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An explanation of the quaternary ammonium compound-Eosin Y equilibria in the presence and absence of Triton X-100

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Abstract

The quaternary ammonium compound (QAC)-Eosin-Y equilibria were examined in the presence, and absence, of micelles of Triton X-100. At pH 8.0 and 2.2, an ion-pair and molecular associate, respectively, were observed between QAC and Eosin. The absorbance of these forms was enhanced by partitioning into micelles of Triton X-100.

Keywords: Quaternary ammonium compound (QACs); Ion-pair; Triton X-100; Eosin-Y; Micelle

Quaternary ammonium compounds (QAC) are cationic, organically substituted ammonium compounds which possess strong bactericidal but weak detergent properties (Hugo and Russell, 1982). The primary cidal activity is against Gram-positive bacteria, however, cidal activity is also exhibited against Gram-negative bacteria. The QAC possess antifungal properties, although they are fungistatic rather than fungidal (D'Arcy, 1971; Hugo and Russell, 1982), and also trypanocidal activity (D'Arcy and Taylor, 1962). The uses of QAC are many and varied but usually involve surface disinfection, antisepsis and preservation (Hugo and Russell, 1982).

There have been several approachs employed in the analysis of QACs including ultraviolet spectroscopy in the presence or absence of anionic agents (Petrocci, 1983), gas chromatography (Suzuki et al., 1989), liquid scintillation counting (Bonesvoll and Gjermo, 1978) and high performance liquid chromatography (Collins and Deasy, 1990). However these reported methods were often prohibitive due to cost, thermal degradation or lack of sensitivity. We recently reported a colorimetric method for the analysis of three QAC, cetylpyridinium chloride, cetrimide and benzalkonium chloride, which involved the formation of an ion-pair with an anionic dye (Eosin-Y) in the presence of micelles of the non-ionic surfactant Triton X-100. This method provided greater sensitivities than those reported for other methods and, in addition, was unaffected by the presence of microbial cytoplasmic materials there-

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Table 1
The effect of Eosin-Y concentration and quaternary ammonium compound concentration on the absorbance and λ_{max} of the QAC-Eosin-Y ion-pair, in the presence and absence of Triton X-100 (250 μl ml^{-1}) at pH 2.2

QAC	Description	Triton X-100	λ_{max}	Absorbance
CPC	Excess Eosin	Present	538	0.256
CPC	Excess CPC	Present	538	0.742
CPC	Excess Eosin	Absent	520	0.114
CPC	Excess CPC	Absent	538	0.425
CTAB	Excess Eosin	Present	538	0.227
CTAB	Excess CTAB	Present	538	0.724
CTAB	Excess Eosin	Absent	521	0.116
CTAB	Excess CTAB	Absent	538	0.403
BKC	Excess Eosin	Present	538	0.193
BKC	Excess BKC	Present	538	0.500
BKC	Excess Eosin	Absent	521	0.094
BKC	Excess BKC	Absent	538	0.284

CPC, cetylpyridinium chloride; CTAB, cetrimide; BKC, benzalkonium chloride

fore rendering the method useful in the evaluation of the adsorption of these QACs onto the surface of micro-organisms (Schep et al., 1995a; Schep et al., 1995b).

This study was performed to examine the various equilibria present in the QAC-Eosin-Y system in the presence and absence of micelles of Triton X-100 and hence obtain a greater understanding of the equilibria operative in our analytical method.

Cetylpyridinium chloride, cetrimide, benzalkonium chloride and Tetrabromo-(R) fluorescein (Eosin-Y) were purchased from Sigma Chemicals (St. Louis, USA). Iso-octylphenoxypolyethoxyethanol (Triton X-100), borate buffer tablets and disodium hydrogen orthophosphate anhydrous were obtained from BDH (Poole, England). Citric acid was purchased from Ajax Chemicals Ltd. (Auburn, Australia). All chemicals were AnalaR, or equivalent, grade.

All spectrophotometric measurements were performed using a Hewlett Packard 8452 diode array spectrophotometer linked to a NEC Powermate 1 plus 286 computer. A Hannah HI 1280 pH meter was used to measure pH.

To investigate the nature of the QAC-Eosin-Y equilibria, the λ_{max} and absorbance of the following solutions were determined: (a) Eosin-Y (9.0 μg ml^{-1}) and QAC (1 μg ml^{-1}), i.e., excess Eosin-Y, in the presence and absence of Triton X-100 (250 μl ml^{-1}), at both pH 2.2 and 8.0; (b) Eosin-Y (9.0 μg ml^{-1})/QAC (25 μg ml^{-1}), i.e., excess QAC, in the presence and absence of Triton X-100 (250 μl ml^{-1}), at pH 2.2 and 8.0.

