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Metal Ion Catalysis in the Hydrolysis of Esters of 2-Hydroxy-1,10-phenanthroline: The Effects of Metal Ions on Intramolecular Carboxyl Group Participation

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Rate constants have been determined for hydrolysis of the acetate, glutarate, and phthalate monoesters of 2-hydroxy-1,10-phenanthroline in water at 30°C and $\mu = 0.1$ M with KCl. The hydrolysis reactions of the esters are hydroxide ion catalyzed at pH > 9. The phthalate and glutarate monoesters have in addition pH-independent reactions from pH 5.5 to 9 that involve intramolecular participation by the neighboring carboxylate anion. The pH-independent reaction of the glutarate monoester is ~5-fold faster than that of the phthalate monoester. The plots of log k_{obsd} vs pH for hydrolysis of the carboxyl substituted esters are bell shaped at pH < 5, which indicates a rapid reaction of the zwitterionic species (carboxyl anion and protonated phenanthroline nitrogen). The divalent metal ions, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}, complex strongly with the esters; saturation occurs at metal ion concentrations less than 0.01 M. The 1:1 metal ion complexes have greatly enhanced rates of hydrolysis; the second-order rate constants for the OH^- reactions are increased by factors of 10^5 to 10^8 by the metal ion. The pH-rate constant profiles for the phthalate and glutarate ester metal ion complexes have a sigmoidal region below pH 6 that can be attributed to a metal ion-promoted carboxylate anion nucleophilic reaction. The carboxyl group reactions are enhanced 10^2- to 10^3-fold by the metal ions, which allows the neighboring group reaction to be competitive with the favorable metal ion-promoted OH^- reaction at pH < 6, but not at pH > 6. The half-lives of the pH-independent neighboring carboxyl group reactions of the Cu(II) complexes at 30°C are ~2 s. The other metal ion complexes are only slightly less reactive (half-lives vary from 2.5 to 40 s). These are the most rapid neighboring carboxyl group reactions that have been observed in ester hydrolysis.

INTRODUCTION

A carboxyl group has been implicated in the catalytic action of the esterase and peptidase Zn(II) metalloenzyme carboxypeptidase A (*1–5*). A nucleophilic mechanism involving the γ-carboxyl group of Glu-270 has been proposed for the enzyme-catalyzed hydrolysis of ester substrates (*3,6,7*), although trapping experiments designed to demonstrate the presence of an anhydride intermediate have not been successful (*8*). In addition to the intrinsic chemical interest, knowledge of the mechanisms and the magnitude of the rate constants for coordinated metal ion and carboxyl group participation in the hydrolysis of acyl derivatives is necessary for the meaningful assessment of proposed enzyme mechanisms.

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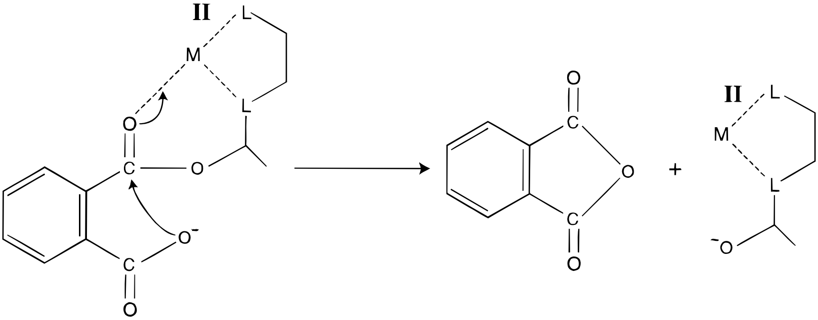
A neighboring carboxyl group can participate in the hydrolysis of esters as an intramolecular nucleophile without assistance from metal ions (*9–15*). However, the rate constants in the nucleophilic reactions are considerably less than those in carboxypeptidase A esterase reactions (*16,17*). The intramolecular carboxyl group reactions of esters continue to be of great interest as examples of “steric fit” in nucleophilic reactions (*15*).

