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The effects of ionic strength and temperature on the dissociation constants of adefovir and cidofovir used as antiviral drugs

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Abstract: The effects of ionic strength and temperature on the dissociation constants of adefovir (PMEA) and cidofovir (HPMPC) used as antiviral drugs were studied at 298 K, 308 K, and 318 K in aqueous media and at different ionic strength backgrounds of NaCl potentiometrically. The dissociation constants of the ligands were determined via the calculation of the titration data with the SUPERQUAD computer program. The thermodynamic parameters (ΔG, ΔH, and ΔS) for all species were calculated. The dissociation order of nitrogen and oxygen atoms in the ligands according to proton affinities values were obtained using PM6 semiempirical methods. Moreover, pK_a values of the ligands were determined at 0.00, 0.10, 0.15, 0.20, and 0.5 mol dm^{-3} ionic strength (NaCl) at 298 K. Consequently, when the ionic strength and temperature in the titration cells were increased, the obtained dissociation constants of PMEA (pK_{a3}, pK_{a4}, and pK_{a5}) and HPMPC (pK_{a2} and pK_{a3}) decreased.

Key words: Adefovir, cidofovir, proton affinities, dissociation constants, thermodynamic parameters

1. Introduction

Viruses are small infectious agents that can replicate only inside the living cells of an organism.[1] Some diseases such as Ebola, AIDS, influenza, herpes, and SARS are caused by viruses and these diseases are described as viral diseases. Treatment of viral diseases is difficult because viruses are highly resistant to extreme environmental conditions. Therefore, few drugs are known for the treatment of viral diseases. One type of antiviral drugs are acyclic nucleotide analogues (ANPs) such as adefovir, cidofovir, famciclovir, tenofovir, and penciclovir.[2,3] The basic chemical structure of ANP compounds consists of a purine base (i.e. adenine, guanine, cytosine) or a pyrimidine base attached to an acyclic side chain that ends in a phosphonate group. In this study, adefovir and cidofovir were investigated with respect to ionic equilibria in aqueous solution. The chemical structures of the PMEA and HPMPC are given in Figures 1a and 1b.

Adefovir [{[2-(6-amino-9H-purin-9-yl)ethoxy]methyl} phosphonic acid] (PMEA) has antiviral activity against the hepatitis B virus.[4,5] Its oral pro-drug is adefovir dipivoxil, which is a butyl ester of PMEA.[6] Therefore, PMEA is used in the treatment of chronic hepatitis B.[7,8] In addition, in vitro PMEA has been shown to be highly effective against hepadnaviruses, retroviruses, and herpesviruses.[9] Moreover, anti-HIV activity of PMEA has been obtained against AIDS.[10]

Cidofovir {HPMPC, 1-(S)-[3-hydroxyl-2-(phosphonomethoxy)propyl]cytosine} is an important anti-DNA virus agent.[11–13] HPMPC prevents the spread of all DNA viruses[14] and it is used in the treatment of

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cytomegalovirus retinitis in AIDS patients,[15] but it is also used in the treatment of papillomatosous infections,[16] progressive multifocal leukoencephalopathy,[17] adenovirus infections,[18] and some severe infections caused by poxviruses.[19] According to anecdotal reports and some studies, HPMPC has been proposed as an auxiliary therapy for highly active antiretroviral therapy (HAART) in AIDS.[20,21]

