Appendix A Grouped titration data

Test Number	1			2			3		
Salt Stoich.									
Na	0			0			0		
K	0			1			1		
Cl	0			1			1		
NO3	0			0			0		
SO4	0			0			0		
Ionic Str	0			0.01			0.05		
Mass	0			1.49			7.46		
Temperature	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**
in °C	24.68	5.40	21.66	24.62	5.23	21.83	24.45	4.34	20.85
Sample tube	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**
1	1.86	98.7	98.4	1.82	98.5	98.5	1.83	98.6	98.5
2	1.91	98.6	98.3	1.88	98.7	98.5	1.93	98.4	98.7
3	1.99	98.1	98.1	1.97	98.3	98.2	1.98	98.1	98.1
4	2.02	98	98	2.06	98	98	2.04	98.1	98.1
5	2.06	98.1	97.9	2.21	97.9	97.6	2.2	97.7	98
6	2.1	98	97.8	2.34	97.4	97	2.4	97.1	97.3
7	2.24	97.8	97.5	2.47	96.9	96.9	2.43	96.9	96.8
8	2.36	97.2	97	2.55	96.5	96.4	2.55	96.5	97
9	2.61	96.7	96.4	2.73	96.2	95.9	2.64	96	96.2
10	2.71	96.1	95.8	2.81	96.7	95.4	2.77	95.8	95.9
11	2.79	95.8	95.5	2.93	95	94.8	2.87	95	95.2
12	2.97	95.1	94.9	3.09	94.8	94.1	2.95	94.9	95.3
13	3.08	94.5	94.2	3.11	94.1	94.3	3.12	94	94.5
14	3.16	94.5	94.3	3.66	89.9	90.5	3.3	92.2	93.1
15	3.21	93.9	94	3.7	89.8	90	3.33	92.8	93.2
16	3.46	92	92.2	4.02	85.6	86.4	3.73	88.7	89.8
17	3.58	91.1	91.2	4.15	84	84.8	3.82	86.8	88.2
18	3.82	88.6	88.8	4.2	83.1	84	3.89	86.2	87.8
19	4.04	86.3	87	4.36	80.3	81.5	3.92	85.8	87
20	4.28	82.2	83	4.57	77.5	78.5	4.2	81.4	83
21	4.39	80	81.4	4.61	77.5	78.3	4.28	80.9	82.5
22	4.6	77.9	78.5	4.65	76.8	77.5	4.6	76.1	78
23	4.83	74.6	75.2	4.77	75	75.9	4.79	74	75.9
24	4.99	72.9	73.4	4.97	72.9	73.5	4.94	72.3	73.8
25	5.27	69.4	69.6	5.14	70.8	71.2	5.2	68.5	69.4
26	5.4	67.8	67.5	5.35	67.9	68	5.46	64.6	65.2
27	5.65	63	62.8	5.62	62.7	62.2	5.64	61	61
28	5.89	56.1	55.7	5.82	58	57.2	5.79	57.2	57.2
29	5.93	56	55.6	5.94	53.6	53.3	6.05	49	48.9
30	6.19	47.9	47.8	6.18	46	45.6	6.22	43.3	43
31	6.38	40.5	40.3	6.42	39	38.5	6.39	39	39
32	6.51	38.3	38	6.68	32.1	31.8	6.53	35	34.9
33	6.78	31	31.3	6.89	28.4	28.2	6.79	29.5	29.5
34	6.99	26.9	27	6.98	27	27.1	7.01	26	26.4
35	7.97	20.6	21.3	8.04	20.4	21.3	7.94	20.5	21.2
36	8.89	20.1	21	9	19.8	20.7	9.05	19.8	21.2
37	10.05	19.8	20.7	10.04	19.9	20.2	9.93	19.8	21.2
38	10.99	19.5	20.3	11.01	19.5	20.1	11.1	19.4	20
39	11.91	19.5	20.3	12.03	19.6	20.2	12.02	19.6	20.1
40	13.02	20.6	21.2	13.01	20.6	21	12.95	20.3	21

