

The Effect of pH and Various Additives on Extinction Coefficients for p-Nitrophenol^{*}

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Summary: The molar extinction coefficients for p-nitrophenol at pHs in the region of 8.0-9.2 in the presence and absence of various additives were determined. The data were statistically analysed. The validity and reproducibility of these values were discussed. The study reveals that pH has a considerable effect on molar extinction coefficient values. These values are however not influenced by such additives like cetyltrimethyl ammonium bromide (CTAB), various strengths of carbonate-bicarbonate buffer and potassium bromide at the same pH.

Introduction

Ester hydrolysis can occur in acid, alkaline or neutral solutions and a comprehensive review of these reactions has been compiled by Bamford and Tipper [1]. Lowry and Richardson [2] have given a detailed account of the possible mechanism involved in ester hydrolysis. Pertinent informations on such topics as the applicability of assay techniques, special assay conditions and reactions, and analytical details of some esters important to pharmacy, have also been recently provided in standard text [3,4]. Quite recently, Landini and Rolla [5] have reported a method for converting tert-butyl esters advantageously into carboxylic acids in a two-phase system at room temperature. This seems to be necessary to emphasize here that tert-butyl esters are highly stable under neutral and basic conditions and are indeed hydrolysed under acidic conditions.

The effect of a cationic surfactant, CTAB, on the base-catalysed hydrolysis

of various p-substituted esters including those of p-nitrophenyl acetate (PNPA) under various conditions have been studied in detail in this school [6-8]. In this regard a few papers have also been published [9-11]. During the study the breakdown product of the ester, i.e. p-nitrophenol was assayed spectrophotometrically from which the residual concentration of the ester was calculated. Such studies however require the knowledge of extinction coefficient values of the degradation product under the same conditions used during the hydrolytic studies of the ester. This paper therefore describes the results of the molar extinction coefficients determined for p-nitrophenol itself as well as for that obtained from the complete hydrolysis of PNPA at pHs 8.0, 8.6 and 9.2 in non-buffer systems and pH 9.2 in bicarbonate-carbonate buffer. pH-stat technique was used to maintain the pH in the absence of buffer. Full details of the experimental techniques are available [11].

^{*}This work was mainly carried out at the department of Pharmaceutics, School of Pharmacy & Pharmacology, University of Bath, England.

Material and Methods

p-Nitrophenol

This was of A.R. Grade supplied by Koch-Light Laboratories.

p-Nitrophenyl Acetate (PNPA)

In this work PNPA was prepared synthetically from *p*-nitrophenol by the method of Chattaway [12]. The product so obtained was washed with water and twice recrystallised from 50% ethanol; m.p. 77°, lit. 77.5 - 78° [13].

Cetyl Trimethyl Ammonium Bromide (CTAB)

Commercial samples of surfactants often contain impurities including homologues and unreacted starting materials. Such impurities cause minima in surface tension curves which are widely accepted as an indicator of surfactant purity [14].

The reagent grade sample of CTAB (BDH) was therefore purified according to the process given elsewhere [8]. m.p. 231.5°; lit. 230-4° [15]. The purity of the recrystallised sample was further checked by surface tension, NMR and mass spectrometry [8].

Buffer Solutions

Carbonate-bicarbonate buffer of single, double and triple strength at pH 9.2 was used. This was made according to Documenta Geigy [16].

Water

Freshly double distilled water through an all glass still was used in this work.

Spectrophotometer

Pye Unicam SP 1800 fitted with an SP 1805 Programme Controller and a Unicam AR 25 Linear Recorder.

Determination of Extinction Coefficient Values

Various concentrations of *p*-nitrophenol were prepared over the range $0.5-7.5 \times 10^{-5} \text{ M}$ at pH 8.0, 8.6 and 9.2 in water using the pH-stat and in various strengths of carbonate-bicarbonate buffer pH 9.2. Absorbance was then recorded on SP 1800 at the λ_{max} of *p*-nitrophenol (400 nm) and plotted according to Beer's Law. The data were submitted to a computerised least squares regression analysis which gave the slopes i.e. extinction coefficients (ϵ), standard deviation of slopes, intercepts and standard deviation of intercepts. All extinctions were determined in triplicate.

In order to check that these values were valid for the hydrolysis of the ester, the same series of concentrations of the ester (PNPA) ($0.5 - 7.5 \times 10^{-5} \text{ M}$) were subjected to a temperature of about 80° for 30 minutes to ensure complete hydrolysis. Preliminary studies showed no increase in absorbance after this time. Absorbances were then determined at 400 nm and were found to be essentially equal to those for *p*-nitrophenol at their corresponding concentrations. A typical Beer-Lambert plot for *p*-nitrophenol and 100% hydrolysed ester is shown in figure 2.

