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ORIGINAL PAPER

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Solvent extraction of amino acids into a room temperature ionic liquid with dicyclohexano-18-crown-6

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Abstract Amino acids Trp, Gly, Ala, Leu are extracted efficiently from aqueous solution at pH 1.5–4.0 (Lys and Arg at pH 1.5–5.5) into the room temperature ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ($BmimPF_6$) with dicyclohexano-18-crown-6 (CE). The most hydrophilic amino acids such as Gly are extracted as efficiently as the less hydrophilic (92–96%). The influence of pH, amino acid and crown ether concentration, volume ratio of aqueous and organic phases, and presence of some cations on amino acid recovery were studied. The ratio of amino acid to crown ether in the extracted species is 1:1 for cationic Trp, Leu, Ala, and Gly and to 1:2 for dicationic Arg and Lys. This ionic liquid extraction system was used successfully for the recovery of amino acids from pharmaceutical samples and fermentation broth, and was followed by fluorimetric determination.

Keywords Room temperature ionic liquid · Liquid–liquid extraction · Amino acids · Dicyclohexano-18-crown-6

Introduction

Amino acids are vital components of a variety of biological, industrial, and environmental samples. Amino acids find their principal commercial applications in human foods, animal feed additives, and in pharmaceuticals; they are also used as intermediates in the synthesis of specialty chemicals. Development of the methods for recovery and determination of amino acids is an problem in analytical chemistry and biotechnology. The diversity, speed, and technological simplicity of liquid–liquid extraction attracts interest to this method.

Amino acids are hydrophilic and are therefore difficult compounds for conventional solvent extraction. However, the extractive potential of the organic solvent can be enhanced by addition of lipophilic cationic or anionic extractants, which, at certain pH, can form extractable complexes with amino acids [1, 2, 3, 4, 5, 6, 7]. However, extraction is typically not efficient, and other substances might interfere.

Another way to facilitate extraction is by use of macrocyclic compounds which form stable hydrophobic "host–guest" complexes with amino acids. The most popular reagents are crown ethers, which form complexes by hydrogen bonding of protonated amino groups [8, 9, 10, 11]; different types of carrier molecule have been designed and studied [12, 13, 14, 15, 16, 17, 18]. Creating optimum conditions for recovery of amino acids is usually achieved by use of hydrophobic counter-ions. For example, the present authors have proposed extraction of amino acids into chloroform with crown ether and anionogenic extractants, dinonylnaphthalenesulfonic acid, and di(2-ethylhexyl)phosphoric acid [19, 20]. However, even then extraction of amino acids, in particular, the most hydrophilic (e.g. Gly) is not very efficient.

Recently, considerable attention has been drawn to room-temperature ionic liquids (RTIL) as "green" alternatives to common solvents. RTIL are organic salts with melting points at or below room temperature. Typically, RTIL consist of a nitrogen- or phosphorus-containing organic cation (such as quaternary ammonium, pyridinium, imidazolium, phosphonium) and a large organic or inorganic anion [21, 22, 23]. Modification of both cation and anion enables fine tuning of solvent properties [24, 25, 26]. RTIL have a wide temperature range in which they are liquid, high heat capacity, high density, and high thermal and chemical stability. They have low vapor pressure and are non-harmful [21, 23, 27, 28]. They also have good electrochemical properties, for example high conductivity and wide electrochemical window [29, 30].

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Ionic liquids are extensively investigated as a replacement for volatile organic solvents in chemistry in general [23, 29] and chemical analysis, in particular. RTIL have been used as stationary phases in gas chromatography [31], mobile phases in liquid chromatography [22], and in the separation of phenolic compounds by capillary electrophoresis [32]. RTIL have substantial potential as diluents in separations; their application is an important step in the design of environmentally safe separation processes, because RTIL are non-flammable, non-toxic and non-volatile. The first reports of the use of RTIL in liquid–liquid separations both of organic substances [33, 34, 35] and of metal ions [36, 37, 38] have appeared very recently. Dai et al. [36] reported highly efficient extraction of strontium ion from water ($D_{Sr}>10^4$) with dicyclohexano-18-crown-6 into different water-immiscible 3-methylimidazolium-based RTIL. Armstrong et al. [35] reported the partition coefficients of a large variety of organic compounds bearing different functionality. The last authors mentioned the moderately effective extraction of relatively less hydrophilic aromatic amino acids (Trp, Phe) in the presence of dibenzo-18-crown-6.

