Simultaneous determination of solubility, dissolution and dilution enthalpies of a substance from a single calorimetric experiment

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Abstract

The thermodynamics of dissolution in water of a set of substances has been studied calorimetrically. The examined substances were: potassium chloride, (glycyl-glycyl)diketopiperazine, (alanyl-alanyl)diketopiperazine, (leucyl-glycyl)diketo-piperazine. They were chosen on the basis of their solubilities, going from a highly soluble electrolyte to the sparingly soluble diketopiperazines. It is shown that, using a commercially available calorimeter, it is possible to perform in a single calorimetric experiment the simultaneous determination of all thermodynamic parameters characterizing dissolution of a substance in a given solvent, i.e. solubility, dissolution enthalpy and dilution enthalpy. The solubility values in water obtained through the proposed method are in good agreement with those reported in the literature and obtained by other techniques. © 1998 Elsevier Science B.V.

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1. Introduction

A knowledge of the thermodynamic properties of aqueous solutions of simple organic substances is particularly useful because these compounds may be looked upon as simple models for biochemical systems. For instance, the energetics of transfer of cyclic dipeptides (i.e. diketopiperazines) from the crystal into water has been especially studied, since these model solid compounds seem appropriate in an attempt to understand the energetics of protein stability [1–8].

Most of the studies concerning model compounds require the determination of dissolution properties of sparingly soluble substances. Solubility and heats of solution as a function of temperature provide the key quantities to evaluate changes in free energy, enthalpy and heat capacity. The low solubility of many solids often does not allow the determination of reliable values for this parameter. When a suitable chromophore is present in the molecule, optical techniques such as UV absorbance are appropriate. In the absence of these chromophores, other techniques are required. One such techniques is a dry-weight determination, in which the dried residue from a known volume of saturated solution is weighed [2,9]. Another technique, the differential refractive index determination, is more versatile and yields solubilities over a range of temperatures [5]. On the other hand, enthalpies of dissolution have been measured by flow calorimetry, where a precise weight of a dry sample is dissolved in

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a continuous flow of solvent [10,11]. Alternatively, the phase-equilibrium perturbation calorimetry utilizes a titration calorimeter [1,12]. To know the complete energetics of the dissolution process, therefore, a separate determination of the solubility is required.

On the contrary, a calorimetric method reported here allows the simultaneous determination of solubility, dissolution and dilution enthalpies. This method does not require separate techniques to determine the solubility, and is independent of the nature of the examined solute and/or solvent. It allows through a single calorimetric experiment to obtain the complete energetics of the dissolution process. The examined substances were: potassium chloride, (glycyl-glycyl)-diketopiperazine c(Gly-Gly), (alanyl-alanyl)diketopiperazine c(Ala-Ala), and (leucyl-glycyl)-diketopiperazine c(Leu-Gly). These were chosen on the basis of their solubilities in water, going from a highly soluble electrolyte to the sparingly soluble diketopiperazines. Their different natures and the wide range of solubilities put in evidence the great versatility of the method, whose simplicity must be stressed. The method uses a commercially available batch calorimeter and does not require reference curves for the substance and the solvent employed. That leads to a rapid determination of the free energy of transfer from one solvent to another.

2. Experimental

The dissolution process for a pure phase going to an infinitely dilute solution can be divided into two steps: transfer of a molecule from the pure phase to a saturated solution, and transfer of a molecule from the saturated solution to an infinitely dilute solution. Here, the first process is referred to as dissolution and the second as dilution. All experiments were run at (298±0.01) K in a Thermal Activity Monitor (TAM), model 2277 from Thermometric (Sweden), equipped with a steel perfusion–titration vessel with an effective volume of about 4 mL. The temperature of the calorimeter was maintained by a water bath to within 0.001 K using a Grant LTD6 temperature control unit. Additions of titrant to the solution into the cell are performed through a syringe controlled by a Hamilton Microlab M. In a typical experiment, the reaction cell was loaded with ca. $(0.5-1.0) \times 10^{-3}$ L of the saturated