In order to determine the effect of CTAB on extinction coefficients, all glass-ware first aged with $9.6 \times 10^{-2} \text{ M}$ CTAB (the highest concentration of surfactant used during a previous kinetic study in this school) [11]. Glass and silica surfaces can acquire a negative charge when in contact with aqueous solution [17]. Due to this substantial negative charge density, glass surfaces have a strong affinity for cationic surface active agents. There are numerous reports on adsorption of surface active agent on to glass surfaces. Ter-Minassian-Saraga [18] has extensively stu-

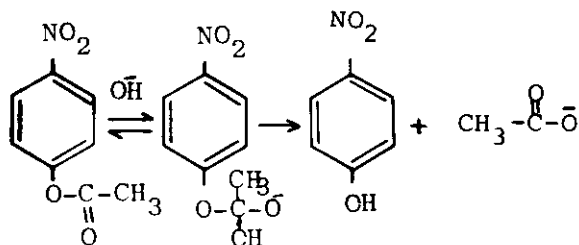
died the adsorption of alkyl-trimethylammonium ions on glass and silica surfaces. Blackman and Harrop [19] examined the reaction of alkyltrimethylammonium bromides of C_{4-20} chain length with silica surfaces using infra-red spectroscopy and reported that they were strongly bonded to the silica surface particularly those with C_{7-20} chain length.

Thus adsorption by the glass surfaces can be an important factor in studies of cationic surface active agents, especially at low concentrations. Therefore it was extremely important to pre-saturate all glass-ware with the surface active agent used [20]. Experience showed that measurements of surface tension and bromide ion concentration were not stable if experiments were performed in unaged glassware, whereas stable reproducible results were obtained when the glassware was treated to the ageing process given elsewhere [8].

After the process of ageing appropriate dilutions of p-nitrophenol and PNPA were made from their respective stock solutions containing 9.6×10^{-2} M CTAB and the extinction values were obtained in a similar manner.

Results and Discussion

In the system under study in this work, one component is the ester, p-nitrophenyl acetate (PNPA) and the second component is its degradation product, p-nitrophenol. On hydrolysis in alkaline medium one mole of PNPA gives one mole of p-nitrophenol: (Scheme 1) and therefore it was desirable to determine the relative contributions of PNPA and phenol to the observed absorbance. The hydrolysis kinetics of PNPA were studied by following the formation of the breakdown product, p-nitrophenol spectrophotometrically at 400 nm. The ultraviolet absorption



SCHEME-I

spectra of PNPA in distilled water and p-nitrophenol in carbonate-bicarbonate buffer at pH 9.2 were determined and are shown in Figure 1. Due to the fact that PNPA is existing in neutral form, therefore, the UV plot in water was considered to be valid for comparison. Figure 1 shows the ester does not absorb at the λ_{\max} of p-nitrophenol

(400 nm) and the data plotted in Figure 2 as well as table 1 which compare absorbances and extinction coefficients respectively for p-nitrophenol and fully hydrolysed PNPA is consistent with p-nitrophenol, being the sole breakdown product which absorbs at 400 nm, and also validates the assay technique employed during previous kinetic studies [11].

Molar extinction coefficients obtained from the slope of the Beer-Lambert plot appear to be insignificantly different from the values of p-nitrophenol. Replicate values of extinction coefficients of p-nitrophenol and 100% hydrolysed ester under various conditions and their associated slopes, standard deviation of slopes, intercepts, standard deviation of intercepts, together with calculated and tabulated Bartlett-Test values at a 0.05 probability level are given in table 1.

The yellow colour which results in the absorbance at 400 nm is due to the formation of the p-nitrophenolate anion. The pK_a of p-nitrophenol is 7.15 at 30° [21] (the temperature of the kinetic run, studied previously)[11]

