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THE WATER-SOLUBLE ROSE BENGAL DERIVATIVES AND THEIR SPECTROSCOPIC BEHAVIOURS

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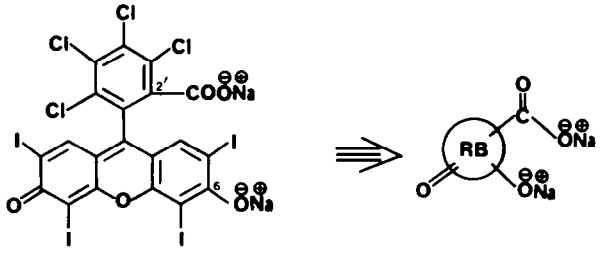
Abstract - The peper presents a series of novel water-soluble derivatives of Rose bengal. Solubility in water of the C-2 Rose bengal esters has been obtained by the attachment of the proper water-soluble moiety to the dye molecule. It is shown that size of the attached molecule has a significiant influence on the spectroscopic properties of the derivatives. The long side chain strongly influences on the position of the absorption λ_{max} and emission spectra. The side chain creates the microenvironment for the molecule to which is attached. This makes it more difficult the diffusion of $H_3O^{\oplus}$ ions into the dye molecule and causes an apparent decrease of pK value for protonation of phenol group of xanthene residue. These types of derivatives make possible to carry out the photooxidation in water even at low pHs.

INTRODUCTION

Rose bengal /3',4',5', 6'- tetrachloro-2'- /2,4,5,7-tetra-iodo-6-hydroxy-3-oxo-3H-xanthen-9-yl/ benzoic acid bis /sodium salt// is one of the most efficient and most used singlet oxygen sensitizers. Though this sensitizer is very useful

SCHEME I



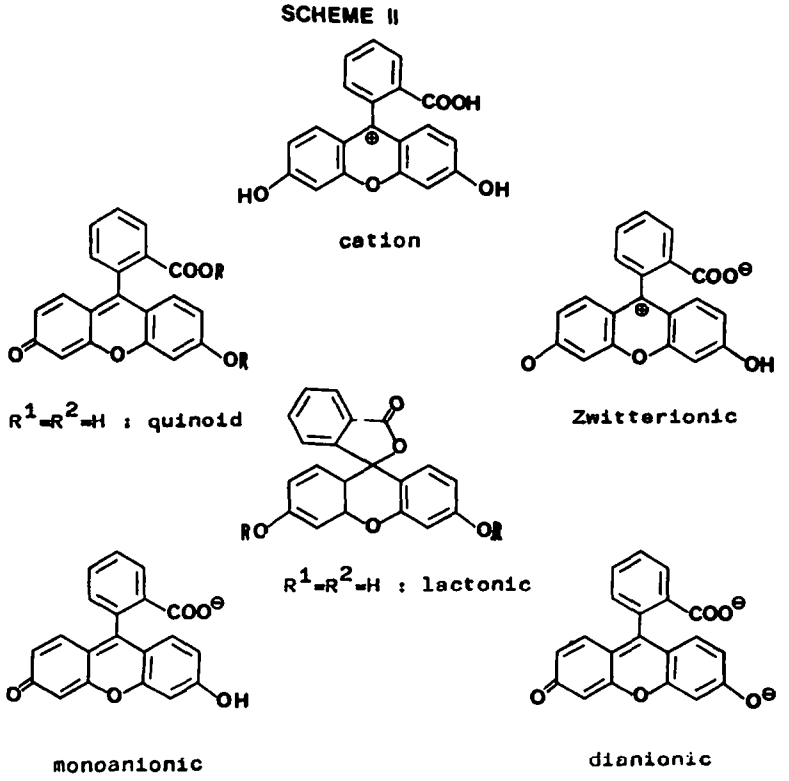
Rose Bengal

in polar solvents, it is essentially insoluble in typical organic solvents such as pentane, chloroform or methylene chloride. The solubility problem of Rose bengal derivatives as well as their fundamental photophysical properties has been carefully studied by Lamberts and Neckers [1-4] . The compatebility of Rose

bengal with non-polar solvents can be improved by immobilization of the dye to the proper polymeric carrier [5-12] , or by immobilization to specific organic molecules [13-15] . The general solution of these problems is based on the chemical properties of Rose bengal, which may form derivatives via nucleophilic displacement at C-2′position, whereas C-6 position is nearly unreactive [16] .

