Biobased ionic liquids as solvents of paramylon

Frédérica Feuzing, Jean-Pierre Mbakidi, Florica Lazar, Luc Marchal, Eric Leroy, Sandrine Bouquillon

Biobased Ionic liquids as Solvents of Paramylon

Frédérica Feuzing,[a,c] Jean-Pierre Mbakidi,[a] Florica Lazar,[b] Luc Marchal,[c] Eric Leroy,[c] Sandrine Bouquillon*[a]

[a]Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, Université de Reims Champagne-Ardenne, BP 1039, 51687 Reims Cedex, France

[b]Matériaux et Ingénierie Mécanique, MATIM, UFR Sciences, Université Reims Champagne Ardenne, 51687 Reims Cedex, France

[c]Université de Nantes, Oniris, CNRS, GEPEA, UMR 6144, F- 44470 Carquefou, France.

Correspondence: sandrine.bouquillon@univ-reims.fr; Phone: +33-(0)-3-26-91-89-73

Keywords

Paramylon, solubility, ionic liquids, microalgae, choline, amino acids.

Abstract

Paramylon is a linear glucan produced by *Euglena gracilis* microalgae as water insoluble intracellular granules. Its macromolecular structure contains only β(1-3) linkages, resulting in a stable triple helix conformation and a high crystallinity. It is soluble in very few solvents such as DMSO, harsh alkaline or acid solutions and imidazolium ionic liquids. In this paper we prepared biosourced more ecofriendly ionic liquids which can tolerate high amounts of water as a co-solvent and which are recyclable. The solubility of the paramylon was then evaluated depending of the nature of the ionic liquid and the structure and thermal properties of the biopolymer before and after solubilisation and regeneration were also discussed.

Introduction

Paramylon is a storage polysaccharide, produced by *Euglena Gracilis* in autotrophic, heterotrophic or mixotrophic aerobic conditions [1]. It is accumulated as highly crystalline granules dispersed in the cytosol [2]. Paramylon is a linear polymer of glucose containing only β (1-3) glycosidic linkages. The macromolecules are assembled as triple helices, further packed in hexagonal crystalline cells arranged tangentially in the granules. The crystallinity approaches 90% and granules are insoluble in water [3]. The name paramylon actually comes from the fact that they are not colored blue-violet by iodinated water, contrary to starch.[1] Due to the strong intermolecular interactions between polymeric chains in the triple helices,

paramylon is only soluble in specific solvents like other linear polyglucans such as cellulose or starch [4]. Paramylon can be dissolved in basic [5] or acidic [6] aqueous solutions and also in organic solvents as DMSO [5a,7] or N,N-Dimethylacetamide these last being most of the time associated to salts like LiCl [5b,8]. Imidazolium ionic liquids (ILs) have also been used for the shaping of PM films for biomaterials applications by a process of solubilization followed by a regeneration [9]. Indeed, at the beginning of the 2000's, Rogers and Seddon reported the dissolution of natural polymers (cellulose) by using ionic liquids (ILs) [10]. 1-butyl-3-methyl-imidazolium chloride and 1-ethyl-3-methyl-imidazolium acetate were able to dissolve up to 15-20 % weight of cellulose. Indeed, ionic liquids show adapted physical and chemical properties such as tunable hydrophilic-lipophilic balance and basic properties that play an important role in the dissolution process of natural polymers [11,12]. But the major inconvenient of these ionic liquids is their eco- and cyto-toxicity [13].

In order to enhance a greener character, ionic liquids are for many years now prepared from renewable resources as starting materials such as acids, amino acids, amino alcohols, and sugars [14].

In this paper, we will use choline, fatty alcohols and amino acids as starting materials for the preparation of BIOBILs which will be used as solvents of PM dissolution.

Experimental section

Materials

Choline chloride, methanesulfonic acid, hexanoic, octanoic, decanoic acids, sodium perchlorate, potassium hydroxide, levulinic acid, potassium L-lactate solution 60%, Sigma, Glycine (Gly), L-alanine (Ala), L-serine (Ser), L-lysine (Lys), L-histidine (His), L-aspartic acid (Asp), L-valine (Val), L-threonine (Thr), L-arginine (Arg) (purity ≥ 99 %), Choline hydroxide (aqueous solution 46 wt%, Sigma) was purchased from Sigma Aldrich. Ethanol was of analytical grade and used without any further purification.

^{1}H and ^{13}C NMR spectra were recorded at 298 K on a Bruker AVANCE Digital operating at 400 MHz. Solutions were prepared by dissolving 20-30 mg of each IL in 0.5 mL of D_2O or DMSO. IR spectra were recorded on a FTIR spectrometer (name) in the 4000–400 cm^{-1} range. The decomposition temperatures (Td) were measured with a thermal gravimetric analyzer, with a heating rate of 10 °C/min under nitrogen. The Td is calculated from the intersection of the baseline weight (after the drying step) and the tangent line derived from the

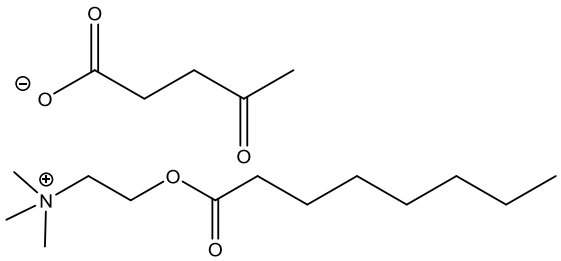
decomposition curve. For reactions under ultrasounds, an ultrasonic bath Elmasonic S15 H is used and the power is 95W.

Synthesis

Choline ester derivatives (CholC8X)

In a 500 mL two-necked flask, Choline chloride (40 g; 0.286 mol; 1 eq.) and methane sulfonic acid (55.73 mL; 0.8589 mol; 3 eq.) were added with octanoic acid (90.65 mL; 0.572 mol). The reaction medium was heated at 110 ° C under reduced pressure (50-100 mbar) for 1 hour and a half. A brown solution was thus obtained and cooled at room temperature. Water (10 ml) was added to the reaction; the resulting mixture was washed several times first with diethyl ether (200 ml) and then with ethyl acetate (50 ml) to remove the excess of carboxylic acid. In a second step, the aqueous phase recovered was introduced into a 250 ml Erlenmeyer flask. An excess of sodium perchlorate (3 eq.), previously dissolved in a minimum of water (20 mL), was then added. A white precipitate was obtained immediately and the reaction medium was left under stirring for 24 h in order to optimize the anionic metathesis between the chloride and perchlorate ions. Ethyl acetate was added to the reaction medium to dissolve the precipitate. After several washes with water to remove unreacted choline chloride and excess of methane sulfonic acid and sodium perchlorate, the organic phase was evaporated under reduced pressure. Finally, the addition of diethyl ether led to the precipitation of the perchlorate salt; after filtration, this white powder was dried under vacuum. This one was dissolved in 100 ml of ethanol. An aqueous solution of potassium levulinate or lactate (1 eq.; 10 mL water) was added and the mixture was stirred at room temperature for 24 h. After evaporation of the ethanol, the aqueous phase is washed with ethyl acetate to remove the unreacted product. The resulting aqueous phase was reduced under vacuum and CholC8Lev was obtained as a viscous liquid of yellow-brown color.