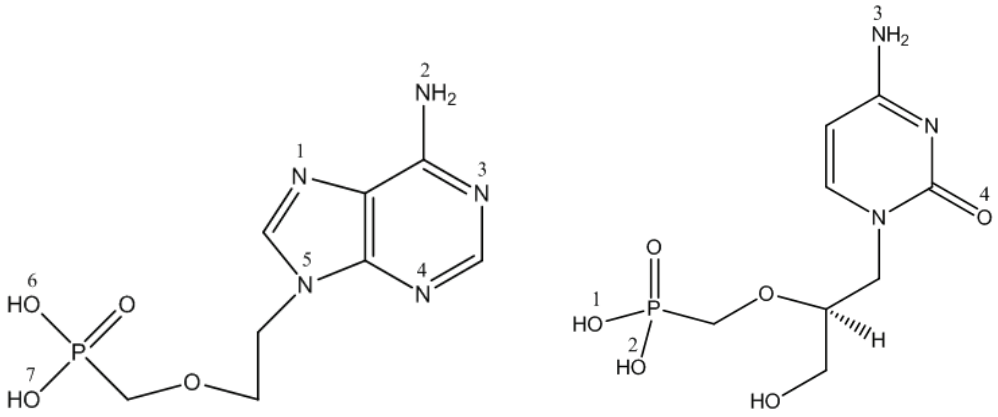

Figure 1. Chemical structures of the ligands (a) PMEA (b) HPMPC.

Consequently, PMEA and HPMPC are extremely important compounds for human health. Therefore, the complexes of ligands with Cu(II), Ni(II), Zn(II), Co(II), Ca(II), and Mg(II) metal ions were characterized in our previous study.[22] However, ionic strength and temperature are 2 important variants in the human body. Therefore, in the present study, the effects of ionic strength and temperature on the pK_a values of PMEA and HPMPC were investigated in aqueous solution using a potentiometric titration method that is frequently used in this field.[23–27]

2. Results and discussion

Dissociation constants were calculated by potentiometric titration from a series of several measurements, where LH_7^{5+} and LH_4^{2+} denote the fully protonated form of PMEA and HPMPC, respectively. All PMEA species are LH_6, LH_5, LH_4, LH_3, LH_2, and LH and the HPMPC species occurring in aqueous solution according to the pH under our experimental conditions are LH_3, LH_2, and LH. Their dissociation equilibrium is as follows:

$$LH_n + H_2O \rightleftharpoons LH_{n-1} + H_3O^+ K_n = [LH_{n-1}][H_3O^+]/[LH_n] \quad (1)$$

Proton affinity gives some information about protonation order. In other words, it reflects the extent of the basicity of donor atoms within the whole ligand. Therefore, the calculations of the proton affinity for the ligands were carried out according to semiempirical molecule orbital (SE-MO) methods based on quantum mechanical principles for examination of the structure of the species formed in the solution and to determine the protonation order of both nitrogen and oxygen atoms in PMEA and HPMPC. SE-MO methods are utilized over a wide area to determine the protonation sequence in polyprotic compounds.[28,29] The MOPAC 2009 software package was used in all theoretical calculations. The formation heats (H_f) and total energies (TE) of the ligands and monoprotonated species were calculated by semiempirical PM6 methods.[30–32] In addition, the proton affinity (PA) of each ionizable atom in the ligands was found according to the following equation and is given in Table 1:

$$PA = 1536.345 + \Delta H_f^\circ(B) - \Delta H_f^\circ(BH^+), \quad (2)$$

where PA is the proton affinity of B types, $\Delta H_f^\circ(B)$ is the formation heat of B molecule, $\Delta H_f^\circ(BH^+)$ is the formation heat of BH^+ molecule, and 1536.345 is the formation heat of H^+.[33]

Table 1. The calculated formation heat (H_f), total energy (TE), and PA values with PM6 methods for PMEA and its monoprotonated forms.

Species	TE (kJ mol^{-1})	H_f (kJ mol^{-1})	PA
PMEA	–323.433	–648	-
1 N - H^+	–324.718	–627	1515
2 N - H_3^+	–324.655	–565	1453
3 N - H^+	–324.718	–586	1474
4 N - H^+	–324.722	–632	1520
5 N - H^+	–324.613	–523	1411
6 O - H_2^+	–324.588	–435	1323
7 O - H_2^+	–324.718	–594	1482
HPMPC	–347.785	–1213	-
1 O - H_2^+	–348.773	–866	1819
2 O - H_2^+	–349.020	–1113	1436
3 N - H_3^+	–348.890	–983	1306
4 O - H^+	–349.070	–1163	1486