The spectroscopic properties of the xanthene dyes are dependent on solvent [17-19] and on microenvironment [20-21] . The structurally complex fluorescein dyes, all exist in different structural forms at different pHs in solution. In the case of fluorescein the derivatives are shown as a function of pHs.

SCHEME II



Protonation equilibria of fluorescein type of dyes

The absorption maximum undergoes consecutively a large, a small and another large red shift as the pH increases. This corresponds to formation of the neutral molecule, the monoanion and dianion of fluorescein respectively. As a consequence one concludes that the ionization of the phenol residues of the xanthene moiety has the largest influence on the position of the absorption maximum. From the similar observations made with eosin, it was possible to conclude that the phenolic function of eosin is more acidic than the carboxylic function [22] .

Rose bengal is widely used and is an efficient sensitizer for the formation of singlet oxygen. Rose bengal has potential applications in the photochemical treatment of excessive algal growth in water [23] and the degradation of organic phosphate pesticides in waste water. In the latter case model studies have been carried out [24,25] .

Rose bengal is comercially available as the disodium salt, forms the lactone at low pH handling it insoluble. Functionalization at the C-2′position makes

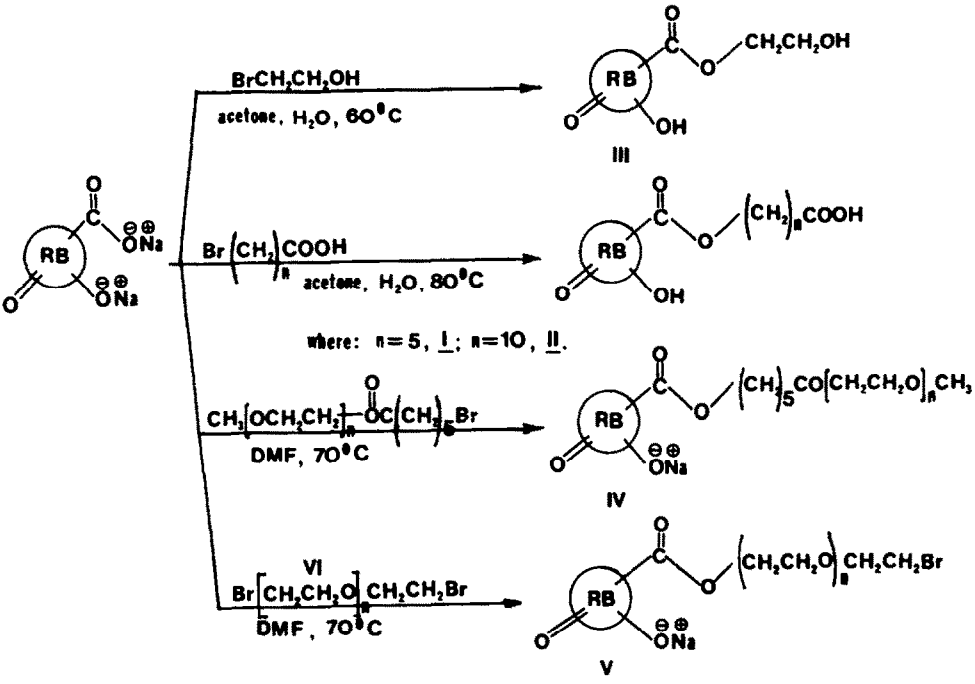
obviates lactone formation though functionalization at C-2′ also decreases solubility in water even at high pH.

