factor in determining the effectiveness of the extraction solvent. The DES synthesized in this study had a remarkably fast extraction reaction and reached equilibrium in less than 5 min.

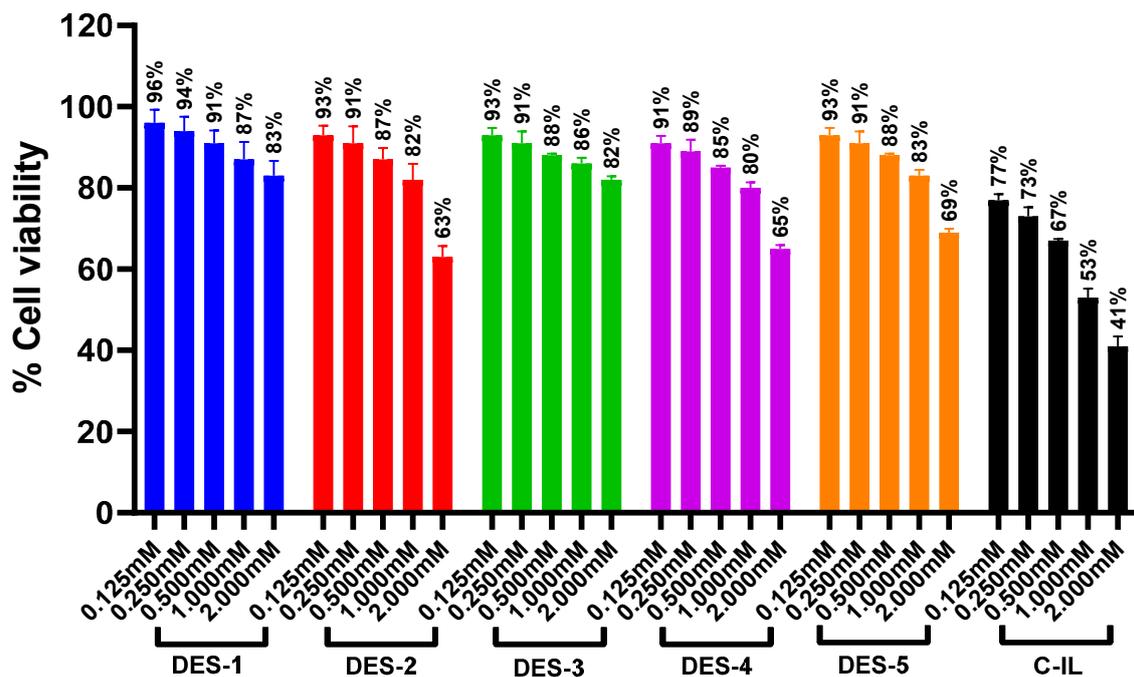


Figure 11. Effects of the five synthesized DESs on human cells at various concentrations. T = 25.0 °C.

Table 4. Methylene blue extraction efficiency comparison of synthesized DES with other DES- and IL-based extraction solvents.

Method	Extraction Solvent	Full Name	Abbreviations	Extraction Efficiency (%)	References
L-L extraction	IL	1-hexyl-2-phenyl-imidazolium bis(trifluoromethanesulfonyl) imide	[HPhIm][NTf <sub>2</sub> ]	97.9	[40]
		1-benzyl-3-methyl-imidazolium bis(trifluoromethanesulfonyl) imide	[BnMIm][NTf <sub>2</sub> ]	94.5	
		1-butyl-3-methyl-imidazolium bis(trifluoromethanesulfonyl) imide	[BMIm][NTf <sub>2</sub> ]	91.5	
		1-hexyl-3-methyl-imidazolium bis(trifluoromethanesulfonyl) imide	[HMIm][NTf <sub>2</sub> ]	90.0	
		1-octyl-3-methyl-imidazolium bis(trifluoromethanesulfonyl) imide	[OMIm][NTf <sub>2</sub> ]	85.0	
Aqueous two-phase system	DES	Choline chloride–glycerol	ChCl-G	>95.0	[26]
L-L Extraction	IL	1-butyl-3-methylimidazolium hexafluorophosphate	[C4mim]PF <sub>6</sub>	91.5	[41]
L-L Extraction	DES	Trioctylphosphine–salicylic acid	DES-1	99.3	This study
		Trioctylphosphine–malonic acid	DES-2	44.0	
		Trioctylamine–salicylic acid	DES-3	29.9	
		Trioctylamine–malonic acid	DES-4	17.8	
		Trihexylamine–salicylic acid	DES-5	6.5	

## 5. Conclusions

Five hydrophobic DESs using three HBAs (TOP, TOA, and THA) and two HBDs (SA and MA) were prepared and studied for MB aqueous phase extraction. All of the DESs

extracted MB from aqueous solutions but DES-1's extraction efficiency was remarkable, reaching up to 99.3%. The MB extraction parameters were thus optimized using DES-1. The extraction attained equilibrium in 5 min at 25.0 °C. The extraction efficiency decreased with HBD concentrations and the  $V_{DES}:V_W$  ratio. The thermodynamic parameters confirmed the spontaneous and exothermic nature of MB extraction and explained the drop in extraction efficiency with temperature. Additionally, DES-1 exhibited a minimal cytotoxicity. In summary, this study showed that DES-1 has the potential to efficiently and safely extract MB from wastewaters.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr10102152/s1>, Figure S1: Spectra of aqueous MB solutions at concentrations of 10.0, 15.0, and 30.0 mg dm<sup>-3</sup> were recorded in the range of 450–750 nm. Spectra of MB after extraction with DES-1 are also shown. It was apparent that DES-1 did not modify the spectrum; i.e., MB's spectral properties were unaffected by its transfer from the aqueous to the DES phase.

**Author Contributions:** M.F.H. performed the experiments and wrote the manuscript; N.Y. assisted in the experimental work; A.S.K. supervised the experimental work; T.H.I., M.I.K. and F.H.J. analyzed the data and reviewed the manuscript; N.A., R.S. and N.A.K. were responsible for the cytotoxicity studies. All authors have read and agreed to the published version of the manuscript.

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