Herein, we report a detailed study of amino acid extraction from aqueous solutions into the room-temperature ionic liquid 1-methyl-3-butylimidazolium hexafluorophosphate ($BmimPF_6$) containing the crown ether dicyclohexano-18-crown-6 (CE). Importantly, nearly quantitative extraction is observed, without any additional counterions, even for the most hydrophilic amino acids. This might give rise to novel promising separation and analysis schemes; we successfully utilized this extraction process for recovery of amino acids from fermentation production solution, with subsequent fluorimetric analysis.

Experimental

Chemicals and apparatus

1-Butyl-3-methylimidazolium hexafluorophosphate ($BmimPF_6$) was synthesized as described elsewhere [33]. Its purity was checked by elemental analysis. Dicyclohexano-18-crown-6 (DC18C6) (a mixture of stereoisomers) was of chemical purity grade. The amino acids arginine, glycine, tryptophan, leucine, alanine, lysine, and valine were all of analytical grade; all except DL-alanine were the L isomer. *o*-Phthalaldehyde (OPA) and *N*-acetyl-L-cysteine were Merck (Germany) products. Other chemicals and reagents were of analytical grade.

pH was measured with an "Expert-001"pH-meter (Econix, Russia). Fluorescence measurements were carried out using a "Fluorat-02" (Lumex, Russia) fluorimeter with 1.00 cm quartz cell; excitation and emission wavelengths were 340 nm and 450 nm, respectively. Spectrophotometric measurements were carried out using a UV-2201 (Shimadzu, Japan) spectrophotometer with matched quartz cells.

Procedure

Extraction was performed at room temperature (21±1 °C). A known amount of amino acids was dissolved in distilled water (concentration range 1×10^{-5}–2×10^{-3} mol L^{-1}). This solution (3 mL) was placed in stoppered vessels with 1 mL $BmimPF_6$. In experiments on the effect of crown ether the CE concentration was 5×10^{-3}–5×10^{-1} mol L^{-1} (1.86–37.25 g L^{-1}). The pH of the aqueous phase was adjusted with HNO_3 and LiOH. After equilibrium was achieved the phases were separated. The distribution ratio of amino acids (D) between water and ionic liquid phases was calculated as $D=(C_{aq}^0-C_{aq})/C_{aq}$, where C_{aq}^0 and C_{aq} are the concentrations of amino acids in aqueous phase before and after extraction, respectively. The recovery (R, %) was defined as $R=(C_{aq}^0-C_{aq})\times100/C_{aq}^0$.

The concentration of amino acids in aqueous solution after extraction (C_{aq}) was determined by fluorescence reaction with *o*-phthalaldehyde in the presence of reducing agent, *N*-acetyl-L-cysteine [39]. For this purpose 3 mL of fluorescent reagent was added to 1 mL amino acid aqueous solution, the vessel was shaken for 1 min and after 5 min the fluorescence was measured. The fluorescent reagent – 60 mL borate buffer (0.05 mol L^{-1}, pH 9) was mixed with 1 mL OPA (10.0 mg mL^{-1} in ethanol) and 1 mL *N*-acetyl-L-cysteine (12.1 mg mL^{-1} in ethanol) – was prepared directly before determination. The linear range for Trp, Phe, Ala, Gly, and Arg was 5×10^{-6}–1×10^{-3} and for Lys 1×10^{-5}–1×10^{-3} mol L^{-1}. All statistical calculations were based on seven replicates. The limit of detection (mol L^{-1}) for Trp, Leu, Ala, Gly, Arg, and Lys was 5.03×10^{-7}, 5.25×10^{-7}, 5.29×10^{-7}, 4.59×10^{-7}, 6.65×10^{-7}, and 9.48×10^{-7}, respectively.

For determination of the ionic liquid content of the aqueous phase UV absorption spectra of the aqueous phase were recorded before and after contact with the ionic liquid phase (15 min). Initial amino acid concentration in the aqueous solution varied from 1×10^{-3} to 1×10^{-2} mol L^{-1} (pH 2); the concentration of CE in the RTIL was 1×10^{-1} mol L^{-1}. The absorption peak of 1-butyl-3-methylimidazolium at ca. 300 nm was used for measurement.