Table 1 and Table 2 show the effect of Eosin-Y concentration and QAC concentration (cetylpyridinium chloride, benzalkonium chloride and cetrimide) on the absorbance and λ_{max} of the QAC-Eosin-Y ion-pair/associate, in the presence and absence of micelles of Triton X-100 (250 μl ml^{-1}) at pH 8.0 and 2.2, respectively. Four major λ_{max} values may be identified from these tables, namely circa 516, 520, 530 and 538 nm. The QAC used in this study were ionised (> 99%) at both pH 2.2 and 8.0 (Proudfoot, 1988). However, Eosin (pK_a 4.2) is an anionic dye and was 99.98 and 0.99% ionised at pH 8.0 and 2.2, respectively (Fompeydie et al., 1979; Mchedlov-Petrosyan et al., 1985). Therefore, the effects of pH, and hence ionisation of Eosin-Y, on the equilibria may be followed. The QAC when combined with excess Eosin-Y at pH 8.0 showed similar λ_{max} (circa 516 nm) and absorbance both in the presence, and absence, of micelles of Triton X-100. However, at pH 2.2, the λ_{max} shifted from circa 520 nm in the absence of Triton X-100 to 538 nm in its presence.

Eosin-Y, alone, and when combined with excess QAC at pH 8.0 (Table 1) resulted in a shift in λ_{max} to 530 nm both in the presence and absence of Triton X-100. However, the absorbance was

Table 2
The effect of Eosin-Y concentration and quaternary ammonium compound concentration on the absorbance and λ_{max} of the QAC-Eosin-Y ion-pair, in the presence and absence of Triton X-100 (250 μl ml^{-1}) at pH 8.0

QAC	Description	Triton X-100	λ_{max}	Absorbance
CPC	Excess Eosin	Present	518	0.754
CPC	Excess CPC	Present	529	0.833
CPC	Excess Eosin	Absent	516	0.739
CPC	Excess CPC	Absent	530	0.746
CTAB	Excess Eosin	Present	518	0.732
CTAB	Excess CTAB	Present	527	0.808
CTAB	Excess Eosin	Absent	516	0.765
CTAB	Excess CTAB	Absent	529	0.690
BKC	Excess Eosin	Present	516	0.790
BKC	Excess BKC	Present	528	0.733
BKC	Excess Eosin	Absent	516	0.766
BKC	Excess BKC	Absent	N.D.	N.D.

CPC, cetylpyridinium chloride; CTAB, cetrimide; BKC, benzalkonium chloride;N.D., not determined

greater in the presence of micelles of this surfactant. At pH 2.2 the λ_{max} was unchanged both in the presence and absence of Triton X-100 (538 nm), however the absorbance was greater in the presence of Triton X-100.

This study was designed to evaluate the contribution of individual ions and ion pairs/associates to the equilibria and, therefore, this will allow an overall picture of the various equilibra to be proposed (Fig. 1). Throughout this investigation, Triton X-100 was employed at a concentration which exceeded the critical micelle concentration (Schep et al., 1995a). The λ_{max} of Eosin-Y, both alone and in the presence of minimal concentrations of QAC, were examined and determined as 516 nm. The presence of micelles of Triton X-100 did not affect either the λ_{max} or the absorbance, and hence it can be suggested that Eosin-Y, as the ionised molecule, does not partition into micelles of Triton X-100. The addition of excess QAC to Eosin-Y at pH 8.0 produced a shift in λ_{max} which, given the charge disparity of the two molecules, is suggested to be due to the QAC-Eosin-Y ion-pair. The presence of micelles of Triton X-100 did not alter this λ_{max}, however, the absorbance values were noticeably greater in the presence rather than in the absence of this surfactant. As the QAC-Eosin-Y ion-pair is effectively an electrically neutral molecule then it is suggested that this will partition into the lipophilic region of the micelle. Hence, in the presence of micelles of Triton X-100, the equilibrium is shifted in the direction of the micelles. Therefore, more ion-pairs are formed in the presence than in the absence of Triton X-100 and a greater absorbance is noted (Fig. 1).

At pH 2.2, Eosin-Y will exist primarily as the unionised molecule and, therefore, the shift of λ_{max} from 516 nm (at pH 8.0) to 520 nm (at pH 2.2) in the presence of excess Eosin-Y is due to this alteration in ionisation state. Such changes in λ_{max} values due to altered ionisation are common (Jenkins, 1975). In the presence of micelles of Triton X-100 at pH 2.2, there is a shift in the λ_{max} of Eosin-Y to 538 nm which is suggested to be due to the partitoning of the un-ionised molecules of Eosin-Y into the lipophilic regions of the micelles. Alterations in λ_{max} values of dyes (of which Eosin-Y is an example) in the presence of micelles has been previously reported and was suggested to be due to the existence of the dye within the hydrocarbon core of the micelle (Mulley, 1964).

