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Quantitative analysis of *in vitro* compatibility of binary and ternary mixtures of nitroimidazole and macrolides in combination with omeprazole using a calorimetric technique

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In the present study, *in vitro* interactions between nitroimidazoles, macrolides and omeprazole in binary and ternary mixtures were examined by measuring their enthalpy of solution ($\Delta_{sol}H$) using a calorimetric technique. A comparison of the enthalpy of solution of the pure drugs with those of binary and ternary mixtures at pH 2 and 6 was made to indicate the magnitude of interaction between them. The $\Delta_{sol}H$ for all the nitroimidazoles is endothermic at pH 2 and 6 but both the macrolides show exothermic behavior, whereas the enthalpy of solution of omeprazole changes from -40.52 to $4.35\ kJmol^{-1}$ as the pH changes from 2 to 6. The results have been quantified by determining the excess enthalpy of solution for both binary and ternary systems. The small deviations from ideality for all the binary systems are attributed to various non-bonding interactions between different functional groups on both the drug molecules. The results suggest compatibility of drug pairs in their binary mixtures. However, ternary mixtures show somewhat larger interactions. The magnitude of interaction enthalpy of a ternary mixture comprising tinidazole, clarithromycin and omeprazoles which are available as a marketed kits has been calculated to be significant, suggesting that the three drugs cannot be co-formulated.

1. Introduction

Triple therapy employing a proton pump inhibitor (P), clarithromycin (CLM) or amoxycillin (AMC) and metronidazole (MET) is accepted worldwide as a first-line therapy for *H. pylori* infection. The combination therapy probably attacks the organism through different mechanisms of action producing at least additive or perhaps synergistic anti-Helicobacter effects (Midolo et al. 1997) Although there are reports of synergism when using MET and CLM together and in combination with omeprazole (OME) (Kearney 2003; Meyer et al. 1999; Goddard et al. 1996; Calafatti et al. 2000; Veldhuyzen et al. 1996; Andersen et al. 2000; Chen et al. 2002), as far as we know, no detailed study of *in vitro* interaction between two of the active principles has been reported. Compatibility of drugs in a combined preparation or combined therapy is a critical factor for the development of pharmaceutical formulations. It is important that the drugs do not interact in a way that is likely to reduce their efficacy and increase their toxicity (Mure et al. 2002). Interactions in the solid or liquid state between the active ingredients in pharmaceutical dosage forms can give rise to changes in stability, solubility, dissolution rate and bioavailability of drugs (Lloyd et al. 1999). Although the higher cure rate with the triple therapy is undisputed, the large number of pills make it a complicated regimen, and it can be made more patient friendly by combining two of the drugs together in a mixed formulation. So it is useful to evaluate the compatibility of these drugs in co-formulation

Thermal methods are efficient for detection of unexpected interactions between the two drugs and their suitability for a combined preparation. Drug-drug and drug-water interactions in aqueous solution can be investigated by determining a wide range of thermodynamic properties such as partial molar volume, partial molar heat capacity and excess enthalpy of solution (Bucci et al. 2000; Giron 2003; Marini et al. 2003; Chadha et al. 2004). The technique of calorimetry finds extensive use in modern drug research (Royall and Gaisford 2005, Urakmi 2005), ranging from control of raw materials to stability, compatibility and preformulation, as well as in quantitative microbiology, and is independent of sample form (Lloyd et al. 1999; Phipps and Macken 2000; Seizer et al. 1999; O'Neill et al. 2003). The technique can be used successfully to clarify the nature of interactions between the two and three component systems (Mura et al. 2002).

The present work is a part of our ongoing research programme on the possible applications of solution calorimetry in predicting any specific or non-specific interactions between the drugs prescribed in combined dosage forms by determining their enthalpy of solution. Besides this, the

Table 1: Molar enthalpy of solution at 310.15 K and fractions of various species of drugs at pH 2 and 6

pH	Drug	f^+	f^-	$f^\pm$	$\Delta_{sol}H$ ($kJ \cdot mol^{-1}$)
1	Metronidazole[a]	0.9693	0.0307	–	25.13
		–	–	–	25.23
2		0.7597	0.2403	–	27.69
		–	–	–	27.70
3		0.2403	0.7597	–	34.03
		–	–	–	34.04
4		0.0307	0.9693	–	36.60
		–	–	–	36.60
6		0.0003	0.9997	–	36.31
		–	–	–	36.91
1	Tinidazole[b]	0.8685	0.1315	–	36.34
		–	–	–	36.35
2		0.3978	0.6022	–	41.62
		–	–	–	41.65
3		0.0620	0.9380	–	45.42
		–	–	–	45.40
4		0.0066	0.9934	–	46.03
		–	–	–	46.05
6		–	–	–	46.11
		–	–	–	46.10
1	Ornidazole[c]	0.9617	0.0383	–	24.56
		–	–	–	24.55
2		0.7153	0.2847	–	27.45
		–	–	–	27.44
3		0.2008	0.7992	–	33.56
		–	–	–	33.53
4		0.0245	0.9755	–	35.63
		–	–	–	35.65
6		0.0002	0.9998	–	35.92
		–	–	–	35.93
1	Secnidazole[d]	0.5743	0.4257	–	28.87
		–	–	–	28.89
2		0.1189	0.8811	–	33.65
		–	–	–	33.64
3		0.0133	0.9867	–	34.74
		–	–	–	34.75
4		0.0013	0.9987	–	34.87
		–	–	–	34.88
6		–	–	–	34.94
		–	–	–	34.93
2	Clarithromycin	1	0	–	−69.95
		–	–	–	−70.55
6		0.999	0.001	–	−70.19
		–	–	–	−70.31
2	Roxithromycin	1	0	–	−66.63
		–	–	–	−66.23
6		0.9987	0.0013	–	−66.37
		–	–	–	−66.49
1	Omeprazole	0.9990	0.0010	–	
		–	–	–	
2		0.9901	0.0099	–	−40.33
		–	–	–	−40.72
3		0.9091	0.0909	–	
		–	–	–	
4		0.5	0.5	–	
		–	–	–	
6		0.0099	0.9885	0.0016	4.29
		–	–	–	4.41

