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Viscometric and Conductometric Studies of Doxycycline Hyclate in Water, Aqueous Glycine and L-Alanine Solutions at Different Temperatures

SHASHI KANT SHARMA*, POONAM and NISHA SHARMA

Department of Chemistry, Himachal Pradesh University, Summer Hill, Shimla-171005, India

*Corresponding author: E-mail: drsklomesh@rediffmail.com

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To investigate the behaviour of doxycycline hyclate in water, aqueous glycine and aqueous L-alanine solutions, the viscometric and conductometric studies have been conducted at different temperatures. Viscosity data has been used to derive the Jones-Dole viscosity B-coefficient, temperature derivative of B-coefficient (dB/dT), viscosity B-coefficient of transfer ($\Delta_{tr}B$), free energy of activation of viscous flow per mole of solvent ($\Delta\mu_1^{o*}$) and solute ($\Delta\mu_2^{o*}$) respectively, activation entropy (ΔS_2^{o*}) and activation enthalpy (ΔH_2^{o*}). Conductance data has been used to compute Walden product ($\Lambda_m^o\eta_o$) and temperature coefficient of Walden product ($d\Lambda_m^o\eta_o/dT$) for doxycycline hyclate in water, and in aqueous glycine and aqueous L-alanine solution. The positive values of B-coefficient, $\Delta_{tr}B$ indicate the prevailing of hydrophilic-ionic interactions in the systems under examination. The negative values of dB/dT and positive values of temperature coefficient of Walden product infer structure maker tendency of doxycycline hyclate in water, and in aqueous glycine and aqueous L-alanine solution. Transfer energy parameters indicate the breaking of intermolecular bonds in transition state which means that formation of activated complex is unfavourable.

Keywords: Jones-Dole viscosity, Activation entropy, Activation enthalpy, Walden product.

INTRODUCTION

Proteins are long chains of amino acids which are critically important for catalyzing the chemical reactions, transporting materials across the cell and receiving and sending chemical signals. Proteins are essential for the main physiological processes of life and form essential functions throughout the system of human body. The proteins such as receptors, proteinaceous enzymes, transport proteins, membrane proteins and ion channels serve as the target proteins for most of the drugs in clinical use [1,2]. The drug transport and disposition is influenced by drug binding to plasma and tissue proteins. Thus drug- protein binding is one of the most important phenomena during the action of drug. To study the mechanism of the drug-protein molecular interactions, 'amino acids' which are the basic building blocks of proteins and the most important model compound of proteins, are quite appropriate and useful to explore these molecular interactions.

The molecular size, shape, chain length, degree of molecular association and intermolecular interactions has notable effect on viscometric properties of binary/ternary mixtures. Thus, viscometric and conductance study of various bioactive compounds is substantial for understanding the ion-ion, ion-hydrophilic and hydrophobic interactions existing in the solutions. The viscometric properties such as viscosity B-coefficient, the viscosity B-coefficient of transfer, free energy of activation of solute/solvent, change in enthalpy and temperature coefficient of Walden product furnish useful information about solute-solute/solute-solvent interactions occurring in solution phase [3-6]. Knowing this advantage, the viscometric measurements have been used in the present study to analyze interactions of drug with water as well as with amino acids. The biochemical processes mostly take place in aqueous media, therefore studying different properties of drugs in aqueous phase will enrich researchers with useful information. This information has significance in industrial and biological fields [7,8]. As amino acids are the fundamental structural unit of biological macromolecules mainly proteins [9-11], the results obtained from the above study will also be helpful in understanding the absorption of drugs, transport of drugs across the

biological membranes, protein hydration, denaturation and aggregation [12-17]. Moreover, to have deeper knowledge of the mechanism of drug action, the viscometric properties could be examined at different temperatures and at variable concentrations of drug and amino acids. In recent years, researchers are developing appreciable interest in studying thermodynamic properties of model compounds like amino acids and small peptides [18-21]. Different attempts have been made by using different techniques to understand interactions taking place between drug and amino acids but little viscometric data is available [22-24].

In present system, the viscometric properties of doxycycline hyclate (drug) in water and aqueous solution of glycine/L-alanine has been studied. Glycine and L-alanine both are non-essential amino acids and are involved in various biochemical processes occurring in the body [25,26]. Both the amino acids are amphiprotic in nature as they contain carboxylic acid and amine functional groups thus exist in neutral as well as zwitterionic form in solutions [27]. Side chain of glycine contains single hydrogen atom and that of L-alanine contains methyl group. Different side chains interact differently with the drug molecules which in turn affect the viscometric and conductometric properties. Thus different results are obtained for various viscometric parameters. Since variation in temperature and concentration has significant effect on various type of interactions [25], thus viscometric and conductance measurements have been examined at four different temperatures (305.15 to 320.15 K) at an interval of 5 K, in order to understand various intermolecular interactions and structure making/breaking ability of the drug in binary/ternary system (doxycycline hyclate + water and doxycycline hyclate + glycine/L-alanine + water).

EXPERIMENTAL

Doxycyline hyclate ($C_{22}H_{24}N_2O_8{\cdot}HCl{\cdot}0.5H_2O{\cdot}0.5C_2H_6O$, m.w. 512.94 g mol^{-1}), analytical grade, glycine ($C_2H_5NO_2$, m.w. 75.07 g mol^{-1}) and L-alanine ($C_3H_7NO_2$, m.w. 89.09 g mol^{-1}) have been used in the present study. After drying the chemicals over anhydrous $CaCl_2$, these have been used as such without any further purification. The mass fraction purity of the used chemicals is ≥ 99 %.

In the present work, the doxycycline hyclate has been studied in water, (0.002, 0.004 and 0.006) mol kg^{-1} aqueous glycine and (0.002, 0.004 and 0.006) mol kg^{-1} aqueous L-alanine. The concentration range of doxycycline hyclate that has been examined is (0.002 to 0.01) mol kg^{-1} in different solvent systems. For preparing all the solutions the triple distilled water with very low specific conductance has been used and weight measurements have been performed in Shimadzu electronic balance. For viscosity calculations the time of flow has been measured with the jacketed Ostwald viscometer. The density and conductance measurements have been performed using DSA 5000 (Density and Sound Analyzer), Anton Paar and calibrated Digital Conductivity Meter respectively. Both the jacketed viscometer and conductivity meter were connected to water thermostat to maintain the constant temperature. For the calibration of viscometer and density and sound analyzer distilled water was used.

