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# The modifying effects of a cationic surfactant on the rates of base catalysed hydrolysis of esters of different structures

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The effect of a cationic surfactant, cetyltrimethylammonium bromide (CTAB), on the hydrolysis rates of four esters of different structure has been investigated. The esters used were ethyl *p*-aminobenzoate (EPAB), *p*-nitrophenyl acetate (PNPA), ethyl *p*-nitrobenzoate (EPNB) and *p*-aminophenyl acetate (PAPA). Association between the esters and the CTAB occurs so that above its cmc the surfactant modifies the rates of hydrolysis, increasing those of EPNB and PNPA and decreasing those of EPAB and PAPA. It thus appears that the type of group on the *p*-position of the aromatic ring plays a major role in determining the kind of effect brought about by the CTAB. Possible reasons to explain the two different effects that CTAB has on ester hydrolysis are put forward.

Mechanisms of ester hydrolysis are well understood and the modifying influence of many agents on the rates of hydrolysis, including the effect of addition of surfactants, has been examined (see Fendler & Fendler (1970) for review). The interactions of surfactants and esters are of importance to Pharmacy, as the shelf lives of preparations containing esters may well be determined by the esters' susceptibility to hydrolysis.

Much of the published work on drug-surfactant interactions is not easily interpreted and the mechanisms of the modifying action exerted by surfactants are not fully understood. Among the simpler factors shown to affect the interaction are the ionic nature and chain length of both surfactant and drug, and whether the ester is hydrolysed by base or acid catalysis. Menger & Portnoy (1967), using the *p*-nitrophenyl esters of acetic, dodecandioic and octanoic acid, have found that anionic micelles *retard* and cationic micelles *enhance* the rate of base catalysed hydrolysis, and that the magnitude of the modifying effect depends on the chain length of the ester. Non-ionic surfactants always appear to retard hydrolytic reactions (Fendler & Fendler, 1970).

Drug-surfactant interactions occur particularly above the critical micelle concentration (cmc), and the effect on the hydrolysis rate, i.e. an *increase* or *decrease* may depend upon the site and orientation of the drug in the micelle. If the drug is in the hydrophobic interior of the micelle, then hydrolysis should be retarded, irrespective of the ionic nature of the surfactant. The structure and ionic nature of the drug may affect its ability to penetrate to the centre of the micelle and such penetration may be dependent upon the pH of the system and configuration and polarity of substituent groups of the drug molecule. If the drug is in the hydrophilic portion of the micelle, either close enough to the surface for electrostatic effects between the surface and ionic species of water to be operative, or at the surface itself, then the nature of the

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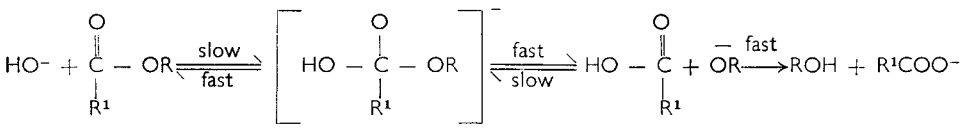
charge on the micelle could well be the major factor in determining the rate of hydrolysis. Simple electrostatic theory would predict that a positive charge on the micellar surface would attract hydroxyl ions and thus base catalysed ester hydrolysis should be enhanced in the presence of a cationic surfactant (cf. Menger & Portnoy, 1967; Romsted & Cordes, 1968; Fendler & Fendler, 1970). These authors have also suggested that the positive charge on the micelle stabilizes the negatively charged tetrahedral intermediate (I) formed according to the BAC 2 mechanism (Gould, 1959):—



(I)

and that this would also be expected to lead to an enhanced rate of hydrolysis.

Simple electrostatic theory cannot, however, account for the decrease in the rate of base catalysed hydrolysis of ethyl *p*-aminobenzoate, ethyl benzoate, diethyl phthallate and propyl benzoate (Mitchell, 1962, 1964) caused by concentrations of cetrimide B.P. above its cmc. It has also been reported (Riegelman, 1960) that the rate of base catalysed hydrolysis of ethyl *p*-aminobenzoate is *increased* by cetyltrimethylammonium bromide (CTAB) in a concentration double its cmc, whilst higher concentrations of CTAB *decrease* the rate to an extent that is proportional to its concentration.

The present work was initiated to investigate the mechanism by which ester structure determines the modifying effect produced by a cationic surfactant on the rates of ester hydrolyses.

