Effects of various salts on the spectral properties of merocyanine 540, a fluorescent probe, in aqueous media

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Abstract. The effects of several biologically-important inorganic salts (NaCl, KCl, $MgCl_2$, $MgSO_4$, $CaCl_2$, $EuCl_3$ and $Er(CH_3COO)_3$) on the electronic absorption and fluorescence spectra of merocyanine 540 (MC-540) have been determined in aqueous media. Depending on both the MC-540 concentration and the nature of salt, a new absorption band is obtained, above a threshold of salt concentration. The cations produce two types of fluorescence quenching according to their charge: in the case of monovalent ions (Na^+, K^+), a nonlinear Stern–Volmer behaviour is observed, while for divalent alkaline-earth (Mg^{2+}, Ca^{2+}) and trivalent lanthanide (Eu^{3+}, Er^{3+}) ions, linear Stern–Volmer relationships are established. Our results, which exclude the presence of protonated MC-540, demonstrate the existence of interactions between the inorganic ions and the MC-540 highly polar zwitterionic structure followed by an ion-induced aggregation of the dye.

Keywords. Merocyanine 540; inorganic salts; absorption and fluorescence spectra; fluorescence quenching.

1. Introduction

Merocyanine 540 (MC-540, 62796-23-0), an anionic fluorescent dye, has been used as a photosensitizer in the photographic industry [1] as well as an external biological probe in the staining of various cellular membranes [2–5] and in the phototreatment of human cancer cells [6–8]. Recently, we have investigated the solvent and concentration effects on the electronic absorption and emission fluorescence spectra of MC-540 [9]. Although one study has been devoted to the interactions of MC-540 with some physiological cations [10], no report has been reported so far on the quantitative effect of salt concentration on the MC-540 fluorescence quenching. The aim of the present study was to determine the quantitative effects of various inorganic salts on the photophysical properties of MC-540, in view of using this fluorescent probe for biophysical and analytical applications.

2. Experimental

2.1 Chemicals

MC-540 (Aldrich, purity 99%) was recrystallized twice in ethanol before use. NaCl, NaBr, NaI, KCl, $MgSO_4$, $MgCl_2$, $CaCl_2$ (Merck). $Er(CH_3COO)_3$ (Johnson Matthey Company) and $EuCl_3$ (Strem Chemical) were used as received. Bidistilled water (pH 5·6) was used as solvent for the preparation of all dye and salt aqueous solutions.
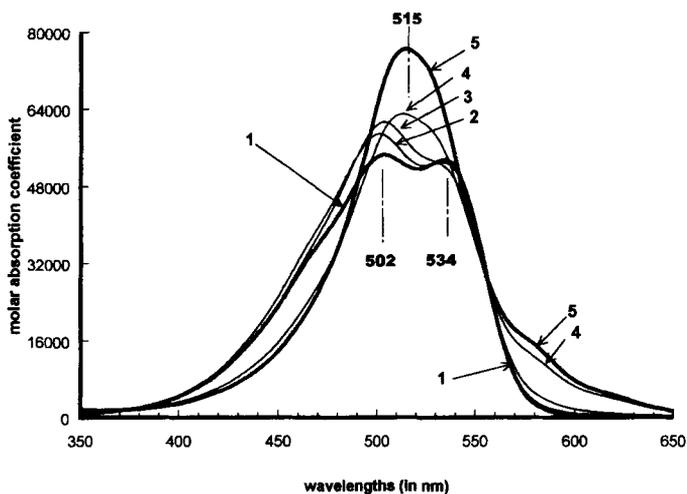
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Figure 1. Effects of NaCl on the MC-540 absorption spectra at 25°C [MC-540] $(M) = 2 \times 10^{-6}$ (1); 2×10^{-5}; (2, 3, 4, 5). [NaCl] (M) = 0·1 (3); 0·2 (4); 0·3 (5)

2.2　*Spectroscopy*

The electronic absorption spectra were recorded at 25·0 ($\pm$ 0·2)°C with a double beam Carry 118 spectrophotometer equipped with a thermostated cell holder. Steady-state fluorescence excitation and emission data were carried out at the same temperature on a Perkin–Elmer LS-50 spectrofluorimeter. The best accuracy of the fluorescence intensity measurements was observed when aliquots of concentrated salt solution were added to a quartz cell firstly filled with an aqueous MC-540 solution.