The demonstration of bifunctional mechanisms in hydrolytic reactions is important because many enzymatic reactions give bell-shaped pH-rate constant profiles that may be due to such mechanisms. Esters with two substituent carboxyl groups can give k_{obsd} vs pH profiles that are bell shaped (*13,14*). The profile for the reaction of salicyl succinate was interpreted in terms of the concerted bifunctional mechanisms **I** and **II** where a carboxylate anion acts as a nucleophile and an un-ionized carboxyl functions as a general acid with proton donation to oxygen (Scheme 1). However, Maugh and Bruice (*14*) considered that the reactions of salicyl succinate involve nucleophilic attack by a carboxylate anion and a changing inductive effect as the second carboxyl group ionizes, or a “series nucleophilic” reaction in which there are two stepwise nucleophilic processes. The same general conclusions were drawn in regard to other proposed bifunctional mechanisms. A properly located chelated metal ion might function with great effect analogous to a general acid in a bifunctional reaction, and there would be no kinetic ambiguity (Eq. [1]).[1]



[1]

Determination of the effects of metal ions in intramolecular nucleophilic reactions is difficult: first, because of the necessity of the metal ion binding to the substrate in a position that is remote from the nucleophile, and secondly, because of the extremely favorable metal ion-promoted OH^- catalysis that is always observed in the hydrolysis of esters having metal ion liganding groups (*19–26*) The facile OH^- reaction restricts competition from other mechanistic pathways (*23*). Rate enhancements in metal ion-promoted OH^- reactions of acyl derivatives are very large (*19,20,23,25–31*), ranging up to 10^8-fold with Cu^{2+} (10^{10}-fold with an acyl phosphate) (*31*). In contrast, a significant effect (10^2- to 10^4-fold) has only been observed in metal ion-promoted carboxyl nucleophilic reactions in cases where the leaving group is an aliphatic alcohol and the metal ion can complex the leaving group oxygen in the transition state (*24,25*). Complexed divalent metal ions were previously found to have little or no

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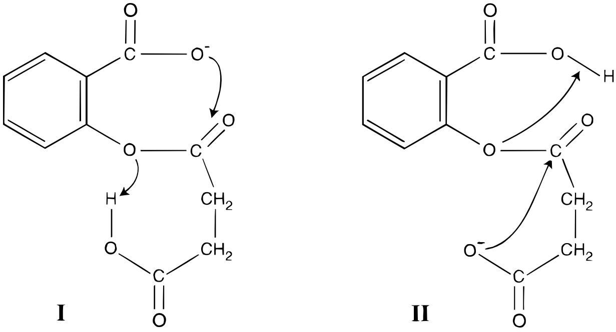
[1] For an analogy of general acid catalysis and metal ion catalysis in acetal hydrolysis see Ref. (*18*).



SCHEME 1

effect in carboxyl group nucleophilic reactions of esters of 8-hydroxyquinoline and 2-carboxy-8-hydroxyquinoline (*22,23*). However, there have been no previous studies of the effects of a metal ion strongly complexed to the carbonyl oxygen of an ester with a carboxyl group substituent capable of functioning as a nucleophile.[2,3] If a carboxyl nucleophilic mechanism is to be considered chemically feasible for an esterase metalloenzyme, then such a mechanism must be capable of generating much larger rate constants and rate enhancements than have hitherto been observed in chemical reactions. It is therefore important to investigate systems that are maximally suited to provide large rate constants.

The 1,10-phenanthrolyl ligand has advantages for the study of metal ion effects, among which is the very strong complexing ability of the phenanthroline nitrogens (*23,31–34*). The position of the metal ion in a 1:1 complex can often be specified. Furthermore, the neutral liganding groups will minimize the charge reduction on the metal ion that results with negatively charged ligands. In esters of 2-hydroxy-1,10-phenanthroline a metal ion will be complexed by the phenanthroline nitrogens and can also complex with the ester carbonyl oxygen in a stable 6-membered chelate ring. A phthalate monoester would allow intramolecular carboxyl group attack via a kinetically favored 5-membered ring transition state in which the strength of metal ion binding should increase as negative charge is generated on oxygen. Therefore, we have determined the effects of the divalent metal ions Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} in the reactions of 2-(1,10-phenanthrolyl) hydrogen phthalate (**III**) and for comparison, the corresponding monoglutarate (**IV**) and acetate (**V**) esters (Scheme 2). The carboxyl group substituent of **III** and **IV** will not be bound to the metal ion in a 1:1 complex because of the unfavorable large ring size required for such chelation.

