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Excitation wavelength dependence of emission spectra of polymer films with doped Acridine orange

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Abstract: *In the present study of the absorption and emission spectra of acridine orange in polyvinyl alcohol (PVA), Polymethyl methacrylate (PMMA) and Polyvinyl acetate (PVAc) have been reported. An interesting part of the study is the excitation wavelength dependence of the emission spectra of these doped polymers.*

Key Words: *Acidine orange, PMMA,PVA, PVAc, excitation wavelength dependence*.

1. INTRODUCTION:

Acridine orange (AO) is one of the basic dyes exhibiting a typical binding to polynucleotide's [1]. When acridine orange is bound to polynucleotide, two types of complexes, complex I and complex II, are formed depending on the concentration ratio of polynucleotide to dye [2-4]. Acridine orange has been identified as a class of molecules which show proton transfer reaction in the excited state. The emission proportion and acid base reaction of acridine orange are well known [5-6]. Acridine orange molecules form hydrogen bonded complexes with solvent molecules. The hydrogen bonded proton can occupy two positions, one near the oxygen of alcohol and the other at nitrogen of acridine orange. Acridine orange shows almost total absence of fluorescence in hydrocarbons relative to alcoholic solvents, which is a well known phenomenon for many nitrogen heterocyclic compounds [4]. The protolytic reactions of excited acridine have been studied extensively by A. Weller [7-8]. The pK_a of acridine orange in the ground state is 5.5 whereas in the excited state it increases to 10.6. Thus it is a much stronger base in the excited state than in the ground state. On the basis of these considerations one would therefore expect that aqueous acridine solutions at around pH=11 will show a sudden change in fluorescence, which is more or less independent of the sudden change in absorption at lower pH. It is obvious that at pH values below 5 the absorption and emission corresponding to acridinium ion. At pH=11 acridine orange only will be the absorbing and emitting species [9-10]. Previously some work has been done about the studies of fluorescence properties of acridine orange in polymer matrix. In polymers, acridine orange absorption and emission spectra show concentration dependence and this has been attributed to the equilibrium between monomers and aggregates [11-13]. Schnidt and Chambers et al. [14] have studied the behavior of the triplet state as a function of dye concentration. It was found that dye molecules aggregate to form a complex in which the molecule are oriented in a fixed direction Komiyama et al. [15] have studied the concentration depolarization of the fluorescence and phosphorescence of acridine orange cations [16]. They have demonstrated the favorable energy transfer process in a system of dye molecules embedded in a polymer matrix by means of depolarization of fluorescence and phosphorescence as a function of dye concentration Ohtani et al. [17] have used acridine orange as photosensitizer to determine the quantum yield of photo reduction of methyl viologen dichloride to its cation in polyvinyl alcohol [18]. In the present work study of acridine orange photophysics in water and methanol with varying pH has been carried out and has been compared with the absorption and emission spectra of acridine orange in polyvinyl alcohol(PVA), Polymethyl methacrylate (PMMA) and Polyvinyl acetate (PVAc). An interesting part of the study is the excitation wavelength dependence of the emission spectra of these doped polymers.

2. EXPERIMENTAL:

Analytical reagent quality acridine orange obtained from Hi-media was used without further purification. PMMA films doped with acridine orange were prepared by dissolving PMMA grains in chloroform and mixing it with the desired concentration of probe molecules. The PVA films were prepared by dissolving PVA in water. However, PVAc films were prepared by dissolving PVAc with toluene. The films of PMMA, PVA and PVAC were obtained by drying the mass in a polypropylene dish.

3. RESULTS AND DISCUSSIONS:

3.1. ABSORPTION SPECTRA:

A mentioned earlier the pK_a values of acridine orange in the ground and first excited singlet states are 5.5 and 10.6 respectively. It is obvious that at pH values below 5 the absorption corresponds to acridinium cation and above

pH=10.6 acridine only will be the absorbing and emitting species. At pH values between 5 and 10.6 the absorption will be due to acridine orange, however, the emitting species may be either acridine or acridinium cation depending upon the rate of the protonation reaction in the excited state. This is seen from fig. 1 and fig.2 for the solution of acridine orange in water and methanol. In fig.1 (a, b, c, d, and e) the absorption spectra are similar, evidently due to the presence of acridinium cation in the ground state. The positions of absorption corresponding to (0-0) transition are at 470.3nm and 491.0nm respectively. With further increase in pH the position of the first absorption band is shifted to the violet by approximately 60nm. This absorption spectrum is characteristic of the presence of acridine in the ground state. The absorption spectra show similar behavior both in water and methanol suggesting that the presence of the acridine or acridinium ion forms are basically pH dependent. However, the differences in the absorption spectra are characteristic of the solvent solute interaction as the peak positions of absorption bands are different for water and methanol. The positions of absorption bands are given in table 1.

