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INVESTIGATION OF MOLECULAR INTERACTIONS OF ANTIBIOTIC DOXYCYCLINE HYCLATE WITH PALMITIC ACID

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Abstract
The Dilute solution viscometric technique is employed to find the molecular interactions between the non-Newtonian Doxycycline hyclate and the essential saturated fatty acid, Palmitic acid in the common solvent Ethyl methyl ketone. The viscometric parameters μ, α and β identifies the molecular interactions at physiological temperatures. The conformity of interaction has been attempted by the ultrasonic velocity; and the related acoustical parameters at temperatures 303, 310 and 313K. The chemical nature of the blend solutions have been identified through refractometric technique. The behaviour of these parameters explains the molecular interactions on the basis of solute-solute and solute-solvent interactions.

Key Words: Palmitic acid; Doxycycline hyclate; Ultrasonic velocity; Viscosity; Refractive index.

Introduction

The antibiotic resistance is a significant threat to public health. The inactivation of the antibiotic molecule arises due to molecular interactions. The investigation of the reasonable molecular interaction for antibiotic resistance has been attempted by many researchers[1,2]. The non-Newtonian molecule, the Doxycycline hyclate a chemically modified synthetic tetracycline was first introduced into chemical practise in 1968[3]. It is known for its bacteriostatic and metalloprotinase activity.

Tetracycline free bases adopt Zwitterionic form in aqueous phase for its bacterial activity and inactive non-ionized free base form is more suitable for lipid membrane solubility with specific molecular conformation. The biological activity may involve both the molecular species upon their interconversion in the thermodynamic environment of aqueous and non-aqueous medium, organic solvent[4]. Therefore, this lipophilic interaction of the macromolecule Doxycycline is expected to make the Doxycycline inactive and it will leads to antibiotic resistance.

The basis of solute-solute attractive interaction among the functional groups of the macromolecules may arise from any specific interactions include hydrogen bonding, dipole-dipole, ion-ion, ion-dipole[5] and dipole-induced dipole interactions. The interest of biophysical chemist in viscosity measurements stem from the fact that the addition of macromolecular solutes alter the viscosity of their solutions.[6] Dilute Solution Viscometry is a simple, low cost and rapid technique[7] for the study of hydrodynamic molecular interaction in the blends. Arun Kumar Misra et.al.,[8] have attempted the interactions of paracetamol with fatty acids through viscometric technique. Confirmity of macromolecular interactions was reported by many workers using ultrasonic technique[9, 10]. Varadarajalu et.al.,[9] and Singh and Singh[11] have suggested the use of ultrasonic velocity and viscosity measurement for investigating polymer interactions. In the present study, the viscosity and ultrasonic techniques were employed in Doxycycline hyclate / Ricinoleic acid blends at the ambient temperature 303K and physiological temperatures 310 and 313K.

Materials and Methods

Doxycycline hyclate ($C_{22}H_{24}N_2O_8$ .HCl.1/2 C_2H_5OH.1/2H_2O) of molecular weight 512.94, manufactured by Yangzhou Huashu Winsome Pharmacy Co. Ltd., China and AR grade Palmitic acid (C_{16} H_{32} O_2)of molecular weight 282.47 g/mol manufactured by s d fine-chem limited, Mumbai have been employed in the present study. Ethyl methyl ketone (CH_2 CO CH_3) of GR

grade is purchased from Merck specialities private LTD, Mumbai is taken as a solvent. The 0.1%w/v binary solutions of antibiotic Doxycycline hyclate (DOX) and fatty acid Palmitic acid (PA) in solvent ethyl methyl ketone (EMK) were prepared. Using binary solutions, blends of composition 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 were prepared. Ubbelohde suspended level viscometer, is employed to measure the relative viscosity of blend at different compositions, at different temperatures 303, 310 and 313K. The ultrasonic velocity measurements have been measured by an ultrasonic interferometer operating at frequency 2MHz supplied by mittal enterprises, New Delhi. The temperature is maintained at 303, 310 and 313K by circulating water from a thermostat, with a thermal stability of ±0.05°C. The density of the solutions was measured using 10ml standard specific gravity bottle.