solution and excess solid of the examined substance, and the injection syringe was filled with doubly distilled water. The system was equilibrated under constant stirring until a steady baseline was obtained. The injection volumes were 15, 25 or 50 µL, depending on the heat generated by the dissolution of the substance. The mixing is ensured through a helical stirrer. The reaction required ca. 80 min to return to a steady baseline, and the heat generated as the system returns to equilibrium was measured. A computer controlled the injections and collected the data. Approximately 10–30 injections of the added solvent were made in each experiment, and at least three experiments were performed for each substance. When the solid was completely solubilized, injections of pure solvent to the homogeneous solution were continued while measuring the dilution heats.

Substances were obtained from Bachem (diketopiperazines) and Carlo Erba (potassium chloride): they were ground using a mortar and pestle to ensure maximum surface area. Saturated solutions were prepared in doubly distilled deionized water by adding sufficient substance to ensure an excess of solid.

The basis of the method is the determination of the heat upon the addition of solvent to a saturated solution, until a homogeneous solution is formed. This solution is further diluted to evaluate the dilution heats. From the determination of these heats and from the knowledge of the quantities of solute and solvent into the cell it is possible to evaluate the solubility, dissolution enthalpy, and enthalpic interaction coefficients of the excess enthalpy. In Figs. 1–4, the registered heat for each addition vs. the volume of the added solvent is reported for the four substances examined. In the presence of two phases, namely in the presence of saturated solution and solid and for constant additions of solvent, invariant heats are obtained: that occurs up to the ith addition. The $(i+1)$th addition induces a deviation from the almost invariant value, since only part of the solvent in the addition dissolves the solid, while the other dilutes the resulting saturated solution. From the $(i+2)$th addition of solvent, heats generated or absorbed represent only heats of dilution, which can be interpolated by the following function:

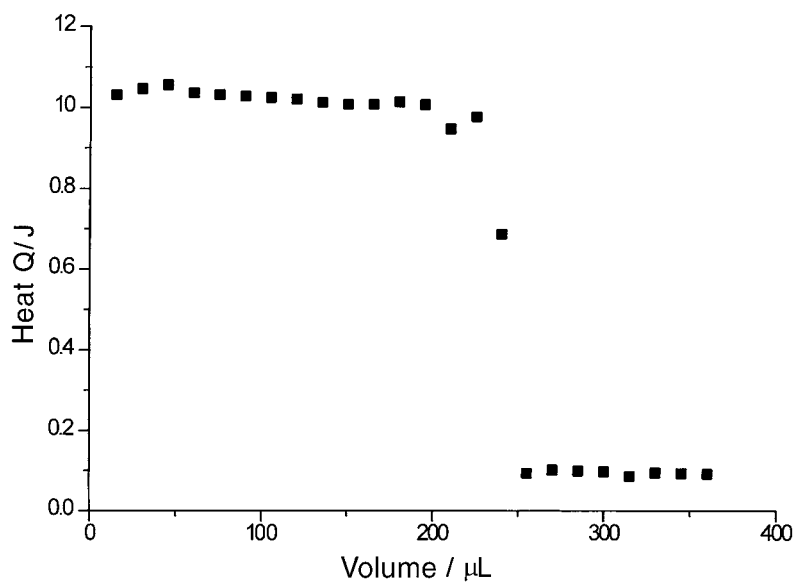$$[a + b(i + n)^x + c(i + n)^y + d(i + n)^z + \ldots] \tag{1}$$

