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Aqueous two-phase systems based on hexafluoroisopropanol and hydrophilic organic solvents: phase diagrams and extraction studies

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This study explores a novel type of the aqueous two-phase system (ATPS) consisting of hexafluoroisopropanol (HFIP) and hydrophilic organic solvent as a phase separation inducing agent. Six solvents (acetone, acetonitrile, dimethyl sulfoxide, ethanol, methanol and tetrahydrofuran) were tested as HFIP-based ATPS inducing agents. Only aprotic solvents induced the formation of ATPS. The results suggest that the hydrogen bonding interaction between HFIP and aprotic solvent is the main driving force of phase separation. The phase separation ability of solvents increased with their log *P* values: less hydrophilic solvents induced phase separation at lower concentrations. HFIP/acetonitrile ATPS was evaluated as an extractant for organic compounds from various classes in aqueous solutions. Obtained extraction efficiencies can be ordered according to the following sequence: amines > esters ≈ aromatic hydrocarbons > hydroxy esters ≈ phenols > carboxylic acids. Interestingly, the proposed system shows an exceptionally good extractability of relatively hydrophilic neutral and positively charged basic compounds. The water immiscible phase possesses a high volatility, a higher than water density and a low viscosity. These properties make ATPS very promising as an extractant for conventional liquid–liquid extraction and particularly well-suited for liquid–liquid microextraction techniques.

Keywords: aqueous two-phase system, hexafluoroisopropanol, aprotic solvents, extraction

INTRODUCTION

Liquid–liquid extraction (LLE) is one of the oldest and still among the most popular techniques in the preparation of samples for analysis [1]. In conventional LLE, hydrophobic sample constituents are extracted from aqueous samples with a water-immiscible organic solvent, such as hexane, diethyl ether, ethyl acetate, chloroform, and some others. However, the limited polarity range of these solvents restricts their use for the extraction of more hydrophilic compounds. Over the last two decades, several new classes of solvents, such as ionic liquids [2], deep eutectic solvents [3] and aqueous two-phase systems (ATPS) [4], have been designed and introduced in LLE and miniaturised its techniques as alternatives to traditional organic solvents. Among the new generation extractants, ATPSs are perhaps most promising due to their wide polarity range, tunable physicochemical properties and simplicity of preparation [5].

ATPSs are formed by mixing at least two water-soluble compounds, traditionally, for example,

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polymer–polymer, polymer–salt, ionic liquid–salt and organic solvent–salt, which above a critical concentration are separated into two distinct phases, each one rich in one of the compounds [4]. ATPSs have been widely used for the preparative separation and purification of proteins, enzymes, nucleic acids, and other biomolecules [5–7]. In recent years, these systems have received extensive attention in the field of analytical chemistry. Both, conventional ATPS-based LLE [4, 8] and its miniaturised version, called homogeneous liquid–liquid microextraction [9], have been successfully applied for the preconcentration of various pollutants from aqueous samples. Although these alternative systems are generally simple, fast, inexpensive, and have potential for the extraction of compounds covering a wide polarity range, they also present certain limitations. Most of the proposed ATPS-based solvents are non-volatile, less dense than water, and highly viscous. These properties limit their use in conventional LLE, particularly in its miniaturised versions. Their low volatility makes them unsuitable for gas chromatography. Solvents less dense than water form a thin film on the surface after phase separation, which is difficult to collect. Additionally, their high viscosity complicates extract handling before analysis and hampers mass transfer between phases, leading to reduced extraction efficiency.

Hexafluoroisopropanol (HFIP) has recently become a very popular solvent with applications in various fields of chemistry [10]. Khaledi et al. [11, 12] reported that perfluorinated alcohols induce ATPS formation in aqueous solutions of various types of amphiphilic molecules such as synthetic surfactants, phospholipids and polyelectrolytes. Later, several ATPSs based on HFIP/Brij-35 [13], HFIP/salt [14] and HFIP-based deep eutectic solvent/salt [15] have been developed and their potential as extraction solvents has been assessed.