Theory

Viscometric Analysis:

Based on additive law Huggin's equation expresses the specific viscosity as a function of concentration if one of the components is alone in solution,[6]

$$\eta_{spe}/C = [\eta] + K_H[\eta]^2 C \quad \text{--------- (1)}$$

Where $[\eta]$ is the intrinsic viscosity, η_{spe}/C is the specific viscosity at concentration C, and K_H is the Hugggin's constant. If $K_H[\eta]^2 = b$, where b is the interaction coefficient, then eq. (1) becomes

$$\eta_{spe}/C = [\eta] + bC \quad \text{----------(2)}$$

Chee[7] extended the Huggin's equation for a ternary system of solute A and solute B in common solvent and gave the interaction parameter

$$\Delta B = \frac{b - \bar{b}}{2W_2W_3} \quad \text{----------(3)}$$

Where $\bar{b} = W2b22 + W3b33$

and $\mu = \Delta B / ([\eta]_2 - [\eta]_1)^2$ ----------(4)

The parameter ∆B can be used to predict solute-solute interaction in general. Where, b22, b33 and b23 are the interaction parameters of the two polymer solutions and blends respectively. $[\eta]_1$ and $[\eta]_2$ are the intrinsic viscosities for the pure component solutions. The blend is miscible if $\mu \geq 0$ and immiscible when $\mu < 0$.

Sun. et. al.,[12] Suggested a new criterion, 'α' based on the classical Huggins equation and Huggin's coefficient K_m in the blends. According to Sun. et.al,[12] for a ternary system, there are three types of interaction that might contribute to the value of K_m: long-range hydrodynamic interaction of pairs of single molecules given by K_{m1}; the formation of double molecules given by K_{m2}; and intermolecular attraction or repulsion given by K_{m3}. Thus, the overall K_m turns out to be:

$$K_m = K_{m1} + K_{m2} + K_{m3} \quad \text{----------(5)}$$

In the absence of strong special interactions that would encourage aggregation, and at sufficiently low concentrations, the second term K_{m2} can be neglected. Reabbrevating, K_{m3} as α and rearranging the final equation then yields.

$$\alpha = K_m - K_{m1} \quad \text{----------(6)}$$

$$\alpha = K_m - \left[\frac{K_A[\eta]_A^2 W_A^2 + K_B[\eta]_B^2 W_B^2 + 2\sqrt{K_A K_B}[\eta]_A[\eta]_B W_A W_B}{\left([\eta]_A W_A + [\eta]_B W_B\right)^2} \right] \quad \text{--------(7)}$$

Where, K_m is the experimentally obtained Huggins constant. The parameter α indicates the nature and strength of the intermolecular interaction. As a criteria the sign of the parameter α can be used to predict the interactions, $\alpha \geq 0$ claims solute-solute interaction and $\alpha < 0$ indicates blend-solvent interaction.

Jiang and Han[13] revised Sun's criterion by substituting an expression for total interaction existing in the system K_m, illustrated as in equation (7).

$$K_m = \frac{K_A[\eta]_A^2 W_A^2 + K_B[\eta]_B^2 W_B^2 + 2K_{AB}[\eta]_A[\eta]_B W_A W_B}{\left([\eta]_A W_A + [\eta]_B W_B\right)^2} \quad \text{----------(8)}$$

Then the new criterion β for the resultant interaction

$$\beta = \frac{2\Delta K[\eta]_A[\eta]_B W_A W_B}{\left(W_A[\eta]_A + W_B[\eta]_B\right)^2} \quad \text{----------(9)}$$

The criteria $\beta \geq 0$ indicates solute-solute interactions and $\beta < 0$ is for blend-solvent interactions.

Ultrasonics Studies

Ultrasonic velocity (u) and density (ρ) are the observed parameters. These parameters were employed to calculate some of the thermoacoustic parameters.