HPLC analysis of the mixture of amino acids

The HPLC system consisted of a Mightsil RP18 (150 mm×4.6 mm) separation column, Aquilon pump, and Biotronic-400 amperometric detector. After post-column derivatization of amino acids with OPA the amperometric measurements were performed with a three-electrode cell by means of a glassy-carbon working electrode, an Ag/AgCl (sat. KCl) reference electrode, and the a body of the cell as auxiliary electrode. The potential used for detection of amino acid derivatives was 0.7 V. The mobile phase was a mixture of acetonitrile (25%) and Na_2HPO_4 (75%). A 20-μL aliquot of the aqueous phase was injected for analysis (chromatographic experiments were performed by Dr E.N. Shapovalova and M. Chernobrovkin, Moscow University).

Analysis of the pharmaceutical samples and fermentation broth

Pharmaceutical "Glicin" (MNPK "Biotiki", Russia) was dissolved in purified water by shaking for about 30 min, and diluted to volume in a volumetric flask with purified water. Appropriate aliquots were taken in such a way that the final concentrations of the amino acid were 4.99, 9.98, and 99.75 mg L^{-1} in each vessel. Drug "Vitamax" (GlaxoWellcome, Egypt; 0.1 g) was dissolved in 50 mL purified water with added HCl and filtered through filter paper. The filtrate was diluted in five times. The amount of arginine and lysine in aliquots were each 8 mg L^{-1}. The extraction of amino acids was performed directly from aqueous solution or solution obtained by dilution of the initial mixture (e.g. for fermentation broth) – 3 mL aqueous solution (pH 1.2–2.0) was brought into contact with 1 mL $BmimPF_6$ containing CE (0.1 mol L^{-1}). After establishing the equilibrium (typically 15 min) the phases were separated. For back-extraction each ionic liquid phase was placed in a stoppered vessel containing distilled water with LiOH (3 mol L^{-1}, 0.1 mL) and shaken for 15 min. After establishing the equilibrium phases were separated and the aqueous phase was used for fluorescence determination of amino acids.

Calibration graphs were constructed for fluorescence determination of amino acids. Under optimum conditions the fluorescence intensity of the fluorescent adduct formed as a result of reaction of the amino acid with OPA was proportional to the concentration of

amino acid, with a good linear relationship. Linearity was assessed by adding amino acid solution in the concentration range 1×10^{-5}–1×10^{-3} mol L^{-1} for Trp, Leu, Ala, and Gly and 3×10^{-5}–1×10^{-3} mol L^{-1} for Arg and Lys. The regression equations were:

Trp	$I = 18312C + 0.2263$	$R^2 = 0.9973$
Leu	$I = 18051C + 0.3341$	$R^2 = 0.9979$
Ala	$I = 17965C + 0.2271$	$R^2 = 0.9981$
Gly	$I = 20159C + 0.2588$	$R^2 = 0.9958$
Arg	$I = 16622C + 0.1590$	$R^2 = 0.9965$
Lys	$I = 11580C + 0.2451$	$R^2 = 0.9974$

where I is the fluorescence intensity and C the concentration in mol L^{-1}. The satisfactory correlation coefficient values showed that Trp, Phe, Gly, Ala, Lys, and Arg responses were linear in the concentration ranges studied. Limits of detection of 9.37×10^{-7}, 9.50×10^{-7}, 8.51×10^{-7}, 9.55×10^{-7}, 1.46×10^{-6}, and 1.03×10^{-6} mol L^{-1} for Trp, Leu, Gly, Ala, Lys, and Arg, respectively, were calculated on the basis of the 3S criterion.

Results and discussion

Time dependence

Preliminary experiments showed that the equilibrium was achieved within 15 min or less.

Dependence on phase volume ratio

To reduce consumption of RTIL we typically used in extraction 1 mL solvent and 3 mL aqueous solution. As is seen from Table 1, even if the ratio V_{aq}/V_o is much larger, e.g. 20, the distribution coefficients remain rather high. (In principle, this enables concentration of amino acids from large volume of aqueous solutions into the small volume of the organic phase). Some decrease of D at higher volume ratios can be attributed to progressive loss of RTIL, because it is partially soluble in water.