In our recent work [16], we proposed a novel HFIP-based ATPS, using acetonitrile as the phase-separation inducer. The proposed ATPS was utilised for the homogeneous liquid–liquid microextraction of cationic dyes from water samples. In this study, common hydrophilic organic solvents were tested as HFIP-based ATPS inducing agents. Their phase separation ability was evaluated and compared through phase diagram measurements. Finally, the HFIP/acetonitrile ATPS was evaluated as an extractant for organic compounds from various classes.

EXPERIMENTAL

Reagents and solutions

Hexafluoroisopropanol (purity ≥99%), acetone (ACE, purity ≥99.5%), acetonitrile (ACN, purity ≥99.9%), dimethyl sulfoxide (DMSO, purity ≥99.5%), ethanol (EtOH, purity ≥99.8%), methanol (MeOH, purity ≥99.9%) and tetrahydrofuran (THF, purity ≥99.5%) were purchased from Merck Life Science (Merck KGaA, Darmstadt, Germany). Analyte standards were acquired from Sigma-Aldrich (St. Lous, MO, USA) with a purity higher than 95%. Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA). All compounds used as model analytes (purity ≥98%) were purchased from Sigma-Aldrich. Stock solutions (500 mg/L) of all the model analytes were prepared in MeOH or MeOH/H_2O (1:1, v/v) according to the solubility of the selected compound and stored in a refrigerator at 4°C. Working solutions (2.0 mg/L) were prepared by diluting the stock solutions in Milli-Q water.

Chromatographic analysis

High-performance liquid chromatography (HPLC) separations were performed on an Agilent 1290 Infinity II LC system (Agilent, Waldbronn, Germany) equipped with a ternary pump, a thermostatted column compartment, a photodiode array detector and an autosampler. InfinityLab Poroshell 120 EC-C18 (3.0 × 150 mm, 2.7 μm, Agilent) column maintained at 25°C was used in the experiments. Separations were performed at a flow rate of 0.5 mL/min. The injection volume was 10 μL. Data acquisition was performed by Agilent OpenLAB CDS software.

Procedures

The binodal curves were determined gravimetrically ($\pm 10^{-4}$ g) by the cloud point method [17] at 20°C and atmospheric pressure. Briefly, an organic solvent was added dropwise to an aqueous HFIP solution until the visual detection of turbidity (biphasic region). Afterwards, water was dropwise added to the mixture until obtaining a clear solution (monophasic region). The procedure was

performed under constant magnetic stirring and was repeated until enough binodal data were obtained. The Merchuk equation [18] was then used to fit experimental binodal curves:

$$w_1 = A \cdot \exp\left(B \cdot w_2^{0.5} - C \cdot w_2^3\right). \quad (1)$$

Here w_1 and w_2 are, respectively, the mass fractions of HFIP and organic solvent, and A, B and C are the parameters obtained by the regression.

For the extraction efficiency measurements, 5 mL of the aqueous solution of different analytes with concentrations of 2.0 mg/L was placed into a 10 mL glass centrifuge tube, and 0.3 mL of HFIP and 0.3 mL of ACN were sequentially added. The mixture was shaken manually for 30 s, resulting in the formation of an emulsion. The phases were separated by centrifugation and the upper aqueous phase was then analysed using the HPLC technique. The extraction efficiency (EE) of each analyte was calculated according to the following equation:

$$EE(\%) = \frac{c_0 \cdot v_0 - c_i \cdot v_i}{c_0 \cdot v_0} \cdot 100\%. \quad (2)$$

Here c_0 and c_i are the concentration of the analyte in the aqueous phase before and after extraction, respectively. V_0 and V_i represent the volume of the aqueous phase before and after extraction, respectively. The final concentrations of the analytes in the aqueous phase were measured using the HPLC technique.

All measurements were performed in triplicate, and the mean values were reported.

