Investigation on tautomeric equilibrium of schiff base in mixed binary solvent

A Dissertation
Submitted for the partial fulfillment

FOR THE DEGREE OF
MASTER OF SCIENCE IN CHEMISTRY

Under Academic Autonomy
NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

By

Prakash Kumar Malik

**Under the Guidance
Of**

Dr. Sabita Patel

**Department Of Chemistry
NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA – 769008, ORISSA**

Dr. Sabita Patel
Assistant Professor
Department of Chemistry
National Institute of Technology
Rourkela-769008, India

CERTIFICATE

This is to certify that the dissertation entitled "**Investigation on tautomeric equilibrium of schiff base in mixed binary solvent**" being submitted by **Prakash Kumar Malik** to the Department Of Chemistry, National Institute Of Technology, Rourkela-769008, for the award of the degree of Master Of Science in Chemistry, is a record of bonafide research carried out by him under my supervision and guidance. The dissertation report has reached the standard fulfilling the requirements of the regulations relating to the nature of the degree.

I further certify that to the best of my knowledge Mr. Malik bears a good moral character.

NIT-Rourkela
Date: (Sabita Patel)

ACKNOWLEDGEMENTS

CONTENTS

Investigation on tautomeric equilibrium of schiff base in mixed binary solvent

1. Introduction

### 1.1 Enol imine-keto enamine tautomerism

Schiff bases derived from o-hydroxybenzaldehyde has attracted a great interest not only for its promising applications towards linear and non-linear optical properties, biological activity and technological applications[1] but also used as model compounds for the theory of hydrogen bonding.[2] Due to its intramolecular hydrogen bonding, depending on the position of proton in the hydrogen bond o-hydroxy salicylidene Schiff bases exhibit two tautomeric forms, enol-imine (E- form) and keto-enamine (K-form) both in solution[3] and in crystalline state[4] (Figure 1). A zwitterionic structure also appears due to a proton transfer in enol – imine and keto – amine tautomer. These classes of compounds also exhibit thermochromic and photochromic behavior.[5]

| E-form | K-form | Zwitterionic form |
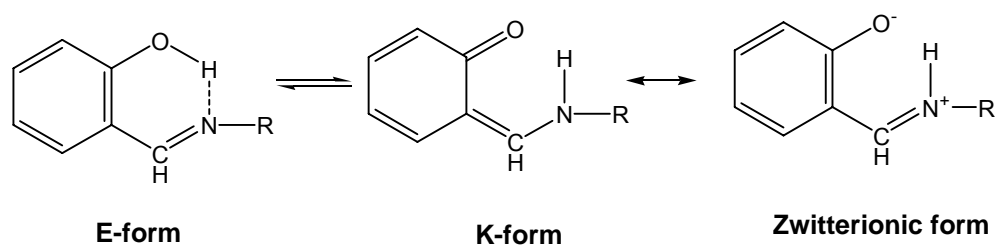
(Figure 1)

The most important intramolecular enol-imine and keto-enamine tautomerism in biological system exists in pyridoxal-5-phosphate (PLP) dependent enzymes. From the crystal structure data of PLP dependent enzymes, it is clear that the cofactor PLP forms a Schiff base with the ε-amino group of a lysine residue or with the amino acid substrates which

are going to be transferred.[6-9] During the catalytic cycle, it is assumed that the E-form of the schiffbase has to be converted to the zwitterionic K-form through intramolecular OHN-hydrogen bonding assisted by protonation of the pyridine ring (Figure 2).[10,11]
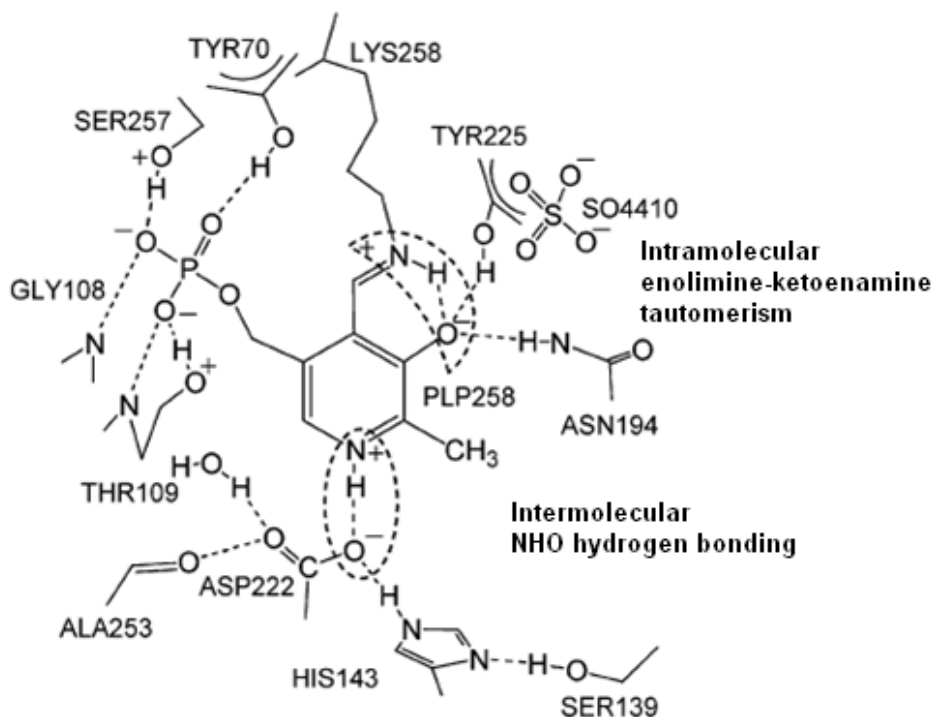
(Figure 2)

