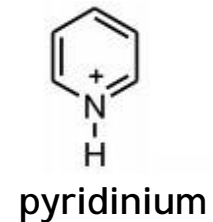
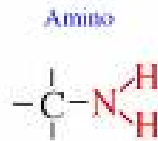
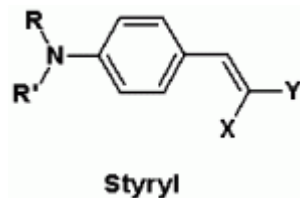
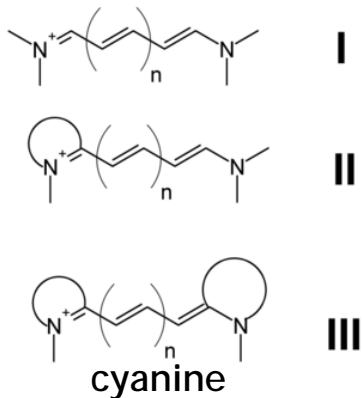

Voltage Sensitivity of the fluorescent probe RH421 in a Model Membrane system

Ronald J. Clarke, Athina Zouni, and Josef F. Holzwarth.
Fritz-Haber-Institut der Max-Planck-Gesellschaft, Berlin, Germany

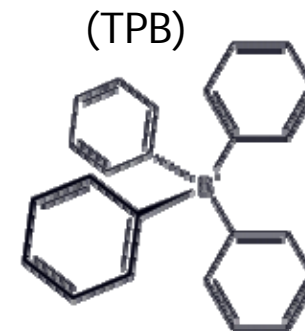
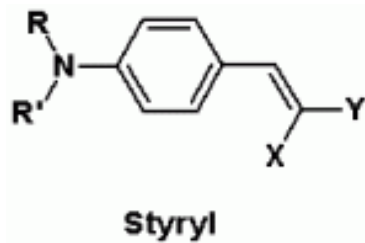


2008-05.22.

Introduction

- Bammel (1990), Pratap and Robinson (1993), Haughland (1992)
 - dyes respond to changes in membrane potential by an electrochromic mechanism
- Waggoner and Grinvald (1977), Loew (1978), Liptay (1969), Clarke (1992)
 - absorbance spectrum
- MLoew (1985) : means of voltage clamp pulse on the giant squid axon (aminostyrylpyridinium)
- Muller (1986) : action potential in heart tissue

- Styryl dye



Investigation of the voltage sensitivity of **styryl dye RH421** in pure lipid membranes.

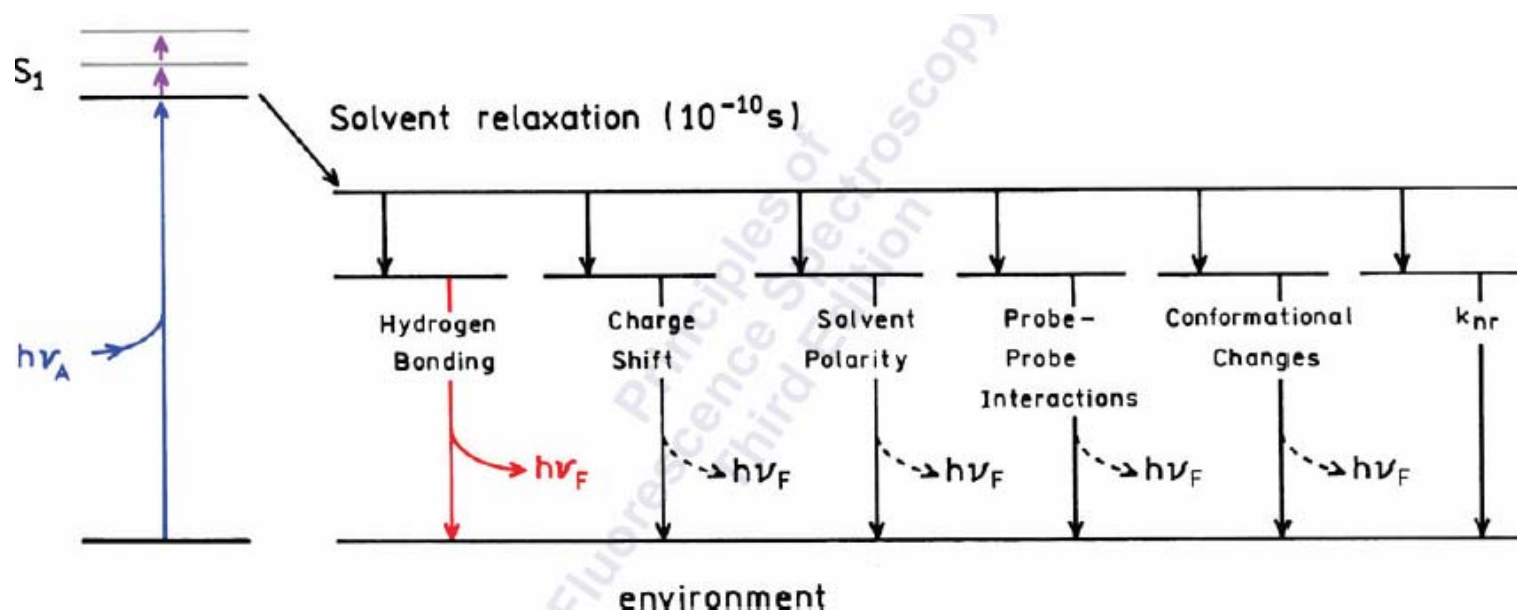
- Intramembrane electrical fields have been

induced by the binding of the hydrophobic ion tetraphenylborate (TPB)