Table 1 Effect of the ratio of aqueous and organic phase volumes on Trp[a] extraction

V_{aq}/V_o	3	5	10	15	20
D	75.9	74.8	73.1	46.6	20.7
Recovery (%)	98.7	98.7	98.7	97.9	95.4

[a]Concentration of Trp 1×10^{-4} mol L^{-1}, CE 1×10^{-1}mol L^{-1}

pH Dependence

Extraction of amino acids with crown ether is illustrated by Fig. 1. Amino acids Trp, Gly, Ala, and Leu are extracted nearly quantitatively from acidic solutions, pH 1.5–4.0 (Lys, Arg at pH 1.5–5.5). The pH values correspond well to the range in which the cationic form of the respective amino acids dominates. With increasing pH (pH>5.5) the amino acids are transformed from cations to zwitterions and then to anions, which results in a progressive decrease of extraction (Fig. 1). It is worth mentioning that arginine and lysine can form both cationic and dicationic species. Dicationic species predominate at low pH whereas the state changes to single-charged cation as pH increases. Obviously, the range of efficient extraction of these amino acids is much broader than for Trp, Gly, Ala, and Leu.

The role of the crown ether

Extraction of amino acids (2×10^{-5}–2×10^{-1} mol L^{-1}) into ionic liquid phase in the absence of crown ether is rather low, 5–10%. However, extraction becomes quantitative in the presence of 0.05–0.10 mol L^{-1} crown ether. This is strongly indicative of the crown ether's critical role as a complexing reagent. Undoubtedly, in a RTIL, as in conventional non-polar media, hydrogen bonding occurs between the ammonium center of the (cationic) amino acid and the polyether.

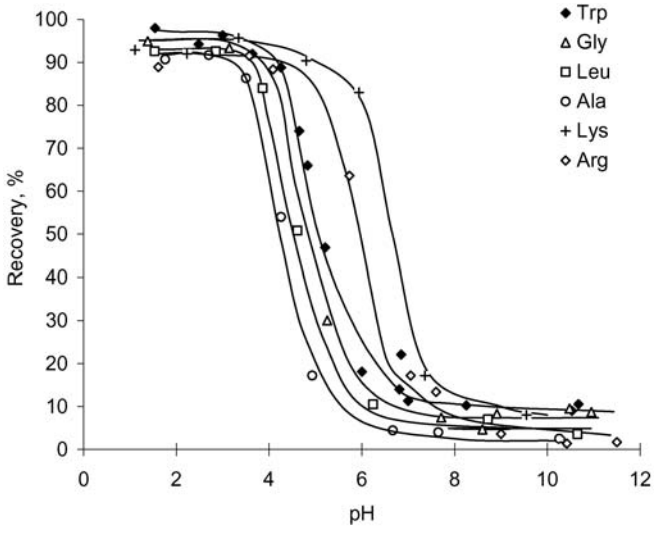

Fig. 1 Dependence on pH of extraction of amino acids (1×10^{-4} mol L^{-1}) with DC18C6 (1×10^{-1}mol L^{-1}) into $BmimPF_6$

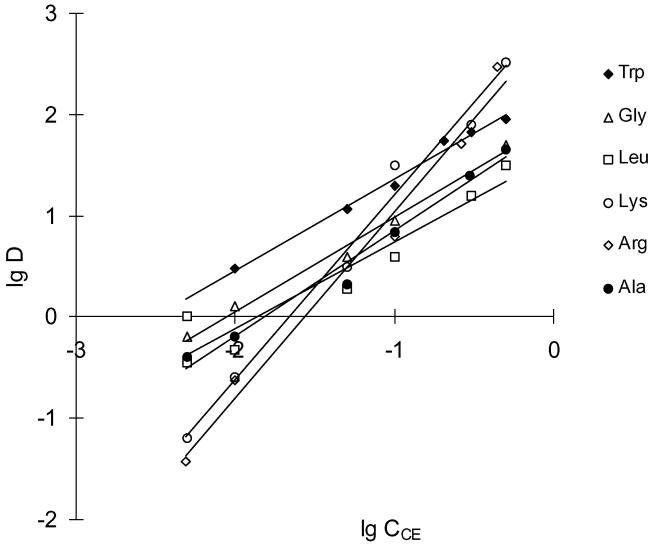

Fig. 2 Dependence on crown ether concentration of extraction of amino acids (1×10^{-4} mol L^{-1}) into $BmimPF_6$

To establish the stoichiometry of interaction we studied the dependence of amino acid extraction on crown ether concentration. The slope of the linear parts of the curves $\lg D = f(\lg C_{CE})$ is 1 for Trp, Leu, Gly, and Ala and 2 for Arg and Lys (Fig. 2). Therefore, the ratio of amino acid to crown ether is 1:1 for cationic amino acids and 1:2 for dicationic amino acids. This confirms the common mechanism involving binding of an ammonium group to the 18-6 crown ether.

