Electronic Supporting Information (ESI)

Monitoring lignin partition in aqueous/alcohol biphasic systems according to pH: influences of the molecular structure and solvent characteristics

Quentin Schmetz [a], Claire Muzyka [b], Thomas Berchem [a] and Aurore Richel [a]

a. University of Liège – Gembloux Agro-Bio Tech, Laboratory of Biomass & Green Technologies, Passage des Déportés, 2, B-5030 Gembloux, Belgium

b. University of Liège – Gembloux Agro-Bio Tech, Passage des Déportés, 2, B-5030 Gembloux, Belgium

Table S1 Lignin partition coefficients of biphasic system according to alcohol type and pH of the aqueous phase

Lignin	Alcohol	pH 12	pH 9.5	pH 7	pH 3.5
Indulin AT	**1-butanol**	-1.238 ± 0.275	-0.019 ± 0.044	0.250 ± 0.060	1.337 ± 0.073
	1-pentanol	-1.482 ± 0.003	-0.275 ± 0.023	0.156 ± 0.028	1.027 ± 0.007
	1-octanol	-2.204 ± 0.022	-1.071 ± 0.008	-0.573 ± 0.012	0.566 ± 0.033
P1000	**1-butanol**	-1.489 ± 0.051	-0.586 ± 0.031	-0.352 0.001	0.947 ± 0.041
	1-pentanol	-1.722 ± 0.069	-0.817 ± 0.026	-0.456 0.069	0.850 ± 0.075
	1-octanol	-1.916 ± 0.145	-1.144 ± 0.035	-0.818 0.090	0.062 ± 0.042
R5000	**1-butanol**	-1.922 ± 0.012	-0.808 ± 0.010	-0.488 ± 0.126	0.600 ± 0.009
	1-pentanol	-1.903 ± 0.02	-1.019 ± 0.029	-0.715 ± 0.005	0.393 ± 0.040
	1-octanol	-2.137 ± 0.05	-1.317 ± 0.001	-1.090 ± 0.017	0.004 ± 0.015

Table S2 FTIR band assignments of Indulin AT, P1000 and R5000

Wavelength (cm^{-1}) - Maximal absorption			**Assignements** (Casas *et al.*, 2012; Faix, 1992; Li and Mcdonald, 2014)
Indulin AT	**P1000**	**R5000**	
3400-3346	3410-3394	3410-3369	OH stretching
2931-2923	2929-2919	2931-2925	CH asymmetric stretching
2869-2835	2869-2840	2871-2854	C-H symmetric stretching
1737-1702	1737-1702	1737-1702	C=O stretching (carboxylic acid unconjugated)
1643-1635	1637-1629	1639-1635	C=O stretching in conjugated
1598-1589	1600-1594	1600-1596	Aromatic skeletal vibrations plus C=O stretch (S>G)
1512-1508	1513-1512	1517-1513	Aromatic skeletal vibrations
1463-1458	1463-1456	1459-1453	Asymmetric bending deformation of methyl and methylene groups
1427-1423	1425	1427-1421	C-H in plane deformation with aromatic ring stretching
-	1330-1324	1324-1321	S unit breathing with C-O stretching and condensed G rings
1267-1263	1270-1267	1269-1267	C-O of guaiacyl ring
1216-1213	1216-1209	1216-1209	C-C plus C-O stretch
1130-1122	-	-	Ether -O-
-	1120-1116	1116-1110	Aromatic C-H deformation in syringyl units
1090-1080	-	-	C-O deformation in secondary alcohols and aliphatic esters
1033-1027	1033-1029	1035-1031	Aromatic C-H in plane deformation (G>S) plus C-O deformation in primary alcohols
858-852	-	-	C-H out of plane in position 2,5 and 6 of G unit
-	833-829	827	C-H out of plane in position 2 and 6 of S and all positions of H unit
815-813	-	-	C–H out-of-plane in positions 2, 5 and 6 of G units

Table S3 ATR-FTIR ratio of C=O unconjugated signal

Solvent	pH	Indulin AT (Softwood)	P1000 (Herbaceous)	R5000 (Hardwood)
		[a] 1724 cm^{-1}	[a] 1720 cm^{-1}	[a] 1716 cm^{-1}
Butanol	9.5	0,14	0,10	0,25
	7	0,14	0,61	0,43
	3.5	0,40	0,87	0,76
	2.5	1,28	1,60	3,69
		[a] 1726 cm^{-1}	[a] 1722 cm^{-1}	[a] 1722 cm^{-1}
Pentanol	9.5	0,12	0,17	0,22
	7	0,60	0,81	0,75
	3.5	0,74	1,06	1,19
	2.5	1,22	1,27	2,28

Ratio = absorbance of the signal of the carbonyl region/absorbance of the signal attributed to ring vibrations (1512 cm^{-1}) (Faix, 1992)
[a] wavenumber of the maximal absorbance of the signal in the unconjugated region for fraction at pH 2.5

