Electronic Supplementary Material (ESI) for RSC Advances.

Peroxide detected in imidazolium-based ionic liquids and approaches for reducing its presence in aqueous and non-aqueous environments

Julie L.L. Carter,[a] Catherine C. Santini,[b] Loïc J. Blum, [a] and Bastien Doumèche,[a]

[a] GEMBAS (Génie Enzymatique, Membranes Biomimétiques, et Assemblage Supramoléculaires) ICBMS UMR 5246 - Université Lyon 1 - CNRS

[b] C2P2 (Chemistry, Catalysis, Polymers and Processes) UMR 5265 - Université Lyon 1 - CNRS - CPE

43 bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

Email : doumeche@univ-lyon1.fr

Fax : + (33) 472 44 79 70

Tel : + (33) 472 43 14 84

Materials and Methods

Chemicals

Methylimidiazole (99%), trimethyl phosphate, β-nicotinamide adenine dinucleotide sodium salt, horseradish peroxidase (E.C. 1.11.1.7, 330 U mL^{-1}) and hydrogen peroxide 30% (w/w) were obtained from Sigma-Aldrich (Saint Q. Fallavier, France). Commercial ILs are: 1,3-dimethylimidiazolium dimethylphosphate (98%) from Iolitec GmbH (Denzlingen, Germany), 1-Ethyl-3-methylimidiazolium diethylphophate (98%) from Solvent Innovation GmbH (Cologne, Germany), 1-Ethyl-3-methylimidiazolium ethylsulfate from Alfa Aesar (Karlsruhe, Germany), 1-Butyl-3-methylimidiazolium hexafluorophosphate (98.5%) and 1-Butyl-3-methylimidiazolium octylsulfate (95%) from Fluka (Steinheim, Germany), 1-Butyl-3-methylimidiazolium acetate (98%), 1-Butyl-3-methylimidiazolium dicyanamide (98%) and 1-Butyl-1-methylpyrrolidinium dicyanamide from Solvionic (Toulouse, France). ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) came from Interchim (Montluçon, France). Catalase (E.C. 1.11.1.6, bovine liver, 200 kU mL^{-1}) and Jacobsen catalyst ((R,R)-(-)-N,N–Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamino-manganese (III) chloride)) was from Fluka (Steinheim, Germany).

Synthesis of N, N-dimethylimidiazolium dimethyl phosphate

Methylimidiazole (1.47 g, 0.018 mmol), used directly from the manufacturer or redistilled under vacuum, was added drop-wise under argon to ice-cold trimethyl phosphate (2.52 g, 0.018 mmol). The reaction mixture was allowed to warm to room temperature before being heated at 80˚C for 24 hours under argon and protected from light. After cooling to room temperature, the remaining substrate was removed under vacuum pressure for 24 h at 80˚C with stirring. The optical quality of the resulting product [MMIm][Me_2PO_4] was analyzed by

UV-vis spectroscopy. The formed product (yield = 97%) was a transparent, colorless, viscous liquid and was characterized by mass spectrometry (ESI^+) with a THERMO LCQ Advantage instrument and by NMR with a Bruker DRX 300. The ^{1}Hand ^{31}P NMR spectra were recorded in CD_3CN. ^{1}H NMR (300 MHz, CD_3CN, 25˚C): δ =3.34 (d, $J_{H,P}$= 10,37 Hz, 6H; CH_3N), 3.85 (s, 6H, CH_3O), 7.72 (d, J_{H-H}= 1.45 Hz, 2H; CH=CH), 9.21 ppm (s, 1H; CH); ^{31}P NMR (400 MHz, CD_3CN, 25˚C): δ = 1.82 ppm; MS (EI^+) : *m/z* 97.1 (100) [$MMIm^+$]: MS (EI^-): m/z 125.1 (100) [$Me_2PO_4^-$].

