Heterocyclic Letters
Vol. 6| No.4 |679-686|Aug-Oct| 2016
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

EFFECT OF SOLVENT ON BINDING OF DIETHYL 4-(4-HYDROXYPHENYL)-2, 6-DIMETHYL-1, 4-DIHYDROPYRIDINE-3, 5-DICARBOXYLATE TO BSA

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ABSTRACT:
This paper presented the binding interaction of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4HDDD) to the BSA by FT-IR spectroscopy and equilibrium dialysis at physiological pH 7.4 in solvents 1,4-dioxane, dimethyl sulphoxide (DMSO) and dimethyl formamide (DMF). In FT-IR experiment, the BSA binds with 4HDDD and amide I band is shifted from 1635 to 1650 cm^{-1} and amide II from 1543 to 1538 cm^{-1}. This proved that the secondary structure of the BSA changes on binding with 4HDDD. Moreover amide I band is more sensitive to the changes of secondary structure than amide II. This indicates that the hydrophobic interactions played a major role in the binding of drug with BSA. An equilibrium dialysis, study shows the non-linear curve obtained from scatchard analysis which suggests the presence of at least two binding sites of 4HDDD to BSA. The association constants in solvents 1, 4-dioxane, DMSO, DMF are 0.802, 0.429 and 0.511 respectively. This shows that binding is more significant in non-polar solvent 1, 4-dioxane than polar solvents DMSO and DMF.

KEYWORDS: Equilibrium Dialysis, FT-IR spectroscopy, protein-drug binding, Scatchard analysis, association constant, BSA protein,

INTRODUCTION:

1, 4-dihydropyridine derivatives shows wide range of biological activities and medicinal properties[i,ii]. Diethyl 4-(4-hydroxyphenyl) 2,6 dimethyl 1,4dihydropyridine3,5dicarboxylate (4HDDD) is a very important moiety of pyridine derivatives specially shows antimicrobial and anticonvulsant activities[iii]. 1, 4- dihydropyridine derivatives synthesized by ethyl acetylene carboxylate, aminoacetaldehyde, dimethyl acetal and different aromatic aldehydes[iv,v] and also from Hantzsch condensation reaction[vi]. These 1, 4-dihydropyridine compounds shows calcium channel blocking activity[vii]. Knowledge of the binding interaction between drugs and plasma protein is very important for us to understand the pharmacokinetics and pharmacodynamics of a drug. Drugs binding influence the metabolic activity of target tissues. Mainly α_1-acidic glycoprotein (α_1-AGP), bovine serum albumin

(BSA) and lipoprotein transports the drugs in the blood. Functional and physiological properties of these proteins extensively studied over several decades[viii]. The main role of albumin is to provide various ligands, such as fatty acids, amino acids and different metal ions in the blood stream to their target organs. The full length BSA precursor protein consists of 583 amino acids in a single polypeptide chain and has many important physiological functions. Hence, in the BSA many binding sites are available to bind with ligands and the nature of binding is different for different ligands[ix,x,xi]. Various techniques are used to monitor the binding interactions of ligands to protein viz. NMR[xii], isothermal titration calorimetry (ITC)[xiii], UV- visible absorbance[xiv] fluroscence[xv] FT-IR and CD spectroscopy[xvi].

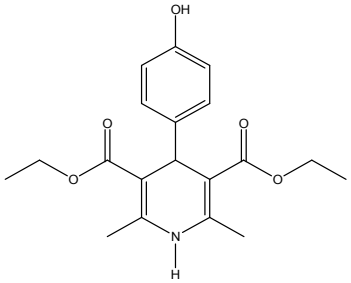


Fig.1: The chemical structure of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

In this paper, we have reported the interaction of 4HDDD with BSA at physiological pH 7.4 by FT-IR spectroscopy and equilibrium dialysis. Binding parameters of 4HDDD to the BSA are determined.

