Electronic Supplementary Material (ESI) for RSC Advances.

Extraction of Active Enzyme by Self-Buffering Ionic Liquids: A Green Medium for Enzymatic Research

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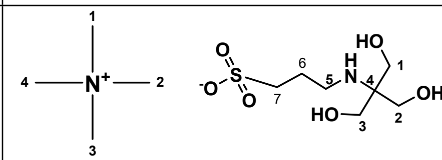
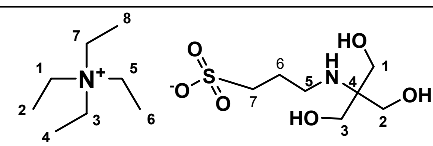
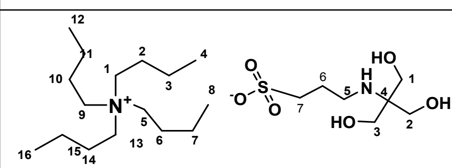
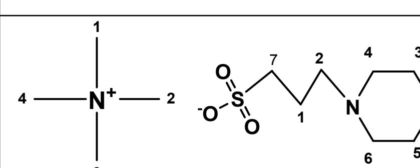
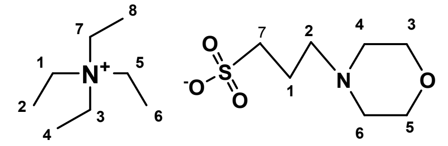
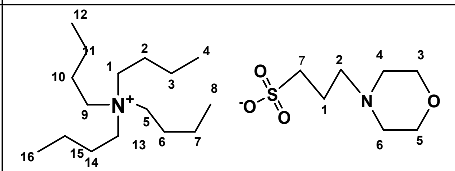
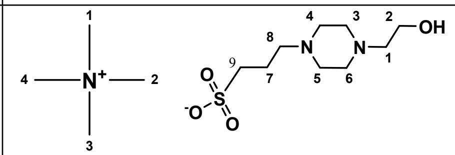
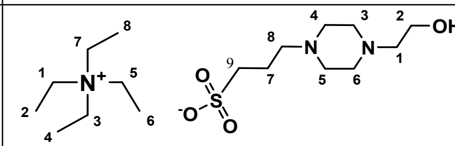
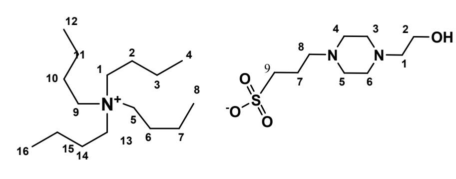
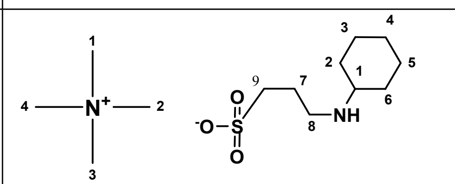
Table S. 1. Results of NMR analysis and the molecular structures of the synthesized GBILs.

Compounds	NMR Analysis	Structure
[TMA][TAPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ [TMA], 3.21(12H, s, C1-C4's H); δ [TAPS], 3.60 (6H, s, C1-C3's H), 1.92 (2H, quin, C6's H), 2.97 (2H, t, C7's H), 2.75 (2H, t, C5's H). *wt%* = 0.01, Analytical Calculations for $C_{11}H_{28}N_2O_6S$ (316.41) C 41.72, H 8.85, and N 8.85; Found C 40.71, H 8.87, and N 8.86.	
[TEA][TAPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TEA]*, 3.27 (8H, *q*, C1C3C5C7's *H*), 1.28 (12H, *m*, C2C4C6C8's *H*); δ *[TAPS]*, 3.60 (6H, *s*, C1-C3's *H*), 1.92 (2H, *quin*, C6's *H*), 2.98 (2H, *t*, C7's *H*), 2.74 (2H, *t*, C5's *H*). *wt%* = 0.2, Analytical calculations for $C_{15}H_{36}N_2O_6S$ (372.52) C 48.31, H 9.66, and N 7.52; Found C 48.37, H 9.85, N 7.38.	
[TBA][TAPS]	^{1}H NMR (500 MHz, DMSO); δ *[TBA]*, 0.93 (12H, *t*, C4C8C12C16's *H*), 1.31 (8H, *sext*, C3C7C11C15's *H*), 1.56 (8H, *quin*, C2C6C10C14's *H*), 3.17 (8H, *t*, C1C5C9C13's *H*); δ *[TAPS]*, 4.18 (6H, *s*, C1-C3's *H*), 1.62 (2H, *quin*, C6's *H*), 2.54 (2H, *t*, C7's *H*), 2.45 (2H, *t*, C5's *H*), 3.32 (1H, *s*, HN), 3.28 (3H, *s*, HO) . *wt%* = 0.02, Analytical calculations for $C_{23}H_{52}N_2O_6S$ (484.73) C 56.94, H 10.72, and N 5.78; Found C 56.47, H 10.73, N 5.84.	
[TMA][MOPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TMA]*, 3.21(12H, *s*, C1-C4's *H*); δ *[MOPS]*, 2.58 (4H, *m*, C4C6's *H*), 1.97 (2H, *quin*, C1's H), 2.53 (2H, *t*, C2's *H*), 2.93 (2H, *t*, C7's *H*), 3.78 (4H,	

	t, C3C5’s *H*). *wt%* = 0.009	
[TEA][MOPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TEA]*, 3.28 (8H, *q*, C1C3C5C7’s *H*), 1.28 (12H, *m*, C2C4C6C8’s *H*); δ *[MOPS]*, 2.59 (4H, *m*, C4C6’s *H*), 1.97 (2H, *quin*, C1’s H), 2.53 (2H, *t*, C2’s *H*), 2.93 (2H, *t*, C7’s *H*), 3.78 (4H, *t*, C3C5’s *H*). *wt%* = 0.25	
[TBA][MOPS]	^{1}H NMR (500 MHz, DMSO); δ *[TBA]*, 0.93 (12H, *t*, C4C8C12C16’s *H*), 1.31 (8H, *sext*, C3C7C11C15’s *H*), 1.57 (8H, *quin*, C2C6C10C14’s *H*), 3.18 (8H, *t*, C1C5C9C13’s *H*); δ *[MOPS]*, 2.31 (4H, *m*, C4C6’s *H*), 1.58 (2H, *quin*, C1’s H), 2.39 (2H, *t*, C2’s *H*), 2.50 (2H, *t*, C7’s *H*), 3.55 (4H, *t*, C3C5’s *H*). *wt%* = 0.3	
[TMA][EPPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TMA]*, 3.21(12H, *s*, C1-C4’s *H*); δ *[EPPS]*, 2.52 (4H, *t*, C4C5’s *H*), 2.59 (4H, *t*, C3C6’s H), 1.20 (2H, *t*, C8’s *H*), 2.93 (2H, *t*, C1’s *H*), 3.66 (2H, *t*, C9’s *H*), 3.75 (4H, *t*, C2’s *H*), 1.95 (2H, *quin*, C7’s H). *wt%* = 0.0085	
[TEA][EPPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TEA]*, 3.27 (8H, *q*, C1C3C5C7’s *H*), 1.27 (12H, *m*, C2C4C6C8’s *H*); δ *[EPPS]*, 2.52 (4H, *t*, C4C5’s *H*), 2.59 (4H, *t*, C3C6’s H), 1.21 (2H, *t*, C8’s *H*), 2.92 (2H, *t*, C1’s *H*), 3.66 (2H, *t*, C9’s *H*), 3.75 (4H, *t*, C2’s *H*), 1.95 (2H, *quin*, C7’s H). *wt%* = 0.15	
[TBA][EPPS]	^{1}H NMR (500 MHz, DMSO); δ *[TBA]*, 0.93 (12H, *t*, C4C8C12C16’s *H*), 1.31 (8H, *sext*, C3C7C11C15’s *H*), 1.58 (8H, *quin*, C2C6C10C14’s *H*), 3.18 (8H, *t*, C1C5C9C13’s *H*); δ *[EPPS]*, 2.29 (4H, *t*, C4C5’s *H*), 2.37 (4H, *t*, C3C6’s H), 1.05 (2H, *t*, C8’s *H*), 2.50 (2H, *t*, C1’s *H*), 3.43 (2H, *t*, C9’s *H*), 3.47 (4H, *t*, C2’s *H*), 1.69 (2H, *quin*, C7’s H). *wt%* = 0.3	
[TMA][CAPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TMA]*, 3.21(12H, *s*, C1-C4’s *H*); δ *[CAPS]*, 1.15-1.31&1.61-1.74 (10H, *m*, C2-C6’s *H*), 2.51 (H, *m*, C1’s *H*), 2.74 (2H, *t*, C8’s *H*), 2.95 (2H, *t*, C9’s *H*) , 1.91 (2H, *quin*, C7’s H). *wt%* = 0.01	

