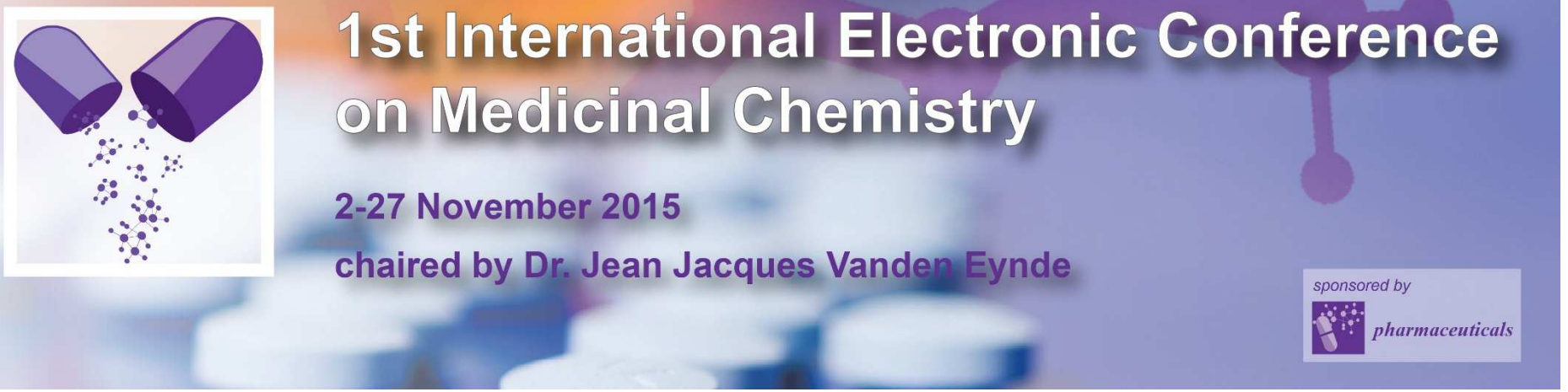


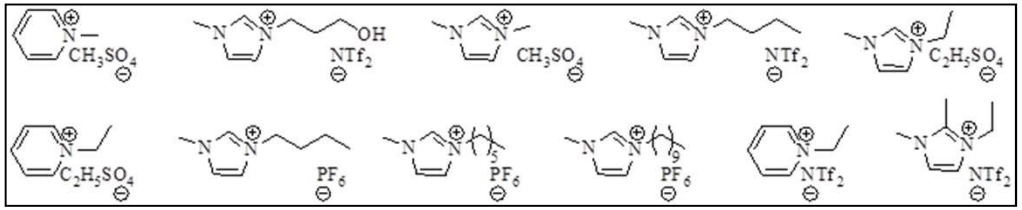
Ionic Liquids Coupled to Microwave Irradiation: an Efficient and Selective Process for secondary lichens metabolites extraction

Ludovic Paquin [1,*], Fabienne Gauffre [2], and Sophie Tomasi [3]

[1] Université de Rennes 1 – Institut de Sciences Chimiques des Rennes – UMR 6226 , Campus de Beaulieu, Bat 10 A 35042 Rennes - France ;
[2] Université de Rennes 1 – Institut de Sciences Chimiques des Rennes - CNRS – UMR 6226, Campus de Beaulieu, Bat 10 A 35042 Rennes - France.
[3] Université de Rennes 1 – Institut de Sciences Chimiques des Rennes – UMR 6226 , Campus de Villejean 35043 Rennes - France
* Corresponding author: ludovic.paquin@univ-rennes1.fr

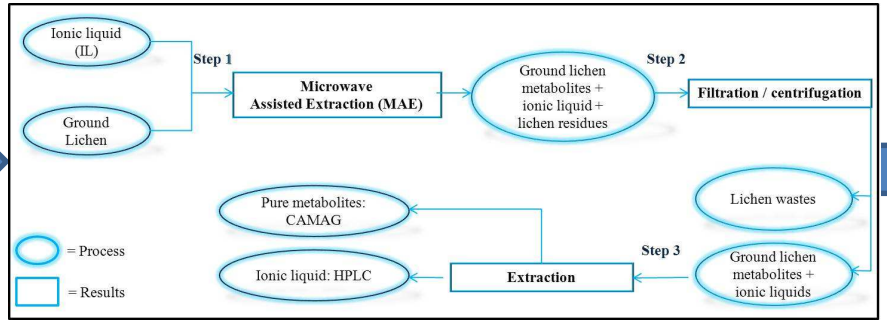
Ionic Liquids Coupled to Microwave Irradiation: an Efficient and Selective Process for secondary lichens metabolites extraction

Graphical Abstract

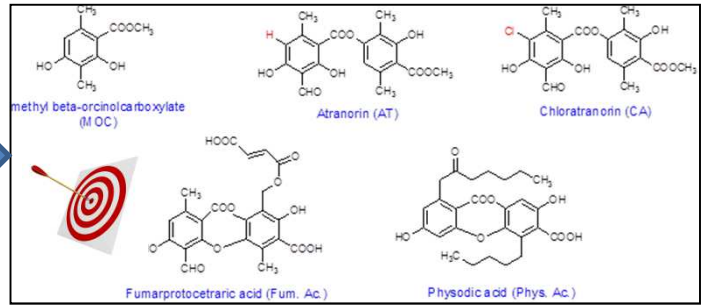


Ionic liquids selected for the extraction

lichen



Process



Secondary metabolites selected

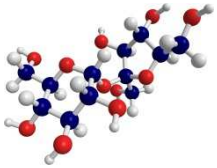
Abstract:
Lichens are particular organisms, consisting of a cyanobacterial or/and an algal symbiotic partnership with a fungus. Their secondary metabolites are usually original molecules which are not found in other types of organisms. These substances are of interest for cosmetics and pharmaceutics as a variety of activities have been reported, antibiotic, anti-inflammatory, antioxidant, UV-A and UV-B filters. The standard methods for the extraction of these secondary metabolites proceed through time consuming, heat reflux protocols with volatile organic solvents. The aim of the current study is to develop innovative methods to accelerate (qualitatively and quantitatively) the selective extraction of lichen compounds. After optimization of microwave assisted extraction (MAE), we applied this method on *Pseudevernia furfuracea* using various ionic liquids (ILS). This extraction was compared to the classical extraction using Volatile Organic Solvents (VOCs). The efficiencies of the extraction methods are evaluated using TLC coupled to a Camag® spectrophotodensitometer and HPLC analysis.

