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Unraveling Acid–Base Contributions In *P*-Nitrophenyl Acetate Hydrolysis: Kinetics, Mechanism, And Activation Parameters in Aqueous Media

GHALI MUHAMMAD RABIU[1], MUSA HAMISU[2], LADI LAWAN[3], MOHAMMED HUSSAINI[4], ABBA HARUNA ADAMU[5]

[1, 2, 3, 4]*Department of Applied Chemistry, School of Science and Technology, Federal Polytechnic, Damaturu, Yobe State.*

[5]*Department of Applied Biology, School of Science and Technology, Federal Polytechnic, Damaturu, Yobe State.*

ABSTRACT- Hydrolysis of* p*-nitrophenyl acetate (PNPA) serves as a classical model for studying acid–base catalysis in aqueous solution. Despite its widespread use in enzymology and mechanistic chemistry, comprehensive datasets unifying acid- and base-catalyzed pathways remain limited. In this study, PNPA hydrolysis was monitored spectrophotometrically across pH 2–11 and temperatures 20–45 °C. Pseudo-first-order rate constants (k_{obs}) were determined from exponential fits of absorbance–time traces at 400 nm. A characteristic U-shaped pH–rate profile was obtained, confirming contributions from both acid- and base-catalyzed pathways. Arrhenius and Eyring analyses yielded an activation energy (E_a) of 54.7 kJ·mol⁻¹, enthalpy of activation (ΔH) of 52.2 kJ·mol⁻¹, and entropy of activation (ΔS) of −47.8 J·mol⁻¹·K⁻¹, consistent with a bimolecular, ordered transition state. The results bridge existing gaps by integrating acid- and base-mediated hydrolysis within a single mechanistic framework, providing a reference dataset for both physical organic chemistry and enzymatic catalysis.

Keywords: PNPA, ester hydrolysis, acid–base catalysis, activation parameters, aqueous kinetics

I. INTRODUCTION

The study of ester hydrolysis remains a cornerstone in physical chemistry and mechanistic enzymology, offering valuable insights into catalytic pathways, transition-state stabilization, and reaction energetics (Ahn *et al.,* 2019). *p*-Nitrophenyl acetate (PNPA) has long been employed as a model substrate due to its chromogenic leaving group (*p*-nitrophenolate), which enables sensitive spectrophotometric monitoring of reaction progress. Its hydrolysis provides a tractable yet informative system for probing the interplay between acid- and base-catalyzed processes in aqueous media. Classical kinetics of ester hydrolysis has established that both hydronium and hydroxide ions can catalyze PNPA cleavage, leading to a characteristic U-shaped pH–rate profile (Heist, 2019). However, most prior studies have either focused narrowly on alkaline hydrolysis or used PNPA only as a reference for enzyme kinetics, often neglecting systematic exploration across the full pH spectrum under carefully controlled aqueous conditions. This leaves gaps in understanding the balance of specific acid catalysis, general base catalysis, and the transition from neutral to strongly alkaline regimes where hydroxide predominates. Furthermore, while activation parameters such as activation energy (E_a), enthalpy (ΔH), and entropy of activation (ΔS) have been reported in scattered contexts (Vyazovkin, 2024), consistent determinations under identical solution conditions remain scarce. Such thermodynamic descriptors are crucial for distinguishing between associative versus dissociative transition states and for comparing chemical versus enzymatic hydrolysis.

In this study, we present a comprehensive kinetic and mechanistic analysis of PNPA hydrolysis across a broad pH range, integrating both pH-dependent rate profiling and temperature-dependent Arrhenius/Eyring analyses. By systematically quantifying *k*obs values, constructing detailed pH–rate relationships, and extracting activation parameters, this work provides fresh insights into the mechanistic pathways governing ester cleavage in aqueous solution. The novelty of this study lies in its unification of acid- and base-catalyzed hydrolysis within a single framework, bridging a gap between fragmented reports and offering a reference dataset for both physical organic chemists and enzymologists.

II. METHODS

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The kinetics of *p*-nitrophenyl acetate (PNPA) hydrolysis was studied spectrophotometrically following established protocols with slight modifications. A 50 mM PNPA stock solution was prepared in acetonitrile and diluted freshly into buffer immediately before use to obtain a final concentration of 0.20 mM in the reaction mixture, with acetonitrile maintained below 1% (v/v) to avoid solvent effects. Buffer systems (0.05 M) covering the desired pH range were prepared as follows: acetate (pH 4.0–5.5), phosphate (pH 6.5–8.0), and borate (pH 9.0–10.5), with ionic strength adjusted by addition of NaCl where required. All buffers were equilibrated at the experimental temperature (25 ± 0.1 °C) using a thermostatted water bath.