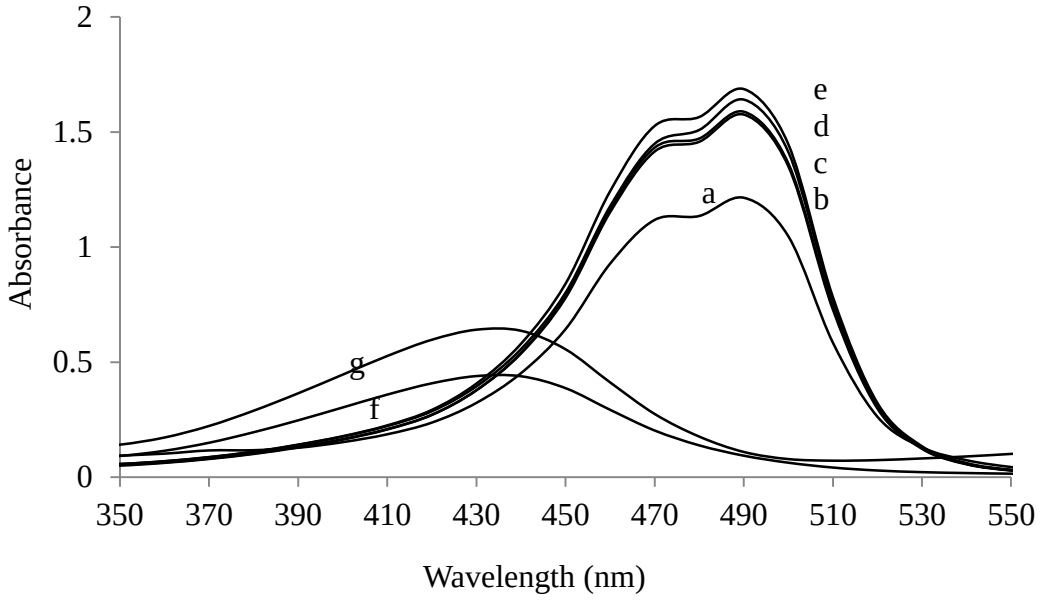

Figure 1. Absorption spectra of acridine orange in water at pH= (a) 1.7 (b) 3.3 (c) 5.0 (d) 7.2 (e) 9.0 (f) 11.0 (g) 12.0.

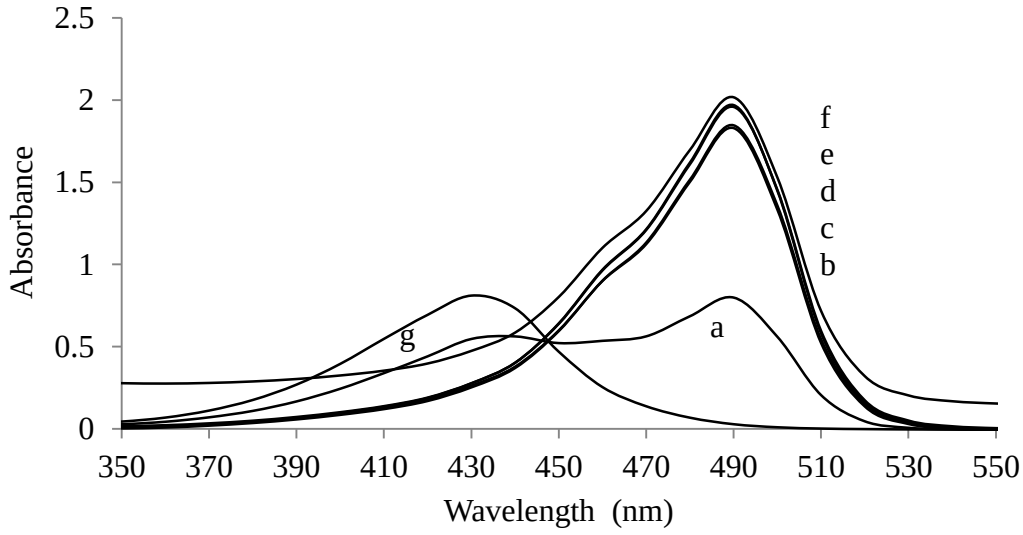

Figure 2. Absorption spectra of acridine orange in methanol at pH= (a) 1.7 (b) 3.3 (c) 5.0 (d) 7.2 (e) 9.0 (f) 11.0 (g) 12.0.

pH	Absorption maximum (0-0)		Emission maximum	
	in water	in methanol	in water	in methanol

1.7	491.05	489.18	530.19	525.36
3.3	491.18	489.89	531.14	524.41
5.0	491.29	489.86	531.72	524.57
7.2	491.07	489.97	531.05	524.83
9.0	491.16	489.89	531.74	515.35
11.0	434.75	489.81	520.02	514.89
12.4	435.10	432.23	515.31	514.78

Table 1 The positions of absorption at different pH for acrtidine orange in water and methanol.

3.2. EMISSION SPECTRA:

Fig.3 and fig.4 show the emission spectra of acridine orange in water and methanol between (pH=1-12.4). The emission spectra of acridine orange at higher pH=12.4 emission at longer wavelength vanishes indicating the absence of acridinium ion and the spectrum corresponding to acridine orange only is observed. The positions of emission band of acridine and acridinium in water at pH=1 and pH=12.4 are at 531.2nm and 515.3nm respectively whereas for methanol they lie at 525.4nm and 514.8nm. The emission bands of acridine and acridinium between pH=1 and pH=12.4 are given in table.1.