$CholC_8Lev$ (76%)



Liquid at room temperature

^{1}H NMR (500 MHz ; DMSO – d_6) δ (ppm) : 0.85 (3 H, t, J = 7.5 Hz) ; 1.24 (8 H, m) ; 1.53 (2 H, t, J = 7.5 Hz) ; 2.06 (3 H, s) ; 2.21 (2 H, t, J = 7.5 Hz) ; 2.33 (2 H, t, J = 7.5 Hz) ; 2.51 (2 H, t, J = 7.5 Hz) ; 3.16 (9 H, s) ; 3.72 (2 H, t, J = 7.5 Hz) ; 4.45 (2 H, t, J = 7.5 Hz)

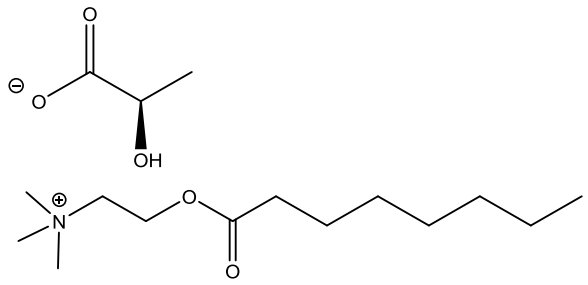
^{13}C NMR (62.5 MHz; DMSO – d_6) δ (ppm): 14.1; 22.4; 24.2; 28.7; 29.2; 29.3; 29.3; 32.4; 40.1; 53.1; 58.4; 63.1; 172.7; 176.6; 208.9

Elemental Analysis calcd.: $C_{18}H_{35}NO_5$: C 62.58; H 10.21; N 4.05%; found: C 62.23; H 9.94; N 4. 27%

IR: υ = 1746 cm^{-1}

T_d: 229°C

CholC_8Lact (80%)



Liquid at room temperature

^{1}H NMR (500 MHz ; DMSO – d_6) δ (ppm) : 0.86 (3 H, t, J=7.5 Hz); 1.07 (3 H, d, J=7.5 Hz); 1.25 (8 H, m); 1.54 (2 H, t, J=7.5 Hz); 2.34 (2 H, t, J=7.5 Hz); 3.16 (9 H, s); 3.47 (1 H, q, J=7.5 Hz); 3.71 (2 H, t, J=7.5 Hz); 4.45 (2 H, t, J=7.5 Hz) ppm

^{13}C NMR (62.5 MHz; DMSO – d_6) δ (ppm): 14.1; 18.2; 21.9; 22.5; 24.5; 29.2; 29.3; 41.2; 53.2; 53.4; 53.7; 67.0; 172.5; 177.8

Elemental Analysis calcd.: $C_{16}H_{33}NO_5$: C 60.16; H 10.41; N 4.38%; found: C 59.61; H 9.92; N 4.26%

IR: υ = 1746 cm^{-1}

T_d: 218°C

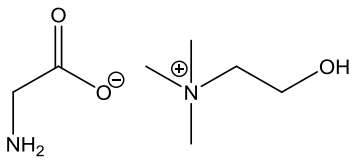
ChAAILs

The choline-based ILs were synthesized *via* an acid/base reaction between commercial Ch(OH) and amino acid (AA) in stoichiometric quantities. Water was added to ensure the complete dissolution of the AA and the reaction mixture was stirred for 24 h at room

temperature under nitrogen atmosphere. Excess of water was removed under vacuum at 50°C. The ChAAILs were then precipitated by adding absolute ethanol. After filtration to remove any unreacted amino acid, the final product was dried under vacuum for 48h at 50°C.

CholGly (95%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ/ppm: 3.04 (s, 9H, $(CH_3)_3N$), 3.07 (s, 2H, CH_2NH_2), 3.39 (m, 2H, CH_2OH), 3.92 (m, 2H, CH_2CH_2N)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 44.7, 53.8, 55.5, 67.4, 180.7

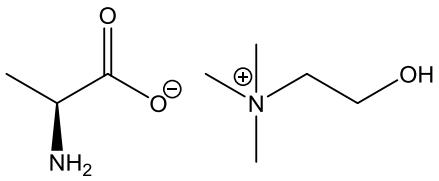
Elemental Analysis calcd.: $C_7H_{18}N_2O_3$: C 47.17; H 10.18; N 15.72%; found: C 46.91; H 9.91; N 15.38%

IR: *v*= 3194, 2920, 1567, 1479, 1391, 1087, 953 cm^{-1}

T_d: 201°C

CholAla (90%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 1.1 (d, 3H, CH_3CH), 3.07 (s, 9H, $(CH_3)_3N$), 3.19 (q, 1H, $CHNH_2$), 3.38 (m, 2H, CH_2OH), 3.92 (m, 2H, CH_2CH_2N)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 20.2, 51.4, 53.8, 55.5, 67.4, 183.6

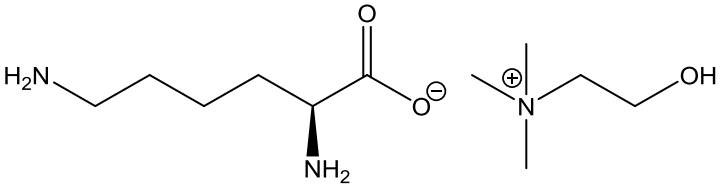
Elemental Analysis calcd.: $C_8H_{20}N_2O_3$: C 49.98; H 10.49; N 14.57%; found: C 49.57; H 9.98; N 14.38%

IR: *v*=3187, 2962, 2863, 1565, 1479, 1395, 1085, 953, 838 cm^{-1}

T_d: 212°C

CholLys (90%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 1.2 (m, 2H, CH_2), 1.36 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 2.29 (t, 2H, CH_2), 3.1 (s, 10H, $(CH_3)_3N$, CH-N), 3.54 (t, 2H, CH_2), 3.96 (m, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 22.4, 31.3, 34.8, 40.3, 53.84, 55.9, 57.3, 67.5, 183.4