[a] $\Delta_{sol}H^+ = 24.75\ kJ \cdot mol^{-1}$ $\Delta_{sol}H^- = 36.98\ kJ \cdot mol^{-1}$
enthalpy of deprotonation of a = 12.23 kj · mol^{-1}
[b] $\Delta_{sol}H^+ = 34.87\ kJ \cdot mol^{-1}$ $\Delta_{sol}H^- = 46.11\ kJ \cdot mol^{-1}$
enthalpy of deprotonation of b = 11.24 kJ · mol^{-1}
[c] $\Delta_{sol}H^+ = 24.10 \cdot kJ\ mol^{-1}$ $\Delta_{sol}H^- = 35.92\ kJ \cdot mol^{-1}$
enthalpy of deprotonation of c = 11.82 kJ · mol^{-1}
[d] $\Delta_{sol}H^+ = 24.44\ kJ \cdot mol^{-1}$ $\Delta_{sol}H^- = 34.89\ kJ \cdot mol^{-1}$
enthalpy of deprotonation of d = 10.45 kJ · mol^{-1}

solubility of these drugs in several buffers (pH 2-6) are also reported. We aim to correlate the magnitude of excess molar enthalpy of solution to the extent of interaction between the various drugs in a combined formulation. We believe that positive excess molar enthalpy of solution which of small magnitude suggests a weak interaction between the drugs. Negative excess enthalpy of solution of the order of nRT (where $n > 1$, R is gas constants, T is temperature) suggests a strong interaction and should be avoided. Moreover for quantitative considerations entropic factors have also to be considered.

2. Investigations, results and discussion

2.1. Enthalpy of solution and other thermodynamic parameters of pure drugs

In order to determine the compatibility of these drugs in the presence of each other by solution calorimetry it is essential to determine the enthalpy of solution of the individual drugs at the desired pH. The molar enthalpy of solution for individual drugs (metronidazole tinidazole, secnidazole, ornidazole, clarithromycin and roxithromycin) at two different concentrations over the pH range 2–8 were determined (Table 1).

The values of molar free energy of solution ($\Delta_{sol}G$) were also calculated using the equation

$$\Delta_{sol}G = RT \ln (s) \quad (1)$$

The values of s, the solubility (mol/L) of the drug determined at a particular pH, $\Delta_{sol}G$ and the calculated values of molar entropy of solution ($\Delta_{sol}S$) are given in Table 2.

The low solubility of nitroimidazoles at both the pH values is due to their relatively unfavorable enthalpy of solution partially offset by more favorable entropies of solution. On the other hand, the solubilities of clarithromycin and roxithromycin and of omeprazole (at pH 2.0) are enthalpically favored. Endothermic behavior was observed for all the nitroimidazoles while clarithromycin shows exothermic behavior over the whole pH range. For omeprazole the heat of solution is exothermic at pH 2 but changes to endothermic with increase in pH. Table 1 shows that the enthalpy of solution of the drugs is independent of concentration, however it changes with pH. Clarithromycin, a fourteen membered ring macrolide, has a pK_a of 9.1 and remains mostly in its protonated form at the experimental pH values and, as expected, its enthalpy of solution re-

Table 2: Solubility and molar free energy and molar entropy of solution of the pure drugs

Drug	pH	Solubility (M)	$\Delta_{sol}G$ ($kJ \cdot mol^{-1}$)	$\Delta_{sol}S$ ($kJ \cdot mol^{-1}K^{-1}$)
Metronidazole	2	0.0603	3.14	0.079
	6	0.0623	3.11	0.108
Tinidazole	2	0.0324	3.84	0.122
	6	0.0301	3.92	0.136
Ornidazole	2	0.0720	2.95	0.079
	6	0.0788	2.84	0.107
Secnidazole	2	0.2178	1.71	0.103
	6	0.2240	1.67	0.107
Clarithromycin	2	0.0233	4.21	−0.240
	6	0.0109	5.06	−0.243
Roxithromycin	2	0.0049	5.95	−0.234
	6	0.0031	6.46	−0.235
Omeprazole	2	0.0072	5.52	−0.149
	6	0.0010	7.78	−0.011

Table 3: Molar enthalpies (kJ/mol) of solution and excess molar enthalpies (kJ/mol) of solution for the binary mixtures of drugs in buffers at pH 2 and 6 at 310.15 K

