Original Research Paper

Volume-8 | Issue-11 | November-2018 | PRINT ISSN No 2249-555X

Chemical Science

# DIELECTRIC PROPERTIES AND FLUID STRUCTURE OF BINARY MIXTURES OF DIMETHYL PHTHALATE AND BENZYLAMINE AT DIFFERENT TEMPERATURES

**A.Mushtaq Ahmed Khan** — Department of Physics, Dr. Zakir Husain College, Ilayangudi, Sivaganga, India

**M.Subramanian*** — Dean (Research), Fatima Michael College of Engineering and Technology, Madurai, India *Corresponding Author

**ABSTRACT** Interactions in the binary mixture of dimethyl phthalate with benzyl amine studied through the orientation of dipoles using dielectric polarization process. This vital information could be obtained from dielectric measurements by virtue of the influence of local order and the overall dipole moment. The information regarding orientation of electric dipoles in polar liquids was obtained from Kirkwood correlation factor, effective correlation factor, and corrective correlation factor. The Bruggeman parameter determination confirms the molecular interaction between the constituents of the binary mixture and thermo dynamical excess parameters were also measured for analysis for three different temperatures (303K, 313K and 323K).

**KEYWORDS :** Dielectric Constant, Refractive Index, Kirkwood Correlation Factor, Bruggeman Factor.

## 1. INTRODUCTION

Thermo dynamical physical properties of liquid systems such as dielectric constant, optical permittivity, Kirkwood correlation factor, effective correlation factor are related to molecular interactions present in binary liquid mixtures. Dimethyl phthalate (DBP) is a commonly used plasticizer. Benzyl amine is found in many agricultural products. It is used in pesticides and fertilizers that helps to protect and grow crops. By studying the properties of these mixtures, we can get valuable information that may be helpful in many ways. Moreover, these compounds are strongly associated in solution because of dipole – dipole interaction and hydrogen bonding.

The main aim of this study is to find out the intermolecular interaction between Dimethyl phthalate and benzylamine molecules through dielectric study at different temperatures 303K, 313K and 323K. Dielectric studies and hydrogen bonding are the key aspects of binary mixture for the determination of structure analysis[1]. These analyses are possible only through dielectric studies. The study of Kirkwood correlation factor has a considerable significance in providing valuable information about the interaction between the like molecules. Many workers had tried to find molecular structure and interaction of alcohols [2-3]and few tried using amines[4-6] as one of the constituent components of the binary mixture.

Dielectric constants, refractive indices and polarizations of binary mixture of polar molecules were estimated by several researchers [7-8]. Many researchers [9-11] used modified expressions for the study of dipolar excess thermodynamic properties of associated liquids in an inert solvent in the study. This work mainly concentrates to calculate the dipolar excess free energy using the values of the linear correction factor 'g' for the following systems, dimethyl phthalate + benzyl amine ( system I ) and dimethyl phthalate + triethylamine ( system II) at 303K , 313K and 323K.

## 2. MATERIALS AND METHODS

The dielectric constants were measured at 1kHz using VLCR-7 meter supplied by Vasavi Electronics, India. Using a water circulating thermostat the temperature was maintained at 303K, 313K and 323K. The refractive indices were measured using an Abbe's refractometer. Densities were determined by using a 10ml specific gravity bottle and a K-Roy microbalance. The liquids were purified by standard methods.

## 3. Theory

The Kirkwood correlation [12] factor (g) is a parameter affording information regarding orientation of electric dipoles in polar liquids. The modified Kirkwood- Frohlich [13] correlation factor (g) for the pure liquid is given by the expression.

$$\frac{4\pi N_A \mu^2 \rho g}{9KTM} = \frac{(\varepsilon_0-\varepsilon_\infty)(2\varepsilon+\varepsilon_\infty)}{\varepsilon_0(\varepsilon_\infty+2)^2} \quad (1)$$

where μ the dipole moment in the gas phase, ρ is the density at a temperature T, M is the molecular weight $\varepsilon_0$ is the dielectric constant of pure liquid, $\varepsilon\infty$ is the dielectric constant of pure liquid at optical frequency(square of the refractive index), K is the Boltzmann constant and $N_A$ is the Avogadro's number. For a mixture of two polar liquids say A and B the equation is modified by Kumbharkhane et al. [14] using some assumptions.