## MATERIALS AND METHODS

*Esters.* The following four esters were used: ethyl *p*-aminobenzoate (EPAB), *p*-nitrophenyl acetate (PNPA), ethyl *p*-nitrobenzoate (EPNB), *p*-aminophenyl acetate (PAPA).

The stability of EPAB and PNPA in the presence of CTAB had been investigated previously (Riegelman, 1960; Mitchell, 1962; Menger & Portnoy, 1967; Romsted & Cordes, 1968), and at concentrations above the cmc the rate of hydrolysis of EPAB was decreased and of PNPA was increased. EPNB and PAPA were chosen as the natural counterparts so that the importance of the type of groups in both the 1- and 4-positions of the aromatic ring in determining the nature of the modifying effect could be established.

*EPAB* was obtained from BDH Ltd., and was of reagent grade. It was twice recrystallized from 50% ethanol; m.p. 89° (lit. 88–90°, Merck Index, 1968).

*PNPA* was prepared from *p*-nitrophenol by the method of Chattaway (1931). The product was washed with water and recrystallized from 50% ethanol; m.p. 77° (lit. 77·5–78°, Bender & Nakamura, 1962).

*EPNB* was prepared by the method of Vogel (1951). The product was recrystallized twice from 70% ethanol; m.p. 57° (lit. 57°, Merck Index, 1968).

*PAPA* was prepared by a three stage reaction. Equimolar quantities of benzaldehyde and *p*-aminophenol were refluxed with dilute acetic acid as solvent for 15 min, to produce benzylidene *p*-aminophenol which was recrystallized from absolute

ethanol. This was then acetylated by the method of Chattaway (1931) and the product was recrystallized from benzene. *p*-Aminophenyl acetate was then prepared from the benzylidene derivative by the method of Galatis (1926). M.p. 75° (lit. 75°, Galatis, 1926).

The structure and purity of all the esters was confirmed by nmr spectroscopy.

*Surfactant.* This was cetyltrimethylammonium bromide (CTAB). To obtain a pure sample, cetyl bromide was reacted with anhydrous trimethylamine in "super dry" ethanol at 110° for 6 h in a high pressure reaction vessel (Baskerville Lindsay, U.K.). The crude CTAB formed was Soxhlet extracted for 12 h with light petroleum (b.p. 40–60°) and twice recrystallized from absolute ethanol. Nmr and mass spectroscopy were used to confirm the structure. No minimum was observed in the surface tension-log concentration curves that were obtained.

The four esters and CTAB were dried in a vacuum oven at 22 torr and 50° and subsequently stored in a desiccator over phosphorus pentoxide.

*Buffer salts and analytical reagents* were all of Analar quality.

*Water* was freshly distilled from an all glass still and had a specific conductivity of $< 10^{-7}$ ohm$^{-1}$ cm$^{-1}$ at 25°.

*Buffer solutions.* Two different systems were used: Sørensen's glycine (glycine 0·1M in 0·1M sodium chloride and 0·1N sodium hydroxide); Delory and King's carbonate bicarbonate (0·2M anhydrous sodium carbonate and 0·2M sodium bicarbonate). The appropriate standard solutions were prepared according to Documenta Geigy (1962).

*pH measurements.* These were made at the temperature of the kinetic run using a Pye Dynacap pH meter fitted with a Pye Ingold 405 combined electrode and 622 thermal resistor. The meter and electrode system were standardized using sodium tetraborate buffer (Manov, DeLollis & others, 1946).

*Spectrophotometric measurements* were made in a Unicam SP500 spectrophotometer using 1 cm cuvettes.

### *Estimation of the critical micelle concentration*

The cmc of the CTAB was measured under all the experimental conditions used during the kinetic runs. The buffer constituents had a marked effect on the value obtained. The cmc was determined by both conductivity and dye solubilisation methods. At 50° in Sørensen's glycine at pH 10·39 the value was $1 \times 10^{-5}$M, while in Delory and King's buffer the values for all systems were between 1 and $2 \times 10^{-4}$M.

### *Kinetic studies*

The appropriate buffer solution (100 ml) containing the desired concentration of CTAB was prepared and equilibrated to the required temperature. The pH of the solution was measured and if necessary adjusted slightly to the correct value. To 96 ml of this solution was added 4 ml of an aqueous solution, of 25 times the required ester concentration. The solution, after mixing, was assayed immediately and then at appropriate intervals throughout the run in order to follow the course of the hydrolysis.

