Electronic Supplementary Material (ESI) for RSC Advances.

Pulsatile Release from pH Triggered Imidazoline Switchable Surfactant Liposomes

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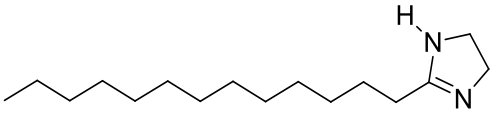
Supplementary Information

1. Synthesis

1.1. 2-Tridecylimidazoline



Ethylene diamine (0.2 mol, 12 mL), tetradecanoic acid (0.1 mol, 22.8 g) and toluene (100 mL) were mixed in a round bottom flask fitted with a Dean-Stark apparatus. The mixture was refluxed at 110 °C until 0.2 mol of water was collected (~ 24 hr). The toluene was removed by rotary evaporation to give a white-yellow crude product. The crude product was dissolved in hot acetone and filtered twice to remove insoluble impurities. The crude was then recrystallised from acetone to give a white crystalline solid.

Yield: 8.12 g, 32.2%

M.P. 87-89 °C

^{1}H NMR: ($CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25-1.29 (m, 20H), 1.60 (qn, *J* = 7.5 Hz, 2H), 2.23 (t, *J* = 7.8 Hz, 2H), 3.58 (s, 4H).

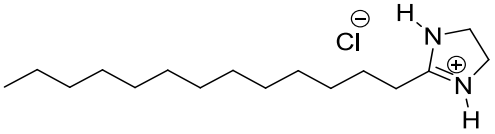
^{13}C NMR: 14.27, 22.84, 26.84, 29.49, 29.50, 29.54, 29.57, 29.64, 29.77, 29.79, 29.81, 29.83, 29.88, 32.07, 168.31.

IR (Diamond Anvil) v: 1638 (m, C=N) cm^{-1}.

Micro. Calc. for $C_{16}H_{32}N_2$ C, 76.13; H, 12.78; N, 11.10.

Found. C, 75.86; H, 12.8; N, 11.10.

1.2. 2-Tridecylimidazoline hydrochloride



2-Tridecylimidazoline (1 g) was added to ether (300 mL) in a three-necked round bottomed flask and was heated until it dissolved. The solution was charged with $HCl_{(g)}$ using a method described by Arnaiz (1995)[1]. Concentrated HCl was placed in a pressure equalising addition funnel attached to a flat bottomed flask containing calcium chloride (50 g). The $HCl_{(l)}$ was added dropwise on the $CaCl_2$ and the evolved $HCl_{(g)}$ was bubbled through the ethereal solution. The product precipitated out of the ether and was recrystallized in acetone.

Yield: 0.61 g, 53.3%

M.P. 121 °C

^{1}H NMR: ($CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.28-1.36 (m, 20H), 1.80 (qn, *J* = 7.6 Hz, 2H) 2.73 (t, *J* = 7.8 Hz, 2H), 3.90 (s, 4H), 10.45 (s, 2H).

^{13}C NMR: ($CDCl_3$) δ 14.28, 22.85, 26.52, 26.80, 29.52, 29.80, 29.82, 29.84, 29.86, 32.08, 44.55, 172.41.

IR (Diamond Anvil) ν: 1599 (m, C=N) cm^{-1}.

Micro calc. for. $C_{16}H_{33}ClN_2$ C, 66.52; H, 11.51; Cl, 12.27; N, 9.70

Found. C, 66.90; H, 11.71; Cl, 11.90; N, 9.63.

2. Surfactant Characterisation measurements

2.1. CMC determination

The CMCs of the chloride salts of C_{13}IDZ was determined conductometrically with treatment of the data by the sigmoidal Boltzmann method.[2] Conductivity measurements were obtained, in triplicate, using a Suntex SC-170 conductivity meter (referenced to 0.100 mol L^{-1} KCl), a water-bath and a jacketed beaker thermostated at 25 ^{o}C. The initial solutions were prepared at a concentration higher than the CMC in 5 mL of Milli-Q™ water. Stepwise dilution of the initial surfactant solutions was undertaken by the addition

of water, to achieve data points sufficient to give two linear plots whose intercept approximates the CMC (Figure 1).