pH 2			pH 6		
x_{clm}	$\Delta_{sol}H_{met:clm}$	$\Delta H^{E}_{met:clm}$	x_{clm}	$\Delta_{sol}H_{met:clm}$	$\Delta H^{E}_{met:clm}$
0.105	16.67	−0.76	0.105	24.92	−0.50
0.198	7.00	−1.34	0.198	14.65	−0.85
0.243	2.36	−1.58	0.243	9.70	−0.99
0.308	−4.28	−1.83	0.306	2.73	−1.16
0.395	−13.12	−2.14	0.395	−6.90	−1.32
0.433	−16.88	−2.21	0.433	−10.97	−1.36
0.524	−25.90	−2.29	0.499	−18.06	−1.39
0.581	−31.49	−2.25	0.581	−26.87	−1.37
0.660	−39.08	−2.10	0.660	−35.21	−1.26
0.706	−43.41	−1.96	0.700	−39.37	−1.19
x_{ome}	$\Delta_{sol}H_{met:ome}$	$\Delta H^{E}_{met:ome}$	x_{ome}	$\Delta_{sol}H_{met:ome}$	$\Delta H^{E}_{met:ome}$
0.095	20.86	−0.38	0.095	32.41	0.91
0.195	13.70	−0.70	0.195	28.71	1.62
0.301	6.22	−0.95	0.308	24.63	2.10
0.406	−1.18	−1.09	0.406	21.19	2.33
0.520	−8.89	−1.13	0.493	18.33	2.38
0.614	−15.27	−1.09	0.520	17.46	2.39
0.709	−21.60	−0.95	0.614	14.60	2.21
0.766	−25.36	−0.83	0.716	11.66	1.87
			0.766	10.27	1.64
x_{ome}	$\Delta_{sol}H_{clm:ome}$	$\Delta H^{E}_{clm:ome}$	x_{ome}	$\Delta_{sol}H_{clm:ome}$	$\Delta H^{E}_{clm:ome}$
0.197	−66.50	−2.10	0.197	−54.20	1.36
0.316	−63.58	−2.73	0.300	−46.14	1.73
0.409	−61.01	−2.93	0.409	−37.82	1.90
0.514	−57.83	−2.87	0.514	−30.00	1.88
0.591	−55.36	−2.67	0.578	−25.33	1.78
0.609	−54.75	−2.59	0.609	−23.13	1.72
0.692	−51.90	−2.24	0.702	−16.47	1.45
0.794	−48.26	−1.60	0.794	−9.99	1.07
0.903	−44.22	−0.80	0.903	−23.72	0.54
x_{clm}	$\Delta_{sol}H_{tin:clm}$	$\Delta H^{E}_{tin:clm}$	x_{clm}	$\Delta_{sol}H_{tin:clm}$	$\Delta H^{E}_{tin:clm}$
0.103	29.70	−0.42	0.103	33.68	−0.32
0.214	16.83	−0.82	0.214	20.51	−0.60
0.302	6.72	−1.08	0.302	9.83	−0.77
0.405	−4.94	−1.31	0.405	−1.95	−0.91
0.521	−18.12	−1.43	0.521	−12.83	−0.97
0.570	−23.52	−1.44	0.570	−21.17	−0.95
0.729	−41.10	−1.23	0.729	−37.62	−0.80
x_{ome}	$\Delta_{sol}H_{tin:ome}$	$\Delta H^{E}_{tin:ome}$	x_{ome}	$\Delta_{sol}H_{tin:ome}$	$\Delta H^{E}_{tin:ome}$
0.097	33.12	−0.56	0.097	40.09	−0.86
0.200	24.21	−1.02	0.200	36.14	−1.53
0.295	16.08	−1.29	0.295	31.17	−1.95
0.377	9.27	−1.43	0.377	28.16	−2.14
0.487	0.17	−1.49	0.487	22.85	−2.23
0.593	−8.48	−1.41	0.593	23.38	−2.08
0.718	−18.49	−1.16	0.718	14.69	−1.70
0.787	−23.96	−0.94	0.787	11.86	−1.36
x_{clm}	$\Delta_{sol}H_{orn:clm}$	$\Delta H^{E}_{orn:clm}$	x_{clm}	$\Delta_{sol}H_{orn:clm}$	$\Delta H^{E}_{orn:clm}$
0.098	16.82	−1.05	0.098	24.69	−0.83
0.204	5.60	−1.97	0.204	12.84	−1.48
0.301	−4.50	−2.57	0.301	2.25	−1.89
0.404	−14.99	−2.96	0.404	−9.10	−2.12
0.499	−24.36	−3.10	0.499	−18.87	−2.16
0.601	−34.28	−3.00	0.601	−29.93	−2.03
0.708	−44.36	−2.63	0.708	−40.90	−1.71
0.755	48.65	−2.36	0.755	−45.73	−1.51
x_{ome}	$\Delta_{sol}H_{orn:ome}$	$\Delta H^{E}_{orn:ome}$	x_{ome}	$\Delta_{sol}H_{orn:ome}$	$\Delta H^{E}_{orn:ome}$
0.100	19.54	−1.11	0.100	31.25	−1.51
0.203	11.73	−1.94	0.203	26.84	−2.68
0.304	4.28	−2.48	0.300	23.03	−3.42
0.405	−2.81	−2.77	0.405	19.26	−3.89
0.504	−9.65	−2.83	0.507	15.93	−3.99
0.609	−16.60	−2.63	0.609	12.92	−3.77
0.694	−22.00	−2.31	0.692	10.74	−3.35
0.805	−28.92	−1.65	0.805	8.07	−2.43

Table 3: (Continued)

	pH 2			pH 6	
x_{clm}	$\Delta_{sol}H_{sec:clm}$	$\Delta H^E_{sec:clm}$	x_{clm}	$\Delta_{sol}H_{sec:clm}$	$\Delta H^E_{sec:clm}$
0.097	22.88	−0.66	0.097	24.08	−0.58
0.203	11.24	−1.27	0.203	12.42	−1.08
0.290	1.84	−1.68	0.290	2.30	−1.36
0.315	−0.83	−1.78	0.315	0.37	−1.43
0.505	−20.65	−2.19	0.505	−17.81	−1.63
0.590	−29.82	−2.17	0.590	−28.70	−1.56
0.634	−34.30	−2.10	0.634	−33.24	−1.49
0.722	−43.23	−1.86	0.722	−39.57	−1.29
x_{ome}	$\Delta_{sol}H_{sec:ome}$	$\Delta H^E_{sec:ome}$	x_{ome}	$\Delta_{sol}H_{sec:ome}$	$\Delta H^E_{sec:ome}$
0.1	25.39	−0.87	0.1	30.73	−1.11
0.194	17.71	−1.52	0.194	27.06	−1.89
0.313	8.42	−2.05	0.313	28.06	−2.52
0.407	1.19	−2.26	0.407	19.68	−2.77
0.487	−4.79	−2.32	0.487	17.15	−2.81
0.612	−13.93	−2.16	0.612	13.60	−2.59
0.700	−20.16	−1.89	0.700	11.37	−2.24
0.801	−27.14	−1.40	0.801	8.76	−1.68

mains unchanged with pH. However, the pK_a values for omeprazole (pKa_1 4.0 and pKa_2 8.2) (Bruni and Ferreira 2002) and nitroimidazoles (1.16–2.5) (Jukka-Pekka et al. 2003) lead to different concentrations of protonated species of the drugs causing variation of the molar enthalpy of solution with pH.

$$\Delta_{sol}H = \sum_{i=0}^{n} f_i \quad \Delta_{sol}H_i \qquad (2)$$

f_i represents the fraction of species 'i' of the drug at a particular pH calculated from its ionization constants. The quantity $\Delta_{sol}H_i$ corresponds to the molar enthalpy of solution for the ith species. These have been calculated by measuring enthalpy of solution at different pH values (eq. 2) and then solving the simultaneous equations. The difference between corresponding $\Delta_{sol}H_i$ values gives the enthalpy of deprotonation. The values of individual $\Delta_{sol}H_i$ and the enthalpy of deprotonation are given at the bottom of Table 1.