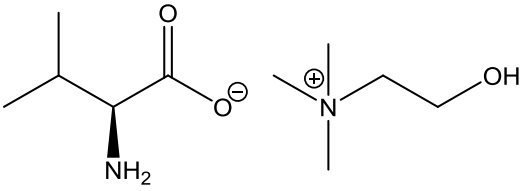
Elemental Analysis calcd.: $C_{11}H_{27}N_3O_3$: C 52.99; H 10.91; N 16.85%; found: C 53.15; H 10.68; N 17.16%

IR: *v*= 3183, 2925, 2855, 1564, 1478, 1393, 1088, 953, 865 cm^{-1}

T_d: 199°C

CholVal (88%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 0.75-0.76 (d, 3H, CH_3), 0.82-0.83 (d, 3H, CH_3), 1.81 (m, 1H, CH), 2.92-2.93 (d, 1H, CH-N), 3.09 (s, 9H, $(CH_3)_3N$), 3.41 (t, 2H, CH_2), 3.94 (m, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 16.7, 19.1, 31.7, 53.8, 55.5, 61.9, 67.4, 182.6

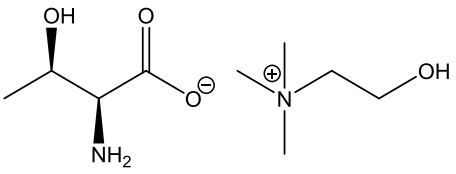
Elemental Analysis calcd.: $C_{10}H_{24}N_2O_3$: C 54.52; H 10.98; N 12.72%; found: C 54.59; H 10.91; N 12.35%

IR: *v*= 3168, 2956, 2869, 1563, 1472, 1396, 1088, 954, 867 cm^{-1}

T_d: 215°C

CholThr (92%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 1.07-1.08 (d, 3H, CH_3), 2.94-2.95 (d, 1H, CH-N), 3.07 (s, 9H, $(CH_3)_3N$), 3.39 (m, 2H, CH_2), 3.8 (m, 1H, CH_2O), 3.94 (m, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 19.3, 55.5, 61.9, 67.4, 69.5, 182.4

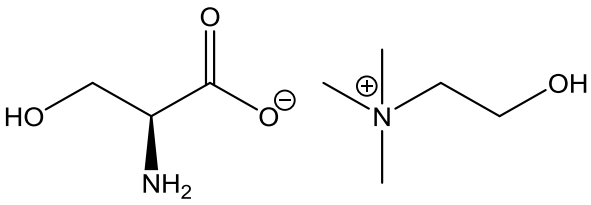
Elemental Analysis calcd.: $C_9H_{22}N_2O_4$: C 48.63; H 9.98; N 12.60%; found: C 49.12; H 10.09; N 12.15%

IR: *v*= 3216, 2967, 2926, 1557, 1478, 1394, 1085, 1060, 953 cm^{-1}

T_d: 212°C

CholSer (90%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 2.95 (s, 9H, $(CH_3)_3N$), 3.09 (t, 1H, CHN), 3.26 (t, 2H, CH_2), 3.42-3.50 (dd, 2H, CH_2), 3.78 (m, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 53.5, 55.3, 57.4, 64.0, 67.2, 178.3

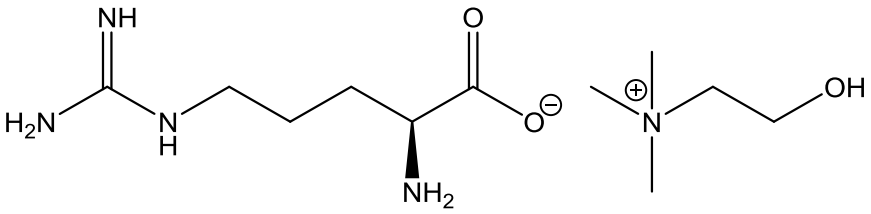
Elemental Analysis calcd.: $C_8H_{20}N_2O_4$: C 46.14; H 9.68; N 13.45%; found: C 46.38; H 10.14; N 12.95%

IR: *v*= 3195, 2856, 1557, 1478, 1398, 1335, 1084, 1044, 953 cm^{-1}

T_d: 210°C

CholArg (75%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 1.20 – 1.40 (t, 4H, CH_2CH_2), 2.85 (m, 11H, $(CH_3)_3N$, CH_2), 2.96 (d, 1H, CH_2N), 3.27 (q, 2H, CH_2), 3.80 (s, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 25.5, 27.7, 32.0, 40.4, 53.5, 55.3, 67.2, 161.1, 182.5

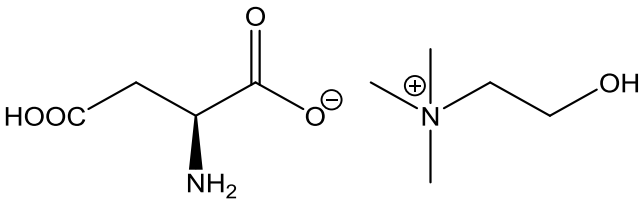
Elemental Analysis calcd.: $C_{11}H_{27}N_5O_3$: C 47.63; H 9.81; N 25.25%; found: C 47.18; H 10.12; N 24.95%

IR: *v*= 3193, 2940, 1566, 1476, 1388, 1087, 952, 549 cm^{-1}

T_d: 203°C

CholAsp (75%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ (ppm): 2.07 – 2.12 (m, 1H, CH_2), 2.42 – 2.46 (m, 1H, CH_2), 3.01 (s, 9H, $(CH_3)_3N$), 3.32 (m, 2H, CH_2), 3.45(q, 1H, CH-N), 3.85 (m, 2H, CH_2)

^{13}C NMR (500 MHz, D_2O) δ (ppm): 16.8, 42.5, 53.6, 55.4, 67.2, 179.2, 180.5

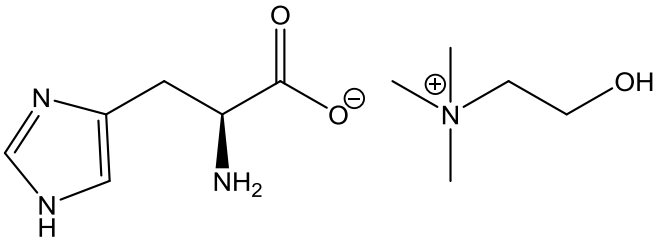
Elemental Analysis calcd.: $C_9H_{20}N_2O_5$: C 47.75; H 8.53; N 11.86%; found: C 47.42; H 8.74; N 11.98%

IR: *v*= 3195, 2962, 1566, 1478, 1375, 1087, 1053, 953, 866 cm^{-1}

T_d: 215°C

CholHis (70%)

Liquid at room temperature



^{1}H NMR (500 MHz, D_2O) δ(ppm): 2.20– 2.25 (m, 1H, CH_2), 2.40– 2.45 (m, 1H, CH_2), 2.51 (s, 9H, $(CH_3)_3N$), 2.82 (m, 3H, CH_2, CH-N), 3.36 (m, 2H, CH_2), 6.35 (s, 1H, =CH), 7.10 (s, 1H, =CH)