The rate of change of the conductivity (*dK*) was divided by the rate of change of the concentration (*dC*) and plotted against concentration to give a sigmoidal Boltzmann distribution. A line of best fit was generated through the data using Origin Pro (Version 8.5 (2010)) with the inflection point (X_o) indicating the greatest rate of change of the conductivity and, hence the CMC.

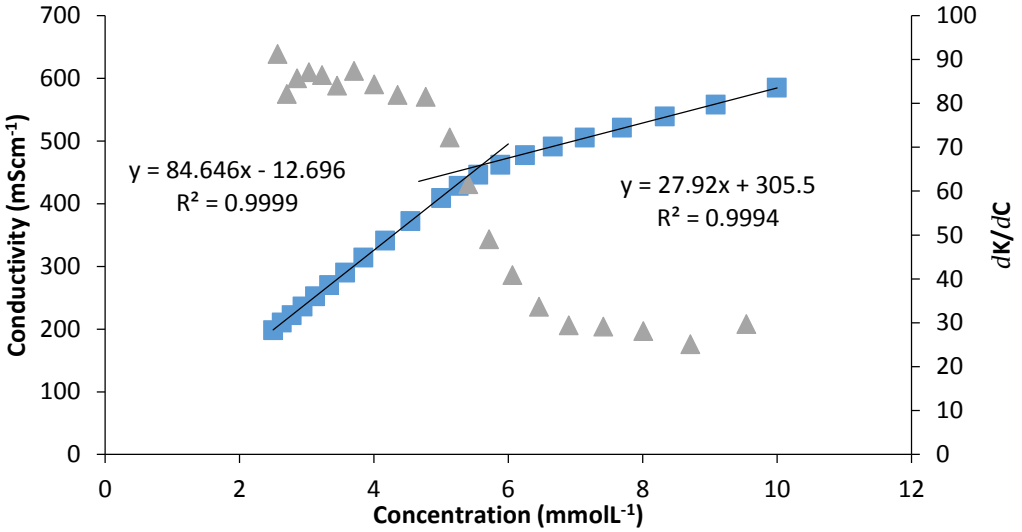


Figure 1. Conductivity versus concentration plot for C_{13}IDZ.HCl with the intercept of the two linear trends indicating the CMC. The *d*K/*d*C vs concentration plot (triangles) gave a sigmoidal Boltzmann distribution with the black line indicating best fit. The midpoint of the sigmoid (X_0) indicates the CMC.

2.2. Calculated Partition Coefficient

The log *D* values of the PS and surfactants were calculated using widely available and commonly used freeware programs. The values were theoretically predicted as an average of three different methods to reduce any error arising from one particular technique. The three models were atomic based VG,[3] fragment based KlogP,[4] and molecular data mining using the Physprop database[5]. For simplicity, log *D* values were calculated with the assumption that the molecules were either fully ionised or un-ionised, as partial ionization would result in a mixture of PS and surfactants and should give log *D* values between the two calculated extremes. The results of the calculations are presented in Table 1.

Table 1. Calculated log *D* of compounds, derived from an average of three different methods (VG, KlogP and Physprop) of calculation. The percentage of the molecule in the hydrophobic phase is given in parentheses.

SS	PS				Surfactant			
	VG	KlogP	Physprop	Average	VG	KlogP	Physprop	Average
C_{13}IDZH	4.8	4.9	5.2	5.0 (99.9)	1.2	2.5	2.9	2.2 (99.4)

2.3. Liposome-water Partition coefficient

The addition of a surfactant to a liposome suspension leads to solubilisation of the lipid bilayer via mixed micelle formation, causing a change in the light scattered. The change in scattering depends on the structures formed by the lipid and surfactant components, which in turn depends on their packing parameter and hydrophobicity.[6] Solubilisation induced changes in the lipid bilayer may therefore be monitored by the variation in the light scattered. This process is governed by the effective detergent to lipid molar ratio (*Re*) in the bilayer, which is defined by Lichtenburg[6] as;

$$Re = \frac{(S_T - S_W)}{(PL - PL_{mono})} \qquad \textbf{Equation 0.1}$$

Where S_T is the total concentration of the surfactant and S_W is the surfactant concentration in the aqueous medium. Due to the low solubility of phospholipid (PL) in water, the concentration of monomeric lipid (PL_{mono}) is assumed to be negligible. Thus, the process can be reduced to;

$$Re = \frac{S_B}{PL} \qquad \textbf{Equation 0.2}$$

where S_B is the concentration of surfactant in the bilayer.