Table S4 ATR-FTIR ratio of the aromatic region (1215 cm^{-1})

Solvent	pH	Indulin AT (Softwood)	P1000 (Herbaceous)	R5000 (Hardwood)
Butanol	9.5	0,90	1,22	1,32
	7	1,17	0,91	1,07
	3.5	1,10	1,36	1,30
	2.5	2,18	1,94	3,73
Pentanol	9.5	0,96	1,13	0,53
	7	1,10	1,24	1,25
	3.5	1,27	1,30	1,99
	2.5	1,57	1,43	3,39

Ratio = absorbance of the signal of the C-C and C-O (1215 cm^{-1})/absorbance of the signal attributed to ring vibrations (1512 cm^{-1}) (Faix, 1992)

Table S5 2D-NMR HSQC assignments of Indulin AT, P1000 and R5000

Label	**δ_C/δ_H**	**Assignement** (Yuan *et al.*, 2011; Heikkinen *et al.*, 2014; Del Río *et al.*, 2012; Constant *et al.*, 2016; Hu *et al.*, 2016b; Crestini *et al.*, 2017)
B_β	53.4/3.05	C_β–H_β in phenylcoumaran substructures
C_β	53.6/3.4	C_β–H_β in β–β′ resinol substructures
-OCH_3	55.6/3.7	C-H in methoxyls
A_γ	59,8/3.7	C_γ–H_γ in γ-hydroxylated β-O-4′ substructures
I_γ	60.12-61.3/4.07-4.1	C_γ–H_γ in cinnamylalcoholend-groups
B_γ	62.7/3.7	C_γ–H_γ in phenylcoumaran substructures
$A_{\alpha(G)}$	70.9/4.7	C_α–H_α in β-O-4′ substructures linked to a G-unit
C_γ	70.9/4.1	C_γ–H_γ in β–β′ resinol substructures
$A_{\alpha(S)}$	71.6/4.8	C_α–H_α in β-O-4′ substructures linked to a S-unit
X2	73/3.1	C_2–H_2 in xylan substructure
X3	74/3.3	C_3–H_3 in xylan substructure
X4	75.7/3.5	C_4–H_4 in xylan substructure
E_α	79.1/5.54	C_α–H_α in α-O-4′ substructures
F_α	81.2/5.0	C_α–H_α in spirodienone substructure
$A_{\beta(G)}$	83.8/4.26	C_β–H_β in β-O-4′ substructures linked to a G unit
C_α	84.6/4.62	C_α–H_α in β–β′ resinol substructures
$A_{\beta\,(S)}$	85.9/4.1	C_β–H_β in β-O-4′ substructures linked to a S unit
B_α	85.9-87.7/5.42-5.5	C_α–H_α in phenylcoumaran substructures
$S_{2,6}$	103.7/6.67	C_2–H_2 and C_6–H_6 in etherified syringyl units
$S'_{2,6}$	106.3/7.3	C_2–H_2 and C_6–H_6 in α-oxidised syringyl units
G_2	110.9/6.96	C_2–H_2 in guaiacyl units
Fer_2	111.0/7.32	C_2-H_2 in ferulate
PCA_β/FA_β	113.6/6.25	C_β–H_β in *p*-coumarate and ferulate
G_5	114.4/6.7	C_5–H_5 in guaiacyl units
$PCA_{3,5}$	115.5/6.77	C_3–H_3 and C_5-H_5 in *p*-coumarate
G_6	118.8/6.75	C_6–H_6 in guaiacyl units
FA_6	123.1/7.1	C_6–H_6 in ferulate
$H_{2,6}$	127.7/7.1-7.2	$C_{2,6}$–$H_{2,6}$ in *p*-hydroxyphenyl units
$PCA_{2,6}$	130.0/7.44	C_2–H_2 and C_6–H_6 in *p*-coumarate
PCA_α/FA_α	144.9/7.5	C_α–H_α in *p*-coumarate and ferulate
ArCHO	191.1/9.78	C_α–H_α in cinnamyl aldehyde end-groups