In this paper the synthesis of new water-soluble derivatives of Rose bengal as well as their spectroscopic properties are described.

RESULTS AND DISCUSSION

The syntheses described in Scheme III and in the Experimental Section in part were presented in the previous papers [14,15] , and are based on the difference in the nucleophility of the carboxylate and the phenolate group [1-4].

SCHEME III



The electronic absorption characteristics of the compounds I and II are given in Figure 1 and in Table 1.

The electronic absorption maxima for I and II occur at different wavelength. Esterification at C-2′ shifts the maximum toward the red by about 4 nm relative to Rose bengal disodium salt /551,5 nm for I/. For essentially an ester with a longer side chain attached at C-2′ /II/, an additional red shift is observed /about 5 nm 557,0 nm/. This suggests that the long chain in RB-COO/CH_2/ $_{10}$COOH resides in such a way that the Rose bengal xanthene residue is surrounded by non-polar side chain /see Scheme IV/.

For RB-COO/CH_2/$_5$-COOH the /CH_2/$_5$ chain is too short and not able to present the dye with essentially a non-polar microenvironment. This might be confirmed also by the measurement of the surface tension of the water solution of I and II. For the concentration $1 \div 2 \times 10^{-4}$ mol/dm^3 , RB-COO/CH_2/$_{10}$COOH decreases the surface tension about 10%; however for RB-COO/CH_2/$_5$-COOH this effect is not observed. The change in surface tension is by the difference in the stereochemistry of I and II. Since II decreases the surface tension of water it is suggested that the hydrophilic parts of the dye /carboxylic side chain groups and phenolic group of the xanthene part/ are proximate, and hydrophilic and

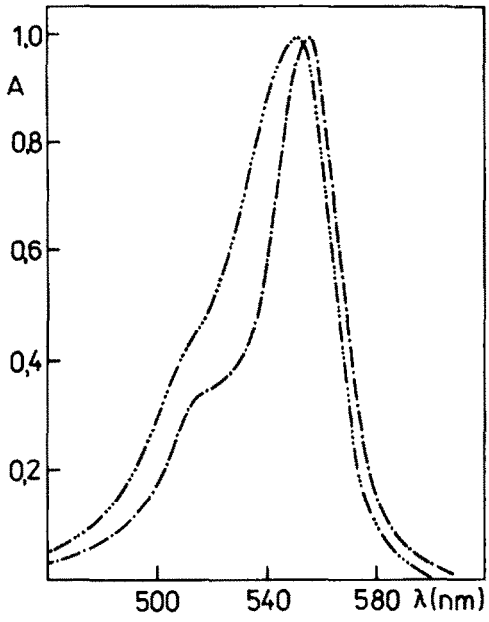
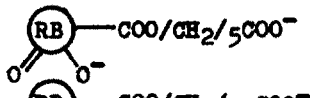


Figure 1 - The electronic absorption spectra in water at pH=9.3

1/ -·-·-·-·- 

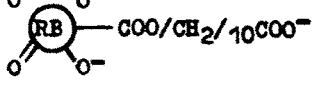
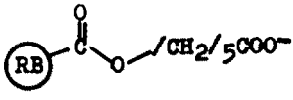
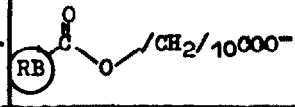
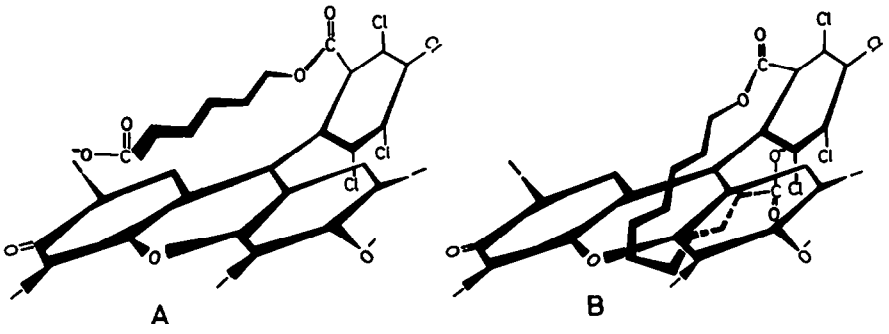
2/ —·—·— 

