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Investigation of drug self-association in aqueous solution using calorimetry, conductivity, and osmometry

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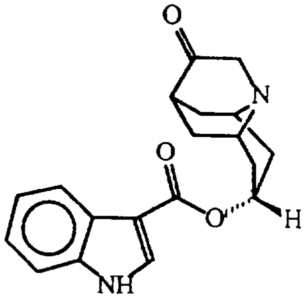
Abstract

Based on heat of dilution, osmotic coefficient and conductivity data, it is evident that the compound of interest in this study undergoes self-association in aqueous solution. The average aggregation number for the association product is small, not greater than 4. The likely association products are cation-cation dimer, cation-cation-anion or all cation trimer, and/or cation-cation-cation-anion tetramer. The observed self-association in aqueous solution is purely enthalpy driven, being entropy disfavored, indicating that the driving force for the self-association is likely to be either hydrogen bonding or π-π interaction or both, but not hydrophobic interaction or pure electrostatic interaction.

Keywords: Self-association; Calorimetry; Heat of dilution; Osmotic coefficient; Conductivity

1. Introduction

During the study of compound **I** (Scheme 1), for its aqueous solubility as a function of pH, it was observed that **I** exhibited much higher-than-expected solubilities at pHs less than 4. From this behavior, the protonated form of **I** was suspected of being involved in a certain degree of self-association, causing the solubility to increase. Therefore, it was of interest to determine whether or not the protonated form of **I** undergoes self-association in aqueous solution and to obtain further information such as average aggregation number and association constant if the self-association is confirmed. In this study, the methane sulfonate salt of **I** (abbreviated as **I**-EF hereafter), because of its high aqueous solubility, was used for the



Scheme 1. Compound **I**. MDL 73,147 (Dolasetron) IH-Indole-3-carboxylic acid, octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl ester.

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study of self-association by the protonated form of **I**.

2. Theoretical

The formation of a self-association product, or an aggregate, from singly-charged monomers can be expressed as the equilibrium in Eq. 1, with the formation constant given by Eq. 2.

$$nM^+ + pX^- \overset{K_A}{\rightleftharpoons} [M_nX_p]^{(n-p)+} \qquad (1)$$

$$K_A = \frac{\gamma_A}{(\gamma_M)^n(\gamma_x)^p} \cdot \frac{c_A}{(c_T - nc_A)^n(c_T - pc_A)^p} \qquad (2)$$

where M^+ represents the monomer, X^- = counteranion, $[M_nX_p]^{(n-p)+}$ represents the association product, K_A = associate formation constant, n = aggregation number, p = number of anions in an aggregate, c_i, γ_i = concentration and activity coefficient, respectively, of species i.

The formation of aggregates in a solution is expected to cause changes in certain physical chemical properties of the solution such as surface tension, osmotic coefficient, light scattering, electrical conductivity (Preston, 1948) and heat of dilution (Streng et al., 1995). In this study, heat of dilution, osmotic coefficient and electrical conductivity data were obtained for **I**-EF in aqueous solution and information such as average association number, associate formation constant, and association enthalpy and entropy was extracted.

2.1. *Heat of dilution*

Since any associated species present in a solution will become completely dissociated as the solution is infinitively diluted, the heat effect associated with such a dilution process should provide a measure for the initial concentration of the associate in the original solution. In practice, the dissociation may be considered complete if the final concentration after dilution is sufficiently low (e.g. < 0.001 M).

