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Enthalpies of solution of ampicillin, amoxycillin and their binary mixtures at 310.15 K

D.V.S. Jain [1], N. Kashid, S. Kapoor, R. Chadha *

University Institute of Pharmaceutical Sciences, Punjab University, Chandigharh, 160014, India

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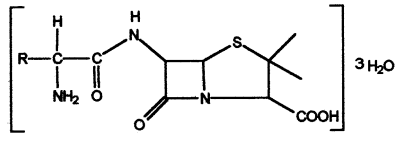
Abstract

Enthalpies of solutions of ampicillin, amoxycillin and their binary mixtures have been determined at pH 2, 5, and 7 using C-80 calorimeter. The systems showed endothermic behaviour; molar enthalpies of solutions of ampicillin were determined to be 13.32, 15.89 and 23.21 kJ mol^{-1} and amoxycillin were 16.32, 18.45 and 26.25 kJ mol^{-1} at pH 2, 5, and 7, respectively. The excess molar enthalpies of solution have also been calculated to find any interaction between these two drugs. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Ampicillin; Amoxycillin; Binary mixture; Calorimeter; Enthalpy of solution; Excess molar enthalpy

1. Introduction

During this decade microcalorimetry has aroused considerable interest as a versatile technique and provides useful information about the thermodynamics (Pikal et al., 1978; Pikal and Dellerman, 1989; Burgot and Burgot, 1990), kinetics (Oliyai and Lindenbaum, 1991) and analytical aspects (Grant and York, 1986; Gao and Rytting, 1997) of a drug molecule. Ampicillin and amoxycillin are important drugs and are widely used for therapeutic purposes. As part of our systematic studies on the thermodynamics and stability of these drugs, we report here the enthalpies of solution of ampicillin trihydrate (**I**), amoxycillin trihydrate (**II**) and their binary mixtures in aqueous buffer solution in the pH range of 2–7 at 310.15 K. The literature survey has revealed that no data are reported for the enthalpies of solutions of these drugs except a report by Tsuji et al. (1978) and Hou and Poole (1969) giving the calculated values from the slope of the classical Van't Hoff plot between solubility and $1/T$ in 0.5 M KCl solution.



(I) **(II)**

* Corresponding author.

[1] Present address: Department of Chemistry, Punjab University, Chandigharh, 160014, India.

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Table 1
Molar enthalpies of solution of ampicillin and amoxycillin at various pH at 310.15 K

pH	[Ampicillin] (M)	ΔH_{sol} (kJ mol^{-1})	[Amoxycillin] (M)	ΔH_{sol} (kJ mol^{-1})
2	0.00148	13.34	0.00143	16.35
	0.00248	13.29	0.00242	16.32
	0.00396	13.32	0.00382	16.30
	0.00495	13.31	0.00485	16.31
		13.32 ± 0.020		16.32 ± 0.021
5	0.00148	15.83	0.00141	18.47
	0.00248	15.84	0.00252	18.43
	0.00396	15.82	0.00392	18.44
	0.00495	15.85	0.00501	18.45
		15.84 ± 0.012		18.45 ± 0.017
7	0.00148	23.24	0.00143	26.23
	0.00248	23.20	0.00255	26.27
	0.00396	23.19	0.00379	26.26
	0.00495	23.21	0.00495	26.24
		23.21 ± 0.020		26.25 ± 0.018
6.5–7		22.77[a]		25.73[b]

[a] Enthalpy of solution estimated by Hou and Poole (1969).
[b] Enthalpy of solution estimated by Tsuji et al. (1978).

2. Material and methods

Amoxycillin trihydrate and ampicillin trihydrate were provided by Morpen Labs Ltd., Parwanoo (India) and were used as supplied without further purification.

The buffers used were citric acid–disodium hydrogen phosphate solutions. Water used for the preparation of buffers was triple-distilled.