Test Number	4			5			6		
Salt Stoich.									
Na	0			0			0		
K	1			1			2		
Cl	0			0			0		
NO3	1			1			0		
SO4	0			0			1		
Ionic Str	0.01			0.05			0.01		
Mass	2.02			10.11			1.16		
Temperature	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**
in °C	24.96	4.05	21.00	24.68	5.40	21.66	24.74	4.47	21.10
Sample tube	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**
1	1.84	98.5	98.7	1.84	98.6	98.5	1.89	98.4	98.5
2	1.96	98.6	98.4	1.86	98.6	98.8	1.9	98.2	98.5
3	2.01	98.5	98.5	1.95	98.6	98.5	2	98.3	98.3
4	2.06	98.1	98	2.01	98.2	98.1	2.01	98	98
5	2.19	98	97.8	2.06	98.1	98.1	2.09	98	97.9
6	2.33	97.6	97.4	2.2	98.1	97.9	2.39	97	97
7	2.43	97	97.1	2.3	97.5	97.2	2.5	96.6	96.9
8	2.53	96.7	96.5	2.49	96.9	97	2.51	96.8	96.8
9	2.65	96.1	96.3	2.67	96.6	96.3	2.52	96.7	96.5
10	2.7	96.1	96.1	2.83	95.8	95.8	2.9	95	95.1
11	2.84	95.4	95.7	2.9	95.5	95.4	2.95	94.9	95
12	2.9	95.3	95.1	3.04	95.2	94.9	3.04	94.5	94.9
13	3.04	94.7	94.7	3.22	93.7	93.9	3.25	93.4	93.7
14	3.13	94.2	94.3	3.37	92.4	92.9	3.39	92.5	92.9
15	3.18	93.6	94	3.42	92	92.8	3.54	91	91.8
16	3.47	91.5	92.2	3.55	91.1	91.2	3.7	89.1	90.1
17	3.58	90.6	91	3.65	90	90.9	3.92	86.7	87.8
18	3.78	88.6	89.4	4	85.5	86.5	4.02	85	86.5
19	3.92	86.5	87.9	4.06	84.5	85.9	4.12	83.5	85
20	4.19	82.6	84.1	4.14	83.4	84.9	4.17	83	84.3
21	4.41	79.1	80.9	4.48	78.1	79.7	4.32	80.3	82.1
22	4.64	76	77.8	4.55	77.2	78.9	4.44	79	80.8
23	4.92	72.6	74.2	4.88	73.3	74.5	4.66	75.9	77.6
24	5.02	71.7	72.9	4.98	72	73	4.95	72.9	73.9
25	5.14	70.1	71.3	5.23	69	69.5	5.19	69.5	70.3
26	5.42	66.3	67	5.48	64.7	64.7	5.4	66.6	67
27	5.67	61.2	61.2	5.59	62.7	62	5.59	63.4	63
28	5.85	56	56	5.81	56.5	56	5.78	58.6	58.5
29	5.98	52.1	52	5.97	52.4	51.6	6.02	51.6	51.1
30	6.16	47.3	46.9	6.28	42.4	41.3	6.18	46.9	46.1
31	6.38	39.5	39	6.43	37.4	36.9	6.46	38.5	37.7
32	6.61	34.1	33.9	6.68	32	31.1	6.54	35.6	35
33	6.83	29	29.2	6.81	29.4	28.7	6.83	29.4	29.4
34	7.02	26.4	26.7	7.01	25.9	25.8	7.02	26.7	26.6
35	8.04	20.4	21.4	7.96	20.6	21.2	7.91	20.7	21.7
36	9.09	19.7	21.1	9.05	19.9	20.7	9.13	20.1	21.5
37	9.85	19.8	21	9.96	19.6	20.6	9.94	19.5	20.6
38	11	19.5	20.2	10.91	19.5	20.4	10.99	19.5	20.5
39	11.97	19.5	20	11.97	19.7	20.3	12	19.5	20
40	12.98	20.5	21.2	12.85	20.2	20.8	12.96	20.5	21.1