Keywords: Extraction; Microwave; Ionic liquids; Secondary metabolites; Lichen

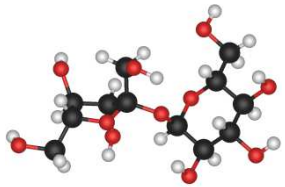
Introduction

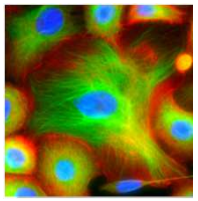

Natural Products

Extraction



Structural Identification





Biological screening

Pure Natural Product

Total Organic synthesis

Bio-inspired compounds

a) *Xanthoria ligulata*

b) *Umbilicaria deusta*

c) *Normandina pulchella*

d) *Pseudevernia furfuracea*

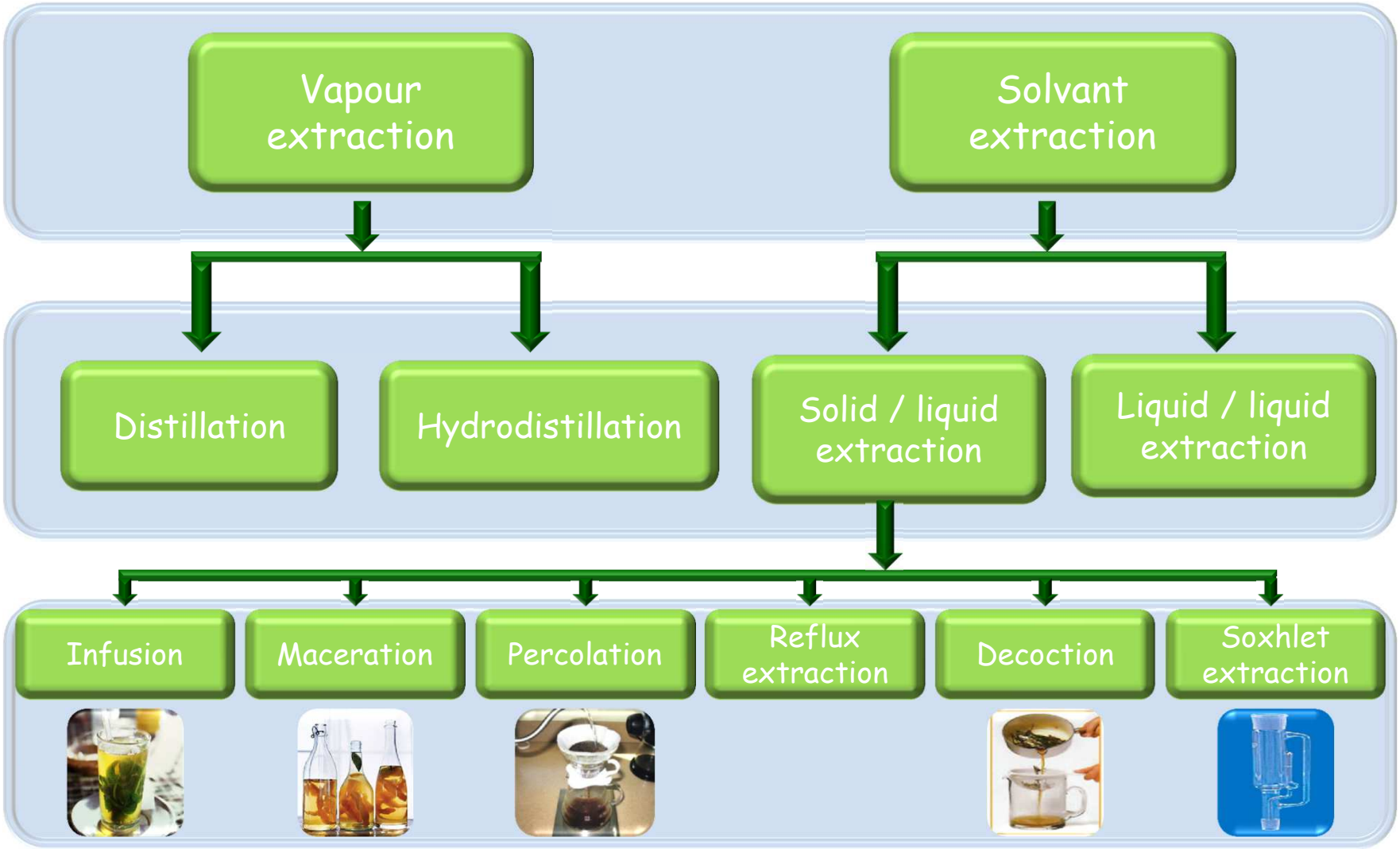
e) *Cladonia Stereocaulon*

f) *Leptogium venustum*

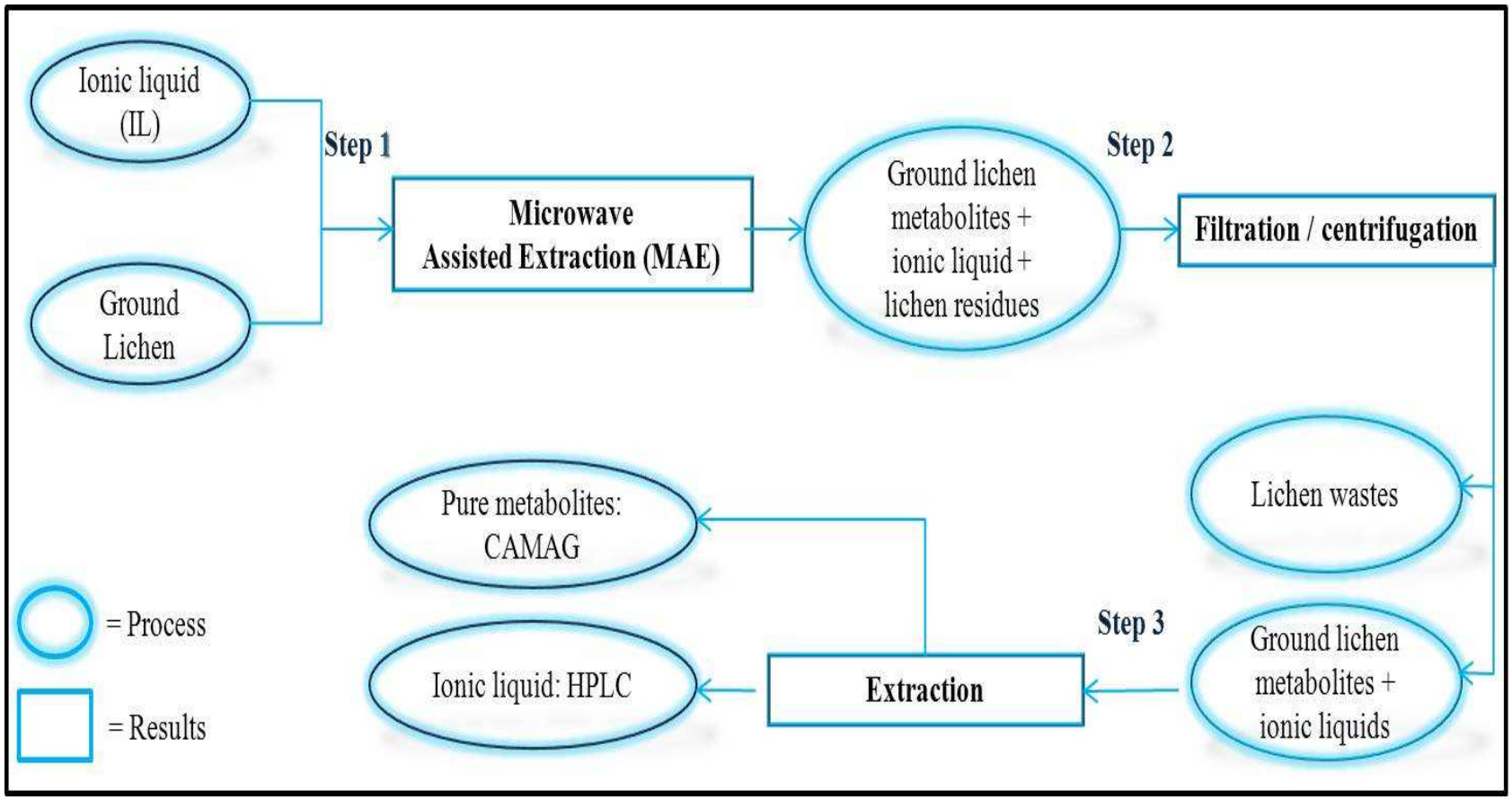
g) *Lepraria lobificans*

Hidalgo *et al.* (1994), *Phytochem.* 37, 1585-1587

Classical process for extraction



Our process for extraction

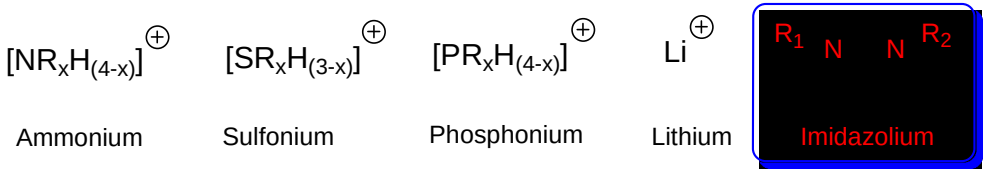


Ionic liquids → selectivity
Microwave irradiation → efficency

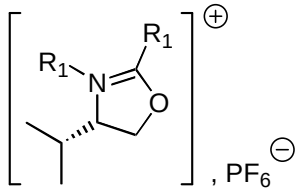
Results and discussion

Ionic liquid= CATION (organic) + ANION (organic or inorganic)

Classical structure:



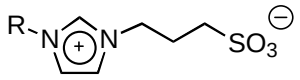
Chiral cation [2]:



(S)-valinol → Oxazolinium

R_1 = Pent, R_2 = Et, mp = 63°C
R_1= Me, R_2 = Et, mp = 79°C

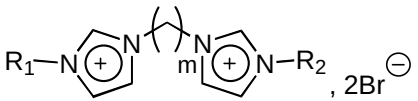
Zwitterion[3] :



R = Et, $CH=CH_2$

$LiNTf_2$ 1 eq. -> **Tg = -16°C**

Poly-cations [4] :



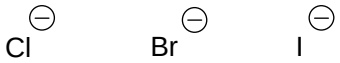
R_1 , R_2 = Me
mp = 67-69°C

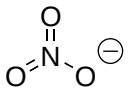
[2] Wassercheid P., *Chem. Commun.* **2001**, 2002

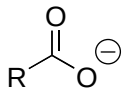
[3] Yoshizawa M., *J. Mater. Chem.* **2001**, *11*, 1057

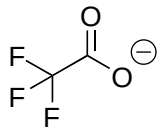
[4] Varma R.S., *Chem.Commun.* **2001**, 643