As both, the molality as well as molarity have been used in calculations therefore for converting molality (m) to molarity (C) using the molecular mass (M) of doxycycline hyclate and density (ρ) of solution following relation has been used:

$$C = \frac{1000m\rho}{1000 + mM} \quad (1)$$

In this equation m is measured in mol kg^{-1}, M in g mol^{-1} and density in kg m^{-3}.

RESULTS AND DISCUSSION

Viscometric studies: An attempt has been made to study the behaviour of doxycycline hyclate in water, aqueous glycine and aqueous L-alanine using viscosity parameters. For viscosity calculations following relation has been used [28,29],

$$\eta_s = \frac{\rho_s t_s \eta_o}{\rho_o t_o} \quad (2)$$

In above equation, ρ_s (kg m^{-3}) is the density, t_s (s) is flow time of solution and η_s (mPa s) is the viscosity of solution. However, ρ_o (kg m^{-3}), t_o (s) and η_o (mPa s) are the respective parameters of solvent, respectively. The values of viscosity have been recorded in Table-1.

The viscosity is found to increase with the increase in concentration of amino acids as well as drug. This may be due to increase in number of molecules which can participate in interactions as a result there is increase in intermolecular interactions which cause more frictional resistance to the flow of solutions. However, the decrease in viscosity with the

TABLE-1
VALUES OF DYNAMIC VISCOSITY (η_s) AND DENSITY (ρ_s) OF DOXYCYCLINE HYCLATE IN WATER, AQUEOUS GLYCINE AND L-ALANINE AT DIFFERENT TEMPERATURES

*m_D (mol kg^{-1})	η_s (mPa s)				$\rho_s \times 10^{-3}$ (kg m^{-3})			
	305.15 K	310.15 K	315.15 K	320.15 K	305.15 K	310.15 K	315.15 K	320.15 K
Doxycycline hyclate + water								
0.000	0.7646	0.6922	0.6302	0.5764	0.995044	0.993310	0.991417	0.989368
0.002	0.7652	0.6928	0.6307	0.5769	0.995312	0.993569	0.991667	0.989610
0.003	0.7654	0.6929	0.6308	0.5770	0.995444	0.993697	0.991791	0.989730
0.004	0.7656	0.6931	0.6310	0.5771	0.995576	0.993824	0.991914	0.989848
0.005	0.7658	0.6932	0.6311	0.5772	0.995706	0.993951	0.992036	0.989966
0.006	0.7659	0.6934	0.6312	0.5773	0.995836	0.994076	0.992157	0.990083
0.007	0.7661	0.6935	0.6314	0.5774	0.995965	0.994201	0.992278	0.990200
0.008	0.7663	0.6937	0.6315	0.5775	0.996093	0.994326	0.992397	0.990316
0.009	0.7665	0.6938	0.6316	0.5776	0.996219	0.994449	0.992516	0.990431
0.010	0.7667	0.6940	0.6318	0.5778	0.996348	0.994572	0.992633	0.990544

Doxycycline hyclate + 0.002 mol kg^{-1} glycine								
0.000	0.7702	0.6985	0.6396	0.5863	0.995253	0.993576	0.991672	0.989573
0.002	0.7706	0.6990	0.6400	0.5867	0.995519	0.993835	0.991922	0.989813
0.003	0.7708	0.6991	0.6401	0.5868	0.995653	0.993963	0.992046	0.989932
0.004	0.7710	0.6993	0.6402	0.5869	0.995786	0.994091	0.992168	0.990050
0.005	0.7711	0.6994	0.6403	0.5870	0.995917	0.994218	0.992290	0.990168
0.006	0.7713	0.6995	0.6405	0.5871	0.996047	0.994344	0.992413	0.990286
0.007	0.7715	0.6997	0.6406	0.5872	0.996177	0.994470	0.992534	0.990404
0.008	0.7717	0.6998	0.6407	0.5873	0.996306	0.994595	0.992655	0.990521
0.009	0.7718	0.7000	0.6408	0.5874	0.996435	0.994719	0.992775	0.990636
0.010	0.7720	0.7001	0.6409	0.5875	0.996562	0.994844	0.992895	0.990753
Doxycycline hyclate + 0.004 mol kg^{-1} glycine								
0.000	0.7727	0.7009	0.6418	0.5882	0.995310	0.993625	0.991733	0.989650
0.002	0.7732	0.7013	0.6422	0.5886	0.995576	0.993882	0.991981	0.989888
0.003	0.7733	0.7014	0.6423	0.5887	0.995708	0.994010	0.992104	0.990006
0.004	0.7735	0.7016	0.6424	0.5888	0.995839	0.994137	0.992227	0.990123
0.005	0.7737	0.7017	0.6426	0.5889	0.995969	0.994263	0.992348	0.990240
0.006	0.7738	0.7019	0.6427	0.5890	0.996099	0.994389	0.992469	0.990357
0.007	0.7740	0.7020	0.6428	0.5891	0.996228	0.994513	0.992589	0.990473
0.008	0.7742	0.7022	0.6429	0.5892	0.996356	0.994638	0.992710	0.990589
0.009	0.7743	0.7023	0.6430	0.5893	0.996483	0.994761	0.992828	0.990704
0.010	0.7745	0.7024	0.6431	0.5894	0.996610	0.994884	0.992948	0.990820
Doxycycline hyclate + 0.006 mol kg^{-1} glycine								
0.000	0.7752	0.7030	0.6438	0.5893	0.995439	0.993745	0.991845	0.989720
0.002	0.7757	0.7034	0.6442	0.5897	0.995702	0.994000	0.992090	0.989955
0.003	0.7758	0.7036	0.6443	0.5898	0.995833	0.994126	0.992212	0.990072
0.004	0.7760	0.7037	0.6444	0.5899	0.995963	0.994252	0.992333	0.990189
0.005	0.7762	0.7037	0.6446	0.5900	0.996091	0.994376	0.992453	0.990305
0.006	0.7764	0.7040	0.6447	0.5901	0.996219	0.994500	0.992573	0.990421
0.007	0.7765	0.7041	0.6448	0.5902	0.996347	0.994624	0.992692	0.990536
0.008	0.7767	0.7043	0.6449	0.5903	0.996474	0.994747	0.992810	0.990651
0.009	0.7769	0.7044	0.6450	0.5904	0.996601	0.994870	0.992928	0.990766
0.010	0.7770	0.7046	0.6452	0.5905	0.996727	0.994991	0.993047	0.990880
Doxycycline hyclate + 0.002 mol kg^{-1} L-alanine								
0.000	0.7727	0.7007	0.6412	0.5880	0.995233	0.993562	0.991646	0.989546
0.002	0.7732	0.7012	0.6416	0.5884	0.995498	0.993819	0.991893	0.989783
0.003	0.7734	0.7013	0.6418	0.5885	0.995630	0.993946	0.992016	0.989901
0.004	0.7736	0.7015	0.6419	0.5886	0.995761	0.994073	0.992138	0.990018
0.005	0.7738	0.7016	0.6420	0.5888	0.995890	0.994199	0.992260	0.990135
0.006	0.7739	0.7018	0.6422	0.5889	0.996020	0.994324	0.992380	0.990253
0.007	0.7741	0.7019	0.6423	0.5890	0.996148	0.994450	0.992501	0.990368
0.008	0.7743	0.7021	0.6424	0.5891	0.996276	0.994575	0.992621	0.990484
0.009	0.7745	0.7022	0.6425	0.5892	0.996403	0.994698	0.992738	0.990601
0.010	0.7747	0.7024	0.6427	0.5893	0.996530	0.994821	0.992858	0.990716
Doxycycline hyclate + 0.004 mol kg^{-1} L-alanine								
0.000	0.7752	0.7030	0.6431	0.5899	0.995303	0.993608	0.991710	0.989598
0.002	0.7757	0.7035	0.6435	0.5903	0.995567	0.993863	0.991955	0.989833
0.003	0.7759	0.7036	0.6437	0.5904	0.995697	0.993990	0.992077	0.989950
0.004	0.7761	0.7038	0.6438	0.5905	0.995827	0.994115	0.992199	0.990067
0.005	0.7763	0.7039	0.6439	0.5906	0.995956	0.994241	0.992320	0.990183
0.006	0.7764	0.7041	0.6440	0.5907	0.996084	0.994366	0.992439	0.990299
0.007	0.7766	0.7042	0.6442	0.5908	0.996212	0.994490	0.992560	0.990414
0.008	0.7768	0.7044	0.6443	0.5909	0.996338	0.994613	0.992678	0.990529
0.009	0.7770	0.7045	0.6444	0.5911	0.996465	0.994736	0.992797	0.990644
0.010	0.7772	0.7047	0.6446	0.5912	0.996590	0.994858	0.992916	0.990758
Doxycycline hyclate + 0.006 mol kg^{-1} L-alanine								
0.000	0.7777	0.7053	0.6451	0.5920	0.995370	0.993674	0.991770	0.989685
0.002	0.7782	0.7058	0.6455	0.5924	0.995632	0.993928	0.992014	0.989919
0.003	0.7784	0.7059	0.6456	0.5925	0.995762	0.994053	0.992136	0.990035
0.004	0.7786	0.7061	0.6458	0.5926	0.995891	0.994179	0.992256	0.990151
0.005	0.7787	0.7062	0.6459	0.5927	0.996020	0.994304	0.992377	0.990267
0.006	0.7789	0.7064	0.6460	0.5928	0.996148	0.994428	0.992497	0.990383
0.007	0.7791	0.7066	0.6461	0.5929	0.996274	0.994552	0.992616	0.990497
0.008	0.7793	0.7067	0.6463	0.5930	0.996400	0.994673	0.992734	0.990611
0.009	0.7795	0.7069	0.6464	0.5931	0.996526	0.994793	0.992852	0.990726
0.010	0.7796	0.7070	0.6465	0.5933	0.996651	0.994916	0.992969	0.990840