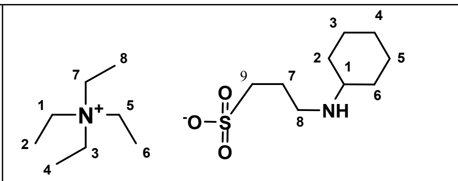
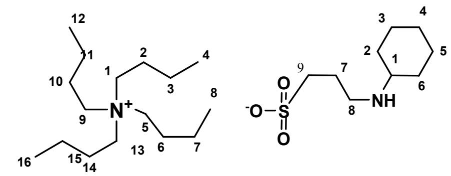
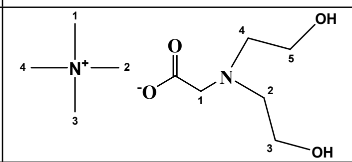
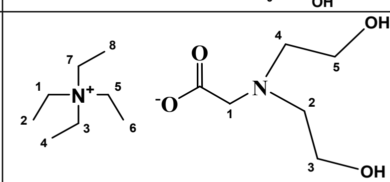
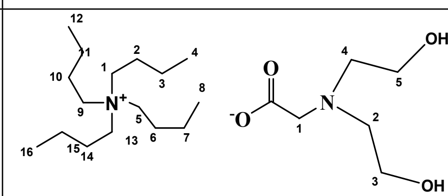
[TEA][CAPS]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TEA]*, 3.28 (8H, *q*, C1C3C5C7's *H*), 1.29 (12H, *m*, C2C4C6C8's *H*); δ *[CAPS]*, 1.07-1.25 &1.62-1.75 (10H, *m*, C2-C6's *H*), 2.49 (H, *m*, C1's *H*), 2.72 (2H, *t*, C8's *H*), 2.93 (2H, *t*, C9's *H*) , 1.92 (2H, *quin*, C7's H). *wt%* = 0.27	
[TBA][CAPS]	^{1}H NMR (500 MHz, DMSO); δ *[TBA]*, 0.93 (12H, *t*, C4C8C12C16's *H*), 1.31 (8H, *sext*, C3C7C11C15's *H*), 1.56 (8H, *quin*, C2C6C10C14's *H*), 3.17 (8H, *t*, C1C5C9C13's *H*); δ *[CAPS]*, 1.08-1.20 &1.62-1.76 (10H, *m*, C2-C6's *H*), 2.29 (H, *m*, C1's *H*), 2.39 (2H, *t*, C8's *H*), 2.51 (2H, *t*, C9's *H*) , 1.59 (2H, *quin*, C7's H), 3.33 (H, *s*, HN). *wt%* = 0.29	
[TMA][BICINE]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TMA]*, 3.21(12H, *s*, C1-C4's *H*); δ *[Bicine]*, 3.67 (4H, *t*, C2C4's *H*), 2.80 (4H, *t*, C3C5's *H*), 3.26 (2H, *s*, C1's *H*). *wt%* = 0.011	
[TEA][BICINE]	^{1}H NMR (500 MHz, D_2O/TSP); δ *[TEA]*, 3.25(8H, *q*, C1C3C5C7's *H*), 1.27 (12H, *m*, C2C4C6C8's *H*); δ *[Bicine]*, 3.65 (4H, *t*, C2C4's *H*), 2.78 (4H, *t*, C3C5's *H*), 3.23 (2H, *s*, C1's *H*). *wt%* = 0.2	
[TBA][BICINE]	^{1}H NMR (500 MHz, DMSO); δ *[TBA]*, 0.93 (12H, *t*, C4C8C12C16's *H*), 1.31 (8H, *sext*, C3C7C11C15's *H*), 1.57 (8H, *quin*, C2C6C10C14's *H*), 3.18 (8H, *t*, C1C5C9C13's *H*); δ *[Bicine]*, 3.44 (4H, *t*, C2C4's *H*), 2.30 (4H, *t*, C3C5's *H*), 2.85 (2H, *s*, C1's *H*). *wt%* = 0.23	

Table S. 2. Experimental mass fraction data for the binodal curve of the systems GBIL (1) + sodium sulphate (2) at 25°C under atmospheric pressure.