EXPERIMENTAL:

Materials and Methods:

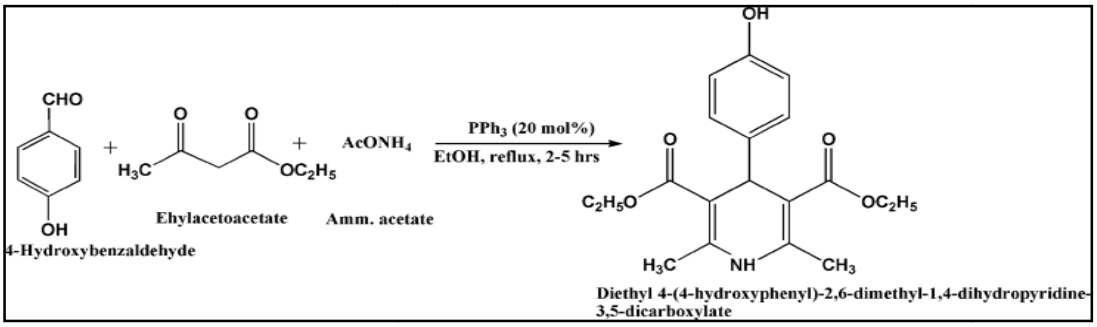
Dialysis membrane (molecular weight Cut off 3500) used in the experiment was purchased from Sigma Chemical Co (USA). UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) and metabolic shaking incubator (REMI RS-24AC) used in the experiment. FT-IR measurements were taken at room temperature on a Bruker FT-IR spectrometer (Alpha model, Germany) equipped with Zn-Se attenuated total reflection (ATR) accessory. All spectra taken via the ATR method with a resolution of 4 cm^{-1} and 60 scans in the region 1800-1300 cm^{-1}. 4HDDD prepared by known method[xvii]. BSA (essential fatty acid free) purchased from Chemsworth Chemicals Ltd (India) and used without further purification. Basic buffer selected to maintain the physiological pH 7.4 of the solution. All other chemicals used in the experiment are of commercial grade.

Optimization study:

4-HDDD is not completely soluble in physiological buffer. Hence mixture of buffer with non aqueous solvent such as 1, 4-dioxane, DMF & DMSO used to dissolve 4-HDDD. The different ratio of buffer: non-aqueous solvent tried, but the complete solubility of 4-HDDD was obtained at optimum ratio 30: 70:: non-aqueous solvent: buffer.

Preparation of 4HDDD :

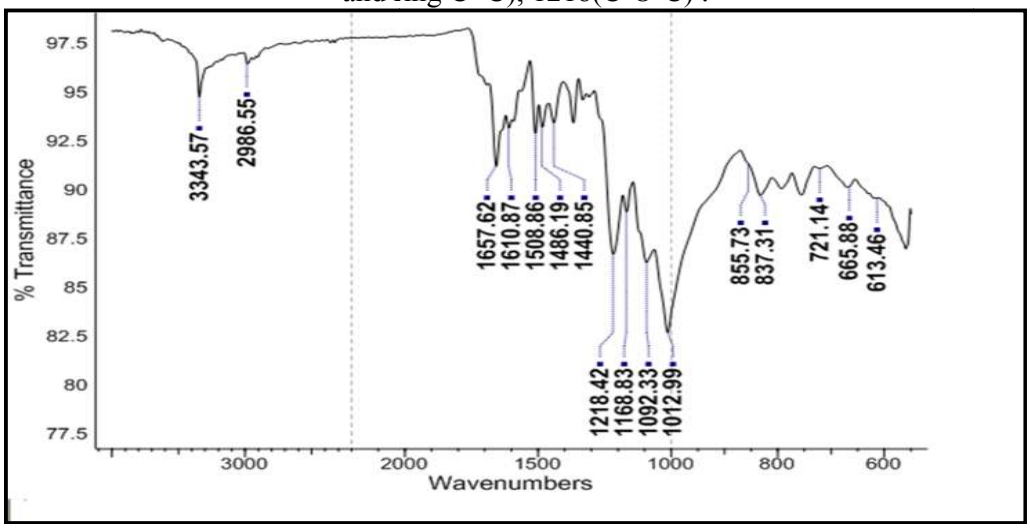
The reaction route is shown in scheme1, R_f : 0.60; % Yield: 93; Melting pt. :241º C.