Heat of dilution for **I**-EF was determined calorimetrically as a function of compound concentration. Details for the heat of dilution experiments and raw data treatment have been reported previously (Streng et al., 1995). In diluting a solution containing associated molecules, the total heat effect is the result of contributions from the aggregate dissociation, the dissociation of protons from the monomers and aggregates, the dilution of the aggregates, and the dilution of the monomers:

$$\begin{aligned} Q_p^{NET} &= Q_p^{Diss,A} + Q_p^{Diss,H} + Q_p^{Dil,A} + Q_p^{Dil,M} \\ &= \Delta n_p^{Diss,A}\Delta H^{Diss,A} + \Delta n_p^{Diss,H}\Delta H^{Diss,H} + \\ &\quad \Delta n_p^{A}\Delta H^{Dil,A} + \Delta n_p^{M}\Delta H^{Dil,M} \end{aligned} \qquad (3)$$

where: $Qp^{Diss,A}$ = heat of aggregate dissociation, $Qp^{Diss,H}$ = heat of proton dissociation, $Qp^{Dil,A}$ = heat of dilution for aggregate, $Qp^{Dil,M}$ = heat of dilution for monomer, $\Delta n_p^{Diss,A}$ = number of moles of aggregate dissociated due to the dilution, $\Delta n_p^{Diss,H}$ = number of moles of proton dissociated due to the dilution, Δn_p^A = number of moles of aggregate diluted, Δn_pM = number of moles of monomer diluted.

If the pK_a of the compound is not too small (e.g. ≥ 6.0) and the concentration of the compound after dilution is not too low (e.g. ≥ 10⁻ 4M), the compound should remain mostly in the protonated form during the dilution process and the heat effect associated with the proton dissociation should be negligible. Therefore, Eq. 3 can be reduced to:

$$\begin{aligned} Q^{NET} &\approx Q^{Diss,A} + Q^{Dil,A} + Q^{Dil,M} \\ &\approx \Delta n^{Diss,A}\Delta H^{Diss,A} + \Delta n^{B}\Delta H^{Dil,A} + \\ &\quad \Delta n^{M}\Delta H^{Dil,M} \end{aligned} \qquad (4)$$

Notice that the subscript p is removed in Eq. 4 for simplicity.

Dividing Q^{NET} by the total number of moles of compound diluted gives the apparent molar heat of dilution. Assuming that the aggregate dissociation is complete after dilution and there is no further heat effect with further dilution, the apparent molar heat of dilution will be equal to the negative of the apparent molar enthalpy of the solution (ϕ^L). Therefore,

$$-\phi_L \approx c_A\frac{\Delta H^{Diss,A}}{c_T} + c_A\frac{\Delta H^{Dil,A}}{c_T} + c_M\frac{\Delta H^{Dil,M}}{c_T} \qquad (5)$$

where: c_A = equilibrium, concentration of aggregate in titrant, c_M = equilibrium concentration of monomer in titrant, c_T = total concentration of the compound in titrant.

According to Eq. 5, in order to obtain $\Delta H^{Diss,A}$, values of $\Delta H^{Dil,M}$ and $\Delta H^{Dil,M}$ must be known. This requirement is rather difficult to fulfill since direct measurement of $\Delta H^{Dil,B}$ and $\Delta H^{Dil,A}$ is impossible. As an approximation, it is assumed that the heat of dilution for the monomer is negligible. Therefore, Eq. 5 becomes:

$$-\phi_L \approx \frac{c_A}{c_T}\Delta H^{DL} \tag{6}$$

where: $\Delta H^{DL} = \Delta H^{Diss,A} + \Delta H^{Dil,A}$.

Combining Eqs. 2 and 6, the unknown parameters n, p, K_A and ΔH^{DL} can be obtained by fitting the experimental $-\phi_L$ vs. c_T profile through a non-linear least squares approach. To take into account the effect of species activities during the equilibrium calculation, Davies' equation may be used to calculate the activity coefficients of the species present in the solution.

$$-\log\gamma_i = A z_i^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \tag{7}$$

where: A = 0.511 in aqueous solution at 25°C, z_i = charge of the species, I = ionic strength, defined as:

$$I = \tfrac{1}{2}\sum_i c_i z_i^2$$

2.2. Osmotic coefficient

Since self-association changes the number of species present in the solution, it should change the osmotic coefficient, a colligative property, of the solution too. In this study, a vapor pressure osmometer was used to determine the apparent osmotic coefficients. The direct output of a vapor pressure osmometer is:

$$\Delta R = k_3 RT \ln a_1 \tag{8}$$

where: ΔR = osmometer output (mV), k = proportional constants, a_1 = solvent activity in sample solution.