Anions structure

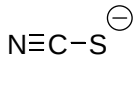




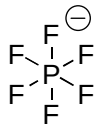




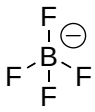
trifluoroacetate



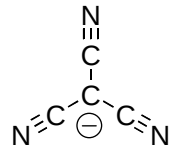
thiocyanate



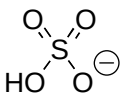
hexafluorophosphate

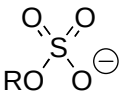


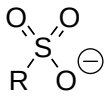
tetrafluoroborate

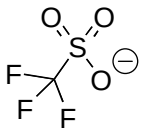


tricyanomethane

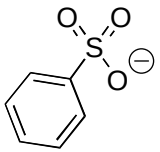




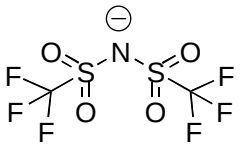




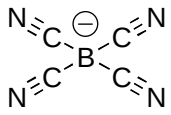
triflate



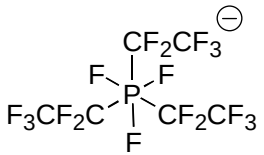
tosylate



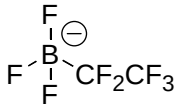
triflimide

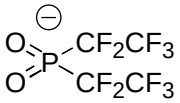


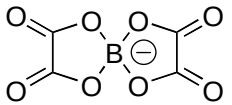
tétracyanoborate



FAP



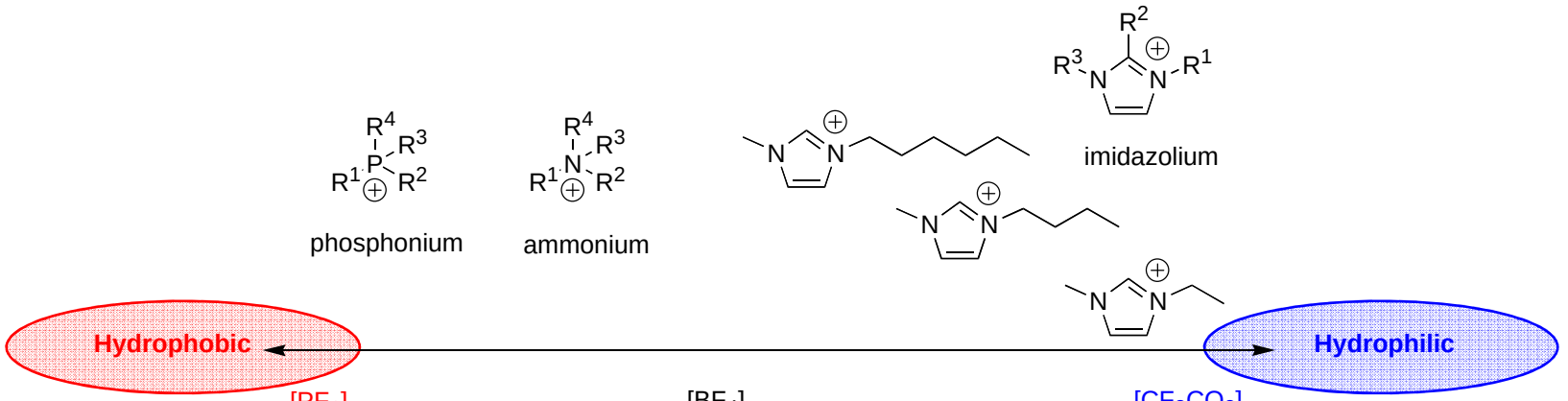


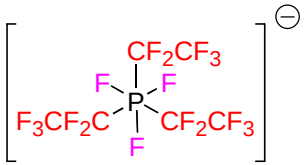


Desirable Properties of "Ionic Liquids"

- Negligible vapor pressure
  - Non-volatile
  - Non-flammable
- High thermal, chemical and electrochemical stability
- Liquid over a wide temperature range (-50°c to 300°c)
- Dissolution of many organic and inorganic compounds
- Variable miscibility with water and organic solvents

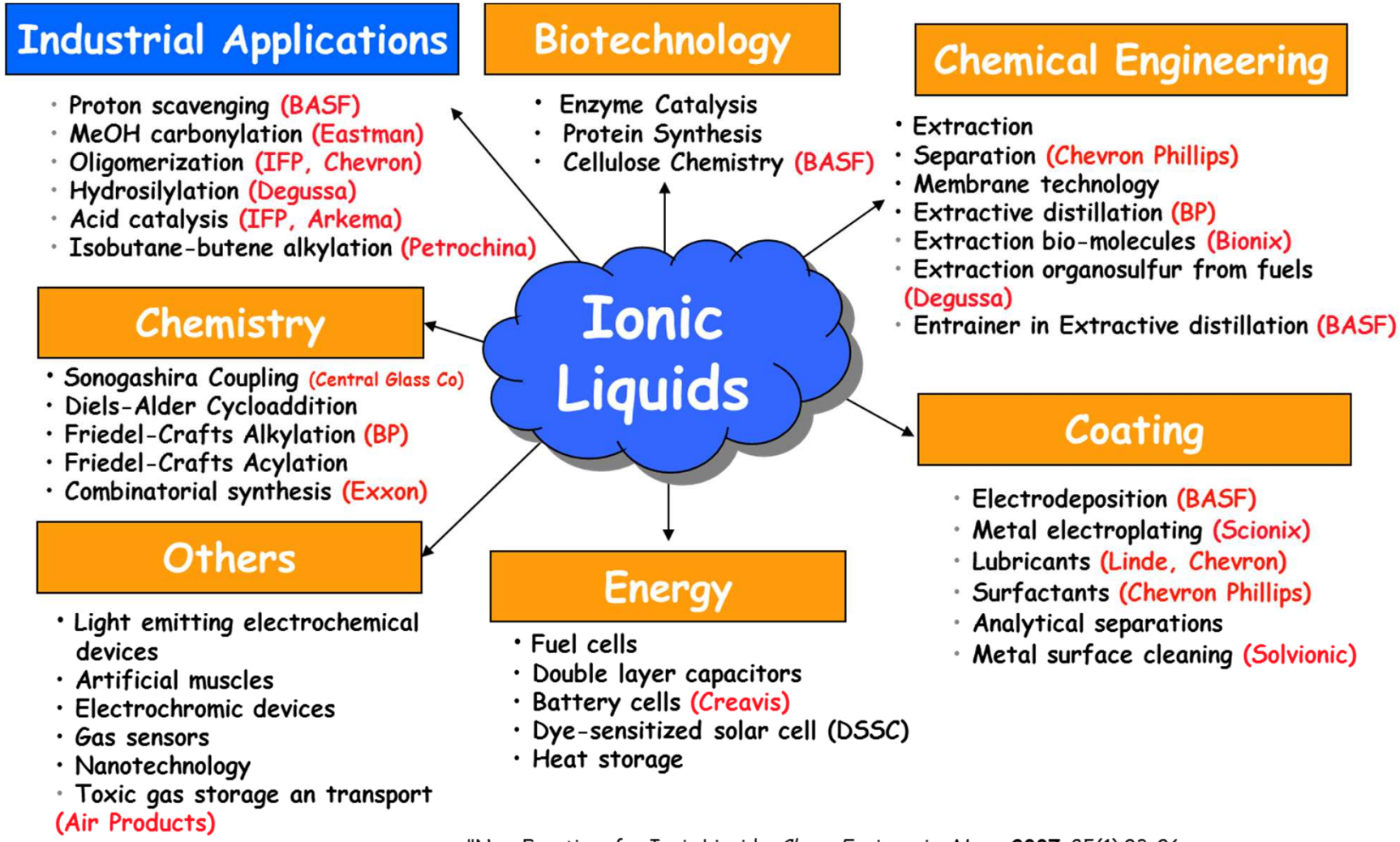
The choice of anion and cation components determines the physicochemical properties





ionic liquid	H_2O in IL / wt %	IL in H_2O / ppm
[hmim][BF_4]	18.1	7.27
[hmim][TCB]	5.39	0.44
[hmim][PF_6]	1.84	-
[Ph_3t][NTF]	1.58	-
[hpyr][NTF]	1.13	0.25
[hmim][NTF]	1.12	0.13
[hmpl][NTF]	0.900	-
[hmim][FAP]	0.195	0.02
[Ph_3t][FAP]	0.180	-
[hmpl][FAP]	0.114	-

