

## Supporting Information

### Extraction of Tetracycline from Fermentation Broth using Aqueous Two-phase Systems composed of Polyethylene Glycol and Cholinium-based Salts

Jorge F. B. Pereira<sup>a</sup>, Filipa Vicente<sup>a</sup>, Valéria C. Santos-Ebinuma<sup>b</sup>, Janete M. Araújo<sup>c</sup>, Adalberto Pessoa<sup>b</sup>, Mara G. Freire<sup>a</sup>, João A. P. Coutinho<sup>a\*</sup>

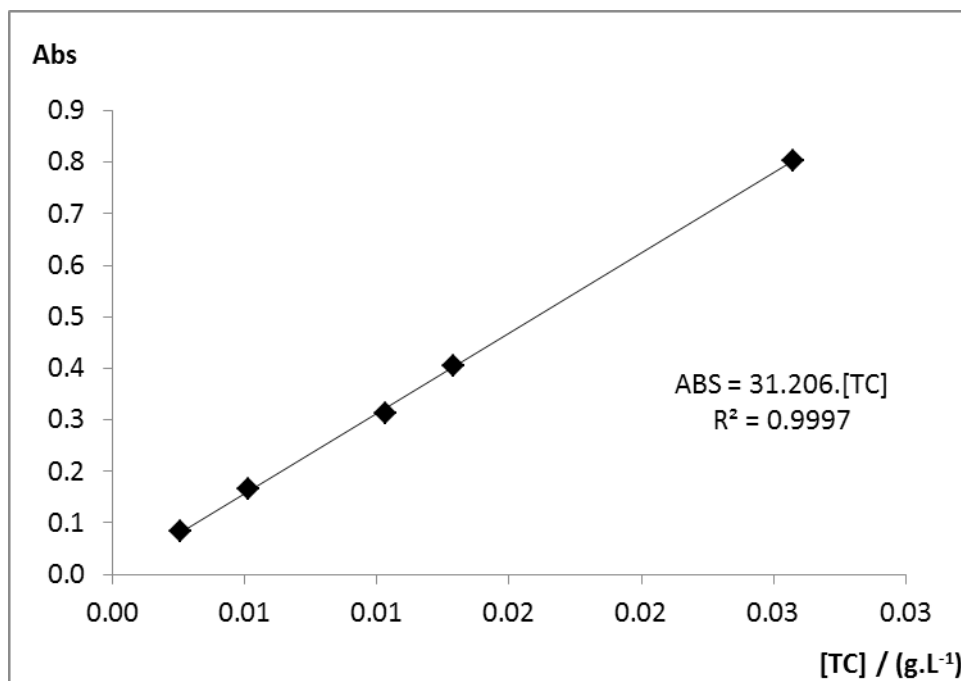
<sup>a</sup>Departamento de Química, CICECO, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

<sup>b</sup>Department of Biochemical and Pharmaceutical Technology, University of São Paulo, Avenida Prof. Lineu Prestes 580, Bl. 16, 05508-900 São Paulo, SP, Brazil