3.　Results and discussion

3.1　*Salt concentration effects on the MC-540 absorption spectra*

Figure 1 shows MC-540 absorption spectra recorded in pure water and in different NaCl aqueous solutions. At low concentration of MC-540 (2×10^{-6} M) the spectrum 1 (recorded in water) exhibits two bands of similar absorbance at 534 and 502 nm, attributed to the presence of monomer forms. Upon increasing the dye concentration 10 times (2×10^{-5} M), the 502 nm band is weakly blue-shifted (2 nm) and its molar absorptivity is slightly enhanced, whereas a molar absorptivity decrease is observed at 534 nm (spectrum 2). These spectral changes are probably due to the increased contribution of MC-540 dimers formed in these more concentrated aqueous solutions [3]. In the presence of NaCl concentrations up to 0·08 M no significant spectral modifications occur, except slight hypochromic and hyperchromic effects produced at 534 nm and 502 nm respectively. In contrast, at NaCl concentrations larger than 0·1 M, a dramatic spectral change takes place (spectrum 5), with the appearance of a main band at 515 nm (the ⟨complexation peak⟩), and two red-shifted, low-absorbing shoulders at about 580 and 620 nm. Moreover, upon adding amounts of NaCl until reaching a saturated salt concentration, the intensities of both, the main band and shoulders are progressively enhanced. A further increase of the salt concentration yields a hypochromic effect, but no modification of the spectral shape, thereby indicating dye precipitation. Similar
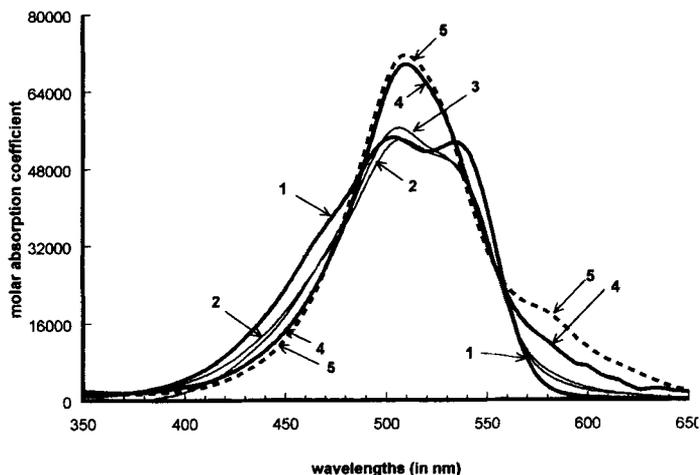
Figure 2. Effects of 1 M electrolytes on the MC-540 absorption spectra at 25°C [MC-540](M) = 2 × 10⁻⁶ (1–4); 2 × 10⁻⁵ (5). NaCl (2); HCl (3); MgCl₂ (4–5).

spectral changes, including the growth of a ⟨complexation peak⟩ at 515 nm at a threshold of salt concentration, are observed with the other salts, In all cases, a *larger* MC-540 concentration (increasing the contribution of aggregated MC-540 forms) requires a *lower* threshold of salt concentration for inducing the intensified 515 nm band. Figure 2 shows the influence of several electrolytes on the MC-540 absorption spectra. As can be seen, the spectral pattern of a 2×10^{-6} M MC-540 solution (spectrum 1) is not significantly modified in the presence of 1 M NaCl (spectrum 3) or 1 M HCl (spectrum 2); while a strong ⟨complexation peak⟩ appears at 515 nm in a 1 M MgCl₂ solution (spectrum 4). Also, the 580 nm and 620 nm shoulders become more intense at high (spectrum 5) than at low (spectrum 4) dye concentration. The absence of any ⟨complexation peak⟩ in very acidic media demonstrates that this 515 nm band cannot be assigned to the MC-540 protonated species, which is possibly present in such media (pH < 1). On the other hand, the formation of the ⟨complexation peak⟩ can be attributed to mutual compensation of the MC-540 concentration and both charge and salt concentrations. As a result, this peak occurs in a very large range of salt concentrations. Finally, the growth of the 515 nm band is also observed when a 2×10^{-5} M MC-540 aqueous solution containing a NaCl concentration slightly lower than the 0·08 M salt threshold is cooled from 25°C to 15°C, proving the thermal reversibility of such effects.