Vacuum evaporation

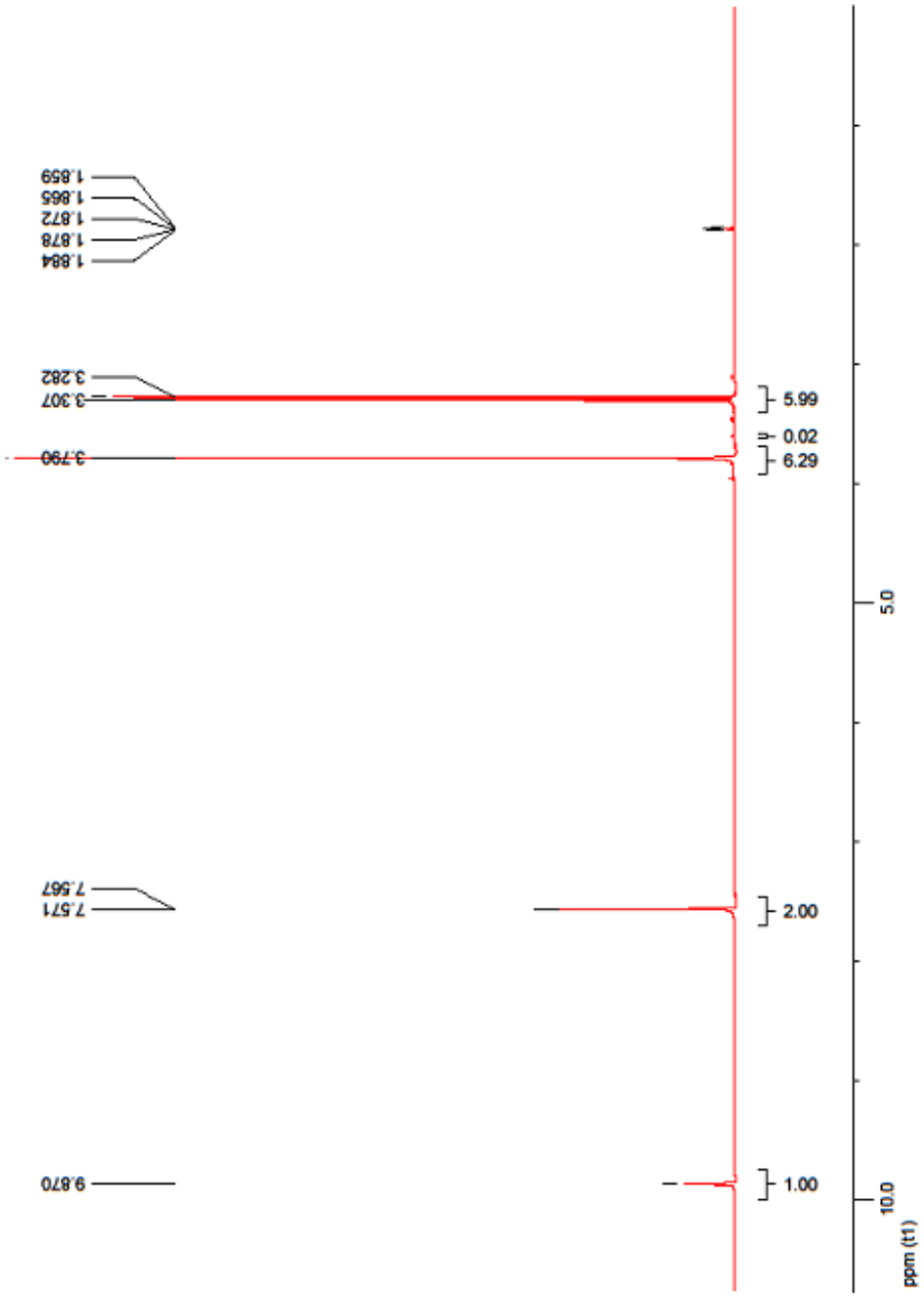
The newly synthesized [MMIm][Me_2PO_4] was subjected to vacuum evaporation while being heated to 80˚C for 12 h under argon and protected from light while stirring in order to remove any excess methylimidiazole or trimethyl phosphate. A sample was characterized by ^{1}H-NMR and ^{31}P-NMR (vide supra).