2.2. Interaction studies of binary mixtures

In aqueous solutions of drugs, the drug-water interactions and the various non-bonding interactions between different functional groups on the two or three drugs in a mixture are important factors that determine the compatibility of the drugs. Therefore heats of solution of the pure drugs are compared with those of the binary mixtures nitroimidazole + macrolide (NM), nitroimidazole + omeprazole (NP), and macrolide + omeprazole (MP) under the same experimental conditions of temperature and pH to find any indication of interaction within the mixture leading to potential compatibility/incompatibility of the drugs in their binary mixtures. The enthalpy of solution data for a mixture of drugs are compared with the superposition of the responses of the pure drugs, which is expected under the no interaction hypothesis. The outcome can be quantified numerically by determining the excess enthalpy of solution. The enthalpy of solution per mole of the binary mixtures was determined over a range of mole fractions and the results are given in Table 3.

The excess molar enthalpy of the binary mixture of nitroimidazole and macrolide is calculated by the equation:

$$\Delta H^E_{(N:M)} = \Delta H_{sol(N:M)} - [x_N\,\Delta H_N + x_M\,\Delta H_M] \qquad (3)$$

Here, $\Delta H_{sol\ (N:M)}$ is the experimental enthalpy of solution of the binary mixture ΔH_N and ΔH_M are the molar enthalpy of nitroimidazole and macrolide, respectively and x_N and x_M are their corresponding mole fractions.

The excess enthalpies of solution for the other binary mixtures (N : P) and (M : P) have also been determined and are given in Table 3.

The values of excess molar enthalpy of solution for each binary mixture was fitted by the least squares method to a Redlich-Kister equation of the form:

$$\Delta H^E = x_i x_j \sum_{k=0}^{n} h_k (x_i - x_j)\,k \qquad (4)$$

Table 4: Parameters h_i of Redlich Eq. (4) for excess molar enthalpy of binary systems

System	pH 2			pH 6		
	h_1 (kJ · mol^{-1})	h_2 (kJ · mol^{-1})	s	h_1 (kJ · mol^{-1})	h_2 (kJ · mol^{-1})	s
MET + CLM	−9.085	1.013	0.019	−5.562	0.278	0.005
MET + OME	−4.539	0.133	0.005	−9.556	−0.971	0.020
CLM + OME	−11.555	−2.928	0.010	7.561	1.712	0.006
TIN + CLM	−5.661	1.326	0.008	−3.839	0.456	0.004
TIN + OME	−5.960	−0.604	0.006	−8.861	−1.203	0.010
ORN + CLM	−12.425	0.587	0.009	−8.631	−0.881	0.006
ORN + OME	−11.286	−1.188	0.010	−16.003	−0.855	0.010
SEC + CLM	−8.709	1.341	0.010	−6.510	−0.244	0.007
SEC + OME	−9.255	−0.707	0.008	−11.230	−1.334	0.010

x_i and x_j are the mole fractions of component i and j and h_k are polynomial coefficients.

It was found that the Redlich-Kister equation (Redlich and Kister 1948) with two parameters, is suitable for all the binary mixtures and reproduces the experimental results within $\pm 0.01\ kJ \cdot mol^{-1}$. The values of h_i for different mixtures are given in Table 4.

The magnitude of ΔH^E results from contributions of several factors, which can be divided into physical, chemical and geometrical.

Tables 3 and 4 show that all the binary mixtures deviate from ideality. Excess enthalpy is positive for clarithromycin + omeprazole at pH 6, and it is negative for all the other systems. The negative value of excess enthalpy indicates a stronger interaction of drugs among themselves compared to the solvent. Similar behavior is obeyed at pH 2 but the absolute values vary in the order: ornidazole > metronidazole > secnidazole > tinidazole.

At pH 2 ornidazole (pK_a 2.4) will be protonated up to 70% and unprotonated at the N^3 position of the imidazole and will interact with the cation obtained by the protonation of the dimethylamino group of clarithromycin. Furthermore, interactions also arise due to hydrogen bonding between the hydroxyl group on the side chain of the ornidazole and the oxygen of the keto group and ester group in clarithromycin. The presence of OH on the $4''$ position may also lead it to interact through a hydrogen bond with the protonated nitrogen at the N3 position of ornidazole. Beside this, there will be additional hydrogen bonding through the chlorine atom present in ornidazole. Metronidazole (pK_a 2.5 and protonated up to 75%) may interact with clarithromycin through H-bonding but the lower value of excess enthalpy of interaction as indicated in the table may be attributed to the absence of a chlorine atom in the molecule. In secnidazole (pK_a 1.16) only 11% of the drug is present in a protonated form and therefore interactions through H-bonding are expected to be much less. The only interaction available between the drugs is through H-bonding between the secondary OH on the side chain of the drug and the oxygen of the keto and ester groups of clarithromycin. In tinidazole (pK_a 1.82) there is no OH-group present in the side chain to interact with the drug through H-bonding. The only interactions present in the system are between the protonated drug (31%) and clarithromycin. Clarithromycin (pK_a 9.1) is a big molecule and the positive charge on the protonated nitrogen is also shielded because of the presence of two methyl groups, suggesting the absence of repulsive interaction. At pH 6 the nitroimidazole is fully unprotonated. Therefore, a decrease in ΔH^E is expected. The decrease is much less in the case of secnidazole and tinidazole as their molecules are already unprotonated (89% and 67% respectively) compared to ornidazole and metronidazole (both 70%).

