

CHAPTER – 4
PHOTOPHYSICAL PROPERTIES OF INDICATOR
MOLECULES IN DIFFERENT DIELECTRIC MEDIA

4.1. Introduction and review of previous works

Solvents possess polar or apolar characteristics. These properties control the reactivity as well as physicochemical characteristics of a process at the molecular level. As a result, solvents play a significant role in chemical and biological processes. One common use of solvent effect is to determine the polarity of the probe-binding site on the macromolecule. This is accomplished by comparison of the emission spectra of the fluorophore when it is bound to the macromolecule and dissolved in solvents of different polarities. However, there are many additional instances where solvent effects are important. When a fluorescent ligand binds to a protein or a fluorophore binds to membranes, the binding is usually accompanied by a spectral shift due to the different environment for the bound ligand or the fluorophore. Compartmentalized water in cells and reverse micelles, microemulsions, liposomes and gels as well as the aqueous environment at the interfaces of normal micelles are known to be physicochemically different from the bulk water¹⁻⁸ with regard to polarity, structure and pH. Reactions performed therein may significantly deviate from the normal courses and are often catalyzed in compartmentalized environments.⁹⁻¹³ It is thus necessary to ascertain the polarity/apolarity of the solvents which are often estimated from the knowledge of their dielectric constant, Kosower-Z value, the charge transfer transition energy, the kinetically derived parameters of Y-man, the Y-value, etc.¹⁴

Overview of solvent effect

When the fluorophores get excited, emission from the fluorophores generally occurs at longer wavelengths as compared to the absorption. This loss of energy is due to a variety of dynamic processes that occur following light absorption (fig.1). The fluorophore is typically excited to the first singlet state (S_1).

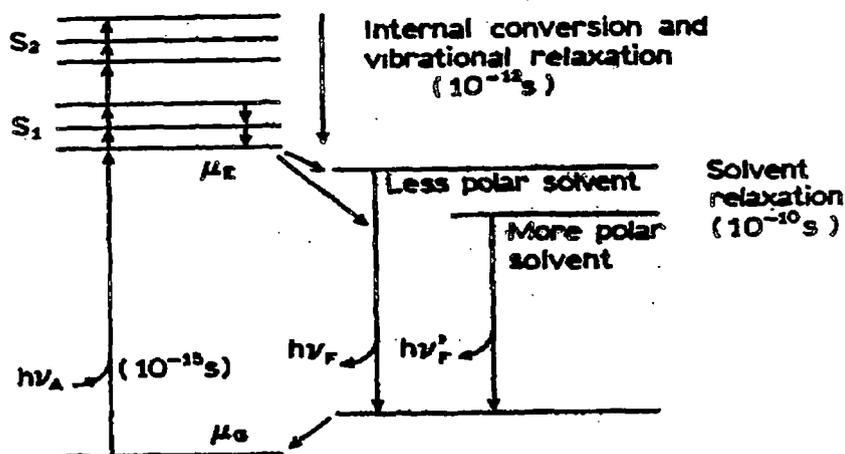


Fig.1. Jablonski diagram for fluorescence with solvent relaxation

The excess vibrational energy is rapidly lost to the solvent. If the fluorophore is excited to the second state (S_2), it rapidly lost to the S_1 state in 10^{-12} s due to the internal conversion. Solvent effect shifts the emission to still lower energy owing to the stabilization of the excited state by the polar solvent molecules. Typically, a fluorophore has a larger dipole moment in the excited state (μ_E) than the ground state (μ_G). Following excitation, the solvent dipoles can reorient or relax to around μ_E , which lowers the energy of the excited state. As the polarity of the solvent is increased, the effect becomes larger, resulting in the emission at lower energies or longer wavelengths. In general, nonpolar molecules are much less sensitive than polar molecules towards the solvent polarity.

Fluorescence life time (1-10 ns) is generally longer than the time required for solvent relaxation (10-100 ns). So, observing the emission spectra, one can understand the solvent relaxation state. Fig 1 shows why the absorption spectra are less sensitive to the solvent polarity than the emission spectra.

Mechanism of the spectral shifts:

It is well known that due to the general solvent effects, fluorescence spectral shifts occur. But this theory is often insufficient because there are some other reasons, one of which is that there is a variety of environment, which changes the nature of the fluorophores. Fluorophores often undergoes specific interactions with the local environment causing a spectral shift by amounts comparable to the general solvent effects. As for instance, when small amount of ethanol is added to indole¹⁵ in a nonpolar solvent cyclohexane, causes a spectral shift more than that caused due to general solvent effects. This is specific because the amount of ethanol added is too small to change the polarity of the solvent. Here, the specificity is due to the hydrogen bonding to the imino group of indole ring.

Besides this specific interaction, another type of interaction has been reported which occurs due to the internal charge-transfer state or a twisted charge-transfer state.¹⁶ This happens in case of fluorophores containing both an electron donating and electron-accepting group. When this type of fluorophores gets excited, there is an increase in charge-separation within it. In polar solvents, the charge-transferred state becomes the lowest energy state, following Kashes' rule¹⁷ which states that emission occurs from the lowest energy excited state. In nonpolar solvents, no charge-transfer takes place, and the species got locally excited which becomes the lowest energy excited state. In some cases, the internal charge transfer state requires rotation of groups on fluorophore to form twisted charge transfer state. Thus, polarity of the solvent sometimes determines which will be the lowest energy excited state due to the charge transfer.

Therefore, there are mainly two types of solvent effects. One is the general solvent effect that is caused due to the interaction of the dipole of the fluorophore with its environment and the other is the specific solvent effect that again may be of two types. One is caused due to fluorophore-solvent

interaction and the other due to the charge transfer or twisted charge transfer state formation depending on the probe structure and the solvent.

General solvent effect – Lippert equation¹⁹

Though there are various types of solvent effects, the general solvent effect is most important. This effect provides many information for solvent dependent spectral shifts. While considering this theory, other effects can be detected as deviation from this theory. According to this general solvent effect theory, the fluorophore is assumed to be a dipole in a continuous medium of uniform dielectric constant (fig.2).

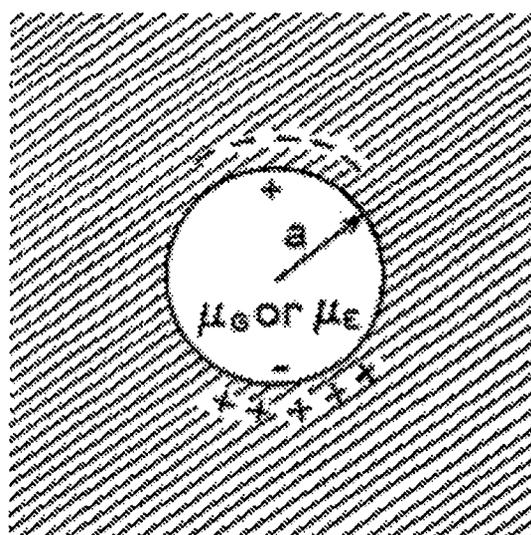


Fig. 2. Dipole in a dielectric medium

The dielectric constant (D) and the refractive index (n) of a solvent affect the emission and absorption.^{18,19} The interactions between the solvent and fluorophore affects the energy difference between the ground state and the excited state. Lippert¹⁹ related this energy difference (in cm^{-1}) to the solvent properties like dipole moment (μ), dielectric constant (D) and refractive index (n) by the equation:

$$\tilde{\nu}_A - \tilde{\nu}_F = \frac{2}{hc} \left(\frac{D-1}{2D+1} - \frac{n^2-1}{2n^2+1} \right) \frac{(\mu_E - \mu_G)^2}{a^3} + \text{const.} \quad (1)$$

In the above equation $\tilde{\nu}_A$ and $\tilde{\nu}_F$ are the wave numbers of absorption and emission respectively, h is the Plank's constant, c is the speed of light, μ_G and μ_E are the dipole moments of the solvent in the ground state and excited state respectively and a is the radius of the cavity in which the fluorophore resides. The eqn. (1) is useful to correlate the observed and calculated energy losses in non-protic solvents. The protic and non-protic solvents are those having hydroxyl groups or other groups that are capable of hydrogen bonding or not.

The refractive index and the dielectric constants have opposite effects on the Stokes' shift. The dielectric constant (D) is a static property, which depends on both electronic and molecular motions. By molecular motions, solvent reorientation around the excited state occurs whereas the refractive index (n) depends on motion of electrons within the solvent molecules. An increase in refractive index decreases the energy losses in the absorption band whereas an increase in dielectric constant results in an increase in Stokes' shift.

The term in the above equation, i.e., $[(D-1)/(2D+1) - (n^2-1)/(2n^2+1)]$ is called the solvent polarity function or the orientation polarizability (Δf). The first term i.e., $(D-1)/(2D+1)$ accounts for the spectral shifts due to the reorientation of the solvent dipoles as well as the redistribution of the electrons in the solvent molecules. The second term, $(n^2-1)/(2n^2+1)$ accounts only for the re-distribution of electrons. The difference between the two terms is called the orientation polarizability or the solvent polarity function (Δf) as it accounts for the spectral shifts due to the reorientation of the solvent molecules. According to this theory, only the solvent reorientation is expected to result in substantial Stokes' shifts.

Hexane, being a nonpolar solvent, does not possess dipole moment. So, there is no question of dipole reorientation around the excited state of the

fluorophore. From the Lippert equation one can calculate the value of Δf , i.e., the orientation polarizability whereas $(\bar{\nu}_A - \bar{\nu}_F)$ is expected to be very small or zero. But in case of non-polar solvents both of their bands do not coincide. Most of the excitation occurs usually at higher vibrational energy level and this energy rapidly (10^{-12} s) got dissipated in the solvents. As the emission occurs to an excited vibrational energy level of the ground state, the absorption and emission bands are shifted by an amount that is at equal to the vibrational energy. These energy losses are accounted for by the constant term of Lippert equation.

When the above experiment is performed in a polar solvent like methanol, substantially larger Stokes' losses are expected. This is due to the large orientation polarizability of methanol for its high dipole moment. This sensitivity of Stokes' shift to the solvent polarity is used to measure the polarity of the environment surrounding the fluorophore by means of fluorescence measurements.

Application of the Lippert equation – the general solvent effect

As the polarity of the solvent affects the emission spectral band, so the emission spectra of the fluorophores are often used to label the macromolecules that are sensitive towards the solvent polarity. Various types of macromolecules of this type are known which show very low fluorescence in water but become highly fluorescent in nonpolar solvents or when bound to proteins and membranes. These probes are highly sensitive towards the solvent polarity and can potentially reveal the polarity of their immediate environments.²⁰

In order to estimate the solvent sensitivity of a fluorophore, Lippert plot is useful. This is a plot of $(\bar{\nu}_A - \bar{\nu}_F)$ versus Δf , i.e., the orientation polarizability. The sensitive fluorophores are chosen from those that undergo largest changes

in dipole moments when excited. It is reported that N-phenyl-N-methyl derivative of 6-amino-naphthalene-2-sulphonic acid is more sensitive than unsubstituted amino derivative of naphthalene sulphonic acid.²¹ In the case when general solvent effect is operative; a linear Lippert plot is obtained. On the other hand, in the case of specific solvent effect, a non-linear Lippert plot is obtained. Thus the solvent effect on fluorescence emission not only depends on dielectric constant and refractive index of the solvents (the general solvent effect) but also on the specific interaction between the solvent molecules and the fluorophores.

Specific solvent effect:

In contrast to the general solvent interactions with the fluorophores, specific interactions are produced by one or more neighboring molecules of the solvent and are determined by the specific chemical properties of both of solvent and the fluorophore.^{22,23} Specific solvent interactions may refer to some factors as mentioned earlier, viz., hydrogen bonding, acid-base reaction or charge transfer interactions. Specific interactions between the solvent and the fluorophore are identified by observing the fluorescence emission spectra of a fluorophore in a variety of solvents. One important example reported is for 2-anilidonaphthalene in hexane²⁴ where addition of low concentration of ethanol (i.e., 2-3%) can change the solvent molecular properties and the change in the spectral shifts which is about 50% as compared to the change when 100% alcohol is added and a full spectral shift is observed. Above change in spectral feature is an important example of specific solvent interaction. The specific solvent effect occurs due to the addition of trace amount of ethanol via hydrogen bond formation between ethanol and the fluorophore.

The specific solvent-fluorophore interaction may occur either in ground state or in the excited state. In the case of interaction occurred in the excited state, the polar additive does not affect the absorption spectra, but if it is

occurred in the ground state, there is some change in the absorption spectra as expected.

Specific solvent effects and Lippert plot:

Like general solvent effect, evidence for the specific solvent-fluorophore interaction can also be seen in the Lippert Plot. In the Lippert Plot of the system, specific solvent-fluorophore interaction occur, the Stokes' shift is generally larger in the event of hydrogen bonding solvents (like water, methanol, ethanol etc.) than in solvents which less readily forms hydrogen bonds.^{25,26} Such behaviour is typical for specific solvent-fluorophore interactions. The excess spectral shifts were explained by hydrogen bonding between the polar solvent and the polar group of the fluorophore.²⁷ The emission spectra of the fluorophores depend on orientation polarizability (the solvent polarity function) of the solvent as well as on the detailed chemical structures of the fluorophores and the solvents.²⁸ In these cases, the Stokes' shifts are approximately proportional to the orientation polarizability of the solvents but larger shifts are found in case of those solvents where hydrogen bond formation is possible. The excess spectral shift is also reported for the ester fluorophores.²⁹ This is due to the localization of a negative charge on the oxygen atom of the ester group and strong hydrogen bond forming ability of this group to the protic solvent.

In the present chapter, studies have been undertaken to understand the behaviour of some organic molecules in water and in a number of selected alkanols (solvent) with varying dielectric constant, refractive index, Kosower-Z values etc. The solutes are, 5-Hydroxyindole, 5-Hydroxy-L-Tryptophan, L-Tyrosine, L-Tyrosinemethylester, 1-Naphthol, 2-Naphthol. The solvents used, are water, Methanol, Ethanol, *iso*-Propyl alcohol, *tert*-Butyl alcohol, Pentanol, Hexanol, Heptanol and Octanol. 1,4 Dioxane-water mixture with varying compositions was used as solvent-water mixture. We have measured the

emission and absorption spectra for the different solutes in water, alkanols and dioxane-water mixtures. The Stokes' shifts calculated from the absorption and emission spectra for all the above solvents have been analyzed in a convincing way to correlate all the studied solvent systems. In this correlation attempt, the roles of Kosower-Z values and the dielectric constant (D) values have also been considered.

4.2. Experimental

The solute molecules, viz., 5-Hydroxyindole, 5-Hydroxy-L-tryptophan, L-Tyrosinemethylester were all Fluka graded and were used as received from Sigma-Aldrich Chemical Co., U.S.A. As mentioned in chapter-3, L-Tyrosine was obtained from Himedia, India and was used without any further purification. 1-Naphthol, 2-Naphthol were supplied by E. Merck, India. These were used after purification twice by vacuum sublimation. The alkanols used were received from Lancaster, England. 1,4-Dioxane was obtained from E. Merck, Germany and was used after purification following procedure mentioned in experimental section of chapter 3. Water used in the present study to prepare stock solutions was double distilled in an all-glass set-up.

The absorption and fluorescence spectra were recorded in double beam uv-visible spectrophotometer (Shimadzu, model UV-240, Japan) and Spectrofluorometer (model: SL- 174, ELICO, India) respectively. Silica cells of 1 cm path length were used in the measurements.

4.3. Results and discussion

The Absorption and Fluorescence spectra were recorded for all the solutes viz., 5-Hydroxyindole, 5-Hydroxy-L-tryptophan, L-Tyrosine, L-Tyrosinemethylester, 1-Naphthol, 2-Naphthol, at a fixed concentration of 1×10^{-4} M separately in different solvents such as water, methanol, ethanol, *iso*-propyl

alcohol, *tert*-butyl alcohol, pentanol, hexanol, heptanol, octanol and 1,4 Dioxane-water mixtures of different compositions viz., 10%, 20%, 30%, 50%, 60% and 80% (w/v) respectively at 298K. Representative spectra are shown in the figures 3 to 54. The spectra are significantly sensitive to the change in the polarity of the medium. Different solutes (indicator molecules) show maximum absorption band at different wavelengths for water and different solvents and also in 1,4-dioxane-water mixtures. As for example, the maximum absorption band of 1-Naphthol in water appears at 318 nm, while the maximum absorption bands in different alkanols with increasing alkyl chain length appear in the range of 320-331 nm and upon excitation, the fluorescence maximum of 1-Naphthol in water appears at 440 nm, but in alkanols, with increasing alkyl chain length, the fluorescence maxima appears in the range of 350-352 nm. The intensities of both of absorption and fluorescence bands also increase with increase in alkyl chain length of the alkanols. The wavelengths at which the other additives show maximum absorption bands and fluorescence maxima in different solvents are also observed.

Spectral features in relation to Solvent parameters:

The transition energies (both absorption and fluorescence) of all the additives with Stokes' shift in different media (in cm^{-1}) are presented in Tables 1 and 11. The solvent parameters like dielectric constant (D), refractive index (n), Kosower-Z value etc. can be correlated with the Stokes' shifts ($\Delta\bar{\nu}$) of all the additives in water and in different alkanols. The plot of Stokes' shift ($\Delta\bar{\nu}$) against the refractive index function, $(n^2 - 1)/(2n^2 + 1)$ and the dielectric constant function, $(D - 1)/(2D + 1)$ of the different solvents (e.g., water and alkanols) are shown in figures 55-59 and of 1,4-Dioxane-water mixtures (Figs. 60-65). Figs. 66-70 show the linear variation of the Stokes' shift with the Kosower-Z value of the same solvent. Similar plots of Stokes' shift against the solvent polarity parameters viz., $(n^2 - 1)/(2n^2 + 1)$ and $(D - 1)/(2D + 1)$ of

mixed solvents of dioxane-water mixtures of various compositions are depicted in figs. 71-75 for different additives. The linearity of these plots is often regarded as the evidence for the dominant importance of general solvent effect in spectral shifts. The plot of Stokes' shift ($\Delta\nu$) versus solvent polarity function $[(D - 1)/(2D + 1) - (n^2 - 1)/(2n^2 + 1)]$ is also found to be linear where the general solvent effect occurred. The non-linearity of the Lippert plot (plot of Stokes' shift versus solvent polarity function) is due to the specific solvent effects. This is because there is a possibility of hydrogen bonding between the polar solvent molecule and polar group on the fluorophore. In the case of L-Tyrosinemethylester, the specific solvent effect is attributed to the fact that there is a localization of the negative charge on the oxygen atom of the ester group.²⁹ In water, Stokes shifts are higher as compared to other solvents, which can be interpreted, as due to the higher value of dielectric constant of water as compared to those solvents. While comparing all the plots of Stokes' shift against orientation polarizability, it is assumed that the Stokes' shifts are approximately proportional to the orientation polarizability i.e., solvent polarity function.

In the case of interactions between the fluorophores and dioxane-water mixtures, excess Stokes' shifts are observed in the order 10% > 20% > 30% > 50% > 60% > 80%. This may also be due to the hydrogen bonding between the protic group of the fluorophore and the solvent mixtures. Here 1-Naphthol, 2-Naphthol, 5-Hydroxyindole, 5-Hydroxy-L-tryptophan contain OH group and L-Tyrosine and L-Tyrosinemethylester and 5-Hydroxy-L-tryptophan contain both OH and NH₂ groups both of which are capable of forming hydrogen bond. In all these cases, the Stokes' shifts increases with increase in the solvent polarity function. The immediate spectral shifts occurs which is attributed to the polar nature of both of the solvent and the fluorophore both are polar and both are associated in the ground state.³⁰ If they were associated in the excited state only these properties would have been dependent on the rates of diffusion of the polar solvent and the fluorophore. In these cases, the dependence on the

concentration of the polar solvent would be similar to that for quenching reactions. From the plot of Stokes' shift and dielectric constant function $(D - 1)/(2D + 1)$ in different solvents in the presence of all the indicators, the Stokes' shift become larger as the dielectric constant function increases (Figs. 55-65). Maximum shift is found in water with maximum dielectric constant function due to its large dielectric constants. Here the spectral shifts are due to both of the orientation of the solvent dipoles and redistribution of electrons in the solvent molecules. While moving towards the other solvents, viz., pentanol, hexanol, heptanol, octanol the shift is less as also the dielectric constant function due to their lower dielectric constant.

In the plots of Stokes' shift verses refractive index function $(n^2 - 1) / (2n^2 + 1)$, the opposite effects are found, i.e., Stokes' shift decreases with the increase in the refractive index function as well as the refractive index of the solvents itself (Figs. 55-65). The term refractive index function accounts only for the redistribution of electrons. In the case of polar solvent water, where the largest shift found has the minimum refractive index function due to minimum refractive index value of water. Actually, the refractive index function affects the Stokes' shift less than the dielectric constant function because according to Lippert theory, only the solvent reorientation is expected to result in the substantial Stokes' shift.

Considering the similar plots of all the additives in dioxane-water mixtures, the maximum shift is observed in 10% dioxane-water mixture due to its maximum dielectric constant value. In all the cases, the variation of Stokes' shift with the dielectric constant function is linear.

Since the solvent properties near the interface of the micelles and reverse micelles of surfactants compare the solvent properties of dioxane-water mixture, these plots are useful to determine the dielectric constant and refractive index of the micelles.

It is also found that the variation of Stokes' shift with the solvent polarity function, i.e., orientation polarizability $[(D - 1)/(2D + 1) - (n^2 - 1)/(2n^2 + 1)]$ of different solvents is an useful way to determine the dipole moment of the solute (fluorophore) both in the ground states as well as in the excited states.^{18,19} The variation often can also be used to estimate the polarity of a new solvent.

On the basis of solvent effect on ultra-violet spectra, Kosower^{31,32} had established the scale of solvent polarity, which he called the Z-values. The scale is based on particularly solvent dependent absorption band, the charge transfer band of the 1-alkyl pyridinium iodides. The high solvent dependence of this band is understandable in terms of the foregoing arguments since the transition, as charge transfer transition, involves a large change in polarity. The scale of Z-value obtained by Kosower is parallel as far as comparable data available to Grunwald and Winstein's Y values, a kinetic scale of solvent polarity.³³

In the present investigation, the plot of Stokes' shift verses Kosower-Z values of our solutes, viz., 1-Naphthol, 2-Naphthol, 5-Hydroxyindole, 5-Hydroxy-L-Tryptophan, L-Tyrosinemethylester, L-Tyrosine and in water and different alkanols are found in good agreement with the Kosower-Z value scale. The absorption maxima are transformed to Kosower-Z values (kcal/mol) using the following relation:

$$Z = 2.859 \times 10^5/\lambda$$

Where λ is the wavelength of the absorption maxima in angstroms.

