A Model Process for Measurement of Solvent Polarity

By **Juan Llor** and **Manuel Cortijo,*** Departamento de Quimica-Fisica, Facultad de Ciencias de la Universidad de Granada, y Departamento de Investigaciones Químicas (Centro Coordinado del C.S.I.C.), Granada, España

The tautomerization of a pyridoxal 5'-phosphate Schiff's base is shown to be an appropiate model process for the measure of solvent polarities. The corresponding polarity scale is compared with scales reported in the literature. This model can be used to calculate the polarities of sites of several proteins at which pyridoxal 5'-phosphate is bound.

THE formulation of many reaction mechanisms can be aided by data on the effect of solvents on the rates and products of the reactions.[1] In order to make use of such data we require some unambiguous way of defining what effect we expect for a given solvent. The dielectric constant is an important parameter of a solution; however, the properties of a solvent are due to a combination of a wide variety of interactions including charge–dipole, charge–charge, dipole–dipole, dipole–induced dipole, hydrogen bonding, *etc.* For this reason correlations between the dielectric constant of a given solvent with its properties are generally poor.

A different approach to studying solvent polarity involves measurement of solvent effects on a given phenomenon (a model process [1]). This gives us a parameter which we can take as a measure of solvent polarity in cases for which such a description is appropriate. Thus, a model process can be taken as a measure of a cybotactic region.

A suitable model process is that which converts an ion-pair or a dipolar ion (maximum interaction with solvent) into a neutral species (zero interaction with solvent) or *vice versa* [equation (1)]. Two types of such

$$D^+A^- \rightleftharpoons DA \qquad (1)$$

processes have been studied, affording the Y-scale of Grunwald and Winstein [2] and the Z-scale of Kosower.[3]

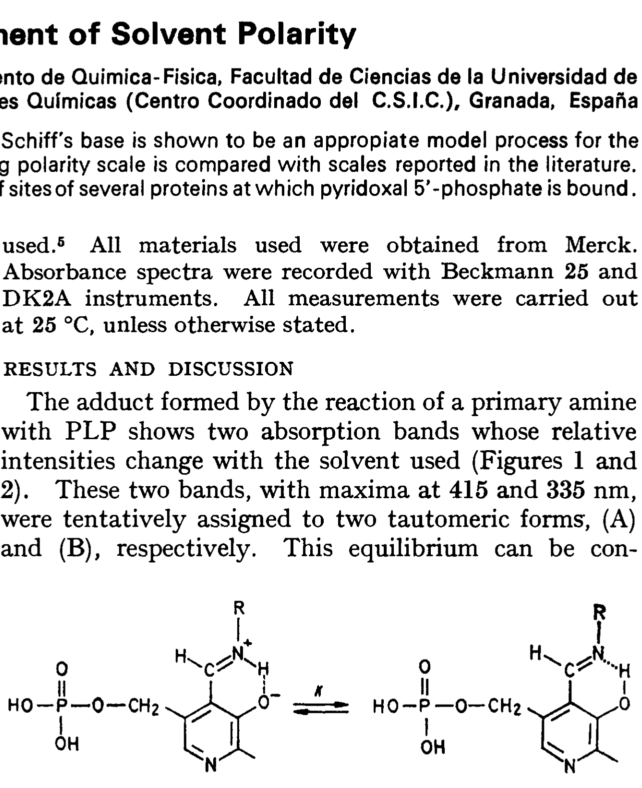
While we were investigating a model for the binding of pyridoxal 5'-phosphate (PLP) to proteins,[4] we discovered a suitable model process for studying solvent polarities.

EXPERIMENTAL

Solutions of PLP Schiff's bases were prepared with an excess of primary amine (100-fold). Blanks were studied before each experiment. PLP concentrations were measured by dilution of recently prepared solutions with 0.1M-sodium hydroxide and measuring their absorptions at 388 nm. A molar absorption coefficient of 6 600 was used.[5] All materials used were obtained from Merck. Absorbance spectra were recorded with Beckmann 25 and DK2A instruments. All measurements were carried out at 25 °C, unless otherwise stated.

