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# Imidazolium-Based Ionic Liquids as Solvents for Analysis of Lipophilic Extractives from Biomass

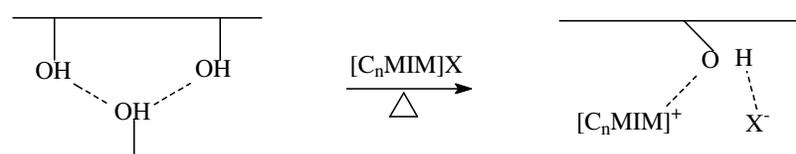
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Titus A.M. Msagati

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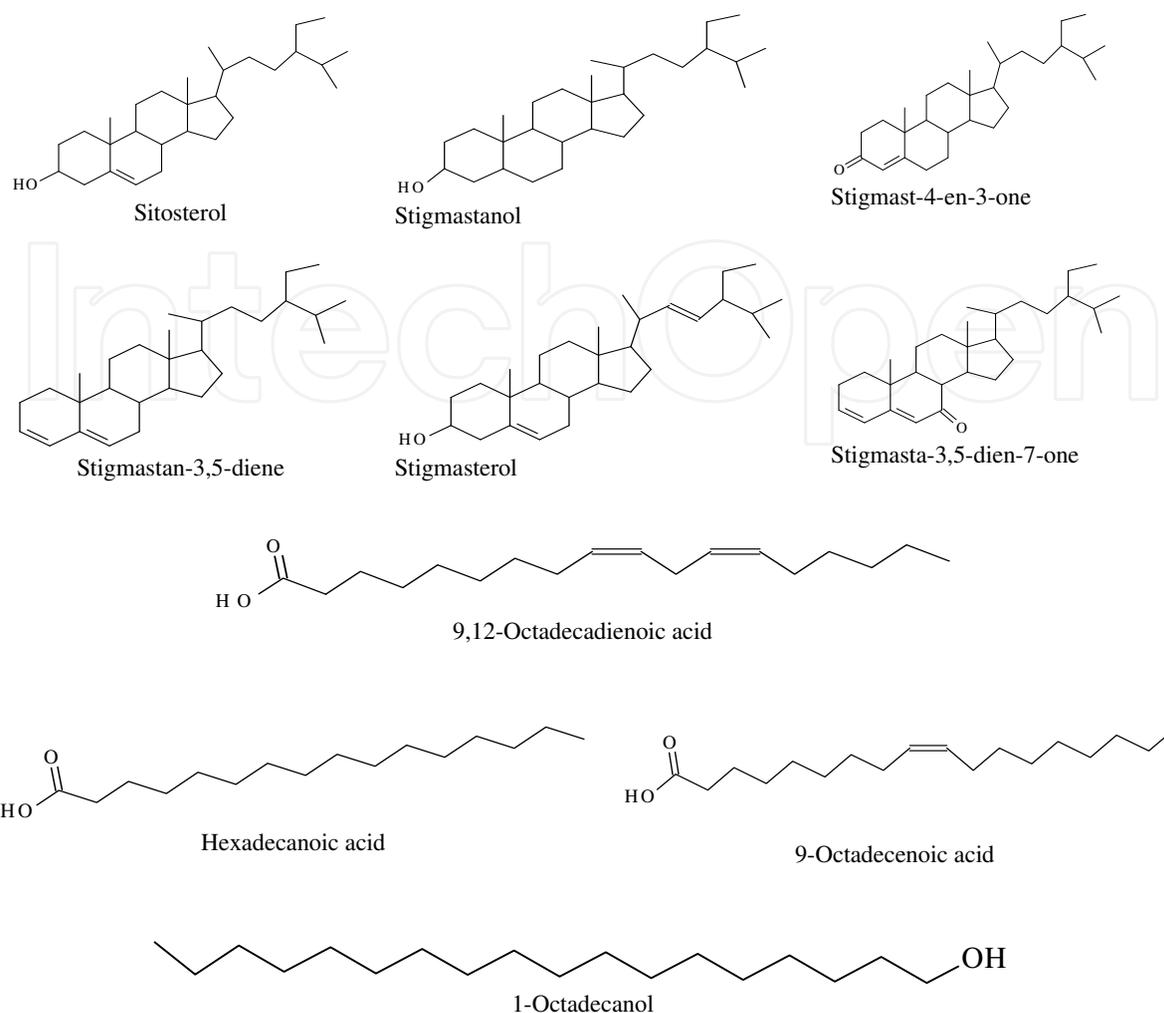
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## 1. Introduction

Ionic liquids are organic salts made of cations and anions of which most of them are liquids at room temperature [1-3]. This is generally a newly emerging class of compounds which so far has been discovered to have numerous applications in chemistry [2] due to their attractive features such as negligible vapour pressure, high thermal stability, biodegradability, ability to solvate compounds of different polarity and miscibility with aqueous and organic solvents [4], [5]. Different classes of ionic liquids have been synthesised so far, such as, imidazolium, ammonium, pyridinium, isoquinolinium, sulfonium, phosphonium, pyrrolidinium and others [6]. Since their discovery ionic liquids have raised a considerable excitement among researchers due to their ability to combine with different reagents in number of applications. The other aspect of ionic liquids is their ability to be recycled which minimizes the cost of usage as well as making them environmentally friendly. Ionic liquids so far have been used as solvents in different areas including for catalysis, synthesis and purification [6]. They have high ability of dissolving biopolymers such as cellulose and other biomass due to their ability to interact with biopolymer matrix forming hydrogen bonding [7] (Scheme 1).