Horseradish peroxidase activity assay

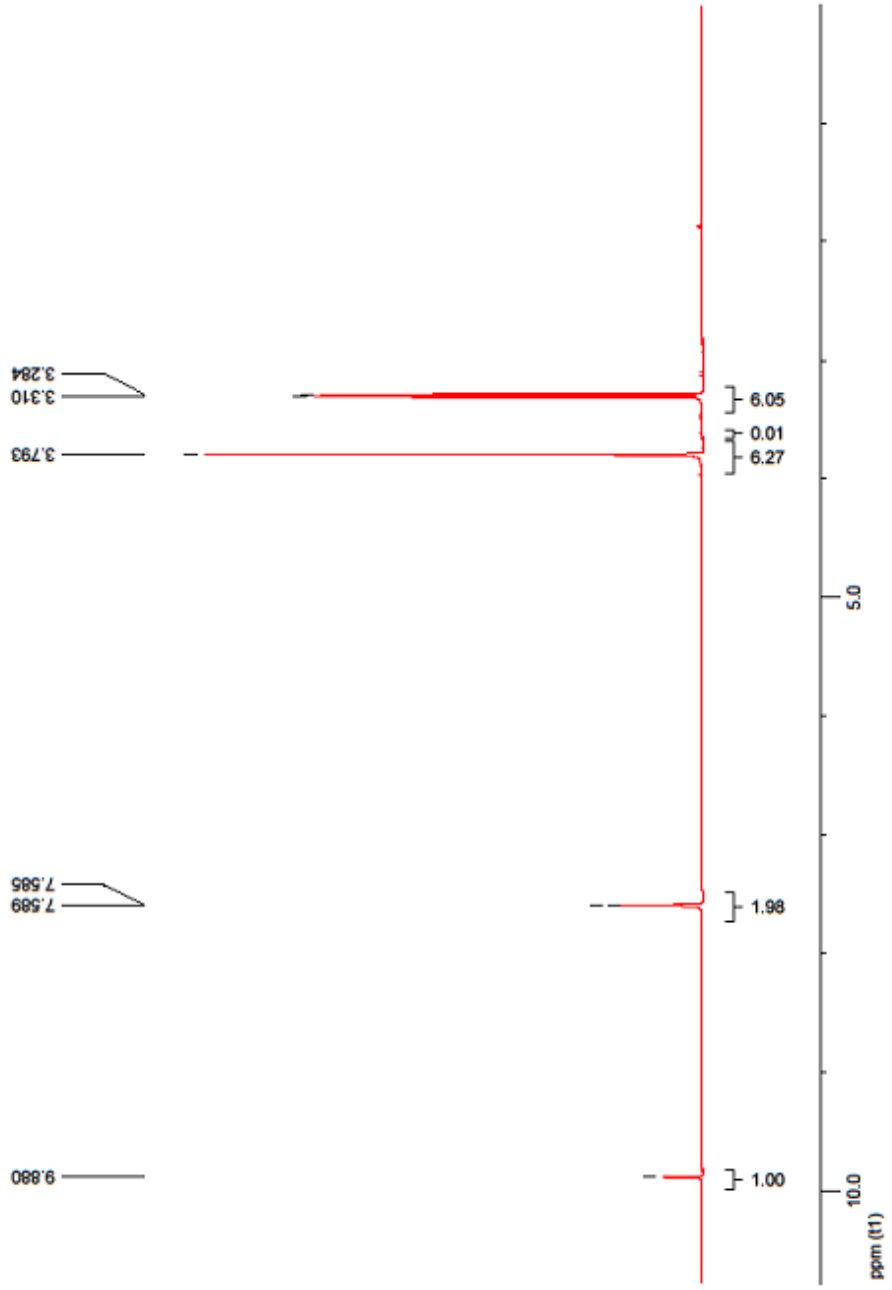
H_2O_2 concentration was measured from the formation of oxidized ABTS in the presence of HRP after 5 minutes by measuring the absorbance at 420 nm ($\varepsilon_M^{420\ nm}$= $2.06 \cdot 10^4$ M^{-1} cm^{-1}) with a Tecan Infinite M200 (Salzburg, Austria) microtitre plate reader at 25˚C. A single well contained enzyme solution (10 µL, 1.3 U mg^{-1}), ABTS (10 µL, 2 mM) and either 15 µL of [MIm], 15 µL [Me_3PO_4] or 15 µL of ionic liquid in potassium phosphate buffer (20 mM, pH 7.6) for a final volume of 100 µL. For solutions containing catalase or the salen-manganese complex, 2 U mg^{-1} of catalase or 80 µg of salen were added using the same protocol as above. The absorbance of a zero standard sample without HRP was measured for each sample. Dilutions ranging from 0 µM – 45 µM of a 30% H_2O_2 solution were prepared and 10 µL of each one added per well in order to calculate initial H_2O_2 concentrations in the samples. The absorbance of the zero assays was subtracted from all other absorbance