*m_D is the molality of doxycycline hyclate in water and in different concentrations of glycine and L-alanine.

increase in temperature may be due to the increase in kinetic energy of molecules present in the solution [30,31].

Jones-Dole equation [32] has been used to examine the variation of ψ with $C^{1/2}$ and is represented as:

$$\psi = \frac{\eta_r - 1}{C^{1/2}} = A + BC^{1/2} \quad (3)$$

where $\eta_r = \eta_s/\eta_o$ is the relative viscosity of the solution, η_s and η_o are the viscosities of solution and solvent respectively and C is the molarity (mol m^{-3}) of the solution. Figs. 1 and 2 give the graphical representation of eqn. 3.

The intercept A is called Falkenhagen coefficient which is an indicative of solute-solute interactions and slope B is called Jones-Dole coefficient which measures solute-solvent interactions [32,33]. The values of $(\eta_r - 1)/C^{1/2}$ are listed in Table-2. The larger positive values of B than A indicates that solute-solvent interactions are stronger than solute-solute interactions in all the systems under consideration [34]. This

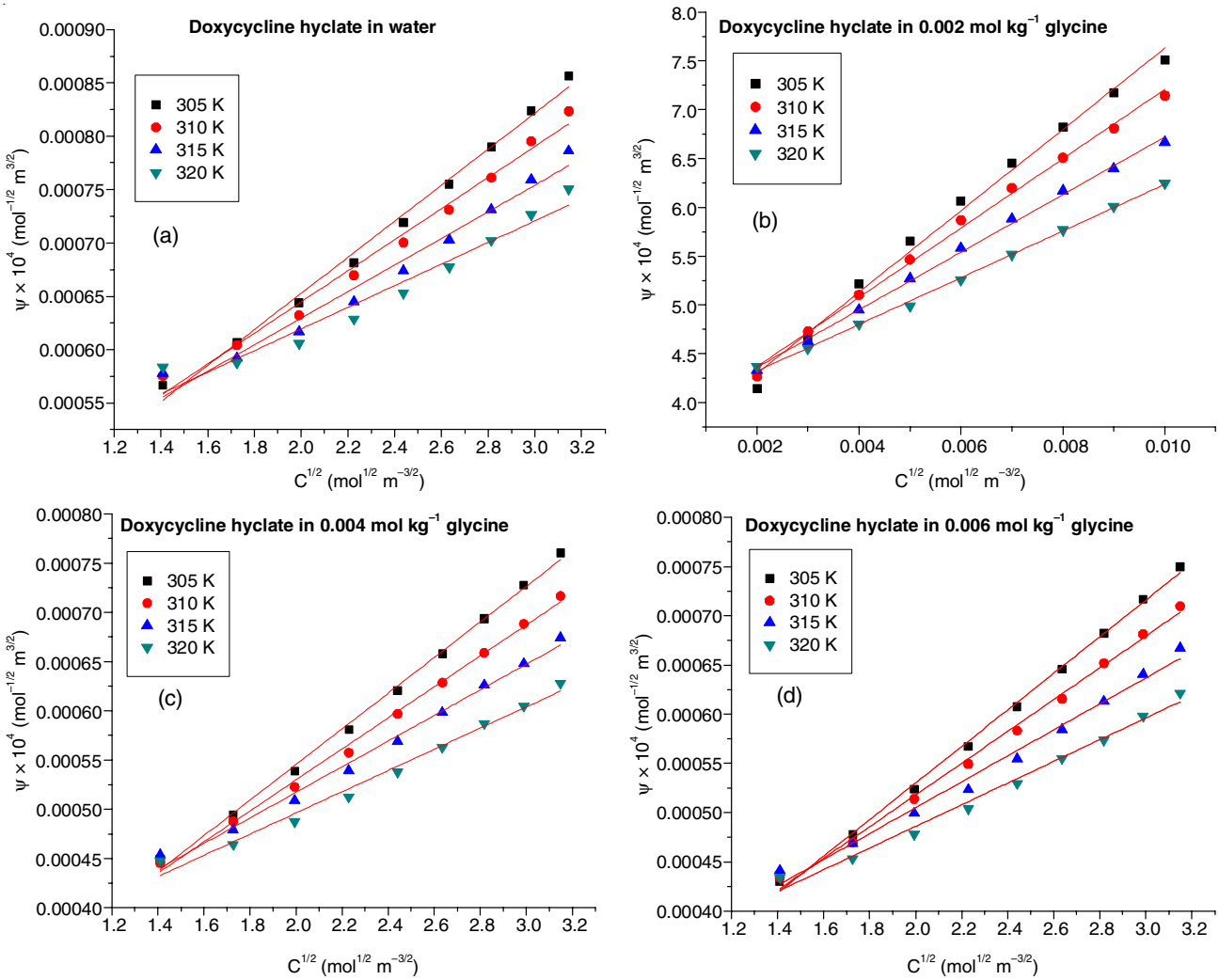

Fig. 1. Plots of $\psi = (\eta_r - 1)/C^{1/2}$ *vs.* concentration ($C^{1/2}$) for doxycycline hyclate in (a) water (b) 0.002 mol kg^{-1} aqueous glycine (c) 0.004 mol kg^{-1} aqueous glycine (d) 0.006 mol kg^{-1} aqueous glycine at different temperatures

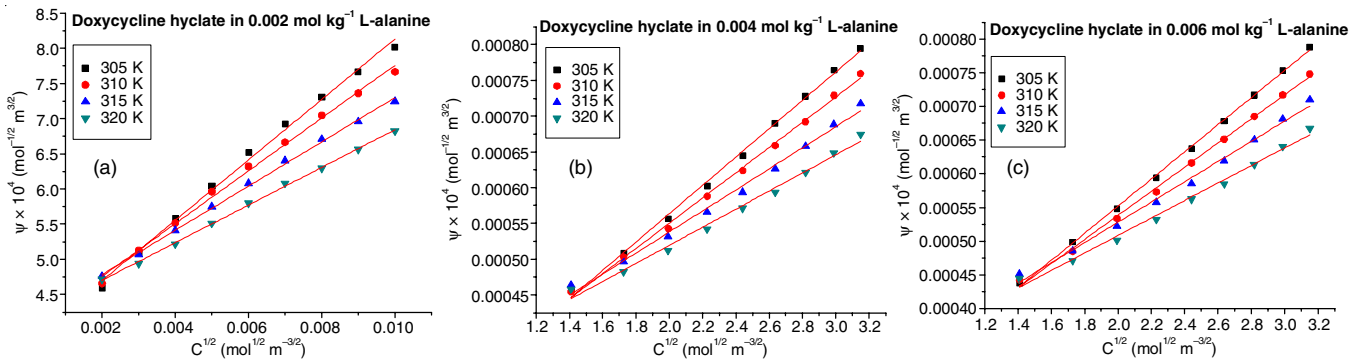

Fig. 2. Plots of $\psi = (\eta_r - 1)/C^{1/2}$ *vs.* concentration ($C^{1/2}$) for doxycycline hyclate in (a) 0.002 mol kg^{-1} aqueous alanine (b) 0.004 mol kg^{-1} aqueous alanine (c) 0.006 mol kg^{-1} aqueous alanine at different temperatures

TABLE-2
VALUES OF $\psi = (\eta_r - 1)/C^{1/2}$ ($\times 10^4$ $mol^{-1/2}$ $m^{3/2}$) OF DOXYCYCLINE HYCLATE IN WATER AND AQUEOUS SOLUTIONS OF GLYCINE AND L-ALANINE AT DIFFERENT TEMPERATURES

$*m_D$ (mol kg^{-1})	305.15 K	310.15 K	315.15 K	320.15 K	305.15 K	310.15 K	315.15 K	320.15 K
	Doxycycline hyclate + water				Doxycycline hyclate + 0.002 mol kg^{-1} glycine			
0.002	5.6638	5.7527	5.7754	5.8338	4.1429	4.2669	4.3314	4.3663
0.003	6.0660	6.0402	5.9187	5.8792	4.6615	4.7290	4.6257	4.5563