3.2   Salt concentration effects on the MC-540 fluorescence emission and excitation spectra

The MC-540 fluorescence excitation and emission spectra have been recorded in aqueous solutions at several dye and salt concentrations (figures 3 and 4). In the absence of salt, the excitation spectra (figure 3) remains approximately homothetic to the absorption spectra in the 2×10^{-6} M $- 10^{-5}$ M MC-540 concentration range (spectra 1 and 2). However, when the MC-540 concentration rises from 10^{-5} M (spectrum 2) to 2×10^{-5} M (spectrum 3), the excitation spectra vary slightly. Upon adding NaCl, the excitation spectrum is apprently split in two bands at 460 nm and 550 nm and
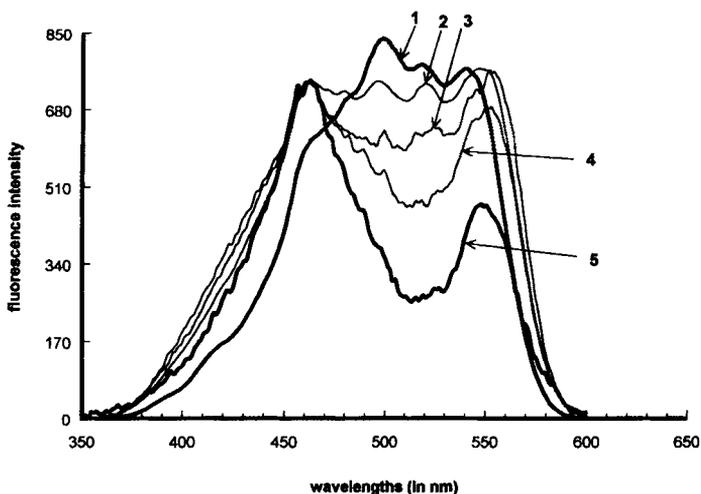
Figure 3. Effects of NaCl on the MC-540 fluorescence excitation spectra at 25°C [MC-540](M) = 2×10^{-6} (1); 10^{-5} (2); 2×10^{-5} (3–5). [NaCl](M) = 0·05(4); 0·1(5) ($\lambda_{em} = 620$ nm)
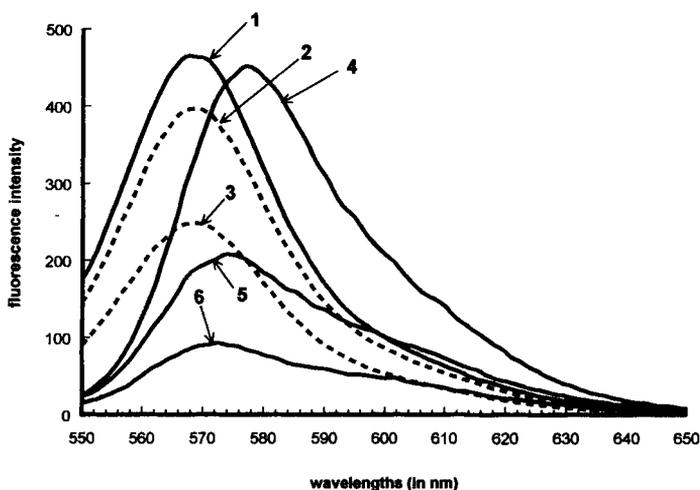

Figure 4. Effects of NaCl on the MC-540 fluorescence emission spectra at 25°C [MC-540](M) = 2×10^{-6} (1–3); 5×10^{-5} (4–6). [NaCl](M) = 0·05(5); 0·1(6) ($\lambda_{ex} = 530$ nm (1); 500 nm (2, 4, 5, 6); 460 nm (3).

simultaneously the intensity of the 550 nm excitation band is strongly reduced relative to the 460 nm one (spectrum 5). The MC-540 fluorescence emission spectra are presented in figure 4. Whatever excitation wavelength is used ($\lambda_{exc} = 460$ nm, 500 nm and 530 nm), a similar fluorescence emission band is observed at 569 nm for a 2×10^{-6} M MC-540 aqueous solution (spectra 1–3). The only difference lies in the intensity, which varies roughly in the same way as the molar absorption coefficient value. This suggests the involvement of vibronic transitions arising from the excitation of the same species present in very dilute solutions, i.e. the monomeric form of the dye.