RESULTS AND DISCUSSION

The adduct formed by the reaction of a primary amine with PLP shows two absorption bands whose relative intensities change with the solvent used (Figures 1 and 2). These two bands, with maxima at 415 and 335 nm, were tentatively assigned to two tautomeric forms, (A) and (B), respectively. This equilibrium can be considered to correspond to equation (1) and appears to be a good model process, and it was thought that its standard Gibbs free energy, $\Delta G^\circ = -RT\ln K$, could be a useful parameter for studying solvent polarities. A previous knowledge of the extinction coefficients of the forms (A) and (B) is necessary in order to evaluate equilibrium constants, and hence the standard Gibbs free energies.



(A) (B)

We have assumed that the extinction coefficients do not change with the solvent and that there are only two forms in equilibrium. These assumptions are confirmed by the presence of clear isosbestic points in Figure 1, except in solutions with water content higher than 90%.

Some Schiff bases of 3-hydroxypyridine-4-carbaldehyde show absorption bands at 400—425 and 265—280 nm which have been assigned to the π_1 and π_2 transitions of a tautomeric ' -enamine ' similar to form (A).[6] For these

---

[1] E. M. Kosower, ' Physical Organic Chemistry,' Wiley, London, 1968.

[2] E. Grunwald and S. Winstein, *J. Amer. Chem. Soc.*, 1948, **70**, 846.

[3] E. M. Kosower, *J. Amer. Chem. Soc.*, 1958, **80**, 3253.

[4] J. Llor, Ph.D. Thesis, University of Murcia, 1973.

[5] E. A. Peterson and H. A. Sober, *J. Amer. Chem. Soc.*, 1954, **76**, 169.

[6] D. Heinert and A. E. Martell, *J. Amer. Chem. Soc.*, 1963, **85**, 183.

compounds, assignments of the 316—330 nm absorptions to the π_1 transition and of the 238—256 nm absorptions to the π_2 transition of an ' enol ' form similar to (B) were made.[6] The equilibrium constant between these two forms changes with solvent polarity.[6] We conclude that the absorption coefficients of form (A) at 335 nm and of form (B) at 415 nm are zero.

Data for solutions with high water content appear to indicate (Figure 1) that this conclusion is unlikely. However the amount of form (B) in these solutions is not negligible and the presence of additional forms cannot be excluded.

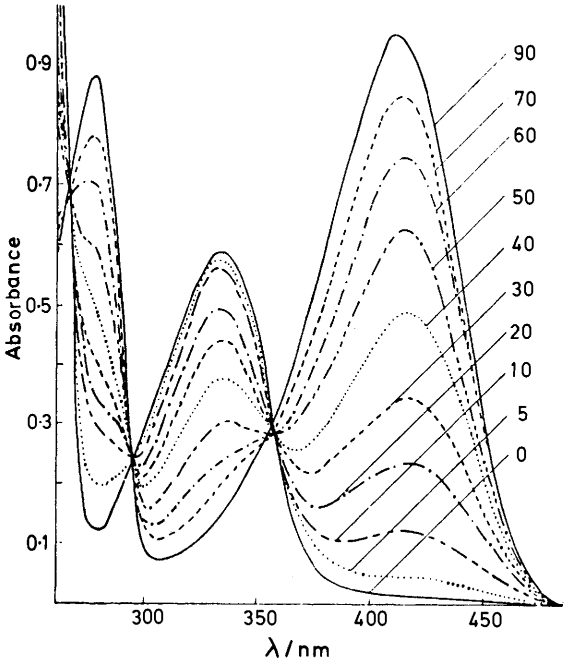


FIGURE 1 Absorption spectra of hexylamine–pyridoxal 5′-phosphate adduct in dioxan–water. PLP (1.5 mM) was treated with an excess of n-hexylamine (0.1M) in dioxan and diluted ten-fold into dioxan–water mixtures. The final solutions had the indicated percentage of water (v/v)

The total concentration, c, can be represented as in equation (2), where ε_A and ε_B are the extinction coefficients of the forms A and B, respectively, and A is the appropriate absorbance. From equation (3) a plot

$$c = c_A + c_B = A_{415}/\varepsilon_A + A_{335}/\varepsilon_B \qquad (2)$$

$$A_{415} = c\varepsilon_A - \varepsilon_A A_{335}/\varepsilon_B \qquad (3)$$

of A_{415} *vs.* A_{335} should give a straight line as long as the total concentration remains constant. There is good agreement with experimental points (Figure 3). A least-squares adjustment gives equation (4), from which

$$A_{415} = 1.282 - 2.11A_{335} \qquad (4)$$

we have obtained the values 8.4×10^3 and 4.0×10^3 for the extinction coefficients of forms (A) and (B), respectively. Additional corroboration is obtained from measurements in other solvent systems (see Figure 2).

