New Journal of Chemistry
Supporting Information

Fast and efficient extraction of DNA from meat and meat derived products using aqueous ionic liquid buffer systems

Anna K. Ressmann[‡a], Eric Gonzalez Garcia[‡b], Diana Khlan[a], Peter Gaertner[a], Robert L. Mach[c], Rudolf Krska[d], Kurt Brunner[*b] and Katharina Bica[*a]

[a] *Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163, 1060 Vienna, Austria Fax: +43 1 58801 16399; Tel: +43 1 58801 163601; E-mail: katharina.bica@tuwien.ac.at*
[b] *Vienna University of Technology, IFA-Tulln, Center of Analytical Chemistry, Konrad Lorenz Str. 20, 3430 Tulln, Austria*
[c] *Vienna University of Technology, Institute for Chemical Engineering, Gumpendorfer Str. 1a, 1060 Vienna, Austria*
[d] *University of Natural Resources and Life Sciences, Department IFA-Tulln, Konrad Lorenz Str. 20, 3430 Tulln, Austria*

1 Material and methods

1.1 General

Commercially available reagents and solvents were used as received from Sigma Aldrich unless otherwise specified. 1-Ethyl-3-methylimidazolium acetate ([C_2mim]OAc), 1-ethyl-3-methylimidazolium chloride ([C_2mim]Cl) were purchased from Iolitec (Heilbronn, Germany).
All other ionic liquids were prepared as previously reported and analytical data were in accordance with literature. Ionic liquids were dried for 24–48 h at 80 °C or at RT and 0.01 mbar with stirring before use and were stored under Argon.
Double-distilled deionised water was obtained from a Millipore Milli-Q water purification system. (Millipore, USA).
^{1}H spectra were recorded on a Bruker AC 400 at 200 MHz, resp., using the solvent peak as reference. J values are given in Hz.
Authentic fresh meat samples from beef (*Bos taurus*), pork (*Sus scrofa*), chicken (*Gallus gallus*), horse (*Equus ferus caballus*), as well as traditional Austrian products like garlic sausage, gouchos, leberkäse, blunzen and knacker were bought in a local supermarket in Vienna, Austria and were kept frozen at -20 °C until further usage. Model sausages were prepared by mixing beef, pork, bacon (20%), salt (2%), pepper (0.7%), Worcester sauce (0.7%), vinegar (0.7%), garlic (0.7%), as well as different herbs and spices. The mixture was thoroughly homogenised with portions of horsemeat fixed at 0%, 0.1%, 0.5%, 1% and 5% by using a meat cutter and filled into sheep gut. Sausages were later cooked for 20 min and were kept frozen at -20 °C until further use.

2 Extraction procedures

2.1 IL/buffer extraction procedure

In a 1.5 ml Eppendorf-vial 100 mg (± 10.0 mg) ionic liquid were dissolved in 900 µl buffer and 200 mg (± 15.0 mg) of meat were added. Optionally, the solution was stirred for 15 min at room temperature before further denaturation. Denaturation was performed at 95 °C for 10 min in a thermo mixer. The mixture was then centrifuged at 13000 rpm for 5 min and 400 µl of the supernatant were transferred to a new Eppendorf tube and kept frozen at -20 °C until further use. All experiments were carried out in triplicates.

2.2 Buffer extraction procedure

Eppendorf-vials (1.5 ml) were charged with 1000 µl buffer and 200 mg (± 15.0 mg) of meat. Optionally, the samples were stirred for 15 min at room temperature before further denaturation. Denaturation was performed at 95 °C for 10 min in a thermo mixer. The mixture was then centrifuged at 13000 rpm for 5 min and 400 µl of the supernatant were transferred to a new Eppendorf tube and kept frozen at -20 °C until further use. All experiments were carried out in triplicates.

2.3 Extraction with commercial kits

2.3.1 SureFood® PREP Animal, from the company r-Biopharm.

400 µl of the lysis buffer and 40 µl of proteinase K were added to an Eppendorf tube containing 50 mg of meat, mixed and incubated at 52 °C for 30 min and 1400 rpm. The sample lysate was later centrifuged for 1 min at 12000 rpm for the pelleting of the unlysated ingredients. 350 µl of the supernatant were transferred to a new Eppendorf tube and 200 µl binding buffer were added to the supernatant. The mixture was later transferred onto a spin filter, incubated at room temperature for 1 min and centrifuged at 12000 rpm for 1 min. The filtrate was later discarded and the remaining ethanol in the filter was removed by another centrifugation step at 12000 rpm for 2 min. Afterwards, the DNA contained in the spin filter was eluted by adding 100 µl of preheated (52 °C) elution buffer directly onto the spin filter, incubated for 3 min at 52 °C and centrifuged at 10000 rpm for 1 min.