The derivative of N-(3,5-dibromosalicylidene)-methylamine (**1**) were taken as a model compound for the PLP to study the intramolecular hydrogen bonding in PLP dependent enzymes by using UV-Vis spectroscopy[12] and $^{13}C,^{13}$ $^{17}O,^{14}$ and $^{15}N^{15}$ NMR spectroscopy. These compounds exhibit a keto-enol tautomerism between an OH- (enol) and an NH-(keto) form, and the position of the equilibrium is affected by the polarity of the solvent.
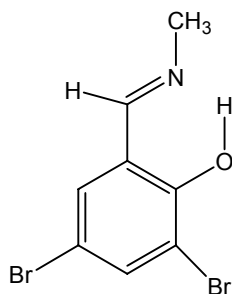
N-(3,5-dibromosalicyliden)methylamine **(1)**

To have a better insight in to the Sharif et al.[11] have extensively studied the intramolecular OHN-hydrogen bonding in model Schiff base of pyridoxal phosphate i.e. N-(3,5-dibromosalicyliden)methylamine (**1**) by means of ^{1}H and ^{15}N NMR in different organic solvent. The OHN- hydrogen bond is extremely sensitive to the local polarity and local hydrogen bonds to the oxygen. With increase in the dielectric constant of the medium, the enol-imine and keto-enamine tautomeric equilibrium shifts toward NH side. A highly polar environment favors the zwitterionic NH-form which is the only reactive state in the enzyme. The position of the proton in the –OH….N- tautomeric equilibrium significantly affects the physical, chemical, and biological properties of o-hydroxy Schiff bases. Most o-hydroxy Schiff bases (90%) exist in crystalline state in a neutral enol – imine form.[16] Presence of substituent in the aromatic ring or in the imine nitrogen changes the position of proton in – OHN- region. In solid 5-nitro-N-salicylideneethylamine the zwitter-ionic canonical structure $[O^-\cdots H-N^+]$ is favored as confirmed from single-crystal X-ray diffraction and ^{13}C magic-angle spinning NMR.[17] The nitro group present in the para position withdraw the negative charge density from the oxygen atom through resonance, thus assisting the proton transfer from the –OH group to the nitrogen atom of the imine group and stabilizing the ionic form.

Krygowski et al. have studied the relationship between π-electron localization/delocalization and H-bond strength in derivatives of o-Hydroxy-Schiff Bases.[18]

For this study they have taken o-hydroxy schiff bases where the phenyl ring is fused with substituted fulvene ring at different position of the phenyl ring (Figure 3). Depending on the position of fusion and also on the substituent attached at exocyclic carbon on the fulvene, π-electron delocalization is taking place as a result of which the bond characteristics of the C1-C2 bond changes. It has been shown that the character of the C1-C2 bond which is common to the phenylic ring and the quasi-ring formed as a result of H-bond formation has a crucial impact on the strength of H-bonding. The strongest H-bonds can be observed for the systems in which the C1-C2 is a double bond thus π-electronic coupling is possible in the quasi-ring for which increases the strength of the hydrogen bond. If the Fulvene is substituted by anion like O^- and NH^- groups, π-electron delocalization taking place over the perimeter and the C1-C2 bond is no more a double bond like thus reducing the hydrogen bond strength.
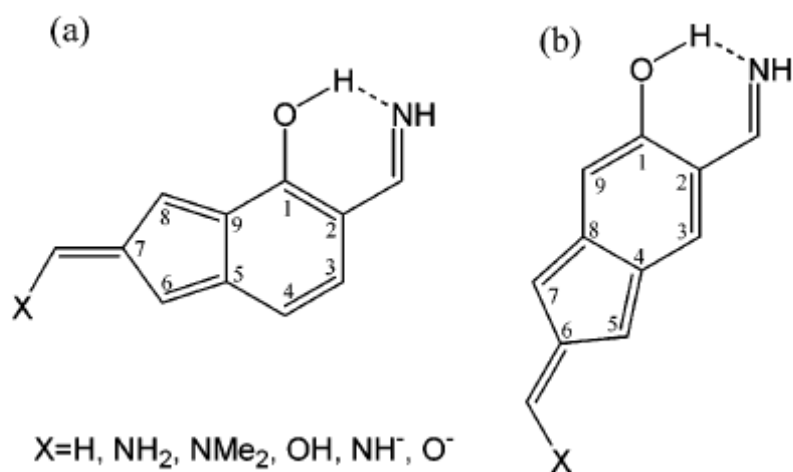

(Figure 3)

With a view to design compounds with large contrast of first hyperpolarizabilities for second order non-linear optical properties, Guillaume et al. have studied the thermodynamics and kinetics of the E / K equilibrium for compounds **2-3** using different computational studies.[19] The two tautomeric forms of **3** were found to coexist in protic