The most interesting point is that extraction with RTIL does not require addition of a counter-ion. Notably, even in the presence of crown ethers amino acids are typically not extracted into conventional solvents without a specially introduced hydrophobic counter-anion. Moreover, even the presence of such a counter- anion does not usually provide efficient extraction [20, 40, 41]. For example, distribution coefficients of amino acids in the presence of 1×10^{-1} mol L^{-1} DC18C6 and 5×10^{-2} mol L^{-1} di(2-ethylhexyl)phosphoric acid are 1.86 for Trp, 1.63 for Phe, 1.04 for Leu, 0.64 for Lys, and 0.42 for Gly (without crown ether: 0.85, 0.67, 0.64, 0.49 and 0.23, respectively) [20].

Extraction equilibria

Given the observed pH-profile for extraction we propose that amino acids are extracted into RTIL in the cationic form. As a common theory suggests, in these circumstances to achieve electroneutrality cations of amino acids should be accompanied by counter-ions [42, 43]. As no special counter-ions were introduced, the counter-ion could be the anion of nitric acid used for pH adjustment. It might thus be expected that partitioning of the amino acids increases with nitric acid concentration. However, the partition coefficients of amino acids with 0.02 and 0.30 mol L^{-1} HNO_3 were practically the same. Moreover, study of the extraction of amino acids from nitric and hydrochloric acids solutions reveals no influence of anions of the aqueous phase on the extraction. Therefore, co-extraction of an aqueous phase anion does not occur and one must suppose that it is hexafluorophosphate anion (component of ionic liquid) which acts as counter-ion for cations of amino acids in the organic phase.

To describe amino acid extraction it is necessary to take into account all the equilibria which might occur in the aqueous and organic phases:

Protolytic equilibrium:

$$AmH_2^+ \overset{K_a}{\rightleftharpoons} AmH + H^+$$

$$AmH + H^+ \overset{K_b}{\rightleftharpoons} AmH_2^+$$

where AmH^+ and AmH are the cation and zwitterion, respectively, of the amino acid.

Partitioning of the crown ether between aqueous and ionic liquid phases:

$$CE_o \overset{K_d}{\rightleftharpoons} CE$$

Partitioning of the ionic liquid into water

$$Bmim_o^+ + PF_{6o}^- \overset{K_D}{\rightleftharpoons} Bmim^+ + PF_6^-$$

(Note that this description is simplified, because one cannot reject the possibility of ion-pair distribution and ion-exchange of RTIL components with cations and anions of the aqueous phase).

The following equilibrium equations can be used to describe extraction, assuming electroneutrality:

$$AmH_2^+ + CE_o + PF_6^- \rightleftharpoons AmH_2^+ \bullet CE\ PF_{6o}^-$$

$$AmH_2^+ + CE_o + Bmim_o^+ \rightleftharpoons AmH_2^+ \bullet CE_o + Bmim^+$$

The first expression corresponds to ion-pair extraction with hexafluorophosphate (RTIL component) as the counter-ion for the amino acid cation. The second expression corresponds to a cation-exchange reaction in which the amino acid cation goes into the organic phase and the dialkylimidazolium ($Bmim^+$) into the aqueous phase. For these equilibrium, the following conditional ion-exchange constant can be written:

$$K = [AmH_2^+\ CE_o [Bmim^+]\ [AmH_2^+]^{-1} [CE]_o^{-1} [Bmim^+]_o^{-1}$$

If ion pair extraction were occurring the distribution ratio should increase on increasing the aqueous concentration of nitric acid (or another acid used to create an acidic medium, e.g. HCl). However, this effect was not observed.

Another indication of an ion exchange mechanism is the significant leakage of the dialkylimidazolim cation into the aqueous phase on extraction of amino acids – confirmed by monitoring the corresponding UV band. After extraction of amino acids the clear absorption peak of the dialkyl imidazolium cation appeared in the spectrum of the aqueous phase at ca. 300 nm (whereas the absorbance peak of Trp at 280 nm disappeared). Increasing the initial amino acid concentration in the aqueous phase from 1×10^{-3} to 1×10^{-2} mol L^{-1} results in an increase of the dialkylimidazolium concentration in water after extraction. Clearly this is caused by ion exchange of the dialkylimidazolium cation with the amino acid cation. Thus the absence of any appreciable anion effect and distribution of dialkylimidazolium cation into water, which accompanies extraction, strongly suggest that extraction of amino acids with DC18C6 into the ionic liquid is an ion-exchange process.

