EFFECT OF SOLVENT ON UV AND VISIBLE SPECTRA OF FORMYLMETHYLFLAVIN AND PHOTOPRODUCTS

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ABSTRACT

The UV and visible absorption maxima and the molar absorptivities of formylmethylfavin and its photoproducts lumichrome and lumiflavin, have been determined in water (pH 7.0), acetonitrile, methanol, ethanol, 1-propanol, 1-butanol, dichloroethane and chloroform. The effect of solvent polarity on the position and intensity of the absorption bands of these compounds has been demonstrated. The variations in the π-π* transitions corresponding to the main absorption bands of flavins are discussed.

Introduction

Formylmethylflavin (1) is an intermediary product in the photodegradation of riboflavin (Smith and Metzler, 1963). It is highly photosensitive and in aqueous solutions yields lumichrome (2) and lumiflavin (3) (McBride and Metzler, 1967; Treadwell *et. al.,* 1968, Heelis *et al.,* 1980). The same products are obtained by alkaline hydrolysis of formylmethylflavin (Song *et al.*, 1965; Ahmad *et al.,* 1980). Recently lumichrome has been shown to be the only photoproduct of formylmethylflavin in organic solvents (Fasihullah, 1988).

The potolysis of formylmethylflavin in aqueous solution can be conveniently followed spectrophotometrically (McBride and Metzler, 1967; Heelis et at, 1980) and it is necessary to know the spectral characteristics of the species involved for kinetic analysis. In the present study we report the effect of solvent on the uv and visible spectra of formylmethylflavin, lumichrome and lumiflavin.

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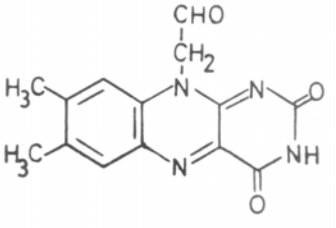

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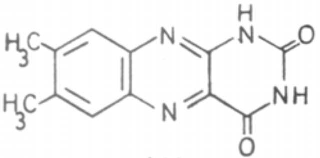

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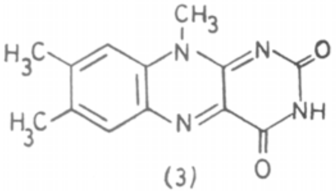

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Materials and Methods

Formylmethylfavin (FMF) was prepared according to the method of Fall and Petering (1956). Lumichrome (LC) and Lumiflavin (LF) were obtained from Sigma Chemical Co. and recrystalised from acetic acid. All solvents were analytical grade or of the purest form available from B.D.H./Merck, and were redistilled before use.

Spectral determinations and absorbance measurements were made on a Shimadzu UV-Visible recording spectrophotometer (Model UV-240) using silica cells of 10 mm path length. The base line was automatically corrected by the built-in memory at the initializing period.

Results and Discussion

The absorption maxima of FMF, LC and LF determined in water (pH 7.0), acetonitrile, methanol, ethanol, 1-propanol, 1-butanol, dichloroethane and chloroform are reported in Table-1. The electronic absorption spectrum of FMF in aqueous solution consists mainly of three bands occurring at 445, 373 and 266 am, and that of LC and LF at 356 and 262 nm and 444, 370 and 263 nm respectively. Al these absorption maxima possess high molar absorptivities (Table-1), indicative of π-π^* type transitions (Heelis, 1982). The precise position of the absorption maxima and the molar absorptivities depends on the environment of the flavin chromophore (Dudley *et. al,* 1964; Penzer and Radda, 1967; Visser and Muller, 1979; Koziolowa, 1979; Koziol *et al,* 1980) and the solvent polarity (Harbury *et. al,* 1959; Koziol and Knobloch, 1965; Koziol, 1966; Yagi *et. al.,* 1980; Eweg *et. al,* 1980). This is evident from the variations observed in the position of the observed in the absorption maxima of FMF, LC and LF in organic solvents of increasing polarity (Table-1).