Table 1

The Electronic Absorption Data for Rose Bengal and Its Water-soluble C-2 Carboxyesters In Water

RB						
λ_{max}	$\varepsilon\lambda_{max}$	λ_{max}	$\varepsilon\lambda_{max}$	λ_{max}	$\varepsilon\lambda_{max}$	
549.0	9.47×10^4	552.5	7.98×10^4	552.0	1.92×10^4	pH=8.0
549.0	9.35×10^4	552.0	7.61×10^4	554.0	4.25×10^4	pH=8.5
548.8	9.45×10^4	551.5	8.21×10^4	557.0	7.92×10^4	pH=9.3
549.0	9.13×10^4	551.7	7.87×10^4	555.0	7.14×10^4	pH=10.0
549.0	9.07×10^4	547.5	8.71×10^4	550.5	7.26×10^4	0.1M NaOH

SCHEME IV



hydrophobic of the dye are distinctly separated. This is not so in the case of RB-COO/CH_2/$_5$-COOH. From these observations one can predict that the spatial structures for I and II are quite different and structures proposed in Scheme III explain the observed phenomena.

The date from Table I indicate an apparent decrease in the molar absorption coefficient for RB-COO/CH_2/$_{10}$-COOH at pH=8,5 and pH=8,0. This is caused by the decreased solubility of the dye. RB-COO/CH_2/$_5$-COOH behaves similarly, but the decrease in solubility occurs at lower pHs /about 7,0 - 7,5/. Since the solubility of aliphatic carboxy acids is determinated by the length of alkyl chain, the difference in the solubility of I and II is likely based on the properties of the respective carboxy acids.

The properties of the Rose bengal carboxy esters indicate that the length of the side chain attached to the dye molecule plays an important role. Shorter side chains make the dye more soluble in water, while the longer side chain causes decrease in solubility. In order to determine how the length of the side chain effects on the solubility in water, the attempt of the synthesis of Rose bengal /C-2/ carboxy esters with shorter side chain has been undertaken. Unfortunately the properties of adequate bromocarboxylic acids make a simple matter more complex. The corresponding carboxy esters are not formed from 3-bromopropionic or 4-bromobutyric acid. These acids are so strong that they protonate the Rose bengal molecule and cause lactone formation. Thus it is possible to obtain derivatives of Rose bengal with long alkyl chains, and in water solubility is observed at high pH, because the carboxy group exists as a salt. Decreasing pH causes the protonation of carboxylic group and Rose bengal /C-2'/ carboxy ester becomes insoluble, because the Rose bengal /C-2'/ esters /e.g. Rose bengal /C-2'/ bensyl or methyl esters/ are insoluble in water.

The properties of poly/ethylene glycol/ monofunctionalized with Rose bengal /IV/ are both more complex and more interesting. This Rose bengal polyglycol is obtained by reaction in Scheme III and is soluble in solvents from toluene to water. The most important photochemical properties of IV in organic solvents have been described in an earlier paper [15] .

The electronic absorption spectra in the visible region for IV in water at various pHs have been measured. Results are shown in Figure 2 and data are summarized in Table 2.

The most noticeable features of the results presented in the Figure 2 and Table 2 are the positions of λ_{max} and molar absorption coefficients at λ_{max}.