[TBA][TAPS]		[TBA][MOPS]		[TBA][EPPS]		[TBA][CAPS]		[TBA][BICINE]	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1
42.41	2.91	44.35	2.10	46.66	1.47	48.21	1.72	46.61	2.02
39.60	4.43	39.18	4.94	43.10	3.39	44.62	3.56	43.46	3.66
36.29	6.22	35.85	6.77	40.29	4.91	42.99	4.39	40.88	5.01
29.01	10.16	33.56	8.04	37.50	6.41	40.55	5.64	39.34	5.81
26.76	11.38	32.07	8.86	35.18	7.66	38.49	6.69	37.68	6.68
25.52	12.04	31.09	9.40	33.34	8.66	37.28	7.31	36.36	7.36
24.30	12.70	30.17	9.90	31.94	9.41	36.08	7.92	34.94	8.10
23.24	13.28	28.81	10.65	30.72	10.07	34.88	8.54	33.92	8.63
22.06	13.92	27.37	11.44	29.60	10.67	33.81	9.08	32.40	9.43
20.26	14.89	26.03	12.18	28.66	11.18	32.89	9.55	30.45	10.44
19.13	15.50	25.16	12.66	27.73	11.68	31.78	10.12	29.13	11.13
18.16	16.03	24.66	12.93	26.39	12.41	30.89	10.58	27.92	11.76
17.15	16.57	23.85	13.38	25.14	13.08	29.69	11.19	26.92	12.28
15.70	17.36	23.10	13.80	24.09	13.65	28.87	11.61	25.87	12.83
14.90	17.79	22.55	14.10	23.05	14.21	27.73	12.19	24.39	13.60
14.12	18.21	21.62	14.61	22.22	14.65	26.29	12.92	23.36	14.14
13.29	18.66	20.97	14.97	21.04	15.29	24.74	13.72	21.89	14.91
12.60	19.03	20.34	15.32	20.20	15.75	24.16	14.02	20.73	15.51
11.62	19.56	19.80	15.61	19.24	16.26	23.61	14.29	19.36	16.23
11.04	19.88	19.18	15.95	18.61	16.60	22.83	14.69	17.84	17.02
10.51	20.16	18.64	16.25	16.51	17.74	22.39	14.92	16.55	17.69
9.72	20.59	18.01	16.60	15.76	18.14	21.15	15.55	14.68	18.66
9.09	20.93	17.59	16.83	15.04	18.53	20.76	15.75	13.03	19.53
8.46	21.27	17.04	17.13	14.43	18.86	20.14	16.07	11.67	20.23
6.92	22.10	16.51	17.42	13.64	19.29	19.58	16.35	10.28	20.96
6.11	22.54	15.98	17.72	11.57	20.41	19.24	16.53	8.72	21.77
42.41	2.91	15.47	18.00	11.07	20.67	18.76	16.78	46.61	2.02
39.60	4.43	15.04	18.23	10.74	20.85	17.99	17.17	43.46	3.66
36.29	6.22	14.53	18.51	10.41	21.03	17.24	17.55	40.88	5.01
29.01	10.16	14.25	18.67	10.03	21.23	16.56	17.90	39.34	5.81
26.76	11.38	13.77	18.93	9.75	21.38	15.95	18.21	37.68	6.68
25.52	12.04	13.44	19.11	9.41	21.57	16.19	18.08	36.36	7.36
24.30	12.70	13.12	19.29	9.02	21.78	15.34	18.52	34.94	8.10
23.24	13.28	12.82	19.46	8.67	21.97	15.12	18.63	33.92	8.63
22.06	13.92	12.48	19.64	8.35	22.14	14.81	18.79	32.40	9.43
20.26	14.89	12.16	19.82	8.02	22.32	14.53	18.94	30.45	10.44
19.13	15.50	11.84	19.99	7.79	22.44	14.25	19.08	29.13	11.13
18.16	16.03	11.52	20.17	7.33	22.69	13.96	19.22	27.92	11.76
17.15	16.57	11.26	20.31	7.18	22.77	13.75	19.33	26.92	12.28
15.70	17.36	10.99	20.46	6.96	22.89	13.40	19.51	25.87	12.83
14.90	17.79	10.73	20.61	6.67	23.05	13.24	19.60	24.39	13.60

Table S. 2. Continued.

[TBA][TAPS]		[TBA][MOPS]		[TBA][EPPS]		[TBA][CAPS]		[TBA][BICINE]	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1
14.12	18.21	10.50	20.73	6.42	23.18	12.98	19.73	23.36	14.14
13.29	18.66	10.16	20.92	6.19	23.31	12.86	19.79	21.89	14.91
12.60	19.03	9.90	21.06	6.01	23.40	12.65	19.89	20.73	15.51
11.62	19.56	9.80	21.12	5.81	23.51	12.50	19.97	19.36	16.23
11.04	19.88	7.30	22.50	5.57	23.64	12.26	20.10	17.84	17.02
10.51	20.16	7.10	22.61	5.44	23.71	12.07	20.19	16.55	17.69
9.72	20.59	6.93	22.70	5.22	23.83	11.85	20.31	14.68	18.66
9.09	20.93	6.75	22.80	5.02	23.94	11.62	20.42	13.03	19.53
8.46	21.27	6.56	22.90	4.83	24.04	11.40	20.54	11.67	20.23
6.92	22.10	6.40	22.99	4.63	24.15	11.28	20.60	10.28	20.96
6.11	22.54	6.20	23.10	4.46	24.24	11.10	20.69	8.72	21.77
		5.99	23.22	4.28	24.33	10.80	20.84		
		5.81	23.32	4.10	24.44	10.57	20.96		
		5.64	23.41	3.93	24.53	10.40	21.05		
		5.49	23.49	3.66	24.67	10.24	21.13		
		5.33	23.58	3.34	24.85	10.05	21.23		
		5.18	23.66	3.02	25.02	9.78	21.36		
		4.90	23.82			9.62	21.45		
		4.77	23.89			9.48	21.52		
		4.61	23.97			9.32	21.60		
		4.51	24.03			9.19	21.67		
		4.29	24.15			9.07	21.73		
		4.10	24.26			8.94	21.79		
		3.93	24.35			8.83	21.85		
		3.79	24.43			8.65	21.94		
		3.69	24.48			8.55	21.99		
		3.54	24.57			8.43	22.05		
		3.42	24.63			8.32	22.11		
		3.29	24.70			8.19	22.17		
		3.14	24.79			8.08	22.23		
		2.88	24.93			7.85	22.35		
		2.78	24.98			7.72	22.41		
						7.63	22.46		
						7.54	22.51		
						7.33	22.61		
						7.14	22.71		
						6.98	22.79		
						6.81	22.88		
						6.68	22.95		
						6.54	23.02		
						6.40	23.09		

Table S. 2. Continued

[TBA][TAPS]		[TBA][MOPS]		[TBA][EPPS]		[TBA][CAPS]		[TBA][BICINE]	
100 w_1	100 w_2	100 w_1	100 w_2	100 w_1	100 w_1	100 w_2	100 w_1	100 w_2	100 w_1
						6.28	23.15		
						6.14	23.22		
						6.02	23.29		
						5.90	23.35		
						5.79	23.40		
						5.65	23.47		
						5.47	23.56		
						5.29	23.66		
						5.11	23.75		
						5.00	23.81		
						4.84	23.89		
						4.64	23.99		
						4.51	24.06		
						4.41	24.11		
						4.29	24.17		
						4.17	24.23		
						4.07	24.28		
						3.95	24.34		
						3.87	24.39		
						3.70	24.47		
						3.59	24.53		
						3.30	24.68		
						2.89	24.88		