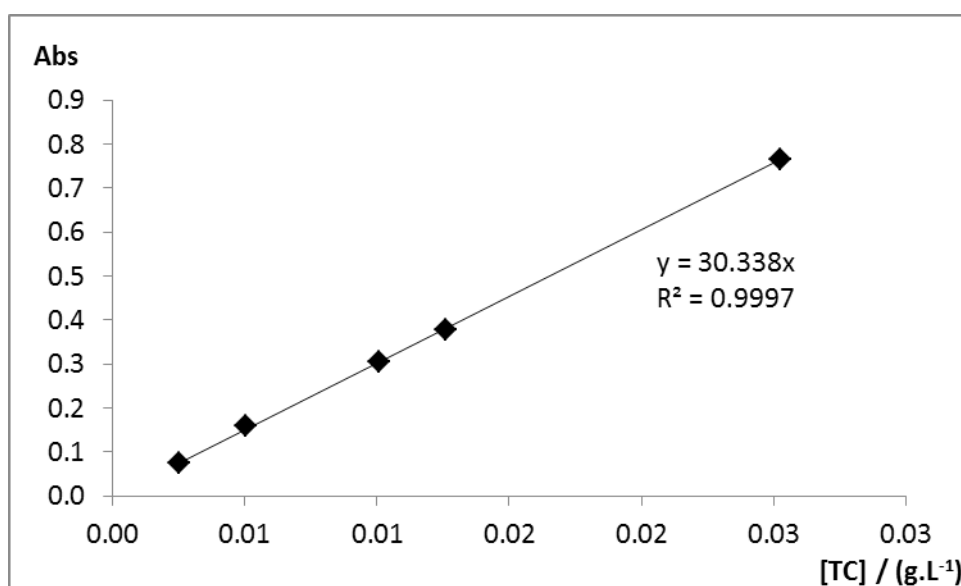
<sup>c</sup>Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Antibióticos. Rua Prof. Artur de Sá, Cidade Universitária, 50670-420 - Recife, PE - Brazil

\*Corresponding author

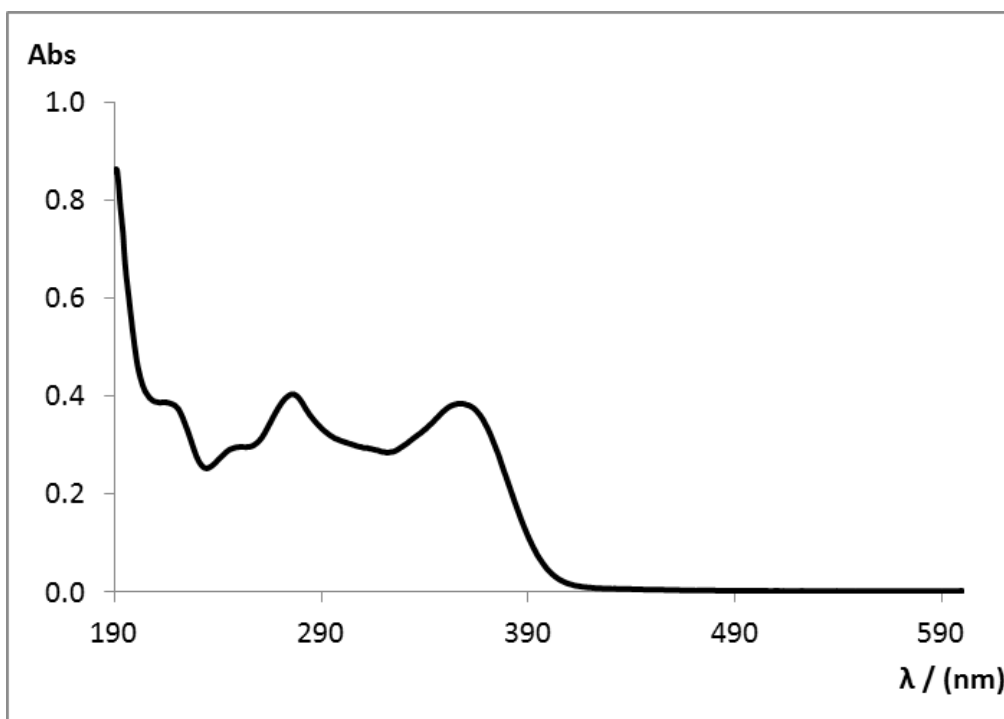
Tel: +351-234-401507; Fax: +351-234-370084; E-mail address: [jcoutinho@ua.pt](mailto:jcoutinho@ua.pt)