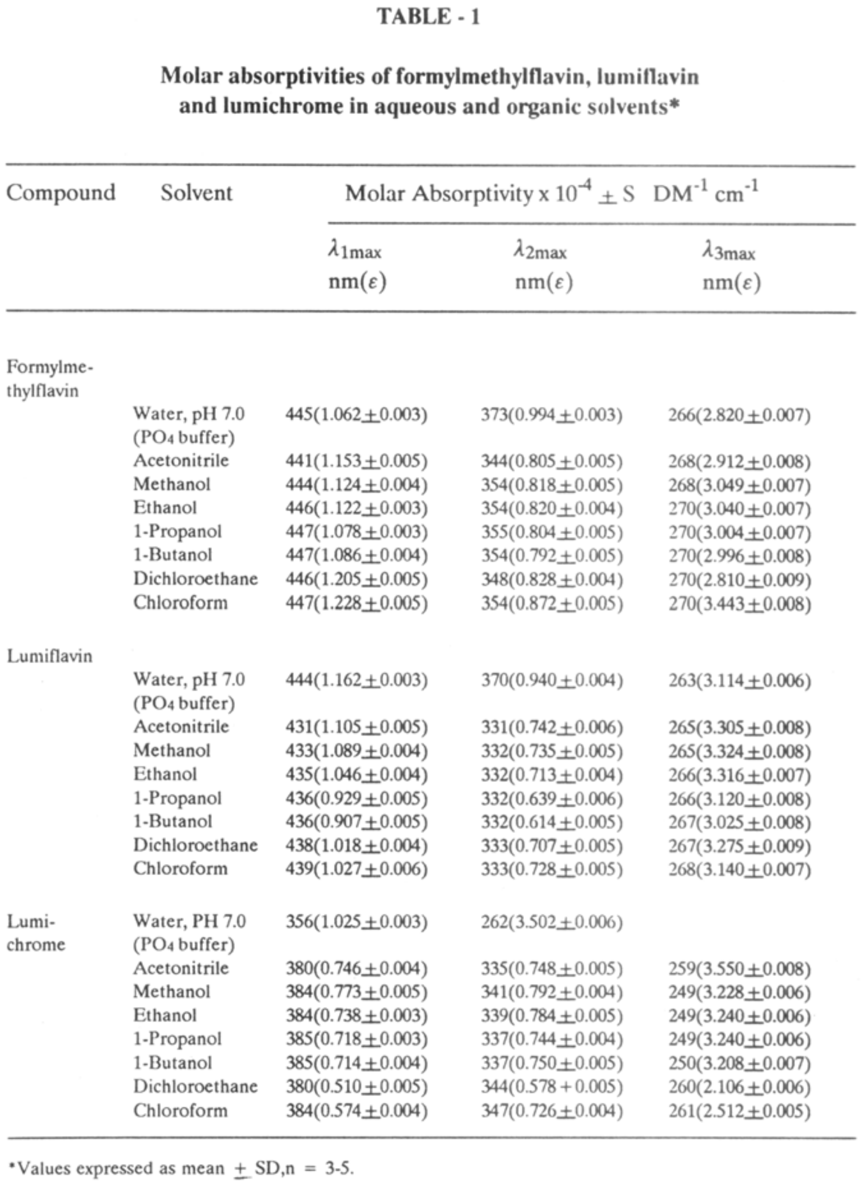

TABLE - 1

Molar absorptivities of formylmethylflavin, lumiflavin and lumichrome in aqueous and organic solvents*