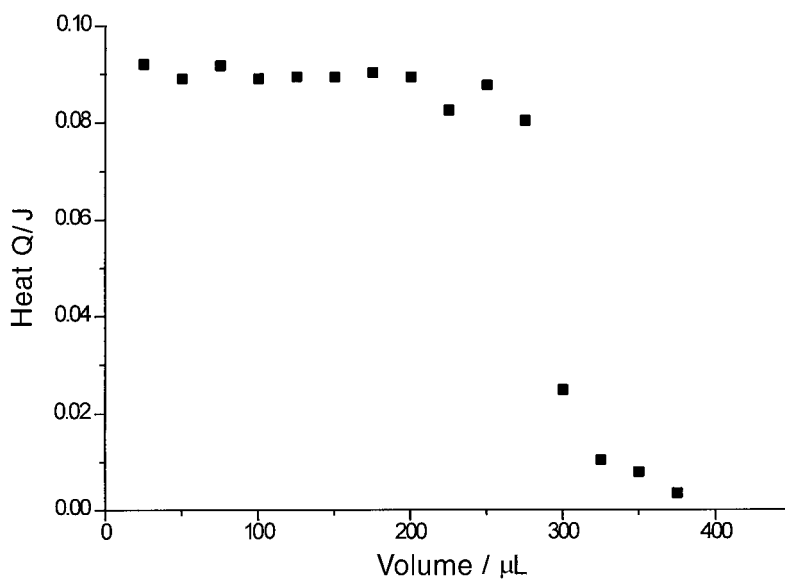Fig. 1. Heat, Q, in J, for each addition against the volume of the added solvent, in µL, for potassium chloride at 298 K.

Fig. 2. Heat, Q, in J, for each addition against the volume of the added solvent, in µL, for c(Gly-Gly) at 298 K.

where $n \geq 2$, x, y, z,... are whole or fractional numbers, and a, b, c, d,... coefficients having no physical meaning. Through the best fitting of the experimental data, the most probable values of these coefficients can be obtained. The crude experimental data are reported in the Appendix A, describing the heat gen-

erated upon the addition of a given volume of solvent to the solution in the cell. Three experiments are reported for KCl, c(Gly-Gly), c(Ala-Ala) and four for c(Leu-Gly): the mass of solvent and substance initially present in the cell for each experiment is also shown.
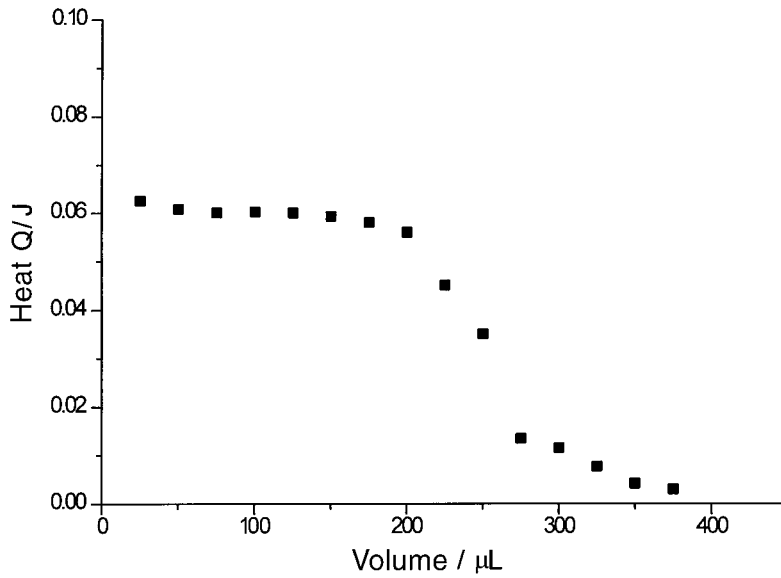
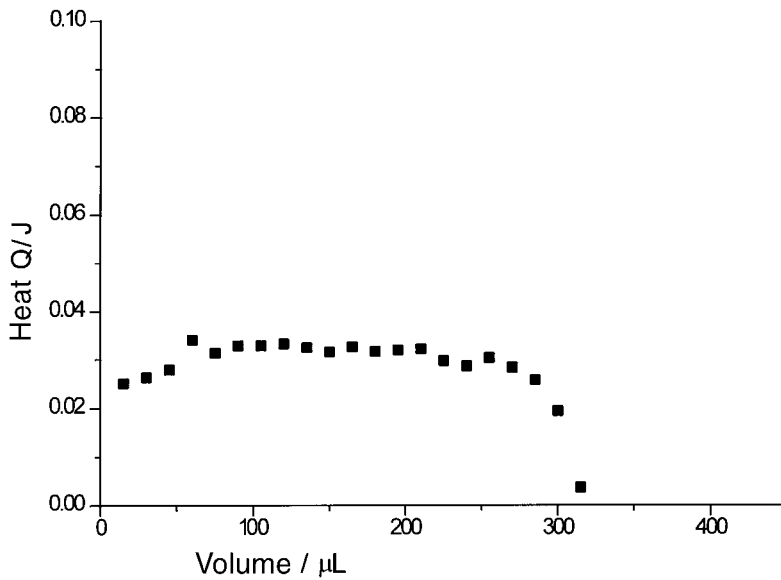Fig. 3. Heat, Q, in J, for each addition against the volume of the added solvent, in µL, for c(Ala-Ala) at 298 K.