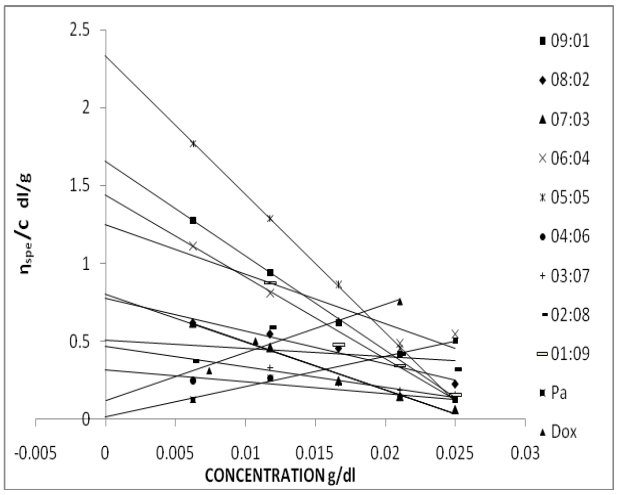
i) Adiabatic Compressibility $\beta = 1/u\rho$ m^2/N
ii) Acoustic impedance $Z = u\rho$ $Kgm^{-2}s^{-1}$.
iii) Free length $L_f = K\sqrt{\beta}$ m

Where k = 93.875 + 0.345* T *10^-8, T is the absolute temperature

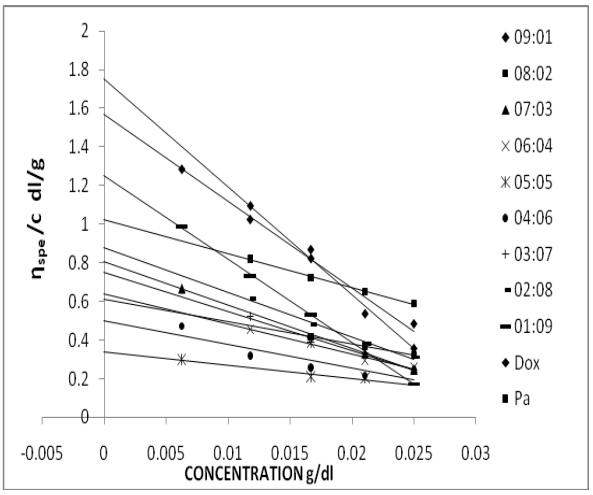
Results and Discussions

Viscosity technique

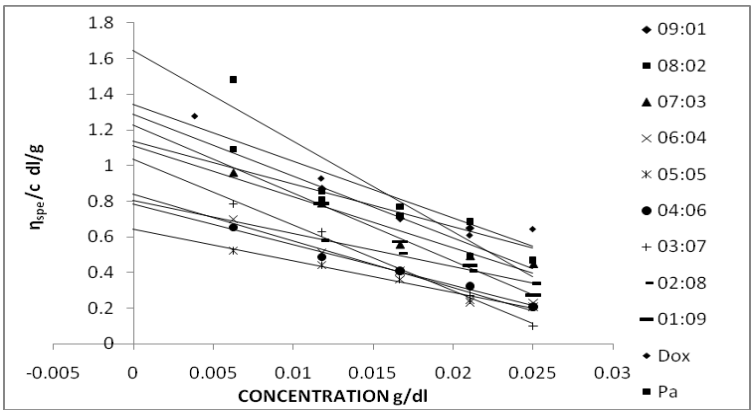
The Huggin's plots of η_{spe}/C versus concentration C for 0.1%w/v solutions of Doxycycline Hyclate and Palmitic acid and for the blends of DOX/PA at compositions for temperatures 303, 310 and 313K were drawn and shown in graphs 1, 2 and 3 respectively. The values of viscometric parameters, the interaction parameter b, intrinsic viscosity $[\eta]$ and the Huggin's constant K_H for 0.1%w/v binary solutions at temperatures 303K, 310K and 313K are summarized in Table I. The values of the derived parameters, the Chee[7] parameter μ, the Sun.et.al,[12] parameter α and the improved parameter β for the blends of DOX/PA at temperatures 303, 310 and 313K are summarized in the Table II.



Graph 1: Variation of Reduced Viscosity (η_{spe}/C) with Concentration at 303 K



Graph 2: Variation of Reduced Viscosity (η_{spe}/C) with Concentration at 310 K



Graph 3. Variation of Reduced Viscosity (η_{spe}/C) with Concentration at 313 K

Table 1. Result of 0.1% solutions of Doxycycline hyclate and Palmitic acid in common solvent EMK.