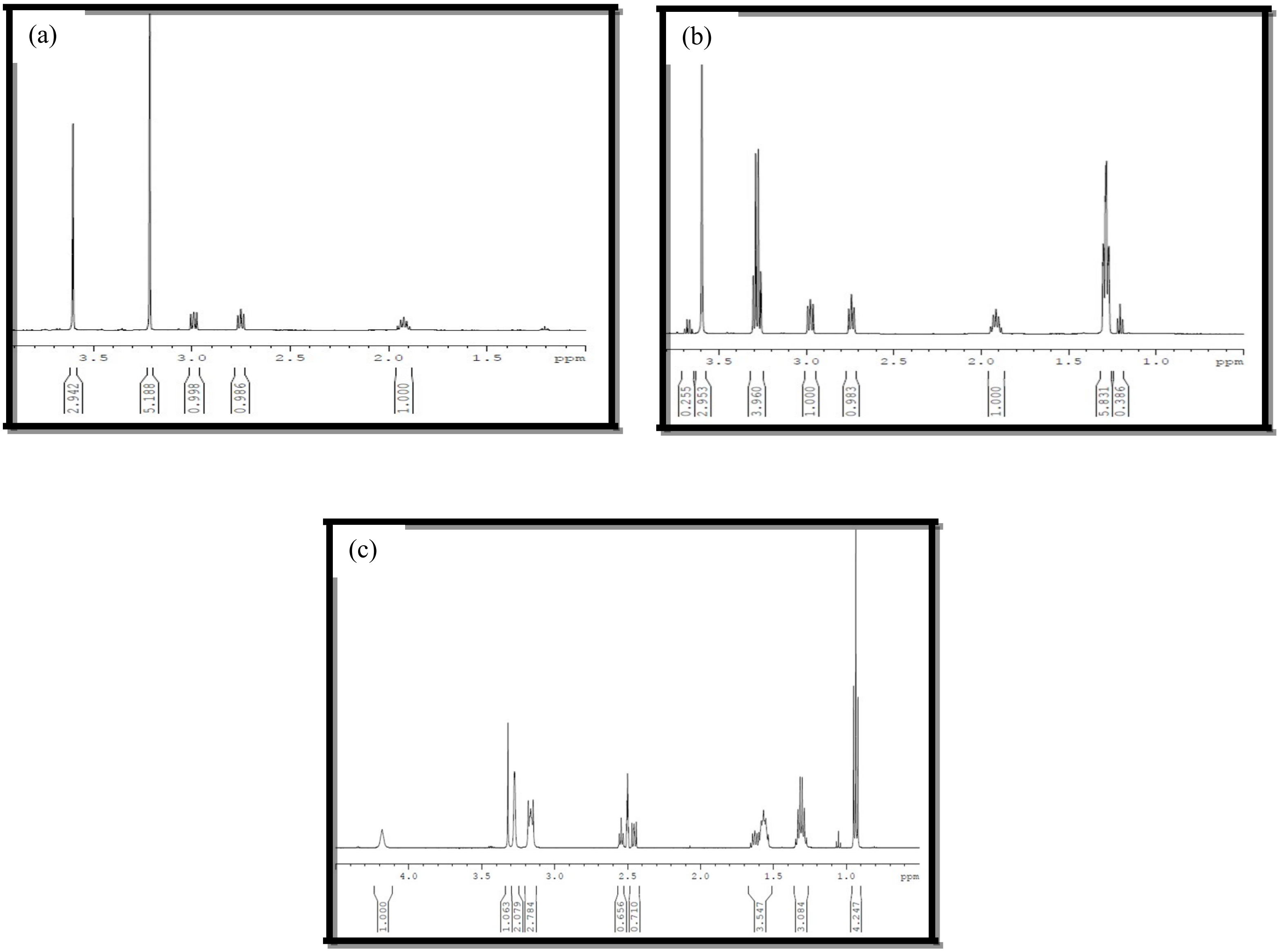


Fig. S. 1. The [1]H NMR spectras of TAPS based GBILs: (a), [TMA][TAPS] in D_2O; (b), [TEA][TAPS] in D_2O; (c); [TBA][TAPS] in DMSO.

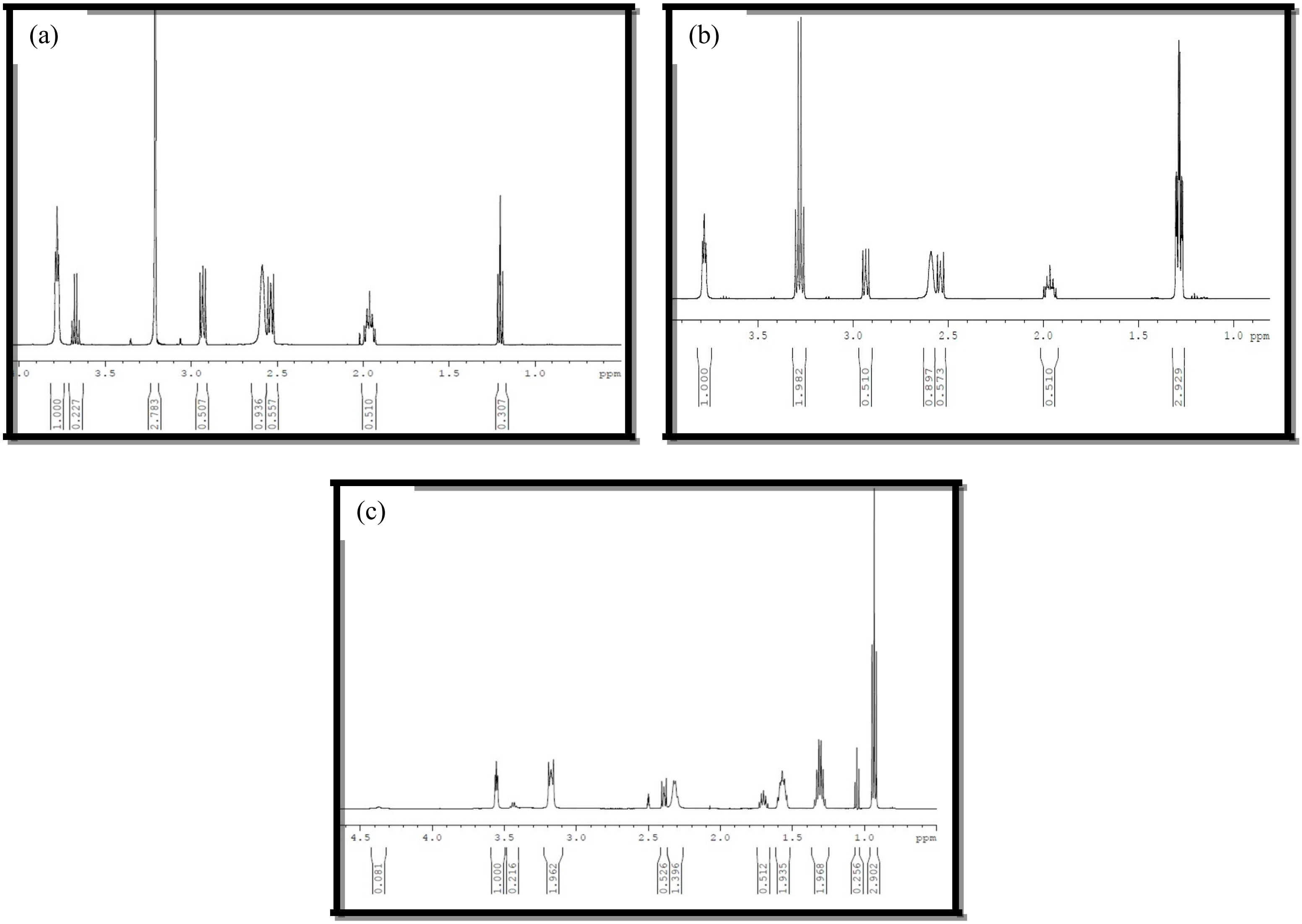


Fig. S. 2. The [1]H NMR spectra of MOPS based GBILs: (a), [TMA][MOPS] in D_2O; (b), [TEA][MOPS] in D_2O; (c); [TBA][MOPS] in DMSO.