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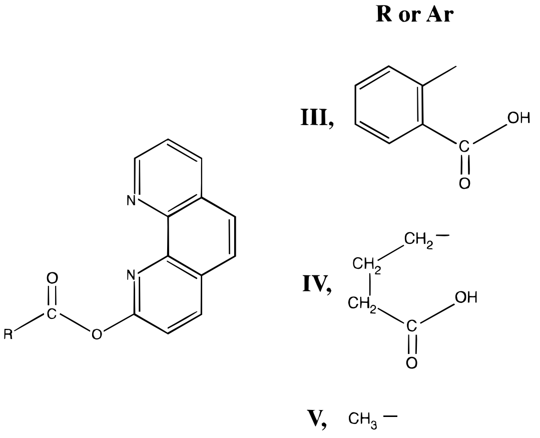
[2] The metal ion complexation of the carbonyl oxygen of salicyl 1,10-phenanthroline-2-carboxylate could be strong, but nucleophilic attack by the carboxylate anion would only result in a reversible rearrangement and is therefore not detected (*23*).

[3] Metal ion complexation of the carbonyl oxygen of amides results in rate retardation in the neighboring carboxyl group reaction (*32*).



SCHEME 2

EXPERIMENTAL

Materials. 2-Hydroxy-1,10-phenanthroline was prepared by the synthetic scheme previously outlined (*35*) and was recrystallized from a benzene-ethanol mixture, mp 159–160 °C; lit. (*35*), mp 159–160 °C. The synthesis of the esters **III** and **IV** was carried out by dissolving freshly prepared 2-hydroxy-1,10-phenanthroline in tetrahydrofuran that had been distilled and dried. An equivalent amount of the appropriate anhydride was added and the solution was refluxed for at least 48 h. The solvent was then removed by rotary evaporation. The oil residue was then extracted several times with dry benzene. The solid that was obtained was recrystallized from dry chloroform or benzene. The acetate ester **V** was prepared similarly except that excess acetic anhydride was employed. 2-(1,10-Phenanthrolyl) hydrogen phthalate (**III**) had mp 142°C after recrystallization. *Anal.* Calcd for $C_{20}H_{12}N_2O_4$: C, 69.76; H, 3.51; N, 8.12. Found: C, 69.94; H, 3.49; N, 8.12. 2-(1,10-Phenanthrolyl) hydrogen glutarate (**IV**) had mp 148 °C after recrystallization. *Anal.* Calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.69; H, 4.53; N, 9.13. 2-(1,10-Phenanthrolyl) acetate (**V**) had mp 152 °C after recrystallization. *Anal.* Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.55; H, 4.30; N, 11.78. Spectra were consistent with the expected structures. The esters are sensitive to air, but can be stored in a desiccator in the dark at low temperature.

Acetonitrile was Eastman Kodak Spectrograde. Amine buffer components were recrystallized or distilled prior to use.

Kinetic measurements. The rates of hydrolysis of **III–V** were measured with a Beckman DU-7500 or a Pye Unicam SP8-100 spectrophotometer by following the absorbance increase at 300–320 nm. The buffers employed were formate, acetate, cacodylate, 2,6-lutidine, *N*-ethylmorpholine, Tris, morpholine, and carbonate. All buffer solutions were maintained at a constant ionic strength of 0.1 M with KCl. The buffer concentration was 0.02 M; buffer catalysis is negligible at that concentration. A typical kinetic run was initiated by injecting 20–30 μL of a 0.004 M stock solution of the ester in acetonitrile into 3 mL of buffer maintained at 30 °C. Product spectra at the conclusion of the reactions matched those of identical concentrations of 2-hydroxy-1,10-phenanthroline and the appropriate carboxylic acid. The solutions employed for kinetic measurements in the absence of metal ions included 2×10^{-5} M EDTA.