^{13}C NMR (500 MHz, D_2O) δ(ppm): 32.1, 54.8, 56.5, 67.1, 117.8, 133.3, 135.1, 180.8

Elemental Analysis calcd.: $C_{11}H_{22}N_4O_3$: C 51.15; H 8.58; N 21.69%; found: C 51.46; H 8.94; N 21.95%

IR: *v*= 3194, 2958, 1342, 1048, 789 cm^{-1}

T_d: 219°C

Dissolution procedure under conventional heating

In a glass pillbox equipped with a cap is introduced the ionic liquid IL (4 mL). This one is heated 5 minutes at 80°C to reduce its viscosity. 80 mg of paramylon is then added (resulting to a concentration of 2%). The mixture is stirred for 1 to 2 hours depending of the nature of the IL. After cooling at room temperature, ethanol (15 mL) is added in order to precipitate the paramylon. After centrifugation (6000 rpm, 10 min), ethanol (15 mL) is added to the residue; the mixture is stirred during 5 minutes and centrifuged; the supernatant is removed. This operation is repeated 2 times. The alcohol solutions are collected and evaporated under reduced pressure to regenerate the ionic liquid. The ionic liquid is recovered with 97 to 99% and can be reused at least 5 times without loss of activity. The white residue is dried at room temperature and lyophilized, then characterized (NMR, IR and XRD). 70 mg of paramylon are finally obtained. Yield: 87.5%.

Dissolution procedure under ultrasounds

In a glass pillbox equipped with a cap is introduced the ionic liquid IL (4 mL). This one is heated 5 minutes at 60°C under ultrasounds (95W) to reduce its viscosity. 80 mg of paramylon is then added (resulting to a concentration of 2%). The mixture is stirred for 2 hours under ultrasounds (95W). Similar procedure as described above for recovering the paramylon is then employed.

72 mg of paramylon is finally obtained. Yield: 90%

X-ray diffraction (XRD)

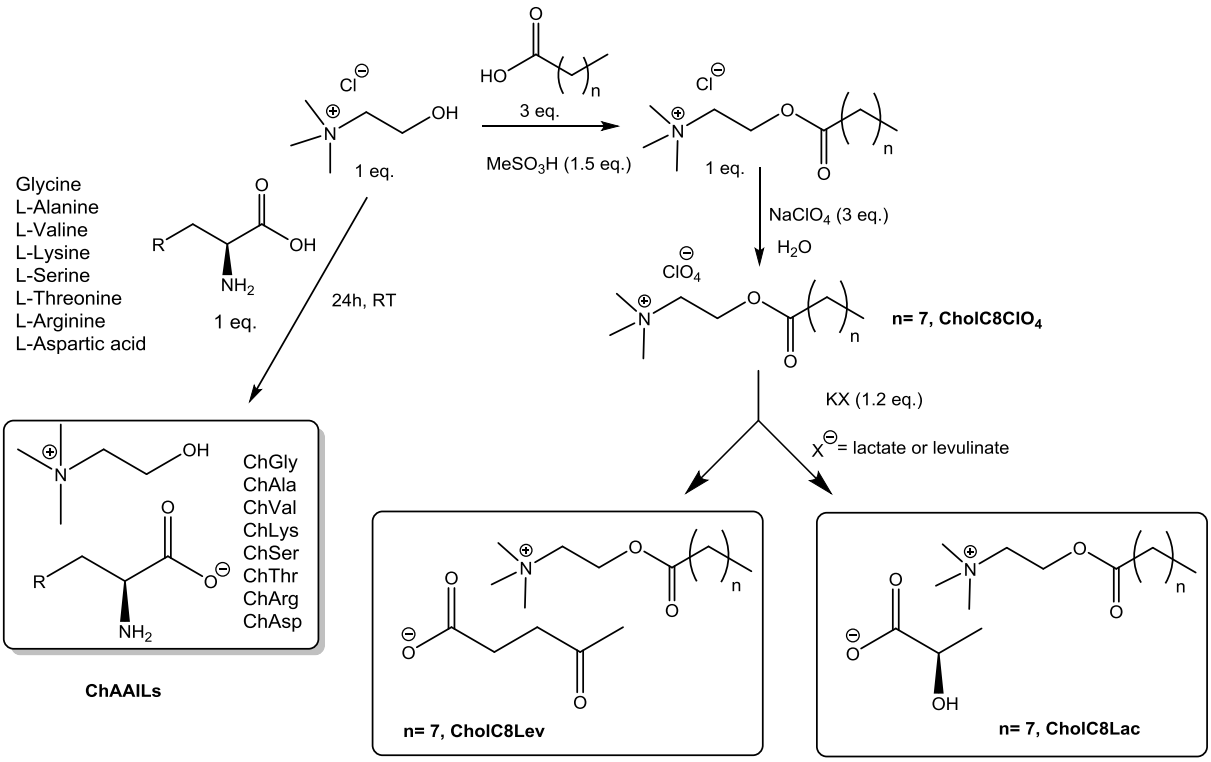
The structure of powders was characterized by X-ray diffraction, using a Bruker D8 Advance X-ray diffractometer with CuKα radiation (λ CuKα = 1.5418 Å), operating voltage of 40 kV and a current of 20 mA. The data were collected using Bragg-Brentano configuration, for 2θ ranging from 4° to 70°, with a step of 0.06° and a scan speed of 2s by step. The XRD analyses were carried out before and after each chemical treatment of the samples.

Results and discussion

ILs and their characterizations

The ILs, i.e. choline ester derivatives CholCnX (n= number of carbon on the ester chain, X= lactate and levulinate) and cholinium aminobases (ChAAILs) were prepared at first.

The choline esters derivatives were prepared in 3 steps according to an easy procedure we recently performed (Scheme 1) [15]. For the present study, we prepared the Cho-C8-Lev and Chol-C8-Lac with high yields; this IL was chosen for his good HLB value and his pH range (9-10).



Scheme 1 – Synthesis of choline ester derivatives

ChAAILs were synthesized *via* an acid-base reaction between commercial Ch(OH) and amino acid in stoichiometric amounts with yields up to 95%. Purification of these ChAAILs was realized by precipitation in absolute alcohol and their characterizations (NMR, IR and TGA) were in total accordance with the ones reported by Zong and al. [16].

All these synthesized ionic liquids are relatively thermostable with degradation temperature above 200°C (Figure S1). Then these ILs and commercial ones were employed for the

solubilization of the PM at a massic concentration of 2% at 80°C for 1 hour. The results are summarized in Table 1.