0.004	6.4365	6.3220	6.1681	6.0584	5.2165	5.1023	4.9495	4.8046
0.005	6.8157	6.6957	6.4496	6.2839	5.6513	5.4646	5.2712	4.9890
0.006	7.1887	7.0038	6.7394	6.5264	6.0642	5.8699	5.5829	5.2560
0.007	7.5509	7.3099	7.0284	6.7740	6.4528	6.1965	5.8829	5.5160
0.008	7.9011	7.6105	7.3126	7.0212	6.8206	6.5094	6.1712	5.7679
0.009	8.2396	7.9525	7.5907	7.2654	7.1705	6.8097	6.3960	6.0116
0.010	8.5668	8.2360	7.8619	7.5055	7.5049	7.1440	6.6660	6.2475
	Doxycycline hyclate + 0.004 mol kg^{-1} glycine				Doxycycline hyclate + 0.006 mol kg^{-1} glycine			
0.002	4.4965	4.4552	4.5379	4.4730	4.2986	4.3404	4.4131	4.3438
0.003	4.9461	4.8787	4.7905	4.6402	4.7802	4.6986	4.6851	4.5327
0.004	5.3878	5.2287	5.0891	4.8745	5.2404	5.1410	4.9948	4.7798
0.005	5.8071	5.5746	5.3932	5.1258	5.6720	5.4934	5.2363	5.0396
0.006	6.2035	5.9674	5.6916	5.3786	6.0771	5.8322	5.5460	5.2986
0.007	6.5791	6.2843	5.9811	5.6274	6.4593	6.1567	5.8439	5.5521
0.008	6.9363	6.5892	6.2607	5.8702	6.8216	6.5181	6.1303	5.7381
0.009	7.2772	6.8827	6.4785	6.0492	7.1666	6.8136	6.4058	5.9806
0.010	7.6038	7.1657	6.7422	6.2814	7.4965	7.0982	6.6713	6.2153
	Doxycycline hyclate + 0.002 mol kg^{-1} alanine				Doxycycline hyclate + 0.004 mol kg^{-1} alanine			
0.002	4.5882	4.6589	4.7637	4.7163	4.5732	4.5425	4.6391	4.5806
0.003	5.0959	5.1280	5.0664	4.9380	5.0792	5.0286	4.9612	4.8237
0.004	5.5824	5.5165	5.4073	5.2182	5.5642	5.4269	5.3131	5.1162
0.005	6.0392	5.9605	5.7487	5.5101	6.0196	5.8769	5.6618	5.4161
0.006	6.5215	6.3200	6.0810	5.7996	6.4474	6.2408	5.9353	5.7113
0.007	6.9226	6.6652	6.4016	6.0822	6.9001	6.5891	6.2644	5.9337
0.008	7.3036	7.0471	6.7102	6.2959	7.2798	6.9227	6.5797	6.2153
0.009	7.6669	7.3624	6.9553	6.5649	7.6419	7.2905	6.8825	6.4869
0.010	8.0146	7.6664	7.2447	6.8251	7.9476	7.5959	7.1737	6.7492
	Doxycycline hyclate + 0.006 mol kg^{-1} alanine							
0.002	4.3761	4.4267	4.5145	4.4441				
0.003	4.9884	4.8475	4.8558	4.7083				
0.004	5.4817	5.3376	5.2186	5.0129				
0.005	5.9424	5.7300	5.5745	5.3206				
0.006	6.3739	6.1619	5.8532	5.6214				
0.007	6.7802	6.5133	6.1860	5.8481				
0.008	7.1650	6.8493	6.5041	6.1329				
0.009	7.5311	7.1712	6.8091	6.4070				
0.010	7.8811	7.4804	7.1021	6.6712				