**Scheme 1.** Schematic diagram showing dissolution of biopolymer in ionic liquid  $[C_nMIM]X$  [3] [5]



**Figure 1.** Chemical structures of some lipophilic extractive components reported from different biomass

Ionic liquids are known to have low toxicity due to their low volatility, a property which makes them to be considered as green and therefore recommended to replace volatile organic solvents in different chemical processes [6, 8].

Lipophilic extractives are a class of non-structural component of the biomass composed of low-molecular weight compounds such as fatty acids, fatty acid esters, sterols, sterol esters, fatty alcohols, triglycerides, hydrocarbons, steroid hydrocarbons and steroid ketones [9-12].

Figure 1 shows some of the common lipophilic extractives obtained from different biomass.

Lipophilic extractives have received attention from researchers partly due to their negative effect in paper and pulp industries as well as their importance in biofuel production and other applications [13, 14]. Lipophilic extractives from wood materials used for the production of pulp and paper affect negatively the pulping process as well as the final product. Whereas lipophilic extractives from other biomass such as blue-green algae are commonly used for other applications such as biofuel production. Thus, effective and quick lipophilic extractives analytical methods are required to characterize and study their qualitative and quantitative

composition in the biomass. Thus, the application of ionic liquids in the analysis of lipophilic extractives is attributed to their ability to dissolve biopolymers and biomass [14] whereby upon adding precipitating solvents (polar solvents), they regenerate pure biopolymers and release non-polymeric materials into the solution. Thus, lipophilic extractives can be easily extracted from the ionic liquid aqueous solution using a small volume of non-polar solvents. The approach is advantageous due to its use of small volume of organic solvent and shorter extraction time. This chapter therefore, reports on the use of imidazolium-based ionic liquids as an effective approach of extracting lipophilic extractives from chemical cellulose and blue-green algae biomass prior to chromatographic analysis.

## 2. Chemical reagents and instrumentation

The following chemicals purchased from Sigma Aldrich (Steinheim, Germany) were used; 1-butyl-3-methylimidazolium chloride [BMIM]Cl (98.0%), 1-ethyl-3-methylimidazolium chloride [EMIM]Cl (98.0%), 1-butyl-3-imidazolium acetate [BMIM]Ac, (97.0%), 1-ethyl-3-imidazolium acetate [EMIM]Ac (97.0%), 1-butyl-3-imidazolium methylsulphate [BMIM]MESO<sub>4</sub>, 1-butyl-3-imidazolium hexafluorophosphate [BMIM]PF<sub>6</sub> (97.0%). Methanol (HPLC grade), acetone (HPLC grade), hexane (HPLC grade), ethyl acetate (99.7%), hydrochloric acid (37.0%), and sodium sulphate anhydrous (99 – 100.5%). Chromatographic instruments used were an Agilent GC-MS, 7890A GC System with a triple-axis detector (5975C MSD), Shimadzu (GC-MS, QP2010, Kyoto, Japan), and GCxGC-TOFMS (Pegasus 4D, LECO Corporation). FT-IR; PerkinElmer Spectrum 100 FT-IR spectrometer with attached diamond attenuated total reflectance (ATR) accessory, TGA measurement was performed on a Perkin Elmer TGA 4000 Thermogravimetric Analyser.

## 3. Experimental

### 3.1. dissolution of chemical cellulose in imidazolium-based ionic liquids

A variety of imidazolium-based ionic liquids were initially screened for their ability to dissolve chemical cellulose at different temperatures. Chemical cellulose samples were accurately weighed and dissolved in molten ionic liquid at 90°C forming a 5 % solution. 25 mL of distilled water at 90°C was added to regenerate cellulose. Then samples were filtered and filtrates extracted using hexane followed by hexane: ethyl acetate by first sonicating for 5 minutes then auto shaking for 20 minutes. The extracts were dried, weighed and dissolved in 0.5 mL of acetone for derivatization before GC-MS analysis.

### 3.2. Dissolution of blue-green algae biomass

A freeze dried algae biomass sample was accurately weighed in duplicate and dissolved in a molten ionic liquid at 90°C forming a non viscous uniform solution of 5% algae biomass. About 5 mL of methanol was added to form slurry followed by addition of derivatization reagent.