Scheme 1: Preparation of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

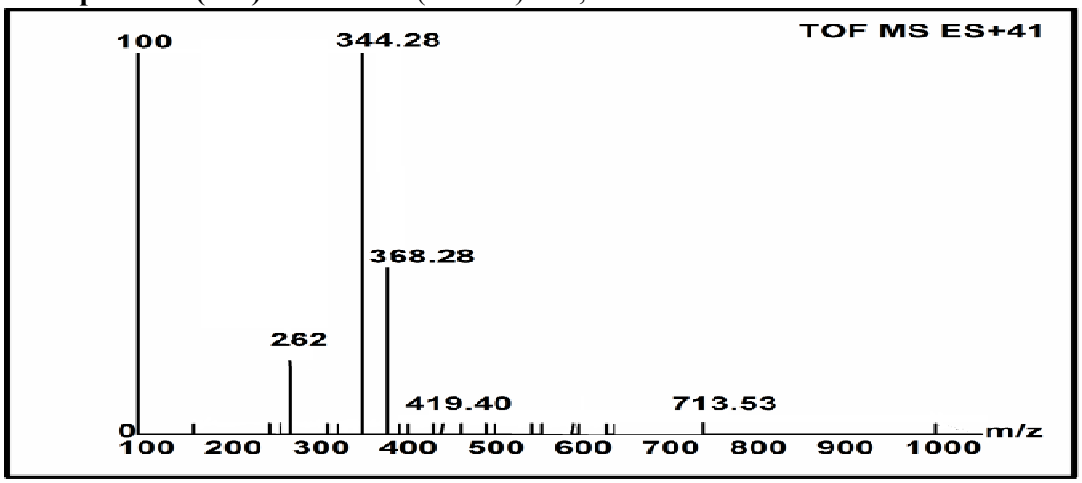
CHARACTERIZATION:

IR spectrum (vmax/cm^{-1}): 3344 (N-H); 2986(C-H); 1792(C=O);1716 (C=O); 1540 (=C-H and ring C=C); 1218(C-O-C) .



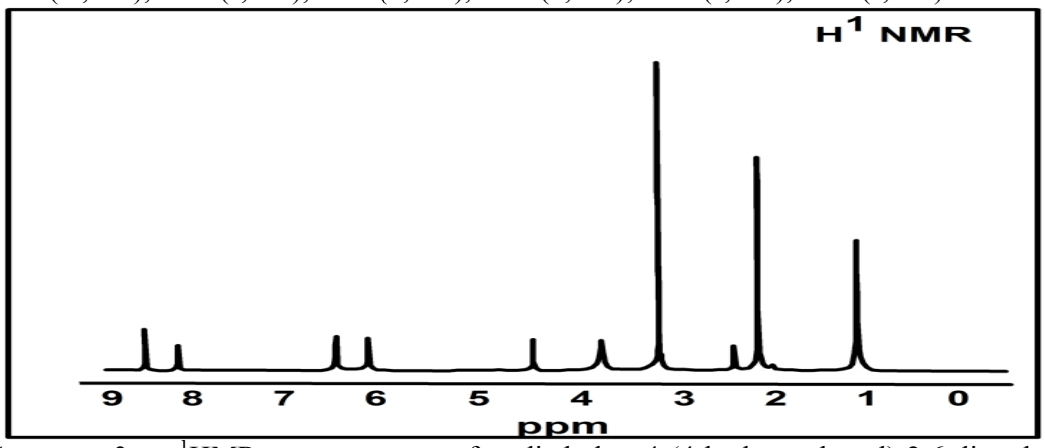
Spectrum- 1: FT-IR spectrum of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

Mass spectrum (m/z): Calculated (M^{++}Na):368, found 368.28.