The partition equilibrium between the membrane and aqueous phase is thought to govern the incorporation of surfactants into a liposome, giving rise to an *Re* sufficient to cause saturation and solubilisation. It has been shown that in sufficiently dilute mixtures of lipid, the distribution of surfactant between membrane and the aqueous phase obeys Shurtenburger's equilibrium partition model[7] represented by the following $P_{mem/w}$;[6,8]

$$P_{\mathrm{mem/w}} = \frac{S_B}{((PL + S_B)S_W)} \qquad \textbf{Equation 0.3}$$

When PL >> S_B then $P_{mem/w}$ can be defined in terms of *Re* as follows;[7]

$$P_{\mathrm{mem/w}} = \frac{S_B}{(PL.S_W)} = \frac{Re}{S_W} \qquad \textbf{Equation 0.4}$$

When the above condition is not met Equation 4.5 can be employed to define $P_{mem/w}$;

$$P_{\mathrm{mem/w}} = \frac{Re}{(S_W(1 + Re))} \qquad \textbf{Equation 0.5}$$

These parameters can be ascertained on the basis of the linear dependence existing between the surfactant concentrations required to achieve these parameters and the phospholipid concentration in the mixed vesicles which, according to de la Maza and Parra,[9] can be described by the following equation;

$$S_T = S_W + Re.PL \qquad \textbf{Equation 0.6}$$

Where *Re* and S_W, in each curve are the slope and the ordinate at zero phospholipid concentration, respectively. By applying Equation 4.6, at intervals of % OD for different phospholipid concentrations, the change in *Re* and S_W can be determined. $P_{mem/w}$ can be obtained from those parameters and Equation 4.5. This particular technique gives the parameters during the solubilisation process.

Table 2. The amount surfactant added to the liposome suspensions.

Surfactant	**Surfactant concentration (mmol L^{-1})**																	
C_{13}IDZ.HCl	0	0.2	0.4	0.6	0.8	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0