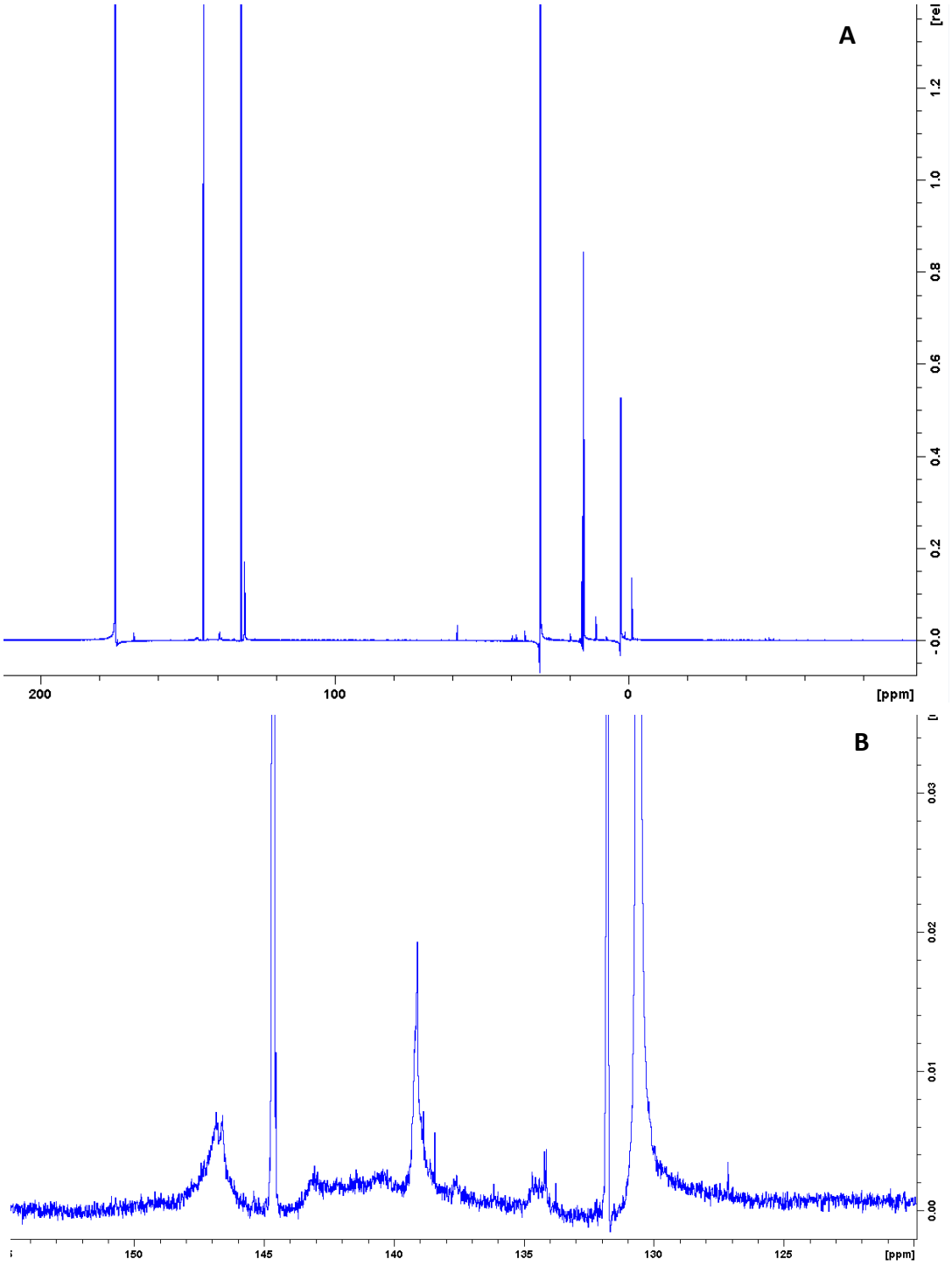

Fig. S1 ^{31}P spectrum of Indulin AT (**A**), magnification on the region of interest for quantification from 125-150 ppm (**B**).