In the presence of excess QAC at pH 2.2, there is a shift in λ_{max} of the system, containing un-ionised Eosin-Y molecules and QAC, to 538 nm. Therefore, it is assumed that the QAC and Eosin-Y (in the un-ionised state) must interact, although not by electrostatic mechanisms, to produce a new molecular associate in solution. The absorbance of

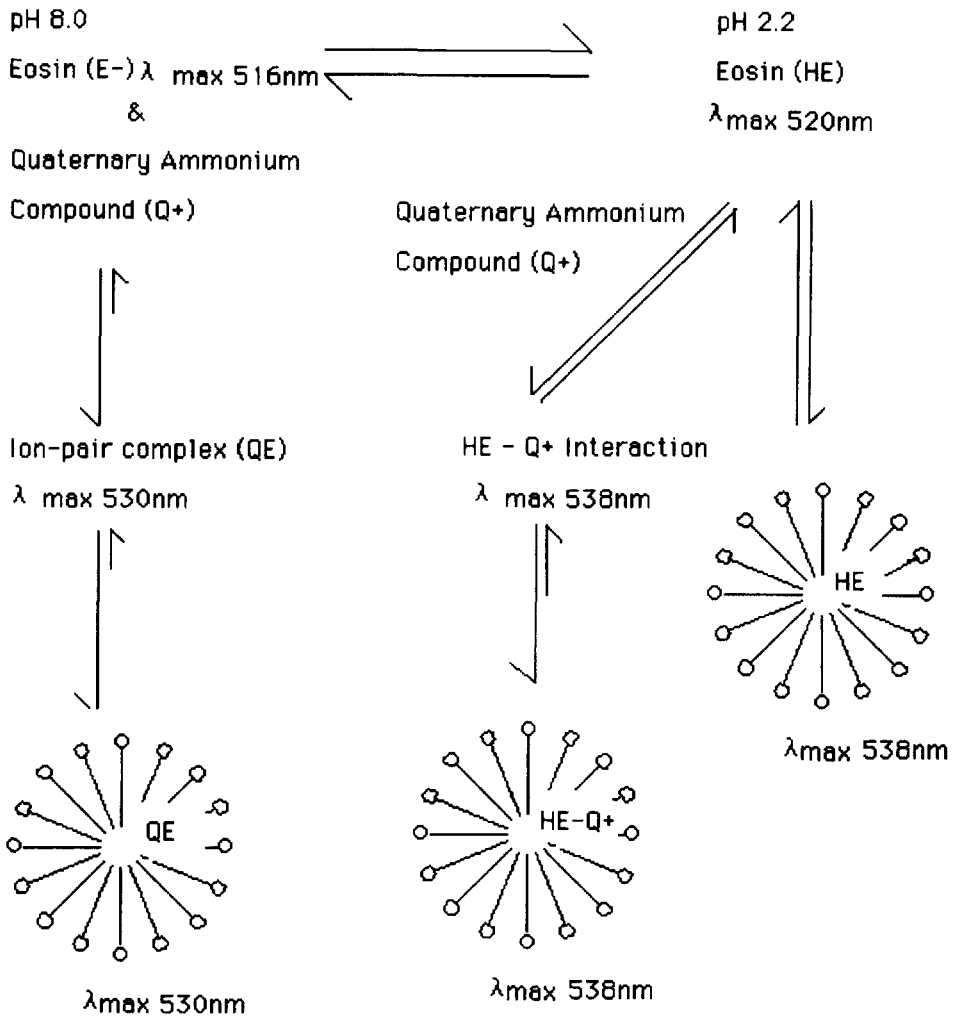

Fig. 1.

this new species is further enhanced by the presence of micelles of Triton X-100. Therefore, in a similar fashion to the proposed scenario at pH 8.0, the micelles are acting as a 'separate phase' into which the un-ionised Eosin/QAC molecule can partition and hence the equilibrium may be shifted towards the formation of molecular associates. Due to the positive charge (associated with the QACs), it is unlikely that the location of this molecular species within the micelles will be the same as that observed at pH 8.0. It is therefore possible that the charged QAC portion may lie towards the outside of the micelle to satisfy the thermodynamic requirements of the system.

Previously we reported a colorimetric method by which these QAC may be analysed in which the pH of the method was 8.0 and the Triton X-100 concentration was 250 μl ml^{-1} (i.e., as used in the current study). Therefore, the current study has shown that, under these conditions, QAC-Eosin-Y ion-pairing occurs and that the micelles act as a sink into which these ion-paired molecules may partition. This suggestion would account for the greater range of concentrations over which linearity was observed and also for the greater sensitivity of the analytical method following inclusion of micelles of Triton X-100 as previously recognised by us (Schep et al., 1995a).

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