Assume that 'g' for the mixture is expressed by an effective average correlation factor($g^{eff}$) such that Kirkwood equation for the mixture can be expressed as

$$\frac{4\pi N_A}{9KT}\left(\frac{\mu_A^2\rho_A}{M_A}X_A + \frac{\mu_B^2\rho_B}{M_B}X_B\right)g^{eff} = \frac{(\varepsilon_{0m}-\varepsilon_{\infty m})(2\varepsilon_{0m}+\varepsilon_{\infty m})}{\varepsilon_{0m}(\varepsilon_{\infty m}+2)^2} \quad (2)$$

where $X_A$ and $X_B$ are the volume fractions and $\mu_A$ and $\mu_B$ are the dipole moment of the liquids A and B respectively and $g^{eff}$ is the effective Kirkwood correlation factor for a binary mixture. The value of $g^{ef}$ in equation(2) will change from gA to gB as the fraction of component B increases from zero to unity.

Another way to visualize the variation in Kirkwood correlation factor is to assume that the correlation factor for molecules A and B in a mixture contribute to effective 'g' in proportion to their pure liquid values $g_A$ and $g_B$. Under this assumption Kirkwood equation for the mixture can be written as

$$\frac{4\pi N_A}{9KT}\left(\frac{\mu_A^2\rho_A g_A}{M_A}X_A + \frac{\mu_B^2\rho_B g_B}{M_B}X_B\right)g_f = \frac{(\varepsilon_{0m}-\varepsilon_{\infty m})(2\varepsilon_{0m}+\varepsilon_{\infty m})}{\varepsilon_{0m}(\varepsilon_{\infty m}+2)^2} \quad (3)$$

where $g_f$ is the correlation factor for the mixture. In equation (3), $g_f$ is unity for pure liquids and will remain close to unity if there is no interaction between A and B.

The Bruggeman equation for a binary mixture [15] is given by the expression

$$f_B = \left(\frac{\varepsilon_{0m}-\varepsilon_{0B}}{\varepsilon_{0A}-\varepsilon_{0B}}\right)\left(\frac{\varepsilon_{0A}}{\varepsilon_{0m}}\right)^{\frac{1}{3}} = (1-\phi_B) \quad (4)$$

where $\varepsilon_{0A}$ ,$\varepsilon_{0B}$ and $\varepsilon_{0m}$ are the dielectric constants of the liquids A B and mixture respectively.

$f_B$ is the Bruggeman dielectric factor, ϕB is the volume fraction of the alcohol

Studying these excess dielectric properties, it can be used to access the useful information regarding the structural changes in binary mixture.

In the present work, excess dielectric properties are determined corresponding to static permittivity and molar volume. The excess permittivity ($\varepsilon^E$) is defined as [16]

$$\varepsilon^E = (\varepsilon_0-\varepsilon_\infty)_m - [(\varepsilon_0-\varepsilon_\infty)_A X_A + (\varepsilon_0-\varepsilon_\infty)_B X_B] \quad (5)$$

where X is the mole fraction and the subscripts m, A and B represent mixture, solvent and solute respectively. The excess permittivity $\varepsilon^E$ may provide qualitative information about the multimer formation in mixtures as follows.