Since the apparent osmotic coefficient ϕ of an electrolyte solution is defined as,

$$\phi' \equiv \frac{-1000 ln a_1}{M_1 v m} \tag{9}$$

where: ϕ' = apparent osmotic coefficient of the sample solution, M_1 = molecular weight of the solvent, v = stoichiometric sum for the eletrolyte, for 1:1 electrolyte, v = 2 m_i = molality concentration of the electrolyte,the osmometer output can be related to the apparent osmotic coefficient as follow:

$$\Delta R = -k_3 RT\frac{M_1}{1000}vm\phi' = Kvm\phi' \tag{10}$$

where: K = the 'instrument constant' or 'cell constant'.

The apparent osmotic coefficient ϕ' can thus be determined through vapor pressure osmometry.

Based on the theory of electrolyte solution thermodynamics (Robinson and Stokes, 1959), the apparent osmotic coefficient ϕ' is related to the concentrations and activity coefficients of all the species present in the solution, as shown below:

$$\phi' = \frac{\sum m_i}{vm}\left(1 + \frac{1}{\sum m_i}\sum_i \int_0^{m_i} m_i d ln\gamma_i\right)$$

where: m_i and γ_i are the molality and activity coefficient, respectively, of species i.

If the electrolyte is 100% dissociated, Σm_i should be equal to vm. If self-association occurs, Σm_i will no longer be equal to vm, and the values of m_i and γ_i are functions of association parameters n, p, and K_A. Fitting of the observed ϕ' vs. m profile based on Eq. 11 leads to the determination of n, p and K_A.

2.3. Conductivity data treatment

If association is occurring, the observed molar conductivity of an electrolyte solution reflects not only the contribution from the unassociated electrolyte, but also the contribution from the association product:

$$\Lambda_{obs} = \alpha\Lambda_M + \frac{1}{n}(1-\alpha)\Lambda_A \tag{12}$$

where: α = percentage fraction of the unassociated electrolyte, Λ_M = molar conductivity of the unassociated electrolyte, n = aggretation number (number of cations in an aggregate), Λ_A = molar conductivity of the association product.

In Eq. 12, α is a function of n, p and K_A. If Λ_M and Λ_A are known, together with the knowledge of Λ_{obs}, α can be calculated and subsequently, n, p and K_A can be calculated. However, Λ_M and Λ_A can not be obtained experimentally since the unassociated and the associated electrolytes can not be separated in solution. Therefore, theoretical expressions for Λ_M and Λ_A are needed. Among the many conductivity expressions available (Horvath, 1985), for higher concentrations, the equation by Robinson and Stokes (Robinson and Stokes, 1959) contains the least number of empirical parameters, and was therefore chosen to represent Λ_M and Λ_A in this study.

$$\Lambda = \Lambda^0 - \frac{(B_1 \Lambda^0 + B_2)\sqrt{I}}{1 + B\mathring{a}\sqrt{I}} \tag{13}$$

where: B_1, B_2 = constants characteristic of the electrolyte, B = 0.329 for water at 25°C, å = closest approach between ions in solution, I = total ionic strength of the solution, which is a function of m, the total concentration of the compound, n, p and K_A.

Substitution of Eq. 13 into Eq. 12 for Λ_M and Λ_A results in:

$$\Lambda_{obs} = \alpha\left(\Lambda_M^0 - \frac{(B_{1m}\Lambda_m^0 + B_{2M})\sqrt{I}}{1 + B\mathring{a}_m\sqrt{I}}\right) + \frac{1}{n}(1-\alpha)\left(\Lambda_A^0 - \frac{(B_{1A}\Lambda_A^0 + B_{2A})\sqrt{I}}{1 + B\mathring{a}_A\sqrt{I}}\right) \tag{14}$$

By fitting the observed Λ_{obs} vs. $\sqrt{m}$ profile based on Eq. 14, values of n, p and K_A may be calculated.