EPAB and PAPA were hydrolysed in flasks kept in a lagged water bath (temperature fluctuations were ±0·1°). PNPA and EPNB were hydrolysed more rapidly and the degradation was followed on a single sample in a 1 cm cuvette kept in the jacketed cell compartment of the spectrophotometer.

EPAB, PNPA and EPNB were hydrolysed in solutions that were not controlled for degree of aeration. With PAPA under these conditions first order kinetics were not obeyed, owing to interference from the oxidation of *p*-aminophenol formed as a degradation product. Thus solutions of PAPA and CTAB were bubbled with oxygen free nitrogen (B.O.C. Ltd.) for 2 h before mixing and the bubbling was continued throughout the experiment.

*Assay procedures*

Only the principles of the methods used are given. Slight modifications were necessary to overcome problems that occurred when the surfactant and ester concentrations were altered, and in all cases the suitability and accuracy of the method employed was confirmed.

EPAB was assayed spectrophotometrically by a method similar to that of Higuchi, Havinga & Busse (1950) at the wavelength of maximum absorption ($\lambda_{max}$) of the ester of 286 nm.

With PNPA the hydrolysis was followed by measuring the production of *p*-nitrophenol at its $\lambda_{max}$, at the pH of the kinetic run, of 400 nm.

The concentration of EPNB was determined by a method similar to that employed for EPAB, at the $\lambda_{max}$ of the ester of 265 nm.

PAPA was assayed by a modified Bratton-Marshall reaction (Tansey, 1969) at 550 nm. This was possible as the degradation product *p*-aminophenol does not form a coloured complex in this reaction.

*Treatment of results*

With one exception, solutions of the esters were found to degrade by apparent first order kinetics. The percentage residual concentration of ester was calculated for each period of heating. Values for the rate constant (k) were obtained from log% concentration-time data by means of a computerized least-squares regression analysis, which gave values of k, its associated standard error, and the correlation coefficient.

## RESULTS AND DISCUSSION

The effect of a range of concentrations of CTAB on the rate of hydrolysis of EPAB in Sørensen's glycine buffer at a pH of 10·39 at 50° is seen from Table 1. The pre-

Table 1. *The effect of CTAB on the rate of hydrolysis of ethyl* p-*aminobenzoate (EPAB) at a concentration of* $1 \times 10^{-3}$M, *at pH* 10·39 *in Sørensen's glycine buffer at 50°.*

| Surfactant concentration (mol litre$^{-1}$) | Observed 1st order rate constant, k (min$^{-1}$) | Standard error of k |
|---|---|---|
| 0 | $1{\cdot}280 \times 10^{-3}$ | $1{\cdot}37 \times 10^{-5}$ |
| $5 \times 10^{-5}$ | $1{\cdot}321 \times 10^{-3}$ | $3{\cdot}15 \times 10^{-5}$ |
| $1 \times 10^{-4}$ | $1{\cdot}177 \times 10^{-3}$ | $1{\cdot}12 \times 10^{-5}$ |
| $2 \times 10^{-4}$ | $1{\cdot}204 \times 10^{-3}$ | $1{\cdot}16 \times 10^{-5}$ |
| $3 \times 10^{-4}$ | $1{\cdot}152 \times 10^{-3}$ | $9{\cdot}8 \times 10^{-6}$ |
| $4 \times 10^{-4}$ | $1{\cdot}166 \times 10^{-3}$ | $1{\cdot}41 \times 10^{-5}$ |
| $1 \times 10^{-3}$ | $1{\cdot}077 \times 10^{-3}$ | $1{\cdot}51 \times 10^{-5}$ |
| $2 \times 10^{-3}$ | $9{\cdot}003 \times 10^{-4}$ | $1{\cdot}29 \times 10^{-5}$ |
| $4 \times 10^{-3}$ | $7{\cdot}388 \times 10^{-4}$ | $1{\cdot}34 \times 10^{-5}$ |
| $1 \times 10^{-2}$ | $6{\cdot}295 \times 10^{-4}$ | $1{\cdot}30 \times 10^{-5}$ |
| $2 \times 10^{-2}$ | $4{\cdot}630 \times 10^{-4}$ | $4{\cdot}71 \times 10^{-6}$ |

cision of the experimental technique was confirmed by four replicate experiments in the absence of CTAB and four in the presence of $1 \times 10^{-4}$M CTAB. The calculated coefficients of variation were 1·39 and 0·87% respectively. With the exception of the lowest value of CTAB used ($5 \times 10^{-5}$M) where the rate constant was not significantly different from the value obtained in the absence of surfactant (t calc. = 0·605, t tab. at $P = 0{\cdot}05 = 2{\cdot}18$), increasing the concentration of CTAB present resulted in a retardation of hydrolysis, the magnitude of which effect increased with surfactant concentration (Fig. 1). Thus, unlike Riegelman (1960), we did not observe