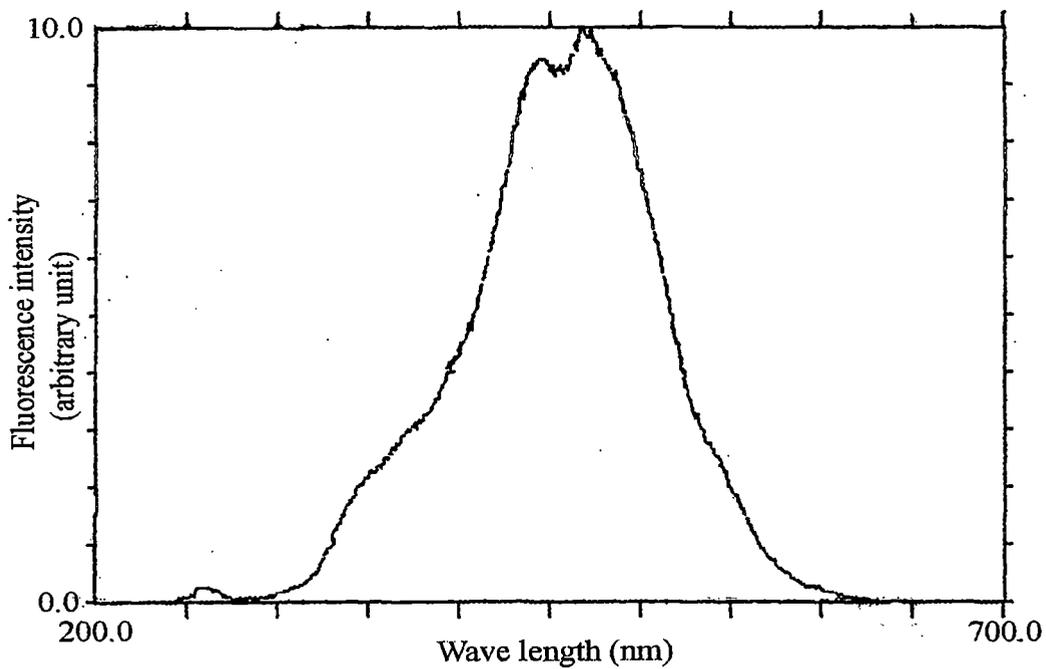


Fig. 3. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in water

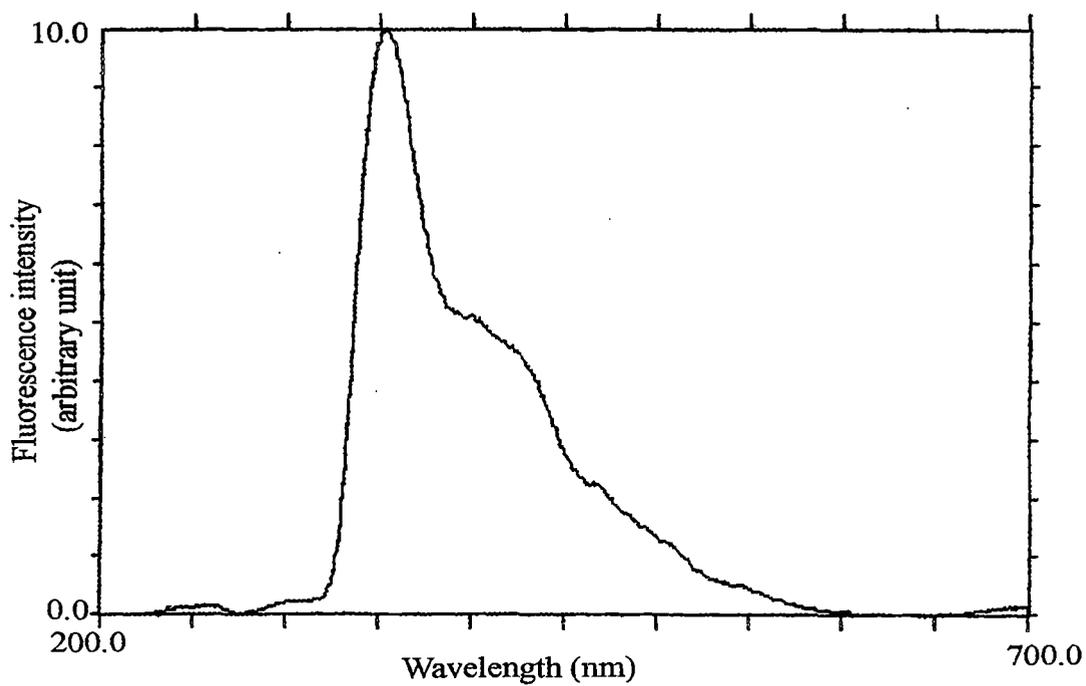


Fig. 4. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in water

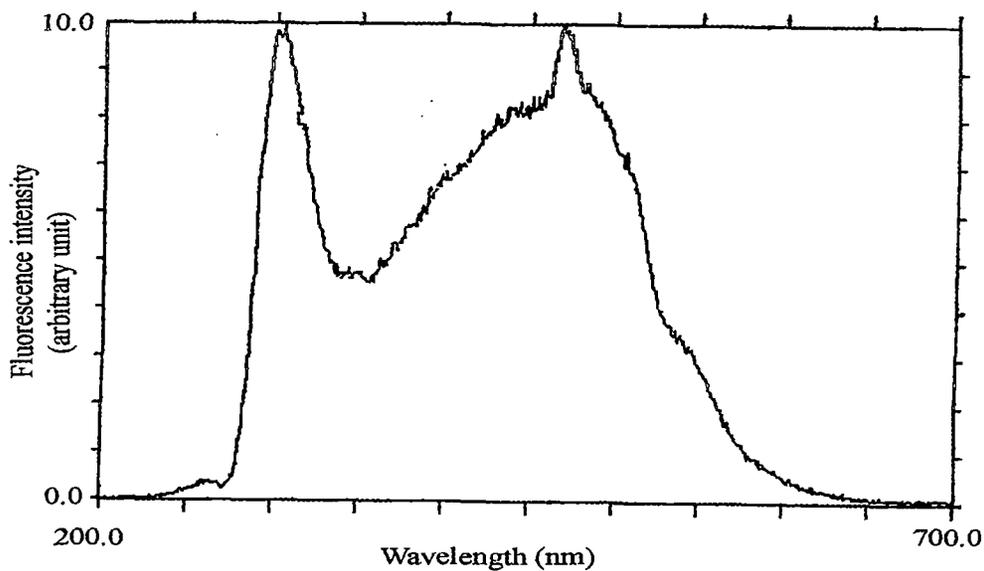


Fig. 5. Emission Spectrum of L-Tyrosine ($1 \times 10^{-4} \text{M}$) in water

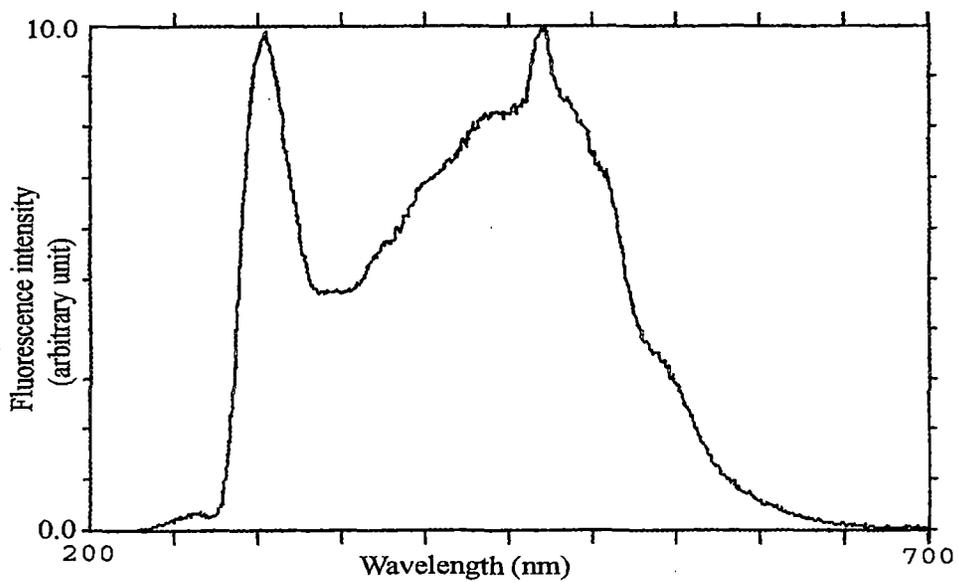


Fig. 6. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in water

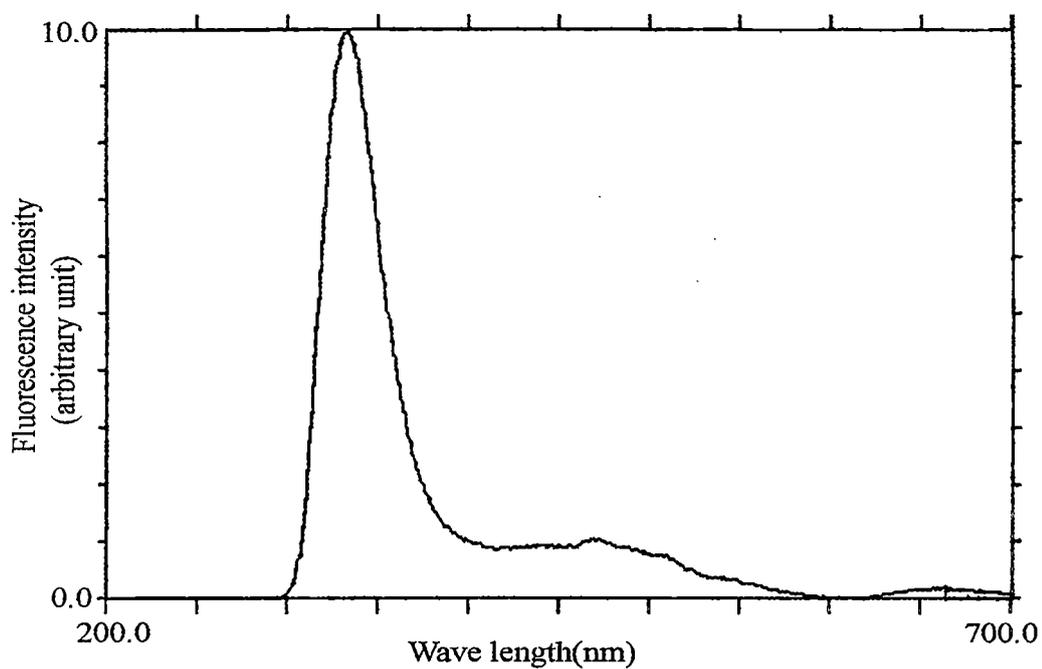


Fig. 7. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in water

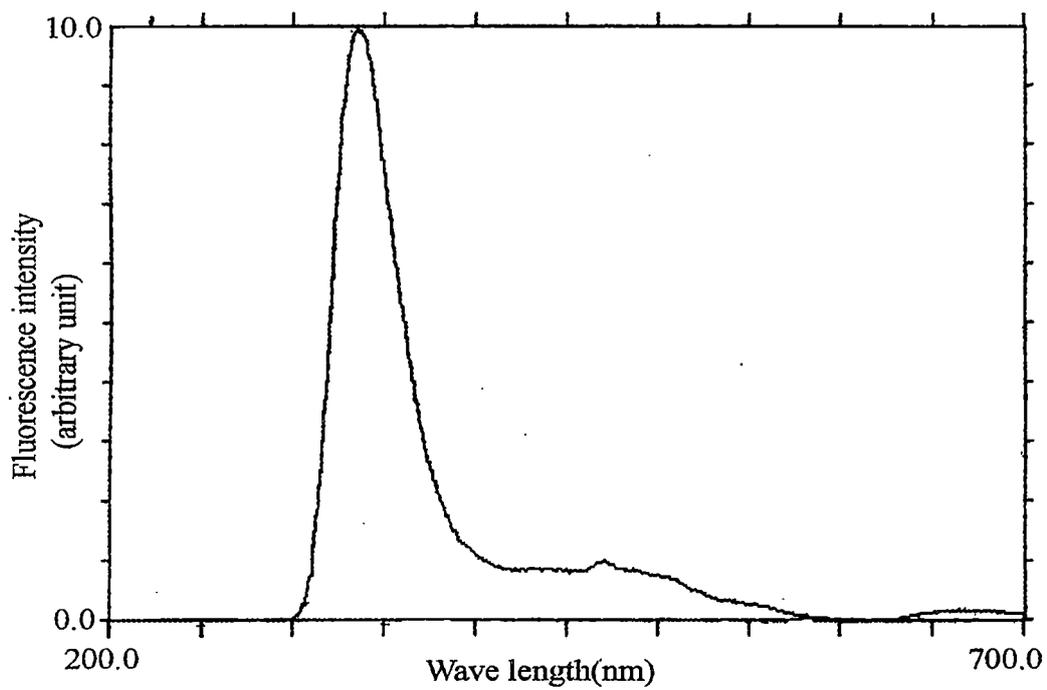


Fig. 8. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in water

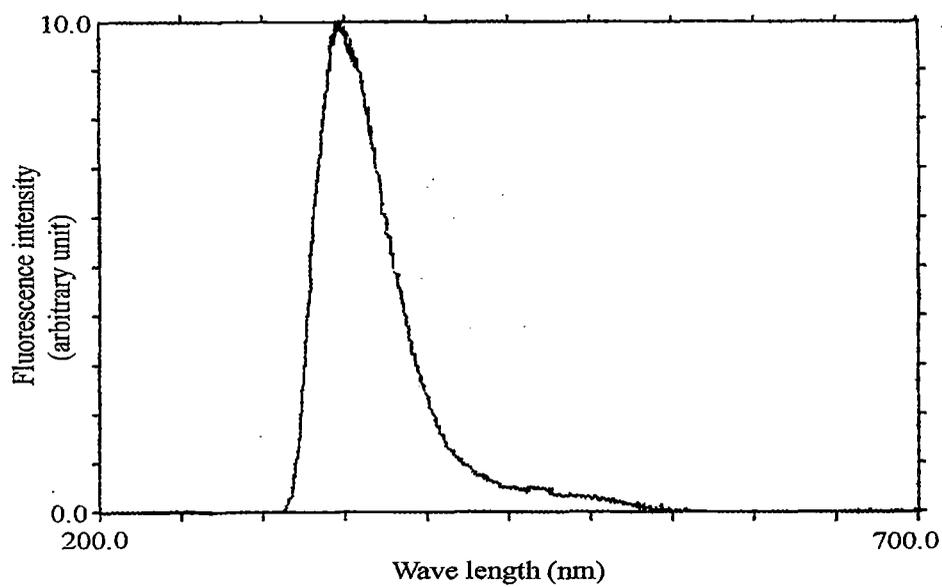


Fig. 9. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Methanol

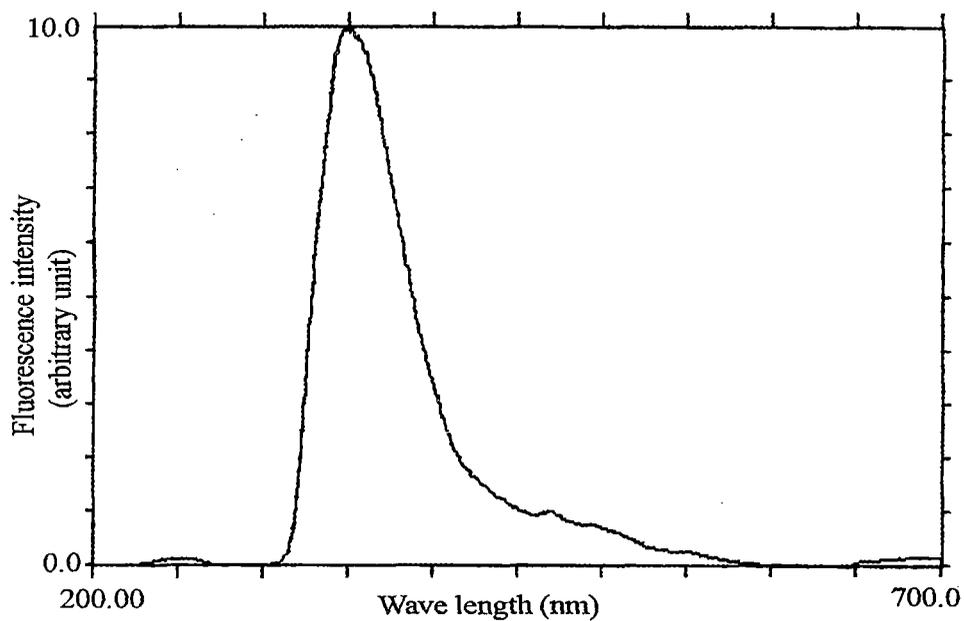


Fig. 10. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Ethanol

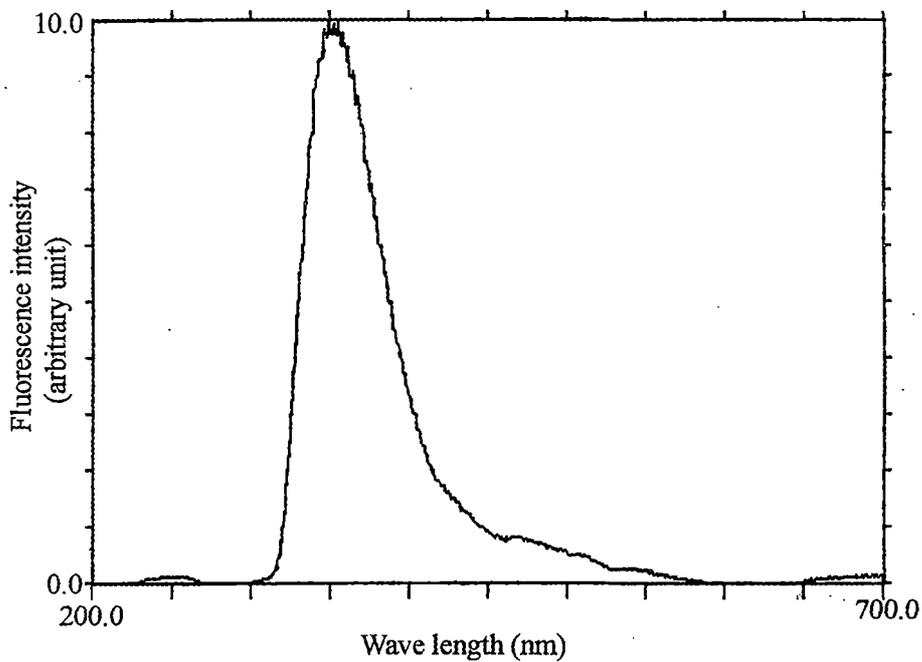


Fig. 11. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in *iso*-propyl alcohol

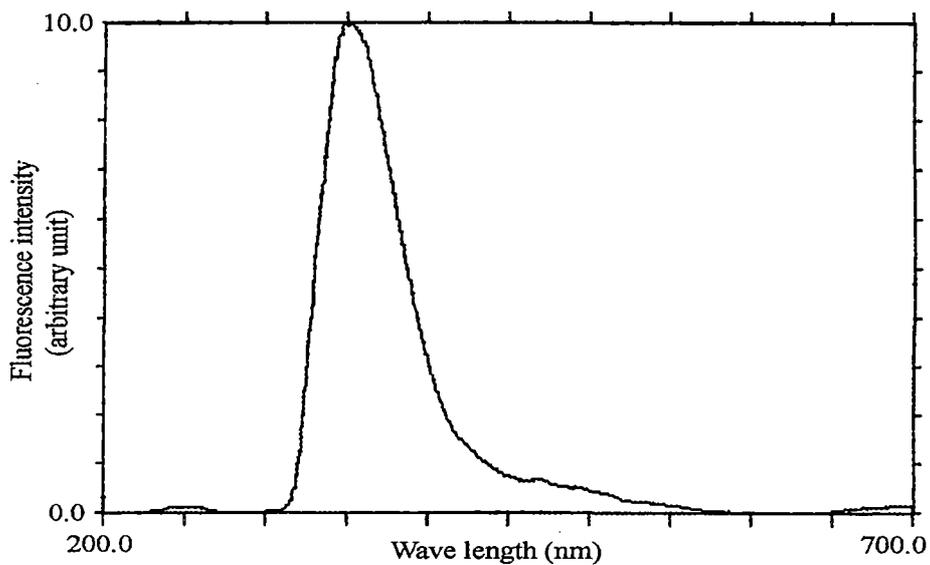


Fig. 12. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in *tert*-butyl alcohol