3. Experimental

3.1. Materials and solutions

The methane sulfonate salt of **I** (**I**-EF) was used in this study. All other chemicals used were of analytical grade and deionized water was used throughout.

3.2. Heat of dilution measurements

The heats of dilution for the aqueous solutions of **I**-EF were determined using an isoperibol titration calorimeter[2]. The experimental procedure was the same as that described earlier (Streng et al., 1995).

3.3. Osmotic coefficient measurements

A vapor pressure osmometer[3] was used for osmotic coefficient measurements. The osmometer was calibrated using standard solutions of NaCl and $BaCl_2$ and the cell constants for the osmometer at the operation temperatures were determined. In order to achieve the best baseline stability, sufficient time should be allowed for the instrument to equilibrate and the room temperature should be controlled 7–9°C below the instrument operation temperature. A strip chart recorder[4] was used to continuously record the output of the osmometer. In each measurement, the testing and reference probes are first wetted with the solvent drops. When baseline is established, the instrument output is zeroed. Then the sample solution (four to five drops) was applied to the testing probe and the stable output thereafter was read as the ΔR value for the sample solution. For each sample solution, the ΔR measurement was repeated several times until consistent readings were obtained. Usually, the ΔR reading after the first sample application (to the testing probe) was not reliable since the previous solution on the sample probe may have not been fully replaced with the new sample solution. Therefore, the first ΔR reading was usually discarded.

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[2] Tronac Model 1250

[3] UIC070

[4] KIPP and ZONEN model BD 40

3.4. Conductivity measurements

The conductivity cell[5] used in this study was calibrated using a 0.1000 M potassium chloride aqueous solution at 15, 25 and 35°C, and the cell constant was found to be 1.0357 cm^{-1}. The experimental procedure for the conductivity measurements of the **I**-EF solutions was as follows. In a 40-mL glass test tube, 20.0 mL of **I**-EF solution (c.a. 0.2 M) was added. The solution was stirred with a magnetic stirrer and was thermostated at the temperature of study. The conductivity of this solution was measured. Either a 1.0 mL or a 2.0 mL aliquot of this solution was removed from the test tube and replaced with exactly the same amount of water. The resulting solution was stirred and thermally equilibrated, and the conductivity of this diluted solution measured. Successive dilution and conductivity measurement continued until the concentration of the last diluted solution was about 0.01 M. In order to obtain a stable conductivity reading, the solutions were equilibrated for 10–15 min.

4. Results and discussion

4.1. Heat of dilution

Heats of dilution for the **I**-EF solutions were determined calorimetrically at 10, 15, 25 and 35°C as a function of compound concentration. The results are listed in Table 1. The concentration vs. heat of dilution profile at 25°C is shown in Fig. 1.

The dilution of **I**-EF results in a significant endothermic heat effect. As seen in Table 1 and Fig. 1, the endothermic heat of dilution is as high as ≈ 8 kJ/mole at 25°C (or ≈ 4 kJ/mole at 35°C) for a 0.2 M **I**-EF solution. The magnitude and the sign of the heats of dilution strongly indicate the presence of ion-ion associations in the solution prior to dilution. Otherwise, this significant endothermic heat of dilution may not be sufficiently explained based on solute solvation and solvent re-structuring.

Table 1
Heats of dilution for **I**-EF in aqueous solutions at different concentrations[a] and temperatures

Conc. (M)	Heat of Dilution - Φ_L (kJ·mol^{-1})			
	10°C	15°C	25°C	35°C
0.0119			1.51	
0.0238			2.47	
0.0202	1.84	1.46		1.46
0.0303	2.68			
0.0474			4.10	
0.0403	4.14	3.43		2.43
0.0713			5.06	
0.0605	5.82	5.15		2.43
0.0806	5.98	5.77		2.64
0.1188			6.57	
0.1013	6.69	7.36		2.97
0.1210	7.24	7.53		3.27
0.1425			7.11	
0.1418	7.91	7.95		3.49
0.1663			7.45	
0.1620	8.11	8.33		3.54
0.1900			7.70	
0.1822	8.49	8.58		3.77
0.2138			8.08	
0.2025	8.62	8.83		4.14
0.2374			8.37	

[a]concentrations before the dilution.