2.3.2 Wizard® Genomic DNA Purification Kit from the Promega Corporation.

20 mg of previously ground tissue tissue (in liquid nitrogen and using a mortar and pestle) were mixed with 600 µl of nuclei lysis solution in a 1.5 ml Eppendorf tube and incubated for 30 min at 65 °C. After this, 17.5 µl of proteinase K (20 mg/ml) were added and incubated for 3 h at 55 °C and 1400 rpm. After this digestion, 3 µl of RNase solution were added to the nuclear lysate and incubated for 30 min at 37 °C. The sample was later cooled down to room temperature during 5 min and 200 µl of protein precipitation solution were added and centrifuged for 4 min at 12500 rpm to form a tight white pellet from protein. 700 µl of the supernatant were then mixed with 600 µl room temperature isopropanol. The mixture was centrifuged for 1 min at 12500 rpm at room temperature, after which the pelleted DNA was visible. The supernatant was decanted and 600 µl of room temperature ethanol was added to wash the DNA. This was later centrifuged for 1 min at 12500 rpm at room temperature. The ethanol was then removed and the pellet dried at 65 °C for 30 min. Afterwards, 100 µl of DNA rehydration solution were added and incubated at 65 °C for 1 h.

2.4 Extraction according to Amani *et al.*

The protocol from Amani *et al*., 2011 was performed with some modifications. 100 mg of the material was put on a 2 ml Eppendorf tube and 500µl of the preheated (70 °C) extraction buffer (200 mM Tris-HCl (pH=7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS) were added. The mixture was incubated at 70 °C and 1200 rpm for 30 min. Afterwards, 500 µl of chloroform:isoamylalcohol (24:1) were added, vortexed and centrifuged for 5 min at 12500 rpm. 300 µl of the supernatant were then transferred to a new Eppendorf tube containing an equal volume of precooled (-20 °C) isopopropanol. The mixture was vortexed and incubated at -20 °C for 90 min. After this, a centrifugation for 5 min at 12500 rpm was carried out. The supernatant was removed and the pellet was dried at 65 °C for 30 min. Later, it was dissolved in 100 µl TE buffer (pH=8).[1]

2.5 Buffer preparation

2.5.1 Phosphate buffer

Phosphate buffer was prepared by dissolving 32 mg NaH_2PO_4 and 3.499 g of Na_2HPO_4 in 200 ml MilliQ water. The pH was adjusted to 8.5.

2.5.2 AMP buffer

AMP buffer was prepared by dissolving 446 mg of 2-amino-2-methyl-1-propanol in 100 ml MilliQ water. The pH was adjusted to 8.5.

2.5.3 TRIS buffer

A 50 mM solution of Tris(hydroxymethyl)-aminomethan in MilliQ water was prepared. The pH was adjusted to 8.5.

2.6 Pretreatement of meat

2.6.1 Pretreatement with heptane

Minced beef meat was stirred in heptane for one hour. The solvent was decanted and the meat was dried at ambient temperature and pressure.

2.6.2 Trituration in mortar

Minced beef meat was frozen with liquid nitrogen and triturated in a mortar.

2.7 Real-time PCR assays

Real time PCR assays were carried out in a RotorGene-Q cycler (Qiagen, Hilden, Germany) according to the following thermal cycling protocol: initial step of 2 min at 95 °C, followed by 50 cycles of 15 sec at 95 °C and 1 min at 62 °C. All amplification reactions were performed in triplicates. For each reaction, 2 µl template DNA were mixed with 13 µl reaction mix having 7.5 µl Kapa™ Probe® Fast (PeqLab, Erlangen, Germany), 4.78 µl sterile and nuclease free water and 0.24 µl for each of the forward and reverse primers and for the FAM-labeled probe (Eurofins MWG Operon, Ebersberg, Germany). Cq values obtained by quantitative PCR were used to assess the amount of amplifiable DNA. Primers and probes for all meat sorts were published by Köppel *et al*.[2]

In addition, calibration curves with 4 points following two or four fold dilutions were performed and linearity and PCR efficiencies were calculated to assess the presence of inhibitors that might hinder the amplification process during the qPCR. qPCR efficiency was determined as:

$$\text{Efficiency} = 10^{\left(\frac{-1}{\text{slope}}\right)} - 1$$

Where slope is the slope of the linear regression in the calibration curve.