The results show that the commercial ionic liquids dissolved the paramylon except [Bmim][Cl]. Concerning the choline esters derived ILs, only the ester containing a C8 carbon chain and a levulination anion led to PM dissolution while all amino acid-based except [Chol][Hist] (more hydrophobic nature of the IL with the unsaturated nitrogen ring) were good solvent for PM. From these observations, we concluded that both viscosity and basicity of ILs could influenced their dissolution power.

Thus as described in Figure S1, we can establish a solubility window of paramylon depending of their viscosity and the pH values of the ILs solutions. Indeed, we began to observe partial solubility between 10 and 13 pH values (corresponding to CholC8Lev and commercials ionic liquids) and a complete PM solubility above a pH value of 13 corresponding to the major ChAAILs. These first results show the importance of both pH of the reaction medium and the nature of the associated anions and cations in ILs governing their viscosities.

Table 1 - PM solubilization

Solvents		Conditions	Viscosity (η/mPa s)		pH	PM Solubilisation[a]
			60°C	80°C		
Formic acid (pure)		15 min, RT	nd	nd	1	+[b]
NaOH (0.5M)		5 min, RT	nd	nd	12	+[c]
DMAc/LiCl		30 min under Argon, 110°C	nd	nd	nd	+[c]
[Bmim] [Cl]		1h, 80°C	225	nd	nd	-
[Bmim] [Ac]	neat	1h, 80°C	54	23	13.8	+[c, d]
	6 mL + H_2O 4 mL	40 min, 80°C				+ [c,d]
[Emim] [Ac]	neat	1h, 80°C	27	15	12	+[c, d]
	6 mL + H_2O					+ [c,d]

	4 mL					
[Chol] [Ac]	neat	1h, 80°C	29	16	10	+ [c,d]
	7 mL + H_2O 3 mL					+ [c,d]
[CholC6] [Lac]		1h, 80°C	42	21	8.3	-
[CholC8] Lac]		1h, 80°C	68	28	9.5	-
[CholC10] [Lac]		1h, 80°C	nd	nd	nd	-
[CholC6] [Lev]		1h, 80°C	53	30	9.7	-
[CholC8] [Lev]	neat	1h, 80°C	73	34	10.3	+ [c,d]
	8 mL + H_2O 2 mL					+ [c,d]
[CholC10] [Lev]		1h, 80°C	nd	nd	nd	-
[Chol] [His]		1h, 80°C	296	nd	13.8	-
[Chol] [Arg]	neat	1h, 80°C	239	84	> 15	+ [c,d]
	9 mL + H_2O 1 mL	1h30, 80°C				+ [c,d]
[Chol] [Val]	neat	1h, 80°C	76	36	14.2	+ [c,d]
	5 mL + H_2O 52 mL					+ [c,d]
[Chol] [Lys]	neat	1h, 80°C	111	47	13.6	+ [c,d]
	7 mL + H_2O 3 mL					+ [c,d]
[Chol] [Ala]	neat	1h, 80°C	56	35	14	+ [c,d]
	4 mL + H_2O 6 mL	45 min, 80°C				+ [c,d]
[Chol] [Gly]	neat	1h, 80°C	54	29	13.2	+ [c,d]
	4 mL	45 min, 80°C				+ [c,d]

	+ H_2O 6 mL					

[a] -: no dissolution, +: dissolution, [b]: chemical modification (^{13}C NMR), [c]: no chemical modification (^{13}C NMR), [d]: XRD modification

Next, to improve our choice of ILs for dissolution, we took into account the viscosity of these solvents because their use is often hindered by their excessive viscosity. So, we characterized the temperature dependence of the viscosities of the different ionic liquids and compared them to commercial ionic liquids commonly used. This relationship between the viscosity and the temperature follows the Arrhenius rule (Viscocity = K x exp (-Ea/RT)) because the ln(viscosity) is inversely proportional to the temperature (Figure 1) in a range of temperature between 60 and 80°C (Table S1).

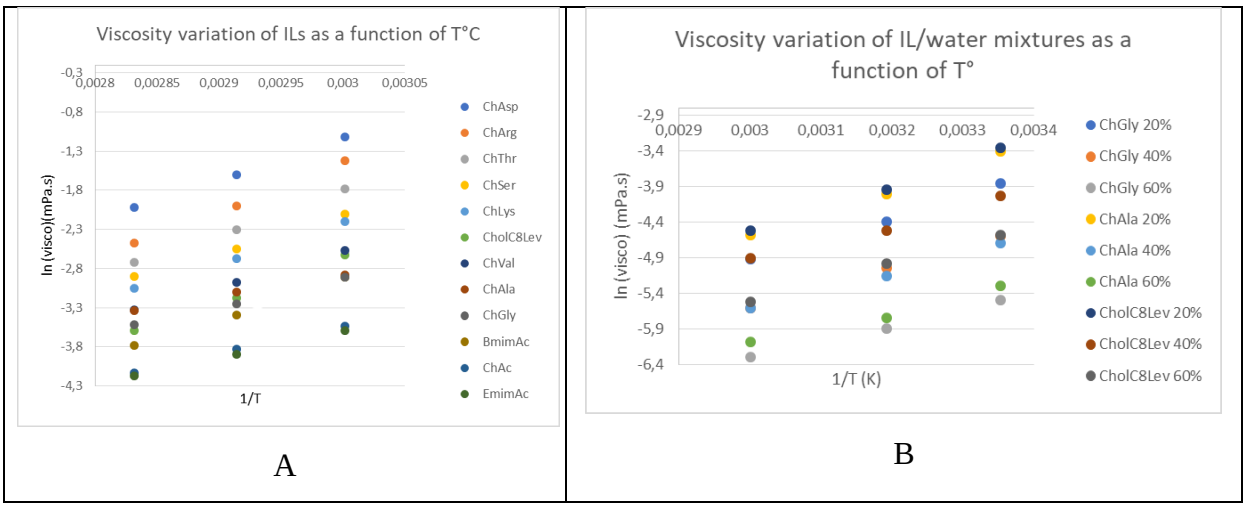


Figure 1 - A -Variation of ln(viscosity) of for pure ILs towards 1/T accordingly to the Arrhenius's equation. B - Variation of ln(viscosity) of mixtures ILs/water towards 1/T accordingly to the Arrhenius's equation

Amoung commercial and "classical" (EmimAc, BmimAc or CholAc) ionic liquids, BmimAc being the most viscous considering the butyl chain on the imidazolium chain; their viscosities are reported in Table 1 to be compared to CholC8Lev and ChAAILs ones. Concerning the ChAAILs, the viscosity is depending on the carbon chain lengths and also on the presence of function like OH, COOH or NH-C(NH)-NH what H bonding in the medium. Indeed, the most viscous is the CholAsp followed by CholArg and CholSer. In general as attempted, the viscosity decreased with the temperature and more drastically for high viscosities even in a small temperature range (60-80°C). CholC8Lev is more viscous than EmimAc, BmimAc,

CholAc, CholAla and CholGly but his viscosity remains relatively low considering the number of carbon atoms on the ester chain. These results followed some previously described observations concerning the viscosities at various temperatures and their relationships towards the nature of cations and anions [17].