For the system nitroimidazole-omeprazole, the excess enthalpy of solution is very small at pH 2 because at this pH, omeprazole developes a positive charge as shown in the Scheme (Kuhler 1995) and there may be electrical repulsion between protonated species of the nitroimidazole and omeprazole due to the presence of similar charges and the small size of the molecules. The small amount of interaction may be due to the presence of H-bonding between the OH group present in the side chain of nitroimidazole and the protonated nitrogen in the pyridine ring of omeprazole. Repulsion will be the highest in the case of metronidazole + omeprazole as metronidazole will be protonated up to 75% and it will be least with secnidazole. The somewhat larger value in the case of ornidazole is probably due to some interaction between the −OH of ornidazole and the protonated nitrogen in the pyridine ring of omeprazole. The smallest value in the case of tinidazole is due to a lack of hydrogen bonding between the protonated nitrogen on the pyridine ring of omeprazole and tinidazole as there is no −OH group on the side chain of the imidazole. At pH 6, the pyridine ring is unprotonated and the molecule is neutral while all the nitroimidazoles are unprotonated and therefore there is less repulsion and also there is interaction due to the NH^+ of the benzimidazole and the −OH on the side chain of the nitroimidazole. In case of roxithromycin, the behavior of all the nitroimidazoles is the same. However the magnitude of the interaction (Tables 5 and 6) is much more because of the presence of a methoxyethoxymethoxy group on the nitrogen atom. Our results show that although all the binary systems deviate from ideality, the magnitude of excess enthalpy of solution is not very high especially for the binary mixture of nitroimidazole and omeprazole. The results suggest that both these drugs can be co-formulated in a combination therapy, which may reduce the number of pills and improve patient compliance. Our results are in agreement with the results of Chen et al. (2002) and Andersen et al. (2000). These workers have suggested that proton pump inhibitors may be effective in the treatment of some infections with metronidazole resistant *H. pylori* strains. They have suggested the effective dose to be 120–160 mg (x_P 0.1062–0.1368) together with the standard dose of 500 mg metronidazole (x_{met} 0.8938–0.8632). The enthalpy of interaction calculated for these doses lies between -0.42 to $-0.52\ kJ \cdot mol^{-1}$ at pH 2 and -0.98 to $+1.21\ kJ \cdot mol^{-1}$ at pH 6 for this combination. The small magnitude of the enthalpy of interaction suggests that they can be safely co-formulated.

2.3. Interaction studies of ternary mixtures

To determine the possible interaction of the drugs with each other in a triple regimen, the enthalpy of solution per

Scheme

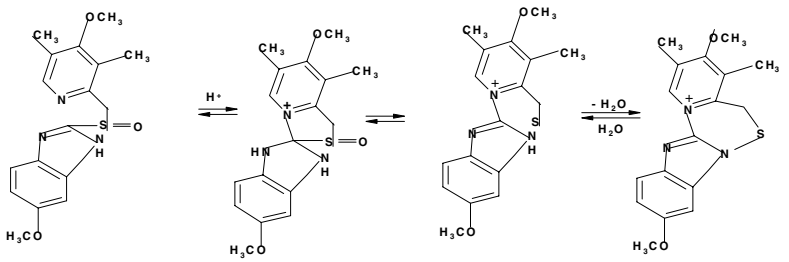

Table 5: Molar enthalpies of solution (kJ/mol) and excess molar enthalpies (kJ/mol) of solution for the binary mixtures of drugs in buffers at pH 2 and 6 at 310.15K

pH 2			pH 6		
x_{rox}	$\Delta_{sol}H_{met:rox}$	$\Delta H^E_{met:rox}$	x_{rox}	$\Delta_{sol}H_{met:rox}$	$\Delta H^E_{met:rox}$
0.104	16.81	−1.08	0.104	25.26	−0.67
0.201	6.94	−1.87	0.193	15.56	−1.14
0.302	−3.19	−2.44	0.302	3.91	−1.56
0.399	−12.71	−2.81	0.403	−6.71	−1.83
0.499	−22.23	−2.92	0.499	−16.77	−1.92
0.590	−30.65	−2.80	0.558	−22.74	−1.91
0.686	−39.37	−2.49	0.686	−35.75	−1.68
0.797	−49.16	−1.87	0.797	−46.75	−1.28
0.896	−57.77	−1.10	0.896	−56.48	−0.74
x_{ome}	$\Delta_{sol}H_{rox:ome}$	$\Delta H^E_{rox:ome}$	x_{ome}	$\Delta_{sol}H_{rox:ome}$	$\Delta H^E_{rox:ome}$
0.200	−63.35	−2.10	0.200	−51.28	0.67
0.309	−61.20	−2.78	0.312	−43.45	0.87
0.393	−59.32	−3.06	0.393	−37.69	0.95
0.481	−57.13	−3.16	0.492	−30.63	0.96
0.598	−53.91	−2.98	0.598	−23.19	0.89
0.708	−50.58	−2.49	0.689	−16.92	0.77
0.804	−47.48	−1.87	0.804	−9.03	0.53
0.893	−44.40	−1.11	0.893	−2.91	0.31
x_{rox}	$\Delta_{sol}H_{tin:rox}$	$\Delta H^E_{tin:rox}$	x_{rox}	$\Delta_{sol}H_{tin:rox}$	$\Delta H^E_{tin:rox}$
0.102	30.06	−0.61	0.102	34.14	−0.43
0.102	29.97	−0.61	0.102	34.04	−0.44
0.301	7.75	−1.37	0.302	10.40	−1.01
0.386	−1.60	−1.54	0.386	1.46	−1.15
0.499	−13.93	−1.61	0.499	−11.77	−1.22
0.610	−25.74	−1.51	0.610	−23.69	−1.16
0.656	−30.65	−1.41	0.702	−34.25	−1.01
0.758	−41.44	−1.14	0.758	−40.14	−0.89
0.879	−53.95	−0.65	0.879	−53.29	−0.52
x_{rox}	$\Delta_{sol}H_{orn:rox}$	$\Delta H^E_{orn:rox}$	x_{rox}	$\Delta_{sol}H_{orn:rox}$	$\Delta H^E_{orn:rox}$
0.106	15.95	−1.59	0.106	24.13	−0.99
0.201	5.91	−2.65	0.199	13.94	−1.70
0.295	−3.56	−3.35	0.295	3.46	−2.30
0.392	−13.14	−3.75	0.405	−8.27	−2.70
0.492	−22.55	−3.86	0.492	−17.21	−2.83
0.619	−34.16	−3.55	0.603	−28.59	−2.76
0.683	−39.84	−3.22	0.683	−36.46	−2.53
0.807	−50.55	−2.28	0.807	−48.50	−1.86
0.903	−58.55	−1.27	0.903	−57.51	−1.06
x_{rox}	$\Delta_{sol}H_{sec:rox}$	$\Delta H^E_{sec:rox}$	x_{rox}	$\Delta_{sol}H_{sec:rox}$	$\Delta H^E_{sec:rox}$
0.098	22.95	−0.88	0.098	24.42	−0.54
0.193	12.80	−1.56	0.205	13.10	−1.02
0.298	1.70	−2.12	0.298	3.35	−1.34
0.403	−9.20	−2.47	0.399	−7.09	−1.58
0.506	−19.54	−2.59	0.506	−18.03	−1.69
0.633	−32.11	−2.42	0.607	−28.27	−1.65
0.746	−42.95	−1.99	0.746	−41.96	−1.34
0.873	−54.86	−1.17	0.873	−54.34	−0.81