The concentration of the DNA extracts were photometrically measured in a NanoVue Plus spectrophotometer (GE Healthcare, Little Chalfont, USA). The yield was calculated as follows:

$$\text{Yield} = \frac{\text{c(DNA)} \cdot \text{V}}{\text{m}}$$

Where c(DNA) is the concentration [ng/µl] of DNA photometrically measured, V is the total sample volume [µl] and m is the mass [mg] of the sample used for extraction. Yield is expressed as µg DNA/g sample.

3 Results

3.1 Influence of untreated and pretreated meat using IL 1

Table 1 Influence of different buffers and pretreatement of meat in the Cq values obtained when extracting mtDNA from beef with **1**

Entry	Buffer	$[C_2mim][Me_2PO_4]$ untreated meat[a]		buffer untreated meat[b]		$[C_2mim][Me_2PO_4]$ treated meat[a]		buffer treated meat[b]	
		Cq[c]	Cq STD	Cq	Cq STD	Cq	Cq STD	Cq	Cq STD
1	phosphate	-	-	34.0	0.5	-	-	35.6	0.9
2	AMP	-	-	43.4	-	-	-	37.2	0.2
3	TRIS	34.1	0.6	35.1	2.1	37.4	0.6	33.6	0.8

[a] Performed with 100 mg (± 10.0 mg) IL, 900 µl corresponding buffer and 200 mg (± 15.0 mg) minced beef for 15 min at room temperature and denaturation for 10 min at 95 °C. [b] Performed with 1000 µl corresponding buffer and 200 mg (± 15.0 mg) minced beef for 15 min at room temperature and denaturation for 10 min at 95 °C. [c] A difference in 1 Cq value corresponds to double the concentration of extracted DNA. The smaller the Cq value, the higher the concentration of extracted DNA. All experiments were carried out in triplicates.

3.3 Ionic liquids tested for minced beef extraction

Table 2 Phosphate choline derivatives, guanidinium derivatives and imidazolium derivatives tested for the extraction of mtDNA from minced beef.

Entry	Ionic liquid[a]	Cq[b]	STD
1	[chol][dhp] **10**	-	-
2	[chol][dbp] **11**	25.67	0.3
3	[chol][dop] **12**	-	-
4	[guan][fom] **13**	-	-
5	[guan][OAc] **14**	-	-
6	[guan][but] **15**	-	-
7	[guan][hex] **16**	27.5	0.5
8	[guan][oct] **17**	25.3	0.4
9	[guan][dec] **18**	-	-
10	[C_2mim][Me_2PO_4] **1**	-	-
11	[C_2mim][OAc] **19**	-	-
12	[C_2mim][Cl] **20**	33.3	1.6
13	[C_4mim][Cl] **21**	41.7	3.3
14	[C_6mim][Cl] **22**	-	-

[a] Performed with 100 mg (± 10.0 mg) IL, 900 µl phosphate buffer and 200 mg (± 15.0 mg) minced beef for 10 min at 95 °C. [b] A difference in 1 Cq value corresponds to double the concentration of extracted DNA. The smaller the Cq value, the higher the concentration of extracted DNA. All experiments were carried out in triplicates.