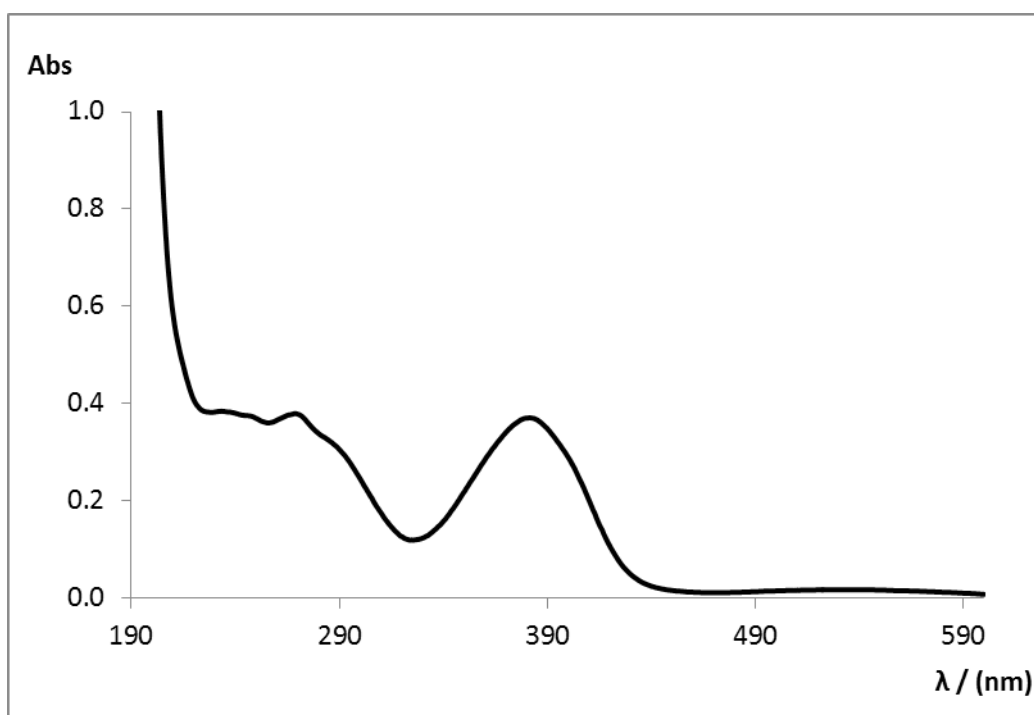
**Fig. A1.** Calibration curve of TC at 25 °C, neutral pH and 276 nm, using a stock solution of 0.258 g.L<sup>-1</sup> of TC.



**Fig. A2.** Calibration curve of TC at 25 °C, alkaline pH and 276 nm, using a stock solution of 0.253 g.L<sup>-1</sup> of TC.



**Fig. A3.** Spectrum of TC absorbance in a diluted aqueous solution (v:v, 1:20) from a TC stock solution of  $0.258 \text{ g.L}^{-1}$ , at  $25^\circ\text{C}$  and a neutral pH.



**Fig. A4.** Spectrum of TC absorbance in a diluted aqueous solution (v:v, 1:20) from a TC stock solution of  $0.253 \text{ g.L}^{-1}$ , at  $25^\circ\text{C}$  and an alkaline pH.

**Table A1.** Validation of the TC quantification.

[Total Protein] / (g.L <sup>-1</sup> ) <sup>a</sup>	6.187
Dilution Factor	1:10
Absorbance at 280 nm due to the protein content <sup>b</sup>	0.281
Total absorbance at 280 nm	0.816
Absorbance of TC at 280 nm	0.535
[TC] at 280 nm / (g.L <sup>-1</sup> ) <sup>c</sup>	0.163
[TC] at 357 nm / (g.L <sup>-1</sup> ) <sup>d</sup>	0.175

<sup>a</sup>obtained from the BCA method; <sup>b</sup>obtained by the calibration curve of BSA: **Absorbance=0.4545\*[Total Protein]**; <sup>c</sup>obtained from the calibration curve of TC at 280 nm (Fig.A1); <sup>d</sup> obtained from calibration curve of TC at 357 nm (Fig. A2)