solvents like methanol as well as in aprotic solvents like acetonitrile. However for substituted N-salicylideneanilines, **2** the E form is the most stable. Substituting the salicylidene ring by an acceptor group or the other ring by a donor makes the E-form more stable. The largest β values are mainly associated with species bearing a donor in the para position of the salicylidene ring and an acceptor on the other ring whereas the largest β values are generally found for the E form.
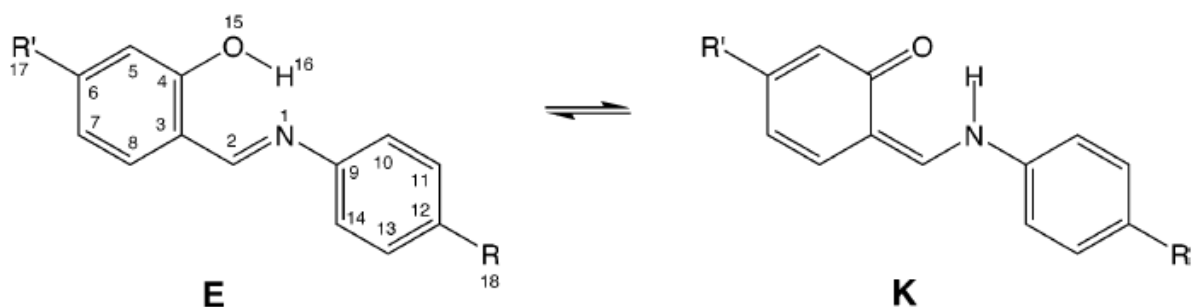
4,4'-substituted N-salicylideneanilines (**2**)
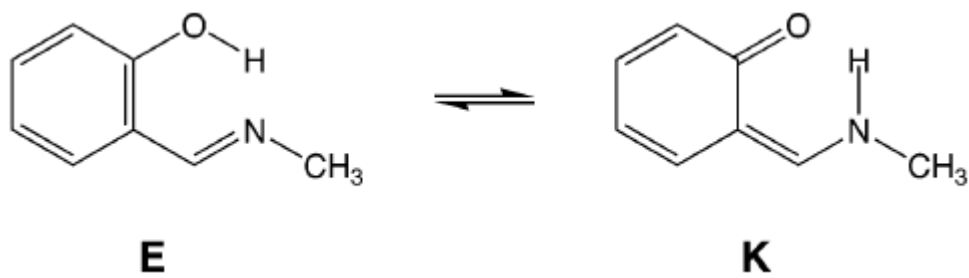
Salicylidenemethylamine (**3**)

The tautomeric equilibrium of the 1-hydroxy-2-naphthaldehyde Schiff base (2-phenyliminomethyl-naphthalen-1-ol), was investigated by density functional theory (B3LYP) and ab initio (MP2) methods in the IEF-PCM polarizable continuum dielectric solvent approximation giving special emphasis on the effect of solvation on this equilibrium by using an apolar (CCl4), polar aprotic (CH3CN), and polar protic (CH3OH) solvent. [20] The predominat tautomer was correactly predicted using these calculations.

Kiruba et al.[21] have investigated the tautomeric equilibria of a series of 3-hydroxypyridine derivatives including pyridoxal-5'-phosphate (PLP), using density functional calculations (B3LYP/6-311+G**//B3LYP/6-31G*) in the gas phase and in different solvents. They have employed three different approaches, namely continuum, discrete, and hybrid (combined discrete/SCRF) to investigate the effects of solvation on the tautomeric equilibria. In all cases, the neutral hydroxy form is found to be significantly more stable than the zwitterionic oxo form (by 43-56 kJ mol^{-1}) in the gas phase. The tautomeric energies reduce substantially in the presence of a polarizable dielectric medium. However, the neutral form is calculated to be the dominant form in nonpolar and aprotic polar solvents. On the other hand, an aqueous medium favors the zwitterionic form. This study highlights the role of both water molecules and bulk solvent effect in influencing the tautomeric equilibria of the PLP related compounds.

Fabian et al.[22] have investigated the enol imine-enaminone tautomerization in 1-hydroxy-2-naphthaldehyde Schiff bases (**4 a-e**) both experimentally(chemometric analysis of UVVis spectra) and computationally (density functional theory calculations (B3LYP/6-311+G**)). The tautomerization constants, K_T = [K form]/ [E form], and thermodynamic parameters, ΔH_T and ΔS_T, are determined by UV/vis spectroscopy. Polar solvents shift the equilibrium toward the quinone form.

	R
a	N(CH$_3$)$_2$
b	OCH$_3$
c	H
d	CN
e	NO$_2$

4 E-form   **4 K-form**
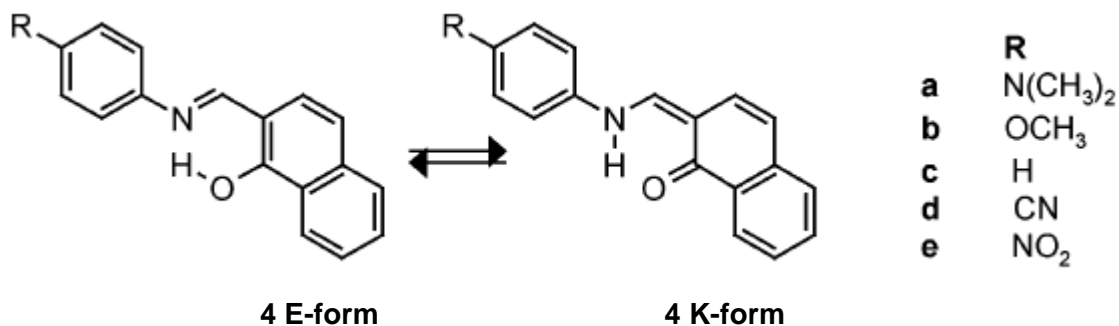
K_T values for all five derivatives were determined in apolar (cyclohexane), polar aprotic (acetonitrile), and polar protic (ethanol) solvents. A bathochromic shifts of the longest wavelength absorption band of both the tautomeric form is observed for both donor and acceptor substituents in the phenyl ring. The band positions of the UV/vis spectra are essentially independent of solvent polarity. The K_T values for **4 a-e** increases in the order cyclohexane < acetonitrile < ethanol, indicating preferential stabilization of the K-form in polar solvents. The UV/vis spectra of compound 4c with respect to change in solvent polarity is shown in Figure 4. Both donor and acceptor substituents in the para position of the N-aryl moiety decrease K^T. Polar solvents shift the equilibria toward the quinone tautomer. Ab initio (HF, MP2) and density functional theory (B3LYP) calculations are used to rationalize the experimental findings. Among the computational procedures, B3LYP calculations yield the best agreement with experimental ΔH_T values. Hartree-Fock and, especially, MP2 overestimate the stability of the phenol tautomers.
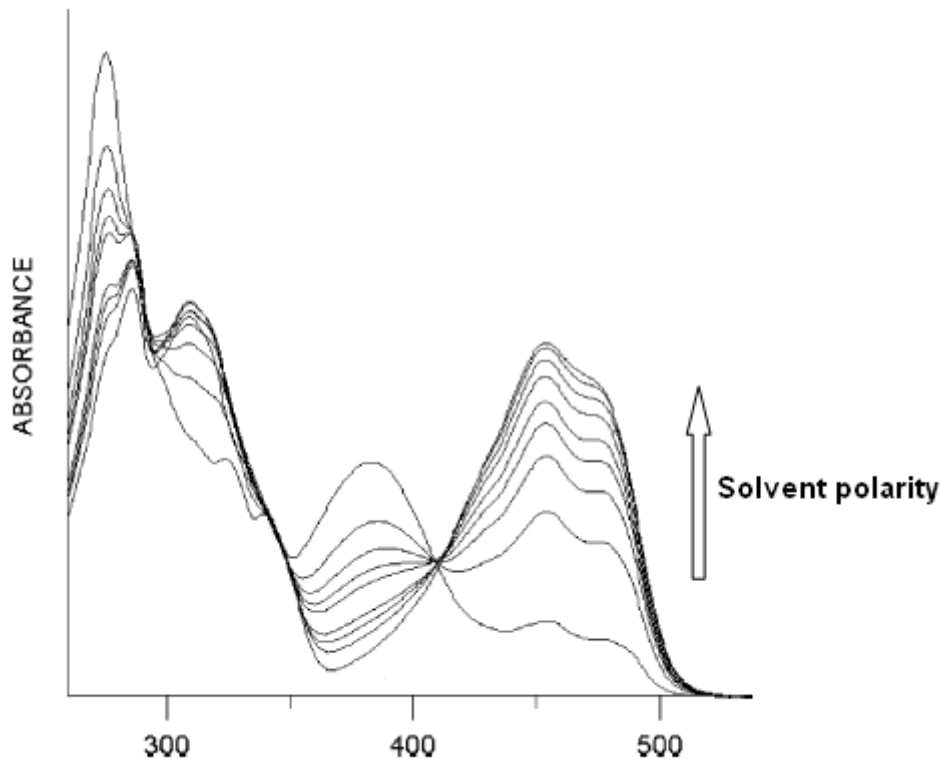
**Figure 4:** UV-Vis spectra of 4c as a function of solvent polarity