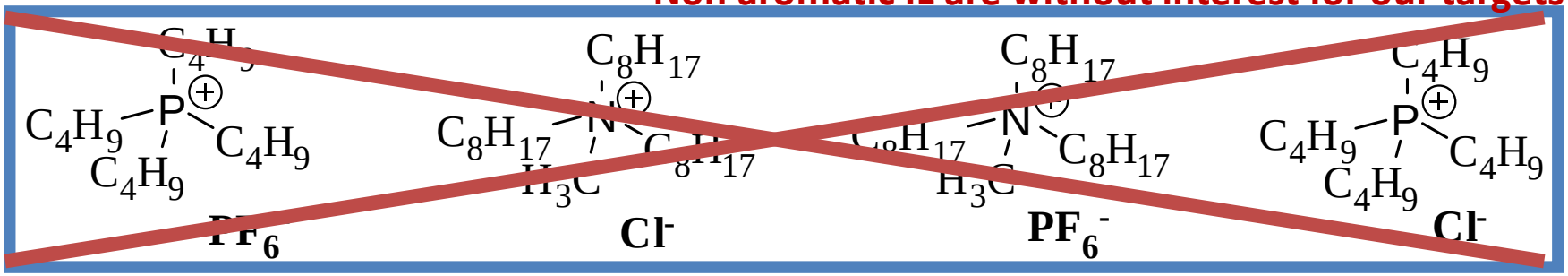
Examples of ionic liquids applications

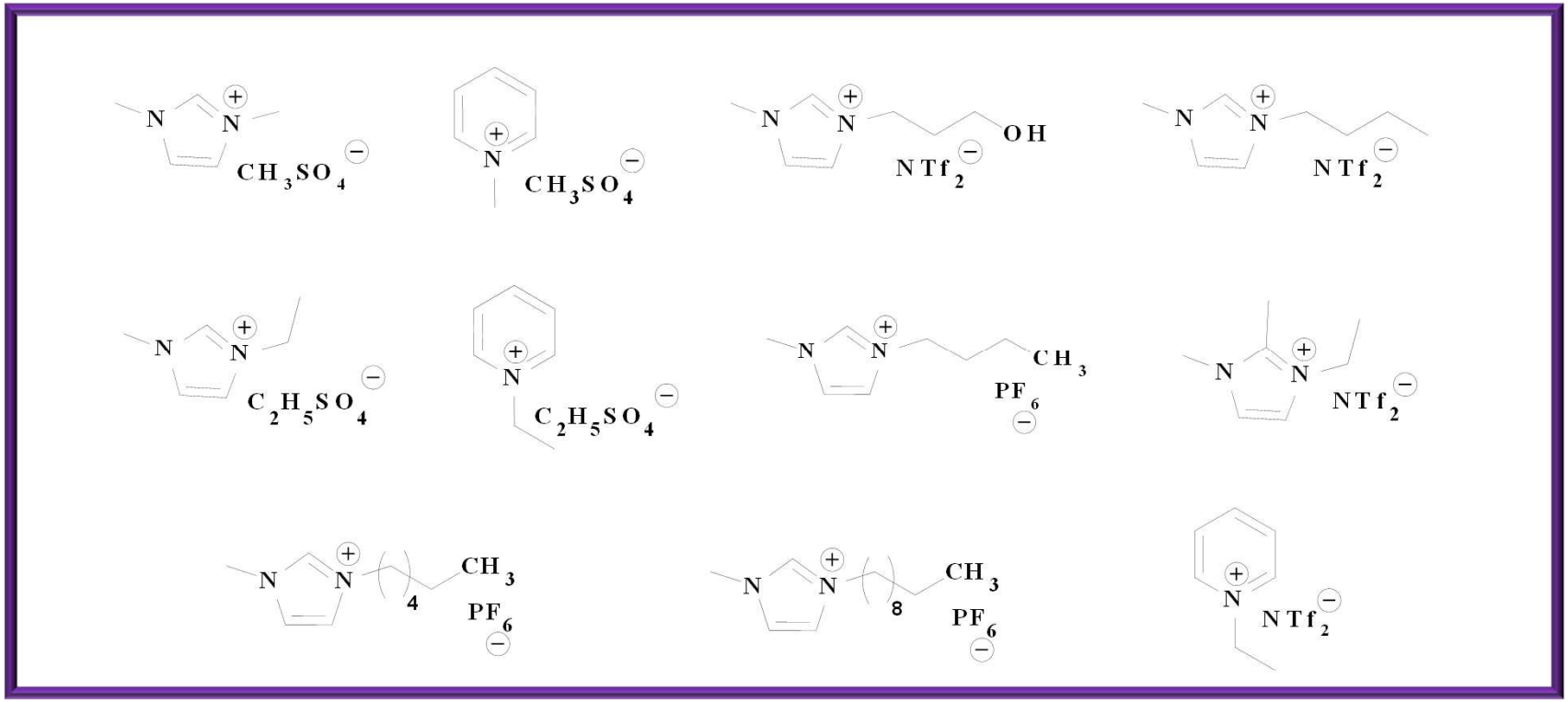


"New Frontiers for Ionic Liquids, *Chem. Engineering News*, **2007**, 85(1) 23-26
"Catalysis in Ionic Liquid", C. Zhang, *Progress in Catal.* **2006**, 153-237
R. Rodgers et al *Science*, **2003**, *302*, 31; *Chemical Engineering News*, **2006**, *84*, 14

Selected ionic liquids for this study

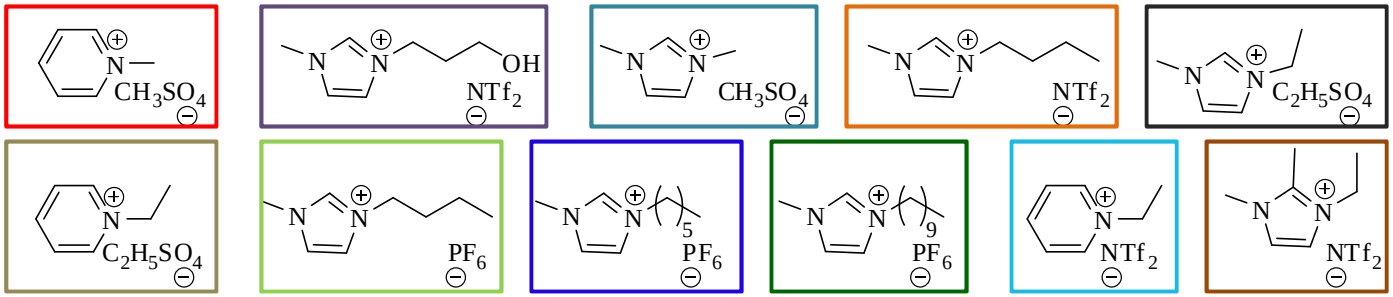
Non aromatic IL are without interest for our targets





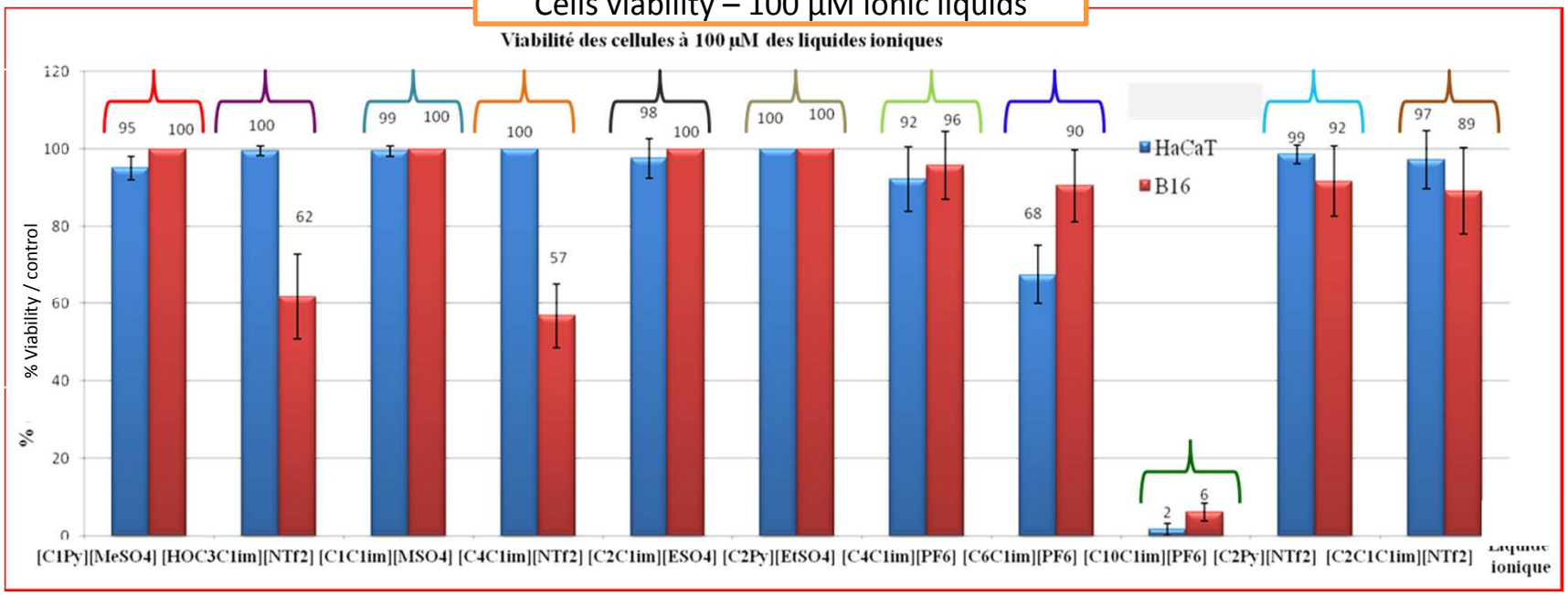
S. Bonny, L. Paquin, D. Carrié, J. Boustie, and S. Tomasi, Anal. Chim. Acta, vol. 707, no. 1–2, pp. 69–75, 2011