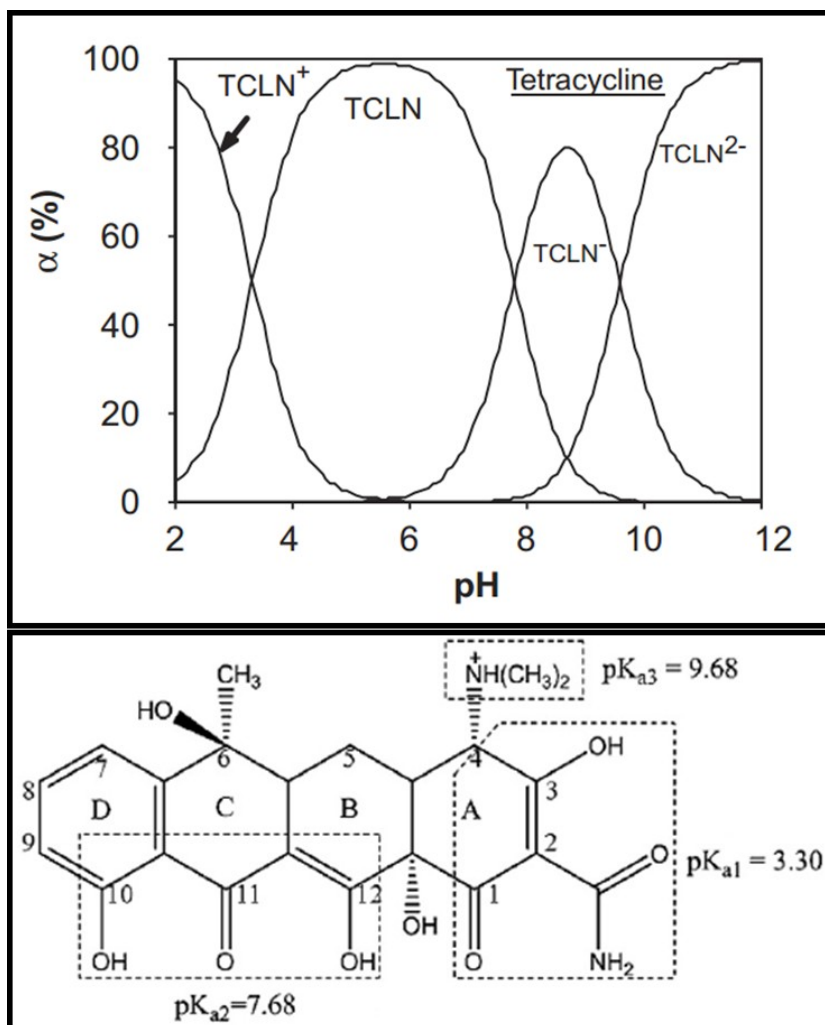
**Table A2.** Initial weight fraction compositions, tie-lines and TLLs in the several ATPS at 25 °C.