According to the calculated results (Table 1), the nitrogen atom in 4 positions in PMEA has the highest PA. Therefore, the first protonated atom is nitrogen in 4 positions in the ligand because of having more basic characters than the others. The most acidic center within the whole ligand is also the oxygen atom in 6 positions. Thus, the protonation order of donor atoms in PMEA is 4N - 1N - 7O - 3N - 2N - 5N - 6O. In other words, the dissociation order of nitrogen and oxygen atoms in PMEA is 6O - 5N - 2N - 3N - 7O - 1N - 4N. In HPMPC, the oxygen atom in 4 positions is the highest PA. Therefore, it has a more basic center than the others. Hence, the first protonated site is 4O in this HPMPC. Moreover, the most acidic center within the whole ligand is 1O. Therefore, the protonation order of potent donor atoms in HPMPC is 4O - 2O - 3N - 1O. In other words, the dissociation order of nitrogen and oxygen atoms in HPMPC is 1O - 3N - 2O - 4O.

Finally, both ligands include phosphoric acid groups containing 2 acidic oxygen atoms. One of the 2 pK_a values of phosphoric acid groups is very low ($pK_a < 2$).[34–38] Therefore, only one pK_a value was calculated for phosphoric acid. Hence, 6 pK_a values for PMEA and 3 pK_a values for HPMPC were determined in this study and our previous study.[22]

2.1. Ionic strength effects on the dissociation constants of the ligands

Ion activity must be used instead of concentrations in all equilibrium calculations because ions in solution interact with each other via Coulomb forces. These ions are not separately treated in the solution because of these interactions. The relationship between concentration and activity has been explained by the Debye–Hückel theory. Therefore, ionic strength changes are affected by the equilibrium constants of the ligands. The effect of ionic strength on the pK_a values of PMEA and HPMPC was investigated and the results obtained are given in Table 2 and Figures 2a and 2b.

In Table 2, pK_{a3}, pK_{a4}, and pK_{a5} values generally decrease because of increasing ionic strength. However, the values for pK_{a1} and pK_{a6} increase while irregular changes are observed for pK_{a2}. Therefore, while the proton release of some N–H or O–H bonds (pK_{a1}, pK_{a6}) decreases, for some bonds in PMEA (pK_{a3},

pK_{a4}, and pK_{a5}) proton release increases. Decreasing values were generally observed for pK_{a2} and pK_{a3} in HPMPC. Nevertheless, disordered changes were observed for pK_{a1} values. Hence, it can be considered that while the proton release of O–H bonds (pK_{a1} and pK_{a3}) increases, it decreases for the N–H bond (pK_{a1}) in HPMPC.

Table 2. Ionic strength effect (I) (NaCl) on dissociation constants of the ligands at 298 K.

	pK_a values					
Ligand	I: 0	I: 0.05	I: 0.1*	I: 0.15	I: 0.2	I: 0.5
PMEA	3.65 ± 0.01	3.66 ± 0.02	3.83 ± 0.02	3.75 ± 0.03	3.83 ± 0.01	3.79 ± 0.03
	4.62 ± 0.02	4.58 ± 0.01	4.49 ± 0.01	4.48 ± 0.02	4.47 ± 0.02	4.86 ± 0.07
	6.96 ± 0.02	6.74 ± 0.02	6.64 ± 0.01	6.56 ± 0.01	6.51 ± 0.01	6.50 ± 0.01
	7.62 ± 0.01	7.43 ± 0.01	7.33 ± 0.01	7.24 ± 0.01	7.26 ± 0.02	6.99 ± 0.02
	10.69 ± 0.03	10.35 ± 0.02	10.41 ± 0.01	10.33 ± 0.01	10.36 ± 0.02	10.17 ± 0.01
	10.96 ± 0.02	11.19 ± 0.03	11.22 ± 0.04	11.39 ± 0.03	11.49 ± 0.06	11.67 ± 0.03
HPMPC	4.87 ± 0.02	4.82 ± 0.01	4.91 ± 0.01	4.78 ± 0.01	4.97 ± 0.02	5.01 ± 0.03
	7.31 ± 0.02	7.10 ± 0.02	7.02 ± 0.03	6.94 ± 0.02	6.93 ± 0.03	6.79 ± 0.03
	10.50 ± 0.03	10.48 ± 0.02	10.31 ± 0.02	10.27 ± 0.02	10.24 ± 0.03	10.13 ± 0.03