Test Number	7			8			9		
Salt Stoich.									
Na	0			0			1		
K	2			0			0		
Cl	0			0			1		
NO3	0			0			0		
SO4	1			0			0		
Ionic Str	0.05			0			0.01		
Mass	5.81			0			1.17		
Temperature	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**
in °C	24.50	4.57	21.09	24.30	4.57	21.16	24.22	4.52	20.53
Sample tube	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**
1	1.99	98.4	98.2	1.96	98.5	98.5	1.94	98.6	98.7
2	2	98.3	98.2	2.06	98.2	98.1	1.95	98.4	98.5
3	2.01	98.4	98.2	2.11	98.2	98.1	2.02	98.1	98.2
4	2.07	98.2	98.1	2.12	98	97.9	2.02	98.5	98.4
5	2.15	98	97.9	2.15	98.2	98	2.08	98	98.1
6	2.23	97.8	97.5	2.46	97	97	2.21	98	97.9
7	2.25	97.9	97.7	2.47	97	96.9	2.29	97.6	97.7
8	2.44	97.1	97	2.57	97	96.8	2.4	97.5	97.3
9	2.53	96.9	96.5	2.8	96	95.9	2.43	97.1	96.9
10	2.65	96.5	96	2.89	95	95	2.62	96.1	96.4
11	2.69	96.3	96.1	2.9	95.3	95.2	2.65	96	96.2
12	2.83	95.9	95.8	3.03	94.9	95	2.81	95.9	95.9
13	2.86	95.6	95.2	3.25	93.6	93.5	2.9	95.1	95.3
14	3	95	94.9	3.38	92.4	92.8	2.97	95	95
15	3.05	94.8	94.4	3.47	92	92.4	3.02	94.9	95
16	3.24	93.8	93.5	3.86	87.4	88.5	3.14	94.2	94.1
17	3.49	92	92	3.87	87.1	88.1	3.44	91.9	92.5
18	3.56	91	91.1	3.88	87.2	88.2	3.7	89.1	90
19	3.8	88.3	88	3.89	87.2	88.1	3.99	85.3	86.8
20	3.97	85.9	86.8	4.27	81.3	82.7	4.15	83	84.4
21	4.24	81.6	83	4.39	79.9	81.2	4.29	80.9	82.5
22	4.44	78.9	80.2	4.46	79	80.2	4.65	76	77.6
23	4.77	74.4	75.8	4.66	76	77.5	4.72	75	76.5
24	5.05	71	72.1	4.81	74	75.3	4.93	72.5	73.8
25	5.23	68.8	69.3	5.03	71.9	72.8	5.11	70.2	71.5
26	5.52	64.2	64.2	5.19	69.6	70.5	5.21	69.1	70
27	5.66	61.2	60.9	5.55	64.7	64.6	5.52	64.4	64.7
28	5.85	56	56.6	5.68	61.8	61.9	5.8	58	57.9
29	5.96	53.1	53	6.01	53	52.3	6.05	50.9	50.1
30	6.17	46	45.9	6.28	44.5	43.6	6.2	46	45.3
31	6.44	38.2	37.3	6.4	40.9	40.7	6.35	41.2	40.5
32	6.58	34	33.8	6.64	33.8	33.4	6.55	35.7	35.2
33	6.75	30.7	30.9	6.78	30.7	30.8	6.76	30.5	30.4
34	7.02	25.9	25.8	7.01	26.8	27	6.99	26.8	27
35	7.81	21	22	7.98	20.3	21.5	7.94	20.3	20.9
36	9.17	19.8	20.8	8.74	19.8	21.1	8.85	19.7	20.2
37	9.87	19.5	20.5	10.02	19.4	20.6	10.11	19.5	20.1
38	11.03	19.4	20	11	19.3	20.1	11.04	19.3	20
39	11.98	19.6	20.1	12.02	19.2	19.9	12.03	19.5	20
40	12.97	20.5	21.1	12.74	20	20.5	12.9	20.3	21

Test Number	10			11			12		
Salt Stoich.									
Na	1			2			2		
K	0			0			0		
Cl	1			0			0		
NO3	0			0			0		
SO4	0			1			1		
Ionic Str	0.05			0.01			0.05		
Mass	5.84			0.95			4.73		
Temperature	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**	**Titration**	**Cold**	**Room**
in °C	25.01	4.51	21.01	25.07	4.55	21.21	25.14	4.58	21.62
Sample tube	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**	**pH**	**% Trans**	**% Trans**
1	1.9	98.4	98.5	1.98	98.5	98.2	2.08	98.4	98
2	1.91	98.5	98.5	1.99	98.4	98.2	2.08	98.3	98.1
3	1.97	98.3	98.4	2.1	98.2	98	2.09	98.1	98.2
4	2.06	98	98.1	2.12	98.1	98.1	2.17	98.2	98
5	2.2	97.8	97.9	2.22	97.9	97.9	2.19	98	98
6	2.27	97.5	97.5	2.32	97.5	97.5	2.23	98	97.9
7	2.34	97.5	97.3	2.46	97	97.1	2.38	97.5	97.3
8	2.47	96.9	97	2.57	96.5	96.7	2.44	97.4	97
9	2.58	96.5	96.5	2.64	96.5	96.2	2.49	97	96.5
10	2.7	95.9	96.1	2.7	96.1	96.2	2.63	96.5	96.7
11	2.84	95.3	95.6	2.72	96	96.2	2.65	96.6	96.4
12	2.94	94.8	95.1	2.78	95.8	95.9	2.71	96.3	96
13	3.06	94.1	94.5	2.96	95.1	95	2.82	95.9	95.9
14	3.11	94	94.3	3.07	94.2	94.1	2.99	95	95
15	3.26	92.9	93.2	3.23	93.5	93.7	3.06	94.7	94.8
16	3.34	92.2	92.9	3.26	93.4	93.8	3.27	93.5	93.2
17	3.61	89.9	91	3.33	92.8	92.9	3.4	92.5	92.7
18	3.7	88.5	89.8	3.57	90.8	91.1	3.52	91.5	91.8
19	4.03	84	85.5	3.77	88.9	89.4	3.8	88	88.8
20	4.17	81.9	83.6	3.91	86.6	87.9	3.89	86.9	87.2
21	4.36	79.2	81	4.08	84.2	85.6	4.21	81.5	83.2
22	4.68	75	76.7	4.38	79.5	81.1	4.61	76	77.8
23	4.86	72.5	74	4.71	75.1	76.8	4.8	73.8	75.1
24	5	70.9	72.1	4.98	72	73.1	5.03	70.9	72.2
25	5.25	67.6	68.6	5.18	69.7	70.5	5.17	69.1	70
26	5.45	64.6	65	5.37	67.2	67.9	5.42	65.5	66
27	5.63	61	61.2	5.55	64	64.2	5.51	63.7	64
28	5.75	57.7	58	5.8	57.9	57.8	5.78	57.4	57.1
29	5.97	51.5	51.2	5.99	52.9	52.5	5.89	54.5	54
30	6.28	41.8	41.2	6.2	45.9	45	6.2	44.6	44
31	6.4	37.7	37	6.38	40.5	40.3	6.33	40.9	40.2
32	6.69	31	30.8	6.58	34.7	34.8	6.67	31.7	31.4
33	6.8	28.6	28.7	6.8	29.8	30	6.88	28	28
34	7.06	25	25.4	7.05	26	26.2	6.96	26.5	26.4
35	7.98	20.4	21.5	8.01	20.3	21.4	7.99	20.5	21.2
36	9.21	19.5	20.9	8.84	19.5	21	9.09	19.5	20.6
37	10.01	19.2	20.4	9.99	19.1	20.4	9.96	19.5	20.5
38	11	19.4	20.2	10.82	19.3	20.1	11.09	19.2	19.9
39	11.97	19.2	20	12	19.3	20	11.93	19.4	20
40	12.73	20.1	20.9	12.69	19.7	20.3	12.79	20.1	20.6