The excess Helmholtz free energy ΔF which is almost equal to the Gibb's free energy ΔG for a condensed matter [16] is given as

$$\Delta F^E = \frac{N_A}{2}\left[\sum_{r=A,B} X_r \mu_r^2 (R_{fr} - R_{fr0})\right] + \frac{N_A}{2}\left[\sum_{r=A,B} X_r^2 \mu_r^2 (g_{rr} - 1)(R_{fr} - R_{fr0})\right]$$

$$+\frac{N_A}{2}\left[X_A X_B \mu_A \mu_B (g_{AB} - 1)(R_{fA} + R_{fB} - R_{fA0} - R_{fB0})\right]$$

$$= \Delta F_0^E + \Delta F_{rr}^E + \Delta F_{AB}^E \qquad (6)$$

where

$$R_{fr} = \frac{8\pi N_A}{9V_r}\left[\frac{(\varepsilon_{0r}-1)(\varepsilon_{\infty r}+2)}{(2\varepsilon_{0r}+\varepsilon_{\infty r})}\right] \qquad (7)$$

$$R_{fr0} = \frac{8\pi N_A}{9V_r}\left[\frac{(\varepsilon_{0m}-1)(\varepsilon_{\infty r}+2)}{(2\varepsilon_{0m}+\varepsilon_{\infty r})}\right] \qquad (8)$$

where V is the molar volume of the components and ε0r is the dielectric constant of the pure liquids.

## 4. Result and Discussion

Table 1 shows the static permittivity ($\varepsilon_0$), the static permittivity at high frequency ($\varepsilon_\infty$) normally calculated from the square of the refractive index for sodium line, effective correlation factor($g^{eff}$) and Kirkwood correlation factor ($g_f$), excess permittivity ($\varepsilon^E$), Bruggemann factor ($f_B$) for the mixture of dimethyl phthalate with benzyl amine for three different temperatures 303K, 313K and 323K. In Table 2, the values of excess dipolar free energy ($\Delta F_0^E$) due to long range electrostatic interaction, $\Delta F_{rr}^E$ the excess dipolar energy due to short range interaction between identical molecules, $\Delta F_{AB}^E$ the excess dipolar energy due to short range interaction between dissimilar molecules and the total excess Helmholtz free energy of mixing $\Delta F^E$ are listed for the binary mixture of dimethyl phthalate and benzyl amine for above the three temperatures.