Reactions that were too fast to be measured with a conventional spectrophotometer were monitored with a Durrum D-110 stopped-flow spectrophotometer equipped with a Hewlitt-Packard Model 1207B storage oscilloscope. The substrate was dissolved at the desired concentration in 10^{-2}–10^{-3} M HCl, $\mu = 0.1$ M with KCl. This solution was introduced into one of two identical drive syringes. The second syringe contained the appropriate buffer maintained at the same ionic strength and the chosen metal ion (Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}). With each buffer three to four reactions were tabulated. The observed reactions followed good first-order kinetics, and infinity points were stable. The hydrolysis of phthalic anhydride or glutaric anhydride has no effect on the observed kinetics in the reactions of **III** and **IV** monitored at 320 nm. Pseudo-first-order rate constants (k_{obsd}) were computer-calculated.

Reaction mixture pH values were measured with a Radiometer-type PHM 22A pH meter equipped with a combination electrode. In calculating second-order rate constants for hydroxide ion catalysis (k_{OH}) the ion product of water, K_w, was taken to be 1.47×10^{-14} at 30°C.

RESULTS

In Fig. 1 a plot is shown of log k_{obsd} vs pH for hydrolysis of 2-(1,10-phenanthrolyl)-hydrogen phthalate (**III**). The plot has a slope of 1.0 at high pH, which indicates a

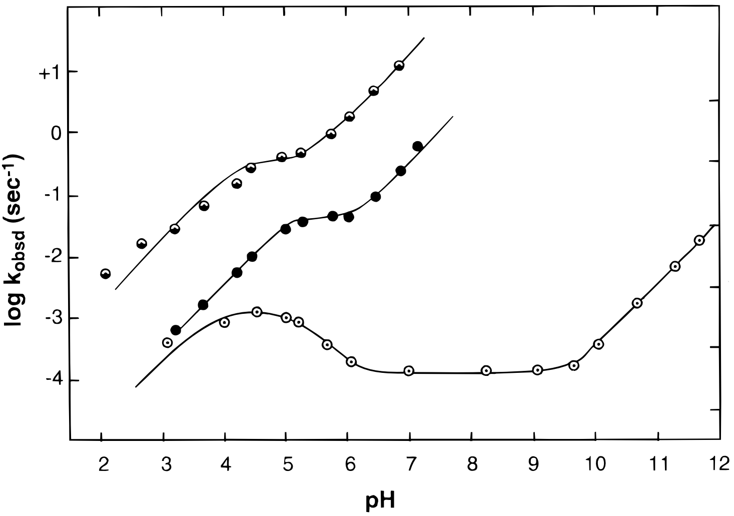
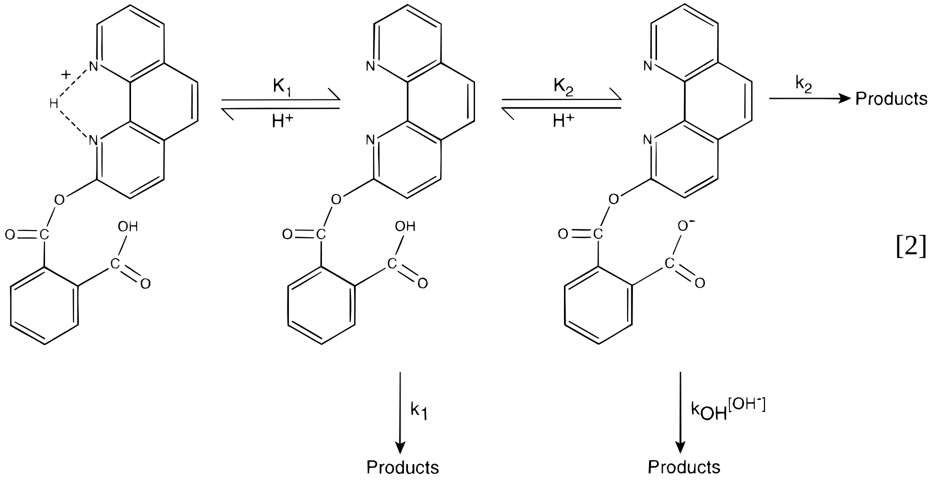