Solvent and environmental effects

The effects of solvent and environment on fluorescence spectra are complex

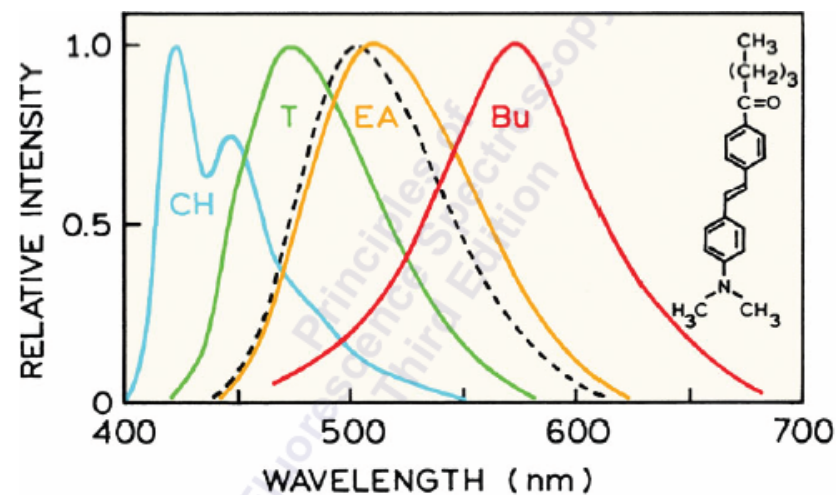
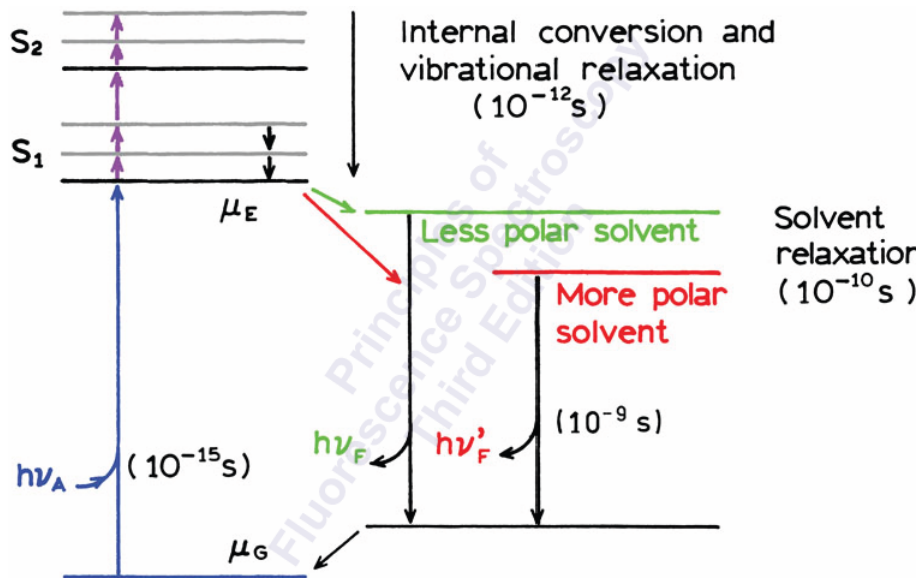
- Solvent polarity and viscosity
- Rate of solvent relaxation
- Probe conformational changes
- Rigidity of the local environment
- Internal charge transfer
- Proton transfer and excited state reaction
- Probe-Probe interactions
- Changes in radiative and nonradiative decay rate



Solvent and environmental effects

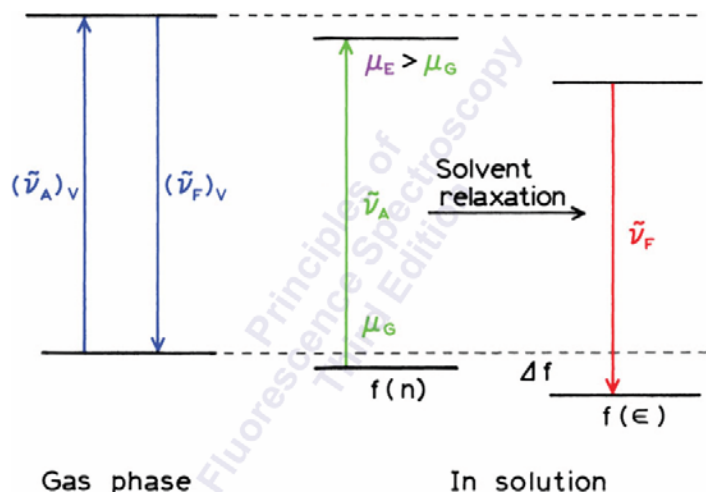
Solvent polarity

Lippert equation partially explains the effect of solvent polarity..