The mixture was heated for 1 h at 60°C in a thermostated water bath then cooled at room temperature followed by extraction of fatty acids methyl esters (FAMES) with 4 mL of hexane under vigorous vortexing. The dried extracts were then dissolved in 1 mL of HPLC grade hexane, filtered using PTFE disc filters for GCxGC-TOFMS analysis.

#### **4. Ultrasonic solid liquid extraction using organic solvent**

For comparison purpose the results of the qualitative and quantitative composition of lipophilic extractives obtained from biomass by ionic liquid based extraction was compared with the results obtained using volatile organic solvent under ultrasonic solid liquid extraction technique (USLE) utilizing the mixture of acetone and methanol for chemical cellulose and chloroform and methanol for blue-green algae biomass. The extraction was carried out at an optimized temperature for 1 h. Extracts were then derivatized and analysed by GC-MS and GCxGC-TOFMS analysis.

#### **5. FT-IR and TGA analysis of extracted and un-extracted biopolymers (cellulose)**

For the investigation of the effect of ionic liquids on the analysed biopolymers the FT-IR and TGA analyses were performed. The extracted and un-extracted biopolymers (cellulose) were thoroughly washed with acetone followed by deionised water then dried in an oven at 90°C prior to FT-IR and TGA analyses.

The FT-IR analysis was achieved using a PerkinElmer Spectrum 100 FT-IR spectrometer with attached diamond attenuated total reflectance (ATR) accessory. Spectra were recorded from 4000  $\text{cm}^{-1}$  to 650  $\text{cm}^{-1}$  in transmittance mode with 4 scans per spectrum at a resolution of 4  $\text{cm}^{-1}$ .

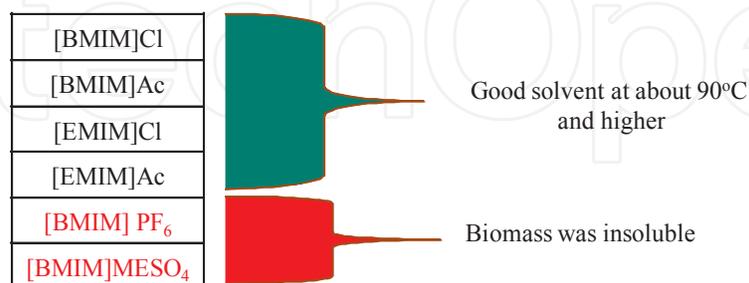
Thermogravimetric analysis (TGA) measurements were performed on a Perkin Elmer TGA 4000 Thermogravimetric Analyser. About 10 mg of sample aliquots were placed in a platinum sample holder pan. The TGA curves were recorded at temperatures ranging between 80°C and 700°C at a ramp-up rate of 10°C/min under nitrogen flow rate of 20 mL/min.

### **6. Results and discussion**

#### **6.1. Dissolution of biomass in ionic liquids**

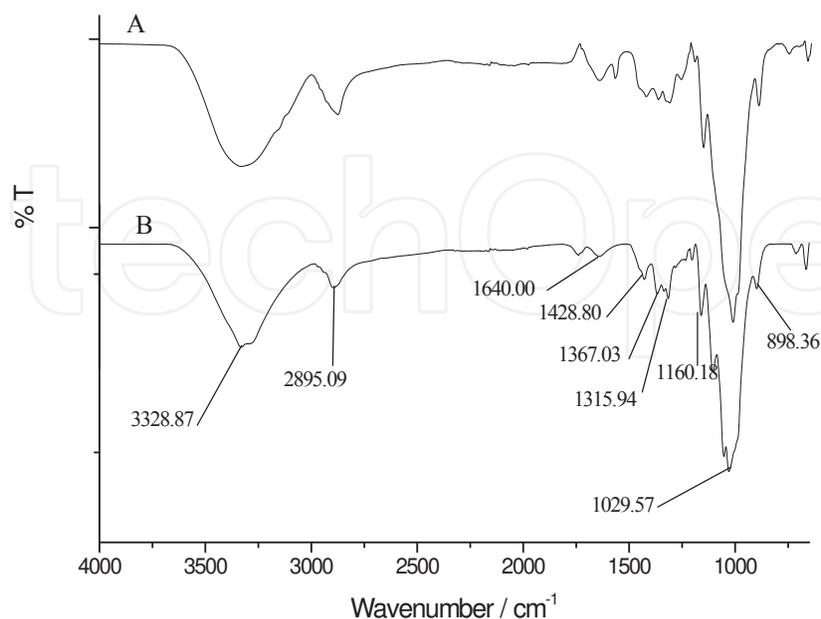
During the screening of ionic liquids on their abilities to dissolve chemical cellulose and blue-green algae biomass it was observed that the dissolution capacity depends on the anions in the ionic liquids and temperature. The dissolution trend was observed to be in the following order: 1-butyl-3-imidazolium acetate > 1-ethyl-3-imidazolium acetate > 1-butyl-3-methylimi-

dazolium chloride > 1-ethyl-3-methylimidazolium chloride whereas those with methyl sulphate and hexafluorophosphate anions were found to be non-solvent, Figure 2. It has to be noted that the chloride (Cl<sup>-</sup>) and acetate (CH<sub>3</sub>COO<sup>-</sup>) anions are all strong hydrogen bonding acceptor and thus have ability to disrupt the hydrogen bonding network in biomass biopolymers leading to their dissolution [7, 15, 16].