The NMR multinuclear studies of D-2-glucosamine Schiff bases (**5**) in DMSO solution and in the solid state show that the a anomeric form predominates for compounds with electron-withdrawing substituents in the aromatic ring. The results suggest that the relative stability of the anomeric forms of glucosamine in the solid state as well as position of the anomeric equilibrium in DMSO solution depend on the position of the proton in the intramolecular hydrogen bond and the electronic structure of the tautomeric NH-form of the Schiff base.[23]

Dziembowska et al.[24] have studied the structure of the Schiff base derived from 2-hydroxy-1-naphthaldehyde and ethylamine (6) by X-ray diffraction, B3LYP/6-31G(d,p) calculations, NMR and FTIR spectroscopy. From the crystal structure it is clear that, **6** forms dimer via two short intramolecular (2.592 and 2.579 Å) and two longer intermolecular (2.957 and 3.053 Å) N–H- - O hydrogen bonds. Tautomeric equilibrium due

to the intramolecular hydrogen transfer between enol and keto forms has been studied in

five solvents on the basis of ^{13}C NMR chemical shifts and equilibrium constants, K_{eq},

determined. The equilibrium constants, Keq, have been found to increase linearly with the

solvent parameter, E_T. Linear correlations have also been established between the

experimental ^{1}H and ^{13}C NMR chemical shifts (δexp) of keto and enol units in five solvents

and the calculated magnetic isotropic shielding tensors (σ_{calc}).