The dilution of a concentrated solution usually causes a certain amount of solvent molecules to break away from bulk solvent structure and join the solvation of the solutes. Breaking up solvent-

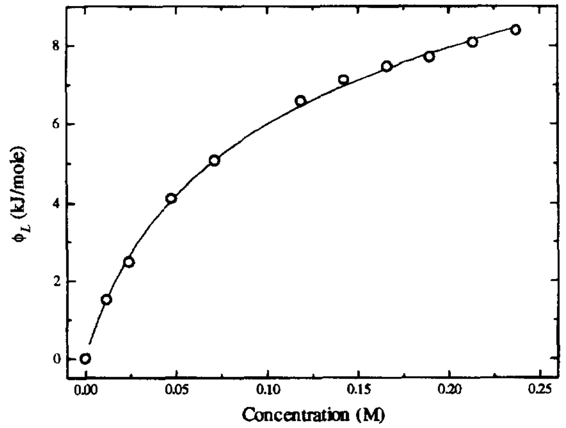

Fig. 1. Heat of dilution as a function of concentration at 25°C (○, experimental result; –, calculated).

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[5] Radiometer CDC 304 coductivity cell and CDM83 conductivity meter

Table 2
Aggregation number, equilibrium constant (K_A), enthalpy change (ΔK_A),and entropy change (ΔS_A) of association by **I**-EF in aqueous solution

Temp. (°C)	Aggregation Number[a]		log K_A	ΔH^b_A (kJ·mol^{-1})	ΔS^b_A(J · K·mol^1)
	n	p			
Results Based on Heat of Dilution Data					
10	2	1	2.51	−22.9	−32.8
10	2	0.5	1.58	−25.4	−59.5
15	2	1	2.42	−24.2	−37.7
25	2	0	0.63	−27.2	−79.2
Results Based on Osmotic Coefficient Data					
25	3	0	1.67		
25	2.5	0.5	2.20		
35	2.5	0.5	2.32		
Results Based on Conductivity Data					
15	2.80	0.75	1.21		
25	2.60	0.80	1.34		

[a] n, p = numbers of cations and anions involved in the formation of an aggregate, respectively. [b] The ΔH_A values (and consequently ΔS_A values) are approximate based on $\Delta H_A \approx -\Delta H^{DL}$. Since ΔH^{DL} also includes the heat of dilution for the associates, which could not be determined experimentally, the exact ΔH_A values could not be calculated.

The n and p values listed are those resulting in best fits of the experimental data. In a few cases, two different sets of n and p can lead to similar quality fits of the experimental data, hence, they are both listed. As to which n and p combination is more correct, there is not a clear-cut criterion for that yet Since it is possible that more than one form of association products are present in the solution at the same time, the calculated n and p values could very well be the apparent or the average n and p values. This is why in many cases the fractional n and p numbers lead to better fits of the experimental data.

It was found that a value of Bå = 1.5 (see eqs 13 and 14) can lead to much better and more reasonable fits of the conductivity data than the value of Bå = 1.0. This indicates that the apparent or average value of the closest approach (å) for **I**-EF in aqueous solution is about 4.6.Å. In fitting the conductivity data, the average values of Λ^0 and $B_1\Lambda^0 + B_2$ (see eq 14) for the associated product were also calculated resulting in: at 15°C, $\Lambda^0 = 100.0$, $(B_1\Lambda^0+B_2) = 157.6$; at 25°C, $\Lambda^0 = 131.2$, $(B_1\Lambda^0+B_2) = 215.1$.

solvent bonds is endothermic while forming solvation shell is exothermic. In most cases, the net heat effect due to solute solvation and solvent re-structuring is exothermic, rather than endothermic. Therefore, the observed endothermic heat of dilution for **I**-EF strongly suggests that some other bonds, presumably the bonds that cause ion-ion association, are broken upon dilution.