Table 1: Molar Extinction Coefficient Values Determined Under Various Conditions

pH	System					Mean Extinction Coefficient	Standard Deviation of the Slope	Intercept	Standard Deviation of the Intercept x 10 ³	Correlation Coefficient	Bartlett Test	
	Buffer	KBr 0.5M	CTAB 9.6 x 10 ⁻²	PNP	PNPA						Calculated	Tabulated
8.0	-	-	-	+	-	16808	15.275	-0.0029	0.03066	0.9999	0.549	7.815
	-	-	-	+	-	16830	66.080	-0.0039	0.21916	0.9999		
	-	-	-	-	+	16811	34.116	-0.0034	0.13420	0.9999		
	-	-	-	+	+	16802	21.348	-0.0031	0.09466	0.9999		
8.6	-	-	-	+	-	18400	70.237	0.0018	0.23300	0.9999	0.945	7.815
	-	-	-	+	-	18370	106.301	0.0007	0.33256	0.9999		
	-	-	-	+	-	18520	153.188	-0.0134	0.50806	0.9999		
	-	-	-	-	+	18419	118.200	-0.0101	0.41367	0.9999		
9.2	-	-	-	+	-	18566	57.517	0.0010	0.17048	0.9999	0.935	11.070
	-	-	-	+	-	18496	100.924	-0.0040	0.30000	0.9999		
	-	-	-	+	-	18361	24.100	-0.0005	0.11540	0.9999		
	-	-	-	+	+	18406	52.054	-0.0008	0.15430	0.9999		
9.2	-	-	-	-	+	18437	60.000	-0.0002	0.17768	0.9999	0.125	11.070
	-	-	-	-	+	18521	35.781	0.0003	0.17137	0.9999		
	+	(S)	-	-	-	18588	51.664	-0.0026	0.29464	0.9999		
	+	(S)	-	+	-	18593	72.400	-0.0030	0.21061	0.9999		
9.2	+	(D)	-	+	-	18650	126.885	-0.0053	0.42083	0.9999	0.051	7.815
	+	(D)	-	+	-	18619	101.523	-0.0040	0.49562	0.9999		
	+	(D)	-	-	+	18635	85.889	-0.0029	0.46751	0.9999		
	+	(D)	-	+	-	18622	115.400	-0.0034	0.44677	0.9999		
9.2	+	(T)	-	+	-	18750	173.877	-0.0080	0.57668	0.9998	0.020	7.815
	+	(T)	-	+	-	18731	151.201	-0.0086	0.51421	0.9999		
	+	(T)	-	-	+	18722	110.604	-0.0078	0.50000	0.9998		
	+	(T)	-	+	-	18737	129.500	-0.0085	0.55214	0.9999		
9.2	+	(S)	+	+	-	18591	95.422	-0.0028	0.26426	0.9999	0.004	7.815
	+	(S)	+	+	-	18598	107.372	-0.0031	0.20300	0.9999		
	+	(S)	+	-	+	18590	80.249	-0.0021	0.22666	0.9999		
	+	(S)	+	+	+	18595	100.796	-0.0030	0.25111	0.9999		
9.2	+	(T)	+	+	-	18753	177.249	-0.0091	0.59460	0.9999	0.004	7.815
	+	(T)	+	+	-	18766	194.500	-0.0088	0.62113	0.9999		
	+	(T)	+	-	+	18759	158.247	-0.0087	0.59881	0.9999		
	+	(T)	+	+	+	18768	198.327	-0.0092	0.64221	0.9999		
9.2	+	(S)	+	+	-	18739	91.000	0.0028	0.30155	0.9999	0.042	7.815
	+	(S)	+	+	-	18722	56.568	0.0006	0.18761	0.9999		
	+	(S)	+	+	+	18746	120.554	-0.0006	0.40000	0.9999		
	+	(S)	+	+	+	18731	66.080	-0.0001	0.22000	0.9999		
9.2	-	-	+	+	-	18480	60.246	-0.0006	0.17421	0.9999	0.044	7.815
	-	-	+	+	-	18471	90.500	-0.0020	0.25242	0.9999		
	-	-	+	-	+	18491	40.000	-0.0004	0.19216	0.9999		
	-	-	+	+	+	18478	71.528	-0.0032	0.16144	0.9999		
9.2	-	-	+	+	-	18498	110.621	-0.0004	0.33391	0.9999	0.033	7.815
	-	-	+	+	-	18511	78.666	-0.0011	0.21300	0.9999		
	-	-	+	+	-	18490	50.000	-0.0005	0.30111	0.9999		
	-	-	+	+	+	18495	90.300	-0.0002	0.11000	0.9999		

N.Y. Double strength buffer
 + Presence of Triple strength buffer
 - Absence of PNP P-nitrophenol
 (S) Single strength buffer
 (D) P-nitrophenyl acetate
 (T) P-nitrophenol