Upon increasing the dye concentration, it occurs again as only one emission band, which is progressively red shifted by ≈ 8 nm ($\lambda_{em} = 577$ nm at 5×10^{-5} M) and is significantly diminished in intensity (spectrum 4). Similar red-shifts of the emission band with increasing MC-540 concentration have been also reported in organic solvents, and have been attributed to aggregation of MC-540 [9]. Upon adding NaCl to this MC-540 concentrated solution, the emission band is blue shifted by about 7 nm and it appears as a shoulder at ~ 610 nm; also, the fluorescence is quenched when the NaCl concentration reaches 0·1 M (spectrum 6). The blue-shift value measured is equivalent to the one observed when a 5×10^{-5} M unsalted MC-540 aqueous solution is diluted to 2×10^{-6} M.

3.3   Quantitative study of the MC-540 fluorescence quenching in the presence of various salts

In order to determine the type of quenching (i.e. static or dynamic), we have analyzed the effects of salt concentration on the MC-540 fluorescence quenching, by means of the Stern–Volmer relation

$$\frac{I_F^0}{I_F} = 1 + K_{SV}[Q]$$

where Q is the quencher concentration, K_{SV} is the Stern–Volmer constant, I_F and I_F^0 denote respectively the intensity of fluorescence in the presence and absence of quencher. A linear plot of I_F^0/I_F vs quencher (i.e. salt) concentration would indicate a dynamic fluorescence quenching, while a nonlinear plot would suggest a static quenching contribution. On the other hand, only a completely fluorescence static quenching would bring a linear plot of log I_F^0/I_F vs quencher concentration [11]. Depending on the nature of the ions, two different types of quenching behaviours have been found.