It should be noted that a similar ion-exchange scheme was proposed as a mode of extraction of strontium into RTIL containing crown ether [37].

Selectivity/various AA

The use of RTIL as diluents for amino acid extraction with crown ether enables high distribution coefficients to be obtained and quantitative recovery to be achieved almost for all the amino acids studied. It is also important that the extraction is quantitative for glycine and other hydrophilic amino acids. Recovery and distribution coefficients are presented in Table 2.

The most hydrophilic amino acids such as Gly are extracted efficiently in the presence of less hydrophilic

Table 2 Recovery and distribution coefficients for amino acid[a] extraction into $BmimPF_6$ with DC18C6[b] (V_o/V_{aq}=1:3, n=8, P=0.95)

Amino acid	D	Recovery (%)
Trp	76	96±3
Gly	58	95±3
Leu	40	93±3
Ala	32	92±3
Lys	47	94±4
Arg	32	92±3

[a]Concentration of amino acids 1×10^{-5}–1×10^{-3} mol L^{-1}
[b]DC18C6 concentration 1×10^{-1} mol L^{-1}

Table 3 Effect of foreign substances on extraction of Trp into $BmimPF_6$ with DC18C6 under optimum conditions

Foreign substance	Recovery[a] (%) for Trp/foreign substance			
	1:1	1:10	1:100	1:1000
K^+, NO_3^-	80	72	64	28
Na^+, NO_3^-	94	89	48	35
Ca^{2+}, NO_3^-	86	76	70	–
Benzylamine hydrochloride	73	28	24	–

[a]In the absence of foreign substances recovery is 96%

amino acids. For instance, recovery of Trp, Val, and Gly is 99, 94, and 93%, respectively, from an equimolar mixture. The extraction was conducted by bringing 3 mL RTIL into contact with 3 mL aqueous solution of amino acids (5×10^{-4} mol L^{-1} each) for 15 min. The pH was 1,80, the CE concentration 0.10 mol L^{-1}

The authors of recent work [35] reported that the distribution coefficients for Phe and Trp into $BmimPF_6$ in the presence of dibenzo-18-crown-6 were, respectively, 0.41 and 1.71 at pH 1.0 (in the presence of 0.1 mol L^{-1} trifluoroacetic acid). It should be noted that our results indicate that extraction of amino acids with dicyclohexano-18-crown-6 is two orders of magnitude better than that obtained with dibenzo-18-crown-6 [35].

The effect of the presence of cations that can also be complexed by the crown ether was investigated further. The extraction experiments were carried out in the presence of the nitrates of sodium, potassium, and calcium and the hydrochloride of benzylamine. In Table 3 we report recovery (R, %) value for extraction of Trp under these conditions.

The potassium ion, which is known to form stable complexes with dicyclohexano-18-crown-6, reduces extraction of the amino acids to a greater extent than other alkali or alkali earth metal ions (recovery is 80, 72, 64, and 28% for Trp/K^+ ratios of 1:1, 1:10, 1:100, and 1:1000, respectively).

Somewhat more intriguing is the relatively low interference of benzylammonium, which is more hydrophobic than amino acid cations. Thus, recovery of Trp is rather high, 73%, in the presence of an equimolar quantity of benzylamine and decreases only to 30% for 1000-fold excess of the latter. A separate study of the extraction of amines (including aniline, *o*-toluidine, and some others; results will be reported elsewhere) showed that amines are well extracted into RTIL in the *neutral* form, the amount of which is negligible in acidic media. The effect of amines on amino acid extraction is therefore relatively weak.

Applications

We used extraction into RTIL to recover amino acids from pharmaceutical samples and fermentation broth and subsequent determination, after back-extraction followed by fluorescence reaction with OPA.

The accuracy of amino acid extraction – fluorimetric determination was confirmed by analysis of model solutions (prepared in Hanks buffer widely used for cell studies in biochemistry). The results shown in Table 4 demonstrate good recovery and low standard deviation.