Furthermore, considering the values obtained at 60, 70 and 80°C, we used the Arrhenius's equation (Viscocity = K x exp (-E/RT)) to determine the activation energy and so to predict the evolution of the viscosity at lower and higher temperatures. The predicted viscosities have been determined at 25°C and 100°C (Table S2) for all ILs and revealed that EmimAc, ChAc, BmimAc and CholC8Lev or CholGly, CholAla, CholVal with respectively 10 or 20 as predicted viscosities, could be used for an industrial development of the PM extraction from microalgae.

As ionic liquids are required to solubilize paramylon from microalgae in a reacting mixture containing often large proportions of water, we measured viscosities of ionic liquids with various percentages of water in order to understand the role of water. 3 ILs were used, CholGly, CholAla and CholC8Lev and 3 water percentages, 20, 40 and 60%. As attempted, the viscosities decreased with increasing water amounts and temperatures (Figure 1 and Table S3) and the predicted viscosities of these mixtures range from 1 to 4 (η/mPa s) (Table S4).

To determine the highest quantity of water necessary to dissolve the paramylon but which could be also tolerated by the different ionic liquids, water content was increased until we obtained a turbid solution. Moderate to high water content can be tolerated by ionic liquids especially for ChAAILs which could tolerate a large amount of water (≥ 50 %) without reducing the dissolution of the PM.

After identifying the most favorable ILs or water/IL mixtures, it was important to show if the nature of the PM could be affected by the dissolution step. Thus, the dissolution of PM in various solvents (acid, basic and commercial ones and commercial and synthetic ILs) was realized during 5 to 120 min at room temperature or at 80°C. After the dissolution, the PM was regenerated by adding EtOH and characterized first of all by ^{13}C NMR. Indeed, we compared the ^{13}C NMR spectra of the native and regenerated PMs. On Figure 2, the superposition of 3 NMR spectra clearly shows that the use of CholC8Lev did not modify chemically the structure of the PM which remains identical to that of the commercial one; in contrast, the dissolution of PM in the formic acid led to the functionalization of the PM which was proved by the detection of a new peak in ^{13}C NMR at 160 ppm relative to a C=O bond. This information have been also confirmed by the infrared spectroscopy, on which we can

identify the characteristic absorption band of the aldehyde for the regenerated paramylon dissolved in formic acid while there was no change with the paramylon dissolved in CholC8Lev (Figure S2).

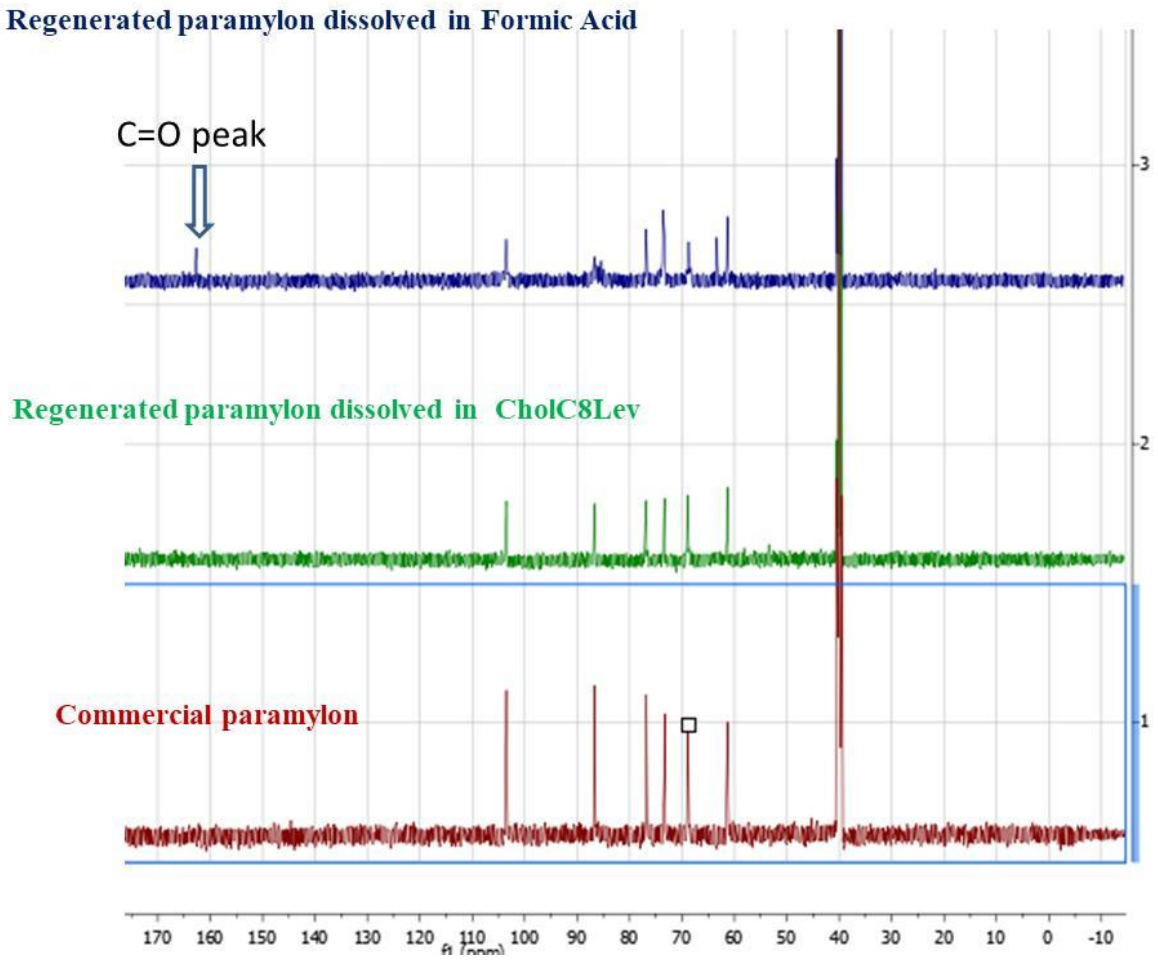


Figure 2 - ^{13}C NMR of PM and regenerated PMs

No chemical modification was also observed with all ILs mentioned in this study (Table 1).

While NMR analysis has shown that the polymer remains chemically unchanged after the dissolution in all ILs and BIOBILs, the XRD highlighted however a modification of the polymer structure (Figure 3).