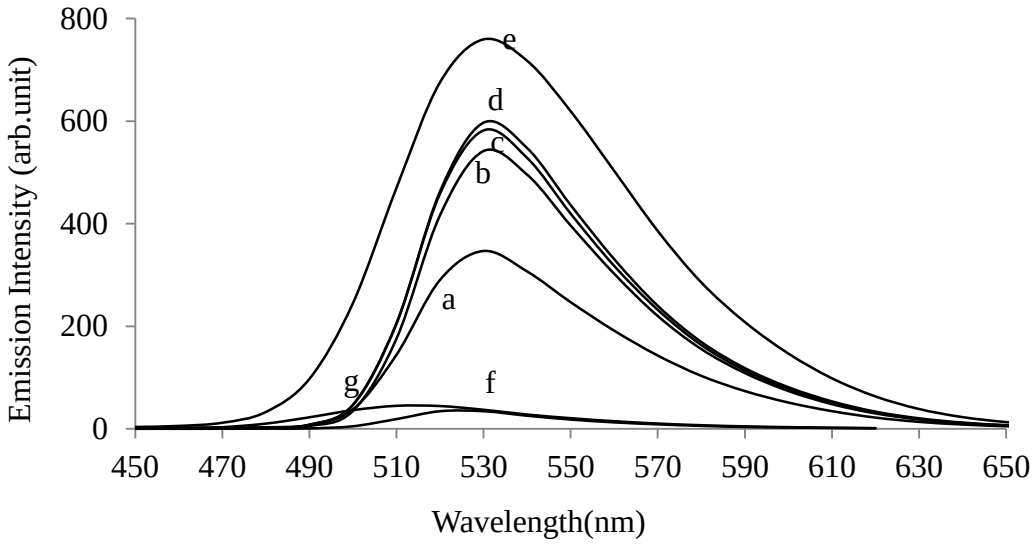

Figure 3. Emission spectra of acridine orange in water at pH= (a) 1.7(b) 3.3(c) 5.0(d) 7.2 (e) 9.0(f) 11.0(g) 12.4.

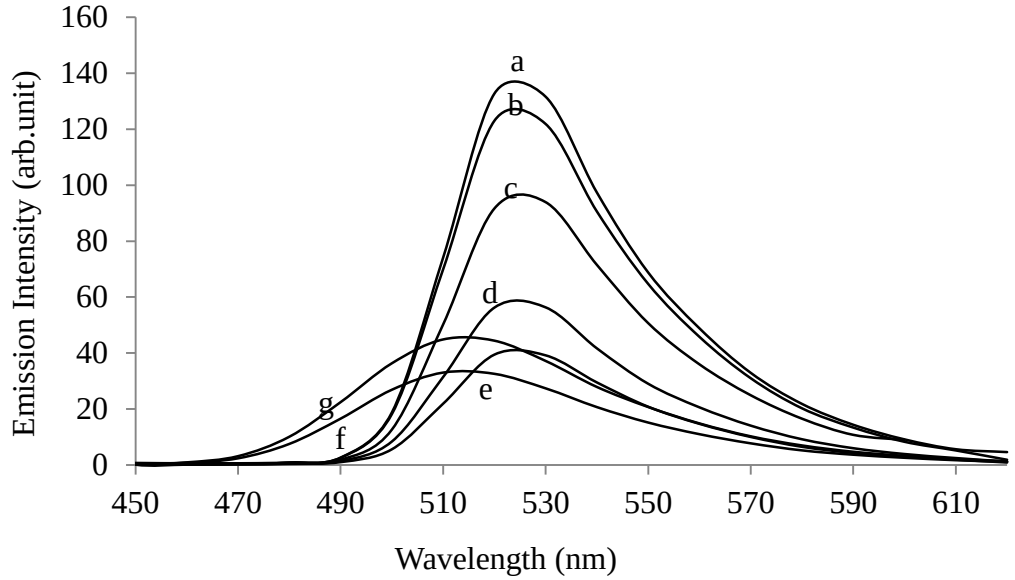

Figure 4. Emission spectra of acridine orange in methanol at pH= (a) 1.7(b) 3.3(c) 5.0(d) 7.2 (e) 9.0 (f) 11.0(g) 12.4.

4. ABSORPTION AND EMISSION SPECTRA OF ACRIDINE ORANGE IN POLYMERS:

a.ACRIDINE ORANGE IN DOPED PVA ABSORPTION SPECTRA

The absorption spectra of PVA film doped with acridine orange (10^{-4M}) is shown in fig 5. It is observed that the 0-0 absorption band has a maximum at 499.79nm as compared to 491.18nm in water. The suggest that in polymer films acridine orange is the absorbing species. The small violet shift of approximately 8 nm in PVA with respect to water is due to the weak interaction between the dye molecule and polymer matrix. The difference of the effect of water molecules surrounding the solvent and the solid polymer matrix also contributes for the violet shift in the absorption spectrum corresponding to the polymer matrix. A weak shoulder also appears at 473nm which is nearly at the same position corresponding to the first excited vibrational band.

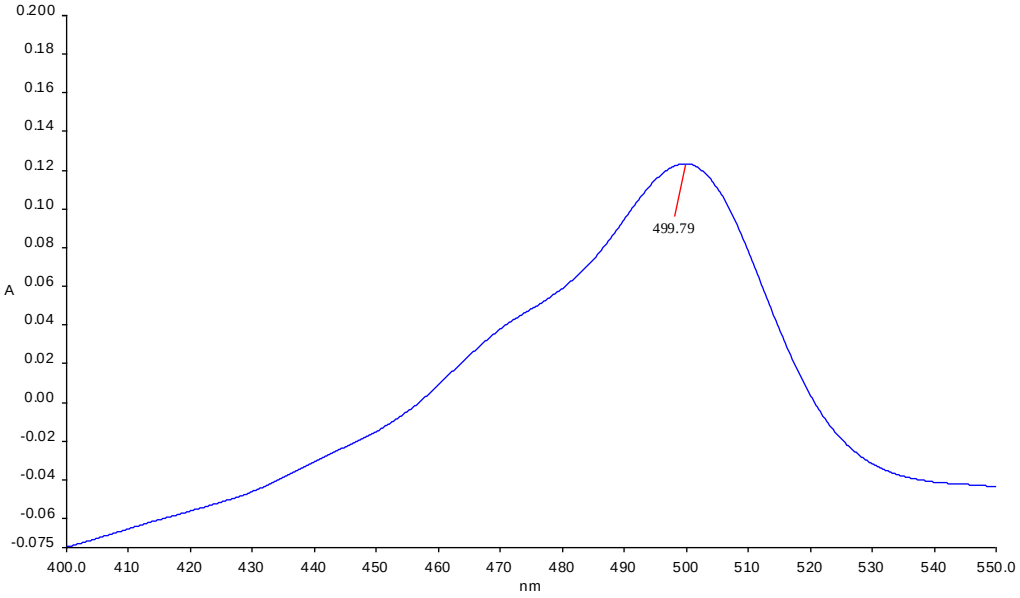

Figure 5. Absorption spectra of acridine orange in PVA film.