We are now in a position to calculate the equilibrium constant for each solvent and its standard Gibbs free energy, ΔG°. If the above assumptions are correct a linear correlation between ΔG° and the corresponding Z values would be expected. An acceptable straight line is obtained (Figure 4). The least-squares adjustment gives equation (5). The scatter of the experimental points could be due to failure of any of the

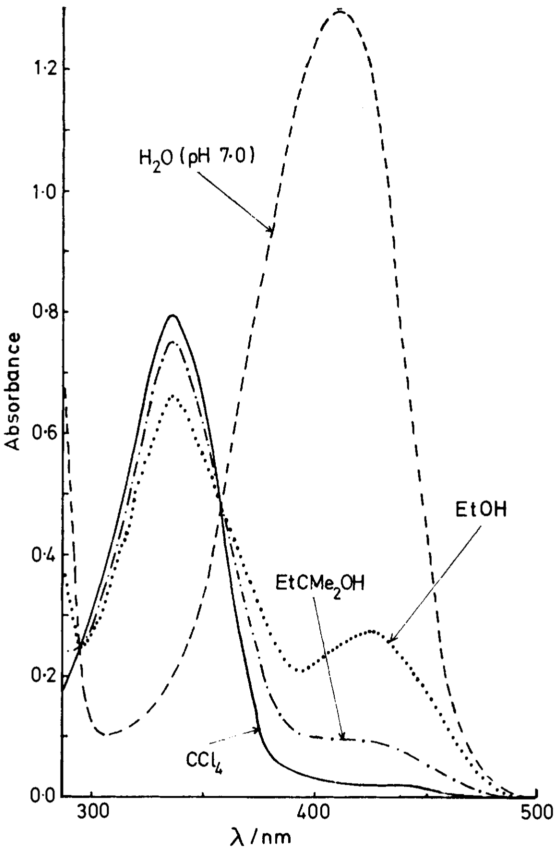


FIGURE 2 Absorption spectra of a pyridoxal 5′-phosphate Schiff's base in several solvents. PLP (2.0×10^{-4}M) was treated with an excess of amine (10^{-2}M) in the indicated media

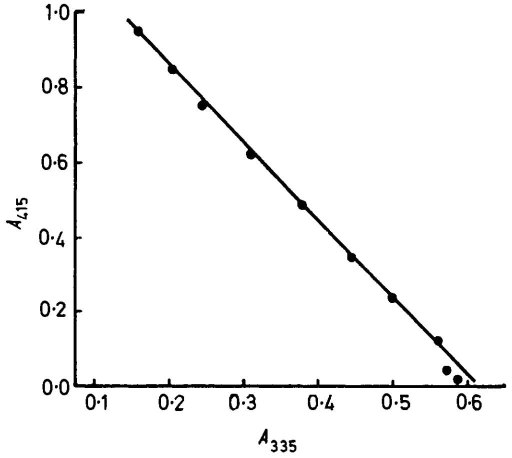


FIGURE 3 Relation between absorbances at 415 and at 335 nm of a PLP Schiff's base in dioxan–water mixtures. Experimental values were taken from Figure 1

previous assumptions. Moreover, we have not made ionic strength corrections and these could be important, especially with low polarity solvents. Therefore, the ΔG^0 values should be taken as apparent and not as

$$4.184Z = 367 + 38.0\Delta G^\circ \text{ (kJ mol}^{-1}\text{)} \qquad (5)$$

thermodynamic values. Equation (5) can be converted into equation (6) where $R = A_{335}/A_{415}$. We have

$$Z = 83.7 - 12.4 \log R \qquad (6)$$

calculated Z values for several commonly used solvents not evaluated by Kosower by use of this equation (see Table).