Temperature	303K			310K			313K		
solutions	[η] dl/g	b	K_H	[η] dl/g	b	K_H	[η] dl/g	b	K_H
0.1% Doxycycline hyclate	0.12	30.82	2191.31	1.57	-44.94	-18.34	1.34	-31.76	-17.63
0.1% Palmitic acid	0.19	2.67	73.96	1.02	-17.42	-16.74	1.28	-34.6	-20.98

The intrinsic viscosity [η] and the interaction parameter b of 0.1% solution of Doxycycline hyclate and Palmitic acid are positive at 303K. The positive value of b suggests the interaction between the groups that are present in the system. The positive value of [η] indicates the solubility of the solute components[6]. At temperatures 310 and 313K, [η] remains positive but the interaction parameter b negative suggesting the dominance of solute-solvent interactions.

Table 2. The calculated Viscometric parameters were tabulated for temperatures 303, 310 and 313K

Doxy : Palmitic acid	303K			310K			313K		
	μ	α	β	μ	α	β	μ	α	β
9:1	8574.90	-1661.9	-97.59	83.2	0.027	0.093	-2738.1	0.676	0.1069
8:2	849.85	-1252.7	-150.1	-66.0	12.82	3.231	5207.5	1.501	0.1633
7:3	2716.48	-929.2	-171.3	-29.7	16.60	5.824	-1373.5	4.627	1.6376
6:4	2909.50	-712.8	-190.9	-52.3	20.43	8.767	-2096.0	18.343	8.5657
5:5	14116.92	-525.9	-182.8	-81.7	42.48	20.382	-4649.9	23.826	11.907
4:6	-1784.01	-317.2	-135.6	-63.8	31.43	15.721	-3164.3	17.481	8.624
3:7	-726.66	-217.9	-114.2	-36.9	18.73	8.888	1078.9	14.475	6.475
2:8	-2239.11	-168.7	-89.21	-27.3	12.80	4.991	-4358.9	8.493	3.127
1:9	7011.73	-97.41	-45.76	40.4	10.72	2.530	1465.6	4.663	1.038

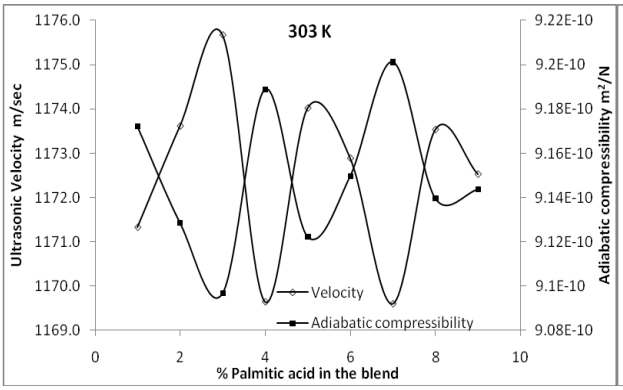
Table 3. The observed and calculated thermoacoustic parameters were tabulated for temperatures 303, 310 and 313K

Comp Dox : Rici	303K			310K			313K		
	U m/s	β $m^2/N\ 10^{10}$	L_f Å	U m/s	β $m^2/N\ 10^{10}$	L_f Å	U m/s	β $m^2/N\ 10^{10}$	L_f Å
9:1	1171.329	9.17	0.600	1143.159	9.68	0.625	1131.631	9.93	0.636
8:2	1173.616	9.13	0.601	1144.530	9.70	0.625	1129.442	9.97	0.637
7:3	1175.672	9.10	0.601	1142.952	9.69	0.625	1131.331	9.93	0.636
6:4	1169.642	9.19	0.600	1143.460	9.71	0.626	1129.999	9.96	0.637
5:5	1174.022	9.12	0.600	1142.772	9.71	0.626	1130.013	9.96	0.637
4:6	1172.887	9.15	0.601	1142.634	9.68	0.625	1130.319	9.94	0.637
3:7	1169.600	9.20	0.598	1143.793	9.68	0.625	1129.440	9.96	0.637
2:8	1173.536	9.14	0.599	1143.784	9.72	0.626	1129.378	9.97	0.637
1:9	1172.528	9.14	0.601	1142.076	9.70	0.626	1128.830	9.97	0.637