Table 6: Parameters h_i for excess molar enthalpy of solution for binary systems

System	pH 2			pH 6		
	h_1 (kJ · mol^{-1})	h_2 (kJ · mol^{-1})	s	h_1 (kJ · mol^{-1})	h_2 (kJ · mol^{-1})	s
MET + ROX	−11.627	−0.045	0.018	−7.650	0.487	0.011
ROX + OME	−12.571	−1.136	0.015	3.822	0.678	0.006
TIN + ROX	−6.402	−0.361	0.008	−4.844	0.057	0.008
ORN + ROX	−15.458	−1.556	0.018	−11.350	0.925	0.014
SEC + ROX	−10.314	0.394	0.009	−6.732	0.765	0.006

Table 7: Experimental and calculated values of excess enthalpies for ternary mixtures at pH 2 and 6 for nitroimidazoles, clarithromycin and omeprazole

x_{met}	x_{clm}	x_{ome}	$\Delta_{sol}H_{met:clm:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{met:clm:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −92.54)						
0.147	0.154	0.699	−39.02	−3.97	−2.47	−3.96
0.219	0.502	0.279	−47.53	−7.06	−4.23	−7.07
0.300	0.232	0.468	−30.35	−7.03	−3.65	−7.05
0.307	0.358	0.335	−34.73	−5.73	−4.26	−5.73
0.475	0.262	0.263	−22.74	−6.84	−3.76	−6.79
0.695	0.154	0.151	−3.64	−5.98	−4.51	−6.00
pH 6 (A = −40.49)						
0.147	0.154	0.699	−3.72	−1.33	−0.69	−1.34
0.223	0.509	0.268	−28.26	−1.80	−0.61	−1.84
0.229	0.252	0.519	−9.44	−2.32	−0.94	−2.15
0.307	0.358	0.335	−14.21	−1.75	−1.13	−1.77
0.496	0.252	0.252	−1.28	−2.83	−1.61	−2.89
0.695	0.154	0.151	13.30	−2.01	−1.36	−2.01

x_{tin}	x_{clm}	x_{ome}	$\Delta_{sol}H_{tin:clm:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{tin:clm:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ\ mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −63.95)						
0.150	0.153	0.697	−36.35	−3.58	−2.55	−3.57
0.252	0.493	0.255	−40.29	−5.84	−3.87	−5.90
0.257	0.254	0.489	−32.51	−5.51	−3.47	−5.51
0.338	0.335	0.327	−29.01	−6.30	−3.86	−6.23
0.486	0.260	0.254	−13.74	−5.47	−3.40	−5.46
0.701	0.146	0.153	9.55	−3.21	−2.23	−3.23
pH 6 (A = −22.81)						
0.150	0.153	0.697	−1.65	−0.80	−0.48	−0.84
0.244	0.256	0.500	−6.54	−1.92	−1.22	−1.93
0.244	0.507	0.249	−24.59	−1.20	−0.39	−1.09
0.338	0.335	0.327	−8.15	−1.59	−0.68	−1.52
0.494	0.249	0.257	4.87	−1.51	−0.87	−1.59
0.701	0.146	0.153	21.29	−1.37	−1.00	−1.36

x_{orn}	x_{clm}	x_{ome}	$\Delta_{sol}H_{orn:clm:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{orn:clm:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −102.74)						
0.151	0.147	0.702	−39.70	−5.03	−3.40	−5.01
0.247	0.509	0.244	−47.45	−8.63	−5.56	−8.72
0.253	0.252	0.495	−39.10	−8.33	−5.16	−8.40
0.374	0.314	0.312	−34.04	−9.61	−5.86	−9.62
0.507	0.251	0.242	−22.16	−8.65	−5.40	−8.56
0.699	0.150	0.151	−3.01	−5.51	−3.86	−5.49
pH 6 (A = −44.77)						
0.151	0.147	0.702	−4.49	−2.60	−1.91	−2.61
0.250	0.257	0.493	−10.49	−3.57	−2.07	−3.49
0.252	0.481	0.267	−27.72	−4.16	−2.67	−4.12
0.374	0.314	0.312	−12.14	−4.90	−3.27	−4.90
0.497	0.248	0.255	−5.40	−3.88	−2.52	−3.92
0.698	0.150	0.152	11.89	−3.32	−2.63	−3.34

x_{sec}	x_{clm}	x_{ome}	$\Delta_{sol}H_{sec:clm:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{sec:clm:ome}$ ($kJ\ mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −75.49)						
0.169	0.143	0.688	−43.31	−11.03	−3.00	−4.26
0.246	0.248	0.506	−36.37	−6.71	−4.41	−6.74
0.249	0.501	0.250	−44.05	−7.12	−4.77	−7.13
0.338	0.328	0.334	−33.01	−7.80	−4.92	−7.71
0.518	0.247	0.235	−15.49	−6.26	−4.22	−6.33
0.700	0.153	0.147	2.58	−4.25	−3.04	−4.23
pH 6 (A = −30.90)						
0.169	0.143	0.688	−3.18	−1.99	−1.47	−1.98
0.247	0.489	0.264	−28.15	−2.76	−1.74	−2.73
0.259	0.263	0.478	−8.98	−2.33	−1.37	−2.38
0.338	0.328	0.334	−13.05	−3.26	−2.11	−3.26
0.489	0.249	0.262	−1.33	−2.04	−1.10	−2.08
0.700	0.153	0.147	12.08	−2.24	−1.72	−2.21