Solvent and environmental effects

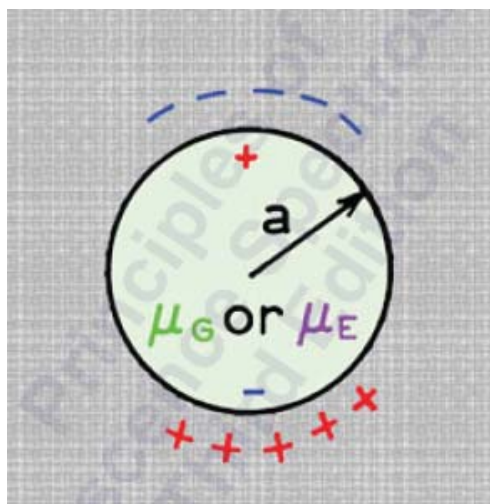
1 Cm = 2.9979 × 10²⁹ D.



Lippert equation

$$\nu_A - \nu_F = \frac{2}{hca^3} \left(\frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) (\mu_E - \mu_G)^2 + \text{constant}$$

↑
Orientation polarizability (Δf)



The energy of the dipole in an electric field

$$E_{dipole} = -\mu R$$

R : electric field, and is relative reactive field in the dielectric induced by the dipole

$$R = \frac{2\mu}{a^3} f$$

f : polarizability of the solvent
a : cavity radius

n : high-frequency response and depends on the motion of electrons within the solvent molecules

ε : static property, depend on both electronic and molecular motion

Solvent and environmental effects

Polarizability of the solvent is a result of both the **mobility of electrons in the solvent** and the **dipole moment of the solvent** molecules

- High frequency polarizability

$$f(n) = \frac{n^2 - 1}{2n^2 + 1} \quad : \text{redistribution of electrons of solvents}$$

- Low frequency polarizability

$$f(\varepsilon) = \frac{\varepsilon - 1}{2\varepsilon + 1} \quad : \text{reorientation of the solvent dipole}$$

The difference between these two terms

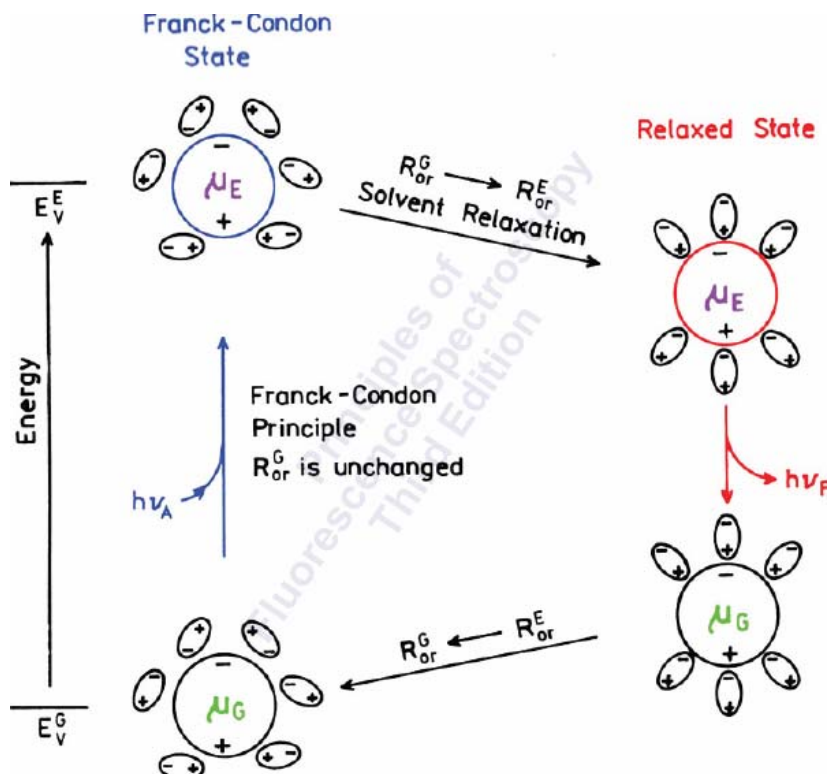
Orientational polarizability

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

	water	Ethanol	Ether	Hexane
ε	78.3	24.3	4.35	1.89
n	1.33	1.35	1.35	1.37
Δf	0.32	0.30	0.17	0.001