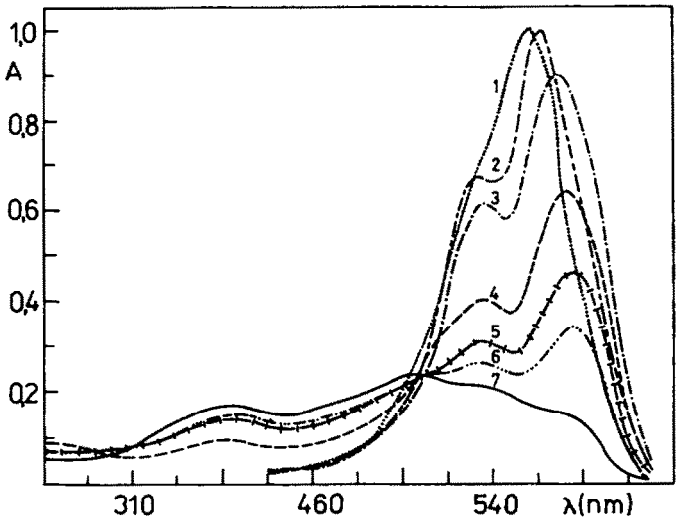
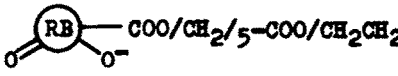


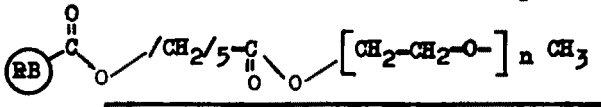
Figure 2 - The electronic absorption of



/IV/ in water at various pHs : 1/ 0.01 n NaOH, 2/ pH=8.0, 3/ pH=6.0, 4/ pH=4.0, 5/ pH=3.0 6/ pH=2.5 7/ 0.01 n HCl

Table 2

Electronic Absorption Data for



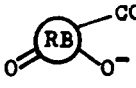
In Water At Various pHs

pH	λ_1 [nm]	λ_2 [nm]	$\varepsilon_{\lambda_{max}}$	$A_{\lambda_1} / A_{\lambda_2}$
0.01 N HCl	505	420	1.38×10^4	-
2.5	576.8	534	1.97×10^4	1.28
3.0	576.1	535	2.67×10^4	1.47
4.0	573.0	536	3.66×10^4	1.60
6.0	569.0	535	5.15×10^4	1.51
8.0	562.4	533	5.78×10^4	1.47
10.0	561.9	535	5.47×10^4	1.47
0.01 N NaOH	556.6	-	5.71×10^4	-

Even at very low pH /pH=2,5/ the phenol residue of the xanthene moiety still exists in part as an anion / λ_{max} = 576,8 nm/. Another observation is also important. The absorption maximum shifts consecutively to longer wavelengths as pH decreases. The total red shift of λ_{max} is about 20 nm /see Table 2/. At pH=2,5 λ_{max} is observed at 576,8 nm. This suggests that the dye molecules are in non-polar environment, and hydrogen-bonding between the dye and solvent molecule becomes more difficult. At wavelengths beyond 570 nm the λ_{max} for Rose bengal were observed only in solvents which are not abble to form hydrogen bonding. [8].
Over 575 nm in a pure non-polar environment /e.g. polymer film/ the λ_{max} was observed [9]. These suggest that Rose bengal molecule forms its own micro-environment by means of the poly/ethylene glycol/ side chain.

To confirm this experimentally, the new Rose bengal derivative /III/ /see Scheme III/ was prepared and its basic spectroscopic properties in water at various pHs have been taken.
The data are summarized in Table 3.