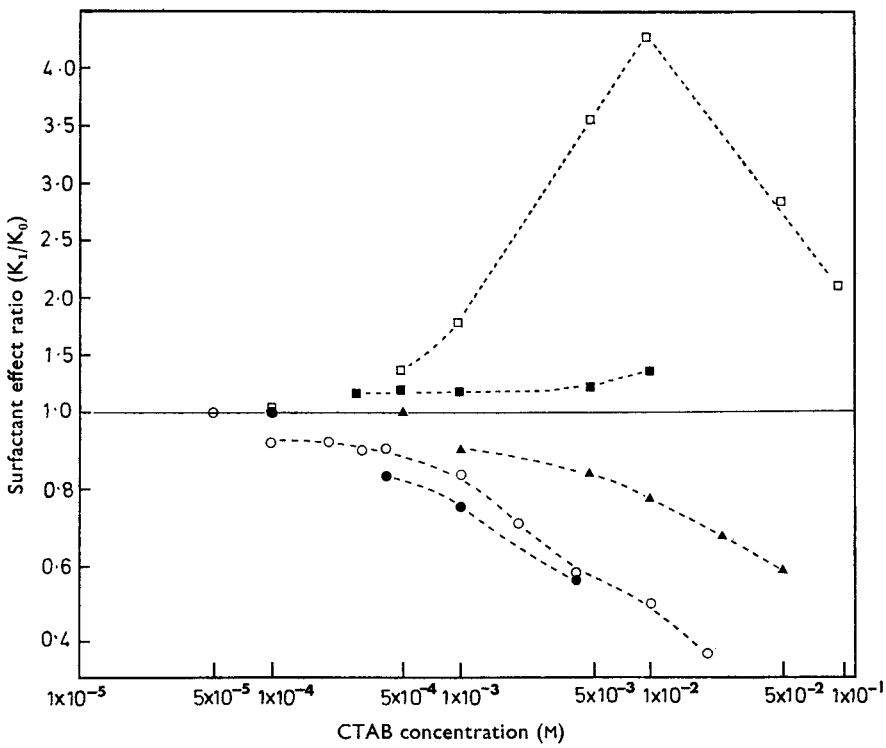



FIG. 1. The effect of CTAB on the hydrolysis of four esters expressed as a ratio ($k_1/k_0$) of the first order rate constants obtained in the presence ($k_1$) and absence ($k_0$) of the surfactant. ●, EPAB; ▲, PAPA; □, PNPA; ■, EPNB; all in Delory and King's buffer; ○, EPAB in Sørensen's glycine buffer.

CTAB, just above its cmc ($1 \times 10^{-5}$M, in glycine buffer) to have a potentiating effect on this ester. The different concentration of ester used by Riegelman ($6 \times 10^{-5}$M) does not explain the difference since we found reduced values of k to be produced by all concentrations of CTAB above the cmc when the experiment was repeated with an ester concentration of $4 \times 10^{-5}$M in bicarbonate buffer (Table 2). The surfactant effect appears to depend upon the formation of micelles, as a concentration of CTAB near the cmc (measured as $1{\cdot}0 \times 10^{-4}$ in bicarbonate buffer) showed no significant modifying action. The reason for the difference between this and Riegelman's findings is not known. Examination of his data suggests the possibility that the reported increase may not be statistically significant.