**TABLE** 1:Dielectric properties of Dimethyl Phthalate with Benzylamine

| Temperature K | $X_2$ | ε static | εinfinity | $g^{eff}$ | $g_f$ | $^E$ | $f_B$ |
|---|---|---|---|---|---|---|---|
| 303 | 0.0 | 8.5140 | 2.2840 | 1.0000 | 1.0000 | 0.0000 | 1.0000 |
| | 0.1 | 8.2593 | 2.2930 | 1.0130 | 1.0190 | 0.1270 | 0.9430 |
| | 0.2 | 7.8638 | 2.3030 | 1.0040 | 1.0010 | 0.0140 | 0.8290 |
| | 0.3 | 7.5183 | 2.3080 | 1.0060 | 1.0270 | 0.1550 | 0.7710 |
| | 0.4 | 7.0319 | 2.3180 | 0.9780 | 1.0080 | 0.0490 | 0.6530 |
| | 0.5 | 6.5514 | 2.3270 | 0.9480 | 0.9890 | -0.0490 | 0.5320 |
| | 0.6 | 6.0766 | 2.3340 | 0.9170 | 0.9680 | -0.1390 | 0.4070 |
| | 0.7 | 5.6075 | 2.3450 | 0.8770 | 0.9400 | -0.2280 | 0.2770 |
| | 0.8 | 5.3750 | 2.3530 | 0.8950 | 0.9770 | -0.0770 | 0.2100 |
| | 0.9 | 5.1439 | 2.3630 | 0.9160 | 1.0230 | 0.0740 | 0.1420 |
| | 1.0 | 4.6858 | 2.3700 | 0.8670 | 1.0000 | 0.0000 | 0.0000 |
| 313 | 0.0 | 8.4468 | 2.2714 | 1.0304 | 1.0000 | 0.0000 | 1.0000 |
| | 0.1 | 8.1921 | 2.2846 | 1.0406 | 1.0163 | 0.1213 | 0.9430 |
| | 0.2 | 7.7966 | 2.2904 | 1.0332 | 1.0162 | 0.1094 | 0.8526 |
| | 0.3 | 7.4511 | 2.2992 | 1.0321 | 1.0233 | 0.1445 | 0.7715 |
| | 0.4 | 6.9647 | 2.3031 | 1.0066 | 1.0074 | 0.0434 | 0.6536 |
| | 0.5 | 6.4842 | 2.3153 | 0.9731 | 0.9844 | -0.0600 | 0.5322 |
| | 0.6 | 6.0094 | 2.3198 | 0.9403 | 0.9634 | -0.1500 | 0.4470 |
| | 0.7 | 5.5403 | 2.3272 | 0.9006 | 0.9368 | -0.2372 | 0.2771 |
| | 0.8 | 5.3078 | 2.3372 | 0.9164 | 0.9709 | -0.0904 | 0.2102 |
| | 0.9 | 5.0767 | 2.3446 | 0.9380 | 1.0166 | 0.0605 | 0.1418 |
| | 1.0 | 4.6186 | 2.3363 | 0.8964 | 1.0000 | 0.0000 | 0.0000 |
| 323 | 0.0 | 8.3796 | 2.2608 | 1.0592 | 1.0000 | 0.0000 | 1.0000 |
| | 0.1 | 8.1249 | 2.2671 | 1.0736 | 1.0209 | 0.1289 | 0.9431 |
| | 0.2 | 7.7294 | 2.2744 | 1.0644 | 1.0203 | 0.1162 | 0.8528 |
| | 0.3 | 7.3839 | 2.2837 | 1.0623 | 1.0276 | 0.1513 | 0.7718 |
| | 0.4 | 6.8975 | 2.2928 | 1.0318 | 1.0086 | 0.0458 | 0.6539 |