Ionic liquids toxicity



No cellular toxicity expected for C10C1 mim

Cells viability – 100 µM ionic liquids

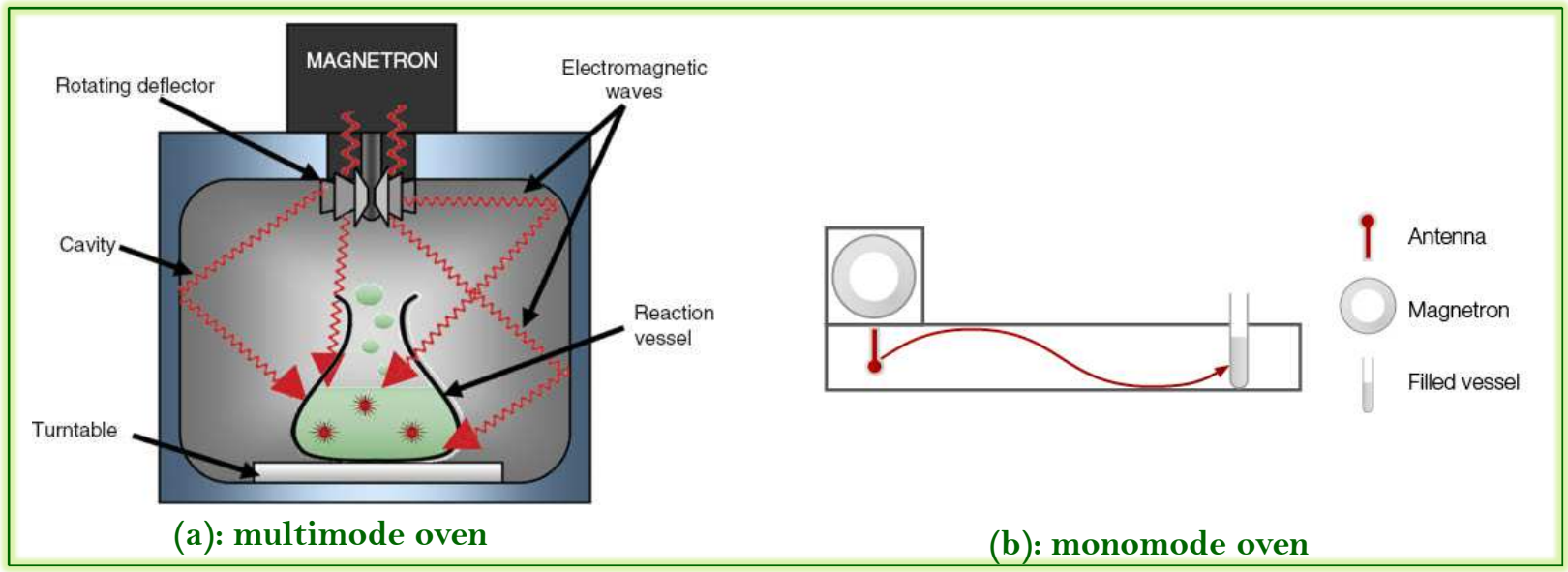


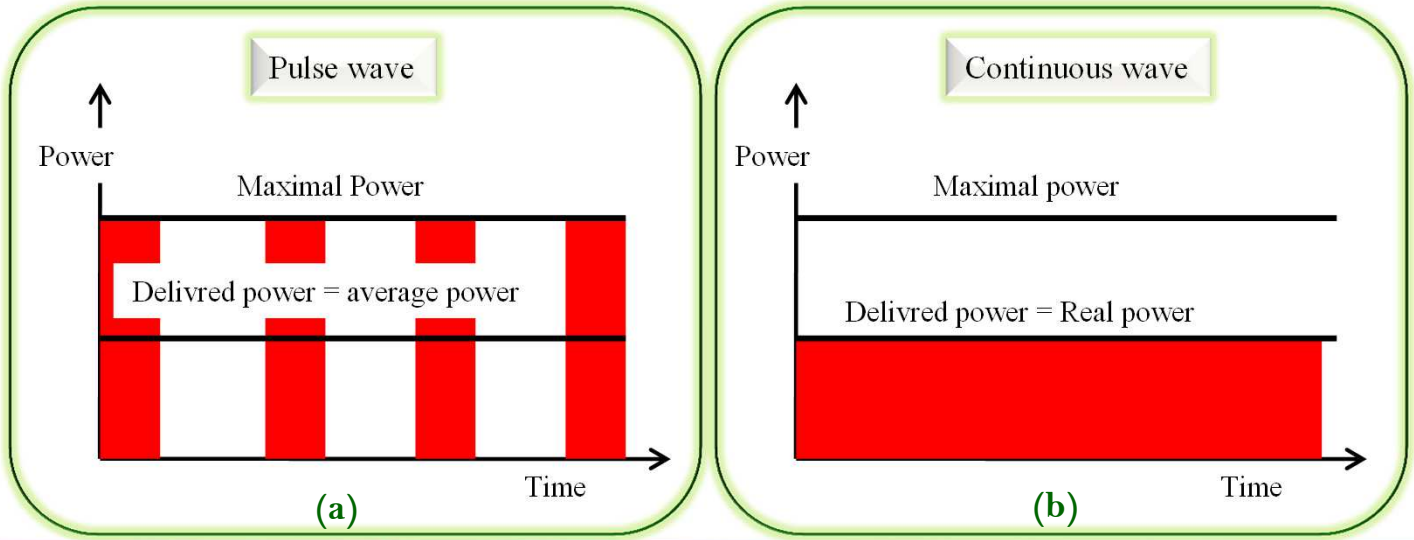
HaCaT: human derived epidermis keratinocytes , **B16:** Lignée cellulaire murine de mélanome

Example of Microwave dedicated to extraction

Plant	Métabolits	Conditions	References
Mango leaves	Mangiférine (Xanthone)	MW : 123 sec, 474 W	(Zhou et al. 2013)
Aquilaria malaccensis (tree)	Essential oil	MW: 10 à 13 min vs 1 h classical conditions	(Bibi Sabrina et al., 2010)
Fruit Luffa usine acutangula (Cucurbitaceae)	Mucilage	MW : 5 min, 640 W vs 1 h classical conditions	(Ravalgi et al. 2012)
Walnut leaves	Essential oil	MW: 1 h vs 3 h classical conditions	(Boukhari et al., 2013)
Star anise	Essential oil	MW : 80 min 80°C	(Yang et al. 2011)
Uncaria sinensis (liana)	Cafeïc acid, flavonoïds…	MW: 20 min 100 °C vs 60 min classical conditions	(Tan et al., 2011)
Camellia oleifera	Polyphenols	MW : 35 min 76°C	(Zhang et al., 2011)
Orange peel	Flavonoïds	MW: 15 min, 482 W	(Yu, 2011)
Lotus Plumula nelumbinis	Flavones	MW : 90 sec, 400 W	(Yu et al., 2011)
Orange	Pigment	MW: 50 sec, 260 W	(Zhang et al., 2010)
Nutshell	Pigment	MW: 50 sec, 260 W	(Zhao et al., 2010)

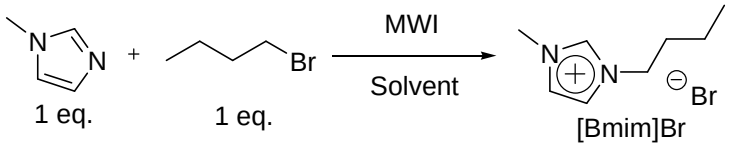
Specificity of microwave irradiation



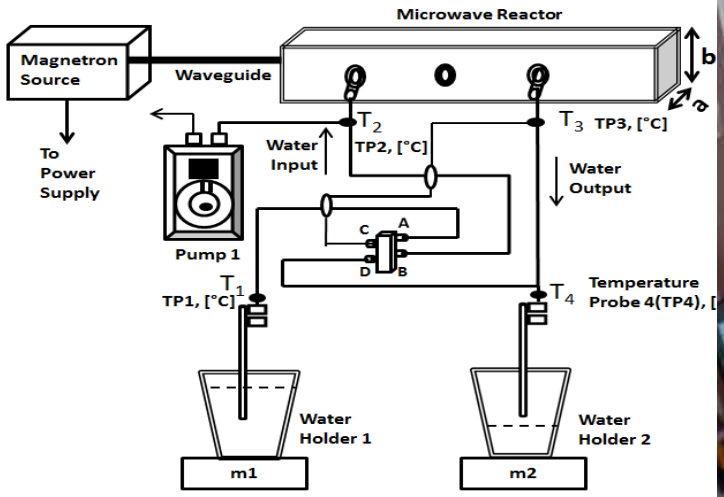


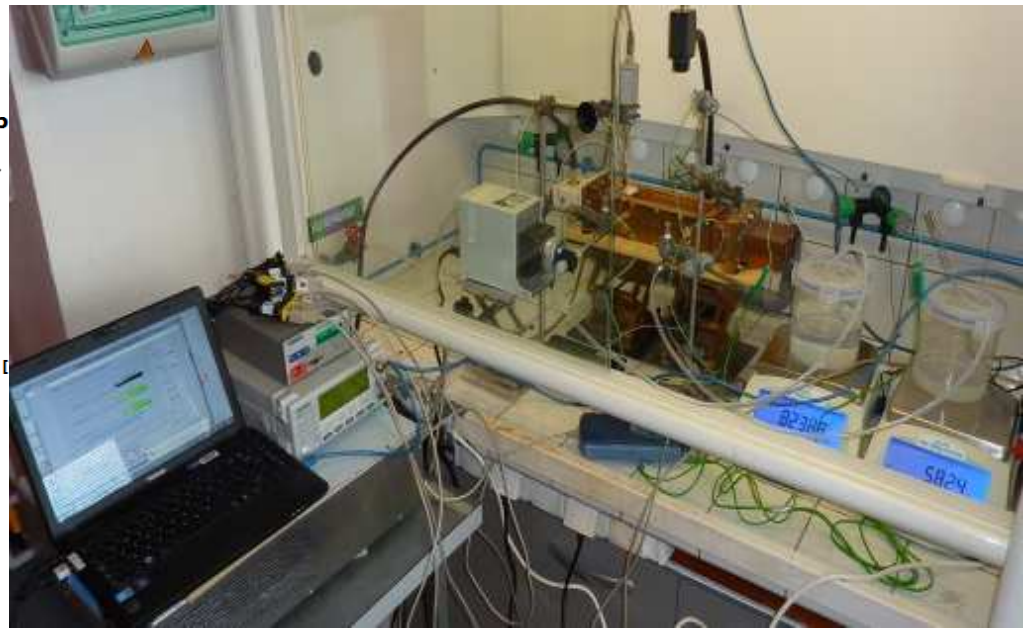
We also can use microwave irradiation in continuous flow to produce ionic liquid

- BmimBr synthesis : 70°C / CH_3CN
- 1mL.min^{-1} / 40W / t_r = 1h → 88%



EUROPEAN COMMISSION





Our equipements

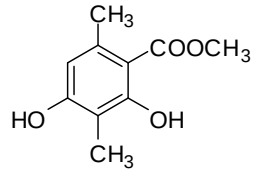
Prolabo S 402
Open vessel

Anton Paar
Monowave 300
close vessel

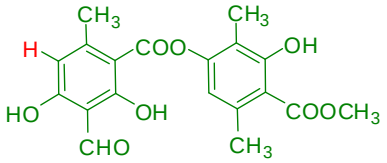
CEM discover +
explorer 24
Open and close
vessel

Prolabo S 1000
Open vessel

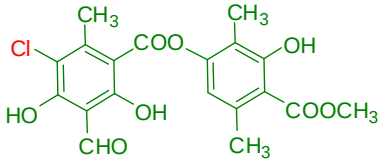
Selected secondary metabolites



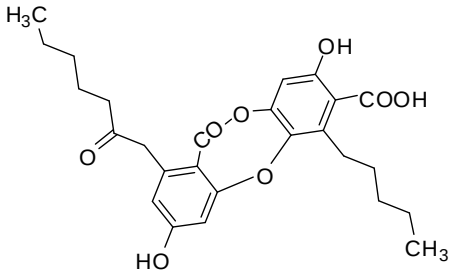
Methyl β-orcinolcarboxylate (MOC)



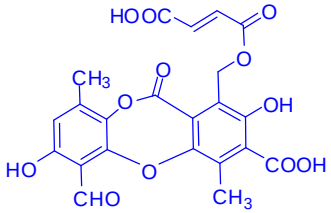
Atranorin (AT)



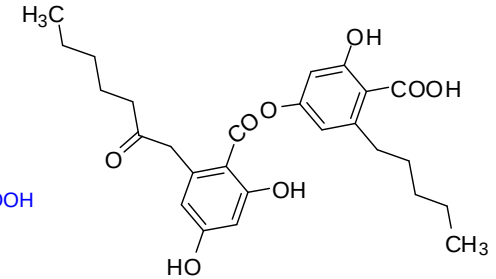
Chloratranorin (CA)



Physodic acid (Ac. Phys.)