Fig. 4. Heat, Q, in J, for each addition against the volume of the added solvent, in µL, for c(Leu-Gly) at 298 K.

2.1. Solubility and dissolution enthalpy

Q_{av} is the average value of the heat associated with the dissolution of solid, when k kg of solvent per every addition is added to the biphasic solution in the cell. Up to the ith addition, all the added solvent are needed

for the dissolution. In the $(i+1)$th addition, however, only a fraction f of the k kg of solvent dissolves the remaining solute, while the $(1-f)$th part dilutes the resulting homogeneous saturated solution. For this addition, the heat associated with the dissolution, namely the heat associated with the formation of

the monophasic system, Q_s, is given by:

$$Q_s = fQ_{av} \tag{2}$$

where $0 \leq f \leq 1$.

Considering that the $(1-f)$th part of the $(i+1)$th addition dilutes the saturated homogeneous solution, the total heat in this step, Q_{i+1}, is given by the sum of Q_s and the heat of dilution, $Q_{dil(i+1)}$:

$$Q_{i+1} \equiv Q_s + Q_{dil(i+1)} = fQ_{av} + [a + b(i+1)^x$$
$$+ c(i+1)^y + d(i+1)^z + \ldots](1-f) \tag{3}$$

Knowing the coefficients $a, b, c, d, \ldots$, the parameter f is obtained through a minimization procedure, taking the value which gives the best agreement between the experimental and calculated heats. The solubility, m_{sat}, and the final molality in the $(i+1)$th step, $m_{f(i+1)}$, are given by:

$$m_{sat} = n_T/(k_c + ik + fk) = n_T/[k_c + (i+f)k] \tag{4}$$

and

$$m_{f(i+1)} = n_T/[k_c + (i+1)k] \tag{5}$$

where $m_{f(i+1)}$ is the final concentration of the homogeneous solution, m_{sat} the solubility in mol kg^{-1} of solvent, n_T the total number of solute moles, k the kilograms of solvent for each addition, i the number of additions before the disappearance of the excess solid, $(i+1)$ the addition that provokes the solubilization of the excess solid and the dilution of the homogeneous solution, and k_c the kilograms of solvent initially present in the cell.

The enthalpy relative to the solubilization of one mole of solid up to the formation of the homogeneous saturated solution, $\Delta H^{0\prime}$, is given by:

$$\Delta H^{0\prime} = (iQ_{av} + fQ_{av})/(n_T - m_{sat}k_c)$$
$$= (i+f)Q_{av}/(n_T - m_{sat}k_c) \tag{6}$$

where $\Delta H^{0\prime}$ is expressed in J mol^{-1}.