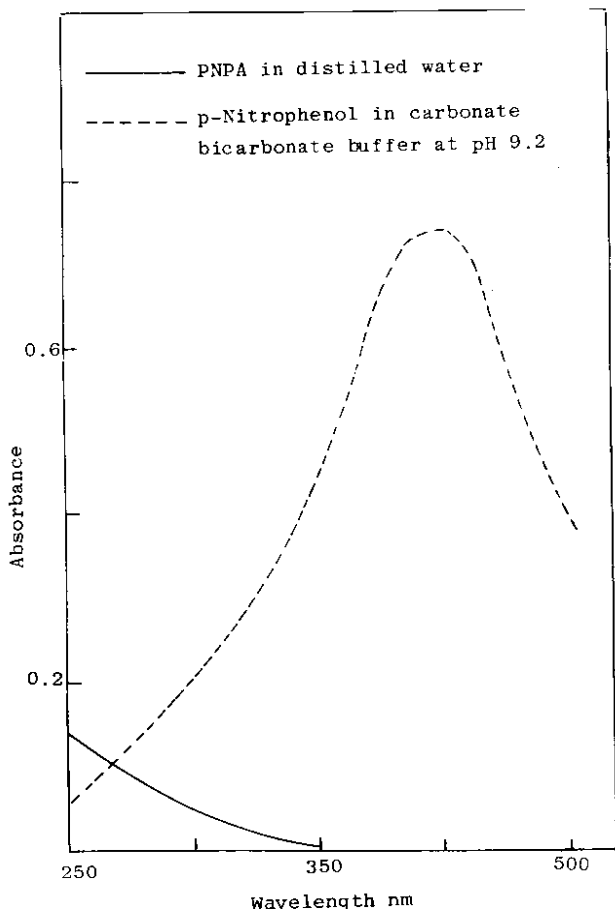


Fig.1: Ultraviolet Absorption Spectra of PNPA and p-Nitrophenol Anion at A Concentration of 4×10^{-5} Molar.

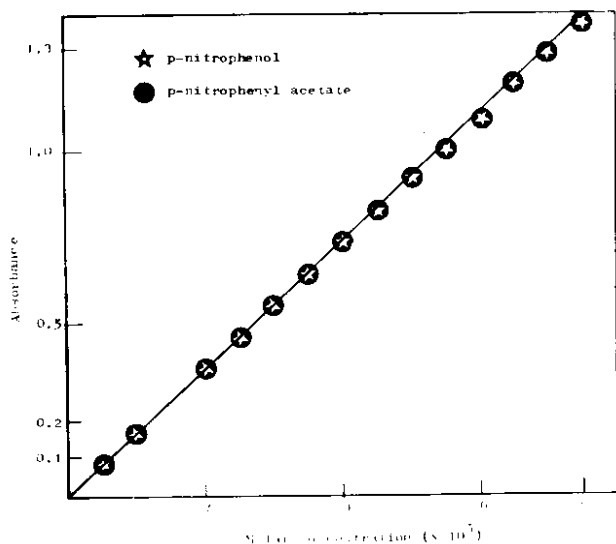


Fig.2: Beer-Lambert Plot For p-Nitrophenol and p-Nitrophenyl Acetate at pH 9.2 in Non-Buffer System and 30°C.

and therefore at the pHs 8.0, 8.6 and 9.2 the phenol will be approximately 87.6%, 98.4% and 99.1% in the union form respectively. These figures are obtained by the use of the Handerson-Hasselbalch equation in its standard form, ignoring activity corrections (equation 1).

$$\text{pH} = \text{pK}_a + \log \text{salt/acid} \quad \text{--(1)}$$

Thus the extinction coefficients for p-nitrophenol can be expected to increase with pH as shown in table 2. However small increase in extinction coefficient between the pHs 8.6 and 9.3 can be explained on the basis of the amount of p-nitrophenolate anion present as also evidenced by the above percental figures. At any given pH the extinction coefficients were reproducible, as evidenced by the X^2 values and coefficients of variation shown in table 2 and were not influenced by the presence of 9.6×10^{-2} M CTAB.

Assuming the degree of ionisation given above is correct, calculation using the experimental coefficients obtained at pH 8.0, 8.6 and 9.2 give values for the extinction coefficient of 100% ionised p-nitrophenolate as 19209, 18727 and 18633 respectively, which are in reasonable agreement, considering the sensitive nature of the calculation to small pH changes.

Table 1 shows that there is a slight increase in extinction coefficient from 18585 to 18735 as the buffer concentration is raised from single to triple strength. However the Bartlett Test shows this is insignificant giving a X^2 value of 0.996. The addition of 0.5 M potassium bromide (the highest concentration used during kinetic experiments) [8] produced a similar insignificant increase. That all these changes in extinction coefficient due to the addition of electrolyte are very small is evidenced by the overall coefficient of varia-

Table-2

Reproducibility of Extinction Coefficients for p-Nitrophenol at a Given pH in The Absence of Additives

pH	Molar Extinction coefficient	$X^2_{\text{calc.}}$	$X^2_{\text{tab.}}$ p=0.05	Coefficient of Variation (%)
8.0	16808	0.549	7.815	0.203
8.6	18427	0.945	7.815	0.607
9.2	18465	0.893	11.070	0.298

tion of 0.468% for all the systems examined at pH 9.2. Table 1 also shows that the extinction coefficients for the 100% hydrolysed ester are not significantly different from the values of p-nitrophenol at their corresponding pHs. This was taken as evidence that PNPA prepared in the laboratory was of a satisfactory purity. These facts therefore confirm that various additives like surfactants, buffer salts and neutral inorganic electrolytes do not have any significant effect on molar extinction coefficient values for p-nitrophenol at the same pH.

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