The pH values of various buffers were determined by pH meter, Elico (India). The system used to determine the enthalpies of solution was a heat flux calorimeter model-C-80 (Setaram, France). In accordance with the Calvet principle, two experimental vessels (reference and sample) were placed in a calorimetric block, which imposes the temperature of experiment as fixed or variable. The temperature control was ± 0.003 K. To determine the enthalpy of solution, the reference cell of calorimeter was loaded with 4 ml of buffer of desired pH and the sample cell with the same amount of the buffer and a definite quantity

Table 2
Fractions of cationic, zwitterionic, and anionic species of ampicillin and amoxycillin at pH 2, 5, and 7

pH	f^+	$f^\pm$	f^-
Ampicillin			
2	0.81706	0.18293	0.00010
5	0.00442	0.99000	0.00557
7	0.00003	0.63988	0.36009
Amoxycillin			
2	0.810090	0.18990	0.00001
5	0.000421	0.98890	0.00684
7	0.000020	0.59038	0.40844

Table 3
Values corresponding to ΔH_s^+, $\Delta H_s^\pm$ and ΔH_s^- for ampicillin and amoxycillin

Drug	ΔH_s^+ (kJ mol^{-1})	$\Delta H_s^\pm$ (kJ mol^{-1})	ΔH_s^- (kJ mol^{-1})
Ampicillin	12.71	15.73	36.46
Amoxycillin	15.84	18.32	37.71

of desired sample partitioned by a 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 calorimeter was tested by measurements on the enthalpy of solution of KCl (Balk and Benson, 1959) in triple-distilled water and for heat of mixing of benzene and methylbenzene at 298.15 K (Murakami et al., 1969). The accuracy of any individual measurement was better than 0.5 J mol^{-1}.

The samples were weighed in the lower container of the calorimetric vessel itself using a single pan Mettler balance with an accuracy of 0.01 mg. Mixtures of ampicillin and amoxycillin were prepared by mass and the possible error in molar fraction was estimated to be less than 10^{-4}.

3. Result and discussion

3.1. Enthalpies of solutions of pure drugs

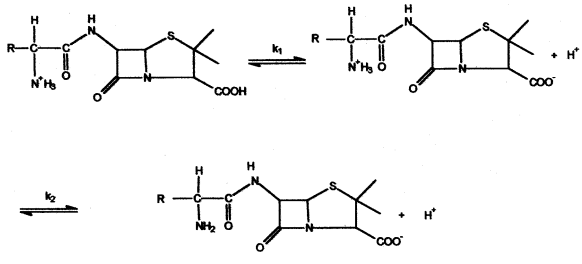
The experimental values of the enthalpies of the solution of both the drugs as a function of pH and concentration are given in Table 1. It can be seen that molar enthalpies of solution (ΔH_{sol}) are independent of concentration but the value changes from 13.31 to 23.21 kJ mol^{-1} for ampicillin and from 16.32 to 26.25 kJ mol^{-1} for amoxycillin as the pH changes from 2 to 7.

As mentioned above no data is available in the literature for direct comparison. Our calorimetrically measured molar enthalpy of solution for ampicillin (23.21 kJ mol^{-1}) and amoxycillin (26.25 kJ mol^{-1}) compares well with the estimated values of Hou and Poole (1969) for ampicillin (22.77 kJ mol^{-1}) and of Tsuji et al. (1978) for amoxycillin (25.23 kJ mol^{-1}) using Van't Hoff equation in 0.5 M KCl solution. (Table 1). It can be seen from Table 1 that agreement between these values and our values at pH 7 is reasonably good, taking into consideration the different experimental conditions.

Both ampicillin and amoxycillin are amphoteric in nature (Hou and Poole, 1969) and exist as zwitterion even in crystalline state (James and Hall, 1968). Thus, the observed enthalpies of solution also include contribution from the protonation/deprotonation of the zwitterion. Accordingly, the observed ΔH_{sol} may be expressed by Eq. (1)

$$\Delta H_{sol} = f^{+}\Delta H_s^{+} + f^{\pm}\Delta H_s^{\pm} + f^{-}\Delta H_s^{-} \quad (1)$$

Where ΔH_{sol} is molar enthalpy of solution and f^{+}, $f^{\pm}$ and f^{-} are the fractions of cationic, zwitterionic and anionic species of these drugs. Their values at pH 2, 5, and 7 were calculated using the literature values of pK_1 and pK_2 (Tsuji et al., 1978) and are given in Table 2. $\Delta H_s^{\pm}$ represents the molar enthalpy of solution for the zwitterionic species of both the drugs, whereas ΔH_s^{+} and ΔH_s^{-} includes the contribution due to protonation and deprotonation of zwitterion at pH 2 and 7 and represents molar enthalpy of solution for cationic and anionic species (Table 3) respectively. It is clear from Table 2 that at pH 5, which is very near to isoelectric point (4.95) of both the penicillins, the predominant species is zwitterion, which is present up to the extent of 99%, and the experimental enthalpy of solution is only due to this species. At pH 2 or 7, the simultaneous equilibria may also occur.