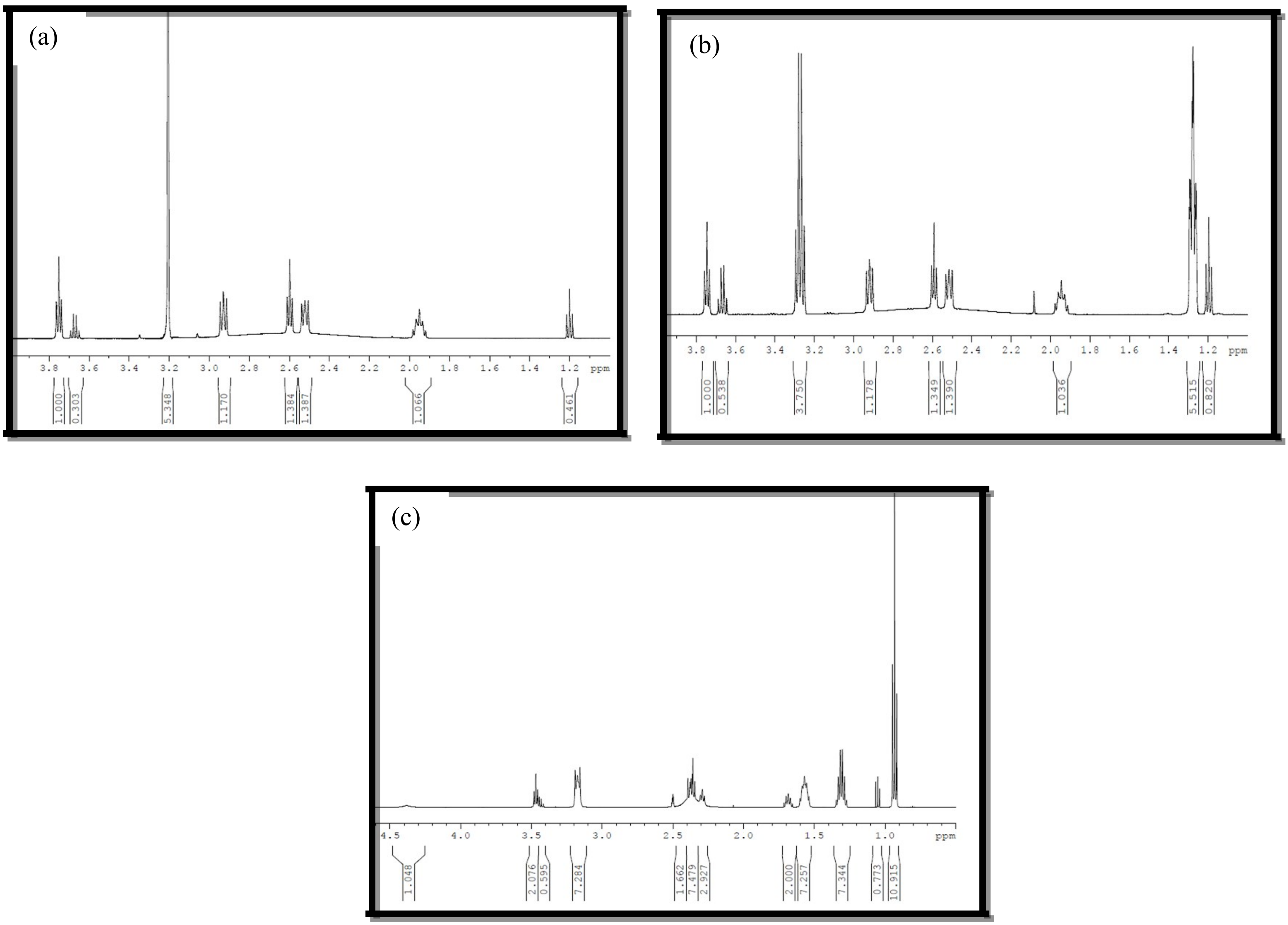


Fig. S. 3. The ^{1}H NMR spectra of EPPS based GBILs: (a), [TMA][EPPS] in D_2O; (b), [TEA][EPPS] in D_2O; (c); [TBA][EPPS] in DMSO.

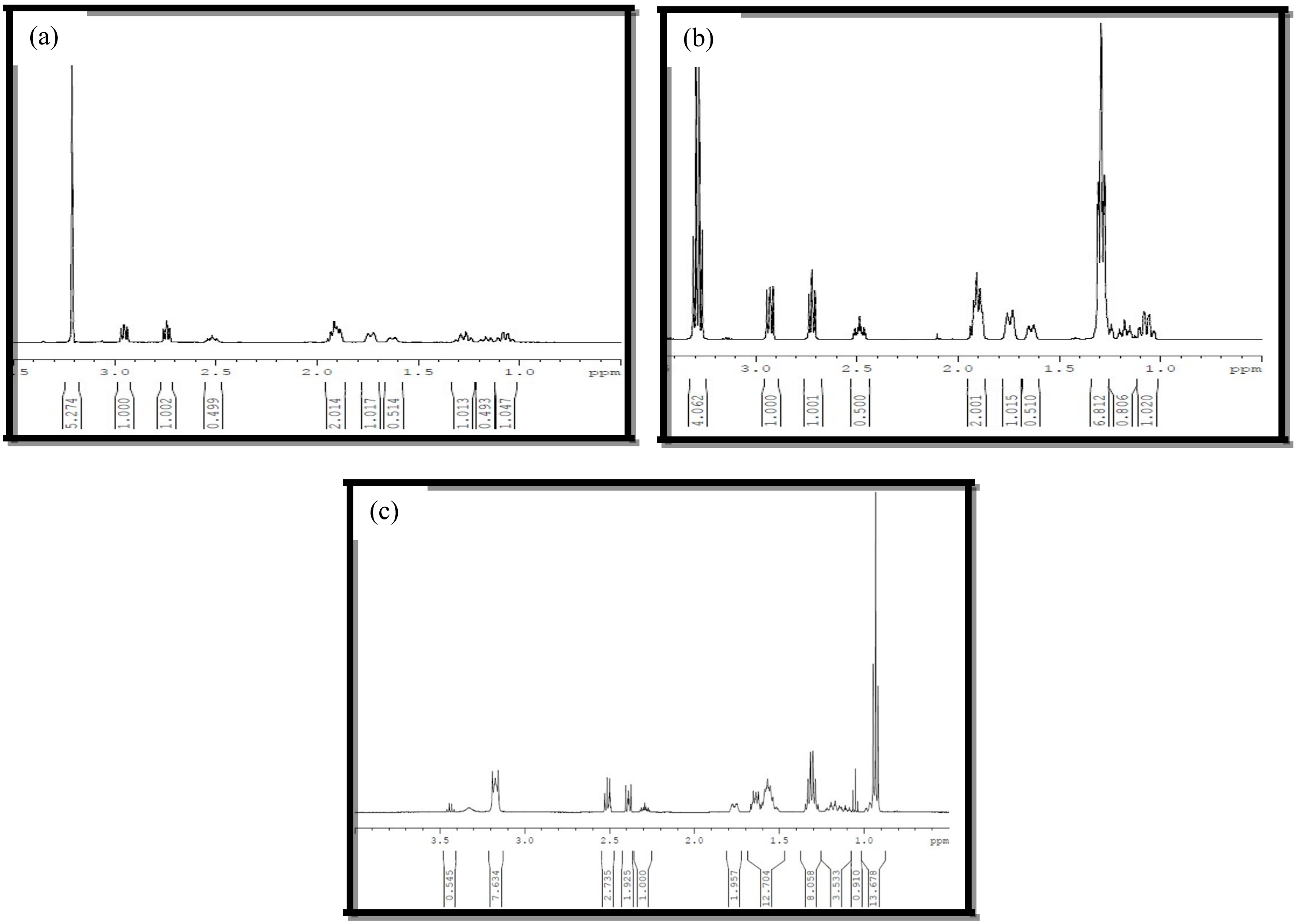


Fig. S. 4. The ^{1}H NMR spectra of CAPS based GBILs: (a), [TMA][CAPS] in D_2O; (b), [TEA][CAPS] in D_2O; (c); [TBA][CAPS] in DMSO.