RESULTS AND DISCUSSION

Six hydrophilic solvents, namely ACE, ACN, DMSO, EtOH, MeOH and THF, were initially tested as HFIP-based ATPS inducing agents. In this experiment, different amounts of an appropriate solvent were added into a glass centrifuge tube containing 5 mL of water and 0.3 mL of HFIP. No phase separation was observed when protic solvents (MeOH and EtOH) were used as inducing agents. In contrast, all four aprotic solvents induced the formation of ATPS with the aqueous phase at the top and the HFIP-rich phase at the bottom. Although the exact mechanism for this phenomenon is unknown, it is believed that the main driving force of phase separation is *competitive hydrogen bonding between water, HFIP, and inducing agent.* HFIP is a strong hydrogen bond donor (even stronger than water) but a weak hydrogen bond acceptor [10]. Similarly, both MeOH and EtOH exhibit a strong hydrogen bond donating ability while, in contrast, aprotic solvents are strong hydrogen bond acceptors. The hydrogen bonding interaction between HFIP and an aprotic solvent is stronger than that between HFIP and water. Thus, solvent molecules displace water molecules from the hydration layer of HFIP. In this case, the HFIP molecules likely cluster and even form micelle-like assemblies with the fluorine groups aggregating toward the centre of the cluster while oriented at the surface hydroxy groups are solvated by an aprotic solvent. Such clusters of HFIP molecules provide a hydrophobic local environment [19]. This results in the formation of the immiscible with water HFIP-rich phase.

Next, the ability of four aprotic solvents to form ATPS when mixed with the aqueous HFIP solution was evaluated. For this purpose, the binodal curves were measured at 20°C and atmospheric pressure. The measured binodal curves are represented in Fig. 1. The binodal curve divides the phase diagram into two regions: below the curve is the monophasic region and above the curve is the biphasic region. As shown in Fig. 1, the phase-formation ability of the solvent used to form ATPS increases in the following order: DMSO < ACN < ACE < THF. The obtained results indicate that for all solvents their phase-formation ability increased with their

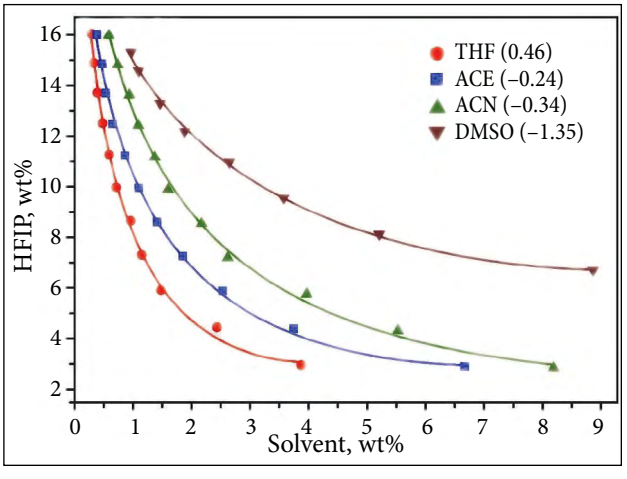

Fig. 1. Binodal curves of HFIP + organic solvent + water ATPSs at 20°C. Log *P* values [20] of the solvents are given in parentheses

log *P* values [20], given in Fig. 1: less hydrophilic solvents induced phase separation at lower concentrations.

The fitting parameters calculated by Eq. (1), standard deviations (σ) and correlation coefficients (R^2) are given in Table 1. Based on the obtained results, it was concluded that Eq. (1) adequately describes the experimental data.

To evaluate the extraction performance of the proposed system, it was tested for the extraction of various organic compounds from aqueous solutions. Acetonitrile was selected as the phase separation agent for the extraction experiments. Six classes of organic compounds, namely aromatic hydrocarbons, esters, hydroxy esters, amines, phenols and carboxylic acids, were used as model analytes. For all compounds, except for carboxylic acids, the pH values at which extractions were carried out were in a range of 7.0 ± 0.5. To suppress the dissociation of carboxylic acids, their solutions were acidified with HCl to pH 2.0. The extraction efficiencies for the compounds tested using the proposed ATPS are presented in Table 2. Figure 2 represents the chromatograms of the aqueous solution of three phthalates before and after extraction. It was not possible to evaluate higher than 98% EE data for most compounds because their concentrations in the aqueous phase after the extraction were below the limit of quantification. It can be observed that for all analytes among the same class their %EE values showed a good correlation with their log *P* values, listed in Table 2. These results indicate that hydrophobicity is the key property ruling the extraction efficiency of compounds with the same functional groups. However, there was no correlation between the %EE and hydrophobicity for the analytes of different classes. For example, the extraction efficiencies obtained for the compounds possessing similar log *P* values, quercetin (log *P* = 1.82), methylparaben (log *P* = 1.96) and 2-nitroaniline (log *P* = 1.85), ranged from 39.7% for quercetin to ≥98% for 2-nitroaniline. Interestingly, the extraction efficiencies of the most hydrophilic caffeine (log *P* = –0.07) and nicotine (log *P* = 1.17) were considerably higher than those obtained for much less hydrophilic carboxylic acids, phenol, quercetin and methylparaben.