Table 2. *The effect of CTAB on the rate of hydrolysis of ethyl* p-*aminobenzoate (EPAB), p-nitrophenyl acetate (PNPA), ethyl* p-*nitrobenzoate (EPNB) and* p-*aminophenyl acetate (PAPA) at a concentration of* $4 \times 10^{-5}$M *in Delory and King's buffer.*

| Surfactant concentration (mol litre$^{-1}$) | EPAB k values (min$^{-1}$) pH 10·55, T = 50° | PNPA k values (min$^{-1}$) pH 9·2 T = 25° | EPNB k values (min$^{-1}$) pH 10·64 T = 25° | PAPA k values (min$^{-1}$) pH 10·64 T = 25° |
|---|---|---|---|---|
| 0 | $2{\cdot}175 \times 10^{-3}$ | $1{\cdot}611 \times 10^{-2}$ | $1{\cdot}212 \times 10^{-2}$ | $2{\cdot}436 \times 10^{-2}$ |
| $9{\cdot}6 \times 10^{-5}$ | $2{\cdot}135 \times 10^{-3}$ | $1{\cdot}591 \times 10^{-2}$ | | |
| $2{\cdot}9 \times 10^{-4}$ | | | $1{\cdot}399 \times 10^{-2}$ | |
| $4{\cdot}8 \times 10^{-4}$ | $1{\cdot}808 \times 10^{-3}$ | $2{\cdot}205 \times 10^{-2}$ | $1{\cdot}440 \times 10^{-2}$ | $2{\cdot}422 \times 10^{-2}$ |
| $9{\cdot}6 \times 10^{-4}$ | $1{\cdot}648 \times 10^{-3}$ | $2{\cdot}817 \times 10^{-2}$ | $1{\cdot}426 \times 10^{-2}$ | $2{\cdot}165 \times 10^{-2}$ |
| $4{\cdot}8 \times 10^{-3}$ | $1{\cdot}209 \times 10^{-3}$ | $5{\cdot}744 \times 10^{-2}$ | $1{\cdot}475 \times 10^{-2}$ | $2{\cdot}043 \times 10^{-2}$ |
| $9{\cdot}6 \times 10^{-3}$ | | $6{\cdot}901 \times 10^{-2}$ | $1{\cdot}625 \times 10^{-2}$ | $1{\cdot}886 \times 10^{-2}$ |
| $2{\cdot}4 \times 10^{-2}$ | | | | $1{\cdot}648 \times 10^{-2}$ |
| $4{\cdot}8 \times 10^{-2}$ | | $4{\cdot}678 \times 10^{-2}$ | | $1{\cdot}423 \times 10^{-2}$ |
| $9{\cdot}6 \times 10^{-2}$ | | $3{\cdot}376 \times 10^{-2}$ | | |

Preliminary experiments showed that the glycine buffer, while having no effect on EPAB, catalysed the hydrolysis of PNPA, and therefore all our subsequent experiments were performed in Delory and King's buffer. The results are in Table 2. For each ester the observed first order rate constant ($k_1$) was used to calculate the *surfactant effect ratio* $k_1/k_0$, where $k_0$ is the value in the absence of CTAB. The values obtained plotted against log CTAB concentration (Fig. 1) illustrate the effect of the surfactant. It is clear that CTAB alters the rate of hydrolysis of the four esters at all concentrations above its cmc. However it increases the rate of hydrolysis of PNPA and EPNB and decreases the rate for EPAB and PAPA.

The magnitude of the modification is much greater for PNPA than for EPNB, possibly because there may be a difference in the association of the two esters with the CTAB. Increasing the concentration of CTAB in the presence of PNPA increases the surfactant effect ratio to a maximum value of 4·2 at a surfactant concentration of $9{\cdot}6 \times 10^{-3}$M. The subsequent fall in the ratio is probably attributable to an increase in counter ion (bromide) concentration causing displacement of hydroxyl ions from the area immediately surrounding the micelle surface, as postulated by Romsted & Cordes (1968). This effect, although operative throughout will become noticeable only after the maximum effect of surfactant has been obtained which, from our results, must lie between $9{\cdot}6 \times 10^{-3}$ and $5 \times 10^{-2}$M. Menger & Portnoy (1967), using n-dodecyltrimethyl ammonium bromide and Romsted & Cordes (1968) using CTAB have also demonstrated the potentiating effect of cationic surfactants on the hydrolysis rate of PNPA. There are quantitative differences between all these results that are probably due to the different buffer solutions used. Unlike our results, no decrease in surfactant effect ratio was reported, probably because the other workers used concentrations of CTAB below that giving a maximum value.

The result obtained with PAPA is similar to that for EPAB but the effect was only noted with PAPA above $1 \times 10^{-3}$M CTAB which could reflect a difference in association constants of the esters with CTAB.

The nature of the ester grouping on the aromatic ring does not therefore determine whether CTAB increases or decreases the hydrolysis rates, although it is still possible that it could influence the magnitude of the response. Rather it is the group on the

*p*-position that appears to determine the kind of modifying effect, probably by determining the site of drug-surfactant interaction, or the orientation of the drug with respect to the micellar surface, or both.