It should be mentioned that **I**-EF has a dissociable proton and the proton dissociation, which has an endothermic heat effect, could occur upon dilution. However, the contribution from proton dissociation to the overall heat of dilution is expected to be rather small and negligible. Based on its pKa (6.16 at 35°C and 6.65 at 15°C), **I** should remain more than 98% protonated even after the dilution (the concentration after dilution is 0.001 M).

Since the heat of dilution for **I**-EF in aqueous solution is strongly endothermic, the breaking of the ion-ion associates must be endothermic too. This will rule out the possibility of hydrophobic ion-ion association by the protonated form of **I** because the hydrophobic ion association usually leads to exothermic heats of dilution (Lindenbaum, 1971; Wood et al., 1967; Levine and Lindenbaum, 1973). Therefore, the possible driving forces for ion-ion association by the protonated form of **I** would be either one (or a combination) of the following: π-π interaction, hydrogen bonding, and electrostatic interaction. All three of these interactions could lead to an endothermic heat of dilution.

In order to obtain information about the stoichiometry and thermodynamic quantities of self-association by **I**-EF, regression analysis of the heats of dilution data using the equations derived in the theoretical section were performed and the results are listed in Table 2. Excellent agreement between the calculated and experimental data was achieved at 10, 15 and 25°C, as seen for 25°C in Fig. 1. It was found that an aggregation number of 3 ($n = 2, p = 1$) gives the best fit of the 10 and 15°C data, while an aggregation number of 2 ($n = 2, p = 0$) leads to the better fit of the 25°C data (Fig. 1). Several other combinations of n and p could also lead to acceptable, though poorer, fits of the experimental data at 10, 15 and 25°C. An aggregation number ($n + p$) greater than 4 results in a much deteriorated and unacceptable fit of the experimental data, i.e. either the whole profile or part of the profile will not correlate. Therefore, it is clear that the self-association by **I**-EF in aqueous solution leads only to small size aggregates, instead of large aggregates or micelles. It was also found that the combination of $n = 1$ and $p = 1$ could not fit the experimental data well at all three temperatures, indicating that the association by **I**-EF in aqueous solution is not simply cation-anion ion pairing. Although the anion involvement in association could help to stabilize the positively charged aggregates, the presence of the anion is not the sole reason that cations can be brought together in forming the association product. It is believed that the cation-cation bonding (π-π interaction or hydrogen bonding) plays an important role in causing the self-association by **I**-EF.

Examining the ΔH_A, and ΔS_A values listed in Table 2, it is seen that the formation of the self-association products by **I**-EF is enthalpically favored and entropically disfavored, i.e. the self-association by **I**-EF is purely enthalpy driven. This provides additional support to the conclusion that the **I**-EF aggregates are formed mainly through π-π interaction or hydrogen bonding, instead of hydrophobic interaction which is usually entropy driven.

It is of interest to compare the ΔH_A values listed in Table 2 for 10 and 15°C with that calculated based on the well known van't Hoff relationship using the two log K_A values listed in Table 2 for 2:1 n/p ratio at 10 and 15°C. Calculation based on the van't Hoff equation leads to a ΔH_A of -28.1 kJ/mole which is slightly greater than the listed ΔH_A values (-22.9 and -24.2 Id/mole). Considering that the listed ΔH_A values in Table 2 are only approximates, and the van't Hoff calcualtion is based on only two data points, the agreement between the listed and the van't Hoff AH_A values is rather good. The good agreement strengthens the validity of the theoretical model used, the calculated log K_A's, and the assumption behind $\Delta H_A \approx \Delta \mathrm{H}^{DL}$.

The heats of dilution for **I**-EF were also determined at 35°C, but the regression analysis was attempted due to poor data consistency. The increased data scattering and uncertainty at 35°C are likely to be caused by deteriorated calorimeter sensitivity and stability at 35°C. Nevertheless, it could still be seen that the heat of dilution for **I**-EF at 35°C is much less endothermic than that at 25°C, indicating that the ion-ion association becomes much weaker at 35°C.