3.4 Different sausages containing horse meat

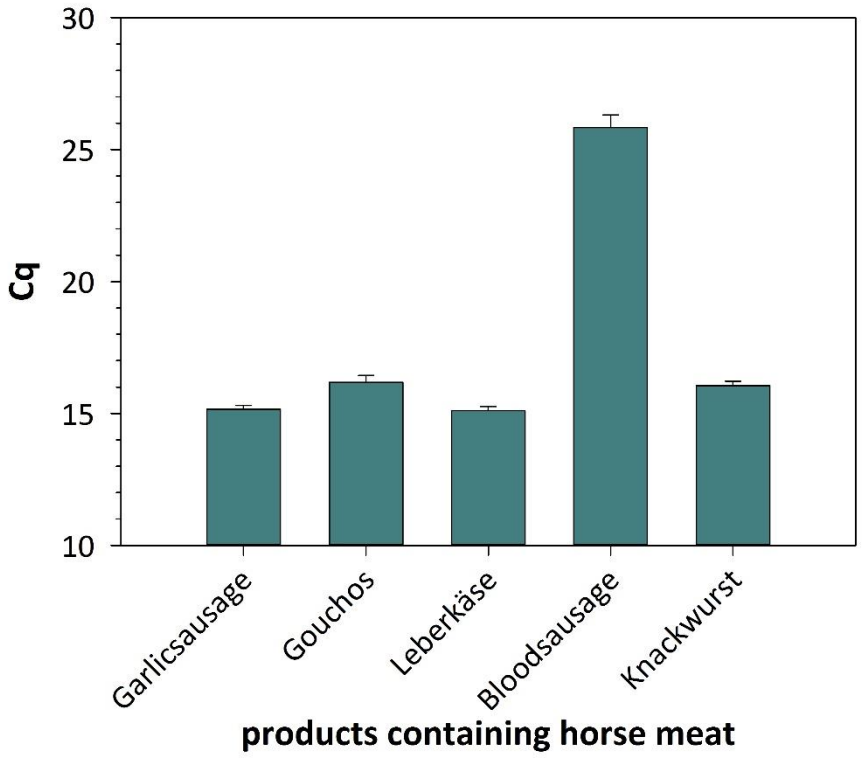

Fig. 1 Extraction of mtDNA from different sausages performed with 100 mg (± 10.0 mg) [chol][hex], 900 µl phosphate buffer and 200 mg (± 15.0 mg) meat for 10 min at 95 °C.

3.5 Influence of ionic liquids on the amplification process during real time PCR

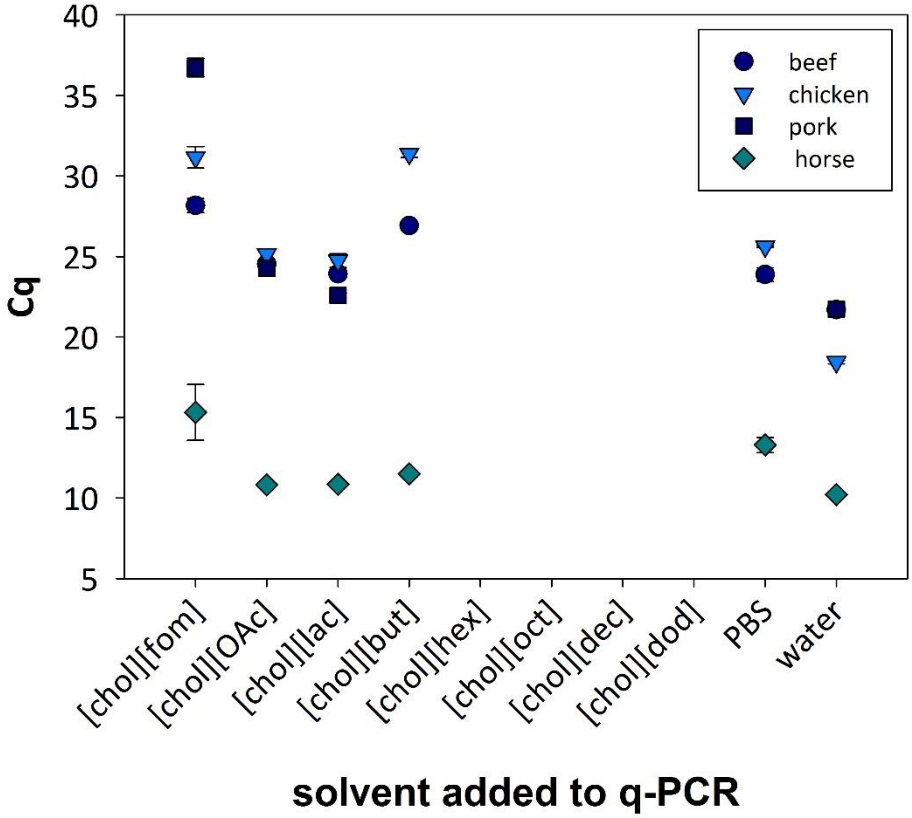

Fig. 2 Influence of the amplification process during real time PCR, c(IL)=33 ng/µl (7.5%).