Where CTAB increases the rate of hydrolysis, the site of association must be the micellar surface, or close enough to it, for the ester linkage to be in a region of high hydroxyl ion concentration such as will be found close to the Stern layer. It would seem logical that the $\delta^-$charge associated with the nitro-group of PNPA and EPNB will cause these esters to orientate themselves with their $NO_2$ groups next to the polar head of the surfactant while the remainder of the molecule projects through the Stern layer into the hydroxyl ion enriched diffuse part of the double layer. Certainly it is unlikely that these esters will penetrate into the hydrocarbon centre of the micelle.

Replacement of the $NO_2$ group by the $NH_2$ group results in a reversal of the CTAB effect. Nmr spectroscopic measurements with a series of organic compounds of different structure (Eriksson & Gillberg, 1966) would suggest that EPAB and PAPA are bound close to the micellar surface, and a similar conclusion for EPAB was reached by Riegelman (1960) on the evidence of ultraviolet spectroscopy. If this is true, then the presence of the $NH_2$ group results, in some way, in the removal of the ester linkage from the region where hydrolysis can readily take place. The $NH_2$ group, with its $\delta+$ charge, will not interact with the micellar surface and it is feasible that it will penetrate into the micelle, thereby drawing the ester group into the viscous region surrounding the micelle into which hydroxyl ions find difficulty in diffusing. It is also feasible that these ester groupings could be brought into the viscous regions as a result of the molecule being adsorbed flat on to the surface of the micelle. An alternative explanation, one analogous to that given by Menger & Portnoy (1967) for the retardation of ester hydrolysis brought about by an anionic surfactant, is that protection is afforded the esters because they are adsorbed into the hydrocarbon interior of the micelles. Menger and Portnoy's conclusion was based on the results of an analysis that indicated that the rate of hydrolysis of the esters in the micelles was zero. In the absence of a similar analysis, which must await measurements on the association constants for micelle-ester interactions, it is not possible to reject a similar explanation for our results, but it would be surprising if the substitution of a $NH_2$ for a $NO_2$ group caused such a profound change in the penetration properties of the esters concerned.

It was necessary to ensure that the results obtained did not merely reflect a slight change in the experimental conditions used, since reported work has indicated that surfactant effects are complex and can be modified by many factors. For example the micellar effect on the base catalysed hydrolysis of *p*-nitrophenyl hexanoate (Romsted & Cordes, 1968) is influenced by the nature and presence of added salts, $NO_3^-$ and $Br^-$ ions changing a *potentiating* micellar effect to a *protective* one at high salt concentrations. In view of this, the effect of CTAB on the four esters was compared under identical conditions of pH, buffer strength and temperature (Table 3). At this lower pH the degradation of PAPA no longer followed first order kinetics. The effect, however, is qualitatively unequivocal and, in order to obtain an approximate quantitative measure of the effect, the initial rate was estimated and it is these values that are given in Table 3. The surfactant effect ratios show that the effect on the four esters is qualitatively the same as already described. However with EPAB, EPNB and PAPA, where the pH has been decreased, the magnitude of the surfactant effect has increased both in its potentiating and in its protective role. This could be

Table 3. *The effect of* 4·8 × $10^{-3}$M *CTAB on the rate of hydrolysis of the four esters at a concentration of* 4 × $10^{-5}$M, *at pH* 9·2 *and* 25°.

| Ester | $k_0$ value ($min^{-1}$) | $k_1$ value ($min^{-1}$) | Surfactant effect ratio ($k_1 : k_0$) |
|---|---|---|---|
| EPAB | 3·739 × $10^{-6}$ | 1·326 × $10^{-6}$ | 0·355 |
| PNPA | 1·611 × $10^{-2}$ | 5·744 × $10^{-2}$ | 3·565 |
| EPNB | 6·023 × $10^{-4}$ | 8·120 × $10^{-4}$ | 1·348 |
| PAPA | 1·674 × $10^{-3}$ | 1·040 × $10^{-3}$ | 0·621 |

because of the change in hydroxyl ion concentration or it could reflect the different ionic constitution of the buffer solutions used.

These results show that stability studies with solubilized systems should be made on the individual preparations and that results for one compound should not be extrapolated even to similar compounds in systems that have not been fully characterized.

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