Fumarprotocetraric acid (Ac. Fum.)



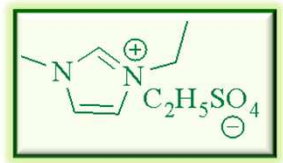
olivetoric Acid (Ac. Oliv.)

compounds	Activity	References
Fumarprotocétraric acid	- transcriptase inhibition - UV Filtre	(Pengsuparp *et al.*, 1995) (Nguyen *et al.*, 2013)
Physodic Acid	Antimicrobial activity	(Türk *et al.*, 2006)
Atranorin	- perfumery - Antimicrobial activity - UVB filtre - Anti-cancer activity	(Heide *et al.*, 1975) (Türk *et al.*, 2006) (Nguyen *et al.*, 2013) (Russo *et al.*, 2012) (Bačkoravà *et al.*, 2012)

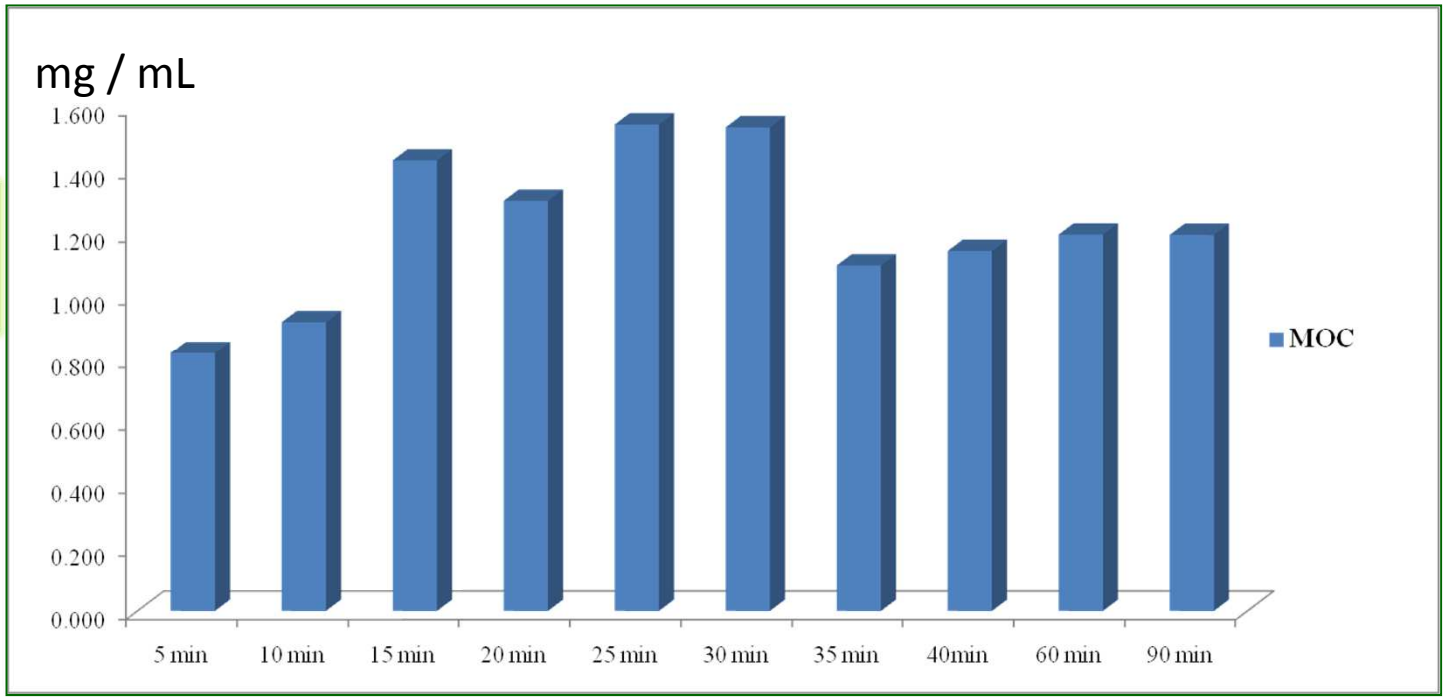
Culberson *et al.* (1980), *Mycologia* 72, 90-109
P. O. Thune and Y. J. Solberg, "Photosensitivity and allergy to aromatic lichen acids, Compositae oleoresins and other plant substances," *Contact Dermatitis*, vol. 6, pp. 64–71, 1980

Time optimisation

IL + Crushed Lichen → MW EXTRACTION → Metabolites + IL → SEPARATION → Pur metabolites



$[C_2C_1im][EtSO_4]$

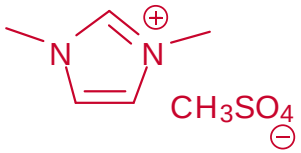


Extraction time

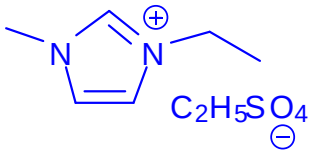
Optimal time : 15 min vs 24 hours under classical conditions

Bonny S., Hitti E., Boustie J., Bernard A., Tomasi S. (2009), *J. chromatogr. A.* **1216**, 7651-7656