5

E   6   K

R =
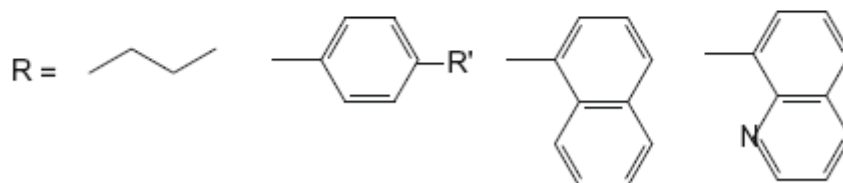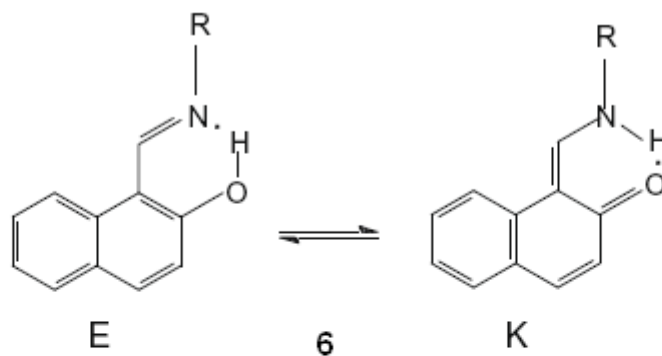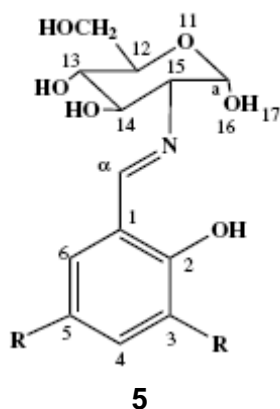
Schiff bases derived from 3-hydroxy-4-pyridinecarboxaldehyde and aniline/substituted aniline have been synthesized as per scheme 1 and characterized by NMR spectroscopy.[25] The ^{1}H, ^{13}C, ^{15}N and ^{17}O NMR data of these compounds are used to discuss the tautomerism. Compounds 7 exist as hydroxy/imino tautomers forming an intramolecular O–H---N hydrogen bond. The OH signals (both in ^{1}H and in ^{17}O NMR) and the CH=N signal (^{15}N NMR) proves the presence of hydroxy/imino tautomers.[25]
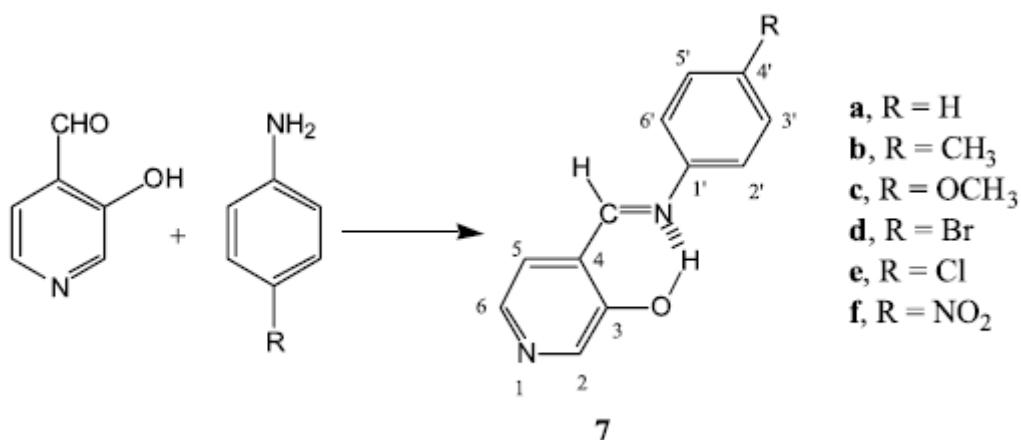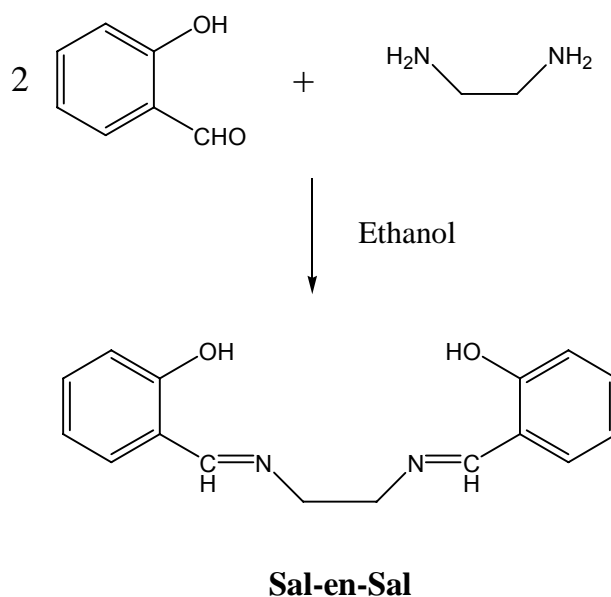

(Scheme 1)
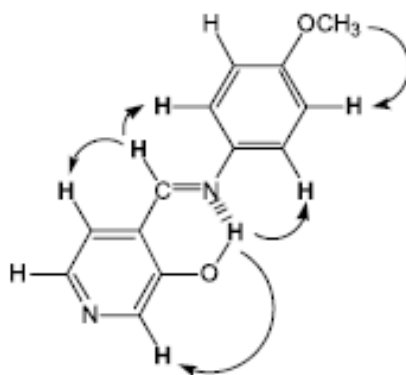
(Figure 5)

To prove the, hydroxy/imino structure with the E-configuration and the O–H - -N intramolecular hydrogen bond, NOESY spectra of **7c** have been recorded. The results obtained clearly establish this structure in solution (Figure 5).[25]

2. Experimental

2.1 Synthesis of bis (o- hydroxy salicylidine) ethylene diamine (sal-en- sal) schiff base

The Schiff base was obtained by refluxing salicylaldehyde (0.02 mol, 2.15 ml) with ethylene diamine (0.01 mole, 0.67 ml) in 25 ml absolute alcohol (Scheme 2). A Yellow solid was obtained which was crystallized twice from alcohol. Melting point = 135^0 C, yield = 90%. IR (cm^{-1}): 3435, 2910, 2840, 1630, 1595, 750; NMR (δ): 13.25 (s, 2H), 6.95(d, 4H), 7.29 (dt, 4H), 6.85 (dt, 4H), 7.25 (dd,4H), 8.24 (S, 2H), 4.0 (s, 4H).

Sal-en-Sal

(Scheme 2)

2.2 Purification of solvents

Dioxane: Dioxane was boiled under reflux for 7 hrs with 10% of its volume of 1N HCl. To remove water, the refluxed dioxane was treated with solid KOH, stirred and the aqueous layer was separated. It was then allowed to stand for one over sodium for 30 hrs and then distilled from sodium. B.P. 100^0 C-102^0 C.

CH$_3$CN: Acetonotrile was purified by distillation.

Aceotne: Acetone was distilled twice before use. B.P. 56^0C.

<u>Ethanol:</u> Ethanol was refluxed with fresh lime (CaO) for several hrs, then it was distilled at 70^0C.

<u>Methanol :</u> Methanol was refluxed with magnesium sulphate (MgSO$_4$) for several hrs, then it was distilled at 65^0C.

Throughout the work triple distilled water was used.

2.3 Spectral measurement

Absorption spectra of Schiff base in different binary aqueous- organic mixtures were recorded spectrophotometrically by using a Shimadzu model UV-VIS spectrophotometer. The binary mixtures were prepared by maintaining various percentage of organic solvents (2%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 90%, 100%) in the Schiff base solution. For the purpose, appropriate aquo- organic mixtures were prepared by mixing water and organic solvents and were left as such for 15 minutes. Stock solution of Schiff bases were prepared (([SB] = 2.5×10^{-3} M) in different organic solvents. From this stock solution 0.1 ml was added to 4.9 ml of water or aquo- organic solvents so that concentration of the Schiff base in binary mixture was always maintained at 5×10^{-5} M. Spectra of all the compounds in different solvent compositions were recorded within 30 seconds of addition of Schiff base to avoid hydrolysis.