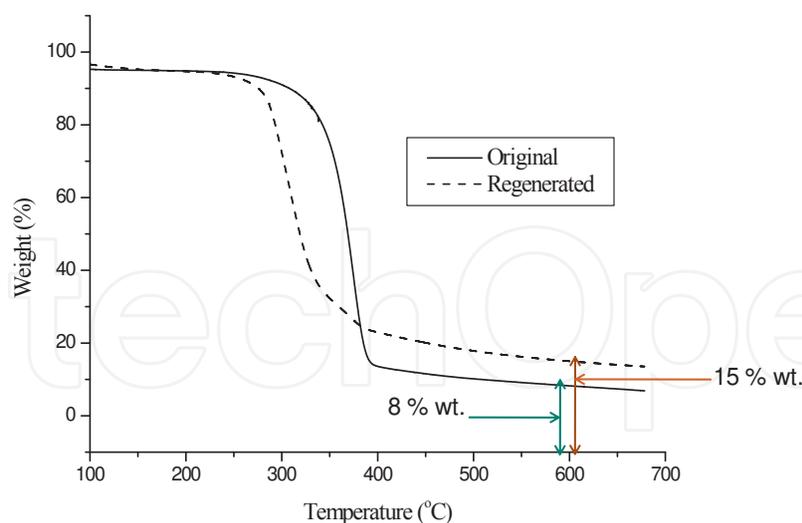


**Figure 2.** Solubility of biomass in different imidazolium based ionic liquids

The effect of ionic liquids on the biomass was investigated by checking their effect on the extracted and un-extracted chemical cellulose. This was examined using FT-IR and TGA and the result showed no significant difference between the original and the regenerated cellulose with an exception of the percentage of mass residues in the TGA profile, which was higher for regenerated cellulose, compared to that of the original cellulose at 600°C, Figures 3 and 4. This confirms that ionic liquids are just solvents because they have no reaction with biopolymers and therefore can be used for analytical processes of biopolymers.



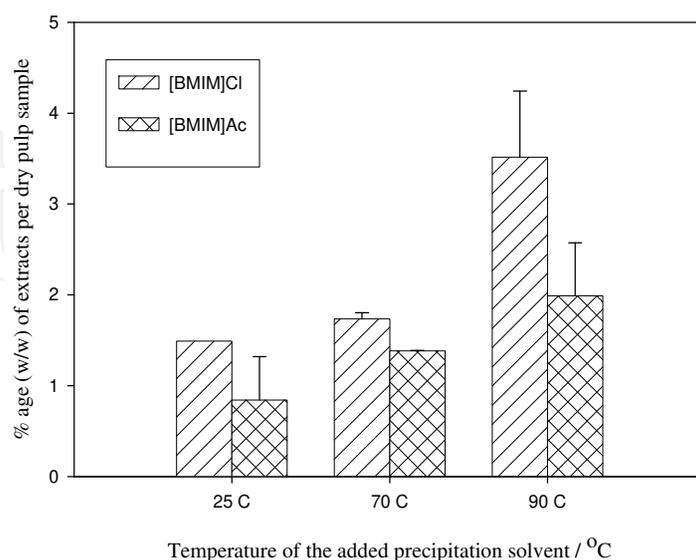
**Figure 3.** FT-IR spectra of regenerated cellulose from ionic liquid (A) and original cellulose (B)



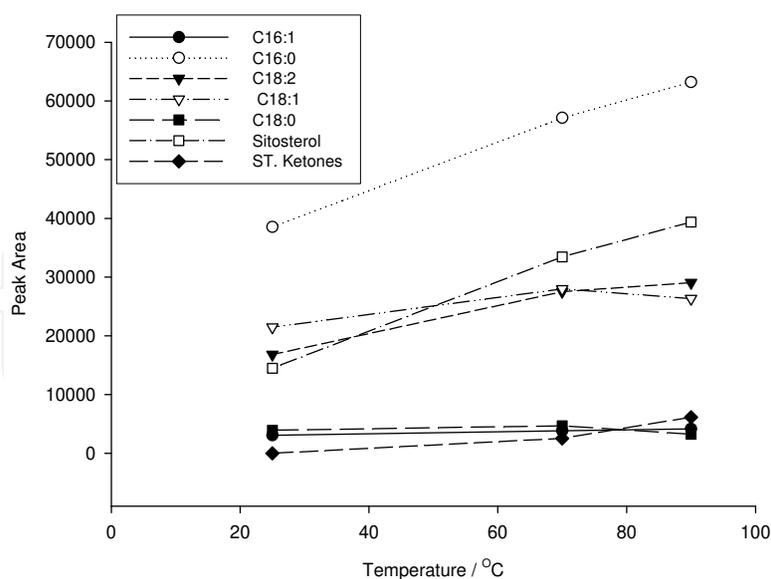
**Figure 4.** TGA analysis of original and regenerated chemical cellulose at a temperature ranging from 80°C to 700°C

Thus, 1-butyl-3-methylimidazolium acetate and 1-butyl-3-methylimidazolium chloride were selected for further investigation through which it was found that ionic liquids with chloride anion was able to recover higher amount of lipophilic extractives.