The enthalpy relative to the dissolution of one mole of solute up to the infinitely dilute solution, ΔH^0, is:

$$\Delta H^0 \equiv \Delta H^{0\prime} - \Phi_L(m_{sat})$$
$$= [(i+f)Q_{av}/(n_T - m_{sat}k_s)]$$
$$- [A_1 m_{sat} + A_2 m_{sat}^2 + \ldots]) \tag{7}$$

where $\Phi_L(m_{sat})$, the apparent molal enthalpy of the saturated solution, is given by the polynomial on the right side of Eq. (7). The value of ΔG^0 is calculated from the solubility as:

$$\Delta G^0 = -RT \ln X_2 \tag{8}$$

and $T\Delta S^0$ as:

$$T\Delta S^0 = \Delta H^0 - \Delta G^0 \tag{9}$$

where X_2 is the solubility mole fraction. The absence of any information about the activity coefficients leads to the evaluation of parameters that are thermodynamically not exactly defined.

In Table 1, solubilities, solution enthalpies, free energies and entropies are reported for the four substances examined. The solubility values are compared with those reported in the literature. The good agreement indicates that the proposed method is as reliable as spectrophotometric and gravimetric methods. It must be underlined that when using this procedure, we are dealing with wet substances. Then, differences between the $\Delta H^{0\prime}$ values obtained through this method and those reported in the literature could eventually be found and used for the determination of the wetting heat.

According to the treatment of solution properties originally proposed by McMillan and Mayer [14] and specifically applied to those of aqueous solutions of nonelectrolytes by Kozak et al. [15] and some other authors [16–18], an excess thermodynamic property, J_T^E can be expressed as a function of molalities of pair and higher order coefficients, j, as follows:

$$J^E = \Sigma_x \Sigma_y j_{xy} m_x m_y + \text{higher terms} \tag{10}$$

Virial coefficients of the power series of the excess enthalpies as a function of molalities can be easily derived from the enthalpies of dilution of binary solutions as follows:

$$\Delta H_{dil}(m_x^i \rightarrow m_x^f) = h_{xx}(m^f - m^i)$$
$$+ h_{xxx}[(m^f)^2 - (m^i)^2] + \ldots \tag{11}$$

where m_x^i and m_x^f are the molalities of the x solute before, and after, the dilution process, respectively. According to the McMillan–Mayer approach [14], the h coefficients represent the enthalpic contributions to the Gibbs free energy coefficients characterizing the interactions between pairs, triplets or higher order

Table 1
Solubilities and dissolution energetics of potassium chloride and cyclic dipeptides at 298 K [a]

System	m_{sat}/(mol kg^{-1})	ΔH^0/(kJ mol^{-1})	$\Delta H^{0\prime}$/(kJ mol^{-1})	$\Delta G^{0\prime}$/(kJ mol^{-1})	$T\Delta S^{0\prime}$/(kJ mol^{-1})
KCl	4.839±0.02	16.3±0.2	14.5±0.2	6.25±0.01	8.2±0.2
	4.76 [b]				
c(Gly-Gly)	0.157±0.003	22.4±0.8	22.3±0.8	14.54±0.05	7.8±0.8
	0.145±0.003 [c,d]		26.2±0.2 [e]	14.7±0.05 [e]	11.5±0.3 [e]
	0.149 [f]		23.4±2 [f]		
c(Ala-Ala)	0.191±0.005	12.24±0.06	12.20±0.06	14.06±0.06	−1.9±0.1
	0.187±0.007 [g]		13.7±0.1 [e]	14.1±0.09 [e]	−0.4±0.2 [e]
	0.179±0.004 [d]				
c(Leu-Gly)	0.056±0.005	10.2±1.2	10.2±1.2	17.1±0.2	−6.9±1.4
	0.061±0.001 [g]		13.3±1.6 [e]	17.0±0.04 [e]	−3.7±2.0 [e]
	0.0481±0.0002 [c]				

[a] Confidence intervals for solubilities and ΔH^0 are the average deviations from three series of measurements, those for ΔG^0 are propagated from the errors in solubility, and those for ΔS^0 are the sum of the errors in ΔH^0 and ΔG^0. The standard state is unit mole fraction.
[b] Ref. [13].
[c] Ref. [4].
[d] Ref. [7].
[e] Ref. [5].
[f] Ref. [8]. In this reference, ΔH^0 is given at 30°C and a final concentration $m=0.0088$.
[g] Ref. [2].

coefficients. They implicitly account also for all variations of solvent–solvent and solute–solvent interactions.