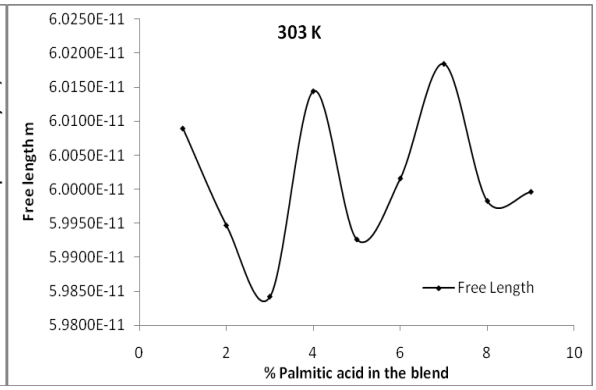
At 303K, the parameters α and β are negative at all compositions. These parameters claim blend-solvent interactions as per the criteria. The above discussed positive and negative values of α, β and μ are in agreement with the reported observations[14, 15]. At 310K and 313K the parameters α and β are positive at all compositions and indicates the solute-solute interaction of DOX/PA. The chee parameter μ exhibits the contradictory results with α and β at all temperatures and compositions. Similar contradictory behavior of μ is common and available in literature[12].

Ultrasonic technique

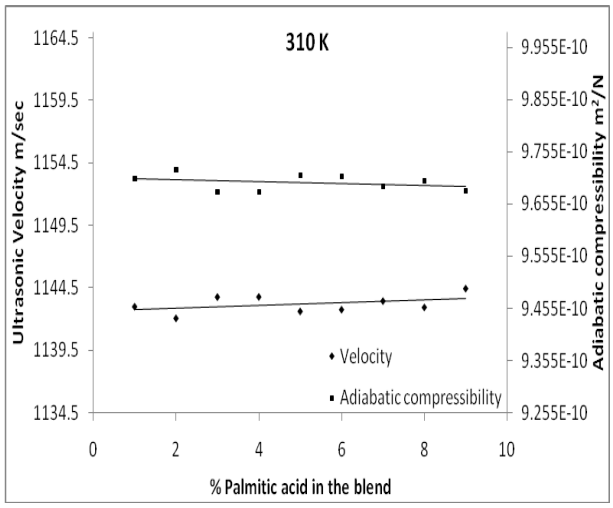
In order to further confirm the identified solute-solute interactions, the measured ultrasonic velocity u and the derived related parameters for the blends at composition are summarized in Table III. The behavior of (u), (κ) and (L_f) of DOX/PA blend solutions are depicted in figures 4, 5, 6, 7, 8 respectively for the temperatures 303K, 310K and 313K.



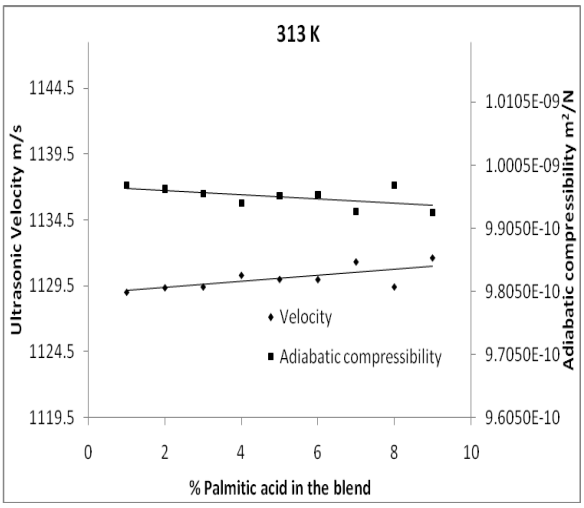
Graph 4: Variation of ultrasonic velocity and adiabatic compressibility with the compositions of DOX/PA blends.