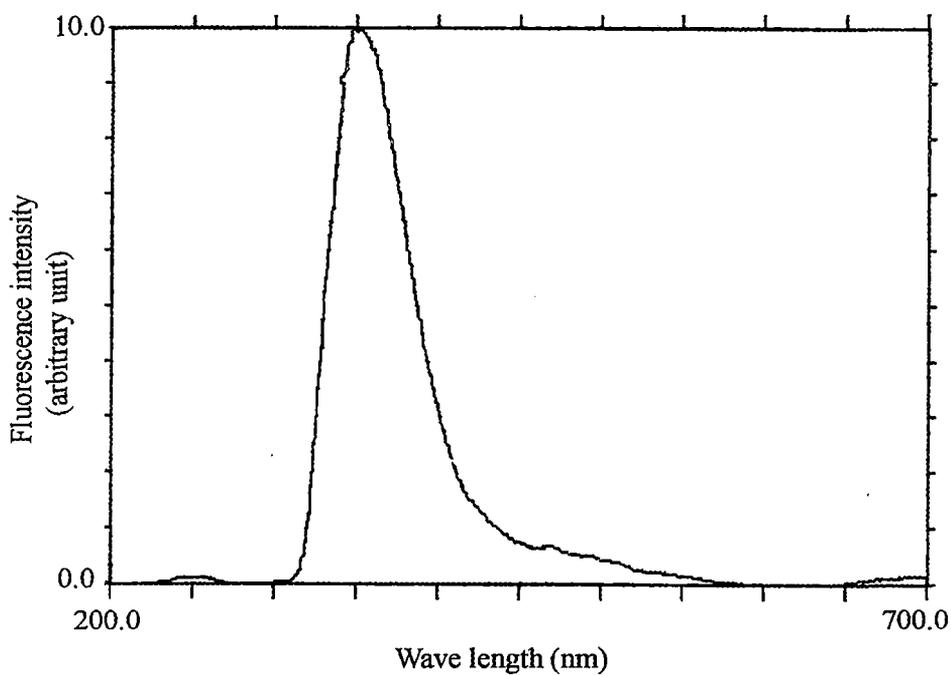


Fig. 13. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Pentanol

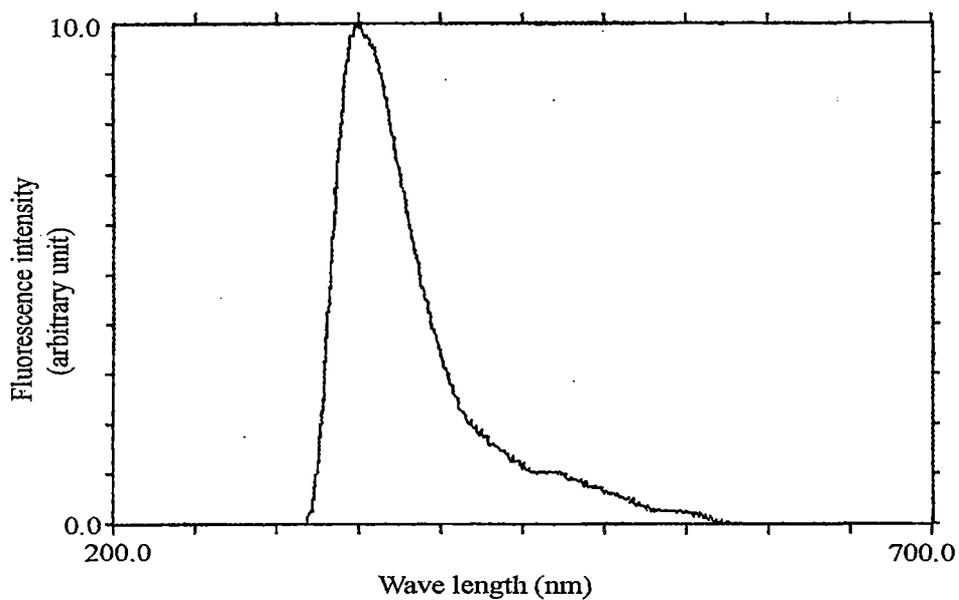


Fig. 14. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Hexanol

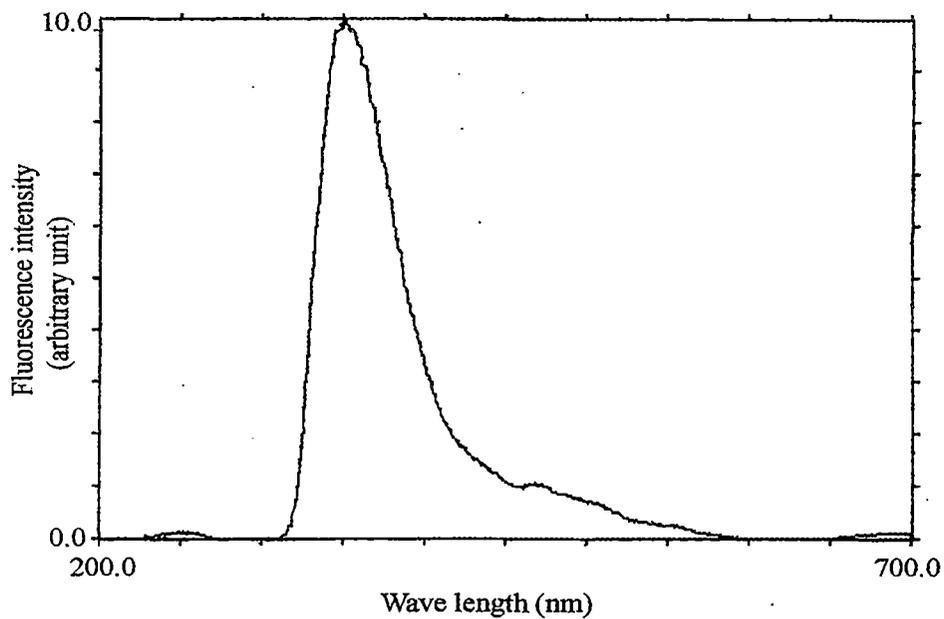


Fig. 15. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Heptanol

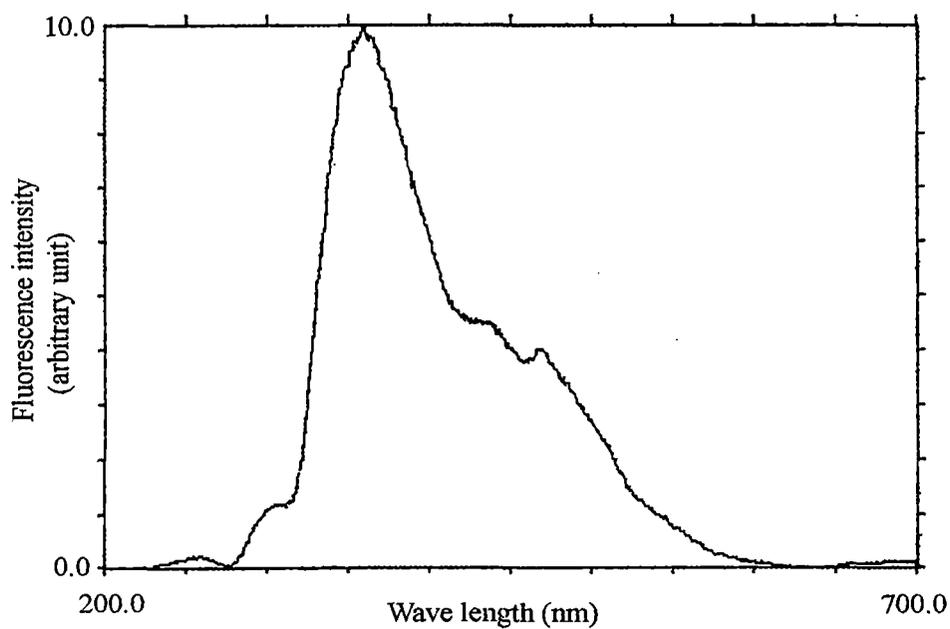


Fig. 16. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in Octanol

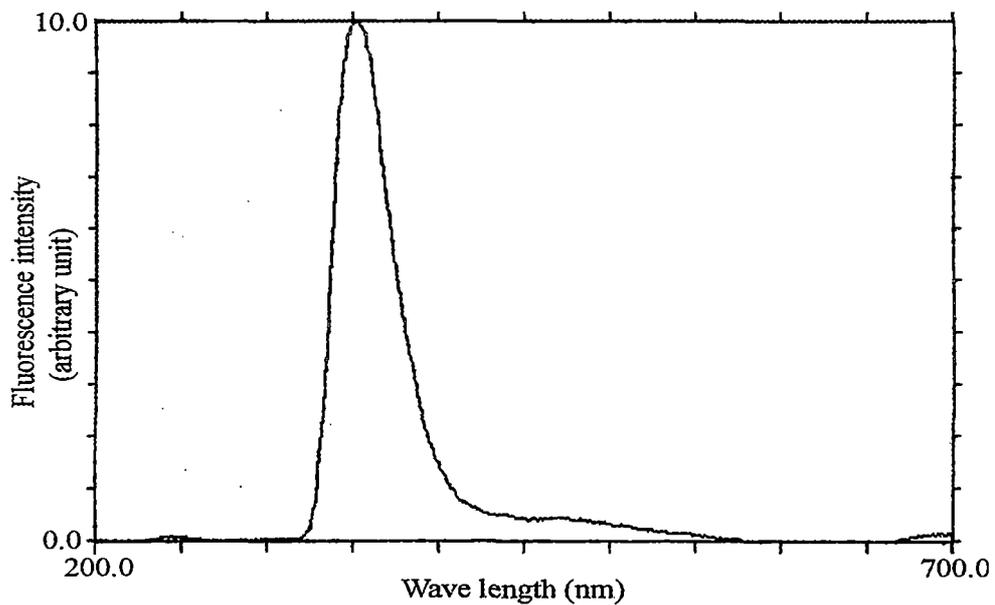


Fig. 17. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Methanol

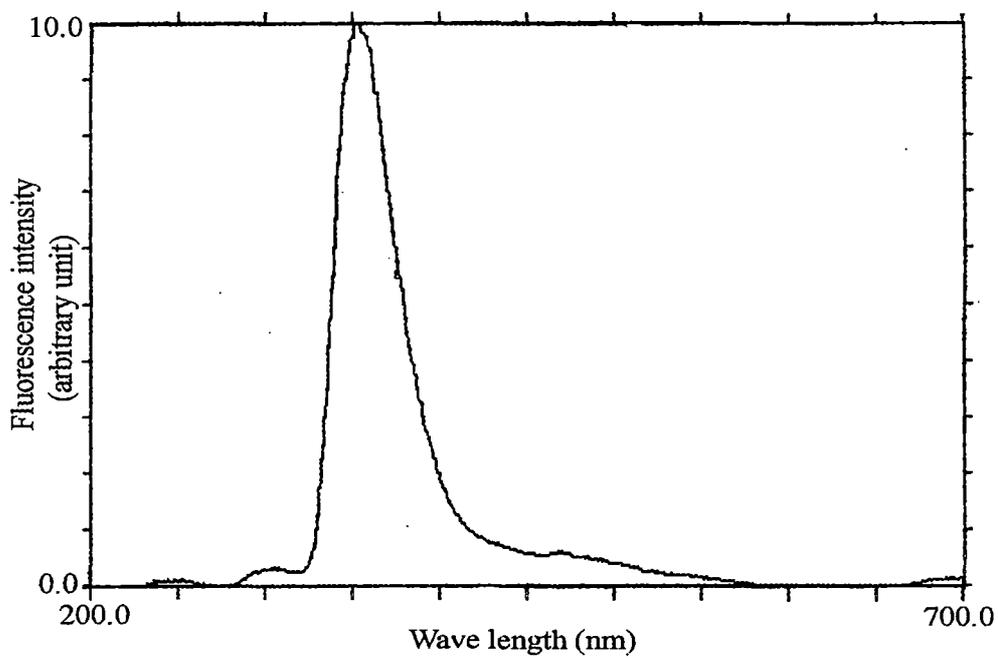


Fig. 18. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Ethanol

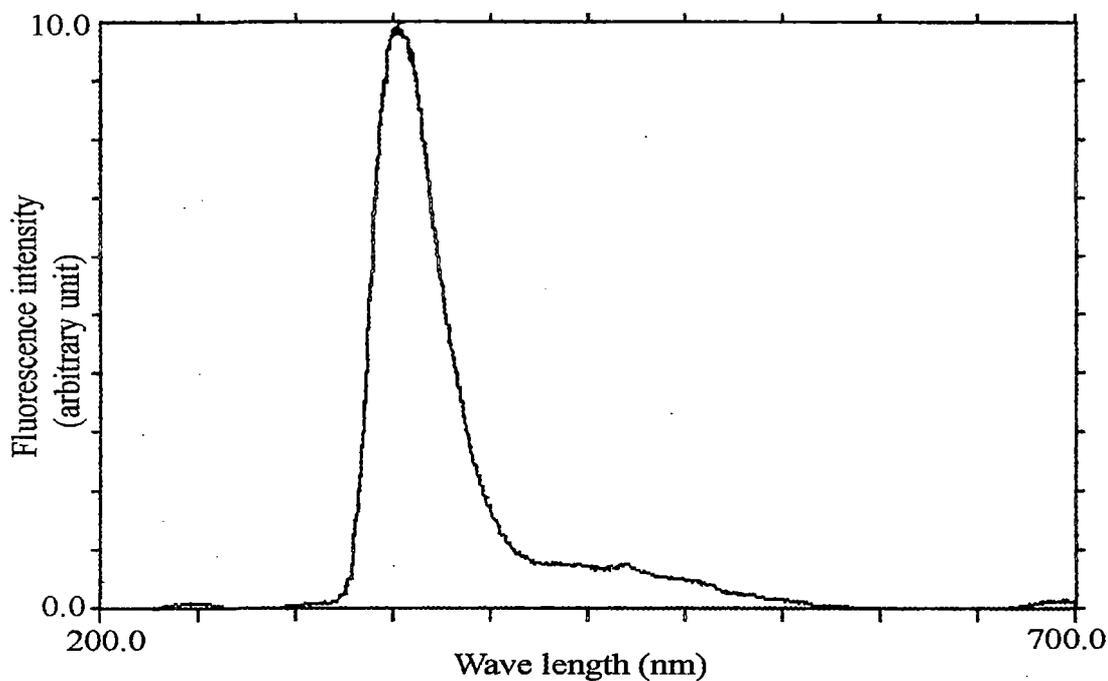


Fig. 19. Emission Spectrum of 2-Naphthol (1×10^{-4} M) in *iso*-Propyl alcohol.

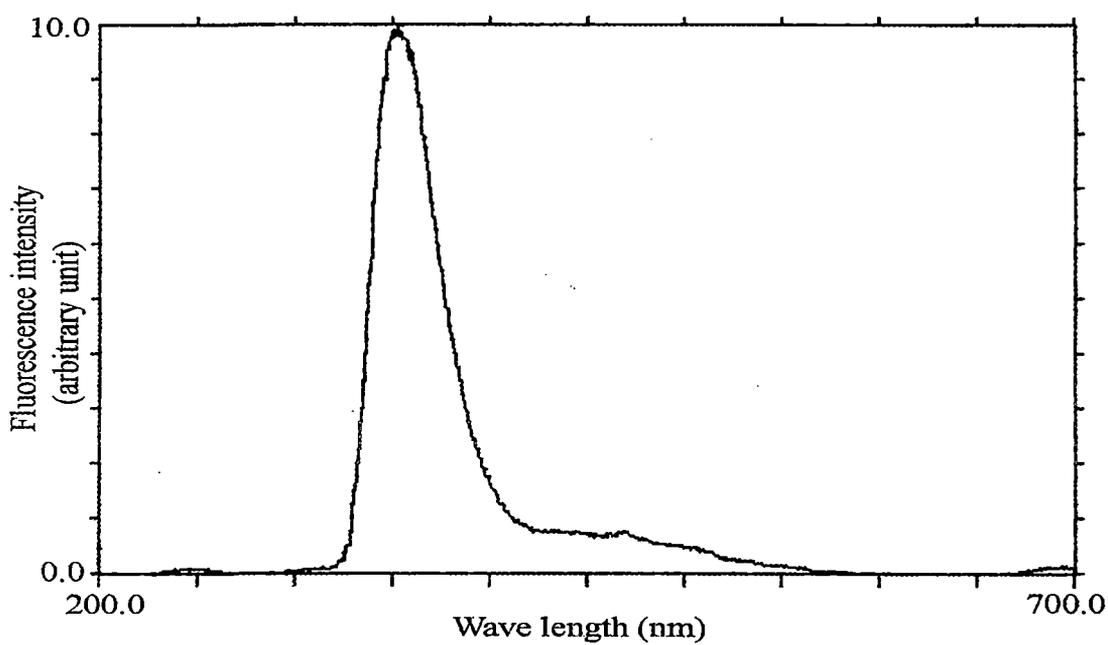


Fig. 20. Emission Spectrum of 2-Naphthol (1×10^{-4} M) in *tert*-Butyl alcohol

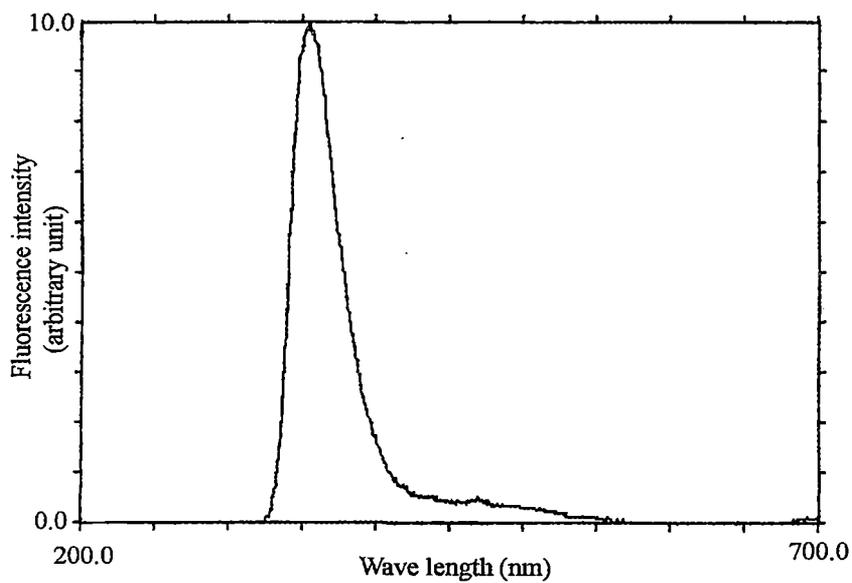


Fig. 21. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Pentanol

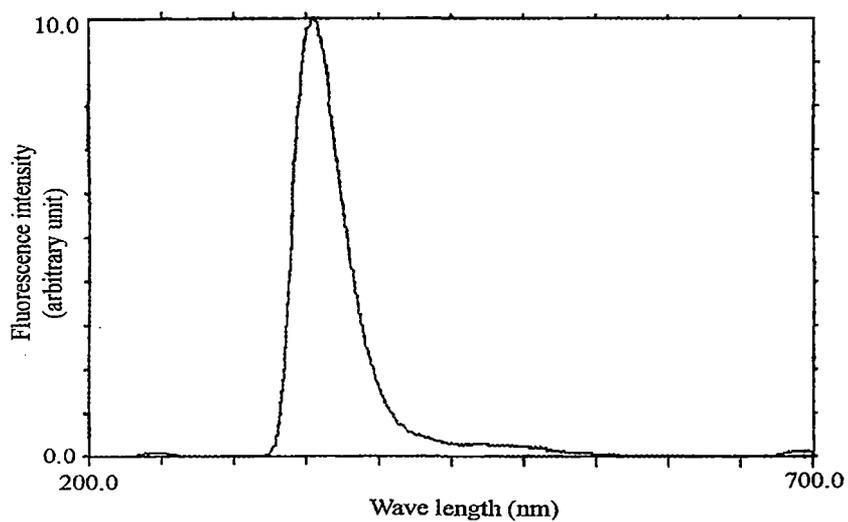


Fig. 22. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Hexanol

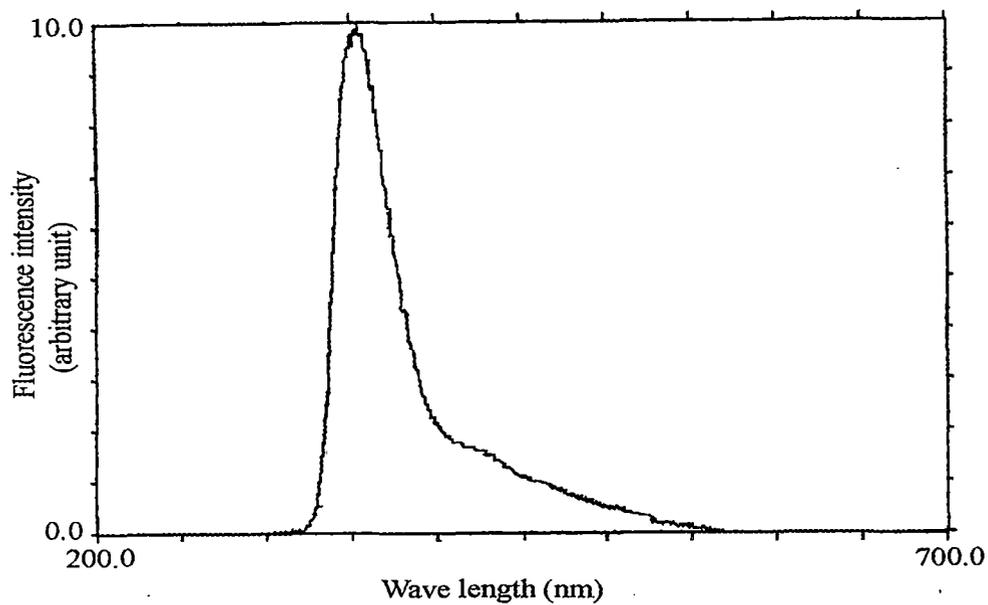


Fig. 23. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Heptanol

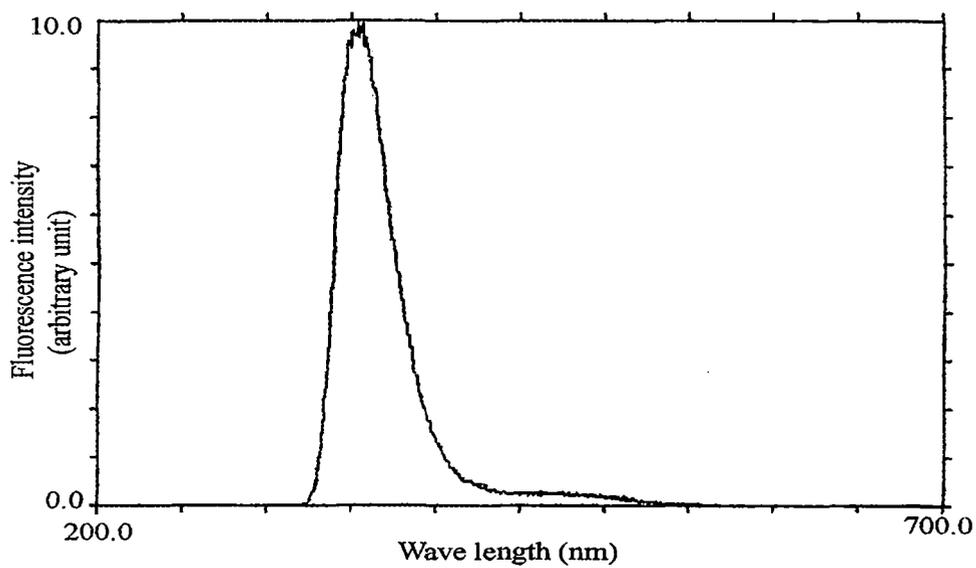


Fig. 24. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in Octanol

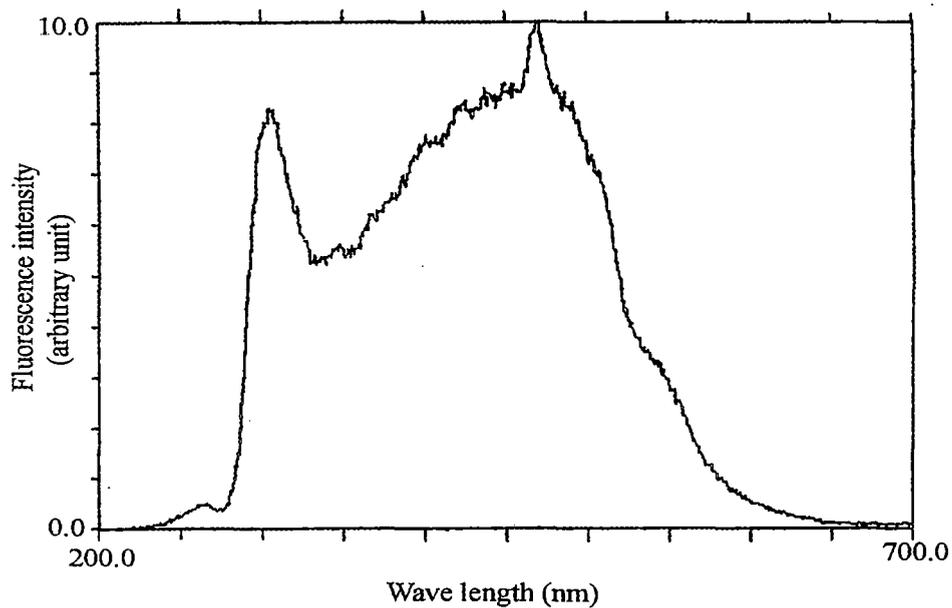


Fig. 25. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Methanol

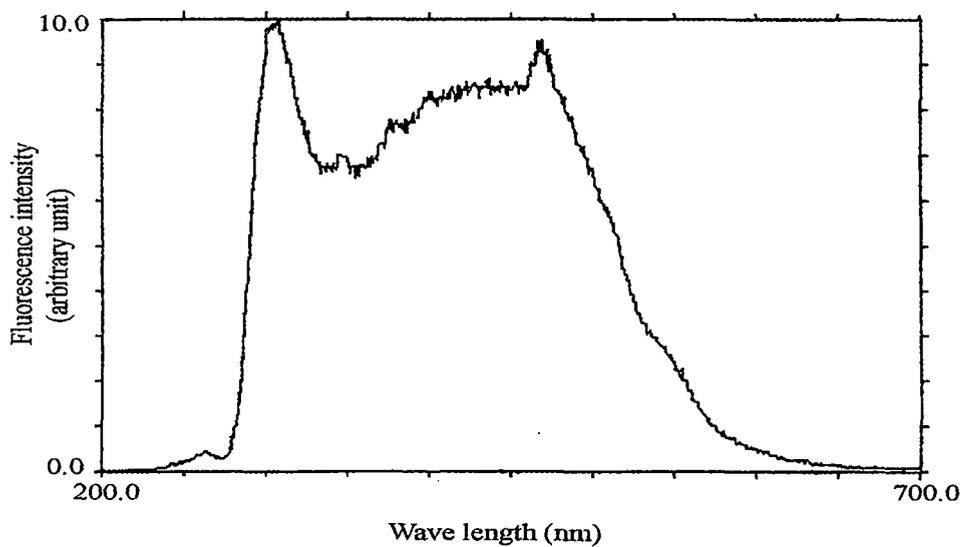


Fig. 26. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Ethanol

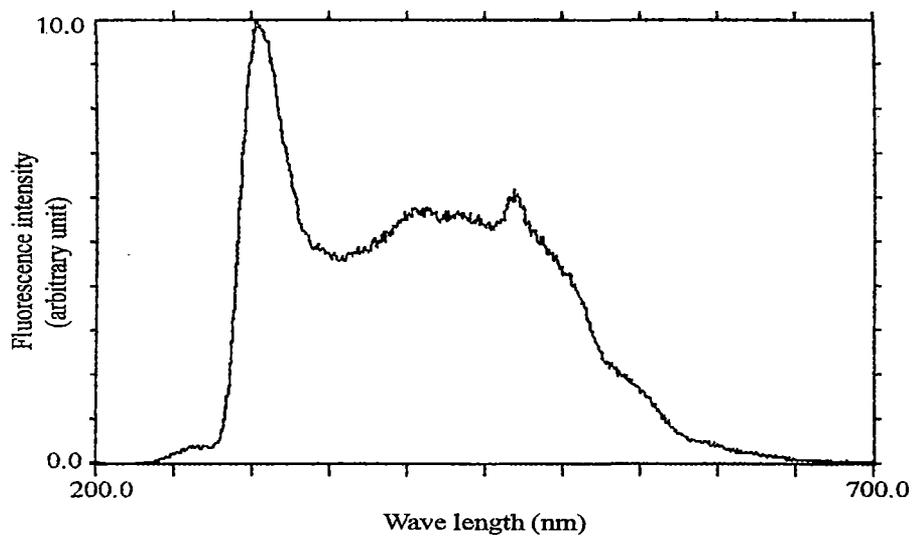


Fig. 27. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in *iso*-Propyl alcohol

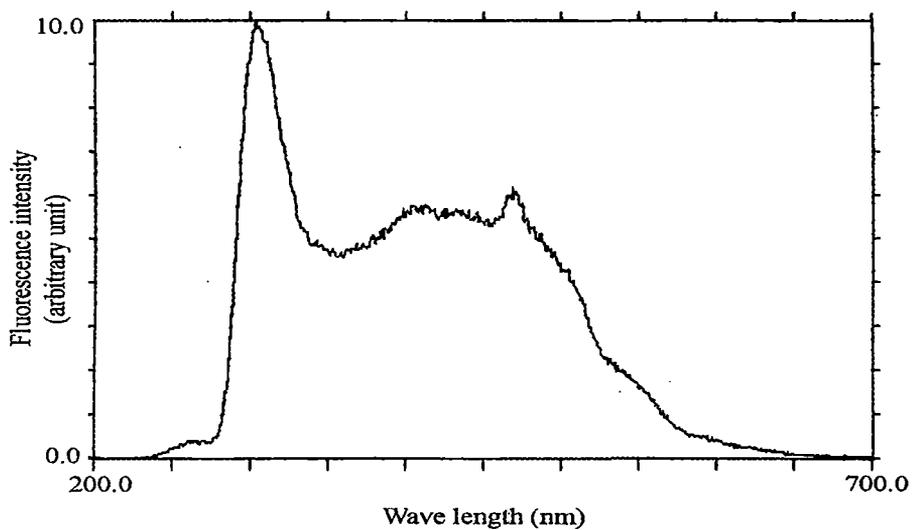


Fig. 28. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in *tert*-Butyl alcohol