Knowing the enthalpic interaction coefficients, the dilution heat for the $(i+1)$th addition can be evaluated also as follows:

$$Q_{dil(i+1)}(J) = n_T[h_{xx}(m_{f(i+1)} - m_{sat}) + h_{xxx}(m_{f(i+1)}^2 - m_{sat}^2) + \ldots] \quad (12)$$

The enthalpy for the dissolution of one mole of solute up to the infinitely dilute solution is:

$$\Delta H^0 \equiv \Delta H^{0\prime} - \Phi_L(m_{sat})$$
$$= [(i+f)Q_{av}/(n_T - m_{sat}k_s)]$$
$$- [h_{xx}m_{sat} + h_{xxx}m_{sat}^2 + \ldots] \quad (13)$$

Conversely, it is possible to evaluate the unknown enthalpic interaction coefficients through the interpolation of the experimental heats of dilution as a function of the difference in molalities.

As a conclusion, a single calorimetric experiment allows to obtain fast and reliable information on the energetics bound to the dissolution of a solute in a given solvent. The results of such studies allow to characterize the interaction between the solute functional groups and the solvent, water in particular. When the examined small-molecule model compounds contains the key elements describing the interaction of amino acid residues in a polypeptide chain, thermodynamic parameters can provide quantitative information on the contributions of the various interactions that determine protein conformation.

Acknowledgements

Appendix A

A.1. Heat generated upon the addition of a given volume of solvent to the solution in the cell

(a) Mass of c(Gly-Gly) in the cell: 0.0138 g; mass of water in the cell: 0.5110 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.09199	25
0.08901	50
0.0917	75
0.08901	100
0.08941	125
0.08936	150
0.09026	175
0.08937	200
0.08248	225
0.08763	250
0.08037	275
0.02483	300
0.01037	325
0.00781	350
0.00342	375

(b) Mass of c(Gly-Gly) in the cell: 0.0127 g; mass of water in the cell: 0.4993 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.09519	25
0.08285	50
0.09448	75
0.08327	100
0.08247	125
0.07893	150
0.07801	175
0.04137	200
0.03344	225
0.03216	250
0.00036	275

(c) Mass of c(Gly-Gly) in the cell: 0.0125 g; mass of water in the cell: 0.5049 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.09189	25
0.08818	50
0.08711	75
0.08705	100
0.08171	125
0.08085	150
0.07475	175
0.04787	200
0.02825	225

0.0002	250
0.00027	275

(d) Mass of c(Ala-Ala) in the cell: 0.0294 g; mass of water in the cell: 0.7354 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.05778	25
0.05826	50
0.05829	75
0.05826	100
0.0577	125
0.05714	150
0.05713	175
0.05693	200
0.05705	225
0.05605	250
0.05647	275
0.05485	300
0.05546	325
0.05419	350
0.04574	375
0.03155	400
0.02199	425
0.01097	450

(e) Mass of c(Ala-Ala) in the cell: 0.0259 g; mass of water in the cell: 0.5408 g; volume for each addition: 50 μL

Q/J	V_{add}/μL
0.12234	50
0.09124	100
0.11539	150
0.11357	200
0.10472	250
0.11439	300
0.11174	350
0.10085	400
0.07221	450
0.04527	500
0.02467	550
0.00151	600