*m_D is the molality of doxycycline hyclate in water and in different concentrations of glycine and L-alanine.

further indicates the presence kosmotropes *i.e.* strongly hydrated solutes in solutions and prevailing of hydrophilic-ionic interactions. It is observed that the magnitude of B-coefficient decrease in the order, L-alanine > glycine > water which indicates that the hydrophilic-ionic interactions are more effective in aqueous glycine/L-alanine solutions than in water. This may be due to the presence of zwitter ionic group in glycine/L-alanine which interacts with charged groups of doxycycline hyclate. The variation of B-coefficient with temperature (T) has been shown in Fig. 3.

The sign of temperature derivative of B *i.e.* dB/dT gives important information about structure making and structure breaking ability of solute in different solvent systems. The negative values of dB/dT in the present system indicate structure making behaviour of doxycycline hyclate in water as well as in aqueous solutions of glycine/L-alanine. Similar results have been obtained by Kaur and Kumar [31] in their study of L-serine in ampicillin and amoxicillin.

Further, viscosity B-coefficient values have also been used to obtain the viscosity B-coefficient of transfer ($\Delta_{tr}B$) from water to aqueous glycine/L-alanine solution and for calculations following equation has been used [35,36]:

$$\Delta_{tr}B = B(\text{aq. glycine/L-alanine}) - B\ (\text{water}) \qquad (4)$$

The values of B-coefficient and dB/dT along with the values of $\Delta_{tr}B$ have been reported in Table-3.

It is revealed from the positive values of $\Delta_{tr}B$ that hydrophilic-ionic interactions are more operative than the hydrophobic-ionic interactions in aqueous glycine and L-alanine. The positive values also indicate that structural order in solution is enhanced in the presence of glycine and L-alanine which may be due to more productive hydrophilic-ionic interactions

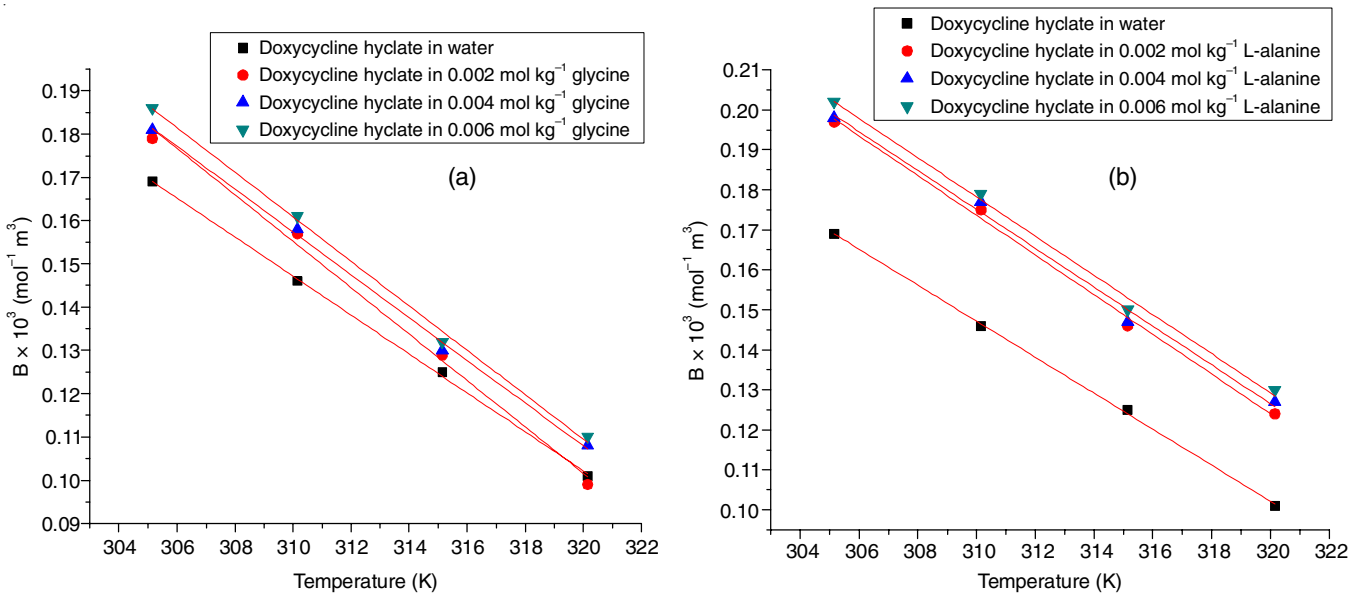

Fig. 3. Plots of B-coefficient *vs.* temperature (T) for doxycycline hyclate in (a) water and (0.002, 0.004 and 0.006) mol kg^{-1} aqueous glycine (b) water and (0.002, 0.004 and 0.006) mol kg^{-1} aqueous L-alanine