Solvent and environmental effects



Reactive field

- Electric motion

$$R_{el}^G = \frac{2\mu_G}{a^3} f(n)$$

$$R_{el}^E = \frac{2\mu_E}{a^3} f(n)$$

- Reorientation motion

$$R_{or}^G = \frac{2\mu_G}{a^3} \Delta f$$

$$R_{or}^E = \frac{2\mu_E}{a^3} \Delta f$$

Absorption the energies of the ground and excited state

$$Energy^E (absorption) = E_V^E - \mu_E R_{or}^G - \mu_E R_{el}^E$$

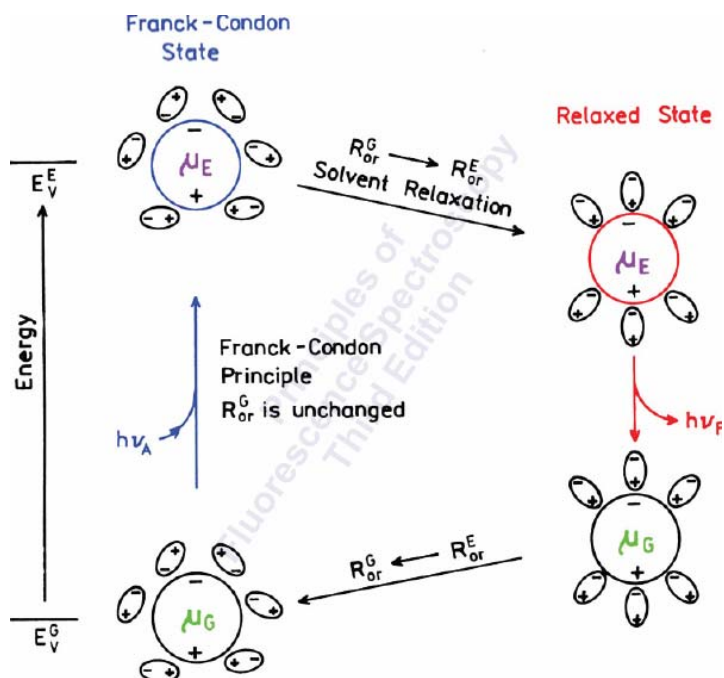
$$Energy^G (absorption) = E_V^G - \mu_G R_{or}^G - \mu_G R_{el}^G$$

$$\nu = \Delta E / hc$$

Energy of absorption

$$\longrightarrow h\nu_A = hc(\nu_A)_V - (\mu_E - \mu_G)(R_{or}^G) - \mu_E R_{el}^E + \mu_G R_{el}^G$$

Solvent and environmental effects



energy of the two levels for emission

$$Energy^E (\text{emission}) = E_V^E - \mu_E R_{or}^E - \mu_E R_{el}^E$$

$$Energy^G (\text{emission}) = E_V^G - \mu_G R_{or}^E - \mu_G R_{el}^G$$

$$\nu = \Delta E / hc$$

$$\longrightarrow h\nu_F = hc(\nu_F)_V - (\mu_E - \mu_G)(R_{or}^E) - \mu_E R_{el}^E + \mu_G R_{el}^G$$

Energy of absorption

$$\longrightarrow h\nu_A = hc(\nu_A)_V - (\mu_E - \mu_G)(R_{or}^G) - \mu_E R_{el}^E + \mu_G R_{el}^G$$

absorption - emission of energy

Solvent and environmental effects

absorption - emission of energy

$$R_{or}^G = \frac{2\mu_G}{a^3} \Delta f$$

$$R_{or}^E = \frac{2\mu_E}{a^3} \Delta f$$

$$\nu_A - \nu_F = -\frac{1}{hc} (\mu_E - \mu_G)(R_{or}^G - R_{or}^E) + \text{constant}$$

Lippert equation

$$\nu_A - \nu_F = \frac{-2}{hca^3} (\mu_E - \mu_G)(\mu_G \Delta f - \mu_E \Delta f) + \text{constant}$$