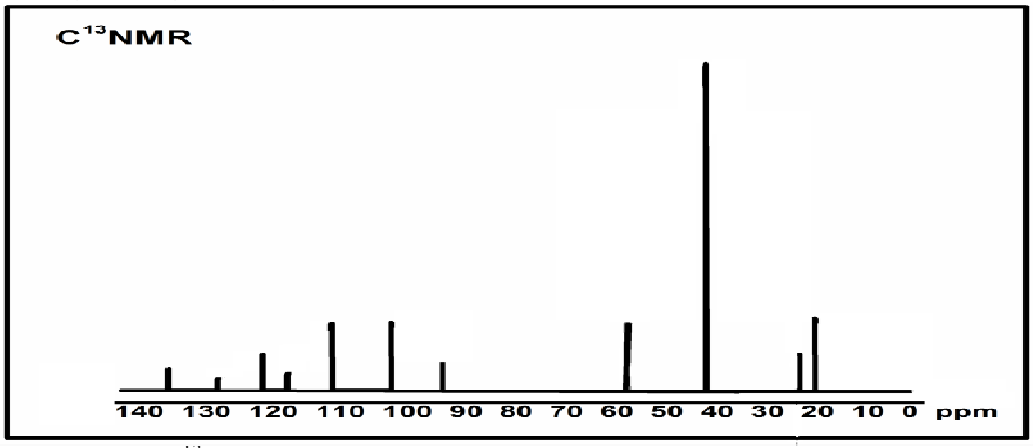
Spectrum-2: LCMS spectrum of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate.

^{1}H NMR Spectrum: (DMSO-d6, 500 MHz): δ (ppm): 1.04-1.19(t, 6H), 2.20 (s, 6H), 3.93-4.01 (m, 4H), 4.73 (s, 1H), 6.56 (d, 2H), 6.91 (d, 2H); 8.68 (s, 1H), 9.05 (s, 1H) .



Spectrum-3: ^{1}HMR spectrum of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

^{13}CMR (DMSO-d6, 500 MHz): δ (ppm): 14.80, 18.79, 59.47, 102.91, 115.12, 128.86, 139.50, 145.33, 156.02, 167.70.



Spectrum-4: ^{13}CMR spectrum of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate

Measurement of binding affinity:

For the Scatchard analysis, binding affinity between BSA and ligand molecule is expressed as an equilibrium constant or association constant which is derived from the law of mass action. BSA (B) interacts with the ligand (L) to form the complex as given below.

$$B + L \longrightarrow BL$$

So the equation for association constant,

$$K_f = \frac{[BL]}{[BL]+[B]}$$

Binding strength of the 4HDDD to BSA is a measure of association constant. Fraction of binding of 4HDDD to BSA is represented in the term of [V] so the equation for [V] is

$$V = \frac{[BL]}{[BL]+[B]}$$

Equation for [V] in terms of association constant (K_f) given as,

$$K_f = \frac{K_f[L]}{K_f[L]+1}$$

Total 'n' available sites on B out of number of site occupied by ligand is represented in terms of [V′ $V' = \frac{nK_f[L]}{K_f[L]+1}$

Scatchards graph of [V] with [L] gives the value for association consant (K_f) while graph [V′] with V′/L is a measure of interacting sites which are available.

Equilibrium dialysis:

Different concentrations (1×10^{-3} M to 3.5×10^{-3} M) of 4-HDDD in solvents 1, 4-dioxane, DMSO, DMF (30:70:: solvent: buffer) were mixed with a BSA solution (1.5×10^{-5}M in 7.4 pH buffer). These solutions were allowed to stand at room temperature for the maximum binding of 4-HDDD to BSA. From each mixture 3.5 ml solution was poured into previously prepared semi-permeable membrane and both the ends were sealed properly. The membrane tubes having 4HDDD-BSA complex solution were immersed in a 100 ml conical flask containing 40 ml buffer solution in each. These conical flasks placed in a metabolic shaker for dialysis for 12 hrs at room temperature. After dialysis, absorbance of bound fraction of 4HDDD to BSA measured on a UV spectrophotometer (λmax 520 nm).

FT-IR:

Different concentrations of 4HDDD and the BSA as mentioned above mixed and allowed to stand at room temperature for maximum binding. FT-IR measurements were carried out at room temperature on FT-IR spectrometer equipped with Zn-Se attenuated total reflection (ATR) method. Absorbance of BSA-4HDDD complex measured at room temperature.