Ternary System	Weigth Fraction Composition (wt %)						$\alpha$	TLL
	$Y_M$	$X_M$	$Y_{PEG}$	$X_{PEG}$	$Y_{Salt}$	$X_{Salt}$		
[Ch]Cl	54.96	35.01	4.41	76.08	94.17	3.14	0.56	115.66
	55.03	34.93	3.90	77.49	92.84	3.46	0.57	115.72
	54.99	34.90	3.48	78.76	91.52	3.80	0.59	115.63
	54.95	30.01	13.31	60.75	89.82	4.26	0.54	95.10
	55.01	29.93	11.73	62.82	88.14	4.76	0.57	95.96
	54.79	30.04	10.15	65.07	86.23	5.37	0.59	96.71
	55.07	24.91	29.85	43.81	74.82	10.10	0.56	56.20
	54.80	25.00	26.73	46.63	72.75	11.18	0.61	58.09
	54.78	24.95	26.04	47.28	71.95	11.61	0.63	58.14
[Ch]Bic	50.01	34.84	91.17	1.12	1.69	72.74	0.54	114.62
	49.86	35.07	91.26	1.11	1.27	74.93	0.54	116.40
	49.72	35.19	91.63	1.05	1.33	74.60	0.54	116.40
	49.71	30.04	89.89	1.34	5.99	61.27	0.52	103.11
	50.05	29.99	87.37	1.82	4.37	64.47	0.55	103.99
	49.84	29.90	85.38	2.26	3.87	65.64	0.56	103.25
	49.72	25.09	76.74	4.94	11.32	53.72	0.59	81.60
	49.99	25.05	76.05	5.21	9.69	55.72	0.61	83.39
	49.95	25.06	77.77	4.55	11.70	53.27	0.58	82.08
[Ch]DHcit	44.90	39.99	19.51	62.83	79.93	8.47	0.58	81.27
	45.00	39.90	19.22	63.18	79.67	8.60	0.57	81.44
	45.08	39.85	18.82	63.67	79.34	8.77	0.57	81.71
	45.70	37.83	26.30	54.39	77.45	9.77	0.63	67.36
	45.90	37.76	28.14	53.16	79.31	8.78	0.65	67.74
	45.76	37.81	27.28	54.07	77.92	9.25	0.64	67.46
	56.76	29.99	74.79	11.30	7.53	81.01	0.73	96.87
	57.06	29.84	77.05	9.99	9.63	76.96	0.70	95.03
	56.84	30.07	77.28	9.86	9.87	76.54	0.70	94.81
[Ch]Ac	49.93	39.97	0.62	77.42	99.68	2.30	0.50	124.38
	49.31	39.47	0.75	76.16	98.29	2.45	0.50	122.25
	49.92	39.93	0.63	77.32	99.64	2.20	0.50	124.28
	49.84	35.10	0.96	74.61	86.63	5.37	0.57	110.15
	49.79	34.97	0.93	74.80	85.74	5.66	0.58	109.42
	49.96	34.92	0.86	75.34	85.35	5.79	0.58	109.44
	49.84	30.03	5.01	61.47	83.53	6.41	0.57	95.90
	49.93	29.99	5.07	61.36	83.77	6.33	0.57	96.04
	49.94	29.97	4.50	62.48	82.24	6.88	0.58	95.58
[Ch]DHph	49.81	29.92	85.81	0.41	0.09	70.67	0.58	110.84
	50.01	29.99	85.07	0.47	0.06	72.05	0.59	111.14
	49.89	29.95	85.95	0.40	0.08	70.76	0.58	111.01
	40.29	29.78	77.24	1.40	0.77	60.13	0.52	96.42
	40.11	30.00	76.03	1.60	0.63	61.22	0.52	96.12
	40.05	29.95	79.66	1.07	1.04	58.40	0.50	97.32
	35.04	29.89	70.37	2.75	1.67	55.52	0.49	86.63
	35.09	29.99	68.15	3.32	1.26	57.29	0.51	85.95
	34.80	30.04	70.14	2.81	1.65	55.58	0.48	86.46