$$\nu_A - \nu_F = \frac{2\Delta f}{hca^3} (\mu_E - \mu_G)^2 + \text{constant}$$

$$\Delta f = \frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

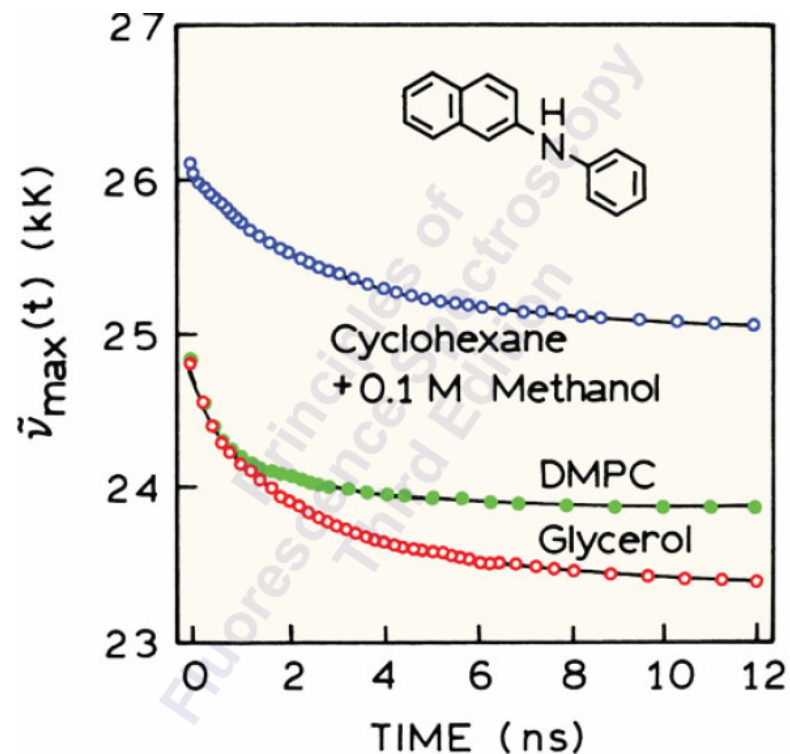
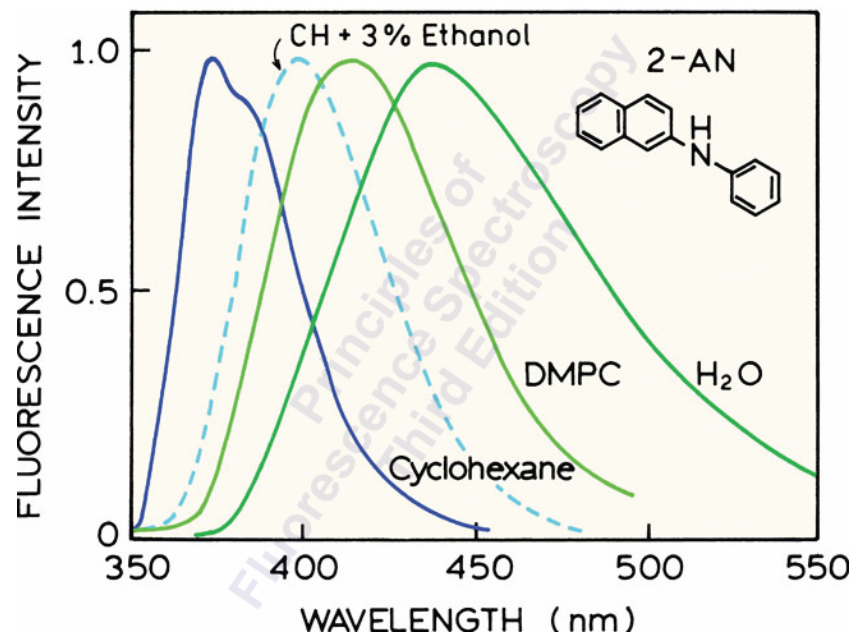
*Lippert equation is only an approximation, and contains many assumptions.
(spherical, specific interaction of solvent, two state dipole moments point in the same direction, etc..)

If dipole moment point in different direction,

$$\nu_A - \nu_F = \Delta b \times \left[\frac{\epsilon - 1}{\epsilon + 2} - \frac{n^2 - 1}{n^2 + 2} \right] \frac{(2n^2 + 1)}{(n^2 + 2)} + \text{constant}$$

$$\text{where, } \Delta b = \frac{2}{hca^3} (\mu_G^2 - \mu_E^2 - \mu_G \mu_E \cos \alpha)$$

Solvent and environmental effects



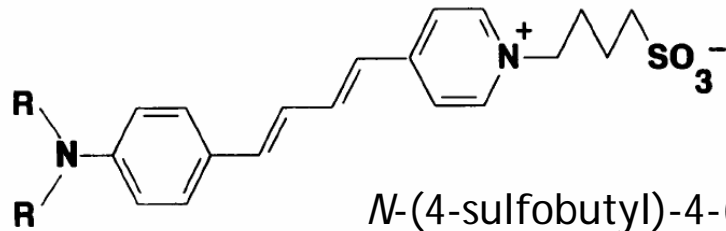
What is the polarity of the environment of 2-AN in the membranes?

~~Mostly nonpolar~~



Hydrogen bonding interactions of 2-AN in membranes is supported by time-resolved

Materials and Methods



Single peak (695nm); independent of the excitation wavelength.

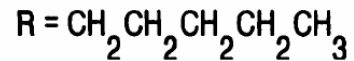


FIGURE 1 Structure of RH421.