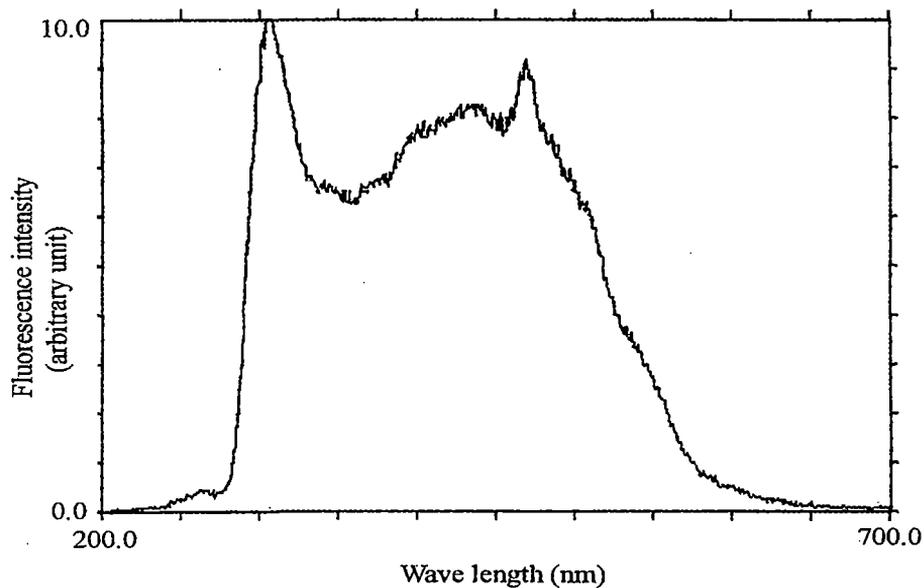


Fig. 29. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Pentanol

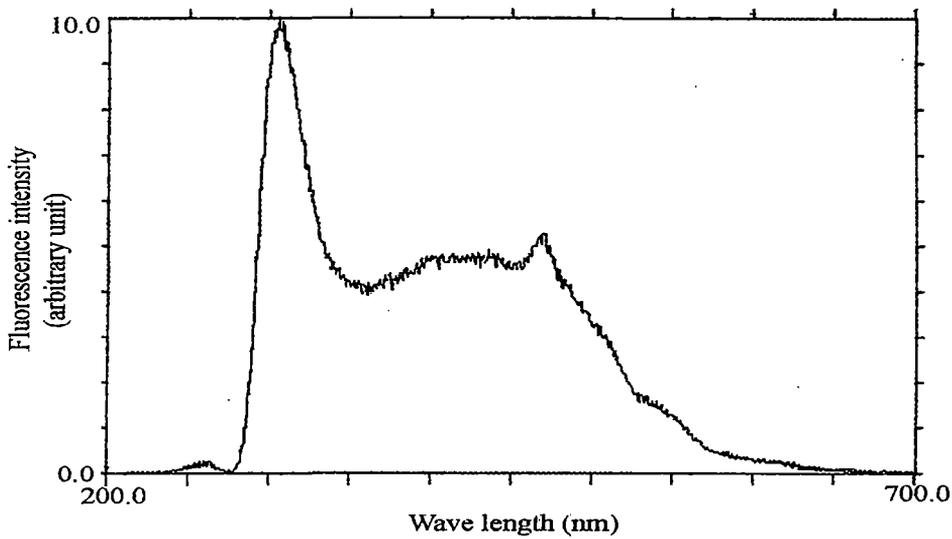


Fig. 30. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Hexanol

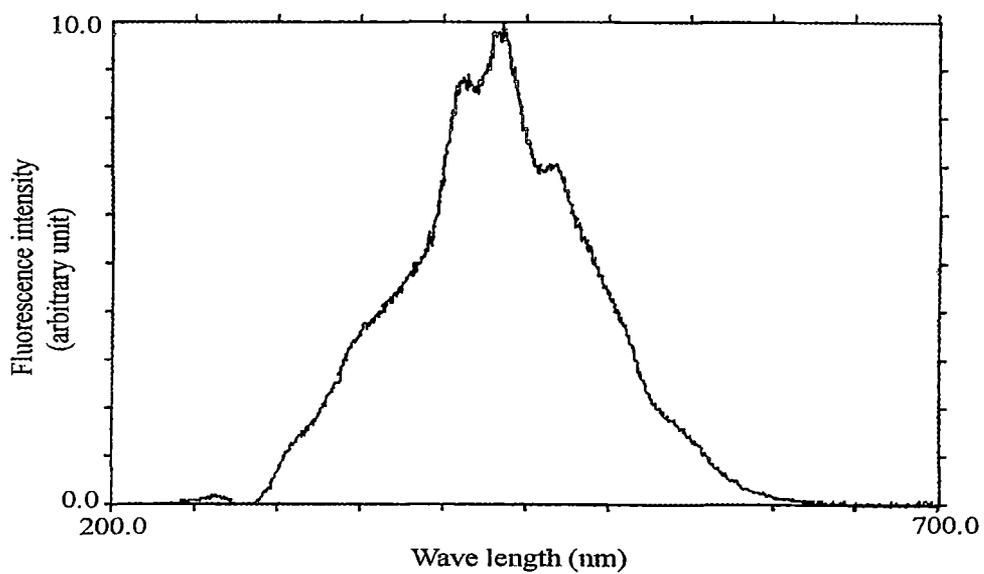


Fig. 31. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Heptanol

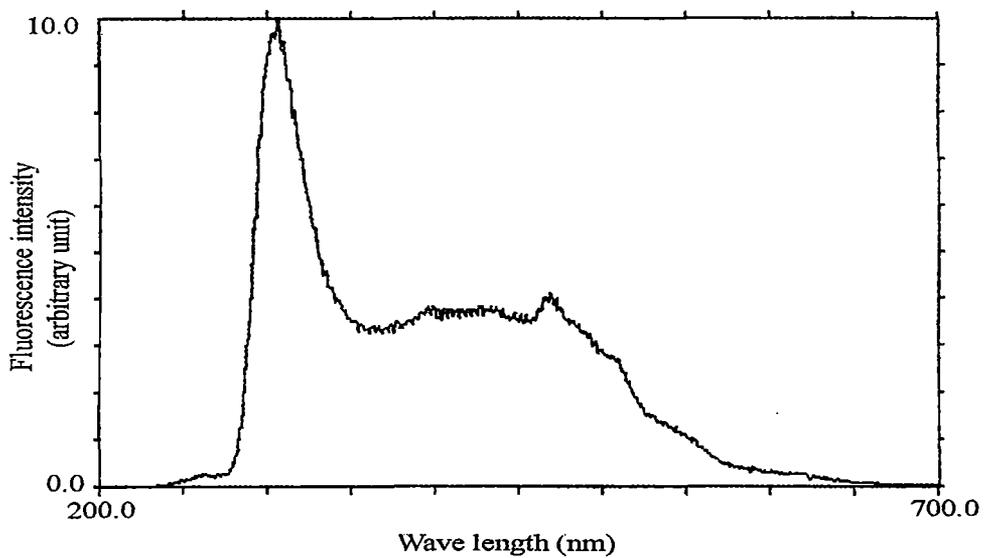


Fig. 32. Emission Spectrum of L-Tyrosinemethylester ($1 \times 10^{-4} \text{M}$) in Octanol

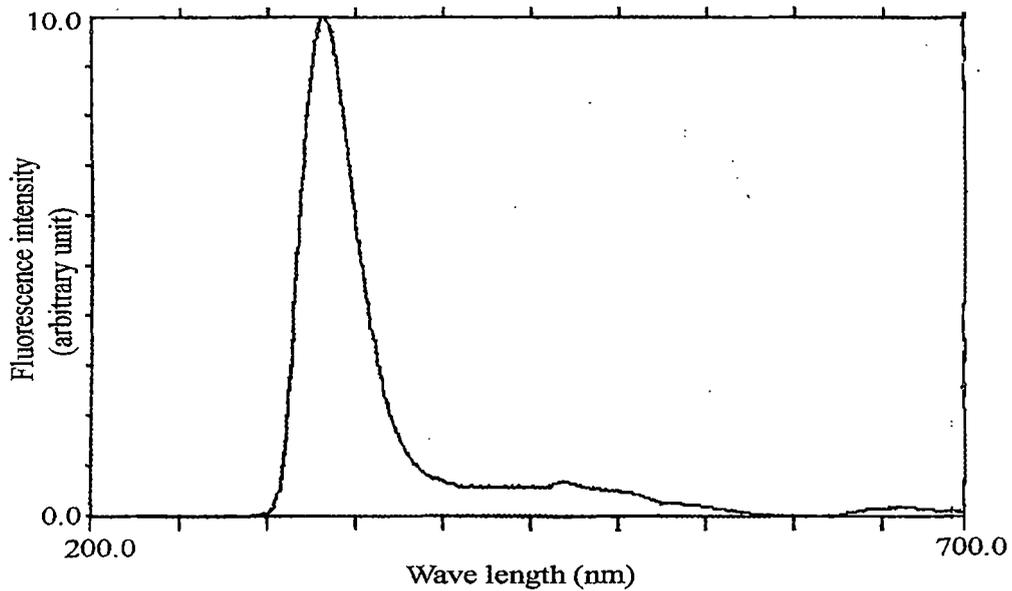


Fig. 33. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Methanol.

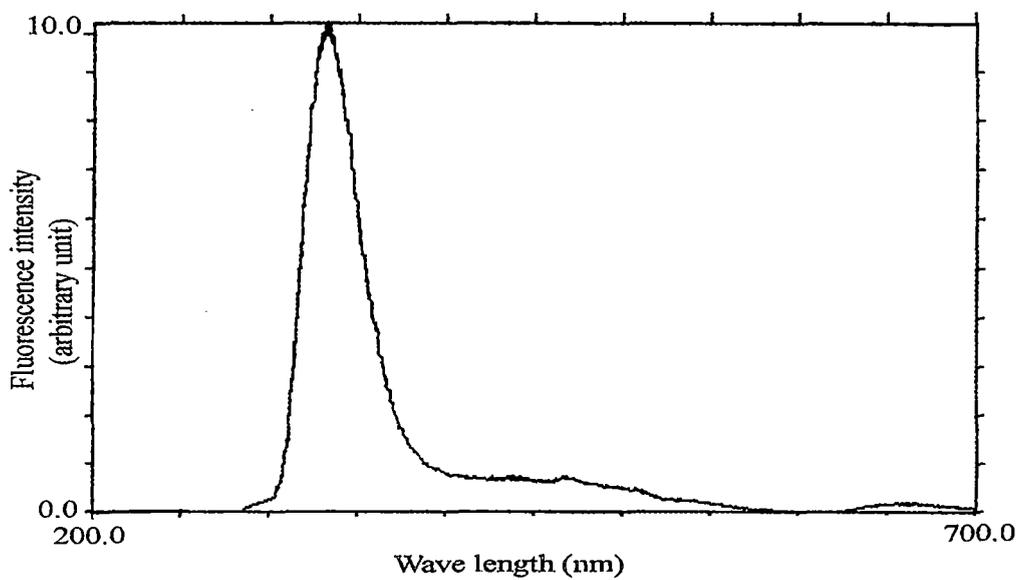


Fig. 34. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Ethanol.

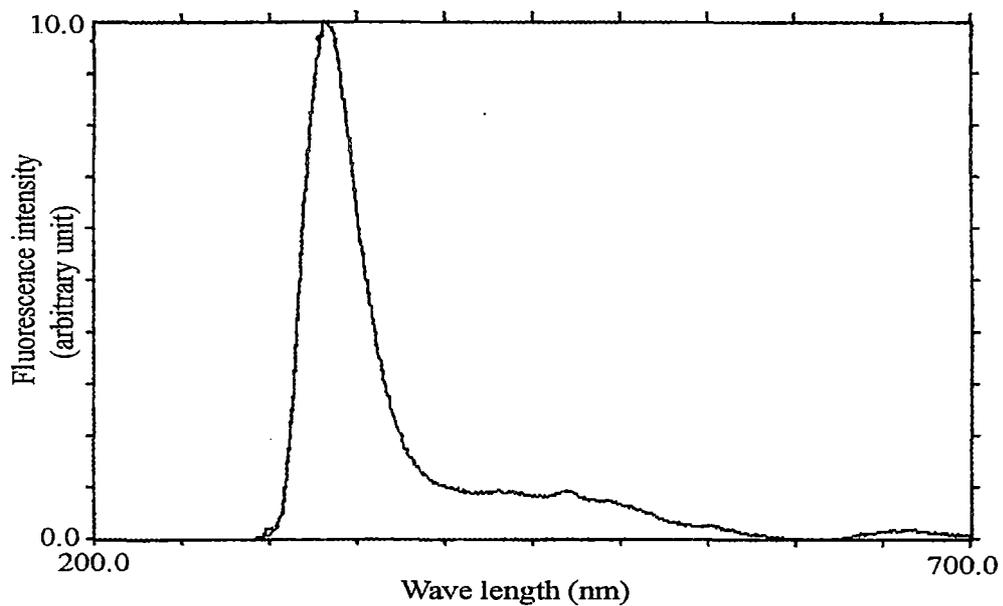


Fig. 35. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in *iso*-Propyl alcohol.

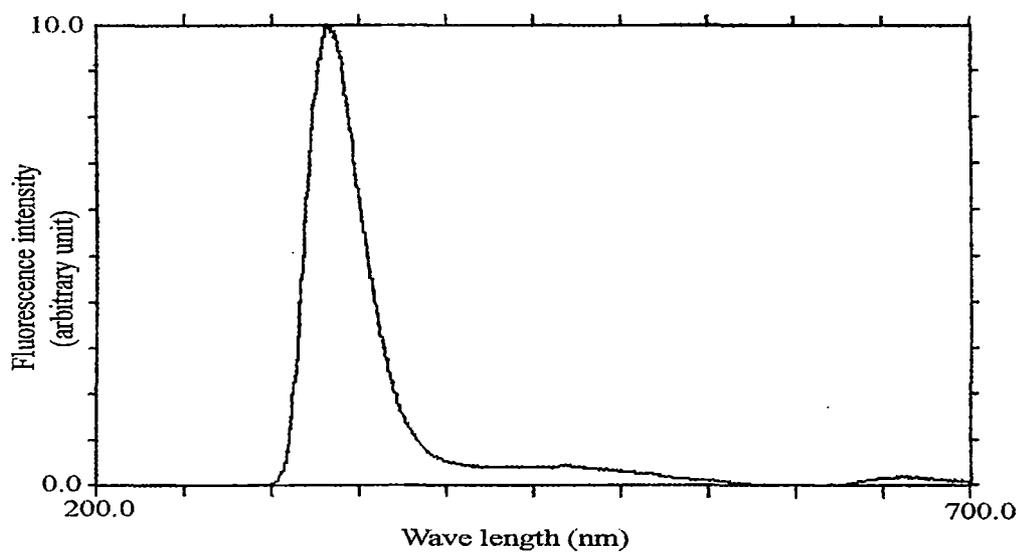


Fig. 36. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in *tert*-Butyl alcohol.

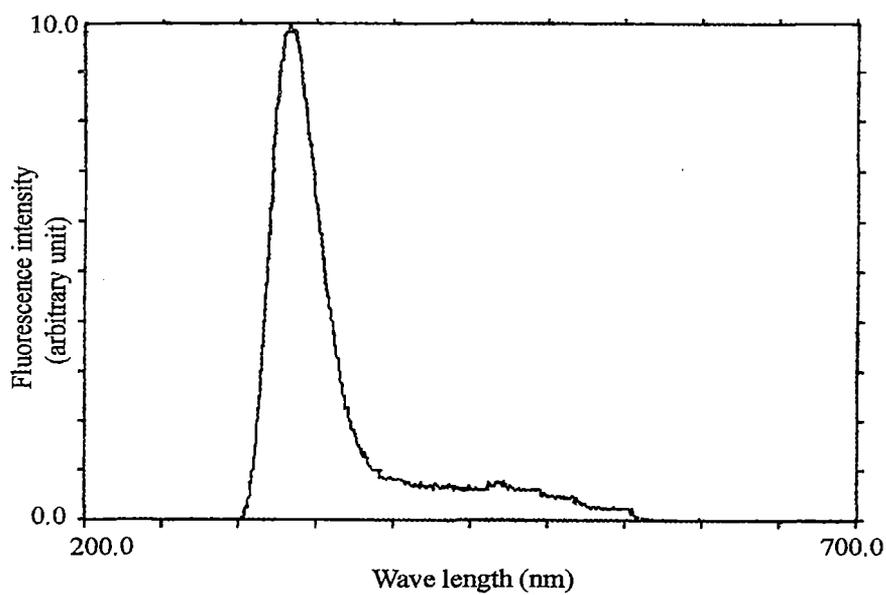


Fig. 37. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Pentanol.

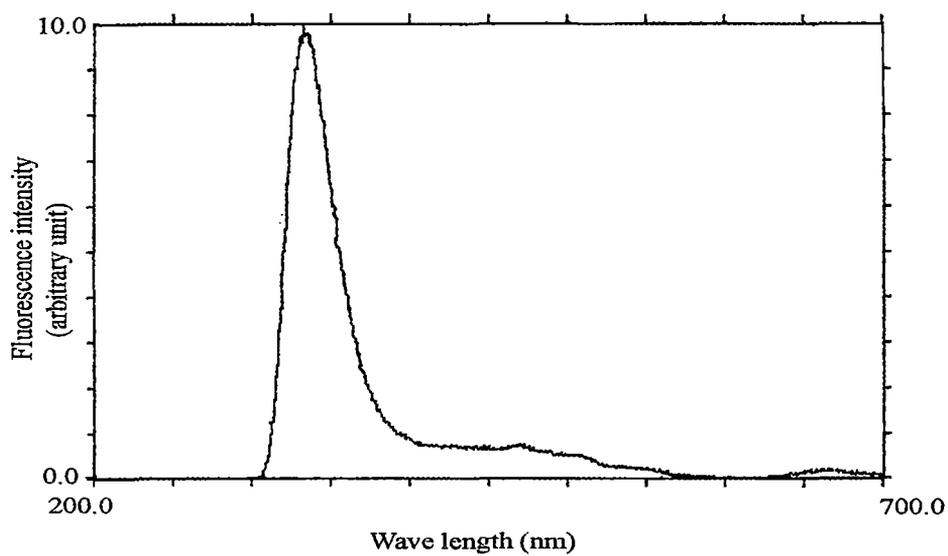


Fig. 38. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Hexanol.

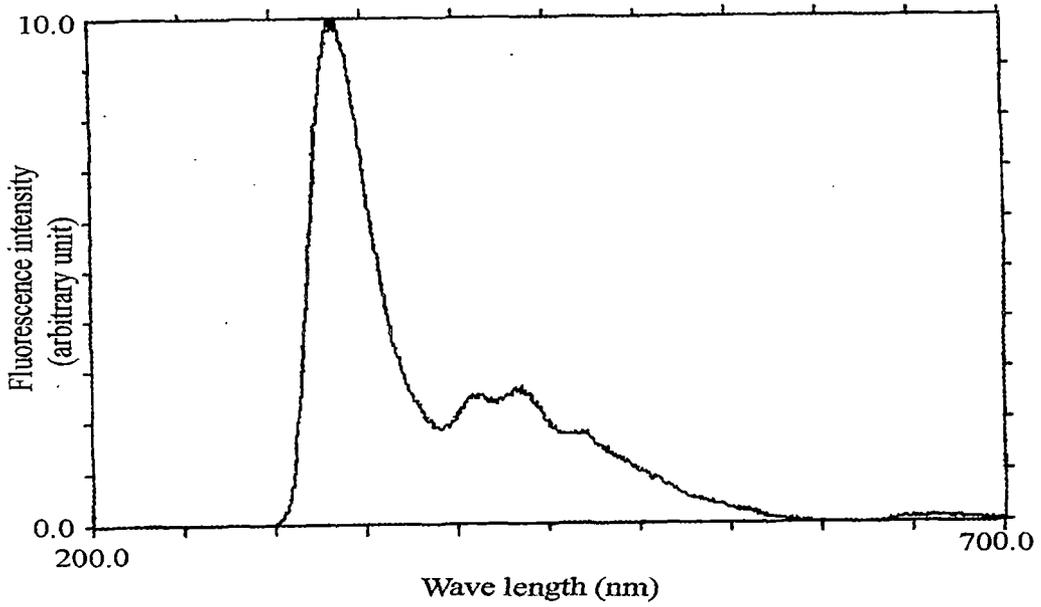


Fig. 39. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Heptanol.

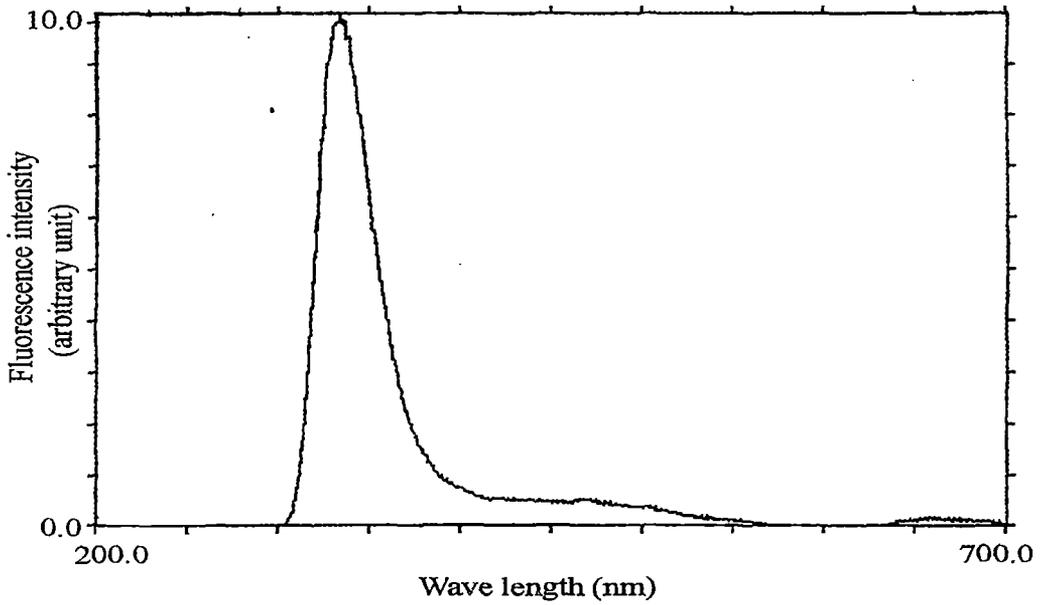


Fig. 40. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in Octanol.

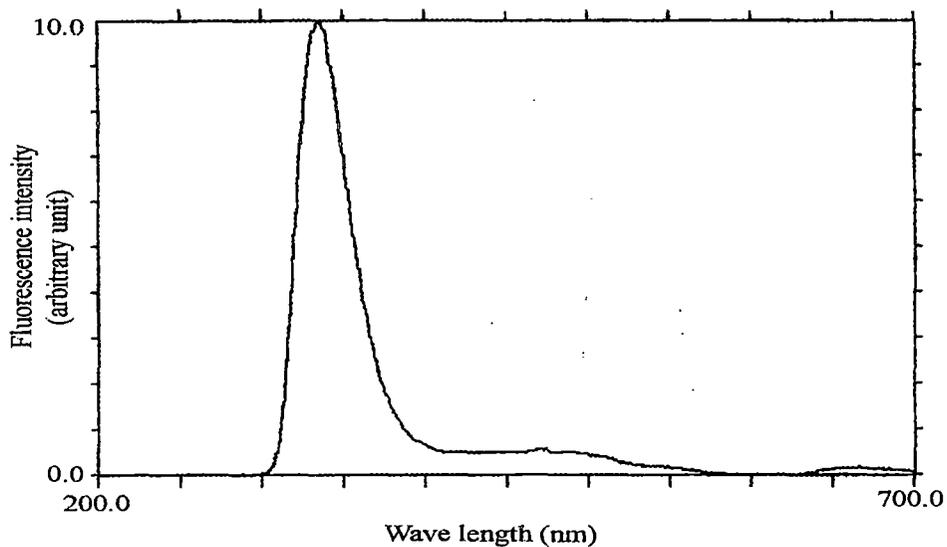


Fig. 41. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Methanol.

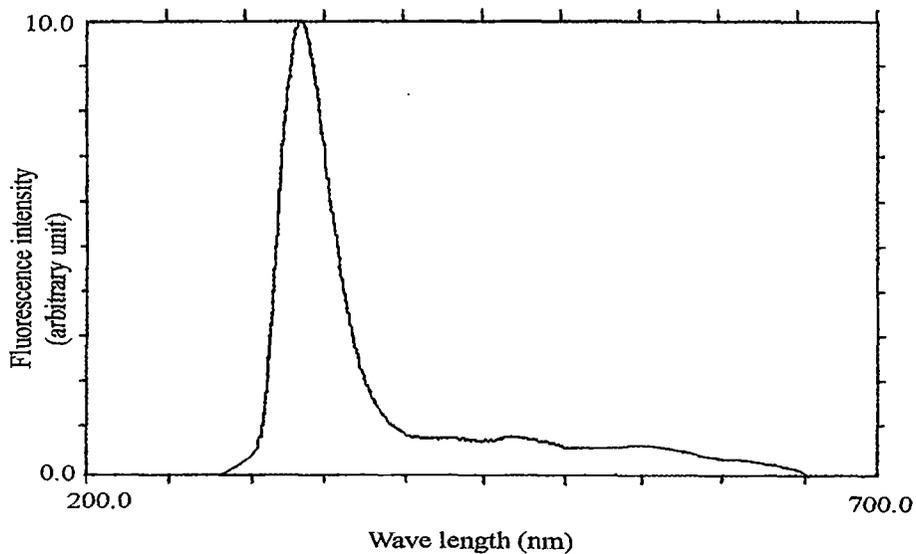


Fig. 42. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Ethanol.

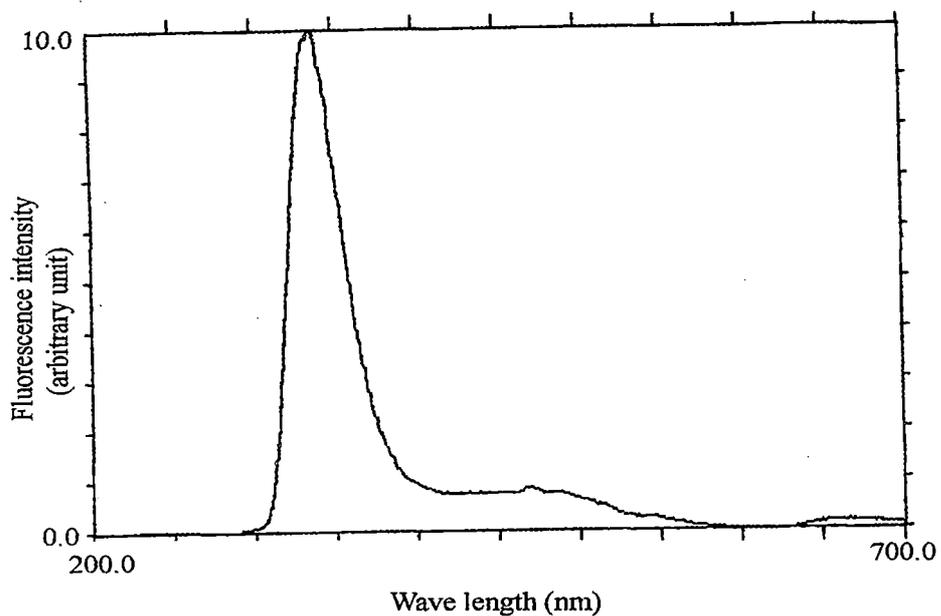


Fig. 43. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in *iso*-Propyl alcohol.