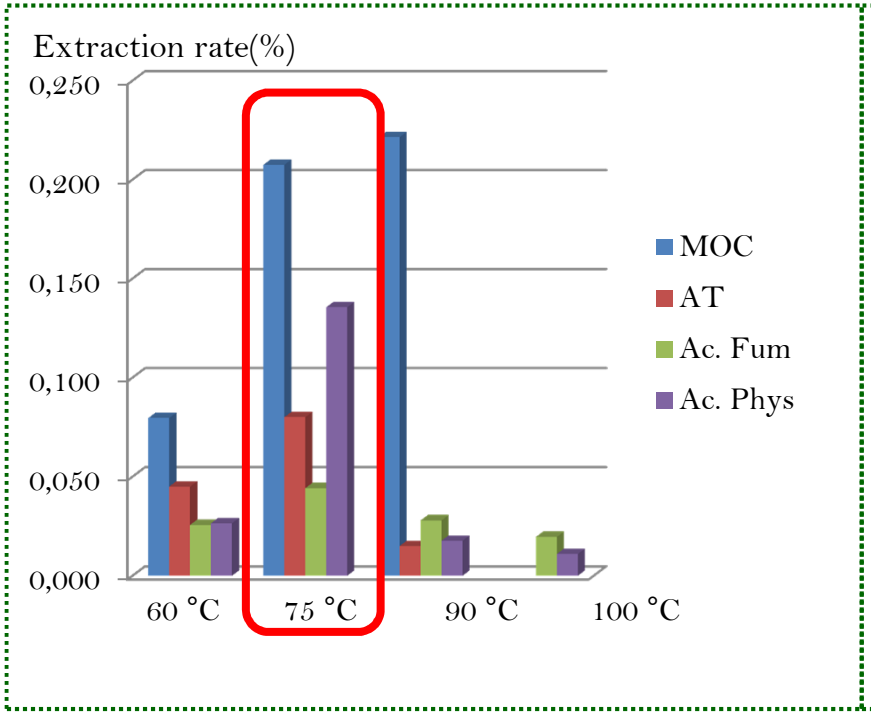
Temperature optimisation

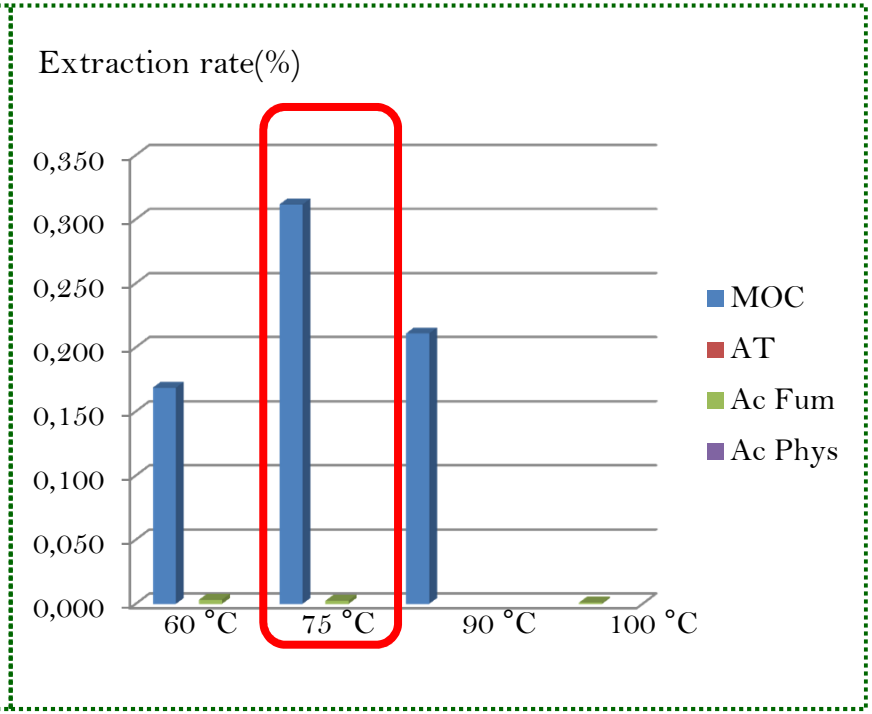


$[C_1C_1im][MeSO_4]$



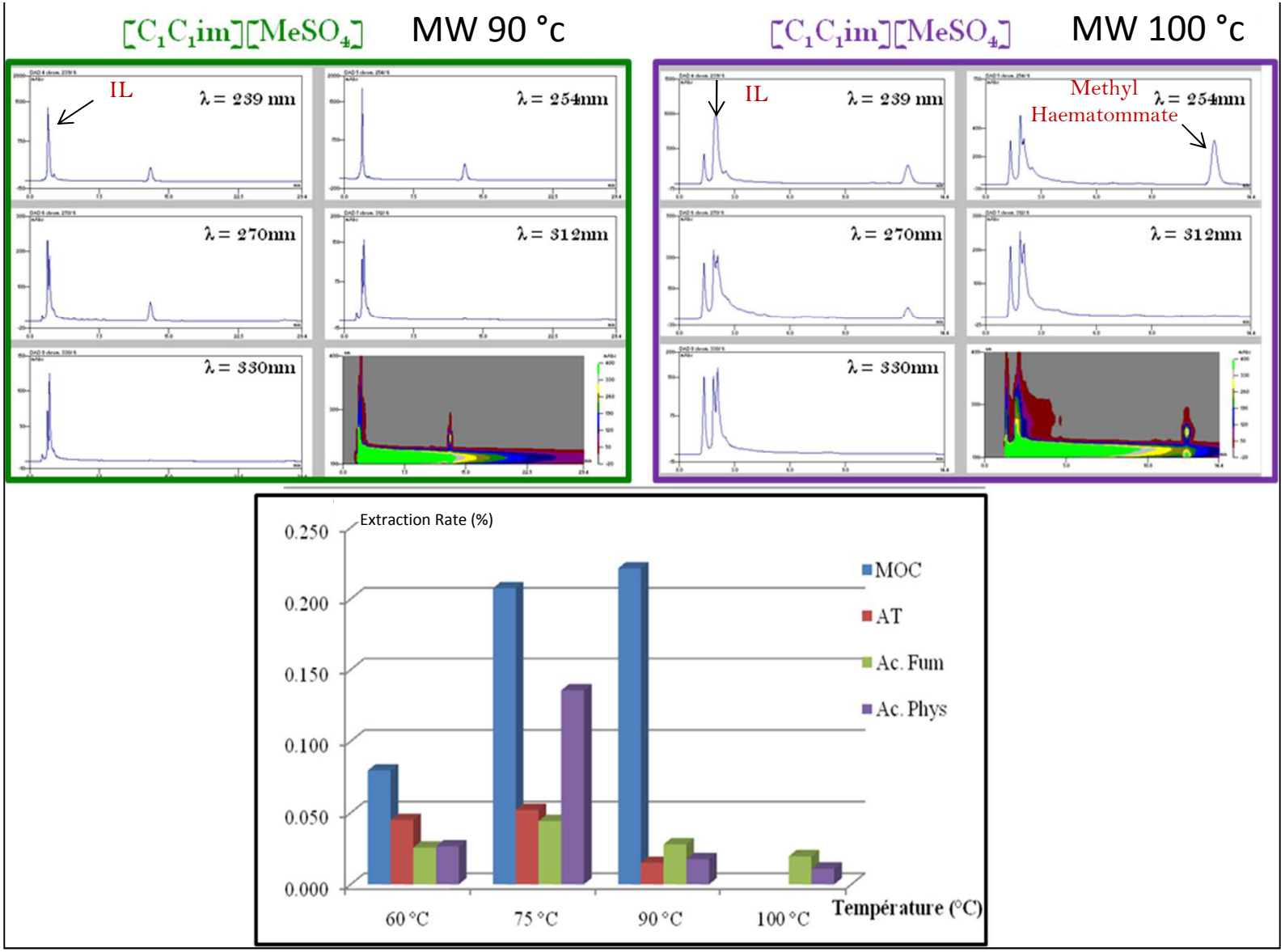
$[C_2C_1im][EtSO_4]$





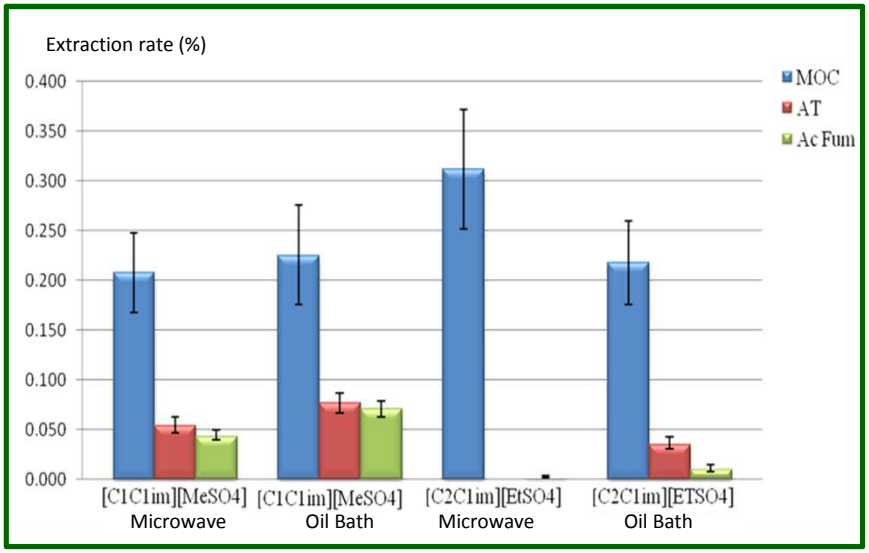
Optimal temperature: 75 °C as classical conditions

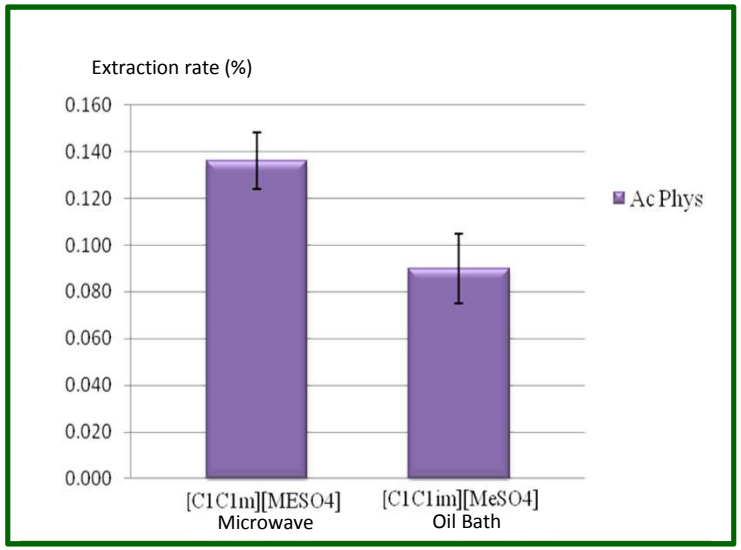
$$\text{Extraction rate} = \frac{\text{amount of metabolite}}{\text{weight of the dried lichen}} \times 100$$