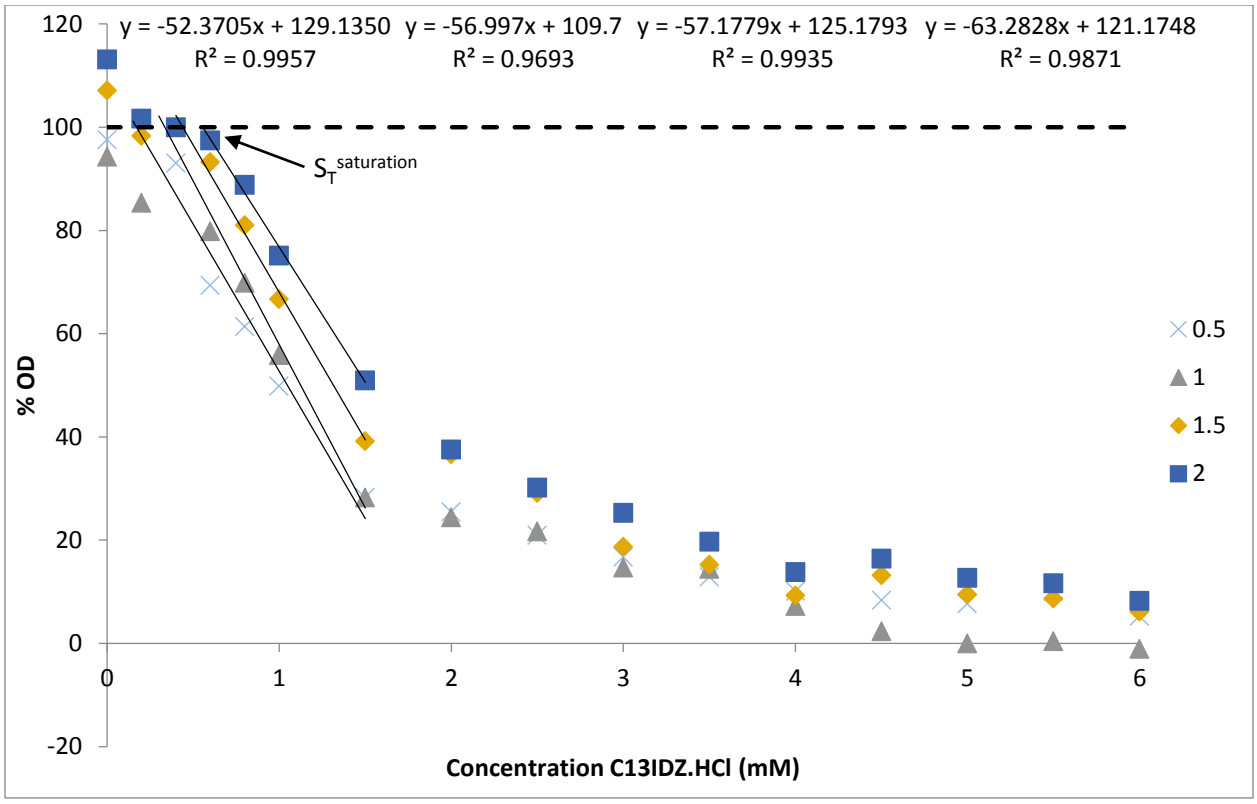


Figure 2. OD profile of solubilisation of increasing concentrations of liposomes (0.5, 1.0, 1.5, 2.0 mmol L^{-1}) resulting from the addition of increasing amounts of C_{13}IDZ.HCl at pH 6.0.

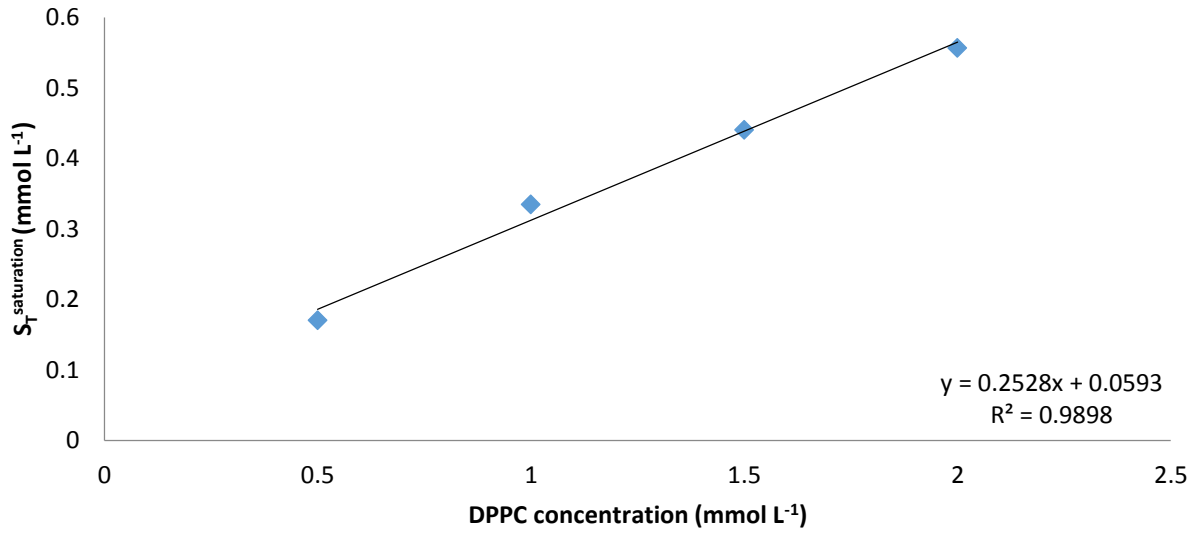


Figure 3. Concentration C_{13}IDZ.HCl (S_T) required to saturate (< 100% OD) control liposomes at concentrations of 0.5, 1.0, 1.5, 2.0 mmol L^{-1} (pH 6.0). The linear trend provides the variables for Eq. 4.6.

2.4. Dynamic light scattering

The hydrodynamic diameter (HD) of the liposome solutions were determined before fluorescent measurement using DLS. DPPC liposome solution (1 ml, 1 mmol L^{-1}) was placed in a plastic disposable sizing cuvette. Each sample was measured in triplicate at 25 ^{o}C, with an equilibration time of 120 s, at a backscatter angle of 173^{o}. The data was processed using Malvern Zetasizer software (Version 6.30 (2011)).