TABLE-3
VALUES OF B-COEFFICIENT, $\Delta_{tr}B$ AND dB/dT OF DOXYCYCLINE HYCLATE IN WATER, AQUEOUS GLYCINE AND L-ALANINE

Temperature (K)	Doxycycline hyclate in water	Doxycycline hyclate in aqueous glycine			Doxycycline hyclate in aqueous L-alanine		
		0.002 mol kg^{-1}	0.004 kg^{-1}mol	0.006 mol kg^{-1}	0.002 mol kg^{-1}	0.004 mol kg^{-1}	0.006 mol kg^{-1}
B × 10^3 (mol^{-1} m^3)							
305.15	0.169	0.179	0.181	0.186	0.197	0.198	0.202
310.15	0.146	0.157	0.158	0.161	0.175	0.177	0.179
315.15	0.125	0.129	0.130	0.132	0.146	0.147	0.150
320.15	0.101	0.099	0.108	0.110	0.124	0.127	0.130
Δ_{tr}B × 10^3 (mol^{-1} m^3)							
305.15	–	0.010	0.012	0.017	0.028	0.029	0.032
310.15	–	0.011	0.012	0.016	0.029	0.031	0.033
315.15	–	0.004	0.005	0.007	0.021	0.023	0.025
320.15	–	0.002	0.007	0.009	0.023	0.025	0.028
dB/dT × 10^3 (mol^{-1} m^3 K^{-1})							
	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005

between zwitter ions of glycine/L-alanine and charged groups of drug. The higher values of $\Delta_{tr}B$ in aqueous solution of L-alanine than glycine indicate that hydrophilic-ionic interactions are more pronounced in aqueous solution of L-alanine due to effective interactions between zwitter ionic centre of L-alanine and charged groups of drug. However, due to the small size of glycine, it occupies the cages formed by water molecules and is not easily available for interactions with doxycycline hyclate.

Thermodynamic activation parameters of viscous flow: The thermodynamic activation parameters of viscous flow consist of $\Delta\mu_1^{o*}$ and $\Delta\mu_2^{o*}$ *i.e.* free energy of activation of viscous flow per mole of solvent and free energy of activation of viscous flow per mole of solute respectively.

Eyring and coworkers [37] gave an equation to calculate and after rearrangement it is written as:

$$\Delta\mu_1^{o*} = RT\ln\left(\frac{\eta_o V_1^o}{hN_A}\right) \quad (5)$$

In this equation, h is the Planck's constant, N_A is Avogadro's number, R is the universal gas constant, T is the temperature and $V_1^o (= \Sigma x_i M_i / d)$ is the partial molar volume of the solvent. In the expression $V_1^o = \Sigma x_i M_i / d$, x_i is the mole fraction of solvent mixture, M_i is the molecular weight of solvent mixture, d is the density of solvent mixture.

Similarly Feakins *et al.* [29,38] used Eyring transition state theory [39] gave an expression to calculate $\Delta\mu_2^{o*}$:

$$\Delta\mu_2^{o*} = \Delta\mu_1^{o*} + [(B - (V_1^o - V_2^o)(RT / V_1^o) \quad (6)$$

In this equation $V_2^o = V_\phi^o$ and the values of V_1^o, V_2^o, $\Delta\mu_1^{o*}$ and $\Delta\mu_2^{o*}$ are reported in Table-4.

The positive and larger values of $\Delta\mu_2^{o*}$ over $\Delta\mu_1^{o*}$ indicate that the hydrophilic-ionic interactions persisting in the different solutions are more effective in ground state than in the transition state. The $\Delta\mu_2^{o*}$ values are found to be larger in aqueous solution of amino acids than in water which may be due to more operative hydrophilic-ionic interactions between zwitter ions of amino acids and charged groups of drug. The comparative studies has also shown that the values of $\Delta\mu_2^{o*}$ are larger in aqueous L-alanine than in aqueous glycine which indicates the effective hydrophilic-ionic interactions in in aqueous L-alanine. This is due to more effective ion-hydrophilic inter-

TABLE-4
VALUES OF V_1^o , V_2^o , $\Delta\mu_1^{o*}$, $\Delta\mu_2^{o*}$, $T\Delta S_2^{o*}$ AND ΔH_2^{o*} OF DOXYCYCLINE HYCLATE IN WATER, AQUEOUS GLYCINE AND L-ALANINE AT DIFFERENT TEMPERATURES

Temperature (K)	$V_1^o \times 10^6$ (m^3 mol^{-1})	$V_2^o \times 10^6$ (m^3 mol^{-1})	$\Delta\mu_1^{o*}$ (kJ mol^{-1})	$\Delta\mu_2^{o*}$ (kJ mol^{-1})	$T\Delta S_2^{o*}$ (kJ mol^{-1})	ΔH_2^{o*} (kJ mol^{-1})
Doxycycline hyclate + water						
305.15	18.130	379.721	9.001	83.263	112.600	195.863
310.15	18.189	384.271	8.901	81.475	114.445	195.920
315.15	18.321	389.136	8.817	79.704	116.290	195.994
320.15	18.413	394.084	8.733	77.698	118.135	195.833
Doxycycline hyclate + 0.002 mol kg^{-1} glycine						
305.15	18.086	379.970	9.013	87.102	142.505	229.607
310.15	18.116	384.483	8.914	84.623	144.840	229.463
315.15	18.151	389.629	8.831	82.279	147.175	229.454
320.15	18.190	395.451	8.746	80.105	149.510	229.615
Doxycycline hyclate + 0.004 mol kg^{-1} glycine						
305.15	18.085	380.331	9.021	85.228	111.685	196.913
310.15	18.115	385.278	8.922	83.624	113.515	197.139
315.15	18.150	390.467	8.840	81.383	115.345	196.728
320.15	18.188	396.441	8.754	79.875	117.175	197.050
Doxycycline hyclate + 0.006 mol kg^{-1} glycine						
305.15	18.082	381.795	9.029	86.138	119.314	205.452
310.15	18.113	386.458	8.930	84.352	121.269	205.621
315.15	18.148	391.941	8.848	81.853	123.224	205.077
320.15	18.187	397.865	8.759	80.450	125.179	205.629
Doxycycline hyclate + 0.002 mol kg^{-1} L-alanine						
305.15	18.086	380.768	9.022	87.987	118.093	206.080
310.15	18.117	385.592	8.922	86.118	120.028	206.146
315.15	18.152	390.956	8.838	83.743	121.963	205.706
320.15	18.190	397.005	8.753	82.335	123.898	206.233
Doxycycline hyclate + 0.004 mol kg^{-1} L-alanine						
305.15	18.085	381.479	9.030	87.832	105.582	193.414
310.15	18.116	386.423	8.930	86.485	107.312	193.797
315.15	18.151	392.026	8.846	84.095	109.042	193.137
320.15	18.189	397.899	8.762	82.864	110.772	193.636
Doxycycline hyclate + 0.006 mol kg^{-1} L-alanine						
305.15	18.084	382.352	9.038	88.410	104.666	193.077
310.15	18.116	386.423	8.939	86.836	106.381	193.218
315.15	18.149	392.383	8.854	84.575	108.096	192.672
320.15	18.188	398.581	8.771	83.441	109.811	193.253