The diffractograms of pristine paramylon present 3 main peaks [18,19]: i) a first and strongest peak at $2\theta \approx 7°$ which corresponds to the [100] planes of the hexagonal crystalline cell, in which paramylon triple helices are packed along the vertical axis; ii) a second strongest peak at $2\theta \approx 19°$ which can be ascribed to the [111] planes; and iii) a third peak at $2\theta \approx 20.7°$ which can be ascribed to [201] planes.

As can be seen on **figures 3a and 3b (zoom of the right side on Figures 3a BIS and 3b BIS in SI)**, the [100] diffraction peak at 7° vanishes for all the regenerated paramylon samples, indicating that no hexagonal lateral packing of triple helices is present after solubilization in ILs. For the paramylon samples solubilized in amino acid based ILs, a new broad diffusive peak appears around $2\theta \approx 6.2°$, corresponding to a characteristic distance of 1.42 nm. It may be ascribed to the presence of individual triple helices in regenerated paramylon. It is noteworthy that for these ILs, the two other characteristic peaks ([111] and [201]) of pristine paramylon also disappear, while a new peak appears at $2\theta \approx 18.5°$ (**Figure 3b**). The occurrence of such a peak was recently reported for paramylon samples hot-pressed above 230°C, and ascribed to the melting and recrystallization of paramylon in an unknown crystalline structure, different from the native hexagonal packing [6]. Regarding the other regenerated paramylon samples (**Figure 3a**), this new peak at 18.5° is also present after solubilization in [Emim][Ace] and [Chol][Ace], while the native [111] peak remains at 19° for [CholC8][Lev] and [Bmim][Ace]. Concurrently, the [201] diffraction peak around 20.7° vanishes for these later, while a broad diffusion peak appears for [Emim][Ace] and [Chol][Ace].

In all cases, the differences between the diffractograms before and after solubilization in ILs suggest a complete disruption of the crystalline structure by the solvents, with a recrystallization with a different crystalline packing after regeneration, and/or the presence of individual triple helices.

Summing up, XRD results showed that after their dissolution in ionic liquids at 80°C, the paramylon samples recrystallize upon regeneration but differently depending on the nature of the ionic liquid. All samples showed partial recrystallization, different from native paramylon but we have not clear correlation with pH values discriminating partial or total solubilization.

Thermal stability of regenerated paramylon samples was also performed by TGA measurements. The pristine paramylon presents a degradation temperature between 290°C and 320°C (Figure 4a). For regenerated paramylon dissolved in ionic liquids, no change was observed with [CholAc] and [CholArg], while lower thermal stability was noticed when imidazolium ILs were used (Figure 4b). Two thermal degradation steps could be noted, one between 250 °C and 290 °C corresponding to the degradation of the mono helices and another one between 290°C and 320°C corresponding to the degradation of the triple helices as described for native curdlan by Okuyama and al. [20].

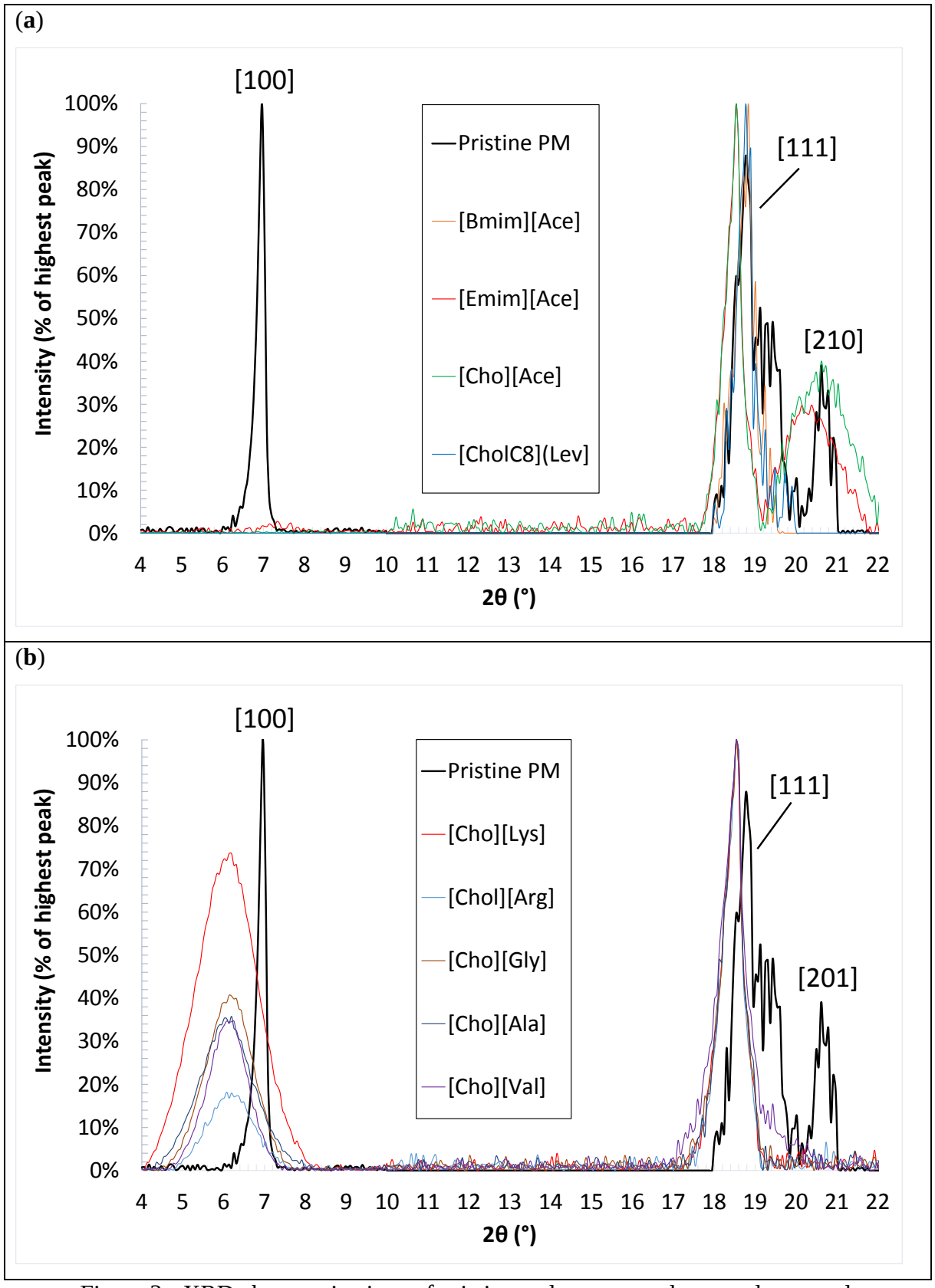


Figure 3 - XRD characterizations of pristine and regenerated paramylon samples.