Appendix B pK_a Calculation steps

The spreadsheet procedure used to find the pK_as and absorptivity factors for fluorescein can be subdivided into a number of steps.

B1 Data entry:

1. Titration data were entered on a worksheet: These data included the test salt quantities, test temperatures, titration pH steps, and transmittance values.
2. Transmittance readings were converted to absorbance values, and these were then corrected for the effects of stray light.

B2 Determine the buffer composition:

1. Temperature corrected ionisation constants were calculated (Equation 23) for each buffer salt (omitting fluorescein) at the buffer measurement temperature.
2. A look-up table was set up with a series of decreasing proton concentration values in the top row.
3. The charge balance at each proton concentration was calculated using the temperature corrected ionisation constants, salt concentrations and an estimated HCl addition, and a precise charge balanced proton concentration was calculated.
4. The activity coefficient was calculated at the charge balanced ionic strength and this was used to calculate the pH.
5. The "Solver" function was used to find the HCl addition concentration where the activity corrected proton concentration was the same as the measured pH.
6. This calculated buffer chemical composition was used in subsequent calculations.

B3 Determine the amount of KOH added at each titration step:

1. Temperature corrected ionisation constants were calculated for each of the test salts (omitting fluorescein) at the titration temperature.
2. The amount of KOH added at each titration step was calculated in a similar manner to that used to find the buffer HCl addition (B2).
3. Once the KOH addition was found for each of the 40-titration steps the precise chemical composition of each titration sample was known.

B4 Determine the fluorescein parameters at low temperature:

1. Temperature corrected ionisation constants for each of the test salts were calculated for the low temperature absorbance test.
2. A look-up table was set up using the corrected ionisation constants and titration sample composition so as to find the exact charge balance pH.
3. Estimated fluorescein pK_as were used to calculate the different fluorescein ionic species concentrations at the calculated pH.
4. Estimated absorptivity factors were used to calculate the total absorbance of the fluorescein after adjusting for the KOH addition dilution effect.
5. The estimated fluorescein pK_as and absorptivity factors were adjusted using the "Solver" function until the best least-squares fit was found for all 40 titration samples.
6. These mixed fluorescein pK_as were activity corrected by adding the appropriate activity correction factor.

B5 Determine the fluorescein parameters at room temperature:

1. The activity corrected pK_as and absorptivity parameters at room temperature were calculated as for the low temperature test (B4).

B6 Calculate the thermodynamic pK_a values of fluorescein:

1. The enthalpy of reaction was calculated for each fluorescein ionisation constant using Equation 24 in combination the low temperature and room temperature activity corrected pK_as.
2. The thermodynamic pK_as were calculated using Equation 23, the reaction enthalpies and the low temperature activity corrected pK_as.