Table 3 The amplification process during real time PCR, c(IL)=5.5 ng/µl (1.25%) and c(IL)=2.2 ng/µl (0.5%)

Entry	meat	c (IL)	[chol][oct]		[chol][dec]		[chol][dod]	
			Cq	Cq STD	Cq	Cq STD	Cq	Cq STD
1	beef	5.5 ng/µl	22.2	0.1	25.28	0.1	-	-
2	chicken	5.5 ng/µl	19.6	0.1	29.1	0.6	-	-
3	pork	5.5 ng/µl	21.9	0.1	30.57	1.5	-	-
4	horse	5.5 ng/µl	9.0	0.1	9.01	0.18	-	-
5	beef	2.2 ng/µl	22.0	0.0	21.8	0.1	-	-
6	chicken	2.2 ng/µl	18.9	0.1	19.2	0.18	28.7	0.9
7	pork	2.2 ng/µl	22.2	0.01	21.8	0.0	-	-
8	horse	2.2 ng/µl	8.9	0.1	8.8	0.0	-	-

4 Synthesis of ionic liquids

4.1 Imidazolium-based ionic liquids

Synthesis of 1-ethyl-3-methylimidazolium dimethyl phosphate [C_2mim][Me_2PO_4]
Trimethylphosphate (38 ml) was added dropwise to 1-ethylimidazol under argon atmosphere. The mixture was heated to 80 °C and stirred for 24 h. The mixture was washed with ethyl acetate (3 × 50 ml). The solvent was evaporated to dryness and the residue was dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h. A yellow oil was obtained in 99 % yield, which started to crystallize upon storage at room temperature.[3]
^{1}H-NMR (200 MHz, $CDCl_3$): δ_H = 1.52 (3H, t, J=7.33, N-CH_2-C*H*$_3$), 3.53 (6H, d, J=10.56 Hz, P(OCH_3)$_2$), 4.00 (3H, s, NCH_3), 4.30 (2H, q, J=7.37 Hz, N-C*H*$_2$-CH_3), 7.38 (2H, m, H-4, H-5), 10.50 (1H, s, H-2).
Analytical data were in accordance with literature values.
Synthesis of 1-butyl-3-methylimidazolium chloride [C_4mim][Cl]
1-Chlorobutane (22.5 g, 0.30 mol) was added dropwise to 20.5 g (0.25 mol) of freshly distilled 1-methylimidazole. The mixture was refluxed at 50 °C for 96 h under argon until NMR indicated complete conversion. After cooling to RT the solution was washed with ethyl acetate (3 × 10 ml) and dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h. A dark yellow oil was obtained that was crystallized from a mixture of ethylacetate and acetonitrile. The product was obtained as colorless crystalls in 94% yield.[4]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 0.88 (3H, t, J =7.2 Hz, N-(CH_2)$_3$-C*H*$_3$), 1.26 (2H, sext, J=7.54 Hz, N-CH_2-CH_2-C*H*$_2$-CH_3), 1.75 (2H, quin, J=7.2 Hz, N-CH_2-C*H*$_2$-CH_2-CH_3), 3.85 (3H, s,N-CH_3), 4.17 (2H, t, J=7.2 Hz, N-C*H*$_2$-CH_2-CH_2-CH_3), 7.79 (2H, d, J=14.6 Hz,H-4,H-5), 9.38 (1H, s, H-2)
Analytical data was in accordance with literature values.

4.2 Choline-based ionic liquids

4.2.1 General procedure for choline-ionic liquids via neutralization

The exact concentration of choline hydrogen carbonate was determined *via* titration prior to use. ILs were prepared by dropwise addition of the base to the corresponding acid (1:1) in an appropriate solvent, *e.g.* water or methanol. The solution was stirred at ambient temperature and pressure for 30 min to 4 h. The solvent was removed and the IL was dried in vacuo (0.01 mbar) over night.

Synthesis of choline formate [chol][fom]
Synthesis was accomplished according to the general procedure using 3.031 g (65.85 mmol) formic acid, choline bicarbonate (77% solution in water) (14.126 g, 65.85 mmol) and 5 ml water. A colorless liquid was obtained in quantitative yield.[5]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H =3.12 (9H, s, N(CH_3)$_3$), 3,40-3.49 (2H, m, N-CH_2), 3.80-3.87 (2H, m, O-CH_2), 8.52 (1H, s, HCOO)
Analytical data was in accordance with literature.