3. Results and Discussion

The sal-en-sal schiff base is insoluble in water and aprotic solvents but soluble in dipolar aprotic solvents, like DMSO, DMF, dioxane, acetone, acetonitrile and protic solvents like methanol and ethanol. The solution of the compounds in organic solvents then go into solution in water till the aquo- organic solvent composition of 98:2 (v/v).

The Schiff base exhibits maximum absorption at 310-330nm in neat organic solvents ascribed to n-π* transitions. In protic solvents like ethanol and methanol a weak broad band also appears around 402nm (Figure 6). Increasing the amount of water in the binary mixture a peak appears around 402 nm which experiences hyperchromism. A simultaneous decrease in the intensity of 310-330 nm peak is also observed. Figure 7 represent these changes in spectral characteristics of the Schiff base by changing the solvent from pure non aqueous to aquo- organic solvent mixtures.
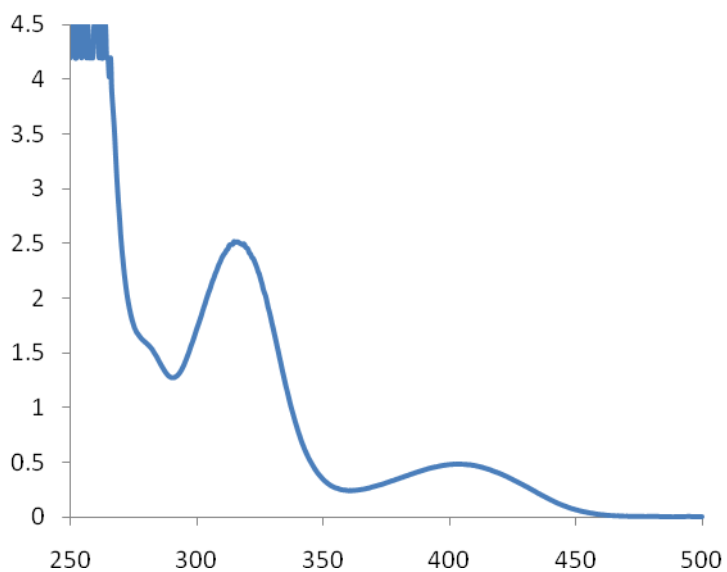

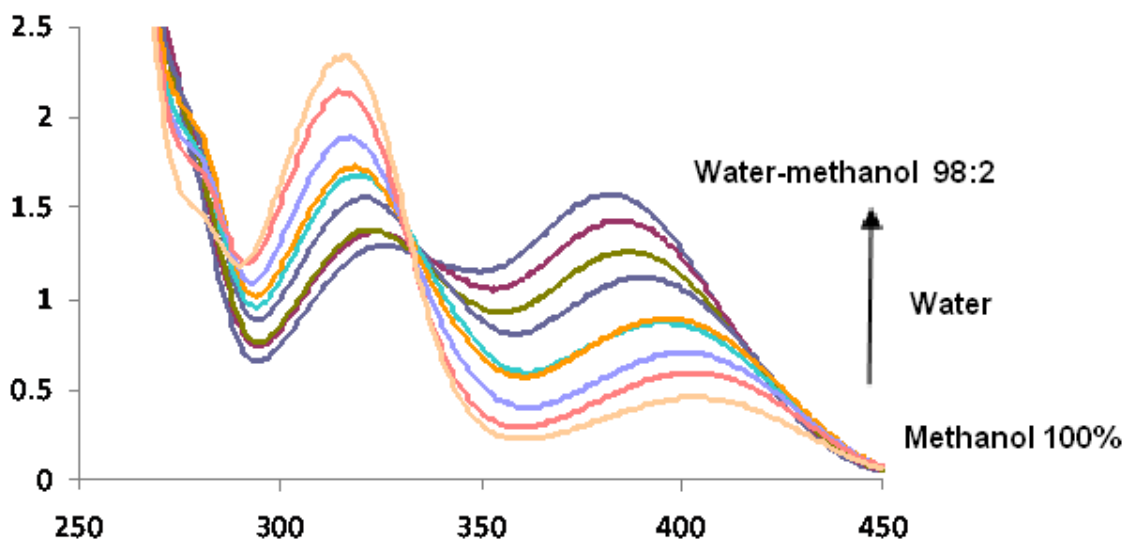**Figure 6** UV-Vis absorption spectrum of sal-en-sal in methanol

Figure 7: Spectral characteristics of sal-en-sal Schiff base in water-methanol solvent mixture as a function of solvent compositions.