Table 8: Experimental and calculated values of excess enthalpies for ternary mixtures at pH 2 and 6 for nitroimidazoles, roxithromycin and omeprazole

x_{met}	x_{rox}	x_{ome}	$\Delta_{sol}H_{met:rox:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{met:rox:ome}$ ($kJ\ mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −77.69)						
0.177	0.142	0.681	−36.30	−4.22	−2.94	−4.26
0.248	0.506	0.246	−43.73	−7.00	−4.64	−7.03
0.262	0.243	0.495	−35.69	−6.72	−4.24	−6.69
0.363	0.319	0.318	−31.70	−7.66	−4.75	−7.61
0.495	0.247	0.258	−19.95	−6.77	−4.27	−6.72
0.659	0.159	0.182	−4.33	−4.66	−3.19	−4.67
pH 6 (A = −33.58)						
0.177	0.141	0.682	−1.02	−2.06	−1.46	−2.04
0.244	0.485	0.271	−24.96	−2.84	−1.79	−2.86
0.277	0.234	0.489	−6.39	−3.10	−2.04	−3.10
0.363	0.319	0.318	−10.12	−3.57	−2.32	−3.55
0.495	0.241	0.264	−0.18	−3.44	−2.43	−3.49
0.659	0.159	0.182	11.57	−2.80	−2.15	−2.79
X_{tin}	x_{rox}	x_{ome}	$\Delta_{sol}H_{tin:rox:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{tin:rox:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −53.13)						
0.151	0.149	0.700	−35.44	−3.44	−2.62	−3.46
0.248	0.504	0.248	−38.83	−5.59	−3.94	−5.58
0.257	0.247	0.496	−31.22	−5.38	−3.80	−5.47
0.334	0.338	0.328	−28.03	−6.15	−4.16	−6.13
0.485	0.248	0.267	−12.55	−5.47	−3.74	−5.44
0.695	0.150	0.155	9.22	−3.47	−2.58	−3.44
pH 6 (A = −23.84)						
0.151	0.149	0.700	−1.22	−1.30	−0.90	−1.27
0.221	0.251	0.528	−6.20	−2.04	−1.27	−1.97
0.242	0.499	0.259	−24.94	−2.02	−1.21	−1.95
0.334	0.338	0.328	−8.21	−2.49	−1.64	−2.53
0.481	0.261	0.258	3.34	−2.62	−1.87	−2.64
0.695	0.150	0.155	−24.25	−1.85	−1.52	−1.91
X_{orn}	x_{rox}	x_{ome}	$\Delta_{sol}H_{orn:rox:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{orn:rox:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −93.72)						
0.150	0.147	0.703	−39.51	−5.36	−3.89	−5.35
0.239	0.486	0.275	−45.88	−9.02	−6.09	−9.08
0.240	0.243	0.517	−38.91	−8.45	−5.65	−8.48
0.321	0.343	0.336	−37.58	−10.02	−6.54	−10.01
0.491	0.258	0.251	−23.02	−9.21	−6.23	−9.21
0.690	0.155	0.155	−3.75	−6.15	−4.57	−6.12
pH 6 (A = −45.77)						
0.150	0.147	0.703	−4.37	−3.06	−2.34	−3.05
0.254	0.238	0.508	−9.34	−4.86	−3.47	−4.88
0.255	0.502	0.243	−27.89	−4.79	−3.30	−4.71
0.321	0.343	0.336	−15.25	−5.46	−3.86	−5.56
0.525	0.252	0.223	−2.21	−5.27	−3.98	−5.33
0.690	0.155	0.155	11.31	−3.90	−3.16	−3.92
x_{sec}	x_{rox}	x_{ome}	$\Delta_{sol}H_{sec:rox:ome}$ ($kJ \cdot mol^{-1}$)	$\Delta H^E_{sec:rox:ome}$ ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (binary contribution) ($kJ \cdot mol^{-1}$)	ΔH^E_{cal} (ternary contribution) ($kJ \cdot mol^{-1}$)
pH 2 (A = −62.68)						
0.151	0.150	0.699	−37.54	−4.33	−3.33	−4.32
0.243	0.506	0.251	−42.62	−6.99	−5.03	−6.96
0.250	0.251	0.499	−35.26	−6.77	−4.83	−6.79
0.353	0.323	0.324	−30.38	−7.66	−5.34	−7.66
0.515	0.245	0.240	−15.28	−6.61	−4.77	−6.67
0.698	0.144	0.158	3.14	−4.40	−3.39	−4.39
pH 6 (A = −30.44)						
0.151	0.150	0.699	−3.54	−1.87	−1.38	−1.86
0.245	0.252	0.503	−9.01	−2.97	−2.01	−2.96
0.253	0.476	0.271	−24.60	−3.01	−1.92	−2.91
0.353	0.323	0.324	−11.09	−3.35	−2.42	−3.54
0.508	0.243	0.249	−0.89	−3.53	−2.55	−3.49
0.698	0.143	0.159	13.08	−2.44	−2.01	−2.49

mole of ternary mixtures (nitroimidazole, macrolides and omeprazole) was determined at various mole fractions. The molar enthalpy of interaction of ternary systems was obtained from the equation:

$$\Delta H^{E}_{(N:M:P)} = \Delta H_{sol} - [x_N \Delta H_N + x_M \Delta H_M + x_P \Delta H_P] \quad (5)$$

The values of excess enthalpy for ternary mixtures of different compositions are given in Tables 7 and 8. If it is assumed that interactions in a ternary system are entirely due to binary interactions then the molar enthalpy of interaction of the drugs can be calculated from those of the constituent binary mixtures. We have calculated the apparent mole fractions for nitroimidazoles, macrolides and omeprazole in the ternary mixtures. The contributions of the binary interactions between any two components (N : M, N : P, M : P) in the ternary mixtures have been calculated from the Redlich-Kister Eq. (3) using the equation:

$$\Delta H^{E}_{cal(N:M,\ N:P,\ M:P)} = (x_N + x_M)\,\Delta H^{E}_{N:M} + (x_N + x_P)\,\Delta H^{E}_{N:P} + (x_M + x_P)\,\Delta H^{E}_{MP} \quad (6)$$