Table 3

Parameters of The Electronic Absorption Spectra for  In Water At Various pHs

pH	λ_{max}	$\varepsilon_{\lambda_{max}}$
2.40	513.3	1.36×10^4
3.80	556.3	2.06×10^4
5.64	554.3	3.54×10^4
7.73	553.1	6.94×10^4
12.60	548.3	7.62×10^4

For III the side chain is short and is not able to form an microenvironment for molecule to which is attached. It is easy to see that for III the total red shift of λ_{max} is only about 5,5 nm, when pH goes from high value /pH=12,17/ to low value /pH=3,81/. The longest wavelength is observed at 555,6 nm, pH=4,15. When this is compared with the λ_{max} observed for Rose bengal /C-2/ esters in MeOH [4,26], it is suggested that III in water shows a strong hydrogen bonding effect / λ_{max}=558,0 nm for Rose bengal in MeOH, λ_{max}=562,0 nm for Rose bengal /C-2/ benzyl ester in MeOH/.

It is possible to conclude that for III and IV in water there are two different hydrogen bonding pathways. For IV there is a barrier which water finds difficult in crossing. In order to confirm this experimentally the pK values for protonation equilibria for phenolate function have been established. For III the phenolate function of the xanthene residue of the dye has a pK of 5,1÷5,2. For IV pK=3,7. Issa and coworkers established for Rose bengal /disodium salt/ pK=3,72 [27]. The convergence of pK values observed for IV and Rose bengal /di-

sodium salt/ seems to be accidental. It is very well known that Rose bengal phenolic function is more acidic than is the carboxylic acid function. In other words before the phenol group becomes protonated Rose bengal lactone is formed and this changes the charge distribution in xanthene residue of the dye and as a result the acidity of phenol groups may be increased. The esterification of C-2′ carboxy group does not permit the Rose bengal lactone formation. It is easy to understand /see Scheme II/ that charge distribution in the xanthene residue is different for the lactone from and C-2′ ester of the dye.

It seems to be clear that the structure of the organic function used for the esterification of C-2′ carboxylic group does not cause the changes of pK value for the protonation of C-6 phenol group. The situation is more complex when attached group is large, long and flexible, and additionally, the polarity of the attached group is different in comparison with polarity of solvent. The long side chain forms a microenvironment for the dye to which is attached. This makes the diffusion of $H_3O^{\oplus}$ ions to the dye molecule more difficult. Under this condition the concentration of protons must be higher for the dye with the microenvironmental layer, and lower for the dye without the microenvironmental cover. It is clear that effect of the microenvironmental layer should be greater for the side chain with higher molecular weight.

In order to confirm this assumption longer derivatives of Rose bengal has been prepared /see Scheme III, compound V/, and their spectroscopic properties in water measured. For the synthesis the poly/ethylene glycol/ with high molecular weight was used /m.w. ≈2000/. Figure 3 shows the protonation curves for the dyes III, IV and V.

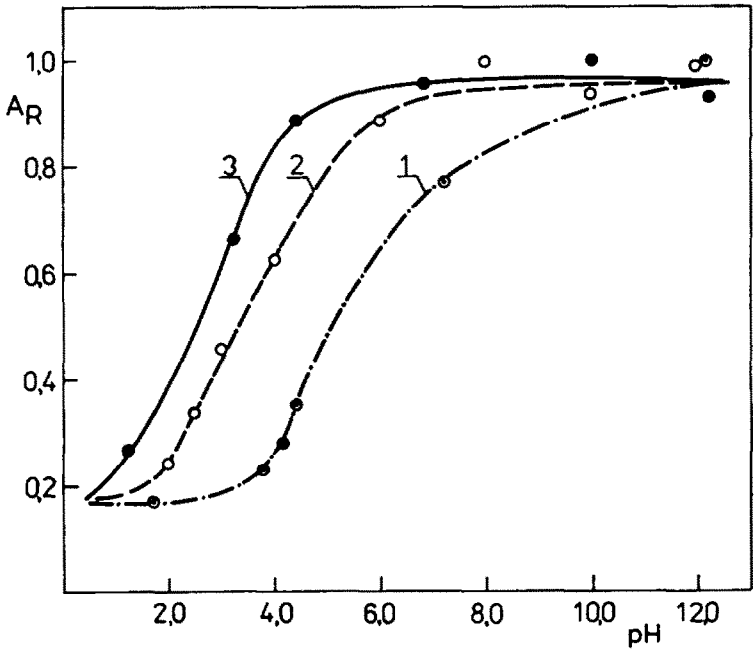
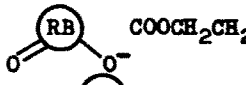
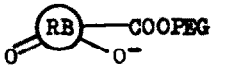
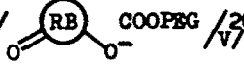