RESULT AND DISCUSSION:

FT-IR analysis:

The amide I band at 1635 cm^{-1} is due to C=O stretching and amide II band at 1543 cm^{-1} is due to C-N stretch coupled with N-H bending. In BSA-4HDDD complex, the peak position of the amide I band is shifted from 1635 to 1650 cm^{-1} in 1, 4-dioxane, 1644 and 1643 cm^{-1} in DMSO & DMF resp. Similarly amide II band is shifted from 1543 to 1538, 1542, 1542 cm^{-1} in 1, 4-dioxane, DMSO, DMF respectively (Figure 2-4). It is observed that amide-I band is more sensitive to the changes of secondary structure of BSA than amide II. As the concentration of 4HDDD increases from 1×10^{-3} to 3.5×10^{-3} M, its binding with BSA is also increased. Increase in binding is attributed to amide I band. However, very small changes in the amide II band are observed in complex. (Table 1).

Table 1: Shifting of amide I & II after binding with 4-HDDD in different concentrations.

Conc. of 4HDDD	**BSA-amide I band shifting from 1635 cm^{-1}**			**BSA-amide II band shifting from 1542 cm^{-1}**		
	1,4-Dioxane	**DMSO**	**DMF**	**1,4-Dioxane**	**DMSO**	**DMF**
1×10^{-3}	1647	1639	1638	1538	1542	1539
1.5×10^{-3}	1648	1643	1639	1542	1540	1542
2×10^{-3}	1649	1643	1639	1538	1539	1538
2.5×10^{-3}	1650	1644	1642	1537	1540	1538
3×10^{-3}	1649	1644	1643	1537	1542	1538
3.5×10^{-3}	1650	1644	1643	1538	1542	1542

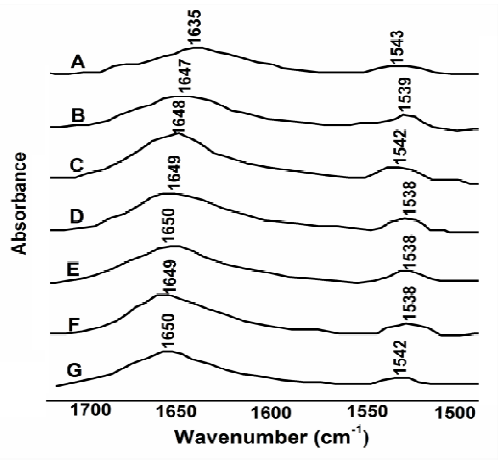


Fig. 2: FT-IR spectra of protein-drug complex in 1,4-dioxane.

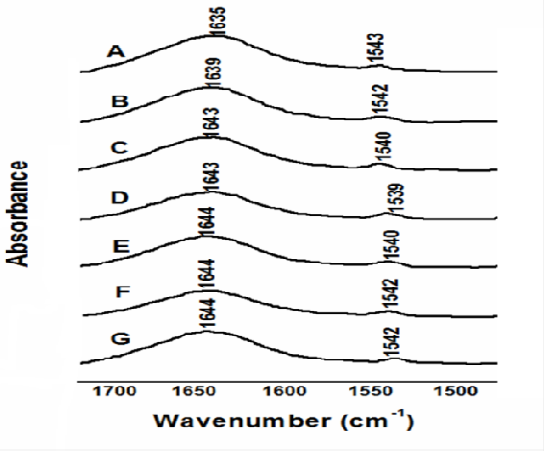


Fig. 3: FT-IR spectra of protein drug complex in DMSO.

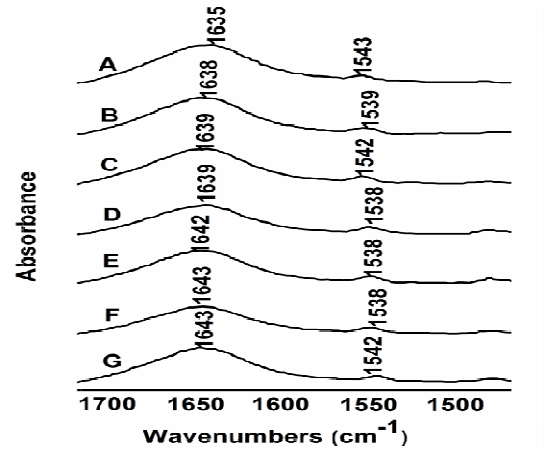


Fig. 4: FT-IR spectra of protein-drug complex in DMF.