Appendix C Buffer calculation example

EXAMPLE OF BUFFER COMPOSITION CALCULATION

Reporter	17.00	17.33	17.67	
Stock for 1 to 28		Liters2	14	Liters
Buffer Stock		MW	Mass (g)	Molarity
NaAc	0.005	82.03	5.74	0.005
KH_2PO_4	0.005	136.09	9.53	0.005
Fluorescein	102.6	mg		
+Container	4.46	g		
Container =	4.36	g		
+ KOH	4.74	g	MW	Molarity
KOH =	0.28	g	56.11	2.68E-04
+ Water	31.53	g		
Fluid mass	27.17	g		
Pre del	40.99	g		
Post del	20.55	g		
Delivered	20.44	g sol	MW	Molarity
Fluorescein	5.51	mg/l	376.28	1.46E-05
Bulk pH	1.88			
HCl added	0.024	mol/l		

Please note that columns O to AA have been compressed.

Test charge balances at different p[H^+] and vary [HCl]

						Screening pH													
				p[H^+]		1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	#				
p[H^+] Increment	0.05			[H^+]		3E-02	3E-02	3E-02	2E-02	2E-02	2E-02	2E-02	1E-02	1E-02	#				
				K_a															
			Acetate	1.76E-05		6E-04	6E-04	7E-04	8E-04	9E-04	1E-03	1E-03	1E-03	1E-03	#				
			$H_2PO_4^-$	7.18E-03		2E-01	2E-01	2E-01	2E-01	3E-01	3E-01	3E-01	3E-01	4E-01	#				
			HPO_4^{2-}	6.21E-08		4E-07	4E-07	5E-07	7E-07	8E-07	1E-06	1E-06	1E-06	2E-06	#				
			PO_4^{3-}	4.46E-13		5E-18	7E-18	1E-17	1E-17	2E-17	3E-17	3E-17	5E-17	6E-17	#				
			[OH-]	9.85E-15		3E-13	3E-13	4E-13	4E-13	5E-13	6E-13	6E-13	7E-13	8E-13	#				Calc.
pH	Acetate	Fixed Pos	HCl (Var)	H3PO4 Tot		1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	#	p[H^+]	Low	High	p[H^+]
1.88	0.0050	0.0103	**0.02407**	0.0050		-2E-02	-1E-02	-1E-02	-7E-03	-5E-03	-3E-03	-5E-04	1E-03	3E-03	#	1.80	-5.1E-04	1.3E-03	1.81
						1.50	1.55	1.60	1.65	1.70	1.75	1.80	1.85	1.90	#				

Calculate activity when charges balance

Charge	1	1	1	-1	-1	-1	-2	-3	-1				
[Proton]	H^+ Test	Potassium	Sodium	Acetate	Hydroxide	$H_2PO_4^-$	HPO_4^{2-}	PO_4^{3-}	Est. HCl	Ionic	Proton		
1.814	1.5E-02	5.3E-03	5.0E-03	5.7E-06	6.4E-13	2.0E-05	6.7E-13	1.7E-25	2.4E-02	Strength	Activity	pH	Diff
Ion contribution	1.5E-02	5.3E-03	5.0E-03	5.7E-06	6.4E-13	2.0E-05	2.7E-12	1.5E-24	2.4E-02	0.025	0.855	1.88	0.000
											Solver Target Value =		5.9E+03

Appendix C Buffer calculation explanation

The worksheet on the previous page shows an example of the buffer composition calculation used in tests 1 to 12.

1. The value in cell B3 is the test number which is used to look up the test data on a separate worksheet and automatically enter the test data as calculation input.
2. The values inside the bordered block A4 to E20 describe the quantities of buffer additives (sodium acetate and potassium dihydrogen phosphate) and the fluorescein concentration calculation, as well as the final result of the HCl calculation (reported from cell D32 later in the worksheet).
3. Block A8 to B17 contains the gravimetric data that describes how a pellet of KOH (which helps dissolve the fluorescein) is added to 102.6 mg fluorescein and is then dissolved in a small quantity of water to give a total fluid mass of 27.17g. 20.44g of this concentrated solution is added to the buffer bulk solution (14 litres).
4. The bordered block A22 to AE33 uses the values in block A4 to E20 (not including cell B20) as data, as well as temperature corrected K_a values (cells E26 to E30) from another worksheet.
5. The bordered block A22 to AE33 is a charge balance calculation so assumes that the activity coefficient is one, and uses the measured pH 1.88 (cell A32) as a reference point to set up a lookup table in cells F24 to AA33.
6. The buffer acetate molarity is read into cell B32 from cell E6 and phosphate molarity is read into cell E32 from cell E7.
7. The total charge of all the completely ionised cations is calculated in cell C32. This calculation combines the sodium from the acetate and fluorescein, and the potassium from the potassium dihydrogen phosphate and KOH.
8. An estimated HCl molarity is used in cell D32 and is used to calculate the chloride concentration. This estimated HCl concentration will be modified by the "Solver" function later.
9. Temperature corrected K_a values for acetate, the phosphate species and water are read into cells E26 to E30. These are used in the charge balance calculations.
10. A series of $p[H^+]$ values is generated in cells F23 to AA23 using an appropriate starting point (the measured pH rounded up, and then reduced by 0.5) and an

increment value (B24). These p[H^+] values are converted to proton concentrations in cells F24 to AA24.