actions between zwitter ionic centres of L-alanine and charged groups of drug in aqueous solution of L-alanine than in aqueous solution of glycine as explained earlier. However the increasing trend in $\Delta\mu_2^{o*}$ values with the rise in concentration of amino acids indicates increase in solute-solvent interactions. The large positive values of $\Delta\mu_2^{o*}$ also suggest structure maker tendency of doxycycline hyclate in different solvent systems.

The activation entropy (ΔS_2^{o*}) can be derived from the following relation:

$$\frac{d(\Delta\mu_2^{o*})}{dT} = -\Delta S_2^{o*} \quad (7)$$

This is the expression for slope of linear plots of ΔS_2^{o*} *vs.* T as shown in Fig. 4.

The activation enthalpy (ΔH_2^{o*}) for viscous flow of doxycycline hyclate has also been calculated using the following relation:

$$\Delta\mu_2^{o*} = \Delta H_2^{o*} + T\Delta S_2^{o*} \quad (8)$$

The values of $T\Delta S_2^{o*}$ and ΔH_2^{o*} at different temperatures have been reported in Table-4.

The positive values of $T\Delta S_2^{o*}$ for all the experimental solutions at all temperatures suggest that transition state is very disordered and the positive ΔH_2^{o*} values indicate that bond breaking takes place in transition state and therefore formation of activated complex is unfavourable. The comparative studies has also shown that more energy is required for the formation of transition state in aqueous L-alanine than in aqueous glycine which may be due to stronger hydrophilic-ionic interactions in aqueous L-alanine than in aqueous glycine.

Conductance studies (Walden product)

The specific conductance (κ) and molar concentration (C) of examined dilute solutions has been used to calculate the molar conductance (Λ_m) by using following relation:

$$\Lambda_m = \frac{\kappa \times 1000}{C} \quad (9)$$

The values of Λ_m are reported in Table-5.

Onsager equation has been used to study the relation between Λ_m and $C^{1/2}$. This equation is given as:

$$\Lambda_m = \Lambda_m^o - KC^{1/2} \quad (10)$$

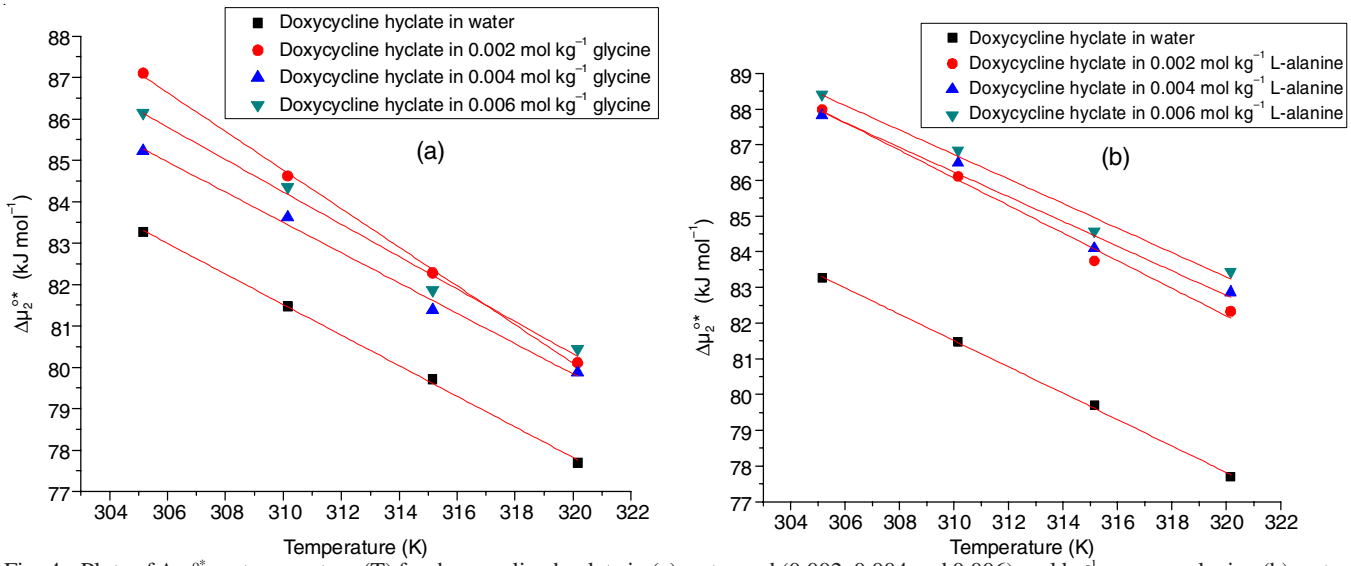

Fig. 4. Plots of $\Delta\mu_2^{o*}$ *vs.* temperature (T) for doxycycline hyclate in (a) water and (0.002, 0.004 and 0.006) mol kg^{-1} aqueous glycine (b) water and (0.002, 0.004 and 0.006) mol kg^{-1} aqueous L-alanine

TABLE-5
VALUES OF MOLAR CONDUCTANCE (Λ_m, milli S m^2 mol^{-1}) OF DOXYCYCLINE HYCLATE IN WATER, AQUEOUS GLYCINE AND L-ALANINE AT DIFFERENT TEMPERATURES