*Values were taken from ref. 22 and each titration was repeated 3 times

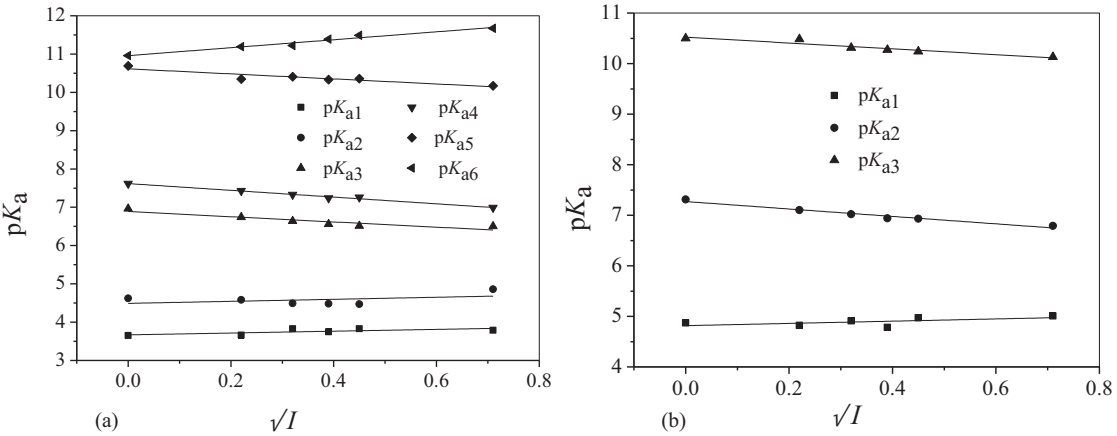

Figure 2. pK_a values versus ionic strength (298 K, as background NaCl) (a) PMEA (b) HPMPC.

2.2. Calculation of the thermodynamic parameters of dissociation constants

The titration curves with NaOH as a titrant in water and at different temperatures and the dissociation constants for the ligands were evaluated at 298 K, 308 K, and 318 K, and are given in Figures 3a and 3b and Table 3.

Figure 3 shows the titration curves for different temperatures (298 K, 308 K, and 318 K, respectively). Comparing the titration curves of PMEA and HPMPC (Figure 3) at different temperatures shows that increasing temperature shifts the titration curves to a more alkali region. This can simply be explained as a result of proton release from the ligands.[39,40] Figures 4a and 4b show the effect of temperature on the dissociation constants of PMEA and HPMPC, and pK_a values of PMEA and HPMPC in different temperatures are given in Table 3.

A dissociation constant of a ligand is a direct consequence of the underlying thermodynamics of the dissociation equilibria. Furthermore, pK_a values directly proportional to the standard Gibbs energy change for the equilibria. Therefore, pK_a changes with temperature can be understood based on Le Chatelier's principle. Namely, when a reaction is endothermic, the pK_a value decreases with increasing temperature; the contrary

is true for exothermic reactions. All the thermodynamic parameters of the dissociation process of PMEA and HPMPC are recorded in Tables 4 and 5.