FIG. 1. Plot of log k_{obsd} vs pH for the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen phthalate in H_2O at 30°C, $\mu = 0.1$ M with KCl, in the presence of 0.002 M Cu^{2+} (◒) and 0.004 M Zn^{2+} (●), and in the absence of metal ion (⊙).

hydroxide ion-catalyzed reaction. At pH 6–9 there is a pH-independent reaction, and at pH $<$ 6 the plot of Fig. 1 is bell shaped. The reaction scheme is that of Eq. [2].



[2]

The equation for k_{obsd} is then

$$k_{obsd} = \frac{k_1K_1a_H + k_2K_1K_2 + k_{OH}K_1K_2\,[OH^-]}{a_H{}^2 + K_1a_H + K_1K_2}, \qquad [3]$$

where K_1 and K_2 are the first and second dissociation constants. Thus, the pH-independent region of Fig. 1 represents a reaction of the anionic species, while the bell shaped portion of the profile is due to a reaction of the neutral species, or more likely a kinetic equivalent zwitterion (ionized carboxyl group and protonated nitrogen). Values of the rate constants are given in Table 1. The apparent pK_a values of 4.0 and 5.0 provide a good fit to the experimental data employing Eq. [3]. There is, however, some positive deviation at low pH ($<$ 3) which indicates the incursion of a protonated species reaction. The solid line in Fig. 1 is theoretical for Eq. [3] and the constants in Table 1.

A similar plot of log k_{obsd} vs pH is also produced in the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen glutarate (**IV**), as seen in Fig. 2, and again Eq. [3] provides a good fit for the experimental data with pK_{app} 3.5 and 4.5. The rate constants for **IV** in Table 1 are several-fold larger than those of **III**.

The divalent metal ions Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} bind strongly to **III–V**. Figure 3 is a plot of k_{obsd} vs the concentration of Zn^{2+} in the hydrolysis of **IV** at pH 3.2. Saturation occurs at Zn^{2+} concentrations less than 0.005 M. The equation for k_{obsd} at constant pH is

$$k_{obsd} = \frac{k_MK_M\,[M^{2+}]}{(1 + K_M\,[M^{2+}])}, \qquad [4]$$

where K_M is the metal ion association constant and k_M is the rate constant at the

TABLE 1

Values of the Rate Constants for Hydrolysis of 2-(1,10-Phenanthrolyl) hydrogen Phthalate (**III**), Glutarate (**IV**), and Acetate (**V**) in Water at 30°C and $\mu = 0.1$ M with KCl

Compound	Metal ion	k_1 (s^{-1})	k_2 (s^{-1})	k_{OH} ($M^{-1}\ s^{-1}$)	pK_{app}
III	None	2×10^{-3}	1.3×10^{-4}	2.5	4.0, 5.0
	Cu^{2+}		4.2×10^{-1}	1.1×10^{8}	4.3
	Ni^{2+}		1.8×10^{-1}	9.0×10^{7}	4.5
	Zn^{2+}		4.5×10^{-2}	2.1×10^{6}	5.0
	Co^{2+}		1.6×10^{-2}	1.0×10^{6}	5.1
IV	None	1×10^{-2}	7.0×10^{-4}	12.2	3.5, 4.5
	Cu^{2+}		2.5×10^{-1}	6.8×10^{7}	4.4
	Ni^{2+}		1.3×10^{-1}	2.7×10^{7}	4.15
	Zn^{2+}		1.3×10^{-1}	4.3×10^{7}	4.15
	Co^{2+}		6.7×10^{-2}	4.0×10^{6}	4.2
V	None			10.2	
	Cu^{2+}			1.2×10^{6}	
	Ni^{2+}			2.7×10^{5}	
	Zn^{2+}			9.0×10^{4}	
	Co^{2+}			8.8×10^{4}	

specified pH for hydrolysis at a saturating concentration of the metal ion. Values of the association constants are given in Table 2.