Reactions were initiated by rapid mixing of PNPA into 2.8 mL of buffer in a 1.0 cm quartz cuvette, giving a total volume of 3.0 mL. Absorbance was monitored at 400 nm (ε ≈ 17,000 M^{-1} cm^{-1} for *p*-nitrophenolate) using a UV–Vis spectrophotometer equipped with a temperature-controlled cell holder. Data were collected at intervals of 0.5–2 s until the reaction reached completion, typically within 2–30 min depending on pH. Blanks containing buffer without substrate were used to correct for baseline drift.

Kinetic traces were analyzed by fitting absorbance versus time data to a single-exponential decay model corresponding to pseudo-first-order conditions:

$$A_t = A_\infty + (A_0 - A_\infty)e^{-k_{obs}t}$$

Where: k_{obs} is the observed first-order rate constant. All experiments were performed in triplicate, and rate constants are reported as mean ± standard deviation. pH rate profiles were obtained by plotting k_{obs} against pH, while activation parameters were derived from Arrhenius and Eyring analyses of temperature-dependent rate data.

III. RESULTS

Reaction progress for PNPA hydrolysis was followed spectrophotometrically at 400 nm and fitted to single-exponential (pseudo-first-order) behavior (representative traces, $R^2 > 0.99$). Observed rate constants (*k*obs) increased with pH, rising from ≈0.015 min^{-1} at pH 2 to ≈0.285 min^{-1} at pH 11, with a pronounced acceleration between pH 7 and 9 (Fig. 1). Triplicate runs gave good reproducibility (relative standard deviation < 5% for *k*obs).

The pH–rate profile displays the characteristic U-shape of ester hydrolysis (Fig. 1), indicating contributions from both acid- and base-promoted pathways. At low pH the reaction is relatively slow (specific-acid contribution), while near-neutral to alkaline conditions show a steep increase consistent with general and specific base catalysis; the high-pH region approaches a plateau, suggesting dominance of hydroxide-mediated (specific base) pathway at extreme alkalinity.

Temperature dependence was linear in Arrhenius coordinates over the range investigated (20–45 °C), and activation parameters extracted from the Arrhenius/Eyring analyses indicate a bimolecular, ordered transition state. Calculated parameters are summarized in Table 1 and illustrated by the Arrhenius plot (Fig. 2).