DMPC (dimyristoylphosphatidylcholine)

- DMPC unilamellar vesicles were prepared by the ethanol injection method (Zouni, 1994)

Buffer containing

- 30mM Tris,
- 1mM EDTA,
- 150mM NaCl
- HCl (0.1M, pH)

Absorbance and
Fluorescence measurements

- Shimadzu (UV-2100 and RF-5000)

Dipole moment of ground-state RH421

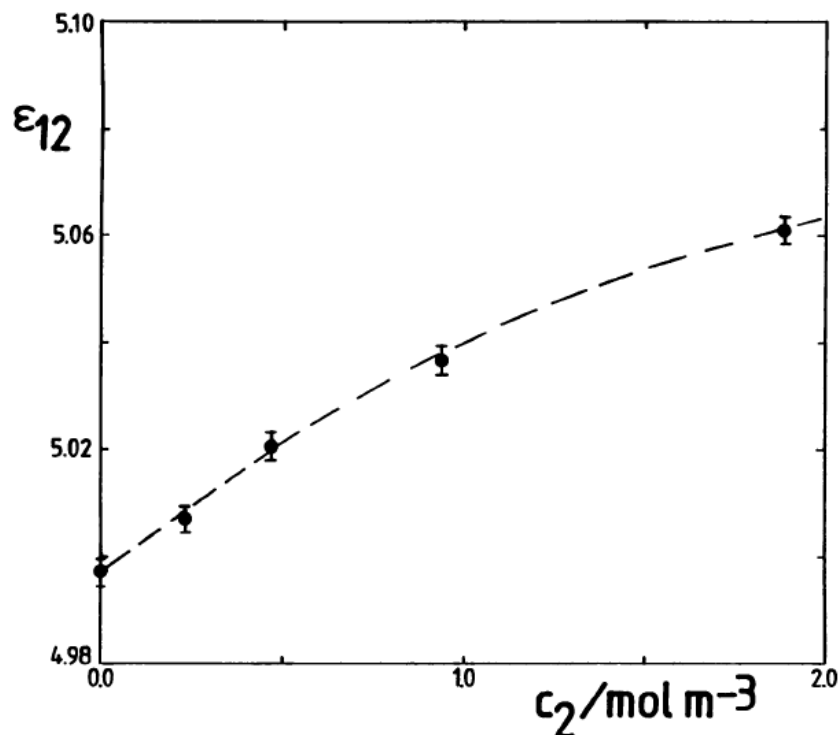


FIGURE 2 Dielectric constant, ϵ_{12} , of RH421 solutions in chloroform as a function of the dye concentration, c_2 .

Dipole meter type DM01

Dipole moment of the solute (Moll and Lippert)

$$\mu^2 = \frac{27kT\epsilon_0}{N_A(\epsilon_1 + 2)^2} \lim_{c_2 \rightarrow 0} \left[\frac{d\epsilon_1}{dc_2} - \frac{dn_{12}^2}{dc_2} \right]$$

T : absolute temp.

ϵ_1 : dielectric constant of the solvent

n_{12} : refractive index of the solution

c_2 : solute concentration

Dipole moment of RH421

4.0×10^{-29} Cm (12Debye)

1 Cm = 2.9979×10^{29} D.

Dipole moment change upon excitation

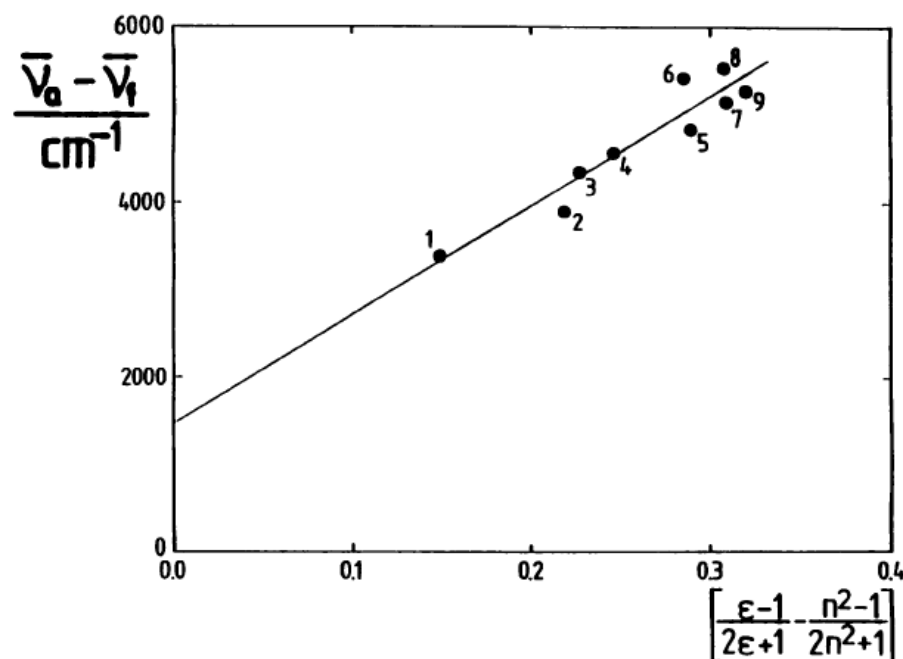


FIGURE 3 Lippert plot of the Stokes shift, $\bar{\nu}_a - \bar{\nu}_f$, of RH421 as a function of the solvent orientation polarizability, $[(\epsilon - 1)/(2\epsilon + 1) - (n^2 - 1)/(2n^2 + 1)]$. The points refer to the following solvents: (1)chloroform, (2) dichloromethane, (3) octanol, (4) hexanol, (5) ethanol, (6) acetone, (7) methanol, (8) acetonitrile, and (9) water.