b EMISSION SPECTRA:

The emission spectra of acridine orange doped in PVA are shown in fig. 6. The emission band is broad banded having a peak at 540.7nm and a weak shoulder approximately at 572.2nm. The difference between the peak and the shoulder is approximately 102cm^{-1}. On changing the excitation wavelength gradually from 415nm to 500nm no change is observed in the emission profile or the position corresponding to peak intensity. The small red shift from 540.7nm to 541.4nm may be considered to be negligible. Comparing the spectrum with water and methanol it is evident that in PVA matrix acridine orange in the emitting species. With a small red shift of approximately 9nm.

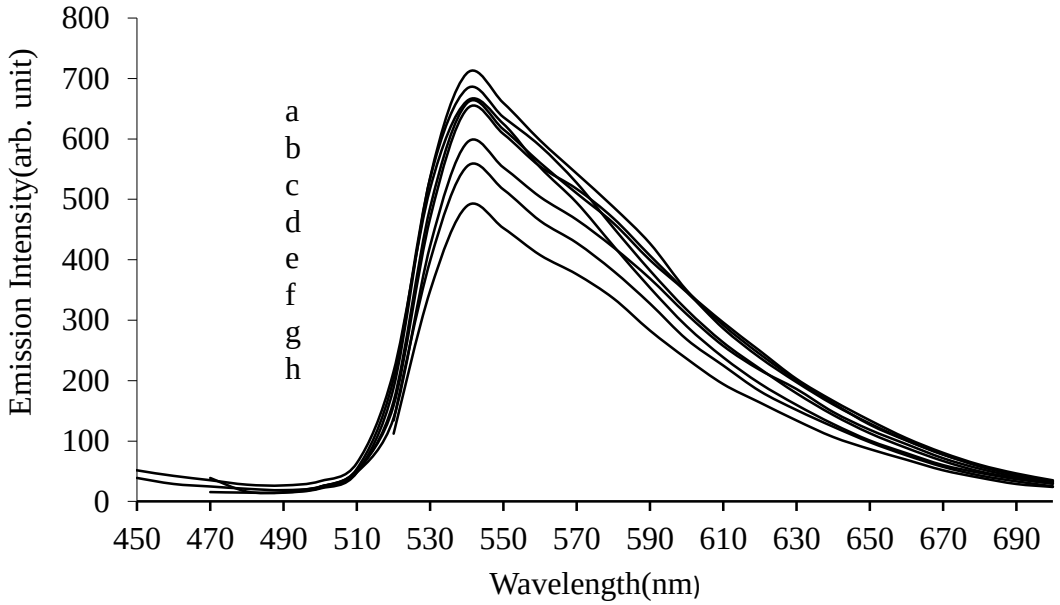

Figure 6. Emission spectra of AO in PVA film (Exactions wavelength (a) 415 (b) 430 (c) 450 (d) 460 (e) 470 (f) 480 (g) 490and (h) 500nm.

c ACRIDINE ORANGE IN DOPED PMMA:

ABSORPTION SPECTRA:

The absorption spectrum of acridine orange in Polymethyl methacrylate film (fig.7) consists of 0-0 band at 503.5nm and the first excited band at 480.0nm. Both the bands have nearly equal intensities. This when compound to the absorption spectra in water (491.0 and 470.3nm and methanol (489.8nm) is suggestive of the fact that acridine orange is the absorbing species in PMMA also. The main difference lies in the relative intensities of the 0-0 and the first excited band of will characteristic of the solvent solute interaction.