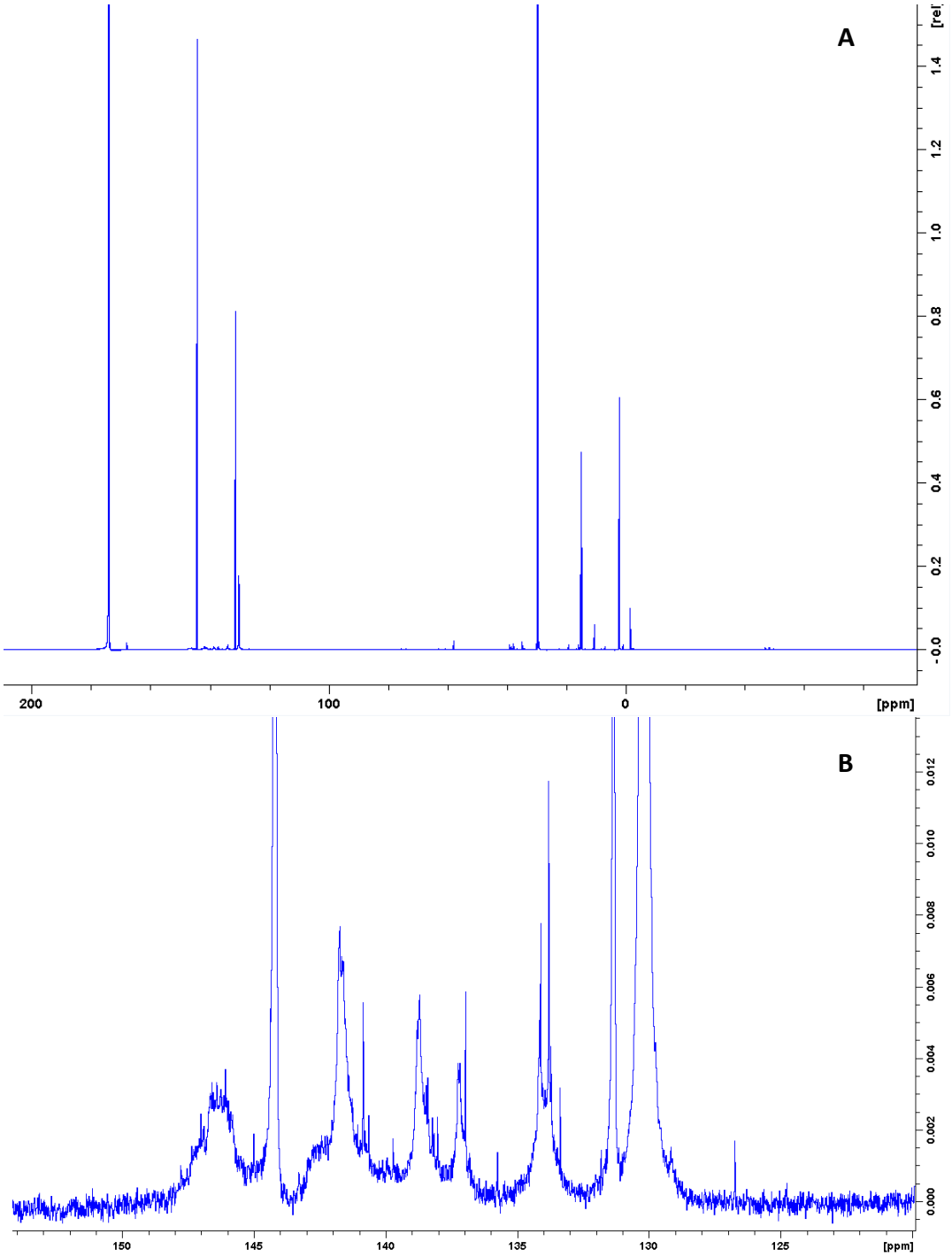

Fig. S2 ^{31}P spectrum of P1000 (**A**), magnification on the region of interest for quantification from 125-150 ppm (**B**).