3.3.1   Quenching of MC-540 fluorescence by monovalent alkaline ions

Figure 5 shows the Stern–Volmer plots for the MC-540 fluorescence quenching by NaCl at several dye concentrations. The resulting fits display a set of curves, each curve consisting of two parts, a plateau region and a nonlinear increase of I_F^0/I_F above a threshold of NaCl concentration. As previously observed in the absorption spectra, the greater is the MC-540 concentration, the lower is the value of the NaCl concentration threshold. Moreover, identical threshold values are obtained for the absorption and fluorescence measurements. These observations also demonstrate that a relation exists between the formation of the ⟨complexation peak⟩ and the MC-540 fluorescence quenching. The slopes, calculated from the tangent of each curve, show a better quenching efficiency of NaCl for aggregated MC-540 (at higher concentration) than for the free dye (at lower MC-540 concentration). No linear plot of log I_F^0/I_F vs quencher concentration is established, allowing to exclude involvement of a purely static quenching, which generally occurs in heterogeneous media. Our results are in agreement with the formation of small aggregates such as dimers or trimers in the MC-540 aqueous solutions [12]. While the nonlinear Stern–Volmer plots obtained with Na$^+$ and K$^+$ display a very similar pattern at fixed chloride counterion, some differences appear when the anion of the salt is interchanged. For instance, an increase of the slope values is observed according to the order: I$^-$ < Br$^-$ < Cl$^-$. Therefore, in the case of
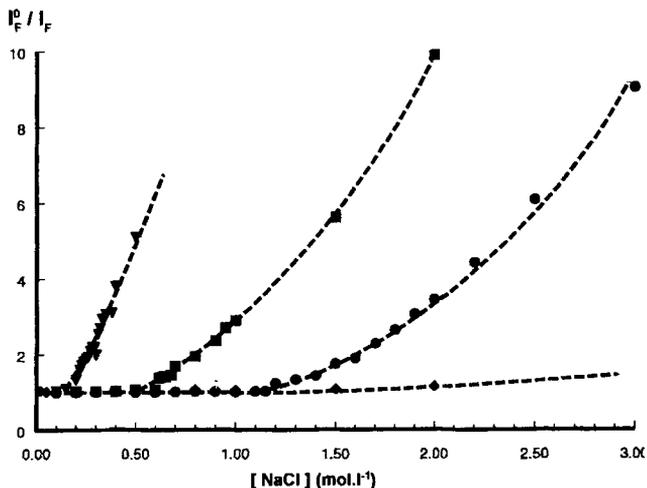
Figure 5. Nonlinear Stern–Volmer plots of the fluorescence quenching of excited MC-540 in water, as a function of NaCl concentrations [MC-540](M) = 5×10^{-7} (♦); 2×10^{-6} (●); 5×10^{-6} (■); 10^{-5} (▼) $\lambda_{ex} = 515$ nm; $\lambda_{em} = 620$ nm; $T = 25°$C
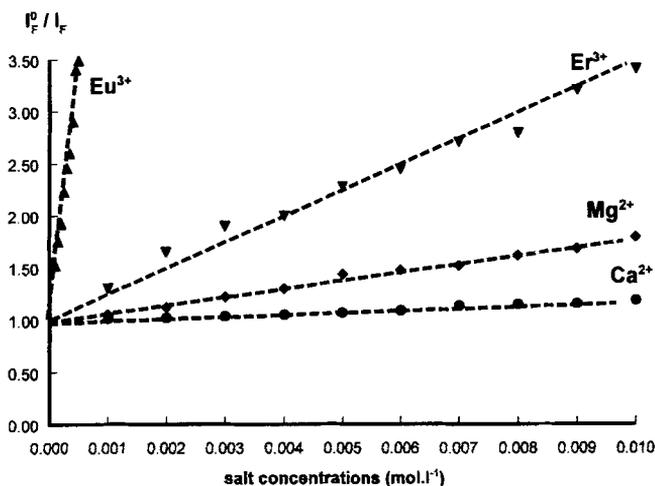

Figure 6. Stern–Volmer plots of the fluorescence quenching of excited MC-540 in water, as a function of $CaCl_2$ (●), $MgCl_2$ (♦), $Er(CH_3COO)_3$ (▼) and $EuCl_3$ (▲) concentrations [MC-540] = 10^{-5} M; $\lambda_{ex} = 515$ nm; $\lambda_{em} = 620$ nm; $T = 25°$C.

monovalent ions, we can conclude that the decrease in the size of the ion provokes an increase of its quenching efficiency.

3.3.2 *Quenching of MC-540 fluorescence by divalent alkaline-earth and trivalent lanthanide ions*

Figure 6 shows the Stern–Volmer plots obtained for the quenching of MC-540 by divalent Mg^{2+} and Ca^{2+} and trivalent Er^{3+} and Eu^{3+} ions. The resulting curves exhibit a good linearity with their slopes increasing according to the order:

$MgCl_2 < CaCl_2 < Er(CH_3COO)_3 < EuCl_3$, with Stern–Volmer constants equal to 21, 81, 222, 5053 $dm^3 mol^{-1}$ respectively. This allows us to conclude that, for ions bearing different charges, the greater is the charge, the stronger is the efficiency quenching. On the other hand, in the case of ions with identical charge, the greater is the size of the ion, the stronger is the fluorescence quenching. Our results are therefore consistent with the occurrence of a dynamic fluorescence quenching in the presence of multivalent ions.

4. Conclusion

We can conclude from our work that the shape and wavelength position of the electronic absorption and fluorescence spectra of MC-540 are extremely sensitive to the presence of various amounts of biologically-important salts in aqueous media and to the concentration of the dye. These spectral changes can be related to the formation of ion-induced, MC-540 aggregated species. Moreover, we have demonstrated the existence of two distinct behaviours in the MC-540 fluorescence quenching, depending on the electrical charge and nature of the ions. These interesting findings could help in developing an ión-sensitive, fluorescent sensor based on the MC-540 photophysical properties which could be applied to biological media.

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