measurements. The absorbance measured was plotted in function of H_2O_2 concentration and the line used to determine the initial H_2O_2 concentration in the sample. The calculated values obtained are the average of at least 3 measurements and are expressed in μM in the neat IL.

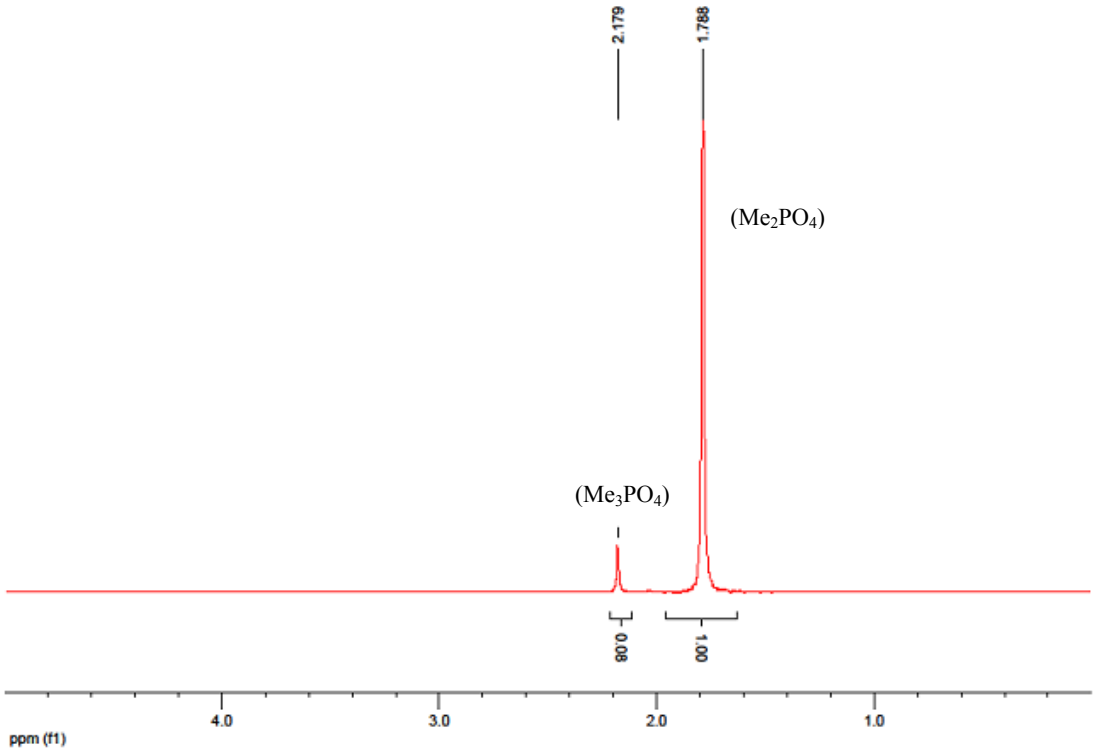
^{1}H-NMR spectra of academic-synthesized [MMIm][Me_2PO_4] using redistilled MIm after vacuum evaporation