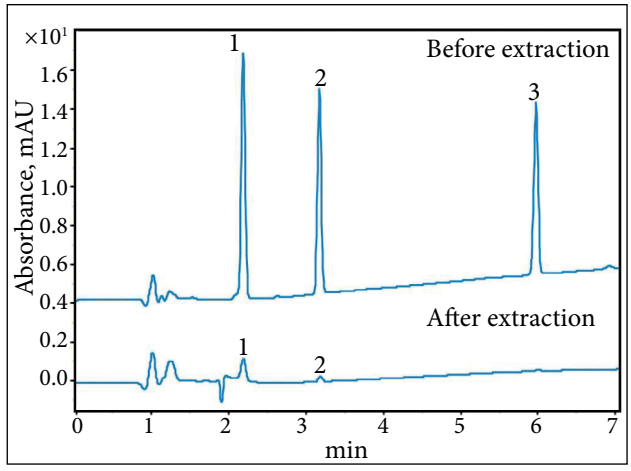

Fig. 2. Chromatograms of the aqueous solution of three phthalates before and after extraction. 1, dimethyl phthalate; 2, diethyl phthalate; 3, dibutyl phthalate

In general, the obtained extraction efficiencies for compounds of different classes can be arranged according to the following sequence: amines > esters ≈ aromatic hydrocarbons > hydroxy esters ≈ phenols > carboxylic acids. These results indicate that the proposed ATPS is the most effective extractant for basic compounds, less effective for neutral ones, and poorly extracts acidic analytes. Since only the basic analytes are strong hydrogen bond acceptors, this is not surprising. Thus, strong hydrogen bonding interactions with HFIP, which displays a high hydrogen bond donor ability, enhance the extraction of

Table 1. **Parameters of Eq. 1 for HFIP + organic solvent + water ATPSs at 20°C**

ATPS	A	B	C	R^2	σ[a]
HFIP + THF + H_2O	35.572	–1.499	–0.0198	0.9971	0.186
HFIP + ACE + H_2O	30.321	–1.061	$-1.40\cdot10^{-3}$	0.9970	0.205
HFIP + ACN + H_2O	32.070	–0.903	$3.73\cdot10^{-4}$	0.9968	0.210
HFIP + DMSO + H_2O	25.213	–0.522	$-3.31\cdot10^{-4}$	0.9976	0.119

[a] $\sigma = \sqrt{\sum (w_{cal} - w_{exp})^2 / n}$, where w_{cal} and w_{exp} are the calculated and experimental mass fractions, respectively.

Table 2. **Extraction efficiencies of selected organic compounds (2.0 mg/L) from aqueous solution (aqueous sample volume 5.0 mL, HFIP volume 0.3 mL, ACN volume 0.3 mL, extraction time 30 s)**

Class	Analyte	Log *P* [20]	EE ± σ, %; *n* = 3
Aromatic hydrocarbons	Benzene	2.13	86.1 ± 1.8
	Toluene	2.73	90.3 ± 1.6
	Naphthalene	3.30	94.7 ± 2.1
Esters	Dimethyl phthalate	1.60	91.5 ± 1.5
	Diethyl phthalate	2.42	96.8 ± 1.9
	Dibutyl phthalate	4.50	≥98
Hydroxy esters	Methylparaben	1.96	40.1 ± 0.6
	Ethylparaben	2.47	57.1 ± 0.6
	Propylparaben	3.04	77.6 ± 1.3
Amines	Caffeine	–0.07	67.7 ± 1.4
	Nicotine	1.17	91.8 ± 1.8
	2-Nitroaniline	1.85	≥98
Carboxylic acids	Sorbic	1.33	25.6 ± 0.9
	Benzoic	1.87	29.2 ± 0.8
	Salicylic	2.26	38.8 ± 1.2
Phenols	Phenol	1.46	31.8 ± 0.7
	Quercetin	1.82	39.7 ± 0.6
	Biochanin A	3.22	81.3 ± 1.6