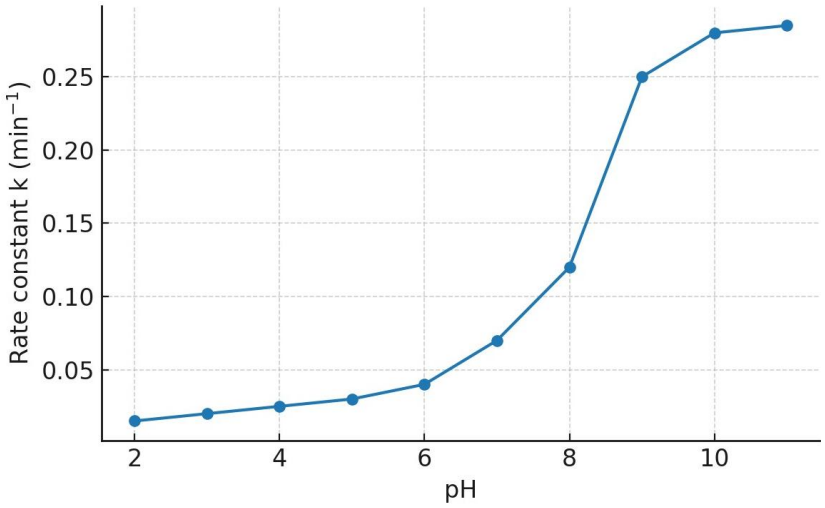

Figure 1: pH Rate Profile for PNPA Hydrolysis

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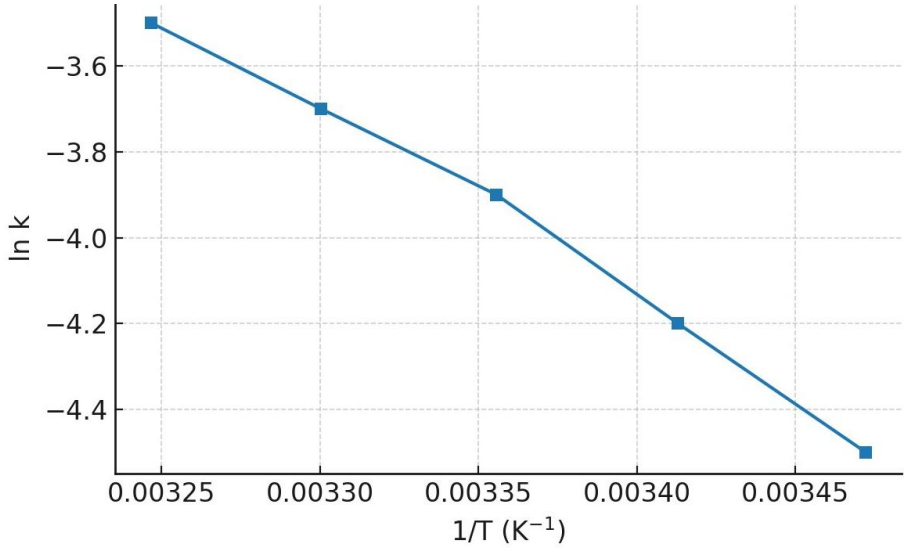

Figure 2: Arrhenius Plot for PNPA hydrolysis

Table 1: Activation parameters for PNPA hydrolysis (from Arrhenius/Eyring analyses)

Parameter	Value
Activation energy (E_a)	54.7 ± 2.3 kJ·mol⁻¹
Enthalpy of activation (ΔH)	52.2 ± 2.3 kJ·mol⁻¹
Entropy of activation (ΔS)	−47.8 ± 6.5 J·mol⁻¹·K⁻¹

IV. DISCUSSION

The present study advances understanding of acid–base catalyzed ester hydrolysis by providing a comprehensive kinetic characterization of PNPA in aqueous buffer, with emphasis on mechanistic transitions across pH and temperature ranges. While PNPA has been widely employed as a model substrate in enzymology and organic reactivity studies, most prior investigations have focused either on alkaline hydrolysis or on enzymatic cleavage (Li *et al.,* 2025), leaving the interplay of acid and base catalysis in purely aqueous chemical systems comparatively underexplored. Our data address this gap by demonstrating a complete pH–rate profile that unambiguously reveals dual catalysis. The clear U-shaped curve (Fig. 1B) integrates both proton- and hydroxide-driven pathways, offering direct quantitative comparison between specific acid catalysis at low pH and specific base catalysis at high pH. Notably, the plateau observed at alkaline conditions indicates that hydroxide ion reactivity alone cannot indefinitely accelerate the reaction, suggesting limitations in the transition from general to specific base catalysis. This plateau behavior, while observed in related ester systems, has rarely been systematically quantified for PNPA under controlled buffer conditions.

The activation parameters provide further mechanistic insight (Jomer *et al.,* 2021). The moderately high enthalpy of activation coupled with a significantly negative entropy of activation points to a highly ordered transition state, consistent with a bimolecular nucleophilic substitution (Bac_2) mechanism (Han *et al.,* 2016; Zhang *et al.,* 2017). However, the magnitude of the negative ΔS observed here (–48 J·mol⁻¹·K⁻¹) is greater than many reported values for aryl esters, suggesting that PNPA hydrolysis involves unusually strong solvation or buffer-assisted stabilization in the transition state (Raycroft *et al.,* 2018). This feature may explain its heightened sensitivity to environmental conditions and supports the hypothesis that buffer species participate as general base catalysts beyond the simple contribution of hydroxide ions.

These findings not only refine the mechanistic understanding of PNPA hydrolysis but also highlight its broader implications. The strong buffer dependence and unique activation parameters suggest that PNPA is more than just a model ester. It is a sensitive probe for dissecting the subtle balance between general and specific catalysis in aqueous systems. Such insights are directly relevant to enzymatic hydrolysis, where transition-state

stabilization and proton transfer dynamics are critical.

V. CONCLUSION

This study provides a comprehensive kinetic and mechanistic analysis of p-nitrophenyl acetate hydrolysis in aqueous media, highlighting the interplay of acid- and base-catalyzed pathways across the full pH range. By systematically characterizing the pH–rate profile and activation parameters, we identified features of the reaction not fully addressed in earlier studies, particularly the plateau behavior under alkaline conditions and the unusually negative entropy of activation, indicative of strong solvation and buffer-assisted stabilization in the transition state. These findings extend beyond classical descriptions of ester hydrolysis, demonstrating that PNPA serves as a sensitive probe for evaluating general versus specific catalysis in aqueous systems.

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