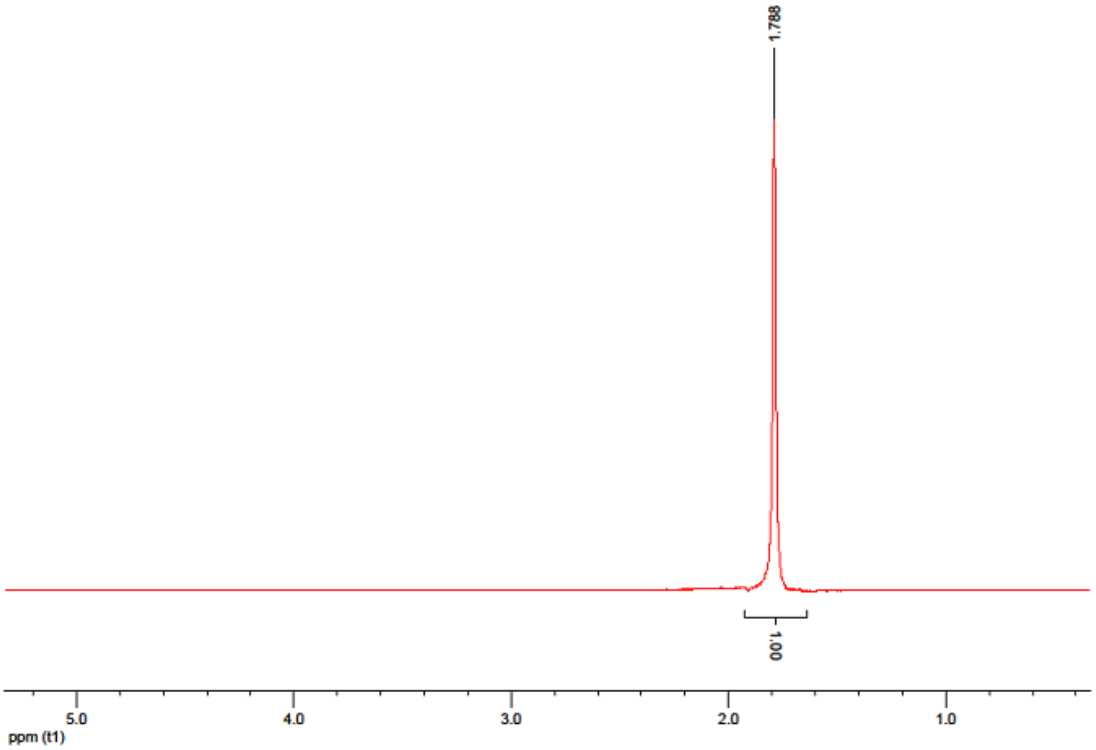
^{1}H-NMR spectra of synthesized [MMIm][Me_2PO_4] synthesized using non-redistilled MIm after vacuum evaporation