| | 0.5 | 6.4170 | 2.2973 | 1.0010 | 0.9906 | -0.0493 | 0.5326 |
| | 0.6 | 5.9422 | 2.3016 | 0.9661 | 0.9700 | -0.1384 | 0.4874 |
| | 0.7 | 5.4731 | 2.3128 | 0.9212 | 0.9409 | -0.2288 | 0.2775 |
| | 0.8 | 5.2406 | 2.3198 | 0.9383 | 0.9787 | -0.0783 | 0.2105 |
| | 0.9 | 5.0095 | 2.3241 | 0.9613 | 1.0292 | 0.0763 | 0.1420 |
| | 1.0 | 4.5514 | 2.3323 | 0.9035 | 1.0000 | 0.0000 | 0.0000 |

**TABLE 2:** Dipolar energy values of the mixture of Dimethyl Phthalate with Benzylamine

| Temperature K | $X_2$ | $\Delta F_0^E$ J/mole | $\Delta F_{rr}^E$ J/ mole | $\Delta F_{AB}^E$ J/ mole | $\Delta F^E$ J/ mole |
|---|---|---|---|---|---|
| 303 | 0.1 | -4.6884 | 0.3627 | -0.5436 | -4.8694 |
| | 0.2 | 2.8006 | 1.3433 | -0.2611 | 3.8828 |
| | 0.3 | 4.0700 | 2.7971 | -0.4294 | 6.4377 |
| | 0.4 | 18.2631 | 4.3604 | 1.4752 | 24.0987 |
| | 0.5 | 30.9841 | 5.7520 | 2.3670 | 39.1032 |
| | 0.6 | 41.9625 | 6.5769 | 1.5460 | 50.0855 |
| | 0.7 | 50.8569 | 6.3409 | -1.0996 | 56.0982 |
| | 0.8 | 28.4313 | 6.4107 | -1.8247 | 33.0173 |
| | 0.9 | 2.6386 | 5.5890 | -1.3536 | 6.8741 |
| 313 | 0.1 | -4.6882 | 0.9043 | -1.7614 | -5.5453 |
| | 0.2 | 2.9106 | 2.3605 | -2.2476 | 3.0235 |
| | 0.3 | 4.2231 | 3.7912 | -2.4772 | 5.5370 |
| | 0.4 | 18.5854 | 5.2767 | -0.4463 | 23.4159 |
| | 0.5 | 31.4626 | 6.3341 | 1.2491 | 39.0459 |
| | 0.6 | 42.5806 | 6.7132 | 1.1158 | 50.4096 |
| | 0.7 | 51.5932 | 6.1002 | -0.9045 | 56.7890 |
| | 0.8 | 28.8793 | 5.6063 | -1.4700 | 33.0157 |
| | 0.9 | 2.7288 | 4.5662 | -1.0103 | 6.2847 |
| 323 | 0.1 | -4.9366 | 1.4902 | -3.2518 | -6.6982 |
| | 0.2 | 2.5730 | 3.5720 | -4.4464 | 1.6985 |
| | 0.3 | 3.7579 | 5.2232 | -4.9054 | 4.0758 |
| | 0.4 | 18.2050 | 6.8950 | -2.1932 | 22.9068 |
| | 0.5 | 31.2195 | 7.8607 | -0.0461 | 39.0340 |
| | 0.6 | 42.5329 | 7.9443 | 0.6557 | 51.1329 |
| | 0.7 | 51.8051 | 6.9250 | -0.7064 | 58.0238 |
| | 0.8 | 28.8507 | 5.8922 | -1.0916 | 33.6512 |
| | 0.9 | 2.4327 | 4.5008 | -0.6379 | 6.2956 |