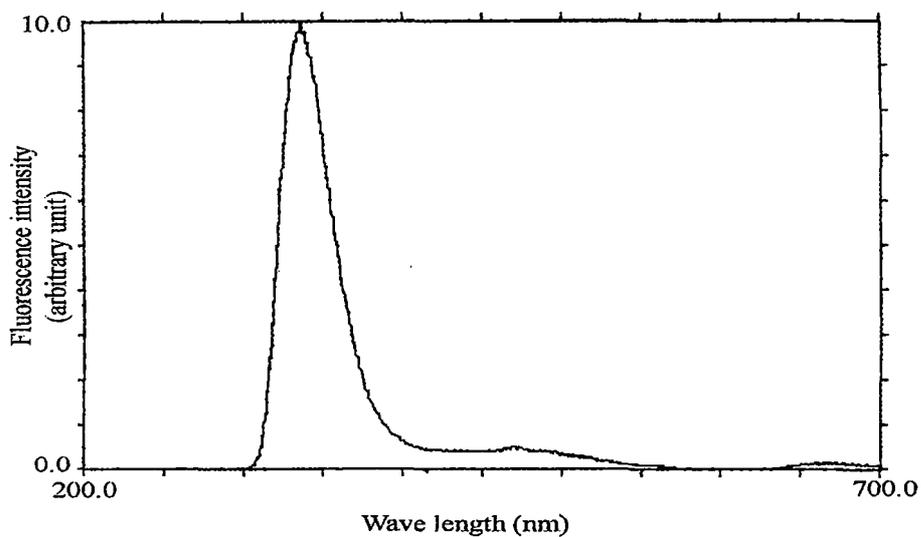


Fig. 44. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in *tert*-Butyl alcohol.

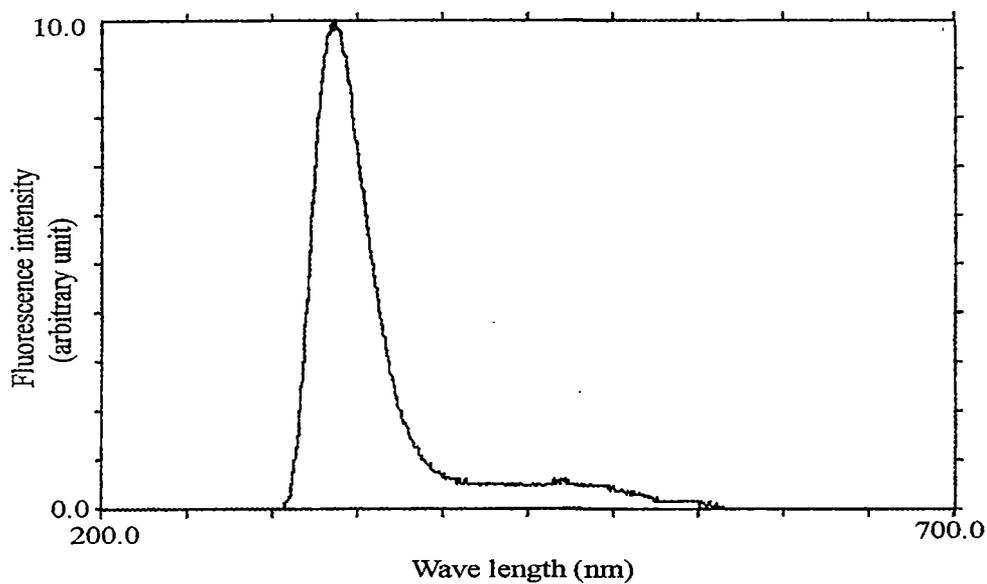


Fig. 45. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Pentanol.

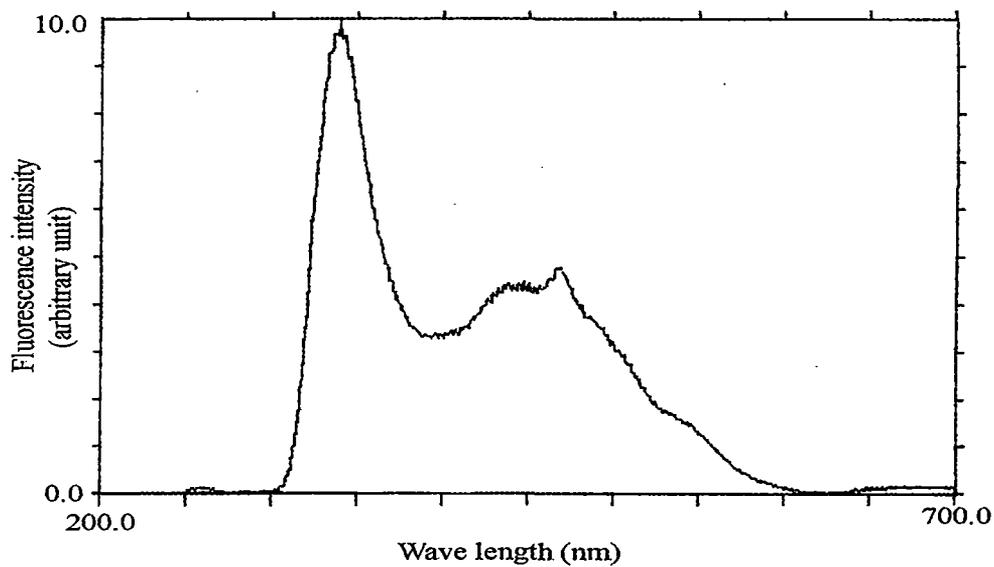


Fig. 46. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Hexanol.

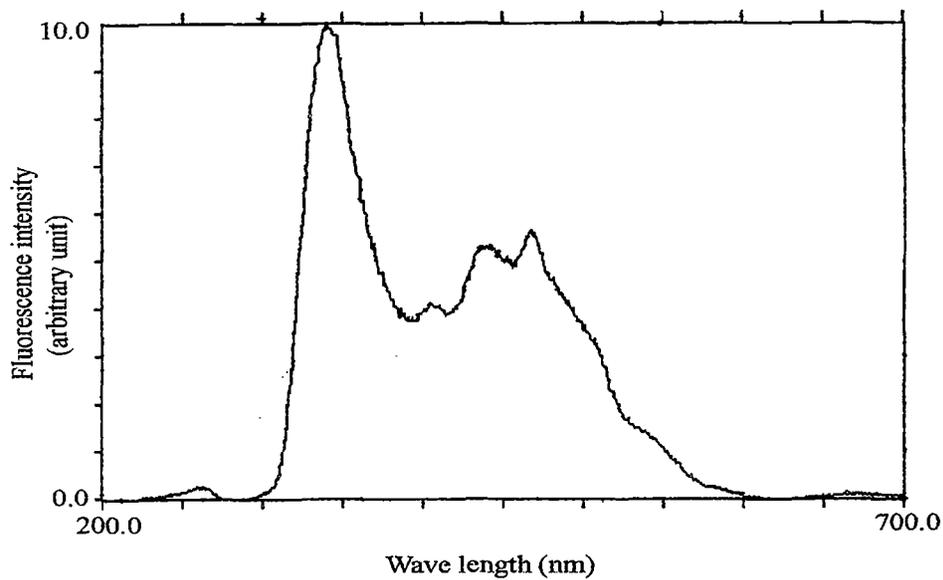


Fig. 47. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Heptanol.

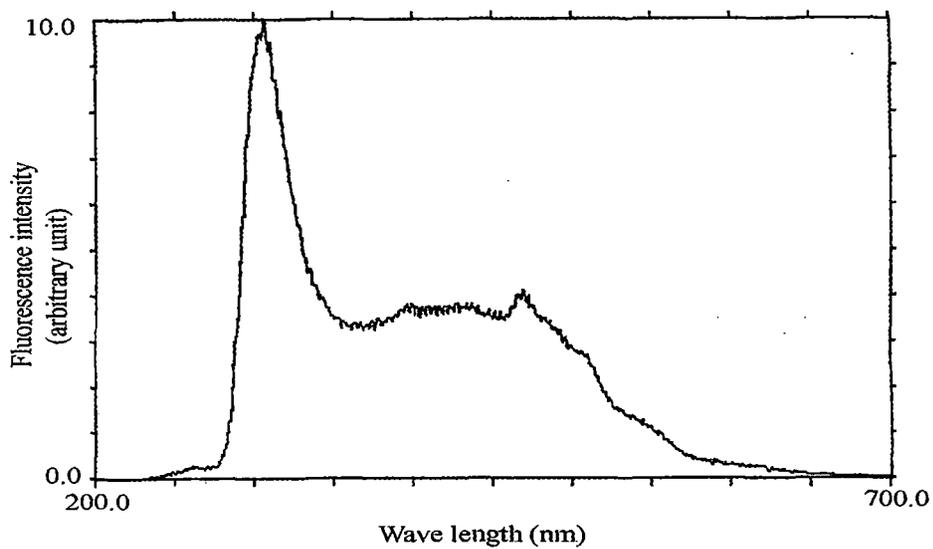


Fig. 48. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in Octanol.

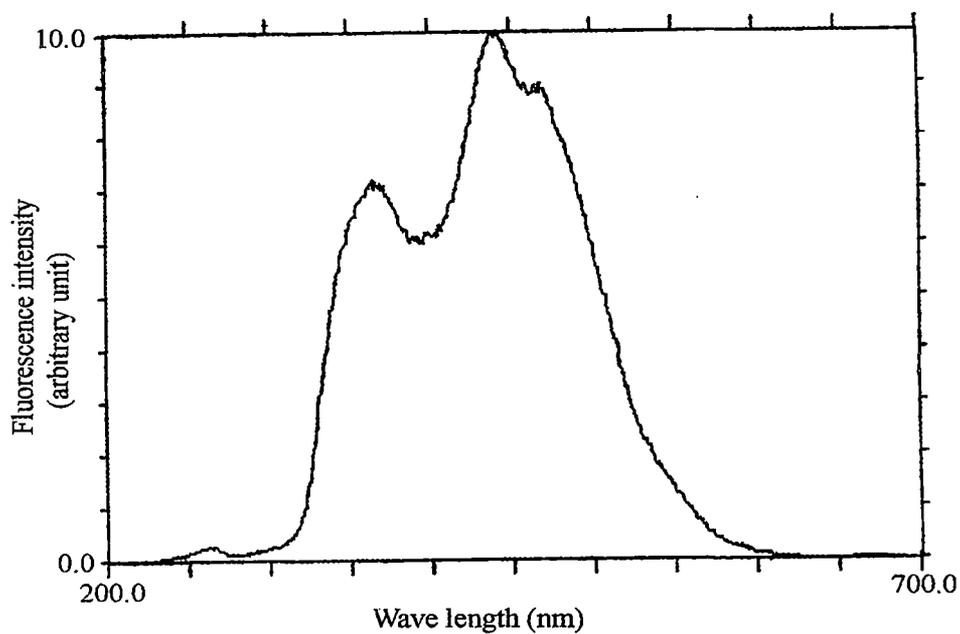


Fig. 49. Emission Spectrum of 1-Naphthol ($1 \times 10^{-4} \text{M}$) in 50% 1,4-Dioxane.

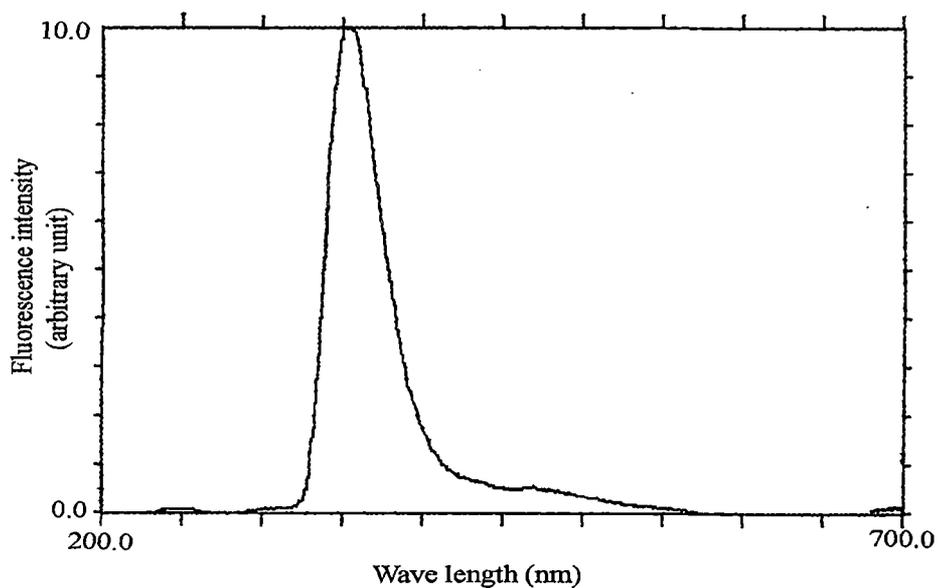


Fig. 50. Emission Spectrum of 2-Naphthol ($1 \times 10^{-4} \text{M}$) in 60% 1,4-Dioxane.

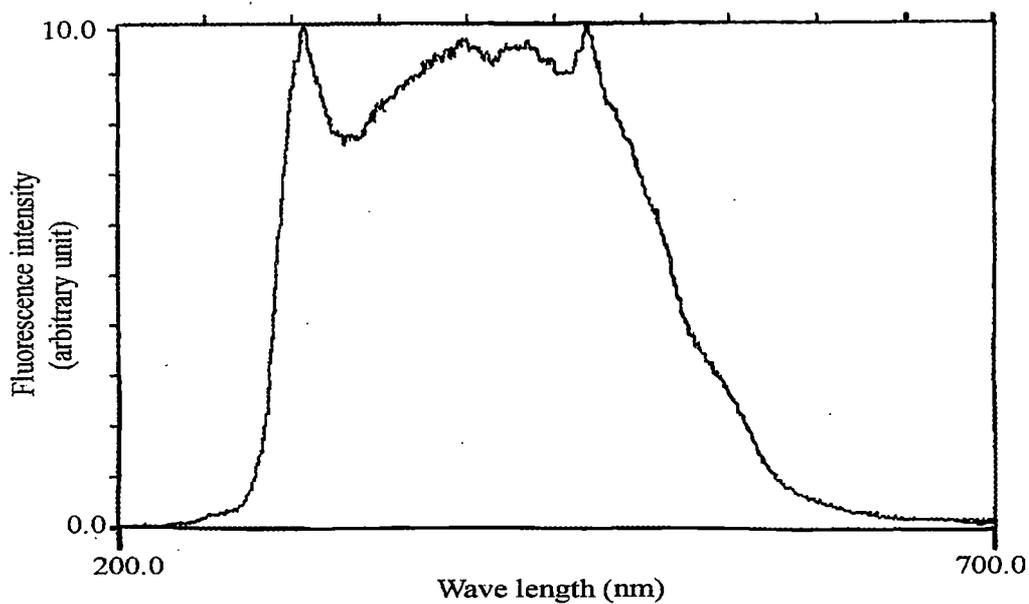


Fig. 51. Emission Spectrum of L-Tyrosine ($1 \times 10^{-4} \text{M}$) in 80% 1,4-Dioxane.

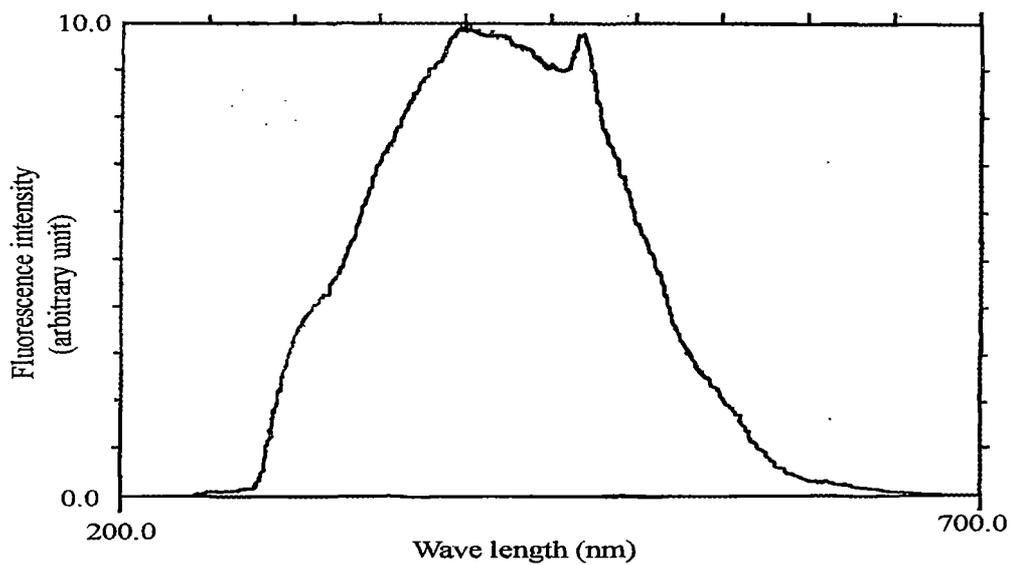


Fig. 52. Emission Spectrum of L-Tyrosinmethylester ($1 \times 10^{-4} \text{M}$) in 50% 1,4-Dioxane.

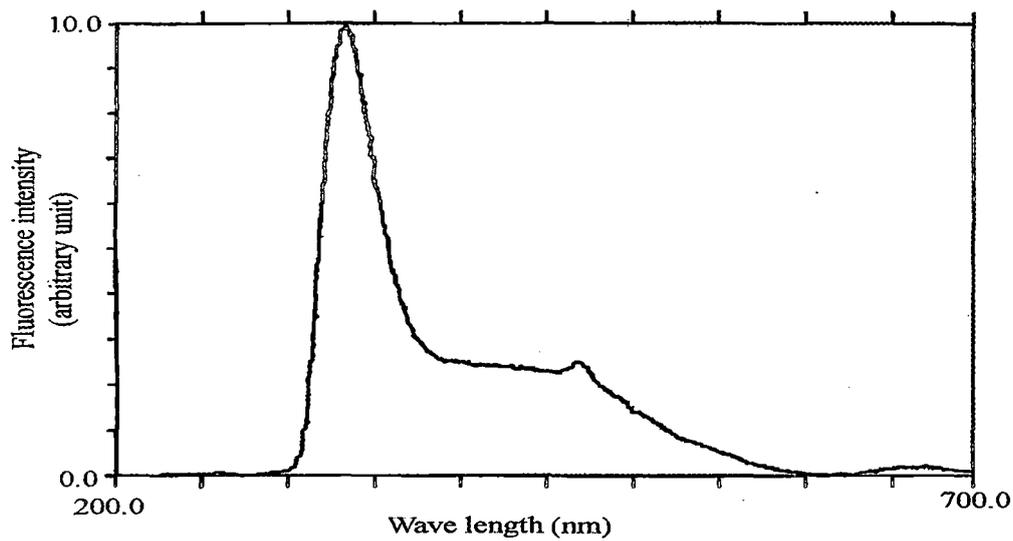


Fig. 53. Emission Spectrum of 5-Hydroxyindole ($1 \times 10^{-4} \text{M}$) in 60% 1,4-Dioxane.

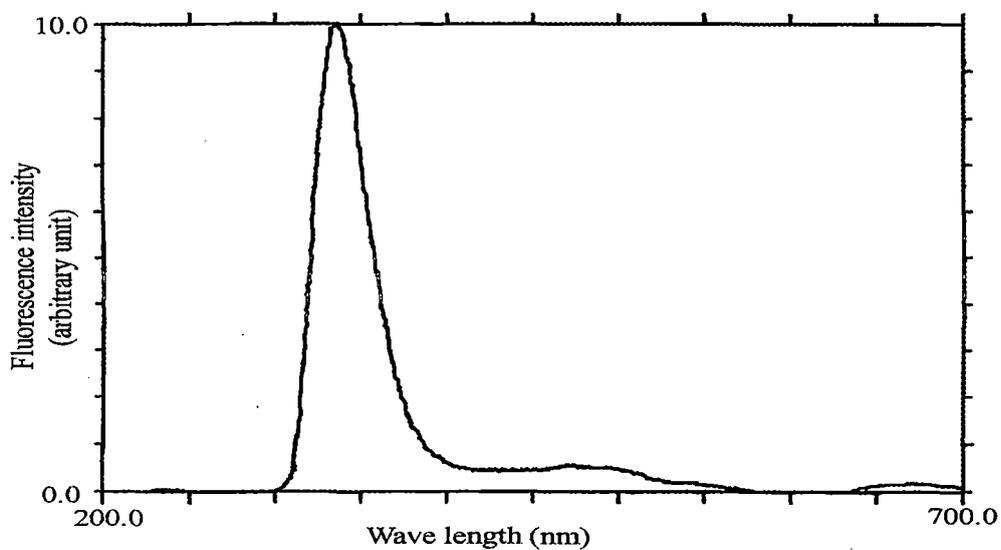


Fig. 54. Emission Spectrum of 5-Hydroxy-L-tryptophan ($1 \times 10^{-4} \text{M}$) in 80% 1,4-Dioxane.

TABLE: 1

Transition energies of absorption and emission of 1-Naphthol in water and different alkanols along with the solvent parameters at 298K

Solvent	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol ⁻¹
	Absorption	Emission				
Water	31447	22727	8720	78.4	1.3333	395.8
Methanol	31250	28571	2679	32.6	1.3288	349.8
Ethanol	31056	28490	2566	24.3	1.3576	333.1
<i>iso</i> -Propyl alcohol	30864	28490	2374	20.1	1.3859	327.6
<i>tert</i> -Butyl Alcohol	30769	28490	2279	17.1	1.3993	325.1
Pentanol	30675	28409	2266	15.2	1.4101	315.5
Hexanol	30488	28409	2079	13.4	1.4198	311.3
Heptanol	30303	28409	1894	11.2	1.4249	304.2
Octanol	30211	28409	1802	10.0	1.4509	303.3

TABLE: 2

Transition energies of absorption and emission of 2-Naphthol in water and different alkanols along with the solvent parameters at 298K

Solvent	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z / kJmol^{-1}
	Absorption	Emission				
Water	30303	28169	2134	78.4	1.3333	395.8
Methanol	30120	28090	2030	32.6	1.3288	349.8
Ethanol	30030	28090	1940	24.3	1.3576	333.1
<i>iso</i> -Propyl alcohol	29940	28090	1850	20.1	1.3859	327.6
<i>tert</i> -Butyl Alcohol	29674	28011	1663	17.1	1.3993	325.1
Pentanol	29586	27933	1653	15.2	1.4101	315.5
Hexanol	29499	27933	1566	13.4	1.4198	311.3
Heptanol	29412	27778	1634	11.2	1.4249	304.2
Octanol	29326	27778	1548	10.0	1.4509	303.3

TABLE: 3

Transition energies of absorption and emission of 5-Hydroxyindole in water and different alkanols along with the solvent parameters at 298K

Solvent	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z / kJmol^{-1}
	Absorption	Emission				
Water	32258	29851	2407	78.4	1.3333	395.8
Methanol	32051	29762	2289	32.6	1.3288	349.8
Ethanol	31874	29674	2173	24.3	1.3576	333.1
<i>iso</i> -Propyl alcohol	31746	29586	2160	20.1	1.3859	327.6
<i>tert</i> -Butyl Alcohol	31646	29586	2060	17.1	1.3993	325.1
Pentanol	31546	29499	2047	15.2	1.4101	315.5
Hexanol	31348	29412	1936	13.4	1.4198	311.3
Heptanol	31259	29412	1838	11.2	1.4249	304.2
Octanol	31056	29412	1644	10.0	1.4509	303.3

TABLE: 4

Transition energies of absorption and emission of 5-Hydroxy-L-Tryptophan in water and different alkanols along with the solvent parameters at 298K

Solvent	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z / kJmol^{-1}
	Absorption	Emission				
Water	31948	29499	2449	78.4	1.3333	395.8
Methanol	31646	29412	2234	32.6	1.3288	349.8
Ethanol	31546	29412	2134	24.3	1.3576	333.1
<i>iso</i> -Propyl alcohol	31447	29240	2207	20.1	1.3859	327.6
<i>tert</i> -Butyl Alcohol	31250	29155	2095	17.1	1.3993	325.1
Pentanol	31152	29155	1997	15.2	1.4101	315.5
Hexanol	31056	29070	1986	13.4	1.4198	311.3
Heptanol	30960	29070	1890	11.2	1.4249	304.2
Octanol	30864	29070	1974	10.0	1.4509	303.3

TABLE: 5

Transition energies of absorption and emission of L-Tyrosinemethylester in water and different alkanols along with the solvent parameters at 298K

Solvent	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z / kJmol^{-1}
	Absorption	Emission				
Water	35714	32258	3456	78.4	1.3333	395.8
Methanol	35416	32258	3203	32.6	1.3288	349.8
Ethanol	35336	32258	3078	24.3	1.3576	333.1
<i>iso</i> -Propyl alcohol	35088	32154	2934	20.1	1.3859	327.6
<i>tert</i> -Butyl Alcohol	34965	32051	2914	17.1	1.3993	325.1
Pentanol	34843	32051	2792	15.2	1.4101	315.5
Hexanol	34722	31949	2773	13.4	1.4198	311.3
Heptanol	34602	31949	2653	11.2	1.4249	304.2
Octanol	34364	31847	2517	10.0	1.4509	303.3