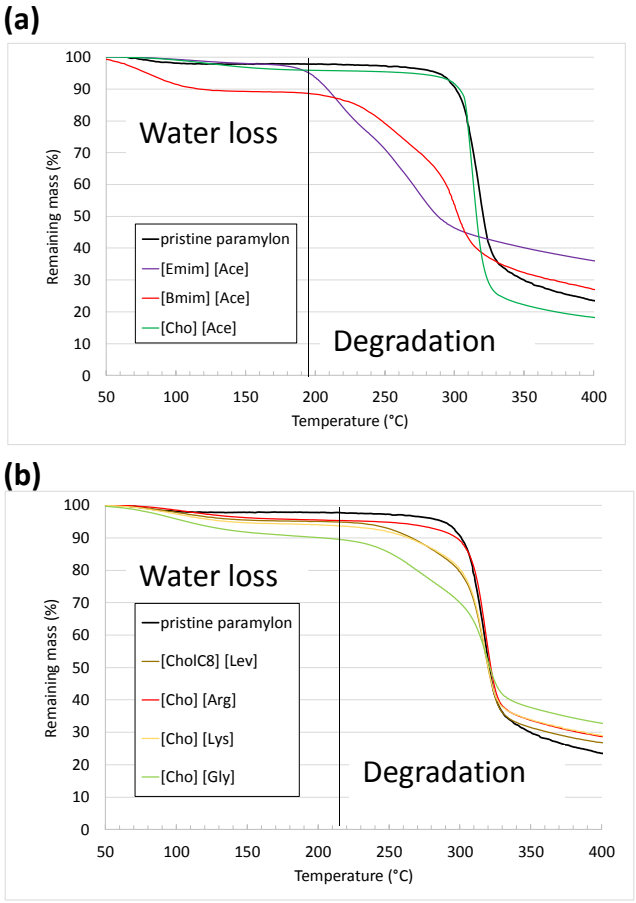


Figure 4 – Thermal stability of pristine paramylon and regenerated paramylon samples.

As the final aim of this study is to extract paramylon from microalgae, we wanted to determine the maximum percentage of water that might not be harmful for the solubilization of this biopolymer. We used imidazolium and cholinium based ILs and optimized the conditions and the highest IL/ratio which could lead to the best dissolution of PM at 80°C. The optimal conditions are summarized in Table 1.

With commercial ILs (BmimAc and EmimAc), at 80°C during respectively 60 or 40 minutes, the solubilization of PM could occur until a ratio IL/ water equal to 6/4. When the amount of water is more important, a precipitate of PM was observed. Concerning the use of CholC8Lev, a lower quantity of water was tolerated (ratio IL/ water 8/2), probably due to the higher hydrophobic character of the IL containing a C8 carbon chain.

The behavior of PM in ChAAILs in the presence of water is really dependent on the nature of the amino carboxylates. Indeed, a higher proportion of water was tolerated with CholGly and CholAla (4/6) and an equal proportion (5/5) of water and CholVal which contains an

additional methyl group. When we considered the solubility of PM in CholAc/water and CholGly or CholAla/water mixtures, we proved the benefit of the NH_2 group on the solubility in IL/water mixtures. Finally, concerning the CholArg, the presence of the guanidine group required a lower water amount. . So moderate to very high water content can be tolerated for the dissolution of paramylon in BIOBILS, especially ChAAILs which could be used with a large amount of water (≥ 50 %) without loss of PM dissolution power.

Finally, in order to minimize the heating temperature as much as possible, activation by ultrasounds (95W during 2 hours) was used, thus allowing the temperature to be reduced from 80 to 60°C. Similar dissolutions were observed with imidazolium ILs, CholAc and CholC8Lev as well as ChAAILs. No chemical modification was observed through NMR and same observations in DRX have been realized. Indeed, the technique using ultrasounds seems to be a very good method for solubilizing the paramylon because firstly, the temperature can be lowered and secondly, no any chemical modification of the paramylon structure occurred.

The last step of our study was to enhance the major property of the ILs, namely their recycling. The recycling procedure is described on Figure 5 and was applied using conventional heating or US activation with all ILs previously mentioned.

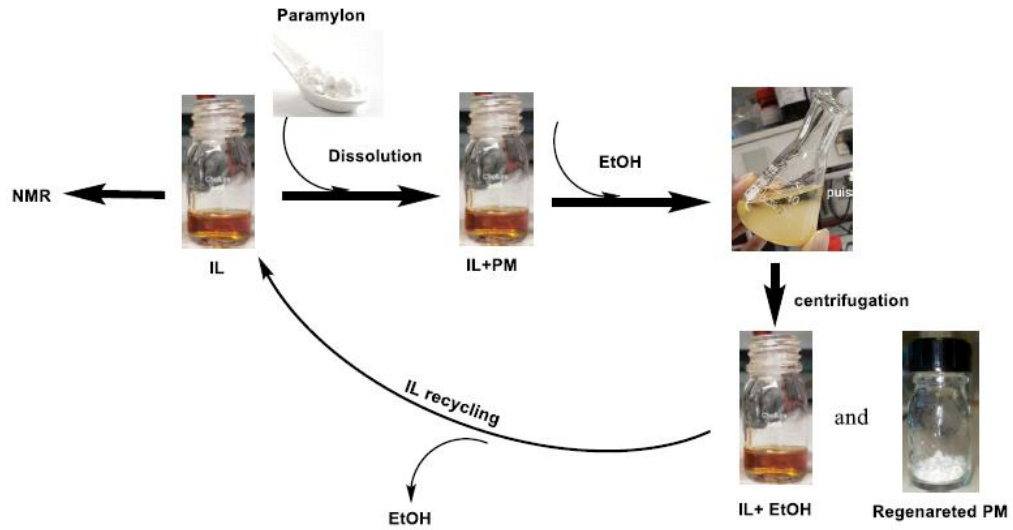


Figure 5 – IL recycling procedure after PM dissolution

After each centrifugation, the supernatant alcohol solutions were collected and evaporated under reduced pressure to regenerate the ionic liquid whose unchanged structure was

confirmed by ^{1}H NMR (Figure S3). The ionic liquid was recovered with 97 to 99% yield and can be reused at least 4 times without loss of activity.

Conclusions

In this paper we described the solubilization of PM in choline based ionic liquids under conventional heating or by using ultrasounds activation. A concentration of 2% in PM was used and the solubilization could occur in various water amounts depending of the nature of the ILs. The recycling of the ionic solvents has been also proved including the non-degradation of the solvents and of the PM after each step by NMR and XRD analyses. The next step is the PM solubilization from microalgae biomass. These tests are currently in progress.

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