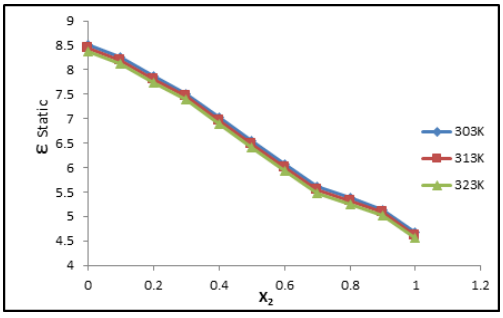


**Figure1:** Plot of static permittivity with mole fraction of dimethyl phthalate + benzyl amine

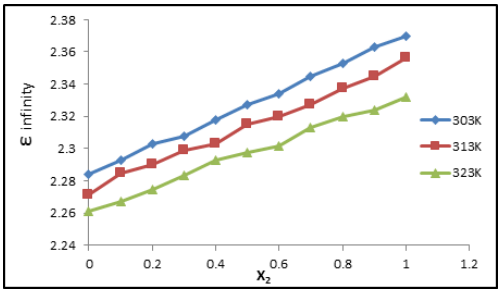


**Figure 2 :** Plot of optical permittivity with mole fraction of dimethyl phthalate + benzyl amine

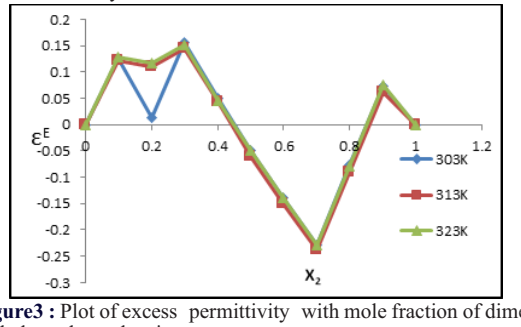


**Figure3 :** Plot of excess permittivity with mole fraction of dimethyl phthalate + benzyl amine

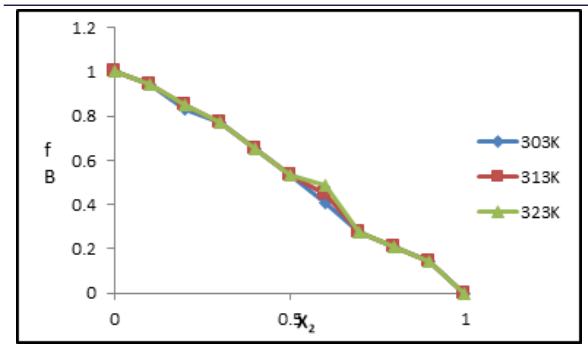


**Figure 4:** Plot of Bruggeman parameter with mole fraction of dimethyl phthalate+benzylamine

For the system of dimethyl phthalate with benzyl amine, there is a non linear variation of dielectric constant ($\varepsilon_0$) with concentration of amines indicating the hetero interaction between the components. The non-linear variation of the static dielectric constant of the mixture indicates the strong interaction exists between phthalate and benzyl amine molecules. In the first system, the value of $g^{eff}$ for pure phthalate is greater than unity at all temperatures indicating that molecular dipoles have parallel orientation among themselves. As the temperature increases, the increase in the value of $g^{eff}$ indicates the strengthening of parallel orientation of dipoles. For pure amine, the values of $g^{eff}$ is less than unity indicating the anti parallel orientation of the molecular dipoles and as the temperature increases the molecular dipoles orientated in such a way that they are disturbed by the temperature and align anti parallel to themselves. But for the mixture, there is a decreasing tendency in the values of $g^{eff}$ by adding amine with phthalate, the parallel orientation of the molecular dipoles of the phthalate disappears and reaches zero nearly at 20 % to 30 % of amines mixed with phthalate at room temperature. The molecular association is poor in this region. On further increasing the concentration of amines, the amine molecules dominate and the parallel orientation completely disappears resulting in the anti parallel orientation of dipoles. On further increasing concentration of amine, the effective number of dipoles increases. Moreover, the values of $g^{eff}$ state that they have anti parallel orientation. On increasing the temperature similar behavior is observed and effective number of dipoles increases at higher temperature indicating the strong interaction between the unlike molecules. The heterogeneous interaction between unlike molecules strength is found to depend on the concentration and temperature of the mixture. This behavior shows the concentration and temperature variation affects the structural properties of phthalate and amines.

Figure 3 shows the variation of excess permittivity for different mole fraction and figure 4 shows the variation Bruggeman parameter for different volume fraction of the solvent. The values of excess permittivity at 303K temperature in the mixture of phthalate and amine is such that, in phthalate rich regions it is positive and in amine rich region it is negative. On mixing amines with phthalate the number of dipoles in the phthalate decreases at this temperature. On increasing the temperature, there is a further decrease in number of dipoles takes place. On increasing the concentration of the amine molecules , the number of dipoles increases indicating the hetero interaction between the amine and phthalate. As a hole there is a strong hetero interaction takes place between these molecules.

The deviation in the value of $g_f$ from unity indicates the magnitude of interaction between the compounds. Greater deviation from unity means a larger strength of interaction [17]. The deviation in the value of $g_f$ from unity also indicates the formation of multimers between the two components. The non -linear variation of Bruggeman factor also indicates the strong interaction between the dipoles. Similar behavior is observed for all temperatures studied.

Table 2 shows the long range and short range interactions between the dipole for the binary mixture of dimethyl phthalate and benzyl amine. These are studied from the excess Helmholtz free energy $\Delta F^E$, a thermodynamically parameter. It is found that, the values of $\Delta F_0^E$ for this system is positive for all the temperatures studied. As the concentration increases, the magnitude of this value increases indicating the existence of long range dipole-dipole interaction [18]. The values $\Delta F_{rr}^E$ predict the information of the short range interaction between similar molecules. The values are low and maximum at 60% concentration of amine indicating strong short range interaction are due to hydrogen bonding. The magnitude of $\Delta F_{AB}^E$ gives the information on the strength of interaction between unlike molecules. For our system, it is found that strength of interaction is very low in phthalate rich region and appreciable in amine rich region. Finally the positive values of $\Delta F^E$ for the present system, for all concentration and at all temperatures indicates the formation of β-clusters with anti parallel alignment[19].

## 5. Conclusion

The values of dielectric constant, dielectric constant at optical frequency, excess permittivity, Kirkwood correlation factor, Bruggeman parameter, long range and short range interaction between like and unlike molecules are given for the combination of binary mixtures namely, dimethyl phthalate and benzyl amine at different temperatures (303K, 313K and 323K). The energy of dimethyl phthalate increases in a rapid way with the addition of triethylamine than benzyl amine. The strength of interaction between the molecules reach a maximum when the concentrations of triethyl amine and phthalate are nearly equal. The above listed data can be used to interpret liquid structure in above mixture.