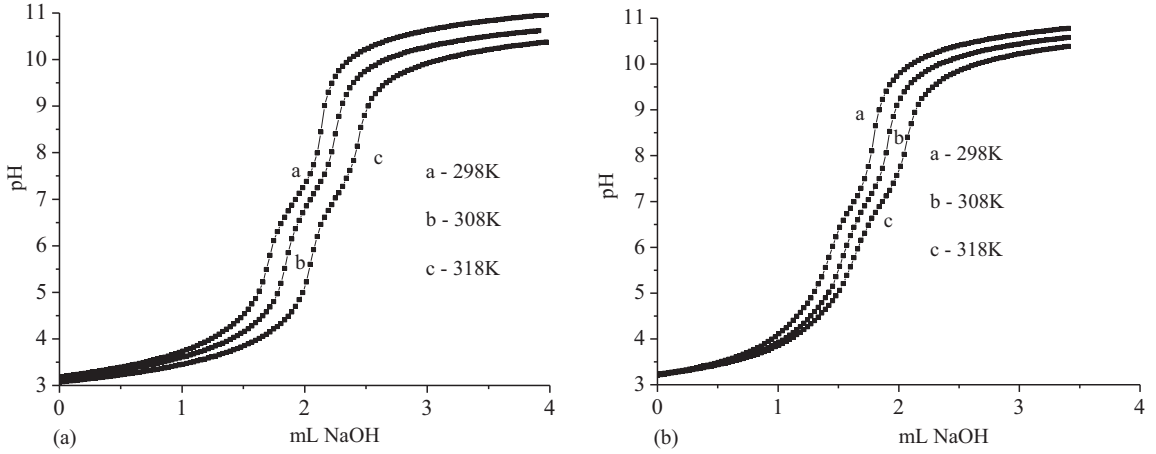

Figure 3. Titration curves in different temperatures for (a) PMEA and (b) HPMPC (I: 0.1 mol dm^{-3} NaCl, 0.03 mmol HCl).

Table 3. pK_a values of PMEA and HPMPC in different temperatures (I: 0.1 mol dm^{-3} NaCl, 0.03 mmol HCl).

	Temperatures (T/K)					
	298 K		308 K		318 K	
	$log_{10}\beta$*	pK_a*	$log_{10}\beta$	pK_a	$log_{10}\beta$	pK_a
PMEA	11.22 ± 0.02	3.83 ± 0.02	11.23 ± 0.02	3.86 ± 0.02	11.42 ± 0.02	3.89 ± 0.01
	21.68 ± 0.03	4.49 ± 0.01	21.62 ± 0.02	4.51 ± 0.02	21.78 ± 0.02	4.28 ± 0.02
	28.96 ± 0.04	6.64 ± 0.01	28.95 ± 0.02	6.62 ± 0.02	29.20 ± 0.02	6.59 ± 0.02
	35.59 ± 0.03	7.33 ± 0.01	35.57 ± 0.02	7.33 ± 0.02	35.79 ± 0.02	7.42 ± 0.02
	40.09 ± 0.04	10.41 ± 0.01	40.07 ± 0.02	10.39 ± 0.02	40.07 ± 0.02	10.36 ± 0.02
	43.92 ± 0.06	11.22 ± 0.04	43.93 ± 0.06	11.23 ± 0.01	43.96 ± 0.01	11.42 ± 0.07
HPMPC	10.31 ± 0.04	4.91 ± 0.01	10.23 ± 0.03	4.64 ± 0.01	9.98 ± 0.03	4.51 ± 0.01
	17.33 ± 0.05	7.02 ± 0.03	17.19 ± 0.03	6.97 ± 0.02	16.86 ± 0.02	6.88 ± 0.01
	22.24 ± 0.06	10.31 ± 0.02	21.83 ± 0.03	10.23 ± 0.02	21.37 ± 0.04	9.98 ± 0.03