The spectra of the Schiff base in various solvent systems show isosbestic points at 342 nm suggesting the existence of equilibrium between two species: one in organic solvent (S_O) and another in water (S_w). Since these species show distinct peaks, their concentrations have been derived from optical density values at respective λ_{max} for all the solvent systems. Two intersecting straight lines are obtained for the plots (Figure 8) with concentration of S_O (=C_O) and concentration of S_w (= C_w) vs volume percentage of organic component in the solvent mixture. The C_w values drop nearly to zero and that of C_O increases to C_T (Total concentration of the Schiff bases) at 100 percent by volume of organic solvent. Values of C_O and C_W, however do not touch zero or C_T respectively at 100% by volume of water. It is observed that the slopes of both the straight lines are same but the intercepts are different. The intercepts I_1 and I_2 refer to the expected concentration of S_w (=C_w) and S_0 (=C_0) in water. Since the expected concentration of S_w becomes zero in pure organic solvent, the value of C_0^0 is also expected to be zero in pure water. Instead the value becomes positive.
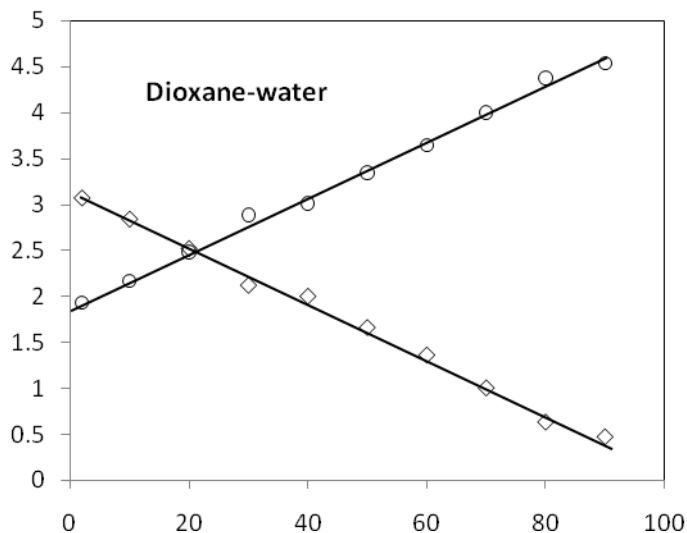
Figure 8 Plot of C_O (o), C_W (◊) vs. volume percent of dioxane in dioxane –water mixtures.

The point of intersection of the linear plots is exactly at $C_0 = C_w$. The values of percentage of organic solvent at the point of interaction i.e. when $C_0 = C_w$ are given in table 1 .

Table 1 Percentage of organic solvents where $C_o = C_w$

Solvent	Percentage of organic solvents
Dioxnae	20
Acetonitrile	10
Acetone	14
Ethanole	50
Methanol	58

The two peaks observed in the spectra of 2-hydroxy Schiff base in polar and non polar solvents are due to the possible existence of this compound in NH/OH tautomerism (scheme-3).

A weak band at 402 nm appears in alcoholic solvents which cannot be due to the presence of a small amount of water since the absorbance that case would correspond to the presence of 12% of water by volume. Hence, alcohols also assist tautomerism to some

extent. Further, the occurrence of isosbestic points at 342 nm in different solvent composition clearly indicate that the intramolecular proton transfer leading to NH/OH tautomerism is independent of nature of bulk solvent composition. It can, therefore, be said that the local environment of the molecule does not change with the bulk solvent composition. The nonzero C_0^0 value in 100% water indicates that the Schiff base exist in both the forms in water.

E-form

K-form
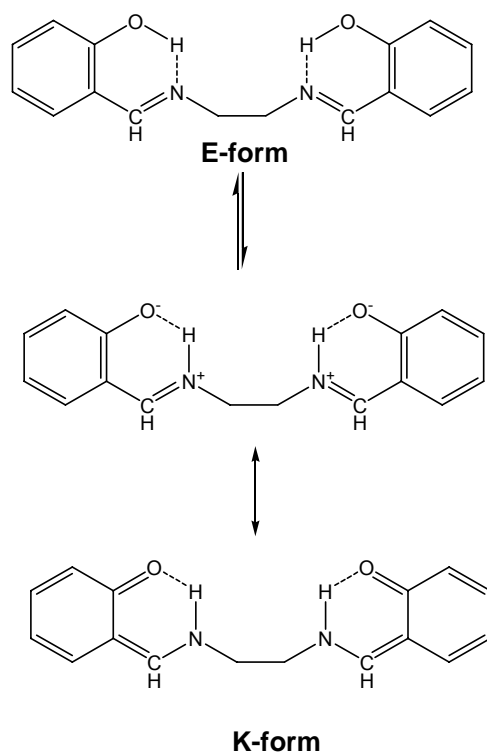
(Scheme 3)

The Schiff bases possesses both hydrophobic and hydrophilic region, the former being in excess of the later. A small amount of protic or dipolar aprotic organic solvent in the mixture shifts the solute systems from hydrophobic – excess type to hydrophilic-excess type for which the compounds go into solution in water. Changing the topology of the molecule such that in water, the hydrophilic surface is exposed whereas in non aqueous

solvent, the hydrophobic surface is exposed. As a result the oil-coated solute molecule goes into solution both in water and in non aqueous solvent.

In view of existence of an equilibrium between two species S_w and S_o, the values of C_o/C_w have been calculated for various solvent compositions as follows (Scheme 4) . The ratio is found to increase with increasing percentage of organic component in the mixture. The ratio is, therefore, plotted against percentage of organic solvent by volume in the solvent mixture (figure 9). Two linear plots are obtained in all cases.
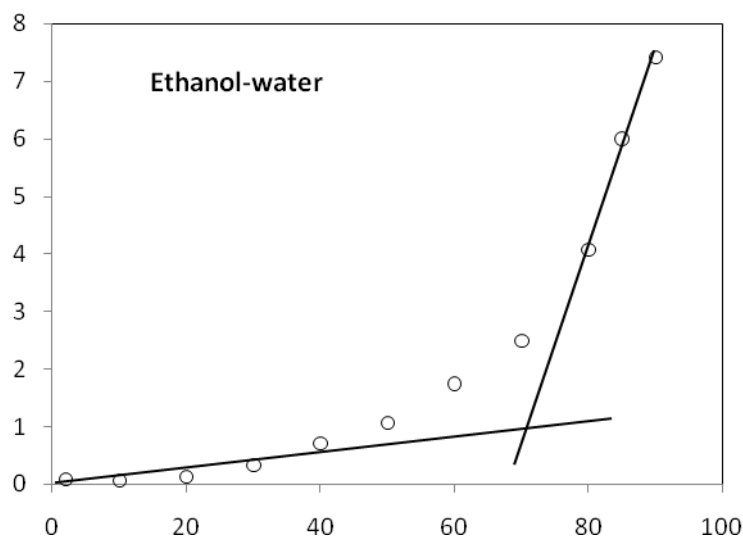
$$S_W + O \overset{K}{\rightleftharpoons} S_O$$
$$K = [S_0] / [S_w][O]$$
$$\text{or, } K[O] = [S_0] / [S_w] = C_0 / C_w.$$
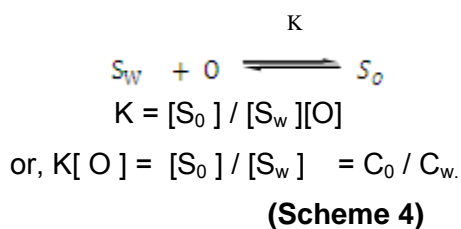
(Scheme 4)