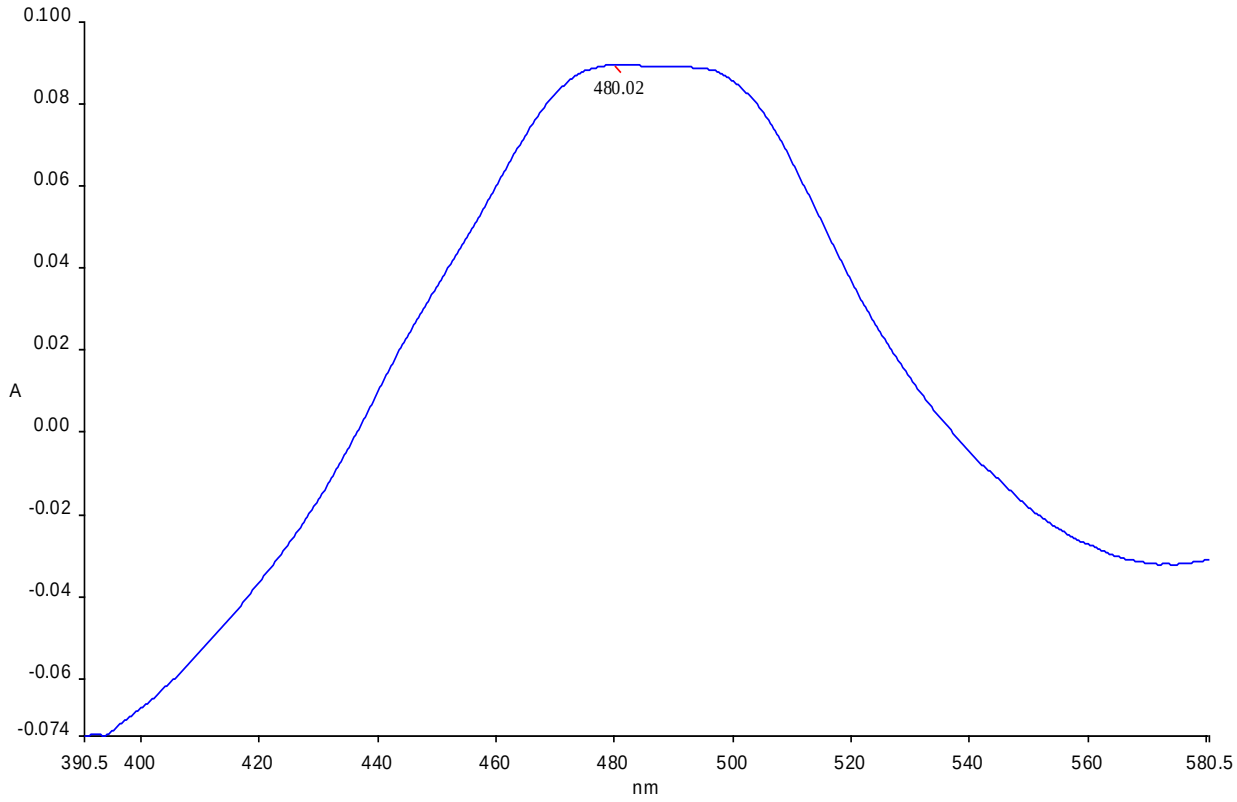

Figure 7. Absorption spectra of acridine orange in PMMA film.

d. EMISSION SPECTRUM OF ACRIDINE ORANGE IN PMMA:

The emission spectrum corresponding to excitation wavelength at 415nm shows two peaks respectively at 534.8nm and 576.1nm. The lower wavelength peak appears sharper with respect to the higher wavelength peak. Corresponding to 415nm excitation the intensities of both the maxima are comparable. The position of 534.8nm peak is close to the 531nm peak in water and 525.4nm peak methanol and evidently the emitter species in PMMA is acridine orange. On gradually increasing the excitation wavelength from 415nm to 500nm the ratio of the intensities of 576.1nmband with respect to the 534.8nm band shows a continues increase. Also red shift, particularly for the 576.1nm band, is observed. The 576.1nm band is red shifted to 584.5nm when excited at 500nm fig.8.The above observations of the excitation wavelength dependence of the emission spectra are indicative of the presence of two types of compositions in the PMMA matrix surrounding the acridine orange ion. The absorption spectra, however, cannot distinguish between these two types of compositions as it is a mixture of the two. But the selection of excitation wavelength enables to excite a particular conformer. From the dependence of excitation wavelength of the emission spectrum if is seen that while corresponding to shorter wavelength excitation both types of conformers are excited with relatively equal number on long wavelength excitation in the population of the conformer absorbing at lounger wavelength is relatively more than the conformer absorbing at lower wavelength. The increase in population of the longer wavelength absorbing conformer is nearly linear as shown in fig. 9.

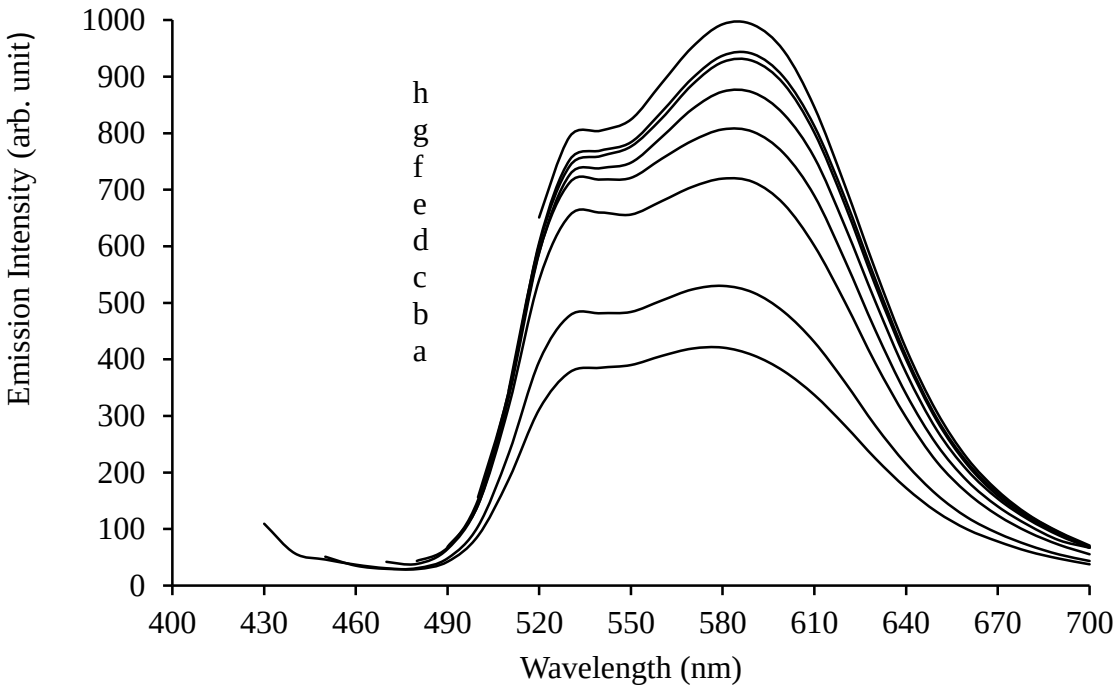

Figure 8 Emission spectra acridine orange in PMMA at different excitation wavelength (a) 415(b) 430(c) 450(d) 460(e) 470 (f) 480 (g) 490and (h) 500nm.