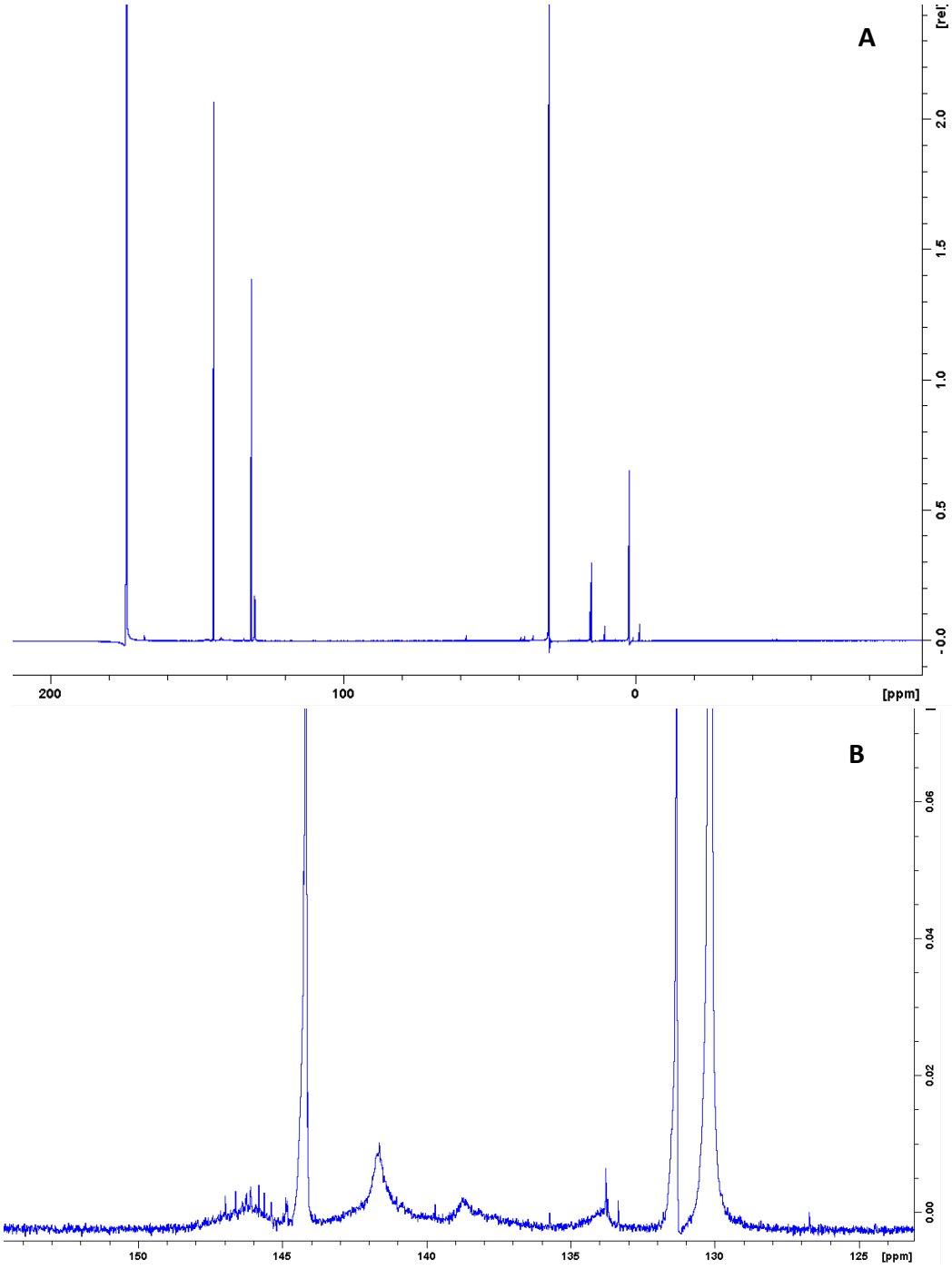

Fig. S3 ^{31}P spectrum of R5000 (**A**), magnification on the region of interest for quantification from 125-150 ppm (**B**).

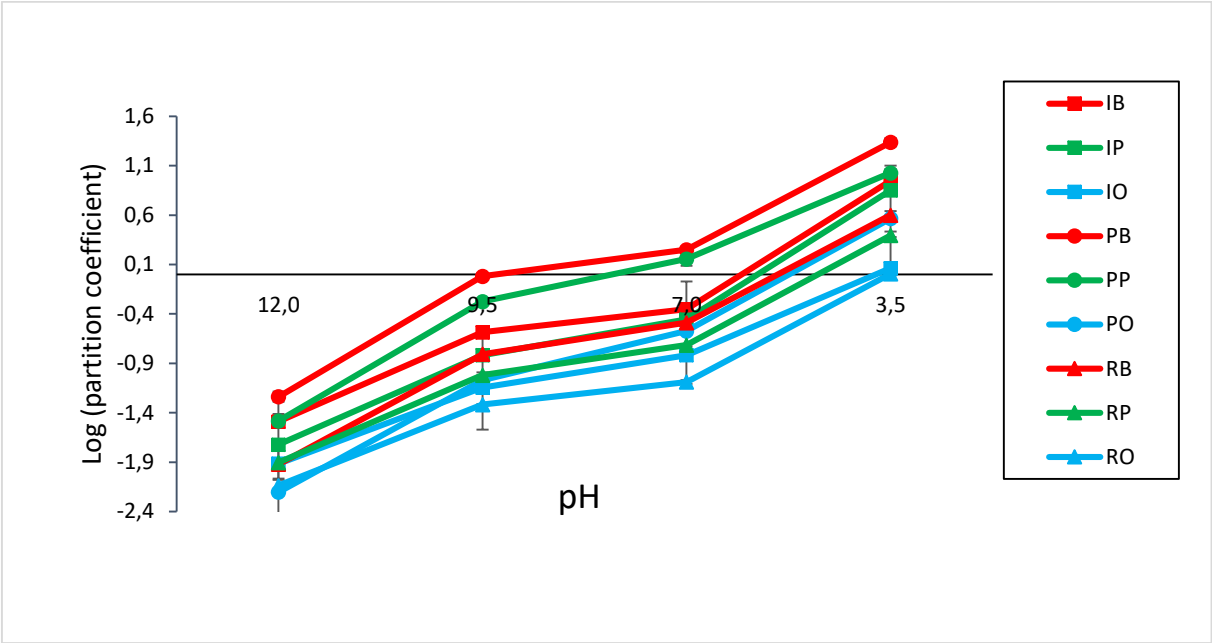

Fig. S4 Partition coefficient evolution of (square) Indulin AT (**I**), (circle) P1000 (**P**) and (triangle) R5000 (**R**) in biphasic system according to pH variation of the aqueous phase and the organic phase (red) butanol (**B**), (green) pentanol (**P**) and (blue) octanol (**O**).