Synthesis of choline acetate [chol][OAc]
Synthesis was accomplished according to the general procedure using 1.001 g (16.66 mmol) acetic acid, choline bicarbonate (77% solution in water) (3.574 g, 16.66 mmol) and 3 ml water. A colorless jelly-like solid was obtained in quantitative yield.[5]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 1.58 (3H, s, CH_3COO), 3.12 (9H, s, $N(CH_3)_3$), 3.39-3.44 (2H, m, N-CH_2), 3.78-3.86 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline lactate [chol][lac]
Synthesis was accomplished according to the general procedure using 0.994 g (1.10 g 90 %, 11.04 mmol) lactic acid, choline bicarbonate (77% solution in water) (2.368 g, 11.04 mmol) and 3 ml water. A colorless oil was obtained in quantitative yield.[5]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 1.05 (3H, d, J= 6.65, CH-C*H*$_3$), 3.10 (10H, s, $N(CH_3)_3$, OH), 3.33-3.45 (3H, m, N-CH_2, C*H*-CH_3), 3.79-3.87 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline butyrate [chol][but]
Synthesis was accomplished according to the general procedure using 0.96 g (10.90 mmol) butyric acid, choline bicarbonate (77% solution in water) (2.337 g, 10.90 mmol) and 3 ml water. A colorless liquid was obtained in quantitative yield.[6]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 0.79 (3H, t, J = 7.33, -CH_2-C*H*$_3$), 1.39 (2H, sex, J =7.32, -C*H*$_2$-CH_3), 1.79 (2H, t, J=7.14, -C*H*$_2$-CH_2-CH_3), 3.12 (9H, s, $N(CH_3)_3$), 3.40-3.48 (2H, m, N-CH_2), 3.80-3.85 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline hexanoate [chol][hex]
Synthesis was accomplished according to the general procedure using 10.371 g (89.28 mmol) caproic acid, choline bicarbonate (77% solution in water) (19.154 g, 89.28 mmol) and 15 ml methanol. A colorless gel was obtained in quantitative yield. [6]
^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 0.83 (3H, t, J = 6.65, -CH_2-CH_3), 1.13-1.20 (4H, m, CH_2-CH_2-CH_3), 1.25-1.45 (2H, m, COO-CH_2- CH_2), 1.79 (2H, t, J=7.34, COO-CH_2-), 3.11 (9H, s, $N(CH_3)_3$), 3.37-3.43 (2H, m, N-CH_2), 3.80-3.94 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline octanoate [chol][oct]
Synthesis was accomplished according to the general procedure using 1.5888 g (10.79 mmol) octanoic acid, choline bicarbonate (77% solution in water) (2.315 g, 10.79 mmol) and 15 ml methanol. A colorless gel was obtained in quantitative yield. [6]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 1.35 (3H, t, J = 6.86, -CH_2-CH_3), 1.77-1.78 (8H, m, -C_4H_8-CH_3), 2.04 (2H, t, J=6.65, COO-CH_2-CH_2), 2.60 (2H, t, J=7.60, COO-CH_2-), 3.67 (9H, s, $N(CH_3)_3$), 3.93-3.95 (2H, m, N-CH_2), 4.44-4.47 (2H, m, O-CH_2)
Analytical data was in accordance with literature. [6]

Synthesis of choline decanoate [chol][dec]
Synthesis was accomplished according to the general procedure using 4.0 g (23.43 mmol) decanoic acid, choline bicarbonate (80% solution in water) (4.839 g%, 23.43 mmol) choline bicarbonate and 15 ml methanol. A colorless solid was obtained in quantitative yield. [6]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H =0.90 (3H, t, J=6.46, -CH_3), 1.30 (12H, s, CH2), 1.60 (2H, t, J=6.95, OOC-CH_2-CH_2-), 2.1 (t, 2H, J=7.53, OOC-CH_2-CH_2-), 3.22 (9H, s, $N(CH_3)_3$), 3.47-3.52 (m, 2H, N-CH_2), 3.96-4.04 (m, 2H, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline dodecanoate [chol][dod]
Synthesis was accomplished according to the general procedure using 3.94 g (19.66 mmol) decanoic acid, choline bicarbonate (80% solution in water) (4.102 g, 19.66 mmol) and 15 ml methanol. A colorless solid was obtained in quantitative yield. [6]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H =0.92 (3H, t, J=3.72, -CH_3), 1.30 (16H, s, CH2), 1.59 (2H, t, J=4.99, OOC-CH_2-CH_2-), 2.16 (t, 2H, J=7.53, OOC-CH_2-CH_2-), 3.22 (9H, s, $N(CH_3)_3$), 3.47-3.52 (m, 2H, N-CH_2), 3.96-4.04 (m, 2H, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline dihydrogen phosphate [chol][dhp]
Synthesis was accomplished according to the general procedure using phosporic acid (89% solution in water) (1.022 g 89%, 9.285 mmol), choline bicarbonate (77% solution in water) (1.99 g, 9.285 mmol) and 5 ml water. A colorless solid was obtained in quantitative yield.[7]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 3.22 (9H, s, $N(CH_3)_3$), 3.51 (2H, m, N-CH_2), 4.01 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline dibutyl phosphate [chol][dbp]
Synthesis was accomplished according to the general procedure using 1.52 g (1.022 g 89%, mmol) phosporic acid and 1.50 g (1.992 77%, mmol) choline bicarbonate and 5 ml water. A colorless solid was obtained in quantitative yield.[7]
^{1}H-NMR (400 MHz, d_4-MeOD): δ_H= 0.94 (t, 6H,J=7.24, -CH_3), 1.43 (4H, sext, -CH_2-CH_3), 1.61 (4H, quint, -CH_2- CH_2-CH3), 3.23 (9H, s, $N(CH_3)_3$), 3.50 (2H, t, J=4.79, N-CH_2), 3.85 (4H, quint, J=6.26, P-O-CH_2), 3.99-4.04 (2H, m, O-CH_2)
Analytical data was in accordance with literature.