Since [BMIM]Cl and [BMIM]Ac were identified to be suitable solvent for biopolymers, an investigation and comparisons on their capacity to extract lipophilic extractives was performed. It was found that [BMIM]Cl extracted higher amount of lipophilic extractives than [BMIM]Ac, Figure 5. This difference could be explained by the nature of the anions of the two ionic liquids in which acetate has basic properties whereas chloride ionic liquid is acidic.



**Figure 5.** Comparison of [BMIM]Cl and [BMIM]Ac in the extraction of lipophilic extractives at 25°C, 75°C and 90°C of the added water for biopolymers precipitation



**Figure 6.** Amount of lipophilic extractives per precipitating solvent temperature

The effect of temperature on the extraction ability of these two types of imidazolium ionic liquids was also considered and found that the amount of lipophilic extractives obtained from biopolymers (cellulose) increased with temperature as presented in Figure 5. Further investigation for effect of precipitating solvent temperature on the amount of lipophilic extractives obtained was performed on the individual compounds as indicated in Figure 6. Thus, the percentage recovery of the individual compounds was also verified to increase with temperature of the added precipitation solvent.

This can be explained by the fact that, at low temperature of the precipitation solvent, lipophilic extractives precipitate on the surface of the cellulose due to their hydrophobicity. In this study water was found to be the best polar solvent for precipitation of dissolved cellulose. When trying to avoid the problem of lipophilic extractives depositing on cellulose surface during regeneration of cellulose, the possibility of using any other polar volatile organic solvents as cellulose precipitating solvents, was considered. However, most of these volatile organic solvents boil at low temperatures while the dissolution temperature in this study was fixed at the optimal temperature of 90°C which was too high for volatile organic solvent to exist as liquids and therefore would result into serious environmental pollution.

In comparing with the conventional volatile organic solvent extraction, it was observed that ionic liquids could quickly extract lipophilic extractives from biomass in which the qualitative and quantitative compositions were found to be similar to that obtained using conventional volatile organic solvents. In blue-green algae biomass the ionic liquid was used to extract fatty acids in which it was confirmed that both ionic liquid extraction and volatile organic solvent extraction obtained similar compositions. Therefore, the result warranted the use of ionic liquids as extraction medium for lipophilic extractives analyses and the process is quick and environmentally friendly.

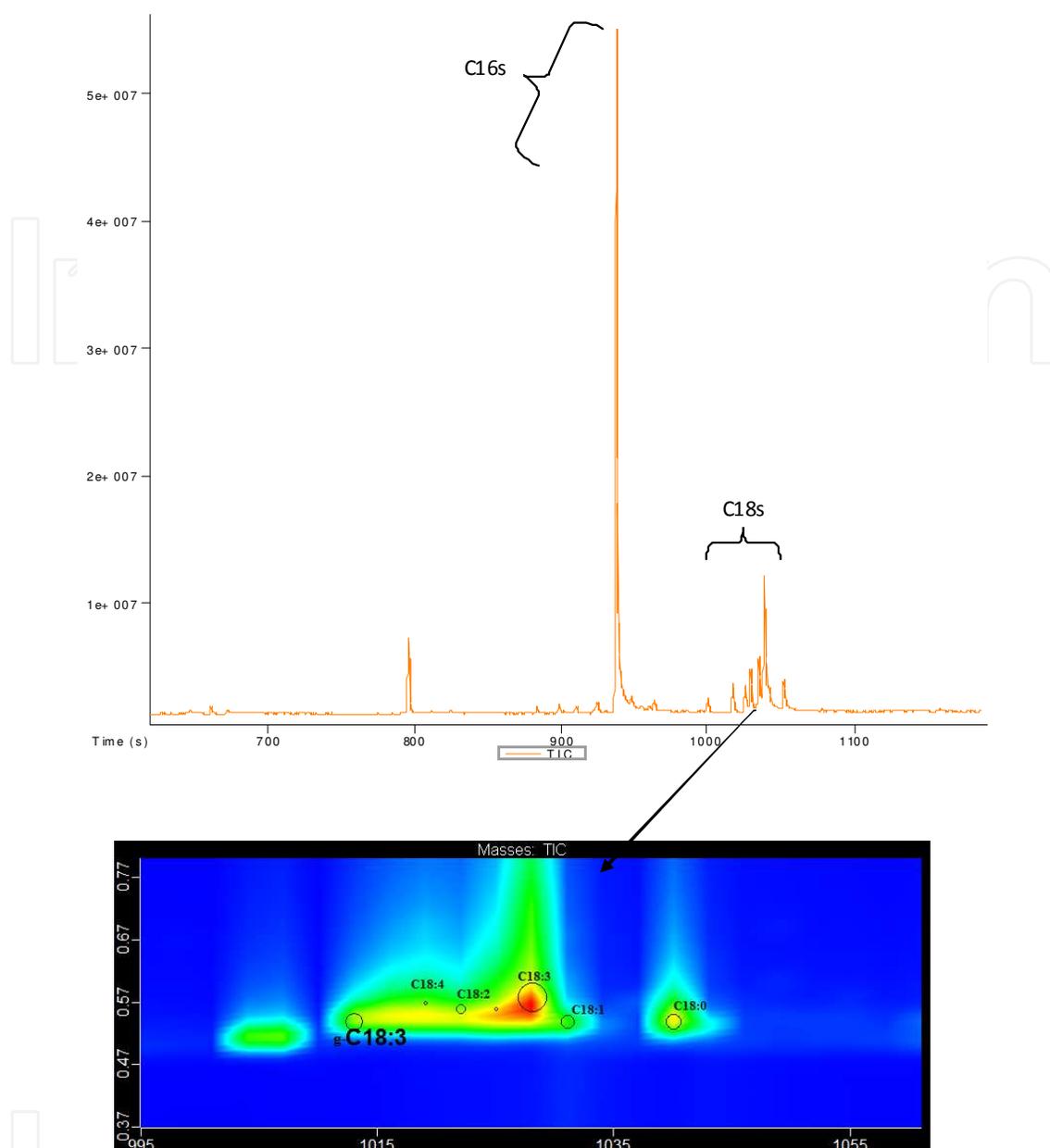
## 6.2. Identification and quantification of lipophilic extractives