basic compounds. The presence of ACN does not appear to have a role in the extraction, other than in generating the ATPS because the same trend was observed for the extraction efficiencies using other inducing agents (Fig. 3). DMSO was not used in this experiment because of its poor phase separation capability. Some differences in the obtained EE% values may be attributed to slight variations (±20 μL) in the formed HFIP-rich phase volumes.

In LLE using traditional organic extractants, the neutral form of an analyte usually exhibits better extractability than its less hydrophobic charged form. The extraction of nicotine at acidic (pH = 3.0) and alkaline (pH = 11.0) conditions was examined to prove this to our system. In an alkaline aqueous solution, nicotine (pK_a = 8.1) is uncharged, while under acidic conditions it exists in a fully protonated cationic form. The EE for the charged form was found to be 94.8%, compared to 87.6% for the neutral form, indicating a comparable or even better extraction of the cationic form. However, this was not observed for the benzoic acid: the %EE obtained for its neutral form at pH 2.0 was approximately 2.5 times higher than that of its anionic form at pH 10.

In order to further explore the role of charge sign of the analyte, we decided to evaluate the extraction efficiency of the two hydrophilic and oppositely charged dyes, namely cationic methylene blue and anionic acid red 1. As shown in Fig. 4, the cationic methylene blue seems to be completely extracted into the bottom HFIP-rich phase whereas the negatively charged acid red 1 almost completely remained in the top aqueous phase.

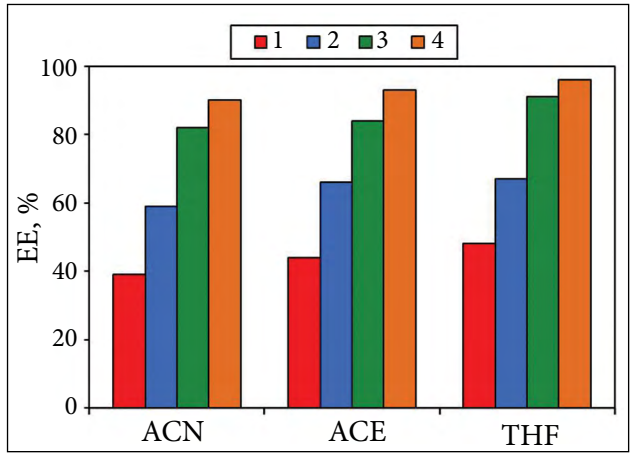

Fig. 3. The effect of ATPS inducing solvent type on the extraction efficiencies of four organic compounds (2.0 mg/L). Aqueous sample volume 5.0 mL; HFIP volume 0.3 mL; inducing agent volume 0.15 mL (THF), 0.20 mL (ACE) and 0.30 mL (ACN); extraction time 30 s. 1, salicylic acid; 2, ethylparaben; 3, biochanin A; 4, nicotine