TABLE: 6

Transition energies of absorption and emission of 1-Naphthol in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/ cm^{-1}		Stokes Shift (ν)/ cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z / kJmol^{-1}
	Absorption	Emission				
10%	31746	22321	9425	69.2	1.3444	392.8
20%	31646	22321	9325	61.9	1.3515	379.1
30%	31546	22371	9175	53.2	1.3675	363.2
50%	31348	22422	8926	40.7	1.3810	347.8
60%	31250	22422	8828	27.2	1.3910	335.6
80%	31056	22472	8584	11.9	1.4070	319.7

TABLE: 7

Transition energies of absorption and emission of 2-Naphthol in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/cm⁻¹		Stokes Shift (ν)/cm⁻¹	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol⁻¹
	Absorption	Emission				
10%	32789	30120	2669	69.2	1.3444	392.8
20%	32680	30211	2469	61.9	1.3515	379.1
30%	32573	30303	2270	53.2	1.3675	363.2
50%	32362	30488	1874	40.7	1.3810	347.8
60%	32258	30581	1677	27.2	1.3910	335.6
80%	32258	30675	1583	11.9	1.4070	319.7

TABLE: 8

Transition energies of absorption and emission of 5-Hydroxyindole in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/cm⁻¹		Stokes Shift (ν)/cm⁻¹	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol⁻¹
	Absorption	Emission				
10%	32258	30120	2138	69.2	1.3444	392.8
20%	32154	30120	2034	61.9	1.3515	379.1
30%	32051	30211	1840	53.2	1.3675	363.2
50%	31847	30303	1544	40.7	1.3810	347.8
60%	31746	30488	1258	27.2	1.3910	335.6
80%	31546	30488	1058	11.9	1.4070	319.7

TABLE: 9

Transition energies of absorption and emission of 5-Hydroxy-L-tryptophan in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/cm⁻¹		Stokes Shift (ν)/cm⁻¹	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol⁻¹
	Absorption	Emission				
10%	32051	29851	2200	69.2	1.3444	392.8
20%	31949	30030	1919	61.9	1.3515	379.1
30%	31746	30120	1626	53.2	1.3675	363.2
50%	31646	30120	1526	40.7	1.3810	347.8
60%	31447	30211	1236	27.2	1.3910	335.6
80%	31250	30303	0947	11.9	1.4070	319.7

TABLE: 10

Transition energies of absorption and emission of L-Tyrosinemethylester in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/cm^{-1}		Stokes Shift (ν)/cm^{-1}	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol⁻¹
	Absorption	Emission				
10%	36496	21231	15265	69.2	1.3444	392.8
20%	36366	21231	15135	61.9	1.3515	379.1
30%	36232	21231	15001	53.2	1.3675	363.2
50%	35971	21277	14694	40.7	1.3810	347.8
60%	35842	21277	14565	27.2	1.3910	335.6
80%	35714	21277	14437	11.9	1.4070	319.7

TABLE: 11

Transition energies of absorption and emission of L-Tyrosine in 1,4-Dioxane-water mixtures along with the medium parameters at 298K

Dioxane % (v/v)	Transition Energy (ν)/cm⁻¹		Stokes Shift (ν)/cm⁻¹	Dielectric Constant(D)	Refractive Index(n)	Kosower-Z /kJmol⁻¹
	Absorption	Emission				
10%	35714	21008	14706	69.2	1.3444	392.8
20%	35461	21008	14453	61.9	1.3515	379.1
30%	35211	21053	14158	53.2	1.3675	363.2
50%	35088	21053	14035	40.7	1.3810	347.8
60%	34965	21097	13868	27.2	1.3910	335.6
80%	34722	21097	13625	11.9	1.4070	319.7

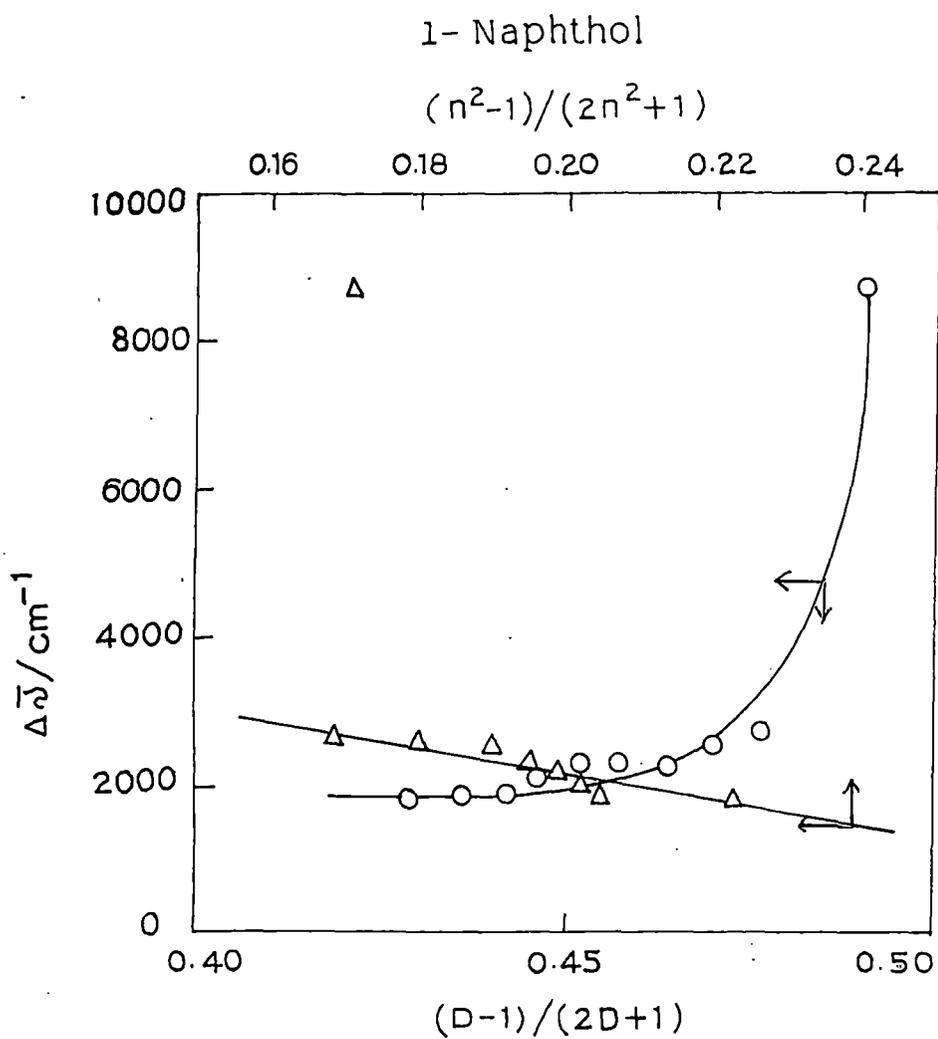


Fig.55. The plot of Stokes' shift, $\Delta\bar{\nu}$ against dielectric constant function and the refractive index function of the solvents (water and alkanols).

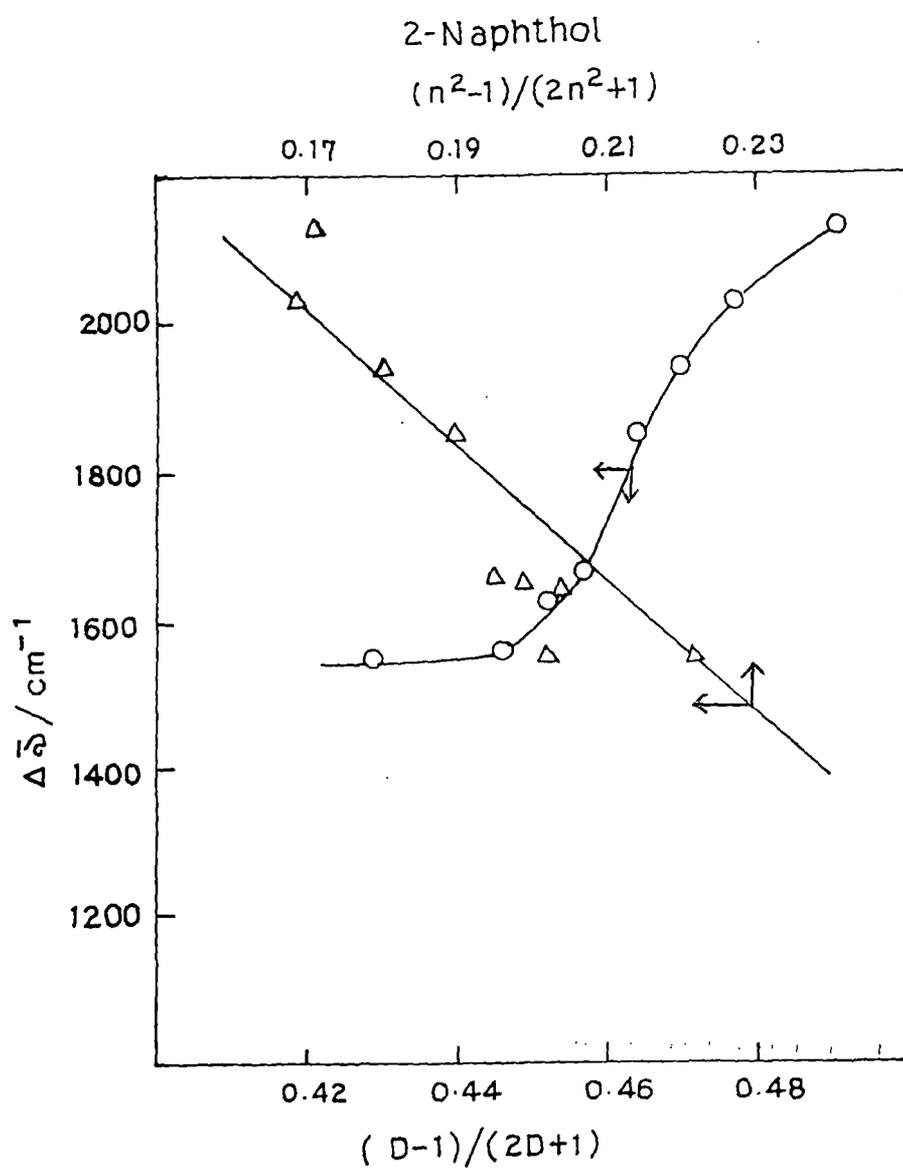


Fig.56. The plot of Stokes' shift, $\Delta\bar{\nu}$ against dielectric constant function and the refractive index function of the solvents (water and alcohols).

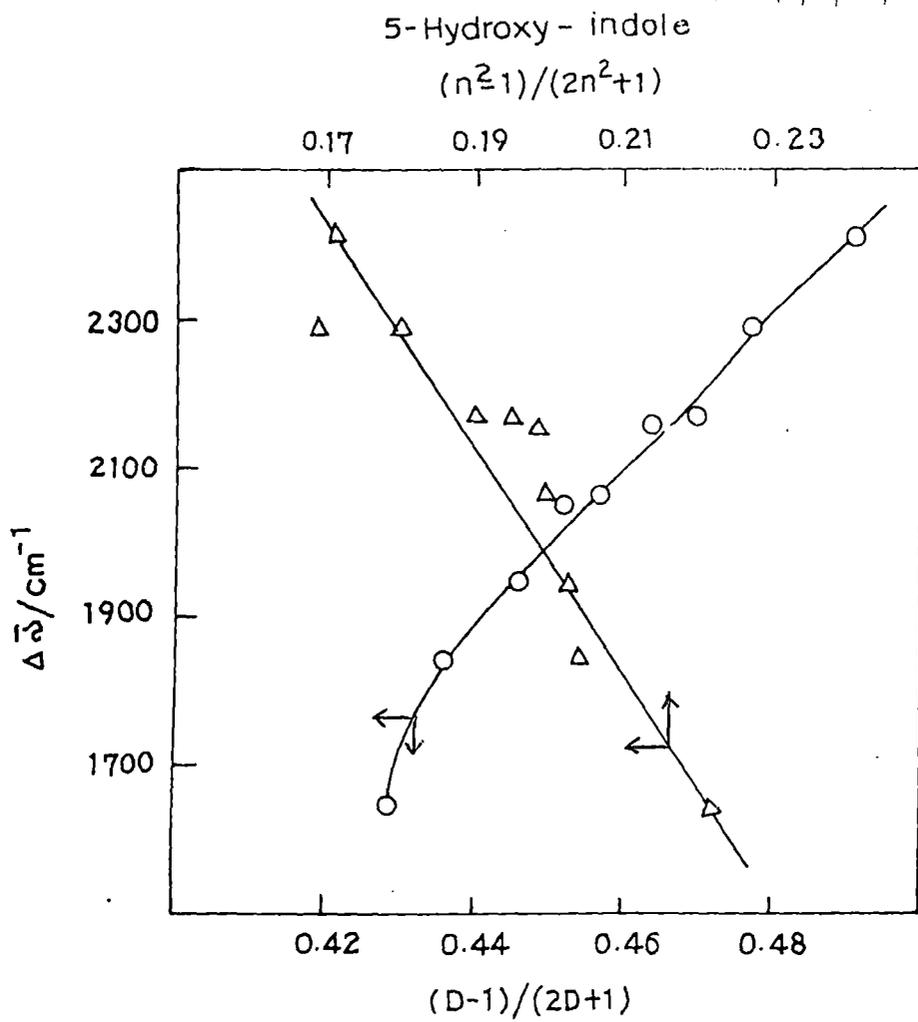


Fig.57. The plot of Stokes' shift, $\Delta\bar{\nu}$ against dielectric constant function and the refractive index function of the solvents (water and alkanols).

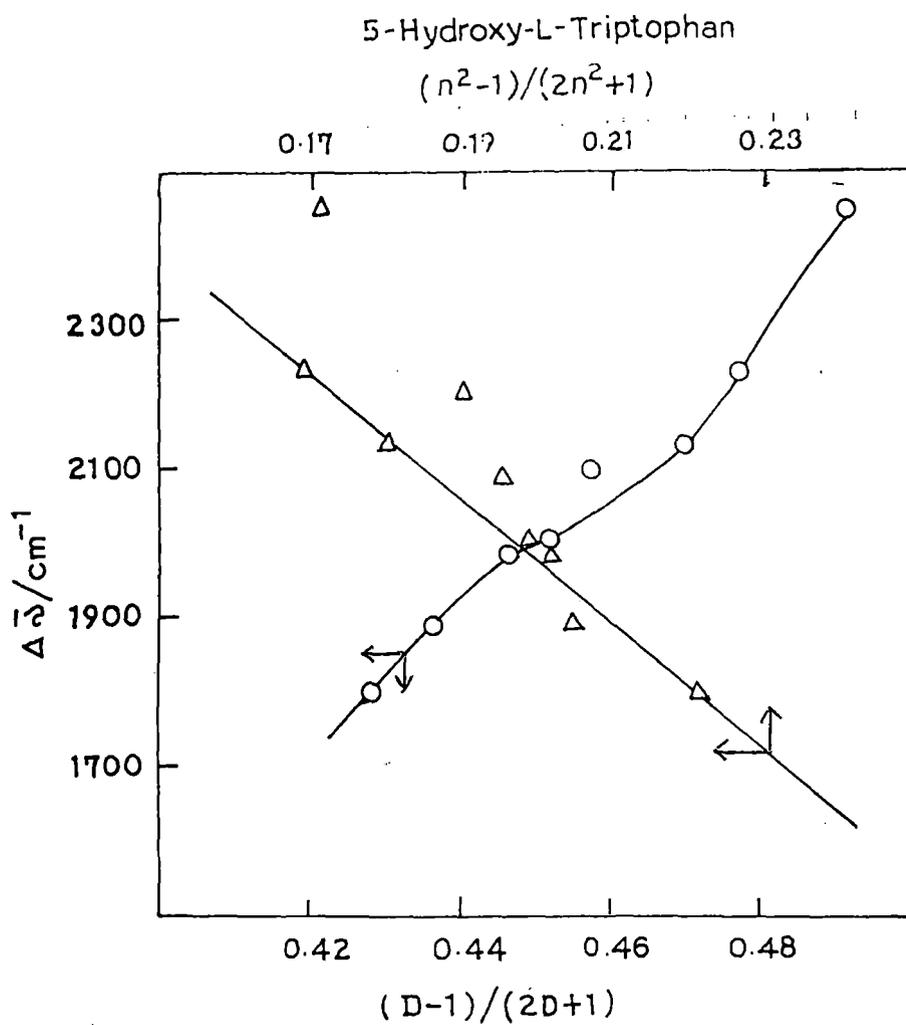


Fig.58. The plot of Stokes' shift, $\Delta\bar{\nu}$ against dielectric constant function and the refractive index function of the solvents (water and alkanols).

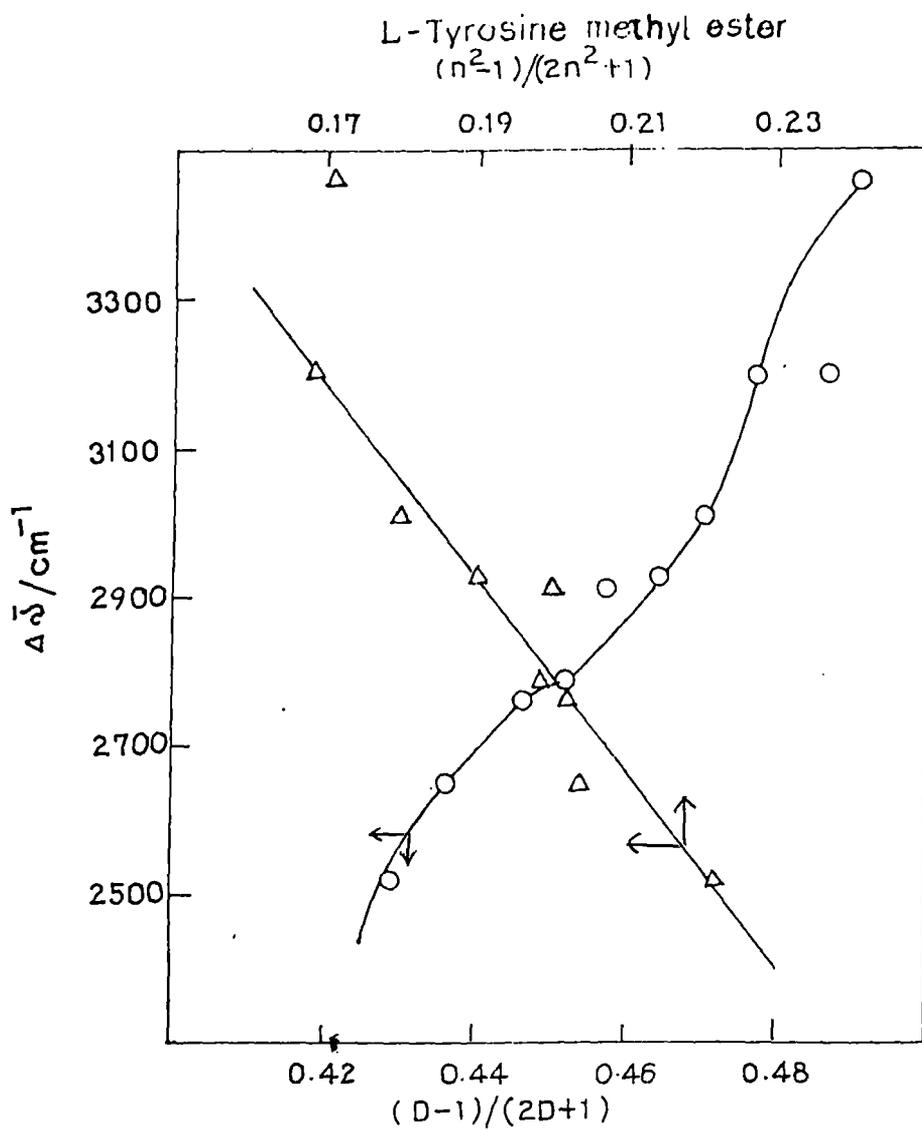


Fig.59. The plot of Stokes' shift, $\Delta\bar{\nu}$ against dielectric constant function and the refractive index function of the solvents (water and alkanols).

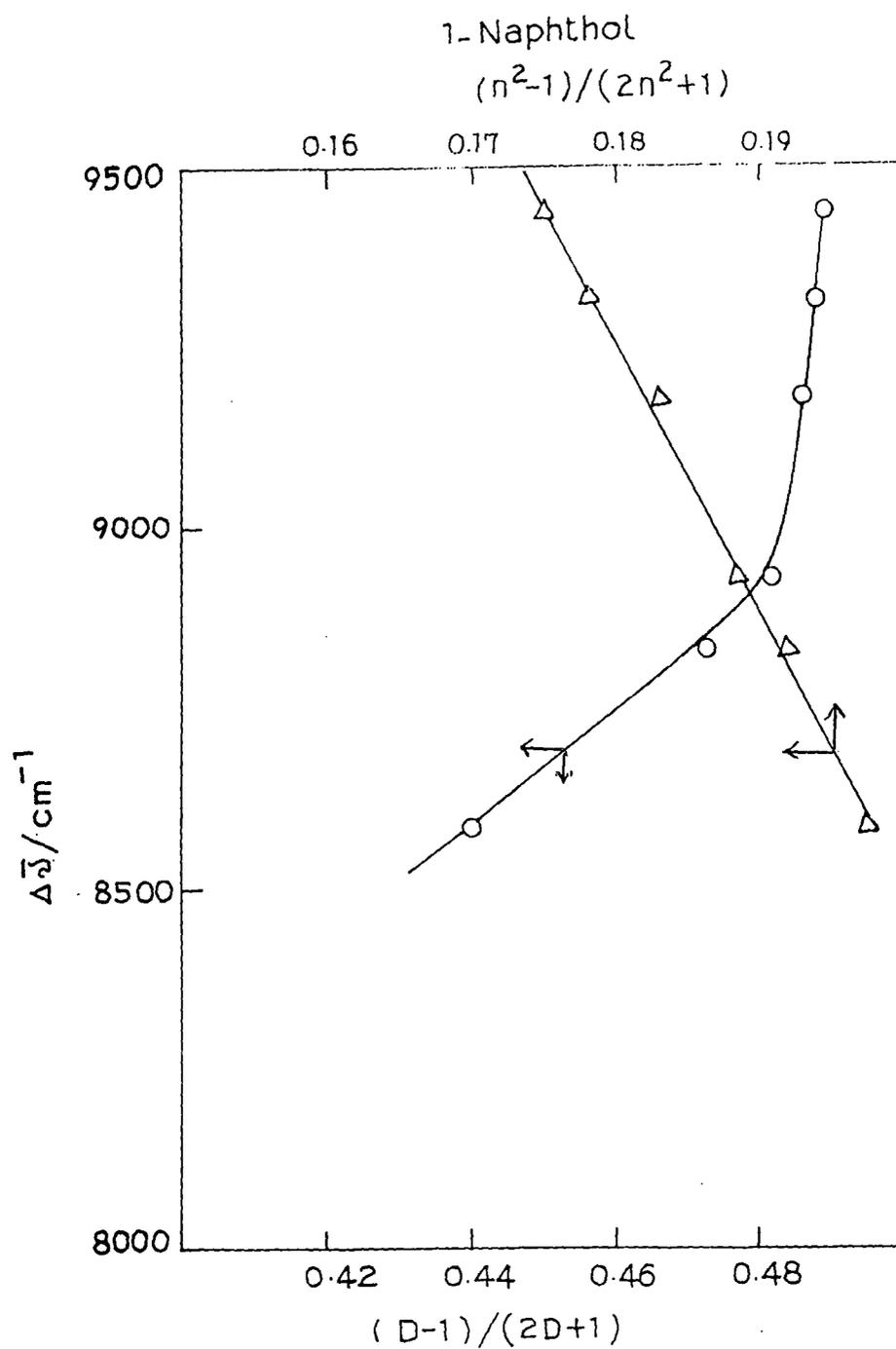


Fig.60. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

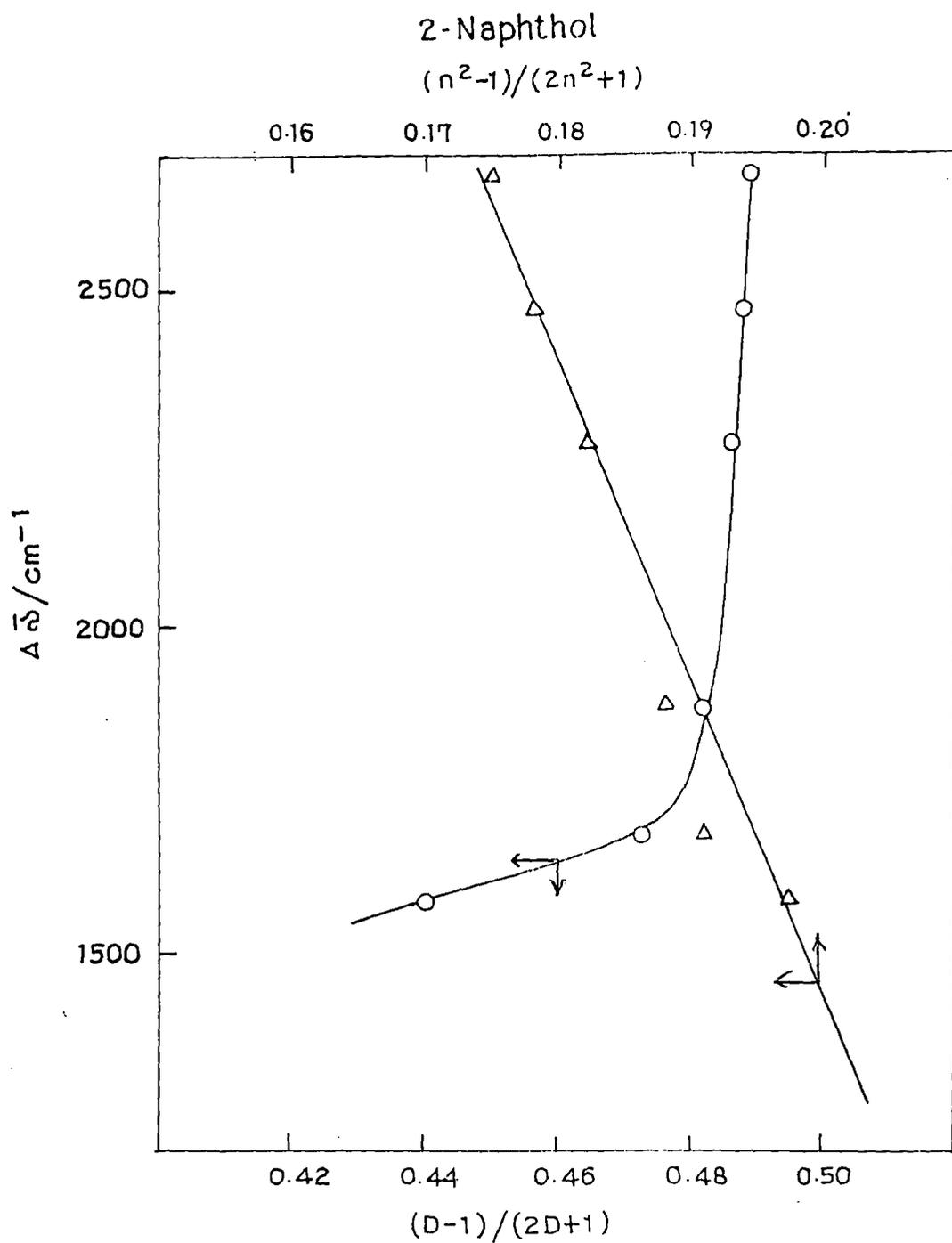


Fig.61. The plot of Stokes' shift, $\Delta \bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