Figure 9 Plot of Co/C$_W$ vs. volume percent of ethanol in ethanol- water mixture

The percent volume of orgnic solvents (= 70% v/v) at which the liner plots intersect, does not vary perceptibly with the nature of aprotic organic solvent. Therefore the biphasic

system has a transition at almost 70% by volume of organic component i.e. the non-aqueous solvent molecules remain within water clusters upto a volume percentage of almost 70, beyond which the water clusters remain in the oil framework.

Hence the bis o-OH Schiff base with a spacer are found to be highly water sensitive. More o-OH Schiff base units in a molecule with intra unit spacers may make the molecule more efficient to detect the presence of a very little amount of water in any environment.

4. Conclusions

Tautomeric equilibrium of bis-salicilidene schiff base with a spacer unit has been studied in aqueous-organic binary solvent mixture. Organic solvent favors the E-form while aqueous solvent favors both the E-form and K-form. The binary solvent mixture has a transition at almost 70% volume of organic solvent. i.e. the non-aqueous solvent molecules remain within water clusters upto a volume percentage of almost 70, beyond which the water clusters remain in the oil framework. The tautomeric equilibrium is very sensitive to water.

5. References

1. M. Guillaume, B. Champagne, N. Markova, V. Enchev, F. Castet, J. Phys. Chem. A 2007, 111, 9914.

2. P. I. Nagy, W. M. F. Fabian, J. Phys. Chem. B 2006, 110, 25026..

3. W. Rodrıguez-Cordoba, J. S. Zugazagoitia, E. Collado-Fregoso and J. Peon, J. Phys. Chem. A 2007, 111, 6241.

4. P. Gilli, V. Bertolasi, L Pretto, A. Lycka, G. Gilli, J. Am. Chem. Soc. 2002, 124, 13554.

5. J. Harada, T. Fujiwara, K. Ogawa, J. Am. Chem. Soc. 2007, 129, 16216.

6. J. Jagar, M. Moser, U. Sauder, J. N. Jansonius, J. Mol. Biol. 1994, 239, 285.

7. D. L. Smith, S. C. Almo, M. D. Toney, D. Ringe, Biochemistry 1989, 28, 8161.

8. J. P. Shaw, G. A. Petsko, D. Ringe, Biochemistry 1997, 36, 1329.

9. C. G. F. Stamper, A. A. Morollo, D. Ringe, Biochemistry 1998, 37, 10438.

10. P. Christen, D. E. Metzler, Transaminases, Wiley: New York, 1985.

11. S. Sharif, G. S. Denisov, M. D. Toney, H-H. Limbach, J. Am. Chem. Soc. 2006, 128, 3375.

12. M. Rospenk, I. Krol-Starzomska, A. Filarowski, A. Koll, Chem. Phys.2003, 287, 113.

13. Z. Rozwadowski, T. Dziembowska, Magn. Reson. Chem. 1999, 37, 274.

14. (a) J. C. Zhuo, Magn. Reson. Chem. 1999, 37, 259. (b) L. Kozerski, R. Kawecki, P. Krajewski, B. Kwiecien, D. W. Boykin, S. Bolvig, P. E. Hansen, Magn. Reson. Chem. 1998, 36, 921.

15. T. Dziembowska, Z. Rozwadowski, A. Filarowski, P. E. Hansen, Magn. Reson. Chem. 2001, 39, 67.

16. P. M. Dominiak, E. Grech, G. Barr, S. Teat, P. Mallinson, K. Woz´niak, Chem. Eur. J. 2003, 9, 963.

17. T. M. Krygowski, K. Woz´niak, R. Anulewicz, D. Pawlak, W. Kołodziejski, E. Grech, A. Szady, J. Phys. Chem. A 1997, 101, 9399.

18. T. M. Krygowski, J. E. Zachara-Horeglad, M. Palusiak, S. Pelloni, P. Lazzeretti, J. Org. Chem. 2008, 73, 2138.

19. M. Guillaume, B. Champagne, N. Markova, V. Enchev, F. Castet, J. Phys. Chem. A 2007, 111, 9914.

20. P. I. Nagy, W. M. F. Fabian, J. Phys. Chem. B 2006, 110, 25026.

21. G. S. M. Kiruba, M. W. Wong, J. Org. Chem. 2003, 68, 2874.

22. W. M. F. Fabian, L. Antonov, D. Nedeltcheva, F. S. Kamounah, P. J. Taylor, J. Phys. Chem. A 2004, 108, 7603.

23. B. Kołodziej, E. Grech, W. Schilf, B. Kamienski, M. Makowski, Z. Rozwadowski, T. Dziembowska, J. Mol. Struc. 2007, 844–845, 32.

24. T. Dziembowska, M. Szafran, A. Katrusiak, Z. Rozwadowski, J. Mol. Struc. 2009, 929, 32.

25. D. Sanz, A. Perona, R. M. Claramunt, Jose´ Elguero, Tetrahedron 2005, 61,145.