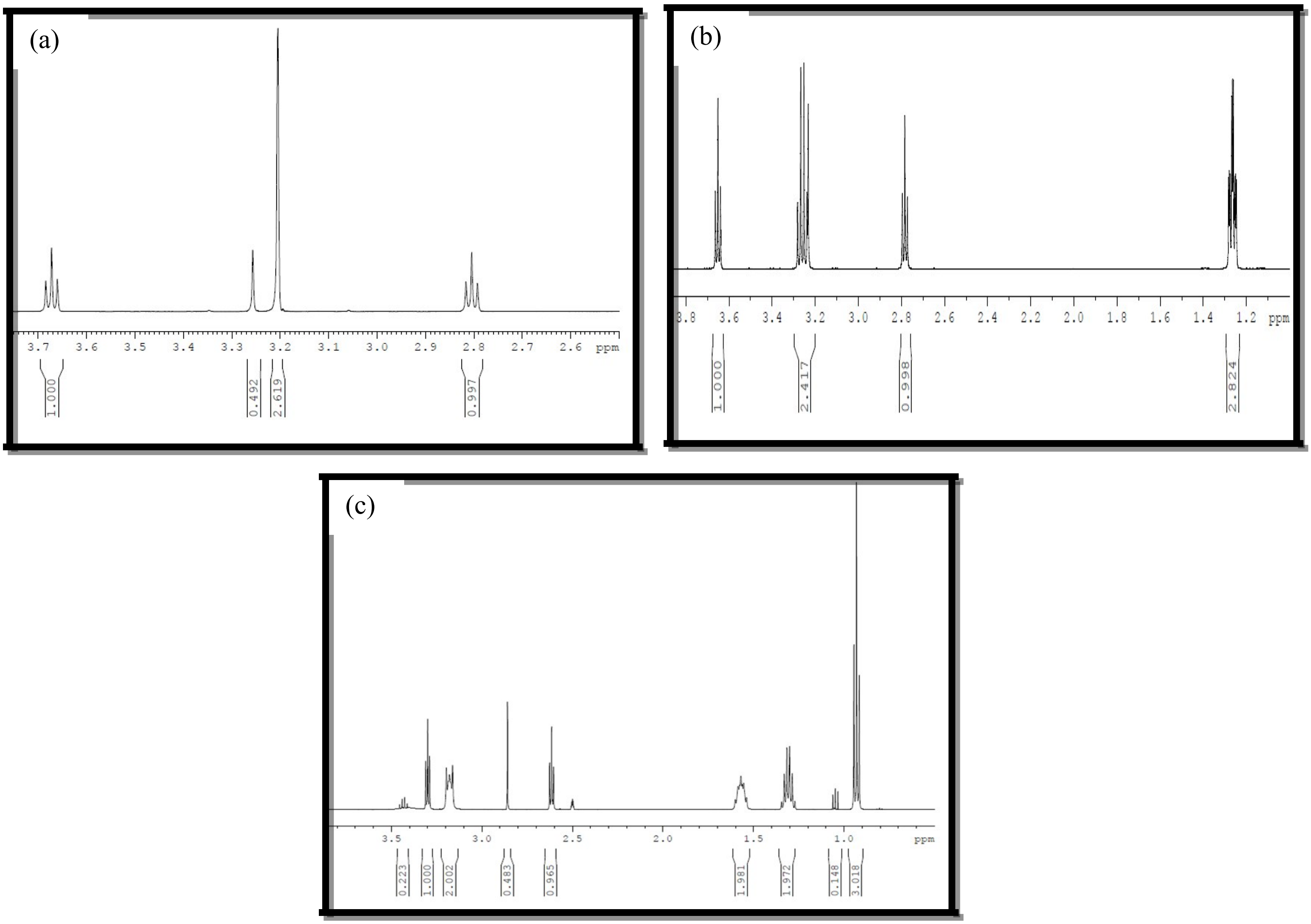


Fig. S. 5. The ^{1}H NMR spectra of CAPS based GBILs: (a), [TMA][CAPS] in D_2O; (b), [TEA][CAPS] in D_2O; (c); [TBA][CAPS] in DMSO.