## REFERENCES


1. Thenappan T, Sankar U, Dielectric studies of hydrogen sorted complexes of alcohols with N, N-dimethyl Formamide. J Mol Liq 2006; 126:1
2. Subramanian M, Uma Maheswari RM, Sathis Kumar K, Microwave Dielectric Relaxation Studies of Ternary liquid mixtures of Dioctyl Phthalate and Diethyl phthalate with isobutanol. Ind J App Res 2018; 8:3
3. Parthipana G, Arivazhagana G, Thenappan T, Dielectric and thermodynamic studies on a binary mixture of anisole with butyric or caprylic acid. Phil Mag Letts 2008; 88: 2
4. Mushtaq Ahmed Khan A, Subramanian M, Dielectric Properties and Fluid Structure of Binary Mixtures of Dimethyl Phthalate and Triethylamine at different temperatures. Int J Current Res in Life Sciences 2018; 7:6;2214-2219
5. Narwade BS, Gawali PG, Rekha Pande P, Kalamse GM, Dielectric studies of binary mixtures of n-propyl alcohol and ethylenediamine. J Che Sci 2005; 117:6
6. Liakath Ali Khan F, Sivagurunathan P, Raja Mohamed Kamil S, Mehrotra SC, Dielectric studies of methyl methacrylate and butyl methacrylate with primary alcohols using time domain reflectometry. Ind J Pure and Appl Phys 2007;45:754
7. Vishwar T, Subramanian V, Subbaiah DV, Murthy VRK, Conformational and microwave dielectric relaxation studies of hydrogen bonded polar binary mixtures of propionaldehyde with isopropyl amine. Mol Phy 2008; 106:295
8. Indira T, Thenappan T, Dielectric and thermodynamic studies on a inary mixture of Ethylbromide with oleic acid. National J Chem Biosis 2010; 1:1
9. Parthipan G, Arivazhagan G, Thenappan T, Dielectric and thermodynamic studies on a binary mixture of anisole with butyric or caprylic acid. Philo Maga Lett 2008;88:125.
10. Partiban G, Aswathaman H, Arivazhagen G, Thenappan T, Philo Magazine Let 2008; 88:251
11. Sengwa RJ, Sankhala S, Shinyashiki N, J Soln Chem, Dielectric parameters and Hydrogen Bond Interaction Study of Binary Alcohol Mixtures. 2008; 37:137
12. Kirkwood JG, The Dielectric Polarization of Polar Liquids. J Chem Phys 1939;7:911.
13. Frohlich H, Theory of Dielectrics. Oxford University Press London 1949
14. Kumbharkhane AC, Joshi YS, Hudge PG, Mehrotra SC, The dielectric relaxation study of 2(2- alkoxyethoxy)ethanol–water mixtures using time domain reflectometry. J Mol Liq 2011; 163:70
15. Bruggeman DAG , Ann Phys 1935; 5: 636
16. Ghanadzadeh A, Ghanadzadeh H, Alinejad M, Dielectric Study on Polar Binary Mixtures of Ester –Alcohol at 298.2K. Iranian J Chem Eng 2012;9:3
17. Pawar VP, Patil AR, Mehrotra SC, Temperature Dependent Dielectric Relaxation Study of Chlorobenzene with n-methyl formamide from 10MHz to 20 GHz. J Mol Liq 2005;121:5
18. Gupta KK, Bansal AK, Singh PJ, Sharma KS, Study of molecular interactions in binary mixtures of acetophenone derivative and cyclohexylamine. Ind J Phys 2005;79:2
19. Vijaya Krishna T, Sreehari Sastry S, Dielectric and Thermodynamic studies on the Hydrogen Bonded Binary System of Isopropy Alcohol and Aniline. J Soln Chem 2010;39:1377.