11. Columns O to AB continue the calculations of columns F24 to N33 but are narrowed here to fit this worksheet example on a single page.
12. The values in cells F31 to AA31 and F33 to AA33 echo F23 to AA23 values and are used for lookup searches later in the calculation.
13. The proton concentrations in cells F24 to AA24 are used in combination with each species ionisation constant and molarity, to calculate the total number of those ions present at each proton concentration. These values are shown in cells F26 to AA30.
14. The sum total of the cations is then subtracted from the sum total of the anions and the resulting charge balance is shown outlined in cells F32 to AA32. (The ionic sign has been reversed to facilitate lookup searches.)
15. Horizontal lookup functions are used to report the p[H^+] just before the charges balance (AB32), the charge balance either side of the charge balance (AC32 and AD32), and to calculate a precise p[H^+] for a zero charge balance (AE32).
16. The charge balance p[H^+] (AE32) is corrected for activity effects in the next bordered block (A35 to N40) and the buffer ion concentrations are calculated.
17. The ionic strength (K39) and the proton activity (L39) is calculated and used in combination with the proton concentration, to determine the pH (M39).
18. The difference between the calculated pH (M39) and measured pH (B19) is shown in cell N39 and exaggerated (multiplied by 10^{10}) in cell N40.
19. The spreadsheet "Solver" function is used to change the HCl concentration (D32) until the exaggerated difference in cell N40 reaches a minimum value, and this final HCl concentration is used in later calculations.

Appendix D Fluorescein parameter calculation

Figure 14 shows how the fluorescein parameters were calculated. The spreadsheet "Solver" was used to find the fluorescein pK_as and absorptivity factors that gave the best least-squares fit between the calculated and measured absorbance values. Only the 31st titration sample of test 5 is shown in Figure 14 but the "Best-fit" parameters are those that gave the best overall fit for all 40 samples in this particular test run. For clarity the formulas are arranged vertically whereas the model used a horizontal arrangement.

A solving method similar to that described in Appendix C was used to determine the precise chemical composition and temperature corrected pH of each of the 40 samples in the 12-titration tests. These data were used to calculate an activity corrected proton concentration that would be equivalent to an accurate pH measurement. As this activity corrected proton concentration (pH) is used in conjunction with uncorrected fluorescein molarities the solving process yields mixed pK_as. These mixed pK_a values were activity corrected and standardised at 25°C to determine thermodynamic pK_as.

The "long-form" of the pK_a activity correction is shown in the last four rows of formulas. These formulas are a combination of Equations 17 to 21 such that only the ionic charge is altered in each case. The proton charge is shown as zero because its activity has been included in the pH calculation. The activity correction was made by adding the appropriate activity correction factor to the mixed pK_a value (listed at the top of the worksheet).

During the titration the ionic strength of the titrated solution increases and this causes the activity correction factors to change too. In this investigation the correction factor used to determine the activity corrected pK_a used that of the pK_a pH.