Plots of log k_{obsd} vs pH are shown in Figs. 1 and 2 at saturating concentrations of Cu^{2+}, Zn^{2+}, and Co^{2+}. Similar plots were also obtained with Ni^{2+}, but were omitted

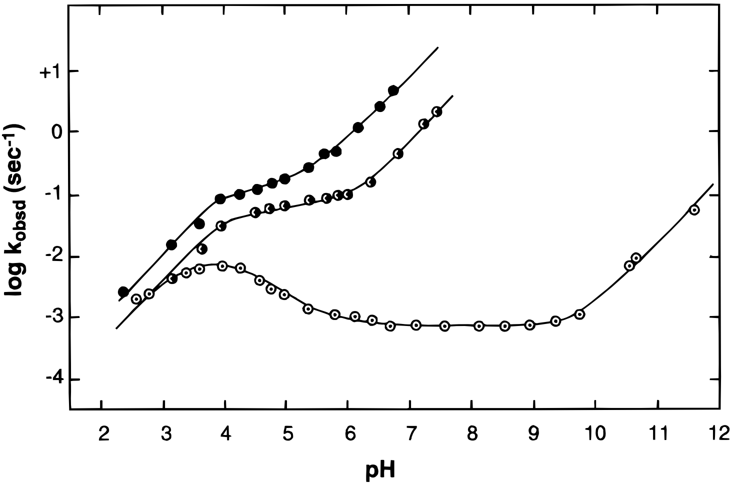


FIG. 2. Plot of log k_{obsd} vs pH for the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen glutarate in H_2O at 30°C, $\mu = 0.1$ M with KCl, in the presence of 0.003 M Zn^{2+} (●) and 0.003 M Co^{2+} (◑), and in the absence of metal ion (⊙).

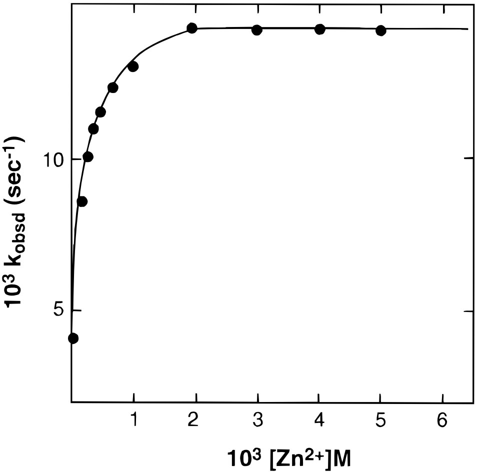


FIG. 3. Plot of k_{obsd} vs the concentration of Zn^{2+} at pH 3.20 in the hydrolysis of 2-(1,10-phenanthrolyl) hydrogen glutarate in H_2O at 30°C, $\mu = 0.1$ M with KCl.

from the figures for clarity. The plots have a slope of 1.0 at pH > 6, and a pH-independent region from pH 4 to 6, which is followed by a downward bend to again give a line of slope 1.0 at lower pH. The reaction scheme for hydrolysis of the 1:1 metal ion complexes **VI–VIII** is given in Eq. [5].

TABLE 2

Values of the Association Constants for Binding of Divalent Metal Ions with 2-(1,10-Phenanthrolyl) hydrogen Phthalate (**III**), Glutarate (**IV**), and Acetate (**V**) in Water at 30°C and $\mu = 0.1$ M with KCl

Compound	Metal ion	$K_M\ M^{-1}$
III	Cu^{2+}	2×10^{4a}
	Ni^{2+}	7×10^{3a}
	Zn^{2+}	2.5×10^{4b}
	Co^{2+}	7×10^{3b}
IV	Cu^{2+}	1×10^{3c}
	Ni^{2+}	3.5×10^{3a}
	Zn^{2+}	5×10^{3a}
	Co^{2+}	4×10^{3a}
V	Cu^{2+}	10^{5d}
	Ni^{2+}	3×10^{4d}
	Zn^{2+}	3×10^{4d}
	Co^{2+}	1×10^{4d}

[a] At pH 3.20.
[b] At pH 4.28.
[c] At pH 2.80.
[d] At pH 6.20.