*Values were taken from ref. 22 and each titration was repeated 3 times

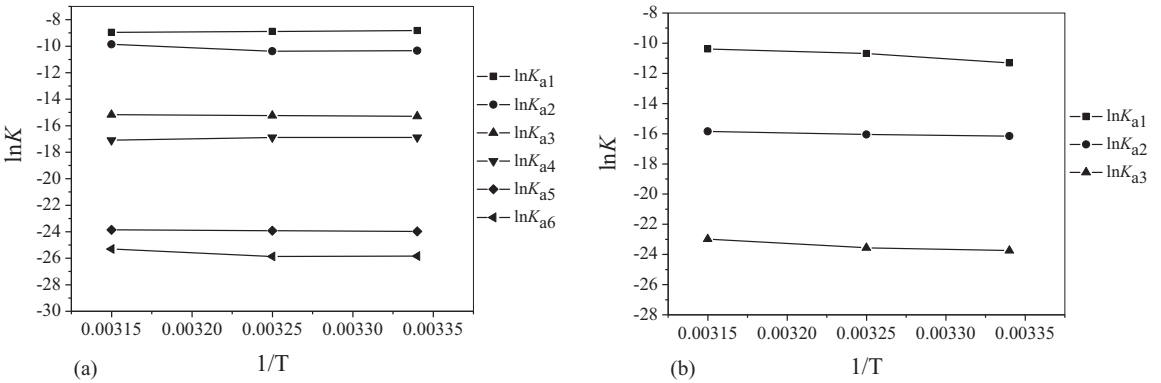

Figure 4. Effect of temperature on K values of the ligands (I: 0.1 mol dm^{-3} NaCl, 0.03 mmol HCl) (a) PMEA (b) HPMPC.

It can be concluded that thermodynamic values can be obtained since the pK^H values of PMEA and HPMPC decrease with increasing temperature (Table 4). If ΔH has a positive value, the dissociation process shows endothermic properties. Conversely, the dissociation process shows exothermic properties. Large positive values for ΔG indicate that the dissociation process is not spontaneous.[41]

The following conclusions can be drawn from this discussion:

- The proton affinities of donor atoms of the ligands were calculated using PM6 semiempirical methods. Hence, the dissociation order of nitrogen and oxygen atoms in the ligands was obtained as 6O - 5N - 2N - 3N - 7O - 1N - 4N for PMEA and 1O - 3N - 2O - 4O for HPMPC.
- The effect of ionic strength effect (background NaCl) on the pK_a values of PMEA and HPMPC was investigated at 298 K in aqueous solution. While systematic changes were observed for some pK_a values, irregular changes were observed in other constants for both ligands.
- The effects of temperature on the dissociation constants of PMEA and HPMPC were studied at 0.1 mol dm^{-3} ionic strength (NaCl) and, according to the obtained data, thermodynamic parameters (ΔH, ΔS, and ΔG) were calculated for 298 K, 308 K, and 318 K temperatures. The results obtained are given in Tables 4 and 5.
- These results could be of considerable assistance for advancing understanding of the drugs' behavior in vivo.

Table 4. Thermodynamic functions of PMEA (I: 0.1 mol dm^{-3} NaCl).

		Gibbs energy	Enthalpy	Entropy
	Dissociation constants	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1} K^{-1}
T/K	pK_{a1} values	ΔG	ΔH	ΔS
298	3.83	21.85		–91.58
308	3.86	22.76	–5.44	–91.56
318	3.89	23.68		–91.58
	pK_{a2} values	ΔG	ΔH	ΔS
298	4.49	25.62		–22.88
308	4.51	26.59	18.80	–25.31
318	4.28	26.06		–22.83
	pK_{a3} values	ΔG	ΔH	ΔS
298	6.64	37.88		–111.93
308	6.62	39.03	4.52	–112.04
318	6.59	40.12		–111.93
	pK_{a4} values	ΔG	ΔH	ΔS
298	7.33	41.82		–167.41
308	7.33	43.22	–8.07	–166.53
318	7.42	45.17		–167.43
	pK_{a5} values	ΔG	ΔH	ΔS
298	10.41	59.39		–184.11
308	10.39	61.26	4.52	–184.22
318	10.39	63.07		–184.10
	pK_{a6} values	ΔG	ΔH	ΔS
298	11.22	64.01		–275.05
308	11.23	66.22	–17.96	–273.29
318	11.42	69.52		–275.09

Table 5. Thermodynamic functions of HPMPC (I: 0.1 mol dm^{-3} NaCl).