Best-fit (solved) values at 21°C	**Value**	**Name**	**Formula**
-Log of mixed Ka_1	2.3046	pKa1_C	
-Log of mixed Ka_2	4.2401	pKa2_C	
-Log of mixed Ka_3	6.3751	pKa3_C	
Cation absorptivity factor	19	Factor_1_C	
Neutral species absorptivity factor	1413	Factor_2_C	
Monoanion absorptivity factor	9361	Factor_3_C	
Dianion absorptivity factor	48225	Factor_4_C	
Experimental conditions			
Fluorescein molarity	1.46E-05	Flu_Conc	
Titration pH (@ 25°C)	6.43		
Measured absorbance	**0.438**	**Abs_Test**	
Solved values (temperature corrected)			
Temperature corrected proton concentration	4.79E-07	Proton_Conc	Uses similar solving method to that shown for buffer composition calculation
Ionic strength	0.081	Ionic_Str.	
Proton activity correction	0.775	Act_Correct	
Dilution factor	0.9954	Dil_Fact	
Calculated values			
First ionisation constant (Ka_1)	5.0E-03	Ka1_C	=10^-pKa1_C
Second ionisation constant (Ka_2)	5.8E-05	Ka2_C	=10^-pKa2_C
Third ionisation constant (Ka_3)	4.2E-07	Ka3_C	=10^-pKa3_C
Temperature corrected pH	6.43	pH_Calc	=-LOG(Act_Correct*Proton_Conc)
Temperature corrected proton activity	3.71E-07	Proton_Act	=10^-pH_Calc
Cation molarity (H_3Flu^+)	3.30E-12	H3Flu	=Flu_Conc/(1+Ka1_C/Proton_Act+Ka1_C*Ka2_C/Proton_Act^2+Ka1_C*Ka2_C*Ka3_C/Proton_Act^3)
Neutral species molarity (H_2Flu)	4.41E-08	H2Flu	=Flu_Conc/(Proton_Act/Ka1_C+1+Ka2_C/Proton_Act+Ka2_C*Ka3_C/Proton_Act^2)
Monoanion molarity ($HFlu^{1-}$)	6.84E-06	HFlu1	=Flu_Conc/(Proton_Act^2/(Ka1_C*Ka2_C)+Proton_Act/Ka2_C+1+Ka3_C/Proton_Act)
Dianion molarity (Flu^{2-})	7.77E-06	Flu2	=Flu_Conc/(Proton_Act^3/(Ka1_C*Ka2_C*Ka3_C)+Proton_Act^2/(Ka2_C*Ka3_C)+Proton_Act/Ka3_C+1)
Fitted absorbance value	**0.437**	**Abs_Fit**	=Dil_Fact*(Factor_1_C*H3Flu+Factor_2_C*H2Flu+Factor_3_C*HFlu1+Factor_4_C*Flu2)
Activity correction values for pKas			Formulas written in "long-form" to aid editing
Reference value to simplify calculations	-0.111	Ref	=-0.5*SQRT(Ionic_Str.)/(1+SQRT(Ionic_Str.))
Add to mixed pKa_1	-0.111		=-LOG((10^(Ref*0^2)*10^(Ref*0^2))/10^(Ref*1^2))
Add to mixed pKa_2	0.111		=-LOG((10^(Ref*1^2)*10^(Ref*0^2))/10^(Ref*0^2))
Add to mixed pKa_3	0.332		=-LOG((10^(Ref*2^2)*10^(Ref*0^2))/10^(Ref*1^2))

Solver adjusts pKas and Factors to minimise difference between measured (Abs_Test) and fitted (Abs_Fit) absorbance values for all 40 readings in the test run.
pKa activity correction values change with titration progress and increasing ionic strength.
pKa correction values adopted are those calculated at the ionic strength prevalent at pKa pH.

Figure 14 Example calculation for reading 31 (pH 6.43) of test 5

Appendix E Data for Beer's law graph

Fluorescein (mg/l)	pH <2.0	Absorb--ance	Fluorescein (mg/l)	pH 5.2	Absorb--ance	Fluorescein (mg/l)	pH >8.0	Absorb--ance
84.52	1.95	0.105	91.46	6.65	1.921	92.87	10.19	1.959
20.48	1.89	0.023	22.57	5.50	0.674	19.58	10.09	1.721
4.91	1.90	0.006	5.62	5.23	0.153	4.09	10.01	0.538
1.21	1.88	0.001	1.41	5.16	0.036	0.86	9.99	0.116
0.30	1.89	0.001	0.35	5.15	0.009	0.18	9.98	0.025
0.07	1.89	0.000	0.09	5.15	0.002	0.04	10.15	0.006
87.49	1.99	0.100	92.77	6.66	1.921	93.56	10.19	2.000
21.16	1.92	0.022	22.97	5.51	0.684	19.60	10.10	1.745
5.16	1.92	0.005	5.71	5.22	0.156	4.10	9.98	0.541
1.25	1.91	0.002	1.43	5.18	0.037	0.91	9.99	0.125
0.30	1.90	0.000	0.36	5.16	0.009	0.19	9.96	0.026
0.07	1.90	0.000	0.09	5.15	0.002	0.04	10.07	0.005
69.89	1.92	0.077	131.88	7.02	1.959	135.22	10.30	2.000
16.73	1.88	0.018	33.22	5.73	1.131	28.47	10.07	1.824
4.03	1.86	0.004	7.93	5.26	0.222	6.02	9.92	0.796
0.98	1.86	0.001	1.89	5.18	0.051	1.26	9.87	0.171
0.24	1.87	0.000	0.45	5.16	0.012	0.27	9.87	0.036
0.06	1.87	0.000	0.11	5.16	0.003	0.06	9.98	0.007
68.06	1.98	0.075	136.77	7.03	1.959	135.48	10.53	2.000
16.41	1.95	0.017	29.72	5.64	0.963	28.25	10.21	1.824
4.05	1.92	0.004	6.63	5.25	0.185	5.91	10.05	0.762
1.01	1.92	0.001	1.46	5.18	0.039	1.24	10.02	0.166
0.25	1.91	0.000	0.33	5.16	0.009	0.26	9.99	0.035
0.06	1.90	0.000	0.07	5.15	0.001	0.05	10.11	0.007
136.57	1.95	0.178	181.02	7.37	2.000	172.99	10.68	2.046
33.71	1.87	0.036	38.02	5.80	1.292	36.55	10.27	1.886
8.44	1.84	0.009	7.96	5.27	0.216	7.62	10.05	0.967
2.05	1.85	0.003	1.66	5.18	0.044	1.60	9.98	0.215
0.50	1.85	0.000	0.35	5.17	0.009	0.34	9.97	0.047
0.12	1.86	0.000	0.07	5.16	0.001	0.07	10.07	0.009
138.26	1.93	0.177	179.01	7.37	2.000	179.45	10.83	2.046
33.62	1.85	0.037	38.23	5.81	1.319	37.59	10.34	1.886
8.29	1.84	0.008	7.88	5.27	0.217	7.89	10.15	0.991
2.06	1.83	0.002	1.66	5.17	0.045	1.65	10.07	0.221
0.51	1.84	0.000	0.35	5.16	0.009	0.35	10.07	0.047
0.12	1.83	0.000	0.07	5.16	0.001	0.07	10.13	0.009