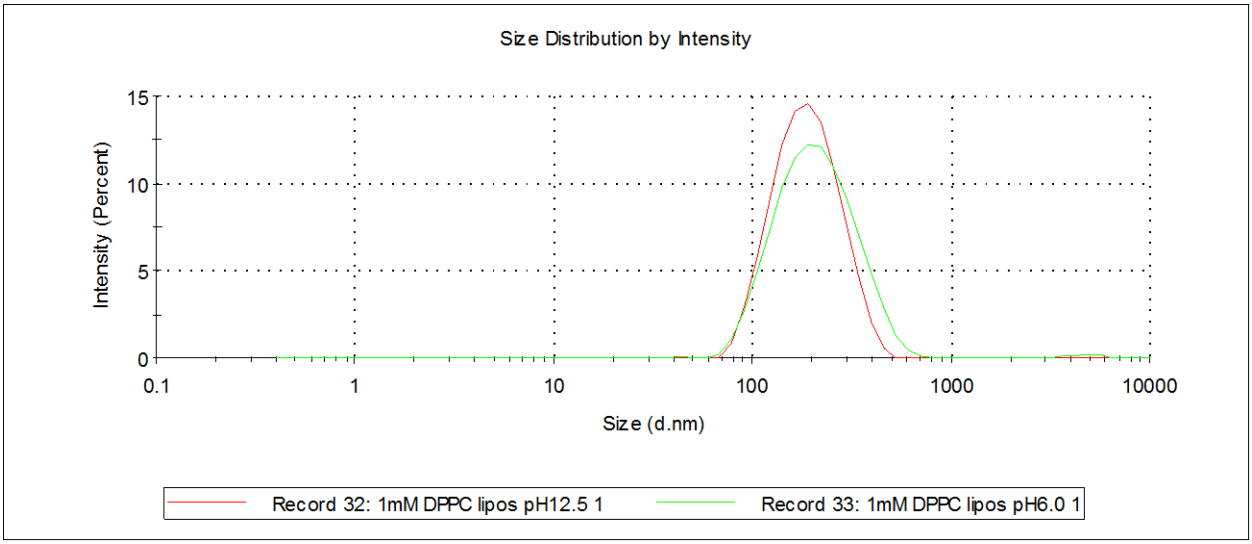


Figure 4. DLS measurements of DPPC liposomes extruded by 200 nm polycarbonate membranes, before (pH12.5, green, PDI 0.17) and after (pH 6.0, green, PDI 0.19) the addition of HCl.