Lipophilic extractives determined by GC-MS analysis on the extracts from chemical cellulose were fatty acids, sterols, fatty alcohols, steroid hydrocarbons and steroid ketones. On the other hand the GCxGC-TOFMS analysis of fatty acids composition of the extracts from blue-green algae biomass was dominated by 7-hexadecenoic acid (C16:1), hexadecanoic acid (C16:0),  $\gamma$ -linolenic acid ( $\gamma$ -C18:3); linoleic acid (C18:2); linolenic acid (C18:3); 6,9,12,15-octadecatetraenoic acid (C18:4); oleic acid (C18:1) and octadecanoic acid (C18:0). The peaks of identified fatty acids in blue-green algae biomass by GCxGC-TOFMS are shown in the one dimension (1D) chromatogram with its corresponding two dimension (2D) contour chromatogram of the C18s fatty acids which were somehow eluting at relatively very close retention times, Figure 7. Thus, the determination of the fatty acids which were analysed as their methyl esters involved the consideration of 1D and 2D chromatography with application of Windows-based ChromaTOF software of the GCxGC-TOFMS.

Generally it can be observed that for the fatty acids with a chain of 18 carbon atoms (C18s) were coeluting in 1D chromatography, however, through the application of deconvolution power of the Pegasus 4D GCxGC-TOFMS they were properly determined, Figure 8. The tendency of C18s fatty acids isomers to coelute and bear the identical mass spectra is actually due to the fact that they all have similar chemical structures which only differs in the number and positions of double bonds. Hence it can be observed in the given chromatograms that C18s fatty acids methyl esters of Linolenic acid (C18:3), Oleic acid (C18:2) and 9-Octadecanoic acid (C18:1) were somehow coeluting in this analysis. But their actual determination was achieved by the employment of the deconvolution algorithm of ChromaTOF software which made the separation of their mass spectra, identification and quantification accurately done. It is this aspect of the GCxGC-TOFMS technique which makes this study to recommend it as a more suitable and advanced technique for analysis of lipophilic extractives if one is to obtain reliable data. Figure 8 shows the 1D GCxGC-TOFMS mass spectra of C18s fatty acids.

The obtained fatty acids composition from blue-green algae biomass using ionic liquids (IL) [BMIM]Cl and that of volatile organic solvent (VOS) was found to be similar, Figure 9. It can be easily observed that the quantitative composition of different fatty acids components obtained by the two extraction techniques were similar with some few exceptions, i.e., the amount of 7-hexadecenoic acid (C16:1),  $\gamma$ -6,9,12-octadecatrienoic acid ( $\gamma$ -C18:3) and 9,12-octadecadienoic acid (C18:2) were relatively higher in ionic liquid extraction. While on the other hand, hexadecanoic acid (C16:0), 6,9,12,15-octadecatetraenoic acid (C18:4), 9,12,15-octadecatrienoic acid (C18:3) and octadecanoic acid (C18:0) were relatively higher in volatile organic solvent extraction technique. To justify the similarity of the quantitative composition of fatty acids one-way analysis of variance (ANOVA) was performed and confirmed that at the 0.05 level there was no significant difference ( $P \gg 0.05$ ) between the means of the data sets obtained by the two extraction techniques. This information indicates that there is no reaction (such as oxidative reaction) between fatty acids and ionic liquid [BMIM]Cl and therefore, ionic liquids are just solvents of fatty acids and lipophilic extractives in general.