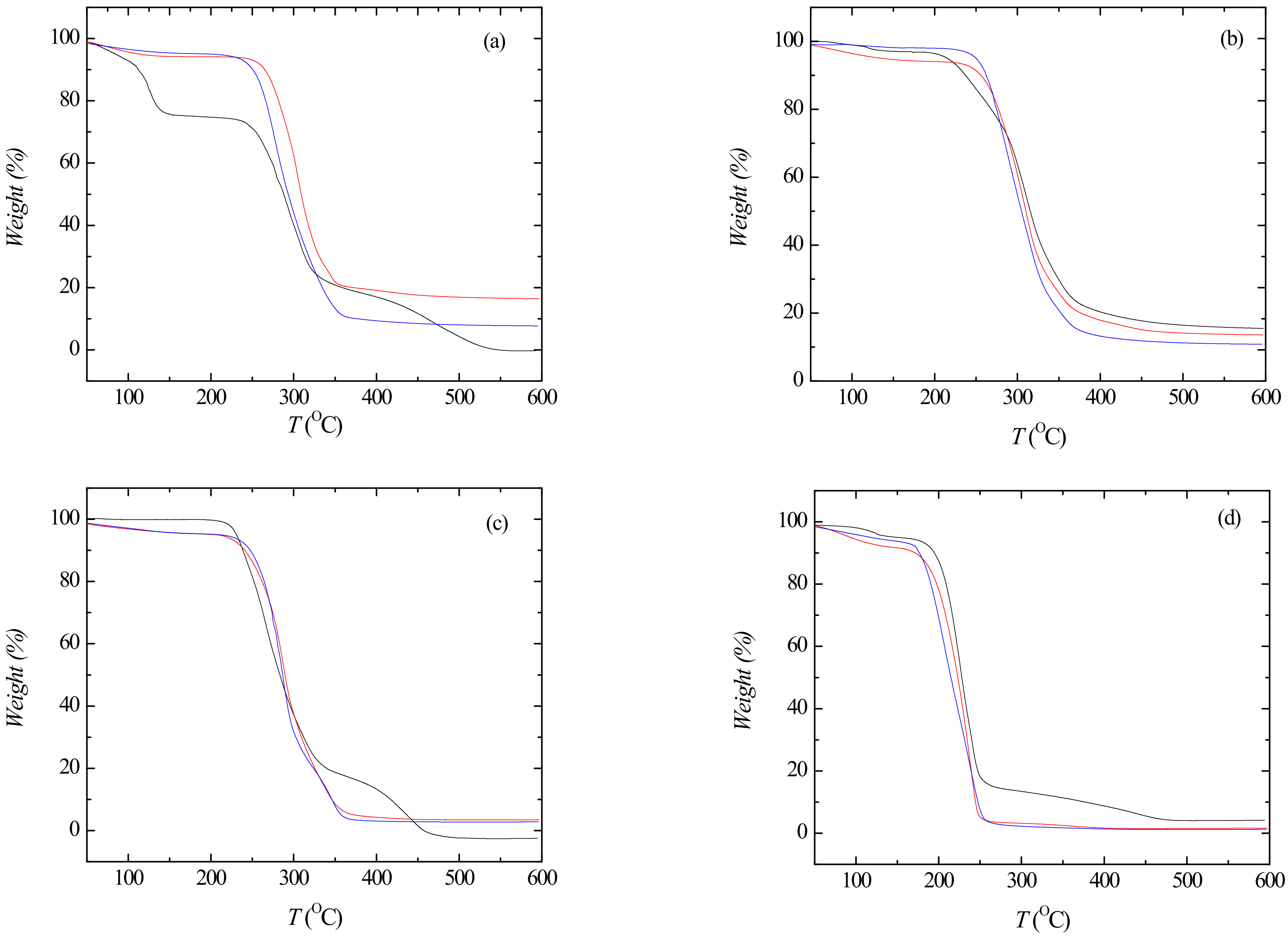


Fig. S. 6. Thermal profiles of the synthesized MOPS based GBILs (a), EPPS based GBILs (b), CAPS based GBILs (c), and BICINE based GBILs (d): (-), TMA based ILs; (-), TEA based ILs; and (-), TBA based ILs.

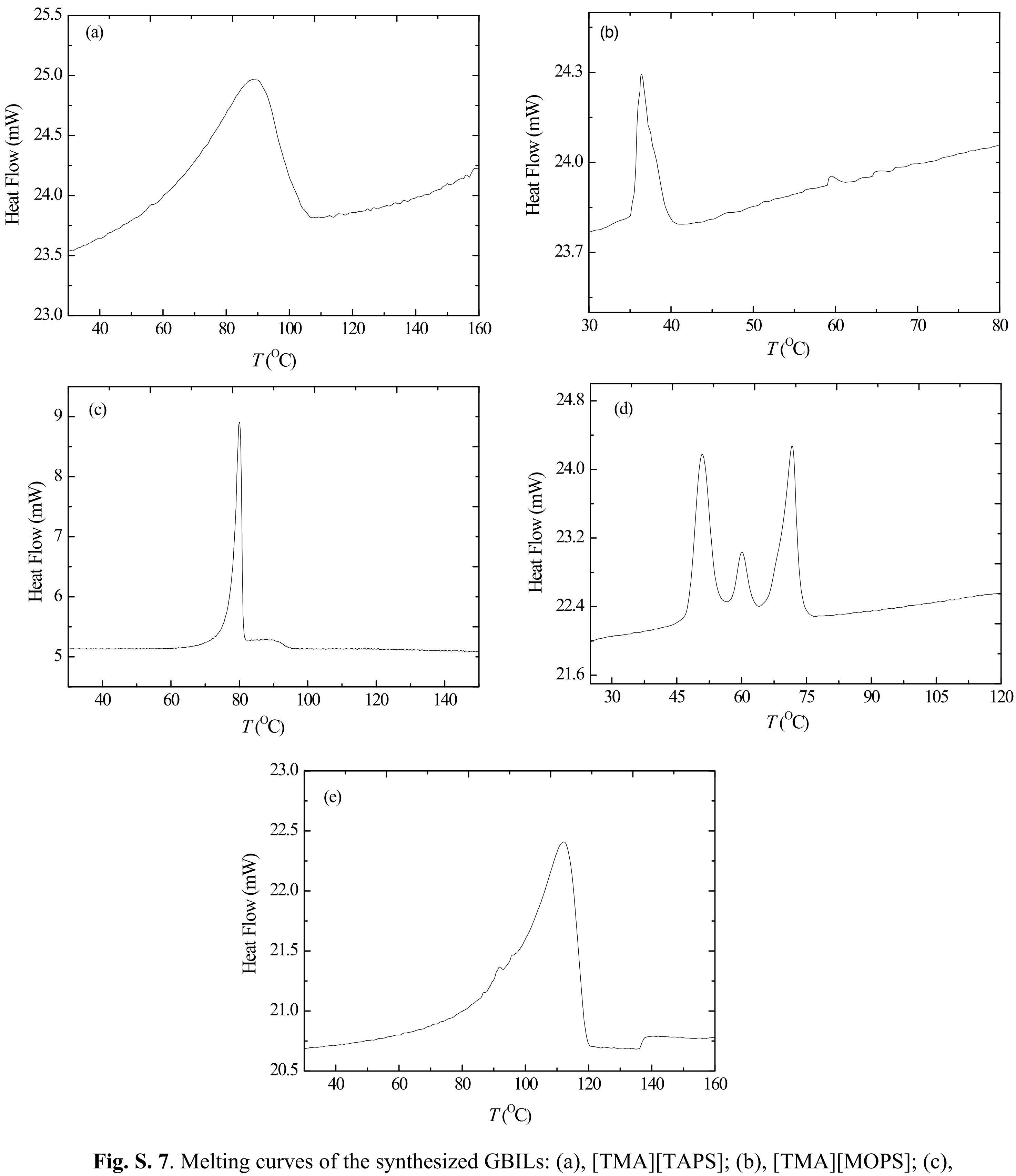


Fig. S. 7. Melting curves of the synthesized GBILs: (a), [TMA][TAPS]; (b), [TMA][MOPS]; (c), [TMA][EPPS]; (d), [TMA][CAPS]; (e), [TMA][BICINE].