(f) Mass of c(Ala-Ala) in the cell: 0.0211 g; mass of water in the cell: 0.5264 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.06258	25
0.0608	50
0.0601	75
0.06025	100
0.06004	125
0.0593	150
0.05807	175
0.05597	200
0.04509	225
0.03512	250
0.01344	275
0.01148	300
0.00766	325
0.00414	350
0.00302	375

(g) Mass of c(Leu-Gly) in the cell: 0.0071 g; mass of water in the cell: 0.5600 g; volume for each addition: 25 μL

Q/J	V_{add}/μL
0.01755	25
0.01747	50
0.01632	75
0.01554	100
0.01363	125
0.00949	150
0.01131	175
0.00899	200
0.00777	225
0.0065	250

(h) Mass of c(Leu-Gly) in the cell: 0.0087 g; mass of water in the cell: 0.7164 g; volume for each addition: 15 μL

Q/J	V_{add}/μL
0.0251	15
0.0263	30
0.0279	45
0.0340	60
0.0313	75
0.0328	90
0.0328	105
0.0332	120
0.0324	135
0.0315	150

0.0326	165
0.0316	180
0.0319	195
0.0321	210
0.0297	225
0.0286	240
0.0303	255
0.0283	270
0.0258	285
0.0194	300
0.00369	315

(i) Mass of c(Leu-Gly) in the cell: 0.0149 g; mass of water in the cell: 0.8670 g; volume for each addition: 50 μL

Q/J	V_{add}/μL
0.0310	50
0.0298	100
0.0283	150
0.0262	200
0.0251	250
0.0282	300
0.0239	350
0.0226	400
0.0261	450
0.0277	500
0.0293	550
0.0243	600
0.0161	650
0.0133	700
0.011	750

(j) Mass of c(Leu-Gly) in the cell: 0.0117 g; mass of water in the cell: 0.5780 g; volume for each addition: 50 μL

Q/J	V_{add}/μL
0.03352	50
0.03335	100
0.03049	150
0.03109	200
0.02845	250
0.0267	300
0.02559	350
0.02708	400
0.02039	450
0.01767	500

0.01489	550
0.01247	600
0.01277	650
0.01281	700
0.01019	750
0.00653	800
0.00804	850
0.00402	900
0.00474	950
0.00152	1000

(k) Mass of KCl in the cell: 0.3015 g; mass of water in the cell: 0.6010 g; volume for each addition: 15 µL

Q/J	V_{add}/µL
1.0309	15
1.0459	30
1.05546	45
1.0359	60
1.03163	75
1.02858	90
1.02459	105
1.02109	120
1.01266	135
1.00816	150
1.00846	165
1.01507	180
1.00768	195
0.94761	210
0.97763	225
0.68698	240
0.09328	255
0.10147	270
0.09924	285
0.09742	300
0.08581	315
0.09488	330
0.09324	345
0.09158	360

(l) Mass of KCl in the cell: 0.3018 g; mass of water in the cell: 0.5755 g; volume for each addition: 15 µL

Q/J	V_{add}/µL
1.10167	15
1.03072	30
1.03453	45
1.04255	60

1.04277	75
1.04652	90
1.0481	105
1.04961	120
1.05956	135
1.05715	150
1.06105	165
1.06171	180
1.06494	195
0.93713	210
1.07622	225
1.05128	240
0.99235	255
0.4589	270
0.10524	285
0.10279	300
0.0916	315
0.09863	330
0.09668	345
0.09487	360
0.09328	375
0.08458	390

(m) Mass of KCl in the cell: 0.2592 g; mass of water in the cell: 0.6239 g; volume for each addition: 15 µL

Q/J	V_{add}/µL
1.14193	15
1.02367	30
1.00419	45
0.9941	60
0.97585	75
0.90831	90
0.4151	105
0.10226	120
0.10012	135
0.09788	150
0.09487	165
0.09343	180
0.09068	195
0.08955	210
0.08727	225
0.08559	240

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