Dipole moment change calculated from the slope

$$\Delta\mu = 25$$

TABLE 1 Wavelengths of the absorbance and fluorescence emission maxima and the Stokes shift of RH421 in various solvents at 20°C and in a DMPC membrane at 30°C

Solvent	Orientation polarizability	$\lambda_{\text{Abs}}^{\text{max}}$ (nm)	$\lambda_{\text{Em}}^{\text{max}}$ (nm)	$\bar{\nu}_A - \bar{\nu}_F$ (cm ⁻¹)
Chloroform	0.149	549	674	3378
Dichloromethane	0.218	545	692	3898
Octanol	0.226	522	676	4364
Hexanol	0.245	522	686	4580
Ethanol	0.289	521	698	4867
Acetone	0.285	502	691	5448
Methanol	0.309	515	702	5172
Acetonitrile	0.308	508	708	5561
Water	0.320	485	652	5281
DMPC membrane		480	646–665	5350–5800
DMPC membrane (+50 μM TPB)		520	658–665	4030–4200

Electric field-induced abs. and flu. changes

- Hydrophobic ions (TPB) bind a short distance from the water-membrane interface inside the membrane phase.
- They produce strong electrical fields within the membrane, to which the dye can react

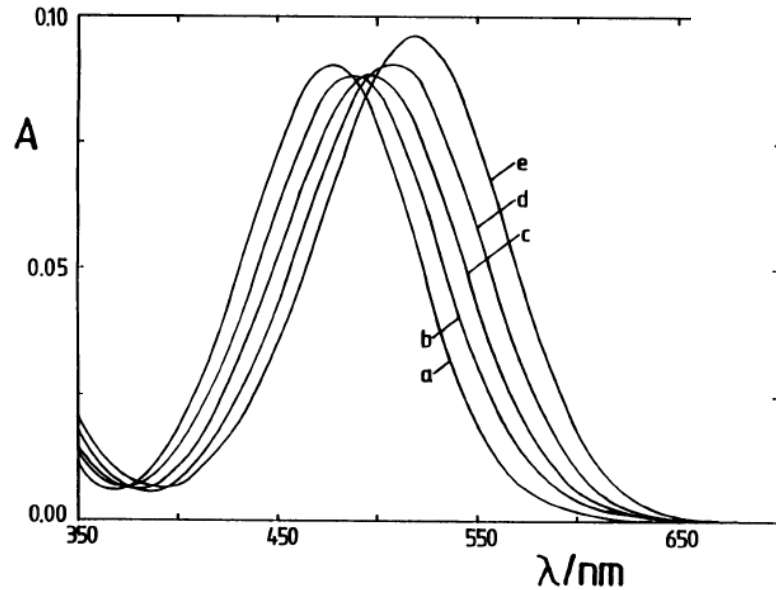
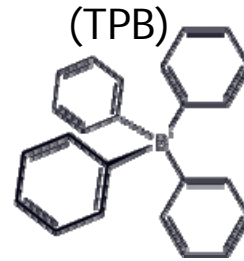


FIGURE 4 Absorbance spectra of 3.7 μM RH421 in the presence of 200 μM of DMPC in the form of unilamellar vesicles as a function of the TPB concentration: (a) 0, (b) 5 μM , (c) 10 μM , (d) 20 μM , and (e) 50 μM . $T = 30^\circ\text{C}$, pH 7.2.