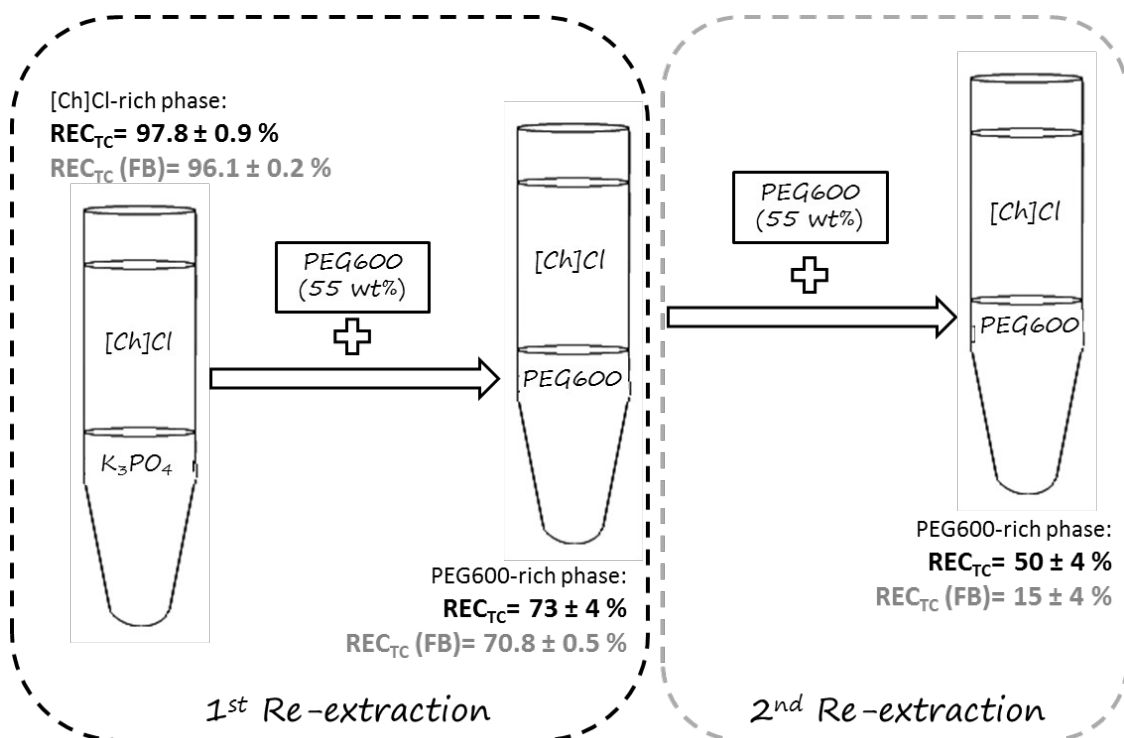
$Y$  and  $X$  are, respectively, the PEG and salt weight percentages, and the subscripts PEG, Salt and M represent the PEG-rich, the Salt-rich and the initial mixture, respectively. The parameter  $\alpha$  is the ratio between the weight of the PEG-rich and the total mass of the mixture.

**Table A3.** Initial mixture compositions and respective values of conductivities at 25°C.

<b>Ternary System</b>	<b>Weight Fraction Composition / (wt %)</b>		<b>Conductivity / (mS.cm<sup>-1</sup>)</b>		<b>Phase</b>	
	<b>PEG 600</b>	<b>IL</b>	<b>Top</b>	<b>Bottom</b>	<b>Top</b>	<b>Bottom</b>
[Ch]Cl	54.69	27.73	10.98	0.507	IL	PEG
[Ch]Bic	45.78	29.49	0.482	22.00	PEG	IL
[Ch]Ac	50.01	29.97	7.760	0.641	IL	PEG
[Ch]DHcit	59.61	27.40	0.364	-	PEG	IL
[Ch]DHph	40.19	30.07	0.092	2.640	PEG	IL



**Fig. A5.** Chemical Structure and speciation' curves of tetracycline as a function of the pH (adapted from Qiang, Z. and C. Adams (2004). Potentiometric determination of acid dissociation constants ( $pK_a$ ) for human and veterinary antibiotics. Water Research 38: 2874-2890).



**Fig. A6.** Illustrative scheme of the extraction of commercial TC and TC obtained from fermented broth to a [Ch]Cl-rich phase using the [Ch]Cl/ $K_3PO_4$  ATPS and re-extraction of TC from the previously formed cholinium-rich phase to a PEG600-rich phase using the PEG600/[Ch]Cl ATPS. The TC recovery ( $REC_{TC}$ ) in the respective PEG- or [Ch]Cl-rich phase is the ratio between the TC concentration in the respective phase to that in the sum of both phases.