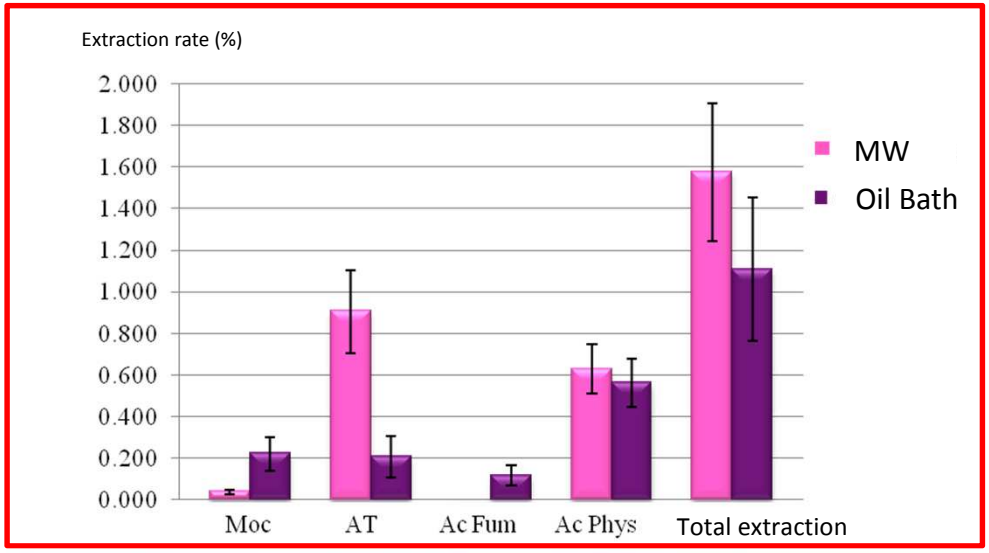


Above 90°C ⇒ metabolites degradation

Oil bath vs microwave irradiation



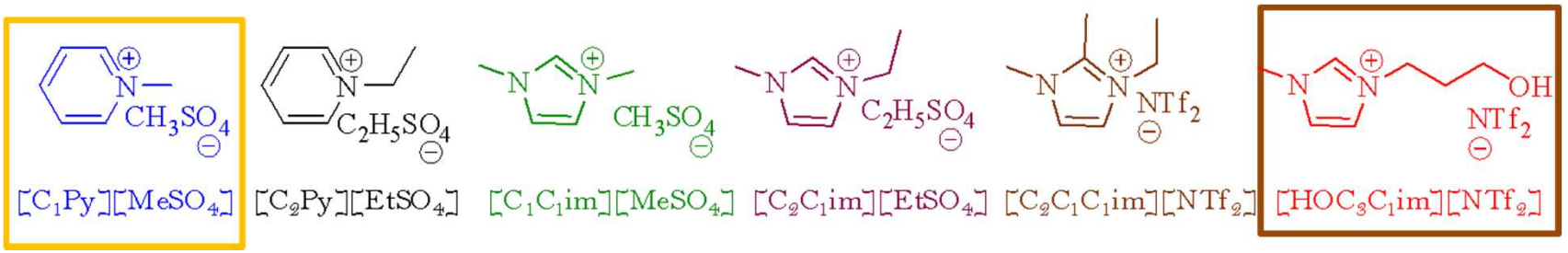


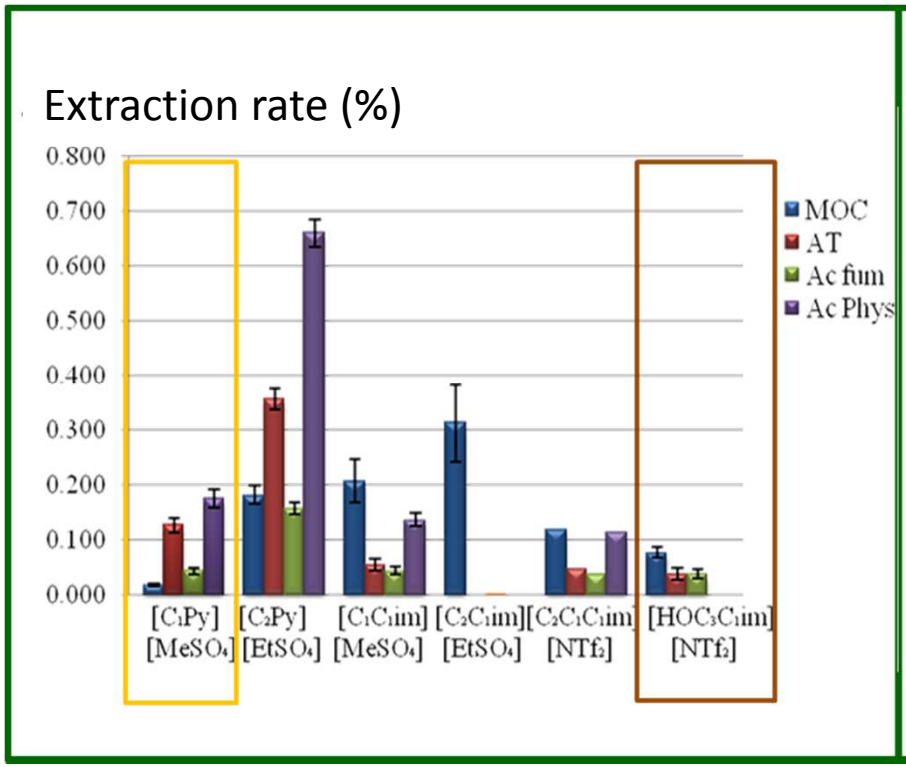


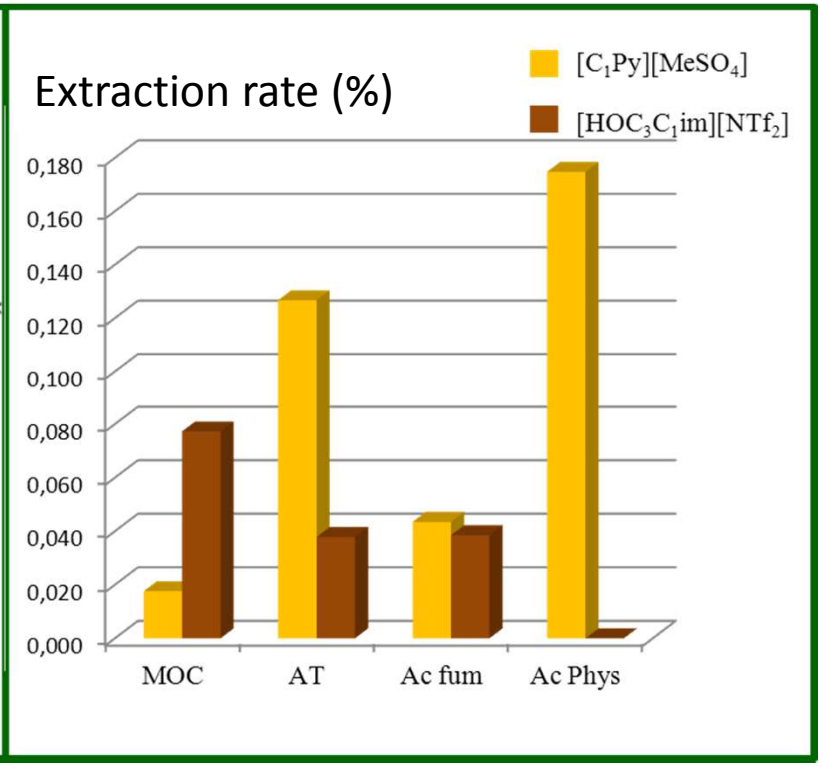
microwaves are generally more efficient

Microwave: 15 min
Oil bath: 24 h

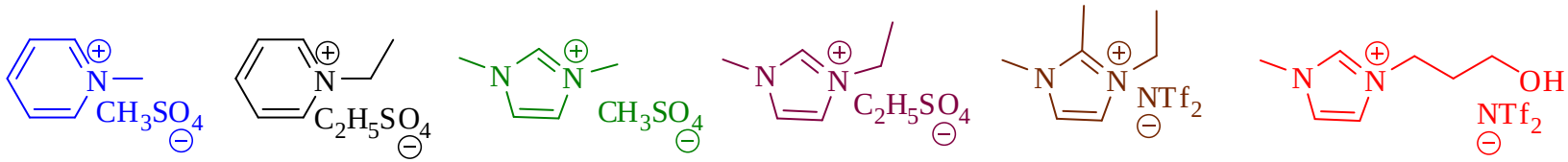
Selectivity

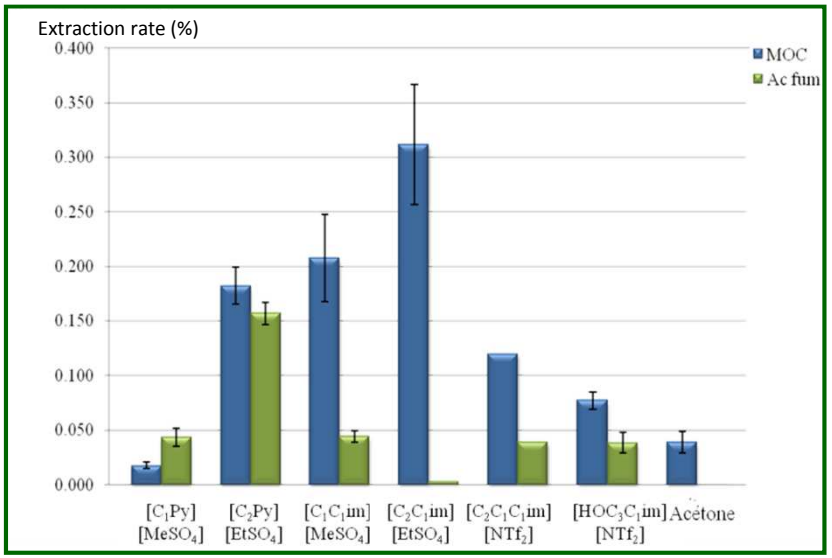


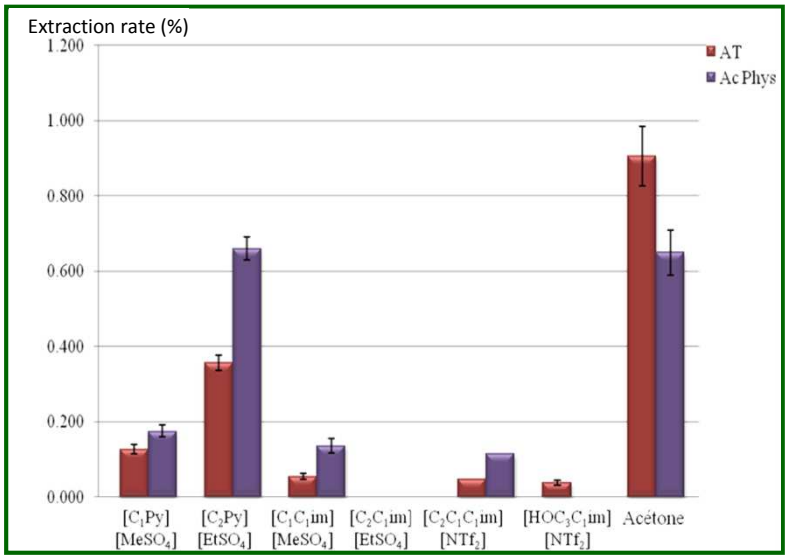




Ionic liquid vs Acetone







Risk of antranorin degradation with IL associated to Microwave extraction

Conclusions

- Efficiency:
  - 15 minutes vs 24h
  - Easily reach high temperatures
  - good and precise temperature control

- Selectivity:
  - correlation between structure and extraction

- Expanded into other applications:
  - Algae
  - Vegetals products that may be inspired by Ivory Coast Pharmacopoeia

Acknowledgments