Analysis of pharmaceutical products

The method was successfully applied to the determination of glycine in the pharmaceutical preparation "Glicin" (MNPK "Biotiki", Russia). The results indicate satisfactory precision and accuracy (Table 4).

Additional validation was performed by the standard addition method. Known amounts of arginine and lysine were added to solutions of the pharmaceutical product "Vitamax" (GlaxoWellcome, Egypt) and the amounts of amino acids found were compared with the manufacturer's declaration. It is apparent from Table 4 that agreement was good.

Analysis of fermentation broth

We used the RTIL containing the crown ether for recovery of amino acids from microbial fermentation broth (samples kindly supplied by Dr Z. Kuvaeva, Minsk Institute of Physical and Organic Chemistry, Belarus). The extraction was performed at room temperature directly from fermentation broth or from aqueous solution obtained by dilution of the initial mixture. For this purpose, 3 mL fermentation broth (or diluted solution) and 3 mL ionic liquid containing 0.13–0.45 mol L^{-1} DC18C6 were shaken for 15 min. Extraction and back-extraction were performed under previously found optimum conditions. The concentrations of the amino acids were determined by fluorimetry with *o*-phthalaldehyde after back-extraction in aqueous solution, as described above. As can be seen from Table 4, excellent results were obtained.

Amino acids are extracted efficiently from dilute fermentation broth into ionic liquid in the presence of crown ether and can be readily back-extracted by alkaline aqueous solution. It should be noted that determination of amino acids in fermentation broth by chromatographic, spectroscopic, and other methods typically requires pre-

Table 4 Determination of amino acids in different samples (n=3, P=0.95)

	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	*RSD* (%)
Model solution Hanks' buffer[a] as a background				
Leu	13.12	12.8±0.4	97±3	1
Leu	131.1	122±17	98±2	6
Leu	10.2	10.0±0.7	98±2	3
Trp	20.4	20.0±0.9	98±2	2
Trp	102.1	102±4	99±2	1
Trp, Leu	20.62	20.0±1.0	99±2	3
Pharmaceutical preparation "Glicin"				
Gly	4.99[b]	4.9±0.4	98±2	1
Gly	9.98[b]	9.8±1.4	98±2	6
Gly	99.75[b]	99.8±0.8	99±2	3
Gly	4.99[b]	4.9±0.4	98±2	1
Pharmaceutical preparation "Vitamax"				
Lys, Arg	16[c]	15.6±1.6	97±2	3
Lys, Arg	56[c]	55.9±0.7	98±2	6
Lys, Arg	112[c]	111±3	99±2	3
Fermentation broth				
Dilution of fermentation broth	Concentration of crown ether (mol L^{-1})	Found (sum of amino acids[d]) (mol L^{-1})	Recovery (%)	*RSD* (%)
1:1000	0.13	0.23±0.03	97±3	5
1:100	0.13	0.21±0.02	94±4	6
1:10	0.13	0.22±0.40	95±3	7
1:1	0.45	0.20±0.02	85±4	4

[a]Hanks' buffer contains (mg): NaCl (26.6), KCl (13.3), $MgSO_4.7H_2O$ (6.7), $Na_2HPO_4.7H_2O$ (0.3), K_2HPO_4 (0.2), $CaCl_2.6H_2O$ (0.2)
[b]Manufacturer's declared data
[c]Manufacturer's declared data plus added amount
[d]Manufacturer's declared data (g L^{-1}): Leu 20, Ala 2.5, Ile 1.0, Glu 5.0, Lys 1.5; sum of amino acids 0.233 mol L^{-1}.

liminary purification from peptides, proteins, and other accompanying substances. Use of ionic liquid extraction enabled avoidance of this purification stage and provide purer back-extracts.

Fermentation broth was analyzed by an independent method (use of a commercial amino acid analyzer). This analysis was performed after preliminary cleaning from peptides and proteins (sedimentation with trichloroacetic acid; the procedure is very time-consuming and difficult to automate). The concentration found (sum of amino acids) was 0.21 mol L^{-1}, in good agreement with the result from our method, 0.20±0.04 mol L^{-1}.

Conclusion

Extraction into a ionic liquid, in sharp contrast with conventional organic solvents, enables quantitative recovery of amino acids from complicated technological samples; no specially introduced counter-ions are required. The use of ionic liquid eliminates emulsion formation, which usually occurs when cationic or anionic extractants are used; consequently, the separation time is reduced.

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