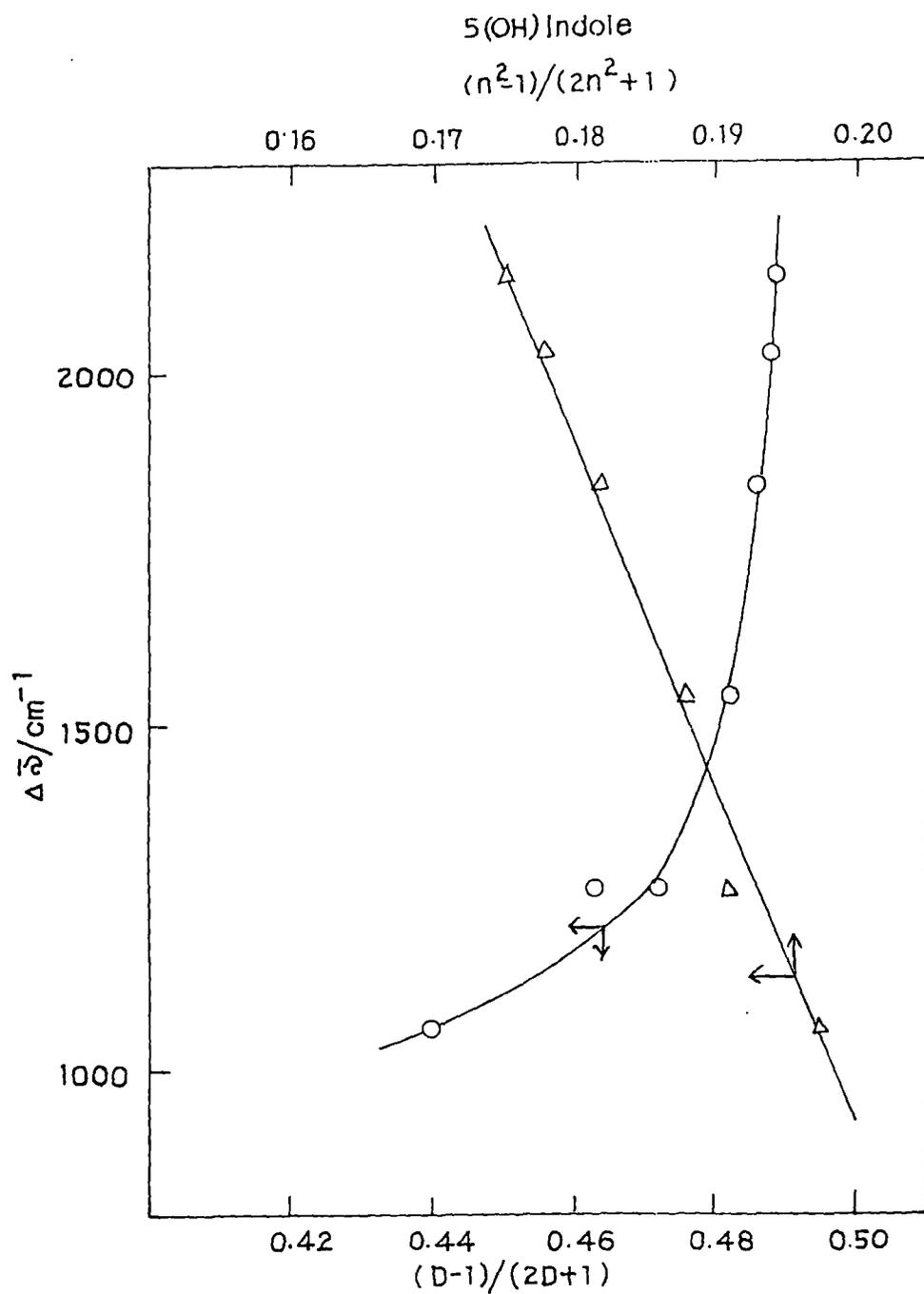


Fig.62. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

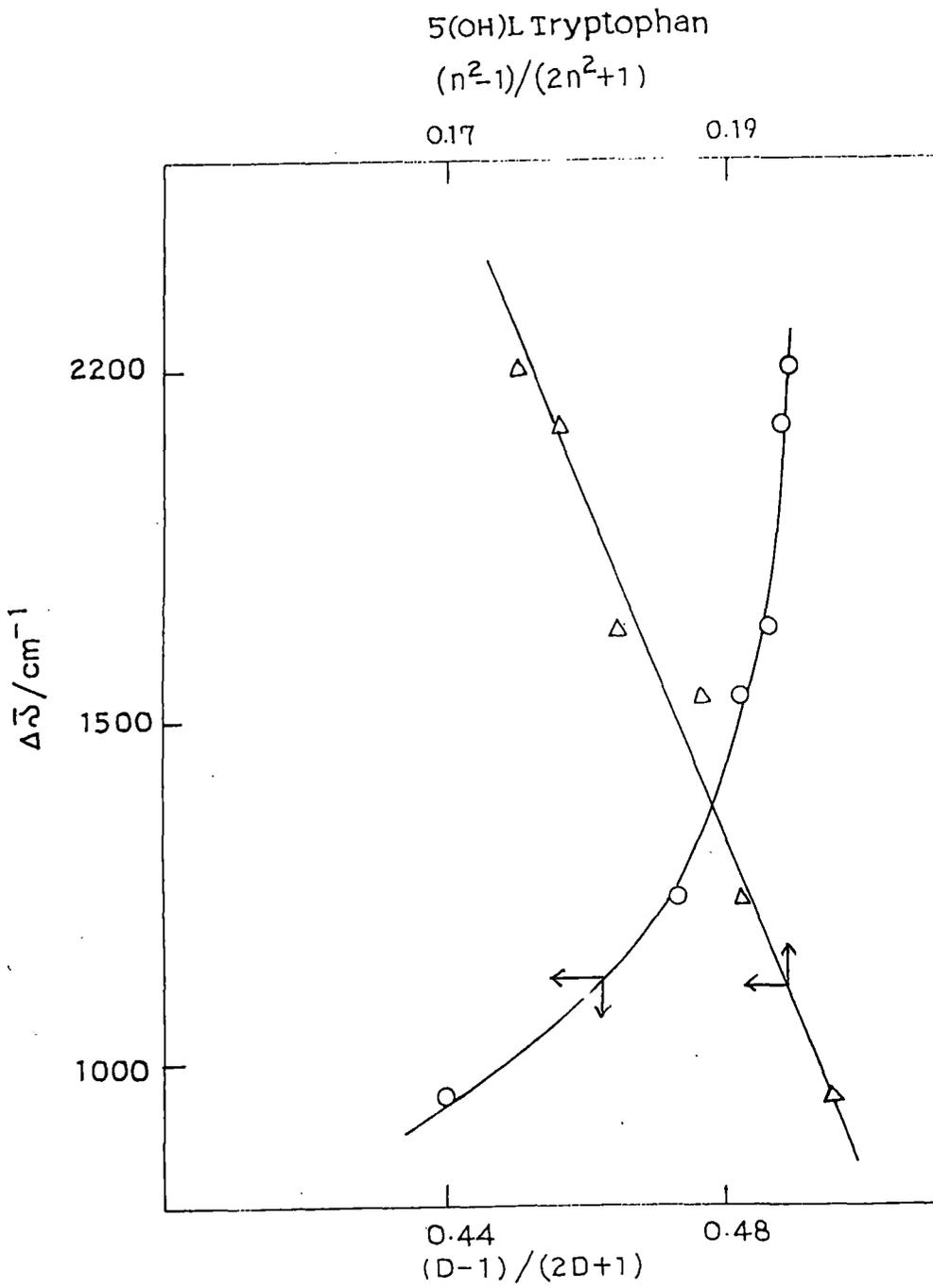


Fig.63. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

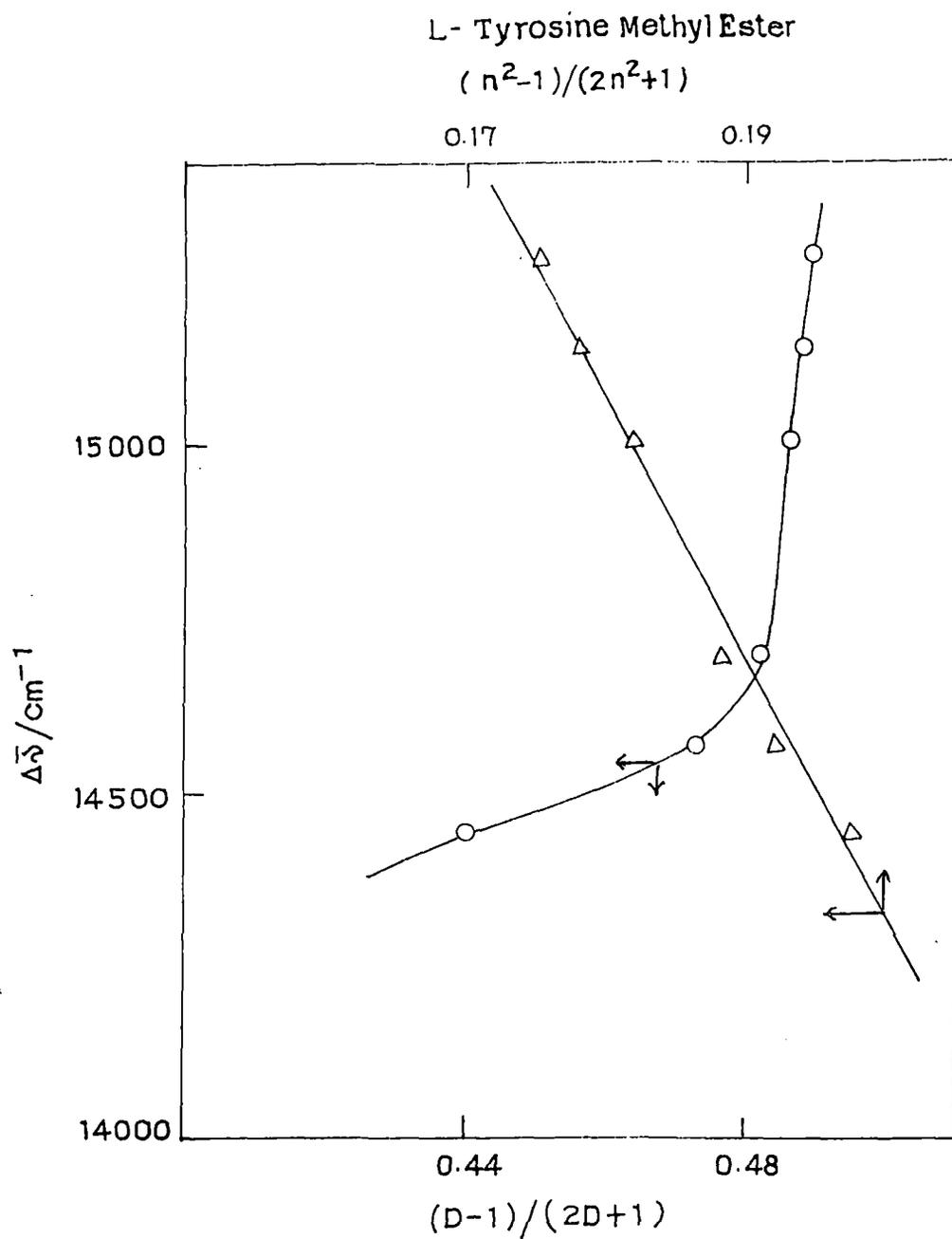


Fig.64. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

L-Tyrosine

$$(n^2-1)/(2n^2+1)$$

0.17

0.19

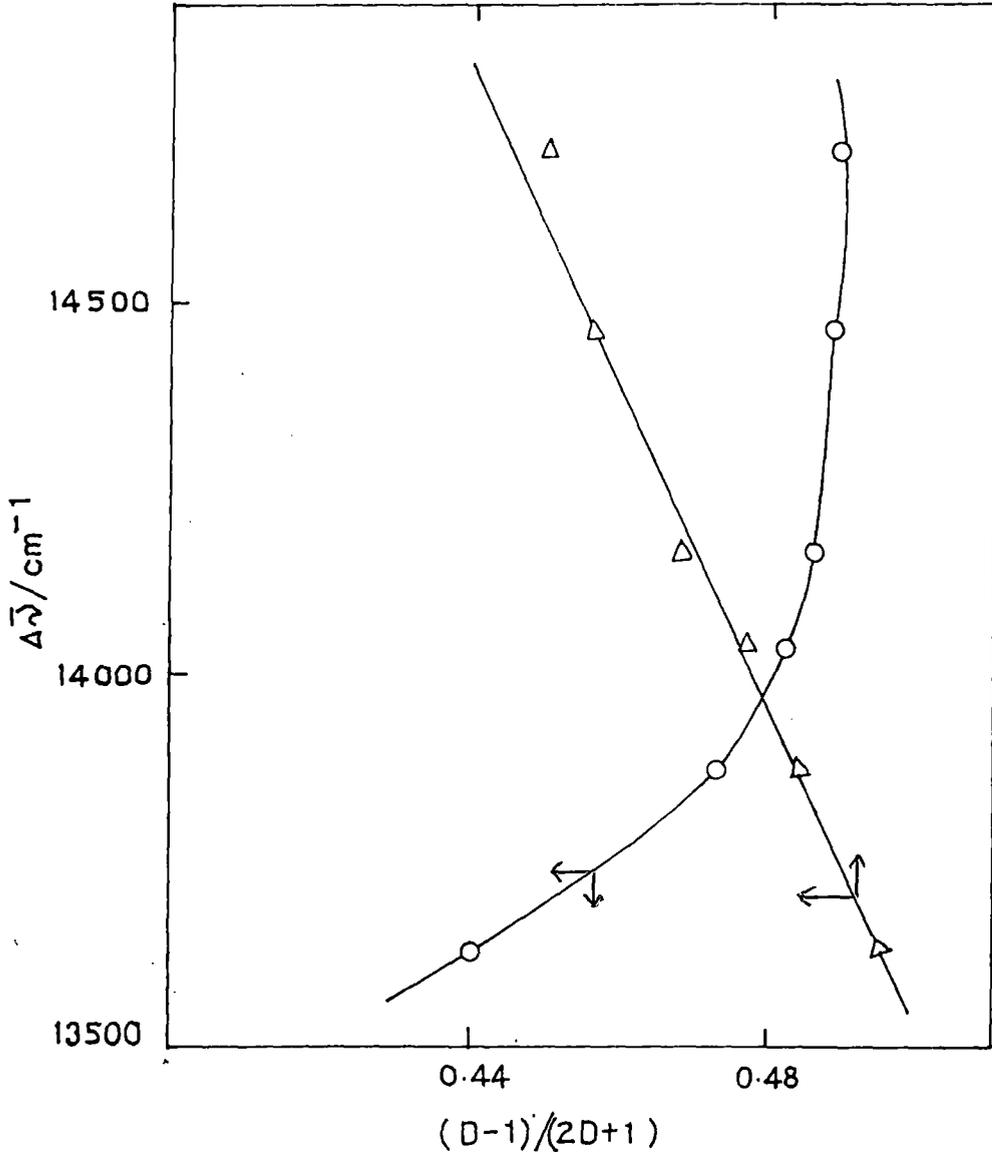


Fig. 65. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the dielectric constant function and the refractive index function of dioxane-water mixtures.

1-Naphthol

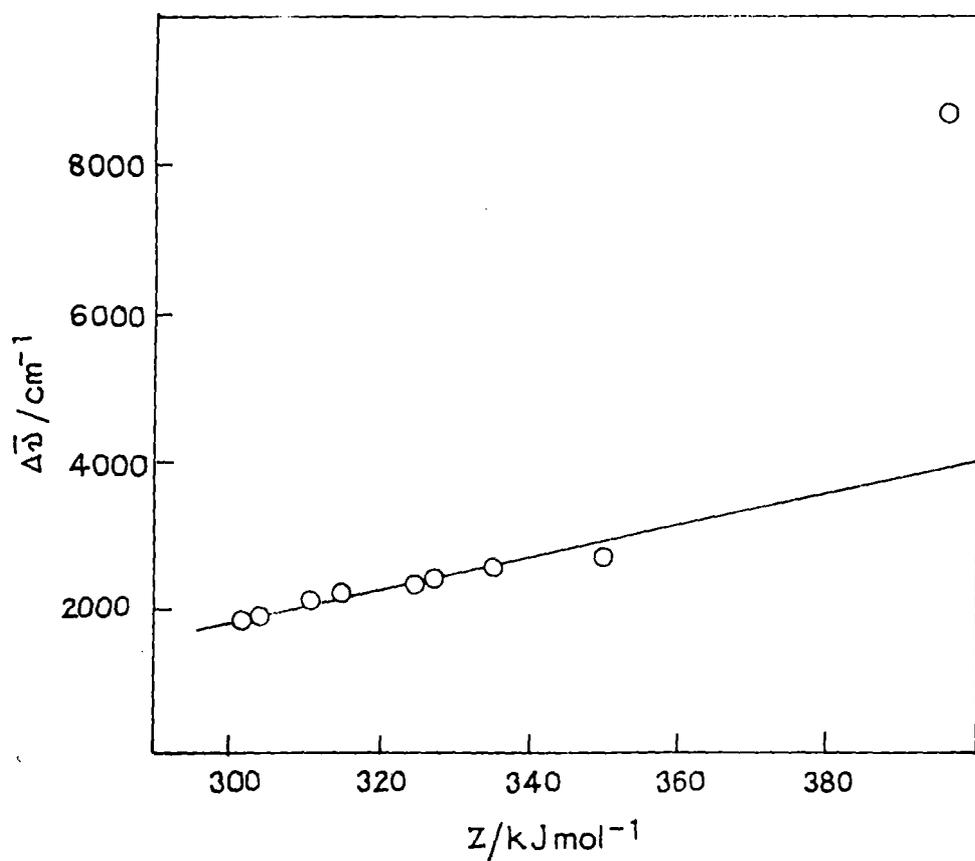


Fig.66. The plot of Stokes' shift, $\Delta\bar{\nu}$ against Kosower-Z values of the solvents (water and alkanols).

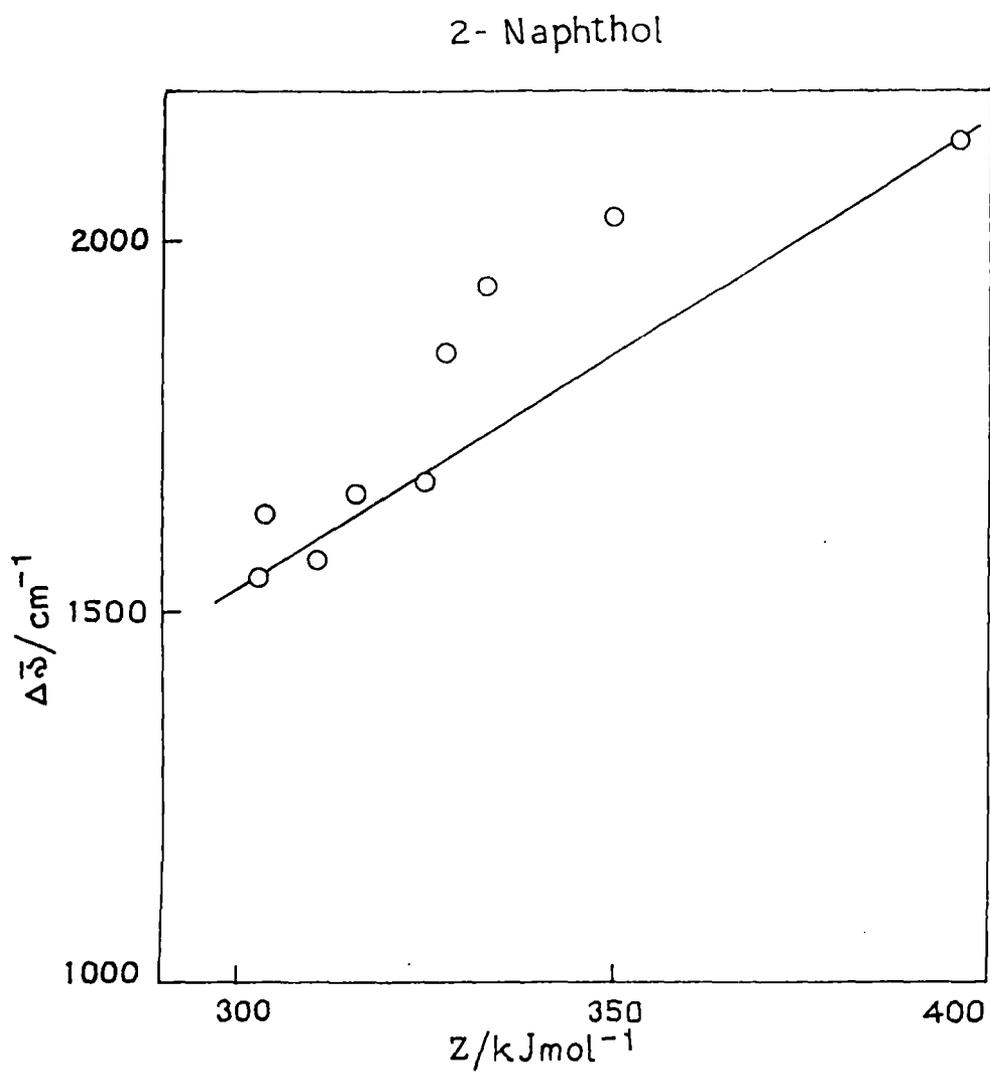


Fig.67. The plot of Stokes' shift, $\Delta\bar{\nu}$ against Kosower-Z values of the solvents (water and alkanols).

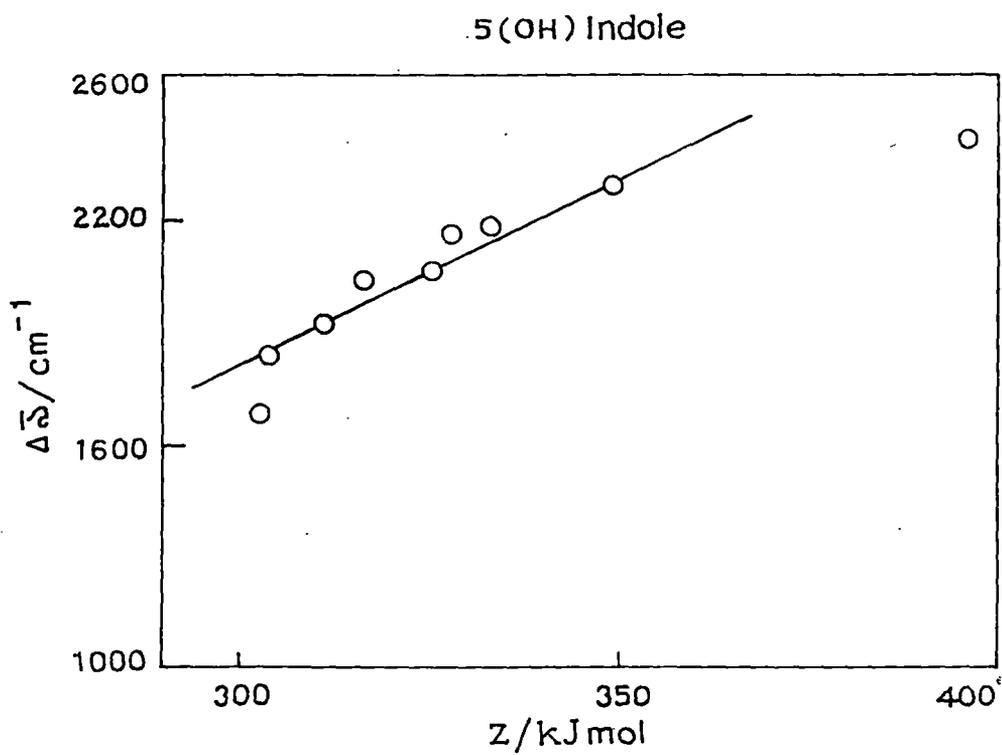


Fig.68. The plot of Stokes' shift, $\Delta\bar{\nu}$ against Kosower-Z values of the solvents (water and alkanols).