4.2. *Osmotic coefficients*

According to Eq. 11, the apparent osmotic coefficient ϕ' should be equal to unity when the solute concentration approaches zero. As the concentration of the solute increases, ion pairs or self-association products begin to form and Σm_i becomes not equal to vm, hence Φ begins to deviate from unity, even if the solution is still ideal.

As seen from Table 3 and Fig. 2, the observed ϕ decreases significantly as the concentration of **I**-EF increases (the first few points as shown in Fig. 2 may not be accurate due to the limitation of instrument sensitivity at low solute concentrations). The extent of ϕ' decrease as a result of solute concentration increase is very significant and could not be fully accounted for by the effect of solution non-ideality alone (Fig. 2). The decrease in ϕ' must be a result of both the increased solution non-ideality and the increased self-association. Theoretical estimation indicates that, as shown in Fig. 2, the effect of solution non-ideality alone accounts only for a small portion of the total observed ϕ' decrease. Therefore, the osmotic coefficient data clearly indicate that **I**-EF does form certain types of self-association products as the concentration increases, and hence results in unusual ϕ' decrease.

Table 3
Apparent Osmotic Coefficients (ϕ') of **I**-EF in aqueous solutions

Conc.(m)	25°C[a]			35°C[a]		
	R (mV)	vmϕ' (Osm)	ϕ'	R (mV)	vmϕ' (Osm)	ϕ'
0.0099	68	0.0185	0.935	29	0.0138	0.695
0.0194	135	0.0368	0.950	61	0.0290	0.745
0.0377	231	0.0629	0.835	122	0.0579	0.770
0.0613	335	0.0912	0.745	196	0.0931	0.760
0.0822	420	0.1144	0.695	240	0.1140	0.695
0.1014	516	0.1405	0.695	296	0.1406	0.695
0.1243	610	0.1661	0.670	343	0.1629	0.655
0.1461	706	0.1923	0.655	387	0.1838	0.630
0.1633	776	0.2113	0.645	420	0.1994	0.610

[a] K = 3672 at 25°C and 2106 at 35°C (calibrated against NaCl and $BaCl_2$).

Regression analysis of the observed ϕ' vs. m profiles based on the equations given in the theoretical section leads to excellent fit of the experimental data at 25°C and a good fit of the ϕ' vs. m profile at 35°C. The calculated n and p values that result in best fits of the experimental data and the corresponding $\log K_A$ values are listed in Table 2. It is seen that the n, p and $\log K_A$ values obtained based on osmotic coefficient data are a little different from, but still reasonably close to, those based on the heat of dilution data. It is again confirmed that the aggregation number for **I**-EF associates is not large.

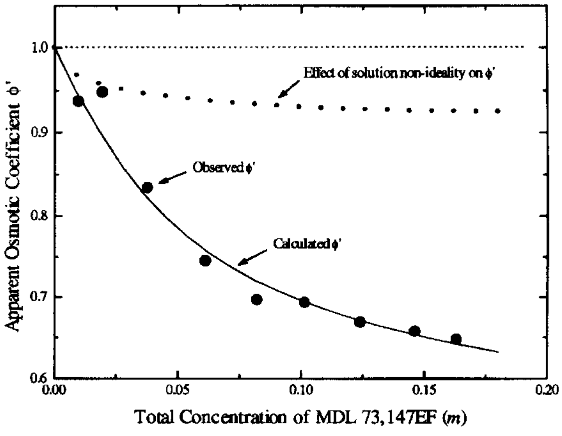

Fig. 2. Observed and calculated ϕ' as a function of concentrationat 25°C.