Synthesis of choline bis(2,4,4-trimethylpentyl) phosphinate [chol][dop]
Synthesis was accomplished according to the general procedure using bis(2,4,4-trimethylpentyl) phosphinate (90%, 1.532 g, 4.746 mmol), choline bicarbonate (77% solution in water) (1.018 g, 4.746 mmol) and 5 ml methanol. A colorless viscous oil/solid was obtained in quantitative yield. [7]
^{1}H-NMR (400 MHz, d_4-MeOD): δ_H= 0.95 (18H, s, -CH-(C*H*$_3$)$_3$), 1.33-1.54 (14H, m, -CH$_2$-CH$_2$-CH-CH$_2$-), 3.23 (9H, s, N(CH$_3$)$_3$), 3.50 (2H, m, N-CH$_2$), 3.76-3.79 (4H, m, P-O-CH$_2$), 3.99-4.04 (2H, m, O-CH$_2$)
Analytical data was in accordance with literature.

4.3 Synthesis of *N,N,N,N*-tetramethylguanidinium ILs

4.3.1 General procedure for N,N,N,N-tetramethylguanidinium ILs

ILs were prepared by dropwise addition of *N,N,N,N*-tetramethylguanidin to the corresponding acid (1:1) in an appropriate solvent or without solvent. The solution was stirred at ambient temperature and pressure for 30 min to 4 h. The solvent was evaporated and the IL dried in HV for 3 h. [8]

Synthesis of *N,N,N,N*-tetramethylguanidinium formate [guan][fom]
Synthesis was accomplished according to the general procedure using 0.406 g (8.69 mmol) formic acid and *N,N,N,N*-tetramethylguanidin (1.015 g, 8.69 mmol). A colorless solid was obtained in quantitative yield. [8]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 2.99 (12H, s, N-CH$_3$), 8.55 (1H, s, HCOO)
Analytical data was in accordance with literature.

Synthesis of *N,N,N,N*-tetramethylguanidinium acetate [guan][OAc]
Synthesis was accomplished according to the general procedure using 0.54 g (8.99 mmol) acetic acid and *N,N,N,N*-tetramethylguanidin (1.036 g, 8.99 mmol). A colorless solid was obtained in quantitative yield. [8]

^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 1.89 (3H, s, -CH$_3$), 2.99 (12H, s, N-CH$_3$),
Analytical data was in accordance with literature.

Synthesis of *N,N,N,N*-tetramethylguanidinium butyrate [guan][but]
Synthesis was accomplished according to the general procedure using 0.960 g (10.91 mmol) butyric acid and *N,N,N,N*-tetramethylguanidin (1.255 g, 10.91 mmol). A colorless solid was obtained in quantitative yield.[8]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 0.94 (3H, t, J=7.34, -CH$_3$), 1.62 (2H, sex, J=7.39, -C*H*$_2$-CH$_3$), 2.14 (2H, t, J=7.43, -C*H*$_2$-CH$_2$-CH$_3$), 2.99 (12H, s, N-CH$_3$)
Analytical data was in accordance with literature.