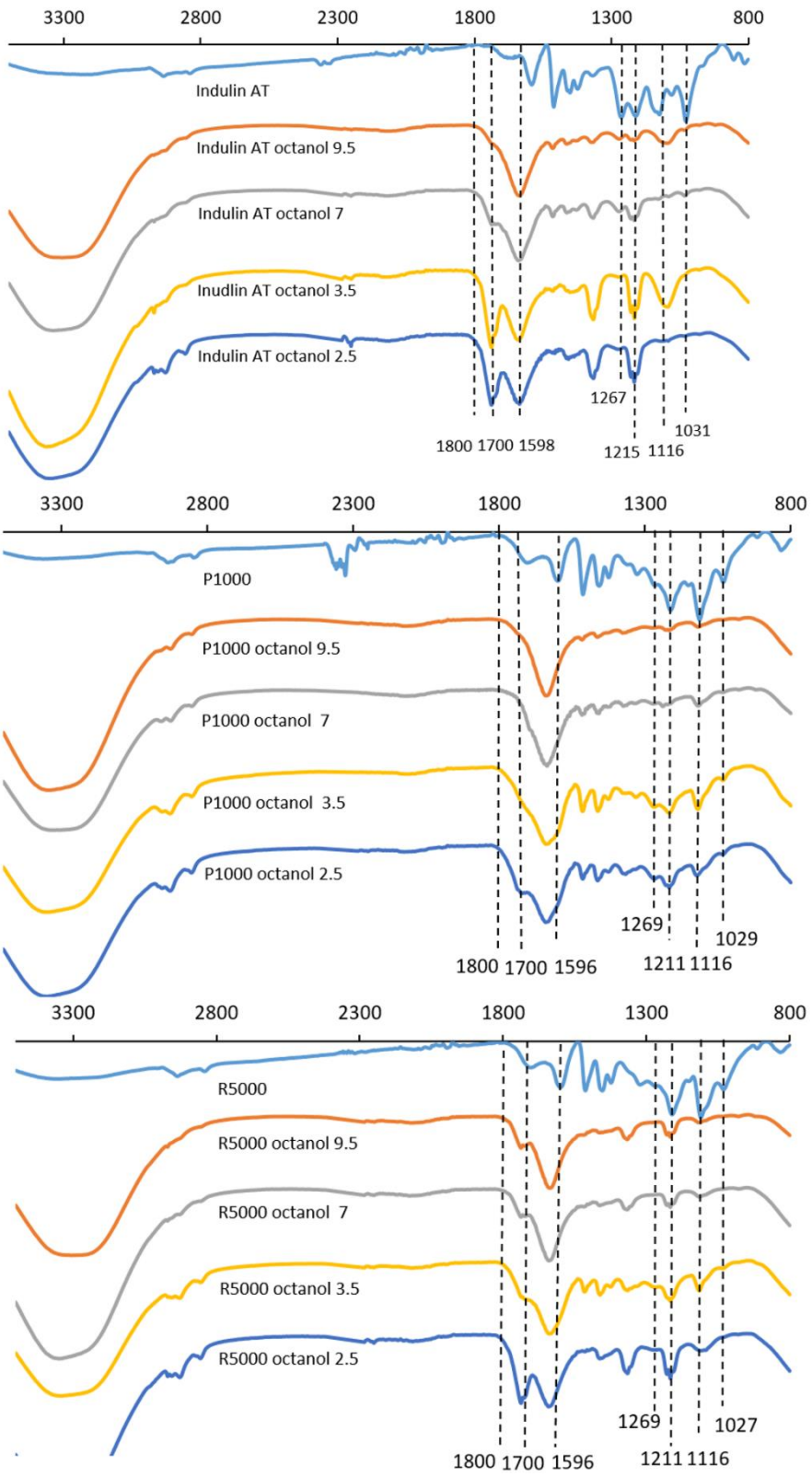

Fig. S5 ATR-FTIR spectra of Indulin AT, P1000 and R5000 parent and migrated fractions in octanol in transmittance (%) as function of wavelength (cm^{-1}).