Equilibrium Dialysis:

Preparation of standard curve:

The binding parameters of 4HDDD-BSA complex have been characterized using scatchard analysis. Solutions of different concentrations (1×10^{-3}M to 3.5×10^{-3}M) of 4HDDD prepared in 3:7:: non aqueous solvents :buffer. Scatchard curve obtained by plotting the absorbance against the corresponding concentrations. The scatchard analysis of binding of 4HDDD with BSA at pH 7.4 in all the solvents provided a non-linear curve. This suggests the presence of at least two binding sites for the binding of 4HDDD to BSA. The association constant (K_f) for BSA-4HDDD complex in solvents 1,4-dioxane, DMSO and DMF are 0.862, 0.429 and 0.511 respectively. Figures 5 to 7 shows the scatchard graphs of BSA-4HDDD binding in 1, 4-dioxane, DMSO and DMF respectively

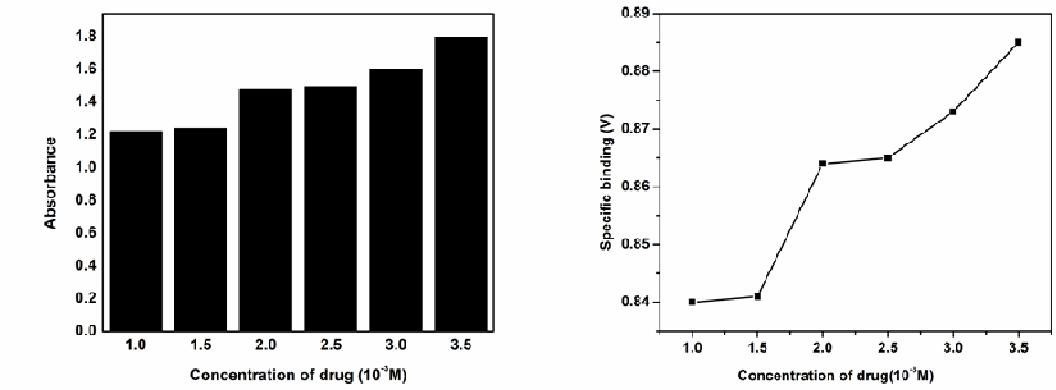


Fig. 5: Graph of absorbance & Specific binding vs conc. ligand in 1, 4-dioxane

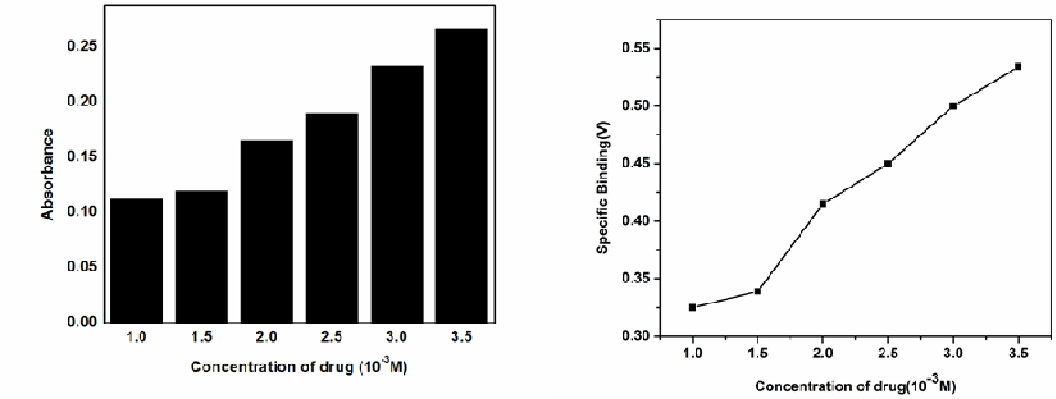


Fig. 6: Graph of absorbance & Specific binding vs conc. ligand in DMSO

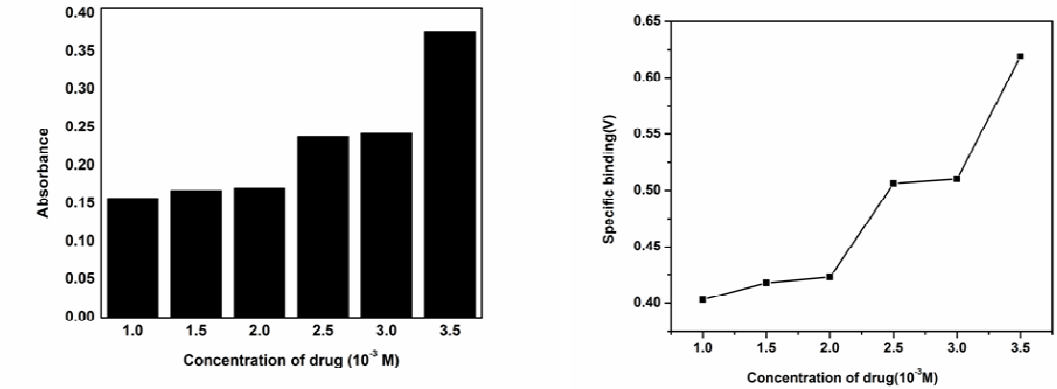


Fig. 7: Graph of absorbance & Specific binding vs conc. ligand in DMF.

CONCLUSION:

In the present study, the interaction of diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5 dicarboxylate (4HDDD) and the BSA has been studied by FT-IR spectroscopy and equilibrium dialysis at physiological pH in various solvents. The experimental result clearly indicates that 4HDDD interact with BSA in amide I site mainly through hydrophobic interaction, which changes the secondary structure of BSA. It is observed that binding affinity increased as the concentration of the drug increases; this probably enhances the pharmacological activity of the drug. BSA-4HDDD binding is also confirmed by equilibrium dialysis. Scatchard analysis gives the association constants for BSA-4HDDD interaction in