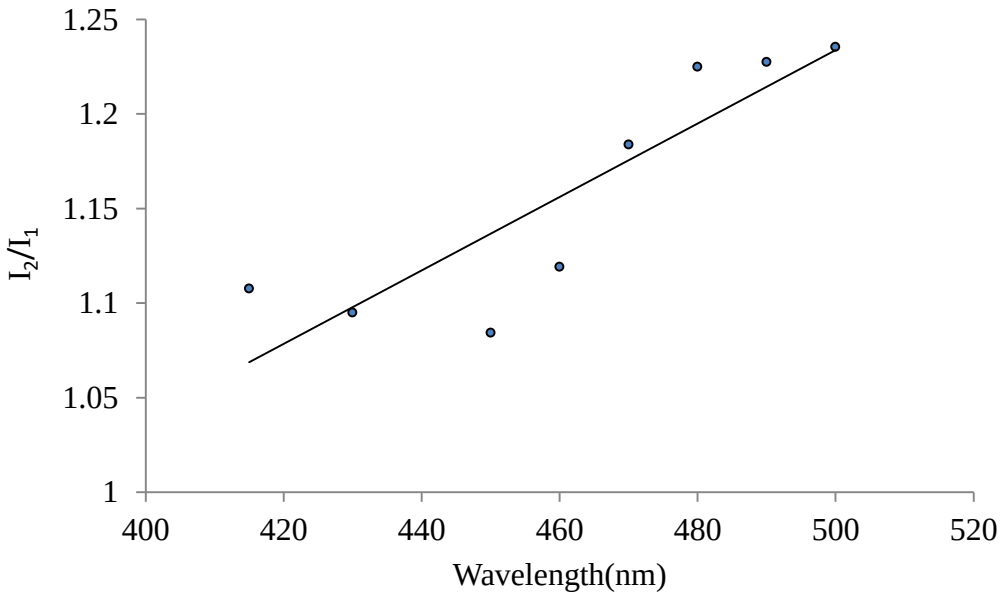

Figure 9. Emission intensity ratio $I_{576.1}/I_{534.8}$ for excitation at different wavelengths for acridine orange in PMMA.

e. ACRIDINE ORANGE IN DOPED PVAC ABSORPTION SPECTRA:

The absorption spectra of polyvinyl acetate doped with acridine orange is shown in fig 10. The main absorption peak 0-0 lies at 499.2nm characteristic of acridine orange. A weak shoulder to appear at 473.3nm the spectrum is similar to these observed in water and methanol.

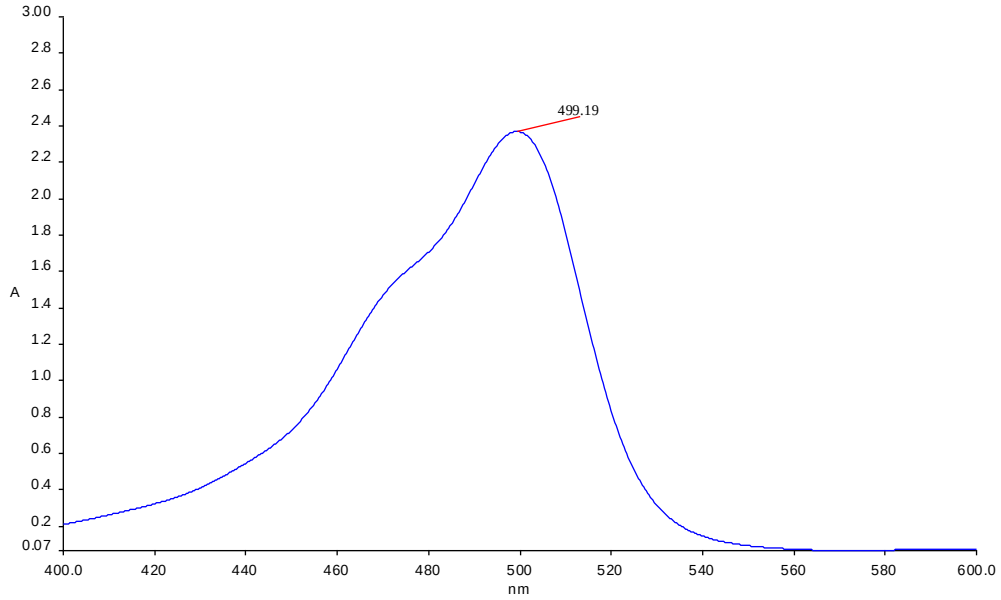

Figure 10. Absorption spectra acridine orange in PVAc.

f. EMISSION SPECTRA:

The emission spectra of acridine orange in PVAc show unique wave length dependence. The general form of the emission spectrum reveals that the emission in peaked at a single wavelength which makes it similar to the spectra observed in water and methanol. However, the position of emission peak corresponding to 415nm excitation is at 599nm which is red shifted markedly with respect water, methanol, PVA and PMMA. There is a gradual red shift in the position of the emission peak on increasing the excitation wavelength from 415nm to 500nm. The excitation wavelength dependence is shown in figs.11 (a) 11(b) and 11(c). The positions of emission peaks for different wavelength of excitation one given in table 5.2. It is observed that the positions corresponding to short wavelength (415nm) and long wavelength excitation (550nm) is red shifted from 599nm to 715.39nm. The graph between excitation wavelength and the emission peak is shown in 11(d). It is seen that the dependence in linear.The above observations of the excitation wavelength dependence of emission spectra indicate the possibility of multiple compositions of PVAc surrounding the acridine orange molecules. The large red shift in comparison to water, methanol PVA and PMMA in the emission is also characteristic of the charge transfer type from the alcoholic group to the nitrogen ring. The acridine orange molecule undergoes charge transfer in the excited state [15]. A possible mechanism for the observed excitation wavelength dependence is that after the process of charge transfer in the excited state a show relaxation of the excited molecule with respect to the surrounding polymer matrix takes place. However, a clear understanding of this can only be obtained by decay time studies. Such excitation wavelength independence of has been observed in the early studies of some fluorophors.

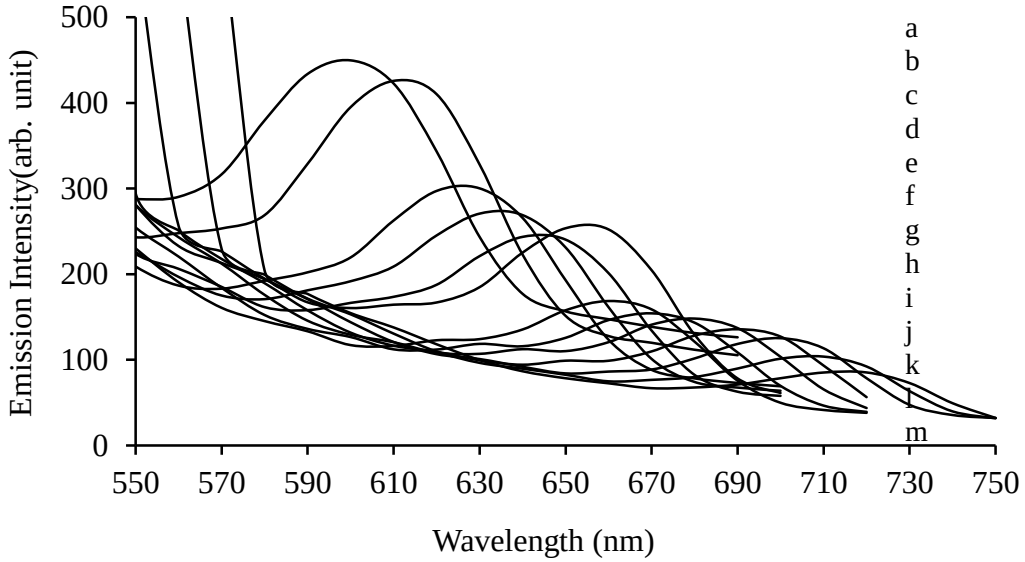

Figure 11a).mission spectra of acridine orange doped in PVAc(a) 415(b) 430(c) 450(d) 460(e) 470 (f)480 (g) 490 (h) 500(i)510 (j) 520(k) 530(l) 540 and (m)550 nm.

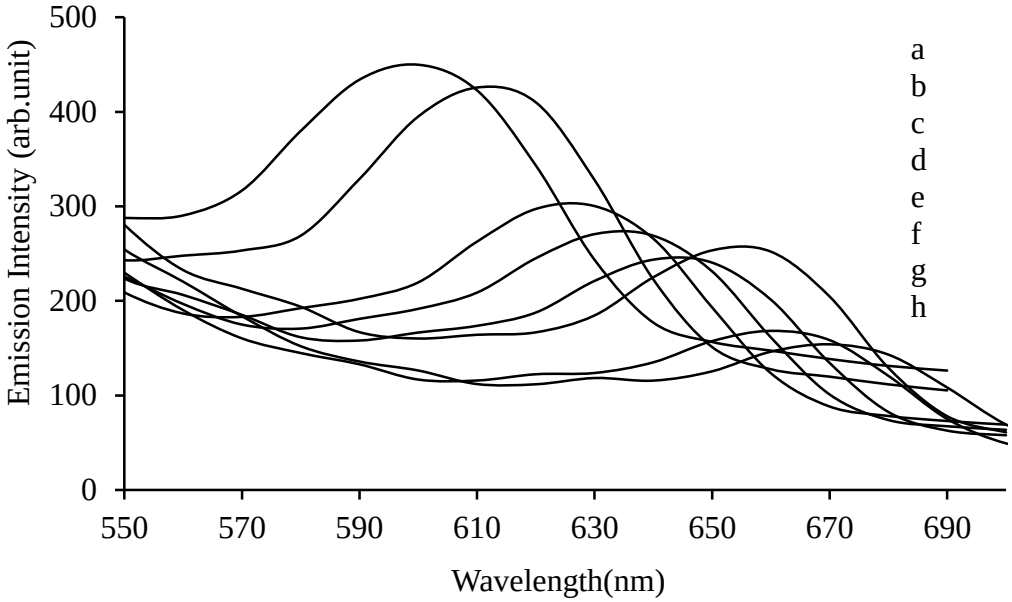

Figure (11b).Emission spectra of acridine orange doped in PVAc Excitation wavelength (a) 415(b) 430(c) 450(d) 460(e) 470 (f)480 (g) 490 (h)500nm.