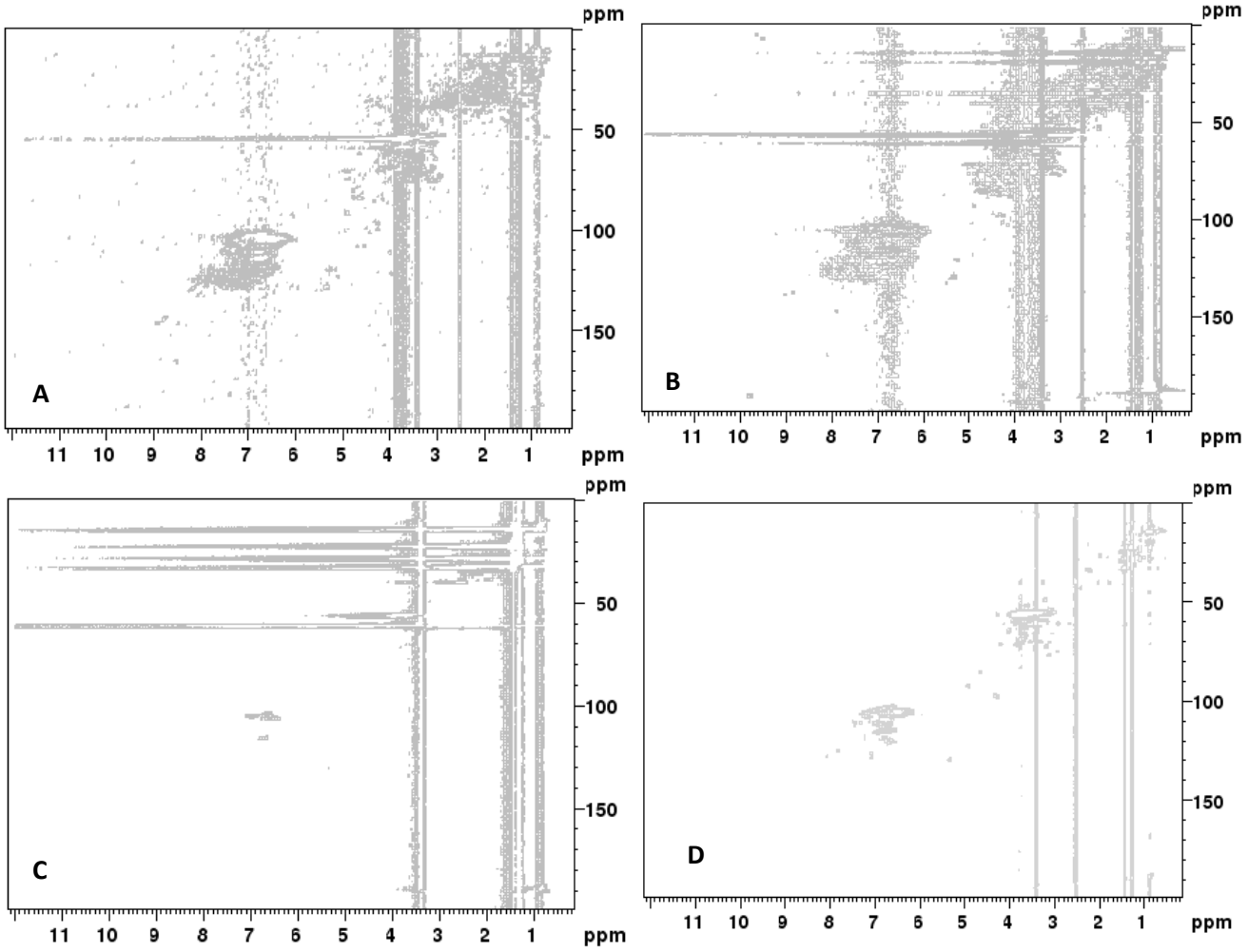

Fig. S6 2D-HSQC spectra of R5000 fraction in butanol at pH 9.5 (**A**) and 2.5 (**B**) as well as fractions in pentanol at pH 9.5 (**C**) and 2.5 (**D**).