Synthesis of *N,N,N,N*-tetramethylguanidinium hexanoate [guan][hex]
Synthesis was accomplished according to the general procedure using 1.785 g (15.37 mmol) hexanoic acid, *N,N,N,N*-tetramethylguanidin (1.770 g, 15.37 mmol) and 5 ml methanol. A colorless solid was obtained in quantitative yield.[8]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 0.91 (3H, t, J=6.65, -CH$_3$), 1.32 (4H, m, -(C*H*$_2$)$_2$-CH$_3$), 1.60 (2H, m, -C*H*$_2$-(CH$_2$)$_2$-CH$_3$), 2.16 (2H, t, J=7.53-C*H*$_2$-(CH$_2$)$_3$-CH$_3$), 2.99 (12H, s, N-CH$_3$)
Analytical data was in accordance with literature.

Synthesis of *N,N,N,N*-tetramethylguanidinium octanoate [guan][oct]
Synthesis was accomplished according to the general procedure using 0.949 g (6.58 mmol) octanoic acid, *N,N,N,N*-tetramethylguanidin (0.758 g, 6.58 mmol) and 5 ml methanol. A colorless solid was obtained in quantitative yield.[8]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 0.90 (3H, m, -CH$_3$), 1.31 (8H, m, -(C*H*$_2$)$_4$-CH$_3$), 1.59 (2H, m, , -C*H*$_2$-(CH$_2$)$_4$-CH$_3$), 2.15 (2H, t, J=7.53, -C*H*$_2$-(CH$_2$)$_5$-CH$_3$), 2.99 (12H, s, N-CH$_3$)
Analytical data was in accordance with literature.[9]

Synthesis of *N,N,N,N*-tetramethylguanidinium decanoate [guan][dec]

Synthesis was accomplished according to the general procedure using 0.906 g (5.14 mmol) decanoic acid, *N*,*N*,*N*,*N*-tetramethylguanidin (0.592 g, 5.14 mmol) and 5 ml methanol. A colorless solid was obtained in quantitative yield.[8]
^{1}H-NMR (200 MHz, d_4-MeOD): δ_H = 0.90 (3H, t, J=6.36, -CH_3), 1.30 (12H, -(CH_2)$_6$-CH_3), 1.59 (2H, m, -CH_2-(CH_2)$_6$-CH_3), 2.15 (2H, t, J=7.53, , -CH_2-(CH_2)$_7$-CH_3), 2.99 (12H, s, N-CH_3); ^{13}C-NMR (400 MHz, d_4-MeOD): δ_H = 14.44, 23.73, 27.72, 30.46, 30.67, 30.71, 30.85, 33.07, 39.05, 39.91, 161.90, 182.60; ν^{max}/cm-1: 2921, 2852, 1549, 1455, 1434, 1407, 1388, 1308, 1238, 1103, 1066, 1035, 884, 722, 539, 518, 506; Elemental analysis: calculated: w-% C: 62.68, w-% H: 11.57, w-%N: 14.62, calculated: 0.60xH_2O: w-% C: 60.14, w-% H: 11.56, w-%N: 14.09, measured: w-% C: 60.59, w-% H: 11.28, w-%N: 13.70

5 References

1. J. Amani, R. Kazemi, A. R. Abbasi and A. H. Salmanian, *Iran. J. Biotechnol.*, 2011, **9**, 69-71.
2. R. Koeppel, F. Zimmerli and A. Breitenmoser, *Eur. Food Res. Technol.*, 2009, **230**, 125-133.
3. E. Kuhlmann, S. Himmler, H. Giebelhaus and P. Wasserscheid, *Green Chem.*, 2007, **9**, 233-242.
4. M. B. Alves, V. O. Santos, Jr., V. C. D. Soares, P. A. Z. Suarez and J. C. Rubim, *J. Raman Spectrosc.*, 2008, **39**, 1388-1395.
5. Z. Li, X. Liu, Y. Pei, J. Wang and M. He, *Green Chem.*, 2012, **14**, 2941-2950.
6. M. Petkovic, J. L. Ferguson, H. Q. N. Gunaratne, R. Ferreira, M. C. Leitao, K. R. Seddon, L. P. N. Rebelo and C. S. Pereira, *Green Chem.*, 2010, **12**, 643-649.
7. K. D. Weaver, H. J. Kim, J. Sun, D. R. MacFarlane and G. D. Elliott, *Green Chem.*, 2010, **12**, 507-513.
8. A. W. T. King, J. Asikkala, I. Mutikainen, P. Jaervi and I. Kilpelaeinen, *Angew. Chem., Int. Ed.*, 2011, **50**, 6301-6305, S6301/6301-S6301/6305.
9. , NL6407547, 1965.