solvents 1, 4-dioxane, DMSO and DMF. They are 0.862, 0.429 and 0.511 in 1, 4-dioxane, DMSO and DMF respectively. Binding is more significant in 1, 4-dioxane than DMSO and DMF. It concludes that binding of 4-HDDD to BSA is more in non polar solvent (1, 4-dioxane) than polar solvents (DMF & DMSO).

ACKNOWLEDGEMENT:

We thanks to department of chemistry, Jankidevi Bajaj College of science for providing necessary research facilities. No funding was received for this study.

REFERENCES:

i. M. Nikoorazm, et al., Scientia Iranica, 20, 603 (2013).
ii. S.A. Sadeek, E.L. Attar and M.S. Ibrahim, Bulletin of the Chemical Society of Ethiopia, 29, 75 (2015).
iii. H.S. Sohal, M. Kaur, R. Khare and K. Singh, American Journal of Organic Chemistry, 4, 21 (2014)
iv. J. Safari, F. Azizi, and M. Sadeghi, New Journal of Chemistry, 39, 1905 (2015).
v. A. Sausins and G. Duburs, Heteroc., 27, 269 (1988).
vi. A. Panduangan and T. Paramasivan, Ind. J. Chem., 47, 1084 (2008).
vii. R. Kumar, B. Kaur and V.K. Bajaj, Journal of Pharmacology and pharmacotherapeutics, 4, 64 (2013).
viii. A.C. Gaudio, A. Korolkovas and Y. Takahata, J. Pharm. Sci., 83, 1110 (1994).
ix. X.M. He and D.C. Carter, Nature, 358, 209 (1992).
x. G. Sudlow, D.J. Brickett, and D.N. Wade, Mol. Pharmacology, 11, 824 (1975).
xi. L. Fielding and S. Rutherford, Magnetic res. Chemistry, 43, 463 (2005).
xii. H.U. Kragh, et al., Pharmacol. Rev., 33, 17 (1981).
xiii. L. Xiangrong, W. Gongke, C. Dejun and Lu. Yan, Mol. BioSyst., 11, 326 (2014).
xiv. S.K. Chaturvedi, E. Ahmad, J.M. Khan, P. Alam and R.H. Khan, Mol. BioSyst., 11, 307 (2015).
xv. S. Baroni, M. Mattu, S. Aime and M. Fasano, Eur. J. Biochem., 268, 6214 (2001).
xvi. J. Tian, J. Liu and X. Chen, FEBS Lett., 347, 995 (2005).
xvii. A. Debache, W. Ghalem, R. Boulcina, A. Belfaitah and S. Rhouati, Tetrahedron letters, 50, 5248 (2009).

Received on September 21, 2016.