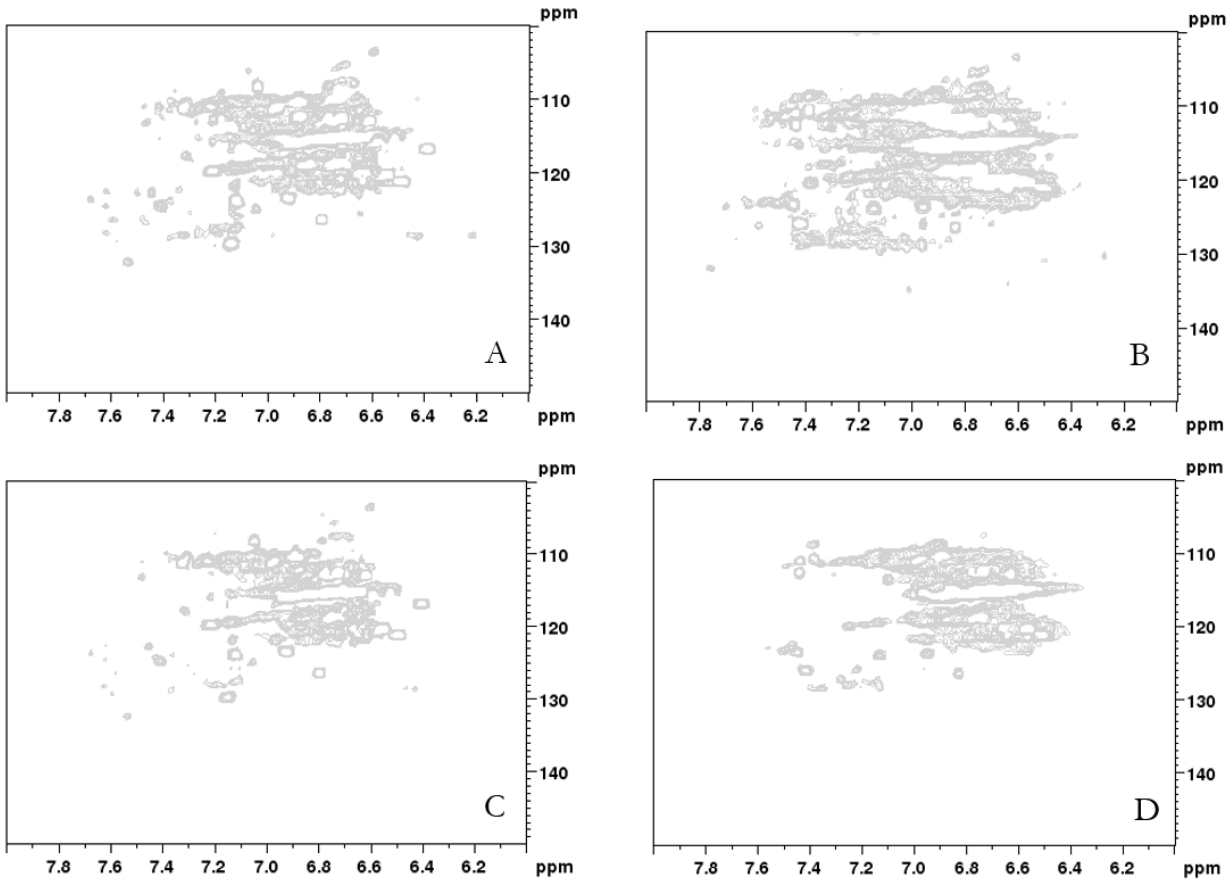

Fig. S7 2D-HSQC spectra of aromatic/unsaturated region of Indulin AT fraction in butanol at pH 9.5 (**A**) and 3.5 (**B**) as well as fractions in pentanol at pH 9.5 (**C**) and 3.5 (**D**).

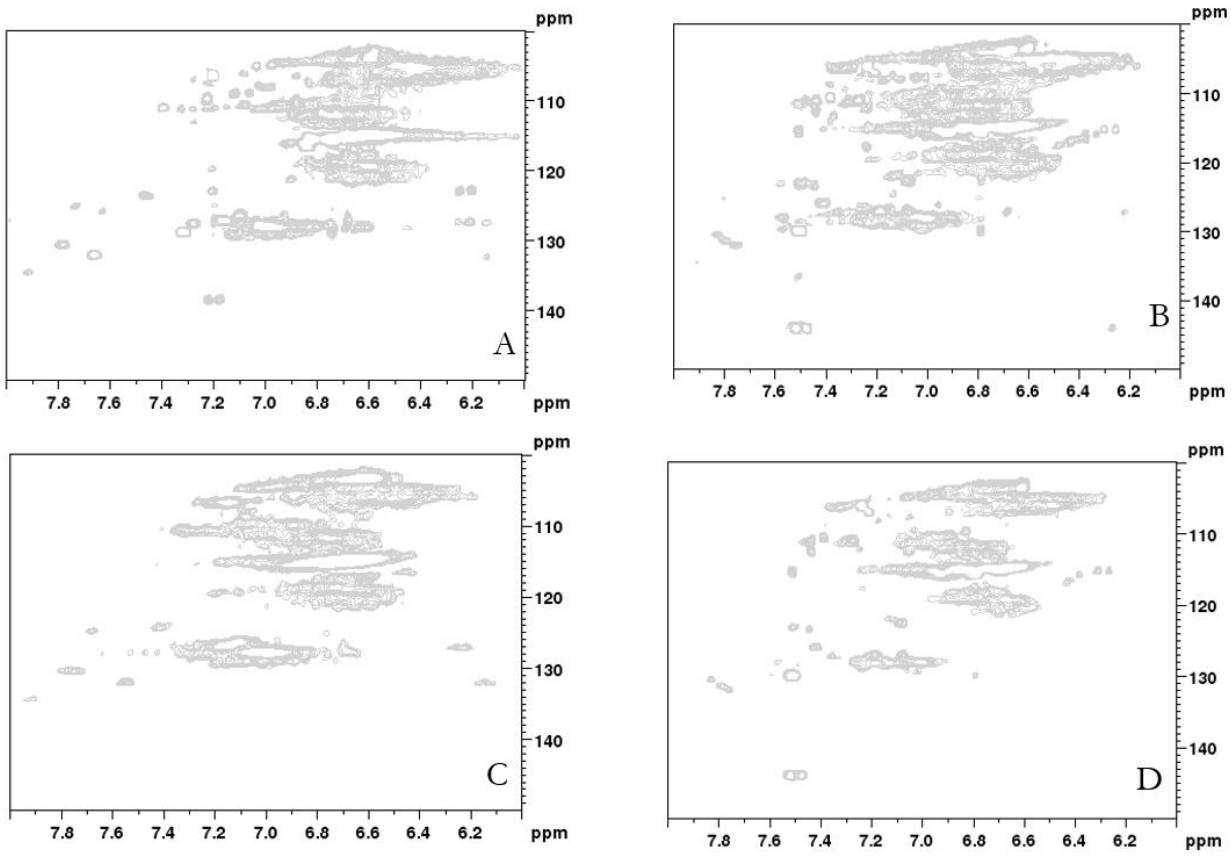

Fig. S8 2D-HSQC spectra of aromatic/unsaturated region of P1000 fraction in butanol at pH 9.5 (**A**) and 3.5 (**B**) as well as fractions in pentanol at pH 9.5 (**C**) and 3.5 (**D**).