The enthalpies of solution for the zwitterionic forms of ampicillin and amoxycillin are 15.73 and 18.32 kJ mol^{-1}, respectively (Table 3). Thus, enthalpies of reaction for carboxyl group or enthalpies of deprotonation (ΔH_1) $RCOOH \Leftrightarrow RCOO^{-} + H^{+}$ have been calculated to be 3.03 and 2.48 kJ mol^{-1} for ampicillin and amoxycillin, respectively, whereas the enthalpies of reaction for charged amino group (ΔH_2) are calculated to be 20.76 and 19.45 kJ mol^{-1} for ampicillin and amoxycillin. The former values (ΔH_1) fall fairly well within the range of characteristic heat of ionization for carboxyl group of amino acids which varies from 0.25 to 4.83 kJ mol^{-1} (Green-

stein and Winitz, 1961). Moreover, there is good agreement between our values and the values calculated by Hou and Poole (1969) for ampicillin (3.347 kJ mol^{-1}) estimated from pK_1 data. However the ΔH_2 values are lower than the characteristic heat of ionization for charged amino group of amino acids varying between 39.32 and 54.39 kJ mol^{-1} (Greenstein and Winitz, 1961). The endothermic behaviour of enthalpies of solution indicates little interaction of the drugs with the solvent.

The free energy of solution of both the drugs was calculated by Eq. (2), used to calculate the free energy of solution for amino acids (Greenstein and Winitz, 1961).

$$\Delta G_{sol} = -RT \ln N_2/0.0177 \quad (2)$$

Where N_2 represents the mole fraction of saturated solution and has been calculated from the literature values of solubilities of ampicillin and amoxycillin (Tsuji et al., 1978) expressed in term of molality of the solution. The values of ΔG_{sol} are given in Table 4 and have further been used to calculate the molar entropies of solution. Molar entropies of ionization of cationic and zwitterionic species of ampicillin have been calculated to be -41.33, -6.12 J K^{-1} mol^{-1} and of amoxycillin to be -43.10, -73.41 J $K^{-1}mol^{-1}$.

3.2. Interaction between ampicillin and amoxycillin

Both ampicillin and amoxycillin have been known to show synergistic effects with other an-

Table 4
Molar free energies and molar entropies of solution for ampicillin and amoxycillin

pH	ΔG_{sol} (kJ mol^{-1})	ΔS_{sol} (J K^{-1} mol^{-1})
Ampicillin		
2	5.22	26.11
5	9.46	20.57
7	7.95	49.20
Amoxycillin		
2	6.47	31.75
5	10.96	24.14
7	09.79	53.07

Table 5
Molar enthalpies of solution [$\Delta H_{sol(m)}$] and excess molar enthalpies of solution [ΔH^E_{sol}] for the binary mixture of ampicillin and amoxycillin at various pH

X	$\Delta H_{sol(m)}$ (kJ mol^{-1})	((ΔH^E_{sol} (kJ mol^{-1})
pH 2		
0.0928	13.5008	−0.0459
0.1508	13.6595	−0.0640
0.2240	13.8376	−0.1089
0.2996	14.0411	−0.1358
0.3762	14.2488	−0.1615
0.4911	14.5699	−0.1905
0.6062	14.9083	−0.2029
0.7629	15.4051	−0.1835
0.8758	15.8081	−0.1245
0.8851	15.8434	−0.1175
pH 5		
0.0928	16.0007	−0.0681
0.1508	16.1141	−0.1021
0.3051	16.4500	−0.1599
0.3762	16.6051	−0.1860
0.4911	16.8716	−0.2125
0.6062	17.1523	−0.2252
0.6837	17.3534	−0.2218
0.7202	17.4520	−0.2162
0.8318	17.7705	−0.1822
0.8851	17.9486	−0.1401
pH 7		
0.0928	23.4109	−0.0822
0.1508	23.5512	−0.1215
0.2240	23.7397	−0.1599
0.2996	23.9481	−0.1858
0.3762	24.1591	−0.2122
0.4911	24.4877	−0.2398
0.6062	24.8341	−0.2502
0.6837	25.0763	−0.2482
0.7629	25.3362	−0.2338
0.8491	25.7725	−0.2022
0.8851	25.6350	−0.1761