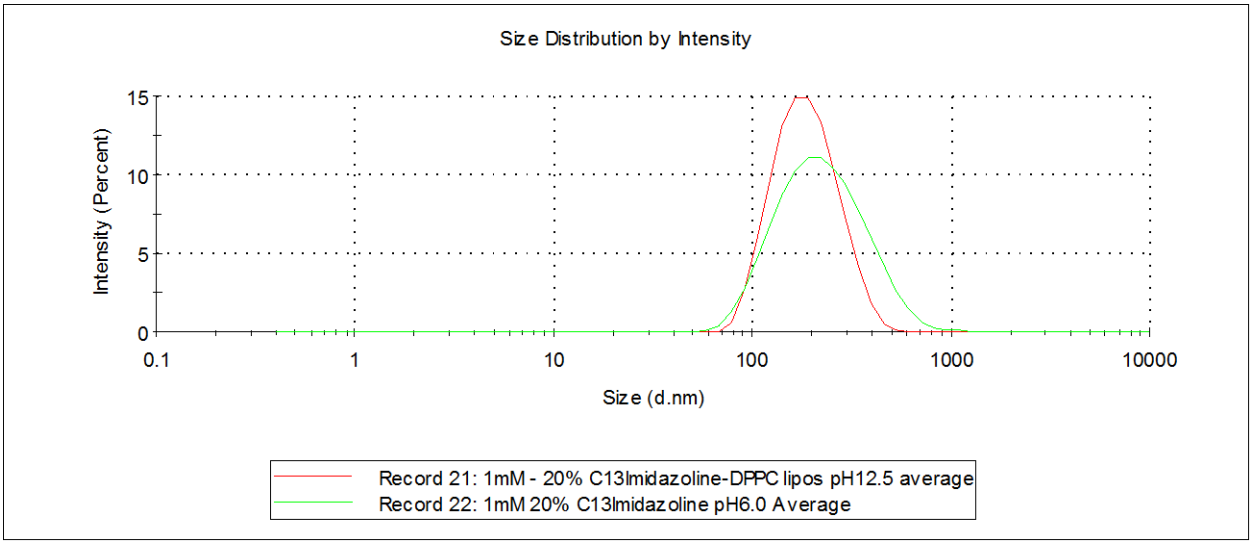


Figure 5. DLS measurements of DPPC liposomes incorporating 20 mol% C_{13}IDZ, extruded by 200 nm polycarbonate membranes, before (pH 12.4, red, PDI 0.12) and after (pH 6.0, green, PDI 0.19) the addition of HCl.

2.5. pK_a determination of C_{13}IDZ

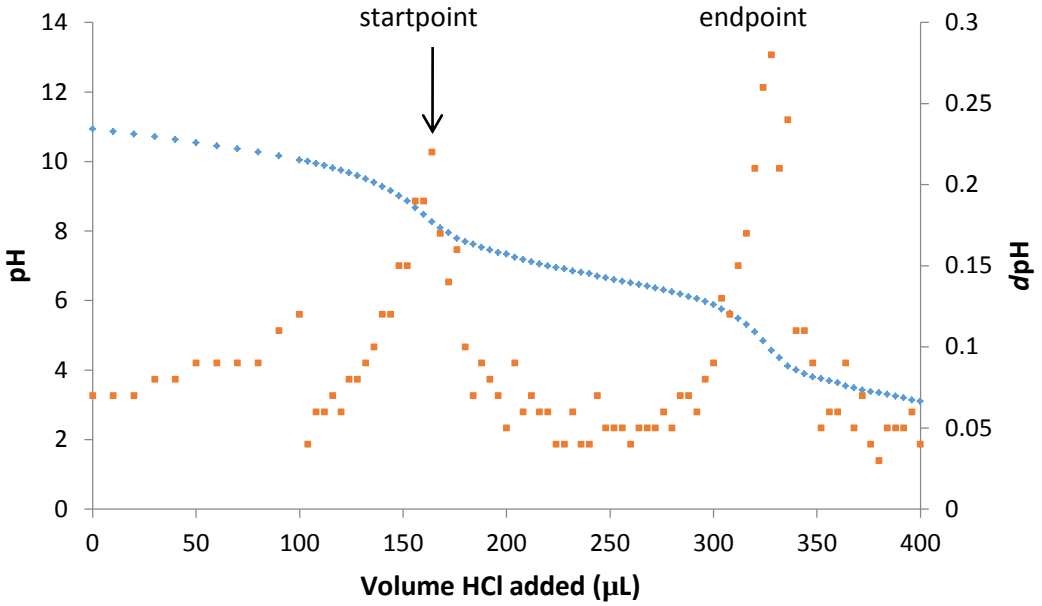


Figure 6. Measurements of pH of 20 mol% C_{13}IDZ-liposomes titrated with HCl (◆). First derivation of the pH showing the start and end points of the titration (■). pK_a = 6.7.