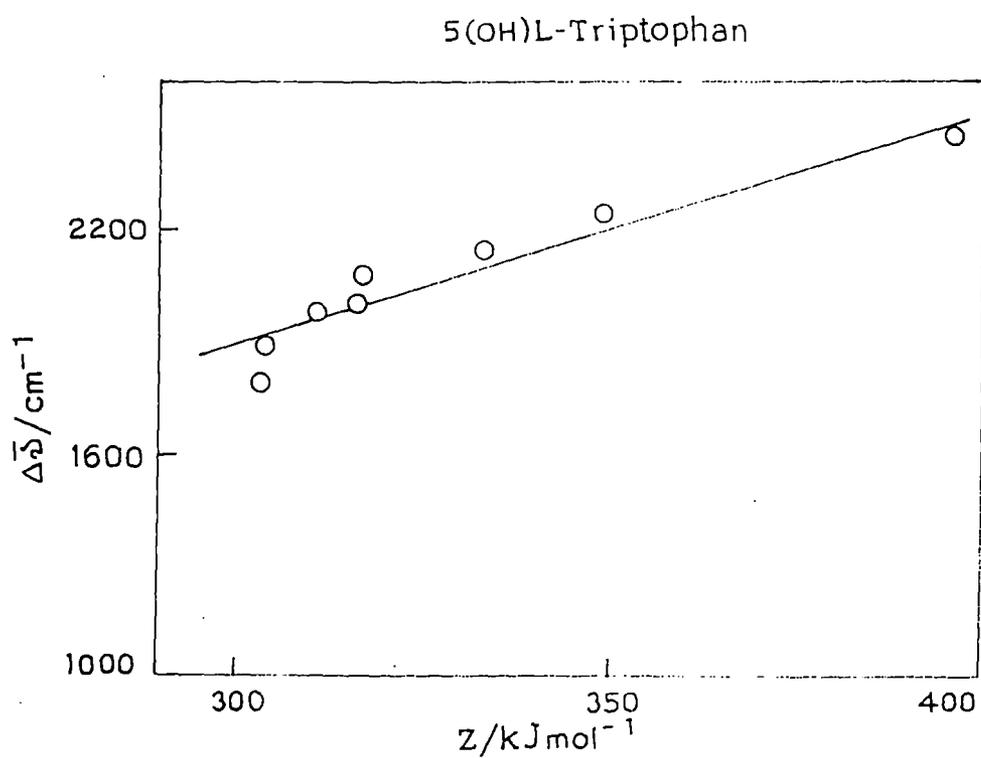


Fig.69. The plot of Stokes' shift, $\Delta\bar{\nu}$ against Kosower-Z values of the solvents (water and alkanols).

L- Tyrosine Methyl Ester

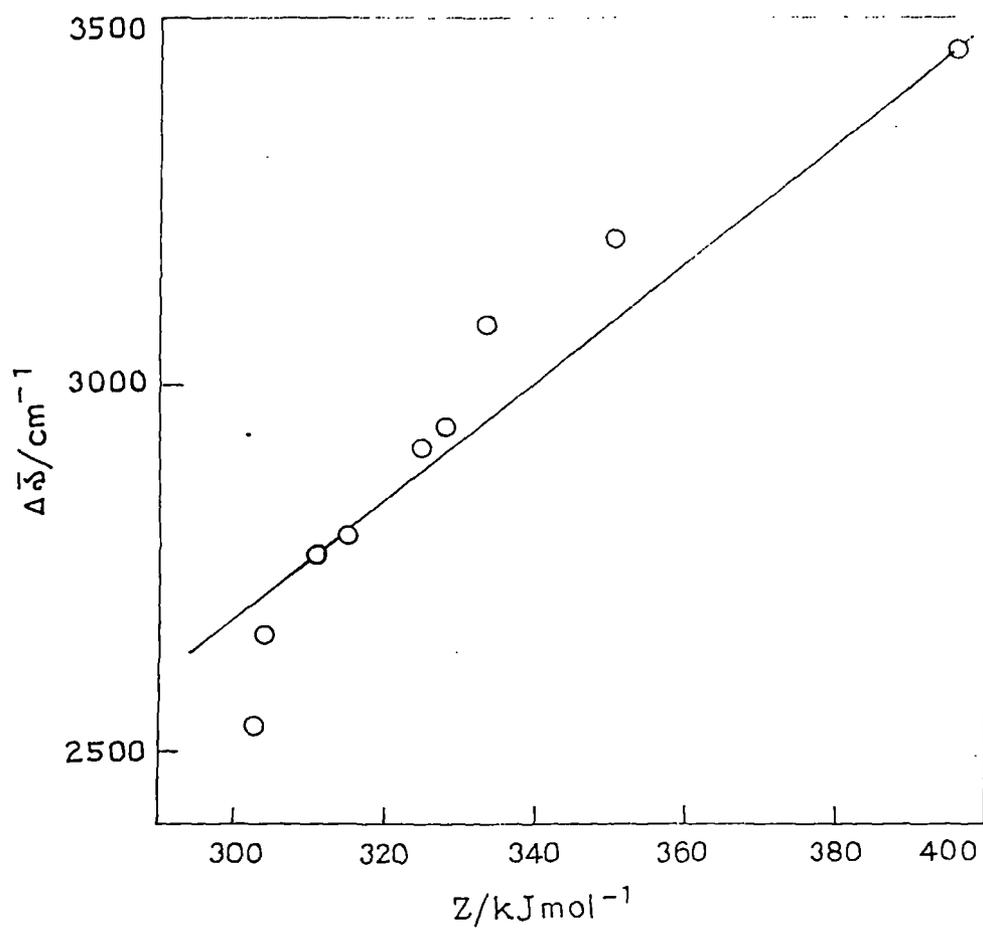


Fig.70. The plot of Stokes' shift, $\Delta\bar{\nu}$ against Kosower-Z values of the solvents (water and alkanols).

1. Naphthol

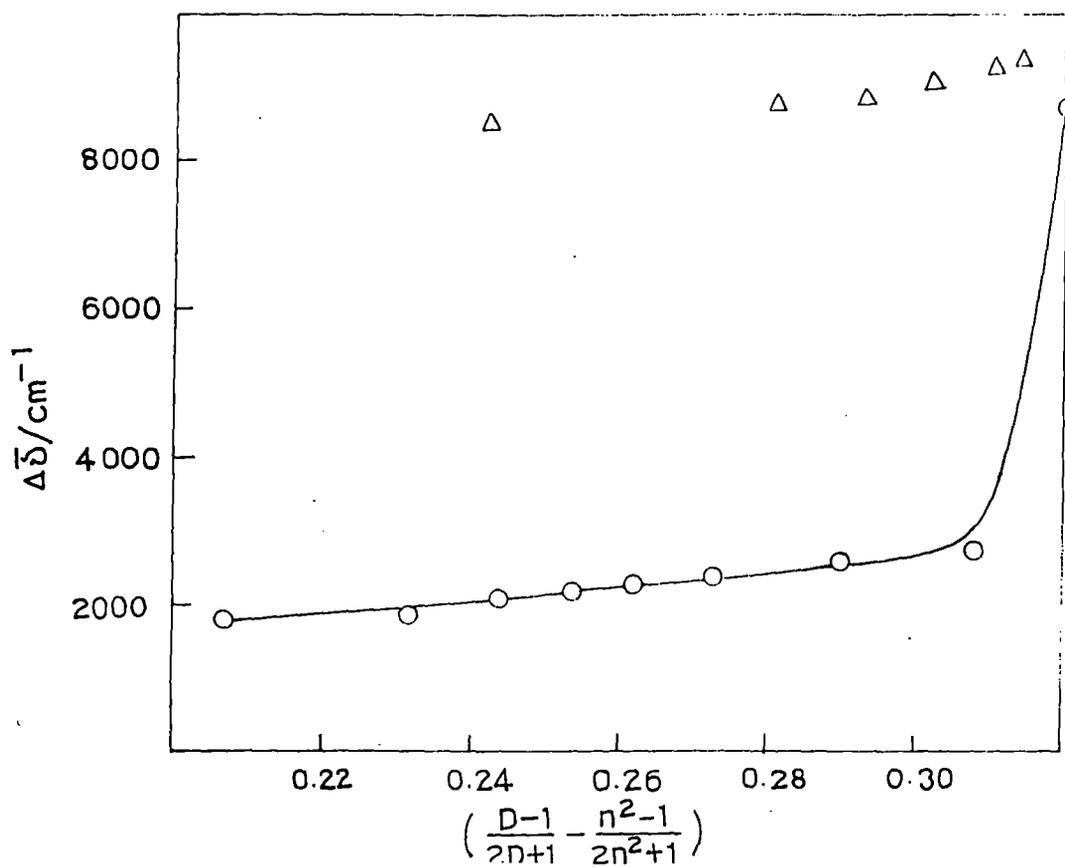


Fig.71. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the solvent polarity function, $\left[\frac{D-1}{2D+1}\right] - \left[\frac{n^2-1}{2n^2+1}\right]$ of different alkanols [O] and dioxane-water mixtures [Δ].

2-Naphthol

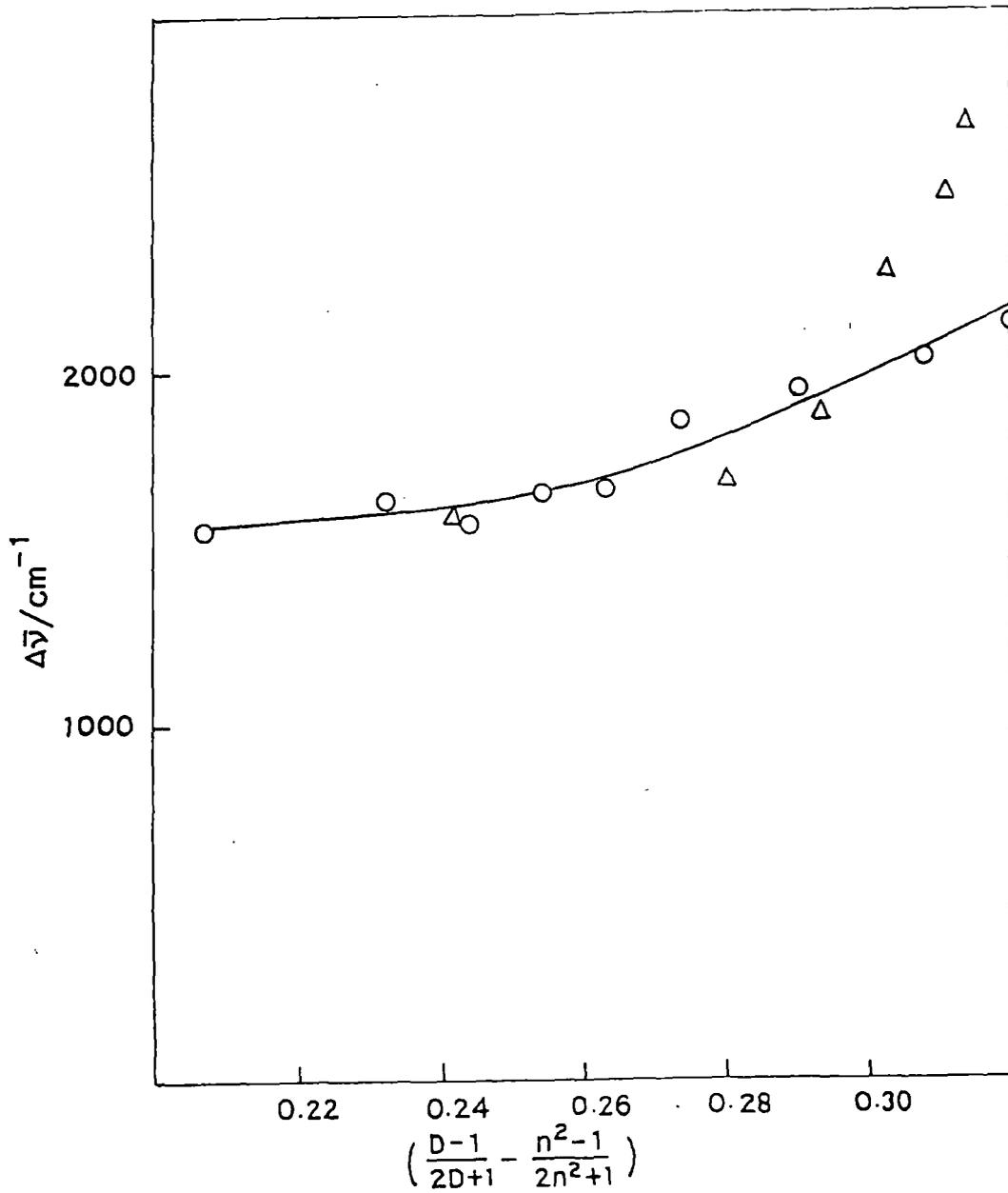


Fig.72. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the solvent polarity function, $\left[\frac{D-1}{2D+1}\right] - \left[\frac{n^2-1}{2n^2+1}\right]$ of different alkanols [O] and dioxane-water mixtures [Δ].

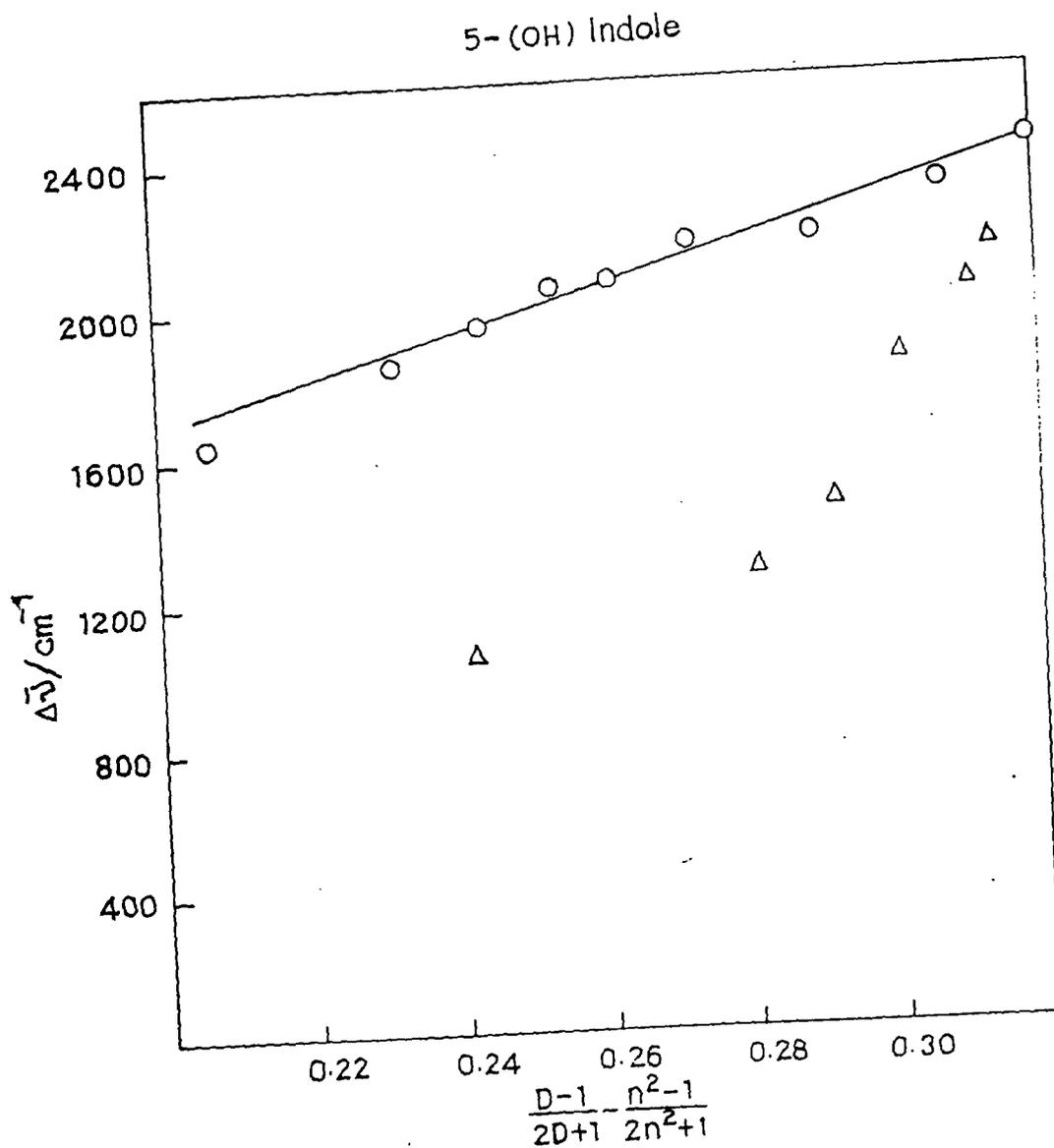


Fig. 73. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the solvent polarity function, $[(D-1)/(2D+1)] - [(n^2-1)/(2n^2+1)]$ of different alkanols [O] and dioxane-water mixtures [Δ].

5 (OH)L-Tryptophan

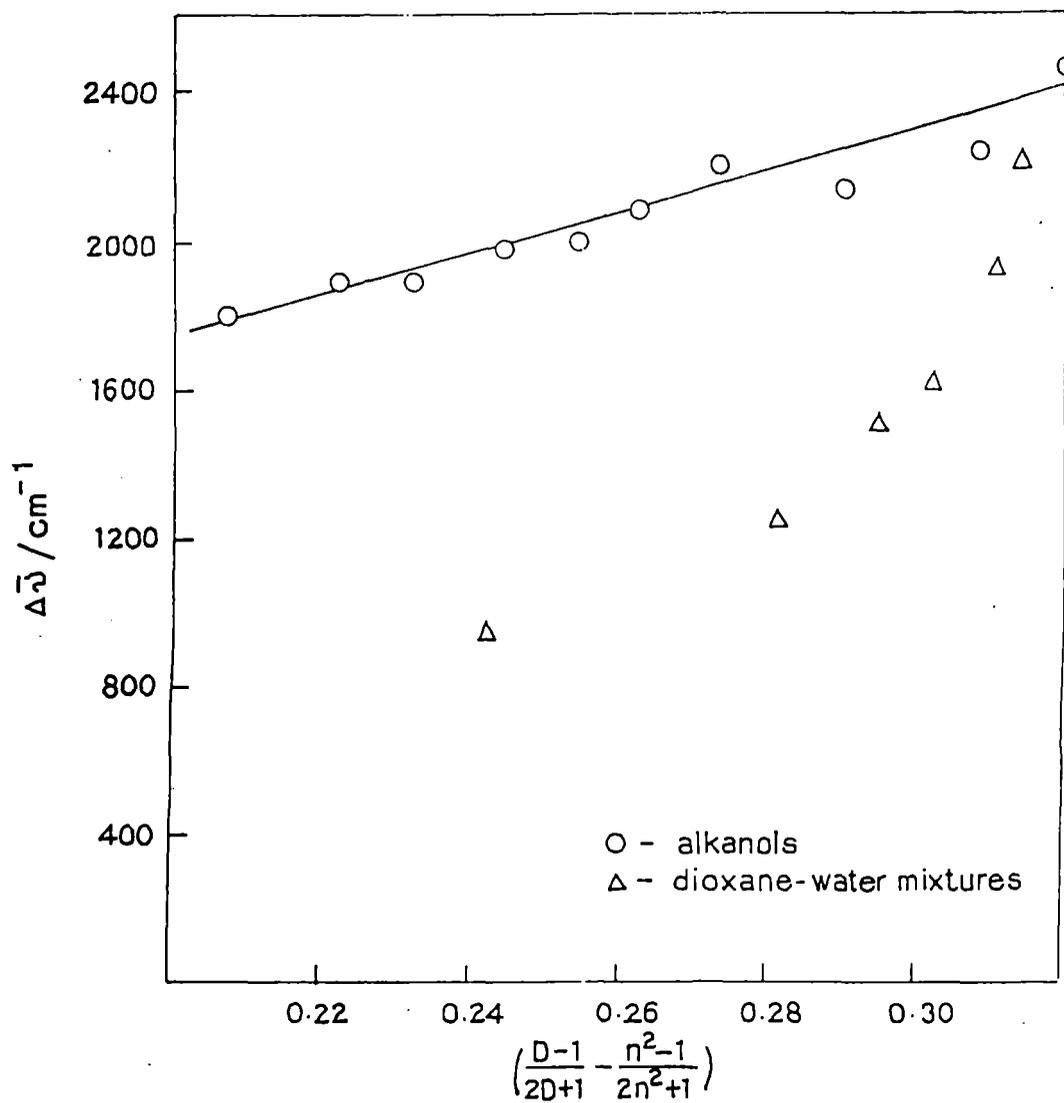


Fig. 74. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the solvent polarity function, $[(D-1)/(2D+1)] - [(n^2-1)/(2n^2+1)]$ of different alkanols [O] and dioxane-water mixtures [Δ].

L-Tyrosine Methyl Ester

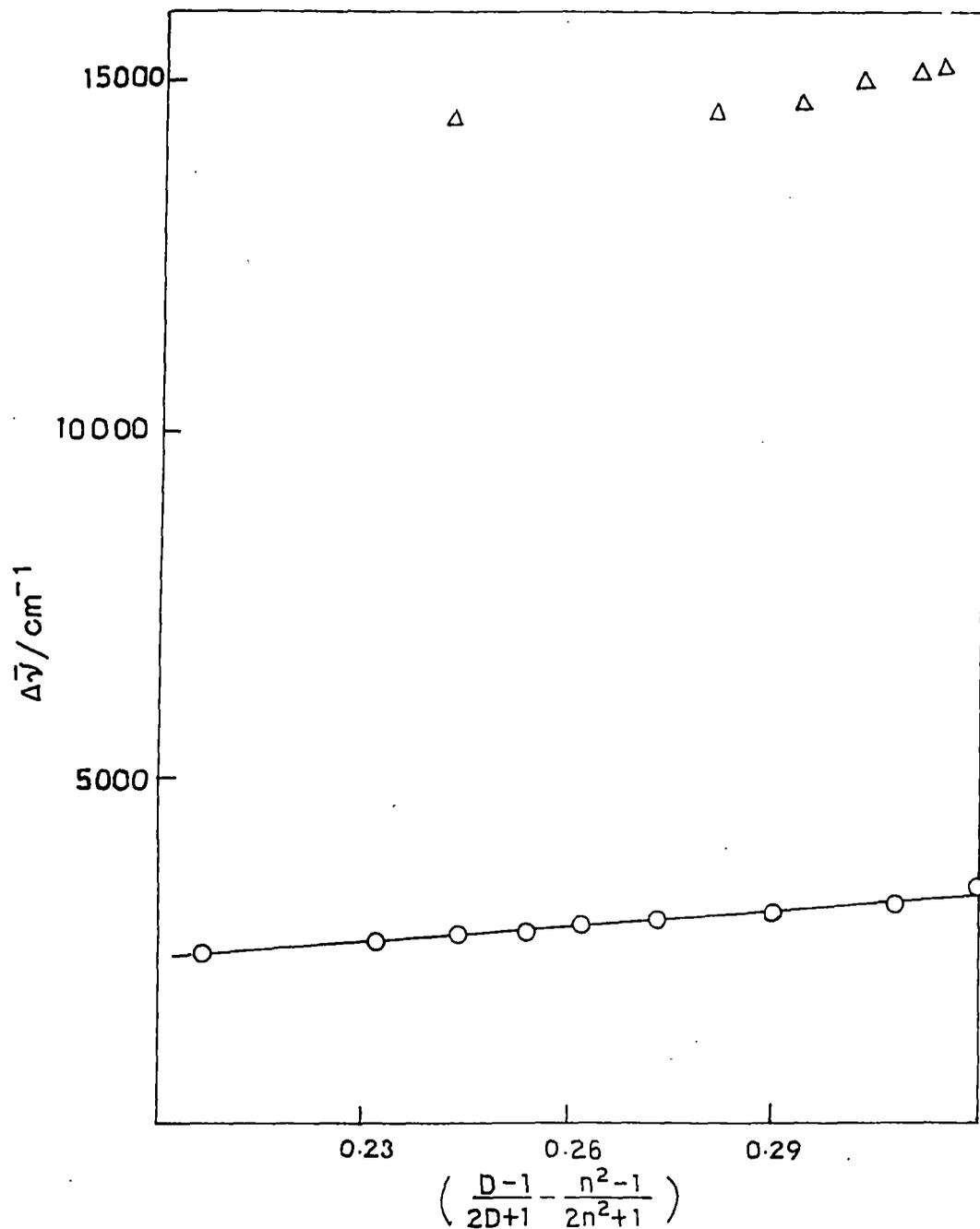


Fig. 75. The plot of Stokes' shift, $\Delta\bar{\nu}$ against the solvent polarity function, $[(D-1)/(2D+1)] - [(n^2-1)/(2n^2+1)]$ of different alkanols [O] and dioxane-water mixtures [Δ].

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