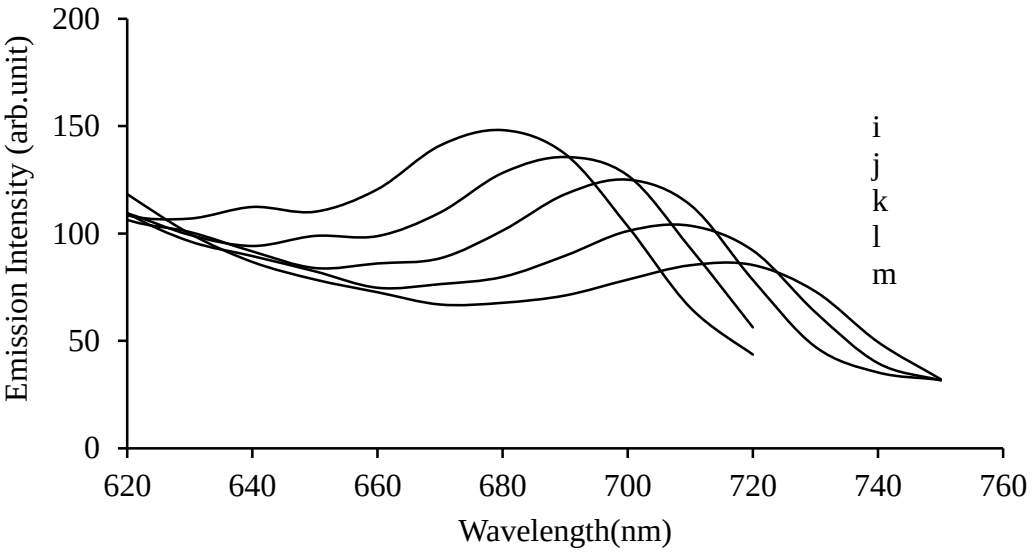

Figure (11c). Emission spectra of acridine orange at Excitation wavelength (i) 510 (j) 520 (k) 530 (l) 540 (m) 550nm.

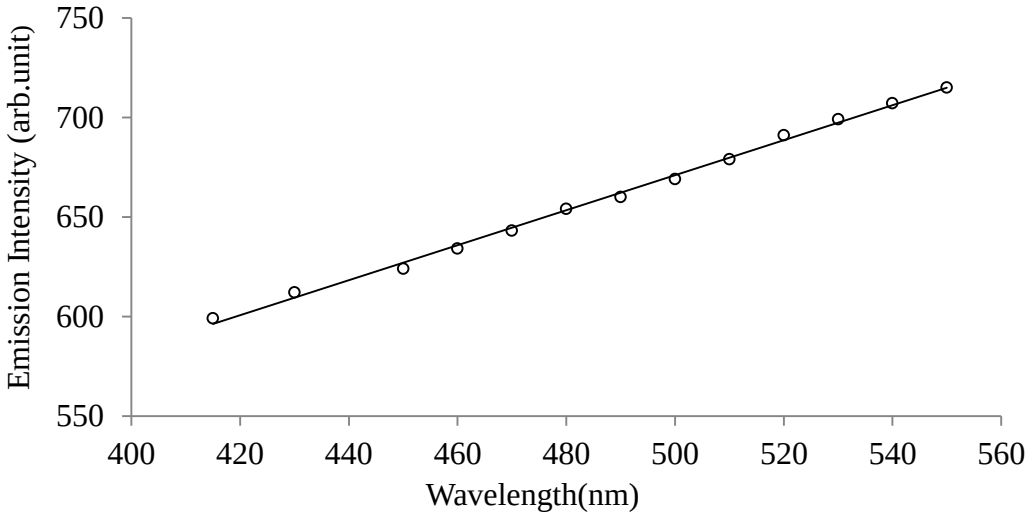

Figure (11d). Wavelength dependence of emission intensity of acridine orange doped in PVAc.

Polymers	Absorption bands (nm)	Emission maximum							
		λ=415 nm	λ=430 nm	λ=450 nm	λ=460 nm	λ=470 nm	λ=480 nm	λ=490 nm	λ=500 nm
Acridine (water)	491.05	531.74	---------	---------	--------				
Acridine (methanol)	489.18	524.41	---------	---------	--------				
PMMA	480.02,503.5	534.80	534.83	534.83	534.97	536.03	536.03	537.53	535.30
		576.06	578.89	583.23	583.30	584.46	585.31	585.71	584.48
PVA	499.19	541.38	540.20	540.78	541.07	541.26	541.00	540.68	540.68
PVAc	499.79	599.33	612.55	624.04	634.49	643.97	654.88	660.91	679.53

Table. 2. Positions of absorption and emission bands of acridine orange in different polymer matrix.

5. CONCLUSION:

The acridine orange spectrum is solutions show pH dependence which is due to the presence of acidic (acridinium) and basic (acridine orange) forms the concentrations of which in the ground state are dependent on the pH of the solution. The absorption spectra of acridine orange in PVA, PMMA and PVAc are characteristic of the presence of acridine in polymer matrix. The emission spectra in PVA specifically showed the presence of acridine orange the absence of any excitation wavelength dependence show the homogenous conformation of the polymer molecules surrounding the fluorophor. In case of emission spectra of acridine orange in PMMA while the emission shows the acridine orange characteristics the possibility of two conformations surrounding the fluorophor is indicated by to the two peaks and the variation is their intensity ratio which is excitation wavelength dependent. In the case of the emission of acridine orange in PVAc the large red shift in the emission peak is indicative of the charge transfer in the excited state. Also excitation wavelength dependent gradual red shift in the position of the emission peak reveals the possibility of excited state relaxation for which the study decay characteristics is also needed.

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