3. CF Release Procedures

3.1. Fluorescence Standard Curves to Determine CF Concentration at Different pH

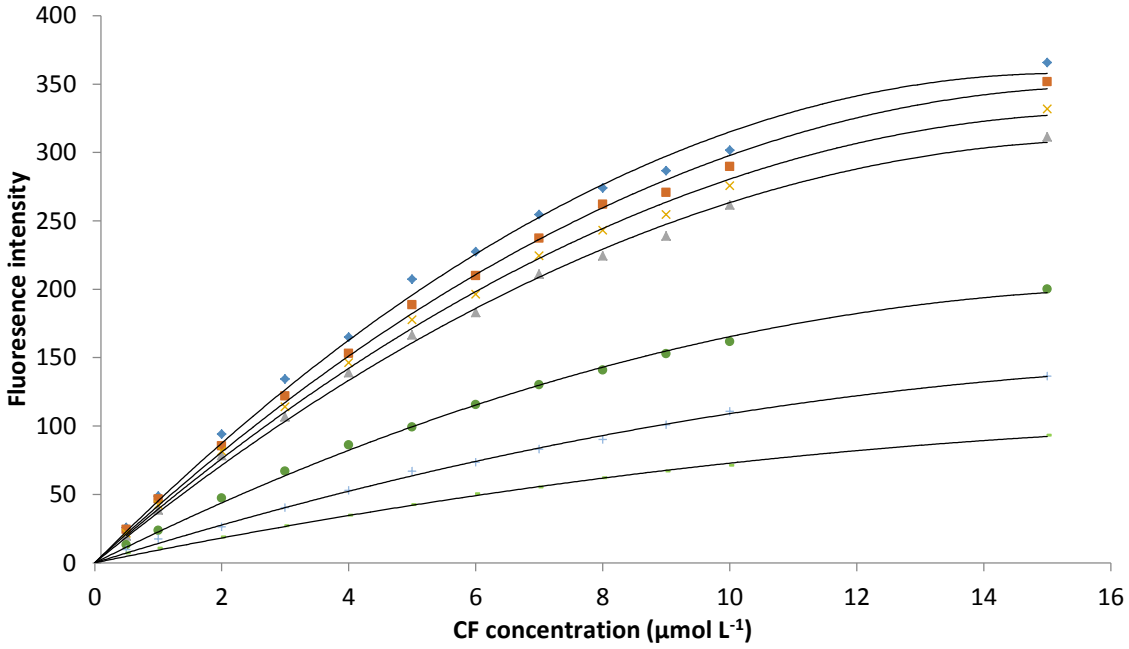


Figure 7. Fluorescence intensity with change in CF concentration at pH 5.5 (–), 6.0(**+**), 6.5 (●), 7.0 (▲), 7.5 (**x**), 8.0 (■) and 9.0 (◆) in 20 mmol L^{-1} phosphate buffer and measured in triplicate.