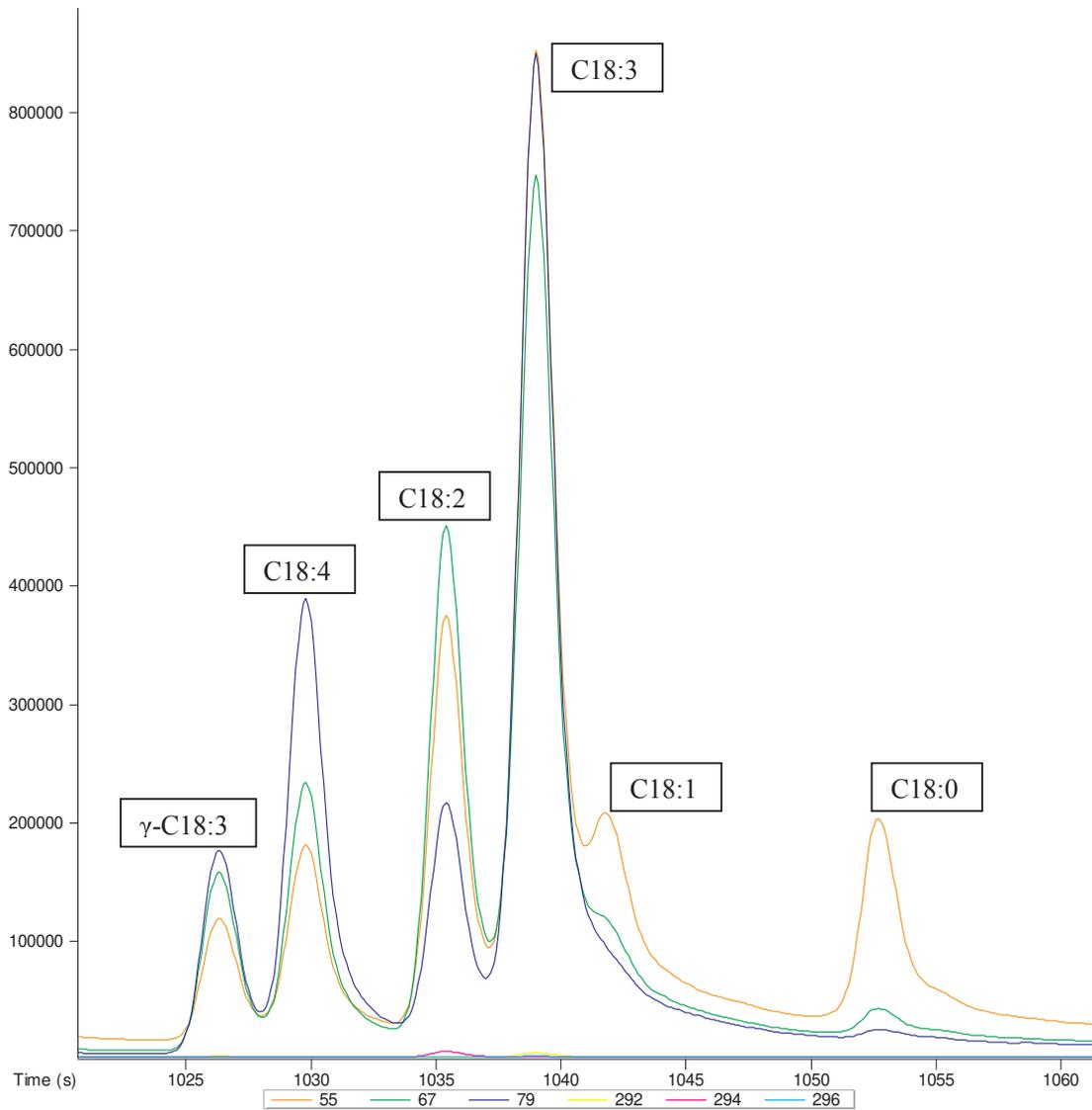
The similarity in qualitative and quantitative composition of fatty acids from blue-green algae biomass obtained using ionic liquid based extraction (IL) and conventional volatile organic



**Figure 7.** 1D and 2D GCxGC-TOFMS chromatogram indicating the main fatty acids from blue-green algae biomass (C16s=C16:1 and C16:0, whereas C18s= $\gamma$ -C18:3, C18:4, C18:2; C18:3, C18:1 and C18:0). The 2D chromatogram shows the corresponding C18s fatty acids methyl esters elution region in which the X-axis is retention time (second) on the primary column and the Y-axis is retention time (second) on the secondary column

solvent extraction (VOS) method justified that ionic liquid is a suitable solvent to replace volatile organic solvent for reduced environmental pollution.