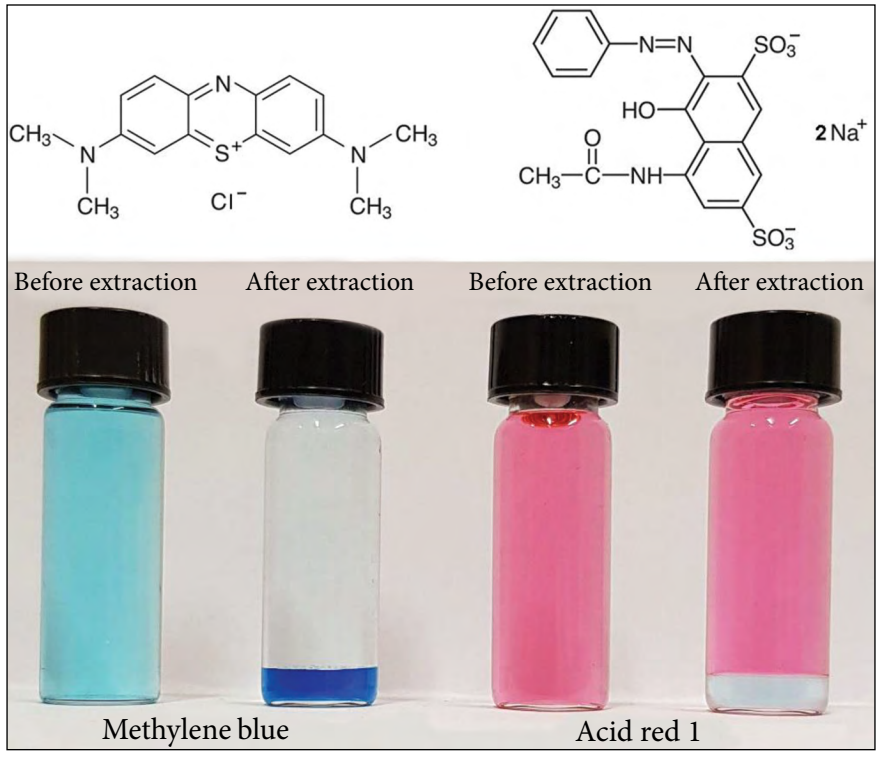

Fig. 4. Partition behaviour of the oppositely charged dyes

CONCLUSIONS

Aprotic hydrophilic solvents induced the formation of ATPS in aqueous HFIP solutions. Compared to conventional ATPSs based on polymers, surfactants, ionic liquids, and deep eutectic solvents, the proposed system has several advantages. The water-immiscible HFIP-rich phase exhibits a high volatility, a higher density than water and a low viscosity. These properties make the ATPS highly promising as an extractant for conventional liquid–liquid extraction, and particularly well-suited for liquid–liquid microextraction techniques. Finally, as the proposed system demonstrates an exceptional extractability for relatively hydrophilic neutral compounds, as well as charged basic compounds, it shows a great promise as a selective extractant for these substances from complex matrices.

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VANDENINĖS DVIFAZĖS SISTEMOS HEKSAFLUORIZOPROPANOLIO IR HIDROFILINIŲ ORGANINIŲ TIRPIKLIŲ PAGRINDU: FAZIŲ DIAGRAMOS IR EKSTRAKCIJOS TYRIMAI

S a n t r a u k a

Šiame darbe parodyta, kad hidrofiliniai organiniai tirpikliai inicijuoja vandeninės dvifazės sistemos (VDS) susidarymą vandeniniuose heksafluorizopropanolio (HFIP) tirpaluose. VDS iniciatoriais buvo palyginti šeši organiniai tirpikliai – acetonas, acetonitrilas, dimetilsulfoksidas, etanolis, metanolis ir tetrahidrofuranas. Nustatyta, kad VDS susidarymą inicijuoja tik aprotoniniai tirpikliai. Tikėtina, kad pagrindinė VDS susidarymo varomoji jėga – stiprus vandenilinis ryšys tarp HFIP ir inicijuojančio tirpiklio. Aprotoninių tirpiklių VDS susidarymą inicijuojančioji geba stiprėja didėjant tirpiklio log *P* vertei: kuo hidrofobiškesnis tirpiklis, tuo mažesnė jo koncentracija reikalinga VDS susidarymui. Įvairių klasių organinių junginių ekstrakcijos iš vandeninių tirpalų tyrimui buvo pasirinkta sistema HFIP/acetonitrilo pagrindu. Nustatyta, kad organinių junginių ekstrakcijos efektyvumas mažėja tokia tvarka: aminai > esteriai ≈ aromatiniai angliavandeniliai > hidroksiesteriai ≈ fenoliai> karboksirūgštys. Ypač efektyviai ekstrahuojami santykinai hidrofiliniai neutralūs ir teigiamą krūvį turintys baziniai junginiai. Susidaranti nesimaišanti su vandeniu fazė yra labai laki, sunkesnė už vandenį bei pasižymi nedidele klampa. Šios savybės rodo, kad pasiūlyta sistema turėtų būti puikia alternatyva tradiciniams organiniams tirpikliams klasikinėje skysčių-skysčių ekstrakcijoje ir ypač miniatiūrizuotoms jos versijoms.