Compound	Solvent	Molar Absorptivity x 10^{-4} ± S DM^{-1} cm^{-1}		
		λ_{1max} nm(ε)	λ_{2max} nm(ε)	λ_{3max} nm(ε)
Formylmethylflavin	Water, pH 7.0 (PO$_4$ buffer)	445(1.062±0.003)	373(0.994±0.003)	266(2.820±0.007)
	Acetonitrile	441(1.153±0.005)	344(0.805±0.005)	268(2.912±0.008)
	Methanol	444(1.124±0.004)	354(0.818±0.005)	268(3.049±0.007)
	Ethanol	446(1.122±0.003)	354(0.820±0.004)	270(3.040±0.007)
	1-Propanol	447(1.078±0.003)	355(0.804±0.005)	270(3.004±0.007)
	1-Butanol	447(1.086±0.004)	354(0.792±0.005)	270(2.996±0.008)
	Dichloroethane	446(1.205±0.005)	348(0.828±0.004)	270(2.810±0.009)
	Chloroform	447(1.228±0.005)	354(0.872±0.005)	270(3.443±0.008)
Lumiflavin	Water, pH 7.0 (PO$_4$ buffer)	444(1.162±0.003)	370(0.940±0.004)	263(3.114±0.006)
	Acetonitrile	431(1.105±0.005)	331(0.742±0.006)	265(3.305±0.008)
	Methanol	433(1.089±0.004)	332(0.735±0.005)	265(3.324±0.008)
	Ethanol	435(1.046±0.004)	332(0.713±0.004)	266(3.316±0.007)
	1-Propanol	436(0.929±0.005)	332(0.639±0.006)	266(3.120±0.008)
	1-Butanol	436(0.907±0.005)	332(0.614±0.005)	267(3.025±0.008)
	Dichloroethane	438(1.018±0.004)	333(0.707±0.005)	267(3.275±0.009)
	Chloroform	439(1.027±0.006)	333(0.728±0.005)	268(3.140±0.007)
Lumichrome	Water, PH 7.0 (PO$_4$ buffer)	356(1.025±0.003)	262(3.502±0.006)	
	Acetonitrile	380(0.746±0.004)	335(0.748±0.005)	259(3.550±0.008)
	Methanol	384(0.773±0.005)	341(0.792±0.004)	249(3.228±0.006)
	Ethanol	384(0.738±0.003)	339(0.784±0.005)	249(3.240±0.006)
	1-Propanol	385(0.718±0.003)	337(0.744±0.004)	249(3.240±0.006)
	1-Butanol	385(0.714±0.004)	337(0.750±0.005)	250(3.208±0.007)
	Dichloroethane	380(0.510±0.005)	344(0.578 + 0.005)	260(2.106±0.006)
	Chloroform	384(0.574±0.004)	347(0.726±0.004)	261(2.512±0.005)

*Values expressed as mean ± SD,n = 3-5.

The sensitivity of flavin spectra to solvent changes is probably due to a greater degree of solute-solvent interaction on increasing the solvent polarity. The band position may be affected by relative stabilisation of the ground and excited states by salvation. The increase in absorption maximum with an increase in the polarity of the solvent implies that the energy necessary for the π-π^* transition is less in the more polar solvent due to stabilisation of the excited state by interaction with the solvent. The electronic displacement during excitation results in an excited state which approaches the polar structure C^+-C=C-C due to intramolecular charge transfer. This is in contrast to the n-π^* carbonyl transition which undergoes a small hypsochromic shift on increasing the polarity of the solvent, since interaction with the solvent stabilizes the lone pair electrons In the ground state (Bladon, 1964; Jaffe and Urchin, 1962)

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Grabe (1972, 1974), on the basis of quantum mechanical calculations, predicted the lowest energy transition of flavins to be close to 450 nm, which is in good agreement with the positions observed for FMF (445 nm) and LF (444 nm) in aqueous solution. This band exhibits minute or no change in positin upon moving from water to less polar solvents, as expected for this π-π^* assignment

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The near UV band at 373 nm probably consists of a π-π^* transition, though quantum mechanical calculations (Eweg *et al,* 1980) suggest that some mixing of an n-π^* transition involving the N-1 non-bonding electron pair with the π-π^* transition may occur. Thus this band may have some contribution from the intramolecular charge-transfer leading to a polar excited state and thereby accounting for the solvent dependence of its position (Heelis, 1982). The bathochromic shift obseved for FMF (354 nm to 373 nm) and LF (333 um to 370 um) on moving to the protic solvents could result from the destabilisation of the N-1 non-bonding electrons by hydrogen bonding (Yagi *et al.,* 1980; Eweg *et al* 1980). A relatively greater decrease in hydrogen bonding in the excited state would lead to a relatively greater increase in the energy level of the excited state compared with that of the ground state. This is possible on account of the higher polarity of the excited state of the solute molecule as in the case of isoalloxazines (i.e. FMF and LF). Thus decrease in the polarity of the solvent is associated with a hypsochromic shift (Ahmad, 1968). LC, being an alloxazinc derivative, does not show this behaviour on changing the solvent polarity. The higher energy π-π^* transitions of FMF (266 nm), LF (263 nm) and LC (262 nm) are in accordance with the theoretical predictions of the overall maximum of flavins at 265 nm (Sun *et. al.,* 1972). Variations in the UV and visible absorption characteristics of flavins due to changes in pH and solvent polarity have been utilized to develop specific analytical methods for photodegradation studies of FMF in different solvent media (Heelis *et. al.,* 1980; Ahmad, 1985; Fasihullah, 1988).