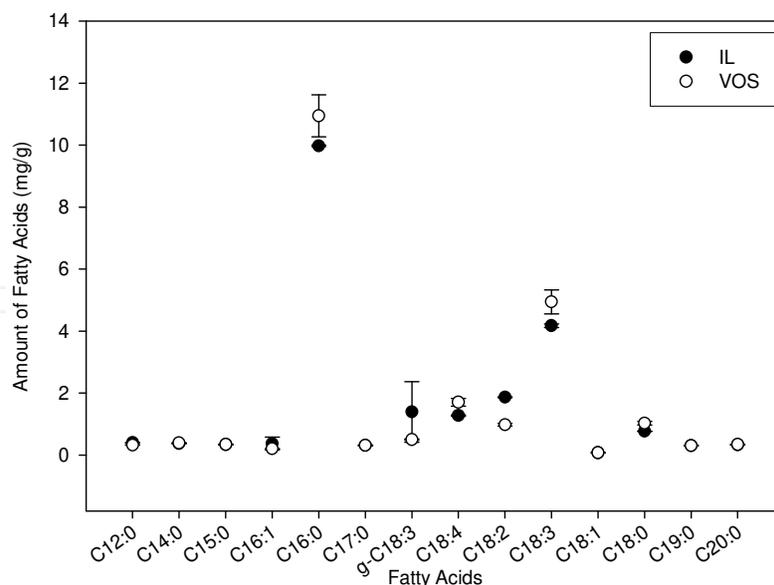
This study also investigated the difference between the amounts of fatty acids extracted from blue-green algae biomass using ionic liquid extraction followed by derivatization stage against the one with direct derivatization. The data showed that the extraction under direct derivatization enhanced the amount of fatty acids obtained. It was observed that the total amount of fatty acids increased from 6.07 mg/g (of dry biomass sample) in ionic liquid extraction with



**Figure 8.** 1D GCxGC-TOFMS mass spectral deconvolution of C18s fatty acids used for identification

separate derivatization step to 21.96 mg/g (of dry biomass sample) in ionic liquid extraction with direct derivatization. It was further observed that for the saturated fatty acids the increase was from 3.64 mg/g (of dry biomass sample) in ionic liquid extraction with separate derivatization step to 12.80 mg/g (of dry biomass sample) in ionic liquid extraction with direct derivatization. For the unsaturated fatty acids the increase was from 2.43 mg/g (of dry biomass sample) in ionic liquid extraction with separate derivatization step to 9.16 mg/g (of dry biomass sample) in ionic liquid extraction with direct derivatization. Therefore in order to exhaust the amount of fatty acids in blue-green algae biomass using ionic liquid extraction approach, direct derivatization method is vital.

The use of ionic liquid can significantly reduce the pollution of the environment not only due to its properties [4, 17-19] but also its miscibility with water, a property which makes it reduce



**Figure 9.** Comparison of fatty acids composition of ionic liquid (IL) extraction and volatile organic solvent extraction methods (VOS) (mg/g of dry algae biomass).

the use of volatile organic solvent in analytical procedures [19]. Due to the ability of ionic liquids in dissolving biomass, the extraction of lipophilic extractives becomes more efficient due to the fact that lipophilic extractives are released into the solution in which cellulose is regenerated by the addition of precipitation solvent such as water. Thus, the extraction of analytes is easily achieved using small amount of volatile organic solvents in the ionic liquid-aqueous filtrate [5].

## 7. Conclusion

This study has demonstrated the application of imidazolium based ionic liquids in the extraction of lipophilic extractives from biomass. Ionic liquids were screened for their dissolution capacity of biomass and their ability to extract lipophilic extractives from the analysed biomass (i.e. blue-green algae and chemical cellulose). It was observed that ionic liquids particularly the ones with anions which are strong hydrogen bonding acceptor such as chloride ( $\text{Cl}^-$ ) and acetate ( $\text{CH}_3\text{COO}^-$ ) can easily dissolve biopolymers and other biomass. [BMIM]Ac and [BMIM]Cl were identified to be the most suitable ionic liquids solvents for the extraction of lipophilic extractives, in which [BMIM]Cl was found to extract more lipophilic extractives than [BMIM]Ac. The study investigated the suitability of imidazolium ionic liquids in extracting lipophilic extractives from biomass in which the composition obtained by using volatile organic solvents and that of ionic liquids were similar. Therefore, imidazolium based ionic liquids is just a solvent and suitable to replace volatile organic solvents for minimized environmental pollution. The ionic liquid based extraction procedure was found to be quick, effective and efficient as compared to volatile organic solvents based extraction procedure. Based on the observed results direct derivatization for the ionic liquid based extraction is

recommended when using ionic liquids for the extraction and analysis of lipophilic extractives from biomass.

## Acknowledgements

The authors are grateful to the Research Unit of Nanotechnology for Water Sustainability, UNISA, South Africa for financial support and instrumentation facilities.

Authors also thank the Chemistry Department, University of Dar es Salaam, Tanzania.

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