This new model process shows little sensitivity to slight modifications in molecular structure. This is a disadvantage that will prevent some further types of treatment. Nevertheless, this method shows the following advantages over previous ones: (i) it is only necessary to measure optical densities at two wavelengths in the near-u.v.; (ii) it is not necessary to know the total product concentration [see equation (6)]; (iii) the Schiff's base is formed very rapidly upon mixing the PLP with a large excess of a primary amine (*ca.* 100-fold) in the given solvent; the reason for using a large excess of amine is to preclude hydrolysis of the Schiff's base; and (iv) the type of primary amine is unimportant (as long as it does not contain a chromophore). We have not found significant differences between data obtained from ethylamine and from octadecylamine. In fact, it is convenient to use different types of amine in different solvents, to avoid solubility problems.

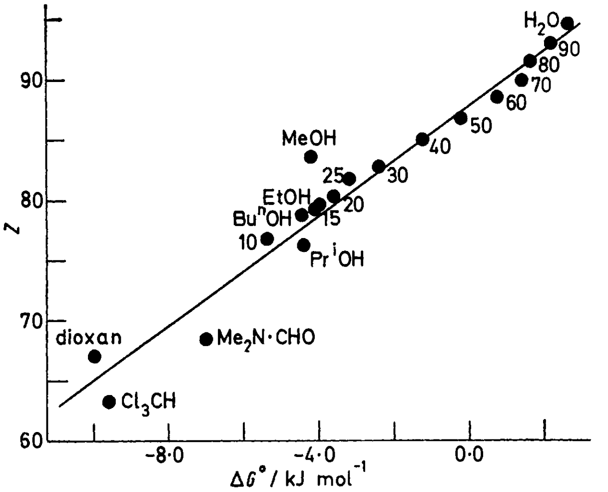


FIGURE 4 Relation between ΔG° and solvent polarity. The ΔG° values were calculated from absorption spectra as described in the text. The Z values were taken from reference 3; the numbers on the figure are the amounts of water (%, v/v) in dioxan–water mixtures

This model is especially useful with PLP enzymes, in which the PLP is bound through a Schiff's base linkage to the terminal amino-group of a lysine residue in the protein. For example, the R value for glycogen phosphorylase b is 13.2,[7] which corresponds to a Z value of 69.8. Reduction of the enzyme with borohydride opens its 'hydrophobic pocket' to give a Z value of *ca.* 81.[8] A similar situation was found in a 'deforming buffer.'[9] The spectral changes with pH observed with other enzymes, such as aspartate aminotransferase,[10] tryptophanase,[11] glutamate decarboxylase,[12] *etc.*, could be interpreted quantitatively in terms of this model process, on the assumption that Schiff's base formation is the mode of binding of PLP to these proteins.

Z Values

Solvent	Z Value (kJ mol^{-1}/4.184)
Carbon tetrachloride	64.7
Cyclohexane	65.3
t-Pentyl alcohol	72.5
Dimethylformamide–water (3.5)[a]	74.7
(5.0)	75.0
(10)	76.9
(15)	78.8
(20)	80.6
(25)	82.4
(30)	84.1
(40)	87.1
(50)	88.0
(60)	90.7
(70)	91.8
(75)	92.6
(80)	93.5
(90)	93.9

[a] Percentage of water (v/v).

This work was supported in part by the Fundacion J. March and the Fomento de la Investigacion en la Universidad.

[6/326 *Received*, 16*th February*, 1976]

---

[7] M. Cortijo, I. Z. Steinberg, and S. Shaltiel, *J. Biol. Chem.*, 1971, **246**, 933.

[8] M. Cortijo and S. Shaltiel, *European J. Biochem.*, 1972, **29**, 134.

[9] J. L. Hedrick, S. Shaltiel, and E. H. Fischer, *Biochemistry*, 1969, **8**, 2422.

[10] R. C. Hughes, W. T. Jenkins, and E. H. Fischer, *Proc. Nat. Acad. Sci. U.S.A.*, 1962, **48**, 1615.

[11] H. Kagamiyama, Y. Morino, and R. E. E. Snell, *J. Biol. Chem.*, 1970, **245**, 2819.

[12] R. Shukuya and G. W. Schwert, *J. Biol. Chem.*, 1960, **235**, 1653.