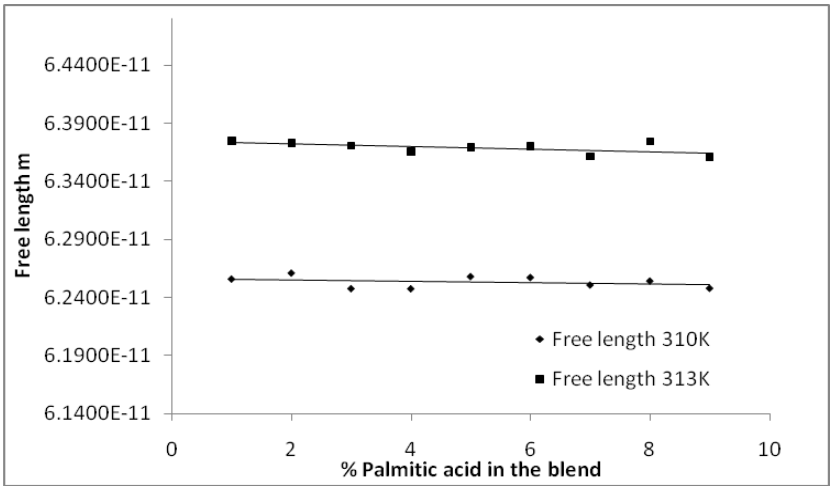
Graph 5: Variation of ultrasonic velocity and adiabatic compressibility with the compositions of DOX/PA blends.



Graph 6: Variation of ultrasonic velocity and adiabatic b compressibility with the compositions of DOX/PA blends.



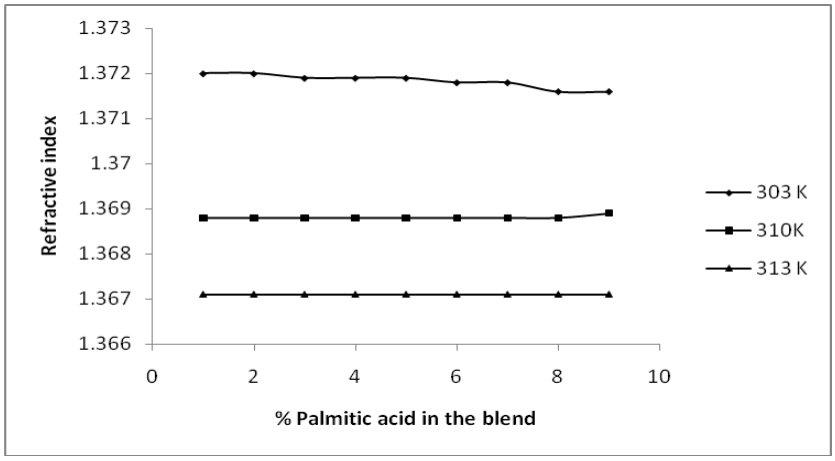
Graph 7: Variation of ultrasonic velocity and adiabatic compressibility with the compositions of DOX/PA blends.



Graph 8: Variation of ultrasonic velocity and adiabatic compressibility with the compositions of DOX/PA blends.

From the behaviours of the parameters it is clearly evident that the variation is non-linear at 303K and indicates solute-solvent interactions in the blend. The criterion of linear behavior in u, κ and L_f in the blends of DOX/PA at temperatures 310 and 313K and establishes solute-solute interaction between DOX and PA. These observations are in agreement with the results obtained through dilute solution viscometric technique.

Varadarajulu et. al.[9] Singh and Singh et. al.,[11] somanathan et. al.,[16] Paladhi and Singh,[17] Murali mohan et.al.,[18] have employed the Viscometric and Ultrasonic techniques to identify the solute-solute and solute-solvent interactions in the polymer blends and established the criteria.



Graph 9: Variation of refractive index with compositions of DOX/PA blends.

Refractometric studies:

The plot of refractive indices against compositions for the blend system DOX/PA for temperatures 303K, 310K and 313K is shown in graph 9. The prepared blend solutions appeared to be homogeneous and highly transparent. The recorded values of refractive indices vary only in the fourth decimal and the variation is linear. The linear variation of refractive index is an indication of homogeneous nature of the solutions without any chemical or structural changes in the system[9].

Conclusion

In the present investigation the DSV, ultrasonics and refractometric techniques have successfully investigated the molecular interactions between Doxycycline hyclate and Palmitic acid. At physiological temperatures 310 and 313K the interactions are favoured between Doxycycline hyclate and Palmitic acid and these interactions are

absent at room temperatures. These molecular interactions are at the physiological temperatures might leads to the formation of antibiotic resistance.

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