Appendix F Transmittance data from sunlight and heat degradation experiment

Run 1	Room Temperature				Hot Controls					Sun Exposed			
Hours	A	B	C	D	Hours	A	B	C	D	A	B	C	D
0.000	24.8	75.3	25.0	69.7	0.000	24.7	75.8	25.0	69.8	24.8	75.4	24.8	67.2
0.085	24.9	75.6	25.0	69.8	0.087	24.5	75.6	24.7	73.8	28.7	76.0	24.9	63.8
0.168	24.5	75.2	24.8	69.8	0.174	24.3	75.2	24.5	73.8	33.5	77.2	25.0	60.0
0.349	24.8	75.4	25.0	70.2	0.333	24.6	75.3	24.9	74.2	45.0	79.4	25.6	57.1
0.672	24.9	75.2	25.1	70.2	0.725	24.4	75.3	25.0	74.4	69.9	82.2	26.2	50.1
1.168	24.8	75.2	25.0	70.1	1.199	24.4	75.3	25.0	74.2	92.2	85.0	27.6	50.9
2.001	24.7	75.3	25.0	69.9	2.083	24.7	75.0	25.0	74.1	97.2	87.6	30.8	62.2

Run 2	Room Temperature				Hot Controls					Sun Exposed			
Time	A	B	C	D	Time	A	B	C	D	A	B	C	D
0.000	23.9	75.5	24.0	71.9	0.000	23.7	75.0	24.2	71.8	23.9	75.9	23.7	71.3
0.084	24.0	75.8	23.9	71.8	0.092	23.8	75.9	24.1	72.8	29.4	77.7	26.3	61.1
0.169	23.8	75.6	23.9	71.9	0.271	23.7	75.4	23.9	72.9	35.2	79.1	27.2	53.0
0.335	23.7	75.9	24.0	71.9	0.439	23.9	75.4	24.3	73.4	47.7	81.1	27.4	44.4
0.673	24.0	75.3	24.0	71.8	0.715	23.8	75.7	24.2	73.7	71.5	85.0	28.7	39.5
1.184	24.0	75.4	24.0	72.0	1.176	23.7	75.7	24.3	73.8	91.8	87.8	30.6	39.4
2.006	24.0	75.9	23.9	71.8	2.011	23.5	75.3	23.9	73.6	97.0	89.0	34.0	44.9

Run 3	Room Temperature				Hot Controls					Sun Exposed			
Time	A	B	C	D	Time	A	B	C	D	A	B	C	D
0.000	24.0	74.0	24.1	66.7	0.000	23.9	74.0	23.9	64.8	23.6	74.0	24.0	64.3
0.084	23.8	74.0	24.1	67.1	0.178	23.6	73.9	23.6	67.2	28.1	75.0	24.3	64.0
0.179	23.8	74.0	24.0	67.0	0.346	23.5	73.9	24.1	68.0	34.0	77.0	24.5	61.3
0.338	23.6	74.1	24.0	67.2	0.519	23.5	73.8	24.0	68.8	45.6	79.5	24.6	58.7
0.671	23.7	74.0	24.0	67.5	0.789	23.5	73.8	23.7	69.8	69.4	82.9	25.0	55.2
1.173	23.5	74.0	24.0	67.1	1.258	23.5	73.5	23.8	70.5	91.4	86.0	27.0	52.5
2.009	23.5	74.0	24.0	67.0	2.068	23.8	73.7	24.0	72.6	97.1	88.9	31.0	59.7