Where,

$$\Delta H^{E}_{N:M} = x_N' x_M' \,(h_{1(N:M)} + h_{2(N:M)}\,(x_N' - x_M') \quad (7)$$

$$\Delta H^{E}_{NP} = x_N'' x_P'' \,(h_{1(N:P)} + h_{2(N:P)}\,(x_N'' - x_P'') \quad (8)$$

$$\Delta H^{E}_{M:P} = x_M''' x_P''' \,(h_{1(M:P)} + h_{2(M:P)}\,(x_M''' - x_P''')] \quad (9)$$

here x_N, x_M & x_O are apparent mole fractions for nitroimidazole, macrolides and omeparazole in the ternary mixtures. The corresponding mole fractions for binary interactions in the Redlich-Kister equation have been calculated using the equations:

$$x_N' = x_N/(x_N + x_M) \quad \text{and} \quad x_M' = x_M/(x_N + x_M)$$

$$x_N'' = x_N/x_N + x_P \quad \text{and} \quad x_P'' = x_P/x_N\,x_P$$

$$x_M''' = x_M/x_M + x_P \quad \text{and} \quad x_P''' = x_P/x_M + x_P$$

Here $\Delta H^{E}_{N:M}$, $\Delta H^{E}_{N:P}$ and $\Delta H^{E}_{M:P}$ are assumed to be the binary contributions to excess enthalpy by the binary mixtures: nitroimidazoles + macrolide, nitroimidazole + omeprazole and macrolide + omeprazole respectively. The contributions calculated using Eq. (6) are given in Tables 4 and 6.

It was found that the calculated values of molar enthalpy of interaction for ternary systems taking into account only binary interactions are less in magnitude than the corresponding experimental values. Therefore a ternary contribution has also been included to minimize the difference between the experimental value for a ternary mixture and that predicted only from contributions by binary mixtures. For this purpose the following equation has been used.

$$\Delta H^{E}_{cal\ (N:M:P)} = (x_N + x_M)\,\Delta H^{E}_{N:M} + (x_N + x_O)\,\Delta H^{E}_{N:O} + (x_M + x_O)\,\Delta H^{E}_{M:O} + x_N x_M x_O\, A_{N:M:O} \quad (10)$$

Here $A_{N:M:O}$ is the interaction parameter for the ternary system. The values of $A_{N:M:O}$ calculated from Eq. (10) by the least squares method are given in Tables 7–8.

As expected, introduction of a ternary contribution, considerably improves the agreement between the calculated and experimental values. The calculated values of $A_{N:M:O}$ have been found to be negative, which indicates that the ternary system deviates more from ideality as compared to its constituent binary systems. This type of behavior suggests some synergistic interaction between the above-mentioned drugs when present in the ternary mixtures. The study suggests that when a nitroimidazole is combined with a mixture of PPI and macrolide, the interaction between the drugs is greater compared to what is expected. The values of ternary interaction parameters have been further used to determine the interaction of drugs in the triple regimen where all the three drugs are prescribed in a kit to be taken twice. The formulations of recommended marketed kits (OTC-HP kit by Biochem Pharma Industries, Pyloban kit by IRM Pharma and Pylobact kit by Rextar) consist of 500 mg tinidazole (x_{tin} 0.8377), 250 mg clarithromycin (x_{clm} 0.1383) and 20 mg omeprazole (x_{ome} 0.024). The molar enthalpy of interaction for the corresponding mole fractions of the drugs has been found to be -1.17 kJ · mol^{-1} at pH 2 and -0.53 kJ · mol^{-1} at pH 6. The magnitude and sign of the interaction enthalpy suggest that it is not safe if all the drugs recommended in a triple regimen involving a PPI are co-formulated in a single capsule.

The results presented above clearly indicate that solution calorimetry has wide scope to study the interaction and compatibility of mixtures of drugs by determining their enthalpy of solution. The extent of compatibility has been quantified between nitroimidazoles, macrolides and omeprazole in solution. Excess molar enthalpy is small and negative for their binary mixtures and has been correlated to the absence of any specific interaction. Deviation of the ternary system more from ideality as compared with the constituent binary systems, found in the present study, predicts that all three drugs should not be co-formulated.

3. Experimental

3.1. Chemicals

Nitroimidazoles (M/s AARTI Drugs Ltd., Mumbai, India), clarithromycin (Ranbaxy Labs Ltd., Gurgaon, India), roxithromycin and omeprazole (Torrent Research Centre, Ahmedabad, India) were procured as donated samples. All the samples were stored as received in airtight plastic containers with desiccators inside. They were used as such without further purification. All the drugs were sieved (#150) and fractions with particle size less than 150 μm were used throughout the study.

3.2. Buffers

Phosphate buffers of pH 2 and 6 and ionic strength 0.1 M were prepared using AR grade mono- and di-sodium salts of phosphoric acid according to a given procedure (Christian 1986). The solutions were freshly prepared using triple distilled water and the pH values were measured using a pH meter (Elico, India) calibrated with standard buffers of pH 4.0, 7.0 and 9.2.

3.3. Preparation of samples

Quantities of dried and sieved samples of drugs were accurately weighed and combined to produce mixtures of varying mole fractions. For example, 2.5 mg of metronidazole and 5.1 mg of clarithromycin were weighed and mixed mechanically for the preparation of a binary mixture with mole fractions 0.6819 and 0.3181, respectively. In calculating the mole fractions in aqueous buffers we did not take into account water and other electrolytes present in the solution and called these apparent mole fractions. For each sample three replicate investigations were performed and results are quoted as mean values.

3.4. Calorimetric studies

A model C-80 heat flux microcalorimeter (SETARAM, France) was used for thermal measurements. In accordance with the Calvet principle, two experimental vessels (reference and sample) were placed in a calorimetric block. Temperature control by the thermostat of the calorimeter was within ±0.001 K. The performance of the calorimeter was tested by measuring the enthalpy of a solution of potassium chloride in triple-distilled water, which has a known enthalpy of solution of 17.322 kJ/mol.

In order to measure the enthalpy of solution, the reference cell was loaded with 5 ml of buffer solution of the desired pH, and the sample cell with a definite quantity of the individual drug or the desired mixture and 5 ml of the same buffer solution separated by a displaceable lid. After stabilization, mixing was done in the calorimeter itself by reversing it, which homogenizes the sample with the buffer solution. The signal was automatically

recorded on the strip chart recorder. The deviation of the sample signal from the base line indicates the rate of heat evolved or absorbed by the sample.

3.5. Solubility studies

Weighed amounts of pure drugs were placed in 25 ml conical flasks containing 10 ml buffer solution of the desired pH. These flasks were equilibrated in a thermostated shaking water bath set at 37 °C for 24 h. Then the samples were filtered through 0.2 μm filters and suitably diluted. Diluted samples were assayed spectrophotometrically at different wavelengths for different drugs.

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