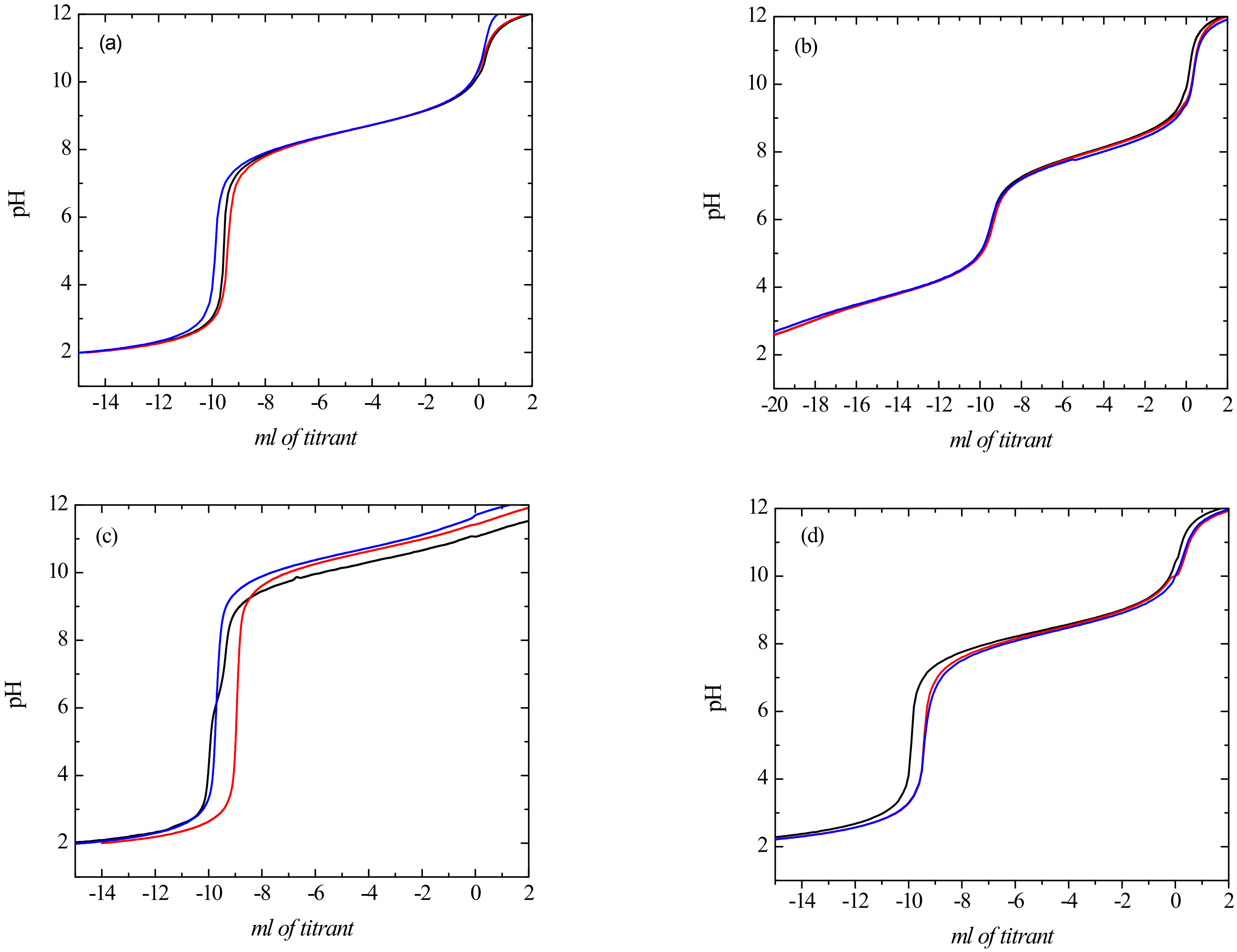


Fig. S. 8. pH profiles of the synthesized TAPS based GBILs (a), EPPS based GBILs (b), CAPS based GBILs (c), and BICINE based GBILs (d): (-), TMA based ILs; (-), TEA based ILs; and (-), TBA based ILs.

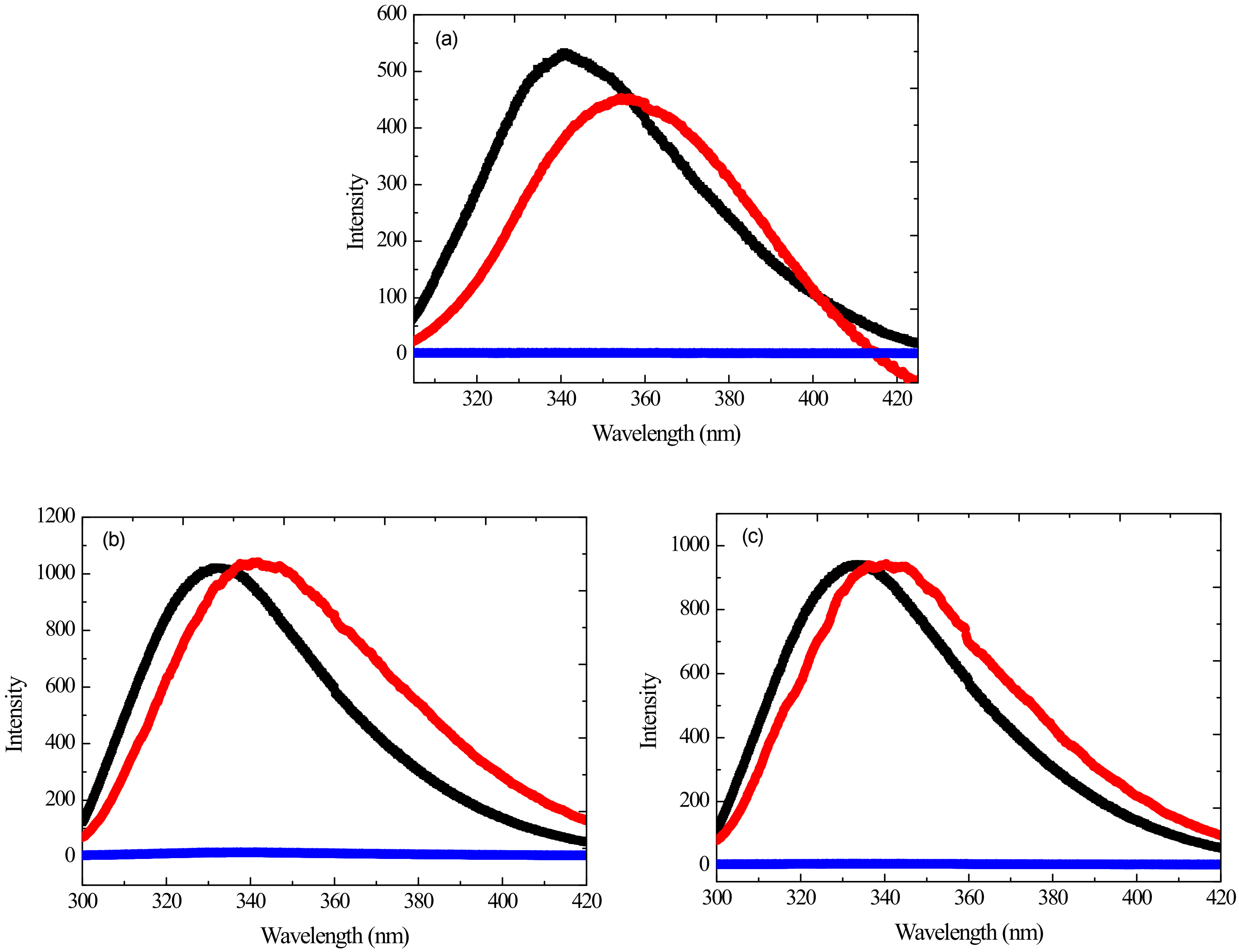


Fig. S. 9. The fluorescence spectra of α-chymotrypsin in [TBA][MOPS] (a), [TBA][EPPS] (b), and [TBA][BICINE] (c) before and after extraction: (■); α-chymotrypsin in aqueous GBIL solution before extraction, (●); α-chymotrypsin in upper phase after extraction, (■); α-chymotrypsin in lower phase after extraction.