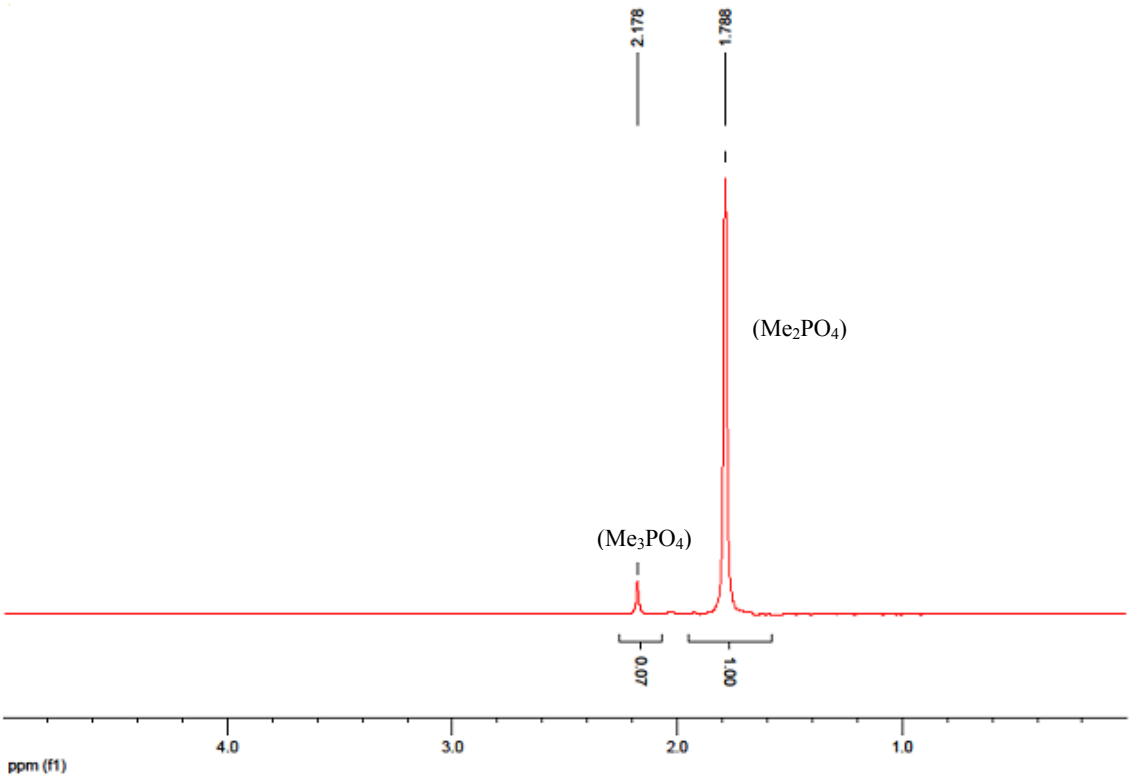
^{31}P-NMR spectra of [MMIm][Me_2PO_4] synthesized using redistilled MIm before vacuum evaporation



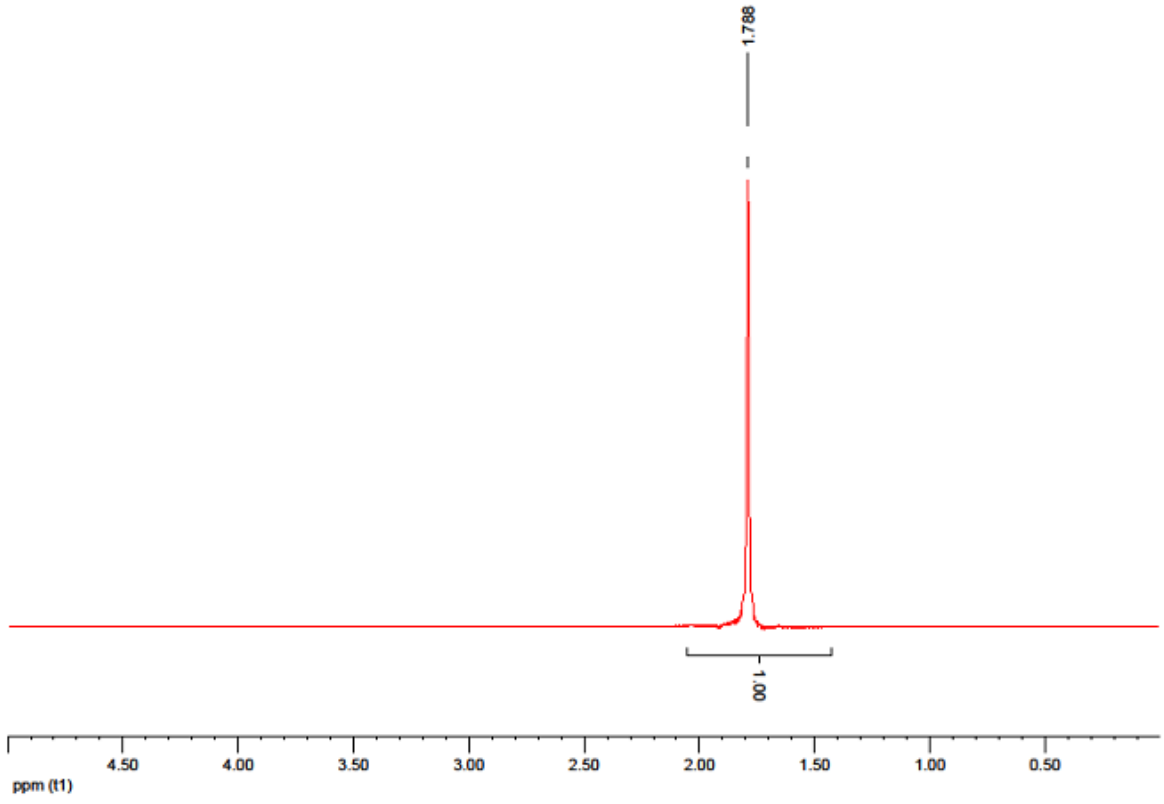
^{31}P-NMR spectra of [MMIm][Me_2PO_4] synthesized using redistilled MIm after vacuum evaporation