4.3. Electrical conductivity

Table 4 lists the observed molar conductivity data for **I**-EF as a function of concentration. The profile of the observed conductivity (Λ_{obs}) verses the square root of concentration ($c^{1/2}$) at 25°C is shown in Fig. 3. As seen in Fig. 3, the Λ_{obs} vs. $c^{1/2}$ profiles at 25°C appears to be linear initially until $c^{1/2}$ becomes greater than 0.25 (or $c > 0.06$ M). The Λ_{obs} vs. $c^{1/2}$ profiles begin to deviate negatively from the original linearity after c exceeds 0.06 M. As seen in Fig. 3, if no association is occurring, the Λ_{obs} vs. $c^{1/2}$ profile should exhibit positive deviation from the original linearity due to increased solution non-ideality. Therefore, the observed negative deviation from linearity is a clear indication that self-association is occurring for **I**-EF in aqueous solution. However, it is also noted that the observed negative deviations for I-EF are rather small, as compared to those observed for MDL 201,346A (Streng et al., 1995) and some detergents (Preston, 1948) which form large aggregates or micelles. Therefore, the conductivity data again confirms that the aggregation numbers for **I**-EF associates are small. Regression analysis of the observed Λ_{obs} vs. $c^{1/2}$ profiles resulted in excellent fits of the experimental data and the calculated n, p and $\log K_A$ values (Table 2) are comparable with those based on heat of dilution data and osmotic coefficient data.

Table 4
Conductivity of **I**-EF in water as a function of concentration

15°C		25°C	
Concentration (M)	Molar Conductivity(S cm^2/mol)	Concentration (M)	Molar Conductivity(S cm^2/mol)
0.0063	48.66	0.0071	61.02
0.0116	47.67	0.0118	59.75
0.0171	46.55	0.0197	57.77
0.0221	45.79	0.0274	56.57
0.0286	44.90	0.0342	55.26
0.0349	44.41	0.0407	54.55
0.0427	43.56	0.0462	53.90
0.0495	42.83	0.0525	52.95
0.0573	41.88	0.0596	52.18
0.0663	41.18	0.0678	51.33
0.0768	40.10	0.0771	50.32
0.0889	39.15	0.0838	49.52
0.0978	38.44	0.0910	48.79
0.1076	37.64	0.0990	47.98
0.1183	36.94	0.1076	47.21
0.1301	36.13	0.1169	46.36
0.1432	35.27	0.1271	45.48
0.1575	34.35	0.1381	44.61
0.1732	33.43	0.1502	43.68
0.1905	32.49	0.1632	42.71
0.2096	31.35	0.1774	41.66
		0.1928	40.66
		0.2096	39.60

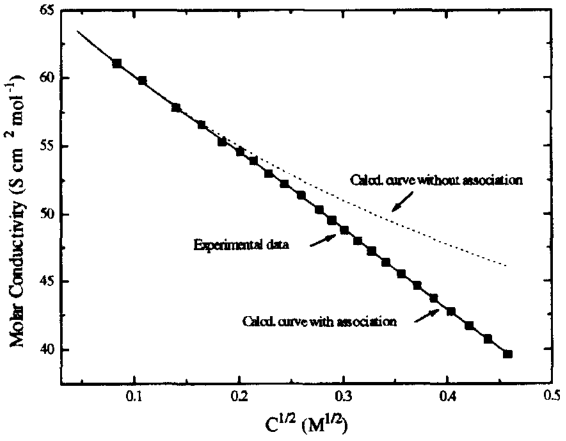

Fig. 3. Molar conductivity vs. square root of concentration for I-EFat 25°C.

5. Conclusions

Based on heats of dilution, osmotic coefficient and conductivity data, it is concluded that **I**-EF undergoes self-association in aqueous solution. The aggregation numbers for the association products are, however, rather small (not greater than 4). The likely association products include cation-cation dimer, cation-cation-anion or all cation trimer, and/or cation-cation-cation-anion tetramer, but not cation-anion ion pair. The self-association by **I**-EF in aqueous solution is purely enthalpy driven and is entropy disfavored, indicating that the driving force for the self-association is likely to be either hydrogen bonding or π-π interaction or both, but not hydrophobic interaction and pure electrostatic interaction.

It should be mentioned that in actual **I**-EF solution, more than one form of association products can be present at the same time, thus causing the determination of exact association stoichiometry (i.e. the n and p numbers) difficult and causing some fractional n and p numbers to be favored over some integer numbers in achieving better fits of the experimental data.

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