Figure 3 - The protonation curves for : 1/  2/  /550/ /IV/, 3/  /2000/ /V/

It is easy to see that for the C-2′ Rose bengal esters with side chains of various lengths, different pK values are observed for the protonation of phenol group. The lowest value is observed for the longest side chain /V/, the highest value for the shortest chain /III/. These observations suggest that dye molecule forms the own microenvironment using the long side chain covalently attached at C-2′ position. The isolation of the dye molecule from the entire environment is better when length of the attached side chain is greater.

Fluorescence tests for III and IV confirm the formation of the micro-environmental cover. Figure 4 and Figure 5 show the fluorescence spectra for III and IV measured in water at various pHs.

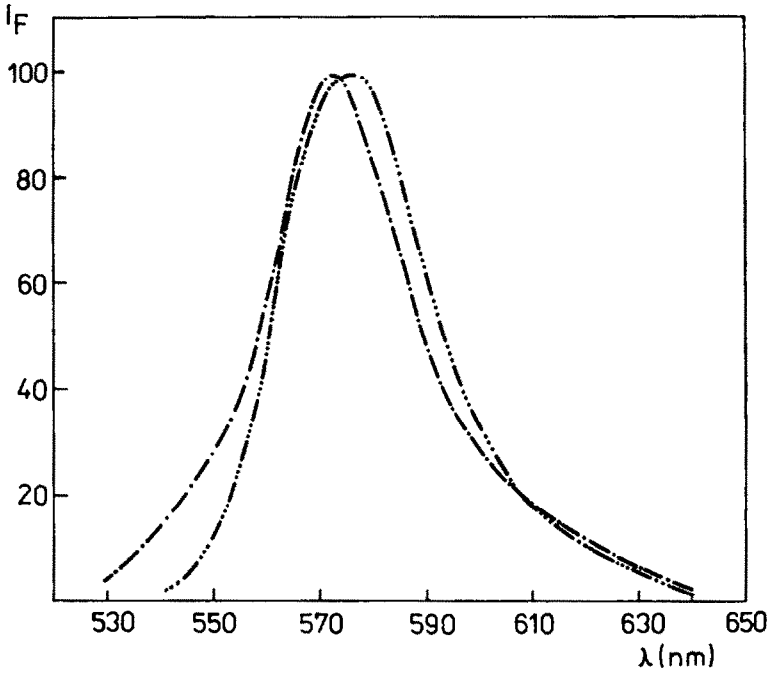
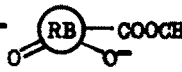


Figure 4 - Fluorescence emission spectra in water : —·—·—  , ····· RB

The comparison of the data presented in Figures 4 and 5 unequivocally indicate different properties for both dyes. For III the pH change essentially does not change the position of the fluorescence maximum, so it is possible to conclude that polarity of the dye environment is not changed when pH undergoes from high to low value. For IV the changes of the fluorescence maximum are very distinct. Supposed own microenvironmental conception very easy explains observed behaviour. The poly/ethylene glycol/ cover is less polar than water and formation of microenvironmental layer decreases polarity of medium surrounding the dye molecule. This causes the red shift of fluorescence maximum.