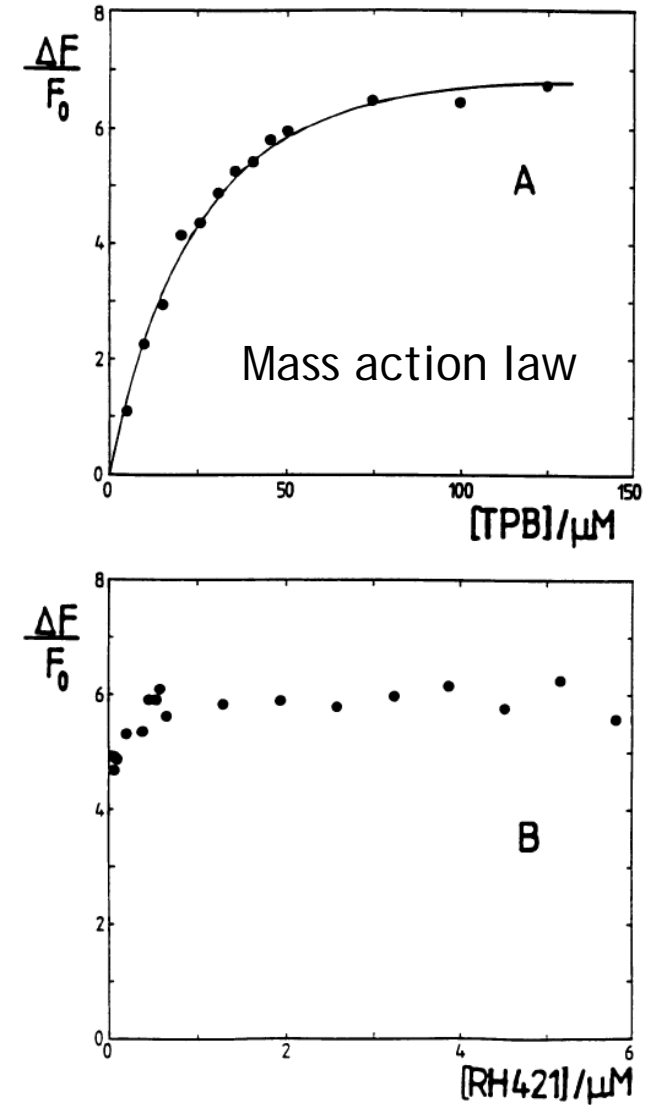


FIGURE 5 (A) Fluorescence change of 3.7 μM RH421 in the presence of 154 μM DMPC in the form of unilamellar vesicles after the addition of TPB as a function of the TPB concentration; $\lambda_{\text{ex}} = 550 \text{ nm}$ (+ OG530 cutoff filter), $\lambda_{\text{em}} = 660 \text{ nm}$ (+ RG645 cutoff filter), bandwidth = 3 nm, $T = 30^\circ\text{C}$, pH 7.2. (B) Fluorescence changes after the addition of 50 μM TPB in the presence of 154 μM DMPC vesicles as a function of the RH421 concentration. All other experimental conditions are as for (A). $\Delta F/F_0$ represent the normalized fluorescence change, whereby F_0 is the fluorescence before the addition of TPB.

Effect of TPB on the acid-base properties of RH421

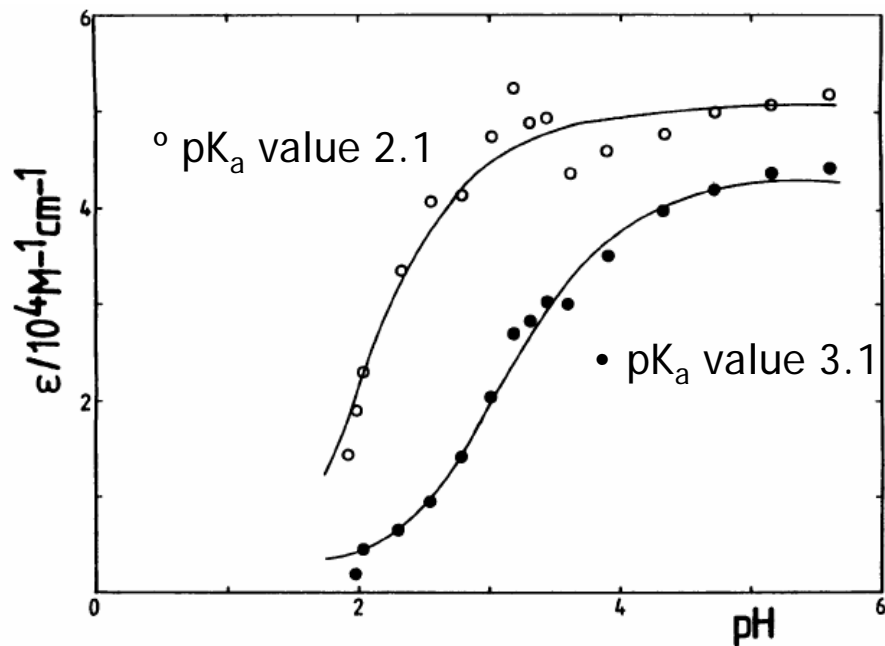


FIGURE 7 Variation of the molar absorptivity, ϵ , of RH421 at 500 nm in the presence of 500 μM of DMPC in the form of unilamellar vesicles and 1.0 M NaCl as a function of pH (●) without TPB and (○) after the addition of 50 μM of TPB. The solid curves represent nonlinear fits of the data to the Henderson-Hasselbalch equation. $[\text{RH421}] = 3.7 \mu\text{M}$, bandwidth = 5 nm, $T = 30^\circ\text{C}$.

Reduction in pK in the presence of TPB is a field-induced reorientation within the membrane.

If the dye is drawn further into the membrane by the induced field, the polarity of its environment would be reduced, so that the protonation would become more difficult and the pK would decrease.