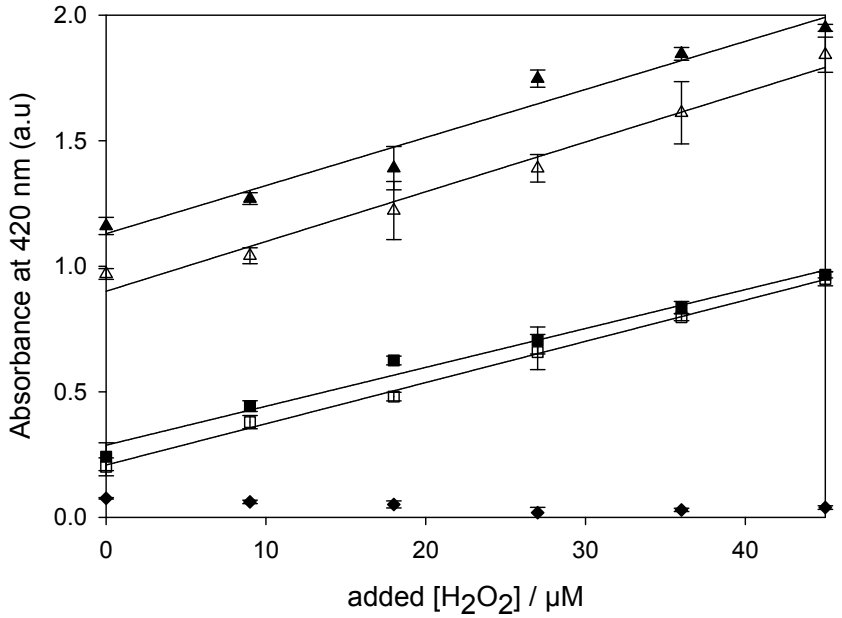
^{31}P-NMR spectra of [MMIm][Me_2PO_4] synthesized using non-redistilled MIm before vacuum evaporation



^{31}P-NMR spectra of [MMIm][Me_2PO_4] synthesized using non-redistilled MIm after vacuum evaporation



Dosage of H_2O_2 using standard curves to calculate the peroxide concentration in [MMIm][Me_2PO_4] synthesized from non-redistilled MIm (▲) and redistilled MIm (■) before vacuum evaporation for 24h at 80°C and [MMIm][Me_2PO_4] synthesized from non-redistilled MIm (∆) and redistilled MIm (□) after vacuum evaporation. H_2O_2 concentration after catalase addition is also shown.All measurements are performed in triplet in 15% (v/v) [MMIm][Me_2PO_4], [HRP] 1.30 U mL^{-1}, [ABTS] 2 mM, [H_2O_2] 0-45 µM and phosphate buffer pH 7.6, 20 mM at 25˚C.



UV-vis spectra of [MMIm][Me_2PO_4] synthesized with (1) non-redistilled MIm and (2) redistilled MIm (IL is synthesized as described in the experimental section).