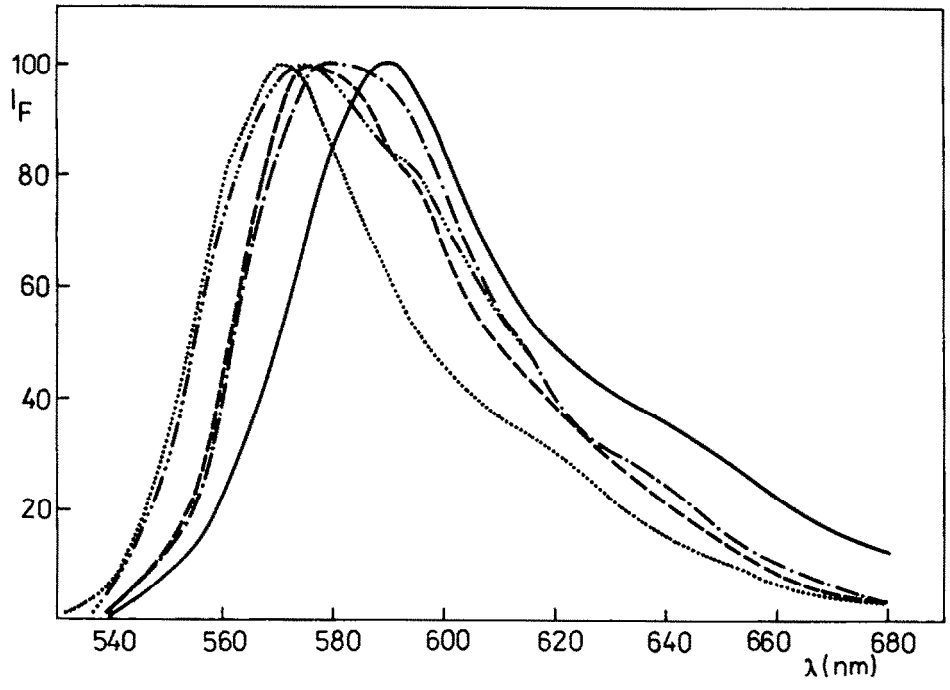


Figure 5 - Fluorescence emission spectra of RB—COO/CH_2/5- COO/CH_2CH_2O/n-CH_3 /IV/ in water at various pHs
——— 0.01 n HCl, — — — pH=3, —·—·— pH=4.0
—···—···— pH=8.0, ····· 0.01 n NaOH

EXPERIMENTAL DETAILS

Electronic absorption spectra were measured on Carl-Zeiss Jena M40 spectrometer. The steady-state luminescence measurement were carried out using multifunctional modular Cobrabid MSF 102 spectrofluorimeter. The pH have been taken using the Mera-Elwro N 517 pH-Meter.

The synthesis of I, II and IV have been described earlier [14,15] .

Rose Bengal /C-2'/ 2-Hydroxyethyl Ester, Molecular Form /III/

Rose bengal /1.0 g., 1 mmol/ was dissolved in water /10 ml/ and a solution of 2-bromoethanol /0.4 g., 3 mmol/ in 10 ml of acetone added. The resulting mixture was refluxed overnight. The solvents were then evaporated in vacuo. The product was purified using column chromatography. IR : 1736 cm^{-1} /C=O ester group/ : Vis : /MeOH/ : 663.0 nm, $\varepsilon = 9.8 \times 10^4$.

Monofunctionalized Poly/ethylene glycol/ 2000 with Rose Bengal /V/

Poly/ethylene glycol/2000/Roth/ was converted to its α, ω dibromo derivative /VI/; and 2.0 g /about 1 mmol/ was dissolved in 50 ml dry DMF, 3.0 g /3 mmol/ of Rose bengal was added. The mixture was stirred at 80°C for 24 h. After the reaction DMF was evaporated in vacuo and final products were separated using column chromatography. For study the monofunctionalized poly/ethylene glycol/ with Rose bengal was used. IR : 1735 cm^{-1} /C=O ester group/.

Acknowledgment : I am very greatful to Dr D.C.Neckers who initiated my interest to Rose bengal and helped me to understand better the chemistry of this dye.

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