Table 6
Values of parameters h_i in Eq. (4) along with the standard deviations (SD) of ΔH^E_{sol} for various mixtures of amoxycillin and ampicillin at pH 2, 5 and 7

pH	h_i J mol^{-1}			SD
	h_0	h_1	h_2	
2	−0.7729	−0.3890	−0.1249	0.005
5	−0.8471	−0.3668	−0.4130	0.003
7	−0.9510	−0.3888	−0.5784	0.006

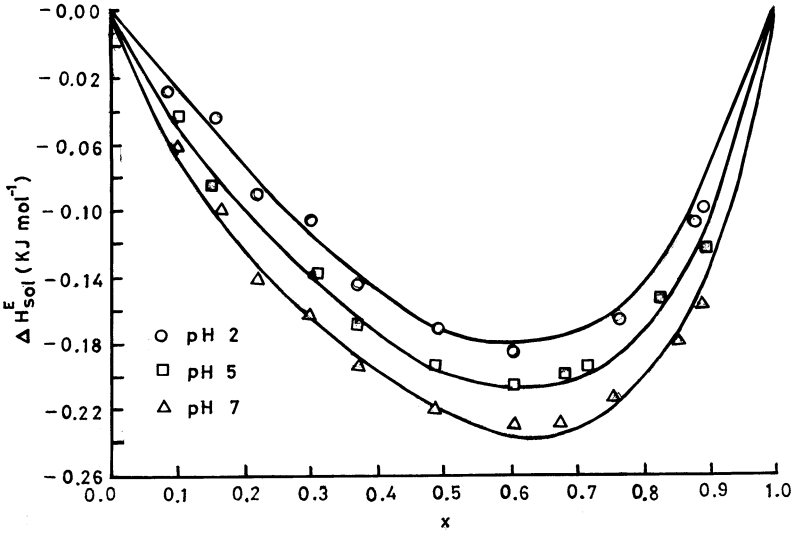


Fig. 1. Excess molar enthalpies of solutions of binary mixtures of ampicillin and amoxycillin at pH 2, 5 and 7. The curves have been calculated from Eq. (4) and points represent experimental results.

tibiotics. Several methods for their analysis (Sabath et al., 1967; Moety et al., 1989; Akanni and Ayim, 1992; Fattah et al., 1992) and stability determination (Girona et al., 1988) in a mixture have been reported. Now in order to find any interaction between the antibiotics, we have studied the enthalpies of solution of the mixture of ampicillin and amoxycillin. The results at various mole fractions are given in Table 5. The excess molar enthalpies of solution of binary mixture were calculated by Eq. (3).

$$\Delta H^{E}_{sol} = \Delta H_{sol(m)} - [_{1-X}\{f_1^{+}\Delta H^{+}_{s_1} + f_1^{\pm}\Delta H^{\pm}_{s_1} + f_1^{-}\Delta H^{-}_{s}\} + _{X}\{f_2^{+}\Delta H^{+}_{s_2} + f_2^{\pm}\Delta H^{\pm}_{s_2} + f_2^{-}\Delta H^{-}_{s_2}\}] \quad (3)$$

$\Delta H_{sol(m)}$, molar enthalpy of solution of binary mixture.

$_{1\text{-}X}$, mole fraction of ampicillin.

$_{X}$, mole fraction of amoxycillin.

The results at various mole fractions are fitted to the equation

$$\Delta H^{E}_{m} = {}_{(1-X)(X)} \sum_{i=0}^{n} h_{i(2X-1)^i} \quad (4)$$

h_i, magnitude of the ith parameter.

The values of the parameters: h_i has been determined by the method of the least squares along with the standard deviations are given in Table 6. The experimental excess molar heats of solutions are plotted in (Fig. 1). The smoothened representations by Eq. (4) are shown as solid curves and experimental values as points in Fig. 1.

The excess molar enthalpies of solution of the binary mixture of ampicillin and amoxycillin are found to be negative over all the mole fractions and the pH range 2–7 (Table 5). The small negative values of excess enthalpies indicate little interaction between the drugs. The estimation of the enthalpy of solution of binary mixture of ampicillin and amoxycilllin and calculation of ΔH^{E}_{sol} gives us a simple and direct procedure for simultaneous determination of both the drugs in a mixture. A conventional spectroscopic determination has been found to be inapplicable because λ_{max} of both the drugs is the same and there is a possibility of interference.

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