References

Ahmad, I. (1968). Ph.D. Thesis, University of London, London.

Ahmad, I. Rapson. H.D.C., Heelis, P.F. and Phillips, G.O. (1980). *J. Org. Chem.* **45**: 731.

Ahmed, I. (1985). In "Proceedings of International Seminar on Policies, Management and Quality Assurance of Pyharmaeeuticals". Ahmad, T. and Khalid, R., Eds., Quality Control Authority, Islamabad, pp.256-270.

Bladon, P. (1964). In "Physical Methods in Organic Chemistry'. Schwarz J.C.Y., Ed., Oliver and Boyd, Edinburgh, pp.126-167.

Dudley, K.H., Ehrenberg, A., Hemmerich, P. and Muller, F. (1904). *Helv. Chim Acta*, **47**: 1354.

Eweg, J.K., Muller, F., van Dam, H., Terpstra, A. and Oskam, A. (1980). *J. Am. Chem. Soc.* **102**: 51.

Fall, H.H. and Petering, H.G. (1956). *J. Am. Chcm. Soc*. **78**: 377.

Fasihullah, Q. (1988). Ph.D. Thesis, University of Karachi.

Grabs, B. (1972). *Acta Chem. Scand., Ser. A*, **26**: 4084.

Grabe, B. (1974). *Acta Chem. Scand., Ser. A*, **28**: 363.

Harbury, H.A., Lallouse, K.F., Loach, P.A. and Amick. R.M. (1959). *Proc. Natl. Acad. Sci. USA,* **45**: 1708.

Heelis, P.F., Philips, (G.O Ahmad, I. and rapson, H.D.C. (1980). *Photobiochem. Photobiophys*. **I**: 125.

Heelis, P.F. (1982). *Chem. Soc. Rev.* **11**: 15.

Jaffe, H.H. and Orchin M. (1962). Theory and Applications of Ultraviolet Spectroscopy, Koziol John Wiley, New York, p.186.

Koziol, J. and Knoblock, E. (1965). *Biochem Biophys. Acta* **102**: 289.

Koziol, J. (1966). *Photochem. Photobiol*. **5**: 41.

Koziol, J., Kozolowa, A., Konarski, .1., Panek- Jane. D. and Dawidowski, J. (1980). In "Flavins and Flavoproteins", Yagi, K. and Yamano, T., Eds., Japan Scientific Societies Press. Tokyo, p.475.

Koziolowa, A. (1979). *Photochem. Photobiol*. **29**: 459.

McBride, M. and Metzler, D.E. (1967). *Photochem. Photobiol*. **6**: 133.

Panzer, G.R. and Radda, G.K. (1967). *Quart. Rev*. **21**: 43.

Smith, E.C. and Metzler, D.E. (1963). *J. Am. Chem. Soc*. **85**: 3285.

Song, P.S., Smith, E.C. and Metzler, D.E. (1965). *J. Am. Chem. Soc.* **87**: 4181.

Sun, N.I., Moore, T.A. and Song, P.S. (1972). *J. Am. Chem. Soc.,* **94**: 1730.

Treadwell, G.E., Cairns, W.L. and Metzler, D.E. (1968). *J. Chromatogr*. **35**: 376.

Visser, A.J.W.G. and Muller, F. (1979). *Helv. Chim. Acta,* **62**: 593.

Yagi, K., Ohishi, N, Nishimoto, K, Choi, J.D. and Song, P.S. (1980). *Biochemistry* **19**: 1553.