3.2 Representative example of Raw Fluorescence data

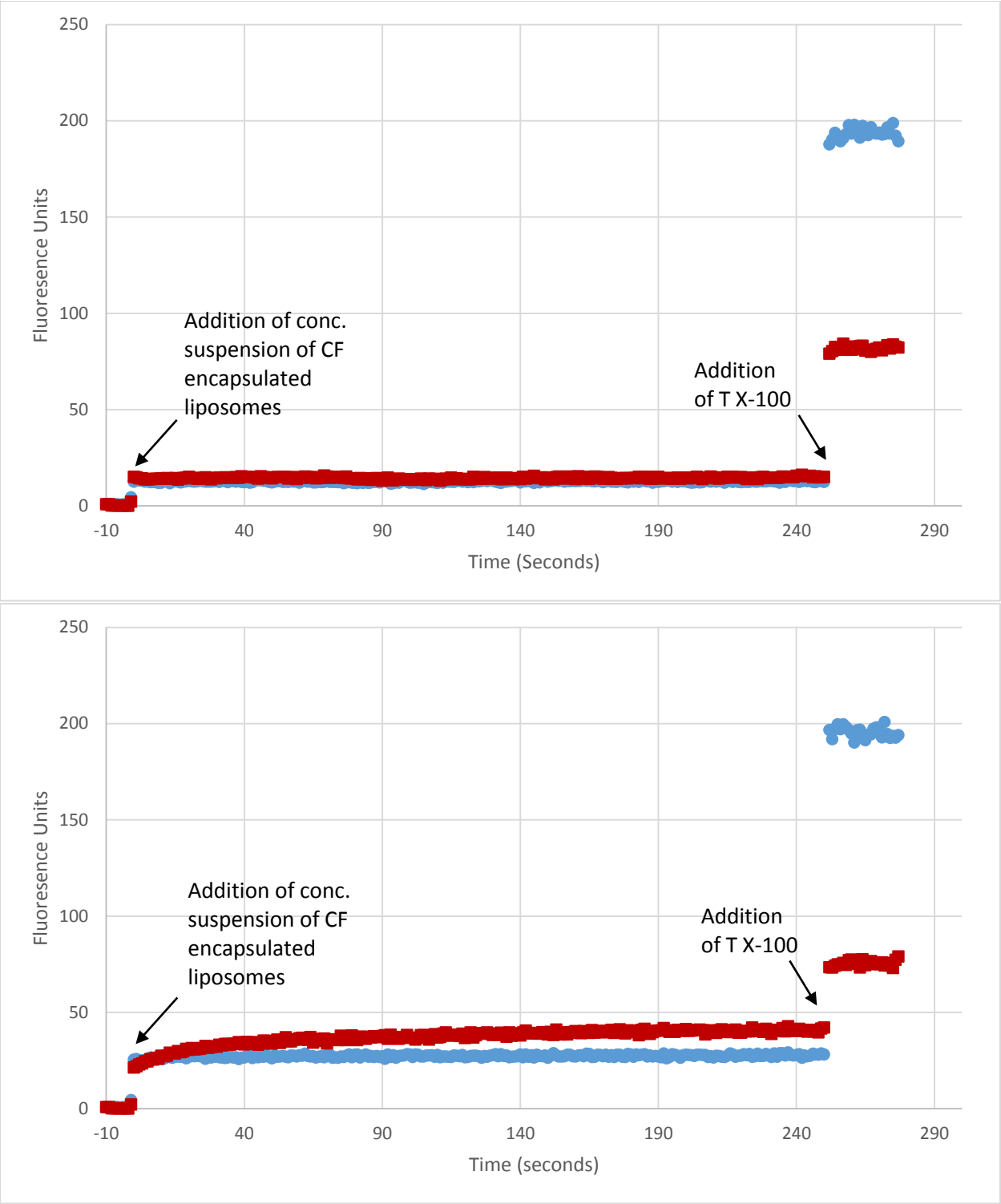


Figure 8. Examples of raw fluorescence curves resulting from the addition of control (Top) and 10% C_{13}IDZ (bottom) liposomes to buffer solutions at pH 12.4 (Blue) and pH 6.0 (Red) followed by the addition of T X-100.

3.3. Longterm release

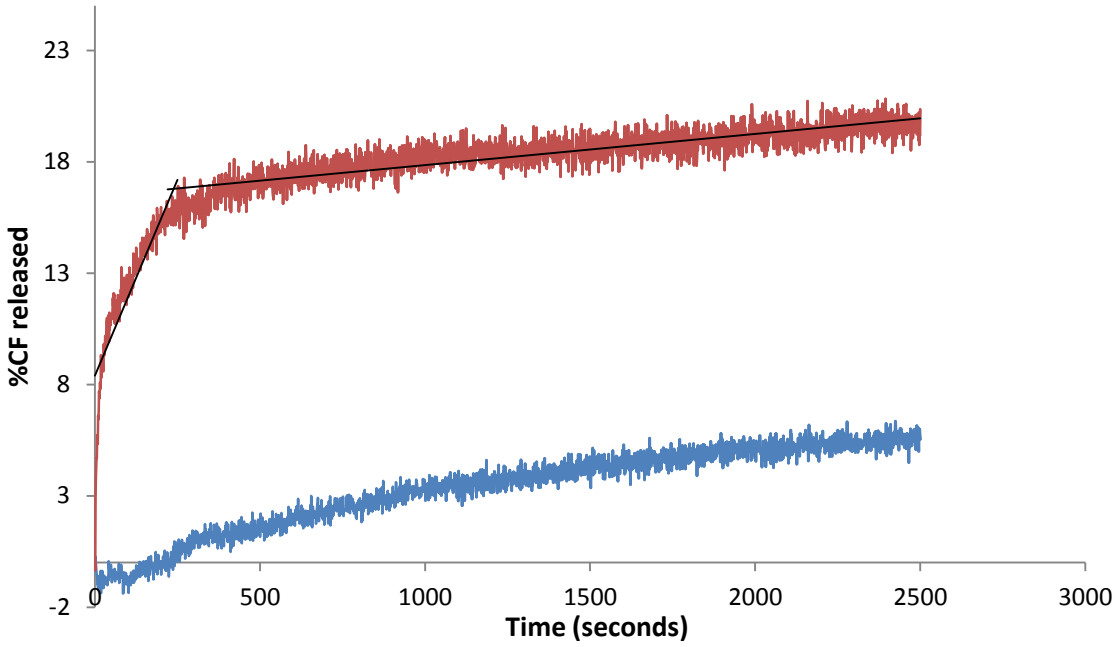


Figure 9. Long term release (approximately 2500 seconds) of CF from control liposomes (blue) and 10% C_{13}IDZ-liposomes (red) from an HCl induced pH change of the suspension from 12.4 to 6.5 at 20 °C.

References

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