		Gibbs energy	Enthalpy	Entropy
	Dissociation constants	kJ mol^{-1}	kJ mol^{-1}	J mol^{-1} K^{-1}
T/K	pK_{a1} Values	ΔG	ΔH	ΔS
298	4.91	28.01		28.18
308	4.64	27.36	36.41	29.38
318	4.51	27.46		28.15
	pK_{a2} values	ΔG	ΔH	ΔS
298	7.02	40.05		–91.92
308	6.97	41.10	12.66	–92.34
318	6.88	41.88		–91.91
	pK_{a3} values	ΔG	ΔH	ΔS
298	10.31	58.82		–97.52
308	10.23	60.32	29.76	–99.23
318	9.98	60.76		–97.48

3. Materials and methods

3.1. Reagents

NaCl ($\geq$99%) used in this research was purchased from Merck, potassium hydrogen phthalate (KHP) ($\geq$99%) and borax ($Na_2B_4O_7$) ($\geq$99%) from Fluka, PMEA (99%) from Watson International Ltd., and HPMPC (98%), 0.1 mol dm^{-3} NaOH, and 0.1 mol dm^{-3} HCl as standard solution from Aldrich. All reagents were of analytical quality and were used without further purification. CO_2-free double-distilled deionized water obtained using an aquaMAX^{TM} - *Ultra* water purification system (Young Lin Inst.) was used throughout all the experiments; its resistivity was 18.2 MΩ cm. pH-metric titrations were performed using a Molspin pH meter with an Orion 8102BNUWP ROSS ultra-combination pH electrode. The temperature in the double-wall glass titration vessel was constantly controlled using a thermostat ($\pm$0.1 °C) (DIGITERM 100, SELECTA). The cell solution was stirred during titration at a constant rate.

3.2. Procedures

First 0.05 mol kg^{-1} potassium hydrogen phthalate (KHP) and 0.01 mol kg^{-1} borax were prepared from the reagents for the calibration of the electrode. Then 1 $\times$ 10^{-3} mol dm^{-3} PMEA and HPMPC solutions were prepared and used in all the experiments. The electrode pairs were calibrated according to the instructions of the Molspin Manual[42] with buffer solutions (KHP) of pH 4.005, 4.018, and 4.038; and ($Na_4B_4O_7$ borax) of pH 9.180, 9.102, and 9.038 at 298 K, 308 K, and 318 K temperatures, respectively.[43]

The potentiometric cell was calibrated to obtain the formal electrode potential E°_{cell} at each ionic strength and temperature change.[44,45] For this purpose, HCl solutions were prepared for each medium with titrated NaOH solutions. For all the conditions examined, the reproducible values of the autoprotolysis constants K_w were calculated from several series of [H$^+$] and [OH$^-$] measurements. Pure nitrogen gas (99.9%) was purged from the solutions in the cell to obtain an inert atmosphere. Next, 1.0 mol dm^{-3} NaCl stock solution was prepared and diluted to 0.00, 0.10, 0.15, 0.20, and 0.50 mol dm^{-3}, which were used as the ionic background to maintain a constant ionic strength. An automatic burette was connected to a Molspin pH-mV-meter. The SUPERQUAD[46] computer program was used for the calculation of the dissociation constants.

A solution containing approximately 0.01 mmol of PMEA/HPMPC was placed into the titration cell. The required amount of 0.1 mol dm^{-3} HCl was added. While thermodynamic studies were carried out at 0.10 mol dm^{-3} ionic strength (NaCl), ionic strength studies were conducted at 298 K. Finally, doubly distilled deionized water was added to the cell to make up the total volume of 50 mL. The pH data were obtained after the addition of 0.03 cm^3 increments of the standardized NaOH solution. Each titration was repeated 3 times and the standard deviations quoted refer to random errors only.

Furthermore, all titration measurements for 298 K, 308 K, and 318 K temperatures were carried out and the thermodynamic parameters of equilibrium constants of PMEA and HPMPC were calculated for each temperature. The slope of the plot pK^H or $\log_{10}K$ vs. $1/T$ was utilized to evaluate the enthalpy change (ΔH) for the dissociation process, respectively

$$\Delta G = -2.30RT \log_{10} K \tag{3}$$

$$\Delta S = (\Delta H - \Delta G)/T \tag{4}$$

or

$$\log_{10}K = (-\Delta H/2.303)(1/T) + (\Delta S/2.303R) \tag{5}$$

From the ΔG and ΔH values, the entropy changes (ΔS) can be calculated using the well-known equations (Eqs. (3), (4), and (5)).

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