*m_D (mol kg^{-1})	305.15 K	310.15 K	315.15 K	320.15 K	305.15 K	310.15 K	315.15 K	320.15 K
	Doxycycline hyclate + water				Doxycycline hyclate + 0.002 mol kg^{-1} glycine			
0.002	227.19	252.50	267.98	294.73	176.42	188.99	202.19	226.95
0.003	216.22	228.91	247.66	261.05	164.57	182.18	188.63	218.43
0.004	196.73	205.19	233.98	238.06	157.66	172.55	183.51	214.61
0.005	189.82	192.74	219.04	219.97	150.12	167.61	177.71	205.90
0.006	181.43	184.33	204.70	205.91	146.78	163.72	169.90	202.42
0.007	173.89	178.33	193.48	194.46	146.14	159.38	165.00	196.74
0.008	167.45	173.85	185.57	189.64	144.91	157.39	159.30	192.00
0.009	165.89	170.38	182.55	185.90	143.97	155.63	157.71	189.88
0.010	160.99	167.61	180.15	182.93	143.23	153.04	155.14	187.20
	Doxycycline hyclate + 0.004 mol kg^{-1} glycine				Doxycycline hyclate + 0.006 mol kg^{-1} glycine			
0.002	189.90	202.63	219.74	227.18	191.06	208.32	218.28	234.67
0.003	181.27	192.32	209.62	220.77	184.62	196.84	210.39	225.54
0.004	178.58	184.15	194.61	210.82	180.91	188.06	200.40	218.28
0.005	172.96	177.57	188.78	201.45	173.5	184.73	192.02	210.14
0.006	169.98	172.36	182.14	195.66	170.97	178.86	186.78	203.67
0.007	167.99	168.65	180.84	185.25	168.44	174.29	182.06	195.49
0.008	165.85	165.89	177.38	180.21	166.19	172.61	176.28	189.13
0.009	162.45	163.75	174.48	174.98	165.23	171.32	174.14	184.31
0.010	161.08	162.05	170.97	171.99	163.48	170.23	172.43	182.86
	Doxycycline hyclate + 0.002 mol kg^{-1} L-alanine				Doxycycline hyclate + 0.004 mol kg^{-1} L-alanine			
0.002	225.29	243.15	263.81	284.06	229.05	248.28	264.8	284.08
0.003	220.97	236.80	253.65	270.00	225.11	241.86	253.73	272.65
0.004	217.19	229.43	244.85	259.1	221.17	235.29	243.95	261.93
0.005	213.40	223.06	237.99	249.92	217.63	228.76	236.5	250.27
0.006	210.90	218.68	232.60	243.30	214.97	223.59	229.37	242.02
0.007	209.14	215.56	228.77	236.88	213.08	219.92	224.29	236.14
0.008	207.83	213.25	225.92	232.83	211.69	217.19	220.51	231.76
0.009	206.84	211.47	223.72	229.700	210.62	215.08	217.58	228.37
0.010	206.05	210.05	221.98	227.22	209.78	213.41	215.26	225.67
	Doxycycline hyclate + 0.006 mol kg^{-1} L-alanine							
0.002	240.14	259.64	275.12	296.58				
0.003	233.31	248.46	262.37	275.45				
0.004	227.93	240.21	249.00	261.64				
0.005	223.12	232.07	239.90	250.39				
0.006	219.60	227.84	234.75	240.00				
0.007	217.11	224.85	227.81	235.43				
0.008	215.26	222.63	225.62	230.75				
0.009	213.84	220.92	223.94	229.46				
0.010	212.72	219.56	220.61	228.45				

*m_D is the molality of doxycycline hyclate in water and in different concentrations of glycine and L-alanine.

TABLE-6
VALUES OF LIMITING MOLAR CONDUCTANCE (Λ_m^o) AND WALDEN PRODUCT ($\Lambda_m^o\eta_o$) FOR DOXYCYCLINE HYCLATE IN WATER, AQUEOUS GLYCINE AND L-ALANINE AT DIFFERENT TEMPERATURES

*m_A (mol kg^{-1})	Λ_m^o (milli S m^2 mol^{-1})				$\Lambda_m^o\eta_o$ (? S m^2 mol^{-1} Pa s)			
	305.15 K	310.15 K	315.15 K	320.15 K	305.15 K	310.15 K	315.15 K	320.15 K
Doxycycline hyclate + water								
0.000	279.00	308.28	338.71	370.90	0.2133	0.2134	0.2135	0.2138
Doxycycline hyclate + aqueous glycine								
0.002	195.90	216.20	237.40	259.50	0.1509	0.1510	0.1518	0.1521
0.004	210.20	232.10	253.90	277.30	0.1624	0.1627	0.1629	0.1631
0.006	212.10	234.20	256.10	279.90	0.1644	0.1646	0.1649	0.1649
Doxycycline hyclate + aqueous L-alanine								
0.002	239.90	269.20	294.60	326.20	0.1854	0.1886	0.1889	0.1918
0.004	244.10	276.80	302.90	331.10	0.1892	0.1946	0.1948	0.1953
0.006	260.30	287.30	314.40	342.90	0.2024	0.2026	0.2028	0.2030

In the above equation Λ_m^o is the limiting molar conductance, K is constant and C is molar concentration.

Walden product ($\Lambda_m^o\eta_o$) has been calculated using the limiting molar conductance Λ_m^o. The related data is shown in Table-6. The variation of Walden product with temperature *i.e.* $d\Lambda_m^o\eta_o/dT$ reveals important information about structure maker and breaker behaviour of solute. The positive values of $d\Lambda_m^o\eta_o/dT$ for doxycycline hyclate in different solvent systems suggest structure maker nature of doxycycline hyclate in different solvent systems which have been supported by the conclusions drawn from the viscosity parameters.

Conclusion

The viscometric and conductance measurement of doxycycline hyclate in water, aqueous glycine and aqueous L-alanine solutions has been carried out and results are analyzed in terms of interactions present in the binary as well as in ternary system. The positive viscosity B-coefficient and $\Delta_{tr}B$ values found to follow the trend L-alanine > glycine > water which indicates more productive hydrophilic-ionic interactions in aqueous amino acid solutions than in water which may be due to the effective hydrophilic-ionic interaction between zwitter ionic group of glycine/L-alanine and charged groups of drug. The negative magnitude of dB/dT indicates structure making behaviour of doxycycline hyclate in water as well as in aqueous solutions of glycine/L-alanine. The higher values of $\Delta\mu_2^{o*}$ than $\Delta\mu_1^{o*}$ indicate difficulty in the formation of transition state which indicates strong intermolecular interactions in the ground state than in transition state. The positive magnitude of $T\Delta S_2^{o*}$ and ΔH_2^{o*} indicates cleavage of intermolecular bonds in the transition state which means formation of transition state is highly unfavourable. The positive magnitude of $d\Lambda_m^o\eta_o/dT$ further support the fact that doxycycline hyclate behaves as structure maker in different solvent systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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